

**VALENT U.S.A. CORPORATION  
VALENT TECHNICAL CENTER  
DUBLIN, CALIFORNIA****DETERMINATION OF S-1283  
IN COTTONSEED  
METHOD RM-37****DATE: JUNE 6, 1997****INTRODUCTION**

This method determines residues of S-1283 [2-(2,6-difluorophenyl)-4-(2-ethoxy-4-tert-butylphenyl)-2-oxazoline] in cottonseed. This method is derived from the crop residue method developed by Sumitomo Chemical Industry Co., Ltd<sup>1</sup>.

Briefly, S-1283 residues are extracted from cottonseed using acetone, then partitioned with dichloromethane/water. The organic phase, containing the S-1283 residues, is evaporated and partitioned with acetonitrile/hexane to remove oils. The acetonitrile from this step is evaporated and the S-1283 is then quantified by gas chromatography using a nitrogen-phosphorus specific flame-ionization detector (NPD) following cleaned-up using a silica gel Sep-Pak. Confirmatory procedures using an alternate GC column or a mass selective detector are included in the method.

**REAGENTS**

Acetone - pesticide quality or equivalent.

Acetonitrile - pesticide quality or equivalent.

Dichloromethane - pesticide quality or equivalent.

Ethyl acetate - pesticide quality or equivalent.

Hexane - pesticide quality or equivalent.

Sodium chloride - reagent grade or equivalent.

Sodium sulfate - anhydrous, granular, AR grade or equivalent.

Water-deionized.

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### REAGENT SOLUTIONS

Ethyl acetate:hexane, 5% (v/v) - Combine 5 parts of ethyl acetate with 95 parts of hexane. For example, add 50 mL of ethyl acetate and 950 mL of hexane sequentially to a reagent bottle. Store at room temperature.

Hexane (saturated with acetonitrile)/Acetonitrile (saturated with hexane) - Combine equal volumes of hexane and acetonitrile in a separatory funnel and shake vigorously for 1 minute. Allow the phases to separate, then drain the acetonitrile (bottom layer) into a bottle or flask and stopper. Pour the hexane phase into another bottle or flask and stopper. Store each solvent at room temperature.

Sodium chloride:water- 5% (w/v) - add 50 grams of sodium chloride to 1 L of deionized water and shake until dissolved.

### REFERENCE STANDARDS

S-1283 - analytical standard of known purity. Prepare a stock solution containing 1 mg/mL in acetone. Prepare a fortifying solution containing 1.0 µg/mL by diluting the stock solution with acetone. Prepare a minimum of four linearity standards by diluting this stock solution with acetone to concentrations ranging from 0.05 to 1.0 µg/mL (See Note 1). Prepare a calibrating solution containing 1.0 µg/mL by diluting the stock solution with acetone. (The calibrating solution may be used as one of the four required linearity standards). All solutions should be kept refrigerated when not in use.

### EQUIPMENT

Baker SPE-12G Column Processor (12-port vacuum manifold) - J.T. Baker Product # 7018-00 or equivalent system.

Büchner funnels - 9 cm diameter.

Filter flasks - 500 mL.

Filter funnels - approximately 10 cm diameter.

Filter paper - Whatman GF/A glass fiber or equivalent, 9 cm diameter.

Gas Chromatograph - Hewlett-Packard Model 5890, equipped with a packed column glass insert for splitless injection (HP Part No. 5080-8732, packed with approximately 5 mm of silanized glass wool), an NP detector, automatic sampler, and HP ChemStation or equivalent system.

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**EQUIPMENT (CONTINUED)**

Glass wool - Pyrex® or equivalent.

Mason jars - 1 pint with plastic screw cap lids or equivalent.

Omni-Mixer - with adapter for use with 1-pint Mason jars.

Pasteur pipets - 5¼" and 9".

Rotary evaporator - Büchi or equivalent, equipped with a temperature controlled water bath.

Round-bottom flasks - 50 mL and 500 mL.

Separatory funnels - 500 mL.

Bakerbond SPE Silica Gel Cartridges - Cat # 7086-03. See Note 2.

Syringe - Yale hypodermic, 10 mL, glass Luer Tip.

Ultrasonic cleaner - Branson 3200 or equivalent.

Vials - 6 dram, with polyethylene lined screw caps or equivalent.

**ANALYTICAL PROCEDURES**

**1. Extraction**

Weigh 10 grams ( $\pm$  0.1 grams) of cottonseed into a one pint Mason jar. At this point, if required by the testing facility, control samples for method recovery should be fortified with S-1283 (See Note 3). Add 150 mL of acetone to the sample and blend on the Omni-Mixer for 10 minutes.

Filter the sample into a 500 mL filter flask using a Büchner funnel and Whatman GF/A glass fiber filter paper. Rinse the Mason jar with three 20 mL portions of acetone and add each portion to the Büchner funnel.

Transfer the combined filtrates to a 500 mL round-bottom flask. Rinse the filter flask with two 20 mL portions of acetone and add to the round-bottom flask. Evaporate the acetone using a rotary-evaporator and water bath set to  $<40^{\circ}\text{C}$ .

