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VALENT USA CORPORATION
VALENT TECHNICAL CENTER
DUBLIN, CALIFORNIA

DETERMINATION OF FLUMIOXAZIN RESIDUES IN CROPS
RM-30A-3

DATE: March 18, 2003

INTRODUCTION

This method describes the procedure for determining residues of flumioxazin, [2-[7-fluoro-3,4-dihydro-3-oxo-4-(2-propynyl)-2H-1,4-benoxazin-6yl]-4,5,6,7-tetrahydro-1H-isoindole-1,3(2H)-dione] in crops. This method is based on Valent Method RM-30A-1. Briefly, the method involves extraction with acetone:water (4/1, v/v), partition of the residues into dichloromethane, cleanup using Florisil column chromatography, and measurement by gas chromatography utilizing a nitrogen-specific flame-ionization detector or by gas chromatography using a mass spectrometer with selective ion monitoring.

This method is a revision of Valent Method RM-30A-1 to add confirmatory GC/MS conditions.

REAGENTS

Acetone - Pesticide Quality or equivalent.

Acetonitrile - Pesticide Quality or equivalent.

Dichloromethane - Pesticide Quality or equivalent.

Ethyl acetate - Pesticide Quality or equivalent.

Florisil - PR grade, U.S. Silica Co. or equivalent. Blend 2 parts 100-200 mesh and 3 parts 60-100 mesh and activate overnight at 130°C. Allow to cool overnight before using.

Hexane - Pesticide Quality or equivalent.

Sodium chloride - Certified ACS grade or equivalent. Prepare a 5% (w/v) solution by dissolving 5 gram analytical grade crystals in 100 mL deionized water.

Sodium sulfate - Anhydrous, granular, reagent grade.

REFERENCE STANDARDS

Flumioxazin - analytical standard of known purity, available from Valent U.S.A. Corporation. Prepare a stock solution containing 1.0 mg/mL of flumioxazin in acetone as follows: weigh 0.100 g

of flumioxazin and quantitatively transfer to a 100 mL volumetric flask using acetone to rinse the weighing vessel. Dilute to volume with acetone, stopper and shake. Prepare a calibrating/ fortifying solution by diluting this stock solution to 1.0 µg/mL with acetone. Prepare a minimum of four linearity solutions ranging from 0.02 µg/mL to 1.0 µg/mL by diluting the stock solution with acetone. See Note 1. All solutions must be refrigerated when not in use.

EQUIPMENT

Eberbach Reciprocating Shaker or equivalent.

Hobart Food Chopper or equivalent.

Glass Chromatography Columns - 300 x 19 mm i.d. with 250 mL reservoir and Teflon® stopcock. Kontes Cat. # K-420280-0232.

Glass wool.

Pasteur pipettes - 5¾" and 9".

Rotary Vacuum Evaporators - Büchi (Brinkman) equipped with a temperature controlled water bath or equivalent system.

Ultrasonic bath.

Büchner Funnels - 10 cm diameter.

Filter Flasks - 500 mL.

Filter Funnels - 10 cm diameter.

Mason jars - 1 pint with plastic screw cap lids or equivalent.

Round-bottom Flasks - 50 mL, 250 mL, and 500 mL capacity with 24/40 ground glass joints.

Filter paper - Whatman #1, 9 cm diameter.

Separatory Funnels - 250 mL and 500 mL, equipped with Teflon® stopcocks.

Gas Chromatograph – Agilent 6890 equipped with temperature programmable on-column inlet, a nitrogen-phosphorus detector, autosampler, and ChemStation software or equivalent system.

Gas Chromatograph - Agilent 6890, equipped with a pulsed splitless injection port, a mass selective

detector, 7683 automatic sampler, and HP ChemStation (or equivalent system).

ANALYTICAL PROCEDURES

Extraction

Thoroughly grind and mix the entire sample received. For coarse samples such as forage, hay, or straw, grind with dry ice using the Hobart Food Chopper. For grain samples, grind using a Wiley Mill, adding dry ice only if needed. Transfer 10 grams (± 0.1 gram) to a 1 pint Mason jar. At this point, if required by the testing facility, control samples may be fortified for method recovery with an appropriate volume of the 1 µg/mL fortifying solution of flumioxazin (e.g. 0.2 mL of this solution would fortify the sample with 0.02 ppm). See Note 2.

