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RICERCA, INC.
DEPARTMENT OF RESIDUE ANALYSIS

REPORT TITLE

# METHOD FOR THE DETERMINATION OF RESIDUES OF SDS-3701 IN MILK AND MEAT - REVISED

DOCUMENT NUMBER: 6442-95-0075-MD-002

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Report/SDS-3701

REPORT

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Report/SDS-3701

# METHOD FOR THE DETERMINATION OF RESIDUES OF SDS-3701 IN MILK AND MEAT - REVISED


## ABSTRACT

An analytical method for the determination of SDS-3701 in milk and cattle tissues is presented in this document. The limit of quantitation is 0.01 ppm in milk, butterfat, skim milk, muscle, kidney, liver and body fat.

Residues are initially extracted from the samples using acidic acetone and then partitioned into diethyl ether. Residues of SDS-3701 are derivatized to the methyl ether and undergo cleanup (optional) by alumina column chromatography prior to quantitation by electron capture gas chromatography.

Ricerca, Inc.
6442-95-0075-MD-002
Report/SDS-3701

TABLE OF CONTENTS


| | PAGE |
|---|---|
| TITLE | 1 |
| DISTRIBUTION | 2 |
| APPROVAL | 3 |
| ABSTRACT | 4 |
| TABLE OF CONTENTS | 5 |
| INTRODUCTION | 6 |
| MATERIALS | 6 |
| Chemicals | 6 |
| Equipment | 8 |
| Chromatography Conditions | 9 |
| PREPARATION OF SDS-3701 STANDARD SOLUTIONS | 12 |
| PREPARATION OF SAMPLES | 13 |
| Muscle | 13 |
| Liver | 13 |
| Kidney | 13 |
| Body Fat | 13 |
| Whole Milk, Butterfat, Skim Milk | 13 |
| EXTRACTION OF RESIDUE - MUSCLE, LIVER, KIDNEY AND FAT | 13 |
| EXTRACTION OF RESIDUE - WHOLE MILK, BUTTERFAT, SKIM MILK | 14 |
| PARTITIONING | 15 |
| DERIVATIZATION OF SDS-3701 WITH DIAZOMETHANE | 16 |
| Preparation of Diazomethane Solution | 16 |
| Derivatization | 17 |
| PREPARATION FOR DIRECT QUANTITATION | 17 |
| SAMPLE CLEANUP OF SDS-3701 | 18 |
| PREPARATION FOR QUANTITATION (AFTER SAMPLE CLEANUP) | 18 |
| QUANTITATION | 18 |
| LIMIT OF QUANTITATION/LIMIT OF DETECTION | 19 |
| RESULTS | 20 |
| APPENDIX I | |
| Representative Chromatograms | 21 |

Ricerca, Inc.
6442-95-0075-MD-002
Report/SDS-3701

## INTRODUCTION

This document provides an analytical procedure for the determination of residues of 2,5,6-trichloro-4-hydroxyisophthalonitrile (SDS-3701) in milk and cattle tissues (muscle, kidney, liver and fat). The methodology presented in this report was utilized in the analytical portion of a previously conducted study, "Meat and Milk Magnitude of Residue Study in Lactating Dairy Cows Dosed With Chlorothalonil and SDS-3701," Ricerca, Inc. Document Number 6007-94-0120-CR-003. The methodology was validated in cattle tissue but could probably be utilized to analyze other types of animal tissue.

Residues are initially extracted from the samples using acidic acetone and partitioned into diethyl ether. Residues of SDS-3701 are derivatized to the methyl ether and undergo cleanup (optional) by alumina column chromatography prior to quantitation by electron capture gas chromatography.

The current report incorporates clarifications and additional details concerning critical steps not detailed in the original method (Ricerca Document Number 6442-95-0075-MD-001). While the methodology remains the same, these clarifications and corrections will allow residue chemists to more easily perform the method.

Sections of the method dealing with solvent evaporation and derivatization are critical to its successful use. Failure to follow the steps as written will result in significant loss of SDS-3701 residues.

## MATERIALS

### Chemicals

Acetic acid, glacial, ACS reagent grade or equivalent

Acetone, ACS certified, or equivalent

Acidic acetone, consisting of 95% acetone and 5% 10N $H_2SO_4$ (v/v)

Alumina (aluminum oxide W200 acid), activated at 120 °C ± 5 °C for at least 24 hours before use, available from Fisher Scientific Company, Cat. No. 5-719-832

Buffer solutions, pH 4 and 7, available from Fisher Scientific Company, Cat. Nos. SB98-500 and SB108-500, or equivalent

Diethyl ether, anhydrous, ACS reagent grade or equivalent

Helium, high purity

Hydrochloric acid (HCl), concentrated, ACS reagent grade, or equivalent

"Keeper" solution, consisting of 2% paraffin oil in petroleum ether

Methanol, ACS certified, or equivalent

Methylene chloride, ACS certified, or equivalent

N-methyl-N'-nitro-N-nitrosoguanidine, available from Aldrich Chemical Co., Catalog No. 12, 994-1

$N_2$, high purity, or equivalent

Paraffin oil, light, available from Fisher Scientific Company, Cat. No. 0121-1

Petroleum ether, distilled in glass, nanograde, or equivalent

Sodium bicarbonate ($NaHCO_3$) solution, 0.8M, prepared by dissolving 67.2 g in 2 L deionized water

Sodium chloride (NaCl), ACS certified, or equivalent

Sodium hydroxide (NaOH) solution, 4N, prepared by dissolving 16 g in 100 mL deionized water

Sodium sulfate ($Na_2SO_4$), anhydrous, ACS certified, or equivalent

Sulfuric acid ($H_2SO_4$), 10N, ACS reagent grade, or equivalent

Sulfuric acid ($H_2SO_4$), 1N, prepared from 10N $H_2SO_4$

2,5,6-Trichloro-4-hydroxyisophthalonitrile (SDS-3701), analytical standard of known purity

Toluene, ACS reagent grade, or equivalent

**Equipment**

Blender, stainless steel, explosion proof or explosion resistant

Bottles, 2-oz, 4-oz and 8-oz narrow mouth glass Qorpak® clear Boston Round with Polyseal-lined caps (Fisher Scientific Company, Cat. Nos. 03-326-5B, 5C, 5D or equivalent) and 32-oz wide mouth glass Qorpak® clear with Teflon® lined caps, (Fisher Scientific Company, Cat. No. 03-321-1D or equivalent)

Büchner funnel, Filter paper: Whatman No. 4, Whatman 934-AH

Centrifuge, IEC Centra-7 or equivalent

Chromatographic glass columns, 200 mm x 9 mm, Kontes Co., Cat. No. K420100-23 or equivalent

Diazomethane Generator, Chrompak, Cat. No. M12421-85-1

Hobart Food Cutter, Model 8181-D with Hobart Chopping End

Micropipettor, Series H, 10 µL, Labindustries, Inc., Cat. No. MP-10H, or equivalent

Needle, 22g B-D No. 5156, or equivalent

pH Meter equipped with standard combination electrode

Rotary evaporator

Shaker, Eberbach reciprocating, or equivalent

Standard laboratory glassware: beakers, round-bottom flasks, separatory funnels, etc.

Stirrer, magnetic

Syringe, 1 cc Tuberculin B-D No. 5602, or equivalent

Water bath, maintained at a temperature of 37° ± 2 °C, or equivalent apparatus

Chromatography Conditions

Residues are quantitated using a gas chromatograph equipped with a $^{63}Ni$ electron capture detector, an autosampler and a data system. Analytical conditions:

Column 1: 6′ x 1/4" o.d. x 2 mm i.d. glass column filled with 3% OV-7 on 100/120 mesh Supelcoport

Column 2: 6′ x 1/4" o.d. x 2 mm i.d. glass column filled with 3% Dexsil 300 on 100/120 mesh Supelcoport

Column 3: DB-608 capillary column, 30 m, 0.32 mm i.d., 0.5 µm film thickness (JW Catalog No. 123-1730), split injection, 20:1 to 40:1 split ratio

Column 4: DB-5 capillary column, 30 m, 0.25 mm i.d., 0.25 µm film thickness (JW Catalog No. 122-5032), splitless injection to 0.5 to 1 minute, split flow: minimum 20:1

Temperature: Column 1: Isothermal - 200 °C
Column 2: Isothermal - 190° to 200 °C
Column 3: Isothermal - 195° to 200 °C

Alternate temperature programs for Column 3:

1) 170 °C for 25 minutes then increasing to 260 °C at 50 °C/minute and held for 5 minutes.

