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ATTACHMENT III

VOLUME 2 OF 2 OF SUBMISSION

FENOXYCARB

STUDY TITLE

ANALYTICAL METHOD FOR THE DETERMINATION
OF FENOXYCARB IN PEARS BY HIGH PERFORMANCE
LIQUID CHROMATOGRAPHY

DATA REQUIREMENT

EPA GUIDELINE NUMBER 171-4 (c)

STUDY DIRECTOR

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STUDY COMPLETED

APRIL 15, 1994

PERFORMING LABORATORY

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LABORATORY PROJECT ID

ANALYTICAL METHOD AG-620

VOLUME 1 OF 1 OF STUDY

PAGE 1 OF 34

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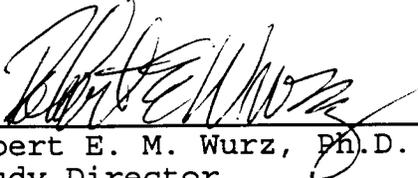
Signature: N. Beth Carroll

Date: 4/15/94

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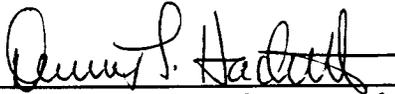
STATEMENT CONCERNING GOOD LABORATORY PRACTICE

The analytical work reported in AG-620 was performed in accordance with Good Laboratory Practice Standards, 40 CFR Part 160.



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**ANALYTICAL METHOD FOR THE DETERMINATION OF FENOXYCARB IN
PEARS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY**

ANALYTICAL METHOD NO. AG-620

Project Number: 343002

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I. SUMMARY AND INTRODUCTION

A. SCOPE

This method is for determination of residues of fenoxycarb in pears. The limit of detection, as determined by the smallest standard injected, is 2.0 ng for fenoxycarb and the limit of quantitation, 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-620 was adapted from ADC Project #1103¹ and is intended for use as a tolerance enforcement method.

B. PRINCIPLE

A 50-g subsample of crop substrate is homogenized with acetone, filtered through a glass fiber filter and rinsed with acetone. The extract is rotary evaporated to remove the acetone. The concentrated extract is diluted with water and saturated salt solution. This aqueous solution is partitioned several times with hexane and the retained organic solution is dried through sodium sulfate. The sample solution is evaporated to dryness and reconstituted with ethyl acetate/toluene for cleanup on a silica column. The final fraction is evaporated to dryness then reconstituted in acetonitrile then buffer. Residue determination is done by HPLC (150 X 4.6 mm C18 column) with UV detection at 228 nm.

II. MATERIALS AND METHODS

A. APPARATUS

- 1.0 Bottles, square amber wide mouth, 16 oz.
- 2.0 Chromatography column, 45 x 1.5 cm i.d. with a 250-ml reservoir
- 3.0 Concentration tubes, 50-ml
- 4.0 Flasks, Erlenmeyer, 500-ml
- 5.0 Flasks, round bottom (RB), 250-ml, 1000-ml
- 6.0 Flasks, side arm, 500-ml
- 7.0 Funnels, Buchner, 7-cm

- 8.0 Funnels, separatory, 1000-ml with Teflon stopcock
- 9.0 Glass microfibre filter (GF/D), Whatman, 7-cm (Fisher Cat. # 09-873F)
- 10.0 Graduated cylinders, 10-ml, 50-ml, 250-ml
- 11.0 Homogenizer, Polytron or equivalent
- 12.0 Pipets, disposable pasteur
- 13.0 Rotary evaporator, Buchi or equivalent
- 14.0 Vials, Wheaton, 2-ml. or equivalent
- 15.0 Ultrasonic Cleaner

B. REAGENTS

- 1.0 Acetone, HPLC grade
- 2.0 Acetonitrile (ACN), HPLC grade
- 3.0 Ethyl acetate (EtOAc), HPLC grade
- 4.0 5% Ethyl acetate/toluene (v/v)
- 5.0 Hexane, HPLC grade
- 6.0 Potassium phosphate monobasic (KH_2PO_4), Certified ACS grade
- 7.0 0.05M Potassium phosphate monobasic, 6.8 g of KH_2PO_4 in 1-L water adjusted to pH of 4.5-5.0.
- 8.0 Silica gel, 70-230 mesh, EM Science (7734-3). Place silica gel in a 130°C oven overnight to activate. One day prior to use, weigh 97 g of silica into a 500-ml Erlenmeyer flask and add 3-ml water. Shake to remove lumps and let equilibrate overnight.
- 9.0 Sodium chloride, Certified ACS grade
- 10.0 Sodium sulfate, Certified ACS grade
- 11.0 Toluene, HPLC grade (Burdick & Jackson)
- 12.0 Water, HPLC grade
- 13.0 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 frozen at $\leq -20^\circ\text{C}$ (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 50-g subsample of substrate into a 16 oz. wide mouth jar. Fortify recovery samples with fenoxycarb standard at this point. Add 200 ml of acetone to the sample and allow to stand for 1 minute. Homogenize the sample with a Polytron homogenizer for approximately five minutes at about 20,000 rpm in an ice bath. Rinse the Polytron blade with 4 X 10-ml acetone and add to the jar. Rinse the Polytron blade with acetone and water between samples.
- 2.2 Filter the sample through Whatman glass microfibre filter (GF/D) seated in a Buchner funnel (preferably 7 cm) into a 500-ml side-arm flask using low vacuum filtration. Rinse the sample bottle with 4 X 20 ml of acetone, pour over the filter cake and filter the rinse. The filter cake is rinsed again with 50 ml of acetone. Combine the extracts and transfer to a 500-ml flat bottom flask with four 15-ml rinses of acetone.
- 2.3 Remove the acetone from the extract by rotary vacuum evaporation in a water bath at $\leq 40^{\circ}\text{C}$ until the volume is approximately 20 ml. (Note: All evaporations are done using rotary vacuum evaporation).

3.0 Partition Cleanup

- 3.1 Quantitatively transfer the aqueous sample to a 1000-ml separatory funnel along with 100 ml of water. Add 200 ml of water to the flask and sonicate to remove sample residue and transfer to the sep. funnel. Rinse the flask with a final 100-ml rinse of water and add to the sep.

funnel. Add 100 ml of hexane and 10-ml saturated sodium chloride solution to the sep. funnel and shake for about one minute, allowing for venting.

- 3.2 Allow the phases to separate then drain the lower, aqueous layer back into the flat-bottom flask. Transfer the upper, hexane layer to a clean 500-ml Erlenmeyer flask, draining it through a glass funnel plugged with glass wool and containing approximately 15 g of sodium sulfate rinsed with hexane. The aqueous layer is partitioned two more times with 50 ml of hexane each by the procedure above, combining the hexane layers in the same flask. Rinse the sep. funnel with 20 ml of hexane and drain through the sodium sulfate. Finally, rinse the sodium sulfate with 50 ml of hexane.

4.0 Silica Gel Chromatography

- 4.1 Weigh a 10-g aliquot of 3% deactivated silica gel into a beaker and add 40 ml of 5% EtOAc/toluene. Degas the slurry for 10 minutes in an ultrasonic bath. Transfer the slurry to a 250-ml reservoir column containing a glass wool plug and drain excess solvent. Caution: Do not allow the bed to go to dryness during any elution.
- 4.2 Evaporate the sample from Section II.C.3.2 to dryness in a water bath at $\leq 40^{\circ}\text{C}$. Reconstitute the sample residue with 4 ml of 5% EtOAc/toluene and load onto the column. Discard the eluate.
- 4.3 Rinse the flask two more times with 4 ml of 5% EtOAc/toluene and load onto the column after the previous rinse has drained to the column bed.

