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CGA-152005

STUDY TITLE

ANALYTICAL METHOD FOR THE DETERMINATION
OF CGA-152005 IN MEAT, MILK, BLOOD, AND EGGS BY
HIGH PERFORMANCE LIQUID CHROMATOGRAPHY
INCLUDING VALIDATION DATA

DATA REQUIREMENT

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LABORATORY PROJECT IDENTIFICATION

ANALYTICAL METHOD AG-592

VOLUME 1 OF 1 OF STUDY

PAGE 1 OF 126

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The Good Laboratory Practices Compliance Statement regarding EPAs GLP Standards (40 CFR Part 160) provided on page twenty-seven (27) of this volume and signed by the Study Director is truthful and accurate.

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TABLE OF CONTENTS

<u>TITLE</u>	<u>PAGE NO.</u>
AG-592: Analytical Method for the Determination of CGA-152005 In Meat, Milk, Blood, and Eggs by High Performance Liquid Chromatography including Validation Data	5
Appendix I: Protocol 26-92 and Amendment 1	58
Appendix II: Separate Documents Accompanying this Report	117
Appendix III: Residue Test Report RI-MV-001-92	118

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ANALYTICAL METHOD AG-592

ANALYTICAL METHOD FOR THE DETERMINATION
OF CGA-152005 IN MEAT, MILK, BLOOD, AND EGGS BY
HIGH PERFORMANCE LIQUID CHROMATOGRAPHY
INCLUDING VALIDATION DATA

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TABLE OF CONTENTS

	<u>PAGE NO.</u>
I. SUMMARY AND INTRODUCTION.....	7
A. Scope	7
B. Principle	7
II. MATERIALS AND METHODS.....	8
A. Apparatus	8
B. Reagents	9
C. Analytical Procedure	10
1. Extraction	10
2. Partitioning	11
3. Alumina-A Sep-Pak Cleanup	12
D. Instrumentation	14
1. Description and Operating Conditions	14
2. Standardization	15
E. Interferences	15
F. Confirmatory Techniques	15
G. Time Required	15
H. Modifications and Potential Problems	15
1. Emulsion Formation	15
2. Heat	16
3. Degradation in Methanol	16

TABLE OF CONTENTS
(Continued)

	<u>PAGE</u> <u>NO.</u>
I. Preparation of Standard Solutions ...	16
1. Preparation of Analytical Standards	16
2. Preparation of Fortification Standards	16
J. Methods of Calculation	17
1. Linear Regression Analysis	17
2. Calculation of Sample Concentration	17
K. Fortification Experiments	18
1. Substrate Fortification	18
2. Calculation of Procedural Recovery	18
III. RESULTS AND DISCUSSION.....	19
IV. CONCLUSION.....	22
V. CERTIFICATION AND CERTIFICATION OF GOOD LABORATORY PRACTICES.....	23
VI. QAU STATEMENT.....	24
VII. LIST OF TABLES AND FIGURES.....	25
TABLE I. LIQUID CHROMATOGRAPHIC OPERATING PARAMETERS FOR THE ANALYSIS OF CGA-152005	25
TABLE II. TYPICAL STANDARDIZATION DATA FOR CGA-152005 (FROM THE ANALYSIS OF POULTRY LEAN MEAT)	26
TABLE III. RECOVERY RESULTS FOR CONTROL AND CGA-152005 FORTIFIED CONTROL SUBSTRATES USING METHOD AG-592	27

TABLE OF CONTENTS
(Continued)

	<u>PAGE</u> <u>NO.</u>
TABLE IV. PRECISION OF ANALYTICAL METHOD AG-592 AS DEMONSTRATED BY THE RESULTS OF ANALYZING ¹⁴ C-CGA-152005 CONTAINING SUBSTRATES	29
TABLE V. SUMMARY TABLE FOR THE EXTRACTABILITY, ANALYSIS, AND ACCOUNTABILITY OF ¹⁴ C-CGA-152005 TREATED SUBSTRATES USING METHOD AG-592	30
FIGURE 1. STRUCTURE AND CHEMICAL NAME OF CGA-152005	31
FIGURE 2. ANALYTICAL PROCEDURE FLOWCHART FOR AG-592	32
FIGURE 3. TYPICAL STANDARD CHROMATOGRAMS (FROM POULTRY LEAN MEAT ANALYSES)	33
FIGURE 4. CALIBRATION PLOT OF THE STANDARDS OBTAINED FROM THE ANALYSIS OF POULTRY LEAN MEAT	34
FIGURE 5. CHROMATOGRAMS OF THE REAGENT BLANKS FROM THE ANALYSIS OF (A) POULTRY LIVER, (B) POULTRY PERITONEAL FAT, (C) BEEF TENDERLOIN, AND (D) BEEF BLOOD	35
FIGURE 6. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF POULTRY LEAN MEAT FOR CGA-152005 USING METHOD AG-592	36

TABLE OF CONTENTS
(Continued)

	<u>PAGE</u> <u>NO.</u>
FIGURE 7. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF EGGS FOR CGA-152005 USING METHOD AG-592	37
FIGURE 8. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF GOAT LIVER FOR CGA-152005 USING METHOD AG-592	38
FIGURE 9. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF GOAT OMENTAL FAT FOR CGA-152005 USING METHOD AG-592	39
FIGURE 10. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF GOAT ROUND FOR CGA-152005 USING METHOD AG-592	40
FIGURE 11. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF GOAT KIDNEY FOR CGA-152005 USING METHOD AG-592	41
FIGURE 12. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF GOAT MILK FOR CGA-152005 USING METHOD AG-592	42
FIGURE 13. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF POULTRY LIVER FOR CGA-152005 USING METHOD AG-592	43
FIGURE 14. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF POULTRY PERITONEAL FAT FOR CGA-152005 USING METHOD AG-592	44

TABLE OF CONTENTS
(Continued)

	<u>PAGE</u> <u>NO.</u>
FIGURE 15. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF BEEF LIVER FOR CGA-152005 USING METHOD AG-592	45
FIGURE 16. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF BEEF KIDNEY FOR CGA-152005 USING METHOD AG-592	46
FIGURE 17. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF BEEF TENDERLOIN FOR CGA-152005 USING METHOD AG-592	47
FIGURE 18. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF BEEF ROUND FOR CGA-152005 USING METHOD AG-592	48
FIGURE 19. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF BEEF OMENTAL FAT FOR CGA-152005 USING METHOD AG-592	49
FIGURE 20. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF BEEF PERITONEAL FAT FOR CGA-152005 USING METHOD AG-592	50
FIGURE 21. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF BEEF BLOOD FOR CGA-152005 USING METHOD AG-592	51
FIGURE 22. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF BEEF MILK FOR CGA-152005 USING METHOD AG-592	52
VIII. REFERENCES	53

I. SUMMARY AND INTRODUCTION

A. SCOPE

This method is for the determination of residues of CGA-152005 (see Figure 1) in animal tissues, milk, blood, and poultry eggs. The limit of detection of this method is 0.60 ng of CGA-152005 and the limit of determination is 0.05 ppm for tissues, eggs, and blood and 0.01 ppm for milk.

B. PRINCIPLE

A 10-g subsample of substrate is homogenized with 90% acetone:10% 0.1% sodium bicarbonate aqueous solution. After filtering, the volume is reduced under vacuum until only the aqueous portion remains. The sample is diluted with 25 ml 0.4% sodium carbonate and partitioned against methyl tert-butyl ether:hexane (1:1). The aqueous phase is acidified with 20 ml 0.8% phosphoric acid, followed by the addition of 20 ml of saturated aqueous salt solution. The compound is extracted by partitioning with dichloromethane:hexane (1:1). The organic portion is dried with sodium sulfate, reduced to about 10 ml and added to a preconditioned alumina-A Sep-Pak column. After column washes with hexane, ethyl acetate, and 5% acetonitrile in ethyl acetate, the compound is eluted with methanol:acetonitrile (60:40). The eluate is concentrated to just dryness. One ml of acetonitrile is added, followed by one ml of picopure water, and the sample is filtered through a 0.45 micron Acrodisc filter. The filter is rinsed with 1 ml of acetonitrile:water (1:1). The sample is analyzed by high performance liquid chromatography (HPLC) using a narrow bore reverse phase column (Supelcosil LC-18-DB) with a mobile phase of acetonitrile:0.05% phosphoric acid in picopure water (40:60). Detection is by UV at 225 nm. A flowchart of the method is shown in Figure 2.

II. MATERIALS AND METHODS

A. APPARATUS

- 1.0 Analytichem Reservoir (Analytichem # 1213-1-1012 or equivalent)
- 2.0 Boston wide mouth round, 8-oz. (Penn Bottle Co.)
- 3.0 Filter, Acrodisc LC PVDF, 0.45 μ m, 25.0 mm (Product #4408)
- 4.0 Filter Paper, Folded Reeve Angel #802, 18.5 cm
- 5.0 Flasks, Erlenmeyer, 250 and 500-ml (Fisher # 10-047C and 10-047E)
- 6.0 Flasks, Round Bottom, 100-ml, 250-ml, and 1000-ml (Fisher #10-067C, 10-067E, and 10-067H)
- 7.0 Funnel, Fluted Pyrex (Fisher # 10-329C or equivalent)
- 8.0 Funnel, Separatory, 125-ml, and 250-ml, with teflon stopcock (Fisher #10-437-10B, and 10-437-10C)
- 9.0 Graduated cylinder, 10-ml, 25-ml, 50-ml, 100-ml, 250-ml (Fisher #08-551A, 08-551B, 08-551C, 08-551D, and 08-551E)
- 10.0 Homogenizer, Polytron (Brinkman Instruments or equivalent)
- 11.0 Pasteur pipets (Kimble 13-678-30B, 13-678-30C)
- 12.0 Pipets, volumetric class A
- 13.0 Rotary Evaporator, Buchii or equivalent, with warm water bath (Buchler Instruments or equivalent), refrigerated recirculating bath (Endocal or equivalent), and aspirator pump (Cole-Parmer or equivalent)
- 14.0 Sep-Pak Alumina-A Cartridges, (Waters #51800)

- 15.0 Syringe, Hamilton Gastight, Luer Tip, 1-ml (Fisher #14-824-24 or equivalent)
- 16.0 Ultrasonicator (Heatsystems Inc. or equivalent)
- 17.0 Vials, crimptop borosilicate (Sun Broker Inc. or equivalent)
- 18.0 Kontes 100 ml Rotary Evaporator Trap (Fisher catalog K570200-0124 or equivalent)

B. REAGENTS

- 1.0 Acetone, OPTIMA grade (Fisher # A949-4 or equivalent)
- 2.0 Acetonitrile, HPLC grade (Fisher # A998-4 or equivalent)
- 3.0 Dichloromethane, HPLC grade (Burdick & Jackson #300-4 or equivalent)
- 4.0 Ethyl Acetate, HPLC grade (Fisher # E195-4 or equivalent)
- 5.0 Hexane, HPLC grade (Fisher # H302-4 or equivalent)
- 6.0 Methanol, HPLC grade (Fisher # A452-4 or equivalent)
- 7.0 Methyl tert-butyl ether, HPLC grade (Fisher #E127-4)
- 8.0 Phosphoric acid, HPLC grade (Fisher #A260 or equivalent)
- 9.0 Sodium bicarbonate, Certified ACS grade (Fisher #S233-3 or equivalent)
- 10.0 Sodium carbonate, Certified ACS grade (Fisher #S263-3 or equivalent)
- 11.0 Sodium chloride, Certified ACS grade (Fisher #S271-3 or equivalent)
- 12.0 Water, HPLC grade (picopure or equivalent)

13.0 CGA-152005, Analytical Standard supplied by CIBA-GEIGY Corporation, 410 Swing Rd., Greensboro, NC 27419.

C. ANALYTICAL PROCEDURE

1.0 Extraction

1.1 Meat, Blood, Milk, and Eggs

Weigh 10 grams of tissue slices, well mixed blood or egg homogenate, or 50 grams milk into a tared 8-oz. glass bottle. Add 90 ml of 90% acetone:10% 0.1% sodium bicarbonate in picopure water. Homogenize for 30 seconds with the polytron at medium speed. Return any tissues remaining on the polytron blade back to the 8-oz. bottle. Filter through a Reeve Angel #802 filter in a longstem funnel into a 250-ml Erlenmeyer flask. NOTE: The second extraction is not required for the milk samples. Remove the filter paper after all solvent has passed through and place it back into the 8-oz. bottle. Add another 90 ml of 90% acetone:10% 0.1% sodium bicarbonate and homogenize with the polytron for 30 seconds. The generator should be at the bottom of the filter paper and will homogenize the substrate left on the paper, but not the filter itself. A polytron setting slower than the one used in the first extraction may be required. Pour the homogenate through a new Reeve Angel #802 filter and collect with the first extract. Measure and record the volume of the combined extracts for later calculations.

2.0 Partitioning

- 2.1 Transfer the extract from section 1.1 to a 1000-ml round bottom flask and reduce the solvent by rotary evaporation under vacuum without the use of the water bath. A rotary evaporator trap is essential during extract evaporation. Then, after about two minutes, lower the flask into a 38 - 40°C warm water bath. CAUTION: While bumping does not typically occur in the early stages of the extract reduction, bubbling will occur when most of the organic solvent has been removed. The addition of 10 ml of ethyl acetate to the sample will help if excessive bubbling occurs. Remove the sample when only the aqueous portion remains. Transfer the solution to a 125-ml separatory funnel.
- 2.2 Add 10 ml of 0.4% sodium carbonate to the 1000-ml round bottom flask from Section C.2.1, swirl and sonicate to dissolve remaining residues. Transfer to the same 125-ml separatory funnel containing the sample. Repeat the rinse of the 1000-ml round bottom flask with another 10 ml of 0.4% sodium carbonate, and a third rinse of 5 ml. Add 30 ml of methyl tert-butyl ether (MtBE):hexane (1:1) to the 125-ml separatory funnel and shake for one minute, taking care to vent the funnel. Allow the two layers to separate, breaking any emulsions that may form by agitating with a glass rod or by applying a small amount of heat. Drain the lower aqueous layer and any persistent

emulsions into a 250-ml Erlenmeyer flask and discard the organic layer. Pour the aqueous phase back into the 125-ml separatory funnel and repeat the extraction with 30 ml of MtBE:hexane (1:1). Collect the aqueous layer in the same Erlenmeyer flask.

- 2.3 Add 20 ml of 0.8% H_3PO_4 (in picopure water) and 20 ml of picopure water saturated with sodium chloride to the aqueous phase in the Erlenmeyer flask from section C.2.2. Transfer to a 250-ml separatory funnel and extract 3 times by ~~partitioning~~ partitioning with 60 ml dichloromethane: hexane (1:1), taking care to vent the funnel. The first 60 ml of dichloromethane:hexane (1:1) used for the partitioning should also be used to rinse the 250-ml Erlenmeyer flask. Treat emulsions that form as was done in section C.2.2, and combine and save the upper organic layers in a separate 500-ml Erlenmeyer flask.

3.0 Alumina-A Sep-Pak Cleanup

- 3.1 Attach a 50-ml Analytichem reservoir to an Alumina-A Sep-Pak column, and condition the column with the following rinses: 25 ml methanol: acetonitrile (60:40), 15 ml ethyl acetate, and 25 ml dichloromethane (DCM):hexane (1:1). Do not let the column dry between rinses and prior to applying the sample.
- 3.2 Add approximately 15 grams of sodium sulfate directly to the organic phase from section C.2.3 to bind any water in the extract, and transfer to a 500

ml round bottom flask. Rinse the sodium sulfate 3 times with small volumes (~3-5 ml) of DCM:hexane (1:1). Reduce the volume of the sample under vacuum by rotary evaporation in a 35°C warm water bath to 5 to 10 ml, taking care not to let the sample go to dryness.

- 3.3 Add the sample from section C.3.2 to the conditioned Alumina-A Sep-Pak column. Rinse the round bottom flask 3 times with 1 to 2 ml of DCM:hexane (1:1), pouring each rinse into the reservoir above the Alumina-A column. Allow the sample and washes to drip through the column by gravity only. The flow may occasionally stop and can be restarted by applying gentle pressure to the top of the reservoir with a pipet bulb. Wash with the following, being careful to insure that the column does not go dry between rinses. Collect the eluate in a 100-ml round bottom flask.

Wash with 15 ml hexane
(discard)

Wash with 25 ml ethyl acetate
(discard)

Wash with 15 ml 5%
acetonitrile in ethyl acetate
(discard)

Elute with 30 ml 60:40
methanol: acetonitrile
(collect)

- 3.4 Reduce the eluate volume from section C.3.3 to just dryness under vacuum by rotary evaporation in a 35°C water bath. This step must be done soon after elution from the

Alumina-A column since preliminary reports indicate that CGA-152005 is unstable in methanol for prolonged periods of time. When the sample has just reached dryness, remove it from the rotary evaporator and add 1 ml acetonitrile. Swirl and sonicate to dissolve all residues. Add 1 ml picopure water and swirl and sonicate a second time.

- 3.5 Prewash a 0.45 μ m, 25mm Acrodisc PVDF HPLC syringe filter with 3 X 1ml acetonitrile: picopure water (1:1). Filter the sample from section C.3.4 slowly through the Acrodisc using a 1-ml glass syringe. Add 1 ml acetonitrile: picopure water (1:1) to the round bottom flask, swirl and pass through the same filter as a rinse. Mix the sample well and transfer 1 to 2 ml to a borosilicate crimpltop vial for analysis by HPLC. Increase the final dilution volume for samples containing higher concentrations of CGA-152005 such that the peak heights obtained by LC analysis will be within the range of the calibration standards discussed in sections I.1.0 and J.1.0.

D. INSTRUMENTATION

1.0 Description and Operating Conditions

Residues of CGA-152005 are determined by HPLC on a narrow bore base deactivated Supelco C-18 column (LC-18-DB). The mobile phase is 60% 0.05% phosphoric acid in picopure water:40% acetonitrile at a flow rate of 0.3 ml per

minute. Detection of CGA-152005 is performed using an ultraviolet detector at a wavelength of 225 nm. The injection volume is 10 μ l. The HPLC conditions are listed in Table 1.

2.0 Standardization

Standardize the HPLC system by injecting 10- μ l aliquots of standard solutions of CGA-152005 in a working range of 0.6-10 ng/injection. Generate a linear regression from the data by comparing detector response and ng injected. See section 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 analysis can be performed overnight using automatic injection.

H. MODIFICATIONS AND POTENTIAL PROBLEMS

1.0/ Emulsion Formation

Some samples may develop emulsions during partitioning (during steps in sections C.2.2 and C.2.3). These may be cleared if allowed to settle out and then gently stirred with a glass rod. Slight heating may help (a hair dryer was used), but care must be taken to avoid temperatures in excess of 40°C. Any emulsions that remain should stay with the aqueous phases during the partitioning steps.

2.0 Heat

During the evaporation of the solvents from the samples in sections C.2.1, C.3.2, and C.3.4, any water bath used must not have a temperature $>40^{\circ}\text{C}$ and the samples should be removed just as soon as they reach dryness. A temperature of 35°C is required for removal of the solvent from the Alumina-A eluate. Excessive temperature, especially when the sample has gone to dryness, may lead to analyte decomposition.

3.0 Degradation in Methanol

After elution from the Alumina-A column, the solvent must be evaporated as soon as possible, since CGA-152005 was reported to be unstable in methanol. Do not store the sample overnight in the methanol:acetonitrile eluent.

I. PREPARATION OF STANDARD SOLUTIONS

1.0 Preparation of Analytical Standards

Weigh 10 mg of CGA-152005 analytical standard into a 100-ml volumetric flask and dilute to the mark with acetonitrile. Make serial dilutions of the 0.1 mg/ml standard solution with 50% acetonitrile: 50% picopure water to give a series of fortification/analytical standards in a range of 0.06 $\mu\text{g}/\text{ml}$ to 1.0 $\mu\text{g}/\text{ml}$ of CGA-152005. Store the standard solutions in amber bottles at 4°C in the dark when not in use.

2.0 Preparation of Fortification Standards

The 1.0 $\mu\text{g}/\text{ml}$ standard used to fortify standards is prepared in section I.1.0 above. See section K.1.0 for fortification procedures.

J. METHODS OF CALCULATION

1.0 Linear Regression Analysis

Inject 10- μ l aliquots of sample extracts onto the HPLC system. Compare the analyte peak height found in the sample extracts to the peak heights determined for the analytical standards by entering them into a linear regression program or by using a computer system (e.g., VG Multichrom) to determine the nanograms of CGA-152005 in the injected aliquot.

2.0 Calculation of Sample Concentration

Calculate the residue results in terms of ppm of CGA-152005 by using the following equation:

$$(1) \text{ ppm} = \frac{(\text{ng CGA-152005 Found})}{(\text{mg sample injected}) (\%R)}$$

Where the ng CGA-152005 is obtained from the linear regression analysis in section J.1.0 and the mg sample injected is calculated as follows: (Equation 2)

$$(2) \text{ mg inj.} = \frac{(G) (V_a) (V_e)}{(V_e) (V_f)}$$

G = milligrams sample extracted

V_a = aliquot volume (from section C.1.1)

V_e = extraction volume (from section C.1.1)

V_f = 180 ml (or 90 ml for milk) +
(sample size in grams)
(sample % moisture expressed as
a decimal)

The % moisture for each substrate used in this work was 80% for tissues, 0% for fat, 90% for blood, 87% for milk, 80% for whole egg, and 48% for egg yolks.

V_i = injection volume (μ l)

V_f = total volume of final injection
solution (μ l)

The %R for the fresh fortifications shown in equation (1) is used as a correction for procedural recoveries, expressed as a decimal, and is shown in equation 3 (section K.2.0).

K. Fortification Experiments

1.0 Substrate Fortification

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 CGA-152005. For example, add 0.5 ml of a 1.0 μ g/ml standard solution of CGA-152005 to 10 g of control tissues or 50 g of milk for a 0.05 ppm or 0.01 ppm fortification, respectively. The volume of the various fortification standards used can be varied from 0.5 to 2.0 ml depending on the desired fortification level. Allow the solvent to evaporate for at least 20 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.

2.0 Calculation of Procedural Recovery

The final ppm value of the control and fortified samples can be obtained using the calculations shown in section J.2.0. Determine the % recovery by first subtracting the background detector response, if any, in the control sample from the CGA-152005 response in the recovery sample. Calculate the

procedural recovery factor as a percentage (R) by the equation:

$$(3) \quad \%R = \frac{\text{ppm CGA-152005 found}}{\text{ppm CGA-152005 added}} \times 100$$

III. RESULTS AND DISCUSSION

The objective of Protocol 26-92¹ was to validate analytical Method AG-592 for the quantification of residues of CGA-152005 in meat, milk, eggs, and blood. This was accomplished, as demonstrated by the results in this report, by analyzing control and CGA-152005 fortified control samples of poultry liver, peritoneal fat, lean meat, and eggs, and 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 poultry lean meat and eggs and goat liver, omental fat, round, kidney, and milk, obtained from metabolism studies^{2,3} in which the animals had been dosed with radiolabelled CGA-152005, were analyzed. The screening level for all the substrates is 0.050 ppm except milk which has a screening level of 0.01 ppm. The limit of detection, defined as the lowest concentration standard injected during a run, is 0.60 ng CGA-152005. The limit of determination (screening level), defined by the lowest fresh fortification level within a run, is 0.05 ppm for all substrates except milk which is 0.01 ppm.

Typical standard chromatograms of CGA-152005 at various concentrations are shown in Figure 3 and were obtained during the analysis of poultry lean meat. The concentrations of CGA-152005 and their respective peak heights from this run are shown in Table II. The calibration plot for this run is shown in Figure 4 and the correlation coefficient of 0.99938 is typical of the calibration plots obtained during this study. The peak heights obtained for the analytical standards were reproducible over the course of the study when injected under similar chromatographic

conditions, thus demonstrating the stability of the injection standards.

Reagent blanks were also analyzed during the analysis of poultry liver (representative of tissues), poultry peritoneal fat (representative of fat samples), beef tenderloin (representative of lean meats), and beef blood (representative of high moisture substrates). The chromatograms of the reagent blanks are shown in Figure 5. As can be seen, there are no peaks near the retention time (about 22 minutes) of CGA-152005 in any of the chromatograms.

