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Determination of DE-570 (Florasulam) and the 5-Hydroxy Florasulam Degradate in Soil by Capillary Gas Chromatography with Mass Selective Detection

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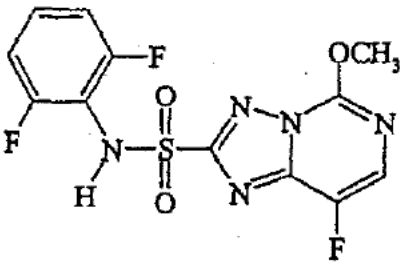
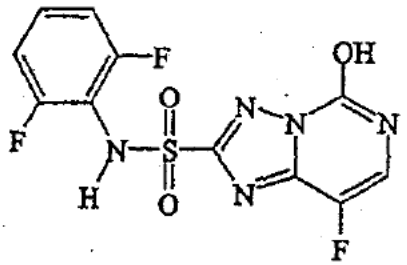
A. Scope

This method is applicable for the determination of residues of florasulam, *N*-(2,6-difluorophenyl)-8-fluoro-5-methoxy[1,2,4]triazolo-[1,5-c]pyrimidine-2-sulfonamide, and the 5-hydroxy florasulam degradate, *N*-(2,6-difluorophenyl)-8-fluoro-5-hydroxy[1,2,4]triazolo-[1,5-c]pyrimidine-2-sulfonamide, in soil. The method was validated over the concentration range of 1.0 to 100 ng/g with a limit of quantitation of 1.0 ng/g.



DE-570
Florasulam (proposed common name)
CAS 145701-23-1



5-Hydroxy Florasulam

B. Principle

Residues of florasulam and the 5-hydroxy florasulam are extracted from soil with acidified acetone. The extract is purified by filtration through a graphitized carbon solid-phase extraction (SPE) column and concentrated to remove acetone. The concentrated extract is diluted with 0.01 N hydrochloric acid and residues are partitioned onto an octadecyl (C_{18}) SPE column. Residues are eluted from the SPE with a solution of acetonitrile in

0.01 N hydrochloric acid. Residues are partitioned into a 1:1 solution of ethyl acetate:toluene and the solvent is concentrated to dryness. Residues of florasulam and 5-hydroxy florasulam are dissolved in acetone and derivatized to the *N*-ethyl florasulam and *N*-ethyl 5-oxo-6-*N*-ethyl florasulam, respectively, (Figure 1) at ambient temperature with triethyloxonium tetrafluoroborate and triethylamine (Note L.1.). The acetone solution is concentrated to dryness and the derivatized residues are dissolved in 1-chlorobutane and partitioned with a 5% sodium chloride in 0.2 M potassium carbonate solution. The 1-chlorobutane layer is removed and concentrated to dryness. Derivatized residues are dissolved in toluene containing the internal standards *N*-methyl florasulam and *N*-methyl 5-oxo-6-*N*-methyl florasulam (Figure 1). Residues of florasulam and 5-hydroxy florasulam as the N-ethyl florasulam and N-ethyl 5-oxo-6-N-ethyl florasulam, respectively, are determined 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-Dow AgroSciences 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, 1-chlorobutane, ethyl acetate, methyl *t*-butyl ether, toluene, and triethylamine are flammable and must be used in well-ventilated areas away from ignition sources.

3. Acetic acid, hydrochloric acid and phosphoric acid solutions are corrosive and can cause severe burns. It is imperative that proper eye and personal protective equipment be used when handling these reagents.

4. (Trimethylsilyl)diazomethane and triethyloxonium tetrafluoroborate are corrosive and alkylating agents. It is imperative that proper eye and personal protective equipment be used when handling these reagents.

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

D. Equipment (Note L.2.)

1. Balance, analytical, Model AE-100, Mettler Instrument Corporation, Hightstown, NJ 08520.

2. Balance, toploading, Model P-1200, Mettler Instrument Corporation.

3. Centrifuge, with rotor to accommodate 45- and 12-mL vials, Model U-5000, 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. Mass selective detector, Model 5971A, Hewlett-Packard, Palo Alto, CA 94304.

