Cover Sheet for

ENVIRONMENTAL CHEMISTRY METHOD

Pestcide Name:	Diclosulam
MRID #:	445878-02
Matrix:	Soil
Analysis:	GC/MS

APPENDIX A

Environmental Chemistry Residue Analytical Method GRM 96.22.R1

GRM.: 96.22.R1
EFFECTIVE: December 5, 1997
SUPERSEDES: GRM 96.22

Determination of Residues of Diclosulam and the Aminosulfonyl Triazolopyrimidine (ASTP) Degradate in Soil by Capillary Gas Chromatography with Mass Selective Detection

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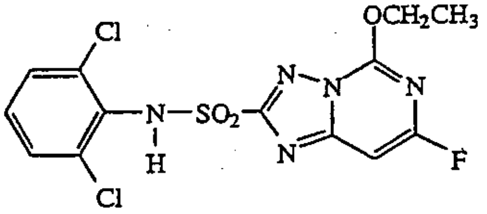
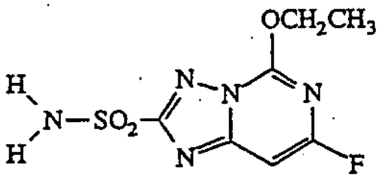
A. Scope

This method is applicable for the quantitation of residues of diclosulam and the ASTP degradate in soil. The method was validated over the concentration range of 1.0 to 120 ng/g with a limit of quantitation of 1.0 ng/g.



Diclosulam
N-(2,6-Dichlorophenyl)-5-ethoxy-7-fluoro[1,2,4]triazolo[1,5-c]pyrimidine-2-sulfonamide
CAS 145701-21-9



ASTP
2-Aminosulfonyl-5-ethoxy-7-fluoro(1,2,4)triazolo(1,5-c)pyrimidine

B. Principle

Soil samples are extracted with acidified acetone containing 10% 0.1 N hydrochloric acid. The extracts are treated with saturated aqueous magnesium acetate, decanted from a viscous aqueous layer, and concentrated to approximately 1 mL. Residues of diclosulam and ASTP are reconstituted with 0.01 N hydrochloric acid and then partitioned on an octadecyl (C_{18}) solid-phase extraction (SPE) column. ASTP and diclosulam are eluted separately from the C_{18} SPE. ASTP is partitioned into ethyl acetate, isolated, and derivatized to its 2,3,4,5,6-pentafluorobenzyl derivative, *N,N*-PFB-ASTP. Diclosulam residues are reconstituted with ethyl acetate, loaded onto a neutral alumina SPE column, and eluted with 3% acetic acid in dichloromethane. Reconstituted residues of diclosulam in acetone are derivatized to its ethyl derivative. After respective workups, residues of *N*-ethyl-diclosulam and *N,N*-PFB-ASTP are reconstituted separately in 5% NaCl and partitioned into a toluene solution containing *N*-methyl-diclosulam as an internal standard and are then quantitated by capillary gas chromatography with mass selective detection (GC/MSD).

C. Safety Precautions

1. Each analyst must 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 (MSDS), 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 in compliance with local, state, and federal laws and regulations.

2. Acetone, acetonitrile, dichloromethane, ethyl acetate, iodoethane, toluene, and triethylamine are flammable and should be used in well-ventilated areas away from ignition sources.

3. Concentrated acetic acid and hydrochloric acid solutions are corrosive and can cause severe burns. It is imperative that proper eye and personal protection equipment be used when handling these reagents.

4. Iodoethane is an alkylating agent. It is imperative that proper eye and personal protection equipment be used when handling this reagent.

5. 2,3,4,5,6-Pentafluorobenzyl bromide is a lachrymator and should be used in a hood.

6. Triethylamine is corrosive. It is imperative that proper eye and personal protection equipment be used when handling this reagent.

D. Equipment (Note M.1.)

1. Balance, analytical, Model AE240, Mettler Instrument Corporation, Hightstown, NJ 08520.

2. Balance, Model PM460, Mettler Instrument Corporation.
3. Centrifuge, with rotor to accommodate 12-mL test tubes and 45-mL vials, Model Centra-8, International Equipment Company, Needham Heights, MA 02194.
4. Evaporator, TurboVap LV, Zymark Corporation, Hopkinton, MA 01748.
5. Gas chromatograph, Model 5890 Series II, Hewlett-Packard, Wilmington, DE 19808.
6. Injector, automatic, Model 7673, Hewlett-Packard.
7. Mass selective detector data system, Model G1034B, Hewlett-Packard, Palo Alto, CA 94304.
8. Mass selective detector, Model 5971A, Hewlett-Packard.
9. Shaker, variable speed reciprocating with box carrier, Model 6000, Eberbach Corporation, Ann Arbor, MI 48106.
10. Ultrasonic bath, Model 1200, Branson Cleaning Equipment Company, Shelton, CT 06484.
11. Vacuum manifold box, Model spe-21, J.T. Baker, Inc., Phillipsburg, NJ 08865.
12. Vial Crimper, catalog number 8710-0979, Hewlett-Packard.
13. Vortex mixer, Model G-560, Scientific Industries, Inc., Bohemia, NY 11716.
14. Water purification system, Model Milli-Q UV Plus, Millipore Corporation, Milford, MA 01757.

E. <u>Glassware and Materials</u> (Note M.1.)

1. Bottle, amber, with PTFE-lined cap, 4-oz. (125-mL), catalog number 03-320-48, Fisher Scientific, Pittsburgh, PA 15219.
2. Caps, for 11-dram (45-mL) vials and 16 x 100 mm screw cap test tube, PTFE-lined, catalog numbers 02-883-3F, and 14-930-15E, Fisher Scientific.
3. Column adapter, PTFE, catalog number 120-1100, Jones Chromatography, Inc., Lakewood, CO 80228.
4. Column, capillary gas chromatography, DB-5 liquid phase, 10 x 0.18 mm i.d., 0.4-µm film thickness, catalog number 122-5032, J&W Scientific, Folsom, CA 95630.
5. Column inlet liner, deactivated, catalog number 5181-3315, Hewlett-Packard.

6. Column, neutral alumina SPE, catalog number 188-2360, J&W Scientific.
7. Column, octadecyl (C_{18}) SPE, catalog number 7020-07, J.T. Baker, Inc.
8. Column reservoir, 25-mL, catalog number 120-1007-E, Jones Chromatography, Inc.
9. Eppendorf Repeater Pipetter, catalog number 21-380-8, Fisher Scientific.
10. Eppendorf Combitip, 1.25-mL and 12.5-mL, catalog number 21-380-8E and 21-380-8C, Fisher Scientific.
11. Filters, charcoal, moisture, and oxygen, catalog numbers 7972, 7971, and 7970, Chrompack, Inc., Raritan, NJ 08869. (Note M.2.)
12. Pipets, 5-mL disposable, catalog number 13-678-25D, Fisher Scientific.
13. Screw-top test tube, 16 x 100 mm (12-mL), catalog number 14-957-86D, Fisher Scientific.
14. Vial, autosampler, 2-mL, catalog number C4011-1, National Scientific Co., Lawrenceville, GA 30243.
15. Vial, 11-dram (45-mL), catalog number 03-339-5D, Fisher Scientific.
16. Vial insert, limited volume, catalog number 03-375-3B, Fisher Scientific.
17. Vial seal, for 2-mL autosampler vial, catalog number C4011-1A, National Scientific Company.

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

1. Reagents

   a. Acetic acid, HPLC grade, catalog number A35-500, Fisher Scientific.

   b. Acetone, Optima grade, catalog number A929-4, Fisher Scientific.

   c. Acetonitrile, Optima grade, catalog number A996-4, Fisher Scientific.

   d. α-Bromo-2,3,4,5,6-pentafluorotoluene (2,3,4,5,6-pentafluorobenzyl bromide) (CAS# 1765-40-8), catalog number 10,105-2, Aldrich Chemical Company, Milwaukee, WI 53233.

   e. Dichloromethane, HPLC grade, catalog number D150-4, Fisher Scientific.

   f. Ethyl acetate, Optima grade, catalog number E196-4, Fisher Scientific.

g. Gas, helium, 99.995% purity, Airco, Murray Hill, NJ 07974.

h. Gas, nitrogen, 99.99% purity, Airco.

i. Hydrochloric acid, 0.1 N, certified concentration, catalog number SA54-4, Fisher Scientific.

j. Hydrochloric acid,0.01 N, certified concentration, catalog number SA62-1, Fisher Scientific.

k. Iodoethane, 99%, catalog number 1-778-0, Aldrich Chemical Company.

l. Magnesium acetate tetrahydrate, reagent grade, catalog number 22,864-8, Aldrich Chemical Company.

m. Sodium chloride, certified ACS, catalog number S271-1, Fisher Scientific.

n. Standards

Diclosulam: *N*-(2,6-dichlorophenyl)-5-ethoxy-7-fluoro[1,2,4]triazolo[1,5-c]pyrimidine-2-sulfonamide

N-ethyl-diclosulam: *N*-ethyl-*N*-(2,6-dichlorophenyl)-5-ethoxy-7-fluoro[1,2,4]triazolo[1,5-c]pyrimidine-2-sulfonamide

N-methyl-diclosulam: *N*-methyl-*N*-(2,6-dichlorophenyl)-5-ethoxy-7-fluoro[1,2,4]triazolo[1,5-c]pyrimidine-2-sulfonamide

ASTP: 2-aminosulfonyl-5-ethoxy-7-fluoro(1,2,4)triazolo(1,5-c)pyrimidine

Obtain from Test Substance Coordinator, Building 306/A1, DowElanco, Indianapolis, IN 46268-1054.

o. Toluene, Optima grade, catalog number T291-4, Fisher Scientific.

p. Triethylamine, 99+%, catalog number 23,962-3, Aldrich Chemical Company.

q. Water, HPLC grade, catalog number W5-4, Fisher Scientific.

2. Prepared Solutions

a. 90% acetone/10% 0.1 N hydrochloric acid

Pipet 100 mL of 0.1 N hydrochloric acid into a 1000-mL volumetric flask and dilute to volume with acetone.

b. 20% acetonitrile/80% 0.01 N hydrochloric acid

Transfer 200 mL of acetonitrile into a 1000-mL volumetric flask and dilute to volume with 0.01 N hydrochloric acid.

c. 40% acetonitrile/60% 0.01 N hydrochloric acid

Transfer 400 mL of acetonitrile into a 1000-mL volumetric flask and dilute to volume with 0.01 N hydrochloric acid.

d. 3% acetic acid/97% dichloromethane

Pipet 30 mL of acetic acid into a 1000-mL volumetric flask and dilute to volume with dichloromethane.

e. Saturated magnesium acetate, aqueous

Add 10 g of magnesium acetate tetrahydrate to 20 mL of deionized water and agitate at ambient temperatures for at least 1 hour.

f. Sodium chloride, aqueous, 5%

Dissolve 10. g of sodium chloride in 200 mL of deionized water.

G. Preparation of Standards

All solutions prepared in Section G should be stored in amber bottles, sealed with PTFE-lined caps.

1. Preparation of ASTP and Diclosulam Fortification Solutions (Note M.3.)

   a. Weigh 0.0100 g of ASTP analytical standard and quantitatively transfer to a 100-mL volumetric flask. Dilute to volume with acetone to obtain a 100-µg/mL stock solution.

   b. Weigh 0.0100 g of diclosulam analytical standard and quantitatively transfer to a 100-mL volumetric flask. Dilute to volume with acetone to obtain a 100-µg/mL stock solution.

   c. Prepare separate fortification solutions by diluting aliquots of 100-µg/mL solutions from Sections G.1.a. and G.1.b. with acetone as follows:

Aliquot of 100 µg/mL Solns.	Final Soln. Volume	Std. Soln. Final Conc.	Equivalent Sample Concentration[a]
mL	mL	ng/mL	ng/g
0.010	100	10.0	1.0
0.050	100	50.0	5.0
0.10	100	100.	10.

