

FOSETYL - AL.

**BUSINESS CONFIDENTIAL**

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Title : Analytical Method for the Determination of Fosetyl-Al in Plants and Replacement of Diazomethane with (Trimethylsilyl)diazomethane

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KEYWORDS

fosetyl-Al. Aliette, diazomethane, (trimethylsilyl)diazomethane, derivatization, registration, plants, cotton, cottonseed

Summary & Conclusions :

A method of analysis was developed to determine the residue of fosetyl-Al in plants. The published method (DFG 522) in the German BBA was modified. (Trimethylsilyl)diazomethane was found to be a safe replacement for potentially explosive diazomethane. The method was verified by fortifying cottonseed, mustard greens, cantaloupe, spinach, lemon, onion, pineapple, and banana at their respective (or proposed) tolerance levels. Recoveries were obtained at the 70-120% level.

Residues are extracted from plants with 1% sulfuric acid, partitioned with hexanes (in the case of cottonseed and fatty substrates), and an aliquot precipitated with isopropanol. After an aliquot is derivatized with (trimethylsilyl)diazomethane, the solvent is evaporated and the residue quantified by gas chromatography with flame photometric detection.


Environmental Chemistry: Method Development

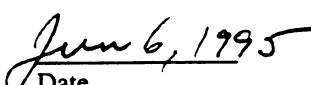
Title: Analytical Method for the Determination of Fosetyl-Al in Plants and Replacement of Diazomethane with (Trimethylsilyl)diazomethane.

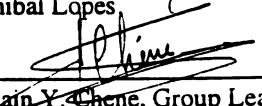
Analyte: Fosetyl-Al

Substrates: Cottonseed, mustard greens (*Brassica* vegetables), cantaloupe (cucurbit vegetables), spinach (leafy vegetables), lemon (citrus), onion (bulb vegetables), pineapple, banana

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Author: 
Anibal Lopes


Date

Approval: 
Alain Y. Chene, Group Leader

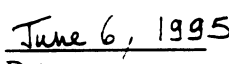
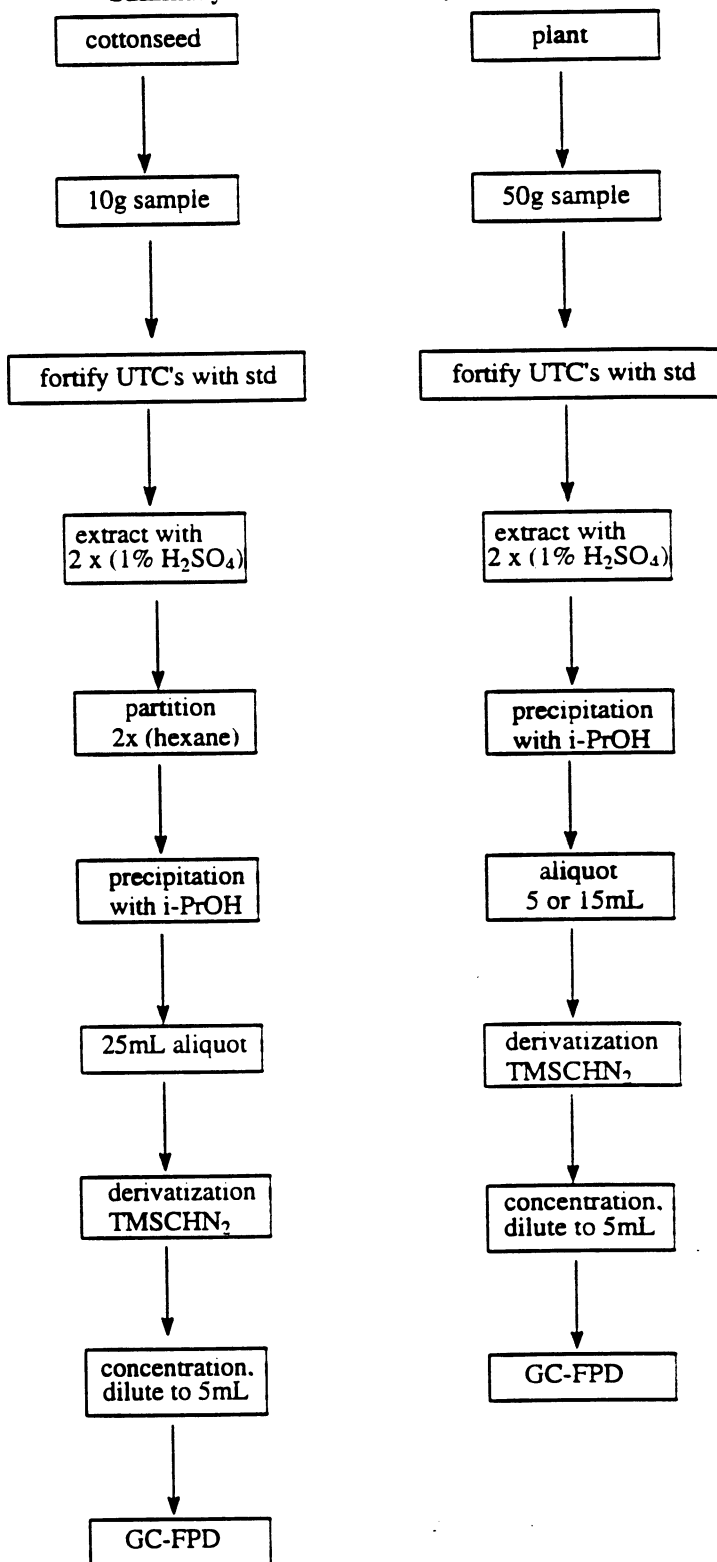

Date

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Summary Flowchart of Analytical Method



Analytical Method for the Determination of Fosetyl-Al in Plants and Replacement of Diazomethane with (Trimethylsilyl)diazomethane.

I. Introduction

A. Scope

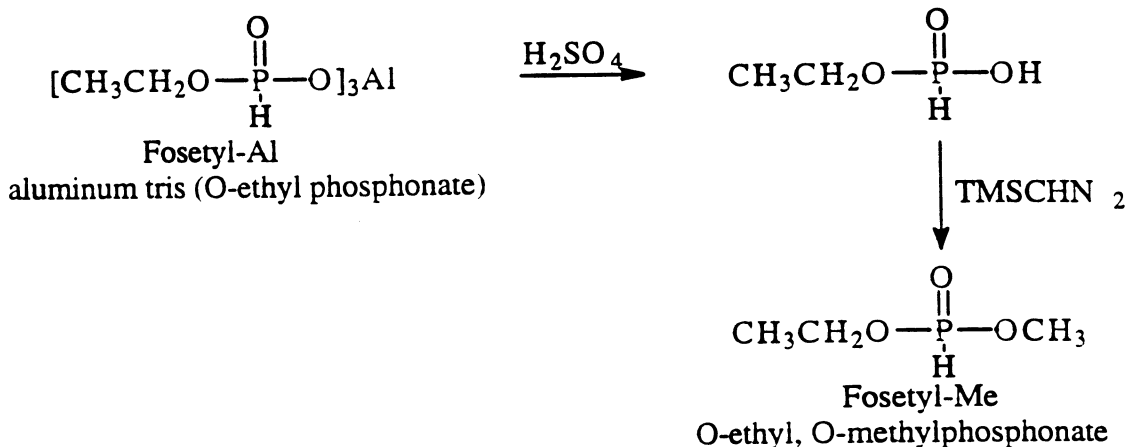
An analytical method is described for the analysis of fosetyl-Al in plants.

B. Principle

Residues are extracted from cottonseed with 1% sulfuric acid and partitioned against hexane. An aliquot of the aqueous phase is diluted with isopropanol and filtered. Derivatization of fosetyl-Al to fosetyl-Me with (trimethyl-silyl)diazomethane for 30 minutes at 35°C was followed by evaporation to ~2mL volume. After dilution to 5mL, fosetyl-Me was quantified by gas chromatography using a flame photometric detector.

Residues are extracted from other plants with 1% sulfuric acid. An aliquot is diluted with isopropanol and filtered. Derivatization of fosetyl-Al to fosetyl-Me with (trimethylsilyl)diazomethane for 30 minutes at 35°C was followed by evaporation to ~2mL volume. After dilution to 5mL, fosetyl-Me was quantified by gas chromatography using a flame photometric detector.

