Study Title

An Analytical Residue Method for the Determination of Tebuconazole and HWG 2061 Residues in Bovine and Poultry Tissues, Milk and Eggs

Data Requirement

EPA Ref.: 171-4 (d), Residue Analytical Method - Animals

Authors

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Performing/Submitting Laboratory

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Mobay Study Number

FR110203

Mobay Report Number

101316

Tebuconazole is the common name for FOLICUR®
FOLICUR® is a Reg. TM of Bayer AG, Germany

101316

Data Confidentially Statement

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA #10(d)(1)(A), (B) or (C).

Company: Mobay Corporation
Agricultural Chemicals Division
Research and Development Department
Environmental Research

Company Agent: _____ Date: 10-15-91
D. R. Flint, Manager

These data are the property of the Agricultural Chemicals Division of Mobay Corporation, and as such, are considered to be confidential for all purposes other than compliance with FIFRA 10. Submission of these data in compliance with FIFRA does not constitute a waiver of any right to confidentiality which may exist under any other statute or in any other country.

Certification of Good Laboratory Practice

The study described in this document meets the requirements of 40 CFR Part 160. A quality assurance statement is presented on page 4 of this report.

Submitter: Mobay Corporation
Agricultural Chemicals Division
Research and Development Department
Environmental Research

D. R. Flint, Manager
Environmental Research
Date: 10-15-91

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J. J. Murphy, Manager
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Date: 10/15/91

Study Director:
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Date: 10/14/91

Certification of Availability of Raw Data

It is hereby certified that the registrant possesses or has access to the raw data identified in Appendix 1 of this report.

Company Agent:
T. J. Olson,
Quality Assurance Specialist
Date: 10/15/91

Quality Assurance Statement

Study Title: An Analytical Residue Method for the Determination of Tebuconazole and HWG 2061 Residues in Bovine and Poultry Tissues, Milk and Eggs

Mobay Study Number: FR110203

Audits of this study were conducted as required by Good Laboratory Practice regulations of FIFRA, Part 160, August 17, 1989. The audits are listed below.

Inspection Date	Phase Inspected	Date Reported to Study Director	Date Reported to Management
03/05/91	Protocol	03/18/91	03/18/91
09/13/91	Sample Set Extraction Bovine Kidney - for Study Number FR060402[1]	10/10/91	10/11/91
09/17/91	Sample Set Extraction Chicken Fat - for Study Number FR060502[1]	10/08/91	10/08/91
10/11/91	Final Report	10/11/91	10/13/91

Based on the audits described above, it is concluded that the results presented in this report accurately describe the methods and standard procedures followed and reflect the raw data generated during the conduct of the study.

Company Agent: _____ Date: 10/15/91

T. J. Olson,
Quality Assurance Specialist

[1] Sample extractions for Study Numbers FR060402 and FR060502 followed procedures in this analytical method.

Certification of Authenticity

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Revisions

Date	Revision

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An Analytical Residue Method for the Determination of Tebuconazole and HWG 2061 Residues in Bovine and Poultry Tissues, Milk and Eggs

1.0 Summary

An analytical residue method has been developed to determine tebuconazole and HWG 2061 residues in animal tissues, milk and eggs. The matrices are extracted by the scheme used in the metabolism experiments, and the extracted conjugated HWG 2061 is hydrolyzed by an overnight acidic reflux. After hydrolysis, tebuconazole and HWG 2061 residues are separated from the sample matrix by gel permeation chromatography, hexane/acetonitrile partitioning and high performance liquid chromatography using both reverse phase and semi-permeable surface columns. Tebuconazole and a t-butyldimethylsilane derivative of HWG 2061 are each analyzed using a medium-polarity capillary gas chromatography column and a nitrogen specific flame ionization detector.

Recovery of tebuconazole and HWG 2061 from bovine and poultry tissues and eggs fortified at 0.1 ppm ranged from 72% to 116%. Recovery of tebuconazole and HWG 2061 ranged from 83% to 106% from milk fortified at 0.05 ppm and from 94% to 102% from milk fortified at 0.1 ppm. Residue levels were less than 0.1 ppm for control tissues and eggs and less than 0.05 ppm for control milk. Thus, the limit of determination for tebuconazole and HWG 2061 in bovine and poultry tissues and eggs is 0.1 ppm. The limit of determination for tebuconazole and HWG 2061 in milk is 0.05 ppm.

2.0 Introduction

The major metabolic pathway for tebuconazole [FOLICUR®, HWG 1608, α-[2-(4-chlorophenyl)ethyl]-α-(1,1-di-methylethyl)-1*H*-1,2,4-triazole-1-ethanol] in lactating goats and chickens was shown to be oxidation to HWG 2061, α-[2-(4-chlorophenyl)-ethyl]-α-[(2-hydroxy-1,1-dimethyl)ethyl]-1*H*-1,2,4-triazole-1-ethanol, with subsequent conjugation of the HWG 2061[1,2].

Based upon these metabolism studies, an analytical residue method[3] was developed for tebuconazole and its major metabolite HWG 2061 in animal tissues milk and eggs.

To decrease the variability and increase the recovery of tebuconazole and HWG 2061 when using this method, significant modifications have been made in sample cleanup and the derivatization of HWG 2061 prior to analysis. The new detailed procedure is presented in this report.

3.0 Experimental

This study was conducted from September, 1990 through September, 1991 at Mobay Research Park located near Stilwell, Kansas. Raw data and the final report are stored at Mobay Corporation.

3.1 Materials

3.1.1 Apparatus

Assorted laboratory glassware.
Dry block heater, 8 x 10 ml, Reacti-Therm or equivalent (Pierce, Rockford, IL).
Fused silica capillary columns: 0.53 mm i.d. x 15 m, DB 17, 1.0 μm film thickness (J & W Scientific, Folsom, CA) and 0.25 mm i.d. x 14 m, DB 17, 0.25 μm film thickness (Quadrex Corporation, New Haven, CT).
Gas chromatograph (glc), Varian 3400 or equivalent, capable of capillary column chromatography and equipped with a "N/P bead" flame ionization detector (Varian Analytical Instruments, Sugar Land, TX).
Gas chromatograph-mass spectrometer, Hewlett Packard 5995C or equivalent (Hewlett Packard, Rolling Meadows, IL)
Gel permeation chromatograph (gpc) equipped with a 60-g Bio-Bead SX-3 column using chloroform/methanol (95:5) as the solvent (ABC Laboratories, Columbia, MO).
High performance liquid chromatograph (hplc), Varian 5000 or equivalent, capable of solvent gradient elution and a variable wavelength uv flow-through detector (uv set at 220 nm). Beckman 163 (Beckman Instruments, Inc., Fullerton, CA) or equivalent.
Hplc semi-permeable surface (SPS) semi-preparative column, 1 cm id x 25 cm, SPS-5PM-100-C8 (Regis Chemical Company, Morton Grove, IL).
Hplc (RP18) guard column, 4.6 mm id x 3 cm Spheri-3, 10 μ (Brownlee Labs Inc., Santa Clara, CA).
Magnetic stirrer/hot plate, Corning or equivalent.
Nelson data processing system or equivalent.
N-Evap analytical evaporator (Organomation Association, Inc., South Berlin, MA) or equivalent.
Rotary vacuum evaporator and water bath.
Tekmar Tissumizer, Model SD-45 (Tekmar Co., Cincinnati, OH) or equivalent.

3.1.2 Reagents/Supplies

Acrodisc® CR PTFE and LC13 PFDF, No. 4219 and No. 4452, respectively, non-sterile, 0.45 μm pore size (Gelman Sciences, Ann Arbor, MI).
Buffer, pH 5. Before use, be sure that buffer salts are in solution. Prepare a 0.6 *M* phosphate buffer in the following manner using sonication to solublize salts. Weigh 163.2 g of potassium dihydrogen phosphate (KH_2PO_4) into a 2-l graduated cylinder, and dilute this solution to volume with distilled water (label this cylinder "A"). Weigh 40.2 g of sodium hydrogen phosphate heptahydrate ($Na_2HPO_4 \cdot 7H2O$) into a 250-ml graduated cylinder, and dilute this solution to volume with distilled water (label this cylinder "B"). Pour 295 ml of solution A into a 500-ml volumetric flask, and add 5 ml of solution B (label flask "C"). Mix the solution thoroughly. Confirm solution C to be pH 5; if not pH 5, adjust solution C with the addition either solution A or B.
Celite filter aid (Fisher Scientific, Pittsburgh, PA) or Hy-Flo Super Cel (Johns Manville, Toledo, OH).
Filter paper, No. 42 (Whatman, Hillsboro, OR).
Glass microfibre filters, GF/A (Whatman).
Glass wool, Pyrex.
Hydrochloric acid (HCl), 2 *N*.
Mega Bond Elute C18 octadecyl disposable column, 10g/60-ml capacity

(Analytichem International, Harbor City, CA)
N-methyl-N-t-butyldimethylsilyl trifluoroacetamide (MTBSTFA) with t-butyldimethylchlorosilane (TBDMCS) silylation reagent (Regis Chemical Company).
Reacti-Vials, 10 ml with Tuf-bond Teflon/silicone septa discs (Pierce Chemical Company).
Sodium hydroxide (NaOH), 19.1 *M*, 50% w/w solution (Mallinckrodt, Paris, KT).
Sodium sulfate, granular, anhydrous, AR grade, No. 8024 (Mallinckrodt).
Solvents, pesticide grade: acetone, acetonitrile, chloroform, hexane and methanol (Burdick & Jackson, Muskegon, MI); silylation grade acetonitrile (Regis Chemical Co.).
Vials, 1.1 ml tapered multipurpose autosampler vial and support sleeve (Varian, Sunnyvale, CA).
Water, distilled or hplc water (Burdick and Jackson).

3.1.3 Standards Required

Analytical standards of tebuconazole and HWG 2061 may be obtained from Mobay Corporation, Agricultural Chemicals Division, Metabolism/Residue Methodology, Mobay Research Park, 17745 S. Metcalf Ave, Stilwell, Kansas 66085.

Tebuconazole Standard: Prepare a primary standard solution of tebuconazole at 500 µg/ml in methanol. From this primary standard, prepare solutions of 5.0 µg/ml, 3.75 µg/ml, 2.5 µg/ml, 1.25 µg/ml and 0.625 µg/ml in methanol.

HWG 2061 Standard: Prepare a primary standard solution of HWG 2061 at 100 µg/ml in methanol. From this primary standard, prepare solutions of 2.0 µg/ml, 1.5 µg/ml, 1.0 µg/ml, 0.5 µg/ml and 0.25 µg/ml in methanol.

Store the standard solutions under refrigerated conditions (2°C); under these conditions, the standard solutions are stable for at least 1 month.

3.2 Method

3.2.1 General Instructions

3.2.1.1 Evaporations

Carry out all evaporations in a 30°C (or less) water bath using a rotary vacuum evaporator except for evaporations in the Reacti-Vials. The Reacti-Vial evaporations are carried out under a stream of nitrogen with the vials placed in an N-Evap water bath at 30°C.

3.2.1.2 SPS Column Calibration

Calibrate the elution parameters of tebuconazole and HWG 2061 from the semi-preparative (SPS) hplc column. Inject 100 µg of each standard in 1 ml of methanol/water (85:15); record the elution volume against uv absorbance. (Note: As the standards are originally in methanol, dilute the tebuconazole standard solution

and concentrate the HWG 2061 standard solution appropriately, and add water to each standard solution to achieve an 85:15, methanol: water ratio). The retention times for HWG 2061 and tebuconazole are approximately 15 min to 19 min and 20 min to 24 min, respectively (Figure 1).

3.2.1.3 GPC Column Calibration

Calibrate the elution parameters of tebuconazole and HWG 2061 from the gpc SX-3 Bio-Bead column. This is achieved by fortifying a liver control extract which has been processed up to the gpc step with 2000 µg of each standard. Evaporate the methanol from the fortified control sample, and re-dissolve the residue in 8 ml of the gpc solvent. Inject 5 ml of the sample onto the gpc column, and collect twenty-three, 10-ml fractions. Evaporate a 1-ml aliquot from each fraction to dryness, and re-dissolve the residue in 1.25 ml of methanol/water (85:15). (Note: Since only 1/10 of each fraction is analyzed, if both standards were in one fraction, the concentration of each standard in the 1.25 ml final volume would be 100 µg/ml.) Analyze each fraction using the hplc analysis conditions described in 3.2.2.6.3 to determine the elution times of the standards from the gpc column eluate.

Establish the "dump" and "collect" range by plotting the percent of the combined standards present in each of the twenty-three, 10-ml fractions (See Figure 2). Starting from the last 10-ml fraction which contained the standards and moving to earlier fractions in the series of fractions, total the percent recovered until at least 90% has been reached. This set of fractions represents the "collect" fraction. In this manner, the majority of the control sample matrix will be excluded from the "collect" fraction and will be in the "dump" fraction.

When the liver extract is chromatographed, fraction 8 (70 ml to 80 ml) or fraction 9 (80 ml to 90 ml) will be dark brown in color. Normally these fractions will not contain any of the standards and will be discarded in the "dump" fraction. The color in fractions 11 through 15 will be gold to light yellow; these fractions usually contain the start of the "collect" fraction. The "wash" fraction is arbitrarily set for a 60 ml volume following the "collect" fraction volume.

3.2.2 Detailed Procedure (see Figure 3 for flow diagram)

3.2.2.1 Extraction

3.2.2.1.1 Liver, Kidney, Muscle, Milk and Egg

1. Weigh a 40-gram sample into a 300-ml tall-form beaker, and add 150 ml of methanol.

2. Blend the sample with a Tekmar blender for 2 min at high speed.

3. Filter the homogenate under vacuum through a No. 42 Whatman filter paper covered with a bed (3 gram) of Hyflo Super Cel into a 500-ml side-arm vacuum flask.

4. Return the filter cake (including filter paper) to the blending jar, and add 150 ml of methanol.

5. Blend the filter cake and filter paper with a Tekmar blender for 2 min at high speed.

6. Filter the homogenate under vacuum through a No. 42 Whatman filter paper covered with a bed (3 gram) of Hyflo Super Cel into the same 500-ml side-arm vacuum flask.

   Note: If the combined filtrate has particulate matter present, filter the combined filtrate under vacuum through No. 42 filter paper (no Super Cel) into another 500-ml side-arm vacuum flask.

7. Transfer the filtrate into a 1000-ml separatory funnel, and add 150 ml of hexane (pre-saturated with acetonitrile) to the funnel.

8. Stopper and shake the funnel for 30 sec; allow the phases to separate.

9. Drain the lower methanol/water fraction into a pre-weighed 1000-ml boiling flask labeled A.

10. Add 150 ml of acetonitrile (pre-saturated with hexane) to the separatory funnel.

11. Stopper and shake the funnel for 30 sec; allow the phases to separate.

12. Drain the lower acetonitrile fraction into the flask labeled A from Step 9.

13. Evaporate the sample until only the water remains.

    Note: This is a very crucial step. Part of the sample may be lost if the sample is allowed to foam up when the solution has evaporated to the water. Watch the sample closely when the volume is low. Higher water bath temperatures seem to make the foaming worse. The final volume of the sample should be concentrated to approximately 35 ml (35 g). This can be determined by weighing the flask and subtracting the pre-weighed flask weight. DO NOT add additional acetonitrile to the sample to aid the evaporation. Use a nitrogen stream to concentrate the sample if foaming is bad.

14. Proceed to Acid Hydrolysis (3.2.2.2).

3.2.2.1.2 Fat and Skin

Note: In the following procedure, the acetonitrile is pre-saturated with hexane, and the hexane is pre-saturated with acetonitrile.

1. Weigh a 40-gram sample into a 300-ml tall form beaker, and add 150 ml of hexane.
2. Blend the sample with a Tekmar blender for 2 min at high speed.
3. Filter the homogenate under vacuum through a No. 42 Whatman filter paper covered with a bed (3 gram) of Hyflo Super Cel into a 500-ml side-arm flask.
4. Return the filter cake (including filter paper) to the blending jar, and add 150-ml of fresh hexane.
5. Blend the filter cake and filter paper with a Tekmar blender for 2 min at high speed.
6. Filter the homogenate under vacuum through a No. 42 Whatman filter paper covered with a bed (3 gram) of Hyflo Super Cel into the same 500-ml side-arm flask.

   Note: If the combined filtrate has particulate matter present, filter the combined filtrate under vacuum through No. 42 filter paper (no Super Cel) into another 500-ml side-arm vacuum flask.

7. Transfer the filtrate to a 1000-ml separatory funnel, and add 300 ml of acetonitrile into the funnel.
8. Stopper and shake the funnel for 30 sec; allow the phases to separate.
9. Drain the lower acetonitrile fraction into a 1000-ml boiling flask (labeled A).
10. Add 300 ml of acetonitrile to the separatory funnel.
11. Stopper and shake the funnel for 30 sec; allow the phases to separate.
12. Drain the lower acetonitrile fraction into the boiling flask A from Step 9.
13. Return the filter cake and filter paper (Step 6) to the blender jar, add 100 ml of methanol, and blend the sample with a Tekmar blender for 2 min at high speed.
14. Filter the homogenate under vacuum through a No. 42 Whatman filter paper covered with a bed (3 gram) of Hyflo Super Cel into the

side-arm flask from Step 6.

Note: If the filtrate has particulate matter present, filter the filtrate under vacuum through No. 42 filter paper (no Super Cel) into another 500-ml side-arm vacuum flask.

15. Combine the methanol fraction from Step 14 with the combined acetonitrile fractions in boiling flask A from Steps 9 and 12.

16. Evaporate the combined sample to dryness.

17. Proceed to Acid Hydrolysis (3.2.2.2).

3.2.2.2 Acid Hydrolysis

1. Add 50 ml of 2*N* HCl to the sample in boiling flask A from the initial extraction (Step 13 of 3.2.2.2.1 or Step 16 of 3.2.2.1.2).

2. For fat or skin samples, add 30 ml of distilled water into boiling flask A.

3. Add five to ten glass boiling beads to the flask.

Note: Use only boiling beads, do not use a stirring bar.

4. Attach flask A to a condenser, bring the sample to reflux and continue to reflux the sample for 16 hours (or overnight).

5. Allow the sample to cool to room temperature, and remove flask A from the condenser.

6. Add 20 ml of pH 5, 0.6 *M* phosphate buffer to the sample in flask A.

7. Place flask A in an ice bath, and allow the solution to cool for 3 to 5 min.

8. While swirling flask A, slowly pipet 5.0 ml of 19.1 *M* NaOH into the aqueous solution.

Note: Before proceeding with the following steps, confirm that the aqueous solution is pH 5 ± 0.5 using a universal pH indicator paper. If the pH is not within this range, adjust pH with 2*N* HCl or 19.1 *M* NaOH.

9. Pour the sample into a 1000-ml separatory funnel labeled A.

10. Rinse flask A with 150 ml of acetone, and pour the acetone into separatory funnel A.

11. Rinse flask A with 225 ml of chloroform, and pour the chloroform into separatory funnel A.

12. Stopper and shake the separatory funnel for 30 sec. Allow the

phases to separate, and drain the lower organic fraction into a second 1000-ml separatory funnel labeled B containing 75 ml of distilled water.

13. Stopper and shake separatory funnel B for 30 seconds. Allow the phases to separate, and drain the lower organic fraction through 100 g of sodium sulfate [pre-rinsed with 50 ml of acetone/chloroform (2:3)] into a 1000-ml boiling flask labeled B.

14. Rinse flask A again with 150 ml of acetone, and pour the acetone into separatory funnel A.

15. Rinse flask A again with 225 ml of chloroform, and pour the chloroform into separatory funnel A.

16. Stopper and shake separatory funnel A for 30 sec. Allow the phases to separate, and drain the lower organic fraction into separatory funnel B.

17. Stopper and shake separatory funnel B for 30 sec. Allow the phases to separate, and drain the lower organic fraction through the same 100 g of sodium sulfate into boiling flask B.

18. Evaporate the combined organic fractions to dryness. Sweep the flask with a nitrogen stream to completely remove any solvent.

19. For liver, kidney and muscle samples:

    a. Add 0.5 ml of methanol to the flask to solublize the sample residue, and transfer the sample to a 13-ml centrifuge tube.

    b. Add 5 ml of chloroform to the flask, swirl the sample to thoroughly mix the solution, and transfer the solution into the tube.

    c. Complete the sample transfer from the flask into the tube with an additional 5 ml of chloroform.

    d. Concentrate the sample to 8.0 ml using a stream of nitrogen.

    e. Proceed to Gel Permeation Chromatography (3.2.2.3)

20. For milk, eggs, fat and skin samples, proceed to Hexane/Acetonitrile Partition (3.2.2.4).

3.2.2.3 Gel Permeation Chromatography

1. Draw the sample from the 13-ml centrifuge tube (Step 19d of 3.2.2.2) into a 10-ml syringe (with a long needle), place a 0.45 µm Acrodisc CR filter on the syringe, and filter the sample into a 25-ml beaker.

   Note: The solution must be free from any particulates.

2. With another syringe, withdraw the sample from the beaker, and inject the sample into a 5-ml sample loop of the gel chromatograph.

3. Place the sample collection dispensing line into a 125-ml boiling flask.

4. Repeat Steps 1 through 3 for each sample.

5. Initiate the chromatography process using the following gpc parameters:

Flow rate:	5 ml/min
Column pressure:	Approximately 5 psi.
Solvent system:	Chloroform/methanol (95:5)
Elution parameters:	Use elution parameters determined by calibration prior to analysis (See 3.2.1.3)

6. Evaporate the "collect" fraction in the 125-ml boiling flask just to an oily film. Sweep the flask with a nitrogen stream to completely remove any solvent.

   Note: If the sample will not be analyzed within the day, store the sample under refrigeration (2°C) until analysis can be performed. The residue is stable for at least 3 days under these conditions.

7. Proceed to Hexane/Acetonitrile Partition (3.2.2.4)

3.2.2.4 Hexane/Acetonitrile Partition

Note: In the following procedure, the acetonitrile is pre-saturated with hexane, and the hexane is pre-saturated with acetonitrile.

1. To the oily residue in the 125-ml boiling flask (Step 6 of 3.2.2.3), add 25 ml of hexane, and swirl the solvent in the flask. Pour the solvent into a 500-ml separatory funnel labeled A. Transfer as much of the remaining sample residue as possible with an additional 75 ml of hexane.

2. Add 25 ml of acetonitrile into the 125-ml boiling flask, and swirl the solvent in the flask. Pour the solvent into separatory funnel A. Complete the quantitative transfer of the sample residue with an additional 75 ml of acetonitrile.

3. Stopper and shake separatory funnel A for 30 sec; allow the phases to separate.

4. Drain the lower acetonitrile fraction into another 500-ml separatory funnel labeled B containing 100 ml of hexane.

5. Stopper and shake separatory funnel B for 30 sec; allow the phases to separate.

6. Drain the lower acetonitrile fraction into a 500-ml boiling flask.

7. Repeat Steps 2 through 6 (combining the acetonitrile fractions in the same flask) twice.

8. Evaporate the combined 300 ml of acetonitrile in the 500-ml boiling flask to dryness.

9. Proceed to Mega Bond Elute Chromatography (3.2.2.5)

3.2.2.5 Mega Bond Elute Chromatography

1. Activate the column as follows:

   a. Place the lower column luer fitting from the on/off valve into the collection needle on a vacuum manifold (or a 250-ml side arm vacuum flask with a rubber stopper containing a long needle with a luer top).

   b. Rinse the column under vacuum with a total of 75 ml of methanol at a flow rate of approximately 15 ml per min (5 min total time).

   c. After the methanol has reached the top of the column bed, rinse the column with 75 ml of distilled water at a flow rate of approximately 15 ml per min.

   d. When the water reaches the top of the column bed, turn the column valve off. Do not allow the column to go dry.

2. Add 25 ml of methanol/water (3:7) to the 125-ml flask (Step 8 of 3.2.2.4). Swirl the solvent in the flask, and pour the extract into the column.

3. Open the valve on the column to allow the solvent to drain through the packing at a rate of approximately 5 ml/min (5 min total time).

4. While the column is draining, add another 25 ml of methanol/water (3:7) to the 125-ml flask. Swirl the solvent in the flask.

5. When the solvent has reached the top of the column bed, pour the additional solvent into the column.

6. Repeat Steps 4 and 5.

7. When the solvent has reached the top of the column bed, turn the column valve off. Discard the 75 ml of methanol/water eluate.

8. Place the column onto another vacuum manifold (or flask) as before.

9. Add 25 ml of methanol/water (85:15) to the 125-ml flask (Step 2). Swirl the solvent in the flask, and pour the solvent into the column.

10. Open the valve on the column to allow the solvent to drain through the packing into the collection flask at a rate of approximately 5 ml/min (5 min total time).

11. While the column is draining, add another 25 ml of methanol/water (85:15) to the 125-ml flask. Swirl the solvent in the flask.

12. When the solvent has reached the top of the column bed, pour the additional solvent into the column.

13. When the solvent has reached the top of the column bed, pour an additional 50 ml of methanol/water (85:15) into the column. When the solvent reaches the top of the column bed, turn the column valve off.

14. Transfer the methanol/water (85:15) eluate into a 250-ml boiling flask.

15. Evaporate the sample to the aqueous residue. Add 150 ml of acetonitrile to azeotrope the water from the sample. Evaporate the sample to dryness.

16. Add 5 ml of methanol to the flask, and swirl (vortex) the solvent to solublize the residue. Transfer the methanol into a 13-ml centrifuge tube using a Pasteur pipet. Repeat the transfer procedure two more times using approximately 2.5 ml of methanol each time.

17. Evaporate the methanol to just less than 2 ml using the N-Evap with a stream of nitrogen.

18. Proceed to SPS Column Chromatography (3.2.2.6).

3.2.2.6 SPS Column Chromatography

3.2.2.6.1 Liver, Kidney and Muscle

1. Dilute the sample in the 13-ml centrifuge tube (Step 17 of 3.2.2.5) to 2.1 ml with methanol. Rotate the solvent to the top of the tube to solublize all the residue. Vortex the sample to mix the solution.

2. Add 0.4 ml of hplc grade water to bring the total volume to 2.5 ml, and swirl the sample. Vortex the sample to mix the solution.

3. Draw the sample from the centrifuge tube into a 5-ml syringe (with a long needle), place a 0.45 µm Acrodisc LC13 filter on the syringe, and filter the sample into another 13-ml centrifuge tube.

   Note: If the filter plugs and creates pressure during filtration, change filters and proceed with filtration.

4. Proceed to Chromatography Process (3.2.2.6.3).

3.2.2.6.2 Milk, Egg, Fat and Skin

1. Dilute the sample in the 13-ml centrifuge tube (Step 17 of 3.2.2.5) to 3.4 ml with methanol. Rotate the solvent to the top of the tube to solublize all the residue. Vortex the sample to mix the solution.

2. Add 0.6 ml of hplc grade water to bring the total volume to 4.0 ml, and swirl the sample. Vortex the sample to mix the solution.

3. Draw the sample from the centrifuge tube into a 5-ml syringe (with a long needle), place a 0.45 µm Acrodisc LC13 filter on the syringe, and filter the sample into another 13-ml centrifuge tube.

   Note: If the filter plugs and creates pressure during filtration, change filters and proceed with filtration.

4. Proceed to Chromatography Process (3.2.2.6.3).

3.2.2.6.3 Chromatography Process

1. Initiate the chromatography process using the following hplc parameters:

   Mobile phase solvents: Methanol and hplc grade water.
   Solvent flow rate: 2 ml/min
   UV detector wavelength: 220 nm.
   Solvent program: Start with a linear gradient of 60% methanol to 80% methanol in 20 min, followed by a linear gradient of 80% methanol to 100% methanol in 15 min. Maintain 100% methanol for 15 min.

   Allow the column to equilibrate at 60% methanol for 30 min before making the next injection.

2. Inject 1.2 ml of the sample solution to be chromatographed (Step 3 of 3.2.2.6.1 or 3.2.2.6.2) into the 1-ml hplc injection loop. Store the remaining sample solution under refrigeration (2°C) as a backup sample.

3. Collect the individual eluents for HWG 2061 and tebuconazole (based upon predetermined elution times, see 3.2.1.2) in 125-ml pear-shaped flasks. Label the flasks appropriately for HWG 2061 and tebuconazole.

4. Evaporate the solvent in each 125-ml flask to the aqueous solution. Add 30 ml of acetonitrile to the flask, and evaporate the sample to dryness.

5. To the flask labeled tebuconazole:

   a. Add 3 to 5 ml of methanol; swirl (vortex) the solvent to solublize the residue. Transfer the methanol into a 13-ml

centrifuge tube using a Pasteur pipet.

b. Repeat the procedure two more times using approximately 2.5 ml of methanol each time.

c. Evaporate the sample to dryness using the N-Evap with a stream of nitrogen.

d. Add 400 µl of methanol to the tube, stopper, and wrap Parafilm around the tube/stopper joint; vortex the solution to thoroughly solubilize the sample.

e. Using a Pasteur pipet, transfer the solution into a 1.1 ml tapered autosampler vial (with support sleeve). Cap and store the sample under refrigeration (2°C) until the sample is analyzed.

f. Proceed to Analysis (3.2.2.8).

6. To the flask labeled HWG 2061:

a. Add 3 to 5 ml of methanol; swirl (vortex) the solvent to solublize the residue. Transfer the methanol into a 10-ml Reacti-Vial using a Pasteur pipet.

b. Repeat the procedure two more times using approximately 2 ml of methanol each time.

c. Evaporate the methanol to dryness using the N-Evap with a stream of nitrogen.

d. Add 0.5 to 1 ml of acetonitrile to the vial, and rotate the solvent to the top of the vial to solublize all residue.

e. Evaporate the acetonitrile to dryness using the N-Evap with a stream of nitrogen.

f. Proceed to Derivatization (3.2.2.7)

3.2.2.7 Derivatization

Note: Start a 0.1 ppm HWG 2061 standard at this step. Pipet 1 ml of a 1 µg/ml HWG 2061 standard (see 3.1.3) into a 10-ml Reacti-Vial (for the total standard curve, pipet 1 ml of the remaining standards described under 3.1.3 into separate vials). Evaporate the methanol in the vial(s) with a nitrogen stream.

If additional auto-sampler vials containing the derivatized 0.1 ppm HWG 2061 standard are needed during analysis when a gc auto-injector is used, pipet 5 ml of the 2.0 µg/ml HWG 2061 standard into a Reacti-Vial. Evaporate the methanol in the vial with a nitrogen stream. After the derivatization

has been completed (Step 6 below), add 4.0 ml of methanol to the Reacti-Vial to provide a sufficient volume to fill each of ten, 1.1 ml gc autosampler vials with 400 µl of the derivatized HWG 2061 standard.

1. Add 0.5 ml of derivatization grade acetonitrile to the Reacti-Vial (Step 6 of 3.2.2.6 and the 0.1 ppm HWG 2061 standard started at this step), and slowly vortex the solution to solublize the residue.

2. Add 0.5 ml of MTBSTFA (with 1% TBDMCS) to the Reacti-Vial, place the septum on the vial (Teflon side down), screw the cap on tightly, and vortex the solution to mix the sample well.

3. Heat the Reacti-Vial at 90°C for 90 min in a heating block (pre-heated at 90°C).

4. Remove the Reacti-Vial from the heating block, and allow the Reacti-Vial to cool to room temperature.

5. Evaporate the reaction solvents completely in the N-Evap under a stream of nitrogen.

   Note: The sample solution in the vial must be taken to dryness. If the sample is not dry, reagent blank peaks may occur during gc chromatography.

6. Add 400 µl of methanol to the vial, place the septum on the vial (Teflon side down), screw the cap on tightly, and vortex the solution to thoroughly solublize the sample.

7. Using a Pasteur pipet, transfer the solution into a 1.1 ml tapered autosampler vial (with support sleeve). Cap and store the sample under refrigeration (2°C) until the sample is analyzed.

8. Proceed to Analysis (3.2.2.8).

3.2.2.8 Analysis

3.2.2.8.1 Standard Procedure

A. Instrument Conditions:

Detector:	Flame ionization "N/P bead detector". Air: 170 ml/min. Hydrogen: 4.5 ml/min.
Column:	Fused silica capillary column, 0.53 mm i.d. x 15 m, DB 17, 1.0 µm film thickness.
Carrier gas:	Nitrogen, 8 ml/min.
Temperatures:	Injection port: 250°C Detector: 300°C

Column program: Hold 70°C for 0.5 min,
Ramp 25°C/min to 125°C,
Hold 125° for 0.5 min,
Ramp 7.5°C/min to 275°C,
Hold 275°C for 6.8 min.

B. Procedure:

1. Inject 5 µl of the 0.1 ppm equivalent standard solution (2.5 µg tebuconazole/ml, 1 µg HWG 2061 derivative/0.4 ml) before and after each sample injection. Determine the area under the tebuconazole or derivatized HWG 2061 peaks at their respective retention times (approximately 22 min or 26 min, respectively).

2. Inject 5 µl of sample (10 g equivalent/0.4 ml). Determine the area of any peak at the retention times of tebuconazole or derivatized HWG 2061.

3. If the sample response is greater than the standard response, dilute the sample appropriately to correspond to the standard, and re-inject the diluted sample.

C. Standard Curves:

1. To show that the detector response is linear, inject 5 µl of each tebuconazole standard of 0.625 µg/ml, 1.25 µg/ml, 2.5 µg/ml, 3.75 µg/ml and 5.0 µg/ml (see 3.1.3); inject each derivatized HWG 2061 standard of 0.25 µg/0.4 ml, 0.5 µg/0.4 ml, 1.0 µg/0.4 ml, 1.5 µg/0.4 ml and 2.0 µg/0.4 ml (see 3.2.2.7).

2. Plot area versus concentration to confirm a linear response. The five standard concentrations above represent 0.025 ppm, 0.05 ppm, 0.10 ppm, 0.15 ppm and 0.20 ppm tebuconazole or HWG 2061 standard equivalents.

D. Calculations:

1. Calculate ppm by comparing the response (peak height, peak area, microvolts, etc.) for a sample to the average response of a corresponding standard (before and after each sample).

$$ppm = \frac{\text{response (spl)}}{\text{response (std)}} \times \frac{\text{ng std injected}}{\text{g spl weight}} \times \frac{\text{final spl vol (µl)}}{\text{spl injected vol (µl)}} \times \frac{\text{g spl weight}}{\text{g final spl weight}} \times \frac{\text{final vol dilution factor}}{1}$$

Note: This equation reduces to the equation below when the sample is compared to the 2.5 µg/ml standards.

$$ppm = \frac{\text{response (spl)}}{\text{response (std)}} \times 0.1 \text{ ppm} \times \text{dilution factor}$$

2. To convert HWG 2061 ppm residues to tebuconazole ppm equivalent residues, use the following equation:

   ppm tebuconazole (equivalent) = ppm HWG 2061 x 0.95

3.2.2.8.2 Confirmatory Procedure

A. Instrument Conditions:

Detector:	MSD SIM	
Ions Monitored:	Tebuconazole:	125, 250, 307
	HWG 2061:	125, 250, 306, 380, 437
Column:	Fused silica capillary column, 0.25 mm i.d. x 14 m, DB 17, 0.25 μm film thickness	
Carrier gas:	Helium, 1.6 ml/min.	
Temperatures:	Injection port:	250°C
	Mass analyzer:	250°C
	Transfer line:	250°C
	Ion source:	250°C
Column purge:	2 min	
Column program:	tebuconazole:	hold 180°C for 2 min, ramp 5°C/min to 210°C, ramp 20°C/min to 250°C,
	HWG 2061:	hold 180°C for 1 min, ramp 10°C/min to 250°C, hold at 250°C for 2 min

B. Procedure:

1. Inject 2 μl of the standard (2.5 μg/ml) before and after each sample injection. Determine the area under the peak at the retention time for tebuconazole (approximately 4 min) and derivatized HWG 2061 (approximately 6 min).

