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Determination of Residues of Cyhalofop-butyl and Metabolites in Water by Capillary Gas Chromatography with Mass Spectrometry Detection and 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, cyhalofop-acid, cyhalofop-amide, cyhalofop-diacid, and cyhalofop-FHPBA in water. For cyhalofop-butyl, the method was validated over the concentration range of 1-1000 ng/mL with a validated limit of quantitation of 1.0 ng/mL. For the metabolites, the method was validated over the concentration range of 1-500 ng/mL with validated limits of quantitation of 1.0 ng/mL for cyhalofop-acid, cyhalofop-amide, and cyhalofop-diacid, and 2.0 ng/mL for cyhalofop-FHPBA.

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

2. PRINCIPLE

Residues of cyhalofop-butyl and its metabolites are extracted from acidified water using a 60% 1-chlorobutane/40% methyl-*tert*-butyl ether (MTBE) solution. The solvent mixture is evaporated to dryness and the residue reconstituted for analysis.

For the determination of cyhalofop-butyl, the residue is constituted with toluene containing cyhalofop ethyl ester (cyhalofop-ethyl) as an internal standard and then analyzed by capillary gas chromatography with mass spectrometry detection (GC/MS).

For the determination of the cyhalofop-metabolites, 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, ethanol, isooctane, methanol, methyl-*tert*-butyl-ether, and toluene are flammable and should be used in well-ventilated areas away from ignition sources.

3.3. Acetic acid, hydrochloric acid, and sulfuric acid 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. Centrifuge, with rotor to accommodate 12- and 40-mL vials, Model Centra-GP8, International Equipment Company, Needham Heights, MA 02194.

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

4.1.4. Heating module, ReactiTherm, catalog number 18835, Pierce Chemical Company, Rockford, IL 61105.

4.1.5. Heating block, ReactiBlock U-1, 21-mm holes, catalog number 18818, Pierce Chemical Company.

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

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

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

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

4.2. GC/MS System Used for Quantitation

4.2.1. Column, capillary gas chromatography, Durabond-5MS liquid phase, 12 m x 0.20 mm i.d., 0.33-µm film thickness, catalog number 128-5512, J & W Scientific, Inc., Folsom, CA 95630.

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

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

4.2.4. Inlet sleeve, double gooseneck splitless, catalog number 20784, Restek Corporation, Bellefonte, PA 16823.

4.2.5. Mass spectrometer, Model 5972A, Hewlett-Packard, Palo Alto, CA 94304.

4.2.6. Mass spectrometer data system, Model G1701AA, Hewlett-Packard.

4.3. LC/MS System Used for Quantitation

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

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

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

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

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

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

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

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

4.4. LC/MS/MS System Used for Confirmation

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

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

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

5. GLASSWARE AND MATERIALS (Note 12.1.)

5.1. Cylinder, graduated mixing, 1000-mL, catalog number 20039-1000, Kimble/Kontes, Vineland, NJ 08360.

5.2. Cylinder, graduated mixing, 2000-mL, catalog number 20039-2000, Kimble/Kontes, Vineland, NJ 08360.

5.3. Filter, charcoal, catalog number 7972, Chrompack, Inc., Raritan, NJ 08869 (Note 12.3.)

5.4. Filter, moisture, catalog number 7971, Chrompack, Inc. (Note 12.3.)

5.5. Filter, oxygen, catalog number 7970, Chrompack, Inc. (Note 12.3.)

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

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

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

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

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

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

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

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

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

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

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

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

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

5.19. Syringe, 100-µL, Model 710N, Hamilton Company, Reno, NV 89502.

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

5.21. Syringe, 500-µL, Model 750N, Hamilton Company.

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

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

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

5.25. 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, Pittsburgh, PA 15219.

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. Ethanol, anhydrous, minimum 99.5% purity, catalog number 45,983-6, Aldrich Chemical Company, Milwaukee, WI 53201.