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## **2. Dichloromethane/Water Partitioning**

Add 40 mL of dichloromethane to the round-bottom flask and sonicate to dislodge any residues adhering to the walls of the flask. Transfer the extract to a 500 mL separatory funnel. Add another 40 mL of dichloromethane to the round-bottom flask, swirl, sonicate briefly, and add to the separatory funnel.

Add 150 mL of 5% (w/v) sodium chloride solution to the separatory funnel and shake vigorously for approximately one minute. Allow the phases to separate, then drain the lower dichloromethane layer into a clean 500 mL round-bottom flask through a filter funnel containing approximately 50 grams of sodium sulfate, suspended on a plug of glass wool.

Re-extract the aqueous phase with an additional 50 mL of dichloromethane. Drain the dichloromethane through the filter funnel into the 500 mL round-bottom flask containing the first extract. Rinse the sodium sulfate with two 10 mL portions of dichloromethane. Evaporate the combined dichloromethane phases to dryness using a rotary-evaporator and water bath set to <math><40^{\circ}\text{C}</math>.

## **3. Hexane/Acetonitrile Partitioning**

Transfer the sample extract to a 500 mL separatory funnel by rinsing the round-bottom flask first with 75 mL of hexane (saturated with acetonitrile) followed by 50 mL of acetonitrile (saturated with hexane). Shake for one minute and allow the phases to separate. Drain the lower, acetonitrile layer back into the 500 mL round-bottom flask. Re-extract the hexane layer with another 50 mL portion of acetonitrile (saturated with hexane) as before. Combine this acetonitrile with the first extract.

Evaporate the combined acetonitrile layers to approximately 10 mL and transfer to a 50 mL round-bottom flask using a Pasteur pipet. Rinse the 500 mL flask three times with 5 mL portions of acetonitrile and add each rinse to the 50 mL round-bottom flask. Evaporate the acetonitrile to dryness using a rotary-evaporator and water bath set to <math><40^{\circ}\text{C}</math>.

## **4. Sep-Pak Silica Gel Cartridge Cleanup (See Note 2)**

Attach a Sep-Pak silica gel cartridge to the Baker-SPE vacuum manifold. Attach a 10 mL glass syringe (plunger removed) to the column and pre-condition the column with 10 mL of 5% (v/v) ethyl acetate:hexane followed by 10 mL of hexane. Do not exceed a flow rate of 5 mL/minute. Do not allow the column to dry before the sample is applied. Remove the 10 mL glass syringe from the column.

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Add 5 mL of hexane to the flask containing the sample extract and sonicate the extract for 15 seconds. Using a Pasteur pipet, transfer the extract to the column, in portions, while applying gentle vacuum to the column. Do not allow the column to dry. Repeat this procedure with another 5 mL of hexane followed by 5 mL of 5% ethyl acetate:hexane. Rinse the sample flask with each solvent before transferring to the column. Discard these eluants.

Place a 6 dram vial under the column and elute the S-1283 from the column as follows: add 5 mL of 5% ethyl acetate:hexane to the original sample flask and sonicate for 15 seconds. Using a Pasteur pipet, transfer this rinse to the column, in portions, while applying gentle vacuum to the column until all the solvent has been eluted from the column.

Transfer the eluate to a 50 mL round-bottom flask using three 1 mL portions of acetone to rinse the vial, then evaporate to dryness using a rotary-evaporator and water bath set to <40°C. Redissolve the extract in 1.0 mL of acetone, sonicate for approximately 15 seconds, then transfer the extract to an autosampler vial and store at ≤0°C until GC analysis.

#### 5. Gas Chromatography Measurement

Analyze the sample, along with the calibrating standard solution, using the following operating conditions:

Column: DB-5 (30 M x 530 μm) wide bore capillary (1.5 μm film thickness, J&W Cat # 125-5032 or equivalent).

Column Oven Temperature: 250°C. Hold Time 10 minutes.

Detector Temperature: 300°C

Injector Temperature: 250°C

Carrier Gas: Helium at 10 mL/min

Make-Up Gas: Helium at 20 mL/min

Air: 102 mL/min

Hydrogen: 3.6 mL/min

Injection Size: 1.0 μl

The GC parameters shown above are given only as a guide. They may be modified as needed to optimize the chromatography or to resolve matrix interferences. Each set of chromatograms must be clearly labeled with the GC parameters used. See Note 4 for alternative GC parameters.

The recommended sequence of samples and standards for analysis is: calibrating standard, sample, sample, sample, calibrating standard, etc. (The calibrating standard vials contain 1.0 μg/mL of S-1283 in acetone). This sequence may, however, be modified if the reproducibility requirement is met. (See Note 5). Each sequence must begin and end with a calibration standard.

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### 6. Calculations

The amount of S-1283 in each sample is calculated using the following formula:

$$\text{ppm S-1283} = \frac{B \times C \times V \times DF}{A \times W}$$

where:

- B = integration counts for S-1283 in the sample.
- C = concentration of S-1283 in the calibrating standard (1.0 µg/mL).
- V = final volume of the sample extract (1.0 mL).
- DF = dilution factor, used if the sample extract is diluted prior to analysis.
- A = mean integration counts for S-1283 in the calibrating standards.
- W = sample weight (10 grams).

