GRM: 95.10
EFFECTIVE: September 14, 1995
SUPERSEDES: New

Determination of Residues of Cloransulam-methyl and Cloransulam in Soil by Capillary Gas Chromatography with Mass Selective Detection

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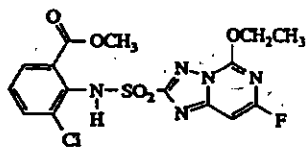
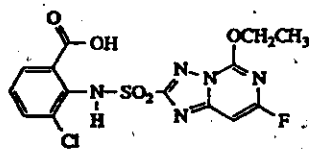
A. Scope

This method is applicable for the quantitation of residues of cloransulam-methyl and cloransulam in soil. The method was validated over the concentration range of 1.0 to 125 ng/g with a limit of quantitation of 1.0 ng/g.



Cloransulam-methyl
CAS 147150-35-4



Cloransulam
CAS 159518-97-5

B. Principle

Residues of cloransulam-methyl and cloransulam in soil are extracted with acidified acetone. Magnesium acetate is added to the extract to precipitate the soil matrix. The extract is concentrated to remove acetone, further acidified and partitioned onto an octadecyl (C_{18}) solid-phase extraction (SPE) column. The residues are eluted with acetonitrile and the acetonitrile is evaporated. Residues of cloransulam-methyl and cloransulam in acetone are derivatized with triethyloxonium tetrafluoroborate and triethylamine to *N*-ethyl-cloransulam-methyl and *N*-ethyl-cloransulam-ethyl, respectively. The acetone is evaporated and the derivatized residues are partitioned from a solution of potassium bicarbonate into a solution of methyl-*t*-butyl ether (MTBE) in hexane. The MTBE/hexane solution is partitioned onto a silica gel SPE column and eluted with a solution of acetone in toluene. The solvent is evaporated and the derivatized residues are dissolved in toluene containing the *N*-methyl-cloransulam-methyl as an internal standard. Samples are analyzed 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, 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. Acetic acid, acetone, acetonitrile, hexane, MTBE, toluene and triethylamine are flammable and should be used in well-ventilated areas away from ignition sources.
3. Concentrated acetic acid, and 1.0 N and 0.1 N hydrochloric acid are corrosive and can cause severe burns. It is imperative that proper eye and personal protection equipment be used when handling these reagents.
4. Triethylamine is corrosive. It is imperative that proper eye and personal protection equipment be used when handling these reagents.
5. Triethyloxonium tetrafluoroborate is corrosive and an alkylating agent. It is imperative that proper eye and personal protection equipment be used when handling this reagent.

D. Equipment (Note N.1.)

1. Automatic sampler, Model 7673, Hewlett-Packard, Wilmington, DE 19808.
2. Balance, analytical, Model AE200, Mettler Instrument Corporation, Hightstown, NJ 08520.
3. Balance, pan, Model BB2440, Mettler Instrument Corporation.
4. Centrifuge, with rotor to accommodate 7-, 12-, and 45-mL vials, Model Centra-8, International Equipment Company, Needham Heights, MA 02194.
5. Evaporator, N-Evap, Model 111, Organomation Associates, Inc., South Berlin, MA 01549.
6. Evaporator, TurboVap LV, Zymark Corporation, Hopkinton, MA 01748.
7. Gas chromatograph, Model 5890 Series II, Hewlett-Packard.
8. Mass selective detector, Model 5971A, Hewlett-Packard, Palo Alto, CA 94304.
9. Mass selective detector data system, Model G1034B, Hewlett-Packard.
10. Shaker, variable speed reciprocating with box carrier, Model 6000, Eberbach Corporation, Ann Arbor, MI 48106.
11. Ultrasonic bath, Model 1200, Branson Cleaning Equipment Company, Shelton, CT 06484.
12. Vacuum manifold box, Model spe-21, J.T. Baker, Inc., Phillipsburg, NJ 08865.
13. Vial crimper, catalog number 8710-0979, Hewlett-Packard, Wilmington, DE 19808.
14. Vortex mixer, Model G-560, Scientific Industries, Inc., Bohemia, NY 11716.
15. Water purification system, Model Milli-Q UV Plus, Millipore Corporation, Milford, MA 01757.

