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Determination of Residues of Cyhalofop-butyl and Metabolites in Sediment and Soil by Liquid Chromatography with Mass Spectrometry Detection

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1. SCOPE

This method is applicable for the quantitative determination of residues of cyhalofop-butyl and its major metabolites in soil. The method measures cyhalofop-butyl (as the cyhalofop-acid equivalent), as well as cyhalofop-acid, cyhalofop-amide, cyhalofop-diacid, and cyhalofop-FHPBA. The method was validated over the concentration range of 10-1000 ng/g with validated limits of quantitation of 10 ng/g for cyhalofop-acid, cyhalofop-amide, and cyhalofop-FHPBA, and 16 ng/g for cyhalofop-diacid.

Common and chemical names for the above compounds are given in Table 1.

2. PRINCIPLE

Residues of cyhalofop-butyl and its major metabolites are extracted from sediment and soil using a 90% acetone/10% 1.0 N hydrochloric acid solution. An aliquot of the extract is concentrated to remove the acetone and is then diluted with 0.1 N sodium hydroxide to hydrolyze any cyhalofop-butyl to cyhalofop-acid. Following hydrolysis, the sample is acidified with hydrochloric acid and then extracted with a 60% 1-chlorobutane/40% methyl-*tert*-butyl ether (MTBE) solution. The 1-chlorobutane/MTBE solution is evaporated to dryness, and the residue reconstituted with an 89.5% hexane/10% acetone/0.5% formic acid solution. This solution is purified using a silica gel solid-phase extraction (SPE) and the column eluate is then evaporated to dryness. The residue is reconstituted with HPLC mobile phase containing compound X-460511 as an internal standard and then analyzed by HPLC with mass spectrometry detection (LC/MS).

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. Acetic acid, acetone, acetonitrile, 1-chlorobutane, ethyl acetate, formic acid, hexane, methanol, and methyl-*tert*-butyl-ether are flammable and should be used in well-ventilated areas away from ignition sources.

3.3. Acetic acid, formic acid, 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 12.1.)

4.1. Laboratory Equipment

4.1.1. Balance, analytical, Model AE200, Mettler-Toledo, Inc., Hightstown, NJ 08520.

4.1.2. Balance, pan, Model BB2440, Mettler-Toledo, Inc.

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

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

4.1.5. Evaporator, N-Evap, Model 111, Organomation Associates, Inc., South Berlin, MA 01549. (Note 12.2.)

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

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

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

4.1.9. Ultrasonic cleaner, Model 1200, Branson Cleaning Equipment Company, Shelton, CT 06484.

4.1.10. Vacuum manifold, Model spe-12G, Mallinckrodt Baker, Inc., Phillipsburg, NJ 08865.

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

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

4.2. LC/MS System Used for Quantitation

4.2.1. Column, analytical, ZORBAX SB-C8 reversed-phase, 75 mm x 4.6 mm i.d., catalog number 866953-906, Hewlett-Packard, Wilmington, DE 19808.

4.2.2. Column, guard, ZORBAX RX-C8 reversed-phase, 12.5 mm x 4.6 mm i.d., catalog number 820950-913, Hewlett-Packard.

4.2.3. Degasser, vacuum, Model 1100, catalog number G1322A, Hewlett-Packard.

4.2.4. Mass spectrometer, Model 1100, catalog number G1946A, Hewlett-Packard.

4.2.5. Mass spectrometer data system, LC/MSD ChemStation v1.0, Hewlett-Packard.

4.2.6. Oven, column, Model 1100, catalog number G1316A, Hewlett-Packard.

4.2.7. Pump, binary, Model 1100, catalog number G1312A, Hewlett-Packard.

4.2.8. Sampler, automatic liquid, Model 1100, catalog number G1313A, Hewlett-Packard.

4.3. LC/MS/MS System Used for Confirmation

In addition to using the same liquid chromatographic instrumentation as above, the following mass spectrometer is used for confirmation.

