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CGA-173506

STUDY TITLE

ANALYTICAL METHOD FOR THE DETERMINATION OF CGA-173506 IN CROPS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY INCLUDING VALIDATION DATA AND AMENDMENT 1

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STUDY COMPLETED

MARCH 9, 1993

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LABORATORY PROJECT IDENTIFICATION

ANALYTICAL METHOD NO. AG-597
AND AMENDMENT 1

VOLUME 1 OF 1 OF STUDY

PAGE 1 OF 154

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STATEMENT CONCERNING GOOD LABORATORY PRACTICE

The Good Laboratory Practices Compliance Statement provided on Page Thirty-Five (35) of this volume and signed by the Study Director is truthful and accurate.

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ANALYTICAL METHOD FOR THE DETERMINATION OF CGA-173506 IN CROPS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY INCLUDING VALIDATION DATA

ANALYTICAL METHOD NO. AG-597

PROJECT NUMBER: 426982 PROTOCOL: 56-92

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I. SUMMARY AND INTRODUCTION

A. SCOPE

This method is for determination of residues of CGA-173506 in crops. The limit of detection of this method as determined by the smallest standard amount injected is 0.5 ng of CGA-173506. The limit of determination as determined by fortification experiments is at 0.01 ppm for all substrates except sorghum grain at 0.05 ppm. The chemical name and structure of CGA-173506 is shown in Figure 1.

B. PRINCIPLE

A 10-g subsample of crop substrate is homogenized twice with fresh 90% acetonitrile (ACN)/water. Both extracts are filtered through Whatmann 2V and Reeve Angel 802 paper. A 40-ml aliquot (2 gram equivalent) is taken and the ACN is evaporated off.

The sample is diluted with a saturated salt solution and partitioned twice with methyl tert-butyl ether (MTBE). For fodder samples only, the sample is, instead, partitioned twice with hexane, then diluted with water and a saturated salt solution, then partitioned twice with a 80% hexane/MTBE solution. Toluene is added to the organic and the MTBE is evaporated off. Hexane is added to the sample.

Grain and tuber samples are cleaned up on a 0.5-gram silica Bond Elut column, pre-conditioned with 10% IPA/hexane and rinsed with hexane. The sample is loaded onto the column and CGA-173506 is eluted from the column using 50% DCM/toluene.

For forage, hay and fodder samples, a preconditioned 1-gram silica Mega-Bond Elut column is used. The sample is loaded onto the column and washed with 50% hexane/toluene. CGA-173506 is eluted with 25% DCM/toluene.

Eluates from all silica columns are evaporated to dryness. The residue remaining is dissolved in methanol and water, then loaded onto a preconditioned 0.5-gram phenyl Bond Elut column. CGA-173506 is eluted with acetone. The acetone solution is evaporated to dryness and the residue dissolved in an appropriate amount of mobile phase. CGA-173506 is determined by normal phase HPLC using a 4.6 mm id X 250 mm Amino column and UV detection at 268 nm. For fodder samples, CGA-173506 is determined by normal phase HPLC using a 4.6 mm id X 250 mm Amino column connected to a 4.6 mm id X 100 mm C1 column to the Amino column.

A flow chart for the analysis of grain and potato tuber substrates is shown in Figure 2. For forage and hay substrates, see Figure 3 and for fodder substrates, see Figure 4 for the flow chart for these substrates.

II. MATERIALS AND ANALYTICAL STANDARDS

A. APPARATUS

1.0 Jars, wide mouth, 16 oz.
2.0 Bottles, Boston Round, narrow mouth, 8 oz.
3.0 Carbon filter tube
4.0 Disposable Pasteur pipets
5.0 Funnel, long stem, 50 mm dia.
6.0 Funnel, long stem, 24 mm dia.
7.0 Funnel, separatory, 60-ml with Teflon stopcock
8.0 Funnel, separatory, 250-ml with Teflon stopcock
9.0 Absorbent cotton
10.0 Filter paper, Whatman 2V, 24 cm
11.0 Filter paper, Whatman reeve angel 802, 24 cm
12.0 Graduated cylinders, 10-ml, 25-ml, 50-ml, 100-ml and 250-ml or equivalent.
13.0 Homogenizer, Polytron or equivalent
14.0 Round bottom flasks, 50-ml, 100-ml and 250-ml
15.0 Rotary evaporator, Buchii or equivalent

16.0 Vials, Wheaton, 2-ml or equivalent
17.0 Vial caps, Teflon lined. (Sunbrokers, IUC, Wilmington, NC. Cat# 200 152)
18.0 Volumetric pipets, 1/2-ml, 1-ml, 2-ml, 4-ml, 8-ml, 10-ml
19.0 Supelco Visiprep solid phase extraction vacuum Manifold (Cat. #5-7030M) or equivalent
20.0 Fisher Scientific Ultrasonic Cleaner (Cat.# 15-336-9) or equivalent

B. REAGENTS AND SOLVENTS

1.0 Acetonitrile (ACN), HPLC grade
2.0 Toluene, HPLC grade
3.0 Dichloromethane (DCM), HPLC grade
4.0 Hexane, HPLC grade
5.0 Methyl tert-butyl ether (MTBE), HPLC grade
6.0 Methanol (MeOH), HPLC grade
7.0 Acetone, HPLC grade
8.0 Isopropanol (IPA), HPLC grade
9.0 N-propanol (NPA), HPLC grade
10.0 Sodium chloride, Certified ACS grade
11.0 Anhydrous sodium sulfate, certified ACS grade
12.0 Saturated solution of sodium chloride in water
13.0 Water, HPLC grade or equivalent
14.0 Bond Elut, silica 0.5 grams (Varian Cat. #601303)
15.0 Bond Elut, Phenyl, 0.5 grams (Varian Cat. #613303)
16.0 Mega-Bond Elut, silica 1.0 grams (Varian Cat. #1225-6008)
17.0 90 % Acetonitrile/Water
18.0 80% Hexane/Toluene
19.0 50% Hexane/Toluene
20.0 25% Dichloromethane/Toluene
21.0 50% Dichloromethane/Toluene
22.0 90% Hexane/Isopropanol
23.0 80% Hexane/methyl tert-butyl-ether
24.0 CGA-173506, Analytical Standard supplied by Ciba-Geigy Corporation, 410 Swing Rd., Greensboro, NC 27419.

C. ANALYTICAL PROCEDURE

1.0 Sample Preparation

1.1. Samples are received and stored frozen at -20°C (SOP 7.20). Samples are prepared under the general guidelines of the U.S. Food and Drug Administration Pesticide Analytical Manual Volume I, Section 141 (Ciba SOP 7.21).

2.0 Extraction

All Substrates

2.1. Weigh a 10-g aliquot of crop substrate into a 16-oz. wide mouth jar. Fortify with CGA-173506 at this point for recovery samples. Add 150-ml 90% ACN/water. Homogenize the sample with a Polytron homogenizer at medium power for 90 seconds. Allow samples to settle for a minimum of 5 minutes.

2.2. Decant the clear solution through a Reeve Angel 802 filter inside a Whatmann 2V filter into a flint glass round or equivalent bottle retaining sample material in the extraction flask.

2.3. Repeat extraction with an additional 50 ml of 90% ACN/water solution for 30 sec. and filter solution through the same set of filter papers combining the filtrates in the same sample extract bottle.

3.0 Grain and Potato Tuber Samples

3.1. Partition Clean-up

3.1.1. Transfer a 40-ml aliquot of the sample extract to a 100-ml or 250-ml round bottom flask. Attach a trap to the rotary vacuum evaporation apparatus and remove the organic solvent until the volume is <4.0 ml (bath temperature <40°C).

3.1.2. Add 10 ml of a saturated sodium chloride solution and 10 ml of MTBE to the round bottom flask. Swirl flask to dissolve any residue. Sonicate solution if necessary to loosen or dissolve any adhering residue. Transfer the solution to a 60-ml separatory funnel. Use an additional 5 mls of the saturated sodium chloride solution to rinse out the round bottom flask.

3.1.3. Stopper the 60-ml separatory funnel and shake for 30 sec. Allow the two layers to separate. Drain the lower, aqueous layer and any emulsion back into the round bottom flask from Section II.C.3.2. Drain the MTBE layer through a small cotton plug which is in a carbon filter tube or funnel into another 100-ml round bottom flask.

3.1.4. Add 10 ml of MTBE to the original round bottom flask, swirl flask and transfer solutions back to the 60-ml separatory funnel. Stopper funnel and shake for 30 sec. Break any emulsion that may form and drain the lower, aqueous layer and any remaining emulsion back into the round bottom flask discarding the aqueous layer. Pass the organic layer through the cotton plug.

3.1.5. Rinse the cotton plug with 2 10-ml portions of MTBE.

3.1.6. Add 3 ml of toluene to the round bottom flask and evaporate off the MTBE on a rotary evaporator. Evaporate in a water bath at 30-35°C to ~1.5-2 ml volume. The toluene should remain in the flask. Do not evaporate to dryness as the analyte might adhere to the sides of the flasks.

3.2. <u>Silica Column Cleanup</u>

3.2.1. Set up a 0.5 gram Bond Elut column with a reservoir on the vacuum apparatus. Precondition column with 10 ml of a 90% Hexane/isopropanol solution. Rinse the column with 10 ml of hexane. Discard solvents.

3.2.2. Add 10 ml of hexane to the round bottom flask, swirl flask to mix solvents and quantitatively transfer sample solution to the preconditioned column. Rinse the flask with an additional 5 ml of 80% hexane/toluene and transfer to column. Discard the hexane/toluene solutions.

3.2.3. Elute CGA-173506 with 10 ml of a 50% DCM/toluene solution. Rinse flask with this solvent prior to elution through column. Collect solution in appropriate glassware and evaporate elution solvent to dryness.

3.2.4. Redissolve residue remaining in 5 mls of methanol. Sonicate samples. Add 15 mls of water to the methanol and mix well. Continue to the phenyl cleanup column, Section II.C.3.3.

3.3. <u>Phenyl Column Cleanup</u>

3.3.1. Set up a 0.5-gram Phenyl Bond Elut column with reservoir on the vacuum apparatus. Precondition column with 10 ml of methanol. Rinse column with 10 ml of water. Discard these rinses.

3.3.2. Load sample solution from Section II.C.3.2.4 onto the column. Rinse round bottom flask with an additional 5 mls of water

and load onto the column. Discard load and rinse eluates.

3.3.3. Elute CGA-173506 from column with 5 mls of acetone rinsed through the round bottom flask. Collect acetone and evaporate to dryness. Ensure that no water remains in the flask. The addition of about 3 mls of isopropanol will aid in the evaporation of the water in the elution solvent.

3.3.4. Redissolve samples in an appropriate volume of 90/6/6 Hexane/MeOH/IPA (v/v/v) to the flask. Sonicate samples for several minutes, turning flask on its sides at periodic intervals.

4.0 Forage and Hay Samples

4.1. Partition Clean-up

4.1.1. Transfer a 40-ml aliquot of the sample extract to a 100-ml or 250-ml round bottom flask. Attach a trap to the rotary vacuum evaporation apparatus and remove the organic solvent until the volume is <4.0 ml (bath temperature <40°C).

4.1.2. Add 10 ml of a saturated sodium chloride solution and 10 ml of MTBE to the round bottom flask. Swirl flask to dissolve any residue. Sonicate solution if necessary to

loosen or dissolve any adhering residue. Transfer the solution to a 60-ml separatory funnel. Use an additional 5 mls of the saturated sodium chloride solution to rinse out the round bottom flask.

4.1.3. Stopper the 60-ml separatory funnel and shake for 30 sec. Allow the two layers to separate. Drain the lower, aqueous layer and any emulsion back into the round bottom flask from Section II.C.3.2. Drain the MTBE layer through a small cotton plug which is in a carbon filter tube or funnel into another 100-ml round bottom flask.

4.1.4. Add 10 ml of MTBE to the original round bottom flask, swirl flask and transfer solutions back to the 60-ml separatory funnel. Stopper funnel and shake for 30 sec. Break any emulsion that may form and drain the lower, aqueous layer and any remaining emulsion back into the round bottom flask discarding the aqueous layer. Pass the organic layer through the cotton plug.

4.1.5. Rinse the cotton plug with 2 10-ml portions of MTBE.

4.1.6. Add 3 ml of toluene to the round bottom flask and evaporate off the

MTBE on a rotary evaporator. Evaporate in a water bath at 30-35°C to ~1.5-2 ml volume. The toluene should remain in the flask. Do not evaporate to dryness as the analyte might adhere to the sides of the flasks.

4.2. Silica Column Clean-up

4.2.1. Set up a 1-gram MEGA Bond Elut silica column with a reservoir on the vacuum apparatus. Precondition the column with 10 ml of 90% Hexane/isopropanol solution. Rinse column with 10 ml of hexane. Discard elutants.

4.2.2. Add 10 ml of hexane to round bottom flask, swirl to mix solvents and quantitatively transfer sample solution to column. Ensure that solution does not have any globs of material. Sonicate flask if need be to break up the globs of material. Rinse the flask with an additional 5 ml of 80% hexane/toluene and transfer to column. Discard hexane/toluene elutants.

4.2.3. Add 10 ml of a 50% hexane/toluene solution to each sample flask. Dissolve by sonication any remaining residue in the flask into this solution. Transfer solution to silica column. Discard eluate.

4.2.4. Elute CGA-173506 from column with 20 ml of a 25% Dichloromethane/ toluene solution. Add elution solvent to flask then to column to dissolve any remaining material in the flask. Collect and evaporate eluate to dryness.

4.2.5. Redissolve residue remaining in 5 ml of methanol. Sonicate the samples to ensure that all of the samples goes into solution. Add 15 ml of water to the methanol. Mix well. Continue to the phenyl column cleanup, Section II.C.4.3.

4.3. <u>Phenyl Column Cleanup</u>

4.3.1. Set up a 0.5-gram Phenyl Bond Elut column with reservoir on the vacuum apparatus. Precondition column with 10 ml of methanol. Rinse column with 10 ml of water. Discard these rinses.

4.3.2. Load sample solution from Section II.C.4.2.5 onto the column. Rinse round bottom flask with an additional 5 ml of water and load onto the column. Discard load and rinse eluates.

4.3.3. Elute CGA-173506 from column with 5 ml of acetone rinsed through the round bottom flask. Collect acetone and evaporate to dryness. Ensure that no water

remains in the flask. The addition of about 3 ml of isopropanol will aid in the evaporation of the water in the elution solvent.

4.3.4. Redissolve samples in an appropriate volume of 90/6/6 Hexane/MeOH/IPA (v/v/v) to the flask. Sonicate samples for several minutes, turning flask on its sides at periodic intervals.

5.0 **Fodder Samples**

5.1. **Partition Clean-up**

5.1.1. Transfer a 40 ml aliquot to a 250-ml separatory funnel. Add 25 ml of hexane and shake funnel for 1 min. Allow layers to separate and transfer lower ACN layer to another 250-ml separatory funnel. Discard hexane layer.

5.1.2. Repeat partition with another 25-ml portion of hexane. Transfer ACN layer to the original separatory funnel. Discard the hexane layer.

5.1.3. Add 150 ml of water and 10 ml of saturated NaCl to the separatory funnel. Add 25 ml of an 80% hexane/methyl tert-butyl ether solution to the separatory funnel. Shake funnel for 1 min.

5.1.4. Drain the lower ACN/water layer into second 250-ml separatory funnel. Drain

the hexane/MTBE layer through a small column of sodium sulfate (@30 grams) into a 100-ml round bottom flask.

5.1.5. Add another 25 ml of 80% hexane/MTBE to separatory funnel and shake for one minute. Allow layers to separate. Discard aqueous layer. Drain the hexane/MTBE layer through the same sodium sulfate column.

