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VOLUME 60 OF 65 OF SUBMISSION

CGA-245704

TITLE

ANALYTICAL METHOD FOR THE DETERMINATION OF TOTAL RESIDUES OF CGA-245704 AS CGA-210007 IN TOBACCO, LEAFY VEGETABLES, AND FRUITING VEGETABLES BY COLUMN SWITCHING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

DATA REQUIREMENT

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PERFORMING LABORATORY

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VOLUME 1 OF 1 OF STUDY

STATEMENT OF NO DATA CONFIDENTIALITY CLAIM

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

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STATEMENT CONCERNING GOOD LABORATORY PRACTICES

The document in this submission, is an analytical method and is not considered a final report because validation of the method is not included Therefore, certification of compliance with Environmental Protection Agency's Good Laboratory Practice Standards (40 CFR Part 160, October 16, 1989) is not required

Robert K. Williams
Agent of Submitter/Sponsor
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3/27/98
Date

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Novartis Crop Protection, Inc
Human Safety Department

Analytical Method for the Determination of Total Residues of CGA-245704 as CGA-210007 in Tobacco, Leafy Vegetables, and Fruiting Vegetables by Column Switching High Performance Liquid Chromatography

Analytical Method No. 671A

Project Number 436001

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I. SUMMARY AND INTRODUCTION

A. Scope

This method is used for the determination of total residues of CGA-245704 as CGA-210007 in tobacco, leafy vegetables, and fruiting vegetables (including processed fractions). The limit of detection (LOD), as determined by the smallest standard concentration injected, is 0 35 ng for CGA-210007 The limit of quantitation (LOQ), as demonstrated by the smallest acceptable fortification level used in the study, is 0 02 ppm CGA-245704 equivalents for all substrates. The chemical names and structures for CGA-245704 and CGA-210007 are presented in Figure 1.

Analytical Method AG-671 was a re-issue of REM 172.02[1] and REM 172 11[2] to incorporate additional substrates, update formatting, lower the LOQ, include significant purification, procedural, and instrument modifications, and to report procedural and radiovalidation data. **Analytical Method AG-671A is a re-issue of AG-671 to incorporate instrumental conditions and supporting data for a confirmatory procedure utilizing HPLC with mass spectrometric detection (LC/MS).** Analytical Method AG-671A supercedes Analytical Method AG-671, all method references herein will be cited as Analytical Method AG-671A.

B. Principle

CGA-245704 and metabolites containing the CGA-210007 moiety are hydrolyzed to CGA-210007 by heating a representative crop sample in the presence of sodium hydroxide. Methanol is added and the mixture shaken. The extract is purified on Chem Elut and C_{18} columns followed by an acidic liquid-liquid partition. Additional purification and final determination are performed using column switching high performance liquid chromatography (HPLC) with UV-detection or conformational HPLC analyses with mass spectrometric detection (LC/MS). A flow diagram for the method is presented in Figure 2.

II. MATERIALS AND METHODS

Below is a compilation of the materials needed to run this method. Standard laboratory equipment is not listed.

A. Apparatus

1.0 Lab Shaker, IKA Labortechnik KS501 Digital, IKA Works, Cat. #2526401, or equivalent

2.0 Lab Oven, Fisher Scientific, Model #506G, Cat. #13-246-506G, or equivalent

3.0 Coors Porcelain with Fixed Perforated Plate (Büchner funnel), Fisher Scientific, Cat. #10-356C, or equivalent

4.0 Filter Flask, Pyrex Brand with Tubulation, Fisher Scientific, Cat. #10-180E, or equivalent

5.0 Glass Microfibre Filters, circles 70 mm, Fisher Scientific, Cat. #1827 070, or equivalent

6.0 Disposable Pasture pipettes with 2-mL bulb

7.0 Graduated Cylinders, 250, 100, 50, 20 mL

8.0 Kontes Recovery Flasks with outer joint, 1000 mL (teardrop flasks), Fisher Scientific, Cat. #K608675-4724, or equivalent

9.0 Volumetric pipettes, 0.5-10 mL

10.0 Graduated pipettes, 0 5-10 mL

11.0 Beakers, 100 mL

12.0 Rotary evaporator, Büchi, Fisher Scientific, Cat. #09-548-105F, or equivalent

13.0 C_{18} Mega Bond Elute SPE column, 5 g/20 cc, Varian, Cat. #1225-6023

14.0 Vacuum Manifold, Waters, Cat. #WAT054760, or equivalent

15.0 Aspirator Pump, Cole-Parmer Instrument Company, Cat. #H07049-02, or equivalent

16.0 Chem Elut 20-mL capacity, Varian, Cat. #12198008

17.0 Erlenmeyer Flasks 250, 125 mL

18.0 Separatory Funnels, 500, 250, 125 mL with glass stopcocks

19.0 Round Bottom Flask, 250 mL

20.0 Pyrex Brand Crucible Holders (carbon filter tubes), Fisher Scientific, Cat. #08-261A, or equivalent

21.0 Sanitary Cotton, Fisher Scientific, Cat #07900, used as a filter, inserted into the neck of a crucible holder, approximately 1" x 1" x 1" in size.

22.0 Vortex Maxi Mix Plus, Fisher Scientific, Cat. #12-815-18, or equivalent

23.0 Vials for LC injection, Wheaton 2-mL glass, or equivalent

24.0 pH Stick, Haake-Büchler, Fisher Scientific, Cat. #13-643-50, or equivalent

25.0 Ultrasonic Cleaner, Cole-Parmer Instrument Company, Cat. #H08895-05, or equivalent

26.0 Rotary Evaporator Trap with inner vapor tube at bottom, Kontes Cat. #K570200-0124, 100 mL, 24/40

B. Reagents

1.0 Sodium Hydroxide (NaOH) 1N Volumetric Solution, reagent grade, J.T. Baker, Cat. #5635-02, or equivalent

2.0 Methanol (MeOH), HPLC grade, Fisher Scientific, Cat. #A452-4, or equivalent

3.0 HPLC-Grade Water either purified in house with a HYDRO™ purification system or obtained from manufacturer

4.0 Sodium Chloride (NaCl), Certified ACS grade, Fisher Scientific Cat #S-421-3, or equivalent

5.0 Saturated Sodium Chloride (Sat. NaCl), add sodium chloride to reagent-grade water until maximum solubility is obtained

6.0 Hydrochloric Acid (HCl) concentrated, reagent grade, Fisher Scientific Cat. #A144-212, or equivalent

7.0 1M HCl, carefully mix 82.5 mL of conc. HCL with reagent-grade water and bring to a 1-liter final volume

8.0 Phosphoric Acid (H_3PO_4) 85%, Certified ACS grade, Fisher Scientific, Cat. #A242, or equivalent

9.0 0.02M H_3PO_4, mix 2.31 g of H_3PO_4 with reagent-grade water and bring to a 1-liter final volume

10.0 Dichloromethane (DCM), HPLC grade, Fisher Scientific, Cat. #D143-4, or equivalent

11.0 Ethyl Acetate (EtOAc), HPLC grade, Fisher Scientific, Cat. #E-195, or equivalent

12.0 9:1 DCM/EtOAc (v/v), combine 3600 mL of DCM with 400 mL of EtOAc

13.0 Acetonitrile (ACN), HPLC grade, Fisher Scientific, Cat. 4998-4, or equivalent

14.0 Mobile Phase: 80.20 0.02M H_3PO_4/ACN (v/v), mix 14.76 g of H_3PO_4 (85%) with reagent-grade water and bring to a volume of 6400 mL. Solution pH range should fall between 2.8-3.0. Combine the 0.02M H_3PO_4 solution with 1600 mL of ACN for a total volume of 8.0 liters. Degas with helium purge.

15.0 Mobile Phase: 75:25 0.02M H_3PO_4/ACN (v/v), mix 13 84 g of H_3PO_4 (85%) with reagent-grade water and bring to a volume of 6000 mL Solution pH range should fall between 2 8-3.0. Combine the 0.02M H_3PO_4 solution with 2000 mL of ACN for a total volume of 8 liters. Degas with helium purge.

16 0 CGA-245704 Analytical Standard, Novartis Crop Protection, Inc., P O. Box 18300, Greensboro, NC 27419

17.0 CGA-210007 Analytical Standard, Novartis Crop Protection, Inc , P. O. Box 18300, Greensboro, NC 27419

C. Analytical Procedure

1 0 Sample Preparation

Samples are received and stored frozen at -20°C. Samples are prepared under the general guidelines of the U S. Food and Drug Administration Pesticide Analytical Manual Volume I, Section 141 and Novartis SOP 7.21 Revision 2[3] (or the current revision).

2.0 Extraction

2.1 Weigh a 4-g subsample from a well-homogenized chopped or ground crop sample into a 250-mL Erlenmeyer flask Fortify samples with CGA-245704 if needed (see Section II.I.3 0 for fortification details).

2.2 Add 36 mL of distilled water and 4 mL of 1N sodium hydroxide to the substrate (the pH should be ≥12, however, it should not be necessary to check this with every sample). Stopper the flask, swirl the mixture, and allow it to shake (mechanical shaker) at high speed for approximately 10 minutes Place the stoppered flask in a preheated oven

(65-70°C) for 30 minutes. Swirl the flask approximately every 10 minutes.

2.3 Allow the mixture to cool to room temperature (this step can be expedited by use of a cold tapwater bath), and add 80 mL of methanol to the flask. Seal the flask and shake (mecnanical shaker) the sample at high speed for 30 minutes. Filter under vacuum by carefully pouring the sample through a glass fiber filter paper inserted into a Büchner funnel assembly. After filtering, rinse the sampling flask with approximately 3 x 20 mL of methanol. Use each rinse to wash the filter cake; stir and compress the filter cake during this rinsing to ensure quantitative transfer.

2.4 Transfer the filtrate to a 1000-mL teardrop flask and remove the methanol, rotating slowly to avoid bumping, with a water aspirated rotary evaporator while maintaining the water bath temperature at approximately 30°C Reduce the sample volume to approximately 50 mL and then increase rotation to full speed until approximately 8-10 mL of sample remains.

3.0 Partition Cleanup

3.1 Chem Elut Column: Add 8 mL of 1M HCl to the sample from Section II.C.2.4, swirl, and load onto a Chem Elut Column. Wait approximately 10-20 minutes for sample absorption, rinse the 1000-mL teardrop flask with 100 mL 9:1 dichloromethane/ethyl acetate, elute the column with the rinse from the flask, and collect into a 250-mL Erlenmeyer flask. (A small amount of pressure may be applied to the top of the Chem Elut column to efficiently drain the column, if necessary.) Transfer the eluant to a 500-mL

separatory funnel. Rinse the 250-mL Erlenmeyer flask with 10 mL of 9:1 dichloromethane/ethyl acetate and add the rinse to the 500-mL separatory funnel.

3.2 Alkaline Partition: Add 20 mL of 1N sodium hydroxide to the column eluant, stopper the separatory funnel, and shake **gently** to minimize emulsions (use frequent venting) Allow 10-15 minutes for the phases to separate **completely**. (See Section II.H.1.0, Modifications and Potential Problems, if emulsions develop from difficult substrates, i.e. cured tobacco.) Drain off the lower, organic layer and discard appropriately

3 3 C_{18} Solid Phase Extraction. Attach the C_{18} Mega Bond Elut column onto a vacuum manifold. Precondition the cartridge by rinsing with 15 mL of methanol, followed by 20 mL of water, and discard appropriately. **Do not let column bed go dry at anytime.** Load the upper, aqueous fraction from the alkaline partition (Section II.C.3.2) and elute quickly through the cartridge under maximum vacuum collecting the filtrate in a 100-mL beaker. Rinse the cartridge with 50 mL of water and collect the rinse in the same 100-mL beaker. (See Section II.H.2.0, Modifications and Potential Problems.)

3.4 Acidic Partition: Transfer all of the filtrate from the beaker back into the 500-mL separatory funnel used for the alkaline partitioning (Section II.C.3.2). Rinse the beaker with 40 mL of saturated sodium chloride solution, and then add the rinse to the separatory funnel. Add 4 mL of phosphoric acid (85%) to the funnel, and shake well (use frequent venting) to mix thoroughly.

3.4.1 Partition the solution with 50 mL of ethyl acetate and wait approximately 10 minutes to allow the phases to separate completely. Drain the lower, aqueous layer into a 125-mL Erlenmeyer flask. Drain the upper, organic layer and any emulsion into a 125-mL separatory funnel. Transfer the aqueous layer back to the 500-mL separatory funnel. Rinse the 125-mL Erlenmeyer flask with 50 mL of ethyl acetate and add the rinse to the 500-mL separatory funnel containing the aqueous layer. Partition and allow the layers to separate, and then drain off the lower, aqueous layer and discard appropriately. Combine the upper, organic layer and any emulsion or residual aqueous material with the organic fraction in the 125-mL separatory funnel. **(Drain and discard appropriately any aqueous layer that may have settled from the combined organic layers upon sitting.)**

3.4.2 Insert a small amount of cotton (1" x 1" x 1") into the stem of a crucible holder (carbon filter tube). Filter the organic layer through the cotton and collect the filtrate in a 250-mL round bottom flask. Rinse the crucible holder with 10 mL of ethyl acetate draining it through the cotton filter

4.0 Analyte Concentration

4.1 Evaporate the contents of the round bottom flask from Section II.C.3.4.2

to **complete** dryness using a rotary evaporator **(equipped with a trap adapter)**, rotating slowly at room temperature (~25°C). Add the appropriate final volume of 0.02 M H_3PO_4 to the sample and vortex or sonicate with an ultrasonic cleaner to ensure dissolution of the analyte.

4.2 Transfer an aliquot of the final fraction to an injection vial for HPLC analysis. Analyze the samples according to Section II.D., Instrumentation or II.F., Confirmatory Technique

D. Instrumentation

1.0 Description and Operating Conditions

An aliquot of the final sample fraction from Section II.C.4.2 is analyzed by column switching high performance liquid chromatography for residues of CGA-210007. The instrumental conditions are described in Table I and a diagram of the switching valve setup is presented in Figure 3 Control of the switching valve is accomplished via time-programmed contact closures of the detector, autoinjector, or other timing source

1.1 Determine the retention time of **CGA-210007 on column 1** (Spherisorb SAX/SB) by connecting the outlet of column 1 directly to the detector (by passing the switching valve) and injecting 25 ng of the analyte. (Inject 50 µl of the 0.5 ng/µl CGA-210007 standard solution prepared in Section II.I.1.1.). Record the starting and ending retention times of the eluting CGA-210007 analyte. Repeat the cuttime determination until the starting and ending retention times are consistent between analyses. The cuttime (ending retention time - starting

retention time) should be ≤ 1 minute if possible.

1.2 Connect the HPLC system as shown in Figure 3 for **column switching** Program the valve to switch to the INJECT position at the beginning of the CGA-210007 analyte peak and return to the LOAD position at the end of the analyte peak, as determined in Section II.D.1.1

1.3 Inject 25 ng of CGA-210007 to determine its retention time through the two columns and to confirm that the valve time programming is working properly.

2.0 Calibration and Standardization

2.1 The HPLC system is calibrated with each analytical run by checking the cuttime and comparing it to the retention time and detector response relative to previous runs. Retention times should not vary by more than 5% and detector response should not vary more than 10% between runs. Otherwise the system should be inspected and proper maintenance or recalibration should be performed to minimize this variation. (See Section II.H.4.0, Modifications and Potential Problems.)

2.2 The HPLC system is standardized by injecting 50-µL aliquots of the standard solutions of CGA-210007 in a working range of 0.35-25 ng/injection during residue analyses. A linear regression function is generated from the data comparing detector response and ng injected. Representative standardization data and chromatograms are presented in Figures 4. The corresponding calibration curve is presented in Figure 5.

E. Interferences

1.0 Analysis of control samples from the tobacco, leafy vegetable, and fruiting vegetable crop groups showed no significant (>0.01 ppm) interferences at the analyte retention time with a limit of quantitation of 0.02 ppm No interferences have been observed in reagent blanks.

F. Confirmatory Technique

A confirmatory procedure for the detection and quantitation of CGA-245704 (as CGA-210007) has been developed that employs HPLC with mass spectrometric detection (LC/MS), in compliance with the confirmatory method requirement of US EPA Residue Chemistry Guidelines, OPPTS 860.1340, "Residue Analytical Methods." This technique utilizes the same extracts produced by the primary method, and therefore can be used in the event of chromatographic or other interferences with the HPLC/UV technique. The LC/MS technique can confirm detections of CGA-210007 to levels of <0.05 ppm in tobacco, and <0.02 ppm in both lettuce and tomatoes (fruit, wet pomace, and paste).

A validation of this technique was performed by analyzing identical sample sets on both detection systems simultaneously. In addition to calibration standards, a typical validation set consisted of one untreated control, two or more fortified controls, and two or more field samples containing incurred residues of CGA-245704*.

After analysis by negative ion electrospray LC/MS (ES-), the peak area measurements of the 179 m/z ion ($m-H^-$) were transcribed into the "Manual Data Entry" mode of the Multichrom Worksheet program for complete calculation of residues in the crop substrates.

Comparative results of the LC/MS detection technique versus HPLC/UV detection in the tobacco, lettuce, and tomato substrates are presented in Table X.

* Samples of flue-cured tobacco were obtained from an in-progress storage stability study and were fortified with CGA-210007 rather than CGA-245704

Representative chromatograms of CGA-210007 in flue-dried tobacco, head lettuce, unwashed tomatoes, wet pomace, and tomato paste are presented in Figures 10-14, respectively. Each set of representative chromatograms includes a calibration standard, an untreated control, a fortified control (procedural recovery), and a treated sample containing field-incurred residues.

Complete HPLC and mass spectrometric conditions are presented in Table XI.

G. Time Required For Analysis

The extraction and cleanup of a set of 6 samples may be completed by a skilled analyst in approximately 6-8 hours. Automated injection of the samples can be performed overnight

H. Modifications and Potential Problems

1.0 Any difficult emulsions formed during the sample work up can be minimized by swirling the emulsion with a glass stirring rod and allowing time for complete separation. If the emulsion is persistent (i.e. Section II.C.3 2), the complete sample should be transferred to a 250-ml polyurethane centrifuge bottle. Cap the bottle and proceed with centrifuging of sample(s) by slowly ramping to approximately 9,000 rpm for approximately 10 minutes and then slowly reduce speed Decant the sample back into the separatory funnel and rinse the centrifuge bottle with an appropriate solvent; add the rinse to the separatory funnel containing the sample. Keep any emulsions with the analyte fraction until the final separation is conducted.

2.0 Use of the C_{18} Mega Bond Elut column was necessary to minimize persistent emulsions encountered in substrates. The procedure is intended to remove unwanted nonpolar and basic components The elution should be

conducted at maximum vacuum to minimize interaction of the ionized analyte with the SPE stationary phase.

3.0 A previous report[2] noted that CGA-245704 is very unstable in acetonitrile solutions stored in light at room temperature. Therefore, the CGA-245704 standard solutions were stored in amber bottles during the validation of this method. The a.i of a stock solution containing 100 µg/mL of CGA-245704 in acetonitrile (stored in the dark at -20°C), should be stable for at least 6 months

4.0 If minimal variability is noted in retention time and detector response between daily cuttime calibrations, then it is not necessary to calibrate on a daily basis. However if this condition changes, then daily calibrations should be begun until stability is achieved.

5.0 If an interference peak is present at the retention time of the CGA-210007 analyte during method validation or field trial sample analyses and this peak prevents accurate quantitation of CGA-210007, then reduce the starting and ending "cuttime" in portions of about 15 seconds each. Proceed until the interference disappears or is reduced to an insignificant height If this does not remove the interference or analyses are being done for tolerance enforcement requirements, then proceed with quantitation by use of the confirmatory method.

6.0 The mobile phases for the HPLC systems if prepared in large quantity, can be recycled during the analyses. However, if problems develop such as increased baseline noise, control interference peak, or shifting retention times, this practice should be discontinued and new mobile phase prepared. The mobile phases should not be recycled during the determination of the analyte cuttime on column 1, as this would result in cross-contamination of the mobile phases.

7.0 LC/MS: Decreased procedural recoveries were observed when a sample injection volume of 100 µL was used during method development. It is suspected that ionization of CGA-210007 is suppressed when injecting more than 50 µL of a crop substrate matrix. The use of a column effluent splitter installed between the analytical HPLC column and the MS detector, with a split ratio optimized for the size of the sample injection, is recommended. Refer to Table XI for a description of the split ratio and injection volume used in this study.

8.0 LC/MS Since this method monitors the CGA-210007 [m-H] ion at 179 m/z, acetic acid (60 m/z) should not be used as a component of the LC/MS mobile phase, as it creates a spectral interference by reassociating at 179 m/z, representing the acetic acid [m-H]+2m negative ion.

I. Preparation of Standard Solutions and Sample Fortification Procedures

1.0 Preparation of Analytical Standard

Weigh 10 mg of CGA-210007 into a 100-mL volumetric flask, and dilute to the 100-mL mark with acetonitrile. The concentration of this stock solution is 100 ng/µL. Store the stock injection solution in the refrigerator when not in use.

1.1 Make serial dilutions of the 100 ng/µL stock solution with 0.02M H_3PO_4 until working solutions containing 0.007 ng/µL to 0.50 ng/µL are obtained. Store these injection standards in the refrigerator when not in use.

2.0 Preparation of Fortification Standard

Weigh 10 mg of CGA-245704 analytical standard into a 100-mL volumetric flask and dilute to the 100-mL mark with acetonitrile. The concentration of this

stock solution is 100 ng/µL. Store the stock fortification standard in the freezer when not in use.

2.1 Make serial dilutions of the 100 µg/mL standard solution with acetonitrile to give a series of fortification standards in a range of 0.02 ng/µL to 1.0 ng/µL of CGA-245704. Store these fortification standards in the refrigerator when not in use.

3.0 Sample Fortification Procedures

The method is validated for each set of samples analyzed by including an untreated control sample and one or more control samples fortified prior to extraction with CGA-245704.

3.1 Add between 0.50 mL to 2.0 mL of the appropriate standard solution of CGA-245704 to 4 g of the crop substrate prior to the addition of the hydrolysis solution (Section II.C 2.1) Allow the sample to sit for a few minutes before adding the aqueous NaOH required for hydrolysis. If a higher concentration spike is desired, adjust the concentration of the fortification solution so that no more than 2 mL of solution is added to the substrate.

3.2 Analyze the fortified samples (procedural recoveries) through the same method procedures as for the treated samples.

3.3 See Section II.J.2.0 for the procedure to calculate the ppm of CGA-245704 in the samples.

C. Methods of Calculations

1.0 Determination of Sample Residues
(Tolerance Enforcement Data)

Inject 50-µL aliquots of the final fractions prepared in Section II.C.4.2 into the HPLC under the same conditions as the standards. Make appropriate dilutions of the samples (if necessary) with 0.02M H_3PO_4 to bring the sample peak heights within the range of the standard curve. Compare the peak heights observed in the treated (unknown analyte quantity) samples to the standard curve or enter the peak height into the least squares program to determine the nanograms of CGA-210007 in the injected aliquot. Representative chromatograms for reagent blanks, control, and procedural recovery crop samples are shown in Figures 4-9.

To calculate the residue results, the mg injected must first be calculated according to Equation 1:

$$\text{(1) Sample weight injected (mg)} = \frac{(G)\,(V_a)\,(V_i)}{(V_f)[V_e + G(M/100)]} \times CF_1 \times CF_2$$

G = sample weight extracted (g)
V_a = aliquot volume (mL)
V_i = injection volume (µL)
V_f = volume of final fraction (mL)
V_e = extraction volume (mL)
M = % moisture of sample
CF_1 = conversion factor to convert grams of sample to milligrams (CF_1 = 1000/1)
CF_2 = conversion factor to convert microliters to milliliters (CF_2 = 1/1000)

In this method, the total extraction aliquot is used for analyses therefore, $V_a = [V_e + G(M/100)]$, and these values cancel out of Equation 1.

Determine the concentration (ppm) of the analyte in residue samples (tolerance enforcement) according to Equation 2

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

To convert the ppm found for CGA-210007 to parent CGA-245704 equivalents, multiply the results from Equation 2 by 1.17 This ratio (F) is determined from the molecular weights as defined below.

$$(3)\ \text{Ratio of molecular weights (F)} = \frac{\text{MW parent (210.28 g/mol)}}{\text{MW of the (180.18 g/mol) CGA-210007 metabolite}} = 1.17$$

2.0 Determination of Procedural Recoveries (**Method Validation and Residue Field Trial Data**)

2.1 Calculate the ng of CGA-210007 and mg sample weight injected using the procedures described in Section II.J.1.0. Calculate the final ppm values of the control and fortified samples according to Equation 2 (Section II.J.1.0).

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 Equation 4:

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

Convert the ppm found of CGA-210007 to parent CGA-245704 equivalents by multiplying by F, the ratio of the molecular weights

3.0 Determination of Corrected Sample Residues (**Method Validation and Residue Field Trial Data**)

3.1 Determine the ng of CGA-210007 injected and calculate the mg injected using the procedures described in Section II.J 1.0.

Calculate the final ppm values of the residue samples according to Equation 2 (Section II.J 1 0). Calculate the ppm found of CGA-210007 to parent CGA-245705 equivalents by multiplying by F, the ratio of the molecular weights. Calculate the method recovery according to Equation 4 (Section II.J 2.1). Use the recovery, or mean recovery if two or more fortified samples are analyzed, to correct the concentration (ppm) of each analyte for the treated sample according to the following equation.

(5) Corrected Residue (ppm) = $\frac{\text{ng analyte found}}{\text{mg sample}} \times \frac{1}{R}$

Note: Recovery adjustments for controls, recovery corrections for small control values ($\leq$0.02 ppm), and recoveries for residue concentrations are not used in tolerance enforcement calculations. The recovery correction is used to correct the amount of analyte found in the fortified (spiked) samples. The recovery correction is utilized for method validation. and for the correction of residue data.

III. RESULTS AND DISCUSSION

Analytical Method AG-671A was evaluated for accuracy, precision, extractability, and accountability by analyses of residue field trial samples and metabolism radiolabeled samples. A comprehensive report of these details is presented in ABR-97117, Final Report Amendment Number 1[4] A summary of the data and conclusions are outlined below.

A. Accuracy

Untreated tobacco, leafy vegetable and fruiting vegetable (including tomato processing fractions) crop group substrates were fortified with

CGA-245704 at levels ranging from 0.02-20 ppm Recovery results from fortified control samples (Table VI) were used to calculate accuracy in terms of a mean, standard deviation (sd), and coefficient of variation (CV(%) = sd/mean x 100). Procedural recovery data from fortified control field trial samples, along with procedural recovery data from validation sets analyzed herein (Table II), were compiled to assess the overall accuracy of Analytical Method AG-671A.

Procedural recoveries were calculated by dividing the concentration of CGA-245704 measured in each fortified sample by the concentration added to the sample Representative Multichrom analysis sheets (spreadsheets) showing data for all of the intermediate calculations are presented in Tables III-V. Substrate, fortification levels, and residues in the fortified controls are presented in Table VI and summarized below.

Commodity	CGA-245704 Mean Recovered (%)	CGA-245704 Mean Standard Deviation
Tobacco (Green, Uncured)	84	9.1 (n=17)
Tobacco (Cured)	88	11 (n= 7)
Head Lettuce	94	10 (n=14)
Leaf Lettuce	90	9.3 (n=14)
Spinach	89	8.5 (n=15)
Celery	90	9.2 (n=17)
Tomato	87	8.1 (n=15)
Tomato Processing	100	12 (n=25)
Bell Peppers	84	4.7 (n= 9)
Hot Peppers	96	12 (n= 9)

No significant interferences (>0.01 ppm) were found at or near the retention time of CGA-210007 in the reagent blanks (Figure 6) or the control samples. An interference (>0.01 ppm) was present at the retention time of CGA-210007 in the tomato processing puree (0.01 ppm) and paste (0.02 ppm) sample sets. This apparent contamination was detection in the tomato fruit of one of the field trials (02-FR-030-96) and increased in magnitude with concentration of the processing fractions Subsequent verification of the tomato puree residue by liquid chromatography/mass spectroscopy indicates the presence of a CGA-210007 contaminate in the control sample. Results from an additional field trial (0W-FR-412-96) showed no indication of any significant (>0.01 ppm) residue or interference in the tomato processing control samples. HPLC

chromatograms for the ^{14}C-tobacco, ^{14}C-lettuce and ^{14}C-tomato samples (control, fortified, and radiolabeled samples) are shown in Figures 7-9. Representative chromatograms of fortified field trial and processing (control and procedural recovery) samples are presented in ABR-97117, Final Report Amendment Number 1.

These acceptable procedural recovery results confirm that the analytical procedure outlined in Analytical Method AG-671A is adequate for recovery of residues and is satisfactory for use as a tolerance enforcement method.

B. Precision

Precision of the method was determined by calculating the mean, standard deviation, and coefficient of variation of results from the analysis of fortified control (Table VI) and ^{14}C-incurred samples. Table VII contains the statistical results for recovery values from fortification experiments. Table VIII contains the statistical results for the triplicate analyses of ^{14}C-incurred tobacco, lettuce, and tomato samples The precision of Analytical Method AG-671A is demonstrated by its CV values and by the close agreement of triplicate sample results.

C. Extractability

The extractability of the TRR by Analytical Method AG-671A was determined by comparing the total ppm of ^{14}C-residue in the sample from combustion analysis to the ppm ^{14}C-residue in the initial sample extract (Section II.C 2.2 3) of the method. The formula for the determination of percent extractability is:

$$\% \text{ extractability} = \frac{\text{Total } ^{14}\text{C-residue (ppm) in sample extract by LSC}}{\text{TRR (ppm)}} \times 100$$

Table VIII summarizes the TRR values, concentration of ^{14}C-residue in sample extracts, and the total extractability. The TRR values were determined by combustion analysis in the metabolism studies and are reported in ^{14}C-CGA-245704 equivalents. The concentration of the ^{14}C-extractable residue was calculated by LSC analyses of the extracts and

reported in ppm ^{14}C-CGA-245704 equivalents The total extractabilities were 108%, 87%, and 99% for the ^{14}C-tobacco, ^{14}C-lettuce, and ^{14}C-tomato, respectively. These extractability results are consistent with quantitation by 2-dimensional thin layer chromatography used in the metabolism studies The validation extractability results are compared with the metabolism extractability results in Table IX.

D. Accountability

The accountability of Analytical Method AG-671A is determined by comparing the total ppm of ^{14}C-residue found in the sample by combustion analysis to the ppm of CGA-245704 equivalent residue found in the final extract by HPLC analysis. The formula for the determination of percent accountability is:

$$\% \text{ accountability} = \frac{\text{Total residue (ppm) in sample extract determined by HPLC analysis}}{\text{TRR (ppm)}} \times 100$$

The total mean accountabilities were 78%, 33%, and 71% for the ^{14}C-tobacco, ^{14}C-lettuce, and ^{14}C-tomato, respectively. These accountability results are consistent with the 2-dimensional thin layer chromatography results obtained in the respective metabolism studies after hydrolysis of the sample to free CGA-210007. In Analytical Method AG-671A, the hydrolysis step is based on treatment of the sample with 1N NaOH/heat and would most likely result in a greater conversion to total CGA-210007[5-7] than the metabolism 0.1N NaOH/room temperature treatment. The accountability results for lettuce were less than the results obtained in the metabolism study after quantifying by 2-dimensional thin layer chromatography The metabolism accountability for lettuce was based on the surface rinse radioactivity and the lettuce head penetrated radioactivity. The hydrolysis conditions for conversion of penetrated radioactivity were conducted in 0.1N NaOH (room temperature) and cellulase. The accountability results reported herein, were based on an unwashed lettuce sample. The difference in magnitude of the lettuce accountability results can be attributed to

the different metabolism profile for lettuce. The validation accountability results are compared with the metabolism accountability results in Table IX.

IV. CONCLUSION

Novartis Analytical Method AG-671A is shown to be a valid method for the determination and confirmation of CGA-245704 in RAC and processing substrates from the tobacco, leafy and fruiting vegetable crop groups. The validity is demonstrated by the acceptable accuracy and precision obtained on numerous procedural recovery samples (radiovalidation and field trial sample sets), and by the extractability and accountability obtained by the analysis of weathered radioactive substrates using Analytical Method AG-671A. Validation results are reported in ABR-97117, Final Report Amendment Number 1[4].

V. CERTIFICATION

The reports and experimental results included in this study, Laboratory Project I.D. Analytical Method AG-671A, are certified to be authentic accounts of the experiments.