2) 220 °C for 5 minutes then increasing to 260 °C at 50 °C/minute and held for 5 minutes.

3) 200 °C for 9 minutes then increasing to 260 °C at 50 °C/minute and held for 5 minutes.

Column 4: Isothermal - 220 °C

Injection Port Temperature: 280 °C (Columns 1, 2)
320 °C (Column 3)
300 °C (Column 4)

Detector Temperature: 350 °C (Columns 1, 2, 3)
300 °C (Column 4)

Carrier Gas: Nitrogen, 30 mL/min (Columns 1, 2)
Helium, 1-2 mL/min (Columns 3, 4)

Make-Up Gas: Nitrogen, 28-30 mL/min (Columns 3, 4)

Injection Volume: 2 to 3 µL (Columns 1, 2)
1 to 1.5 µL (Columns 3, 4)

Run Time: Length of run should be long enough to ensure that no late eluters interfere with subsequent injections.

Column Choice:

| Matrix | Preferred Column |
|---|---|
| Milk, Butterfat or Skim Milk | Column 3 (with oven temperature program 3 or isothermal at 200 °C)<br>Alternates - Columns 1, 2 or 4 |
| Body Fat | Column 1<br>Alternate - Column 2 |
| Muscle | Column 3 (with oven temperature program 1) |
| Kidney | Column 4 (isothermal at 220 °C)<br>Column 3 (isothermal at 195 °C) |
| Liver | Column 3 (with oven temperature program 2) |

**NOTE:** The use of other columns or changes in chromatographic conditions may be made as necessary to obtain adequate resolution and sensitivity.

**PREPARATION OF SDS-3701 STANDARD SOLUTIONS**

**NOTE:** All pipets and volumetric flasks used to prepare or transfer SDS-3701 standards are acid washed by rinsing with 1N $H_2SO_4$, deionized water and acetone and air drying prior to use.

A standard solution of SDS-3701 is prepared by weighing 0.1 g SDS-3701 to the nearest 0.1 mg into a tared container. The SDS-3701 is quantitatively transferred to a 100-mL volumetric flask using acetone.

The contents of the volumetric flask are diluted to volume with acetone to produce a stock solution of 1000 µg SDS-3701 per mL. This stock solution is diluted with acetone to concentrations of 100, 10, 1 and 0.1 µg per mL. One drop of concentrated hydrochloric acid is added to all above solutions.

A 1-mL aliquot of the 10 µg per mL solution is transferred by pipet to a 4-oz bottle which has been rinsed with 1N $H_2SO_4$, deionized water, acetone and allowed to dry prior to use. The solvent is evaporated just to dryness using a heated water bath with a stream of dry air or nitrogen. SDS-3701 is derivatized to the methyl ether as described under **DERIVATIZATION OF SDS-3701 WITH DIAZOMETHANE**. A 100-mL portion of toluene is added to result in a concentration of 0.1 µg SDS-3701 per mL. This methylated standard solution and dilutions (see **QUANTITATION**) are utilized for quantitation by electron capture gas chromatography.

Ricerca, Inc.
6442-95-0075-MD-002
Report/SDS-3701

## PREPARATION OF SAMPLES

### Muscle

Each semi-frozen sample is cut into slices. The slices are fed into a Hobart Chopping End.

### Liver

Each semi-frozen sample is cut into slices. The slices are blended into a puree using a stainless steel blender. The puree is transferred to a 32-oz glass bottle with a Teflon-lined cap.

### Kidney

Each semi-frozen sample is cut into quarters. The quarters are chopped in a Hobart Food Cutter.

### Body Fat

Each semi-frozen sample is cut into small pieces. The small pieces are fed into a Hobart Chopping End.

NOTE: Alternatively, the above matrices may be chopped with dry ice. The dry ice is allowed to sublime before proceeding with sample extraction.

### Whole Milk, Butterfat, Skim Milk

Each sample is thawed and shaken thoroughly.

## EXTRACTION OF RESIDUE - MUSCLE, LIVER, KIDNEY AND FAT

A 10 g chopped/ground subsample is weighed into a blender cup. A 100-mL portion of the extraction solvent is added. (Concurrent recovery samples

are fortified at this step.) For liver samples, the extraction solvent is put into the blender cup before the subsample is added. The sample and solvent are blended for approximately two minutes.

The sample and solvent are quantitatively transferred using an acetone rinse to a Büchner funnel covered with Whatman No. 4 filter paper. The filtrate is poured through the funnel a second time, and the filter cake is rinsed with additional acetone. The filtrate is brought to an appropriate recorded volume (typically 200 mL) using acetone. The total extract is thoroughly mixed. A measured and recorded portion of the filtrate equivalent to approximately 5 g of the sample is transferred to a 250-mL beaker. The filtrate is evaporated overnight in an operating fume hood to near dryness by placing the beaker in the heated water bath with a stream of dry air or nitrogen impinging on the solvent surface. CAUTION: Failure to remove all traces of acetone may result in poor recovery of SDS-3701.

EXTRACTION OF RESIDUE - WHOLE MILK, BUTTERFAT, SKIM MILK

A 10-g subsample of whole milk, butterfat or skim milk is weighed into an 8-ounce bottle. A 100-mL portion of the extraction solvent (10 mL per gram of sample) is added. (Concurrent recovery samples are fortified at this step.) The bottle is sealed. The sample is shaken on a reciprocating platform shaker (high speed) for approximately 2 hours.

The sample is quantitatively transferred using an acetone rinse to a Büchner funnel covered with Whatman No. 4 filter paper. The filtrate is poured through the funnel a second time, and the filter cake is rinsed with additional acetone. An alternate procedure is to centrifuge the sample and decant the supernatant liquid. The total extract is thoroughly mixed, and the volume recorded (typically 200 mL). A measured and recorded portion of the filtrate equivalent to 5 g of the sample is transferred to a 250-mL beaker. The filtrate is evaporated free of acetone by allowing it to stand overnight in an operating fume hood. If traces of acetone remain, the beaker is placed in a heated water bath with a stream of dry air or nitrogen impinging on the solvent surface.

The filtrate is evaporated to near dryness. CAUTION: Failure to remove all traces of acetone may result in poor recovery of SDS-3701.

PARTITIONING

After the acetone has evaporated, an 80-mL[1] portion of 0.4M $NaHCO_3$ is slowly added to the beaker to avoid the sample bubbling over. The pH of the contents of the beaker is adjusted to 4.5 ± 0.3[2] with the aid of a pH meter while stirring on a magnetic stirrer using either 0.4M $NaHCO_3$, 4N NaOH or 10N $H_2SO_4$. The aqueous solution at pH 4.5[2] is quantitatively transferred to a 250-mL separatory funnel using 50 mL petroleum ether. The separatory funnel is vigorously shaken manually for approximately two minutes; the phases are allowed to separate. The lower (aqueous) phase is drained into the beaker and the ether phase discarded.

The aqueous phase is quantitatively transferred to the separatory funnel using an additional 50-mL portion of petroleum ether. The partitioning is repeated as previously described, and the ether phase discarded.

The pH of the aqueous phase is adjusted to less than 2 by the addition of 10 mL of 10N $H_2SO_4$. Sufficient NaCl is added to the beaker containing the aqueous solution to obtain a 30% solution (w/v); this is mixed thoroughly using a magnetic stirrer until the NaCl is dissolved. The resulting solution is quantitatively transferred to the separatory funnel using approximately 10 mL water and 50 mL diethyl ether. The separatory funnel is vigorously shaken manually for two minutes, the phases are allowed to separate and the lower (aqueous) phase is drained into the beaker. The diethyl ether phase is poured from the top of the separatory funnel into a 4-oz glass bottle which has been rinsed with acid water (pH <2), deionized water, acetone and allowed to dry before use. CAUTION: Care must be taken so that no water is transferred with the diethyl ether. The partitioning is repeated. Both ether extracts are combined and concentrated to dryness using a water bath.

