GRM #: 94.07.R1
EFFECTIVE: February 8, 1995
SUPERSEDES: 94.07

Determination of Residues of XDE-565 in Soybean Grain, Forage and Hay by Capillary Gas Chromatography/Mass Spectrometry

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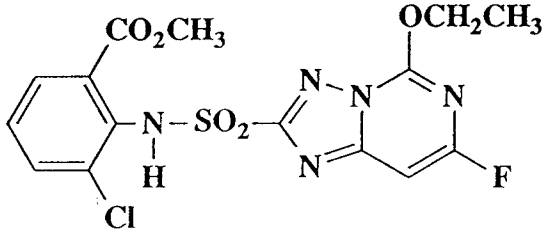
A. Scope

This method is applicable for the quantitative determination of residues of XDE-565, N-(2-carbomethoxy-6-chlorophenyl)-5-ethoxy-7-fluoro[1,2,4]triazolo[1,5-c]-pyrimidine-2-sulfonamide, in soybean grain, forage and hay over the concentration range of 0.01-0.50 µg/g with a validated limit of quantitation (LOQ) of 0.01 µg/g.



XDE-565
CAS No. 147150-35-4

B. Principle

Soybean grain, forage and hay samples are extracted using 90% acetone/10% 0.1 N hydrochloric acid. An aliquot is evaporated to dryness, and the remaining residue is buffered to pH 7.5 and partitioned against n-hexane. The hexane is discarded and the aqueous solution is acidified and purified using C_{18} and neutral alumina solid phase extractions (SPE).

The eluant from the neutral alumina SPE column is evaporated to dryness and derivatized with trimethylsilyldiazomethane (TMS-diazomethane) (Figure 1). Following derivatization, the sample is evaporated to dryness and the N-methyl-XDE-565 derivative is partitioned from an aqueous solution into toluene containing N-ethyl-XDE-565 as the internal standard. Analysis is by capillary gas chromatography with mass spectrometry (GC/MS).

C. Safety Precautions

1. 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 obtained from the container label or from the supplier. Disposal of reagents, reactants, and solvents must be made in compliance with local, state, and federal laws and regulations.

2. Volatile and flammable organic solvents must be used in well-ventilated areas away from ignition sources.

3. Acetic, hydrochloric and phosphoric acids, as well as sodium hydroxide, are corrosive and can cause severe burns.

4. The derivatization reagent, trimethylsilydiazomethane is flammable, is a strong irritant and a neurological hazard.

5. Proper eye protection must be worn while handling these and all reagents. Long laboratory coats and protective gloves must also be worn.

6. Erlenmeyer flasks under vacuum are susceptible to implosion. Use polypropylene flasks or glass flasks wrapped with electrical tape in combination with proper shielding.

D. Equipment (Note L.1.)

1. Automatic sampler, Model 7673A, Hewlett-Packard, Wilmington, DE 19808-9824.

2. Balance, analytical, Model AE200, Mettler Instrument Corporation, Highstown, NJ 08520.

3. Balance, analytical, Model PM600, Mettler Instrument Corporation.

4. Centrifuge, Model IEC Centra-8, or equivalent, equipped with rotor and cups to hold 40-mL vials (27 x 110 mm), International Equipment Company, Needham Heights, MA 02194.

5. Evaporator, Reacti-Vap Evaporator, cat. no. 18780Z, Pierce Chemical Company, Rockford, IL 61105.

6. Evaporator, TurboVap, Zymark Corporation, Zymark Center, Hopkinton, MA 01748.

7. Gas chromatograph, Model 5890 Series II, Hewlett-Packard.

8. Grinder, hammermill, Model 2001, equipped with a 3/16-inch screen, AGVISE Laboratories, Northwood, ND 58267.

9. Heating block, Reacti-Block B-1, 9-hole, cat. no. 18802Z, Pierce Chemical Company.

10. Heating block, Reacti-Therm heating module, Pierce Chemical Company.

11. Homogenizer, Omni Mixer, Model 17105, Omni International, Waterbury, CT 06704.

12. Mass selective detector, Model 5971A, Hewlett-Packard, Palo Alto, CA 94304.

13. Mass spectrometer data system, Model G1034B, Hewlett-Packard.

14. Needles, Reacti-Vap polypropylene, cat. no. 7436-00 or PTFE coated needles, cat. no. 18784Z, Pierce Chemical Company.

15. Pipetter, adjustable digital, 10-100 µL, Eppendorf, cat. no. 53511-584, VWR Scientific, Chicago, IL 60666.

16. Pipetter, adjustable digital, 100-1000 µL, Eppendorf, cat. no. 53511-610, VWR Scientific.

17. Pipetter, adjustable, 1000-5000 µL, Oxford BenchMate, Oxford cat no. 8885-500036, Fisher Scientific, Pittsburgh, PA 15219.

18. Shaker, mechanical variable speed reciprocating with box carrier, Model 6000, Eberbach Corporation, Ann Arbor, MI 48106.

19. Stirring plate, Model 546725, Barnstead/Thermolyne, Dubuque, IA 52001.

20. Thermometer, Reacti-Therm, 0-200 °C, cat. no. 18911Z, Pierce Chemical Company.

21. Timer, cat. no. 06-662-5, Fisher Scientific, Pittsburgh, PA 15219.

22. Ultrasonic cleaning system, Model FS5, Fisher Scientific.

23. Vacuum mainfold box, cat. no. 5-7250, Supelco, Bellefonte, PA 16823.

24. Vial crimper, cat. no. 8710-0979, Hewlett-Packard, Avondale, PA 19311.

25. Vortex Mixer, Genie 2, cat. no. 12-812, Fisher Scientific.

26. Water purification system, Milli-Q UV Plus, Millipore Corporation, Milford, MA 01730.

E. Glassware and Materials (Note L.1.)

1. Bottles, 8-oz. Qorpak cat. no. 7984 with PTFE-lined closures, Fisher cat. no. 03-320-11G.

2. Charcoal scrubber, cat. no. 7972, Chrompack, Inc., Raritan, NJ 08869 (Note L.2.).

3. Column, capillary gas chromatography, DB-5 liquid phase, 10 m x 0.18 mm i.d., 0.4 µm film thickness, cat. no. 121-5013, J &W Scientific, Folsom, CA 95630.

4. Column, neutral alumina SPE, cat. no. P477, Fisher Scientific.

5. Column, C_{18} SPE, cat. no. P479, Fisher Scientific.

6. Filters, glass fiber Acrodisc, cat. no. 4523, Gelman Sciences, Ann Arbor, MI 48106.

7. Gas, helium, 99.995% purity, BOC Gases, Indianapolis, IN 46241.
8. Gas, nitrogen, technical grade, BOC Gases.
9. Inlet sleeve, deactivated, cyclo double gooseneck splitless (4 mm i.d.), cat. no. 20895, Restek, Bellefonte, PA 16823-8812.
10. Moisture trap, cat. no. 7971, Chrompack, Inc. (Note L.2.).
11. Oxygen trap, cat. no. 7970, Chrompack, Inc. (Note L.2.).
12. pH Paper, cat. no. 9590, EM Science, Merck, Gibbstown, NJ 08027.
13. Pipets, serological, 10 x 0.1 mL, disposable, cat. no. 13-678-31J, Fisher Scientific.
14. Pipetter tips, disposable, 1-100 µL, Eppendorf, cat. no. 53511-802, VWR Scientific.
15. Pipetter tips, disposable, 101-1000 µL, Eppendorf, cat. no. 53511-868, VWR Scientific.
16. Pipetter tips, disposable, 1000-5000 µL, Oxford BenchMate, Oxford cat. no. 8885-091408, Fisher Scientific.
17. Septa, crimp-top, cat no. 52-410, The Mullhall Company, Worthington, OH 43085.
18. Syringes, plastic disposable 10 cc, cat. no. 9604, Becton Dickinson & Co., Franklin Lakes, NJ 07417-1884.
19. Vials, 8-mL screw-cap glass with PTFE-lined septa, cat. no. 03-393A, Fisher Scientific.
20. Vials, 40-mL glass, Kimble cat. no. 60958-A with PTFE-lined caps, cat. no. 02-883-3F, Fisher Scientific.
21. Vials, autosampler, for GC, cat. no. 225175SP, Wheaton, Millville, NJ 08332.
22. Vial inserts, 250 µL, cat. no. C4011-631, National Scientific Company, Lawrenceville, GA 30243.

F. Reagents and Chemicals (Note L.1.)

1. Reagents

   a. Acetone, Optima grade, cat. no. A929-4, Fisher Scientific.

   b. Acetonitrile, Optima grade, cat. no. A996-4, Fisher Scientific.

   c. Acetic acid, glacial, HPLC grade, cat no. A35-500, Fisher Scientific.

   d. Hexane, HPLC grade, cat. no. H302-4, Fisher Scientific.

   e. Hydrochloric acid, 0.1 N (certified), cat. no. SA54-1, Fisher Scientific.

   f. Hydrochloric acid, 0.5 N (certified), cat. no. SA50-1, Fisher Scientific.

g. Hydrochloric acid, 2 N (certified), cat. no. SA431-500, Fisher Scientific.

h. Methylene chloride, OmniSolv, cat. no. DX0831-1, EM Science, Gibbstown, New Jersey 08027.

i. Phosphoric acid, 99.99% purity, 85 wt. %, cat. no. 34,524-5, Aldrich Chemical Co., Milwaukee, WI 53233.

j. Potassium dihydrogen phosphate, certified, cat. no. P285-500, Fisher Scientific.

k. Sodium hydroxide, 0.1 N (certified), cat. no. SS276-1, Fisher Scientific.

l. Standards

(1) XDE-565, N-(2-carbomethoxy-6-chlorophenyl)-5-ethoxy-7-fluoro[1,2,4]triazolo[1,5-c]-pyrimidine-2-sulfonamide, Test Substance Coordinator, DowElanco, 9330 Zionsville Road, Indianapolis, IN 46268-1053.

(2) N-ethyl-XDE-565, N-(2-carbomethoxy-6-chlorophenyl)-5-ethoxy-7-fluoro[1,2,4]triazolo[1,5-c]-pyrimidine-2-ethylsulfonamide, DowElanco.

(3) N-methyl-XDE-565, N-(2-carbomethoxy-6-chlorophenyl)-5-ethoxy-7-fluoro[1,2,4]triazolo[1,5-c]-pyrimidine-2-methylsulfonamide, DowElanco.

m. Toluene, OmniSolv, cat. no. TX0737-1, EM Scientific.

n. Trimethylsilyldiazomethane, 2 M solution in hexanes, cat. no. 36,283-2, Aldrich Chemical Co.

2. Prepared Solutions (Note L.3.)

a. Acetic acid, 0.5% (v/v) in methylene chloride is prepared by pipeting 5 mL of glacial acetic acid and diluting to 1000 mL with methylene chloride in a volumetric flask.

b. Acetone (90%)/0.1 N hydrochloric acid (10%), (v/v) is prepared by pipeting 100 mL 0.1 N hydrochloric acid into approximately 800 mL acetone in a 1000-mL volumetric flask and mixing. After the solution equilibrates to ambient temperature, adjust to volume by adding acetone and mixing.

c. 0.5 N hydrochloric acid (80%)/acetonitrile (20%), (v/v) is prepared by pipeting 100 mL of acetonitrile into about 300 mL of 0.5 N hydrochloric acid in a 500-mL volumetric flask and mixing. After the solution equilibrates to ambient temperature, the volume is adjusted to 500 mL by adding 0.5 N hydrochloric acid and mixing.

d. Phosphoric acid, 0.01 M (in acetone), is prepared by placing 0.115 g of phosphoric acid (85 weight %) in a 100-mL volumetic flask and bringing to volume with acetone.

e. Potassium dihydrogen phosphate, 0.1 M, is prepared by dissolving 6.805 g of potassium dihydrogen phosphate (FW=136.09) in deionized water and adjusting to volume in a 500-mL volumetric flask.

f. Potassium dihydrogen phosphate buffer, 0.1 M (pH 7.5) is prepared by adding 41.1 mL of 0.1 N sodium hydroxide to 50 mL of the 0.1 M potassium dihydrogen phosphate in a graduated cylinder and mixing. Verify the pH of the final solution with pH paper.

