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Determination of Residues of Fluroxypyr and Its Major Metabolites in Soil by Capillary Gas Chromatography with Mass Selective Detection

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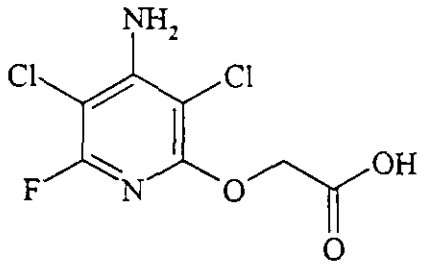
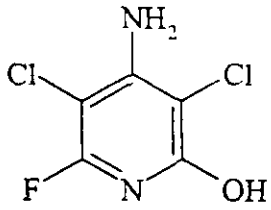
1. SCOPE

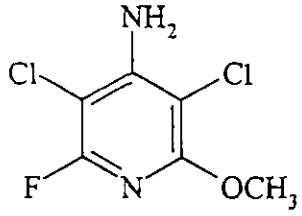
This method is applicable for the quantitative determination of fluroxypyr (((4-amino-3,5-dichloro-6-fluoro-2-pyridinyl)oxy)acetic acid) and its major metabolites, fluroxypyr-DCP (4-amino-3,5-dichloro-6-fluoro-2-pyridinol), and fluroxypyr-MP (4-amino-3,5-dichloro-6-fluoro-2-methoxypyridine) in soil. The method measures total fluroxypyr (as the fluroxypyr acid equivalent), fluroxypyr-DCP, and fluroxypyr-MP in soil over the concentration range of 0.01-1.0 µg/g with a validated limit of quantitation of 0.01 µg/g.



Fluroxypyr
CAS No. 69377-81-7



Fluroxypyr-DCP
CAS No. 94133-62-7



Fluroxypyr-MP
CAS No. 35622-80-1

2. PRINCIPLE (Figure 1)

Soil samples are extracted twice with 90% acetone/10% 0.1 N hydrochloric acid. An aliquot of the extract is hydrolyzed with 1.0 N sodium hydroxide as it is concentrated under a stream of nitrogen. The hydrolyzed samples are acidified, diluted with deionized water, and purified using C_{18} solid phase extraction (SPE). The fluroxypyr and fluroxypyr-DCP are eluted with a solution of 30% acetonitrile/69% water/1% 1.0 N hydrochloric acid into a vial containing sodium chloride. The acetonitrile is evaporated, and the samples are partitioned into 1-chlorobutane. The C_{18} solid SPE column is dried and the fluroxypyr-MP is eluted with 1-chlorobutane. The separate 1-chlorobutane fractions from each sample are combined and concentrated under a stream of nitrogen at 40 °C. The samples are derivatized for 1 hour at 60 °C with *N*-methyl-*N*-(*tert*-butyldimethylsilyl)-trifluoracetamide (MTBSTFA) to form the *tert*-butyldimethylsilyl (TBDMS) derivatives of fluroxypyr and fluroxypyr-DCP (Figure 2). The fluroxypyr-MP is assayed underivatized. Fluroxypyr 1-butyl ester is added as an internal standard to each sample and each calibration standard prior to analysis. The samples are analyzed by capillary gas chromatography with mass selective detection.

3. SAFETY PRECAUTIONS

3.1 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-Dow AgroSciences LLC 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.

3.2 Acetone, acetonitrile, 1-chlorobutane, and decane are flammable and should be used in well-ventilated areas away from ignition sources.

3.3 The derivatization reagent, *N*-methyl-*N*-(*tert*-butyldimethylsilyl)-trifluoracetamide is flammable and a strong eye and skin irritant.

3.4 Hydrochloric acid and sodium hydroxide are corrosive and can cause severe burns. It is imperative that proper eye and personal protection equipment be worn when handling these reagents.

4. EQUIPMENT (Note 14.1)

4.1 Balance, analytical, Model AE100, Mettler Instrument Corporation, Hightstown, NJ 08520.

4.2 Balance, top loading, Model PG2002, Mettler Instrument Corporation.

4.3 Centrifuge, with rotor to accommodate 12- and 40-mL vials, Model Centra-GP8, International Equipment Company, Needham Heights, MA 02194.

4.4 Evaporator, Reacti-Vap, Model 18780Z, Pierce Chemical Company, Rockford, IL 61105.

4.5 Evaporator, TurboVap LV, Model 43750/14, Zymark Corporation, Zymark Center, Hopkinton, MA 01748.

4.6 Gas chromatograph, Model 5890A Series II, Hewlett-Packard, Wilmington, DE19808.

4.7 Hammer mill, with 3/16-inch screen, Model 2001, AGVISE Laboratories, Inc., Northwood, ND 58267.

4.8 Heating block, Reacti-Block B-1, 9-hole, Model 18802Z, Pierce Chemical Company.

4.9 Heating block, Reacti-Therm heating module, Model 18870Z, Pierce Chemical Company.

4.10 Injector, automatic, Model 7673, Hewlett-Packard.

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

4.12 Mass selective detector data system, Model G1701AA, Hewlett-Packard.

4.13 Needles, Reacti-Vap PTFE-coated, Model 18784Z, Pierce Chemical Company.

4.14 Oven, Model OV-490A-2, Blue M Electric Company, Blue Island, IL 60406.

4.15 Pipetter, adjustable, digital, Eppendorf, 10-100 µL, catalog number 53511-584, Brinkmann Instruments Inc., Westbury, NY 11590.

4.16 Pipetter, adjustable, digital, Eppendorf, 10-1000 µL, catalog number 53511-610, Brinkmann Instruments Inc.

4.17 Pipetter, adjustable, digital, Oxford BenchMate, 1000-5000 µL, catalog number 8885-500036, Oxford Labware, St. Louis, MO 63103.

