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TOUCHDOWN®: Determination of Glyphosate and  
Aminomethylphosphonic Acid in Corn Grain, Corn Forage, and  
Corn Fodder by Gas Chromatography and Mass-Selective Detection

Data Requirement

Guideline § 171-4(c)

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Performing Laboratory

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Study Number

GLYP-92-AM-04

Report Number

RR '92-042B

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Study Number: GLYP-92-AM-04  
Report Number: RR 92-042B  
Study Title: TOUCHDOWN®: Determination of Glyphosate and Aminomethylphosphonic Acid in Corn Grain, Corn Forage, and Corn Fodder by Gas Chromatography and Mass-Selective Detection

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study meets the requirements for 40 CFR Part 160, except that a study conduct audit was not performed specifically for this study. An audit was conducted for study 0224-89-MR-02 on July 8, 1992, using the same method and samples used in this study.

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Study No.: GLYP-92-AM-04  
Study Title: Validation of an Analytical Method for Residues of Glyphosate and AMPA in Corn Seed, Corn Forage and Corn Fodder

**QUALITY ASSURANCE STATEMENT**

In accordance with ZENECA 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.

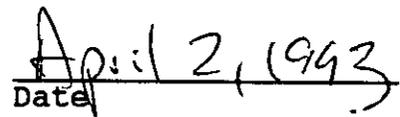
<u>Date</u>	<u>Inspection/Audit</u>	<u>Report Date</u>
September 28, 1992	Protocol	October 7, 1992
March 8, 1993	Data and final report	March 8, 1993

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

July 8, 1992	Procedure	July 24, 1992
May 21, 1992	Lab Facility	June 4, 1992

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

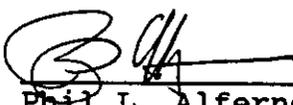
  
Kenneth D. Walburn  
Quality Assurance Specialist

  
Date

Study Number: GLYP-92-AM-04  
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CERTIFICATION OF AUTHENTICITY

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

Study Director  \_\_\_\_\_ Date 4/2/93 \_\_\_\_\_  
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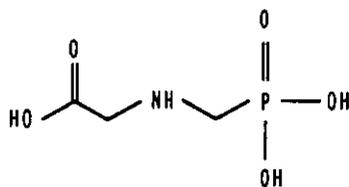
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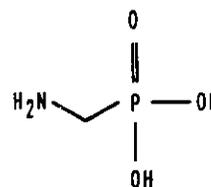
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1 SUMMARY/INTRODUCTION

This method is intended for the determination of residues of glyphosate [N-(phosphonmethyl)glycine, PMG] and aminomethylphosphonic acid (AMPA) in corn commodities. PMG is the 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 crop commodities by maceration with water. The crude extract is partitioned with chloroform and then subjected to a cation-exchange cleanup procedure. The analytes in the purified 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. 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 reagents and volatile by-products are evaporated, and the residuum is dissolved in ethyl acetate. The extract is analyzed using capillary gas chromatography 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 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 sampler/injector.

2.1.2 Mass-selective detector. Hewlett-Packard model 5970A mass-selective detector with Unix ChemStation software, version A.01.04. 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 with a 0.25- $\mu$ m 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 (Restek #20784).

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

2.1.6 Column connectors. Press-tight column unions (J&W Scientific #705-0705).

- 2.1.7 Syringes, gas chromatography (GC) injection. 10- $\mu$ L capacity (Hamilton 701N) for HP 7673 autosampler.
- 2.1.8 Syringes, sample handling. 10-, 25-, 100-, 250- $\mu$ L capacity Gas-Tight syringes (Hamilton 1700 series) for derivatized extract and standard handling.
- 2.1.9 Syringe, crude extract handling. 3-mL plastic, disposable syringe with Luer-Lok fittings (Becton/Dickenson #9585).
- 2.1.10 Eppendorf automatic pipet. Eppendorf Trivolume pipet, with disposable tips; 20-, 25-, and 50- $\mu$ L volumes (Brinkman #022334107, Baxter #P5060-20B); 100-, 200-, and 250- $\mu$ L volumes (Brinkman #022334301, Baxter #P5060-100B); 500-, 750-, and 1000- $\mu$ L volumes (Brinkman #022334506, Baxter #P5060-500B). Eppendorf Variable Volume pipet, 10 to 100  $\mu$ L (Brinkman #22333551; Baxter #P5063-2).
- 2.1.11 Filters. Disposable syringe filter units. 0.45-micron pore size with Luer hub. (Zetapor brand from Alltech #5-8072).
- 2.1.12 Glass pipets. 2-, 5-, and 10-mL disposable, graduated glass pipets for general use.
- 2.1.13 Glass bottles. 4- or 8-oz, wide-mouthed bottles equipped with Poly-Seal caps. 2- and 4-oz, narrow-mouthed bottles with Poly-Seal caps.
- 2.1.14 Autosampler vials. Standard 2.0-mL crimp-top vial (Sunbrokers #200-000) with standard crimp top (Sunbrokers #200-100) and limited-volume (250  $\mu$ L) insert (Sunbrokers #200-228).
- 2.1.15 Derivatization vials. Standard 2.0-mL screw-top vial (Sunbrokers #200-250) with phenolic plastic, open-top cap

(Wheaton #240506), and double-thickness PTFE septum (Sunbrokers #200-338).

- 2.1.16 Evaporation manifold. 12-unit evaporation manifold with aluminum heating/cooling block, 13-mm tube size (Chemical Research Supplies #201188).
- 2.1.17 Solid-phase extraction (SPE) manifold. 12-unit SPE column manifold (Baxter #9400DK).
- 2.1.18 Cation-exchange columns. Poly-Prep sample preparation columns with AG 50W-XB resin, H+ form, 200 - 400 mesh (Bio-Rad #731-6214).
- 2.1.19 Vortex evaporator. Haake Buchler Rotary Evapo-Mix vortex evaporator (Eberbach Corporation, Ann Arbor, MI), equipped to accept 50-mL centrifuge tubes described in section 2.1.20.
- 2.1.20 Centrifuge tubes. 50-mL glass, graduated, centrifuge tubes with #16 ground-glass stopper for mobile-phase solution evaporation.
- 2.2 Reagents
  - 2.2.1 Ethyl acetate. High purity for pesticide residue analysis.
  - 2.2.2 Methanol. ACS Reagent grade.
  - 2.2.3 Hydrochloric acid. ACS Reagent grade.
  - 2.2.4 Water. Millipore grade.
  - 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 Cation exchange mobile-phase solution. 900 mL, 0.16 M HCl (12.1 mL concentrated HCl in 900 mL water) and 100 mL methanol.
- 2.2.10 Acidic modifier. Combine 16 g monobasic potassium phosphate, 160 mL water, 40 mL methanol, 13.4 mL concentrated hydrochloric acid.

### 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: C. Doss, Product Development Department.

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 in a similar manner. Fortification solutions are prepared to fortify untreated (control) samples and demonstrate recovery. Calibration solutions are used to calibrate the instrument. To prepare

each of these solutions, at a nominal concentration of 1.0 mg/mL, place a known quantity ( $\pm 0.1$  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 solutions. 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) = 1.0 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 both PMG and AMPA stock solutions, and diluting the combined aliquots with water. For example, to prepare a 100  $\mu$ g/mL 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. Dilute with water to a total weight

of 50 g. Although dilution by weight is described here, dilution by volume is not precluded. Add to 2 to 3 drops of concentrated HCl as a preservative. 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 ( $\mu\text{g/mL}$ )

C(ss) = concentration of analyte in the stock standard ( $\mu\text{g/mL}$ )

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

W(ws) = final weight of working standard (stock solution and diluent, g)

Serially dilute 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  $\mu\text{g/mL}$  is prepared, as described in section 2.3.3. A requirement of the derivatization method is that the volume of sample extract or standard solution added to the derivatizing reagent remains constant. As a result, a range of concentrations of intermediate calibration solutions are required. Prepare these dilute working standards by serially diluting the 100  $\mu\text{g/mL}$  calibration standard 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 0.012 g of crop per mL of extract, a intermediate calibration solution with an analyte concentration of 0.50  $\mu\text{g/mL}$  is needed. This intermediate calibration solution

will be further diluted to give the daily-use standards, described below.

- 2.3.5 Daily-use calibration standards. The intermediate calibration solutions described in 2.3.4 are further diluted to produce daily-use standards. Prepare these standards by diluting an aliquot of the intermediate standard to a known volume using the mobile-phase solution (section 2.2.9), not water, as the diluent. Using an Eppendorf pipet, dilute 20  $\mu$ L of intermediate standard to a final volume of 3.0 mL. 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 carryover can occur. To quantitate a sample with an analyte concentration of 0.05 mg/kg, and a extract concentration equivalent to extractives from 0.012 g of crop/mL, a series of daily-use standards are prepared by diluting 0.50 and 5.0  $\mu$ g/mL intermediate standards to daily-use standards with concentrations ranging from 3.33 to 33.3 ng/mL.

### 3 ANALYTICAL PROCEDURE

#### 3.1 Crop Extraction

- 3.1.1 Place a 50-g subsample of a homogeneous crop sample into a Waring blender jar. After taking into account the amount of water present in the sample (see reference 1), add an amount of water that would bring the total volume of water to 250 mL. For example, a fruit crop that contains 80% water would require an additional 210 mL of water to be added. A grain with a 10% water content would require 245 mL of water. For high volume crops such as hay or forage, 25 g of sample may be extracted with a total of 125 mL of water. Blend at high speed for 3 minutes. Allow the macerate to settle, and then

transfer 30 to 40 mL of aqueous extract to a 4-oz, wide-mouthed jar. Centrifuge for 10 minutes at about 2000 rpm.

3.1.2 Chloroform partition. Using a disposable pipet, transfer 15 mL of the aqueous extract to a 2-oz, wide-mouthed jar. Add 15 mL of chloroform and shake for 2 minutes. Centrifuge for 10 minutes at about 2000 rpm.

3.1.3 Acidic modifier. Transfer a 10-mL aliquot of the aqueous layer to a 2-oz, narrow-mouthed jar. Add 1.0 mL of the acidic modifier solution (16 g  $\text{KH}_2\text{PO}_4$ , 160 mL water, 40 mL methanol, and 13.4 mL concentrated HCl; section 2.2.10) and shake. A 0.55-mL aliquot of this extract represents 0.10 g of sample.

Any precipitate created by the addition of the acidic modifier may be removed by filtering a 2.0-mL aliquot through a 0.45- $\mu\text{m}$  syringe filter unit.