[1] 120 mL for body fat.
[2] 7.0 ± 0.3 for body fat.

The SDS-3701 residue is converted to methyl SDS-3701 using the derivatization procedure described below.

DERIVATIZATION OF SDS-3701 WITH DIAZOMETHANE

CAUTION: EXTREME CARE IS TAKEN IN HANDLING THE REAGENTS DURING ALL OPERATIONS. THE PRECURSOR IS A CANCER-SUSPECT AGENT, EXTREMELY TOXIC AND POTENTIALLY EXPLOSIVE. THE REACTION PRODUCTS ARE ALSO POTENTIALLY CARCINOGENIC AND EXTREMELY TOXIC. A FULL COMPLEMENT OF PERSONAL PROTECTIVE EQUIPMENT (LAB COAT, SAFETY GLASSES AND GLOVES) IS WORN BY LAB PERSONNEL HANDLING THE REAGENTS AND NO SCRATCHED, CHIPPED OR GLASS-STOPPERED GLASSWARE IS USED. ALL WORK IS PERFORMED IN A FUME HOOD BEHIND A SAFETY SHIELD.

Preparation of Diazomethane Solution

The diazomethane solution is prepared just prior to use with the Diazomethane Generator. A minimum 0.8-g portion of the precursor (N-methyl-N'-nitro-N-nitrosoguanidine) is placed in the inside tube through its screw cap opening along with 0.5 mL of water. Diethyl ether (approximately 20 mL) is placed in the outside tube and the two parts are assembled with a pinch-type clamp. The lower part is immersed in an ice bath and approximately 2.5 mL of 4N NaOH is injected through the silicone rubber septum via a syringe. The addition is done dropwise to prevent the mixture from getting too hot and to control the volume of gas produced. The diazomethane collected in the ether is ready for use after all evidence of gas production has ceased (pale yellow color). Any unused reagent is neutralized/destroyed by adding excess glacial acetic acid.

NOTE: Only diazomethane solution prepared immediately prior to derivatization should be used. Other diazomethane precursors and preparation methods should not be used unless comparable results can be demonstrated.

Derivatization

The SDS-3701 is converted to its methyl ether derivative by the addition of 10 µL of 1:3 concentrated HCl:methanol (v/v) and 4 mL of the diazomethane solution to the dried residue remaining in the bottle after evaporation. The reaction mixture is allowed to set in an operating fume hood at ambient temperature for 30 minutes, after which time the solvent is evaporated in the heated water bath using a gentle stream of dry air or nitrogen. If necessary to ensure complete derivatization, an additional 10 µL of 1:3 concentrated HCl:methanol (v/v) and a 4-mL portion of the diazomethane solution is added after the first evaporation, and the mixture is allowed to set for an additional 10 minutes. The solvent is evaporated as described previously. **CAUTION:** The solution should be evaporated just to dryness. Continued use of the dry air or nitrogen stream after the solvent has evaporated can result in low recovery of methylated SDS-3701.

For preparation of methyl SDS-3701 standard solutions, 100 mL toluene is added to the bottle. Appropriate dilutions in toluene are prepared. For samples, the residue after derivatization is dissolved using 5 mL of methylene chloride and reserved for optional cleanup by column chromatography prior to quantitation by gas chromatography. Any unused reagent is destroyed by adding excess glacial acetic acid.

PREPARATION FOR DIRECT QUANTITATION

If no interfering chromatographic peaks are expected, residues of methyl SDS-3701 may be quantitated without cleanup. A 2 mL aliquot of the methylene chloride extract is pipetted into a bottle, 0.4 mL of "Keeper" solution is added, and the sample is concentrated just to dryness on a heated water bath. The residue is dissolved in 2 mL toluene for analysis by gas chromatography (see **QUANTITATION**). Dilutions are made as needed.

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6442-95-0075-MD-002
Report/SDS-3701

## SAMPLE CLEANUP OF SDS-3701

NOTE: The elution pattern of each batch of alumina is verified using a standard prior to analysis of samples. Modification of the eluant may be necessary to obtain satisfactory recovery and resolution from chromatographic peak interferences.

A glass chromatographic column, 200 mm x 9 mm, is packed bottom to top with a 0.5 cm bed of glass wool, 3 g of the activated alumina (taken directly from the oven and allowed to cool in column) and approximately 1 cm anhydrous sodium sulfate. The column is gently vibrated to settle the packing. The column is washed with approximately 10 mL of methylene chloride. After the methylene chloride has entered the column, a 2-oz glass bottle or 250 mL round-bottom flask is placed under the column. Two mL of the sample (2 gram equivalent) in methylene chloride is transferred to the column using a pipet. After the sample has moved into the column bed, derivatized SDS-3701 is eluted from the column with 40 mL methylene chloride.

## PREPARATION FOR QUANTITATION (AFTER SAMPLE CLEANUP)

A 0.4-mL portion of "Keeper" solution is added to the eluant. The eluant is concentrated just to dryness using a water bath with a stream of dry air or nitrogen or under reduced pressure. The residue is dissolved in 2 mL toluene for quantitation of methyl SDS-3701 by gas chromatography. Dilutions are made as needed.

## QUANTITATION

Portions of the sample extract (approximately 1 mL) are sealed in separate vials designed for use with the autosampler. This includes all samples, the calibration standard, and other standards in the concentration range of interest.

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6442-95-0075-MD-002
Report/SDS-3701

The analysis weight and volume, concentration(s) of the calibration standard(s) and retention time of the compound of interest are entered into the data system. Using these parameters, the instrument reduces the data to ppm values (µg of SDS-3701 per gram of sample) by external standard calibration using the following calculations:

$$\text{Calibration Factor} = \frac{\text{Standard Concentration } (\mu g/mL) \times 10{,}000^{1}}{\text{Standard Peak Area (or Peak Height)}}$$

$$\text{Analysis Weight} = \frac{\text{Sample Weight} \times \text{Aliquot} \times \text{Dilution to Clean Up}}{\text{Extraction Volume}}$$

$$\text{ppm residue} = \frac{\text{Calibration Factor} \times \text{Vol. for GC} \times \text{Sample Peak Area (or Peak Height)}}{\text{Analysis Weight} \times 10{,}000^{1}}$$

A series of varying concentration standards are prepared to establish a linear operating range. Typical standard concentrations might include 0.002 µg/mL, 0.005 µg/mL, 0.01 µg/mL, 0.03 µg/mL and 0.1 µg/mL. Alternatively, residues may also be calculated by a multipoint standard curve.

LIMIT OF QUANTITATION/LIMIT OF DETECTION

The limit of quantitation is equal to the lowest fortification level of 0.01 ppm. The limit of detection is established by the lowest concentration gas chromatography standard analyzed (at least 0.005 µg/mL), which, following method parameters, is equivalent to 0.005 ppm.

[1]The factor of 10,000 is used by a Varian data system.

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6442-95-0075-MD-002
Report/SDS-3701

As this method is written, a 0.01 ppm SDS-3701 residue level in the sample is equivalent to a 0.01 µg/mL SDS-3701 standard:

$$\text{ppm SDS-3701 residue} = \frac{0.01\ \mu g/mL \times 2\ mL\ (\text{final Volume})}{2\ \text{grams (analysis weight)}} = 0.01\ \text{ppm SDS-3701}$$

**RESULTS**

The results of the analysis of fortified milk and meat samples from a previous study (Ricerca, Inc. Document Number 6007-94-0120-CR-003) are shown below. These results demonstrate the analytical method for the determination of SDS-3701 in milk and animal tissues is valid. APPENDIX I contains representative chromatograms.