4.18 Shaker, variable speed reciprocating with box carrier, Model 6000, Eberbach Corporation Ann Arbor, MI 48103.

4.19 Timer, catalog number 06-662-5, Fisher Scientific, Pittsburgh, PA 15219.

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

4.21 Vacuum manifold, catalog number 5-7250, Supelco, Inc., Bellefonte, PA 16823.

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

4.23 Vortex mixer, Model G-560, Scientific Industries, Inc., Bohemia, NY 11716.

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

5. GLASSWARE AND MATERIALS (Note 14.1)

5.1 Column, capillary gas chromatography, Durabond-1701 liquid phase, 10 m x 0.18 mm i.d., 0.4-µm film thickness, catalog number 121-1723, J & W Scientific, Folsom, CA 95630.

5.2 Column, C_{18} SPE, catalog number 6803-0588, Whatman, Fairfield, NJ 07004.

5.3 Cylinder, graduated mixing, 50-mL, with ground glass stopper, catalog number 20036-50, Kimble/Kontes, Vineland, NJ 08360.

5.4 Cylinder, graduated mixing, 1000-mL, catalog number 20036-1000, Kimble/Kontes.

5.5 Filter, charcoal, catalog number 7972, Chrompack, Inc., Raritan, NJ 08869. (Note 14.2)

5.6 Filter, moisture, catalog number 7971, Chrompack, Inc. (Note 14.2)

5.7 Filter, oxygen, catalog number 7970, Chrompack, Inc. (Note 14.2)

5.8 Flask, volumetric, 100-mL, catalog number 161-8987, National Scientific Company, Lawrenceville, GA 30243.

5.9 Flask, volumetric, 200-mL, catalog number 161-8988, National Scientific Company.

5.10 Flask, volumetric, 1000-mL, catalog number 161-8972, National Scientific Company.

5.11 Inlet sleeve, deactivated, cyclo double gooseneck (4 mm ID) splitless, catalog number 20895, Restek Corporation, Bellefonte, PA 16823-8812.

5.12 Pipet, volumetric, 0.5-mL, catalog number 261-6010, National Scientific Company.

5.13 Pipet, volumetric, 1.0-mL, catalog number 261-6011, National Scientific Company.

5.14 Pipet, volumetric, 2.0-mL, catalog number 261-6012, National Scientific Company.

5.15 Pipet, volumetric, 5.0-mL, catalog number 261-6015, National Scientific Company.

5.16 Pipet, volumetric, 8.0-mL, catalog number 261-6018, National Scientific Company.

5.17 Pipet, volumetric, 10-mL, catalog number 261-6020, National Scientific Company.

5.18 Pipet, volumetric, 15-mL, catalog number 261-6025, National Scientific Company.

5.19 Pipet, volumetric, 20-mL, catalog number 261-6030, National Scientific Company.

5.20 Pipet, volumetric, 25-mL, catalog number 261-6035, National Scientific Company.

5.21 Pipet, volumetric, 50-mL, catalog number 261-6050, National Scientific Company.

5.22 Pipet, volumetric, 100-mL, catalog number 261-6065, National Scientific Company.

5.23 Pipet, volumetric, 200-mL, catalog number 261-6070, National Scientific Company.

5.24 Syringe, 10-µL, Model 701N, Hamilton Company, Reno, NV 89520.

5.25 Syringe, 100-µL, Model 710N, Hamilton Company.

5.26 Syringe, 250-µL, Model 725N, Hamilton Company.

5.27 Vial, autosampler, 2-mL, catalog number C4000-1, National Scientific Company.

5.28 Vial, 12-mL, with PTFE-lined screw cap, catalog number B7800-12, National Scientific Company.

5.29 Vial, 40-mL, with PTFE-lined screw cap, catalog number B7800-6, National Scientific Company.

5.30 Vial cap, for autosampler vial, catalog number C4000-54B, National Scientific Company.

5.31 Vial limited volume insert, 200-µL, for autosampler vial, catalog number C4011-631, National Scientific Company.

6. REAGENTS AND CHEMICALS (Note 14.1)

6.1 Reagents

6.1.1 Acetone, OmniSolv grade, catalog number AX0110-1, EM Science, Gibbstown, NJ 08027.

6.1.2 *Acetonitrile, OmniSolv grade, catalog number AX0142-1, EM Science.*

6.1.3 1-Chlorobutane, OmniSolv grade, catalog number CX0914-1, EM Science.

6.1.4 Decane, certified, catalog number 02128-500, Fisher Scientific.

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

6.1.6 Hydrochloric acid, 5.0 N, ACS reagent grade, certified concentration, catalog number LC15360-2, Fisher Scientific.

6.1.7 Hydrochloric acid, 1.0 N, ACS reagent grade, certified concentration, catalog number SA48-1, Fisher Scientific.

6.1.8 Hydrochloric acid, 0.5 N, ACS reagent grade, certified concentration, catalog number SA50-1, Fisher Scientific.

6.1.9 Hydrochloric acid, 0.1 N, ACS reagent grade, certified concentration, catalog number SA54-1, Fisher Scientific.

6.1.10 MTBSTFA (*N*-methyl-*N*-(*tert*-butyldimethylsilyl)-trifluoroacetamide), catalog number 48920, Pierce Chemical Company.

6.1.11 Nitrogen, gas, 99.95% purity, Airco.

6.1.12 Sodium chloride, ACS reagent grade, catalog number S271-1, Fisher Scientific.

6.1.13 Sodium hydroxide, 1.0 N, ACS reagent grade, catalog number SS266-1, Fisher Scientific.

6.1.14 Standards

a. Fluroxypyr: (((4-amino-3,5-dichloro-6-fluoro-2-pyridinyl)oxy)acetic acid)

b. Fluroxypyr-DCP: (4-amino-3,5-dichloro-6-fluoro-2-pyridinol)

c. Fluroxypyr-MP: (4-amino-3,5-dichloro-6-fluoro-2-methoxypyridine)

d. Fluroxypyr 1-butyl ester: ((4-amino-3,5-dichloro-6-fluoro-2-pyridinyl)oxy)acetic acid, 1-butyl ester)

Obtain from Test Substance Coordinator, Dow AgroSciences LLC, 9330 Zionsville Road, Building 306/A1, Indianapolis, IN 46268-1054.

