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TOUCHDOWN®: Determination of Residues of the Trimethylsulfonium  
Cation in Milk, Eggs, and Animal Tissues  
by Gas Chromatography

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Study Number

GLYP-93-AM-03

Report Number

RR 93-100B

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Trimethylsulfonium Cation in Milk, Eggs, and  
Animal Tissues by Gas Chromatography

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study meets the requirements for 40 CFR Part 160.

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**Study Number:** GLYP-93-AM-03

**Report Title:** Touchdown®: Determination of Residues of Trimethylsulfonium Cation in Milk, Eggs, and Animal Tissues

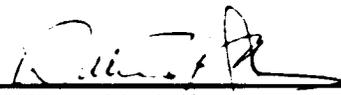
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### QUALITY ASSURANCE STATEMENT

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>
Oct 22, 1993	Protocol	Oct 22, 1993
Oct 22, 1993	Study Conduct	Oct 22, 1993
Oct 26, 1993	Final Report & Raw Data	Oct 28, 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

12/29/93  
\_\_\_\_\_  
Date

Study Number: GLYP-93-AM-03

Study Title: TOUCHDOWN®: Determination of Residues of the Trimethylsulfonium Cation in Milk, Eggs, and Animal Tissues 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  12/29/93  
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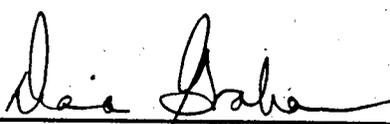
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1 SUMMARY/INTRODUCTION

This method is intended for determining residues of the trimethylsulfonium cation (TMS) in milk, eggs, and animal tissues. The limits of quantitation (LOQ) for (1) milk and eggs, (2) muscle and fat, and (3) liver and kidney are 0.01, 0.03, and 0.1 mg/kg (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 registered trademark, TOUCHDOWN®. The TMS is extracted from samples of milk and eggs by maceration with acetone, and from samples of edible tissues by maceration with 1:9 0.1 N aqueous HCl:acetone (vol./vol.). An aliquot of the sample extract is first cleaned-up by partitioning it with hexane to remove lipids and organosoluble coextractives. After removal of residual organic solvents, the aqueous residuum is cleaned-up by column chromatography using a cation-exchange resin. The cleaned-up extract is heated at 100° C for 1 to 2 hours in the presence of tin(II) chloride, toluene, and potassium hydroxide to dealkylate the TMS to dimethylsulfide (DMS). The amount of DMS, which is formed and retained in the toluene, is quantified by using capillary, gas chromatography and a sulfur-selective detector, such as the sulfur chemiluminescence or flame photometric detectors. Recoveries of TMS from milk and eggs fortified at 0.01 and 0.10 mg/kg, muscle and fat fortified at 0.03 and 0.30 mg/kg, and liver and kidney fortified at 0.1 and 1.0 mg/kg ranged from 76 to 115%, with a mean recovery of 93% (n=36) and coefficient of variation of 12% when analyzed using this method.

This is an updated version of the the method RRC 87-42 entitled "Determination of Sulfosate Cation Residues in Milk, Eggs, and Edible Tissues by Gas Chromatography" (reference 1).

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-pint jars with lids.

2.1.2 Centrifuge. International Equipment Company (IEC) Centra-8 (Needham Heights, MA).

2.1.3 Evaporator. Haake Buchler Rotary Evapo-Mix.

2.1.4 Jars. 4- and 8-oz, clear wide-mouthed and 4-oz, amber narrow-mouthed jars with screwcaps.

2.1.5 Pipets and Pipet Tips. Eppendorf adjustable model pipets for 10-100 uL delivery (catalog no. 22-44-010-1) and 100-1000 uL delivery (catalog no. 22-44-020-9) with corresponding disposable tips.

2.1.6 Centrifuge Tubes and Stoppers. 50-mL Graduated, centrifuge tube (Kontes, catalog no. 411650-0000) with size 16, flat-head stopper (Kontes, catalog no. 850250-0016).

2.1.7 Column Racks. Econo System Racks (Bio-Rad Laboratories, Hercules, CA; catalog no. 731-8200)

- 2.1.8 Columns. (1) 15 by 1.5 cm (i.d.) low-pressure chromatographic columns (Bio-Rad, catalog no. 737-1516). (2) 20 by 1.0 cm (i.d.) low-pressure chromatographic columns (Bio-Rad, catalog no. 737-1021). Columns should come with translucent column ends with male luer lock fittings.
- 2.1.9 Column Connectors. Female luer to female luer (Bio-Rad, catalog no. 731-8228).
- 2.1.10 Beakers. 100-mL capacity.
- 2.1.11 Graduated Cylinders. 10-, 25-, 50-, 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; catalog no. 2-3284).
- 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. 15-cm (5.75-inch) length, glass, Pasteur pipets and 10-mL disposable pipets with associated 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. 200-228). 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 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 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 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 (J&W Scientific; Folsom, CA; catalog no. 125-5032).

## 2.2 Reagents and Materials

2.2.1 Water. Distilled water of a purity that is suitable for use in trace-organic analysis.

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 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 Cation-Exchange Resins. (1) Bio-Rex® 70, 50-100 mesh, sodium form, analytical-grade cation exchange resin (Bio-Rad, Hercules, CA; catalog no. 142-5832). (2) AG® 50W-X2, 100-200 mesh, hydrogen form, analytical-grade cation exchange resin (Bio-Rad, catalog no. 142-1241).
- 2.2.5 Solid Glass Balls. 1.5- to 2-mm diameter glass balls (Jencons (Scientific) Ltd.; sold by Scientific Products (Baxter) catalog no. G6031-15).
- 2.2.6 Potassium Chloride (KCl). Assay  $\geq$  99%; A.C.S. Reagent (Kodak, catalog no. 104-9766).
- 2.2.7 Potassium Chloride Solution. 1 M aqueous solution (74.6 g/L).
- 2.2.8 Organic Solvents: Acetone, Hexane, Toluene. Each is a high-purity grade of solvent sold for use in trace-organic analyses.
- 2.2.9 Extraction Solvent. Mix one volume 0.1 N HCl solution and 9 volumes acetone.
- 2.2.10 Potassium Hydroxide (KOH). Dry solid pellets with a minimum assay of 85%.
- 2.2.11 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 TMS 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 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 ( $\pm 0.1$  mg) of approximately 266 mg, or other appropriate amount, of TMS iodide in a 4-oz amber, glass bottle. Multiply the amount of milligrams weighed out by 0.377 to calculate the weight in grams 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

#### 3.1 Sample Extraction

Samples are extracted either with acetone or acidic aqueous acetone depending on the specific matrix. Because acetone is miscible with water, it is necessary to estimate the amount of water in the sample prior to extraction to determine the total extract volume. This estimation is facilitated by use of the Composition of Foods handbook (reference 2). For example, a value of 87.4% water is given for the entry "Milk, cow: Fluid (pasteurized and raw): Whole: 3.5% fat" in the handbook. If 30 g of milk is assumed to contain 26 mL of water and 64 mL of acetone is used for extraction, the total volume of aqueous extract is 90 mL. The sample:extract ratio is 30 g/90 mL or 0.333 g per milliliter of extract.

3.1.1 Extraction of Milk and Eggs. Place 30 g of milk or egg sample and an appropriate volume of acetone in a blender jar. The total extract volume should be 90 mL. Blend for 5 min.

Note: 1) Representative water contents for cow's milk and whole chicken eggs are 87 and 74%, respectively. An appropriate volume of acetone for these samples would be 64 and 68 mL, respectively. For milk and eggs, the sample to extract ratio is 30 g/90 mL or 0.333 g per mL of extract.  
2) A 'LO' speed and a rheostat setting of 40 is suggested to minimize excessive splashing or foaming.

3.1.2 Extraction of Tissues. Place 25 g of animal tissue and an appropriate volume of acidic aqueous acetone (see 2.2.9) in a blender jar. The total extract volume should be 150 mL. Blend for 10 min.

Note: 1) Representative water contents for beef muscle tissue, beef liver, beef kidney, and beef fat are 60, 70, 76, and 15%, respectively. An appropriate volume of aqueous acetone for these samples would be 135, 132, 131, and 146 mL, respectively. For tissues, the sample to extract ratio is 25 g/150 mL or 0.167 g per mL of extract. 2) A 'LO' speed and a rheostat setting of 40 is suggested to minimize stress on the blender unit and heating of the macerate.

### 3.2 Cleanup with Hexane Partitioning

- 3.2.1 Decant the macerate into a 4- (milk and eggs) or 8-oz (tissue) jar, cap the jar, and centrifuge, e.g., 10 min at 2500 rpm, to settle the larger particulate matter.
- 3.2.2 Place a 30-mL aliquot of the supernate in a 50-mL centrifuge tube. The 30-mL aliquot represents extractives from 5 g of tissues and 10 g of milk or eggs.
- 3.2.3 Add 20 mL of hexane, cap the tube, and mix very gently. Uncap the tube, and discard the major portion of the upper layer using a disposable pipet. Repeat the procedure with a fresh 20-mL portion of hexane.
- 3.2.4 Remove the remaining acetone and hexane using an evaporator until about 5 to 10 mL of aqueous extract remains. The egg extract foams when the solvent is gone, so special care should be given.

Note: 1) The egg extract may need to be centrifuged to aggregate and settle particulates. 2) The amount of aqueous extract remaining after solvent removal can be estimated based on the water content of the sample and the extraction solvent. For example, a 30-mL aliquot of beef muscle

extract represents 30 mL x 0.167 g/mL or 5.0 g of tissue. If the tissue is 60% water, then 0.6 x 5 g or 3 g (mL) of water is contributed by the tissue. The remaining 27 mL is extraction solvent containing 10% water; 27 mL x 0.1 is 3 mL. Therefore, solvent evaporation should be continued until about 6 mL of liquid, i.e., water, remains. 3) If a suitable evaporator is not available, the extract can be transferred to another container, such as a small round-bottomed flask, for solvent evaporation. Acetone can be used to aid the transfer. Alternatively, due to the small amount of solvent that needs to be evaporated, a gentle stream of air can be used for evaporation.

