Biochemistry Department
Ciba Crop Protection
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Greensboro, North Carolina

VALIDATION OF ANALYTICAL METHODOLOGY FOR CGA-329351 WITH LETTUCE SAMPLES TREATED WITH ^{14}C-CGA-329351

Report No.: ABR-95115

Project No.: 435005

Study No.: 236-95

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Study Initiation Date: May 8, 1995

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I. GOOD LABORATORY PRACTICES STATEMENT

This study was performed in accordance with Good Laboratory Practice Standards as required by the EPA-FIFRA Good Laboratory Practice Standard 40 CFR Part 160.

5.1.2.e Woo

1-16-96

Study Director Date

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CIBA-GEIGY
CIBA CROP PROTECTION
QUALITY ASSURANCE UNIT

QUALITY ASSURANCE STATEMENT

Study Title: Validation of Analytical Methodology for CGA-329351 with Lettuce Samples Treated with ^{14}C-CGA-329351

Study Director: [redacted]

Final Report Number: ABR-95115

Study Number: 236-95

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

INSPECTION/AUDIT TYPE	INSPECTION/AUDIT DATES	REPORTING DATE
Study Protocol Audit	05/02/95	05/03/95
In-Progress Inspection: Preparation, application of 2nd foliar spray	06/22/95	06/28/95
Lettuce sampling	07/07/95	07/07/95
Analytical- reflux of samples	10/24/95	10/26/95
Final Report Audit (Includes BIOL-95010 & ANPHI-95006)	12/20,21/95; 1/3-5/96	01/05/96

Prepared By: [redacted] Date: 5 JAN '96

<u>Test Sites</u>

Biological Phase:	Plant Maintenance, Treatment and Sample Collection Ciba-Geigy Corporation Ciba Crop Protection Biochemistry Department 410 Swing Road P. O. Box 18300 Greensboro, NC 27419
Analytical Phase I:	Sample Preparation, Assay of Radioactivity Ciba-Geigy Corporation Ciba Crop Protection Vero Beach Research Center Biological Studies Group 7145 58th Avenue Vero Beach, FL 32967
Analytical Phase II:	Validation of Analytical Methodology Ciba-Geigy Corporation Ciba Crop Protection Biochemistry Department 410 Swing Road P. O. Box 18300 Greensboro, NC 27419

Study Initiation Date:	May 8, 1995
Biological Phase Initiation: (Lettuce Planted)	May 17, 1995
Mature Harvest:	July 13, 1995
Analytical Phase II Experimental Initiation:	April 13, 1995
Analytical Phase II Experimental Completion:	December, 1995

III. GENERAL INFORMATION

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Biologist IV

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Chemist I

Technical Writer: [redacted]
Chemist II

Other Study Participants: [redacted]

<u>Test and Reference Materials</u>

See Section VI.A.

Ciba Study/ Protocol Number: 236-95, Amendments 1-3

Test Facility: Ciba-Geigy Corporation
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Archive Location: The raw data for Analytical Phase I of this study is archived at the Vero Beach Research Center, Vero Beach, FL. The final report for Analytical Phase I, as well as the protocol, amendments, all other raw data, the Biological Report and the ABR are archived in the Ciba Agricultural Group Archives at Ciba-Geigy Corporation, Greensboro, North Carolina. All other study and nonstudy specific data will be archived according to applicable SOP's.

IV. INTRODUCTION

Metalaxyl, CGA-48988, is the active ingredient contained in several Ciba fungicides, including Ridomil® and Apron®. Metalaxyl consists of a mixture of R and S enantiomers. The R-enantiomer, designated as Ciba code CGA-329351, has been shown to exhibit increased biological activity as compared to the S-enantiomer. This increased activity could make it possible to decrease the amount of active ingredient in Metalaxyl fungicides without a decrease in the product effectiveness. As part of the process to register CGA-329351 as an active ingredient for future fungicides, it was necessary to validate the current Metalaxyl methodology using CGA-329351. Therefore, the overall objective of Study 236-95[1] was to validate Analytical Method AG-395[2] for the determination of CGA-329351, the R-enantiomer of Metalaxyl. Analytical Method AG-395 was validated with ^{14}C-Metalaxyl-treated lettuce in 1983. These results are reported in ABR-83033[3].

In order to validate the methodology and demonstrate the extractability and accountability of Analytical Method AG-395 with CGA-329351, it was necessary to analyze ^{14}C-CGA-329351-treated samples. For comparison purposes, it was also deemed necessary to concurrently analyze ^{14}C-CGA-48988-treated samples. Therefore, the purpose of the Biological Phase of this study was to generate the ^{14}C-CGA-329351- and ^{14}C-CGA-48988-treated lettuce samples necessary for analysis. The purpose of Analytical Phase I of this study was to prepare the generated lettuce samples for analysis as well as to determine their total radioactive residues (TRR). The objectives of Analytical Phase II of Study 236-95 were to perform the analyses necessary to validate Analytical Method AG-395 with CGA-329351, as well as to qualitatively determine and compare the metabolism profiles of ^{14}C-CGA-329351 and ^{14}C-CGA-48988 in lettuce.

V. SUMMARY

Analytical Method AG-395 determines the total residues of Metalaxyl and CGA-329351 (the R-enantiomer of Metalaxyl) as 2,6-Dimethylaniline (DMA) in crops by Gas Chromatography with Nitrogen/Phosphorus Detection (GC/NPD). The limit of

detection (LOD) for the method, as determined by the smallest standard amount injected, is 0.02 ng. The limit of quantitation (LOQ), as demonstrated by the lowest fortification successfully recovered, is 0.05 ppm. All results are expressed as CGA-329351 or CGA-48988 equivalents. For chemical names and structures of CGA-329351, CGA-48988 and DMA, see Figure 1.

In order to generate the appropriate samples for validation of Analytical Method AG-395, greenhouse-grown lettuce was treated with either ^{14}C-CGA-329351 or ^{14}C-CGA-48988. The CGA-329351 lettuce samples were treated four times at the Metalaxyl 1X and 2X rates, which in terms of CGA-329351, corresponded to 0.1 lb a.i./acre/treatment and 0.2 lb a.i./acre/ treatment, and was equivalent to 0.4 lb a.i./acre total and 0.8 lb a.i./acre total, respectively. The CGA-48988 lettuce samples were treated four times at the 1X rate, which for Metalaxyl, corresponded to 0.2 lb a.i./acre/treatment and is equivalent to 0.8 lb a.i./acre total. Immature and mature lettuce plant samples were harvested one day and seven days post fourth application. All samples were frozen and shipped for preparation and TRR determination from Ciba Biochemistry, Greensboro, North Carolina to Ciba Vero Beach Research Center (VBRC), Vero Beach, Florida. For more information and results concerning the Biological Phase of Study 236-95, see BIOL-95010[4].

The TRR's determined for both the ^{14}C-CGA-329351- and ^{14}C-CGA-48988-treated lettuce samples were similar at the comparable rates. For example, the TRR determined for the 2X mature ^{14}C-CGA-329351-treated lettuce was 2.03 ppm, while the TRR for the 1X mature ^{14}C-CGA-48988-treated lettuce was 1.89 ppm. The TRR's determined for all mature lettuce samples (day seven post fourth application) were between 36% and 56% lower than those determined for the immature (day one post fourth application) samples. For example, the TRR determined for the immature ^{14}C-CGA-329351-treated lettuce was 1.57 ppm, as compared to 0.87 ppm for the mature ^{14}C-CGA-329351-treated lettuce. For a complete listing of the TRR's determined for both immature and mature harvest lettuce from each treatment group, see Table I.

In an effort to compare the metabolism of CGA-329351 with that of CGA-48988, a metabolism profile was

determined for both ^{14}C-CGA-329351- and ^{14}C-CGA-48988-treated mature lettuce by two-dimensional Thin Layer Chromatography (TLC). The metabolism profiles of the lettuce samples from the two treatment groups were very similar. Between 23% and 31% of the extractable residues from the mature lettuce samples were identified as CGA-329351 (or CGA-48988). Between 6% and 7% of the radioactive residues were identified as CGA-107955, a previously identified metabolite of CGA-48988. Approximately 60% of the extractable radioactive resides (from both treatment groups) remained close to the origin on the TLC plates. The relative mobility of these compounds on the TLC plates appear to match the relative mobility of several polar acidic and glucose conjugate metabolites identified in a previous Metalaxyl Metabolism Study which used the same sample matrix and solvent systems (ABR-91084[5], "Uptake and Metabolism of Metalaxyl in Greenhouse Rotational Crops Following Target Tobacco Grown in Soil Treated with [Phenyl-^{14}C]-Metalaxyl"). In addition to comparing well with one another, the metabolism profiles determined in this study compare well with those reported in ABR-91084. See Table II for specifics on the distribution of metabolites in the ^{14}C-CGA-329351- and ^{14}C-CGA-48988-treated lettuce samples.

To validate Analytical Method AG-395 using CGA-329351, control and CGA-329351-fortified control samples of lettuce samples were analyzed. In addition to the controls, ^{14}C-CGA-329351-treated lettuce samples were also analyzed. For comparison purposes, CGA-48988-fortified controls and ^{14}C-CGA-48988-treated lettuce samples were also analyzed. Recoveries for the method using CGA-329351 range from 73% to 116%. The average recovery with CGA-329351 is 88%, with a standard deviation (SD) of 16% and a coefficient of variation (CV) of 19% (n=6). Recoveries for Analytical Method AG-395 using CGA-48988 range from 70% to 93%. The average recovery with CGA-48988 is 80%, with an SD of 8.4% and a CV of 10% (n=6). See Table IV for complete recovery results and the applicable statistics.

Extractabilities for the ^{14}C-CGA-329351-treated lettuce samples using AG-395 range from 66% to 100%. Accountabilities for these samples range from 52% to 64%. Extractabilities for the ^{14}C-CGA-48988-treated

lettuce samples using AG-395 range from 60% to 96%. Accountabilities for these samples range from 61% to 75%. See Table VI for complete information on extractabilities and accountabilities.

VI. MATERIALS

A. Test and Reference Substances

1.0 Biological Phase

<u>Spray Solution Preparation</u>

(^{14}C-phenyl)-CGA-329351

<u>N</u>-(2,6-dimethylphenyl)-<u>N</u>-(methoxyacetyl)-D-alanine methyl ester
CAS Name: D-Alanine, N-(2,6-dimethylphenyl) -N-(methoxyacetyl)-, methyl ester
CAS Registry No: Unassigned
Lot No: MSR-II-89
Specific Activity: 39.3 µCi/mg
Radiochemical Purity: 99.2%
Reanalysis Date: 11/95
Storage: Room Temperature
Source: Ciba Crop Protection, Chemical Synthesis

(^{14}C-phenyl)-CGA-48988

<u>N</u>-(2,6-dimethylphenyl)-<u>N</u>-(methoxyacetyl)-DL-alanine methyl ester
CAS Name: DL-Alanine, N-(2,6-dimethylphenyl) -N-(methoxyacetyl)-, methyl ester
CAS Registry No: Unassigned
Lot No: MSR-III-45
Specific Activity: 36.0 µCi/mg
Radiochemical Purity: 98.6%
Reanalysis Date: 11/95
Storage: Room Temperature
Source: Ciba Crop Protection, Chemical Synthesis

Ridomil® 2E Blank Formulation

Lot No: FL# 950545
Source: Ciba Crop Protection, Formulations Development Group 1

2.0 Analytical Phase II

Method Validation - Analytical Standards

CGA-329351

N-(2,6-dimethylphenyl)-N-(methoxyacetyl)-D-alanine methyl ester
CAS Name: D-Alanine, N-(2,6-dimethylphenyl)-N-(methoxyacetyl)-, methyl ester
CAS Registry No: 70630-17-0
Lot No: S95-1785
Purity: 99.4%
Reanalysis Date: 11/96
Storage: Freezer
Source: Ciba Crop Protection, Analytical & Product Chemistry Department

CGA-48988

N-(2,6-dimethylphenyl)-N-(methoxyacetyl)-DL-alanine methyl ester
CAS Name: DL-Alanine, N-(2,6-dimethylphenyl)-N-(methoxyacetyl)-, methyl ester
CAS Registry No: 57837-19-1
Lot No: S87-1208
Purity: 95.8%
Reanalysis Date: 12/98
Storage: Room Temperature
Source: Ciba Crop Protection, Analytical & Product Chemistry Department

CGA-72649

2,6-Dimethylaniline
CAS Name: Benzenamine, 2,6-dimethyl-
CAS Registry No: 87-62-7
Lot No: 39254
Purity: 99%
Reanalysis Date: 4/97
Storage: Freezer
Source: Ciba Crop Protection, Analytical & Product Chemistry Department

Method Validation - Metabolism Profiles

CGA-67868

CAS Name: Acetamide, N-(2,6-dimethylphenyl)-2-methoxy-
CAS Registry No: 53823-88-4
Lot No: BPM-X-26
Purity: >99.9%
Reanalysis Date: 1/96
Storage: Refrigerate
Source: Ciba Crop Protection, Chemical Synthesis

CGA-100255

CAS Name: DL-Alanine, N-(3-hydroxy-2,6-dimethylphenyl)-N-(methoxyacetyl)-, methyl ester
CAS Registry No: 96258-85-4
Lot No: WFH-VII-86
Purity: 99.7%
Reanalysis Date: 1/96
Storage: Refrigerate
Source: Ciba Crop Protection, Chemical Synthesis

CGA-107955

CAS Name: DL-Alanine, N-(2,6-dimethylphenyl)-N-(hydroxyacetyl)-
CAS Registry No: 104390-55-8
Lot No: GB-XLVI-7
Purity: >99.9%
Reanalysis Date: 10/97
Storage: Refrigerate
Source: Ciba Crop Protection, Chemical Synthesis

CGA-108905

CAS Name: Bezoic acid, 2-[(methoxyacetyl)(2-methoxy-1-methyl-2-oxoethyl)amino]-3-methyl-,
CAS Registry No: 96258-91-2
Lot No: GB-XLIV-78
Purity: 97.6%

Reanalysis Date: 10/97
Storage: Refrigerate
Source: Ciba Crop Protection, Chemical Synthesis

CGA-94689

CAS Name: DL-Alanine, N-[2-(hydroxymethyl)-6-methylphenyl]-N-(methoxyacetyl)-, methyl ester
CAS Registry No: 85933-49-9
Lot No: NEH-XII-22
Purity: >99.9%
Reanalysis Date: 1/97
Storage: Refrigerate
Source: Ciba Crop Protection, Chemical Synthesis

B. Test Systems

1.0 Biological Test Plants

The lettuce (Lactuca sativa) seeds selected for this study were the Black-Seeded Simpson cultivar variety. This variety was selected because it is known to grow and produce well under greenhouse conditions.