[a] The equivalent sample concentration is based on fortifying a 10-g soil sample with 1.0 mL of the fortification solution. Other equivalent sample concentrations can be obtained by fortifying 10-g samples with varying volumes of the stated solutions.

2. Preparation of ASTP Calibration Standard Solutions (Note M.3.)

    a. To prepare ASTP standard solutions, transfer appropriate aliquots of the 100-µg/mL stock solution (G.1.a.) into a 100-mL volumetric flask and dilute to the volume with acetone to result in concentrations ranging from 10.0 to 500 ng/mL as follows:

    b. Take 0.50 mL of standard solutions through the derivatization procedure in Steps J.2.i. to J.2.q. to prepare the 2,3,4,5,6-pentafluorobenzyl derivative of ASTP. (See Figure 1 for structure.)

    c. Dissolve the derivatized standards in 0.50 mL of toluene containing internal standard to result in equivalent sample concentrations ranging from 10.0 to 500 ng/mL.

Aliquot of Soln. G.1.a	Std. Soln. Final Conc.	Aliquot of Std. Soln. to be Derivatized	Calibrn. Soln. Final Conc.	Equivalent Sample Conc.[a]
mL	ng/mL	mL	ng/mL	ng/g
0.01	10.0	0.50[b]	10.0	0.50
0.02	20.0	0.50	20.0	1.0
0.05	50.	0.50	50.	2.5
0.10	100.	0.50	100.	5.0
0.50	500.	0.50	500.	25.

[a] The equivalent sample concentration is based on taking the 10-g soil sample extract to a final analytical volume of 0.50 mL.

[b] A 0.50-mL aliquot of acetone is added to bring the reaction volume up to 1 mL prior to derivatization.

3. Preparation of *N*-Methyl-Diclosulam Internal Standard Solutions (Note M.3.)

    a. Weigh 0.0100 g of *N*-methyl-diclosulam analytical standard and quantitatively transfer to a 100-mL volumetric flask. Dilute to volume with acetone to obtain a 100-µg/mL stock solution.

b. Pipet 4.0 mL of 100-µg/mL stock solution G.3.a. into a 2000-mL volumetric flask. Dilute to volume with toluene to obtain a 0.20-µg/mL internal standard solution.

4. Preparation of *N*-Ethyl-Diclosulam Calibration Standard Solutions (Note M.3.)

   a. Weigh 0.0107 g of *N*-ethyl-diclosulam analytical standard (equivalent to 0.0100 g of diclosulam, 406.22 g/mole) and quantitatively transfer to a 100-mL volumetric flask. Dilute to volume with acetone to obtain a 100-µg/mL diclosulam equivalent stock solution. (See Figure 1 for structure.)

   b. Calibration solutions were diluted to 100 mL with the internal standard solution from Section G.3.b.

Aliquot of Std. Soln. G.4.a. mL	Calibrn. Soln. Final Volume mL	Calibrn. Soln. Final Conc. ng/mL	Equivalent Sample Conc.[a] ng/g
0.010	100	10.0	0.50
0.020	100	20.0	1.00
0.100	100	100.	5.00
0.200	100	200.	10.0
0.500	100	500.	25.0

[a] The equivalent sample concentration is based on taking the 10-g soil sample extract to a final volume of 0.50 mL.

H. Gas Chromatography/Mass Selective Detection of Diclosulam

1. Column

Install the splitless column insert liner (Section E.5.) and the capillary column (Section E.4.) in the split/splitless injection port of the GC/MSD following the manufacturer's recommended procedure.

2. Typical Operating Conditions

Instrumentation:	Hewlett-Packard Model 5890 Series II gas chromatograph Hewlett-Packard Model 7673 autoinjector Hewlett-Packard Model 5971 mass selective detector 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 1.10 minute 120 °C to 220 °C at 30.0 °C/min. 220 °C to 325 °C at 10.0 °C/min. 325 °C to 120 °C at 70.0 °C/min.
Injector	300 °C
Interface	310 °C

Carrier Gas:	helium
Head Pressure	50 kPa
Linear Velocity	approximately 40 cm/sec at an oven temperature of 310 °C
Injection Mode:	splitless
Purge Delay	1.0 minute
Splitter Flow	60 mL/min.
Septum Purge	1.0 mL/min.
Injection Volume:	3 µL
Detector:	electron impact ionization with selective ion monitoring
Calibration Program	maximum sensitivity autotune
Electron Multiplier	1741 volts (same as autotune)
Ions monitored:	
N-Methyl-diclosulam	*m/z* 174 (internal standard)
N-Ethyl-diclosulam	*m/z* 188 (quantitation) *m/z* 190 (confirmation) (See Section L.2. for additional ions.)
Dwell Time:	75 msec

Typical mass spectra of *N*-ethyl-diclosulam and *N*-methyl-diclosulam are shown in Figure 2.

3. Calibration Curve

A typical calibration curve for the determination of diclosulam as its *N*-ethyl derivative in soil is shown in Figure 3.

4. Typical Chromatograms

Typical chromatograms of a standard, reagent blank, control sample, 1.0-ng/g recovery, and 10-ng/g recovery sample for diclosulam are shown in Figures 4 to 8. No interferences were observed for control samples (Figure 6).

I. Gas Chromatography/Mass Selective Detection of ASTP

1. Column

Install the splitless column insert liner (Section E.5.) and the capillary column (Section E.4.) in the split/splitless injection port of the GC/MSD following the manufacturer's recommended procedure.

2. Typical Operating Conditions

Instrumentation:	Hewlett-Packard Model 5890 Series II gas chromatograph Hewlett-Packard Model 7673 autoinjector Hewlett-Packard Model 5971A mass selective detector 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 1.0 minute 120 °C to 325 °C at 15.0 °C/min. 325 °C to 120 °C at 70.0 °C/min.
Injector	270 °C
Interface	300 °C
Carrier Gas:	helium
Head Pressure	50 kPa
Linear Velocity	approximately 40 cm/sec at an oven temperature of 315 °C
Injection Mode:	splitless
Purge Delay	0.6 minute
Splitter Flow	60 mL/min.
Septum Purge	1.0 mL/min.

Injection Volume:	2 µL
Detector:	electron impact ionization with selective ion monitoring
Calibration Program	maximum sensitivity autotune
Electron Multiplier	1741 volts (same as autotune)
Ions monitored:	
N-Methyl-diclosulam	*m/z* 174 (internal standard)
N,N-PFB-ASTP[a]	*m/z* 376 (quantitation) *m/z* 138 (confirmation) (See Section L.2. for additional ions.)
Dwell Time:	75 msec

A typical mass spectrum of *N,N*-bis(2,3,4,5,6-pentafluorobenzyl)-ASTP is shown in Figure 9.

[a] *N,N*-PFB-ASTP = *N,N*-bis(2,3,4,5,6-pentafluorobenzyl)-ASTP

3. Calibration Curves

A typical calibration curve for the determination of ASTP as its 2,3,4,5,6-pentafluorobenzyl derivative in soil is shown in Figure 10.

4. Typical Chromatograms

Typical chromatograms of a standard, reagent blank, control sample, 1.0-ng/g recovery, and a 10-ng/g recovery sample for ASTP are shown in Figures 11 to 15. No interferences were observed for the quantitation ion (*m/z* 376) (Figure 13).

J. Determination of Recoveries of Diclosulam and ASTP from Soil

1. Preparation and Analysis of Recovery Samples

a. Weigh 10.0 ± 0.1 g of soil into a series of 11-dram (45-mL) glass vials and record weight.

b. For preparing a sample set to be used in the determination of recoveries, fortify some of the control soils with the specified aliquots of the appropriate spiking solutions from Step G.1.c. to obtain concentrations of diclosulam and ASTP ranging from 1.0 to 120 ng/g. Analyze at least one unfortified control soil of each soil type used to determine recoveries for each set. Analyze a reagent blank containing no soil sample with each sample set.

c. Add 15 mL of 90% acetone/10% 0.1 N hydrochloric acid to each sample vial and seal with a PTFE-lined cap. Shake at low speed on a reciprocating shaker at approximately 140 excursions per minute for at least 30 minutes.

d. Centrifuge each vial at 2000 rpm for 5 minutes.

e. Decant each extract to a clean 11-dram vial.

f. Repeat Step J.1.c. with a shaking interval of 15 minutes, and Step J.1.d. Combine the extracts.

g. Add 0.5 mL of aqueous saturated magnesium acetate solution to each extract. Seal each vial with a PTFE-lined cap and shake on a reciprocating shaker at approximately 140 excursions per minute for 5 minutes.

h. Centrifuge each vial at 2000 rpm for 5 minutes.

i. Carefully decant the extract to a clean 11-dram vial. Avoid transferring the viscous aqueous residues at the bottom of the vial.

j. Wash down the vial walls with 1 to 2 mL of acetone, and combine the acetone rinse with the solution from Step J.1.i.

k. Concentrate the extract to 1 to 2 mL using a TurboVap evaporator at 40 °C with approximately 8-15 psi stream of nitrogen (Note M.4.).

l. Add 15 mL of 0.01 N hydrochloric acid solution to each extract and cap the vial with a PTFE-lined cap. Sonicate for approximately 1 minute and vortex briefly.

m. Concentrate and purify the samples using the following C_{18} SPE procedure (see Section L.4.a. and L.4.b.):

   (1) Place a C_{18} SPE column on the vacuum manifold box.

   (2) Attach a 25-mL reservoir to the top of the column using a SPE column adapter.

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

   (4) Condition the SPE column with 5 mL of the 0.01 N hydrochloric acid solution. (Do not allow the column bed to dry.)

   (5) Fill the reservoir with the sample solution from Step J.1.l. With the aid of vacuum, pull the sample through the column at a flow rate of approximately 2 to 4 mL/min. Discard the eluate.

   (6) Place an 11-dram collection vial in vacuum box.

(7) Rinse the sample vial with 5 mL of the 20% acetonitrile/80% 0.01 N hydrochloric acid, and add the rinse to the reservoir. With the aid of vacuum, pull the solution through the column at a flow rate of approximately 2 to 4 mL/min, collecting the eluate in an 11-dram vial.

(8) Continue to elute ASTP with an additional 15 mL of the 20% acetonitrile/80% 0.01 N hydrochloric acid solution, collecting the eluate in the same 11-dram vial.

(9) Remove the collection vial containing ASTP residues and seal the vial with a PTFE-lined cap. Save the vial for Step J.2.

(10) Rinse the SPE column with 5 mL of the 40% acetonitrile/60% 0.01 N hydrochloric acid, and pull it through the column at a flow rate of approximately 2 to 4 mL/min with the aid of vacuum. Discard the eluate.

(11) Increase the vacuum to the maximum that is safely recommended by the vacuum manifold box manufacturer. Maintain the vacuum for a minimum of 10 minutes to facilitate air drying of the column.

(12) Place a screw-cap test tube in the vacuum box.

(13) Elute the diclosulam with 6 mL of acetonitrile at a flow rate of approximately 2 to 4 mL/minute.

(14) Seal vial with PTFE-lined cap and take to Step J.3.