Discard the eluate.

- 4.4 Add 28 ml of 5% EtOAc/toluene to the flask and transfer to the column so as not to disturb the column bed and drain at approximately 2 ml/min. Discard the eluate.
- 4.5 Place a 250-ml round-bottom flask under the column. Elute the column with 155 ml of 5% EtOAc/toluene at approximately 2 ml/min and collect.
- 4.6 Evaporate the sample to dryness in a water bath at $\leq 40^{\circ}\text{C}$. Dissolve the sample in 20 ml of acetone. Quantitatively transfer the sample solution to a concentration tube with two 5-ml rinses of acetone. Evaporate the sample to dryness again in a water bath at $\leq 40^{\circ}\text{C}$.
- 4.7 Reconstitute in an appropriate volume of 50:50 acetonitrile:0.05M potassium phosphate buffer by adding the appropriate volume of acetonitrile first. Mix thoroughly with a vortex mixer, then add an equal volume of buffer and vortex again.

D. INSTRUMENTATION

1.0 Description and Operating Conditions

- 1.1 Install the HPLC system according to Table I.
- 1.2 Determine the retention time of Fenoxycarb on the column by injecting 2.0 ng of the analyte. (Inject 50 ul of the 0.04 ng/ul standard solution prepared in Section II.I.1.0)

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 50- μ l aliquots of standard solutions of Fenoxycarb in a working range of 2.0 - 100 ng/injection. Generate a linear regression from the data by comparing detector response and ng injected.

E. INTERFERENCES

None.

F. CONFIRMATORY TECHNIQUES

Ciba Analytical Method AG-609A, "ANALYTICAL METHOD FOR THE DETERMINATION OF FENOXYCARB IN PASTURE GRASSES AND CROPS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH COLUMN SWITCHING", may be tried to analyze pear samples. Although the cleanup procedures of AG-609 are more thorough, there is no validation data for pears with this method.

G. TIME REQUIRED

A skilled analyst can complete the extraction and analysis of a set of 6-8 samples in 12 working hours.

H. MODIFICATIONS AND POTENTIAL PROBLEMS

Samples may be filtered with a 0.45 μ m Acrodisc filter before HPLC analysis.

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 50:50 ACN:0.05M KH_2PO_4 to give a series of fortification/analytical standards in a range of 0.04 ug/ml to 2.0 ug/ml of Fenoxycarb. Store the standard solutions in amber bottles at 4°C in the dark when not in use. See Table II and Figure 3 for data and chromatograms of standards.

J. METHODS OF CALCULATION

1.0 Determination of Sample Residues

- 1.1 Inject 50- μl aliquots of sample extracts from Section II.C.4.7 into the HPLC (Table I) under the same conditions as for standards. Make appropriate dilutions of the samples in Mobile Phase 1 (50:50 ACN:0.05M KH_2PO_4 buffer) 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 Figure 4.
- 1.2 To calculate the residue results in terms of ppm of fenoxycarb, the mg sample injected must be first calculated as follows (Equation 1):

$$(1) \text{ mg inj.} = \frac{(G)(V_i)}{(V_f)}$$

G = milligrams sample extracted
V_i = injection volume (ul)
V_f = total volume of final injection
solution (ul)

To determine the ppm analyte found
in the sample, use Equation 2.

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

R% = recovery ratio given by
equation 4

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 0.50 ng/μl standard solution of fenoxycarb to 50 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:

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

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:

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

III. RESULTS AND DISCUSSION

The accuracy and precision of Analytical Method AG-620 are demonstrated by the mean recovery of 87% and a standard deviation of 5.5%. The range of recoveries was 81-95%. See Table III for a summary of the recovery data. This data was generated during the ruggedness trial of ADC Project #1103 and is reported in ABR-92087². Analytical Method AG-620 was adapted from ADC Project #1103 as a result of modifications made during the ruggedness trial.

IV. CONCLUSION

Analytical Method AG-620 is a valid and accurate method for the determination of parent residues of fenoxycarb in pears.

V. CERTIFICATION

The reports and experimental results included in this study, AG-620, are certified to be authentic accounts of the experiments.



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Residue Chemistry
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910-632-2391

4/15/94
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TABLE I. LIQUID CHROMATOGRAPHIC OPERATING CONDITIONS
FOR THE DETERMINATION OF FENOXYCARB

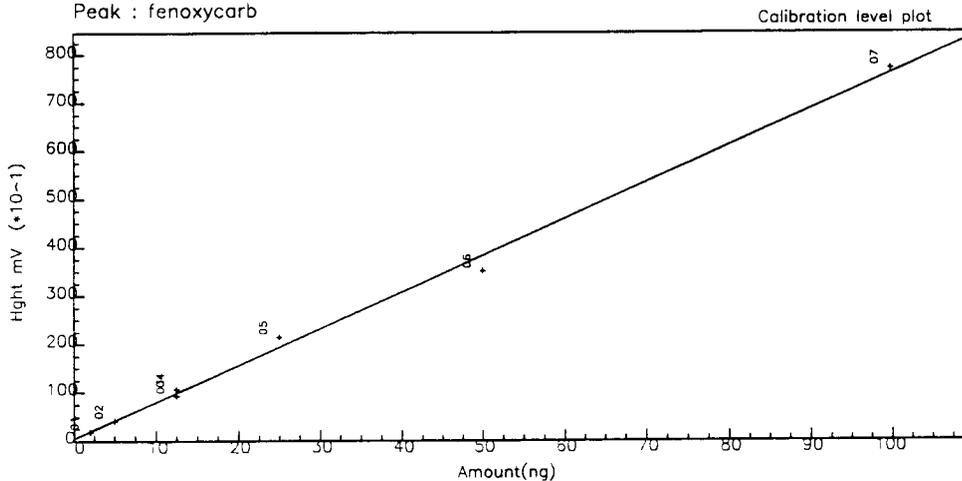
Instrument:	Perkin-Elmer Model ISS-200 Automatic HPLC sampler or equivalent Kratos-Spectroflow 400 LC Pumps or equivalent
Column :	Supelcosil LC-18-DB, 150 mm x 4.6 mm, 5 μ m particle size (Cat. # 5-8348) or equivalent
Precolumn:	Spherisorb, Reverse Phase Guard Cartridge, 30 mm x 4.6 mm, 5 μ m particle size (Fisher Scientific Cat. # 05-692- 450) or equivalent
Mobile Phase	50:50 ACN:0.05M KH ₂ PO ₄ /water (v:v)
Retention Time:	~13.5 min.
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
Injection Volume:	50 μ l
Run Time:	35 min/injection

TABLE II. TYPICAL STANDARDIZATION DATA FOR FENOXYCARB STANDARDS

Std. Soln. ng/ μ l	Std. Wt. Inj. ng	Peak Height μ V
0.04	2.00	186
0.10	5.00	419
0.25	12.5	932
0.25	12.5	1071
0.50	25.0	2140
1.00	50.0	3509
2.00	100.0	7708

Ciba-Geigy Multichrom 2.0 (Q)

