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Determination of Residues of Triclopyr, 3,5,6-Trichloro-2-pyridinol, and 2-Methoxy-3,5,6-trichloropyridine in Sediment and Soil by Capillary Gas Chromatography with Mass Selective Detection

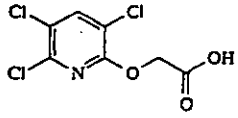
E. L. Olberding and D. R. Foster
North American Environmental Chemistry Laboratory
DowElanco
Indianapolis, Indiana 46268-1053

and

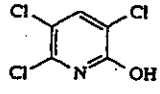
D. A. McNett
Health and Environmental Sciences Laboratory
The Dow Chemical Company
Midland, Michigan 48674

A. Scope

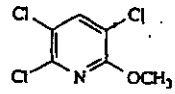
This method is applicable for the quantitative determination of residues of triclopyr (((3,5,6-trichloro-2-pyridinyl)oxy)acetic acid) and its metabolites, 3,5,6-trichloro-2-pyridinol (3,5,6-TCP), and 2-methoxy-3,5,6-trichloropyridine (2-MP) in sediment and soil over the concentration range 0.01-1.0 µg/g with a validated limit of quantitation of 0.01 µg/g.



Triclopyr
CAS No. 55335-06-3



3,5,6-TCP
CAS No. 6515-38-4



2-MP
CAS No. 31557-34-3

B. Principle

Residues of triclopyr, 3,5,6-TCP, and 2-MP are extracted from soil using a 90% acetone/10% 1.0 N hydrochloric acid solution.

For the determination of triclopyr and 3,5,6-TCP, a portion of the acetone/hydrochloric acid extract is concentrated to remove the acetone. Following evaporation of the acetone, the sample is diluted with 0.1 N hydrochloric acid and purified using a C_{18} solid-phase

extraction (SPE). The eluate from the C_{18} SPE is extracted with 1-chlorobutane, concentrated to less than 5 mL, and then combined with the eluate from the silica gel SPE from the 2-MP purification.

For the determination of 2-MP, a portion of the acetone/hydrochloric acid extract is diluted with water, basified, and then extracted with hexane. The hexane extract is purified using a silica gel SPE. The hexane eluate from the SPE is concentrated to approximately 5 mL, and then combined with the 1-chlorobutane from the triclopyr and 3,5,6-TCP purification.

The 1-chlorobutane/hexane mixture is concentrated to less than 1 mL, and an acetone solution containing fluroxypyr analogs as internal standards is added. The sample is then derivatized with *N*-methyl-*N*-(*tert*-butyldimethylsilyl)-trifluoroacetamide (MTBSTFA) to form the *tert*-butyldimethylsilyl (TBDMS) derivatives of triclopyr and 3,5,6-TCP. The sample is then analyzed by capillary gas chromatography with mass selective detection (GC/MSD).

C. Safety Precautions

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

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

3. 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.

D. Equipment (Note N.1.)

1. Balance, analytical, Model AE200, Mettler Instrument Corporation, Hightstown, NJ 08520.

2. Balance, pan, Model BB2440, Mettler Instrument Corporation.

3. Centrifuge, with rotor to accommodate 12-, 16-, and 40-mL vials, Model Centra-8, International Equipment Company, Needham Heights, MA 02194.

4. Desiccator, 250-mm i.d., catalog number 08-595E, Fisher Scientific, Pittsburgh, PA 15219.

5. Evaporator, N-Evap, Model 111, Organomation Associates, Inc., South Berlin, MA 01549. (Note N.2.)

6. Gas chromatograph, Model 5890A Series II, Hewlett-Packard, Wilmington, DE 19808.

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

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

9. Mass selective detector data system, Model G1034C, Hewlett-Packard.

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

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

12. Ultrasonic cleaner, Model 1200, Branson Ultrasonics Corporation, Danbury, CT 06813.

13. Vacuum manifold, Model spe-12G, J. T. Baker Chemical Company, Phillipsburg, NJ 08865.

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

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

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

1. Column, capillary gas chromatography, Durabond-1701 liquid phase, 10 m x 0.18 mm i.d., 0.4-µm film thickness, catalog number 121-0713, J & W Scientific, Folsom, CA 95630.

2. Column, capillary guard, deactivated, 5 m x 0.53 mm i.d., catalog number 10045, Restek Corporation, Bellefonte, PA 16823.