G. Preparation of Standards (Note L.3.)

1. Internal Standard Preparation

a. Accurately weigh 0.1000 g of N-ethyl-XDE-565 analytical standard. Transfer to a 100-mL volumetric flask and dilute to volume with acetone to obtain a 1000 µg/mL stock solution.

b. Pipet 5.0 mL of the 1000 µg/mL solution from Section G.1.a. above to a 500-mL volumetric flask and dilute to volume with toluene to obtain a 10.0 µg/mL solution.

c. Pipet 40.0 mL of the 10.0 µg/mL solution from Section G.1.b. above to a 2000-mL volumetric flask and dilute to volume with toluene to obtain a 0.2 µg/mL solution. This is enough internal standard solution to prepare the calibration solutions (G.3.c. and d.) and dilute approximately 800 samples for analysis.

2. XDE-565 Spiking Solutions

a. Accurately weigh 0.1000 g of XDE-565 analytical standard. Tranfer to a 100-mL volumetric flask and dilute to volume with acetone to obtain a 1000 µg/mL stock solution.

b. Pipet 5.0 mL of the 1000 µg/mL solution from Section G.2.a. above to a 500-mL volumetric flask and dilute to volume with acetone to obtain a 10.0 µg/mL solution.

c. Pipet 50.0 mL of the 10.0 µg/mL solution from Section G.2.b. above to a 500-mL volumetric flask and dilute to volume with acetone to obtain a 1.00 µg/mL solution.

d. Prepare additional fortification solutions to cover the validation range by pipeting the prescribed solution into a volumetric flask and bringing to volume with acetone according to the following table:

Initial Soln. Conc.	Aliquot of Initial Soln.	Final Soln. Vol.	Spiking Soln. Final Conc.	Equivalent XDE-565 Sample Conc.[a]
µg/mL	mL	mL	µg/mL	µg/g
1.00	5.0	100	0.050	0.005
1.00	10.0	100	0.100	0.010
1.00	25.0	100	0.250	0.025
1.00	50.0	100	0.500	0.050
1.00	--	--	1.00	0.100
10.0	25.0	100	2.50	0.250
10.0	50.0	100	5.00	0.500

[a]The equivalent sample concentration is based on fortifying a 10.0-g tissue sample with 1.0 mL of the appropriate fortification solution.

3. N-methyl-XDE-565 Calibration Standards

a. Accurately weigh 0.1033 g of N-methyl-XDE-565 analytical standard. Tranfer to a 100-mL volumetric flask and dilute to volume with acetone to obtain a 1033 µg/mL solution (equivalent to 1000 µg/mL XDE-565).

b. Pipet 5.0 mL of the 1033 µg/mL N-methyl-XDE-565 solution from Section G.3.a. above and 10.0 mL of the 10.0 µg/mL internal standard solution (N-ethyl XDE-565) placing both in a 500-mL volumetric flask. Dilute to volume with toluene to obtain a 10.3 µg/mL calibration standard solution (equivalent to 10.0 µg/mL XDE-565) plus 0.2 µg/mL internal standard.

c. Pipet 50.0 mL of the 10.3 µg/mL N-methyl-XDE-565 solution from Section G.3.b. above containing the 0.2 µg/mL internal standard and place in a 500-mL volumetric flask. Dilute to volume with the 0.2 µg/mL internal standard solution as prepared in section G.1.c. to obtain a 1.03 µg/mL calibration standard solution (equivalent to 1.00 µg/mL XDE-565).

d. Prepare additional calibration curve solutions as needed. Pipet the indicated concentration and volume of each N-methyl-XDE-565 solution containing the 0.2 µg/mL internal standard into 100-mL volumetric flasks. Dilute to volume with internal standard solution (0.2 µg/mL in toluene) as prepared in section G.1.c. according to the following table:

Initial Soln. Conc.	Aliquot of Initial Soln	Final Soln. Vol.	Calibration Soln. Final Conc.[a]	Equivalent XDE-565 Sample Conc.[b]
µg/mL	mL	mL	µg/mL	µg/g
1.03	1.0	100	0.010	0.005
1.03	2.0	100	0.020	0.010
1.03	5.0	100	0.050	0.025
1.03	10.0	100	0.100	0.050
1.03	20.0	100	0.200	0.100
1.03	50.0	100	0.500	0.250
1.03	--	--	1.00	0.500
10.3	20.0	100	2.00	1.00

[a] The XDE-565 concentration equivalent to the N-methyl XDE-565 standard.
[b] The equivalent XDE-565 concentration based on comparison of the N-methyl-XDE-565 standard to a 2.0-g equivalent matrix sample.

H. Gas Chromatography/Mass Spectrometry

1. Typical Operating Conditions

Instrumentation: Hewlett-Packard Model 5890 GC
Hewlett-Packard Model 5971A Mass Selective Detector
Hewlett-Packard Model 7673A Autoinjector
Hewlett-Packard Model G1034B Data System Software

Column:	J&W Scientific fused silica capillary DB-5 liquid phase 10 m x 0.18 mm i.d. 0.4 µm film thickness
Temperatures:	
Column	120 °C for 1.1 min 120 to 325 °C at 20 °C/min 325 °C for 2.0 min Total run time of 13.83 min
Injector:	300 °C
Transfer Line:	310 °C
Carrier Gas:	Helium
Head Pressure:	43 kPa
Linear Velocity:	Approximately 49.7 cm/sec
Injection Mode:	Splitless
Purge Delay:	1.0 min
Septum Purge:	1.0 mL/min
Splitter Flow:	50 mL/min
Injection Port Liner:	Cyclo Double Gooseneck, Splitless (4 mm i.d.)
Injection Volume:	3 µL
Ionization Mode:	Electron impact
Acquisition Mode:	Selected Ion Monitoring
Ions Monitored: N-methyl-XDE-565 N-ethyl-XDE-565	 *m/z* 166 (quantitation), *m/z* 198 (confirmation) *m/z* 212 (quantitation) Tuned using Maximum Sensitivity Autotune (Note L.4. and 5.)
Electron Multiplier:	2100 volts (absolute); tuning was at approximately 2000 volts
Dwell Time:	50 msec

2. Full Scan Spectra

Typical full scan spectra for N-methyl-XDE-565 and the N-ethyl-XDE-565 (internal standard) are shown in Figures 2 and 3.

3. Calibration Curve

A typical calibration curve for the determination of XDE-565 in soybean grain, forage or hay is shown in Figure 4.

4. Typical Chromatograms

Typical chromatograms of a standard, a control sample, and a 0.01 µg/g (LOQ) recovery sample for soybean grain, forage and hay are shown in Figures 5-11, respectively.

I. Determination of Recovery of XDE-565 from Soybean Grain, Forage and Hay

1. Preparation of Recovery Samples

a. Grind the bulk sample of soybean grain, forage or hay thoroughly using a hammermill.

b. Weigh 10-g portions of the prepared soybean samples into a series of 8-oz wide-mouth bottles with PTFE-lined caps.

c. For preparation of fortified samples, add 1.0-mL aliquots of the appropriate spiking solutions to control matrix to obtain concentrations ranging from 0.01 to 0.5 µg/g. (See Section G.2.) A reagent blank, containing no soybean matrix, should be carried through the method with the samples.

d. Add 100 mL of 90% acetone/10% 0.1 N hydrochloric acid solution to the 10-g samples.

e. Blend each sample at high speed for approximately one minute using an Omni-Mixer homogenizer.

f. Cap the bottles and place on a reciprocating shaker at approximately 250 excursions/min for a minimum of 2 hours.

g. Centrifuge the extracted soybean grain, forage or hay at 2500 rpm for 5 minutes.

h. Carefully transfer 20-mL aliquots of each sample using a volumetric pipet, and avoid picking up solids. Place each aliquot in a clean 40-mL vial.

i. Evaporate the extracts to dryness under nitrogen at 60 °C using the TurboVap until only an oily residue remains in each vial.

j. Add 10 mL of 0.1 M potassium dihydrogen phosphate buffer (pH 7.5) to each vial, sonicate and vortex mix thoroughly.

k. Add 10 mL of hexane to each vial, sonicate and then vortex mix thoroughly.

l. Centrifuge the vials for 5 minutes at 2500 rpm and carefully draw off and discard the hexane (top) layer using a disposable pipet. Avoid removal of solids found at the interface.

m. Place the vials under a stream of nitrogen at 60 °C on the TurboVap for about 10 minutes to evaporate any remaining hexane.

n. Add 2 mL of 2 N hydrochloric acid to each sample vial and vortex mix.

o. Allow the samples to stand until the aqueous portion clears and solid particles coagulate. Solid particles will further be removed by filtration in step p.(3).

p. Purify the sample by C_{18} SPE as follows: (Note L.6.)

(1) With the column on a vacuum manifold box, rinse the C_{18} column with 5 mL acetonitrile. (Do not allow the column bed to dry.)

(2) Condition the C_{18} column with 5 mL 0.5 N hydrochloric acid. (Do not allow the column bed to dry.)

(3) Add the sample to the top of the C_{18} column by slowly passing it dropwise through an Acrodisc glass fiber filter attached to a 10 cc disposable syringe to remove all of the solids.

(4) Add 10 mL 80% 0.5 N hydrochloric acid/20% acetonitrile directly to the top of the C_{18} column. With the aid of vacuum, maintain a flow rate of 1-3 mL/min. Discard the eluant.

(5) Thoroughly dry the column by drawing air through it for a minimum of 30 minutes. (The dry column should no longer feel cold to the touch.)

(6) Add 2 mL of hexane to the top of the C_{18} column as a final wash. Discard the eluant.

(7) Dry the C_{18} SPE column for an additional 10 minutes on the vacuum manifold by pulling air through it. (Again, the column should no longer feel cold to the touch when it is dry.)

(8) Elute the XDE-565 with 5 mL acetonitrile into a clean 8-mL vial at a flow rate of 1-3 mL/min. Save this eluant for further purification by alumina SPE (Note L.7.).

q. Purify the sample further by neutral alumina SPE as follows: (Note L.6.)

(1) Place the alumina SPE column on the vacuum manifold and wash with 5 mL acetonitrile. (Do not allow the column bed to dry.) (Note L.7.)

(2) Pass the acetonitrile eluent from the C_{18} SPE column through the alumina column at a flow rate of 1-3 mL/min. Discard the column eluant.

(3) Rinse the alumina SPE column with 5 mL acetonitrile.

(4) Dry the alumina column under vacuum for 10-15 minutes.

(5) Wash the alumina SPE column with 4 mL of 99.5% methylene chloride/0.5% acetic acid. (Do not allow the column bed to dry. Do not exceed 4 mL for the wash.) Discard the column eluant.

(6) Elute with 8 mL of 99.5% methylene chloride/0.5% acetic acid at a flow rate of 1-3 mL/min, collecting the eluant in a 8-mL vial.

(7) Evaporate the eluent from the alumina SPE column to complete dryness under nitrogen at 60 °C.

r. Derivatize the samples for analysis as follows:

(1) Add 1.0 mL of acetone, 10 µL of 0.01M phosphoric acid solution (in acetone) and 50 µL of TMS-diazomethane to each vial.

(2) Cap the samples with a PTFE-lined septa, vortex for 10-15 seconds, and allow to react at ambient temperature for 30 minutes.

(3) Evaporate all the solvents to dryness from each derivatized sample by placing under a stream of nitrogen at 60 °C.

(4) Add 4 mL deionized water, sonicate and vortex mix thoroughly.

(5) Add 1.0 mL of the 0.2 µg/mL N-ethyl XDE-565 internal standard solution (Section G.1.c.) to each vial, sonicate and vortex mix thoroughly.

(6) Carefully transfer a portion of the organic (top) layer to a 2-mL autoinjector vial containing a 250 µL glass insert and seal.

(7) Analyze the samples by capillary GC/MS as described in Section H. (Note L.8.).