2.0 Samples for Analysis

Plants were sampled from each treatment group one day (immature) and again seven days (mature) post fourth application. The inventory number of the plants taken on day one was MET00479.1. The inventory number of the plants taken at maturity was MET00479.2. The immature and mature lettuce plants from the control group, the 1X ^{14}C-CGA-32931-treated group and the ^{14}C-CGA-48988-treated group were analyzed to validate Analytical Method AG-395[2]. The ^{14}C-CGA-32931- and ^{14}C-CGA-48988-treated mature lettuce samples were used for determination of metabolism profiles.

VII. TEST METHODS

A. Biological Methods and Experimental Design

1.0 Biological Phase I

Plant Maintenance and Treatment and Sample Collection

Twenty-eight day old lettuce plants were transplanted into forty pots containing an amended sandy loam soil mix on 6/14/95. The lettuce plants were maintained in three separate cubicles in a temperature controlled greenhouse; one for each test substance and one for the control plants. The plants within the cubicles were also separated into four different treatment groups, consisting of the following: Control, 1X ^{14}C-CGA-329351, 2X ^{14}C-CGA-329351 and 1X ^{14}C-CGA-48988.

The test substances were formulated (2E) immediately prior to use and applied as foliar spray one day post transplanting and again seven, fourteen and twenty-one days post first application. Ten control lettuce plants received a foliar spray with a blank formulation. Ten plants received formulated ^{14}C-CGA-329351 at the 1X rate, which is equivalent to approximately 0.1 lb a.i./acre (0.4 lb a.i./acre/total) and ten plants received formulated ^{14}C-CGA-329351 at the 2X rate, which is equivalent to approximately 0.2 lb a.i./acre (0.8 lb a.i./acre/total). Ten plants received formulated ^{14}C-CGA-48988 at the 1X rate, which is equivalent to approximately 0.2 lb a.i./acre (0.8 lb a.i./acre/total). Whole plant samples were taken one day (immature) and seven days (mature) post fourth application. Temperature and relative humidity were continuously monitored through-out the test period. The plants received scheduled nutrient applications via an automatic watering system. All plant samples were placed into the greenhouse facility freezer (-20°C) immediately after weighing. The samples were later transferred while frozen to the Biochemistry Group freezer storage

facility for storage until shipment. Samples were shipped overnight in dry ice via Federal Express to Ciba VBRC for sample processing and TRR determination. See BIOL-95010[4] for complete information on Biological Phase methods.

Preparation of Spray Solutions

For the 1X metalaxyl rate, ^{14}C-CGA-48988 was weighed into a 20-mL scintillation vial. For the 1X and 2X CGA-329351 rates, the appropriate amount of ^{14}C-CGA-329351 stock solution was added to 20-mL scintillation vials and the solvent evaporated with a gentle stream of nitrogen. Ridomil 2E formulant was dispensed directly into the scintillation vials. These steps were performed on the day before application. On the day of application, the appropriate amount of water was added to yield the stock spray solutions, which were dispensed into ten vials for each treatment group.

2.0 Methods of Calculation

Equation 1 is used to calculate the total test substance requirement for each treatment group.

$$\text{1) Total Application Rate} = (\text{lb ai/A})(\text{A}/43560\ \text{ft}^2)(453600\ \text{mg/lb})(\text{ft}^2/\text{plant})(\#\text{plants/trmt})\ (\text{mg/trmt})$$

lb a.i./A = total rate including all applications
ft^2/plant = area of plant pot or pail
trmt = treatment group
A = acre

To determine the amount of test substance required for each application, the total application rate, as determined above, should be divided by the number of applications.

NOTE: In this study, an excess test substance equivalent in amount to that required for two additional pails was formulated to allow for volume lost to glassware, radioassay and other analyses of the formulated test substance. Therefore, in

calculating the total application rates using Equation 1, 12 plants were used instead of 10 plants.

Equation 2 is used to calculate the actual application rates, per application, as verified by analyzing aliquots of the spray solutions by LSC.

2) $$\text{Actual Application Rate} = \frac{\text{(DPM/mL counted)(mL applied/plant)(43560 ft}^2\text{/A)(lb/453600 mg)}}{\text{(2,220,000 DPM/}\mu\text{Ci)(Sp Act }\mu\text{Ci/mg)(ft}^2\text{ area/plant)}} = \text{lb/A}$$

DPM = Disintegrations per Minute
Sp Act = Specific Activity of Test Substance
area/plant = 0.64 ft^2
^{14}C-CGA-329351 = 39.3 nCi/µg
^{14}C-CGA-48988 = 36.0 nCi/µg

The total actual application rate can be calculated by multiplying the actual application rate by the number of applications.

B. Analytical Methods and Experimental Design

1. Analytical Phase I

Plant samples were homogenized using a Thomas Wiley Mill fitted with a 2-mm diameter screen. The samples were passed through the mill along with dry ice. Samples were collected in properly labeled sample bags and returned to the freezer, where the left-over dry ice was allowed to dissipate.

In order to determine the TRR's of the ^{14}C-incurred lettuce samples, triplicate subsamples of plant material were combusted using an automated Harvey Oxidizer. The generated $^{14}CO_2$ was captured in liquid scintillation cocktail and disintegrations per minute (DPM) were determined by liquid scintillation counting.

For more information regarding the methods used for preparation of the samples and determination of TRR's, see Analytical Report, ANPHI-95006[6].

2.0 Analytical Phase II

Homogeneity and Purity of the Formulation

The homogeneity of each batch of stock spray solution for each treatment group and each application was determined. To accomplish this, a total of nine aliquots were taken from each stock spray solution during the course of dispensing this solution into the vials for individual plants. Specifically, while dispensing the stock spray solution into ten vials, (the number of plants in each treatment group) three aliquots were taken before dispensing vial one, after dispensing vial five and after dispensing vial ten. These aliquots were analyzed by liquid scintillation counting (LSC) to confirm the homogeneity of the stock spray solution.

The radiochemical purity of the stock spray solutions were also determined. This was accomplished by analyzing a portion of one of each set of three aliquots taken for the homogeneity test (described above). The samples were analyzed by TLC with a 95% Chloroform/5% Methanol solvent system. Radioactivity on the developed plates was detected by radioanalytic imaging. Non-radioactive standards were detected by ultraviolet (UV) absorbance.

Metabolite Profiles

Metabolite profiles were determined for the 1X ^{14}C-CGA-329351- and ^{14}C-CGA-48988-treated immature lettuce. Sample extracts from the ^{14}C-incurred lettuce samples were analyzed by two-dimensional TLC. The first solvent system consisted of 83% Chloroform/13% Methanol/1% Formic Acid/1% Water. The second solvent system was made up of 80% Ethyl Acetate/19% Ethanol/1% Acetic Acid. These solvent systems were the same systems used in the ^{14}C-Metalaxyl Metabolism Study, the results of which are reported in ABR-91084. The sample extracts were analyzed along with CGA-329351 and CGA-48988 analytical standards, as well as the following available

metabolite standards: CGA-100255, CGA-108905, CGA-67868, CGA-94689 and CGA-107955. Radioactivity on the developed plates was detected by radioanalytic imaging. Non-radioactive standards were detected by UV absorption. The metabolism profiles of the immature lettuce samples were qualitatively determined by comparison to the standards listed above, as well as by comparison to the metabolism results reported in ABR-91084.

Validation of Methodology

Analytical Method AG-395 (with modifications) was used to determine the total residues of CGA-329351 and its metabolites as 2,6-Dimethylaniline in lettuce. The modifications listed below were used in this study. The principle of the method, as used in this study, follows the list of modifications.

Modifications to AG-395

1) Extraction - Wet Crop, Section 5.3: This extraction procedure not used in this study; the extraction method for dry crops (Section 5.4) was used for all samples.

2) Methanesulfonic Acid Reflux, Section 5.5.4: The water and base volumes were doubled.

3) Steam Distillation, Section 5.6.1: Glass wool plugs were not placed at the top of the condensers.

4) Steam Distillation, Section 5.6.3: Most of the lower aqueous phase was removed through the solvent withdrawal tube first, followed by the hexane phase. The hexane phase only was collected into a scintillation vial and stored in the freezer, as stipulated in AG-395.

5) Silica Sep-Pak Cartridge Clean-up, Sections 5.7.6 and 5.7.7: These steps were omitted. The sample

from Section 5.7.5 was concentrated in dicloromethane (DCM) for GC analysis.

6) Standardization, Section 6.1.1:
GC injection standards were prepared by weighing 100 mg of DMA into 100 mL of DCM for a 1.0 µg/mL stock solution. Serial dilutions were made from this stock standard using DCM.

7) Standardization, Section 6.1.2 and Table I, Chromatographic Conditions:
A DB-Wax capillary column was used for chromatographic analysis. Oven temperature program was modified as follows:
Initial Value = 50°C
Initial Time = 1 minute
Rate = 4°C/minute
Final Value = 160°C
Final Time = 0 minutes
Post Value = 240°C
Post Time = 0 minutes
Equilibration Time = 3 minutes

8) Detection of Sample Residues, Section 6.2.2:
Residues were detected as DMA and were converted to CGA-329351 or CGA-48988 equivalents using the conversion factor of 2.305.

Principle of AG-395

The sample is extracted by refluxing with methanol/water for two hours. The resulting solution is filtered and a portion of that is evaporated to dryness, then refluxed in the presence of methanesulfonic acid for fifteen minutes. After cooling, water, hexane and aqueous sodium hydroxide are added to the solution. The basic solution is then attached to a steam distillation apparatus and refluxed for approximately one hour and fifteen minutes. The distilled aqueous phase is discarded and the distilled aqueous/hexane phase is collected and frozen for separation of the phases. The hexane phase is then subjected to silica solid phase extraction

(SPE). DMA is eluted with DCM, concentrated and analyzed by GC/NPD. For a complete description of the analytical procedures, see Analytical Method AG-395[2].

The validation experiments included analysis of lettuce controls and lettuce controls fortified with CGA-329351 and CGA-48988. The 1X rate ^{14}C-CGA-329351- and ^{14}C-CGA-48988-treated lettuce were also analyzed. (The 2X ^{14}C-CGA-329351-treated lettuce was not analyzed because analysis of the 1X samples provided sufficient data.) The procedural recoveries were fortified at the method LOQ, as well as at several other higher levels. The fortification levels included 0.05 ppm, 1.0 ppm and 5.0 ppm. All ppm values were corrected with the CGA-329351(CGA-48988)/DMA molecular weight (MW) ratio of 2.305 and expressed in CGA-329351 or CGA-48988 equivalents. Procedural recovery values were corrected for control ppm values, where present. Sample residues were corrected for concurrently analyzed procedural recovery values <100%.

The accuracy and precision of Analytical Method AG-395 was determined from the recovery results obtained from the analysis of fortified control samples. The precision was also determined by the reproducibility of the amount of CGA-329351 or CGA-48988 found in the ^{14}C-incurred lettuce samples. The extractability of AG-395 was determined by comparing the TRR with the radioactive residue found in the ^{14}C-incurred sample extract. The accountability was determined by comparing the TRR with the final concentration of CGA-329351 or CGA-48988 (as determined by GC/NPD) found in the ^{14}C-incurred samples.

Representative worksheets containing sample specific information such as samples weights, extract, final and injection volumes, peak heights and extraction and analysis dates can be seen in Tables VII-X.

3.0 Methods of Calculation

Determination of Sample Residues

To calculate the residue results in terms of ppm of CGA-329351 or CGA-48988, the mg of sample injected must first be calculated using Equation 3.

$$3)\ \text{mg inj} = \frac{(\text{mg sample extracted})(\text{aliquot vol})(\text{inj vol})}{\{\text{extract vol} + [\text{g sample}(\%M/100)]/D\}(\text{final vol})}$$

%M = % Moisture
D = Density of Water, 1.0 g/cm^3 (mL = cm^3)
inj = injection

To determine the corrected value of ppm CGA-329351 or CGA-48988 found in the sample, use Equation 4. (R is not used for tolerance enforcement purposes.)

$$4)\ \text{ppm CGA-329351} = \frac{(\text{ng CGA-329351 found})(2.305)}{(\text{mg sample inj})\ (R)}$$

2.305 = molecular weight ratio: CGA-329351/DMA
R = recovery ratio given by Equation 6, expressed in decimal form
inj = injected

Fortification Experiments

The ppm value of CGA-329351 or CGA-48988 found in the controls and fortified controls can be calculated using Equation 5.

$$5)\ \text{ppm CGA-329351} = \frac{(\text{ng DMA found})(2.305)}{(\text{mg sample inj})}$$

2.305 = molecular weight ratio: CGA-329351/DMA
inj = injected

The recovery factor (R) can be calculated by subtracting the background detector response in the control sample, if any, from the analyte response in the recovery sample. The recovery factor can be calculated as a percentage with Equation 6.

6) $R\% = \dfrac{\text{ppm in Recovery} - \text{ppm in Control}}{\text{ppm amount Fortified}} \times 100$

ppm = ppm CGA-329351 or CGA-48988

Extractability

Aliquots of the sample extracts were taken and dpm's obtained. The total dpm in the extracts were calculated and converted to ppm, as shown in Equation 7.

7) $\dfrac{(\text{DPM})\{\text{Extract mL} + (\text{g Sample})(\%\text{M}/100)/\text{D}\}}{(\text{mL Counted})(\text{g Sample})(\text{Sp Acty nCi}/\mu\text{g})(2220\ \text{DPM/nCi})}$

$= \mu g/g => \text{ppm}$

%M = % Moisture
D = Density of Water, 1.0 g/cm^3 (mL = cm^3)
Sp Acty = Specific Activity of the test substance in the spray solutions:
^{14}C-CGA-329351 = 39.3 nCi/µg
^{14}C-CGA-48988 = 36.0 nCi/µg

Extractabilities were then calculated using Equation 8.

8) $\dfrac{\text{ppm found in extract}}{\text{TRR (ppm)}} \times 100$

Accountability

Accountabilities were calculated by comparing the corrected residues of CGA-329351 or CGA-48988, as determined by GC analysis, (see calculations above, Determination of Sample Residues) with the previously determined TRR's, as shown in Equation 9.

9) $\dfrac{\text{ppm CGA-329351}^{1}}{\text{TRR (ppm)}} \times 100$

[1] Corrected for concurrently analyzed fortified controls <100% and with molecular weight ratio of 2.305.

When determining the ppm amount of ^{14}C residues found by LSC, the final sample weight must be determined using Equation 10, a variation of Equation 3.

10) g Sample = $\frac{\text{(g sample extracted)(mL aliquot)(mL counted)}}{\text{\{extract vol + [g sample(\%M/100)]/D\}(final vol)}}$

%M = % Moisture
D = Density of Water, 1.0 g/cm^3 (mL = cm3)

Equation 11 can then be used to calculate the ppm value of the ^{14}C residues as determined by LSC.

