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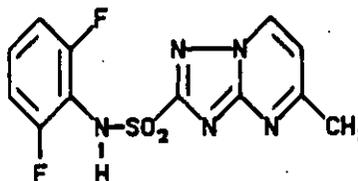
DETERMINATION OF RESIDUES OF DE-498 IN SOYBEANS
 BY
 CAPILLARY GAS CHROMATOGRAPHY/MASS SPECTROMETRY

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This supplement is for the determination of DE-498 in corn grain, forage, and fodder. The only significant modification to the original method is the use of a larger volume of extraction solvent for the analysis of corn forage and fodder.

1. Scope

This method is applicable for the quantitative determination of DE-498 (N-(2,6-difluorophenyl)-5-methyl(1,2,4)triazolo-[1,5a]-pyrimidine-2-sulfonamide) in corn grain, forage, and fodder at a validated lower level of quantitation of 0.005 ppm.



N-(2,6-difluorophenyl)-5-methyl(1,2,4)triazolo-[1,5a]-
 pyrimidine-2-sulfonamide (DE-498)

2. Principle

DE-498 residues are extracted from corn grain, forage, and fodder using a 90% acetone/10% 0.1 N hydrochloric acid solution. Following evaporation of the acetone, the sample is diluted with 0.005 N hydrochloric acid and washed with hexane. The sample is then

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purified using C18 and alumina solid-phase extractions (S-P-E). The eluent from the alumina S-P-E is evaporated to dryness, and the residue reconstituted with acetonitrile. The sample is then derivatized with methyl iodide to form the N-methyl derivative. The derivatized sample solution is evaporated to dryness, reconstituted with toluene containing N-d3-methyl DE-498 as an internal standard, and analyzed by capillary gas chromatography/mass spectrometry (GC/MS).

3. Safety Precautions

- a. Each analyst should be acquainted with the potential hazards of the reagents, products, and solvents used in this method before commencing laboratory work. SOURCES OF INFORMATION INCLUDE: MATERIAL SAFETY DATA SHEETS, LITERATURE, AND OTHER RELATED DATA. Safety information on non-DowElanco products should be requested from the supplier. Disposal of reagents, reactants, and solvents must be in compliance with local, state, and federal laws and regulations.
- b. Acetone, acetonitrile, hexane, methanol, methyl-t-butyl ether, toluene, and triethylamine are flammable and should be used in well-ventilated areas away from ignition sources.

4. Equipment (Note 15.a.)

- a. Gas chromatograph, Model 5890A, Hewlett-Packard, Avondale, PA 19311.
- b. Automatic sampler, Model 7673A, Hewlett-Packard, Avondale, PA 19311.
- c. Mass selective detector, Model 5971A, Hewlett-Packard, Palo Alto, CA 94304.
- d. Mass spectrometer data system, Model 59970, Hewlett-Packard, Palo Alto, CA 94304.
- e. Balance, analytical, Model AE200, Mettler Instrument Corp., Hightstown, NJ 08520.
- f. Balance, pan, Model BB2440, Mettler Instrument Corp.
- g. Centrifuge, with rotor to accommodate 8-ounce wide-mouth glass bottles, Model CU-5000, International Equipment Company, Needham Heights, MA 02194.
- h. Centrifuge, with rotor to accommodate 10-dram vials, Model Centra-8, International Equipment Company.
- i. Evaporator, N-Evap, Model 111, Organomation Associates, Inc., South Berlin, MA 01549. (Note 15.b.)

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- j. Food Cutter, Model 84141-D, with number 12 chopper attachment, Hobart Manufacturing Co., Troy, OH 45373.
 - k. Homogenizer, Polytron, Catalog Number PT-10/35, Brinkmann Instruments, Inc., Westbury, NY 11590.
 - l. Mill, Wiley Model 4, with 3 mm screen, Arthur H. Thomas Co., Philadelphia, PA 19105.
 - m. Shaker, variable-speed reciprocating with box carrier, Model 6000, Eberbach Corp., Ann Arbor, MI 48106.
 - n. Ultrasonic bath, Model 1200, Branson Cleaning Equipment Company, Shelton, CT 06484.
 - o. Vacuum manifold box, Model spe-21, J. T. Baker Chemical Company, Phillipsburg, NJ 08865.
 - p. Vial crimper, Part Number 8710-0979, Hewlett-Packard, Avondale, PA 19311.
 - q. Vortex mixer, Model G-560, Scientific Industries, Inc., Bohemia, NY 11716.
5. Glassware and Materials (Note 15.a.)
- a. Bottle, 8 ounce, round, wide-mouth, clear, with poly(tetrafluoroethylene)-lined screw caps, Catalog Number B7784-1, National Scientific Company, Lawrenceville, GA 30245.
 - b. Column, capillary gas chromatography, Durabond-17 liquid phase, 10 m x 0.18 mm i.d., 0.3 μ m film thickness, Catalog Number 121-1713, J&W Scientific, Folsom, CA 95630.
 - c. Column inlet liner, deactivated, Catalog Number 5181-3315, Hewlett-Packard, Avondale, PA 19311.
 - d. Column, alumina S-P-E, Catalog Number 7214-07, J. T. Baker Chemical Company. (Note 15.c.)
 - e. Column, Cis S-P-E, Catalog Number 7020-07, J. T. Baker Chemical Company. (Note 15.d.)
 - f. Column connector, S-P-E, Catalog Number 7122-0, J. T. Baker Chemical Company.
 - g. Column reservoir, 20 mL S-P-E, Catalog Number 5-7021, Supelco, Inc., Bellefonte, PA 16823.
 - h. Cylinder, graduated, 2000 mL, Catalog Number 131-9058, National Scientific Company.
 - i. Gas, helium, 99.995% purity, Scott Specialty Gases, Troy, MI 48083.

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- j. Gas, nitrogen, technical grade, Scott Specialty Gases.
 - k. Moisture trap, Catalog Number 7971, Chrompack, Inc., Raritan, NJ 08869. (Note 15.e.)
 - l. Charcoal scrubber, Catalog Number 7972, Chrompack, Inc. (Note 15.e.)
 - m. Oxygen trap, Catalog Number 7970, Chrompack, Inc. (Note 15.e.)
 - n. Microdispenser, Model 310, 10 μ L, Drummond Scientific Company, Broomall, PA 19008.
 - o. Microdispenser tubes, 10 μ L, Catalog Number 510G-310G-210G, Drummond Scientific Company.
 - p. Syringes, 50 and 250 μ L, Model 700 Series, Hamilton Company, Reno, NV 89520.
 - q. Vials, 2 dram, with poly(tetrafluoroethylene)-lined screw caps, Catalog Number B7800-3, National Scientific Company.
 - r. Vials, 10 dram, with poly(tetrafluoroethylene)-lined screw caps, Catalog Number B7800-6, National Scientific Company.
 - s. Vials, autosampler, 2 mL, Catalog Number C4011-2, National Scientific Company.
 - t. Vial seals, Catalog Number C4011-1A, National Scientific Company.
6. Reagents and Chemicals (Note 15.a.)
- a. Acetone, acetonitrile, hexane, methanol, methyl-*t*-butyl ether, methylene chloride, and toluene (Optima Grade), Fisher Scientific, Pittsburg, PA 15219.
 - b. Acetic acid, glacial, reagent grade, Fisher Scientific.
 - c. 1% Acetic acid/99% Methylene chloride (v/v).
Prepare by diluting 10 mL of glacial acetic acid to volume in a 1000-mL volumetric flask with methylene chloride.
 - d. 3% Acetic acid/97% Methylene chloride (v/v).
Prepare by diluting 30 mL of glacial acetic acid to volume in a 1000-mL volumetric flask with methylene chloride.
 - e. Hydrochloric acid, 0.1 N, reagent grade, certified concentration, Fisher Scientific.