2. Inject 2 μl of sample (10 g equivalent/0.4 ml). Determine the area of any peak at the retention time for tebuconazole or derivatized HWG 2061.

3. If the sample response is greater than the standard response, dilute the sample appropriately to correspond to the standard response.

C. Standard Curves:

1. To show that the response is linear, inject 2 μl of each

tebuconazole standard of 0.625 µg/ml, 1.25 µg/ml, 2.5 µg/ml; 3.75 µg/ml and 5.0 µg/ml (see 3.1.3); inject each derivatized HWG 2061 standard of 0.25 µg/0.4 ml, 0.5 µg/0.4 ml, 1.0 µg/0.4 ml, 1.5 µg/0.4 ml and 2.0 µg/0.4 ml (see 3.2.2.7).

2. Plot the response versus concentration to confirm a linear response. The five standard concentrations above represent 0.025 ppm, 0.05 ppm, 0.10 ppm, 0.15 ppm and 0.20 ppm tebuconazole or HWG 2061 standard equivalents.

D. Calculations:

See calculation procedure under Standard Analysis Procedure.

3.3 Method Validation

3.3.1 Requirements

1. Duplicate recoveries at 0.1 ppm in all tissues and eggs and duplicate recoveries at 0.05 ppm in milk for both tebuconazole and HWG 2061 are required.

2 Each recovery sample is analyzed with the appropriate 0.1 ppm standard.

3.3.2 Procedure

1. Add 1 ml of the tebuconazole standard solution (4 µg/ml methanol) and 1 ml of the HWG 2061 standard solution (4 µg/ml methanol) to each recovery sample just prior to adding the blending solvent to the weighed tissue or egg sample (3.2.2.1). In the case of milk, add 0.5 ml of each standard solution.

2. Run two control samples for each sample matrix. Run a reagent blank with each matrix set.

3. Run standard curves from 0.025 ppm to 2.0 ppm for tebuconazole and HWG 2061 to show linearity of response.

4. Run the method as written with no modifications; each "cleanup step" was needed in this method to a achieve an adequate control residue.

4.0 Results and Discussion

A flow diagram of the analytical residue method is presented in Figure 3.

The initial extraction (methanol or hexane/methanol) and hydrolysis procedures that were used in the bovine and poultry metabolism studies[1,2] were used in this analytical residue method. Under these conditions, tebuconazole and the major metabolite, HWG 2061 or HWG 2061 conjugate, were extracted from the tissues, milk and eggs, and the HWG 2061 conjugate was hydrolyzed. Good extraction efficiency was shown using aged [^{14}C] tebuconazole and [^{14}C] HWG 2061 residues from the metabolism study animal tissues, milk and eggs (Addendum 1).

The acid reflux hydrolysis procedure that was used produced many low molecular weight nitrogen containing organic compounds from the animal tissues particularly from liver and kidney. This created a siginificant matrix cleanup problem, because the detection of tebuconazole and HWG 2061 was based upon the nitrogen response for these compounds using a flame ionization "nitrogen/phosphorous bead" (FID N/P) detection system.

Thus, an elaborate cleanup procedure was required to separate these natural control interferences from the tebuconazole and HWG 2061 residues. After hydrolysis, the majority of the sample matrix was successfully removed by gel permeation chromatography, a hexane/acetonitrile partition and reverse phase column chromatography. A final purification combined with the separate collection of the tebuconazole and HWG 2061 residues utilized hplc semi-permeable surface column chromatography prior to derivatization of the HWG 2061 and glc analysis.

In the previous analytcal method[3], Regisil, bis (trimethylsilyl)-trifluoro-acetamide (BSTFA), was used to form the trimethylsilyl (TMS) derivative of HWG 2061. Because of low and variable recovery of the TMS HWG 2061 derivative in some tissues, another derivative was selected for this method. N-methyl-N-t-butyl-dimethlylsilyl trifluoroacetamide (MTBSTFA), a reagent reported to produce a derivative more stable to hydrolysis than the TMS derivative, was selected. The t-butyl-dimethylsilyl (TBDMS) derivative of HWG 2061 was formed within 90 min and was shown to be stable with reproducible recovery.

The retention times for tebuconazole and derivatized HWG 2061 from the medium polarity capillary column were approximately 19 min and 23 min, respectively (Figure 4). The instrumental response and linearity of tebuconazole and HWG 2061 is presented in Figure 5. The response for both compounds may increase or decrease from from day to day, most likely from small changes in the hydrogen flow in the flame detector. However, this variation does not affect the linearity of the response for these compounds. The response for both tebuconazole and the TBDMS HWG 2061 derivative in the presence of tissue, milk and egg matrices was shown to be linear over the range (0.025 ppm to 0.2 ppm) tested (Figures 6 through 8).

Recovery of tebuconazole from bovine tissues fortified at 0.1 ppm ranged from 76% to 101% (Table 1). Recovery of tebuconazole from milk fortified at 0.05 ppm was 105% and 106%, and recovery of tebuconazole from milk fortified at 0.1 was 94% and 101%. In bovine tissues, recovery of HWG 2061 fortified at 0.1 ppm ranged from 72% to 89%. Recovery of HWG 2061 from milk fortified at 0.05 ppm was 83% and 93% and at 0.1 ppm was 102% and 102%. At the retention times for both compounds, control bovine tissues and milk samples showed residue levels of less than 0.1 ppm and 0.05 ppm, respectively.

Recovery of tebuconazole from poultry tissues and eggs fortified at 0.1 ppm ranged from 87% to 116% (Table 2). Recovery of HWG 2061 from tissues and eggs fortified at 0.1 ppm ranged from 71% to 114%. Control values at the retention times for both compounds in poultry tissues and eggs were less than 0.1 ppm.

Based upon these data, the limit of determination for tebuconazole and HWG 2061 in bovine and poultry tissues and eggs is 0.1 ppm. The limit of determination for tebuconazole and HWG 2061 in milk is 0.05 ppm.

Gas chromatography/mass spectrometry (gc/ms) using selected ion monitoring (SIM) was used for the confirmatory analysis procedure. Based upon the mass spectra of tebuconazole and the TBDMS HWG 2061 derivative (Figure 9), mass ions 125, 250 and 307 were used for tebuconazole and mass ions 125, 250, 306, 380 and 437 were used for the HWG 2061 derivative. The retention times for tebuconazole and the derivatized HWG 2061 were approximately 3.9 min and 6.2 min, respectively (Figure 10). The instrumental responses of tebuconazole and HWG 2061 were shown to be linear from 0.05 ppm to 2.0 ppm (Figure 11).

An independent laboratory method validation using this method was successful (Addendum 2). Recoveries for tebuconazole and HWG 2061 ranged from 72% to 109% for liver and from 82% to 107% for milk.

The method was shown to be specific for tebuconazole and HWG 2061 with respect to all the other compounds which have been registered by EPA for tolerances in bovine and poultry meat, fat and by-products, milk and milk fat, and eggs (Addendum 3).

5.0 Conclusions

A successful gas chromatographic method has been developed for the determination of tebuconazole and HWG 2061 in bovine and poultry tissues, milk and eggs as proven by acceptable recovery of tebuconazole and HWG 2061, by good extraction efficiency of aged residues, and by being specific for tebuconazole and HWG 2061 residues.

The method required extensive sample cleanup following extraction and rigorous acid hydrolysis. In addition, the instrumental response was minimal for the nitrogens in tebuconazole and HWG 2061 using the nitrogen specific detector. Hence, a limit of determination less than 0.1 ppm for tissues and 0.05 ppm for milk would be very difficult to obtain for tebuconazole and HWG 2061.

Because of the overnight hydrolysis, lengthy cleanup procedures, and derivatization prior to analysis, this method takes approximately 4 days to complete a set of six samples. Eight different individuals have successfully run this method and have recorded acceptable recoveries by this method.

The limit of determination for tebuconazole and HWG 2061 for tissues and eggs is 0.1 ppm and for milk is 0.05 ppm.

6.0 Bibliography

1. Lee, S. G. K. and S. E. Wood. 1987. The metabolism of FOLICUR in dairy goats. Mobay Ag Chem Report No. 94882. MRID 41717402

2. Lee, S. G. K. and L. A. Hanna, K. Johnston, S.E. Wood and W. M. Leimkuehler. 1988. The metabolism of ^{14}C FOLICUR in chickens. Mobay Ag Chem Report No. 87156. MRID 41714404

3. Leimkuehler, W. M., B. P. Smyzer, S. E. Wood, C. A. Lenz and J. L. Delk. 1988. Analytical method for the determination of FOLICUR and HWG 2061 residues in bovine and poultry tissues, milk and eggs. Mobay Ag Chem Report No. 97468. MRID 40995931.

Table 1. Recovery[1] of tebuconazole and HWG 2061 from bovine liver, kidney, muscle, fat and milk.

Matrix	Compound	Ppm Fortification	Control Residue (ppm) Sample Recovery (%)
Liver	Control	none	<0.1, <0.1
	tebuconazole	0.10	89, 91
	Control	none	<0.1, <0.1
	HWG 2061	0.10	72, 78
Kidney	Control	none	<0.1, <0.1
	tebuconazole	0.10	76, 78
	Control	none	<0.1, <0.1
	HWG 2061	0.10	77, 89
Muscle	Control	none	<0.1, <0.1
	tebuconazole	0.10	91, 101
	Control	none	<0.1, <0.1
	HWG 2061	0.10	89, 81
Fat	Control	none	<0.1, <0.1
	tebuconazole	0.10	91, 84
	Control	none	<0.1, <0.1
	HWG 2061	0.10	87, 78
Milk	Control	none	<0.1, <0.1
	tebuconazole	0.10	94, 101
	tebuconazole	0.05	105, 106
	Control	none	<0.1, <0.1
	HWG 2061	0.10	102, 102
	HWG 2061	0.05	83, 93

[1]For raw data and chromatograms, see Appendices 2 to 6.

Table 2. Recovery[1] of tebuconazole and HWG 2061 from poultry liver, muscle, fat, skin and eggs.

Matrix	Compound	Ppm Fortification	Control Residue (ppm) Sample Recovery (%)
Liver	Control	none	<0.1, <0.1
	tebuconazole	0.10	98, 90
	Control	none	<0.1, <0.1
	HWG 2061	0.10	95, 86, 117
Muscle	Control	none	<0.1, <0.1
	tebuconazole	0.10	116, 113
	Control	none	<0.1, <0.1
	HWG 2061	0.10	114, 94
Fat	Control	none	<0.1, <0.1
	tebuconazole	0.10	87, 89
	Control	none	<0.1, <0.1
	HWG 2061	0.10	74, 92
Skin	Control	none	<0.1, <0.1
	tebuconazole	0.10	96, 106
	Control	none	<0.1, <0.1
	HWG 2061	0.10	95, 95
Eggs	Control	none	<0.1, <0.1
	tebuconazole	0.10	87, 91
	Control	none	<0.1, <0.1
	HWG 2061	0.10	86, 71

[1]For raw data and chromatograms, see Appendices 7 to 11.

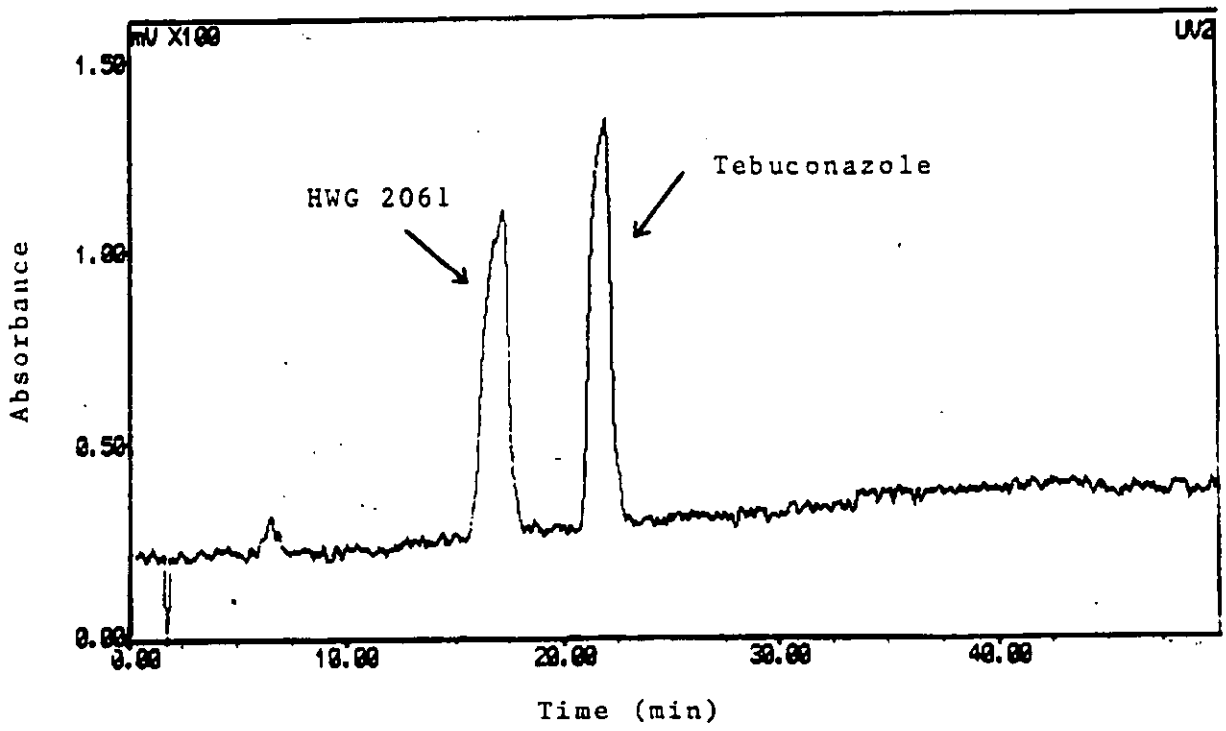


Figure 1. Hplc chromatogram of the elution profile of tebuconazole and HWG 2061 from the semi-permeable surface preparative column.

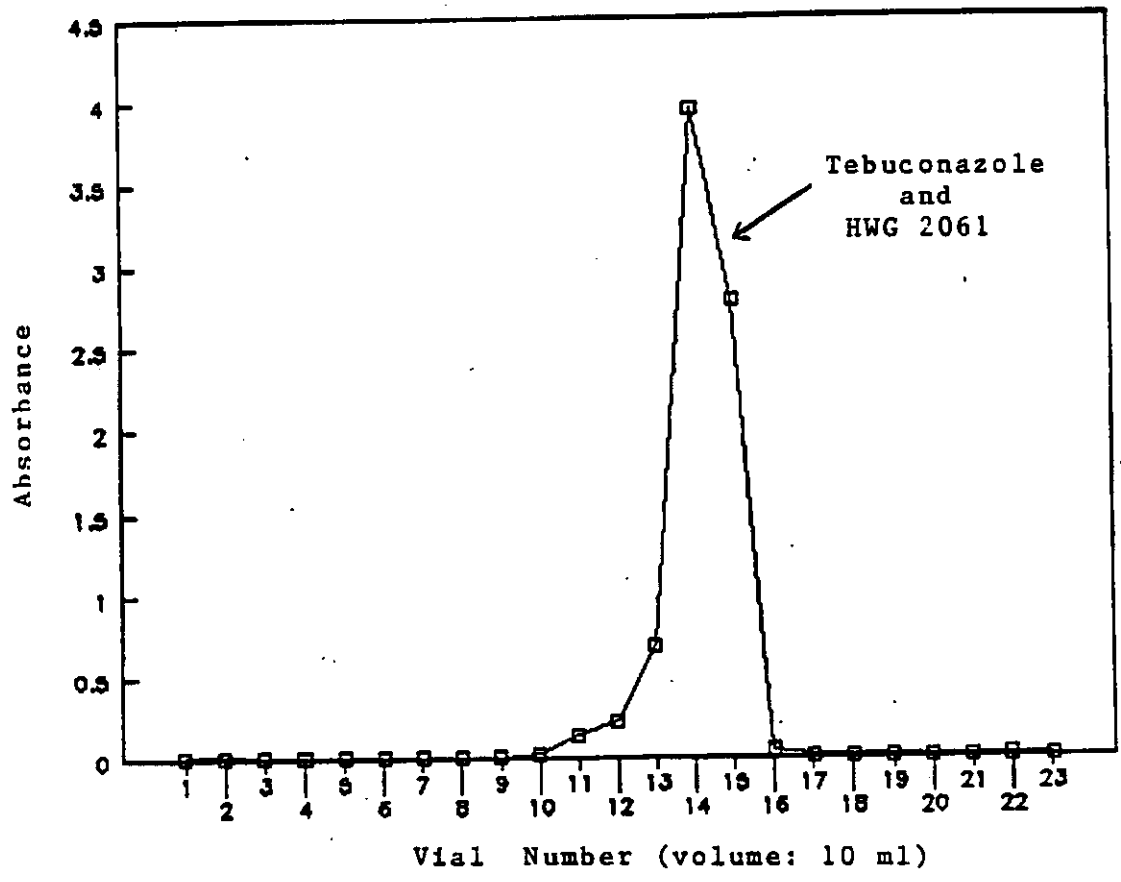


Figure 2. Gpc elution profile of tebuconazole and HWG 2061 from the Bio-Bead SX-3 permeation column.

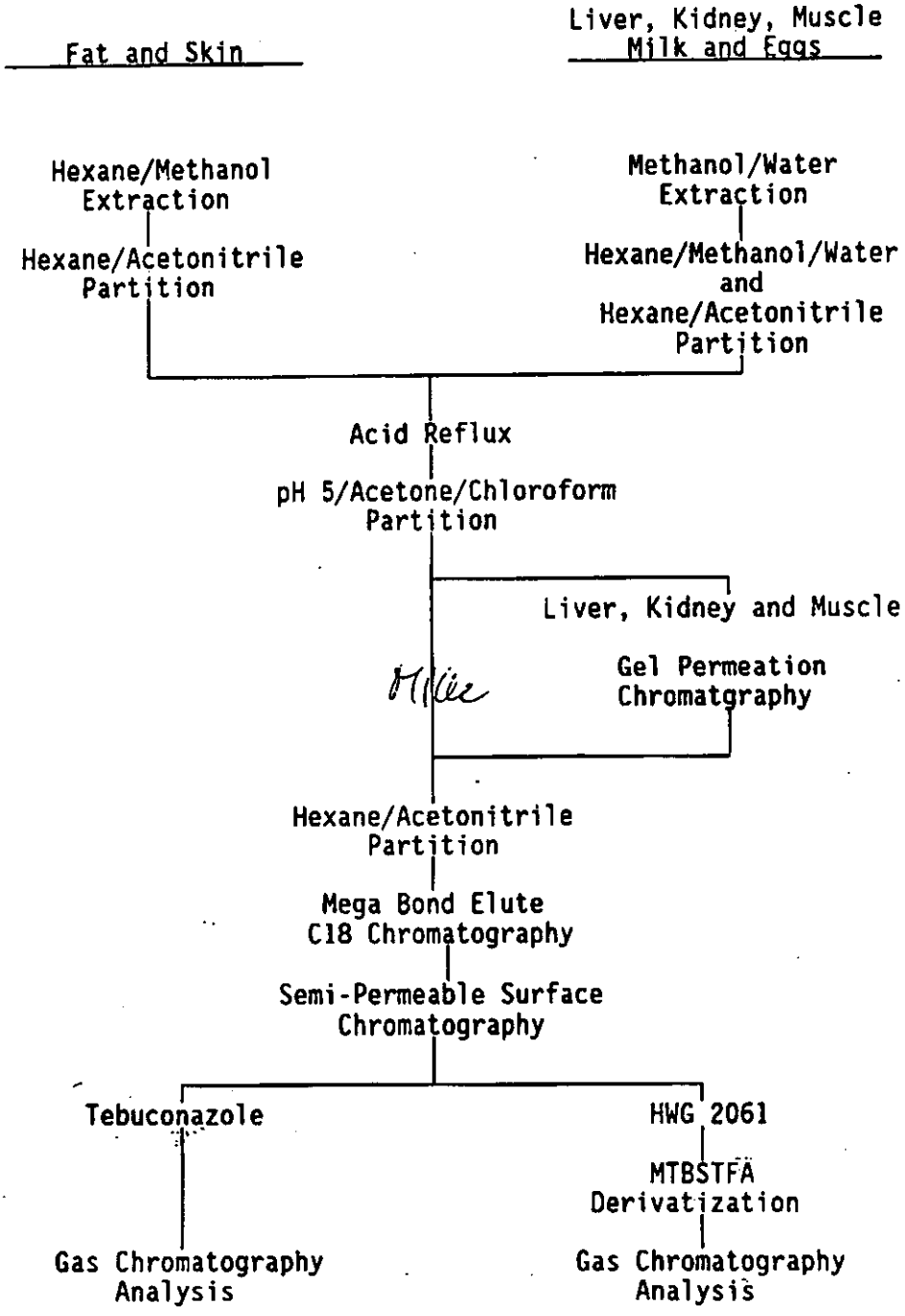


Figure 3. Flow diagram of the analytical residue method used for the analysis of tebuconazole and HWG 2061 in animal tissues, milk and eggs.

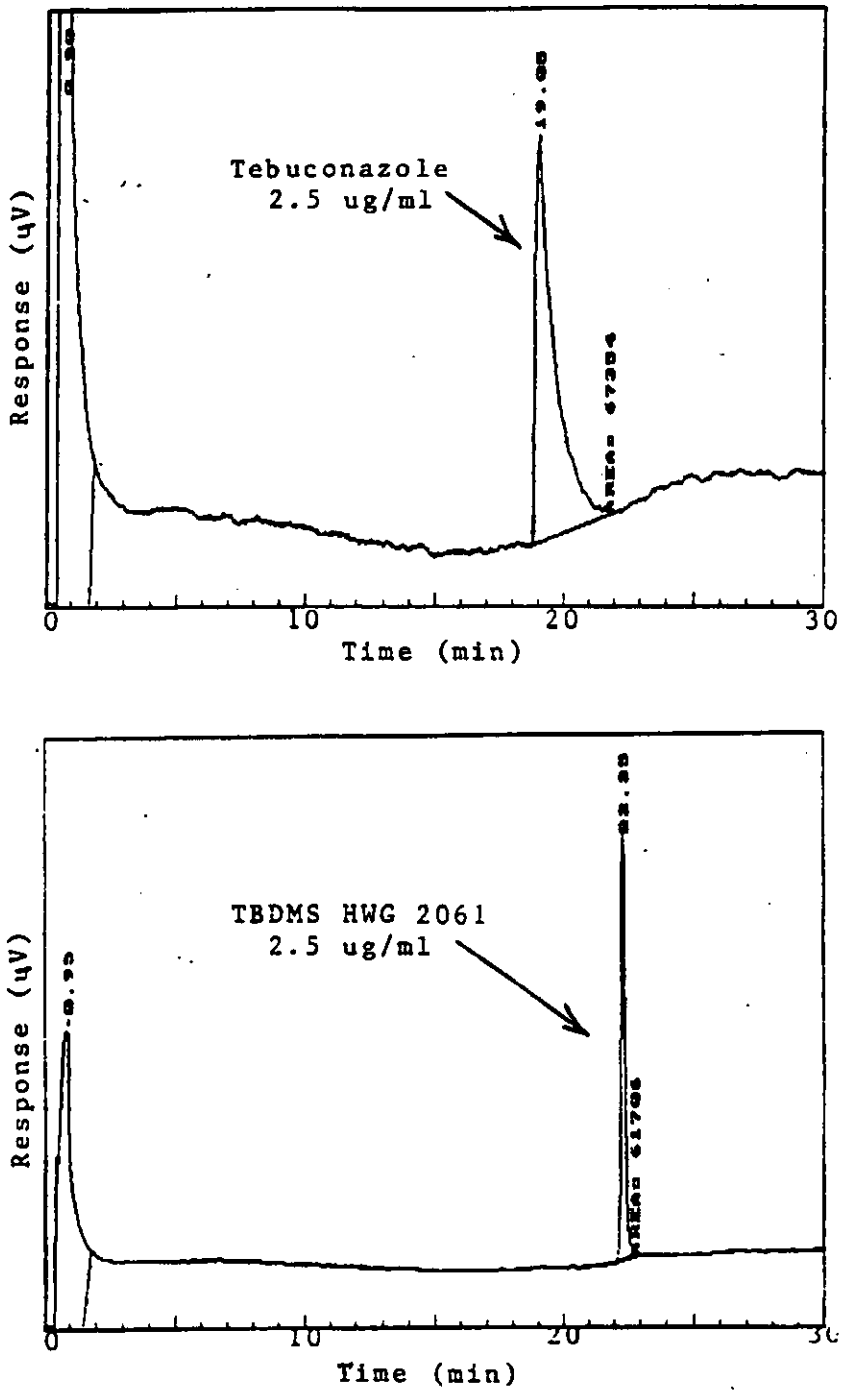


Figure 4. Representative gc chromatogram of tebuconazole and the TBDMS HWG 2061 derivative.

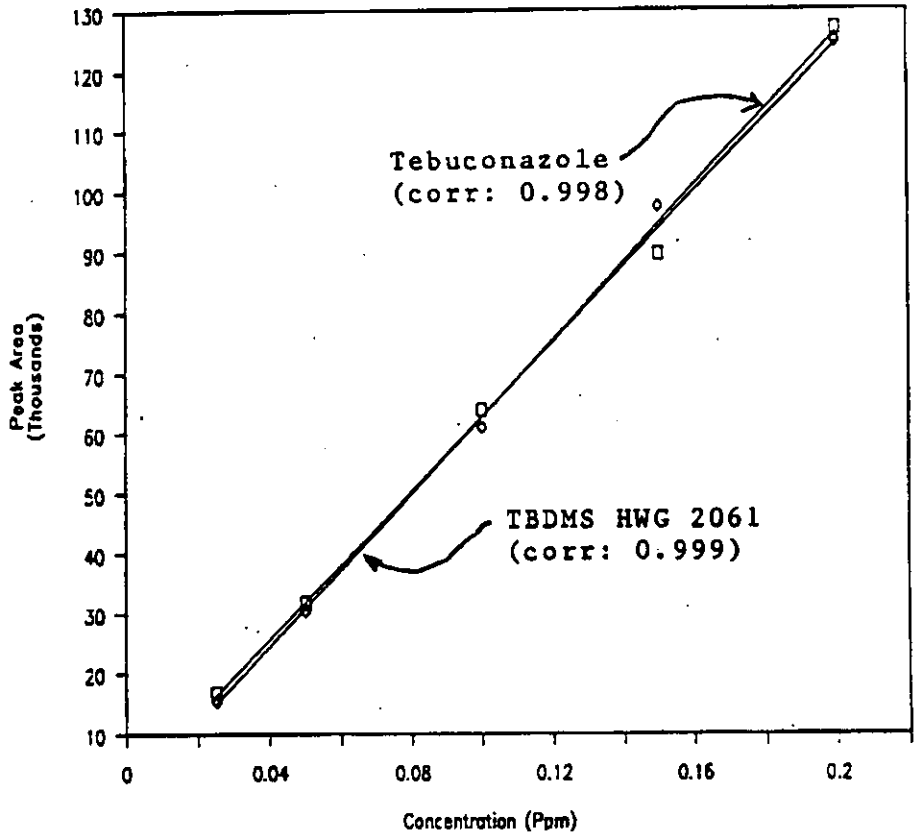
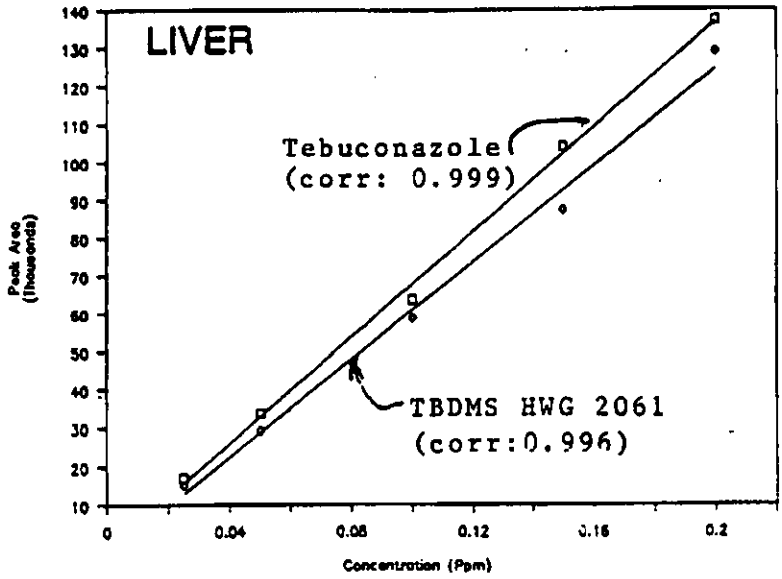


Figure 5. Linearity curves for the instrumental response of tebuconazole and the TBDMS HWG 2061 derivative.



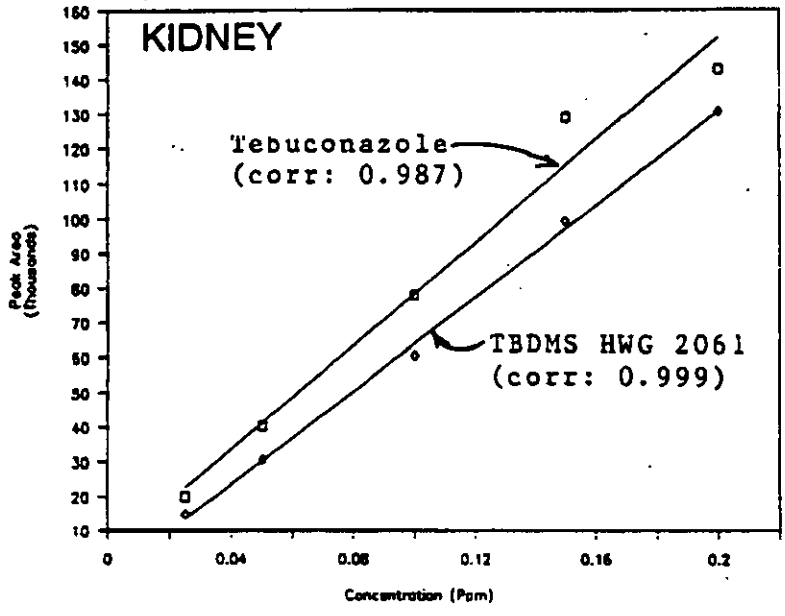
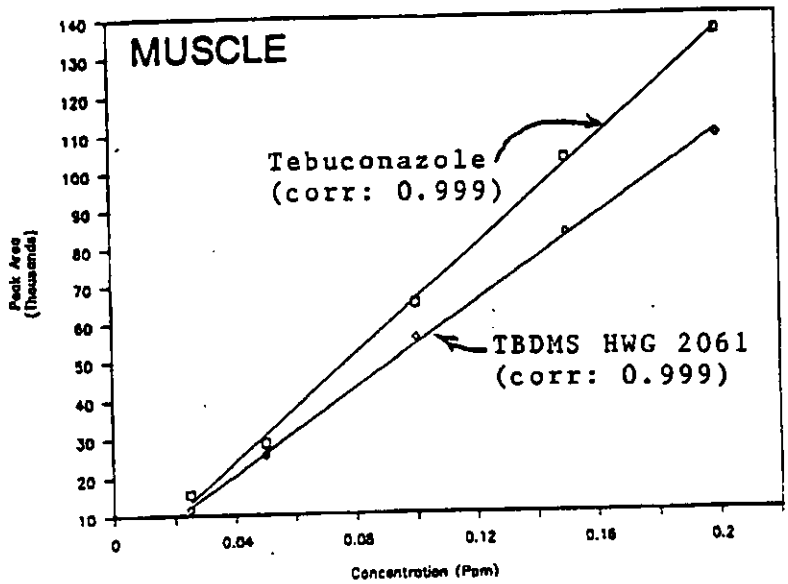


Figure 6. Linearity curves for the response of tebuconazole and the TBDMS HWG 2061 derivative in the presence of liver and kidney extracts.



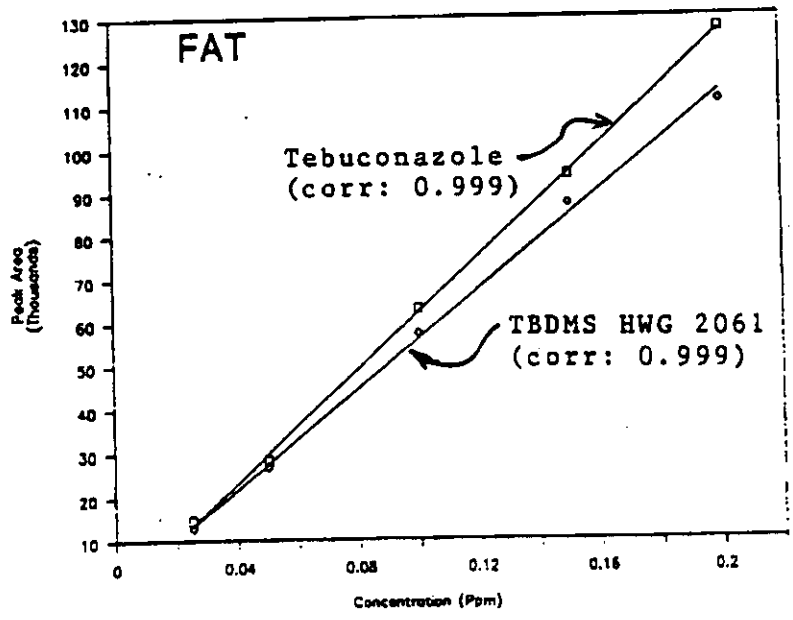
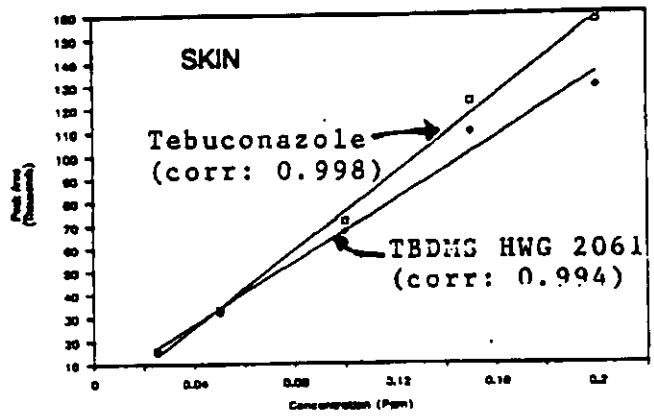
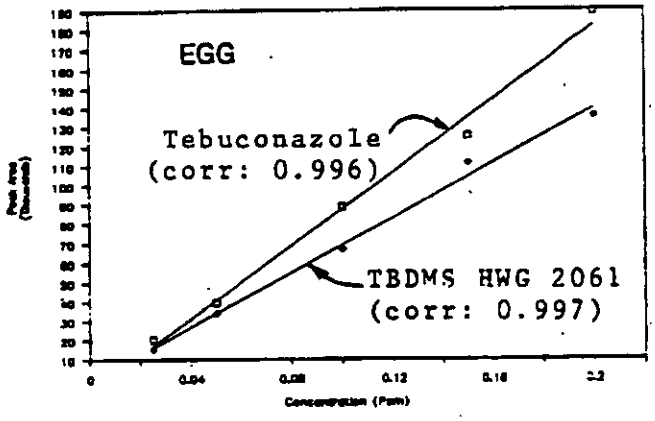


Figure 7. Linearity curves for the response of tebuconazole and the TBDMS HWG 2061 derivative in the presence of muscle and fat extracts.