2. <u>Continuation of Analysis for ASTP in a Recovery Sample Set</u>

a. Add approximately 6 g of sodium chloride to the 11-dram vials from Step J.1.m.(9).

b. Add 5 mL of ethyl acetate and seal the vial with a PTFE-lined cap.

c. Shake the vials for approximately 10 minutes on a reciprocating shaker at approximately 140 excursions per minute.

d. Centrifuge the vials at approximately 2000 rpm for approximately 5 minutes.

e. Using a 5-mL disposable pipet, transfer the ethyl acetate extract (top layer) to a screw-cap test tube.

f. Repeat Steps J.2.b. through J.2.e. and combine the ethyl acetate extracts.

g. Evaporate the solvent to dryness using a TurboVap evaporator at 40 °C with approximately 8-15 psi stream of nitrogen (Note M.4.). It is acceptable to begin evaporation of the first ethyl acetate extract while performing Step J.2.f. It is critical to evaporate all traces of liquids to facilitate the following derivatization of ASTP.

h. Add 1 mL of acetone to each sample.

i. Using an Eppendorf pipettor with a 1.25 mL Combitip, add 25 µL of triethylamine (TEA) and 25 µL of α-bromo-2,3,4,5,6-pentafluorotoluene (2,3,4,5,6-pentafluorobenzyl bromide). Seal with a PTFE-lined cap, sonicate for 15 to 30 seconds, and vortex for approximately 15 seconds.

j. Using the same procedure (Step J.2.i.), concurrently derivatize 0.50-mL acetone aliquots containing known concentrations of ASTP for calibration standards (Section G.2.a.).

k. To complete the derivatization, place the samples in the TurboVap water bath set at approximately 35 °C for approximately 30 minutes.

l. AT AMBIENT TEMPERATURE, evaporate the solvent under a stream of nitrogen at 10 to 15 psi until only an oily residue remains. (Note M.5.)

m. Add 1 mL of an aqueous 5% NaCl solution.

n. Add 0.50 mL of toluene containing 200-ng/mL of *N*-methyl diclosulam as an internal standard.

o. Seal the vial with a PTFE-lined cap. Sonicate the vial for approximately 15 seconds and pulse vortex for approximately 15 seconds. Centrifuge at 2000 rpm for 5 minutes.

p. Carefully transfer a portion of the toluene layer to a 2-mL autosampler vial equipped with a limited volume vial insert, and seal with a cap and crimper. (Note M.6.)

q. Analyze the samples and calibration standards by GC/MSD as described in Section I.2. If the ASTP concentration in a sample is outside the range of the calibration curve, dilute the sample with toluene containing 200 ng/mL of *N*-methyl-diclosulam as an internal standard to yield a response within the range of the calibration curve, and reanalyze. Determine the suitability of the chromatographic system using the following performance criteria:

   (1) Standard curve linearity: Determine that the correlation coefficient equals or exceeds 0.995 for the power regression equation describing the detector response as a function of the standard curve concentration.

   (2) Peak resolution: Visually determine that sufficient resolution has been achieved for ASTP relative to background interferences.

   (3) Detector sensitivity: Visually determine that a minimum signal-to-noise ratio of 10:1 has been attained for ASTP in the 20-ng/mL calibration standard (equivalent to 1.0 ng/g in soil samples).

3. Continuation of Analysis for Diclosulam in a Recovery Sample Set

   a. Evaporate the solvent to dryness in the vial from Step J.1.m.(14) using a TurboVap at approximately 60 °C with an approximate 10 psi stream of nitrogen (Note M.4.).

   b. Add 5 mL of ethyl acetate, seal the vial with a PTFE-lined cap, and sonicate for approximately 1 minute.

   c. Purify the samples using the following neutral alumina SPE procedure (See Section L.4.c.):

      (1) Place a neutral alumina SPE column on the vacuum manifold box.

      (2) Condition the SPE column with 5 mL of ethyl acetate. (Do not allow the column bed to dry.)

      (3) Transfer the sample solution from Step J.3.b. to the column.

      (4) With the aid of vacuum, pull the sample through the column at a flow rate of approximately 2 to 4 mL/min without allowing the column to go dry.

      (5) Place 12-mL screw-top test tubes in the vacuum box to collect the eluate.

      (6) Rinse the vial with 2 mL of 3% acetic acid in dichloromethane.

      (7) Add the rinse to the SPE column, and pull the rinse through the column at a flow rate of approximately 2 to 4 mL/min with the aid of vacuum, collecting the eluate in a 12-mL screw-top test tube.

      (8) Continue to elute the diclosulam with an additional 6 mL of 3% acetic acid in dichloromethane, collecting the eluate in the same 12-mL screw-top test tube.

   d. Evaporate the solvent to dryness using a TurboVap at 60 °C with approximately 7 psi stream of nitrogen (Note M.4.). It is critical to evaporate all traces of acetic acid to facilitate the following derivatization of diclosulam.

   e. Add 1 mL of acetone.

   f. Using an Eppendorf pipettor with a 1.25-mL Combitip, add 25 µL of TEA, and 25 µL of iodoethane to the screw-top test tube. Seal the tube with a PTFE-lined cap.

   g. Sonicate the vial for approximately 30 seconds, and vortex for approximately 15 seconds to mix. Allow the derivatization reaction to proceed at room temperature for 30 minutes.

   h. Evaporate the solvent to dryness using a TurboVap set at 40 °C with a 15 psi stream of nitrogen (Note M.4.).

i. Add 1 mL of the 5% sodium chloride solution and 0.50 mL toluene containing 200-ng/mL of *N*-methyl-diclosulam as an internal standard.

j. Sonicate for approximately 15 seconds, then pulse vortex for approximately 15 seconds.

k. Centrifuge the vial at 2000 rpm for approximately 5 minutes and transfer the toluene to a 2-mL autosampler vial equipped with a limited volume insert. Seal the vial with a cap and crimper.

l. Analyze samples and calibration standards from Step G.4.b. for diclosulam by GC/MSD with selected ion monitoring, using the conditions specified in Section H.2. If the diclosulam concentration in a sample is outside the range of the calibration curve, dilute the sample with toluene containing 200 ng/mL of *N*-methyl-diclosulam as an internal standard to yield a response within the range of the calibration curve, and reanalyze. Determine the suitability of the chromatographic system using the following performance criteria:

   (1) Standard curve linearity: Determine that the correlation coefficient equals or exceeds 0.995 for the power regression equation describing the detector response as a function of the standard curve concentration.

   (2) Peak resolution: Visually determine that sufficient resolution has been achieved for *N*-ethyl-diclosulam relative to background interferences.

   (3) Detector sensitivity: Visually determine that a minimum signal-to-noise ratio of 10:1 has been attained for *N*-ethyl-diclosulam in the 20-ng/mL calibration standard (equivalent to 1.0 ng/g in soil samples).

4. Calculations

a. Using the data collected for the series of calibration standards analyzed in Sections J.3.l. and J.2.q., determine the peak areas for *N*-ethyl-diclosulam (*m/z* 188, 190), *N*-methyl-diclosulam (*m/z* 174), and *N,N*-bis(2,3,4,5,6-pentafluoro-benzyl)-ASTP (*m/z* 376, 138), respectively.

b. For each sample, calculate the diclosulam and ASTP quantitation ratios.

$$\text{Quantitation Ratio} = \left[\frac{\text{peak area of quantitation ion}}{\text{peak area of internal standard ion}}\right]$$

For example, using the data for diclosulam from Figure 7:

$$\text{Quantitation Ratio} = \left[\frac{\text{peak area at } m/z \text{ 188}}{\text{peak area at } m/z \text{ 174}}\right]$$

$$\text{Quantitation Ratio} = \left[\frac{1016}{11837}\right]$$

$$\text{Quantitation Ratio} = 0.08583$$

c. Prepare a standard curve for diclosulam and for ASTP by plotting the equivalent concentration on the abscissa (x-axis) and the respective quantitation ratio on the ordinate (y-axis) as shown in Figures 3 and 10, respectively. Using power regression (1) analysis, determine the equation for the curve with respect to the abscissa.

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

For example, using the diclosulam data from Figures 3 and 7:

$$\begin{array}{l}\text{Diclosulam, Gross} \\ \text{Sample Conc. (ng/mL)}\end{array} = \left[\frac{\begin{array}{c}\text{Diclosulam} \\ \text{quantitation ratio}\end{array}}{\text{constant}}\right]^{1/\text{exponent}}$$

$$= \left[\frac{0.08583}{0.0039}\right]^{1/1.05}$$

$$= 18.78 \text{ ng/mL}$$

d. Determine the net concentration of diclosulam in each recovery sample by multiplying the gross sample concentration (ng/mL) by the method factor (mL/g) and subtracting any net concentration of diclosulam found in the control sample.

For example, using the diclosulam data from Figures 3 and 7:

$$\text{Method Factor} = \left[\frac{\text{final volume (mL)}}{\text{sample weight (g)}}\right]$$

$$= \left[\frac{0.48 \text{ mL}}{9.91 \text{ g}}\right]$$

$$= 0.048 \text{ mL/g}$$

Diclosulam, Sample Conc. (ng/mL) = Gross Sample Conc. (ng/mL) x method factor (mL/g)

= 18.78 (ng/mL) x 0.048 (mL/g)

= 0.91

Diclosulam, Net Sample Conc. (ng/g) = Recovery Sample Conc. (ng/g) - Control Sample Conc. (ng/g)

= 0.91 - 0.00

= 0.91

e. Determine the concentration in each diluted recovery sample by multiplying the diluted concentration by the appropriate dilution factor. For example, for a 10-fold dilution:

Conc. (ng/g) = Found Conc. (ng/g) x 10

f. Determine the percent recovery by dividing the net concentration found in each recovery sample by the theoretical concentration added.

ng Found = Net Sample Conc. (ng/g) x Sample wt. (g)

$$\text{Percent Recovery} = \left[\frac{\text{ng Found}}{\text{ng Added}}\right] \times 100$$

For example, using the diclosulam data from Section J.4:

$$\text{Percent Recovery} = \left[\frac{9.12\text{ ng Found}}{10.0\text{ ng Added}}\right] \times 100$$

$$= 91\%$$

If desired, use the average recovery of all the recovery samples within a given set to correct individual sample results for method efficiency.

Values were calculated based on more digits than are displayed.

K. Determination of Diclosulam and ASTP Residues in Soil

1. Prepare reagent blank, control, recovery, and treated samples as described in Sections J.1. through J.3.

2. Prepare standard calibration curves for diclosulam and ASTP. Determine the percentage recoveries as described in Section J.4.

3. Determine the concentration of diclosulam and ASTP in each treated sample as described in Section J.4.

4. Determination of Soil Moisture

   a. If correction for soil moisture is desired, weigh at least 10 g of soil into an aluminum or glass container and record the weights of the empty container and the container with the wet soil.

   b. Place the sample in an oven at approximately 110 °C and allow to dry for a minimum of 16 hours.

   c. Remove the sample from the oven, place in a desiccator until the sample has cooled to ambient temperature, and then re-weigh and record the weight of the container with the dry soil.

   d. Calculate the percent moisture on a dry-weight basis as follows:

$$\text{Percent Moisture} = \left[\frac{\text{water weight (g)}}{\text{soil dry weight (g)}}\right] \times 100$$

$$= \left[\frac{\begin{array}{c}\text{soil weight}\\ \text{before drying}\end{array} - \begin{array}{c}\text{soil weight}\\ \text{after drying}\end{array}}{\begin{array}{c}\text{soil weight}\\ \text{after drying}\end{array}}\right] \times 100$$

5. Determination of Corrected Diclosulam and ASTP Concentration in Soil

   a. Determine the gross diclosulam and ASTP concentration in the soil as described in Section J.4.

   b. Determine the soil moisture as described in Section J.5.

   c. Determine the corrected diclosulam and ASTP concentrations in soil samples as follows:

$$\begin{array}{c}\text{Corrected}\\ \text{Dry Weight}\\ \text{Conc. (ng/g)}\end{array} = \begin{pmatrix}\text{Net Diclosulam}\\ \text{Residue Conc. (ng/g)}\end{pmatrix} * \left(\frac{100}{\text{\%Recovery}}\right) * \left(1 + \frac{\text{\% Moisture}}{100}\right)$$

* Correction for percent recovery is optional.

