US EPA ARCHIVE DOCUMENT

Ciba-Geigy Corporation
Ciba Plant Protection
Biochemistry Department
P. O. Box 18300
410 Swing Road
Greensboro, NC 27419

Validated

ANALYTICAL METHOD FOR THE DETERMINATION OF FENOXYCARB IN PASTURE GRASSES AND CROPS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH COLUMN SWITCHING

ANALYTICAL METHOD NO. AG-609A

Project Number: 343002

SUBMITTED BY:
R. E. M. Wurz Title: Scientist III

Signature: R E M Wurz

Stacey E. Boyette Title: Scientist I

Signature: Stacey E Boyette

APPROVED BY:
W. T. Beidler Title: Manager, Residue Chemistry

Signature: W. T. Beidler

Date: 5-11-94

TABLE OF CONTENTS

		Page No.
I.	SUMMARY AND INTRODUCTION	5
	A. Scope	5
	B. Principle	5
II.	MATERIALS AND METHODS	6
	A. Apparatus	6
	B. Reagents	6
	C. Analytical Procedure	7
	1.0 Sample Preparation	7
	2.0 Extraction	7
	3.0 Partition Cleanup	8
	4.0 Silica Column Cleanup	10
	5.0 Florisil Column Cleanup	10
	D. Instrumentation	11
	1.0 Description and Operating Conditions	11
	2.0 Standardization	12
	E. Interferences	12
	F. Confirmatory Techniques	12
	G. Time Required	12
	H. Modifications and Potential Problems	12
	I. Preparation of Standard Solutions	13
	J. Methods of Calculation	14
	1.0 Determination of Sample Residues	14
	2.0 Fortification Experiments	15

TABLE OF CONTENTS
(Continued)

		Page No.
III.	RESULTS AND DISCUSSION	16
IV.	CONCLUSION	16
V.	CERTIFICATION	17
VI.	TABLES AND FIGURES	
TABLE I.	LIQUID CHROMATOGRAPH OPERATING CONDITIONS FOR THE DETERMINATION OF FENOXYCARB	18
TABLE II.	TYPICAL STANDARDIZATION DATA FOR FENOXYCARB	19
TABLE III.	SUMMARY OF RECOVERY DATA FOR FENOXYCARB FOR FORTIFIED GRASS SAMPLES	20
TABLE IV.	SUMMARY OF RECOVERY DATA FOR FENOXYCARB FORTIFIED HAY SAMPLES USING AG-609 WITH MODIFICATIONS	23
TABLE V.	SUMMARY OF RECOVERY DATA FOR FENOXYCARB FOR FORTIFIED CROP SAMPLES	24
Figure 1.	Chemical Name and Structures	25
Figure 2.	Schematic Diagram of the HPLC Column Switching System	26
Figure 3.	Flow Diagram for Analytical Method AG-609A	27
Figure 4.	Representative Chromatograms for Fenoxycarb Standards	29

TABLE OF CONTENTS
(Continued)

		Page No.
Figure 5.	Representative Chromatograms for Control and Fortified Control Forage Samples	31
Figure 6.	Representative Chromatograms for Control and Fortified Control Hay Samples	33
Figure 7.	Representative Chromatograms for Control and Fortified Control Hay Samples Using AG-609 with Modifications	35
Figure 8.	Representative Chromatograms for Control and Fortified Control Summer Squash Samples	37
Figure 9.	Representative Chromatograms for Control and Fortified Control Cucumber Samples	38
Figure 10.	Representative Chromatograms for Control and Fortified Control Cantaloupe Samples	39
VII.	REFERENCES	40

I. SUMMARY AND INTRODUCTION

A. SCOPE

This method is for determination of residues of fenoxycarb in pasture grass hay and forage and in crops. The limit of detection, as determined by the smallest standard injected, is 2.0 ng for fenoxycarb and the limit of determination, as demonstrated by the smallest acceptable recovery level, is 0.01 ppm. The chemical name and structure of fenoxycarb is shown in Figure 1.

Analytical Method AG-609A was adapted from Analytical Method AG-609[1]. Analytical Method AG-609 was revised in order to incorporate method modifications for the extraction of grass hay samples. Additionally, corrections have been made to the recovery values in Table III to incorporate a change in the percent moisture. Data in Table IV summarizes the results for hay samples analyzed using the method modifications detailed in Section II.H.1.0.

B. PRINCIPLE

A 25-g subsample of crop substrate is wetted with dilute aqueous H_3PO_4 then homogenized with acetone. The extract is filtered through a glass fiber filter and rinsed with acetone. The extract is rotary evaporated to remove the acetone. The concentrated extract is diluted to consist of 60% acetonitrile (ACN).

This aqueous solution is partitioned with 50 ml hexane and the organic solution is discarded. The sample solution is evaporated to remove ACN and then diluted with water and ammonium hydroxide. The analyte is partitioned into hexane with three 50-ml separations. The combined hexane fractions are evaporated and the residue cleaned up on a silica PrepSep. The eluate containing the analyte is evaporated and further cleanup is done on a Florisil PrepSep. The final fraction is evaporated to dryness then reconstituted in ACN/buffer. Residue determination is done by HPLC with column switching (100-mm C1 column to a 150-mm

Supelcosil LC-18-DB column) with UV detection at 228 nm.

II. MATERIALS AND METHODS

A. APPARATUS

1.0 Bottles, square amber wide mouth, 16 oz.
2.0 Flasks, Erlenmeyer, 500-ml
3.0 Flasks, round bottom (RB), 50-ml, 100-ml, 250-ml, 1000-ml
4.0 Flasks, side arm, 500-ml
5.0 Funnels, Buchner, 7-cm
6.0 Funnels, separatory, 125-ml and 250-ml with Teflon stopcock
7.0 Glass microfibre filter (GF/D), Whatman, 7-cm (Fisher Cat. # 09-873F)
8.0 Graduated cylinders, 10-ml, 50-ml, 250-ml
9.0 Homogenizer, Polytron or equivalent
10.0 Pipets, disposable pasteur
11.0 PrepSep, Florisil, Fisher Scientific (Cat. # P476)
12.0 PrepSep, Silica, Fisher Scientific (Cat. # P478)
13.0 Rotary evaporator, Buchi or equivalent
14.0 Vials, Wheaton, 2-ml. or equivalent

B. REAGENTS

1.0 Acetone, HPLC grade
2.0 Acetonitrile (ACN), HPLC grade
3.0 60% Acetonitrile/Water (v/v)
4.0 Ammonium hydroxide (NH_4OH), ACS Reagent grade
5.0 Dichloromethane (DCM), HPLC grade
6.0 49:50:1 DCM:Hexane:THF (v/v/v)
7.0 Ethyl acetate (EtOAc), HPLC grade
8.0 8% Ethyl acetate/Hexane (v/v)
9.0 Hexane, HPLC grade
10.0 Phosphoric acid (H_3PO_4), Certified ACS grade
11.0 1% Phosphoric acid/water (v/v)
12.0 Sodium sulfate, Certified ACS grade
13.0 Water, HPLC grade
14.0 Fenoxycarb, Analytical Standard supplied by Ciba-Geigy Corporation, 410 Swing Road, Greensboro, NC 27419

C. ANALYTICAL PROCEDURE

1.0 Sample Preparation

Samples are received and stored according to SOP 7.20. Samples are prepared under the general guidelines of the U.S. Food and Drug Administration Pesticide Analytical Manual Volume I, Section 141 and SOP 7.21.

2.0 Extraction

2.1 Weigh an approximately 25-g subsample of substrate into a 16-oz. wide mouth jar. Fortify recovery samples with fenoxycarb standard at this point. Add 20 ml of 1% H_3PO_4 and allow to stand for twenty minutes. Add 200 ml of acetone to the sample and allow to stand for 1 minute. Homogenize the sample with a Polytron homogenizer at a rate of 17,000 to 20,000 rpm for approximately two minutes.

Note: Hay samples may require the alternative extraction procedure outlined in Section II.H.1.0 to reduce interferences and increase recoverability of fenoxycarb.

2.2 Filter the sample through Whatman glass microfibre filter (GF/D) seated in a Buchner funnel (preferably 7-cm) into a 500-ml Erlenmeyer flask, using gravity filtration (or into a 500-ml side-arm flask using vacuum filtration). Rinse the sample bottle with 50 ml of acetone and pour over the filter cake and filter the rinse, removing excess liquid from filter cake. Combine the extract and rinse in a 1-L round bottom (RB) flask.

2.3 Remove the acetone from the extract by rotary vacuum evaporation in a water bath at ≤35°C until the volume is approximately 18 ml. (Note: All evaporations are done using rotary vacuum evaporation). Remaining aqueous volume may be more or less than 18 ml, depending upon the moisture content of the substrate. Whatever the volume may be, all samples should be evaporated to the same volume. Measure the volume of one sample using a graduated cylinder. Add enough acetonitrile to the aqueous liquid to make the solution 60% acetonitrile.

3.0 Partition Cleanup

3.1 Transfer the sample to a 250-ml separatory funnel with 2 X 10-ml rinses of 60% acetonitrile/ water. Rinse the 1-L RB flask with 50 ml of hexane then add to the separatory funnel. Vent the separatory funnel and shake for one minute. Allow layers to separate and drain the lower, aqueous layer back into the original 1-L RB flask.

3.2 Back partition the remaining hexane twice with 10-ml portions of 60% acetonitrile/ water. Shake for one minute, adding the lower layer to the original 1-L RB flask each time.

Note: A disposable pipet may be used to break up emulsions in the final separation. Any emulsion formed during the partitions is retained in the separatory funnel until the final partition when it is

drained with the aqueous layer and carried on to the next step.

Remove the acetonitrile from the sample by rotary evaporation with a bath temperature ≤35°C until the first drops of aqueous come off.

Note: The aqueous volume should be approximately the same as it was before the addition of acetonitrile in Section II.C.2.3.

3.3 Add 2 ml of ammonium hydroxide and 20 ml of water to the sample. Transfer to a 125-ml separatory funnel. Rinse the flask with 50 ml of hexane before transferring to the separatory funnel. Shake for one minute. The lower, aqueous layer is drained into the original 1-L RB flask. The hexane layer is poured out the top of the separatory funnel into a 250-ml round bottom flask, retaining any emulsion in the separatory funnel. The aqueous layer is returned to the separatory funnel and partitioned twice more with 50-ml portions of hexane using the same procedure. Discard the aqueous layer. Evaporate the sample to dryness in a water bath at ≤35°C.

3.4 For cucurbits, the silica and florisil steps may not be necessary. Following the partition, the sample is evaporated and reconstituted in an appropriate final volume for injection (Section II.C.5.2).