Add 50 mL of acetone:water (4/1, v/v), cap securely and shake on the reciprocating shaker for 10 minutes. Allow this mixture to soak overnight at room temperature then filter the sample into a 500 mL filter flask using a 10 cm Büchner funnel and Whatman #1 filter paper.

Return the filter cake to the Mason jar, add 50 mL of acetone:water (4/1, v/v), cap securely and shake on the reciprocating shaker for 10 minutes. Filter the sample through the Büchner funnel/Whatman #1 filter paper, combining this extract with the first. Rinse the filter cake with two 20-mL portions of extraction solvent.

Water/Dichloromethane Partition

Transfer the filtrate to a 500 mL separatory funnel containing 150 mL of 5% aqueous sodium chloride solution. Add 80 mL of dichloromethane to the separatory funnel in two portions, using each portion to rinse the filter flask. Shake for approximately 1 minute.

Filter the dichloromethane extract through a 10 cm filter funnel containing approximately 50 grams of sodium sulfate (suspended on a plug of glass wool) and collect the extract in a 500 mL round bottom flask.

Repeat the partition and filtration steps with an additional 60 mL portion of dichloromethane. Rinse the sodium sulfate cake with 20 mL of dichloromethane. Evaporate the combined dichloromethane extracts to dryness using a rotary vacuum evaporator equipped with a water bath set at $\leq 40^{\circ}C$.

Hexane/Acetonitrile Partition

Dissolve the residue in 50 mL of hexane saturated with acetonitrile and transfer to a 500 mL separatory funnel. Rinse the round-bottom flask with 50 mL of acetonitrile saturated with hexane and add to the hexane in the separatory funnel. Shake for approximately one minute. Drain the lower acetonitrile phase into the same 500 mL round-bottom flask.

Re-extract the remaining hexane phase with an additional 50 mL of acetonitrile saturated with hexane and drain the lower acetonitrile phase into the round-bottom flask containing the first extract. Discard the hexane. Evaporate the combined acetonitrile extracts to dryness using a rotary vacuum evaporator equipped with a water bath set at $\leq 40^{\circ}C$.

Florisil Column Cleanup (See Note 3)

Place a glass wool plug at the bottom of a 300 mm x 19 mm i.d. glass chromatographic column. Close the column stopcock and add 40 mL hexane/ethyl acetate (2/1, v/v) to the column. Slowly add 15 grams of activated Florisil to the column while gently tapping the side of the column. Rinse the sides of the column with a small amount of hexane/ethyl acetate (2/1, v/v) (typically two or three 3 mL portions). Add 2-4g. of glass wool to the top of the column. The glass wool should be packed in the portion of the column occupied by the solvent. Open the stopcock and allow the solvent to drain to the top of the glass wool if necessary.

Redissolve the concentrated sample residue in 1 mL of ethyl acetate, dilute with 2 mL of hexane, and sonicate for approximately 15 seconds. Transfer the extract to the top of the column. Rinse the round bottom flask with three 3-mL portions of hexane/ethyl acetate (2/1, v/v). Transfer each rinse to the column, allowing each portion to drain to the top of the column bed before adding the next rinse.

Elute the column with an additional 18 mL of hexane/ethyl acetate (2/1, v/v) (total volume 30 mL). Discard this eluate. Place a 250 mL round bottom flask under the column and elute the flumioxazin with 70 mL of hexane/ethyl acetate (2/1, v/v).

Evaporate this eluate to dryness using a rotary vacuum evaporator equipped with a water bath set at $\leq 40^{\circ}C$.

Measurement

Redissolve the residue in 5 mL acetone (for a 0.01 ppm limit of detection). The sample may be diluted with acetone if the flumioxazin concentration is expected to exceed the highest linearity standard. Quickly transfer the sample to an autosampler vial using a Pasteur pipette and seal immediately to minimize evaporation losses.