Carol G Hayworth 3/25/98

Carol G Hayworth, B.S. Date
Study Director
Scientist I, Residue Chemistry
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(336-632-6000)

VI. TABLES AND FIGURES

TABLE I LIQUID CHROMATOGRAPHIC OPERATING CONDITIONS FOR ANALYSIS OF CGA-210007

Instrument Conditions[1,2]	
Column 1/Typical Retention Time	Phenomenex Spherisorb™ SAX/S3, 150 x 3.2 mm I.D , 5 µm, HPLC Column Cat. #00F-0110-R0 Retention Time ~7 min.
Mobile Phase 1/Flow Rate	80 20 0.02M H_3PO_4/ACN Flowrate 0.30 mL/min
Column 2	YMC-Pack C8 250 x 4.6 mm I.D., S-5um, 120A, HPLC Column, Cat #OC12S05-2546WT A-203-5
Mobile Phase 2/Flow Rate	75:25 0.02M H_3PO_4/ACN Flowrate 1.0 mL/min
Typical Retention Time Through Column 1 & Column 2.	Retention Time ~22 min
Column Oven/Temperature:	Eppendorf column heater CH-30 Cat. #4301-000-028 and gauge TC-50 Cat. #4300-000-008 or equivalent Set at 30 °C
Detection/UV Wavelength	Perkin Elmer LC-95 UV/Visible Spectrophotometer Detector, or equivalent Wavelength, 235 nm
Pumps(2):	Perkin Elmer Isocratic LC Pump Model 250, or equivalent
Column Switching Valve:	VICI EQ60 HPLC Switching Valve, or equivalent
Auto Injector·	Perkin Elmer Model ISS 100, or equivalent
Volume Injected	50 µL
Data Acquisition.	MicroVAXII (Q) Operating System, VMS Version 5.3-1 Application Software VG Multichrom Version 2 0 Worksheet Version. 1.6.0, or equivalent

[1] HPLC columns should be converted from the storage solvent to the appropriate mobile phase according to the manufacturer's instructions HPLC columns should be equilibrated until the retention time of the standard (CGA-210007) is stabilized

[2] Retention times listed are approximate and will vary depending on instrument setup

TABLE II. SOURCE OF ACCURACY AND PRECISION DATA

Project #	Study #	Commodity	Field Trial # or Analytical Set Name
436001	491-97	^{14}C-Tobacco	Reference 5
436001	491 97	^{14}C-Lettuce	Reference 6
436001	491 97	^{14}C-Tomato	Reference 7
436001	91-96	Tobacco	0S-FR 607 96
436001	91-96	Reagent Blank	91 96 BBR
436001	91 96	Tobacco	NE FR 211 96
436001	91-96	Tobacco	NE FR 212-96
436003	92 96	Head Lettuce	02 FR-028 96
436003	92 96	Reagent Blank	92 96-BBG
436003	92 96	Head Lettuce	0S FR-008 96
436003	92 96	Head Lettuce	0W-FR 533-96
436003	92 96	Head Lettuce	0W-FR 534 96
436003	92 96	Head Lettuce	0W FR 535-96
436003	92-96	Head Lettuce	FL-FR-402-96
436003	92 96	Leaf Lettuce	02-FR-027 96
436003	92-96	Reagent Blank	92-96-BAO
436003	92-96	Leaf Lettuce	05-FR-010-96
436003	92-96	Leaf Lettuce	07-FR-006-96
436003	92-96	Leaf Lettuce	0W FR-536-96
436003	92 96	Leal Lettuce	0W FR 537-96
436003	92 96	Leal Lettuce	0W FR 538 96
436003	92-96	Spinach	02 FR-028 96
436003	92 96	Reagent Blank	92 96 BAG2
436003	92 96	Spinach	0S FR 318 96
436003	92 96	Spinach	0W FR 543 96
436003	02 96	Spinach	MW FR-317 96
436003	92 96	Spinach	NE-FR 304 96
436003	92 96	Spinach	NE FR 501 96
436003	92-96	Celery	0W FR 539-96
436003	92 96	Celery	0W FR 540-96
436003	92 96	Celery	0W-FR 541-96
436003	92-96	Celery	0W FR-542-96
436003	92 96	Celery	FL FR 401 96
436003	92-96	Celery	NE-FR 727 96
436004	93 96	Tomato, Whole	02 FR 030 96
436004	93 96	Tomato, Whole	0W-FR 412 96
436004	93 96	Tomato, Whole	FL FR 404 96
436004	93-96	Reagent Blank	Contract Method Trial Data[1] FL FR 404 96
436004	93 96	Tomato, Wet Pomace	Contract Method Trial Data[1] 02 FR-030-96
436004	93 96	Reagent Blank	93 96-BA2
436004	93-96	Tomato, Wet Pomace	0W FR 412-96
436004	93 96	Reagent Blank	93-96 BH
436004	93-96	Tomato Juice	02 FR-030-96
436004	93-96	Tomato Juice	0W-FR-412 96
436004	93-96	Tomato Puree	02 FR-030-96
436004	93 96	Tomato Puree	0W-FR-412-96
436004	93 96	Tomato Paste	02-FR 030-96
436004	93-96	Tomato Paste	0W FR 412 96
436004	93-96	Bell Pepper	02-FR-071 96
436004	93 96	Hot Pepper	Contract Method Trial Data[1] 0S FR 323 96 Contract Method Trial Data[1]

[1] Contract method trial data (Reference 10) was generated by Ricerca, Inc

TABLE III. ^{14}C-TOBACCO MULTICHROM WORKSHEET

Worksheet : 491 97-UA4
Ana Set Name : 491-97 UA
Analysis : 4919/UA4
Subject : TOMATO/TOBACCO/LETTUCE

Limit of Determ : 0 020 ppm
Corr Factor A : 1 0000
Corr Factor B : 1 1700

Extracted 4-Sep-1997
Analyzed 22-Sep-1997
Channel : 24

Product : COA-245/04
Analyte : COA-210007
Project : 491-97

Bt No	T	G	Field Test	Smp Code	Moist %	Smp wt ext g	Ext vol ml	Aliq 1 vol ml	Interm vol ml	Aliq 2 vol ml	Final wt g	Final vol ml	Inj vol ul	Smp Wt Inj mg
1	S												50 00	
2	S												50 00	
3*	C	1	RIHV0169/	000274	0 00	4 00	172 00	172 00						
4	S										4 000	4.0	50 00	50 000
5	S												50 00	
6	R	1	-	-	0 00	4 00	172 00	172 00					50 00	
7	R	1	*	*	0 00	4 00	172 00	172.00			4 000	4 0	50 00	50 000
8	S										4 000	20 0	50 00	10 000
9	X	1	*	000273	0 00	4 00	172 00	170 00					50 00	
10	X	1	-	*	0 00	4 00	172 00	170 00			3 953	15 u	50 00	13 178
11	X	1	*	*	0 00	4 00	172 00	170 00			3 953	15 0	50 00	13 178
12	S										3 953	15 0	50 00	13 178
13	S												50 00	
													50 00	

Bt No	T	G	Std soln ng/ul	Std Wt Inj ng	Peak Height uV	Analyte Found ng	Residue Found ppm	Value corrt for control	Added Amount ppm	Recov %	Avg Recov %	Corrt Residue ppm	Dry Wt/ (Soil) ppm	Comments
1	S		0 20000	10 0000	1514	9 882								STANDARD
2	S		0 04000	2 0000	295	1 791								STANDARD
3*	C	1			15	-0 062	-0 001							CONTROL TUL-C1
4	S		0 50000	25 0000	3755	24 749					98 21	<0 020		STANDARD
5	S		0 00700	0.3500	56	0 210								STANDARD
6	R	1			163	0 904	0 021	0 021	0 020	105 76				CONTROL+0 02PPM TUL-R1
7	R	1			1777	11 624	1 360	1 360	1 500	90 67				CONTROL+1 50PPM TUL-R2
8	S		0 10000	5 0000	768	4 933								STANDARD
9	X	1			1872	12.253	1 088				98 21	1.108		SAMPLE TUL-I1
10	X	1			1799	11 770	1 045				98 21	1 064		SAMPLE TUL-I2
11	X	1			1803	11 801	1 048				98 21	1 067		SAMPLE TUL-I3
12	S		0 01000	0 5000	94	0.458								STANDARD
13	S		0 20000	10 0000	1657	10.827								STANDARD

Substrate: LEAVES, UNCURED CONTROL
LEAVES, UNCURED COA-245/04

Notes: Study 491-97 Radiovalidation of AG-671
Substrate tobacco, lower leaves, uncured
Source Basic Metabolism Study 94WA04, Harvest Interval 5

B Blank C Control F Freezer R Recovery S Standard V Solvent X : Sample
* Control used for this group Negative controls are treated as 0 0 Underline, Strikethrough Manual override Rejected

TABLE IV ^{14}C-LETTUCE MULTICHROM WORKSHEET

Worksheet 491 97-HK 2 | Limit of Determ 0 020 ppm | Extracted 24 Sep-1997 | Product : CGA-245704
Ana Sut Name 491 97-HC | Corr Factor A 1 0000 | Analyzed 24-Sep-1997 | Analyte CGA-210007
Analysis 4919/HK2 | Corr Factor B : 1 1700 | Channel 24 | Project 491-97
Subject TOMATO/TOBACCO/LETTUCE

Dt Rm	T	G	Field Test	Smp Code	Moist %	Smp wt ext g	Ext vol ml	Aliq 1 vol ml	Interm vol ml	Aliq 2 vol ml	Final wt g	Final vol ml	Inj vol ul	Smp Wt Inj ng
1	S												50 00	
2	S												50 00	
3*	C	1	RIMV01697	000278	0 00	4 00	174 00	174 00					50 00	
4	S										4 000	4 0	50 00	50 000
5	R	1	-	-	0 00	4 00	174 00	174 00					50 00	
6	R	1	-	-	0 00	4 00	174 00	174 00			4 000	4 0	50 00	50 000
7	S										4 000	10 0	50 00	20 000
8	S												50 00	
9	X	1	-	000277	0 00	4 00	174 00	172 00					50 00	
10	X	1	-	-	0 00	4 00	174 00	172 00			3 954	10 0	50 00	19 770
11	X	1	-	-	0 00	4 00	174 00	172 00			3 954	10 0	50 00	19 770
12	S										3 954	10.0	50 00	19 770
13	S												50 00	
													50 00	

Dt Rm	T	G	Std soln ng/ul	Std Wt Inj ng	Peak Height uV	Analyte Found ng	Residue Found ppm	Value corrt for control	Added Amount ppm	Recov %	Avg Recov %	Corrt Residue ppm	Dry Wt/ (Soil) ppm	Comments
1	S		0 20000	10 0000	1513	9 703								STANDARD
2	S		0 01000	0 5000	86	0 511								STANDARD
3*	C	1			0	0 000	0 000							CONTROL LT-C1
4	S		0 50000	25 0000	3909	25 131					95 23	<0.020		STANDARD
5	R	1			131	0 801	0 019	0 019	0 020	93.68				CONTROL+0 02PPM LT-R1
6	R	1			4575	16 542	0 968	0 968	1 000	96 77				CONTROL+1 0PPM LT-R2
7	S		0 04000	2 0000	285	1 709								STANDARD
8	S		0 00700	0 3500	119	0 718								STANDARD
9	X	1			871	5 565	0 329				95 23	0 346		INCURRED SAMPLE LT-11
10	X	1			796	5 085	0 301				95 23	0 316		INCURRED SAMPLE LT-12
11	X	1			715	4 562	0 270				95 23	0 284		INCURRED SAMPLE LT-13
12	S		0 10000	5 0000	783	5.000								STANDARD
13	S		0 20000	10 0000	1559	9 998								STANDARD

Substrates LETTUCE CONTROL
LETTUCE CGA-245704

Notes Study 491-97, Radiovalidation of AU-671
Lettuce, no washing,
Source Basic Metabolism Study 96HK02 1, Intv 2

B Blank C Control F : Freezer R Recovery S . Standard V Solvent X : Sample
* Control used for this group Negative controls are treated as 0 0 Underline Strikethrough : Manual override Rejected

TABLE V. ^{14}C-TOMATO MULTICHROM WORKSHEET

Worksheet 491-97-BB
Ana Set Name 491-97-BB
Analysis : 49197BB
Subject . : TOMATO

Limit of Determ 0 020 ppm
Corr Factor A 1 0000
Corr Factor B : 1 1700

Extracted 9-Sep-1997
Analyzed 10-Sep-1997
Channel 23

Product CGA-245704
Analyte : CGA 210007
Project 491-97

Bl No	T	G	Field Test	Smp Code	Moist %	Smp wt ext g	Ext vol ml	Aliq 1 vol ml	Interm vol ml	Aliq 2 vol ml	Final wt g	Final vol ml	Inj vol ul	Smp Wt Inj mg
1	S												50 00	
2	S												50 00	
3*	C	1	02FRO3096	001008AD01	0 00	4 00	172 00	172 00					50 00	
4	S										4 000	4 0	50 00	50 000
5	R	1	"	"	0 00	4 00	172 00	172 00					50 00	
6	R	1	"	"	0 00	4 00	172 00	172 00			4 000	4 0	50 00	50 000
7	R										4 000	4 0	50 00	50 000
8	S												50 00	
9	X	1	RIMV01697	0002/2	0 00	4 00	172 00	170 00					50 00	
10	X	1	"	"	0 00	4 00	172 00	170 00			3 953	4 0	50 00	49 419
11	X	1	"	"	0 00	4 00	172 00	170 00			3 953	4 0	50 00	49 419
12	S										3 953	4 0	50 00	49 419
13	S												50.00	
													50 00	

Bl No	T	G	Std Soln ng/ul	Std Wt Inj ng	Peak Height uV	Analyte Found ng	Residue Found ppm	Value corrt for control	Added Amount ppm	Recov %	Avg Recov %	Corrt Residue ppm	Dry Wt/ (Soil) ppm	Comments
1	S		0 20000	10.0000	3223	10 197								STANDARD
2	S		0 01000	0 5000	177	0 534								STANDARD
3*	C	1			55	0 147	0 003							CONTROL TO-C1
4	S		0 04000	2 0000	652	2 041					92 24	<0 020		STANDARD
5	R	1			315	0 970	0 023	0 019	0 020	96 27				CONTROL+0 02PPM TO R1
6	R	1			5996	18 995	0 444	0 441	0 500	88 21				CONTROL+0 5PPM TO-R2
7	S		0 50000	25 0000	7877	24 961								STANDARD
8	S		0 00700	0 3500	100	0 289								STANDARD
9	X	1			2420	7 650	0 181							INCURED SAMPLE TO-11
10	X	1			2780	8 791	0 208				92 24	0 196		INCURED SAMPLE TO-12
11	X	1			2965	9 377	0 222				92 24	0 246		INCURED SAMPLE TO 13
12	S		0 10000	5 0000	1544	4 871					92 24	0 241		STANDARD
13	S		0 20000	10 0000	3147	9 957								STANDARD

Substrate : UNWASHED TOMATOES
TOMATOES CGA-245704

Notes: Study 491-97, Radiovalidation of AG-671
Substrate Tomato, after washing
Source Basic Metabolism Study 94WA05, Interval 4

B Blank C : Control F Freezer R Recovery S Standard V Solvent X Sample
* : Control used for this group Negative controls are treated as 0 0 Underline, Strikethrough Manual override, Rejected

TABLE VI. **RECOVERY DATA** FROM CONTROL SUBSTRATES FORTIFIED WITH CGA-245704 AND ANALYZED BY ANALYTICAL METHOD AG-671A

Commodity	Substrate	Sample Code	Control (ppm)	CGA 245704 Added (ppm)		CGA-245704 Found (ppm)		Recovery CGA-245704 Corrected (%)[1,2]		Mean Recovery (%)	Standard Deviation	Mean CV (%)
--	Reagent Blank					<0 001		<0 02	--			
Tobacco	Lower Leaves, Uncured	TUL R1, TUL R2	<0.02	0.02	1.5	0.021	1.360	105	91			
	Green Uncured	1 2-A	<0 02	0 50	-	0 452	-	90	-			
	"	1 4 A	<0 02	0 10	0 02	0 074	0.015	74	76			
	"	1-6 A	<0 02	1 5	3.0	1 330	2 692	89	90			
	"	1 8 A	<0.02 (0 001)	0 02	0 10	0 016	0.093	75	92			
	"	"		0 50		0 402		80	-			
	"	1 10-A	<0.02	0 50		0 382		76	-			
	"	1-4-A	<0.02 (0.003)	0 02	0.20	0.020	0 178	84	87			
	"	"		1.0	-	0 859		86	-			
	"	1 4 A	<0 02	0.02	0 10	0.014	0.076	70	76			
	"	"	-	0.50		0 453		91	-			
										84	9 1 (n=17)	11
	Cured	1-3-A	<0.02 (0.002)	5 0	-	3.917		78	-			
	"	1 5 A	<0 02	4.0	-	3.036		76	-			
	"	1 7-A	<0.02	20	-	17.289	-	86	-			
	"	1-9 A	<0 02	2 0	-	1 786	-	88	-			
	"	1 11 A	<0 02	0 02		0 019		93	-			
	"	1 5 A	<0 02	0 02	-	0 022	-	108	-			
	"	1 5 A	<0.02	0.50	-	0 427	-	85	-			
										88	11 (n=7)	12
	Reagent Blank	"			-	<0.001		<0.02	-			
Head Lettuce	With Wrapper Leaves	LT R1, LT-R2	<0 02	0.02	1 0	0.019	0 868	94	97			
	"	1 5 A	<0.02	0 02	0 10	0 023	0.093	117	93			
	"	"	<0 02	0 02	0.02	0 022	0 014	108	71			
	"	"		0 02	0 02	0 018	0.019	91	97			
	"	"		0 10	0 10	0 088	0.093	88	93			
	"	"		0 10	-	0 092		92				
	"	"	--	0 50	0.50	0 463	0 458	93	92			
	"	"		0 50	--	0 454	--	91				
										94	10 (n=14)	11

[1] Minor discrepancies in residue recoveries (%) between raw data and that reported in this table are a function of round-off differences

[2] Results are corrected for control residues, if detected

TABLE VI. RECOVERY DATA FROM CONTROL SUBSTRATES FORTIFIED WITH CGA-245704 AND ANALYZED BY ANALYTICAL METHOD AG-671A (Continued)

Commodity	Substrate	Sample Code	Control (ppm)	CGA-245704 Added (ppm)		CGA 245704 Found (ppm)		Recovery CGA-245704 Corrected (%)[1,2]		Mean Recovery (%)	Standard Deviation	Mean CV (%)
--	Reagent Blank	---	---	--	--	<0 001	--	<0.02				
Leaf Lettuce	Leaves	1 2 A	<0.02 (0.005)	0 20	1.5	0 169	1.269	81	84			
	"	1-3 A	<0.02 (0 003)	0 10		0 089	--	81	"			
	"	1 4 A	<0 02	0 02	0 50	0.023	0 458	115	91			
	"	1-5 A	<0 02	0 02	0 10	0.020	0.094	90	94			
	"	1-6-A	<0.02	0 02	0 10	0.017	0.090	84	90			
	"	1-3-A	<0 02	0.02		0 017	--	85				
	"	1-3 A	<0.02 (0.008)	0 02	-	0.023		78				
	"	1-3 A	<0 02 (0 001)	0 10	-	0.091	-	90	-			
	"	1 3 A	<0 02	0.02	-	0.049	-	94	-			
	"	1-3-A	<0.02	0.02	-	0.018	-	91	--			
										90	9.3 (n=14)	10
--	Reagent Blank	---	--	--	---	<0 001	-	<0.02	-			
Spinach	Leaves	1 2-A	<0.02	0 02	0 10	0.020	0.095	99	95			
	"	1 3 A	<0.02	0 10	0 50	0.095	0.501	95	100			
	"	1 4 A	<0.02 (0 003)	0 02	0.50	0 018	0 500	78	100			
	"	1 5 A	<0.02 (0 003)	0 10	1.5	0.085	1.365	92	91			
	"	1-6 A	<0.02	0 02	0 10	0.015	0.084	73	84			
	"	1 3 A	<0 02	0 02	-	0 018	--	90	"			
	"	1 3 A	<0.02 (0 005)	0 02	-	0.020		78				
	"	1 3 A1	<0.02	0.02		0 017	--	85	-			
	"	1-3-A	<0.02 (0.008)	0 02	--	0.025		85				
	"	1-3-A	<0 02	0.02	--	0 024	-	94				
										89	8.5 (n=15)	9 5

[1] Minor discrepancies in residue recoveries (%) between raw data and that reported in this table are a function of round-off differences
[2] Results are corrected for control residues if detected

TABLE VI. RECOVERY DATA FROM CONTROL SUBSTRATES FORTIFIED WITH CGA-245704 AND ANALYZED BY ANALYTICAL METHOD AG-671A (Continued)

Commodity	Substrate	Sample Code	Control (ppm)	CGA-245704 Added (ppm)		CGA 245704 Found (ppm)		Recovery CGA-245704 Corrected (%)[1,2]		Mean Recovery (%)	Standard Deviation	Mean CV (%)
Celery	Leaf Stalk	1-2-B	<0.02	0.02	0.10	0.020	0.095	98	95			
	"	1-3 A	<0.02 (0.002)	0.10	0.02	0.092	0.020	90	92			
	"	1-4 A	<0.02 (0.01)	0.02	0.10	0.017	0.093	80	92			
	"	1-5-A	<0.02	0.10	0.50	0.094	0.483	94	97			
	"	1-6 A	<0.02 (0.002)	0.02	0.50	0.019	0.488	85	97			
	"	1-3 A	<0.02	0.02		0.020	--	102	--			
	"	1-3-A	<0.02	0.02	-	0.015		74	-			
	"	1 3 A	<0.02	0.02	0.10	0.014	0.082	72	82			
	"	1-3 A	<0.02, <0.02	0.10	0.02	0.086	0.021	86	105			
	"	1 3 A	<0.02	0.02	-	0.018	--	90	-			
										99	9.2 (n=17)	10
Tomato	Fruit	TO-R1, TO-R2	<0.02 (0.003)	0.02	0.50	0.023	0.444	98	88			
	Fruit Mature, Unwashed	1-6-AB-1	<0.02 (0.007)	0.10	0.50	0.102	0.370	94	73			
	"	1-6 AB 1	<0.02 (0.004)	0.50	1.0	0.501	0.859	99	86			
--	Reagent Blank	--	--	--	--	<0.001	--	<0.02	-			
	Fruit Mature, Unwashed	1-4 A	<0.02 (0.005)	0.02	0.02	0.021	0.021	92	90			
	"	"	<0.02 (0.001)	0.02	0.20	0.020	0.145	84	71			
	"	"		0.20	0.20	0.165	0.177	81	87			
	"	"		1.0	1.0	0.826	0.974	83	97			
	"	"		1.0	--	0.878	--	87	-			
										87	6.1 (n=15)	9.3
	Reagent Blank				-	<0.001	-	<0.02				
	Reagent Blank			-	-	<0.001	-	<0.02				
	Wet Pomace	1-8 AB 4	<0.02	0.02	1.0	0.025	0.078	83	87			
	"	1 8-AB-4	<0.02	0.10	1.5	0.099	1.478	99	98			
	Fresh Juice	1 8 AD-5	<0.02 (0.009)	0.10	1.0	0.110	0.760	100	75			
	"	"	<0.02 (0.004)	1.0	--	0.862	--	86	--			
	"	1-8-AB-5	<0.02	0.10	0.50	0.099	0.510	99	102			
	Puree	1-8 AB 6	<0.02 (0.011)	0.02	0.10	0.038	0.114	141	103			
	"	"	--	0.50	1.0	0.531	1.000	104	99			
	"	1-6 AB-6	<0.02	0.02	0.50	0.018	0.519	92	104			
	"	"	--	1.0	1.5	1.014	1.504	101	107			
	Paste	1 8 AB 7	0.02	0.02	0.10	0.041	0.129	90	108			
	"	"	-	0.50	1.0	0.540	1.061	103	104			
	"	1 0-AB 7	<0.02 (0.002)	0.02	0.10	0.022	0.105	101	103			
	"	"	--	0.50	1.0	0.529	1.052	105	105			
										100	12 (n=25)	12

[1] Minor discrepancies in residue recoveries (%) between raw data and that reported in this table are a function of round-off differences.
[2] Results are corrected for control residues, if detected

TABLE VI **RECOVERY DATA** FROM CONTROL SUBSTRATES FORTIFIED WITH CGA-245704 AND ANALYZED BY ANALYTICAL METHOD AG-671A (Continued)

Commodity	Substrate	Sample Code	Control (ppm)	CGA-245704 Added (ppm)		CGA 245704 Found (ppm)		Recovery CGA 245704 Corrected (%)[1,2]		Mean Recovery (%)	Standard Deviation	Mean CV (%)
Bell Pepper	Pepper	1 4 A	<0 02 (0.003)	0 02	0.02	0 019	0.020	83	87			
	"	"	<0.02 (0.003)	0 02	0 20	0.019	0 171	84	84			
	"	"		0 20	0.20	0 192	0 161	95	79			
	"	"		1 0	1.0	0.813	0 819	81	82			
	"	"		1 0		0.809	"	81				
										84	4 7 (n=9)	5 6
Hot Pepper	Pepper	1-4 A	<0.02 (0 009)	0.02	0 02	0.026	0.032	88	114			
	"	"	<0 02 (0.008)	0.02	0.20	0.032	0 196	118	93			
	"	"		0.20	0.20	0 202	0 189	97	95			
	"	"		1 0	1.0	0.811	0 950	80	95			
	"	"		1.0	"	0 866	"	86	"			
										98	12 (n=9)	13
									n.	142		
									Overall Range.	79-141		

[1] Minor discrepancies in residue recoveries (%) between raw data and that reported in this table are a function of round-off differences
[2] Results are corrected for control residues, if detected

TABLE VII. **STATISTICAL RESULTS** FROM THE ANALYSIS OF FORTIFIED CONTROLS BASED ON FORTIFICATION LEVEL

Fortification Level (ppm)		Tobacco Green	Tobacco Cured	Head Lettuce	Leaf Lettuce	Spinach	Celery	Tomato	Tomato Processing	Bell Pepper	Hot Pepper	Overall[1] Mean	Standard Dev.	Mean CV	n
0.02	mean =	82	101	96	92	85	88	91	101	85	107	92	14	15	52
	standard dev =	14	11	16	12	8.8	12	5.0	23	2.1	16				
	mean CV =	17	11	16	13	10	13	5.5	23	2.5	15				
	n =	5	2	6	7	8	9	4	5	3	3				
0.10	mean =	81		92	69	92	91	94	102			92	7.6	8.2	28
	standard dev =	10		2.4	5.5	5.2	4.8		2.8						
	mean CV =	12		2.6	8.2	5.7	5.3		2.8						
	n =	3		4	4	4	6	1	6						
0.20	mean =	87			81			80		86	95	86	8.1	9.4	11
	standard dev =							6.1		8.2	2.6				
	mean CV =							10		9.5	2.1				
	n =	1			1			3		3	3				
0.50	mean =	84	85	92	91	100	97	87	104			93	9.2	9.9	21
	standard dev =	7.4		1.0		0	0	13	1.1						
	mean CV =	8.8		1.1		0	0	15	1.1						
	n =	4	1	3	1	2	2	3	5						
1.0	mean =	86		97				88	94	81	87	89	8.9	9.9	19
	standard dev =							6.1	11	0.6	7.5				
	mean CV =							6.9	12	0.7	8.7				
	n =	1		1				4	7	3	3				
1.5	mean =	90			84	91			103			93	8.1	8.6	6
	standard dev =	1.4							5.4						
	mean CV =	1.6							5.2						
	n =	2			1	1			2						
2.0	mean =		89									89			1
	standard dev =														
	mean CV =														
	n =		1												
3.0	mean =	90										90			1
	standard dev =														
	mean CV =														
	n =	1													

[1] Overall statistics are a compilation of individual data points from Table VI

TABLE VII **STATISTICAL RESULTS** FROM THE ANALYSIS OF FORTIFIED CONTROLS BASED ON FORTIFICATION LEVEL (Continued)

Fortification Level (ppm)		Tobacco Green	Tobacco Cured	Head Lettuce	Leaf Lettuce	Spinach	Celery	Tomato	Tomato Processing	Bell Pepper	Hot Pepper	Overall Mean	Overall Standard Dev	Overall Mean CV	Overall n
4.0	mean =	---	76	---		--	-	--	--	--	--	76	---	---	1
	standard dev =	--	---	--		---	--	---	---	---					
	mean CV =		--		--				--	--	-				
	n =	--	1	---	--	---	--	-	-						
5.0	mean =		78		---	--	--	--	--	--	--	78	--	--	1
	standard dev =	--	---	---	--	---		--	--	-	--				
	mean CV =	-	---	--	-	---	--	--	--	---	-				
	n =	--	1	-	--	-		--	---	-	--				
20	mean =	---	86	---	-	--		-	--	-	-	86	--	--	1
	standard dev =	-	--		-	--		-	---	-	---				
	mean CV =	---		---		--		---	--	---	---				
	n =	--	1						---	---	---				
	Overall[1]														
	mean =	84	88	94	90	89	90	87	100	84	95	91	11	12	142
	standard dev =	9.1	11	10	9.3	8.5	9.2	8.1	12	4.7	12.4				
	mean CV =	11	12	11	10	9.5	10	9.3	12	5.6	13				
	n =	17	7	14	14	15	17	15	26	9	9				

[1] Overall statistics are a compilation of individual data points from Table VI

TABLE VIII. **EXTRACTABILITY AND ACCOUNTABILITY DATA** FROM ANALYSES OF RADIOLABELED TOBACCO, LETTUCE, AND TOMATO ANALYZED BY ANALYTICAL METHOD AG-671A

Commodity	Substrate	Sample Code	Extraction Date	Analysis Date	Total Radioactive Residue (TRR)[2] (ppm)	^{14}C Liquid Scintillation Analyses[1]				HPLC Analyses		
						Extractability[3] Initial Extract (ppm)	Extractability[4] Initial Extract (%)	Extractability[5] Final Fraction (ppm)	Extractability[6] Final Fraction (%)	Final Fraction[7] (ppm Found)	Final Fraction[8] (ppm Corrected)	Accountability[9] (%)
Tobacco	Lower Leaves, uncured	TUL-I1	9/4/97	9/22/97	1 388	1.500	108	1.094	79	1.088	1 106	80
		TUL-I2	9/4/97	9/22/97	1.388	1.544	111	1 095	79	1.045	1.064	77
		TUL-I3	9/4/97	9/22/97	1.388	1 478	106	1.080	78	1.048	1 067	77
						mean =	108				mean =	78
						std.dev =	2.5				std dev =	1 7
						Mean CV=	2.3				Mean CV=	2 2
						n =	3				n =	3
Head Lettuce	With Wrapper Leaves	LT-I1	9/24/97	9/24/97	0 960	0.850	90	0.505	52	0 329	0 346	36
		LT I2	9/24/97	9/24/97	0.960	0.849	88	0 497	52	0 301	0 316	33
		LT I3	9/24/97	9/24/97	0 960	0 784	82	0 439	46	0 270	0 284	30
						mean =	87				mean =	33
						std.dev =	4 2				std.dev =	3 0
						Mean CV=	4.8				Mean CV=	9.1
						n =	3				n =	3
Tomato	Fruit	TO I1	9/9/97	9/10/97	0 312	0.287	92	0 208	67	0 181	0 196	63
		TO I2	9/9/97	9/10/97	0.312	0.321	103	0 231	74	0 208	0 226	72
		TO-I3	9/9/97	9/10/97	0.312	0 317	102	0.241	77	0.222	0.241	77
						mean =	99				mean =	71
						std.dev =	6 1				std.dev =	7 1
						Mean CV=	6.1				Mean CV=	10
						n =	3				n =	3

[1] Minor discrepancies in residue recoveries (%) between raw data and that reported in this table are a function of round-off differences.
[2] Total radioactive residues (TRR) as determined by combustion (LSC) analyses in the metabolism study (Reference 5, 6, 7)
[3] ^{14}C-CGA-245704 residues determined by liquid scintillation counting of aliquots of the sample extract (AG-671A Section II.C 2.2.3)
[4] Extractability of initial extract (%) = (Total ^{14}C-CGA 245704 residues in sample initial extract (ppm) ÷ sample TRR (ppm)) x 100
[5] ^{14}C-CGA-245704 residues determined by liquid scintillation counting of aliquots of the sample final fraction (AG 671A Section II.C.3.4.2)
[6] Extractability of final fraction (%) = (^{14}C CGA-245704 residues in sample final fraction (ppm) ÷ sample TRR (ppm)) x 100
[7] Residue (ppm) determined by High Performance Liquid Chromatography (HPLC)
[8] Residue (ppm) determined by HPLC corrected by mean recovery (%) from fortified controls in respective sample set (Tables III V).
[9] HPLC Accountability (%) = (Total CGA-245704 residues in sample final fraction (ppm) ÷ sample TRR (ppm)) x 100

TABLE 1X. COMPARISON OF VALIDATION EXTRACTABILITY AND ACCOUNTABILITY WITH QUANTITATION OF METABOLITE FRACTIONS USING 2-DIMENSIONAL THIN LAYER CHROMATOGRAPHY

Commodity	Substrate	Sample Code	Total Radioactive Residue (TRR)[1] (ppm)	^{14}C Liquid Scintillation Analyses Extractability[2] (%)	^{14}C Liquid Scintillation Analyses 2-D TLC Quantitation[3] (%)	HPLC Analyses Accountability[4] (%)	^{14}C Liquid Scintillation Analyses 2-D TLC Accountability[5] (%)
Tobacco	Lower Leaves, Uncured	TUL-I1	1.388	106	—	80	
		TUL-I2	1.388	111	- -	77	-
		TUL-I3	1.388	106	- -	77	- -
		TUL Average	1.388	108	- -	78	
		2-D TLC	1.388		93	--	67
Head Lettuce	With Wrapper Leaves	LT-I1	0.960	90	-	36	-
		LT I2	0.960	88		33	
		LT-I3	0.960	82	- -	30	
		LT Average	0.960	87	- -	33	
		2 D TLC	0.960		98		37[6]
Tomato	Fruit	TO-I1	0.312	92	-	63	-
		TO I2	0.312	103	- -	72	- -
		TO-I3	0.312	102	- -	77	-
		TO Average	0.312	99	-	71	
		2 D TLC	0.312		97	-	64

[1] Total radioactive residues (TRR) as determined by combustion (LSC) analysis in the metabolism study (Reference 5, 6, 7)
[2] Extractability of initial extract (%) = (Total ^{14}C-CGA-245704 residues in sample initial extract (ppm) ÷ sample TRR (ppm)) x 100
[3] Extractability data obtained in metabolism study (References 5, 6, 7)
[4] HPLC Accountability (%) = (Total ^{14}C-CGA 245704 residues in sample final fraction (ppm) ÷ sample TRR (ppm)) x 100
[5] CGA-210007 quantitation results from metabolism study (References 5 & 7) after treatment with 0.1N NaOH (1 hr @ room temperature)
[6] Comparable metabolism hydrolysis conditions were not available for the head lettuce. Metabolism 2-D-TLC quantitation for head lettuce was conducted on the penetrated radioactivity after treatment with 0.1N NaOH and cleavage with cellulase (Reference 6). Extractability and accountability residue results reported herein were determined on the unwashed lettuce substrate (combined surface & penetrated radioactivity). The metabolism 2-D TLC surface and penetrated radioactivity results were combined for comparison with the residue accountability results. The TRR of the unwashed lettuce was 0.960 ppm.