### LIMITS OF DETECTION AND QUANTITATION

The validated limit of detection (LOD) of S-1283 in cottonseed analyzed by this method is 0.005 ppm (5 ppb). The validated limit of quantitation (LOQ) is 0.01 ppm (10 ppb).

### ANALYSIS TIME

A trained analyst, familiar with this method, can complete the analysis of a set of eight samples for S-1283 in approximately 8 hours. The results are available within 24 hours of initiating the analysis.

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**NOTES**

1. At Valent, linearity of the gas chromatograph must be determined each day that samples are analyzed (Valent SOP #VR-007). Linearity is determined by analyzing a series of linearity standards containing 0.05 to 1.0 µg/mL of S-1283. The response for each standard is normalized to response per 1.0 µg/mL by dividing the response of each standard by its concentration. The coefficient of variation (CV) of these responses must be 10% or less. Sample extracts must be diluted to bring the concentration of S-1283 within the range of linearity established.
2. Each lot of Sep-Pak disposable cartridges must be checked for recovery of S-1283 as follows: Transfer 1.0 mL of the 1.0 µg/mL S-1283 calibrating standard solution to a 50 mL round-bottom flask and evaporate using a rotary-evaporator with a water temperature bath set to ≤40°C. Re-dissolve in 5 mL of hexane and transfer to a Sep-Pak silica gel disposable cartridge and elute the S-1283 as described under Step 4, **Sep-Pak Silica Gel Cartridge Cleanup**.  
  
Evaporate the eluate just to dryness using a rotary-evaporator and water bath set to <40°C. Re-dissolve the eluate in 1.0 ml of acetone and analyze with the 1.0 µg/mL calibrating standard as described under Step 5, **Gas Chromatography Measurement**. If the S-1283 peak for the eluate is less than 90% of the calibrating standard, then the elution profile of S-1283 must be determined.
3. At Valent, a standard operating procedure (SOP# VR-002) requires that a fortified control sample be analyzed with each set of samples. If the testing facility does not require concurrent analysis of fortified control samples, or if a UTC sample is not available, this method requirement may be waived.

The level of fortification is generally 0.01 ppm (the LOQ of the method) and/or 0.05 ppm. These fortifications are made by adding 0.10 mL and 0.5 mL, respectively, of the 1.0 µg/mL fortifying solution to a 10 gram sample. Method recoveries must be 70% to 120% to be acceptable unless approved by the chemist responsible for the analysis.

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**NOTES (CONTINUED)**

4. If matrix interferences are encountered during the analysis of S-1283, sample extracts may be re-injected using the following alternate GC parameters:

Column: DB-17 (15 M x 530  $\mu$ m) wide bore capillary (1.0  $\mu$ m film thickness, J & W Scientific Cat # 125-1312 or equivalent).  
Column Oven Temperature: 240°C. Hold Time: 20 minutes  
Detector Temperature: 300°C  
Injector Temperature: 250°C  
Carrier Gas: Helium at 20 mL/min  
Detector Makeup Gas: Helium at 10 mL/min  
Air: 110 mL/min  
Hydrogen: 3.6 mL/min  
Injection Size: 1.0  $\mu$ l

If the alternate GC parameters are unsuccessful at resolving matrix interferences or if confirmation of residues is required, sample extracts may be analyzed by GC/MS using the following parameters:

Instrument - Hewlett-Packard Model 5890 gas chromatograph equipped with a 2 mm I.D. glass insert for splitless injection (Restek Part No. 20713, packed with approximately 5 mm of silanized glass wool), an HP 5970A Mass Selective Detector with a direct capillary interface, automatic sampler, and HP ChemStation or equivalent system.  
Column: DB-5MS (15 M x 250  $\mu$ m, 0.25  $\mu$ m film thickness)  
Splitless Injection: 1.0 min purge  
Injector Temperature: 280°C  
Column Oven Temperature: 150°C. Hold time 1.0 minutes.  
Column Temperature Program Rate: 30°C/minute  
Final Column Temperature: 260°C. Hold Time: 5.0 minutes.  
Transfer Line Temperature: 280°C  
Carrier Gas: Helium at approximately 1 mL/min (5 psig column head pressure)  
Injection Size: 2.0  $\mu$ l  
Acquisition Mode: SIM, 3 ions (359.3, 300.2, and 330.2 m/z)\*  
Solvent Delay: 2 minutes  
Dwell Time per ion: 80 msec.  
Retention Time: 5.8 minutes

\*The target ion is 359.3 (molecular ion). The 300.2 and 330.2 ions are used as qualifiers. See Figure 7.

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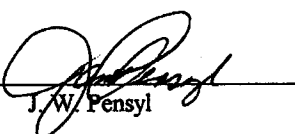
**NOTES (CONTINUED)**

5. At Valent, reproducibility of an analytical run is determined by calculating the CV from the peak units obtained for the calibrating standards analyzed during the run. For a run to be acceptable, these CV's must be 10% or less unless approved by the chemist responsible for the analysis (Valent SOP #VR-013).

