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FENOXYCARB

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TITLE

ANALYTICAL METHOD FOR THE DETERMINATION OF RESIDUES OF  
FENOXYCARB AND HYDROXYLATED METABOLITES, Ro-16-8797 AND  
Ro-17-3192, IN MEAT, MILK, BLOOD, AND EGGS BY COLUMN  
SWITCHING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY  
AMENDMENT 1

DATA REQUIREMENT

EPA GUIDELINE NUMBER 171-4 (d)

STUDY DIRECTOR

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AUGUST 16, 1995

PERFORMING LABORATORY

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LABORATORY PROJECT IDENTIFICATION

ANALYTICAL METHOD NO. AG-608 AMENDMENT 1

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VOLUME 1 OF 1 OF STUDY

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**STATEMENT CONCERNING GOOD LABORATORY PRACTICES**

The Good Laboratory Practices Compliance Statement regarding the U. S. Environmental Protection Agency's Good Laboratory Practice Standards (40 CFR Part 160) provided on page thirty-two (32) of this volume and signed by the Study Director is truthful and accurate.

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ANALYTICAL METHOD FOR THE DETERMINATION OF RESIDUES OF  
FENOXYCARB AND ITS HYDROXYLATED METABOLITES, Ro-16-8797 AND  
Ro-17-3192, IN MEAT, MILK, BLOOD, AND EGGS BY COLUMN  
SWITCHING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

ANALYTICAL METHOD NO. AG-608

Amendment 1

Protocol No.: 357-92

Project No.: 343002

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Study Initiation Date: November 19, 1992

Explanation: This amendment is to replace page 42 only of  
method AG-608. The original page 42  
contained typographical errors, and no data  
is effected by the changes.

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I. SUMMARY AND INTRODUCTION

A. SCOPE

This method is for the determination of residues of Fenoxycarb and its hydroxylated metabolites, Ro-16-8797, and Ro-17-3192 (see Figure 1) in animal tissues, milk and poultry eggs. The limit of detection of this method, defined as the lowest standard concentration injected, is 2.5 ng and the limit of quantitation, defined as the lowest fortification level used in the study, is 0.01 ppm for each of the three analytes.

B. PRINCIPLE

For fat, muscle, and blood, a 10-g subsample of substrate is homogenized in acetonitrile. For all other substrates (liver, kidney, milk, eggs), homogenization of the 10-g subsample of substrate is done in 20% H<sub>2</sub>O/80% acetonitrile. After filtering, the extract is transferred to a separatory funnel and partitioned with hexane. The acetonitrile phase for fat, muscle and blood is evaporated to dryness using vacuum rotary evaporation. For liver, kidney, eggs and milk, the water present in the extract is removed from the acetonitrile by adding toluene and water saturated with sodium chloride and partitioning prior to evaporation of the organic phase. The samples are reconstituted in aqueous sodium carbonate, and the analytes are partitioned into hexane:ethyl acetate. The samples are cleaned up on a Bakerbond Silica SPE column. Parent fenoxycarb and Ro-16-8797 are eluted together while Ro-17-3192 is eluted in a separate fraction. Analysis is performed using column switching high performance liquid chromatography (HPLC). A cyano column (Supelco LC-CN) is used for the preparatory column with a mobile phase of 70% 0.02 M KH<sub>2</sub>PO<sub>4</sub> (pH = 3)/30% methanol. A C1 column (Supelcosil LC-1) is used for the analytical column with a mobile phase of 60% 0.02 M KH<sub>2</sub>PO<sub>4</sub> (pH = 3)/25% methanol/15% acetonitrile. The flow rate through both columns is 1.5 mL/minute. Detection is by UV at 235 nm.

## II. MATERIALS AND METHODS

### A. APPARATUS/EQUIPMENT

- 1.0 Bottles, Owens-Brockway round amber, 32-oz. with 53 mm wide mouth, or equivalent (Penn Bottle Co.)
- 2.0 Filter, Acrodisc LC PVDF, 0.2  $\mu$ m, 13.0 mm (Product # 4455 or equivalent)
- 3.0 Filter Paper, Whatman Qualitative #1, 7 cm, (Whatman # 1001070, or equivalent)
- 4.0 Flasks, KIMAX with side-arm, 500-mL (Fisher #10-181E, or equivalent)
- 5.0 Flasks, Boiling with flat bottom, 250-mL, and 500-mL (Fisher #09-552B, and 09-552C, or equivalent)
- 6.0 Funnel, Buchner porcelain (Fisher # 10-356-C or equivalent)
- 7.0 Funnel, Separatory, 125-mL, and 500-mL (Fisher #10-437-10B, and 10-437-10D, or equivalent)
- 8.0 Glass Wool (Fisher #11-390 or equivalent)
- 9.0 Graduated cylinder, 10-mL, 50-mL, 100-mL, 250-mL (Fisher #08-551A, 08-551C, 08-551D, and 08-551E or equivalent)
- 10.0 Homogenizer, Polytron (Brinkman Instruments or equivalent)
- 11.0 Pasteur pipettes (Kimble 13-678-30B, 13-678-30C or equivalent)
- 12.0 Pipettes, volumetric class A, 1-mL (Fisher # 13-650-2B, or equivalent)
- 13.0 Reservoir, 15-mL for solid phase extraction, (J. T. Baker # 7119-01 or equivalent), with adapter (J. T. Baker # 7122-00 or equivalent). Note - see Section II.H.4.0.
- 14.0 Rotary Evaporator, Buchii or equivalent, with warm water bath (Buchler Instruments

or equivalent), and aspirator pump (Cole-Parmer or equivalent)

- 15.0 Solid Phase Extraction, Bakerbond SPE SiOH, Silica Gel, 1 gram/6 cc, (J. T. Baker # 7086-07)
- 16.0 Syringe, Hamilton Gastight, 1 mL (Fisher #14-815-127 or equivalent)
- 17.0 Tubes, Carbon Filter, 32 mm x 160 mm, (Fisher # 08-261B or equivalent)
- 18.0 Tubes, Concentration, 50-mL, (Fisher # 05-538-20B or equivalent)
- 19.0 Ultrasonicator (Heatsystems Inc. or equivalent)
- 20.0 Vials, crimp top borosilicate (Sun Broker, Inc. or equivalent)

B. REAGENTS

- 1.0 Acetonitrile, HPLC grade (Fisher # A998-4 or equivalent)
- 2.0 Diclorodimethylsilane (Aldrich # D6082-6 or equivalent)
- 3.0 Dichloromethane, HPLC grade (Burdick & Jackson #300-4 or equivalent)
- 4.0 Ethyl Acetate, OPTIMA grade (Fisher # E-196-4 or equivalent)
- 5.0 Hexane, HPLC grade (Fisher # H302-4 or equivalent)
- 6.0 Methanol, HPLC grade (Fisher # A452-4 or equivalent)
- 7.0 Phosphoric acid, Certified ACS grade (Fisher # A242 or equivalent)
- 8.0 Potassium Phosphate, monobasic, Certified ACS Grade (Fisher # P382-500 or equivalent)
- 9.0 Sodium carbonate, Certified ACS grade (Fisher # S263-3 or equivalent)

- 10.0 Sodium Chloride, Certified ACS grade (Fisher # S271-3 or equivalent)
- 11.0 Sodium Sulfate, Certified ACS grade (Fisher # S421-3)
- 12.0 Toluene, High Purity Solvent grade (Burdick and Jackson # 347-4 or equivalent)
- 13.0 Water, HPLC grade (picopure or equivalent)
- 14.0 Fenoxycarb, CGA-114597, Analytical Standard supplied by Ciba-Geigy Corporation, 410 Swing Rd., Greensboro, NC, 27419
- 15.0 Ro-16-8797, CGA-294850, Analytical Standard supplied by Ciba-Geigy Corporation, 410 Swing Rd., Greensboro, NC, 27419
- 16.0 Ro-17-3192 (no CGA-number), Analytical Standard supplied by Ciba-Geigy Corporation, 410 Swing Rd., Greensboro, NC, 27419

C. ANALYTICAL PROCEDURE

1.0 Extraction

1.1 Extraction of Fat, Muscle and Blood Samples

Weigh a 10-gram sample of tissue homogenate or slices, or well mixed blood into a tared extraction bottle. For recovery samples, fortify as specified in section II.I.2.0 and II.J.2.0. Add 200 mL of acetonitrile and homogenize for 2 minutes with the Polytron at high speed (>17000 rpm). Return any tissue remaining on the Polytron blade back into the extraction bottle. Filter under vacuum through a Whatman #1 filter paper in a long stem Buchner funnel into a 500-mL side-arm flask. Rinse the Polytron blade with 50 mL of acetonitrile into the extraction bottle. Pour the rinsate through the tissue homogenate remaining on the filter paper. Pour the extract into a 500-mL separatory funnel when

filtration is complete, and continue with section II.C.2.1.

1.2 Extraction of Liver, Kidney, Milk and Egg Samples

Weigh a 10-gram sample of tissue homogenate or slices, egg homogenate or well mixed milk into a tared extraction bottle. For recovery samples, fortify as specified in section II.I.2.0 and II.J.2.0. Add 200 mL of 20% water:80% acetonitrile (note: the extraction solvent must be at room temperature) and homogenize for 2 minutes with the Polytron at high speed (>17000 rpm). Return any tissue remaining on the Polytron blade back into the extraction bottle. Filter under vacuum through a Whatman #1 filter paper in a long stem Buchner funnel into a 500-mL side-arm flask. Rinse the Polytron blade with 50 mL of 20% water:80% acetonitrile into the extraction bottle. Pour the rinsate through the tissue homogenate remaining on the filter. Pour the extract into a 500-mL separatory funnel when filtration is complete, and continue with section II.C.2.2.

2.0 Partitions

2.1 Hexane Partition for Fat, Muscle and Blood Samples

Add 50 mL of hexane to the extract in the separatory funnel from section II.C.1.1, stopper, and shake for 1 minute. Allow the phases to separate and drain the lower acetonitrile phase back into the side-arm flask used in section II.C.1.1. Discard the top hexane layer. Decant the acetonitrile phase back into the separatory funnel and partition again by adding a fresh 50 mL of hexane and shaking for 1 minute. Collect the lower acetonitrile layer in a 500-mL flat bottom boiling flask (discard the hexane) and remove the acetonitrile by

rotary evaporation in a water bath at 35-40 °C.

2.2 Hexane Partition for Liver, Kidney, Milk and Egg Samples

Add 50 mL of hexane to the extract in the separatory funnel from section II.C.1.2, stopper, and shake for 1 minute. Allow the phases to separate and drain the lower water/acetonitrile phase back into the side-arm flask used in section II.C.1.2. Discard the top hexane layer. Decant the water/acetonitrile phase back into the separatory funnel and partition again by adding a fresh 50 mL of hexane and shaking for 1 minute. Collect the lower 20% water:80% acetonitrile layer again in the same side-arm flask and discard the hexane.

2.2.1 Addition of Water Saturated with Sodium Chloride and Toluene

Decant the 20% water:80% acetonitrile phase back into the 500-mL separatory funnel. Add 25 mL of water saturated with sodium chloride, followed by 50 mL of toluene. Stopper the separatory funnel and shake for 1 minute. Allow the layers to separate, and discard the lower aqueous layer. Collect the upper layer, which will contain the acetonitrile, toluene, and a small amount of water, into a 500-mL flat-bottom boiling flask. Evaporate to dryness using rotary evaporation in a water bath at 35-40 °C.

2.3 50% Hexane:50% Ethyl Acetate Partition for All Substrates

Add 25 mL of 0.5% sodium carbonate to the flat-bottom flask from section II.C.2.1 or II.C.2.2.1, swirl, and sonicate. Transfer this aqueous phase

to a 125-mL separatory funnel. Repeat the rinsing of the round bottom flask with another 25 mL of 0.5% sodium carbonate and pour into the separatory funnel. Add 25 mL of 50% hexane:50% ethyl acetate to the flat-bottom flask, swirl, and also transfer to the same 125-mL separatory funnel. Repeat the rinse with another 25 mL of 50% hexane:50% ethyl acetate. Stopper the 125-mL separatory funnel and shake for 1 minute. Allow the layers to separate and drain the lower aqueous layer into the 500-mL flat-bottom flask used previously.

Prepare a carbon filter tube which contains a glass wool plug and is approximately one-half filled with sodium sulfate, and pre-rinse the sodium sulfate with 25 mL of 50% hexane:50% ethyl acetate (discard the rinse). Pour the 50% hexane: 50% ethyl acetate extract through the carbon filter tube. Collect the 50% hexane: 50% ethyl acetate in a 250-mL flat-bottom boiling flask, which has been silanized using the procedure outlined in Table VI.

Pour the aqueous layer from above back into the 125-mL separatory funnel and partition a second time by adding a fresh 50 mL of 50% hexane:50% ethyl acetate. Discard the lower aqueous layer. Pour the 50% hexane:50% ethyl acetate phase through the carbon filter tube containing sodium sulfate that was used with the first 50% hexane: 50% ethyl acetate extract. Rinse the sodium sulfate with approximately 25 mL of 50% hexane/50% ethyl acetate. Remove the 50% hexane/50% ethyl acetate by rotary evaporation in a water bath at 35 - 40°C.

### 3.0 Silica Solid Phase Extraction for All Substrates

#### 3.1 Column Conditioning

Attach a 15-mL reservoir to a Bakerbond Silica (1 gram, 6 cc) solid phase extraction (SPE) column with an adapter. Condition the column by flushing with 15 mL of ethyl acetate followed by 10 mL of dichloromethane. Let each solvent pass through the column until it reaches the top of the silica packing, and do not let the packing become dry. The flow rate through the SPE column should be about 1 to 2 drops per second with gravity alone, and a vacuum manifold system should not be used. A needle and stopcock can be attached to the bottom of the column to control the flow.

#### 3.2 Sample Addition

Add 5 mL of dichloromethane to the flat-bottom boiling flask from section II.C.2.3. Swirl vigorously and sonicate well. **Note: the dihydroxylated metabolite, Ro-17-3192, will dissolve in dichloromethane only after vigorous shaking and sonication.** Add the sample to the pre-conditioned SPE column detailed in section II.C.3.1. Rinse the round bottom flask with another 5 mL of dichloromethane and add to the SPE. Let the solvent pass through the column and just to the top of the silica packing. Do not let the column become dry.

#### 3.3 Column Washes

Add 10 mL of hexane to the 250-mL flat-bottom boiling flask from section II.C.3.2, swirl, and add to the column. Let the solvent pass through the column and just to the top of the silica packing. Add 6 mL of 20% ethyl acetate/80% hexane to the 250-mL flat-bottom boiling flask, swirl, and add to the column. Let the solvent pass

through the column and just to the top of the silica packing. Discard these washes.

### 3.4 Sample Elution

Add 8 mL of 50% hexane: 50% ethyl acetate to the 250-mL flat-bottom boiling flask from section II.C.3.3, swirl, and add to the column. Parent fenoxycarb and Ro-16-8797 will elute in this solvent. Let the solvent pass through the column and just to the top of the silica packing. Collect in a 50-mL concentration tube (note: to ensure that the 50-mL concentration tubes are clean, pre-rinse them sequentially with water and acetone and let dry prior to use). After elution of fenoxycarb and Ro-16-8797 is complete, add 15 mL ethyl acetate to the 250-mL flat-bottom boiling flask, swirl, and add to the column. Ro-17-3192 will elute in this solvent. Collect in a separate 50-mL concentration tube. Two separate fractions result from the silica elution; one containing Fenoxycarb and Ro-16-8797, and the other containing only the Ro-17-3192.

### 3.5 Preparation of Final Sample

Remove the solvent from the eluents collected in section II.C.3.4 by vacuum rotary evaporation. Reconstitute in an appropriate volume of 50% acetonitrile:50% 0.02 M  $\text{KH}_2\text{PO}_4$  in picopure water (adjusted to pH=3.0 with phosphoric acid) using a volumetric pipette. Swirl and sonicate well. Filter through an Acrodisc filter attached to a syringe if any particulates are visible in the sample. Transfer to a crimp top vial for injection and analysis by HPLC. Note that 2 final fractions result from each sample being analyzed; one which will be injected for analysis of fenoxycarb and Ro-16-8797, and one which will be injected and analyzed for Ro-17-3192. The volume in the

final samples can be increased with higher concentrations of residues to ensure that the analyte peak heights are bracketed by the standards discussed in section II.I.1.0 and II.J.1.0.

#### D. INSTRUMENTATION

##### 1.0 Description and Operating Conditions

Residues of fenoxycarb and the two hydroxylated metabolites are determined by HPLC with column switching. The preparatory column is a Supelcosil LC-CN (3.3 mm x 4.6 mm), with a mobile phase of 70% 0.02 M  $\text{KH}_2\text{PO}_4$  (pH=3.0±0.1), 30% methanol. The analytical column is a Supelcosil LC-1 (250 mm x 4.6 mm), with a mobile phase of 60% 0.02 M  $\text{KH}_2\text{PO}_4$  (pH=3.0±0.1)/25% methanol/15% acetonitrile. The flow rate through both columns is 1.5 mL/minute. Mobile phase recycling is recommended only if large volumes are being used, or if a switching valve is placed at the effluent of both systems with the purpose of preventing crossover between the mobile phases. Detection of the analytes is by ultraviolet detection at a wavelength of 235 nm and the injection volume is 50  $\mu\text{l}$ . The HPLC conditions and column switching setup are described in Table I and Figure 3.

The sample is first injected onto the preparatory column (cyano column), which is initially vented to waste (position A in Figure 3). The switching valve changes the flow of the mobile phase effluent from waste to the analytical column (C1 column) as the analyte(s) begin to elute (position B in Figure 3). After all of the analyte(s) has eluted from the preparatory column, the switching valve returns to the original position where the effluent is vented to waste. The sample will then be separated on the analytical column and elute through the detector.

The time that the analytes elute from the preparatory column must be determined prior to analysis. This is done by injecting a

standard from section II.I.1.0 through the cyano column with the effluent running through the detector. Three peaks result, with Ro-17-3192 eluting first, followed by Ro-16-8797, and then parent fenoxycarb. The switching time, or cut time, for the fraction being analyzed for Ro-16-8797 and fenoxycarb will start when Ro-16-8797 begins to elute, and end when fenoxycarb has completely eluted off of the preparatory column. The cut time for the fraction being analyzed for Ro-17-3192 will begin as this analyte starts to elute and end just after it has completely eluted off of the preparatory column. An example of a standard injection on the cyano column used to determine the cut time is shown in Figure 3.

## 2.0 Standardization

Standardize the HPLC system by injecting 50- $\mu$ l aliquots of solutions containing fenoxycarb, Ro-16-8797, and Ro-17-3192 in a working range of 2.5 to 25 ng/injection. Generate a linear regression from the data by comparing detector response and ng injected. See section II.I.1.0 for preparation of analytical standards.

### E. INTERFERENCES

None.

### F. CONFIRMATORY TECHNIQUES

None.

### G. TIME REQUIRED

The extraction and cleanup of a set of 6 samples may be completed within a time period of 8 hours. HPLC analyses can be performed overnight using automated injection.

### H. MODIFICATIONS AND POTENTIAL PROBLEMS

#### 1.0 SPE Volumes

The silica SPE is a normal phase cleanup of the sample extracts where the analytes are separated and selectively eluted from the

column using specific volumes and solvent strengths. The reproducibility from different lots of Bakerbond silica cartridges has been excellent when the exact volumes described in section II.C.3.4 are used. Measurement of the volumes with a graduated cylinder is suggested.

## 2.0 Solubility of Ro-17-3192 in Dichloromethane

Care must be taken when reconstituting the extract in dichloromethane just prior to SPE cleanup since Ro-17-3192 does not readily dissolve in this solvent. Sonication at this step must be done with every sample. Use of a silanized 250-mL boiling flask in section II.C.2.2 for collection and evaporation of the 50% hexane:50% ethyl acetate extract is essential for subsequent reconstitution of Ro-17-3192. The silanization procedure should be repeated for this boiling flask after approximately 15-20 uses. Applying the SPE washes and elution solvents to the silanized 250-mL boiling flask prior to adding them to the column is also suggested.

