

Flumioxazin

VALENT USA CORPORATION
VALENT TECHNICAL CENTER
DUBLIN, CALIFORNIA

DETERMINATION OF FLUMIOXAZIN
RESIDUES IN CROPS
RESIDUE METHOD RM-30A-1

DATE: JANUARY 23, 1990
REVISED: JANUARY 8, 1996

INTRODUCTION

This method describes the procedure for determining residues of flumioxazin, [V-53482, 7-fluoro-6-{{(3,4,5,6-tetrahydro)phthalimido}-4-(2-propynyl)-1,4-benzoxazin-3(2H)-one}] in crops. This method is based on a method developed by Sumitomo Chemical Co., Ltd¹. 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.

This method was revised on August 5, 1991 to add details regarding the preparation of standard solutions, the grinding and homogenizing of samples, and the fortification of samples for determining method recovery. The GC column parameters were also changed at that time. The method was further revised on November 19, 1992 to make minor changes to the text and to change the company name from Chevron to Valent. The January 8, 1996 revision was made to add confirmatory GC column conditions and to make other changes requested by EPA following their validation of the method.

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 $\mu\text{g/mL}$ with acetone. Prepare a minimum of four linearity solutions ranging from 0.1 $\mu\text{g/mL}$ to 2.0 $\mu\text{g/mL}$ 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.

Pastuer pipets - 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.

EQUIPMENT (CONTINUED)

Gas Chromatograph - Hewlett-Packard 5890A equipped with packed column injector port with megabore adaptor, a nitrogen-phosphorus flame ionization detector, autosampler, and integrator or equivalent system.

Wiley Mill or equivalent.

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 $\mu\text{g}/\text{mL}$ fortifying solution of flumioxazin (e.g. 0.1 mL of this solution would fortify the sample with 0.01 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 and add 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 freshly washed with 20 mL of dichloromethane) 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 $\leq 40^\circ\text{C}$. See Note 3.

Hexane/Acetonitrile Partition

Dissolve the residue in 50 mL of hexane saturated with acetonitrile and transfer to a 250 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 $\leq 40^{\circ}\text{C}$. See Note 3.

Florisil Column Cleanup (See Note 4)

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). Open the stopcock and allow the solvent to drain to the top of the column bed.

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 28 mL of hexane/ethyl acetate (2/1, v/v) (total volume 40 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 $\leq 40^{\circ}\text{C}$. Transfer the residue to a 50 mL round bottom flask using three 5-mL portions of acetone (sonicate the flask for approximately 15 seconds if necessary) and evaporate to dryness using a rotary vacuum evaporator equipped with a water bath set at $\leq 40^{\circ}\text{C}$. See Note 3.

MEASUREMENT

Redissolve the residue in 1.0 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. See Note 1. Quickly transfer the sample to an autosampler vial using a Pasteur pipet and seal immediately to minimize evaporation losses. Load the autosampler with the sample and reference standard vials and analyze using the following parameters:

Column: DB-17 (15 M x 0.53 mm ID, 1.0 μ m film thickness)
J & W Scientific Cat # 125-1712 or equivalent.

Column Temperature Program:
Initial Temperature: 250°C
Initial Hold Time: 1 minute
Program Rate: 20°C/minute
Final Column Temperature: 280°C
Final Hold Time: 8 minutes

Carrier Gas Flow Rate: 10 mL/min (He)
Auxiliary Gas Flow Rate: 25 mL/min (He)
Hydrogen Flow Rate: 3.5 mL/min
Air Flow Rate: 110 mL/min
Injector Temperature: 275°C
Detector Temperature: 300°C
Injection volume: 1 μ l
Retention Time: 5.7 min. (See Figure 1)

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 labelled with the GC parameters used. See Note 5 for alternative GC parameters.

The recommended sequence of analysis of samples and standards is: conditioning sample, calibrating standard, sample, calibrating standard, sample, calibrating standard, sample, etc. This sequence may be modified if the reproducibility requirement is met. See Note 6.

CALCULATION

Use the following formula to calculate the amount of flumioxazin present in the samples:

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

Where

- B = integration counts for flumioxazin in the sample.
- C = concentration of flumioxazin in the calibrating standard (1.0 μ g/mL).
- V = final volume of the sample extract (1.0 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).