**REFERENCE**

1. Tani, T., Wakabayashi, S., Takimoto, Y., and Kato, T., *Residue Analytical Method for S-1283 in Apples*, Report No. ER-MT-9512, Sumitomo Chemical Co., Ltd., May 30, 1995.

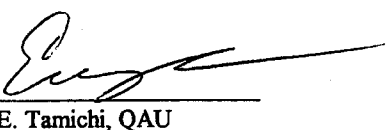
**METHOD APPROVAL**

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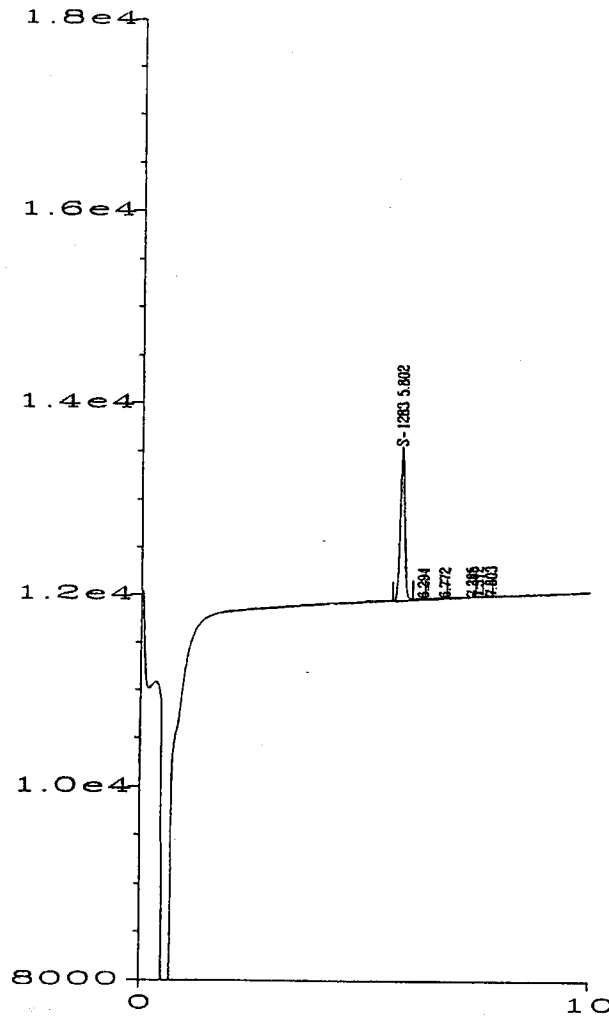


Figure 1. S-1283 Calibrating Standard  
1.0 µg/mL in acetone (1.0 ng injected)  
DB-5 Column

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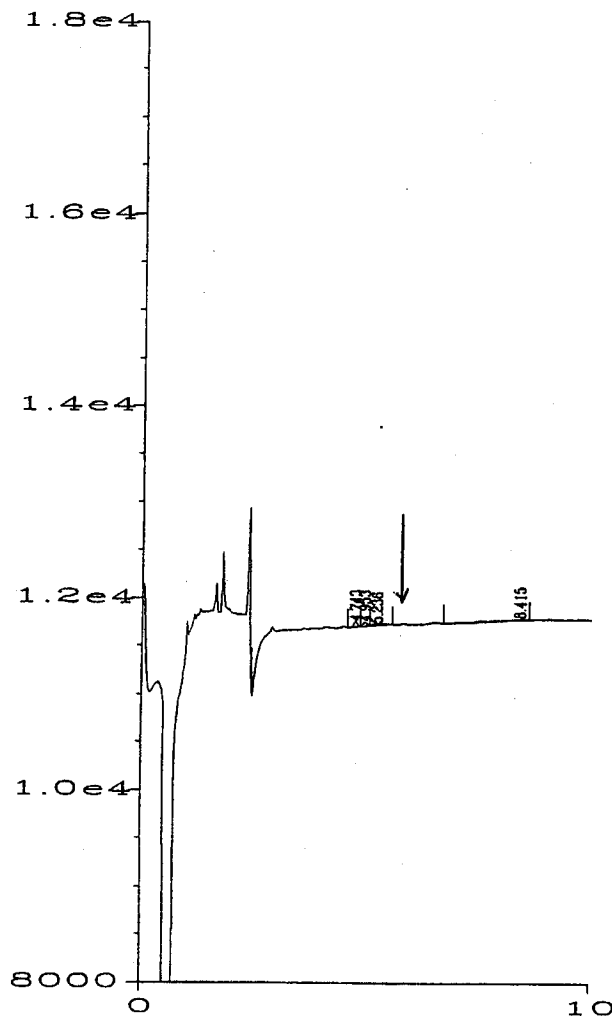


Figure 2. Untreated Control Cottonseed  
10 mg Crop Equivalents Injected  
DB-5 Column

