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DETERMINATION OF RESIDUES OF FLUROXYPYR ((4-AMINO-3,5-DICHLORO-6 FLUORO-2-OXY ACETIC ACID) PYRIDINE) , METHOXYPYRIDINE ((4-AMINO-3,5-DICHLORO-6-FLUORO-2-METHOXY) PYRIDINE) AND 3,5-DICHLOROPYRIDINOL ((4-AMINO-3,5-DICHLORO-6-FLUORO-2-HYDROXY) PYRIDINE) IN SOIL BY CAPILLARY GAS CHROMATOGRAPHY USING MASS SELECTIVE DETECTION.

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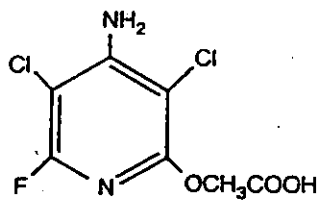
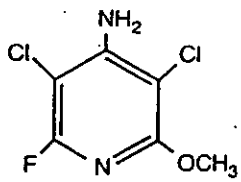
A. Scope:

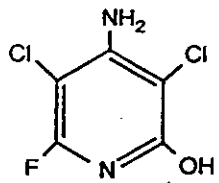
This method is applicable for the quantitative determination of fluroxypyr ((4-amino-3,5-dichloro-6-fluoro-2-oxy acetic acid) pyridine) and the metabolites methoxypyridine ((4-amino-3,5-dichloro-6-fluoro-2-methoxy) pyridine) and 3,5-dichloropyridinol ((4-amino-3,5-dichloro-6-fluoro-2-hydroxy) pyridine) in soil at a validated lower limit of quantitation of 0.01 µg/g.



Fluroxypyr Acid
FW=256
CAS# 69377-81-7



Methoxypyridine
FW=211
CAS# 35622-80-1



3,5 -Dichloropyridinol
MW=197
CAS# 94133-62-7

B. Principle:

Residues of fluroxypyr, methoxypyridine and 3,5-dichloropyridinol are extracted from soil using a 90:10 acetone:0.1 N hydrochloric acid solution. The acetone is evaporated and the methoxypyridine is partitioned from the remaining aqueous solution into hexane. The hexane is then added to a silica gel solid phase extraction (SPE) column and methoxypyridine is eluted from the silica gel SPE with methyl-*tert*-butyl ether (MTBE). Decane is added to the MTBE as a keeper solvent. The MTBE is evaporated, leaving the methoxypyridine concentrated in the decane. The solution is analyzed for methoxypyridine by capillary gas chromatography/mass selective detection (GC/MSD).

The fluroxypyr and 3,5-dichloropyridinol residues are extracted from the aqueous portion of the original extract using a C_{18} SPE. The residues are then eluted with a 30:70 (v/v) mixture of ethyl ether:hexane. The eluting solution is diluted with MTBE to a known volume and split into two equal portions for fluroxypyr and 3,5-dichloropyridinol analysis.

Decane is added as a keeper solvent to the MTBE aliquot used for analysis of 3,5-dichloropyridinol residues. The MTBE is evaporated leaving the 3,5-dichloropyridinol concentrated in decane. N,O-bis(trimethylsilyl)acetamide (BSA) is added to the decane to form the trimethylsilyl (TMS) derivative of 3,5-dichloropyridinol. The TMS derivative is analyzed by GC/MSD and used to quantitate residues of 3,5-dichloropyridinol.

The MTBE solution for the analysis of fluroxypyr is exchanged with acetone. The acetone solution is acidified with dilute phosphoric acid and trimethylsilyl (TMS) diazomethane is added to form the fluroxypyr methyl ester derivative. Dilute sulfuric acid is added and the acetone is evaporated. The fluroxypyr methyl ester is partitioned into toluene. The toluene solution is analyzed for fluroxypyr methyl ester by GC/MSD.

The method was validated and has been practiced in this laboratory using gravimetric techniques (verification of critical liquid volumes by weight). It has been our experience that the use of gravimetric techniques not only improves precision and productivity, but also provide documentation of individual sample raw data. Volumetric techniques would be expected to yield similar results. (Equations for calculation of sample preparation factors are included for both gravimetric and volumetic techniques.)

C. Safety Precautions:

1. Each analyst should be acquainted with the potential hazards of the reagents, products, and solvents used in this method before commencing laboratory work. Sources of information include: MATERIAL SAFETY DATA SHEETS, LITERATURE, AND OTHER RELATED DATA. Safety information on non-DowElanco products should be requested from the supplier. Disposal of reagents, reactants, and solvents must be in compliance with local, state, and federal laws and regulations.

2. Acetone, hexane, methyl-*tert* -butyl ether, ethyl ether, toluene, methanol and decane are flammable and should be used in well-ventilated areas away from ignition sources.

3. Hydrochloric acid, phosphoric acid, sulfuric acid and sodium hydroxide are corrosive and should be handled in a way to minimize the risk of personal contact.

4. Derivitization reagents such as BSA and TMS diazomethane should be handled in a well-ventilated area so as to minimize potential exposure. Both reagents are potential irritants and should be handled with proper protective clothing.

D. Equipment: (See Note P.1.)

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

2. Balance, analytical, Model AE200, Mettler Instrument Corp., Hightstown, NJ 08520.

3. Balance, pan, Model 400, Mettler Instrument Corp.

4. Centrifuge, with head to accommodate 11-dram vials, Model Centra-8, International Equipment Company, Needham Heights, MA 02194.