2. Calculation of Percent Recovery

a. Inject the calibration standards as described in Section G.3., and determine the peak areas for the *m/z* 166 and *m/z* 198 ions for the N-methyl-XDE-565 and for the *m/z* 212 ion for the N-ethyl-XDE-565 internal standard.

b. For each calibration standard, calculate the confirmation ratio. The average confirmation ratio for the XDE-565 calibration standards will be used to verify the presence of XDE-565 in soybean grain, forage and hay samples.

For example, using the data from Figure 5:

$$\text{Confirmation Ratio} = \frac{\text{peak area of confirmation ion}}{\text{peak area of quantitation ion}}$$

$$\text{Confirmation Ratio} = \frac{\text{peak area at } m/z \text{ 198}}{\text{peak area at } m/z \text{ 166}}$$

$$\text{Confirmation Ratio} = \frac{1042}{1539}$$

$$\text{Confirmation Ratio} = 0.6771$$

Positive confirmation of the presence of XDE-565 is indicated when the confirmation ratio for the samples is in the range of ±10% of the average found for the standards.

c. Prepare a standard curve by plotting the XDE-565 concentration on the abscissa (x-axis) and the *m/z* 166/212 peak area ratio on the ordinate (y-axis) as shown in Figure 4. Using a power regression analysis, determine the equation for the curve with respect to the abscissa (1).

For example, using a power regression with the data for XDE-565 from Figure 4:

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

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

$$\underset{(\mu g/mL)}{\text{XDE-565 Conc.}} = \left(\frac{m/z \text{ 166/212 peak area ratio}}{\text{constant}}\right)^{1/\text{exponent}}$$

$$\text{XDE-565 Conc. } (\mu g/mL) = \left(\frac{m/z\ 166/212 \text{ peak area ratio}}{10.0272}\right)^{1/1.1159}$$

d. Determine the net concentration in each recovery sample by substituting the peak area ratio obtained in the equation above and solve for the concentration, taking into account the final sample dilution volume and gross sample weight.

For example, using the data from Figure 7:

$$\text{XDE-565 Conc. } (\mu g/mL) = \left(\frac{\text{net } m/z\ 166/212 \text{ peak area ratio}}{10.0272}\right)^{1/1.1159}$$

$$\text{XDE-565 Conc. } (\mu g/mL) = \left(\frac{0.1120}{10.0272}\right)^{1/1.1159}$$

$$\text{XDE-565 Conc. } (\mu g/mL) = 0.0178\ \mu g/mL$$

$$\text{XDE-565 Conc. } (\mu g/g) = \frac{0.0178\ \mu g/mL \times 1.00\ mL \text{ (final volume)}}{2.0\ g \text{ sample weight}}$$

$$\text{XDE-565 Conc. } (\mu g/g) = 0.0089\ \mu g/g$$

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

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

$$\text{Recovery} = \frac{0.0089\ \mu g/g}{0.0100\ \mu g/g} \times 100\%$$

$$\text{Recovery} = 89.0\%$$

J. Determination of XDE-565 in Soybean Grain, Forage and Hay

1. Prepare reagent blank, control, recovery, and treated samples as described in Section I.1.

2. Prepare a standard calibration curve for XDE-565, and determine the percent recovery in the fortified samples as described in Section I.2.

3. Determine the concentration in each treated sample by substituting the *m/z* 166/212 peak area ratio obtained into the equation for the standard curve and solving

for concentration, taking into account the final sample dilution volume and actual sample weight.

For example, using the data from Figure 7:

$$\text{XDE-565 Conc.}_{(\mu g/mL)} = \left(\frac{m/z\ 166/212\ \text{peak area ratio}}{\text{constant}}\right)^{1/\text{exponent}}$$

$$\text{XDE-565 Conc.}_{(\mu g/mL)} = \left(\frac{0.1120}{10.0272}\right)^{1/1.1159}$$

$$\text{XDE-565 Conc.}_{(\mu g/mL)} = 0.0178\ \mu g/mL$$

$$\text{XDE-565 Conc.}_{(\mu g/g)} = \frac{0.0178\ \mu g/mL \times 1.00\ mL\ (\text{final volume})}{2.0\ g\ \text{equivalent sample weight}}$$

$$\text{XDE-565 Conc.}_{(\mu g/g)} = 0.0089\ \mu g/g$$

4. For the analysis of soybean grain, forage and hay samples in which results will be used for tolerance enforcement, the XDE-565 concentrations as determined in Section J.3. are reported without correction. For those analyses that require correction for method recovery, the following procedure is used:

   a. Determine XDE-565 concentrations in the samples as described in Section J.3.

   b. Determine the corrected XDE-565 concentration in the samples as follows:

$$\text{XDE-565 Conc.}_{(\text{corrected } \mu g/g)} = \text{XDE-565 Conc.}_{(\mu g/g)} \times \frac{100}{\text{Avg. \% Recovery}}$$

$$\text{XDE-565 Conc.}_{(\text{corrected } \mu g/g)} = 0.0089\ \mu g/g \times \frac{100}{90}$$

$$\text{XDE-565 Conc.}_{(\text{corrected } \mu g/g)} = 0.0099\ \mu g/g$$

K. Results and Discussion

1. Method Validation

   a. Recovery Levels and Precision Statement

   A method validation study was conducted to determine the recovery levels and the precision of the method for XDE-565 in soybean grain, forage and hay. The results are summarized in Tables I-III.

   Recovery values for XDE-565 from soybean grain over the concentration range of 0.01 to 0.50 µg/g averaged 85% with one standard deviation equal to 6% (Table I).

   Recovery values for XDE-565 from soybean forage over the concentration range of 0.01 to 0.50 µg/g averaged 87% with one standard deviation equal to 7% (Table II).

Recovery values for XDE-565 from soybean hay over the concentration range of 0.01 to 0.50 µg/g averaged 89% with one standard deviation equal to 9% (Table III).

b. Standard Curve Linearity

The average correlation coefficient (r^2) for the power least squares regression equations describing the detector response as a function of the standard calibration curve concentration was greater than 0.99 for each validation set.

c. Calculated Limits of Detection and Quantitation

The limits of quantitation (LOQ) and detection (LOD) for XDE-565 in soybean grain, forage and hay are calculated using the standard deviation from 0.01 µg/g (the targeted LOQ) recovery results. The limit of detection was calculated as three times the standard deviation, and the limit of quantitation was calculated as ten times the standard deviation following a published technique (2). Results are summarized in Tables IV-VI.

The calculated statistical values support an LOQ between 0.0021 and 0.0086 µg/g in soybean grain, forage and hay which is lower than the targeted method LOQ of 0.01 µg/g. However, results should not be quantified at levels below the 0.01 µg/g value unless samples have been analyzed to support that LOQ.

Similarly, in soybean grain, forage and hay, the calculated statistical values support an LOD between 0.0006 and 0.0026 µg/g. Since this is lower than the targeted LOQ, the instrumental conditions chosen for this method validation (i.e., injection volume, electron multiplier voltage, integration minimum area, etc.) were chosen to support the attainment of a signal to noise ratio of approximately 10:1 for a sample fortified at 0.005 µg/g.

Recovery values for residues found at levels below the validated LOQ should not be calculated or reported.

2. Extraction Efficiency

Plant metabolism studies were conducted on soybeans treated with ^{14}C-XDE-564, both pre- and post-emergence. Residues of the parent compound at or above 0.01 µg/g were found in soybean forage from the post-emergence treatment only (3,4). Therefore, only soybean forage samples containing quantifiable radiolabeled residues of parent compound were analyzed as described in the method. The total recovery of XDE-565 by GC/MS compared favorably to that of the theoretical found in concurrent metabolism assays (Table VII).

3. Confirmation of Residue Identity

Confirmation is by comparison of the retention times (gas chromatography) as well as by selected ion monitoring for obtaining confirmation ratios (mass spectrometry) as shown in Section I.2.b. Positive confirmation of the presence of XDE-565 is indicated when the confirmation ratio for the samples is in the range of ±10% of the average found for the calibration standards.

4. Assay Time

A typical analytical run would consist of a minimum of five standards encompassing the expected range of the gross sample concentrations, a reagent blank, a control (a non-fortified sample), a minimum of two fortified controls (one of which must be fortified at the LOQ), and fourteen samples. This procedure requires about 12 hours to complete with overnight injection of samples using an autosampler.

There are several acceptable "stopping points" in the method where sample preparation may be suspended without compromising the sample analysis results. These are indicated as follows:

a. Step I.1.f. It is possible to hold the samples overnight before taking aliquots for preparation as long as the samples remain tightly capped.

b. Step I.1.h. If samples are to be stored after aliquots are taken, they should be capped using PTFE-lined caps.

c. Step I.1.o. Following the addition of the 2 mL of 2 N hydrochloric acid, the samples can be held for several days. Stability of XDE-565 under acidic conditions for relately short periods of time is quite good.

d. Step I.1.p.(9) If samples are to be stored after aliquots are taken, they should be capped using PTFE-lined caps.

e. Step I.1.q.(6) If samples are to be stored after aliquots are taken, they should be capped using PTFE-lined caps.

f. Step I.1.q.(7) If samples are to be stored after aliquots are taken, they should be capped using PTFE-lined caps.

5. Standardization of SPE Elution Profiles (Note L.6.)

Variation in the C_{18} and alumina SPE columns may influence the elution profile of XDE-565. It is necessary to obtain an elution profile for each lot of SPE column used to ensure optimum recovery and purification efficiency. The following procedures can be used:

a. C_{18} SPE Profile

(1) Place 10 µL of the 1000 µg/mL XDE-565 spiking solution from Section G.2.a. in a 40-mL vial and evaporate to dryness at 60 °C under a stream of nitrogen.

(2) Add 10 mL of 0.1 M potassium dihydrogen phosphate buffer (pH 7.5) and 2 mL of 2 N hydrochloric acid to the vial. Sonicate and vortex mix well.

(3) With the C_{18} SPE column on a vacuum manifold, rinse with 5 mL acetonitrile. (Do not allow the column bed to dry.)

(4) Condition the C_{18} column with 5 mL 0.5 N hydrochloric acid. (Do not allow the column bed to dry.)

(5) Add the solution from Step (2) to the top of the C_{18} column using vacuum to pull the solution through the column and maintain the flow rate at 1-3 mL/min. (Do not allow the column bed to dry.) Discard the eluant.

(6) Add 10 mL 80% 0.5 N hydrochloric acid/20% acetonitrile to the top of the C_{18} column with the aid of vacuum to pull the wash through while keeping a flow rate of 1-3 mL/min. Discard the eluant.

(7) Throughly dry the C_{18} column by leaving it attached to the vacuum manifold and drawing air through it for 30-45 minutes. (The C_{18} should no longer feel cold to the touch.)

(8) Add 2 mL hexane to the top of the C_{18} SPE column, with the aid of vacuum, as a final wash. Discard any eluent from the column.

(9) Dry the C_{18} for an additional 10 minutes on the vacuum manifold by pulling air through it. (The C_{18} column should not feel cold to the touch when it is dry.)

(10) Elute the C_{18} SPE column with acetonitrile, collecting 1-mL individual fractions up to a total volume of 10 mL. The flow rate should be 1-3 mL/min.

(11) Evaporate fractions to dryness at 60 °C under a stream of nitrogen.

(12) For each fraction, derivatize and analyze by GC/MSD as described in Section I.1.r.

(13) Calculate the percent XDE-565 recovered for each fraction as described in Section I.2.

A typical elution profile is illustrated in Figure 12.

b. Alumina SPE Profile

(1) Place 10 µL of the 1000 µg/mL XDE-565 spiking solution in an 8-mL vial and evaporate to dryness at 60 °C under a stream of nitrogen (Section G.2.a.).

(2) Add 5 mL of acetonitrile to the vial, and sonicate and vortex mix well.

(3) With the alumina SPE column on a vacuum manifold, rinse with 5 mL acetonitrile. (Do not allow the column bed to dry.)