TABLE X. COMPARATIVE RESULTS OF LC/MS DETECTION VERSUS HPLC/UV DETECTION IN TOBACCO , LETTUCE, AND TOMATO SUBSTRATES

			LC-MS Detection		HPLC - UV Detection	
Commodity	Substrate	Sample Type	CGA-210007, ppm	Recovery %	CGA-210007, ppm	Recovery %
TOBACCO	FLUE-CURED	CTRL	<0.05		<0.10	
		REC + 1 0 PPM		76		83
		REC + 1 0 PPM		105		85
		FREEZER	0 95		0 96	
		FREEZER	0 97		0.94	
			CGA-245704, ppm	Recovery %	CGA-245704, ppm	Recovery %
LETTUCE	HEAD	CTRL	<0.02		<0 02	
		REC + 0 50 PPM		92		95
		REC + 0 50 PPM		94		87
		1X, 0-DAY PHI	0 47		0.44	
		1X 0-DAY PHI	0 50		0 48	
	HEAD MATURE	CTRL	<0 02		<0 02	
		REC + 0.50 PPM		91		94
		REC + 0 50 PPM		92		91
		1X 7-DAY PHI	0 08		0.09	
		1X 7-DAY PHI	0 09		0.09	
TOMATOES	FRUIT	CTRL	<0.02		<0.02	
	(UNWASHED)	REC + 0 10 PPM		94		98
		REC + 0.50 PPM		101		103
		1X, 14-DAY PHI	0.28		0.27	
		3X 14-DAY PHI	0.64		0.64	
		5X, 14-DAY PHI	0.75		0.71	
	WET POMACE	CTRL	<0.02		<0.02	
		REC + 0.50 PPM		79		74
		REC + 0.50 PPM		91		87
		1X 14-DAY PHI	0.20		0 19	
		3X 14-DAY PHI	0.50		0 48	
		5X 14-DAY PHI	0.76		0.79	
	PASTE	CTRL	0 05		0 03	
		REC - 0 02 PPM		81		93
		REC + 0 10 PPM		74		94
		REC + 0 50 PPM		72		95
		REC + 1 0 PPM		84		91
		1X, 14-DAY PHI	1 5		1 3	
		3X, 14-DAY PHI	3 8		3 4	
		5X, 14-DAY PHI	5 8		5 2	

* Field-incurred residues of CGA-245704 from a representative sample of flue-cured tobacco were not available Instead this data shows the results of analysis of flue-cured tobacco that has been lab-fortified with 1 00 ppm of CGA-210007 as part of an in-progress storage stability study.

TABLE XI. OPERATING CONDITIONS FOR THE CONFIRMATION OF CGA-245704 (AS CGA-210007) BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH MASS SPECTROMETRIC DETECTION

System Description

ESI/APCI quadrupole LC/MS, Platform-LC™ model, equipped with HP-1100 high-performance liquid chromatography inlet system (autosampler, quaternary HPLC pump, column oven, and in-line UV detector).

- Micromass UK Ltd., Wythenshawe, United Kingdom

Liquid Chromatographic Conditions

Analytical HPLC Column:	ZORBAX LC-MS SB-C18, 50 mm x 2.1 mm I.D., 5.0 µm, Part No. 86095.902
Mobile Phase Composition:	80/20 0.5% formic acid in water/acetonitrile
Mobile Phase Flow Rate.	0.20 mL/min
Typical Retention Time:	~5.0 min
Column Oven Temperature:	25 °C
Volume Injected.	50 µL
Column Effluent/Detector Split Ratio:	~0.05 mL/min to waste ~0.18 mL/min to detector

MS Detector Setpoints

Ionization Mode:	electrospray	Source Heater:	90°C
Polarity.	negative ion	Low Mass Res.	15.0 amu
Monitor Ion	179 m/z [m-H+]	High Mass Res	15.0 amu
Capillary/Needle/Probe.	3.6 kV	Multiplier	650 V
Sample Cone:	30 V	Analyzer Vacuum	1.5e-5 torr
Skimmer Lens Offset:	5 V	N_2 Drying Gas Flow	410 L/hr.
Ion Energy:	1.1 V		

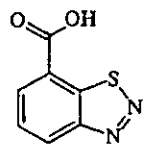
Software Version

Acquisition, Control, and Quantitation:	Masslynx v2.3, Build 007
Operating System.	Windows NT, v4.0, Build 1381

FIGURE 1. STRUCTURES AND CHEMICAL NAMES OF CGA-245704 AND CGA-210007

CGA-245704

Benzo[1,2,3]thiadiazole-7-carbothioic acid S-methyl ester
CAS Registry Number 135158-54-2
Molecular Weight = 210 28 g/mol



CGA-210007

Benzo[1,2,3]thiadiazole-7-carboxylic acid
CAS Registry Number 35272-27-6
Molecular Weight = 180.18 g/mol

FIGURE 2. FLOW DIAGRAM FOR ANALYTICAL METHOD AG-671A

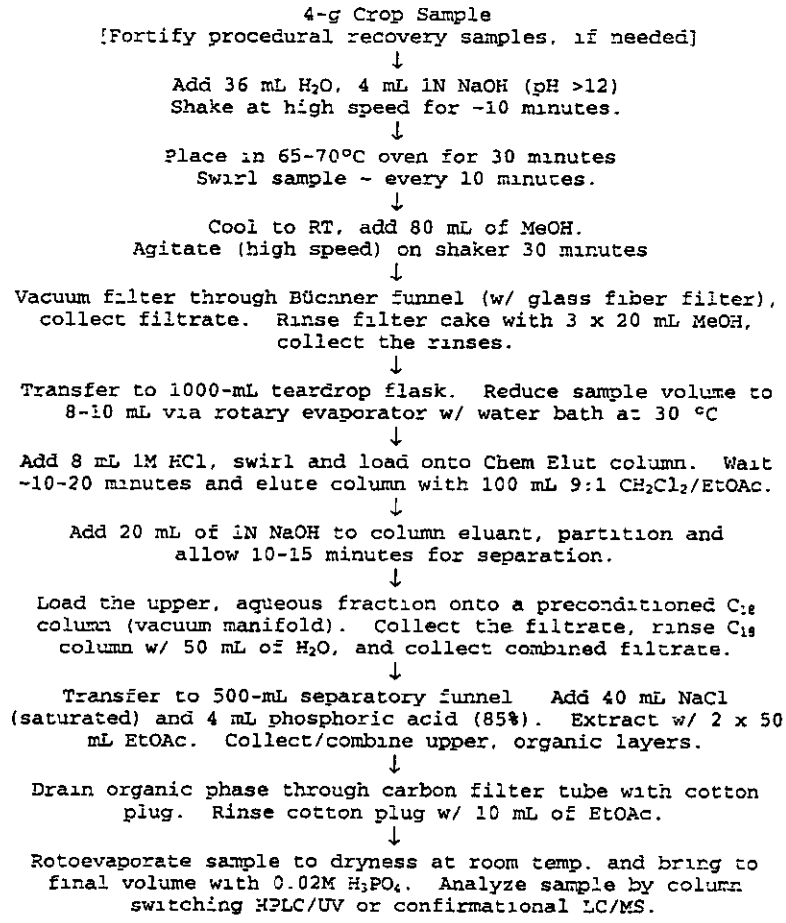
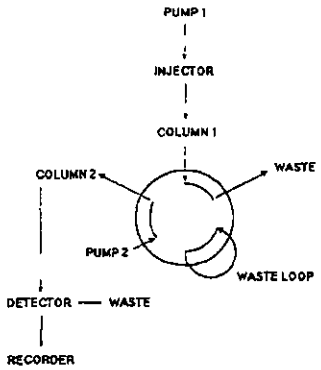
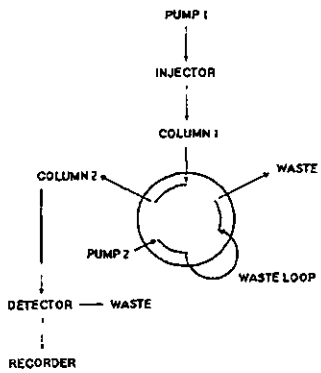


FIGURE 3. SCHEMATIC DIAGRAM OF THE HPLC COLUMN SWITCHING SYSTEM AND REPRESENTATIVE CHROMATOGRAM OF STANDARD PROFILE FROM COLUMN 1

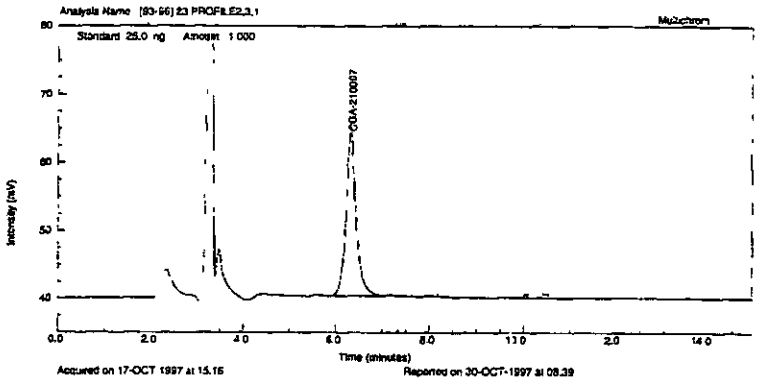


INJECT position (dual column) Direction of flows during injection of the sample onto column 1 (Spherisorb™ SAX/SB) Typical retention window ~7 minutes.



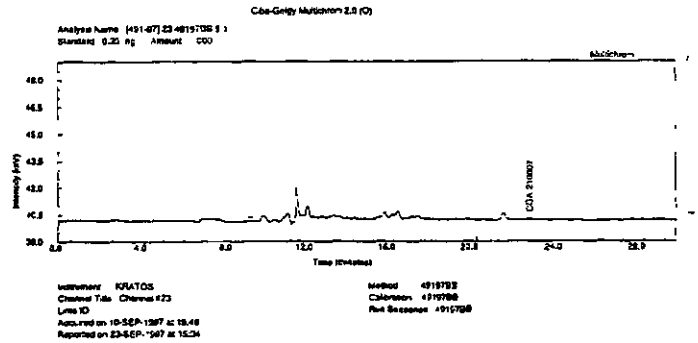
LOAD position (dual column) Direction of flows during switch (cut) from column 1 (Spherisorb™ SAX/SB) onto column 2 (YMC-Pack C8) Typical retention window ~22 minutes.

FIGURE 3. SCHEMATIC DIAGRAM OF THE HPLC COLUMN SWITCHING SYSTEM AND REPRESENTATIVE CHROMATOGRAM OF STANDARD PROFILE FROM COLUMN 1 (Continued)

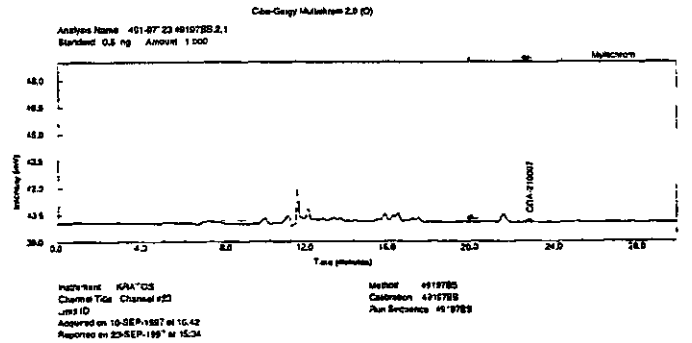


Representative chromatogram of CGA-210007 (25 ng/µl, 24,904 µV) standard elution from column 1 (Spherisorb® SAX/S3). The valve switch (cuttime) was set at 6.0-6.8 minutes

FIGURE 4. REPRESENTATIVE HPLC CHROMATOGRAMS OF CGA-210007 STANDARDS (^{14}C-Tomato Analytical Set)

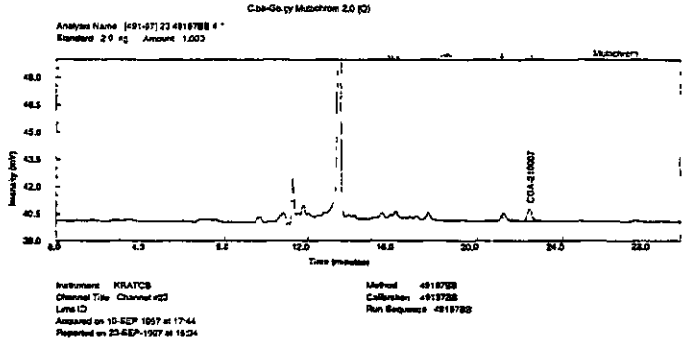


1. Standard, CGA-210007, 0.35 ng, 100 µV

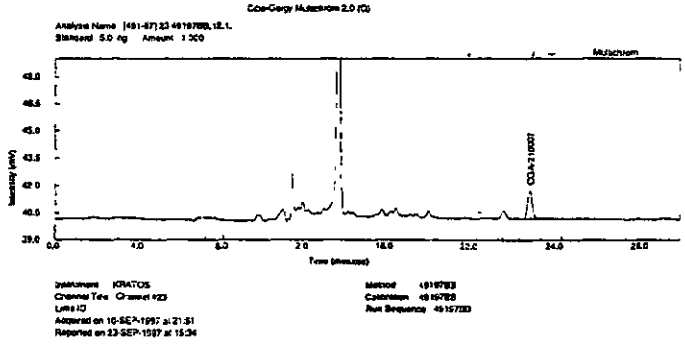


2. Standard, CGA-210007, 0.50 ng, 177 µV

FIGURE 4. REPRESENTATIVE HPLC CHROMATOGRAMS OF CGA-210007 STANDARDS (^{13}C-Tomato Analytical Set) (Continued)

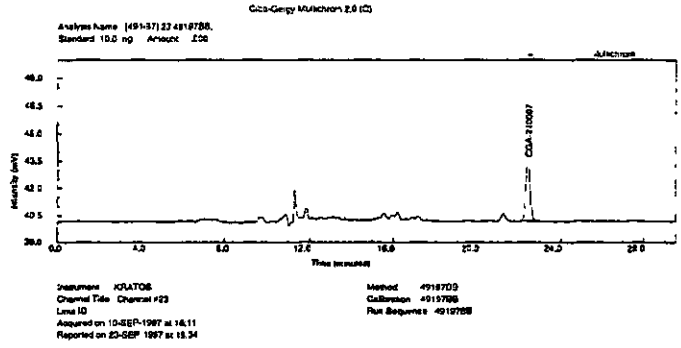


3. Standard, CGA-210007, 2.0 ng, 652 µV

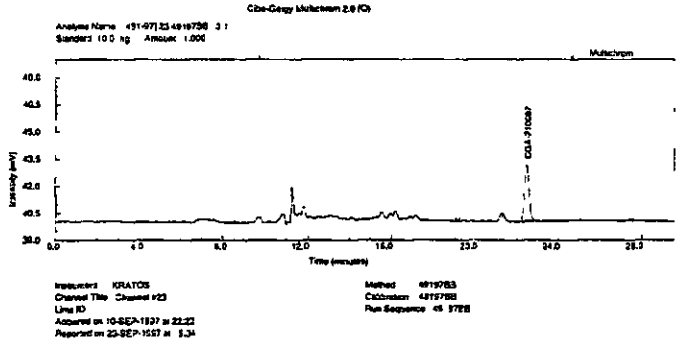


4. Standard, CGA-210007, 5 ng, 1544 µV

FIGURE 4. REPRESENTATIVE HPLC CHROMATOGRAMS OF CGA-210007 STANDARDS (^{14}C-Tomato Analytical Set) (Continued)

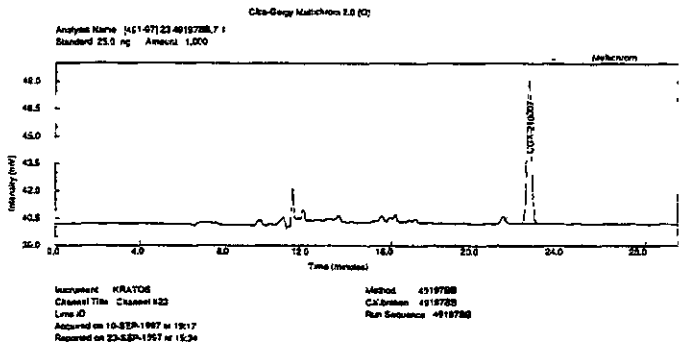


5. Standard, CGA-210007, 10 ng, 3223 µV



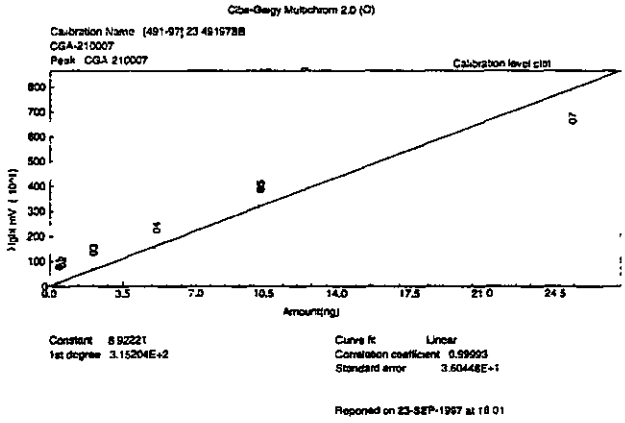
6. Standard, CGA-210007, 10 ng, 3147 µV

FIGURE 4. REPRESENTATIVE HPLC CHROMATOGRAMS OF CGA-210007 STANDARDS (^{14}C-Tomato Analytical Set) (Continued)



7 Standard, CGA-210007, 25 ng, 7877 µV

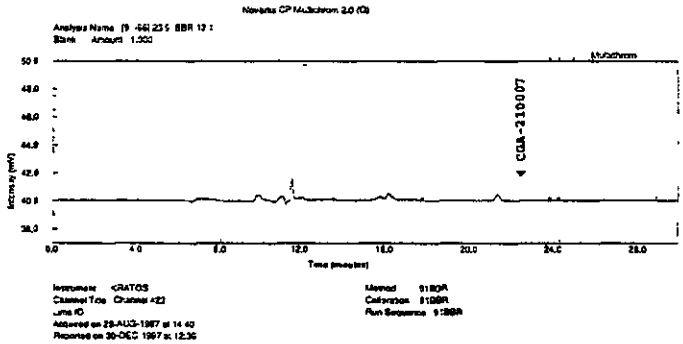
FIGURE 5. REPRESENTATIVE CALIBRATION CURVE USING THE CGA-210007 STANDARDS FROM FIGURE 4



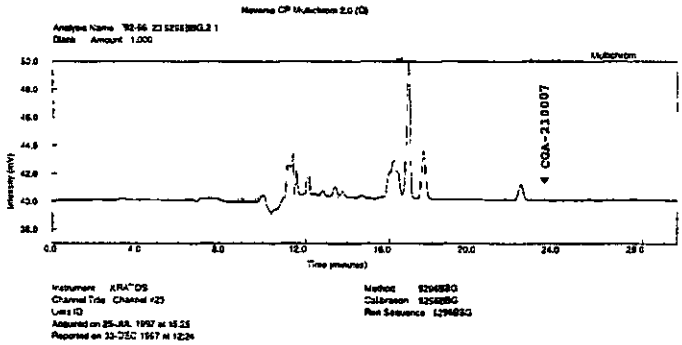
CGA-210007 Calibration Data

ng injected	peak height, µV
0.35	100
0.50	177
2.0	652
5.0	1544
10	3223
10	3147
25	7877

FIGURE 6. REAGENT BLANK CHROMATOGRAMS

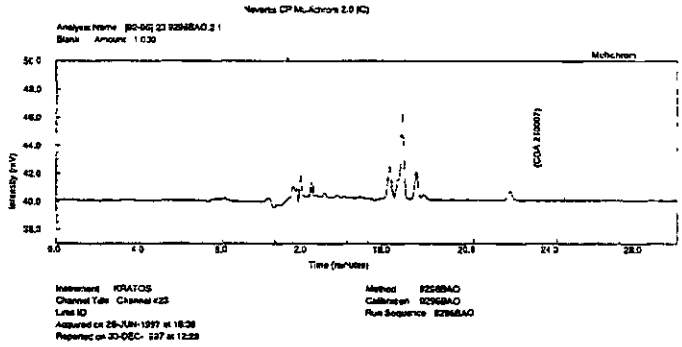


1. Reagent Blank, Tobacco Analytical Set 91-96-BBR, Protocol 91-96

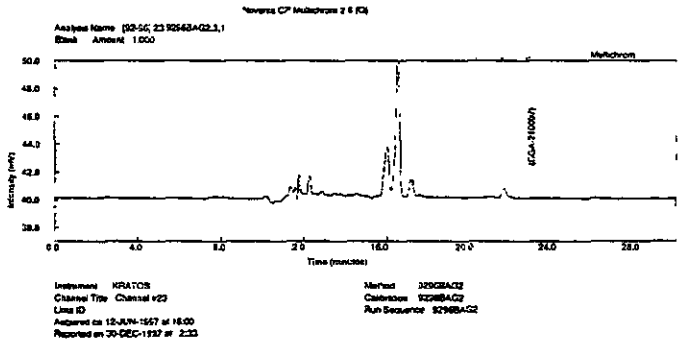


2. Reagent Blank, Head Lettuce Analytical Set 92-96-BBG, Protocol 92-96

FIGURE 6 REAGENT BLANK CHROMATOGRAMS (Continued)



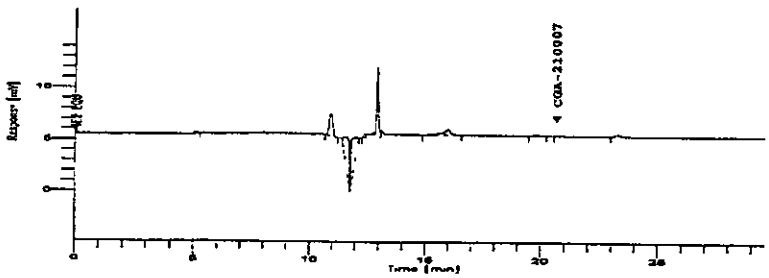
3. Reagent Blank, Leaf Lettuce Analytical Set 92-96-BAO, Protocol 92-96



4. Reagent Blank, Spinach Analytical Set 92-96-BAG2, Protocol 92-96

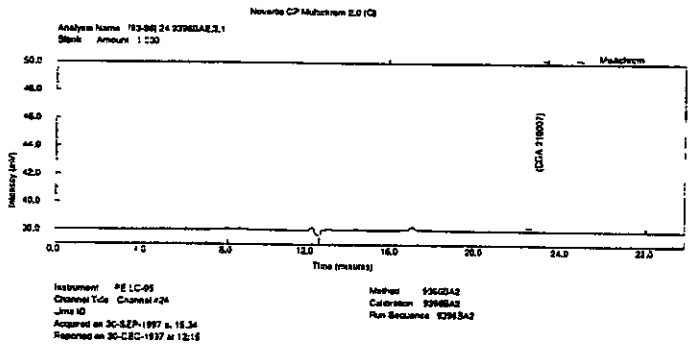
FIGURE 6. REAGENT BLANK CHROMATOGRAMS (Continued)

Sample Name reagent blank
Data File C:\TC4\NOVA\NMV7019A.RAW Date 11/6/97 01.30 AM
Sequence File: C:\TC4\NOVA\NOV1104.SEQ



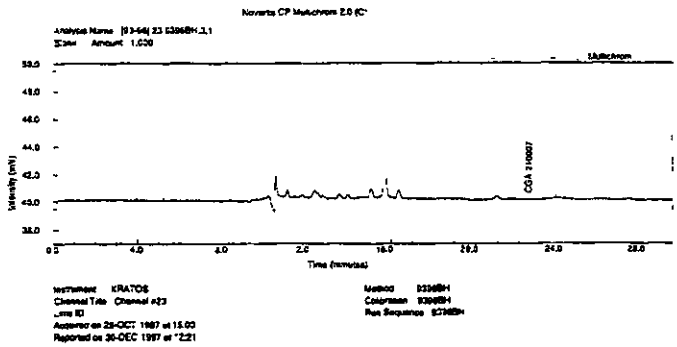
CGA-210007 - TOMATOES - METHOD VALIDATION

5. Reagent Blank, Whole Tomato, Ricerca, Inc. Method Trial Data, Protocol 93-96



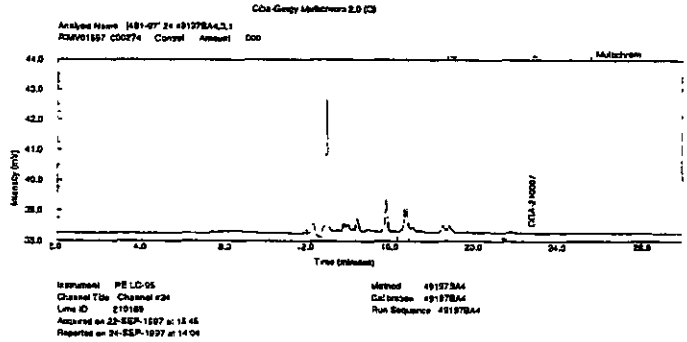
6. Reagent Blank, Tomato Pomace Analytical Set 93-96-BA2, Protocol 93-96

FIGURE 6. REAGENT BLANK CHROMATOGRAMS (Continued)

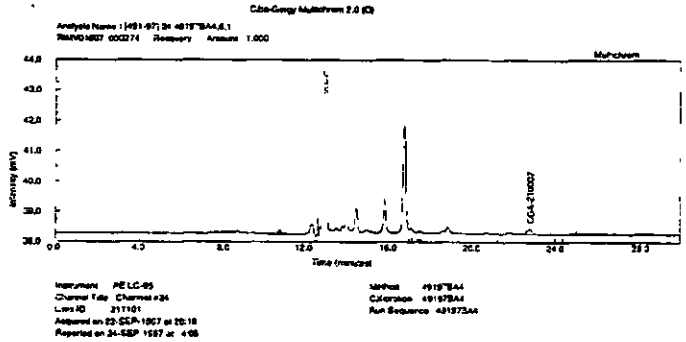


7. Reagent Blank, Tomato Pomace Analytical Set 93-96-BH, Protocol 93-96

FIGURE 7. HPLC CHROMATOGRAMS FROM THE ANALYSIS OF ^{14}C-TOBACCO (Control, Procedural Recovery, and Radiolabeled Samples)

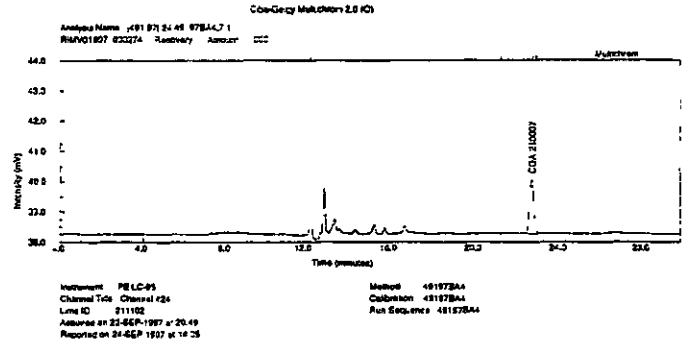


1. TUL-C1, control, 50 000 mg injected, <0.35 ng CGA-210007 quantitated, <0 02 ppm CGA-245704 determined

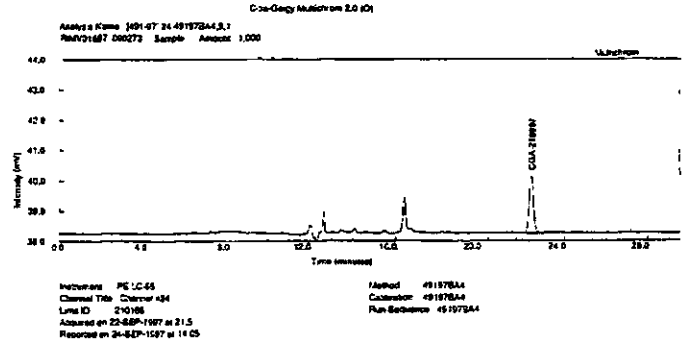


2. TUL-R1, control + 0.02 ppm CGA-245704, 50.000 mg injected, 0.904 ng CGA-210007 quantitated, 0.021 ppm CGA-245704 determined, 106% CGA-245704 recovered

FIGURE 7. HPLC CHROMATOGRAMS FROM THE ANALYSIS OF ^{14}C-TOBACCO (Control, Procedural Recovery, and Radiolabeled Samples) (Continued)

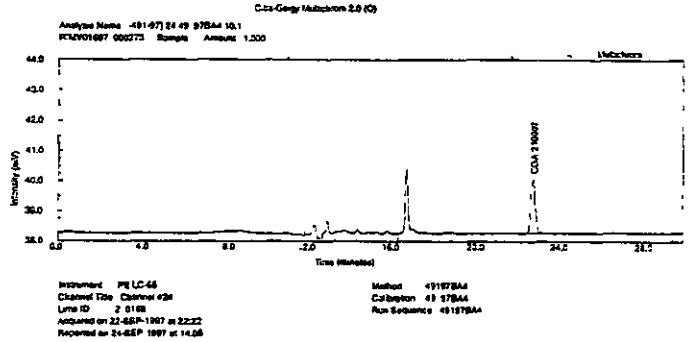


3. TUL-R2, control + 1.5 ppm CGA-245704, 10.000 mg injected, 11.624 ng CGA-210007 quantitated, 1.360 ppm CGA-245704 determined, 91% CGA-245704 recovered