5. Evaporator, TurboVap Model LV, Zymark Corp., Hopkinton, MA 01748.

6. Gas chromatograph, Model 5890A, Hewlett-Packard, Wilmington.

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

8. Mass spectrometer data system, DOS ChemStation, Hewlett-Packard, Palo Alto.

9 SPE Processing Station, vacuum manifold box, Model SPE-21, J.T. Baker Chemical Company, Phillipsburg, NJ 08865.

10. Shaker, variable-speed reciprocating with box carrier, Model 6000, Eberbach Corp., Ann Arbor, MI 48106.

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

12. Vial crimper, part no. 8710-0979, Hewlett-Packard, Wilmington.

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

E. Glassware and Materials: (See Note P.1.)

1. Caps, PTFE Lined, catalog no. 028833F, Fisher Scientific.

2. Charcoal scrubber, catalog no. 7972, Chrompack, Inc., Raritan, NJ 08869 (see Note P.2.).

3. Column, capillary gas chromatography, DB-5 liquid phase, 15 m x 0.25 mm i.d., 0.25 µm film thickness, catalog no. 122-1012, J&W Scientific, Folsom, CA 95630.

4. Column, capillary gas chromatography, DB-Wax liquid phase, 15 m x 0..25 mm i.d., 0.25 µm film thickness, catalog no. 122-7012, J&W Scientific.

5. Column inlet liner, deactivated, catalog no. 5181-3315, Hewlett-Packard, Avondale, PA 19311.

6. Gas, helium, 99.995% purity, Scott Specialty Gases, Troy, MI 48083 (see Note P.2.).

7. Gas, nitrogen, High Purity, Scott Specialty Gases (see Note P.2.).

8. Glass inserts, catalog #1191, Sunbrokers, Wilmington, NC 27489.

9. Moisture trap, catalog no. 7971, Chrompack, Inc. (see Note P.2.).

10. Oxygen trap, catalog no. 7970, Chrompack, Inc. (see Note P.2.).

11. SPE column, silica, catalog no. 7086-03, J. T. Baker Chemical Company.

12. SPE column, C_{18}, catalog no. 7020-13, J.T. Baker Chemical Company.

13. Syringe, 10-µL, Model 700 Series, Hamilton Company, Reno, NV 89520.

14. Syringe, 100-µL, Model 700 Series, Hamilton Company, Reno, NV 89520.

15. Tubes, 16 x 100 mm disposable culture, part no. 14-962-10-D, Fisher Scientific, Pittsburgh, PA

16. Vials, 11-dram, catalog no. 033395D, Fisher Scientific, 585 Alpha Drive Pittsburgh, PA 15238

17. Vials, autosampler, 2-mL, catalog no. C4011-2, National Scientific Company.

18. Vial seals, catalog no. C4011-1A, National Scientific Company.

F. Reagents and Chemicals:

1. Acetone, ethyl ether, methanol, methyl-*t*-butyl ether, hexane, toluene, and decane, all HPLC grade or higher, Fisher Scientific.

2. Derivatizing agent, trimethylsilyl diazomethane, catalog no: 36283-2, Aldrich Chemical Company, Milwaukee, WI 53233.

3. Derivatizing reagent [N,O-bis(trimethylsilyl)acetamide] (BSA), catalog no. 3-3036, Pierce Chemical Company, Rockford IL 61105.

4. Hydrochloric acid, 0.1 N, reagent grade, certified concentration, Fisher Scientific.

5. 90% acetone/10% 0.1 N hydrochloric acid solution.

   Prepare by pouring 200 mL of 0.1 N hydrochloric acid into a 2000-mL graduated cylinder. Add 1500 mL of acetone, swirl the cylinder, and allow to equilibrate to room temperature. Adjust to volume with acetone.

6. o-Phosphoric Acid, reagent grade 85%, catalog no. A-242-500, Fisher Scientific.

7. Phosphoric acid, 0.01M, prepared as follows:

   Weigh 1.15 g of 85% phosphoric acid into a 1000-mL volumetric flask containing 900 mL of deionized water. Swirl to mix and dilute to volume with deionized water.

8. Sodium hydroxide, catalog no. S318-100, Fisher Scientific.

9. Sodium hydroxide, 1.0N, prepared as follows:

   Weigh 40.0 g of anhydrous sodium hydroxide into a 1000-mL volumetric flask and add 400 mL deionized water to dissolve pellets. After dissolution is complete cool to room temperature then dilute to volume with deionized water.

10. Sulfuric acid, concentrated, optima grade, 96.9%, catalog no. A468-250, Fisher Scientific.

11. Sulfuric acid, 0.5 N, prepared as follows:

Weigh 25.77 g of concentrated H_2SO_4 into a 1000-mL volumetric flask containing approximately 500 mL of deionized water. Shake to mix and dilute to volume.

12. 30:70 (v/v) ethyl ether:hexane.

Prepare 1 L by pouring 300 mL of ethyl ether into a flask and add 700 mL of hexane. Swirl to mix.

13. Water, distilled/deionized, Corning MEGA-PURE Still, Model MP-12A, Corning Glass Works, Science Products Division, Corning, NY 14831.

14 Standards:

Fluroxypyr, CAS# 69377-81-7, AGR218256, purity 99.2%. Obtain standard from Sample Coordinator, DowElanco, 9330 Zionsville Road, Indianapolis, IN 46268.