C. Structures



II. Materials

Reagents and Solvents were used as received from supplier, unless otherwise noted. Equivalent reagents and equipment may be substituted where appropriate.

A. Reagents

1. Celite[®]545, , CAS No. 68855-54-9. J. T. Baker, Cat. No. 3371-05
2. (Trimethylsilyl)diazomethane, 2.0M solution in hexanes. CAS No. 18107-18-1, Aldrich, Cat. No. 36283-2

B. Solvents

1. Sulfuric Acid, , CAS No. 7664-93-9, reagent ACS, Fisher, Cat. No. A300S-500
2. Isopropanol, CAS No. 67-63-0, HPLC grade, EM Science, Cat. No. PX1838-1
3. Water, purified by Millipore Milli-Q water purification system

C. Solutions

1. 1% Sulfuric Acid Solution, add 10mL sulfuric acid to 990mL purified water
2. Isopropanol:Water, (9:1), add 900mL Isopropanol to 100mL purified water

D. Equipment

1. Aluminum Crimp-Top Seal, 11mm TFE/RUB Septum, Sun Brokers, Inc., Cat. No. 200100
2. Analytical Balance
3. Autosampler Vials, 1mL, clear, Wheaton, Cat. No. 223682
4. Blender, Waring, and Blender Jars (Small Size, ~500mL), Eberbach, Cat. No. 8470
5. Buchner Funnels, Coors, Cat. No. 30310-120
6. Filter Paper, Whatman glassfibre, 9.0cm, Cat. No. 1822090
7. Glass wool
8. Disposable Pasteur Pipets
9. Graduated cylinders, calibrated to deliver, appropriate sizes
10. Mechanical Shaker
11. Nalgene[®]250mL screw-capped bottles, Nalge Co., Cat. No. 2105-0008
12. Separatory Funnels, 500mL
13. Rotary Evaporator, Buchi, and flat- or round-bottomed flasks in appropriate sizes
14. Volumetric flasks, 5mL, 10mL, 25mL, 50mL, and 100mL, class A
15. Volumetric pipets, appropriate sizes, class A
16. Carlo Erba Fisons 8000 Series Gas Chromatograph with Flame Photometric Detector FPD-700
17. Capillary Megabore Column, DB-Wax, 15m length, 0.53mm ID, 1 μ film, J & W Scientific, Cat. No. 125-7012

E. Analytical Standards

Analytical Standards available from Rhone-Poulenc Ag Company

1. Fosetyl-Al; Aluminum tris (O-ethylphosphonate)

III. Standard Solution Preparation

A. General

1. The stated concentrations of standard solutions should be adjusted to account for the purity of the neat solid standards.
2. After preparation, fortification standards should be transferred from the volumetric flasks into screw-capped amber bottles to prevent possible photodegradation.

3. Store fortification standard solutions in the freezer at or below -20°C when not in use.

B. Fortification Solutions

The following is provided as an example of how standard solutions may be prepared. Other concentrations may be used as appropriate.

1. Weigh 0.2000g ($\pm 5\text{mg}$) of the fosetyl-Al analytical standard into a 50mL volumetric flask. Dissolve in 45mL purified water, sonicating for ~ 50 minutes to help dissolution. Add water to the mark and mix well. The concentration of this standard is $4000\mu\text{g/mL}$ fosetyl-Al in water. This solution should be discarded within a week as there are potential stability problems in water.

Alternatively,

1. Weigh 0.0100g ($\pm 1\text{mg}$) of the fosetyl-Al analytical standard into a 100mL volumetric flask. Dissolve in 10mL purified water, sonicating for ~ 20 minutes to help dissolution. Dilute to the mark with isopropanol and mix well. The concentration of this standard is $100\mu\text{g/mL}$ fosetyl-Al in isopropanol:water (9:1).
2. By further dilution of the $100\mu\text{g/mL}$ fortification standard with isopropanol:water (9:1), prepare a series of fortification standards.

C. Calibration Solutions

The following is provided as an example of how standard solutions may be prepared. Other concentrations may be used as appropriate. These calibration standards are prepared daily along with the samples being analyzed. Stability has not been determined beyond 24 hours.

1. Weigh 0.0100g ($\pm 1\text{mg}$) of the fosetyl-Al analytical standard into a 100mL volumetric flask. Dissolve in 10mL purified water, sonicating for ~ 20 minutes to help dissolution. Add isopropanol to the mark and mix well. The concentration of this standard is $100\mu\text{g/mL}$ fosetyl-Al in isopropanol:water (9:1).
2. Withdraw a 10mL aliquot and add to a 100mL volumetric flask. Add $25\mu\text{L}$ conc. sulfuric acid. Dilute to the mark with isopropanol:water (9:1). The concentration of this standard is $10\mu\text{g/mL}$ fosetyl-Al equivalents in isopropanol:water (9:1).
3. Withdraw a 5mL aliquot and add to a 50mL flat-bottomed flask. Add 2mL (trimethylsilyl)diazomethane (2M in hexanes) under a nitrogen atmosphere and stopper the flask. Heat in a 35°C water bath for 30 minutes. Evaporate to $\sim 2\text{mL}$ volume on a rotary evaporator (water bath temperature below 35°C).
4. Transfer to a 5mL volumetric flask with isopropanol. Dilute to the mark and mix well. The concentration of this calibration standard of fosetyl-Me is $10\mu\text{g/mL}$ fosetyl-Al equivalents in isopropanol.

SAME SOLUTION

This solution is stable (w/ acid) nothing else is

5. By further dilution of the 10 µg/mL calibration standard with isopropanol, prepare a series of calibration standards. Withdraw a 1 mL aliquot and add to a 100 mL volumetric flask. Dilute to the mark with isopropanol and mix well. The concentration of this calibration standard of fosetyl-Me is 0.1 µg/mL fosetyl-Al equivalents in isopropanol.
6. Withdraw 0.5, 1, 2, 5, and 8 mL aliquots from the 0.1 µg/mL standard and add to 10 mL volumetric flasks. Dilute to the mark with isopropanol and mix well. The concentrations of these calibration standards of fosetyl-Me are 0.005, 0.01, 0.02, 0.05, and 0.08 µg/mL fosetyl-Al equivalents in isopropanol.

IV. Methods of Analysis

Samples should be allowed to warm/thaw prior to use. The analysis can be done in one day.

The tilde (~) indicates 'approximately.'

A. Cottonseed

1. Sample Preparation

Grind the cottonseed and then mill or grind.

2. Extraction

- 2.1 Weigh ~ 10g of substrate into a labeled 250 mL Nalgene[®] screw cap bottle.
- 2.2 Fortify untreated control samples (for determination of recovery) with fortification standard solution and let stand at least 10 minutes.
- 2.3 Add 75 mL of 1% sulfuric acid to the bottle.
- 2.4 Shake for ~30 minutes on a mechanical shaker.
- 2.5 Add ~10g Celite[®] to a glassfibre filter (wet with 1% sulfuric acid) in a Buchner funnel.
- 2.6 Under vacuum, press and smooth the Celite[®] pad down using the bottom of a 100 mL beaker.
- 2.7 Wash the Celite[®] pad with 50 mL 1% sulfuric acid. Do not filter to dryness. Allow the filter pad to remain wet. Discard the filtrate.
- 2.8 Filter the sample through the Celite[®] pad under vacuum into a tared 250 mL filter flask. Press the filter cake with a spatula or the bottom of a 100 mL beaker in order to squeeze out as much liquid as possible. If the filter is clogged, scrape the top layer of the filter cake and Celite[®] pad, redistribute and resume filtration.
- 2.9 Scrape the filter cake and the top layer of the Celite[®] pad back into the bottle.
- 2.10 Add 75 mL of 1% sulfuric acid to the bottle.
- 2.11 Shake for ~30 minutes on a mechanical shaker.
- 2.12 Filter back into the same Celite[®] pad. Press the filter cake with a spatula or the bottom of

a 50mL beaker in order to squeeze out as much liquid as possible. If the filter is clogged, scrape the top layer of the filter cake and Celite[®] pad, redistribute and resume filtration.