4.0 Silica Gel Chromatography

4.1 Profile separate lots of silica PrepSeps as the elution volume for fenoxycarb (usually between 15 to 25 ml) can change between lots.

4.2 Reconstitute the sample residue from Section II.C.3.3 in 2 ml of hexane. Precondition silica column with a small amount (2-3 ml) of hexane. Add the sample to the PrepSep using a disposable pipet. Rinse the 250-ml RB flask with 2 X 2 ml of hexane. Add the rinses to

the PrepSep. Add 7 ml of 49:50:1 DCM:Hexane:THF to the PrepSep and discard the flow. Add the appropriate volume (Section II.C.4.1) of 49:50:1 DCM:Hexane:THF to the PrepSep and collect the eluant in a 50-ml RB flask. Evaporate the sample to dryness in a water bath at ≤35°C.

5.0 Florisil Chromatography

5.1 Place a 5-mm deep layer of sodium sulfate on top of the packing of a Florisil PrepSep. Precondition the sodium sulfate and florisil with a small amount (2-3 ml) of 8% EtOAc/hexane. Reconstitute the sample residue from Section II.C.4.0 in 2 ml of hexane. Add the sample to the PrepSep using a disposable pipet. Rinse the 50-ml RB flask with 2 X 2 ml of hexane and add the rinses to the PrepSep. Add 50 ml of 8% EtOAc/hexane to the PrepSep and collect the eluant in 100-ml RB flask.

5.2 Evaporate the sample to dryness in a water bath at ≤35°C. Reconstitute the sample in an appropriate final volume in Mobile Phase 1 (40:60 ACN: 0.05M KH_2PO_4/water) for injection. Sonicate and vortex mix final fraction to ensure proper reconstitution.

Note: For cucurbits, the silica and florisil steps can be omitted. After partition, the sample is evaporated, brought to final volume in Mobile Phase 1 (40:60 ACN:0.05M KH_2PO_4/ water), and injected onto the HPLC system.

D. INSTRUMENTATION

1.0 Description and Operating Conditions

1.1 Install the HPLC system according to Table I and Figure 2. Control of the switching valve is accomplished via time-programmed contact closures of an appropriate instrument.

1.2 Determine the retention time of Fenoxycarb on Column #1 by connecting Column #1 directly to the detector and injecting 20 ng of the analyte. (Inject 40 ul of the 0.5 ng/ul standard solution prepared in Section II.I.1.0)

1.3 Reconnect the system as shown in Figure 2. Program the valve to switch to the INJECT POSITION at the beginning of the Fenoxycarb analyte peak and to return to the LOAD POSITION at the end of the analyte peak of Fenoxycarb.

1.4 Inject 20 ng of Fenoxycarb to

determine its retention time through the two columns and to confirm that the valve time programming is correct.

2.0 Standardization

2.1 Calibrate the HPLC system with each analytical run by checking the retention time and detector response relative to previous runs. Retention times must not vary more than 2% and detector response must not vary more than 5% between runs.

2.2 Standardize the HPLC system by injecting 40-ul aliquots of standard solutions of Fenoxycarb in a working range of 2.0 - 40 ng/injection. Generate a linear regression from the data by comparing detector response and ng injected.

E. INTERFERENCES

Hay samples may contain significant interferences. See Section II.H.1.0 for method modifications.

F. CONFIRMATORY TECHNIQUES

None.

G. TIME REQUIRED

A skilled analyst can complete the extraction and analysis of a set of 6-8 samples in 14 working hours.

H. MODIFICATIONS AND POTENTIAL PROBLEMS

1.0 Interference problems in hay samples can be avoided using the following alternative extraction procedure. In Section II.C.2.1, add 40 ml of 1% H_3PO_4 and allow to stand for twenty minutes. Add 400 ml of acetone to the sample and allow to stand for one

minute. Homogenize and filter as described in Section II.C.2.1 and II.C.2.2, rinsing the sample bottle with 50 ml of acetone and pouring over the filter cake. Transfer a 245-ml aliquot of the extract to a 1-L round bottom (RB) flask and proceed to Section II.C.2.3. Note that a smaller aliquot volume may be used as long as the milligrams injected are adequate to screen the sample at the required level.

2.0 Some samples may develop emulsions after shaking (Section II.C.2.2 and II.C.3.3). These may be cleared if allowed to settle out slightly and then gently stirred with a glass rod.

3.0 During the evaporation of samples solutions in Sections II.C.3.1 and II.C.4.2 any water bath used must not have a temperature >40°C and the samples should be removed as soon as they are ready. Excessive temperature, especially when the sample has gone to dryness, leads to analyte volatilization.

I. PREPARATION OF STANDARD SOLUTIONS

1.0 Preparation of Standard Fenoxycarb Solutions

1.1 Weigh 10 mg of Fenoxycarb analytical standard into a 100-ml volumetric flask and dilute to the mark with ACN.

1.2 Make serial dilutions of the 0.1 mg/ml standard solution with Mobile Phase 1 (40:60 ACN:0.05M KH_2PO_4) to give a series of fortification/ analytical standards in a range of 0.05 ug/ml to 2.5 ug/ml of Fenoxycarb. Store the standard solutions in amber bottles at

4°C in the dark when not in use.

J. METHODS OF CALCULATION

1.0 Determination of Sample Residues

1.1 Inject 40-ul aliquots of sample final fractions from Section II.C.5.2 into the HPLC under the same conditions as for standards. Make appropriate dilutions of the samples in Mobile Phase 1 (40:60 ACN:0.05M KH_2PO_4/water) to bring the sample peak heights within the range of the standard curve. Compare the peak heights of the unknown samples to the standard curve or enter the peak height into a least squares program to determine the nanograms of Fenoxycarb in the injected aliquot. Typical chromatograms for control and procedural recovery samples are shown in Figures 5 through 10.

1.2 To calculate the residue results, the mg injected must first be calculated as follows:

$$(1)\ \text{mg inj.} = \frac{(G)\ (V_a)\ (V_i)}{(V_e + W\ (M/100))\ (V_f)}$$

G = milligrams sample extracted
V_a = aliquot volume
V_e = extraction volume
V_i = injection volume (ul)
V_f = total volume of final injection solution (ul)
R% = recovery ratio given by equation 4
M = % moisture of sample
W = weight of sample in grams

To determine ppm analyte found in samples, use Equation 2:

$$(2)\ \text{ppm} = \frac{(\text{ng Fenoxycarb Found})\ (100)}{(\text{mg sample injected})\ (R\%)}$$

2.0 Fortification Experiments

This method is validated for each set of samples analyzed by including an untreated control sample and one or more control samples fortified immediately prior to extraction with Fenoxycarb.

2.1 Add 1.0 ml of a 0.25-ng/µl standard solution of Fenoxycarb to 25 g of control crop or grass prior to the addition of extraction solvent for a 0.01 ppm fortification. Use correspondingly larger amounts of standards (volume should not exceed 2 ml) for higher fortifications. Analyze control and freshly fortified samples along with the treated samples according to the procedures of the method.

2.2 Calculate the final ppm value of the control and fortified samples according to the following equation:

$$\text{(3) ppm Fenoxycarb} = \frac{\text{ng Fenoxycarb found}}{\text{mg sample injected}}$$

Determine the recovery factor by first subtracting the background detector response, if any, in the control sample from the Fenoxycarb response in the recovery sample. Calculate the recovery factor as a percentage (R) by the equation:

$$\text{(4) } R\% = \frac{\text{(ppm Fenoxycarb found - ppm control)}}{\text{ppm Fenoxycarb added}} \times 100\%$$

III. RESULTS AND DISCUSSION

Control and fenoxycarb fortified control samples were analyzed during the course of the analytical phase of Residue Studies 28-92-B (ABR-94025)[2] and 64-92-B (ABR-93016)[3]. The sample substrates analyzed by the procedures of Analytical Method AG-609A included pasture grass forage and hay, cucumbers, summer squash and cantaloupes. Ciba Analytical Method AG-609A was issued to include method modifications which improve the analysis of hay samples.

Cucurbit samples (cucumbers, summer squash and cantaloupes) may be analyzed by AG-609A without being subjected to column cleanup steps. The cucurbit mean recovery at the limit of determination was 102% (s.d.=15, n=9) while the mean of all cucurbit samples was 99% (s.d.=15, n=17). The pasture grass mean recovery at the limit of determination was 82% (s.d.=12, n=39) while the mean of all pasture grass samples was 81% (s.d.=10, n=108). The mean recovery for hay samples analyzed using the modifications to the method previously noted in Section II.H.1.0 was 88% (s.d.=11, n=23). The above means and standard deviations demonstrate that the accuracy and precision of AG-609A are adequate for tolerance enforcement purposes.

IV. CONCLUSION

Analytical Method AG-609A is a valid and accurate method for the determination of parent residues of fenoxycarb in grasses and crops.

V. CERTIFICATION

The reports and experimental results included in this study, AG-609A, are certified to be authentic accounts of the experiments.

Robert E. M. Wurz
Residue Chemistry
Biochemistry Department
910-632-2391

5/11/94
Date

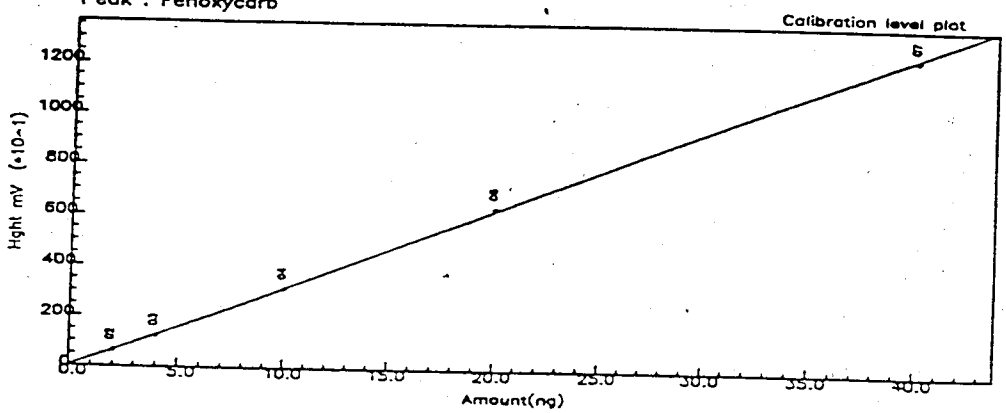
TABLE I. LIQUID CHROMATOGRAPHIC OPERATING CONDITIONS FOR THE DETERMINATION OF FENOXYCARB