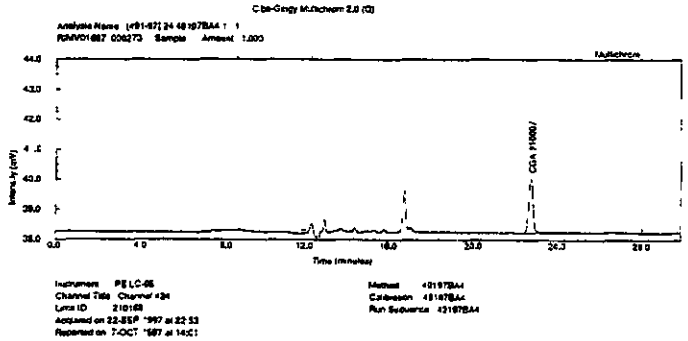


4 TUL-I1, radiolabeled incurred substrate, 13.178 mg injected, 12.253 ng CGA-210007 quantitated, 1.086 ppm CGA-245704 determined, 1.1 ppm CGA-245704 (corrected for average recovery) determined

FIGURE 7. HPLC CHROMATOGRAMS FROM THE ANALYSIS OF ^{14}C-TOBACCO (Control, Procedural Recovery, and Radiolabeled Samples) (Continued)

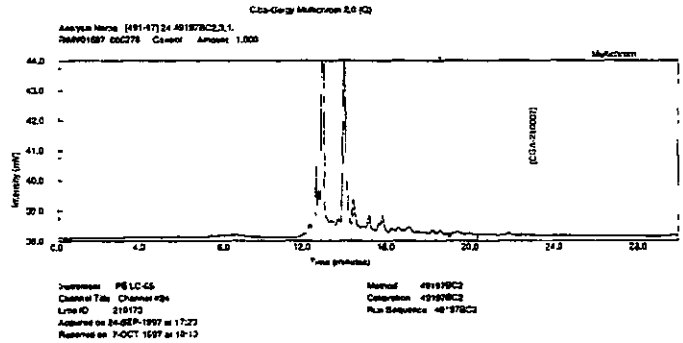


5. TUL-I2, radiolabeled incurred substrate, 13.178 mg injected, 11 770 ng CGA-210007 quantitated, 1 045 ppm CGA-245704 determined, 1.1 ppm CGA-245704 (corrected for average recovery) determined

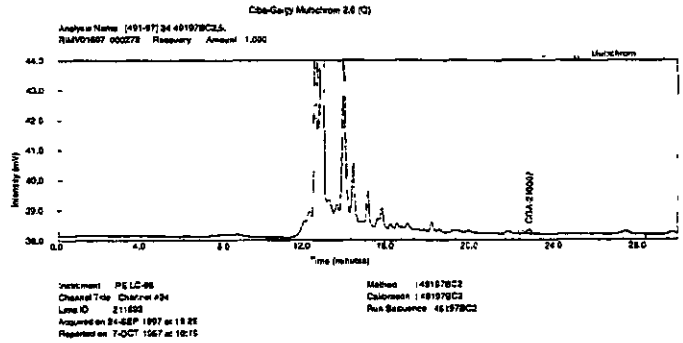


6. TUL-I3, radiolabeled incurred substrate, 13.178 mg injected, 11.801 ng CGA-210007 quantitated, 1.048 ppm CGA-245704 determined, 1 1 ppm CGA-245704 (corrected for average recovery) determined

FIGURE 8. HPLC CHROMATOGRAMS FROM THE ANALYSIS OF ^{14}C-LETTUCE (Control, Procedural Recovery, and Radiolabeled Samples)

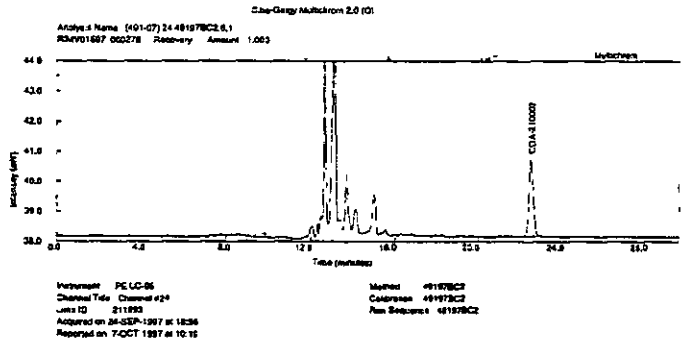


1. LT-C1, control, 50.000 mg injected, <0.35 ng CGA-210007 quantitated, <0.02 ppm CGA-245704 determined

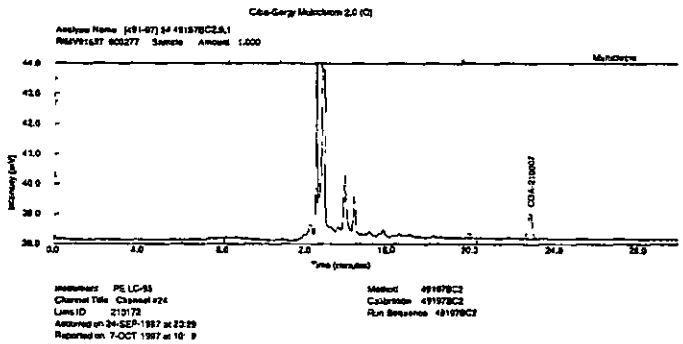


2. LT-R1, control + 0.02 ppm CGA-245704, 50.000 mg injected, 0.801 ng CGA-210007 quantitated, 0.019 ppm CGA-245704 determined, 94% CGA-245704 recovered

FIGURE 8. HPLC CHROMATOGRAMS FROM THE ANALYSIS OF ^{14}C-LETTUCE (Control, Procedural Recovery, and Radiolabeled Samples) (Continued)

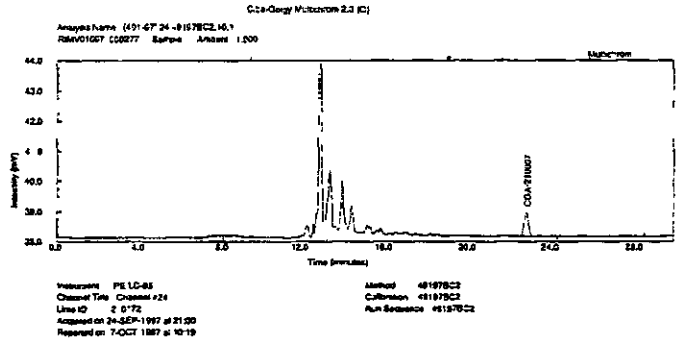


3. LT-R2, control + 1 0 ppm CGA-245704, 20.000 mg injected, 16.542 ng CGA-210007 quantitated, 0.968 ppm CGA-245704 determined, 97% CGA-245704 recovered

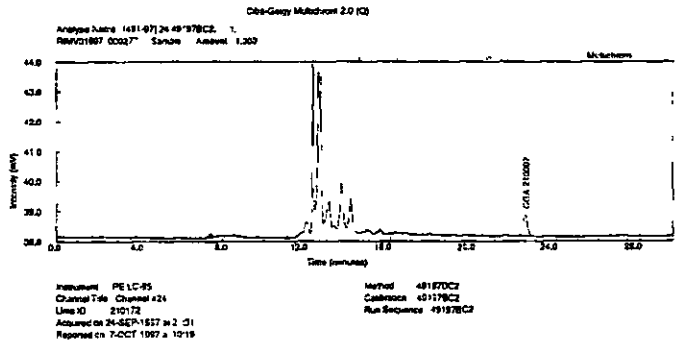


4. LT-I1, radiolabeled incurred substrate, 19.770 mg injected, 5 565 ng CGA-210007 quantitated, 0 329 ppm CGA-245704 determined, 0.346 ppm CGA-245704 (corrected for average recovery) determined

FIGURE 8. HPLC CHROMATOGRAMS FROM THE ANALYSIS OF ^{14}C-LETTUCE (Control, Procedural Recovery, and Radiolabeled Samples) (Continued)

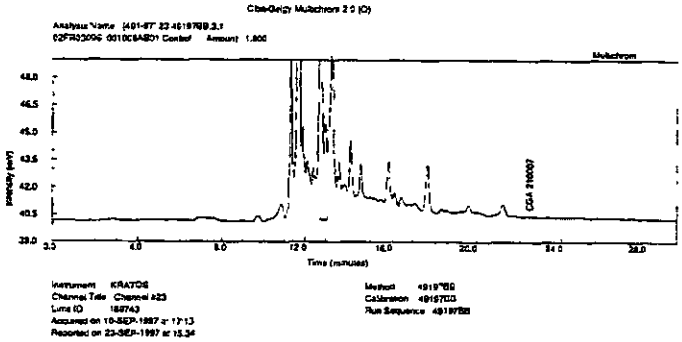


5. LT-I2, radiolabeled incurred substrate, 19.770 mg injected, 5 085 ng CGA-210007 quantitated, 0.301 ppm CGA-245704 determined, 0 316 ppm CGA-245704 (corrected for average recovery) determined

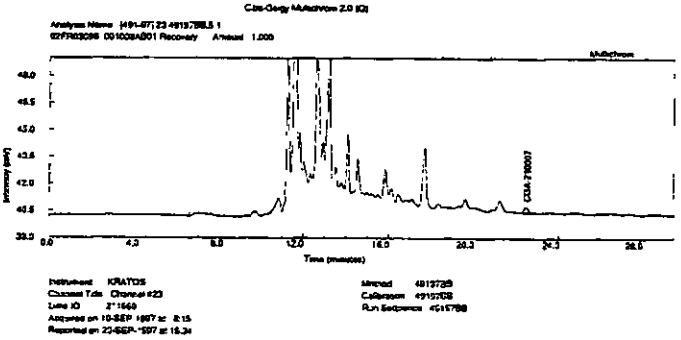


6 LT-I3, radiolabeled incurred substrate, 19.770 mg injected, 4 562 ng CGA-210007 quantitated, 0.270 ppm CGA-245704 determined, 0.284 ppm CGA-245704 (corrected for average recovery) determined

FIGURE 9. HPLC CHROMATOGRAMS FROM THE ANALYSIS OF ^{14}C-TOMATO (Control, Procedural Recovery, and Radiolabeled Samples)

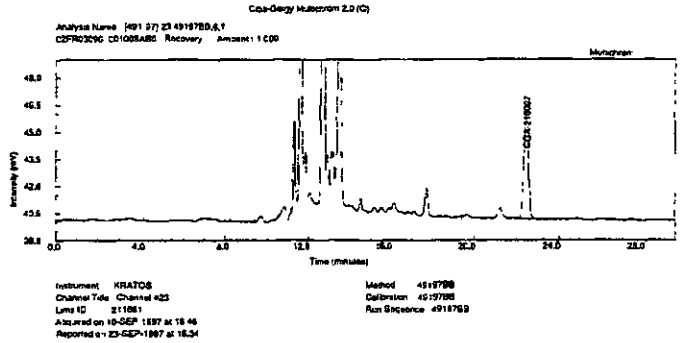


1. TO-C1, control, 50 000 mg injected, <0.35 ng (0.147 ng) CGA-210007 quantitated, <0.02 ppm (0.003) CGA-245704 determined

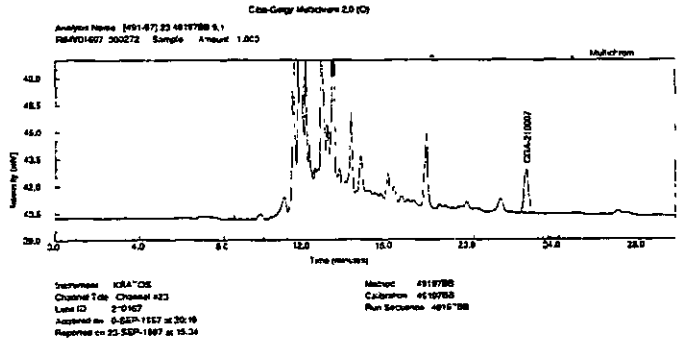


2. TO-R1, control + 0.02 ppm CGA-245704, 50.000 mg injected, 0.970 ng CGA-210007 quantitated, 0.023 ppm CGA-245704 determined, 0.019 ppm CGA-245704 (corrected for control) determined, 96% CGA-245704 recovered

FIGURE 9. HPLC CHROMATOGRAMS FROM THE ANALYSIS OF ^{14}C-TOMATO (Control, Procedural Recovery, and Radiolabeled Samples) (Continued)

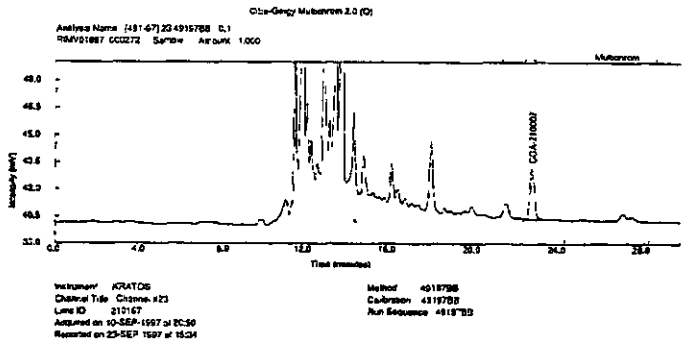


3. TO-R2, control - 0.50 ppm CGA-245704, 50.000 mg injected, 18.995 ng CGA-210007 quantitated, 0.444 ppm CGA-245704 determined, 0 441 ppm CGA-245704 (corrected for control) determined, 88% CGA-245704 recovered

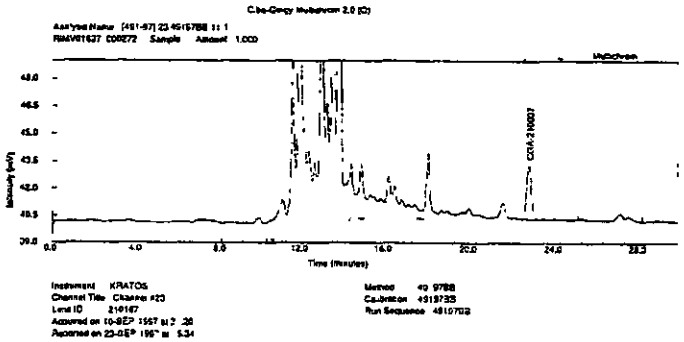


4. TO-I1, radiolabeled incurred substrate, 49.419 mg injected, 7.650 ng CGA-210007 quantitated, 0.181 ppm CGA-245704 determined, 0 196 ppm CGA-245704 (corrected for average recovery) determined

FIGURE 9. HPLC CHROMATOGRAMS FROM THE ANALYSIS OF ^{14}C-TOMATO (Control, Procedural Recovery, and Radiolabeled Samples) (Continued)

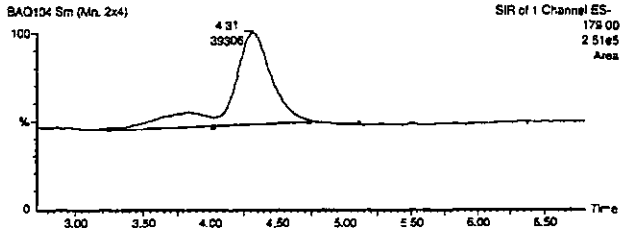


5 TO-I2, radiolabeled incurred substrate, 49 419 mg injected, 8 791 ng CGA-210007 quantitated, 0.208 ppm CGA-245704 determined, 0 226 ppm CGA-245704 (corrected for average recovery) determined

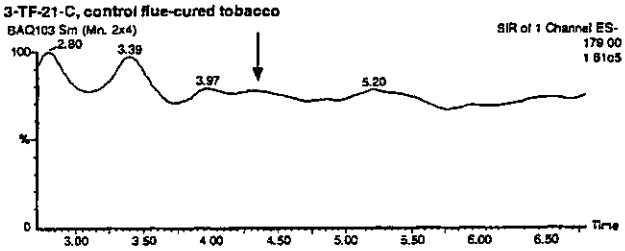


6. TO-I3, radiolabeled incurred substrate, 49 419 mg injected, 9 377 ng CGA-210007 quantitated, 0 222 ppm CGA-245704 determined, 0.241 ppm CGA-245704 (corrected for average recovery) determined

FIGURE 10. REPRESENTATIVE ION CHROMATOGRAMS FROM THE CONFIRMATORY ANALYSIS OF FLUE-CURED TOBACCO*



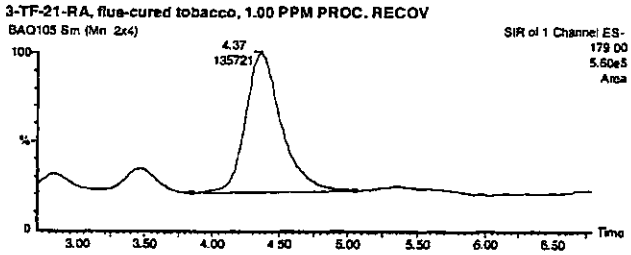
1. Calibration Standard, 2.00 ng injected.



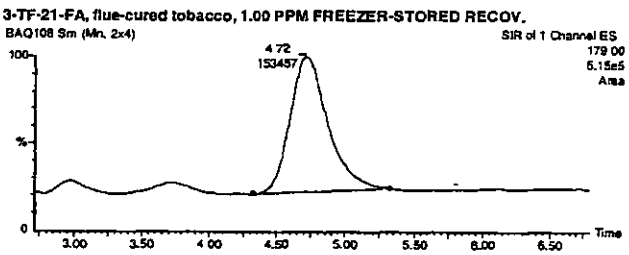
2. 3-TF-21-C, control, 10.0 mg injected, <0.50 ng CGA-210007 quantitated, <0.05 ppm CGA-210007 determined.

* Samples of flue-cured tobacco analyzed during development of the LC/MS confirmatory procedure were obtained from an in-progress storage stability study and were fortified with CGA-210007 rather than CGA-245704

FIGURE 10 REPRESENTATIVE ION CHROMATOGRAMS FROM THE CONFIRMATORY ANALYSIS OF FLUE-CURED TOBACCO* (Continued)



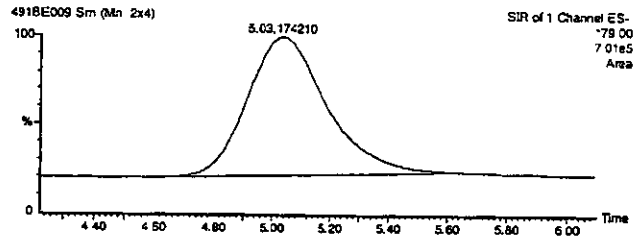
3. 3-TF-21-R-A, control + 1.00 ppm CGA-210007, 10.0 mg injected, 7.593 ng CGA-210007 quantitated, 0.759 ppm CGA-210007 determined, 76% CGA-210007 recovered.



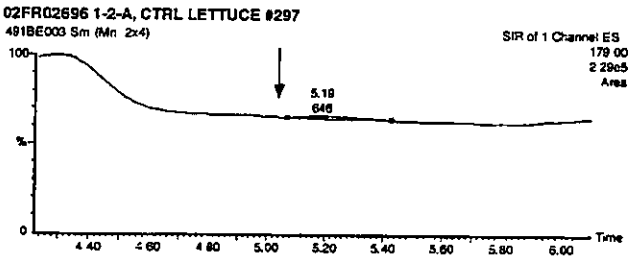
4. 3-TF-21-F-A, 1.00 PPM 2-Month freezer-stored sample, 10.0 mg injected, 8.599 ng CGA-210007 quantitated, 0.860 ppm CGA-245704, 0.95 ppm CGA-210007 determined (corrected for 91% average CGA-210007 recovery)

* Samples of flue-cured tobacco analyzed during development of the LC/MS confirmatory procedure were obtained from an in-progress storage stability study and were fortified with CGA-210007 rather than CGA-245704.

FIGURE 11. REPRESENTATIVE ION CHROMATOGRAMS FROM THE CONFIRMATORY ANALYSIS OF HEAD LETTUCE (0-Day PHI)

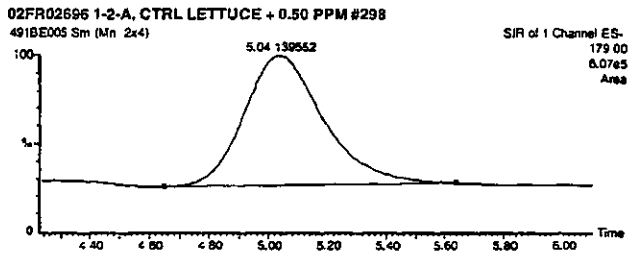


1. Calibration Standard, 10.00 ng injected.

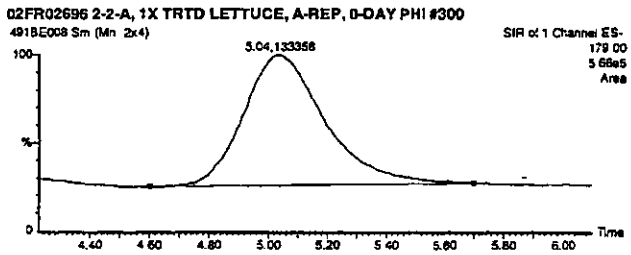


2 1-2-A, control, 50.0 mg injected, <0.35 ng CGA-210007 quantitated, <0.02 ppm CGA-245704 equivalent determined.

FIGURE 11. REPRESENTATIVE ION CHROMATOGRAMS FROM THE CONFIRMATORY ANALYSIS OF HEAD LETTUCE (0-Day PHI) (Continued)

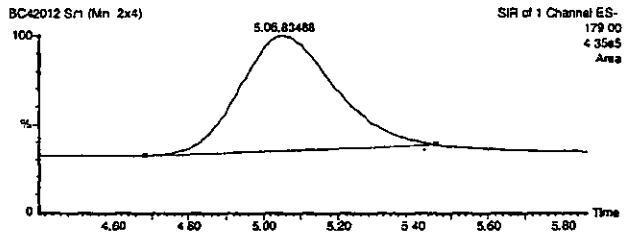


3. 1-2-A, control + 0 50 ppm CGA-245704, 20.0 mg injected, 7.841 ng CGA-210007 quantitated, 0.459 ppm CGA-245704 equivalent determined, 92% CGA-245704 recovered.

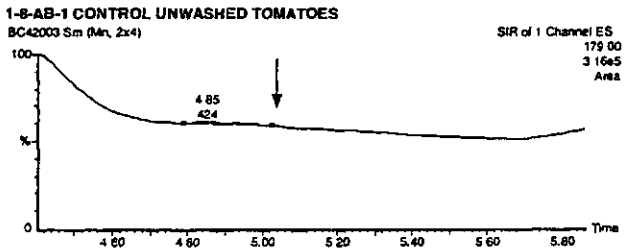


4. 2-2-A, 1X treated head lettuce, 20.0 mg injected, 7.491 ng CGA-210007 quantitated, 0 438 ppm CGA-245704, 0 47 ppm CGA-245704 equivalent determined (corrected for 93% average recovery)

FIGURE 12. REPRESENTATIVE ION CHROMATOGRAMS FROM THE CONFIRMATORY ANALYSIS OF UNWASHED TOMATOES

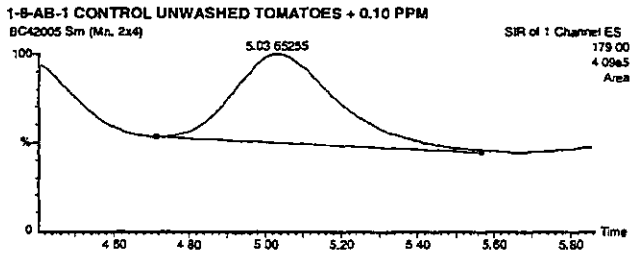


1. Calibration Standard, 5.00 ng injected.

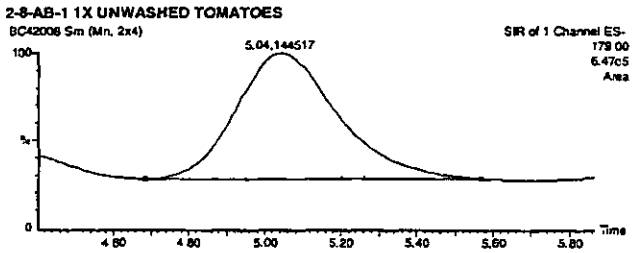


2. 1-8-AB-1, control, 50.0 mg injected. <0.50 ng CGA-210007 quantitated, <0.02 ppm CGA-245704 equivalent determined

FIGURE 12. REPRESENTATIVE ION CHROMATOGRAMS FROM THE CONFIRMATORY ANALYSIS OF UNWASHED TOMATOES (Continued)

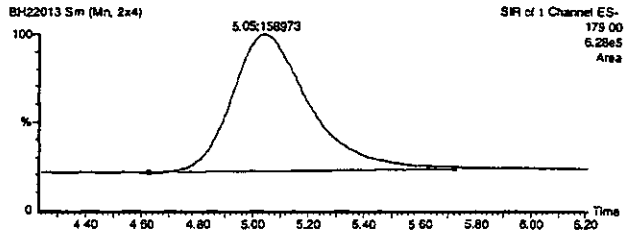


3. 1-8-AB-1, control + 0.10 ppm CGA-245704, 50.0 mg injected, 4.034 ng CGA-210007 quantitated, 0.094 ppm CGA-245704 equivalent determined, 94% CGA-245704 recovered.

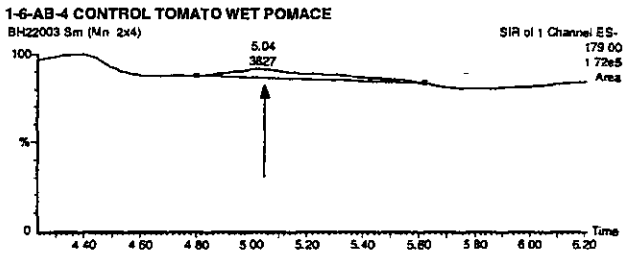


4. 2-8-AB-1, 1X treated unwashed tomato, 40.0 mg injected, 9.178 ng CGA-210007 quantitated, 0.268 ppm CGA-245704, 0.28 ppm CGA-245704 equivalent determined (corrected for 98% average recovery)

FIGURE 13. REPRESENTATIVE ION CHROMATOGRAMS FROM THE CONFIRMATORY ANALYSIS OF TOMATO WET POMACE

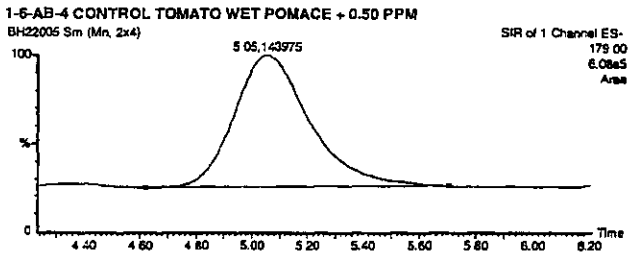


1. Calibration Standard, 10.0 ng injected.

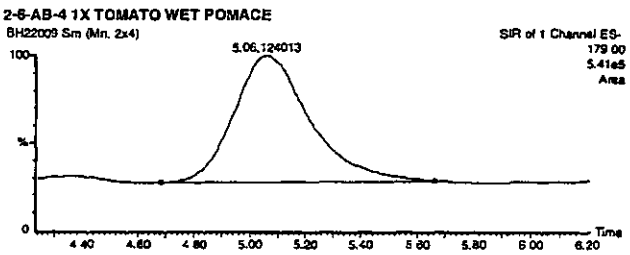


2. 1-6-AB-4, control, 50.0 mg injected, <0.50 ng (0.243 ng) CGA-210007 quantitated, <0.02 ppm (0.006 ppm) CGA-245704 equivalent determined.

FIGURE 13. REPRESENTATIVE ION CHROMATOGRAMS FROM THE CONFIRMATORY ANALYSIS OF TOMATO WET POMACE (Continued)

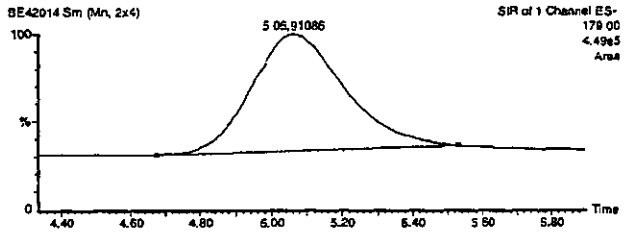


3. 1-6-AB-4, control + 0.50 ppm CGA-245704, 25.0 mg injected, 8.561 ng CGA-210007 quantitated, 0.395 ppm CGA-245704 equivalent determined (corrected for control residue), 79% CGA-245704 recovered

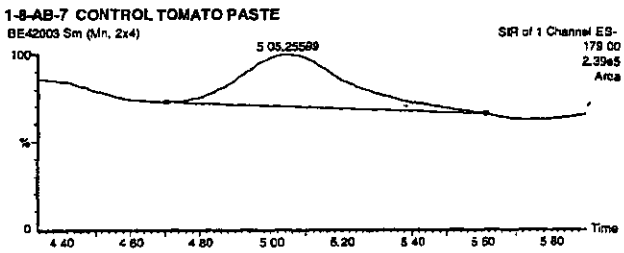


4. 2-6-AB-4, 1X treated tomato wet pomace, 50.0 mg injected, 7.377 ng CGA-210007 quantitated, 0.173 ppm CGA-245704, 0.20 ppm CGA-245704 equivalent determined (corrected for 85% average recovery)

FIGURE 14. REPRESENTATIVE ION CHROMATOGRAMS FROM THE CONFIRMATORY ANALYSIS OF TOMATO PASTE

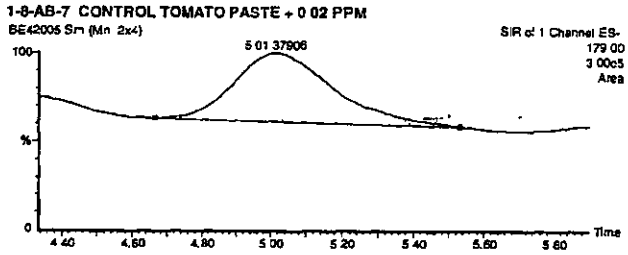


1. Calibration Standard, 5.0 ng injected.

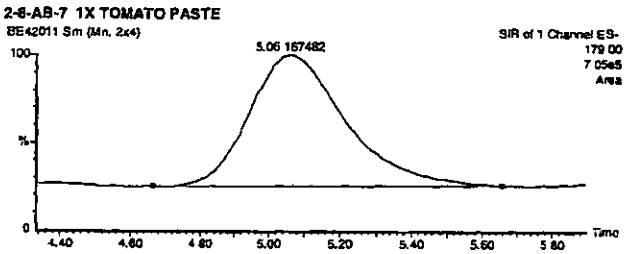


2. 1-8-AB-7, control, 50.0 mg injected, 1.649 ng CGA-210007 quantitated, 0.039 ppm CGA-245704, 0.05 ppm CGA-245704 equivalent determined (corrected for 78% average recovery)

FIGURE 14. REPRESENTATIVE ION CHROMATOGRAMS FROM THE CONFIRMATORY ANALYSIS OF TOMATO PASTE (Continued)



3 1-8-AB-7, control + 0.02 ppm CGA-245704, 50 0 mg injected, 2.342 ng CGA-210007 quantitated, 0 055 ppm CGA-245704 equivalent determined, 0 016 ppm CGA-245704 (corrected for control), 81% CGA-245704 recovered.



4. 2-8-AB-7, 1X treated tomato paste, 10.0 mg injected, 9.640 ng CGA-210007 quantitated, 1.128 ppm CGA-245704, 1.5 ppm CGA-245704 equivalent determined (corrected for 78% average recovery)

VII. REFERENCES

1. Formica, G., REM 172.02, "Determination of Total Residues of CGA-245704 as CGA-210007 by High Performance Liquid Chromatography (HPLC)," Cereals (Grain, Straw, and Green Aerial Part), December 6, 1993.

2. Formica, G., REM 172.11, "Determination of Total Residues of CGA-245704 as CGA-210007 by High Performance Liquid Chromatography," Plant Material and Milk, October 20, 1995.

3. Moore, M., "Preparation of Crop Samples for Residue Analysis," Product Safety Group SOP Number 7.21, Revision 2, Novartis Crop Protection, Inc., March 5, 1997.