5.1.6. Rinse the sodium sulfate column with 2 X 10-ml portions of 80% hexane/ MTBE solution.

5.1.7. Add 3 ml of toluene to the round bottom flask and evaporate off the hexane/MTBE solvents on a rotary evaporator. Evaporate in a water bath at 30-35°C to ~1.5 - 2.5 ml volume. The toluene should remain in the flask. Do not evaporate to dryness as the analyte might adhere to the sides of the flask.

5.2. <u>Silica Column Clean-up</u>

5.2.1. Set up a 1-gram MEGA-Bond Elut column with a reservoir on the vacuum apparatus. Precondition the column with 10 ml of 90% hexane/isopropanol solution. Rinse column with 10 ml of hexane. Discard solvents.

5.2.2. Add 10 ml of hexane to the round bottom flask, swirl flask to mix

solvents, and quantitatively transfer sample solution to column. Ensure that solution does not have any globs of material. Sonicate flask if need be to break up the globs of material. Rinse the flask with an additional 5 ml of 80% hexane/toluene and transfer to column. Discard hexane/toluene solutions. Discard hexane/toluene elutants.

5.2.3. Add 10 ml of a 50% hexane/toluene solution to each sample flask. Dissolve by sonication any remaining residue in the flask into this solution. Transfer solution to silica column. Discard eluate.

5.2.4. Elute CGA-173506 from column with 20 ml of a 25% Dichloromethane/toluene solution. Add elution solvent to flask then to column to dissolve any remaining material in the flask. Collect and evaporate eluate to dryness.

5.2.5. Redissolve residue remaining in 5 ml of methanol. Sonicate the samples to ensure that all of the sample goes into solution. Add 15 ml of water to the methanol. Mix well. Continue to the phenyl column cleanup, Section II.C.5.3.

5.3. Phenyl Column Cleanup

5.3.1. Set up a 0.5-gram Phenyl Bond Elut column with reservoir on the vacuum apparatus. Precondition column with 10 ml of methanol. Rinse column with 10 ml of water. Discard these rinses.

5.3.2. Load sample solution from from Section II.C.5.2.5 onto the column. Rinse round bottom flask with an additional 5 ml of water and load onto the column. Discard load and rinse eluates.

5.3.3. Elute CGA-173506 column with 5 ml of acetone rinsed through the round bottom flask. Collect acetone and evaporate to dryness. Ensure that no water remains in the flask. The addition of about 3 ml of isopropanol will aid in the evaporation of the water in the elution solvent.

5.3.4. Redissolve samples in an appropriate volume of 90/10/10 hexane/n-propanol/isopropanol (v/v/v) to the flask. Sonicate samples for several minutes, turning flask on its sides at periodic intervals.

D. INSTRUMENTATION

1.0 Description and Operating Conditions

See Table I and II for HPLC operating conditions.

2.0 Standardization

2.1. Calibrate the HPLC system with each analytical run by checking the retention time and detector response relative to previous runs. Retention times must not vary more than 2% within a run and detector response should not vary more than 10% between runs.

2.2. Standardize the HPLC system by injecting 100-µl aliquots of standard solutions of CGA-173506 in a working range of 0.5-20 ng/injection. Generate a linear regression from the data by comparing detector response and ng injected.

E. INTERFERENCES

It has been observed that use of the orange silicone rubber vial caps can give extraneous peaks in the chromatograms. It is strongly recommended that teflon lined vial caps be used in this method.

F. CONFIRMATORY TECHNIQUES

None.

G. TIME REQUIRED

A skilled analyst can complete the extraction and analysis of a set of 6-8 samples in 8-10 working hours.

H. MODIFICATIONS AND POTENTIAL PROBLEMS

1.0 CGA-173506 has a solubility of 10 µg/ml in hexane. Stock solutions must be prepared in methanol. Analytical

working Standards must be prepared in the mobile phase being used. Exercise caution if refrigeration of the samples is required. Ensure sample extracts have been well mixed before taking aliquot during the sample work-up as the analyte may precipitate out of solution.

2.0 Sonication of the round bottom flasks is required to maintain the analyte in solution in Sections 3.1.6, 4.1.6 and 5.1.7. Do not allow samples to sit for a long period of time after the sonication before doing the silica column cleanup.

3.0 Take care to ensure the samples do not go to dryness after the partitioning step in Sections 3.1.6, 4.1.6 and 5.1.7. Toluene is added as a keeper solution to prevent the samples from going to dryness. It has been observed that material will adhere to the walls of the flask when samples are taken to dryness, trapping the analyte. If for some reason the sample does go to dryness, redissolve sample in MTBE, add 3 ml of toluene, sonicate samples to dissolve residues in the solvent and reevaporate as specified in the method.

4.0 The operation of the vacuum apparatus should be capable of drawing a vacuum of at least 10 mm for the phenyl column. Do not operate vacuum apparatus in such a way that the flow through the column is faster than dropwise.

I. PREPARATION OF STANDARD SOLUTIONS

1.0 Preparation of Standard CGA-173506 Solutions

1.1. Weigh 25 or 50 mg of CGA-173506 analytical standard into a 25 or 50-ml volumetric flask, respectively, and dilute to the

mark with methanol. This will yield a 1 mg/ml solution of the standard. Store the stock solution in the refrigerator when it is not being used.

1.2. Make serial dilutions of the 1 mg/ml standard solution with the appropriate mobile phase solution to give a series of fortification/analytical standards in a range of 0.005 ng/µl to 20 ng/µl of CGA-173506. Store the standard solutions in amber bottles in the refrigerator when not in use.

J. METHODS OF CALCULATION

1.0 Determination of Sample Residues

Inject 100-µl aliquots of sample final fractions from Sections 3.3.4. 4.3.4 or 5.3.4 into the HPLC under the same conditions as for the standards. Make appropriate dilutions of the samples in the mobile phase being used to bring the sample peak heights within the range of the standard curve. Compare the peak heights of the unknown samples to the standard curve or enter the peak height into a least squares program to determine the nanograms of CGA-173506 in the injected aliquot. Typical chromatograms for control and procedural recovery samples are shown in Figures 6-12.

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

(1)

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

G = milligrams sample extracted
V_a = aliquot volume (ml)
V_e = extraction volume (ml)
V_i = injection volume (µl)
V_f = total volume of final injection (µl)

R = recovery ratio given by equation 4 in Section 2.2.

M = % moisture of sample

W = weight of sample in grams

To determine ppm CGA-173506 found in samples, use Equation 2.

(2)

$$\text{ppm CGA-173506} = \frac{(\text{ng CGA-173506 found})\ (100)}{(\text{mg sample injected})\ (R\%)}$$

2.0 Fortification Experiments

This method is validated for each set of samples analyzed by including an untreated control sample and one or more control samples fortified immediately prior to extraction with CGA-173506.

2.1. Add 1.0 ml of 0.10 ng/µl standard solution of CGA-173506 to 10 g of a control crop prior to the addition of extraction solvent for a 0.01 ppm fortification. Use correspondingly larger amounts of standards for higher fortifications levels. The fortification volume should not exceed 2 ml. Analyze control and freshly fortified samples

along with the treated samples according to the procedures of the method.

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

$$(3) \quad \text{ppm analyte} = \frac{\text{ng CGA-173506 found}}{\text{mg sample injected}}$$

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

$$(4) \quad R\% = \frac{(\text{ppm CGA-173506 found} - \text{ppm control})}{\text{ppm CGA-173506 added}} \times 100$$

III. RESULTS AND DISCUSSION CONTENT - VALIDATION

1.0 Accuracy

Statistical methods for regression analysis are used to generate a standard curve for quantitation of residues. The Standard curve is then used to quantitate the residues as described in Section J. A typical standard curve is shown in Table III. Representative standard chromatograms are shown in Figure 5.

Recovery results for fortified control samples were used to calculate accuracy in terms of a mean, standard deviation (sd) and coefficient of variation (CV) for the limit of determination, and for all recovery results included in the study.

The average recovery for the samples fortified with CGA-173506 at the limit of determination of 0.01 ppm (sorghum grain has a limit of determination of 0.05 ppm) was 88% (SD=14, CV=16%, N=16). The average recovery for all fortification levels was 87% (SD=13, CV=15%, N=41). See Table IV for a detailed listing of all recovery values. Representative chromatograms of control and fortified control samples are found in Figures 6-14.

2.0 Precision

Precision of the method was determined by calculating the Coefficient of Variation of all cold fortified substrates and each three replicate analysis set of ^{14}C-CGA-173506 treated rice grain and stalk samples. The range of CV for rice samples ranged from 2.7% to 16% with the 1X treated rice stalks having the highest CV. The CV for all fortified samples was 15%. This demonstrates that this method has an acceptable level of precision for residues of CGA-173506.

3.0 Extractability

The radioactivity in the sample extracts and final fractions was determined by liquid scintillation counting and expressed as ppm of CGA-173506. Samples were prepared for LSC by measuring an aliquot of extract into a scintillation vial and adding 10-15 ml of Beckman Ready Safe Liquid Scintillation cocktail. The results obtained were in units of disintegrations per minute (dpm) and were converted into ppm as shown by the following representative calculations.

Sample RS.IA had a 1.0 ml aliquot of the extract solution counted out of 200 ml total. The sample size extracted was 10 grams and the

specific activity of the applied ^{14}C-CGA-173506 was 24.6 nCi/µg.[1] The LSC measured 6405 dpm for the sample aliquot (Ciba SOP 4.6).

Using the above information, one calculates the ppm value as follows:

$$\frac{(6405\ \text{dpm/1 ml aliquot})}{(10\ \text{g/200 ml})\ (2.22 \times 10^{3}\ \text{dpm/nCi})\ (24.6\ \text{nCi/µg})} = 2.36\ \text{µg/g} = 2.36\ \text{ppm}$$

The extraction efficiency of the analytical method is determined by comparing the total ppm ^{14}C-residue found in the sample, from combustion analysis, to the ppm ^{14}C-residue found in the initial sample extract from Section 2.0. The equation used for the determination of % extractability is:

$$\%\ \text{extrac.} = \frac{\text{ppm}\ ^{14}\text{C-residue extract}}{^{14}\text{C-residue sample}} \times 100\%\text{ppm}$$

The average extractabilities for greenhouse grown, spray treated rice substrates were 53% (sd=3.9, CV=5.7%, n=3) for 1X treated grain, 90% (sd=0.6, CV=0.6%, n=3) for 1X treated stalks), 119% (sd=9.3, CV=7.8%, n=3) for 5X treated rice grain and 86% (sd=3.2, CV=3.7%, n=3) for 5X treated rice stalks.

The extractability of CGA-173506 was good on both treatments of rice stalks and the 5X treated rice grain. The extractability was poor for the 1X treated rice grain. See Table V for further information and data.

4.0 Accountability

The accountability of an Analytical Method is determined by comparing the total ppm ^{14}C-residue found in the sample, the ppm ^{14}C-residue found in the final fraction and the ppm analyte

found in the final fraction to each other. The determinations of CGA-173506 by HPLC and of ^{14}C by LSC in the final fraction solutions correlated very well and showed that the cleanup procedures isolate analyte, CGA-173506, from other metabolites or degradates.

Accountability of ^{14}C-residues by Analytical Method AG-597 was good in 1X treated stalks and 5X treated stalks and grain and ranged from 60-109%. Accountability in 1X-treated grain was poor, 29-39%, and was due to low extractability. Overall, the accountability for AG-597 is adequate.

5.0 Discussion

Control of bias was accomplished by the analysis of control samples in all analytical sets.

This method has been validated under Protocol No. 56-92[2] and used for the analysis of control, CGA-173506 fortified control and treated rice samples. The objective of Protocol 56-92 was to validate "Draft" Analytical Method AG-597 for the quantitation of residues of CGA-173506 in crops at a 0.01 ppm level of determination. Problems were experienced with sorghum grain at the 0.01 ppm level. Different analysis schemes were tried in an attempt to validate this method at the 0.01 ppm level in this substrate. Results at the 0.01 ppm level were variable and values up to 0.008 ppm were found in the control.

Fortification at the 0.05 ppm level gave consistent and acceptable values. Based on these results, the level of determination for sorghum grain was raised to 0.05 ppm (See Figure 15).

Results of these analyses are shown in Tables III & IV and are reported in Residue Test Report RI-MV-007-92, No. 1.[3]

Test substance ID, test system ID, protocol amendments, protocol deviations and circumstances affecting quality and integrity of data are also reported in Residue Test Report RI-MV-007-92, No. 1. All raw data associated with this study, retained samples and the original final report and protocol are archived in the Metabolism and Residue Chemistry Archives or freezer storage facility at Ciba-Geigy Corporation, Greensboro, NC. Non-study specific raw data will be archived according to applicable SOP's.

IV. CONCLUSION

Analytical Method AG-597 is a valid and accurate method for the determination of parent residues of CGA-173506 in corn, sorghum, potato tubers and rice raw agricultural commodities.

I. CERTIFICATION

The reports and experimental results included in this study, Laboratory Project I.D. AG-597, are certified to be authentic accounts of the experiments.

Robert E. M. Wurz, Ph.D.
Research Scientist
Residue Studies
Residue Chemistry Department
919-632-2391

3/9/93
Date

CERTIFICATION OF GOOD LABORATORY PRACTICES

The analytical work reported in AG-597 was performed in accordance with Good Laboratory Practice Standards, 40 CFR Part 160.