A. ACCURACY

Table III shows the results of analyzing control and freshly fortified control samples. The recoveries for all samples at all fresh fortification levels ranged from 70 to 111 % except for the single 0.50 ppm fortification level for beef blood which was 58%. The mean recoveries for each substrate ranged from 75% (SD=4.0) for goat omental fat to 104% (SD=3.8 and 5.4, respectively) for goat liver and beef liver. The average recovery for all substrates was 90% with a standard deviation of 11% over a CGA-152005 fortification range of 0.01 to 5.0 ppm and the control samples showed no background residues at or above the limit of determination of the method in any substrate. These recovery results demonstrate the accuracy of the method. Representative chromatograms for all the substrates listed in Table III are shown in Figures 6 through 22.

B. PRECISION

The results of subjecting the ¹⁴C-CGA-152005 containing substrates to analysis using Method AG-592 are shown in Table IV. The narrow range and relatively small standard deviations of the ppm found for each substrate demonstrate the precision of the method.

C. EXTRACTABILITY AND ACCOUNTABILITY

The ¹⁴C analyses were performed by transferring an aliquot portion of the appropriate sample (extract or final fraction) to a scintillation vial, adding 15 ml of Beckman Ready-Safe liquid scintillation cocktail, followed by measurement on a Beckman LS 7800 liquid scintillation counting (LSC) instrument. The results are obtained in units of disintegrations per minute (dpm) per volume of sample measured. The % extractabilities were calculated from data obtained by subjecting an aliquot portion of the sample extract to LSC, converting the dpm/ml data to parts per million (ppm), and comparing the results (by percentages) to the total ¹⁴C residue in the sample, as determined by combustion analysis (as reported in the metabolism studies^{2,3}). The % accountabilities of Method AG-592 were calculated from data obtained by the method (liquid chromatography analysis) compared (by percentages) to the total ¹⁴C residue in the sample. The following calculation was performed to convert the dpm/ml value obtained by LSC from one of the poultry lean meat extracts into units of ppm. The activity of the ¹⁴C-CGA-152005 fed to the animal was 40.2 μCi/mg. A 10 gram sample was extracted with 180 ml 90% acetone/10% 0.1% sodium bicarbonate and 3 ml of the extract contained 12,219 dpm. Therefore,

$$\left(\frac{12,219 \text{ dpm}}{3 \text{ ml}}\right) \left(\frac{1,000 \mu\text{g}}{\text{mg}}\right) \left(\frac{\text{mg}}{40.2 \mu\text{Ci}}\right) \left(\frac{1 \times 10^6 \mu\text{Ci}}{\text{Ci}}\right) \left(\frac{\text{Ci}}{3.7 \times 10^{10} \text{ dps}}\right) \left(\frac{\text{dps}}{60 \text{ dpm}}\right) \left(\frac{180 \text{ ml}}{10 \text{ g}}\right)$$

= 0.82 $\frac{\mu\text{g}}{\text{g}}$ or ppm

The extractability and accountability data are shown in Table V. The % extractabilities ranged from a low of 72% for eggs to a high of 148% for goat omental fat. The accountabilities ranged from a low of 28% for eggs to a high of

142% for goat omental fat. It should be noted that these ^{14}C -CGA-152005 containing substrates were obtained from metabolism studies^{2,3} which are still in progress and that the combustion results measure only the total ^{14}C radioactivity in the sample. Therefore, the lower accountabilities may result from a decrease in the quantity of CGA-152005 in these samples due to metabolism to other compounds. The results obtained by liquid chromatographic analysis using method AG-592 are in reasonably good agreement with the results obtained from the LSC measurements of the same final fractions as shown in columns G and I of Table V. Representative chromatograms obtained by subjecting the ^{14}C -CGA-152005 containing substrates to the analytical procedures outlined in Method AG-592 are shown in Figures 6 through 12.

Test substance ID, test system ID, protocol amendments, protocol deviations, and circumstances affecting the quality and integrity of the data are also reported in Residue Test Report RI-MV-001-92⁴. All raw data associated with this study and the original final AG-592 method and protocol are archived in the Residue Chemistry Archives at CIBA-GEIGY Corporation, Greensboro, NC. No specimens remain to be retained.

IV. CONCLUSION

Method AG-592 is a valid and accurate method for the determination of CGA-152005 in meat, milk, eggs, and blood. This conclusion is based on the accuracy, precision, extractability, and accountability of the method as demonstrated by the results in this study.

V. CERTIFICATION

The reports and experimental results included in this study, Laboratory Project I.D. AG-592, are certified to be authentic accounts of the experiments.

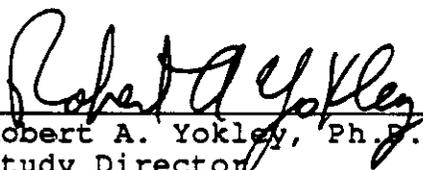


Robert A. Yokley, Ph.D.
Method Development
Residue Chemistry Department
919-632-2295

3-31-92
Date

CERTIFICATION OF GOOD LABORATORY PRACTICES

The analytical work reported in AG-592 was performed in accordance with Good Laboratory Practice Standards, 40 CFR Part 160.



Robert A. Yokley, Ph.D.
Study Director

3-31-92
Date



Robert K. Williams, Manager
Method Development
Residue Chemistry Department
Submitter/Sponsor

3/31/92
Date

VI. QUALITY ASSURANCE STATEMENT

Method Title: ANALYTICAL METHOD FOR THE DETERMINATION OF
CGA-152005 IN MEAT, MILK, BLOOD, AND EGGS BY
HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Study Director: R. Yokley Project Number: 168982

Protocol Number: 26-92 Final Report No.: AG-592

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(s) by the Quality Assurance Unit on the dates listed below.

<u>Audit Type</u>	<u>Inspection/Audit Date</u>	<u>Reporting Date</u>
Protocol	2/07/92	2/07/92
In-Progress	2/28/92	3/02/92
Final Report	3/26, 27, 30/92	3/30/92

Prepared by: Lynda A. Sayers
Date: 3/30/92

VII. LIST OF TABLES AND FIGURES

TABLE I. LIQUID CHROMATOGRAPHIC OPERATING
PARAMETERS FOR THE ANALYSIS OF
CGA-152005

Instrument: Perkin-Elmer Model Series-4 Solvent
Delivery System or equivalent.
Perkin-Elmer Model ISS-100 Automatic
HPLC sampler or equivalent.

Column Oven: BioRad HPLC column heater, model
number 125-0425

Oven Temp.: 30°C

Column: Supelcosil LC-18-DB, 250mm x 2.1 mm,
5 µm particle size (Supelco cat.
#5-7940) with Supelguard LC-18-DB
guard column.

Mobile Phase: 60% 0.05% phosphoric acid in
picopure water:40% acetonitrile

Retention Time: ~22 minutes

Detection: ABI Kratos Spectroflow Model 783
Programmable Absorbance Detector or
equivalent variable wavelength
detector.

Wavelength: 225 nm

Attenuation: 0.006 AUFS

Flow Rate: 0.3 ml/min

Volume Injected: 10 µl

Chart Speed: 0.25 cm/min

Run Time: 30 min/injection

Data Acquisition: Microvax II (Bones or Q)
Operating System, VMS Version 5.3-1
Application Software:
VG Multichrom Version 1.8
Worksheet Version: Ws.pas 1.3.1

TABLE II. TYPICAL STANDARDIZATION DATA FOR
CGA-152005 (FROM THE ANALYSIS OF
POULTRY LEAN MEAT). SEE ALSO FIGURES 3
AND 4

<u>CGA-152005 ng Injected</u>	<u>Peak Height (μV)</u>
0.60	102
1.0	204
2.0	404
4.0	831
6.0	1224
10.0	2175

Correlation Coefficient	0.99938
Slope	217.9
Intercept	-33.85

TABLE III. RECOVERY RESULTS FOR CONTROL AND CGA-152005 FORTIFIED CONTROL SUBSTRATES USING METHOD AG-592

(1) SUBSTRATE	PROTOCOL 26-92 SAMPLE #	CGA-152005 FORTIFICATION (ppm)	CGA-152005 (ppm)	(2) % RECOVERY (AG-592)	% RECOVERY	
					MEAN	STANDARD DEVIATION
Poultry	02	0	<0.05	-		
Liver	03	0.05	0.051	103	99	3.0
	04	0.05	0.048	96		
	05	0.20	0.20	99		
	06	0.50	0.48	96		
Poultry	08	0	<0.05	-		
Pentoneal	09	0.05	0.044	89	88	3.1
Fat	10	0.05	0.045	91		
	11	0.20	0.18	88		
	12	0.50	0.42	83		
Beef	13	0	<0.05	-		
Liver	14	0.05	0.053	106	104	5.4
	15	0.05	0.055	111		
	16	0.20	0.20	101		
	17	0.50	0.49	99		
Beef	18	0	<.05	-		
Kidney	19	0.05	0.047	83	91	6.1
	20	0.05	0.050	90		
	21	0.20	0.19	92		
	22	0.50	0.50	98		
Beef	24	0	<0.05	-		
Tenderloin	25	0.05	0.047	93	98	6.6
	26	0.05	0.047	94		
	27	0.20	0.22	108		
	28	0.50	0.48	97		
Beef Round	29	0	<0.05	-		
	30	0.05	0.046	91	79	10
	31	0.05	0.042	84		
	32	0.20	0.139	70		
	33	0.50	0.363	73		
Beef	34	0	<0.05	-		
Omental Fat	35	0.05	0.041	82	82	6.5
	36	0.05	0.039	78		
	37	0.20	0.16	77		
	38	0.50	0.46	91		
Beef	39	0	<0.05	-		
Perirenal Fat	40	0.05	0.040	79	87	6.3
	41	0.05	0.047	94		
	42	0.20	0.17	85		
	43	0.50	0.45	90		

(1) All samples are from Test Number RI-MV-001-92 - overall mean of 90% with a SD of 11% (n=61).

(2) Recovery samples were corrected for control residues where present.

TABLE III. RECOVERY RESULTS FOR CONTROL AND CGA-152005 FORTIFIED CONTROL SUBSTRATES USING METHOD AG-592 (Continued)

(1) SUBSTRATE	PROTOCOL 26-92 SAMPLE #	CGA-152005 FORTIFICATION (ppm)	CGA-152005 (ppm)	(2) % RECOVERY (AG-592)	% RECOVERY	
					MEAN	STANDARD DEVIATION
Beef Blood	45	0	<0.05	-		
	46	0.05	0.046	93	78	16
	47	0.05	0.044	88		
	48	0.20	0.15	74		
	49	0.50	0.29	58		
50	0	<0.01	-			
Beef Milk	51	0.01	0.0082	82	86	8.2
	52	0.01	0.010	97		
	53	0.10	0.084	84		
	54	0.50	0.393	79		
	55	0	<0.05	-		
Poultry Lean Meat	56	0.05	0.049	97	97	5.1
	57	0.05	0.051	102		
	58	0.50	0.46	91		
Eggs	62	0	<0.05	-	87	2.3
	63	0.05	0.045	89		
	64	0.05	0.042	85		
	65	0.50	0.43	86		
Goat Liver	69	0	<0.05	-	104	3.8
	70	0.05	0.054	108		
	71	0.05	0.051	102		
	72	1.00	1.02	102		
Goat Ornental Fat	76	0	<0.05	-	75	4.0
	77	0.05	0.036	72		
	78	0.05	0.037	74		
Goat Round	79	0.10	0.079	79	100	2.3
	83	0	<0.05	-		
	84	0.05	0.051	101		
	85	0.05	0.051	101		
Goat Kidney	86	0.20	0.19	97	102	3.8
	90	0	<0.05	-		
	91	0.05	0.050	100		
	92	0.05	0.053	107		
Goat Milk	93	5.00	5.0	100	86	15
	97	0	<0.05	-		
	98	0.05	0.043	87		
	99	0.05	0.05	100		
	100	0.10	0.071	71		

- (1) All samples are from Test Number RI-MV-001-92 - overall mean of 90% with a SD of 11% (n=61)
 (2) Recovery samples were corrected for control residues where present.

TABLE IV. PRECISION OF ANALYTICAL METHOD AG-592 AS DEMONSTRATED BY THE RESULTS OF ANALYZING ¹⁴C-CGA-152005 CONTAINING SUBSTRATES

SUBSTRATE	PROTOCOL 26-92 SAMPLE #	(1) FOUND BY AG-592 (ppm)	MEAN (ppm)	RANGE (ppm)	STANDARD DEVIATION
Poultry Lean Meat	59, 60, 61	0.64, 0.62, 0.65	0.63	0.62-0.65	0.015
Eggs	66, 67, 68	0.099, 0.087, 0.088	0.091	0.087-0.099	0.006
Goat Liver	73, 74, 75	1.37, 1.44, 1.42	1.41	1.37-1.44	0.037
Goat Oriental Fat	80, 81, 82	0.082, 0.081, 0.084	0.082	0.081-0.084	0.002
Goat Round	87, 88, 89	0.21, 0.23, 0.22	0.22	0.21-0.23	0.008
Goat Kidney	94, 95, 96	5.4, 5.5, 5.3	5.4	5.3-5.5	0.053
Goat Milk	101, 102, 103	0.055, 0.052, 0.037	0.048	0.037-0.055	0.010

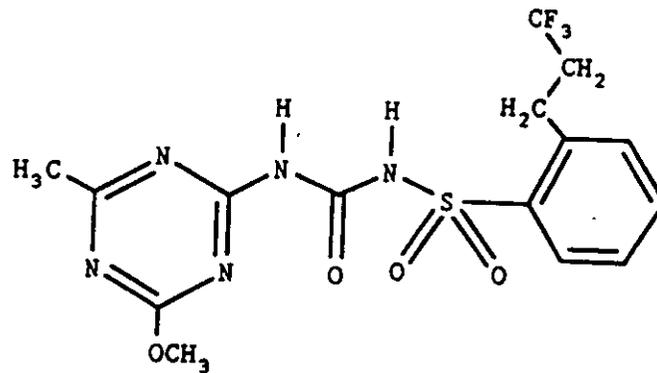
(1) Corrected by % recovery from fresh fortification as shown by Table III for the fresh fortification concentration nearest that of the total ¹⁴C residues.

TABLE V. SUMMARY TABLE FOR EXTRACTABILITY, ANALYSIS, AND ACCOUNTABILITY OF ¹⁴C-CGA-152005 TREATED SUBSTRATES USING METHOD AG-592

A SUBSTRATE	B SAMPLE CODE	C (1) TOTAL RESIDUE	D EXTRACTABILITY		E (3) % EXT	F ANALYSIS		G HPLC		J (7) ACCOUNTABILITY %
			(2) ppm	(4) FINAL FRACTION ¹⁴ C (ppm)		(5) FRACTION ¹⁴ C CORRECTED (ppm)	(6) FINAL FRACTION (ppm)	(5) FINAL FRACTION CORRECTED (ppm)		
Poultry Lean Meat	1191260507	0.705	0.82, 0.82, 0.69	110	0.55, 0.53, 0.55	0.60, 0.58, 0.60	0.58, 0.56, 0.59	0.64, 0.62, 0.65	90	
Eggs	1191260533	0.326	0.24, 0.21, 0.24	72	0.083, 0.081, 0.082	0.096, 0.093, 0.095	0.085, 0.075, 0.076	0.099, 0.087, 0.088	28	
Goat Liver	G90480033	1.371	1.44, 1.38, 1.53	106	1.17, 1.21, 1.22	1.17, 1.21, 1.22	1.37, 1.44, 1.42	1.37, 1.44, 1.42	103	
Goat Omental Fat	G90480024	0.058	0.080, 0.088, 0.090	148	0.064, 0.062, 0.068	0.080, 0.078, 0.086	0.065, 0.064, 0.067	0.082, 0.081, 0.084	142	
Goat Round	G90480031	0.209	0.25, 0.24, 0.24	116	0.20, 0.20, 0.20	0.20, 0.21, 0.21	0.21, 0.22, 0.22	0.21, 0.23, 0.22	106	
Goat Kidney	G90480026	5.048	4.7, 5.2, 5.6	103	4.7, 4.7, 4.6	4.7, 4.7, 4.6	5.4, 5.5, 5.3	5.4, 5.5, 5.3	107	
Goat Milk	G90480012	0.069	0.054, 0.055, 0.055	79	0.025, 0.025, 0.015	0.035, 0.036, 0.22	0.039, 0.037, 0.026	0.055, 0.052, 0.037	70	

- (1) As determined by combustion analysis in metabolism studies^{2,3}
- (2) Determined by liquid scintillation counting of aliquots of the extract from Section III C.1.1 of AG-592
- (3) Average of three extractions shown in column D divided by the Total Residue shown in Column C, times 100.
- (4) Determined by liquid scintillation counting of aliquots of the final fraction from Section II C.3.5
- (5) Corrected by % Recovery from fresh fortification as shown in Table III for the fresh fortification concentration nearest that of the total ¹⁴C residue
- (6) Determined by high performance liquid chromatography.
- (7) Average of Column I, divided by Column C, and multiplied by 100

FIGURE 1. STRUCTURE AND CHEMICAL NAME OF
CGA-152005



CGA-152005

N-[[(4-Methoxy-6-methyl-1,3,5-triazin-2-yl) amino]
carbonyl]-2-(3,3,3-trifluoropropyl)-
benzenesulfonamide

FIGURE 2. ANALYTICAL PROCEDURE FLOWCHART FOR AG-592

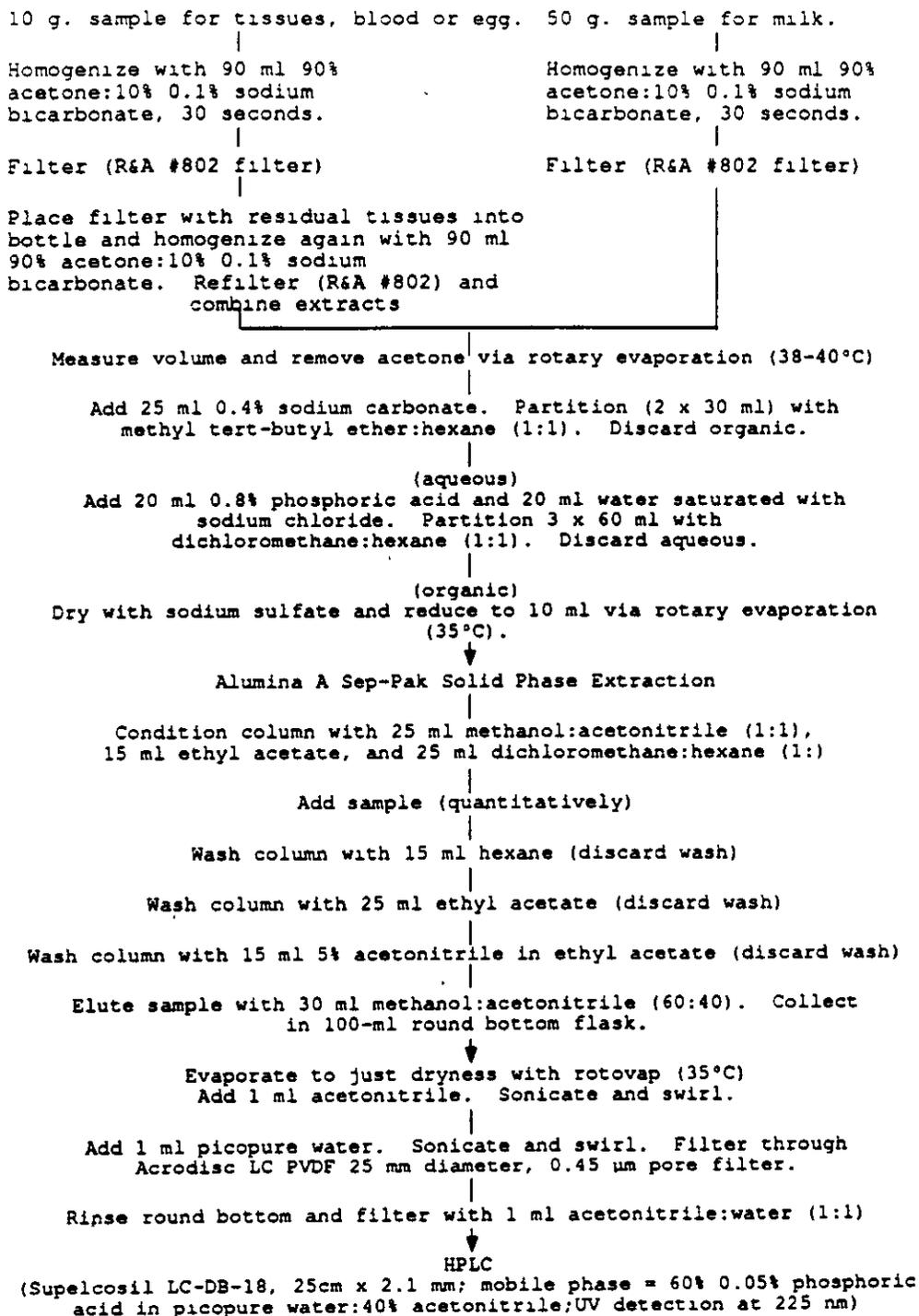
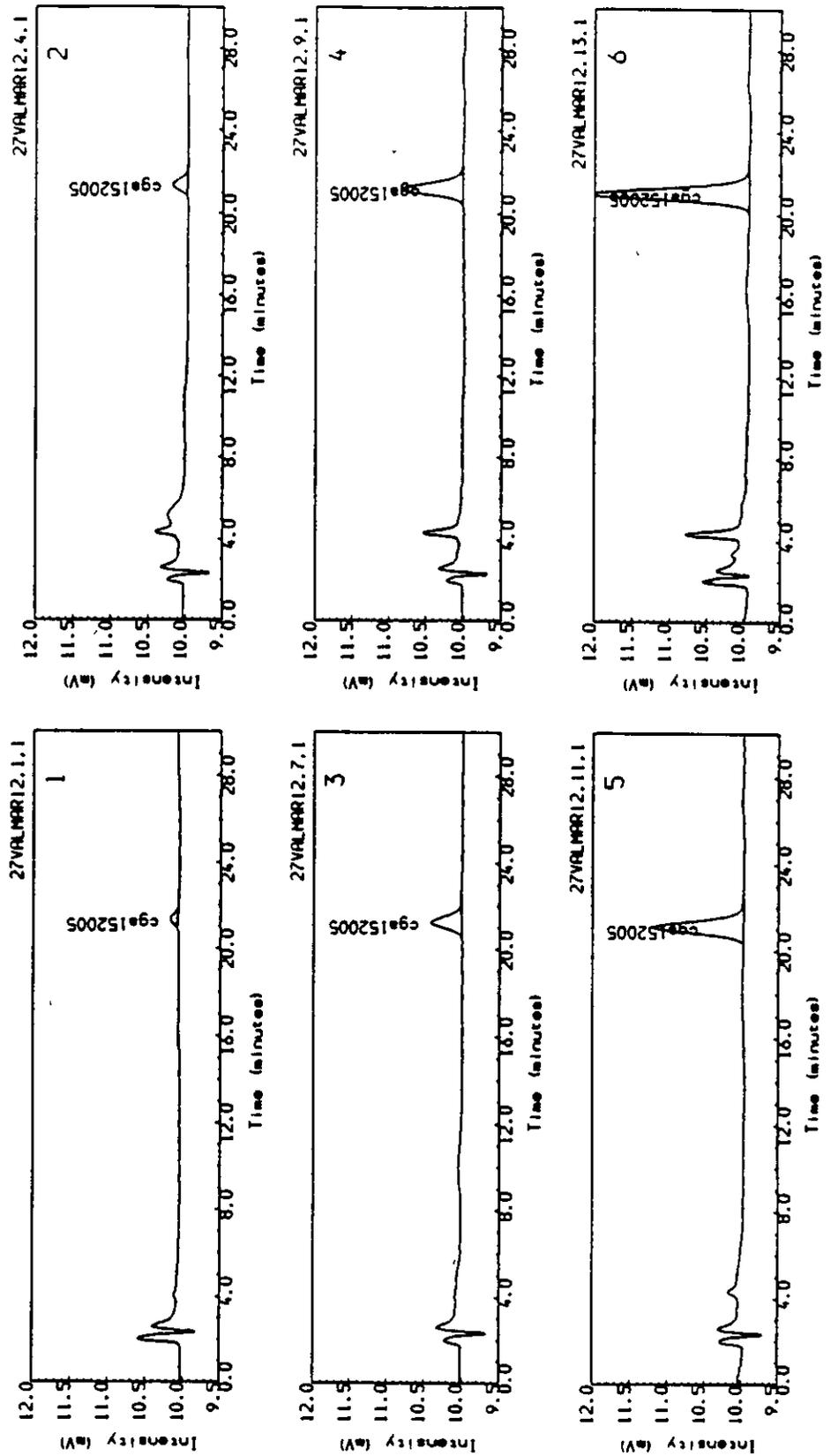
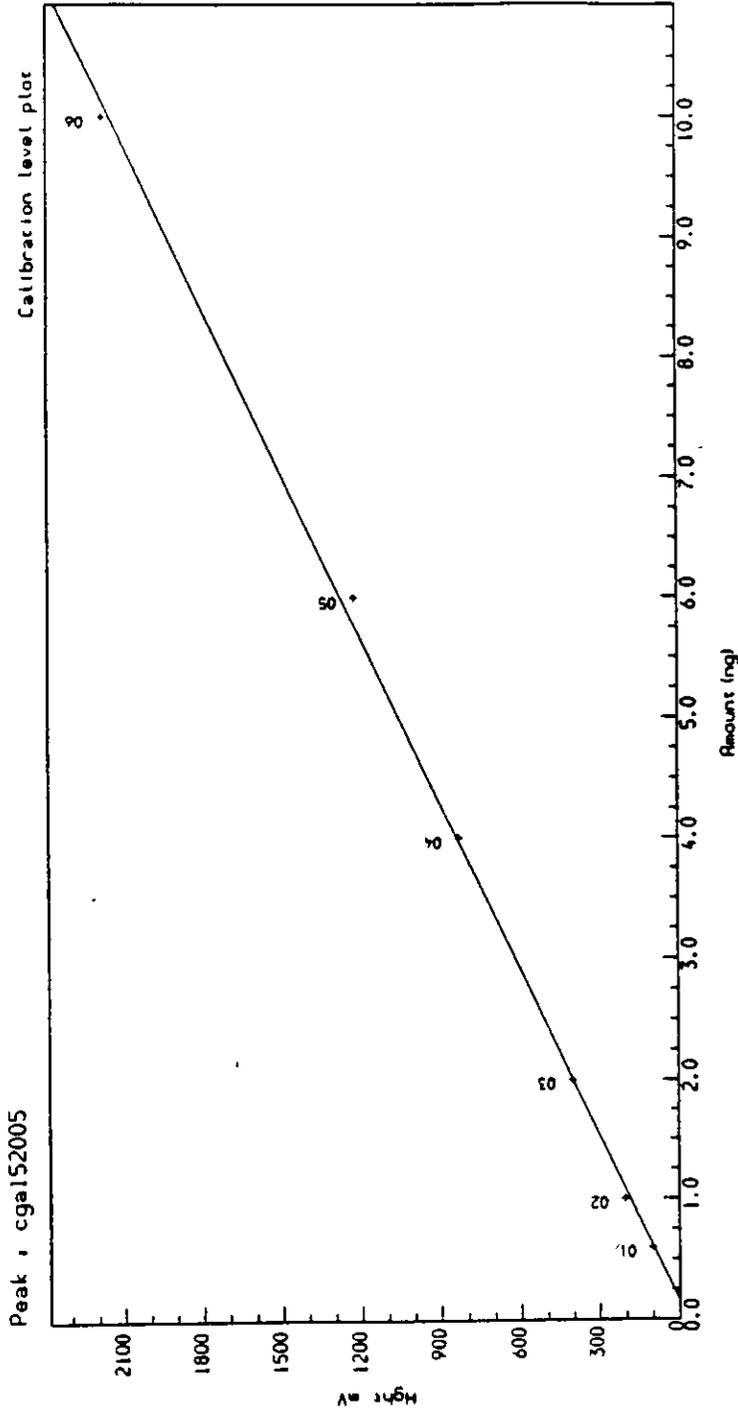