### 3.3 Column Cleanup with Cation-Exchange Resin

3.3.1 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 quickly 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 bottom end of the

column is capped and the resin bed is kept filled with water.

- 3.3.2 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."

- 3.3.3 Add the aqueous extract to the cleanup column packed with the Bio-Rex® 70 resin using a disposable Pasteur pipet; minimize transferring any particulates. Allow all of the extract to enter the column. Use 10 mL of water to rinse the container, and add the rinsate to the column.

- 3.3.4 Wash the Bio-Rex® 70 column with 50 mL of 0.02 N HCl solution using roughly 10-, 15-, 15-, and 10-mL portions. Allow each portion to enter the column prior to addition of another portion.

- 3.3.5 After the last portion has entered the Bio-Rex® 70 column, connect the outlet of the Bio-Rex® 70 column such that the eluate enters directly into the concentration column packed with AG® 50W-X2 resin. Use a female-to-female luer connector.

- 3.3.6 Elute the Bio-Rex® 70 cleanup column with 40 mL of 0.1 N HCl solution using about 15-, 15-, and 10-mL portions.

Note: 1) The two columns should be connected tightly so that the inflow matches the outflow in the AG® 50W-X2 column. 2) Add each portion as soon as the liquid level reaches the top of the glass balls of the Bio-Rex® 70, otherwise air could be drawn into the Bio-Rex® 70 column if the AG® 50W-X2 column continues to drip.

- 3.3.7 Disconnect the columns from each other as soon as the last portion enters the glass-ball bed of the Bio-Rex® 70 column.
- 3.3.8 Elute the AG® 50W-X2 column with 7 mL of 1 M KCl solution, and collect the eluate in a 15-mL dealkylation vial.

#### 3.4 Dealkylation

- 3.4.1 Place an appropriate volume of TMS calibration solution and about 5 mL of 1 M KCl solution in a 15-mL dealkylation vial for use as a standard (high level). For analysis of milk and eggs at the LOQ of 0.01 mg/kg and analysis of muscle and fat tissues at the LOQ of 0.03 mg/kg, addition of 180 uL of a 10 ug TMS/mL solution (1.8 ug TMS) is suggested. For analysis of liver and kidney tissues at the LOQ of 0.1 mg/kg, addition of 300 uL of a 10 ug TMS/mL solution (3.0 ug TMS) is suggested.
- 3.4.2 Add between 200 and 250 mg of tin(II) chloride dihydrate to all the 15-mL dealkylation vials, and suspend the material by gently swirling the vial.
- 3.4.3 Add toluene to each vial. For analysis at the method's LOQs, using an Eppendorf pipet, add 0.50 mL of toluene to milk, eggs, fat, and muscle tissue extracts; add 1.00 mL of toluene to liver and kidney extracts. For analysis at ten times the methods LOQs, add 2.00 mL to milk and egg

extracts, 3.00 mL to fat and muscle tissue extracts, and 2.50 mL to liver and kidney extracts. Add 3.00 mL to the vial containing the calibration solution. For sample extracts, 3 mL is the maximum volume of toluene that can be added to the vial due to volume constraints.

- 3.4.4 Add between 5 and 5.5 g of KOH pellets to the vial. Securely cap the vial immediately, and shake until the pellets dissolve. Hold the vial by the cap because the glass will become hot as a result of the very exothermic heat of solution.

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 the pellets are of a size that will pass into the vial easily. There can be bottle to bottle variations in the pellet size.

- 3.4.5 Heat the vial at 100° C for 1 to 2 hr in an electric heating module. Heat all vials in a set for the same amount of time.

Note: 1) The amount of KOH used and the heating involved are indications of the chemical stability of TMS. 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 integrity of the seal can be verified by

inspection of the impression of the vial's rim that is left on the septum.

- 3.4.6 At least two or three times during heating, shake the vial to facilitate the partitioning of the DMS from the caustic into the toluene. This should be done very carefully in the improbable event that the vial leaks.
- 3.4.7 Remove the vials from the heating block. Cool the vials by immersion in an ice/water bath. Alternatively, allow the vial and its contents to cool to ambient temperature by locating the vial in a high draft area of the fume hood. The DMS solution in toluene is stable at ambient temperature for at least two weeks if it is left in the firmly-capped dealkylation vial.
- 3.4.8 Unscrew the cap from the vial. Using a disposable pipet, place an aliquot of the toluene in an autosampler vial or in a limited-volume insert inside an autosampler vial when the toluene volume is  $\leq 1$  mL. Crimp seal the vial immediately. For analysis of liver and kidney extracts at 10x the LOQ, the toluene extract must be diluted 1:2. This can be done by placing 0.5 mL of toluene and 0.5 mL of dealkylated extract in an autosampler vial without a limited-volume insert.

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 toluene solution be placed into the autosampler vials only on the day of analysis, that is, don't store the solution in the autosampler vials. 4) It is not necessary to use a

limited-volume insert when the available toluene volume is greater than 1 mL.

### 3.5 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). The second fortified sample should be fortified at five to ten times the LOQ or at a level that is expected in the unknown sample.

To fortify a sample at the LOQ, add 300 uL of a 1 ug TMS/mL fortification solution to 30 g of milk or eggs (LOQ=0.01 ppm), 750 uL of a 1 ug TMS/mL fortification solution to 25 g of fat or muscle tissue (LOQ=0.03 ppm), or 250 uL of a 10 ug TMS/mL fortification solution to 25 g of liver or kidney (LOQ=0.1 ppm), for example.

## 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 auxiliary helium supplied to the detector to the values recommended by the detector manufacturer. Use a 3- $\mu$ L 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  $\mu$ L. 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 3.4. Dilute the dealkylated standard (high level: 0.6 or 1.0  $\mu$ g TMS/mL) 1:3 and 2:3 to prepare the low-level (0.2 or 0.33  $\mu$ g TMS/mL) and intermediate-level (0.4 or 0.67  $\mu$ g TMS/mL) standards for use in verifying linearity of response. 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.

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 high-, intermediate-, and low-level standards to establish the calibration curve.  
  
For example inject the 0.6, 0.4, and 0.2 ug TMS equivalents/mL standards for analyses involving milk, eggs, muscle, and fat extracts, and 1.0, 0.67, and 0.33 ug TMS equivalents/mL standards for analyses involving liver and kidney extracts.
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 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 calculations 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 ( $\mu\text{g}/\text{mL}$ )

$R$  = 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 Animal product 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 ( $\mu\text{g/mL}/\text{cm}$ )  
R = average sample response from detector for sample (cm)  
C = concentration of animal product 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 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 = animal product concentration in extract, from section 5.1.2 ( $\text{g/mL}$ ).

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 3). Animal tissues, especially those derived from herbivores and omnivores, are also highly likely to contain Vitamin U.

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. However, due to loss of peak resolution in the transfer line of the SCD, the FPD is the superior detector for ensuring that the eluting peak is DMS.

8 DISCUSSION

8.1 Scope

This method is suitable for the determination of TMS in milk, eggs, and edible animal tissues of muscle, fat, liver, and kidney. Recovery data given in Table I reflect the methodology described herein.

8.2 Precision and Accuracy

Fortified samples were prepared as described under section 3.5, and analyzed according to this method to establish recoveries. Recoveries of TMS from milk and eggs fortified at 0.01 and 0.10 mg/kg, muscle and fat fortified at 0.03 and 0.30 mg/kg, and liver and kidney fortified at 0.1 and 1.0 mg/kg ranged from 76 to 115%, with a mean recovery of 93% (n=36) and coefficient of variation of 12%. Table I lists the individual recoveries obtained from milk, eggs, and four edible tissues, 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 matrix 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 0.01, 0.03, and 0.1 mg TMS/kg for milk and eggs; muscle and fat; and liver and kidney, respectively, were obtained from work conducted for this report.

#### 8.5 Matrix Effects

The absence of chromatographic matrix effects was verified by the analysis of extracts fortified just prior to dealkylation. Results are listed in Table II.

#### 8.6 Extract Analysis

Analyses were performed using a sulfur chemiluminescence detector due to its highly selective and linear response to sulfur. Sample chromatograms are given in Figures 1 through 6. A sample standard curve is given in Figure 7. Sample calculations are given in Appendix A. Because the SCD is not widely used in residue analysis, the traditional flame photometric detector can also be used. Sample chromatograms are given in Figures 8, 9, and 10. A sample standard curve is given in Figure 11. A sample calculation is given in Appendix A.

#### 8.7 Wet-Weight Basis

This method determines the residues of TMS in milk, eggs, and edible tissues 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 <sup>14</sup>C-residues. The data are presented in the reports on TMS metabolism studies involving hens (study 92JH142) and goat (study 92JH144).

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

The method is selective for the analysis of TMS residues in milk, eggs, and edible animal tissues. 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 the method at that concentration.