## 3.2 Extract Cleanup

3.2.1 Cation-exchange column preparation. Prepare the disposable cation-exchange (CAX) cleanup column (Bio-Rad Poly-Prep AG 50W-8X resin, H<sup>+</sup> form) by shaking the column and allowing all the resin to settle to the bottom. After settling, remove the column cap and snap the seal off the bottom of the column. Monitor the level of liquid in the reservoir; stop the flow as soon as the liquid reaches the top of the column bed. Wash the resin with two 5-mL volumes of deionized or distilled water; stop the flow as soon as the liquid reaches the top of the column bed.

3.2.2 Sample addition and elution. Using an Eppendorf pipet, add 0.550 mL of extract (representing 0.10 g of crop) to the column reservoir. Elute to the top of the column bed;

discard the eluant. Add 2.0 mL of the mobile-phase solution (160 mL water, 2.7 mL concentrated HCl, and 40 mL methanol; section 2.2.9) to the reservoir and elute; again discard the eluant. Elute the analytes with 10.5 mL of the mobile-phase solution. Collect eluant in a 50-mL centrifuge tube.

Evaporate eluant to dryness using a vortex evaporator. The temperature of the water bath should not exceed 40 °C. Dissolve the residuum in 1.5 mL of the mobile-phase solution.

### 3.3 Analyte Derivatization

- 3.3.1 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 (total volume 20 to 40 mL). Cap the container and shake gently. Carefully loosen the cap to release any pressure. Due to the potential for pressure buildup, do not fill to more than 75% of capacity. The reagent mixture should be prepared fresh daily. The use of latex gloves when preparing and handling the reagent mixture is strongly recommended.
- 3.3.2 Add 1.6-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 less than -60 °C, as measured by a thermometer placed in the aluminum block. Prepare enough vials to derivatize each standard or sample extract in duplicate.

- 3.3.3 Add a 36- $\mu$ L aliquot of the daily-use calibration standard or sample extract to the prechilled derivatizing reagent in the following manner. Using a variable-volume Eppendorf pipet, withdraw 36  $\mu$ L 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 sample aliquot is added to the reagent mixture, cap and manually shake the vial, and return it to the chilled aluminum block. After all samples 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 samples should remain under the stream of nitrogen for an additional 20 to 30 minutes. Residual derivatization reagents or trifluoroacetic acid can degrade the chromatography of the analytes.

- 3.3.4 Dissolve the residuum in 200  $\mu$ L of ethyl acetate containing 2.0  $\mu$ L citral/mL. Cap the vial, and shake to dissolve contents. Transfer contents to a crimp-top autosampler vial containing a limited volume (250  $\mu$ L) 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) crop residues are high. The injection of more dilute extracts (<0.012 g

crop/mL extract) will improve ruggedness and reliability of the method.

### 3.4 Fortification

If possible, analyze fortified- and unfortified-control samples with each sample set to demonstrate method recovery. For example, add 25 or 250  $\mu$ L of working fortification solution (100  $\mu$ g/mL) to separate control samples (50 g) to produce fortification levels of 0.05 or 0.50 ppm. Extract as detailed in section 3.1. It is recommended that one unfortified and 2 fortified samples be analyzed with each set of 10 field samples. One of the two fortified 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 specific 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 5890 II
Column:	J&W DB-5.625, 30 meter, 0.25 mm i.d., 0.25- $\mu$ m film thickness
Carrier:	Helium, 7.5 lb/square inch 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.

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: 1.5 min

Injection Solvent: ethyl acetate (2.0 µL citral/mL added)

Total Run Time: 10.5 min

4.1.3 Mass-selective detector.

Model: Hewlett-Packard 5970

Software Version: ChemStation A.01.04

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  
(Primary ions) 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, an alternative to the normal "AutoTune" sequence is employed. Using perfluorotributyl-amine (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, an EXTENDED TUNE is performed (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. AMU gain is reduced to increase the bandwidth of the three tuning masses to between 1.8 and 2.4 amu. The bandwidths can be observed 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 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). 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 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, the possibility of 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 3.3.2. For crop extracts equivalent to extractives from 0.012 g of crop/mL, and controls fortified at 0.05 and 0.50 mg/kg, calibration standards at 0.6, 2.0, and 6.0 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 to 5) of a fortified extract in order to equilibrate the columns.
2. Replicate low-level standards to assure constant response.
3. Ethyl acetate blank.
4. Control (injection of duplicates not required).
5. Low-level standard.
6. Low-level, fortified-control extracts.
7. Low-level standard.
8. High-level standard.
9. High-level, fortified-control 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 the analytes in the original sample is calculated by using the external standard method; i.e., the response obtained for the analytes in the sample extract is compared to the response obtained for a separate injections of a known amount of analyte (calibration solution). To use the calculations shown below, the injection volumes for all calibration solutions and sample extracts 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 those samples. The average response of the standard injections before and after the samples of interest is used to determine the calibration factor for those samples.

### 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,  $\mu\text{g/mL}$

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

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

$$C = \frac{W \text{ (sample)}}{V \text{ (solvent)}} \times \frac{V \text{ (crude)}}{V \text{ (crude)} + V \text{ (mod)}} \times \frac{V \text{ (CAX aliquot)}}{V \text{ (CAX final)}} \times \frac{V \text{ (deriv)}}{V \text{ (final)}}$$

Where:

- C = concentration of crop (g/mL)
- W (sample) = weight of crop extracted (g)
- V (solvent) = volume of extracting solvent used (mL)
- V (crude) = volume of crude extract aliquot in section 3.1.3
- V (mod) = volume of acidic modifier in section 3.1.3
- V (CAX aliquot) = volume of extract aliquot subjected to CAX cleanup in section 3.2.2
- V (CAX final) = volume used to dissolve residual material in section 3.2.2
- V (deriv) = volume of cleaned extract subjected to derivatization in section 3.3.3
- V (final) = final volume used to dissolve residuum in section 3.3.4

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 ( $\mu\text{g/g}$  or ppm)
- F = response factor, ( $\mu\text{g/mL}$ )/response unit
- R = average sample response unit from detector for sample
- C = concentration of crop in final extract, g/mL

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:

$R (\mu\text{g/g or ppm}) = Y/C$

Where

Y = calculated extract concentration from the curve fit equation ( $\mu\text{g/mL}$ )

C = crop concentration in extract, from section 5.1.2 ( $\text{g/mL}$ ).

6 MATRIX EFFECT

The composition and characteristics of the crop extract matrix can affect several aspects of the analysis. These include the analyte elution from the CAX column, 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 Cation-Exchange Elution Verification

Specific matrices may affect the elution of the analytes from the CAX column. To test or confirm the recommended elution scheme with a specific matrix, the control matrix (after addition of acidic modifier, section 3.1.3) is fortified to a known level by addition of a small aliquot of analyte standard. The fortified control is then added to the CAX column, eluted, and derivatized as usual.

If recoveries are not satisfactory, adjustment of the CAX elution scheme may be needed to eliminate analyte losses. Collect the 2.0-mL wash fraction, the 10.5-mL target fraction, and an additional postelution fraction, and analyze the individual fractions. These fractions may need to be further subdivided to obtain an accurate elution profile. Once the elution profile is determined, adjustments to the elution scheme can be made to increase the recovery of the problem analyte(s).

Note: The fortification of the control extract should be done at a level that is high enough to allow accurate quantitation of small percentages (5%) of the total fortified amount. A level equivalent to 0.25 mg/kg is recommended (final analyte concentration = 0.003  $\mu\text{g/mL}$ ).

#### 6.2 Derivatization Matrix Standard

Low analyte recovery that cannot be improved by adjusting the CAX elution scheme may be a result of a matrix-induced derivatization deficiency. To evaluate this effect, a derivatized matrix standard (DMS) can be prepared by

fortifying a post-CAX control extract. A known amount of analyte is added to a known amount of the control extract (obtained in section 3.2.2). The recovery here is independent of the extraction efficiency or CAX cleanup efficiency, and is primarily dependent on the derivatization efficiency. Prepare this standard at a level equivalent to 0.25 mg/kg (final analyte concentration = 0.003  $\mu\text{g/mL}$ ).

### 6.3 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 3.3.4) is fortified with high-level derivatized standard (usually 10:1, control:standard). The response from the FCE is compared to that obtained from the nonmatrix standard. The nonmatrix standard should be prepared by diluting the high-level standard with a derivatized reagent blank extract. The FCE is usually analyzed when differences are noted in the chromatographic behavior of analytes in standards and 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.

### 6.4 Matrix Effects - Inlet System Reliability

The derivatized extract matrix has a number of effects on the inlet liner and column. These are not well understood, but may relate to the residual adsorptivity of the columns and how the matrix and analytes can affect, or be affected

by it. In section 4.3, step 1 requires the injection of a fortified-control extract in order to equilibrate the column. Especially significant when a new inlet liner has been installed, it has been noted that the response of standards is increased after several injections of samples extracts, controls, or fortified controls. Since it is not known whether matrix species 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 (1-3 ft) can be limited. The number of injections that can be made before chromatography deteriorates ranges from 20 to 200. The deterioration is due to buildup of nonvolatiles and other polar species, that can lead to an increase in adsorptive sites. For best results use extracts with a crop concentration of <0.012 g/mL whenever possible.

It is recommended that each analysis set starts with a new inlet liner and the removal of 1 to 3 feet of the column. Initial equilibration, as described above (and 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 crops and reagents could result in interferences with retention times coincident with or near that of the analytes.

Satisfactory resolution can usually be achieved with appropriate oven-temperature manipulation. If resolution cannot be achieved, an alternate ion may be monitored (see section 8).

#### 7.1 Determination of Interference Sources

Extracts from untreated plots should always be analyzed to demonstrate the absence of significant interferences from crop matrices, if possible. However, if significant interferences are detected in the untreated control, three 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 an aliquot of deionized water added to the derivatization reagent mixture. Contaminated derivatization reagents can be determined with this sample. The second reagent blank consists of an aliquot of the CAX mobile-phase solution added to the reagent mixture. The mobile-phase solution, as indicated earlier, is used to dilute standards and dissolve the evaporated extract residuum. The third reagent blank consists of a 0.55-mL aliquot of mobile-phase solution carried through the CAX cleanup and subsequent derivatization. Since the concentration factor of the CAX eluant in section 3.2.2 is 7:1 (10.5 mL:1.5 mL), this reagent blank can detect trace interferences or contaminants not detectable in the second reagent blank.