3. Column, C_{18} SPE, catalog number 7020-07, J. T. Baker Chemical Company.

4. Column, silica gel SPE, catalog number 7086-03, J. T. Baker Chemical Company.

5. Column connector, Press-Tight capillary, catalog number 20446, Restek Corporation.

6. Cylinder, graduated mixing, 50-mL, catalog number 20036-50, Kimble/Kontes, Vineland, NJ 08360.

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

8. Dessicant, Drierite adsorbent, catalog number 24001, W. A. Hammond Drierite Company, Xenia, OH 45385.

9. Dish, aluminum weighing, catalog number 08-732, Fisher Scientific.

10. Filter, charcoal, catalog number 7972, Chrompack, Inc., Raritan, NJ 08869. (Note N.3.)

11. Filter, moisture, catalog number 7971, Chrompack, Inc. (Note N.3.)

12. Filter, oxygen, catalog number 7970, Chrompack, Inc. (Note N.3.)

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

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

15. Flask, volumetric, 2000-mL, catalog number 161-8993, National Scientific Company.
16. Gas, helium, 99.995% purity, Airco, Murray Hill, NJ 07974.
17. Gas, nitrogen, 99.99% purity, Airco.
18. Inlet sleeve, double gooseneck splitless, catalog number 20784, Restek Corporation.
19. Pipet, volumetric, 1.0-mL, catalog number 261-6011, National Scientific Company.
20. Pipet, volumetric, 2.0-mL, catalog number 261-6012, National Scientific Company.
21. Pipet, volumetric, 2.5-mL, catalog number 261-6084, National Scientific Company.
22. Pipet, volumetric, 3.0-mL, catalog number 261-6013, National Scientific Company.
23. Pipet, volumetric, 4.0-mL, catalog number 261-6014, National Scientific Company.
24. Pipet, volumetric, 5.0-mL, catalog number 261-6015, National Scientific Company.
25. Pipet, volumetric, 8.0-mL, catalog number 261-6018, National Scientific Company.
26. Pipet, volumetric, 10-mL, catalog number 261-6020, National Scientific Company.
27. Pipet, volumetric, 15-mL, catalog number 261-6025, National Scientific Company.
28. Pipet, volumetric, 20-mL, catalog number 261-6030, National Scientific Company.
29. Pipet, volumetric, 25-mL, catalog number 261-6035, National Scientific Company.
30. Pipet, volumetric, 200-mL, catalog number 261-6070, National Scientific Company.
31. Syringe, 50-µL, Model 705N, Hamilton Company, Reno, NV 89520.
32. Syringe, 100-µL, Model 710N, Hamilton Company.
33. Syringe, 250-µL, Model 725N, Hamilton Company.
34. Syringe, 500-µL, Model 750N, Hamilton Company.
35. Vial, 12-mL, with PTFE-lined screw cap, catalog number B7800-12, National Scientific Company.
36. Vial, 16-mL, with PTFE-lined screw cap, catalog number B7800-3, National Scientific Company.
37. Vial, 40-mL, with PTFE-lined screw cap, catalog number B7800-6, National Scientific Company.
38. Vial, autosampler, 2-mL, catalog number C4000-1, National Scientific Company.
39. Vial cap, for autosampler vial, catalog number C4000-54B, National Scientific Company.

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

1. Reagents

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

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

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

d. Hexane, OmniSolv grade, catalog number HX0295-1, EM Science.

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

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

g. MTBSTFA (*N*-methyl-*N*-(*tert*-butyldimethylsilyl)-trifluoroacetamide), catalog number 48920, Pierce Chemical Company, Rockford, IL 61105.

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

i. Sodium hydroxide, 2.5 N, ACS reagent grade, certified concentration, catalog number SS414-1, Fisher Scientific.

j. Standards

(1) triclopyr (((3,5,6-trichloro-2-pyridinyl)oxy)acetic acid)

(2) 3,5,6-trichloro-2-pyridinol (3,5,6-TCP)

(3) 2-methoxy-3,5,6-trichloropyridine (2-MP)

(4) fluroxypyr (((4-amino-3,5,-dichloro-6-fluoro-2-pyridinyl)oxy)acetic acid)

(5) 4-amino-3,5-dichloro-6-fluoro-2-pyridinol (fluroxypyr-DCP)

(6) 4-amino-3,5-dichloro-6-fluoro-2-methoxypyridine (fluroxypyr-MP)

Obtain from Test Substance Coordinator, DowElanco, 9330 Zionsville Road, Building 306/A1, Indianapolis, IN 46268-1053.

2. Prepared Solutions

a. 90% acetone/10% 1.0 N hydrochloric acid solution (v/v).

Pipet 200 mL of 1.0 N hydrochloric acid into a 2000-mL volumetric flask containing approximately 1500 mL of acetone. Swirl the flask, and allow to equilibrate to room temperature. Dilute to volume with acetone.

b. 80% acetonitrile/19% water/1% 1.0 N hydrochloric acid solution (v/v/v).