TABLES AND FIGURES

- Table I. Recoveries of TMS from Milk, Eggs, and Tissues
- Table II. Recoveries of TMS from Fortified-Control Extracts: Determination of Chromatographic Matrix Effects
- Figure 1. Sample SCD chromatograms of milk fortified at the LOQ of 0.01 mg/kg
- Figure 2. Sample SCD chromatograms of eggs fortified at the LOQ of 0.01 mg/kg
- Figure 3. Sample SCD chromatograms of beef muscle fortified at the LOQ of 0.03 mg/kg
- Figure 4. Sample SCD chromatograms of beef liver fortified at the LOQ of 0.1 mg/kg
- Figure 5. Sample SCD chromatograms of beef kidney fortified at the LOQ of 0.1 mg/kg
- Figure 6. Sample SCD chromatograms of beef fat fortified at the LOQ of 0.03 mg/kg
- Figure 7. Sample SCD calibration curve for DMS, as TMS equivalents, based on injections of 0.2-, 0.4-, and 0.6-ug/mL toluene solution
- Figure 8. Sample FPD chromatograms of milk and eggs fortified at the LOQ of 0.01 mg/kg
- Figure 9. Sample FPD chromatograms of beef muscle and fat fortified at the LOQ of 0.03 mg/kg
- Figure 10. Sample FPD chromatograms of beef liver and kidney fortified at the LOQ of 0.1 mg/kg
- Figure 11. Sample FPD calibration curve for DMS, as TMS equivalents, based on injections of 0.2-, 0.4-, and 0.6-ug/mL toluene solution

Table I. Recoveries of TMS from Milk, Eggs, and Tissues.

<u>Commodities<sup>a</sup></u>	<u>Trial No.</u>	<u>Sample No.</u>	<u>Amount Added (ppm)</u>	<u>Amount Found<sup>b</sup> (%)</u>	<u>Average (%)</u>
Cow's Milk	99CA93-4031	J4031-04	0.01	108	107
		J4031-05	0.01	105	
		J4031-06	0.01	103	
		J4031-07	0.10	109	
		J4031-08	0.10	114	
		J4031-09	0.10	104	
Chicken Eggs	99CA93-4032	J4032-04	0.01	87	84
		J4032-05	0.01	82	
		J4032-06	0.01	84	
		J4032-07	0.10	81	
		J4032-08	0.10	88	
		J4032-09	0.10	83	
Beef Muscle	99CA93-4033	J4033-04	0.03	91	95
		J4033-05	0.03	94	
		J4033-06	0.03	86	
		J4033-07	0.30	95	
		J4033-08	0.30	106	
		J4033-09	0.30	98	
Beef Liver	99CA93-4034	J4034-04	0.1	76	84
		J4034-05	0.1	86	
		J4034-06	0.1	84	
		J4034-07	1.0	86	
		J4034-08	1.0	85	
		J4034-09	1.0	88	
Beef Kidney	99CA93-4035	J4035-04	0.1	90	83
		J4035-05	0.1	79	
		J4035-06	0.1	77	
		J4035-07	1.0	90	
		J4035-08	1.0	80	
		J4035-09	1.0	80	
Beef Fat	99CA93-4036	J4036-04	0.03	113	105
		J4036-05	0.03	100	
		J4036-06	0.03	115	
		J4036-07	0.30	100	
		J4036-08	0.30	103	
		J4036-09	0.30	101	

a) All samples were purchased from local retail outlets.

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

Table I (continued).

Recoveries of TMS from Milk, Eggs, and Tissues.

	Data Summary			
	<u>Average Recovery<sup>a</sup></u> <u>(%)</u>	<u>CV (%)</u>	<u>N</u>	<u>Range (%)</u>
Cow's Milk	107	3.7	6	103-114
Chicken Eggs	84	3.3	6	81- 88
Beef Muscle	95	7.1	6	86-106
Beef Liver	84	5.1	6	76- 88
Beef Kidney	83	7.2	6	77- 90
Beef Fat	105	6.5	6	100-115

CV = coefficient of variation.

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

Table II. 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<sup>a</sup> (%)</u>
Cow's Milk	99CA93-4031	J4031-10	0.1	112
Chicken Eggs	99CA93-4032	J4032-10	0.1	101
Beef Muscle	99CA93-4033	J4033-10	0.3	109
Beef Liver	99CA93-4034	J4034-10	1.0	105
Beef Kidney	99CA93-4035	J4035-10	1.0	115
Beef Fat	99CA93-4036	J4036-10	0.3	109