4.3.1. Mass spectrometer, Model API2000, Perkin Elmer/Sciex Instruments, Thurnhill, ON L3T 1P2.

4.3.2. Mass spectrometer data system, Analyst v1.0, Perkin Elmer/Sciex Instruments.

5. GLASSWARE AND MATERIALS (Note 12.1.)

5.1. Column, silica gel SPE, catalog number 7086-07, Mallinckrodt Baker, Inc.

5.2. Cylinder, graduated mixing, 50-mL, catalog number 20039-0050, Kimble/Kontes, Vineland, NJ 08360.

5.3. Cylinder, graduated mixing, 1000-mL, catalog number 20039-1000, Kimble/Kontes.

5.4. Cylinder, graduated mixing, 2000-mL, catalog number 20039-2000, Kimble/Kontes.

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

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

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

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

5.9. Flask, volumetric, 1000-mL, catalog number 161-8992, National Scientific Company.

5.10. Flask, volumetric, 2000-mL, catalog number 161-8993, National Scientific Company.

5.11. Pipet, serological, 1.0-mL, catalog number P4760-1C, National Scientific Company.

5.12. Pipet, serological, 10-mL, catalog number P4760-10, National Scientific Company.

5.13. Pipet, volumetric, 0.50-mL, catalog number 261-6010, National Scientific Company.

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

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

5.16. Pipet, volumetric, 2.5-mL, catalog number 261-6084, National Scientific Company.

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

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

5.19. Pipet, volumetric, 9.0-mL, catalog number 261-6019, National Scientific Company.

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

5.21. Pipet, volumetric, 11-mL, catalog number 261-6021, National Scientific Company.

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

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

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

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

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

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

5.28. Syringe, 10.0-µL, Model 701N, Hamilton Company, Reno, NV 89502.

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

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

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

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

6. REAGENTS, STANDARDS, AND PREPARED SOLUTIONS (Note 12.1.)

6.1. Reagents

6.1.1. Acetic acid, glacial, 99.7% purity, ACS reagent grade, catalog number A38-500, Fisher Scientific.

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

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

6.1.4. 1-Chlorobutane, OmniSolv grade, catalog number CX0914-1, EM Science.

6.1.5. Ethyl acetate, OmniSolv grade, catalog number EX0241-1, EM Science.

6.1.6. Formic acid, 88% purity, ACS reagent grade, catalog number A118P-500, Fisher Scientific.

6.1.7. Hexane, OmniSolv grade, catalog number HX0297-1, EM Science.

6.1.8. Hydrochloric acid, 6.0 N, catalog number LC15370-2, Fisher Scientific.

6.1.9. Hydrochloric acid, 1.0 N, certified concentration, catalog number SA48-1, Fisher Scientific.

6.1.10. Methanol, OmniSolv grade, catalog number MX-0480-1, EM Science.

6.1.11. Methyl-*tert*-butyl ether, OmniSolv grade, catalog number MX-0826-1, EM Science.

6.1.12. Nitrogen, refrigerated liquid, catalog number LQNI, BOC Gases, Murray Hill, NJ 07974.

6.1.13. Nitrogen, gas, 99.95% purity, BOC Gases.

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

6.1.15. Sodium hydroxide, 0.1 N, certified concentration, catalog number SS276-1, Fisher Scientific.

6.1.16. Water, OmniSolv grade, catalog number WX0004-1, EM Science.

6.2. Standards

6.2.1. cyhalofop-acid ((*R*)-2-[4-(4-cyano-2-fluorophenoxy)phenoxy]propanoic acid)

6.2.2. cyhalofop-amide (2-[4-[4-(aminocarbonyl)-2-fluorophenoxy]phenoxy]propanoic acid)

6.2.3. cyhalofop-butyl (butyl (*R*)-2-[4-(4-cyano-2-fluorophenoxy)phenoxy]propionate)

6.2.4. cyhalofop-diacid (4-[4-(1-carboxyethoxy)phenoxy]-3-fluorobenzoic acid)

6.2.5. cyhalofop-FHPBA (3-fluoro-4-(4-hydroxyphenoxy)benzoic acid)

6.2.6. X-460511 ((*R*)-2-[4-(2,4-dichlorophenoxy)phenoxy]propanoic acid)

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

6.3. Prepared Solutions

6.3.1. 95% acetone/5% formic acid (v/v)

Pipet 10.0 mL of formic acid into a 200-mL volumetric flask and dilute to volume with acetone.

6.3.2. 94.9% acetone/5.0% water/0.1% acetic acid (v/v/v)

Pipet 100.0 mL of distilled/deionized water into a 2000-mL volumetric flask. Pipet 2.0 mL of acetic acid into the same flask; then add approximately 1800 mL of acetone. Swirl the flask and allow to equilibrate to room temperature. Dilute to volume with acetone.

6.3.3. 90% acetone/10% 1.0 N hydrochloric acid (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.

6.3.4. 99.5% acetonitrile/0.5% acetic acid/ (v/v)

Pipet 10.0 mL of acetic acid into a 2000-mL volumetric flask and dilute to volume with acetonitrile.

