III. MATERIALS

A. Test/Reference Substance

Flumioxazin (V-53482)

7-fluoro-6-[(3,4,5,6-tetrahydro)phthalimido]-4-(2-propynyl)-1,4-benzoxazin-3(2H)-one

Lot AS 1663g, 99.8% pure, expiration date July 11, 1997.

The flumioxazin was received on dry ice from Valent USA and was placed in desiccated freezer storage (nominal -20 °C).

B. Test System

Untreated control soil was provided by Valent and was identified as V1011-2U1. It was received frozen on dry ice, and was placed in freezer storage at nominal -20 °C.

IV. METHODOLOGY

A. Reagents

Acetone, high purity, B&J
Methylene chloride, high purity, B&J
Ethyl acetate, high purity, B&J
Florisil, 2 parts 100-200 mesh (Fisher) mixed with 3 parts 60-100 mesh (Fisher), activated overnight at 130 °C, and allowed to cool overnight before use
Hexane, high purity, B&J
Hydrochloric acid, 12 N, Mallinckrodt
Sodium chloride, Fisher
Sodium sulfate, Aldrich; washed with methylene chloride just prior to use
Deionized water (D.I.), in house

B. Solutions

All solutions were stored at ambient conditions.

1. 0.1 N HCl Solution

A 0.1 N solution was prepared by addition of 40 mL of HCl to 4 liters of deionized water.

2. 5% NaCl

A 5% solution of NaCl was prepared by diluting 60 g of NaCl to 1,200 mL with deionized water.

3. Acetone/0.1 N HCl (5/1, v/v)

A 1,000-mL aliquot of acetone was mixed with 200 mL of 0.1 N HCl.

4. Hexane/Ethyl Acetate (2/1, v/v)

A 600-mL aliquot of hexane was diluted to 900 mL with ethyl acetate (i.e., 300 mL of ethyl acetate was added).

C. Equipment

Reciprocating shaker, Eberbach
Liquid chromatography columns, 300 x 19 mm i.d. with Teflon stopcocks
Rotary vacuum evaporators (Buchi), with heated water bath (<40 °C)
Ultrasonic bath, Branson
Drying oven, Thelco, Model 15
Büchner funnels, 10 cm
Filter flasks, 500 mL
Filter funnels, 10 cm
Flat-bottom flasks, 25 mL, 250 mL, and 500 mL
Filter paper, Whatman #1
Separatory funnels, 500 mL
Pipets, fixed volume
Syringe, Glenco 500 µL, calibrated to deliver 100 µL of acetone
Volumetric flasks

Autoinjector vials (12 x 32 mm); autoinjector vial crimp caps with Teflon-faced septa
General laboratory glassware
Pasteur pipets, borosilicate glass disposable
GC system (HP 5890A) equipped with a DB-17 megabore column and nitrogen-phosphorus detector (refer to Section IV.F.1 for parameters).

D. Standard Solutions

1. Preparation of Stock Solutions

A 0.0100-g aliquot of the flumioxazin was weighed, quantitatively transferred to a 10-mL volumetric flask, and diluted to volume with acetone to yield a 1 mg/mL solution. The stock solution was stored in the refrigerator (nominal 5 °C). A 20 µg/mL secondary solution was prepared by aliquoting 0.5 mL of the 1 mg/mL stock into a 25-mL volumetric flask and diluting to volume with acetone.

2. Dilutions for Fortification Standard

A 1 µg/mL standard was prepared by diluting 0.5 mL of the 20 µg/mL standard to 10 mL with acetone.

Addition of 100 µL (*via* calibrated syringe) of the 1 µg/mL standard to 10 g of soil yields a 0.01 ppm fortification. Addition of 0.5 mL of the 1 µg/mL standard to 10 g of soil yields a 0.05 ppm fortification.

3. Dilutions for Quantitation and Linearity Standards

a. 2 µg/mL

A 1-mL aliquot of the 20 µg/mL solution was further diluted to 10 mL with acetone. This standard was only used for verifying linearity.

b. 1 µg/mL

A 2.5-mL aliquot of the 20 µg/mL solution was further diluted to 50 mL with acetone. This standard was used for verifying linearity and for quantitation of the samples.

c. 0.5 µg/mL

A 2.5-mL aliquot of the 2 µg/mL solution was further diluted to 10 mL with acetone. This standard was used for verifying linearity.

d. 0.1 µg/mL

A 1-mL aliquot of the 1 µg/mL solution was further diluted to 10 mL with acetone. This standard was used for verifying linearity.