(4) Pass the solution prepared in Step (2) through the alumina column at a flow rate of 1-3 mL/min. Discard the column eluant.

(5) Rinse the alumina column with 5 mL acetonitrile.

(6) Dry the alumina SPE column under vacuum for 10-15 minutes.

(7) Wash the alumina with 4 mL of 99.5% methylene chloride/0.5% acetic acid. (Do not allow the column to dry. Do not exceed 4 mL for the wash.) Discard the eluant.

(8) Elute the alumina SPE column with 99.5% methylene chloride/0.5% acetic acid collecting 1 mL individual fractions up to a total volume of 15 mL. The flow rate should be 1-3 mL/min.

(9) These fractions are evaporated to dryness at 60 °C under a stream of nitrogen.

(10) For each fraction, derivatize and analyze by GC/MSD as described in Section I.1.r.

(11) Calculate the percent XDE-565 recovered for each fraction as described in Section I.2.

A typical elution profile is illustrated in Figure 13.

L. Notes

1. Equipment, glassware, reagents, and chemicals considered to be equivalent to those specified may be substituted with the understanding that their performance must be confirmed by appropriate testing. Common laboratory supplies are assumed to be readily available and therefore not listed in this method.

2. The scrubber/traps are used in the gas supply lines to purify the helium entering the GC/MS system.

3. All standard solutions and reagents are stored at ambient temperature in amber glass bottles with PTFE-lined closures.

4. The MSD was tuned using maximum sensitivity autotune software and the standard perfluorotributylamine calibration compound, selecting the filament and polarity that gives the greatest abundances. The GC oven temperature was set to 270 °C. Tuning with a mid-mass autotune conducted at *m/z* 69, 131, 219 may used as an alternative.

5. Sensitivity may be improved by optimizing SIM ions by performance of a dynamic mass calibration acquiring multiple ions in 0.1 AMU intervals and bracketing the nominal mass for the analyte.

6. An elution profile for each lot of C_{18} and alumina SPE columns should be performed to ensure optimum clean-up efficiency and recovery.

7. Depending on the number of samples that are to be prepared, XDE-565 can be eluted individually from each SPE column using either gravity feed or pressurized elution, or as a group, using the vacuum manifold box.

8. During the course of analyzing several hundred samples, it was noted that the N-methyl XDE-565 and N-ethyl XDE-565 chromatographic peaks would remain sharper and retain their level of sensitivity for much longer periods of time when in the presence of sample matrix vs. the absence of sample sample matrix (i.e., calibration standards). Removing a small section (approximately 20-40 cm) of the capillary column from the injection port side and/or replacing the injection port liner was found to remedy the problem.

M. References

1. *HP-41C/41 CV Standard Applications Handbook*, Hewlett-Packard Publication No. 00041-90402, 1982, pp 42-48.

2. Keith, L. H.; Crummett, W.; Deegan, J.; Libby, R.A.; Taylor, J.K.; Wentler, G., "Principles of Environmental Analysis", *Anal. Chem.* 55, 1983, 2210-2218.

3. Lewer, P.; Finney-Brink, K.L.; Brink, D.L., "[^{14}C]XDE-565: Nature of the Residue in Soybeans Following Postemergent Application", 1994, MET93060, unpublished report of DowElanco.

4. Stafford, L.E.; Lardie, T.S.; Brink, D.L., "[^{14}C]XDE-565: Nature of the Residue in Soybeans Following Preplant Incorporation," 1994, MET93061, unpublished report of DowElanco.

Table I. Recovery of XDE-565 From Soybean Grain

Sample Number	Date of Analysis	XDE-565, µg/g Added	XDE-565, µg/g Found	Percent Recovery
12077601	15-July-1994	0.000	ND[a]	NA[b]
12098701	15-July-1994	0.000	ND	NA
12025101	15-July-1994	0.000	ND	NA
12097901	17-July-1994	0.000	ND	NA
12025101	15-July-1994	0.005	<0.01	NA
12097901	22-July-1994	0.005	<0.01	NA
12076601	15-July-1994	0.010	0.0089	89
12076601	15-July-1994	0.010	0.0087	87
12076601	15-July-1994	0.010	0.0089	89
12098701	15-July-1994	0.010	0.0091	91
12098701	15-July-1994	0.010	0.0092	92
12098701	15-July-1994	0.010	0.0094	94
12025101	15-July-1994	0.010	0.0090	90
12025101	15-July-1994	0.010	0.0089	89
12025101	15-July-1994	0.010	0.0089	89
12097901	17-July-1994	0.025	0.0197	79
12097901	17-July-1994	0.025	0.0205	82
12097901	17-July-1994	0.050	0.0397	79
12097901	17-July-1994	0.050	0.0420	84
12097901	17-July-1994	0.100	0.0819	82
12097901	17-July-1994	0.100	0.0837	84
12097901	17-July-1994	0.250	0.194	78
12097901	17-July-1994	0.250	0.197	79
12097901	17-July-1994	0.500	0.370	74
12097901	17-July-1994	0.500	0.379	76
			$\bar{x}$ =	85
			s =	6
			n =	19

[a] ND = not detected.

[b] NA = not applicable. Recovery values for residues found below 0.01 µg/g (the LOQ of the method) are not calculated or reported.

Table II. Recovery of XDE-565 From Soybean Forage

Sample Number	Date of Analysis	XDE-565, µg/g Added	XDE-565, µg/g Found	Percent Recovery
14736201	19-July-1994	0.000	ND[a]	NA[b]
14736201	22-July-1994	0.000	ND	NA
14736201	19-July-1994	0.005	<0.01	NA
14736201	22-July-1994	0.005	<0.01	NA
14736201	19-July-1994	0.010	0.0086	86
14736201	19-July-1994	0.010	0.0085	85
14736201	19-July-1994	0.010	0.0082	82
14736201	19-July-1994	0.010	0.0084	84
14736201	19-July-1994	0.010	0.0087	87
14736201	19-July-1994	0.010	0.0071	71
14736201	19-July-1994	0.010	0.0089	89
14736201	19-July-1994	0.010	0.0099	99
14736201	19-July-1994	0.010	0.0099	99
14736201	22-July-1994	0.025	0.0236	94
14736201	22-July-1994	0.025	0.0245	98
14736201	22-July-1994	0.050	0.0462	92
14736201	22-July-1994	0.050	0.0402	80
14736201	22-July-1994	0.100	0.0829	83
14736201	22-July-1994	0.100	0.0911	91
14736201	22-July-1994	0.250	0.207	83
14736201	22-July-1994	0.250	0.212	85
14736201	22-July-1994	0.500	0.409	82
			$\bar{x}$ =	87
			s =	7
			n =	18

[a] ND = not detected.

[b] NA = not applicable. Recovery values for residues found below 0.01 µg/g (the LOQ of the method) are not calculated or reported.

Table III. Recovery of XDE-565 From Soybean Hay

Sample Number	Date of Analysis	XDE-565, µg/g Added	XDE-565, µg/g Found	Percent Recovery
13900902	23-July-1994	0.000	ND[a]	NA[b]
13900902	23-July-1994	0.000	ND	NA
13900902	23-July-1994	0.005	<0.01	NA
13900902	23-July-1994	0.005	<0.01	NA
13900902	23-July-1994	0.010	0.0089	89
13900902	23-July-1994	0.010	0.0091	91
13900902	23-July-1994	0.010	0.0091	91
13900902	23-July-1994	0.010	0.0090	90
13900902	23-July-1994	0.010	0.0099	99
13900902	23-July-1994	0.010	0.0104	104
13900902	23-July-1994	0.010	0.0085	85
13900902	23-July-1994	0.010	0.0094	94
13900902	23-July-1994	0.025	0.0238	95
13900902	23-July-1994	0.025	0.0239	96
13900902	23-July-1994	0.050	0.0468	94
13900902	23-July-1994	0.050	0.0497	99
13900902	23-July-1994	0.100	0.0864	86
13900902	23-July-1994	0.100	0.0905	91
13900902	23-July-1994	0.250	0.195	78
13900902	23-July-1994	0.250	0.186	74
13900902	23-July-1994	0.500	0.370	74
13900902	23-July-1994	0.500	0.399	80
			$\bar{x}$ =	89
			s =	9
			n =	18

[a] ND = not detected.

[b] NA = not applicable. Recovery values for residues found below 0.01 µg/g (the LOQ of the method) are not calculated or reported.

Table IV. Calculated Limits of Detection and Quantitation of XDE-565 in Soybean Grain

Sample Number	XDE-565, µg/g Added	Found
12076601	0.010	0.0089
12076601	0.010	0.0087
12076601	0.010	0.0089
12098701	0.010	0.0091
12098701	0.010	0.0092
12098701	0.010	0.0094
12025101	0.010	0.0090
12025101	0.010	0.0089
12025101	0.010	0.0089
	$\bar{x}$ =	0.0090
	s =	0.0002
	LOD[a] ($3s$) =	0.0006
	LOQ[b] ($10s$) =	0.0021
	n =	9

[a]LOD = Limit of Detection.
[b]LOQ = Limit of Quantitation.

Table V. Calculated Limits of Detection and Quantitation of XDE-565 in Soybean Forage

Sample Number	XDE-565, µg/g Added	Found
14736201	0.010	0.0086
14736201	0.010	0.0085
14736201	0.010	0.0082
14736201	0.010	0.0084
14736201	0.010	0.0087
14736201	0.010	0.0071
14736201	0.010	0.0089
14736201	0.010	0.0099
14736201	0.010	0.0099
	$\bar{x}$ =	0.0087
	s =	0.0009
	LOD[a] ($3s$) =	0.0026
	LOQ[b] ($10s$) =	0.0086
	n =	9

[a]LOD = Limit of Detection.
[b]LOQ = Limit of Quantitation.

Table VI. Calculated Limits of Detection and Quantitation of XDE-565 in Soybean Hay

Sample Number	XDE-565, µg/g Added	Found
13900902	0.010	0.0089
13900902	0.010	0.0091
13900902	0.010	0.0091
13900902	0.010	0.0090
13900902	0.010	0.0099
13900902	0.010	0.0104
13900902	0.010	0.0085
13900902	0.010	0.0094
	$\bar{x}$ =	0.0093
	s =	0.0006
	LOD[a] ($3s$) =	0.0018
	LOQ[b] ($10s$) =	0.0060
	n =	8

[a]LOD = Limit of Detection.
[b]LOQ = Limit of Quantitation.

Table VII. Total Recovery of XDE-565 from Soybean Forage Treated Post-Emergence

Compound Identity[a]	[^{14}C] XDE-565 Found, µg/g [From Metabolism Study (3)][b]	XDE-565 Found, µg/g by GC/MSD[c]
XDE-565 "A" Labeled	0.015 (1993)	0.013
	0.012 (1994)	0.012
XDE-565 "TP" Labeled	0.012 (1993)	0.013
	0.010 (1994)	0.013

a "A" is the aniline labeled XDE-565 and"TP" is the triazolopyrimidine labeled XDE-565.

b Forage samples from soybeans treated post-emergence with ^{14}C-XDE-565 during the plant metabolism study were characterized in 1993 and again in 1994. Levels of ^{14}C-XDE-565 were determined by thin layer chromatography of a fraction isolated by preparative silica gel column chromatography from the ethyl acetate-soluble fraction.

c The same ^{14}C forage samples as analyzed for XDE-565 during the metabolism study were analyzed in duplicate as described in DowElanco method GRM 94.07.