6.3.5. 20% acetonitrile/20% methanol/59% water/1% acetic acid (v/v/v/v)

Pour 400 mL of acetonitrile and 400 mL of methanol into a 2000-mL graduated mixing cylinder. Pipet 20.0 mL of acetic acid into the same cylinder; then add approximately 1000 mL of distilled/deionized water. Swirl the cylinder and allow to equilibrate to room temperature. Dilute to volume with distilled/deionized water.

6.3.6. 60% 1-chlorobutane/40% MTBE (v/v)

Pour 400 mL of methyl-*tert*-butyl ether into a 1000-mL graduated mixing cylinder; then add approximately 500 mL of 1-chlorobutane. Swirl the cylinder and allow to equilibrate to room temperature. Dilute to volume with 1-chlorobutane.

6.3.7. 99% ethyl acetate/1.0% acetic acid

Pipet 10.0 mL of acetic acid into a 1000-mL volumetric flask and dilute to volume with ethyl acetate.

6.3.8. 89.5% hexane/10% acetone/0.5% formic acid (v/v/v)

Pipet 100.0 mL of acetone into a 1000-mL volumetric flask. Pipet 5.0 mL of formic acid into the same flask; then add approximately 800 mL of hexane. Swirl the flask and allow to equilibrate to room temperature. Dilute to volume with hexane.

6.3.9. 99.5% water/0.5% acetic acid (v/v)

Pipet 10.0 mL of acetic acid into a 2000-mL volumetric flask and dilute to volume with glass-distilled water.

7. PREPARATION OF STANDARDS

7.1. Preparation of Spiking Solutions

7.1.1. Weigh 0.1000 g of cyhalofop-acid or 0.1186 g cyhalofop-butyl (0.1000 g cyhalofop-acid equivalent) analytical standard and quantitatively transfer to a 100-mL volumetric flask. Dilute to volume with 94.9% acetone/5.0% water/0.1% acetic acid (v/v/v) to obtain a 1000-µg/mL stock solution.

7.1.2. Weigh 0.1000 g of cyhalofop-amide analytical standard and quantitatively transfer to a 100-mL volumetric flask. Dilute to volume with 94.9% acetone/5.0% water/0.1% acetic acid (v/v/v) to obtain a 1000-µg/mL stock solution.

7.1.3. Weigh 0.1000 g of cyhalofop-diacid analytical standard and quantitatively transfer to a 100-mL volumetric flask. Dilute to volume with 94.9% acetone/5.0% water/0.1% acetic acid (v/v/v) to obtain a 1000-µg/mL stock solution.

7.1.4. Weigh 0.1000 g of cyhalofop-FHPBA analytical standard and quantitatively transfer to a 100-mL volumetric flask. Dilute to volume with 94.9% acetone/5.0% water/0.1% acetic acid (v/v/v) to obtain a 1000-µg/mL stock solution.

7.1.5. Pipet 20.0 mL of each of the 1000-µg/mL stock solutions in Sections 7.1.1-7.1.4 into a single 200-mL volumetric flask and adjust to volume with 94.9% acetone/5.0% water/0.1% acetic acid (v/v/v) to obtain a solution containing 100.0 µg/mL of each compound.

7.1.6. Pipet 10.0 mL of the 100.0-µg/mL solution in Section 7.1.5 into a 200-mL volumetric flask and adjust to volume with 94.9% acetone/5.0% water/0.1% acetic acid (v/v/v) to obtain a solution containing 5.00 µg/mL of each compound.

7.1.7. Prepare solutions for spiking soil samples by diluting the 5.00-µg/mL solution from Section 7.1.6 with 94.9% acetone/5.0% water/0.1% acetic acid (v/v/v) as follows:

Aliquot of 5.00-µg/mL Soln.	Final Soln. Volume	Spiking Soln. Final Conc.	Equivalent Sample Conc.[a]
mL	mL	µg/mL	ng/g
1.00	200	0.025	5.0
1.00	100	0.050	10.0
2.50	100	0.125	25.0
5.00	100	0.250	50.0
10.0	100	0.500	100.0
25.0	100	1.25	250
50.0	100	2.50	500
--	--	5.00	1000

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

7.2. Preparation of the X-460511 Internal Standard Solution for Cyhalofop-butyl Metabolite Determination

7.2.1. Weigh 0.0100 g of X-460511 standard and quantitatively transfer to a 100-mL volumetric flask. Dilute to volume with 20% acetonitrile/20% methanol/59% water/1% acetic acid (v/v/v/v) to obtain a 100.0-µg/mL stock solution.