Analyze a range of linearity standards with the analytical sequence. The recommended sequence of samples and standards for analysis is: calibrating standard, sample, sample, sample, calibrating standard, etc. This sequence may, however, be modified if the reproducibility requirement is met. (See Note 1). Each sequence must begin and end with a calibration standard.

Analyze using the following GC parameters:

Column: RTX-200 (15 M x 0.53 mm ID, 0.5 µm film thickness)
Restek or equivalent.

Column Temperature Program:
Initial Temperature: 40°C
Initial Hold Time: 0 minute
Program Rate: 30°C/minute
Final Column Temperature: 300°C
Final Hold Time: 10 minutes

Injection
Mode: on Column
Injector Temperature Program:
Initial Temp: 45°C
Hold time: 0.1 minutes
Prog Rate: 250°C/minute
Final Temp: 300°
Mode: Constant Pressure
Flow: 2 mL/minute
Carrier gas: helium

Detector
Temp.: 300°C
Hydrogen Flow: 3 mL/min.
Air Flow: 60 mL/min.
Mode: Constant makeup flow of 4 mL/min. helium

Injector
Volume: 1 µL

The parameters shown are given only as a guide. They may be modified as needed to optimize the chromatography or to resolve matrix interferences. Each set of chromatograms must be clearly labeled with the GC parameters used.

If matrix interferences are encountered during the analysis of flumioxazin, sample extracts may be re-injected using the following alternate GC/MS parameters:

Condition the instrument with at least six injections of sample extract and analyze using the following GC/MS conditions:

Column: HP-1MS capillary, (30 M x 320 µm, 0.30 µm film thickness).

Column Temperature Program:
- Initial Temperature: 40°C
- Initial Hold Time: 0.5 minute
- Program Rate: 30°C/minute
- Final Column Temperature: 300°C
- Final Hold Time: 2 minutes

Injection
- Mode: Pulsed Splitless
- Temp: 300°C
- Pressure: 1.54 psi
- Pulse Pressure: 30.0 psi
- Pulse Time: 0.50 min.
- Purge Flow: 5.4 mL/min.
- Purge Time: 0.50 min.
- Total Flow: 9.7 mL/min.
- Carrier gas: helium
- Injection Volume: 0.5 µL

Detector:	EI / MS
Transfer Line Temperature:	280°C
Quadrapole Temperature:	150°C
Source Temperature:	230°C
Acquisition mode:	Selective Ion Monitoring (SIM)

Flumioxazin

Retention Time:	10.03 minutes
Acquisition Interval:	9.0 – 11.0 minutes
Molecular Ion:	354.0
Qualifier Ion:	287.0
Dwell Time:	80 msec (each ion)

The GC/MS parameters shown above are given only as a guide. They may be modified as needed to optimize the chromatography, to resolve matrix interferences, or to utilize other mass spectrometers. Each set of chromatograms must be clearly labeled with the GC/MS parameters used.

CALCULATION

Use the following formula to calculate the amount of flumioxazin present in the samples analyzed by GC/NP:

$$ppm = \frac{B \times C \times V \times DF}{A \times W}$$

Where

B = integration counts for flumioxazin in the sample.
C = concentration of flumioxazin in the calibrating standard (0.5 µg/mL).
V = final volume of the sample extract (5 mL).
DF = dilution factor, used if the sample extract is diluted prior to analysis.
A = mean integration counts for the flumioxazin calibrating standards.
W = sample weight (10 grams).

When samples are quantitated by GC/MS the amount of flumioxazin present may be calculated as above or by utilizing a linearity curve incorporating five or more data points to determine the instrument response over a range of analyte concentrations.