Figure 3. Typical Standard Chromatograms (From Poultry Lean Meat Analyses).
Quantity Injected in ng CGA-152005 (1) 0.60 (2) 1.0 (3) 2.0
(4) 4.0 (5) 6.0 (6) 10.0.



C-G Multichrom 1.8 (BONES)

Calibration Name : 27 VALMAR12.

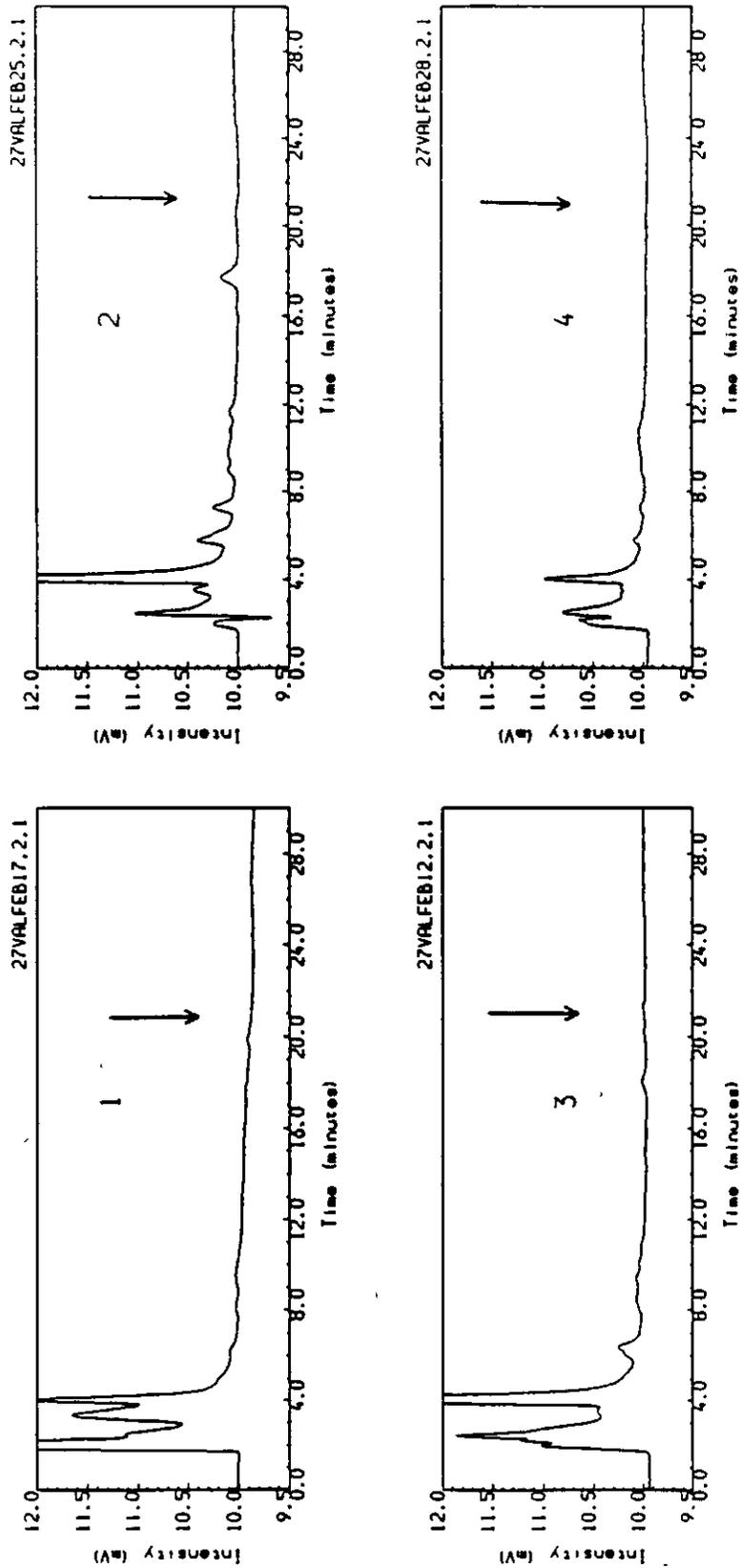


Curve fit : Linear
Correlation coefficient : 0.99938
Standard error : 3.08029E+1
Reported on 13-MAR-1992 at 07.17

Constant : -3.385802E+1
1st degree : 2.17901E+2

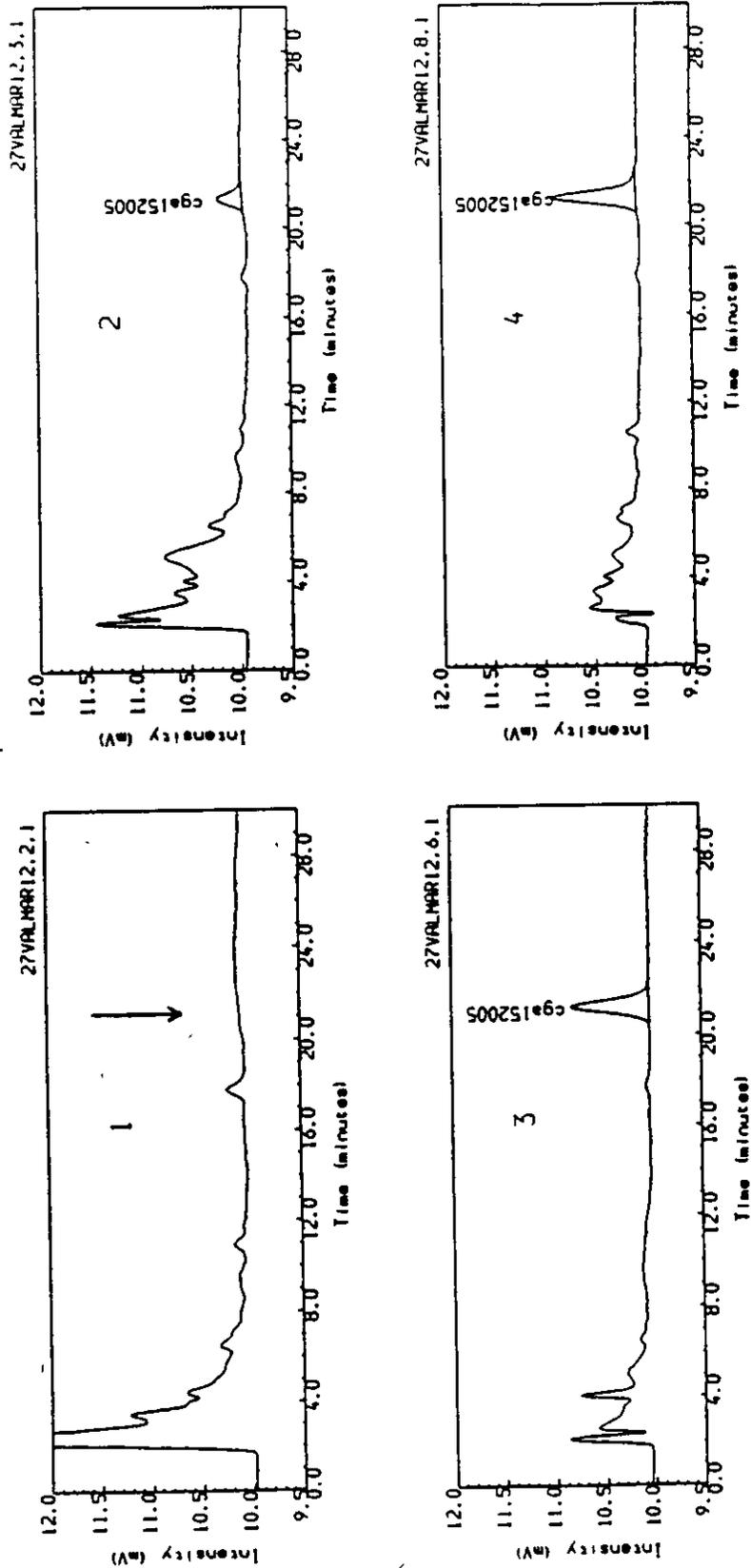
FIGURE 4. CALIBRATION PLOT OF THE STANDARDS OBTAINED FROM THE ANALYSIS OF ROULTRY LEAN MEAT

Figure 5. Chromatograms Of The Reagent Blanks Obtained During The Analyses Of (1) Poultry Liver, (2) Poultry Peritoneal Fat, (3) Beef Tenderloin, and (4) Beef Blood.



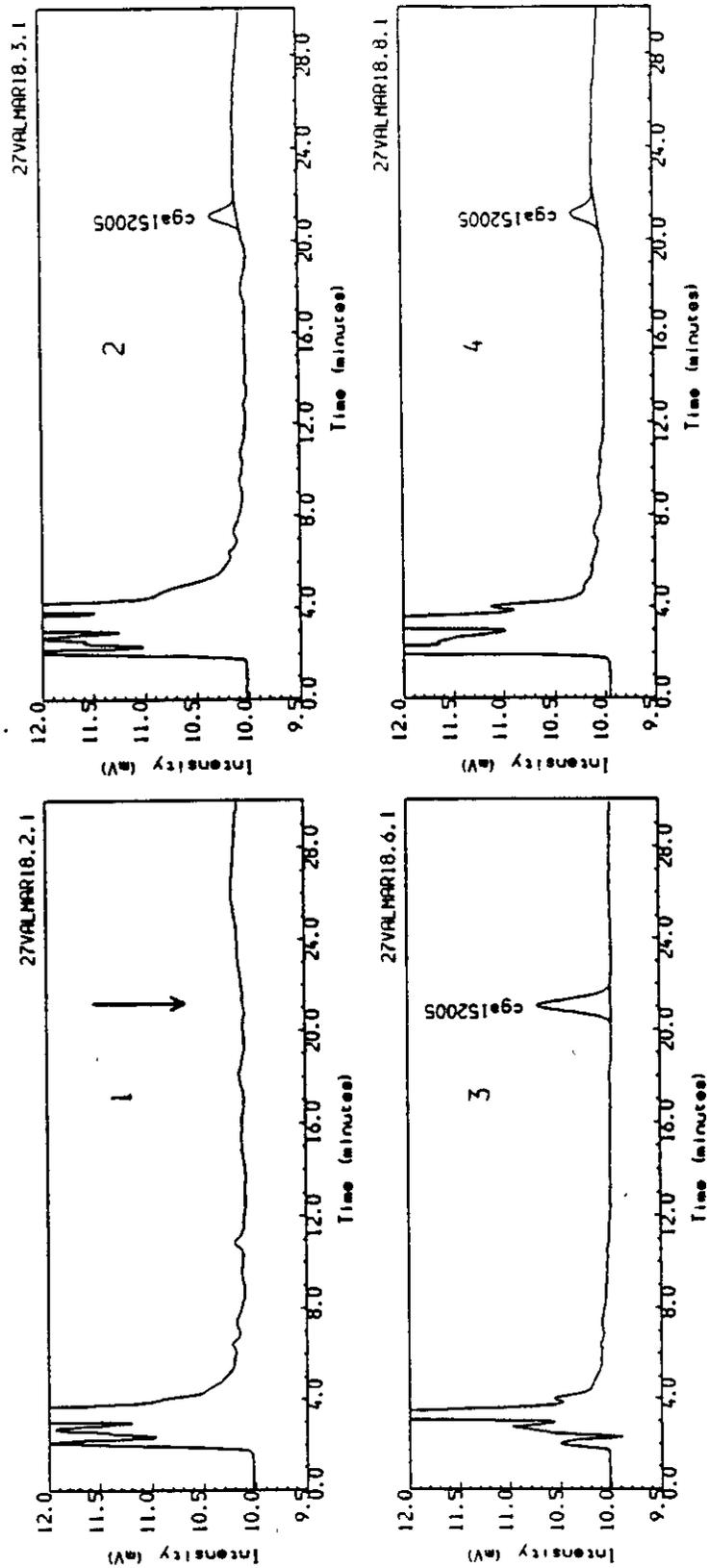
1. 28.77 mg equivalent injected, (0.60 ng CGA-152005, (0.050 ppm
 2. 28.77 mg equivalent injected, (0.60 ng CGA-152005, (0.050 ppm
 3. 29.30 mg equivalent injected, (0.60 ng CGA-152005, (0.050 ppm
 4. 28.42 mg equivalent injected, (0.60 ng CGA-152005, (0.050 ppm

Figure 6. Representative Chromatograms From The Analysis Of Radiolabelled CGA-152005 Containing Poultry Lean Meat Using Method AG-592.



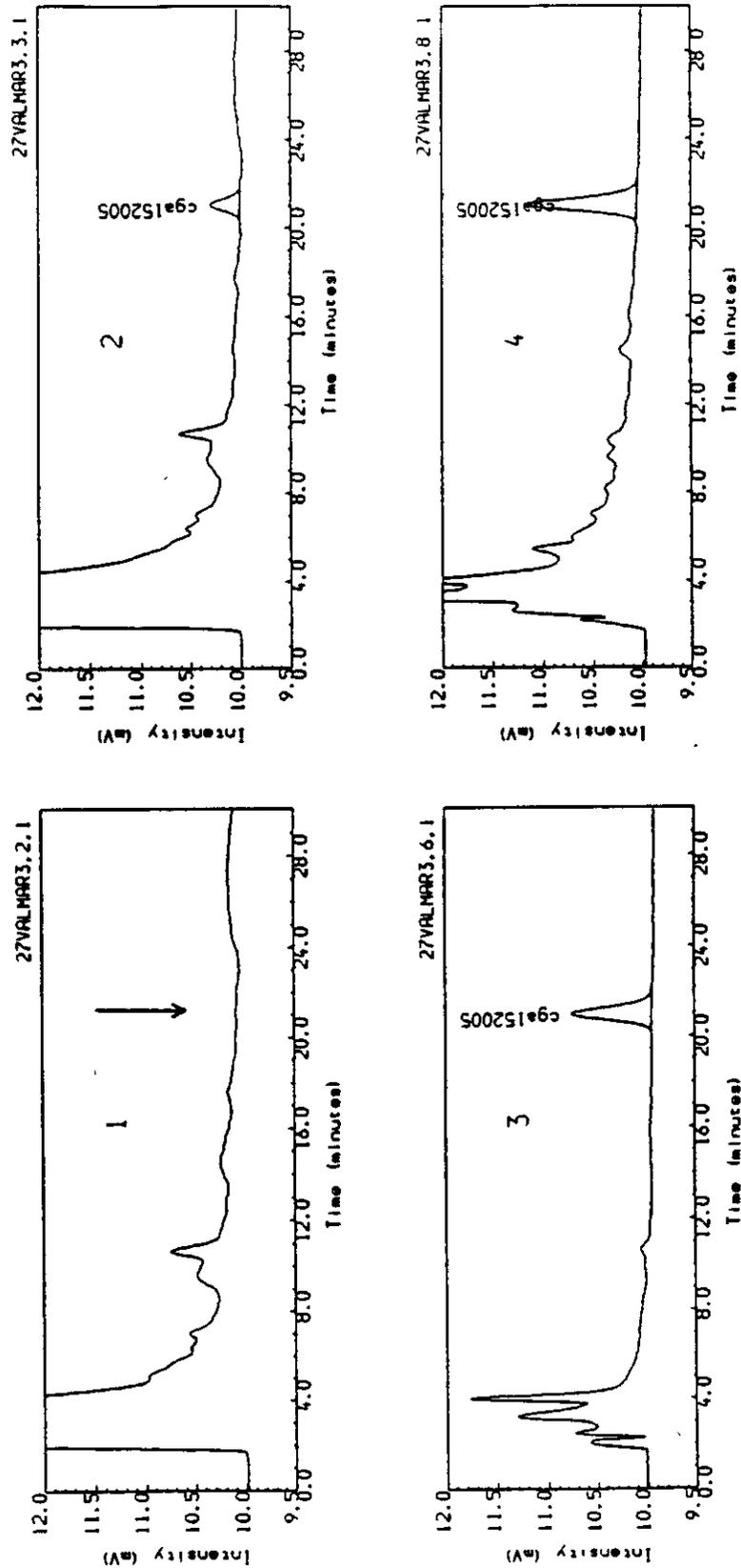
1. Control, 27.31 mg injected, (0.60 ng CGA-152005, 0.050 ppm found)
2. 0.050 ppm fortification, 27.31 mg injected, 1.33 ng CGA-152005, 0.049 ppm found, 97 % Recovery.
3. 0.50 ppm fortification, 8.30 mg injected, 3.79 ng CGA-152005, 0.46 ppm found, 91 % Recovery.
4. 0.71 ppm as ¹⁴C-CGA-152005, 6.81 mg injected, 3.97 ng CGA-152005, 0.58 ppm found, 83 % Accountability.

Figure 7. Representative Chromatograms From The Analysis Of Radiolabelled CGA-152005 Containing Eggs Using Method AG-592.



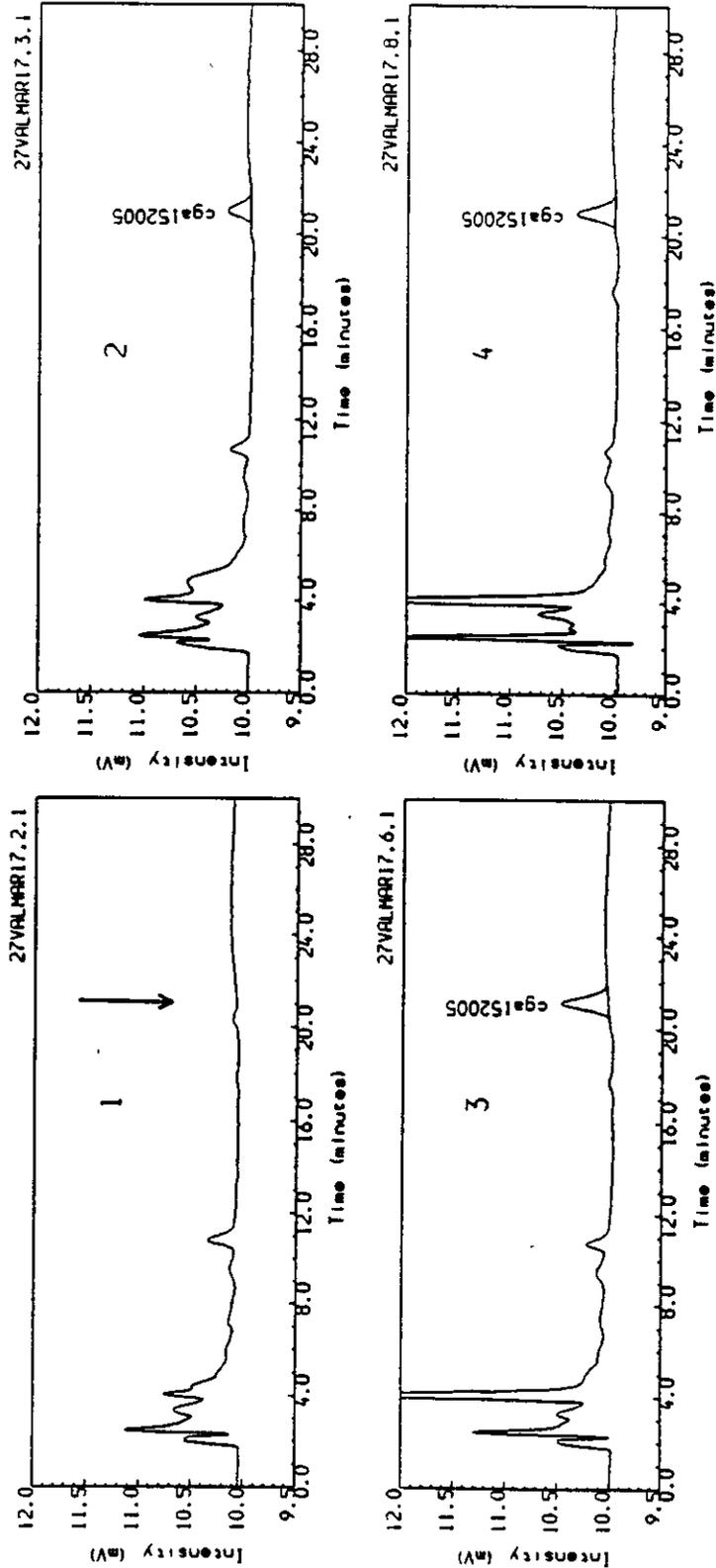
1. Control, 28.37 mg injected, 0.60 ng CGA-152005, 0.050 ppm
2. 0.050 ppm fortification, 28.37 mg injected, 1.27 ng CGA-152005, 0.045 ppm found, 89 % Recovery.
3. 0.50 ppm fortification, 8.51 mg injected, 3.67 ng CGA-152005, 0.43 ppm found, 86 % Recovery.
4. 0.326 ppm as 14C-CGA-152005, 14.35 mg injected, 1.22 ng CGA-152005, 0.085 ppm found, 26 % Accountability.