2.13 Weigh the filtrate.

3. Partitioning with 1% Sulfuric Acid/Hexane

3.1 Add the filtrate to a 500mL separatory funnel and wash twice with 100mL hexanes. Do not shake the separatory funnel too vigorously. *The organic phase is an emulsion. It is not necessary to wait for the emulsion to clear since an aliquot of the aqueous phase will be taken later.* Clean the separatory funnel with a water wash between hexane washes to remove residual emulsified organic phase.

4. Precipitation

4.1 Add a 5mL aliquot of the aqueous phase to a 50mL volumetric flask (since the density of the aqueous phase is 1.0, there is no need to weigh the aliquot).

4.2 Dilute to the mark with isopropanol and mix well.

4.3 Filter through a plug of glass wool into a 125mL Erlenmeyer flask. *It is not necessary to filter the complete sample since an aliquot will be withdrawn later.*

5. Derivatization

5.1 Withdraw a 25mL aliquot of the filtrate and add to a 125mL flat-bottomed flask.

5.2 Add 7-8mL of (trimethylsilyl)diazomethane (2M in hexanes) ~one mL at a time under a stream of nitrogen. The yellow color should remain. Swirl the flasks, stopper, and place in a 35°C water bath for 30 minutes.

5.3 Evaporate the solution to ~ 2mL volume on the rotary evaporator (until most of the yellow color is removed), maintaining the water bath temperature below 35°C.

5.4 Transfer to a 5mL volumetric flask with isopropanol. Dilute to the mark and mix well.

5.5 Analyze by gas chromatography using flame photometric detection.

Since the tolerance on cottonseed is low (0.2ppm) and the sample size is small (10g), it is advisable to analyze reagent (1% sulfuric acid, 100mL) spiked at 0.2ppm and correct the recoveries accordingly. Follow steps 4.1 through 5.5 above.

B. Plants

1. Sample Preparation

Thoroughly chop the frozen sample into small pieces with a knife. Grind with Dry Ice (~50g) in a blender if necessary. If Dry Ice is used, allow the samples to warm to room temperature before fortification.

2. Extraction

- 2.1 Weigh ~ 50g of substrate into a blender.
 - 2.2 Fortify untreated control samples (for determination of recovery) with fortification standard solution and let stand at least 10 minutes.
 - 2.3 Add 50mL of 1% sulfuric acid to the blender and blend for 2 minutes.
 - 2.4 Add 10g Celite[®] to a glassfibre filter (wet with 1% sulfuric acid) in a Buchner funnel.
 - 2.6 Under vacuum, press and smooth the Celite[®] pad down using the bottom of a 100mL beaker.
 - 2.7 Wash the Celite[®] pad with 50mL of 1% sulfuric acid. Do not filter to dryness. Allow the filter pad to remain wet. Discard the filtrate.
 - 2.8 Filter the sample through the Celite[®] pad under vacuum into a tared 250mL filter flask. Press the filter cake with a spatula or the bottom of a 100mL beaker in order to squeeze out as much liquid as possible. If the filter is clogged, scrape the top layer of the filter cake and Celite[®] pad, redistribute and resume filtration.
 - 2.9 Scrape the filter cake and the top layer of the Celite[®] pad back into the blender. Add 50mL of 1% sulfuric acid to the blender and blend for 2 minutes.
 - 2.10 Filter back into the same Celite[®] pad. Press the filter cake with a spatula or the bottom of a 100mL beaker in order to squeeze out as much liquid as possible. If the filter is clogged, scrape the top layer of the filter cake and Celite[®] pad, redistribute and resume filtration.
 - 2.11 Weigh the filtrate and mix well. Alternatively, one may dilute the filtrate to a convenient volume with 1% sulfuric acid and mix well.
3. Partitioning with 1% Sulfuric Acid/Hexane (for onion and high fat substrates (eg. avocado))
This partitioning step may be excluded for substrates with low fat content.
- 3.1 Add the filtrate to a 500mL separatory funnel and wash twice with 100mL hexanes. Do not shake the separatory funnel too vigorously. *The organic phase is an emulsion. It is not necessary to wait for the emulsion to clear since an aliquot of the aqueous phase will be taken later.* Clean the separatory funnel with a water wash between hexane washes to remove residual emulsified organic phase.
4. Precipitation
- 4.1 Add a 5mL aliquot of the filtrate from step 2.11 (or aqueous phase from step 3.1 if the partitioning step was included) to a 50mL volumetric flask.
 - 4.2 Dilute to the mark with isopropanol and mix well.
 - 4.3 Filter through a plug of glass wool into a 125mL Erlenmeyer flask. *It is not necessary to filter the complete sample since an aliquot will be withdrawn later.*

5. Derivatization

- 5.1 Withdraw a 5mL aliquot of the filtrate and add to a 125mL flat-bottomed flask (a 15mL aliquot is recommended for substrates with a tolerance of 0.1ppm).
- 5.2 Add 2mL (5mL is necessary for samples containing a 15mL aliquot) of (trimethylsilyl)-diazomethane (2M in hexanes) one mL at a time under a stream of nitrogen. The yellow color should remain. Swirl the flasks, stopper, and place in a 35°C water bath for 30 minutes.
- 5.3 Evaporate the solution to ~ 2mL volume on the rotary evaporator (until most of the yellow color is removed), maintaining the water bath temperature below 35°C.
- 5.4 Transfer to a 5mL volumetric flask with isopropanol. Dilute to the mark and mix well.
- 5.5 Withdraw appropriate aliquots and dilute accordingly to the range spanned by the calibration standards prepared simultaneously. Analyze by gas chromatography using flame photometric detection.

C. General Notes

1. If the untreated samples are shown to contain fosetyl-Me by GC-FPD analysis, reanalyze the sample and include the partition step (3.1) to remove fatty residues.
2. Some samples (eg. lemon) may take ~ 1 hour to filter. Increasing the vacuum does not necessarily hasten filtration. It is important to be patient and ensure that the filter cakes are squeezed of as much liquid as possible.
3. Some of the filtrates foam and care must be taken to avoid sucking the filtrate into the vacuum line. The vacuum needs to be adjusted to regulate the foaming.
4. The samples should be analyzed within 24 hours. The stability of the samples beyond 24 hours has not been determined.

V. Gas Chromatography

A. Instrumentation

- | | | |
|-----------------------|-------------------|---|
| 1. Gas Chromatograph: | Fisons Carlo Erba | Series 8000 GC, or equivalent |
| | Fisons Carlo Erba | Autosampler, CTC A200S, or equivalent |
| 2. Detector: | Fisons Carlo Erba | Flame Photometric Detector FPD-700, or equivalent |
| 3. Data Acquisition: | Waters | 860 Data Capture System, or equivalent |
| 4. Column: | J&W Scientific | DB-WAX 15m x 0.53mm id x 1µm film |

B. GC Conditions

1. Detector Make-Up Gas: Helium, 5kPa, ~30mL/min
2. Carrier Gas: Helium, ~3mL/min

60°C
90°C
7.5 psi = 23 ml/min

90°C
7.5 psi = 19.5 ml/min
2 psi = 3 ml/min

AL
best
settings

Makeup = 0.5 kPa
H_f = 150 kPa
air = 45 kPa

7 psi Col press

MWP

- | | | | |
|---|-----------------|--|---------------------------------|
| 3. Inlet Liner: | | Glass liner, 3mm id, tapered
(Fisons Cat. No. 45320032) | |
| 4. Injector Temperature: | 200 | 160°C | |
| 5. Detector Temperature: | | 220°C ← on FPD ^{and on} Zone 2 Zone 2 temp). | |
| 6. Oven Temperature: | 5 min | Equilibration Time: 1 minute | |
| | 10 - 6 min | Initial: 90°C, hold 1 minute | |
| | 25°C/min - 240° | Ramp 3°/min to 105°C, hold 1 minute | |
| | 15 min | Ramp 20°C/min to 220°C, hold 6 minutes | Time for temp program 18.75 min |
| 7. Injection Volume | 3 | 4.0µL | |
| 8. Splitless injection with split vent off for 30 seconds | | | |

C. General Notes

1. The carrier gas flow cannot be measured with the FPD-700 detector installed. It was estimated based on retention time of unreturned peak (solvent front and volume of column).
2. The injector temperature can be set at 100°C if adequate sensitivity is achieved. Increasing the injector temperature above 160°C usually does not increase sensitivity.
3. A silver seal (Fisons Cat. No. 29003629) can be used at the interface of the glass liner and the graphite seal (Fisons Cat. No. 29003406).
4. The GC parameters are guidelines and can be optimized for the instrument and column actually used. Record the actual GC conditions used for data acquisition and include in report.
5. The initial 6-12 inches of the column may need to be cut off after repeated column use (~300 samples) due to contamination from crop matrices.