SUMMARY OF CONCURRENT RECOVERIES

| Matrix | Fortified Range (ppm) | No. of Samples Analyzed | % Recovery | | |
|---|---|---|---|---|---|
| | | | Range | Mean | Std Deviation |
| Milk | 0.01 - 1.00 | 116 | 70 - 120 | 99 | 9.6 |
| Skim Milk | 0.01 - 0.80 | 16 | 86 - 112 | 97 | 6.9 |
| Butterfat | 0.01 - 0.50 | 16 | 84 - 110 | 97 | 6.9 |
| Omental Fat | 0.01 - 0.50 | 3 | 82 - 100 | 91 | 9.0 |
| Perirenal Fat | 0.01 - 0.50 | 4 | 71 - 96 | 83 | 10.4 |
| Loin Muscle | 0.01 - 0.20 | 4 | 78 - 103 | 89 | 12.0 |
| Round Muscle | 0.01 - 0.50 | 4 | 90 - 110 | 102 | 8.7 |
| Liver | 0.01 - 0.50 | 4 | 76 - 106 | 94 | 14.2 |
| Kidney | 0.01 - 5.00 | 4 | 94 - 120 | 101 | 13.0 |
| All matrices | 0.01 - 5.00 | 171 | 70 - 120 | 96 | 15.0 |

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6442-95-0075-MD-002
Report/SDS-3701

# APPENDIX I

## REPRESENTATIVE CHROMATOGRAMS

Pages I-1 through I-14

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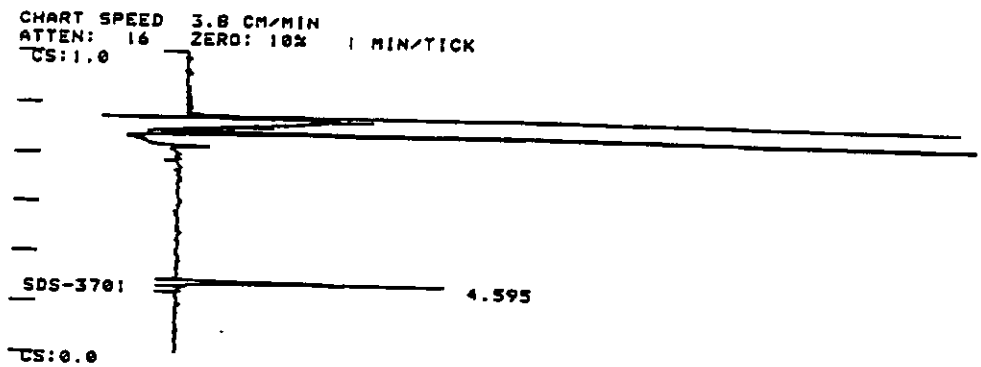
6442-95-0075-MD-002
Report/SDS-3701

I-1

FIGURE I-1

Calibration Standard, 0.01 µg/mL SDS-3701




TITLE: SDS-3701 IN COW MILK 14:22 15 JUL 94

CHANNEL NO: 1 SAMPLE: 0.01 UG/ML METHOD: 3701M-CAPU

| PEAK NO | PEAK NAME | RESULT FACTOR | TIME (MIN) | TIME OFFSET | HEIGHT COUNTS | SEP CODE | W1/2 (SEC) |
|---|---|---|---|---|---|---|---|
| 1 | SDS-3701 | 0.020004U | 4.595 | -0.005 | 4999 | BB | 2.60 |
| TOTALS: | | | | -0.005 | 4999 | | |

UNIDENT AREA: 0

DETECTED PKS: 1 REJECTED PKS: 0

AMT STD: 1.00000

NOISE: 72.8 OFFSET: -29

RACK: 1 VIAL: 3 INJ: 1

SAVED FILE: MILK001

NOTES:
SDS-3701 IN COW MILK: 6007-94-0120
6007- PRECEDES ALL SAMPLE I.D.'S IN SEC.=9
DB-608 CAPILLARY COLUMN: 30 M, 0.32 MM ID
COL TEMP = 225°C; INJECTION VOL = 1 UL
HE FLOWS: COL=1.5, SPLIT=42 ML/MIN (29:1)
MAKEUP FLOW = 28.5 ML/MIN (NITROGEN)
GC: HP-237A; COLUMN SERIAL NO.: 1279722
ANALYSTS: R. S. DVORAK, P. C. HAYES JR.

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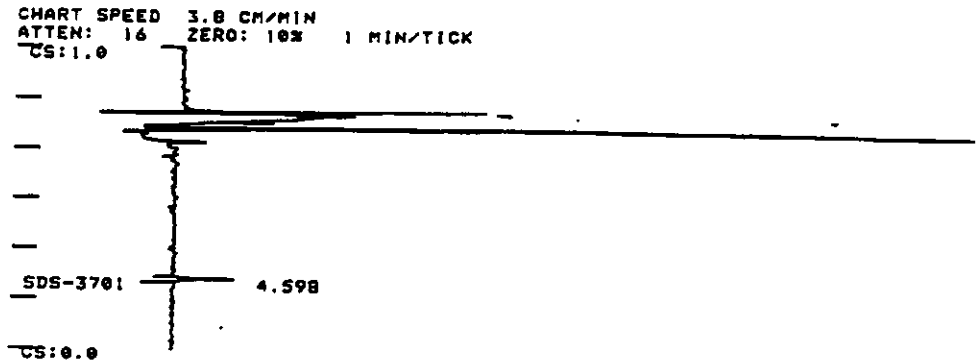
6442-95-0075-MD-002
Report/SDS-3701

I-2

**FIGURE I-2**

Analytical Standard, 0.002 µg/mL SDS-3701




TITLE: SDS-3701 IN COW MILK 14:30 15 JUL 94

CHANNEL NO: 1 SAMPLE: 0.002 UG/ML METHOD: 3701M-CAPU

| PEAK NO | PEAK NAME | RESULT PPM | TIME (MIN) | TIME OFFSET | HEIGHT COUNTS | SEP CODE | W1/2 (SEC) |
|---|---|---|---|---|---|---|---|
| 1 | SDS-3701 | 0.002 | 4.598 | -0.002 | 1176 | BB | 2.35 |
| TOTALS: | | 0.002 | | -0.002 | 1176 | | |

UNIDENT AREA: 0

DETECTED PKS: 1 REJECTED PKS: 0

DIVISOR: 1.00000 MULTIPLIER: 1.00000

NOISE: 72.8 OFFSET: -24

RACK: 1 VIAL: 5 INJ: 1

SAVED FILE: MILK002

NOTES:
SDS-3701 IN COW MILK: 6007-94-0120
6007- PRECEDES ALL SAMPLE I.D.'S IN SEC.29
DB-608 CAPILLARY COLUMN: 30 M, 0.32 MM ID
COL TEMP = 225°C; INJECTION VOL = 1 UL
HE FLOWS: COL=1.5, SPLIT=42 ML/MIN (29:1)
MAKEUP FLOW = 28.5 ML/MIN (NITROGEN)
GC: HQ-237A; COLUMN SERIAL NO.: 1279722
ANALYSTS: R. S. DVORAK, P. C. HAYES JR.

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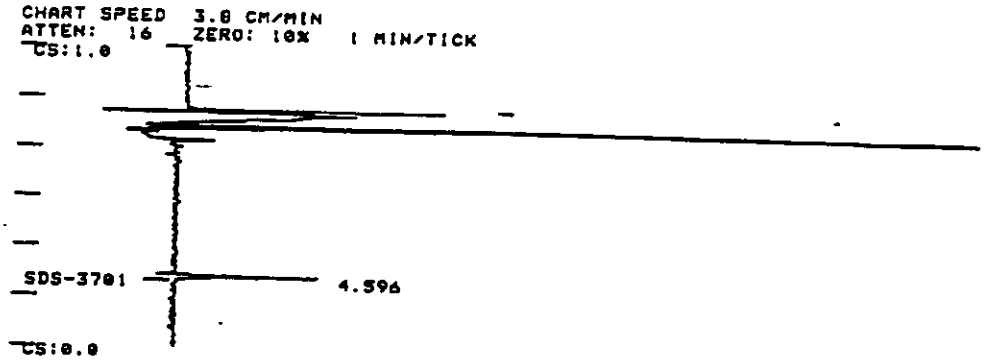
6442-95-0075-MD-002
Report/SDS-3701