7. Mass selective detector data system, Model G1034B, Hewlett-Packard.

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

9. Ultrasonic bath, Model 1200, Branson Cleaning Equipment Company, Shelton, CT 06484.

10. Vacuum manifold, Model spe-21, Mallinckrodt Baker, Inc., Phillipsburg, NJ 08865.

11. Vial crimper, catalog number 8710-0979, Hewlett-Packard.

12. Vortex mixer, Model K-550-G, Scientific Industries, Inc., Bohemia, NY 11716.

E. Glassware and Materials (Note L.2.)

1. Bottle, amber, 4 oz (125-mL), with PTFE-lined cap, catalog number 03-320-4B, Fisher Scientific, Pittsburgh, PA 15219.

2. Column adapter, SPE, PTFE, catalog number 120-1100, Jones Chromatography, Inc., Lakewood, CO 80228.

3. Column, capillary gas chromatography, DB-5MS liquid phase, 12 m x 0.2 mm i.d., 0.33-µm film thickness, catalog number 128-5512, J&W Scientific, Folsom, CA 95630.

4. Column inlet liner, deactivated, catalog number 5181-3315, Hewlett-Packard.

5. Column, graphitized carbon SPE, catalog number 57092, Supelco, Inc., Bellefonte, PA 16823

6. Column, octadecyl (C_{18}) SPE, catalog number WAT023635, Waters Corp., Milford, MA 01757

7. Column reservoir, 25- and 70-mL, catalog numbers 120-1007-E and 120-1009-F, Jones Chromatography, Inc.

8. Filters, charcoal, moisture, and oxygen, catalog numbers 7972, 7971, and 7970, Chrompack, Inc., Raritan, NJ 08869. (Note L.3.)

9. Jug, 133 oz (3990 mL), with PTFE-lined cap, catalog number 03-442-2H, Fisher Scientific.

10. Pipetter, Drummond microdispensers, 50- and 100-µL capacity, catalog numbers 21-170-15C and 21-170-15D, Fisher Scientific.

11. Pipetter bores, for Drummond microdispensers, 50- and 100-µL bores, catalog numbers 21-169D and 21-169F, Fisher Scientific.

12. Tube, 16 x 100 mm (12-mL) screw-cap with PTFE-lined cap, catalog number 14-930-10B, Fisher Scientific.

13. Vial, 45-mL, clear, catalog number 03-339-5D, Fisher Scientific.

14. Vial, autosampler, 2-mL, catalog number C4011-1, National Scientific Company, Lawrenceville, GA 30243.

15. Vial, cap, PTFE-lined, for 45-mL vial, catalog number 03-391-12F, Fisher Scientific.

16. Vial, limited volume insert, 200-µL capacity, catalog number 03-375-3B, Fisher Scientific.

17. Vial seal, for 2-mL autosampler vial, catalog number C4011-1A, National Scientific Company.

18. Water purification system, Model Milli-Q UV Plus, Millipore Corporation, Milford, MA 01757.

F. Reagents and Prepared Solutions (Note L.2.)

1. Reagents

a. Acetic acid, glacial, certified ACS plus grade, catalog number A38-500, Fisher Scientific.

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

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

d. 1-Chlorobutane, HPLC grade, catalog number B429-4, Fisher Scientific.

e. Ethyl acetate, HPLC grade, catalog number E195-4, Fisher Scientific.

f. Helium gas, 99.995% purity, Airco, Murray Hill, NJ 07974.

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

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

i. Methyl *t*-butyl ether, HPLC grade, catalog number E127-4, Fisher Scientific.

j. Nitrogen gas, 99.99% purity, Airco.

k. o-Phosphoric acid (85%), HPLC grade, catalog number A260-500, Fisher Scientific.

l. Potassium carbonate (sesquihydrate), certified ACS grade, catalog number *P*179-500, Fisher Scientific.

m. Standards

Florasulam: *N*-(2,6-Difluorophenyl)-8-fluoro-5-methoxy[1,2,4]triazolo-[1,5-c]pyrimidine-2-sulfonamide

5-Hydroxy Florasulam: *N*-(2,6-Difluorophenyl)-8-fluoro-5-hydroxy[1,2,4]triazolo-[1,5-c]pyrimidine-2-sulfonamide

Obtain from Test Substance Coordinator, Building 306, Dow AgroSciences, Indianapolis, IN 46268-1054.

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

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

p. Triethyloxonium tetrafluoroborate, 1.0 M solution in dichloromethane, catalog number 17,623-0, Aldrich Chemical Company, Milwaukee, WI 53233.

q. (Trimethylsilyl)diazomethane, 2.0 M solution in hexanes, catalog number 36,283-2, Aldrich Chemical Company.