6.2 Prepared Solutions

6.2.1 Acetone/0.1 N hydrochloric acid, (90:10) (v/v)

Pour 900 mL of acetone into a 1000-mL graduated mixing cylinder. Pipet 100 mL of 0.1 N hydrochloric acid into the same cylinder. Invert the cylinder several times to mix. After allowing the solution to equilibrate to ambient temperature, adjust the volume to 1000 mL with 0.1 N hydrochloric acid, if needed.

6.2.2 Acetonitrile/water/1.0 N hydrochloric acid, (30:69:1) (v/v/v)

Pour 690 mL of distilled/deionized water to a 1000-mL volumetric flask. Pipet 10 mL of 1.0 N hydrochloric acid and 300 mL of acetonitrile into the same flask. Invert the flask several times to mix. After allowing the solution to equilibrate to ambient temperature, adjust the volume to 1000 mL with distilled/deionized water, if needed.

7. PREPARATION OF STANDARDS

7.1 Preparation of Spiking Solutions/Calibration Standards

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

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

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

7.1.4 Pipet 2.0 mL of each of the three 1000-µg/mL stock solutions prepared in Sections 7.1.1-7.1.3 into a single 200-mL volumetric flask and adjust to volume with acetone to obtain a solution containing 10.0 µg/mL of each compound.

7.1.5 Prepare solutions for spiking soil samples by diluting the 10.0 µg/mL combination solution from Section 7.1.4 with acetone as follows:

Aliquot of 10.0 µg/mL Soln.	Final Soln. Volume	Spiking Soln. Final Conc.	Equivalent Sample Conc.[a]
mL	mL	µg/mL	µg/g
0.50	200	0.025	0.005
1.00	200	0.050	0.010
5.00	100	0.50	0.10
25.0	200	1.25	0.25
25.0	100	2.50	0.50
50.0	100	5.00	1.00

[a] The equivalent sample concentration is based on fortifying a 5.0-g soil sample with 1.0 mL of spiking solution.

7.1.6 Prepare calibration standards by dispensing 200 µL of the solutions from Step 7.1.5 into 12-mL vials containing 550 µL of 1-chlorobutane and 50 µL of decane. Derivatize the calibration standards along with the samples according to the procedure described in

Step 9.3.24 through 9.3.29. The concentration range of these calibration standards is from 0.005 to 1.00 µg/mL. (Note 14.3)

7.2 Preparation of Internal Standard Solutions

7.2.1 Weigh 0.1000 g of fluroxypyr 1-butyl ester analytical standard and quantitatively transfer to a 100-mL volumetric flask. Dilute to volume with acetone to obtain a 1000-µg/mL stock solution.

7.2.2 Pipet 1.0 mL of the 1000-µg/mL stock solution prepared in Section 7.2.1 into a 100-mL volumetric flask and adjust to volume with acetone to obtain a solution containing 10.0 µg/mL of fluroxypyr 1-butyl ester.

8. GAS CHROMATOGRAPHY/MASS SPECTROMETRY

8.1 Column

Install the splitless column inlet sleeve and the capillary column in the split/splitless injection port of the gas chromatograph following the manufacturer's recommended procedures.

8.2 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 G1034C data system
Column:	J&W Scientific fused silica capillary DB-1701 liquid phase 10 m x 0.18 mm i.d. 0.4-µm film thickness
Temperatures:	
Column	90 °C for 1.0 min 90 °C to 255 °C at 10 °C/min 255 °C to 280 °C at 20 °C/min 280 °C for 2.00 min
Injector Interface	260 °C 280 °C
Carrier Gas:	Helium
Constant Flow	On

Vacuum Compensation	On
Head Pressure	16 kPa
Linear Velocity	approximately 37.4 cm/sec
Injection Mode:	splitless
Purge Delay	0.9 min
Splitter Flow	35 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; usertune
Electron Multiplier	2200 volts (≅300 volts above autotune) (Note 14.4)
Ions Monitored:	
Fluroxypyr-TBDMS	*m/z* 311 (quantitation) and *m/z* 253 (for confirmation) (Note 14.5)
Fluroxypyr-DCP-TBDMS	*m/z* 253 (quantitation) and *m/z* 257 (for confirmation) (Note 14.5)
Fluroxypyr-MP	*m/z* 210 (quantitation) and *m/z* 181 (for confirmation) (Note 14.5)
Fluroxypyr 1-butyl ester	*m/z* 310 (internal standard)
Dwell Time	75 msec

Typical mass spectra of fluroxypyr-TBDMS and fluroxypyr-DCP-TBDMS are shown in Figure 3. Typical mass spectra of fluroxypyr-MP and fluroxypyr 1-butyl ester are shown in Figure 4.

Typical calibration curves for fluroxypyr, fluroxypyr-DCP and fluroxypyr-MP are shown in Figures 5-7, respectively.

Typical chromatograms for fluroxypyr, fluroxypyr-DCP and fluroxypyr-MP are shown in Figures 8-16, respectively.

9. DETERMINATION OF RECOVERY OF FLUROXYPYR AND METABOLITES FROM SOIL

9.1 Method Validation

Validate the analytical procedure given in Section 9.3 by analyzing the following with each sample set:

At least one reagent blank.
At least two unfortified controls.
At least two controls fortified at the limit of quantitation.
At least two controls fortified at a level of the expected residue concentration in the samples.

9.2 Sample Preparation

Prepare the samples for analysis by crushing with a hammer, blending with dry ice, and grinding using a hammer mill equipped with a 3/16-inch size screen. Prepared samples are stored frozen at < -20 °C prior to analysis.