4. Hayworth, C. and Grunenwald, M., ABR-97117, Final Report Amendment Number 1, "Validation of Draft Analytical Method AG-671A for the Determination of Total Residues of CGA-245704 as CGA-210007 in Tobacco, Leafy Vegetables, and Fruiting Vegetables by Column Switching High Performance Liquid Chromatography."

5. Nicollier, G., CIBA GEIGY Ltd., Basel, Switzerland, Plant Metabolism, "Behavior and Metabolism of CGA 245704 in Greenhouse Grown Tobacco after Foliar Spray Application of [U-^{14}C-Phenyl]-CGA 245704," Study 94WA04.

6. Kiffe, M., CIBA GEIGY Ltd., Basel, Switzerland, Plant Metabolism, "Metabolism of CGA 245704 in Greenhouse Grown Lettuce after Treatment with [Phenyl-U-^{14}C] Labelled Material, Study 96MK02.1," In-progress.

7. Nicollier, G., CIBA GEIGY Ltd., Basel, Switzerland, Plant Metabolism, "Metabolism of CGA 245704 in Greenhouse Grown Tomatoes after Treatment [Phenyl-U-^{14}C] Labeled Material," Study 94WA05.

8. Hayworth, C., Protocol 91-96, "CGA-245704 - Magnitude of the Residues in or on Tobacco," Novartis Crop Protection, Inc., April 17, 1996

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9. Hayworth, C , Protocol 92-96, "CGA-245704 - Magnitude of the Residues in or on Crop Group 4: Leafy Vegetables," Novartis Crop Protection, Inc , February 16, 1996.

10. Hayworth, C., Protocol 93-96, "CGA-245704 - Magnitude of the Residues in or on Crop Group 8: Fruiting Vegetables," Novartis Crop Protection, Inc , March 8, 1996.

APPENDIX I

SEPARATE DOCUMENTS ACCOMPANYING THIS REPORT

Formica, G., REM 172.02, "Determination of Total Residues of CGA-245704 as CGA-210007 by High Performance Liquid Chromatography (HPLC)," Cereals (Grain, Straw, and Green Aerial Part), December 6, 1993

Formica, G , REM 172 11, "Determination of Total Residues of CGA-245704 as CGA-210007 by High Performance Liquid Chromatography," Plant Material and Milk, October 20, 1995

CGA 245704	RESIDUE METHOD VALIDATED	REM 172.02
DETERMINATION OF TOTAL RESIDUES OF CGA 245704 AS CGA 210007 BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)	CEREALS (GRAIN, STRAW, AND GREEN AERIAL PART	DEC. 06, 1993 PP 2.53/Gi

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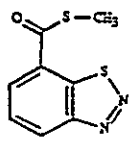
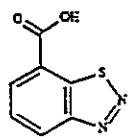
CHEMICAL STRUCTURES

CGA 245704



CGA 210007



1. INTRODUCTION

1.1 Scope of the Method

The method allows the quantitative determination of the combined residues of CGA 245704 and its metabolites which contain the CGA 210007 moiety in cereal (grain, straw and green aerial part or abbreviated simply "plant"). (See section 6.1, Figure 1 for structures and chemical names).

The lower practical level of quantitation by this method is 0.019 mg/kg for grain and 0.047 mg/kg for straw and plant calculated as CGA 245704 equivalents.

1.2 Principle of the Method

CGA 245704 and possible metabolites containing the CGA 210007 moiety are hydrolyzed to CGA 210007 by heating a homogenized subsample in the presence of sodium hydroxide. Methanol is added and the mixture shaken. An aliquot of the extract is cleaned up by acidic/alkaline/acidic partition. The reextract is cleaned up by a silica column. Additional clean up and final determination are performed by a two column HPLC switching system with UV-detection.

2. MATERIALS AND METHODS

Standard laboratory equipment is not listed. All equipment and chemicals mentioned herein can be substituted by suitable products of any origin. Prove suitability of reagents by analyzing reagent blanks.

2.1 Equipment

2.1.1 Rotating evaporator, Büchi, Rotavapor RE, Büchi AG, Flawil, CH.
2.1.2 Vacuum system, Terno Duo 500 S, Terno AG, Küblis, CH or commercial water aspirator.
2.1.3 Circulation cooler, mgw Lauda WK 450, Meßgeräte-Werke-Lauda, Dr. R. Wobser KG, Lauda-Königshofen, FRG.
2.1.4 Lab-shaker, A. Kühner AG, 4000 Basel, CH.
2.1.5 Folded filter paper, 15 cm diameter, Macherey-Nagel, 5160 Düren, FRG, Cat. No. MN 713 1/4.
2.1.6 Glass wool.
2.1.7 Ultrasonic bath, Bransonic 220, Branson Cleaning Equipment Co., Parrot Drive, Shelton, CT 06484-0768, USA.
2.1.8 Vortex mixer, Model Vortex-Genie, Scientific Industries Inc., Springfield, Massachusetts, USA.
2.1.9 Empty borosilicate glass cartridge, 8 mL volume, J. T. Baker Inc., Phillipsburg, N.J. 08865, USA, represented in Switzerland by P. H. Stehelin and Cie AG, 4003 Basel, Cat. No. 7328-06.
2.1.10 PTFE frits for 8 ml borosilicate glass cartridge, J. T. Baker Inc., Cat. No. 7329-06.
2.1.11 Adapter for 8 mL glass cartridge, J. T. Baker Inc., Cat No. 4528.
2.1.12 Glass reservoirs built in-house of about 25 ml content to be attached to the cartridges or equivalent (e.g. 25 mL Bond-Elut reservoirs, Varian, Harbor City CA, USA, Cat. No. A-1213-1011).
2.1.13 Vacuum manifold to accomodate solid-phase extraction cartridges, built in-house or commercial equivalent, e.g. VISIPREP, Supelco Inc., Bellefonte, Pennsylvania, USA, represented in Switzerland by Supelco SA, Gland, CH, Cat. No. 5-7030.
2.1.14 High Performance Liquid Chromatograph: refer to section 2.4.

2.2 Reagents and Standard

Main suppliers' addresses: E. Merck AG, 6100 Darmstadt, FRG.
Fluka Chemie AG, 9470 Buchs, CH.
J. T. Baker Inc., Phillipsburg, N.J. 08864, USA.

2.2.1 Water, demineralized and HPLC-grade, prepared in house, Ciba or HPLC-grade, J. T. Baker Inc., Cat. No. 4218.
2.2.2 Methanol for extraction, distilled in house, Ciba or analytical grade, E. Merck, Cat. No. 6009.
2.2.3 n-Hexane for residue analysis, Merck, Cat. No. 4371.
2.2.4 tert.-Butyl methyl ether (TBME), puriss., analytical grade, Fluka, Cat. No. 20249.
2.2.5 Methanol, LiChrosolv, HPLC-grade, Merck, Cat. No. 6018.
2.2.6 Acetonitrile, LiChrosolv, chromatography grade, Merck, Cat. No. 14291.
2.2.7 Hydrochloric acid 1 M, Ciba, Cat. No. 24 or J. T. Baker Inc., Cat. No. 7088 or about 1 M prepared by diluting of an analytical grade concentrated hydrochloric acid with HPLC-water .
2.2.8 ortho-Phosphoric acid, 85%, analytical grade, E. Merck, Cat. No. 573. Prepare a solution containing about 0.02 mole of phosphoric acid per liter HPLC water by diluting 2.3 g concentrated acid to 1 liter with water for HPLC.
2.2.9 Sodium hydroxide 1 M, Ciba, internal Cat. No. 17 or J. T. Baker Inc., Cat. No. 7097.
2.2.10 Silica Gel for flash chromatography, J. T. Baker Inc., Cat. No. 7024-01
2.2.11 Calcium chloride dihydrate, analytical grade, Merck, Cat. No. 2382.
2.2.12 Sodium chloride, analytical grade, Merck, Cat. No. 6404. Prepare a saturated solution of sodium chloride in water for HPLC (brine).
2.2.13 Ammonium chloride, analytical grade, Merck, Cat. No. 1145. Prepare a 0.05 M solution of ammonium chloride by dissolving 2.7 g salt in one liter of 0.02 M phosphoric acid (see above step 2.2.8).
2.2.14 CGA 245704 reference substance for recovery experiments. Prepare a stock solution containing 200 µg CGA 245704/mL acetonitrile Lichrosolv.
2.2.15 CGA 210007 reference substance for standardization. Prepare a stock solution containing 200 µg CGA 210007/mL acetonitrile LiChrosolv.

2.3 Analytical Procedure

2.3.1 Preparation of samples and subsamples

Prepare representative laboratory samples from the field samples (e.g. mix grain with dry ice and mill in a cross beater mill. Mix straw and plant with dry ice and chop using a suitable cutter). Analyze the samples immediately after preparation or store at about -20 °C until analysis.

For analysis weigh subsamples of e.g. 5 g of grain or e.g. 2 g of straw or plant into a 250 mL wide mouth jar. Avoid thawing of the frozen laboratory samples but preferably scratch off or drill out the subsample needed for analysis.

2.3.2 Fortification

To regularly check the performance of the method, analyze also at least two fortified control samples with each series of analyses. To prepare these samples, add known amounts of CGA 245704 to control samples prior to extraction.

Select fortification levels to be one and ten times the lower practical level of determination or in the range of the expected residue levels. Make sure that control samples neither are contaminated nor show interfering signals.

To fortify samples with 0.02 and 0.2 mg/kg for grain or 0.05 and 0.5 mg/kg for straw and plant, prepare solutions of CGA 245704 containing 0.1 and 1.0 µg per mL acetonitrile LiChrosolv by appropriate dilution of the stock solution (cf section 2.2.14). To prepare fortified samples, add 1 mL of one of the solutions to an untreated subsample. Proceed as described in section 2.3.3.

2.3.3 Hydrolysis/Extraction

Add as much water to the weighed subsample (cf. section 2.3.1) as to achieve a total water volume of 18 mL taking into account the crop solubles content being 1 mL for grain, 0.4 mL for straw and 1.6 mL for plant (see section 3.6 for moisture content). Add 2 mL of sodium hydroxide 1 M, seal the jar tightly, swirl the mixture and allow the jar to stand for about 30 minutes in an oven preheated to 50 - 55 °C. Swirl the jar occasionally. Allow the mixture to cool, add 80 mL (79 mL to fortified samples) of distilled methanol and 2 g of calcium chloride (the addition of the salt is necessary for grain and optionally for plant and straw. The salt facilitates the following filtration and reduces the formation of emulsion during the acidic partition, section 2.3.4). Shake the tightly sealed jar for about 30 minutes. Total volume of the extract is 100 mL. Filter immediately about 50 mL extract through a folded filter paper of 15 cm diameter into a 100 mL Erlenmeyer flask and continue according to section 2.3.4.

Remark: To avoid formation of emulsion during the following partition step, expecially for wheat grain samples, filtration and subsequent partition cleanup (section 2.3.4) have to be performed without interruption immediately after shaking with methanol. If this is not possible, the extraction mixtures are to be immediately stored at about -18 °C until filtration and partition can be done. After aliquoting for analysis, the remaining filtrates are to be immediately stored at about -18 °C, as reserve for potential further analysis.

2.3.4 Cleanup by Acidic Partition

Transfer 20 mL of filtrate, corresponding to 1 g grain or 0.4 g straw and plant material to a 250 mL separatory funnel. Add 50 mL water, 40 mL saturated sodium chloride solution and 8 mL 1 M hydrochloric acid. Shake the mixture to homogenize. Add 50 mL of a solvent mixture of hexane + tert.-butyl methyl ether 7 vol. + 3 vol. solvent mixture A). Shake and allow phases to separate. Drain the aqueous phase into a 250 mL Erlenmeyer flask. Filter the organic phase including emulsion, if present, into a clean 250 mL separatory funnel through a funnel plugged up with a loose plug of glass wool. Transfer the aqueous phase back to the separatory funnel. Add 50 mL of the solvent mixture A and shake Allow phases to separate. Drain and discard the aqueous phase. Filter the organic phase into the separatory funnel containing the first reextract through the funnel plugged with glass wool. Observe if water is present below the organic phase. In this case, drain and discard the water.

2.3.5 Cleanup by Alkaline/Acidic Partition

Add 20.0 mL of 1 N sodium hydroxide to the organic phase of the acidic partition clean up (section 2.3.4), stopper the separatory funnel and shake well. Allow phases to separate. Drain 15.0 mL of the alkaline phase, corresponding to 0.75 g of grain or to 0.3 g plant/straw, into a graduated 25 mL test tube and discard the rest. Add 2 mL of 85% phosphoric acid and 10 mL of the solvent mixture A (see section 2.3.4). Shake and allow phases to separate. Transfer the organic phase into a 100 mL round bottom flask by means of a Pasteur pipette. Repeat the partition two times more with portions of 10 mL of the same solvent mixture, each. Transfer the organic phase each time into the round bottom flask. Discard the aqueous phase. Evaporate the combined organic phases to dryness under reduced pressure using a rotating evaporator (water bath temp.: about 50 °C).

2.3.6 Cleanup by Silica Cartridge

Remark: To pack the cartridges used for this cleanup proceed as follows:

Insert a PTFE frit on the bottom of a borosilicate glass tube of 8 ml content, fill 1 g of silica gel into the tube and place a second frit on the top of the filling. Vibrate for ~1 minute for the material to settle, using a Vortex mixer and press the upper frit on the top of the adsorbent. Add 2 g of anhydrous sodium sulfate. The cartridge is ready for use.

Attach the cartridge packed as described above, to the vacuum manifold. Tightly connect a 25 mL glass reservoir to the top of the cartridge. Precondition the cartridge by passing through 5 mL of a solvent mixture of TBME + methanol LiChrosolv 85 vol. + 15 vol. by suction.

Dissolve the residue of the alkaline/acidic partition (section 2.3.5) in 5 mL of a solvent mixture of TBME + methanol LiChrosolv 85 vol. + 15 vol. and transfer the solution to the reservoir. Pass the mixture through the cartridge at a rate of 5 - 7 mL per minute by suction. Discard the eluate. Wash the flask with 5 mL of the same solvent mixture, transfer the wash to the reservoir and pass it through the cartridge. Discard the eluate. Elute CGA 210007 with 10 mL of a solvent mixture of TBME + methanol LiChrosolv 6 vol. + 4 vol. into a 25 mL round bottom flask by suction.

2.3.7 Preparation of the Final Solution

Evaporate the eluate of the silica cartridge cleanup (section 2.3.6) to dryness under reduced pressure using a rotating evaporator (water bath temp.: about 50 °C). Dissolve the residue in 3 mL of 0.02 N phosphoric acid in HPLC water using an ultra sonic bath (final solution).

2.4 Instrumentation

2.4.1 High Performance Liquid Chromatographic Systems (HPLC)

For the determination of CGA 210007 use a HPLC two-column switching system with UV-detector, pumps, autosampler-injector, columns and conditions as follows:

Detector:	Spectroflow 783 (ABI Analytical Kratos Division, Ramsey, NJ07446, USA) or equivalent.
Pumps:	Two Shimadzu Solvent Delivery Modules LC-9A, (Shimadzu Corporation, Analytical Instruments Division, Kyoto, Japan) or equivalent.
Valves:	Multiport Streamswitch "MUST" equipped with two low dead volume valves for column switching (Spark Holland, 7800 AJ Emmen, Holland) in connection with an automatic injection unit PROMIS II (Spark Holland) or equivalent.
Recorder:	Strip chart SE 120, dual channel (ABB, Goerz Metrawatt, 1101 Vienna, A) or equivalent.
Column oven:	Modell STH 585. Temperature range: 5 - 80 °C (Gynkotek GmbH) or equivalent.
REMARK:	Column oven is absolutely necessary in non thermostated laboratories to ensure constant elution times and reproducible cut of the a.i. from column to column (see below "Determination of Switching Interval").

Optional (for system automation):

Control and data collection unit:	HP 3350A Laboratory Automation System (Hewlett-Packard, Palo Alto, CA 94304, USA).
Column 1:	Stainless steel tube, 10 cm length, 2 mm i.d., packed with Nucleosil SB (strongly basic anion exchanger), particle size 5 µm (Dr. H. Knauer KG, 6370 Oberursel, FRG, Cat. No.: B45 - Y91) or equivalent.
Remark:	A new column has to be conditioned with the mobile phase 1 (see below) for about 8 hours or over night at a flow rate of 0.2 mL/min to obtain reproducible chromatograms.
Column 2:	Stainless steel tube, 10 cm length, 2 mm i.d., packed with Nucleosil 100 C_{18}, particle size 5 µm (Dr. H. Knauer KG, Cat. No.: B45 - Y76) or equivalent.

For the HPLC system, connect pumps, injector, columns, valves and detector according to Figure 3 (section 6.1).

Mobile phase 1:	0.05 M ammonium chloride in 0.02 M phosphoric acid + acetonitrile Lichrosolv 85 vol. + 15 vol.
Mobile phase 2:	0.02 M phosphoric acid in Water for HPLC + acetonitrile Lichrosolv 76 vol. + 24 vol.
Flow rate 1:	0.2 ml/min

Retention time: about 13 min (column 1)

Flow rate 2: 0.2 ml/min

Retention time: about 22 min (columns 1 and 2)

Injection vol.: 50 µL

Recorder: 10 mV full scale

Column oven: 23 °C

Chart speed: 0.5 - 1.0 cm/min

Detector: Wave length: 235 nm
sensitivity: 0.005 aufs

Determination of Switching Interval

Determine actual switching times each time a series of samples is to be quantitated. Connect the outlet of column 1 directly to the UV-detector. Inject 50 µL of a 0.08 µg CGA 210007/mL standard solution (see below section 2.4.2) and measure the begin and the end times of the peak of CGA 210007. Thereafter, connect column 2 to the detector and switch that portion of eluate from column 1 which contains CGA 210007, e.g. portion between 12 min 00 s and 13 min 24 s to column 2 (see section 6.2.7, chromatogram A for an example of measurement of switching times).

2.4.2 Preparation of Standard Solution

Standardize the chromatographic system each time a series of samples is to be quantitated. The range of the concentrations is depending on the range of residues to be determined, in particular, the lowest standard concentration is depending on the lower practical level.

Calculate the lowest standard concentration (C) as follows:

$$C = \frac{L \cdot F \cdot A}{V_f} \quad [\mu g/mL]$$

L = lower practical level [mg/kg] or [µg/g]
F = conversion factor from CGA 245704 to CGA 210007
A = aliquot of crop cleaned up through silica [g]
V_f = volume of the final solution [mL]

With the values proposed in this method, the lowest standard concentration is 0.004 µg CGA 210007/mL as presented below:

$$C = \frac{0.019 \times 0.857 \times 0.75}{3} = 0.004 \ \mu g/mL \quad \text{for grain and}$$

$$C = \frac{0.047 \times 0.857 \times 0.30}{3} = 0.004 \ \mu g/mL \quad \text{for plant/straw}$$

Prepare at least four standard solutions of different concentrations by appropriately diluting the stock solution of CGA 210007 (section 2.2.15) with 0.02 M phosphoric acid in water for HPLC.

Select the concentrations as required; typical values are 0.08, 0.04, 0.01 and 0.004 µg/mL.

The conversion factor from CGA 210007 to CGA 245704 is: 1.17

2.4.3 Quantitation of Residues

As the detector response may change on injection of coextractives, it is recommended to inject standards and final solutions alternately. Inject 50 µL of standard solutions (corresponding to 4.0, 2.0, 0.5 and 0.2 ng CGA 210007, respectively) and final solutions. Measure the response of the analyte at the characteristic retention time and calculate response function and residues as detailed in REM 119.04 (Ciba, Plant Protection Division, Residue Analysis, 1991).

2.5 Interferences

No interferences which disturbed the determination of the CGA 210007 were encountered so far.

2.6 Confirmatory Techniques

No specific conditions have been worked out so far.

2.7 Time Required for Analysis

A total of about 15 hours is required to work up a set of 12 samples to the point of HPLC injection. Automated HPLC analysis can be performed overnight.

2.8 Modifications and Potential Problems

a) The filtration of the extraction mixtures (section 2.3.3) may be slow if calcium chloride is not added. Furthermore, the filtrate can be turbid.

b) Extracts and filtrates (see section 2.3.3) must not remain at room temperature before partition, otherwise an emulsion appears during the partition cleanup.

3. RESULTS AND DISCUSSION

3.1 Accuracy and Precision

The overall accuracy and precision of the method is: (status as of Nov. 1993)

$\bar{x}$ = 91%, s_{abs} = 6.6%, n = 50.

Find typical recovery percentages in section 6.3.

3.2 Limit of Determination

The ultimate limit of determination of this method was not determined. The lower practical level of CGA 245704 quantitated by this method is: 0.019 mg/kg for grain and 0.047 mg/kg for plant and straw.

3.3 Ruggedness

The method was tested by three technicians using fortified samples. The analyses were performed on different days with different instrumentations.

Results obtained were comparable with regard to recovery percentages and chromatographic profile.

3.4 Limitations

None known, so far.

3.5 Method Validation

The performance of the method has been checked with recovery experiments, section 6.3.

3.6 Correction for the Increase of the Effective Extraction Volume by Soluble Sample Constituents

The soluble part of the sample (i.e. water and low molecular plant constituents) has to be considered for correct results. Exact values for this contribution are not easily available. In view of the relatively high imprecision of residue analytical results, rough estimates of the volume of the soluble portion of the samples are acceptable, as long as the total volume is known with reasonable accuracy.

In view of an overall precision of ± 2% for the total volume of the extract, it is sufficient to know the volume contribution of the solubles by ± 20%, provided that the ratio sample size/solvent added is 0.1, e.g. sample size 10 g, solvent added 100 mL (specific weight of the soluble = 1 is supposed, the volume contraction on mixing is neglected).

For the calculation procedure of this method, the unknown exact volume of the solubles are replaced by rough estimates:

The various crop materials are classified into three groups. The volumes of soluble constituents are estimated using group factors (G) of 0.8, 0.5 and 0.2 mL/g, respectively. The following classification bases on average values of solubles.

Group I: Volume contribution 0.8 mL per gram matrix.
With the limits ± 20% as given above, the group value of 0.8 mL/g covers "true values" between 0.6 and 1.0 mL/g with sufficient precision.
e.g.: - Fruit
- Vegetables
- Green parts
- Fodder
- Meat

Group II: Volume contribution 0.5 mL per gram matrix.
"True values" between 0.3 and 0.7 mL/g are covered with sufficient precision.
e.g.: - Nuts
- Oil seeds

Group III: Volume contribution 0.2 mL per g matrix.
"True values" between 0 and 0.4 mL/g are covered with sufficient precision.
e.g.: - Cereals
- Straw
- Peas and beans
- Other dried crops
- Soil

4. CERTIFICATION

The experimental results included in this method REM 172.02 are certified to be authentic accounts of the experiments. All raw data of results mentioned have bee audited by the internal Quality Assurance Unit (QUA) and are stored in archives of Residue Analysis PP 2.53.

Statement of compliance with Good Laboratory Practice (GLP).

The recovery studies were performed in compliance with "Procedure and Principles of GLP in Switzerland", issued by the Department of the Interior, Bern, 1986, equivalent to "OECD Principles of Good Laboratory Practice", Paris, 1981. The facility was accredited by Swissauthorities to be in compliance with OECD GLP-principles.

5. REFERENCES

- REM 119.04: General Method for Calculation of Residue Analytical Results, Ciba, Plant Protection Division, Residue Analysis, 4002 Basel, CH.
- REM 172.03: "CGA 245704, DETERMINATION OF TOTAL RESIDUES AS CGA 210007 BY HPLC IN SOIL", Dr. G. Formica and C. Giannone, Sep. 10, 1993, CIBA, Plant Protection Division, Residue Analysis, 4002 Basel, CH.

Collaborator: H Borell

Formica

Dr. G. Formica
Ciba
Plant Protection Division
Residue Analysis (PP 2.53)
R-1098.P.09
CH-4002 Basel
Tel. (061) 697 27 85

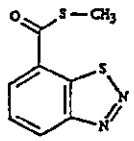
C. Giannone

6. FIGURES AND TABLES

6.1 Figures

Figure 1: Structures and Chemical Names

CGA 245704

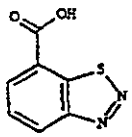


$C_8H_6N_2OS_2$

Molecular mass: 210.28

7-S-methyl-1,2,3-benzothiadiazolethioate

CGA 210007

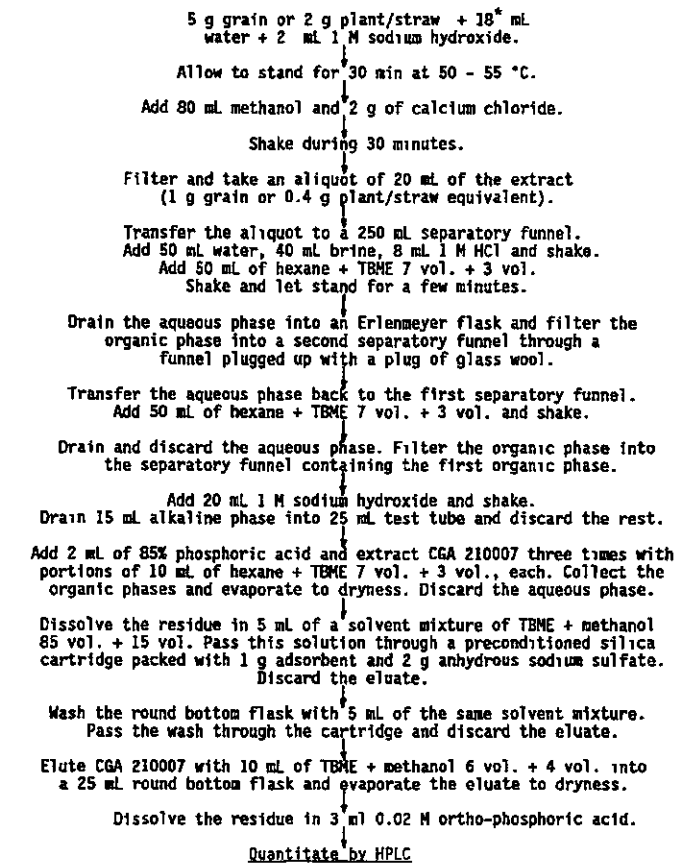


$C_7H_4N_2O_2S$

Molecular mass: 180.18

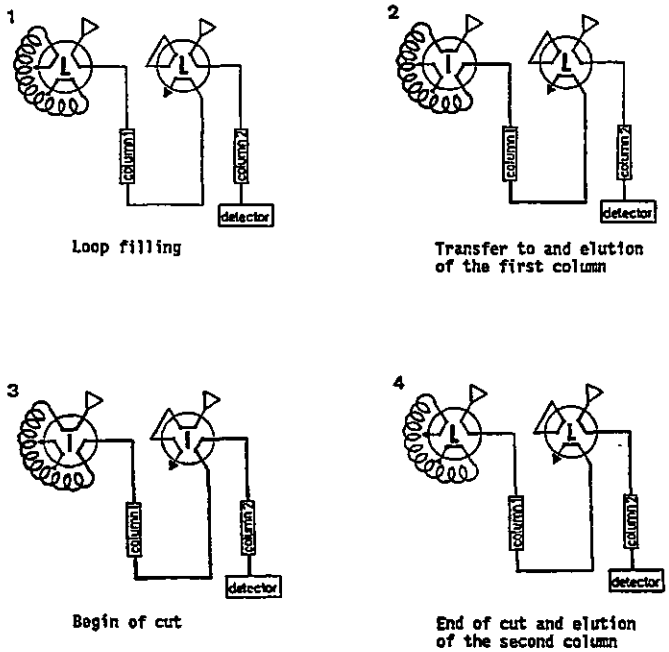
1,2,3-Benzothiadiazol-7-carboxylic acid

Figure 2: Procedure Flow Diagram



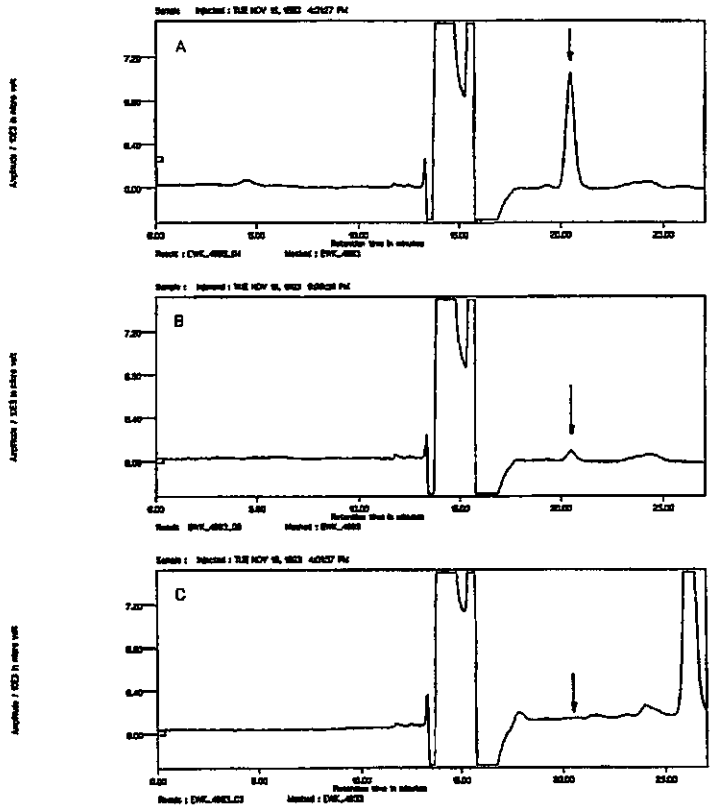
* The moisture content of crop is to be taker into consideration (see section 2.3 3 and section 3.6)

Figure 3: Chromatographic System for Column Switching



6.2 Representative Chromatograms

6.2.1 Representative chromatograms for wheat grain.
File: E. Wilhelm; book 10; Nov. 17, 1993

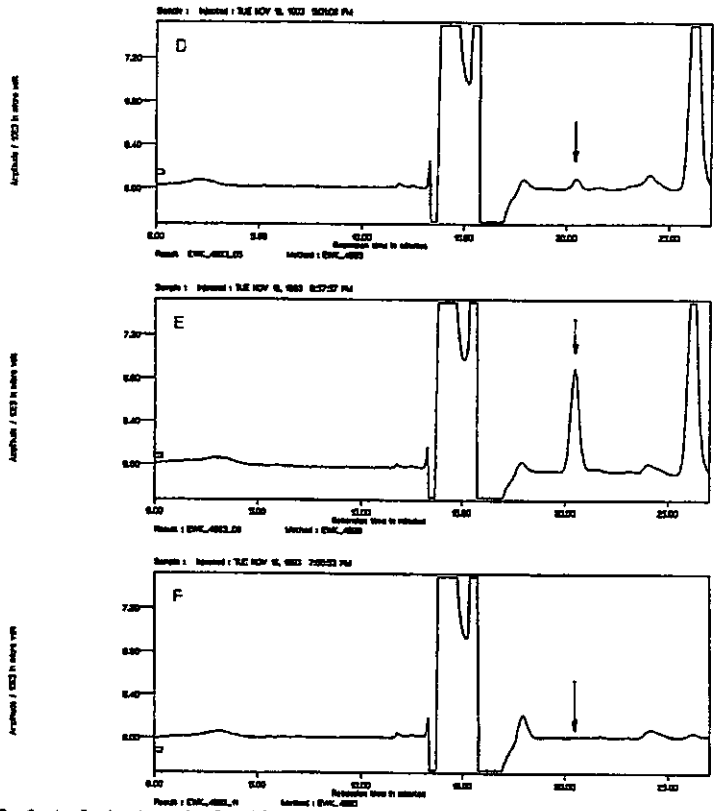


A: Standard solution of CGA 210007. Volume injected corresponds to 2.0 ng a.i.

B: Standard solution of CGA 210007. Volume injected corresponds to 0.2 ng a.i.

C: Control wheat grain Aliquot injected corresponds to 12.5 mg grain.

6.2.1 (cont.)