Instrument:	Perkin-Elmer Model ISS-200 Automatic HPLC sampler or equivalent 2 - Kratos-Spectroflow 400 LC Pumps or equivalent ABI Model 783 Variable Wavelength UV Detector 6-port Switching Valve - Valco Instruments Co., Inc., Houston, TX or equivalent
Column 1:	Spherisorb C1 Cartridge Column, 100 mm x 4.6 mm, 5 µm particle size (Fisher Scientific cat. #05-692-547) or equivalent
Column 2:	Spherisorb ODS2 Cartridge Column, 150 mm x 4.6 mm, 3 µm particle size (Fisher Scientific cat. #05-692-536) or equivalent
Mobile Phase 1:	40:60 ACN:0.05M KH_2PO_4/water (v:v)
Mobile Phase 2:	65:35 ACN:0.05M KH_2PO_4/water (v:v)
Retention Time:	~7 min. (Column 1) ~12 min. (through both columns)
Detection:	ABI Model 783 Variable Wavelength UV Detector or equivalent variable wavelength detector.
Wavelength:	228 nm
Attenuation:	0.004 AUFS
Flow Rate:	1.0 ml/min (both pumps)
Injection Volume:	40 ul
Run Time:	20 min/injection

TABLE II. TYPICAL STANDARDIZATION DATA FOR FENOXYCARB

Std. Soln. ng/µl	Std. Wt. Inj. ng	Peak Height µV
0.05	2.0	645
0.05	2.0	638
0.10	4.0	1236
0.25	10.0	3136
0.50	20.0	6372
0.50	20.0	6375
1.00	40.0	12451

Ciba—Geigy Multichrom 2.0 (Q)
Calibration Name : [28—92] 39 2892BAF4.
Fenoxycarb — Pasture Grasses
Peak : Fenoxycarb



Constant : 3.33403E+1
1st degree : 3.12436E+2

Curve fit : Linear
Correlation coefficient : 0.99988
Standard error : 7.26443E+1
Reported on 16—JUN—1993 at 08:33

(Field Test 0S-IR-601-92, Forage)

Table III. Summary of Recovery Data for Fenoxycarb for Fortified Grass Samples

Protocol 28-92

Substrate	Residue Test Report Number (Report 1)	Fortification (ppm)	Recovery (%)
Forage	0W-IR-205-92	0.01	61
	0S-IR-601-92		62
	02-IR-005-92		64
	0S-IR-601-92		66
	0S-IR-201-92		67
	02-IR-005-92		69
	0W-IR-205-92		71
	02-IR-005-92		72
	0S-IR-201-92		73
	04-IR-001-92		73
	0W-IR-205-92		74
	"		74
	02-IR-005-92		75
	0S-IR-601-92		75
	"		76
	"		77
	02-IR-005-92		77
	0W-IR-205-92		78
	0S-IR-811-92		81
	0S-IR-601-92		82
	0W-IR-205-92		84
	0S-IR-811-92		85
	02-IR-005-92		86
	04-IR-001-92		88
	"		90
	02-IR-005-92		90
	0S-IR-105-92		91
	04-IR-001-92		92
	0S-IR-904-92		92
	0S-IR-811-92		93
	"		93
	0S-IR-105-92		94
	"		94
	0S-IR-601-92		95
	0S-IR-811-92		95
	0S-IR-105-92		95
	0S-IR-904-92		97
	"		99
	02-IR-005-92		109

Table III Summary of Recovery Data for Fenoxycarb for Fortified Grass Samples (Continued)

Protocol 28-92

Substrate	Residue Test Report Number (Report 1)	Fortification (ppm)	Recovery (%)
Forage	0S-IR-201-92	0.05	66
	02-IR-005-92		68
	0S-IR-601-92		68
	0W-IR-205-92		69
	0S-IR-601-92		72
	02-IR-005-92		73
	0S-IR-601-92		75
	0S-IR-904-92		81
	0W-IR-205-92		86
	0S-IR-811-92		87
	0S-IR-601-92		87
	0S-IR-201-92		88
	02-IR-005-92		89
	0S-IR-811-92		90
	02-IR-005-92		92
	"		92
Forage	0S-IR-601-92	0.1	70
	0S-IR-201-92		71
	0W-IR-205-92		72
	0S-IR-601-92		85
	02-IR-005-92		88
	04-IR-001-92		88
	02-IR-005-92		89
	0S-IR-904-92		92
Forage	0S-IR-601-92	0.5	70
	"		74
	02-IR-005-92		76
	0W-IR-205-92		79
	0S-IR-201-92		82
	04-IR-001-92		87
	0S-IR-811-92		87
	0W-IR-205-92		87
	02-IR-005-92		88
	0S-IR-105-92		89
	0S-IR-904-92		92
	0S-IR-811-92		92

Table III Summary of Recovery Data for Fenoxycarb for Fortified Grass Samples (Continued)

Protocol 28-92

Substrate	Residue Test Report Number (Report 1)	Fortification (ppm)	Recovery (%)
Forage	0W-IR-205-92	1.0	66
	0S-IR-811-92		80
	0S-IR-904-92		82
	0S-IR-601-92		86
	02-IR-005-92		88
	0S-IR-811-92		89
	0S-IR-105-92		89
Forage	02-IR-005-92	2.0	83
	0S-IR-601-92		84
Hay	0W-IR-205-92	0.01	64
	0S-IR-601-92		70
	0S-IR-811-92		75
	02-IR-005-92		75
	0S-IR-601-92		75
	0S-IR-201-92		75
	02-IR-005-92		77
	0S-IR-904-92		77
	"		78
	0S-IR-811-92		78
	0W-IR-205-92		82
	0S-IR-601-92		83
	02-IR-005-92		91
Hay	0S-IR-105-92	0.05	90
Hay	0S-IR-811-92	0.1	62
	0W-IR-205-92		69
	0S-IR-601-92		73
	"		73
	"		74
	02-IR-005-92		77
	0W-IR-205-92		79
	0S-IR-105-92		79
	0S-IR-811-92		80
	02-IR-005-92		84

Table IV. Summary of Recovery Data for Fenoxycarb for Fortified Hay Samples Using AG-609A with Modifications

Protocol 28-92

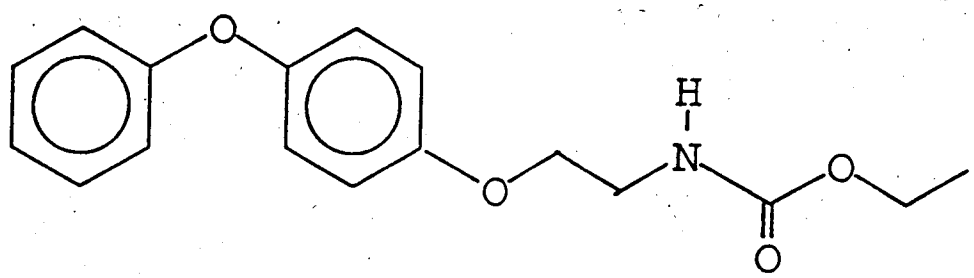
Substrate	Residue Test Report Number (Report 1)	Fortification (ppm)	Recovery (%)
Hay	OW-IR-214-93	0.01	61
	04-IR-001-92		73
	OW-IR-519-93		76
	OS-IR-821-93		86
	OS-IR-201-92		90
	OW-IR-519-93		92
	OS-IR-821-93		95
	"		95
	OW-IR-214-93		116
Hay	04-IR-001-92	0.05	89
	OS-IR-201-92		92
	FL-IR-402-93		94
	OW-IR-213-93		94
	04-IR-001-92		104
Hay	OS-IR-501-93	0.10	76
	OS-IR-603-93		91
Hay	OS-IR-822-93	0.20	80
Hay	OW-IR-214-93	0.50	77
	FL-IR-402-93		86
	OW-IR-213-93		92
	OS-IR-822-93		96
Hay	OS-IR-603-93	1.00	84
	OS-IR-501-93		89

Table V. Summary of Recovery Data for Fenoxycarb for Fortified Crop Samples

Protocol 64-92

Substrate	Residue Test Report Number (Report 1)	Fortification (ppm)	Recovery (%)
Summer Squash	0S-IR-603-92	0.01	97
	"	0.01	102
	"	0.01	108
	"	0.05	118
	"	0.50	103
Cucumbers	0S-IR-602-92	0.01	111
	"	0.01	118
	02-IR-006-92	0.01	122
	"	0.01	77
	"	0.05	77
	0S-IR-602-92	0.10	85
	02-IR-006-92	0.50	77
	0S-IR-602-92	2.00	109
Cantaloupes	02-IR-007-92	0.01	85
	"	0.01	101
	"	0.05	92
	"	0.50	100