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

2. Prepared Solutions

a. 0.1% acetic acid in acetone, (v/v)

Transfer 1.0 mL of acetic acid (glacial) into a 1000-mL volumetric flask and dilute to volume with acetone.

b. acetone:0.1 N hydrochloric acid (9:1), (v/v)

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

c. 30% acetonitrile in 0.01 N hydrochloric acid, (v/v)

Transfer 300 mL of acetonitrile into a 1000-mL volumetric flask and dilute to volume with 0.01 N HCl.

d. ethyl acetate:toluene (1:1), (v/v)

Transfer 1500 mL of ethyl acetate and 1500 mL of toluene into a 133-oz jug.

e. 0.1 M phosphoric acid in acetone

Transfer 1.15 g of phosphoric acid (85%) into a 100-mL volumetric flask and dilute to volume with acetone.

f. 5% sodium chloride in 0.2 M potassium carbonate, aqueous (w/w)

Transfer 50 g of sodium chloride and 33 g of potassium carbonate sesquihydrate to a 1000-mL volumetric flask. Dissolve the salts in 800 mL of deionized water (DI) water, swirl to dissolve and dilute to volume with DI water.

G. Preparation of Standards

1. Preparation of a Combined Florasulam and 5-Hydroxy Florasulam Stock Solution

Weigh 0.0100 g each of the florasulam and 5-hydroxy florasulam analytical standards. Quantitatively transfer each into a single 100-mL volumetric flask. Dilute to volume with 0.1% acetic acid in acetone to obtain a 100-µg/mL of each florasulam and 5-hydroxy florasulam combined stock solution.

2. Preparation of Florasulam and 5-Hydroxy Florasulam Spiking and Calibration Solutions

a. Pipet 10 mL of the stock solution from Section G.1. into a 100-mL volumetric flask. Dilute to volume with 0.1% acetic acid in acetone to obtain a 10-µg/mL solution of florasulam and 5-hydroxy florasulam.

b. Pipet 5.0 mL of the 10-µg/mL solution from Section G.2.a. into a 100-mL volumetric flask. Dilute to volume with 0.1% acetic acid in acetone to obtain a 500-ng/mL solution of florasulam and 5-hydroxy florasulam.

c. Pipet 10.0 mL of the 500-ng/mL solution from Section G.2.b. into a 100-mL volumetric flask. Dilute to volume with 0.1% acetic acid in acetone to obtain a 50.0-ng/mL solution of florasulam and 5-hydroxy florasulam.

d. Pipet 2.0 mL of the 500-ng/mL solution from Section G.2.b. into a 100-mL volumetric flask. Dilute to volume with 0.1% acetic acid in acetone to obtain a 10.0-ng/mL solution of florasulam and 5-hydroxy florasulam.

e. Pipet 10.0 mL of the 50.0-ng/mL solution from Section G.2.c. into a 100-mL volumetric flask. Dilute to volume with 0.1% acetic acid in acetone to obtain a 5.0-ng/mL solution of florasulam and 5-hydroxy florasulam.

f. Pipet 5.0 mL of the 50.0-ng/mL solution from Section G.2.c. into a 100-mL volumetric flask. Dilute to volume with 0.1% acetic acid in acetone to obtain a 2.5-ng/mL solution of florasulam and 5-hydroxy florasulam.

3. Preparation of *N*-Methyl Florasulam and *N*-Methyl 5-Oxo-6-*N*-Methyl Florasulam Internal Standard Solution

a. Weigh 0.0100 g of the 5-hydroxy florasulam analytical standard and quantitatively transfer into a 100-mL volumetric flask. Dilute to volume with 0.1% acetic acid in acetone to obtain a 100-µg/mL 5-hydroxy florasulam solution.

b. Transfer 2.0 mL of the 100-µg/mL 5-hydroxy florasulam solution into a 12-mL screw-cap tube.

c. Add 25 µL of 0.1 M phosphoric acid in acetone and 50 µL of (trimethylsilyl)diazomethane.

d. Seal the vial with a PTFE-lined cap and allow to react for 30 minutes at ambient temperature.

e. Concentrate to dryness in a Turbo Vap evaporator. The water bath temperature should be set at ~60 °C and the nitrogen pressure set at 10 psi.

f. Add 4 mL of methyl *t*-butyl ether (MTBE).

g. Add 2 mL of 5% sodium chloride in 0.2 M potassium carbonate.

h. Seal the vial with a PTFE-lined cap and shake vigorously by hand for 15 seconds.

i. Centrifuge the vial for 5 minutes at 2000 rpm.

j. Using a disposable Pasteur pipet, transfer the top MTBE layer into a 12-mL screw-cap tube.

k. Repeat Step G.3.f. and Steps G.3.h. through G.3.j. combining the MTBE layers in the 12-mL screw-cap tube.

l. Concentrate to dryness in a Turbo Vap evaporator. The water bath temperature should be set at ~60 °C and the nitrogen pressure set at 10 psi.

m. Add 4 mL of toluene.

n. Seal the vial with a PTFE-lined cap. Vortex briefly and sonicate for 10 seconds.

o. Transfer the toluene to a 1000-mL volumetric flask.

p. Rinse the vial with 4 mL of toluene and transfer the toluene rinse to the 1000-mL flask.

q. Dilute the flask to volume with toluene to obtain an ~100 ng/mL combined standard of *N*-methyl florasulam and *N*-methyl 5-oxo-6-*N*-methyl florasulam.