11) $\frac{\text{DPM}}{\text{(g Sample)(Sp Acty nCi/µg)(2220 DPM/nCi)}} = \text{µg/g} => \text{ppm}$

g Sample = g Sample from Equation 10
Sp Acty = Specific Activity of Test Substance in the spray solutions:
^{14}C-CGA-329351 = 39.3 nCi/µg
^{14}C-CGA-48988 = 36.0 nCi/µg

VIII. CIRCUMSTANCES AFFECTING THE STUDY

There were no circumstances that adversely affected the quality or integrity of the study.

IX. RESULTS AND DISCUSSION

A. Biological Phase

Black-Seeded Simpson lettuce was successfully grown to maturity under greenhouse conditions after foliar spray applications with ^{14}C-CGA-329351 and ^{14}C-CGA-48988 at the desired nominal rates. The lettuce plants were divided into four treatment groups of ten plants each. The treatment groups consisted of a control group, a 1X rate ^{14}C-CGA-329351 group, a 2X rate ^{14}C-CGA-329351 group and a 1X rate ^{14}C-CGA-48988 group. The 1X rate for ^{14}C-CGA-329351 was equivalent to 0.1 lb a.i./acre/treatment (0.4 lb a.i./acre/ total) and the 2X rate for ^{14}C-CGA-329351 was equivalent to 0.2 lb a.i./acre/ treatment (0.8 lb a.i./acre/total). The 1X rate for CGA-48988 was equivalent to 0.2 lb a.i./ acre/treatment (0.8 lb a.i./acre/total). To see actual application rates for each treatment group and each application, see BIOL-95010.

The lettuce plants did not exhibit any symptoms of phytotoxicity from the radiolabeled foliar spray applications. Immature and mature lettuce plants were harvested one day and seven days post fourth application. All samples were frozen and shipped for preparation and TRR determination from Ciba Biochemistry, Greensboro, North Carolina to Ciba VBRC, Vero Beach, Florida. For more information and results concerning the Biological Phase of Study 236-95, see BIOL-95010.

B. Analytical Phase I

The TRR's determined for both the ^{14}C-CGA-329351- and ^{14}C-CGA-48988-treated lettuce samples were similar at the comparable rates. For example, the TRR determined for the 2X mature ^{14}C-CGA-329351-treated lettuce was 2.03 ppm, while the TRR for the 1X mature ^{14}C-CGA-48988-treated lettuce was 1.89 ppm. The TRR's determined for all mature lettuce samples were between 36% and 56% lower than those determined for the immature samples. For example, the TRR determined for the immature ^{14}C-CGA-329351-treated lettuce was 1.57 ppm, as compared to 0.87 ppm for the mature ^{14}C-CGA-329351-treated lettuce. For a complete listing of the total radioactive residues, see Table I.

C. Analytical Phase II - Homogeneity, Purity and Metabolism Profiles

1.0 Homogeneity and Purity of the Test Substance

In order to demonstrate the radiochemical integrity of the test substance and the homogeneity of the stock spray solutions during the course of the study, aliquots were taken and analyzed during the preparation of the spray solutions. The formulations were prepared on 6/14/95, 6/20/95, 6/27/95 and 7/5/95. Aliquots for homogeneity and purity testing were taken on 6/15/95, 6/22/95, 6/29/95 and 7/6/95. The analyses to determine homogeneity were done either on the same day that the aliquots were taken or one day after. The purity analyses were performed one to six days after the initial aliquot was taken. If the analyses were not

performed on aliquot day, the samples were stored refrigerated.

As determined by LSC, the homogeneity of the spray solutions ranged from 83% to 103%. See Table III for a complete listing of the homogeneity results. The radiochemical purity of the ^{14}C-CGA-329351 and the ^{14}C-CGA-48988 was 99.2% and 98.1%, respectively as determined on 5/4/95. As determined by TLC, the radiochemical purities of the aliquots taken during the study (6/19/95 - 7/11/95) ranged from 99.0% to 99.5%. Figures 2 and 3 contain the developed TLC plates used to determine the radiochemical purity of the test substances.

2.0 Metabolism Profiles

In an effort to compare the metabolism of CGA-329351 with that of CGA-48988, a metabolism profile was determined for both ^{14}C-CGA-329351- and ^{14}C-CGA-48988-treated immature lettuce by two-dimensional TLC. The metabolism profiles of the lettuce samples from the two treatment groups were very similar. In addition to comparing well with one another, the metabolism profiles determined in this study also compare well with those reported in ABR-91084 ("Uptake and Metabolism of Metalaxyl in Greenhouse Rotational Crops Following Target Tobacco Grown in Soil Treated with [Phenyl-^{14}C]-Metalaxyl").

Figures 4 and 5 show the developed TLC plates of the analyzed ^{14}C-CGA-329351- and ^{14}C-CGA-48988-treated immature lettuce samples. As can be seen in these Figures, between 23% and 31% of the extractable residues were identified as CGA-329351 or CGA-48988. Between 6% and 7% of the radioactive residues were identified as CGA-107955, a previously identified metabolite of CGA-48988. Approximately 60% of the extractable radioactive resides remained close to the origin on the TLC plates. The relative mobility of these compounds appear to match the relative mobility of several polar acidic

and glucose conjugate metabolites previously identified in lettuce (using the same solvent systems) and reported in ABR-91084. During this study, there were no analytical standards available of these metabolites that would have allowed a direct comparison. Therefore, it can only be postulated that this group of polar compounds near the origin of the TLC plates could include those polar acidic and glucose conjugate metabolites identified in ABR-91084. For chemical names and structures of CGA-329351, CGA-48988 and CGA-107955, see Figure 1. For structures of other postulated and identified metalaxyl metabolites, see ABR-91084.

D. Analytical Phase II - Validation of Methodology

The LOD of Analytical Method AG-395, as determined by the smallest standard amount injected, is 0.02 ng. The LOQ, as demonstrated by the lowest fortification successfully recovered, is 0.05 ppm.

Typical chromatograms of DMA standards, which were run during the analysis of immature lettuce, are shown in Figure 7. The corresponding calibration curve along with standardization data, is shown in Figure 6. The peak heights and correlation coefficient shown are typical of those obtained throughout the study.

Representative chromatograms from reagent blanks run throughout the study are shown in Figure 8. Reagent blanks were included in each analysis set. A very small peak occurred in the reagent blank that was run with the ^{14}C-CGA-48988-treated immature lettuce, but the height of this interference peak was below the method LOD. No peaks occurred at the analyte retention time in any of the other reagent blanks.

1.0 Accuracy and Precision

Table IV shows the results from analyzing controls and control samples fortified with CGA-329351 and CGA-48988 over a range of 0.05 ppm to 5.0 ppm. Recoveries for the method using CGA-329351 range from 73% to 116%. The average recovery with CGA-329351

is 88%, with an SD of 16% and a CV of 19% (n=6). Recoveries for Analytical Method AG-395 using CGA-48988 range from 70% to 93%. The average recovery with CGA-48988 is 80%, with an SD of 8.4% and a CV of 10% (n=6).

In this study, the control that was analyzed along with the 1X CGA-48988 immature lettuce samples contained an interference peak at the analyte retention time that was equivalent to 0.27 ppm. Since the purpose of this study was to validate current methodology with CGA-329351, (the results of which are unaffected by the interference peak) the 1X CGA-48988 results associated with the control interference peak will be reported. No detachable residues (LOQ = 0.05 ppm) were found in the other control samples. See Table IV for complete recovery results and the applicable statistics. Representative chromatograms from analyses of controls and fortified controls are shown in Figures 9-12.

Table V lists the results from replicate analyses of immature and mature lettuce from ^{14}C-CGA-329351- and ^{14}C-CGA-48988-treated samples. The standard deviations of the residues found by GC for these substrates range from 0.040 ppm to 0.28 ppm and the CV's range from 3.7% to 11%. See Table V for individual residues and the applicable statistics. Representative chromatograms resulting from these analyses are shown in Figures 9-12.

2.0 Extractability

The extractability of ^{14}C-CGA-329351 and ^{14}C-CGA-48988 and metabolites from ^{14}C-CGA-329351- and ^{14}C-CGA-48988-treated lettuce was determined for Analytical Method AG-395. This was determined by comparing the concentration of the radioactive residue in the ^{14}C-incurred lettuce extracts with the previously determined TRR's. See Section VII.B.3.0 for calculations. The extractability of ^{14}C-CGA-329351 from immature and mature lettuce averages 102% and

90%, respectively. The extractability of ^{14}C-CGA-48988 from immature and mature lettuce averages 82% and 101%, respectively. See Table VI for a complete listing of the extractability results.

3.0 Accountability

The accountability of Analytical Method AG-395 was determined by comparison of the TRR with the ^{14}C-incurred sample residues as determined by GC analysis. The accountability of ^{14}C-CGA-329351 in immature and mature lettuce averages 57% and 56%, respectively. The accountability of ^{14}C-CGA-48988 in immature and mature lettuce averages 67% and 72%, respectively. See Table VI for a complete listing of total residues found in the final fractions by both LSC and GC.

X. CONCLUSIONS

The purpose of determining metabolite profiles of the ^{14}C-CGA-329351- and ^{14}C-CGA-48988-treated lettuce samples was to demonstrate the similarity of the metabolism of CGA-329351 with that of CGA-48988. As can be seen in Tables I and II, the total radioactive residues and metabolite profiles of the two groups of treated lettuce compare well.

Analytical Method AG-395 is a valid and accurate method for the determination of total residues of CGA-329351 as 2,6-Dimethylaniline in lettuce. This conclusion is based on the acceptable accuracy, precision, extractability and accountability results obtained in this study.

XI. TABLES AND FIGURES

TABLE I. SUMMARY OF TOTAL RADIOACTIVE RESIDUES FOUND IN ^{14}C-CGA-329351- AND ^{14}C-CGA-48988-TREATED LETTUCE

Substrate	TRR (ppm)
1X ^{14}C-CGA-329351	
Immature Lettuce	1.57
Mature Lettuce	0.87
2X ^{14}C-CGA-329351	
Immature Lettuce	3.17
Mature Lettuce	2.03
1X ^{14}C-CGA-48988	
Immature Lettuce	3.77
Mature Lettuce	1.89

TABLE II. DISTRIBUTION OF METABOLITES IN ^{14}C-CGA-329351- AND ^{14}C-CGA-48988-TREATED IMMATURE LETTUCE EXTRACTS AS DETERMINED BY TWO-DIMENSIONAL TLC

Treatment and Substrate	% CGA-329351	% CGA-48988	% CGA-107955	% Glucose Conj Metabolites[5]	% Acidic Metabolites[5]	Unidentified Polar Compounds[6]
1X[1] ^{14}C-CGA-329351 Mature Lettuce[2]	23.4	--	5.8	--	--	66.6
1X[3] ^{14}C-CGA-48988 Mature Lettuce[2]	--	31.0	6.7	--	--	60.5
^{14}C-CGA-48988[4] 50% Mature Lettuce Foliage	--	16.8	1.4	37.0	39.7	--

This table is for comparison purposes only, these numbers represent the distribution of metabolites in the ^{14}C that was extracted and are not directly related to those numbers representing the percent of total radioactive residue (%TRR).

1 Total application: 0.4 lb a.i./acre/total.
2 Whole plants extracted as described in AG-395[2] (Dry Crop Extraction).
3 Total application 0.8 lb a.i./acre/total.
4 Target crop of tobacco was grown in soil treated once with 3 lbs a.i./acre. Lettuce was grown as a rotational crop and the foliage was harvested when the plants were at 50% maturity. Values given are those from both organic and aqueous extracts. See ABR-91084[5] for details.
5 Refers to results reported in Metabolism ABR-91084[5].
6 Refers to results obtained during this study and is an average of the results from two separate TLC plates.

TABLE III. HOMOGENEITY OF SPRAY SOLUTIONS AS DETERMINED BY DIRECT LIQUID SCINTILLATION COUNTING

Spray Solution and Application	Aliquot Time[1]	Average DPM	Theoretical DPM[2]	% of Actual DPM Relative to Theoretical
1st Application				
1X ^{14}C-CGA-329351	Begin	277,527	290,820	95
	Middle	277,822	290,820	96
	End	285,671	290,820	98
2X ^{14}C-CGA-329351	Begin	575,432	581,640	99
	Middle	584,696	581,640	101
	End	528,876	581,640	91
1X ^{14}C-CGA-48988	Begin	500,922	532,800	94
	Middle	473,502	532,800	89
	End	501,075	532,800	94
2nd Application				
1X ^{14}C-CGA-329351	Begin	128,819	145,410	89
	Middle	137,839	145,410	95
	End	133,561	145,410	92
2X ^{14}C-CGA-329351	Begin	283,169	290,820	97
	Middle	263,520	290,820	91
	End	285,908	290,820	98
1X ^{14}C-CGA-48988	Begin	231,215	266,400	87
	Middle	246,666	266,400	93
	End	238,253	266,400	89

1) Three aliquots were taken at three separate times from the stock spray solutions while dispensing into the (ten) vials for the individual plants. The aliquots were taken before dispensing vial one (Begin), after dispensing vial five (Middle) and after dispensing vial ten (End).

2) Theoretical DPM calculated using the following equation:

(µg test substance)(Specific Activity nCi/µg)(2220 DPM/nCi)(final vol)(vol counted) = Ther. DPM

where: µg test substance = test substance in stock spray solution
final vol = final volume of stock spray solution
vol counted = volume of each homogeneity aliquot taken for counting

TABLE III. HOMOGENEITY OF SPRAY SOLUTIONS AS DETERMINED BY DIRECT LIQUID SCINTILLATION COUNTING (Continued)

3rd Application				
1X ^{14}C-CGA-329351	Begin	141,198	145,410	97
	Middle	143,709	145,410	99
	End	149,558	145,410	103
2X ^{14}C-CGA-329351	Begin	275,719	290,820	95
	Middle	288,730	290,820	99
	End	290,516	290,820	100
1X ^{14}C-CGA-48988	Begin	259,698	266,400	98
	Middle	267,431	266,400	100
	End	263,061	266,400	99
4th Application				
1X ^{14}C-CGA-329351	Begin	114,369	116,330	98
	Middle	118,824	116,330	102
	End	119,453	116,330	103
2X ^{14}C-CGA-329351	Begin	231,106	232,660	99
	Middle	239,095	232,660	103
	End	239,881	232,660	103
1X ^{14}C-CGA-48988	Begin	207,100	213,120	97
	Middle	213,556	213,120	100
	End	216,270	213,120	102

1) Three aliquots were taken at three separate times from the stock spray solutions while dispensing into the (ten) vials for the individual plants. The aliquots were taken before dispensing vial one (Begin), after dispensing vial five (Middle) and after dispensing vial ten (End).