7.2.2. Pipet 20.0 mL of the 100.0-µg/mL stock solution in Section 7.2.1 into a 200-mL volumetric flask and adjust to volume with 20% acetonitrile/20% methanol/59% water/1% acetic acid (v/v/v/v) to obtain a 10.0-µg/mL stock solution.

7.2.3. Pipet 5.0 mL of the 10.0-µg/mL solution in Section 7.2.2 into a 1000-mL volumetric flask and dilute to volume with 20% acetonitrile/20% methanol/59% water/1% acetic acid (v/v/v/v) to obtain a 0.050-µg/mL solution.

7.3. Preparation of Calibration Standards for Cyhalofop-butyl Metabolite Determination

7.3.1. Pipet 10.0 mL of each of the 1000-µg/mL stock solutions in Sections 7.1.1-7.1.4 into a single 100-mL volumetric flask and adjust to volume with 20% acetonitrile/20% methanol /59% water/1% acetic acid (v/v/v/v) to obtain a solution containing 100.0 µg/mL of each compound.

7.3.2. Pipet 10.0 mL of the 100.0-µg/mL stock solution in Section 7.3.1 into a 100-mL volumetric flask and adjust to volume with 20% acetonitrile/20% methanol/59% water/1% acetic acid (v/v/v/v) to obtain a 10.0-µg/mL stock solution.

7.3.3. Pipet 10.0 mL of the 10.0-µg/mL stock solution in Section 7.3.2 into a 100-mL volumetric flask and adjust to volume with 20% acetonitrile/20% methanol/59% water/1% acetic acid (v/v/v/v) to obtain a 1.00-µg/mL stock solution.

7.3.4. Prepare calibration standards by diluting the 1.00-µg/mL stock solution from Section 7.3.3 with 20% acetonitrile/20% methanol/59% water/1% acetic acid (v/v/v/v) as follows:

Aliquot of 1.00-µg/mL Soln.	Final Soln. Volume	Calibration Solution Conc.	Equivalent Sample Conc.
mL	mL	µg/mL	ng/g
0.500	200	0.0025	2.5
1.00	200	0.005	5.0
2.00	200	0.010	10.0
5.00	200	0.025	25.0
5.00	100	0.050	50.0
10.00	100	0.100	100.0
25.0	100	0.250	250
50.0	100	0.500	500
--	--	1.000	1000

8. INSTRUMENTAL CONDITIONS

8.1. Liquid Chromatography/Mass Spectrometry Conditions for Quantitation

8.1.1. Assemble the various components of the liquid chromatographic system (Section 4.2.) following the manufacturer's recommended procedures.

8.1.2. Typical Operating Conditions (Note 12.3)

Instrumentation: Hewlett-Packard Model 1100 vacuum degasser
Hewlett-Packard Model 1100 binary pump
Hewlett-Packard Model 1100 automatic liquid sampler
Hewlett-Packard Model 1100 column oven
Hewlett-Packard Model 1100 mass selective detector
Hewlett-Packard Model 1100 data system

Columns:

Guard: ZORBAX RX-C8
12.5 mm x 4.6 mm i.d.

Analytical: ZORBAX SB-C8
75 mm x 4.6 mm i.d.

Temperature: 30 °C

Solvent Composition: A: 0.5% acetic acid in water
B: 0.5% acetic acid in acetonitrile

Flow Rate: 1.0 mL/min

Gradient:

Time, min	A, %	B, %
0.00	75	25
1.00	75	25
9.00	25	75
10.00	15	85
11.00	15	85
11.01	75	25

Run Time: 13.5 min

Injection Volume: 100 µL

Detector:

Interface	electrospray
Polarity	negative ion selected ion monitoring
Gain	10

SIM parameters:	Time, min	Fragmentor	Ion
Cyhalofop-amide	4.00	80	*m/z* 246 (quantitation) *m/z* 318 (alternate)
Cyhalofop-FHPBA			*m/z* 247 (quantitation) *m/z* 203 (alternate)
Cyhalofop-diacid	6.04	100	*m/z* 247 (quantitation) *m/z* 319 (alternate)
Cyhalofop-acid		100	*m/z* 228 (quantitation) *m/z* 300 (alternate)
X-460511 (Int. Std.)	8.80	80	*m/z* 325 (quantitation)

Mass spectra of the above compounds are shown in Figure 1.

8.1.3. Calibration Curves

Typical calibration curves for the determination of cyhalofop-acid, cyhalofop-amide, cyhalofop-diacid, and cyhalofop-FHPBA in soil are shown in Figures 2-5.