4. Preparation of *N*-Ethyl Florasulam and *N*-Ethyl 5-Oxo-6-*N*-Ethyl Florasulam Calibration Standard Solutions

With each sample set, prepare the *N*-ethyl florasulam and *N*-ethyl 5-oxo-6-*N*-ethyl florasulam calibration standard solutions for each of the standards from Step G.2.b. through G.2.f. as follows:

Spiking Soln. G.2.	Florasulam and 5-OH Florasulam Calibration Soln. Conc.[a]	Florasulam and 5-OH Florasulam Equiv. Sample Conc.
ng/mL	ng/mL	ng/g
2.5	5.0	0.5
5.0	10.0	1.0
10.0	20.0	2.0
50.0	100.0	10.0
500.0	1000	100.0

[a] The calibration solution concentration is based on derivatizing 1.0 mL of the appropriate spiking solution from Step G.2. and diluting to a final volume of 0.5 mL.

a. Pipet 1.0 mL of the appropriate standard into a 12-mL screw-cap vial.

b. Proceed with Steps I.1.r.(1) through I.1.ab.

H. Gas Chromatography/Mass Spectrometry

1. Column

Install the splitless column insert liner (Section E.4.) and the capillary column (Section E.3.) 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
Column:	J&W Scientific fused silica capillary DB-5MS liquid phase 12 m x 0.2 mm i.d. 0.33-µm film thickness
Temperatures:	
Column	120 °C for 1.0 minute 120 °C to 325 °C at 15 °C/min. 325 °C for 5.33 minutes
Injector	270 °C
Interface	300 °C
Carrier Gas:	Helium
Head Pressure	50 kPa
Linear Velocity	approximately 40 cm/sec at an oven temperature of 280 °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 Mode:	electron impact ionization with selected ion monitoring
Calibration Program	maximum sensitivity autotune
Electron Multiplier	1671 volts (tune voltage + 200)
Ions Monitored:	
N-Methyl florasulam	*m/z* 142 (internal standard)
N-Ethyl florasulam	*m/z* 156 (florasulam quantitation and confirmation) *m/z* 140 (florasulam confirmation) *m/z* 138 (florasulam confirmation)
N-Methyl 5-oxo-	

6-*N*-methyl florasulam	*m/z* 142 (internal standard)
N-Ethyl 5-oxo-6-*N*-Ethyl florasulam	*m/z* 156 (5-hydroxy florasulam quantitation and confirmation) *m/z* 140 (5-hydroxy florasulam confirmation) *m/z* 154 (5-hydroxy florasulam confirmation)
Dwell Time	100 msec

Typical mass spectra of *N*-methyl florasulam (Internal Standard) and *N*-ethyl florasulam are shown in Figure 2. Typical mass spectra of *N*-methyl 5-oxo-6-*N*-methyl florasulam (Internal Standard) and *N*-ethyl 5-oxo-6-*N*-ethyl florasulam are shown in Figure 3.

3. Calibration Curve

Demonstrate that the calibration curves for florasulam and 5-hydroxy florasulam fit a least squares power regression equation (1) over the equivalent soil concentration range of 0.5 to 100 ng/g. The least squares coefficient of determination (r^2) must be greater than or equal to 0.995. Representative calibration curves for the determination of florasulam and 5-hydroxy florasulam in soil are shown in Figures 4 and 5, respectively.

4. Typical Chromatograms

Typical chromatograms of a 1.0-ng/g florasulam equivalent soil standard, soil control, and soil fortified at 1.0 ng/g with florasulam are shown in Figures 6-8. Typical chromatograms of a 1.0-ng/g 5-hydroxy florasulam equivalent soil standard, soil control, and soil fortified at 1.0 ng/g with 5-hydroxy florasulam are shown in Figures 9-11.

I. Determination of Recovery of Florasulam and 5-Hydroxy Florasulam from Soil

1. Preparation of Recovery Samples

a. Weigh 5.0 g of the appropriate soil sample into each of a series of 45-mL vials. For preparing fortified samples, use some of the samples as controls and fortify the remaining samples by adding 1.0 mL of the appropriate spiking solution (Section G.2.) to obtain concentrations ranging from 0.50 to 100 ng/g.