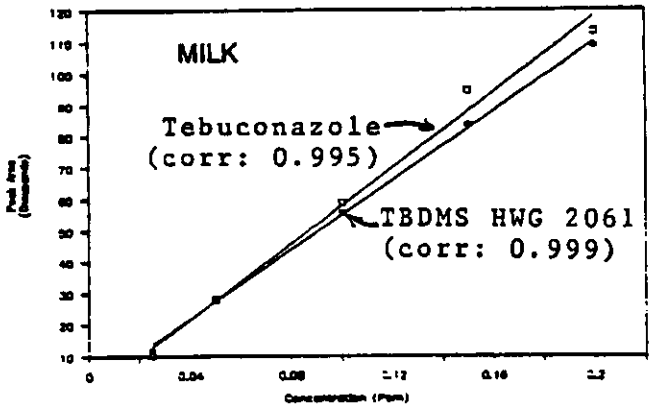


Figure 8. Linearity curves for the response of tebuconazole and the TBDMS HWG 2061 derivative in the presence of skin, egg and milk extracts.

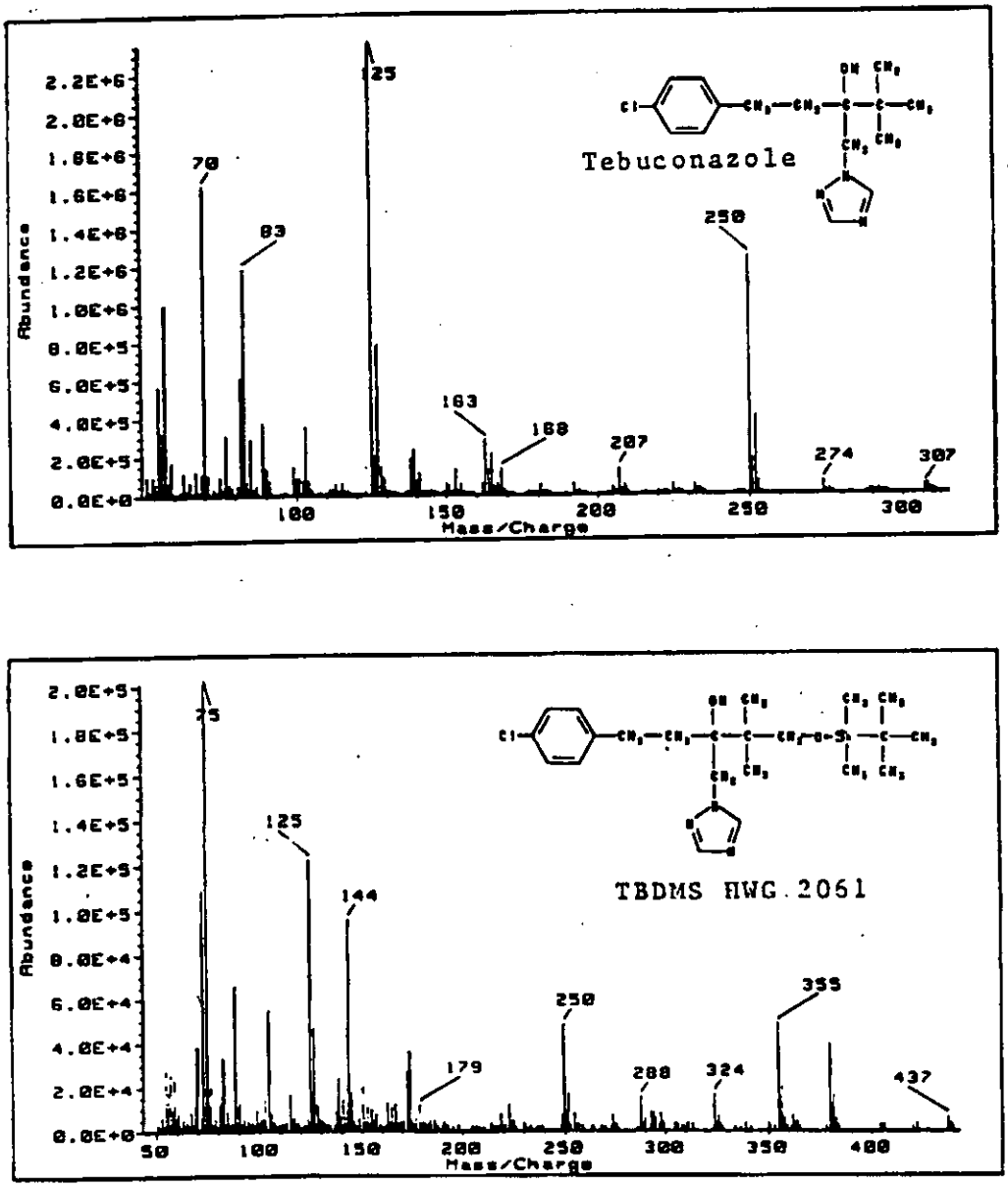


Figure 9. Mass spectrum of tebuconazole and the TBDMS HWG 2061 derivative.

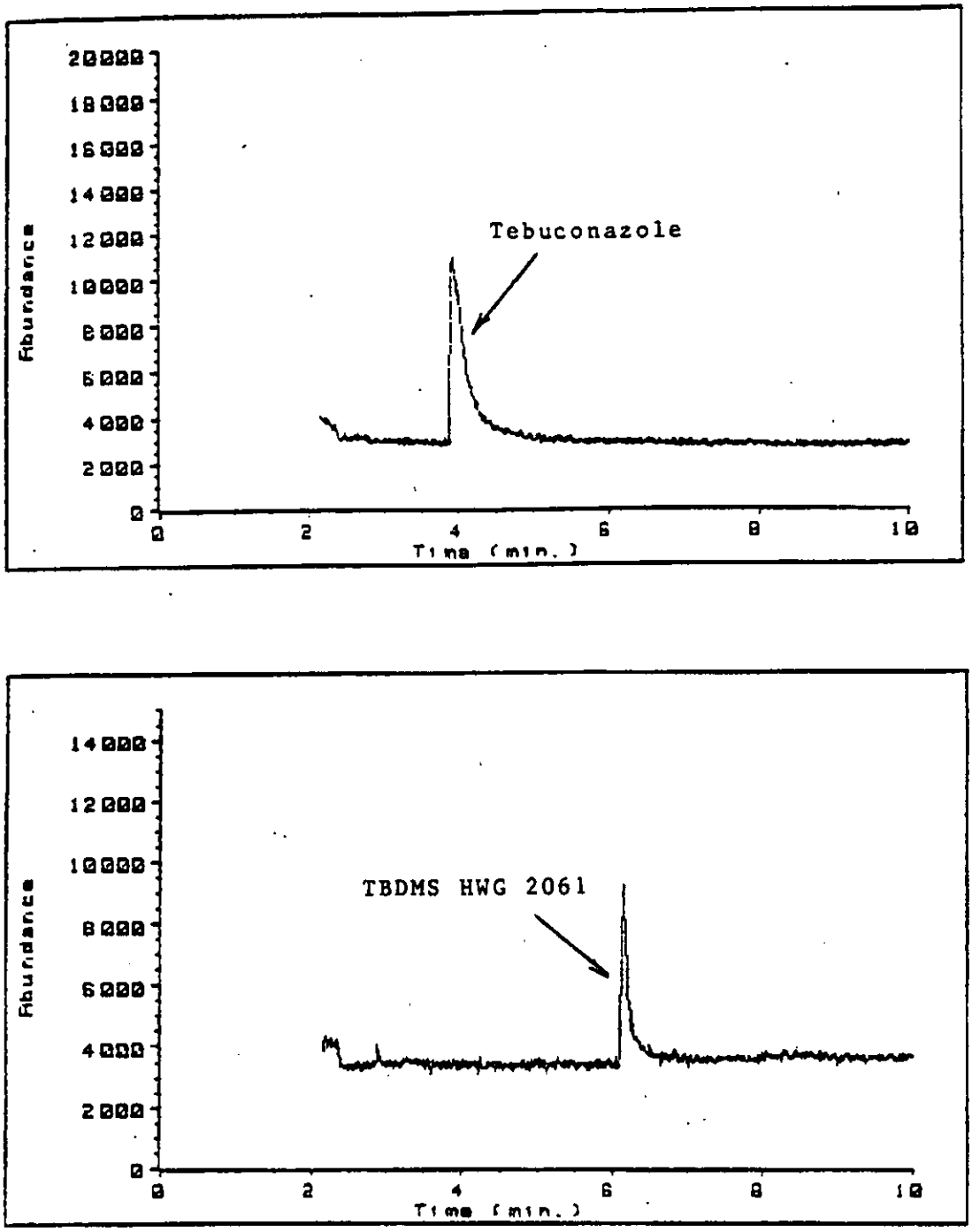


Figure 10. Gc/ms selected ion chromatograms of tebuconazole and the TBDMS HWG 2061 derivative.

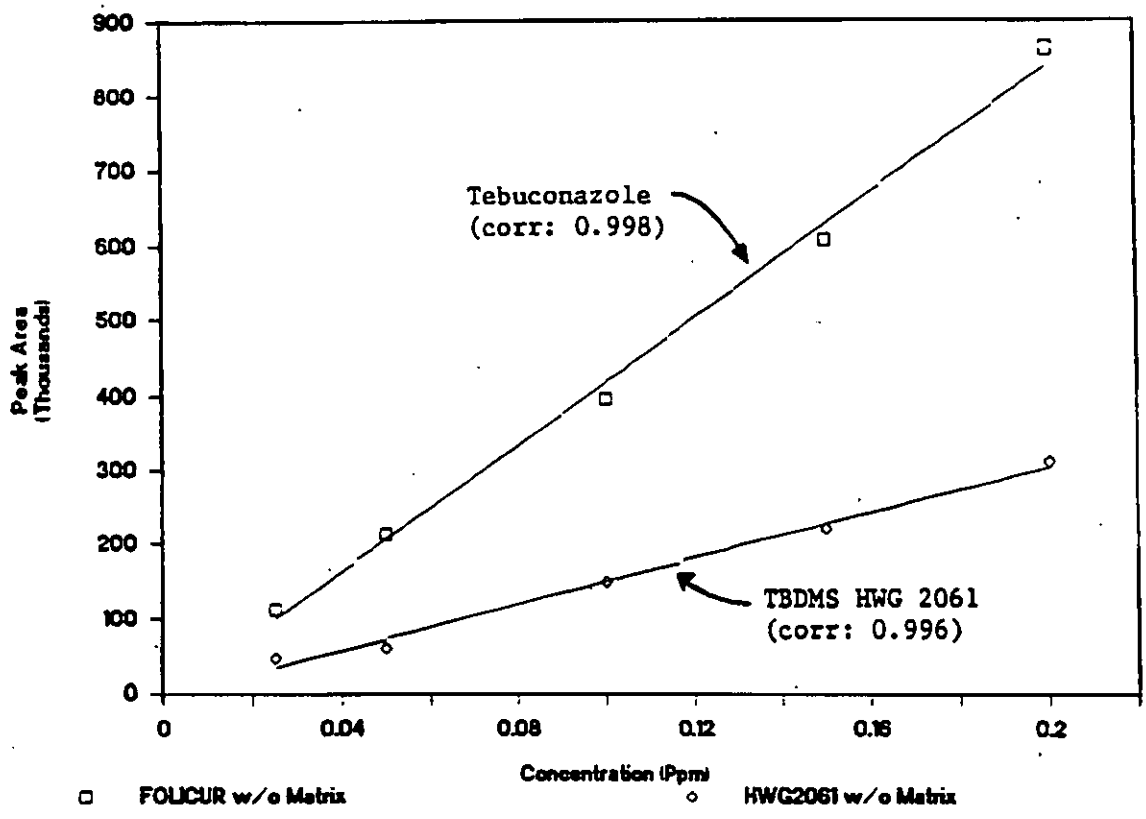


Figure 11. Linearity curves for the instrumental response of tebuconazole and the TBDMS HWG 2061 derivative using gc/ms selected ion monitoring (confirmatory procedure).

Appendix 1. Archive listing of notebooks and project personnel.

Notebook References

Notebook Number	Name	Year Issued	Page Numbers
90-R-197	R. R. Gronberg, V. J. Lemke	1990	All pages
91-R-104	H. W. Chopade	1991	All pages
91-R-103	A. E. Mathew	1991	All pages

Project Personnel

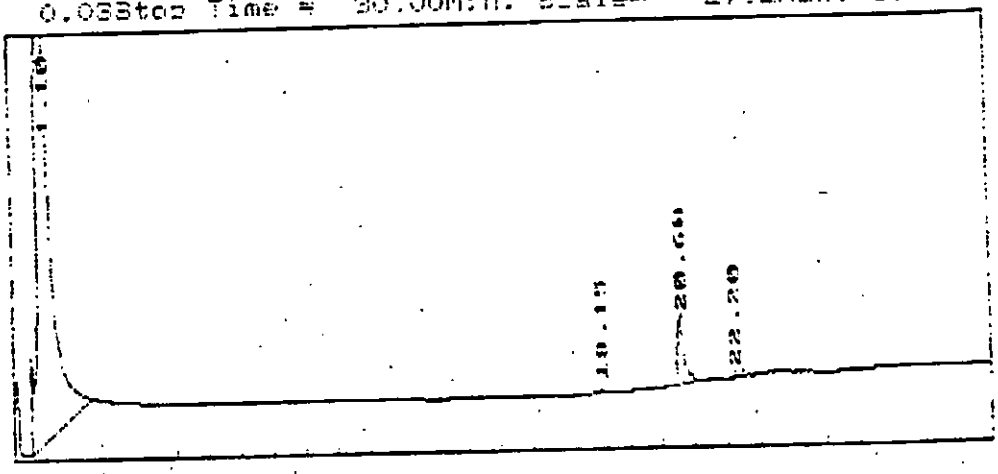
Name	Duties
R. R. Gronberg	Study director; participated in generating experimental data for method development; prepared method report.
H. M. Chopade	Chemist; participated in generating experimental data for method development.
A. E. Mathew	Chemist; participated in generating experimental data for method development.
V. J. Lemke	Technician; participated in generating experimental data for method development.
C. M. Blum	Technician; participated in generating experimental data for fortified standard recovery samples.
T. L. Fitzpatrick	Technician; participated in generating experimental data for fortified standard recovery samples.
T. J. McLaughin	Technician; participated in generating experimental data for fortified standard recovery samples.
D. J. Unruh	Technician; participated in generating experimental data for fortified standard recovery samples.

Appendix 2. Raw data and chromatograms for the recovery of tebuconazole and HWG 2061 in bovine liver.

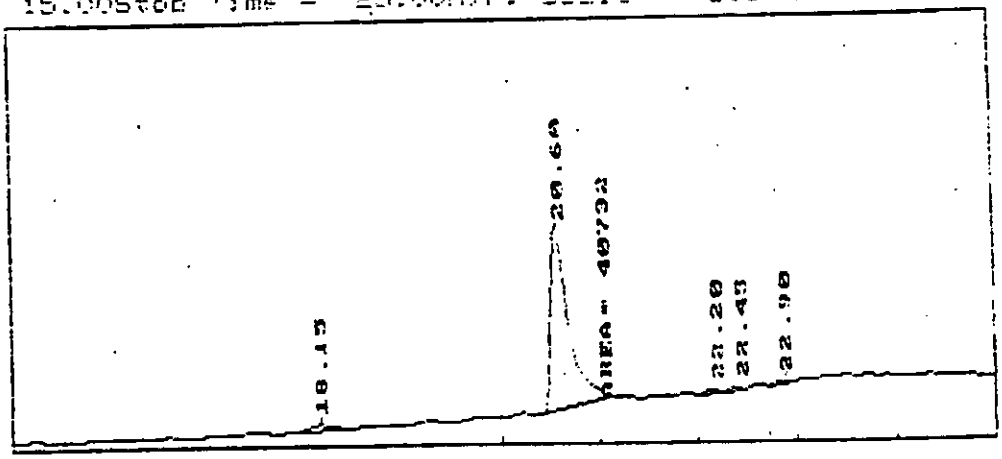
Sample Description	Date ('91) Ext.	Date ('91) Inj.	GC Response(mv)	Residue ppm Gross	Residue ppm Net	Rec %	Chart No.
Tebuconazole							
0.1 ppm Standard	-	08/27	40732	-	-	-	F827#24
Control Rep. #1	08/20	08/27	2719	0.0068	-	-	F827#25
0.1 ppm Standard	-	08/27	38940	-	-	-	F827#26
Control Rep. #2	08/20	08/27	3892	0.0099	-	-	F827#27
0.1 ppm Standard	-	08/27	39448	-	-	-	F827#28
Control + 0.1 ppm	08/20	08/27	38418	0.0997	0.0913	91	F827#29
0.1 ppm Standard	-	08/27	37647	-	-	-	F827#30
Control + 0.1 ppm	08/20	08/27	36585	0.0974	0.0890	89	F827#31
0.1 ppm Standard	-	08/27	37513	-	-	-	F827#32
HWG 2061							
0.1 ppm Standard	-	08/27	38313	-	-	-	F827#33
Control Rep. #1	08/20	08/27	1842	0.0046	-	-	F827#34
0.1 ppm Standard	-	08/27	41725	-	-	-	F827#35
Control Rep. #2	08/20	08/27	1178	0.0028	-	-	F827#36
0.1 ppm Standard	-	08/27	42676	-	-	-	F827#37
Control + 0.1 ppm	08/20	08/27	31200	0.0755	0.0718	72	F827#38
0.1 ppm Standard	-	08/27	39973	-	-	-	F827#39
Control + 0.1 ppm	08/20	08/27	34576	0.0816	0.0779	78	F827#40
0.1 ppm Standard	-	08/27	44740	-	-	-	F827#41

Appendix 2.

Plot of cata file: C:F827#24.PTS
Date: 01-01-1980 Time: 04:07:28
Sample Name: 0.1 PPM FOLICUR STANDARD SAMPLE NO.91R104-26-3R
Start Time= 0.03Stop Time = 30.00Min. Scale= 2702Max. Scale= 23617

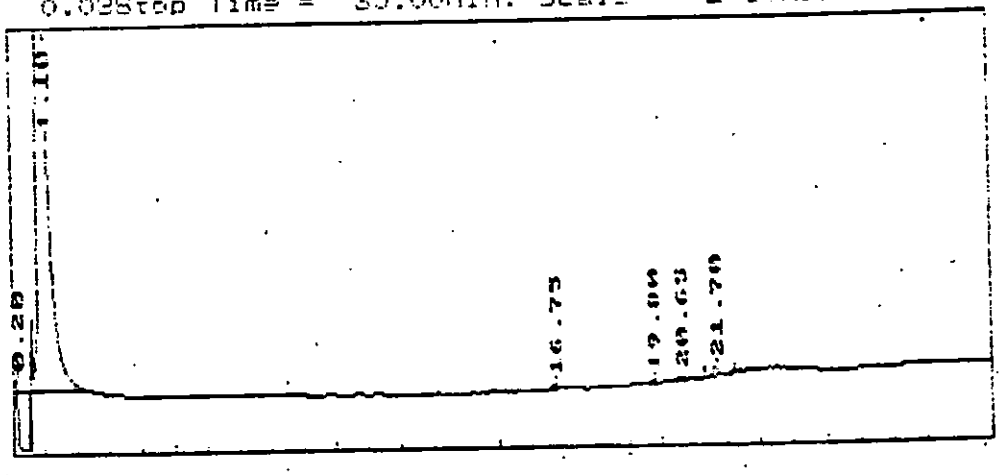


START TIME= 20.400 START HEIGHT= 5530
STOP TIME= 21.125 STOP HEIGHT= 5832
AREA = 40732
Plot of data file: C:F827#24.PTS
Date: 01-01-1980 Time: 04:08:46
Sample Name:
Start Time= 15.00Stop Time = 25.00Min. Scale= 5064Max. Scale= 18256

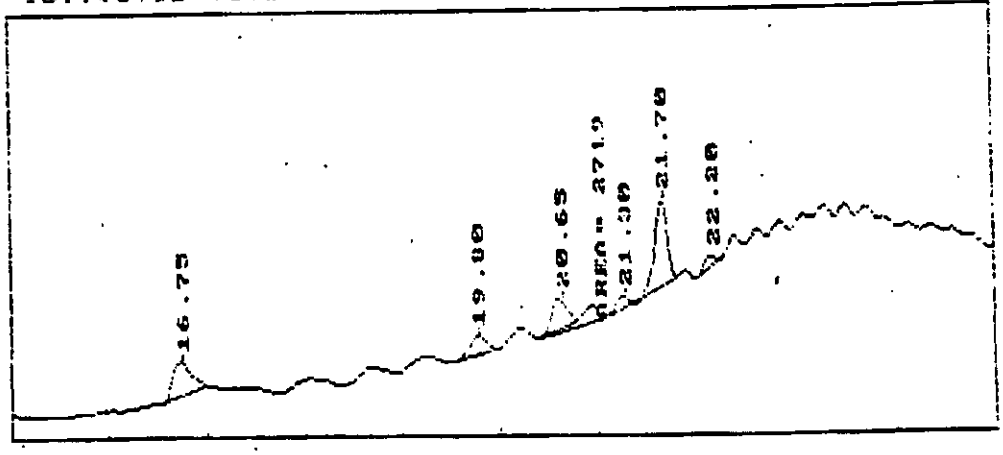


Appendix 2.

Plot of data file: C:F827#25.PTS
Date: 01-01-1980 Time: 04:10:06
Sample Name: CONTROL BOVINE LIVER REP.#1 SAMPLE NO. 91R104-26-3N
Start Time= 0.02Stop Time = 30.00Min. Scale= 2719Max. Scale= 23310

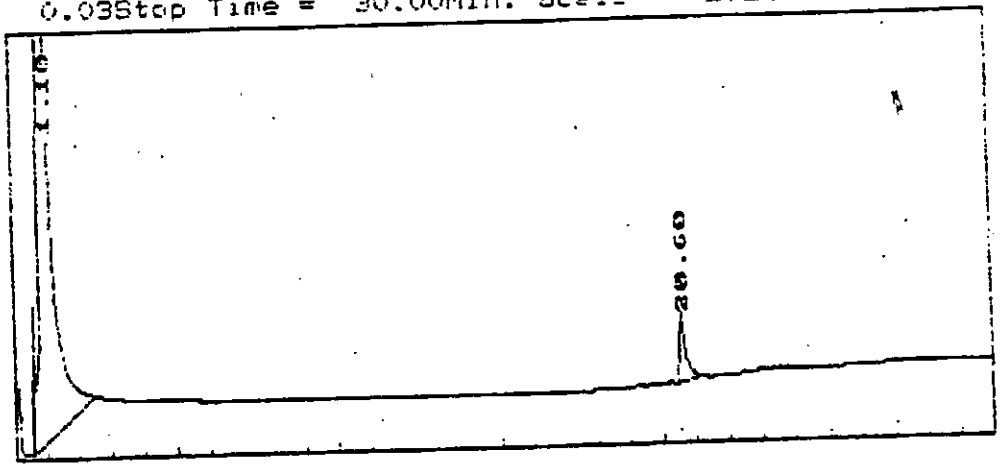


START TIME= 20.425 START HEIGHT= 5626
STOP TIME= 21.100 STOP HEIGHT= 5734
AREA = 2719
Plot of data file: C:F827#25.PTS
Date: 01-01-1980 Time: 04:12:49
Sample Name:
Start Time= 15.00Stop Time = 25.00Min. Scale= 5144Max. Scale= 7364

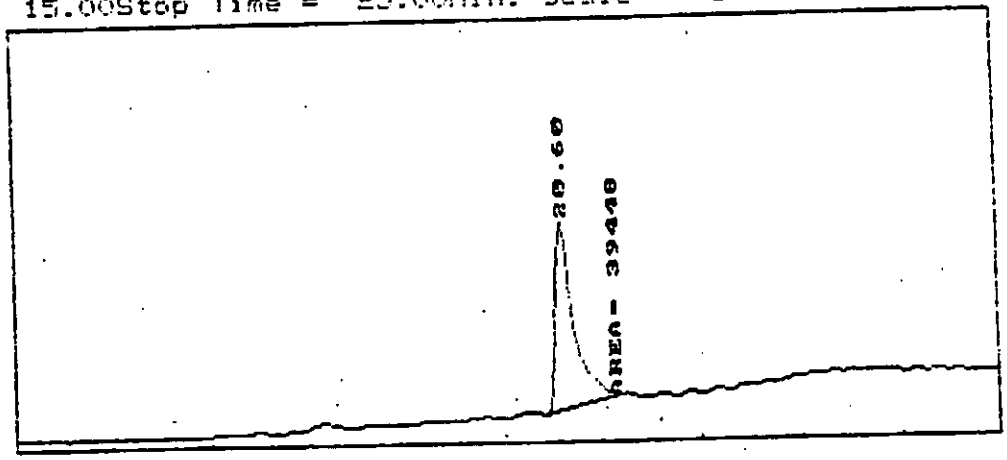


Appendix 2.

Plot of data file: C:F827#28.PTS
Date: 09-10-1991 Time: 17:21:21
Sample Name: 0.1 PPM FOLICUR STANDARD SAMPLE NO. 91R104-26-3R
Start Time= 0.03Stop Time = 30.00Min. Scale= 2723Max. Scale= 2325-

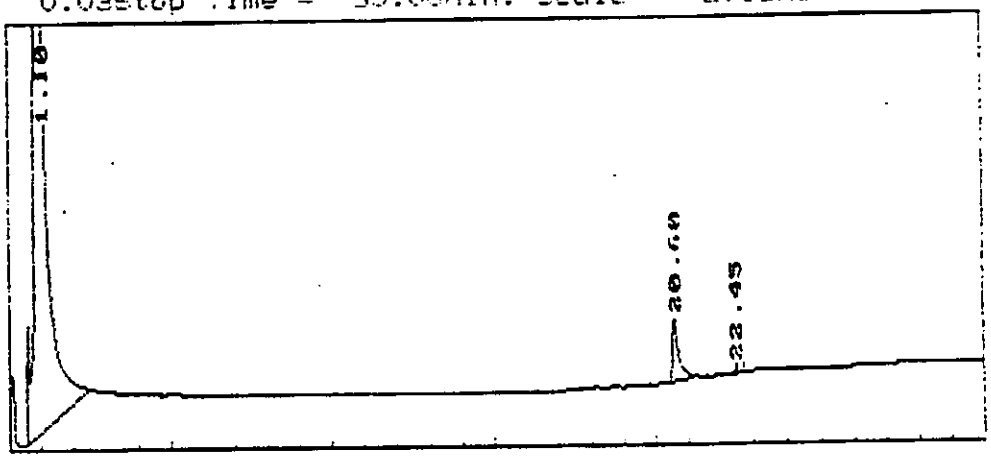


START TIME= 20.400 START HEIGHT= 5462
STOP TIME= 21.200 STOP HEIGHT= 5773
AREA = 39448
Plot of data file: C:F827#28.PTS
Date: 09-10-1991 Time: 17:23:12
Sample Name:
Start Time= 15.00Stop Time = 25.00Min. Scale= 5045Max. Scale= 1174

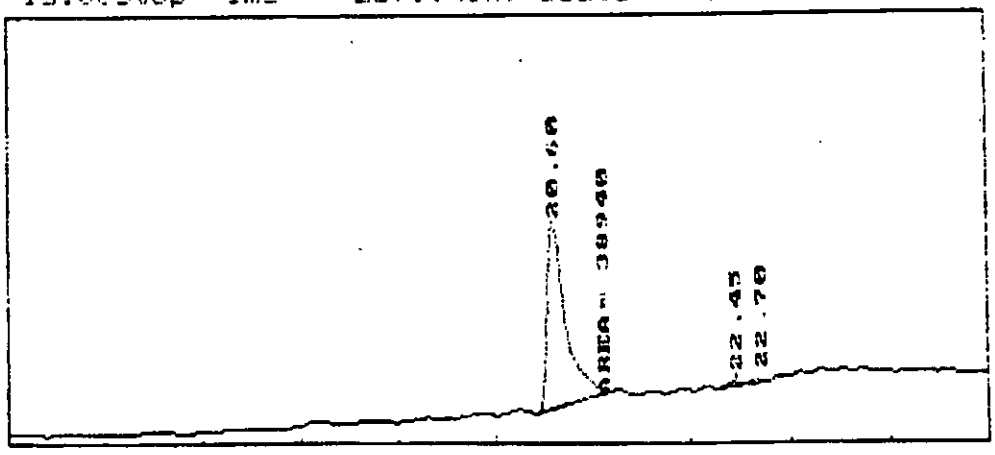


Appendix 2.

Plot of data file: C:F827#26.PTS
Date: 01-01-1980 Time: 04:14:16
Sample Name: 0.1 PPM FOLICUR STANDARD SAMPLE NO. 91R104-26-3R
Start Time= 0.02Stop Time = 30.00Min. Scale= 2716Max. Scale= 23375

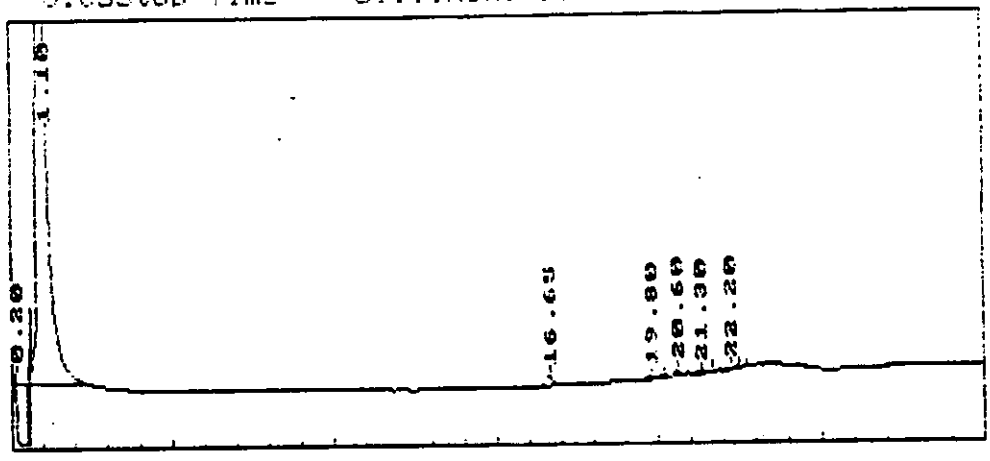


START TIME= 20.425 START HEIGHT= 5525
STOP TIME= 21.175 STOP HEIGHT= 5865
AREA = 38940
Plot of data file: C:F827#26.PTS
Date: 01-01-1980 Time: 04:15:29
Sample Name:
Start Time= 15.00Stop Time = 25.00Min. Scale= 5067Max. Scale= 11753

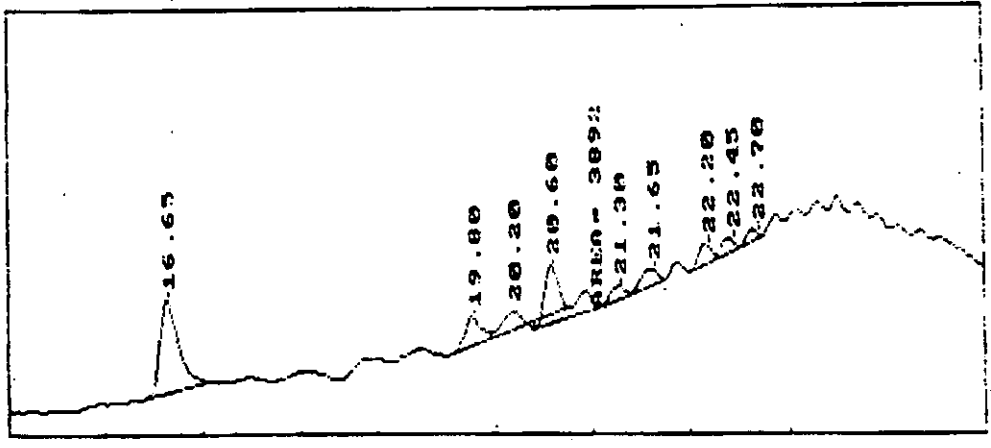


Appendix 2.

Plot of data file: C:F827#27.PTS
Date: 09-10-1991 Time: 16:46:47
Sample Name: CONTROL BOVINE LIVER REP.#2 SAMPLE NO. 91R104-26-30
Start Time= 0.03Stop Time = 30.00Min. Scale= 2721Max. Scale= 23190

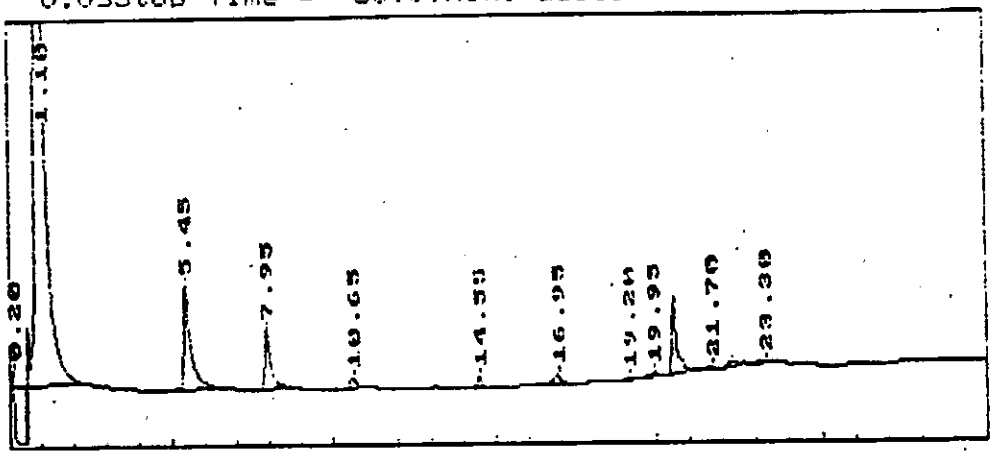


START TIME= 20.400 START HEIGHT= 5682
STOP TIME= 21.100 STOP HEIGHT= 5792
AREA = 3892
Plot of data file: C:F827#27.PTS
Date: 09-10-1991 Time: 16:49:53
Sample Name:
Start Time= 15.00Stop Time = 25.00Min. Scale= 5185Max. Scale= 7193

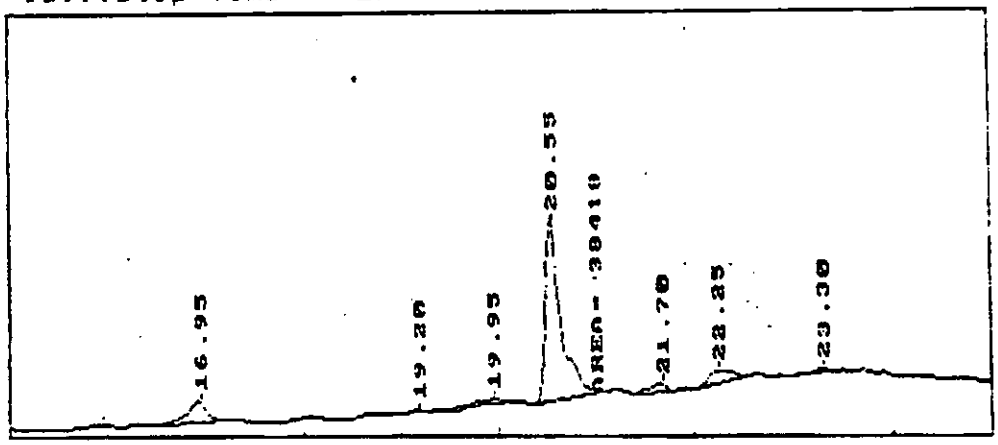


Appendix 2.