Figure 8. Representative Chromatograms From The Analysis Of Radiolabelled CGA-152005 Containing Goat Liver Using Method AG-592.



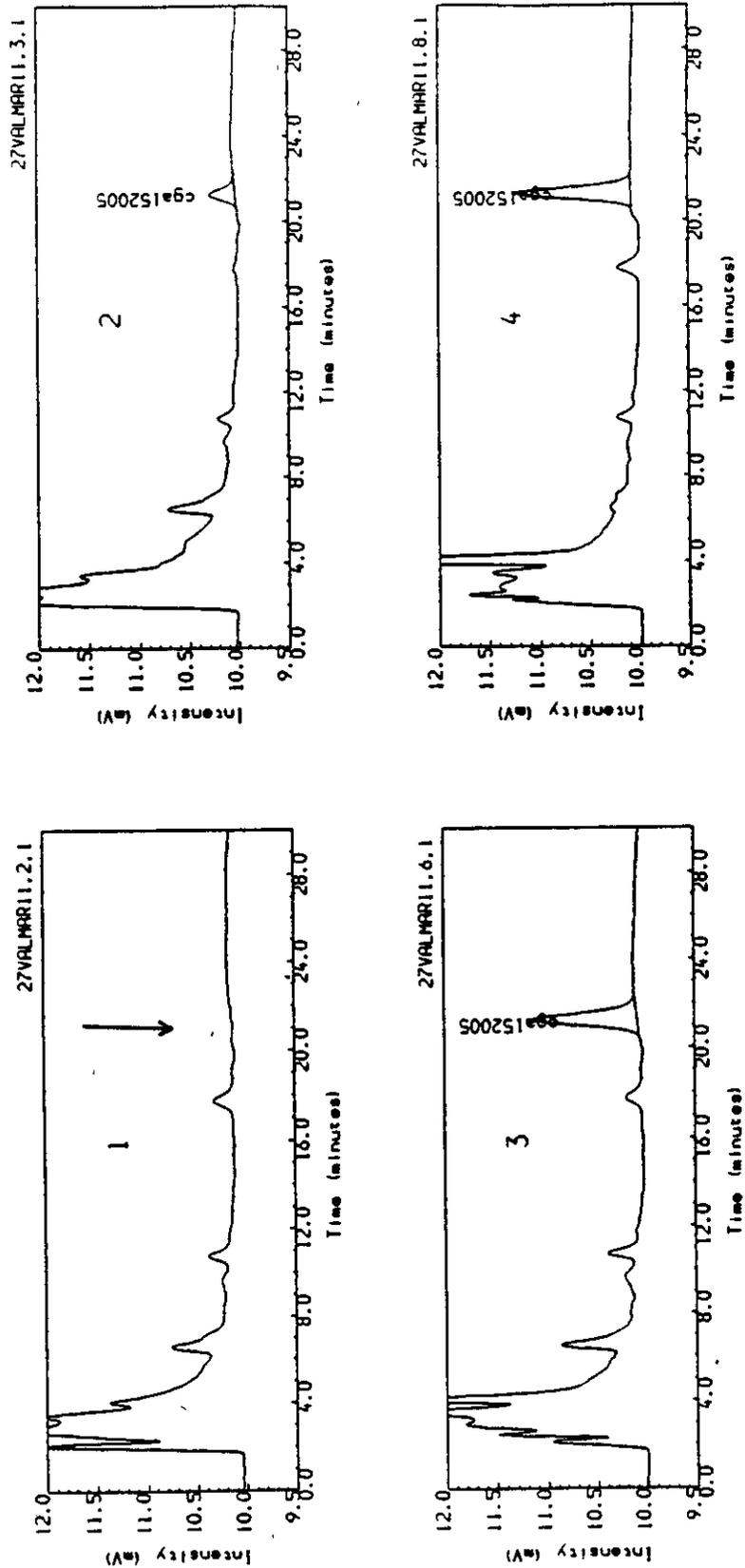
- 1. Control, 27.31 mg injected, 0.60 ng CGA-152005, 0.050 ppm
- 2. 0.050 ppm fortification, 27.31 mg injected, 1.48 ng CGA-152005, 0.054 ppm found, 108 % Recovery.
- 3. 1.00 ppm fortification, 4.12 mg injected, 4.21 ng CGA-152005, 1.02 ppm found, 102 % Recovery.
- 4. 1.37 ppm as 14C-CGA-152005, 4.20 mg injected, 5.76 ng CGA-152005, 1.37 ppm found, 100% Accountability.

Figure 9. Representative Chromatograms From The Analysis Of Radiolabelled CGA-152005 Containing Goat Omental Fat Using Method AG-592.



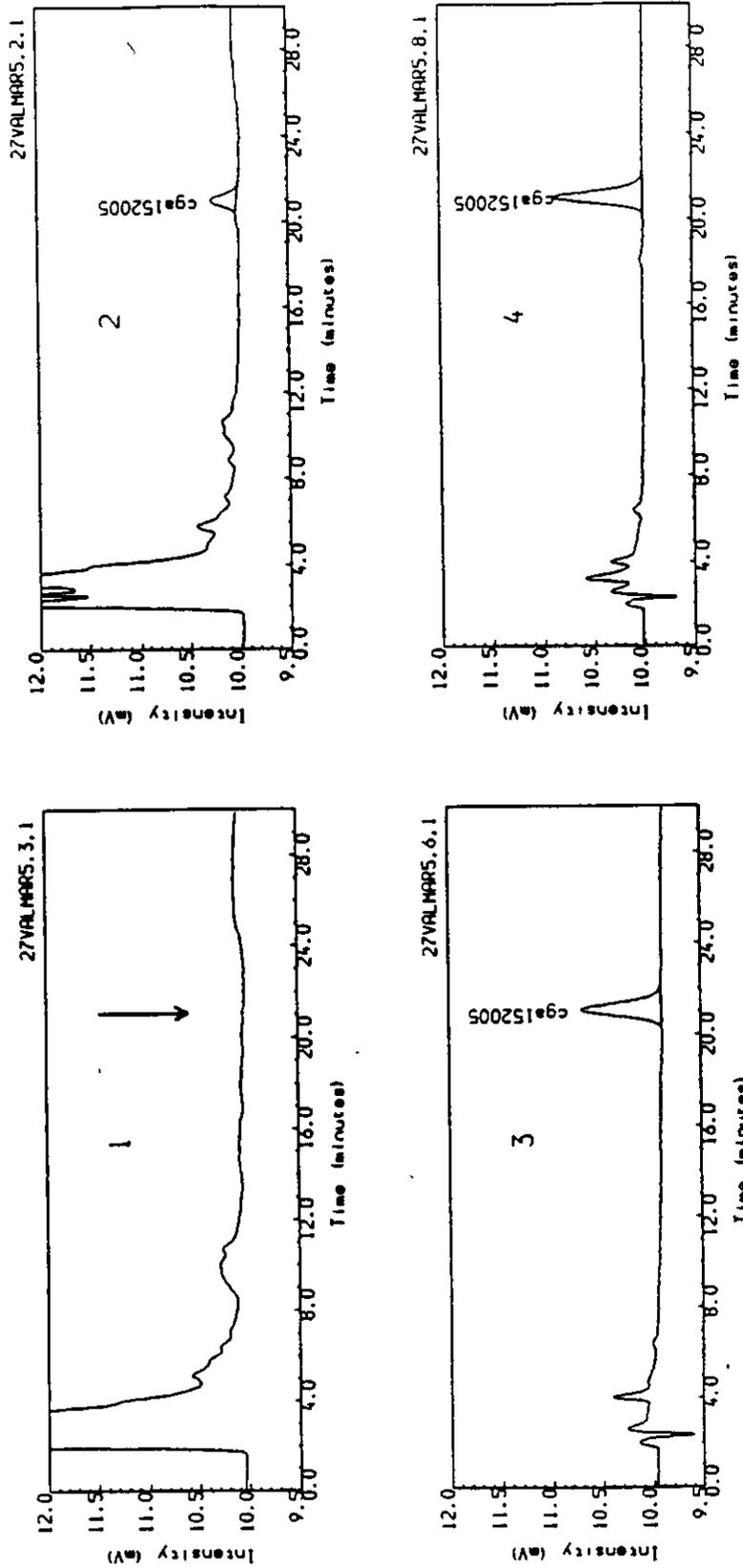
- 1. Control, 27.04 mg injected, 0.60 ng CGA-152005, 0.050 ppm
- 2. 0.050 ppm fortification, 27.59 mg injected, 0.99 ng CGA-152005, 0.036 ppm found, 72 % Recovery.
- 3. 0.10 ppm fortification, 27.04 mg injected, 2.15 ng CGA-152005, 0.079 ppm found, 79 % Recovery.
- 4. 0.058 ppm as 14C-CGA-152005, 26.85 mg injected, 1.74 ng CGA-152005, 0.065 ppm found, 112 % Accountability.

Figure 10. Representative Chromatograms From The Analysis Of Radiolabelled CGA-152005 Containing Goat Round Using Method AG-592.



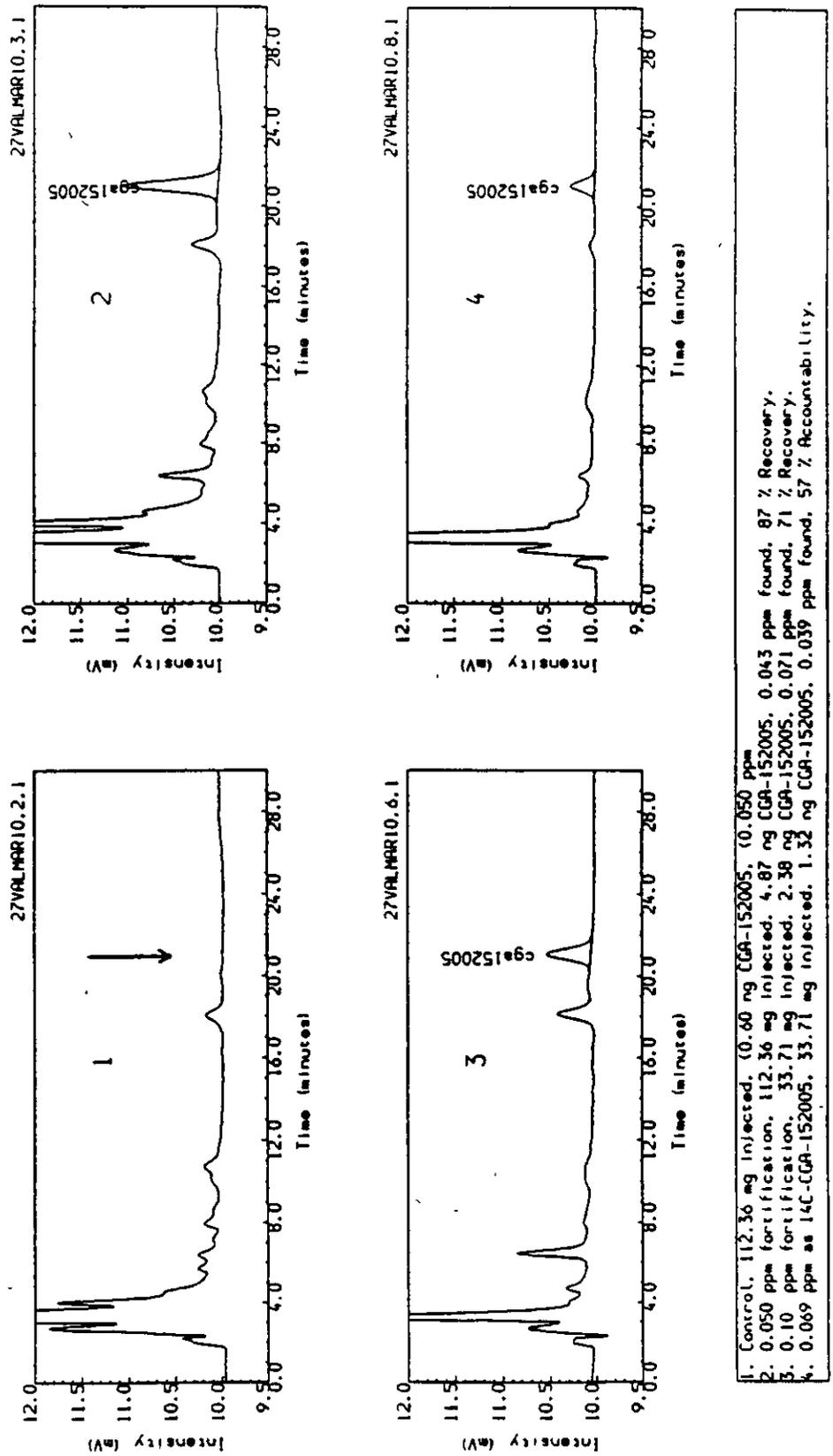
- 1. Control, 26.95 mg injected, 0.60 ng CGA-152005, 0.050 ppm
- 2. 0.050 ppm fortification, 26.95 mg injected, 1.36 ng CGA-152005, 0.051 ppm found, 101 % Recovery.
- 3. 0.20 ppm fortification, 26.95 mg injected, 5.23 ng CGA-152005, 0.19 ppm found, 97 % Recovery.
- 4. 0.21 ppm 14C-CGA-152005, 26.95 mg injected, 5.66 ng CGA-152005, 0.21 ppm found, 99% Accountability.

Figure 11. Representative Chromatograms From The Analysis Of Radiolabelled CGA-152005 Containing Goat Kidney Using Method AG-592.



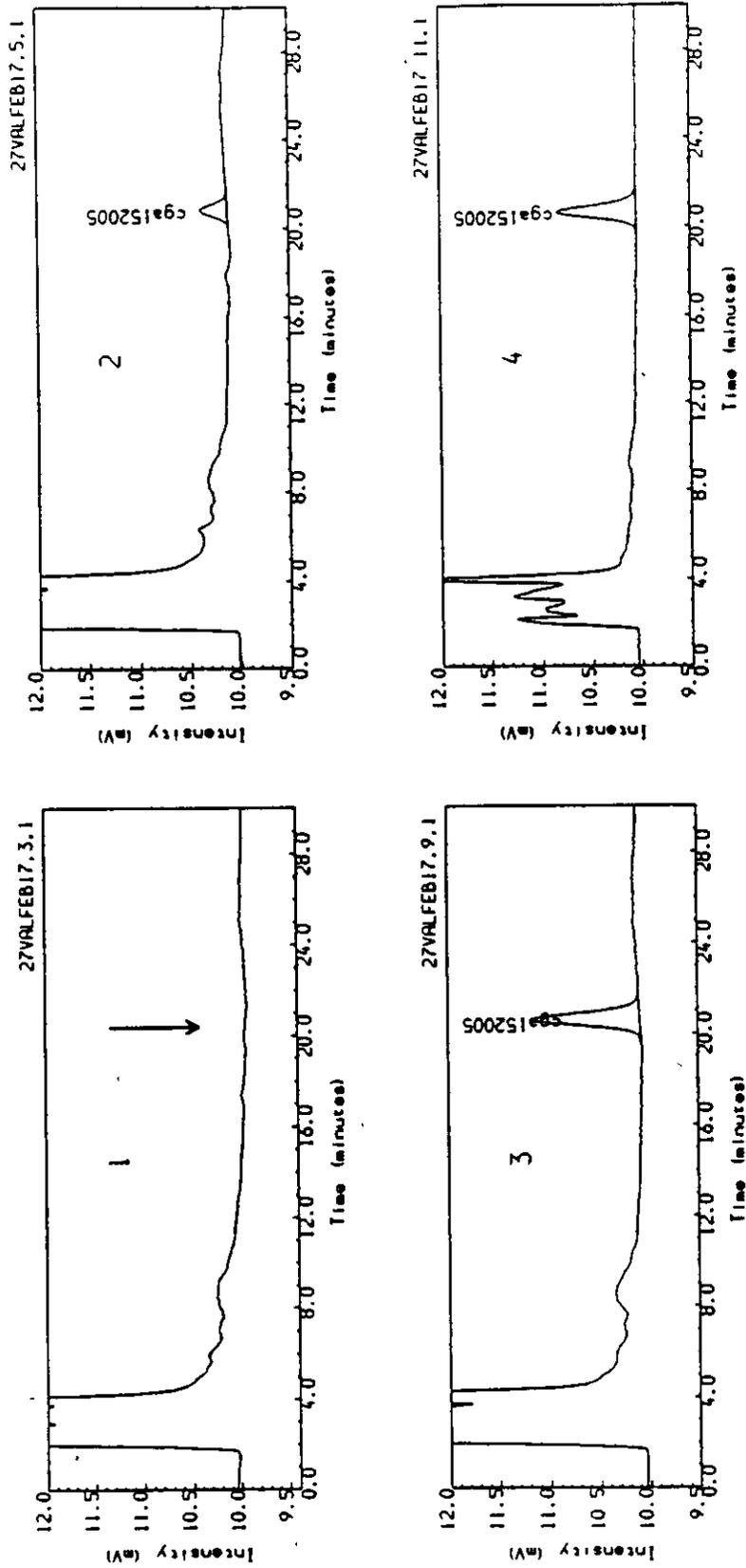
- 1. Control. 27.31 mg injected. (0.60 ng CGA-152005. (0.050 ppm
- 2. 0.050 ppm fortification. 27.66 mg injected. 1.38 ng CGA-152005. 0.050 ppm found. 100 % Recovery.
- 3. 5.00 ppm fortification. 0.82 mg injected. 4.14 ng CGA-152005. 5.02 ppm found. 100 % Recovery.
- 4. 5.05 ppm as 14C-CGA-152005. 0.84 mg injected. 4.55 ng CGA-152005. 5.41 ppm found. 107 % Accountability.

Figure 12. Representative Chromatograms From The Analysis Of Radiolabelled CGA-152005 Containing Goat Milk Using Method AG-592.



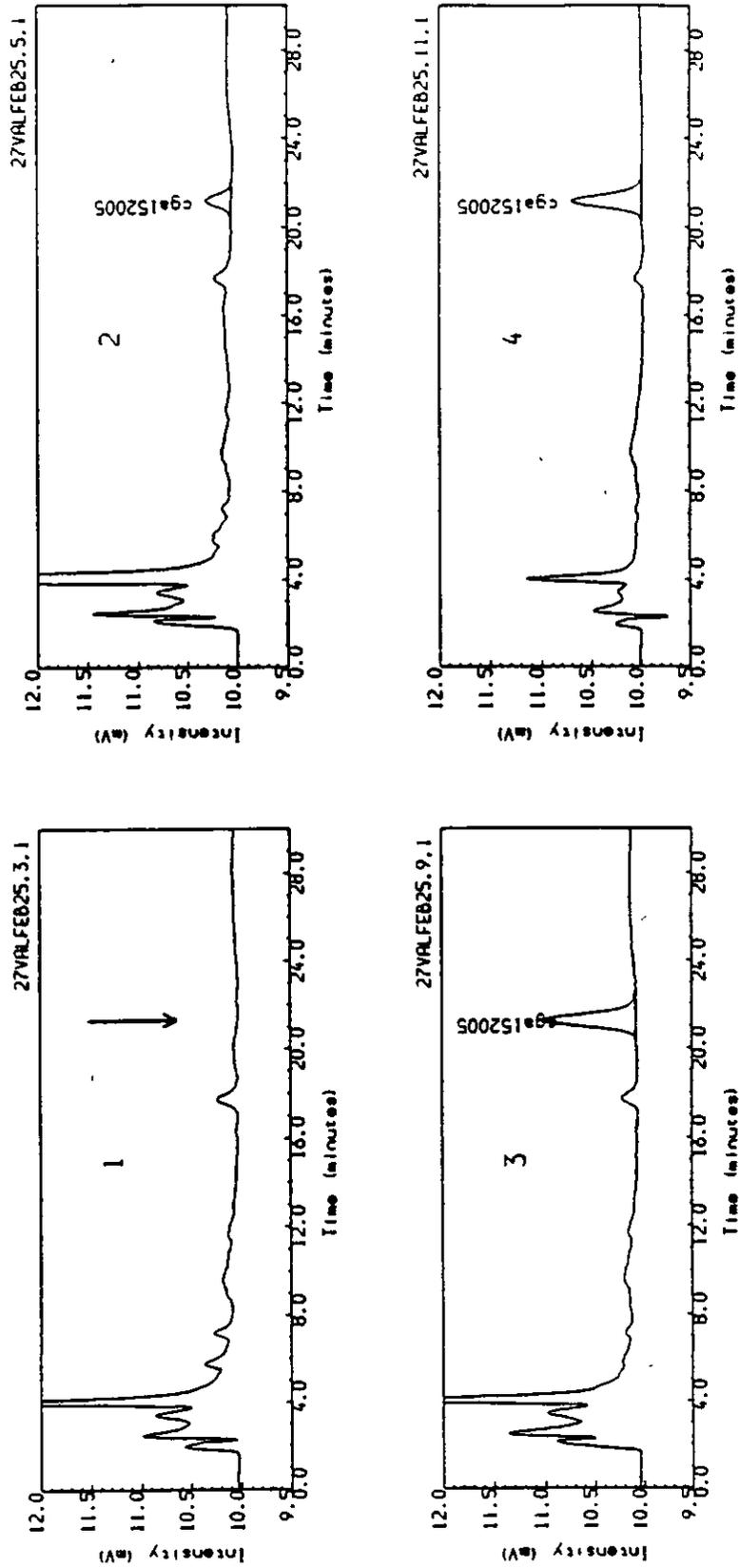
1. Control, 112.36 mg injected, 0.60 ng CGA-152005, 0.050 ppm
 2. 0.050 ppm fortification, 112.36 mg injected, 4.87 ng CGA-152005, 0.043 ppm found, 87 % Recovery.
 3. 0.10 ppm fortification, 33.71 mg injected, 2.38 ng CGA-152005, 0.071 ppm found, 71 % Recovery.
 4. 0.069 ppm as 14C-CGA-152005, 33.71 mg injected, 1.32 ng CGA-152005, 0.039 ppm found, 57 % Accountability.

Figure 13. Representative Chromatograms From The Analysis Of Poultry Liver For CGA-152005 Using Method AG-592.



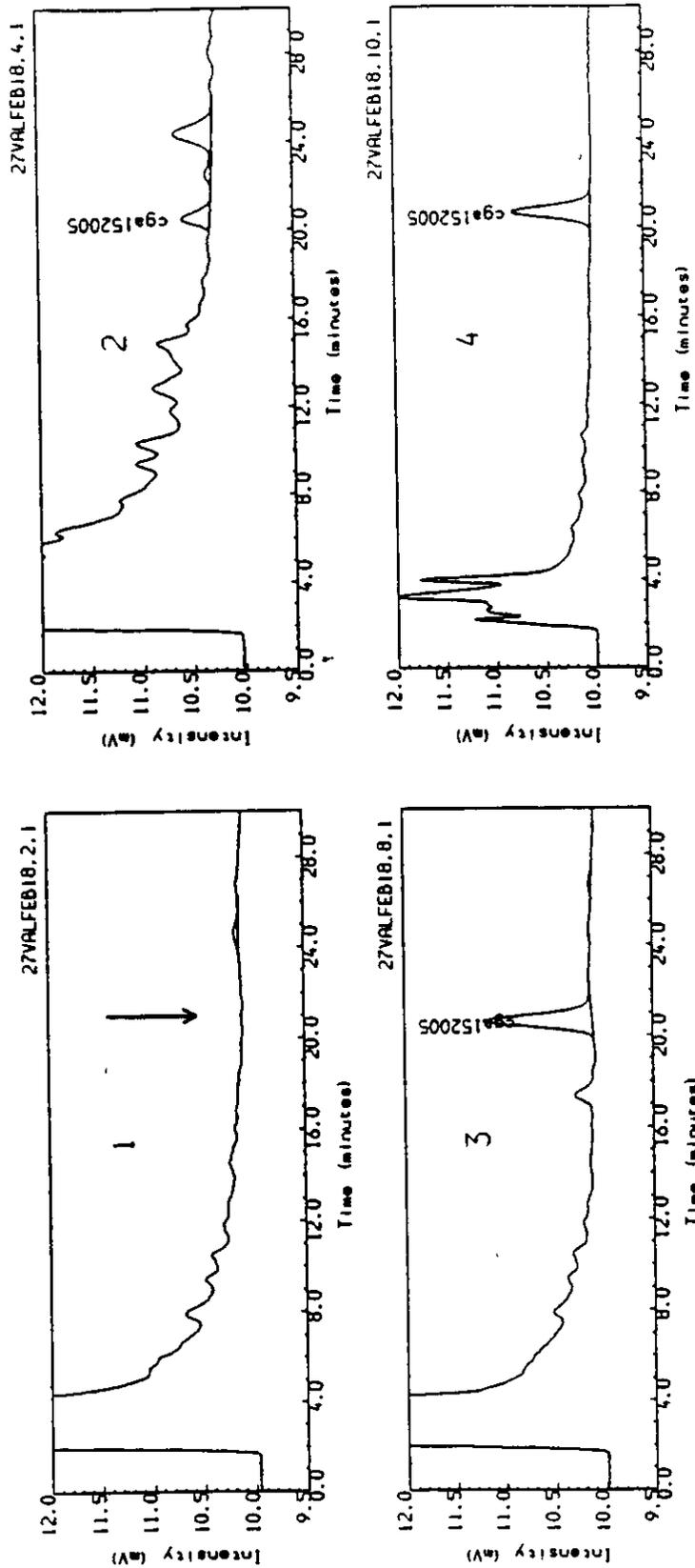
1. Control, 28.01 mg injected, 0.60 ng CGA-152005, 0.050 ppm
 2. 0.050 ppm fortification, 27.66 mg injected, 1.62 ng CGA-152005, 0.051 ppm found, 103 % Recovery.
 3. 0.20 ppm fortification, 28.19 mg injected, 5.61 ng CGA-152005, 0.20 ppm found, 99 % Recovery.
 4. 0.50 ppm fortification, 8.40 mg injected, 4.05 ng CGA-152005, 0.48 ppm found, 96 % Recovery.