8.1.4. Typical Chromatograms

Typical chromatograms of a standard, control sample, and a 10.0-ng/g recovery sample for the determination of the above compounds in soil are illustrated in Figures 6-8.

8.2. Liquid Chromatography/Mass Spectrometry Conditions for Confirmation

8.2.1. Assemble the various components of the liquid chromatographic system (Section 4.3.) following the manufacturer's recommended procedures.

8.2.2. Typical Operating Conditions (Note 12.3)

Instrumentation:
Hewlett-Packard Model 1100 vacuum degasser
Hewlett-Packard Model 1100 binary pump
Hewlett-Packard Model 1100 automatic liquid sampler
Hewlett-Packard Model 1100 column oven
PE/Sciex Model API2000 mass spectrometer
PE/Sciex Model Analyst v1.0 mass spectrometer data system

Columns:

Guard: ZORBAX RX-C8, 12.5 mm x 4.6 mm i.d.

Analytical: ZORBAX SB-C8, 75 mm x 4.6 mm i.d.

Temperature: ambient

Solvent Composition:
A: 0.5% acetic acid in water
B: 0.5% acetic acid in acetonitrile

Flow Rate:
Column: 0.9 mL/min
Interface: 0.2 mL/min

Gradient:

Time, min	A, %	B, %
0.00	75	25
1.00	75	25
8.00	15	85
9.00	15	85
9.10	75	25
10.50	75	25

Run Time: 10.5 min

Injection Size: 50 µL

Detector:
Interface: electrospray
Polarity: negative ion multiple reaction monitoring (MRM)
Resolution: quadropole 1 – unit, quadropole 3 – low

MRM parameters:	Precursor Ion	Product Ion	Time, msec
Cyhalofop-amide	*m/z* 318	*m/z* 246	150
Cyhalofop-FHPBA	*m/z* 247	*m/z* 183	150
Cyhalofop-diacid	*m/z* 319	*m/z* 247	150
Cyhalofop-acid	*m/z* 300	*m/z* 228	150
X-460511 (Int. Std.)	*m/z* 325	*m/z* 253	150

8.2.3. Typical Chromatograms

Typical chromatograms of a standard, control sample, and a 10-ng/g recovery sample for the confirmation of cyhalofop-amide, cyhalofop-FHPBA, cyhalofop-diacid, and cyhalofop-acid in soil are illustrated in Figures 9-11.

9. DETERMINATION OF RECOVERY OF CYHALOFOP-BUTYL AND METABOLITES FROM SEDIMENT AND 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 freezing with liquid nitrogen and then grinding or chopping using a hammer mill with a 3/16-inch screen size. Prepared samples are stored frozen < -20 °C prior to analysis.

9.3. Sample Analysis

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

9.3.2. For preparing fortified samples, add 1.0-mL aliquots of the appropriate spiking solutions to obtain concentrations ranging from 10.0 to 1000 ng/g.

9.3.3. Add 25 mL of the 90% acetone/10% 1.0 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 vial 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 2200 rpm.

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

9.3.8. Repeat Steps 9.3.3-9.3.6 with 15 mL of the 90% acetone/10% 1.0 N hydrochloric acid extraction solution and a 30-minute shaking time.

9.3.9. Combine the acetone/hydrochloric acid solution from Step 9.3.8 with the 25 mL from Step 9.3.7, adjust to 40.0 mL with additional extraction solution and mix thoroughly.

9.3.10. Transfer an 8.0-mL portion of the acetone/hydrochloric acid solution from Step 9.3.9 into a clean 40-mL vial.

9.3.11. Evaporate the acetone using an N-Evap evaporator set at 40 °C and a nitrogen flow rate of approximately 500 mL/min. (There will be approximately 0.8 mL of liquid remaining in the sample vial.)

9.3.12. Add 11.0 mL of 0.10 N sodium hydroxide to the sample vial. Cap the vial with a PTFE-lined cap, vortex the sample for 5-10 seconds, and then sonicate the sample for 5-10 seconds.

9.3.13. Allow the sample to sit for 20 minutes at room temperature. (During this time, cyhalofop-butyl is hydrolyzed to cyhalofop-acid.)

9.3.14. Centrifuge the sample vial for 5 minutes at 2200 rpm.

9.3.15. Transfer the aqueous supernatant solution to a clean 40-mL vial, being careful to leave the residue pellet in the bottom of the vial. Discard the sample vial containing the residue pellet.

9.3.16. Add 1.05 mL of 6.0 N hydrochloric acid, 4 g of sodium chloride, and 5.0 mL of the 60% 1-chlorobutane/40% MTBE extraction solution to the sample vial.