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

1. Bottles, amber, 4 oz., 8 oz. and 32 oz., with TFE-lined caps, catalog numbers 03-320-4B, 03-320-4C and 03-320-4E, Fisher Scientific, Pittsburgh, PA 15219.
2. Cap, for 7- and 12-mL vials, PTFE-lined, catalog number 02-883-3B, Fisher Scientific.
3. Cap, for 45-mL vial, PTFE-lined, catalog number 02-883-3F, Fisher Scientific.
4. Column, capillary gas chromatography, DB-5 liquid phase, 10 m x 0.18 mm i.d., 0.4 µm film thickness, catalog number 121-5013, J&W Scientific, Folsom, CA 95630.
5. Column, C_{18} SPE, catalog number 7020-07, J.T. Baker, Inc.
6. Column, silica gel SPE, catalog number 7086-07, J.T. Baker, Inc.
7. Column adapter, PTFE, catalog number 120-1100, Jones Chromatography, Inc., Lakewood, CO 80228.
8. Column inlet liner, deactivated, catalog number 5181-3315, Hewlett-Packard.
9. Column reservoir, 25 mL, catalog number 120-1007-E, Jones Chromatography, Inc.
10. Filter, charcoal, catalog number 7972, Chrompack, Inc., Raritan, NJ 08869. (Note N.2.)
11. Filter, glass fiber prefilters, 1.0 µm pore size, catalog number 4523, Gelman Sciences, Inc., Ann Arbor, MI 48106.
12. Filter, moisture, catalog number 7971, Chrompack, Inc. (Note N.2.)
13. Filter, oxygen, catalog number 7970, Chrompack, Inc. (Note N.2.)
14. Gas, helium, 99.995% purity, Airco, Murray Hill, NJ 07974.
15. Gas, nitrogen, 99.99% purity, Airco.
16. Microdispensers, 25 µL and 100 µL, Drummond Dialamatic Microdispenser, catalog numbers 300225 and 300275, Drummond Scientific Company, Broomall, PA 19008.
17. Microdispenser replacement bores, 25 µL and 100 µL, catalog numbers 300225G and 300275G, Drummond Scientific Company.
18. Syringes, 100, 250, and 500 µL capacity, catalog numbers 80600, 80700, and 80800, Hamilton Co., Reno, NV 89520.
19. Vial, autosampler, 2-mL, catalog number C4011-1, National Scientific Co., Lawrenceville, GA 30243.
20. Vials, 7-, 12-, and 45-mL, catalog numbers 03-337-26, 03-338-29C, and 03-339-5D, Fisher Scientific.
21. Vial seal, for 2-mL autosampler vial, catalog number C4011-1A, National Scientific Company.

F. Reagents and Chemicals (Note N.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. Hexane, Optima grade, catalog number H303-4, Fisher Scientific.

e. Hydrochloric acid, 0.1 N, ACS reagent grade, certified concentration, catalog number SA54-4, Fisher Scientific.

f. Hydrochloric acid, 1.0 N, ACS reagent grade, certified concentration, catalog number SA48-4, Fisher Scientific.

g. Magnesium acetate tetrahydrate, catalog number 22,864-8, Aldrich Chemical Co., Milwaukee, WI, 53233.

h. Methyl-t-butyl ether, MTBE, HPLC grade, catalog number E127-4, Fisher Scientific.

i. Potassium bicarbonate, certified ACS grade, catalog number P184-500, Fisher Scientific.

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

k. Triethylamine, catalog number 13,206-3, Aldrich Chemical Co.

l. Triethyloxonium tetrafluoroborate, 1.0 M solution in dichloromethane, catalog number 17,623-0, Aldrich Chemical Co.

m. Standards.

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

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

(3) *N*-Methyl-cloransulam-methyl: *N*-Methyl-(2-carbomethoxy-6-chlorophenyl)-5-ethoxy-7-fluoro[1,2,4]triazolo[1,5-c]pyrimidine-2-sulfonamide

(4) *N*-Ethyl-cloransulam-methyl: *N*-Ethyl-(2-carbomethoxy-6-chlorophenyl)-5-ethoxy-7-fluoro[1,2,4]triazolo[1,5-c]pyrimidine-2-sulfonamide

(5) *N*-Ethyl-cloransulam-ethyl: *N*-Ethyl-(2-carboethoxy-6-chlorophenyl)-5-ethoxy-7-fluoro[1,2,4]triazolo[1,5-c]pyrimidine-2-sulfonamide

Obtain from Test Substance Coordinator, DowElanco, 9330 Zionsville Road, Indianapolis, IN 46268-1053.