9.3 Sample Analysis

9.3.1 Weigh 5.0-g portions of the prepared soil sample into a series of 40-mL vials.

9.3.2 For preparing fortified samples, add 1.0-mL aliquots of the appropriate spiking solutions from Section 7.1.5 to untreated control soil to obtain concentrations ranging from 0.01 to 1.0 µg/g.

9.3.3 Add 25 mL of the 90% acetone/10% 0.1 N hydrochloric acid extraction solution to the sample vial.

9.3.4 Cap the vial with a PTFE-lined cap, and sonicate the sample for approximately 5 minutes.

9.3.5 Shake the sample for a minimum of 60 minutes on a reciprocating shaker at approximately 180 excursions/minute.

9.3.6 Centrifuge the sample vial for 5 minutes at 2500 rpm.

9.3.7 Transfer the extract to a 50-mL graduated mixing cylinder and close the cylinder with a ground glass stopper.

9.3.8 Repeat Steps 9.3.3 through 9.3.6. using only 15 mL of the 90% acetone/10% 0.1 N hydrochloric acid extraction solution and shake for a minimum of 30 minutes on a reciprocating shaker.

9.3.9 Combine the extract from Step 9.3.8. with the extract from Step 9.3.7. in the same graduated cylinder and adjust the final volume to 40 mL with more of the 90% acetone/10% 0.1 N hydrochloric acid extraction solution. Mix the sample extract well by inverting the cylinder.

9.3.10 Transfer an 8.0-mL aliquot of the extract from Step 9.3.9. to a 40-mL vial.

9.3.11 Add a 2-mL aliquot of 1.0 N sodium hydroxide to the sample vial and vortex mix for 10-15 seconds.

9.3.12 Concentrate the sample to just under 3 mL under a stream of nitrogen on a TurboVap evaporator set at a water bath temperature of 40 °C and the pressure for the nitrogen flow set at approximately 5 psi. (Critical Step: Evaporate to just under 3 mL. Do not allow the evaporation to proceed to dryness.)

9.3.13 Add 2 mL of 5.0 N hydrochloric acid and 15.0 mL of distilled/deionized water to the sample vial and vortex mix for 10-15 seconds. Cap the vial and sonicate for 10-15 seconds and vortex mix for 10-15 seconds.

9.3.14 Purify the sample using the following C_{18} SPE procedure (Note 14.6):

a. Place a C_{18} SPE column on the vacuum manifold.

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

c. Condition the SPE column with 5 mL of 0.5 N hydrochloric acid. (Do not allow the column bed to dry.)

d. Transfer the sample solution from Step 9.3.13 to the SPE column, and slowly pull the sample through the column at a flow rate of 1-2 mL/min with the aid of vacuum. Discard the eluate. (Do not allow the column bed to dry.)

e. Rinse the sample vial with 2.0 mL of 0.5 N hydrochloric acid, and transfer to the SPE column. Slowly pull the rinse solution through the column at a flow rate of 1-2 mL/min with the aid of vacuum, and discard the eluate.

f. Dry the SPE column under vacuum for approximately 1 minute at 20 inches of Hg.

g. Elute the fluroxypyr and the fluroxypyr-DCP from the SPE column by passing 14 mL of a 30% acetonitrile/69% water/1% 1.0 N hydrochloric acid solution through the column with the aid of vacuum at a flow rate of 1-2 mL/min, collecting the eluate in a 40-mL vial containing 5 g of sodium chloride. Retain this solution for further preparation in Step 9.3.15 below.

h. Dry the SPE column under vacuum for approximately 5 to 10 min at 20 inches of Hg. (Critical Step: If any of the delivery needles from the SPE columns have drops of water clinging to them, carefully dry them with a tissue before proceeding to Step 9.3.14.i. below. Water in the sample will inhibit the derivatization of the fluroxypyr and the fluroxypyr-DCP.) (Note 14.7)

i. Elute the fluroxypyr-MP from the SPE column by passing 5 mL of 1-chlorobutane through the column with the aid of vacuum at a flow rate of 1-2 mL/min, collecting the eluate in a 12-mL vial.

j. Using an adjustable pipetter, add 50 µL of decane to the sample vial to act as a keeper for the fluroxypyr-MP during the evaporation.

k. Concentrate the 1-chlorobutane solution to about 2 mL under a stream of nitrogen using a TurboVap evaporator set at a water bath temperature of 40 °C and the pressure for the nitrogen flow set at approximately 5 psi. Retain this solution to combine with the solution obtained in Step 9.3.20 below. (Critical Step: Avoid allowing the evaporation to proceed to dryness.)

9.3.15 Vortex mix the sample collected in Step 9.3.14.g. for about 1-2 minutes to super-saturate the solution with the sodium chloride.

9.3.16 Centrifuge the sample vial for 5 minutes at 2500 rpm. (Two solvent layers will appear.)

9.3.17 Completely evaporate the acetonitrile (upper layer) from the sample under a stream of nitrogen using a TurboVap evaporator set at a water bath temperature of 40 °C and the pressure for the nitrogen flow set at approximately 5 psi. Just under 10 mL of liquid will remain in the vial.

9.3.18 Add 5 mL of 1-chlorobutane to the sample vial. Cap the vial, and shake the sample for 20 minutes on a reciprocating shaker set at approximately 180 excursions/min.