### 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.

Alternate m/z ions for the derivatives of AMPA and PMG are given below.

<u>AMPA Derivative</u>		<u>PMG Derivative</u>	
<u>Ion (m/z)</u>	<u>Abundance*</u>	<u>Ion (m/z)</u>	<u>Abundance</u>
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.

## 9 DISCUSSION

### 9.1 Scope

This method is suitable for the determination of AMPA and PMG in a corn grain, corn forage, and corn fodder. However, due to the nature of some crops, variations in the method may be needed to maintain the accuracy and precision of the method. Recovery data given in Tables I and II reflect the methodology described herein.

### 9.2 Precision and Accuracy

Fortified crop samples were prepared as described under section 3.4, and analyzed according to this method to establish recovery. Recoveries of PMG from corn commodities

fortified at 0.05 and 0.50 mg/kg ranged from 75 to 116%, with a mean recovery of 90% (n=18) and coefficient of variation of 11.4%. Recoveries of AMPA from corn commodities fortified at 0.05 and 0.50 mg/kg ranged from 77 to 108%, with a mean recovery of 95% (n=18) and a coefficient of variation of 8.9%. Tables I and II list the recoveries obtained from corn grain, corn forage, and corn fodder.

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 coefficient of variations given in Tables I and II are a measure of precision.

### 9.3 Detection Limit

The detection limit for a specific analyte in a specific crop commodity is based on the minimum detectability of the analyte, and the crop concentration in the extract. The minimum detectable amount has been established as a response large enough that a 25% change can be distinguished. Also required is a single-to-noise ratio of at least 10. The detection limit for a specific crop is obtained by dividing the minimum detectable concentration by the crop concentration in the extract. 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.

### 9.4 Lower Limit of Quantitation

The lower limit of quantitation (LOQ) 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 exhibit some interlaboratory variation. LOQ values of 0.05 mg/kg for PMG and for AMPA were obtained from work conducted for this report.

#### 9.5 Matrix Effects

The absence of chromatographic matrix effects was verified by the analysis of fortified-control extracts, as described in section 6.3. Results are listed in Table III. Sample chromatograms are shown in Figures 8 and 9.

#### 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 m/z) exist for the quantitation of PMG. While 446 and 611 provide the greatest response for AMPA and PMG, respectively, the abundance of the other ions is great enough for accurate quantitation. In this work, corn grain and corn forage were analyzed while monitoring the 502 and 611 m/z fragments for AMPA and PMG, respectively. Corn fodder was analyzed monitoring the 446 and 611 m/z fragments. In addition, the corn fodder extracts were reanalyzed, monitoring the 502 and 584 m/z fragments.

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

9.7 Dry-Weight Basis

This method determines the residues of AMPA and PMG in corn grain, corn forage, and corn fodder 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. Percent moisture can be determined by drying a subsample at 105 °C for 18 hours.

9.8 Extraction Efficiency

The extraction efficiency for both analytes using the solvents given in this method was tested by analyzing fortified crop commodities. The results are listed in Tables I and II.

9.9 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 Good Laboratory Practices 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.

9.9.1 Flammable solvents. Ethyl acetate, trifluoroacetic anhydride, and methanol are flammable.

9.9.2 Pressure buildup. Mixing trifluoroacetic anhydride and heptafluorobutanol generates 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. Latex gloves are strongly recommended when handling trifluoroacetic anhydride.

10 CONCLUSION

The method is specific for the analysis of AMPA and PMG residues in corn grain, corn forage, and corn fodder. 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. Untreated and fortified samples should be extracted and analyzed with each set of samples to demonstrate absence of interferences and adequate recovery, if possible. 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 matrices 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 ten times the LOQ. The absence of significant matrix effects should be demonstrated by the analysis of fortified-control extracts.

## 11 METHOD NOTES

### 11.1 CAX Cleanup Procedure

The majority of unwanted sample extract components are discarded in the first 2.5 mL of eluant (0.55 mL of sample and 2.0 mL of wash). PMG elutes in the next 6 mL of the eluant (0 to 6 mL of the 10.5-mL collected eluant fraction), while AMPA elutes in the last 6 mL of the eluant (4.5 to 10.5 mL of the 10.5-mL collected eluant fraction).

While there is overlap in the CAX elution of the analytes, there can also be some overlap in the CAX elution of the unwanted sample components and PMG. These components can manifest themselves during the GC analysis by interfering with the detection of AMPA. There can be a general rise in the baseline, and additional peaks may also be present. Ions 502 or 372 m/z may be used as an alternate detection method for AMPA. The GC-MSD detection of PMG is rarely affected by the CAX overlap of the PMG and these unwanted components.

In severe cases, the wash volume can be increased to 2.5 mL in order to eliminate some of the interfering components; however, this may reduce recovery of PMG. The additional 0.5 mL of wash should be collected as a separate fraction and analyzed to determine the exact elution profile.

### 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 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, resulting in low recovery of the PMG and AMPA derivatives. To prevent losses due to analyte adsorption, drying of the aqueous extracts is eliminated in this procedure. Instead, a small aliquot (36  $\mu$ L) 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 heptafluorobutanol and trifluoroacetic anhydride reduces some of the danger. Addition to a chilled, mixed reagent further reduces this risk. No more than 50  $\mu$ L of aqueous sample or standard should be added to 1.6 mL of reagent. The method of sample addition and pipet tip rinsing ensures quantitative transfer of the sample aliquot. The method 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 carryover. 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 to volume ratios. To avoid carryover, 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 sections 3.2.2 and 3.3.3, eliminates the possibility of syringe carryover. Derivatives of PMG and AMPA are not subject to adsorption and may be handled with glass syringes.

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 2.1.15). Open-top caps made with nonphenolic plastic 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 cleaned up within four hours of extraction. Stock and working 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. The procedure may be interrupted after the CAX cleanup (section 3.2.2); continue with the derivatization procedure on the following day.

### 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  $\mu\text{L}/\text{mL}$ . The addition of citral helps decrease losses due to analyte adsorption and increase peak symmetry.

11.4.2 Noncrop matrix considerations. Considerations should be given to the components of the final extract other than those contributed by the crop commodity. The amounts of water, HCl, and phosphate that are derivatized make significant, chromatographic-related contributions to the final extract. In order to keep the final concentration of these components consistent between all standards and sample extracts, the following guidelines are offered.

1. Daily-use standards must be prepared by diluting intermediate standards with the CAX mobile-phase solution, or the same solution used to dilute the extract residuum in section 3.2.2.
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 must remain constant for the entire analysis sample set.
3. The amount of ethyl acetate (containing 2.0  $\mu\text{L}$  citral/mL) used to dissolve the residuum in section 3.3.4 must remain constant for all samples and standards within the sample set.
4. Individual samples may be diluted with derivatized control or reagent blank extract in order to bring the unexpectedly high residue samples 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 of the detection system to offset the limitations imposed by the method's direct derivatization of aqueous 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  $\mu\text{L}$  is used as a direct method for increasing sensitivity. Optimal chromatographic bandwidths can be maintained with this volume, if other considerations are made, including inlet liner, column inlet, and oven-temperature profile.

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  $\mu\text{L}$ .

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/insertion tool (Restek #20114). Fused-silica wool is recommended over glass wool due to its inherent inertness. Pack the wool to a moderate density in a 15- to 20-mm plug at the center of the liner. Install the inlet end of the column so that it approaches the wool packing. This can be achieved by extending the column end about 23 to 25 mm from the tip of the ferrule.

11.5.4 Oven-temperature profile. The use of 5- $\mu$ L injection volumes requires special considerations be given the oven-temperature profile and injection solvent. In general, the initial oven temperature 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 of 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 oven 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.

- 11.5.5 Injector temperature. Increasing the injector 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 a 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: injector temperatures in excess of 320 °C have been used with no apparent analyte degradation.)

12 TABLES AND FIGURES

- |            |   |
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Table I. Recovery of PMG from Corn Commodities

<u>Commodities</u>	<u>Trial No.</u>	<u>Sample No.</u>	<u>Amount Added (ppm)</u>	<sup>1</sup> <u>Amount Found (%)</u>	<u>Average (%)</u>
Corn Grain	99CA-92-9001	H9001-04	0.05	91	101
		H9001-05	0.05	116	
		H9001-06	0.05	96	
	99CA-92-9001	H9001-07	0.50	75	81
		H9001-08	0.50	78	
		H9001-09	0.50	89	
Corn Forage	99CA-92-9002	H9002-04	0.05	97	95
		H9002-05	0.05	92	
		H9002-06	0.05	96	
	99CA-92-9002	H9002-07	0.50	95	99
		H9002-08	0.50	103	
		H9002-09	0.50	100	
Corn Fodder	99CA-92-9003	H9003-04	0.05	80	82
		H9003-05	0.05	80	
		H9003-06	0.05	86	
	99CA-92-9003	H9003-07	0.50	85	83
		H9003-08	0.50	86	
		H9003-09	0.50	77	

<u>Data Summary</u>	<u>Average Recovery (%)</u>	<sup>2</sup> <u>CV (%)</u>	<u>N</u>	<u>Range (%)</u>
Corn Grain	91	14.8	6	75-116
Corn Forage	97	3.6	6	92-103
Corn Fodder	82	4.2	6	77-86
All 0.05 mg/kg Fortifications	93	11.1	9	80-116
All 0.50 mg/kg Fortifications	88	10.9	9	75-103
Overall	90	11.4	18	75-116

<sup>1</sup> Based on peak height response

<sup>2</sup> CV is coefficient of variation

Table II. Recovery of AMPA from Corn Commodities

<u>Commodities</u>	<u>Trial No.</u>	<u>Sample No.</u>	<u>Amount Added (ppm)</u>	<sup>1</sup> <u>Amount Found (%)</u>	<u>Average (%)</u>
Corn Grain	99CA-92-9001	H9001-04	0.05	89	95
		H9001-05	0.05	108	
		H9001-06	0.05	89	
	99CA-92-9001	H9001-07	0.50	87	93
		H9001-08	0.50	87	
		H9001-09	0.50	104	
Corn Forage	99CA-92-9002	H9002-04	0.05	106	104
		H9002-05	0.05	103	
		H9002-06	0.05	104	
	99CA-92-9002	H9002-07	0.50	94	98
		H9002-08	0.50	97	
		H9002-09	0.50	103	
Corn Fodder	99CA-92-9003	H9003-04	0.05	95	95
		H9003-05	0.05	96	
		H9003-06	0.05	93	
	99CA-92-9003	H9003-07	0.50	91	84
		H9003-08	0.50	83	
		H9003-09	0.50	77	