FIGURE 1. CHEMICAL NAME AND STRUCTURE



Fenoxycarb

ethyl (2-[4-phenoxy]phenoxy) ethyl carbamate

FIGURE 2. SCHEMATIC DIAGRAM OF THE HPLC COLUMN SWITCHING SYSTEM

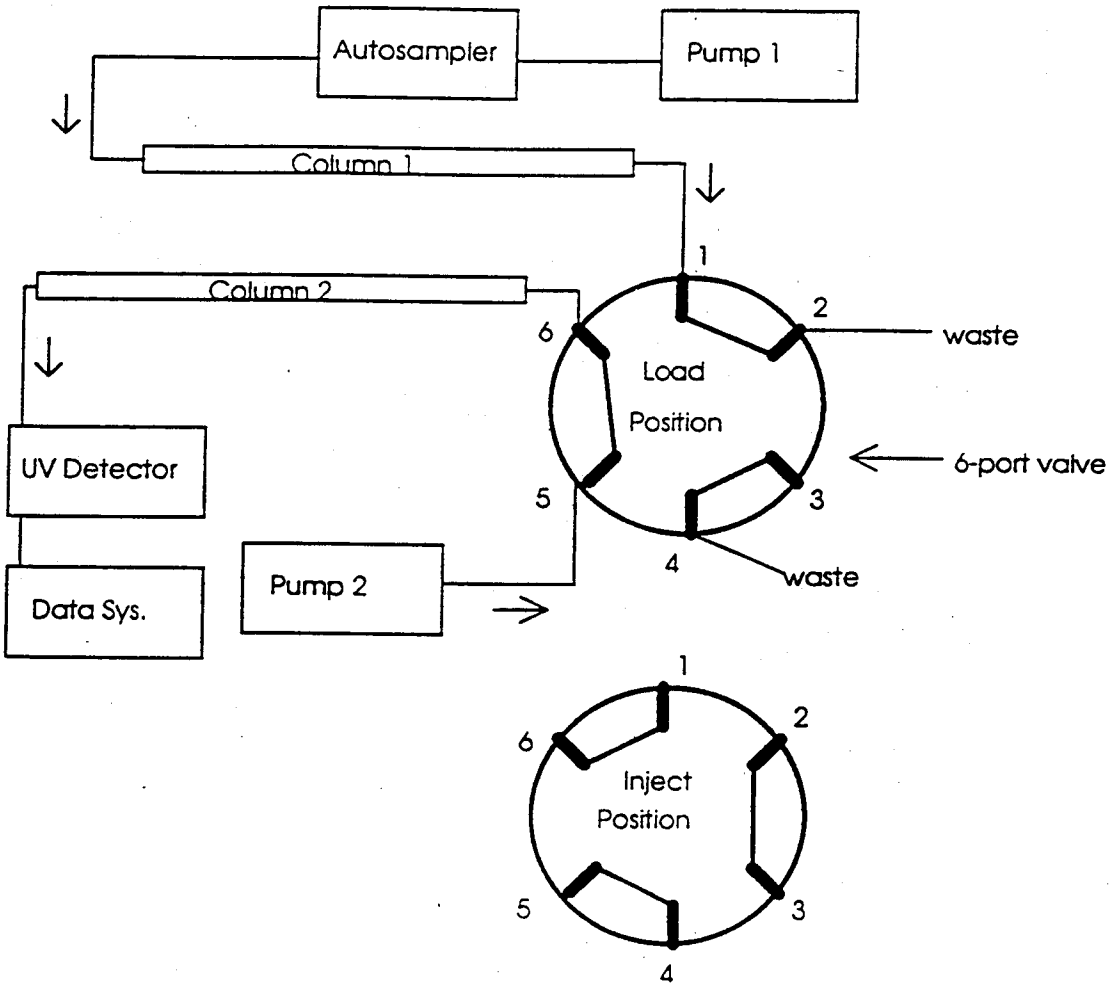


FIGURE 3. FLOW DIAGRAM FOR ANALYTICAL METHOD AG-609A

25-g sample

|

Add 20-ml 1% H_3PO_4/water
Steep for 20 minutes

|

Add 200-ml acetone
Steep for 1 minute

|

Homogenize for 2 minutes

|

Filter extract through glass microfibre filter
Rinse jar and filter cake with 50-ml acetone
Collect extract and rinse in 500-ml Erlenmeyer

|

Remove acetone by rotary evaporation (Bath ≤35°C)

|

Measure remaining aqueous volume
Add enough ACN to constitute 60% ACN/water

|

Transfer to 250-ml separatory funnel with
2 x 10 ml rinses of 60% ACN/water

|

Add 50-ml hexane, transfer to sep. funnel
Shake for 1.0 minute

|

Back-partition hexane with
2 x 10-ml 60% ACN/water (1 min.)

|

Discard hexane layer

Collect aqueous layers in
the original 500-ml
Erlenmeyer flask

|

Remove ACN by rotary
evaporation (Bath ≤35°C)

|

Add 2-ml conc. NH_4OH +
20-ml water Transfer to
125-ml separatory funnel

|

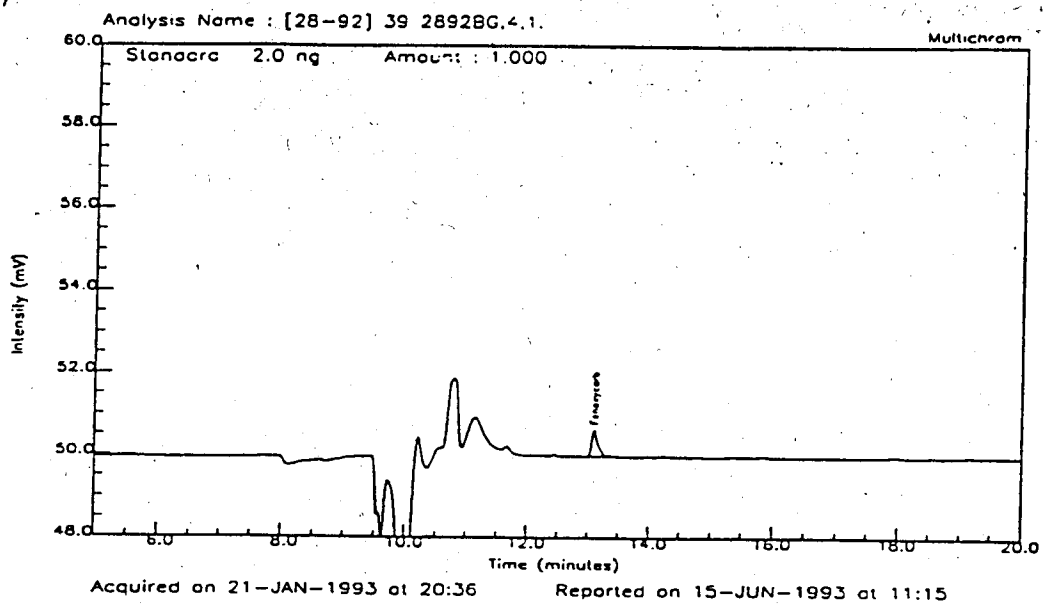
Discard lower aqueous layers _ Partition with 3 x 50-ml
hexane (1 min each)

FIGURE 3. FLOW DIAGRAM FOR ANALYTICAL METHOD AG-609A
(Continued)

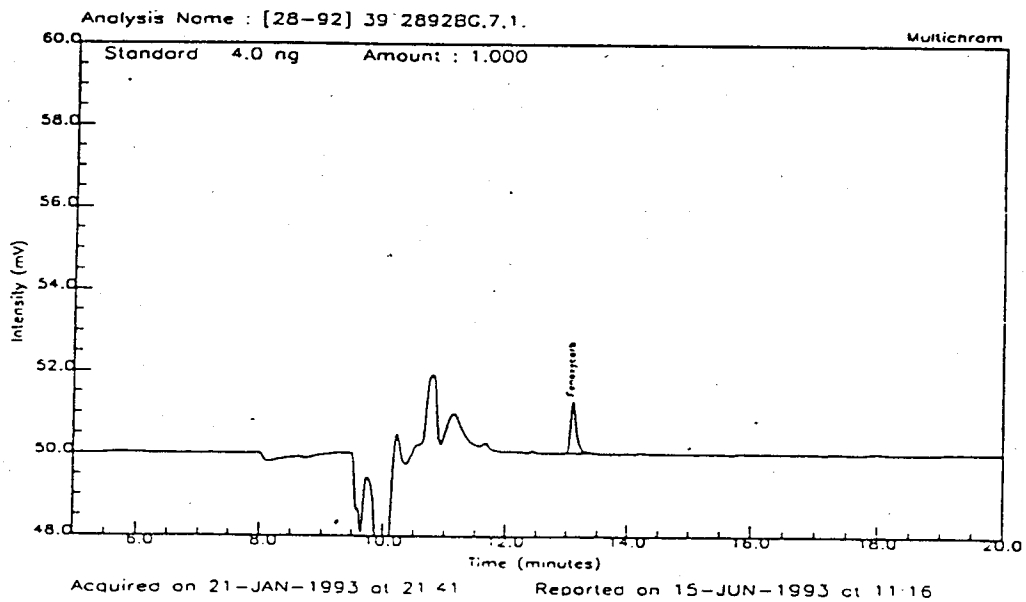
Collect hexane layers in
a 250-ml RB Flask
|
Evaporate sample to
dryness (Bath ≤35°C);
Reconstitute residue in
2-ml hexane
|
Precondition silica PrepSep with 2-3 ml hexane
Load sample onto column using disposable pipet
Rinse 250-ml RB flask with 2 x 2-ml hexane
Add rinses to silica PrepSep
|
Add 7-ml 49:50:1 DCM:Hexane:THF to Si PrepSep
Discard Eluate
Add 15-ml 49:50:1 DCM:Hexane:THF to Si PrepSep
Collect eluate in 50-ml RB flask
|
Evaporate sample to dryness (Bath ≤35°C)
Reconstitute residue in 2-ml hexane
|
Place 5-mm layer of Na_2SO_4 in top of Florisil PrepSep
Precondition column with 2-3 ml 8% EtOAc/hexane
Load sample onto column using disposable pipet
Rinse 50-ml RB flask with 2 x 2-ml hexane
Load rinses onto florisil PrepSep
|
Add 50-ml 8% EtOAc/hexane to florisil PrepSep
Collect flow in 100-ml RB flask
|
Evaporate sample to dryness (Bath ≤35°C)
Reconstitute residue in 40:60 ACN:0.05M KH_2PO_4

Figure 4. Representative Chromatograms for Fenoxycarb Standards

A)



B)

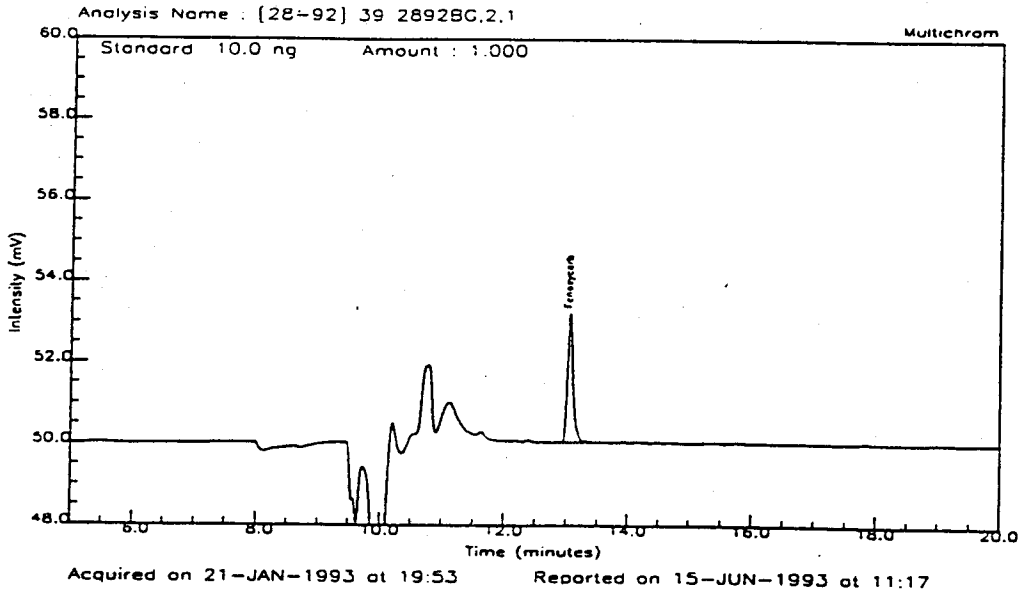


(Field Test: OW-IR-205-92)

