

XII. APPENDICES

A. Analytical Method

**A ROBOTIC PROCEDURE FOR
SAMPLE PREPARATION AND ANALYSIS OF
BIFENTHRIN AND 4'-HYDROXY-BIFENTHRIN**

The original method which formed the basis for this procedure is found in Report No. P-2011M (see Section X, Reference 3).

Modifications to the method are as follows:

I. GENERAL INFORMATION

Separate methods were developed and validated manually for bifenthrin and 4'-hydroxy-bifenthrin, taking into account the requirements of architecture and reduced volumes for an automated (robotic) environment. The methods were then combined into a single sample work-up for both components followed by two separate chromatographic procedures for quantitation. The bifenthrin samples were injected by an integrated procedure with the robotic system and gas chromatograph. The final step for 4'-hydroxy-bifenthrin (solvent exchange and vial preparation) was performed manually and injected after the automated procedure was completed.

II. MATERIALS

A. Apparatus

Analytic Balance, PM 2000, Mettler
Containers, stainless steel, 400 mL, Omni
Corporation International (OCI)
Tubes, 25 x 150 mm, capped, Kimax
Tubes, 25 x 150 mm, uncapped, Pyrex®
Tips, pipette, 1 mL and 5 mL, Zymark
Homogenizer, OCI Omni-Mixer, Omni Corporation
International

Dishwasher, Forma Scientific, Model 8890
Solid Phase Extraction Columns, Fisher
Florisisil Prep-Sep[®] (1 g)
N-EVAP[®] Evaporator
Septa, double-faced Teflon[®] -coated
silicone,
Restek Corp.
Injection port sleeve, Hewlett-Packard
Gooseneck Splitless (4mm I.D.), Restek
Corp.
Syringe, Hamilton, 50, 100, 250, 500 uL

Zymate[®] Robotic System (FMC-2)

Capacity - 20 Samples/Run

Zymate Robot
System V Controller
Printer, Okidata[®]

Turntable for the following:

1. 2 sample container racks for stainless steel (SS) 400 mL OCI containers (24 positions total; first 20 positions used)
 2. 25 x 150 mm capped tubes (80 positions)
 3. 25 x 150 mm uncapped tubes (80 positions)
 4. 15 mL centrifuge tubes (80 positions)
 5. GC vial racks (50 positions)
 6. Pipette hand (0.2-1 mL)
 7. Pipette hand (1-5 mL)
- PM 2000 Mettler top-loading balance with holder for stainless steel 400 mL container

High volume pump dispenser for extraction solvent

Centrifuge

Vortex Stations,

1. Centrifuge tubes (15 mL)
2. Centrifuge tubes (25 x 150 mm)

Vial preparation station

Disposal station

Liquid/Solid station

Evaporation station for 15 mL centrifuge tubes

Pipette tip rack (5 mL)

Liquid/Liquid station

Evaporation station for 25 x 150 tubes

Capping/Uncapping station

Omni Homogenizer (OCI) station
Laserscan Barcode Reader station
Filtration station
Vortex station (25 x 150 mm tubes) and liquid
dispenser nozzles
Pipette tip rack (1 mL)
Hand A
Hand B
Hand OCI
Hand G
Hand H
Hand for GC vial injection
3 Power and Event Controllers (PEC)
3 Master Lab Stations (MLS) with 3 syringes
each
2 Masterflex[®] pumps
AT&T[®] Uninterruptible Power Supply
Hand-held operator terminal

Computer System

386 AT Personal Computer, Compuadd Multisync
II Monitor, NEC
Epson[®] LQ 500 printer

Gas Chromatographic System

Hewlett-Packard 5890 GC (2)
Hewlett-Packard 7673A Autosampler (2)
Hewlett-Packard Barcode Reader
Hewlett-Packard 3396 Integrator (2)

B. Reagents and Standards

Acetone, Resi-Analyzed, JT Baker
Acetonitrile, Resi-Analyzed, JT Baker
Analytical Standards, FMC Corporation, ACG,
Princeton, NJ:
1. Bifenthrin (FMC 54800)
2. 4'-hydroxy-bifenthrin (FMC 78128)
Sodium Chloride, Reagent Grade, JT Baker
Water, double-deionized
Ethyl Acetate, Resi-Analyzed, JT Baker
Hexane, Byrdick & Jackson
Versatone[®] Glassware Cleaning Agent, VWR
Cyclohexane, Resi-Analyzed, JT Baker
Methyl t-Butyl Ether, OmniSolv
Methanol, Resi-Analyzed, JT Baker
Hexanes, Resi-Analyzed, JT Baker

C. Analytical Procedure

1. Glassware Preparation

All glassware was thoroughly washed in a Forma Scientific dishwasher (Model 8890) with the following wash cycle using a non-phosphorous detergent: pre-wash, wash, two tap water rinses, and three final distilled water rinses. The cleaned glassware was then hand-rinsed with acetone prior to use.