<u>Data Summary</u>	<u>Average Recovery (%)</u>	<sup>2</sup> <u>CV (%)</u>	<u>N</u>	<u>Range (%)</u>
Corn Grain	94	9.2	6	87-108
Corn Forage	101	4.2	6	94-106
Corn Fodder	89	7.7	6	77-96
All 0.05 mg/kg Fortifications	98	7.0	9	89-108
All 0.50 mg/kg Fortifications	91	9.3	9	77-104
Overall	95	8.9	18	77-108

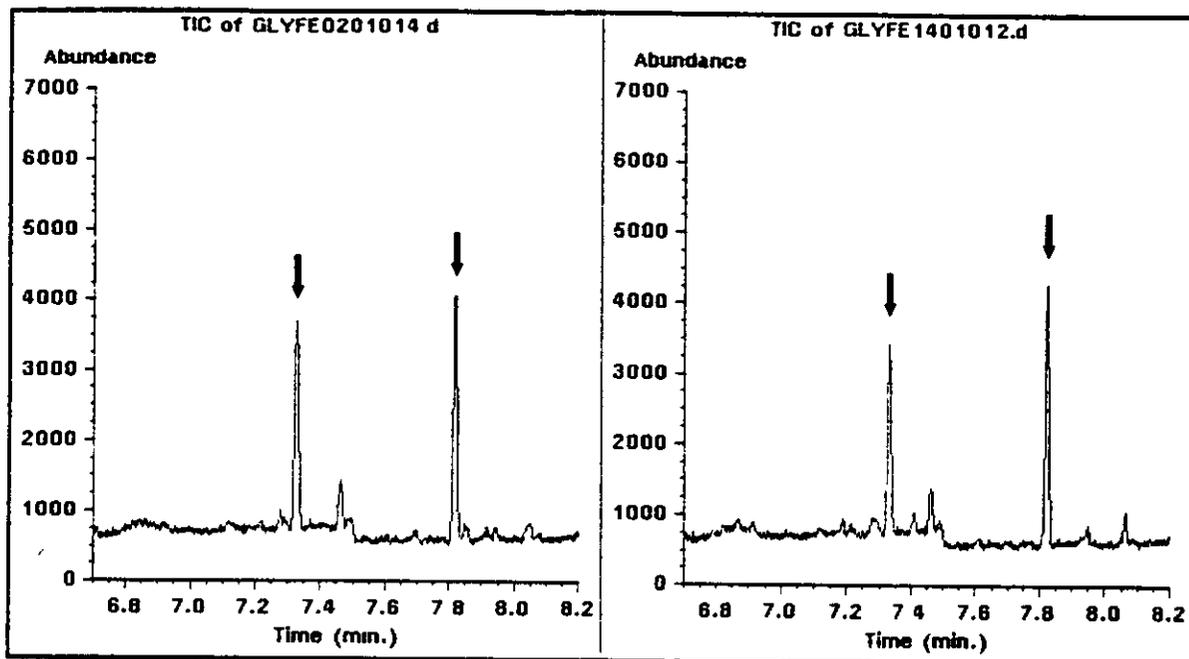
<sup>1</sup> Based on peak height response

<sup>2</sup> CV is coefficient of variation

Table III. Recovery of PMG and AMPA from Fortified-Control Extracts (determination of chromatographic matrix effect)

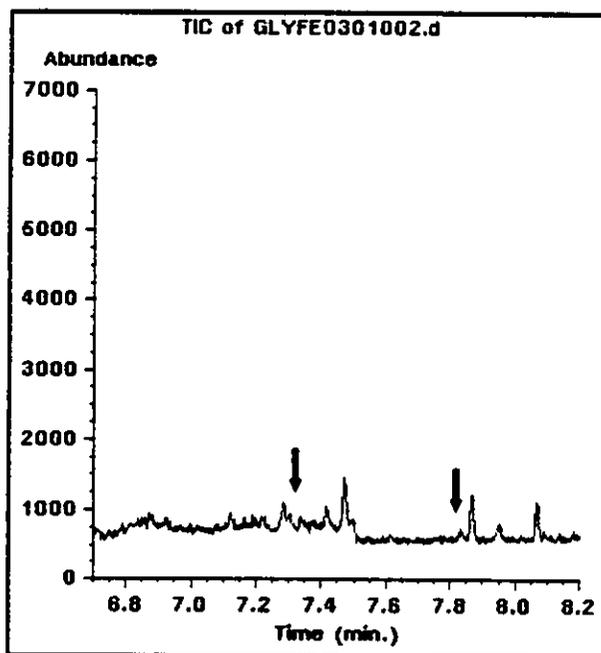
<u>Commodities</u>	<u>Trial No.</u>	<u>Sample No.</u>	<u>Amount Added (ppm)</u>	<u>Amount PMG Found (%)</u>	<u>Amount AMPA Found (%)</u>
Corn Grain	99CA-92-9001	H9001-10	0.05	93	85
		H9001-11	0.05	107	107
Corn Forage	99CA-92-9002	H9002-10	0.10	109	99
		H9002-11	0.10	116	103
Corn Fodder	99CA-92-9003	H9003-10	0.05	113	106
		H9003-11	0.05	107	102
Average Recovery:				108	100

Figure 1. Sample chromatograms - corn grain fortified at 0.05 mg/kg



AMPA/PMG STANDARD - 0.60 ng/mL  
Rt: AMPA-7.33, PMG-7.82 min.

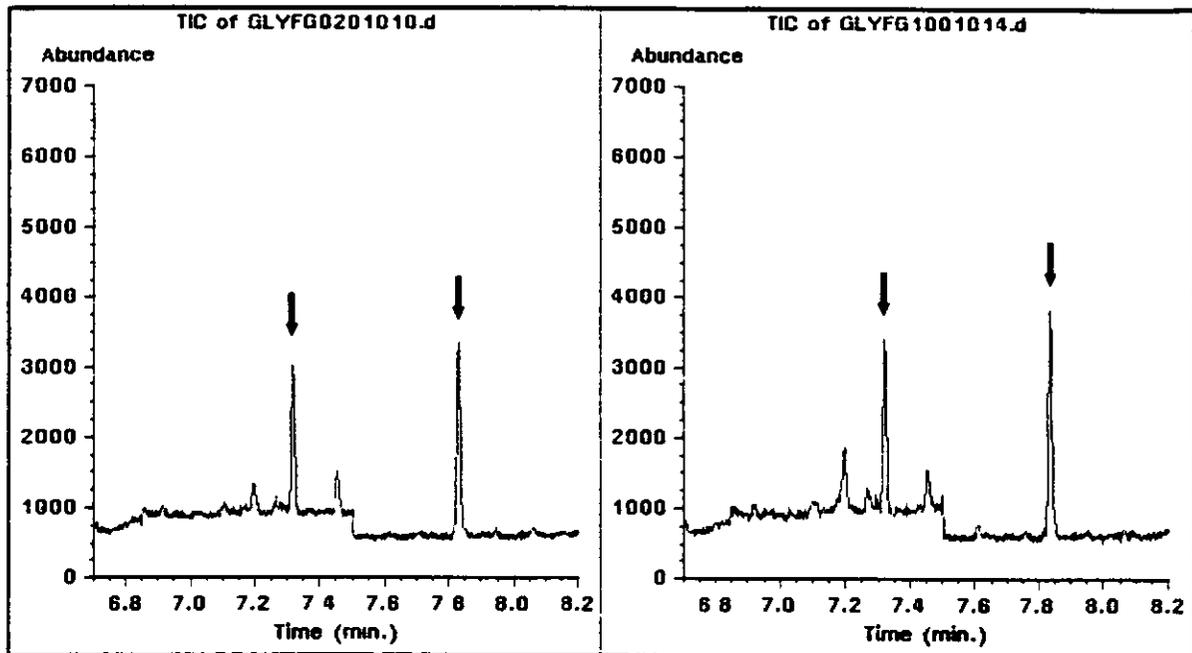
CORN GRAIN, FORTIFIED - 0.05 mg/kg  
H9001-06 0.012 g/mL



CORN GRAIN, CONTROL  
H9001-02 0.012 g/mL

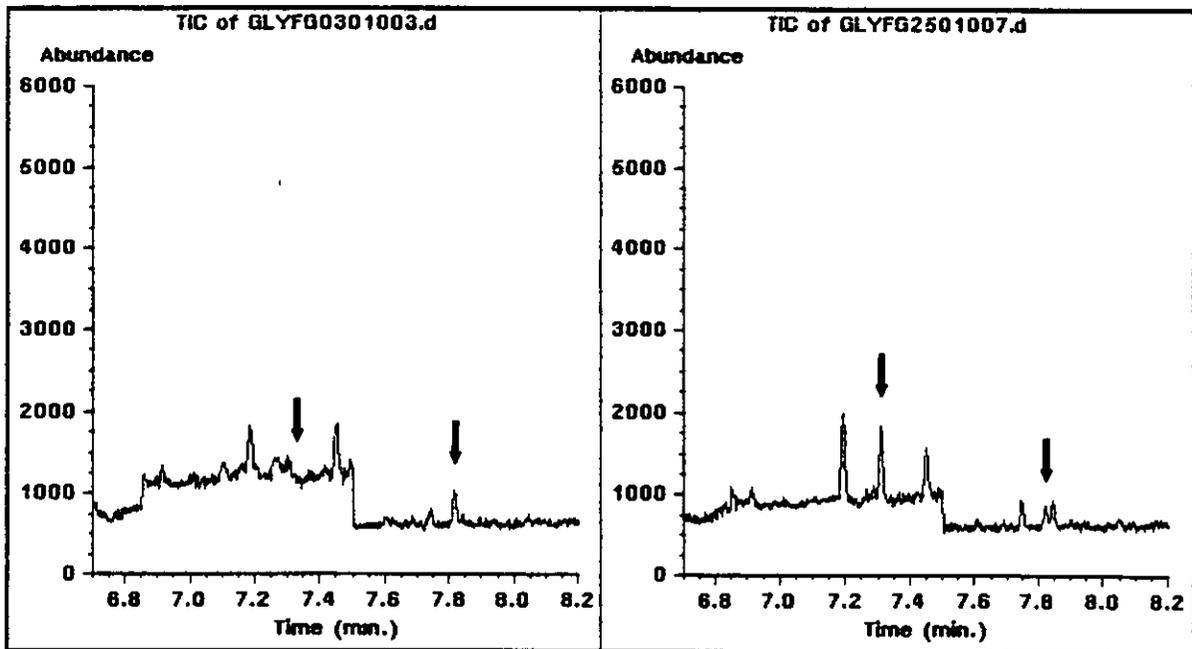
Note: AMPA detected using 502 m/z, PMG detected using 611.5 m/z.

