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**TOUCHDOWN®: Determination of Residues of the Trimethylsulfonium
Cation in Agricultural Crops by Gas Chromatography**

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Author

432736-04

Y. Iwata

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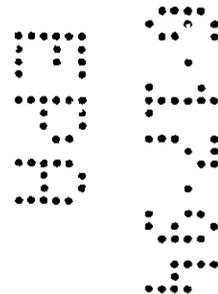
ZENECA Ag Products
Western Research Center
Environmental Chemistry Section
1200 South 47th Street
Box 4023
Richmond, CA 94804-0023

Study Number

GLYP-93-AM-04

Report Number

RR 93-105B



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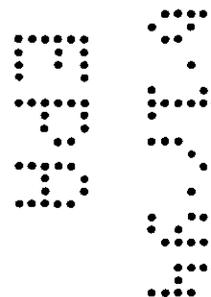
Submitter M. E. Rhodes 12/22/93
Date

Regulatory Product Manager M. E. Rhodes
(Title) (Signature)

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by Gas Chromatography

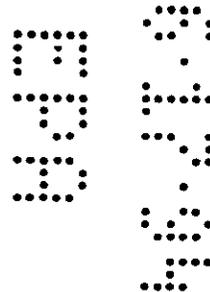
GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study meets the requirements for 40 CFR Part 160.

Submitter M. E. Rhodes 12/22/93
M. E. Rhodes Date
Regulatory Product Manager
ZENECA Ag Products
Telephone: (615) 982-9076

Sponsor M. G. Kleinschmidt by Diana Baba 12/30/93
M. G. Kleinschmidt, Ph.D. Date
Manager,
Environmental Chemistry Section
ZENECA Ag Products

Study Director Y. Iwata 12/30/93
Y. Iwata Date
Research Associate
ZENECA Ag Products



Study Number: GLYP-93-AM-04

Report Title: Touchdown®: Determination of Residues of Trimethylsulfonium Cation in Agricultural Crops by Gas Chromatography

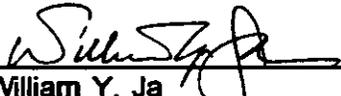
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QUALITY ASSURANCE STATEMENT

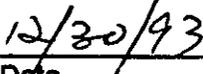
In accordance with Zeneca Ag Products (Zeneca Inc.) policy and procedures for Good Laboratory Practice, the conduct of this study was inspected/audited by the Quality Assurance Unit at the Western Research Center, Richmond, California, United States of America.

<u>Date</u>	<u>Inspection/Audit</u>	<u>Report Date</u>
Nov 29, 1993	Protocol	Nov 29, 1993
Nov 29, 1993	Study Conduct	Nov 29, 1993
Dec 28, 1993	Final Report & Raw Data	Dec 29, 1993

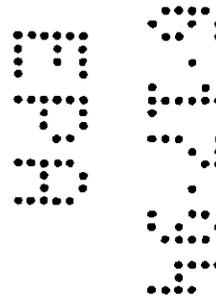
So far as can be reasonably established, the methods described and results incorporated in this report accurately reflect the raw data produced during the study



William Y. Ja
Group Leader, Quality Assurance Unit



Date



Study Number: GLYP-93-AM-04
Study Title: TOUCHDOWN®: Determination of Residues of the Trimethylsulfonium Cation in Agricultural Crops by Gas Chromatography

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 *Y. Iwata* 12/30/93
Y. Iwata Date
Research Associate

The following ZENECA Ag Products personnel performed work on this study:

Y. Iwata Protocol Preparation and Sample Analysis
Sharon Patterson Sample Tracking

Approved By:

D. G. Graham 12/30/93
D. G. Graham, Ph.D. Date
Group Leader,
Environmental Chemistry Section

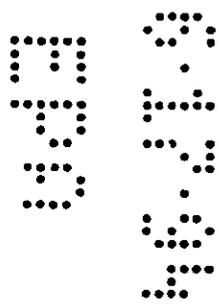


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1 SUMMARY/INTRODUCTION

This method is intended for determining residues of the trimethylsulfonium cation (TMS) in animal feed and food crops at levels of 0.1 to 1 ppm and 0.05 to 0.5 ppm, respectively. Glyphosate-trimesium, which is composed of a 1:1 mixture of the trimethylsulfonium cation [CAS Registry No. 676-84-6] and the glyphosate(-1) anion, is the active herbicidal ingredient in the formulated product marketed by ZENECA Ag Products under the trademark, TOUCHDOWN®. The TMS is extracted from finely chopped or milled samples by maceration with an aqueous phosphate buffer. The aqueous extract is recovered by filtration through a glass-fiber filter. An aliquot of the extract is (1) treated with basic anion-exchange resin to neutralize acids and then with (2) phenylisocyanate and barium hydroxide to remove amino acids. The mixture is filtered. An aliquot of the cleaned and filtered extract is further cleaned-up on columns packed with cation-exchange resin. The eluate, if necessary, is heated overnight in the presence of tin(II) chloride at 100° C to decompose residual interfering compounds. The eluate is then heated at 100° C in the presence of potassium hydroxide and toluene to dealkylate the TMS to dimethylsulfide (DMS). The DMS, that is trapped in the toluene, is quantitated by using capillary, gas chromatography and a sulfur chemiluminescence detector (SCD) or a flame photometric detector (FPD). Recoveries of TMS from corn forage and fodder fortified at 0.1 and 1.0 mg/kg, and corn grain fortified at 0.05 and 0.50 mg/kg, ranged from 82 to 118%, with a mean recovery of 99% (n=18) and coefficient of variation of 11.9%, when analyzed using this method.

2 **MATERIAL/METHODS**

The recommended equipment and reagents are described. Equipment with equivalent performance specifications and reagents of comparable purity can be substituted.

2.1 **Apparatus**

2.1.1 **Blender and Jar.** Waring brand; 1-pt jars with lids.

2.1.2 **Pipets and Pipet Tips.** Eppendorf adjustable model pipets for 10-100 uL delivery (catalog # 22-44-010-1) and 100-1000 uL delivery (catalog # 22-44-020-9) with corresponding disposable tips.

2.1.3 **Jars.** 4-oz, wide-mouthed jars with screwcaps.

2.1.4 **Filter Funnel with Filter Flask.** (a) Porcelain, Buchner funnel with 91-mm diameter at plate (Coors 60243) and a 250-mL filter flask with filter adapter. (b) Porcelain, Buchner funnel with 43-mm diameter at plate (Coors 60239) and a 125-mL filter flask with filter adapter.

2.1.5 **Filters.** (a) Whatman GF/D glass microfiber, 9-cm diameter (Whatman, catalog # 1823-090). (b) Whatman No. 1 paper, 4.25-cm diameter (Whatman, cat. # 1001-042).

2.1.6 **Column Rack.** Econo System Rack (Bio-Rad, Hercules, CA; catalog no. 731-8200).

2.1.7 **Columns.** (a) 15 by 1.5 cm i.d. chromatographic columns (Bio-Rad, cat. # 737-1516), (b) 20 by 1.0 cm i.d. chromatographic columns (Bio-Rad, cat. # 737-1021).

- 2.1.8 Connectors. Female Luer to female Luer (Bio-Rad, cat. # 731-8228).
- 2.1.9 Beakers. 100-mL capacity, borosilicate glass beakers.
- 2.1.10 Pipets and Pipet Filler. Disposable, 5- and 10-mL capacity. Red pipet filler (Baxter Scientific Products, cat. # P5311-1).
- 2.1.11 Graduated Cylinders. 10-, 25-, 50-, 100-, 250- and 1000-mL capacity.
- 2.1.12 15-mL Glass Vials with Screw-Caps. Each cap has a hole and contains a silicone septum lined with a perfluoroethylene polymer (Supelco, Bellefonte, PA; cat. # 2- M3284). *Now 2-7159
Nud*
- 2.1.13 Electric Heating-Module. A Multi-Blok Heater (115 V, 50/60 Hz, 100 watts) made by Lab-Line Instruments, Inc. (Melrose Park, IL). The unit is equipped with an aluminum heating block (Supelco; catalog no. 3-3316) drilled with 8 holes (21 mm wide, 31 mm deep) to accept glass vials for heating.
- 2.1.14 Disposable Pipets. 5.75-inch, glass Pasteur pipets (VWR, cat. # 14672-200) and pipet bulbs.
- 2.1.15 Autosampler Vials, Inserts, and Caps. Standard 2-mL crimp-top vials (Sunbrokers, catalog no. 200-000) with standard crimp top (Sunbrokers, catalog no. 200-100), and limited-volume (250 uL) inserts (Sunbrokers, catalog no. 200-225). A crimper is also required.
- 2.1.16 Gas-Chromatographic System. A Hewlett-Packard (HP) model 5880A, Level 4, gas chromatograph equipped with a HP model 7673A autosampler/injector. The autosampler is equipped with a 10-uL syringe with a 23-gauge needle (Hamilton 701N).

The instrument is equipped with a 350B Sulfur Chemiluminescence Detector made by Sievers (Boulder, CO) and a HP 3394A Integrator.

Alternatively, A HP model 5890A gas chromatograph equipped with a HP model 7673A high-speed autosampler/injector. The autosampler is equipped with a 10-uL syringe with a 23-gauge needle (Hamilton 701N). The instrument is equipped with a flame photometric detector with a sulfur bandpass filter and a HP 3396A integrator.

2.1.17 Gas-Chromatographic Column. A 30 m by 0.53 mm i.d., fused-silica, capillary column bonded with a 1.5-um thickness of (95%) -dimethyl-(5%) -diphenylsiloxane, Durabond-5 (cat. # 125-5032, J&W Scientific; Folsom, CA).

2.2 Reagents and Materials

2.2.1 Water. Distilled or deionized water.

2.2.2 1.0 N Hydrochloric Acid (HCl). Aqueous solution; available from Mallinckrodt (catalog no. 6388). Note: Dilution of 83 mL of concentrated reagent HCl to 1.0 liter will yield a 1 N HCl solution.

2.2.3 Hydrochloric Acid Solutions. 0.1 and 0.02 N acid solutions are used for column chromatography. To prepare a 0.1 N HCl solution, dilute one part of 1.0 N HCl with 9 parts of distilled water. To prepare a 0.02 N HCl solution, dilute one part of 0.1 N HCl with 4 parts of distilled water.

2.2.4 85% Phosphoric Acid. Fisher, cat. # A-242.

2.2.5 10% Phosphoric Acid. Dilute 100 mL of 85% phosphoric acid solution to 850 mL total volume.

- 2.2.6 Disodium Hydrogen Phosphate. Mallinckrodt, cat. # 7917.
- 2.2.7 0.1 M Disodium Hydrogen Phosphate. 14.2 g/liter.
- 2.2.8 Methanol. A high-purity grade suitable for trace analysis.
- 2.2.9 Phosphate Buffer (Extraction Solution). Prepare 1-liter of aqueous solution containing 100 mL 10% phosphoric acid, 48 mL 0.1 M disodium hydrogen phosphate, and 40 mL of methanol.
- 2.2.10 Anion-Exchange Resin. AG 1-X8, 20-50 mesh, hydroxide form (Bio-Rad Labs., Richmond, CA; cat. # 140-1422).
- 2.2.11 Cation-Exchange Resin for Cleanup. Bio-Rex 70, 50-100 mesh, sodium form, analytical-grade cation exchange resin (Bio-Rad Labs.; cat. # 142-5832).
- 2.2.12 Cation-Exchange Resin for Concentration. AG 50W-X2, 100-200 mesh, hydrogen form, analytical-grade cation exchange resin (Bio-Rad Labs.; cat. # 142-1241).
- 2.2.13 Solid Glass Balls. 1.5- to 2-mm diameter glass balls (Jencons Ltd.; marketed by Scientific Products (Baxter) catalog no. G6031-15).
- 2.2.14 Barium Hydroxide Monohydrate. Reagent powder (Matheson Coleman and Bell).
- 2.2.15 Phenylisocyanate. Aldrich Chemical Company, cat. # 18,535-3.
- 2.2.16 Potassium Chloride. 99+ % purity (Kodak, cat. # 104-9766).
- 2.2.17 1 M Potassium Chloride Solution. 74.56 g/L

- 2.2.18 Toluene. A high-purity grade of solvent suitable for trace-organic analyses.
- 2.2.19 Potassium Hydroxide. Dry solid pellets with a minimum assay of 85%. Note: KOH must pass through mouth of dealkylation vial.
- 2.2.20 Tin(II) Chloride Dihydrate ($\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$). Assay $\geq 98\%$; A.C.S. Reagent.

2.3 Reference Materials

- 2.3.1 Reference Standard. Trimethylsulfonium iodide, assay $\geq 99\%$, is available from Zeneca, Inc., 1200 South 47th Street, Box Number 4023, Richmond, CA 94804-0023; Attention: Manager, Environmental Sciences Department.

The Material Identification number of the TMS iodide used in this study was ASW 1441A (Request number was 1993-I156).

- 2.3.2 Calibration and Fortification Stock Solutions. Prepare two separate stock solutions. Calibration solutions are used to calibrate the instrument. Fortification solutions are used to fortify samples and demonstrate procedural recoveries, if required. All solutions are prepared with 0.1 N HCl solution in order to inhibit microbial growth.

Prepare a stock solution containing 2.66 mg of trimethylsulfonium iodide per milliliter of 0.1 N HCl. [The formula weights of TMS, iodide, and TMS iodide are 77, 127, and 204, respectively; therefore the TMS/TMS iodide ratio is 0.377, and 2.66 mg TMS iodide/mL multiplied times 0.377 equals 1.00 mg TMS/mL].