The concentration of flumioxazin in the sample extracts is calculated on the basis of peak area using a second order polynomial equation. The equation is automatically generated through the use of the graphing functions of an Excel spreadsheet (See Note 5). The data is presented graphically as concentration of the linearity standards verses the peak areas of the linearity standards which results in the following equation:

$$Y=Ax^2+Bx+C$$

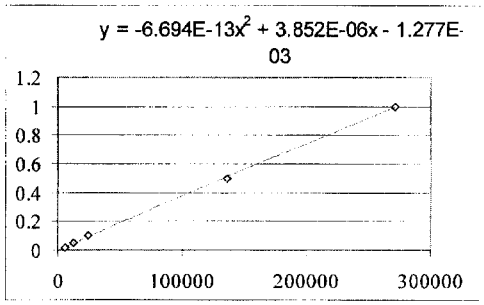
The data is weighted inversely proportional to the concentration of each standard. The weighting is accomplished by incorporating each data point into the graph with a frequency equal to (1/concentration). For example, a data point for a linearity standard with a concentration of 0.01 µg/mL would be entered into the graph 100 times while a data point for a linearity standard with a concentration of 1.0 µg/mL would be entered into the same graph 1 time.

Example:

For a linearity area response of:

µg/mL	Area
1.0	271173
0.5	136711
0.1	24051
0.05	12963
0.02	6044

The resulting graph from the Excel spreadsheet is as follows:



$Y = Ax^2 + Bx + C$

A = -6.694E-13

B = 3.852E-06

C = -1.277E-03

To ensure that the equation is appropriate, the areas of the linearity standards are entered into the equation of the line and calculated as samples. The standards must calculate within 20% of their known concentration. An example of this from the above data is the 1.0 µg/mL standard which has an area of 271173. The calculated concentration would be 0.9941 µg/mL which is 99% of the known concentration.

A sample extract with an area response of 10260 would have a concentration as follows:

$$\mu g/mL = Ax^2 + Bx + C$$

$$\mu g/mL = (-6.694E\text{-}13 \times 10260 \times 10260) + (3.852E\text{-}06 \times 10260) + -1.277E\text{-}03$$

$$\mu g/mL = 0.0382$$

The amount of flumioxazin in the sample is calculated using the following

formula:

$$ppm = \frac{C \times FV \times TV \times DF}{W \times AV}$$

where:

C = Calculated concentration of analyte found in the sample extract. (µg/mL)
FV = Final volume of sample extract. (5 mL)
TV = Total volume of sample extract. (100 mL)
DF = Dilution factor, used if sample extract is diluted prior to analysis.
W = Sample weight in grams. (10 g)
AV = Aliquot volume of sample extract used. (100 mL)

LIMITS OF DETECTION AND QUANTITATION

The limit of detection for flumioxazin in crops analyzed by this method is 0.01 ppm. The validated limit of quantitation for flumioxazin in crops analyzed by this method is 0.02 ppm.

ANALYSIS TIME

A trained analyst can complete the analysis of a set of eight samples for flumioxazin in approximately 8 hours. The results are available within 24 hours of initiating the analysis.

NOTES

1. At Valent, the linearity of the gas chromatographic system must be verified each day that samples are analyzed. Linearity is determined by analyzing at least four linearity standards ranging in concentration from 0.02 µg/mL to 1.0 µg/mL. The concentration of these standards and the final volume of the sample extract may be changed to take advantage of individual instrument conditions. The mean of the response factors (response equivalent to 1 µg/mL) should have a coefficient of variation of ±10% or less for GC/NPD and ±15% or less for GC/MS. Deviations to this requirement require the approval of the chemist responsible for the analysis. Sample extracts must be diluted to bring the concentration of flumioxazin within the range of linearity established.

2. At Valent, a standard operating procedure requires that at least one fortified control sample be analyzed with each set of samples. If the testing facility does not require concurrent analysis of fortified control samples, or if a UTC sample is not available, this method requirement may be waived.

The level of fortification is generally 0.02 ppm (the LOQ of the method) and/or 0.10 ppm. These fortifications are made by adding 0.2 mL of the 1.0 µg/mL fortifying solution and 0.1 mL of the 10 µg/mL fortifying solution to a 10 gram sample. Method recovery must be between 70% to 120% to be acceptable unless approved by the chemist responsible for the analysis. Method recovery results are only used to verify method performance; they are never used to correct results found in field samples.