## 3.0 HPLC Mobile Phase Recycling

During the switching time when the sample is being loaded from the preparatory column to the analytical column, cross contamination of the two mobile phases will occur. Recycling of the HPLC mobile phase is not suggested unless care is taken to minimize the effect of this cross contamination. One way to do this is by using a large enough volume of mobile phase so that the contamination does not change the overall composition of each mobile phase. Another way is by using a second switching valve that will switch the effluent from both mobile phases to a separate waste flask, and not to recycle, during the time that the sample is being loaded from the preparatory column to the analytical column.

#### 4.0 SPE Reservoirs

Interferences appeared during the validation study that were attributed to the SPE reservoirs listed in section II.A.13.0. The problem was eliminated by replacing these reservoirs with 30 cc glass reservoirs (e.g., glass syringe barrel from 30 cc Yale Luer Lok Syringe, VWR Scientific product #BD2314).

### I. PREPARATION OF STANDARD SOLUTIONS

#### 1.0 Preparation of Analytical Standard

Weigh 10 mg of fenoxycarb analytical standard into a 100-mL volumetric flask and dilute to the mark with acetonitrile. Repeat for Ro-17-3192 and Ro-16-8797 to obtain three separate stock solutions of 100 ng/ $\mu$ L. Add 1 mL of each to a 100-mL volumetric flask and dilute to the mark with 50% acetonitrile:50% 0.02M  $\text{KH}_2\text{PO}_4$  in picopure water (adjusted to  $\text{pH}=3.0\pm 0.1$ ) to give a stock solution containing 1 ng/ $\mu$ L of all three analytes. Alternatively, weigh the three analytes into the same 100-mL volumetric flask to obtain a stock containing 100 ng/ $\mu$ L of all 3 analytes. Make serial dilutions of the stock solution with 50% acetonitrile:50% 0.02M  $\text{KH}_2\text{PO}_4$  in picopure water ( $\text{pH}=3$ ) to give a series of fortification/analytical standards in a range of 0.05 to 1.0 ng/ $\mu$ L. Store the standards in a refrigerator in capped amber bottles when not in use.

#### 2.0 Fortification Standards

The 0.1 ng/ $\mu$ L standard prepared in section II.I.1.0 is used to fortify samples at the screening level of 0.01 ppm. Other standards prepared in II.I.1.0 may be used for fortification at other sample concentrations as necessary. See section II.J.2.0 for fortification procedures.

### J. METHODS OF CALCULATION

#### 1.0 Determination of Sample Residues

Inject 50- $\mu$ L aliquots of the final fractions prepared in section II.C.3.5 onto

the HPLC system under the same conditions as for the standards. Make appropriate dilutions of the samples in 50% acetonitrile:50% 0.02M  $\text{KH}_2\text{PO}_4$  in picopure water (adjusted to  $\text{pH}=3.0\pm 0.1$ ) 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 heights into a least squares program to determine the nanograms of fenoxycarb, Ro-16-8797, and Ro-17-3192 in the injected aliquot. Typical chromatograms for control and procedural recovery samples are shown in Figures 7 - 36.

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  
 $V_f$  = total volume of final injection solution ( $\mu\text{L}$ )  
 $R\%$  = recovery ratio given by equation 4  
 $M$  = % moisture of sample  
 $W$  = weight of sample in grams

To determine ppm of each analyte found in samples, use equation 2.

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

If necessary, convert the ppm found for Ro-16-8797 and Ro-17-3192 to parent fenoxycarb equivalents, multiply the results from equation 2 by the ratio of the molecular weights, defined as F:

$$F = \text{the ratio of the molecular weights} = \frac{\text{MW parent}}{\text{MW metabolite}}$$

See Figure 1 for the molecular weights of these compounds.

## 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, Ro-16-8797, and Ro-17-3192.

2.1 Add 1 mL of 0.1 ng/ $\mu$ l to 10 grams of substrate to obtain a sample fortified at 0.01 ppm. Allow the solvent to evaporate for at least 15 minutes prior to addition of extraction solvent. 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:

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

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

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

### III. RESULTS AND DISCUSSION

The objective of Protocol 357-92<sup>1</sup> was to validate Analytical Method AG-608 for the quantitation of residues of fenoxycarb (CGA-114597), and its hydroxylated metabolites, Ro-16-8797 (CGA-294850) and Ro-17-3192 (no CGA- number) in meat, milk, blood and eggs. This was accomplished by analyzing control samples and fenoxycarb, Ro-16-8797, and Ro-17-3192 fortified control samples of poultry liver, peritoneal fat, lean meat, and eggs, beef liver, kidney, tenderloin, round, omental fat, perirenal fat, blood and dairy milk, and goat liver, omental

fat, round, kidney, and milk. In addition, samples of goat liver, kidney, fat, meat, and milk obtained from metabolism study 126-92<sup>2</sup> were analyzed, in which the animals had been dosed with radiolabelled fenoxycarb. The limit of detection (LOD), as determined by the smallest standard injected, is 2.5 ng, and the limit of quantitation (LOQ), as demonstrated by the smallest acceptable recovery level, is 0.01 ppm for each of the three analytes.

Typical standard chromatograms of fenoxycarb, Ro-16-8797, and Ro-17-3192 (injected separately from the other two analytes), which were run during the analysis of poultry lean meat, are shown in Figure 4. The concentrations for fenoxycarb, Ro-16-8797, and Ro-17-3192 and their respective peak heights are shown in Table II, and the calibration plots are shown in Figure 5. The correlation coefficients of 0.99921, 0.99980, and 0.99985 for Fenoxycarb, Ro-16-8797, and Ro-17-3192, respectively, were typical of the calibration plots obtained during this study, and the peak heights for all analytes were reproducible over the course of the study.

Chromatograms from reagent blanks which were included in the analysis of poultry liver, poultry peritoneal fat, beef tenderloin, and beef blood are shown in Figure 6. No peaks occurred at the retention times of Fenoxycarb, Ro-16-8797, and Ro-17-3192.

#### A. ACCURACY

Table III shows the results from analyzing control and control samples fortified with fenoxycarb, Ro-16-8797, and Ro-17-3192 over a range of 0.01 ppm to 0.5 ppm. For the parent fenoxycarb, recoveries ranged from 61% to 128%, with an overall average of 95% (standard deviation (SD) = 11). The mean recovery for fenoxycarb for each substrate ranged from 80% for beef kidney to 111% for goat kidney (SD=11 and 12, respectively). For the metabolite Ro-16-8797, recoveries ranged from 68% to 98%, with an overall average of 86% (SD=6.4). The mean recovery for Ro-16-8797 for each substrate ranged from 76% for goat milk to 93% for beef omental fat (SD=6.0 and 4.1, respectively). For the metabolite Ro-17-3192, recoveries ranged from 51% to 85%, with an overall average of 69% (SD=6.3). The mean recovery for Ro-17-3192 for each substrate ranged from 56% for dairy milk to 76% for beef perirenal fat (SD=5.5 and 4.0,

respectively). Representative chromatograms of control and fortified control samples are shown in Figures 7-36.

#### B. PRECISION

The results from analyzing the  $^{14}\text{C}$ -fenoxycarb treated goat substrates are shown in Table IV. The mean, range, standard deviation, and coefficient of variation (CV) resulting from triplicate analysis of each substrate are shown where residues were detected. Ro-17-3192 was only detected in the goat liver and was not present in any other  $^{14}\text{C}$ -fenoxycarb treated goat substrates. A CV of 15% resulted. Ro-16-8797 was detected above LOD in goat liver and kidney, where the CV were 8.7% and 0.6%, respectively. Fenoxycarb was detected above LOD in all substrates except milk, and the CV ranges from 1.1% (round) to 18% (fat). These results, along with the relatively narrow ranges and acceptable standard deviations resulting from the triplicate analyses, demonstrate the precision of method AG-608. Chromatograms obtained from the analysis of the  $^{14}\text{C}$ -fenoxycarb treated goat substrates are included in Figures 27-36.

#### C. EXTRACTABILITY

Percent extractability results were obtained by transferring an aliquot portion of each extract of the  $^{14}\text{C}$ -fenoxycarb treated goat samples to a scintillation vial, adding approximately 12 mL of Beckman Ready-Safe liquid scintillation cocktail, and measuring the disintegrations per minute (dpm) present in the aliquot on a Beckman LS 7800 liquid scintillation counting (LSC) instrument. The total dpm in the extract was calculated from the aliquot result (dpm/mL of the aliquot multiplied by total mL of the extract) and converted to parts per million (ppm). The % extractability was determined by comparing the ppm result to the total radioactive residue (TRR) in the sample, as determined by the combustion analyses reported in metabolism study 126-92<sup>2</sup>. As an example, 3 mL of goat kidney sample #90 was pipetted from the 250 mL extract following homogenization of a 10 gram sample. LSC determined that the aliquot contained 12154 dpm. The specific activity of the  $^{14}\text{C}$ -fenoxycarb fed to the goat was 35.5  $\mu\text{Ci}/\text{mg}$ . Therefore,

$$\left( \frac{12154 \text{ dpm}}{3 \text{ mL}} \right) \left( \frac{250 \text{ mL}}{10 \text{ g}} \right) \left( \frac{1 \text{ mg}}{35.5 \text{ } \mu\text{Ci}} \right) \left( \frac{1 \text{ } \mu\text{Ci}}{2.22 \times 10^6 \text{ dpm}} \right) \left( \frac{1000 \text{ } \mu\text{g}}{1 \text{ mg}} \right)$$

$$= 1.285 \frac{\mu\text{g}}{\text{g}} \text{ or ppm}$$

The TRR for the kidney of goat #204 is 1.377 ppm<sup>2</sup>, and the % extractability for sample #90 is therefore 93%. Average extractability results from all of the <sup>14</sup>C-fenoxycarb treated substrates are shown in columns (2) and (3) of Table V, and range from 55% in omental fat to 94% in kidneys.

D. ACCOUNTABILITY

Accountability results are calculated by comparing the total ppm obtained from HPLC, as determined by method AG-608, to the total ppm of <sup>14</sup>C-residue in the initial sample (TRR). In Table V, columns (7) -(10) the ppm determined by HPLC is first corrected for recovery, converted to fenoxycarb equivalents, and the results for the three analytes are summed for each sample analyzed. The mean of the total ppm for the triplicate analysis is calculated and expressed as a percentage of the TRR. In addition, aliquot portions of the final fractions obtained in section II.C.3.5 were transferred for LSC, and the ppm of <sup>14</sup>C-residue found in the final fractions were determined using calculations similar to the ppm extractability determined above. These results are shown in column (5) of Table V. Note that fraction 1 contains Ro-16-8797 and fenoxycarb, and fraction 2 contains Ro-17-3192.

Percent accountabilities ranged from 0% in milk to 24% in liver. It is noted that the <sup>14</sup>C-fenoxycarb treated substrates were obtained from metabolism study 126-92<sup>2</sup> which is still in progress, and that the combustion measurements measure only the <sup>14</sup>C radioactivity in the sample. The low accountabilities observed likely result from a decrease in the presence of Ro-17-3192, Ro-16-8797, and parent fenoxycarb due to metabolism to other compounds. Since method AG-608 is specific for recovery of Ro-17-3192, Ro-16-8797, and fenoxycarb, losses of other <sup>14</sup>C-metabolites would be expected to occur in the

fractions discarded during cleanup procedures of method AG-608. Aliquot portions of the discarded fractions from one of the three samples analyzed from each substrate were assayed by LSC to determine the ppm  $^{14}\text{C}$ -residue present. For liver, round, kidney, and milk, the majority of the radioactivity partitioned into the aqueous phases from steps II.C.2.2.1 and/or II.C.2.3. A large amount of the  $^{14}\text{C}$ -residues in fat and round partitioned into the hexane phase in section II.C.2.2. These results indicate the presence of  $^{14}\text{C}$ -metabolites with chemistries different than Ro-17-3192, Ro-16-8797, and fenoxycarb.

The results obtained by HPLC analysis are in reasonably good agreement with results obtained from LSC measurement of the same final fractions. The LSC measurement is higher in all cases, especially with liver and kidney. This suggests that  $^{14}\text{C}$ -metabolites other than the three analyzed for by this method are present in the final fraction.

Test substance ID, test system ID, protocol amendments, protocol deviations, and circumstances affecting the quality and integrity of the data are reported in Residue Test Report RI-MV-024-92<sup>3</sup>. All raw data associated with this study and the original final AG-608 method and protocol are archived in the Biochemistry Group Archives at Ciba Corporation, Greensboro, NC. No specimens remain to be retained.

#### IV. CONCLUSION

Method AG-608 is a valid and accurate method for the determination of Ro-17-3192, Ro-16-8797, and fenoxycarb in meat, milk, blood and eggs. This conclusion is based on the accuracy, precision, extractability, and accountability results in this study.

V. CERTIFICATION

The reports and experimental results included in this study, Laboratory Project ID. AG-608, are certified to be authentic accounts of the experiments.

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Chemist  
Residue Chemistry  
Biochemistry Department

5-23-94  
Date

CERTIFICATION OF GOOD LABORATORY PRACTICES

The analytical work reported in AG-608 was performed in accordance with Good Laboratory Practice Standards, 40 CFR Part 160.

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QUALITY ASSURANCE UNIT

QUALITY ASSURANCE STATEMENT

Study Title: ANALYTICAL METHOD FOR THE DETERMINATION  
OF RESIDUES OF FENOXYCARB AND ITS HYDROXYLATED  
METABOLITES, Ro-16-8797 AND Ro-17-3192, IN MEAT,  
MILK, BLOOD, AND EGGS BY COLUMN SWITCHING HIGH  
PERFORMANCE LIQUID CHROMATOGRAPHY

Study Director: D. D. Campbell

Protocol No.: 357-92

Final Method: AG-608

Pursuant to Good Laboratory Practice Regulations, this statement verifies that the aforementioned study was inspected and/or audited and the findings reported to Management and to the Study Director by the Quality Assurance Unit on the dates listed below.

<u>INSPECTION/AUDIT TYPE</u>	<u>INSPECTION/AUDIT DATE(S)</u>	<u>DATE REPORTED</u>
Protocol Audit	11/19/92	11/20/92
In-progress	10/12-18/93	10/18/93
Final Method Audit	1/31-2/15/94	2/15/94

Prepared by: Jennifer A. Gann  
Jennifer Gann

Date: 2/15/94

VII. TABLES AND FIGURES

TABLE I.

LIQUID CHROMATOGRAPHY OPERATING PARAMETERS FOR THE  
DETERMINATION OF FENOXYCARB RESIDUES

HPLC Pumps:	2 Waters 501 HPLC pumps or equivalent
Autosampler:	Perkin-Elmer Model ISS-100 Automatic HPLC sampler or equivalent.
Column Switching Valve:	VICI EQ60 HPLC Switching Valve or equivalent
Column Oven:	BioRad HPLC column heater, model number 125-0425 or equivalent
Oven Temp.:	30-35°C
Preparatory Column: particle	Supelcosil LC-CN 3.3 mm x 4.6 mm, 5 µm size (Supelco # 5-8979M)
Prep. Mobile Phase:	70% 0.02 M KH <sub>2</sub> PO <sub>4</sub> , pH adjusted to 3.0±0.1 with H <sub>3</sub> PO <sub>4</sub> /30% Methanol
Analytical Column: particle	Supelcosil LC-1, 250 mm x 4.6 mm, 5 µm size (Supelco # 5-8296M)
Analyt. Mobile Phase:	60% 0.02 M KH <sub>2</sub> PO <sub>4</sub> , pH adjusted to 3.0±0.1 with H <sub>3</sub> PO <sub>4</sub> /25% Methanol/15% Acetonitrile
Flow Rate:	1.5 mL/minute
Detection:	UV detection at 235 nm using a ABI Kratos Spectroflow model 783 Programmable Absorbance Detector or equivalent.
Injection Volume:	50 µL
Run Times:	
-Fenoxycarb/Ro-16-8797 Analysis:	30 minutes/injection
-Ro-17-3192 Analysis:	15 minutes/injection
Retention Time Ranges:	Fenoxycarb = 14 - 27 min., Ro-16-8797 = 10 - 16 min, Ro-17-3192 = 5.6 - 8 min. (note: longer retention times were observed when a new analytical column was installed)
Data Acquisition:	Microvax II (Q) Operating System, VMS Version 5.3-1 Application Software VG Multichrom Version 2.0 Worksheet Version: Ws.pas 1.3.1

TABLE II.

TYPICAL STANDARDIZATION DATA FOR FENOXYCARB, Ro-16-8797,  
and Ro-17-3192 (FROM THE ANALYSIS OF POULTRY LEAN MEAT)

<u>ng of Standard Injected</u>	<u>Ro-17-3192 Peak Height (uV)</u>	<u>Ro-16-8797 Peak Height (uV)</u>	<u>Fenoxycarb Peak Height (uV)</u>
2.5	293	248	112
3.0	354	292	152
5.0	623	515	273
10.0	1169	985	486
25.0	3006	2585	1325
Correlation coefficient:	0.99985	0.9998	0.99921
Slope:	120.2	103.8	53.3
Intercept:	-4.522	-20.06	-15.56

TABLE III.

RECOVERY RESULTS FOR CONTROL AND FENOXYCARB, Ro-16-8797, and Ro-17-3192 FORTIFIED CONTROL SUBSTRATES USING METHOD AG-608

(1) Substrate	Protocol Sample #	Fortification PPM	Fenoxycarb				Ro-16-8797				Ro-17-3192									
			Determined PPM	% Recovery	% Mean	% S.D.	Determined PPM	% Recovery	% Mean	% S.D.	Determined PPM	% Recovery	% Mean	% S.D.						
Poultry Liver	2	CONTROL	<0.01																	
	3	0.01	0.01	114	94	12	<0.01	95	92	2.7	0.01	82	71	6.6						
	4	0.01	0.01	89			0.01	94			0.007	74								
	5	0.20	0.18	87			0.18	91			0.13	66								
	5a	0.20	0.17	87			0.18	88			0.14	67								
	6	0.50	0.48	95			0.46	92			0.34	67								
Poultry Peritoneal Fat	8	CONTROL	<0.01																	
	9	0.01	0.01	95	96	2.2	<0.01	88	86	1.8	0.007	68	68	1.0						
	10	0.01	0.01	98			0.01	84			0.007	69								
	11	0.20	0.19	97			0.17	86			0.13	67								
	11a	0.20	0.19	95			0.17	83			0.13	67								
	12	0.50	0.46	93			0.43	87			0.33	67								
Poultry Lean Meat	13	CONTROL	<0.01																	
	14	0.01	0.01	108	92	13	<0.01	88	88	0.9	0.008	75	74	2.7						
	15	0.01	0.01	95			0.01	89			0.007	73								
	16	0.20	*	76			*	87			*	71								
	16a	0.20	0.15	88			0.17	88			0.14	77								
	17	0.50	0.44				0.44				0.38									
Poultry Eggs	18	CONTROL	<0.01																	
	19	0.01	0.01	113	101	13	<0.01	95	93	2.1	0.007	74	75	3.1						
	20	0.01	0.01	111			0.01	94			0.007	71								
	21	0.20	**	89			**	91			**	75								
	21a	0.20	0.18	92			0.18	91			0.15	78								
	22	0.50	0.46				0.46				0.39									

\*Sample 16 lost during analysis

\*\*Sample 21 lost during analysis

TABLE III. (Continued)