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

Figure 1. Sample SCD chromatograms of milk fortified at the LOQ of 0.01 mg/kg

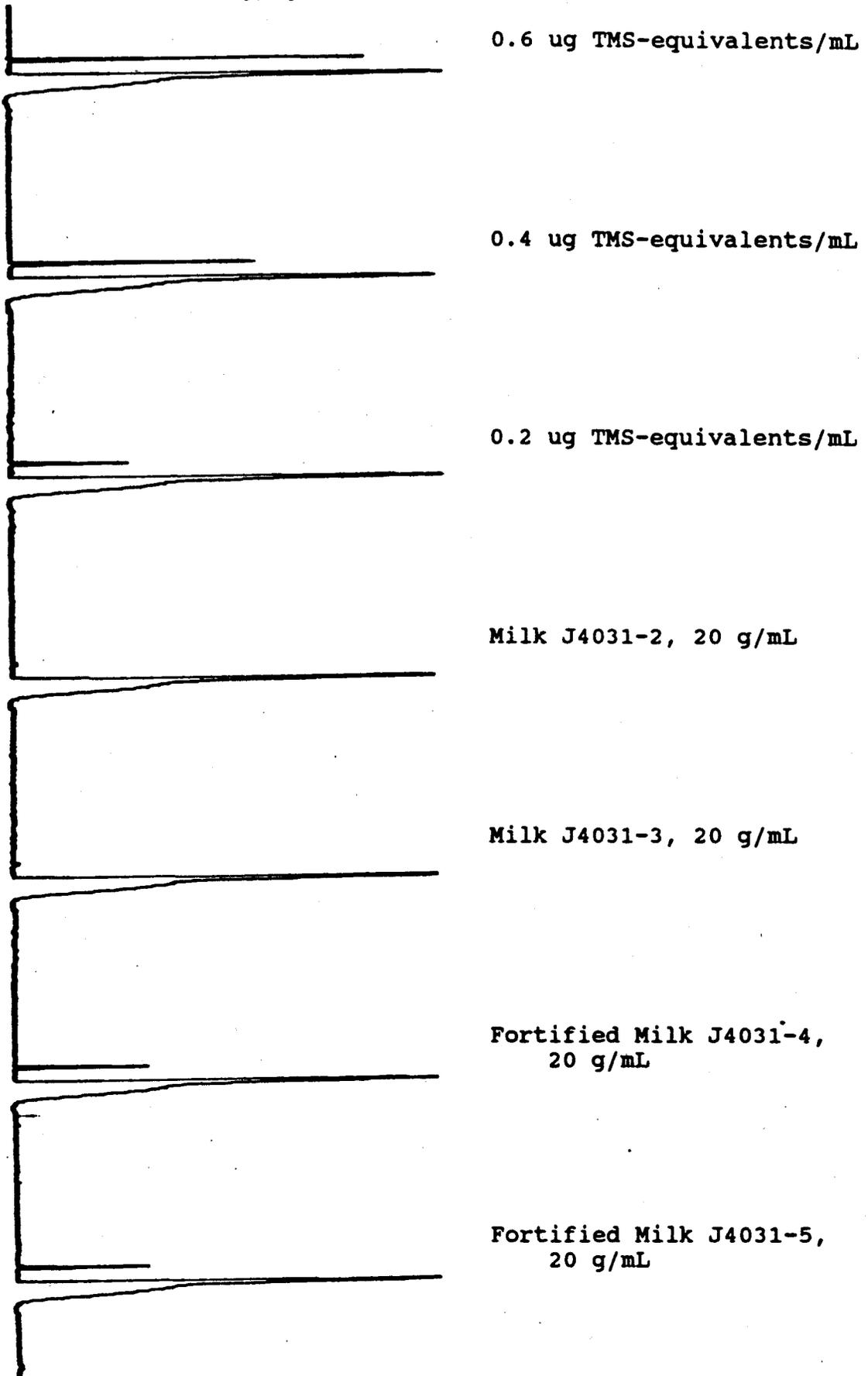


Figure 2. Sample SCD chromatograms of eggs fortified at the LOQ of 0.01 mg/kg

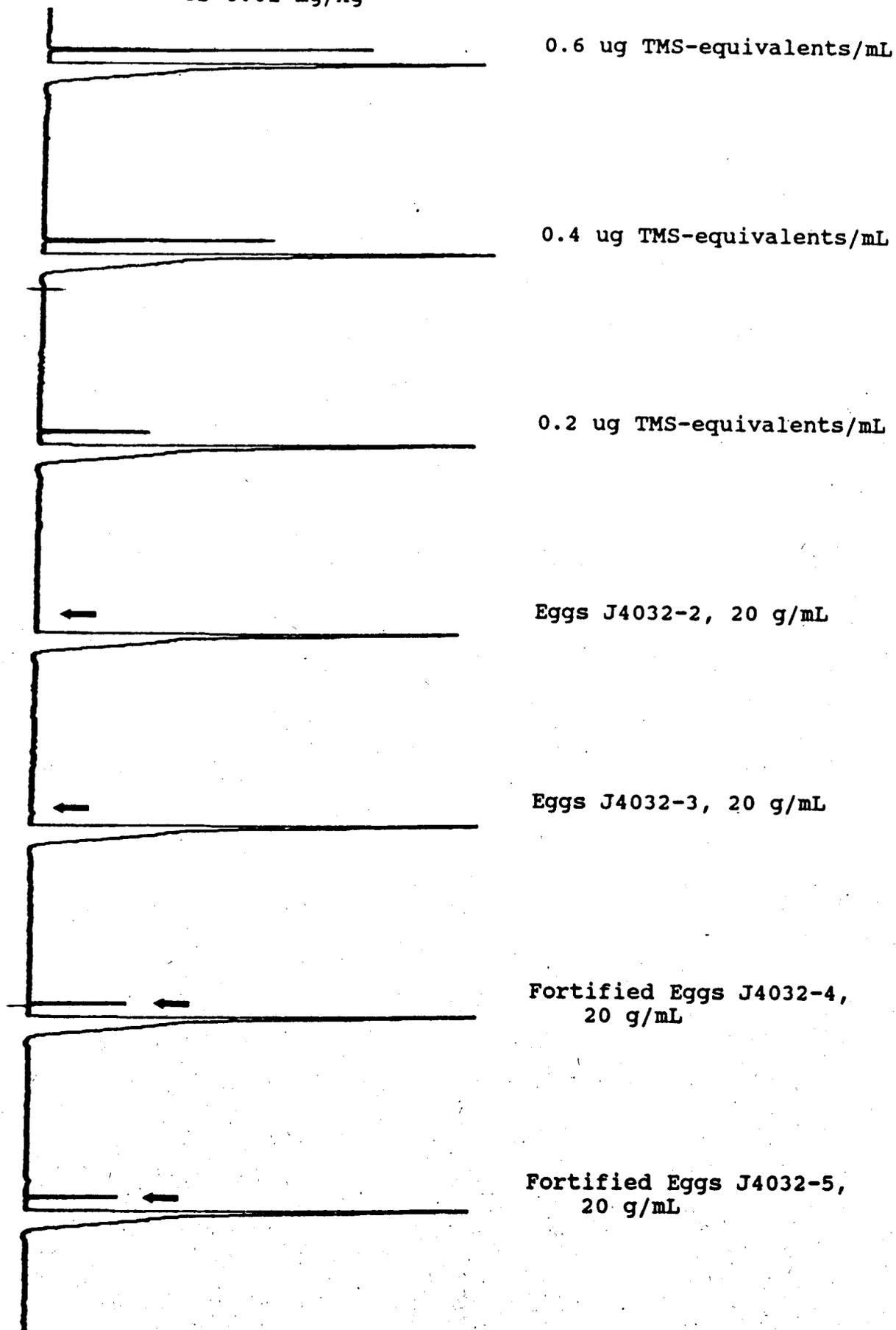


Figure 3. Sample SCD chromatograms of beef muscle fortified at the LOQ of 0.03 mg/kg

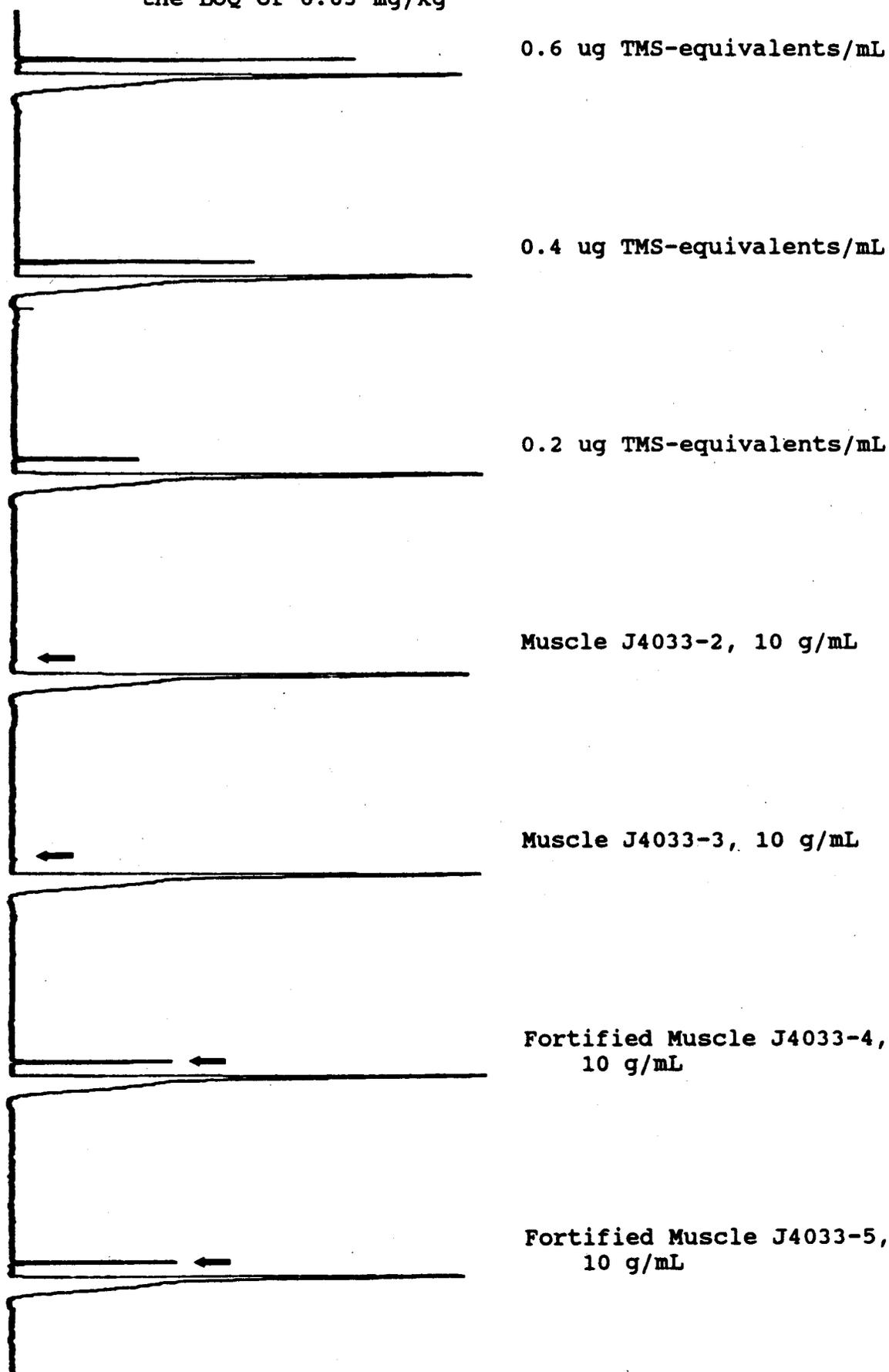


Figure 4. Sample SCD chromatograms of beef liver fortified at the LOQ of 0.1 mg/kg

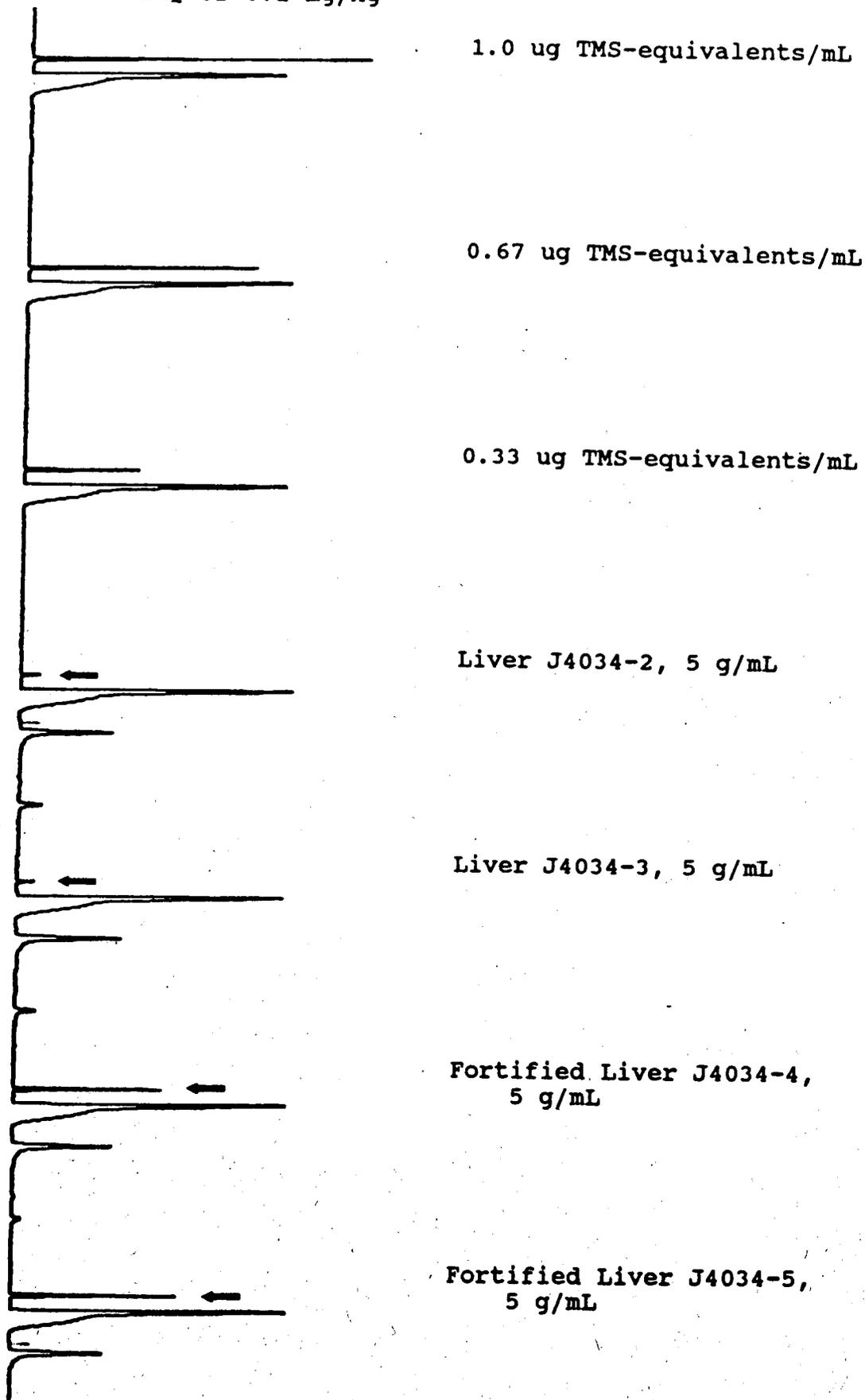


Figure 5. Sample SCD chromatograms of beef kidney fortified at the LOQ of 0.1 mg/kg