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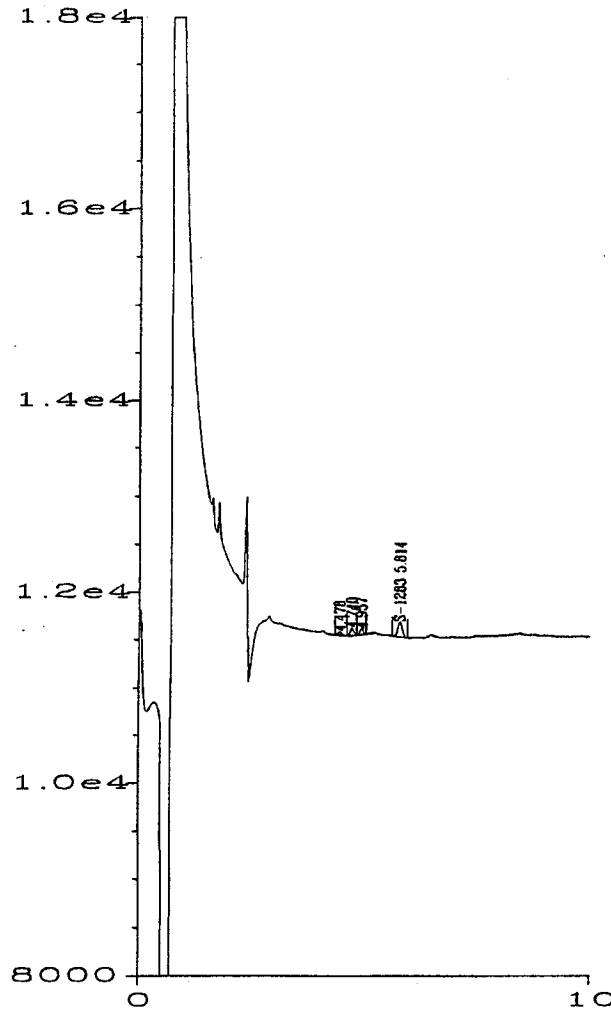


Figure 3. Cottonseed Fortified with 0.01 ppm S-1283  
10 mg Crop Equivalents Injected  
DB-5 Column

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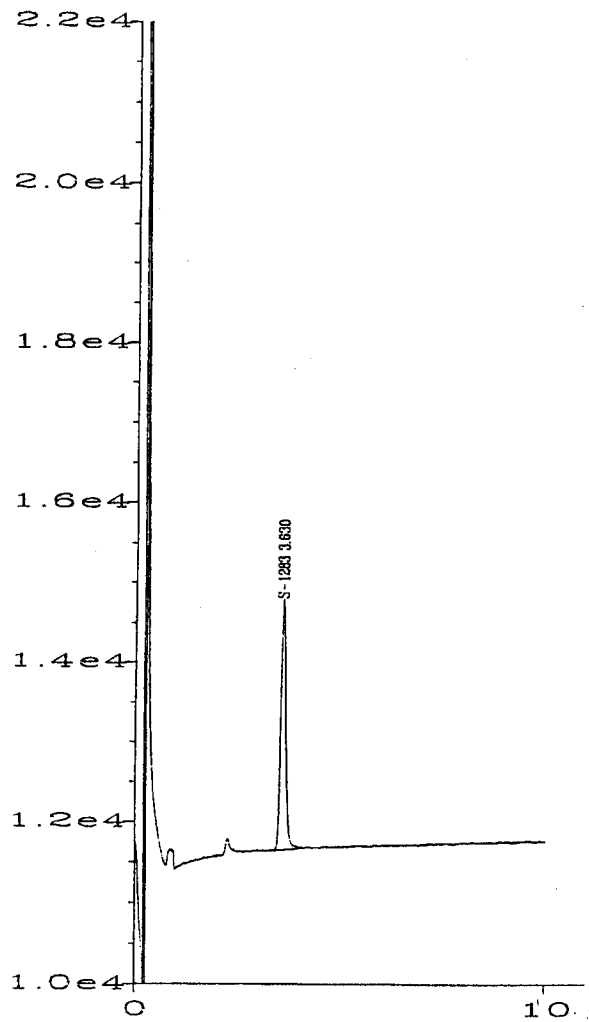


Figure 4. S-1283 Calibrating Standard  
1.0 µg/mL in acetone (1.0 ng injected)  
DB-17 Column