Calibration Name : [363-92] 39 FENOXYP5.
Fenoxycarb First Pass
Peak : fenoxycarb



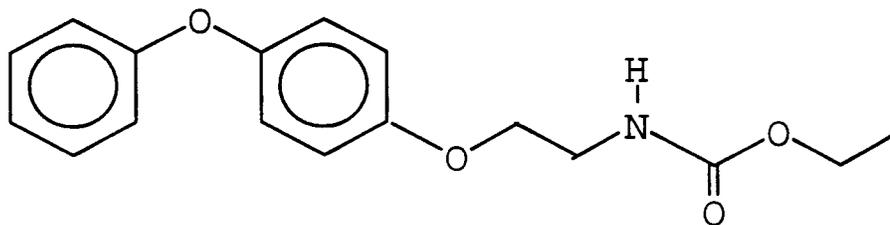
Constant : 4.57302E+1
1st degree : 7.55782E+1

Curve fit : Linear
Correlation coefficient : 0.99807
Standard error : 1.80168E+2
Reported on 13-APR-1994 at 10:51

Table III. SUMMARY OF RECOVERY DATA FOR FENOXYCARB FOR FORTIFIED PEAR SAMPLES

<u>Sample</u>	<u>Code #</u>	<u>ppm Found</u>	<u>% Recovery</u>
Reagent Blank	---	<0.01	---
Control Pear	1A-3	<0.01	---
Control Pear	1B-3	<0.01	---
Control Pear + 0.01 ppm fenoxycarb	P-R-1A-3	0.008	82
Control Pear + 0.01 ppm fenoxycarb	P-R-1B-3	0.008	81
Control Pear + 0.05 ppm fenoxycarb	P-R-2A-3	0.042	84
Control Pear + 0.05 ppm fenoxycarb	P-R-2B-3	0.047	95
Control Pear + 0.10 ppm fenoxycarb	P-R-3A-3	0.091	91
Control Pear + 0.10 ppm fenoxycarb	P-R-3B-3	0.087	87

Mean = 87%, s.d. = 5.5%, CV = 6.3%

FIGURE 1. CHEMICAL NAME AND STRUCTURE

Fenoxycarb

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

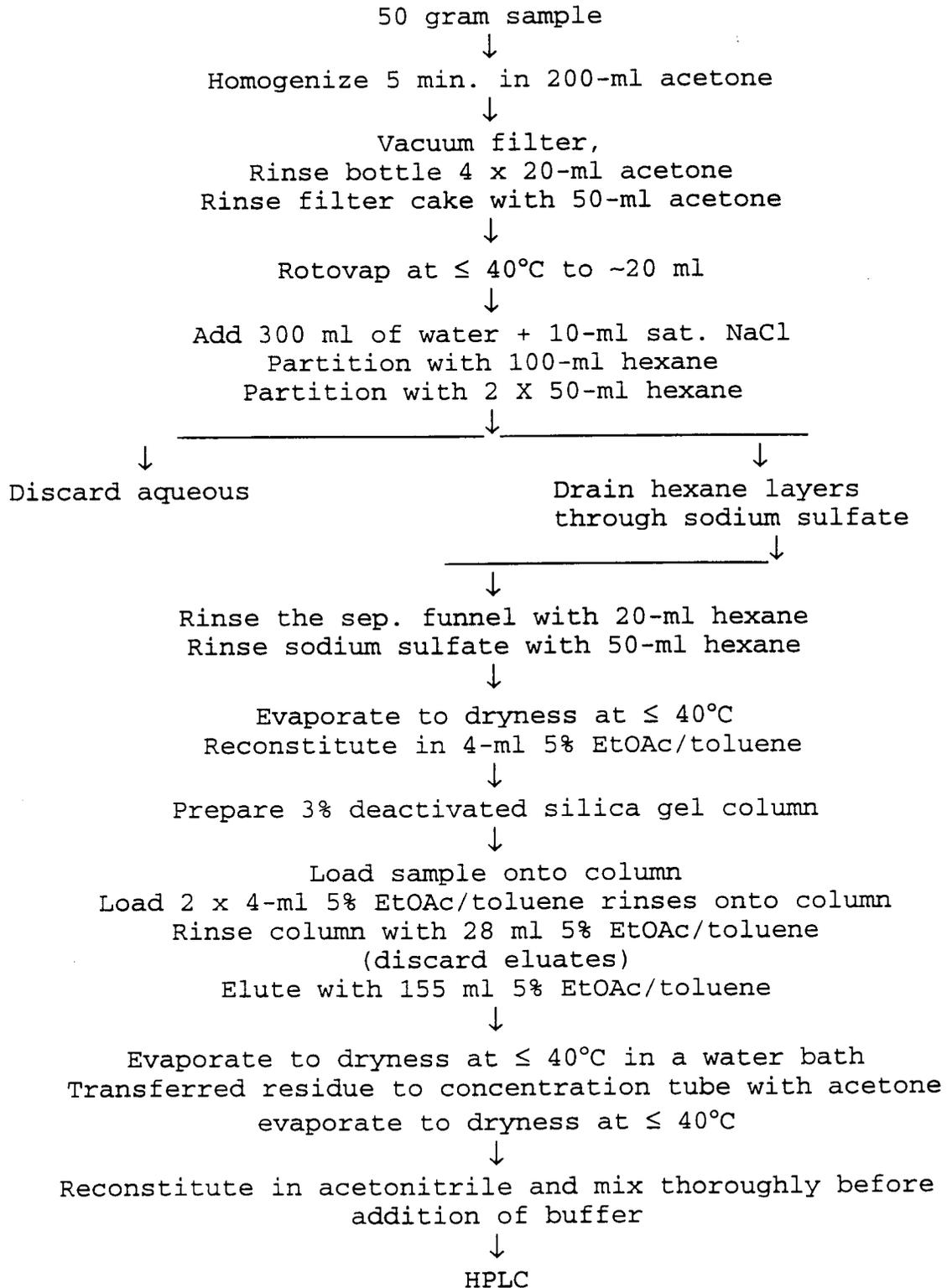
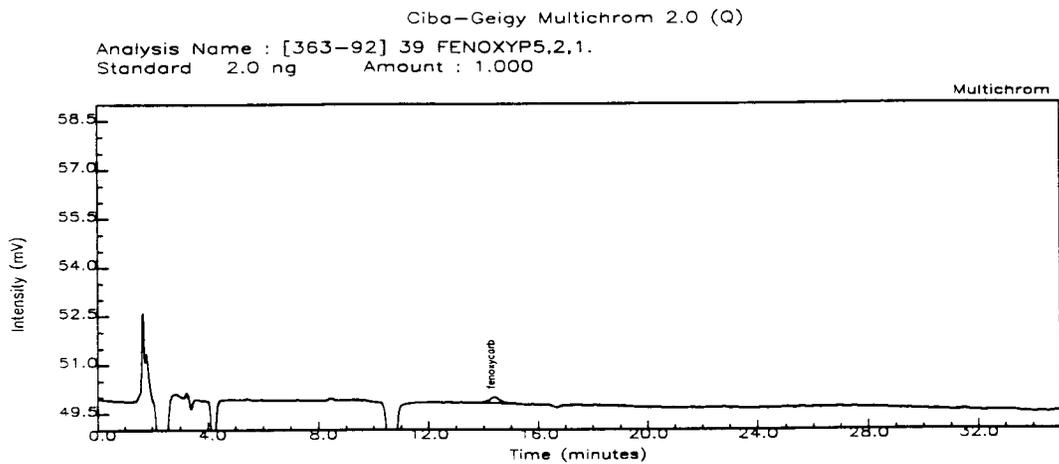
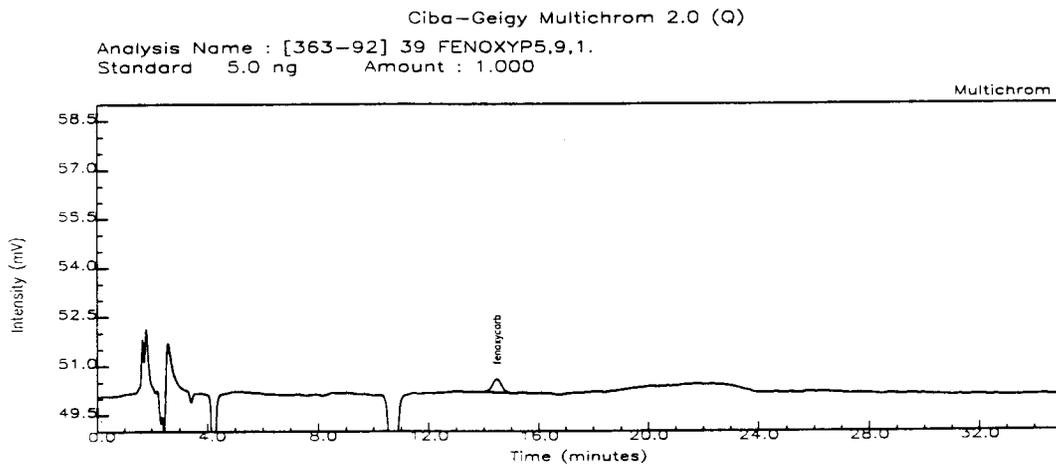
FIGURE 2. FLOW DIAGRAM FOR ANALYTICAL METHOD AG-620