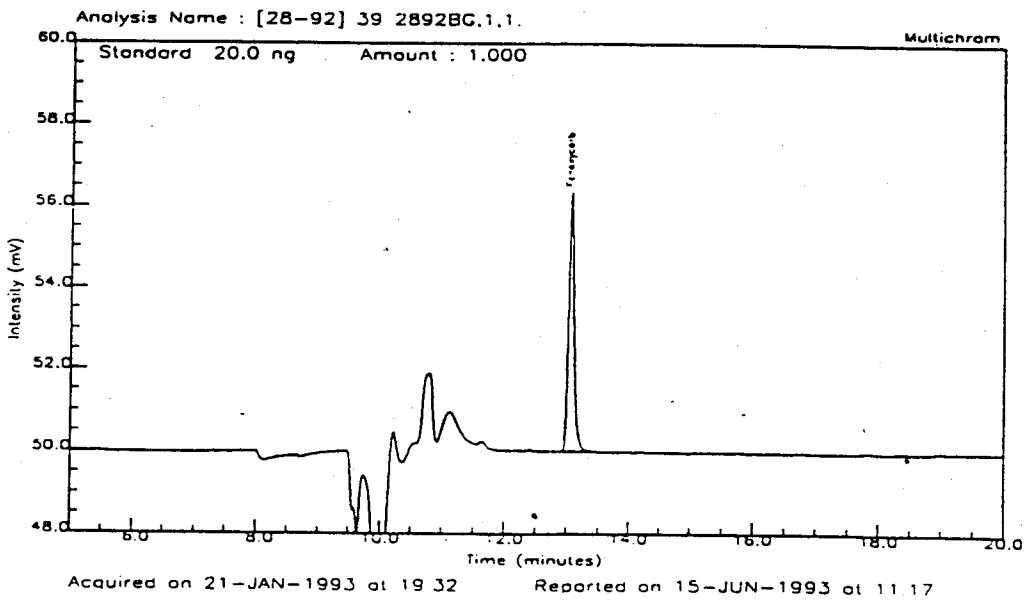
A: 2.0 ng injected
B: 4.0 ng injected

Figure 4. Representative Chromatograms for Fenoxycarb Standards (Continued)

C)



D)

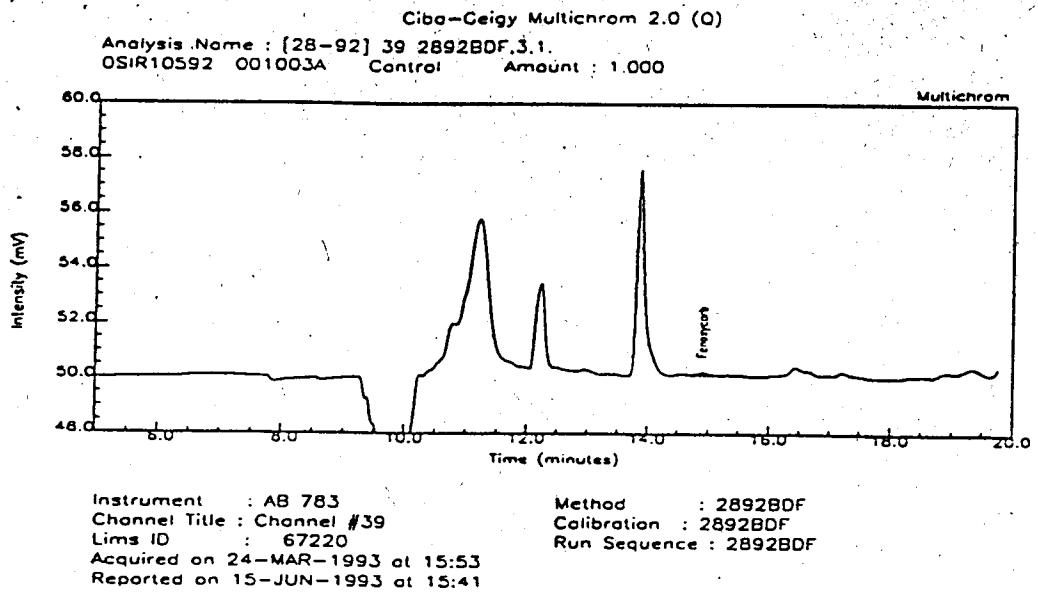


(Field Test: OW-IR-205-92)

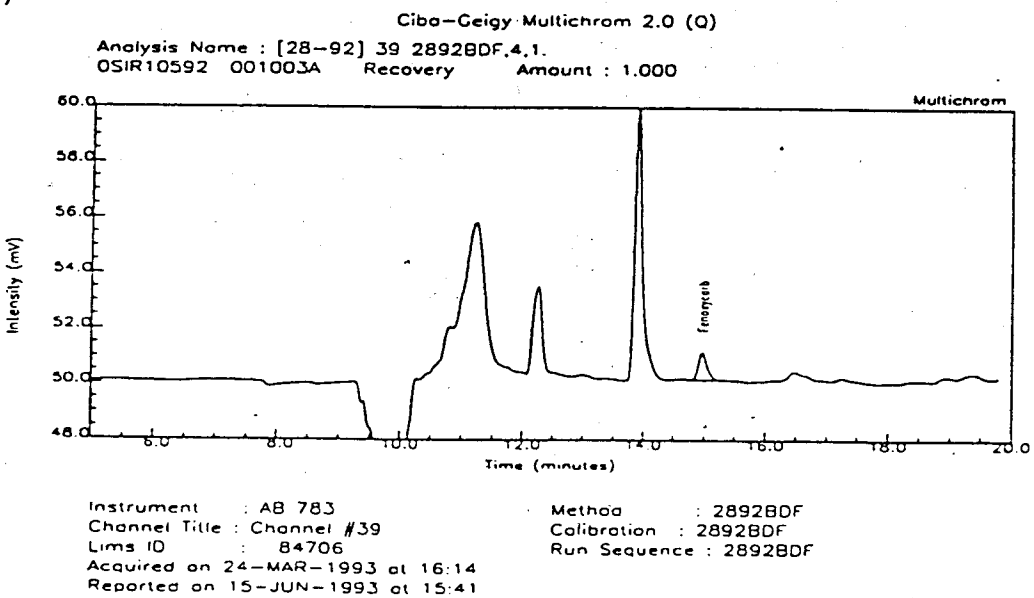
C: 10.0 ng injected
D: 20.0 ng injected

Figure 5. Representative Chromatograms for Control and Fortified Control Forage Samples

A)



B)

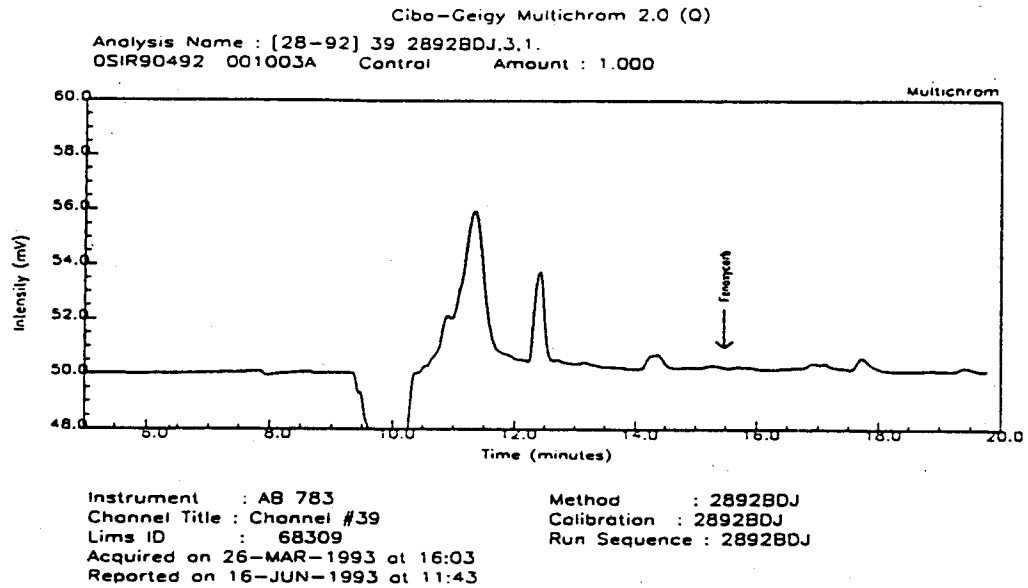


*Recoveries corrected for control values.
(Field Test: OS-IR-105-92)

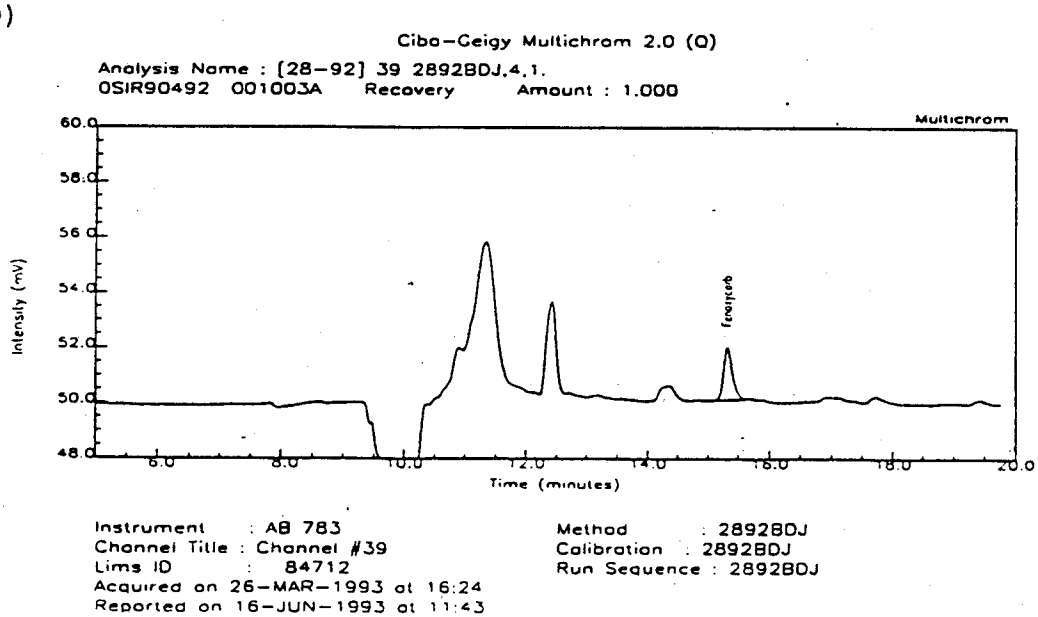
A: Control, 500 mg injected, -0.066 ng found, <0.01 ppm
B: Control + 0.01 ppm Fenoxycarb, 500 mg injected, 4.709 ng found, 0.0094 ppm, 94% recovery*

Figure 5. Representative Chromatograms for Control and Fortified Control Forage Samples (Continued)

C)



D)