To prepare each stock solution place a known quantity (± 0.1 mg) of approximately 266 mg of TMS iodide in a 4-oz amber, glass bottle. Multiply the amount of milligrams weighed out by 0.377 to calculate the weight of 0.1 N HCl to add to the TMS iodide, e.g., if exactly 266 mg are weighed out, add 100 g of 0.1 N HCl. Add the calculated weight of 0.1 N HCl to the 4-oz bottle. Close the bottle with a Poly-Seal cap or a cap lined with a fluorocarbon polymer, such as Teflon®. Mix the contents thoroughly to dissolve the TMS iodide. The concentration of the stock solution is 1.0 mg or 1000 ug TMS/mL. Starting with the stock solution, make 1:10 serial dilutions to prepare 100, 10 and 1.0 ug TMS/mL solutions. Use 0.1 N HCl to prepare all solutions.

3 ANALYTICAL PROCEDURE

Crop samples are extracted with an extraction solution consisting of an aqueous phosphate buffer. Therefore, it is necessary to estimate the amount of water in the sample prior to extraction. This estimation is facilitated for food items by use of the Composition of Foods handbook (reference 1). For example, a value of 13.8% water is given for "corn, field, whole-grain, raw" in the handbook. If 50 g of corn grain is assumed to contain 7 mL of water and 243 mL of water are used for extraction, the total volume of aqueous extract is 250 mL. The crop:extract ratio is 0.2 g per milliliter of extract. The water content of forage and fodder are typically 70 and 60%, respectively. If desired, a subsample for analysis can be oven-dried at a low temperature, for example, 75° C, to a constant weight to obtain a better estimate of water content.

3.1 Sample Extraction of Non-Oily Crops

- 3.1.1 Place 25 g of crop sample and an appropriate volume (125 mL less moisture in sample) of phosphate buffer extraction solution in a blender jar, and blend for 10 min. Let the macerate stand for at least 0.5 hr.

Note: 1) If untreated controls are available, fortify samples prior to addition of the phosphate buffer. 2) The acidic phosphate buffer appears to be required to reduce adsorption on low moisture feed items, such as corn forage and corn fodder. 4) Water can be used instead of the phosphate buffer for fruit samples, such as citrus, pome and stone fruits, and grapes. 5) A 'LO' speed and a rheostat setting of 40 is suggested to minimize stress on the blender assembly and heating of the macerate.

- 3.1.2 Vacuum-filter the extract through a Buchner funnel lined with a 9-cm, glass-fiber filter.

Note: 1) Do not use a filter aid, such as diatomaceous earth, as it will likely adsorb the TMS cation and remove it from the extract. 2) Avoid filter paper because it will likely clog. 3) Only 40 mL of extract is needed to continue with this method.

- 3.1.3 Proceed to section 3.3.

3.2 Sample Extraction of Oily Crops

- 3.2.1 Place 50 g of crop sample and an appropriate volume (250 mL less moisture in sample) of phosphate buffer extraction solution in a blender jar, and blend for 10 min. Let the macerate stand for at least 0.5 hr.

Note: If untreated controls are available, fortify samples prior to addition of the phosphate buffer. 2) A 'LO' speed and a rheostat setting of 40 is suggested to minimize stress on the blender assembly and heating of the macerate.

- 3.2.2 Place the crop macerate in a 16-oz jar. Cap and centrifuge the jar for 20 min to separate the solid, aqueous, and oil phases. Remove the floating oil phase using a spoon.

Note: 1) The separation, for nutmeats, is only partially effective in that the aqueous phase retains a milk-like appearance due to suspended particles and oil. 2) It is not possible to remove all of the oil phase due to a mechanical breakup of the layer during removal. 3) Do not use a filter aid, such as diatomaceous earth, as it will likely adsorb the TMS cation and remove it from the extract. 4) Avoid vacuum filtration because the filter paper will likely clog.

- 3.2.3 Place 150 mL of the milky aqueous phase and 50 mL of dichloromethane in an 8-oz jar. Stir the mixture with a spoon. Cap and centrifuge the jar.

- 3.2.4 Remove 100 mL of the aqueous phase and place it in a 250-mL separatory funnel with 50 mL of toluene. Gently invert the funnel several times to mix. Allow the layers to separate. Drain the aqueous phase into a clean 250-mL separatory funnel. Discard the toluene phase.

Note: 1) The 100-mL aliquot should be removed with a pipet. Decantation will likely result in a sudden release of the entire dichloromethane phase when the tilt of the jar reaches a critical angle. 2) Do not shake the separatory funnel too briskly or copious quantities of relatively persistent bubbles will form. 3) Because only 60 mL of

aqueous extract needs to be recovered at the end of the next step, the toluene emulsion phase can be liberally discarded.

3.2.5 Add 50 mL of hexane to the separatory funnel. Gently invert the funnel several times to mix. Discard the hexane phase.

3.2.6 Proceed to section 3.3.

3.3 Anion Exchange and Phenylisocyanate Cleanup

The purpose of this cleanup step is (1) to reduce the acidity of the extract and remove phosphate anions and (2) to reduce the amino acids present in the extract, especially S-methyl methionine (Vitamin U) that can produce dimethylsulfide (DMS) when heated with alkali.

Phenylisocyanate reacts with amino acids to produce insoluble products that can be removed by filtration. The Cation Exchange Column cleanup step described below does not perform reproducibly if an excessive amount of amino acids are loaded onto the column.

3.3.1 For non-oily crops, place a 40-mL aliquot of the extract in a 4-oz jar, add 7 g of AG® 1-X8 resin to the jar, and swirl the jar to mix. For oily crops, place a 60-mL aliquot of the extract in a 4-oz jar, add 10 g of AG® 1-X8 resin to the jar, and swirl the jar to mix. Let the mixture stand for at least 0.5 hr with occasional swirling to mix.

Note: If in section 3.1.1, water was used instead of the phosphate buffer for the extraction, addition of the AG 1-X8 resin can be omitted.

3.3.2 Add to the 4-oz jar about 0.20 to 0.25 g of barium hydroxide. For non-oily crops add 3 mL of phenylisocyanate and for oily crops add 1 mL of phenylisocyanate in a well

ventilated hood. Tightly cap the jar. Shake vigorously for at least 30 sec to disperse the phenylisocyanate throughout the extract. Allow the mixture to stand for at least 1 hr. Shake the mixture manually at least 3 times within the first 15 to 20 min. Place a paper towel over the cap, and loosen it slowly as the contents are under a slight amount of positive pressure.

Note: 1) Phenylisocyanate is a lachrymator, i.e, causing tearing. 2) Barium hydroxide is used due to its insolubility in water. As few cations as possible should be introduced into the extract solution. 3) Use of PTFE-lined caps is unnecessary; its use may give a poor seal and cause leakage.

3.3.3 For oily crop extracts only, centrifuge the jar at 2500 rpm for 15 min.

3.3.4 Vacuum filter the extract through a Buchner funnel lined with 4.25-cm, filter paper. Continue the evacuation of the flask for at least one to two minutes after the filtration is completed. The filtrate in the flask will probably become cloudy.

Note: The filtration should be done in a well-ventilated hood in the unlikely case that some phenylisocyanate remains unreacted.

3.3.5 Pour the filtrate back into the 4-oz jar and refilter through the same filter funnel and into the same flask.

3.4 Cation Exchange Column Cleanup

3.4.1 Prepare Cleanup Column. Use a 15 cm by 1.5-cm (i.d.) Bio-Rad chromatographic column. Place 10 g of Bio-Rex® 70 resin

in a 100-mL beaker, and add between 20 and 25 mL of water. Pour the resin-water slurry into the column. It will be necessary to gently tap the column with a plastic object to dislodge fines that obstruct the pores in the frit. After the water has drained from the column, wash the column with about 25 mL of water by adding roughly 5-, 5-, and 15-mL portions using a disposable Pasteur pipet. Adding the water forcefully by rapidly squeezing the pipet bulb and disturbing the resin surface enhances flow through the column. After the last wash water has been added and most of the resin has settled, but before the last of the water has entered the resin bed, place about 5 g of glass balls on top of the resin.

Note: 1) Water, in addition to that specified above, can be used for resin transfer, column washing, etc. 2) The column can be prepared several days in advance of sample cleanup. It is only necessary that the end of the column is capped and the resin bed is kept filled with water.

3.4.2 Prepare Concentration Column: Use a 20 by 1.0-cm (i.d.) Bio-Rad chromatographic column. Place 2.5 g of AG® 50W-X2 resin in a 100-mL beaker and add about 15 mL of water. Pour the resin-water slurry into the column. After the water has drained from the column, wash the column with about 15 mL of water using roughly 5-mL portions. After the last wash water has been added and most of the resin has settled, but before the last of the water has entered the resin bed, place about 2 g of glass balls on top of the resin.

See above note to "Prepare Cleanup Column."

3.4.3 Using a 25-mL graduate, add 25 mL of extract to the cleanup column packed with Bio-Rex® 70. This represents 5 g of original crop sample for a 0.2 crop:solvent ratio and 2.5 g

of original crop sample for a 0.1 crop:solvent ratio. Allow all of the extract to enter the column.

Note: The column does not have enough space to take all 25 mL at once.

- 3.4.4 Wash the Bio-Rex® 70 column with 50 mL of 0.02 N HCl solution using roughly 5-, 15-, 15-, and 15-mL portions. Allow each portion to enter the column prior to addition of another portion.
- 3.4.5 After the last portion has entered the Bio-Rex® 70 column, tightly connect the outlet of the Bio-Rex® 70 cleanup column such that the eluate enters directly into concentration column packed with AG® 50W-X2 resin. Use a female-to-female Luer connector.
- 3.4.6 Elute the Bio-Rex® 70 cleanup column with 40 mL of 0.1 N HCl solution using about 10-, 15-, and 15-mL portions.
- Note: 1) The two columns should be connected tightly so that the inflow closely matches the outflow in the lower column. 2) Add each portion as soon as the liquid level reaches the top of the glass bead bed, otherwise the AG® 50W-X2 column may draw air into the top column if it continues to drip.
- 3.4.7 Disconnect the columns from each other as soon as the last portion enters the glass bead bed of the Bio-Rex® 70 column.
- 3.4.8 Elute the AG® 50W-X2 concentration column with 7 mL of 1 M KCl solution, and collect the eluate directly in a 15-mL dealkylation vial.

Note: This is a convenient stopping point; cap the vial.

3.5 Thermal Cleanup

Omit this step, and continue with section 3.6 for tolerance enforcement analyses. For method testing purposes, this step is usually essential to minimize background DMS for calculation of TMS recovery only from leafy matrices, such as forage and fodder samples, fortified at the LOQ.

- 3.5.1 Add 200-250 mg of tin(II) chloride to the vial. Cap the vial tightly, and heat at 100° C for about 15 hr, that is, overnight.
- 3.5.2 Remove the cap carefully from the hot vial, and continue the heating for about 0.5 hr. Periodically exchange the air in the vial using a gentle stream of air to remove any dimethylsulfide that may have formed.

3.6 Dealkylation

- 3.6.1 Place 500 uL of a 10 ug TMS/mL calibration solution in a 15-mL dealkylation vial for use as a standard. Add 5 mL of 1 M KCl solution.

Note: Dealkylation of TMS is not affected by the KCl present in the sample extracts.

- 3.6.2 Add about 200 - 250 mg of tin(II) chloride dihydrate to the 15-mL vials containing the calibration solution and the cleaned-up extracts. Gently swirl the vials to suspend the tin chloride. Add 5.0 mL of toluene to the vial containing the calibration solution and 1 mL to the vials containing the cleaned-up extracts.

Note: 1) The recommended amount of toluene added is based on samples containing residues near or below the LOQ. If

high residues are anticipated, the amount of toluene should be increased; however, 3 mL is the maximum that can be comfortably added to the 15-mL dealkylation vial. 2) The tin(II) chloride is added as an antioxidant to minimize oxidation of the DMS to dimethylsulfoxide. If it has been added in a previous step, do not add more.

- 3.6.3 Add 5 to 5.5 g of KOH pellets to the vial. Firmly cap the vial immediately and shake to dissolve the added KOH.

Note: The KOH pellets should be added smoothly to prevent loss of toluene through splashing. They should be added quickly because of the rapid generation of heat. Ensure that all pellets are of a size that will pass into the vial easily. There can be bottle to bottle variations in the pellet size.

- 3.6.4 Heat the vial at 100° C for 1 to 2 hr in an electric heating module.

Note: 1) The amount of KOH used and the heating involved are indications of the chemical stability of TMS. 2) All vials should be heated for the same amount of time. 2) Occasionally, the septum in the cap could pop out; this is built into the procedure as a safety feature to relieve excessive pressure buildup due to unforeseen circumstances, e.g., excessive heating block temperatures. If this occurs, discard the sample and repeat with a new aliquot of sample extract. Do not loosen the cap because the volatile DMS could escape. Appearance can be deceiving in that the seal is generally maintained even when the cap is a bit off of center. The adequacy of the seal can be confirmed after heating by inspection of the indentation left on the septum by the rim of the glass vial.

3.6.5 During heating, shake the vial periodically, at least 2 to 3 times, to facilitate the partitioning of the DMS from the caustic into the toluene.

3.6.6 Remove the vial from the heating block. Cool the vial in an ice water bath. Alternately, allow the vial and its contents to cool to ambient temperature by locating the vial in a high draft area of the fume hood.

Note: The DMS solution in toluene is stable at ambient temperature for at least two weeks if it is maintained tightly sealed in the original dealkylation vial.

3.6.7 Unscrew the cap from the vial, and place an aliquot of the toluene in an autosampler vial fitted with a limited-volume insert. Crimp seal the vial immediately.

Note: 1) Refer to the "Calibration" section 4.2 below for the need to make serial dilutions of the dealkylated calibration solution. 2) Avoid areas that use or store carbon disulfide, which is used as a solvent for many applications. Carbon disulfide has a retention time nearly coincident to that for DMS. 3) It is recommended that extract solution be placed into the autosampler vials only on the day of analysis, i.e., don't store the solution in the autosampler vials. 4) If high residues are anticipated, the toluene solution can be diluted prior to DMS analysis by using microliter autopipets.