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- f. Hydrochloric acid, 0.005 N.
Prepare by diluting 50 mL of 0.1 N hydrochloric acid to volume in a 1000-mL volumetric flask with distilled/deionized water.
- g. 90% acetone/10% 0.1 N hydrochloric acid solution.
Prepare by pouring 200 mL of 0.1 N hydrochloric acid into a 2000-mL graduated cylinder. Add 1500 mL of acetone, swirl the cylinder, and allow to equilibrate to room temperature. Adjust to volume with acetone.
- h. Sodium chloride, ACS reagent grade, Fisher Scientific.
- i. Sodium chloride, 5% (w/v).
Prepare by dissolving 50 grams of sodium chloride in distilled/deionized water in a 1000-mL volumetric flask. Adjust to volume with distilled/deionized water.
- j. Methyl iodide, minimum 99.5% purity, Catalog Number 28,956-6, Aldrich Chemical Company, Milwaukee, WI 53233.
- k. Methyl iodide, stable-isotope labeled, $^{12}\text{CD}_3\text{I}$, Catalog Number 29,675-9, Aldrich Chemical Company.
- l. Triethylamine, minimum 99% purity, Catalog Number 13,206-3, Aldrich Chemical Company.
- m. Water, distilled/deionized, Corning MEGA-PURE Still, Model MP-12A, Corning Glass Works, Science Products Division, Corning, NY 14831.
- n. Standard
N-(2,6-difluorophenyl)-5-methyl(1,2,4)triazolo-[1,5a]-pyrimidine-2-sulfonamide (DE-498), analytical standard.^{a/}

7. Preparation of Standards

a. Preparation of Calibration Standards/Spiking Solutions

- (1) Dissolve 0.1000 gram of DE-498 analytical standard in acetone in a 100-mL volumetric flask. Dilute to volume to obtain a 1000 $\mu\text{g/mL}$ stock solution.
- (2) Dilute 10 mL of the above 1000 $\mu\text{g/mL}$ solution to 1000 mL with acetone in a 1000-mL volumetric flask to obtain a 10.0 $\mu\text{g/mL}$ (10.0 $\text{ng}/\mu\text{L}$) initial solution.

^{a/} Obtain from Sample Coordinator, DowElanco, P.O. Box 1706, Midland, Michigan 48641-1706.

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- (3) Solutions for spiking corn samples are prepared by diluting the initial solution from Section 7.a.(2) above with acetone as follows:

Aliquot of Initial Soln.	Final Soln. Volume	Spiking Soln. Final Conc.	Equivalent Sample Conc.
mL	mL	µg/mL	ppm
5.0	1000	0.050	0.005
10.0	1000	0.100	0.010
25.0	1000	0.250	0.025
50.0	1000	0.500	0.050

The equivalent sample concentration is based on using a 10-gram corn sample for analysis.

- (4) Solutions for calibration standards are prepared by dispensing 200 µL of the DE-498 spiking solutions in Section 7.a.(3) above into 2-dram vials and derivatizing according to the procedure described in Section 9, steps p through bb.

b. Preparation of Internal Standard Solution

- (1) Pipet 2.0 mL of the 1000 µg/mL DE-498 stock solution from Section 7.a.(1) into a 2-dram vial.
- (2) Evaporate the solution to dryness using an N-Evap evaporator.
- (3) Add 1.0 mL of acetonitrile, cap the vial, and sonicate for 5-10 seconds.
- (4) Add 50 µL of triethylamine and 50 µL of stable-isotope labeled methyl iodide (Section 6.k), cap the vial, and sonicate for 5-10 seconds.
- (5) Allow the mixture to react with the methyl iodide for 30 minutes at room temperature.
- (6) Evaporate the solution to dryness using an N-Evap evaporator.
- (7) Add 1.0 mL of a 5% sodium chloride solution, cap the vial, and sonicate for 5-10 seconds.
- (8) Add 5.0 mL of methyl-*t*-butyl ether, cap the vial, and vortex the sample for 5-10 seconds.
- (9) Centrifuge the vial for 5 minutes at 2500 rpm.
- (10) Carefully transfer the methyl-*t*-butyl ether layer to a clean 10-dram vial.
- (11) Repeat Steps 8-9 three additional times, combining the methyl-*t*-butyl ether layers in the 10-dram vial.
- (12) Evaporate the methyl-*t*-butyl ether to dryness using an N-Evap evaporator
- (13) Add 20 mL of acetone, cap the vial, and sonicate for 5-10 seconds.

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- (14) Transfer the acetone to a 200-mL volumetric flask.
- (15) Rinse the 10-dram vial again with 20 mL of acetone, and transfer the acetone to the 200-mL volumetric flask.
- (16) Dilute the solution to volume with acetone. This solution contains 10.0 $\mu\text{g/mL}$ N-d₃-methyl DE-498.
- (17) Dilute 10.0 mL of the above 10.0 $\mu\text{g/mL}$ solution to 1000 mL with toluene in a 1000-mL volumetric flask to obtain a 0.100 $\mu\text{g/mL}$ (0.100 $\text{ng}/\mu\text{L}$) solution.

8. Gas Chromatography/Mass Spectrometry

a. Column

Install the splitless liner (Section 5.c.) and the capillary column (Section 5.b.) on the split/splitless injection port of the GC/MS following the manufacturer's recommended procedure.

b. Typical operating conditions for the determination of DE-498 by capillary GC/MS:

Instrumentation: Hewlett-Packard Model 5890A Gas Chromatograph / Model 5971A Mass Selective Detector

Column: J&W Scientific fused silica capillary Durabond-17 liquid phase
10 m x 0.18 mm i.d.
0.30 μm film thickness

Temperatures:

Column 120°C for 1.1 minutes
120°C to 325°C at 20°C/minute
325°C for 5.65 minutes

Injector 320°C

Interface 310°C

Carrier Gas: helium

Head Pressure 100 kPa

Linear Velocity 25 cm/sec

Injection Mode: splitless

Purge Delay 1.0 minutes.

Splitter Flow 50 mL/min

Septum Purge: 1.0 mL/min

Injection Volume: 2 μL

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Ions Monitored: N-methyl DE-498
m/z 134 (base peak ion)
(M⁺-205; see Figure 1)
m/z 142 (M⁺-197; see Figure 1)
N-d3-methyl DE-498 (internal standard)
m/z 145 (M⁺-197; see Figure 2)

Electron Multiplier: 1800 volts

- c. A typical calibration curve is shown in Figure 3.
- d. Typical chromatograms of a standard, control sample, and a 0.005 recovery sample for corn grain, forage, and fodder are shown in Figures 4-12, respectively.

9. Recovery of DE-498 from Corn

- a. Use the Wiley Mill or Hobart Food Cutter to thoroughly grind or chop the bulk sample.
- b. Weigh 10.0-gram portions of the prepared control corn samples into a series of 8-ounce wide-mouth bottles.
- c. For preparing fortified samples, use part of the samples as controls and fortify the remaining samples by adding 1.0-mL aliquots of the appropriate spiking solutions (Section 7.a.(3)) in acetone to obtain concentrations ranging from 0.005 to 0.050 ppm.
- d. For forage and fodder samples, add 10 mL of 0.1 N hydrochloric acid and allow to set for approximately one hour.
- e. For grain samples, add 100 mL of a 90% acetone/10% 0.1 N hydrochloric acid extracting solution.
For forage and fodder samples, add 140 mL of a 90% acetone/10% 0.1 N hydrochloric acid extracting solution.
- f. Blend the sample at high speed for 1 minute using a Polytron homogenizer.
- g. Cap the bottle and shake the sample for a minimum of 2 hours on a reciprocating shaker at approximately 180 excursions/minute.
- h. Centrifuge the sample container for 10 minutes at 2500 rpm.
- i. For grain samples, transfer a 20-mL aliquot of the acetone/hydrochloric acid solution to a clean 10-dram vial.
For forage and fodder samples, transfer a 30-mL aliquot of the acetone/hydrochloric acid solution to a clean 10-dram vial.