Pour 800 mL of acetonitrile into a 1000-mL graduated mixing cylinder. Pipet 10.0 mL of 1.0 N hydrochloric acid into the same cylinder; then add approximately 150 mL of water. Swirl the cylinder, and allow to equilibrate to room temperature. Dilute to volume with water.

c. 40% acetonitrile/59% water/1% 1.0 N hydrochloric acid solution (v/v/v).

Pour 400 mL of acetonitrile into a 1000-mL graduated mixing cylinder. Pipet 10.0 mL of 1.0 N hydrochloric acid into the same cylinder; then add approximately 500 mL of water. Swirl the cylinder, and allow to equilibrate to room temperature. Dilute to volume with water.

G. Preparation of Standards

1. Preparation of Spiking Solutions/Calibration Standards

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

b. Weigh 0.1000 g of 3,5,6-trichloro-2-pyridinol analytical standard and quantitatively transfer to a 100-mL volumetric flask. Dilute to volume with acetone to obtain a 1000-µg/mL stock solution.

c. Weigh 0.1000 g of 2-methoxy-3,5,6-trichloropyridine analytical standard and quantitatively transfer to a 100-mL volumetric flask. Dilute to volume with acetone to obtain a 1000-µg/mL stock solution.

d. Pipet 20.0 mL of each of the stock solutions in Sections G.1.a.-c. into a single 200-mL volumetric flask and adjust to volume with acetone to obtain a solution containing 100.0 µg/mL of each compound.

e. Prepare solutions for spiking soil samples by diluting the solution from Section G.1.d with acetone as follows:

Aliquot of Initial Soln.	Final Soln. Volume	Spiking Soln. Final Conc.	Equivalent Sample Conc.[a]
mL	mL	µg/mL	µg/g
0.050	200	0.025	0.005
0.100	200	0.050	0.010
0.250	200	0.125	0.025
0.500	200	0.250	0.050
1.00	200	0.500	0.100
2.50	200	1.25	0.250
5.00	200	2.50	0.500
10.00	200	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.

f. Prepare calibration standards by dispensing 200 µL of the solutions from Section G.1.e. into 12-mL vials containing 0.5 mL of 1-chlorobutane and derivatizing according to the procedure described in Section I.1.gg.-kk. The concentration range of these calibration standards is from 0.005-1.0 µg/mL.

Chemical structures of the underivatized and derivatized triclopyr, 3,5,6-TCP, and 2-MP are shown in Figure 1.

2. Preparation of Internal Standard Solution

a. 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.

b. Weigh 0.1000 g of 4-amino-3,5-dichloro-6-fluoro-2-pyridinol analytical standard and quantitatively transfer to a 100-mL volumetric flask. Dilute to volume with acetone to obtain a 1000-µg/mL stock solution.

c. Weigh 0.1000 g of 4-amino-3,5-dichloro-6-fluoro-2-methoxypyridine analytical standard and quantitatively transfer to a 100-mL volumetric flask. Dilute to volume with acetone to obtain a 1000-µg/mL stock solution.

d. Pipet 2.5 mL of each of the stock solutions in Sections G.2.a.-c. into a single 200-mL volumetric flask and adjust to volume with acetone to obtain a solution containing 12.5 µg/mL of each compound.

Chemical structures of the underivatized and derivatized fluroxypyr, fluroxypyr-DCP, and fluroxypyr-MP are shown in Figure 2.

H. Gas Chromatography/Mass Spectrometry

1. Column

Connect the guard column (Section E.2.) to the capillary column (Section E.1.) using a Press-Tight column connector (Section E.5.). Install the splitless column inlet sleeve (Section E.18.) and capillary column assembly in the split/splitless injection port of the GC/MSD following the manufacturer's recommended procedures.

2. Typical Operating Conditions

Instrumentation:	Hewlett-Packard Model 5890A gas chromatograph Hewlett-Packard Model 7673 automatic injector Hewlett-Packard Model 5971A mass selective detector Hewlett-Packard Model G1034C data system software
Columns:	
Guard	Restek fused silica capillary 5 m x 0.53 mm i.d. deactivated