Figure 14. Representative Chromatograms From The Analysis Of Poultry Peritoneal Fat For CGA-152005 Using Method AG-592.



- 1. Control, 28.89 mg injected, 0.60 ng CGA-152005, 0.050 ppm
- 2. 0.050 ppm fortification, 29.82 mg injected, 1.32 ng CGA-152005, 0.044 ppm found, 89 % Recovery.
- 3. 0.20 ppm fortification, 28.70 mg injected, 5.04 ng CGA-152005, 0.18 ppm found, 88 % Recovery.
- 4. 0.50 ppm fortification, 8.94 mg injected, 3.73 ng CGA-152005, 0.42 ppm found, 83 % Recovery.

Figure 15. Representative Chromatograms From The Analysis of Beef Liver For CGA-152005 Using Method AG-592.



- 1. Control. 26.95 mg injected, 0.60 ng CGA-152005, 0.050 ppm found
- 2. 0.050 ppm fortification, 27.66 mg injected, 1.47 ng CGA-152005, 0.053 ppm found, 106 % Recovery
- 3. 0.20 ppm fortification, 27.66 mg injected, 5.59 ng CGA-152005, 0.20 ppm found, 101 % Recovery
- 4. 0.50 ppm fortification, 27.66 mg injected, 13.98 ng CGA-152005, 0.49 ppm found, 99 % Recovery

Figure 16. Representative Chromatograms From The Analysis Of Beef Kidney For CGA-152005 Using Method AG-592.

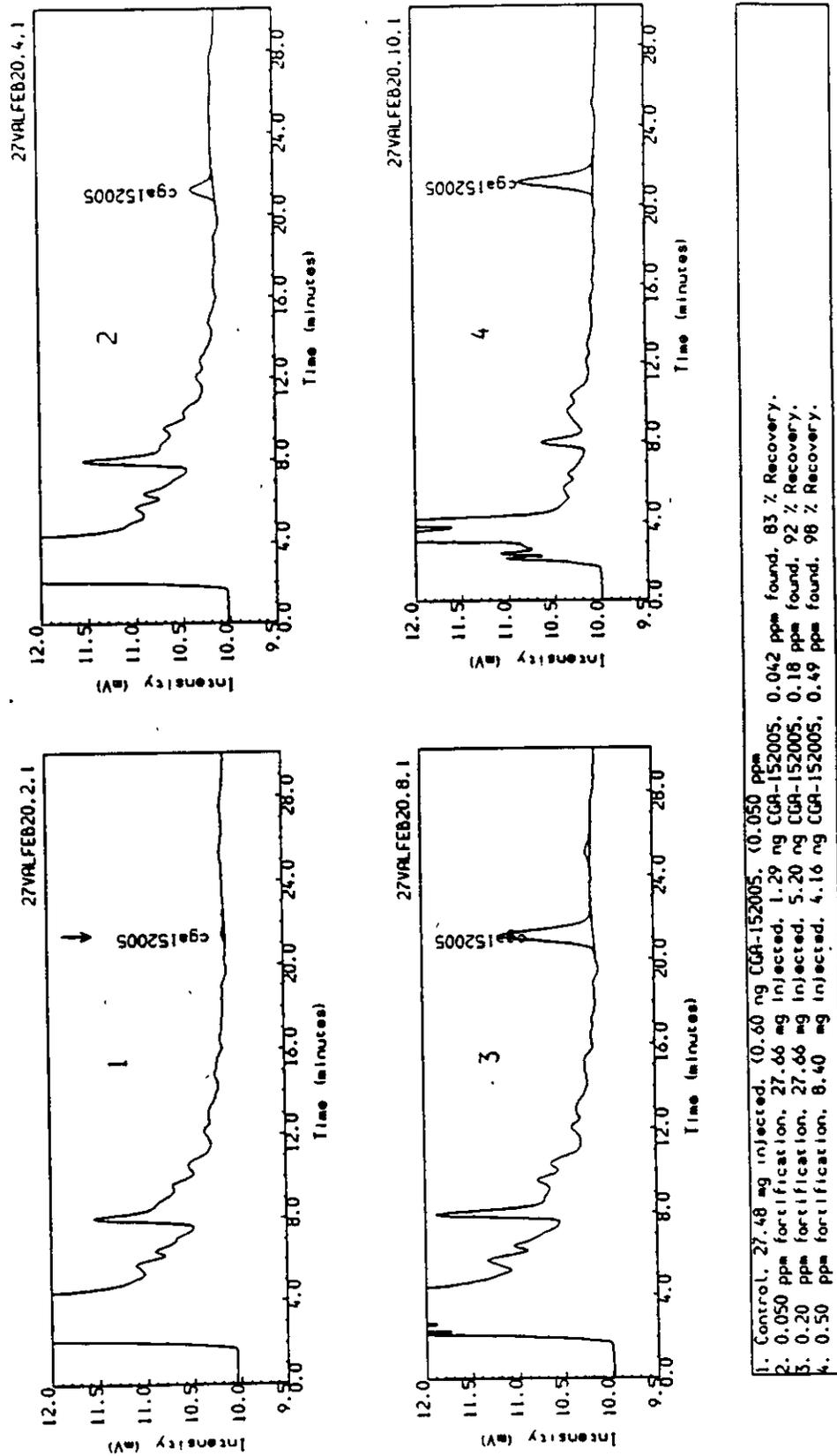
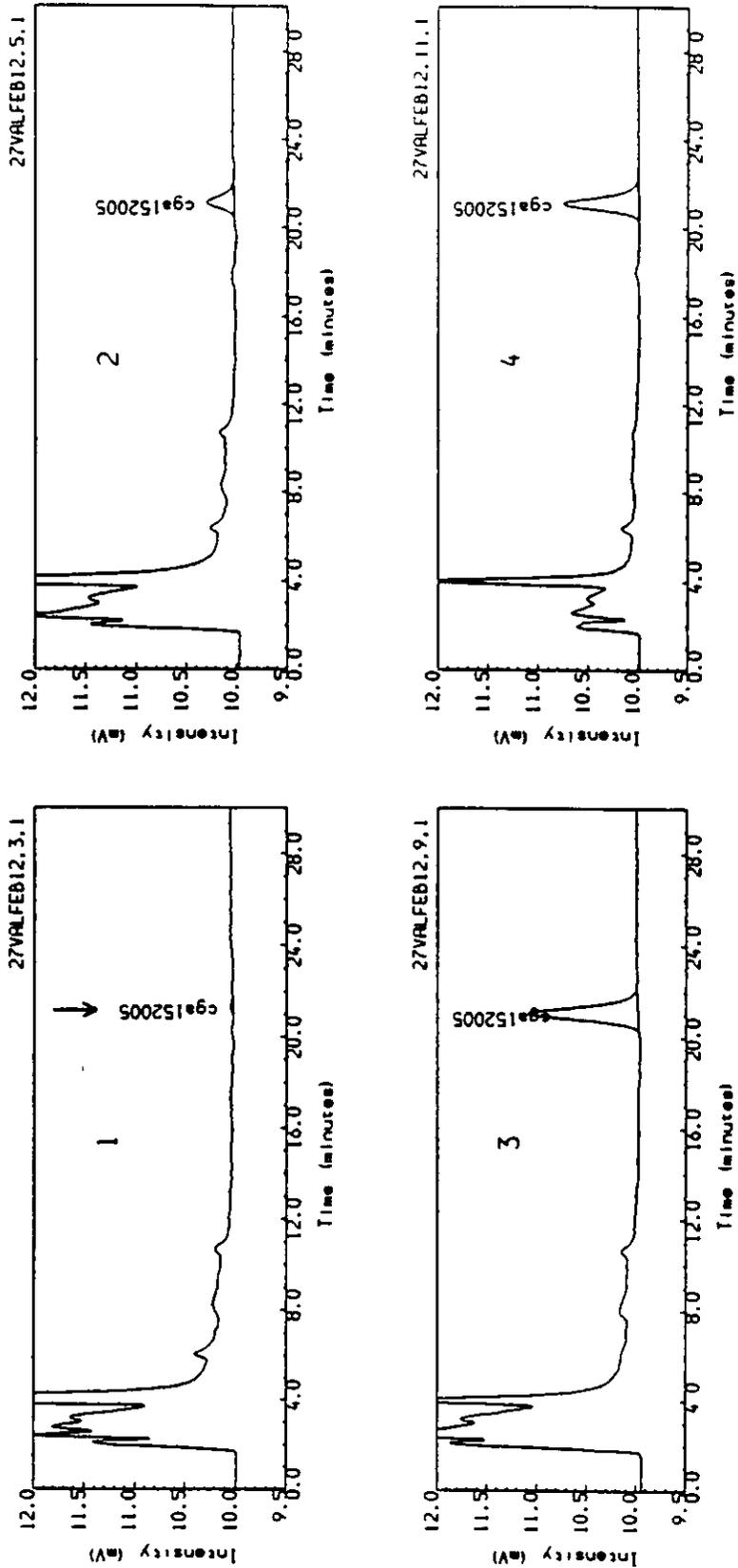


Figure 17. Representative Chromatograms From The Analysis Of Beef Tenderloin For CGA-152005 Using Method AG-592.



- 1. Control, 28.01 mg injected, 0.60 ng CGA-152005, 0.050 ppm
- 2. 0.050 ppm fortification, 28.72 mg injected, 1.34 ng CGA-152005, 0.047 ppm found, 93 % Recovery.
- 3. 0.20 ppm fortification, 28.55 mg injected, 6.14 ng CGA-152005, 0.22 ppm found, 108 % Recovery.
- 4. 0.50 ppm fortification, 8.51 mg injected, 4.11 ng CGA-152005, 0.48 ppm found, 97 % Recovery.

Figure 18. Representative Chromatograms From The Analysis Of Beef Round
 For CGA-152005 Using Method AG-592.

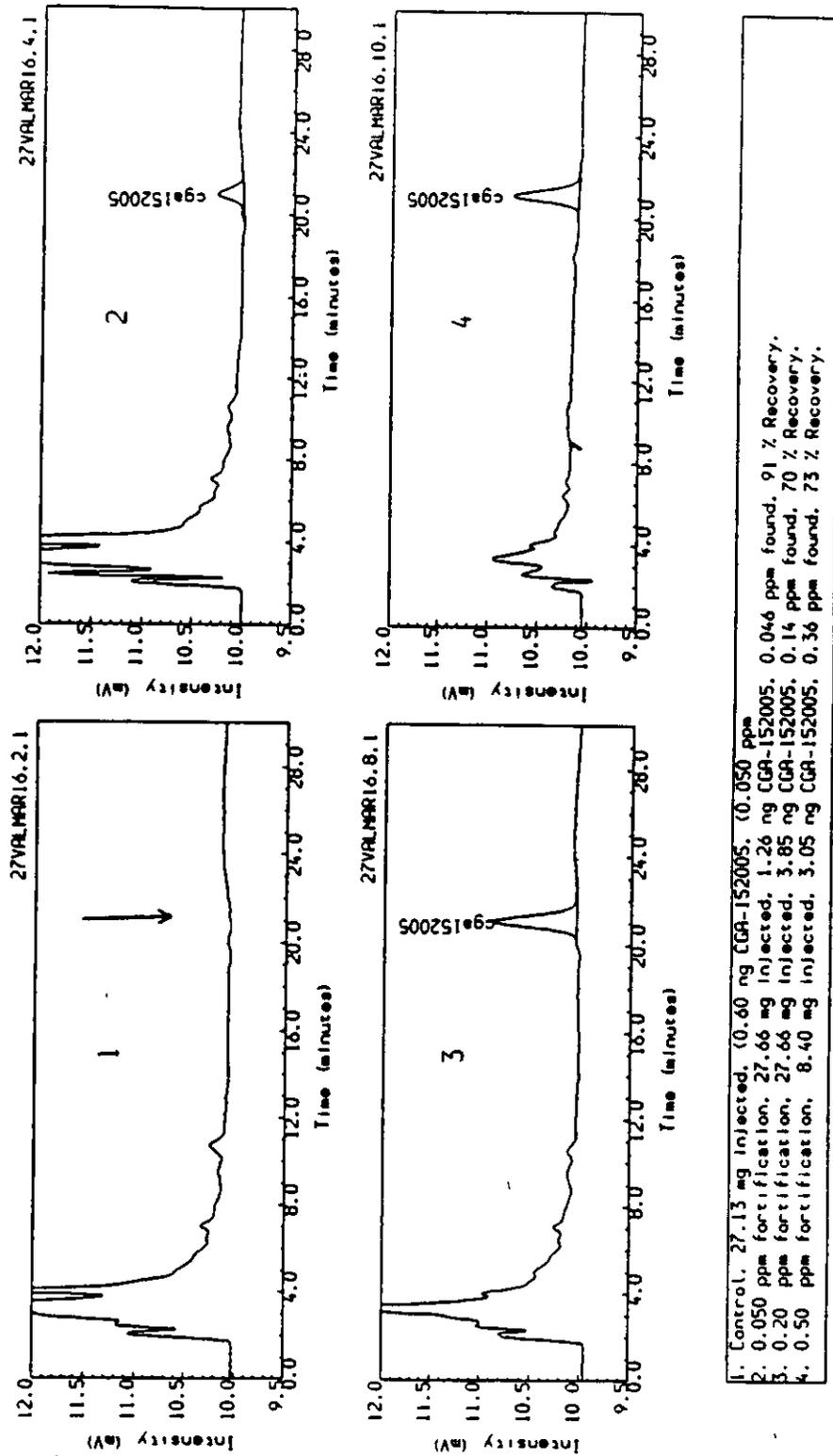
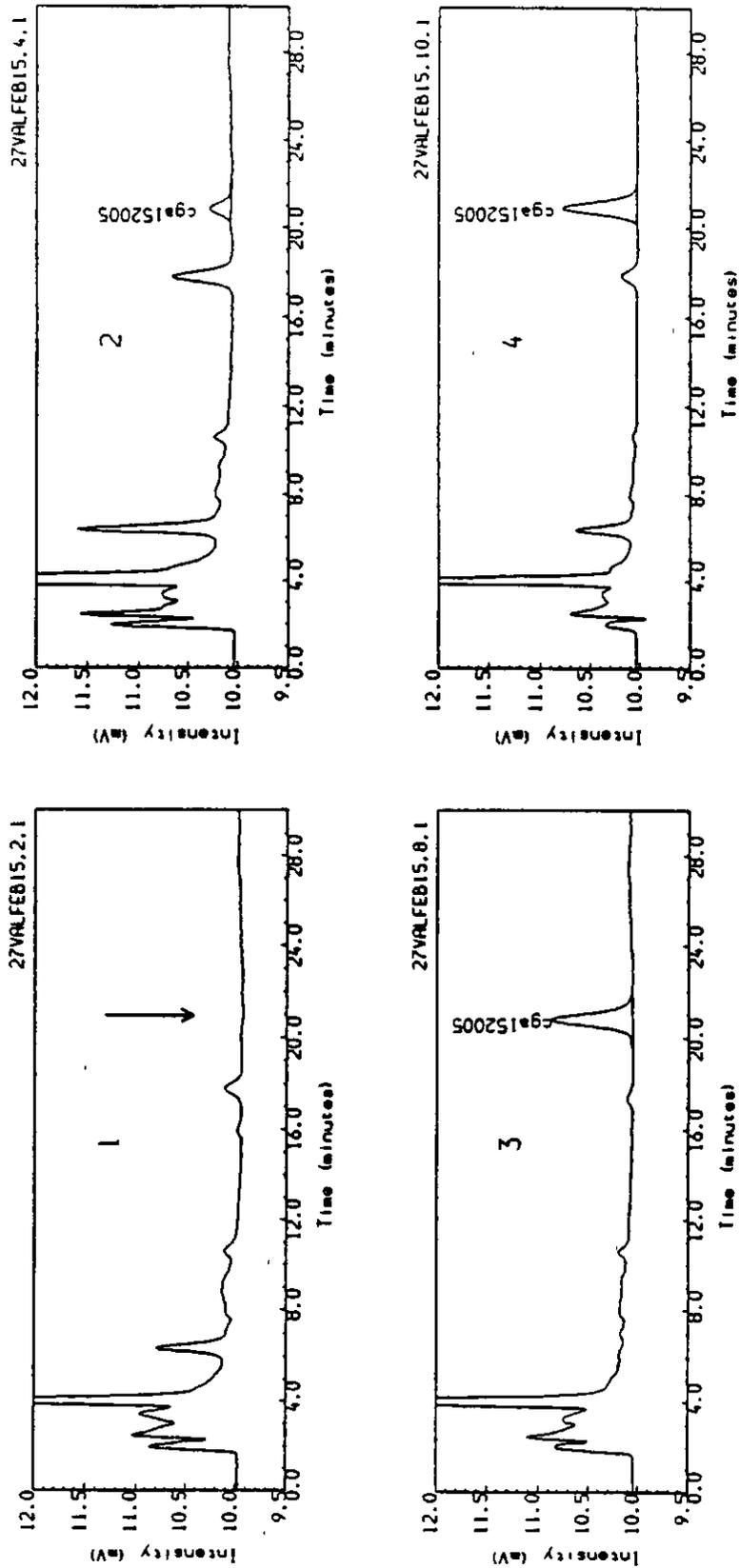


Figure 19. Representative Chromatograms From The Analysis of Beef Omental Fat For CGA-152005 Using Method AG-592.



1. Control, 27.41 mg injected, 0.60 ng CGA-152005, 0.050 ppm
2. 0.050 ppm fortification, 27.41 mg injected, 1.13 ng CGA-152005, 0.041 ppm found, 82 % Recovery.
3. 0.20 ppm fortification, 27.41 mg injected, 4.25 ng CGA-152005, 0.16 ppm found, 77 % Recovery.
4. 0.50 ppm fortification, 8.56 mg injected, 3.90 ng CGA-152005, 0.46 ppm found, 91 % Recovery.

Figure 20. Representative Chromatograms From The Analysis Of Beef Perirenal Fat For CGA-152005 Using Method AG-592.

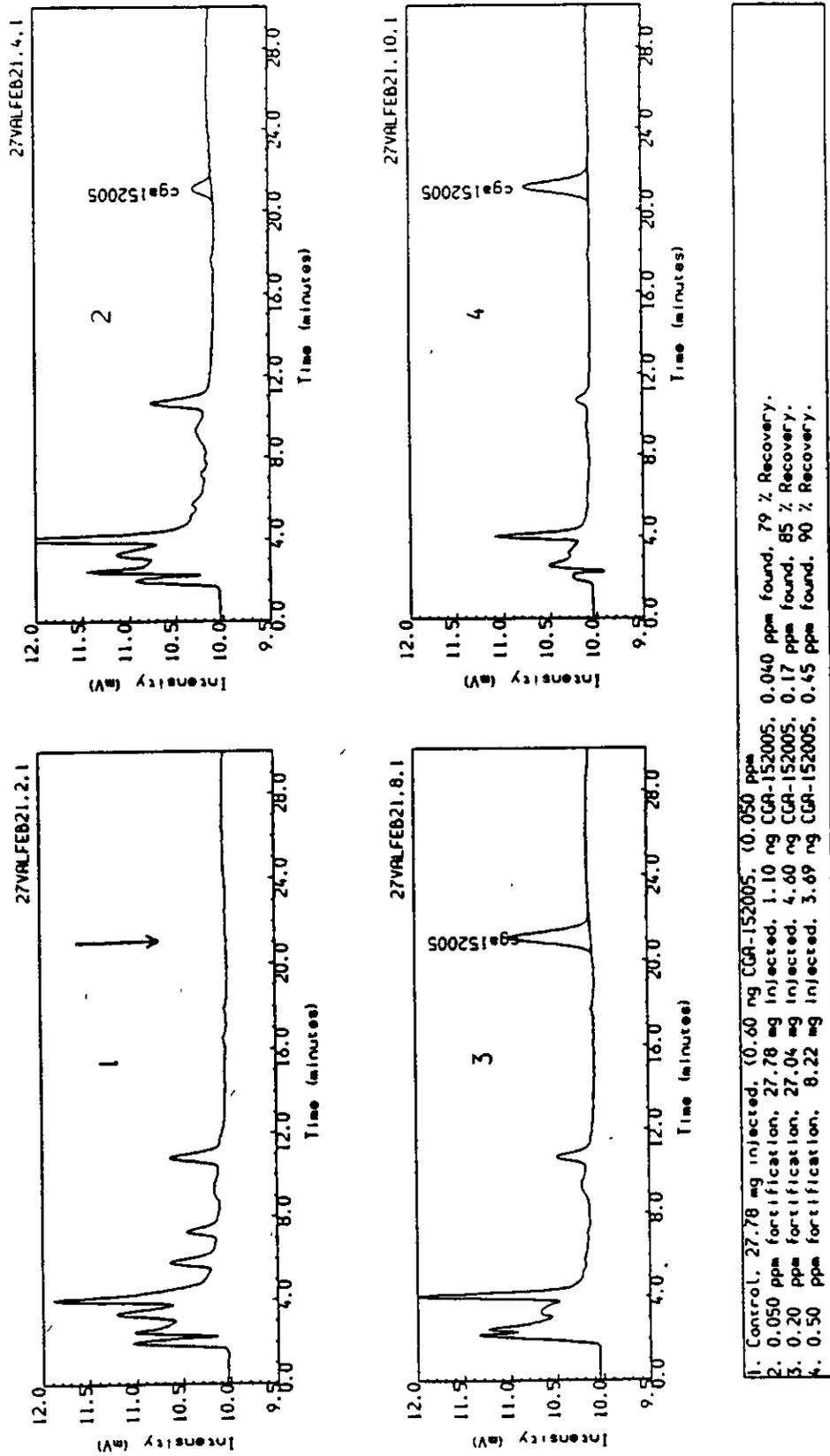


Figure 21. Representative Chromatograms From The Analysis Of Beef Blood For CGA-152005 Using Method AG-592.