Robert E. M. Wurz, Ph.D.
Study Director

3/9/93
Date

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

3/9/93
Date

CIBA-GEIGY CORPORATION
PLANT PROTECTION
QUALITY ASSURANCE UNIT

QUALITY ASSURANCE STATEMENT

Report Title: ANALYTICAL METHOD FOR THE DETERMINATION OF CGA-173506 IN CROPS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY INCLUDING VALIDATION DATA

Study Director: R. E. M. Wurz

Study No.: 56-92

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

INSPECTION/AUDIT TYPE	INSPECTION/AUDIT DATE	REPORTING DATE
Protocol Audit	4/10,13/92	4/13/92
In-progress Inspection	4/21/92	4/22/92
Report Audit	3/1-3/93	3/3/93

Prepared By: Faye M. Wilhite
Faye M. Wilhite

Date: March 3, 1993

TABLE I. LIQUID CHROMATOGRAPHIC OPERATING CONDITIONS FOR DETERMINATION OF CGA-173506 IN GRAIN, HAY, FORAGE AND POTATO TUBER SAMPLES

Instrument:	Waters 501 HPLC pump or equivalent Perkin-Elmer Model ISS-100 Automatic HPLC sampler or equivalent ABI Kratos Spectroflow Model 783 Variable Wavelength UV Detector or equivalent
Column Oven:	BioRad HPLC column heater, model number 125-0425 or equivalent.
Oven Temp.:	30°C
Guard column:	Normal Phase, 3 cm X 4.6 mm i.d. (Phase Separations Cat. #839451) or equivalent
Column 1:	Spherisorb Amino(NH_2) 250 mm x 4.6 mm i.d., 5 um particle size (Phase Separations Cat. #839530) or equivalent
Mobile Phase:	Hexane/Methanol/Isopropanol (90/6/6) (v/v/v)
Retention Time:	~10 min.
Wavelength:	268 nm
Attenuation:	0.003 AUFS
Flow Rate:	1.0 ml/min
Volume Injected:	100 µl
Run Time:	20 min/injection
Data Collection:	VAX / Multichrom

TABLE II. LIQUID CHROMATOGRAPHIC OPERATING CONDITIONS FOR DETERMINATION OF CGA-173506 IN FODDER SAMPLES

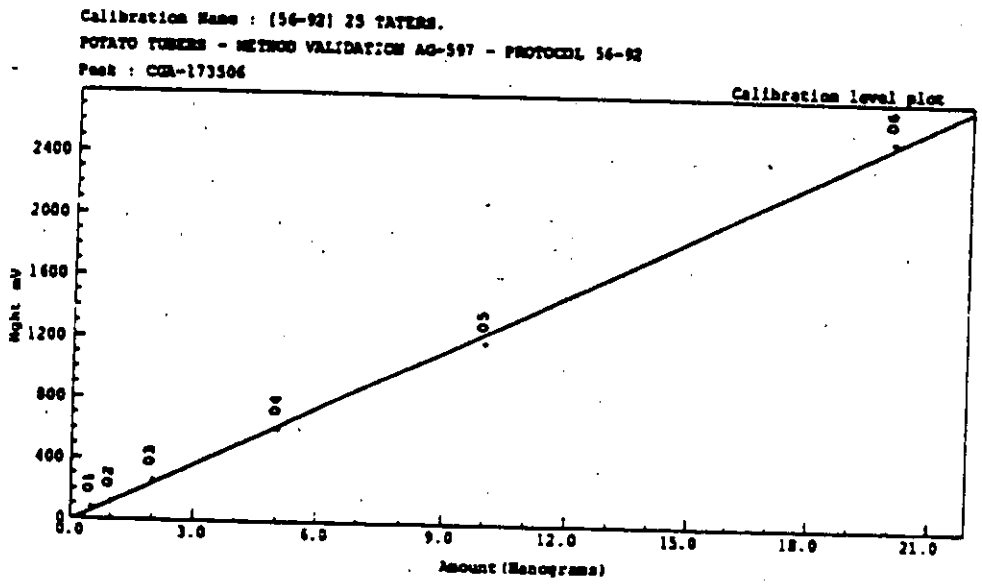
Instrument:	Waters 501 HPLC pump or equivalent Perkin-Elmer Model ISS-100 Automatic HPLC sampler or equivalent ABI Kratos Spectroflow Model 783 Variable Wavelength UV Detector or equivalent
Column 1:	Spherisorb C_1, 100mm X 4.6mm (Phase Separations Cat. #839567) or equivalent, connected to Spherisorb Amino(NH_2) 250 mm x 4.6 mm i.d., 5 um particle size (Phase Separations cat. #839530) or equivalent
Mobile Phase:	Hexane/N-Propanol/Isopropanol (90/10/10) (v/v/v)
Retention Time:	~10 min.
Wavelength:	268 nm
Attenuation:	0.003 AUFS
Flow Rate:	1.0 ml/min
Volume Injected:	100 µl
Run Time:	20 min/injection
Data Collection:	VAX / Multichrom

TABLE III. TYPICAL STANDARDIZATION DATA FOR CGA-173506

HPLC system for grain, forage, hay and potato tubers

STD Soln ng/ul	STD wt. Inj. ng	Peak Height UV
0.005	0.5	76
0.01	1.0	117
0.02	2.0	262
0.05	5.0	603
0.10	10.0	1187
0.20	20.0	2538

Correlation Coefficient = 0.99933



Constant : -9.90044
1st degree : 1.25801E+2

Curve fit : Linear
Correlation coefficient : 0.99933
Standard error : 3.8667BE+1
Reported on 23-FEB-1993 at 11:31

TABLE IV. SUMMARY OF RECOVERY DATA FOR CROP SAMPLES FORTIFIED WITH CGA-173506

Sample Number	Substrate	Fortification Level (ppm)	% Recovery
RBO	Reagent Blank	0	(<0.01 ppm)
Corn			
G.00	Grain	0 (Control)	(<0.01 ppm)
G.01A	Grain	0.01	114
G.01B	Grain	0.01	109
G.01C	Grain	0.01	105
G.05	Grain	0.05	97
G.10	Grain	0.10	95
F.00	Forage	0 (Control)	(<0.01 ppm)
F.01A	Forage	0.01	79
F.01B	Forage	0.01	86
F.05	Forage	0.05	86
F.10	Forage	0.10	86
F.20	Forage	0.20	83
D.00	Fodder	0 (Control)	(<0.01 ppm)
D.01A	Fodder	0.01	70
D.01B	Fodder	0.01	73
D.05	Fodder	0.05	89
D.10	Fodder	0.10	92
D.20	Fodder	0.20	97
Sorghum*			
SG.00	Grain	0 (Control)	(<0.01 ppm)
SG.05	Grain	0.05	88
SG.10	Grain	0.10	102
SH.00	Hay	0 (Control)	(<0.01 ppm), (<0.01 ppm)
SH.01A	Hay	0.01	95
SH.01B	Hay	0.01	96
SH.05	Hay	0.05	98
SH.10	Hay	0.10	76
SH.20	Hay	0.20	70,72
SD.00	Fodder	0 (Control)	(<0.01 ppm)
SD.01A	Fodder	0.01	75
SD.01B	Fodder	0.01	80
SD.05	Fodder	0.05	70
SD.10	Fodder	0.10	86
SD.20	Fodder	0.20	81
Potato			
PT.00	Mature Tuber	0 (Control)	(<0.01 ppm)
PT.01A	Mature Tuber	0.01	101
PT.01B	Mature Tuber	0.01	75
PT.01C	Mature Tuber	0.01	80
PT.05	Mature Tuber	0.05	92
PT.10	Mature Tuber	0.10	79
Rice			
RG.00A	Grain	0 (Control)	(<0.01 ppm)
RG.01A	Grain	0.01	72
RG.05	Grain	0.05	109
RG.00B	Grain	0 (Control)	(<0.01 ppm)
RG.01B	Grain	0.01	94
RG.10	Grain	0.10	117
RS.0A	Stalks	0 (Control)	(<0.01 ppm)
RS.0B	Stalks	0 (Control)	(<0.01 ppm)
RS.05	Stalks	0.05	79
RS2.0	Stalks	2.0	85
RS.10	Stalks	0.10	94
RS8.0	Stalks	8.0	94
Mean = 87%	Std. Dev. = 13	CV = 15.%	N = 41

*Samples SG.01A, SG.01B, and SG.01C were analyzed but not reported because the limit of determination for sorghum grain was raised to 0.05 ppm.

TABLE V. SUMMARY OF RESULTS FOR ^{14}C-CGA-173506 TREATED RICE

Sample Code	Study Number 214-89 Code No.	PHI	Incurred ^{14}C Level (ppm)[a]	% ^{14}C Extracted	ppm ^{14}C Found in Final Fraction	HPLC ppm Found	% HPLC Accountability
1X Treated Sample[1]							
(Grain)							
RG.IA	P89060060	52	0.076	56	0.033	0.028	37
RG.IB	P89060060	52	0.076	53	0.034	0.030	39
RG.IC	P89060060	52	0.076	50	0.024	0.022	29
						(CV=16%)	
(Stalks)							
RS.IA	P89060058	52	2.59	91	1.81	2.00	77
RS.IB	P89060058	52	2.59	90	1.77	1.92	74
RS.IC	P89060058	52	2.59	90	1.96	1.90	73
						(CV=2.7%)	
5X Treated Samples[2]							
(Grain)							
RG.VA	P89060080	50	0.363	116	0.353	0.361	99
RG.VB	P89060080	50	0.363	129	0.377	0.396	109
RG.VC	P89060080	50	0.363	111	0.401	0.411	113
						(CV=6.6%)	
(Stalks)							
RS.VA	P89060078	50	9.75	84	6.19	5.99	61
RS.VB	P89060078	50	9.75	90	6.84	6.84	70
RS.VC	P89060078	50	9.75	85	6.66	6.66	68
						(CV=6.9%)	

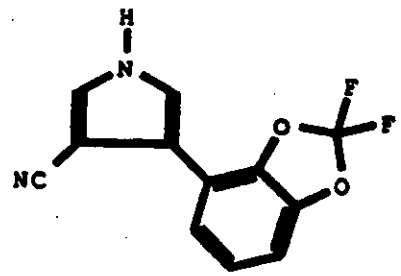
[a] ^{14}C incurred levels determined by combustion/LSC by Metabolism Department. Reference lab notebook 3721 page 8, Metabolism Department Protocol 214-89.

[1] 1X treatment = 0.25 lb. a.i./A foliar spray applied twice with a 14 day interval in between applications.

[2] 5X treatment = 1.25 lb. a.i./A foliar spray applied twice with a 14 day interval in between applications.

Comment: Results are corrected for procedural recoveries <100%.
PHI = pre-harvest interval

FIGURE 1. CHEMICAL NAME AND STRUCTURE



CGA-173506

4-(2,2-difluoro-1,3-benzdioxol-4-yl)-1H-pyrrole-3-carbonitrile

FIGURE 2. FLOWCHART FOR ANALYTICAL METHOD AG-597, GRAIN AND POTATO TUBER SUBSTRATES

Grain and Tubers

10 g of solid substrate

Add 150 ml 90% ACN/Water
Homogenize for 90 seconds
Let solution sit for minimum of 5 min.

Decant solvent through Reeve Angel 802 paper inside Whatmann 2V filter

Add 50-ml 90% ACN/water
Homogenize for 30 seconds

Filter through same filter paper combining extracts

Transfer 40-ml aliquot to 100-ml RB flask
Evaporate to < 4.0-ml volume

Add 10 ml of saturated sodium chloride solution and 10 ml of MTBE to RB flask

Transfer solutions to 60-ml separatory funnel
Shake sep funnel for 30 sec.

Drain aqueous layer back into original RB flask

Drain organic layer through small cotton plug into 100-ml RB

Add 10 ml of MTBE to original flask
Transfer to sep funnel
Shake sep funnel for 30 sec

Aqueous-Discard

Drain organic through same cotton plug

Rinse cotton plug with 2 x 10 ml of MTBE

(Continued)

(Continued)

FIGURE 2. FLOWCHART FOR ANALYTICAL METHOD AG-597, GRAIN AND POTATO TUBER SUBSTRATES (Continued)

Add 3 ml of toluene
Evaporate off MTBE

Add 10 ml of hexane

Sonicate sample

Precondition 0.5 gram bond elut silica column with 10 ml of 10% IPA/hexane, followed by 10 ml of hexane

Load sample onto column

Rinse flask with 5-ml of 80% hexane/toluene

Elute CGA-173506 with 10 ml 50% DCM/toluene

Evaporate solution to dryness
Redissolve residue in 5-ml MeOH

Add 15 ml of water

Condition 0.5 gram bond elut phenyl column with 10 ml MeOH followed by 10 ml of water

Load sample onto column

Elute sample with 5-ml acetone

Evaporate sample to dryness

Add appropriate volume of mobile phase

Sonicate samples for 5 min.

Take aliquot for HPLC determination.

FIGURE 3. FLOWCHART FOR ANALYTICAL METHOD AG-597, FORAGE AND HAY SUBSTRATES

Forage and Hay Substrates

Weigh out 10 g of solid substrate

Add 150 ml 90% ACN /Water
Homogenize for 90 seconds
Let solution sit for minimum of 5 min.

Decant solvent through Reeve Angel 802 Paper inside a Whatmann 2V filter

Add 50-ml 90% ACN /water
Homogenize for 30 seconds

Filter through same filter paper combining extracts

Transfer 40 ml aliquot to 100 ml RB flask
Evaporate to <4.0 ml volume

Add 10 ml saturated sodium chloride solution and 10 ml of MTBE to RB

Transfer solutions to 60-ml separatory funnel
Shake sep funnel for 30 sec.

Drain aqueous layer back into original RB flask

Drain organic through small cotton plug into 100 ml RB

Add 10 ml of MTBE to original flask
transfer to sep funnel
shake sep funnel for 30 sec.

Aqueous-Discard

Drain organic through same cotton plug

Rinse cotton plug with 2 x 10 ml of MTBE

(continued)

FIGURE 3. FLOWCHART FOR ANALYTICAL METHOD AG-597, FORAGE AND HAY SUBSTRATES (Continued)

Add 3 ml of toluene
Evaporate off MTBE

Add 10 ml of hexane
Sonicate sample

Precondition 1.0 gram bond elut silica column with 10 ml of 10% IPA/hexane followed by 10 ml of hexane

Load sample onto column
Rinse flask with 5 ml of 80% hexane/toluene, add to column

Wash column with 10 ml of 50% hexane/toluene

Elute CGA-173506 with 20 ml 25% DCM/toluene

Evaporate solution to dryness

Redissolve residue in 5 ml MeOH, then add 15-ml water

Condition 0.5 gram bond elut phenyl column with 10-ml MeOH, followed by 10 ml of water

Load sample onto column.

Elute sample with 5-ml acetone
Evaporate sample to dryness

Add appropriate volume of mobile phase

Sonicate samples for 5 min.

Take aliquot for HPLC determination

FIGURE 4. FLOWCHART FOR ANALYTICAL METHOD AG-597, FODDER SUBSTRATES

Fodder Substrates

Weigh out 10 g of solid substrate

Add 150-ml 90% ACN/Water
Homogenize for 90 seconds
Let solution sit for minimum of 5 min.

Decant solvent through Reeve Angel 802 Paper inside a Whatmann 2V filter

Add 50-ml 90% ACN /water
Homogenize for 30 seconds

Filter through same filter paper combining extracts

Transfer 40 ml aliquot to 250 ml separatory funnel

Add 10 ml hexane to separatory funnel
Shake sep funnel for 1 min

Drain ACN layer to second 250-ml sep funnel → Discard Hexane

Add 10-ml hexane to sep funnel
Shake sep funnel for 1 min.

Drain ACN layer to original sep funnel → Discard Hexane

Add 150-ml H_2O, 10-ml saturated sodium chloride solution and 25-ml 80% hexane/MTBE to sep funnel. Shake sep funnel for 30 sec.

Drain aqueous layer into 2nd sep funnel

Drain hexane/MTBE layer through sodium sulfate column into 100-ml RB flask

(Continued)

FIGURE 4. FLOWCHART FOR ANALYTICAL METHOD AG-597, FODDER SUBSTRATES (Continued)

Add 25 mls of 80% hexane/MTBE to sep funnel
Shake sep funnel for 1 min.