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 soil 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 (Valent SOP #VR-007). Linearity is determined by analyzing at least four linearity standards ranging in concentration from 0.1 $\mu\text{g/mL}$ to 2.0 $\mu\text{g/mL}$. The mean of the response factors (response equivalent to 1 $\mu\text{g/mL}$) should have a coefficient of variation of $\pm 10\%$ or less. 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 (SOP# VR-002) 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 and 1.0 mL, respectively, of the 1.0 $\mu\text{g/mL}$ 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. Samples must be removed from the rotary evaporator immediately after the solvent has evaporated to avoid loss of flumioxazin.
4. Each batch of Florisil must be checked for recovery of flumioxazin as follows: Transfer 1.0 mL of the 1.0 $\mu\text{g/mL}$ flumioxazin fortifying solution to a 50 mL round-bottom flask and evaporate to dryness using a rotary-evaporator and water bath set to $<40^\circ\text{C}$. 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 $\mu\text{g/mL}$ calibrating 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.

NOTES (CONTINUED)

5. If matrix interferences are encountered during the analysis of flumioxazin, the following GC parameters may be used:

Column: DB-1 (30 M x 0.53 mm ID, 1.5 μ m film thickness)
J & W Scientific Cat # 125-1032 or equivalent.

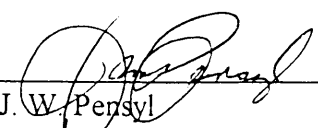
Column Temperature Program:
Initial Temperature: 250°C
Initial Hold Time: 1 minute
Program Rate: 20°C/minute
Final Column Temperature: 275°C
Final Hold Time: 8 minutes

Carrier Gas Flow Rate: 20 mL/min (He)
Auxiliary Gas Flow Rate: 10 mL/min (He)
Hydrogen Flow Rate: 3.8 mL/min
Air Flow Rate: 100 mL/min
Injector Temperature: 300°C
Detector Temperature: 300°C
Injection volume: 2 μ l

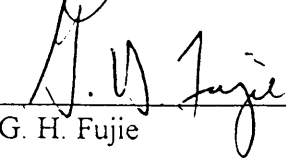
6. At Valent, the reproducibility of an analytical run is determined by calculating the CV from the peak units obtained for the calibrating standards analyzed during the run. For a run to be acceptable, this CV must be 10% or less unless approved by the chemist responsible for the analysis (Valent SOP #VR-013).

REFERENCE

1. Ohnishi, J., Hirota, M. and Yamada, H., Report No. ER-MT-8940, "Residue Analytical Method for S-53482 in Soybean Forage and Seed," November 24, 1989.