L. Results and Discussion

1. Method Validation

a. Recovery Levels and Precision

Method was validated by using the stated procedure to analyze several hundred samples for diclosulam and ASTP. Some representative recovery results for diclosulam and ASTP over a concentration range of 1.0 to 120 ng/g are given in Table I and Table II, respectively. Statistical summaries of recoveries for diclosulam and ASTP are 87 ±10% and 84 ±9%, respectively.

b. Calculated Limits of Detection and Quantitation

The calculated LOD and LOQ were determined using the standard deviation from the recovery results at the validated LOQ (1.0 ng/g). Following a published technique (2), the LOD was calculated as 3x the standard deviation and the LOQ was calculated as 10x the standard deviation. The calculated results for diclosulam and ASTP are presented in Tables I and II, respectively.

Calculated LOQ values for diclosulam and ASTP were 0.92 and 0.43 ng/g, respectively. These calculated LOQ values support the target LOQ of 1.0 ng/g. Similarly, the calculated LOD values for diclosulam and ASTP were 0.28 and 0.13 ng/g, respectively. These calculated LOD values support the target LOD of 0.3 ng/g. Results that are less than the method LOD should be reported as not detected.

c. Standard Curve Linearity

The average correlation coefficient (r^2) of the power regression equations describing the detector response as a function of concentration was equal to or greater than 0.995 for each set used as validation data for diclosulam and ASTP.

2. Confirmation of Residues of Diclosulam and ASTP

For diclosulam and ASTP, confirmation can be obtained from comparison of the retention times and the confirmation ratios resulting from selected ion monitoring (mass spectrometry) to those of standards. If additional confirmation is required beyond that discussed in this method, the mass spectrum of *N*-ethyl-diclosulam (Figure 2) contains ions at *m/z* 172 or *m/z* 174 that may be used for confirmation. For ASTP, the mass spectrum of *N,N*-bis(2,3,4,5,6-pentafluorobenzyl)-ASTP (Figure 9) contains an ion at *m/z* 154 for additional confirmation.

3. Assay Time

A typical analytical run would consist of a minimum of five standards encompassing the linear range of sample concentrations, a reagent blank, a control (a non-fortified sample), a minimum of two fortified controls (one of which must be at the LOQ), and ten samples. This typical analytical run could be completed in less than two working days.

There are several acceptable stopping points in the method where sample preparation (Section J) may be suspended without adverse effects on the sample analysis. If the samples are to be stored overnight, it is recommended that the samples be stored at cool temperatures in low pH value solutions (to reduce hydrolysis), or preferably in organic solutions. The vials should be capped with PTFE-lined caps. The recommended stopping points are the following:

a. Step J.1.c. (shake overnight)
b. Step J.1.j.
c. Step J.1.m.(9) (for ASTP)
d. Step J.1.m.(14) (for diclosulam)
e. Step J.2.f. (for ASTP)
f. Step J.2.h. (for ASTP)
g. Step J.2.n. (for ASTP)
h. Step J.2.p. (for ASTP)
i. Step J.3.b. (for diclosulam)
j. Step J.3.c.(8) (for diclosulam)
k. Step J.3.e. (for diclosulam)
l. Step J.3.i. (for diclosulam)
m. Step J.3.k. (for diclosulam)

4. Standardization of SPE Elution Profiles

Variation in the C_{18} and neutral alumina SPE columns may influence the elution profile of diclosulam and ASTP. It is recommended to obtain an elution profile for each lot of SPE columns used to ensure optimum recovery and clean-up efficiency. The following procedures can be used:

a. C_{18} SPE Profile - ASTP

(1) Add 50 µL of the 10-µg/mL ASTP solution (Step G.1.c.) to an 11-dram vial containing 15 mL of 0.01 N hydrochloric acid.

(2) Proceed as described in Steps J.1.m.(1) through J.1.m.(5) and collect the eluate in an 11-dram vial.

(3) Remove the column reservoir and column adapter. Elute the analyte from the column with the 20% acetonitrile/80% 0.01 N hydrochloric acid, collecting 2.0-mL aliquots in a series of ten to twelve 12-mL screw-top culture tubes (Note M.7.).

(4) Add 1 g and 6 g of sodium chloride to each screw-top culture tube and 11-dram vial, respectively.

(5) Add 2 mL and 5 mL of ethyl acetate to each of the 12-mL screw-top culture tubes and 11-dram vials, respectively. Seal with PTFE-lined caps and shake the vials for 10 minutes on a reciprocating shaker at approximately 140 excursions/minute. Centrifuge the vials for 3 minutes at 2500 rpm.

(6) Transfer the ethyl acetate extracts to a series of 12-mL screw cap test tubes.

(7) Repeat Steps L.4.a.(5) and L.4.a.(6) and combine the extracts.

(8) Proceed as described in Steps J.2.g. through J.2.q.

(9) Calculate the percentage recoveries for each aliquot as described in Section J.4.

A typical elution profile is shown in Figure 16. If the elution profile differs from that shown, adjust the volume of eluting solvent in Step J.1.m.(8).

b. C_{18} SPE Profile - Diclosulam

(1) Add 50 µL of the 10-µg/mL diclosulam solution (Step G.1.c.) to an 11-dram vial containing 15 mL of 0.01 N hydrochloric acid.

(2) Proceed as described in Steps J.1.m.(1) through J.1.m.(6) and collect the eluate in an 11-dram vial.

(3) Add 6 g of sodium chloride and 5 mL of ethyl acetate to the vial from Step L.4.b.(2.).

(4) Seal with a PTFE-lined cap and shake the vial for 10 minutes on a reciprocating shaker at approximately 140 excursions/minute. Centrifuge the vial for 3 minutes at 2500 rpm.

(5) Transfer the ethyl acetate extract to a 12-mL screw-top culture tube.

(6) Repeat Steps L.4.b.(3) to L.4.b.(5) and combine the extracts.

(7) Rinse the SPE column with 5 mL of 40% acetonitrile/60% 0.1 N hydrochloric acid and collect the eluate.

(8) Extract the eluate from Step L.4.b.(7) as in Steps L.4.b.(3) to L.4.b.(6). Save the combined extracts.

(9) Remove the column reservoir and column adapter. Elute diclosulam from the column with acetonitrile, collecting 1.0-mL aliquots in a series of ten 12-mL screw-top culture tubes (Note M.7.).

(10) Proceed as in Steps J.3.d. to J.3.l with samples from Steps L.4.b.(6), L.4.b.(8), and L.4.b.(9).

(11) Calculate the percentage recoveries for each aliquot as described in Section J.4.

A typical elution profile is shown in Figure 17. If the elution profile differs from that shown, adjust the volume of eluting solvent in Step J.1.m.(13).

c. Neutral Alumina SPE Profile - Diclosulam

(1) Add 50 µL of the 10-µg/mL diclosulam solution (Step G.1.c.) to a 12-mL screw-top culture tube.

(2) Proceed with Steps J.3.a. through J.3.c.(5) and collect the eluate.

(3) Elute the analyte with the 3% acetic acid in dichloromethane solution, collecting 1.0-mL aliquots in a series of ten 12-mL screw-top culture tubes (Note M.7.).

(4) Proceed with Steps J.3.d. through J.3.l. for samples collected in Steps L.4.c.(2) and L.4.c.(3).

(5) Calculate the percentage recoveries for the vial rinse and each aliquot as described in Section J.4.

A typical elution profile is shown in Figure 17. If the elution profile differs from that shown, adjust the volume of eluting solvent in Step J.3.c.(8). Alternatively, the percent of acetic acid may be varied from 1% to 5% by volume (Note M.8.).

M. Notes

1. 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.

2. The filters are used in the carrier gas supply lines to purify the helium entering the gas chromatograph.

3. Aqueous diclosulam and ASTP solutions are susceptible to hydrolysis, especially at high pH values. Therefore, diclosulam and ASTP standard solutions are prepared in acetone or toluene.
4. Evaporation of all solutions, except Step J.2.1., can be carried out on a TurboVap set at 40 °C with an approximate 8-15 psi stream of nitrogen. Step J.2.1. requires ambient temperature to prevent substantial losses of derivatized ASTP residues. However, the stated temperatures were actually used to expedite sample analysis without substantial losses of residues.
5. Heating during concentration may result in substantial losses of derivatized ASTP residues.
6. If any of the aqueous layer is transferred, the extract should be carefully transferred to a new vial because GC/MSD performance can be adversely affected by non-volatile residues in the aqueous phase and water.
7. The collection of aliquots from the SPE columns can be accomplished as follows:
    a. Pipet the appropriate aliquot of the eluting solution into a 12-mL screw cap test tube.
    b. Remove the SPE column from the vacuum manifold box.
    c. Add 1.0-mL aliquots of the eluting solution to the column, and pull the solution through the column using a SPE vacuum box (Section D.11.) until the liquid is at the top of the column packing. Collect the eluate in a 12-mL screw cap test tube.
    d. Add additional eluting solution as needed to collect the prescribed number of aliquots for the elution profile.
8. In order to obtain satisfactory diclosulam elution profiles, the percent of acetic acid in dichloromethane may be varied from 1% to 5% by volume because of the variability of different lots of neutral alumina SPE columns.

N. References

1. Freund, J. E.; Williams, F. J. *Dictionary/Outline of Basic Statistics*, Dover Publications: Mineola, NY, 1991; p 170, eq L3a.
2. Keith, L. H.; Crummett, W.; Deegan, J.; Libby, R. A.; Taylor, J. K.; Wentler, G., *Anal. Chem.*, **1983**, 55, 2210-2218.

Table I. Recovery Data for Diclosulam in Soils

Sample Number	Date of Analysis	Diclosulam, ng		Percent Recovery
		Added	Found	
18235401	27-Jan-97	0.0	ND[a]	–
18235401	29-Jan-97	0.0	ND	–
18238901	27-Jan-97	0.0	ND	–
18238901	29-Jan-97	0.0	ND	–
18241901	05-Feb-97	0.0	ND	–
18238901	05-Feb-97	0.0	ND	–
18235401	27-Jan-97	3.0	< 10.0	NA[b]
18235401	29-Jan-97	3.0	< 10.0	NA
18238901	27-Jan-97	3.0	< 10.0	NA
18238901	29-Jan-97	3.0	< 10.0	NA
18235401	27-Jan-97	10.0	9.02	90
18235401	27-Jan-97	10.0	9.12	91
18235401	29-Jan-97	10.0	9.70	97
18235401	29-Jan-97	10.0	9.31	93
18238901	27-Jan-97	10.0	8.38	84
18238901	27-Jan-97	10.0	7.82	78
18238901	29-Jan-97	10.0	9.63	96
18238901	29-Jan-97	10.0	9.40	94
18241901	05-Feb-97	10.0	9.47	95
18241901	05-Feb-97	10.0	10.0	100
18238901	05-Feb-97	10.0	7.09	71
18238901	05-Feb-97	10.0	7.69	77
		$\bar{x}$ =	8.88	
		s =	0.92	
		LOD ($3s$)[c] =	2.77 ng	
		LOQ ($10s$)[d] =	9.22 ng	
18235401	27-Jan-97	50.0	41.8	84
18235401	29-Jan-97	50.0	42.9	86
18238901	27-Jan-97	50.0	36.7	73
18238901	29-Jan-97	50.0	43.6	87
18235401	27-Jan-97	100	98.7	99
18235401	29-Jan-97	100	103	103
18238901	27-Jan-97	100	86.7	87
18238901	29-Jan-97	100	86.9	87
18241901	05-Feb-97	1200	986	82
18238901	05-Feb-97	1200	746	62
			$\bar{x}$ =	87
			s =	10
			n =	22

---

[a] ND = Not detected at target LOD of 0.3 ng/g.

[b] NA = Not applicable to this sample because residue was below the target LOQ of 1.0 ng/g.