I-3

**FIGURE I-3**

Analytical Standard, 0.005 µg/mL SDS-3701




TITLE: SDS-3701 IN COW MILK 14:39 15 JUL 94

CHANNEL NO: 1 SAMPLE: 0.005 UG/ML METHOD: 3701M-CAPU

| PEAK NO | PEAK NAME | RESULT PPM | TIME (MIN) | TIME OFFSET | HEIGHT COUNTS | SEP CODE | W1/2 (SEC) |
|---|---|---|---|---|---|---|---|
| 1 | SDS-3701 | 0.005 | 4.596 | -0.004 | 2671 | BB | 2.45 |
| TOTALS: | | 0.005 | | -0.004 | 2671 | | |

UNIDENT AREA: 0

DETECTED PKS: 1 REJECTED PKS: 0

DIVISOR: 1.00000 MULTIPLIER: 1.00000

NOISE: 72.8 OFFSET: -44

RACK: 1 VIAL: 7 INJ: 1

SAVED FILE: MILK003

NOTES:
SDS-3701 IN COW MILK: 6007-94-0120
6007- PRECEDES ALL SAMPLE I.D.'S IN SEC.#9
DB-608 CAPILLARY COLUMN: 30 M, 0.32 MM ID
COL TEMP = 225°C; INJECTION VOL = 1 UL
HE FLOWS: COL=1.5, SPLIT=42 ML/MIN (29:1)
MAKEUP FLOW = 28.5 ML/MIN (NITROGEN)
GC: HO-237A1 COLUMN SERIAL NO.: 1279722
ANALYSTS: R. S. DVORAK, P. C. HAYES JR.

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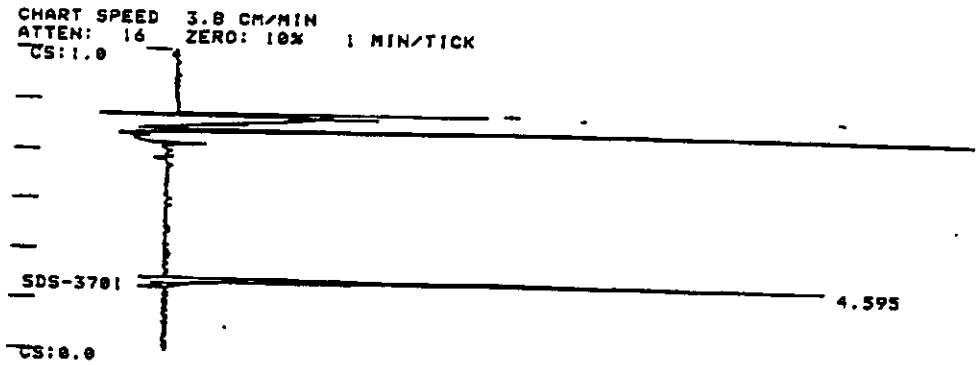
Report/SDS-3701

I-4

FIGURE I-4

Analytical Standard, 0.03 µg/mL SDS-3701




TITLE: SDS-3701 IN COW MILK 14:55 15 JUL 94

CHANNEL NO: 1 SAMPLE: 0.03 UG/ML METHOD: 3701M-CAPU

| PEAK NO | PEAK NAME | RESULT PPM | TIME (MIN) | TIME OFFSET | HEIGHT COUNTS | SEP CODE | W1/2 (SEC) |
|---|---|---|---|---|---|---|---|
| 1 | SDS-3701 | 0.024 | 4.595 | -0.005 | 12095 | BV | 2.75 |
| TOTALS: | | 0.024 | | -0.005 | 12095 | | |

UNIDENT AREA: 350

DETECTED PKS: 2 REJECTED PKS: 0

DIVISOR: 1.00000 MULTIPLIER: 1.00000

NOISE: 72.8 OFFSET: -69

RACK: 1 VIAL: 11 INJ: 1

SAVED FILE: MILK005

NOTES:
SDS-3701 IN COW MILK: 6007-94-0120
6007- PRECEDES ALL SAMPLE I.D.'S IN SEC.#9
DB-608 CAPILLARY COLUMN: 30 M, 0.32 MM ID
COL TEMP = 225°C; INJECTION VOL = 1 UL
HE FLOWS: COL=1.5, SPLIT=42 ML/MIN (29:1)
MAKEUP FLOW = 28.5 ML/MIN (NITROGEN)
GC: HO-237A; COLUMN SERIAL NO.: 1279722
ANALYSTS: R. S. DVORAK, P. C. HAYES JR.

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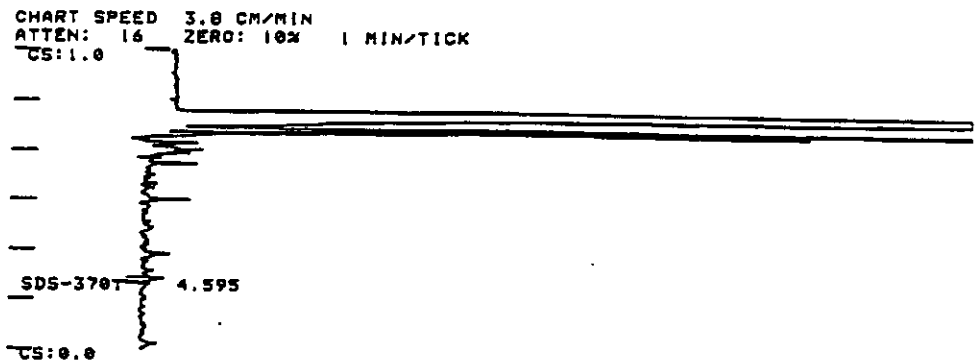
Report/SDS-3701

I-5

**FIGURE I-5**

Milk, Control Sample




TITLE: SDS-3701 IN COW MILK 15:03 15 JUL 94

CHANNEL NO: 1 SAMPLE: MK-02-01 METHOD: 3701M-CAPU

| PEAK NO | PEAK NAME | RESULT PPM | TIME (MIN) | TIME OFFSET | HEIGHT COUNTS | SEP CODE | W1/2 (SEC) |
|---|---|---|---|---|---|---|---|
| 1 | SDS-3701 | 0.001 | 4.595 | -0.005 | 369 | BV | 2.40 |
| TOTALS: | | 0.001 | | -0.005 | 369 | | |

UNIDENT AREA: 0

DETECTED PKS: 2 REJECTED PKS: 1

DIVISOR: 2.00000 MULTIPLIER: 2.00000

NOISE: 72.8 OFFSET: -59

RACK: 1 VIAL: 13 INJ: 1

SAVED FILE: MILK006

NOTES:
SDS-3701 IN COW MILK: 6007-94-0120
6007- PRECEDES ALL SAMPLE I.D.'S IN SEC.#9
DB-608 CAPILLARY COLUMN: 30 M, 0.32 MM ID
COL TEMP = 225°C; INJECTION VOL = 1 UL
HE FLOWS: COL=1.5, SPLIT=42 ML/MIN (29:1)
MAKEUP FLOW = 28.5 ML/MIN (NITROGEN)
GC: HO-237A; COLUMN SERIAL NO.: 1279722
ANALYSTS: R. S. DVORAK, P. C. HAYES JR.