Plot of data file: C:F827#29.PTS
Date: 09-10-1991 Time: 17:13:04
Sample Name: CONTROL BOVINE LIVER+0.1 PPM FOLICUR STD.#1 SAMPLE NO. 91R104-26-3P
Start Time= 0.03Stop Time = 30.00Min. Scale= 2711Max. Scale= 23422

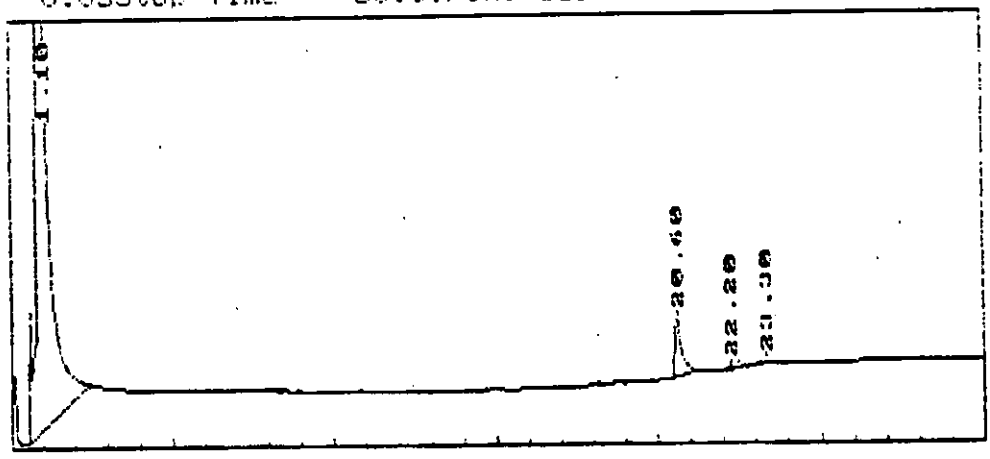


START TIME= 20.400 START HEIGHT= 5790
STOP TIME= 21.075 STOP HEIGHT= 6050
AREA = 38418
Plot of data file: C:F827#29.PTS
Date: 09-10-1991 Time: 17:14:16
Sample Name:
Start Time= 15.00Stop Time = 25.00Min. Scale= 5226Max. Scale= 13246

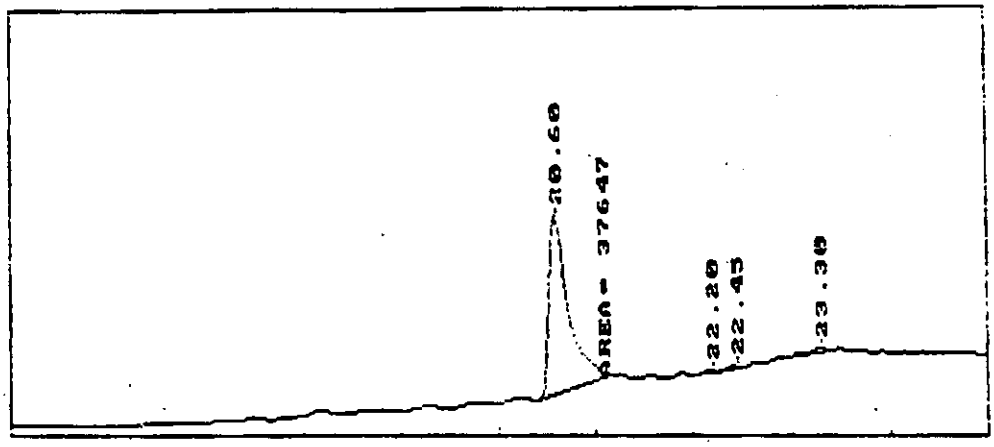


Appendix 2.

Plot of data file: C:FE27#30.PTS
Date: 09-10-1991 Time: 17:10:30
Sample Name: 0.1 PPM FOLICUR STANDARD SAMPLE NO. (IR104-26-3R
Start Time= 0.03Stop Time = 30.00Min. Scale= 8750Max. Scale= 32775

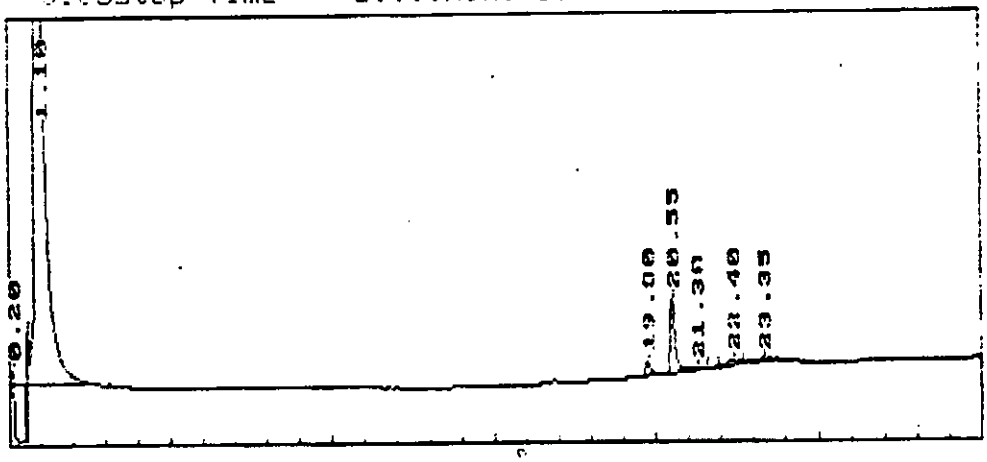


START TIME= 20.275 START HEIGHT= 5615
STOP TIME= 21.150 STOP HEIGHT= 5980
AREA = 37647
Plot of data file: C:F827#30.PTS
Date: 09-10-1991 Time: 17:11:52
Sample Name:
Start Time= 15.00Stop Time = 25.00Min. Scale= 5105Max. Scale= 11497

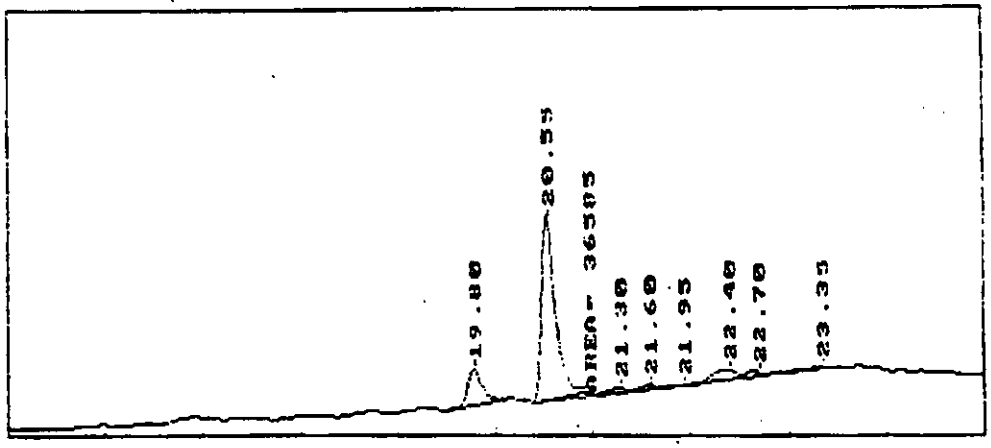


Appendix 2.

Plot of data file: C:F827#31.PTS
Date: 09-10-1991 Time: 17:08:36
Sample Name: CONTROL BOVINE LIVER+0.1 PPM FOLICUR STANDARD #2 SAMPLE NO. 91R104-26-3Q
Start Time= 0.03Stop Time = 30.00Min. Scale= 2722Max. Scale= 23352

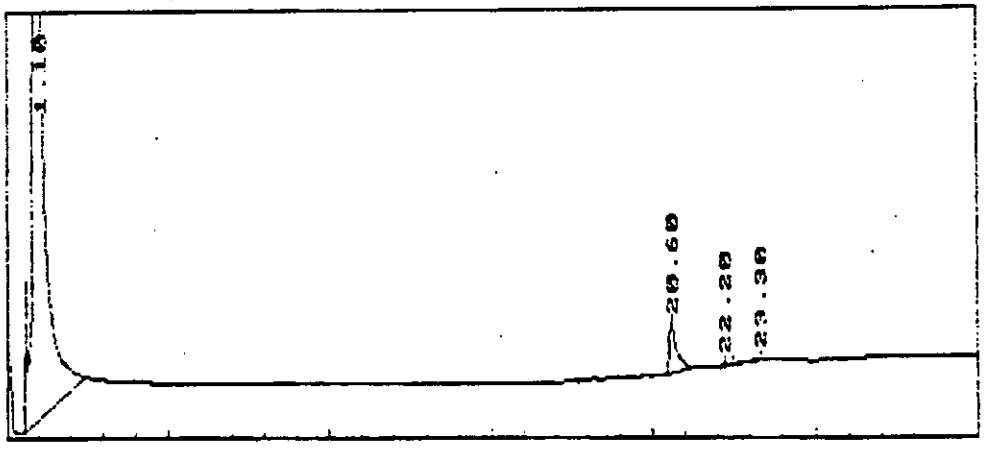


START TIME= 20.400 START HEIGHT= 5798
STOP TIME= 21.050 STOP HEIGHT= 6001
AREA = 36585
Plot of data file: C:F827#31.PTS
Date: 09-10-1991 Time: 17:09:38
Sample Name:
Start Time= 15.00Stop Time = 25.00Min. Scale= 5185Max. Scale= 13425

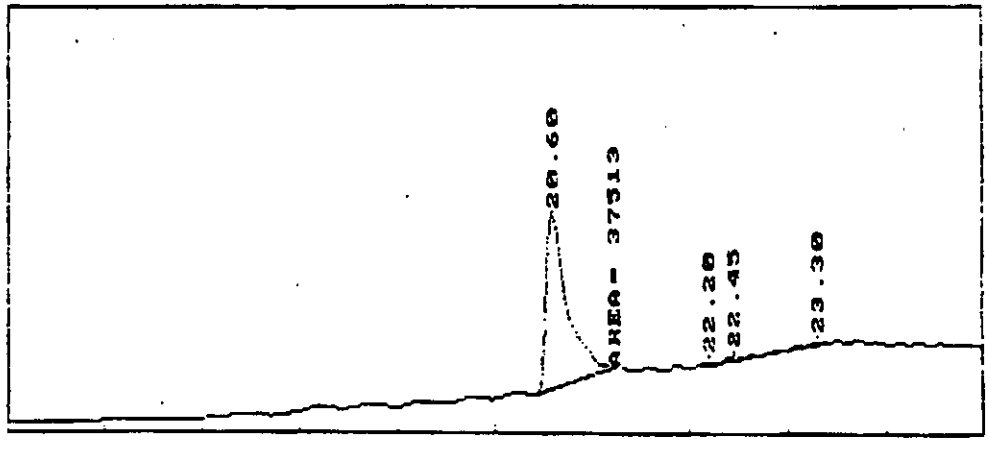


Appendix 2.

Plot of data file: C:F827#32.PTS
Date: 09-10-1991 Time: 17:15:08
Sample Name: 0.1 PPM FOLICUR STANDARD SAMPLE NO. 91R104-26-3R
Start Time= 0.03Stop Time = 30.00Min. Scale= 2691Max. Scale= 23595

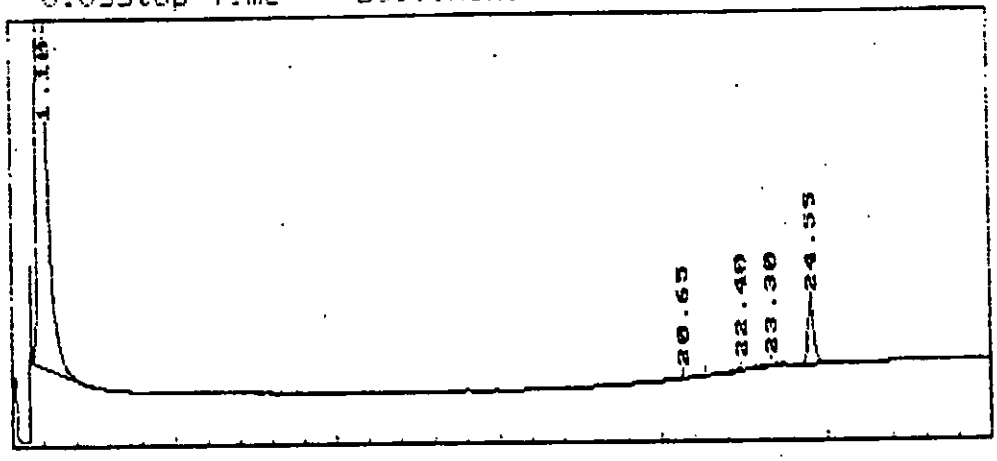


START TIME= 20.425 START HEIGHT= 5566
STOP TIME= 21.300 STOP HEIGHT= 5964
AREA = 37513
Plot of data file: C:F827#32.PTS
Date: 09-10-1991 Time: 17:16:19
Sample Name:
Start Time= 15.00Stop Time = 25.00Min. Scale= 5064Max. Scale= 10944

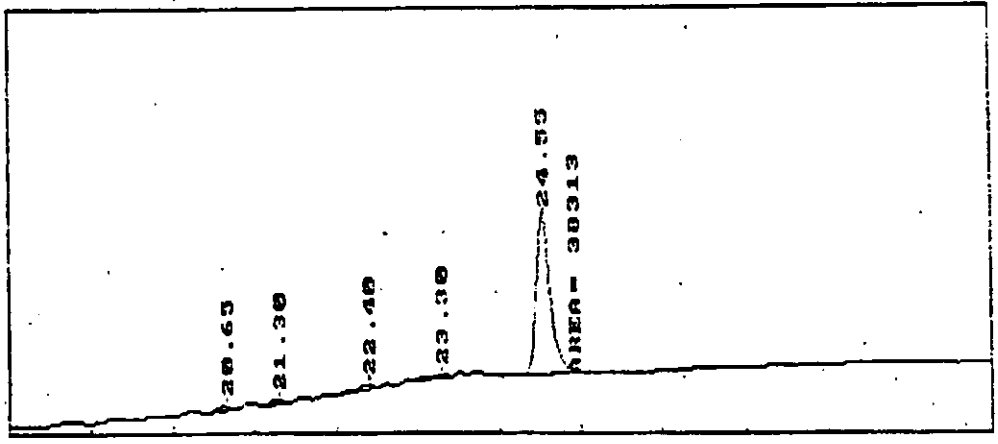


Appendix 2.

Plot of data file: C:F827#33.PTS
Date: 09-10-1991 Time: 17:26:10
Sample Name: 0.1 PPM HWG 2061 STANDARD SAMPLE NO. 91R104-26-3M
Start Time= 0.03Stop Time = 30.00Min. Scale= 2705Max. Scale= 24064

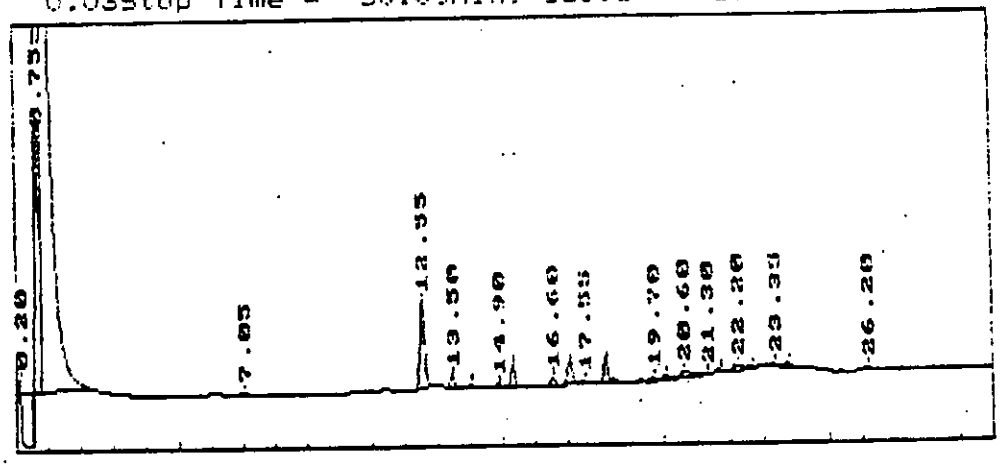


START TIME= 24.275 START HEIGHT= 6320
STOP TIME= 25.000 STOP HEIGHT= 5390
AREA = 38313
Plot of data file: C:F827#33.PTS
Date: 09-10-1991 Time: 17:27:13
Sample Name:
Start Time= 18.00Stop Time = 30.00Min. Scale= 5241Max. Scale= 13491

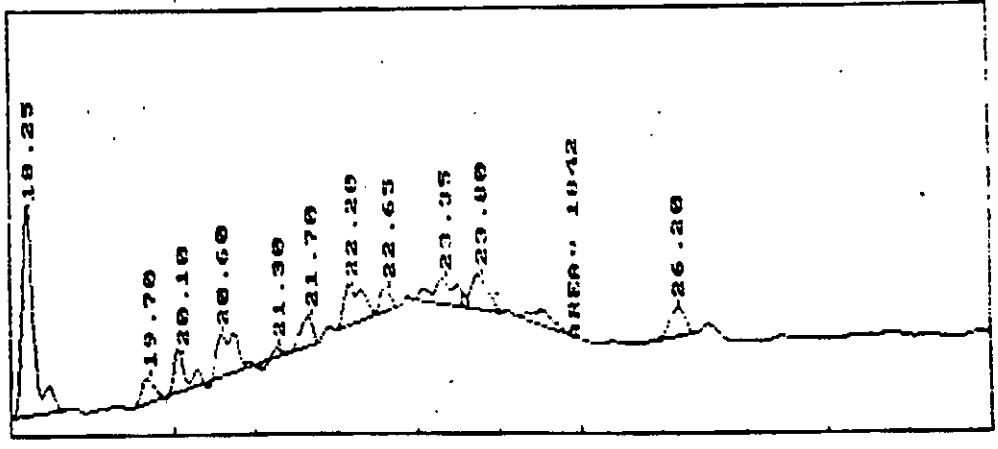


Appendix 2.

Plot of data file: C:F827#34.PTS
Date: 09-10-1991 Time: 17:28:06
Sample Name: CONTROL BOVINE LIVER REP.#1 SAMPLE NO. 91R104-26-3I
Start Time= 0.03Stop Time = 30.00Min. Scale= 2671Max. Scale= 2511

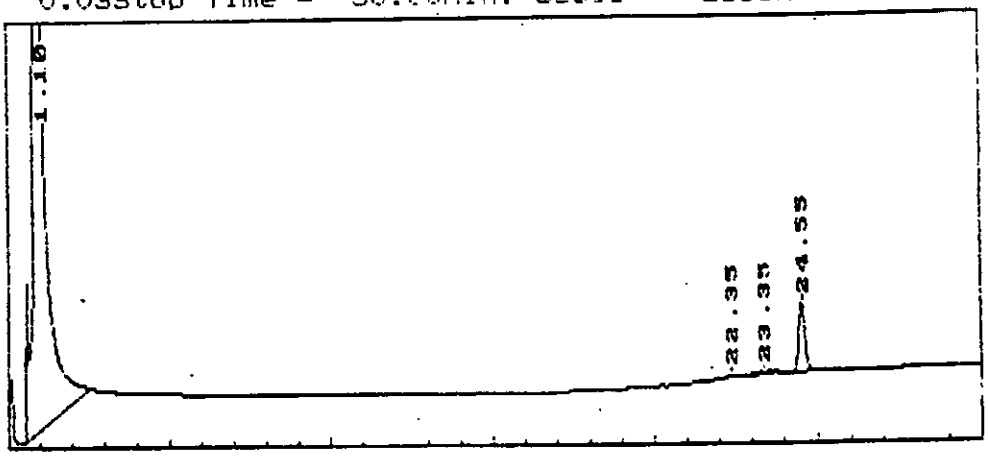


START TIME= 24.200 START HEIGHT= 6405
STOP TIME= 25.000 STOP HEIGHT= 6253
AREA = 1842
Plot of data file: C:F827#34.PTS
Date: 09-10-1991 Time: 17:29:10
Sample Name:
Start Time= 18.00Stop Time = 30.00Min. Scale= 565=Max. Scale= 3364

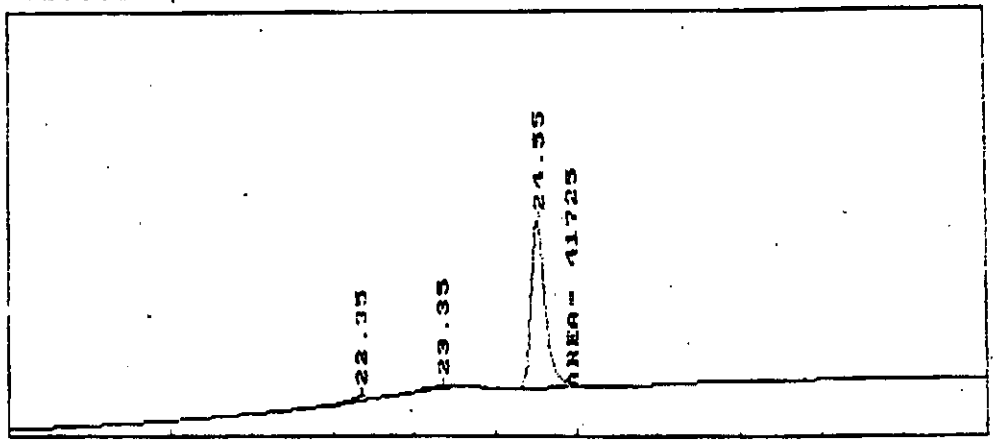


Appendix 2.

Plot of data file: C:F827#35.PTS
Date: 09-10-1991 Time: 17:30:06
Sample Name: 0.1 PPM HWG 2061 STANDARD SAMPLE NO. 91R104-26-3M
Start Time= 0.03Stop Time = 30.00Min. Scale= 2656Max. Scale= 84649

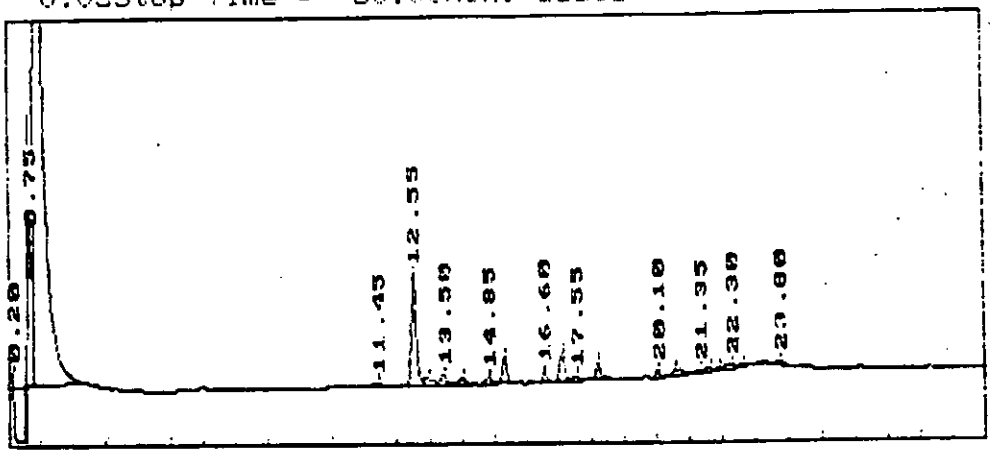


START TIME= 24.275 START HEIGHT= 6115
STOP TIME= 25.025 STOP HEIGHT= 6181
AREA = 41725
Plot of data file: C:F827#35.PTS
Date: 09-10-1991 Time: 17:31:22
Sample Name:
Start Time= 18.00Stop Time = 30.00Min. Scale= 5174Máx. Scale= 13832

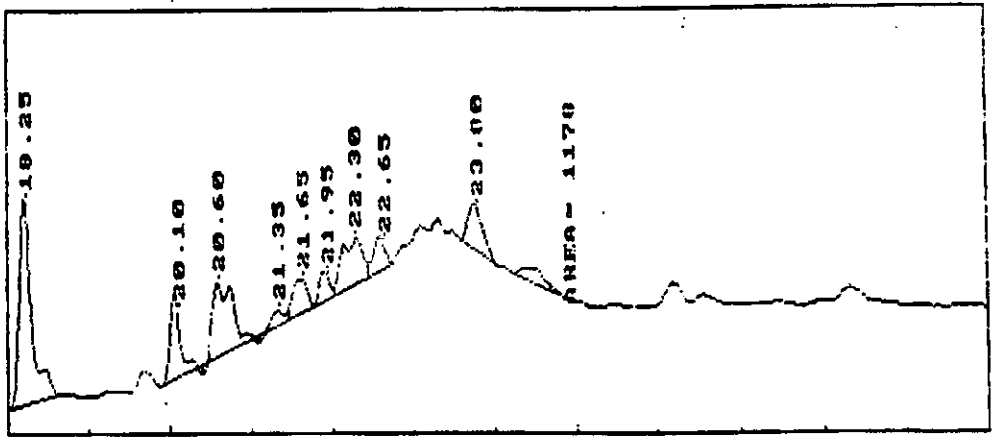


Appendix 2.

Plot of data file: C:F827#36.PTS
Date: 09-10-1991 Time: 17:32:13
Sample Name: CONTROL BOVINE LIVER REP.#2 SAMPLE NO. 91R104-26-3J
Start Time= 0.03Stop Time = 30.00Min. Scale= 2696Max. Scale= 24667

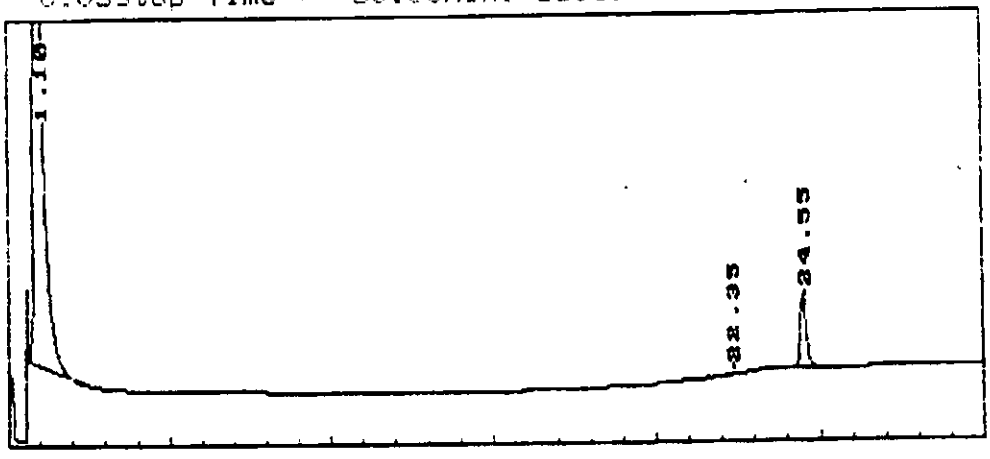


START TIME= 24.250 START HEIGHT= 6385
STOP TIME= 25.000 STOP HEIGHT= 6262
AREA = 1178
Plot of data file: C:F827#36.PTS
Date: 09-10-1991 Time: 17:33:10
Sample Name:
Start Time= 18.00Stop Time = 30.00Min. Scale= 5706Max. Scale= 7500

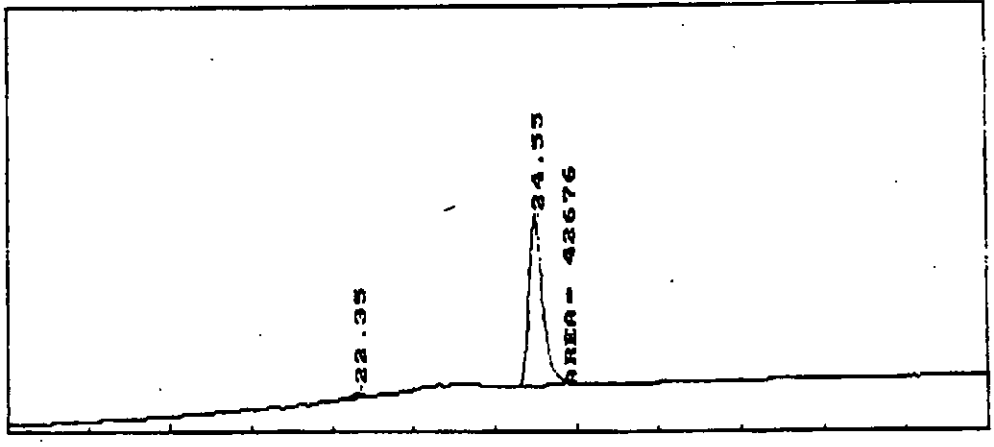


Appendix 2.

Plot of data file: C:F827#37.PTS
Date: 09-10-1991 Time: 17:34:14
Sample Name: 0.1 PPM HWG 2061 STANDARD SAMPLE NO. 91R104-26-3M
Start Time= 0.03Stop Time = 30.00Min. Scale= 2687Max. Scale= 24164

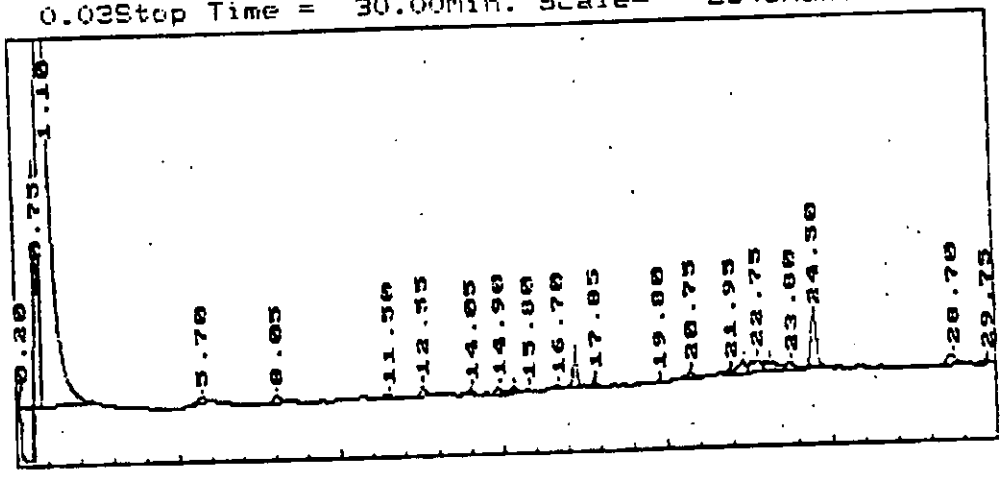


START TIME= 24.275 START HEIGHT= 6122
STOP TIME= 25.000 STOP HEIGHT= 6187
AREA = 42676
Plot of data file: C:F827#37.PTS
Date: 09-10-1991 Time: 17:35:28
Sample Name:
Start Time= 18.00Stop Time = 30.00Min. Scale= 5197Max. Scale= 14141

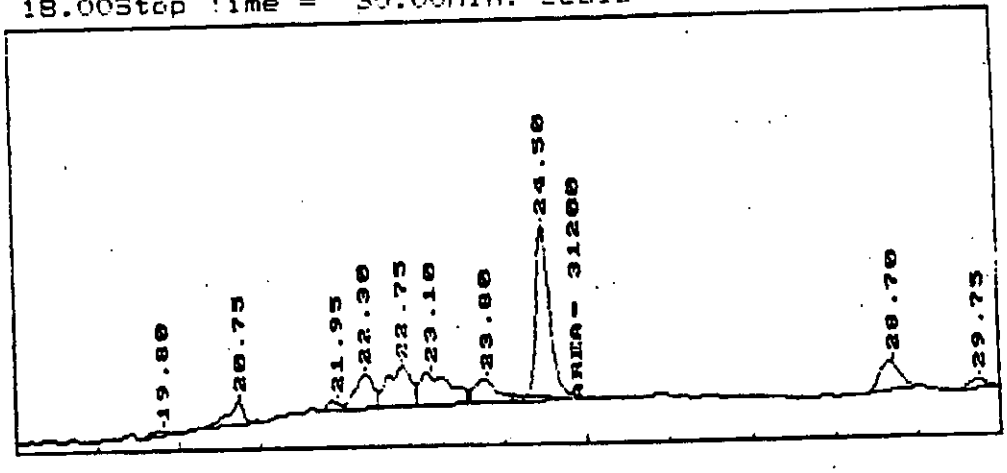


Appendix 2.