Figure 2. Sample chromatograms - corn forage fortified at 0.05 mg/kg



AMPA/PMG STANDARD - 0.60 ng/mL  
Rt. AMPA-7.33, PMG-7.82 min.

CORN FORAGE, FORTIFIED - 0.05 mg/kg  
H9002-06 0.012 g/mL

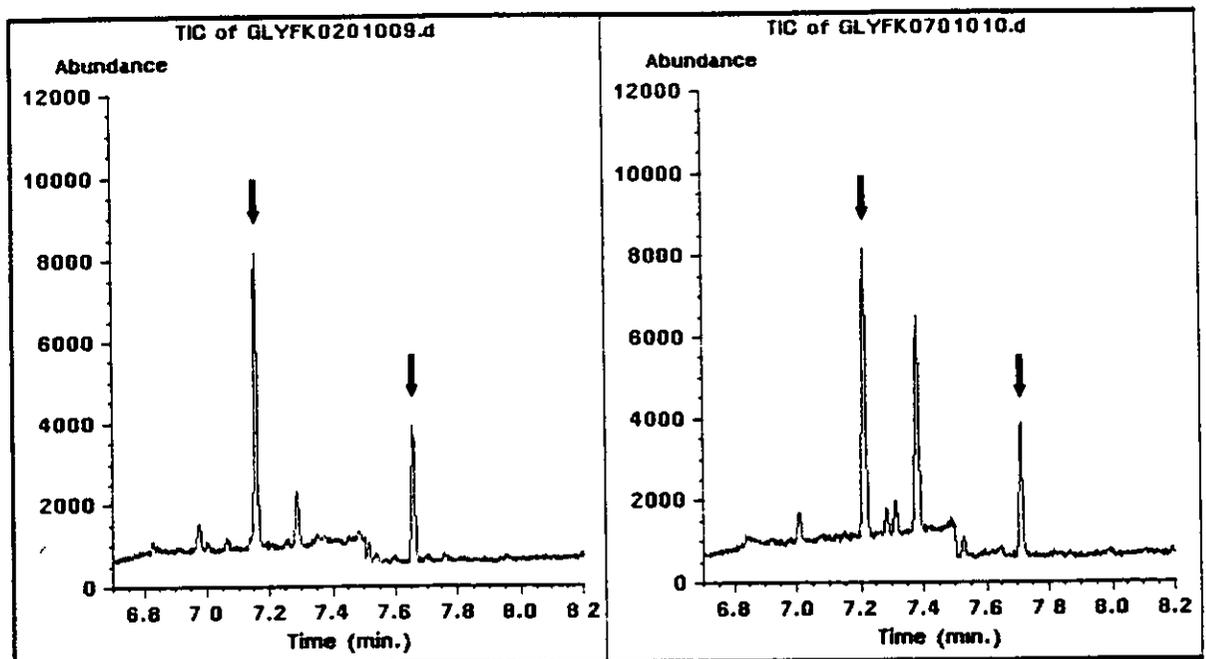


CORN FORAGE - CONTROL  
H9002-02 0.012 g/mL

CORN FORAGE, TREATED-10 lb A.I./acre  
E383-2 0.012 g/mL

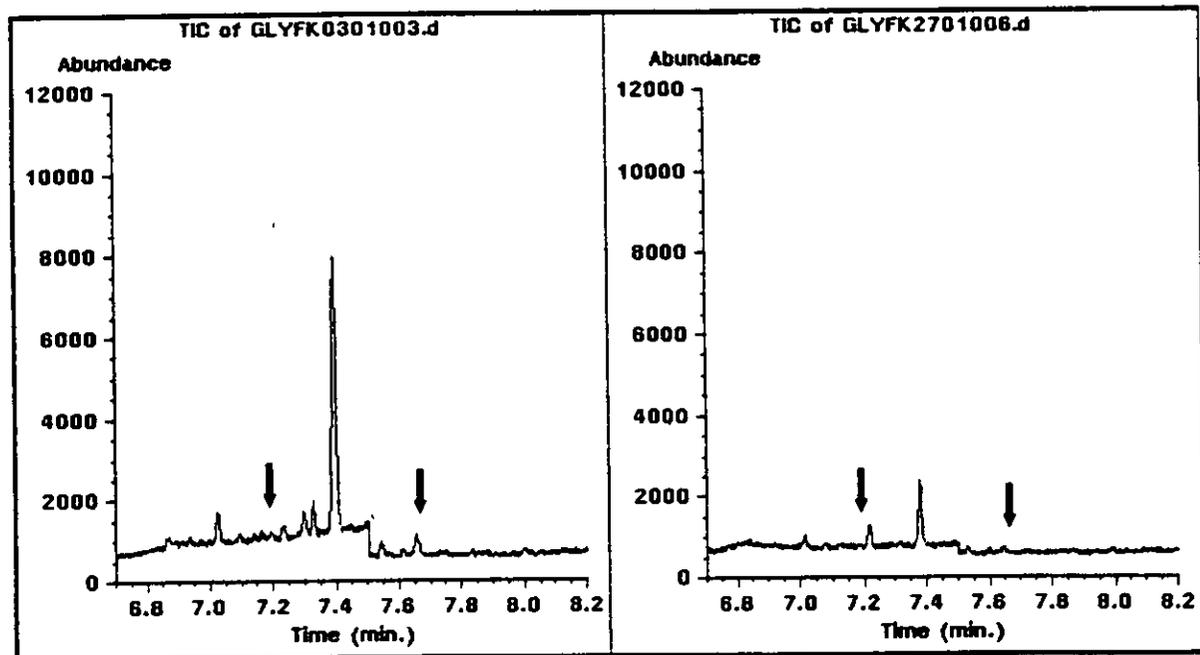
Note: AMPA detected using 502 m/z, PMG detected using 611.5 m/z.

Figure 3. Sample chromatograms - corn fodder fortified at 0.05 mg/kg



AMPA/PMG STANDARD - 0.60 ng/mL  
Rt: AMPA-7.18, PMG-7.69 min.

CORN FODDER, FORTIFIED - 0.05 mg/kg  
H9003-04 0.012 g/mL

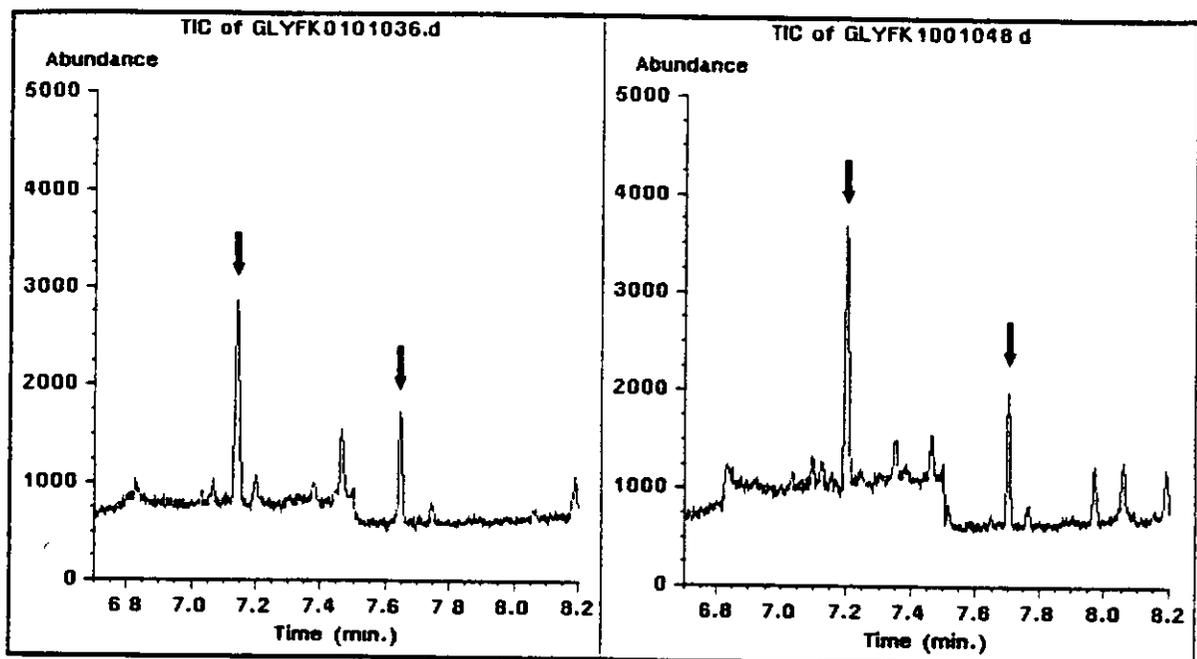


CORN FODDER, CONTROL  
H9003-02 0.012 g/mL

CORN FODDER, TREATED-10 lb A.I./acre  
E383-4 0.012 g/mL

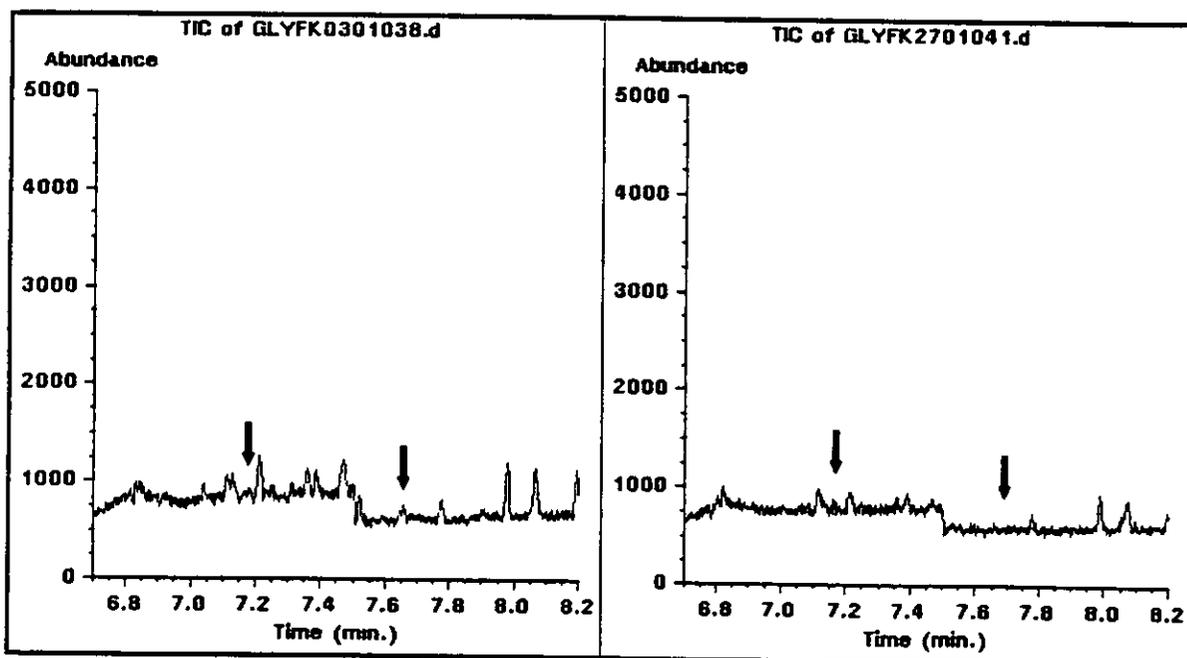
Note: AMPA detected using 445.5 m/z, PMG detected using 611.5 m/z.