RECOVERY RESULTS FOR CONTROL AND FENOXYCARB, Ro-16-8797, and Ro-17-3192 FORTIFIED CONTROL SUBSTRATES USING METHOD AG-608

(1) Substrate	Protocol 357-92 Sample #	Fortification PEM	Fenoxycarb				Ro-16-8797				Ro-17-3192			
			Determined PEM	% Recovery	% Mean	% S.D.	Determined PEM	% Recovery	% Mean	% S.D.	Determined PEM	% Recovery	% Mean	% S.D.
Beef Tenderloin	24	CONTROL	<0.01	108	101	15	<0.01	74	79	3.5	<0.01	69	70	3.0
	25	0.01	0.01	123			0.007	77			0.007	73		
	26	0.01	0.18	90			0.008	81			0.13	66		
	27	0.20	0.18	88			0.16	82			0.14	69		
	27a	0.20	0.48	95			0.16	82			0.36	73		
28	0.50					0.41	82			0.36	73			
Beef Round	29	CONTROL	<0.01	103	102	15	<0.01	82	85	4.8	<0.01	61	66	4.7
	30	0.01	0.01	96			0.01	90			0.006	66		
	31	0.01	0.19	97			0.01	91			0.007	74		
	32	0.20	0.17	87			0.18	83			0.15	64		
	32a	0.20	0.64	128			0.17	81			0.13	64		
	33	0.50					0.40	81			0.32	63		
	33	0.50					0.40	81			0.32	63		
Beef Omental Fat	34	CONTROL	<0.01	116	99	13	<0.01	90	93	4.1	<0.01	73	75	6.7
	35	0.01	0.01	108			0.01	91			0.007	85		
	36	0.01	0.17	85			0.01	90			0.01	70		
	37	0.20	0.18	91			0.18	97			0.14	69		
	37a	0.20	0.47	93			0.19	98			0.14	76		
	38	0.50					0.49	98			0.38	76		
	38	0.50					0.49	98			0.38	76		
Beef Perirenal Fat	39	CONTROL	<0.01	105	94	6.9	<0.01	95	93	3.4	<0.01	76	76	4.0
	40	0.01	0.01	94			0.01	94			0.008	72		
	41	0.01	0.18	90			0.01	87			0.007	73		
	42	0.20	0.17	86			0.18	92			0.15	75		
	42a	0.20	0.48	96			0.17	97			0.15	82		
	43	0.50					0.48	97			0.41	82		

TABLE III. (Continued)

RECOVERY RESULTS FOR CONTROL AND FENOXYCARB, Ro-16-8797, and Ro-17-3192 FORTIFIED CONTROL SUBSTRATES USING METHOD AG-608

Protocol (1) Substrate	357-92 Sample #	Fortification PPM	Fenoxycarb				Ro-16-8797				Ro-17-3192			
			Determined PPM	% Recovery	% Mean	% S.D.	Determined PPM	% Recovery	% Mean	% S.D.	Determined PPM	% Recovery	% Mean	% S.D.
Beef Blood	45	CONTROL	<0.01	93	90	2.7	<0.01	87	83	3.1	<0.01	68	64	4.7
	46	0.01	0.01	86		0.01	80		0.006		0.007	56		
	47	0.01	0.18	90		0.17	85		0.13		0.13	67		
	48	0.20	0.18	89		0.16	80		0.13		0.13	65		
	49	0.50	0.46	92		0.41	82		0.32		0.32	65		
Dairy Milk	50	CONTROL	<0.01		90	4.3	<0.01		80	1.3	<0.01		56	5.5
	51	0.01	***			***			***					
	52	0.01	0.01	87		0.01	79		0.006		0.006	63		
	53	0.20	0.18	89		0.16	82		0.12		0.12	58		
	54	0.50	0.48	97		0.40	79		0.10		0.10	51		
Beef Liver	55	CONTROL	<0.01	112	93	11	<0.01	95	92	4.2	<0.01	66	67	2.4
	56	0.01	0.01	91		0.01	97		0.006		0.007	64		
	57	0.01	0.17	84		0.18	88		0.14		0.14	71		
	58	0.20	0.17	85		0.18	88		0.14		0.14	69		
	59	0.50	0.46	91		0.45	91		0.34		0.34	67		
Beef Kidney	60	CONTROL	0.004		80	11	0.002		91	1.4	<0.01		73	2.4
	61	0.01	0.01	81		0.01	92		0.008		0.008	76		
	62	0.01	0.01	61		0.01	92		0.007		0.007	73		
	63	0.20	0.17	84		0.18	89		0.14		0.14	71		
	64	0.50	0.45	88		0.18	89		0.14		0.14	71		

\*\*\* sample 51 rejected due to suspected bad silica SPE cartridge.

TABLE III. (Continued)  
RECOVERY RESULTS FOR CONTROL AND FENOXYCARB, Ro-16-8797, and Ro-17-3192 FORTIFIED CONTROL SUBSTRATES USING METHOD AG-608

(1) Substrate	Protocol 357-92 Sample #	Fortification PPM	Fenoxycarb				Ro-16-8797				Ro-17-3192						
			Determined PPM	% Recovery	% Mean	% S.D.	Determined PPM	% Recovery	% Mean	% S.D.	Determined PPM	% Recovery	% Mean	% S.D.			
Goat Liver	65	CONTROL	<0.01														
	65a	CONTROL	<0.01														
	66	0.01	0.01	86	4.2	87	76	2.1	79	67	63	63	3.6				
	67	0.01	0.01	81			78			66							
	68	0.20	0.17	86			79			59							
	68a	0.20	0.18	89			81			63							
68b	0.50	0.46	93			81			60								
Goat Omental Fat	72	CONTROL	<0.01														
	73	0.01	0.01	94	6.2	88	87	3.4	84	67	71	71	3.8				
	74	0.01	0.01	81			85			70							
	75	0.15	0.13	89			80			75							
Goat Round	79	CONTROL	<0.01														
	80	0.01	0.01	101	3.8	99	91	1.9	89	73	75	75	2.9				
	81	0.01	0.01	94			87			75							
	82	0.02	0.02	101			90			79							
Goat Kidney	86	CONTROL	<0.01														
	87	0.01	0.01	125	12	111	96	4.7	91	67	71	71	3.2				
	88	0.01	0.01	109			87			71							
	89	0.50	0.51	101			90			73							



TABLE IV.

PRECISION OF ANALYTICAL METHOD AG-608 AS DEMONSTRATED BY  
THE RESULTS OF ANALYZING <sup>14</sup>C-FENOXYCARB TREATED SUBSTRATES

Substrate and Sample Code	Sample Number	HPLC Corrected Final Fraction (ppm)		
		Ro-17-3192	Ro-16-8797	Fenoxycarb
Goat	69	0.03	0.70	0.11
Liver	70	0.02	0.59	0.09
78230	71	0.03	0.67	0.11
	Mean:	0.03	0.66	0.10
	Range:	0.02 - 0.03	0.59 - 0.70	0.09 - 0.11
	Standard Deviation:	0.004	0.06	0.01
	CV (%):	15	8.7	11
Goat	76	<0.01	<0.01	0.04
Ornamental	77	<0.01	<0.01	0.03
Fat	78	<0.01	<0.01	0.04
78231				
	Mean:	<0.01	<0.01	0.04
	Range:	<0.01	<0.01	0.03 - 0.04
	Standard Deviation:	<0.01	<0.01	0.007
	CV (%):	ND	ND	18
Goat	83	<0.01	<0.01	<0.01 (0.004)
Round	84	<0.01	<0.01	<0.01 (0.005)
78232	85	<0.01	<0.01	<0.01 (0.005)
	Mean:	<0.01	<0.01	<0.01 (0.005)
	Range:	<0.01	<0.01	0.004 - 0.005
	Standard Deviation:	<0.01	<0.01	0.00005
	CV (%):	ND	ND	1.1
Goat	90	<0.01	0.010	<0.01 (0.007)
Kidney	91	<0.01	0.010	<0.01 (0.008)
78229	92	<0.01	0.010	<0.01 (0.007)
	Mean:	<0.01	0.010	<0.01 (0.007)
	Range:	<0.01	0.010 - 0.010	0.007 - 0.008
	Standard Deviation:	<0.01	0.0001	0.001
	CV (%):	ND	0.6	7.9
Goat	97	<0.01	<0.01	<0.01
Milk	98	<0.01	<0.01	<0.01
78233	99	<0.01	<0.01	<0.01
	Mean:	<0.01	<0.01	<0.01
	Range:	<0.01	<0.01	<0.01
	Standard Deviation:	<0.01	<0.01	<0.01
	CV (%):	ND	ND	ND

(1) Corrected by mean % recovery from fortified controls as shown in Table III of AG-608. ND = none detected.

TABLE V.  
SUMMARY TABLE FOR EXTRACTABILITY, ANALYSIS, AND ACCOUNTABILITY OF  
<sup>14</sup>C-FENOXYCARB TREATED SUBSTRATE USING AG-608

Substrate and Sample Code	Sample No	Extractability			Radiolabel Analysis			HPLC Analysis				(10) Mean % Accountability
		(1) Total Radioactive Residue	(2) Extractable PPM	(3) % Extractability	(4) Fraction	(5) Final Fraction <sup>14</sup> C PPM	(6) Analyte	(7) Final Fraction PPM	(8) Final Fraction Corrected	(9) Fenoxycarb Equivalents		
Goat Liver 78230	69	3.15	2.911	92	Fraction 1	0.97	Ro-17-3192	0.02	0.03	0.03	0.03	24
					Fraction 2	0.32	Ro-16-8797	0.56	0.70	0.67		
					Total	1.3	Fenoxycarb Total	0.10	0.11	0.11		
70	3.15	2.591	82	Fraction 1	0.86	Ro-17-3192	0.02	0.02	0.02	0.02		
				Fraction 2	0.27	Ro-16-8797	0.47	0.59	0.56			
				Total	1.1	Fenoxycarb Total	0.08	0.09	0.09			
71	3.15	2.886	92	Fraction 1	0.99	Ro-17-3192	0.02	0.03	0.03	0.03		
				Fraction 2	0.29	Ro-16-8797	0.53	0.67	0.64			
				Total	1.3	Fenoxycarb Total	0.09	0.11	0.11			
Goat Omental Fat 78231	76	0.43	0.234	54	Fraction 1	0.044	Ro-17-3192	<0.01	<0.01	<0.01	8.8	
					Fraction 2	0.002	Ro-16-8797	<0.01	<0.01	<0.01		
					Total	0.046	Fenoxycarb Total	0.04	0.04	0.04		
77	0.43	0.239	56	Fraction 1	0.039	Ro-17-3192	<0.01	<0.01	<0.01	<0.01		
				Fraction 2	0.002	Ro-16-8797	<0.01	<0.01	<0.01			
				Total	0.040	Fenoxycarb Total	0.03	0.03	0.03			
78	0.43	0.241	55	Fraction 1	0.045	Ro-17-3192	<0.01	<0.01	<0.01	<0.01		
				Fraction 2	0.003	Ro-16-8797	<0.01	<0.01	<0.01			
				Total	0.048	Fenoxycarb Total	0.04	0.04	0.04			
		average %:										

TABLE V. (Continued)  
SUMMARY TABLE FOR EXTRACTABILITY, ANALYSIS, AND ACCOUNTABILITY OF  
<sup>14</sup>C-FENOXYCARB TREATED SUBSTRATE USING AG-608

Substrate and Sample Code	Sample No	(1) Total Radioactive Residue	Extractability			Radiolabel Analysis				HPLC Analysis				(10) Mean % Accountability
			(2) Extractable PPM	(3) % Extractability	(4) Eraction	(5) Final Fraction <sup>14</sup> C PPM	(6) Analyte	(7) Final Fraction PPM	(8) Final Fraction Corrected	(9) Fenoxycarb Equivalents				
Goat Round 78232	83	0.062	0.046	73	Fraction 1	0.008	Ro-17-3192	<0.01	<0.01	<0.01	<0.01	<0.01	7.3	
					Fraction 2	0.003	Ro-16-8797	<0.01	<0.01	<0.01	<0.01			
					Total	0.011	Fenoxycarb	<0.01 (0.004)	<0.01 (0.004)	<0.01 (0.004)	<0.01 (0.004)			
84	0.062	0.046	75	Fraction 1	0.009	Ro-17-3192	<0.01	<0.01	<0.01	<0.01	<0.01	1.2		
				Fraction 2	0.003	Ro-16-8797	<0.01	<0.01	<0.01	<0.01				
				Total	0.012	Fenoxycarb	<0.01 (0.004)	<0.01 (0.005)	<0.01 (0.005)	<0.01 (0.005)				
85	0.062	0.042	68	Fraction 1	0.008	Ro-17-3192	<0.01	<0.01	<0.01	<0.01	<0.01	1.2		
				Fraction 2	0.004	Ro-16-8797	<0.01	<0.01	<0.01	<0.01				
				Total	0.012	Fenoxycarb	<0.01 (0.004)	<0.01 (0.005)	<0.01 (0.005)	<0.01 (0.005)				
Goat Kidney 78229	90	1.38	1.285	72	average %:		0.025	<0.01	<0.01	<0.01	<0.01	<0.01	1.2	
					Fraction 1	0.010	Ro-17-3192	<0.01	<0.01	<0.01	<0.01			
					Total	0.036	Fenoxycarb	<0.01 (0.007)	<0.01 (0.007)	<0.01 (0.007)	<0.01 (0.007)			
91	1.38	1.307	95	Fraction 1	0.024	Ro-17-3192	<0.01	<0.01	<0.01	<0.01	<0.01	1.2		
				Fraction 2	0.012	Ro-16-8797	<0.01 (0.009)	<0.01 (0.009)	<0.01 (0.009)	<0.01 (0.009)				
				Total	0.036	Fenoxycarb	<0.01 (0.008)	<0.01 (0.008)	<0.01 (0.008)	<0.01 (0.008)				
92	1.38	1.282	93	Fraction 1	0.025	Ro-17-3192	<0.01	<0.01	<0.01	<0.01	<0.01	1.2		
				Fraction 2	0.012	Ro-16-8797	<0.01 (0.009)	<0.01 (0.009)	<0.01 (0.009)	<0.01 (0.009)				
				Total	0.037	Fenoxycarb	<0.01 (0.007)	<0.01 (0.007)	<0.01 (0.007)	<0.01 (0.007)				
94	1.38	1.282	94	average %:		0.025	<0.01	<0.01	<0.01	<0.01	<0.01	1.2		
				Fraction 1	0.012	Ro-17-3192	<0.01	<0.01	<0.01	<0.01				
				Total	0.037	Fenoxycarb	<0.01 (0.007)	<0.01 (0.007)	<0.01 (0.007)	<0.01 (0.007)				

TABLE V. (Continued)  
SUMMARY TABLE FOR EXTRACTABILITY, ANALYSIS, AND ACCOUNTABILITY OF  
<sup>14</sup>C-FENOXYCARB TREATED SUBSTRATE USING AG-608

Substrate and Sample Code	Sample No	Extractability			Radiolabel Analysis			HPLC Analysis			(10) Mean % Account-ability
		(1) Total Radioactive Residue	(2) Extractable PPM	(3) % Extractability	(4) Fraction	(5) Final Fraction <sup>14</sup> C PPM	(6) Analyte	(7) Final Fraction PPM	(8) Final Fraction Corrected	(9) Fenoxycarb Equivalents	
Goat Milk 78233	97	0.53	0.273	52	Fraction 1	0.001	Ro-17-3192	<0.01	<0.01	<0.01	ND
					Fraction 2	0.0001	Ro-16-8797	<0.01	<0.01	<0.01	
					Total	0.001	Fenoxycarb Total	<0.01	<0.01	<0.03	
	98	0.53	0.321	61	Fraction 1	0.001	Ro-17-3192	<0.01	<0.01	<0.01	
					Fraction 2	0.0001	Ro-16-8797	<0.01	<0.01	<0.01	
					Total	0.002	Fenoxycarb Total	<0.01	<0.01	<0.03	
	99	0.53	0.316	60	Fraction 1	0.001	Ro-17-3192	<0.01	<0.01	<0.01	
					Fraction 2	0.0001	Ro-16-8797	<0.01	<0.01	<0.01	
					Total	0.001	Fenoxycarb Total	<0.01	<0.01	<0.03	
			average %:	58							

- (1) As determined by combustion analysis in the metabolism study (see reference 2)
  - (2) Determined by liquid scintillation counting of aliquots of the extract from section II.C.1.0 of AG-608
  - (3) Average of the three extractions shown (Extractable PPM) divided by the Total Radioactive Residue, multiplied by 100.
  - (4) Data is shown for each of the two fractions that result from section II.C.3.4 of AG-608, where Fenoxycarb and Ro-16-8797 are eluted in fraction 1, and Ro-17-3192 is eluted separately in fraction 2. Total is sum of fraction 1 and fraction 2.
  - (5) Determined by liquid scintillation counting of aliquots of the final fractions from section II.C.3.5 of AG-608.
  - (6) Data is shown for each analyte. Total is the sum of the three analytes.
  - (7) Determined by column-switching high performance liquid chromatography (HPLC).
  - (8) Corrected by mean % recovery from fortified controls as shown in Table III of AG-608.
  - (9) Corrected Final Fraction results converted to Fenoxycarb equivalents as described in section II.J.1.0 of AG-608.
  - (10) Average of the Total ppm of the three analyses in Fenoxycarb equivalents, divided by the Total Radioactive Residue, multiplied by 100. ND = none detected.
- Note: Total HPLC residue values totaled for the purpose of calculating Accountability only.

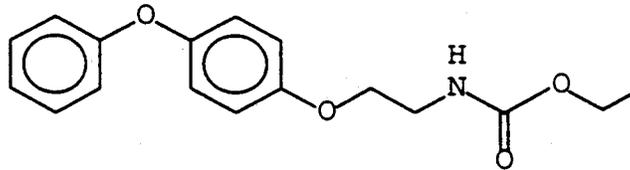
TABLE VI.

GLASSWARE SILANIZATION PROCEDURE

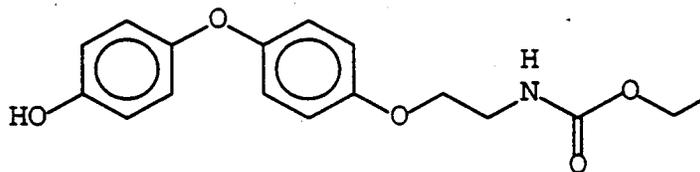
Note: This procedure must be performed in a fume hood. Once silanized, the 250-mL flat-bottom boiling flask can typically be used for approximately 15 - 20 analyses before needing another treatment.

1. Make a 5% dilution of the dichlormethylsilane in methylene chloride.
2. Fill the 250-mL flat-bottom boiling flask with this solution and let stand for 15 - 30 minutes at room temperature.
3. Decant the silane solution out of the boiling flask. The solution can be re-used for the next 250-mL flat-bottom boiling flask, stored in the refrigerator for use at another time, or discarded into a waste container containing methanol.
4. Rinse the 250-mL flat-bottom boiling flask 3 times with approximately 50 mL methanol per rinse. Discard each rinse into a waste container containing methanol.
5. Rinse 250-mL flat-bottom boiling flask with 50 mL dichloromethane. Discard the rinse into the same waste container.
6. Place the 250-mL flat-bottom boiling flask in an oven heated to approximately 110°C for at least two hours. Cool prior to use.