[c] Calculated limit of detection, LOD = 0.28 ng/g. (2.77 ng/10 g sample wt. = 0.28 ng/g)

[d] Calculated limit of quantitation, LOQ = 0.92 ng/g. (9.22 ng/10 g sample wt. = 0.92 ng/g)

Table II. Recovery Data for ASTP in Soils

Sample Number	Date of Analysis	ASTP, ng Added	ASTP, ng Found	Percent Recovery
18235401	27-Jan-97	0.0	ND[a]	–
18235401	29-Jan-97	0.0	ND	–
18238901	27-Jan-97	0.0	ND	–
18238901	29-Jan-97	0.0	ND	–
18241901	05-Feb-97	0.0	ND	–
18238901	05-Feb-97	0.0	ND	–
18235401	27-Jan-97	3.0	< 10.0	NA[b]
18235401	29-Jan-97	3.0	< 10.0	NA
18238901	27-Jan-97	3.0	< 10.0	NA
18238901	29-Jan-97	3.0	< 10.0	NA
18235401	27-Jan-97	10.0	8.75	88
18235401	27-Jan-97	10.0	8.85	89
18235401	29-Jan-97	10.0	8.43	84
18235401	29-Jan-97	10.0	7.96	80
18238901	27-Jan-97	10.0	8.73	87
18238901	27-Jan-97	10.0	7.73	77
18238901	29-Jan-97	10.0	8.41	84
18238901	29-Jan-97	10.0	8.70	87
18241901	05-Feb-97	10.0	8.90	89
18241901	05-Feb-97	10.0	9.12	91
18238901	05-Feb-97	10.0	8.11	81
		$\bar{x}$ =	8.52	
		s =	0.43	
		LOD ($3s$)[c] =	1.30 ng	
		LOQ ($10s$)[d] =	4.32 ng	
18235401	27-Jan-97	50.0	40.7	81
18235401	29-Jan-97	50.0	40.7	81
18238901	27-Jan-97	50.0	34.2	68
18238901	29-Jan-97	50.0	38.7	77
18235401	27-Jan-97	100	105	105
18235401	29-Jan-97	100	100	100
18238901	27-Jan-97	100	89.5	90
18238901	29-Jan-97	100	85.3	85
18241901	05-Feb-97	1200	864	72
18238901	05-Feb-97	1200	897	75
			$\bar{x}$ =	84
			s =	9
			n =	21

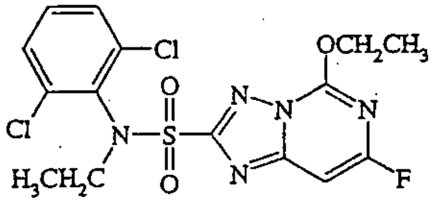
---

[a] ND = Not detected at target LOD of 0.3 ng/g.

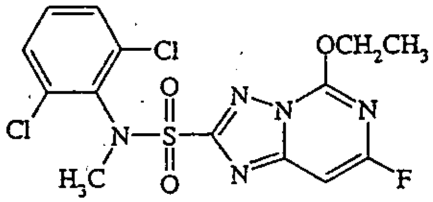
[b] NA = Not applicable to this sample because residue was below the target LOQ of 1.0 ng/g.

[c] Calculated limit of detection, LOD = 0.13 ng/g. (1.30 ng/10 g sample wt. = 0.13 ng/g)

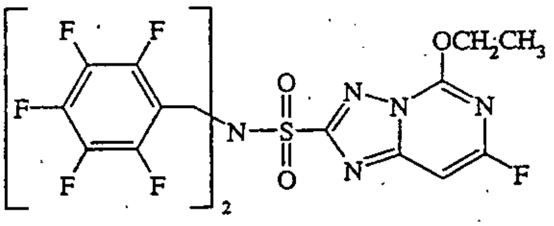
[d] Calculated limit of quantitation, LOQ = 0.43 ng/g. (4.32 ng/10 g sample wt. = 0.43 ng/g)



N-Ethyl-diclosulam
Formula: $C_{15}H_{14}Cl_2FN_5O_3S$
Formula Weight: 434.29
Molecular Weight: 433



N-Methyl-diclosulam
Formula: $C_{14}H_{12}Cl_2FN_5O_3S$
Formula Weight: 420.26
Molecular Weight: 419



N,N-bis(2,3,4,5,6-pentafluorobenzyl)-ASTP
Formula: $C_{21}H_{10}F_{11}N_5O_3S$
Formula Weight: 621.39
Molecular Weight: 621

Figure 1. Structures of Analytes Derivatized as *N*-Ethyl-diclosulam, *N*-Methyl-diclosulam, and *N,N*-bis(2,3,4,5,6-pentafluorobenzyl)-ASTP

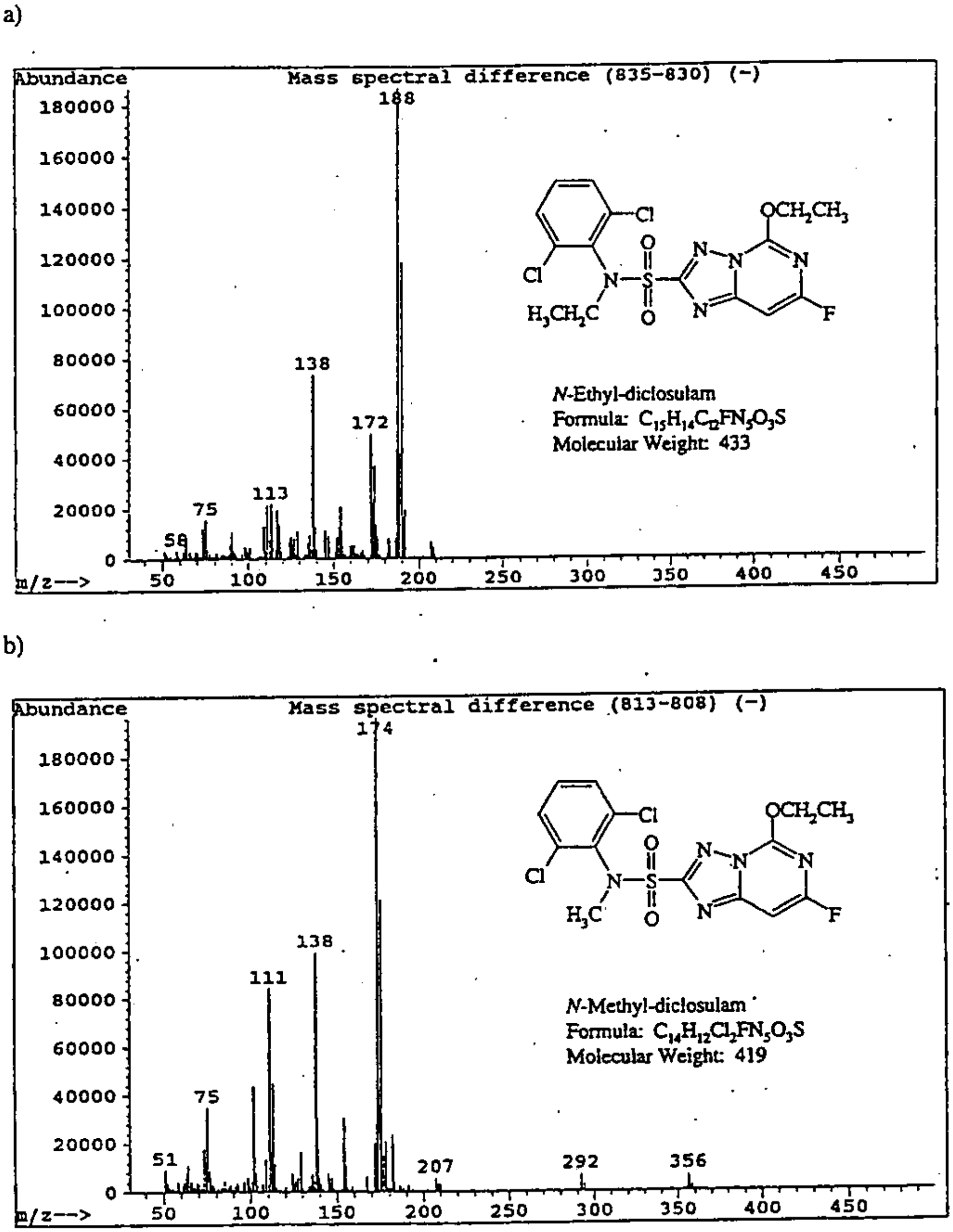
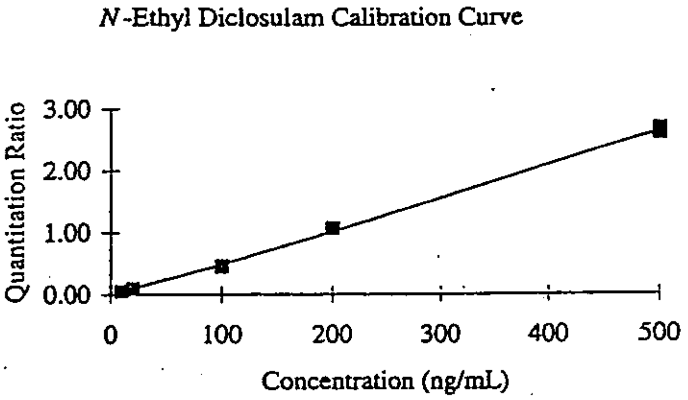


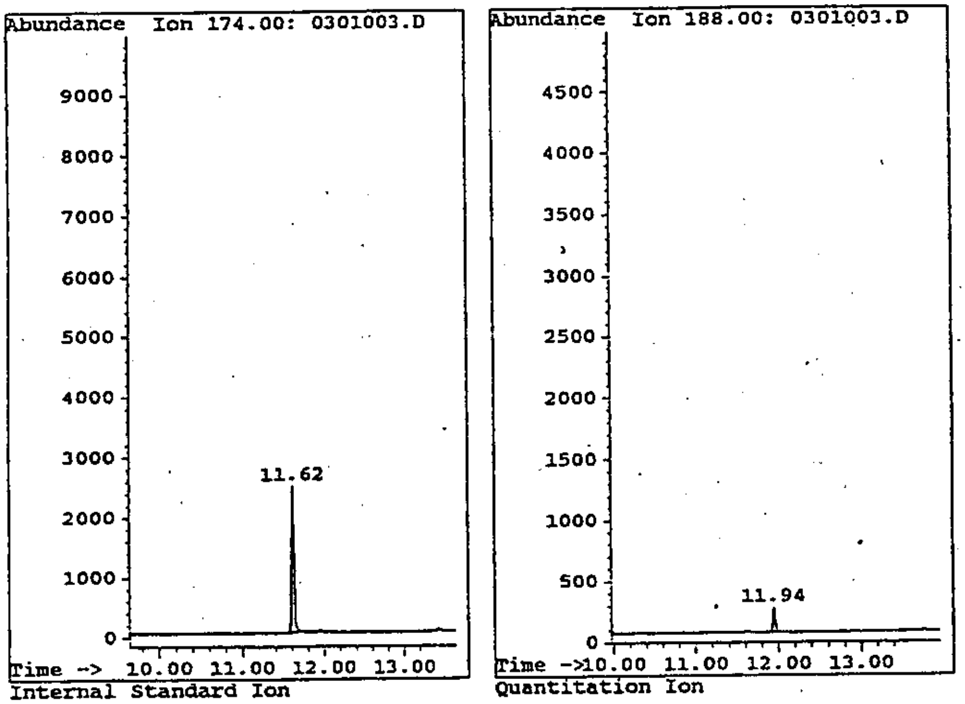
Figure 2. Electron Impact Mass Spectra of (a) *N*-Ethyl-diclosulam and, (b) *N*-Methyl-diclosulam



Diclosulam Concentration (ng/mL)	Equivalent Sample Concentration (ng/g)	Diclosulam Quantitation Ratio *m/z* 188/*m/z* 174
10.0	0.50	0.043
10.0	0.50	0.047
20.0	1.00	0.086
20.0	1.00	0.095
100	5.00	0.445
100	5.00	0.464
200	10.0	1.057
200	10.0	1.068
500	25.0	2.676
500	25.0	2.598