Figure 4. Sample chromatograms - corn fodder (alternate ion) fortified at 0.05 mg/kg



AMPA/PMG STANDARD - 0.60 ng/mL  
Rt: AMPA-7.16, PMG-7.66 min.

CORN FODDER, FORTIFIED - 0.05 gm/kg  
H9003-05 0.012 g/mL

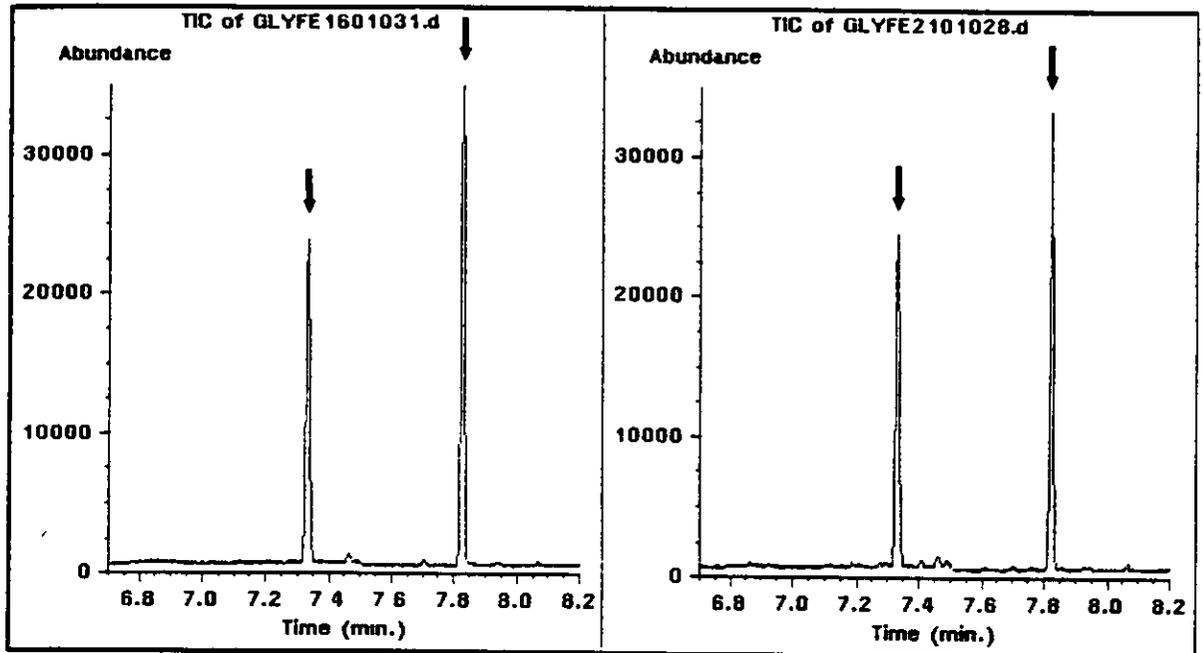


CORN FODDER, CONTROL  
H9003-02 0.012 g/mL

CORN FODDER, TREATED-10 lb A.I./acre  
E383-4 0.012 g/mL

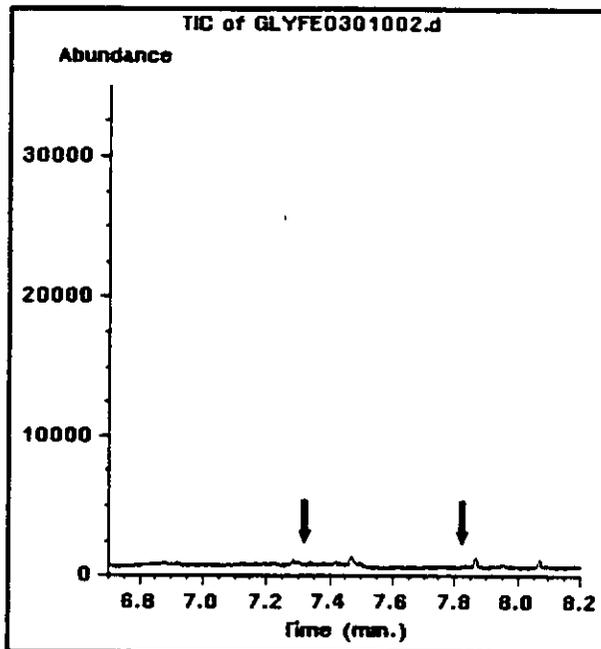
Note: AMPA detected using 502 m/z, PMG detected using 584 m/z.

Figure 5. Sample chromatograms - corn grain fortified at 0.50 mg/kg



AMPA/PMG STANDARD - 6.0 ng/mL  
Rt: AMPA-7.32, PMG-7.82 min.

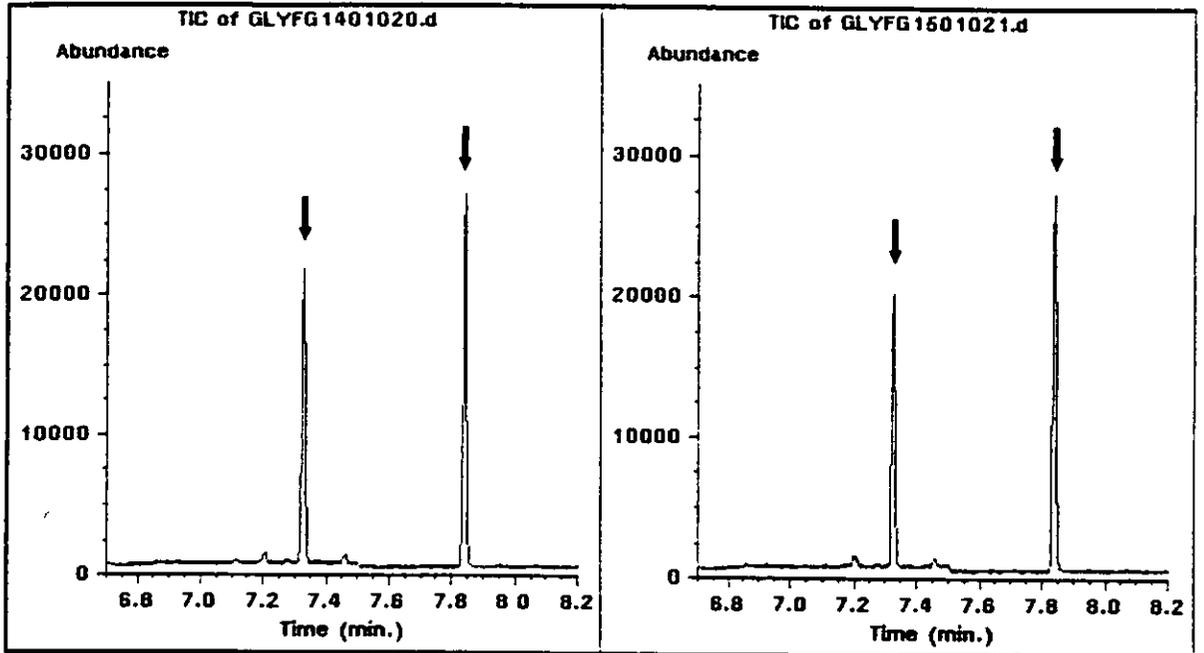
CORN GRAIN, FORTIFIED - 0.50 mg/kg  
H9001-09 0.012 g/mL



CORN GRAIN, CONTROL  
H9001-02 0.012 g/mL

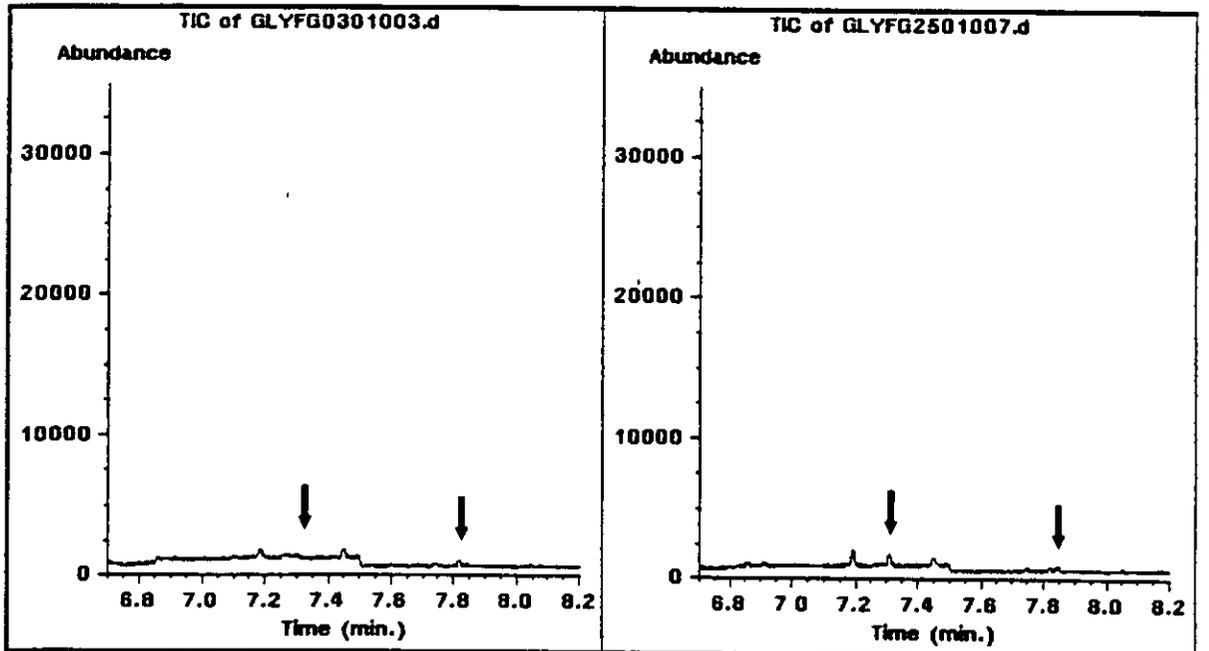
Note: AMPA detected using 502 m/z, PMG detected using 611.5 m/z.