3.7 Fortifications

Analyze residue-free control samples and fortified, residue-free control samples whenever possible along with any sample analysis. It is recommended that one control sample and two fortified control samples be analyzed each time for every

set consisting of ten samples or less. One of the fortified samples should be fortified at the method's lower limit of quantitation (LOQ) of 0.05 (or 0.1) ppm. To prepared an 0.05- or 0.10-ppm, fortified sample, add 125 or 250 uL of a 10 ug TMS/mL fortification solution, respectively, to 25 g of sample. The second fortified sample should be fortified at twice the LOQ or at a level expected in the unknown sample.

4 INSTRUMENTAL ANALYSIS CONDITIONS

Follow the manufacturer's instructions for operation of the gas chromatograph, autosampler/injector, sulfur chemiluminescence detector (SCD), and flame photometric detector (FPD). 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 For the SCD, use 115° C for the inlet and isothermal column temperatures. Use helium as the carrier gas; set the column flow rate to about 7 mL/min. Set the flow rates of air (e.g., 280 mL/min), hydrogen (e.g., 200 mL/min), and auxillary helium supplied to the detector to the values recommended by the detector manufacturer. Use a 3-uL injection volume with a splitless single-piece liner with -2 mm i.d. straight bore. The retention time of DMS is about 1 min; a large toluene peak will appear after about 2 min.
- 4.1.2 For the FPD, the column flow was 6 mL/min of helium. A splitless single-piece liner with -2 mm i.d. straight bore was used. The inlet and column temperatures were 150° and 100° C isothermal, respectively. The air and hydrogen flows to the detector were 94 and 64 mL/min, respectively. The

volume injected was 2 uL. Due to quenching by residual toluene, at least a 5-min run time between injections is recommended. The DMS retention time is about 1 min.

4.2 Calibration and Analysis

Calibrate the gas chromatograph by using the appropriate TMS calibration standard prepared in section 2.3.2 and dealkylated to DMS in section 3.6. Dilute the dealkylated 1 ug/mL standard (high level), 1:4, and 2:4 to prepare the 0.25 ug/mL (low level) and 0.5 ug/mL (intermediate level) standards, respectively, for use in generating a calibration curve. Even though the TMS has been dealkylated to DMS, it is simplest to continue expressing all concentrations in TMS equivalents. Depending on the overall precision of the chromatographic system, the analyst may opt to make duplicate injections of all calibration standards and sample extracts. The identity of the analyte peak in the sample chromatogram is assigned based upon the coincidence of retention times with the DMS peak of the calibrant chromatogram. Sample extracts containing residues that are higher than the 1 ug/mL calibrant solution must be diluted and reanalyzed.

Following is a suggested analytical scheme. Injections can be made in the following order:

1. Replicate injections (3 to 5) of the high-level standard to equilibrate the column.
2. Injections of the 1.0, 0.50, and 0.25 ug TMS equivalent/mL (high-, intermediate-, and low-level) standards to establish the calibration curve.
3. Injection of up to 7 samples. These samples can be extracts of untreated controls, fortified controls, treated-field samples, etc. Sample extracts containing residues that are higher than the High-level calibrant solution must be diluted and reanalyzed.

4. Injections of the 1.0, 0.50, and 0.25 ug TMS equivalent/mL (high-, intermediate-, and low-level) standards to establish the calibration curve.
5. Repeat steps 3 and 4 until all samples have been injected.

5 CALCULATIONS

The concentration of the analytes in the original sample is calculated by using the external standard method, that is, the response (peak height) obtained for the analytes in the sample extract is compared to the response obtained for a separate injection(s) of a known amount of analyte in the 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 average response obtained for all TMS standards used during the run is used for calculating the concentration of TMS in the samples.

5.1 Linear Response Calculation Methods

Appendix A gives sample calculation; using both the calibration factor method described in 5.1.1 and the linear regression method described in 5.2.2.

- 5.1.1 Calibration factor. Calculate the average response factor, $F(\text{avg})$, for injection of high-, intermediate- and low-level calibration solutions;

$$F(\text{avg}) = [F(\text{high}) + F(\text{intermediate}) + F(\text{low})]/3$$

Individual-level response factors, F , are calculated as follows:

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

Where

- F = response factor
C = concentration of calibration solution ($\mu\text{g/mL}$)
R = average response units from detector for calibration solution (cm)

5.1.2 Linear Regression Analysis. Alternatively, perform a linear regression analysis on the results of the injections of the calibration standards using TMS concentration (x-axis) versus the SCD detector response (y-axis). The regression analysis will provide the constants m and b for the linear equation, $y = mx + b$, where m is the slope and b is the y-intercept at $x = 0$. Calculate the TMS concentration, X, in each sample extract using the equation $x = (y - b)/m$. If any background corrections are applied, subtract the detector responses first. Calculate the analyte in the sample as in 5.2.2.

5.1.3 Crop in extract. Calculate the concentration of the matrix; that is, the amount of matrix that the extract represents, as follows:

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

Where:

- C = concentration of matrix (g/mL)
W (sample) = weight of matrix extracted (g)
V (solvent) = volume of extracting solvent used (mL); volume includes endogenous water in the matrix.
V (aliquot) = volume of crude-extract aliquot removed for cleanup.
V (toluene) = volume of toluene used to trap the DMS; volume includes any toluene dilutions.

5.1.4 Analyte in sample. Calculate the analyte concentration, A, in the original sample as follows:

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

Where

A = concentration of analyte in original sample ($\mu\text{g/g}$, mg/kg , or ppm)

F = response factor ($\mu\text{g/mL/cm}$)

R = average sample response from detector for sample (cm)

C = concentration of crop in final extract (g/mL)

5.2 Nonlinear Response Calculation Methods

For detector responses that significantly deviate from linearity, such as the FPD, the following procedure can be used to calculate extract concentrations. Appendix A gives an example calculation for the linear regression analysis method.

5.2.1 Calculation of analyte concentration in extract.

Perform a linear regression analysis on the results of the injections of the calibration standards using TMS concentration (x-axis) versus the square root of the FPD detector response (y-axis). The regression analysis will provide the constants m and b for the linear equation, $y = mx + b$, where m is the slope and b is the y-intercept at $x = 0$. Calculate the TMS concentration, X, in each sample extract using the equation $x = (y - b)/m$. Note that y is the square root of the detector response. If any background corrections are applied, subtract the square root of the responses, rather than the responses themselves.

Alternatively, the calibration data can be plotted on graph paper, and the TMS concentration, X, in each sample extract can be determined from the calibration curve.

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

$$A (\mu\text{g/g or ppm}) = X/C$$

Where

X = analyte concentration in the final extract calculated from the curve fit equation or determined from a graphical standard curve ($\mu\text{g/mL}$)

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

6 INTERFERENCES

6.1 Carbon Disulfide

Because analytical laboratories sometimes stock and use large amounts of carbon disulfide, contamination by carbon disulfide is a potential problem. Contaminations have occurred from 1) using toluene from a bottle stored in a cabinet containing a bottle of carbon disulfide and 2) using a centrifuge immediately after someone else had used it to centrifuge bottles containing carbon disulfide as the extraction solvent. Carbon disulfide does resolve from DMS if both are at low concentrations.

6.2 Dimethylsulfide (DMS)

TMS is not the only source of DMS in the final solution subjected to dealkylation. S-Methylmethionine, called Vitamin U in the Merck Index, is believed to be at least one source of DMS in the samples. Under alkaline conditions, Vitamin U can readily produce DMS. It has been isolated

from cabbage and subsequently detected in the foliage of a range of higher plants, including parsley, pepper, onion, lettuce, and turnip. Its presence has been established in tomato foliage and fruit, potato, and green tea. Cabbage leaves and kohlrabi were found to contain relatively high levels of the compound, corresponding to as much as 0.2 and 0.1% of the tissue dry weight, respectively. Vitamin U is probably ubiquitous in all plant tissues (reference 2).

7 **CONFIRMATORY TECHNIQUES**

Unexpected positive results, as in untreated controls or preapplication samples, should generally be confirmed by other means. However, if analysis is conducted by a sulfur-selective detector and the retention time of the peak is coincident with that of DMS, the peak is probably due to DMS, so confirmatory work is not productive. The origin of the DMS is likely from endogenous precursors in the sample matrix. Because some peak resolution is lost in the SCD transfer line, the FPD is the better detector for ensuring the identity of DMS based on retention time.

8 **DISCUSSION**

This method is a revised version of the crop portion of the method "Determination of SC-0224 Cation Residues in Crops, Water, and Soil by Gas Chromatography," (reference 3). An acidic phosphate buffer, instead of water, is used for extraction to minimize adsorptive losses of TMS. The sample size that is cleaned up has been increased to allow easier quantitation at the LOQ. Cleanup procedures have been expanded to lower background DMS originating from endogenous coextractives, and thus give lower "apparent" TMS residues. A more efficient column technique is used to concentrate the TMS prior to dealkylation. A capillary column is used for

the gas chromatography. The choice for the detector on the chromatograph has been expanded to include both a flame photometric detector (FPD) and a sulfur chemiluminescence detector (SCD). The SCD has been added because the FPD responds nonlinearly to sulfur and requires more complex data handling than the linearly responding SCD.

8.1 Scope

This method is suitable for the determination of TMS in agricultural crops. Recovery data given in Table I reflect the methodology described herein. The use of the raw agricultural commodities of field corn tested the method on a high chlorophyll matrix, forage; a low-moisture matrix, fodder; and a grain matrix containing 3.9% fat (reference 1).

Recovery data given in Table II were generated during the analysis of samples for other studies and reflect the methodology described herein very closely. Samples were extracted at a crop to solvent ratio of 0.2, except for dry matrices such as prunes, almond hulls, corn fodder, and dry apple pomace which were extracted at a crop to solvent ratio of 0.1. The fruit and pomace samples were extracted with water instead of with phosphate buffer. The nutmeats and soybean oil were extracted as described in the oily crop section 3.2 of this method. The soybean oil did not require the additional partitioning with toluene and hexane nor the barium hydroxide and phenylisocyanate treatment. The soybean soapstock was a unique matrix. Two grams were placed in a dealkylation vial with 4 mL of water. It was acidified using about 15 drops of concentrated hydrochloric acid. The aqueous mixture was extracted six times with 6 mL of toluene each time. After ensuring that all the toluene

has been removed by heating at 100° and directing a gentle stream of air into the vial, the extract was dealkylated.

8.2 Precision and Accuracy

Fortified corn samples were prepared as described under section 3.11, and analyzed according to this method to establish recoveries. Recoveries of TMS from corn forage and fodder fortified at 0.1 and 1.0 mg/kg, and corn grain fortified at 0.05 and 0.50 mg/kg, ranged from 82 to 118%, with a mean recovery of 99% (n=18) and coefficient of variation of 11.9%. Table I lists the individual recoveries obtained from corn forage, fodder, and grain, and the values reflect the accuracy of the method.

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

8.3 Detection Limit

The detection limit for a specific analyte in a specific matrix is based on the minimum detectability of the analyte, and the matrix 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 signal-to-noise ratio of at least 10. The detection limit for a specific matrix is obtained by dividing the minimum detectable amount by the amount of crop represented by the extract. Because the detection limit depends on the amount of sample cleaned up, the amount of time and effort expended on removing interfering

coextractives, level of instrumental performance, etc., no effort was made to establish detection limits for the various matrices.

8.4 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 0.1 mg TMS/kg for corn forage and fodder and 0.05 mg TMS/kg for corn grain were obtained from work conducted for this report (see Table I). In general, the LOQ values for this method are 0.05 mg/kg for food crops and 0.1 mg/kg for feed items.

8.5 Matrix Effects

The absence of chromatographic matrix effects, that is, excessively enhanced recoveries, was verified by the analysis of extracts fortified just prior to dealkylation. Results are listed in Table III.

8.6 Extract Analysis

Analyses were performed using a sulfur chemiluminescence detector (SCD) due to its highly selective and linear response to sulfur. Sample SCD chromatograms are given in Figures 1, 2, and 3 for the analyses performed for this study. A sample SCD standard curve is given in Figure 20. Sample calculations are given in Appendix A. Because the SCD is not widely used in residue analysis, the traditional flame photometric detector (FPD) can also be used. Sample FPD chromatograms, corresponding to Figures 1, 2, and 3, are given in Figures 17, 18, and 19. A sample FPD standard

curve is given in Figure 21. A sample calculation is given in Appendix A.

Sample SCD chromatograms for other crops are given in Figures 4 through 16.

8.7 Wet-Weight Basis

This method determines the residues of TMS 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.

8.8 Radiovalidation of Extraction Efficiency

The extraction efficiencies for TMS using the solvents given in this method were tested by analyzing samples with incurred residues. The data have been reported and submitted to the US EPA (reference 4).

8.9 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 (MSDS) accompanying the chemical or available from the supplier. 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 **CONCLUSION**

This method is selective for the analysis of TMS residues in food and feed crops. 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. If possible, untreated and fortified samples should be extracted and analyzed with each set of samples to demonstrate absence of interferences and adequate recovery. If determination of TMS residues at a concentration other than the LOQ and ten times the LOQ is required, suitably fortified samples must be analyzed to validate this 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.