9.3.19 Centrifuge the sample vial for 5 minutes at 2500 rpm.

9.3.20 Transfer and combine the 1-chlorobutane layer (upper layer) in the 12-mL vial with that retained from Step 9.3.14.k. above. (Critical Steps: Avoid picking up any water or particulates found at the solvent interface. Water will inhibit the derivatization of the fluroxypyr and the fluroxypyr-DCP.) (Note 14.7)

9.3.21 Concentrate the solution from Step 9.3.20 to about 2 mL under a stream of nitrogen using a TurboVap evaporator set at a water bath temperature of 40 °C and the pressure for the nitrogen flow set at approximately 5 psi. (Critical Step: Do not allow the evaporation to proceed to dryness.) (Note 14.8)

9.3.22 Repeat Steps 9.3.18 through 9.3.20, and then proceed to Step 9.3.23.

9.3.23 Concentrate the solution from Step 9.3.22 to about 0.8 mL under a stream of nitrogen using a TurboVap evaporator set at a water bath temperature of 40 °C and the pressure for the nitrogen flow set at approximately 5 psi. (Critical Steps: Do not allow the evaporation to proceed to dryness. If it is necessary to adjust the volume back up to about 0.8 mL, use 1-chlorobutane as the solvent and gauge the volume with a direct comparison tube.) (Note 14.8)

9.3.24 Using an adjustable pipetter, add 100 µL of MBTSTFA derivatization reagent to the sample vial and to the appropriate calibration standards (as prepared in Step 7.1.6) which encompass the concentration range of the recovery samples. Vortex mix the sample for approximately 10-15 seconds followed by sonication for about 10-15 seconds.

9.3.25 Place the sample, along with the calibration standards in an oven or reaction block set at 60 °C for 1 hour for derivatization of the fluroxypyr acid and the fluroxypyr-DCP.

9.3.26 Remove the sample and calibration standards from the oven and allow the reaction mixture to completely cool to ambient temperature before opening the vials.

9.3.27 Using an adjustable pipetter, add 100 µL of the 10 µg/mL fluroxypyr 1-butyl ester internal standard solution (in acetone) to the sample vial and the calibration standards. Sonicate the sample and standards for about 5-10 seconds followed by vortex mixing for about 5-10 seconds.

9.3.28 Transfer a portion of the sample and the calibration standards to a limited-volume insert inside of a 2-mL autosampler vial and seal with a cap.

9.3.29 Analyze the calibration standards and the samples by capillary gas chromatography/mass spectrometry as described in Section 8. Determine the suitability of the chromatographic system using the following performance criteria:

a. Standard curve linearity: Determine that the correlation coefficient equals or exceeds 0.995 for the least squares equation which describes the detector response as a function of analyte concentration plotted on a standard curve. If power regression is used, the power exponent should be between 0.90-1.10.

b. Peak resolution: Visually determine that sufficient resolution has been achieved for the analyte and internal standard relative to background interferences.

c. Appearance of chromatograms: Visually determine that the chromatograms resemble those shown in Figures 8-16 with respect to peak response, baseline noise, and background interference. Visually determine that a minimum signal-to-noise ratio of 10:1 has been attained for each analyte in the 0.005-µg/mL calibration standard.

9.4 Calculation of Percent Recovery

9.4.1 Inject the series of calibration standards as described in Section 8. and determine the peak areas for the analytes and the internal standard as indicated below:

Fluroxypyr-TBDMS	*m/z* 311 (quantitation), *m/z* 253 (confirmation) (Note 14.5)
Fluroxypyr-DCP-TBDMS	*m/z* 253 (quantitation), *m/z* 257 (confirmation) (Note 14.5)
Fluroxypyr-MP	*m/z* 210 (quantitation), *m/z* 181 (confirmation) (Note 14.5)
Fluroxypyr 1-Butyl Ester	*m/z* 310 (internal standard)

9.4.2 For each standard, calculate the quantitation ratio.

For example, using the data for fluroxypyr from Figure 8:

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

$$\text{Quantitation Ratio} = \frac{\text{peak area at } m/z \text{ 311}}{\text{peak area at } m/z \text{ 310}}$$

$$\text{Quantitation Ratio} = \frac{10762}{186409}$$

$$\text{Quantitation Ratio} = 0.0577$$

9.4.3 Prepare a standard curve by plotting the equivalent fluroxypyr, fluroxypyr-DCP, or fluroxypyr-MP concentration on the abscissa (x-axis) and the respective quantitation ratio with respect to the fluroxypyr 1-butyl ester internal standard on the ordinate (y-axis) as shown in Figures 5-7. Using regression analysis, determine the equation for the curve with respect to the abscissa.

For example, using power regression (15.1) with the fluroxypyr data from Figure 5:

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

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

$$\text{Fluroxypyr Conc. } (\mu g/g) = \left(\frac{\text{Fluroxypyr-TBDMS quantitation ratio}}{\text{constant}}\right)^{1/\text{exponent}}$$

$$\text{Fluroxypyr Conc. } (\mu g/g) = \left(\frac{\text{Fluroxypyr-TBDMS quantitation ratio}}{5.4941}\right)^{1/0.9832}$$

9.4.4 Determine the gross concentration in each recovery sample by substituting the quantitation ratio obtained into the above equation and solving for the concentration.

For example, using the fluroxypyr data from Figure 10:

$$\text{Fluroxypyr Conc. (gross } \mu g/g) = \left(\frac{\text{Fluroxypyr-TBDMS quantitation ratio}}{5.4941}\right)^{1/0.9832}$$

$$\text{Fluroxypyr Conc. (gross } \mu g/g) = \left(\frac{0.0489}{5.4941}\right)^{1/0.9832}$$

$$\text{Fluroxypyr Conc. (gross)} = 0.0082\ \mu g/g$$

9.4.5 It is acceptable to background subtract any peaks found in the control sample from the sample fortified with fluroxypyr, fluroxypyr-DCP, or fluroxypyr-MP for use in the calculation of percent recovery. Find the relative concentration of any background peaks found in the control sample at the retention time of the analyte using the corresponding standard calibration curve. The net concentration of the analyte found in the recovery sample is then determined by subtracting the concentration of the background peak found in the control sample from that of the gross analyte concentration found in the recovery sample.

$$\text{Fluroxypyr Conc. (net } \mu g/g) = \text{Fluroxypyr Conc. (gross } \mu g/g) - \text{Background Conc. (control } \mu g/g)$$

For example, using the fluroxypyr data from Figures 9 and 10:

$$\text{Fluroxypyr Conc. (net } \mu g/g) = 0.0082\ \mu g/g - 0.0000\ \mu g/g$$

$$\text{Fluroxypyr Conc. (net)} = 0.0082\ \mu g/g$$

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

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

$$\text{Recovery} = \frac{0.0082\ \mu g/g}{0.01\ \mu g/g} \times 100\%$$

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

10. DETERMINATION OF FLUROXYPYR AND ITS METABOLITES IN SOIL

10.1 Prepare reagent blank, control, recovery, and treated samples as described in Section 9.

10.2 Prepare a standard calibration curve for fluroxypyr, fluroxypyr-DCP, and fluroxypyr-MP and determine the percent recovery for each analyte as described in Section 9.4.