Written by: 
J. W. Pensyl

Date: 1/9/96

Reviewed by: 
G. H. Fujie

Date: 1/11/96

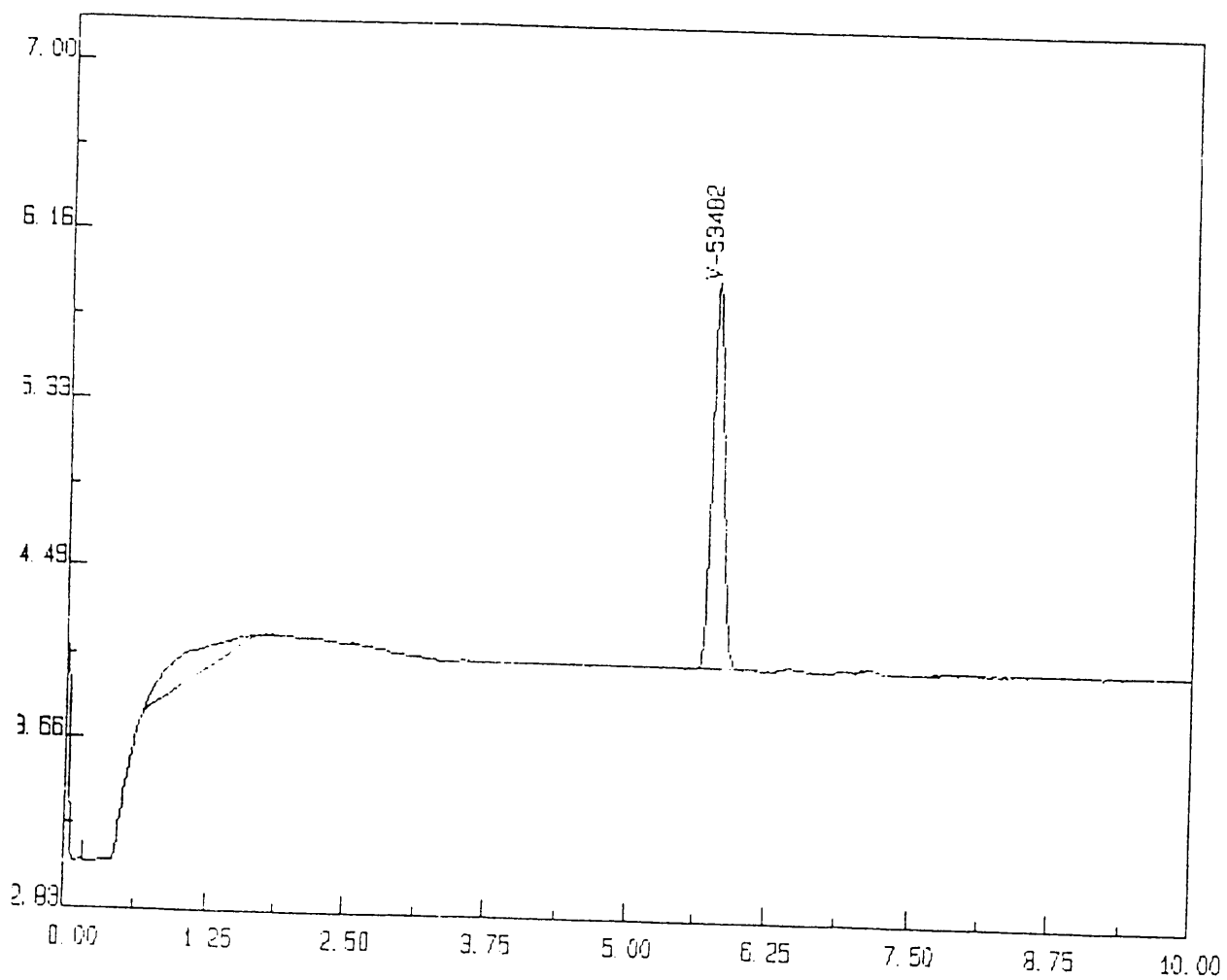


Figure 1. Calibration Standard
1.0 $\mu\text{g/mL}$ of Flumioxazin
1.0 μl injected (1 ng) on DB-17 column