2) Theoretical DPM calculated using the following equation:

(µg test substance)(Specific Activity nCi/µg)(2220 DPM/nCi)(final vol)(vol counted) = Ther. DPM

where: µg test substance = test substance in stock spray solution
final vol = final volume of stock spray solution
vol counted = volume of each homogeneity aliquot taken for counting

TABLE IV. ACCURACY AND PRECISION OF AG-395 AS DEMONSTRATED BY RECOVERY RESULTS FOR CONTROL AND CGA-329351- AND CGA-48988-FORTIFIED LETTUCE CONTROL SUBSTRATES

Fortified Substrate	Sample ID	PPM Fortification	PPM Determined	% Recovery[1]
CGA-329351				
Immature Lettuce	--	Reagent Blank	<0.05	
	130850	Control	<0.05	
	150735	0.05	0.048	96
	150736	0.05	0.058	116
	150737	1.0	0.73	73
Mature Lettuce	--	Reagent Blank	<0.05	
	130851	Control	<0.05	
	151217	0.05	0.074	80
	151218	1.0	0.91	87
	151219	5.0	3.75	74

Overall Average Recovery: 88%
Standard Deviation: 16%
Coefficient of Variation: 19%

Fortified Substrate	Sample ID	PPM Fortification	PPM Determined	% Recovery[1]
CGA-48988				
Immature Lettuce	--	Reagent Blank	<0.05	
	130850	Control	0.27	
	152054	1.0	1.00	74
	152055	1.0	0.97	70
	152056	5.0	4.37	82
Mature Lettuce	--	Reagent Blank	<0.05	
	130851	Control	0.043	
	151614	0.05	0.082	76
	151615	1.0	0.97	93
	151616	5.0	4.30	85

Overall Average Recovery: 80%
Standard Deviation: 8.4%
Coefficient of Variation: 10%

1) Recovery amounts corrected with control residues, where present and with molecular weight conversion factor of 2.305.

TABLE V. PRECISION OF AG-395 AS DEMONSTRATED BY REPLICATE ANALYSES OF ^{14}C-CGA-329351- AND ^{14}C-CGA-48988-TREATED LETTUCE

Substrate	Sample Code	TRR (ppm)	Residue in ppm CGA-329351[1]	Average Residue (ppm)	SD	% CV
1X ^{14}C-CGA-329351						
Immature Lettuce	130852	1.57	0.81			
	130852	1.57	0.86	0.89	0.098	11
	130852	1.57	1.00			
Mature Lettuce	130853	0.87	0.50			
	130853	0.87	0.52	0.49	0.040	8
	130853	0.87	0.45			
1X ^{14}C-CGA-48988						
Immature Lettuce	130856	3.77	2.47			
	130856	3.77	2.84	2.53	0.28	11
	130856	3.77	2.29			
Mature Lettuce	130857	1.89	1.36			
	130857	1.89	1.40	1.35	0.050	3.7
	130857	1.89	1.30			

1) Residues determined by GC/NPD an corrected for concurrently analyzed fortified control recoveries <100% and with the MW conversion factor of 2.305.

TABLE VI. SUMMARY OF THE EXTRACTABILITLY AND ACCOUNTABILITY OF ^{14}C-CGA-329351 - AND ^{14}C-CGA-48988-TREATED LETTUCE AS DETERMINED BY AG-395

Substrate	Sample Code	TRR ppm	PPM Extracted	Mean[1] % Extractibility	LSC Total Residue ^{14}C (ppm)	GC Residue[2] in ppm CGA-329351	GC Corrected[3] Residue in ppm CGA-329351	Mean[4] % Accountability
1X ^{14}C-CGA-329351								
Immature	130852	1.57	1.61		0.85	0.77	0.81	
Lettuce	130852	1.57	1.54	102	0.84	0.82	0.86	57
	130852	1.57	1.66		0.96	0.95	1.00	
Mature	130853	0.87	0.76		0.40	0.40	0.50	
Lettuce	130853	0.87	0.63	90	0.34	0.42	0.52	56
	130853	0.87	0.95		0.42	0.37	0.45	
1X ^{14}C-CGA-48988								
Immature	130856	3.77	2.47		1.83	1.86	2.47	
Lettuce	130856	3.77	3.55	82	2.54	2.13	2.84	67
	130856	3.77	3.23		1.96	1.73	2.29	
Mature	130857	1.89	1.89		1.14	1.15	1.36	
Lettuce	130857	1.89	1.88	101	1.22	1.18	1.40	72
	130857	1.89	1.93		1.14	1.10	1.30	

1) Extractability is calculated from the mean of the extractable ppm. Specific Activity of ^{14}C-CGA-329351: 39.3 nCi/µg
Specific Activity of ^{14}C-CGA-48988: 36.0 nCi/µg
2) Molecular weight conversion factor : 2.305
3) Sample residues corrected for concurrently analyzed fortified control recoveries <100% and MW conversion factor of 2.305.
4) Accountability is calculated from the mean of the corrected residues found by GC.

TABLE VII. REPRESENTATIVE RAW DATA AND SAMPLE INFORMATION - IMMATURE ^{14}C-CGA-329351-TREATED LETTUCE

Worksheet ...: 236-95-BA
Ana Set Name : 236-95-BA
Analysis ....: 1X3
Subject .....: LETTUCE

Limit of Determ : 0.050 ppm
Corr Factor A ..: 1.0000
Corr Factor B ..: 2.3050

Extracted : 9-Oct-1995
Analyzed .: 11-Oct-1995
Channel ..: 11

Product .: CGA-329351
Analyte .: DMA
Project .: 236-95

Bt Nm	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												2.00	
2*	C	1	1	001000	94.00	10.00	100.00	20.00			1.828	2.0	2.00	1.828
3	S												2.00	
4	R	1	"	"	94.00	10.00	100.00	20.00			1.828	2.0	2.00	1.828
5	R	1	"	"	94.00	10.00	100.00	20.00			1.828	2.0	2.00	1.828
6	S												2.00	
7	R	1	"	"	94.00	10.00	100.00	20.00			1.828	8.0	2.00	0.457
8	S												2.00	
9	X	1	2	002000	94.00	10.00	100.00	20.00			1.828	10.0	2.00	0.366
10	X	1	"	"	94.00	10.00	100.00	20.00			1.828	10.0	2.00	0.366
11	X	1	"	"	94.00	10.00	100.00	20.00			1.828	13.0	2.00	0.281
12	S												2.00	
13	B	1	REAGENT	BLANK	94.00	10.00	100.00	20.00			1.828	2.0	2.00	1.828
14	S												2.00	

Bt Nm	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		0.05000	0.1000	681	0.113								
2*	C	1			0	0.000	0.000				95.00	<0.050		CONTROL
3	S		0.01000	0.0200	109	0.018								
4	R	1			229	0.038	0.048	0.048	0.050	95.73				0.05 ppm recovery
5	R	1			278	0.046	0.058	0.058	0.050	116.32				0.05 ppm recovery
6	S		0.10000	0.2000	1151	0.192								
7	R	1			868	0.145	0.729	0.729	1.000	72.94				1. ppm recovery
8	S		0.10000	0.2000	1142	0.190								
9	X	1			736	0.123	0.773				95.00	0.814		1X 14-C CGA-329351
10	X	1			779	0.130	0.818				95.00	0.861		1X 14-C CGA-329351
11	X	1			697	0.116	0.952				95.00	1.002		1X 14-C CGA-329351
12	S		0.20000	0.4000	2432	0.406								
13	B	1			0	0.000	0.000				95.00	<0.050		REAGENT BLANK
14	S		0.02500	0.0500	306	0.051								

Substrates : WHOLE PLANT

Notes : CGA-329351 in greenhouse grown lettuce
1X treatment - Immature Harvest

Analyst: Lisa Swaim

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 VIII. REPRESENTATIVE RAW DATA AND SAMPLE INFORMATION - MATURE ^{14}C-CGA-329351-TREATED LETTUCE

Worksheet ...: 236-95-BD
Ana Set Name : 236-95-BD
Analysis ....: 1X3W
Subject .....: LETTUCE

Limit of Determ : 0.050 ppm
Corr Factor A ..: 1.0000
Corr Factor B ..: 2.3050

Extracted : 17-Oct-1995
Analyzed .: 20-Oct-1995
Channel ..: 11

Product .: CGA-329351
Analyte .: DMA
Project .: 236-95

Bt Nm	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												2.00	
2*	C	1	1	001000	94.00	10.00	100.00	20.00			1.828	2.0	2.00	1.828
3	R	1	"	"	94.00	10.00	100.00	20.00			1.828	2.0	2.00	1.828
4	S												2.00	
5	R	1	"	"	94.00	10.00	100.00	20.00			1.828	8.0	2.00	0.457
6	R	1	"	"	94.00	10.00	100.00	20.00			1.828	35.0	2.00	0.104
7	S												2.00	
8	X	1	2	002000	94.00	10.00	100.00	20.00			1.828	8.0	2.00	0.457
9	X	1	"	"	94.00	10.00	100.00	20.00			1.828	8.0	2.00	0.457
10	S												2.00	
11	X	1	"	"	94.00	10.00	100.00	20.00			1.828	8.0	2.00	0.457
12	B	1	REAGENT	BLANK	94.00	10.00	100.00	20.00			1.828	2.0	2.00	1.828
13	S												2.00	

Bt Nm	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		0.10000	0.2000	1399	0.212								
2*	C	1			138	0.027	0.034				80.51	<0.050		C-3W
3	R	1			355	0.059	0.074	0.040	0.050	80.09				R4-3W
4	S		0.02500	0.0500	308	0.052								
5	R	1			1179	0.179	0.905	0.871	1.000	87.08				R5-3W
6	R	1			1115	0.170	3.753	3.718	5.000	74.37				R6-3W
7	S		0.20000	0.4000	2680	0.399								
8	X	1			496	0.080	0.401				80.51	0.498		1X4-3W
9	X	1			523	0.084	0.421				80.51	0.523		1X5-3W
10	S		0.01000	0.0200	152	0.029								
11	X	1			447	0.072	0.366				80.51	0.454		1X6-3W
12	B	1			0	0.000	0.000				80.51	<0.050		RB-3W
13	S		0.05000	0.1000	485	0.078								

Substrates : WHOLE PLANT

Notes : CGA-329351 in greenhouse grown lettuce
1X treatment - Mature Harvest

Analyst: Lisa Swaim

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 IX. REPRESENTATIVE RAW DATA AND SAMPLE INFORMATION - IMMATURE ^{14}C-CGA-48988-TREATED LETTUCE

Worksheet ...: 236-95-BF
Ana Set Name : 236-95-BF
Analysis ....: 23695BF
Subject .....: LETTUCE

Limit of Determ : 0.050 ppm
Corr Factor A ..: 1.0000
Corr Factor B ..: 2.3050

Extracted : 1-Nov-1995
Analyzed .: 6-Nov-1995
Channel ..: 11

Product .: METALAXYL
Analyte .: DMA
Project .: 236-95

Bt Nm	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												2.00	
2*	C	1	1	001000	94.00	10.00	100.00	20.00			1.828	2.0	2.00	1.828
3	S												2.00	
4	R	1	"	"	94.00	10.00	100.00	20.00			1.828	8.0	2.00	0.457
5	R	1	"	"	94.00	10.00	100.00	20.00			1.828	8.0	2.00	0.457
6	S												2.00	
7	R	1	"	"	94.00	10.00	100.00	20.00			1.828	35.0	2.00	0.104
8	X	1	4	004000	94.00	10.00	100.00	20.00			1.828	25.0	2.00	0.146
9	S												2.00	
10	X	1	"	"	94.00	10.00	100.00	20.00			1.828	25.0	2.00	0.146
11	X	1	"	"	94.00	10.00	100.00	20.00			1.828	25.0	2.00	0.146
12	S												2.00	
13	B	1	REAGENT	BLANK	94.00	10.00	100.00	20.00			1.828	2.0	2.00	1.828
14	S												2.00	

Bt Nm	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		0.02500	0.0500	2587	0.064								
2*	C	1			10184	0.211	0.267				75.23	0.354		M-C CONTROL
3	S		0.20000	0.4000	20319	0.409								
4	R	1			9543	0.199	1.003	0.737	1.000	73.67				M-R1 (1 ppm RECOVERY)
5	R	1			9173	0.192	0.967	0.700	1.000	70.04				M-R2 (1 ppm RECOVERY)
6	S		0.01000	0.0200	968	0.032								
7	R	1			9487	0.198	4.365	4.099	5.000	81.98				M-R3 (5 ppm RECOVERY)
8	X	1			5373	0.118	1.856				75.23	2.468		M-1X1 (1X CGA-48988)
9	S		0.10000	0.2000	9114	0.191								
10	X	1			6274	0.135	2.133				75.23	2.835		M-1X2 (1X CGA-48988)
11	X	1			4945	0.109	1.725				75.23	2.293		M-1X3 (1X CGA-48988)
12	S		0.05000	0.1000	3858	0.088								
13	B	1			73	0.015	0.018				75.23	<0.050		M-RB REAGENT BLANK
14	S		0.05000	0.1000	3783	0.087								

Substrates : WHOLE PLANT

Notes : CGA-48988 in greenhouse grown lettuce
1X treatment - Immature Harvest

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 X. REPRESENTATIVE RAW DATA AND SAMPLE INFORMATION - MATURE ^{14}C-CGA-48988-TREATED LETTUCE

Worksheet ...: 236-95-BE
Ana Set Name : 236-95-BE
Analysis ....: 1XMW
Subject .....: LETTUCE

Limit of Determ : 0.050 ppm
Corr Factor A ..: 1.0000
Corr Factor B ..: 2.3050

Extracted : 23-Oct-1995
Analyzed .: 24-Oct-1995
Channel ..: 11

Product .: METALAXYL
Analyte .: DMA
Project .: 236-95

Bt Nm	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												2.00	
2*	C	1	1	001000	94.00	10.00	100.00	20.00			1.828	2.0	2.00	1.828
3	S												2.00	
4	R	1	"	"	94.00	10.00	100.00	20.00			1.828	2.0	2.00	1.828
5	R	1	"	"	94.00	10.00	100.00	20.00			1.828	8.0	2.00	0.457
6	S												2.00	
7	R	1	"	"	94.00	10.00	100.00	20.00			1.828	35.0	2.00	0.104
8	X	1	4	004000	94.00	10.00	100.00	20.00			1.828	15.0	2.00	0.244
9	S												2.00	
10	X	1	"	"	94.00	10.00	100.00	20.00			1.828	15.0	2.00	0.244
11	X	1	"	"	94.00	10.00	100.00	20.00			1.828	15.0	2.00	0.244
12	S												2.00	
13	B	1	REAGENT	BLANK	94.00	10.00	100.00	20.00			1.828	2.0	2.00	1.828
14	S												2.00	