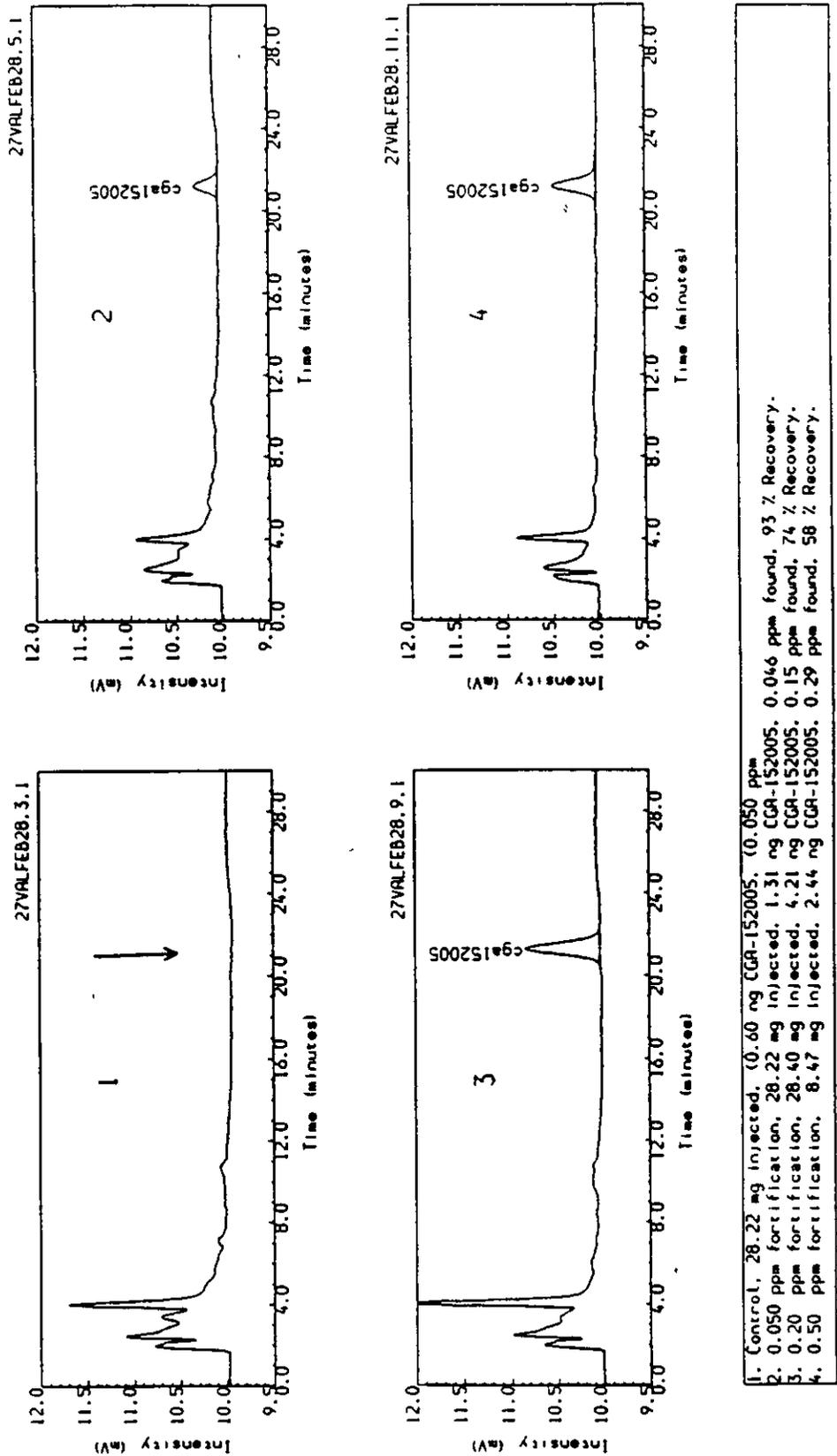
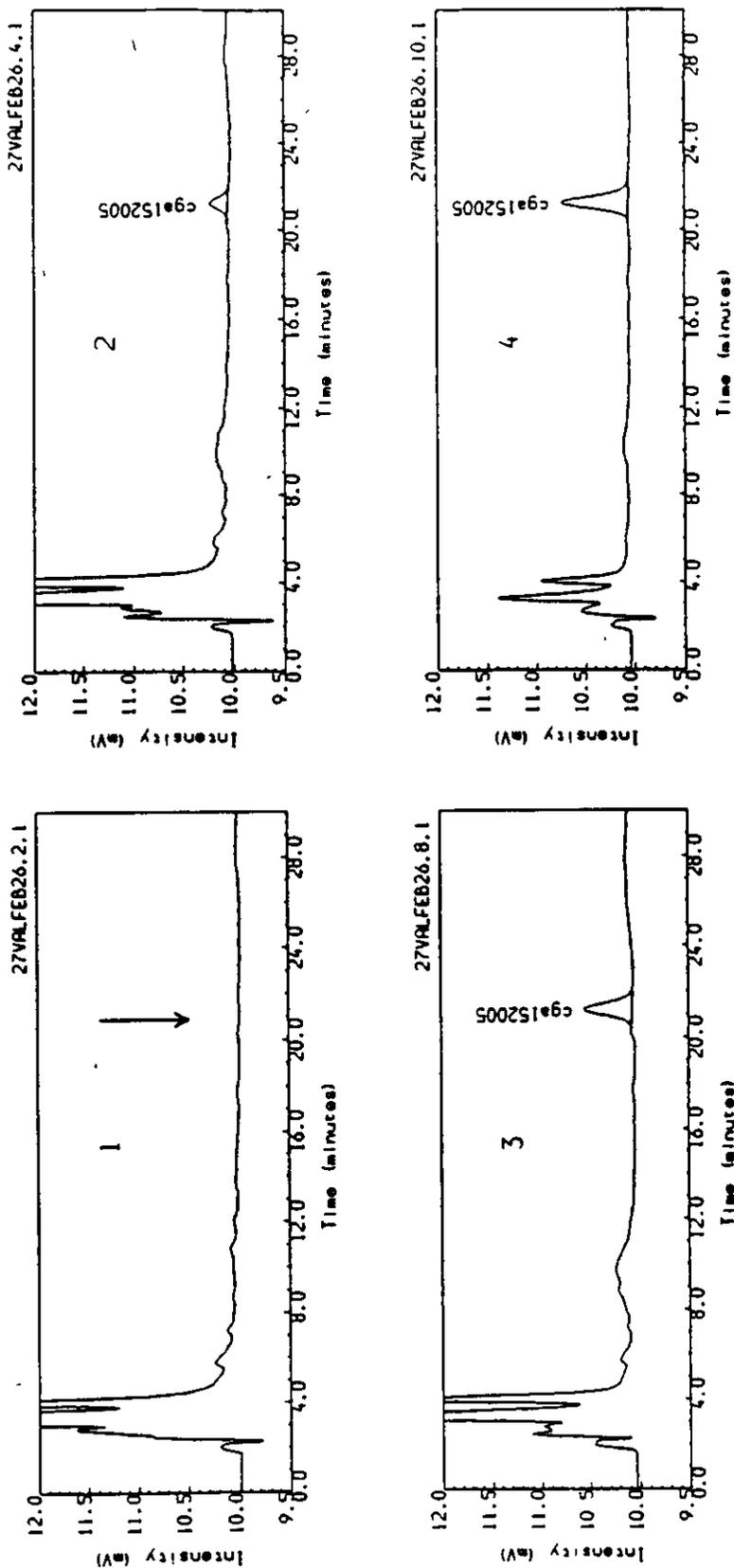


Figure 22. Representative Chromatograms From The Analysis Of Dairy Milk For CGA-152005 Using Method AG-592.



- 1. Control, 112.36 mg injected, 0.60 ng CGA-152005 (0.010 ppm)
- 2. 0.010 ppm fortification, 112.36 mg injected, 0.93 ng CGA-152005, 0.008 ppm found, 82 % Recovery.
- 3. 0.10 ppm fortification, 29.72 mg injected, 2.49 ng CGA-152005, 0.084 ppm found, 84 % Recovery.
- 4. 0.50 ppm fortification, 8.92 mg injected, 3.50 ng CGA-152005, 0.39 ppm found, 79 % Recovery.

VIII. REFERENCES

1. Yokley, R. A., "Validation of 'Draft' Analytical Method AG-592 for the Determination of CGA-152005 in Meat, Milk, Blood, and Eggs by High Performance Liquid Chromatography," CIBA-GEIGY Protocol 26-92, February, 1992.
2. Itterly, W., "Metabolism of [Triazine-¹⁴C]-CGA-152005 in Lactating Goat," Metabolism Study No. M90-168-001A, Protocol No. 169-90, In Progress.
3. Fisher, G. D., "Metabolism of [Triazine-¹⁴C]-CGA-152005 in Poultry," F-00115, Metabolism Study No. M91-168-002A, Protocol No. 196-90, In Progress.
4. Yokley, R. A., Residue Test Report RI-MV-001-92, Report No. 1, Project No. 168982, March, 1992.

APPENDIX I

RESIDUE CHEMISTRY DEPARTMENT PROTOCOL NUMBER 26-92
AND AMENDMENT 1

SUBMITTER/SPONSOR:
Ciba Plant Protection
Ciba-Geigy Corporation
Post Office Box 18300
Greensboro, NC 27410

AGRICULTURAL DIVISION
CIBA-GEIGY CORPORATION

RESIDUE CHEMISTRY DEPARTMENT
PROTOCOL 26-92

VALIDATION OF "DRAFT" ANALYTICAL METHOD AG-592 FOR THE
DETERMINATION OF CGA-152005 IN MEAT, MILK, BLOOD, AND
EGGS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Study/Project No.: 168982

STUDY DIRECTOR:

Robert A. Yokley

SPONSOR APPROVAL:

R. K. Williams

TITLE: Project Scientist,
Method Development

TITLE: Manager,
Method
Development

SIGNATURE: *Robert A. Yokley*

SIGNATURE: *R. K. Williams*

DATE: February 10, 1992

DATE: 2/10/92

QUALITY ASSURANCE UNIT AUDIT

AUDITOR: *[Signature]*

DATE: 2/6/92

SPONSOR:

CIBA-GEIGY Corporation
Agricultural Division
410 Swing Road
Post Office Box 18300
Greensboro, NC 27419

TESTING FACILITY:

CIBA-GEIGY Corporation
Agricultural Division
Method Development Laboratory
410 Swing Road
Post Office Box 18300
Greensboro, NC 27419

PROPOSED EXPERIMENTAL STARTING DATE: February 10, 1992

PROPOSED EXPERIMENTAL COMPLETION DATE: March 20, 1992

PROPOSED STUDY COMPLETION DATE: March 31, 1992

STUDY OBJECTIVES

The objective of this study is to validate "Draft" Analytical Method AG-592¹, shown in Appendix I, for the determination of residues of CGA-152005 in meat, blood, and eggs at a screening level of 0.05 ppm and in milk at a screening level of 0.01 ppm. This validation will be accomplished by analysis of control and fortified control samples to demonstrate the accuracy and precision of the method. Results of the determination of CGA-152005 in meat, milk, blood, and eggs will be reported in Analytical Method AG-592 and in Residue Test Report RI-MV-001-92².

TEST AND REFERENCE SUBSTANCES

CGA-152005: Lot No. S90-1490; B No. 06775; Purity, 97.1%; Reassay Date, 1/93; Storage Condition, room temperature.

A stock standard of CGA-152005 and serial dilutions of the stock standard are stored in the L-2074 refrigerator when not in use. Dilutions of the stock standard will be used for both fortification and quantification.

TEST SYSTEM

Control, fortified control, and ¹⁴C-CGA-152005 containing substrates will be analyzed as described in Table I. The various substrates used in this study are listed below and will be referenced under Test Number RI-MV-001-92.

- (1) Beef liver, kidney, tenderloin, round, perirenal and omental fat, blood, and milk (Inventory No. 13355.1).
- (2) Goat liver, omental fat, round, kidney, and milk (Inventory No. 13355.2).
- (3) Goat liver, omental fat, round, kidney, and milk from Metabolism Study M90-168-001A³ (Inventory No. 13355.4).
- (4) Poultry liver and peritoneal fat (Inventory No. 13355.5).

- (5) Poultry lean meat and eggs from Metabolism Study M91-168-002A⁴ (Inventory No. 13355.3).

JUSTIFICATION OF TEST SYSTEM

Analysis of control and fortified control beef liver, kidney, tenderloin, round, perirenal and omental fat, blood, and milk as well as poultry liver and peritoneal fat by "Draft" Analytical Method AG-592 (Appendix I) will be performed to determine the accuracy and precision of the method for CGA-152005. Further, analysis of ¹⁴C-CGA-152005 containing goat liver, omental fat, round, kidney, and milk³ as well as ¹⁴C-CGA-152005 containing poultry lean meat and eggs⁴ will be performed using AG-592 to determine the extractability and accountability of the method for CGA-152005.

EXPERIMENTAL DESIGN

CIBA-GEIGY "Draft" Analytical Method AG-592 (Appendix I) will be used to determine CGA-152005.

Fortified Samples - "Draft" Analytical Method AG-592 (Appendix I). See also "TEST SYSTEM" Section.

Modifications - Any modifications will be documented with protocol amendments.

The experiments will consist of the analysis of control and fortified control meat, blood, milk, and egg samples, fortified at or above the screening level of "Draft" Analytical Method AG-592 (Appendix I). Several ¹⁴C-CGA-152005 containing substrates will also be analyzed.

The sets of samples to be analyzed in this study are outlined in Table I. The accuracy of the method used in this study will be confirmed by the recovery results from the analyses of fortified control samples. The precision of the method will be determined by the reproducibility of the amount of CGA-152005 determined by the method. The accuracy and precision of the method will also be evaluated by

comparing the results of the method (by LC) for the ^{14}C -CGA-152005 containing substrates to the combustion analysis results of the same substrates.

The control of bias in the study will be accomplished by the use of control samples for all fortification experiments. Other experimental design details may be found in Appendix I, "Draft" Analytical Method AG-592.

ROUTE OF ADMINISTRATION

Preparation of standards and fortification of control samples will be performed according to procedures in "Draft" Analytical Method AG-592 (Appendix I). Radiolabeled CGA-152005 was administered to goats and poultry to provide substrates for metabolism studies M90-168-001A³ and M91-168-002A⁴.

RECORDS TO BE MAINTAINED

All personnel involved in the study will maintain laboratory notebooks or worksheets in which all data for the project will be recorded as required by Good Laboratory Practice according to the procedures outlined in Metabolism and Residue Chemistry SOP 8.1, Revision 4. Original chromatograms, computer print-outs, etc. will be clearly marked as Test Number RI-MV-001-92. All data placed in this file will be clearly labeled as to origin and referenced to the notebook and page of the corresponding work description. Raw data will be archived in the Residue Chemistry Archives under Residue Test Report RI-MV-001-92². Results of the method validation will be reported in Residue Test Report format. Laboratory notebooks will remain in the possession of the analyst until the study is completed and then transferred to the Residue Department Archives (see Table II).

PROPOSED STATISTICAL METHODS

Statistical methods for regression analysis for a standard curve and quantification of residues are described in "Draft" Analytical Method AG-592 (Appendix I).

Recovery results for fortified control samples will be used to calculate accuracy in terms of a mean and standard deviation for the screening level and for all recovery results included in the study.

The precision of the method will also be determined by calculating the mean, range, and standard deviation of triplicate analyses of the ^{14}C -CGA-152005 containing goat and poultry substrates.

The extractability of the method will be determined by performing liquid scintillation counting measurements of aliquot portions of the extract and comparing those results to the total radioactive residue (TRR) as determined by combustion analysis of an aliquot portion of the same substrate^{3,4}. The accountability of the method will be determined by comparing the results obtained using Method AG-592 to those determined by liquid scintillation counting measurements of aliquot portions of the final fraction used for analysis by liquid chromatography.

PERSONNEL

1. Study Director:
Robert A. Yokley, Project Scientist.
2. Project Analyst:
Dan Campbell, Associate Chemist

RESIDUE CHEMISTRY DEPARTMENT
 PROTOCOL NUMBER 26-92
 AGRICULTURAL DIVISION
 CIBA-GEIGY CORPORATION
 PAGE 6 OF 12

TABLE I: SAMPLES TO BE ANALYZED

POULTRY LIVER

<u>Sample No.</u>	<u>Code No.</u>	<u>Fortification Level</u> <u>(ppm)</u>	<u>Replicates</u>
1	RI-MV-001-92	Reagent Blank	1
2	RI-MV-001-92	0 (Control)	1
3, 4	RI-MV-001-92	0.05	2
5	RI-MV-001-92	0.20	1
6	RI-MV-001-92	0.50	1

POULTRY PERITONEAL FAT

<u>Sample No.</u>	<u>Code No.</u>	<u>Fortification Level</u> <u>(ppm)</u>	<u>Replicates</u>
7	RI-MV-001-92	Reagent Blank	1
8	RI-MV-001-92	0 (Control)	1
9, 10	RI-MV-001-92	0.05	2
11	RI-MV-001-92	0.20	1
12	RI-MV-001-92	0.50	1

BEEF LIVER

<u>Sample No.</u>	<u>Code No.</u>	<u>Fortification Level</u> <u>(ppm)</u>	<u>Replicates</u>
13	RI-MV-001-92	0 (Control)	1
14, 15	RI-MV-001-92	0.05	2
16	RI-MV-001-92	0.20	1
17	RI-MV-001-92	0.50	1

BEEF KIDNEY

<u>Sample No.</u>	<u>Code No.</u>	<u>Fortification Level</u> <u>(ppm)</u>	<u>Replicates</u>
18	RI-MV-001-92	0 (Control)	1
19, 20	RI-MV-001-92	0.05	2
21	RI-MV-001-92	0.20	1
22	RI-MV-001-92	0.50	1

BEEF TENDERLOIN

<u>Sample No.</u>	<u>Code No.</u>	<u>Fortification Level</u> <u>(ppm)</u>	<u>Replicates</u>
23	RI-MV-001-92	Reagent Blank	1
24	RI-MV-001-92	0 (Control)	1
25, 26	RI-MV-001-92	0.05	2
27	RI-MV-001-92	0.20	1
28	RI-MV-001-92	0.50	1

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 PAGE 7 OF 12

TABLE I: SAMPLES TO BE ANALYZED
 (Continued)

<u>BEEF ROUND</u>			
<u>Sample No.</u>	<u>Code No.</u>	<u>Fortification Level</u> <u>(ppm)</u>	<u>Replicates</u>
29	RI-MV-001-92	0 (Control)	1
30, 31	RI-MV-001-92	0.05	2
32	RI-MV-001-92	0.20	1
33	RI-MV-001-92	0.50	1

<u>BEEF OMENTAL FAT</u>			
<u>Sample No.</u>	<u>Code No.</u>	<u>Fortification Level</u> <u>(ppm)</u>	<u>Replicates</u>
34	RI-MV-001-92	0 (Control)	1
35, 36	RI-MV-001-92	0.05	2
37	RI-MV-001-92	0.20	1
38	RI-MV-001-92	0.50	1

<u>BEEF PERIRENAL FAT</u>			
<u>Sample No.</u>	<u>Code No.</u>	<u>Fortification Level</u> <u>(ppm)</u>	<u>Replicates</u>
39	RI-MV-001-92	0 (Control)	1
40, 41	RI-MV-001-92	0.05	2
42	RI-MV-001-92	0.20	1
43	RI-MV-001-92	0.50	1

<u>BEEF BLOOD</u>			
<u>Sample No.</u>	<u>Code No.</u>	<u>Fortification Level</u> <u>(ppm)</u>	<u>Replicates</u>
44	RI-MV-001-92	Reagent Blank	1
45	RI-MV-001-92	0 (Control)	1
46, 47	RI-MV-001-92	0.05	2
48	RI-MV-001-92	0.20	1
49	RI-MV-001-92	0.50	1

<u>MILK</u>			
<u>Sample No.</u>	<u>Code No.</u>	<u>Fortification Level</u> <u>(ppm)</u>	<u>Replicates</u>
50	RI-MV-001-92	0 (Control)	1
51, 52	RI-MV-001-92	0.01	2
53	RI-MV-001-92	0.10	1
54	RI-MV-001-92	0.50	1

TABLE I: SAMPLES TO BE ANALYZED
 (Continued)

The following sets also contain triplicate analyses of ¹⁴C-CGA-152005 containing substrates.

POULTRY LEAN MEAT

<u>Sample No.</u>	<u>Code No.</u>	<u>Fortification Level</u> <u>(ppm)</u>	<u>Replicates</u>
55	RI-MV-001-92	0 (Control)	1
56, 57	RI-MV-001-92	0.05	2
58	RI-MV-001-92	0.50 (1)	1
59, 60, 61	H91260507	0.705 (2)	3

EGGS

<u>Sample No.</u>	<u>Code No.</u>	<u>Fortification Level</u> <u>(ppm)</u>	<u>Replicates</u>
62	RI-MV-001-92	0 (Control)	1
63, 64	RI-MV-001-92	0.05	2
65	RI-MV-001-92	0.50	1
66, 67, 68	H91260533	0.326	3

GOAT LIVER

<u>Sample No.</u>	<u>Code No.</u>	<u>Fortification Level</u> <u>(ppm)</u>	<u>Replicates</u>
69	RI-MV-001-92	0 (Control)	1
70, 71	RI-MV-001-92	0.05	2
72	RI-MV-001-92	0.10 (1)	1
73, 74, 75	G90480033	0.058 (3)	3

GOAT OMENTAL FAT

<u>Sample No.</u>	<u>Code No.</u>	<u>Fortification Level</u> <u>(ppm)</u>	<u>Replicates</u>
76	RI-MV-001-92	0 (Control)	1
77, 78	RI-MV-001-92	0.05	2
79	RI-MV-001-92	1.50 (1)	1
80, 81, 82	G90480024	1.335 (3)	3

- (1) A fresh fortification near the value of the ¹⁴C-CGA-152005 containing samples.
- (2) Preliminary results from Metabolism Study M91-168-002A, Protocol 196-90, In Progress.
- (3) Preliminary results for Total Radioactive Residue from Metabolism Study M90-168-001A, Protocol No 169-90, In Progress

RESIDUE CHEMISTRY DEPARTMENT
 PROTOCOL NUMBER 26-92
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 PAGE 9 OF 12

TABLE I: SAMPLES TO BE ANALYZED
 (Continued)

GOAT ROUND

<u>Sample No.</u>	<u>Code No.</u>	<u>Fortification Level</u> <u>(ppm)</u>	<u>Replicates</u>
83	RI-MV-001-92	0 (Control)	1
84, 85	RI-MV-001-92	0.05	2
86	RI-MV-001-92	0.20 (1)	1
87, 88, 89	G90480031	0.209 (2)	3

GOAT KIDNEY

<u>Sample No.</u>	<u>Code No.</u>	<u>Fortification Level</u> <u>(ppm)</u>	<u>Replicates</u>
90	RI-MV-001-92	0 (Control)	1
91, 92	RI-MV-001-92	0.05	2
93	RI-MV-001-92	5.0 (1)	1
94, 95, 96	G90480026	5.048 (2)	3

GOAT MILK

<u>Sample No.</u>	<u>Code No.</u>	<u>Fortification Level</u> <u>(ppm)</u>	<u>Replicates</u>
97	RI-MV-001-92	0 (Control)	1
98, 99	RI-MV-001-92	0.01	2
100	RI-MV-001-92	0.10 (1)	1
101, 102, 103	G90480012	0.069 (2)	3

- (1) A fresh fortification near the value of the ¹⁴C-CGA-152005 containing samples.
 (2) Preliminary results for Total Radioactive Residue from Metabolism Study M90-168-001A, Protocol No 169-90, In Progress.

TABLE II

GENERAL INFORMATION

Study Director: Robert A. Yokley,
Project Scientist
Method Development,
(919) 632-2142

Project Analyst: Dan Campbell, Associate Chemist

Test Substance: CGA-152005 for this study (See
Test and Reference Substances
Section). The metabolism study
substrates (goat and poultry)
contain ¹⁴C-CGA-152005.

Testing Facility: CIBA-GEIGY Corporation
Agricultural Division
Method Development Laboratory
Residue Chemistry Department
Post Office Box 18300
410 Swing Road
Greensboro, NC 27419

Archive Location: The protocol, raw data, final
report, and Residue Test Report
will be archived in the Residue
Chemistry Department Archive at
CIBA-GEIGY Corporation,
Greensboro, NC. No specimen will
remain to be retained.

Project Number: 168982

Test Number: RI-MV-001-92

RESIDUE CHEMISTRY DEPARTMENT
PROTOCOL NUMBER 26-92
AGRICULTURAL DIVISION
CIBA-GEIGY CORPORATION
PAGE 11 OF 12

RADIATION SAFETY COMMITTEE

PROTOCOL FOR VALIDATION OF ANALYTICAL METHOD AG-592

Study/Project No. 168982

Radiolabeled samples from poultry tissues and eggs dosed with ^{14}C -CGA-152005 according to Metabolism Study M91-168-002A and goat tissues and milk dosed with ^{14}C -CGA-152005 according to Metabolism Study M90-168-001A are approved for use in this study under Radioactive Materials Project RMP-2.