Discard Aqueous Layer

Drain hexane/MTBE layer through same sodium sulfate column into 100 ml RB flask

Rinse sodium sulfate column with 2 x 10 ml of 80% hexane/MTBE

Add 3 ml of toluene
Evaporate off hexane/MTBE solution

Add 10 ml of hexane
Sonicate sample

Precondition 1.0 gram bond elut silica column with 10 ml of 10% IPA/hexane followed by 10 ml of hexane

Load sample onto column
Rinse flask with 5 ml of 80% hexane/toluene

Wash column with 10 ml of 50% hexane/toluene

Elute CGA-173506 with 20 ml 25% DCM/toluene

Evaporate solution to dryness

Redissolve residue in 5-ml MeOH, then add 15-ml water

Condition 0.5 gram bond elut phenyl column with 10 mls MeOH, followed by 10 ml of water

Load sample onto column

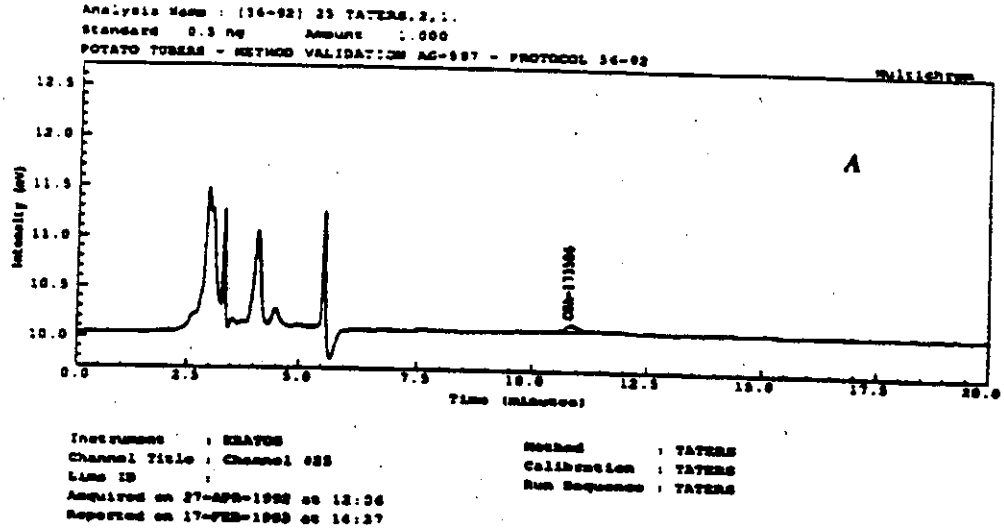
Elute sample with 5-ml acetone
Evaporate sample to dryness

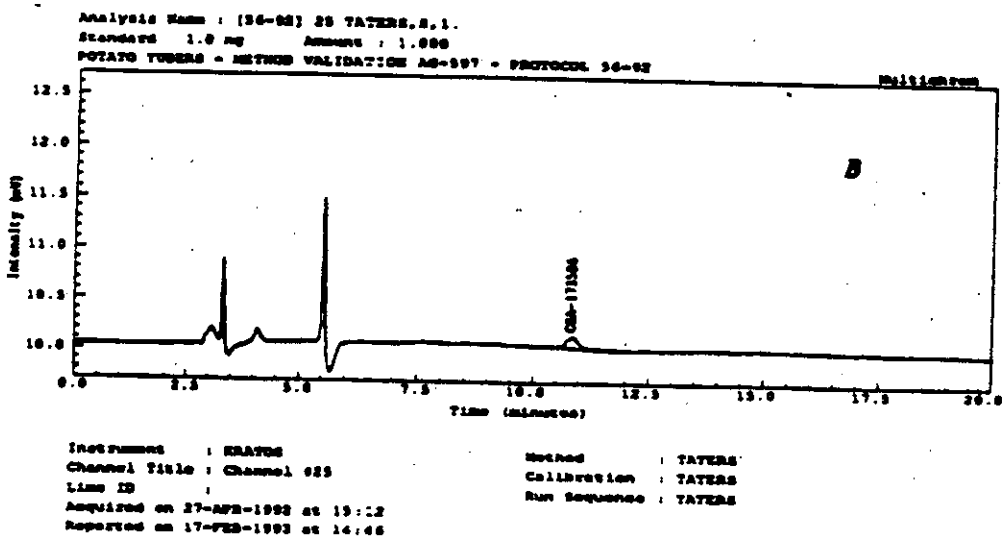
Add appropriate volume of mobile phase

Sonicate samples for 5 min.

Take aliquot for HPLC determination

FIGURE 5. REPRESENTATIVE CHROMATOGRAMS FOR CGA-173506 STANDARDS

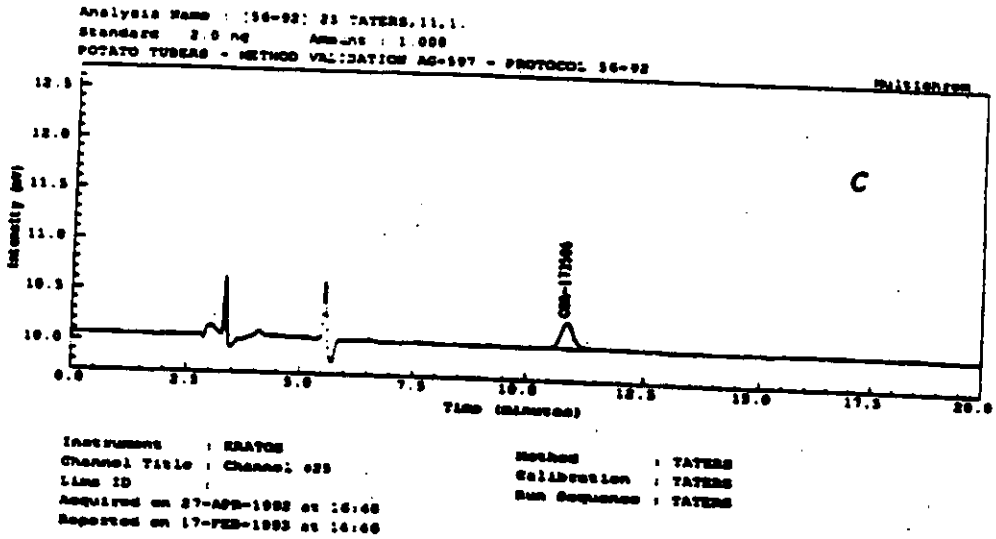


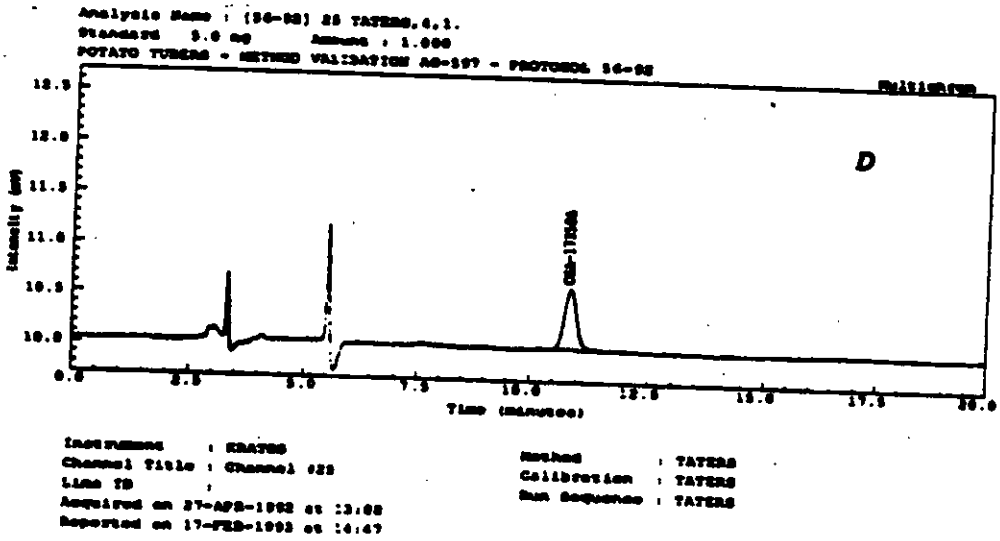


A. 0.50 ng injected

B. 1.0 ng injected

FIGURE 5. REPRESENTATIVE CHROMATOGRAMS FOR CGA-173506 STANDARDS (Continued)

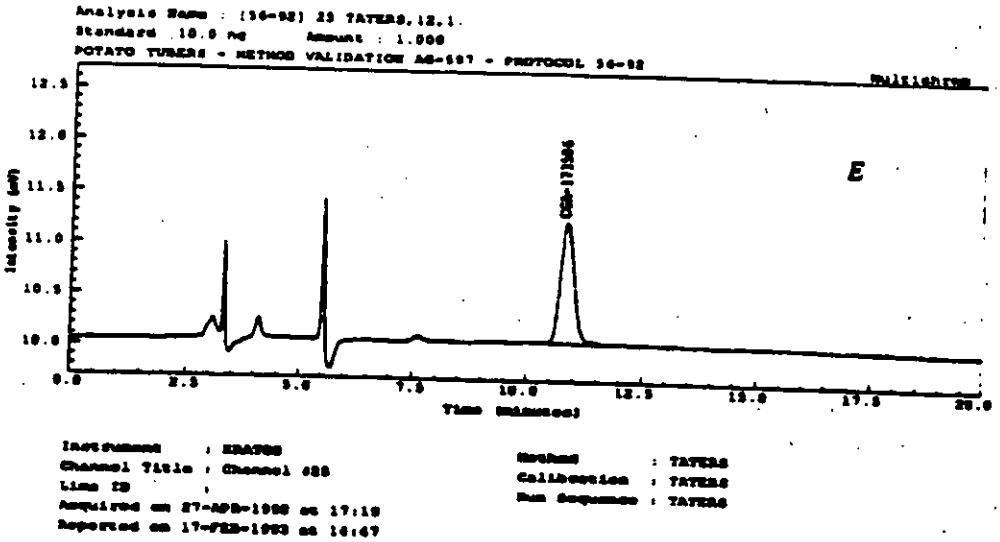


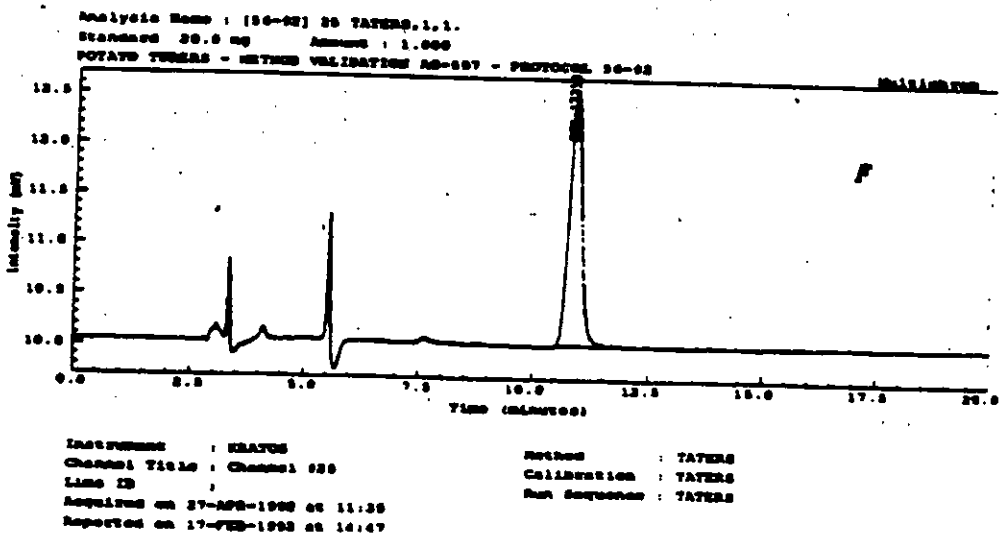


C. 2.0 ng injected

D. 5.0 ng injected

FIGURE 5. REPRESENTATIVE CHROMATOGRAMS FOR CGA-173506 STANDARDS (Continued)

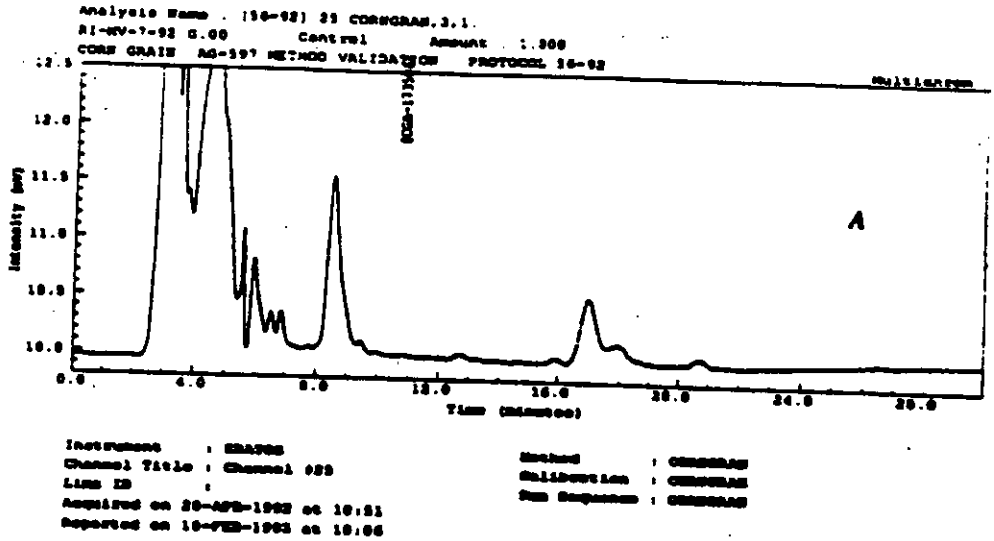


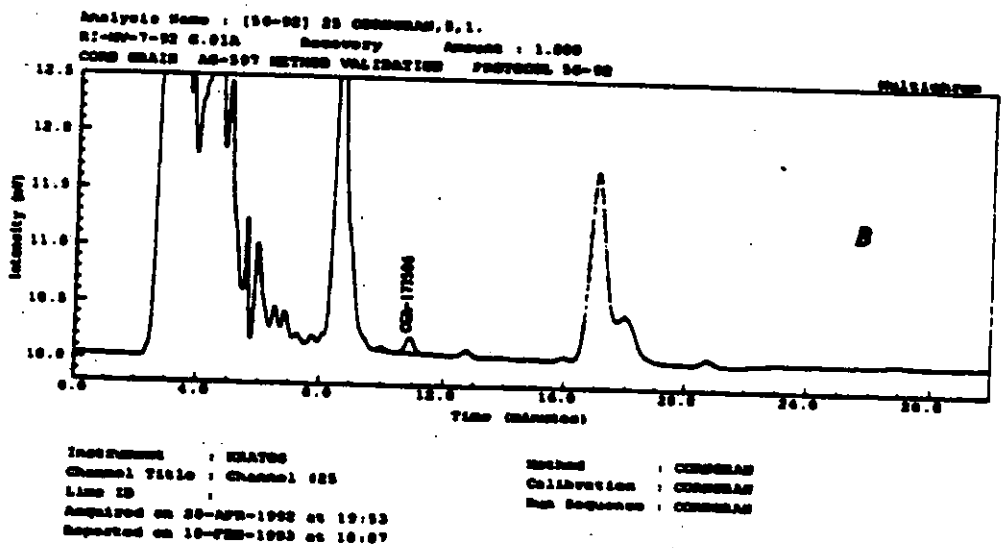


E. 10.0 ng injected

F. 20.0 ng injected

FIGURE 6. REPRESENTATIVE CHROMATOGRAMS FOR CONTROL AND FORTIFIED CONTROL CORN GRAIN SAMPLES



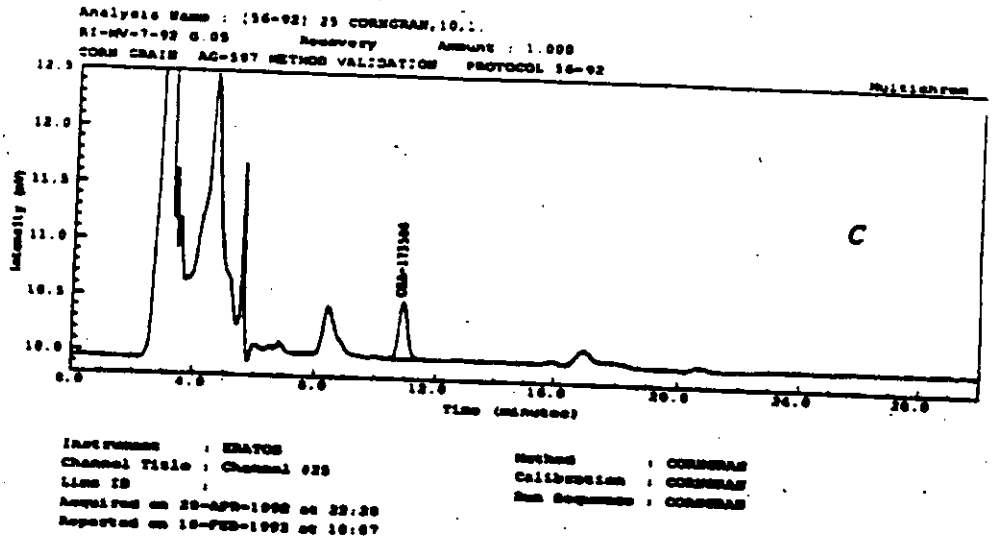


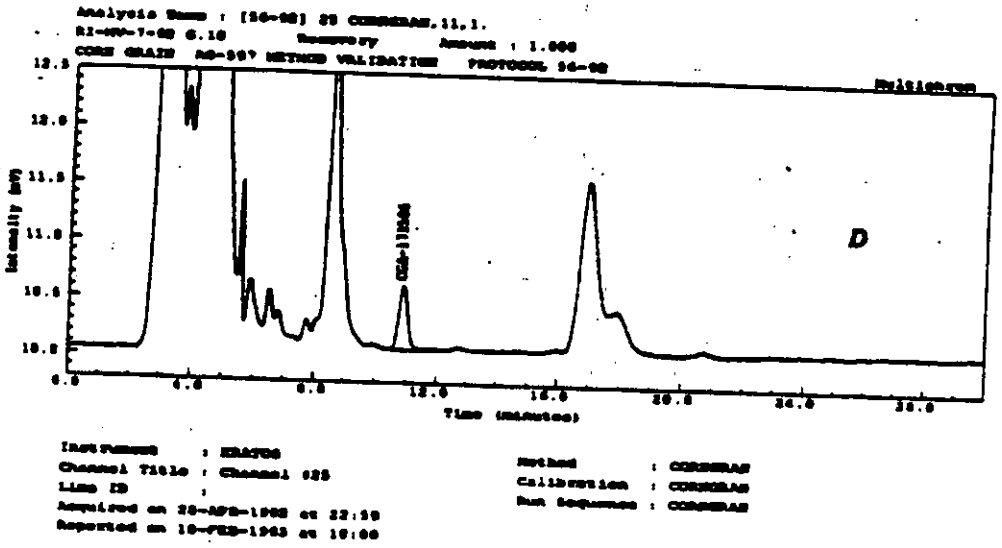
A. Control; 99.5 mg injected, <0.5 ng found, <0.01 ppm.