3. Each batch of Florisil must be checked for recovery of flumioxazin as follows: Transfer 1.0 mL of the 1.0 µg/mL flumioxazin fortifying solution to a 50 mL round-bottom flask and evaporate to dryness using a rotary-evaporator and water bath set to <40°C. Transfer the residue to a Florisil column and elute the flumioxazin as described under Florisil Column Cleanup. Evaporate the eluate to dryness, add 1.0 mL of acetone and swirl to completely dissolve the residue. Analyze this eluant and the 1.0 µg/mL standard as described under Measurement. If the flumioxazin peak for the eluant is less than 90% of the calibrating standard, then the elution profile of flumioxazin must be determined.

4. At Valent, the reproducibility of an analytical run is determined by calculating the CV from the peak units obtained for the calibrating standards analyzed during the run. For a run to be acceptable, this CV must be 10% or less unless approved by the chemist responsible for the analysis.

5. There are other programs that can calculate a weighted regression graph such as Curve Expert 1.3 (Hyams Development, Starkville, MS).

REFERENCE

1. Pensyl, J., Determination of Flumioxazin Residues in Crops, Residue Method RM-30A-1, January 8, 1996.

Written by: _____ Date: 18 MAR 03
T. Schreier

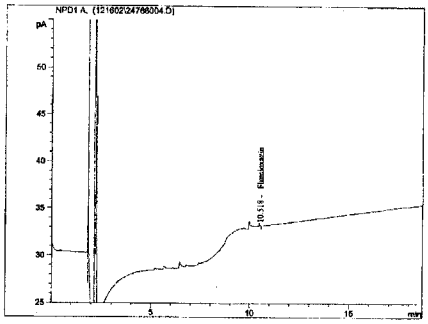
Reviewed by: _____ Date: 3/18/03
G. H. Fujie

Reviewed by: _____ Date: 3/18/03
L. Massey, QAU

Figure 1

Reference Standard
Flumioxazin
0.02 µg/mL

GC/NPD



GC/MS

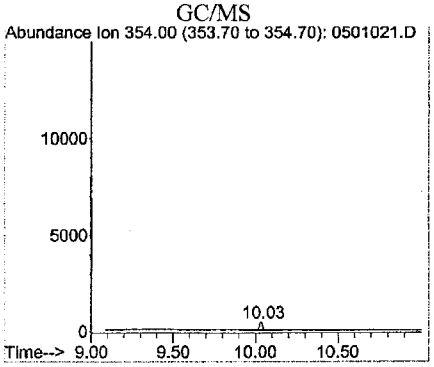
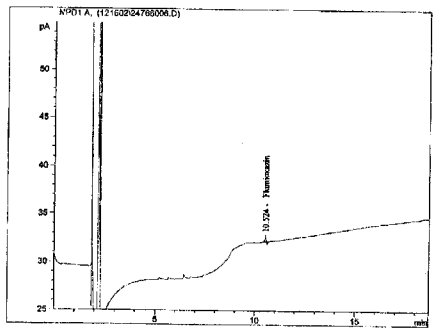


Figure 2

Reference Standard
Flumioxazin
0.1 µg/mL

GC/NPD



GC/MS

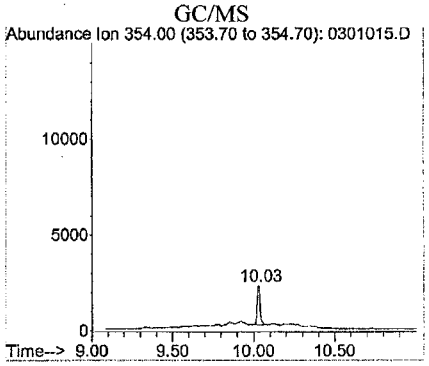
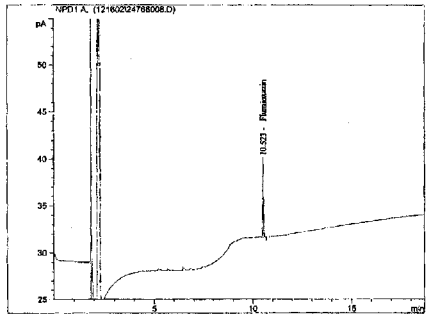