Figure 3. Representative Chromatograms for Fenoxycarb Standards

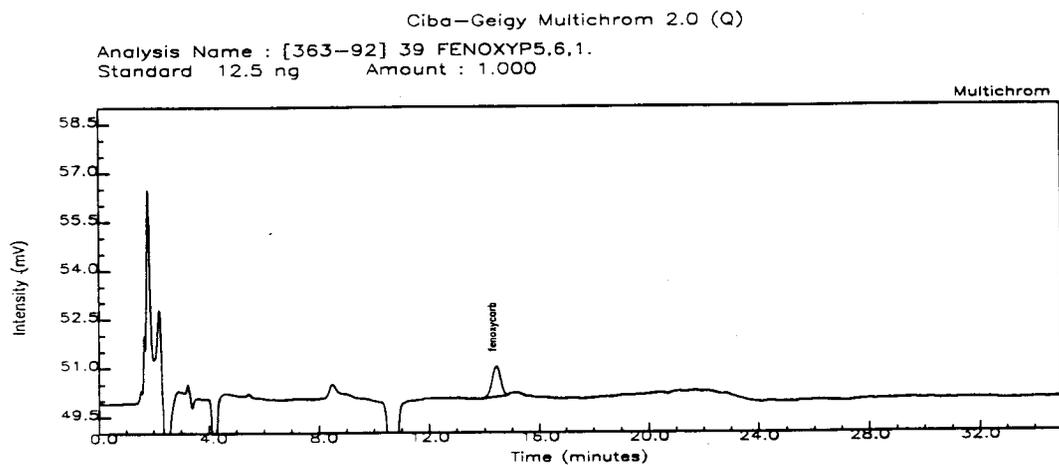


2.0 ng injected

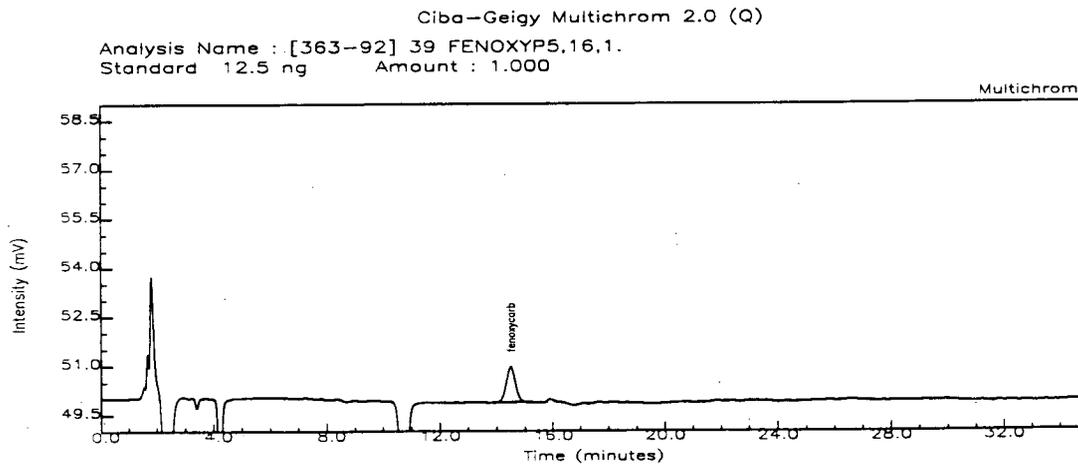


5.0 ng injected

Figure 3. Representative Chromatograms for Fenoxycarb Standards (Continued)