2. Prepared Solutions

a. 1:20:79 (acetic acid:acetonitrile:0.005 N hydrochloric acid) solution

Pipet 10 mL of acetic acid and 200 mL of acetonitrile into a 1000-mL volumetric flask and dilute to volume with 0.005 N hydrochloric acid.

b. 9:1 (acetone:1.0 N hydrochloric acid) solution

Pipet 200 mL of 1.0 N hydrochloric acid into a 2000-mL volumetric flask containing approximately 1000 mL of acetone. Swirl the flask and allow to equilibrate to room temperature. Dilute to volume with acetone.

c. 5% acetone in toluene solution

Pipet 50 mL of acetone into a 1000-mL volumetric flask and dilute to volume with toluene.

d. 0.005 N hydrochloric acid solution

Pipet 50 mL of 0.1 N hydrochloric acid into a 1000-mL volumetric flask and dilute to volume with deionized water.

e. magnesium acetate solution

Weigh 10 g magnesium acetate tetrahydrate into a 45-mL vial and dissolve in 20 mL of deionized water.

f. 20% MTBE in hexane solution

Pipet 200 mL of MTBE into a 1000-mL volumetric flask and dilute to volume with hexane.

g. 0.1 M potassium bicarbonate solution

Transfer 10 g of potassium bicarbonate to a 1000-mL volumetric flask and dissolve in 500 mL of deionized water. Dilute to volume with deionized water.

G. Preparation of Standards

All solutions prepared in Section G. should be stored in amber bottles sealed with PTFE-lined caps (Section E.1.).

1. Preparation of Cloransulam-methyl and Cloransulam Stock Solutions

a. Weigh 0.1000 g of cloransulam-methyl analytical standard and quantitatively transfer to a 100-mL volumetric flask. Dilute to volume with acetone to obtain a 1000 µg/mL stock solution.

b. Weigh 0.1000 g of cloransulam analytical standard and quantitatively transfer to a 100-mL volumetric flask. Dilute to volume with acetone to obtain a 1000 µg/mL stock solution.

2. Preparation of Cloransulam-methyl and Cloransulam Spiking Solutions

a. Pipet 1.0 mL of each of the stock solutions in Sections G.1.a. and G.1.b. into a 100-mL volumetric flask and bring to volume with acetone to obtain an initial solution of 10.0 µg/mL for cloransulam-methyl and cloransulam.

b. Solutions for spiking soil samples are prepared by adding 10.0 mL of acetone and 10 µL of acetic acid to a volumetric flask. The flask is agitated to allow acetic acid to contact the glass surface. The appropriate aliquot of the initial solution from Section G.2.a. is then added and diluted to volume with acetone as follows:

Aliquot of Soln. G.2.a.	Final Soln. Volume	Spiking Soln. Final Conc.	Equivalent Sample Conc.[a]
mL	mL	ng/mL	ng/g
0.10	100	10.0	1.0
0.20	100	20.0	2.0
0.50	100	50.0	5.0
5.0	200	250.	25.0
25.0	200	1250.	125.

[a] The equivalent sample concentration is based on fortifying a 10-g soil sample with 1.0 mL of spiking solution.

3. Preparation of *N*-Methyl-Cloransulam-methyl, *N*-Ethyl-Cloransulam-methyl, and *N*-Ethyl-Cloransulam-ethyl Stock Solutions

a. Weigh 0.1000 g of *N*-methyl-cloransulam-methyl analytical standard and quantitatively transfer to a 100-mL volumetric flask. Dilute to volume with acetone to obtain a 1000 µg/mL stock solution.

b. Weigh 0.1065 g of *N*-ethyl-cloransulam-methyl analytical standard and quantitatively transfer to a 100-mL volumetric flask. Dilute to volume with acetone to obtain a 1065 µg/mL (1000 µg/mL cloransulam-methyl equivalent) stock solution.

c. Weigh 0.1135 g of *N*-ethyl-cloransulam-ethyl analytical standard and quantitatively transfer to a 100-mL volumetric flask. Dilute to volume with acetone to obtain a 1135 µg/mL (1000 µg/mL cloransulam equivalent) stock solution.