10.3 Determine the gross concentration of fluroxypyr, fluroxypyr-DCP, and fluroxypyr-MP in each treated sample by substituting the quantitation ratio obtained into the equation for the standard calibration curve, and calculating the residue result as described in Section 9.4.4.

10.4 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 fluroxypyr data from Figure 10 and Table 1 for the samples analyzed on 14-Nov-1998:

10.4.1 Determine the gross analyte concentrations in the soil sample as described in Section 9.4.4.

10.4.2 Determine the corrected analyte concentration in the soil sample as follows:

$$\text{Fluroxypyr Conc. (corrected } \mu g/g) = \text{Fluroxypyr-TBDMS Conc. (gross } \mu g/g) \times \left(\frac{100}{\text{Average Percent Recovery}}\right)$$

$$\text{Fluroxypyr Conc. (corrected } \mu g/g) = 0.0082\ \mu g/g \times \frac{100}{79}$$

$$\text{Fluroxypyr Conc. (corrected } \mu g/g) = 0.0104\ \mu g/g$$

11. DETERMINATION OF SOIL MOISTURE

11.1 To correct for soil moisture, accurately weight approximately 10 g of soil into a tared aluminum or glass container and record the weight.

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

11.3 Remove the sample from the oven, place in a desiccator until the sample has cooled to ambient temperature, and then weigh the dried soil sample and record the weight.

11.4 Calculate the percent soil moisture on a dry-weight basis as follows:

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

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

12. DETERMINATION OF FLUROXYPYR AND METABOLITE CONCENTRATIONS IN SOIL CORRECTED FOR SOIL MOISTURE

12.1 Determine the fluroxypyr, fluroxypyr-DCP, and fluroxypyr-MP concentrations in the soil samples as described in Section 10.

12.2 Determine the soil moisture as described in Section 11.

12.3 Determine the fluroxypyr, fluroxypyr-DCP, or fluroxypyr-MP concentration in the soil samples corrected on a dry-weight basis as follows:

$$\text{Analyte Conc. (dry weight } \mu\text{g/g)} = \text{Analyte Conc. (gross } \mu\text{g/g)} \times \left(1 + \frac{\%\ \text{Moisture}}{100}\right)$$

13.5 Standardization of C_{18} SPE Column Elution Profile

13.5.1 Add 20 mL of 0.5 N hydrochloric acid to a 40-mL vial.

13.5.2 Add 10 µL of the 100-µg/mL calibration standard stock solution (in acetone) to the vial and vortex mix for 10-15 seconds.

13.5.3 Profile using the following C_{18} SPE procedure:

a. Place a C_{18} SPE column on the vacuum manifold.

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

c. Condition the SPE column with 5 mL of 0.5 N hydrochloric acid. (Do not allow the column bed to dry.)

d. Transfer the solution from Step 13.5.2 to the SPE column, and slowly pull the sample through the column at a flow rate of 1-2 mL/min with the aid of vacuum. Discard the eluate. (Do not allow the column bed to dry.)

e. Rinse the 40-mL vial with 2.0 mL of 0.5 N hydrochloric acid, and transfer to the top of the SPE column. Slowly pull the rinse solution through the column at a flow rate of 1-2 mL/min with the aid of vacuum, and discard the eluate.

f. Dry the SPE column under vacuum for approximately 1 minute at 20 inches of Hg.

g. Elute the fluroxypyr and the fluroxypyr-DCP from the SPE column, collecting 18 individual 1-mL fractions in separate 40-mL vials.

Pass the 30% acetonitrile/69% water/1% 1.0 N hydrochloric acid solution through the column with the aid of vacuum at a flow rate of 1-2 mL/min.

h. Add 13 mL of a 30% acetonitrile/69% water/1% 1.0 N hydrochloric acid solution and 5 g of sodium chloride to each vial. Retain these solutions for further preparation in Step 13.5.4 below.

i. Dry the SPE column under vacuum for 5 to 10 min at 20 inches of Hg. (Critical Step: If any of the delivery needles from the SPE columns have drops of water clinging to them, carefully dry them with a tissue before proceeding to Step 13.5.3.j.)

j. Elute the fluroxypyr-MP from the SPE column collecting 8 individual 1-mL fractions in separate 12-mL vials. Pass the 1-chlorobutane through the column with the aid of vacuum and a flow rate of 1-2 mL/min.

k. Add 10 µL of the 100 µg/mL fluroxypyr 1-butyl ester internal standard solution (in acetone) to each vial. Sonicate the vials for about 5-10 seconds followed by vortex mixing for about 5-10 seconds.

l. Transfer an aliquot from each vial to a limited-volume insert inside of a 2-mL autosampler vial and seal with a cap.

m. Retain these fractions for analysis along with the calibration standards. Assay by capillary gas chromatography/mass spectrometry as described in Section 8.

13.5.4 Vortex mix the fractions from Step 13.5.3.h. for about 1 to 2 minutes to super-saturate each fraction with the sodium chloride.

13.5.5 Centrifuge the vials for 5 minutes at 2500 rpm. (Two solvent layers will appear.)

13.5.6 Completely evaporate the acetonitrile (upper layer) from each fraction under a stream of nitrogen using a TurboVap evaporator set at a water bath temperature of 40 °C and the pressure for the nitrogen flow set at approximately 5 psi. Just under 10 mL of liquid will remain in the vial.