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- j. Evaporate the acetone using an N-Evap evaporator. (The volume of liquid in the vial at this point should be less than 2 mL for grain samples, and less than 4 mL for forage and fodder samples.)
- k. Add 15.0 mL of 0.005 N hydrochloric acid, cap the vial, and sonicate the sample for 10-15 seconds.
- l. Add 10 mL of hexane, cap the vial, sonicate the sample for 10-15 seconds, and then vortex the sample for 10-15 seconds.
- m. Centrifuge the vial for 5 minutes at 2500 rpm and discard the hexane (top) layer. Be careful not to remove solids at the hexane/hydrochloric acid interface.
- n. The sample is then purified using the following S-P-E procedure (Note 15.d.):
 - (1) Place a C₁₈ S-P-E column on the vacuum manifold box.
 - (2) Rinse the C₁₈ column with 5 mL of methanol.
 - (3) Condition the C₁₈ column with 5 mL of 0.005 N hydrochloric acid. (Do not allow the column bed to dry.)
 - (4) Connect a 20-mL reservoir to the top of the C₁₈ column using an S-P-E column connector.
 - (5) Transfer the sample solution from Step 9.m to the reservoir and, with the aid of vacuum, slowly pull the sample through the column. Without allowing the column bed to dry, wash the sample vial with a 10-mL aliquot of 0.005 N hydrochloric acid and transfer the wash to the reservoir.
 - (6) Dry the sample vial using an N-Evap evaporator and save for Step 9.o.
 - (7) Thoroughly dry the C₁₈ column and attached reservoir by drawing air through them for approximately 45 minutes.
 - (8) Remove the C₁₈ column and attached reservoir from the vacuum manifold box and save for the second S-P-E described below.
- o. The sample is purified further using the following alumina S-P-E procedure (Note 15.c.):
 - (1) Place an alumina S-P-E column on the vacuum manifold box.
 - (2) Rinse the alumina column with 5 mL of methanol. (Do not allow the column bed to dry.)
 - (3) Connect the C₁₈ S-P-E column and attached reservoir from Step 9.n.(8) to the top of the alumina S-P-E column using a S-P-E column connector.
 - (4) Add 10.0 mL of methanol to the dried sample vial from Step 9.n.(6). Sonicate the vial for 10-15 seconds and then vortex the vial for 10-15 seconds, making sure to remove material adhering to the vial walls.

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- (5) Transfer the methanol from Step 9.o.(4) to the reservoir and, with the aid of vacuum, slowly pull the methanol through the C₁₈ and alumina S-P-E columns. Without allowing the column beds to dry, rinse the reservoir with 5.0 mL of clean methanol.
 - (6) Dry the S-P-E columns and attached reservoir by drawing air through them for approximately 10 minutes.
 - (7) Remove the C₁₈ column and attached reservoir from the alumina column. Discard the C₁₈ S-P-E column.
 - (8) Rinse the alumina column with 1.5 mL of a 1% acetic acid/99% methylene chloride solution. (Do not allow the column bed to dry.)
 - (9) Remove the alumina column from the vacuum manifold box and elute the DE-498 with 5.0 mL of a 3% acetic acid/97% methylene chloride solution. Collect the eluent in a 2-dram vial. (Note 15.f.)
- p. Evaporate the solution to dryness using an N-Evap evaporator.
 - q. Add 500 μ L of acetonitrile, cap the vial, and sonicate for 5-10 seconds.
 - r. Add 10 μ L of triethylamine and 10 μ L of methyl iodide, cap the vial, and sonicate for 5-10 seconds.
 - s. Allow the sample to react with the methyl iodide for 30 minutes at room temperature.
 - t. Evaporate the solution to dryness using an N-Evap evaporator.
 - u. Add 1.0 mL of a 5% sodium chloride solution, cap the vial, and sonicate for 5-10 seconds.
 - v. Add 5.0 mL of methyl-*t*-butyl ether, cap the vial, and vortex the sample for 5-10 seconds.
 - w. Centrifuge the vial for 5 minutes at 2500 rpm.
 - x. Carefully transfer the methyl-*t*-butyl ether layer to a 2-dram vial.
 - y. Evaporate the solution to dryness using an N-Evap evaporator.
 - z. Add 1.0 mL of toluene containing the N-d₃-methyl DE-498 internal standard, cap the vial, and sonicate for 5-10 seconds.
 - aa. Centrifuge the vial for 5 minutes at 2600 rpm.
 - bb. Transfer the solution to a 2-mL autosampler vial. Seal the vial with a cap and crimper.
 - cc. Analyze the sample by capillary gas chromatography/mass spectrometry as described in Section 8.

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10. Determination of Percent Recovery of DE-498

- a. Inject the calibration standards described in Section 7.a.(4) and determine the peak areas at m/z 134 and m/z 142 for methylated DE-498 and at m/z 145 for d_3 -methylated DE-498.

For each standard calculate the DE-498 confirmation ratio. The average confirmation ratio for all of the calibration standards will be used to confirm the presence of DE-498 in the corn samples.

For example, using the data from Figure 4:

$$\text{Confirmation Ratio} = \frac{\text{peak area at } m/z \text{ 142}}{\text{peak area at } m/z \text{ 134}}$$

$$\text{Confirmation Ratio} = \frac{80802}{142543}$$

$$\text{Confirmation Ratio} = 0.5669$$

Positive confirmation of the presence of DE-498 is indicated when the confirmation ratio for the samples is in the range of $\pm 10\%$ of the average found for the standards.

- b. Prepare a standard curve by plotting the equivalent DE-498 concentration on the abscissa (x-axis) and the m/z 134/145 peak area ratio on the ordinate (y-axis) as shown in Figure 3. Using regression analysis, determine the equation for the curve with respect to the abscissa.

For example, using power regression with the data for Figure 3:

$$Y = \text{constant} \times X^{\text{(exponent)}}$$

$$X = \left(\frac{Y}{\text{constant}} \right)^{1/\text{exponent}}$$

$$\text{DE-498 Conc. (ppm)} = \left(\frac{m/z \text{ 134/145 peak area ratio}}{\text{constant}} \right)^{1/\text{exponent}}$$

$$\text{DE-498 Conc. (ppm)} = \left(\frac{m/z \text{ 134/145 peak area ratio}}{27.5282} \right)^{1/0.94779}$$

- c. Determine the net concentration in each recovery sample by first subtracting the average DE-498 peak area ratio in the control samples from that of the recovery sample. Substitute the peak area ratio obtained into the above equation and solve for the concentration.

For example, using the data from Figures 5 and 6:

$$\text{DE-498 Conc. (ppm)} = \left(\frac{\text{net } m/z \text{ 134/145 peak area ratio}}{27.5282} \right)^{1/0.94779}$$

$$\text{DE-498 Conc. (ppm)} = \left(\frac{0.17627 - 0.00000}{27.5282} \right)^{1/0.94779}$$

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DE-498 Conc. = 0.00485 ppm

- d. Determine the percent recovery by dividing the net concentration of each recovery sample by the theoretical concentration added.

$$\text{Recovery} = \frac{\text{Concentration Found}}{\text{Concentration Added}} \times 100\%$$

$$\text{Recovery} = \frac{0.00485 \text{ ppm}}{0.00502 \text{ ppm}} \times 100\%$$

$$\text{Recovery} = 97\%$$

11. Determination of DE-498 in Corn Samples

- a. Prepare control, recovery, and treated samples as described in Section 9.
- b. Prepare a standard curve and determine the DE-498 concentration in the recovery samples as described in Section 10.
- c. Determine the concentration in each treated sample by substituting the DE-498 m/z 134/145 peak area ratio obtained into the equation for the standard curve and solving for the concentration.

For example, using the data from Figure 6:

$$\text{DE-498 Conc. (ppm)} = \left(\frac{\text{m/z 134/145 peak area ratio}}{\text{constant}} \right)^{1/\text{exponent}}$$

$$\text{DE-498 Conc. (ppm)} = \left(\frac{0.17627}{27.5282} \right)^{1/0.94779}$$

$$\text{DE-498 Conc.} = 0.00485 \text{ ppm}$$

12. Determination of Corrected DE-498 in Corn

- a. Determine the DE-498 concentration in the corn samples as described in Section 11.
- b. Determine the corrected DE-498 concentration in corn samples as follows:

$$\text{DE-498 Conc. (corrected ppm)} = \text{DE-498 Conc. (ppm)} \times \frac{100}{\% \text{ Recovery}}$$

13. Precision Statement

Recovery values of DE-498 from samples of corn grain fortified over the concentration range of 0.005 to 0.050 ppm averaged 97% with one standard deviation equal to 7% (Table I).

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Recovery values of DE-498 from samples of corn forage fortified over the concentration range of 0.005 to 0.050 ppm averaged 92% with one standard deviation equal to 6% (Table II).

Recovery values of DE-498 from samples of corn fodder fortified over the concentration range of 0.005 to 0.050 ppm averaged 91% with one standard deviation equal to 3% (Table III).