Plot of data file: C:F827#38.PTS
Date: 09-10-1991 Time: 17:40:21 SAMPLE NO. 91R104-26-3K
Sample Name: CONTROL BOVINE LIVER + 0.1 PPM HWG 2061 STD. REP.#1
Start Time= 0.03Stop Time = 30.00Min. Scale= 2646Max. Scale= 24822

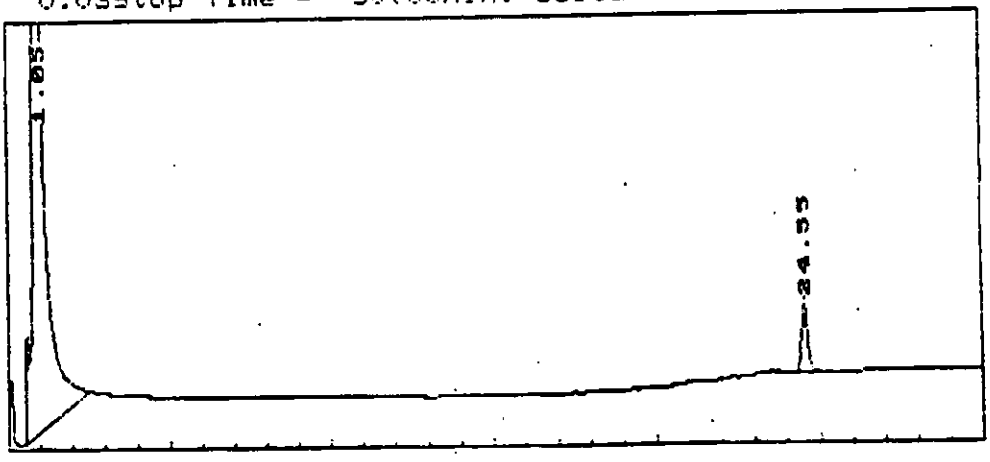


START TIME= 24.200 START HEIGHT= 6483
STOP TIME= 24.975 STOP HEIGHT= 6442
AREA = 31200
Plot of data file: C:F827#38.PTS
Date: 09-10-1991 Time: 17:42:41
Sample Name:
Start Time= 18.00Stop Time = 30.00Min. Scale= 5751Max. Scale= 1240

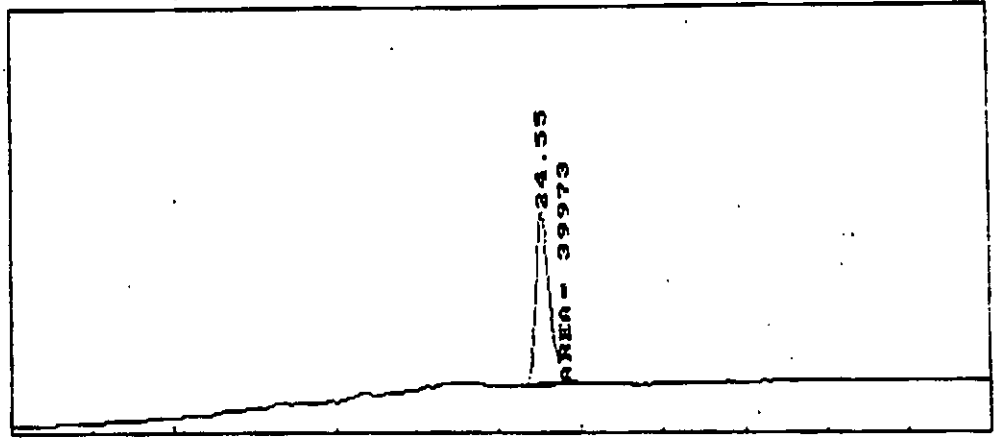


Appendix 2.

Plot of data file: C:F827#39.PTS
Date: 09-10-1991 Time: 17:43:39
Sample Name: 0.1 PPM HWG 2061 STANDARD SAMPLE NO. 91R104-26-3M
Start Time= 0.03Stop Time = 30.00Min. Scale= 2677Max. Scale= 24687

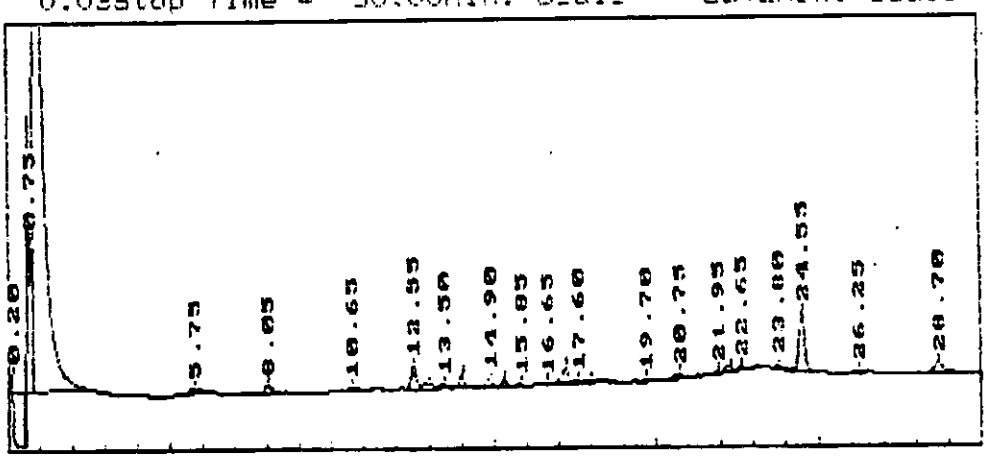


START TIME= 24.325 START HEIGHT= 6104
STOP TIME= 24.900 STOP HEIGHT= 6227
AREA = 39973
Plot of data file: C:F827#39.PTS
Date: 09-10-1991 Time: 17:45:05
Sample Name:
Start Time= 15.00Stop Time = 30.00Min. Scale= 5179Max. Scale= 13829

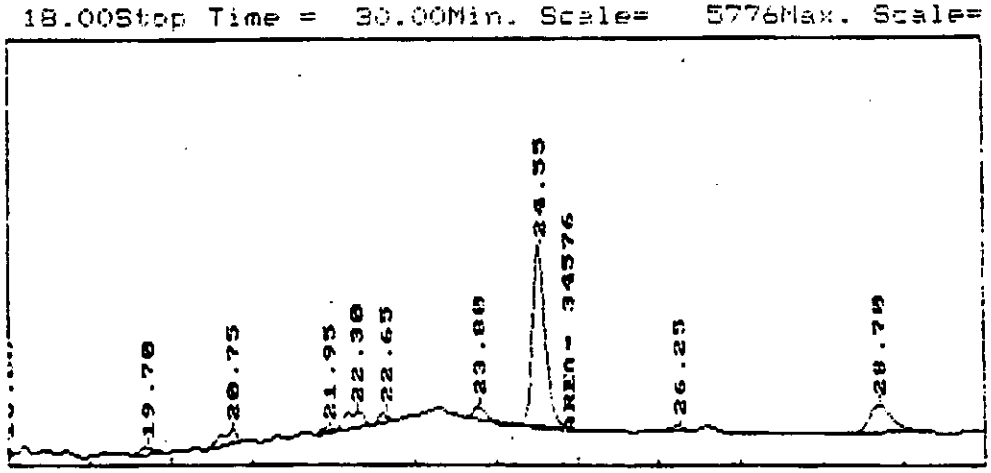


Appendix 2.

Plot of data file: C:F827#40.PTS
Date: 09-10-1991 Time: 17:46:17 SAMPLE NO. 91R104-26-3L
Sample Name: CONTROL BOVINE LIVER + 0.1 PPM HWG 2061 STD. REP.#2
Start Time= 0.03Stop Time = 30.00Min. Scale= 2692Max. Scale= 24868

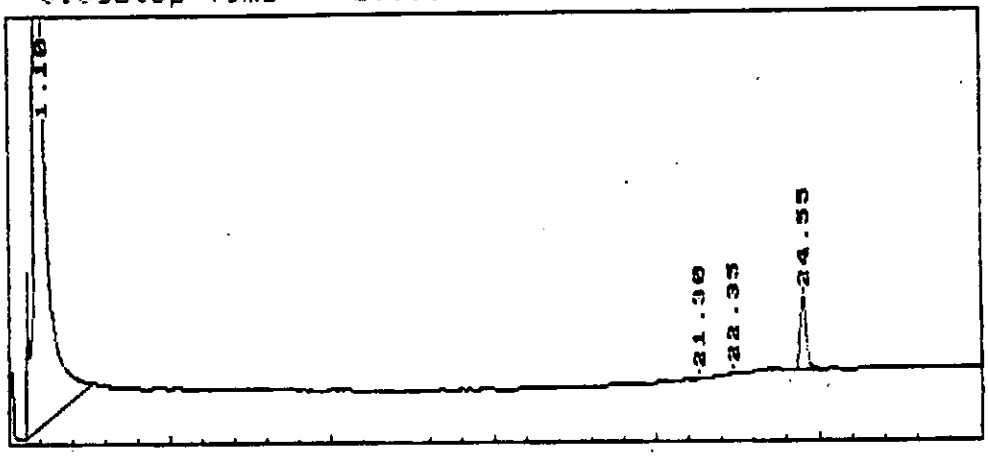


START TIME= 24.250 START HEIGHT= 6414
STOP TIME= 24.975 STOP HEIGHT= 6353
AREA = 34576
Plot of data file: C:F827#40.PTS
Date: 09-10-1991 Time: 17:49:26
Sample Name:
Start Time= 18.00Stop Time = 30.00Min. Scale= 5776Max. Scale= 12948

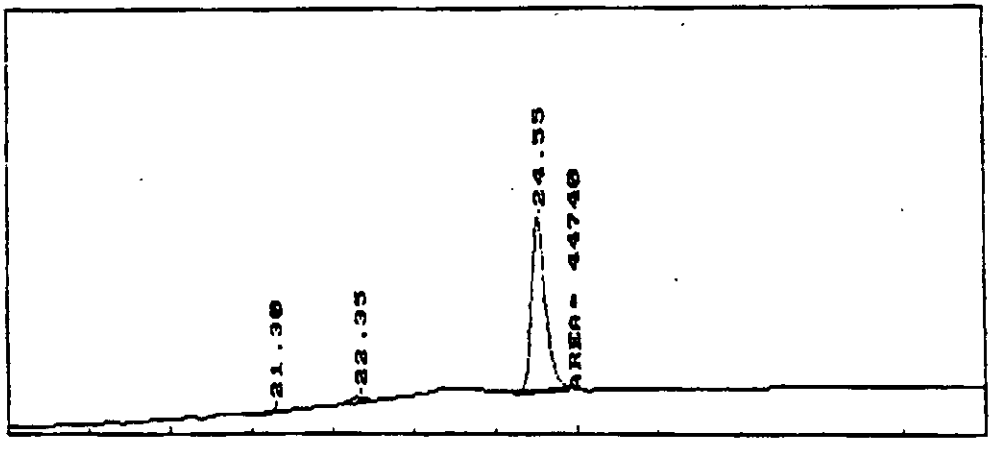


Appendix 2.

Plot of data file: C:F827#41.PTS
Date: 09-10-1991 Time: 17:51:30
Sample Name: 0.1 PPM HWG 2061 STANDARD SAMPLE NO. 91R104-26-3M
Start Time= 0.03Stop Time = 30.00Min. Scale= 2597Max. Scale= 24446



START TIME= 24.225 START HEIGHT= 5991
STOP TIME= 25.050 STOP HEIGHT= 6057
AREA = 44740
Plot of data file: C:F827#41.PTS
Date: 09-10-1991 Time: 17:52:45
Sample Name:
Start Time= 18.00Stop Time = 30.00Min. Scale= 5163Max. Scale= 14083

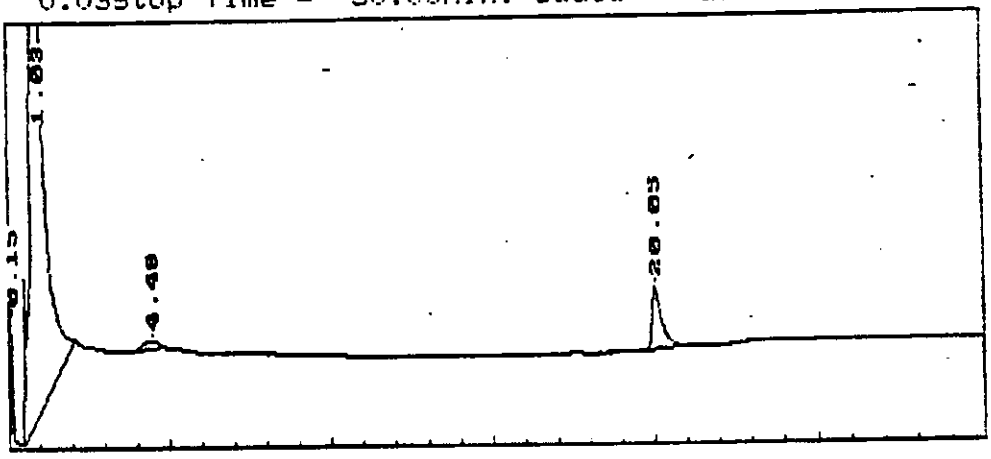


Appendix 6. Raw data and chromatograms for the recovery of tebuconazole and HWG 2061 in milk.

Sample Description	Date ('91) Ext.	Date ('91) Inj.	GC Response(mv)	Residue ppm Gross	Residue ppm Net	Rec %	Chart No.
Tebuconazole							
0.1 ppm Standard	-	08/13	41148	-	-	-	F813#2
Control Rep. #1	08/01	08/13	2420	0.0057	-	-	F813#3
0.1 ppm Standard	-	08/13	43813	-	-	-	F813#4
Control Rep. #2	08/01	08/13	1096	0.0026	-	-	F813#5
0.1 ppm Standard	-	08/13	38964	-	-	-	F813#6
Control + 0.1 ppm	08/01	08/13	39031	0.0982	0.0940	94	F813#7
0.1 ppm Standard	-	08/13	40535	-	-	-	F813#8
Control + 0.1 ppm	08/01	08/13	39673	0.1051	0.1009	101	F813#9
0.1 ppm Standard	-	08/13	34931	-	-	-	F813#11
0.1 ppm Standard	-	09/14	37110	-	-	-	F913#29
Control Rep. #3	09/09	09/14	1945	0.0052	-	-	F913#30
0.1 ppm Standard	-	09/14	37693	-	-	-	F913#31
0.1 ppm Standard	-	09/14	33816	-	-	-	F913#35
Control + 0.05 ppm	09/09	09/14	19179	0.0583	0.0531	106	F913#36
0.1 ppm Standard	-	09/14	31934	-	-	-	F913#37
Control + 0.05 ppm	09/09	09/14	18726	0.0577	0.0525	105	F913#38
0.1 ppm Standard	-	09/14	32952	-	-	-	F913#39
HWG 2061							
0.1 ppm Standard	-	08/14	48933	-	-	-	F814#1
Control Rep. #1	08/06	08/14	282	0.0006	-	-	F814#2
0.1 ppm Standard	-	08/14	43519	-	-	-	F814#3
Control Rep. #2	08/06	08/14	538	0.0013	-	-	F814#4
0.1 ppm Standard	-	08/14	41037	-	-	-	F814#5
Control + 0.1 ppm	08/06	08/14	42921	0.1030	0.1021	102	F814#6
0.1 ppm Standard	-	08/14	42267	-	-	-	F814#7
Control + 0.1 ppm	08/06	08/14	45127	0.1025	0.1016	102	F814#8
0.1 ppm Standard	-	08/14	45784	-	-	-	F814#9
0.1 ppm Standard	-	09/14	34068	-	-	-	F914#1
Control Rep. #3	09/09	09/14	583	0.0016	-	-	F914#2
0.1 ppm Standard	-	09/14	38719	-	-	-	F914#3
0.1 ppm Standard	-	09/14	43423	-	-	-	F914#7
Control + 0.05 ppm	09/09	09/14	17749	0.0431	0.0415	83	F914#8
0.1 ppm Standard	-	09/14	38974	-	-	-	F914#9
Control + 0.05 ppm	09/09	09/14	18975	0.0481	0.0465	93	F914#10
0.1 ppm Standard	-	09/14	39844	-	-	-	F914#11

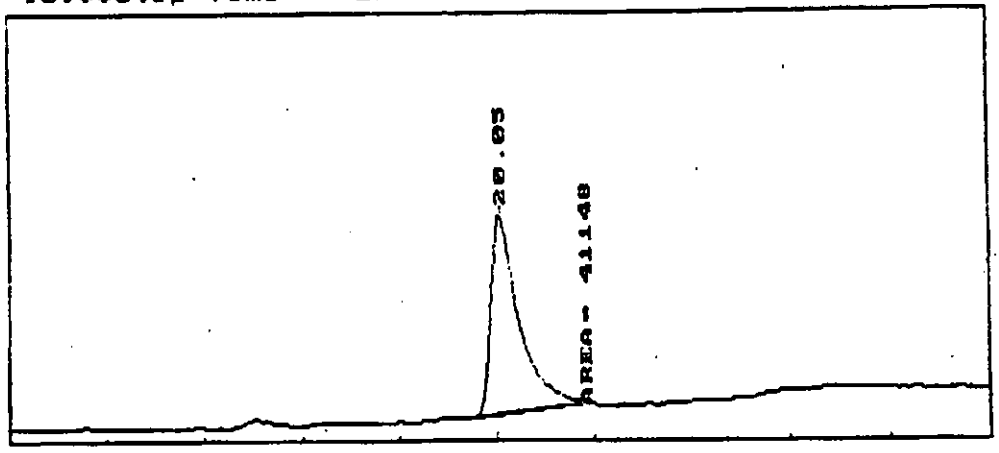
Appendix 6.

Plot of data file: C:F813#2.PTS
Date: 08-14-1991 Time: 14:07:03 SAMPLE NO. 91R104-24-3R
Sample Name: 0.1 PPM FOLICUR STANDARD
Start Time= 0.03Stop Time = 30.00Min. Scale= 2223Max. Scale= 16203



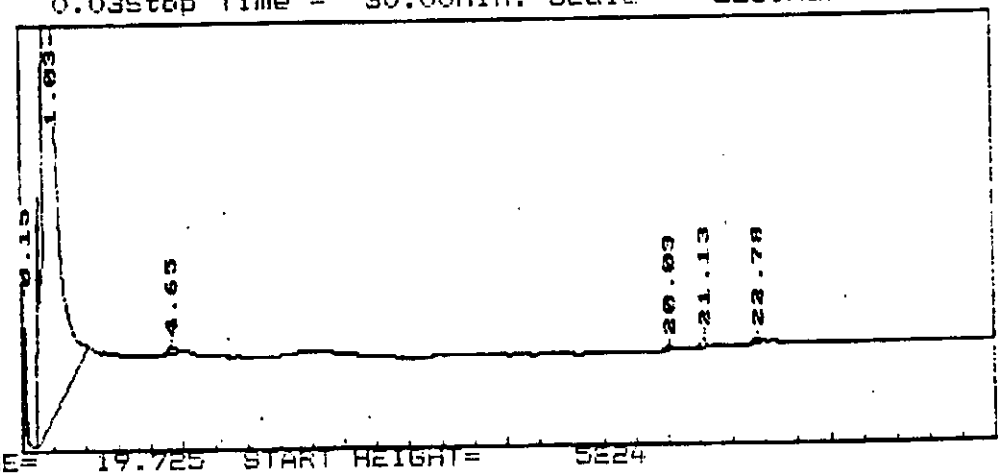
START TIME= 19.775 START HEIGHT= 5177
STOP TIME= 20.975 STOP HEIGHT= 5323
AREA = 41148

Plot of data file: C:F813#2.PTS
Date: 08-14-1991 Time: 14:08:41
Sample Name:
Start Time= 15.00Stop Time = 25.00Min. Scale= 4960Max. Scale= 9304



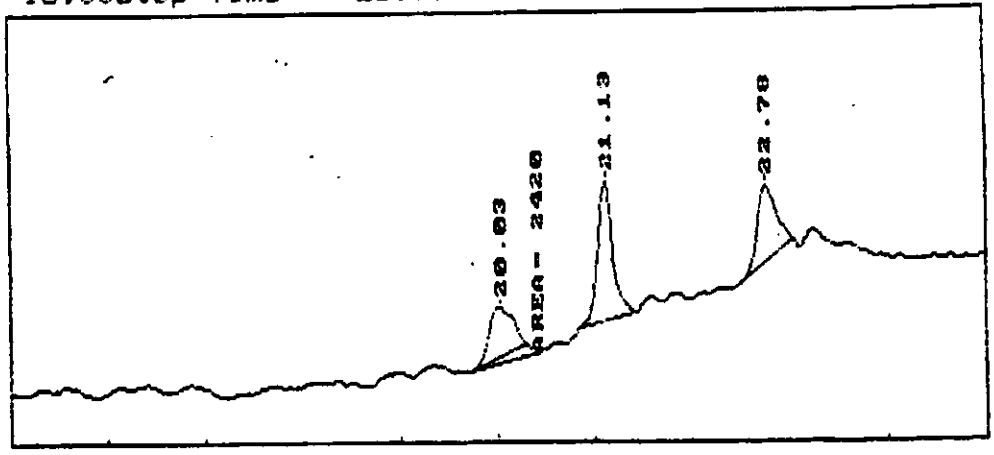
Appendix 6.

Plot of data file: C:F813#3.PTS
Date: 08-14-1991 Time: 14:12:49 **SAMPLE NO. 91R104-24-3N**
Sample Name: **CONTROL BOVINE MILK REP.#1**
Start Time= 0.03Stop Time = 30.00Min. Scale= 2231Max. Scale= 16483



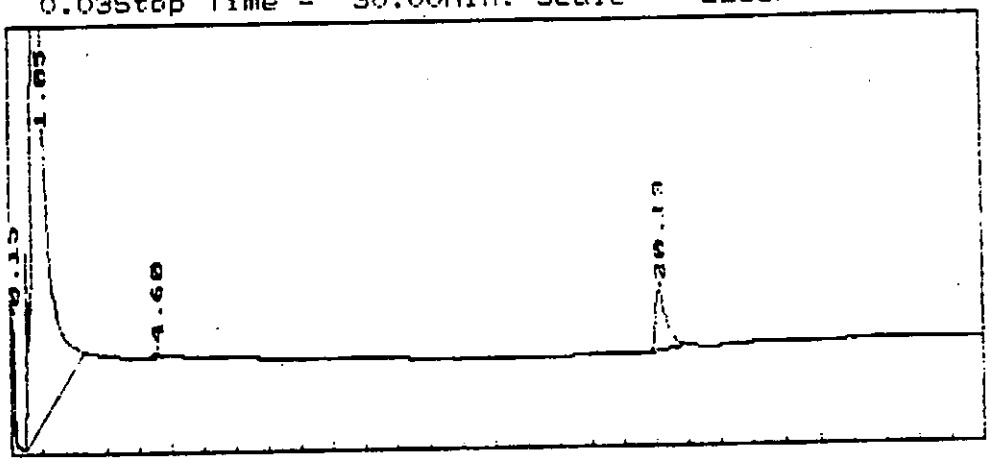
START TIME= 19.725 START HEIGHT= 5224
STOP TIME= 20.475 STOP HEIGHT= 5271
AREA = 2420

Plot of data file: C:F813#3.PTS
Date: 08-14-1991 Time: 14:14:33
Sample Name:
Start Time= 15.00Stop Time = 25.00Min. Scale= 5068Max. Scale= 6060

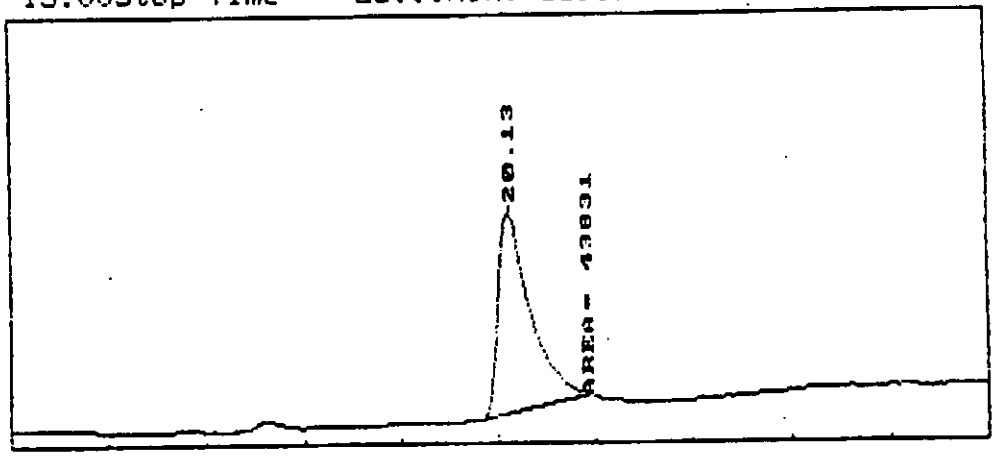


Appendix 6.

Plot of data file: C:FS13#4.PTS
Date: 08-14-1991 Time: 14:16:36 **SAMPLE NO. 91R104-24-3R**
Sample Name: **0.1 PPM FOLICUR STANDARD**
Start Time= 0.03Stop Time = 30.00Min. Scale= 2285Max. Scale= 15821

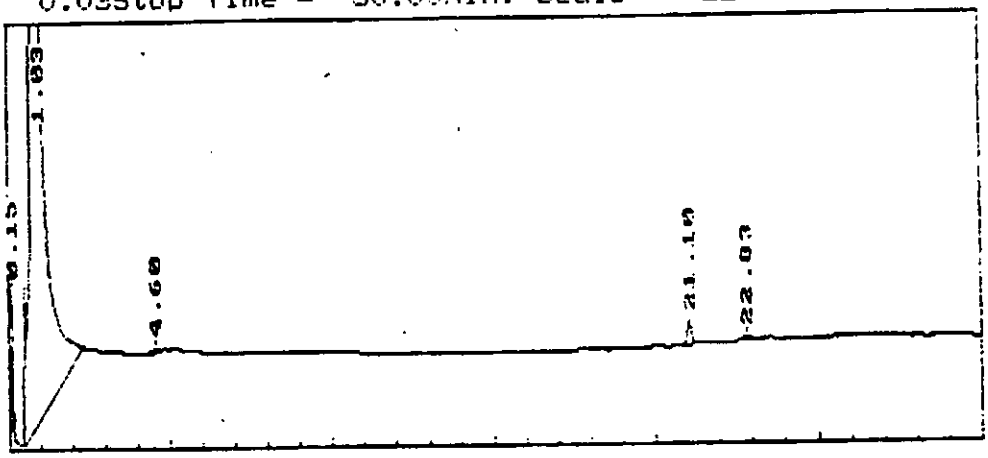


START TIME= 19.825 START HEIGHT= 5145
STOP TIME= 20.975 STOP HEIGHT= 5377
AREA = 43831
Plot of data file: C:FS13#4.PTS
Date: 08-14-1991 Time: 14:17:52
Sample Name:
Start Time= 15.00Stop Time = 25.00Min. Scale= 4946Max. Scale= 9046

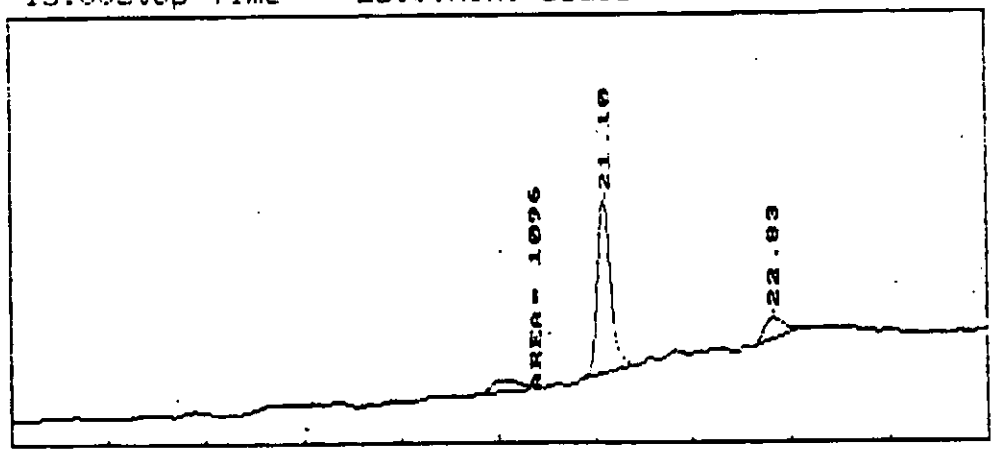


Appendix 6.

Plot of data file: C:F813#5.PTS
Date: 08-14-1991 Time: 14:20:49 **SAMPLE NO. 91R104-24-30**
Sample Name: **CONTROL BOVINE MILK REP.#2**
Start Time= 0.03Stop Time = 30.00Min. Scale= 2276Max. Scale= 15864

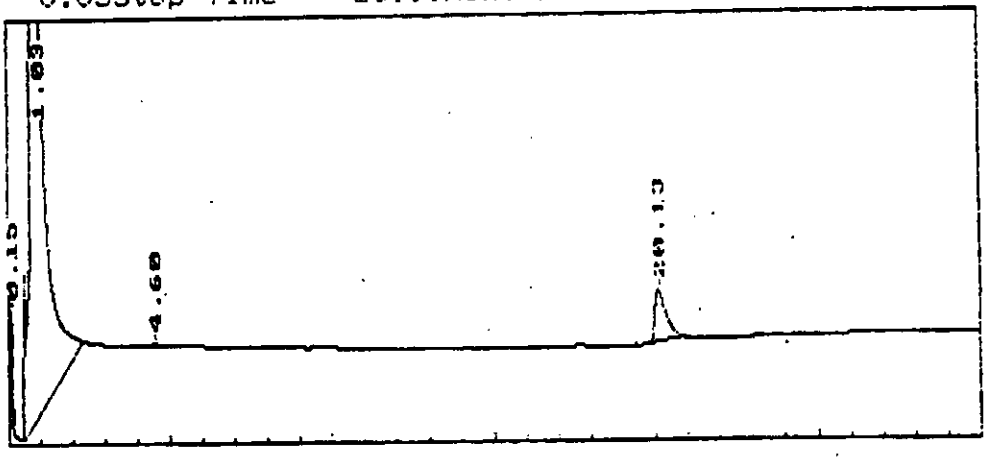


START TIME= 19.825 START HEIGHT= 5209
STOP TIME= 20.450 STOP HEIGHT= 5236
AREA = 1096
Plot of data file: C:F813#5.PTS
Date: 08-14-1991 Time: 14:22:21
Sample Name:
Start Time= 15.00Stop Time = 25.00Min. Scale= 5005Max. Scale= 6835

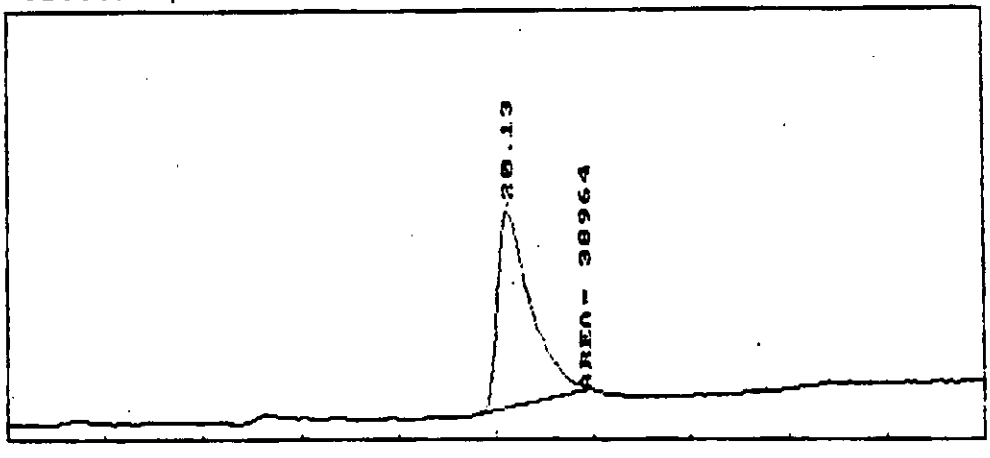


Appendix 6.

Plot of data file: C:F813#6.PTS
Date: 08-14-1991 Time: 14:23:46 **SAMPLE NO. 91R104-24-3R**
Sample Name: **0.1 PPM FOLICUR STANDARD**
Start Time= 0.03Stop Time = 30.00Min. Scale= 2291Max. Scale= 15670

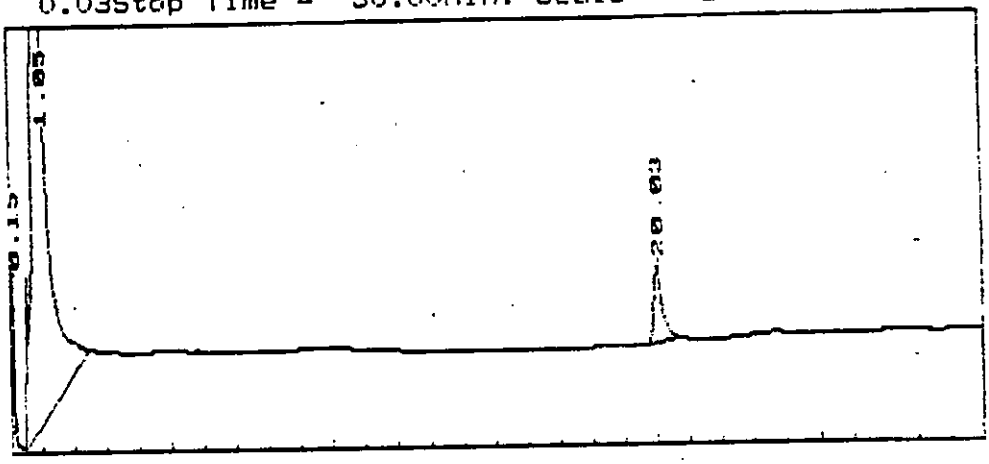


START TIME= 19.775 START HEIGHT= 5147
STOP TIME= 20.975 STOP HEIGHT= 5372
AREA = 38964
Plot of data file: C:F813#6.PTS
Date: 08-14-1991 Time: 14:25:13
Sample Name:
Start Time= 15.00Stop Time = 25.00Min. Scale= 4959Max. Scale= 8559

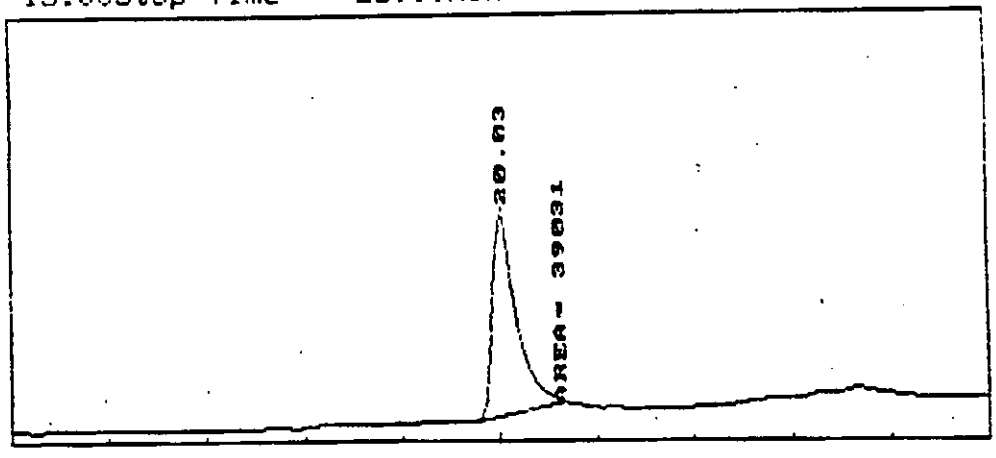


Appendix 6.