Fluroxypyr methyl ester, AGR 218601, purity 98.3%. Obtain standard from Sample Coordinator, DowElanco.

Methoxypyridine, CAS# 35622-80-1, AGR 250194, purity 99.9%. Obtain standard from Sample Coordinator, DowElanco.

3,5-Dichloropyridinol, CAS# 94133-62-7, AGR 251053, purity 99.5%. Obtain standard from Sample Coordinator, DowElanco.

G. Preparation of Standards:

1. Preparation of stock solutions for preparation of spiking solutions and calibration standards.

Identical fortification solutions were prepared for fluroxypyr, methoxypyridine and 3,5-dichloropyridinol.

a. For fluroxypyr, methoxypyridine and 3,5-dichloropyridinol dissolve 0.1000 g of standard in acetone in a 100-mL volumetric flask. Dilute to volume to obtain a 1000 µg/mL stock solution.

b. For fluroxypyr, methoxypyridine and 3,5-dichloropyridinol transfer, using a volumetric pipette, a 10 mL aliquot to a 100-mL volumetric flask. Dilute to volume to obtain a 100 µg/mL stock solution.

2. Preparation of fortification solutions:

Aliquots of the stock solutions, prepared above, are diluted with acetone according to the table below to obtain solutions with the listed concentrations.

Identical fortification solutions are prepared for fluroxypyr, methoxypyridine and 3,5-dichloropyridinol. Unless otherwise specified the dilutions are made from the 100 µg/mL stock solutions using volumetric pipettes.

Aliquot of Stock (mL)	Final Volume (mL)	Spiking Soln. Conc. (µg/mL)	Spiking Volume (mL)	Equivalent 5 g Sample Conc. (µg/g)
5.0	100	5.0	--	1.0
2.5	100	2.5	1.0	0.5
1.0	100	1.0	--	0.2
0.5	100	0.5	1.0	0.1
10[a]	100	0.25	--	0.05
10[b]	100	0.10	1.0	0.02
10[c]	100	0.05	1.0	0.01

[a] A 10 mL aliquot of the 2.5 µg/mL spiking solution is diluted 1/10 to obtain the 0.25 µg/mL spiking solution.

[b] A 10 mL aliquot of the 1.0 µg/mL spiking solution is diluted 1/10 to obtain the 0.10 µg/mL spiking solution.

[c] A 10 mL aliquot of the 0.5 µg/mL spiking solution is diluted 1/10 to obtain the 0.05 µg/mL spiking solution.

3. Preparation of calibration solutions used to quantitate µg/mL for samples:

   a. The formula weight difference between fluroxypyr and the methyl ester derivative require the calibration solutions to be prepared in terms of fluroxypyr equivalents. Dissolve 0.1055 g of fluroxypyr methyl ester standard in acetone in a 100-mL volumetric flask. Dilute to volume to obtain a 1000 µg/mL fluroxypyr equivalent stock solution. Transfer, with a volumetric pipette, 10 mL of the 1000 µg/mL solution to a 100-mL volumetric flask and dilute to volume with acetone to obtain a 100 µg/mL stock solution of fluroxypyr equivalent.

   b. Calibration solutions for fluroxypyr equivalent, methoxypyridine and 3,5-dichloropyridinol are prepared by diluting the respective stock solutions prepared in G.3.a. for fluroxypyr equivalent and G.1. for methoxypyridine and 3,5-dichloropyridinol according to the chart below, substituting toluene for a diluent for fluroxypyr equivalent and decane as a diluent for methoxypyridine and 3,5-dichloropyridinol

Aliquot of Stock (mL)	Final Volume (mL)	Calibration Soln. Conc. (µg/mL)
5.0	100	5.0
2.5	100	2.5
1.0	100	1.0
0.5	100	0.5
10[a]	100	0.25
10[b]	100	0.10
10[c]	100	0.05

[a] A 10 mL aliquot of the 2.5 µg/mL spiking solution is diluted 1/10 to obtain the 0.25 µg/mL spiking solution.

[b] A 10 mL aliquot of the 1.0 µg/mL spiking solution is diluted 1/10 to obtain the 0.10 µg/mL spiking solution.

[c] A 10 mL aliquot of the 0.5 µg/mL spiking solution is diluted 1/10 to obtain the 0.05 µg/mL spiking solution.

H. Gas Chromatography/Mass Selective Detection:

1. Columns:

For the analysis of fluroxypyr methyl ester use the DB-Wax column (E.3.). For the determination of methoxypyridine and 3,5-dichloropyridinol use the DB-5 column (E.2.).

Install the inlet liner and the capillary column on the split/splitless injection port of the GC/MSD following the manufacturer's recommended procedure.

2. The same GC operating conditions are used for the analysis of all three compounds. Typical operating conditions for the determination of the analytes by GC/MSD:

Instrumentation: Hewlett-Packard Model 5890A Gas Chromatograph/Model 5971A Mass Selective Detector

Temperatures:

Column: 120 °C for 1 min
120 to 280 °C at 20 °C/min

Injector: 290 °C

Interface: 295 °C

Carrier Gas: Helium (see Note P.2.)