Bt Nm	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		0.10000	0.2000	1714	0.204								
2*	C	1			284	0.034	0.043				84.75	0.051		C-MW
3	S		0.02500	0.0500	415	0.050								
4	R	1			539	0.065	0.082	0.038	0.050	76.18				R4-MW
5	R	1			1623	0.193	0.973	0.930	1.000	92.96				R5-MW
6	S		0.05000	0.1000	850	0.101								
7	R	1			1639	0.195	4.299	4.255	5.000	85.10				R6-MW
8	X	1			1023	0.122	1.152				84.75	1.360		1X4-MW
9	S		0.05000	0.1000	847	0.101								
10	X	1			1050	0.125	1.183				84.75	1.395		1X5-MW
11	X	1			975	0.116	1.099				84.75	1.297		1X6-MW
12	S		0.01000	0.0200	129	0.016								
13	B	1			0	0.000	0.000				84.75	<0.050		RB-MW
14	S		0.20000	0.4000	3354	0.398								

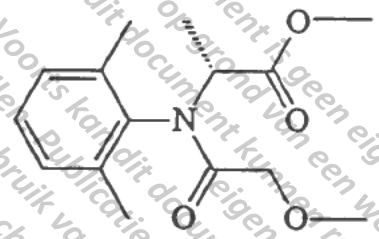
Substrates : WHOLE PLANT

Notes : CGA-48988 in greenhouse grown lettuce
1X treatment - Mature Harvest

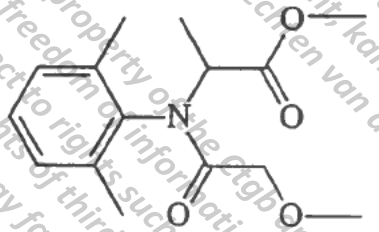
Analyst:

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~~

FIGURE 1. CHEMICAL NAMES AND STRUCTURES

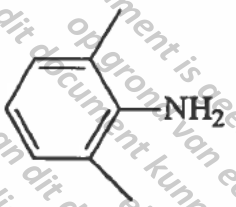


CGA-329351
D-Alanine, N-(2,6-dimethylphenyl)-N-(methoxyacetyl)-, methyl ester
CAS Registry No: 70630-17-0

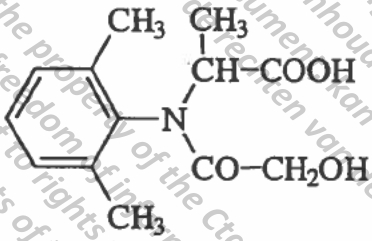


CGA-48988
DL-Alanine, N-(2,6-dimethylphenyl)-N-(methoxyacetyl)-, methyl ester
CAS Registry No: 57837-19-1

FIGURE 1. CHEMICAL NAMES AND STRUCTURES (Continued)



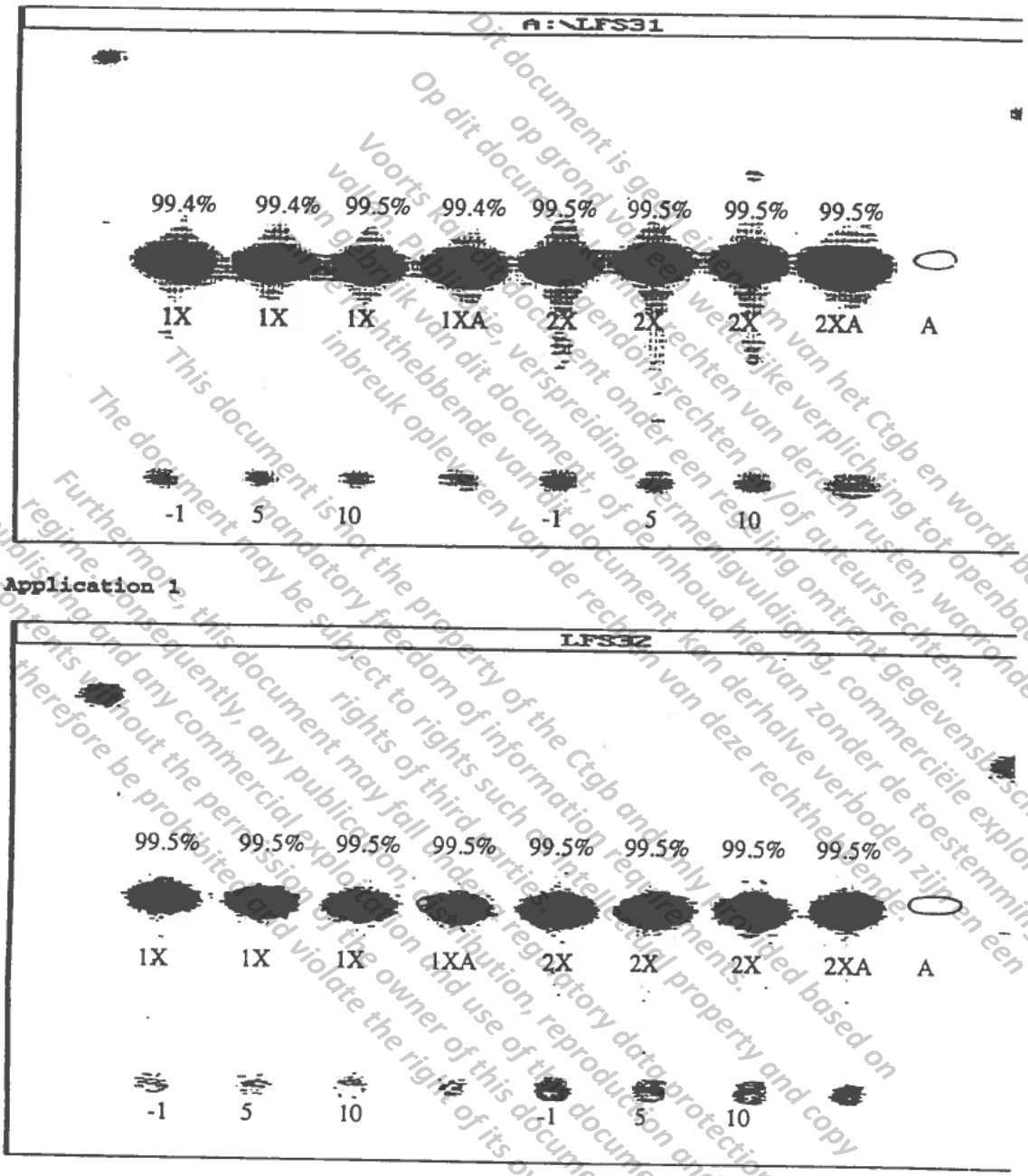
CGA-72649
Benzenamine, 2,6-dimethyl-
CAS Registry No: 87-62-7



CGA-107955
DL-Alanine, N-(2,6-dimethylphenyl)-N-(hydroxyacetyl)-
CAS Registry No: 104390-55-8

See ABR-91084[5] for structures of other postulated and identified CGA-48988 metabolites.

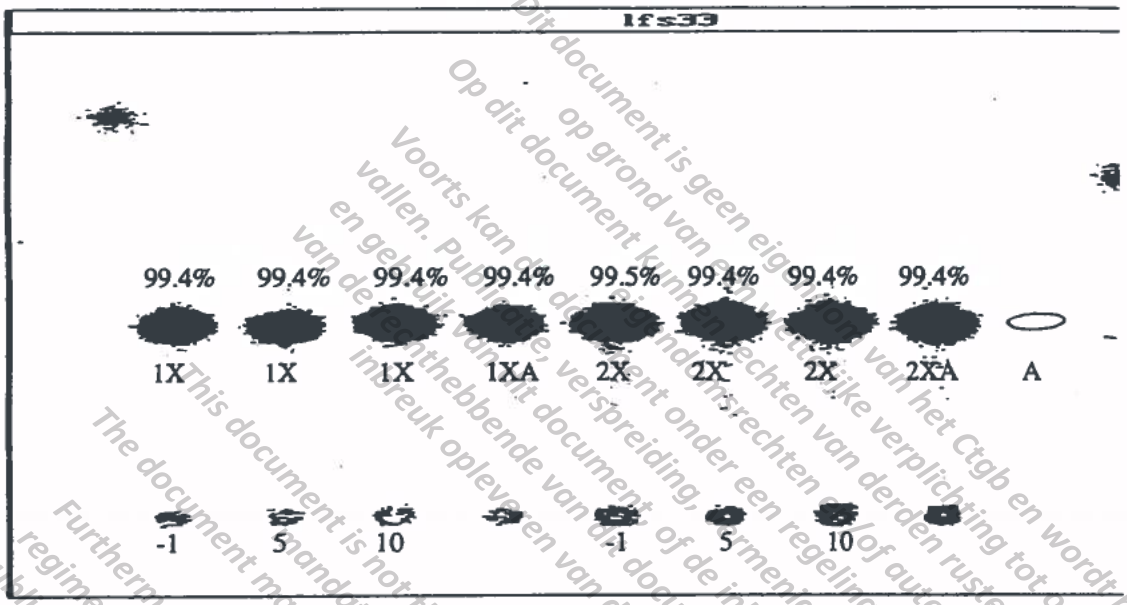
FIGURE 2. PURITY OF ^{14}C-CGA-329351 SPRAY SOLUTIONS AS DETERMINED BY TLC WITH UV DETECTION AND RADIOANALYTIC IMAGING



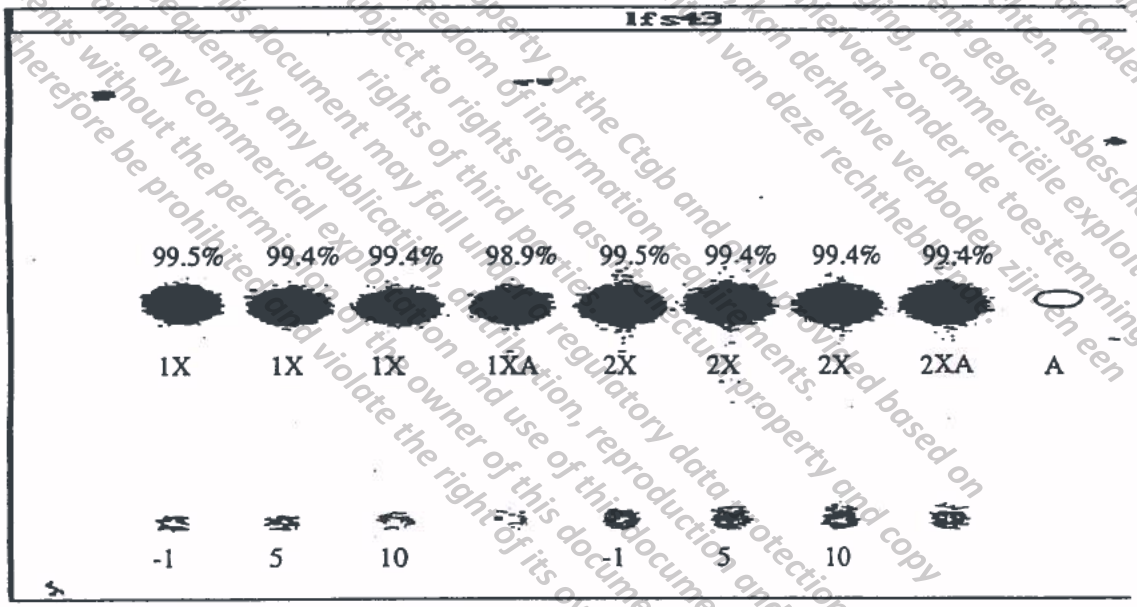
1X: 1X ^{14}C-CGA-329351 Spray Solution
2X: 2X ^{14}C-CGA-329351 Spray Solution
A: CGA-329351 Standard UV spot
1XA: 1X ^{14}C-CGA-329351 Spray Solution with CGA-329351 Standard
2XA: 2X ^{14}C-CGA-329351 Spray Solution with CGA-329351 Standard

The spot descriptions, -1, 5 and 10, represent aliquots taken while dispensing stock spray solutions into the vials for individual plants. The sample in the "-1" column is a portion of one of the aliquots taken before dispensing vial one. The sample in the "5" column is a portion of one of the aliquots taken after dispensing vial five and the "10" sample is a portion of one of the aliquots taken after dispensing vial ten. See Sections VII.B.2.0 for further clarification on aliquots taken for purity analyses.

FIGURE 2. PURITY OF ^{14}C-CGA-329351 SPRAY SOLUTIONS AS DETERMINED BY TLC WITH UV DETECTION AND RADIOANALYTIC IMAGING (Continued)



Application 3

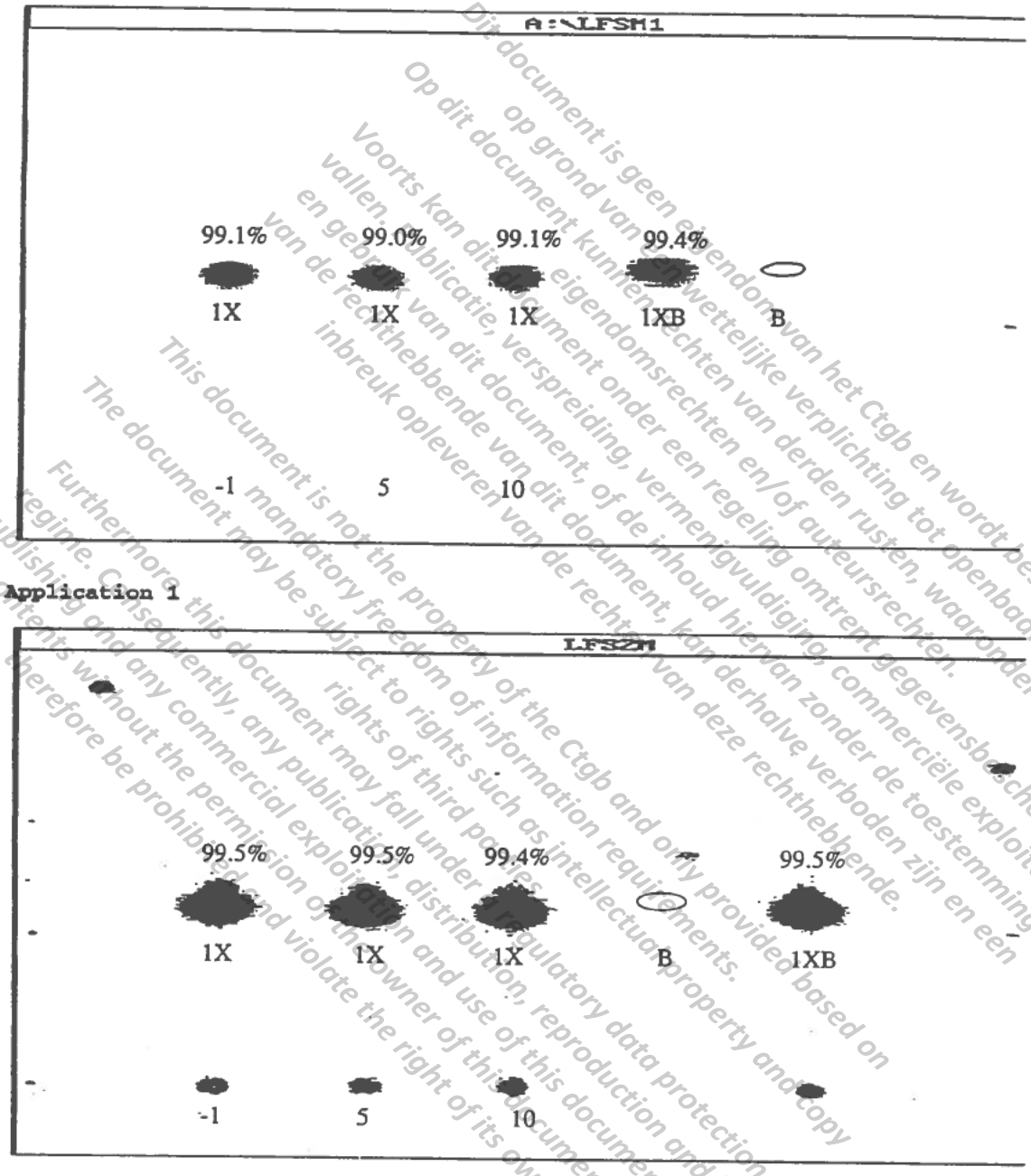


Application 4

1X: 1X ^{14}C-CGA-329351 Spray Solution 2X: 2X ^{14}C-CGA-329351 Spray Solution
A: CGA-329351 Standard UV spot
1XA: 1X ^{14}C-CGA-329351 Spray Solution with CGA-329351 Standard
2XA: 2X ^{14}C-CGA-329351 Spray Solution with CGA-329351 Standard