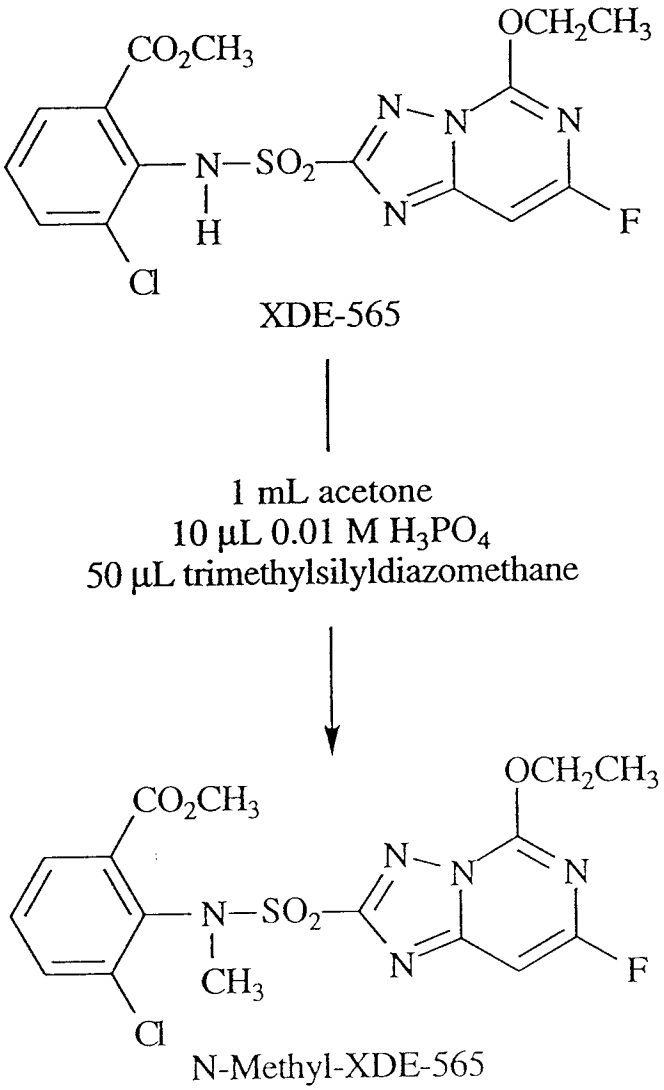
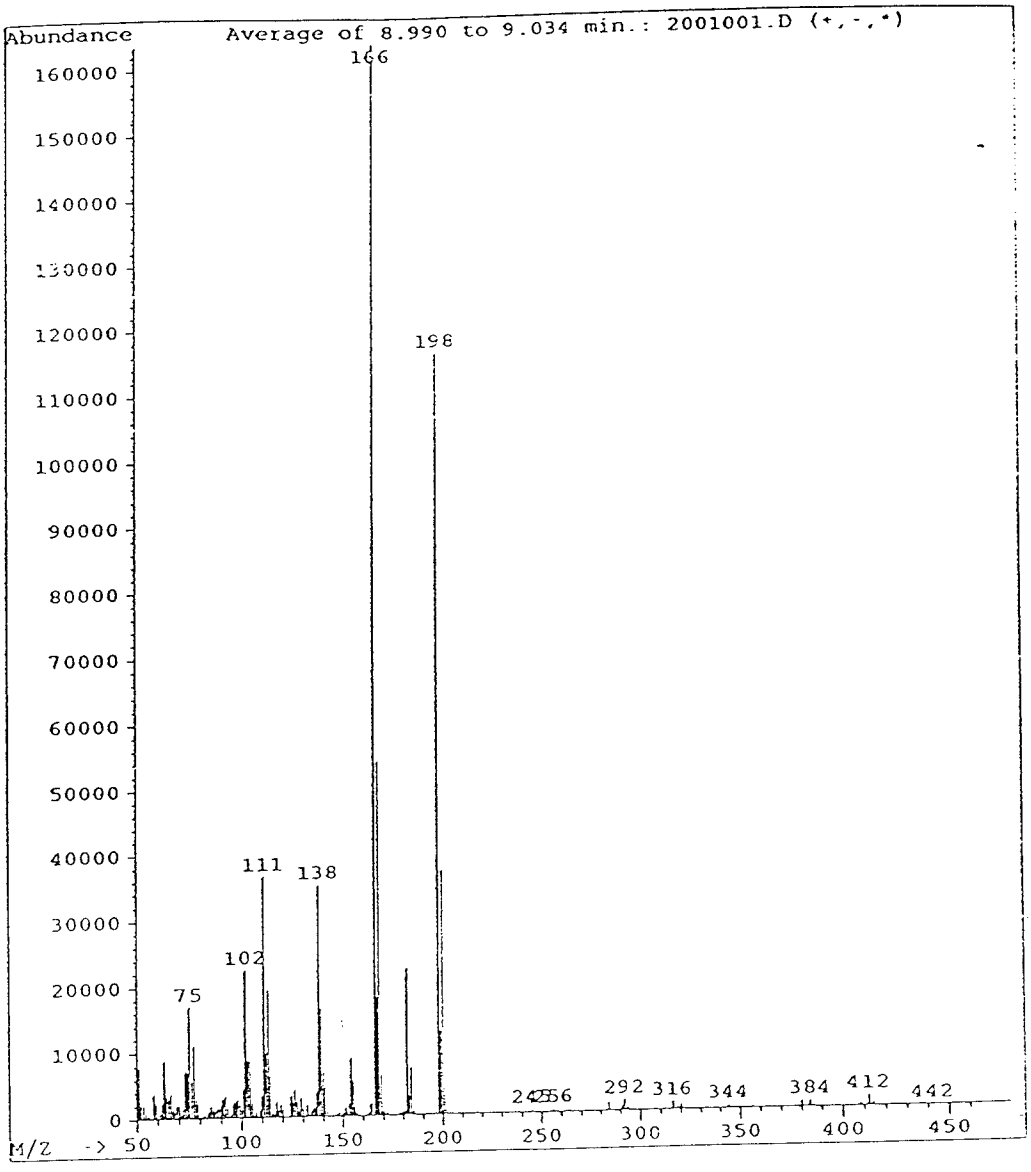
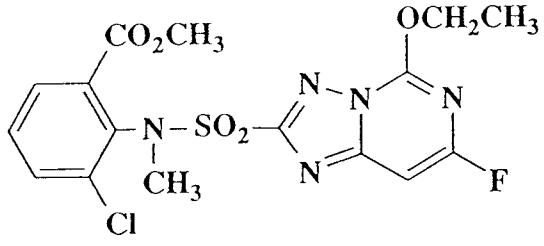


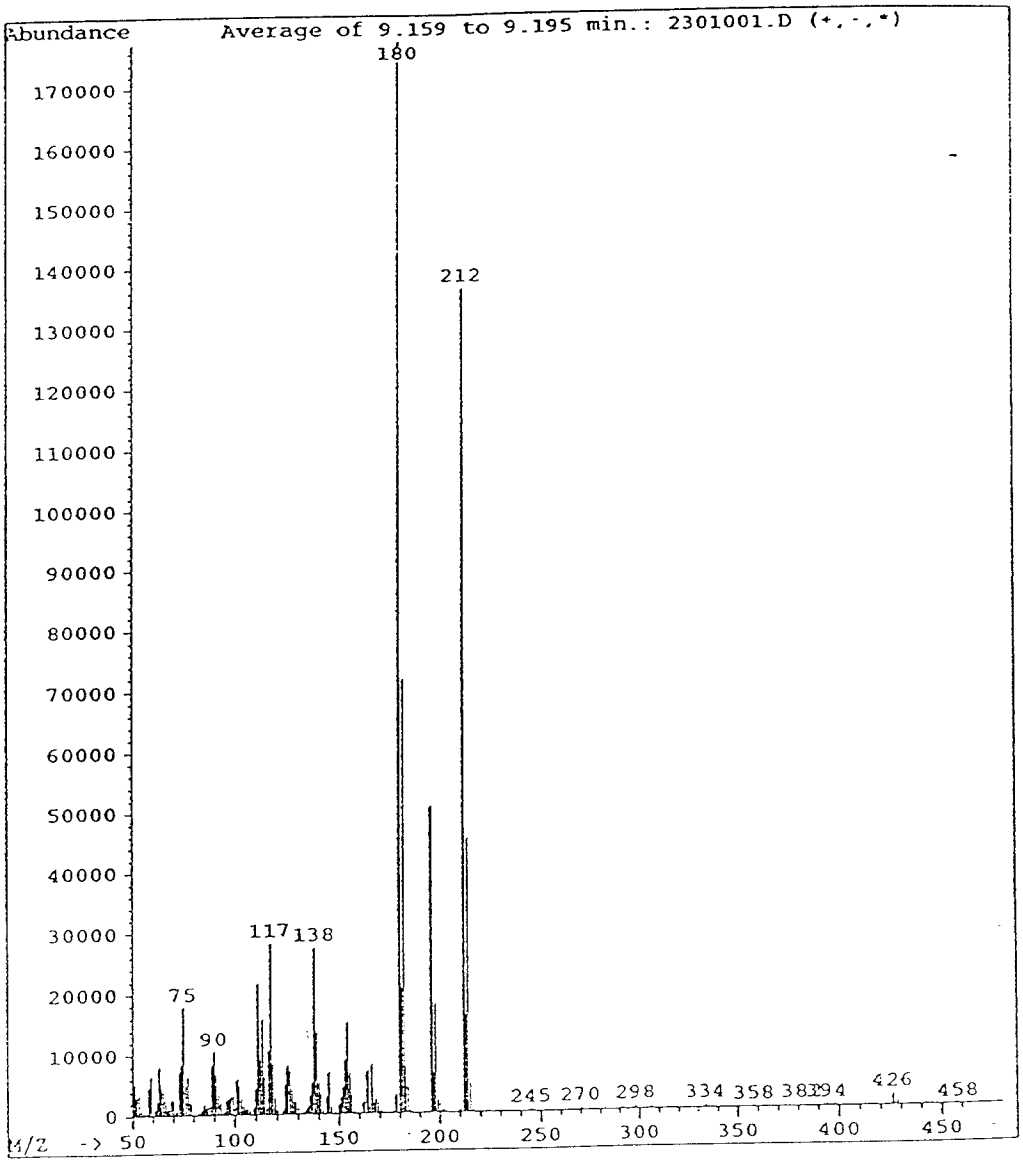
Figure 1. Derivatization of XDE-565 to N-methyl-XDE-565 with Trimethylsilyldiazomethane