4. Preparation of Cloransulam-methyl and Cloransulam Calibration Solutions

a. Pipet 1.0 mL of the stock solution in Section G.3.a. into a 100-mL volumetric flask and bring to volume with toluene to obtain an initial solution of 10.0 µg/mL for *N*-methyl-cloransulam-methyl.

b. Pipet 1.0 mL of the stock solutions in Sections G.3.b. and G.3.c. into a 100-mL volumetric flask and bring to volume with toluene to obtain an initial solution equivalent to 10.0 µg/mL for cloransulam-methyl and cloransulam.

c. Solutions for calibration are prepared by adding the appropriate aliquot of the solution from Section G.4.a. and the appropriate aliquot of the solution from G.4.b. to a volumetric flask and diluting to volume with toluene as follows:

Aliquot of Soln. G.4.a.	Aliquot of Soln. G.4.b.	Final Soln. Volume	Calibn. Soln. Final Conc.	Equivalent Sample Conc.[a]
mL	mL	mL	ng/mL	ng/g
2.0	0.10	100	10.0	0.50
2.0	0.20	100	20.0	1.0
2.0	1.0	100	100.	5.0
2.0	5.0	100	500.	25.0

[a] The equivalent sample concentration is based on taking the 10-g soil sample extract to a final volume of 0.5 mL.

5. Preparation of *N*-Methyl-Cloransulam-methyl Internal Standard Solution

   a. Pipet 20.0 mL of the solution from Section G.4.a. into a 1000-mL volumetric flask and dilute to volume with toluene.

H. Gas Chromatography/Mass Spectrometry

1. Column

Install the splitless column inlet liner (Section B.8.) and the capillary column (Section B.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 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 min 120 °C to 325 °C at 15 °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 260 °C
Injection Mode:	splitless
Purge Delay	0.7 min
Splitter Flow	60 mL/min
Septum Purge	1.0 mL/min
Injection Volume:	3 µL
Detector:	electron impact ionization with selected ion monitoring
Calibration Program	maximum sensitivity autotune
Electron Multiplier	1647 volts (tune voltage plus 200)
Ions Monitored:	
N-Methyl-cloransulam-methyl	*m/z* 198 (internal standard)
N-Ethyl-cloransulam-methyl	*m/z* 212 (quantitation), *m/z* 180 (confirmation)
N-Ethyl-cloransulam-ethyl	*m/z* 226 (quantitation), *m/z* 180 (confirmation)
Dwell Time	100 msec

Typical mass spectra of *N*-methyl-cloransulam-methyl, *N*-ethyl-cloransulam-methyl, and *N*-ethyl-cloransulam-ethyl are shown in Figures 1-3, respectively. Nominal *m/z* 198, 212 and 226 ions result from loss of the sulfonyl triazolopyrimidine radical (mass 245) from the respective molecular ions. The nominal *m/z* 180 ion monitored results from loss of methanol (mass 32) and ethanol (mass 46) from the *m/z* 212 and *m/z* 226 ions, respectively.

3. Calibration Curves

Typical calibration curves for the determination of cloransulam-methyl and cloransulam in soil are shown in Figures 4 and 5, respectively.

4. Typical Chromatograms

Typical chromatograms of a standard, control sample, and a 1.0-ng/g recovery sample for cloransulam-methyl and cloransulam in soil are shown in Figures 6-11.

I. Determination of Recovery of Cloransulam-methyl and Cloransulam from Soil

To minimize the potential for cross contamination, equipment used to process samples and reusable glassware should be thoroughly rinsed with the 9:1 (acetone:1.0 N hydrochloric acid) solution followed by acetone prior to use.

1. Preparation of Recovery Samples

a. Weigh 10.0 g of control soil into a series of 45-mL glass vials.

b. For preparing fortified samples, use some of the samples as controls and fortify the remaining samples by adding the specified aliquots of the appropriate spiking solutions (Section G.2.b.) in acetone to obtain concentrations ranging from 1.0 to 125 ng/g. A reagent blank, containing no soil sample, should be carried through the method with the samples.

c. Add 15.0 mL of the 9:1 (acetone:1.0 N hydrochloric acid) solution to each sample vial and seal with a PTFE-lined cap.

d. Vortex the samples briefly and sonicate 10-15 seconds.

e. Shake the samples for a minimum of 30 minutes on a reciprocating shaker at approximately 180 excursions/minute.

f. Centrifuge each sample for 5 minutes at 2500 rpm.

g. Attach a glass fiber prefilter (Section E.11.) to a 25-mL reservoir. Decant the extract to the reservoir and collect the filtered extract in a 45-mL vial. The glass fiber prefilter may be secured to a vacuum manifold box.

h. Extract each sample a second time by repeating Steps I.1.c., d. and f. Combine the extracts by decanting to the reservoir in Step I.1.g.

i. Precipitate the soil matrix using the following procedure:

(1) Add 50 µL of the magnesium acetate solution and vortex briefly.