Head Pressure: 10 psi

Linear Velocity: 25 cm/sec

Injection Mode: Splitless

Purge Delay: 1.5 minutes

Splitter Flow: 75 mL/min

Septum Purge: 1.0 mL/min

Injection Volume: 1 µL

Ions Monitored:

fluroxypyr methyl ester

m/z 209 (base peak), 268 (molecular ion) (Figure 1)

methoxypyridine

m/z 209, 210 (molecular ion) (Figure 2)

3,5-dichloropyridinol (trimethyl silyl derivative)

m/z 253 (base peak), 255 (Figure 3)

3. Typical calibration curves are shown in Figures 4-6 for the fluroxypyr methyl ester, methoxypyridine, and 3,5-dichloropyridinol analytes, respectively.

4. Typical chromatograms of calibration standards, control samples and fortified controls samples are shown in Figures 7-30, respectively.

I. Recovery of Analytes From Soil:

1. Weigh 5-g portions of soil into a series of 11-dram vials. Record the mass to 0.01 g (see Note P.3.). This value is the sample weight, M, used in calculations (N.2.).

2. Prepare procedural recovery samples by fortifying 5-g portions of a control sample with 1.0-mL aliquots, using volumetric pipettes, of the spiking solutions of all three compounds to obtain concentrations ranging from 0.01 to 0.50 µg/g.

3. Add 25 mL of the 90% acetone/10% 0.1 N hydrochloric acid extracting solution. Record the mass of the volume to 0.01g (see Note P.3.).

4. Cap the vial and sonicate the sample for a minimum of two minutes.

5. Shake the sample for a minimum of 45 minutes in a reciprocating shaker at approximately 180 excursions/minute (see Note P.4.).

6 Sonicate the sample for a minimum of two minutes.

7. Centrifuge the sample at approximately 2500 rpm to produce a particulate free supernatant (see Note P.5.).

8. Transfer 20 mL of the extracting solution to a clean, tared 11-dram vial.

9. Add 15 mL of the 90% acetone/10% 0.1 N hydrochloric acid extracting solution to the vial containing the original soil sample. Record the mass of this volume to 0.01 g (see Note P.3.). Add this value to the weight obtained in Section I.3. This is the value of the total extraction volume added, EA, used in calculations (N.2.).

10. Repeat steps 4-7.

11. Decant the extracting solution to the 11-dram vial containing the first extract. Record the mass of this extract to 0.01 g (see Note P.3.). This is the extract volume recovered, ER, used in calculations (N.2.) (see Note P.6.).

12. Concentrate the extract under nitrogen at 25 °C using a TurboVap to a final volume of approximately 4 mL (see Note P.7.).

13. Add 1 mL of 1N NaOH.

14. Add 10 mL of hexane using a 10 mL volumeteric pipette. Record the mass of this hexane to 0.01 g (see Note P.3.). This is the hexane added, HA, used in calculations (N.2.).

15. Cap the vial and shake the sample for 20 minutes in a reciprocating shaker at approximately 180 excursions/minute.

16. Transfer at least 5 mL of the hexane layer into a clean 16 x 100 mm tube. Record the mass of the hexane to 0.01 g (see Note P.3.). This is the hexane recovered, HR, used in calculations (N.2.).

17. Add 5 mL 0.5N H_2SO_4 to the 11 dram vial containing the extract.

18. Place the sample in a TurboVap at 25 °C and evaporate any remaining hexane that might be on top of the aqueous layer (see Note P.7.).

SPE Cleanup of the Organic and Aqueous Extracts

19. Set up the SPE processing manifold (see Notes P.8.,9.).

20. Residues of methoxypyridine are isolated from the hexane solution (I.16.) using the following silica SPE procedure.

    a. Condition the silica gel column with 10 mL of hexane. Do not allow the column to go dry.

    b. When the conditioning solvent reaches the top of the column bed, load the hexane fraction (I.16.) onto the column. Discard the conditioning and loading fractions. Do not allow the colmn to go dry.

    c. When the loading fraction reaches the top of the column bed, elute the methoxypyridine from the column with 10 mL of MTBE into a clean 16 x 100 mm tube. Record the weight of the empty tube (see Note P.3.). This is the decane tube tare weight, DT, used in I.23.

    d. Add 0.5 mL of decane to this tube.

21. Residues of fluroxypyr and 3,5-dichloropyridinol are isolated from the aqueous solution (I.18.) using the following C_{18} SPE procedure:

    a. Condition the column with 10 mL of methanol. Do not allow the column to go dry.

    b. When the methanol reaches the top of the column bed, condition the column with 10 mL of 0.5N H_2SO_4. Do not allow the column to go dry.

    c. When the acid reaches the top of the column bed, load the aqueous sample (I.18.) onto the column.

    d. Dry the column with air by pulling a vacuum through the columns for 30 minutes.

    e. Elute the fluroxypyr and 3,5-dichloropyridinol from the column with 10 mL of 30:70 ethyl ether:hexane into a 16 x 100 mm tube.

Methoxypyridine Analysis

22. Evaporate the MTBE (I.20.d.) at room temperature using a TurboVap, leaving 0.5 mL of decane. Record the mass to 0.01 g. This is the weight of the decane volume, DW, used in I.23.

23. Determine final volume of the decane solution using the following equation (see Note P.3.):

$$\text{methoxypyridine decane volume (MDV)(mL)} = \frac{(DW - DT)}{\text{density of decane}}$$

    Density of decane 0.7300 g/mL

24. Transfer the solution to a 2-mL autosampler vial. Seal the vial with a cap.

25. Analyze the sample for methoxypyridine by capillary GC/MSD.

3,5-Dichloropyridinol Analysis

26. Transfer the 30:70 ethyl ether:hexane solution (L21.e.) to a tared, graduated cylinder and adjust the volume to 20 mL with MTBE. Record the mass to 0.01 g (see Note P.3.). This is the MTBE added, MA, value used in calculations (N.2.).