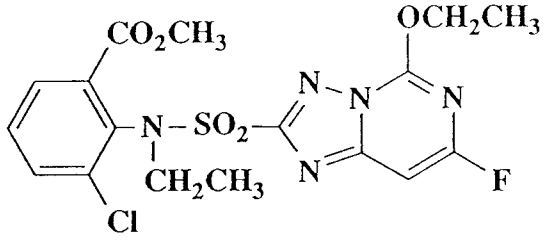




N-Methyl-XDE-565
$C_{16}H_{15}N_5O_5ClFS$, MW=443

Figure 2. Mass Spectrum of N-methyl-XDE-565

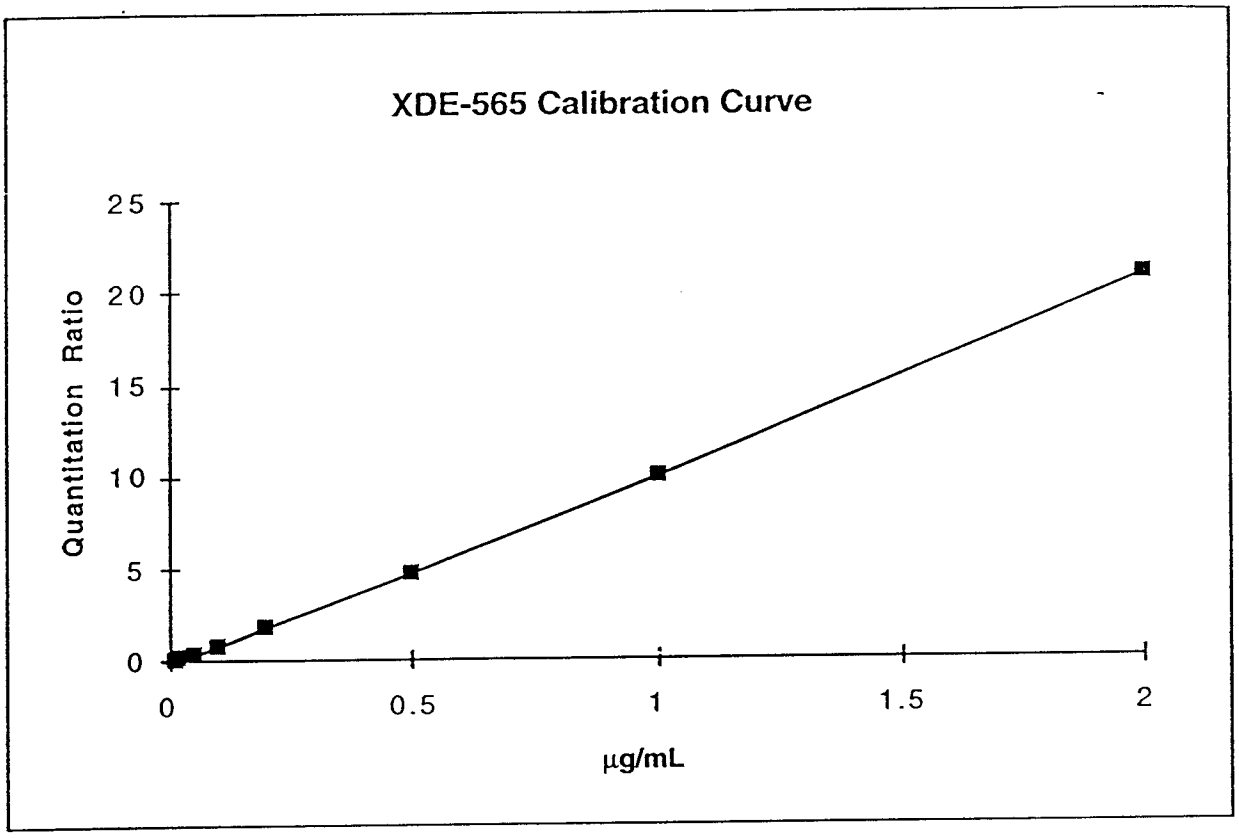




N-Ethyl-XDE-565

$C_{17}H_{17}N_5O_5ClFS$, MW= 457

Figure 3. Mass Spectrum of N-ethyl-XDE-565

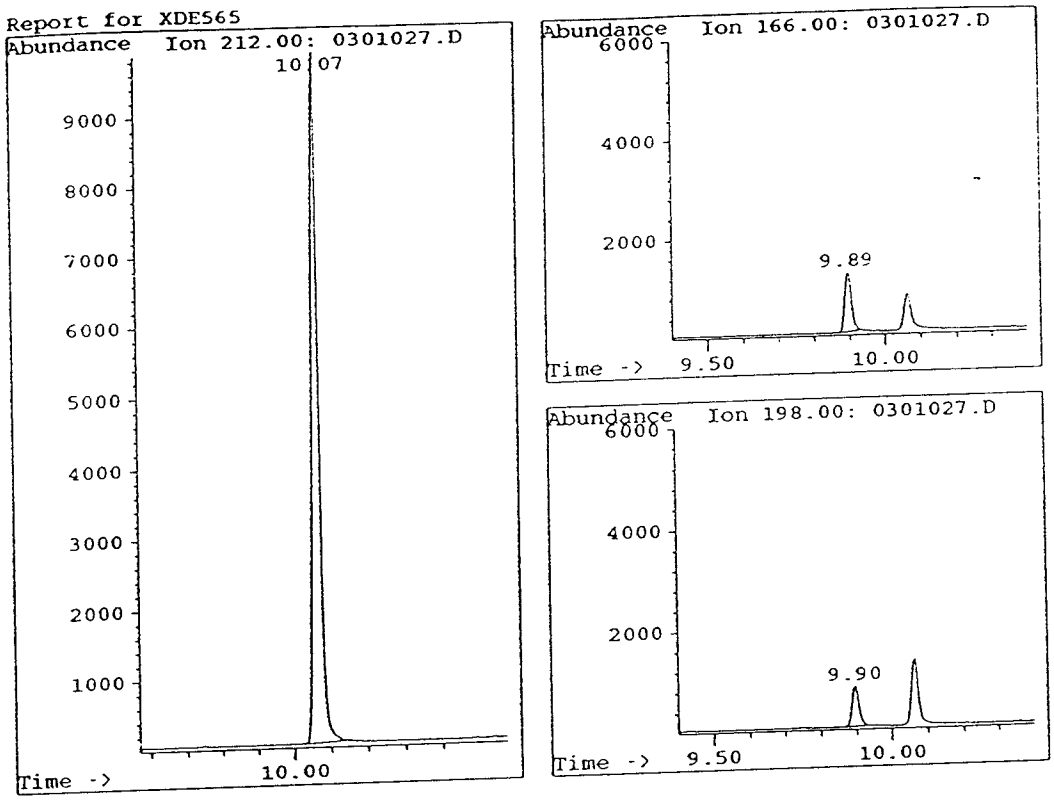


XDE-565 µg/mL	XDE-565 Quantitation Ratio
0.01	0.0590
0.02	0.1220
0.05	0.3550
0.10	0.7821
0.20	1.7292
0.50	4.7347
1.00	10.0351
2.00	20.8321

$$X = \left(\frac{Y}{10.0272}\right)^{1/1.1159}$$

$$r^2 = 0.9998$$

Figure 4. Typical Calibration Curve for the Determination of XDE-565 in Soybean Grain, Forage or Hay



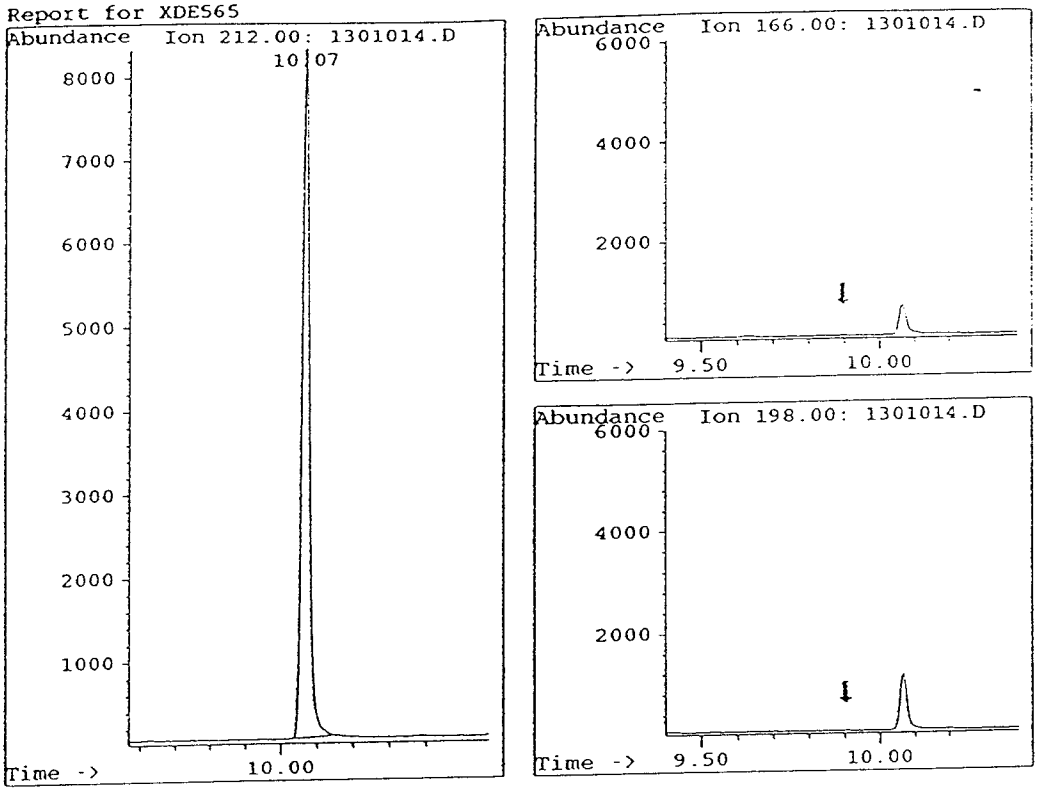
Data File : 0301027.D
ALS Bottle : 3
Date : 15 Jul 94 6:52 pm
Data Path : C:\HPCHEMPC\DATA\DS071594\
Instrument : GC/MSD S/N 3218A15556

Sample Name: B635:03 0.02 ug/ml Std. (0.2 ug/mL)
Sample Info: xde565.m (0.2 ug/mL internal std)
Operator : Darcy Shackelford

INTSTD RETENTION TIME:	10.07
PEAK AREA (M/Z 212):	12610
XDE565 RETENTION TIME:	9.89
PEAK AREA (M/Z 166):	1539
PEAK AREA (M/Z 198):	1042
XDE565 CONFIRMATION RATIO OF M/Z 198/166:	0.6771
XDE565 QUANTITATION RATIO OF M/Z 166/212:	0.1220

Average Confirmation Ratio: 0.672

Figure 5. Typical Chromatogram of a 0.02 µg/mL N-methyl-XDE-565 Standard Equivalent to 0.01 µg/g in Soybean Grain, Forage or Hay



Data File : 1301014.D
ALS Bottle : 13
Date : 15 Jul 94 3:14 pm
Data Path : C:\HPCHEMPC\DATA\DS071594\
Instrument : GC/MSD S/N 3218A15556

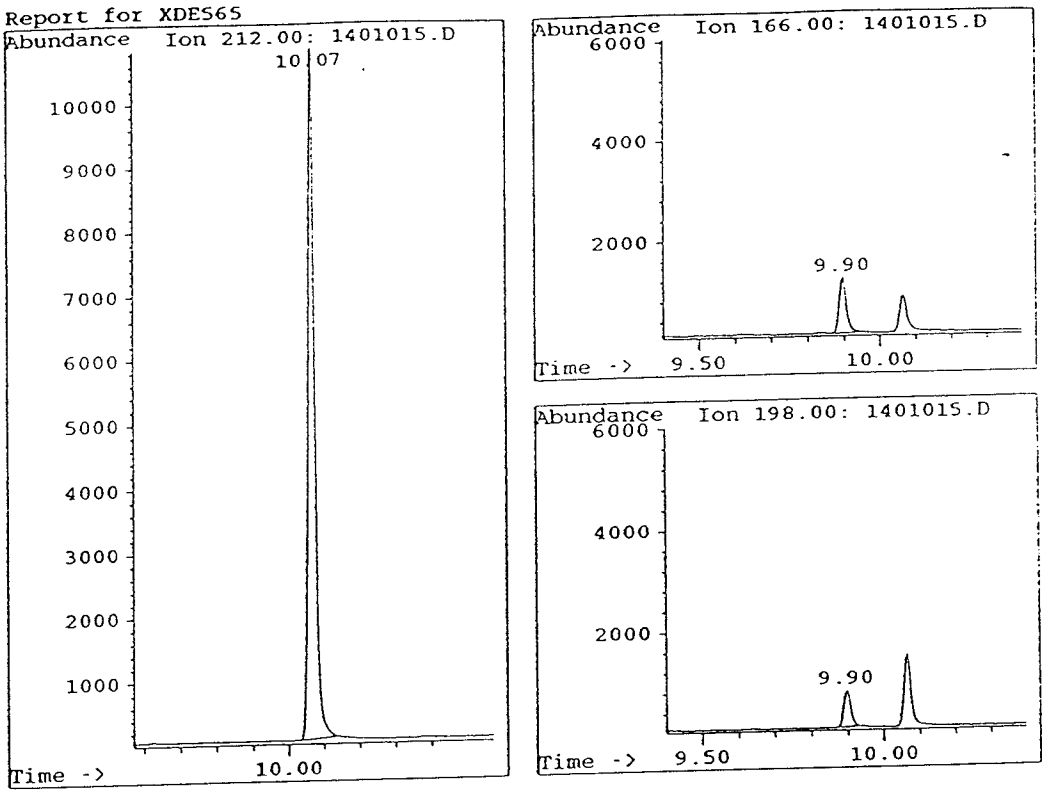
Sample Name: B651:1-4 2g eq CONTROL SOYBEANS SN#12025101
Sample Info: xde565.m (0.2 ug/mL int.std)
Operator : Darcy Shackelford