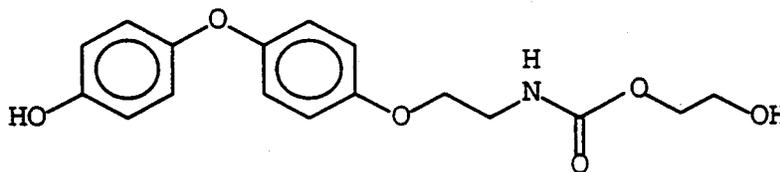
FIGURE 1. STRUCTURES AND CHEMICALS NAMES OF  
FENOXYCARB, Ro-16-8797, AND Ro-17-3192



Fenoxycarb, CGA-114597  
ethyl [2-(4-phenoxyphenoxy)ethyl] carbamate  
MW = 301.3



Ro-16-8797, CGA-294850  
ethyl N-2-[4-(4-hydroxyphenoxy)phenoxyethyl] carbamate  
MW = 317.3



Ro-17-3192, No CGA - number  
• (2-hydroxyethyl)N-2-[4-(4-hydroxyphenoxy)phenoxyethyl]  
carbamate  
MW = 333.3

FIGURE 2. ANALYTICAL PROCEDURE FLOWCHART FOR AG-608

For Fat, Muscle and Blood Samples:

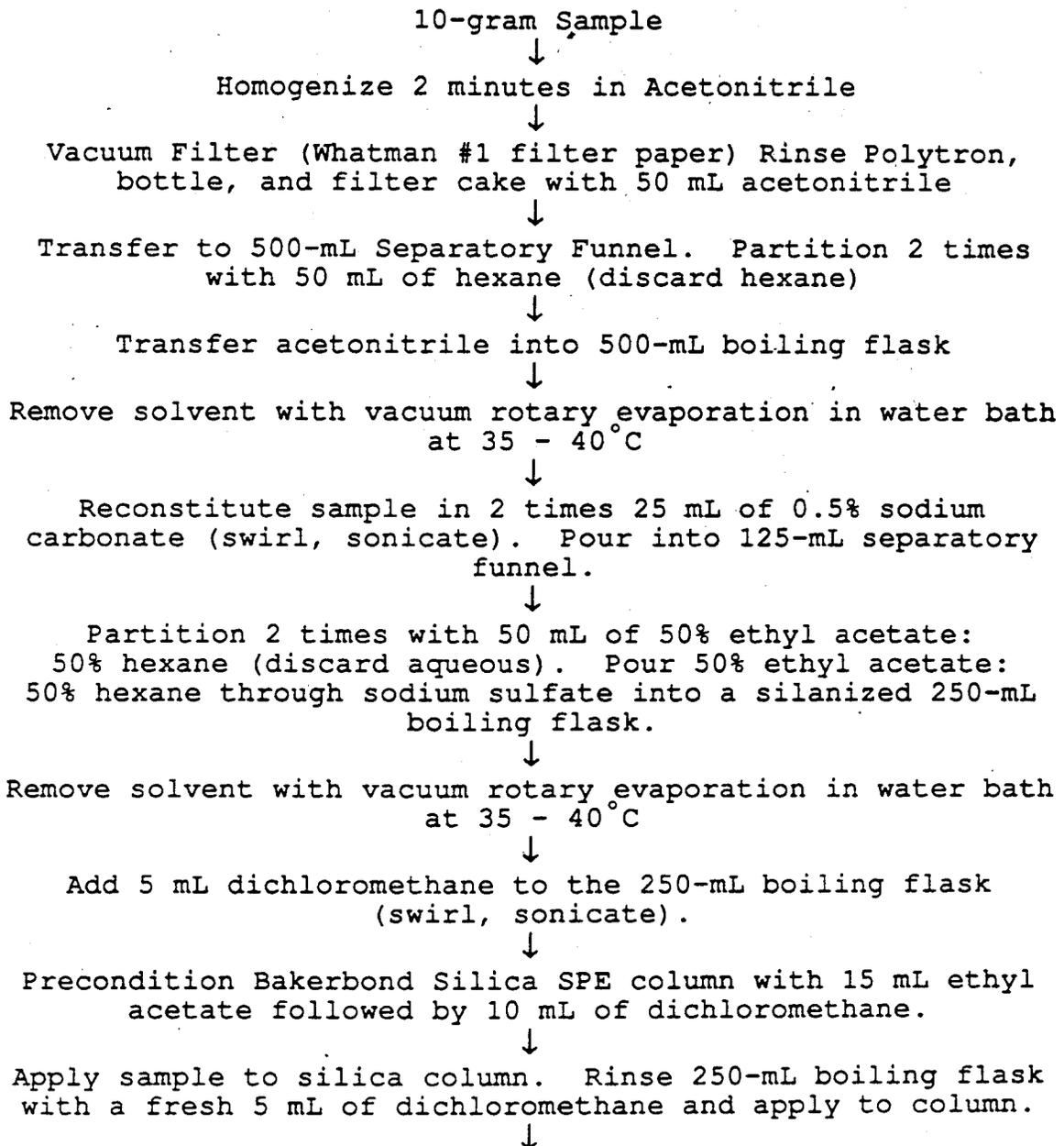


FIGURE 2. ANALYTICAL PROCEDURE FLOWCHART FOR AG-608  
(continued)

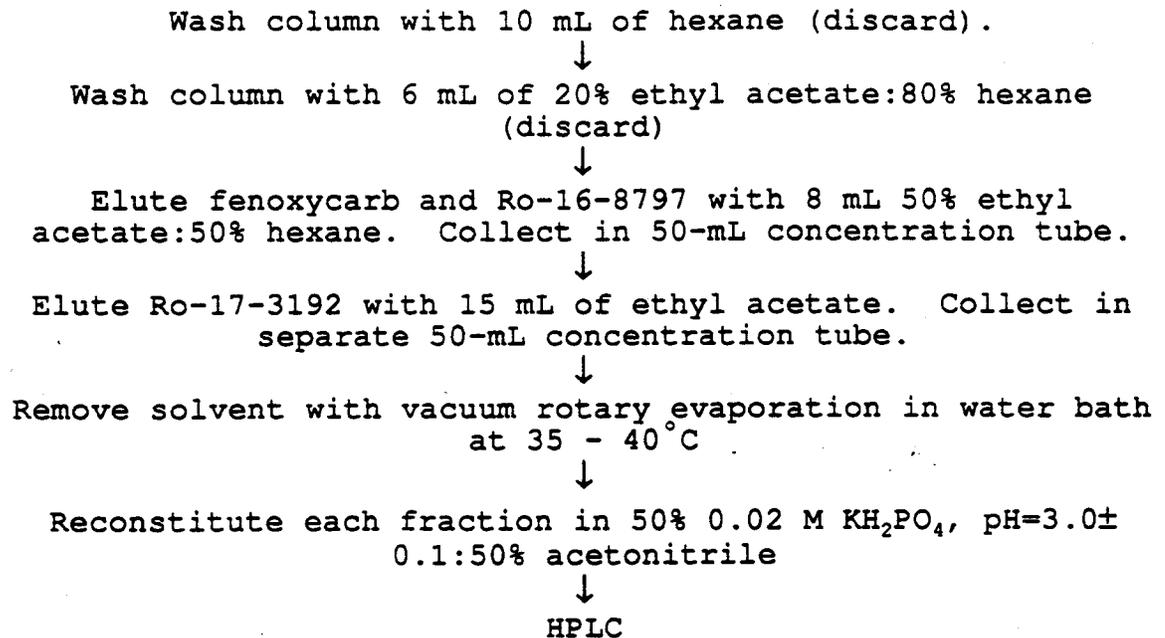


FIGURE 2. ANALYTICAL PROCEDURE FLOWCHART FOR AG-608  
(continued)

For Liver, Kidney, Milk and Eggs:

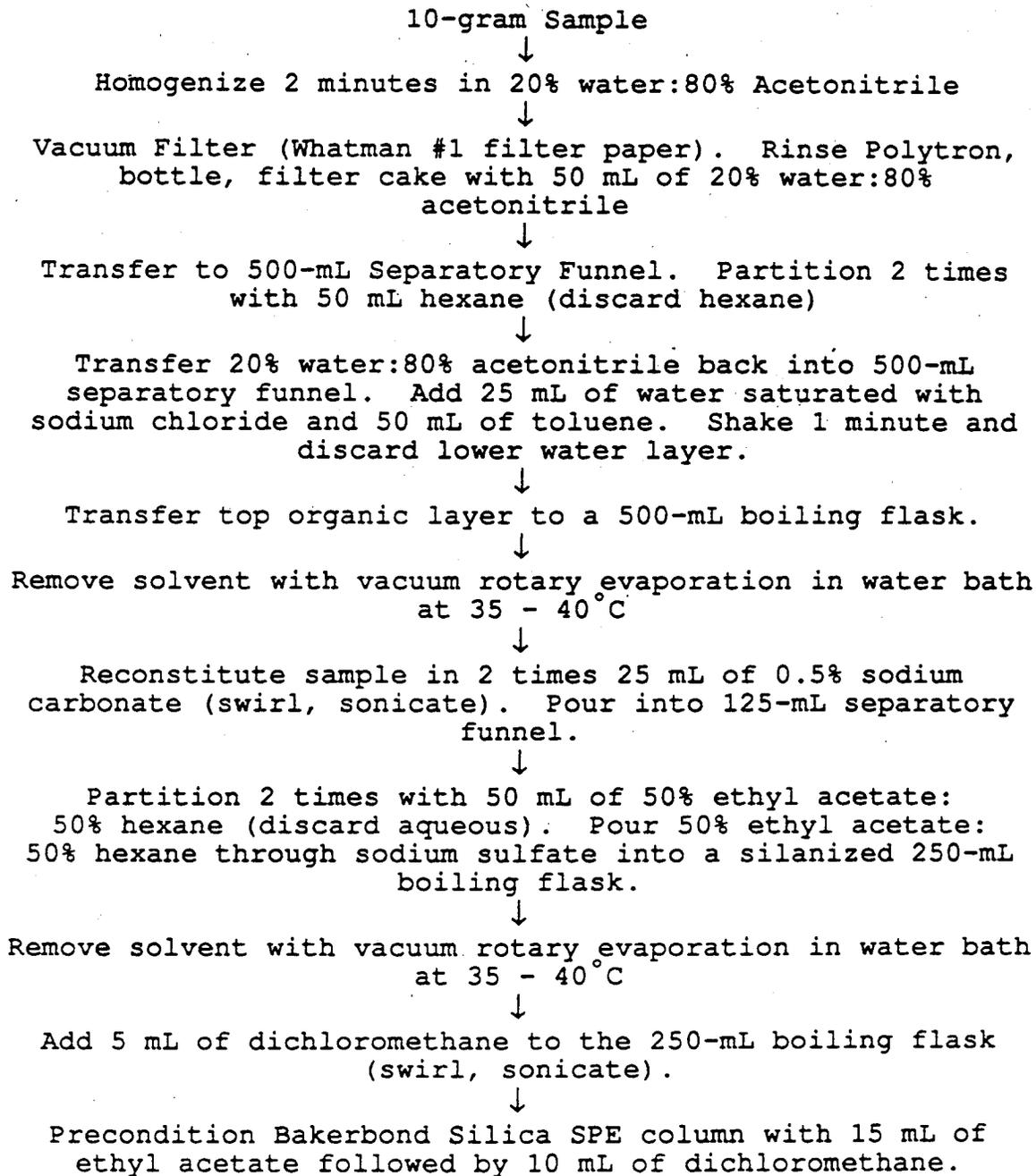


FIGURE 2. ANALYTICAL PROCEDURE FLOWCHART FOR AG-608  
(continued)

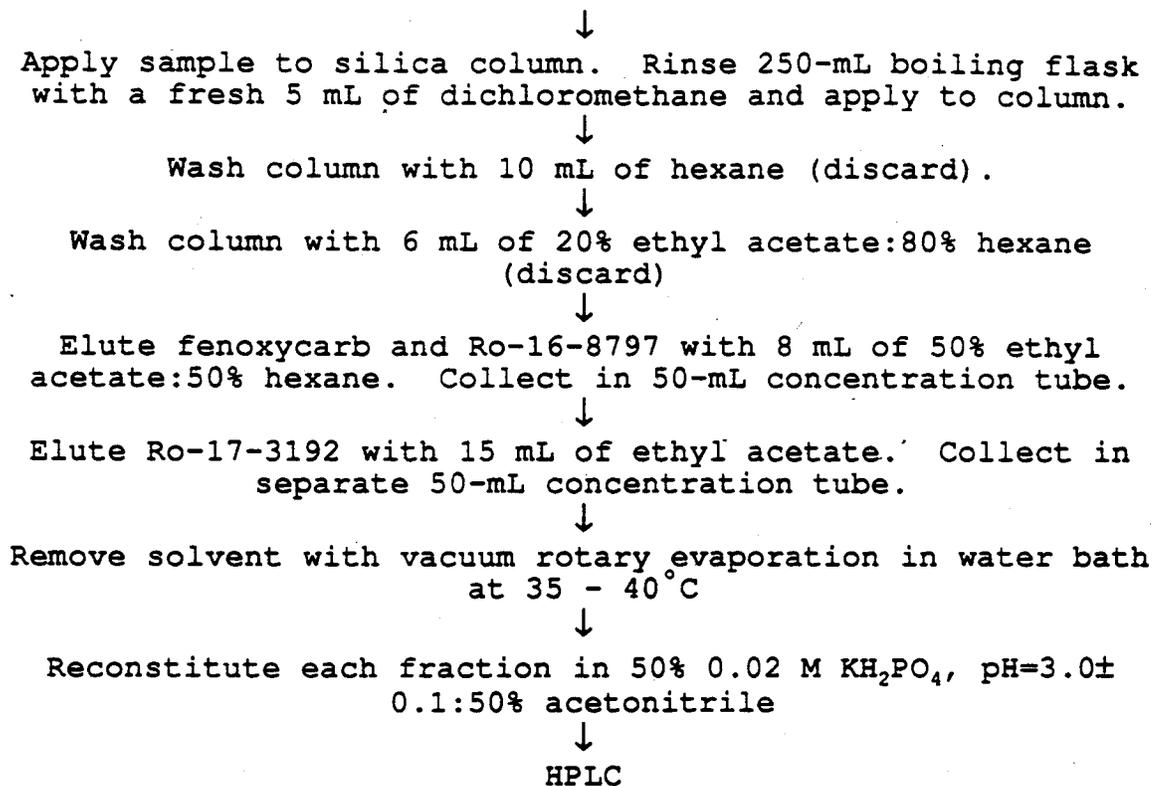
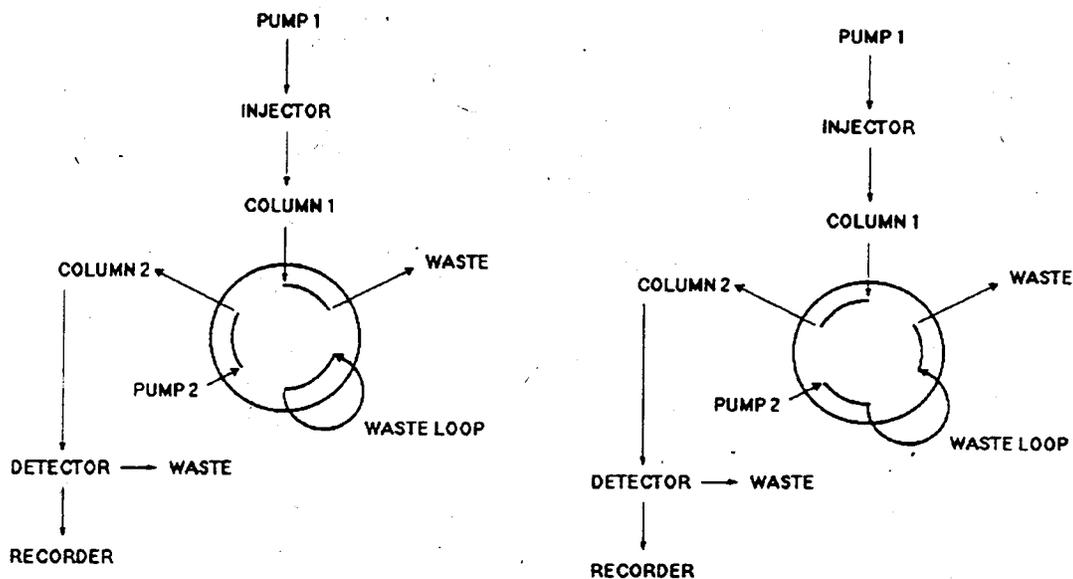
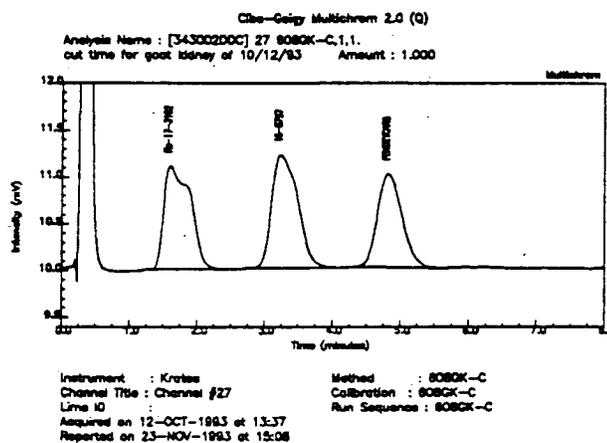


FIGURE 3. DIAGRAM OF COLUMN SWITCHING SYSTEM AND EXAMPLE OF A STANDARD INJECTION USED TO DETERMINE THE CUT TIME



Direction of flows during analysis and injection of the sample onto the cyano column (= column 1)

Direction of flows during the switch from the cyano column to the C1 analytical column(= column 2)

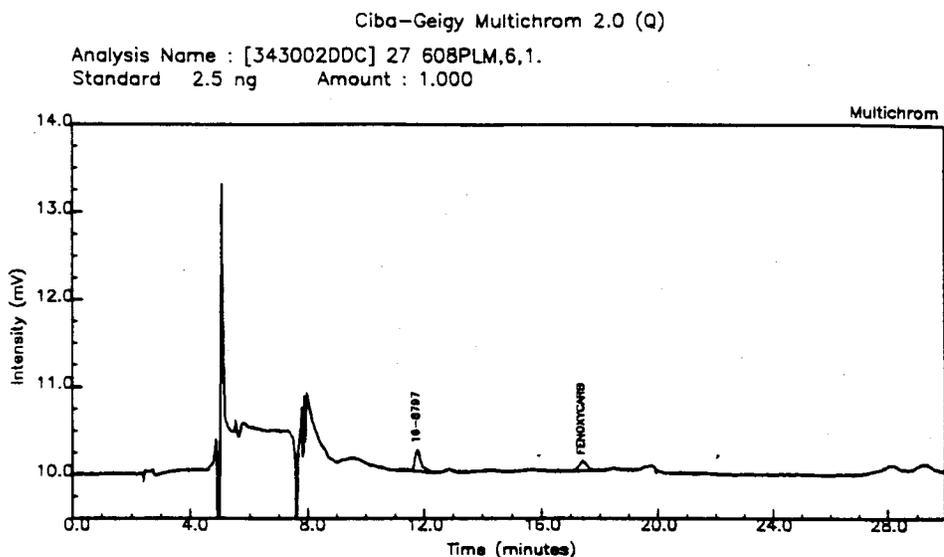


Cut times:

<u>Fraction #</u>	<u>Analytes in Fraction</u>	<u>cut start time (min)</u>	<u>cut end time (min)</u>
Fraction 1	Ro-16-8797 and Fenoxycarb	2.9	5.4
Fraction 2	Ro-17-3192	1.4	2.2

FIGURE 4. TYPICAL STANDARD CHROMATOGRAMS (FROM THE ANALYSIS OF LEAN POULTRY MEAT): Representative Chromatograms for Fenoxycarb, Ro-16-8797, and Ro-17-3192 Standards

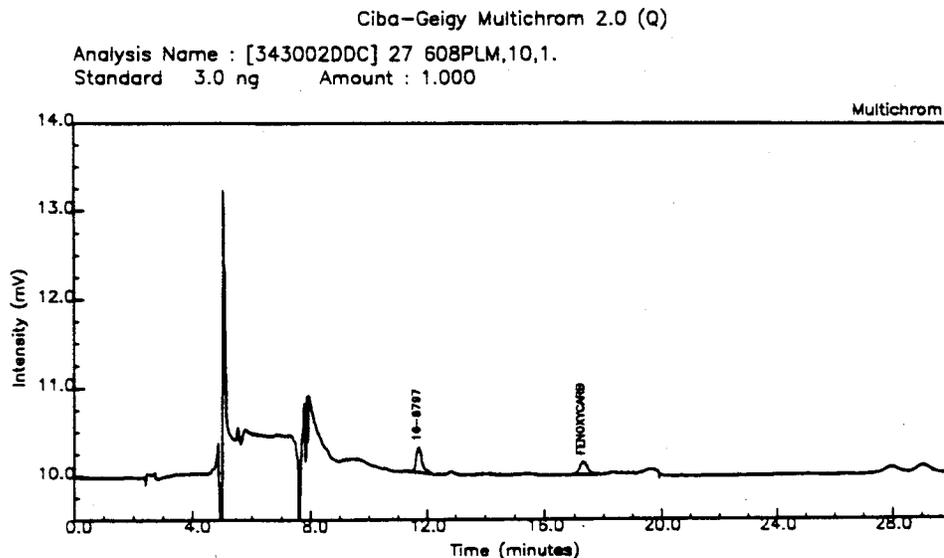
1)  
2.5 ng  
standard  
for  
Ro-16-8797  
and  
Fenoxycarb