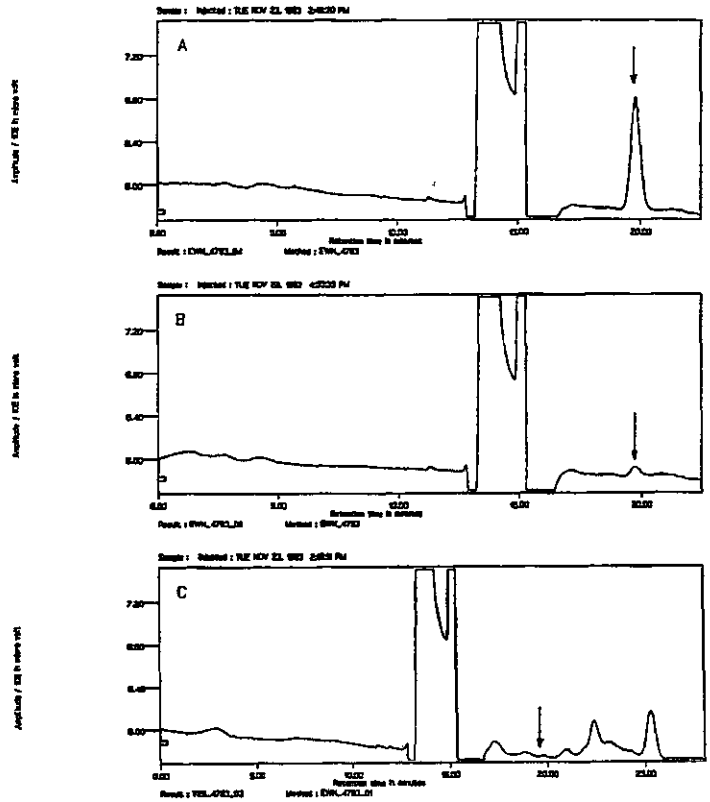


D: Control wheat grain fortified with 0 02 mg CGA 245704 per kg.
Aliquot injected corresponds to 12.5 mg grain or to 0.214 ng CGA 210007.
Value found: 0.191 ng CGA 210007, corresponding to 90%.

E: Control wheat grain fortified with 0.20 mg CGA 245704 per kg
Aliquot injected corresponds to 12.5 mg grain or to 2.14 ng CGA 210007.
Value found: 1.86 ng CGA 210007, corresponding to 87%.

F: Sample from treated field. Aliquot injected corresponds to 12.5 mg grain.

6.2.2 Representative chromatograms for wheat straw.
File: E. Wilhelm; book 10; Nov. 24, 1993

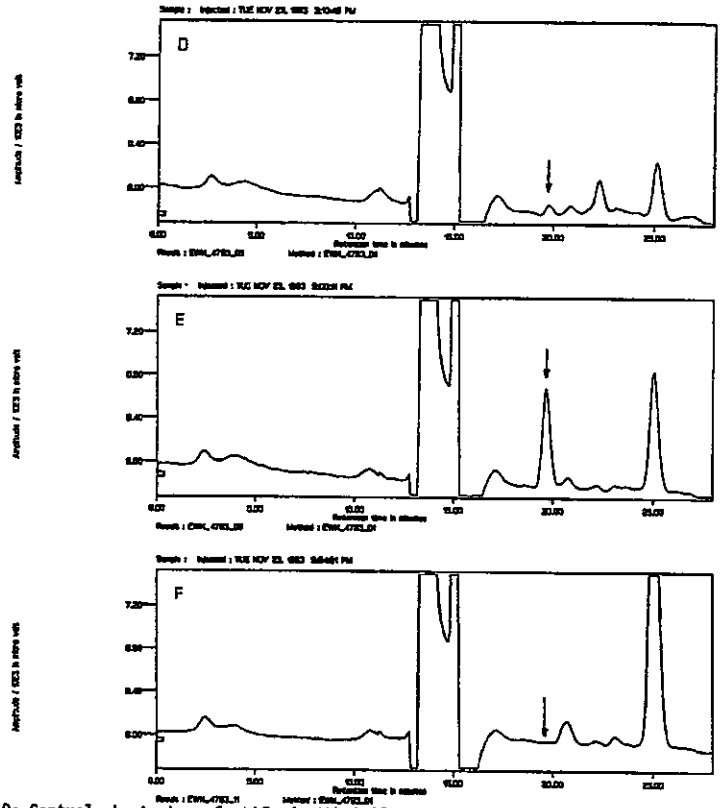


A Standard solution of CGA 210007. Volume injected corresponds to 2.0 ng a.i

B: Standard solution of CGA 210007. Volume injected corresponds to 0.2 ng a.i.

C: Control wheat straw. Aliquot injected corresponds to 5 mg straw.

6.2.2 (cont.)

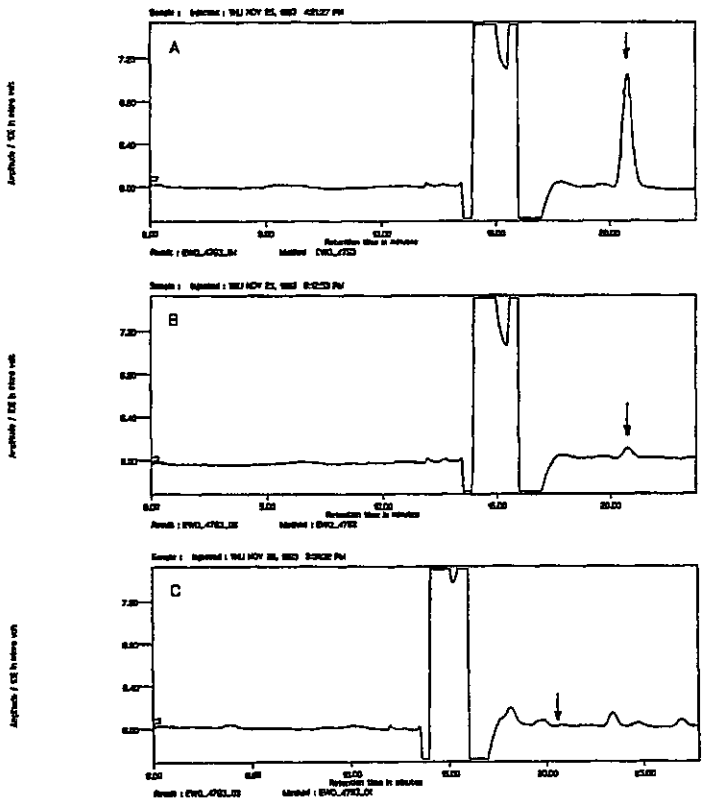


D: Control wheat straw fortified with 0.05 mg CGA 245704 per kg.
Aliquot injected corresponds to 5 mg straw or to 0.214 ng CGA 210007.
Value found: 0.192 ng CGA 210007, corresponding to 90%.

E: Control wheat straw fortified with 0.50 mg CGA 245704 per kg.
Aliquot injected corresponds to 5 mg straw or to 2.14 ng CGA 210007.
Value found: 1.73 ng CGA 210007, corresponding to 81%.

F: Sample from treated field. Aliquot injected corresponds to 5 mg straw.

6.2.3 Representative chromatograms for wheat plant.
File: E. Wilhelm; book 10; Nov. 26, 1993

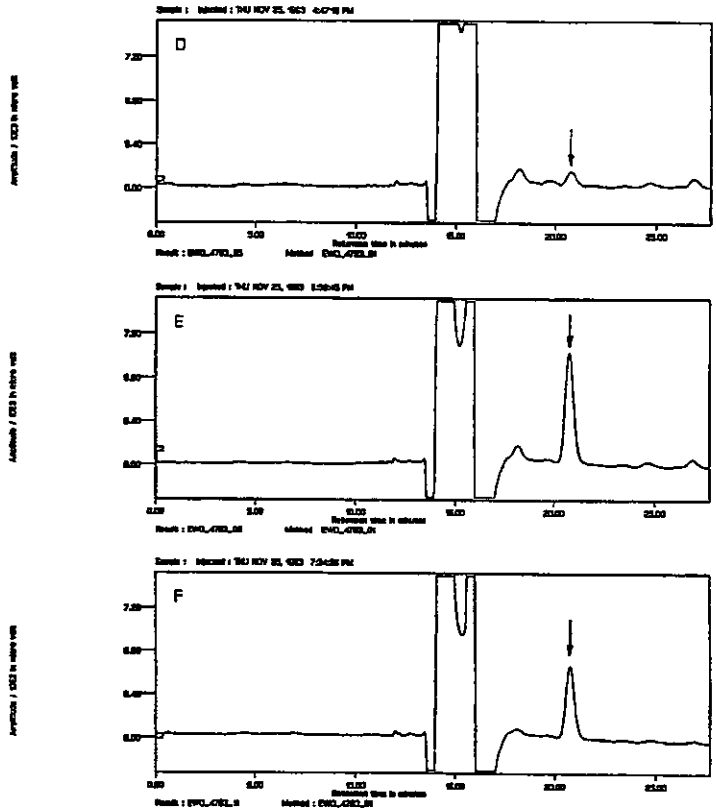


A: Standard solution of CGA 210007. Volume injected corresponds to 2.0 ng a.i.

B: Standard solution of CGA 210007. Volume injected corresponds to 0.2 ng a.i.

C: Control wheat plant. Aliquot injected corresponds to 5 mg plant.

6.2.3 (cont.)

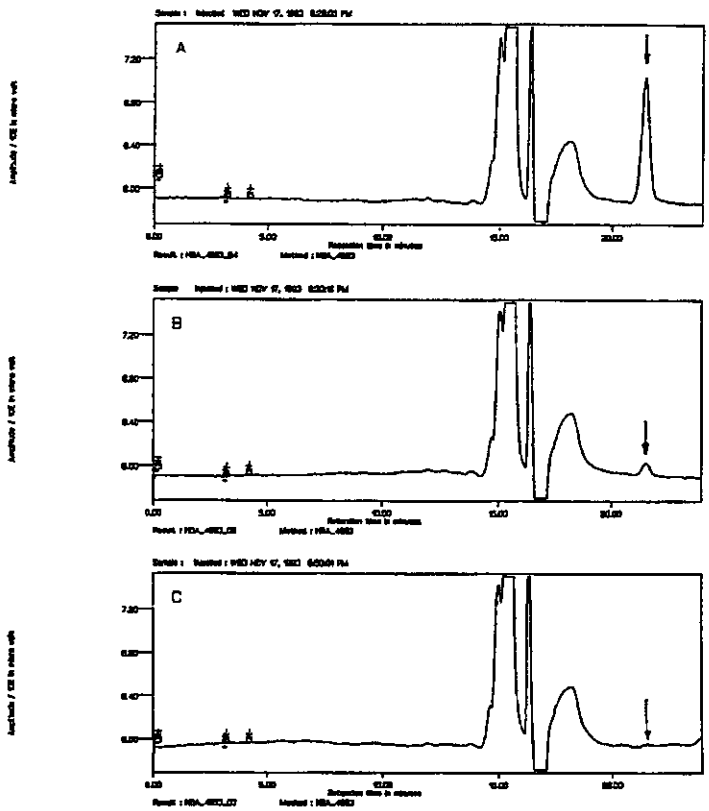


D: Control wheat plant fortified with 0.05 mg CGA 245704 per kg.
Aliquot injected corresponds to 5 mg plant or to 0.214 ng CGA 210007.
Value found: 0.23 ng CGA 210007, corresponding to 107%.

E: Control wheat plant fortified with 0.50 mg CGA 245704 per kg.
Aliquot injected corresponds to 5 mg plant or to 2.14 ng CGA 210007.
Value found: 1.92 ng CGA 210007, corresponding to 90%.

F: Sample from treated field. Aliquot injected corresponds to 5 mg plant.
Value found: 1.27 ng CGA 210007, corresponding to 1.49 mg CGA 245704/kg.

6.2.4 Representative chromatograms for barley grain.
File: H. Borell; book 12; Nov. 18, 1993

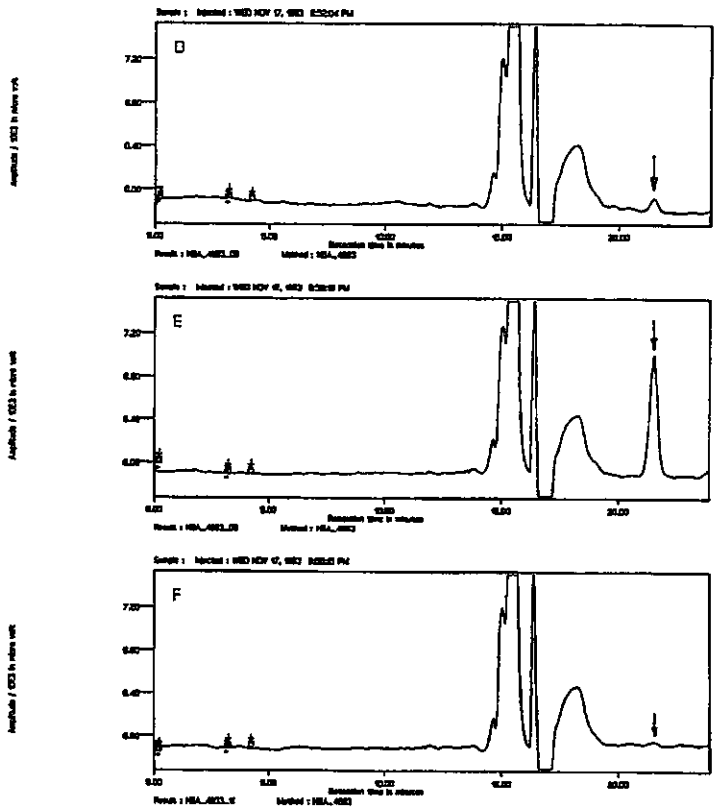


A: Standard solution of CGA 210007. Volume injected corresponds to 2.0 ng a.i.

B: Standard solution of CGA 210007. Volume injected corresponds to 0.2 ng a.i.

C: Control barley grain. Aliquot injected corresponds to 12.5 mg grain.

6.2.4 (cont.)

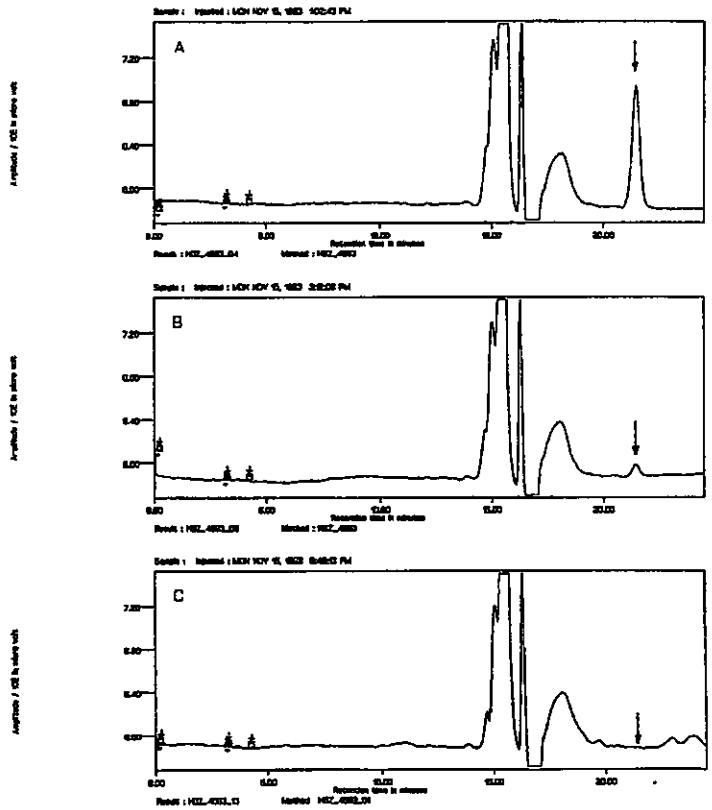


D: Control barley grain fortified with 0.02 mg CGA 245704 per kg.
Aliquot injected corresponds to 12.5 mg grain or to 0.214 ng CGA 210007.
Value found: 0.206 ng CGA 210007, corresponding to 97%.

E: Control barley grain fortified with 0.20 mg CGA 245704 per kg.
Aliquot injected corresponds to 12.5 mg grain or to 2.14 ng CGA 210007.
Value found: 1.95 ng CGA 210007, corresponding to 91%.

F: Sample from treated field. Aliquot injected corresponds to 12.5 mg grain.

6.2.5 Representative chromatograms for barley straw.
File: H. Borell; book 12; Nov. 16, 1993

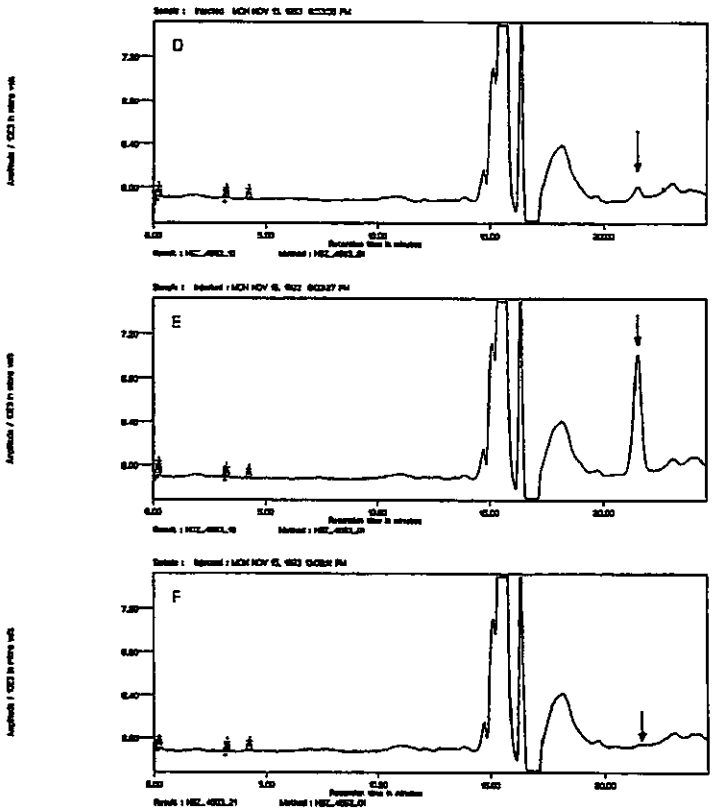


A: Standard solution of CGA 210007. Volume injected corresponds to 2.0 ng a.i.

B: Standard solution of CGA 210007. Volume injected corresponds to 0.2 ng a.i.

C: Control barley straw. Aliquot injected corresponds to 5 mg straw.

6.2.5 (cont.)

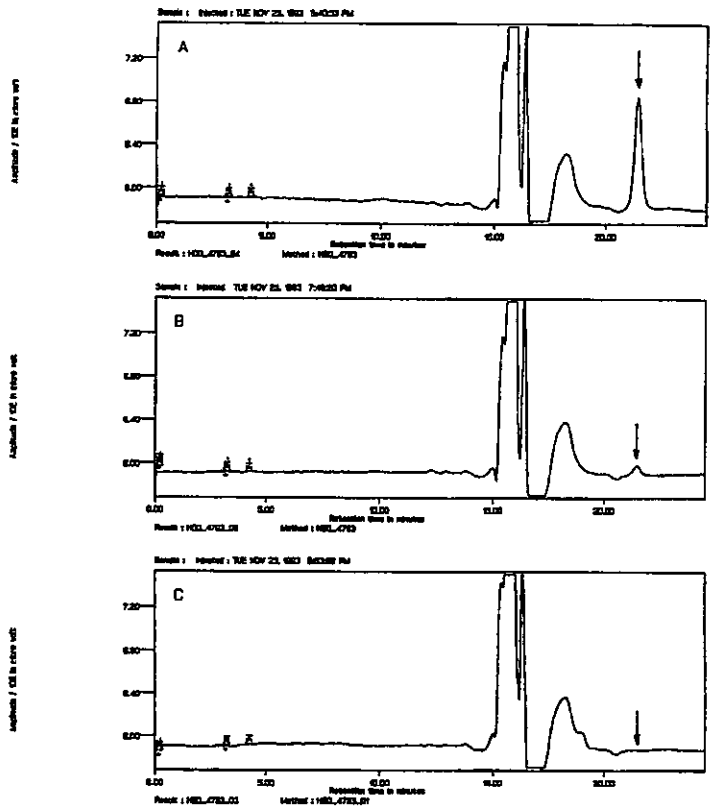


D: Control barley straw fortified with 0.05 mg CGA 245704 per kg.
Aliquot injected corresponds to 5 mg straw or to 0.214 ng CGA 210007.
Value found: 0.203 ng CGA 210007, corresponding to 95%.

E: Control barley straw fortified with 0.50 mg CGA 245704 per kg.
Aliquot injected corresponds to 5 mg straw or to 2.14 ng CGA 210007.
Value found: 1.93 ng CGA 210007, corresponding to 90%.

F: Sample from treated field. Aliquot injected corresponds to 5 mg straw.

6.2.6 Representative chromatograms for barley plant.
File: H. Borell; book 12; Nov. 24, 1993

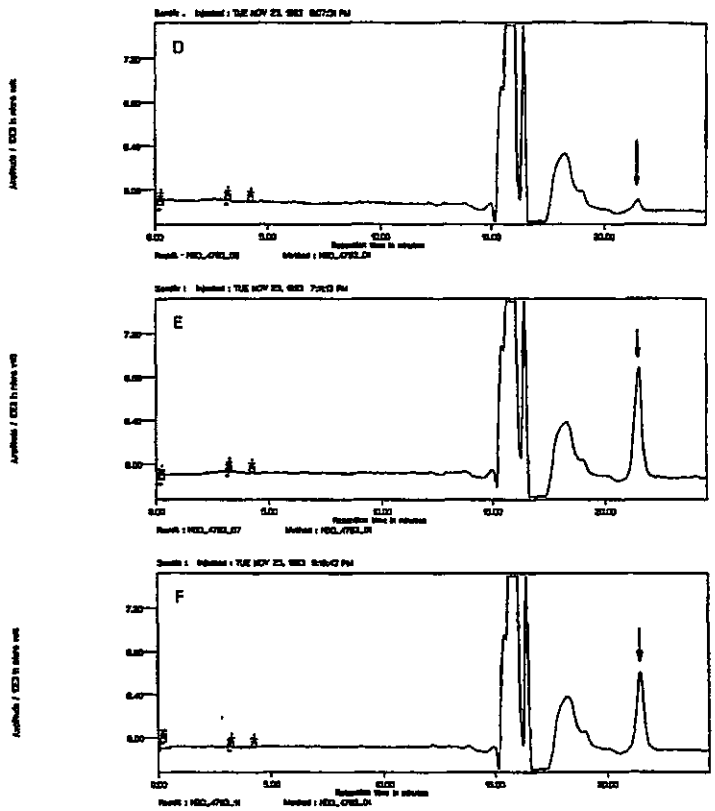


A: Standard solution of CGA 210007. Volume injected corresponds to 2.0 ng a.i.

B: Standard solution of CGA 210007. Volume injected corresponds to 0.2 ng a.i.

C: Control barley plant. Aliquot injected corresponds to 5 mg plant.

6.2.6 (cont.)

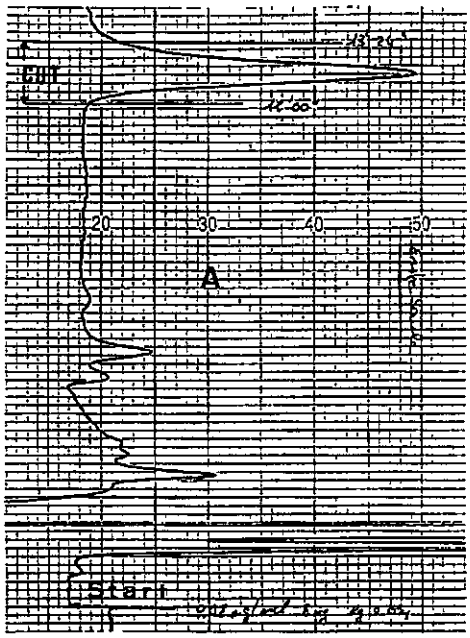


D: Control barley plant fortified with 0.05 mg CGA 245704 per kg.
Aliquot injected corresponds to 5 mg plant or to 0.214 ng CGA 210007.
Value found: 0.215 ng CGA 210007, corresponding to 100%.

E: Control barley plant fortified with 0.50 mg CGA 245704 per kg.
Aliquot injected corresponds to 5 mg plant or to 2.14 ng CGA 210007.
Value found: 1.99 ng CGA 210007, corresponding to 93%.

F: Sample from treated field. Aliquot injected corresponds to 5 mg plant.
Value found: 1.46 ng CGA 210007, corresponding to 0.34 mg CGA 245704/kg.

6.2.7 Typical chromatogramm for the determination of the switching interval.



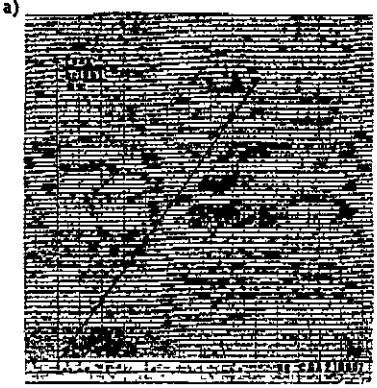
A: Example of standard solution chromatographed only through column 1 to determine the switching interval (cut). In this case the "cut" is between 12 min 00 s and 13 min 24 s. Amount of CGA 210007 injected: 4 ng.

6.2.8 Typical calibration curves of CGA 210007.

a) File: H. Borell; Book 12; Oct. 27, 1993.
Wheat grain; Sequence: HBU_4393.

b) File: H. Borell; Book 12; Nov. 18, 1993.
Barley grain; Sequence: HBA_4693.

a)

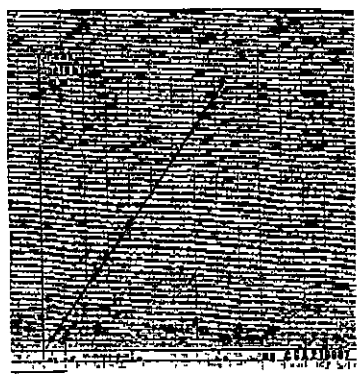


CGA 210007	Peak height	
ng	x	x̄*
4.0	121/114/109	113
2.0	60/56/63	56.8
0.5	15/14/13.5	14.3
0.2	6.0/5.8/5.5	5.75

* Mean values calculated according to REN 119.04 (see References, section 5).

Absolute standard deviation: 4.6%.

b)



CGA 210007	Peak height	
ng	x	x̄*
4.0	120/122	120
2.0	60.4/60	60.0
0.5	14.7/14.9	15.1
0.2	6.30/6.16	6.18

* Mean values calculated according to REN 119.04 (see References, section 5).

Absolute standard deviation: 1.7%.

6 3 RECOVERIES

Status: As of Nov. 1993

TYPICAL PERCENT RECOVERIES USING METHOD REM 172.02

Substrate	Fortification level [mg/kg]	Percent recovered
Wheat grain	0.02	98/98/97/90/93
	0.04	98/86/93/88/92
	0.20	94/89/88/87/84
Wheat plant	0.05	75/94/107
	0.10	80/97/95
	0.50	93/98/90
Wheat straw	0.05	98/78/88/90/92
	0.10	93/86/87/80/76
	0.50	93/80/89/81/82
Barley grain	0.02	97/97
	0.04	92/93
	0.20	91/89
Barley plant	0.05	100
	0.50	93
Barley straw	0.05	95
	0.10	91
	0.50	90

Average recovery: $\bar{X}$ = 91%, s_{abs} = 6.6%, n = 50

Acceptable recovery percentages: 71 - 111%

Further recovery values may be added and the range of acceptable recoveries may be updated accordingly.

The above recoveries are documented in the following files:

H. Borell; Book 12; Oct. 27, 1993. REPORT ON RESIDUE STUDY: 2067/92
H. Borell; Book 12; Oct. 27, 1993. REPORT ON RESIDUE STUDY: 2068/92
H. Borell; Book 12; Nov. 02, 1993. REPORT ON RESIDUE STUDY: 2067/92
H. Borell; Book 12; Nov. 08, 1993. REPORT ON RESIDUE STUDY: 2068/92
H. Borell; Book 12; Nov. 24, 1993. REPORT ON RESIDUE STUDY: 2027/93
H. Borell; Book 12; Nov. 18, 1993. REPORT ON RESIDUE STUDY: 2027/93
H. Borell; Book 12; Nov. 16, 1993. REPORT ON RESIDUE STUDY: 2027/93
H. Borell; Book 12; Nov. 16, 1993. REPORT ON RESIDUE STUDY: 2026/93
H. Borell; Book 12; Nov. 22, 1993. REPORT ON RESIDUE STUDY: 2026/93
H. Borell; Book 12; Nov. 22, 1993. REPORT ON RESIDUE STUDY: 2028/93

C. Giannone; Book 3R; Oct. 20, 1993. REPORT ON RESIDUE STUDY: 2067/92
C. Giannone; Book 3R; Oct. 20, 1993. REPORT ON RESIDUE STUDY: 2068/92

E. Wilhelm; Book 10; Nov. 26, 1993. REPORT ON RESIDUE STUDY: 2023/93
E. Wilhelm; Book 10; Nov. 24, 1993. REPORT ON RESIDUE STUDY: 2023/93
E. Wilhelm; Book 10; Nov. 24, 1993. REPORT ON RESIDUE STUDY: 2024/93
E. Wilhelm; Book 10; Nov. 17, 1993. REPORT ON RESIDUE STUDY: 2024/93
E. Wilhelm; Book 10; Nov. 17, 1993. REPORT ON RESIDUE STUDY: 2023/93

For all experiments mentioned, a Quality Assurance Statement was released by the internal QUA.

FILE /REP/CG/REM172.02

Dr. G. Formica
Residue Analysis
R-1098 P.09
Tel. 72785/72795

15 June 1994

AMENDMENT 1 *

also filed REM 1993

TO CGA 245704, REM 172.02

Page 4 of the above Method has been revised.

Reason

Special attention must be paid to the word "preated". In the revised page, the word " preheated" has been emphasized

15. Jun 1994 Formica
date Dr. G Formica

* Revised page enclosed

File Am to REM172 02

Select fortification levels to be one and ten times the lower practical level of determination or in the range of the expected residue levels. Make sure that control samples neither are contaminated nor show interfering signals.

To fortify samples with 0.02 and 0.2 mg/kg for grain or 0.05 and 0.5 mg/kg for straw and plant, prepare solutions of CGA 245704 containing 0.1 and 1.0 µg per mL acetonitrile LiChrosolv by appropriate dilution of the stock solution (cf section 2.2.14). To prepare fortified samples, add 1 mL of one of the solutions to an untreated subsample. Proceed as described in section 2.3.3.

2.3.3 Hydrolysis/Extraction

Add as much water to the weighed subsample (cf. section 2.3.1) as to achieve a total water volume of 18 mL taking into account the crop solubles content being 1 mL for grain, 0.4 mL for straw and 1.6 mL for plant (see section 3.6 for moisture content). Add 2 mL of sodium hydroxide 1 M, seal the jar tightly, swirl the mixture and allow the jar to stand for about 30 minutes in an oven preheated to 50-55 °C. Swirl the jar occasionally. Allow the mixture to cool, add 80 mL (79 mL to fortified samples) of distilled methanol and 2 g of calcium chloride (the addition of the salt is necessary for grain and optionally for plant and straw. The salt facilitates the following filtration and reduces the formation of emulsion during the acidic partition, section 2.3.4). Shake the tightly sealed jar for about 30 minutes. Total volume of the extract is 100 mL. Filter immediately about 50 mL extract through a folded filter paper of 15 cm diameter into a 100 mL Erlenmeyer flask and continue according to section 2.3.4.

Remark: To avoid formation of emulsion during the following partition step, expecially for wheat grain samples, filtration and subsequent partition cleanup (section 2.3.4) have to be performed without interruption immediately after shaking with methanol. If this is not possible, the extraction mixtures are to be immediately stored at about -18 °C until filtration and partition can be done. After aliquoting for analysis, the remaining filtrates are to be immediately stored at about -18 °C, as reserve for potential further analysis.

2.3.4 Cleanup by Acidic Partition

Transfer 20 mL of filtrate, corresponding to 1 g grain or 0.4 g straw and plant material to a 250 mL separatory funnel. Add 50 mL water, 40 mL saturated sodium chloride solution and 8 mL 1 M hydrochloric acid. Shake the mixture to homogenize. Add 50 mL of a solvent mixture of hexane + tert.-butyl methyl ether 7 vol + 3 vol. solvent mixture A). Shake and allow phases to separate. Drain the aqueous phase into a 250 mL Erlenmeyer flask. Filter the organic phase including emulsion, if present, into a clean 250 mL separatory funnel through a funnel plugged up with a loose plug of glass wool. Transfer the aqueous phase back to the separatory funnel. Add 50 mL of the solvent mixture A and shake. Allow phases to separate. Drain and discard the aqueous phase. Filter the organic phase into the separatory funnel containing the first reextract through the funnel plugged with glass wool. Observe if water is present below the organic phase. In this case, drain and discard the water.