27. Record the weight to 0.01 g (see Note P.3.) of a clean 16 x 100 mm tube. This is the 3,5-dichloropyridinol tube tare, PTT.

28. Transfer 10 mL of MTBE to the tared tube for 3,5-dichloropyridinol analysis. Record the mass to 0.01 g (see Note P.3.). The difference between this value and PTT is the weight of the MTBE removed for 3,5-dichloropyridinol analysis, MRP, used in calculations (N.2.).

29. To the tube from I.28., add 0.5 mL of decane using a volumetric pipette.

30. Evaporate the solution at room temperature using a TurboVap (see Note P.7.), leaving 0.5 mL of decane. Record the mass to 0.01 g (see Note P.3.). This is the evaporated decane weight, ED, used below. Determine final volume of the decane solution using the following equation (see Note P.3.):

$$\text{3,5-dichloropyridinol decane volume (PDV)(mL)} = \frac{\text{(ED-PTT)}}{\text{density of decane}}$$

Density of decane = 0.7300 g/mL

31. Transfer the solution to a 2-mL autosampler vial. Using a microliter syringe add 5 microliters of BSA. Shake and seal the vial with a cap (Note P.10.).

32. Analyze the sample for the trimethylsilyl derivative of the 3,5-dichloropyridinol by capillary GC/MSD.

Fluroxypyr Analysis

33. Transfer the remaining MTBE from I.26. to a clean, tared 16 x 100 mm tube for fluroxypyr analysis. Record the mass to 0.01 g (see Note P.3.). This is the MTBE removed for fluroxpyr analysis, MRF, used in calculations (N.2.).

34. Add approximately 1 mL of acetone to the tube from I.33.

35. Evaporate the solution at room temperature using a TurboVap (see Note P.7.) until approximately 1 mL remains. Add 1 mL of acetone and evaporate until approximately 1 mL remains.

36. Using a microliter syringe add 10 microliters of 0.01M H_3PO_4.

37. Using a microliter syringe add 50 microliters of trimethylsilyl-diazomethane.

38. Shake the solution to mix. A stable, persistent yellow color should form. If no yellow is observed or if the solution is lighter in a shade of yellow add the trimethlsilyl diazomethane in 10-µL quantities until the persistent yellow color is developed.

39. Allow the solution to stand at room temperature for no less than 20 minutes.

40. Add 5 mL of 0.5N H_2SO_4 to the acetone.

41. Evaporate the acetone out of the solution using a TurboVap (see Note P.7.) until approximately 5 mL of solution remains.

42. Using a volumetic pipette add 0.5 mL of toluene and sonicate for 30 seconds.

43. Hand vortex the solution at maximum vortex speed for 30 seconds. It is important to use a pulsed technique of on for three seconds and off for one second.

44. Remove a portion of the toluene layer and analyze for fluroxypyr methyl ester by GC/MSD.

J. Determination and Interpretation of Confirmation Ratio:

The mass selective detector (MSD) works on the principle that the molecule of interest is fragmented by an electron beam. The mass spectrum that is generated from this process is a consistent and unique identifying mechanism for qualitative and quantitative determination of the molecule of interest. It is also accepted that the ratios of the spectral peaks in a mass spectrum occur at consistent and discrete ratios to one another. The fact that molecules fragment in a consistent and reproducible fashion allows for the calculation of compound-specific confirmation ratios. The confirmation ratio of a compound is calculated by dividing the peak areas for two mass ions collected in an analysis. The confirmation ratio should remain constant within experimental error for an analytical set. If an analysis yields a peak where the confirmation ratio is within the acceptable range of the confirmation ratio of the analytical standards then the peak is considered to be "confirmed."

Using the data from the method validation the average confirmation ratios for fluroxypyr methyl ester, methoxypyridine and 3,5-dichloropyridinol are:

Analyte	Average CR	+15%	-15%
Fluroxypyr methyl ester	0.4292	0.4936	0.3648
Methoxypyridine	1.2854	1.4782	1.0926
3,5-Dichloropyridinol	0.6677	0.7679	0.5675

Samples that have a CR in the range of ± 15% of the average CR of the calibration standards are reported as the analyte.

K. Calculation of Percent Recovery of Analytes:

1. Inject the calibration standards described in G.3. and determine the peak areas at *m/z* 209 and *m/z* 268 for fluroxypyr methyl ester, *m/z* 209 and *m/z* 210 for methoxypyridine and *m/z* 253 and *m/z* 255 for 3,5-dichloropyridinol.

   For each standard calculate each analyte's confirmation ratio (CR). Average the CRs generated for the calibration standards in an analytical set for each analyte. The average confirmation ratios will be used to confirm the presence of residues in the samples. Analyte-specific confirmation ratios are calculated using the following equations:

$$\text{Fluroxypyr methyl ester CR} = \frac{\text{peak area at } m/z\ 268}{\text{peak area at } m/z\ 209}$$

$$\text{Methoxypyridine CR} = \frac{\text{peak area at } m/z\ 210}{\text{peak area at } m/z\ 209}$$

$$\text{3,5-Dichloropyridinol CR} = \frac{\text{peak area at } m/z\ 255}{\text{peak area at } m/z\ 253}$$

For soil analyses analyte specific confirmation ratios are calculated using the above equations.