Instrument : Kratos  
Channel Title : Channel #27  
Lims ID :  
Acquired on 29-SEP-1993 at 18:45  
Reported on 17-NOV-1993 at 13:51

Method : 608PLM  
Calibration : 608PLM  
Run Sequence : 608PLM

2)  
3.0 ng  
standard  
for  
Ro-16-8797  
and  
Fenoxycarb

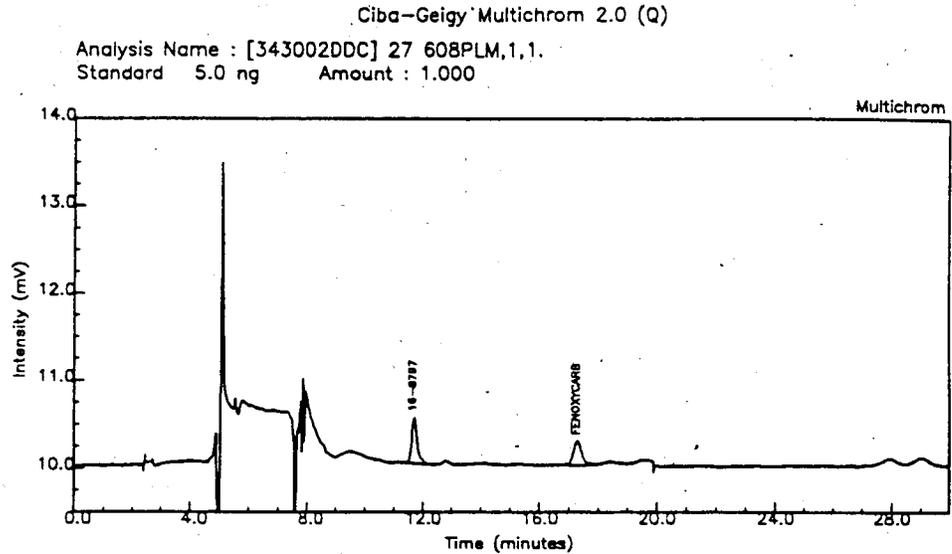


Instrument : Kratos  
Channel Title : Channel #27  
Lims ID :  
Acquired on 29-SEP-1993 at 20:49  
Reported on 17-NOV-1993 at 13:52

Method : 608PLM  
Calibration : 608PLM  
Run Sequence : 608PLM

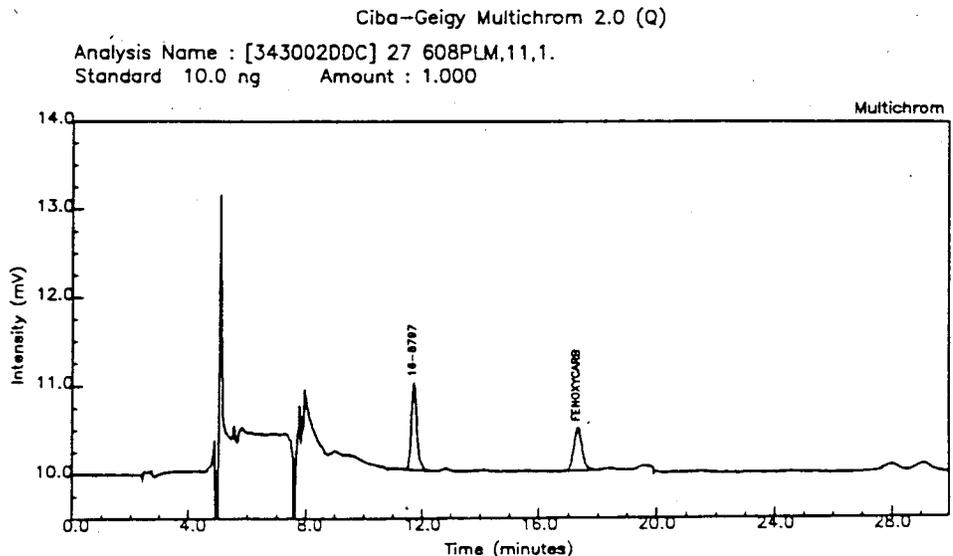
FIGURE 4. TYPICAL STANDARD CHROMATOGRAMS (FROM THE ANALYSIS OF LEAN POULTRY MEAT): Representative Chromatograms for Fenoxycarb, Ro-16-8797, and Ro-17-3192 Standards (Continued)

3)  
5.0 ng  
standard  
for  
Ro-16-8797  
and  
Fenoxycarb



Instrument : Kratos Method : 608PLM  
Channel Title : Channel #27 Calibration : 608PLM  
Lims ID : Run Sequence : 608PLM  
Acquired on 29-SEP-1993 at 16:10  
Reported on 17-NOV-1993 at 13:52

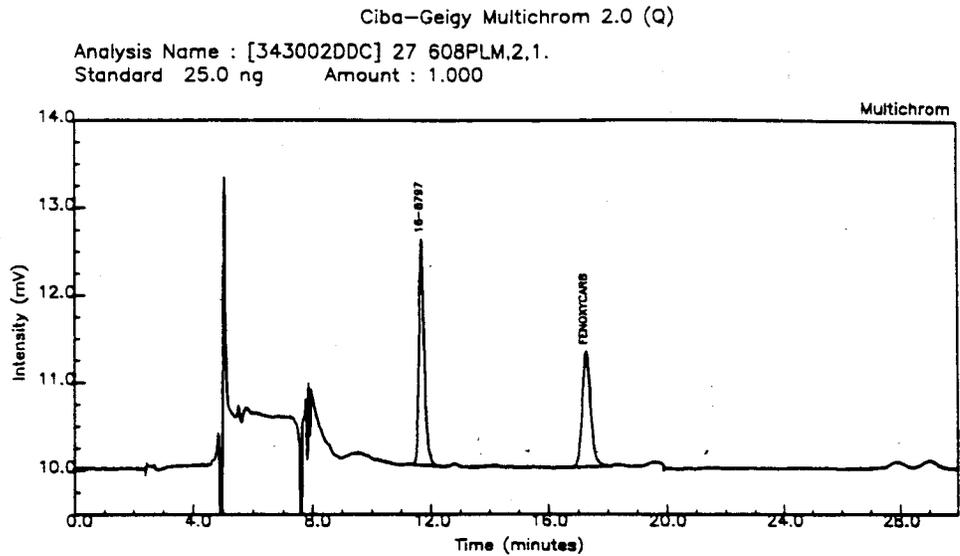
4)  
10.0 ng  
standard  
for  
Ro-16-8797  
and  
Fenoxycarb



Instrument : Kratos Method : 608PLM  
Channel Title : Channel #27 Calibration : 608PLM  
Lims ID : Run Sequence : 608PLM  
Acquired on 29-SEP-1993 at 21:20  
Reported on 17-NOV-1993 at 13:53

FIGURE 4. TYPICAL STANDARD CHROMATOGRAMS (FROM THE ANALYSIS OF LEAN POULTRY MEAT): Representative Chromatograms for Fenoxycarb, Ro-16-8797, and Ro-17-3192 Standards (Continued)

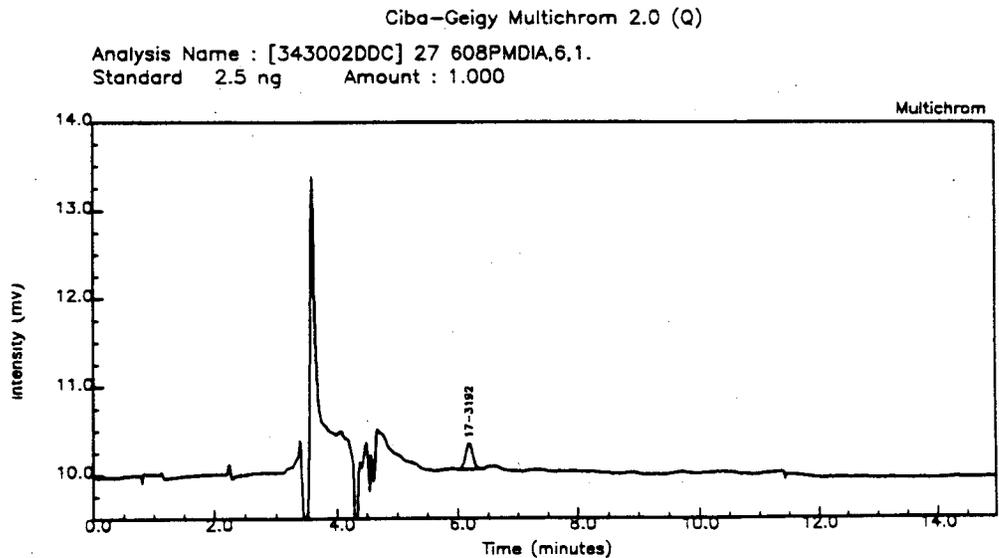
5)  
25.0 ng  
standard  
for  
Ro-16-8797  
and  
Fenoxycarb



Instrument : Kratos  
Channel Title : Channel #27  
Lims ID :  
Acquired on 29-SEP-1993 at 16:41  
Reported on 17-NOV-1993 at 13:53

Method : 608PLM  
Calibration : 608PLM  
Run Sequence : 608PLM

6)  
2.5 ng  
standard  
for  
Ro-17-3192

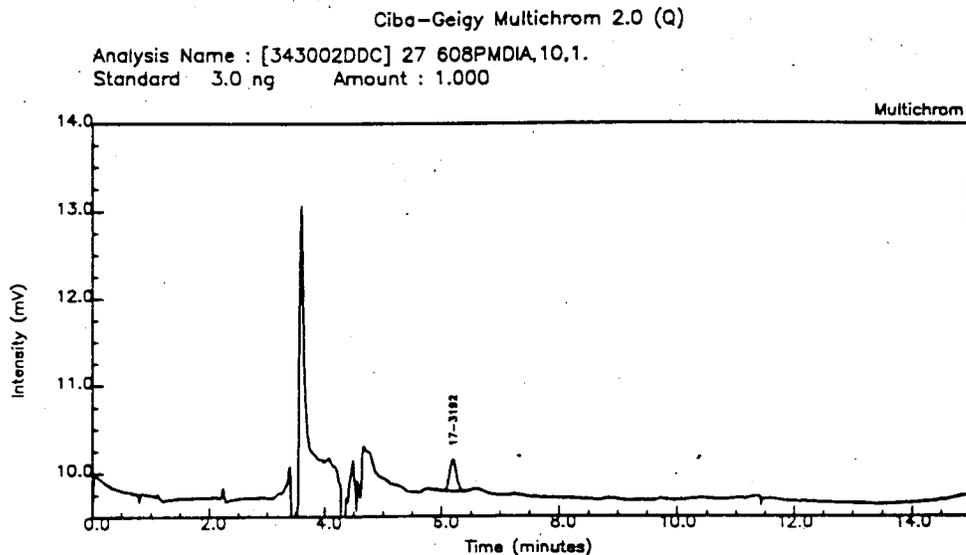


Instrument : Kratos  
Channel Title : Channel #27  
Lims ID :  
Acquired on 30-SEP-1993 at 09:31  
Reported on 17-NOV-1993 at 14:14

Method : 608PMDIA  
Calibration : 608PMDIA  
Run Sequence : 608PMDIA

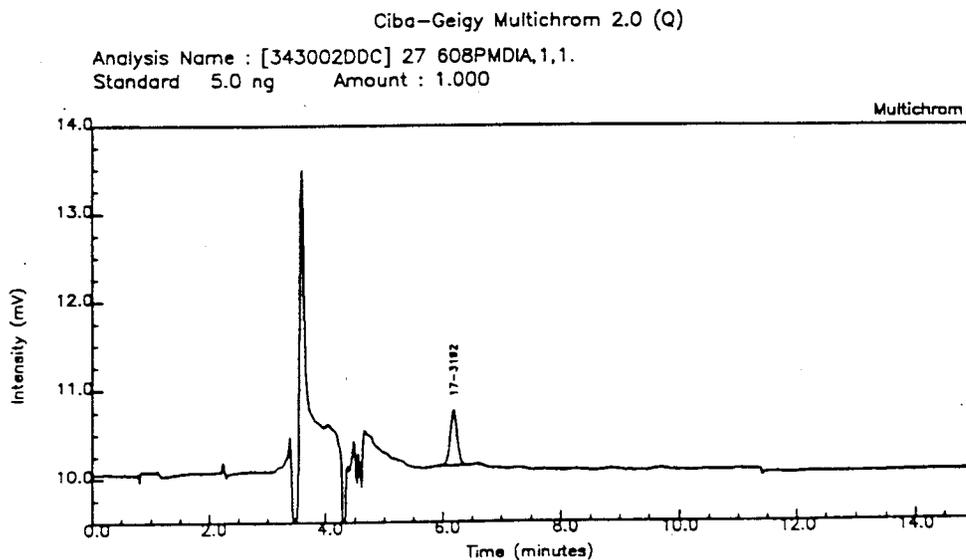
FIGURE 4. TYPICAL STANDARD CHROMATOGRAMS (FROM THE ANALYSIS OF LEAN POULTRY MEAT): Representative Chromatograms for Fenoxycarb, Ro-16-8797, and Ro-17-3192 Standards (Continued)

7)  
3.0 ng  
standard  
for  
Ro-17-3192



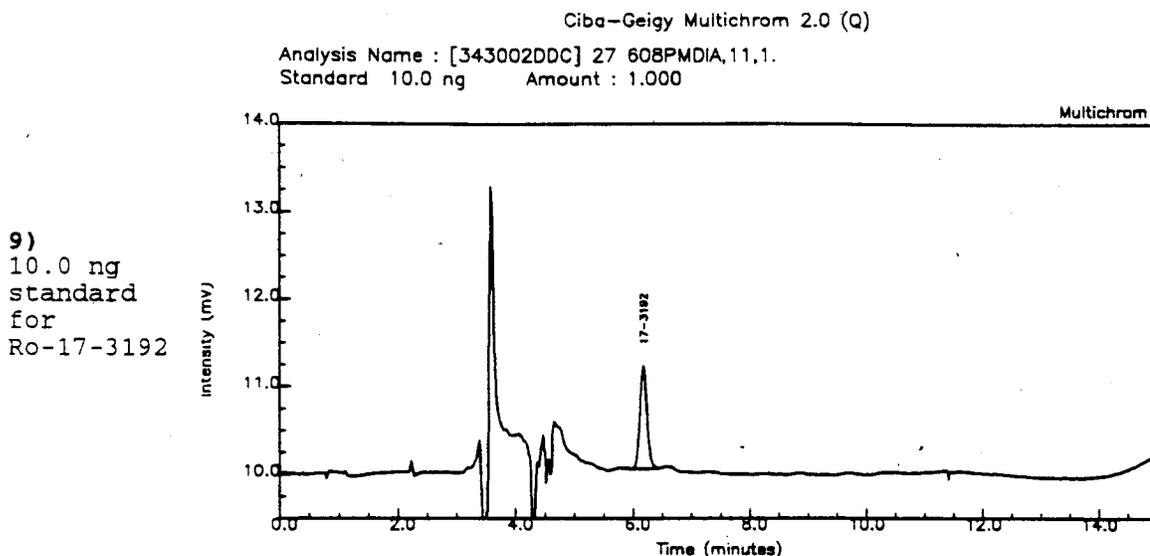
Instrument : Kratos Method : 608PMDIA  
Channel Title : Channel #27 Calibration : 608PMDIA  
Lims ID : Run Sequence : 608PMDIA  
Acquired on 30-SEP-1993 at 10:35  
Reported on 17-NOV-1993 at 14:15

8)  
5.0 ng  
standard  
for  
Ro-17-3192



Instrument : Kratos Method : 608PMDIA  
Channel Title : Channel #27 Calibration : 608PMDIA  
Lims ID : Run Sequence : 608PMDIA  
Acquired on 30-SEP-1993 at 08:04  
Reported on 17-NOV-1993 at 14:16

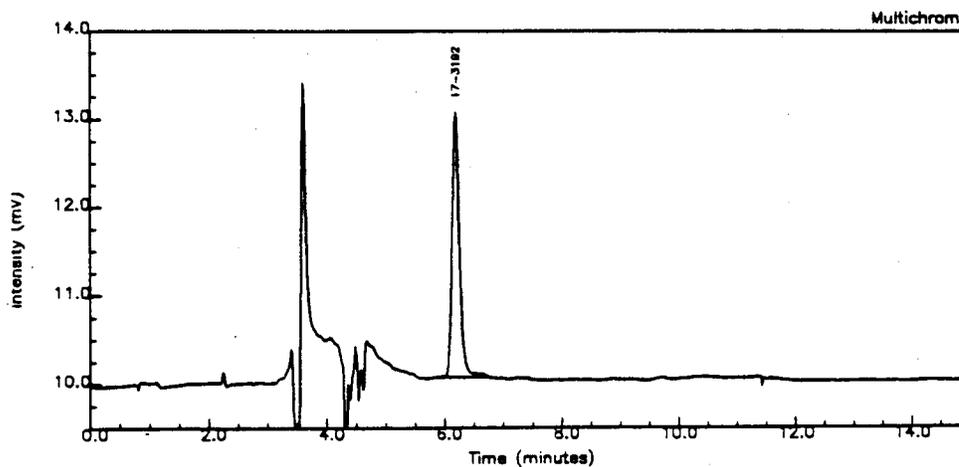
FIGURE 4. TYPICAL STANDARD CHROMATOGRAMS (FROM THE ANALYSIS OF LEAN POULTRY MEAT): Representative Chromatograms for Fenoxycarb, Ro-16-8797, and Ro-17-3192 Standards (Continued)



Instrument : Kratos Method : 608PMDIA  
Channel Title : Channel #27 Calibration : 608PMDIA  
Lims ID : Run Sequence : 608PMDIA  
Acquired on 30-SEP-1993 at 10:51  
Reported on 17-NOV-1993 at 14:16  
Ciba-Geigy Multichrom 2.0 (Q)

Analysis Name : [343002DDC] 27 608PMDIA,2,1.  
Standard 25.0 ng Amount : 1.000

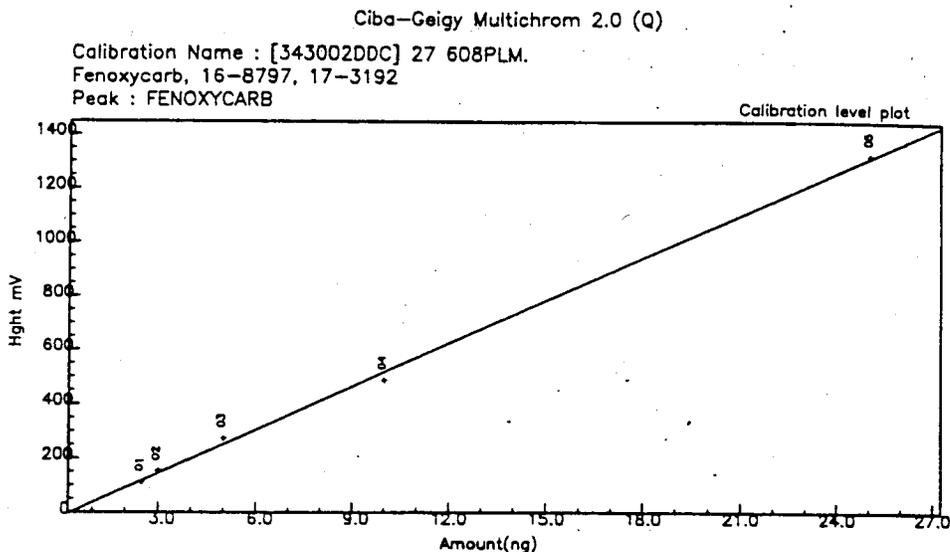
10)  
25.0 ng  
standard  
for  
Ro-17-3192



Instrument : Kratos Method : 608PMDIA  
Channel Title : Channel #27 Calibration : 608PMDIA  
Lims ID : Run Sequence : 608PMDIA  
Acquired on 30-SEP-1993 at 08:20  
Reported on 17-NOV-1993 at 14:16