Analytical	J & W Scientific fused silica capillary Durabond-1701 liquid phase 10 m x 0.18 mm i.d. 0.4-µm film thickness
Temperatures:	
Column	60 °C for 1.0 min 60 °C to 255 °C at 10 °C/min 255 °C to 290 °C at 20 °C/min 290 °C for 2.75 min
Injector Interface	260 °C 280 °C
Carrier Gas:	helium
Head Pressure Linear Velocity	50 kPa approximately 25 cm/s
Injection Mode:	splitless
Purge Delay Splitter Flow Septum Purge	0.9 min 50 mL/min 1.0 mL/min
Injection Volume:	2 µL
Detector:	electron impact selected ion monitoring
Calibration Program Electron Multiplier	maximum sensitivity autotune (Note N.4.) 1775 volts (= 280 volts above autotune)
Ions Monitored:	
Triclopyr-TBDMS	*m/z* 312 (quantitation) *m/z* 254, 256, 314 (confirmation) (Section M.2.)
3,5,6-TCP-TBDMS	*m/z* 254 (quantitation) *m/z* 256 (confirmation)
2-MP	*m/z* 211 (quantitation) *m/z* 182, 210, 212, 213 (confirmation) (Section M.2.)
Fluroxypyr-TBDMS Fluroxypyr-DCP-TBDMS Fluroxypyr-MP	*m/z* 311 (internal standard for triclopyr-TBDMS) *m/z* 253 (internal standard for 3,5,6-TCP-TBDMS) *m/z* 210 (internal standard for 2-MP)
Dwell Time:	75 ms

Mass spectra of the above triclopyr and fluroxypyr compounds are shown in Figures 3-8, respectively.

3. Calibration Curves

Typical calibration curves for the determination of triclopyr, 3,5,6-TCP, and 2-MP are shown in Figures 9-11, respectively.

4. Typical Chromatograms

Typical chromatograms of a standard, control sample, and a 0.010-µg/g recovery sample for the determination of triclopyr, 3,5,6-TCP, and 2-MP in sediment are illustrated in Figures 12-20, respectively. None of the control samples in the method validation study contained interference peaks at the retention times of the analytes or internal standards.

I. Determination of Recovery of Triclopyr and Metabolites from Sediment and Soil

1. Preparation of Recovery Samples

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

b. For preparing fortified samples, use some of the samples as controls and fortify the remaining samples by adding 1.0-mL aliquots of the appropriate spiking solutions (Section G.1.e.) in acetone to obtain concentrations ranging from 0.005 to 1.0 µg/g. A reagent blank, containing no soil, should be carried through the method with the samples.

c. Add 25 mL of the 90% acetone/10% 1.0 N hydrochloric acid extraction solution to the vial.

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

e. Shake the sample for a minimum of 2 hours on a reciprocating shaker at approximately 180 excursions/minute.

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

g. Transfer the acetone/hydrochloric acid solution into a clean 50-mL graduated mixing cylinder.

h. Repeat Steps I.1.c.-f. with 15 mL of the 90% acetone/10% 1.0 N hydrochloric acid extraction solution and a 30-minute shaking time.

i. Combine the acetone/hydrochloric acid solution from Step I.1.h. with the 25 mL from Step I.1.g. and adjust to 40.0 mL with additional extraction solution.

2-Methoxy-3,5,6-trichloropyridine

j. Transfer an 8.0-mL portion of the acetone/hydrochloric acid solution from Step I.1.i. into a clean 40-mL vial.

k. Add 10 mL of distilled/deionized water and 1.0 mL of 2.5 N sodium hydroxide to the sample vial. Cap the vial with a PTFE-lined cap, and vortex the sample for 10-15 seconds.

l. Add 5.0 mL of hexane to the sample vial. Cap the vial, and shake the sample for 20 minutes on a reciprocating shaker at approximately 180 excursions/minute.

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

n. Transfer the hexane (top) layer into a clean 12-mL vial.

o. Add an additional 5.0 mL of hexane to the sample vial. Cap the vial, and shake the sample for 20 minutes on a reciprocating shaker at approximately 180 excursions/minute.

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

q. Combine the hexane layer from Step I.1.p. with the hexane extract from Step I.1.n. and mix thoroughly.

r. Purify the sample using the following silica gel SPE procedure (Section M.4.a.):

(1) Place a silica gel SPE column on the vacuum manifold.

(2) Rinse the SPE column with 2.5 mL of 1-chlorobutane. (Do not allow the column bed to dry.)

(3) Condition the SPE column with 2.5 mL of hexane. (Do not allow the column bed to dry.)

(4) Place a 16-mL vial in the vacuum manifold to collect the eluant in the following step.

(5) Transfer the sample solution from Step I.1.q. to the SPE column, and slowly pull the sample through the column with the aid of vacuum. Collect the eluate in the 16-mL vial. (Do not allow the column bed to dry.)

(6) Rinse the sample vial with 4 mL of hexane, and when the sample solution in Step I.1.r.(5) is within 2 mm of the top of the column bed, transfer the rinse to the SPE column. Slowly pull the rinse solution through the column with the aid of vacuum, collecting the eluate in the same 16-mL vial.

s. Concentrate the hexane from Step I.1.r.(6) to approximately 5 mL using an N-Evap evaporator. (This extract, containing the 2-MP, will be further treated as described in Step I.1.ff.)