Plot of data file: C:F813#7.PTS
Date: 08-14-1991 Time: 14:26:56 SAMPLE NO. 91R104-24-3P'
Sample Name: CONTROL BOVINE MILK + 0.1 PPM FOLICUR STANDARD REP.#1
Start Time= 0.03Stop Time = 30.00Min. Scale= 2307Max. Scale= 15497

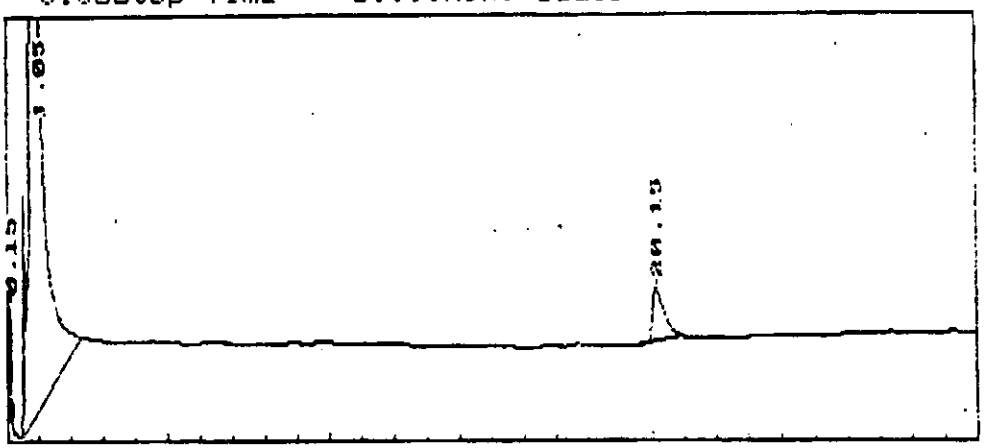


START TIME= 19.775 START HEIGHT= 5255
STOP TIME= 20.675 STOP HEIGHT= 5487
AREA = 39031
Plot of data file: C:F813#7.PTS
Date: 08-14-1991 Time: 14:28:07
Sample Name:
Start Time= 15.00Stop Time = 25.00Min. Scale= 5040Max. Scale= 9970

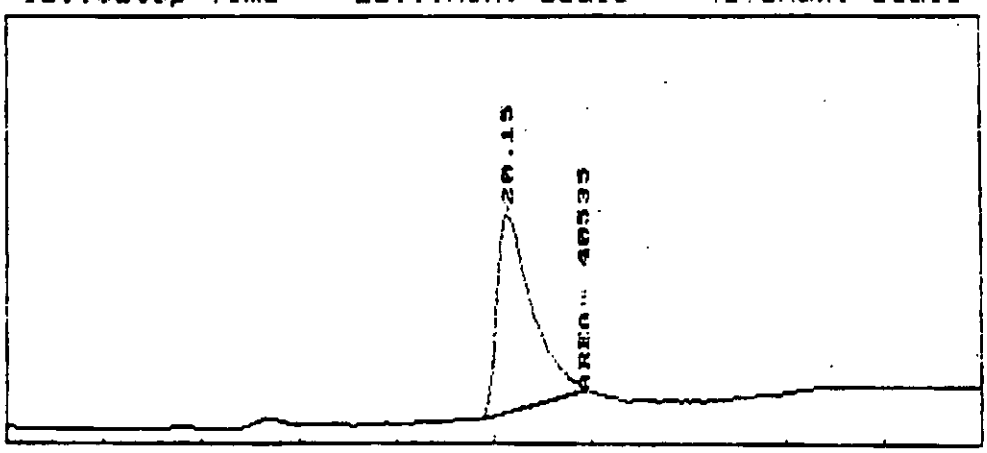


Appendix 6.

Plot of data file: C:F813#8.PTS
Date: 08-14-1991 Time: 14:29:28 **SAMPLE NO. 91R104-24-3R**
Sample Name: **0.1 PPM FOLICUR STANDARD**
Start Time= 0.03Stop Time = 30.00Min. Scale= 3241Max. Scale= 15230

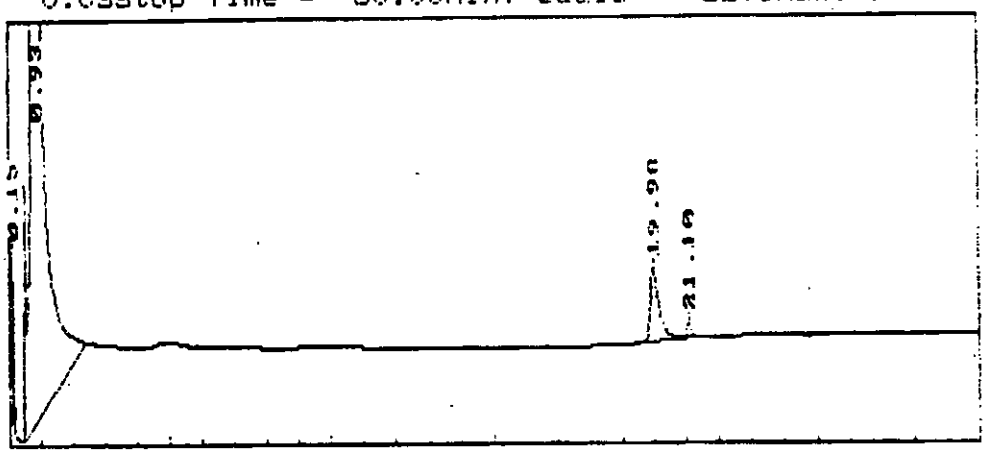


START TIME= 19.875 START HEIGHT= 5093
STOP TIME= 20.975 STOP HEIGHT= 5328
AREA = 40535
Plot of data file: C:F813#8.PTS
Date: 08-14-1991 Time: 14:30:30
Sample Name:
Start Time= 15.00Stop Time = 25.00Min. Scale= 4895Max. Scale= 8399

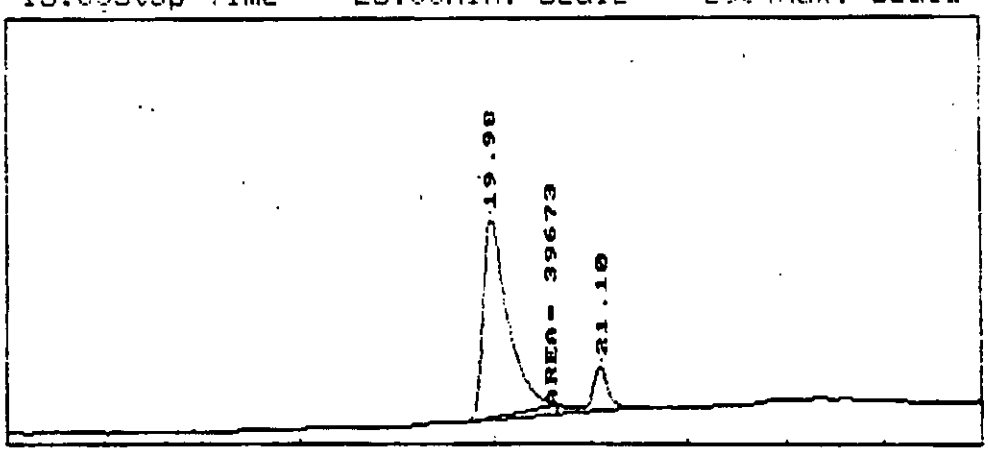


Appendix 6.

Plot of data file: C:F813#9.PTS
Date: 08-14-1991 Time: 14:31:53 SAMPLE NO. 91R104-24-3Q
Sample Name: CONTROL BOVINE MILK + 0.1 PPM FOLICUR STANDARD REP.#2
Start Time= 0.03Stop Time = 30.00Min. Scale= 2291Max. Scale= 15441

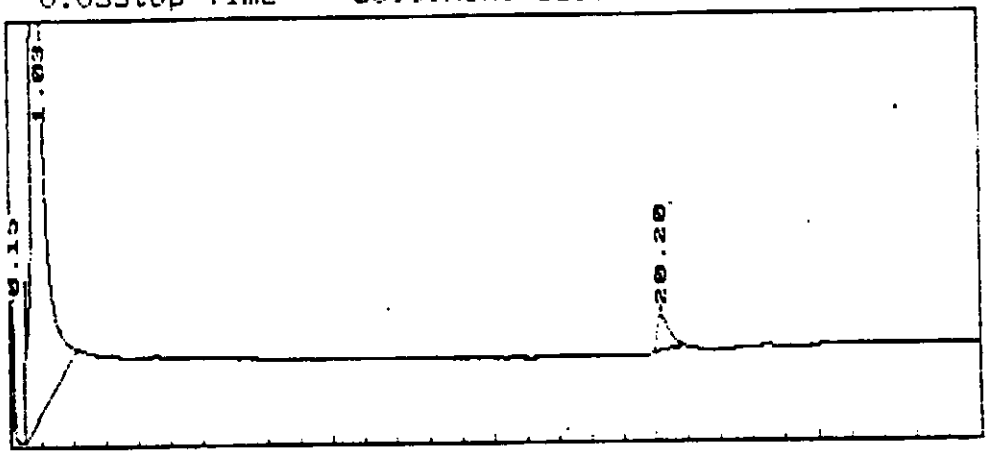


START TIME= 19.725 START HEIGHT= 5252
STOP TIME= 20.650 STOP HEIGHT= 5456
AREA = 39673
Plot of data file: C:F813#9.PTS
Date: 08-14-1991 Time: 14:33:24
Sample Name:
Start Time= 15.00Stop Time = 25.00Min. Scale= 5004Max. Scale= 9920

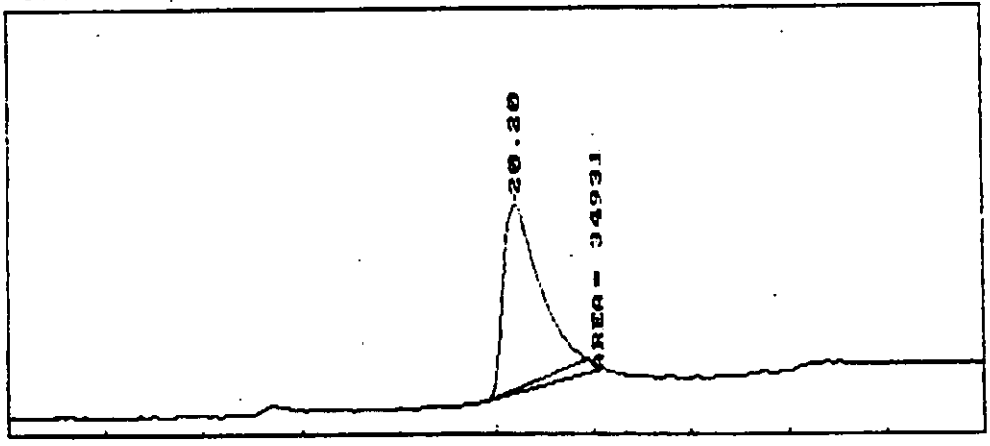


Appendix 6.

Plot of data file: C:F813#11.PTS
Date: 08-14-1991 Time: 14:50:22 **SAMPLE NO. 91R104-24-3R**
Sample Name: **0.1 PPM FOLICUR STANDARD**
Start Time= 0.03Stop Time = 30.00Min. Scale= 2268Max. Scale= 17043

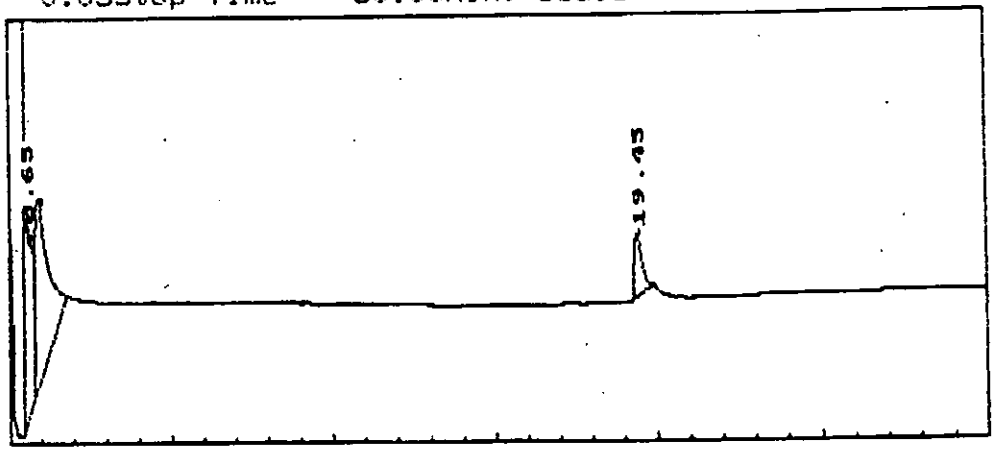


START TIME= 19.900 START HEIGHT= 5146
STOP TIME= 20.975 STOP HEIGHT= 5416
AREA = 32422
START TIME= 19.900 START HEIGHT= 5146
STOP TIME= 21.100 STOP HEIGHT= 5369
AREA = 34931
Plot of data file: C:F813#11.PTS
Date: 08-14-1991 Time: 14:52:24
Sample Name:
Start Time= 15.00Stop Time = 25.00Min. Scale= 4949Max. Scale= 7751

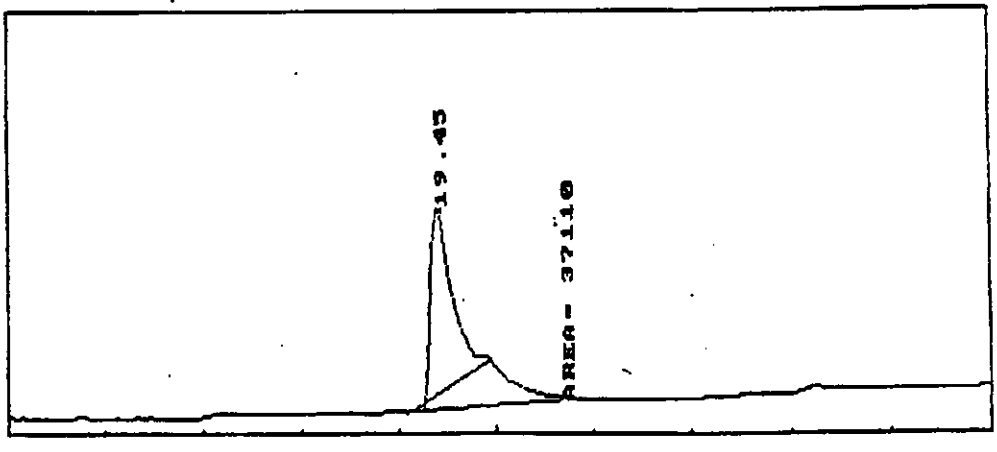


Appendix 6.

Plot of data file: C:f913#29.PTS SAMPLE NO. 91R104-28-2V
Date: 09-20-1991 Time: 14:21:50
Sample Name: 0.1 PPM FOLICUR STANDARD
Start Time= 0.03Stop Time = 30.00Min. Scale= 1712Max. Scale= 12019

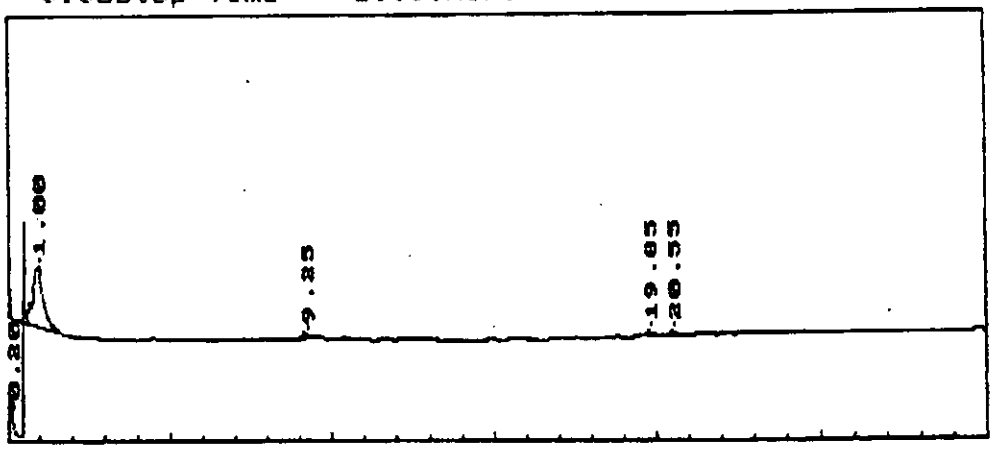


START TIME= 19.225 START HEIGHT= 4971
START TIME= 19.225 START HEIGHT= 4971
STOP TIME= 20.800 STOP HEIGHT= 5080
AREA = 37110
Plot of data file: C:f913#29.PTS
Date: 09-20-1991 Time: 14:23:57
Sample Name:
Start Time= 15.00Stop Time = 25.00Min. Scale= 4820Max. Scale= 8120

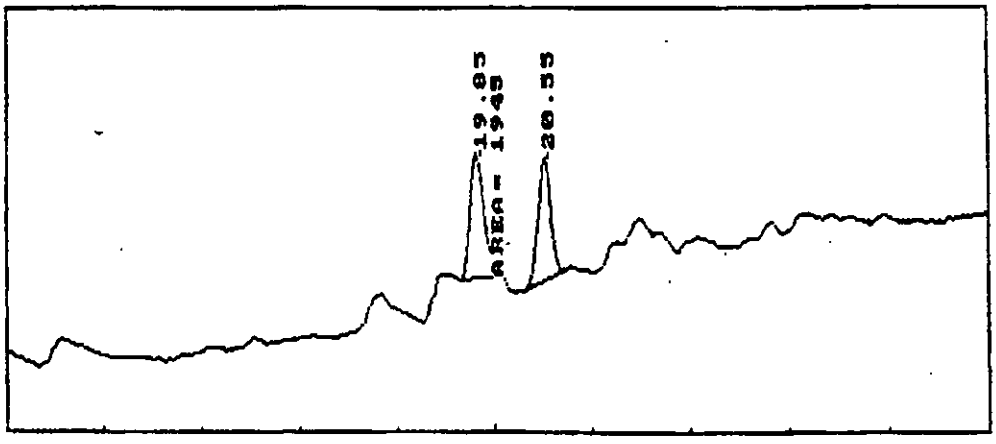


Appendix 6.

Plot of data file: C:F913#30.PTS
Date: 09-20-1991 Time: 14:25:17 SAMPLE NO. 91R104-28-2Q
Sample Name: CONTROL BOVINE MILK
Start Time= 0.03Stop Time = 30.00Min. Scale= 1693Max. Scale= 16031

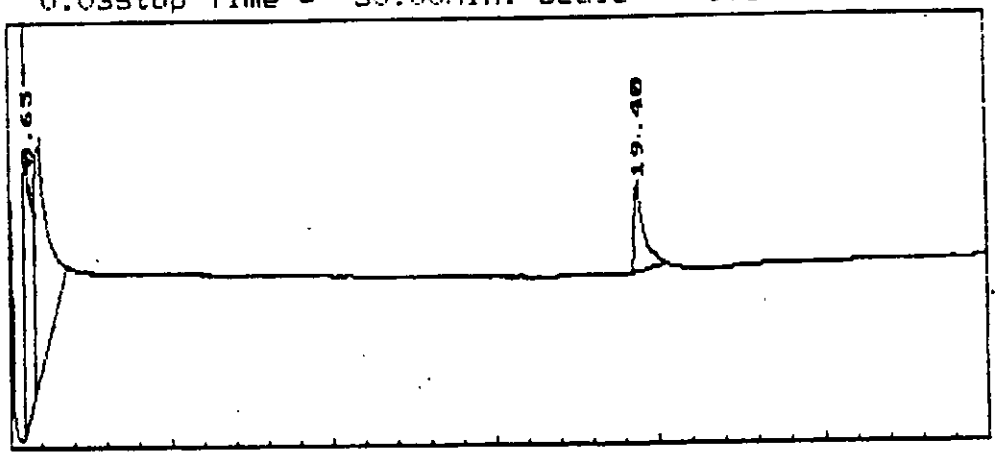


START TIME= 19.675 START HEIGHT= 5121
STOP TIME= 20.100 STOP HEIGHT= 5127
AREA = 1945
Plot of data file: C:F913#30.PTS
Date: 09-20-1991 Time: 14:29:25
Sample Name:
Start Time= 15.00Stop Time = 25.00Min. Scale= 4884Max. Scale= 5540



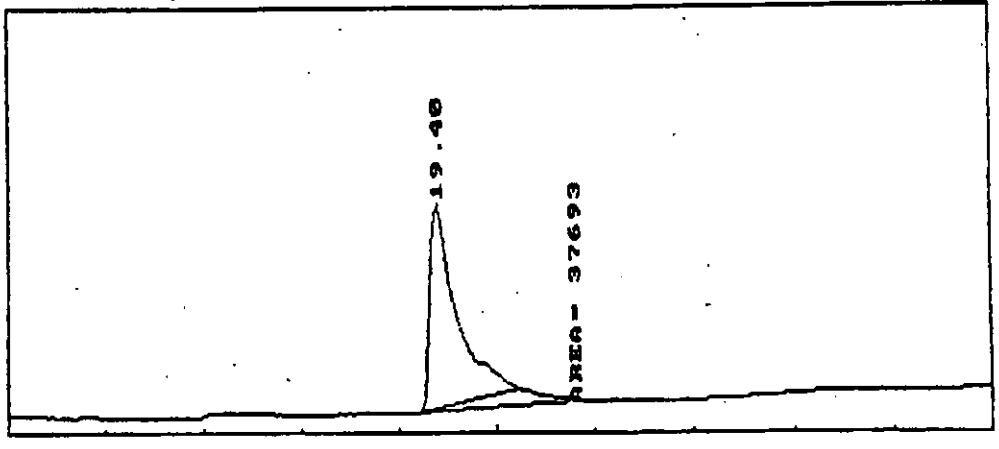
Appendix 6.

Plot of data file: C:F913#31.PTS
Date: 09-20-1991 Time: 14:30:51 SAMPLE NO. 91R104-28-2V
Sample Name: 0.1 PPM FOLICUR STANDARD
Start Time= 0.03Stop Time = 30.00Min. Scale= 1739Max. Scale= 10089



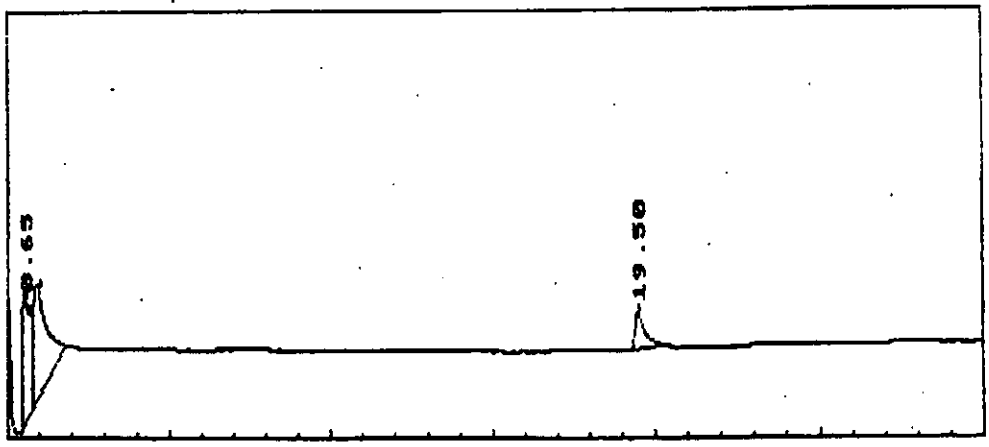
START TIME= 19.150 START HEIGHT= 4999
STOP TIME= 20.875 STOP HEIGHT= 5106
AREA = 37693

Plot of data file: C:F913#31.PTS
Date: 09-20-1991 Time: 14:32:25
Sample Name:
Start Time= 15.00Stop Time = 25.00Min. Scale= 4850Max. Scale= 8290

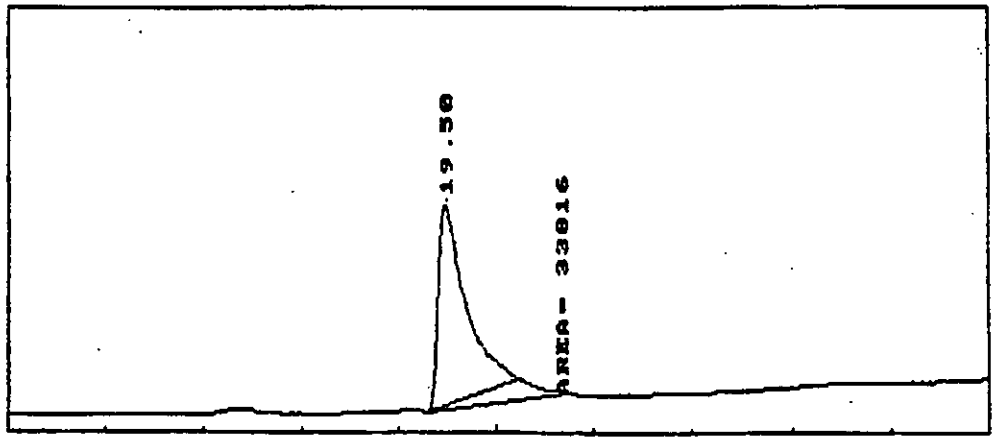


Appendix 6.

Plot of data file: C:F913#35.PTS
Date: 09-20-1991 Time: 14:44:50 SAMPLE NO. 91R104-28-2V
Sample Name: 0.1 PPM FOLICUR STANDARD
Start Time= 0.03Stop Time = 30.00Min. Scale= 1716Max. Scale= 18124

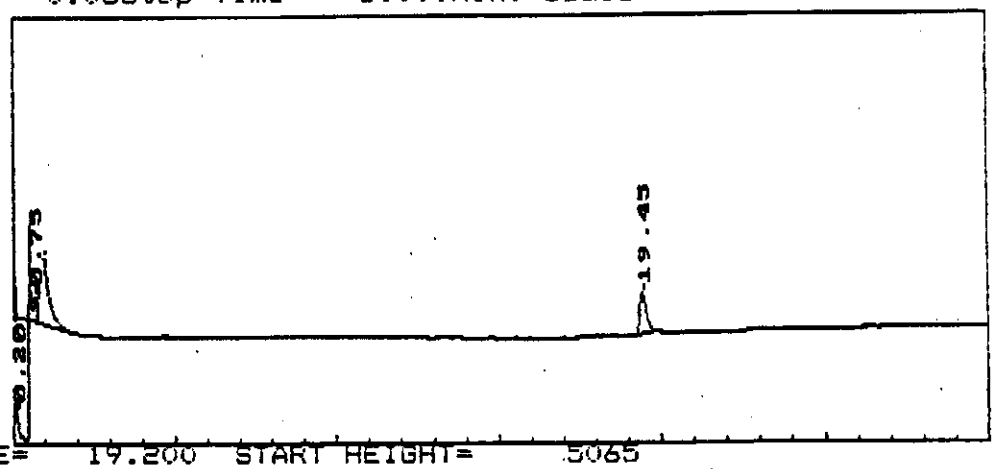


START TIME= 19.300 START HEIGHT= 4936
STOP TIME= 20.750 STOP HEIGHT= 5073
AREA = 33816
Plot of data file: C:F913#35.PTS
Date: 09-20-1991 Time: 14:47:15
Sample Name:
Start Time= 15.00Stop Time = 25.00Min. Scale= 4803Max. Scale= 7767



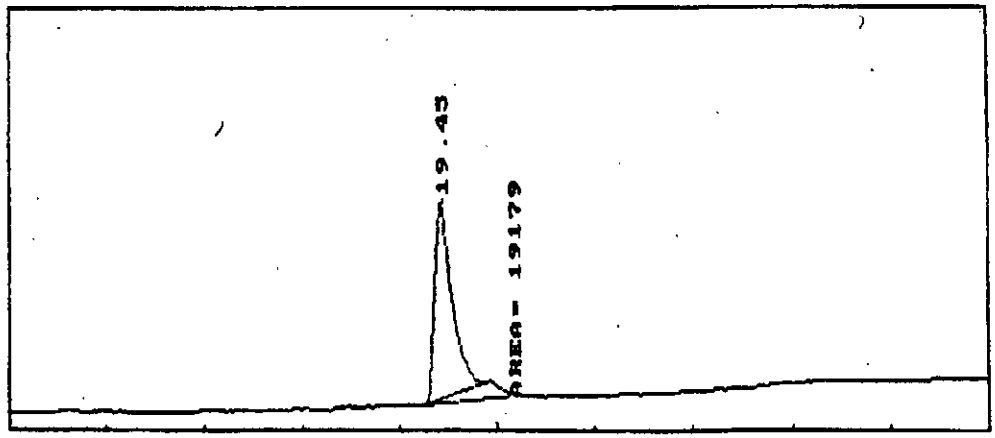
Appendix 6.

Plot of data file: C:F913#36.PTS
Date: 09-20-1991 Time: 14:49:02 Sample No. 91R104-28-2T
Sample Name: CONTROL BOVINE MILK + 0.05 PPM FOLICUR STD. REP.#1
Start Time= 0.03Stop Time = 30.00Min. Scale= 1769Max. Scale= 15181



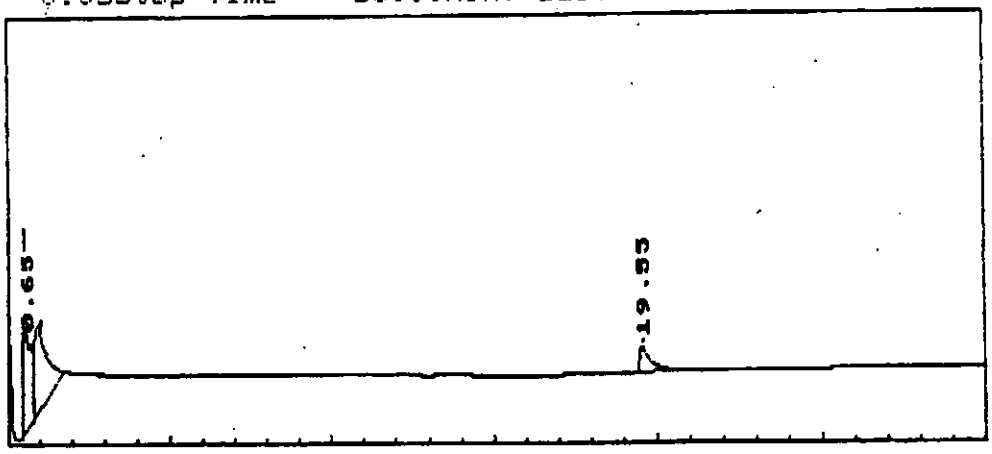
START TIME= 19.200 START HEIGHT= 5065

START TIME= 19.225 START HEIGHT= 5063
STOP TIME= 20.250 STOP HEIGHT= 5128
AREA = 19179
Plot of data file: C:F913#36.PTS
Date: 09-20-1991 Time: 14:50:23
Sample Name:
Start Time= 15.00Stop Time = 25.00Min. Scale= 4909Max. Scale= 7687

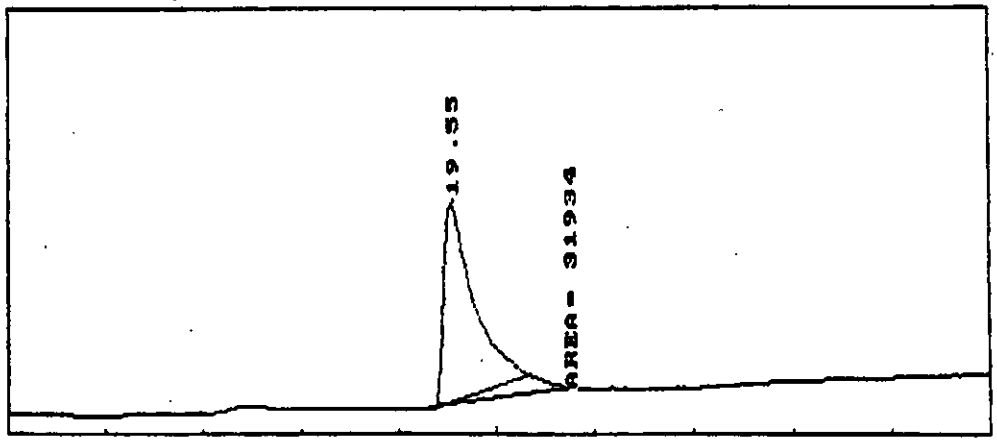


Appendix 6.

Plot of data file: C:F913#37.PTS
Date: 09-20-1991 Time: 14:51:29 **SAMPLE NO. 91R104-28-2V**
Sample Name: **0.1 PPM FOLICUR STANDARD**
Start Time= 0.03Stop Time = 30.00Min. Scale= 1725Max. Scale= 22793

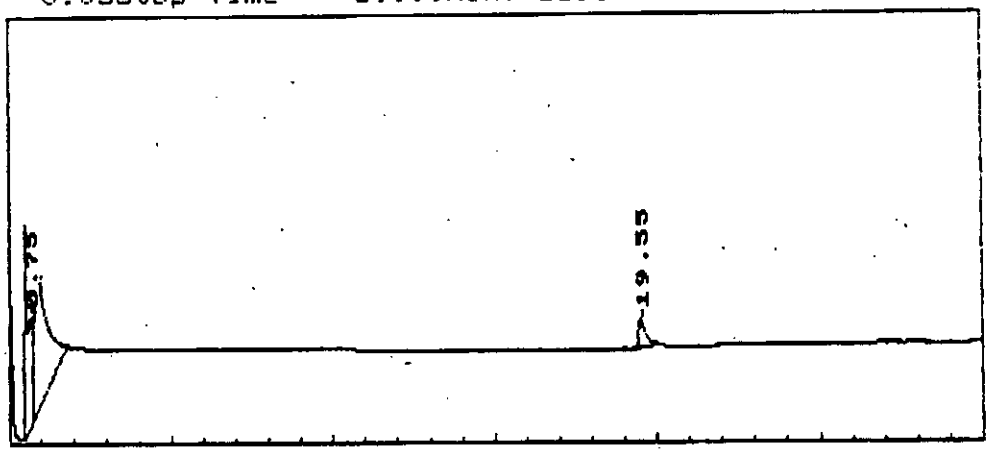


START TIME= 19.325 START HEIGHT= 4971
STOP TIME= 20.825 STOP HEIGHT= 5100
AREA = 31934
Plot of data file: C:F913#37.PTS
Date: 09-20-1991 Time: 14:54:18
Sample Name:
Start Time= 15.00Stop Time = 25.00Min. Scale= 4817Max. Scale= 7499

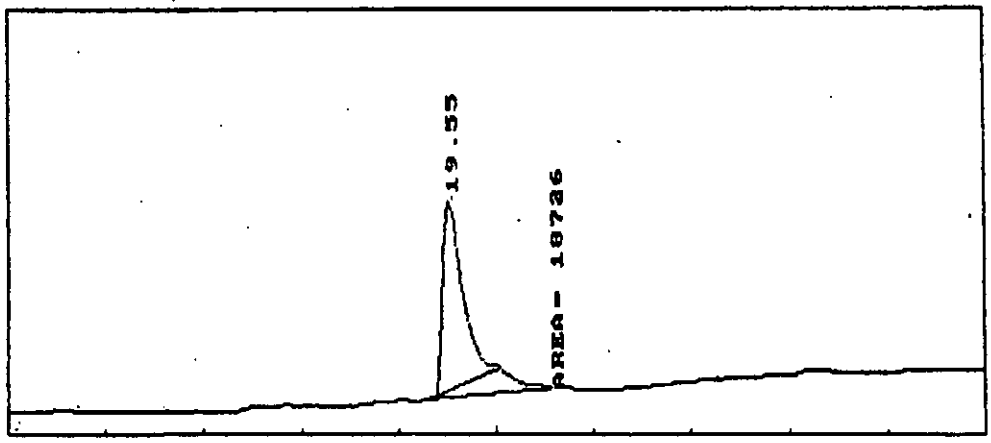


Appendix 6.