Figure 3

Reference Standard
Flumioxazin
1.0 µg/mL

GC/NPD



GC/MS

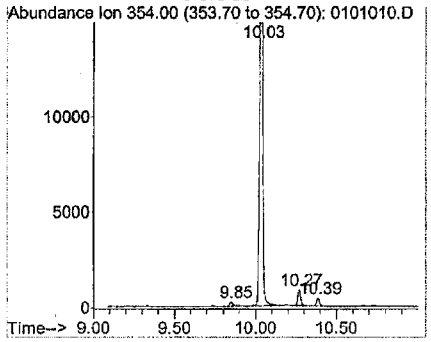
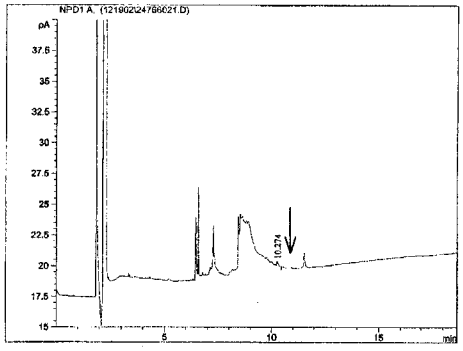


Figure 4

Control Peanuts
(2 mg crop equivalent/µL in acetone)

GC/NPD



GC/MS

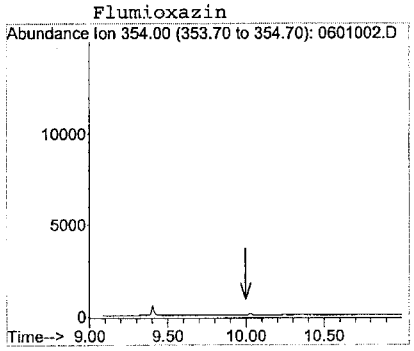
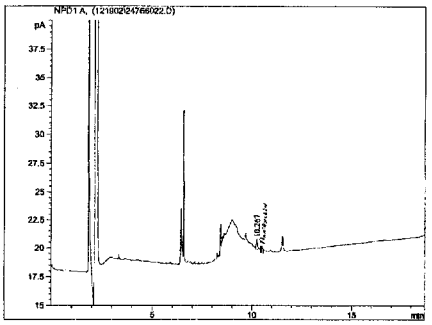


Figure 5

Control Peanuts
Fortified at 0.02 ppm
(2 mg crop equivalent /µL in acetone)

GC/NPD



GC/MS

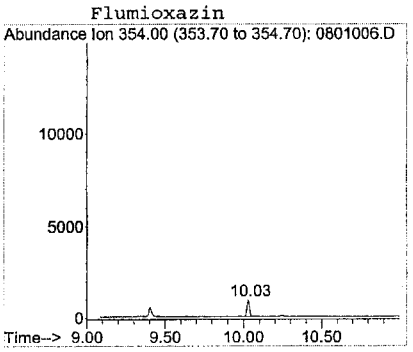
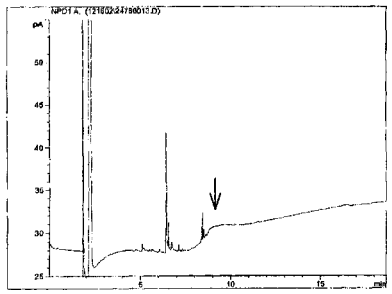


Figure 6

Control Soybeans
(2 mg crop equivalent /µL in acetone)

GC/NPD



GC/MS

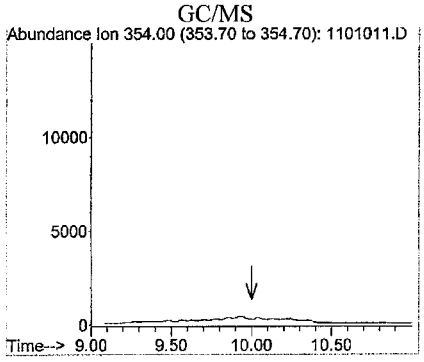
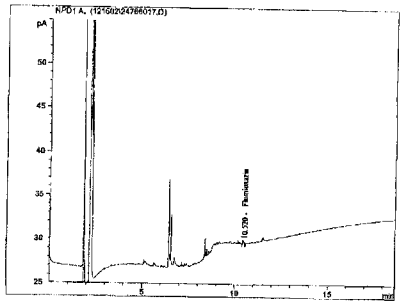