(2) Repeat Step I.1.i.(1) until flocculation of soil matrix occurs. Soil extracts have typically required at least 200 µL of the magnesium acetate solution to onset flocculation.

(3) Centrifuge for 7 minutes at 2500 rpm. If the extract still displays an orange color repeat Step L1.i.(1) until the the extract is yellow after centrifugation. The amount required for precipitation should be consistent for soils from the same site and depth. If solution is cloudy after centrifugation, filter the solution through a glass fiber prefilter (Section E.11.) when decanting to the vial in Step L1.j.

j. Decant extract to a clean 45-mL vial.

k. Add 5 mL of the 9:1 (acetone:0.1 N hydrochloric acid) solution to the precipitate, vortex briefly and sonicate for 10 seconds. Centrifuge for 7 minutes at 2500 rpm and combine extracts.

l. Evaporate the acetone in the extract by placing the vial in a Turbo-Vap evaporator set at 50 °C. Concentrate the sample under nitrogen to approximately 2 mL.

m. Add 7.5 mL of the 1:20:79 (acetic acid:acetonitrile: 0.005 N hydrochloric acid) solution and 5 mL of 0.1 N HCl to the vial and seal with a PTFE-lined cap.

n. Vortex the samples briefly and sonicate for 10-15 seconds. Repeat this step if necessary to dissolve residues adhering to the vial walls.

o. Centrifuge for 5 minutes at 2500 rpm. Use care in handling the centrifuged sample to prevent disturbing the sediment.

p. The samples are then concentrated and purified using the following C_{18} SPE procedure:

(1) Place a C_{18} SPE column (Section E.5.) on the vacuum manifold box.

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

(3) Rinse the reservoir and SPE column with 5 mL of acetonitrile. (Do not allow the column bed to dry.)

(4) Condition the reservoir and SPE column with 5 mL of 0.005 N hydrochloric acid solution. (Do not allow the column bed to dry.)

(5) Carefully transfer the sample solution from Step L1.o. to the reservoir and, with the aid of vacuum pull the sample through the column at a flow rate of approximately 2 mL/min.

(6) Rinse the sample vial with 7.5 mL of the 1:20:79 (acetic acid:acetonitrile: 0.005 N hydrochloric acid) solution. Repeat Steps L1.n. and L1.o. Carefully transfer the rinse to the reservoir after the entire sample has passed through the column. With the aid of vacuum, pull the rinse through the column at a flow rate of approximately 2 mL/min.

(7) Rinse the reservoir and column with 10 mL of 0.005 N hydrochloric acid solution. With the aid of vacuum, pull the rinse through the column at a flow rate of approximately 2 mL/min.

(8) Remove the reservoir and column adaptor. Allow the column to dry under vacuum for 30 minutes.

(9) Elute the cloransulam-methyl and cloransulam with 5.0 mL of acetonitrile, collecting the eluent in a 7-mL vial. Discard the SPE column.

r. Evaporate the sample to dryness by placing the vial in an N-Evap evaporator set at 50 °C.

s. Allow the vial to cool and add 1 mL of acetone.

t. Add 25 µL triethylamine to the vial and seal with a PTFE-lined cap. Vortex the vial briefly to mix.

u. Add 100 µL triethyloxonium tetrafluoroborate solution to the vial and seal with a PTFE-lined cap. Vortex the vial briefly and shake the vial for 20 minutes on a reciprocating shaker at approximately 180 excursions/minute.

v. Repeat Steps L1.s. and L1.t. adding 200 µL triethyloxonium tetrafluoroborate solution.

w. Evaporate the sample to dryness by placing the vial in an N-Evap evaporator set at 50 °C.