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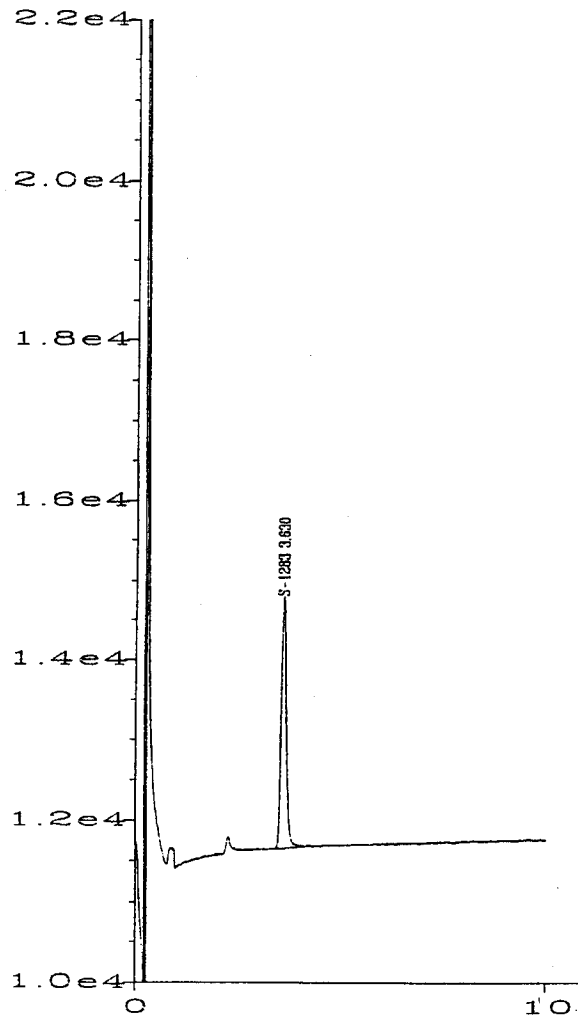


Figure 4. S-1283 Calibrating Standard  
1.0  $\mu\text{g}/\text{mL}$  in acetone (1.0 ng injected)  
DB-17 Column

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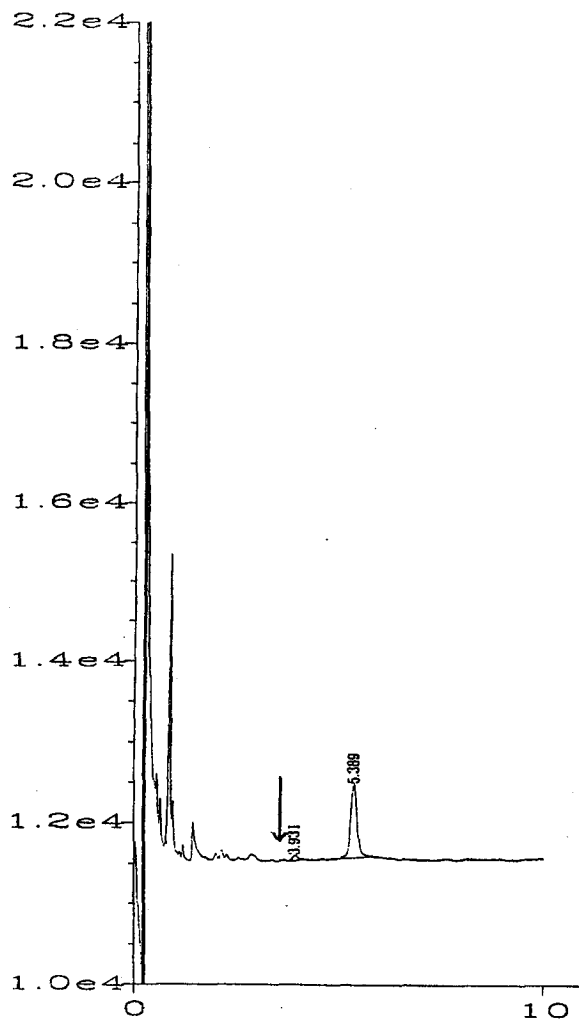


Figure 5. Untreated Control Cottonseed  
10 mg Crop Equivalents Injected  
DB-17 Column

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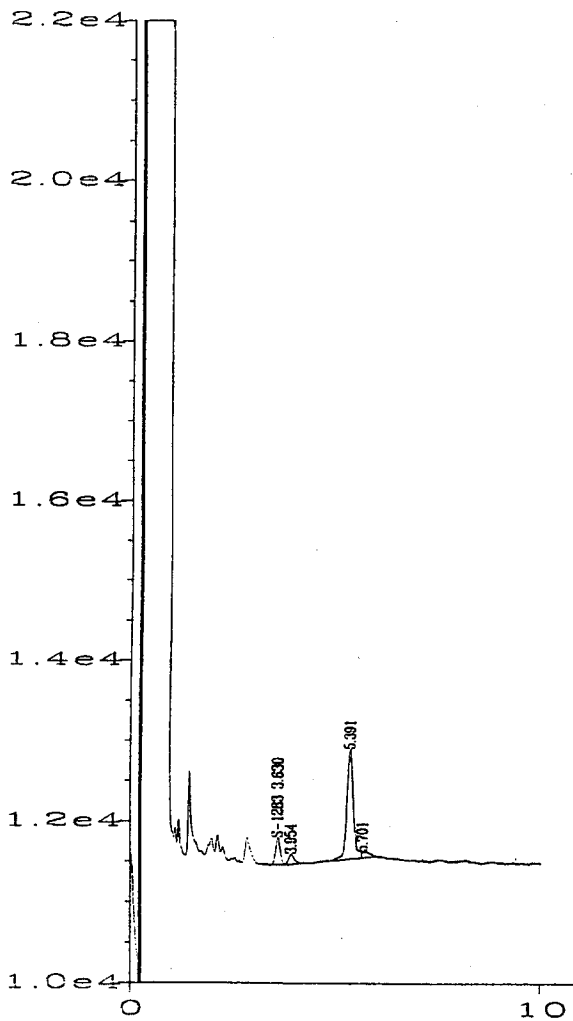