13.5.7 Add 5 mL of 1-chlorobutane to each vial. Cap the vials, and shake the vials for 20 minutes on a reciprocating shaker set at approximately 180 excursions/min.

13.5.8 Centrifuge the vials for 5 minutes at 2500 rpm.

13.5.9 Transfer the 1-chlorobutane layer (upper layer) from each vial processed in Step 13.5.8 to a separate 12-mL vial. (Critical Steps: Avoid picking up any water or particulates found at the solvent interface. Water will inhibit the derivatization of the fluroxypyr acid and the fluroxypyr-DCP.) (Note 14.7)

13.5.10 Concentrate the fractions from Step 13.5.9 to about 2 mL under a stream of nitrogen using a TurboVap evaporator set at a water bath temperature of 40 °C and the pressure for the nitrogen flow set at approximately 5 psi. Retain these as separate fractions to be combined with the like fractions collected in Step 13.5.12. (Critical Step: Avoid allowing the evaporation to proceed to dryness.) (Note 14.8)

13.5.11 Repeat Steps 13.5.7 through 13.5.9. Transfer the 1-chlorobutane layer from each vial and combine with the complementary fractions collected in Step 13.5.9, and proceed to Step 13.5.12.

13.5.12 Concentrate the fractions from Step 13.5.11 to about 0.7 mL under a stream of nitrogen using a TurboVap evaporator set at a water bath temperature of 40 °C and the pressure for the nitrogen flow set at approximately 5 psi. (Critical Step: Avoid allowing the evaporation to proceed to dryness.) (Note 14.8)

13.5.13 Adjust the volume of each fraction back to 0.8 mL with 1-chlorobutane using a comparison tube to gauge the volume.

13.5.14 Add 100 µL of MBTSTFA derivatization reagent to each vial. Vortex mix the vials for approximately 1 min followed by sonication for about 1 minute.

13.5.15 Place the elution profile fractions, along with the appropriate calibration standards which encompass the concentration range of the fractions in an oven or reaction block set at 60 °C for 1 hour for derivatization of the fluroxypyr acid and the fluroxypyr-DCP.

13.5.16 Remove the fractions and calibration standards from the oven and allow the reaction mixture to completely cool to ambient temperature before opening the vials.

13.5.17 Add 100 µL of the 10-µg/mL fluroxypyr 1-butyl ester internal standard solution (in acetone) to each vial and to each calibration standard. Sonicate the fractions and standards for about 5-10 seconds followed by vortex mixing for about 5-10 seconds.

13.5.18 Transfer a portion of each fraction and the calibration standards to a limited-volume insert inside of a 2-mL autosampler vial and seal with a cap.

13.5.19 Analyze the calibration standards and the elution profile fractions by capillary gas chromatography/mass spectrometry as described in Section 8.

13.5.20 Calculate the concentration of each analyte in each fraction as described in Section 9.4. Determine the percent of each analyte that is found in each fraction. If necessary, adjust the volume of elution solvent collected during sample purification. (Typical elution profiles for fluroxypyr, fluroxypyr-DCP, and fluroxypyr-MP are shown in Figures 17-18.)

14. NOTES

14.1 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.

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

14.3 The final concentration of each calibration standard solution will be a 1:5 dilution of the concentration of the original fortification solution that was dispensed. For example: By dispensing 200 µL of the 0.05-µg/mL fortification solution for preparation, a calibration standard of 0.01 µg/mL will be the final result.

14.4 Several tuning, or calibration, options are available for the Model 597X series of MSDs. The "Maximum Sensitivity Autotune" calibration feature was found to consistently yield approximately 5-10 times the sensitivity compared to that of the "Standard Autotune" calibration.

14.5 For confirmation of the presence of fluroxypyr, monitoring ions *m/z* 255 and/or *m/z* 313 in addition to *m/z* 253 is acceptable if there is need for additional confirmation ions. For confirmation of the presence of fluroxypyr-DCP, monitoring ion *m/z* 255 in addition to *m/z* 257 is acceptable if there is need for additional confirmation ions. For confirmation of the presence of fluroxypyr-MP, monitoring ions *m/z* 180, *m/z* 209, and/or *m/z* 212 in addition to *m/z* 181 is acceptable if there is need for additional confirmation ions.

14.6 An elution profile for each new lot of C_{18} SPE columns should be performed to ensure optimum recovery of each analyte.

14.7 In transferring the 1-chlorobutane layers to the 12-mL vial in which the sample will be derivatized or when eluting the fluroxypyr-MP from the C_{18} SPE with 1-chlorobutane, it is important not to introduce water into the sample. Contaminating the sample at these points with water may have deleterious effects on sample derivatization and compromise the subsequent GC/MSD analysis and recovery values.

14.8 Once the solution has been concentrated, it is important not to transfer it to a new vial for any reason prior to derivatization. During concentration, the solubility of fluroxypyr in 1-chlorobutane is reduced and recovery may be jeopardized.