10 **TABLES AND FIGURES**

- | | |
|------------|--|
| Table I. | Recoveries of TMS from Corn Forage, Fodder, and Grain |
| Table II. | Recoveries of TMS from Crops. |
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- Figure 3. Sample SCD chromatograms of corn grain fortified at the LOQ of 0.05 mg/kg
- Figure 4. Sample SCD chromatograms of peach fruit (Reprinted from reference 5)
- Figure 5. Sample SCD chromatograms of prunes (Reprinted from reference 6)
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- Figure 15. Sample SCD chromatograms of soybean oil (Reprinted from reference 11)
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- Figure 17. Sample FPD chromatograms of corn forage fortified at the LOQ of 0.1 mg/kg
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- Figure 19. Sample FPD chromatograms of corn grain fortified at the LOQ of 0.05 mg/kg

- Figure 20. Sample SCD calibration curve for DMS, as TMS equivalents, based on injections of 1.0, 0.50, and 0.25-ug/mL toluene solutions
- Figure 21. Sample FPD calibration curve for DMS, as TMS equivalents, based on injections of 1.0, 0.75-, 0.50-, and 0.25 ug/mL toluenes solution

Table I. Recoveries of TMS from Corn Forage, Fodder, and Grain.

<u>Commodities</u>	<u>Trial No.</u>	<u>Sample No.^a</u>	<u>Amount Added (ppm)</u>	<u>Amount Found (%)</u>	<u>Average^b (%)</u>
Corn forage	99CA93-4037	J4037-04	0.1	89	89
		J4037-05	0.1	82	
		J4037-06	0.1	87	
		J4037-07	1.0	85	
		J4037-08	1.0	97	
		J4037-09	1.0	95	
Corn fodder	99CA93-4038	J4038-04	0.1	89	97
		J4038-05	0.1	85	
		J4038-06	0.1	89	
		J4038-07	1.0	114	
		J4038-08	1.0	104	
		J4038-09	1.0	99	
Corn grain	99CA93-4039	J4039-04	0.05	111	111
		J4039-05	0.05	106	
		J4039-06	0.05	111	
		J4039-07	0.50	105	
		J4039-08	0.50	118	
		J4039-09	0.50	114	

- a) Samples generated from the field trial 41-NE-89-379 of study 0224-89-MR-02 were redefined and reused for this study.
b) Analysis by SCD. Calculation using calibration factor method.

Data Summary

	<u>Average Recovery (%)</u>	<u>CV (%)</u>	<u>N</u>	<u>Range (%)</u>
Corn forage	89	5.8	6	82- 97
Corn fodder	97	11.1	6	85-114
Corn grain	111	4.9	6	105-118
All samples	99	11.9	18	82-118

CV = coefficient of variation.

Table II. Recoveries of TMS from Crops.

<u>Commodity</u>	<u>TMS Added (mg/kg)</u>	<u>TMS Recovered (%)</u>	<u>Origin of Data^a</u>
peach fruit	0.05	86	reference 4
	0.05	86	
	0.05	91	
	0.05	87	
	0.05	82	
cherry fruit	0.05	104	reference 4
	0.05	88	
	0.05	101	
	0.05	108	
	0.05	118	
plum fruit	0.05	125	reference 4
	0.05	127	
	0.05	130	
	0.1	117	
	0.1	115	
	0.1	138	
plum fruit	0.05	136	reference 5
	0.1	132	
prunes	0.05	102	reference 5
	0.05	108	
almond hulls	0.1	60	reference 6
	0.1	78	
	0.3	78	
almond nutmeat	0.1	84	reference 6
	0.1	117	
walnut nutmeat	0.05	90	reference 6
	0.1	82	
	0.1	88	
	0.1	85	
pecan nutmeat	0.05	64	reference 6
	0.1	62	
	0.1	75	
pear fruit	0.05	77	reference 7
	0.05	65	
	0.1	64	

Table II continued. Recoveries of TMS from Crops.

<u>Commodity</u>	<u>TMS Added (mg/kg)</u>	<u>TMS Recovered (%)</u>	<u>Origin of Data^a</u>
apple fruit	0.05	78	reference 7
	0.05	79	
	0.05	80	
	0.1	67	
apple fruit	0.05	96	reference 8
	0.1	95	
wet apple pomace	0.05	83	reference 8
dry apple pomace	0.05	70	reference 8
apple juice	0.05	67	reference 8
	0.5	96	
corn forage	0.1	92	reference 9
	0.1	88	
	0.1	83	
corn fodder	0.1	85	reference 9
	0.1	92	
	0.1	86	
corn grain	0.05	85	reference 9
	0.05	89	
	0.05	81	
soybean oil	0.05	76	reference 10
	0.15	84	
soybean soapstock	0.05	117	reference 10
	0.1	107	

a) See Reference Section for complete citation.

Table III. Recoveries of TMS from Fortified-Control Extracts: Determination of Chromatographic Matrix Effects.

<u>Commodities</u>	<u>Trial No.</u>	<u>Sample No.</u>	<u>Amount Added (ppm)</u>	<u>Amount Found^a (3)</u>
Corn forage	99CA93-4037	J4037-11	1.0	115
Corn fodder	99CA93-4038	J4038-10	1.0	115
Corn grain	99CA93-4039	J4039-11	0.5	117

a) Analysis by SCD. Calculation using calibration factor method.

Figure 1. Sample SCD chromatograms of corn forage fortified at the LOQ of 0.1 mg/kg

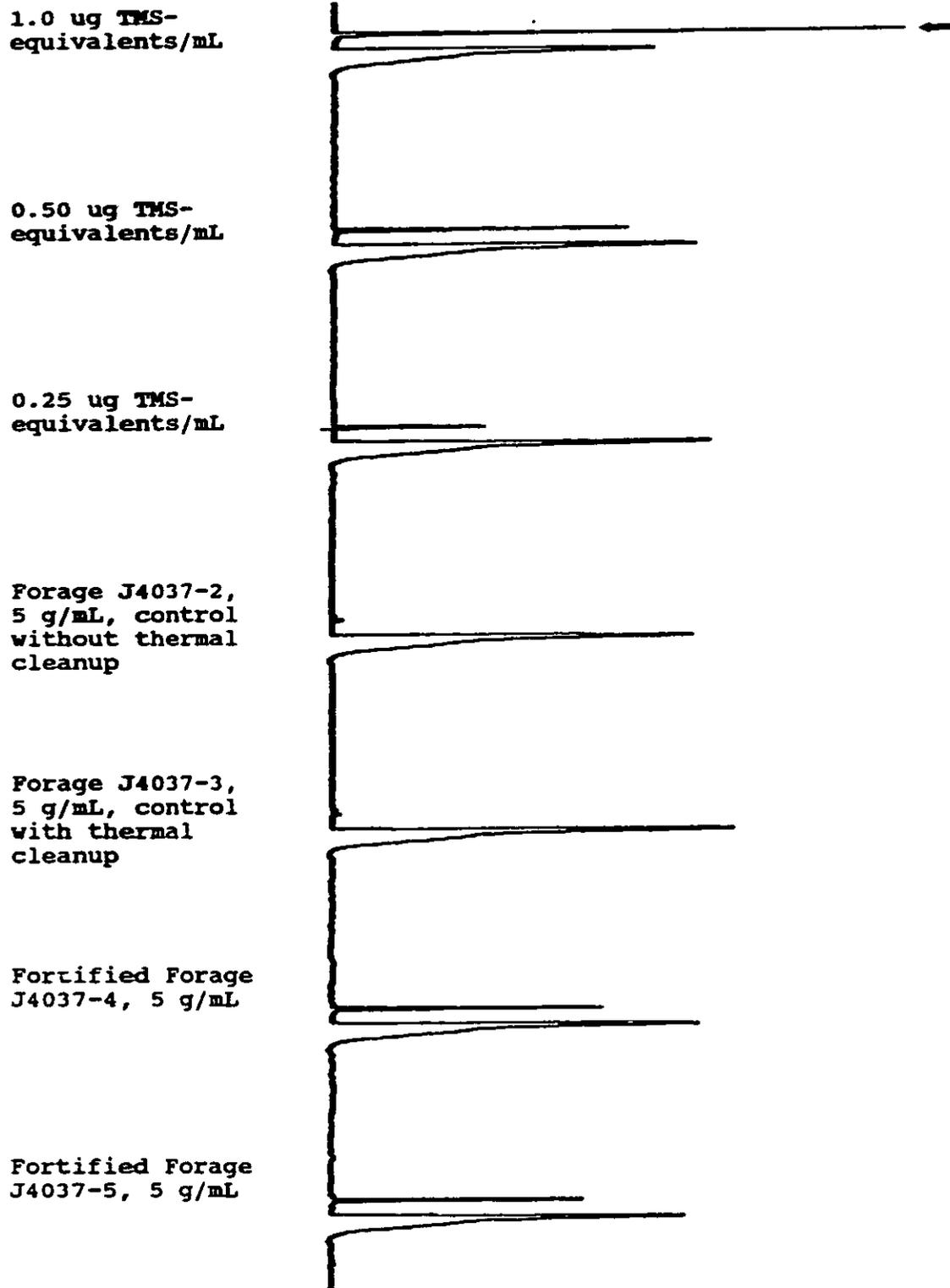


Figure 2. Sample SCD chromatograms of corn fodder fortified at the LOQ of 0.1 mg/kg

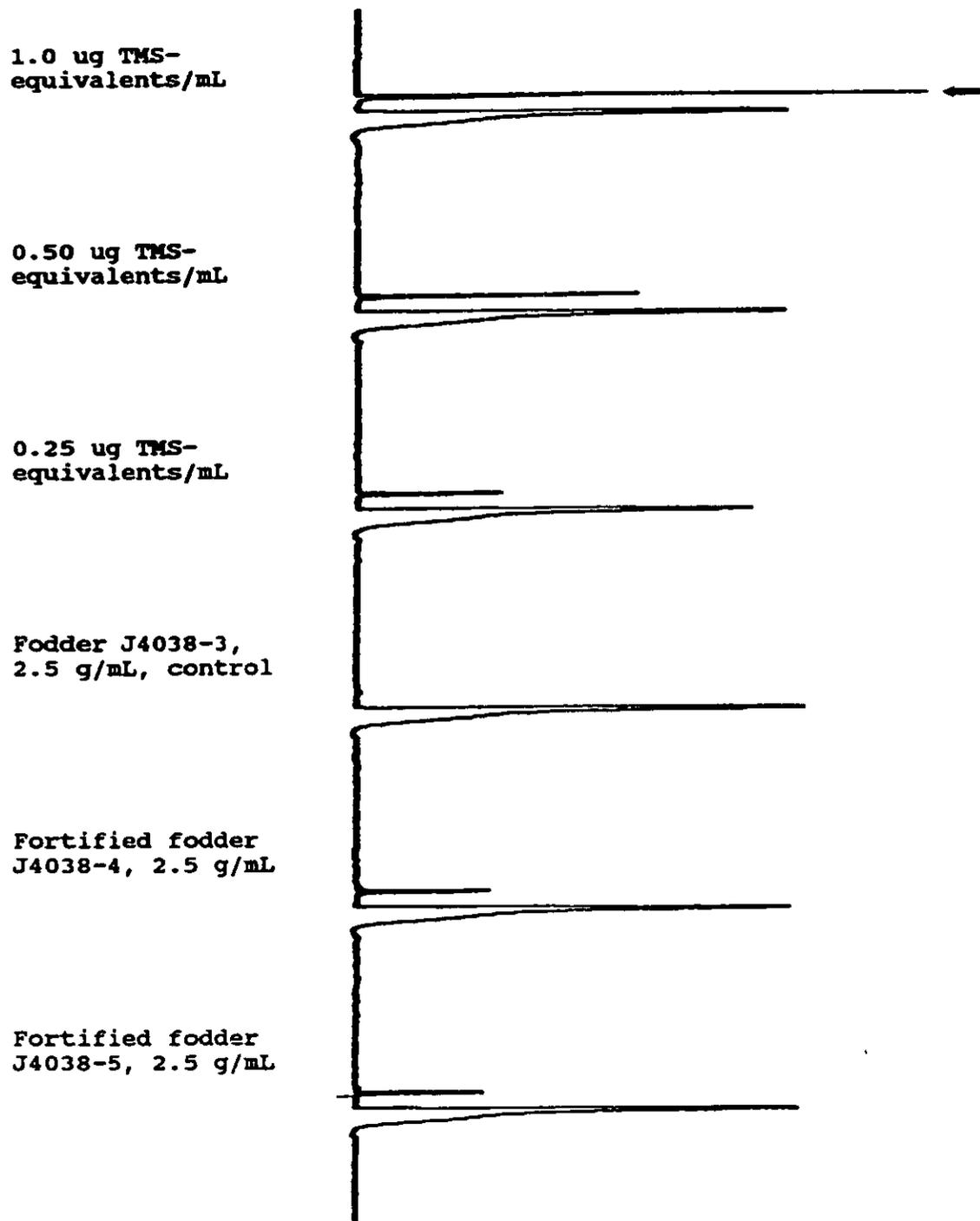


Figure 3. Sample SCD chromatograms of corn grain fortified at the LOQ of 0.05 mg/kg