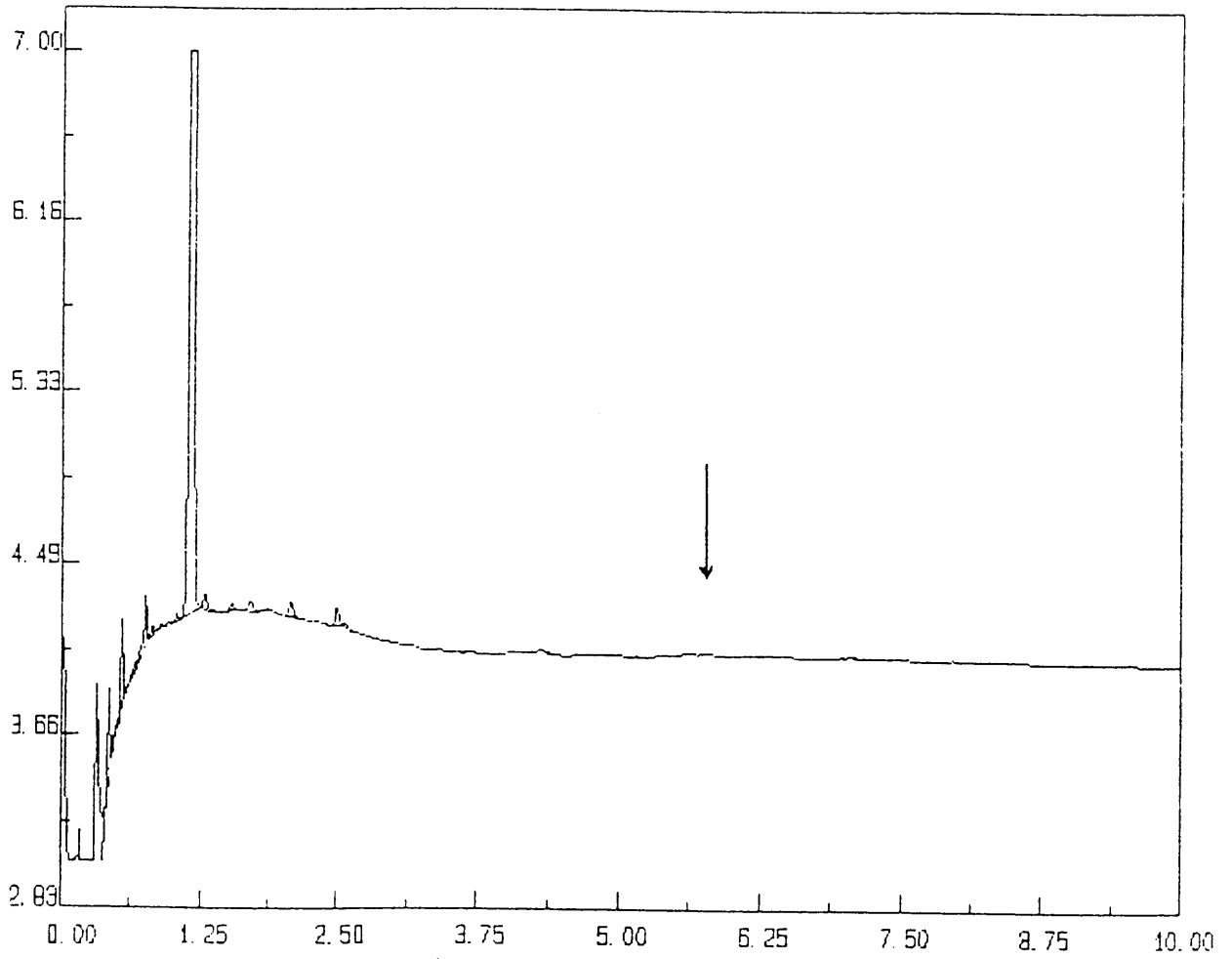


Figure 2. Untreated Control Soybean Seed
1.0 μ l injected on DB-17 column
(10 mg sample equivalent)

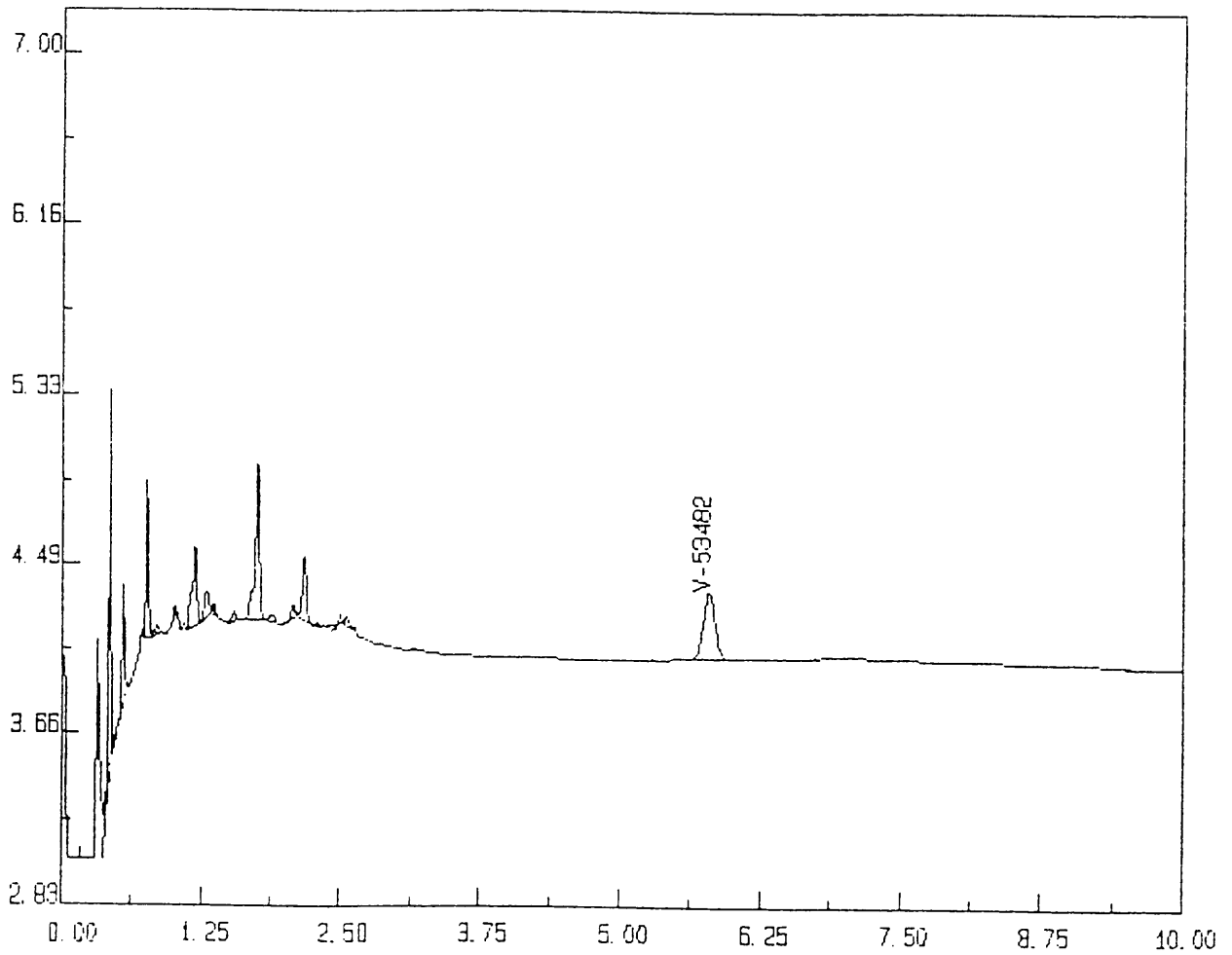


Figure 3. Soybean Seed Fortified with 0.02 ppm of Flumioxazin
1.0 μ l injected on DB-17 column (10 mg sample equivalent)