Plot of data file: C:F913#38.PTS
Date: 09-20-1991 Time: 14:55:38 SAMPLE NO. 91R104-28-2U
Sample Name: CONTROL BOVINE MILK + 0.05 PPM FOLICUR STD. REP.#2
Start Time= 0.03Stop Time = 30.00Min. Scale= 1733Max. Scale= 17299

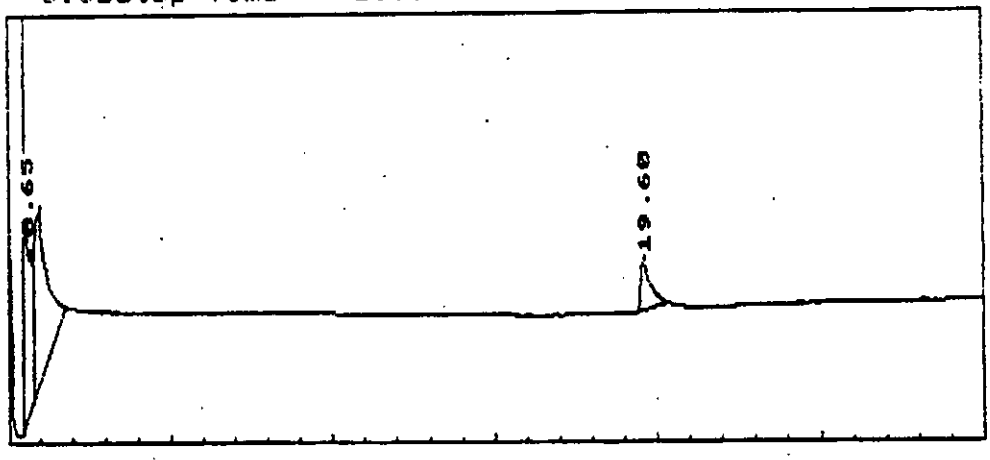


START TIME= 19.250 START HEIGHT= 5039
STOP TIME= 20.700 STOP HEIGHT= 5112
AREA = 18726
Plot of data file: C:F913#38.PTS
Date: 09-20-1991 Time: 14:57:19
Sample Name:
Start Time= 15.00Stop Time = 25.00Min. Scale= 4872Max. Scale= 7090

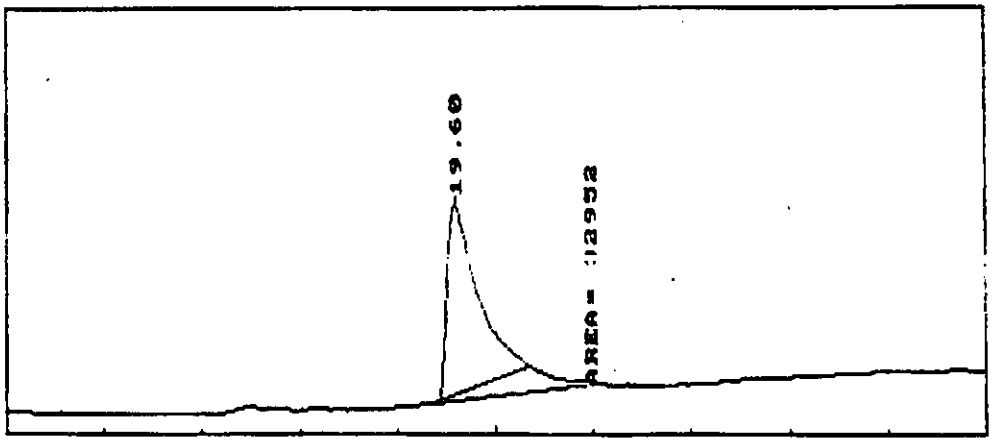


Appendix 6.

Plot of data file: C:F913#39.PTS
Date: 09-20-1991 Time: 14:59:04 SAMPLE NO. 91R104-28-2V
Sample Name: 0.1 PPM FOLICUR STANDARD
Start Time= 0.03Stop Time = 30.00Min. Scale= 1784Max. Scale= 12774

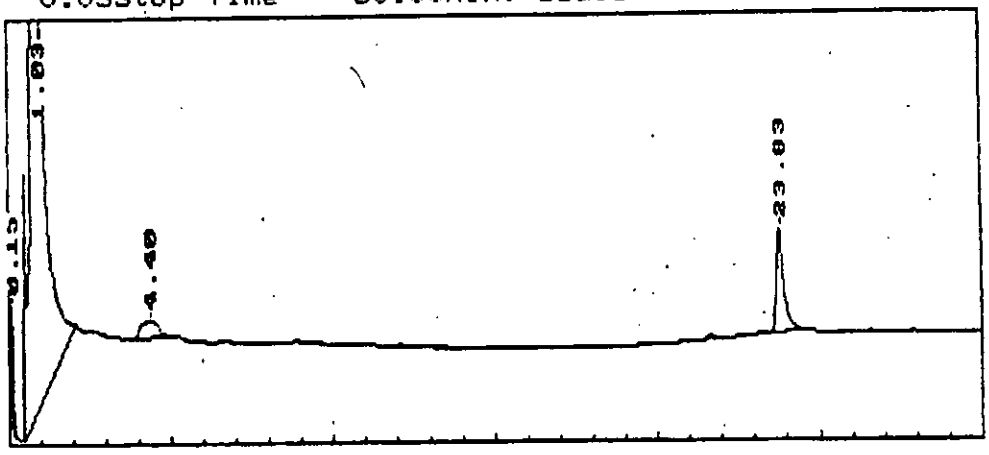


START TIME= 19.375 START HEIGHT= 5031
STOP TIME= 21.050 STOP HEIGHT= 5157
AREA = 32952
Plot of data file: C:F913#39.PTS
Date: 09-20-1991 Time: 15:00:14
Sample Name:
Start Time= 15.00Stop Time = 25.00Min. Scale= 4865Max. Scale= 7433

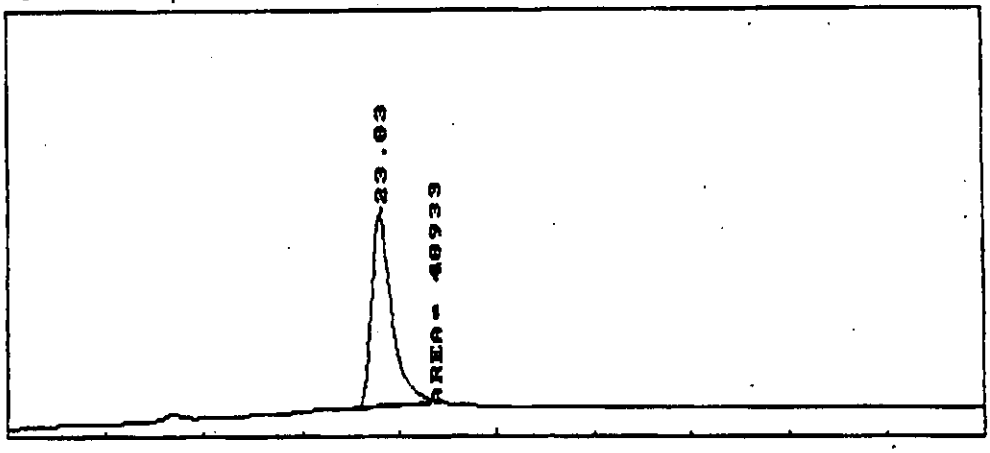


Appendix 6.

Plot of data file: C:F814#1.PTS
Date: 08-20-1991 Time: 13:22:17 SAMPLE NO. 91R104-24-3M
Sample Name: 0.1 PPM HWG 2061 STANDARD
Start Time= 0.03Stop Time = 30.00Min. Scale= 2301Max. Scale= 16020

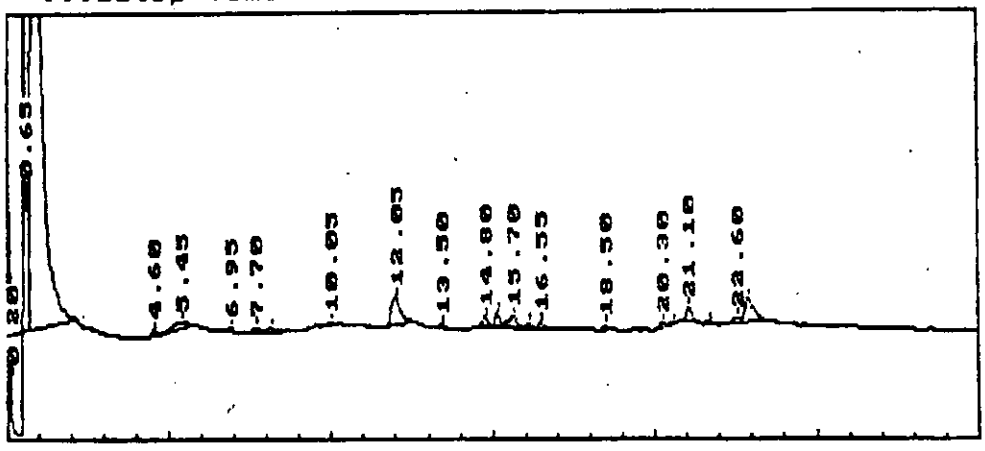


START TIME= 23.550 START HEIGHT= 5680
STOP TIME= 24.450 STOP HEIGHT= 5791
AREA = 48933
Plot of data file: C:F814#1.PTS
Date: 08-20-1991 Time: 13:23:21
Sample Name:
Start Time= 20.00Stop Time = 30.00Min. Scale= 5217Max. Scale= 12561

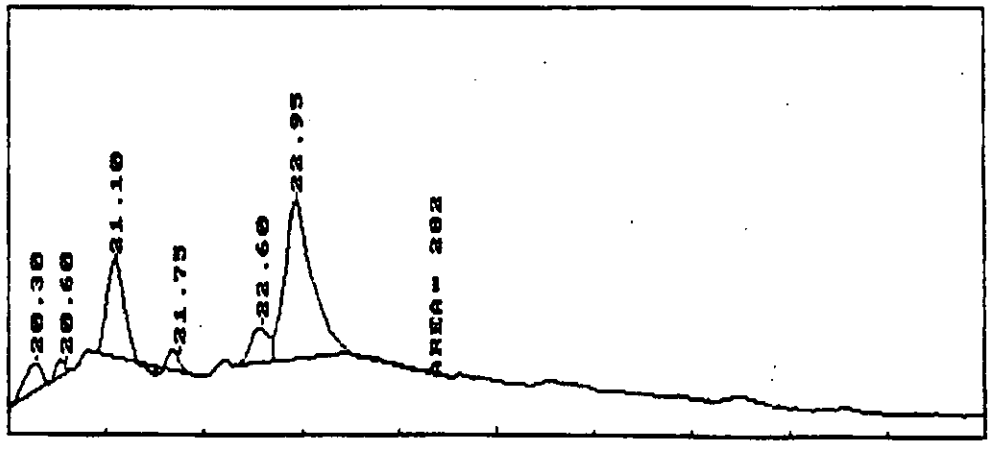


Appendix 6.

Plot of data file: C:F814#2.PTS
Date: 08-20-1991 Time: 13:24:44 SAMPLE NO. 91R104-24-3I
Sample Name: CONTROL BOVINE MILK REP.#1
Start Time= 0.03Stop Time = 30.00Min. Scale= 2267Max. Scale= 16478

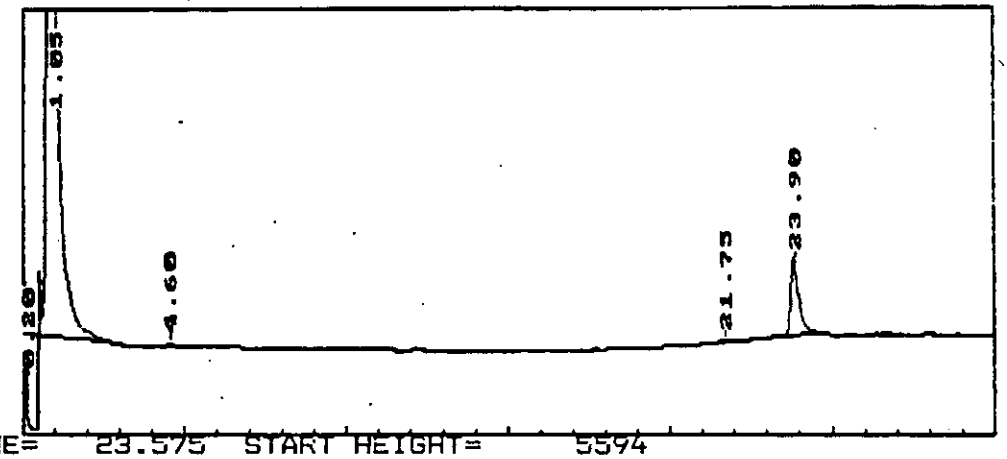


START TIME= 23.550 START HEIGHT= 6069
STOP TIME= 24.450 STOP HEIGHT= 5967
AREA = 282
Plot of data file: C:F814#2.PTS
Date: 08-20-1991 Time: 13:26:02
Sample Name:
Start Time= 20.00Stop Time = 30.00Min. Scale= 5660Max. Scale= 7888



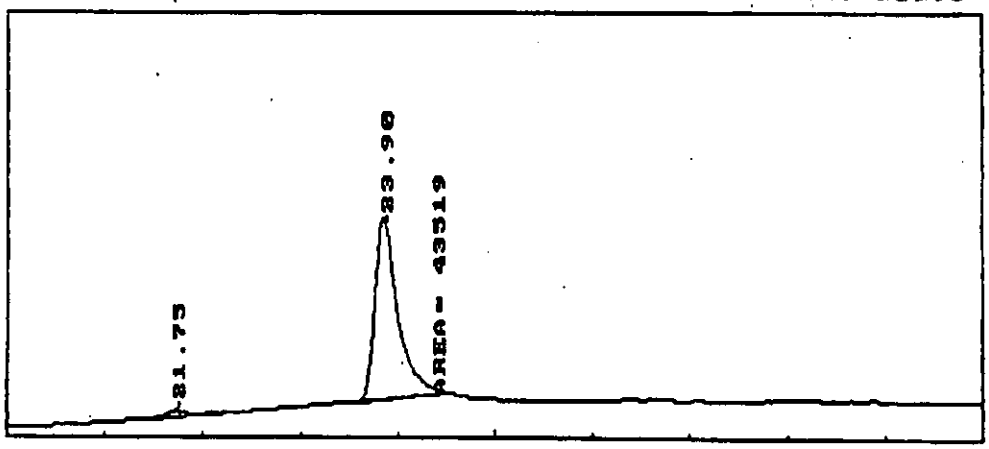
Appendix 6.

Plot of data file: C:F814#3.PTS
Date: 08-20-1991 Time: 13:27:10 SAMPLE NO. 91R104-24-3M
Sample Name: 0.1 PPM HWG 2061 STANDARD
Start Time= 0.03Stop Time = 30.00Min. Scale= 2239Max. Scale= 17026



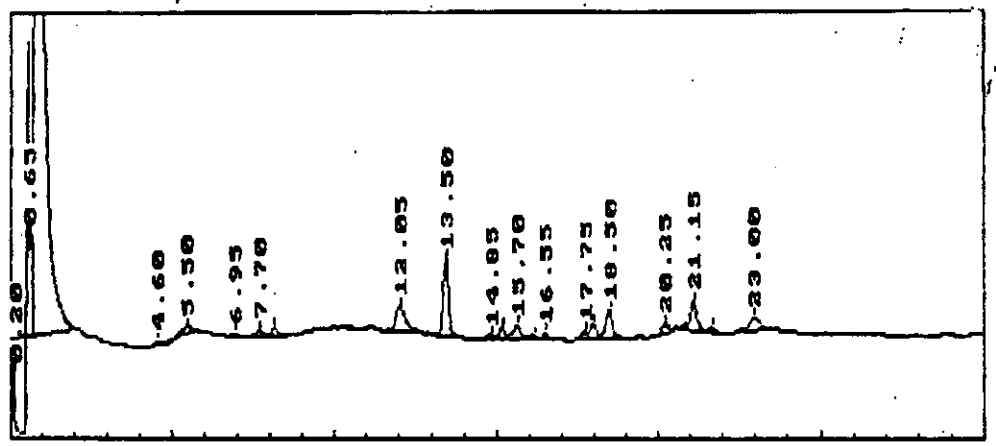
START TIME= 23.575 START HEIGHT= 5594

START TIME= 23.600 START HEIGHT= 5600
STOP TIME= 24.500 STOP HEIGHT= 5748
AREA = 43519
Plot of data file: C:F814#3.PTS
Date: 08-20-1991 Time: 13:28:36
Sample Name:
Start Time= 20.00Stop Time = 30.00Min. Scale= 5114Max. Scale= 11222

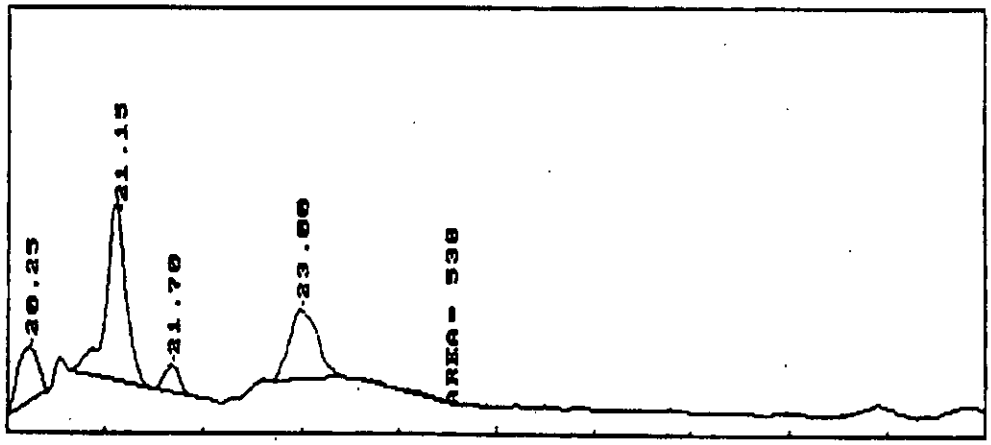


Appendix 6.

Plot of data file: C:F814#4.PTS
Date: 08-20-1991 Time: 13:29:44 SAMPLE NO. 91R104-24-3J
Sample Name: CONTROL BOVINE MILK REP.#2
Start Time= 0.03Stop Time = 30.00Min. Scale= 2275Max. Scale= 17426

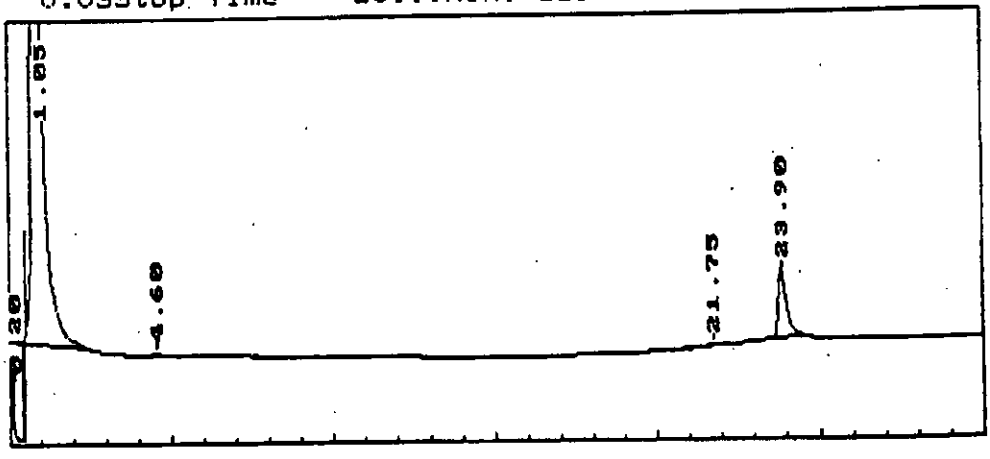


START TIME= 23.600 START HEIGHT= 6049
STOP TIME= 24.600 STOP HEIGHT= 5897
AREA = 538
Plot of data file: C:F814#4.PTS
Date: 08-20-1991 Time: 13:31:09
Sample Name:
Start Time= 20.00Stop Time = 30.00Min. Scale= 5728Max. Scale= 8302

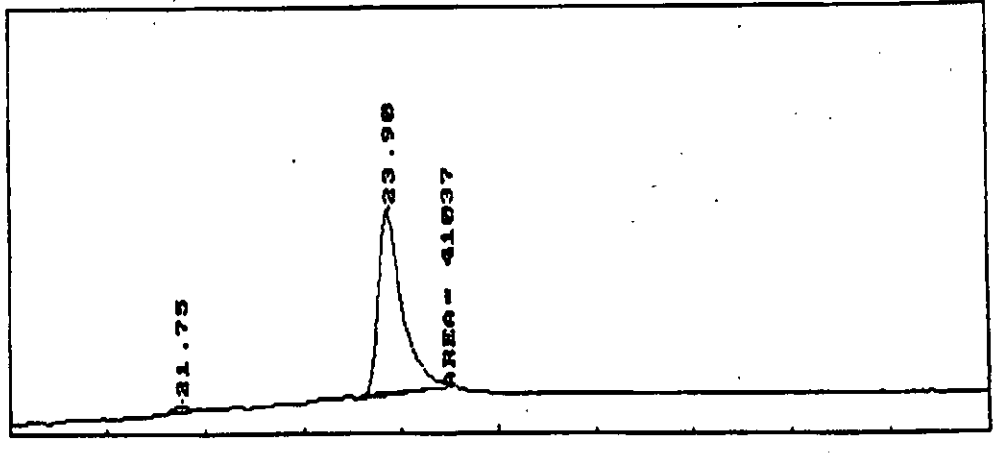


Appendix 6.

Plot of data file: C:F814#5.PTS
Date: 08-20-1991 Time: 13:32:09 SAMPLE NO. 91R104-24-3M
Sample Name: 0.1 PPM HWG 2061 STANDARD
Start Time= 0.03Stop Time = 30.00Min. Scale= 2202Max. Scale= 16939

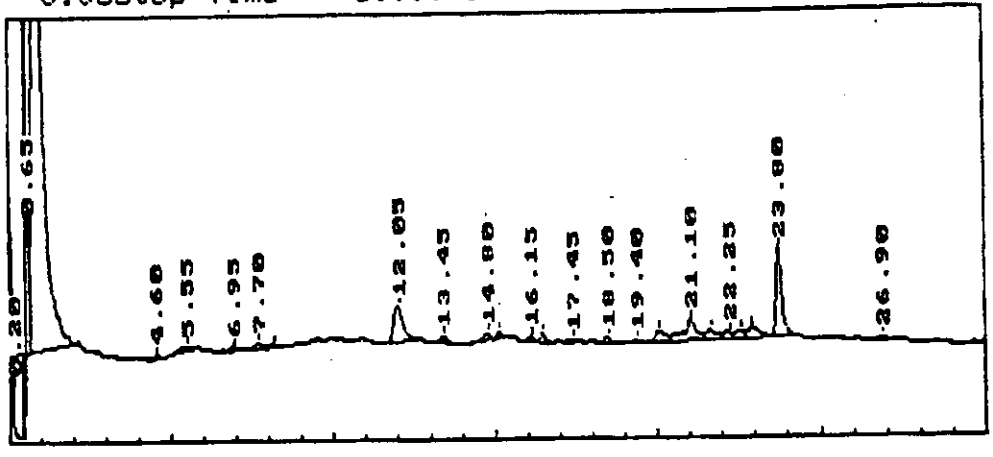


START TIME= 23.600 START HEIGHT= 5523
STOP TIME= 24.550 STOP HEIGHT= 5666
AREA = 41037
Plot of data file: C:F814#5.PTS
Date: 08-20-1991 Time: 13:33:15
Sample Name:
Start Time= 20.00Stop Time = 30.00Min. Scale= 5072Max. Scale= 10632

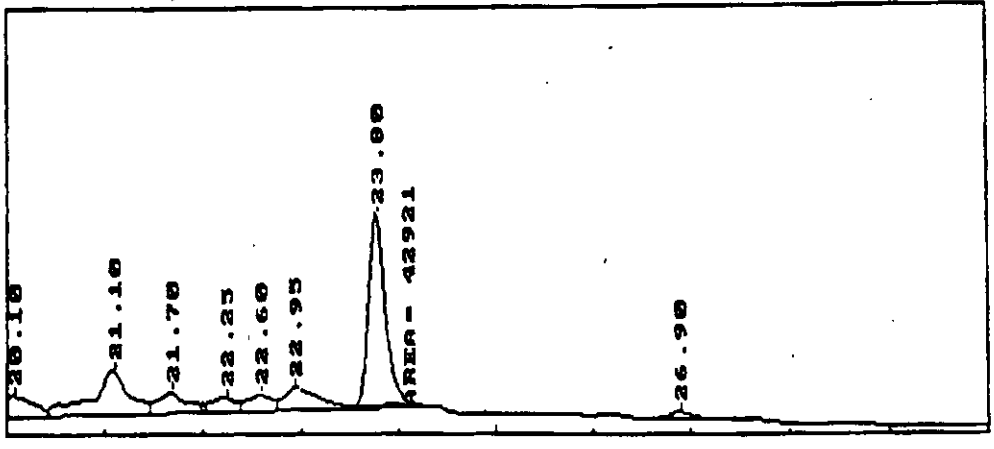


Appendix 6.

Plot of data file: C:FB14#6.PTS
Date: 08-20-1991 Time: 13:40:00 SAMPLE NO. 91R104-24-3K
Sample Name: CONTROL BOVINE MILK + 0.1 PPM HWG 2061 STD. REP.#1
Start Time= 0.03Stop Time = 30.00Min. Scale= 2270Max. Scale= 19841

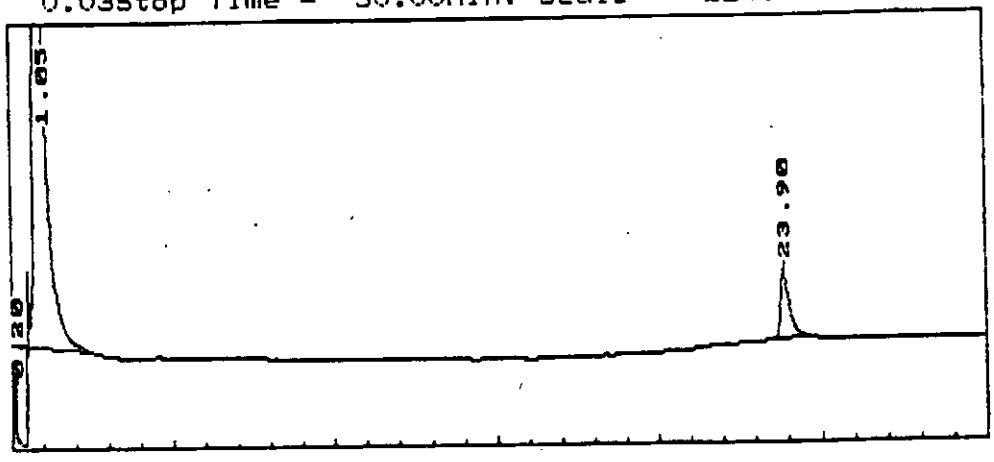


START TIME= 23.525 START HEIGHT= 6260
STOP TIME= 24.175 STOP HEIGHT= 6322
AREA = 42921
Plot of data file: C:FB14#6.PTS
Date: 08-20-1991 Time: 13:42:22
Sample Name:
Start Time= 20.00Stop Time = 30.00Min. Scale= 5784Max. Scale= 13848



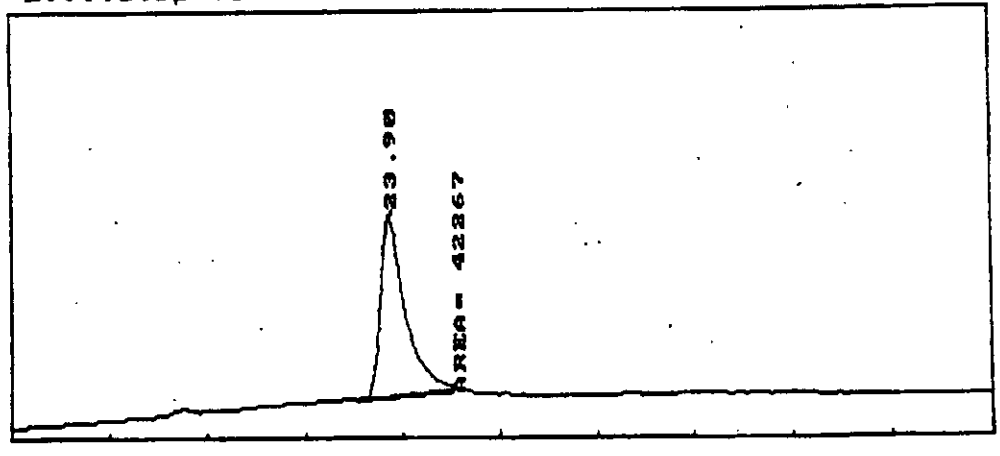
Appendix 6.

Plot of data file: C:F814#7.PTS
Date: 08-21-1991 Time: 14:06:45 SAMPLE NO. 91R104-24-3M
Sample Name: 0.1 PPM HWG 2061 STANDARD
Start Time= 0.03Stop Time = 30.00Min. Scale= 2240Max. Scale= 16816



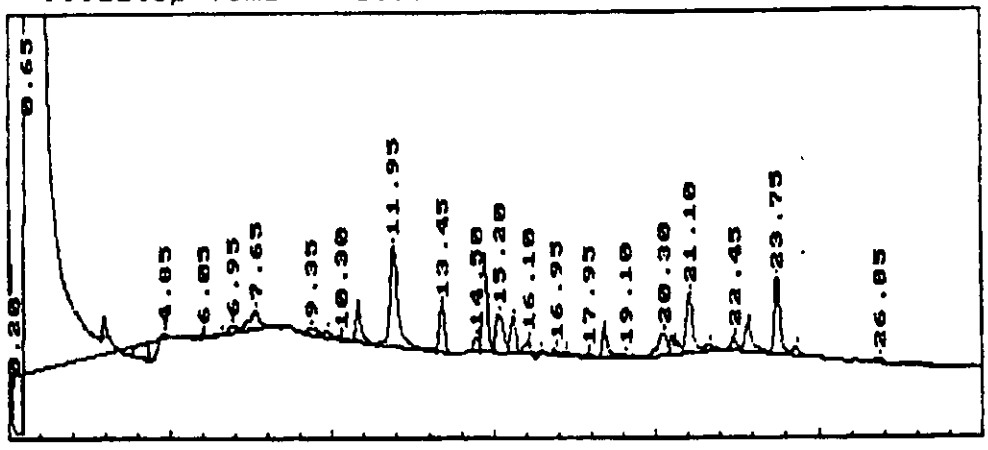
START TIME= 23.550 START HEIGHT= 5608
STOP TIME= 24.650 STOP HEIGHT= 5735
AREA = 42267

Plot of data file: C:F814#7.PTS
Date: 08-21-1991 Time: 14:08:18
Sample Name:
Start Time= 20.00Stop Time = 30.00Min. Scale= 5127Max. Scale= 10677

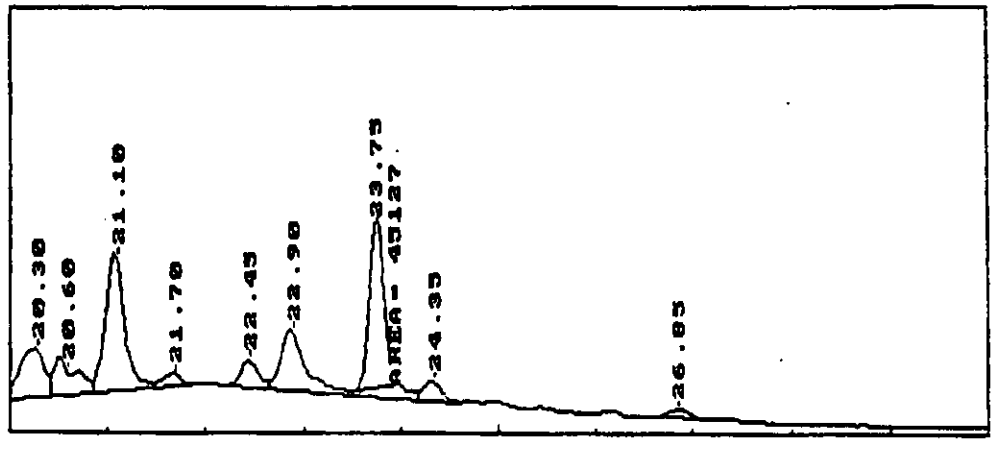


Appendix 6.

Plot of data file: C:F814#8.PTS
Date: 08-21-1991 Time: 14:10:39 SAMPLE NO. 91R104-24-3L
Sample Name: CONTROL BOVINE MILK + 0.1 PPM HWG 2061 STD. REP.#2
Start Time= 0.03Stop Time = 30.00Min. Scale= 2284Max. Scale= 27224

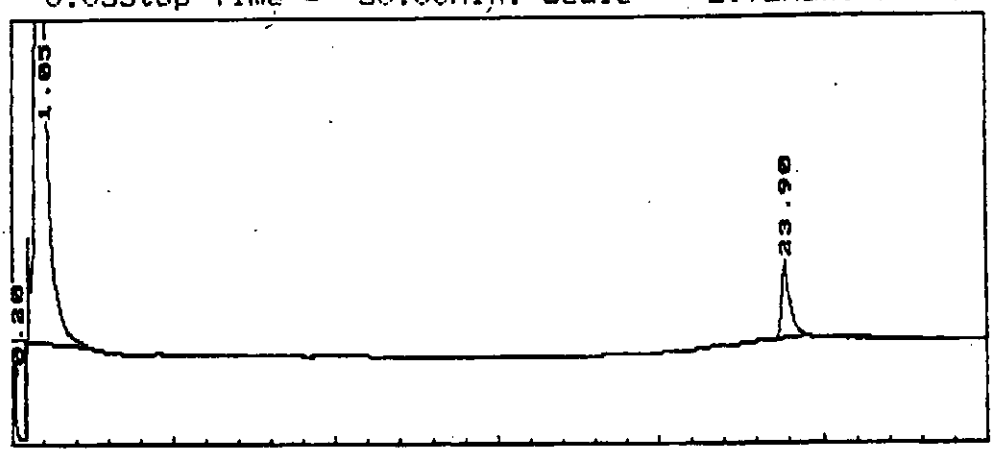


START TIME= 23.550 START HEIGHT= 7111
STOP TIME= 24.000 STOP HEIGHT= 7269
AREA = 45127
Plot of data file: C:F814#8.PTS
Date: 08-21-1991 Time: 14:11:50
Sample Name:
Start Time= 20.00Stop Time = 30.00Min. Scale= 6050Max. Scale= 16890

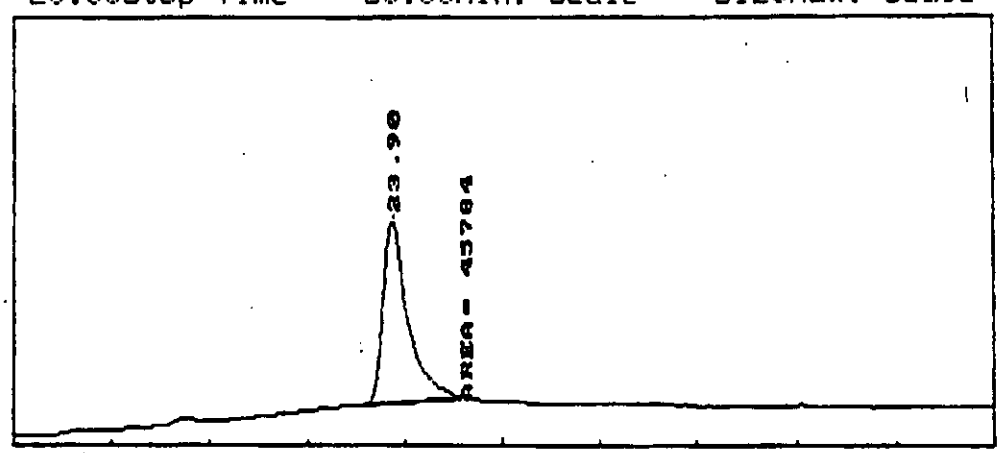


Appendix 6.