VI. Quantification of Residues

A. Calibration Curves

1. Linear regression should be used to generate calibration curves for the analyte. At least five different standard concentrations should be run with each set of samples. Standards should be interspersed with samples to compensate for any minor change in instrument response. Extracts should be diluted such that the peak areas obtained are within the area range between the lowest and highest standards injected.
2. Linear regression coefficients should be calculated from response (peak area or height) versus concentration of the standard (µg/mL). Data from the derivatized calibration standards should be fit to the linear equation,

$$y = a + bx$$

where: y = peak area or height
a = calibration line intercept

b = calibration line slope

x = conc of analyte in inj soln

3. When the calculated values of the coefficient of determination (r^2) begin to decline, it is usually an indication that the GC inlet liner needs to be changed or that the front end of the column needs to be cut.

B. Quantification of Residues

1. Fosetyl-Al should be quantified as fosetyl-Me by comparison to the standard curves obtained from a linear regression analysis of the data.

2. Equations

2.1 Concentration of analyte in sample in ppm (parts per million).

$$z = \frac{10(y - a)ce}{bdf}$$

where:

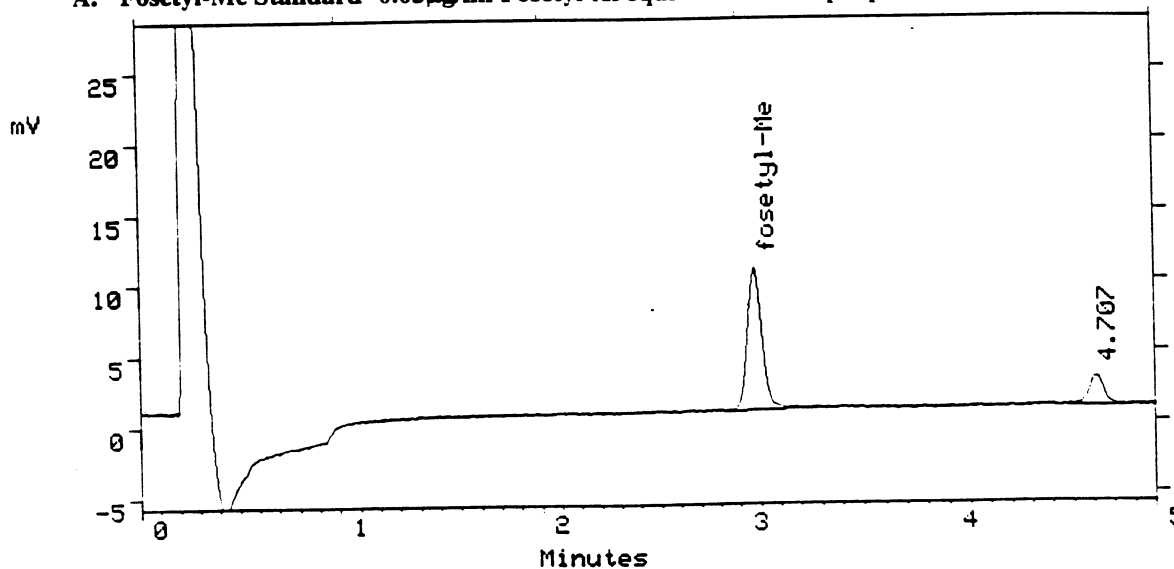
- y = peak area (or height), response of analyte of interest
- a = intercept of calibration line from linear regression ($\mu\text{g/mL}$)
- b = slope of calibration curve from linear regression (response per $\mu\text{g/mL}$)
- c = final volume of sample (mL)
- d = sample weight (g)
- e = weight of filtrate (g), or volume of filtrate (mL)
- f = volume of aliquot taken from 50mL dilution (precipitation) step
- z = conc of analyte in sample (ppm)

2.2 Percent recovery

$$\% \text{ recovery} = \frac{(\text{ppm found in fortified sample} - \text{ppm found in UTC}) \times 100}{\text{actual fortification level in ppm}}$$

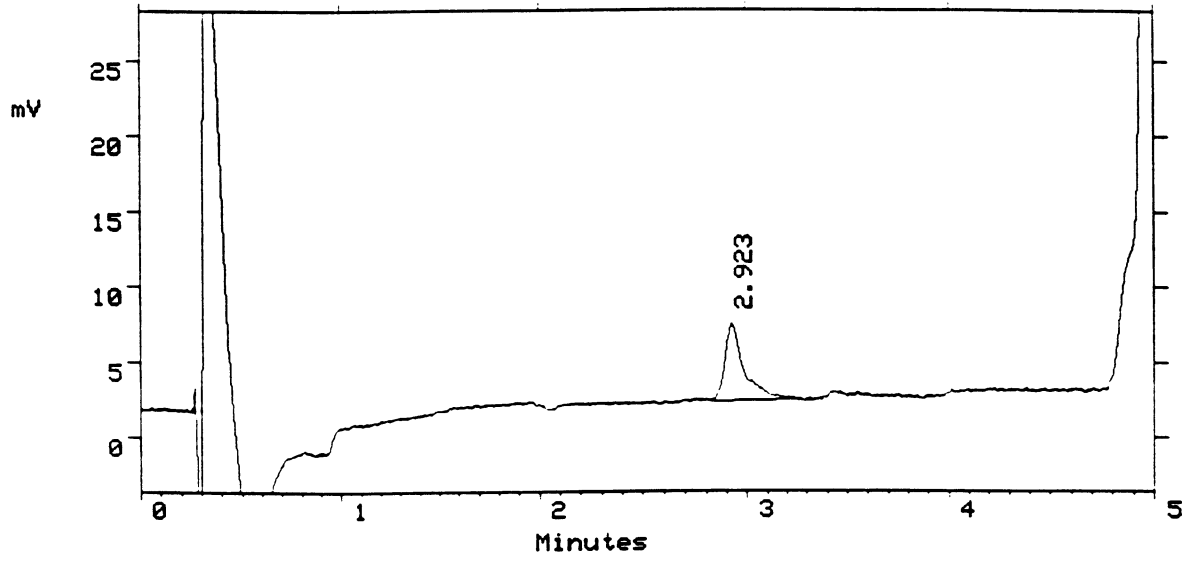
VII. Example Chromatograms (Waters 860 Data Capture)