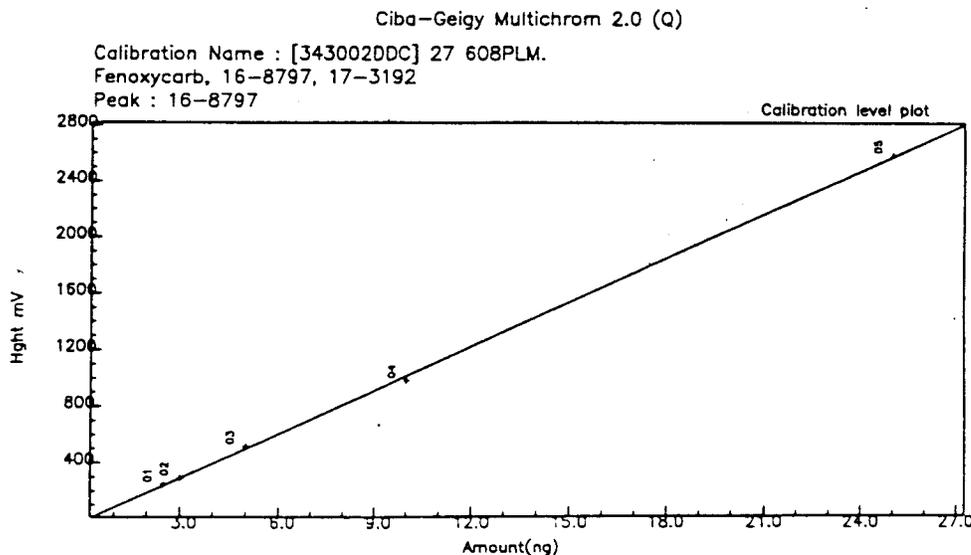
FIGURE 5. CALIBRATION PLOTS OBTAINED FROM THE ANALYSIS OF POULTRY LEAN MEAT

1)  
Calibration  
Plot for  
Fenoxycarb:



Constant : -1.555626E+1  
1st degree : 5.33006E+1  
Curve fit : Linear  
Correlation coefficient : 0.99921  
Standard error : 2.29699E+1  
Reported on 17-NOV-1993 at 13:20

2)  
Calibration  
Plot for  
Ro-16-8797:



Constant : -2.006337E+1  
1st degree : 1.03836E+2  
Curve fit : Linear  
Correlation coefficient : 0.99980  
Standard error : 2.26356E+1  
Reported on 17-NOV-1993 at 13:19

FIGURE 5. CALIBRATION PLOTS OBTAINED FROM THE ANALYSIS OF  
POULTRY LEAN MEAT (Continued)

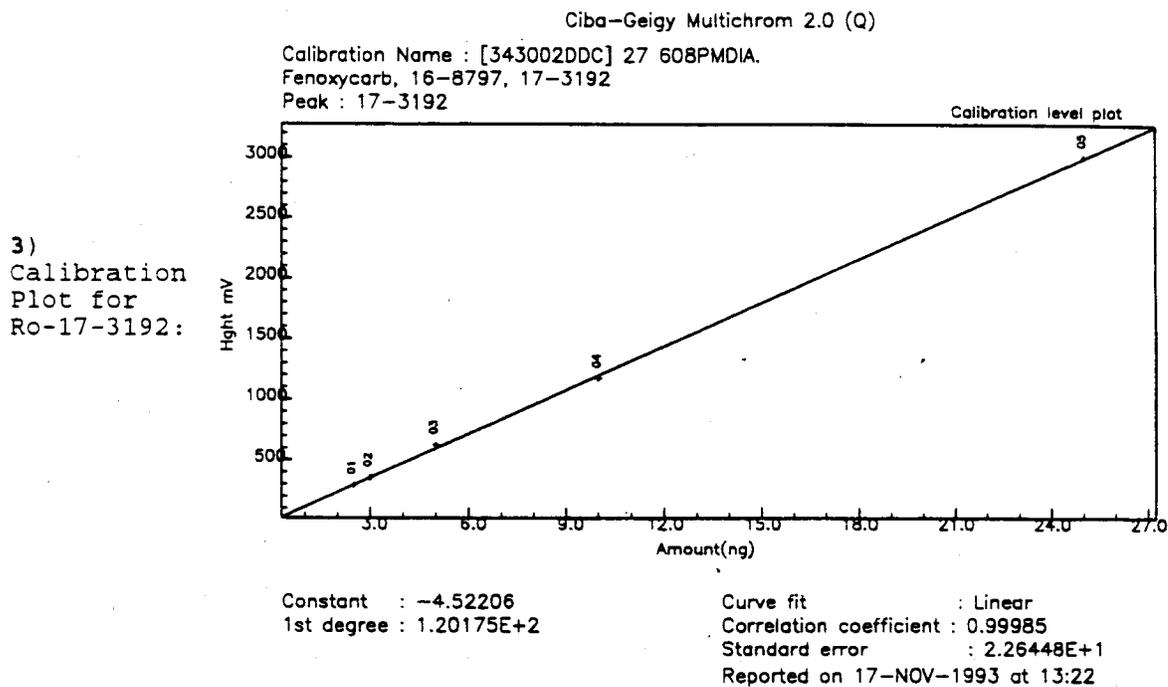


FIGURE 6. CHROMATOGRAMS OF THE REAGENT BLANKS FROM THE ANALYSIS OF (A) POULTRY LIVER, (B) POULTRY PERITONEAL FAT, (C) BEEF TENDERLOIN, AND (D) BEEF BLOOD.

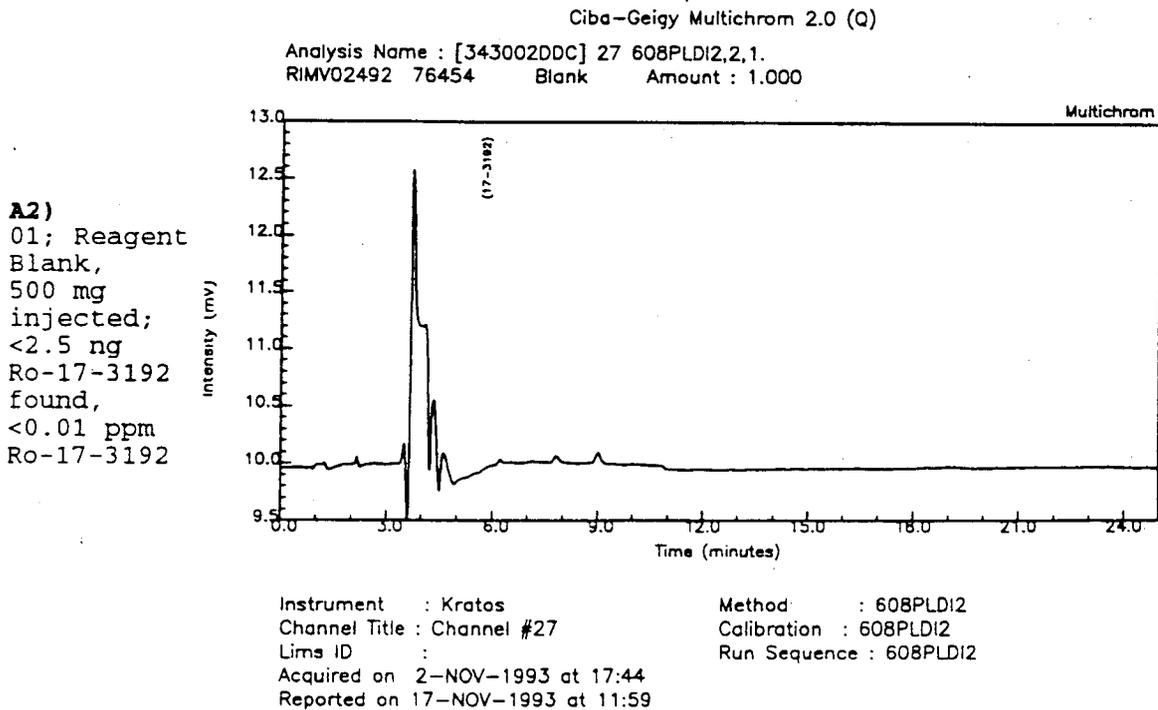
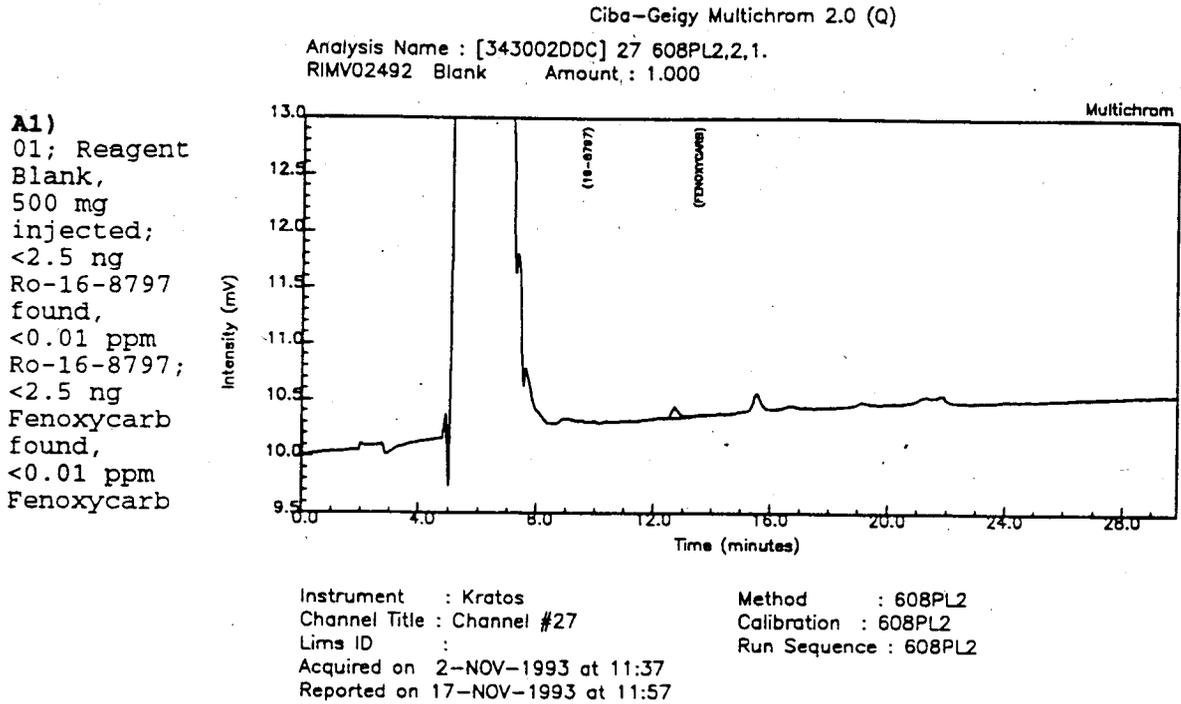




FIGURE 6. CHROMATOGRAMS OF THE REAGENT BLANKS FROM THE ANALYSIS OF (A) POULTRY LIVER, (B) POULTRY PERITONEAL FAT, (C) BEEF TENDERLOIN, AND (D) BEEF BLOOD. (Continued)

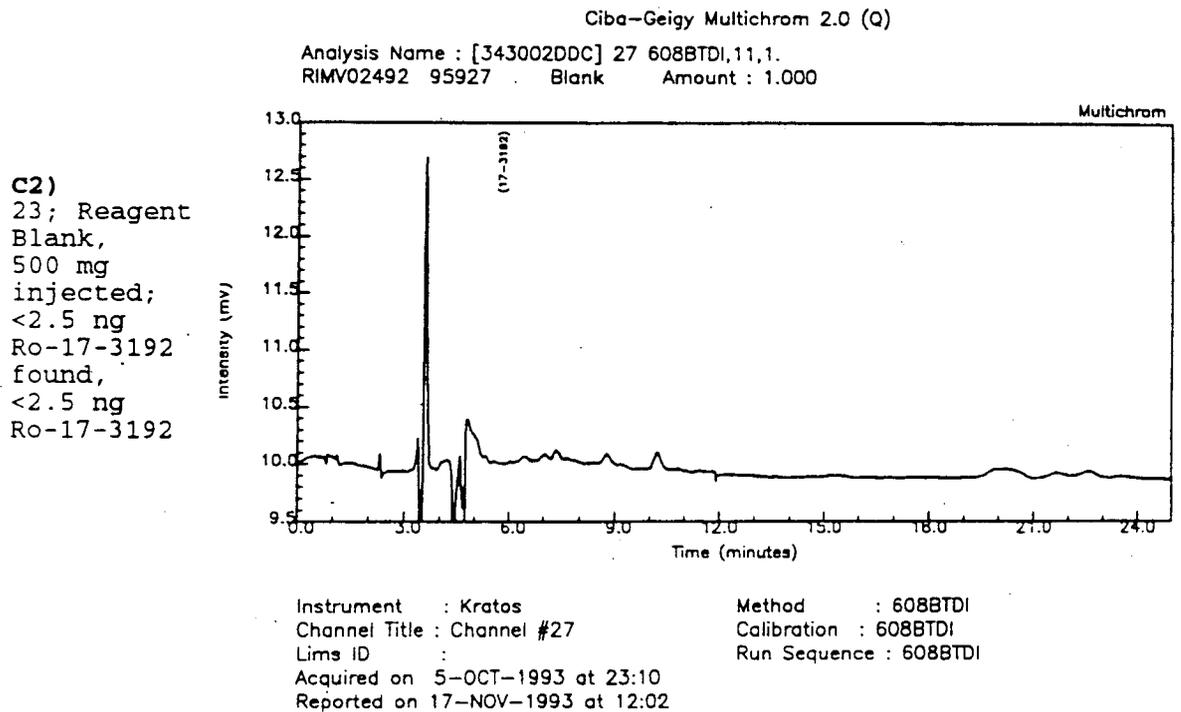
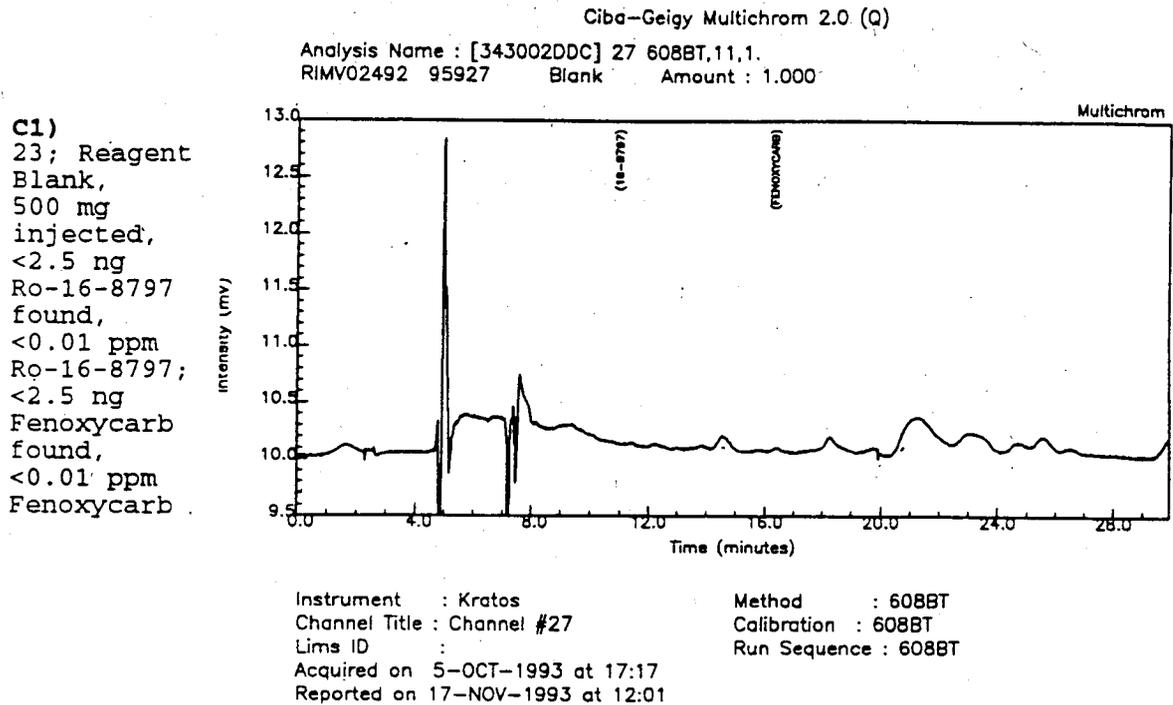


FIGURE 6. CHROMATOGRAMS OF THE REAGENT BLANKS FROM THE ANALYSIS OF (A) POULTRY LIVER, (B) POULTRY PERITONEAL FAT, (C) BEEF TENDERLOIN, AND (D) BEEF BLOOD. (Continued)

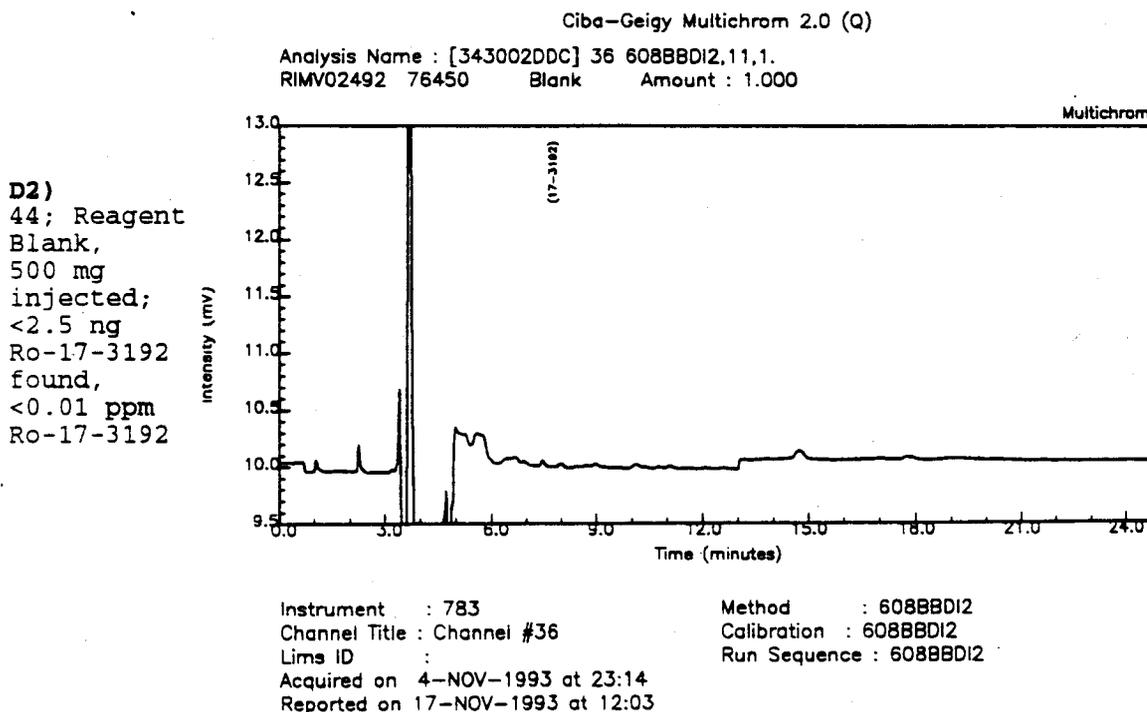
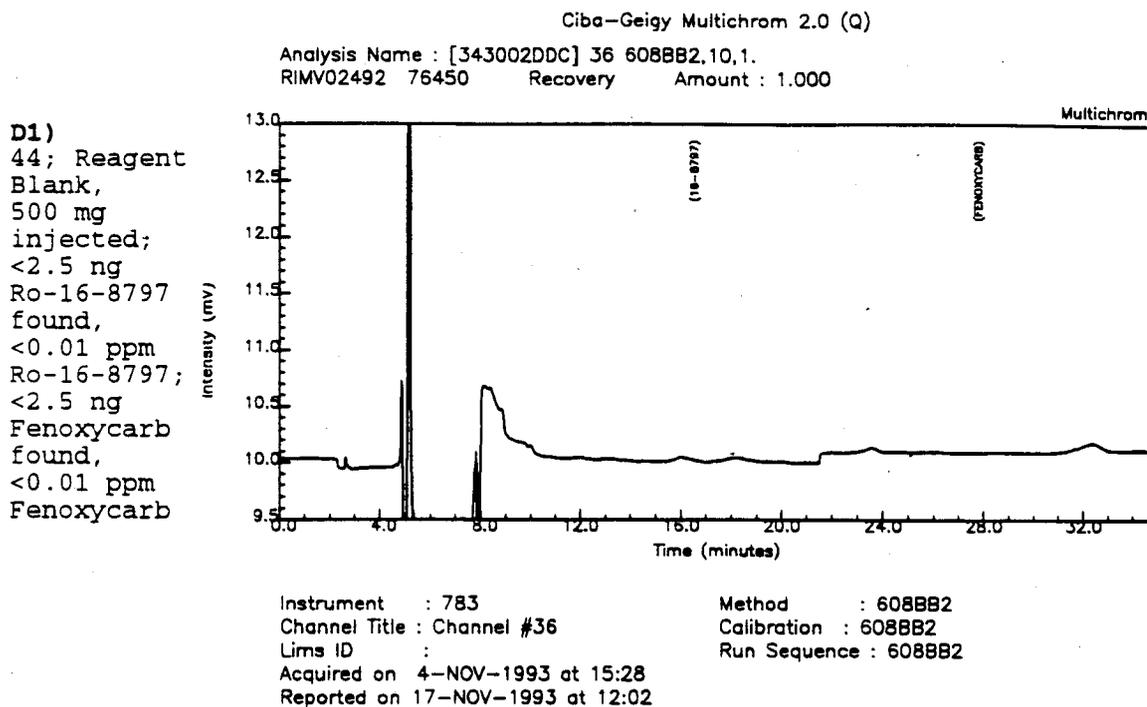