6.1.6. Helium, gas, 99.995% purity, BOC Gases, Murray Hill, NJ 07974.

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

6.1.8. Isooctane (2,2,4-trimethylpentane), OmniSolv grade, catalog number TX1389-1, EM Science.

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

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

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

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

6.1.13. Sulfuric acid, concentrated (approximately 36 N), ACS reagent grade, catalog number A300-500, Fisher Scientific.

6.1.14. Toluene, OmniSolv grade, catalog number TX0737-1, EM Science.

6.1.15. 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. 0.5% acetic acid/99.5% acetonitrile (v/v)

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

6.3.2. 0.5% acetic acid/99.5% water (v/v)

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

6.3.3. 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.

6.3.4. 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 flask; 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.

6.3.5. 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.

6.3.6. 2 N sulfuric acid in ethanol

Weigh 10.2 g of concentrated sulfuric acid into a 12-mL vial. Carefully transfer the acid to a 100-mL volumetric flask containing approximately 60 mL of ethanol (the flask should be chilled in an ice bath). Rinse the vial several times with ethanol, transferring each wash to the volumetric flask. Swirl the flask, and let it remain in the ice bath for approximately five minutes. Remove the flask from the ice bath and allow to equilibrate to room temperature. Dilute to volume with ethanol.

7. PREPARATION OF STANDARDS

7.1. Preparation of Spiking Solutions

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

7.1.2. Weigh 0.1000 g of cyhalofop-acid 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-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.

7.1.4. 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.

7.1.5. 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.

7.1.6. Pipet 20.0 mL of each of the stock solutions in Sections 7.1.1-7.1.5 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.

7.1.7. Prepare solutions for spiking water samples by diluting the 100 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 Initial Soln.	Final Soln. Volume	Spiking Soln. Final Conc.	Equivalent Sample Conc.[a]
mL	mL	µg/mL	ng/mL
0.100	200	0.050	0.50
0.200	200	0.100	1.00
0.500	200	0.250	2.50
1.00	200	0.500	5.00
2.00	200	1.00	10.0
5.00	200	2.50	25.0
5.00	100	5.00	50.0
10.00	100	10.0	100.0
25.0	100	25.0	250.0
50.0	100	50.0	500.0
--	--	100.0	1000.0

[a] The equivalent sample concentration is based on fortifying a 10.0-mL water sample with 100 µL of spiking solution.

7.2. Preparation of the Cyhalofop Ethyl Ester Internal Standard Solution for Cyhalofop-butyl Determination

7.2.1. Pipet 1.0 mL of the 1000-µg/mL cyhalofop-acid analytical standard from Section 7.1.2 into a 12-mL vial.

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

7.2.3. Add 1.0 mL of the 2 N sulfuric acid/ethanol derivatizing solution 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.

7.2.4. Place the sample vial in a heating block set at 100 °C and allow the mixture to react for 30 minutes.

7.2.5. Remove the sample vial from the heating block and allow the reaction mixture to cool to room temperature.

7.2.6. Evaporate the ethanol using an N-Evap evaporator set at a water bath temperature of 40 °C and a nitrogen flow rate of approximately 500 mL/min. (After evaporation of the ethanol, there will be approximately 0.1 mL of sulfuric acid remaining in the vial.)

7.2.7. Add 5.0 mL of water and 3.0 mL of isooctane to the sample vial. Vortex the sample for 5-10 seconds, and then sonicate the sample for 5-10 seconds.

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

7.2.9. Transfer the isooctane (top) layer into a clean 100-mL volumetric flask.

7.2.10. Repeat Steps 7.2.7-7.2.9 two additional times (adding isooctane only), combining the isooctane layers in the same 100-mL volumetric flask containing the isooctane from Step 7.2.9.

7.2.11. Evaporate the isooctane to dryness using an N-Evap evaporator set at a water bath temperature of 40 °C and a nitrogen flow rate of approximately 200 mL/min.

7.2.12. Reconstitute the standard to volume with toluene. This solution contains 10.9-µg/mL of cyhalofop-ethyl, equivalent to 10.0-µg/mL of cyhalofop-acid.

7.2.13. Pipet 25.0 mL of the 10.0-µg/mL solution in Section 7.2.12 into a 1000-mL volumetric flask and dilute to volume with toluene to obtain a 0.250-µg/mL solution.