Triclopyr and 3,5,6-Trichloropyridinol

t. Transfer an 8.0-mL portion of the acetone/hydrochloric acid solution from Step I.1.i. into a clean 16-mL vial.

u. Concentrate the solution from Step I.1.t. to less than 2 mL (but not to dryness) using an N-Evap evaporator. (Note N.2.)

v. Add 15 mL of 0.1 N hydrochloric acid to the sample vial. Cap the vial, and sonicate the sample for 10-15 seconds.

w. Purify the sample using the following C_{18} SPE procedure (Section M.4.b.):

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

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

(3) Condition the SPE column with 5 mL of 0.1 N hydrochloric acid. (Do not allow the column bed to dry.)

(4) Transfer the sample solution from Step I.1.v. to the SPE column, and slowly pull the sample through the column with the aid of vacuum. (Do not allow the column bed to dry.)

(5) Rinse the sample vial with 2 mL of 0.1 N hydrochloric acid, and transfer the rinse to the SPE column. Slowly pull the rinse solution through the column with the aid of vacuum.

(6) Dry the SPE column under vacuum for 1 minute.

(7) Rinse the SPE column with 4.0 mL of a 40% acetonitrile/59% water/1% 1.0 N hydrochloric acid solution, discarding the eluate.

(8) Elute the triclopyr and 3,5,6-TCP with 3.0 mL of an 80% acetonitrile/19% water/1% 1.0 N hydrochloric acid solution, collecting the eluate in a 40-mL vial.

x. Add 10 mL of 0.1 N hydrochloric acid, 5 g of sodium chloride (enough to saturate the solution), and 5.0 mL of 1-chlorobutane to the sample vial.

y. Cap the vial with a PTFE-lined cap, and shake the sample for 30 minutes on a reciprocating shaker at approximately 180 excursions/minute.

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

aa. Transfer the 1-chlorobutane (top) layer into a clean 12-mL vial. (Note N.5.)

bb. Add an additional 5.0 mL of 1-chlorobutane to the sample vial. Cap the vial, and shake the sample for 30 minutes on a reciprocating shaker at approximately 180 excursions/minute.

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

dd. Combine the 1-chlorobutane layer from Step I.1.cc. with the 1-chlorobutane extract from Step I.1.aa. and mix thoroughly. (Note N.5.)

ee. Concentrate the solution from Step I.1.dd. to approximately 5 mL using an N-Evap evaporator.

ff. Transfer the hexane solution from Step I.1.s. to the above vial containing the 1-chlorobutane extract. Continue concentrating the solution to less than 0.8 mL (but not to dryness) using an N-Evap evaporator. (Note N.2.)

gg. Add 100 µL of the internal standard solution (Section G.2.d.) and 100 µL of MTBSTFA derivatizing reagent to the sample vial.

hh. Adjust the volume in the sample vial to 1.0 mL with 1-chlorobutane and firmly seal with a PTFE-lined cap. Vortex the sample for 5-10 seconds, and then sonicate the sample for 5-10 seconds.

ii. Place the sample vial in an oven set at 60 °C and allow the mixture to react for 60 minutes.

jj. Remove the sample vial from the oven and allow the reaction mixture to cool to room temperature.

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

ll. Analyze the calibration standards from Section G.1.f. and samples by capillary gas chromatography/mass spectrometry as described in Section H.2. Determine the suitability of the chromatographic system using the following performance criteria:

(1) Standard curve linearity: Determine that the correlation coefficient equals or exceeds 0.99 for the least squares equation which describes the detector response as a function of standard curve concentration. If power regression is used, the power exponent should be between 0.90-1.10.

(2) Peak resolution: Visually determine that sufficient resolution has been achieved for the analytes and internal standards relative to background interferences.

(3) Appearance of chromatograms: Visually determine that the chromatograms resemble those shown in Figures 12-20 with respect to peak response, baseline noise, and background interference. Visually determine that a minimum signal-to-noise ratio of 5:1 has been attained for each analyte in the 0.01-µg/mL calibration standard (equivalent to 0.01 µg/g in soil samples).

2. Calculation of Percent Recovery

a. Inject the series of calibration standards described in Section G.1.f. and determine the peak areas for the analytes and internal standards as indicated below.