Figure 6. Sample chromatograms - corn forage fortified at 0.50 mg/kg



AMPA/PMG STANDARD - 6.0 ng/mL  
Rt: AMPA-7.33, PMG-7.84 min.

CORN FORAGE, FORTIFIED - 0.50 mg/kg  
H9002-07 0.012 g/mL

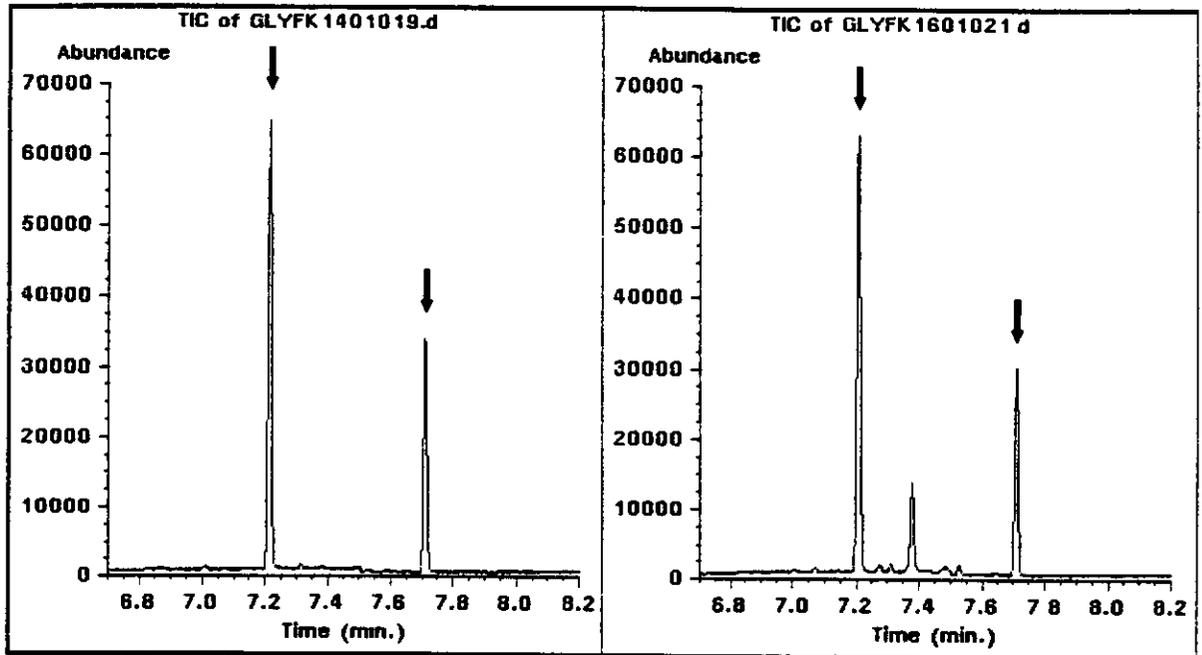


CORN FORAGE, CONTROL  
H9002-02 0.012 g/mL

CORN FORAGE, TREATED-10 lb A.I./acre  
E383-02 0.012 g/mL

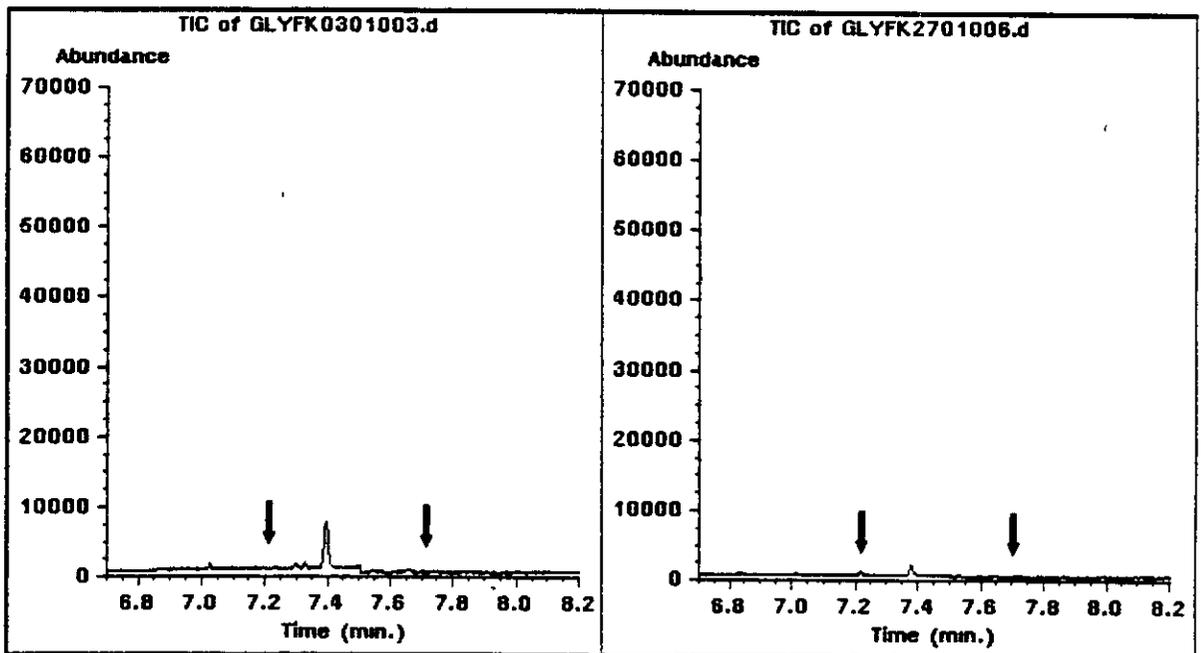
Note: AMPA detected using 502 m/z, PMG detected using 611.5 m/z.

Figure 7. Sample chromatograms - corn fodder fortified at 0.50 mg/kg



AMPA/PMG STANDARD - 6.0 ng/mL  
Rt: AMPA-7.21, PMG-7.71 min.