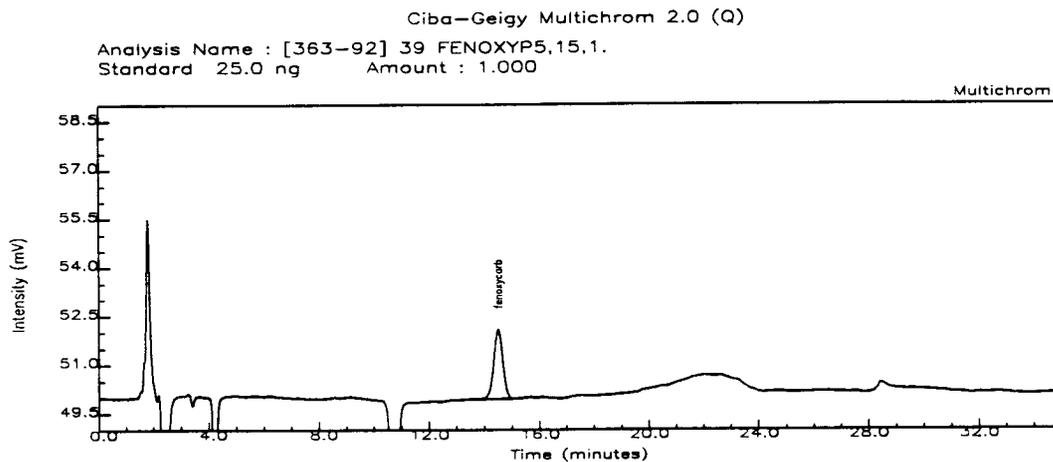


12.5 ng injected

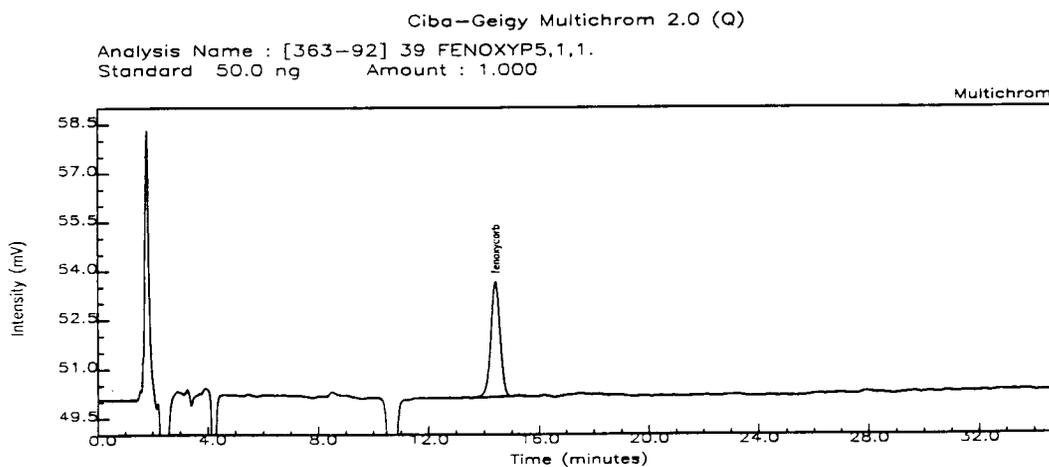


12.5 ng injected

Figure 3. Representative Chromatograms for Fenoxycarb Standards (Continued)

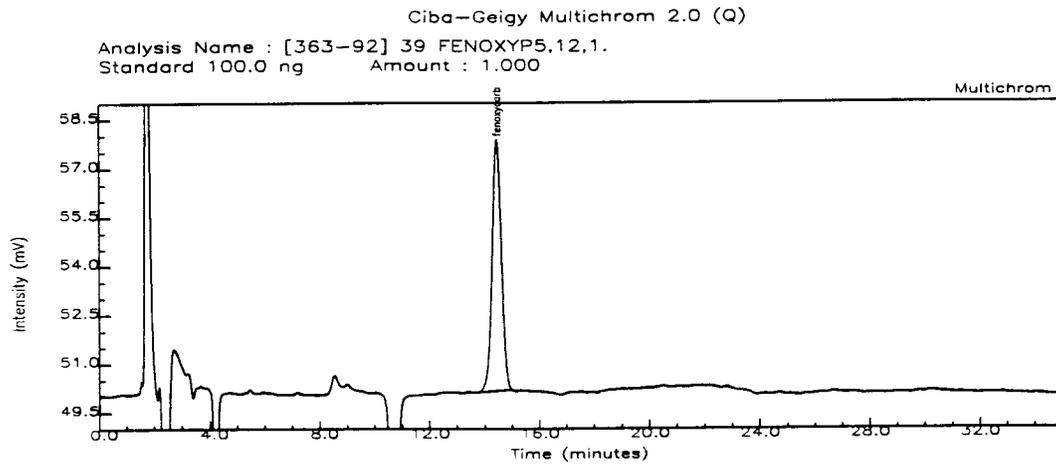


25.0 ng injected



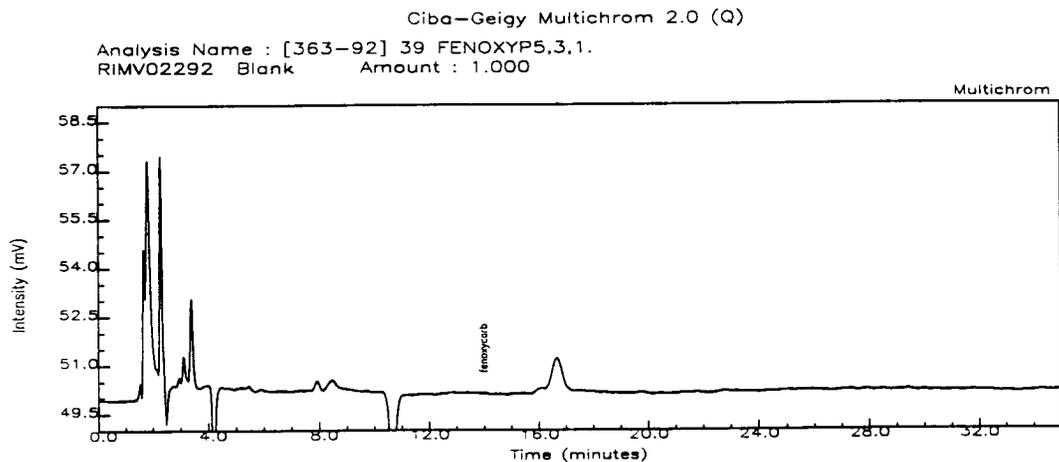
50.0 ng injected

Figure 3. Representative Chromatograms for Fenoxycarb Standards (Continued)

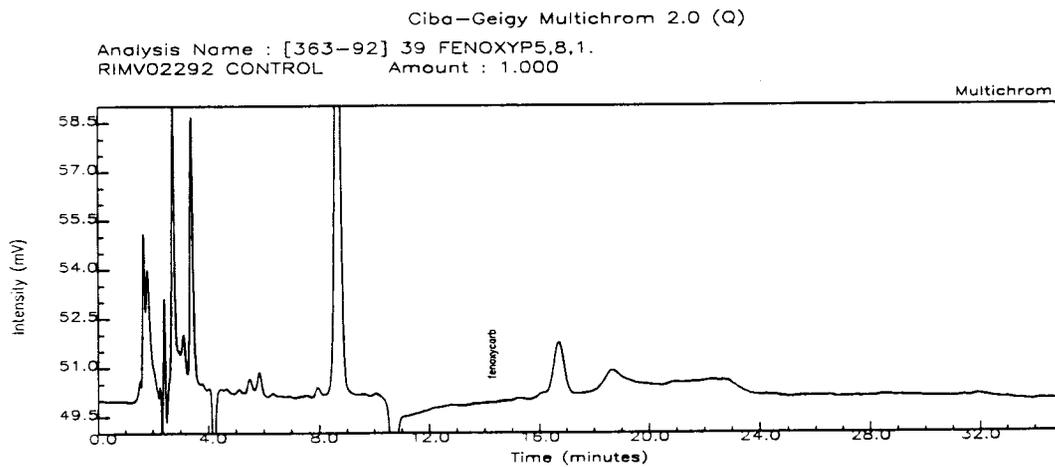


100.0 ng injected

Figure 4. Representative Chromatograms for Control and Fortified Control Pear Samples