Florasulam and 5-OH Florasulam Spiking Soln. Conc.	Volume of Soln.	Florasulam and 5-OH Florasulam Equiv. Sample Conc.[a]
ng/mL	mL	ng/g
2.5	1.0	0.5
5.0	1.0	1.0
10.0	1.0	2.0
50.0	1.0	10.0
500.0	1.0	100.0

[a] The equivalent sample concentration is based on fortifying a 5-g sample with the indicated amount of the appropriate solution.

A reagent blank, containing no soil sample, is carried through the method with the samples.

b. Add 15 mL of the acetone:0.1 N hydrochloric acid (9:1) extraction solution.

c. Seal the vial with a PTFE-lined cap and shake the sample for a minimum of 30 minutes using a reciprocating shaker set at approximately 180 excursions/minute.

d. Centrifuge the vial for 5 minutes at ~2000 rpm.

e. Decant each extract to a clean 45-mL vial.

f. Repeat Steps I.1.b. through I.1.e. with a shaking time of 5 minutes. Combine the extracts.

g. Purify the sample using a graphitized carbon SPE column as follows:

(1) Place a graphitized carbon SPE column on the vacuum manifold box.

(2) Attach a 70-mL reservoir to the top of the column using an SPE column adapter.

(3) Condition the SPE column with 5 mL of the acetone:0.1 N hydrochloric acid (9:1) solution.

(4) Transfer the combined extract from Step I.1.f. to the reservoir. With the aid of vacuum, pull the sample through the column at a flow rate of ~4 mL/min. Collect the eluate in a 45-mL vial.

(5) Rinse the sample vial with 5 mL of the acetone:0.1 N hydrochloric acid (9:1) solution. When the entire sample has passed through the column, add the rinse to the reservoir. With the aid of vacuum, pull the rinse through the column at a flow rate of ~4 mL/min. Collect and combine the eluate in the 45-mL vial.

h. Concentrate the sample using a Turbo Vap evaporator to ~2 mL. The water bath temperature should be set at ~60 °C and the nitrogen pressure set at 10 psi.

i. Add 3 mL of 0.01 N hydrochloric acid. Seal the vial with a PTFE-lined cap and vortex briefly to mix.

j. Further purify the sample using a C_{18} SPE column as follows:

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

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

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

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

(5) Position a 45-mL vial inside the vacuum manifold box to collect the eluate from the Steps I.1.j.(6) through I.1.j.(8).

(6) Transfer the sample solution from Step I.1.i. to the reservoir. With the aid of vacuum, pull the sample through the column at a flow rate of ~4 mL/min. Collect the eluate in the 45-mL vial.

(7) Rinse the sample vial with 5 mL of the 30% acetonitrile in 0.01 N hydrochloric acid solution. After the entire sample has passed through the column, add the sample rinse to the reservoir. With the aid of vacuum, pull the rinse through the column at a flow rate of ~4 mL/min. Continue to collect the eluate in the 45-mL vial.

(8) After the rinse has passed through the column, add 20 mL of the 30% acetonitrile in 0.01 N hydrochloric acid solution to the reservoir. With the aid of vacuum, pull the rinse through the column at a flow rate of ~4 mL/min. Continue to collect the eluate in the 45-mL vial.

k. Add ~8 g of sodium chloride to the vial.

l. Add 10 mL of the ethyl acetate:toluene (1:1) solution to the vial.

m. Seal the vial with a PTFE-lined cap and shake for ~10 minutes on a reciprocating shaker at ~180 excursions/minute.

n. Centrifuge the vial for 5 minutes at ~2000 rpm.

o. Using a Pasteur pipet, transfer the top organic layer to a 45-mL vial.

p. Repeat Steps I.1.l. through I.1.o. and combine the organic layers.

q. Concentrate the samples to dryness in a Turbo Vap evaporator. The water bath temperature should be set at ~60 °C and the nitrogen pressure set at 10 psi.

r. Add 2 mL of acetone and derivatize residues of florasulam and 5-hydroxy florasulam (Figure 1) to the *N*-ethyl florasulam and *N*-ethyl 5-oxo-6-*N*-ethyl florasulam, respectively, as follows:

(1) Add 25 µL of triethylamine.

(2) Add 100 µL of triethyloxonium tetrafluoroborate.

(3) Seal the vial with a PTFE-lined cap, sonicate briefly and swirl to rinse the sides of the vial.