Figure 6. Cottonseed Fortified with 0.01 ppm S-1283  
10 mg Crop Equivalents Injected  
DB-17 Column

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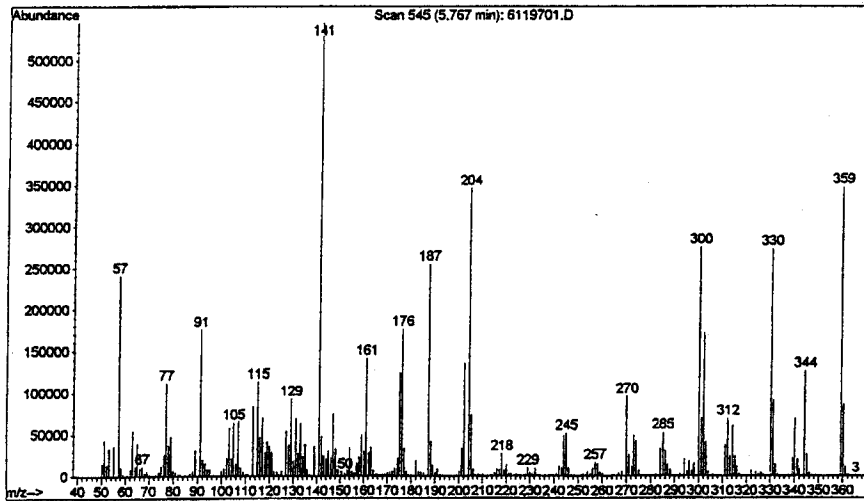
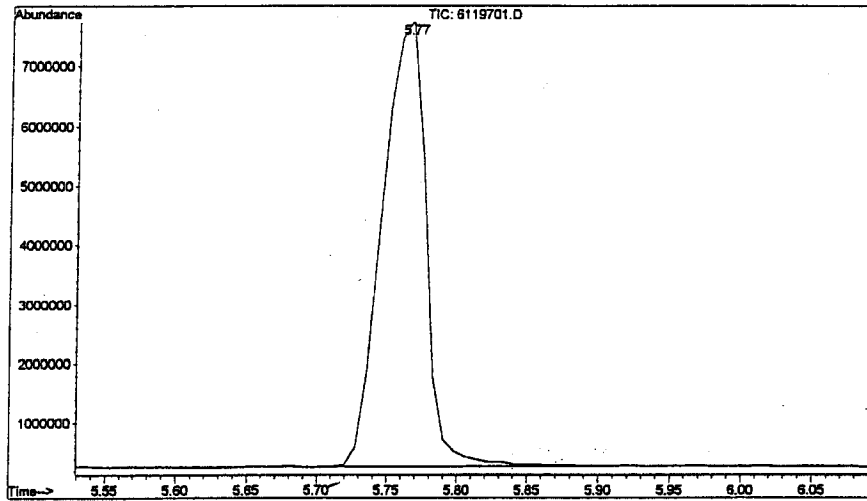


Figure 7. Total Ion Chromatogram and  
Full Scan Mass Spectrum of the 5.8 minute peak (S-1283)  
0.2 µg injected

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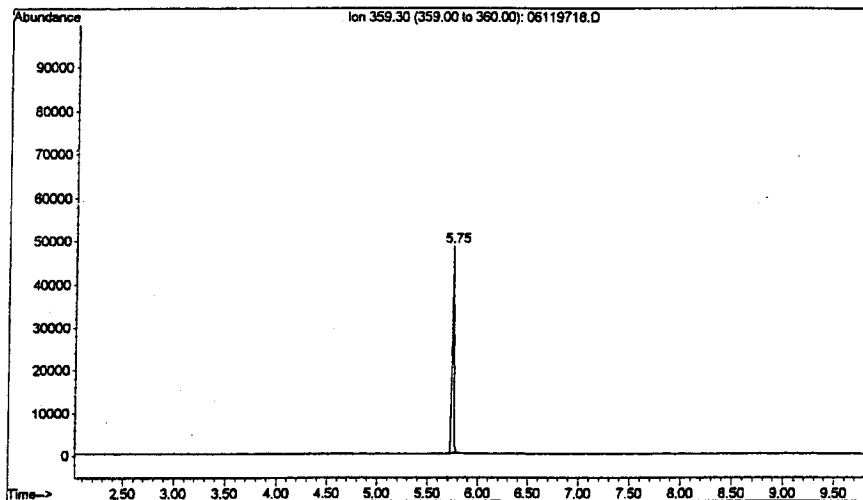


Figure 8. S-1283 Calibrating Standard  
1.0  $\mu\text{g}/\text{mL}$  in acetone (2.0 ng injected)  
Analyzed on MSD