E. Procedure

The method was used as written with the following exceptions.

Extraction

A 1-pint French square bottle was used rather than a Mason jar.

Partition

A flat-bottom flask was used rather than a round-bottom flask.

Florisil Column Cleanup

Method note #4 describes a testing procedure to determine the elution profile of flumioxazin from each batch of Florisil. A 1-mL aliquot of the 1 µg/mL flumioxazin standard was transferred into a 25-mL flask (the method called for use of a 50-mL flask) and rotary evaporated just until dry. The residue was dissolved in 3 mL of hexane/ethyl acetate (2/1), ultrasonicated for 30 seconds, and applied to the column. After the solvent drained to the top of the bed, three 3-mL rinses of the flask using hexane/ethyl acetate (2/1) were applied to the column. The entire 12-mL application volume was collected in one flask. Another receiver flask was placed under the column and the first 20 mL of the 28-mL wash described in the method was collected. The receiver flask was replaced and the last 8 mL of the 28-mL wash was collected separately. The receiver flask was replaced and the column was eluted with 70 mL of hexane/ethyl acetate (2/1), described in the method as the fraction containing the flumioxazin. Three additional 10-mL aliquots of hexane/ethyl acetate (2/1) eluant were applied to the column and collected separately.

Fractions to be evaluated for flumioxazin content were rotary evaporated just until dry. An appropriate volume of acetone was added to each fraction (1 mL for the 70 mL fraction normally collected, and 0.5 mL for all other eluate fractions evaluated); the extracts were injected and quantitated by direct comparison to a 1 µg/mL standard injection.

The results indicated that the 12-mL application volume and the first 28-mL wash volume were to be discarded, and a 90-mL aliquot of hexane/ethyl acetate was to be collected to ensure total collection of flumioxazin. The columns used for samples of run 1 were eluted using this optimized scheme.

When the Florisil column eluate was collected, a 250-mL flat-bottom flask was used rather than a 250-mL round-bottom flask. After evaporation of the eluate just to dryness, the residue was transferred to a 25-mL round-bottom flask rather than a 50-mL flask as described in the method for the second evaporation.

F. Quantitation

After verification of the linearity of the system (refer to Section V), the samples were injected with the 1.0 µg/mL quantitation standard interspersed throughout the run; the run began and ended with a standard injection.

1. Instrumentation

Only the hydrogen flow rate was modified from the parameters described in the method; the method allows for modification of parameters for optimal performance.

Primary Column:	DB-17 (J & W), 15 m x 0.53 mm, 1.0-µm film
Confirmatory Column:	DB-5 (J & W), 15 m x 0.53 mm, 1.5-µm film

Instrument:	Hewlett Packard 5890A
Temperatures:	Detector: 300 °C
	Inlet: 275 °C
	Oven Program:
	Initial: 250 °C
	Hold: 1 min
	Ramp: 20 °C/min to 280 °C
	Hold: 8 min

Flows:	Carrier Gas:	Helium (UHP) at 7 psi head pressure, 10 mL/min
	Makeup Gas:	Helium at 25 mL/min
	Detector Gases:	Air at 110 mL/min
		Hydrogen at 4 mL/min
	Purge on at 1.5 min	
Insert:	Splitless, silylated, narrow bore	
Injector:	Hewlett Packard 7673A, 1-µL injection volume	
Detector:	Hewlett Packard 19234B NP Detector	

2. Calculations

Data for the standards and samples were collected and integrated with Nelson Analytical Chromatography Model 2600 Software (Version 5.1.5).

The 1.0 µg/mL flumioxazin standard solution was injected before the first sample and after every sample, ending the run with a standard. The average peak height of this standard was used for calculating sample concentrations.

$$\text{ppm} = \frac{\text{peak height sample}}{\text{average peak height standard}} \times \frac{1\ \mu\text{g/mL} \times \text{final volume*}}{\text{sample weight (10 g)}}$$

* 0.5-mL final volume for samples

$$\%\ \text{Recovery} = \frac{\text{ppm fortified} - \text{ppm control}}{\text{ppm fortification}} \times 100$$