14. Discussion

- a. Since the mass spectrum of N-methyl DE-498 shows that primarily lower mass fragments are formed, the mass selective detector tuning was optimized for lower mass sensitivity. Using the "USERTUNE" feature of the mass selective detector and the standard perfluorotributylamine tuning compound, the mass spectrometer tuning was conducted at m/z 69, 100, and 131. Although not usually necessary for obtaining the sensitivity required to analyze samples at the low end of the validated range, tuning the instrument in this manner often doubled the sensitivity for the ions monitored in this method.
- b. During the course of analyzing several hundred corn samples using the method described above, it was found that the DE-498 chromatographic peak shape would remain sharper for longer periods of time when sample matrix was present vs. the absence of sample matrix (i.e. calibration standards). Breaking off a small section (approx. 25-50 cm) of the capillary column from the injection port side of the column was found to remedy the problem. An alternative solution that also worked very well was to fortify control corn sample extracts with the appropriate DE-498 standard after the alumina S-P-E (Section 9.o.(9)). This technique had the advantage of minimizing any sample matrix effects on the chromatographic process and subsequent quantitation of DE-498. It must be noted, however, that this technique is not suitable for the analysis of enforcement samples.

15. Notes

- a. Equipment, glassware, materials, reagents, and chemicals considered to be equivalent to those specified may be substituted with the understanding that their performance must be confirmed by appropriate tests. Common laboratory supplies are assumed to be readily available and are, therefore, not listed here.
- b. The N-Evap evaporator should be set at a water bath temperature of 40°C and a nitrogen flow rate of 200 mL/min.
- c. Variation in the alumina S-P-E columns may influence the elution profile of the DE-498. It is necessary to obtain an elution profile of each lot of S-P-E used with both of the acetic acid/

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methylene chloride solutions to ensure optimum clean-up efficiency.

The purpose of the 1% acetic acid/99% methylene chloride solution is to elute impurities from the alumina column prior to the elution of the DE-498. Consequently, after generating the elution profile for DE-498 using this solution, the actual volume of solution used in Section 9.o.(8) should be ca. 0.5 mL less than the volume at which DE-498 begins to elute from the alumina column.

- d. Variation in the C18 S-P-E columns may influence the elution profile of the DE-498. It is necessary to obtain an elution profile of each lot of S-P-E column used to ensure optimum clean-up efficiency.
- e. The scrubber/traps are used in the gas supply lines to purify helium entering the gas chromatograph.
- f. Depending on the number of samples being prepared, one may elute the DE-498 from each S-P-E column individually, using either gravity-feed or pressurized elution, or as a group, using the vacuum manifold box.

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TABLE I. RECOVERY OF DE-498 FROM CORN GRAIN.

Control Grain Sample Number ^{a/}	ppm		Percent Recovery
	Added	Found	
289833-289842 ^{b/}	0.00502	0.00571	114
290149	0.00502	0.00539	107
289807	0.00502	0.00487	97
289421	0.00502	0.00442	88
290261	0.00502	0.00451	90
289833-289842	0.00502	0.00485	97
290149	0.00502	0.00475	95
289807	0.00502	0.00483	96
289833-289842	0.01004	0.01007	100
290149	0.01004	0.01127	112
289807	0.01004	0.00976	97
289403	0.01004	0.00933	93
289843	0.01004	0.00963	96
290267	0.01004	0.00971	97
290276	0.01004	0.00947	94
290645	0.01004	0.00975	97
289415	0.01004	0.00903	90
289807	0.01004	0.00897	89
289807	0.01004	0.00990	99
289397	0.02510	0.02307	92
289409	0.02510	0.02345	93
289807	0.02510	0.02519	100
289285	0.05020	0.04995	100
289807	0.05020	0.05052	101
			97 ± 7 ^{c/}

^{a/} Several samples of corn grain were used for controls. There was no DE-498 detected in any of the corn grain samples used for spiking.

^{b/} Ten bags, each containing approximately 30 lb of grain, were composited.

^{c/} Mean ± 1 standard deviation (n=24).

EFFECTIVE: September 4, 1992

ACR #: 91.6.15

TABLE II. RECOVERY OF DE-498 FROM CORN FORAGE.

Control Forage Sample Number ^{a/}	ppm		Percent Recovery
	Added	Found	
289279	0.00502	0.00482	96
290273	0.00502	0.00469	94
289279	0.00502	0.00476	95
290273	0.00502	0.00468	93
289282	0.00502	0.00475	95
289279	0.00502	0.00495	99
287556	0.01004	0.00965	96
287686	0.01004	0.00906	90
288012	0.01004	0.00915	91
289045	0.01004	0.00957	95
289282	0.01004	0.00930	93
287556	0.01004	0.00947	94
287686	0.01004	0.00951	95
289279	0.01004	0.00925	92
289279	0.01004	0.00902	90
288009	0.02510	0.02302	92
288090	0.02510	0.02267	90
289282	0.02510	0.02252	90
289279	0.02510	0.02381	95
287271	0.05020	0.04466	89
287282	0.05020	0.03532	70
			92 ± 6 ^{b/}

^{a/} Several samples of corn forage were used for controls. There was no DE-498 detected in any of the corn forage samples used for spiking.

^{b/} Mean ± 1 standard deviation (n=21).

EFFECTIVE: September 4, 1992

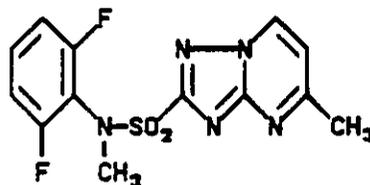
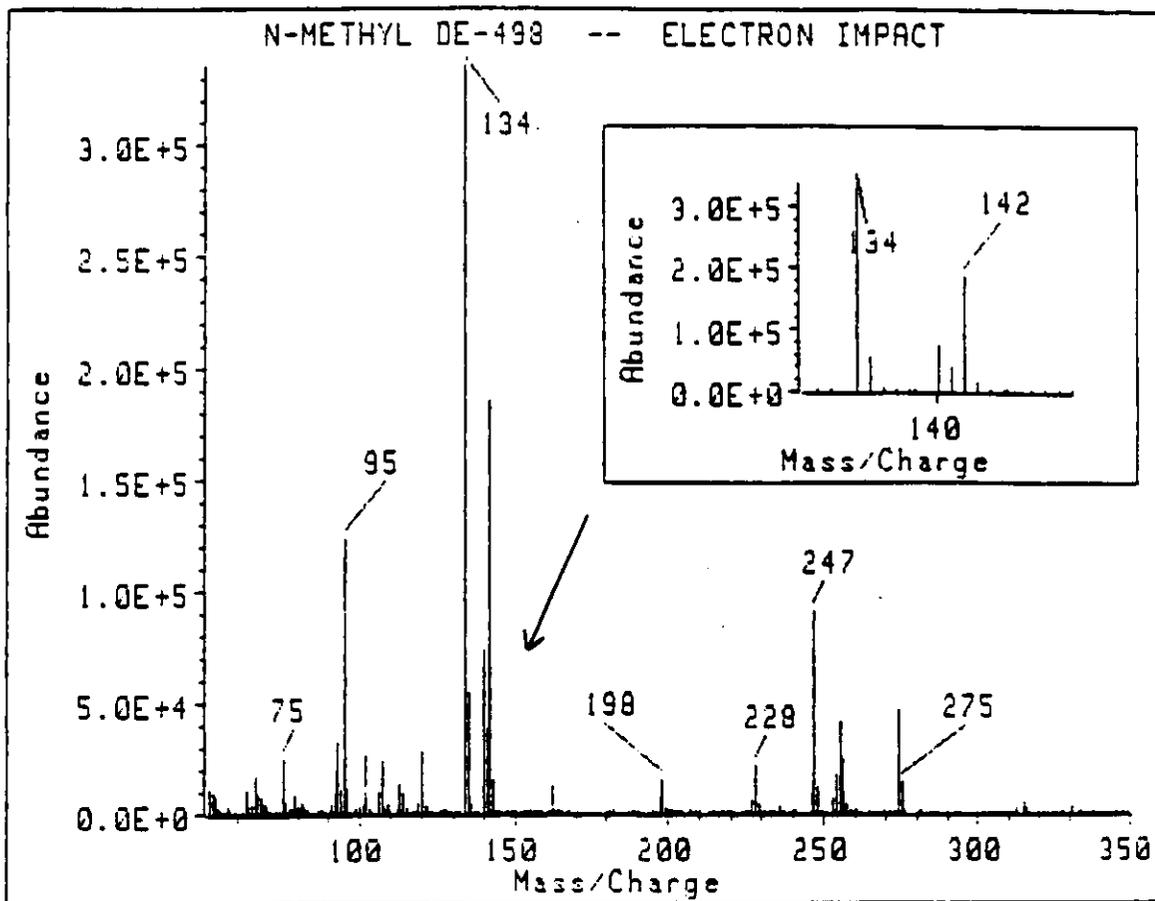
ACR #: 91.6.1S

TABLE III. RECOVERY OF DE-498 FROM CORN FODDER.