Power Regression Equation: X = (Y/0.0039)^(1/1.05)
Coefficient of Determination (r^2): 0.9988

Figure 3. Typical Calibration Curve for the Determination of Diclosulam as *N*-Ethyl-diclosulam



Internal Standard Ion

Quantitation Ion

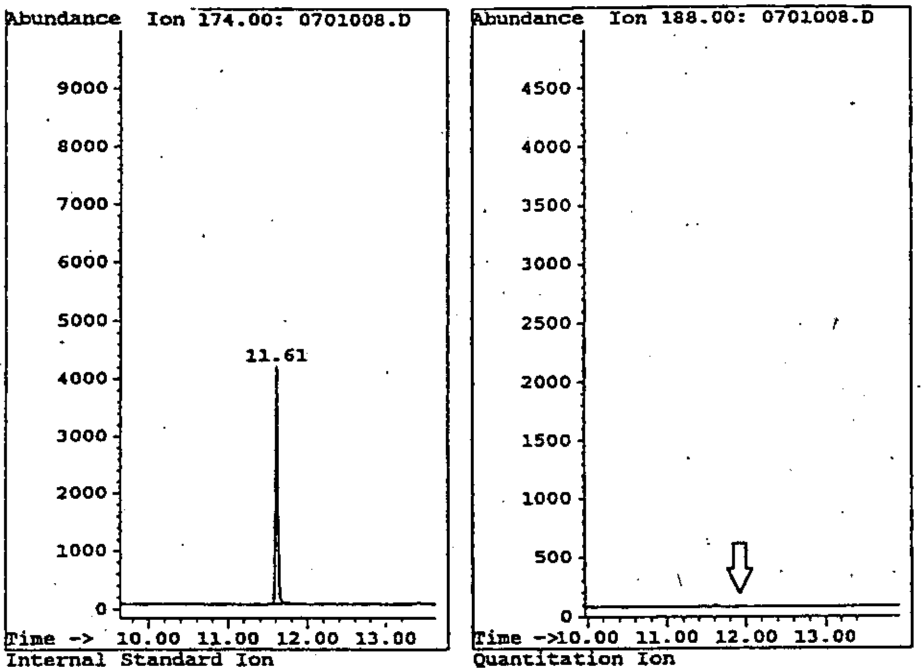
```
Data File  : 0301003.D                          Acquisition: 29 Jan 97
ALS Bottle : 3                                  Operator   : B. A. Blakeslee
Method     : diclo.M                            Integration: 01/30/97
Instrument : GC S/N 3203A40952                  Integrator : Blakeslee
Data Path  : C:\CHEMPC\DATA\ENV96021\GADO\

Sample Name: Std 20. ng/mL Diclosulam,
```

Compound	Ion	Retention Time	Peak Area
Internal Standard	174	11.62	5117
N-ethyl-Diclosulam	188	11.94	442

Equivalent Diclosulam Concentration: 1.00 ng/g

Figure 4. Typical Chromatogram of a 20-ng/mL *N*-Ethyl-diclosulam Standard, Equivalent to 1.0 ng/g of Diclosulam in Soil



Internal Standard Ion

Quantitation Ion

Data File : 0701008.D
ALS Bottle : 7
Method : diclo.M
Instrument : GC S/N 3203A40952
Data Path : C:\CHEMPC\DATA\ENV96021\GAD0\

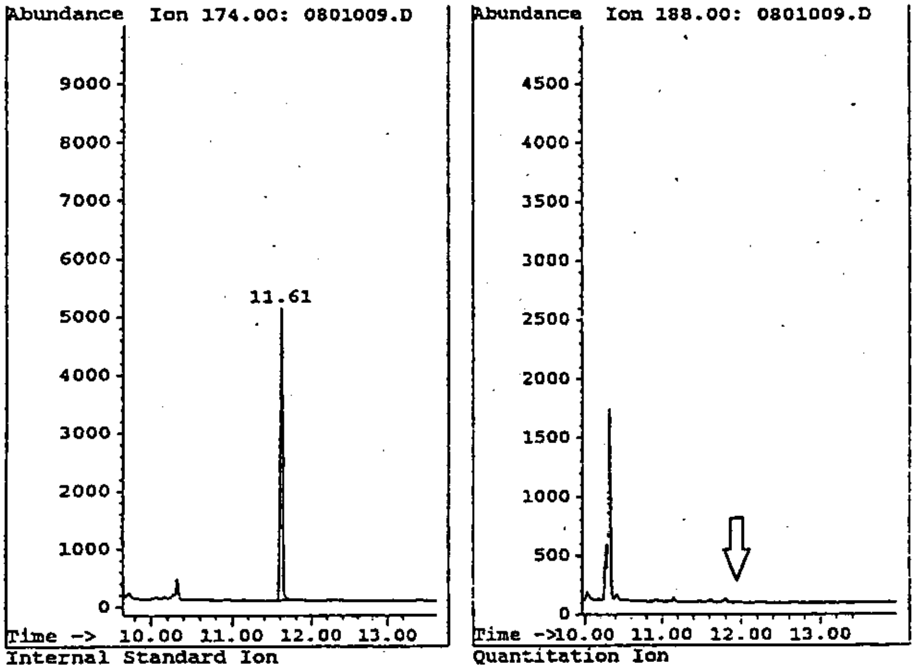
Acquisition: 29 Jan 97
Operator : B. A. Blakeslee
Integration: 01/30/97
Integrator : Blakeslee

Sample Name: Reagent Blank

Compound	Ion	Retention Time	Peak Area
Internal Standard	174	11.61	8620
N-ethyl-Diclosulam	188	Not Found	Not Found

Diclosulam Concentration: 0.00 ng/g

Figure 5. Typical Chromatogram of a Reagent Blank Sample for the Determination of Diclosulam



Internal Standard Ion

Quantitation Ion

Data File : 0801009.D
ALS Bottle : 8
Method : diclo.M
Instrument : GC S/N 3203A40952
Data Path : C:\CHEMPC\DATA\ENV96021\GAD0\

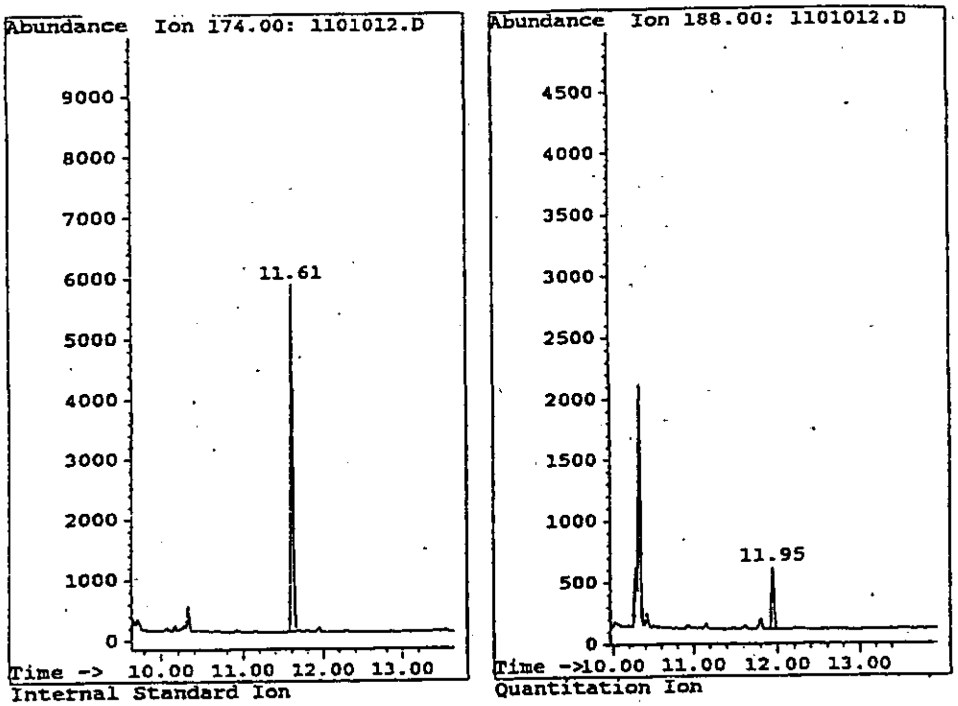
Acquisition: 29 Jan 97
Operator : B. A. Blakeslee
Integration: 01/30/97
Integrator : Blakeslee

Sample Name: 18235401

Compound	Ion	Retention Time	Peak Area
Internal Standard	174	11.61	10241
N-ethyl-Diclosulam	188	Not Found	Not Found

Diclosulam Concentration: 0.00 ng/g

Figure 6. Typical Chromatogram of a Control Soil Sample for the Determination of Diclosulam



Internal Standard Ion

Quantitation Ion

Data File : 1101012.D
ALS Bottle : 11
Method : diclo.M
Instrument : GC S/N 3203A40952
Data Path : C:\CHEMPC\DATA\ENV96021\GAD0\

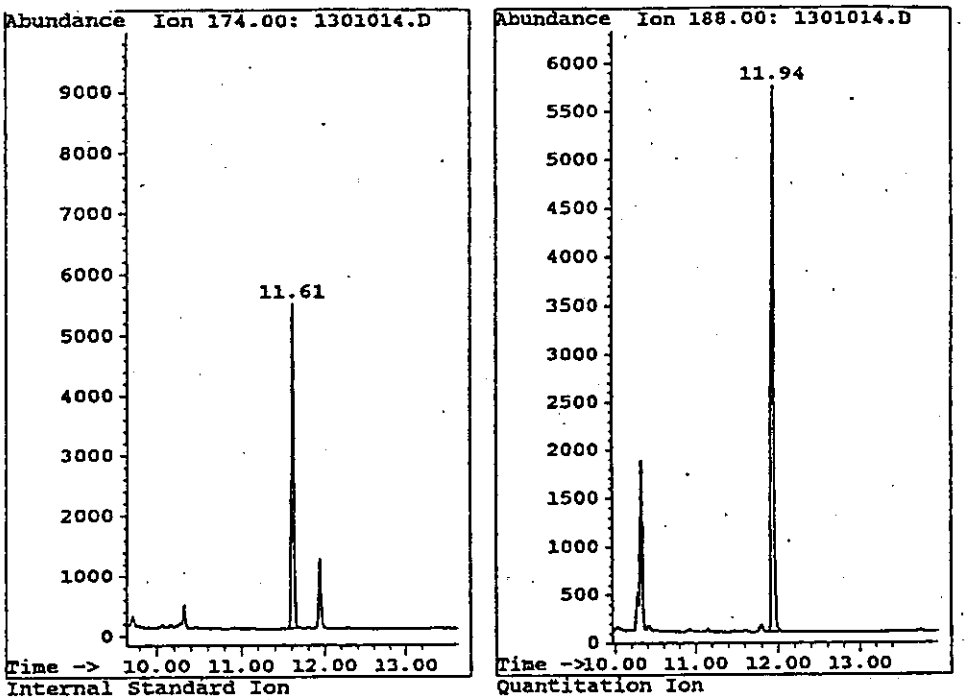
Acquisition: 29 Jan 97
Operator : B. A. Blakeslee
Integration: 01/30/97
Integrator : Blakeslee

Sample Name: 18235401 + 1.0 ng/g B

Compound	Ion	Retention Time	Peak Area
Internal Standard	174	11.61	11837
N-ethyl-Diclosulam	188	11.95	1016

Diclosulam Concentration: 0.91 ng/g
Recovery: 91%

Figure 7. Typical Chromatogram of a Control Soil Sample Fortified with 1.0 ng/g Diclosulam



Internal Standard Ion

Quantitation Ion

```
Data File  : 1301014.D                    Acquisition: 29 Jan 97
ALS Bottle : 13                           Operator   : B. A. Blakeslee
Method     : diclo.M                      Integration: 01/30/97
Instrument : GC S/N 3203A40952            Integrator : Blakeslee
Data Path  : C:\CHEMPC\DATA\ENV96021\GADO\

Sample Name: 18235401 + 10. ng/g
```