9.3.17. Cap the vial with a PTFE-lined cap, and shake the sample for 20 minutes on a reciprocating shaker at approximately 180 excursions/minute.

9.3.18. Centrifuge the sample vial for 5 minutes at 2000 rpm.

9.3.19. Transfer the 1-chlorobutane/MTBE (top) layer into a clean 12-mL vial. (Note 12.4.)

9.3.20. Add an additional 5.0 mL of the 60% 1-chlorobutane/40% MTBE extraction solution to the sample vial containing the aqueous phase. Cap the vial, and shake the sample for 20 minutes on a reciprocating shaker at approximately 180 excursions/minute.

9.3.21. Centrifuge the sample vial for 5 minutes at 2000 rpm.

9.3.22. Combine the 1-chlorobutane/MTBE layer from Step 9.3.21 with the 1-chlorobutane/MTBE extract from Step 9.3.19 and mix thoroughly. (Note 12.4.)

9.3.23. Evaporate the solution from Step 9.2.22 to dryness using an N-Evap evaporator set at 40 °C and a nitrogen flow rate of approximately 500 mL/min.

9.3.24. Reconstitute the sample with 1.0 mL of a 95% acetone/5% formic acid solution and firmly seal the sample vial with a PTFE-lined cap. Vortex the sample for 5-10 seconds, and then sonicate the sample for 5-10 seconds.

9.3.25. Add 9.0 mL of hexane to the sample vial and firmly seal with a PTFE-lined cap. Vortex the sample for 5-10 seconds, and then sonicate the sample for 5-10 seconds.

9.3.26. Purify the sample using the following silica gel SPE procedure:

a. Place a silica gel SPE column on the vacuum manifold.

b. Condition the SPE column with 5 mL of an 89.5% hexane/10% acetone/0.5% formic acid solution. (Do not allow the column bed to dry.)

c. Transfer the sample solution from Step 9.3.25 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 without allowing the column bed to dry.

d. Rinse the sample vial with 5.0 mL of an 89.5% hexane/10% acetone/0.5% formic acid solution. When the sample solution in Step 9.3.26.c is within 2 mm of the top of the column bed, transfer the rinse to the SPE column. With the aid of vacuum, slowly pull the rinse solution through the column, and discard the eluate.

e. Elute the analytes from the SPE column by passing 10.0 mL of a 99% ethyl acetate/1% acetic acid solution through the column, collecting the eluate in a 12-mL vial.

9.3.27. Evaporate the solution to dryness using an N-Evap evaporator set at 40 °C and a nitrogen flow rate of 500 mL/min.

9.3.28. Add 1.0 mL of HPLC mobile phase containing the X-460511 internal standard to the sample vial. Cap the vial, vortex the sample for 5-10 seconds, and then sonicate the sample for 5-10 seconds.

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

9.3.30. For quantitation, analyze the calibration standards (Section 7.3.4.) and samples by liquid chromatography/mass spectrometry as described in Sections 8.1. 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 standard curve concentration. 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 6-8 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 10.0-ng/mL calibration standards.

9.3.31. For quantitation, analyze the calibration standards (Section 7.3.4.) and samples by liquid chromatography/mass spectrometry as described in Section 8.2.

10. CALCULATIONS

10.1. Calculation of Standard Calibration Curve

10.1.1. Inject the series of calibration standards described in Section 7.3.4 and determine the peak areas for the analytes and internal standards as indicated below.

cyhalofop-acid	*m/z* 228 (quantitation)
cyhalofop-amide	*m/z* 246 (quantitation)
cyhalofop-diacid	*m/z* 247 (quantitation)
cyhalofop-FHPBA	*m/z* 247 (quantitation)
X-460511	*m/z* 325 (internal standard)

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

For example, using the data for cyhalofop-amide from Figure 6:

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

$$\text{Quantitation Ratio} = \frac{\text{peak area at } m/z \text{ 246}}{\text{peak area at } m/z \text{ 325}}$$

$$\text{Quantitation Ratio} = \frac{19654}{156106}$$

$$\text{Quantitation Ratio} = 0.1259$$

10.1.3. Prepare a standard curve for each analyte by plotting the equivalent analyte concentration on the abscissa (x-axis) and the respective quantitation ratio on the ordinate (y-axis) as shown in Figures 2-5. Using regression analysis, determine the equation for the curve with respect to the abscissa.