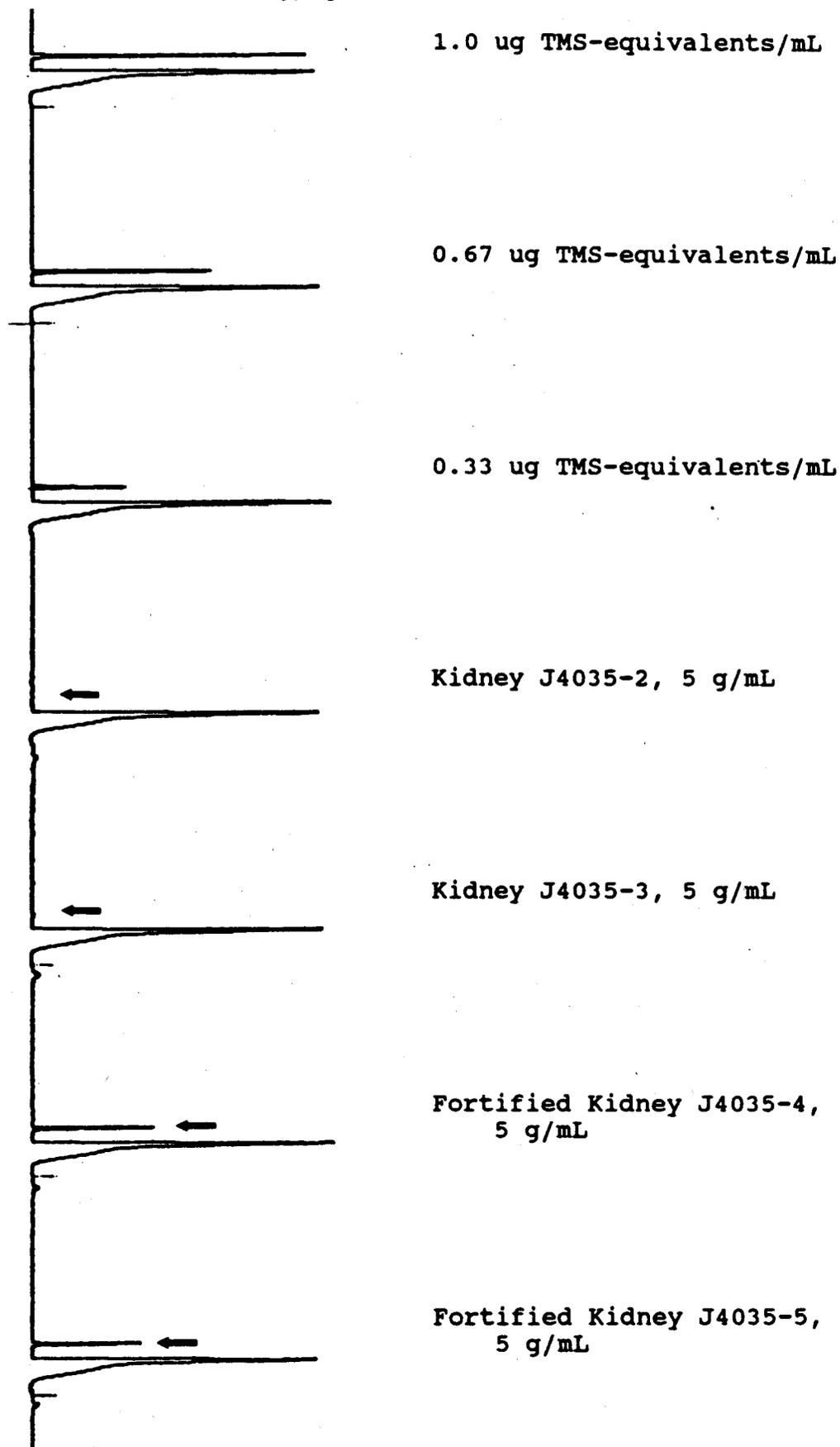


Figure 6. Sample SCD chromatograms of beef fat fortified at the LOQ of 0.03 mg/kg

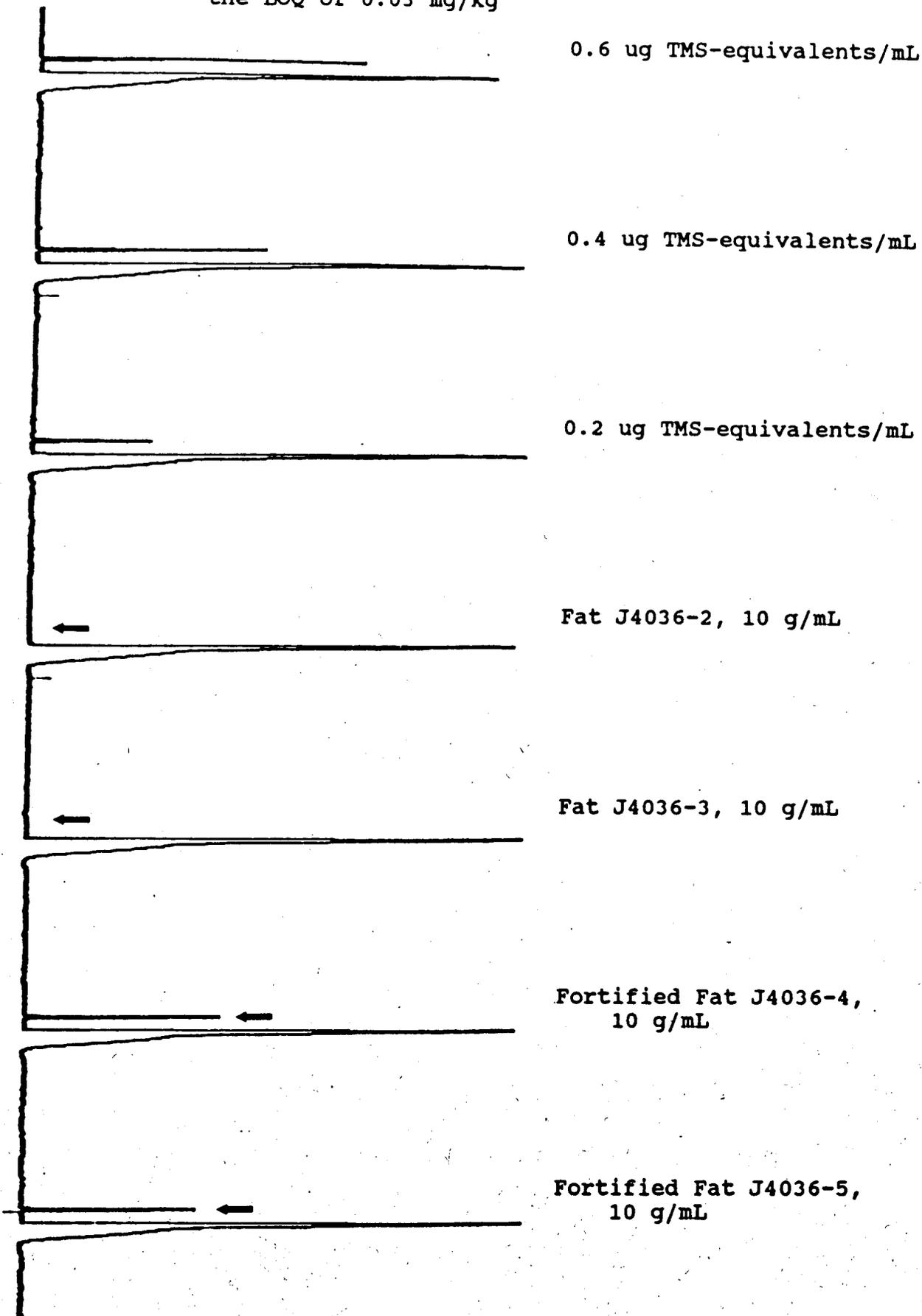


Figure 7. Sample SCD calibration curve for DMS, as TMS equivalents, based on injections of 0.2-, 0.4-, and 0.6-ug/mL toluene solution.