* Revised page according to Amendment 1

Ciba Crop Protection

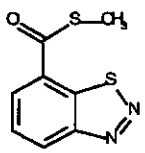
ciba
Ciba-Geigy Limited
CH-4002 Basel

CGA 245704	RESIDUE METHOD VALIDATED	REM 172.11
DETERMINATION OF TOTAL RESIDUES OF CGA 245704 AS CGA 210007 BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY	PLANT MATERIAL AND MILK	Oct. 20, 1995 PP 2 533/Fo

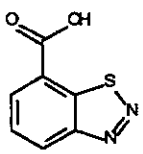
TABLE OF CONTENTS

CHEMICAL STRUCTURES

CGA 245704



CGA 210007



1. INTRODUCTION

1.1 Scope of the Method

The method allows the quantitative determination of the combined residues of CGA 245704 and its metabolites which contain the CGA 210007 moiety in plant material and milk (see section 6.1, Figure 1 for structures and chemical names)
The limits of quantitation of this method are 0.02 mg/kg for plant material and 0 005 mg/kg for milk, calculated as CGA 245704 equivalents

1.2 Principle of the Method

CGA 245704 and possible metabolites containing the CGA 210007 moiety are hydrolyzed to CGA 210007 by heating a homogenized subsample in the presence of sodium hydroxide. Methanol is added and the mixture shaken. An aliquot of the extract is cleaned up by acidic/alkaline/ acidic partition A further cleanup by a silica column (see section 2.3.6) may be necessary for some crop materials Final determination is performed by a two column HPLC switching system with UV-detection
The principle of this method and the analytical procedure described are based on REM 172 02 (see reference 5.1)

2. MATERIALS AND METHODS

Standard laboratory equipment is not listed All equipment and chemicals mentioned herein can be substituted by suitable products of any origin Prove suitability of reagents by analyzing reagent blanks

2.1 Equipment

2.1.1 Rotating evaporator, Büchi, Rotavapor RE, Büchi AG, Flawil, CH
2.1.2 Vacuum system, Temo Duo 500 S, Temo AG, Kübis, CH or commercial water aspirator
2 1.3 Circulation cooler, mgw Lauda WK 450, Messgeräte-Werke-Lauda, Dr R. Wobser KG, Lauda-Königshofen, FRG, Cat No. 7329-06.
2.1.4 Lab-shaker, A. Kühner AG, 4000 Basel, CH
2.1.5 Folded filter paper, 15 cm diameter, Macherey-Nagel, 5160 Düren, FRG, Cat. No MN 713 1/4
2 1.6 Ultrasonic bath, Bransonic 220, Branson Cleaning Equipment Co., Par rot Drive, Shelton, CT 06484-0768, USA.
2 1 7 High Performance Liquid Chromatograph, refer to section 2.4

The following equipment is needed only when the silica column cleanup (see section 2.3 6) is necessary

2.1.8 Vacuum manifold to accomodate solid-phase extraction cartridges, built in-house or commercial equivalent, e g. VISIPREP, Supelco, Bellefonte, Pennsylvania, USA, represented in Switzerland by Supelco SA, 9471 Buchs, CH, Cat. No 5-7030
2 1.9 Vortex mixer, Model Vortex-Genie, Scientific Industries Inc , Spring field, Massachusetts, USA.
2.1.10 Empty borosilicate glass tube, 8 mL volume, J T Baker Inc., Phillipsburg, N.J. 08865, USA, represented in Switzerland by P. H Stehelin and Cie AG, 4003 Basel, Cat. No 7328-06.
2.1.11 PTFE frits for 8 ml borosilicate glass cartridge, J T. Baker Inc., Cat. No 7329-06
2 1 12 Adapter for 8 mL glass tube, J T Baker Inc., Cat No 4528.
2 1 13 Glass reservoirs built in-house of about 25 ml content to be attached to the cartridges or equivalent (e.g. 25 mL Bond-Elut reservoirs, Varian, Harbor City CA, USA, Cat. No. A-1213-1011)

2.2 Reagents and Standard

Main suppliers' addresses. E. Merck AG, 6100 Darmstadt, FRG.
Fluka Chemie AG, 9470 Buchs, CH.
J T Baker Inc., Phillipsburg, N.J 08864, USA

2.2 1 Water, HPLC-grade, prepared in house, Ciba or J T. Baker Inc., Cat. No 4218
2.2.2 Methanol for extraction, distilled in house, Ciba or analytical grade, E. Merck, Cat. No 6009
2.2.3 n-Hexane for residue analysis, Merck, Cat. No 4371
2.2.4 tert -Butyl methyl ether (TBME), puriss., analytical grade, Fluka, Cat. No 20249.

2.2.5 Methanol, LiChrosolv, HPLC-grade, Merck, Cat. No. 6018
2.2.6 Acetonitrile, LiChrosolv, chromatography grade, Merck, Cat No. 14291.
2.2.7 Hydrochloric acid 1 M, Ciba, Cat. No. 24 or J. T. Baker Inc., Cat. No. 7088 or about 1 M prepared by diluting analytical grade concentrated hydrochloric acid with HPLC-water
2.2.8 ortho-Phosphoric acid, 85%, analytical grade, E. Merck, Cat. No 573. Prepare a solution containing about 0 02 mole of phosphoric acid per liter HPLC water by diluting 2.3 g concentrated acid to 1 liter with water for HPLC
2.2 9 Sodium hydroxide 1 M, Ciba, Internal Cat. No. 17 or J T. Baker Inc., Cat. No 7097.
2 2 10 Silica Gel for flash chromatography, J T. Baker Inc , Cat. No. 7024-01
2.2 11 Calcium chloride dihydrate, analytical grade, Merck, Cat. No. 2382.
2.2 12 Sodium chloride, analytical grade, Merck, Cat. No 6404 Prepare a saturated solution of sodium chloride in water for HPLC (brine).
2.2.13 Ammonium sulfate, analytical grade, Merck, Cat. No. 1217. Prepare a 0.05 M solution of ammonium sulfate by dissolving 6.61 g salt in one liter of 0 02 M phosphoric acid (see section 2 2.8)
2 2 14 CGA 245704 reference substance for recovery experiments. Prepare a stock solution containing 200 µg CGA 245704/mL acetonitrile Lichrosolv.
2 2 15 CGA 210007 reference substance for standardization. Prepare a stock solution containing 200 µg CGA 210007/mL acetonitrile LiChrosolv.

REMARK

CGA 245704 solutions in acetonitrile have been demonstrated to rapidly degrade if exposed to day light at room temperature Therefore prepare solutions of CGA 245704 in brown bottles. However, if a stock solution of 200 µg /mL acetonitrile is stored in the dark at -20 °C, the a.i. is stable at least for 6 months.

2.3 Analytical Procedure

2.3.1 Preparation of Specimens and Subspecimens

Prepare representative laboratory specimens from the field specimens (e.g. mix plant material with dry ice and chop using a suitable cutter).
Analyze the specimens immediately after preparation or store them at about -20 °C until analysis.
For analysis let milk specimens thaw and shake vigorously Weigh subspecimens of e g 10 g of plant material or 20 g milk into a 250 mL wide mouth jar Avoid thawing of the frozen laboratory plant material specimens, but preferably scratch off or drill out the subspecimens needed for analysis

2.3 2 Fortification

To regularly check the performance of the method, analyze also at least two fortified control specimens with each series of analyses. To prepare these specimens, add known amounts of CGA 245704 to control specimens prior to extraction
Select fortification levels to be one and ten times the limit of quantitation (LOQ) or in the range of the expected residue levels Make sure that control specimens neither are contaminated nor show interfering signals.

To fortify specimens with 0 02 and 0.2 mg/kg (plant material) or 0 005 and 0.05 mg/kg (milk), prepare standard solutions of CGA 245704 containing 0 2 and 2.0 µg/mL acetonitrile LiChrosolv by appropriate dilution of the stock solution (see section 2.2.14) To prepare 0 02 and 0 2 mg/kg fortified specimens (for plant material) add 1 mL of one of the standard solutions to an uncontaminated plant material subspecimen To prepare 0 005 and 0.05 m/kg fortified specimens (for milk) , add 0.5 mL of one of the standard solutions to an uncontaminated milk subspecimen. Proceed as described in section 2.3 3.

REMARK

The standard solutions used for the fortification were demonstrated to be chemically stable for at least 24 days, if they are kept in brown volumetric flasks and stored in a refrigerator at 4 °C.

2.3.3 Hydrolysis/Extraction

2.3.3.1 Plant Material

Add as much water to the weighed plant material subspecimens (cf. section 2.3.1) as to achieve a total water volume of 18 mL, taking into account the soluble part of the subspecimens (see section 2.10). Add 2 mL of sodium hydroxide 1 M, seal the jar tightly, swirl the mixture and allow the jar to stand for about 30 minutes in an oven preheated to 55-60 °C. Swirl the jar occasionally. Allow the mixture to cool, add 80 mL (79 mL to fortified subspecimens) of distilled methanol and 2 g of calcium chloride (the addition of the salt may improve, for some materials, the following filtration and reduces the formation of an emulsion during the acidic partition, see section 2.3.4). Shake the tightly sealed jar for about 30 minutes The total volume of the extract is 100 mL. Filter immediately about 50 mL extract through a folded filter paper of 15 cm diameter into a 100 mL Erlenmeyer flask and continue according to section 2.3.4

2.3.3.2 Milk

Add 4 mL of sodium hydroxide 1 M, seal the jar tightly, swirl the mixture and allow the jar to stand for about 30 minutes in an oven preheated to 55-60 °C. Swirl the jar occasionally Allow the mixture to cool, add 101 mL of distilled methanol (100.5 mL to fortified milk subspecimens) and 2 g of calcium chloride. Shake the tightly sealed jar for about 30 minutes. The total volume of the extract is 125 mL. Filter immediately about 50 mL extract through a folded filter paper of 15 cm diameter into a 100 mL Erlenmeyer flask and continue according to section 2.3.4

2.3.4 Cleanup by Acidic Partition

Transfer 10 mL (plant material) and 25 mL (milk) of filtrate, corresponding to 1 g plant material and 4 g milk, respectively, to a 500 mL separatory funnel Add 50 mL water, 40 mL saturated sodium chloride solution and 8 mL 1 M hydrochloric acid. Shake the mixture to homogenize. Add 50 mL of a solvent mixture of hexane + tert.-butyl-methyl ether 7 vol. + 3 vol (solvent mixture A) Shake and allow phases to separate. Drain the aqueous phase and the emulsion between both phases, if there is any, into a 250 mL Erlenmeyer flask . Drain the organic phase into a clean 250 mL separatory funnel. Transfer the content of the Erlenmeyer flask back to the separatory funnel. Add 50 mL of the solvent mixture A and shake Allow phases to separate Drain and discard the aqueous phase. Drain the organic phase into the separatory funnel containing the first reextract. Observe if water is present below the organic phase. If this is the case, drain and discard the water.

2.3.5 Cleanup by Alkaline/Acidic Partition

Add 20.0 mL of 1 M sodium hydroxide to the organic phase of the acidic partition clean up (section 2.3.4), stopper the separatory funnel and shake well. Allow phases to separate Drain 15.0 mL of the alkaline phase, corresponding to 0.75 g of plant material or 3 g of milk, into a graduated 25 mL test tube and discard the rest. Add 2 mL of 85% phosphoric acid and 10 mL of the solvent mixture A (see section 2.3.4). Shake and allow phases to separate. Transfer the organic phase into a 100 mL round bottom flask by means of a Pasteur pipette, taking care not to take along water drops! Repeat the partition two times more with portions of 10 mL of the same solvent mixture, each. Transfer the organic phase each time into the round bottom flask and discard the aqueous phase Observe again the combined organic phase to make sure that no water drops have been taken along Evaporate the organic phase to dryness under reduced pressure using a rotating evaporator (water bath temp . about 50 °C)

REMARK

The following described cleanup by silica is not necessary for the crops tested in this method and for milk. For these materials, it can be omitted However, for other, not yet analyzed plant materials , this additional silica cleanup step may be necessary.

2.3 6 Cleanup by Silica Cartridge

To pack the cartridges used for this cleanup, proceed as follows: Put down a PTFE frit onto the bottom of a borosilicate glass tube of 8 mL content, fill 1 g of silica gel into the tube and place a second frit onto the top of the filling Using a Vortex mixer, vibrate for approx. 1 minute for the material to settle. Press the upper frit onto the top of the adsorbent. Add 2 g of anhydrous sodium sulfate and place a third frit on the top Vibrate as before for about 30 s and press the upper frit on the top The cartridge is ready for use.
Attach the cartridge packed as described above, to the vacuum manifold Tightly connect a 25 mL glass reservoir to the top of the cartridge Precondition the cartridge by sucking through 5 mL of a solvent mixture of TBME + methanol LiChrosolv 85 vol. + 15 vol.
Dissolve the residue of the alkaline/acidic partition (see section 2.3 5) in 5 mL of a solvent mixture of TBME + methanol LiChrosolv 85 vol. + 15 vol and transfer the solution to the reservoir. Pass the mixture through the cartridge at a rate of 5 - 7 mL per minute by suction Discard the eluate Wash the flask with 5 mL of the same solvent mixture, transfer the wash to the reservoir and pass it through the cartridge, too. Discard the eluate. Elute CGA 210007 with 10 mL of a solvent mixture of TBME + methanol LiChrosolv 6 vol. + 4 vol. into a 25 mL round bottom flask by suction. Evaporate the eluate to dryness under reduced pressure using a rotating evaporator (water bath temp.: about 50 °C).

2 3 7 Preparation of the Final Solution

Dissolve the residue obtained according to the procedure described in section 2 3.5 or 2 3 6 in 3 mL of 0 02 M phosphoric acid in HPLC water using an ultra sonic bath (final solution)

2.4 Instrumentation

2.4.1 High Performance Liquid Chromatographic System (HPLC)
For the determination of CGA 210007 use a HPLC two-column switching system with UV-detector, pumps, autosampler-injector, columns and conditions as follows

Detector:	Spectroflow 783 (ABI Analytical, Kratos Division, Ramsey, J07446, USA) or equivalent.
Pumps:	Pumps must allow constant, pulsefree flowrate of 0.2 mL/min. Two Shimadzu Solvent Delivery Modules LC-9A (Shimadzu Corporation, Analytical Instruments Division, Kyoto, Japan) or equivalent
Valves.	Multiport Streamswitch "MUST" equipped with two low dead volume valves for column switching (Spark Holland, 7800 AJ Emmen, Holland) in connection with an automatic injection unit PROMIS II (Spark Holland) or equivalent.
Recorder	Strip chart SE 120, dual channel (ABB, Goerz Metrawatt, 1101 Vienna, A) or equivalent.
Column oven	Temperature range 5 - 80 °C. Modell STH 585 (Gynkotek GmbH) or equivalent.
REMARK	A column oven is absolutely necessary in non thermostated laboratories to ensure constant elution times and a reproducible cut of the a.i. from column to column (see below "Determination of Switching Interval")

Optional (for system automation)

Control and collection unit	HP 3350A Laboratory Automation System (Hewlett-Packard, Palo Alto, data CA 94304, USA).
Column 1:	Stainless steel tube, 10 cm length, 2 mm i.d., packed with Nucleosil SB, strongly basic anion exchanger, particle size 5 µm, Cat. No : B45 - Y91, ("Wissenschaftliche Gerätebau" Dr. H. Knauer GmbH, 14163 Berlin, FRG) or equivalent.
REMARK	A new column 1 has to be conditioned with the mobile phase 1 (see below) overnight at a flow rate of 0.2 mL/min The retention time of CGA 210007 has to range between 10 and 15 min. If the retention time is above this range, the elution strengt of mobile phase 1 has to be adjusted accordingly. For example, if the measured retention time is 20 min, the amount of acetonitrile in the mobile phase 1 has to be gradually increased to a maximum of 20%.(Remember peak broadening occurs with an increasing amount of acetonitrile in the mobile phase 1, which results in a loss of overall sensitivity. Therefore keep the amount of acetonitrile in the mobile phase 1 as low as possible. The retention time on the column 1 has to be checked at each start of a new sequence and, if necessary, the concentration of acetonitrile has to be adjusted accordingly.
Column 2	Stainless steel tube, 10 cm length, 2 mm I d , packed with Nucleosil 100 C_{18}, particle size 5 µm, Cat No : B45 - Y76) ("Wissenschaftliche Gerätebau" Dr H. Knauer GmbH, 14163 Berlin, FRG) or equivalent

For the HPLC system, connect pumps, injector, columns, valves and detector according to Figure 3 (see section 6 1).

Mobile phase 1.	0 05 M ammonium sulfate in 0 02 M phosphonc acid + acetonitrile LiChrosolv 85 vol. + 15 vol. The composition of this mobile phase has to be adjusted as explained in the remark above
Mobile phase 2	0 02 M phosphonc acid in water for HPLC + acetonitrile LiChrosolv 76 vol + 24 vol
Flow rate 1:	0 2 ml/min.
Retention time	10-15 min (column 1)
Flow rate 2:	0.2 ml/min.
Retention time	15-19 min. (columns 1 and 2)
Injection vol .	50 µL
Recorder	10 mV full scale
Column oven	23 °C
Chart speed	0.5 - 1 0 cm/min
Detector:	Wave length: 235 nm; sensitivity: 0 004 aufs

Determination of Switching Interval

Determine actual switching times each time a series of specimens is to be quantitated. Connect the outlet of column 1 directly to the UV-detector. Inject 50 µL of a 0.08 µg CGA 210007/mL standard solution (see section 2.4 2) and measure the begin and the end times of the CGA 210007 peak . Thereafter, connect column 2 to the detector and switch that portion eluate 1 which contains CGA 210007, e.g the portion between 11 min 30 s and 13 min 30 s from column 1 to column 2 (in this case the cut time is 2 min.). See section 6.2.2 for an example of the measurement of switching times.

REMARK

If an interference peak, which disturbs the quantitation of CGA 210007, appears in the control sample, reduce the "cut time" in portions of about 15 s each, until the interference disappears or is reduced to an insignificant height (below the limit of quantitation)

2 4 2 Calibration of the Chromatographic System by External Standards

Standardize the chromatographic system each time a series of specimens is to be quantitated The range of the concentrations is depending on the range of residues to be determined, in particular, the lowest standard concentration is depending on the limit of quantitation

Calculate the lowest standard concentration (C) as follows:

$$C = \frac{LOQ * F * A}{VF} [\mu g/mL]$$

LOQ	Limit of quantitation [mg/kg] (0 02 for plant material or 0 005 for milk)
F	Conversion factor from CGA 245704 to CGA 210007 (0 857)
VF.	Volume of the final solution [mL]
A	Aliquot of crop cleaned up in g (0 75 g for plant or 3.0 g for milk)

With the values proposed in this method, the lowest standard concentration is 0 004 µg CGA 210007/mL, as calculated below

$$C = \frac{0\ 02\ (0.005) \times 0.857 \times 0\ 75\ (3\ 0)}{3} = 0\ 004\ [\mu g/mL]$$

Prepare at least four standard solutions of different concentrations by appropriately diluting the stock solution of CGA 210007 (see section 2.2 15) with 0 02 M phosphonc acid in water for HPLC.
Select the concentrations as required, typical values are 0 08, 0 04, 0.01 and 0 004 µg/mL. As the detector response may change on injection of coextractives, it is recommended to inject standards and final solutions alternately Inject 50 µL of standard solutions (corresponding to 4 0, 2.0, 0.5 and 0.2 ng CGA 210007, respectively) and final solutions.

The conversion factor from CGA 210007 to CGA 245704 is 1.17

2.5 Interferences

No interferences which hindered the determination of the CGA 210007 have been encountered so far

2.6 Confirmatory Techniques

No specific conditions have been worked out so far.

2.7 Time Required for Analysis

A total of about 2 working days is required to work up a set of 12 specimens to the point of HPLC injection. Automated HPLC analysis can be performed overnight.

2.8 Modifications and Potential Problems

See last remark on page 6

2.9 Calculation Procedure

Measure the response of the analyte at the characteristic retention time and calculate response function and residues as detailed in REM 119 06 (refence 5.2).

2.10 Estimation of the Effective Extraction Volume

The soluble part of the specimen (i.e. water and low molecular plant constituents) has to be considered for correct results. Exact values for this contribution are not easily available. In view of the relatively high imprecision of residue analytical results, rough estimates for the volume of the solubles are acceptable as long as the total volume is known with reasonable accuracy

In view of an overall accuracy of ±2% for the total extraction volume it is sufficent to know the volume contribution of the solubles by ±20%, provided that the ratio specimen size/solvent added is 0 1, e.g. specimen size 10 g and solvent added 100 mL (supposing the specific weight of the solubles to be 1 g/mL and the volume contraction on mixing to be neglected)

For the calculation procedure of this method, the unknown exact volumes of the solubles are replaced by rough estimates:

The various crop materials are classified into three groups. The volumes of soluble constituents are estimated using group-factors (G) of 0 8, 0.5 and 0.2 mL/g The following classification is based on average values of solubles [5.3]:

Group I: Volume contribution of 0.8 mL per gram matrix
With the limits ±20 % as given above, the group value of 0.8 mL/g covers "true values" between 0 6 and 1 0 mL/g with sufficient accuracy
E g.. Fruits, vegetables, green parts, fodder, meat

Group II Volume contribution of 0 5 mL per gram matrix.
"True values" between 0 3 and 0 7 mL/g are covered with sufficient accuracy
E.g · Nuts, oil seed.

Group III: Volume contribution of 0.2 mL per gram matrix.
"True values" between 0 and 0.4 mL/g are covered with sufficient accuracy
E.g · Cereals, straw, peas and beans, other dried crops, often also soil but check water content first.

3. RESULTS AND DISCUSSION

3.1 Accuracy and Precision

The overall accuracy and precision of the method is (status as of Sep.28, 1995)

$\overline{x}$ = 93.5% Sabs = 7.2% n = 76

Acceptable recovery percentages 73 - 113 %

3 2 Limit of Quantitation

The limit of quantitation LOQ of this method is 0 02 mg/kg for plant material and 0.005 mg/kg for milk.

3.3 Ruggedness

The method was successfully tested by two technicians using fortified specimens. The analyses were performed on different days with different instrumentation
The results obtained were comparable with regard to recovery percentages and chromatographic profile.

3.4 Limitations

None known so far.

3.5 Method Validation

The method has been validated by performing recovery experiments. The report on this validation study (130/95) including QA-statement is attached to this method as section 7 1

4. CERTIFICATION

The experimental results included in this method REM 172.11 are certified to be authentic accounts of the experiments.

Origin of data: Special Study 130/95.

5. REFERENCES

5.1 REM 172.02. "CGA 245704, DETERMINATION OF TOTAL RESIDUES AS CGA 210007 BY HPLC IN CEREALS", Dr. G. Formica and C Giannone, Dec. 06, 1993, CIBA, Crop Protection Division, Residue Analysis, 4002 Basel, CH.

5.2 REM 119.06· General Method for Calculation of Residue Analytical Results, Ciba, Crop Protection Division, Residue Analysis, 4002 Basel, CH

5 3 Documenta Geigy, Wissenschaftliche Tabelle, Ed J R. Geigy AG, CH-Basel, 7th ed.1968, p. 494-511

Collaborator: H. Borell

Formica

Dr G. Formica
Ciba
Crop Protection Division
Residue Analysis (PP 2 533)
R-1098.P 09
CH-4002 Basel
Tel. (061) 697 27 85

H Baell

H. Borell

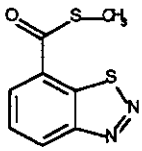
File: REM17211

6. FIGURES AND TABLES

6.1 Figures

Figure 1. Structures and Chemical Names

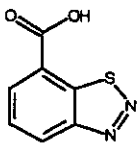
CGA 245704



Benzo[1,2,3]thiadiazole-7-carbothioic acid S-methyl ester

$C_9H_6N_2OS_2$ Molecular mass: 210.28

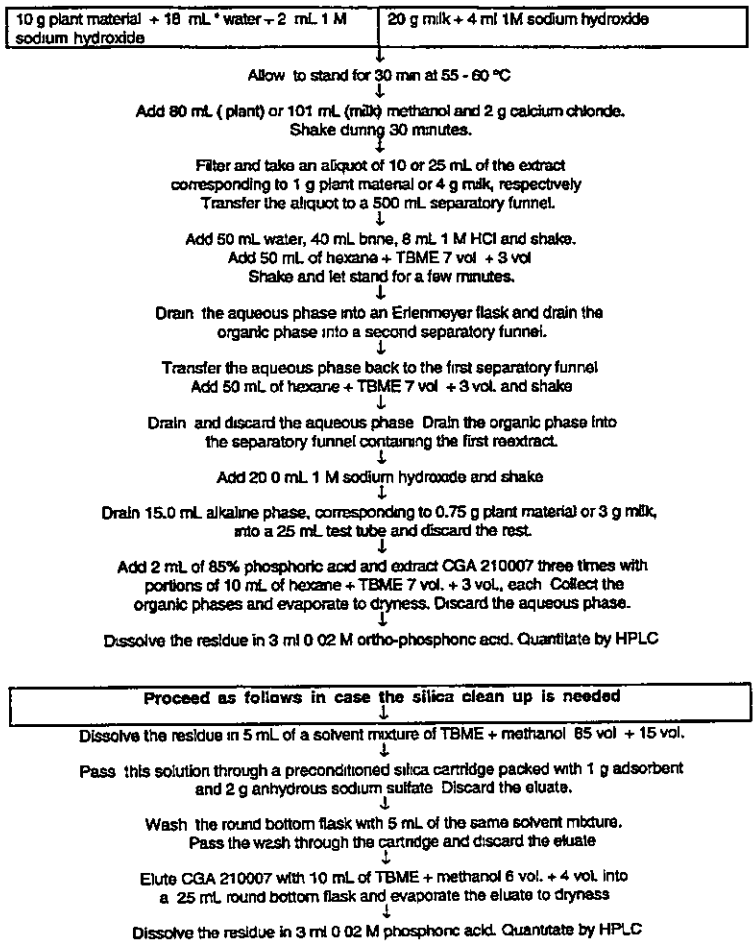
CGA 210007



Benzo[1,2,3]thiadiazole-7-carboxylic acid

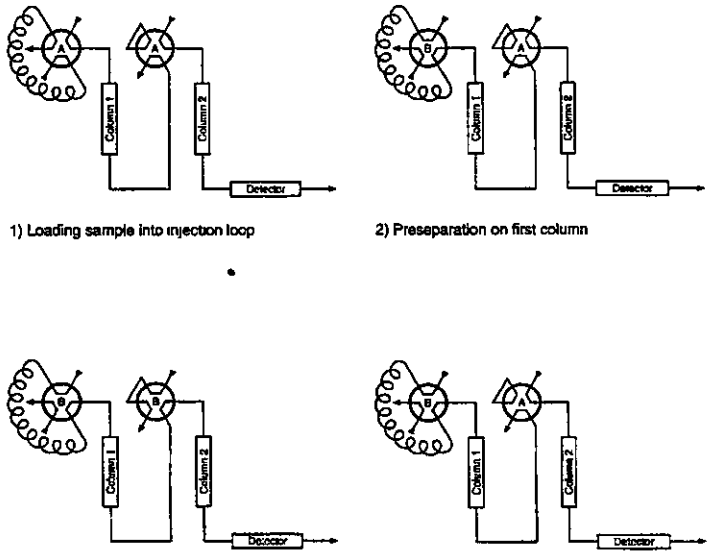
$C_7H_4N_2O_2S$ Molecular mass: 180.18

Figure 2: Procedure Flow Diagram



* The moisture content of the crop has to be taken into consideration (see section 2.3.3 and section 2 10)

Figure 3. Setup of the Column Switching System



1) Loading sample into injection loop

2) Preseparation on first column

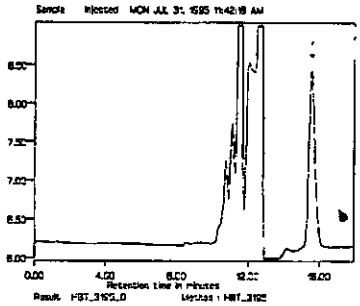
3) Transfer of heart cut to second column

4) Analysis of heart cut on second column

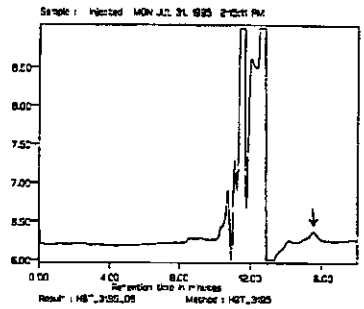
6.2 Representative Chromatograms

All chromatograms shown were recorded during the validation study (see section 7.1) Peak responses are expressed in Peak Height Units (PHU). However, all peak heights in the following Tables are given in mm as measured on the chart recorder

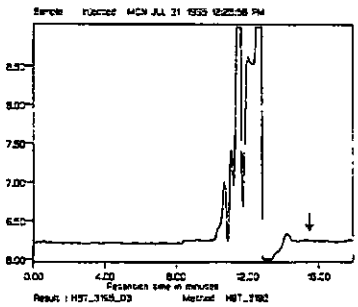
6.2.1 CGA 210007 in banana peel



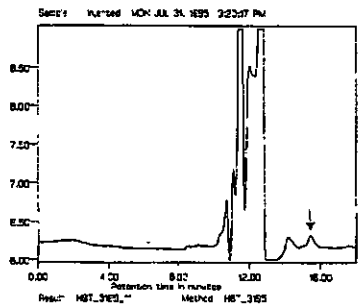
Standard solution 0 08 µg/mL CGA 210007 (4 ng injected), peak. 120 mm



Standard solution 0.004 µg/mL CGA 210007 (0.2 ng injected), peak.6 mm

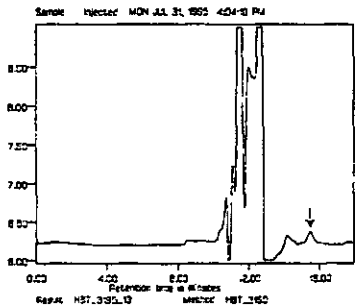


Untreated control specimen (banana peel), formal specimen size injected: 12 5 mg, peak 0 mm

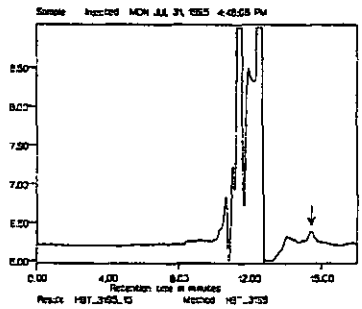


Control specimen fortified with 0.02 mg/kg CGA 245704, formal specimen size injected 12 5 mg, corresponding to 0 214 ng CGA 210007 Peak found 6 5 mm, corresponding to 0.22 ng CGA 210007 or to 102%

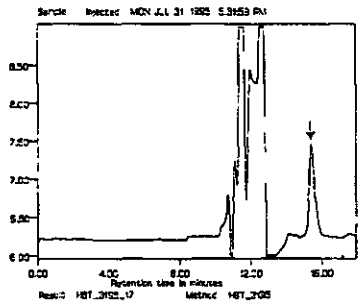
6.2.1 CGA 210007 in banana peel – Continued



Control specimen fortified with 0.02 mg/kg CGA 245704, formal specimen size injected 12.5 mg , corresponding to 0.214 ng CGA 210007 Peak found: 7.3 mm, corresponding to 0.24 ng CGA 210007 or to 114%



Control specimen fortified with 0 02 mg/kg CGA 245704, formal specimen size injected. 12.5 mg , corresponding to 0.214 ng CGA 210007. Peak found. 7 0 mm, corresponding to 0.23 ng CGA 210007 or to 109%



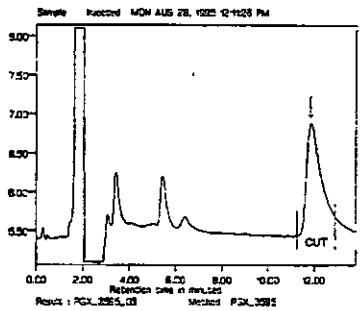
Control specimen fortified with 0 2 mg/kg CGA 245704, formal specimen size injected 12.5 mg, corresponding to 2,14 ng CGA 210007 Peak found 62 9 mm, corresponding to 2 10 ng CGA 210007 or to 98%.