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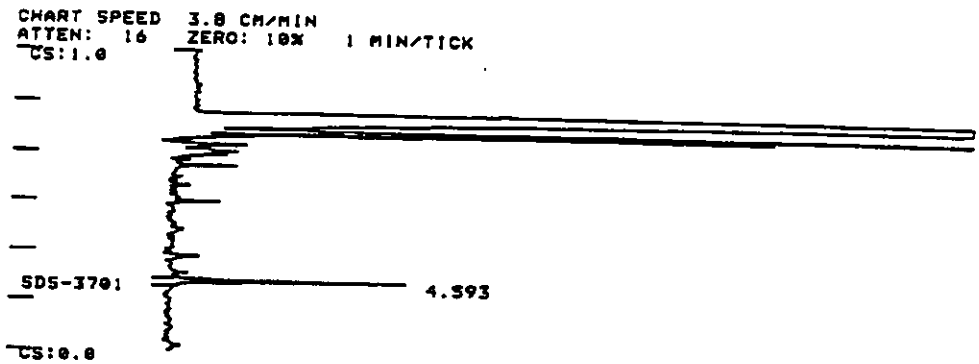
Report/SDS-3701

I-6

FIGURE I-6

Milk, 0.01 ppm SDS-3701 Fortified Control Sample, 80% Recovery




TITLE: SDS-3701 IN COW MILK 15:15 15 JUL 94

CHANNEL NO: 1 SAMPLE: MK-02F-01 METHOD: 3701M-CAPU

| PEAK NO | PEAK NAME | RESULT PPM | TIME (MIN) | TIME OFFSET | HEIGHT COUNTS | SEP CODE | W1/2 (SEC) |
|---|---|---|---|---|---|---|---|
| 1 | SDS-3701 | 0.008 | 4.593 | -0.007 | 4246 | BV | 2.50 |
| TOTALS: | | 0.008 | | -0.007 | 4246 | | |

UNIDENT AREA: 0

DETECTED PKS: 2 REJECTED PKS: 1

DIVISOR: 2.00000 MULTIPLIER: 2.00000

NOISE: 72.8 OFFSET: 67

RACK: 1 VIAL: 1 INJ: 1

SAVED FILE: MILK007

NOTES:
SDS-3701 IN COW MILK: 6007-94-0120
6007- PRECEDES ALL SAMPLE I.D.'S IN SEC.89
DB-608 CAPILLARY COLUMN: 30 M, 0.32 MM ID
COL TEMP = 225°C; INJECTION VOL = 1 UL
HE FLOWS: COL=1.5, SPLIT=42 ML/MIN (29:1)
MAKEUP FLOW = 28.5 ML/MIN (NITROGEN)
GC: HP-237A; COLUMN SERIAL NO.: 1279722
ANALYSTS: R. S. DVORAK, P. C. HAYES JR.

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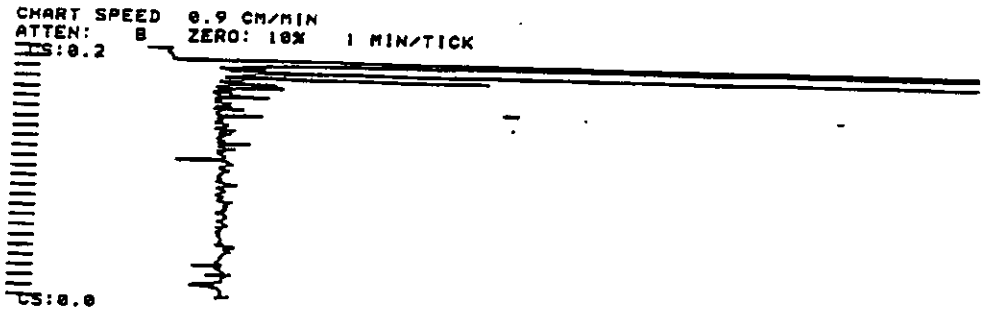
Report/SDS-3701

I-7

**FIGURE I-7**

**Loin Muscle, Control Sample**




TITLE: SDS-3701 ON LOIN MUSCLE 18:57 5 OCT 94

CHANNEL NO: 1 SAMPLE: LM-01-28 METHOD: 3701LM170-CA

| PEAK NO | PEAK NAME | RESULT PPM | TIME (MIN) | TIME OFFSET | AREA COUNTS | SEP CODE | W1/2 (SEC) |
|---|---|---|---|---|---|---|---|
| TOTALS: | | 0.000 | | | 0 | | |

UNIDENT AREA: 0

DETECTED PKS: 0 REJECTED PKS: 0

DIVISOR: 2.00000 MULTIPLIER: 2.00000

NOISE: 7.3 OFFSET: -67

RACK: 1 VIAL: 4 INJ: 1

SAVED FILE: LM008

ERRORS:
NO PEAKS

NOTES:
PROJECT 6007-94-0120
6007- PRECEDES ALL MUSCLE SAMPLE ID'S
COL FLOW = 1.5 ML/MIN. HELIUM
TCOL:170°C(25MIN)->240°C@50°C/MIN,HOLD5MIN
MAKEUP FLOW = 28.5 ML/MIN. NITROGEN
COLUMN: DB-608, 30M, 0.32 MM ID, 1 UL INJ
GC: HP-237A: COLUMN SERIAL NO.: 1279722
ANALYSTS: R.S. DVORAK, P.C. HAYES JR.

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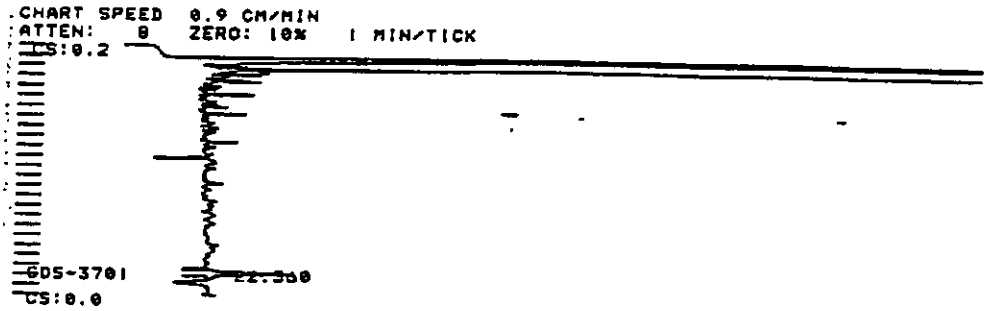
6442-95-0075-MD-002
Report/SDS-3701

I-8

FIGURE I-8

Loin Muscle, 0.01 ppm SDS-3701 Fortified Control Sample, 80% Recovery




TITLE: SDS-3701 ON LOIN MUSCLE 20:10 5 OCT 94

CHANNEL NO: 1 SAMPLE: LM-01F-2B METHOD: 3701LM170-CA

| PEAK NO | PEAK NAME | RESULT PPM | TIME (MIN) | TIME OFFSET | AREA COUNTS | SEP CODE | W1/2 (SEC) |
|---|---|---|---|---|---|---|---|
| 1 | SDS-3701 | 0.008 | 22.698 | -0.012 | 10779 | VB | 13.80 |
| TOTALS: | | 0.008 | | -0.012 | 10779 | | |

UNIDENT AREA: 0

DETECTED PKS: 2 REJECTED PKS: 1

DIVISOR: 2.00000 MULTIPLIER: 2.00000

NOISE: 7.3 OFFSET: -113

RACK: 1 VIAL: 8 INJ: 1

SAVED FILE: LM010

NOTES:
PROJECT 6007-94-0120
6007- PRECEDES ALL MUSCLE SAMPLE ID'S
COL FLOW = 1.5 ML/MIN, HELIUM
TCOL:170°C(25MIN)->260°C@50°C/MIN,HOLD5MIN
MAKEUP FLOW = 28.5 ML/MIN, NITROGEN
COLUMN: DB-608, 30M, 0.32 MM ID, 1 UL INJ
GC: HG-237A: COLUMN SERIAL NO.: 1279722
ANALYSTS: R.S. DVORAK, P.C. HAYES JR.

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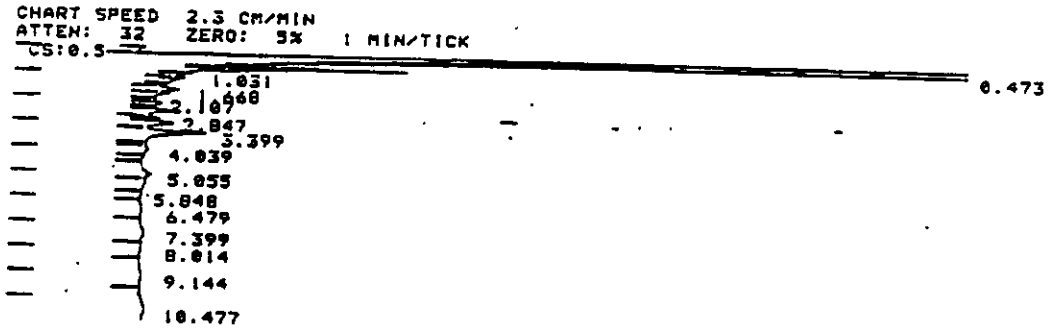
Report/SDS-3701

I-9

**FIGURE I-9**

**Omental Fat, Control Sample**




TITLE: SDS-3701 RESIDUES 12:52 19 AUG 94

CHANNEL NO: 1 SAMPLE: OF-01-28 METHOD: SDS-3701

| PEAK NO | PEAK NAME | RESULT PPM | TIME (MIN) | AREA COUNTS | SEP CODE |
|---|---|---|---|---|---|
| TOTALS: | | 0.000 | | 0 | |

UNIDENT AREA: 629344

DIVISOR: 2.00000 MULTIPLIER: 2.00000

RACK: 2 VIAL: 8 INJ: 1

NOTES:
ANALYSTS: KING, PRINCE AND QUINN
3% OV-7, ZUL INJ., HQ-235A
PROJECT: 6007-94-0120
6007 PRECEDES THE SAMPLE ID IN SECTION 9.