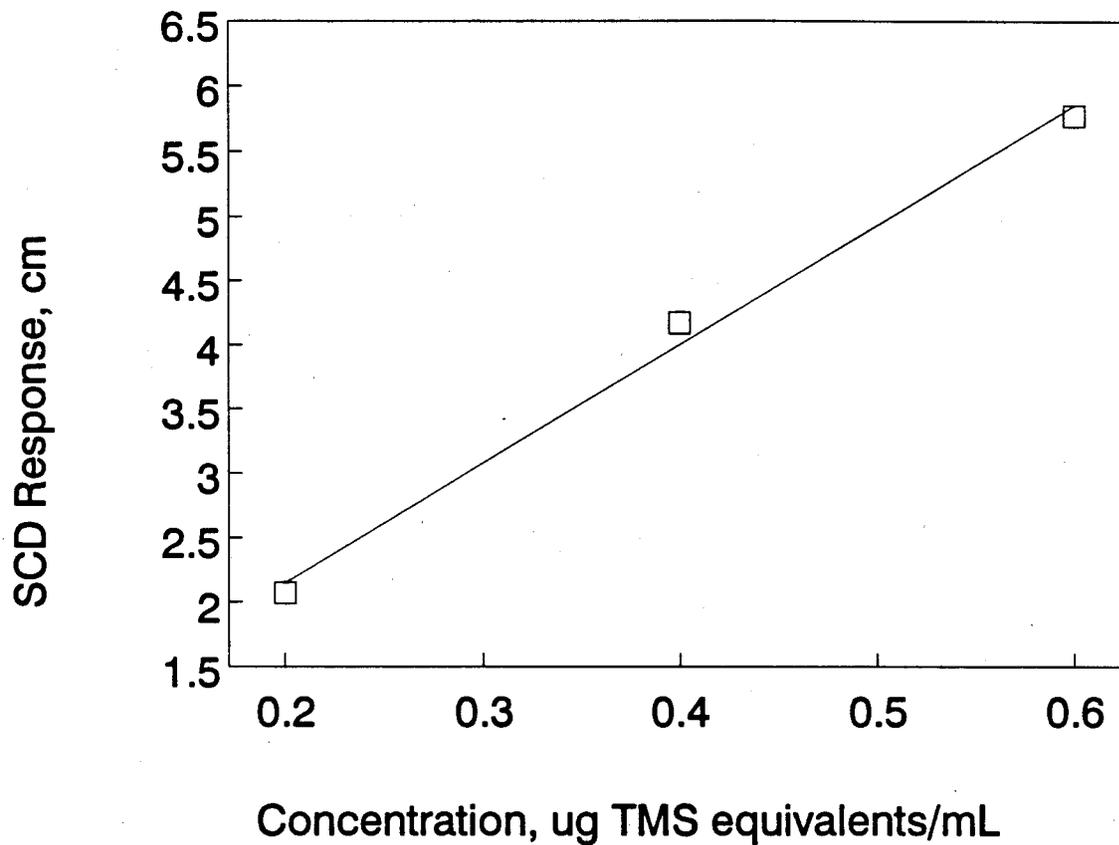
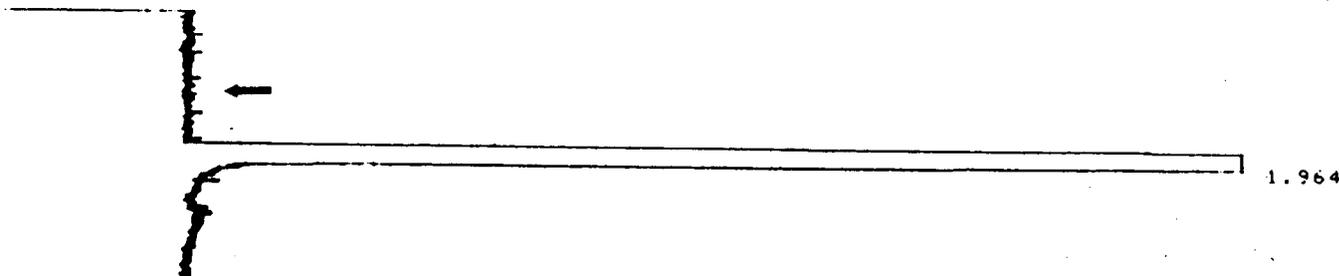


Figure 8. Sample FPD chromatograms of milk and eggs fortified at the LOQ of 0.01 mg/kg. (Response of standards are shown in Figure 11.)

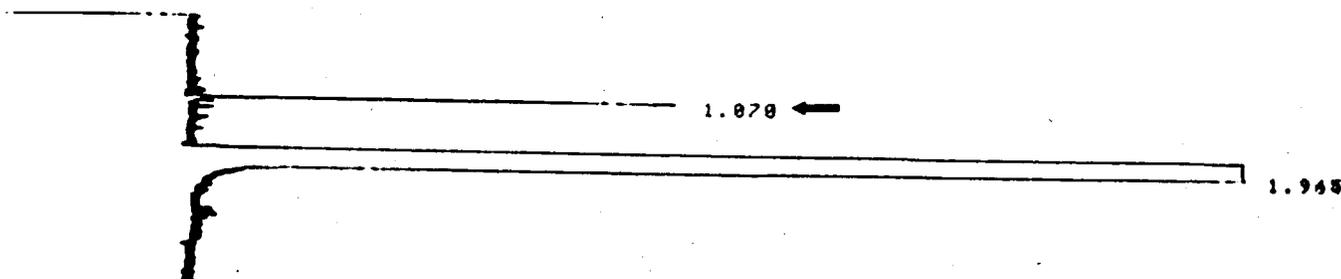
Untreated extract equivalent to 20 g of milk/mL of toluene.

RUN # 172 NOV 11, 1993 12:25:38  
START



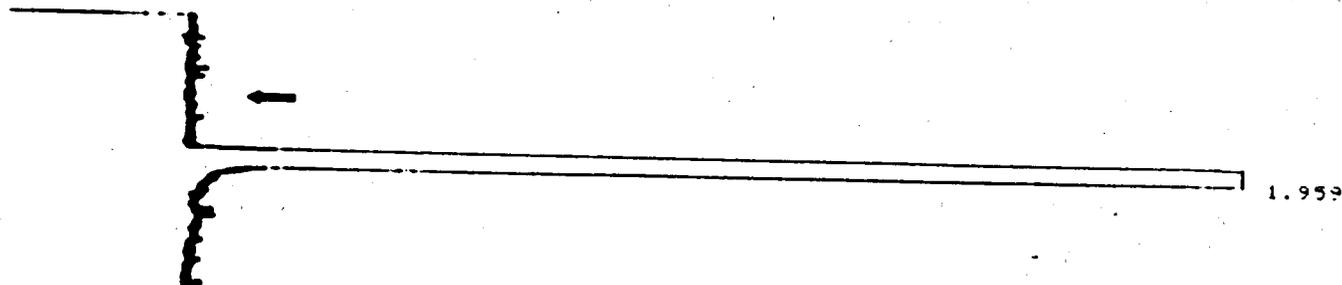
Fortified cleaned extract equivalent to 20 g of milk/mL of toluene.

RUN # 173 NOV 11, 1993 12:31:55  
START



Untreated extract equivalent to 20 g of eggs/mL of toluene.

RUN # 174 NOV 11, 1993 12:38:19  
START



Fortified cleaned extract equivalent to 20 g of eggs/mL of toluene.

RUN # 175 NOV 11, 1993 12:44:43  
START

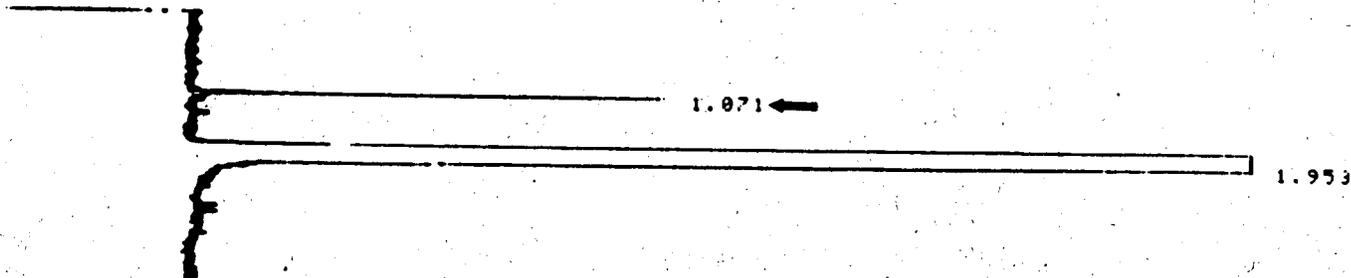
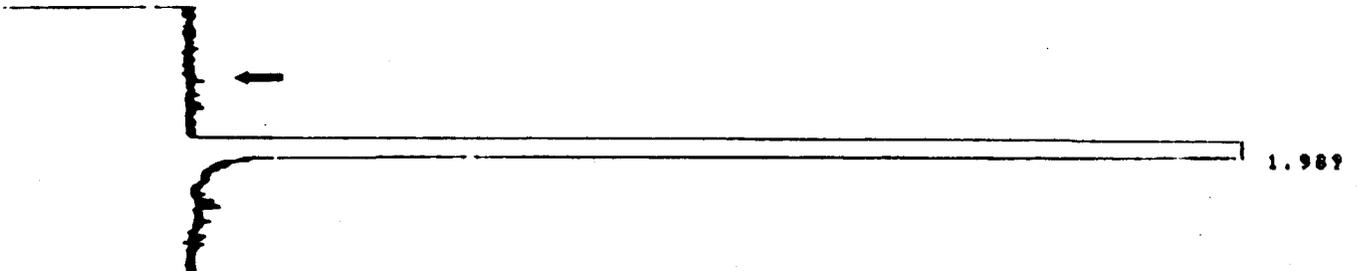


Figure 9. Sample FPD chromatograms of beef muscle and fat fortified at the LOQ of 0.03 mg/kg (Response of standards are shown in Figure 11.)

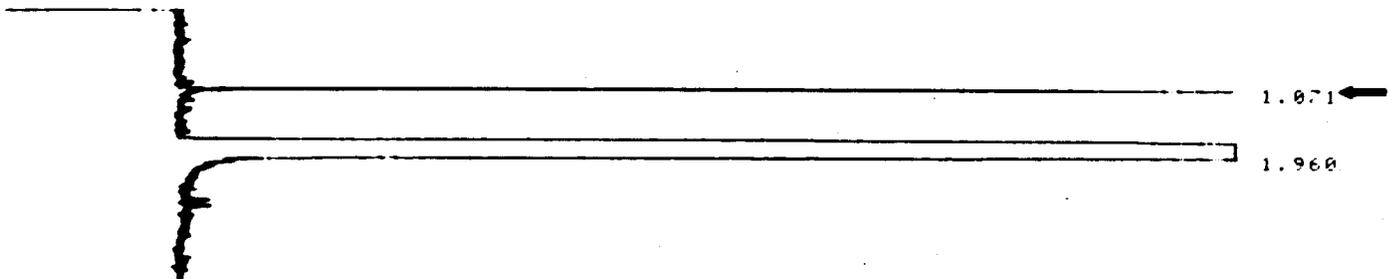
Untreated extract equivalent to 10 g of muscle/mL of toluene.