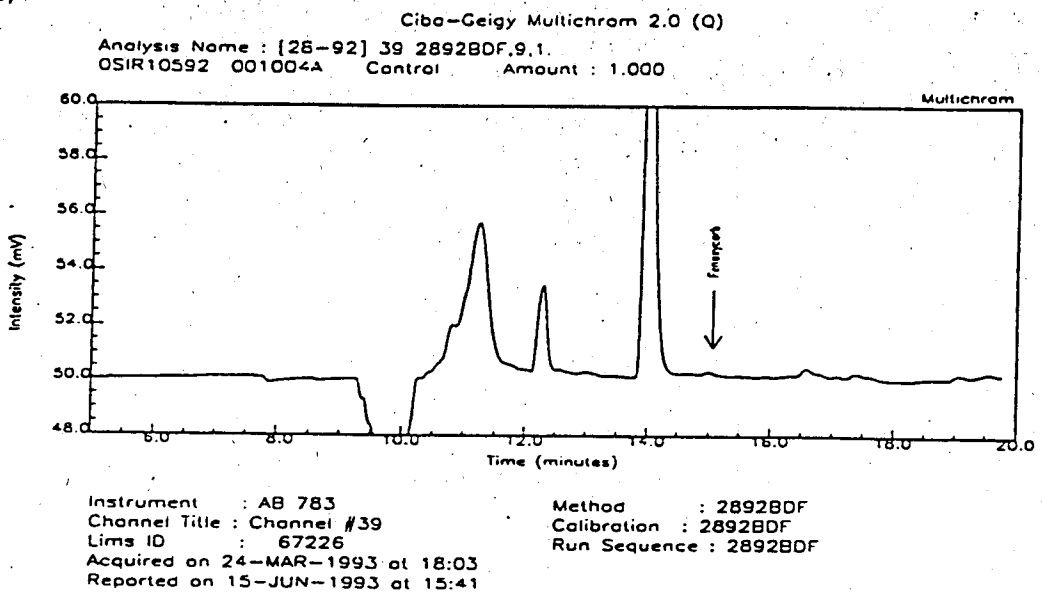


*Recoveries corrected for control values.
(Field Test: 0S-IR-904-92)

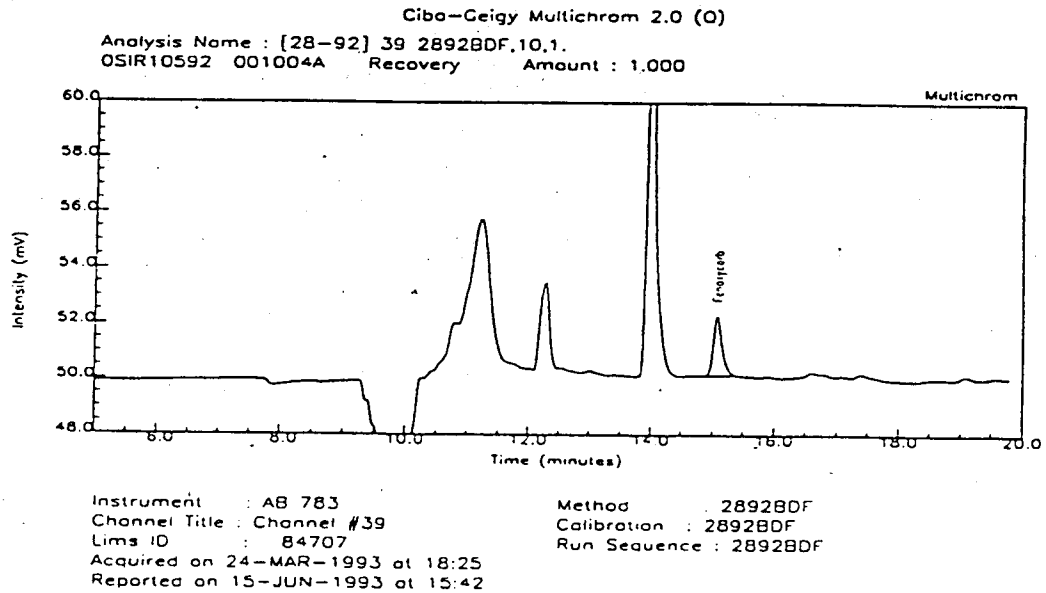
C: Control, 500 mg injected, 0 ng found, <0.01 ppm
D: Control + 0.05 ppm Fenoxycarb, 250 mg injected, 10.12 ng found, 0.0405 ppm, 81% recovery*

Figure 6. Representative Chromatograms for Control and Fortified Control Hay Samples

A)



B)

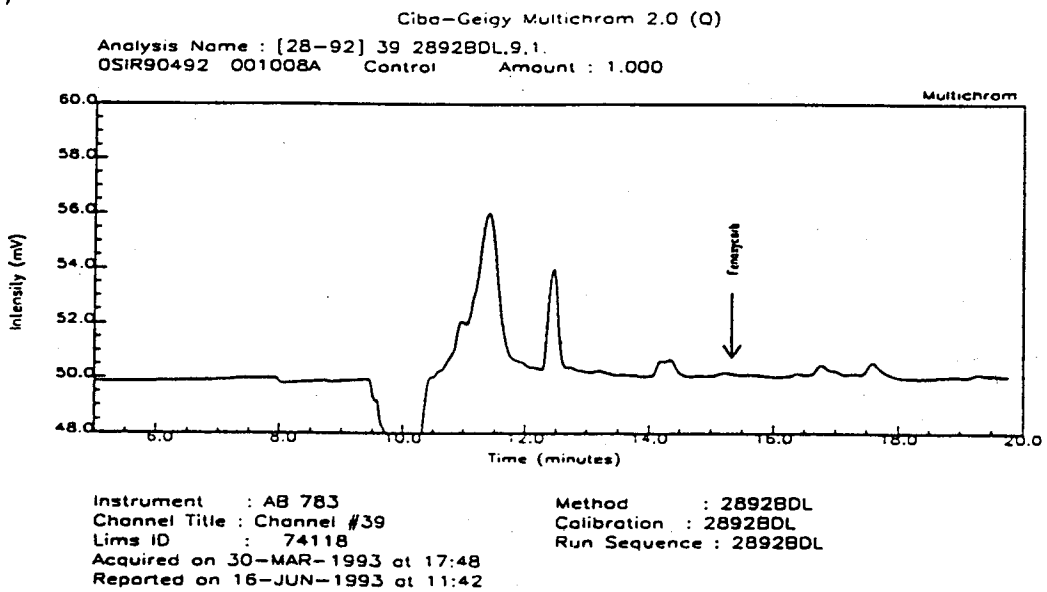


*Recoveries corrected for control values.
(Field Test: OS-IR-105-92)

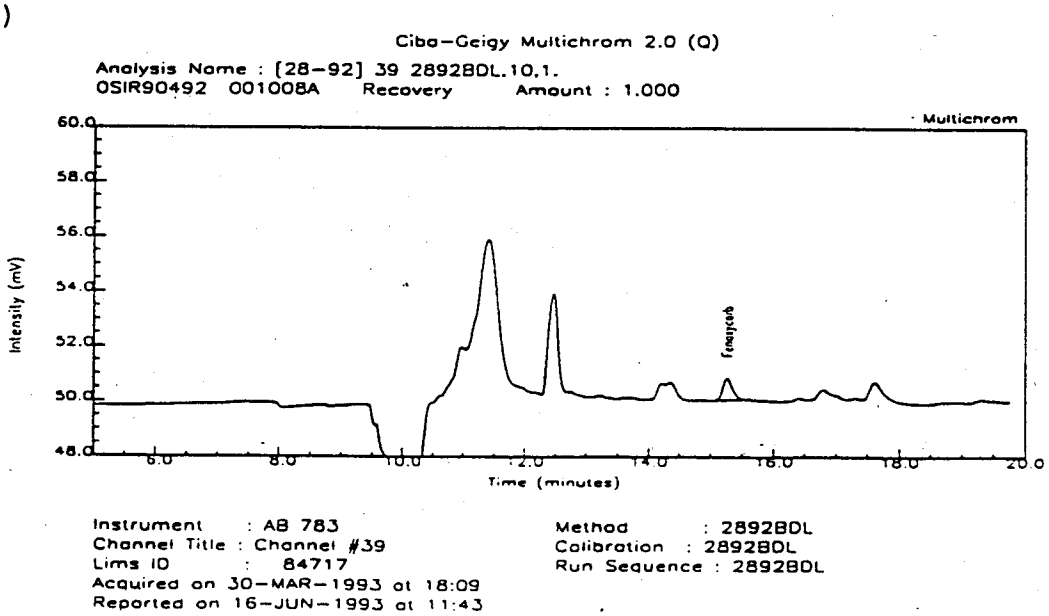
A: Control, 500 mg injected, 0 ng found, <0.01 ppm
B: Control + 0.05 ppm Fenoxycarb, 250 mg injected, 11.26 ng found, 0.045 ppm, 90% recovery*

Figure 6. Representative Chromatograms for Control and Fortified Control Hay Samples (Continued)

C)



D)

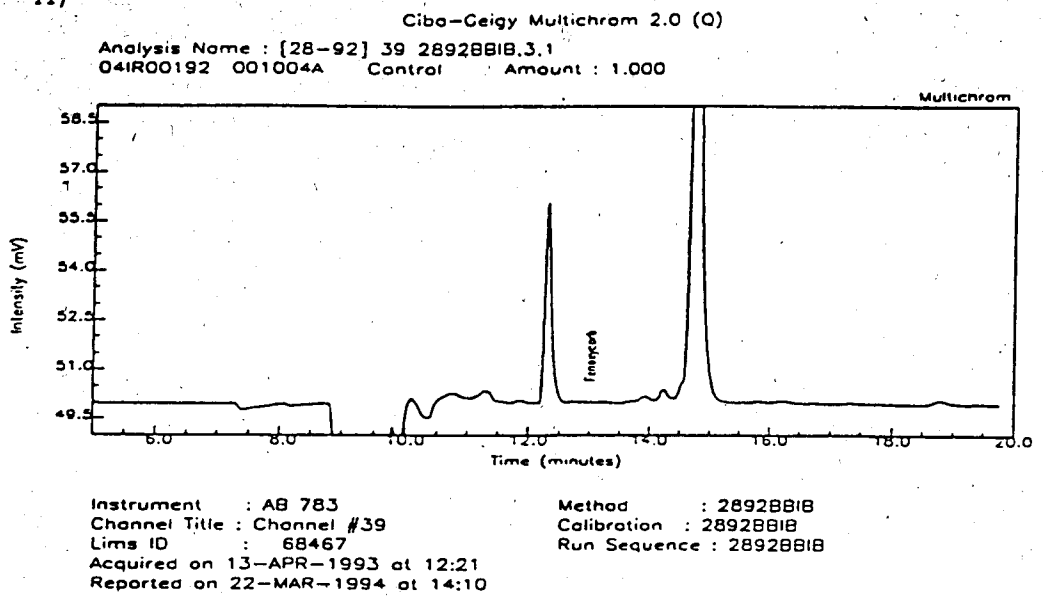


*Recoveries corrected for control values.
(Field Test: OS-IR-904-92)