For example, using power regression (13.1.) with the cyhalofop-amide data from Figure 3:

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

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

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

$$\text{Cyhalofop-amide Conc. (ng/g)} = \left(\frac{\text{cyhalofop-amide quantitation ratio}}{0.0124}\right)^{1/1.0220}$$

10.2. Calculation of Percent Recovery

10.2.1. 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 cyhalofop-amide data from Figure 8:

$$\text{Cyhalofop-amide Conc. (gross ng/g)} = \left(\frac{\text{cyhalofop-amide quantitation ratio}}{0.0124}\right)^{1/1.0220}$$

$$\text{Cyhalofop-amide Conc. (gross ng/g)} = \left(\frac{0.1029}{0.0124}\right)^{1/1.0220}$$

$$\text{Cyhalofop-amide Conc. (gross)} = 7.90 \text{ ng/g}$$

10.2.2. Determine the net concentration in each recovery sample by subtracting the equivalent cyhalofop-amide concentration found in the control sample from that of the gross cyhalofop-amide concentration in the recovery sample.

For example, using the cyhalofop-amide data from Figures 7 and 8:

$$\text{Cyhalofop-amide Conc. (net ng/g)} = \text{Cyhalofop-amide Conc. (gross ng/g)} - \text{Cyhalofop-amide Conc. (control ng/g)}$$

$$\text{Cyhalofop-amide Conc. (net ng/g)} = 7.90 \text{ ng/g} - 0.00 \text{ ng/g}$$

$$\text{Cyhalofop-amide Conc. (net)} = 7.90 \text{ ng/g}$$

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

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

$$\text{Recovery} = \frac{7.90 \text{ ng/g}}{10.0 \text{ ng/g}} \times 100\%$$

$$\text{Recovery} = 79\%$$

10.3. Determination of Cyhalofop-butyl and Metabolites in Soil

10.3.1. 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 10.2.1.

10.3.2. 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 cyhalofop-amide data from Figure 8 and Table 2 for the samples analyzed on 11-Dec-1998:

$$\underset{\text{(corrected ng/g)}}{\text{Cyhalofop-amide Conc.}} = \underset{\text{(gross ng/g)}}{\text{Cyhalofop-amide Conc.}} \times \left(\frac{100}{\%\ \text{Recovery}}\right)$$

$$\underset{\text{(corrected ng/g)}}{\text{Cyhalofop-amide Conc.}} = 7.90\ \text{ng/g} \times \frac{100}{78}$$

$$\underset{\text{(corrected)}}{\text{Cyhalofop-amide Conc.}} = 10.2\ \text{ng/g}$$

10.4. Determination of Soil Moisture

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

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

10.4.3. 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.

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

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

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

10.5. Determination of Dry Weight Concentrations of Cyhalofop-butyl and Metabolites in Sediment and Soil

10.5.1. Determine the analyte concentrations in the sample as described in Section 10.3.

10.5.2. Determine the soil moisture as described in Section 10.4.

10.5.3. Determine the dry weight analyte concentrations in the samples as follows:

$$\underset{\text{(dry weight ng/g)}}{\text{Cyhalofop-amide Conc.}} = \underset{\text{(ng/g)}}{\text{Cyhalofop-amide Conc.}} \times \left(1 + \frac{\%\ \text{Moisture}}{100}\right)$$

11.4. Standardization of Silica Gel SPE Elution Profiles

11.4.1. To a 12-mL vial, add 1.0 mL of the 2.50-µg/mL spiking solution from Section 7.1.7.

11.4.2. Evaporate the solution to dryness using an N-Evap evaporator set at 40 °C and a nitrogen flow rate of approximately 500 mL/min.

11.4.3. Reconstitute the sample with 1.0 mL of a 95% acetone/5% formic acid solution and firmly seal the sample vial with a PTFE-lined cap. Vortex the sample for 5-10 seconds, and then sonicate the sample for 5-10 seconds.

11.4.4. Add 9.0 mL of hexane to the sample vial and firmly seal with a PTFE-lined cap. Vortex the sample for 5-10 seconds, and then sonicate the sample for 5-10 seconds.

11.4.5. Place a silica gel SPE column on the vacuum manifold.

11.4.6. Condition the SPE column with 5 mL of an 89.5% hexane/10% acetone/0.5% formic acid solution. (Do not allow the column bed to dry.)

11.4.7. Transfer the sample solution from Step 11.4.4 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. Collect the eluate in a 12-mL vial. (Do not allow the column bed to dry.)

11.4.8. Rinse the sample vial with 5.0 mL of an 89.5% hexane/10% acetone/0.5% formic acid solution. When the sample solution in Step 11.4.7 is within 2 mm of the top of the column bed, transfer the rinse to the SPE column. With the aid of vacuum, slowly pull the rinse solution through the column, collecting the eluate in a 12-mL vial.