Signed: W. L. Secrest, Radiation Safety Officer
W. L. Secrest

Date: 2/10/92

REFERENCES:

1. Campbell, D. D. and Yokley, R. A., "Analytical Method For the Determination of CGA-152005 in Meat, Milk, and Eggs By High Performance Liquid Chromatography," "Draft" Method No. AG-592.
2. Yokley, R. A., Residue Test Report RI-MV-001-92 Report No. 1, Protocol Number 26-92, Project Number 168982.
3. Itterly, W., "Metabolism of [Triazine-¹⁴C]-CGA-152005 in Lactating Goat," Metabolism Study No. M90-168-001A, Protocol No. 169-90, In Progress.
4. Fisher, G. D., Metabolism of [Triazine-¹⁴C]-CGA-152005 in Poultry," Metabolism Study No. M91-168-002A, Protocol No. 196-90, In Progress.

APPENDIX I

"DRAFT" ANALYTICAL METHOD FOR THE DETERMINATION
OF CGA-152005 IN MEAT, MILK, BLOOD, AND EGGS BY
HIGH PERFORMANCE LIQUID CHROMATOGRAPHY
INCLUDING VALIDATION DATA

ANALYTICAL METHOD NO. 592

CIBA-GEIGY CORPORATION
AGRICULTURAL DIVISION
RESIDUE CHEMISTRY DEPARTMENT
410 SWING ROAD
P. O. BOX 18300
GREENSBORO, NC 27419

PROJECT NUMBER: 168982 PROTOCOL: 26-92

SUBMITTED BY: Daniel D. Campbell

TITLE: Associate Chemist

SIGNATURE:

APPROVED BY: R. K. Williams

TITLE: Manager, Method Development

SIGNATURE:

DATE:

STUDY DIRECTOR: Robert A. Yokley, Ph.D.

SIGNATURE:

COMPLETION DATE:

I.	SUMMARY AND INTRODUCTION	>
	A. Scope	>
	B. Principle	>
II.	MATERIALS AND METHODS	>
	A. Apparatus	>
	B. Reagents	>
	C. Analytical Procedure	>
	1. Extraction	>
	2. Partitioning	>
	3. Alumina-A Sep-Pak Cleanup	>
	D. Instrumentation	>
	1. Description and Operating Conditions	>
	2. Standardization	>
	E. Interferences	>
	F. Confirmatory Techniques	>
	G. Time Required	>
	H. Modifications and Potential Problems	>
	1. Emulsion Formation	>
	2. Heat	>
	3. Degradation in Methanol	>
	I. Preparation of Standard Solutions	>
	1. Preparation of Analytical Standards	>

TABLE OF CONTENTS
(Continued)

	<u>PAGE</u> <u>NO.</u>
2. Preparation of Fortification Standards	>
J. Determination of Sample Residues	>
1. Analysis by Linear Regression	>
2. Calculation of Sample Concentration	>
K. Fortification Experiments	>
1. Substrate Fortification	>
2. Calculation of Procedural Recovery	>
III. RESULTS AND DISCUSSION	>
IV. CONCLUSION	>
V. CERTIFICATION	>
VI. QAU STATEMENT	>
VII. LIST OF TABLES AND FIGURES	>
TABLE I. LIQUID CHROMATOGRAPH OPERATING PARAMETERS FOR THE ANALYSIS OF CGA-152005	>
TABLE II. TYPICAL STANDARDIZATION DATA FOR CGA-152005	>
TABLE III. RECOVERY RESULTS FOR CONTROL AND CGA-152005 FORTIFIED CONTROL SUBSTRATES AND ¹⁴ C-CGA-152005 CONTAINING SUBSTRATES	>

TABLE OF CONTENTS
(Continued)

	<u>PAGE</u> <u>NO.</u>
TABLE IV. SUMMARY TABLE FOR THE EXTRACTABILITY, ANALYSIS, AND ACCOUNTABILITY OF ¹⁴ C-CGA-152005 TREATED SUBSTRATES USING AG-592	>
FIGURE 1. STRUCTURE AND CHEMICAL NAME OF CGA-152005	>
FIGURE 2. ANALYTICAL PROCEDURE FLOWCHART FOR AG-592	>
FIGURE 3. TYPICAL STANDARD CHROMATOGRAMS (FROM THE ANALYSIS OF POULTRY LEAN MEAT)	>
FIGURE 4. CALIBRATION PLOT OF THE STANDARDS OBTAINED FROM THE ANALYSIS OF POULTRY LEAN MEAT	>
FIGURE 5. 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. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF POULTRY LEAN MEAT FOR CGA-152005 USING METHOD AG-592	>
FIGURE 7. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF EGGS FOR CGA-152005 USING METHOD AG-592	>
FIGURE 8. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF GOAT LIVER FOR CGA-152005 USING METHOD AG-592	>

TABLE OF CONTENTS
(Continued)

	<u>PAGE</u> <u>NO.</u>
FIGURE 9. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF GOAT OMENTAL FAT FOR CGA-152005 USING METHOD AG-592	>
FIGURE 10. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF GOAT ROUND FOR CGA-152005 USING METHOD AG-592	>
FIGURE 11. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF GOAT KIDNEY FOR CGA-152005 USING METHOD AG-592	>
FIGURE 12. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF GOAT MILK FOR CGA-152005 USING METHOD AG-592	>
FIGURE 13. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF POULTRY LIVER FOR CGA-152005 USING METHOD AG-592	>
FIGURE 14. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF POULTRY PERITONEAL FAT FOR CGA-152005 USING METHOD AG-592	>
FIGURE 15. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF BEEF LIVER FOR CGA-152005 USING METHOD AG-592	>
FIGURE 16. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF BEEF KIDNEY FOR CGA-152005 USING METHOD AG-592	>

TABLE OF CONTENTS
(Continued)

	<u>PAGE</u> <u>NO.</u>
FIGURE 17. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF BEEF TENDERLOIN FOR CGA-152005 USING METHOD AG-592	>
FIGURE 18. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF BEEF ROUND FOR CGA-152005 USING METHOD AG-592	>
FIGURE 19. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF BEEF OMENTAL FAT FOR CGA-152005 USING METHOD AG-592	>
FIGURE 20. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF BEEF PERITONEAL FAT FOR CGA-152005 USING METHOD AG-592	>
FIGURE 21. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF BEEF BLOOD FOR CGA-152005 USING METHOD AG-592	>
FIGURE 22. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF BEEF MILK FOR CGA-152005 USING METHOD AG-592	>

I. SUMMARY AND INTRODUCTION

A. SCOPE

This method is for the determination of residues of CGA-152005 (see figure 1) in animal tissues, milk, blood, and poultry eggs. The limit of detection of this method is 0.06 ng of CGA-152005 and the limit of determination is 0.01 ppm.

B. PRINCIPLE

A 10-g subsample of substrate is homogenized with 90% acetone:10% 0.1% sodium bicarbonate aqueous solution. After filtering, the volume is reduced under vacuum until only the aqueous portion remains. The sample is diluted with 25 ml 0.4% sodium carbonate and partitioned against methyl tert-butyl ether:hexane (1:1). The aqueous phase is acidified with 20 ml 0.8% phosphoric acid, followed by the addition of 20 ml of saturated aqueous salt solution. The compound is extracted by partitioning with dichloromethane:hexane (1:1). The organic portion is dried with sodium sulfate, reduced to about 10 ml and added to a preconditioned alumina-A Sep-Pak column. After column washes with hexane, ethyl acetate, and 1% acetonitrile in ethyl acetate, the compound is eluted with methanol:acetonitrile (60:40). The eluent is concentrated to just dryness. One ml of acetonitrile is added, followed by one ml of picopure water, and the sample is filtered through a 0.45 micron Acrodisc filter. The filter is rinsed with 1 ml of acetonitrile:water (1:1). The sample is analyzed by high performance liquid chromatography (HPLC) using a narrow bore reverse phase column (Supelcosil LC-18-DB) with a mobile phase of acetonitrile:0.05% phosphoric acid in picopure water (40:60). Detection is by UV at 225 nm.

- 15.0 Syringe, Hamilton Gastight, Luer Tip, 1-ml (Fisher #14-824-24 or equivalent)
- 16.0 Ultrasonicator (Heatsystems Inc. or equivalent)
- 17.0 Vials, crimpltop borosilicate (Sun Broker Inc. or equivalent)

B. REAGENTS

- 1.0 Acetone, OPTIMA grade (Fisher # A929-4 or equivalent)
- 2.0 Acetonitrile, HPLC grade (Fisher # A998-4 or equivalent)
- 3.0 Dichloromethane, HPLC grade (Burdick & Jackson #300-4 or equivalent)
- 4.0 Ethyl Acetate, HPLC grade (Fisher # E195-4 or equivalent)
- 5.0 Hexane, HPLC grade (Fisher # H302-4 or equivalent)
- 6.0 Methanol, HPLC grade (Fisher # A452-4 or equivalent)
- 7.0 Methyl tert-butyl ether, HPLC grade (Fisher #E127-4)
- 8.0 Phosphoric acid, Certified ACS grade (Fisher #A242 or equivalent)
- 9.0 Sodium bicarbonate, Certified ACS grade (Fisher #233-3 or equivalent)
- 10.0 Sodium carbonate, Certified ACS grade (Fisher #S263-3 or equivalent)
- 11.0 Sodium chloride, Certified ACS grade (Fisher #5271-3 or equivalent)
- 12.0 Water, HPLC grade (picopure or equivalent)
- 13.0 CGA-152005, Analytical Standard supplied by CIBA-GEIGY Corporation, 410 Swing Rd., Greensboro, NC 27419.

C. ANALYTICAL PROCEDURE1.0 Extraction1.1 Meat, Blood, Milk, and Eggs

Weigh 10 grams of tissue slices, well mixed blood or egg homogenate, or 50 grams milk into a tared 8-oz. glass bottle. Add 90 ml of 90% acetone:10% 0.1% sodium bicarbonate in picopure water. Homogenize for 30 seconds with the polytron at medium speed. Return any tissues remaining on the polytron blade back to the 8-oz. bottle. Filter through a Reeve Angel #802 filter in a longstem funnel into a 250-ml Erlenmeyer flask. NOTE: The second extraction is not required for the milk samples. Measure and record the volume of the extract for later calculations. Remove the filter paper after all solvent has passed through and place it back into the 8-oz. bottle. Add another 90 ml of 90% acetone:10% 0.1% sodium bicarbonate and homogenize with the polytron for 30 seconds. The generator should be at the bottom of the filter paper and will homogenize the substrate left on the paper, but not the filter itself. A polytron setting slower than the one used in the first extraction may be required. Pour the homogenate through a new Reeve Angel #802 filter and collect with the first extract.

2.0 Partitioning

- 2.1 Transfer the extract from section 1.1 to a 1000-ml round bottom flask and reduce the solvent by rotary evaporation under vacuum. Lower the flask into a 38 - 40°C warm water bath after about 10 minutes. CAUTION: While bumping does not typically occur in the early stages of the extract reduction, bubbling will occur when most of the organic solvent has been removed. The addition of 10 ml of ethyl acetate to the sample will help if excessive bubbling occurs. Remove the sample when only the aqueous portion remains. Transfer the solution to a 125 ml separatory funnel.
- 2.2 Add 10 ml of 0.4% sodium carbonate to the 1000-ml round bottom flask, swirl and sonicate to dissolve remaining residues. Transfer to the 125 ml separatory funnel containing the sample. Repeat with another 10 ml of 0.4% sodium carbonate, and end with a third rinse of 5 ml. Add 30 ml of methyl tert-butyl ether (MtBE):hexane (1:1) and shake for one minute, taking care to vent the funnel. Allow the two layers to separate, breaking any emulsions that may form by agitating with a glass pipet or by applying a small amount of heat. Drain the lower aqueous layer and any persistent emulsions into a 250-ml Erlenmeyer flask and discard the organic layer. Pour the aqueous phase back into the 125-ml separatory funnel and repeat the extraction with 30 ml of

MtBE:hexane (1:1). Collect the aqueous layer into the same Erlenmeyer flask.

- 2.3 Add 20 ml of 0.8% H_3PO_4 (in picopure water) and 20 ml of picopure water saturated with sodium chloride to the aqueous phase in the Erlenmeyer flask from section C.2.2. Transfer to a 250-ml separatory funnel and extract 3 times by partitioning with 60 ml dichloromethane: hexane (1:1), taking care to vent the funnel. The first 60 ml of dichloromethane:hexane (1:1) used for the partitioning should also be used to rinse the 250-ml Erlenmeyer flask. Treat emulsions that form as was done in section C.2.2, and combine and save the upper organic layers in a separate 500-ml Erlenmeyer flask.

3.0 Alumina-A Sep-Pak Cleanup

- 3.1 Attach a 50-ml Analytichem reservoir to an Alumina-A Sep-Pak column, and condition the column with the following rinses: 25 ml methanol: acetonitrile (60:40), 15 ml ethyl acetate, and 25 ml dichloromethane (DCM):hexane (1:1). Do not let the column dry between rinses and prior to applying the sample.
- 3.2 Add approximately 15 grams of sodium sulfate directly to the organic phase from section C.2.3 to bind any water in the extract, and transfer to a 500 ml round bottom flask. Rinse the sodium sulfate 3 times with small volumes (~3-5 ml) of DCM:hexane (1:1). Reduce the volume of the sample under

vacuum by rotary evaporation in a 35°C warm water bath to 5 to 10 ml, taking care not to let the sample go to dryness.

- 3.3 Add the sample from section C.3.2 to the conditioned Alumina-A Sep-Pak column. Rinse the round bottom flask 3 times with 1 to 2 ml of DCM:hexane (1:1), pouring each rinse into the reservoir above the Alumina-A column. Allow the sample and washes to drip through the column by gravity only. The flow may occasionally stop and can be restarted by applying gentle pressure to the top of the reservoir with a pipet bulb. Wash with the following, being careful to insure that the column does not go dry between rinses. Collect the eluent in a 100-ml round bottom flask.

Wash with 15 ml hexane
(discard)

Wash with 25 ml ethyl acetate
(discard)

Wash with 15 ml 1:1
acetonitrile in ethyl acetate
(discard)

Elute with 30 ml 60:40
methanol: acetonitrile
(collect)

- 3.4 Reduce the eluant volume from section 3.3 to just dryness under vacuum by rotary evaporation in a 35°C water bath. This step must be done soon after elution from the Alumina-A column since preliminary reports indicate that CGA-152005 is unstable in methanol for prolonged periods

of time. When the sample has just reached dryness, remove it from the rotary evaporator and add 1 ml acetonitrile. Swirl and sonicate to dissolve all residues. Add 1 ml picopure water and swirl and sonicate a second time. For milk samples at 0.01 ppm, increase the volumes to 3 ml acetonitrile and 3 ml water.

- 3.5 Prewash a 0.45 um, 25mm Acrodisc PVDF HPLC syringe filter with 3 X 1ml acetonitrile: picopure water (1:1). Filter the sample from section 3.4 slowly through the Acrodisc using a 1-ml glass syringe. Add 1 ml acetonitrile: picopure water (1:1) to the round bottom flask, swirl and pass through the same filter as a rinse. For 0.01 ppm milk samples, increase the volume of the rinse to 4 ml for a total of 10 ml in the final sample. Mix the sample well and transfer 1 to 2 ml to a borosillcate crimptop vial for analysis by HPLC.

D. INSTRUMENTATION

1.0 Description and Operating Conditions

Residues of CGA-152005 are determined by HPLC on a narrow bore base deactivated Supelco C-18 column (LC-18-DB). The mobile phase is 60% 0.05% phosphoric acid in picopure water:40% acetonitrile at a flow rate of 0.3 ml per minute. Detection of CGA-152005 is

performed using an ultraviolet detector at a wavelength of 225 nm. The injection volume is 10 ul. The HPLC conditions are listed in Table 1.

2.0 Standardization

Standardize the HPLC system by injecting 10-ul aliquots of standard solutions of CGA-152005 in a working range of 0.6-10 ng/injection. Generate a linear regression from the data by comparing detector response and ng injected. See section I.2.0 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 analysis can be performed overnight using automatic injection.

H. MODIFICATIONS AND POTENTIAL PROBLEMS

1.0 Emulsion Formation

Some samples may develop emulsions during partitioning (during steps in sections C.2.1 and C.2.2). These may be cleared if allowed to settle out and then gently stirred with a glass rod. Slight heating may help (a hair dryer was used), but care must be taken to avoid temperatures in excess of 40°C. Any emulsions that remain should stay with the aqueous phases during the partitioning.

2.0 Heat

During the evaporation of the solvents from the samples in sections C.2.1, C.3.2, and C.3.4, any water bath used must not have a temperature $>40^{\circ}\text{C}$ and the samples should be removed just as soon as they reach dryness. A temperature of 35°C is required for removal of the solvent from the Alumina-A eluent. Excessive temperature, especially when the sample has gone to dryness, may lead to analyte decomposition.

3.0 Degradation in Methanol

After elution from the Alumina-A column, the solvent must be evaporated as soon as possible, since CGA-152005 was reported to be unstable in methanol. Do not store the sample overnight in the methanol:acetonitrile eluent.

I. PREPARATION OF STANDARD SOLUTIONS

1.0 Preparation of Analytical Standards

Weigh 10 mg of CGA-152005 analytical standard into a 100-ml volumetric flask and dilute to the mark with acetonitrile. Make serial dilutions of the 0.1 mg/ml standard solution with 50% acetonitrile: 50% picopure water to give a series of fortification/analytical standards in a range of 0.06 ug/ml to 1.0 ug/ml of CGA-152005. Store the standard solutions in amber bottles at 4°C in the dark when not in use.

2.0 Preparation of Analytical Standards

The 1.0 ug/ml standard used to fortify standards is prepared in section I.1.0 above. See section K.1.0 for fortification procedures.

J. DETERMINATION OF SAMPLE RESIDUES

1.0 Linear Regression Analysis

Inject 10-ul aliquots of sample extracts onto the HPLC system. Compare the analyte peak height found in the sample extracts to the peak heights determined for the analytical standards by entering them into a linear regression program or by using a computer system (e.g. VG Multichrom) to determine the nanograms of CGA-152005 in the injected aliquot.

2.0 Calculation of Concentration

Calculate the residue results in terms of ppm of CGA-152005 by using the following equation:

$$(1) \text{ ppm} = \frac{(\text{ng CGA-152005 Found})}{(\text{mg sample injected})}$$

Where the ng CGA-152005 is obtained from the linear regression analysis in section J.1.0 and the mg sample injected is calculated as follows:
(Equation 2)

$$(2) \text{ mg inj.} = \frac{(G) (V_a) (V_i)}{(V_e) (V_f)}$$

G = milligrams sample extracted

V_a = aliquot volume (from section C.1.1)

V_e = extraction volume (from section C.1.1; usually 90 ml for milk and 180 ml for other substrates)

V_i = injection volume (ul)

V_f = total volume of final injection solution (ul)

K. Fortification Experiments

1.0 Substrate Fortification

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 CGA-152005. Add 0.5 ml of a 1.0 ug/ml standard solution of CGA-152005 to 10 g. of control tissues or 50 g of milk for a 0.05 ppm or 0.01 ppm fortification, respectively. Allow the solvent to evaporate for at least 20 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.

2.0 Calculation of Procedural Recovery

The final ppm value of the control and fortified samples can be obtained using the calculations shown in section J.2.0. Determine the % recovery by first subtracting the background detector response, if any, in the control sample from the CGA-152005 response in the recovery sample. Calculate the recovery factor as a percentage (R) by the equation:

$$(4) \quad R\% = \frac{\text{ppm CGA-152005 found}}{\text{ppm CGA-152005 added}} \times 100$$

III. RESULTS AND DISCUSSION

IV. CONCLUSION

V. CERTIFICATION

The reports and experimental results included in this study, Laboratory Project I.D. AG-592, are certified to be authentic accounts of the experiments.

Robert K. Williams, Manager
Method Development
Residue Chemistry Department
919-632-2295

Date

CERTIFICATION OF GOOD LABORATORY PRACTICES

The analytical work reported in AG-592 was performed in accordance with Good Laboratory Practice Standards, 40 CFR Part 160.

Robert A. Yokley, Ph.D
Study Director

Date

VI. QAU STATEMENT

TABLE I. LIQUID CHROMATOGRAPHIC OPERATING
PARAMETERS FOR THE ANALYSIS OF
CGA-152005

Instrument: Perkin-Elmer Model Series-4 Solvent
Delivery System or equivalent.
Perkin-Elmer Model ISS-100 Automatic
HPLC sampler or equivalent.

Column Oven: BioRad HPLC column heater, model
number 125-0425

Oven Temp.: 30°C

Column: Supelcosil LC-18-DB, 250mm x 2.1 mm,
5 um particle size (Supelco cat.
#5-7940M) with Supelguard LC-18-DB
guard column.

Mobile Phase: 60% 0.05% phosphoric acid in
picopure water:40% acetonitrile

Retention Time: ~22 minutes

Detection: ABI Kratos Spectroflow Model 783
Programmable Absorbance Detector or
equivalent variable wavelength
detector.