Triclopyr-TBDMS	*m/z* 312 (quantitation), *m/z* 256 (confirmation)
3,5,6-TCP-TBDMS	*m/z* 254 (quantitation), *m/z* 256 (confirmation)
2-MP	*m/z* 211 (quantitation), *m/z* 182 (confirmation)
Fluroxypyr-TBDMS	*m/z* 311 (internal standard for triclopyr-TBDMS)
Fluroxypyr-DCP-TBDMS	*m/z* 253 (internal standard for 3,5,6-TCP-TBDMS)
Fluroxypyr MP	*m/z* 210 (internal standard for 2-MP)

b. For each standard, calculate each analyte's confirmation ratio. Use the average confirmation ratio for each analyte to confirm the presence of the analyte in the soil samples.

For example, using the data for triclopyr from Figure 12:

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

$$\text{Confirmation Ratio} = \frac{\text{peak area at } m/z \text{ 256}}{\text{peak area at } m/z \text{ 312}}$$

$$\text{Confirmation Ratio} = \frac{2702}{1965}$$

$$\text{Confirmation Ratio} = 1.3751$$

Confirmation of the presence of the analyte is indicated when the confirmation ratio for the sample is within the range of ±15% of the average found for the standards.

c. For each standard, calculate each analyte's quantitation ratio.

For example, using the data for triclopyr from Figure 12:

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

$$\text{Quantitation Ratio} = \frac{\text{peak area at } m/z\ 312}{\text{peak area at } m/z\ 311}$$

$$\text{Quantitation Ratio} = \frac{1965}{241431}$$

$$\text{Quantitation Ratio} = 0.00814$$

d. Prepare a standard curve for each analyte by plotting the equivalent analyte concentration (as µg/g) on the abscissa (x-axis) and the respective quantitation ratio on the ordinate (y-axis) as shown in Figures 9-11. Using regression analysis, determine the equation for the curve with respect to the abscissa.

For example, using power regression (1) with the triclopyr data from Figure 9:

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

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

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

$$\text{Triclopyr Conc. } (\mu g/g) = \left(\frac{\text{triclopyr quantitation ratio}}{0.72423}\right)^{1/0.98471}$$

e. 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 triclopyr data from Figure 14:

$$\text{Triclopyr Conc. (gross } \mu g/g) = \left(\frac{\text{triclopyr quantitation ratio}}{0.72423}\right)^{1/0.98471}$$

$$\text{Triclopyr Conc. (gross µg/g)} = \left(\frac{0.00747}{0.72423}\right)^{1/0.98471}$$

$$\text{Triclopyr Conc.} = 0.0096\ \mu g/g$$

f. Determine the net concentration in each recovery sample by subtracting any apparent triclopyr concentration in the control sample from that of the gross triclopyr concentration in the recovery sample.

For example, using the triclopyr data from Figures 13 and 14:

$$\text{Triclopyr Conc. (net µg/g)} = \text{Triclopyr Conc. (gross µg/g)} - \text{Triclopyr Conc. (control µg/g)}$$

$$\text{Triclopyr Conc. (net µg/g)} = 0.0096\ \mu g/g - 0.0000\ \mu g/g$$

$$\text{Triclopyr Conc. (net)} = 0.0096\ \mu g/g$$

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

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

$$\text{Recovery} = \frac{0.0096\ \mu g/g}{0.0100\ \mu g/g} \times 100\%$$

$$\text{Recovery} = 96\%$$

J. Determination of Triclopyr and Metabolites in Sediment and Soil

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

2. Prepare a standard calibration curve for triclopyr, 3,5,6-TCP, and 2-MP, and determine the percent recovery for each analyte as described in Section I.2.

3. Determine the gross concentration of each analyte in each treated 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.e.

4. For those analyses that require correction for method recovery, use the average recovery of all the recovery samples to correct for method efficiency. The following procedure is used:

   a. Determine the gross analyte concentrations in the sample as described in Section I.2.e.

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

$$\text{Triclopyr Conc. (corrected µg/g)} = \text{Triclopyr Conc. (gross µg/g)} \times \left(\frac{100}{\text{Average Percent Recovery}}\right)$$

$$\text{Triclopyr Conc. (corrected µg/g)} = 0.0096\ \mu g/g \times \frac{100}{106}$$

$$\text{Triclopyr Conc. (corrected)} = 0.0091\ \mu g/g$$

K. Determination of Soil Moisture

1. Accurately weigh a 10-g portion of soil into a tared aluminum weighing dish.
2. Place the sample in an oven at 110 °C and allow to dry for a minimum of 16 hours.
3. Remove the sample from the oven and place in a dessicator containing Drierite adsorbent. Re-weigh the sample when it has cooled to room temperature.
4. Calculate the percent moisture (dry weight basis) as follows:

$$\text{Percent Moisture (dry weight basis)} = \frac{\text{water, g}}{\text{dry soil, g}} \times 100$$

$$\text{Percent Moisture (dry weight basis)} = \frac{\left(\text{sample weight before drying, g}\right) - \left(\text{sample weight after drying, g}\right)}{\text{sample weight after drying, g}} \times 100$$