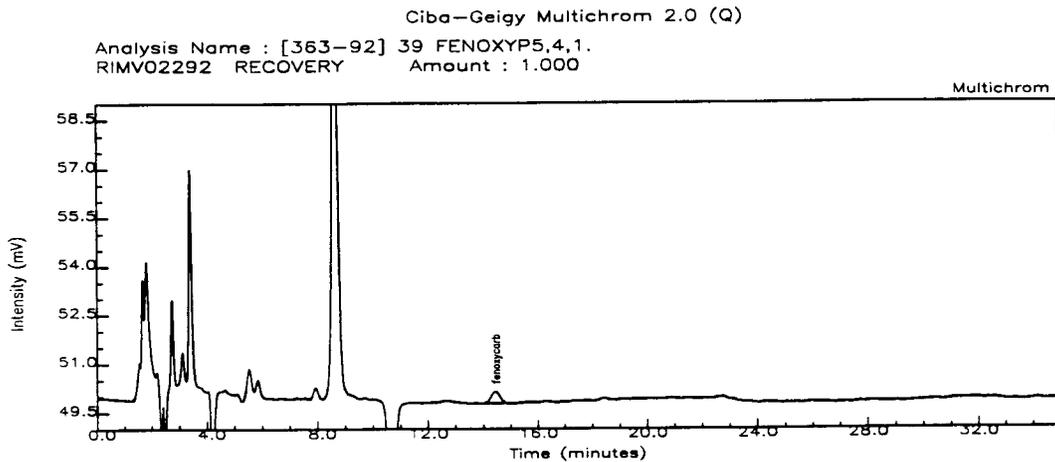
Reagent Blank, 500 mg injected, <2.0 ng found, <0.01 ppm



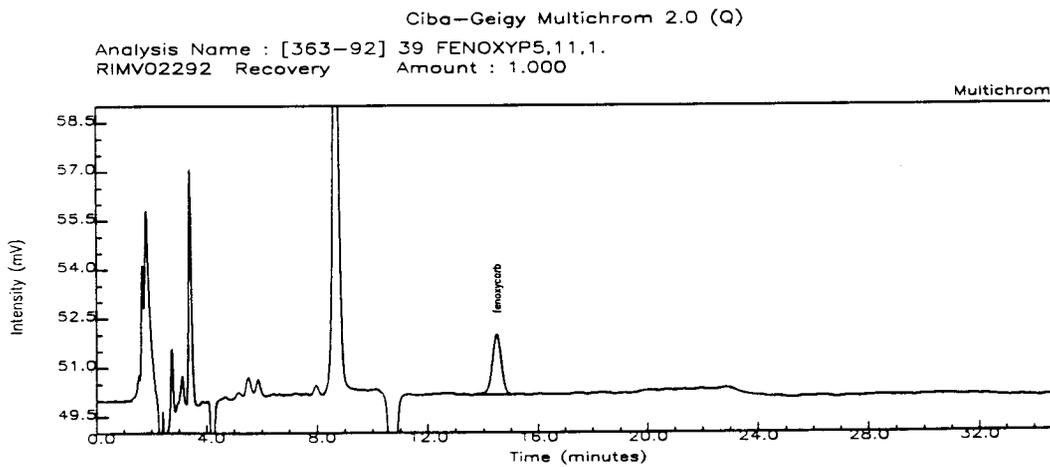
P-C-1B-3, Control, 500 mg injected, <2.0 ng found, <0.01 ppm

(Full Chromatograms)

Figure 4. Representative Chromatograms for Control and Fortified Control Pear Samples (Continued)



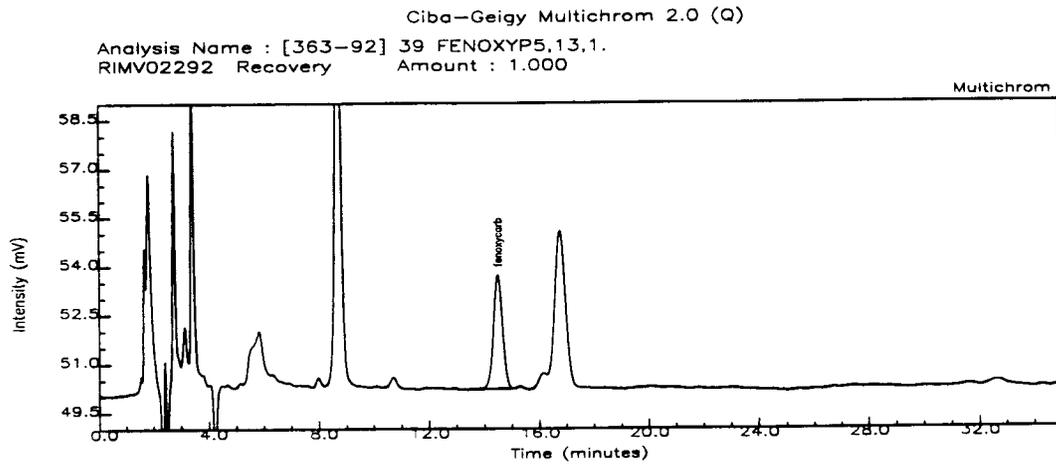
P-R-1A-3, Recovery, 0.01 ppm added, 500 mg injected,
4.083 ng found, 0.008 ppm, 82%



P-R-2B-3, Recovery, 0.05 ppm added, 500 mg injected,
23.66 ng found, 0.047 ppm, 95%

(Full Chromatograms)

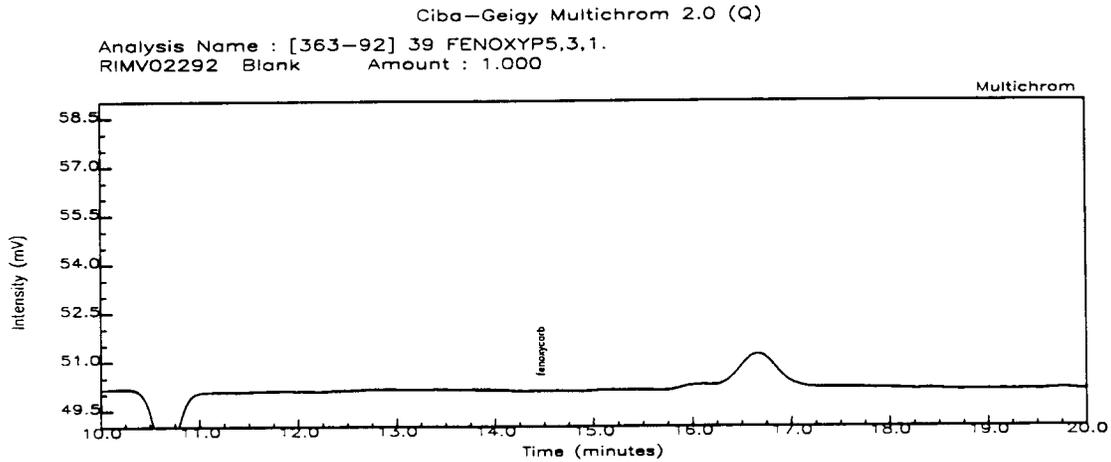
Figure 4. Representative Chromatograms for Control and Fortified Control Pear Samples (Continued)