(4) Allow the reaction to proceed for at least 30 minutes at ambient temperature.

s. Using a disposable Pasteur pipet, transfer the acetone from Step I.1.r. to a 12-mL screw-cap tube. Rinse the vial with 2 mL of acetone and combine with the acetone in the tube.

t. Concentrate the solvent to dryness in a Turbo Vap evaporator. The water bath temperature should be set at ~60 °C and the nitrogen pressure set at 10 psi.

u. Add 4.0 mL of 1-chlorobutane.

v. Add 2 mL of the 5% sodium chloride in 0.2 M potassium carbonate solution

w. Seal the tube with a PTFE-lined cap and shake for ~5 minutes using a reciprocating shaker set at approximately 180 excursions/minute.

x. Centrifuge the tube for ~5 minutes at 2000 rpm and using a disposable Pasteur pipet, transfer the top 1-chlorobutane to a clean 12-mL screw-cap tube.

y. Repeat Steps I.1.u., I.1.w. and I.1.x., combining the 1-chlorobutane layers.

z. Concentrate the solvent to dryness in a Turbo Vap evaporator. The water bath temperature should be set at ~60 °C and the nitrogen pressure set at 10 psi.

aa. Add 0.50 mL of toluene containing the internal standards *N*-methyl florasulam and *N*-methyl 5-oxo-6-*N*-methyl florasulam from Step G.3.q. (Figure 1). Seal the vial with a PTFE-lined cap and sonicate briefly, then swirl to rinse the sides of the vial.

ab. Place a limited volume insert in an autosampler vial. Using a disposable Pasteur pipet, transfer an aliquot of the toluene from Step I.1.aa. to the limited volume insert and seal the autosampler vial with a cap and crimper.

ac. Analyze the samples and calibration standards (Step G.4.) by GC/MSD as described in Section H. Determine the suitability of the chromatographic system using the following performance criteria:

(1) Standard curve linearity: Determine that the coefficient of determination (r^2) for the least squares power regression equation which describes the detector response as a function of the concentration of calibration standards is equal to or greater than 0.995.

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

(3) Detector sensitivity: Visually determine that a minimum signal-to-noise ratio of 10:1 has been attained for the *m/z* 156 florasulam and 5-hydroxy florasulam ion peaks of the 1.0-ng/g equivalent soil concentration calibration standard.

2. Calculation of Percent Recovery

a. Using the data for the series of calibration standards analyzed in Section I.1.ac., determine the peak areas for *N*-ethyl florasulam (*m/z* 156, 140, and 138), *N*-methyl florasulam internal standard (*m/z* 142), *N*-ethyl 5-oxo-6-*N*-ethyl florasulam (*m/z* 156, 140, and 154), and *N*-methyl 5-oxo-6-*N*-methyl florasulam internal standard (*m/z* 142).

b. For each standard, calculate the quantitation ratio of florasulam and 5-hydroxy florasulam. The ions used to calculate the quantitation ratio are the same for both florasulam and 5-hydroxy florasulam, including their respective internal standards. The peaks representing florasulam and 5-hydroxy florasulam are separated by chromatography with the florasulam peak and its internal standard eluting prior to the 5-hydroxy florasulam.

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

$$\text{Quantitation Ratio} = \frac{\text{peak area at } m/z \text{ 156}}{\text{peak area at } m/z \text{ 142}}$$

For example, using the data for florasulam from Figure 6:

$$\text{Quantitation Ratio} = \frac{8638}{54463}$$

$$\text{Quantitation Ratio} = 0.159$$

c. Prepare a standard curve by plotting the florasulam soil equivalent concentration on the abscissa (*x*-axis) and the respective quantitation ratio on the ordinate (*y*-axis) as shown in Figure 4. Using regression analysis, determine the equation for the curve with respect to the abscissa. Prepare a standard curve for 5-hydroxy florasulam in the same manner, as shown in Figure 5.

For example, using power regression with the florasulam data from Figure 4:

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

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

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

$$\text{Florasulam Conc. (ng/g)} = \left[\frac{\text{florasulam quantitation ratio}}{0.1622}\right]^{1/1.0432}$$

d. Determine the gross concentration of florasulam and 5-hydroxy florasulam in each recovery sample by substituting the respective quantitation ratio obtained into the appropriate calibration curve equation and solving for the concentration.

For example, using the florasulam data from Figure 8 and the standard curve information from Figure 4:

$$\text{Florasulam Conc. (gross ng/g)} = \left[\frac{\text{florasulam quantitation ratio}}{0.1622}\right]^{1/1.0432}$$

$$\text{Florasulam Conc. (gross ng/g)} = \left[\frac{0.132}{0.1622}\right]^{1/1.0432}$$

$$\text{Florasulam Conc. (gross)} = 0.822 \text{ ng/g}$$

e. Determine the net concentration in each recovery sample by subtracting the florasulam and 5-hydroxy florasulam concentration in the control sample from that of the gross florasulam and 5-hydroxy florasulam concentration in the recovery sample.