Compound	Ion	Retention Time	Peak Area
Internal Standard	174	11.61	10881
N-ethyl-Diclosulam	188	11.94	11298

Diclosulam Concentration: 9.87 ng/g
Recovery: 99%

Figure 8. Typical Chromatogram of a Control Soil Sample Fortified with 10 ng/g Diclosulam

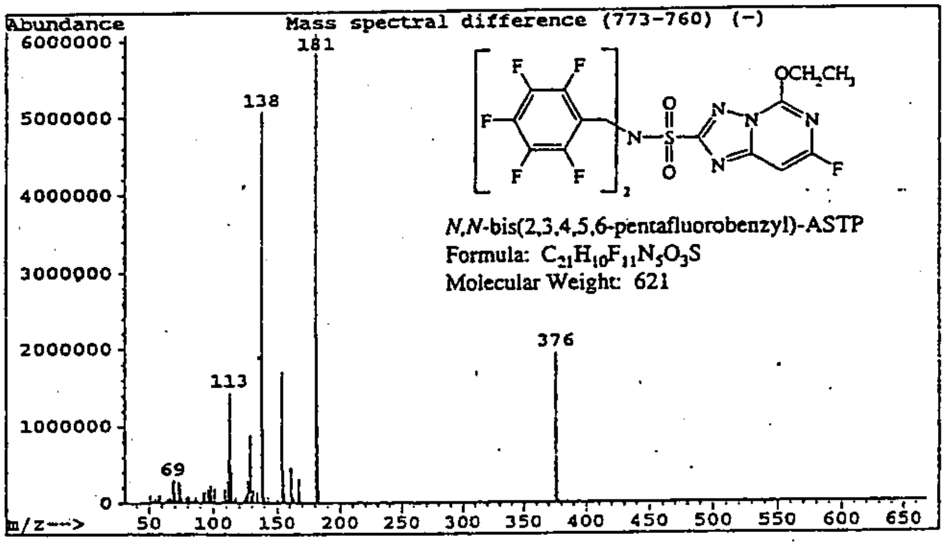
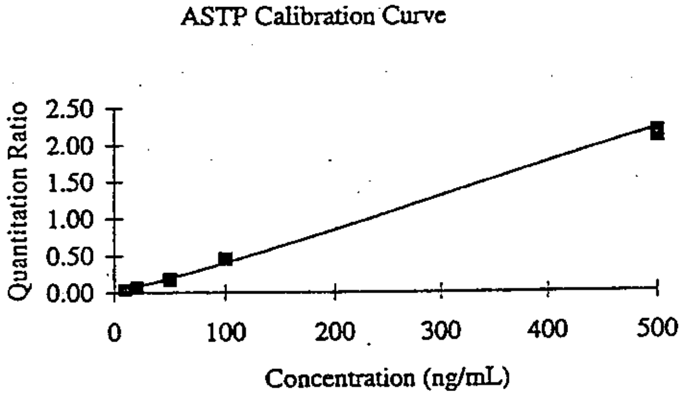


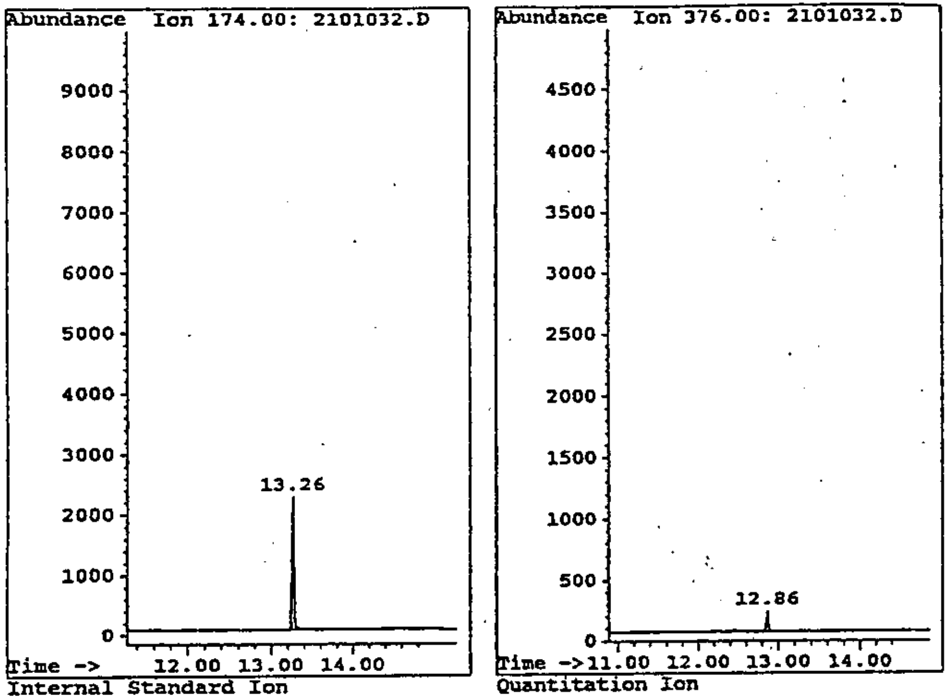
Figure 9. Electron Impact Mass Spectrum of *N,N*-bis(2,3,4,5,6-pentafluorobenzyl)-ASTP



ASTP Concentration (ng/mL)	Equivalent Sample Concentration (ng/g)	ASTP Quantitation Ratio *m/z* 376/*m/z* 174
10.0	0.50	0.036
10.0	0.50	0.035
20.0	1.00	0.073
20.0	1.00	0.069
50.0	2.50	0.183
50.0	2.50	0.168
100	5.0	0.461
100	5.0	0.449
500	25	2.163
500	25	2.088

Power Regression Equation: $X = (Y/0.0030)^\wedge(1/1.06)$
Coefficient of Determination (r^2): 0.9971

Figure 10. Typical Calibration Curve for the Determination of ASTP as its Pentafluorobenzyl Derivative



Internal Standard Ion

Quantitation Ion

Data File : 2101032.D
ALS Bottle : 21
Method : ASTP.M
Instrument : GC S/N 3203A40952
Data Path : C:\CHEMPC\DATA\ENV96021\GAD0\

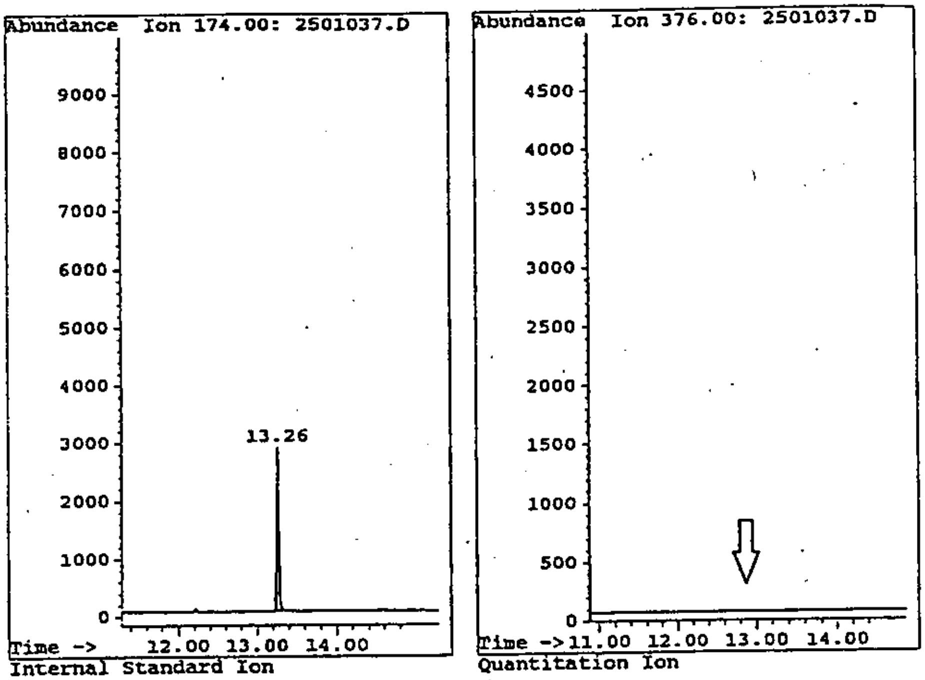
Acquisition: 30 Jan 97
Operator : B. A. Blakeslee
Integration: 01/30/97
Integrator : Blakeslee

Sample Name: Std 20. ng/mL ASTP

Compound	Ion	Retention Time	Peak Area
Internal Standard	174	13.26	3723
ASTP	376	12.86	270

Equivalent ASTP Concentration: 1.00 ng/g

Figure 11. Typical Chromatogram of a 20-ng/mL Standard, Equivalent to 1.0 ng/g of ASTP in Soil



Internal Standard Ion

Quantitation Ion

Data File : 2501037.D
ALS Bottle : 25
Method : ASTP.M
Instrument : GC S/N 3203A40952
Data Path : C:\CHEMPC\DATA\ENV96021\GAD0\

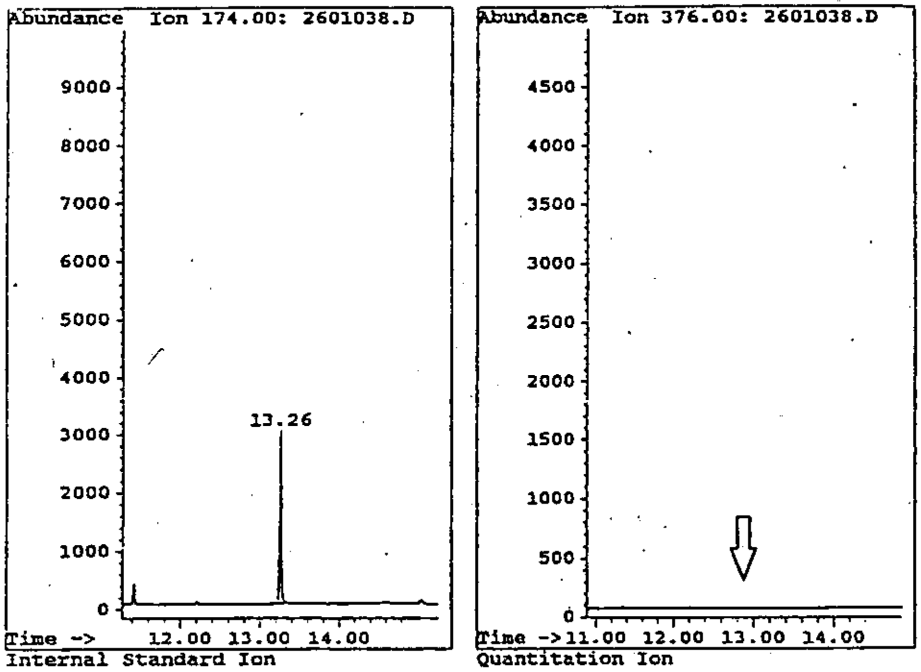
Acquisition: 30 Jan 97
Operator : B. A. Blakeslee
Integration: 01/30/97
Integrator : Blakeslee

Sample Name: Reagent Blank

Compound	Ion	Retention Time	Peak Area
Internal Standard	174	13.26	4471
ASTP	376	Not Found	Not Found

ASTP Concentration: 0.00 ng/g

Figure 12. Typical Chromatogram of a Reagent Blank Sample for the Determination of ASTP