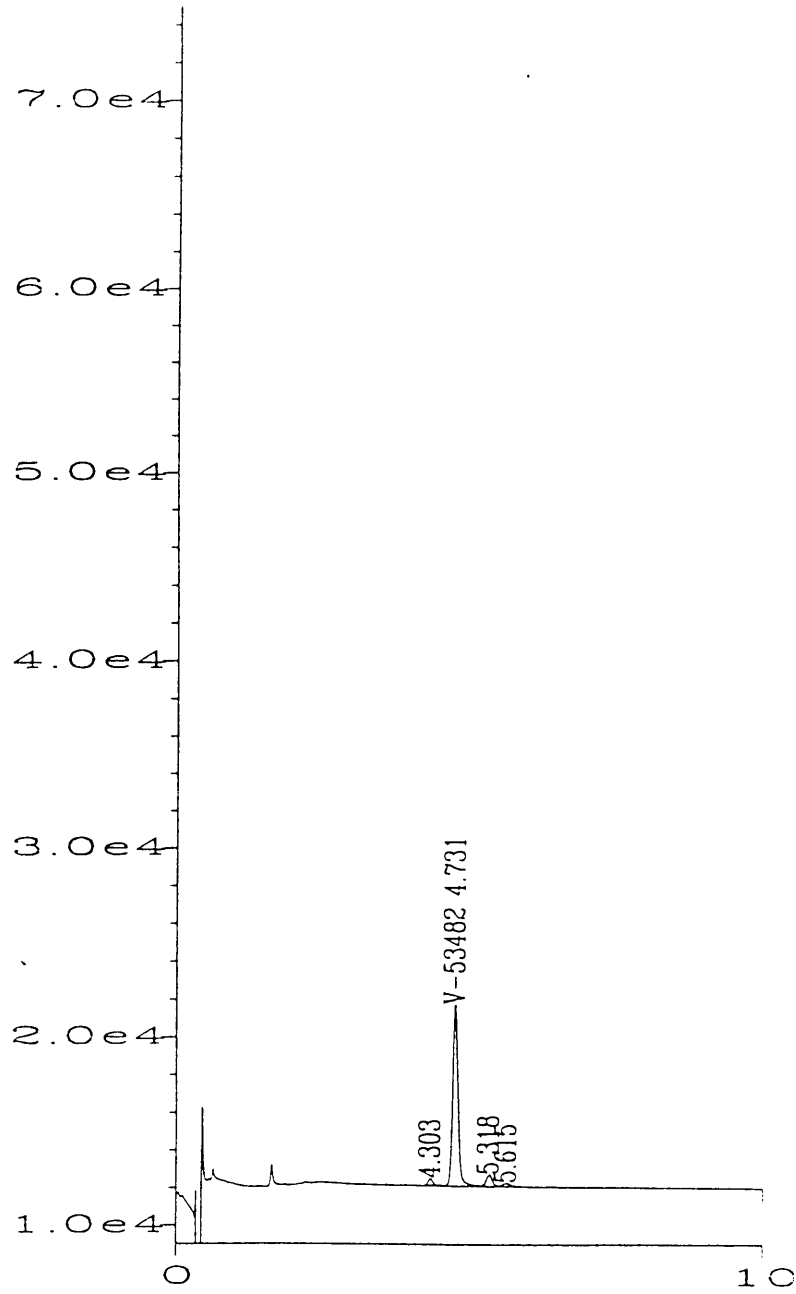


Figure 4. Calibration Standard
1.0 $\mu\text{g/mL}$ of Flumioxazin
2.0 μl injected (2 ng) on DB-1 column