INTSTD RETENTION TIME: 10.07
PEAK AREA (M/Z 212): 11380

NO XDE565 FOUND

XDE-565 Concentration: 0.0000 µg/g
Average Confirmation Ratio: 0.672

Figure 6. Typical Chromatogram of a Control Sample of Soybean Grain Containing No Detectable Residue of XDE-565



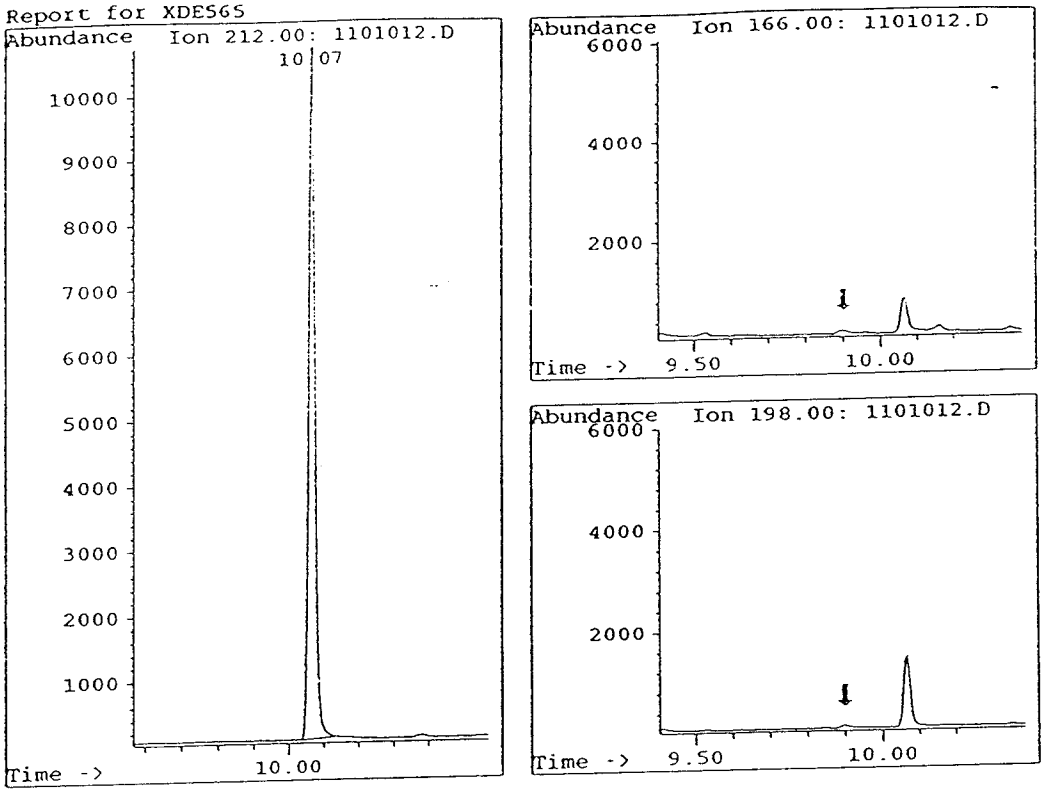
Data File : 1401015.D
ALS Bottle : 14
Date : 15 Jul 94 3:31 pm
Data Path : C:\HPCHEMPC\DATA\DS071594\
Instrument : GC/MSD S/N 3218A15556

Sample Name: B651:1-5 2g eq SOYBEAN SN#12076601+0.01 UG/G
Sample Info: xde565.m (0.2 ug/mL int.std)
Operator : Darcy Shackelford

INTSTD RETENTION TIME:	10.07
PEAK AREA (M/Z 212):	13729
XDE565 RETENTION TIME:	9.90
PEAK AREA (M/Z 166):	1537
PEAK AREA (M/Z 198):	944
XDE565 CONFIRMATION	
RATIO OF M/Z 198/166:	0.6142
XDE565 QUANTITATION	
RATIO OF M/Z 166/212:	0.1120

XDE-565 Concentration: 0.0089 µg/g
Recovery: 89%
Average Confirmation Ratio: 0.672

Figure 7. Typical Chromatogram of a Control Sample of Soybean Grain Fortified With 0.01 µg/g XDE-565



Data File : 1101012.D
ALS Bottle : 11
Date : 19 Jul 94 8:11 pm
Data Path : C:\HPCHEMPC\DATA\DS071994\
Instrument : GC/MSD S/N 3218A15556

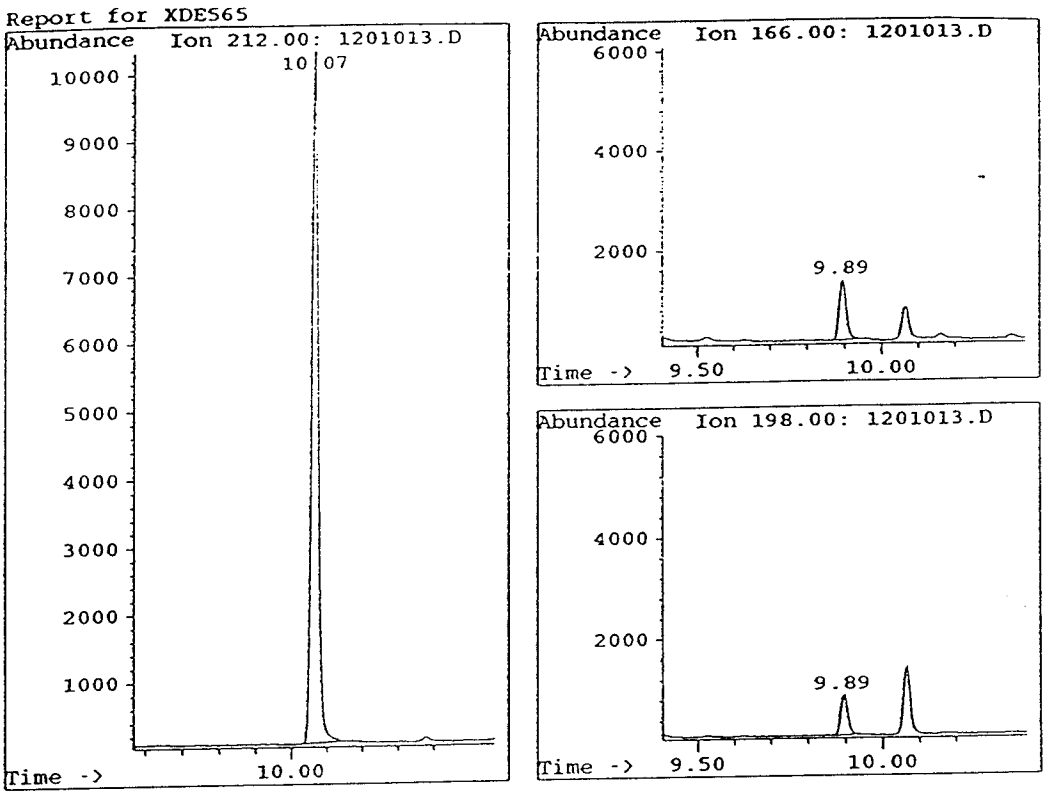
Sample Name: B651:8-2 2g eq SOYBEAN FORAGE SN#14736201
Sample Info: xde565.m (0.2 ug/mL int.std) CONTROL
Operator : Darcy Shackelford

INTSTD RETENTION TIME: 10.07
PEAK AREA (M/Z 212): 13063

NO XDE565 FOUND

XDE-565 Concentration: 0.0000 µg/g
Average Confirmation Ratio: 0.663

Figure 8. Typical Chromatogram of a Control Sample of Soybean Forage Containing No Detectable Residue of XDE-565



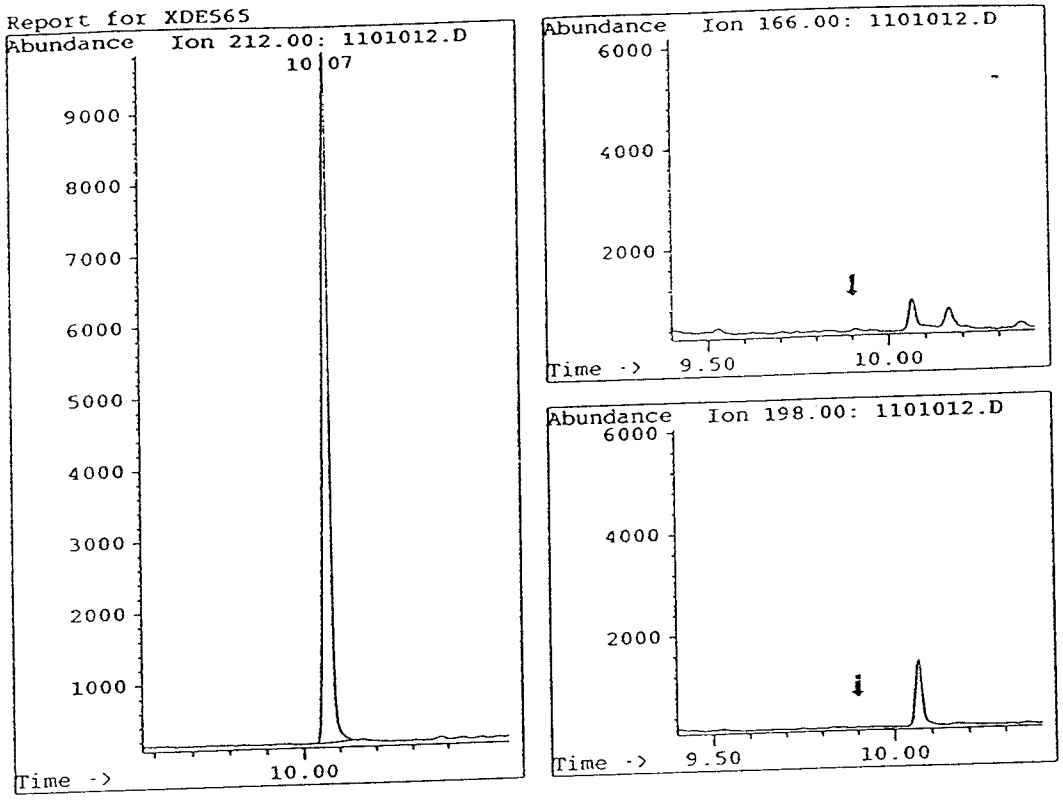
Data File : 1201013.D
ALS Bottle : 12
Date : 19 Jul 94 8:27 pm
Data Path : C:\HPCHEMPC\DATA\DS071994\
Instrument : GC/MSD S/N 3218A15556

Sample Name: B651:8-3 2g eq FORAGE SN#14736201+0.01 UG/G
Sample Info: xde565.m (0.2 ug/mL int.std)SOYBEAN FORAGE
Operator : Darcy Shackelford

INTSTD RETENTION TIME:	10.07
PEAK AREA (M/Z 212):	13057
XDE565 RETENTION TIME:	9.89
PEAK AREA (M/Z 166):	1565
PEAK AREA (M/Z 198):	1022
XDE565 CONFIRMATION RATIO OF M/Z 198/166:	0.6530
XDE565 QUANTITATION RATIO OF M/Z 166/212:	0.1199

XDE-565 Concentration: 0.0086 µg/g
Recovery: 86%
Average Confirmation Ratio: 0.663

Figure 9. Typical Chromatogram of a Control Sample of Soybean Forage Fortified With 0.01 µg/g XDE-565



Data File : 1101012.D
ALS Bottle : 11
Date : 23 Jul 94 2:13 pm
Data Path : C:\HPCHEMPC\DATA\BS072294\
Instrument : GC/MSD S/N 3218A15556