B. Control + 0.01 ppm CGA-173506; 99.5 mg injected, 1.13 ng found, 0.011 ppm, 114% recovery.

(Recovery results corrected for control values.)

FIGURE 6. REPRESENTATIVE CHROMATOGRAMS FOR CONTROL AND FORTIFIED CONTROL CORN GRAIN SAMPLES (Continued)



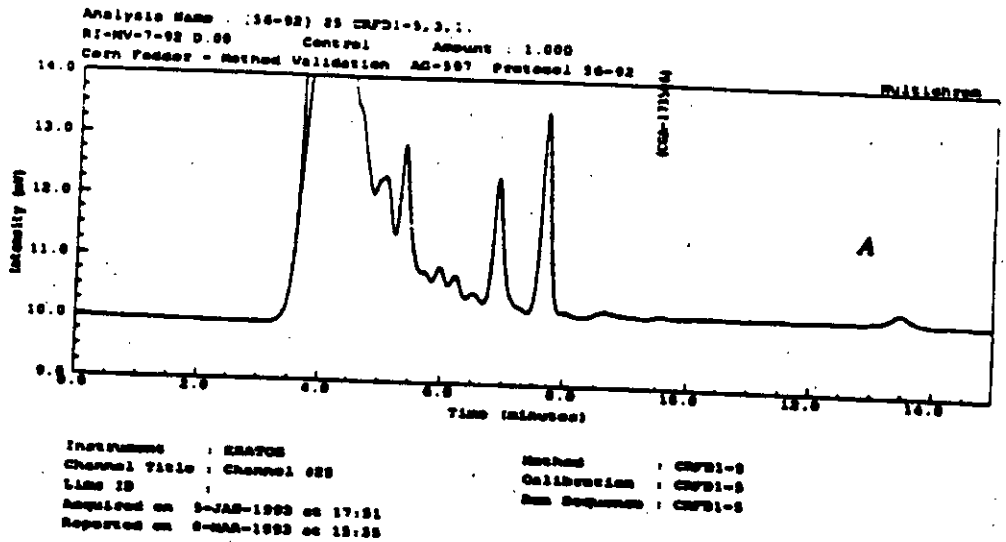


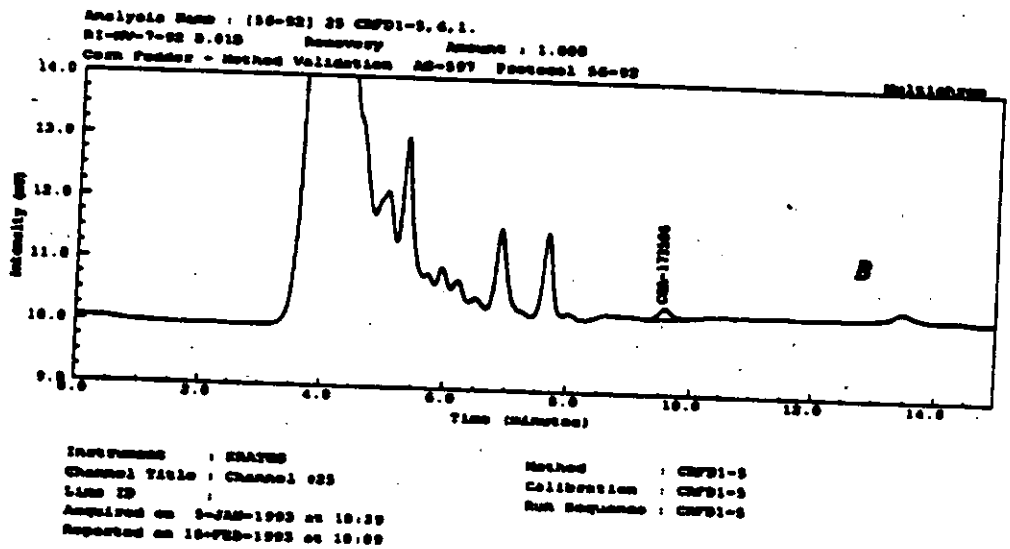
C. Control + 0.05 ppm CGA-173506; 99.5 mg injected, 4.34 ng found, 0.044 ppm, 87% recovery.

D. Control + 0.10 ppm CGA-173506; 49.8 mg injected, 4.75 ng found, 0.095 ppm, 95% recovery.

(Recovery results corrected for control values.)

FIGURE 7. REPRESENTATIVE CHROMATOGRAMS FOR CONTROL AND FORTIFIED CONTROL CORN FODDER SAMPLES



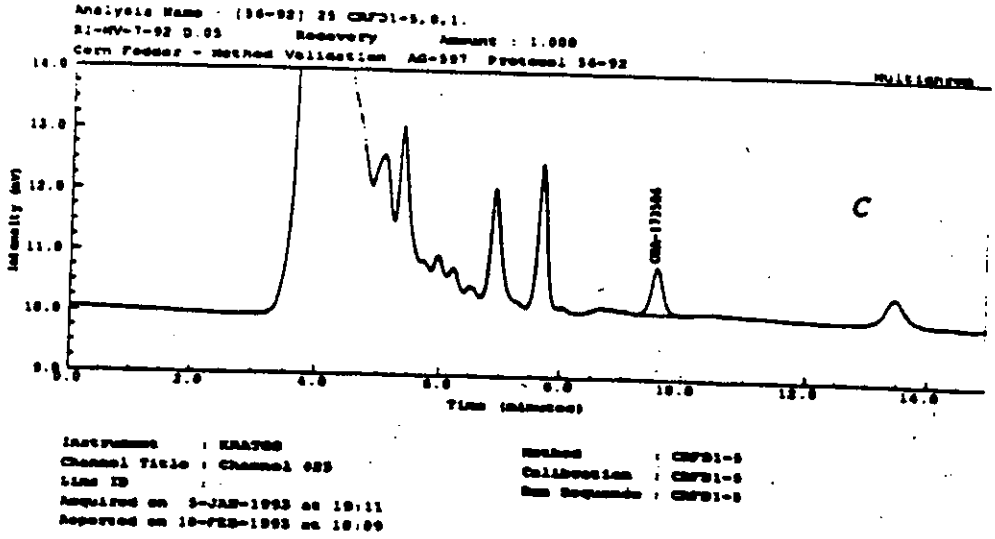


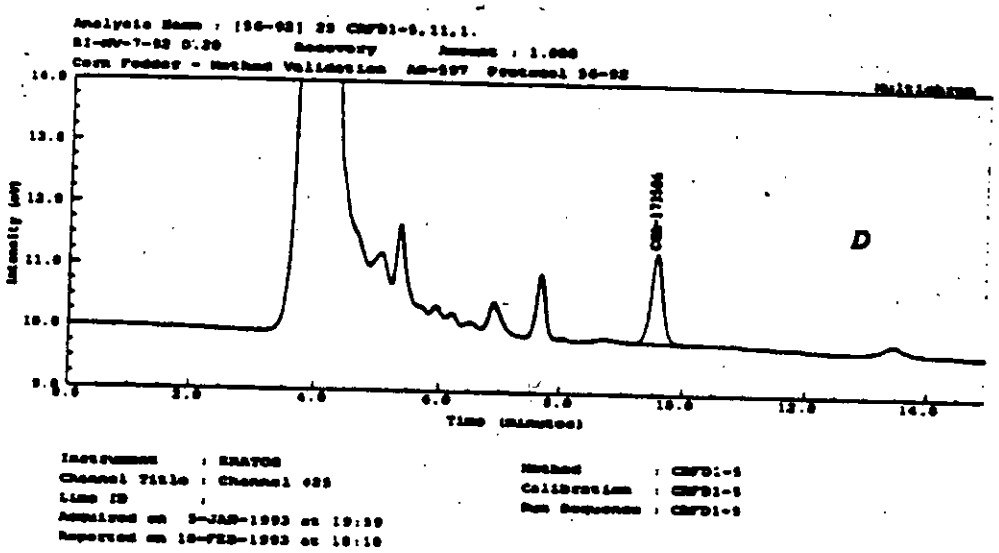
A. Control; 98.8 mg injected, <0.50 ng found, <0.01 ppm.

B. Control + 0.01 ppm CGA-173506; 98.8 mg injected, 0.72 ng found, 0.007 ppm, 73% recovery.

(Recovery results corrected for control values.)

FIGURE 7. REPRESENTATIVE CHROMATOGRAMS FOR CONTROL AND FORTIFIED CONTROL CORN FODDER SAMPLES (Continued)



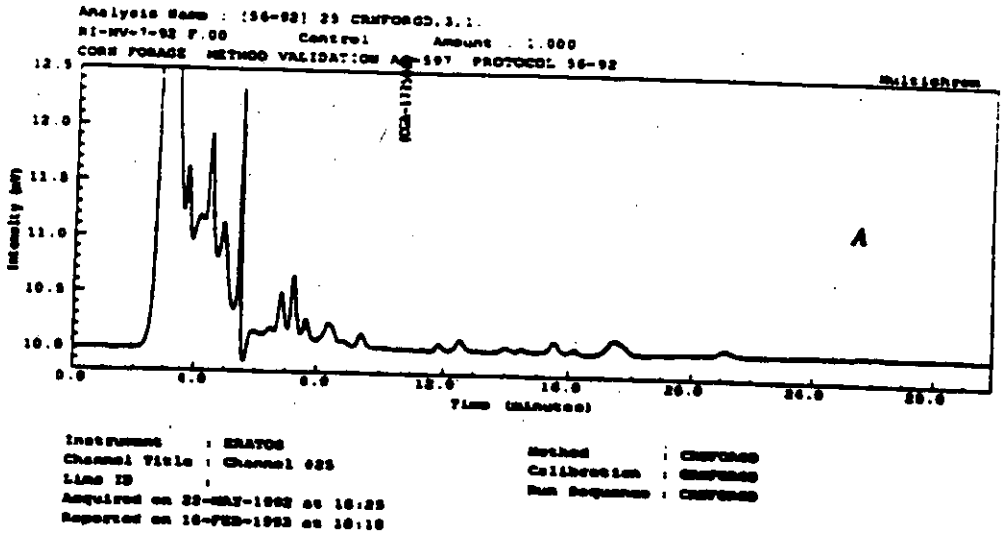


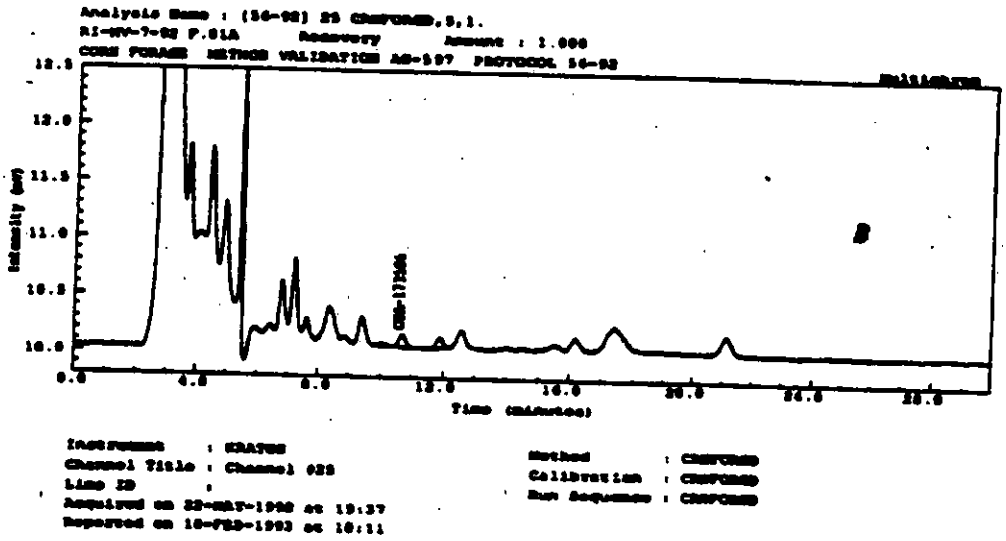
C. Control + 0.05 ppm CGA-173506; 98.8 mg injected, 4.4 ng found, 0.045 ppm, 89% recovery.

D. Control + 0.20 ppm CGA-173506; 49.4 mg injected, 8.6 ng found, 0.175 ppm, 87% recovery.

(Recovery results corrected for control values.)