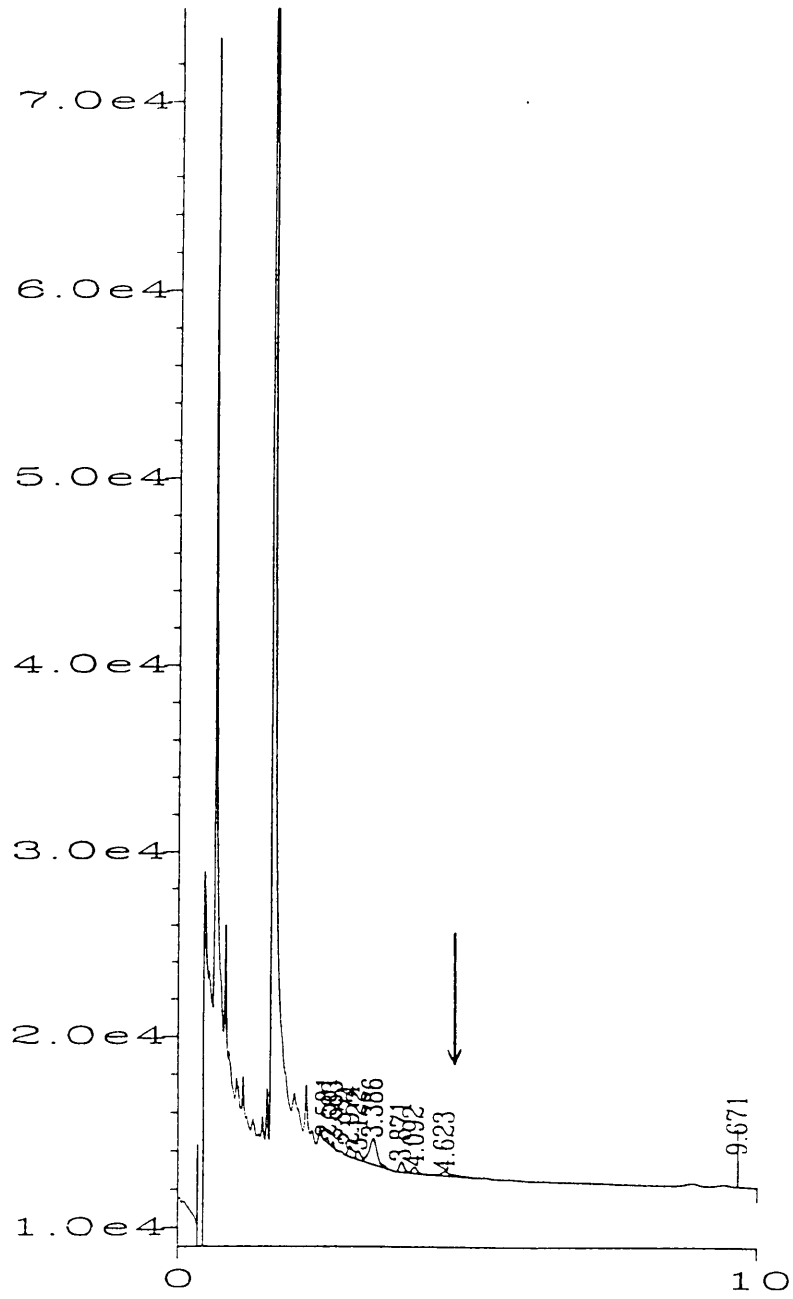


Figure 5. Untreated Control Soybean Seed
2.0 μ l injected on DB-1 column
(20 mg sample equivalent)