7.3. Preparation of Calibration Standards for Cyhalofop-butyl Determination

7.3.1. Prepare calibration standards by dispensing 100 µL of the solutions from Section 7.1.7 into 12-mL vials.

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

7.3.3. Add 1.0 mL of toluene containing the cyhalofop-ethyl internal standard (Section 7.2.13.) to the sample vial. Cap the vial, vortex the sample for 5-10 seconds, and then sonicate the sample for 5-10 seconds.

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

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

7.4.1. 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.

7.4.2. Pipet 20.0 mL of the stock solution in Section 7.4.1 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.

7.4.3. Pipet 5.0 mL of the 10.0-µg/mL solution in Section 7.4.2 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.

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

7.5.1. Pipet 10.0 mL of each of the stock solutions in Sections 7.1.2-7.1.5 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.

7.5.2. Pipet 10.0 mL of the stock solution in Section 7.5.1 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.

7.5.3. Pipet 20.0 mL of the stock solution in Section 7.5.2 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 1.00-µg/mL stock solution.

7.5.4. Prepare calibration standards by diluting the stock solution from Section 7.5.3 with 20% acetonitrile/20% methanol/59% water/1% acetic acid (v/v/v/v) as follows:

Aliquot of Initial Soln.	Final Soln. Volume	Calibration Solution Conc.	Equivalent Sample Conc.
mL	mL	µg/mL	ng/mL
0.500	200	0.0025	0.025
1.00	200	0.005	0.05
2.00	200	0.010	1.00
5.00	200	0.025	2.50
5.00	100	0.050	5.00
10.00	100	0.100	10.0
25.0	100	0.250	25.0
50.0	100	0.500	50.0
--	--	1.000	100.0

8. INSTRUMENTAL CONDITIONS

8.1. Gas Chromatography/Mass Spectrometry Conditions for Quantitation

8.1.1. Column

Install the splitless column inlet sleeve (Section 4.2.4.) and the capillary column (Section 4.2.1.) in the split/splitless injection port of the gas chromatograph following the manufacturer's recommended procedures.

8.1.2. Typical Operating Conditions (Note 12.4.)

Instrumentation:	Hewlett-Packard Model 5890A Series II gas chromatograph Hewlett-Packard Model 7673 autoinjector Hewlett-Packard Model 5972A mass selective detector Hewlett-Packard Model G1701AA data system
Column:	J&W Scientific fused silica capillary DB-5MS liquid phase 12 m x 0.2 mm i.d. 0.33-µm film thickness
Temperatures:	
Column	125 °C for 5.0 min 125 °C to 310 °C at 15 °C/min 310 °C for 2.67 min
Injector	280 °C
Interface	300 °C
Carrier Gas:	helium
Constant Flow	on
Vacuum Compensation	on
Head Pressure	25 kPa
Linear Velocity	approximately 40 cm/s
Injection Mode:	splitless
Purge Delay	4.9 min
Splitter Flow	50 mL/min
Septum Purge	1.0 mL/min
Injection Volume:	1 µL
Detector Mode:	electron impact ionization with selected ion monitoring
Calibration Program	maximum sensitivity autotune; usertune (Note 12.5.)
Electron Multiplier	1700 volts (same as autotune)
Ions Monitored:	
Cyhalofop-ethyl	*m/z* 329 (internal standard)
Cyhalofop-butyl	*m/z* 357 (quantitation) *m/z* 229, 256 (confirmation)
Dwell Time:	75 msec

Mass spectra of cyhalofop-ethyl and cyhalofop-butyl are shown in Figure 1.

8.1.3. Calibration Curves

A typical calibration curve for the determination of cyhalofop-butyl in water is shown in Figure 2.

8.1.4. Typical Chromatograms

Typical chromatograms of a standard, control sample, and a 1.00-ng/mL recovery sample for the determination of cyhalofop-butyl in water are illustrated in Figures 3-5.

8.2. Liquid Chromatography/Mass Spectrometry Conditions for Quantitation

8.2.1. Typical Operating Conditions (Note 12.4.)

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 (SIM)
Gain	10

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

Mass spectra of the above compounds are shown in Figure 6.

8.2.2. Calibration Curves

Typical calibration curves for the determination of cyhalofop-acid, cyhalofop-amide, cyhalofop-diacid, and cyhalofop-FHPBA in water are shown in Figures 7-10.