Control Fodder Sample Number ^{a/}	ppm		Percent Recovery
	Added	Found	
289424	0.00502	0.00460	92
290264	0.00502	0.00463	92
289424	0.00502	0.00454	90
290264	0.00502	0.00454	90
289406	0.01004	0.00896	89
289846	0.01004	0.00944	94
290270	0.01004	0.00951	95
290279	0.01004	0.00932	93
289418	0.01004	0.00862	86
290648	0.01004	0.00919	92
289400	0.02510	0.02180	87
289412	0.02510	0.02098	84
289288	0.05020	0.04667	93
289301	0.05020	0.04624	92
			91 ± 3 ^{b/}

^{a/} Several samples of corn fodder were used for controls. There was no DE-498 detected in any of the corn fodder samples used for spiking.

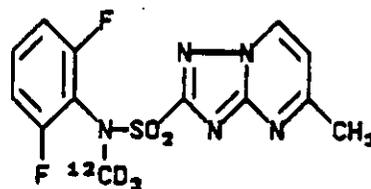
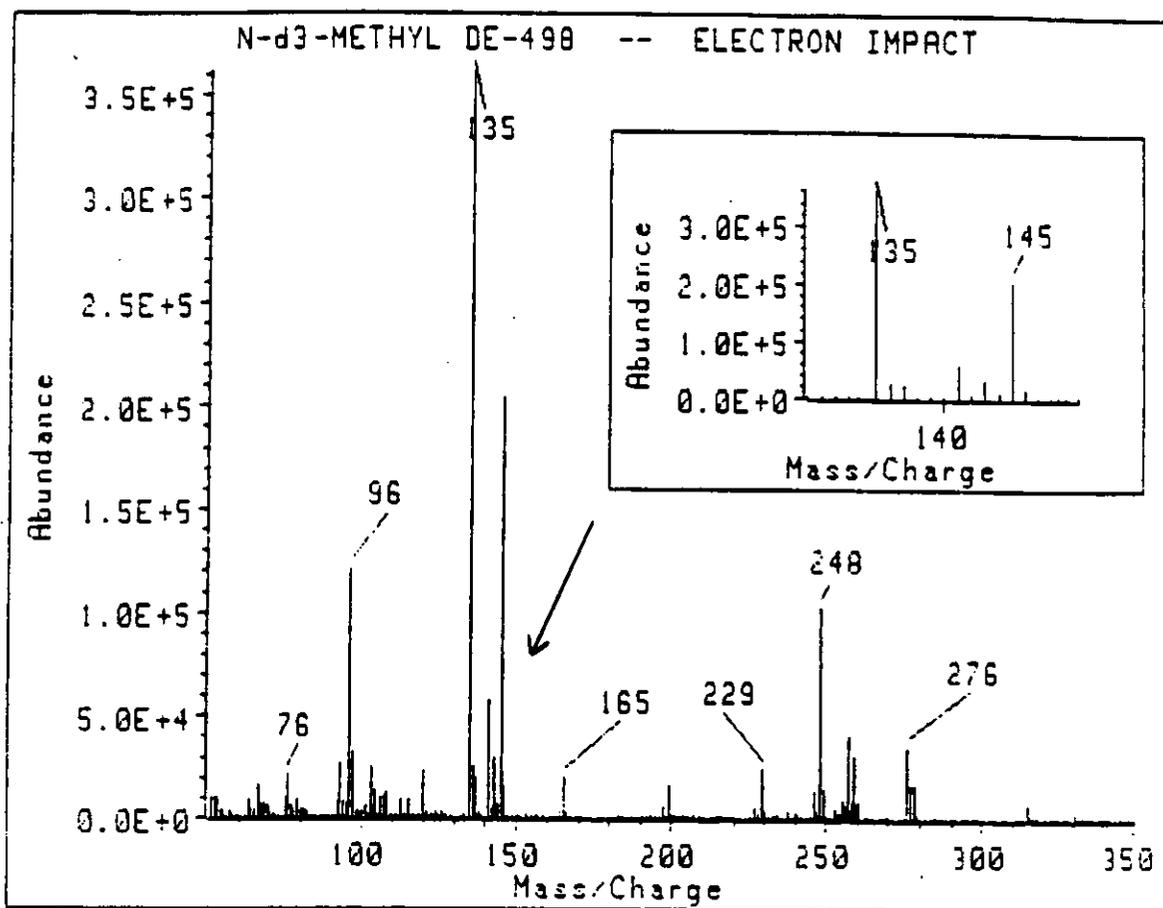
^{b/} Mean ± 1 standard deviation (n=14).



N-Methyl DE-498

Molecular Weight: 339

FIGURE 1. MASS SPECTRUM OF THE N-METHYL DERIVATIVE OF DE-498.

N-d₃-Methyl DE-498

Molecular Weight: 342

FIGURE 2. MASS SPECTRUM OF THE N-d₃-METHYL DERIVATIVE OF DE-498.