FIGURE 7. REPRESENTATIVE CHROMATOGRAMS OBTAINED FROM THE ANALYSIS OF POULTRY LIVER FOR FENOXYCARB AND Ro-16-8797 USING METHOD AG-608

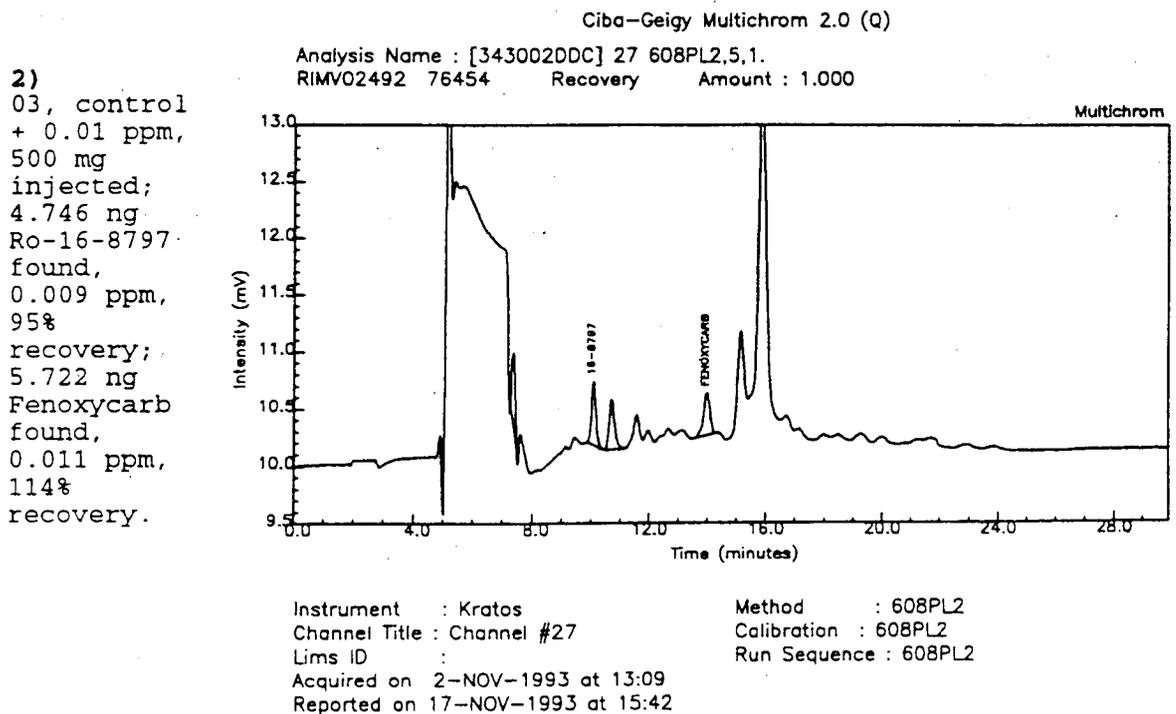
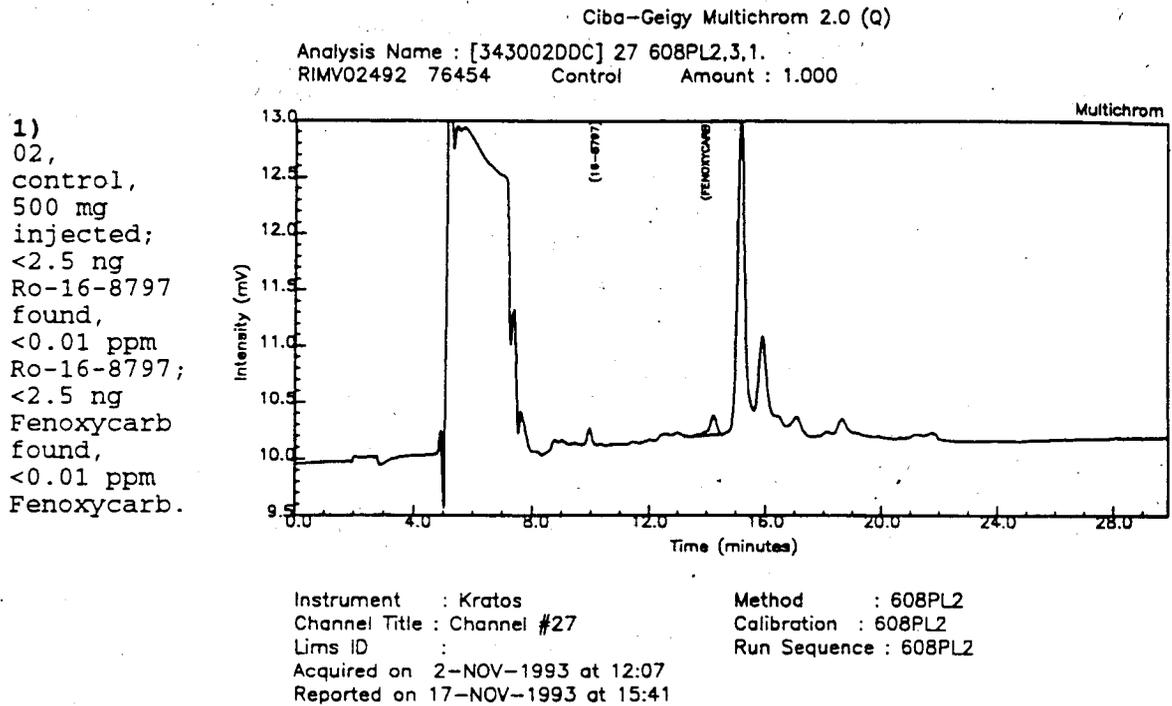
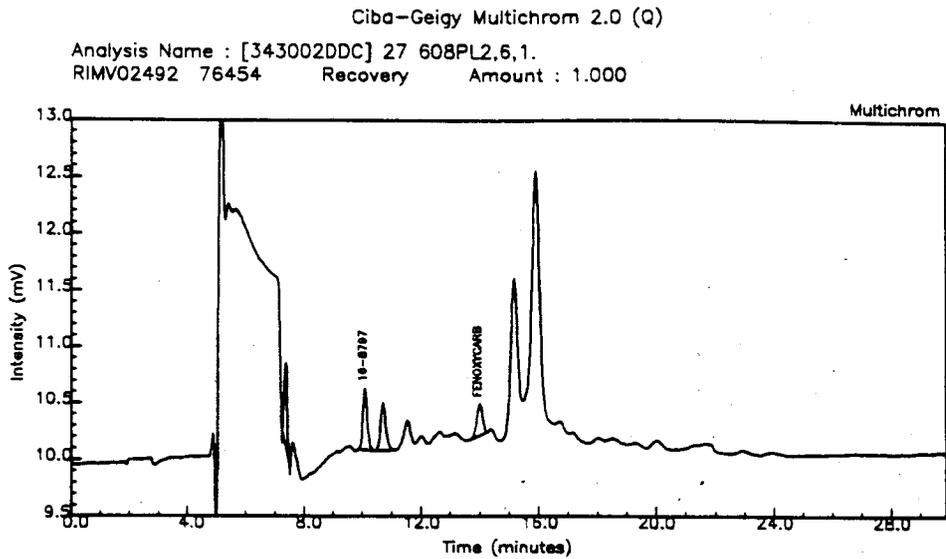


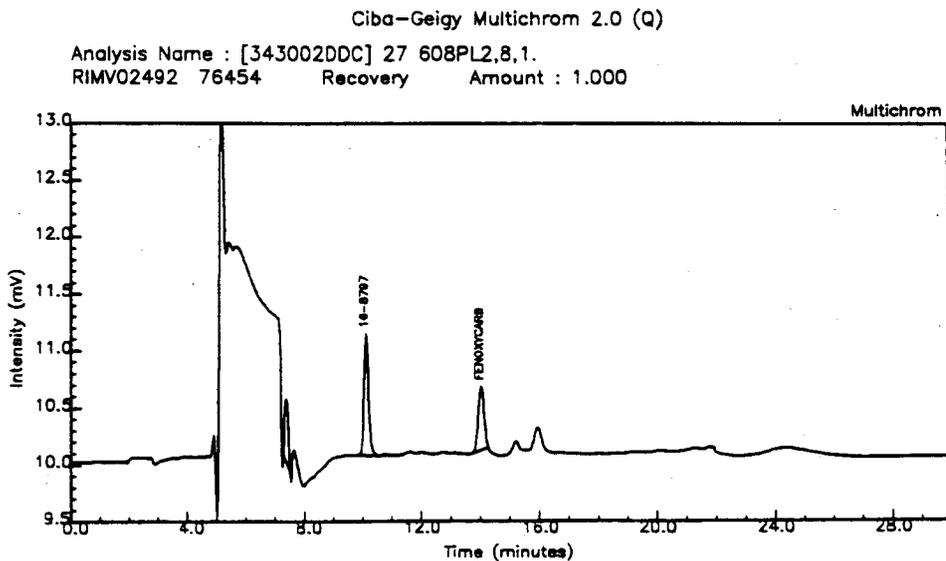
FIGURE 7. REPRESENTATIVE CHROMATOGRAMS OBTAINED FROM THE ANALYSIS OF POULTRY LIVER FOR FENOXYCARB AND Ro-16-8797 USING METHOD AG-608 (Continued)

3)  
04, control  
+ 0.01 ppm,  
500 mg  
injected;  
4.690 ng  
Ro-16-8797  
found,  
0.009 ppm,  
94%  
recovery;  
4.435 ng  
Fenoxycarb  
found,  
0.009 ppm,  
89%  
recovery



Instrument : Kratos Method : 608PL2  
Channel Title : Channel #27 Calibration : 608PL2  
Lims ID : Run Sequence : 608PL2  
Acquired on 2-NOV-1993 at 13:40  
Reported on 17-NOV-1993 at 15:42

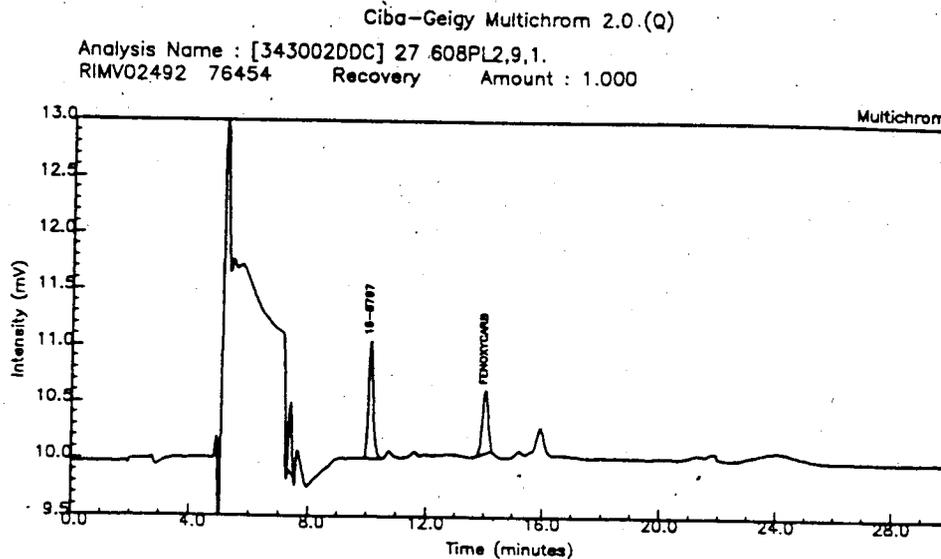
4)  
05, control  
+ 0.20 ppm,  
50 mg  
injected;  
9.064 ng  
Ro-16-8797  
found,  
0.181 ppm,  
91%  
recovery;  
8.734 ng  
Fenoxycarb  
found,  
0.175 ppm,  
87%  
recovery



Instrument : Kratos Method : 608PL2  
Channel Title : Channel #27 Calibration : 608PL2  
Lims ID : Run Sequence : 608PL2  
Acquired on 2-NOV-1993 at 14:42  
Reported on 17-NOV-1993 at 15:42

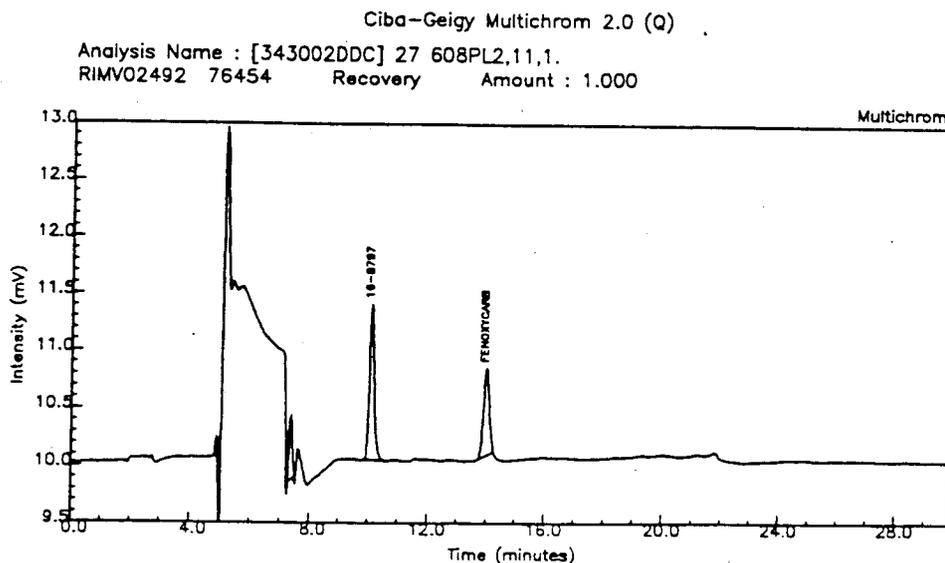
FIGURE 7. REPRESENTATIVE CHROMATOGRAMS OBTAINED FROM THE ANALYSIS OF POULTRY LIVER FOR FENOXYCARB AND Ro-16-8797 USING METHOD AG-608 (Continued)

5)  
05a,  
control +  
0.20 ppm,  
50 mg  
injected;  
8.801 ng  
Ro-16-8797  
found,  
0.176 ppm,  
88%  
recovery;  
8.675 ng  
Fenoxycarb  
found,  
0.173 ppm,  
87%  
recovery.



Instrument : Kratos Method : 608PL2  
Channel Title : Channel #27 Calibration : 608PL2  
Lims ID : Run Sequence : 608PL2  
Acquired on 2-NOV-1993 at 15:14  
Reported on 17-NOV-1993 at 15:42

6)  
06, control  
+ 0.50 ppm,  
25 mg  
injected;  
11.505 ng  
Ro-16-8797  
found,  
0.460 ppm,  
92%  
recovery;  
11.875 ng  
Fenoxycarb  
found,  
0.475 ppm,  
95%  
recovery.



Instrument : Kratos Method : 608PL2  
Channel Title : Channel #27 Calibration : 608PL2  
Lims ID : Run Sequence : 608PL2  
Acquired on 2-NOV-1993 at 16:16  
Reported on 17-NOV-1993 at 15:43