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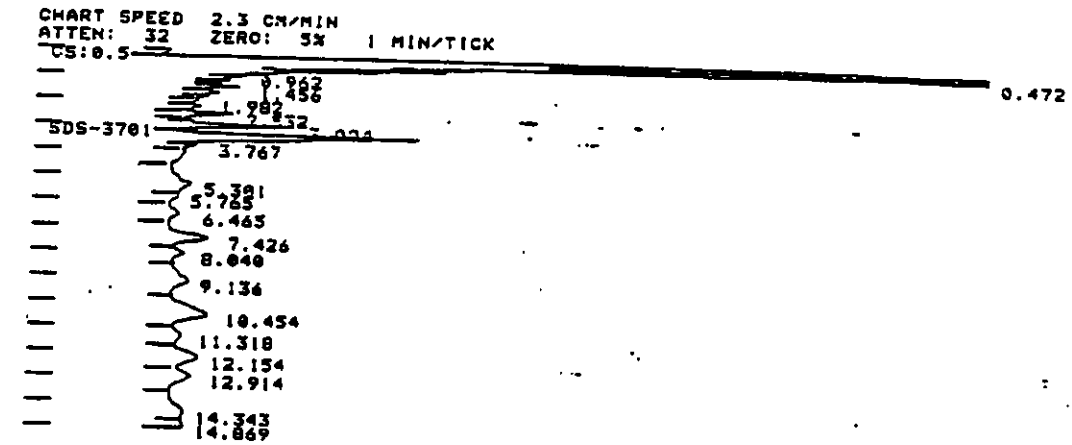
Report/SDS-3701

I-10

FIGURE I-10

Omental Fat, 0.01 ppm SDS-3701 Fortified Control Sample, 100% Recovery




TITLE: SDS-3701 RESIDUES 13:18 19 AUG 94

CHANNEL NO: 1 SAMPLE: OF-01F-28 METHOD: SDS-3701

| PEAK NO | PEAK NAME | RESULT PPM | TIME (MIN) | AREA COUNTS | SEP CODE |
|---|---|---|---|---|---|
| 13 | SDS-3701 | 0.010 | 3.004 | 28118 | BB |
| TOTALS: | | 0.010 | | 28118 | |

UNIDENT AREA: 1329280

DIVISOR: 2.00000 MULTIPLIER: 2.00000

RACK: 2 VIAL: 10 INJ: 1

NOTES:
ANALYSTS: KING, PRINCE AND QUINN
3% OV-7, 2UL INJ., HQ-235A
PROJECT: 6007-94-0120
6007 PRECEDES THE SAMPLE ID IN SECTION 9.

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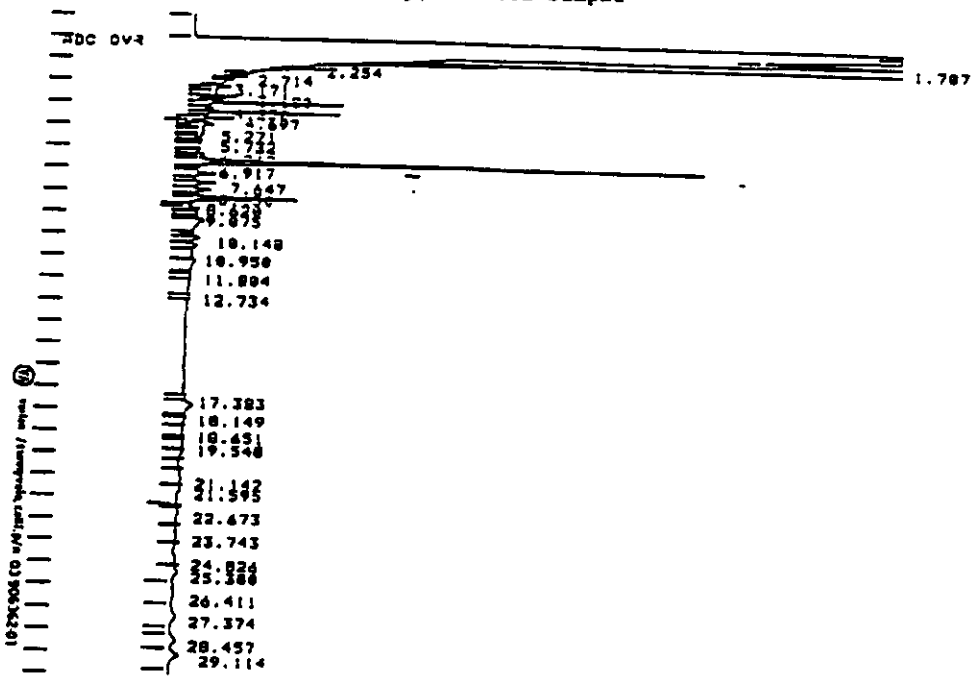
Report/SDS-3701

I-11

FIGURE I-11

Kidney, Control Sample




TITLE: SDS-3701 RESIDUES

CHANNEL NO: 1 SAMPLE: KD-01-28 18:26 1 SEP 94 METHOD: SDS-3701

| PEAK NO | PEAK NAME | RESULT PPM | TIME (MIN) | TIME OFFSET | AREA COUNTS | SEP CODE | W1/2 (SEC) |
|---|---|---|---|---|---|---|---|
| 49 | SDS-3701 | 0.005 | 9.339 | 0.019 | 12991 | VB | 9.70 |
| TOTALS: | | 0.005 | | 0.019 | 12991 | | |

UNIDENT AREA: 0481060

DETECTED PKS: 77 REJECTED PKS: 7

DIVISOR: 2.00000 MULTIPLIER: 2.00000

NOISE: 18.2 OFFSET: -294

RACK: 1 VIAL: 7 INJ: 1

ERRORS:
ADC OVERRANGE

NOTES:
ANALYSTS: KING, PRINCE AND QUINN
DB-608 (30M X 0.32MM I.D. 0.5 FILM THICK)
INSTRUMENT: HP-235C, 1.5UL INJ. VOL.
PROJECT: 6007-94-0128
6037 PRECEDES THE SAMPLE ID IN SECTION 9

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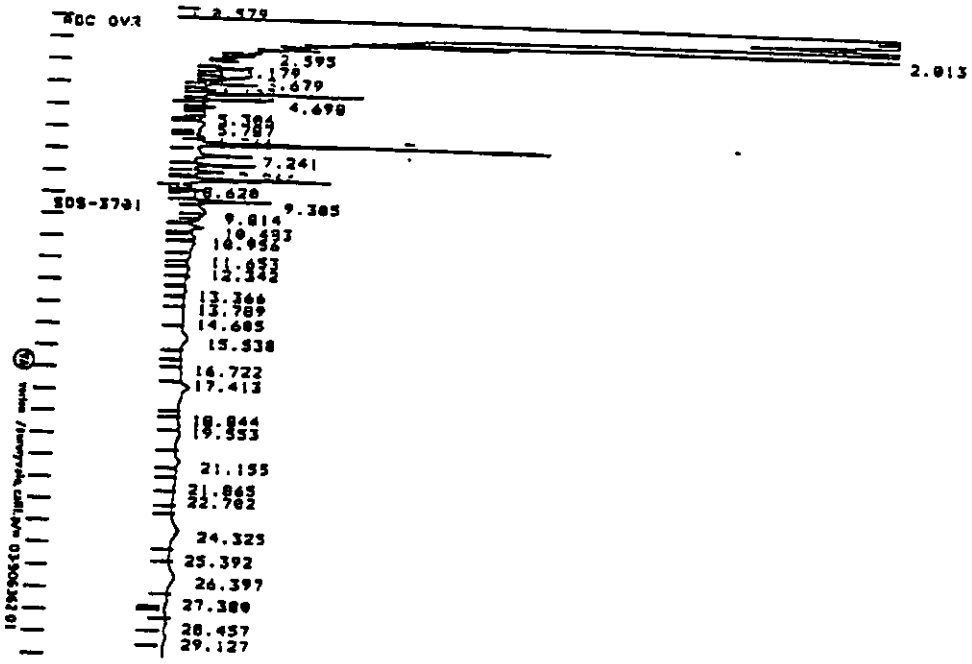
Report/SDS-3701