L. Determination of Dry Weight Concentrations of Triclopyr and Metabolites in Sediment and Soil

1. Determine the analyte concentrations in the sample as described in Section J.
2. Determine the soil moisture as described in Section K.
3. Determine the dry weight analyte concentrations in the samples as follows:

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

3 Assay Time

A typical analytical run consists of a minimum of four standards encompassing the expected range of sample concentrations, a reagent blank, a control (a non-fortified sample), a minimum of two fortified controls (one of which must be at the LOQ), and ten samples. This typical analytical run can be prepared in approximately ten hours, followed by the chromatographic analysis.

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

a. Step I.1.e. and Step I.1.h. It is possible to perform either shaking operation for an extended period of time. In fact, greatest productivity will occur when the first shaking operation (Step I.1.e.) is done overnight.

b. Step I.1.i.

c. Step I.1.j.

d. Step I.1.q.

e. Step I.1.r.(6).

f. Step I.1.s.

g. Step I.1.t.

h. Step I.1.v.

i. Step I.1.w.(8).

j. Step I.1.dd.

k. Step I.1.ee.

l. Step I.1.ff.

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

4. Standardization of SPE Elution Profiles

Variation in the silica gel and C_{18} SPE columns may influence the elution profiles of triclopyr, 3,5,6-TCP, and 2-MP. It is necessary to obtain an elution profile for each lot of SPE columns used to ensure optimum recovery and clean-up efficiency. The following procedures can be used:

a. Silica SPE Profile for 2-MP

(1) To a 12-mL vial containing 10 mL of hexane, add 10 µL of the 100-µg/mL spiking solution (Section G.1.d.).

(2) Place a silica gel SPE column on the vacuum manifold.

(3) Rinse the SPE column with 2.5 mL of 1-chlorobutane. (Do not allow the column bed to dry.)

(4) Condition the SPE column with 2.5 mL of hexane. (Do not allow the column bed to dry.)

(5) Transfer the sample solution from Step M.4.a.(1) to the SPE column, and slowly pull the sample through the column with the aid of vacuum, collecting 2-mL aliquots in 12-mL vials. (Do not allow the column bed to dry.)

(6) Rinse the sample vial with 6 mL of hexane, and when the sample solution in Step M.4.a.(5) is within 2 mm of the top of the column bed, transfer the rinse to the SPE column. Slowly pull the rinse solution through the column with the aid of vacuum, and continue to collect 2-mL aliquots in 12-mL vials.

(7) For each fraction collected, add 2.0 mL of 1-chlorobutane to the sample vial.

(8) Concentrate the solutions to less than 1 mL (but not to dryness) using an N-Evap evaporator. (Note N.2.)

(9) Proceed as described in Section I.1.gg. through I.1.ll.

(10) Calculate the percent recovery for 2-MP as described in Section I.2.

A typical elution profile is illustrated in Figure 21. If the elution profile differs from that shown, adjust the volume of hexane to be collected in Step I.1.r.(6).

b. C_{18} SPE Profile for Triclopyr and 3,5,6-TCP

(1) To a 16-mL vial containing 15 mL of 0.1 N hydrochloric acid, add 10 µL of the 100-µg/mL spiking solution (Section G.1.d.).

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

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

(4) Condition the SPE column with 5 mL of 0.1 N hydrochloric acid. (Do not allow the column bed to dry.)

(5) Transfer the sample solution from Step M.4.b.(1) to the SPE column, and slowly pull the sample through the column with the aid of vacuum. (Do not allow the column bed to dry.)

(6) Rinse the sample vial with 2 mL of 0.1 N hydrochloric acid, and transfer the rinse to the SPE column. Slowly pull the rinse solution through the column with the aid of vacuum.

(7) Dry the SPE column under vacuum for 1 minute.

(8) Elute the triclopyr and 3,5,6-TCP with the acetonitrile/hydrochloric acid solution.

For the 40% acetonitrile/59% water/1% 1.0 N hydrochloric acid solution, elute with 8 mL of the solution, collecting 1.0-mL aliquots in 40-mL vials.

For the 80% acetonitrile/19% water/1% 1.0 N hydrochloric acid solution, elute with 8 mL of the solution, collecting 1.0-mL aliquots in 40-mL vials.

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

(10) Calculate the percent recovery for triclopyr and 3,5,6-TCP as described in Section I.2.