For example, using the florasulam data from Table I and Figures 7 and 8:

$$\begin{matrix}\text{Florasulam Conc.} \\ \text{(net ng/g)}\end{matrix} = \begin{matrix}\text{Florasulam Conc.} \\ \text{(gross ng/g)}\end{matrix} - \begin{matrix}\text{Background Conc.} \\ \text{(control ng/g)}\end{matrix}$$

$$\begin{matrix}\text{Florasulam Conc.} \\ \text{(net ng/g)}\end{matrix} = 0.822 \text{ ng/g} - 0.00 \text{ µg/g}$$

$$\text{Florasulam Conc. (net)} = 0.822 \text{ ng/g}$$

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

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

$$\text{Recovery} = \frac{0.822 \text{ ng/g}}{1.0 \text{ ng/g}} \times 100\%$$

$$\text{Recovery} = 82\%$$

J. Determination of Florasulam and 5-Hydroxy Florasulam in Soil

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

2. Prepare the appropriate standard calibration curves for florasulam and 5-hydroxy florasulam and determine the power regression equation that fits the curve as described in Section I.2.

3. Determine the gross concentration of florasulam and 5-hydroxy florasulam in each sample by substituting the quantitation ratio obtained into the equation for the standard calibration curve, and calculating the uncorrected residue result as described in Section I.2.d.

4. For those analyses that require correction for method recovery, use the average recovery of all the recovery samples from a given sample set to correct for method efficiency.

   For example, using the florasulam data from Figure 8 and Table I for the samples analyzed on July 16-23, 1997:

   a. Determine the gross analyte concentration in the sample as described in Section I.2.d.

b. Determine the corrected analyte concentration in the sample as follows:

$$\text{Florasulam Conc. (corrected ng/g)} = \text{Florasulam Conc. (gross ng/g)} \times \left[\frac{100}{\text{Ave. Percent Recovery of Sample Set}}\right]$$

$$\text{Florasulam Conc. (corrected ng/g)} = 0.822 \text{ ng/g} \times \frac{100}{85}$$

$$\text{Florasulam Conc. (corrected)} = 0.967 \text{ ng/g}$$

5. Determination of Soil Moisture

a. If correction for soil moisture is desired, weigh approximately 10 g of soil into a tared aluminum or glass container and record the weight.

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.

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

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

$$\text{Percent Moisture} = \left[\frac{\text{soil weight before drying} - \text{soil weight after drying}}{\text{soil weight after drying}}\right] \times 100$$

a. Samples may be allowed to shake overnight at Step I.1.c.

b. After combining the extracts and sealing the vial with a PTFE-lined cap, sample preparation may be suspended at Step I.1.p.

c. Samples sealed in the autosampler vial (Step I.1.ab.) may be allowed to set overnight prior to performing Step I.1.ac.

5. Standardization of C_{18} SPE Cartridge Elution Profile

Variation in the C_{18} SPE cartridges can influence the elution profile of florasulam. It is necessary to obtain an elution profile for each lot of SPE cartridges used to ensure optimum recovery and clean-up efficiency. The following procedure may be used.

a. Pipet 1.0 mL of the 500 ng/mL fortification solution (100 ng/g equivalent soil concentration) from Section G.2.b. into a 45-mL vial and concentrate the solvent to dryness in a Turbo Vap evaporator. The water bath temperature should be set at ~60 °C and the nitrogen pressure set at 10 psi.

b. Add 5 mL of 0.01 N hydrochloric acid solution and seal the vial with a PTFE-lined cap. Sonicate the vial for ~5 seconds and vortex briefly.

c. Proceed with Steps I.1.j.(1) through I.1.j.(5). Transfer the contents of the vial from Step K.5.b. to the reservoir. With the aid of vacuum, pull the sample through the column at a flow rate of ~4 mL/min. Collect the eluate in the 45-mL vial and label as load fraction.

d. Proceed with Step I.j.(7) collecting the eluate in a separate 45-mL vial labeled fraction 1.

e. Proceed with Step I.j.(8) collecting 5-mL fractions in a series of 45-mL vials labeled fractions 2-5.

f. Proceed with Step I.1.k. through I.1.ac. for each vial.

g. Based upon a 100-ng/g equivalent sample load, calculate separate percent recoveries of florasulam for the load and each fraction.

h. Typical florasulam and 5-hydroxy florasulam elution profiles from a C_{18} SPE cartridge are presented in Figure 12.