I-12

FIGURE I-12

Kidney, 0.01 ppm SDS-3701 Fortified Control Sample, 120% Recovery




TITLE: SDS-3701 RESIDUES

CHANNEL NO: 1 SAMPLE: KD-01F-2B 19:31 1 SEP 94 METHOD: SDS-3701

| PEAK NO | PEAK NAME | RESULT PPM | TIME (MIN) | TIME OFFSET | AREA COUNTS | SEP CODE | W1/2 (SEC) |
|---|---|---|---|---|---|---|---|
| 45 | SDS-3701 | 0.012 | 9.385 | -0.015 | 34638 | VV | 5.10 |
| TOTALS: | | 0.012 | | -0.015 | 34638 | | |

UNIDENT AREA: 9022870

DETECTED PKS: 76 REJECTED PKS: 3

DIVISOR: 2.00000 MULTIPLIER: 2.00000

NOISE: 18.2 OFFSET: -172

RACK: 1 VIAL: 9 INJ: 1

ERRORS:
ADC OVERRANGE

NOTES:
ANALYSTS: KING, PRINCE AND QUINN
DB-608 (30M X 0.32MM I.D. 0.5 FILM THICK)
INSTRUMENT: HP-235C, 1.5UL INJ. VOL.
PROJECT: 6007-94-0120

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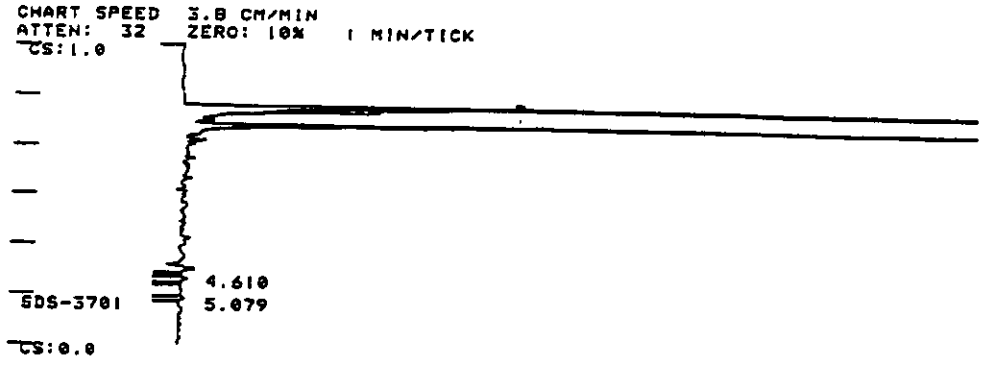
Report/SDS-3701

I-13

FIGURE I-13

Liver, Control Sample




TITLE: SDS-3701 IN COW LIVER 17:06 3 OCT 94

CHANNEL NO: 1 SAMPLE: 01-20 METHOD: 3701L-CAP

| PEAK NO | PEAK NAME | RESULT PPM | TIME (MIN) | TIME OFFSET | AREA COUNTS | SEP CODE | W1/2 (SEC) |
|---|---|---|---|---|---|---|---|
| 3 | SDS-3701 | 0.000 | 5.079 | -0.001 | 410 | BB | 2.50 |
| TOTALS: | | 0.000 | | -0.001 | 410 | | |

UNIDENT AREA: 1301

DETECTED PKS: 4 REJECTED PKS: 1

DIVISOR: 2.00000 MULTIPLIER: 2.00000

NOISE: 29.1 OFFSET: -40

RACK: 1 VIAL: 5 INJ: 1

SAVED FILE: L043

NOTES:
SDS-3701 IN COW LIVER: 6007-94-0120
6007-4 PRECEDES ALL SAMPLE I.D.'S IN SEC.59 [illegible] 10/5/94
DB-608 CAPILLARY: 30M, 0.32MM ID: 1 UL INJ
COL TEMP:220°C(6MIN)->260°C@50C°/MIN(5MIN)
HE FLOWS: COL=1.5, SPLIT=42 ML/MIN (29:1)
MAKEUP FLOW = 28.5 ML/MIN (N2)
GC: HP-237A: COLUMN SERIAL NO.: 1279722
ANALYSTS: R.S. DVORAK, P.C. HAYES JR.

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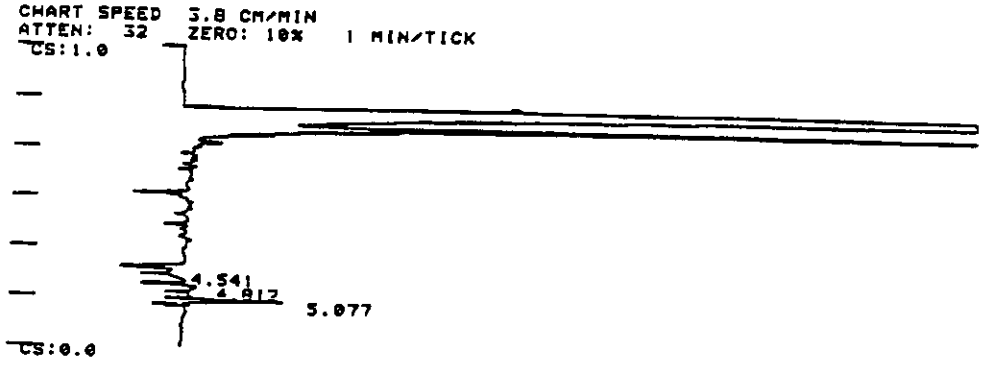
Report/SDS-3701

I-14

**FIGURE I-14**

Liver, 0.01 ppm SDS-3701 Fortified Control Sample, 90% Recovery




TITLE: SDS-3701 IN COW LIVER 17:38 3 OCT 94

CHANNEL NO: 1 SAMPLE: 01F-28 METHOD: 3701L-CAP

| PEAK NO | PEAK NAME | RESULT PPM | TIME (MIN) | TIME OFFSET | AREA COUNTS | SEP CODE | W1/2 (SEC) |
|---|---|---|---|---|---|---|---|
| 4 | SDS-3701 | 0.009 | 5.077 | -0.003 | 11487 | VV | 3.10 |
| TOTALS: | | 0.009 | | -0.003 | 11487 | | |

UNIDENT AREA: 15189

DETECTED PKS: 6 REJECTED PKS: 2

DIVISOR: 2.00000 MULTIPLIER: 2.00000

NOISE: 29.1 OFFSET: -36

RACK: 1 VIAL: 9 INJ: 1

SAVED FILE: L045

NOTES:
SDS-3701 IN COW LIVER: 6007-94-0120
6007-/PRECEDES ALL SAMPLE I.D.'S IN SEC.#9 [illegible] Green Pobbe 11/17/94
DB-608 CAPILLARY: 30M, 0.32MM ID: 1 UL INJ
COL TEMP:220°C(6MIN)->260°C@50C°/MIN(5MIN)
HE FLOWS: COL=1.5, SPLIT=42 ML/MIN (29:1)
MAKEUP FLOW = 28.5 ML/MIN (N2)
GC: HO-237A: COLUMN SERIAL NO.: 1279722
ANALYSTS: R.S. DVORAK, P.C. HAYES JR.