11.4.9. Elute the analytes from the SPE column by passing 12.0 mL of a 99% ethyl acetate/1% acetic acid solution through the column, collecting 2.0-mL aliquots in 12-mL vials.

11.4.10. For each fraction collected, proceed as described in Section 9.3.27 through 9.3.30.

11.4.11. Calculate the percent recovery for each analyte as described in Section 10.2.

Typical elution profiles are illustrated in Figure 12. If the elution profiles differ from those shown, adjust the volume of the 99% ethyl acetate/1% acetic acid solution to be collected in Step 9.3.26.e.

12. Notes

12.1. 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.

12.2. The N-Evap evaporator should be set at a water bath temperature of 40 °C and a nitrogen flow rate of approximately 500 mL/min, or enough to dimple the surface of the solution being evaporated.

12.3. If necessary, modify the typical operating conditions to obtain optimum performance or to meet the system suitability criteria specified in Step 9.3.30.

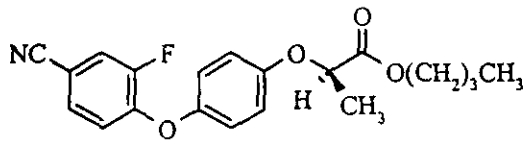
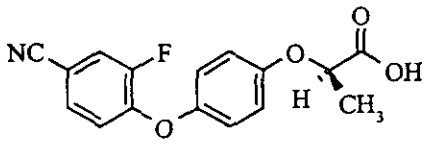
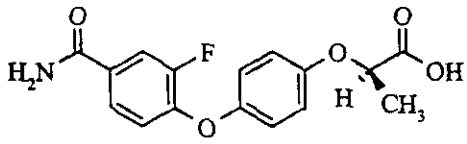
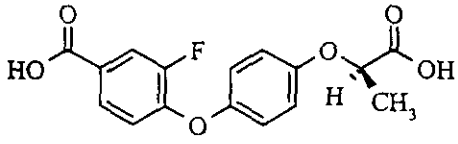
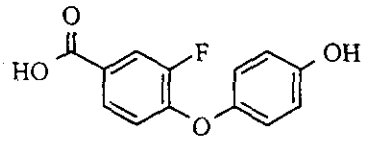
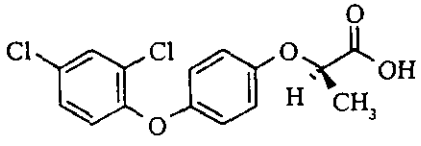
12.4. In transferring the 1-chlorobutane/MTBE layer, it is important not to transfer any water. Contaminating the 1-chlorobutane/MTBE with water may have deleterious effects on the derivatization and subsequent GC/MSD analysis.

13. REFERENCES

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

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

Table 1. Identity and Structures of Cyhalofop-butyl and Metabolites

Common Name of Compound	Structure and CAS Name
Cyhalofop-butyl Molecular Formula: $C_{20}H_{20}FNO_4$ Formula Weight: 357.38 Molecular Weight: 357 CAS Number: 122008-85-9	 butyl (*R*)-2-[4-(4-cyano-2-fluorophenoxy)-phenoxy]propionate
Cyhalofop-acid Molecular Formula: $C_{16}H_{12}FNO_4$ Formula Weight: 301.274 Molecular Weight: 301 CAS Number: 122008-78-0	 (*R*)-2-[4-(4-cyano-2-fluorophenoxy)-phenoxy]propanoic acid
Cyhalofop-amide Molecular Formula: $C_{16}H_{14}FNO_5$ Formula Weight: 319.289 Molecular Weight: 319 CAS Number: not available	 2-[4-[4-(aminocarbonyl)-2-fluorophenoxy]-phenoxy]propanoic acid
Cyhalofop-diacid Molecular Formula: $C_{16}H_{13}FO_6$ Formula Weight: 320.274 Molecular Weight: 320 CAS Number: not available	 4-[4-(1-carboxyethoxy)phenoxy]-3-fluorobenzoic acid
Cyhalofop-FHPBA Molecular Formula: $C_{13}H_9FO_4$ Formula Weight: 248.213 Molecular Weight: 248 CAS Number: not available	 3-fluoro-4-(4-hydroxyphenoxy)benzoic acid
Compound X-460511 Molecular Formula: $C_{15}H_{12}Cl_2O_4$ Formula Weight: 327.163 Molecular Weight: 326 CAS Number: not available	 (*R*)-2-[4-(2,4-dichlorophenoxy)-phenoxy]propanoic acid