FIGURE 8. REPRESENTATIVE CHROMATOGRAMS FOR CONTROL AND FORTIFIED CONTROL CORN FORAGE SAMPLES



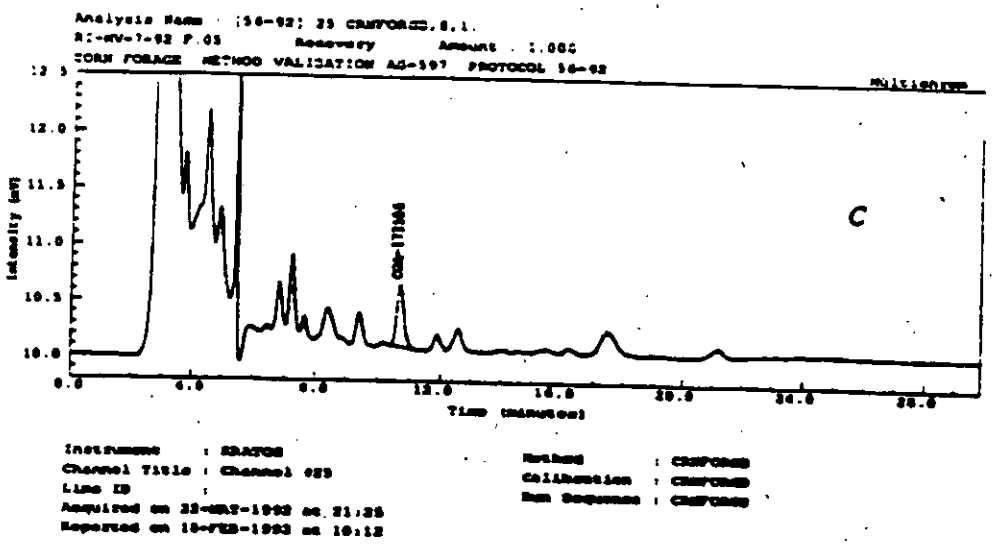


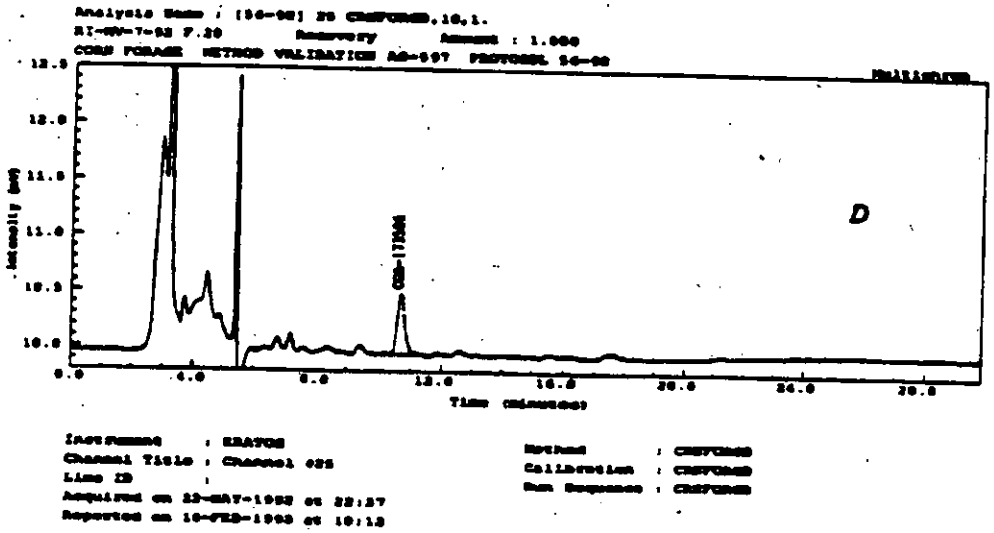
A. Control; 96.4 mg injected, <0.5 ng found, <0.01 ppm.

B. Control + 0.01 ppm CGA-173506; 96.4 mg injected, 0.76 ng found, 0.008 ppm, 79% recovery.

(Recovery results corrected for control values.)

FIGURE 8. REPRESENTATIVE CHROMATOGRAMS FOR CONTROL AND FORTIFIED CONTROL CORN FORAGE SAMPLES (Continued)



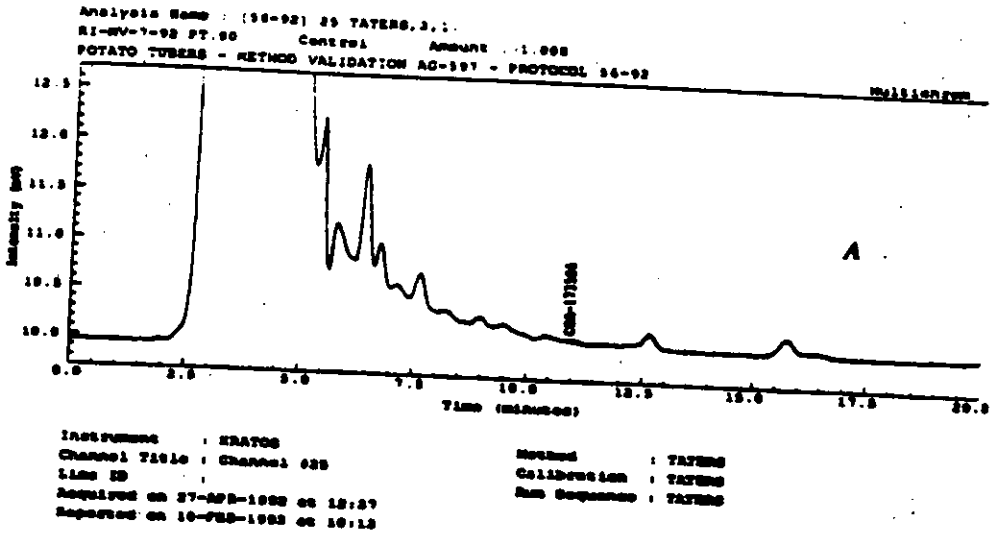


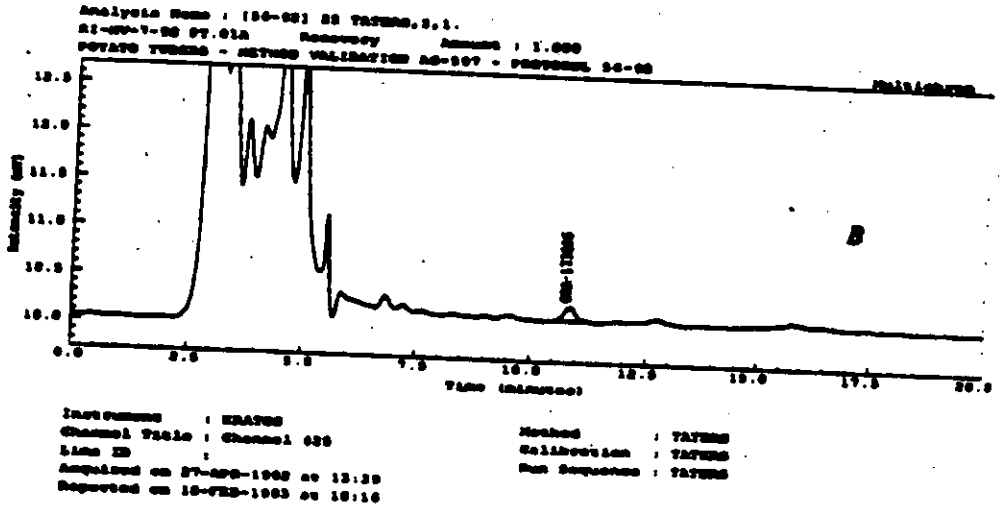
C. Control + 0.05 ppm CGA-173506; 96.4 mg injected, 4.17 ng found, 0.043 ppm, 86% recovery.

D. Control + 0.20 ppm CGA-173506; 24.1 mg injected, 3.99 ng found, 0.165 ppm, 83% recovery.

(Recovery results corrected for control values.)

FIGURE 9. REPRESENTATIVE CHROMATOGRAMS FOR CONTROL AND FORTIFIED CONTROL POTATO TUBER PIECES



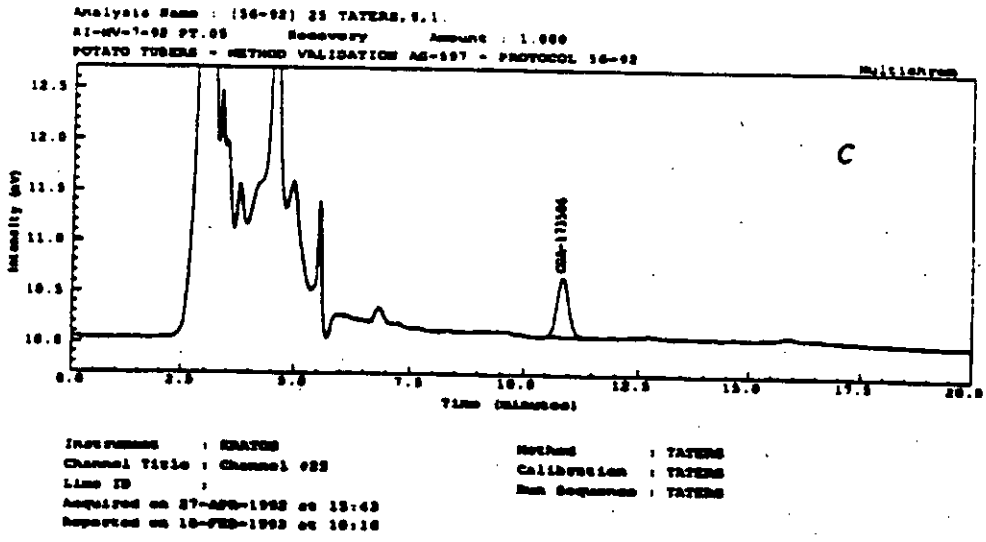


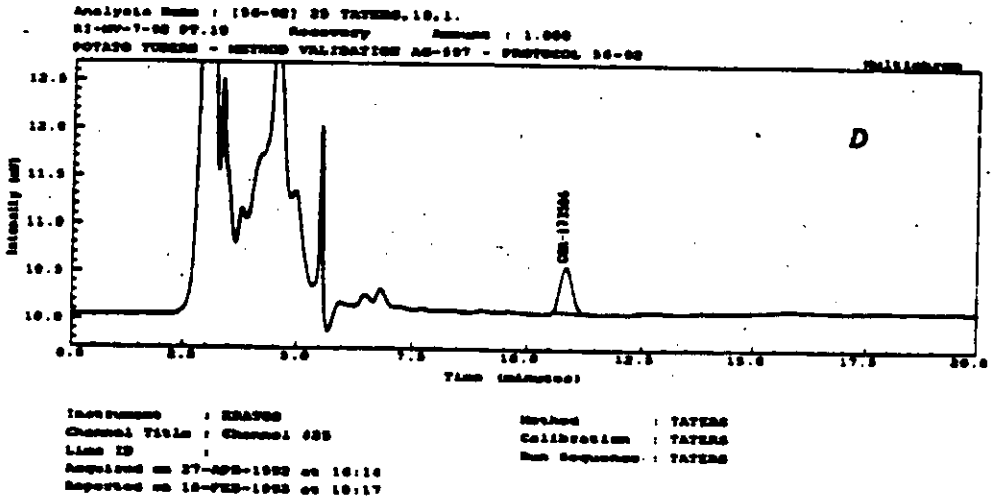
A. Control; 96.2 mg injected, 0.28 ng found, <0.01 ppm (0.003 ppm).

B. Control + 0.01 ppm CGA-173506; 96.2 mg injected, 1.26 ng found, 0.013 ppm, 101% recovery.

(Recovery results corrected for control values.)

FIGURE 9. REPRESENTATIVE CHROMATOGRAMS FOR CONTROL AND FORTIFIED CONTROL TUBER PIECES (Continued)



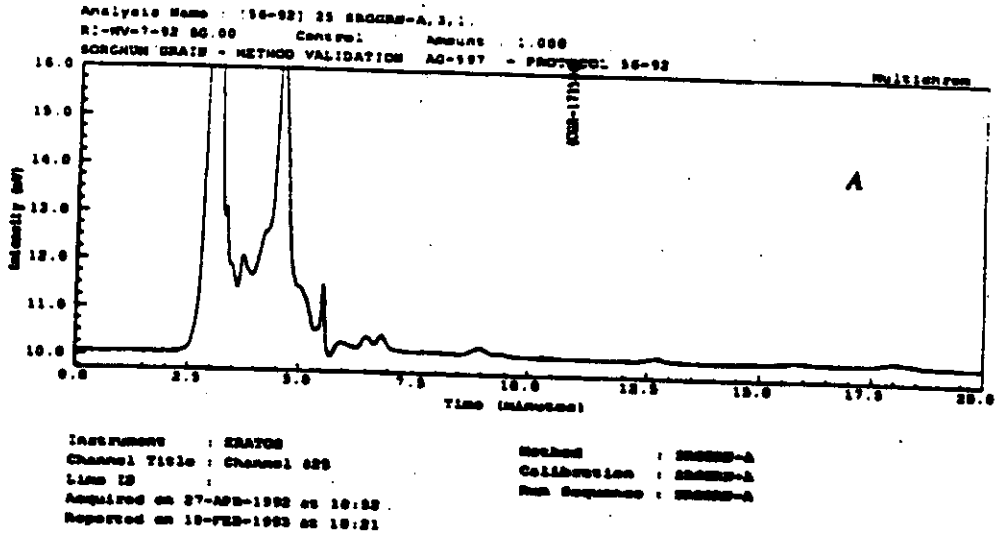


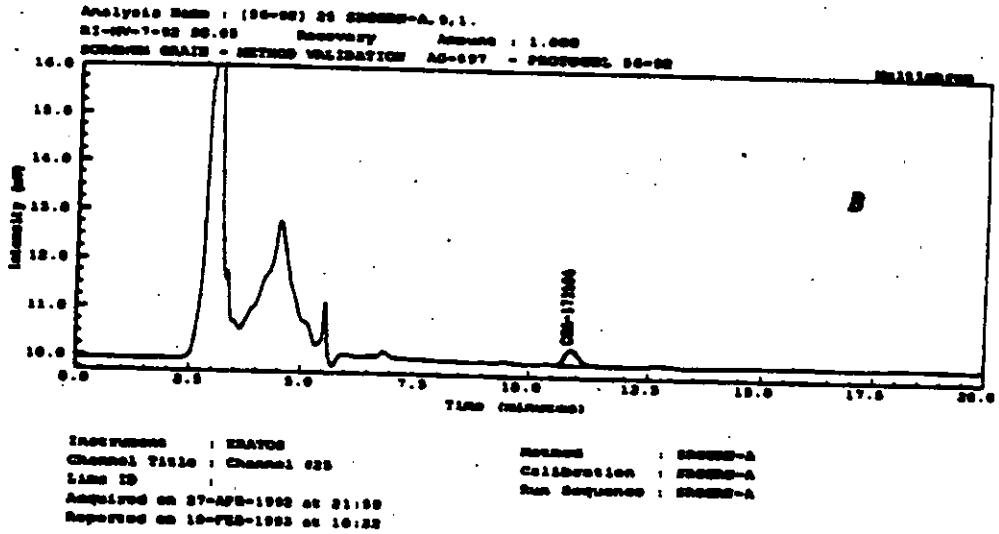
C. Control + 0.05 ppm CGA-173506; 96.2 mg injected, 4.72 ng found, 0.049 ppm, 92% recovery.

D. Control + 0.10 ppm CGA-173506; 48.1 mg injected, 3.93 ng found, 0.082 ppm, 79% recovery.

(Recovery results corrected for control values.)

FIGURE 10. REPRESENTATIVE CHROMATOGRAMS FOR CONTROL AND FORTIFIED CONTROL SORGHUM GRAIN SAMPLES



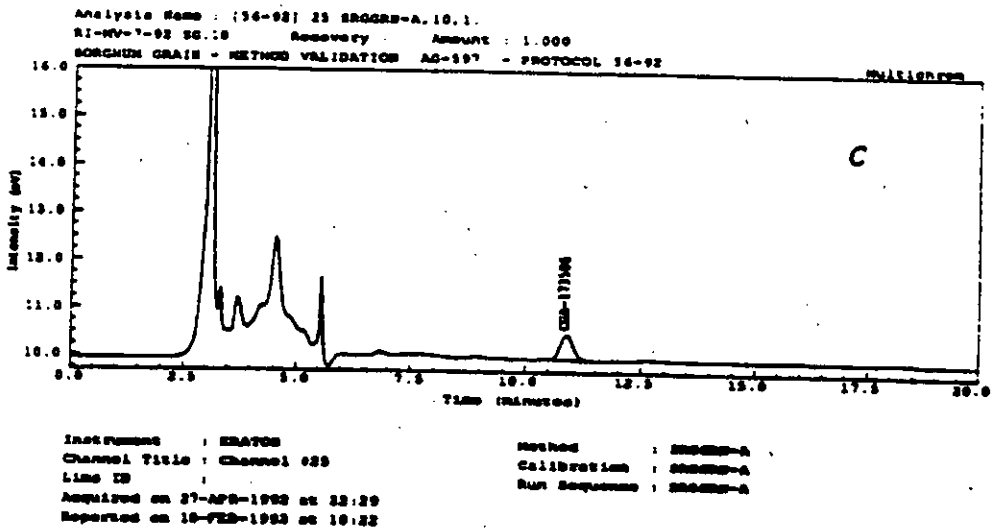


A. Control; 99.5 mg injected, <2.0 ng found, <0.05 ppm.

B. Control + 0.05 ppm CGA-173506; 99.5 mg injected, 3.27 ng found, 0.033 ppm, 66% recovery.

(Recovery results corrected for control values.)