For example using the data for fluroxypyr methyl ester from Figure 12:

$$\text{Fluroxypyr methyl ester CR} = \frac{\text{peak area at } m/z \text{ 268}}{\text{peak area at } m/z \text{ 209}}$$

Fluroxypyr methyl ester CR = (peak area at *m/z* 268/peak area at *m/z* 209)

Fluroxypyr methyl ester CR = (112278/255643)

Fluroxypyr methyl ester CR = 0.439

The CR is within the acceptable confirmation range for fluroxypyr methyl ester indicating the presence of fluroxypyr residues.

Using data for methoxypyridine from Figure 20

$$\text{Methoxypyridine CR} = \frac{\text{peak area at } m/z \text{ 210}}{\text{peak area at } m/z \text{ 209}}$$

Methoxypyridine CR = (peak area at *m/z* 210/peak area at *m/z* 209)

Methoxypyridine CR = (315080/244248)

Methoxypyridine CR = 1.29

The CR is within the acceptable confirmation range for methoxypyridine indicating the presence of methoxypyridine residues.

Using the data for 3,5-dichloropyridinol from Figure 28:

$$\text{3,5-Dichloropyridinol CR} = \frac{\text{peak area at } m/z \text{ 255}}{\text{peak area at } m/z \text{ 253}}$$

3,5-Dichloropyridinol CR = (peak area at *m/z* 255/peak area at *m/z* 253)

3,5-Dichloropyridinol CR = (4573348/6675759)

3,5-Dichloropyridinol CR = 0.685

The CR is within the acceptable confirmation range for 3,5-dichloropyridinol indicating the presence of 3,5-dichloropyridinol residues.

2. Determine the analyte concentrations for the recovery samples using log-linear transform, N.1., as follows:

$$\text{peak area} = \text{constant} \times (\text{concentration})^{(\text{exponent})}$$

$$\text{concentration} = (\text{peak area}/\text{constant})^{1/\text{exponent}}$$

For example, using the data for methoxypyridine, *m/z* 210, from Figure 20:

Methoxypyridine concentration

Methoxypyridine µg/mL = (*m/z* 210 peak area/constant)$^{1/exponent}$ (µg/mL)

Methoxypyridine Conc. (µg/mL) (*m/z* 210) = (315080/4797418)$^{(1/1.045)}$

Methoxypyridine Conc. (µg/mL) (*m/z* 210) = 0.073 µg/mL

3. Correct the analyte concentration by applying the appropriate sample preparation correction factor (N.2.).

   For example, using the data for methoxypyridine for the sample from Figure 20:

   Methoxypyridine Conc. (µg/mL) = (0.073)µg/mL * (0.121)mL/g (N.2.f)

   Methoxypyridine Conc. (µg/mL) = 0.0088 µg/g

4. Determine the net concentration of each analyte in the recovery samples by subtracting the average concentration of each analyte in the control samples from the concentrations determined for the recovery samples.

   Net Conc. = Sample Conc. - Control Conc.

   For example, using the data for methoxypyridine for the sample from Figure 20:

   Net Conc. = 0.0088 - 0.0000

   Net Conc. = 0.0088

5. Determine the percent recovery for each analyte fortified into the recovery samples by dividing the net concentration by the fortification concentration of the analyte in the recovery sample as follows:

   % Recovery = (Net Concentration/Fortified Concentration) x 100

   For example, using the data for methoxypyridine for the sample from Figure 20:

   % Recovery = (0.0088 µg/g Recovered/0.010 µg/g Added) * 100

   % Recovery = 88

6. Determine the average percent recovery for each analyte by averaging the percent recovery for all of the fortified samples.

L. Determination of Fluroxypyr, Methoxypyridine and 3,5-dichloropyridinol in Soil Samples:

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

2. Calculate log-linear transform regression coefficients from the peak areas measured for the analytes in the calibration standards as described in N.1.

3. Determine the average percent recovery for the sample set for fluroxypyr, methoxypyridine and 3,5-dichloropyridinol in the recovery samples as described in K.

4. Inject the sample solutions and determine the peak areas at *m/z* 209 and *m/z* 268 for fluroxypyr methyl ester, *m/z* 209 and *m/z* 210 for methoxypyridine and *m/z* 253 and *m/z* 255 for 3,5-dichloropyridinol.

5. Determine the µg/mL concentrations of fluroxypyr, methoxypyridine and 3,5-dichloropyridinol in each treated sample by substituting the *m/z* 268, *m/z* 210, and *m/z* 253 peak areas, respectively, into the log-linear transform regression equation using the coefficients generated as described in N.1.

6. Determine the µg/g concentration of fluroxypyr, methoxypyridine and 3,5-dichloropyridinol in each treated sample by applying the appropriate analyte specific method factor (N.2.).

7. Determine the percent soil moisture as described in Section M.

8. Determine the dry-weight concentration of the analytes in soil samples as follows:

$$\text{Soil Concentration } (\mu g/g) = \frac{\mu g/g \text{ Found}}{\% \text{ Recovery} * 100} \times (1+(\%\text{Moisture} * 100)$$

M. Determination of Soil Moistures:

1. Accurately weigh a 10-g portion of soil into a tared weighing dish.

2. Place the sample in an oven at 110 °C and allow to dry for a minimum of 15 to 16 hours.

3. Remove the sample from the oven, place in a desiccator until the sample has cooled to room temperature, and then re-weigh.