A. Fosetyl-Me Standard- 0.05 $\mu\text{g/ml}$ Fosetyl-Al equivalents in isopropanol

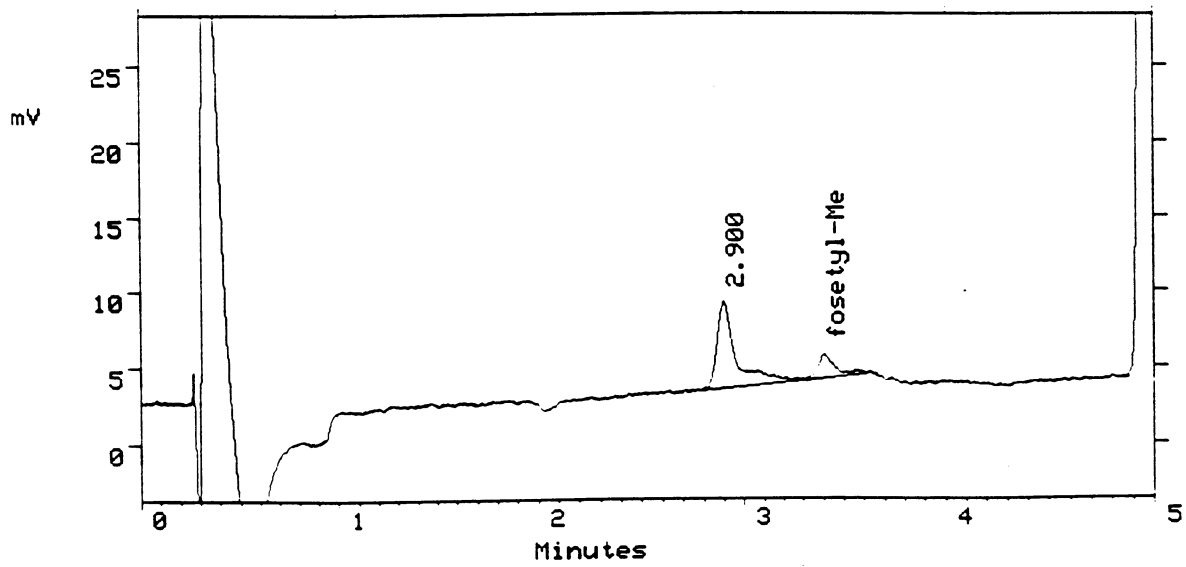


B. Cottonseed Samples

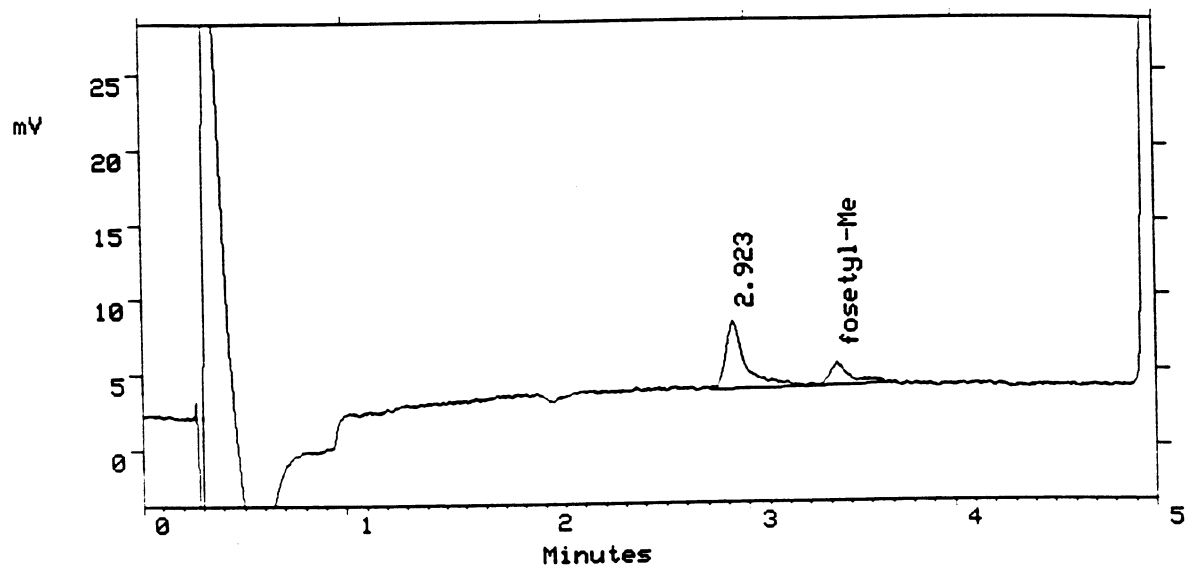
1. Cottonseed, Untreated Control



2. Cottonseed, Fortified at 0.2ppm Level

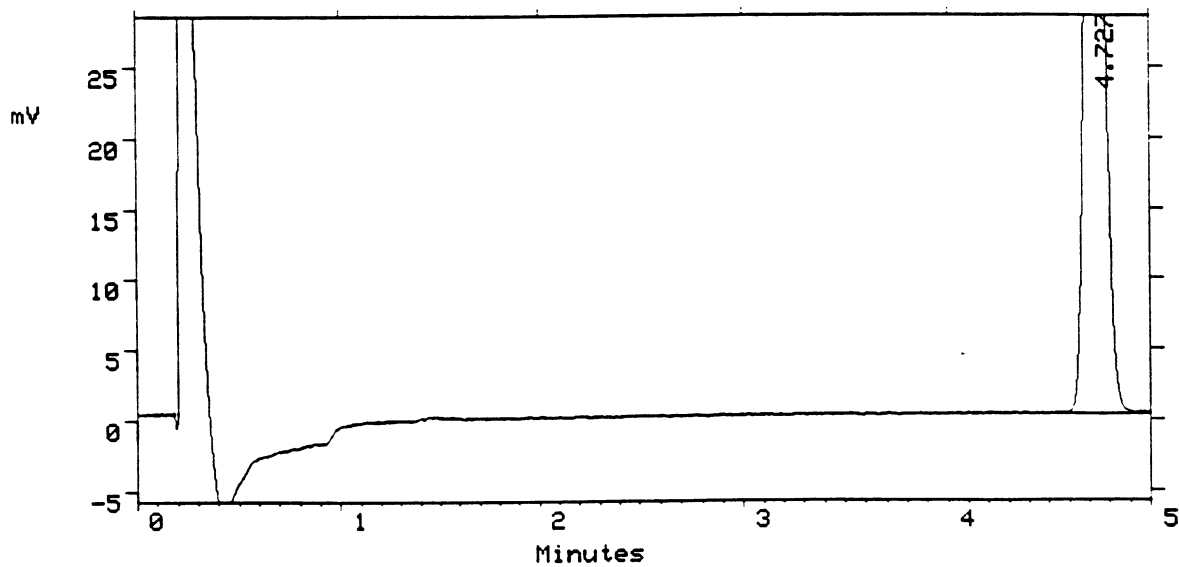


3. Cottonseed, Reagent Spike at 0.2ppm Level