8.2.3. Typical Chromatograms

Typical chromatograms of a standard, control sample, and a 1.00-ng/mL recovery sample for the determination of the above compounds in water are illustrated in Figures 11-13.

8.3. Liquid Chromatography/Mass Spectrometry Conditions for Confirmation

8.3.1. Typical Operating Conditions (Note 12.4.)

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 (approximately 1:4 split ratio)

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

8.3.2. Typical Chromatograms

Typical chromatograms of a standard, control sample, and an LOQ recovery sample for the confirmation of cyhalofop-amide, cyhalofop-FHPBA, cyhalofop-diacid, and cyhalofop-acid in water are illustrated in Figures 14-16.

9. DETERMINATION OF RECOVERY OF CYHALOFOP-BUTYL AND METABOLITES FROM WATER

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

Samples are stored refrigerated or frozen prior to analysis.

9.3. Sample Analysis

9.3.1. For preparing fortified samples, dispense 100-µL aliquots of the appropriate spiking solutions encompassing the necessary concentration range into a series of 40-mL vials and evaporate the solution to dryness using an N-Evap evaporator set at 40 °C and a nitrogen flow rate of approximately 500 mL/min.

9.3.2. Add 10.0-mL of control water to the sample vial and seal with a PTFE-lined cap. Vortex the sample for 5-10 seconds, and then sonicate the solution for 2 minutes.

9.3.3. For non-fortified samples, transfer a 10.0-mL portion of the water sample into a 40-mL vial.

9.3.4. Add 1.0 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.

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

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

9.3.7. Transfer the 1-chlorobutane/MTBE (top) layer into a clean 12-mL vial. (Note 12.6.)

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

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

9.3.10. Combine the 1-chlorobutane/MTBE layer from Step 9.3.9 with the 1-chlorobutane/MTBE extract from Step 9.3.7 (Note 12.6.). Add 100 µL of acetic acid to the solution and mix thoroughly.

9.3.11. Evaporate the solution from Step 9.3.10 to dryness using an N-Evap evaporator set at 40 °C and a nitrogen flow rate of approximately 500 mL/min.

9.3.12. For the determination of cyhalofop-butyl only:

a. Add 1.0 mL of toluene containing the cyhalofop-ethyl internal standard to the sample vial from Step 9.3.11. Cap the vial, vortex the sample for 5-10 seconds, and then sonicate the sample for 5-10 seconds.

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

c. Analyze the calibration standards (Section 7.3.) and samples by capillary gas chromatography/mass spectrometry as described in Section 8.1.

9.3.13. For the determination of cyhalofop-butyl metabolites only:

a. Add 1.0 mL of HPLC mobile phase containing the X-460511 internal standard to the sample vial Step 9.3.11. Cap the vial, vortex the sample for 5-10 seconds, and then sonicate the sample for 5-10 seconds.

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

c. Analyze the calibration standards (Section 7.5.) and samples by liquid chromatography/mass spectrometry as described in Section 8.2.

9.3.14. 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 3-5 and Figures 11-13 with respect to peak response, baseline noise, and background interference. Visually determine that a minimum signal-to-noise ratio of 10:1 has been attained for each analyte in the 0.005-µg/mL calibration standards.

10. CALCULATIONS

10.1. Calculation of Standard Calibration Curve

10.1.1. Inject the series of calibration standards described in Section 7.3 (cyhalofop-butyl) and Section 7.5 (cyhalofop-butyl metabolites) and determine the peak areas for the analytes and internal standards as indicated below.

cyhalofop-butyl	*m/z* 357 (quantitation)
cyhalofop-ethyl	*m/z* 329 (internal standard)
cyhalofop-acid	*m/z* 228 (quantitation)
cyhalofop-amide	*m/z* 318 (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-butyl from Figure 3:

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

$$\text{Quantitation Ratio} = \frac{\text{peak area at } m/z\ 357}{\text{peak area at } m/z\ 329}$$

$$\text{Quantitation Ratio} = \frac{5354}{177107}$$

$$\text{Quantitation Ratio} = 0.03023$$

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 Figure 2 and Figures 7-10. Using regression analysis, determine the equation for the curve with respect to the abscissa.