4. Calculate the percent moisture (dry weight basis) as follows:

Percent Moisture (dry weight basis):

$$\%\text{Moisture} = \frac{(\text{Sample Mass, before drying}) - (\text{Sample Mass, after drying})}{\text{Sample Mass, after drying}} * 100$$

N. Calculations:

1. Log-linear transform regression coefficients

Regression analysis can be accomplished by a variety of methods. It has been observed in our laboratories that one of the more valuable types is log-transform linear least squares or log-linear transform regression.

Calculate the log-linear transform regression coefficients using the concentrations and peak areas determined for the calibration standards using the following equation:

$$y = \text{constant} * x^{(\text{exponent})}$$

where:

$$\text{exponent} = \frac{N*\Sigma(\ln x*\ln y)-\Sigma(\ln x)*\Sigma(\ln y)}{N*\Sigma((\ln x)^2)-\Sigma(\ln x))^2}$$

$$\text{constant} = e^{(\Sigma(\ln y)-\text{exponent}*\Sigma(\ln x))}$$

x = concentration

y = peak area

N = number of data points

Calculate individual constants and exponents for fluroxypyr methyl ester, *m/z* 268; methoxypyridine, *m/z* 210; and 3,5-dichloropyridinol, *m/z* 253.

2. Method factors for fluroxypyr, methoxypyridine and 3,5-dichloropyridinol.

Method factors appear in the result calculations to compensate for variations in individual sample preparations. Method factors incorporate dilution and unit conversion factors. Eleven parameters are involved in calculating the method factors for fluroxypyr, methoxypyridine and 3,5-dichloropyridinol. These parameters are:

Extraction Specific
M Soil mass (I.1.)
EA extraction volume added (I.9.)
ER extraction volume recovered (I.11.)

Fluroxypyr Specific
MA MTBE Added (I.26.)
MRF MTBE removed for fluroxypyr analysis. (I.33.)
FTV Fluroxypyr toluene final volume (I.42.)

Methoxypyridine Specific
HA Hexane Added (I.14.)
HR Hexane Recovered (I.16.)
MDV Methoxypyridine decane final volume (I.23.)

3,5 dichloropyridinol Specific

MA MTBE added (I.26.)
MRP MTBE removed for 3,5 dichloropyridinol analysis (I.28.)
PDV 3,5 dichloropyridinol decane final volume (I.30.)

a. Extraction Volume Ratio (EVR)

The EVR is the ratio of the extraction volume added (EA) to extraction volume recovered (ER) for analysis of residues. Calculation of the ratio of the volume of extraction solution added to the extraction solution recovered is required because the total 40 mL extraction volume added is not recovered. Up to 5 mL may be absorbed depending upon soil characteristics.

Calculate the EVR:

$$EVR = \frac{EA}{ER}$$

b. The fluroxypyr MTBE volume ratio (FMR)

The eluant from C_{18} SPE column (I.21.e.) contains the residues of fluroxypyr and 3,5-dichloropyridinol. An aliquot of the eluent is analyzyed for fluroxypyr methyl ester. The fluroxypyr MTBE volume ratio (FMR) is the ratio of MTBE added (MA) to MTBE removed for fluroxypyr methyl ester analysis (MRF).

Calculate FMR:

$$FMR = \frac{MA}{MRF}$$

c. Hexane Volume Ratio (HVR)

The HVR is the ratio of hexane added (HA) to hexane recovered (HR) for analysis of methoxypyridine. Calculation of the hexane added to hexane recovered ratio is required for two reasons. Because of absorption of hexane into the extraction solution, quantitative transfer of the hexane added is not possible. Also, while the volume of hexane removed is linearly correlated to the method sensitivity for methoxypyridine, the SPE cleanup is moisture sensitive. In order to minimize the possibility of aqueous contamination, quantitative transfer of the hexane extract is not recommended. In this laboratory 5 mL of the hexane extract was shown to be an adequate volume to achieve neccesary instrument sensitivity

Calculate the HVR:

$$HVR = \frac{HA}{HR}$$

d. 3,5-Dichloropyridinol MTBE volume ratio (PMR)

The eluant from C_{18} SPE column (I.21.e.) contains the residues of fluroxypyr and 3,5-dichloropyridinol. An aliquot of the eluent is analyzed for 3,5-dichloropyridinol. The PMR is the ratio of MTBE added (MA) to MTBE removed for 3,5-dichloropyridinol analysis (MRP).

Calculate PMR:

$$PMR = \frac{MA}{MRP}$$

e. The Fluroxypyr Method Factor (FMF) converts a µg/mL value of fluroxypyr methyl ester to a µg/g value and compensates for individual sample dilutions and sample preparation factors specific to the analysis of fluroxypyr methyl ester.

Calculate the FMF using the following equation:

$$FMF = \frac{FTV}{M} * EVR * FMR$$

where:

EVR = Extraction Volume Ratio (N.2.a.)
FMR = Fluroxypyr MTBE volume Ratio (N.2.b.)

f. The Methoxypyridine Method Factor (MMF) converts a µg/mL value of methoxypyridine to a µg/g value and compensates for individual sample dilutions and sample preparation factors specific to the analysis of methoxypyridine.