x. Allow the vial to cool and add 2.5 mL of 20% MTBE in hexane and 3 mL of 0.1 M potassium bicarbonate solution. Seal the vial with a PTFE-lined cap.

y. Shake the vial for 3 minutes on a reciprocating shaker at approximately 180 excursions/minute.

z. Centrifuge the vial at 2500 rpm for 2 minutes.

aa. Transfer the top organic layer to a clean 7-mL vial.

bb. Extract the aqueous solution with a second 2.5 mL of 20% MTBE in hexane and repeat Steps L1.y. and L1.z. Combine extracts.

cc. The samples are then purified using the following silica gel SPE procedure:

(1) Place a silica gel SPE column (Section E.6.) on the vacuum manifold box.

(2) Rinse the SPE column with 5 mL of toluene.

(3) Condition the SPE column with 5 mL of hexane. (Do not allow the column bed to dry.)

(4) Transfer the sample solution from Step L1.z. to the SPE column and, with the aid of vacuum, pull the sample through the column at a flow rate of approximately 2 mL/min.

(5) Rinse the sample vial with 2.5 mL of 20% MTBE in hexane and transfer the rinse to the SPE column. With the aid of vacuum, pull the rinse through the column at a flow rate of approximately 2 mL/min.

(6) Elute the SPE column with 10 mL of 5% acetone in toluene. Collect the eluent in a 12-mL vial.

dd. Evaporate the sample to dryness by placing the vial in an N-Evap evaporator set at 50 °C.

ee. Allow the vial to cool and add 0.5 mL of toluene containing the internal standard from Step G.5.a.

ff. Vortex and sonicate the vial briefly and centrifuge at 2500 rpm for 5 minutes.

gg. Transfer the sample to a 2-mL autosampler vial and seal with a cap and crimper.

hh. Analyze the samples and calibration standards from Step G.4.c. by capillary gas chromatography/ mass spectrometry as described in Section H.

hh. Samples showing levels of cloransulam-methyl or cloransulam above 25 ng/g are diluted 6-fold and reanalyzed as follows:

(1) Transfer 100 µL of the derivatized sample to a 2-mL autosampler vial.

(2) Add 0.5 mL of toluene containing the internal standard from Step G.5.a.

(3) Seal the vial with a cap and crimper and swirl gently to mix.

(4) Reanalyze the sample by GC/MSD as described in Section H.

2. Calculation of Percent Recovery

a. Using the data for the series of calibration standards analyzed in Section I.1.gg., determine the peak areas for *N*-methyl-cloransulam-methyl (*m/z* 198), *N*-ethyl-cloransulam-methyl (*m/z* 212,180), and *N*-ethyl-cloransulam-ethyl (*m/z* 226,180).

b. For each standard, calculate the cloransulam-methyl and cloransulam confirmation ratios. The average confirmation ratio for all calibration standards will be used to confirm the presence of cloransulam-methyl and cloransulam in the soil samples.

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

For example, using the data for cloransulam-methyl from Figure 6:

$$\text{Cloransulam-methyl Confirmation Ratio} = \frac{\text{peak area at } m/z \text{ 212}}{\text{peak area at } m/z \text{ 180}}$$

$$\text{Cloransulam-methyl Confirmation Ratio} = \frac{4336}{5531}$$

$$\text{Cloransulam-methyl Confirmation Ratio} = 0.7839$$

For example, using the data for cloransulam from Figure 9:

$$\text{Cloransulam Confirmation Ratio} = \frac{\text{peak area at } m/z \text{ 226}}{\text{peak area at } m/z \text{ 180}}$$

$$\text{Cloransulam Confirmation Ratio} = \frac{3694}{5813}$$

$$\text{Cloransulam Confirmation Ratio} = 0.6355$$

Confirmation of the presence of cloransulam-methyl and cloransulam is indicated when the confirmation ratio for the samples is within the range of ±15% of the average found for the standards.

c. For each standard, calculate the cloransulam-methyl and cloransulam quantitation ratios.