The spot descriptions, -1, 5 and 10, represent aliquots taken while dispensing stock spray solutions into the vials for individual plants. The sample in the "-1" column is a portion of one of the aliquots taken before dispensing vial one. The sample in the "5" column is a portion of one of the aliquots taken after dispensing vial five and the "10" sample is a portion of one of the aliquots taken after dispensing vial ten. See Sections VII.B.2.0 for further clarification on aliquots taken for purity analyses.

FIGURE 3. PURITY OF ^{14}C-CGA-48988 SPRAY SOLUTIONS AS DETERMINED BY TLC WITH UV DETECTION AND RADIOANALYTIC IMAGING

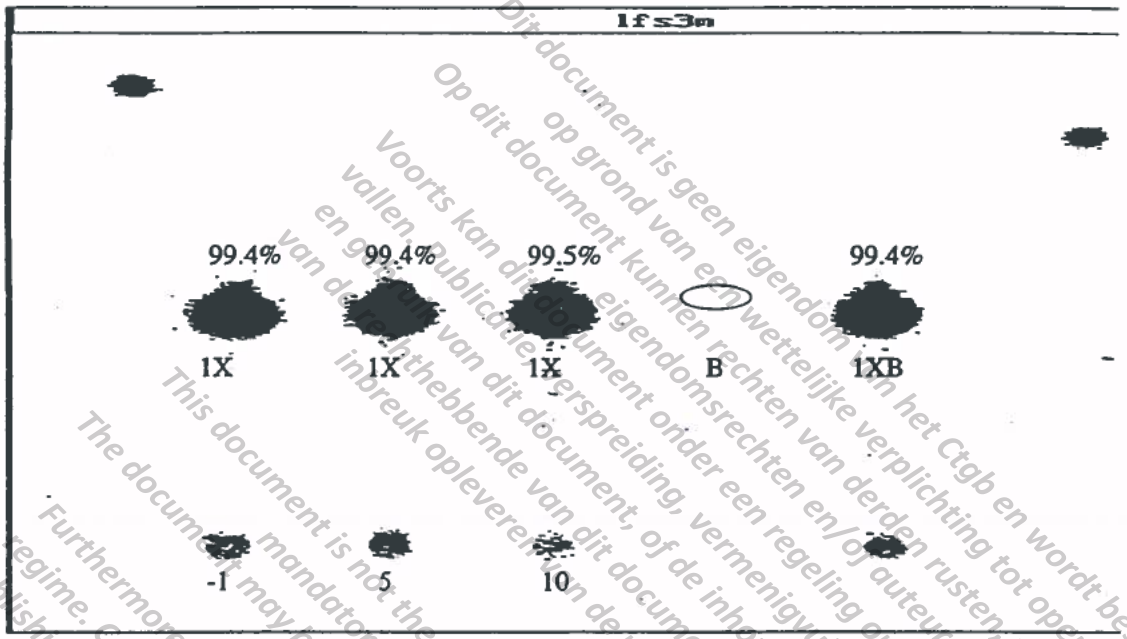


Application 2

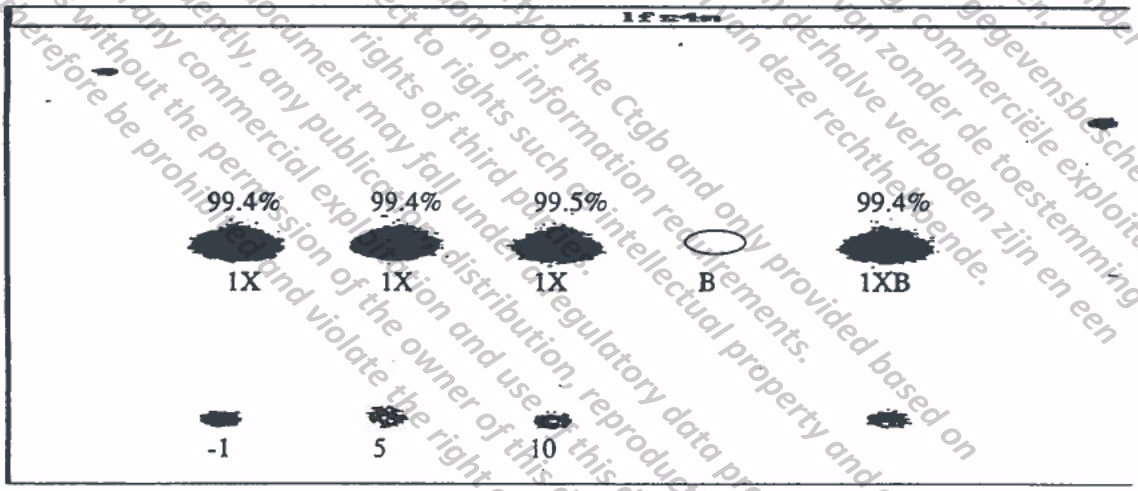
1X: 1X ^{14}C-CGA-48988 Spray Solution
B: CGA-48988 Standard UV spot
1XB: 1X ^{14}C-CGA-48988 Spray Solution with CGA-48988 Standard

The spot descriptions, -1, 5 and 10, represent aliquots taken while dispensing stock spray solutions into the vials for individual plants. The sample in the "-1" column is a portion of one of the aliquots taken before dispensing vial one. The sample in the "5" column is a portion of one of the aliquots taken after dispensing vial five and the "10" sample is a portion of one of the aliquots taken after dispensing vial ten. See Sections VII.B.2.0 for further clarification on aliquots taken for purity analyses.

FIGURE 3. PURITY OF ^{14}C-CGA-48988 SPRAY SOLUTIONS AS DETERMINED BY TLC WITH UV DETECTION AND RADIOANALYTIC IMAGING (Continued)



Application 3



Application 4

1X: 1X ^{14}C-CGA-48988 Spray Solution
B: CGA-48988 Standard UV spot
1XB: 1X ^{14}C-CGA-48988 Spray Solution with CGA-48988 Standard

The spot descriptions, -1, 5 and 10, represent aliquots taken while dispensing stock spray solutions into the vials for individual plants. The sample in the "-1" column is a portion of one of the aliquots taken before dispensing vial one. The sample in the "5" column is a portion of one of the aliquots taken after dispensing vial five and the "10" sample is a portion of one of the aliquots taken after dispensing vial ten. See Sections VII.B.2.0 for further clarification on aliquots taken for purity analyses.

FIGURE 4. METABOLITE PROFILE OF ^{14}C-CGA-329351-TREATED IMMATURE LETTUCE AS DETERMINED BY TWO DIMENSIONAL TLC WITH UV DETECTION AND RADIOANALYTIC IMAGING

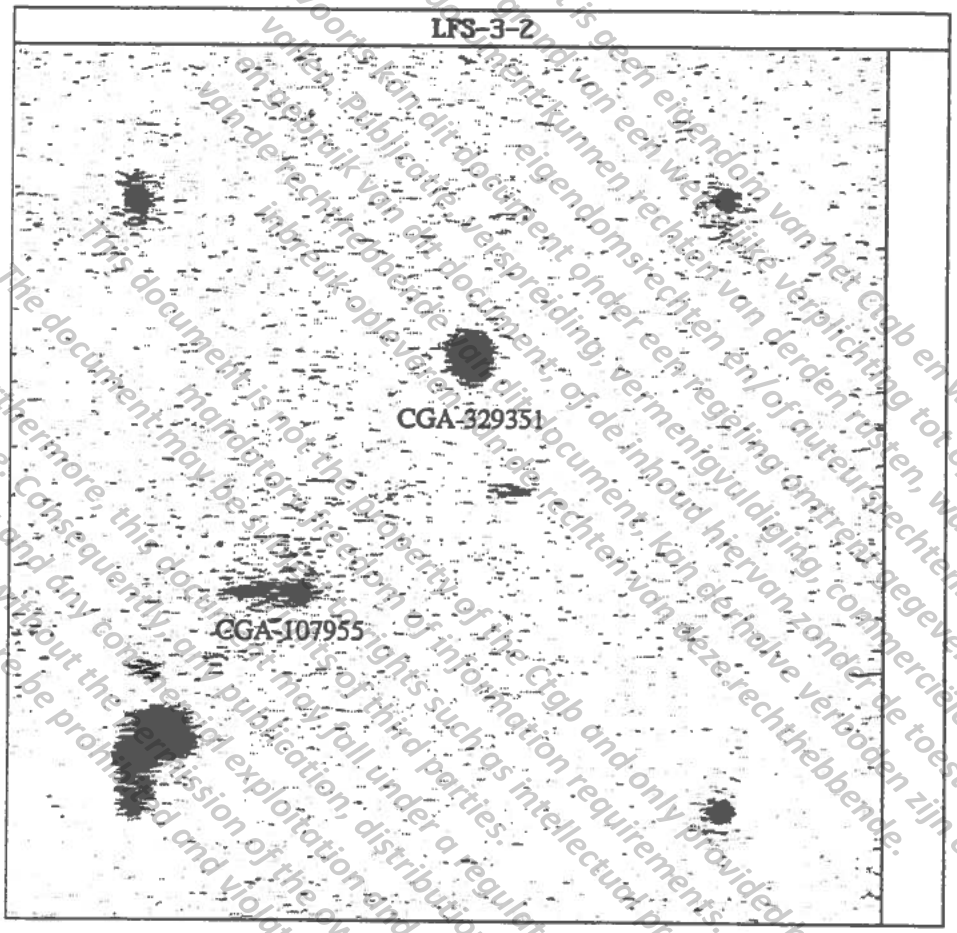


FIGURE 5. METABOLITE PROFILE OF ^{14}C-CGA-48988-TREATED IMMATURE LETTUCE AS DETERMINED BY TWO DIMENSIONAL TLC WITH UV DETECTION AND RADIOANALYTIC IMAGING