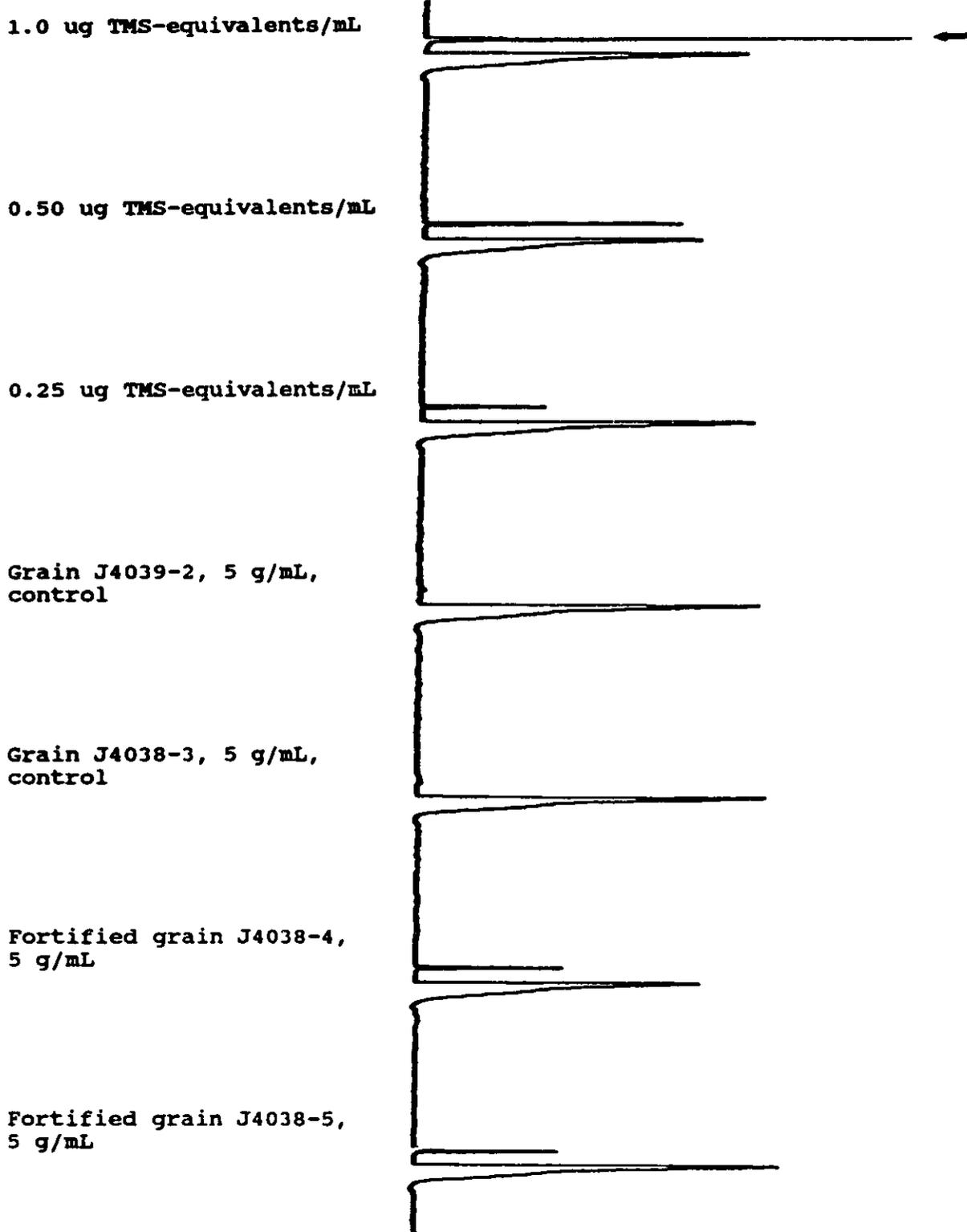
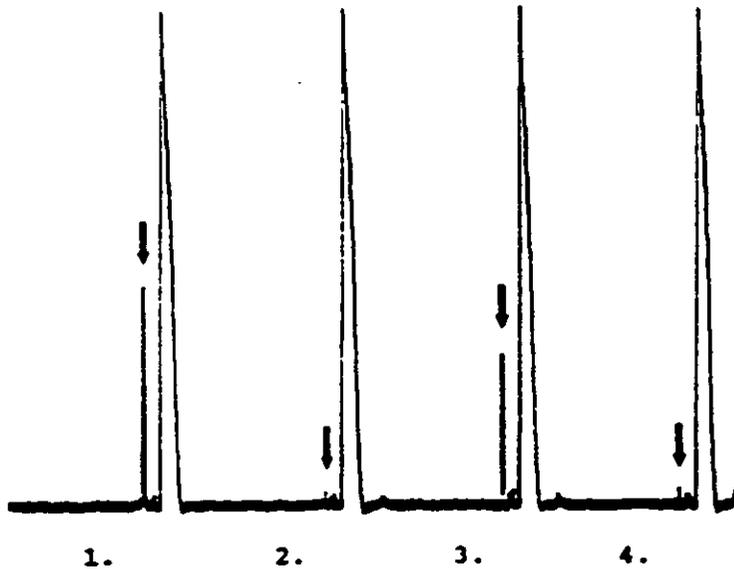


Figure 4. Sample SCD chromatograms of peach fruit (Reprinted from reference 5)



1. Calibration solution containing 0.2 $\mu\text{g/mL}$ of TMS
2. Untreated control peach (G936-1; 4.0 g/mL)
3. Untreated control peach (G936-1; 4.0 g/mL) fortified at 0.05 ppm TMS. The recovery was 82%.
4. Treated peach (G936-2; 4.0 g/mL)

Figure 5. Sample SCD chromatograms of prunes (Reprinted from reference 6)

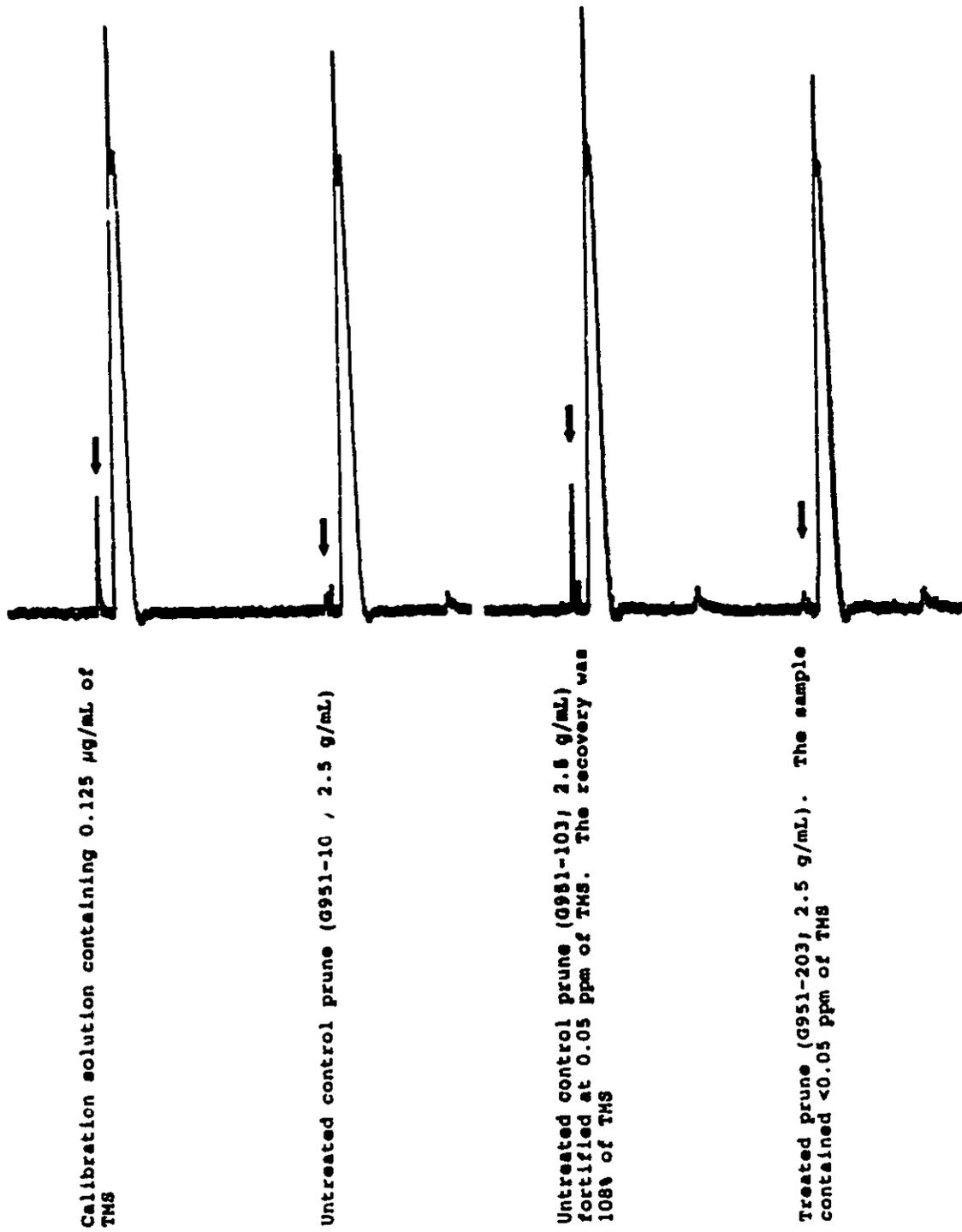


Figure 6. Sample SCD chromatograms of almond hulls (Reprinted from reference 7)

Calibration
solution
containing 0.5
 $\mu\text{g/mL}$ of TMS



Untreated control
almond hulls
(F866-11; 2.5
g/mL)



Untreated control
almond hulls
(F866-11; 2.5
g/mL) fortified at
0.3 ppm TMS. The
recovery was 78%.



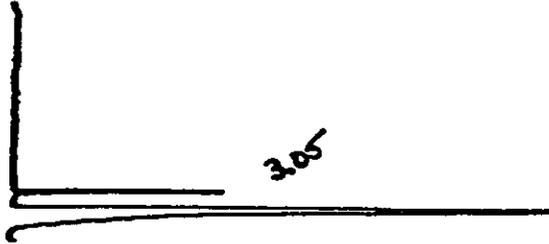
Treated almond
hulls (F866-31;
2.5 g/mL). The TMS
residue was 0.20
ppm.



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Figure 7. Sample SCD chromatograms of almond nutmeat (Reprinted from reference 7)

Calibration
solution
containing 0.25
µg/mL of TMS



Untreated control
almond nutmeat
(F862-1; 5.0 g/mL)



Untreated control
almond nutmeat
(F862-1; 5.0 g/mL)
fortified at 0.1
ppm TMS. The
recovery was 84%.

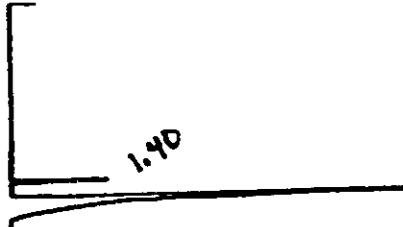


Treated almond
nutmeat (F862-20;
5.0 g/mL). The
TMS residue was
<0.05 ppm.



Figure 8. Sample SCD chromatograms of walnut nutmeat (Reprinted from reference 7)

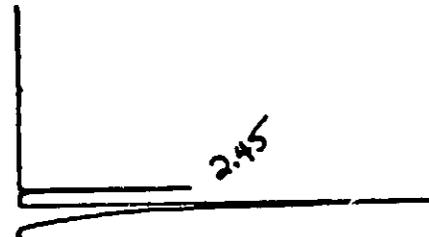
Calibration
solution
containing 0.25
 $\mu\text{g/mL}$ of TMS



Untreated control
walnut nutmeat
(F882-1; 5.0 g/mL)



Untreated control
walnut nutmeat
(F882-1; 5.0 g/mL)
fortified at 0.1
ppm TMS. The
recovery was 82%.



Treated walnut
nutmeat (F882-2;
5.0 g/mL). The
TMS residue was
<0.05 ppm.

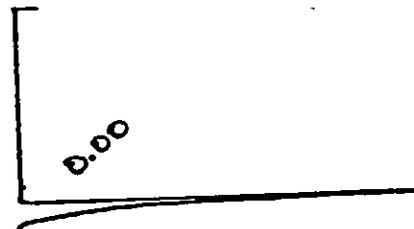


Figure 9. Sample SCD chromatograms of pecan nutmeat (Reprinted from reference 7)

Calibration
solution
containing 0.25
µg/mL of TMS



Untreated control
pecan nutmeat
(F875-1; 5.0 g/mL)



Untreated control
pecan nutmeat
(F875-1; 5.0 g/mL)
fortified at 0.05
ppm TMS. The
recovery was 64%.



Treated pecan
nutmeat (F876-2;
5.0 g/mL). The
TMS residue was
<0.05 ppm.