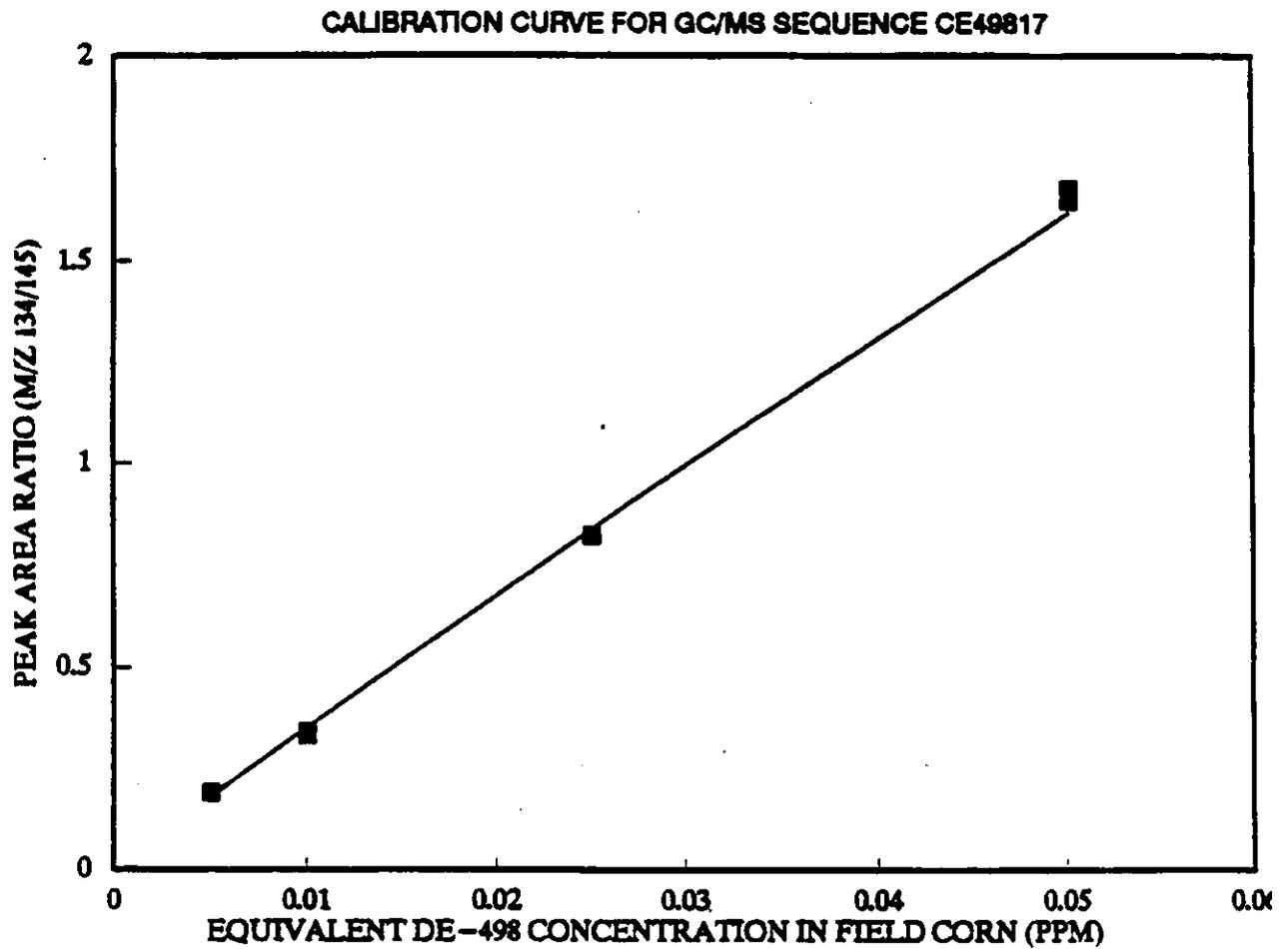
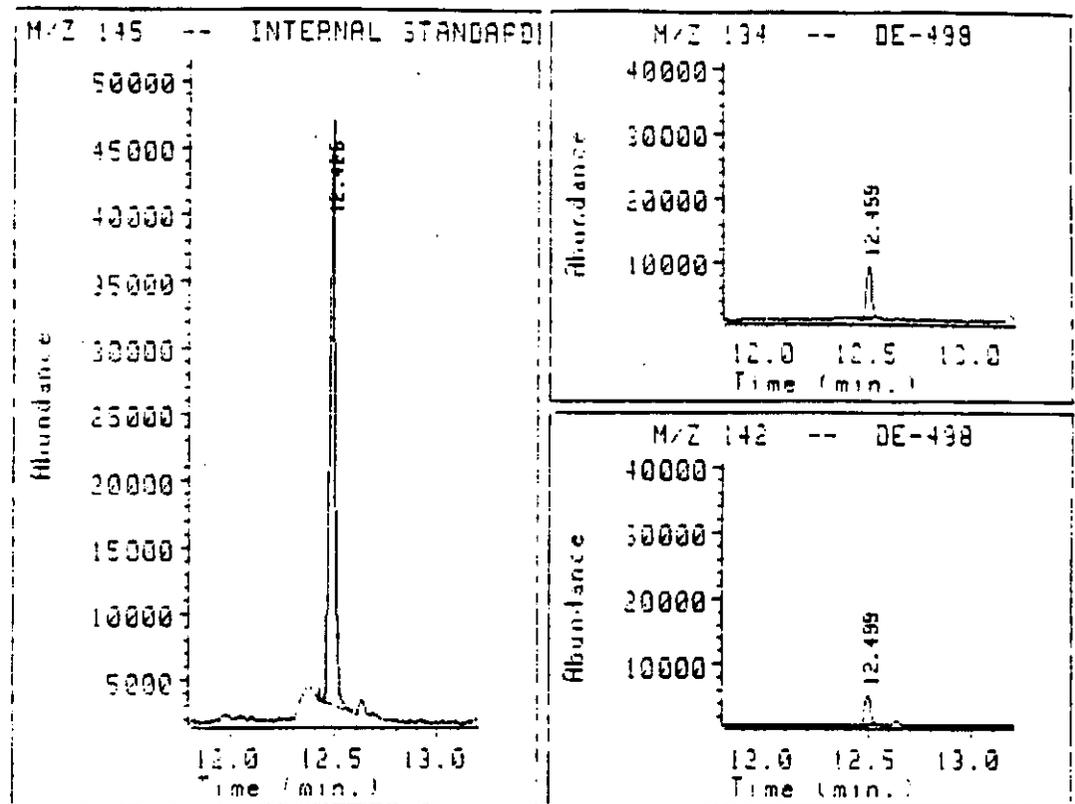


FIGURE 3. TYPICAL CALIBRATION CURVE FOR THE DETERMINATION OF DE-498 IN CORN.

EFFECTIVE: September 4, 1992

ACR #: 91.6.1S



Data File : DATA2:CE17A05A.D
 Date : 17 Apr 91 2:49 pm
 Instrument : MS_5971A - S/N 2749A00149

Sample Name: DE-498 STANDARD - 010.04 ng/~~wt~~ - EQUIVALENT TO 0.00502 PPM
 Sample Info: mL 5/20/91
 Operator : E. L. OLBERDING DOD

INTSTD RETENTION TIME IS : 12.49
 PEAK AREA (M/Z 145) : 756424

DE-498 RETENTION TIME IS : 12.50
 PEAK AREA (M/Z 134) : 142543
 PEAK AREA (M/Z 142) : 80802

DE-498 CONFIRMATION
 RATIO OF M/Z 142/134: 0.5669

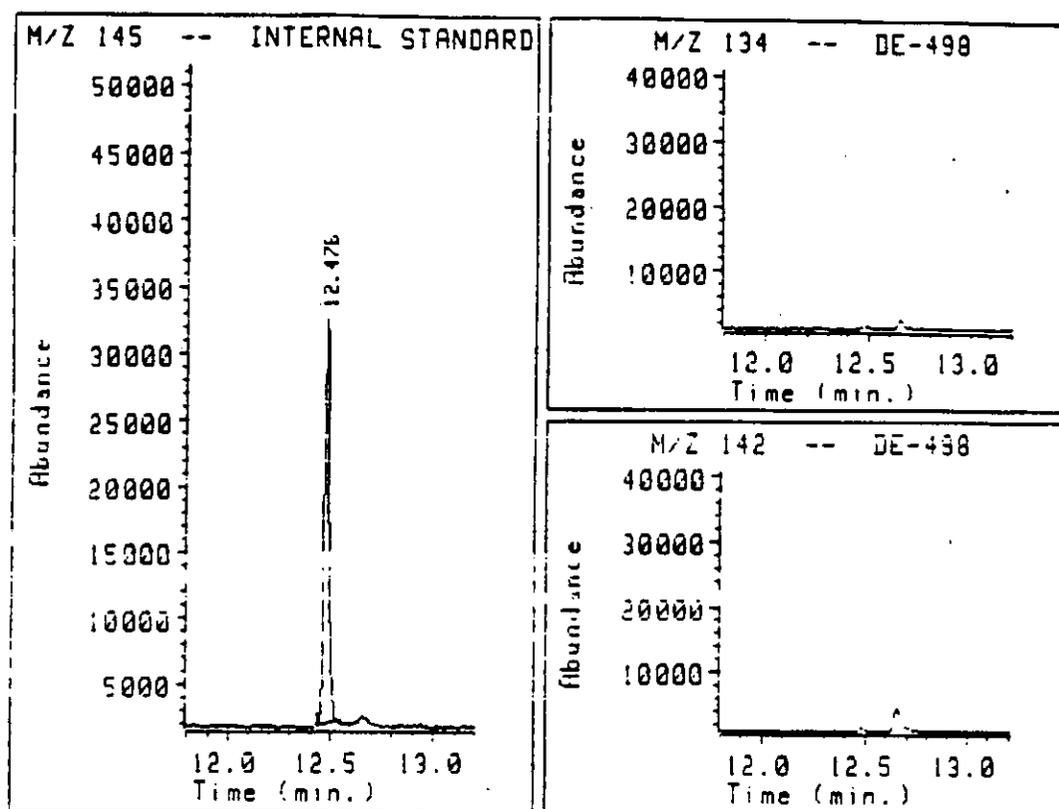
DE-498 QUANTITATION
 RATIO OF M/Z 134/145: 0.1884

EQUIVALENT DE-498 CONCENTRATION: 0.00502 ppm
 AVERAGE CONFIRMATION RATIO: 0.5269

FIGURE 4. TYPICAL CHROMATOGRAM OF A 10.0 ng/mL STANDARD EQUIVALENT TO 0.005 ppm DE-498 IN CORN GRAIN.