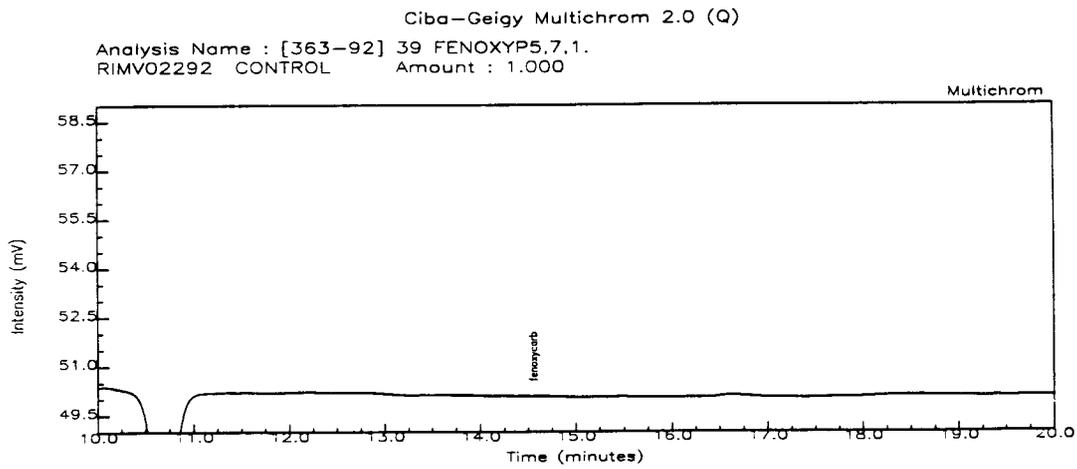
P-R-3A-3, Recovery, 0.10 ppm added, 500 mg injected,
45.50 ng found, 0.091 ppm, 91%

(Full Chromatograms)

Figure 4. Representative Chromatograms for Control and Fortified Control Pear Samples (Continued)

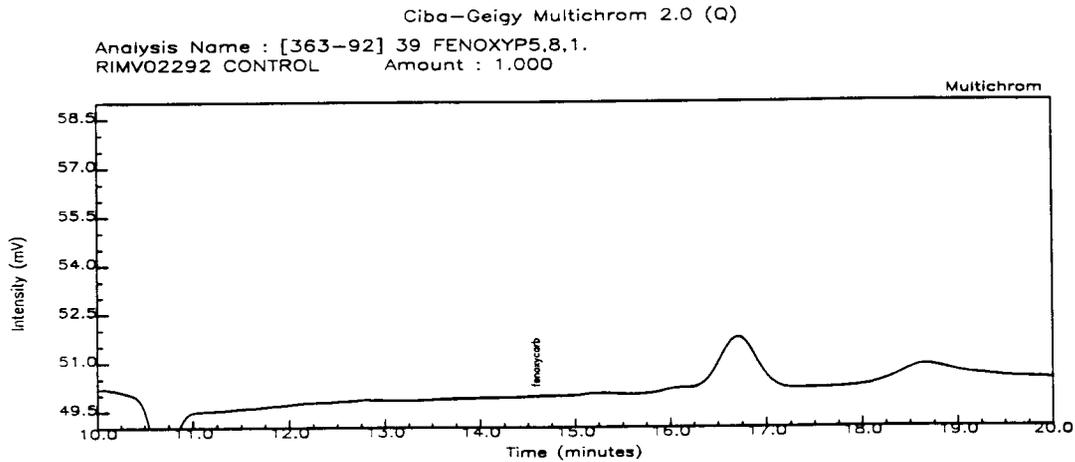


Reagent Blank, 500 mg injected, <2.0 ng found, <0.01 ppm

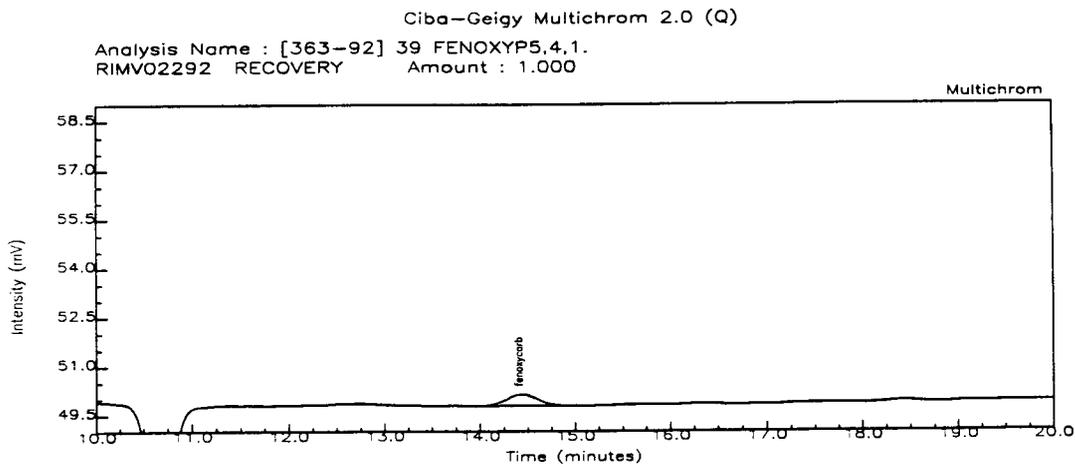


P-C-1A-3, Control, 500 mg injected, <2.0 ng found, <0.01 ppm

Figure 4. Representative Chromatograms for Control and Fortified Control Pear Samples (Continued)

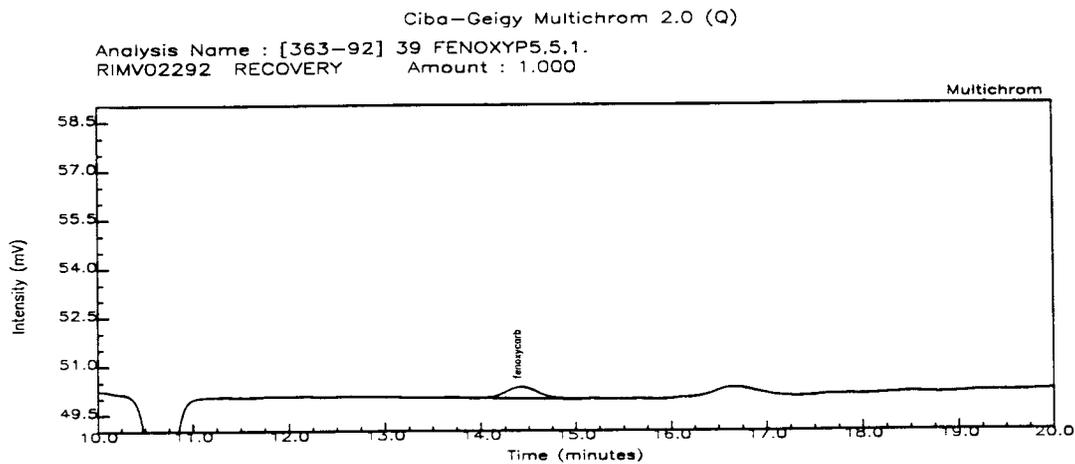


P-C-1B-3, Control, 500 mg injected, <2.0 ng found, <0.01 ppm

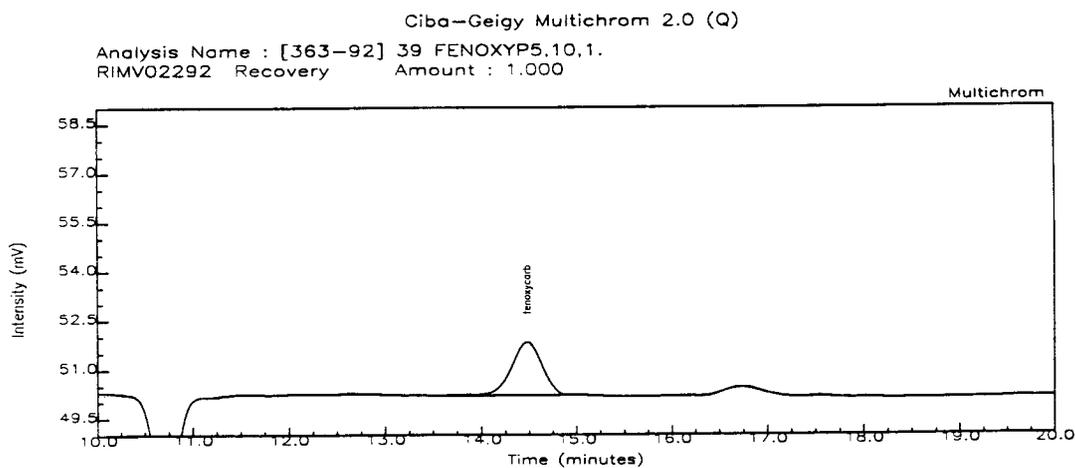


P-R-1A-3, Recovery, 0.01 ppm added, 500 mg injected,
4.083 ng found, 0.008 ppm, 82%