RUN # 176 NOV 11, 1993 12:51:06  
START



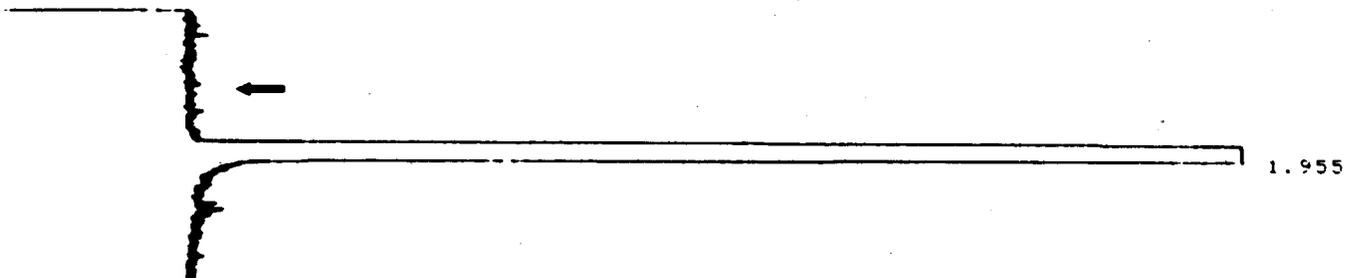
Fortified cleaned extract equivalent to 10 g of muscle/mL of toluene.

RUN # 177 NOV 11, 1993 12:57:33  
START



Untreated extract equivalent to 10 g of beef fat/mL of toluene.

RUN # 186 NOV 11, 1993 13:55:09  
START



Fortified cleaned extract equivalent to 10 g of fat/mL of toluene.

RUN # 185 NOV 11, 1993 13:48:47  
START

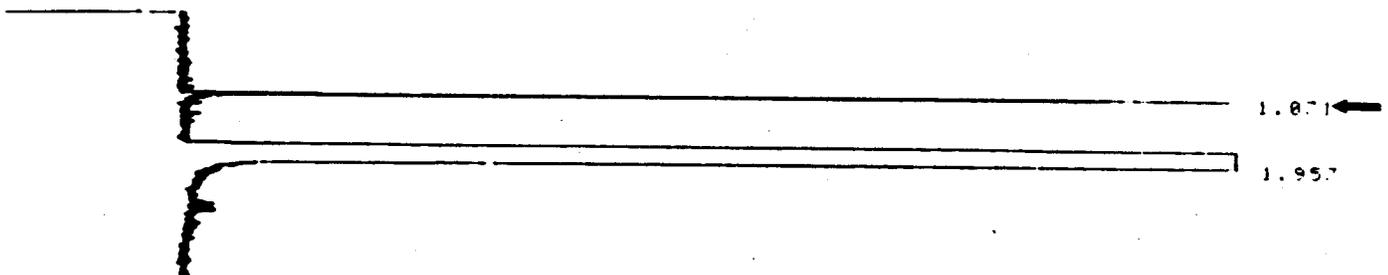
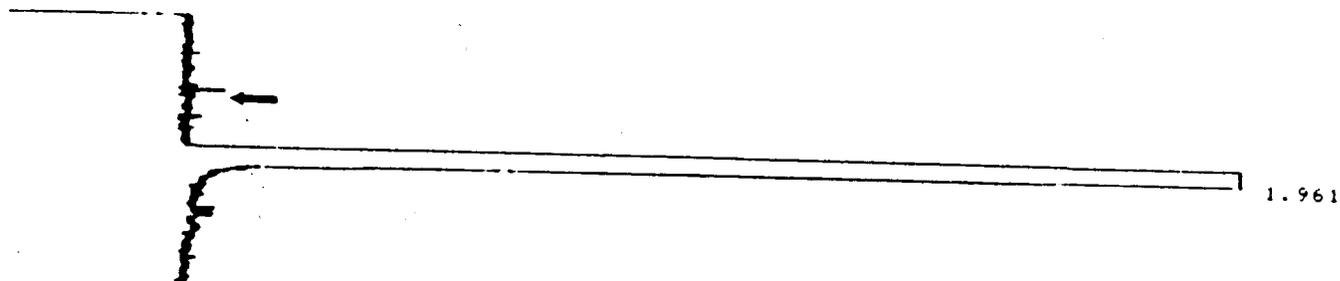


Figure 10. Sample FPD chromatograms of beef liver and kidney fortified at the LOQ of 0.1 mg/kg (Response of standards are shown in Figure 11.)

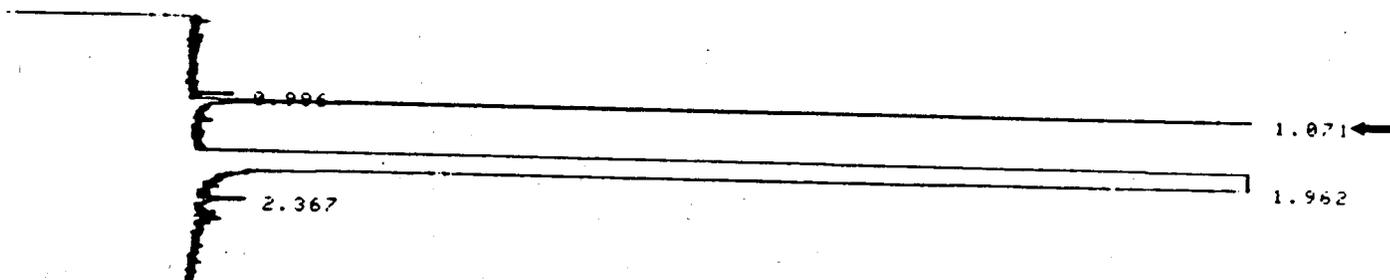
Untreated extract equivalent to 5 g of liver/mL of toluene.

RUN # 181 NOV 11, 1993 13:23:18  
START



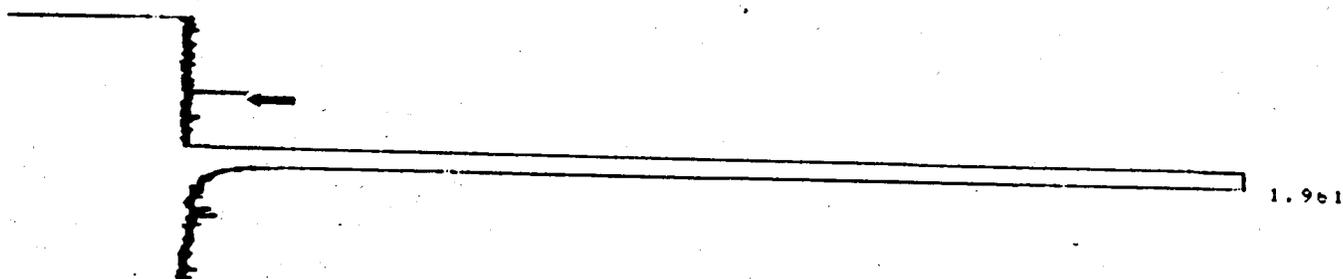
Fortified cleaned extract equivalent to 5 g of liver/mL of toluene.

RUN # 182 NOV 11, 1993 13:29:33  
START



Untreated extract equivalent to 5 g of kidney/mL of toluene.

RUN # 183 NOV 11, 1993 13:36:00  
START



Fortified cleaned extract equivalent to 5 g of kidney/mL of toluene.

RUN # 184 NOV 11, 1993 13:42:21  
START

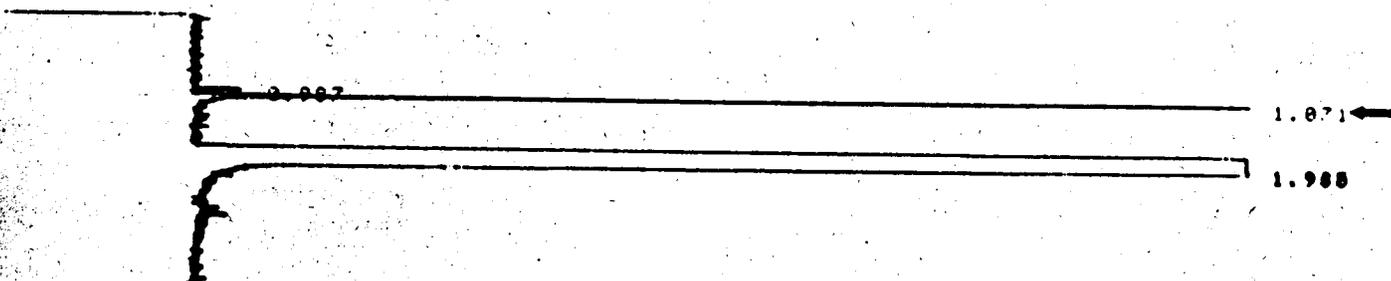
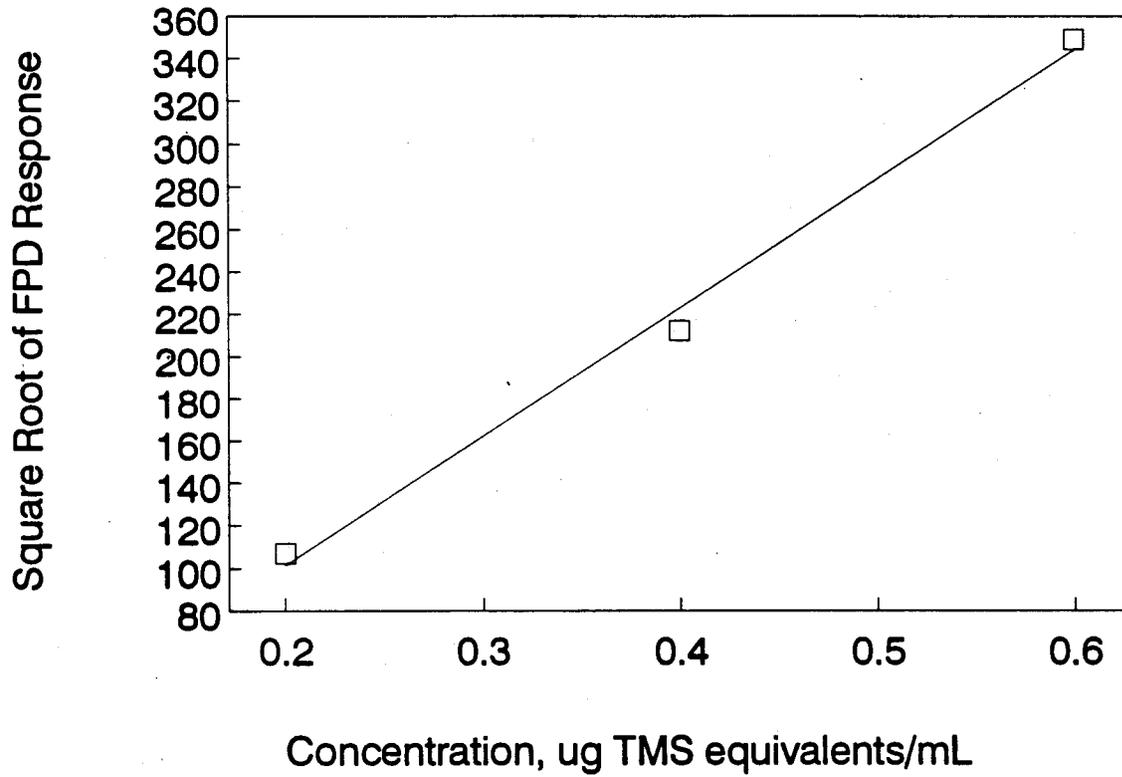