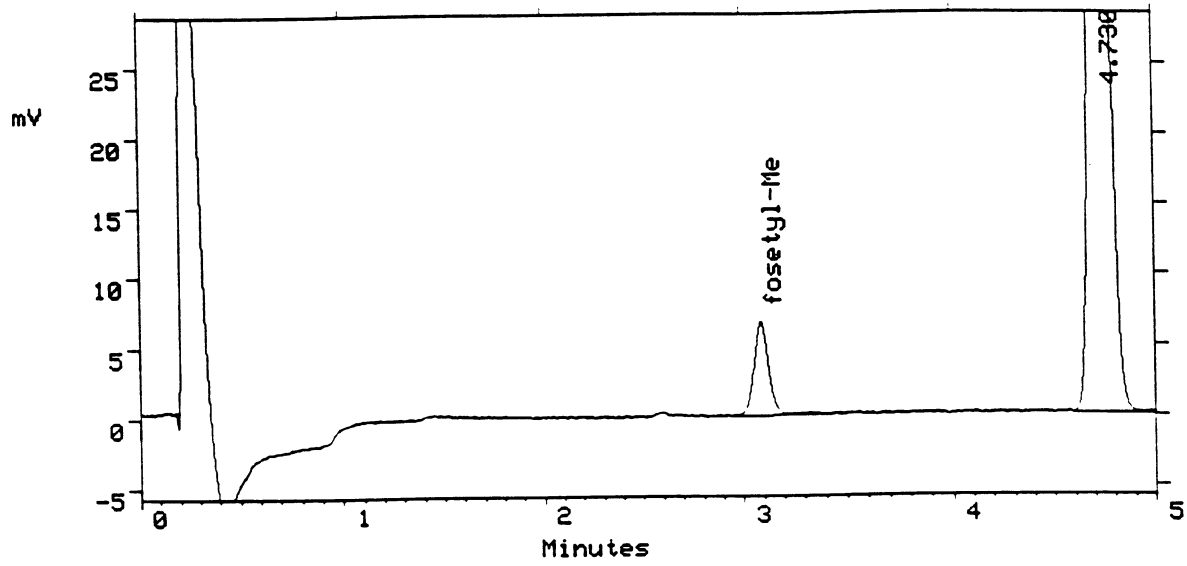


C. Brassica vegetables

1. Mustard Greens, Untreated Control

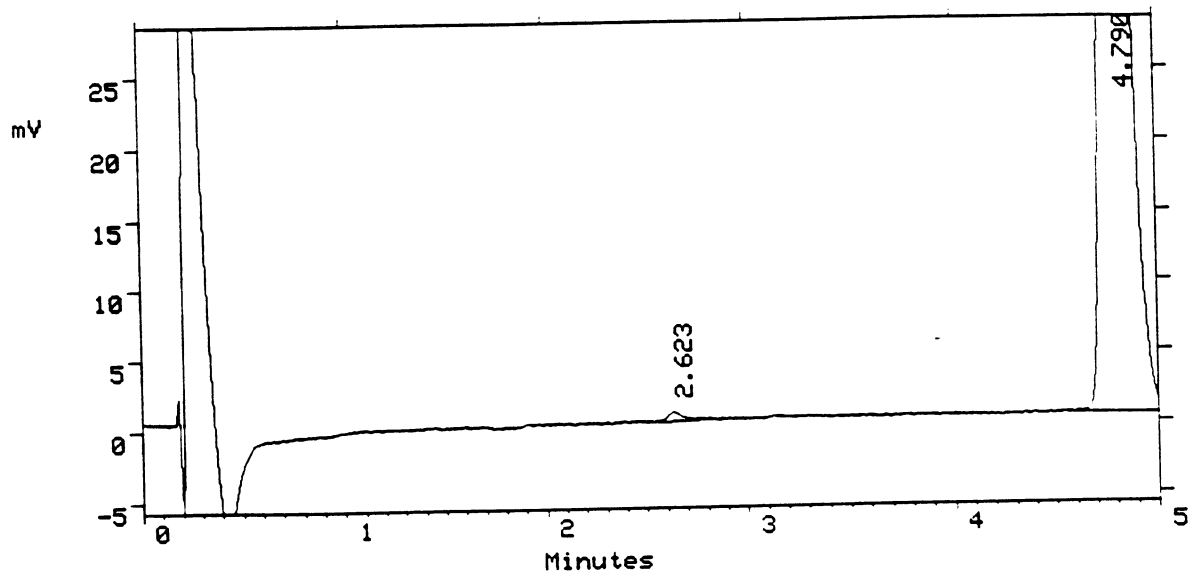


2. Mustard Greens, Fortified at 54.7ppm Level

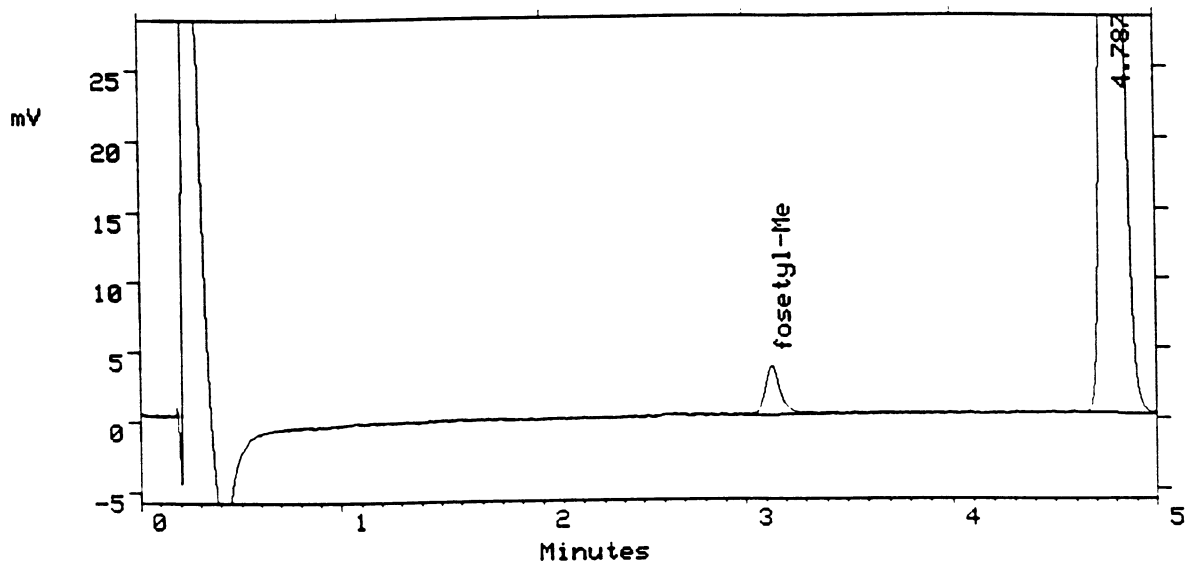


D. Curcubit vegetables

1. Cantaloupe, Untreated Control

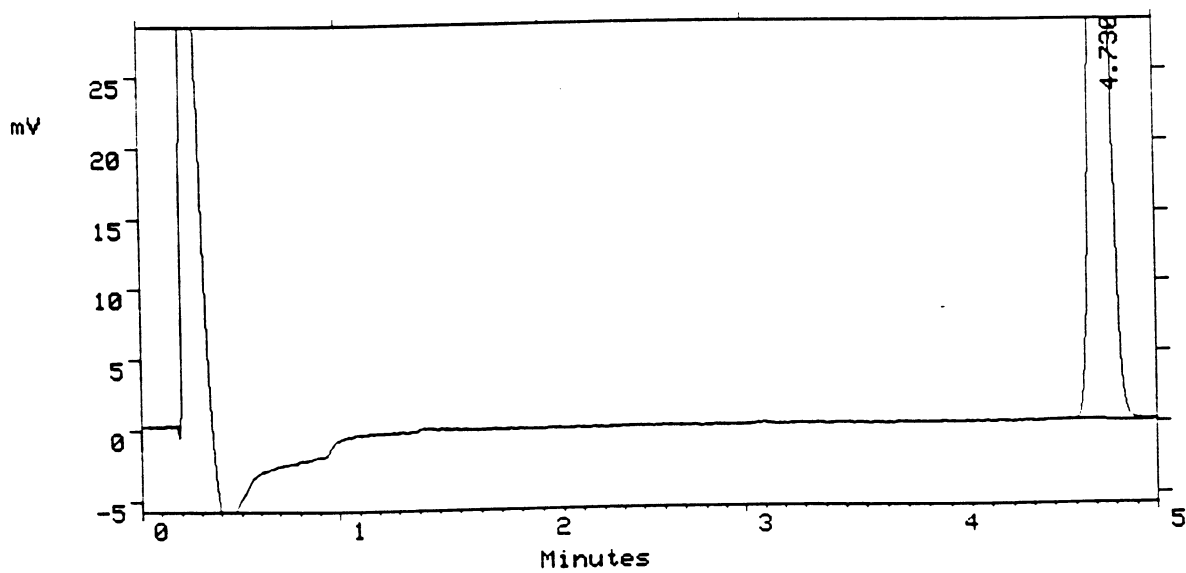


2. Cantaloupe, Fortified at 15ppm Level