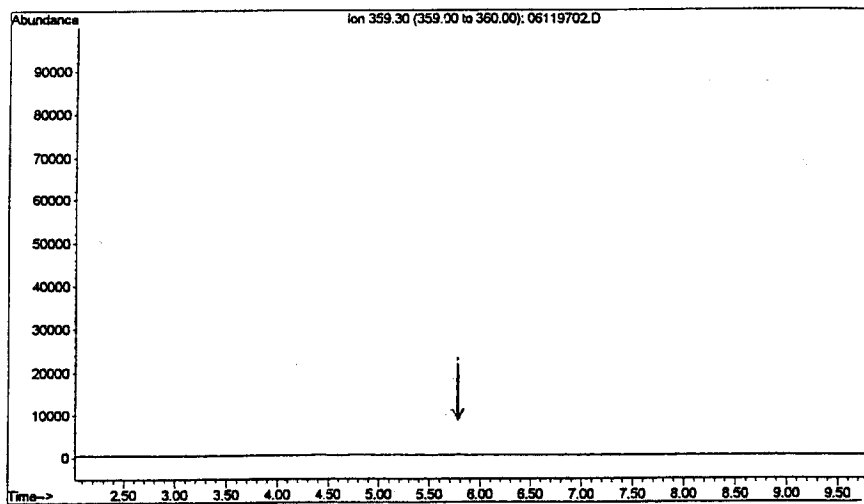


Figure 9. Untreated Control Cottonseed  
20 mg Crop Equivalents Injected  
Analyzed on MSD

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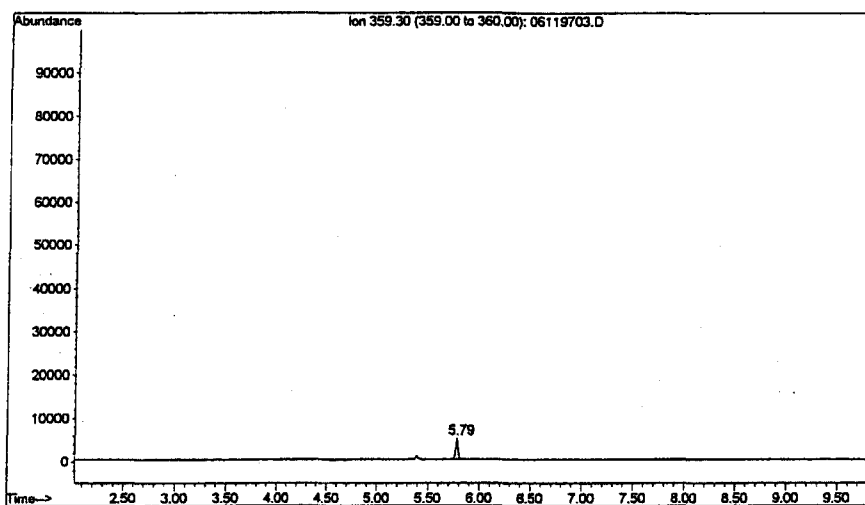


Figure 10. Cottonseed Fortified with 0.01 ppm S-1283  
20 mg Crop Equivalents Injected  
Analyzed on MSD

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Validation Report For Residue Method RM-37  
Reproducibility of Analysis (SOP # VR-002)

ANALYTE: S-1283

Sample Matrix	Fortification Level ppm	Amount Found ppm	% Recovery
Cottonseed	0.010	0.0092	92
Cottonseed	0.010	0.0098	98
Cottonseed	0.010	0.0094	94
		Mean Recovery	95
		CV	3.23
		n	3
Cottonseed	0.050	0.0444	89
Cottonseed	0.050	0.0443	89
Cottonseed	0.050	0.0444	89
Cottonseed	0.050	0.0454	91
Cottonseed	0.050	0.0404	81
Cottonseed	0.050	0.0442	88
		Mean Recovery	88
		CV	3.97
		n	6

Reference: Raw Data File 11794, pages 001-002. This data obtained by re-injecting samples on alternate GC column (DB-17).

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Date  
Approved [Signature] 6/24/97  
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Date

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Validation Report For Residue Method RM-37  
Reproducibility of Analysis (SOP # VR-002)

ANALYTE: S-1283

Sample Matrix	Fortification Level ppm	Amount Found ppm	% Recovery
Cottonseed	0.010	0.0115	115
Cottonseed	0.010	0.0119	119
Cottonseed	0.010	0.0101	101
		<b>Mean Recovery</b>	<b>112</b>
		<b>CV</b>	<b>8.46</b>
		<b>n</b>	<b>3</b>
Cottonseed	0.050	0.0507	101
Cottonseed	0.050	0.0491	98
Cottonseed	0.050	0.0495	99
Cottonseed	0.050	0.0526	105
Cottonseed	0.050	0.0500	100
Cottonseed	0.050	0.0533	107
		<b>Mean Recovery</b>	<b>102</b>
		<b>CV</b>	<b>3.50</b>
		<b>n</b>	<b>6</b>

Reference: Raw Data File 11750, pages 001- 002. This data obtained by re-injecting sample extracts on MSD.

11794  
KOE 1/20/98

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Reviewed by	Date