Figure 11. Sample FPD calibration curve for DMS, as TMS equivalents, based on injections of 0.2-, 0.4-, and 0.6-ug/mL toluene solution.



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.      Graham, D.G. (1987) Determination of sulfosate cation residues in milk, eggs, and edible tissues by gas chromatography. Stauffer Chemical Co., Report No. RRC 87-42, submitted in MRID 41462103 and 4146204.
2.      Watt, B. K. and Merrill, A. L. in *Composition of Foods; Agricultural Handbook No. 8*; U.S. Department of Agriculture: revised 1963.
3.      "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.

14      APPENDICES

Appendix A. Sample calculations

**Appendix A. Sample calculations**

Calculation Method per Section 5.1.1

Calibration Factor Method

TMS solution, ug/mL	Mean peak height, cm	(ug/mL)/cm ratio	Regression Output:	
0.60	5.77	0.1040	Constant	0.3
0.40	4.17	0.0960	Std Err of Y Est	0.204124
0.20	2.07	0.0968	R Squared	0.993949
Mean:	0.0989		No. of Observations	3
			Degrees of Freedom	1
			X Coefficient(s)	9.25
			Std Err of Coef.	0.721687

\*\*\*\*\*GC/SCD ANALYSIS OF BEEF MUSCLE\*\*\*\*\*

Sample solution	Peak height, cm	Percent of previous value	Bkgrd. corrected peak height, cm	Calc'd TMS conc., ug/mL	Tissue conc., g/mL	Calc'd TMS conc., ppm	TMS conc. added, ppm	TMS found, ppm or %
0.6 ug TMS/mL	6.00	-	-	-	-	-	-	-
0.4 ug TMS/mL	4.15	-	-	-	-	-	-	-
0.2 ug TMS/mL	2.10	-	-	-	-	-	-	-
J4033-02 control	0.00	-	-	-	10.0	-	-	<0.01
J4033-03 control	0.00	-	-	-	10.0	-	-	<0.01
J4033-04 0.03 ppm	2.75	-	2.75	0.272	10.0	0.0272	0.030	91%
J4033-05 0.03 ppm	2.85	-	2.85	0.282	10.0	0.0282	0.030	94%
J4033-06 0.03 ppm	2.60	-	2.60	0.257	10.0	0.0257	0.030	86%
0.6 ug TMS/mL	5.70	95	-	-	-	-	-	-
0.4 ug TMS/mL	4.30	104	-	-	-	-	-	-
0.2 ug TMS/mL	2.05	98	-	-	-	-	-	-
J4033-07 0.3 ppm	4.80	-	4.80	0.475	1.67	0.2844	0.300	95%
J4033-08 0.3 ppm	5.35	-	5.35	0.529	1.67	0.3170	0.300	106%
J4033-09 0.3 ppm	4.95	-	4.95	0.490	1.67	0.2933	0.300	98%
J4033-10 0.3 ppm	5.50	-	5.50	0.544	1.67	0.3259	0.300	109%
0.6 ug TMS/mL	5.60	98	-	-	-	-	-	-
0.4 ug TMS/mL	4.05	94	-	-	-	-	-	-
0.2 ug TMS/mL	2.05	100	-	-	-	-	-	-

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Average Percent Recovery for Matrix-Fortified Samples: 95  
Coefficient of Variation, %: 7.1

Calculation Method per Section 5.1.2

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

Calculation of TMS Concentrations in Beef Muscle from SCD Data

Sample solution analyzed	Manually-measured peak height, cm	Bkgrd-subtracted peak height, cm	TMS conc. found, ug/mL*	Sample matrix conc., g/mL	TMS conc. found, ppm	TMS conc. added, ppm	TMS found, ppm or %
0.6 ug TMS/mL	6.00	-	-	-	-	-	-
0.4 ug TMS/mL	4.15	-	-	-	-	-	-
0.2 ug TMS/mL	2.10	-	-	-	-	-	-
J4033-02 control	0.00	-	-	-	-	-	-
J4033-03 control	0.00	-	-	-	-	-	-
J4033-04 0.03 ppm	2.75	2.75	0.265	10.0	0.026	0.030	88%
J4033-05 0.03 ppm	2.85	2.85	0.276	10.0	0.028	0.030	92%
J4033-06 0.03 ppm	2.60	2.60	0.249	10.0	0.025	0.030	83%
0.6 ug TMS/mL	5.70	-	-	-	-	-	-
0.4 ug TMS/mL	4.30	-	-	-	-	-	-
0.2 ug TMS/mL	2.05	-	-	-	-	-	-
J4033-07 0.3 ppm	4.80	4.80	0.486	1.67	0.291	0.30	97%
J4033-08 0.3 ppm	5.35	5.35	0.546	1.67	0.327	0.30	109%
J4033-09 0.3 ppm	4.95	4.95	0.503	1.67	0.301	0.30	100%
J4033-10 0.3 ppm	5.50	5.50	0.562	1.67	0.337	0.30	112%
0.6 ug TMS/mL	5.60	-	-	-	-	-	-
0.4 ug TMS/mL	4.05	-	-	-	-	-	-
0.2 ug TMS/mL	2.05	-	-	-	-	-	-

\*X = (Y-constant)/(x coefficient) derived from the regression analysis.

LOTUS 1-2-3 Regression Analysis of Data

		Regression Output	
TMS Conc., ug/mL	Peak height, cm	Constant	0.3
		Std Err of Y Est	0.204124
		R Squared	0.993949
		No. of Observations	3
		Degrees of Freedom	1
0.60	5.77	X Coefficient(s)	9.25
0.40	4.17	Std Err of Coef.	0.721687
0.20	2.07		

Calculation Method per Section 5.2

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

Calculation of TMS Concentrations from FPD Data

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 %
0.6 ug TMS/mL	136520	369	-	-	-	-	-	-
0.4 ug TMS/mL	51358	227	-	-	-	-	-	-
0.2 ug TMS/mL	12699	113	-	-	-	-	-	-
Milk, control	0	0	-	-	20.0	-	-	-
Milk, extract fortified	14331	120	120	0.23	20.0	0.012	0.01	115%
Eggs, control	0	0	-	-	20.0	-	-	-
Eggs, extract fort.	13267	115	115	0.17	20.0	0.008	0.01	84%
Muscle, control	0	0	-	-	10.0	-	-	-
Muscle, extract fort.	32725	181	181	0.28	10.0	0.028	0.03	92%
0.6 ug TMS/mL	118290	344	-	-	-	-	-	-
0.4 ug TMS/mL	42450	206	-	-	-	-	-	-
0.2 ug TMS/mL	11154	106	-	-	-	-	-	-
Liver, control	0	0	-	-	5.0	-	-	-
Liver, extract fort.	109121	330	330	0.52	5.0	0.105	0.10	105%
Kidney, control	0	0	-	-	5.0	-	-	-
Kidney, extract fort.	94384	307	307	0.49	5.0	0.097	0.10	97%
Fat, control	0	0	-	-	10.0	-	-	-
Fat, extract fortified	30490	175	175	0.27	10.0	0.027	0.03	89%
0.6 ug TMS/mL	111798	334	-	-	-	-	-	-
0.4 ug TMS/mL	41493	204	-	-	-	-	-	-
0.2 ug TMS/mL	10422	102	-	-	-	-	-	-
Mean % Recovery:								97.0
Coefficient of Variation, %:								11.7

\*  $x = y - b/m$ , where  $b$  is "constant" and  $m$  is "x coefficient" from regression analysis.

LOTUS 1-2-3 Regression Analysis of Data

TMS concn. ug/mL	Mean Square root of FPD response	Regression Output:	
0.60	349	Constant	-19.7383
0.40	212	Std Err of Y Est	12.99062
0.20	107	R Squared	0.994291
		No. of Observations	3
		Degrees of Freedom	1
		X Coefficient(s)	606.1590
		Std Err of Coef.	45.92881

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