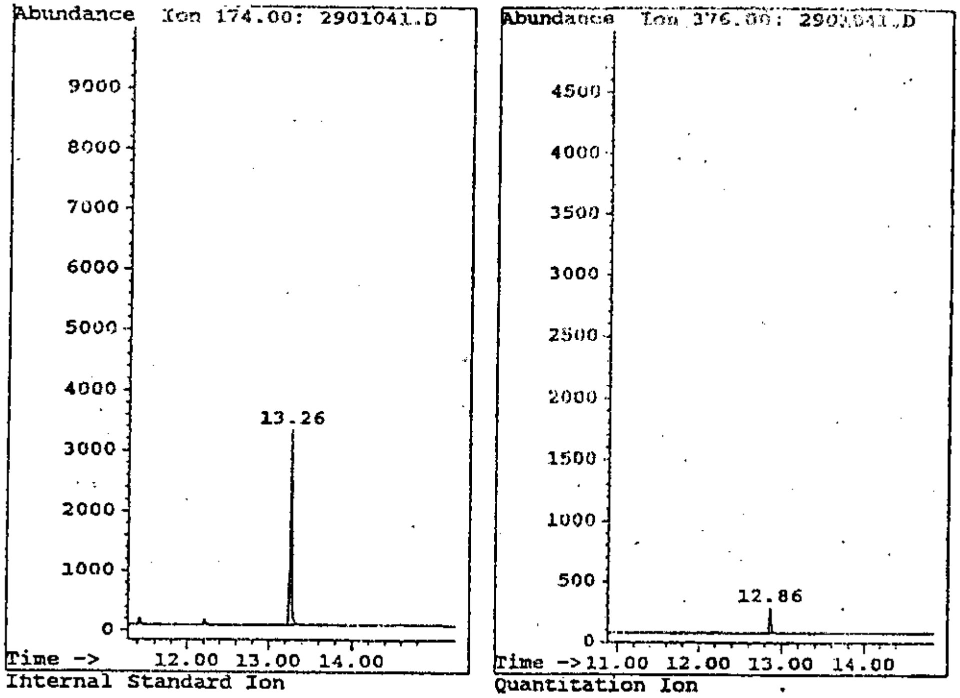
```
Data File  : 2601038.D                          Acquisition: 30 Jan 97
ALS Bottle : 26                                 Operator   : B. A. Blakeslee
Method     : ASTP.M                             Integration: 01/30/97
Instrument : GC S/N 3203A40952                  Integrator : Blakeslee
Data Path  : C:\CHEMPC\DATA\ENV96021\GAD0\

Sample Name: 18235401
```

Compound	Ion	Retention Time	Peak Area
Internal Standard	174	13.26	4632
ASTP	376	Not Found	Not Found

ASTP Concentration: 0.00 ng/g

Figure 13. Typical Chromatogram of a Control Soil Sample for the Determination of ASTP



Internal Standard Ion

Quantitation Ion

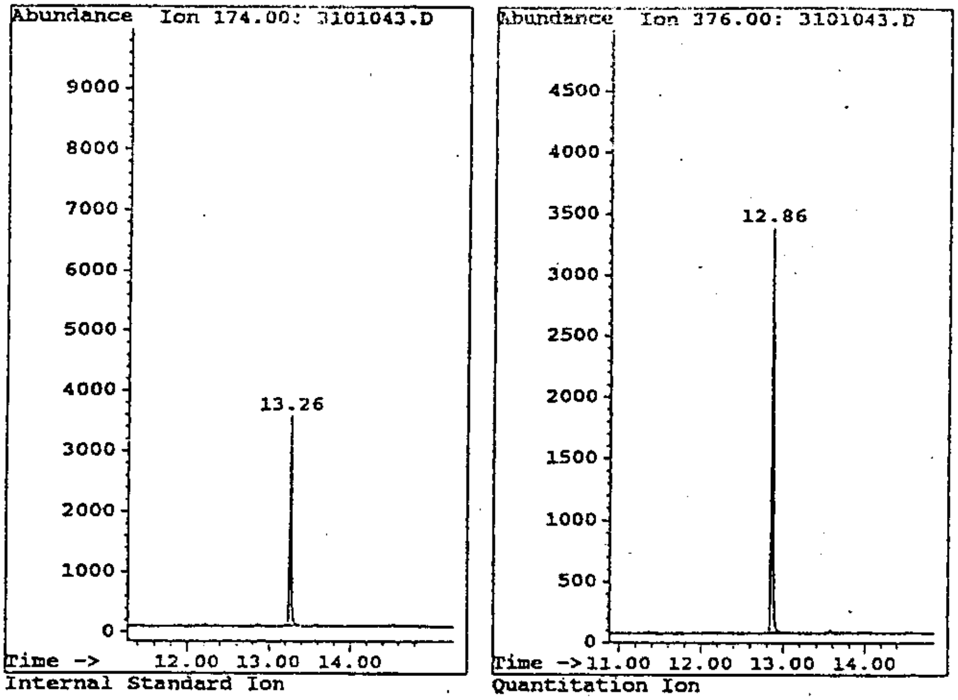
```
Data File   : 2901041.D                    Acquisition: 30 Jan 97
ALS Bottle  : 29                           Operator   : B. A. Blakeslee
Method      : ASTP.M                       Integration: 01/30/97
Instrument  : GC S/N 3203A40952            Integrator : Blakeslee
Data Path   : C:\CHEMPC\DATA\ENV96021\GAD0\

Sample Name: 18235401 + 1.0 ng/g B
```

Compound	Ion	Retention Time	Peak Area
Internal Standard	174	13.26	5216
ASTP	376	12.86	332

ASTP Concentration: 0.885 ng/g
Recovery: 89%

Figure 14. Typical Chromatogram of a Control Soil Sample Fortified with 1.0 ng/g ASTP



Internal Standard Ion

Quantitation Ion

```
Data File   : 3101043.D                      Acquisition: 30 Jan 97
ALS Bottle  : 31                             Operator   : B. A. Blakeslee
Method      : ASTP.M                         Integration: 01/30/97
Instrument  : GC S/N 3203A40952              Integrator : Blakeslee
Data Path   : C:\CHEMPC\DATA\ENV96021\GAD0\

Sample Name: 18235401 + 10. ng/g
```

Compound	Ion	Retention Time	Peak Area
Internal Standard	174	13.26	5624
ASTP	376	12.86	4935

ASTP Concentration: 10.5 ng/g
Recovery: 105%

Figure 15. Typical Chromatogram of a Control Soil Sample Fortified with 10 ng/g ASTP

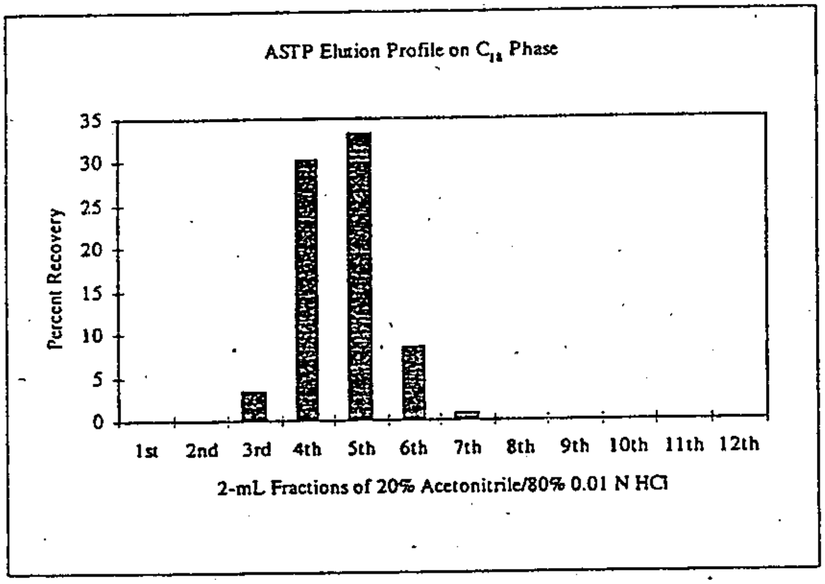


Figure 16. Elution Profile of ASTP on C_{18} SPE Column with 20% Acetonitrile/80% 0.01 N HCl

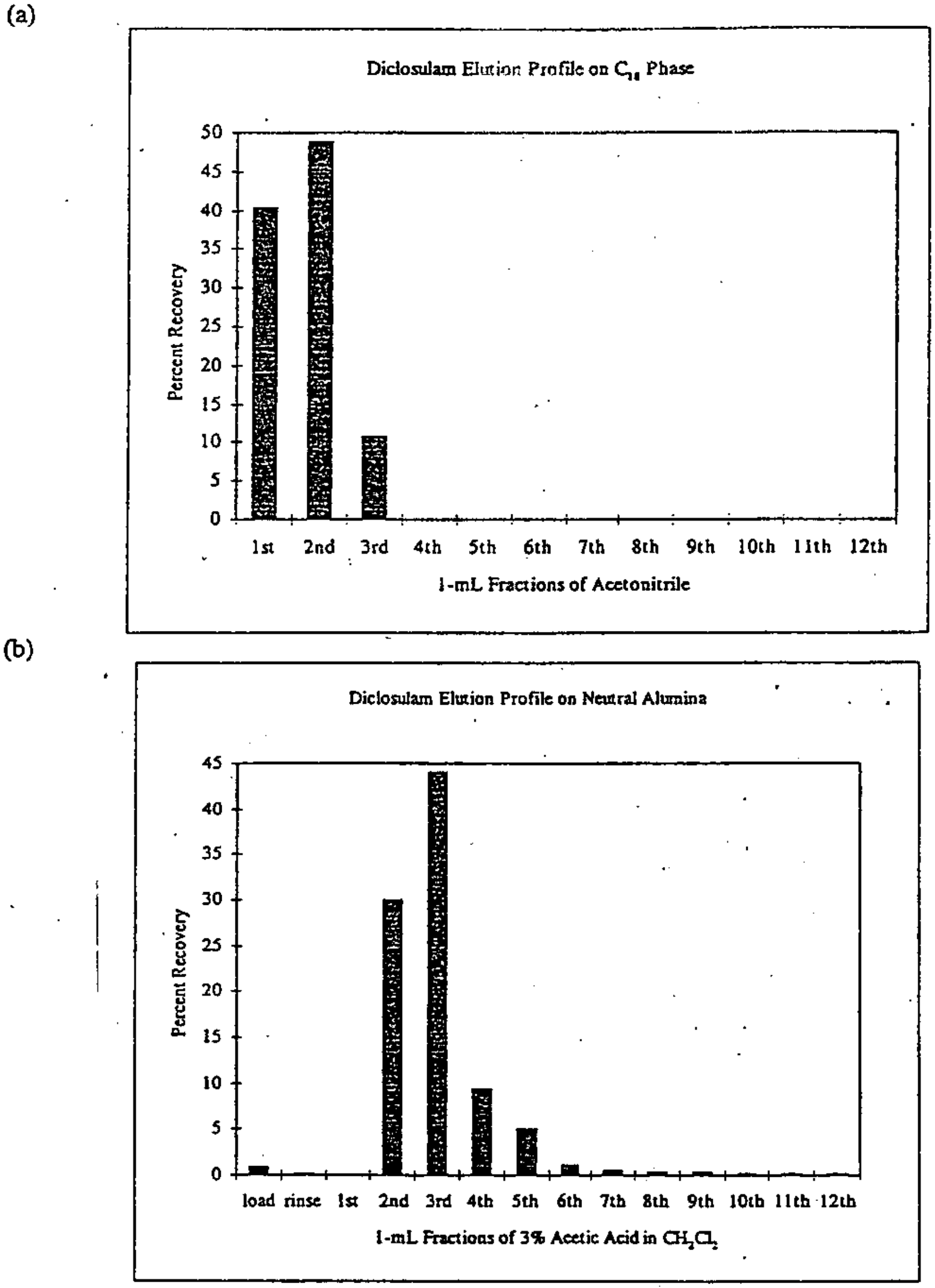


Figure 17. Elution Profile of Diclosulam: (a) C_{18} SPE Column with Acetonitrile, and (b) Neutral Alumina with 3% Acetic Acid in Dichloromethane