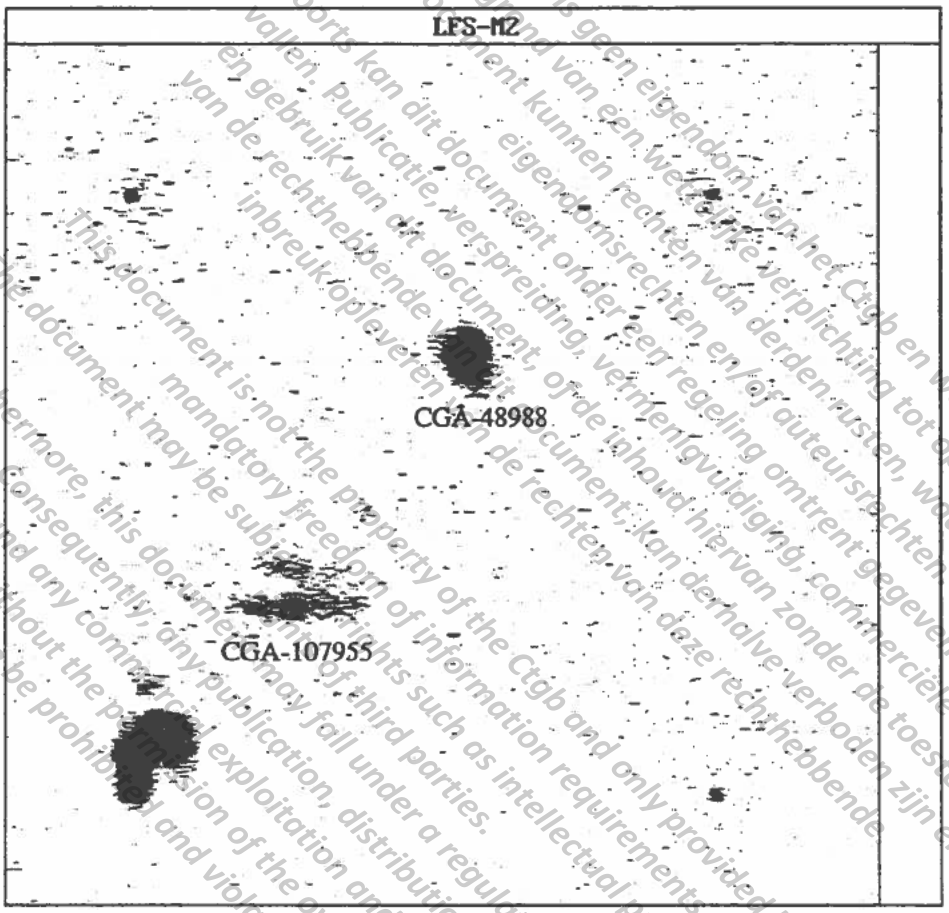
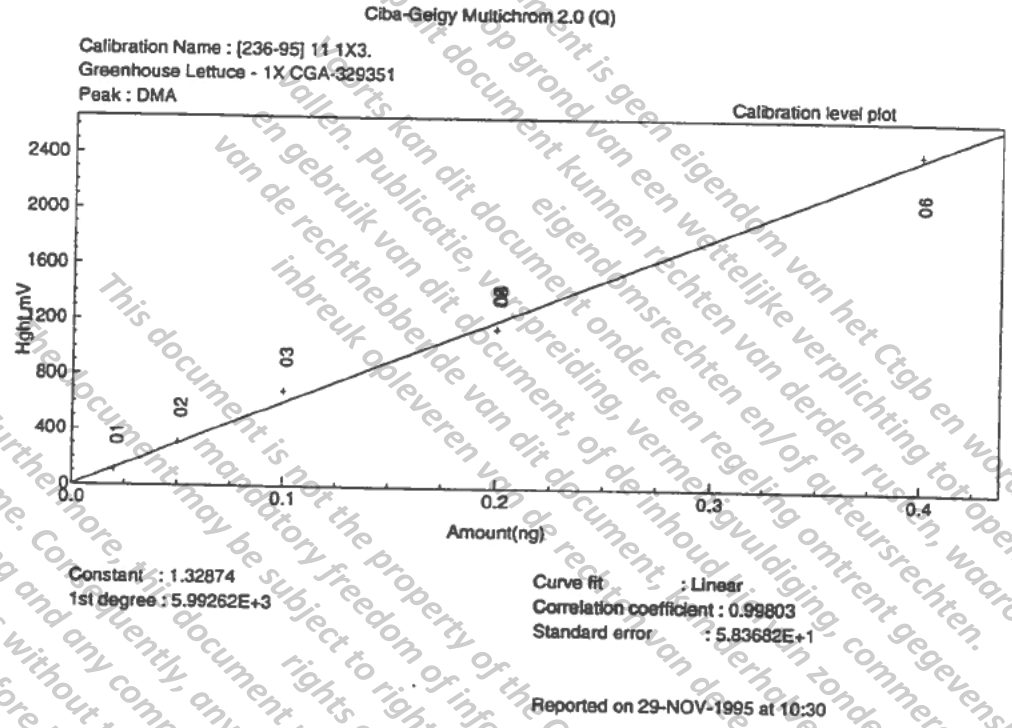
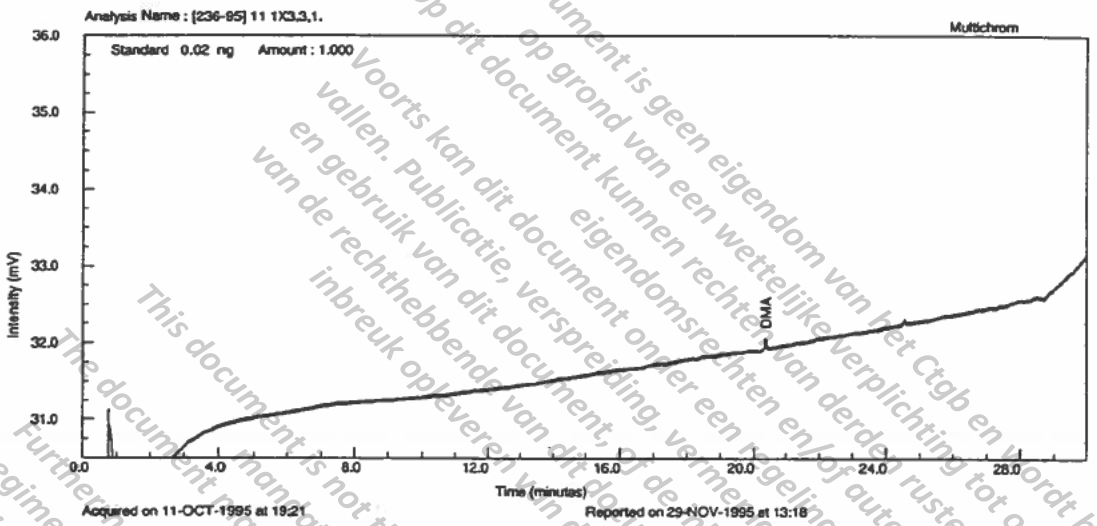


FIGURE 6. TYPICAL CALIBRATION CURVE AND STANDARDIZATION DATA FOR AG-395 OBTAINED FROM THE ANALYSIS OF IMMATURE LETTUCE

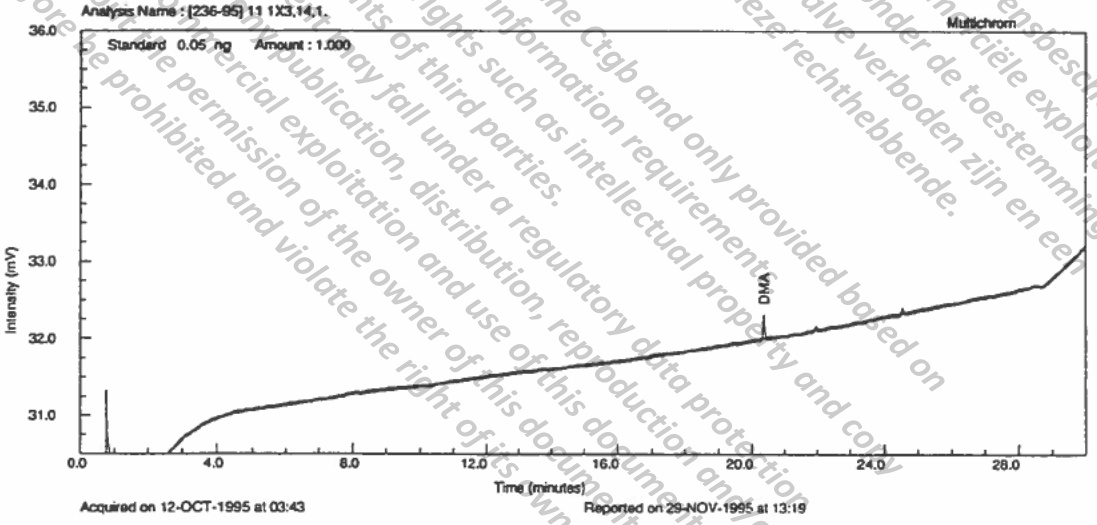


NG	PEAK HEIGHT
0.02	109
0.05	306
0.10	681
0.20	1151
0.20	1142
0.40	2432

FIGURE 7. TYPICAL DMA STANDARD CHROMATOGRAMS FOR AG-395 OBTAINED FROM THE ANALYSIS OF IMMATURE LETTUCE

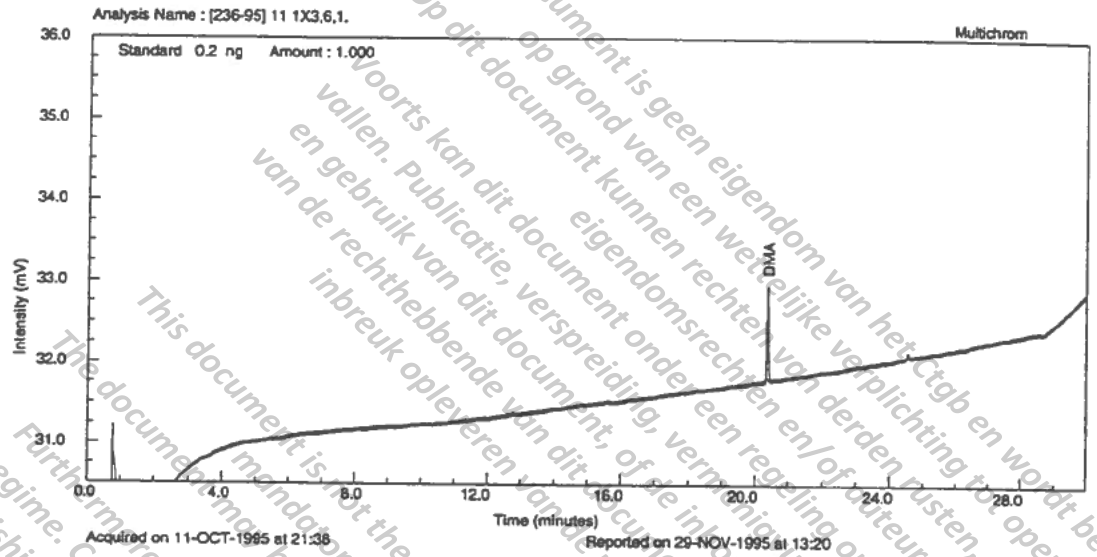


0.02 ng 2,6-Dimethylaniline Standard

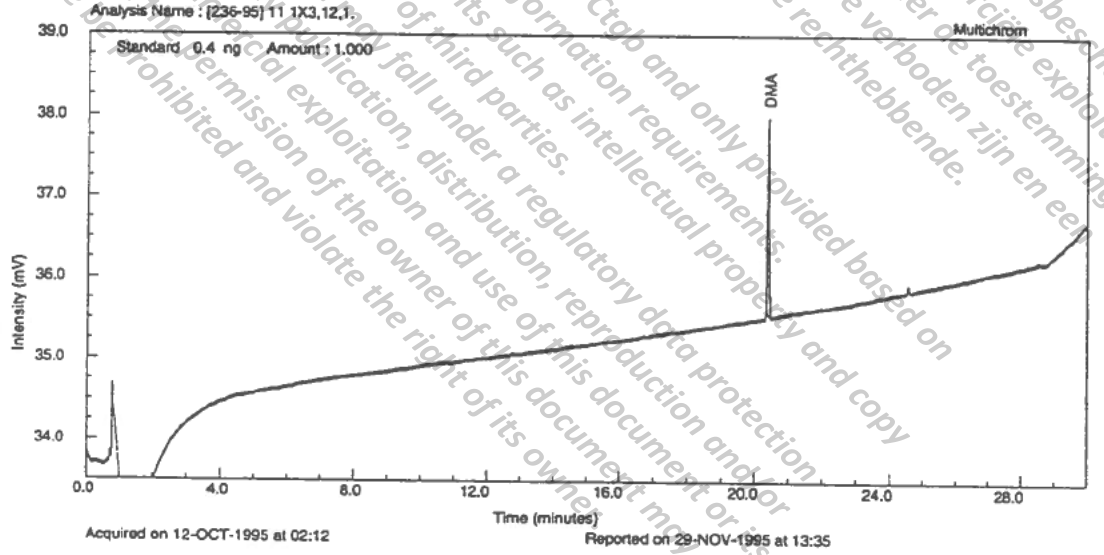


0.05 ng 2,6-Dimethylaniline Standard

FIGURE 7. TYPICAL DMA STANDARD CHROMATOGRAMS FOR AG-395 OBTAINED FROM THE ANALYSIS OF IMMATURE LETTUCE (Continued)

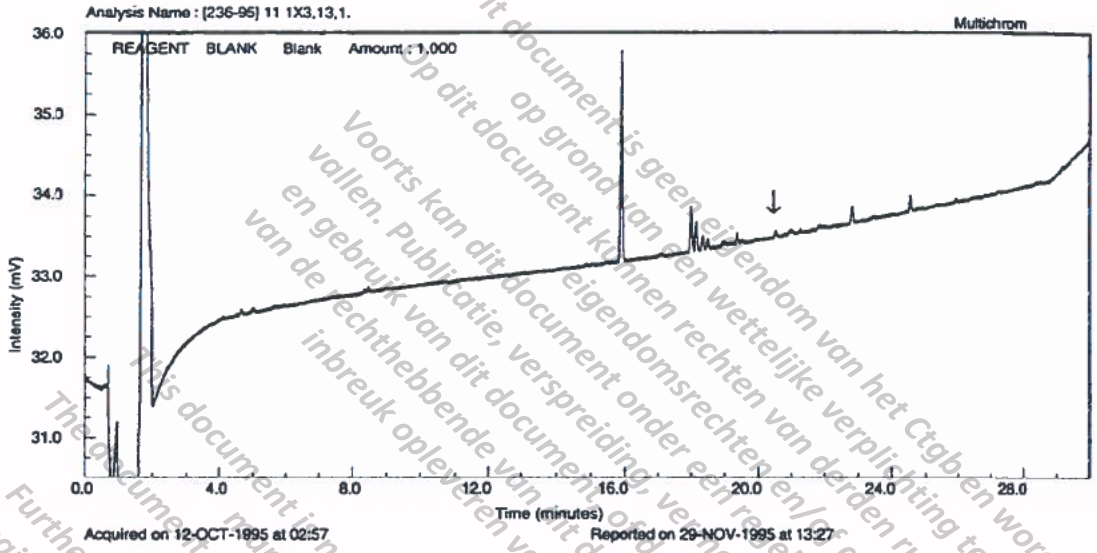


0.2 ng 2,6-Dimethylaniline Standard

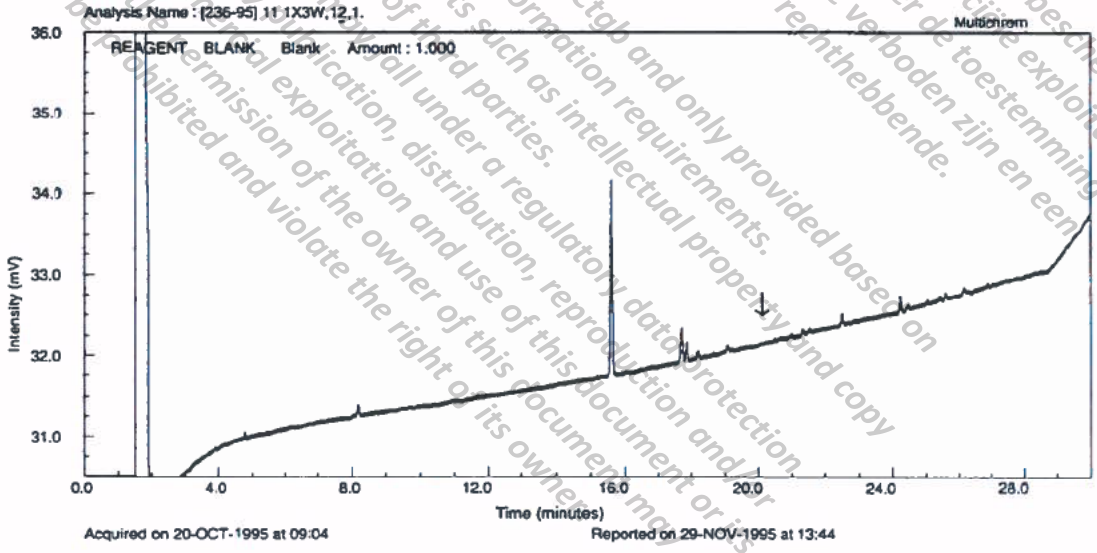


0.4 ng 2,6-Dimethylaniline Standard

FIGURE 8. REPRESENTATIVE REAGENT BLANK CHROMATOGRAMS USING AG-395

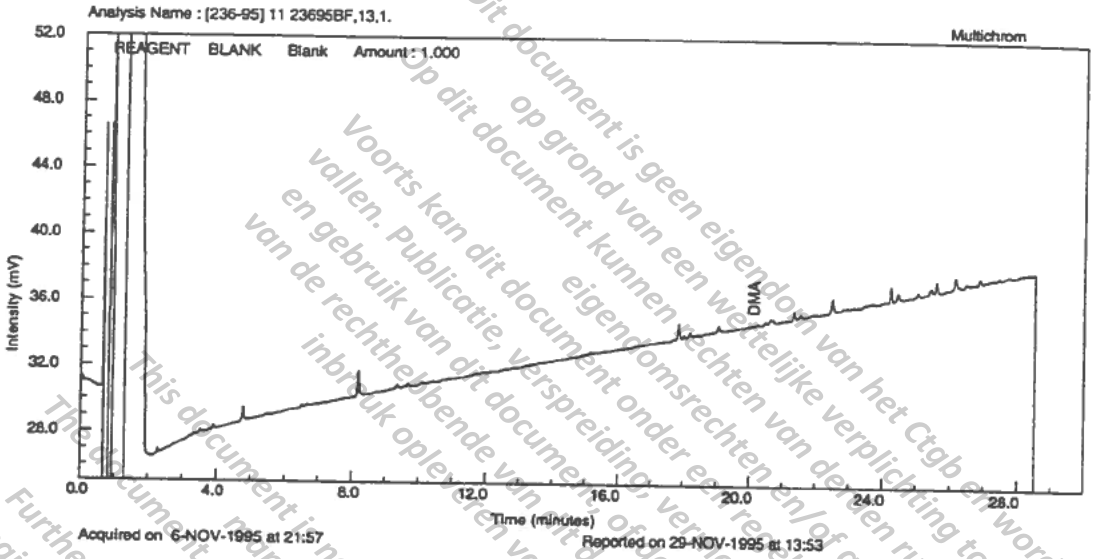


Blank run with 1X ^{14}C-CGA-329351 treated immature lettuce, 1.83 mg eq inj, <0.02 ng found, <0.05 ppm CGA-329351