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

For example, using the data for cloransulam-methyl from Figure 6:

$$\text{Cloransulam-methyl Quantitation Ratio} = \frac{\text{peak area at } m/z \text{ 212}}{\text{peak area at } m/z \text{ 198}}$$

$$\text{Cloransulam-methyl Quantitation Ratio} = \frac{4336}{54393}$$

$$\text{Cloransulam-methyl Quantitation Ratio} = 0.07972$$

For example, using the data for cloransulam from Figure 9:

$$\text{Cloransulam Quantitation Ratio} = \frac{\text{peak area at } m/z \text{ 226}}{\text{peak area at } m/z \text{ 198}}$$

$$\text{Cloransulam Quantitation Ratio} = \frac{3694}{54393}$$

$$\text{Cloransulam Quantitation Ratio} = 0.06791$$

d. Prepare a standard curve for cloransulam-methyl and for cloransulam by plotting the equivalent concentration on the abscissa (x-axis) and the respective quantitation ratio on the ordinate (y-axis) as shown in Figures 4 and 5. Using power regression (1) analysis, determine the equation for the curve with respect to the abscissa.

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

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

For example, using the cloransulam-methyl data from Figure 4:

$$\text{Cloransulam-methyl Conc. (ng/g)} = \left(\frac{\text{Cloransulam-methyl quantitation ratio}}{\text{constant}}\right)^{1/\text{exponent}}$$

$$\text{Cloransulam-methyl Conc. (ng/g)} = \left(\frac{\text{Cloransulam-methyl quantitation ratio}}{0.07447}\right)^{1/1.1238}$$

For example, using the cloransulam data from Figure 5:

$$\text{Cloransulam Conc. (ng/g)} = \left(\frac{\text{Cloransulam quantitation ratio}}{\text{constant}}\right)^{1/\text{exponent}}$$

$$\text{Cloransulam Conc. (ng/g)} = \left(\frac{\text{Cloransulam quantitation ratio}}{0.06536}\right)^{1/1.1545}$$

e. Determine the net concentration of cloransulam-methyl and cloransulam in each recovery sample by first subtracting the average quantitation ratios in the control sample from the respective ratios of the recovery sample. Substitute the quantitation ratio obtained into the appropriate equation above, and solve for the concentration.

For example, using the cloransulam-methyl data from Figures 7 and 8:

$$\text{Cloransulam-methyl Conc. (ng/g)} = \left(\frac{\text{net Cloransulam-methyl quantitation ratio}}{0.07447}\right)^{1/1.1238}$$

$$\text{Cloransulam-methyl Conc. (ng/g)} = \left(\frac{0.06856 - 0.0000}{0.07447}\right)^{1/1.1238}$$

$$\text{Cloransulam-methyl} = 0.929 \text{ ng/g}$$

For example, using the cloransulam data from Figures 10 and 11:

$$\text{Cloransulam Conc. (ng/g)} = \left(\frac{\text{net Cloransulam quantitation ratio}}{0.06536}\right)^{1/1.1545}$$

$$\text{Cloransulam Conc. (ng/g)} = \left(\frac{0.06397 - 0.0000}{0.06536}\right)^{1/1.1545}$$

$$\text{Cloransulam} = 0.982 \text{ ng/g}$$

f. Determine the concentration in each diluted recovery sample by multiplying the diluted concentration by the dilution factor of 6.

$$\text{Conc. (ng/g)} = \text{diluted Conc. (ng/g)} \times 6$$

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

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

For example, using the cloransulam-methyl data from Section I.2.e:

$$\text{Recovery} = \frac{0.929 \text{ ng/g}}{1.003 \text{ ng/g}} \times 100\%$$

$$\text{Recovery} = 93\%$$

For example, using the cloransulam data from Section I.2.e:

$$\text{Recovery} = \frac{0.982 \text{ ng/g}}{1.001 \text{ ng/g}} \times 100\%$$

$$\text{Recovery} = 98\%$$

The average recovery of all the recovery samples can be used to correct individual sample results for method efficiency.

J. Determination of Cloransulam-methyl and Cloransulam in Soil

1. Prepare reagent blank, control, recovery, and treated samples as described in Section I.1.
2. Prepare standard calibration curves for cloransulam-methyl and cloransulam and determine the percentage recoveries as described in Section I.2.
3. Determine the concentration of cloransulam-methyl and cloransulam in each treated sample as described in Section I.2.