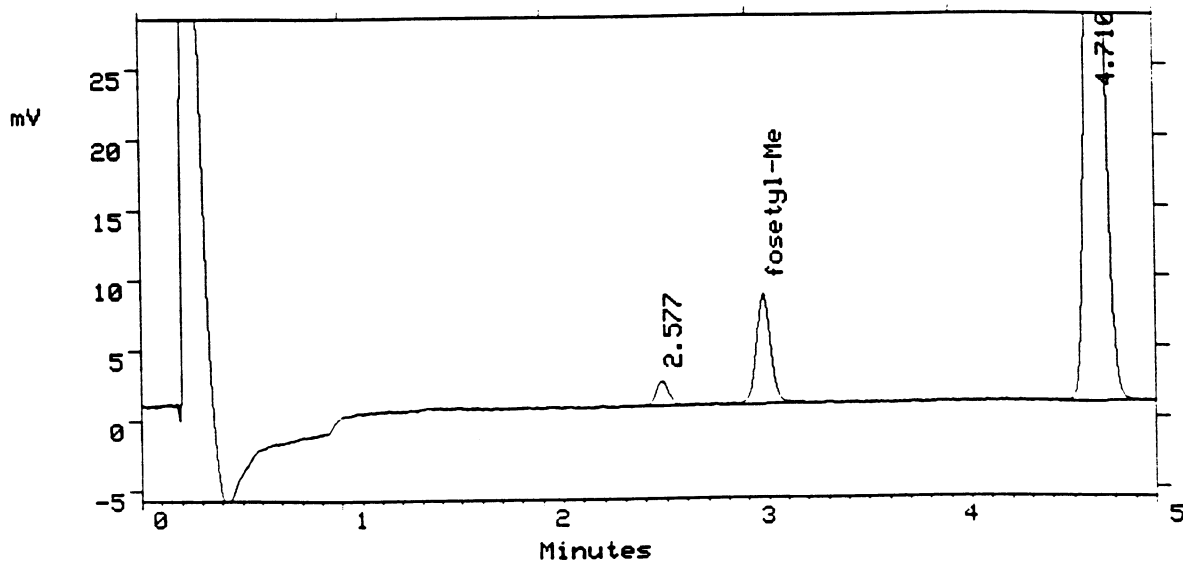


E. Leafy vegetables

1. Spinach, Untreated Control

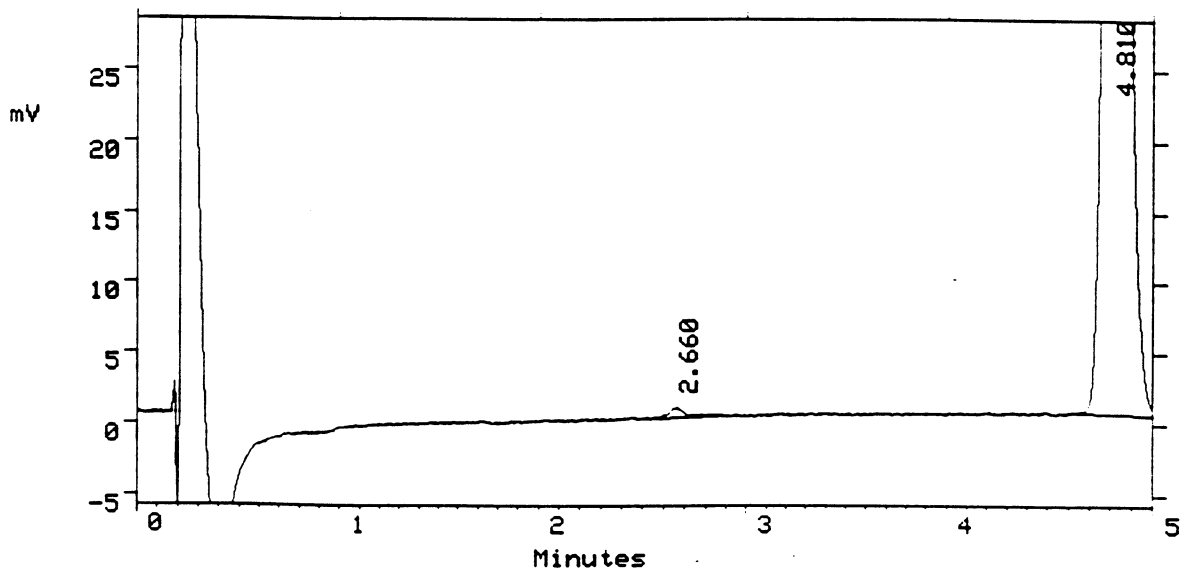


2. Spinach, Fortified at 78.2ppm Level

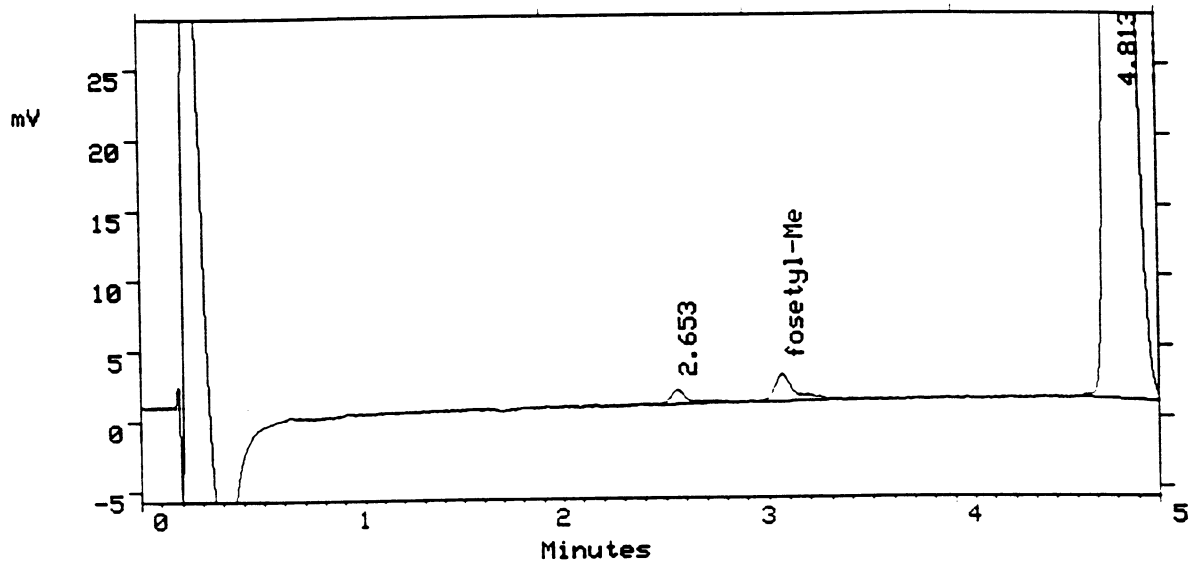


G. Bulb Vegetables

1. Onion, Untreated Check

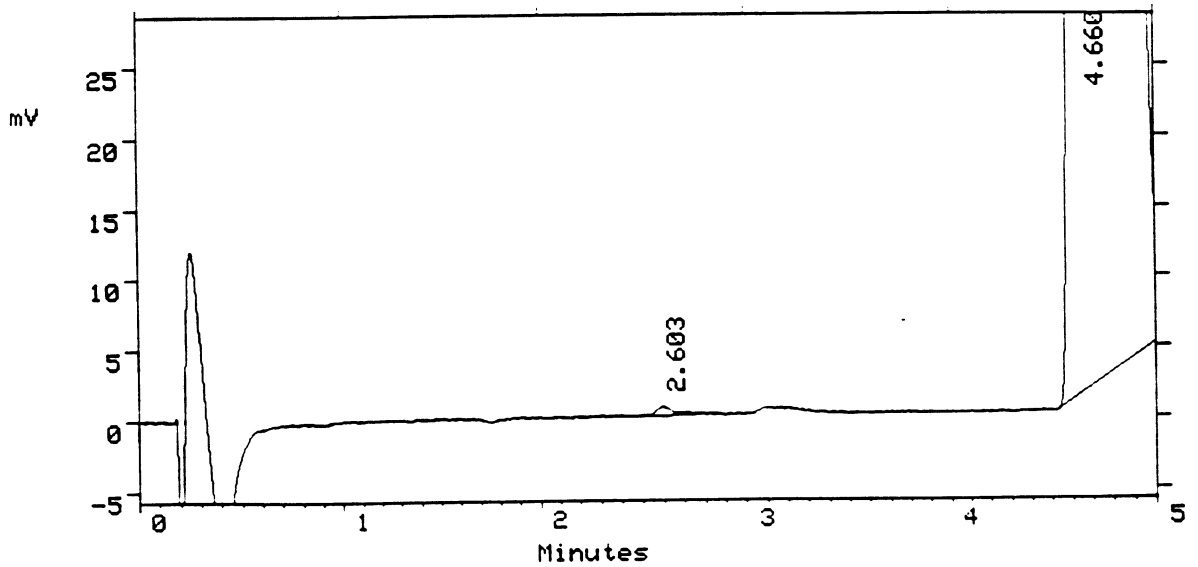


2. Onion, Fortified at 0.5ppm Level

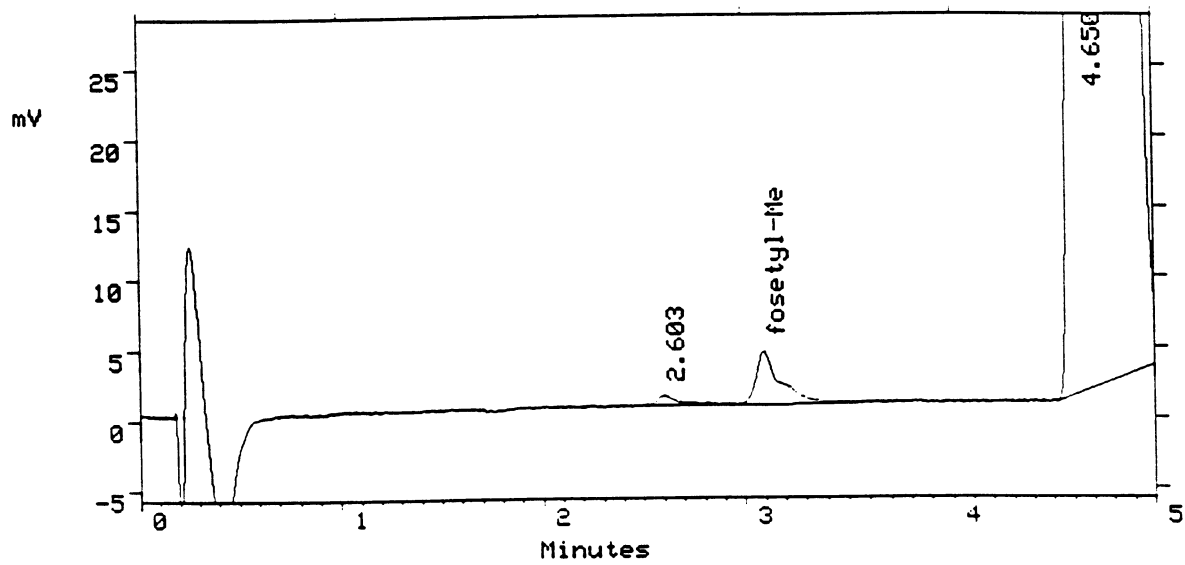