Wavelength: 225 nm

Attenuation: 0.006 AUFS

Flow Rate: 0.3 ml/min

Volume Injected: 10 ul

Chart Speed: 0.25 cm/min

Run Time: 30 min/injection

Data Acquisition: Microvax II (Bones)
Operating System, VMS Version 5.3-1
Application Software:
VG Multichrom Version 1.8
Worksheet Version: Ws.pas 1.3.1

TABLE II. TYPICAL STANDARDIZATION DATA FOR
CGA-152005

TABLE III. RECOVERY RESULTS FOR CONTROL AND
CGA-152005 FORTIFIED CONTROL SUBSTRATES
AND ¹⁴C-CGA-152005 CONTAINING SUBSTRATES

TABLE IV. SUMMARY TABLE FOR EXTRACTABILITY
ANALYSIS AND ACCOUNTABILITY OF
¹⁴C-CGA-152005 TREATED SUBSTRATE USING
AG-592

FIGURE 1. STRUCTURE AND CHEMICAL NAME OF
CGA-152005

CGA-152005

1-(4-Methoxy-6-methyl-triazin-2-yl)-3-[2-(3,3,3-
trifluoropropyl)-phenylsulfonyl]-urea

FIGURE 2. ANALYTICAL PROCEDURE FLOWCHART FOR AG-592

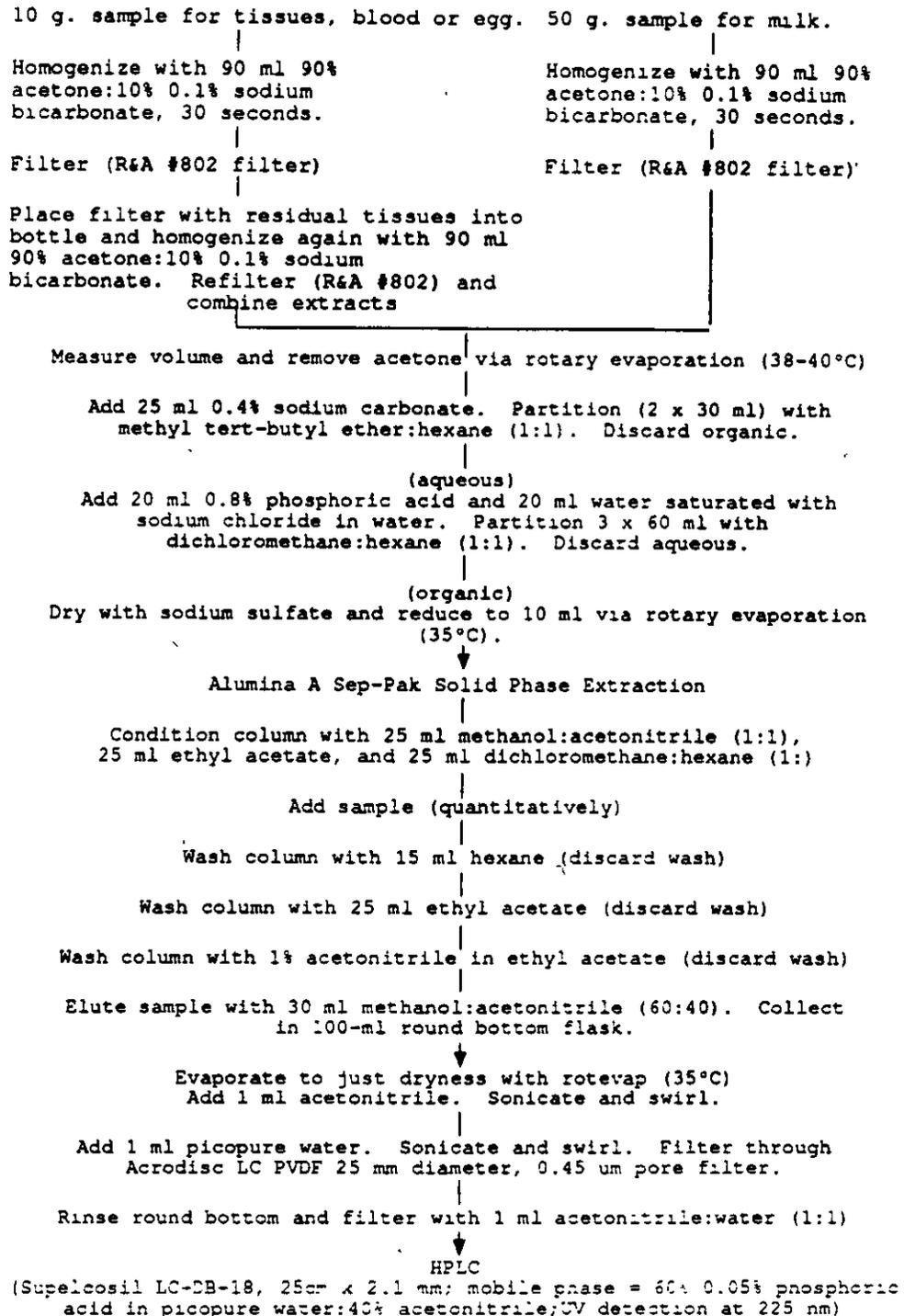


FIGURE 3. TYPICAL STANDARD CHROMATOGRAMS (FROM
THE ANALYSIS OF POULTRY LEAN MEAT)

FIGURE 4. CALIBRATION PLOT OF THE STANDARDS
OBTAINED FROM THE ANALYSIS OF POULTRY
LEAN MEAT

FIGURE 5. CHROMATOGRAMS OF REAGENT BLANKS FROM
THE ANALYSIS OF (A) POULTRY LIVER, (B)
POULTRY PERIONEAL FAT, (C) BEEF
TENDERLOIN, AND (D) BEEF BLOOD

FIGURE 6. REPRESENTATIVE CHROMATOGRAMS FROM THE
ANALYSIS OF POULTRY LEAN MEAT FOR
CGA-152005 USING METHOD AG-592

FIGURE 7. REPRESENTATIVE CHROMATOGRAMS FROM THE
ANALYSIS OF EGGS FOR CGA-152005 USING
METHOD AG-592

FIGURE 8. REPRESENTATIVE CHROMATOGRAMS FROM THE
ANALYSIS OF GOAT LIVER FOR CGA-152005
USING METHOD AG-592

FIGURE 9. REPRESENTATIVE CHROMATOGRAMS FROM THE
ANALYSIS OF GOAT OMENTAL FAT FOR
CGA-152005 USING METHOD AG-592

FIGURE 10. REPRESENTATIVE CHROMATOGRAMS FROM THE
ANALYSIS OF GOAT ROUND FOR CGA-152005
USING METHOD AG-592

FIGURE 11. REPRESENTATIVE CHROMATOGRAMS FROM THE
ANALYSIS OF GOAT KIDNEY FOR CGA-152005
USING METHOD AG-592

FIGURE 12. REPRESENTATIVE CHROMATOGRAMS FROM THE
ANALYSIS OF GOAT MILK FOR CGA-152005
USING METHOD AG-592

FIGURE 14. REPRESENTATIVE CHROMATOGRAMS FROM THE
ANALYSIS OF POULTRY PERITONEAL FAT FOR
CGA-152005 USING METHOD AG-592

FIGURE 15. REPRESENTATIVE CHROMATOGRAMS FROM THE
ANALYSIS OF BEEF LIVER FOR CGA-152005
USING METHOD AG-592

FIGURE 17. REPRESENTATIVE CHROMATOGRAMS FROM THE
ANALYSIS OF BEEF TENDERLOIN FOR
CGA-152005 USING METHOD AG-592

FIGURE 18. REPRESENTATIVE CHROMATOGRAMS FROM THE
ANALYSIS OF BEEF ROUND FOR CGA-152005
USING METHOD AG-592

FIGURE 19. REPRESENTATIVE CHROMATOGRAMS FROM THE
ANALYSIS OF BEEF OMENTAL FAT FOR
CGA-152005 USING METHOD AG-592

FIGURE 20. REPRESENTATIVE CHROMATOGRAMS FROM THE
ANALYSIS OF EGG AND BEEF PERITONEAL FAT
CGA-152005 USING METHOD AG-592

FIGURE 21. REPRESENTATIVE CHROMATOGRAMS FROM THE
ANALYSIS OF BEEF BLOOD FOR CGA-152005
USING METHOD AG-592

RESIDUE CHEMISTRY PROTOCOL AMENDMENT

AMENDMENT NUMBER: 1

PROTOCOL NUMBER: 26-92

TITLE: Validation of "Draft" Analytical Method AG-592 for the Determination of CGA-152005 in Meat, Milk, Blood, and Eggs By High Performance Liquid Chromatography.

PROJECT NUMBER (S): 168982

CHANGE(S):

(1) The following statement should be added to Protocol 26-92 on page 5 after the Proposed Statistical Methods Section.

MODIFICATIONS - Any modifications which alter the chemical procedure, solvent ratios, sample size, or sample identity will be documented with protocol amendments. It is expected that editorial alterations and changes in format will be made to the "Draft" Analytical Method before it is issued. These changes will not require amendment documentation since they will be documented in the validated Analytical Method which will be approved as a final report.

(2) The 14C-CGA-152005 concentrations listed in Table I on page 8 are listed as 0.058 ppm for goat liver and 1.335 ppm for goat omental fat. These are incorrect. The correct values are 1.371 ppm for goat liver and 0.058 ppm for goat omental fat.

(3) A footnote should be added for the egg samples listed in Table I on page 8. The fresh fortifications are on whole eggs whereas only the yolks were used for the 14C-CGA-152005 containing samples.

(4) The sample size is listed as 10 grams in the method. However, the sample size for the 14C-CGA-152005 containing egg yolk samples listed in Table I on page 8 was 5 grams during the method validation.

(5) A Submitter/Sponsor signature and date section will be added to the certification page.

(6) In Section C.3.3 of AG-592, the third wash of the alumina A column is listed as 1% ACN in ethyl acetate. This is incorrect. The correct percentage is 5% ACN in ethyl acetate.

(7) A statement was added to Section C.3.5 to address the final dilution volumes for samples containing high concentrations of CGA-152005 so that the samples are sufficiently diluted to insure peak heights within the calibration range of the injected standards.

(8) The final dilution volume for the 0.010 ppm fortification level milk samples was listed as 4 ml on page 14. This should be listed as 3 ml.

(9) It was listed in the protocol that the goat milk fortification (Table I, page 9) would be 0.010 ppm for the low fortification level. The actual fortification during the method validation was 0.050 ppm for the low fortification level.

REASON(S):

(1) This statement will allow editorial changes to be made to the method without requiring a protocol amendment for every minor change that does not affect the quality and/or integrity of the study.

(2) We reported the numbers supplied to us by metabolism personnel in the protocol. We realized the error when we analyzed the 14C-CGA-152005 containing goat liver and the peaks were much larger than expected. A review of the combustion data revealed that we had been supplied the wrong numbers for goat liver and goat omental fat. The goat liver analyses had to be repeated.

(3) The yolks were available and contained sufficient activity for the method validation.

(4) Only about 18 grams were available so the sample size had to be reduced to perform triplicate analyses.

(5) The absence of this submitter/sponsor signature and date section was cited in an In-Progress audit of another study. It has been added here to maintain GLP compliance.

(6) This was a proof reading oversight. The wash actually used was the 5% ACN in ethyl acetate solution.

(7) This was inadvertently omitted from the method and should be included.

(8) An oversight. The correct volume is 3 ml.

(9) We intended to fortify goat milk at the 0.010 ppm level but the samples were inadvertently fortified at the 0.050 ppm level instead. However, This does not adversely affect the results of this study since the radiolabelled samples contained CGA-152005 concentrations greater than 0.05 ppm. In

addition, the results of the analysis of dairy milk demonstrated the ability of the method to screen at the 0.010 ppm level.

EFFECTIVE DATE(S): March 20, 1992

CHANGE INITIATED BY: Robert A. Yorkley DATE: 3-20-92
CURRENT STUDY DIRECTOR

STUDY ACCEPTED BY: _____ DATE:

(IF APPLICABLE) NEW STUDY DIRECTOR

CHANGE AUTHORIZED BY: _____ DATE:

(IF APPLICABLE) MANAGEMENT SIGNATURE

CONTRACT LABORATORY
STUDY COORDINATOR: _____ DATE:
(IF APPLICABLE)

AMENDMENTS TO BE DISTRIBUTED PER PROTOCOL DISTRIBUTION LIST

PAGE 1 OF 3

APPENDIX II

SEPARATE DOCUMENTS ACCOMPANYING THIS REPORT

1. Itterly, W. Protocol No. 169-90, ABR-93041, "Metabolism of [Triazine-14C]CGA-152005 in Lactating Goats after Multiple Oral Administrations."
2. Fisher, G. D., Protocol No. 196-90, "Metabolism of [Triazine-14C]-CGA-152005 in the Chicken."

SUBMITTER/SPONSOR:
Ciba Plant Protection
Ciba-Geigy Corporation
Post Office Box 18300
Greensboro, NC 27410

APPENDIX IV

RESIDUE TEST REPORT RI-MV-001-92 REPORT NO. 1

SUBMITTER/SPONSOR:
Ciba Plant Protection
Ciba-Geigy Corporation
Post Office Box 18300
Greensboro, NC 27410

RESIDUE CHEMISTRY DEPARTMENT
AGRICULTURAL DIVISION
CIBA-GEIGY CORPORATION
GREENSBORO, NORTH CAROLINA

RESIDUE TEST REPORT

FIELD TEST NUMBER: RI-MV-001-92

REPORT NO.: 01

PROJECT NUMBER: 168982

PROTOCOL NUMBER: 26-92 and Amendment #1

TEST SUBSTANCE: CGA-152005

TEST SYSTEM: Poultry, Goat, and Beef Tissues, Milk, and Blood

LOCATION: Not Applicable

AG: 592 NO. OF ANALYSES: 99

LABORATORY: CIBA-GEIGY Method Development

DESCRIPTION: "Draft" Method AG-592 was validated for the determination of CGA-152005 by analyzing control samples, fortified control samples, and samples treated/dosed with ¹⁴C-CGA-152005 which were obtained from metabolism studies. Radioactive extractability and accountability determinations were also performed on the incurred ¹⁴C-CGA-152005 residues in treated poultry and goat substrates.

STUDY DIRECTOR: Robert A. Yokley, Ph.D.
Project Scientist

SIGNATURE: *Robert A. Yokley*

DATE: 3-31-92

APPROVED BY: R. K. Williams
Manager, Method Development

SIGNATURE: *R. K. Williams*

APPROVAL DATE: 3/31/92

DISTRIBUTION: D. D. Campbell
R. A. Kahrs
R. K. Williams
R. A. Yokley
Main File

RESIDUE TEST REPORT

FIELD TEST NUMBER: RI-MV-001-92PROTOCOL NUMBER: 26-92REPORT NUMBER: 01PROJECT NUMBER: 168982BIOLOGY SECTION

The various substrates used in this study are referenced under Test Number RI-MV-001-92 and include the below listed substrates.

- (1) Beef liver, kidney, tenderloin, round, perirenal and omental fat, blood, and milk (Inventory Number 13355.1).
- (2) Goat liver, omental fat, round, kidney, and milk (Inventory Number 13355.2).
- (3) Poultry liver and peritoneal fat (Inventory Number 13355.5).
- (4) Goat liver, omental fat, round, kidney, and milk from Metabolism Study M90-168-001A, Protocol Number 169-90, In-Progress (Inventory Number 13355.4).
- (5) Poultry lean meat and eggs from Metabolism Study M91-168-002A, Protocol Number 196-90, In-Progress (Inventory Number 13355.3).

CIRCUMSTANCES AFFECTING THE QUALITY OR INTEGRITY OF THE DATA

None

SAMPLE IDENTIFICATION NUMBERS

Each sample was assigned a specific sample code number in Protocol 26-92. This sample code was used in laboratory notebook 4235.

STUDY PERSONNEL

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RESIDUE TEST REPORT

FIELD TEST NUMBER: RI-MV-001-92
 REPORT NUMBER: 01

PROTOCOL NUMBER: 26-92
 PROJECT NUMBER: 168982

ANALYTICAL SECTIONMETHODOLOGYMETHOD NUMBER

AG-592

COMMENTS

All data were acquired using a VG Multichrom Version 1.8 or 2.0 data acquisition system. Liquid scintillation counting was performed according to SOP 4.6, Revision Number 2 and the data were calculated manually. An example calculation is shown in AG-592.

TEST SUBSTANCES AND CALIBRATION STANDARDS

<u>ANALYTICAL STANDARD</u>	<u>LOT NUMBER</u>	<u>PURITY</u>	<u>REANALYSIS DATE</u>
CGA-152005	S90-1490 (B06775)	97.1%	1/93

SUMMARY

The validity of "Draft" Analytical Method AG-592 for the determination of CGA-152005 was tested by analyzing control samples, fortified control samples, and samples of animal tissues, milk, and blood treated/dosed with ¹⁴C-CGA-152005 from metabolism studies M90-168-001A and M91-168-002A (in-progress) in order to determine the extractability, accountability, precision, and accuracy of the method.

CONCLUSION

Method AG-592 is a valid method for the determination of CGA-152005 in meat, milk, eggs, and blood.

RESIDUE TEST REPORT

FIELD TEST NUMBER: RI-MV-001-92PROTOCOL NUMBER: 26-92REPORT NUMBER: 01PROJECT NUMBER: 168982ANALYTICAL SECTION

RESULTS

The accuracy of the method is demonstrated by the results shown in Table I in terms of fresh fortification level, quantity found, and % recovery. The mean for each set of recoveries ranged from 75% to 104%. The average recovery for all substrates was 90% with a standard deviation of 11%.

The precision of the method is demonstrated by the results shown in Table II in which the ppm found from triplicate analyses of the ¹⁴C-CGA-152005 containing substrates are tabulated. The standard deviations range from 0.002% for goat omental fat (mean = 0.082 ppm) to 0.053% for goat kidney (mean = 5.4 ppm).

Table III shows the extractability and accountability data. The extractability was calculated by comparing (by percentages) the concentration of the total radioactive residue found in the extract, as determined by liquid scintillation counting, with the total radioactive residue in the sample, as preliminarily reported in metabolism studies M90-168-001A and M91-168-002A (In-progress). The extractabilities ranged from 72% for eggs to 148% for goat omental fat.

The % accountability was calculated by comparing (by percentages) the concentration of CGA-152005 in the final fractions, as determined by liquid chromatography, with the ¹⁴C-CGA-152005 concentration in the sample, as reported in metabolism studies M90-168-001A and M91-168-002A (In-progress). The accountabilities ranged from 28% for eggs to 142% for goat omental fat.

The limit of detection is 0.60 ng CGA-152005 and the limit of determination (screening level) is 0.05 ppm CGA-152005 for all substrates except milk which has a screening level of 0.01 ppm.

TABLE I. RECOVERY RESULTS FOR CONTROL AND CGA-152005 FORTIFIED CONTROL SUBSTRATES USING METHOD AG-592

(1) SUBSTRATE	PROTOCOL 26-92 SAMPLE #	CGA-152005 FORTIFICATION (ppm)	CGA-152005 (ppm)	(2) % RECOVERY (AG-592)	% RECOVERY	
					MEAN	STANDARD DEVIATION
Poultry	02	0	<0.05	.		
Liver	03	0.05	0.051	103	99	3.0
	04	0.05	0.048	96		
	05	0.20	0.20	99		
	06	0.50	0.48	96		
Poultry	08	0	<0.05	.		
Pentoneal	09	0.05	0.044	89	88	3.1
Fat	10	0.05	0.045	91		
	11	0.20	0.18	88		
	12	0.50	0.42	83		
Beef	13	0	<0.05	.		
Liver	14	0.05	0.053	106	104	5.4
	15	0.05	0.055	111		
	16	0.20	0.20	101		
	17	0.50	0.49	99		
Beef	18	0	<0.05	.		
Kidney	19	0.05	0.047	83	91	6.1
	20	0.05	0.050	90		
	21	0.20	0.19	92		
	22	0.50	0.50	98		
Beef	24	0	<0.05	.		
Tenderloin	25	0.05	0.047	93	98	6.6
	26	0.05	0.047	94		
	27	0.20	0.22	108		
	28	0.50	0.48	97		
Beef Round	29	0	<0.05	.		
	30	0.05	0.046	91	79	10
	31	0.05	0.042	84		
	32	0.20	0.139	70		
	33	0.50	0.363	73		
Beef	34	0	<0.05	.		
Omental Fat	35	0.05	0.041	82	82	6.5
	36	0.05	0.039	78		
	37	0.20	0.16	77		
	38	0.50	0.46	91		
Beef	39	0	<0.05	.		
Perirenal Fat	40	0.05	0.040	79	87	6.3
	41	0.05	0.047	94		
	42	0.20	0.17	85		
	43	0.50	0.45	90		

(1) - All samples are from Test Number RI-MV-001-92 - overall mean of 90% with a SD of 11% (n=61).
(2) Recovery samples were corrected for control residues where present.

RI-MV-001-92-01 [CENTERL-DOC.RESIDUE.RI] ms/lca:3/25/92

TABLE I. RECOVERY RESULTS FOR CONTROL AND CGA-152005 FORTIFIED CONTROL SUBSTRATES USING METHOD AG-592
(Continued)

(1) SUBSTRATE	PROTOCOL 26-92 SAMPLE #	CGA-152005 FORTIFICATION (ppm)	CGA-152005 (ppm)	(2) % RECOVERY (AG-592)	% RECOVERY	
					MEAN	STANDARD DEVIATION
Beef Blood	45	0	<0.05	-		
	46	0.05	0.046	93	78	16
	47	0.05	0.044	88		
	48	0.20	0.15	74		
	49	0.50	0.29	58		
Beef Milk	50	0	<0.01	-		
	51	0.01	0.0082	82	86	8.2
	52	0.01	0.010	97		
	53	0.10	0.084	84		
	54	0.50	0.393	79		
Poultry Lean Meat	55	0	<0.05	-		
	56	0.05	0.049	97	97	5.1
	57	0.05	0.051	102		
	58	0.50	0.46	91		
Eggs	62	0	<0.05	-		
	63	0.05	0.045	89	87	2.3
	64	0.05	0.042	85		
	65	0.50	0.43	86		
Goat Liver	69	0	<0.05	-		
	70	0.05	0.054	108	104	3.8
	71	0.05	0.051	102		
	72	1.00	1.02	102		
Goat Omental Fat	76	0	<0.05	-		
	77	0.05	0.036	72	75	4.0
	78	0.05	0.037	74		
	79	0.10	0.079	79		
Goat Round	83	0	<0.05	-		
	84	0.05	0.051	101	100	2.3
	85	0.05	0.051	101		
	86	0.20	0.19	97		
Goat Kidney	90	0	<0.05	-		
	91	0.05	0.050	100	102	3.8
	92	0.05	0.053	107		
	93	5.00	5.0	100		
Goat Milk	97	0	<0.05	-		
	98	0.05	0.043	87	86	15
	99	0.05	0.05	100		
	100	0.10	0.071	71		

(1) All samples are from Test Number RI-MV-001-92 - overall mean of 90% with a SD of 11% (n=61).
(2) Recovery samples were corrected for control residues where present.

TABLE II. PRECISION OF ANALYTICAL METHOD AG-592 AS DEMONSTRATED BY THE RESULTS OF ANALYZING ¹⁴C-CGA-152005 CONTAINING SUBSTRATES

SUBSTRATE	PROTOCOL 26-92 SAMPLE #	(1) FOUND BY AG-592 (ppm)	MEAN (ppm)	RANGE (ppm)	STANDARD DEVIATION
Poultry Lean Meat	59, 60, 61	0.64, 0.62, 0.65	0.63	0.62-0.65	0.015
Eggs	66, 67, 68	0.099, 0.087, 0.088	0.091	0.087-0.099	0.006
Goat Liver	73, 74, 75	1.37, 1.44, 1.42	1.41	1.37-1.44	0.037
Goat Oriental Fat	80, 81, 82	0.082, 0.081, 0.084	0.082	0.081-0.084	0.002
Goat Round	87, 88, 89	0.21, 0.23, 0.22	0.22	0.21-0.23	0.008
Goat Kidney	94, 95, 96	5.4, 5.5, 5.3	5.4	5.3-5.5	0.053
Goat Milk	101, 102, 103	0.055, 0.052, 0.037	0.048	0.037-0.055	0.010

(1) Corrected by % recovery from fresh fortification as shown by Table III for the fresh fortification concentration nearest that of the total ¹⁴C residues.

TABLE III. SUMMARY TABLE FOR EXTRACTABILITY, ANALYSIS, AND ACCOUNTABILITY OF ¹⁴C-CGA-152005 TREATED SUBSTRATES USING METHOD AG-592

A SUBSTRATE	B SAMPLE CODE	C (1) TOTAL RESIDUE	D EXTRACTABILITY		E (3) % EXT	F ANALYSIS		G (5) FRACTION ¹⁴ C CORRECTED (ppm)	H (6) FINAL FRACTION (ppm)	I IIPLC (5) FINAL FRACTION CORRECTED (ppm)	J (7) ACCOUNTABILITY %
			(2) ppm	(4) FINAL FRACTION ¹⁴ C (ppm)							
Poultry Lean Meat	1191260507	0.705	0.82, 0.82, 0.69	110	0.55, 0.53, 0.55	0.60, 0.58, 0.60	0.58, 0.56, 0.59	0.64, 0.62, 0.65	90		
Eggs*	1191260533	0.326	0.24, 0.21, 0.24	72	0.083, 0.081, 0.082	0.096, 0.093, 0.095	0.085, 0.075, 0.076	0.099, 0.087, 0.088	28		
Goat Liver	G90480033	1.371	1.44, 1.38, 1.53	106	1.17, 1.21, 1.22	1.17, 1.21, 1.22	1.37, 1.44, 1.42	1.37, 1.44, 1.42	103		
Goat Oriental Fat	G90480024	0.058	0.080, 0.088, 0.090	148	0.064, 0.062, 0.068	0.080, 0.078, 0.086	0.065, 0.064, 0.067	0.082, 0.081, 0.084	142		
Goat Round	G90480031	0.209	0.25, 0.24, 0.24	116	0.20, 0.20, 0.20	0.20, 0.21, 0.21	0.21, 0.22, 0.22	0.21, 0.23, 0.22	106		
Goat Kidney	G90480026	5.048	4.7, 5.2, 5.6	103	4.7, 4.7, 4.6	4.7, 4.7, 4.6	5.4, 5.5, 5.3	5.4, 5.5, 5.3	107		
Goat Milk	G90480012	0.069	0.054, 0.055, 0.055	79	0.025, 0.025, 0.015	0.035, 0.036, 0.22	0.039, 0.037, 0.026	0.055, 0.052, 0.037	70		

- (1) As determined by combustion analysis in metabolism studies
- (2) Determined by liquid scintillation counting of aliquots of the extract from Section #11 C 11 of AG-592.
- (3) Average of three extractions shown in column D divided by the Total Residue shown in Column C, times 100
- (4) Determined by liquid scintillation counting of aliquots of the final fraction from Section #11 C.3.5
- (5) Corrected by % Recovery from fresh fortification as shown in Table III for the fresh fortification concentration nearest that of the total ¹⁴C residue
- (6) Determined by high performance liquid chromatography.
- (7) Average of Column I, divided by Column C, and multiplied by 100