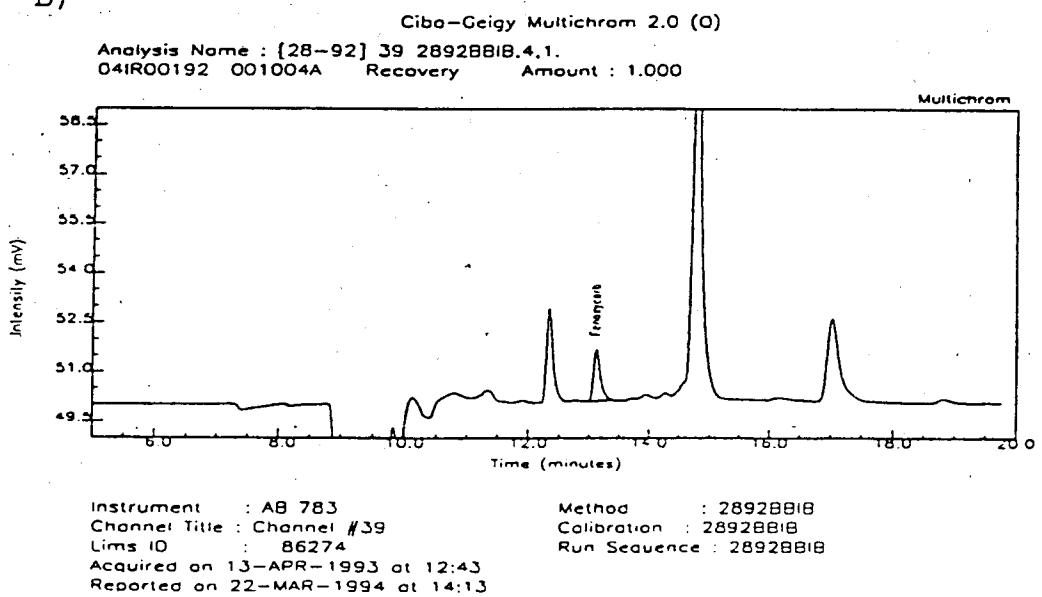
C: Control, 500 mg injected, 0 ng found, <0.01 ppm
D: Control + 0.01 ppm Fenoxycarb, 500 mg injected, 3.89 ng found, 0.0078 ppm, 78% recovery*

Figure 7. Representative Chromatograms for Control and Fortified Control Hay Samples Using AG-609A with Modifications

A)



B)

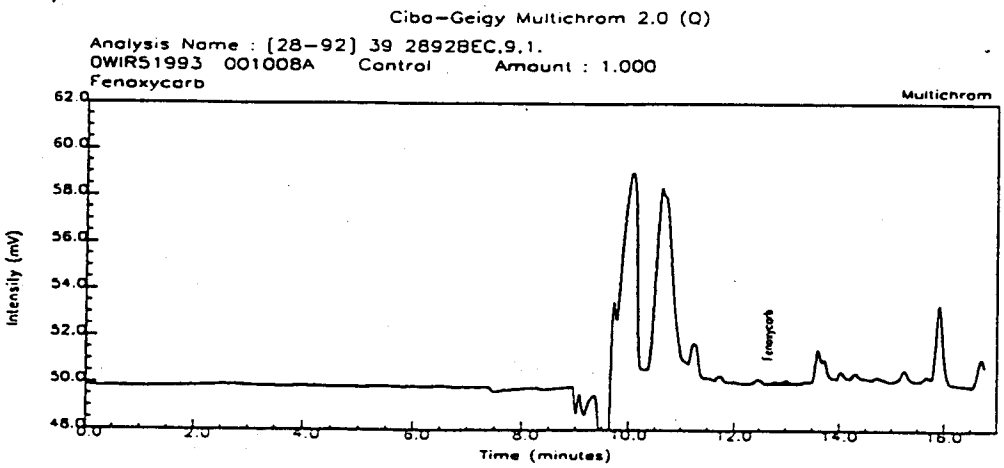


*Recoveries corrected for control values.
(Field Test: 04-IR-001-92)

A: Control, 497.2 mg injected, 0.057 ng found, <0.01 ppm
B: Control + 0.05 ppm Fenoxycarb, 124.3 mg injected, 6.50 ng found, 0.052 ppm, 0.0518 ppm (corrected for control), 104% recovery*

Figure 7. Representative Chromatograms for Control and Fortified Control Hay Samples Using AG-609A with Modifications (cont.)

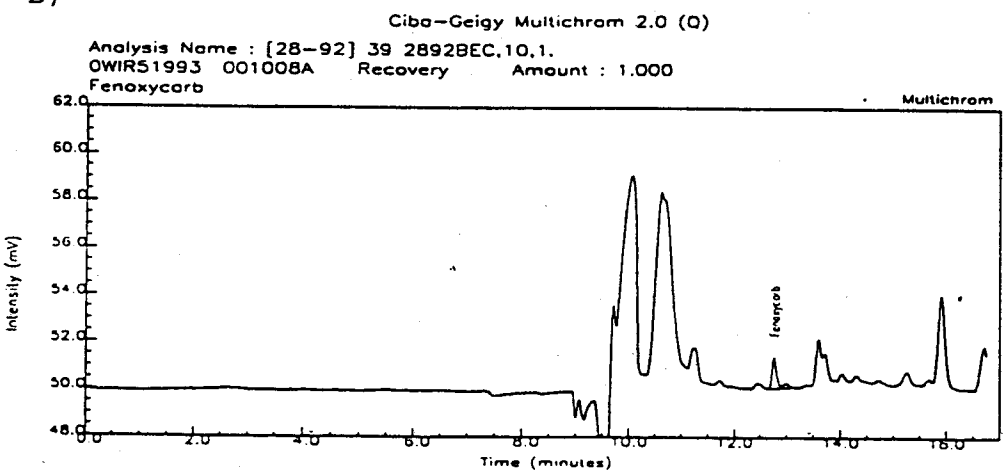
C)



Instrument : AB 783
Channel Title : Channel #39
Lims ID : 98289
Acquired on 3-JAN-1994 at 17:49
Reported on 22-MAR-1994 at 15:11

Method : 2892BEC
Calibration : 2892BEC
Run Sequence : 2892BEC

D)



Instrument : AB 783
Channel Title : Channel #39
Lims ID : 101593
Acquired on 3-JAN-1994 at 18:08
Reported on 22-MAR-1994 at 15:11

Method : 2892BEC
Calibration : 2892BEC
Run Sequence : 2892BEC

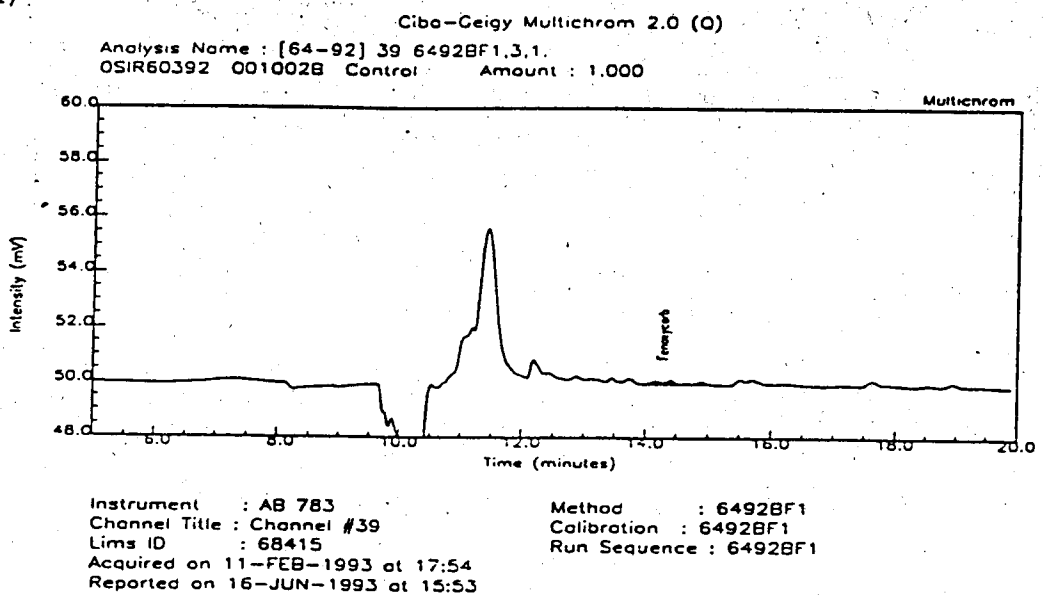
*Recoveries corrected for control values.
(Field Test: OW-IR-519-93)

C: Control, 497.2 mg injected, 0.021 ng found, <0.01 ppm (0.00004 ppm)

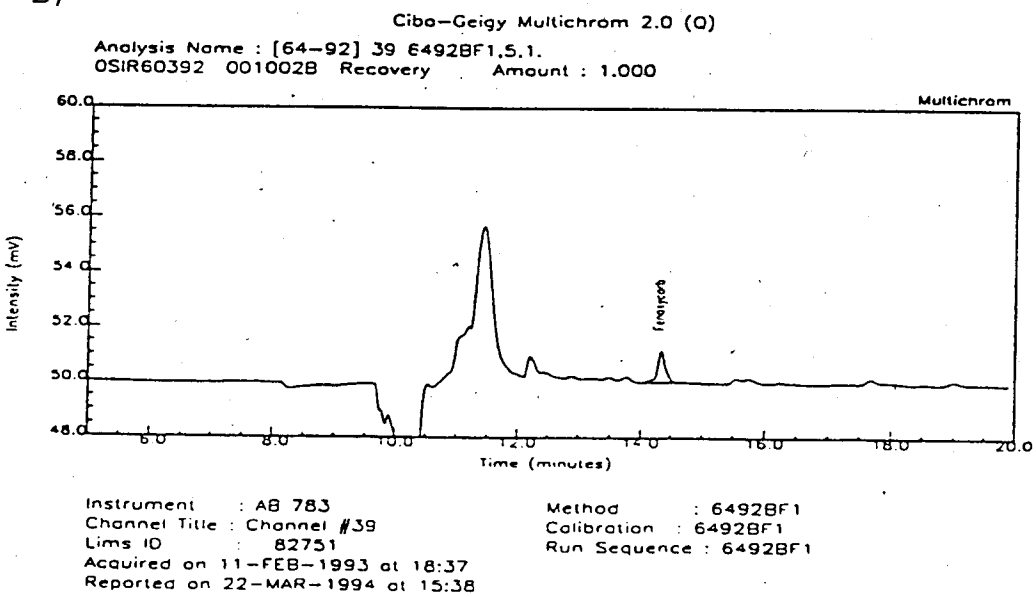
D: Control + 0.01 ppm Fenoxycarb, 497.2 mg injected, 3.791 ng found, 0.008 ppm, 0.0076 ppm (corrected for control), 76% recovery*