2. Extraction

Sample ID barcoded (Assay No.) extraction of 50 g of soil was carried out in a stainless steel (SS) container using an OCI Omni-mixer for 2 minutes with 250 mL of solvent (acetonitrile:water, 70:30). A 10 g aliquot mixture was centrifuged at 300 rpm for 90 seconds.

3. Partition

A 2 g aliquot (10 mL) was pipetted from the 10 g centrifuged aliquot into a 25 x 150 mm tube; 10 mL of 5% NaCl in water was added. The mixture was partitioned with 3 x 10 mL hexane by vortexing 3 times for 30 seconds each time with a 15 second pause between each vortex. The combined hexane was evaporated in a 25 x 150 mm tube, down to 1-2 mL.

4. Sample Clean-Up - Florisil (SPR) Purification by Robotics

a. Column Conditioning:

5 mL Acetone

15 mL Hexane

b. Add sample to column.

c. Rinse tube with 0.5 mL cyclohexane, add to column

d. Elution Solvents:

Bifenthrin - 2 mL 10% Ethyl Acetate
in Hexane

4'-Hydroxy-Bifenthrin - 10 mL 60:39:1
Hexane:MTBE:Methanol

5. Injection

- a. The bifenthrin elution is ready to be placed in vial for injection.
- b. The 4'-hydroxy-bifenthrin elution (10 mL) must be evaporated to 0.5 mL; 5 mL of hexane is added and vortexed. The sample is then evaporated to 2 mL for injection.

6. Instrument Parameters

A. Bifenthrin

Instrument: Hewlett-Packard Model 5890
Gas Chromatograph equipped
with Hewlett-Packard Model
7673 Auto Sampler

Column: 15 m x 0.53 mm, 1.5 μ m
film thickness, DB-1
capillary column (J & W
Scientific).

Inlet: Fitted with a Gooseneck
Splitless Sleeve (4mm
I.D.)
Temperature = 250°

Injection
Mode: Splitless; Direct

Oven
Program:
Initial Temp. = 210°C
Initial Time = 0 min
Program Rate = 5°C/min
Final Temp. = 260°C
Final Time = 10 min

Carrier Gas: Helium, -10 mL/min

Make-up Gas: 10% Methane in Argon
-30 mL/min

Injection
Volume: 4 μ L

Chart Speed: 0.5 cm/min

Detector: Electron Capture, ⁶³Ni,
Hewlett-Packard

B. 4'-Hydroxy-bifenthrin

Instrument: Hewlett-Packard Model 5890
Gas Chromatograph equipped
with Hewlett-Packard Model
7673 Auto Sampler

Column: 15 m x 0.53 mm, 1.5 μ m
film thickness, DB-5
capillary column (J. & W
Scientific).

Inlet: Fitted with a Gooseneck
Splitless Sleeve (4mm
I.D.)
Temperature = 275°C

Injection
Mode: Splitless; Direct

Oven

Program:
Initial Temp. = 230°C
Initial Time = 0 min
Program Rate = 5°C/min
Final Temp. = 280°C
Final Time = 10 min

Carrier Gas: Helium, -10 mL/min

Make-up Gas: 10% Methane in Argon
-30 mL/min

Injection
Volume: 2 μ L

Chart Speed: 0.5 cm/min

Detector: Electron Capture, ⁶³Ni,
Hewlett-Packard

7. Quantitation

A Hewlett-Packard 3396A Integrator was used to quantitate both bifenthrin and 4'-hydroxy-bifenthrin by peak area integration and external standard calibration. Prior to analysis, the HP 5890A was calibrated with the proper standards and showed a linear response between ng injected and area counts. An external standard calibration method was entered at the start of analysis of a series of samples. The method was verified by injecting a standard after every two samples. The amount of both bifenthrin and 4'-hydroxy-bifenthrin in each sample was reported in parts per million (ppm) using appropriate dilution factors calculated from sample aliquot, amount injected, and final volume of samples.