Figure 10. Sample SCD chromatograms of pear fruit (Reprinted from reference 8)

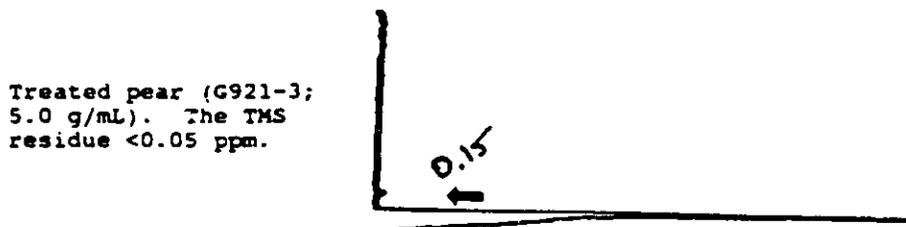
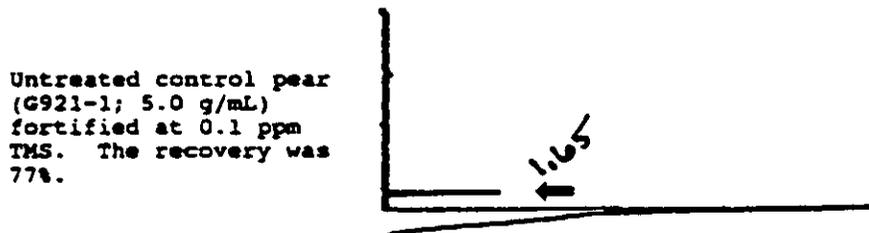
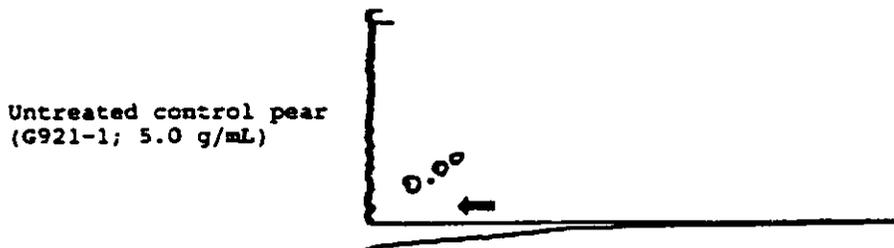
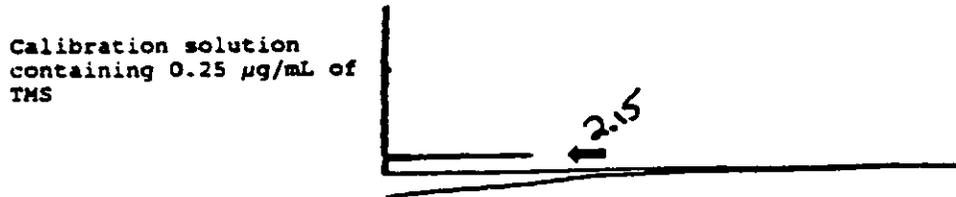
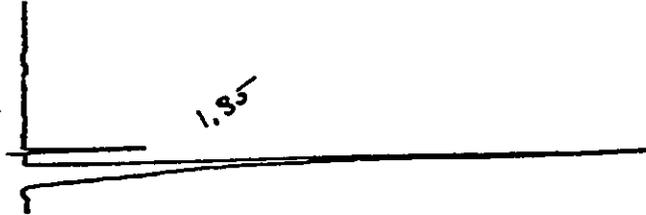


Figure 11. Sample SCD chromatograms of apple fruit (Reprinted from reference 9)

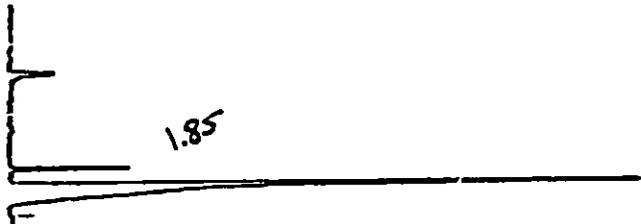
Calibration solution
containing 0.25 $\mu\text{g/mL}$ of
TMS



Untreated control apple
(G916-10; 5.0 g/mL)



Untreated control apple
(G916-10; 5.0 g/mL)
fortified at 0.05 ppm
TMS. The recovery was
96%.



Treated apple (G916-201;
5.0 g/mL). The TMS
residue was <0.05 ppm.



Figure 12. Sample SCD chromatograms of wet apple pomace
(Reprinted from reference 9)

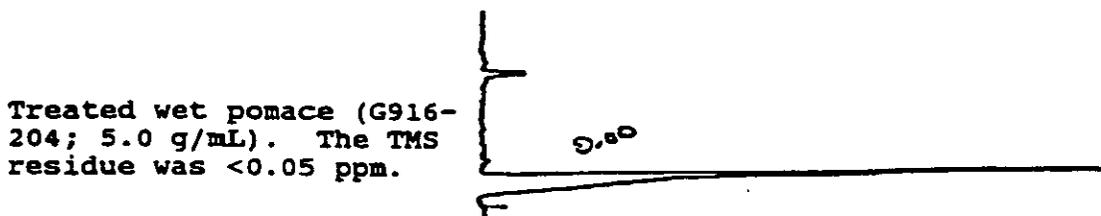
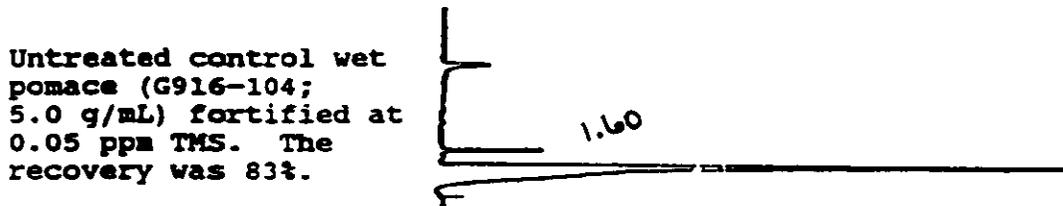
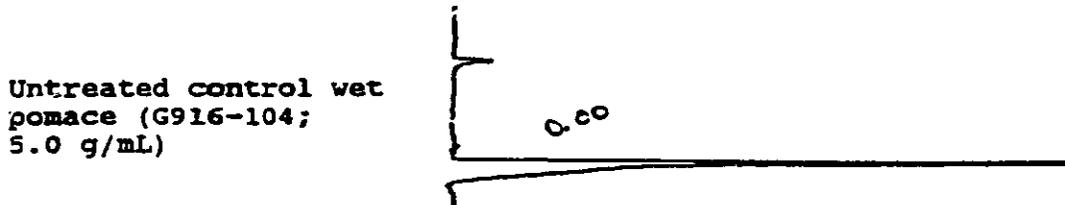
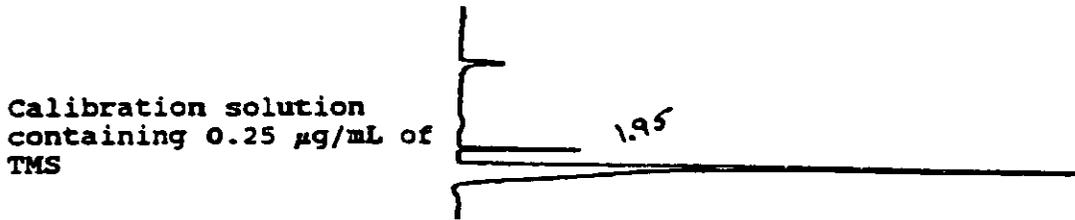


Figure 13. Sample SCD chromatograms of dry apple pomace
(Reprinted from reference 9)

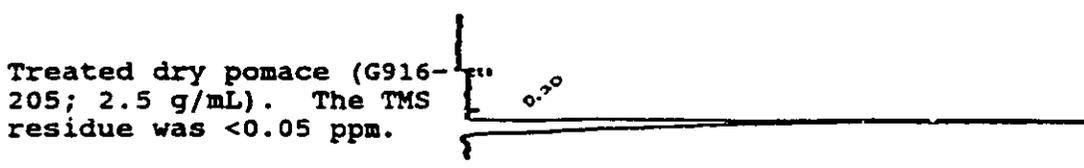
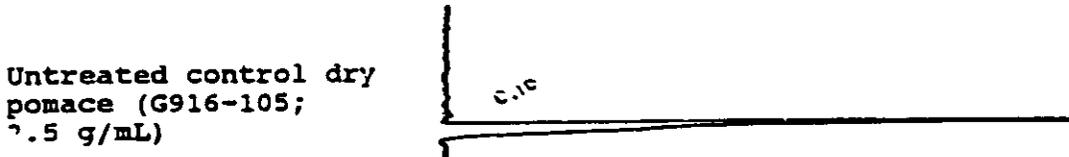
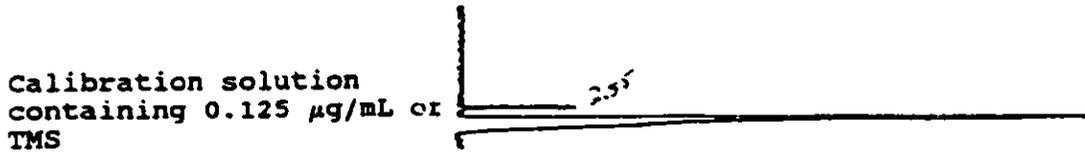
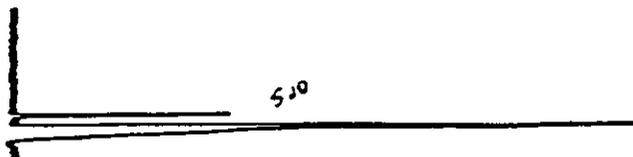


Figure 14. Sample SCD chromatograms of apple juice (Reprinted from reference 9)

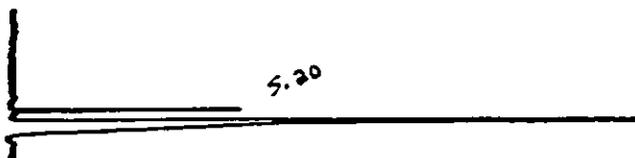
Calibration solution
containing 0.25 $\mu\text{g/mL}$ of
TMS



Untreated control
clarified, pasteurized
juice (G916-106;
5.0 g/mL)



Untreated control
clarified, pasteurized
juice (G916-106;
5.0 g/mL) fortified at
0.05 ppm TMS. The
recovery was 96%.



Treated clarified,
pasteurized juice (G916-
206; 5.0 g/mL). The TMS
residue was <0.05 ppm.



Figure 15. Sample SCD chromatograms of soybean oil (Reprinted from reference 11)

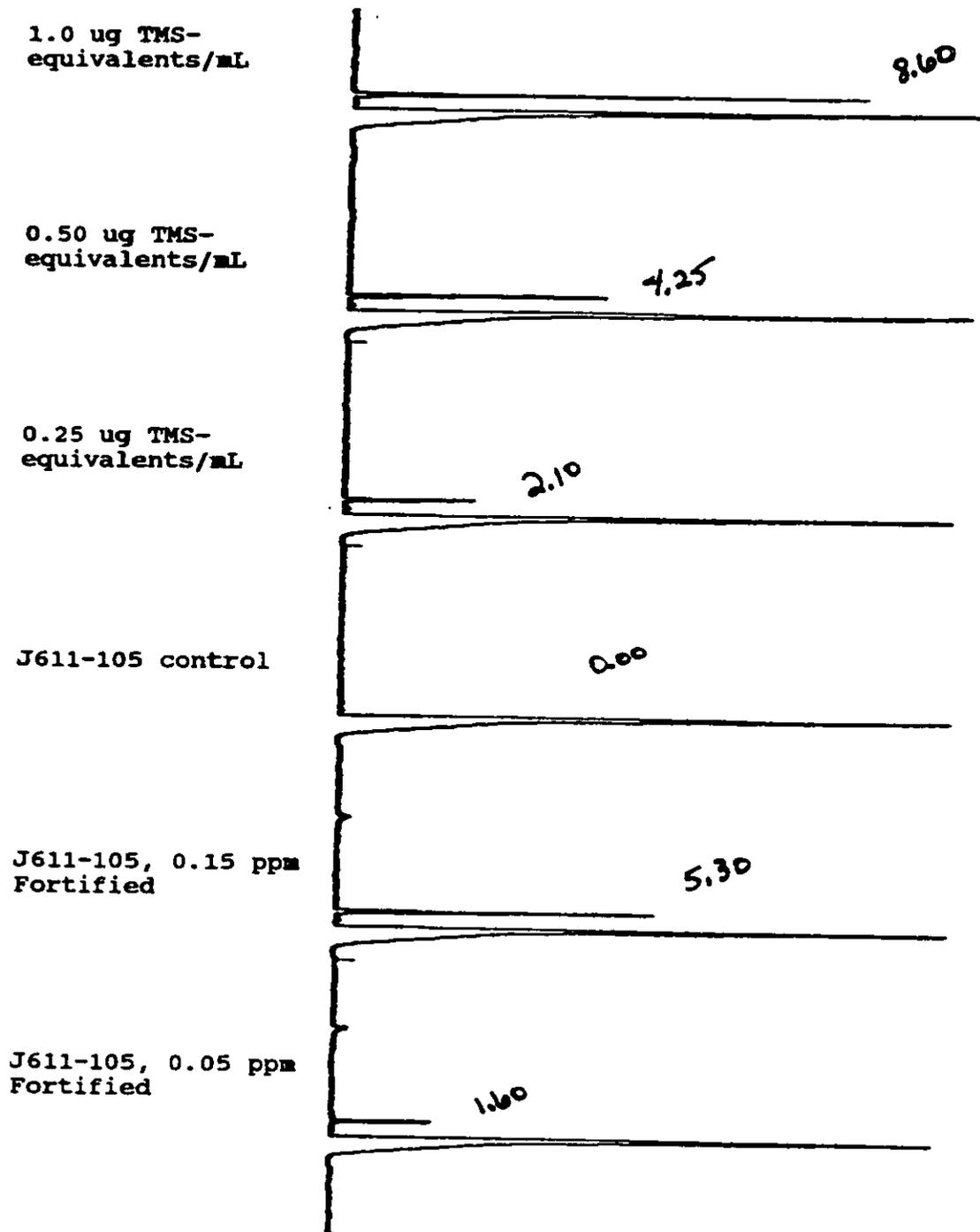
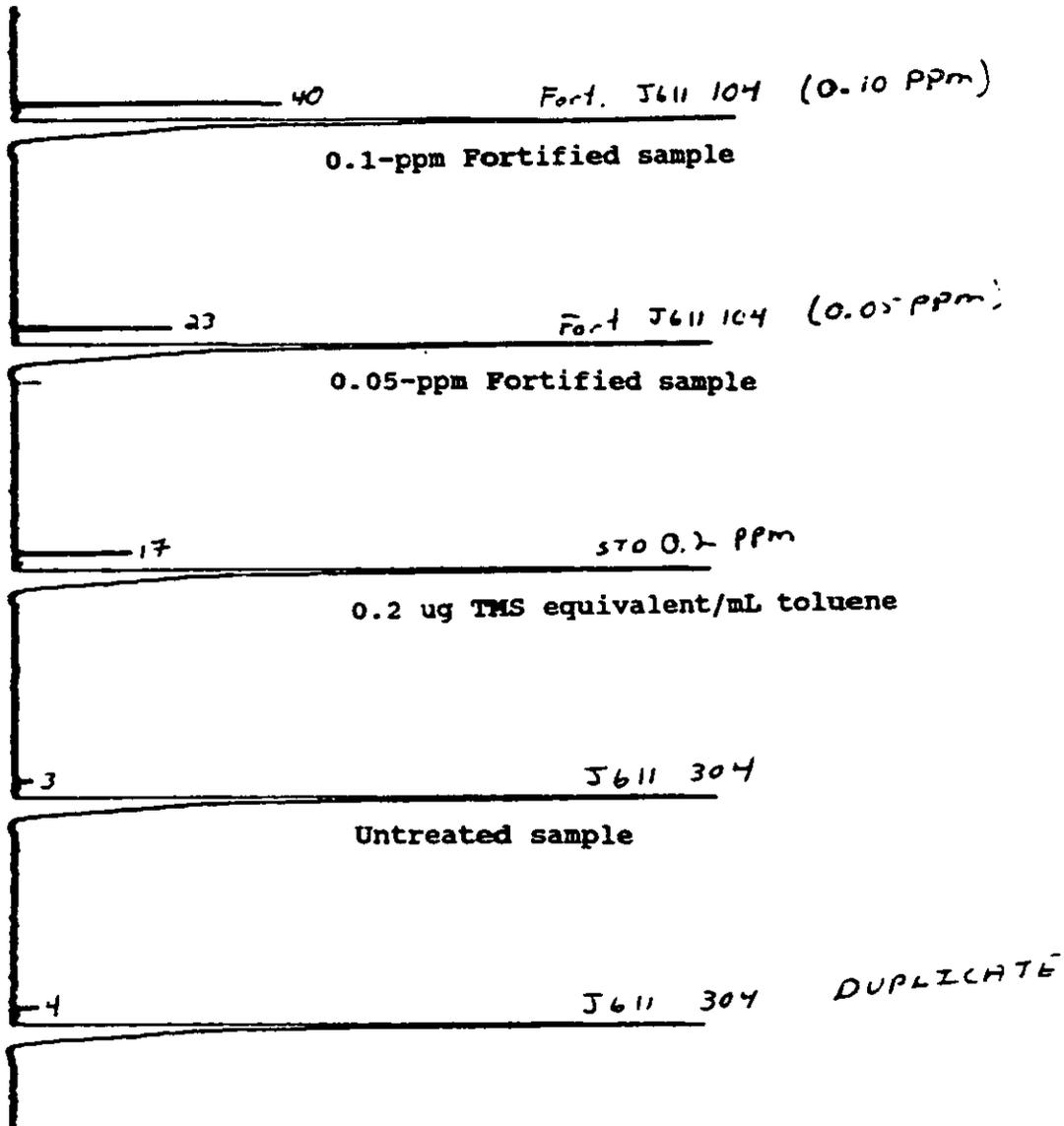