K. Determination of Soil Moisture

1. Weigh 10.00 g of soil into an aluminum or glass container.
2. Place the sample in an oven at approximately 110 °C and allow to dry for a minimum of 16 hours.
3. Remove the sample from the oven, place in a desiccator until the sample has cooled to ambient temperature, and then re-weigh.
4. Calculate the percent moisture on a dry weight basis as follows:

$$\text{Percent Moisture} = \frac{\text{soil moisture weight (g)}}{\text{dehydrated soil weight (g)}} \times 100$$

$$\text{Percent Moisture} = \frac{\left(\text{soil weight}_{\text{before drying}} - \text{soil weight}_{\text{after drying}}\right)}{\text{soil weight}_{\text{after drying}}} \times 100$$

L. Determination of Corrected Cloransulam-methyl and Cloransulam in Soil

1. Determine the cloransulam-methyl and cloransulam concentration in the soil samples as described in Section J.
2. Determine the soil moisture as described in Section K.
3. Determine the corrected cloransulam-methyl and cloransulam concentrations in soil samples as follows:

$$\text{Corrected Dry Weight Conc. (ng/g)} = (\text{Conc. (ng/g)})\left(\frac{100}{\text{\% Recovery}}\right)^{*}\left(1 + \frac{\text{\% Moisture}}{100}\right)$$

* Correction for percent recovery is optional.

3. Assay Time

A typical analytical run would consist of a minimum of four 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 prepared in approximately 12 hours, and the chromatographic analysis take place the same evening.

There are several acceptable "stopping points" in the method where sample preparation (Section I) may be suspended without deleterious effects on the sample analysis. These are indicated below:

If the samples are to be stored overnight, the vials should be capped with PTFE-lined caps.

a. Step I.1.h.

b. Step I.1.m.

c. Step I.1.p.(9)

d. Step I.1.r.

e. Step I.1.aa.

4. Standardization of SPE Elution Profiles

Variation in the C_{18} and silica gel SPE columns may influence the elution profile of cloransulam-methyl and cloransulam. It is necessary to obtain an elution profile for each lot of SPE column used to ensure optimum recovery and clean-up efficiency. The following procedures can be used:

a. C_{18} SPE Profile

(1) In a 45-mL vial, add 1.0 mL of the 1250 ng/mL spiking solution (Section G.2.b.).

(2) Evaporate the acetone by placing the vial in a Turbo-Vap evaporator set at 50 °C.

(3) Proceed as described in Section I.1.m and I.1.n.

(4) Proceed as described in Section I.1.p.(1) through I.1.p.(4).

(5) Transfer the sample solution from Step M.4.a.(4) to the SPE column and, with the aid of vacuum, pull the sample through the column at a flow rate of approximately 2 mL/min. (Do not allow the column bed to dry.)

(6) Rinse the sample vial with 7.5 mL of the 1:20:79 (acetic acid:acetonitrile: 0.005 N hydrochloric acid) solution. Transfer the rinse to the reservoir after the entire sample has passed through the column. With the aid of vacuum, pull the rinse through the column at a flow rate of approximately 2 mL/min.

(7) Proceed as described in Section I.1.p.(7) and I.1.p.(8).

(8) Elute the column with 8 mL of acetonitrile, collecting 1.0-mL aliquots in 7-mL vials.

(9) For each fraction collected, proceed as described in Section I.1.q through I.1.aa.

(10) Proceed as described in Section I.1.cc through I.1.gg.

(11) Calculate the percent recovery in each fraction for each analyte as described in Section I.2.

Typical elution profiles for cloransulam-methyl and cloransulam are illustrated in Figure 12.

b. Silica Gel SPE Profile

(1) Add 50 µL of the 10 µg/mL equivalent solution (Section G.4.b.) to a 7-mL vial.

(2) Evaporate the toluene to dryness by placing the vial in a Turbo-Vap evaporator set at 50 °C.

(3) Allow the vial to cool and add 2.5 mL of 20% MTBE in hexane and 3 mL of deionized water. Seal the vial with a PTFE-lined cap.

(4) Proceed as described in Section I.1.x through I.1.bb.(5).

(5) Elute the column with 10 mL of 5% acetone in toluene, collecting 1.0-mL aliquots in 7-mL vials.

(6) For each fraction collected, proceed as described in Section I.1.cc through I.1.gg.

(7) Calculate the percent recovery in each fraction for each analyte as described in Section I.2.

Typical elution profiles for *N*-ethyl-cloransulam-methyl and *N*-ethyl-cloransulam-ethyl are illustrated in Figure 13.

N. 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.