Calculate the MMF using the following equation:

$$MMF = \frac{MDV}{M} * EVR * HVR$$

where:

EVR = Extraction Volume Ratio (N.2.a.)
HVR = Hexane Volume Ratio (N.2.c.)

g. The 3,5-Dichloropyridinol Method Factor (PMF) converts a µg/mL value of 3,5-dichloropyridinol to a µg/g value and compensates for individual sample dilutions and sample preparation factors specific to the analysis of 3,5-dichloropyridinol.
Calculate the PMF using the following equation:

$$PMF = \frac{PDV}{M} * EVR * PMR$$

where:

EVR = Extraction Volume Ratio (N.2.a.)
PMR =3,5-Dichloropyridinol MTBE Volume Ratio (N.2.d.)

h. An example of these calculations for methoxypyridine from Figure 20

The values for the analysis are:

M	Soil mass (I.1.)	5.00 g
EA	extraction volume added (I.9.)	33.07 g
ER	extraction volume recovered (I.11.)	31.33 g
	Methoxypyridine Specific	
HA	Hexane Added (I.14.)	6.9891 g
HR	Hexane Recovered (I.16.)	6.0427 g
MDV	Methoxypyridine decane final volume (I.23.)	0.5 mL

Calculate the EVR:

$$EVR = \frac{EA}{ER}$$

$$EVR = \frac{(33.07\ g)}{(31.33\ g)}$$

$$EVR = 1.055$$

Calculate the HVR:

$$HVR = \frac{HA}{HR}$$

$$HVR = \frac{(6.9891\ g)}{(6.0427\ g)}$$

$$HVR = 1.1566$$

Calculate the MMF:

$$MMF = \left(\frac{MDV}{M}\right) * EVR * HVR$$

$$MMF = \left(\frac{(0.5\ mL)}{(5.00\ g)}\right) * 1.055 * 1.1566$$

$$MMF = 0.121\ mL/g$$

P. Notes:

1. The equipment listed was used to validate the method. It is the recommended equipment for routine operation, but equivalent equipment may be substituted. It is the responsibility of the analyst to ensure that such equipment generates reliable data.

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

3. Gravimetric methods improve the accuracy and precision of the method. Volumetric calculations may be substituted; however, it is the responsibility of the analyst to ensure that the volumes are accurate and the calculations are correct.

4. Pulsed vortexing may be substituted for shaking. The sample should be vortexed at a speed sufficient to suspend the sample in the extract solution for approximately three seconds and and then removed from the vortex for approximately one second. This cycle should be repeated for a minimum of two minutes.

5 Depending on the centrifuge, this time may vary from 30 seconds to several minutes.

6. Calculation of the ratio of the volume of extraction solution added to the extraction solution recovered is required because the total 40-mL extraction volume added is not recovered. Up to 5 mL may be absorbed depending upon soil characteristics.

7. As the volume decreases, the nitrogen pressure should be increased to maintain even evaporation.

8. Solvents were passed through the SPE manifold using a regulated vacuum to achieve a flow of approximately 2-3 mL/min.

9. Variations in the C_{18} columns may influence the elution profile of fluroxypyr and 3,5-dichloropyridinol. If low recoveries are encountered it may be necessary to obtain an elution profile for both fluroxypyr and 3,5-dichloro-pyridinol to ensure that optimum clean-up is occuring.

   An elution profile can be generated in the following manner:

   a. Using a 100-µL syringe, add 20 µL of 5 µg/mL fluroxypyr or 3,5-dichloropyridinol solution to a 16 x 100 mm test tube filled with 10 mL of 0.5 N H_2SO_4 solution.

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

   c. Rinse the column with 5 mL of methanol.

   d. Rinse the column with 5 mL of 0.5 N H_2SO_4.

   e. Transfer the solution from step a. above to the column and with aid of the vacuum slowly pull through the column.

   f. Dry the C_{18} column by drawing air through the column for at least 30 minutes.

   g. Elute the fluroxypyr or 3,5-dichloropyridinol with 10 mL of 70:30 hexane:ethyl ether, collecting 1 mL aliquots in 16 x 100 mm test tubes.

h. For each fraction collected continue the preparation as described in I.34. for fluroxypyr and I.29. for 3,5-dichloropyridinol.

Variations in the silica columns may influence the elution profile of methoxypyridine. If low recoveries are encountered it may be necessary to obtain an elution profile for methoxypyridine to ensure that optimum clean-up is occuring.

An elution profile can be generated in the following manner:

a. Using a 100-µL syringe, add 20 µL of of the 5 µg/mL methoxypyridine solution to a 16 x 100 mm test tube filled with 10 mL of 0.5 N H_2SO_4 solution.

b. Place a silica column on the vacuum manifold box.

c. Rinse the column with 10 mL of hexane.

d. Transfer the solution from step a. above to the column and with aid of the vacuum slowly pull through the column.

e. Elute the methoxypyridine with 10 mL of MTBE, collecting 1 mL aliquots in 16 x 100 mm test tubes.

f. To each fraction collected add 0.5 mL of decane and continue the preparation as described in I.22. for methoxypyridine.

10. The calibration standards for 3,5-dichloropyridinol must also be derivatized with BSA prior to analysis. The silylated derivative is stable in a non-aqueous environment. Stability is reduced in the presence of water.

11. Lehmann, R.G., "Extraction of Fluroxypyr and its Metabolites From Aged Soil," GH-C 2048, 1988, unpublished report of DowElanco.