A typical elution profile is illustrated in Figure 22. If the elution profile differs from that shown, adjust the volume of the 40% acetonitrile/59% water/1% 1.0 N hydrochloric acid solution to be discarded in Step I.1.w.(7) or the volume of

the 80% acetonitrile/19% water/1% 1.0 N hydrochloric acid solution to be collected in Step I.1.w.(8).

N. Notes

1. Equipment, glassware, materials, reagents, and chemicals considered to be equivalent to those specified may be substituted with the understanding that their performance must be confirmed by appropriate tests. Common laboratory supplies are assumed to be readily available and are, therefore, not listed.

2. The N-Evap evaporator should be set at a water bath temperature of 30 °C and a nitrogen flow rate of approximately 200 mL/min. At elevated water bath temperatures, the 3,5,6-TCP and 2-MP may volatilize, thereby reducing recoveries.

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

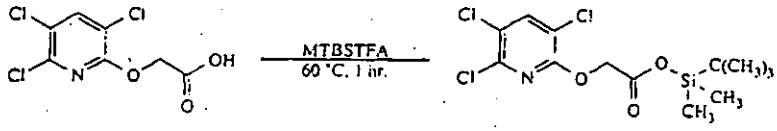
4. Several tuning, or calibration, options are available for the Model 597X series of MSDs. The "Maximum Sensitivity Autotune" feature was found to consistently yield approximately 5-10 times the sensitivity compared to that of the "Standard Autotune".

5. In transferring the 1-chlorobutane layer, it is important not to remove any water from the lower layer. Contaminating the 1-chlorobutane with water will have deleterious effects on the derivatization and subsequent GC/MSD analysis.

O. References

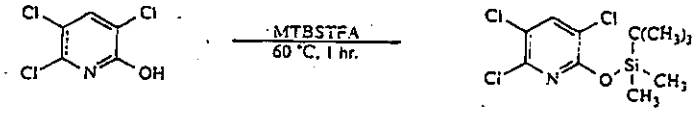
1. *HP-41C/41CV Standard Applications Handbook*, Hewlett-Packard Publication No. 00041-90402, 1982, pp 42-48.

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



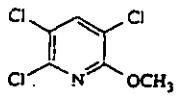
Triclopyr
Formula: $C_7H_4Cl_3NO_3$
Molecular Weight: 255

Triclopyr-TBDMS
Formula: $C_{13}H_{18}Cl_3NO_3Si$
Molecular Weight: 369



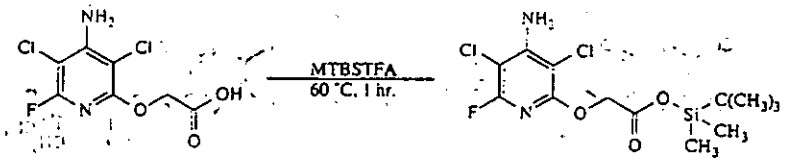
3,5,6-TCP
Formula: $C_5H_2Cl_3NO$
Molecular Weight: 197

3,5,6-TCP-TBDMS
Formula: $C_{11}H_{16}Cl_3NOSi$
Molecular Weight: 311



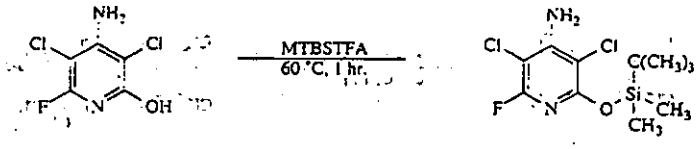
2-MP
Formula: $C_6H_4Cl_3NO$
Molecular Weight: 211

Figure 1. Chemical Structures of Triclopyr, 3,5,6-Trichloro-2-pyridinol and their TBDMS Derivatives, and 2-Methoxy-3,5,6-trichloropyridine.



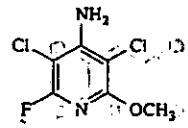
Fluroxypyr
Formula: $C_7H_5Cl_2FN_2O_3$
Molecular Weight: 254

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



Fluroxypyr-DCP
Formula: $C_5H_3Cl_2FN_2O$
Molecular Weight: 196

Fluroxypyr-DCP-TBDMS
Formula: $C_{11}H_{17}Cl_2FN_2OSi$
Molecular Weight: 310



Fluroxypyr-MP
Formula: $C_6H_5Cl_2FN_2O$
Molecular Weight: 210

Figure 2. Chemical Structures of Fluroxypyr, 4-Amino-3,5-dichloro-6-fluoro-2-pyridinol and their TBDMS Derivatives, and 4-Amino-3,5-dichloro-6-fluoro-2-methoxypyridine