15. REFERENCES

15.1 Freund, J. E.; Williams, F. J. *Dictionary/Outline of Basic Statistics*; Dover: New York, 1991; p 170.

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

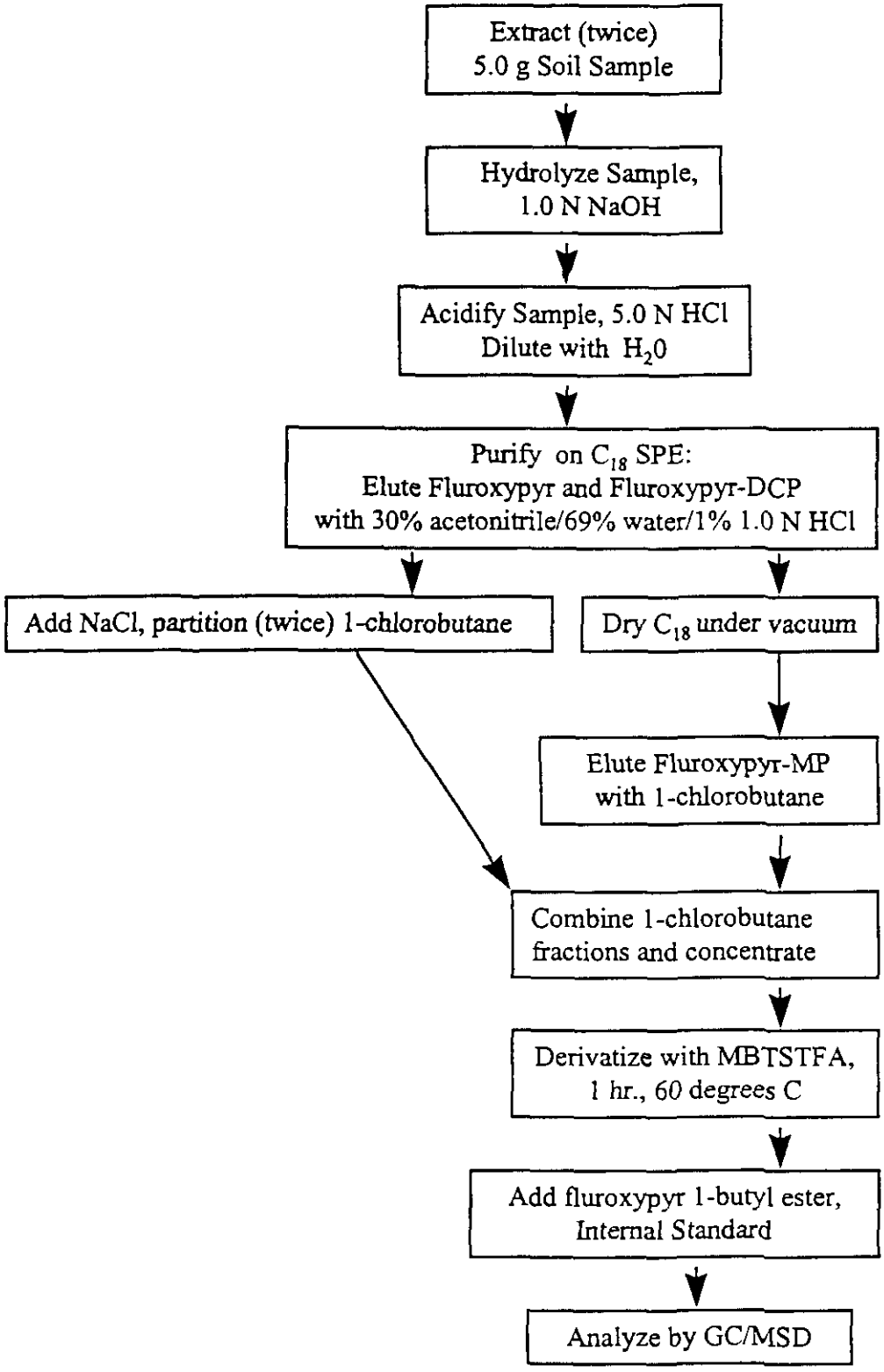
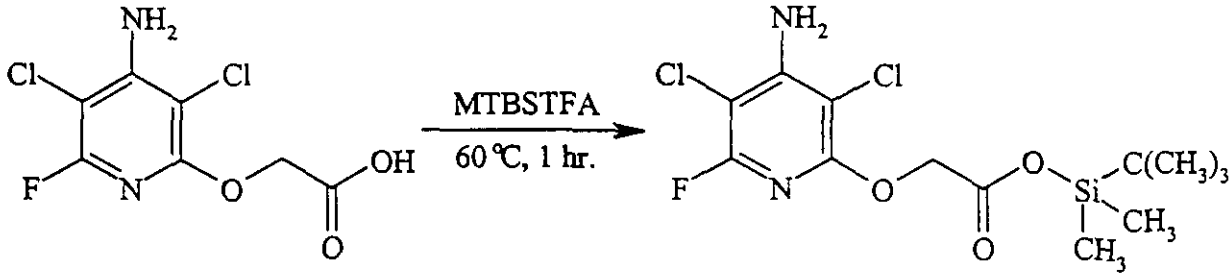
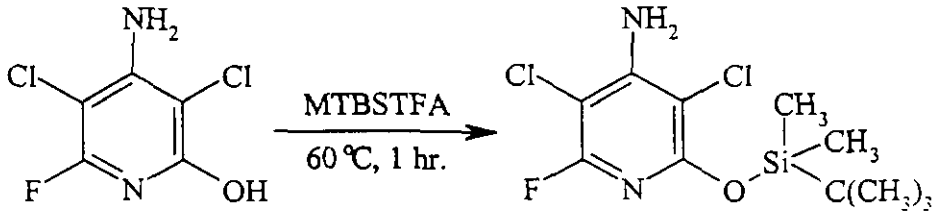


Figure 1. Flow Chart for the Determination of Fluroxypyr, Fluroxypyr-DCP, and Fluroxypyr-MP in Soil



Fluroxypyr
Formula: $C_7H_5Cl_2FN_2O_3$
Formula Weight: 255.03
Molecular Weight: 254

Fluroxypyr-TBDMS
Formula: $C_{13}H_{19}Cl_2FN_2O_3Si$
Formula Weight: 369.30
Molecular Weight: 368



Pyridinol degradate
of Fluroxypyr
Formula: $C_5H_3Cl_2FN_2O$
Formula Weight: 197.00
Molecular Weight: 196

Pyridinol degradate
of Fluroxypyr-TBDMS
Formula: $C_{11}H_{17}Cl_2FN_2OSi$
Formula Weight: 311.26
Molecular Weight: 310

Figure 2. Chemical Structures of Fluroxypyr and Fluroxypyr-DCP and their TBDMS Derivatives