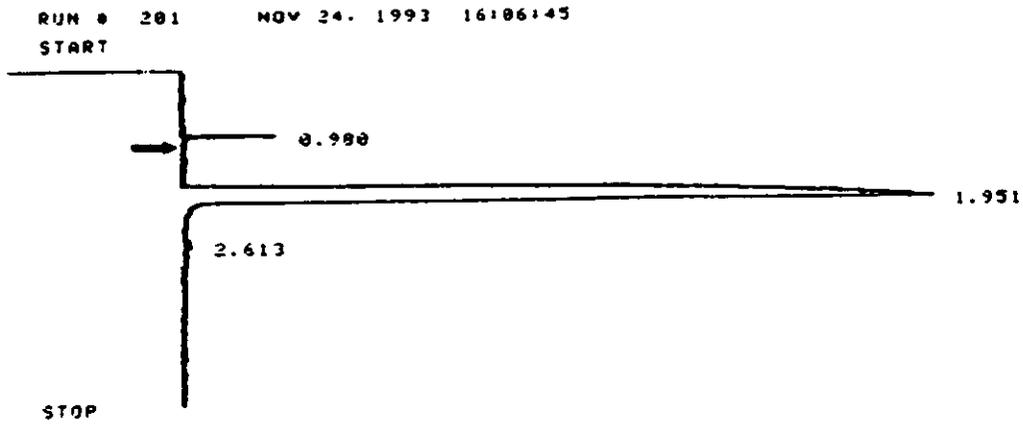
Figure 16. Sample SCD chromatograms of soybean soapstock
(Reprinted from reference 11)



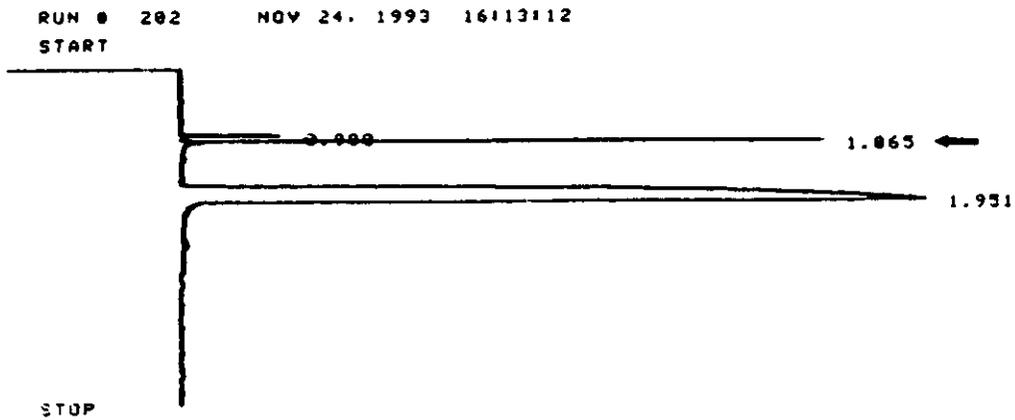
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Figure 17. Sample FPD chromatograms of corn forage fortified at the LOQ of 0.1 mg/kg (Response of standards is shown in Figure 21)

Untreated sample J4037-3 equivalent to 5 g of forage/mL of toluene.



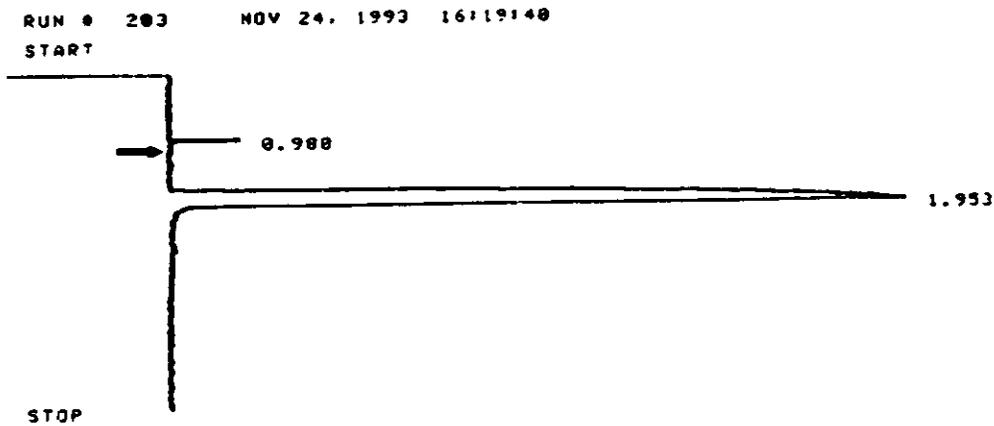
Fortified sample J4037-4 equivalent to 5 g of forage/mL of toluene.



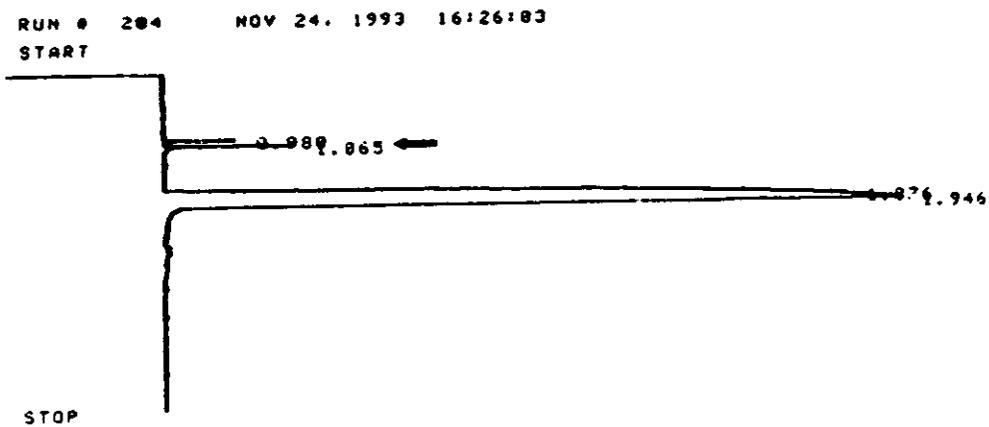
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Figure 18. Sample FPD chromatograms of corn fodder fortified at the LOQ of 0.1 mg/kg (Response of standards is shown in Figure 21)

Untreated sample J4038-3 equivalent to 2.5 g of fodder/mL of toluene.



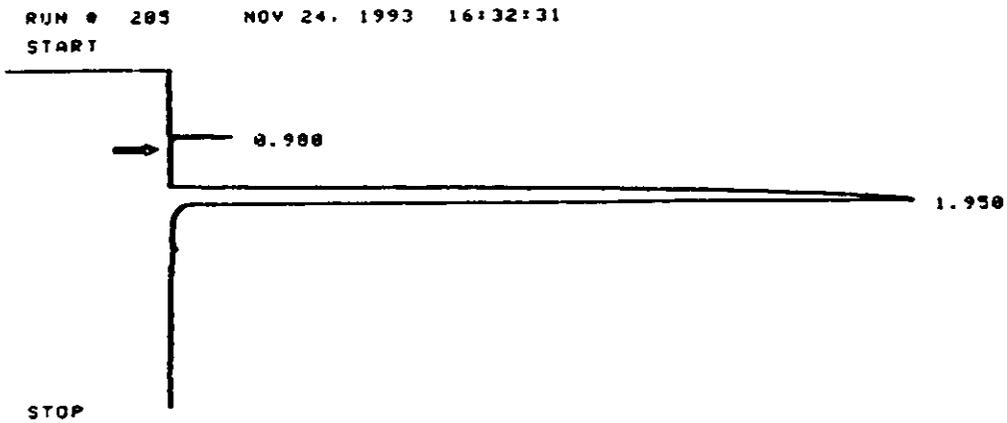
Fortified sample J4038-4 equivalent to 2.5 g of fodder/mL of toluene.



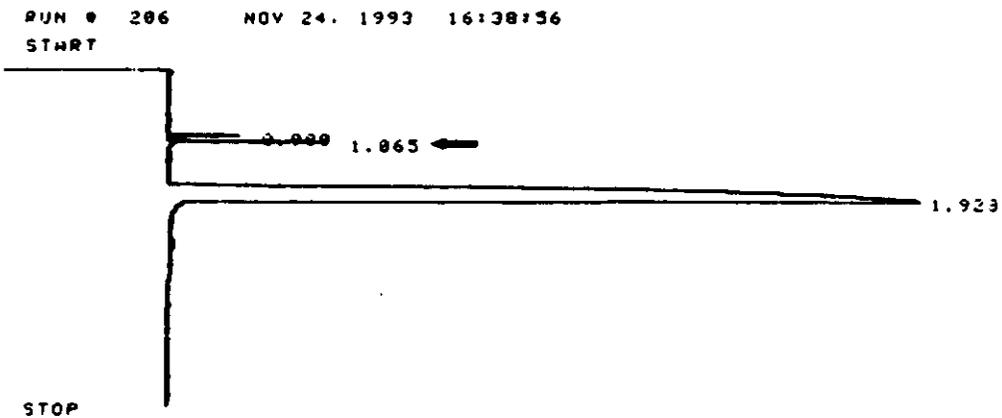
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Figure 19. Sample FPD chromatograms of corn grain fortified at the LOQ of 0.05 mg/kg (Response of standards is shown in Figure 21)

Untreated sample J4039-3 equivalent to 5 g of grain/mL of toluene.



Fortified sample J4039-4 equivalent to 5 g of grain/mL of toluene.



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Figure 20. Sample SCD calibration curve for DMS, as TMS equivalents, based on injections of 1.0-, 0.50-, and 0.25-ug/mL toluene solutions.