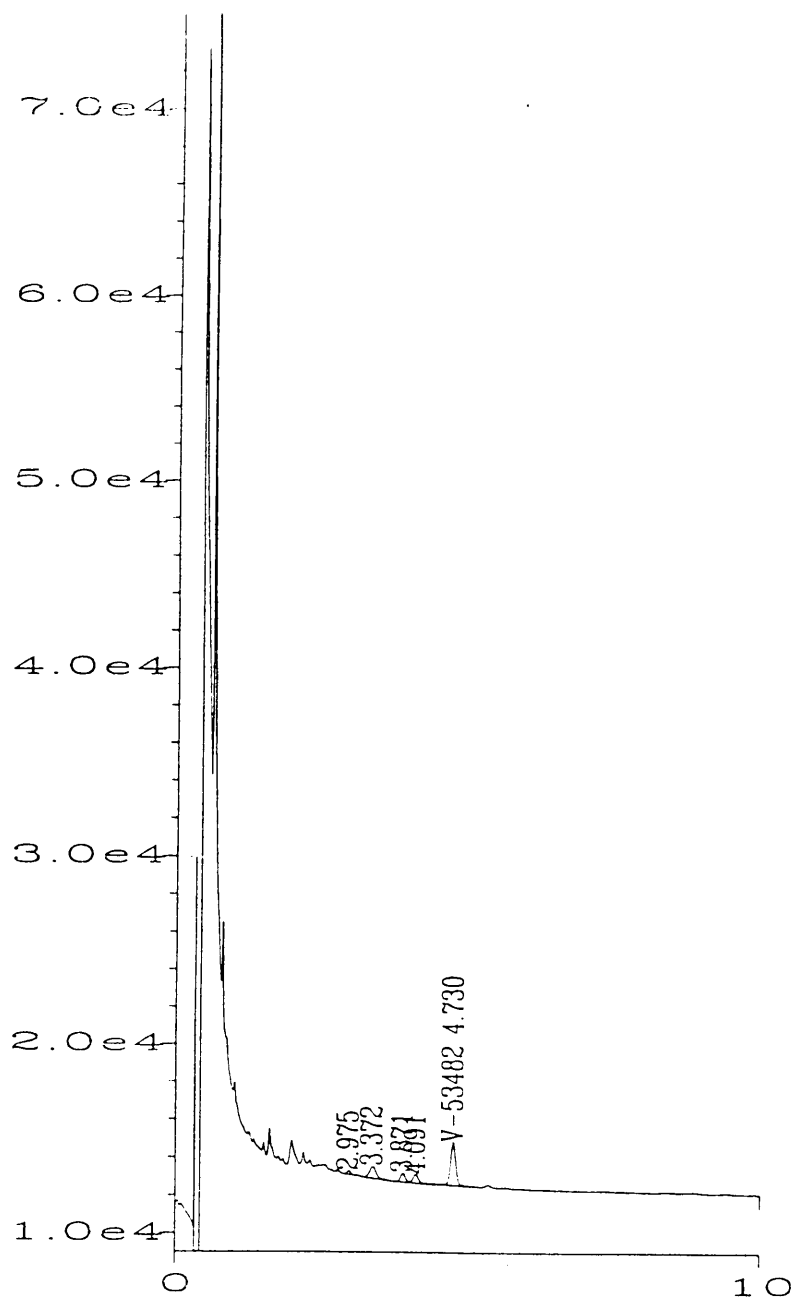


Figure 6. Soybean Seed Fortified with 0.02 ppm of Flumioxazin
2.0 μ l injected on DB-1 column (20 mg sample equivalent)

CHEVRON CHEMICAL COMPANY
RESIDUE CHEMISTRY LABORATORY

ANALYTICAL METHOD NO. PM 35A VALIDATION REPORT

SOP RE-02-1	Reproducibility	Reproducibility (aged)	L.O.D.
Sample Matrix	<u>Corn Grain</u>		
Level:	<u>0.1 ppm</u>	_____	<u>0.01 ppm</u>
X =	<u>0.0917 mg</u>		<u>0.01 mg</u>
% of Nominal =	<u>91.7</u>		<u>100</u>
C.V. =	<u>4.6</u>		<u>0</u>
n =	<u>6</u>		<u>3</u>
Notebook Reference:	<u>3230-0014</u>		<u>3230-0013</u>

mg

Analyst(s)

[Signature]

Approved

1/18/90

Date

1/18/90

Date

Comments:

Efficacy of Extraction Procedure

Procedure used: Column Recovery Check 96.8 99.2 $\bar{x} = 98\% \text{ rec}$

Notebook Reference: 10134-09

mg

Analyst(s)

[Signature]

Approved

1/18/90

Date

1/18/90

Date

Comments:

Reviewed by: [Signature]

CHEVRON CHEMICAL COMPANY
RESIDUE CHEMISTRY LABORATORY

ANALYTICAL METHOD NO. RM-30A VALIDATION REPORT

SOP RE-02-1	Reproducibility	Reproducibility (aged)	L.O.D.
Sample Matrix	SOYBEAN HAY		
Level:	<u>0.1 PPM</u>	_____	_____
X =	9.70×10^{-2}	_____	_____
% of Nominal =	97.0 %	_____	_____
C.V. =	$\pm 2.4 \%$	_____	_____
n =	3	_____	_____

Notebook Reference: 3230-0031

WGL

Analyst(s)

3/23/90

Date

[Signature]
Approved

3/23/90

Date

Comments:

Recalculation only

Efficacy of Extraction Procedure

Procedure used:

Notebook Reference:

Analyst(s)

Date

Approved

Date

Comments:

Reviewed by: _____

CHEVRON CHEMICAL COMPANY
RESIDUE CHEMISTRY LABORATORY

ANALYTICAL METHOD NO. RM-30A VALIDATION REPORT

SOP RE-02-1	Reproducibility	Reproducibility (aged)	L.O.D.
Sample Matrix	CORN GRAIN		
Level:	<u>0.1 PPM</u>	_____	_____
X =	0.0830		
% of Nominal =	83 %		
C.V. =	5.6		
n =	3		