Further details are exactely described in this method and are given again below here They are valid for this sequence as well as for all mentioned sequences in this method (except for milk, where further details are given in the pages containing the corresponding chromatograms):

Weight of plant: 10 g
Total volume for extraction: 100 mL
Aliquot: 0.75 g plant. Final volume. 3 mL
Volume injected. 50 µL

Details on the calibration curve are given on the section 7

6.2.2 Chromatogram of the Analyte on the First Column of the HPLC System, used for the determination of the switching interval

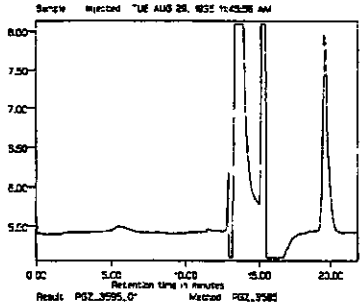


Standard solution 0.08 µg/mL CGA 210007, 50 µL injected, corresponding to 4 ng

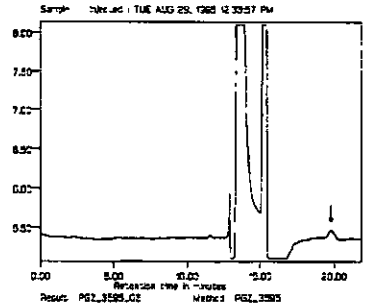
The two vertical bars on the peak of the analyte, mark a possible choice for the cutting interval (CUT). In this case the interval is between 11 min. 30 s and 13 min 30 s The peak fraction within this interval is to be switched to the second column, as described in the section 2.4.1

The given chromatogram was recorded just before the start of the sequence PGZ_3595 for the validation of the method for tomatoes (see also the chromatograms recorded of this sequence on the following page).

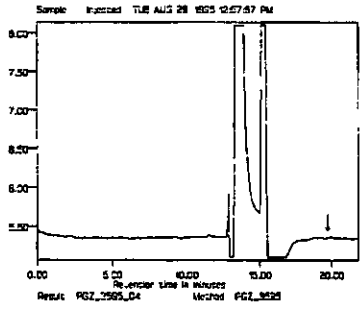
6.2.3 Analysis for CGA 210007 in tomatoes



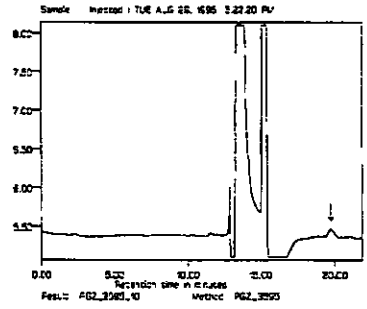
Standard solution 0.08 µg/mL CGA 210007 (4 ng injected), peak: 123 mm



Standard solution 0 004 µg/mL CGA 210007 (0.2 ng injected), peak: 6 mm

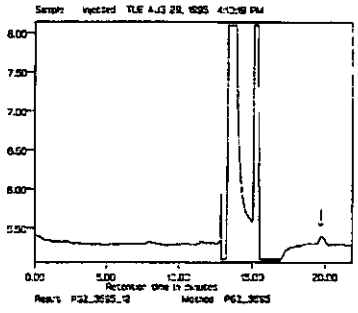


Untreated control specimen (tomatoes), formal specimen size injected. 12.5 mg, peak: 0 mm

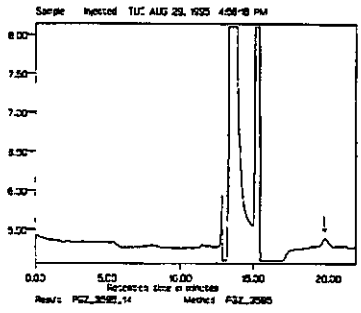


Control specimen fortified with 0 02 mg/kg CGA 245704, formal specimen size injected. 12.5 mg , corresponding to 0.214 ng CGA 210007. Peak found 4 8 mm, corresponding to 0.20 ng CGA 210007 or to 92 %

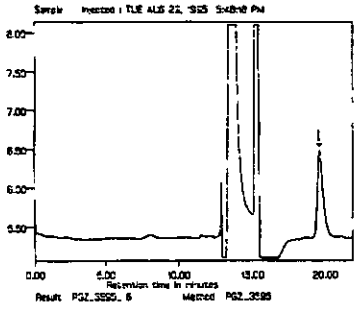
6.2.3 CGA 210007 in tomatoes – Continued



Control specimen fortified with 0 02 mg/kg CGA 245704, formal specimen size injected 12.5 mg , corresponding to 0 214 ng CGA 210007 Peak found. 5.5 mm, corresponding to 0.22 ng CGA 210007 or to 102 %

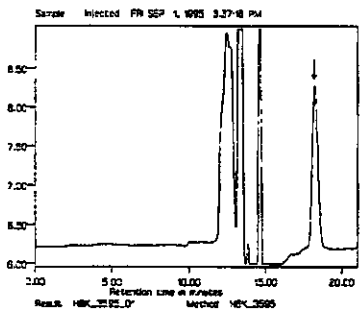


Control specimen fortified with 0 02 mg/kg CGA 245704, formal specimen size injected 12.5 mg , corresponding to 0 214 ng CGA 210007. Peak found: 5.5 mm, corresponding to 0 22 ng CGA 210007 or to 102 %

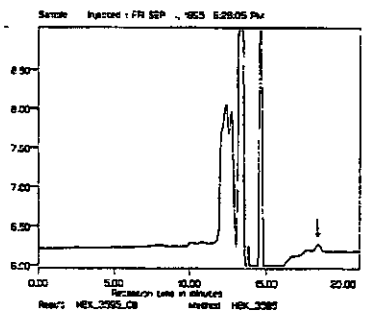


Control specimen fortified with 0.2 mg/kg CGA 245704, formal specimen size injected 12.5 mg, corresponding to 2.14 ng CGA 210007 Peak found. 58 mm, corresponding to 1 9 ng CGA 210007 or to 89% recovery

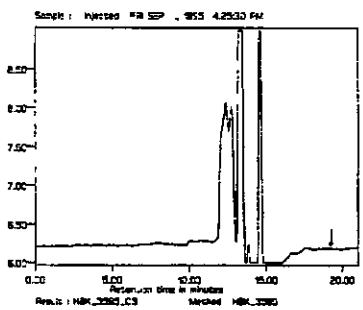
6.2.4 CGA 210007 in cucumbers



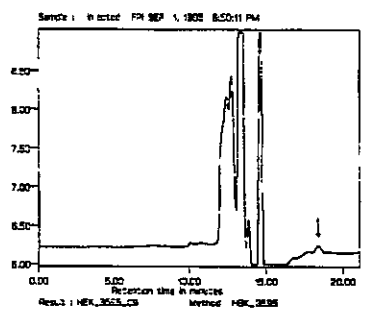
Standard solution 0.08 µg/mL CGA 210007 (4 ng injected), peak: 108 mm



Standard solution 0.004 µg/mL CGA 210007 (0.2 ng injected), peak: 4.7 mm

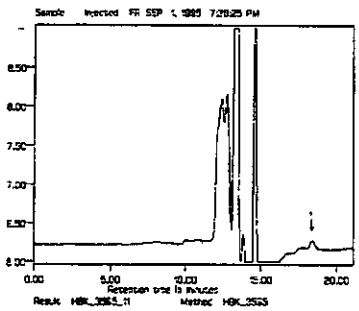


Untreated control specimen (cucumbers), formal specimen size injected 12.5 mg, peak: 0 mm

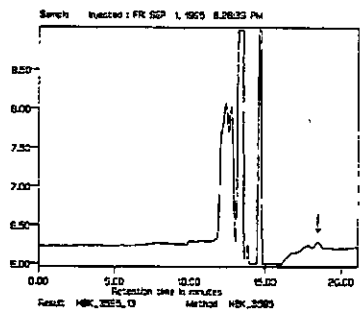


Control specimen fortified with 0.02 mg/kg CGA 245704, formal specimen size injected 12.5 mg , corresponding to 0 214 ng CGA 210007. Peak found 4 9 mm, corresponding to 0.20 ng CGA 210007 or to 95%

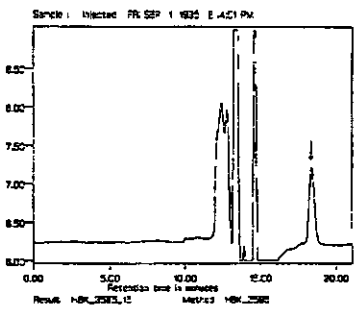
6.2.4 CGA 210007 in cucumbers – Continued



Control specimen fortified with 0 02 mg/kg CGA 245704, formal specimen size injected· 12 5 mg , corresponding to 0 214 ng CGA 210007 Peak found· 5 3 mm, corresponding to 0.22 ng CGA 210007 or to 102 %

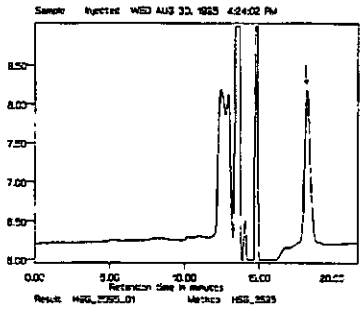


Control specimen fortified with 0 02 mg/kg CGA 245704, formal specimen size injected: 12.5 mg, corresponding to 0.214 ng CGA 210007 Peak found· 4 2 mm, corresponding to 0.18 ng CGA 210007 or to 83%

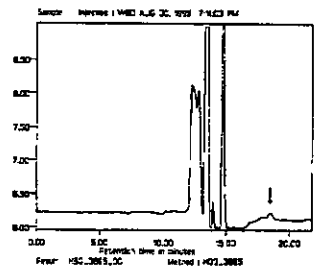


Control specimen fortified with 0.2 mg/kg CGA 245704, formal specimen size injected 12.5 mg, corresponding to 2.14 ng CGA 210007 Peak found. 52 mm, corresponding to 2.0 ng CGA 210007 or to 91% recovery

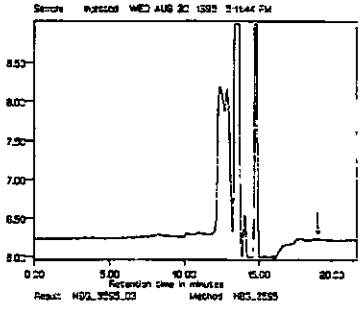
6.2.5 CGA 210007 in milk



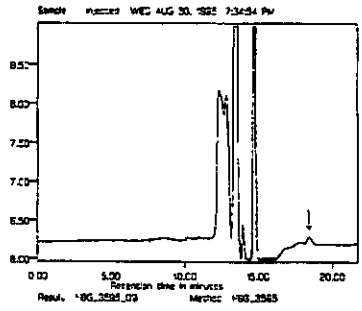
Standard solution 0.08 µg/mL CGA 210007 (4 ng injected), peak: 104 mm



Standard solution 0 004 µg/mL CGA 210007 (0.2 ng injected), peak. 5 mm

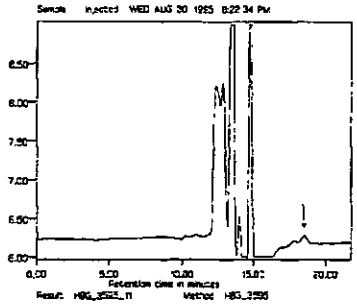


Untreated control specimen (milk), formal specimen size injected 50 mg, peak: 0 mm

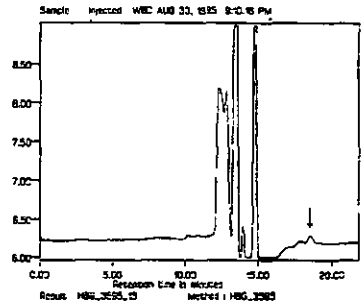


Control specimen fortified with 0.005 mg/kg CGA 245704, formal specimen size injected 50 mg , corresponding to 0.214 ng CGA 210007. Peak found: 5 mm, corresponding to 0.20 ng CGA 210007 or to 95%

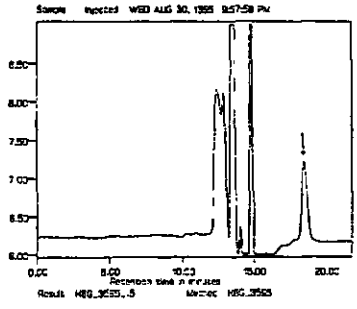
6.2.5 CGA 210007 in Milk – Continued



Control specimen fortified with 0 005 mg/kg CGA 245704, formal specimen size injected: 50 mg , corresponding to 0.214 ng CGA 210007. Peak found: 5 mm, corresponding to 0.20 ng CGA 210007 or to 95 %



Control specimen fortified with 0 005 mg/kg CGA 245704, formal specimen size injected. 50 mg , corresponding to 0.214 ng CGA 210007 Peak found. 5 mm, corresponding to 0 20 ng CGA 210007 or to 95%



Control specimen fortified with 0.05 mg/kg CGA 245704, formal specimen size injected. 50 mg , corresponding to 2.14 ng CGA 210007 Peak found. 54 mm, corresponding to 2.10 ng CGA 210007 or to 98 %

Further details for milk sequences:
Weight of milk: 20 g
Total volume for extraction: 125 mL
Aliquot: 3 g Final volume. 3 mL
Volume injected 50µL

7 APPENDICES

7.1 Report on Validation Study

Report on Special Study 130/95	**VALIDATION of Method REM 172.11** Validation by Analysis of Fortified Specimens and Determination of Recoveries

STUDY DIRECTOR Dr G Formica, R-1098.P.09, Ciba, Crop Protection
Product Safety/Dietary Exposure/Residue Analysis
Date Oct 20, 95 Signature Formica

TEST SUBSTANCES CGA 245704 and CGA 210007
TEST SYSTEMS bananas; tomatoes; cucumbers and milk

1 GENERAL

testing facility	Ciba-Geigy AG, Crop Protection/Residue Analysis CH-4002 Basel
sponsor	Ciba-Geigy AG, Crop Protection, Dietary Exposure Assessment, CH-4002 Basel, Dr S. Dennis
quality assurances	Ciba-Geigy AG, Crop Protection/Product Safety, CH-4002 Basel
test substance	Ciba code CGA 245704 Chemical name Benzo[1,2,3]thiadiazole-7-carbothioic acid S-methyl ester Ciba code: CGA 210007 Chemical name: 1,2,3-Benzothiadiazol-7-carboxylic acid
study dates	Protocol issued 12 Jul 1995 Experimental Date 25 July. 95 - 9 Sep. 95
archives	Ciba, Crop Protection/Residue Analysis. All raw data (including draft method) and original report.
guidelines	- Procedures and Principles of GLP (OECD, Paris 1981, CH-Bern 1986) - European Directive EEC 91/414, European Uniform Principles

2 OBJECTIVE

- The objective of this study is to establish the performance of method REM 172.11 by determining recovery values obtained by analyses of fortified specimens in two Laboratories according to the procedure described in the method.
- The report on validation including the results is issued as part of the final method REM 172.11.

3 RESULTS

See Table on section 7 of this validation
Average recovery (Banana, Tomatoes, Cucumbers and Milk) 93.5%, Sabs=7.2%, n = 76
Acceptable recovery percentage: 73.5 - 113.5
Limit of Quantitation (LOQ) 0 02 mg/Kg for Plant and 0 005 mg/Kg for Milk

4 EXPERIMENTAL DESIGN FOR RECOVERIES

Determination of residues of fortified specimens under repeatability and reproducibility conditions, including checking of performance criteria according to validation requirements of EEC 91/414 Details are as follows.

- **Laboratory 1** (P Gänswein, R-1098 P 05), **substrate group 1** (banana pulp)
  8 specimens at limit of quantitation (LOQ) of 0 02 mg/kg
  8 specimens at 10 times LOQ
- **Laboratory 2** (H Borell, R-1098 P 05), **substrate group 1** (banana pulp)
  3 specimens at limit of quantitation (LOQ) of 0.02 mg/kg
- **Laboratory 1** (P Gänswein), **substrate group 2** (tomatoes)
  3 specimens at limit of quantitation (LOQ) of 0 02 mg/kg
  3 specimens at 10 times LOQ
- **Laboratory 1** (P Gänswein), **substrate group 3** (cucumbers)
  3 specimens at limit of quantitation (LOQ) of 0.02 mg/kg
  3 specimens at 10 times LOQ
- **Laboratory 1** (P. Gänswein), **substrate group 4** (milk)
  3 specimens at limit of quantitation (LOQ) of 0.005 mg/kg
  3 specimens at 10 times LOQ

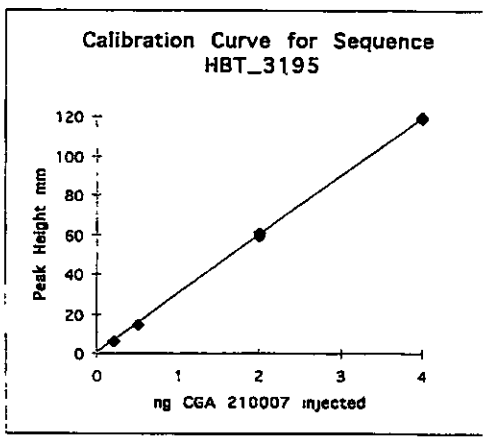
5 ANALYTICAL METHOD

Method REM 172.11, draft issued before start of validation, no modifications to this draft were made during the study.

6 Preparation of the Calibration Curve

The chromatograms given in section 6.2.1 and originate from the same sequence: HBT_3195 All the responses of the standard solutions injected for the preparation of the calibration curve are summarized in the following table

Concentration of standard solution [µg/mL]	Amount of standard injected [ng]	Peak response calculated [mm]	Peak responses measured [mm]
0 08	4	120	120, 119; 119
0 04	2	59 9	60; 60; 61
0 01	0 5	15.0	15, 15, 15
0 004	0 2	6 0	6, 6; 6



Response function

y=A + Bx .

A and B are parameter of the response function

In this case the function is:

y= -0.008553 + 29.98 * x

(linear curve)

y= mm (read on the original Chromatogram)

x= ng injected

s = 1.2%

(s = standard deviation #)

Calculation according to REM 119.06)

 # Standard deviation

Quality criterion for standard curve, standard deviation s (square root of the variance of all relative deviations of the measured response from the corresponding value of the response function), calculated according to REM 119 06 (see reference given in section 5 2)

7 RESULTS (TABLE OF ALL RECOVERIES)

The following table and section 6 2 1 and 6 2.5 contain all information, which is necessary to calculate the individual recoveries.

Substrate	Fortificat. level (mg/kg)	Laboratory 1 Person 1 P. Gänswein (not involved in the Method development)				Laboratory 2 Person 2: H. Borell			
		recovery %	peak height mm	sequence *, calibration curve y=A + Bx	average and standard deviation	recovery %	peak height mm	sequence, calibration curve y=A + Bx	average and standard deviation
banana peel	0.02	95 78 82	5.0 5.0 5.2	PGU_3485 A=0.5625 B=25.62	$\bar{x}$ =88.5% s=7 1 n=6	102 114 109	6.5 7.3 7.0	HBT_3195 A=-0.008553 B=29.98	$\bar{x}$ =103.7% s=5.5% n=6
	0.2	94 89 92	54 51 53	s=6.1		98 98 101	63 63 65	s=1.2%	
banana pulp	0.02	98 95 98	5.0 4.8 5.0	PGP_3395 A=-0.8690 B=28.15	$\bar{x}$ =92.3% s=7.2% n=16	89 86 94	5.2 5.0 5.5	HBU_3195 A=-0.3690 B=29.32	$\bar{x}$ =91.3% s=3.3% n=6
	0.2	91 101 91 87 82	54 60 54 52 49	s=2.7%		95 92 92	59 58 57	s=2.1%	
	0.02	86 76 95 89 91	5.5 5.0 6.0 5.7 5.8	PGW_3485 A=0.8690 B=25.33 s=6.2%					
	0.2	102 100 96	65 65 62						
tomatoes	0.02	92 102 102	4.8 5.5 5.5	PGZ_3595 A=-1 303 B=31 12	$\bar{x}$ =93 7% s=6.6% n=6	112 95 95	6.0 5.0 5.0	HBL_3695 A=-0.4601 B=26.92	$\bar{x}$ =93.0% s=13.0% n=6
	0.2	89 88 89	58 56 58	s=3.5%		72 95 89	41 55 50	s=3.0%	
cucumbers	0.02	96 98 104	5.0 5.0 5.5	PGE_3695 A=-0.6046 B=27.34	$\bar{x}$ =95.0% s=5.1% n=6	95 102 83	4.8 5.3 4.2	HBK_3595 A=-0.5673 B=28.98	$\bar{x}$ =93.2% s=6.2% n=6
	0.2	90 91 93	52 53 54	s=3.8%		91 95 93	52 54 53	s=1 7%	
milk	0 005	91 82 82	5.0 4.5 4.5	PGG_3595 A=-0.2135 B=28.82	$\bar{x}$ =90.2% s=6.6% n=6	103 95	5.5 5.0	HBD_3495 A= -0.5383 B=27.39	$\bar{x}$ =95.3% s=3 0% n=12
	0.05	96 95 95	55 54 54	s=4.6%		97 93	56 54	s=2.4	
	0.005					92	5.0	HBF_3595 A=-0.1883 B=25.70 s=1.2%	
	0.05					95	52		
	0.005					95 95 98	5.0 5.0 5.0	HBG_3595 A=-0.2354 B=25.83	
	0.05					98 92 92	54 51 51	s=2.4%	

* Name of the file containing the chromatograms in the related substrate
No residues were found in all control specimens

8 CRITERIA FOR METHOD VALIDATION

To check the criteria described in the Annex VI of European Directive 91/414/EEC for validation of this method only the necessary recovery values have been considered in the following tables:

8.1 FIRST SUBSTRATE GROUP (banana pulp)

Recovery Table (Results)

Lab 1 Substrate 1	con-trol	recoveries [%] found (individual data) 1	2	3	4	5	6	7	8
0.02 mg/kg	<0.02	98	95	98	86	76	95	89	91
0.20 mg/kg	<0.02	91	101	91	87	82	102	100	94

Recovery-check

Lab 1 Substrate 1	N	average	*EEC-criterion*	standard deviation	*EEC-criterion*	Decision/Comments
0.02 mg/kg	8	91 %	*70-110 %*	7.4 %	*≤20 %*	EEC criterion fulfilled
0.20 mg/kg	8	94 %	*70-110 %*	7 1 %	*≤20 %*	EEC criterion fulfilled

Repeatability Table at LOQ

Lab 1 Substrate 1	N	Recoveries found at LOQ minimum	maximum	difference	Decision/Comments *criterion: difference ≤ 50 %*
0 02 mg/kg (LOQ)	8	76 %	98 %	22 %	EEC criterion fulfilled

Generation of results under repeatability conditions (same lab, operator, within short interval of time).Exception 2 different equipments were used, i e the results were obtained under worst conditions and nevertheless the ECC criterion was fulfilled

Recovery Table (Results)

Substrate 1 at LOQ	con-trol	recoveries [%] found at LOD (individual data) 1	2	3
Lab 2	<0 02	89	86	94

Generation of results under reproducibility conditions (different lab, operator, equipment with exception of 3 results)

Reproducibility Table at LOQ

Lab 1 + 2 Substrate 1	N	Combined recoveries at LOQ minimum	maximum	difference	Comment *criterion: difference ≤ 100 %*
0.02 mg/kg (LOQ)	11	76 %	98 %	22 %	EEC criterion fulfilled

8.2 SECOND SUBSTRATE GROUP (tomatoes)

Recovery Table (Results)

Lab 1 Substrate 2	con-trol	recoveries [%] found (individual data) 1	2	3
0 02 mg/kg	<0 02	92	102	102
0.20 mg/kg	<0 02	89	88	89

Recovery-check

Lab 1 Substrate 2	N	average	*EEC-criterion*	standard deviation	*EEC-criterion*	Decision/Comments
0 02 mg/kg	3	99 %	*70-110 %*	6 %	*≤20 %*	EEC criterion fulfilled
0.20 mg/kg	3	89 %	*70-110 %*	1 %	*≤20 %*	EEC criterion fulfilled

8.3 THIRD SUBSTRATE GROUP (cucumbers)

Recovery Table (Results)

Lab 1 Substrate 3	con-trol	recoveries [%] found (individual data) 1	2	3
0 02 mg/kg	<0 02	96	98	104
0.20 mg/kg	<0 02	90	91	93

Recovery-check

Lab 1 Substrate 3	N	average	*EEC-criterion*	standard deviation	*EEC-criterion*	Decision/Comments
0 02 mg/kg	3	99 %	*70-110 %*	5 %	*≤ 20 %*	EEC criterion fulfilled
0.20 mg/kg	3	91 %	*70-110 %*	2 %	*≤ 20 %*	EEC criterion fulfilled

8.4 FOURTH SUBSTRATE GROUP (milk)

Recovery Table (Results)

Lab 1 Substrate 4	con-trol	recoveries [%] found (individual data) 1	2	3
0.005 mg/kg	< 005	91	82	82
0 05 mg/kg	<0 05	96	95	95

Recovery-check

Lab 1 Substrate 4	N	average	*EEC-criterion*	standard deviation	*EEC-criterion*	Decision/Comments
0.005 mg/kg	3	85 %	*70-110 %*	5 %	*≤ 20 %*	EEC criterion fulfilled
0 05 mg/kg	3	95 %	*70-110 %*	1%	*≤ 20 %*	EEC criterion fulfilled

8.5 OVERALL RECOVERY TABLE (all fortification levels, four substrate groups, two labs)

Labs 1 + 2	N	data of recoveries from above minimum	maximum	difference	average *criterion 70-110 %*	standard deviation
Substrate 1	19	76%	102 %	26 %	92 %	7 %
Substrate 2	6	88 %	102 %	14 %	94 %	7 %
Substrate 3	6	90 %	104 %	14 %	95 %	5 %
Substrate 4	6	82 %	96 %	14 %	90 %	7 %
Substrate 1 + 2 + 3 + 4	37	76 %	104 %	28 %	92%	6 %

9 Collaborators

H Borell, P Gänswein

10 Conversion Factor

The conversion factor from CGA 210007 to CGA 245704 is: 1.17

11 CONCLUSION

According to the criteria described in the Annex VI of European Directive 91/414/EEC this method has been successfully validated.

Statement of Compliance

This study was performed in a facility which was certified to be in compliance with GLP. The study was conducted in full compliance with procedures and principles of GLP in Switzerland, and OECD principles of GLP.

Quality Assurance Statement

See next page.

Quality Assurance Statement
Ciba-Geigy Ltd , GLP Quality Assurance Product Safety, 4002 Basel

Project	130/95
Test Substance	CGA 245704
Study Title	Recovery Validation of Method REM 172.11 "CGA 245704, Determination of Total Residues of CGA 245704 as CGA 210007 in Plant Material and Milk"
Study Director	Dr Giuseppe Formica
QA-Inspector	Pierre Fontana

I hereby certify that the following Quality Assurance activities were performed:

Activity	Performed	Reported
Facility Inspection	May 11, 1995	May 29, 1995
Protocol Audit	July 18, 1995	July 18, 1995
Final Report Audit	October 19, 1995	October 20, 1995

October 20, 1995
Date
Form GESTAT11

Pierre Fontana
Inspector Quality Assurance

APPENDIX II

SEPARATE DOCUMENTS ACCOMPANYING THIS SUBMISSION

Hayworth, C. and Grunenwald, M , ABR-97117, Final Report Amendment Number 1, "Validation of Draft Analytical Method AG-671A for the Determination of Total Residues of CGA-245704 as CGA-210007 in Tobacco, Leafy Vegetables, and Fruiting Vegetables by Column Switching High Performance Liquid chromatography."

Kiffe, M , CIBA GEIGY Ltd , Basel, Switzerland, Plant Metabolism, "Metabolism of CGA 245704 in Greenhouse Grown Lettuce after Treatment with [Phenyl-U-^{14}C] Labelled Material, Study 96MK02 1," In-progress.

Nicollier, G., CIBA GEIGY Ltd., Basel, Switzerland, Plant Metabolism, "Metabolism of CGA 245704 in Greenhouse Grown Tomatoes after Treatment [Phenyl-U-^{14}C] Labeled Material," Study 94WA05.

APPENDIX III

PREVIOUSLY SUBMITTED DOCUMENTS WITH EPA MRID NUMBERS

Nicollier, G , CIBA GEIGY Ltd , Basel, Switzerland, Plant Metabolism, "Behavior and Metabolism of CGA 245704 in Greenhouse Grown Tobacco after Foliar Spray Application of [U-^{14}C-Phenyl]-CGA-245704," Study 94WA04 MRID No. 44014255

EPA ADDENDUM

PP#8F4974

Acibenzolar-s-methyl on Bananas, Spinach, Tomatoes, and Tomato Paste

Conditions and Substitutions used by ACB

1 ACB used a Hewlett-Packard (HP) 1100 Series High Performance Liquid Chromatograph (HPLC) equipped with a HP 1100 Series variable wavelength ultraviolet/visible (UV/VIS) detector (HPLC-UV) The detector was set at 235 nm. The detector was interfaced to a TurboChrom (ver 6 1 1) data system Two analytical columns were used. The columns were connected to a column switching valve on the HPLC, therefore, only one instrument (pump) was used. The column temperatures were programmed according to the method (30 °C). Column 1 was a Phenomenex® Sphereclone SAX, 150 x 4 6 mm i.d., 5 µm, Cat. #OOF-4149-EO. The retention time for this column was ~8 minutes. Column 2 was a Phenomenex® Luna C_8, 250 x 4.6 mm i.d., 5 µm, Cat. #OOG-4040-EO The retention time for this column (Col.1 + Col. 2) was ~42 minutes. The total analysis time was 65 minutes. The flow rate through both columns was 0.6 mL/min using mobile phase 1 and 2 as prepared in the method. However, when the flow was switched to Col. 2, 2% acetonitrile was added from another reservoir into the mixing chamber (2% acetonitrile + 98% mobile phase 2) to bring the analyte into a retention time window free from endogenous peaks. A 100 µL injection volume was used instead of 50 µL as suggested in the method. The method called for Column 1 to be a Phenomenex® Spherisorb SAX/SB, 150 x 3 2 mm i.d., 5 µm with a flow rate fo 0.3 ml/min. The method called for Column 2 to be a YMC-Pack C_8, 250 x 4.6 mm i.d., 5 µm with a flow rate of 1 0 ml/min. By using two columns of the same i.d. (inside diameter) on one instrument, the same flow rate could be used with good chromatographic results. With a flow rate of 0.6 mL/min, back pressure was controlled and the amount of mobile phase used was minimized (hence hazardous waste reduced).

2. The TurboVap®II (Zymark Corporation, Hopkinton, MA) operated at 60 °C under a nitrogen stream was used in place of the rotary vacuum evaporators to concentrate samples during the methanol evaporation. ACB highly recommends this substitution be incorporated into the procedure This substitution eliminated the need to monitor the samples during this step against any bumping and/or foaming that was forewarned in the method had the rotary evaporators been used. In addition, TurboVap® glassware has graduated volumes This was important because the final volume was recommended to be 8-10 mL An 8 mL aliquot of acid is added to the 8-10 mL remaining volume and the mixture is loaded onto a Chem Elute™ column. This was a critical step because the Chem Elute™ columns have a finite capacity of 20 mL.

3a. ACB found no information in the method regarding the time that the separatory funnels were to be shaken during the partitioning steps. ACB shook each sample for one minute following venting procedures.

3b ACB found no information concerning stopping points in the procedure. ACB found that storing the extracts stoppered in the refrigerator overnight following the elution from the Chem Elute™ columns (100 mL 9.1 Dichloromethane/ethyl acetate) did not diminish recoveries. ACB found that storing the extracts stoppered in the refrigerator overnight following the final partitioning with ethyl acetate prior to evaporating to dryness did not diminish recoveries.

3c. ACB found no information concerning the stability of the final extracts contained in auto-sampler vials awaiting analysis. ACB found that the extracts were stable at room temperature for a least 48 hours in capped auto-sampler vials.

4. The method suggested the use of calibration curves to determine sample concentrations (peak height vs concentration). ACB ran calibration curves to demonstrate linearity over the dynamic range of the fortified samples. ACB determined sample concentrations from a ratio of sample responses to the average of standard responses that bracketed the samples during analysis. The bracketing standards were made to be the same concentration as the expected concentration of the fortified samples if 100% recovery were obtained. Prior to analyzing a sample set, the highest concentration of standard was injected onto column 1 to determine the retention time of the standard. After the retention time was established, the timetable of the method was revised to optimize the column switching event.