EFFECTIVE: September 4, 1992

ACR #: 91.6.1S



Data File : DATA2:CE17A66A.D
 Date : 18 Apr 91 3:30 pm
 Instrument : MS_5971A - S/N 2749A00149

Sample Name: AGR290149 - CORN GRAIN - CONTROL
 Sample Info: SAMPLE FROM COLO. IOWA
 Operator : E. L. OLBERDING

INTSTD RETENTION TIME IS : 12.48
 PEAK AREA (M/Z 145) : 535486

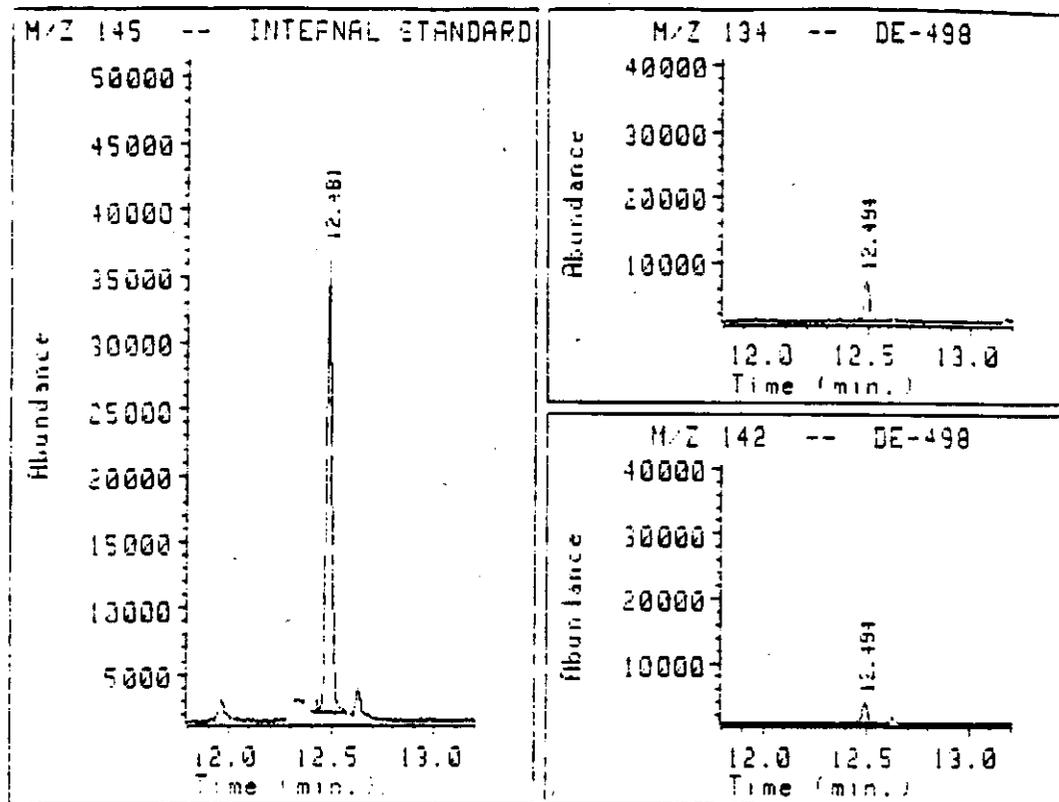
NO DE-498 FOUND

GROSS DE-498 CONCENTRATION: 0.00000 ppm
 CORRECTED DE-498 CONCENTRATION: 0.00000 ppm
 AVERAGE CONFIRMATION RATIO: 0.5269

FIGURE 5. TYPICAL CHROMATOGRAM OF A CONTROL SAMPLE OF CORN GRAIN CONTAINING NO DETECTABLE RESIDUE OF DE-498.

EFFECTIVE: September 4, 1992

ACR #: 91.6.15



Data File : DATA2:CE17A12A.D
 Date : 17 Apr 91 5:38 pm
 Instrument : MS_5971A - S/N 2749A00149

Sample Name: AGR289842 - CORN GRAIN - SPIKED AT 0.00502 PPM - BEFORE - A
 Sample Info: SAMPLE FROM GENESEO, ILLINOIS (DOW)
 Operator : E. L. OLBERDING

INTSTD RETENTION TIME IS : 12.48
 PEAK AREA (M/Z 145) : 640276

DE-498 RETENTION TIME IS : 12.49
 PEAK AREA (M/Z 134) : 112860
 PEAK AREA (M/Z 142) : 61558

DE-498 CONFIRMATION
 RATIO OF M/Z 142/134: 0.5454

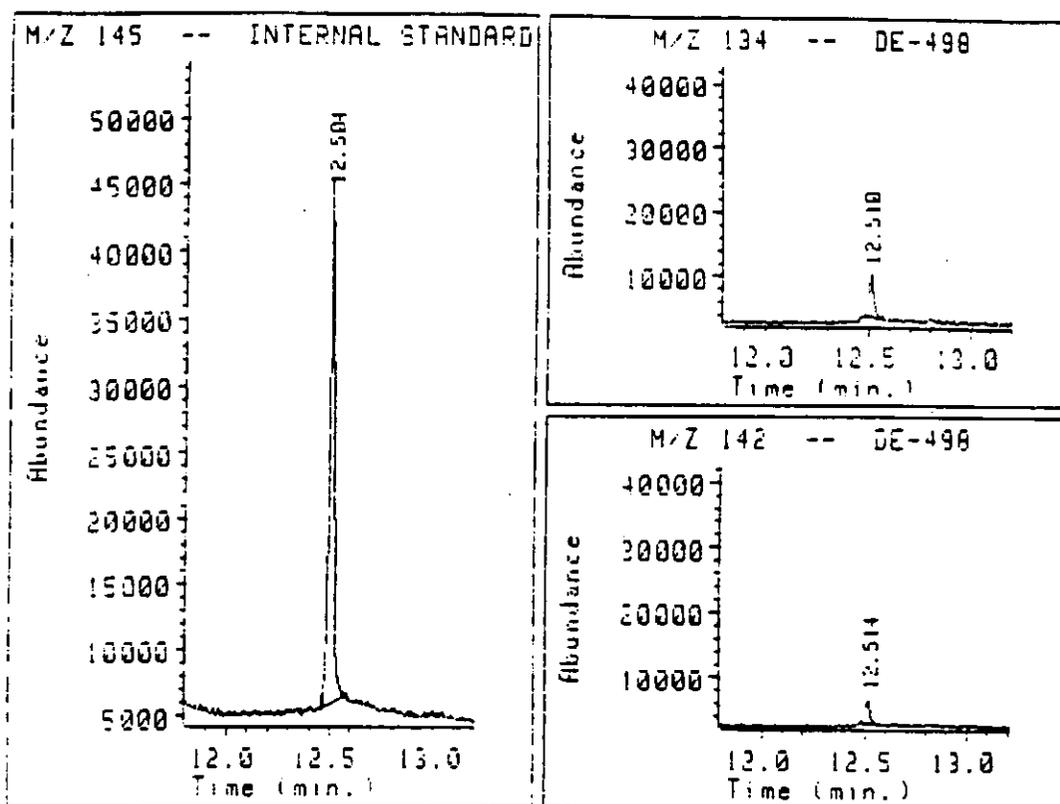
DE-498 QUANTITATION
 RATIO OF M/Z 134/145: 0.1763

GROSS DE-498 CONCENTRATION: 0.00485 ppm
 CORRECTED DE-498 CONCENTRATION: 0.00485 ppm
 RECOVERY: 97%
 AVERAGE CONFIRMATION RATIO: 0.5269

FIGURE 6. TYPICAL CHROMATOGRAM OF A CONTROL SAMPLE OF CORN GRAIN FORTIFIED WITH 0.005 ppm DE-498.