FIGURE 8. REPRESENTATIVE CHROMATOGRAMS OBTAINED FROM THE ANALYSIS OF POULTRY LIVER FOR Ro-17-3192 USING METHOD AG-608

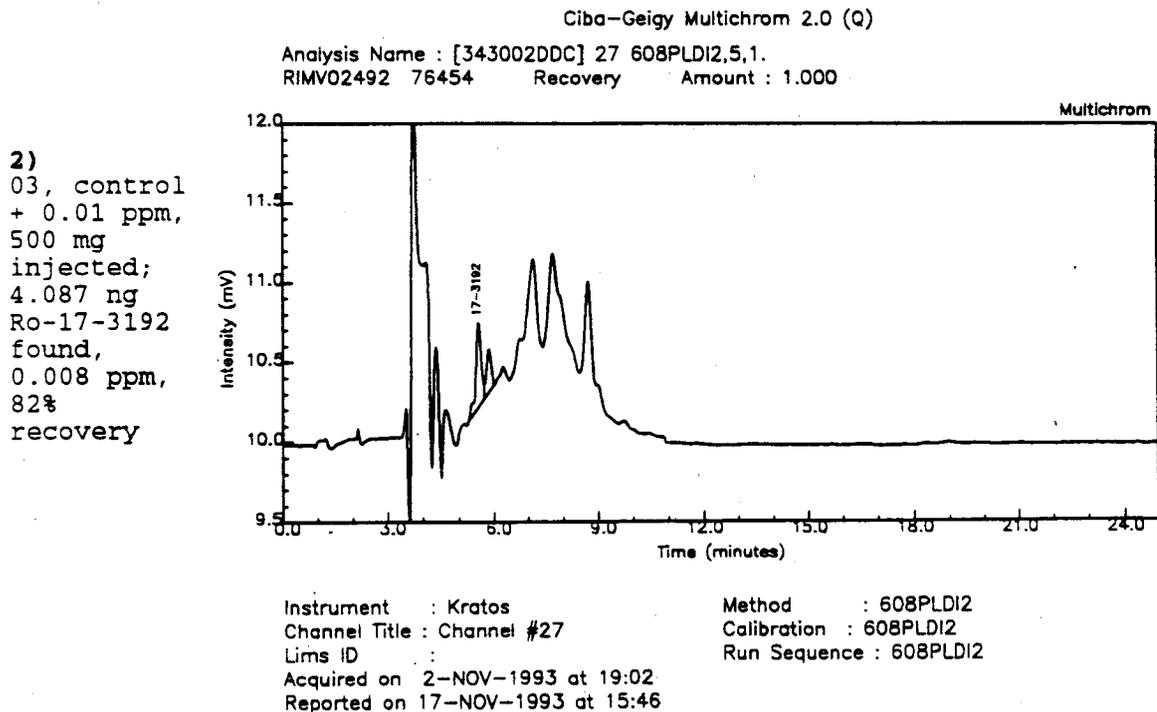
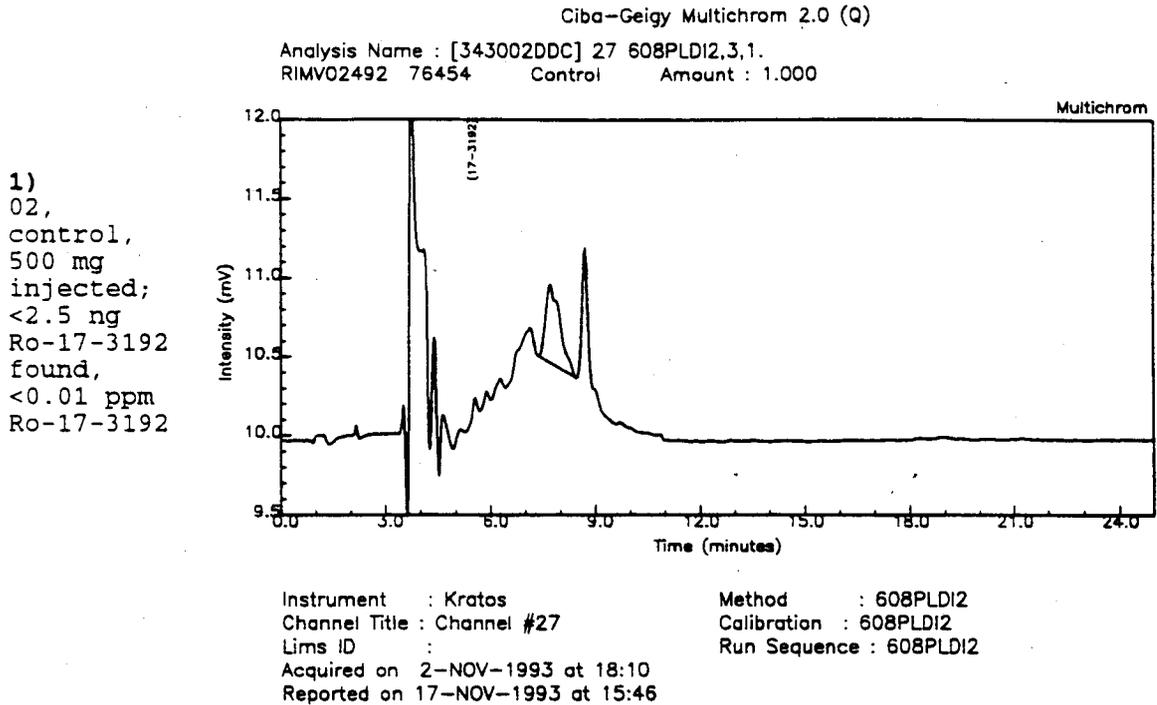
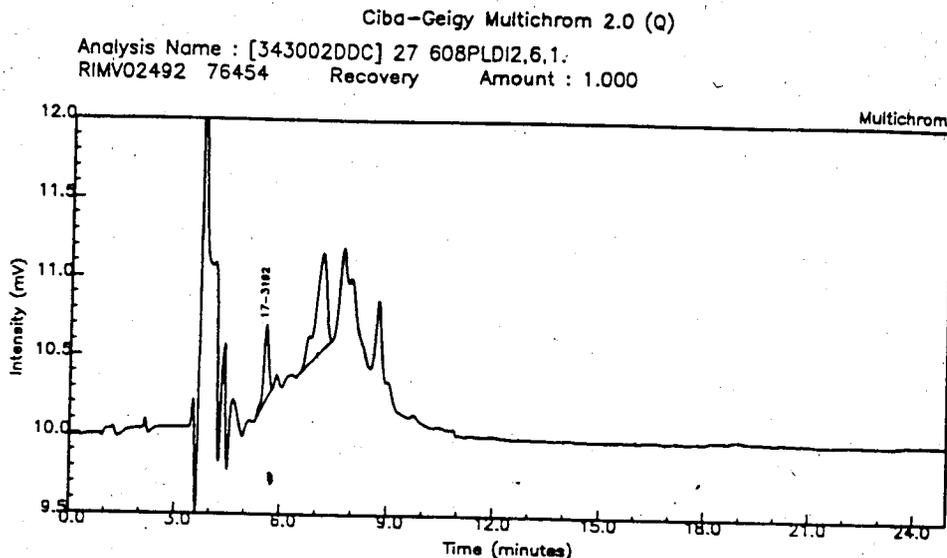


FIGURE 8. REPRESENTATIVE CHROMATOGRAMS OBTAINED FROM THE ANALYSIS OF POULTRY LIVER FOR Ro-17-3192 USING METHOD AG-608 (Continued)

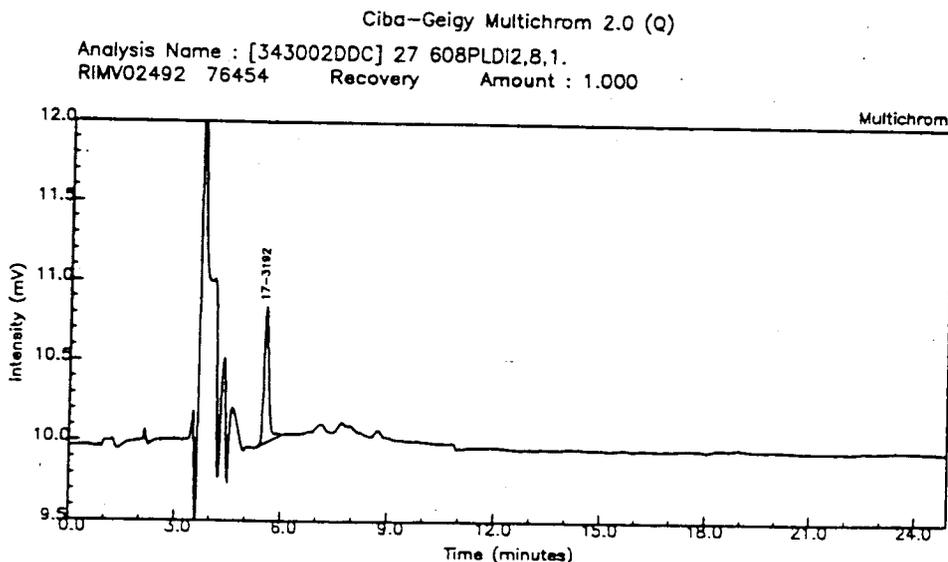
3)  
04, control  
+ 0.01 ppm,  
500 mg  
injected;  
3.682 ng  
Ro-17-3192  
found,  
0.007 ppm,  
74%  
recovery



Instrument : Kratos  
Channel Title : Channel #27  
Lims ID :  
Acquired on 2-NOV-1993 at 19:28  
Reported on 17-NOV-1993 at 15:47

Method : 608PLD12  
Calibration : 608PLD12  
Run Sequence : 608PLD12

4)  
05, control  
+ 0.20 ppm,  
50 mg  
injected;  
6.601 ng  
Ro-17-3192  
found,  
0.132 ppm,  
66%  
recovery

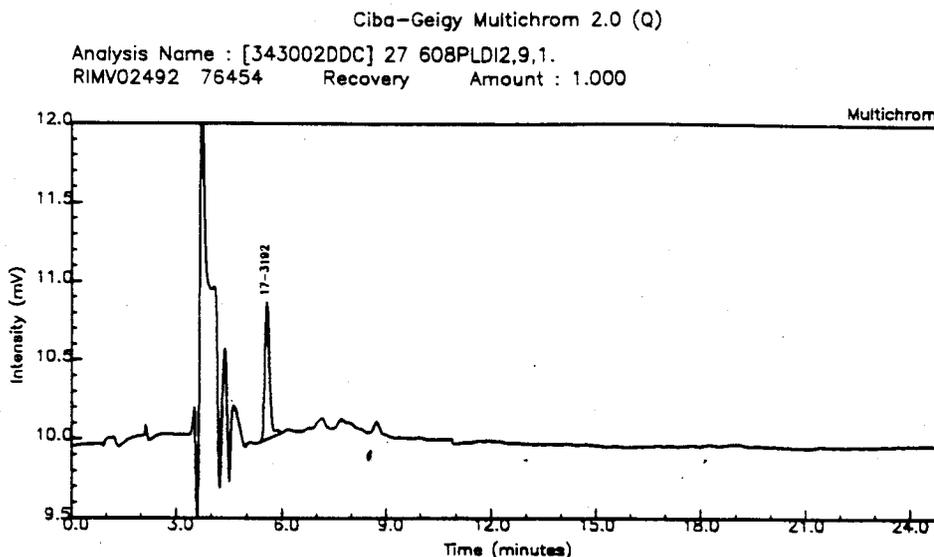


Instrument : Kratos  
Channel Title : Channel #27  
Lims ID :  
Acquired on 2-NOV-1993 at 20:20  
Reported on 17-NOV-1993 at 15:47

Method : 608PLD12  
Calibration : 608PLD12  
Run Sequence : 608PLD12

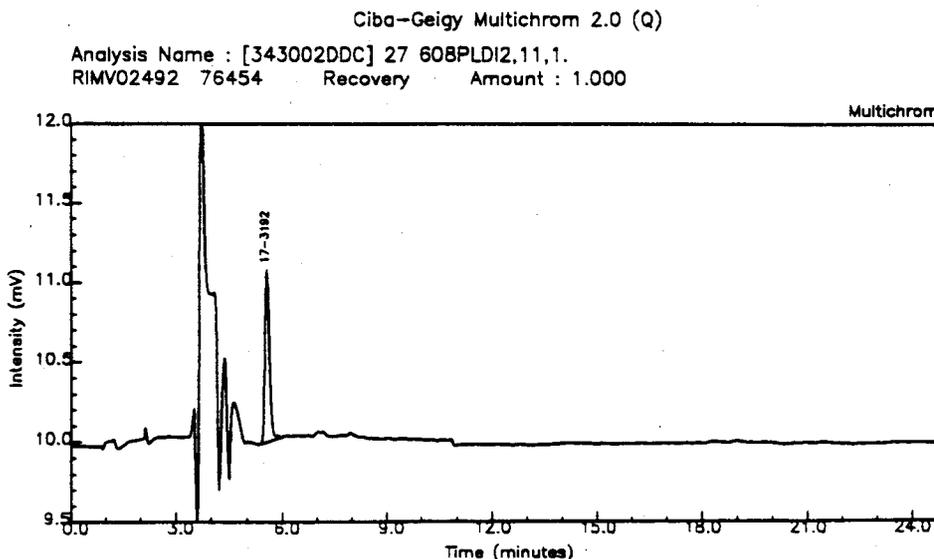
FIGURE 8. REPRESENTATIVE CHROMATOGRAMS OBTAINED FROM THE ANALYSIS OF POULTRY LIVER FOR Ro-17-3192 USING METHOD AG-608 (Continued)

5)  
05a,  
control +  
0.20 ppm,  
50 mg  
injected;  
6.741 ng  
Ro-17-3192  
found,  
0.135 ppm,  
67%  
recovery



Instrument : Kratos Method : 608PLD12  
Channel Title : Channel #27 Calibration : 608PLD12  
Lims ID : Run Sequence : 608PLD12  
Acquired on 2-NOV-1993 at 20:46  
Reported on 17-NOV-1993 at 15:48

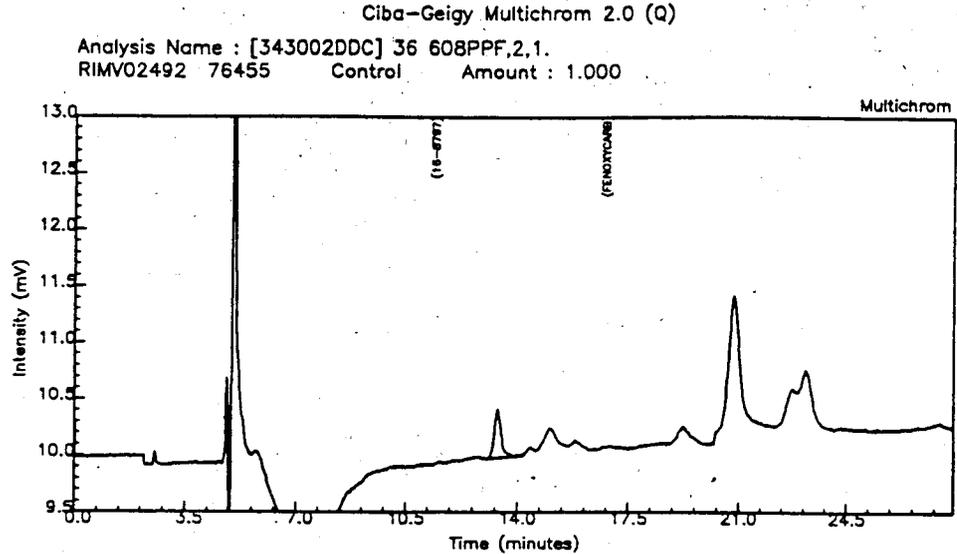
6)  
06, control  
+ 0.50 ppm,  
25 mg  
injected;  
8.394 ng  
Ro-17-3192  
found,  
0.336 ppm,  
67%  
recovery



Instrument : Kratos Method : 608PLD12  
Channel Title : Channel #27 Calibration : 608PLD12  
Lims ID : Run Sequence : 608PLD12  
Acquired on 2-NOV-1993 at 21:38  
Reported on 17-NOV-1993 at 15:48

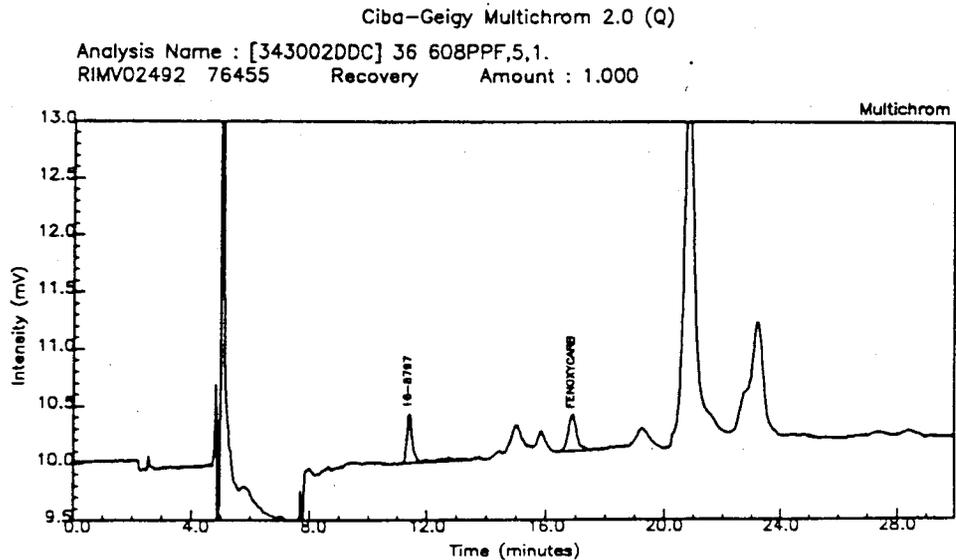
FIGURE 9. REPRESENTATIVE CHROMATOGRAMS OBTAINED FROM THE ANALYSIS OF POULTRY PERITONEAL FAT FOR FENOXYCARB AND Ro-16-8797 USING METHOD AG-608

1)  
08,  
control,  
500 mg  
injected;  
<2.5 ng  
Ro-16-8797  
found,  
<0.01 ppm;  
<2.5 ng  
Fenoxycarb  
found,  
<0.01 ppm.



Instrument : 783 Method : 608PPF  
Channel Title : Channel #36 Calibration : 608PPF  
Lims ID : Run Sequence : 608PPF  
Acquired on 29-SEP-1993 at 14:31  
Reported on 18-NOV-1993 at 08:29

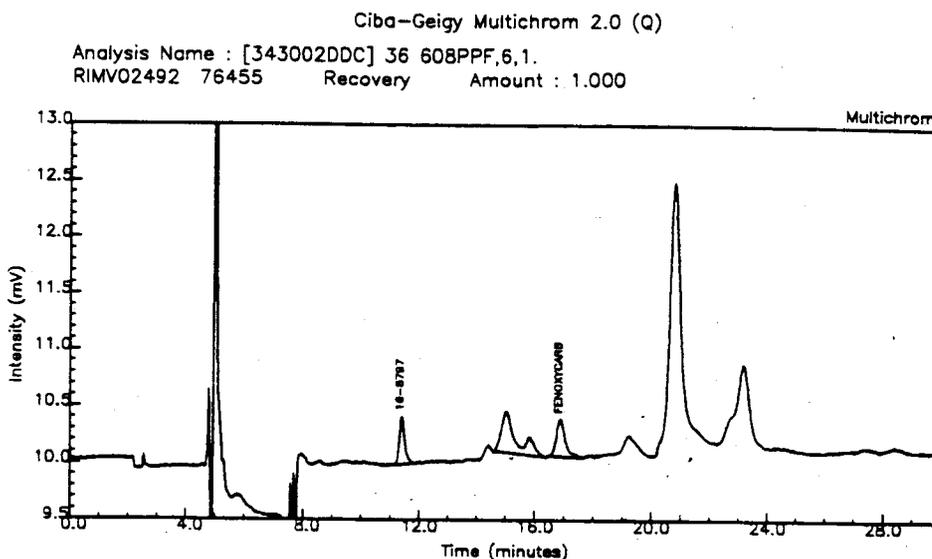
2)  
09, control  
+ 0.01 ppm,  
500 mg  
injected;  
4.408 ng  
Ro-16-8797  
found,  
0.009 ppm,  
88%  
recovery;  
4.750 ng  
Fenoxycarb  
found,  
0.010 ppm,  
95%  
recovery.



Instrument : 783 Method : 608PPF  
Channel Title : Channel #36 Calibration : 608PPF  
Lims ID : Run Sequence : 608PPF  
Acquired on 29-SEP-1993 at 16:07  
Reported on 18-NOV-1993 at 08:29

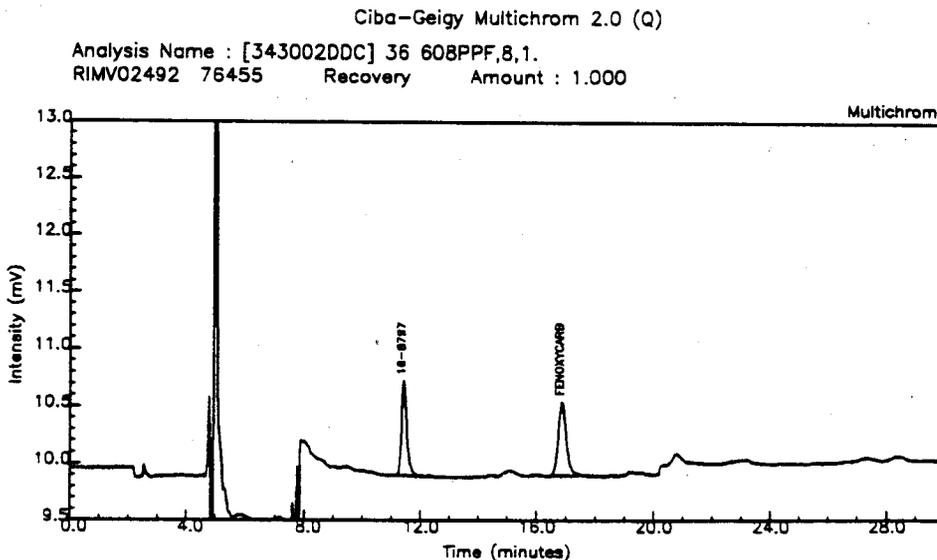
FIGURE 9. REPRESENTATIVE CHROMATOGRAMS OBTAINED FROM THE ANALYSIS OF POULTRY PERITONEAL FAT FOR FENOXYCARB AND Ro-16-8797 USING METHOD AG-608 (Continued)

3)  
10, control  
+ 0.01 ppm,  
500 mg  
injected;  
4.223 ng  
Ro-16-8797  
found,  
0.008 ppm,  
84%  
recovery;  
4.921 ng  
Fenoxycarb  
found,  
0.010 ppm,  
98%  
recovery.



Instrument : 783 Method : 608PPF  
Channel Title : Channel #36 Calibration : 608PPF  
Lims ID : Run Sequence : 608PPF  
Acquired on 29-SEP-1993 at 16:38  
Reported on 18-NOV-1993 at 08:30

4)  
11, control  
+ 0.20 ppm,  
50 mg  
injected;  
8.584 ng  
Ro-16-8797  
found,  
0.172 ppm,  
86%  
recovery;  
9.669 ng  
Fenoxycarb  
found,  
0.193 ppm,  
97%  
recovery.



Instrument : 783 Method : 608PPF  
Channel Title : Channel #36 Calibration : 608PPF  
Lims ID : Run Sequence : 608PPF  
Acquired on 29-SEP-1993 at 17:42  
Reported on 18-NOV-1993 at 08:30