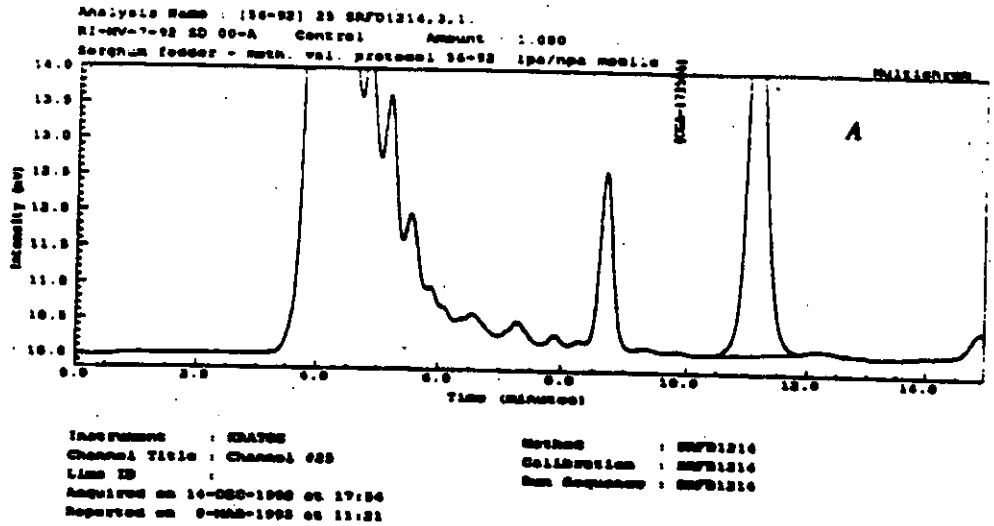
FIGURE 10. REPRESENTATIVE CHROMATOGRAMS FOR CONTROL AND FORTIFIED CONTROL SORGHUM GRAIN SAMPLES (Continued)

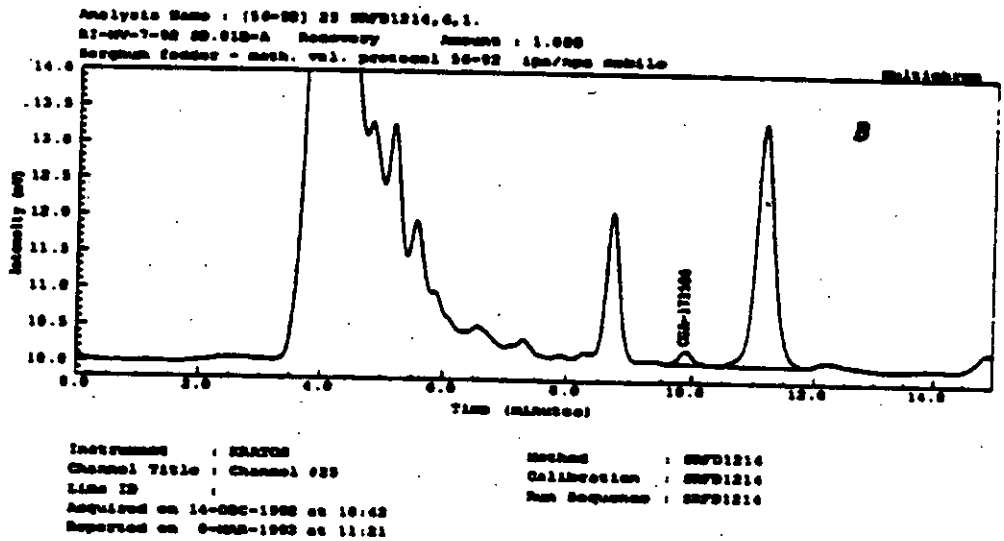


C. Control + 0.10 ppm CGA-173506; 49.7 mg injected, 5.09 ng found, 0.102 ppm, 102% recovery.

(Recovery results corrected for control values.)

FIGURE 11. REPRESENTATIVE CHROMATOGRAMS FOR CONTROL AND FORTIFIED CONTROL SORGHUM FODDER SAMPLES



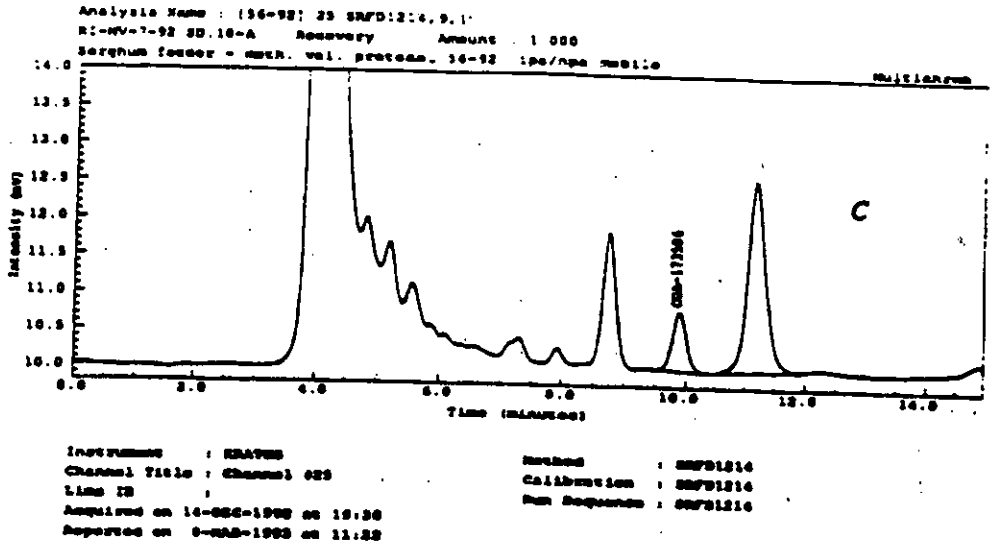


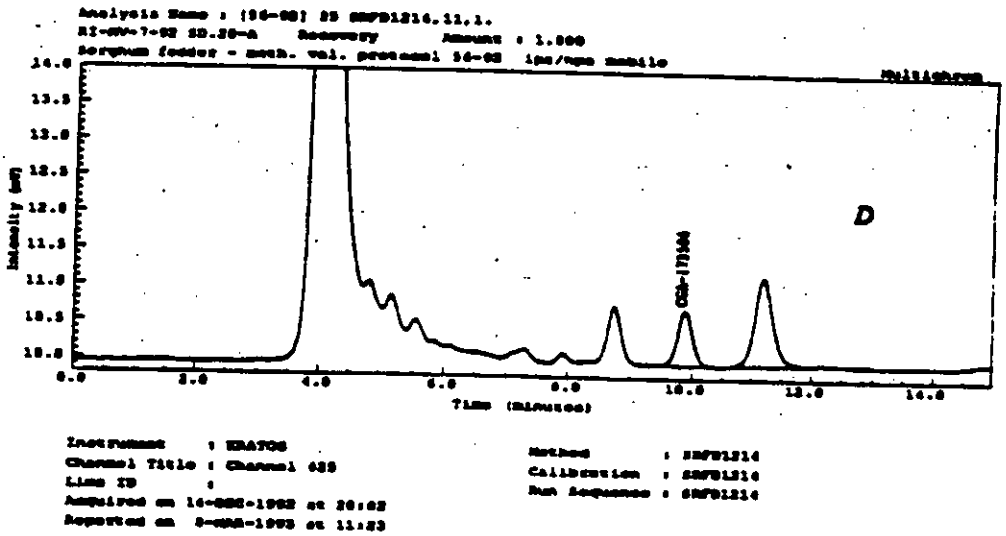
A. Control; 98.8 mg injected, <0.5 ng found, <0.01 ppm.

B. Control + 0.01 ppm CGA-173506; 98.8 mg injected, 0.74 ng found, 0.007 ppm, 75% recovery.

(Recovery results corrected for control values.)

FIGURE 11. REPRESENTATIVE CHROMATOGRAMS FOR CONTROL AND FORTIFIED CONTROL SORGHUM FODDER SAMPLES (Continued)



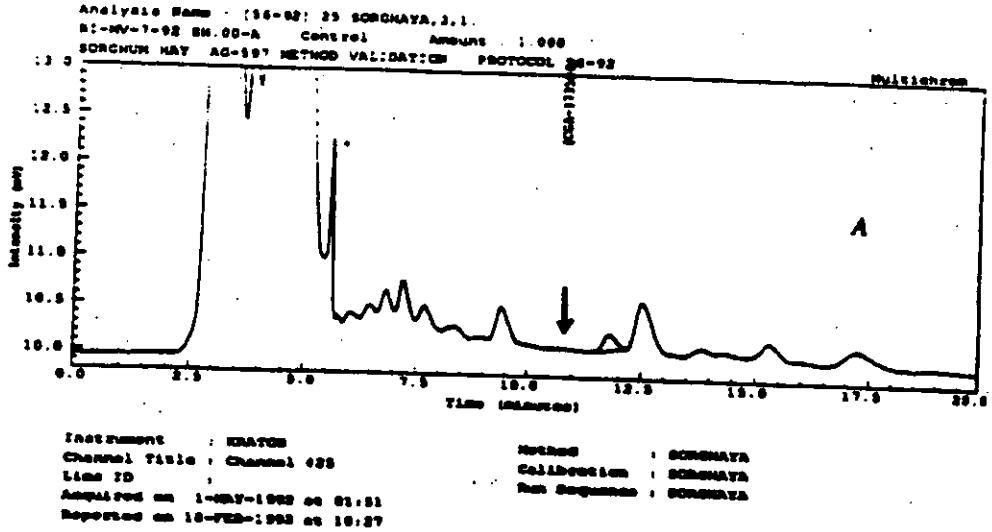


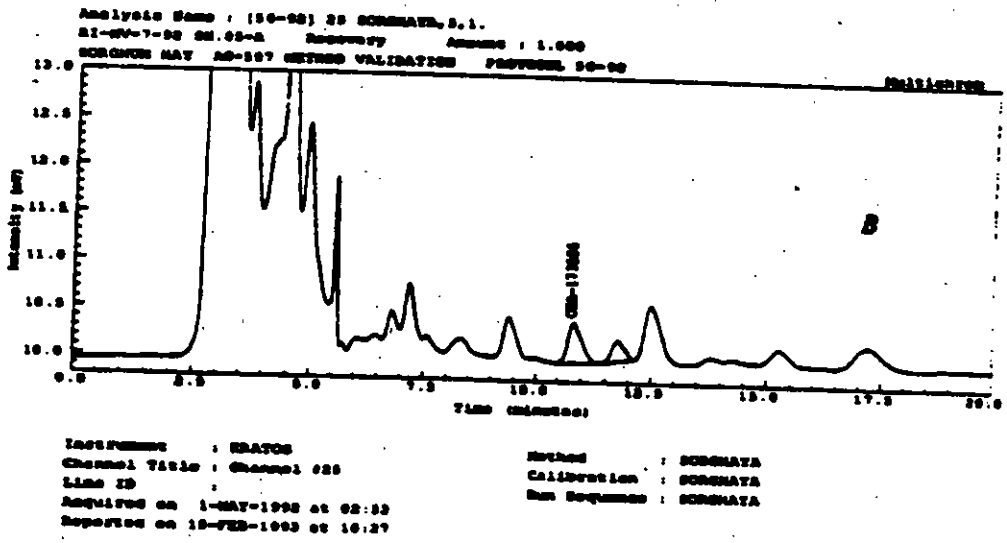
C. Control + 0.1 ppm CGA-173506; 49.4 mg injected, 4.23 ng found, 0.0826 ppm, 86% recovery.

D. Control + 0.20 ppm CGA-173506; 24.7 mg injected, 4.01 ng found, 0.162 ppm, 81% recovery.

(Recovery results corrected for control values.)

FIGURE 12. REPRESENTATIVE CHROMATOGRAMS FOR CONTROL AND FORTIFIED CONTROL SORGHUM HAY SAMPLES



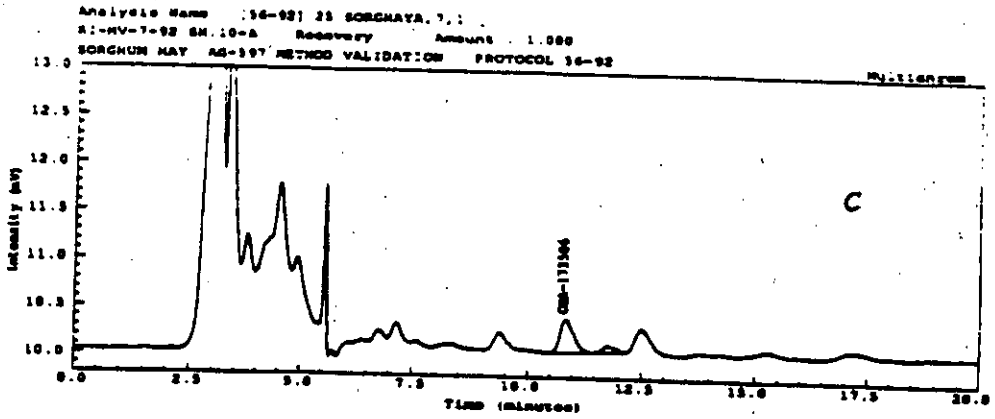


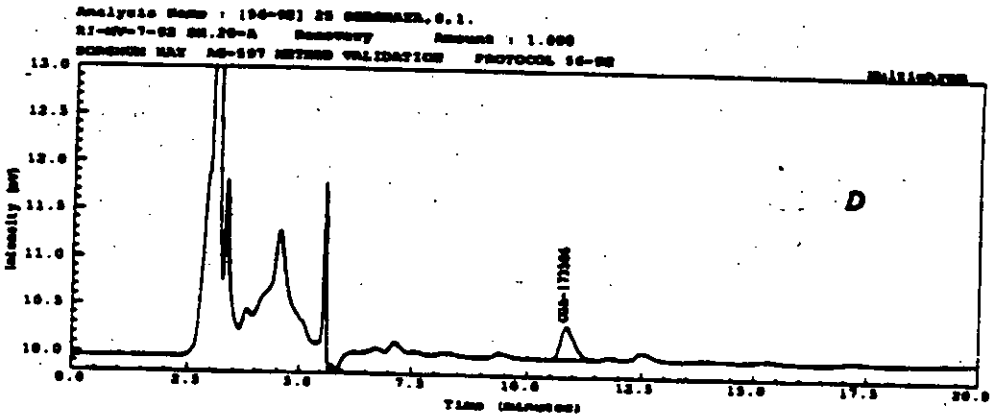
A. Control; 99.5 mg injected, <0.5 ng found, <0.01 ppm.

B. Control + 0.01 ppm CGA-173506; 99.5 mg injected, 4.5 ng found, 0.045 ppm, 90% recovery.

(Recovery results corrected for control values.)