EFFECTIVE: September 4, 1992

ACR #: 91.6.1S



Data File : DATA2:CE12A05A.D
 Date : 7 Apr 91 3:13 pm
 Instrument : MS_5971A - S/N 2749A00149

Sample Name: DE-498 STANDARD - 010.04 ng/mL - EQUIVALENT TO 0.00502 PPM
 Sample Info: *5/20/91 DOD*
 Operator : E. L. OLBERDING

INTSTD RETENTION TIME IS : 12.50
 PEAK AREA (M/Z 145) : 649556

DE-498 RETENTION TIME IS : 12.51
 PEAK AREA (M/Z 134) : 112767
 PEAK AREA (M/Z 142) : 61866

DE-498 CONFIRMATION
 RATIO OF M/Z 142/134: 0.5486

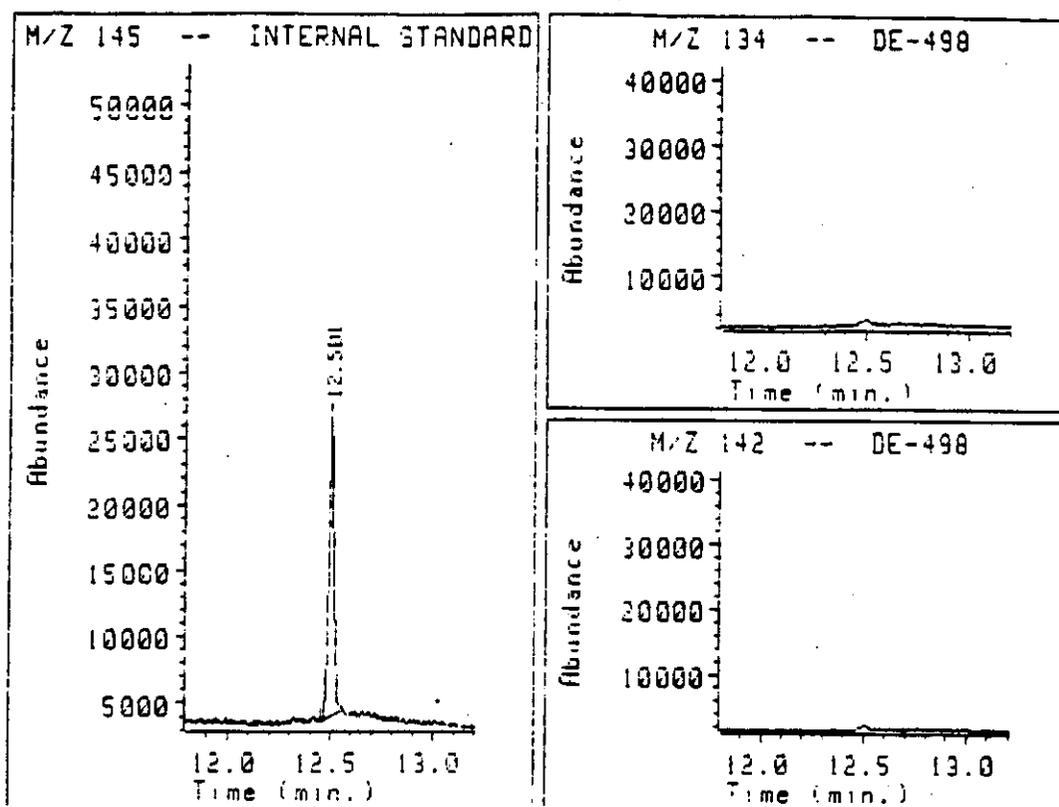
DE-498 QUANTITATION
 RATIO OF M/Z 134/145: 0.1736

EQUIVALENT DE-498 CONCENTRATION: 0.00502 ppm
 AVERAGE CONFIRMATION RATIO: 0.5119

FIGURE 7. TYPICAL CHROMATOGRAM OF A 10.0 ng/mL STANDARD EQUIVALENT TO TO 0.005 ppm DE-498 IN CORN FORAGE.

EFFECTIVE: September 4, 1992

ACR #: 91.6.15



Data File : DATA2:CE12A24A.D
Date : 7 Apr 91 11:09 pm
Instrument : MS_5971A - S/N 2749A00149

Sample Name: AGR287271 - CORN FORAGE - CONTROL
Sample Info: SAMPLE FROM PINEHURST, GEORGIA
Operator : E. L. OLBERDING

INTSTD RETENTION TIME IS : 12.50
PEAK AREA (M/Z 145) : 405117

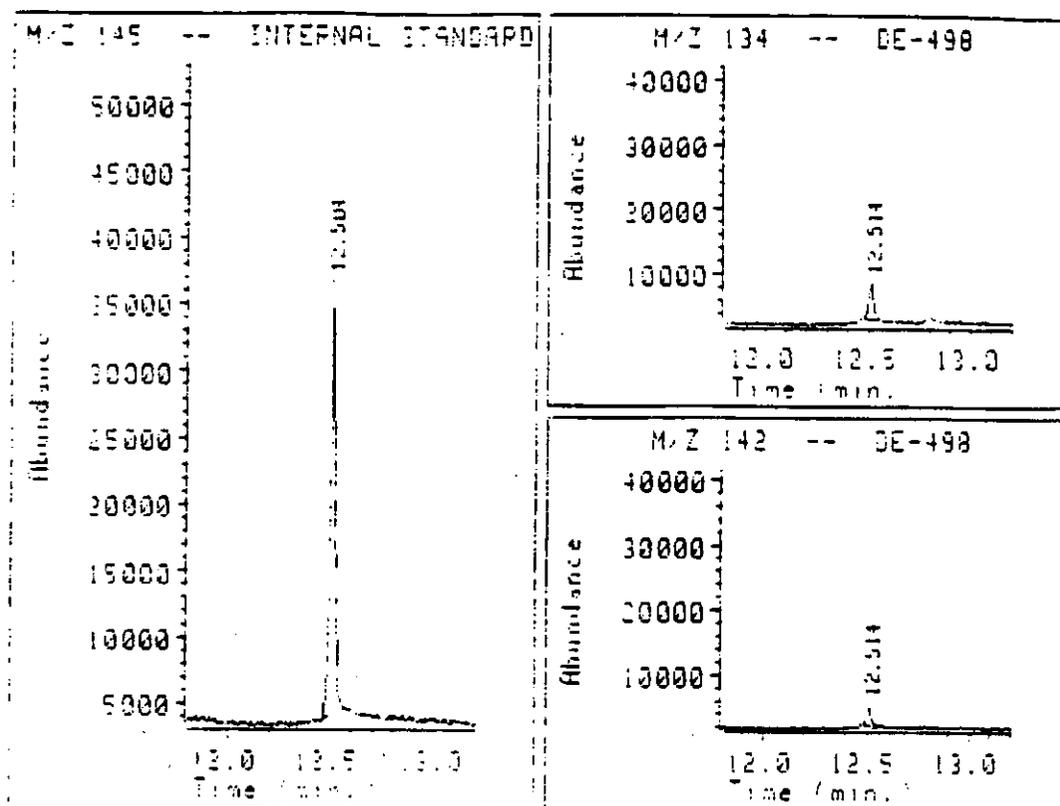
NO DE-498 FOUND

GROSS DE-498 CONCENTRATION: 0.00000 ppm
CORRECTED DE-498 CONCENTRATION: 0.00000 ppm
AVERAGE CONFIRMATION RATIO: 0.5119

FIGURE 8. TYPICAL CHROMATOGRAM OF A CONTROL SAMPLE OF CORN FORAGE CONTAINING NO DETECTABLE RESIDUE OF DE-498.

EFFECTIVE: September 4, 1992

ACR #: 91.6.15



Data File : DATA2:CE12A13A.D
 Date : 7 Apr 91 6:42 pm
 Instrument : MS_5971A - S/N 2749A00149

Sample Name: AGR290273 - CORN FORAGE - SPIKED AT 0.00502 PPM - BEFORE B
 Sample Info: SAMPLE FROM DANVILLE, IOWA
 Operator : E. L. OLBERDING

INTSTD RETENTION TIME IS : 12.50
 PEAK AREA (M/Z 145) : 528111

DE-498 RETENTION TIME IS : 12.51
 PEAK AREA (M/Z 134) : 83872
 PEAK AREA (M/Z 142) : 45277

DE-498 CONFIRMATION
 RATIO OF M/Z 142/134: 0.5393

DE-498 QUANTITATION
 RATIO OF M/Z 134/145: 0.1588

GROSS DE-498 CONCENTRATION: 0.00468 ppm
 CORRECTED DE-498 CONCENTRATION: 0.00468 ppm
 RECOVERY: 93%
 AVERAGE CONFIRMATION RATIO: 0.5119

FIGURE 9. TYPICAL CHROMATOGRAM OF A CONTROL SAMPLE OF CORN FORAGE FORTIFIED WITH 0.005 ppm DE-498.