Plot of data file: C:F814#9.PTS
Date: 08-21-1991 Time: 14:13:12 SAMPLE NO. 91R104-24-3M
Sample Name: 0.1 PPM HWG 2061 STANDARD
Start Time= 0.03Stop Time = 30.00Min. Scale= 2072Max. Scale= 17335

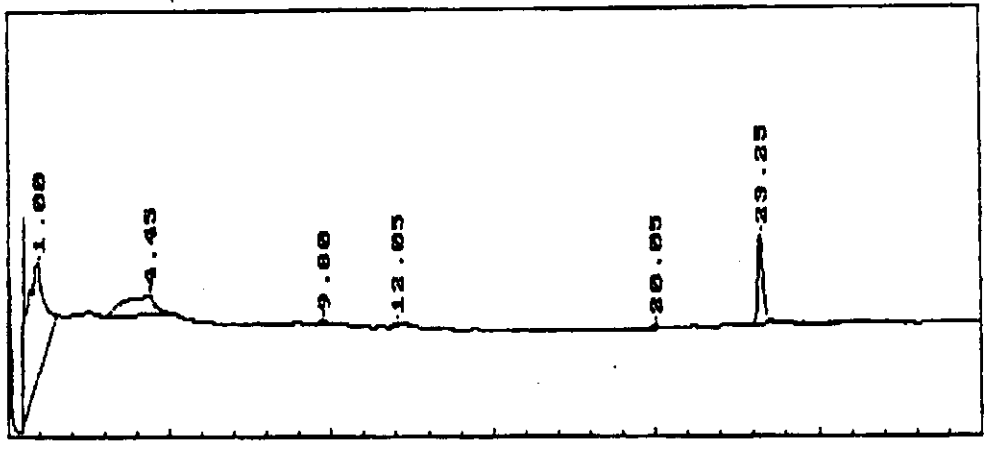


START TIME= 23.550 START HEIGHT= 5661
STOP TIME= 24.700 STOP HEIGHT= 5763
AREA = 45784
Plot of data file: C:F814#9.PTS
Date: 08-21-1991 Time: 14:14:30
Sample Name:
Start Time= 20.00Stop Time = 30.00Min. Scale= 5120Max. Scale= 11066

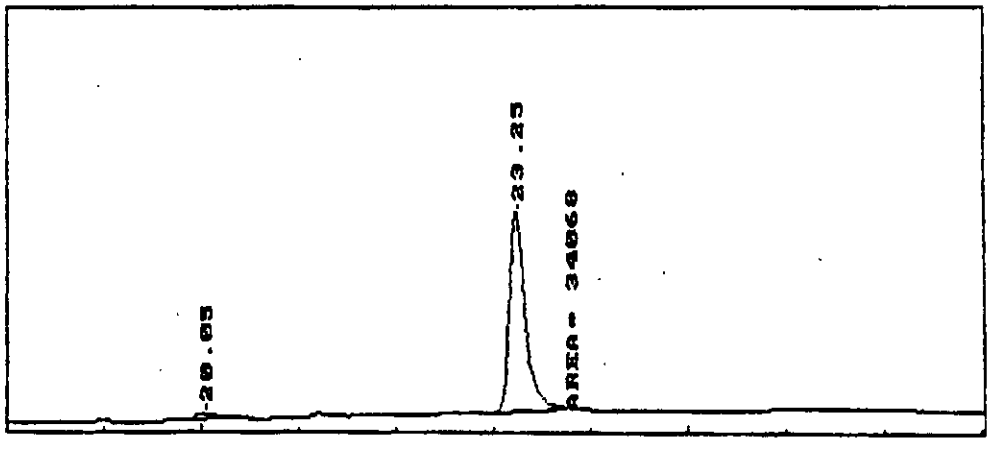


Appendix 6.

Plot of data file: C:F914#1.PTS
Date: 09-20-1991 Time: 15:02:15 SAMPLE NO. 91R104-28-2P
Sample Name: 0.1 PPM HWG 2061 STANDARD
Start Time= 0.03Stop Time = 30.00Min. Scale= 1567Max. Scale= 15735

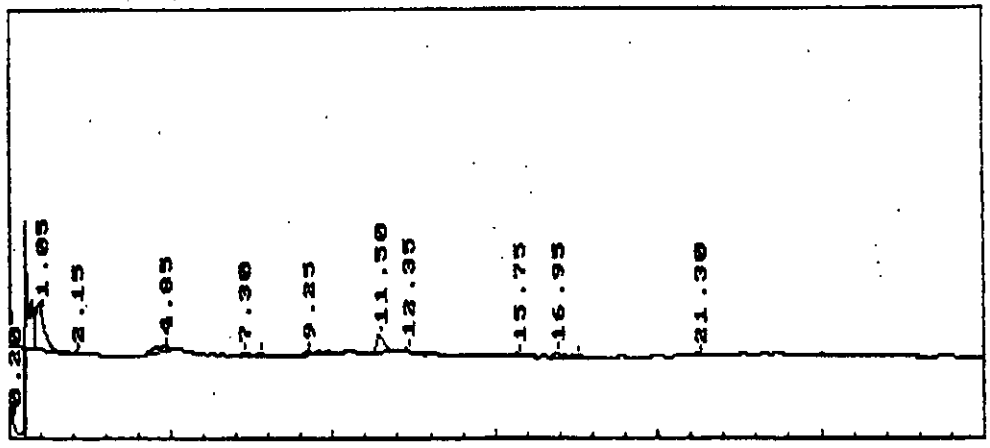


START TIME= 22.950 START HEIGHT= 5132
STOP TIME= 23.875 STOP HEIGHT= 5197
AREA = 34068
Plot of data file: C:F914#1.PTS
Date: 09-20-1991 Time: 15:03:45
Sample Name:
Start Time= 18.00Stop Time = 28.02Min. Scale= 4860Max. Scale= 11160

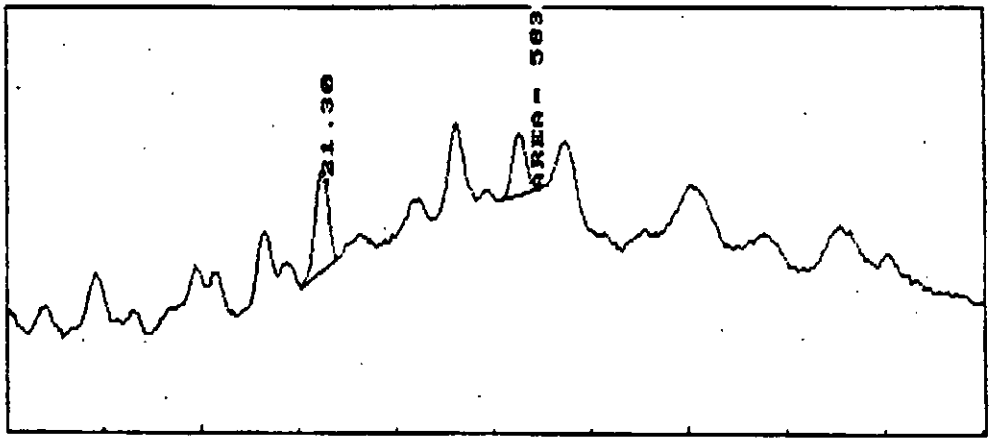


Appendix 6.

Plot of data file: C:F914#2.PTS
Date: 09-20-1991 Time: 15:04:41 SAMPLE NO. 91R104-28-2K
Sample Name: CONTROL BOVINE MILK
Start Time= 0.03Stop Time = 30.00Min. Scale= 1635Max. Scale= 21791

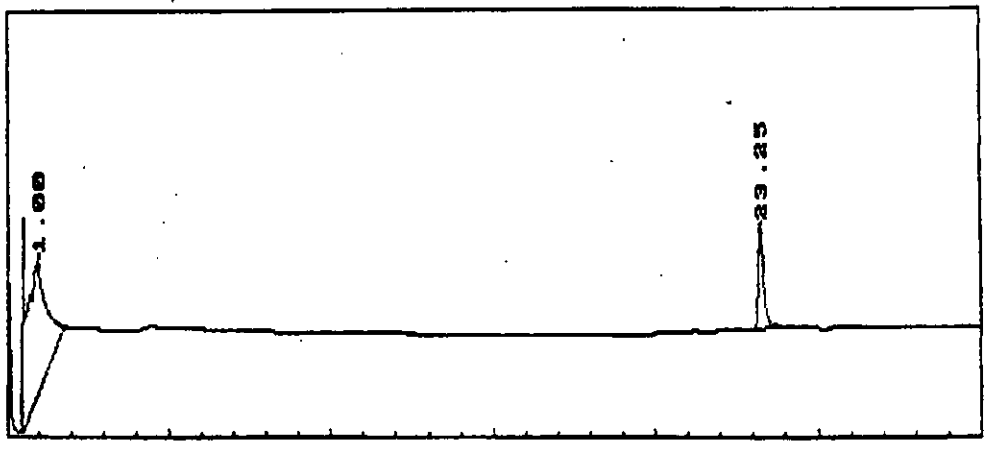


START TIME= 23.075 START HEIGHT= 5421
STOP TIME= 23.525 STOP HEIGHT= 5435
AREA = 583
Plot of data file: C:F914#2.PTS
Date: 09-20-1991 Time: 15:05:55
Sample Name:
Start Time= 18.00Stop Time = 28.02Min. Scale= 5175Max. Scale= 5625

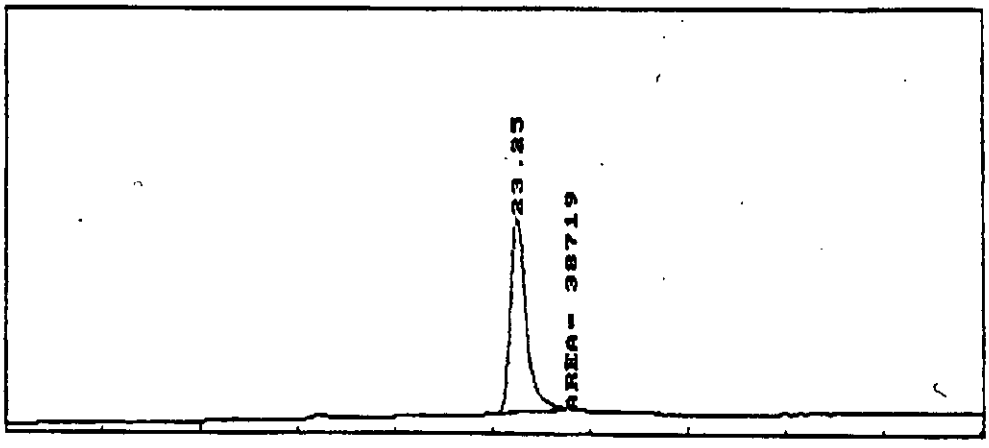


Appendix 6.

Plot of data file: C:F914#3.PTS
Date: 09-20-1991 Time: 15:06:51 SAMPLE NO. 91R104-28-2P
Sample Name: 0.1 PPM HWG 2061 STANDARD
Start Time= 0.03Stop Time = 30.00Min. Scale= 1641Max. Scale= 16011

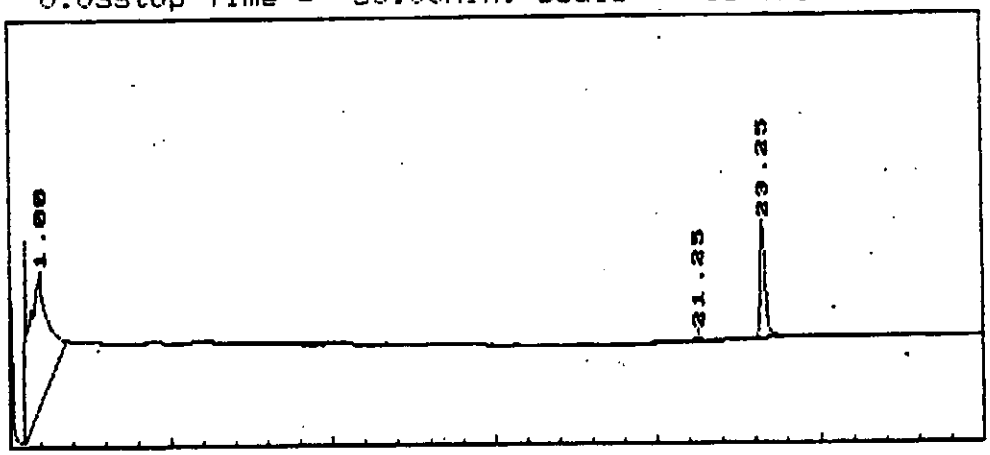


START TIME= 23.025 START HEIGHT= 5187
STOP TIME= 23.875 STOP HEIGHT= 5279
AREA = 38719
Plot of data file: C:F914#3.PTS
Date: 09-20-1991 Time: 15:07:58
Sample Name:
Start Time= 18.00Stop Time = 28.02Min. Scale= 4890Max. Scale= 12218

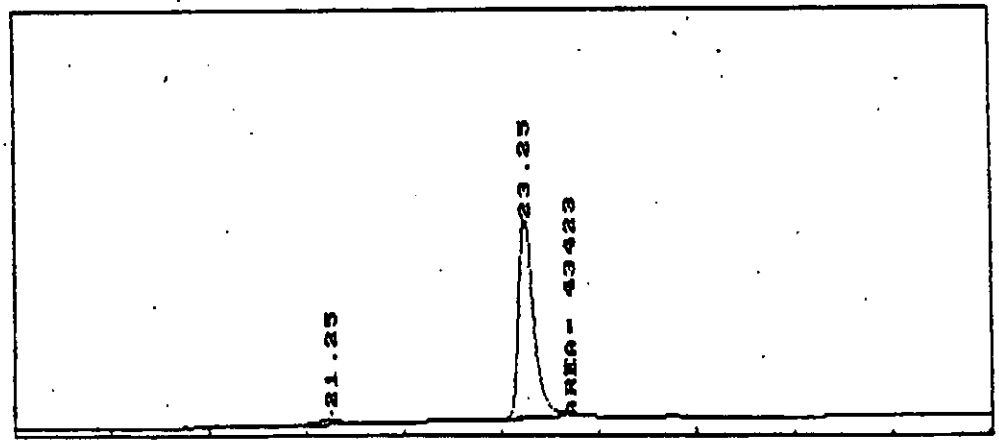


Appendix 6.

Plot of data file: C:F914#7.PTS
Date: 09-20-1991 Time: 15:18:14 SAMPLE NO. 91R104-28-2P
Sample Name: 0.1 PPM HWG 2061 STANDARD
Start Time= 0.03Stop Time = 30.00Min. Scale= 1647Max. Scale= 16533

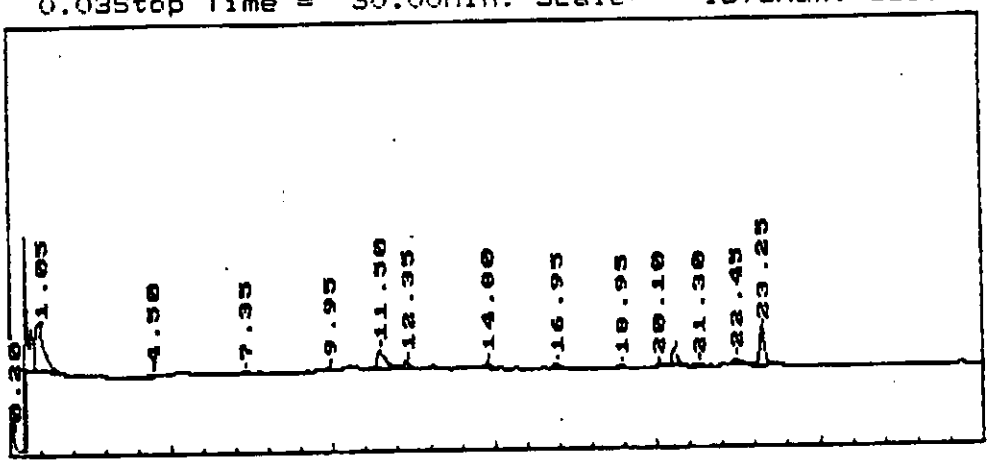


START TIME= 23.025 START HEIGHT= 5125
STOP TIME= 23.775 STOP HEIGHT= 5227
AREA = 43423
Plot of data file: C:F914#7.PTS
Date: 09-20-1991 Time: 15:19:14
Sample Name:
Start Time= 18.00Stop Time = 28.02Min. Scale= 4861Max. Scale= 13319

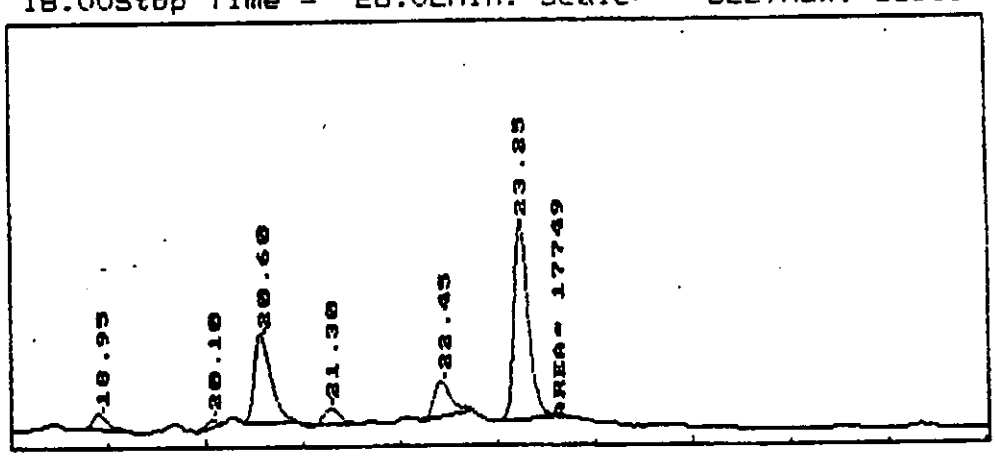


Appendix 6.

Plot of data file: C:F914#8.PTS
Date: 09-20-1991 Time: 15:20:02 SAMPLE NO. 91R104-28-2N
Sample Name: CONTROL BOVINE MILK + 0.05 PPM HWG 2061 STD. REP.#1
Start Time= 0.03Stop Time = 30.00Min. Scale= 1678Max. Scale= 22202

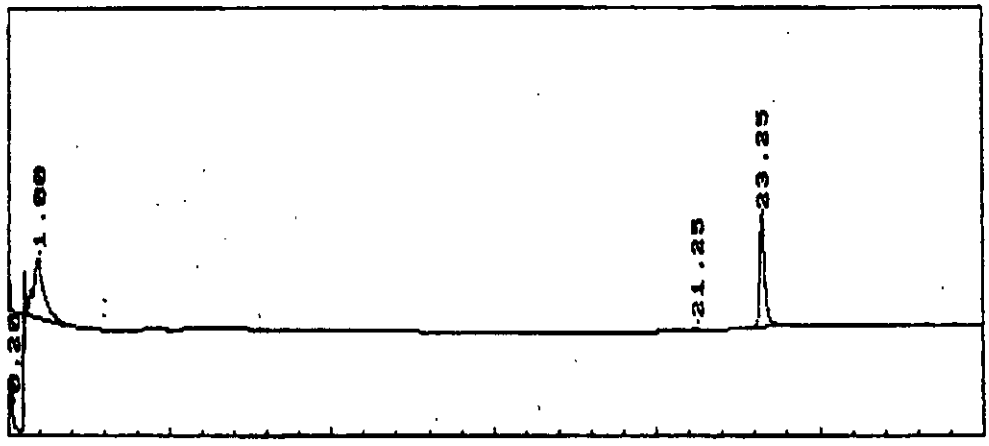


START TIME= 23.000 START HEIGHT= 5427
STOP TIME= 23.700 STOP HEIGHT= 5447
AREA = 17749
Plot of data file: C:F914#8.PTS
Date: 09-20-1991 Time: 15:20:50
Sample Name:
Start Time= 18.00Stop Time = 28.02Min. Scale= 5227Max. Scale= 8929

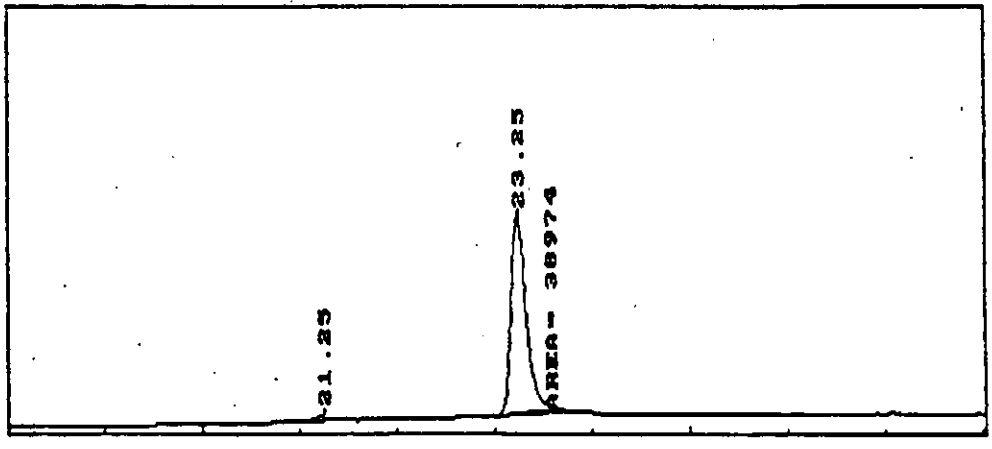


Appendix 6.

Plot of data file: C:F914#9.PTS
Date: 09-20-1991 Time: 15:21:43 SAMPLE NO. 91R104-28-2P
Sample Name: 0.1 PPM HWG 2061 STANDARD
Start Time= 0.03Stop Time = 30.00Min. Scale= 1675Max. Scale= 15897

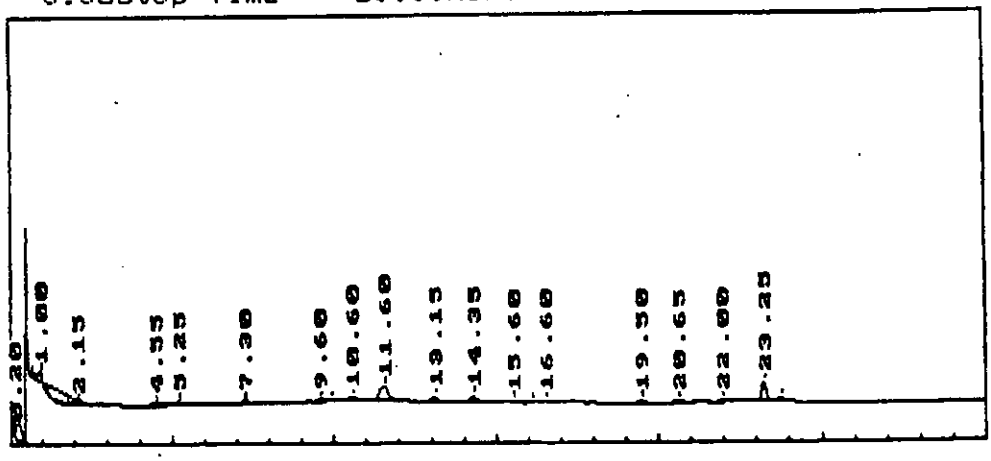


START TIME= 23.025 START HEIGHT= 5173
STOP TIME= 23.650 STOP HEIGHT= 5307
AREA = 38974
Plot of data file: C:F914#9.PTS
Date: 09-20-1991 Time: 15:22:49
Sample Name:
Start Time= 18.00Stop Time = 28.02Min. Scale= 4890Max. Scale= 12682

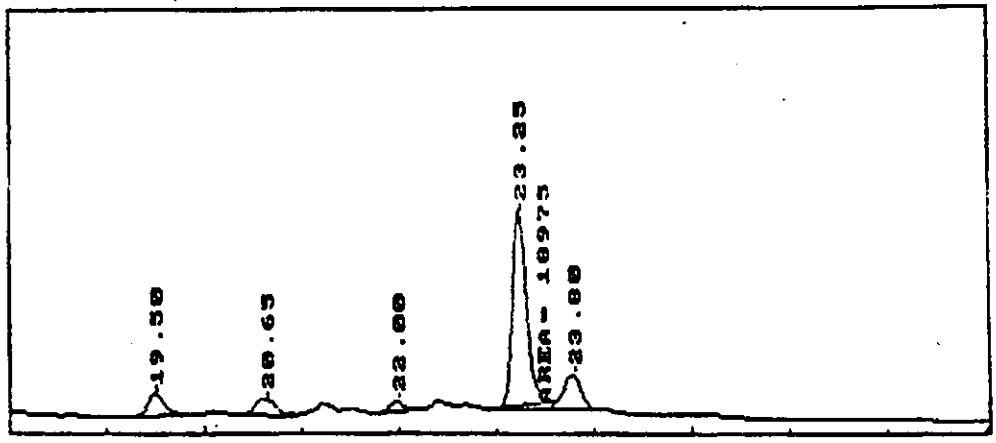


Appendix 6.

Plot of data file: C:F914#10.PTS
Date: 09-20-1991 Time: 15:24:05 SAMPLE NO. 91R104-28-20
Sample Name: CONTROL BOVINE MILK + 0.05 PPM HWG 2061 STD. REP.#2
Start Time= 0.03Stop Time = 30.00Min. Scale= 1678Max. Scale= 44950

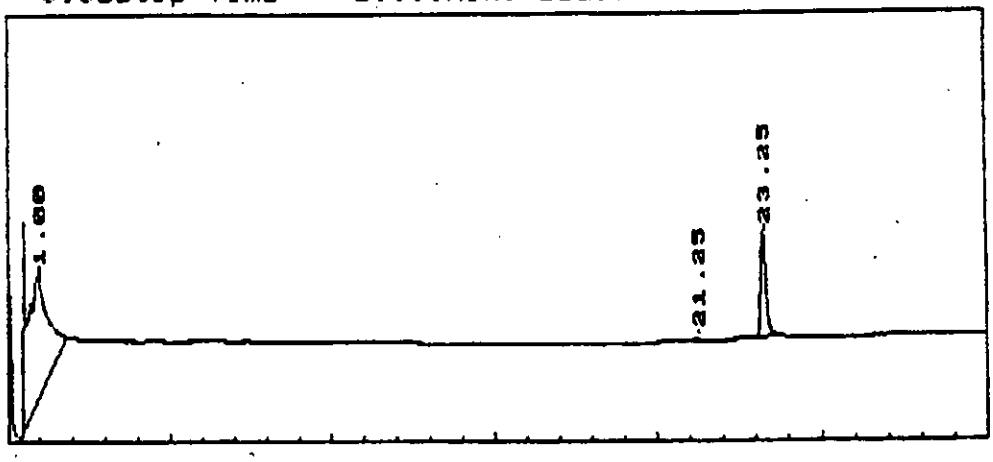


START TIME= 22.950 START HEIGHT= 5455
STOP TIME= 23.550 STOP HEIGHT= 5527
AREA = 18975
Plot of data file: C:F914#10.PTS
Date: 09-20-1991 Time: 15:25:00
Sample Name:
Start Time= 18.00Stop Time = 28.02Min. Scale= 5231Max. Scale= 9331

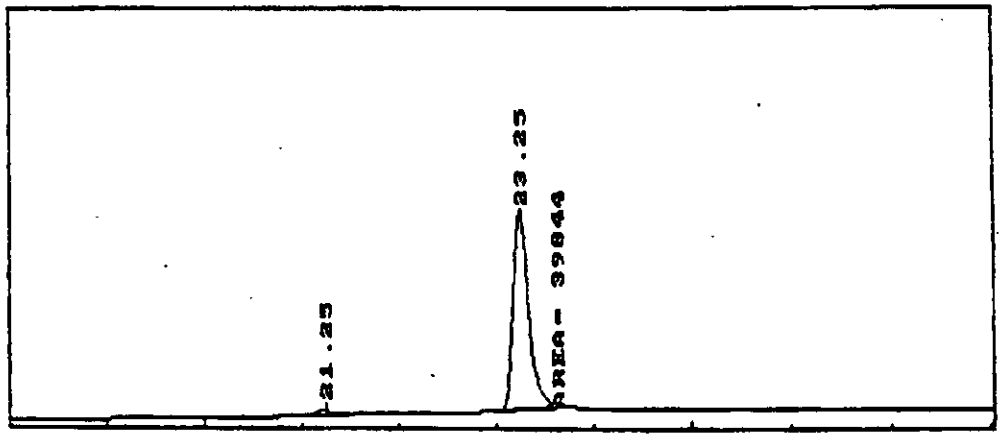


Appendix 6.

Plot of data file: C:F914#11.PTS
Date: 09-20-1991 Time: 15:26:03 SAMPLE NO. 91R104-28-2P
Sample Name: 0.1 PPM HWG 2061 STANDARD
Start Time= 0.03Stop Time = 30.00Min. Scale= 1640Max. Scale= 16600



START TIME= 22.975 START HEIGHT= 5111
STOP TIME= 23.700 STOP HEIGHT= 5222
AREA = 39844
Plot of data file: C:F914#11.PTS
Date: 09-20-1991 Time: 15:27:41
Sample Name:
Start Time= 18.00Stop Time = 28.02Min. Scale= 4850Max. Scale= 12594



Addendum 1. Extraction efficiency of the analytical residue method for tebuconazole and HWG 2061 in animals (Report No. 101339; Study No. FR110205).

Introduction

Conventional recovery experiments do not necessarily reflect the efficiency with which "aged residues" are extracted from animal sample matrices.

To show that the aged total toxic residues or aged residues of concern which were reported in the goat and metabolism metabolism studies are efficiently extracted by the analytical method (Mobay Report No. 101316), the very same radiolabeled samples from these metabolism studies were extracted by the analytical residue method.

The study was conducted from June, 1991 through September, 1991.

Results

The goat and poultry tissues, milk, and eggs were extracted by the procedure described in the analytical residue method. The samples were processed through the acid hydrolysis portion of the analytical residue procedure which converted the conjugated residues to the free residues. These data were compared to the metabolism data in which the [^{12}C] tebuconazole residues were found free and in conjugated forms.

		Tebuconazole Residue (ppm)			HWG 2061[1] Residue (ppm)		
Tissue		Metb[2]	Meth[3]	% Eff	Metb	Meth	% Eff
Liver	(g)[4]	0.69	0.78	113	3.44	2.60	76
	(p)[5]	0.46	0.63	137	9.05	4.47	49
Kidney	(g)	0.23	0.76	330	3.33	2.59	78
Muscle	(g)	0.00	0.01	>100	0.04	0.03	75
	(p)	0.09	0.12	133	0.13	0.12	92
Fat	(g)	0.01	0.05	500	0.14	0.13	108
	(p)	3.78	4.70	124	0.44	0.52	118
Eggs		0.83	0.83	100	0.52	0.56	108
Milk		0.00	0.01	100	0.06	0.06	100

1 The metb. values are the total of free HWG 2061 residue and conjugated HWG 2061 residue extracted by the procedure in the metabolism study. The meth. values are the total of free HWG 2061 residue and un-conjugated (hydrolyzed) HWG 2061 residue extracted by the procedure in the residue method.
2 Metb: Metabolism study.
3 Meth: Method study.
4 g: goat.
5 p: poultry.

Conclusion

The aged tebuconazole and HWG 2061 residues in animal tissues, milk and eggs which were reported in the metabolism studies are extracted efficiently by the analytical residue method (Mobay Report No. 101316).

Addendum 2. Independent laboratory validation of the analytical residue method for tebuconazole and HWG 2061 in animals (Report No. 101348; Study No. FR110204)

Introduction

A Mobay Corporation laboratory at Mobay Research Park near Stilwell, Kansas was selected to validate the analytical residue procedure for the determination of tebuconazole and HWG 2061 residues in bovine and poultry tissues, milk and eggs according to the provisions outlined in PR Notice 88-5.

The study was conducted from July, 1991 through September, 1991.

Results

The standard gc analysis conditions were used in this analysis. Good recovery (>70%, see table below) was achieved for tebuconazole and HWG 2061 in liver and milk fortified at 0.1 ppm and 0.5 ppm. Control samples showed no interference (<0.02 ppm) at the tebuconazole and HWG 2061 retention times. Linearity curves for tebuconazole and HWG 2061 showed a linear response from 0.5 ppm to 4.0 ppm.

	Tebuconazole		HWG 2061	
Tissue	0.1 ppm	0.5 ppm	0.1 ppm	0.5 ppm
Liver	71%, 82%	93%, 117%	89%, 109	92%, 95%
Milk	91%, 107%	94%, 86%	82%, 103%	84%, 92%

Conclusion

The independent laboratory successfully validated the analytical residue method for the determination of tebuconazole and HWG 2061 residues in bovine and poultry tissues, milk and eggs.

Addendum 3. A competitor product interference study for the analytical residue method for tebuconazole and HWG 2061 in animals (Report No. 101950; Study No. FR140202)

Introduction

As of September 1991, 144 compounds have a registered tolerance in bovine and poultry meat, fat and by-products, milk and milk fat, and eggs as described in the Pesticide Chemical News Guide (Food Chemical News, Inc., Duggan and Duggan Editors, Washington, D. C.).

To prove the specificity of the analytical residue method (Mobay Report No. 101316) to detect and measure residues of tebuconazole and HWG 2061 in animal matrices, these competitor compounds were processed through the chemical altering steps and selected cleanup portions of the method. Because the analytical method utilized a flame ionization thermionic detector specific for compounds containing nitrogen or phosphorous, only 114 (those containing nitrogen and phosphorous) of the 144 registered compounds were tested.

This study was conducted in during September and October, 1991.

Results

A total of 12 groups containing 1 to 12 competitor standards in each group were analyzed by selected portions of the analytical procedure. There were no interferences (>0.05 ppm) from the 114 competitor compounds using the gas chromatographic (gc) conditions stated in the analytical method. Only one group of compounds gave a gc response near the tebuconazole retention time. This group was analyzed by the gc/ms selected ion confirmatory procedure and was shown not to have any response at the tebuconazole gc retention time.

Conclusion

None of the nitrogen and/or phosphorous containing compounds (114) which have a registered tolerance in bovine and poultry products as listed in the Pesticide Chemical News Guide dated September, 1991, showed any potential to interfer with the analysis of tebuconazole and HWG 2061 residues when using the designated analytical residue method (Mobay Report No. 101316).