Figure 4. Representative Chromatograms for Control and Fortified Control Pear Samples (Continued)

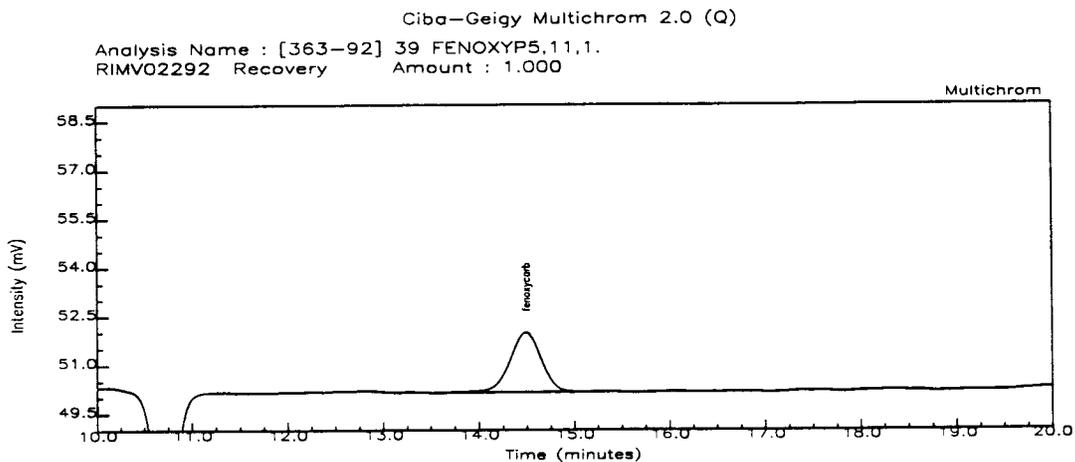


P-R-1B-3, Recovery, 0.01 ppm added, 500 mg injected,
4.066 ng found, 0.008 ppm, 81%

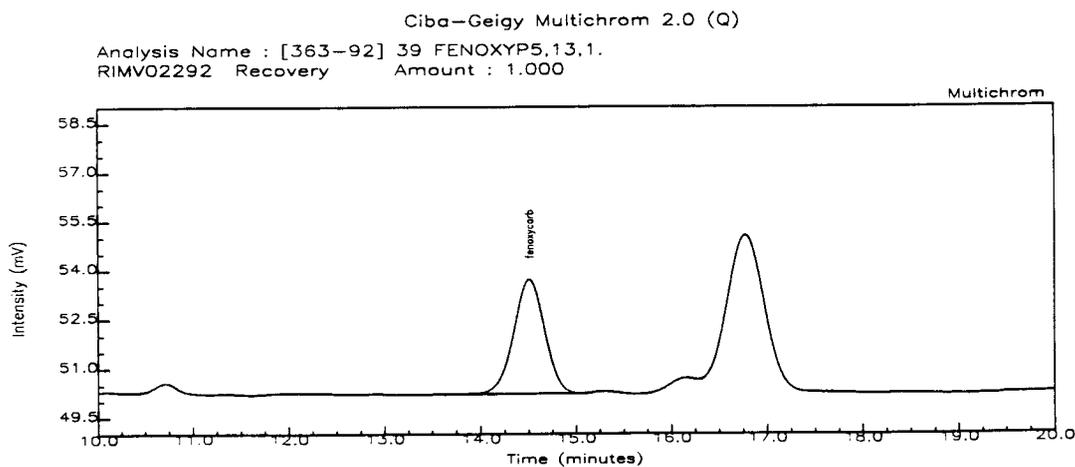


P-R-2A-3, Recovery, 0.05 ppm added, 500 mg injected,
20.89 ng found, 0.042 ppm, 84%

Figure 4. Representative Chromatograms for Control and Fortified Control Pear Samples (Continued)

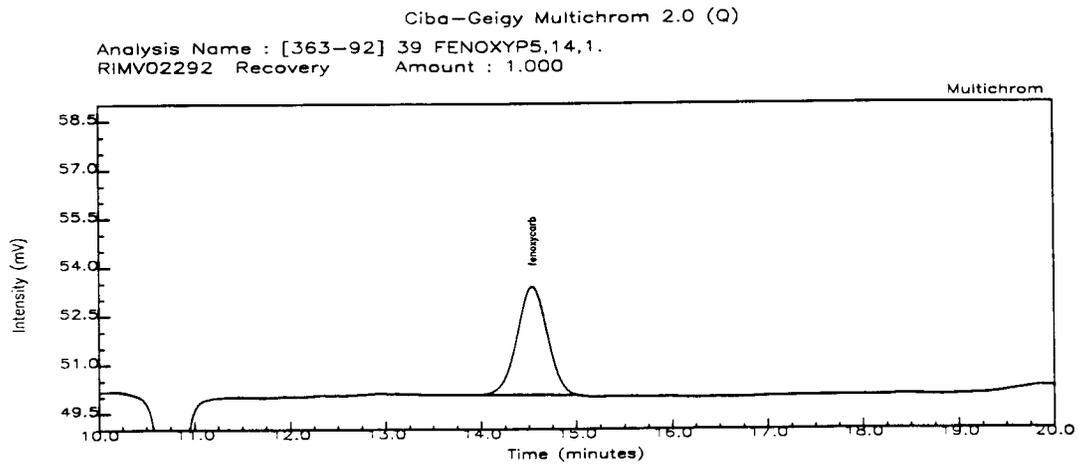


P-R-2B-3, Recovery, 0.05 ppm added, 500 mg injected,
23.66 ng found, 0.047 ppm, 95%



P-R-3A-3, Recovery, 0.10 ppm added, 500 mg injected,
45.50 ng found, 0.091 ppm, 91%

Figure 4. Representative Chromatograms for Control and Fortified Control Pear Samples (Continued)



P-R-3B-3, Recovery, 0.10 ppm added, 500 mg injected,
43.45 ng found, 0.087 ppm, 87%

VII. References

1. K. Martin-Pollock, Validation Report, ADC PROJECT #1103 "THE DETERMINATION OF FENOXYCARB RESIDUES IN PEARS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY", Analytical Dev. Corp.

2. R. E. M. Wurz, ABR-92087 "Method Validation Ruggedness TRIAL FOR THE DETERMINATION OF FENOXYCARB IN PEARS USING ADC PROJECT #1103 "THE DETERMINATION OF FENOXYCARB RESIDUES IN PEARS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY"", Ciba Plant Protection.