For example, using power regression (13.1.) with the cyhalofop-butyl data from Figure 2:

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

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

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

$$\text{Cyhalofop-butyl Conc. (ng/mL)} = \left(\frac{\text{cyhalofop-butyl quantitation ratio}}{0.0308}\right)^{1/1.10098}$$

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-butyl data from Figure 5:

$$\text{Cyhalofop-butyl Conc. (gross ng/mL)} = \left(\frac{\text{cyhalofop-butyl quantitation ratio}}{0.0308}\right)^{1/1.10098}$$

$$\text{Cyhalofop-butyl Conc. (gross ng/mL)} = \left(\frac{0.0288}{0.0308}\right)^{1/1.0098}$$

$$\text{Cyhalofop-butyl Conc. (gross)} = 0.94 \text{ ng/mL}$$

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

For example, using the cyhalofop-butyl data from Figures 4 and 5:

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

$$\text{Cyhalofop-butyl Conc. (net ng/mL)} = 0.94\ \text{ng/mL} - 0.00\ \text{ng/mL}$$

$$\text{Cyhalofop-butyl Conc. (net)} = 0.94\ \text{ng/mL}$$

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

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

$$\text{Recovery} = \frac{0.94\ \text{ng/mL}}{1.00\ \text{ng/mL}} \times 100\%$$

$$\text{Recovery} = 94\%$$

10.3. Determination of Cyhalofop-butyl and Metabolites in Water

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-butyl data from Figure 5 and the average percent recovery from Table 2 for the samples analyzed on 26-Jun-1998:

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

$$\text{Cyhalofop-butyl Conc. (corrected ng/mL)} = 0.94\ \text{ng/mL} \times \frac{100}{94}$$

$$\text{Cyhalofop-butyl Conc. (corrected)} = 1.00\ \text{ng/mL}$$

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

12.4. If necessary, modify the typical operating conditions to obtain optimum performance or to meet the system suitability criteria specified in Step 9.3.14.

12.5. Several tuning, or calibration, options are available for the Model 597X series of MSDs. The "Maximum Sensitivity Autotune" and "Usertune" calibration features were found to consistently yield approximately 5-10 times the sensitivity compared to that of the "Standard Autotune" calibration. In addition, either of these calibrations should be followed with a "Usertune" calibration at *m/z* 264, 314, and 414.

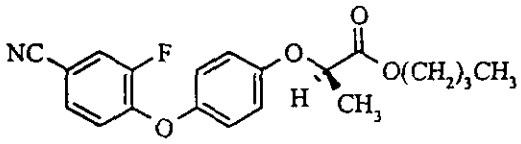
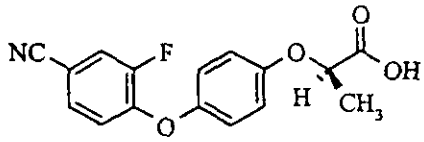
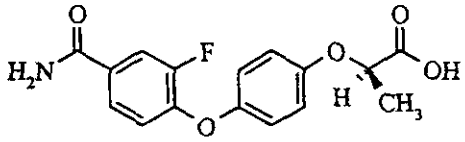
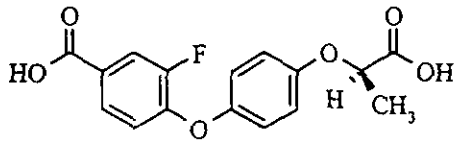
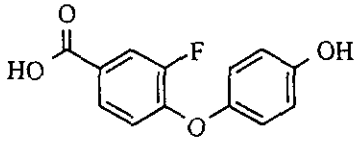
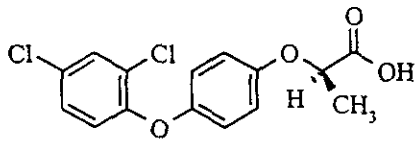
12.6. In transferring the 1-chlorobutane/MTBE layer, it is important not to transfer any water. Contaminating the 1-chlorobutane/MTBE with water may have deleterious effects on the derivatization and subsequent GC/MSD analysis.

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