Figure 8. Representative Chromatograms for Control and Fortified Control Summer Squash Samples

A)



B)

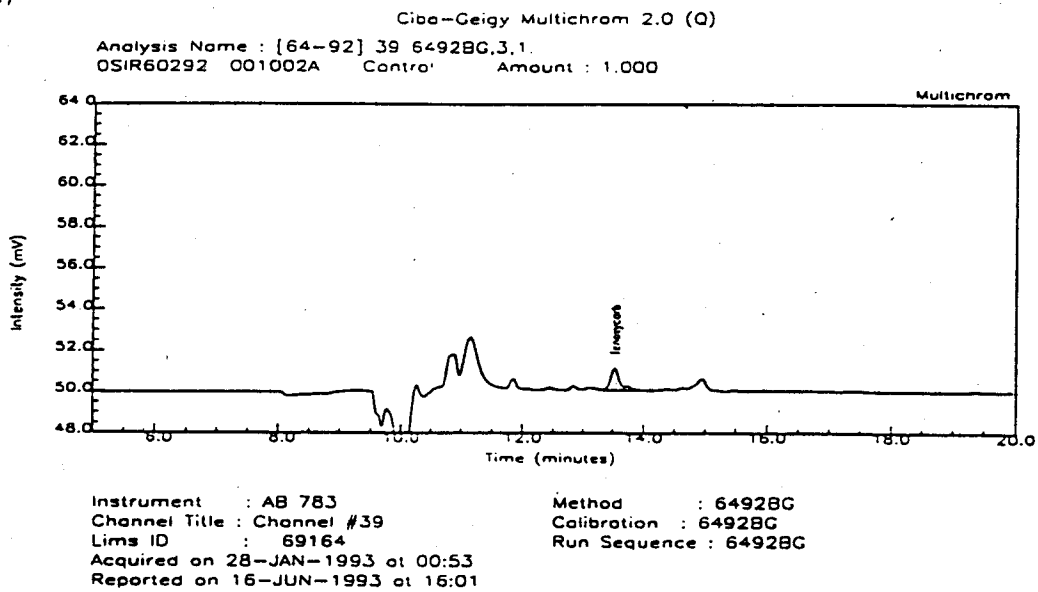


*Recoveries corrected for control values.
(Field Test: OS-IR-603-92)

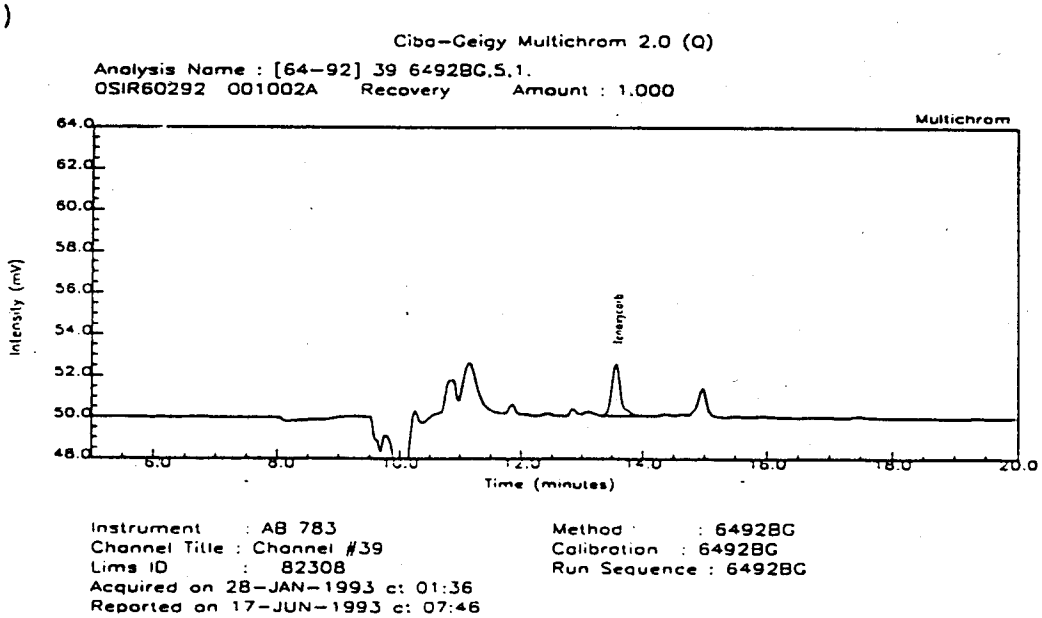
A: Control, 460.0 mg injected, -0.142 ng found, <0.01 ppm
B: Control + 0.01 ppm Fenoxycarb, 460.0 mg injected, 4.985 ng found, 0.0108 ppm, 108% recovery*

Figure 9. Representative Chromatograms for Control and Fortified Control Cucumber Samples

A)



B)



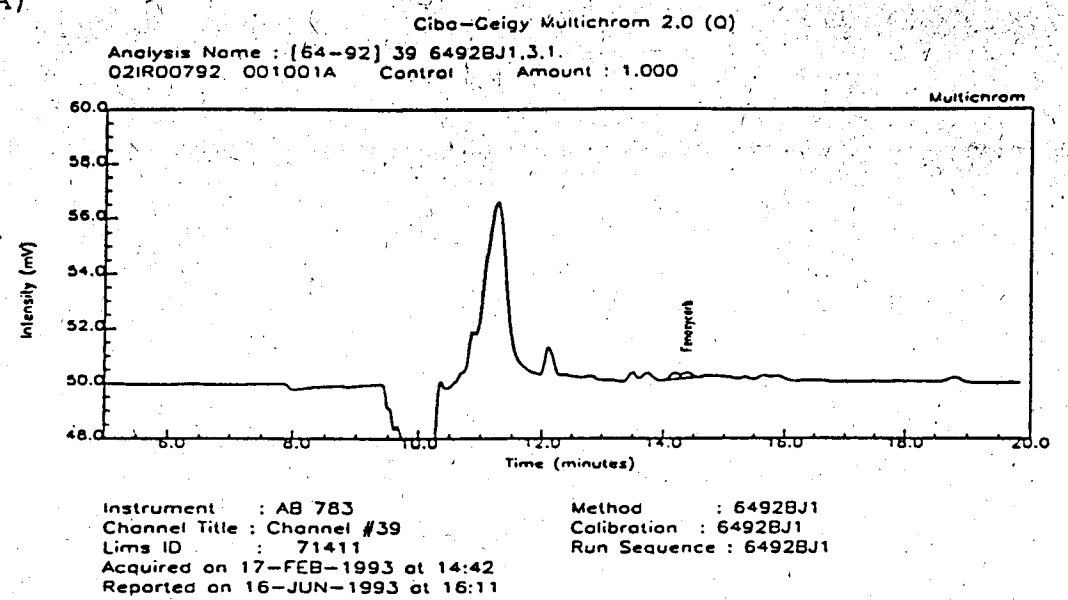
*Recoveries corrected for control values.
(Field Test: OS-IR-602-92)

A: Control, 459.5 mg injected, 3.861 ng found, <0.01 ppm (0.0084 ppm)

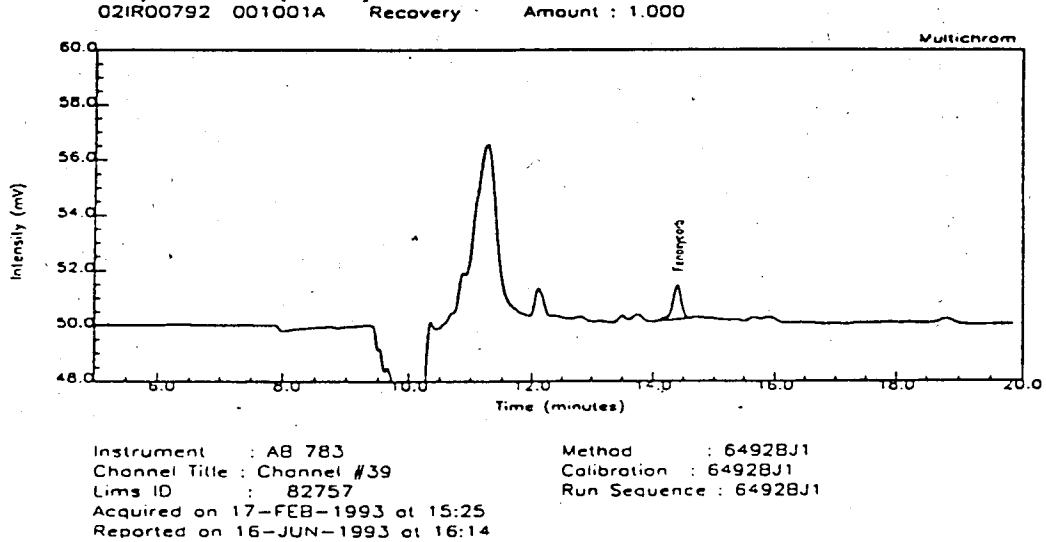
B: Control + 0.01 ppm Fenoxycarb, 459.5 mg injected, 9.295 ng found, 0.0202 ppm, 0.0118 ppm (corrected for control), 118% recovery*

Figure 10. Representative Chromatograms for Control and Fortified Control Cantaloupe Samples

A)



B)



*Recoveries corrected for control values.
(Field Test: 02-IR-007-92)

A: Control, 461.1 mg injected, 0.866 ng found, <0.01 ppm (0.0019 ppm)

B: Control + 0.01 ppm Fenoxycarb, 461.1 mg injected, 5.522 ng found, 0.012 ppm, 0.0101 ppm (corrected for control), 101% recovery*

VII. References

1. R. E. M. Wurz and S. E. Boyetté, Analytical Method No. AG-609, "Analytical Method for the Determination of Fenoxycarb in Pasture Grasses and Crops by High Performance Liquid Chromatography with Column Switching", Ciba-Geigy Corp.

2. S. E. Boyette and R. D. Rollins, ABR-94025, "Fenoxycarb - Magnitude of the Residues in or on Pasture Grasses Following Applications of LOGIC", Ciba-Geigy Corp., in progress.

3. R. E. M. Wurz and S. E. Boyette, ABR-93016, "Fenoxycarb - Magnitude of the Residues in or on Representative Commodities of the Cucurbit Crop Group Following Post Foliar Applications", Ciba-Geigy Corp.