Notebook Reference: 3230-0057

mm

Analyst(s)

4/16/90

Date

[Signature]

Approved

4/16/90

Date

Comments:

ACETONITRILE REPLACED WITH METHANOL

Efficacy of Extraction Procedure

Procedure used:

Notebook Reference:

Analyst(s)

Date

Approved

Date

Comments:

Reviewed by: _____

VALENT U.S.A. CORPORATION
DUBLIN LABORATORY

ANALYTICAL METHOD NO. RM-30A VALIDATION REPORT

SOP VR-002-00 Reproducibility

Sample Matrix Peanut Vines

Analyte	<u>V-53482</u>	<u>V-53482</u>
Level =	0.020 ppm	0.100 ppm
x =	0.018 ppm	0.095 ppm
% of Nominal =	90.89% <u>CEP 5/19/94</u>	95.9%
c. v. =	4.62 5.56	3.97
n =	3	3

Notebook Reference: 1040-004

G. Poser
Analyst(s)

11/2/92
Date

[Signature]
Approved

11/18/92
Date

Comments:

Reviewed by: M. M. [Signature] 11-18-92

VALENT U.S.A. CORPORATION
DUBLIN LABORATORY

ANALYTICAL METHOD NO. RM-30A VALIDATION REPORT

SOP VR-002-00

Reproducibility

Sample Matrix Peanut Hay

Analyte	<u>V-53482</u>	<u>V-53482</u>
Level =	0.020 ppm	0.100 ppm
x =	0.017 ppm	0.086 ppm
% of Nominal =	82 ³ 70 (CE) 5/19/94	86% ⁷⁰
c. v. =	4.39 3.46	2.33
n =	3	3

Notebook Reference: 1040-005

S. Posny
Analyst(s)

11/2/92
Date

G. P. [Signature]
Approved

11/18/92
Date

Comments:

Reviewed by: [Signature] 11/18/92

VALENT U.S.A. CORPORATION
DUBLIN LABORATORY

ANALYTICAL METHOD NO. RM-30A VALIDATION REPORT

SOP VR-002-00

Reproducibility

Sample Matrix Peanut Meat

Analyte

V-53482

V-53482

Level = 0.100 ppm

0.020 ppm

X = 0.091 ppm

0.018 ppm

% of Nominal = 91%

89%

C. V. = 4.06

~~1.46~~ 2.51 CE 5/19/94

n = 6

5

Notebook Reference: 1040-001 & 1040-002

Goldwanda Posey
Analyst(s)

11/2/92
Date

Sam Posey
Approved

11/18/92
Date

Comments:

Reviewed by: 9/18/92 11-18-92

VALENT U.S.A. CORPORATION
DUBLIN LABORATORY

ANALYTICAL METHOD NO. RM-30A VALIDATION REPORT

SOP VR-002-00

Reproducibility

Sample Matrix Peanut Hulls

Analyte	<u>V-53482</u>	<u>V-53482</u>
Level =	0.020 ppm	0.100 ppm
X =	0.020 ppm	0.090 ppm
% of Nominal =	101%	90%
c. v. =	1.87 2.84 ^{CE} _{5/19/94}	1.28
n =	3	3

Notebook Reference: 1040-003

J. Poser
Analyst(s)

11/2/92
Date

Jim Poser
Approved

11/18/92
Date

Comments:

Reviewed by: M. H. H. H. H. 11-11-92

VALENT U.S.A. CORPORATION
VALENT TECHNICAL CENTER

VALIDATION REPORT FOR RESIDUE METHOD RM-30A-1
REPRODUCIBILITY OF ANALYSIS (REF. SOP VR-002)

ANALYTE: FLUMIOXAZIN

Sample Matrix	Fortification Level (ppm)	Result (ppm)	% Recovery
Soybean Seed	2.00E-02	2.10E-02	103
Soybean Seed	2.00E-02	2.20E-02	112
Soybean Seed	2.00E-02	2.00E-02	102
		Mean =	106
		CV =	5.21
		n =	3
Soybean Seed	1.00E-01	1.11E-01	111
Soybean Seed	1.00E-01	9.30E-02	93
Soybean Seed	1.00E-01	1.04E-01	104
		Mean =	103
		CV =	8.84
		n =	3

Notebook Reference: 10719-070

Comments:

* Data for these samples obtained on alternate GC column (DB-1).

M. Clark
Analyst 1/9/96
Date

[Signature]
Approved 1/9/96
Date

[Signature]
Reviewed by 1/11/96
Date