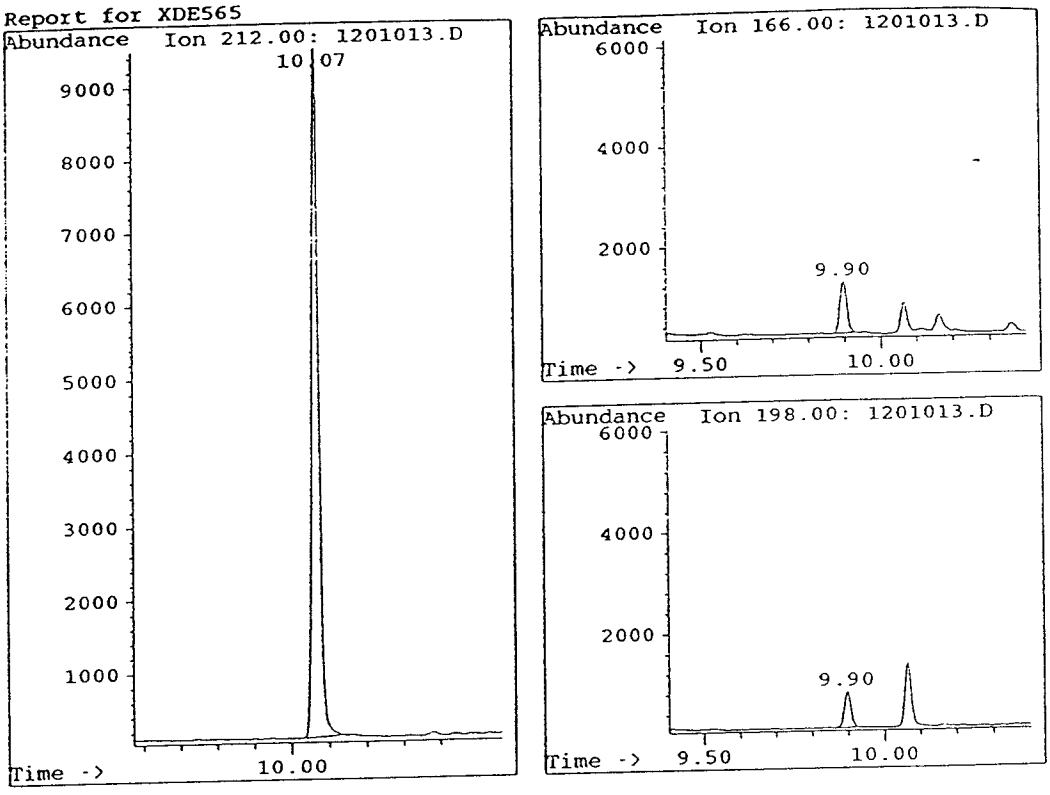
Sample Name: A1078:6-2 2g eq SOYBEAN HAY SN#13900902
Sample Info: xde565.m (0.2 ug/mL int.std) CONTROL
Operator : Brian E. Snell

INTSTD RETENTION TIME: 10.07
PEAK AREA (M/Z 212): 12253

NO XDE565 FOUND

XDE-565 Concentration: 0.0000 µg/g
Average Confirmation Ratio: 0.651

Figure 10. Typical Chromatogram of a Control Sample of Soybean Hay Containing No Detectable Residue of XDE-565



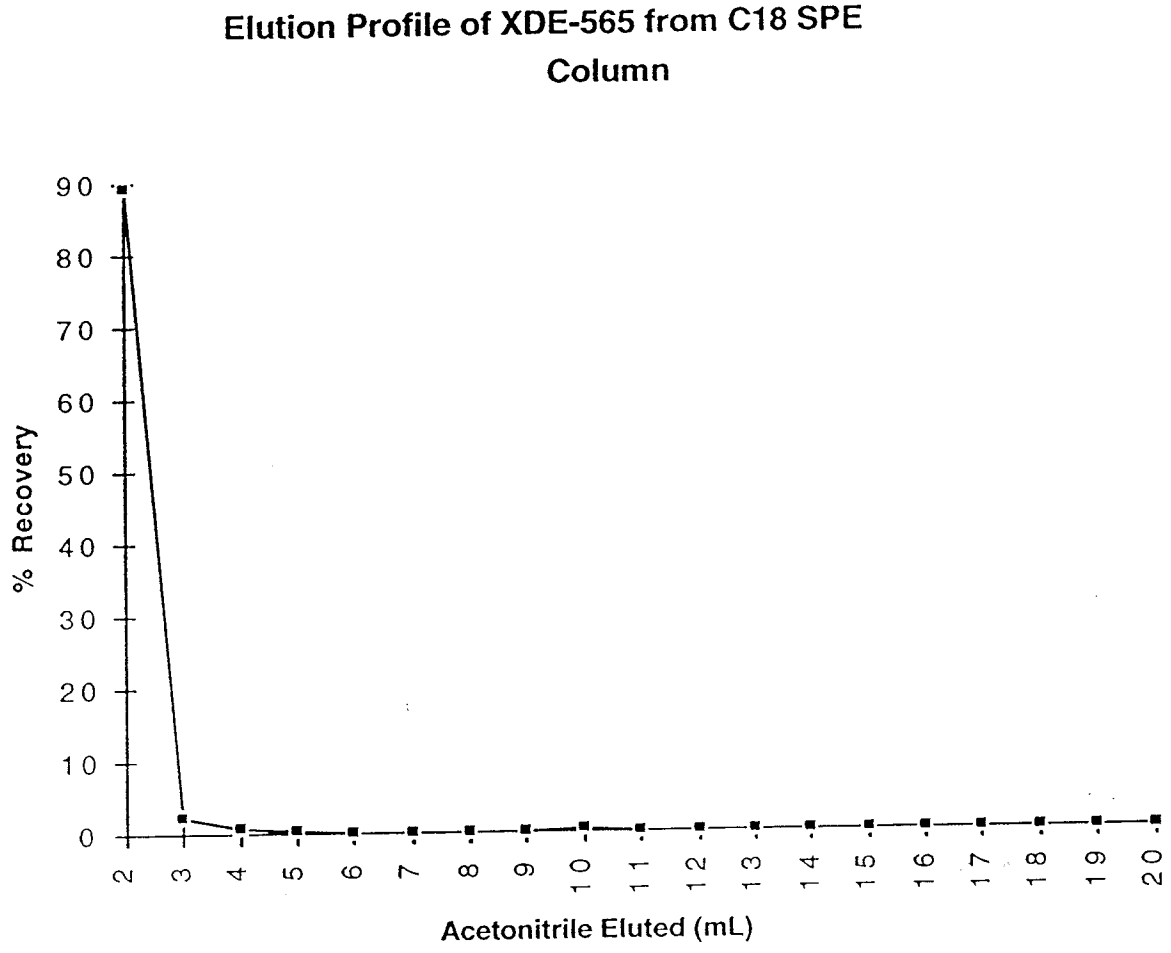
Data File : 1201013.D
ALS Bottle : 12
Date : 23 Jul 94 2:29 pm
Data Path : C:\HPCHEMPC\DATA\BS072294\
Instrument : GC/MSD S/N 3218A15556

Sample Name: A1078:6-3 2g eq HAY SN#13900902+0.01 UG/G
Sample Info: xde565.m (0.2 ug/mL int.std)SOYBEAN HAY
Operator : Brian E. Snell

INTSTD RETENTION TIME:	10.07
PEAK AREA (M/Z 212):	11790
XDE565 RETENTION TIME:	9.90
PEAK AREA (M/Z 166):	1355
PEAK AREA (M/Z 198):	903
XDE565 CONFIRMATION RATIO OF M/Z 198/166:	0.6664
XDE565 QUANTITATION RATIO OF M/Z 166/212:	0.1149

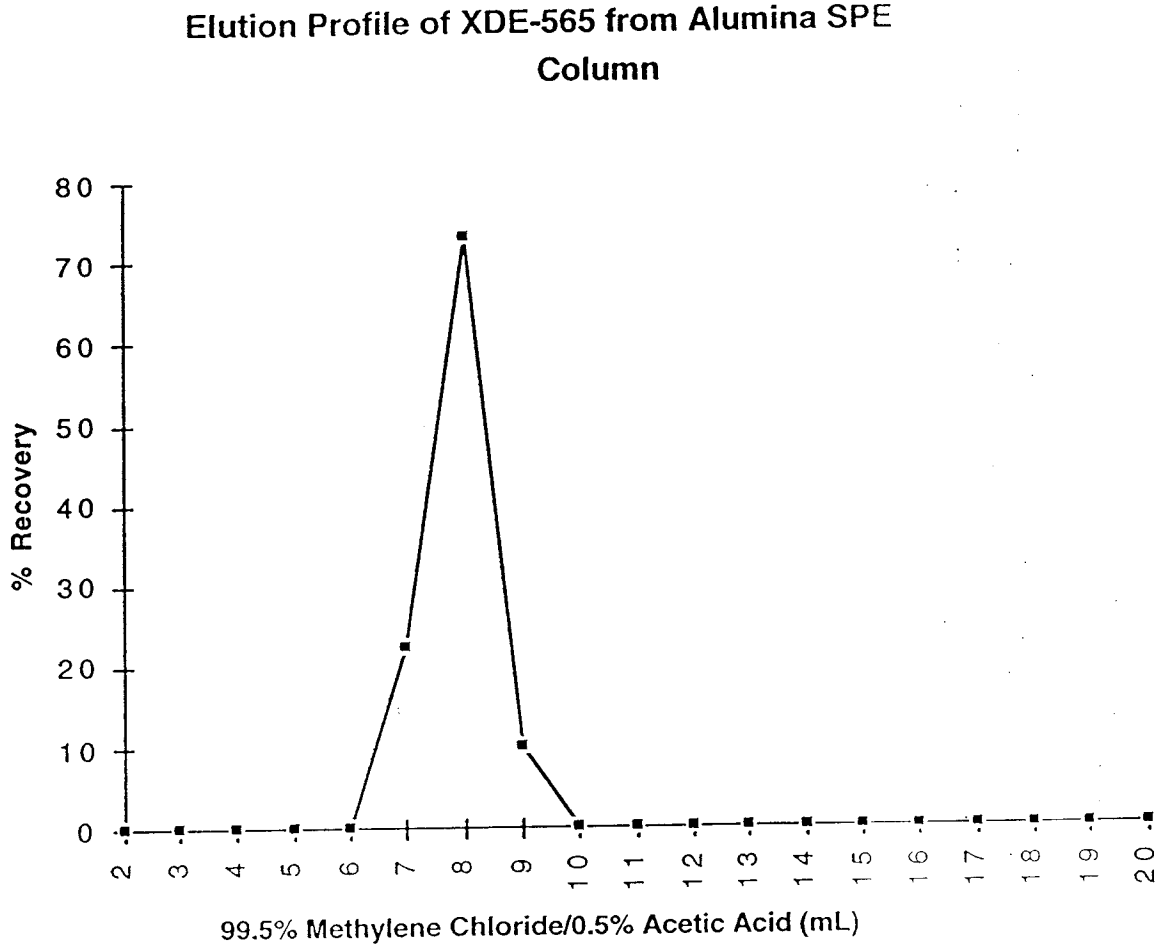
XDE-565 Concentration: 0.0089 µg/g
Recovery: 89%
Average Confirmation Ratio: 0.651

Figure 11. Typical Chromatogram of a Control Sample of Soybean Hay Fortified With 0.01 µg/g XDE-565



Total Recovery = 94%

Figure 12. Typical Elution Profile for 10 µg XDE-565 from C_{18} SPE Column with 20 mL of Acetonitrile



Total Recovery = 105%

Figure 13. Typical Elution Profile for 10 µg XDE-565 from Neutral Alumina with 20 mL 99.5% Methylene Chloride/0.5% Acetic Acid

EPA ADDENDUM
(cloransulam-methyl)

1) The method suggests using an exponential calibration curve to determine sample concentration. ACLB determined sample concentration by comparing analyte response in samples to analyte response in bracketing standards which were close to the expected sample concentrations.

2) The method calls for monitoring one ion for quantitation (m/z 166) and one additional ion for "confirmation" (m/z 198). It is generally accepted in the field of mass spectrometry that at least three ions (with correct retention time and relative intensities) are needed to constitute confirmation of residue. Therefore ACLB recommends that two additional, albeit less abundant, ions (m/z 138 & 182) also be monitored to provide additional confidence of residue confirmation.