TMS Concentration (ug/mL)	Average Peak Ht. (cm)	Calc'd Peak Ht. (cm)
1.00	9.78	9.77
0.50	4.82	4.85
0.25	2.42	2.39

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Regression Output:	
Constant	-0.06666
Std Err of Y Est	0.044543
R Squared	0.999929
No. of Observations	3
Degrees of Freedom	1
X Coefficient(s)	9.638095
Std Err of Coef.	0.082478

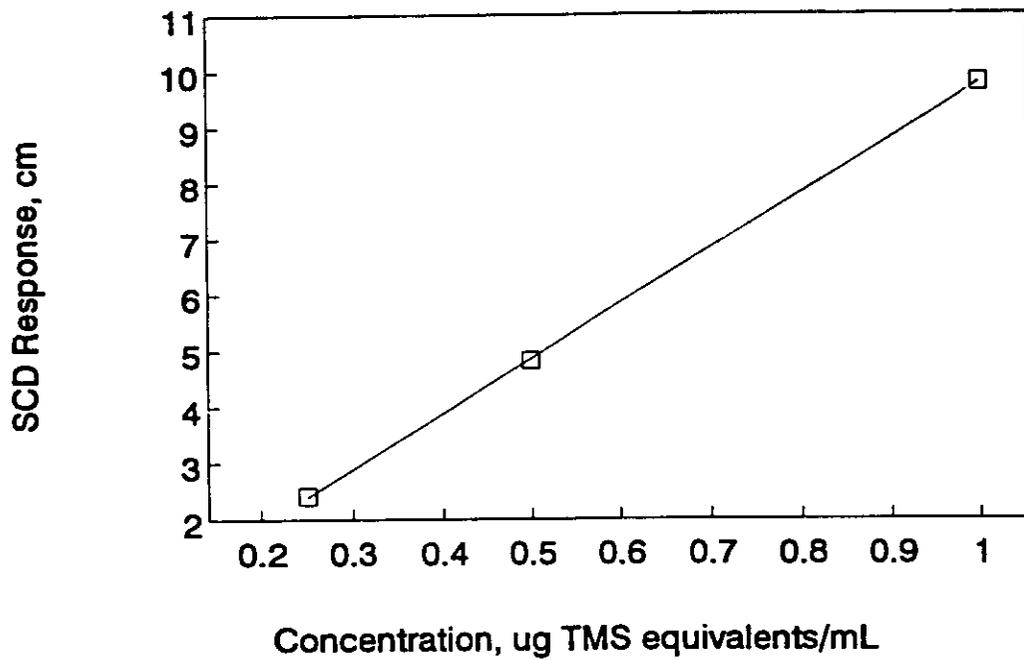
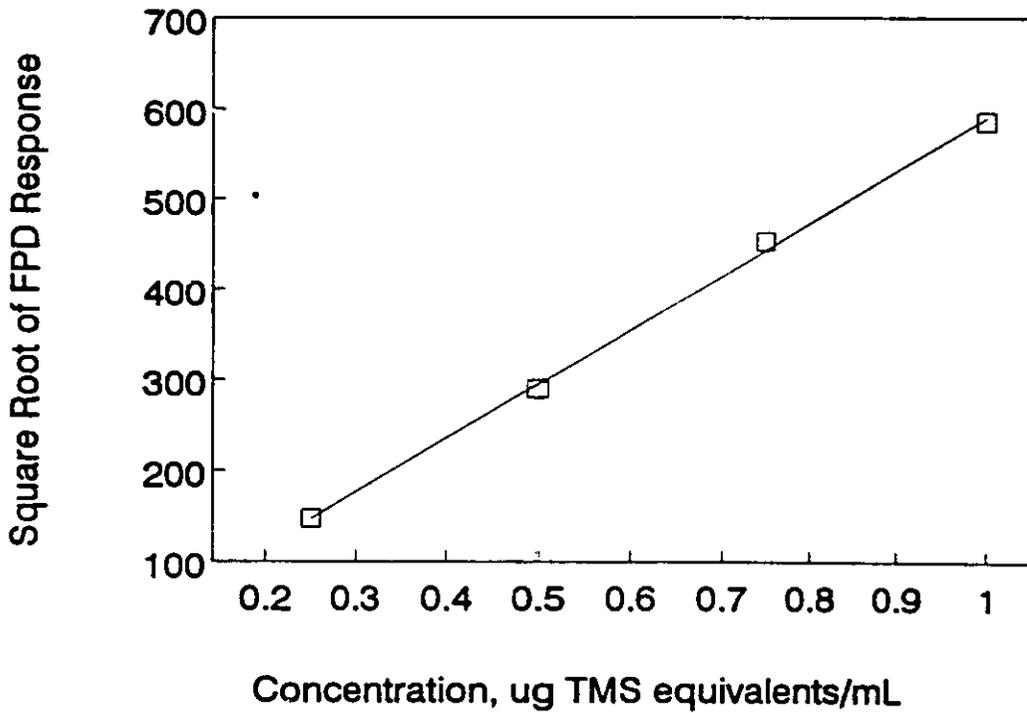


Figure 21. Sample FPD calibration curve for DMS, as TMS equivalents, based on injections of 1.0-, 0.75-, 0.50, and 0.25-ug/mL toluene solutions.

TMS Concentration (ug/mL)	Average Electronic Peak Ht.	Square Root of Peak Ht.	Calc'd Square Root of Peak Ht.
1.00	343298	586	591
0.75	205043	453	443
0.50	83812	290	295
0.25	21812	148	147

Regression Output:	
Constant	-0.51844
Std Err of Y Est	8.738098
R Squared	0.998603
No. of Observations	4
Degrees of Freedom	2
X Coefficient(s)	591.1977
Std Err of Coef.	15.63118

GLYP93AM04
RCW 14643-4-3



11 **RETENTION OF RECORDS**

All of the raw data, the protocol, and original 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.

12 **REFERENCES**

- 1 Watt, B. K. and Merrill, A. L. in *Composition of Foods; Agricultural Handbook No. 8*; U.S. Department of Agriculture: revised 1963.
- 2 "The Biochemistry of Sulphonium Salts" by G.A. Maw in The Chemistry of the Sulphonium Group, Part 2; edited by C.J.M. Stirling; John Wiley & Sons; 1981.
- 3 Katague, D. B. (1989) Determination of SC-0224 Cation Residues in Crops, Water, and Soil by Gas Chromatography. Stauffer Chemical Co., Report RRC 85-33R, MRID 40046206.
- 4 Iwata, Y. (1991) Residue Analysis of Grapes Treated with ¹⁴C-Labelled Glyphosate-Trimesium. ICI Americas, Inc. Report TMS0330B, MRID 42394002.
- 5 Wiebe, L. A. (1993) Glyphosate-Trimesium: Magnitude-of-the-Residue Study on Cherries, Peaches, and Plums from Trials Conducted in the USA During 1991. Zeneca Ag Products, Report RR 92-093B, MRID 42848703.
- 6 Wiebe, L. A. (1993) Touchdown®: Processing Study for Residues of Glyphosate-Trimesium on Plums. Zeneca Ag Products, Report RR 92-100B, MRID 42848704.
- 7 Wiebe, L. A. (1993) Touchdown®: Magnitude-of-the-Residue Study for Residues of Glyphosate-Trimesium on Almonds, Pecans, and Walnuts from Trials Conducted in the USA During 1990. Zeneca Ag Products, Report RR 93-058B.
- 8 Wiebe, L. A. (1993) Touchdown®: Magnitude-of-the-Residue of Glyphosate-Trimesium on Apples and Pears from Trials Conducted in the USA During 1991. Zeneca Ag Products, Report RR 93-075B.
- 9 Wiebe, L. A. (1993) Touchdown®: Processing Study for Residues of Glyphosate-Trimesium on Apples. Zeneca Ag Products, Report RR 93-097B.

- 10 Graham, D. G., Wiebe, L. A. (1994) Touchdown®: Magnitude-of-the-Residue of Glyphosate-Trimesium on Corn from Trials Conducted in the USA During 1989. Zeneca Ag Products, Report RR 93-106B, To be issued.
- 11 Wiebe, L. A. (1994) Touchdown®: Processing Study for Residues of Glyphosate-Trimesium on Soybeans. Zeneca Ag Products, Report RR 93-112B, To be issued.

13 **APPENDICES**

Appendix A. Sample calculations

Appendix A. Sample calculations

Calculation Method per Section 5.1.1

Calibration Factor Method

GC/SCD ANALYSIS OF CORN FORAGE, FODDER, AND GRAIN

TMS solution, ug/mL	Mean peak height, cm	(ug/mL)/cm ratio	Regression Output:	
			Constant	-0.06666
			Std Err of Y Est	0.044543
			R Squared	0.999929
			No. of Observations	3
			Degree of Freedom	1
1.00	9.78	0.1022		
0.50	4.82	0.1038	X Coefficient(s)	9.838095
0.25	2.42	0.1034	Std Err of Coef.	0.082478
	Mean:	0.1032		

Sample solution	Peak height, cm	Percent of previous value	Bkgrd. corrected peak height, cm	Calc'd TMS conc., ug/mL	Crop conc., g/mL	Calc'd TMS conc., ppm	TMS conc. added, ppm	TMS found, ppm or %
1.00 ug TMS/mL	9.75	-	-	-	-	-	-	-
0.50 ug TMS/mL	4.80	-	-	-	-	-	-	-
0.25 ug TMS/mL	2.45	-	-	-	-	-	-	-
J4038-03 control	0.05	-	-	-	2.5	-	-	<0.1
J4038-04 0.1 ppm	2.20	-	2.15	0.222	2.5	0.0887	0.10	89%
J4038-05 0.1 ppm	2.10	-	2.05	0.211	2.5	0.0846	0.10	85%
J4038-06 0.1 ppm	2.20	-	2.15	0.222	2.5	0.0887	0.10	89%
1.00 ug TMS/mL	9.80	101	-	-	-	-	-	-
0.50 ug TMS/mL	4.85	101	-	-	-	-	-	-
0.25 ug TMS/mL	2.40	98	-	-	-	-	-	-
J4037-11 1 ppm	5.60	-	5.59	0.577	0.50	1.1533	1.00	115%
J4038-10 1 ppm	2.80	-	2.80	0.288	0.25	1.1533	1.00	115%
J4039-11 0.5 ppm	2.85	-	2.84	0.293	0.50	0.5859	0.50	117%
J4037-3 control	0.10	-	-	-	2.50	-	-	<0.1
J4039-3 control	0.10	-	-	-	5.00	-	-	<0.05
1.00 ug TMS/mL	9.80	100	-	-	-	-	-	-
0.50 ug TMS/mL	4.80	99	-	-	-	-	-	-
0.25 ug TMS/mL	2.40	100	-	-	-	-	-	-

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Calculation Method per Section 5.1.2

Formula Method ($y = mx + b$; y is detector response)

GC/SCD ANALYSIS OF CORN FORAGE, FODDER, AND GRAIN

TMS solution, ug/mL	Mean peak height, cm	Regression Output	
1.00	9.78	b= Constant	-0.06666
0.50	4.82	Std Err of Y Est	0.044543
0.25	2.42	R Squared	0.999929
		No. of Observations	3
		Degrees of Freedom	1
		m= X Coefficient(s)	9.838095
		Std Err of Coef.	0.082478

Sample solution	Peak height, cm	Percent of previous value	Blkgrd. corrected peak height, cm y	Calc'd TMS conc., ug/mL $(y-b)/m$	Crop conc., g/mL	Calc'd TMS conc., ppm	TMS conc. added, ppm	TMS found, ppm or %
1.00 ug TMS/mL	9.75	-	-	-	-	-	-	-
0.50 ug TMS/mL	4.80	-	-	-	-	-	-	-
0.25 ug TMS/mL	2.45	-	-	-	-	-	-	-
J4038-03 control	0.05	-	-	-	2.5	-	-	<0.1
J4038-04 0.1 ppm	2.20	-	2.15	0.225	2.5	0.0901	0.10	90%
J4038-05 0.1 ppm	2.10	-	2.05	0.215	2.5	0.0861	0.10	86%
J4038-06 0.1 ppm	2.20	-	2.15	0.225	2.5	0.0901	0.10	90%
1.00 ug TMS/mL	9.80	101	-	-	-	-	-	-
0.50 ug TMS/mL	4.85	101	-	-	-	-	-	-
0.25 ug TMS/mL	2.40	98	-	-	-	-	-	-
J4037-11 1 ppm	5.60	-	5.59	0.575	0.50	1.1498	1.00	115%
J4038-10 1 ppm	2.80	-	2.80	0.291	0.25	1.1634	1.00	116%
J4039-11 0.5 ppm	2.85	-	2.84	0.295	0.50	0.5909	0.50	118%
J4037-3 control	0.10	-	-	-	2.50	-	-	<0.1
J4039-3 control	0.10	-	-	-	5.00	-	-	<0.05
1.00 ug TMS/mL	9.80	100	-	-	-	-	-	-
0.50 ug TMS/mL	4.80	99	-	-	-	-	-	-
0.25 ug TMS/mL	2.40	100	-	-	-	-	-	-

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Calculation Method per Section 5.2

Formula Method ($y = mx + b$; y is square root of detector response)

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RCW 14843-4-3

GC/FPD ANALYSIS OF CORN FORAGE, FODDER, AND GRAIN

Sample solution analyzed	Electronic peak height	Square root of electronic peak height	Bkgrd-subtracted electronic peak height for sample.	TMS conc. found, ug/mL*	Sample matrix conc., g/mL	TMS conc. found, ppm	TMS conc. added, ppm	TMS found, ppm or %
1.00 ug TMS/mL	366041	605	--	--	--	--	--	--
0.75 ug TMS/mL	220942	470	--	--	--	--	--	--
0.50 ug TMS/mL	90613	301	--	--	--	--	--	--
0.25 ug TMS/mL	22016	148	--	--	--	--	--	--
J4037-3 forage	0	0	--	--	5.0	--	--	<0.10
J4037-4 0.1 ppm	90767	284	284	0.48	5.0	0.10	0.10	96%
J4038-3 fodder	0	0	--	--	2.5	--	--	<0.10
J4038-4 0.1 ppm	16730	129	129	0.22	2.5	0.09	0.10	87%
J4039 grain	0	0	--	--	5.0	--	--	<0.05
J4039-4 0.05 ppm	20629	144	144	0.24	5.0	0.05	0.05	97%
1.00 ug TMS/mL	320550	566	--	--	--	--	--	--
0.75 ug TMS/mL	189143	435	--	--	--	--	--	--
0.50 ug TMS/mL	77011	278	--	--	--	--	--	--
0.25 ug TMS/mL	21607	147	--	--	--	--	--	--

* $X = (Y - b)/m$. Formula constants are derived from the regression analysis. Alternatively, one can use a graph.

TMS concn. ug/mL	Square root of: ug TMS/mL cm	LOTUS 1-2-3 Regression Analysis of Data	
1.00	586	Regression Output:	
0.75	452	b = Constant	-0.47847
0.50	289	Std Err of Y Est	8.708600
0.25	148	R Squared	0.998611
		No. of Observations	4
		Degrees of Freedom	2
		m = X Coefficient(s)	590.7724
		Std Err of Coef.	15.57841