L. Notes

1. Derivatization of 5-hydroxy florasulam with (trimethylsilyl)diazomethane yields an ~1:1 mixture of *N*-methyl florasulam and *N*-methyl 5-oxo-6-*N*-methyl florasulam.

Derivatization of 5-hydroxy florasulam with triethyloxonium tetrafluoroborate yields ~90% *N*-ethyl 5-oxo-6-*N*-ethyl florasulam.

2. 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.

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

M. References

1. Freund, J. E.; Williams, F. J. *Dictionary/Outline of Basic Statistics*, Dover Publications: Mineola, NY, 1991; p 170, eq I.3a.

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

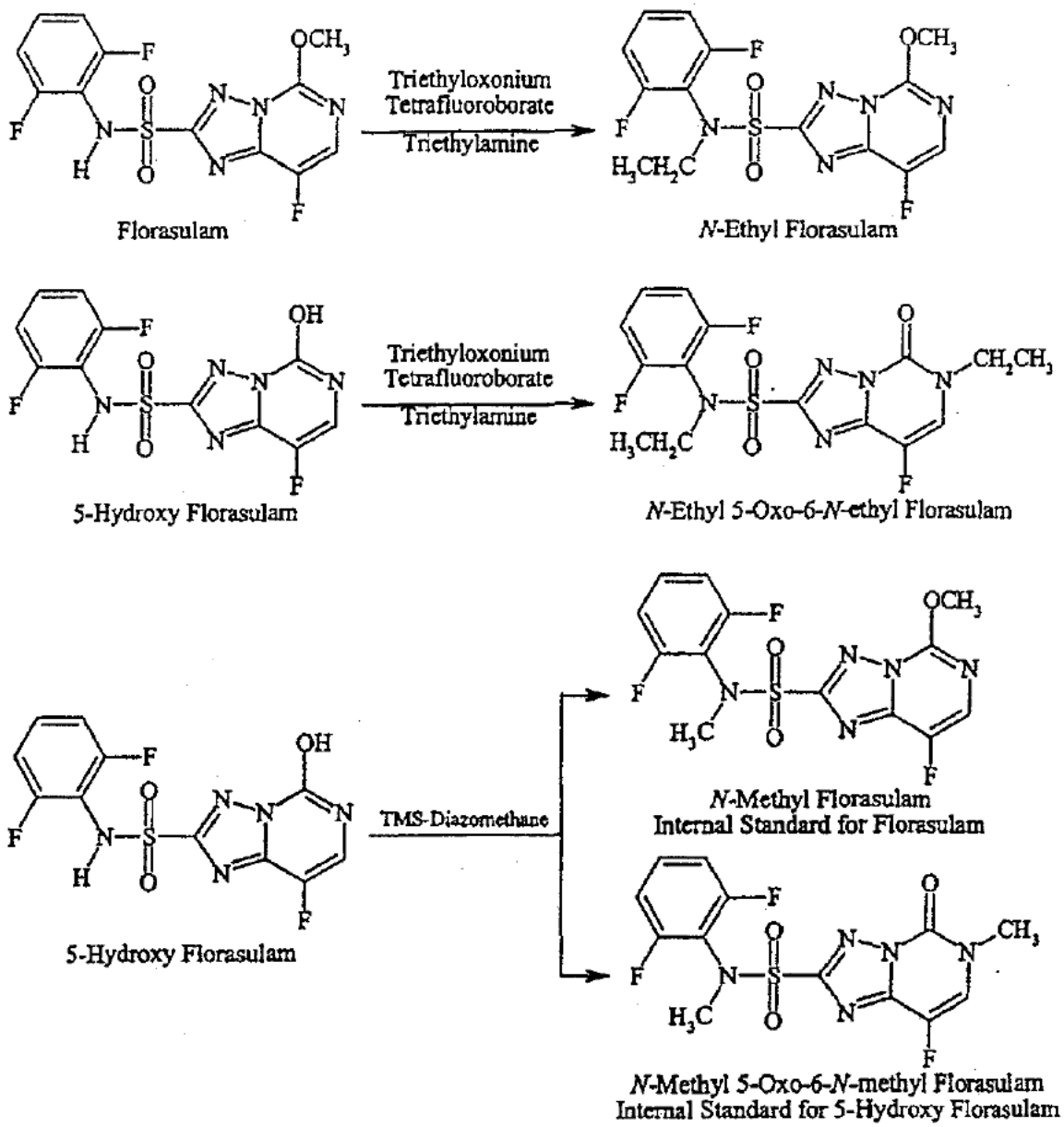


Figure 1. Reactions Showing Structures of Derivatives Used in the Determination of Florasulam and 5-Hydroxy Florasulam in Soil