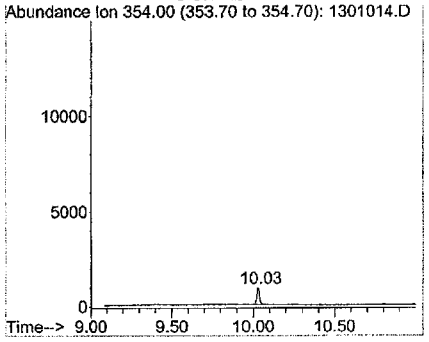
Figure 7

Control Soybeans
Fortified at 0.02 ppm
(2 mg crop equivalent /µL in acetone)

GC/NPD



GC/MS



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VALENT TECHNICAL CENTER

Validation Report For Residue Method RM-30A-3
Reproducibility of Analysis

ANALYTE: Flumioxazin

Sample Matrix	Fortification Level ppm	Amount Recovered ppm	% Recovery
Control	---	---	---
Peanuts	0.02	0.020	100
Peanuts	0.02	0.022	109
Peanuts	0.02	0.023	114

Mean Recovery:	108 %
Standard Deviation:	6.6
n:	3

Reference: V-25611, page 1.
GC/NPD

Julian Kovalsky 3/20/03
Analyst Date

Thom Schmi 18 MAR 2003
Reviewed by Date

3/18/03
Approved Date

Lindy Massey 3/18/03
Reviewed (QAU) Date

VALENT U.S.A. CORPORATION
VALENT TECHNICAL CENTER

Validation Report For Residue Method RM-30A-3
Reproducibility of Analysis

ANALYTE: Flumioxazin

Sample Matrix	Fortification Level ppm	Amount Recovered ppm	% Recovery
Control	---	---	---
Peanuts	0.02	0.019	94
Peanuts	0.02	0.019	95
Peanuts	0.02	0.022	108

Mean Recovery:	99 %
Standard Deviation:	7.9
n:	3

Reference: V-25611, page 1.
GC/MS

Julian Kornbluh 3/20/03
Analyst Date

Thomas Schmidt 18 MAR 2003
Reviewed by Date

[signature] 3/18/03
Approved Date

Linda Massey 3/18/03
Reviewed (QAU) Date

VALENT U.S.A. CORPORATION
VALENT TECHNICAL CENTER

Validation Report For Residue Method RM-30A-3
Reproducibility of Analysis

ANALYTE: Flumioxazin

Sample Matrix	Fortification Level ppm	Amount Recovered ppm	% Recovery
Control	---	---	---
Soybeans	0.02	0.018	90
Soybeans	0.02	0.019	95
Soybeans	0.02	0.019	95

Mean Recovery:	93 %
Standard Deviation:	3.1
n:	3

Reference: V-24766, page 1.
GC/NPD

Julian Kowalsky 3/20/03
Analyst Date

18 MAR 2003
Reviewed by Date

3/18/03
Approved Date

Linda Massey 3/18/03
Reviewed (QAU) Date

VALENT U.S.A. CORPORATION
VALENT TECHNICAL CENTER

Validation Report For Residue Method RM-30A-3
Reproducibility of Analysis

ANALYTE: Flumioxazin

Sample Matrix	Fortification Level ppm	Amount Recovered ppm	% Recovery
Control	---	---	---
Soybeans	0.02	0.019	93
Soybeans	0.02	0.018	92
Soybeans	0.02	0.019	94

Mean Recovery:	93 %
Standard Deviation:	1.1
n:	3

Reference: V-24766, page 1.
GC/MS

Julianne Kowalsky 3/20/03
Analyst Date

[signature] 18 MAR 2003
Reviewed by Date

[signature] 3/18/03
Approved Date

Linda Marcy 3/18/03
Reviewed (QAU) Date