CORN FODDER, FORTIFIED - 0.50 mg/kg  
H9003-07 0.012 g/mL

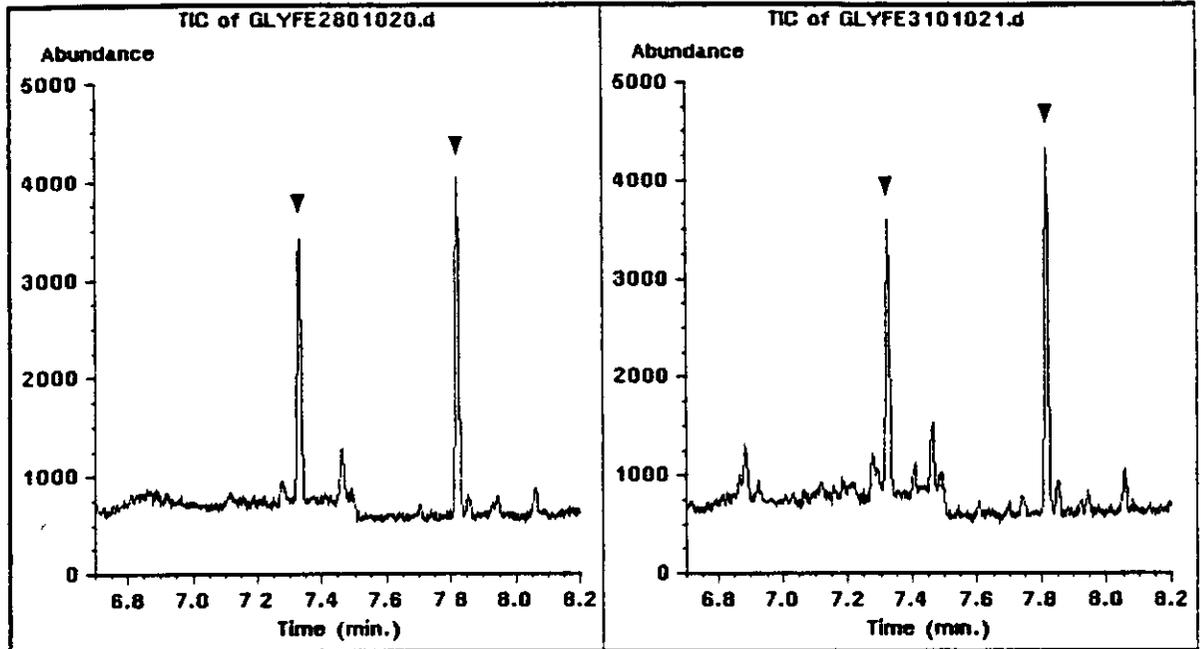


CORN FODDER, CONTROL  
H9003-02 0.012 g/mL

CORN FODDER, TREATED-10 lb A.I./acre  
E383-04 0.012 g/mL

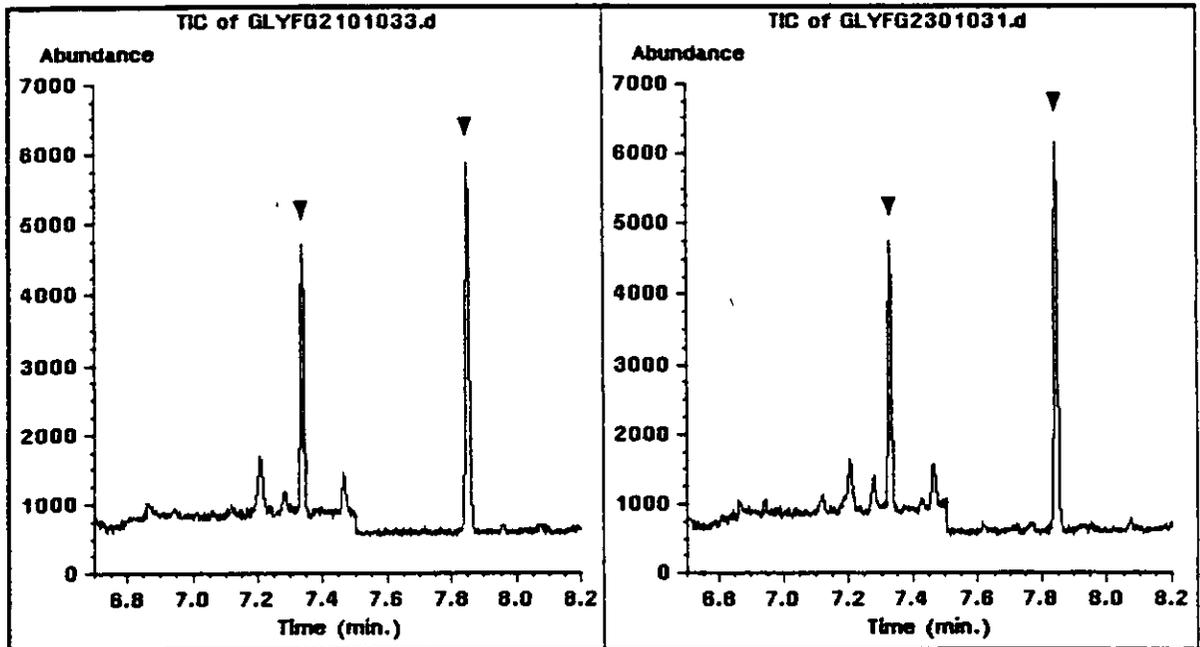
Note. AMPA detected using 445.5 m/z. PMG detected using 611.5 m/z.

Figure 8. Sample chromatograms - fortified-control extracts, corn grain and corn forage



AMPA/PMG FCE STD. - 0.60 ng/mL  
Rt: AMPA-7.33, PMG-7.82 min.

FORT. CONTROL EXTRACT-CORN GRAIN,  
0.60 ng/mL (H9001-10 0.012 g/mL)

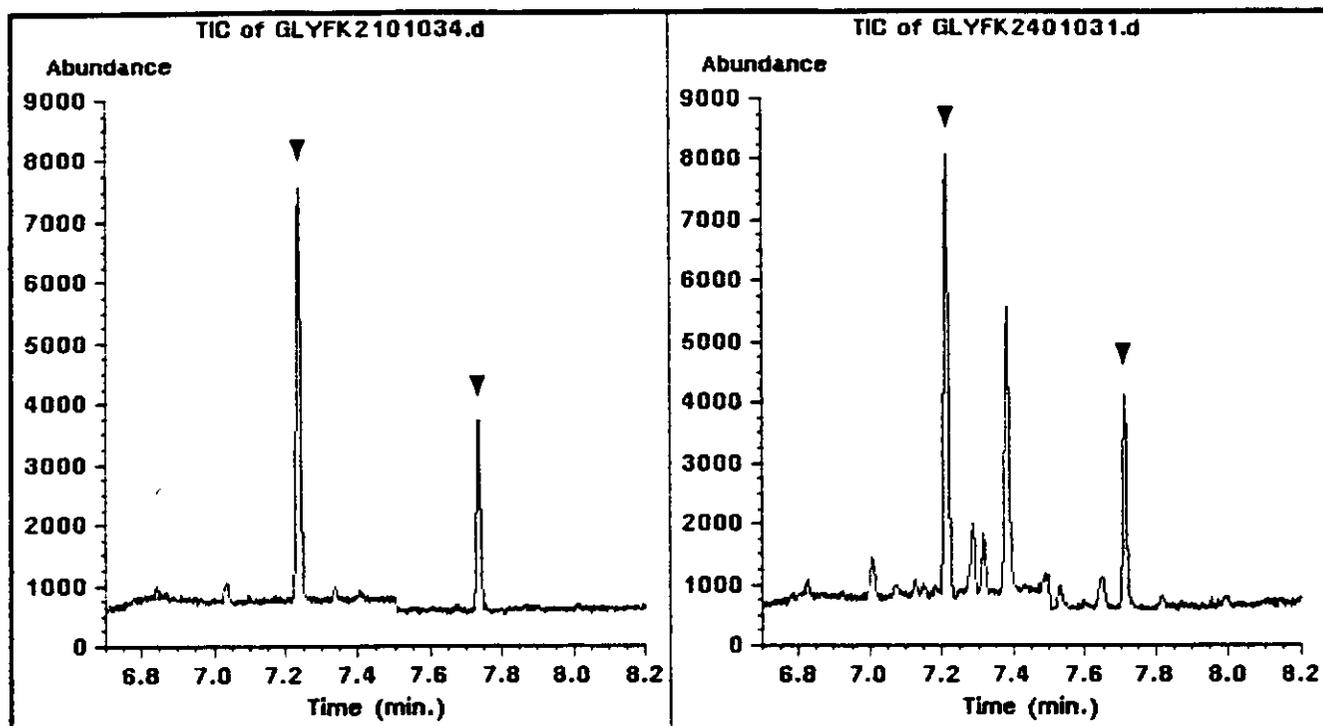


AMPA/PMG FCE STD. - 1.2 ng/mL  
Rt: AMPA-7.34, PMG-7.85 min.

FORT. CONTROL EXTRACT-CORN FORAGE,  
1.2 ng/mL (H9002-10 0.012 g/mL)

Note: AMPA detected using 502 m/z, PMG detected using 611.5 m/z

Figure 9. Sample chromatograms - fortified-control extracts, corn fodder

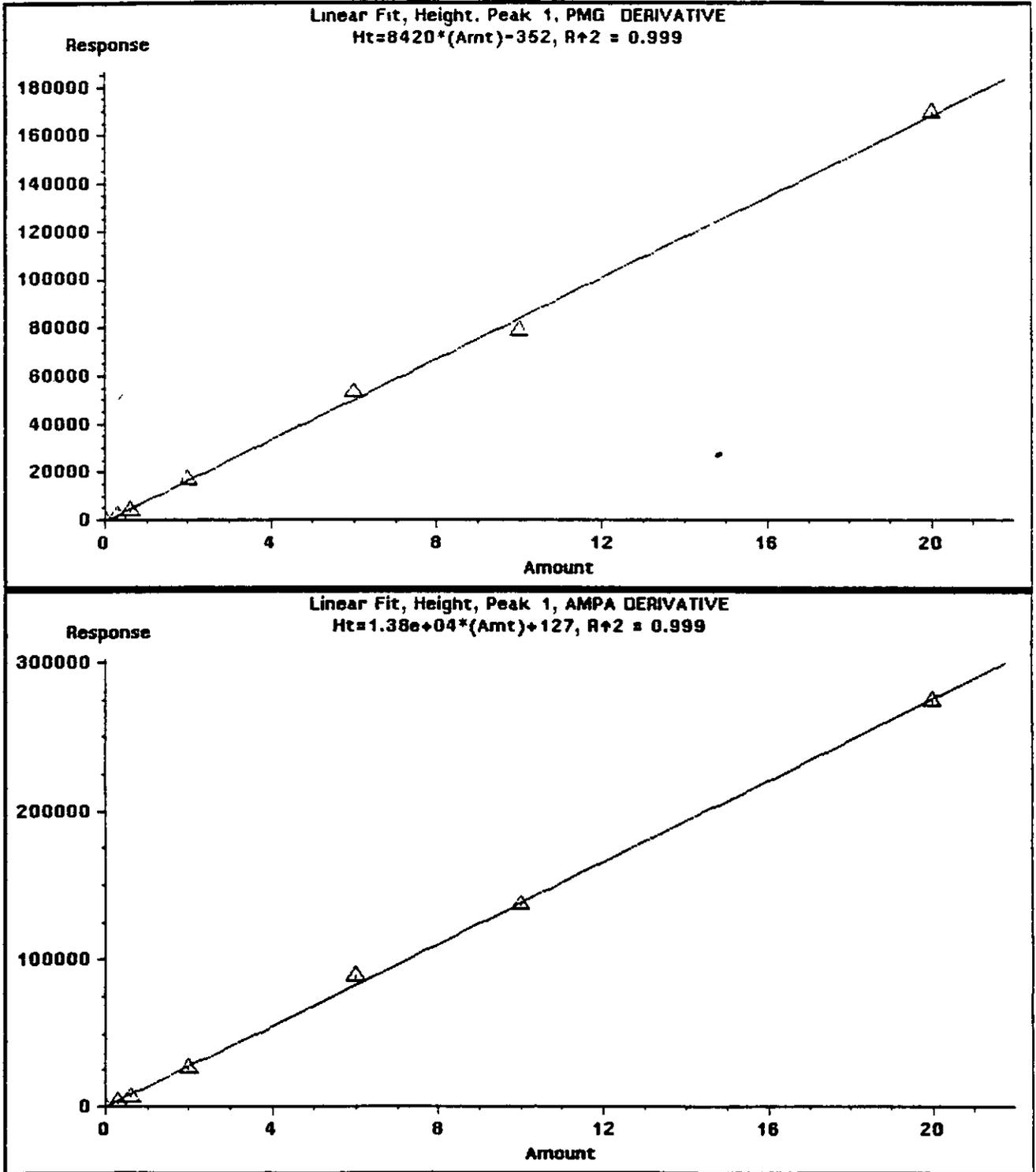


AMPA/PMG FCE STD. - 0.60 ng/mL  
Rt: AMPA-7.22, PMG-7.72 min.

FORT. CONTROL EXTRACT-CORN FODDER,  
0.60 ng/mL (H9003-10 0.012 g/mL)

Note: AMPA detected using 445.5 m/z, PMG detected using 611.5 m/z

Figure 10. Calibration curve for PMG and AMPA derivatives based on injections of 0.3-, 0.6-, 2.0-, 6.0, 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 Good Laboratory Practices Archive at the Western Research Center of ZENECA Ag Products, 1200 South 47th Street, Box 4023, Richmond, California 94804-0023.

**14**      **REFERENCES**

1.      Watt, B. K. and Merrill, A. L. in *Composition of Foods*; Agricultural Handbook No. 8; U.S. Department of Agriculture: revised 1963.

dje/m92-042b.pla/March 29, 1993

**15**      **APPENDICES**

Appendix A. Mass-selective detector manual tune procedures

Appendix B. Analyte derivatization: structures and mass spectral data

Appendix C. Sample calculations