F. Citrus

1. Lemon, Untreated Control

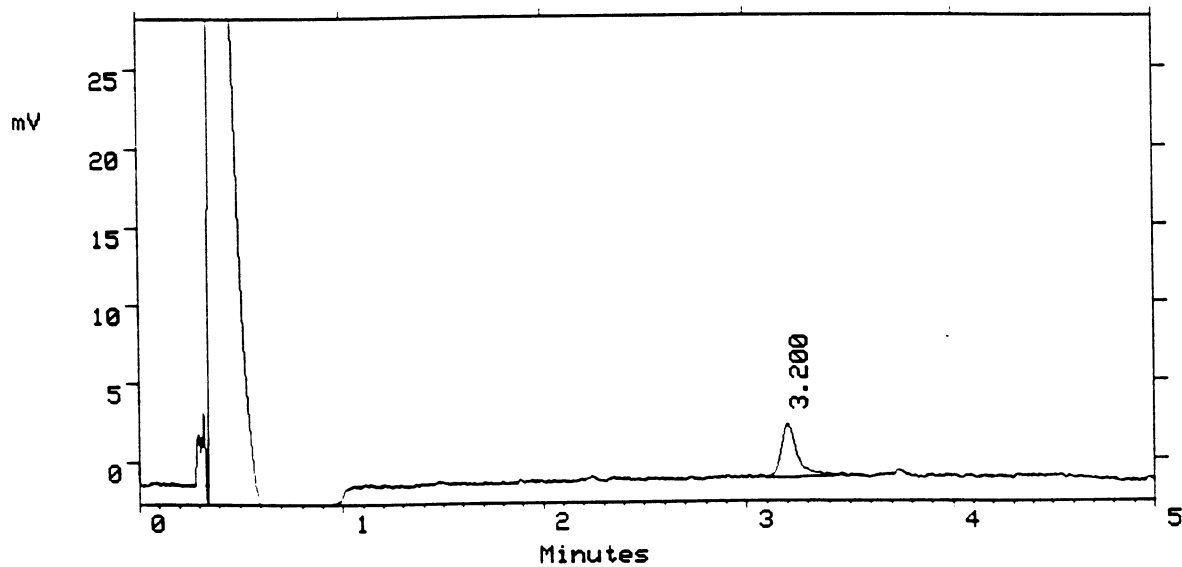


2. Lemon, Fortified at 0.5ppm Level

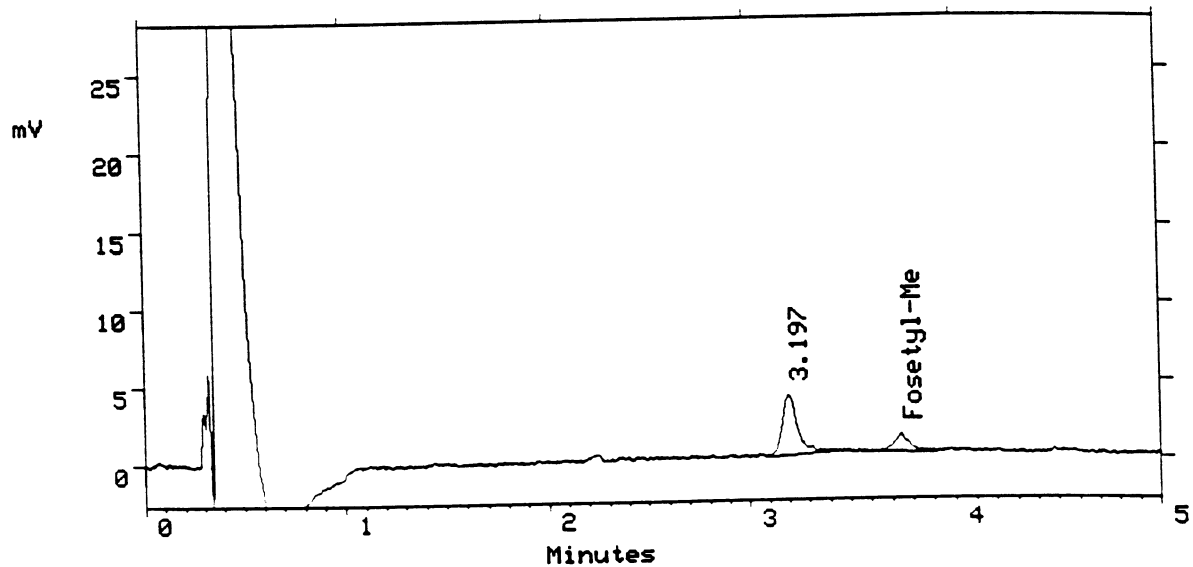


H. Pineapple

1. Pineapple, Untreated Check

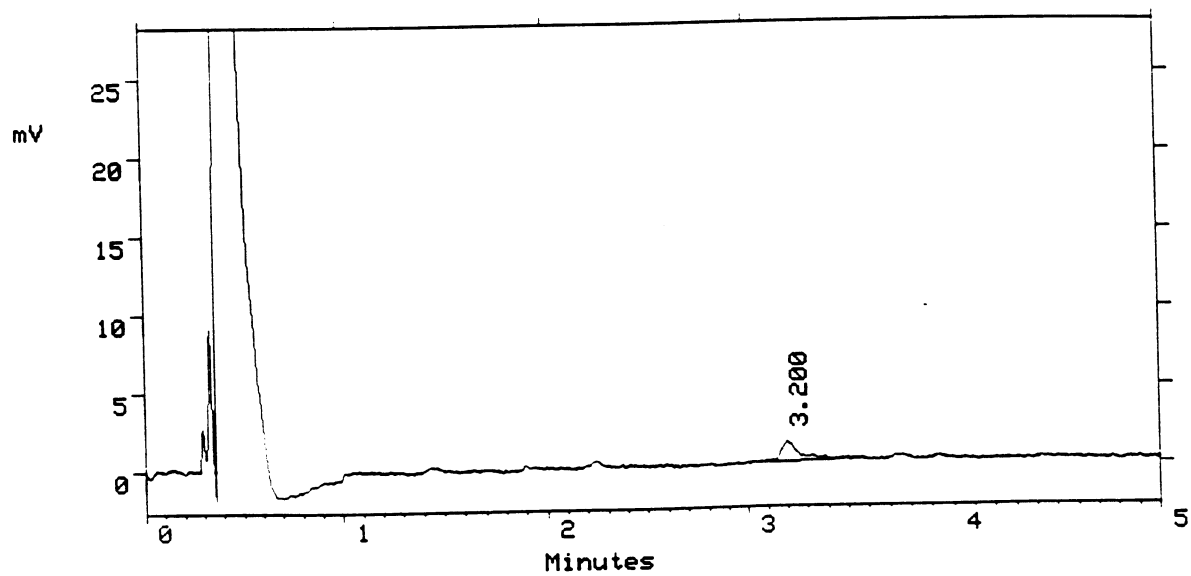


2. Pineapple, Fortified at 0.1ppm Level



I. Banana

1. Banana, Untreated Check



2. Banana, Fortified at 0.1ppm Level