FIGURE 12. REPRESENTATIVE CHROMATOGRAMS FOR CONTROL AND FORTIFIED CONTROL SORGHUM HAY SAMPLES (Continued)



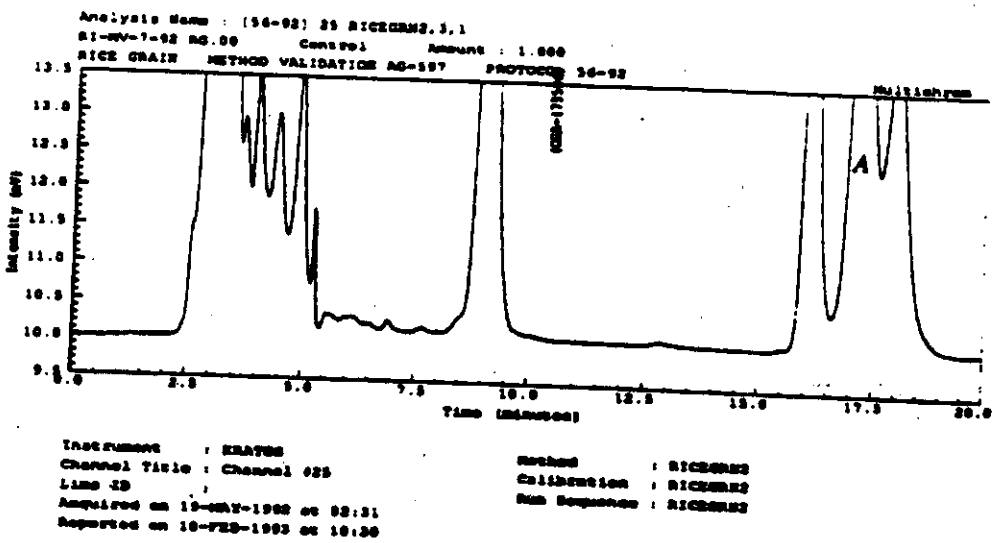


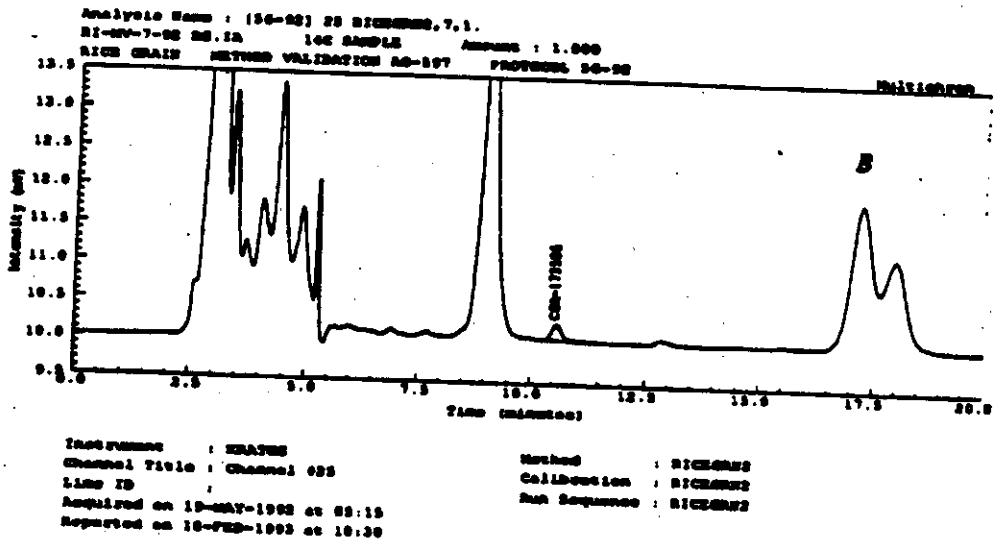
C. Control + 0.10 ppm CGA-173506; 49.8 mg injected, 3.77 ng found, 0.076 ppm, 76% recovery.

D. Control + 0.20 ppm CGA-173506; 24.9 mg injected, 3.58 ng found, 0.144 ppm, 72% recovery.

(Recovery results corrected for control values.)

FIGURE 13. REPRESENTATIVE CHROMATOGRAMS FOR CONTROL AND ^{14}C-CGA-173506 TREATED RICE GRAIN SAMPLES



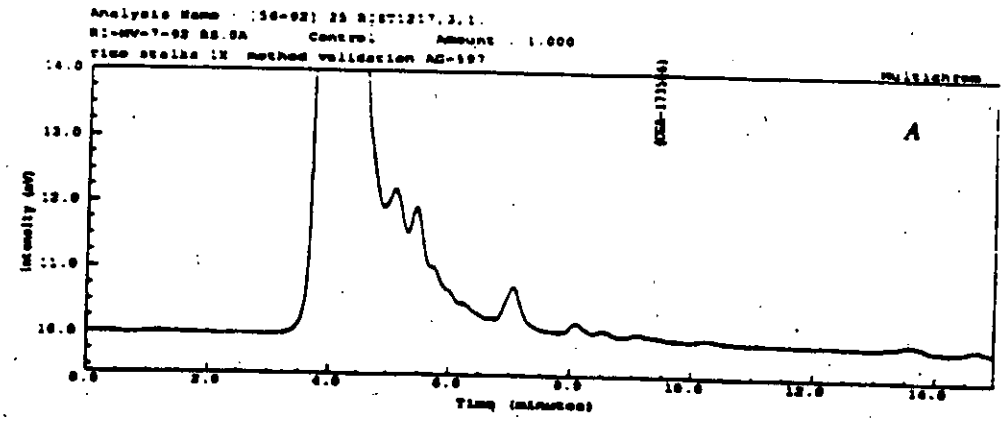


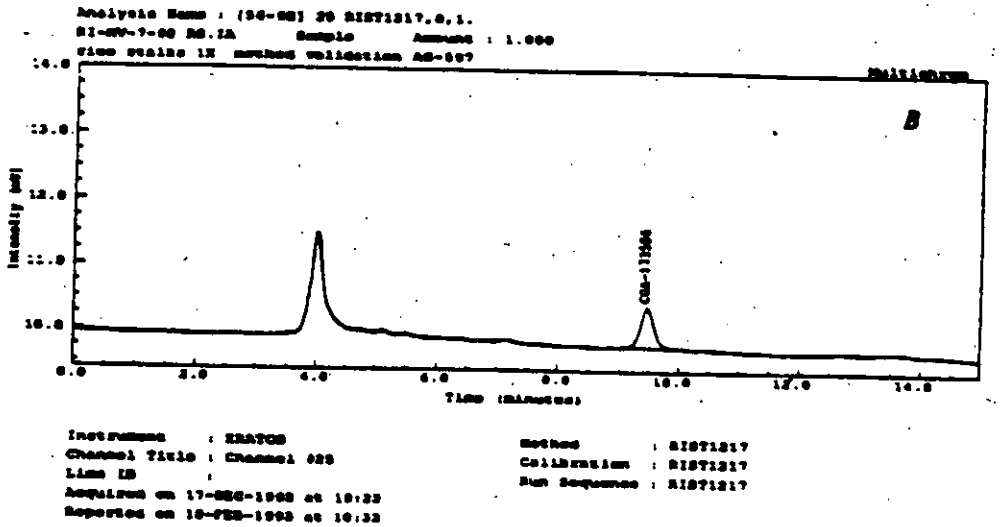
A. Control; 99.4 mg injected, <0.5 ng found, <0.01 ppm.

B. CGA-173506 treated sample, 49.7 mg injected, 1.22 ng found, 0.028 ppm.

(Residues corrected for procedural recovery value.)

FIGURE 14. REPRESENTATIVE CHROMATOGRAMS FOR CONTROL AND ^{14}C-CGA-173506 TREATED RICE STALKS SAMPLES



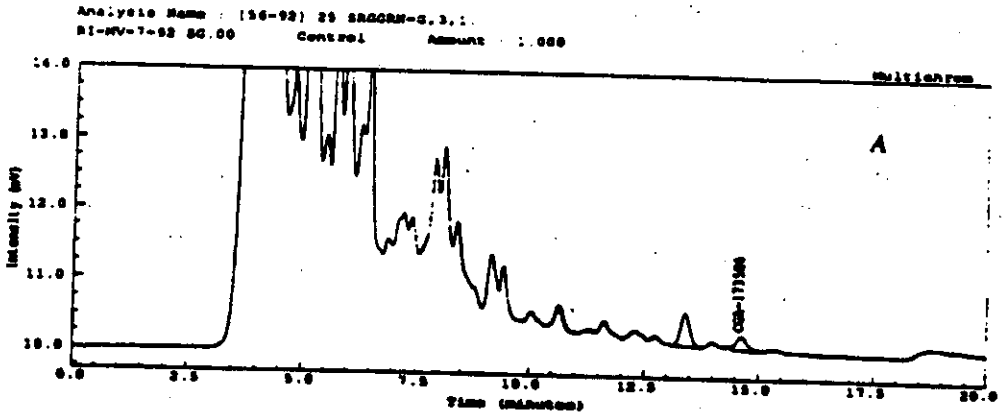


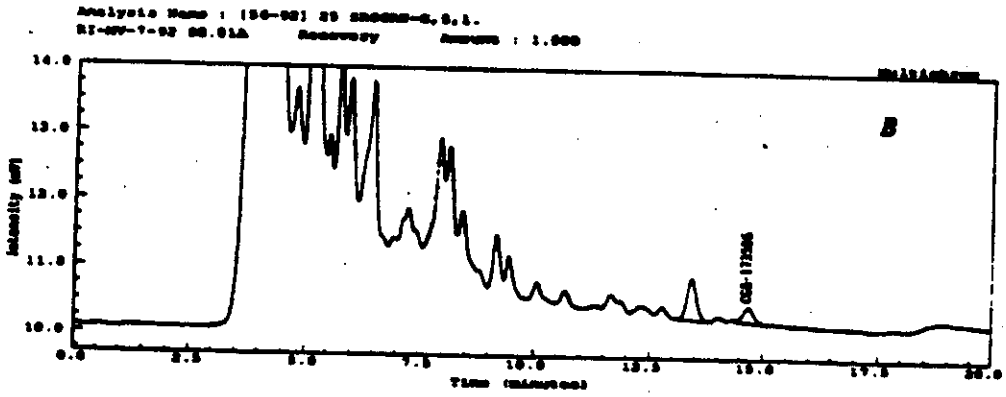
A. Control; 99.5 mg injected, <0.50 ng found, <0.01 ppm.

B. ^{14}C-CGA-173506 treated sample, 1.99 mg injected 3.27 ng found, 2.0 ppm.

(Residue value corrected for procedural recovery.)

FIGURE 15. REPRESENTATIVE CHROMATOGRAMS FOR CONTROL AND FORTIFIED CONTROL SORGHUM GRAIN SAMPLES AT THE 0.01 PPM & 0.05 PPM LEVEL



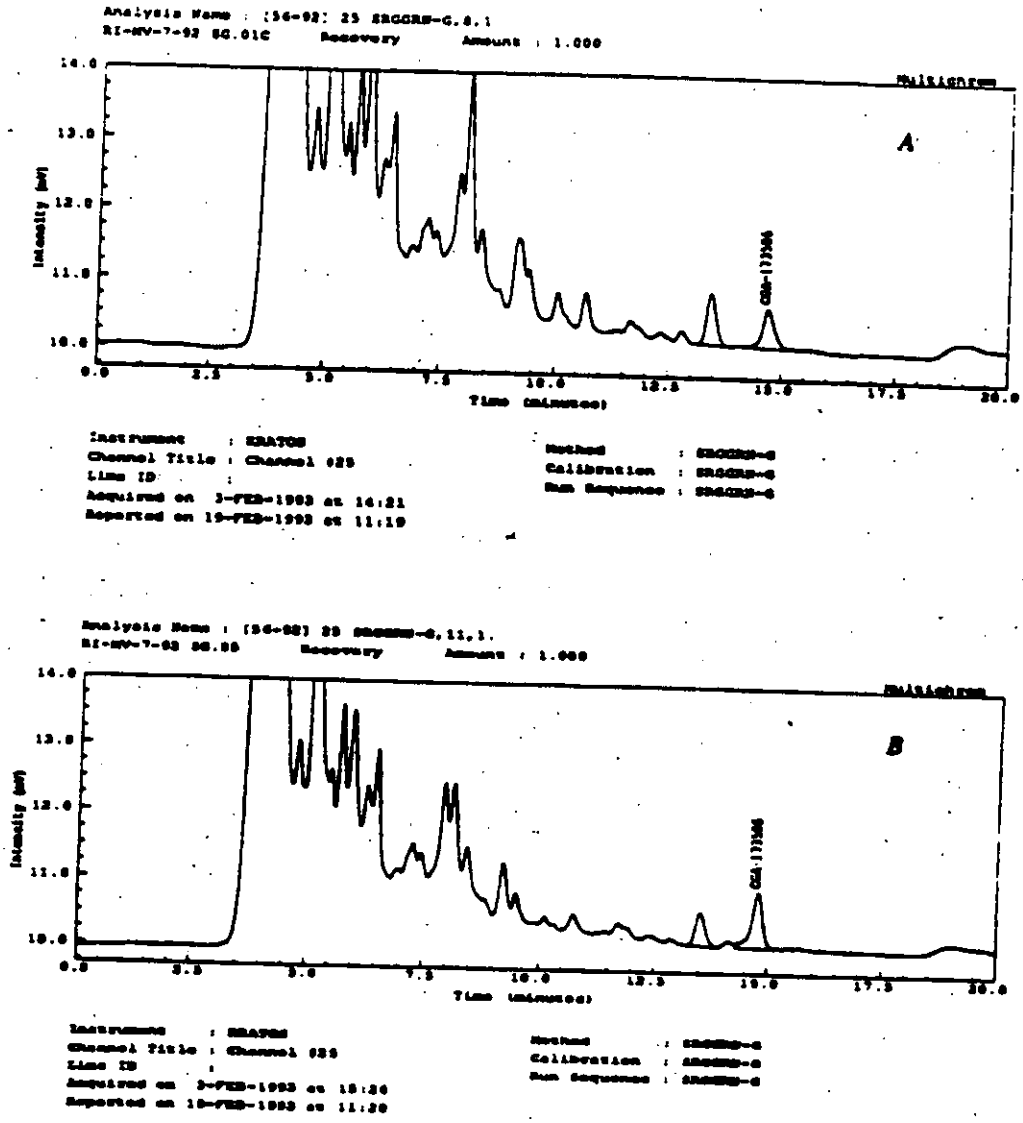


A. Control; 99.5 mg injected, 0.76 ng found, <0.01 ppm (0.008 ppm).

B. Control + 0.01 ppm CGA-173506; 99.5 mg injected, 1.0 ng found, 0.011 ppm, 29% recovery.

(Recovery value corrected for control value.)

FIGURE 15. REPRESENTATIVE CHROMATOGRAMS FOR CONTROL AND FORTIFIED CONTROL SORGHUM GRAIN SAMPLES AT THE 0.01 PPM & 0.05 PPM LEVEL (Continued)



A. Control + 0.01 ppm CGA-173506, 99.5 mg injected, 0.3 ng found, 0.03 ppm, 228% recovery.

B. Control + 0.05 ppm CGA-173506; 99.5 mg injected, 4.7 ng found, 0.047 ppm, 79% recovery.

(Recovery value corrected for control value.)

VII. REFERENCES

1. Fleischmann, T. J., Ciba-Geigy Metabolism Department Protocol Number 214-89, Uptake and Metabolism of ^{14}C-CGA-173506 in Greenhouse Grown Rice.

2. Wurz, R.E.M., Ciba-Geigy Residue Chemistry Department Protocol Number 56-92, Validation of "Draft" Analytical Method AG-597, Analytical Method for the Determination of CGA-173506 in Crops by High Performance Liquid Chromatography Including Validation Data.

3. Slatner, J. W., Residue Test Report, RI-MV-007-92.