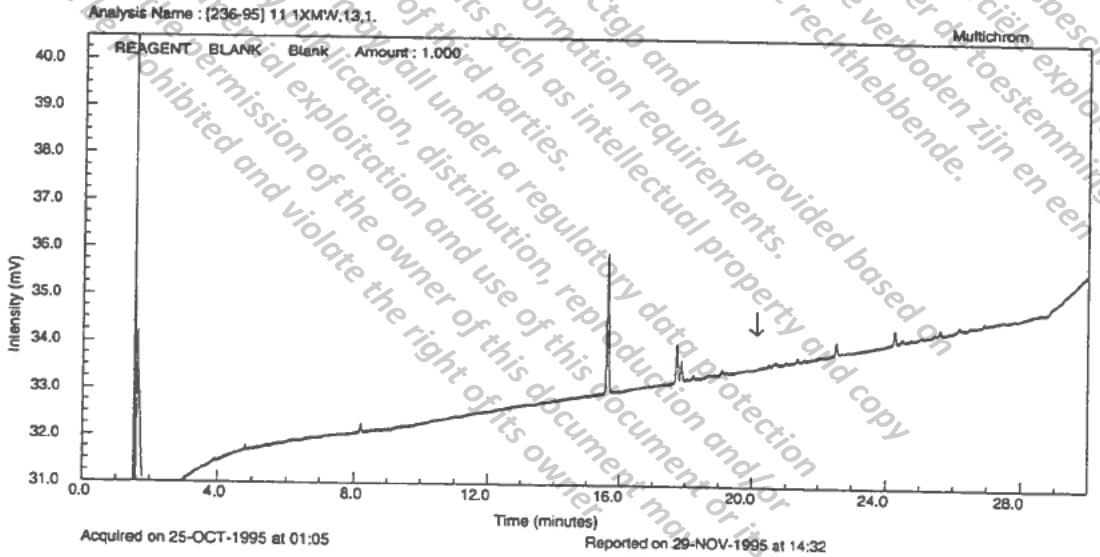


Blank run with 1X ^{14}C-CGA-329351 treated mature lettuce, 1.83 mg eq inj, <0.02 ng found, <0.05 ppm CGA-329351

FIGURE 8. REPRESENTATIVE REAGENT BLANK CHROMATOGRAMS USING AG-395 (Continued)

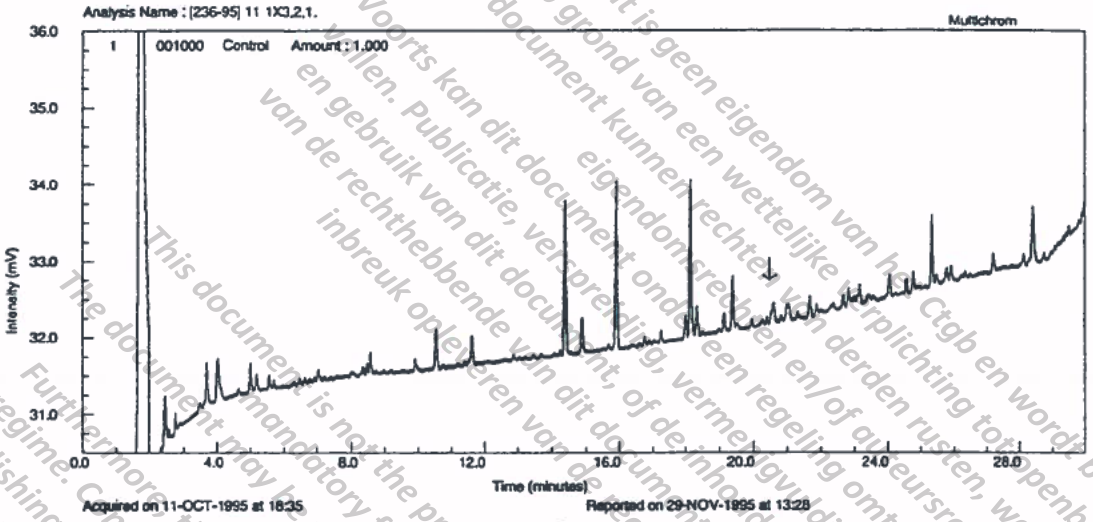


Blank run with 1X ^{14}C-CGA-48988 treated immature lettuce, 1.83 mg eq inj, <0.02 ng found (0.015 ng), <0.05 ppm CGA-48988



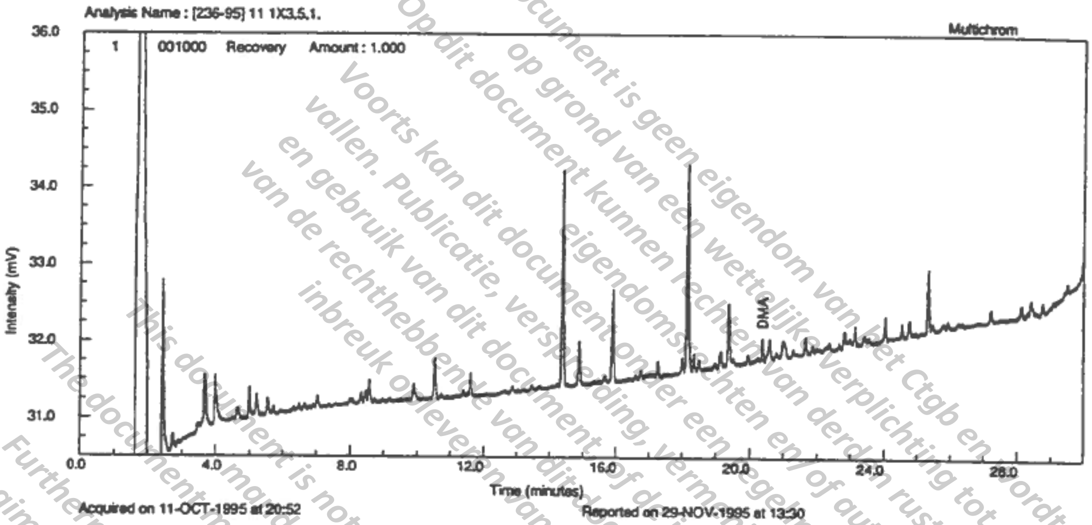
Blank run with 1X ^{14}C-CGA-48988 treated mature lettuce, 1.83 mg eq inj, <0.02 ng found, <0.05 ppm CGA-48988

FIGURE 9. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF CONTROL, CGA-329351-FORTIFIED AND ^{14}C-CGA-329351-TREATED IMMATURE LETTUCE USING AG-395

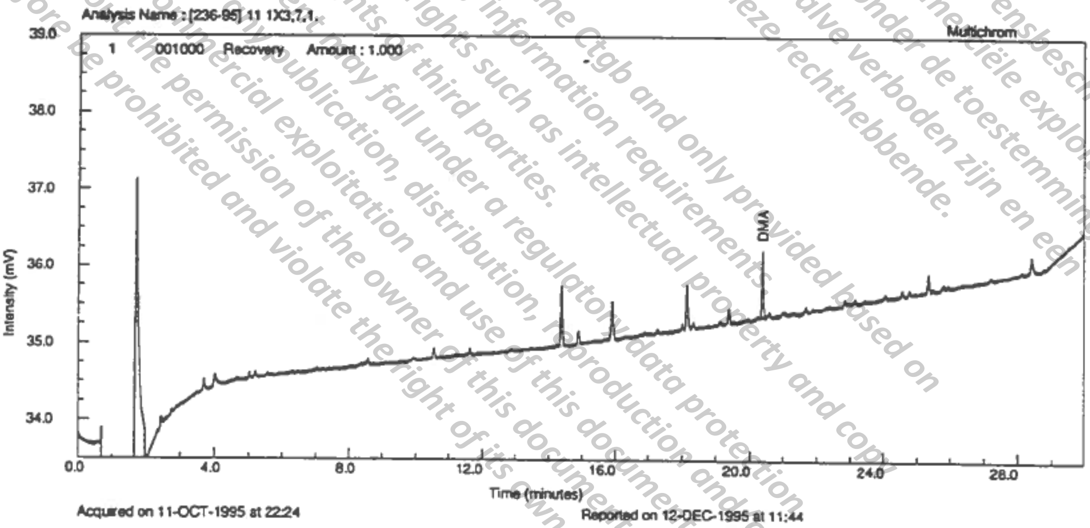


Sample 130850, Control, 1.83 mg inj, <0.02 ng found, <0.05 ppm CGA-329351

FIGURE 9. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF CONTROL, CGA-329351-FORTIFIED AND ^{14}C-CGA-329351-TREATED IMMATURE LETTUCE USING AG-395 (Continued)



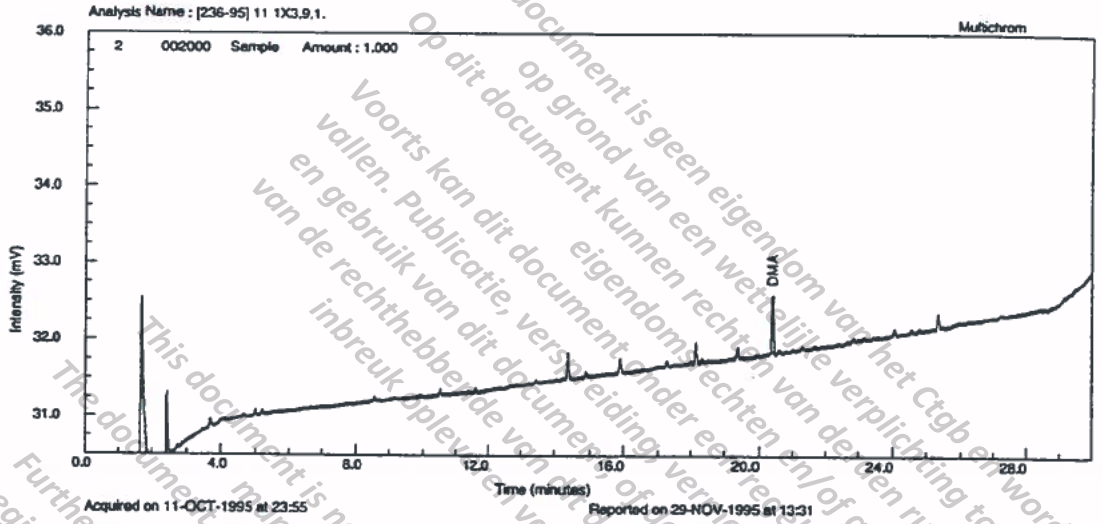
Sample 150736, 0.05 ppm CGA-329351 fortification, 1.83 mg inj, 0.046 ng found, 0.025 ppm uncorrected, 0.058 ppm CGA-329351, 116% Recovery



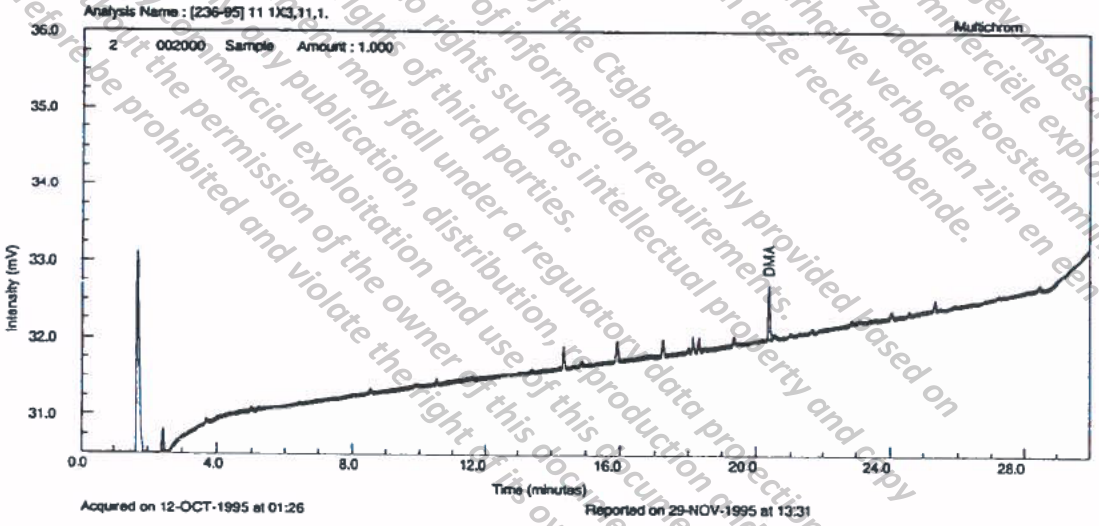
Sample 150737, 1.0 ppm CGA-329351 fortification, 0.46 mg inj, 0.15 ng found, 0.32 ppm uncorrected, 0.73 ppm CGA-329351, 73% Recovery

Recovery results corrected for control ppm values and with MW conversion factor of 2.305.

FIGURE 9. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF CONTROL, CGA-329351-FORTIFIED AND ^{14}C-CGA-329351-TREATED IMMATURE LETTUCE USING AG-395 (Continued)



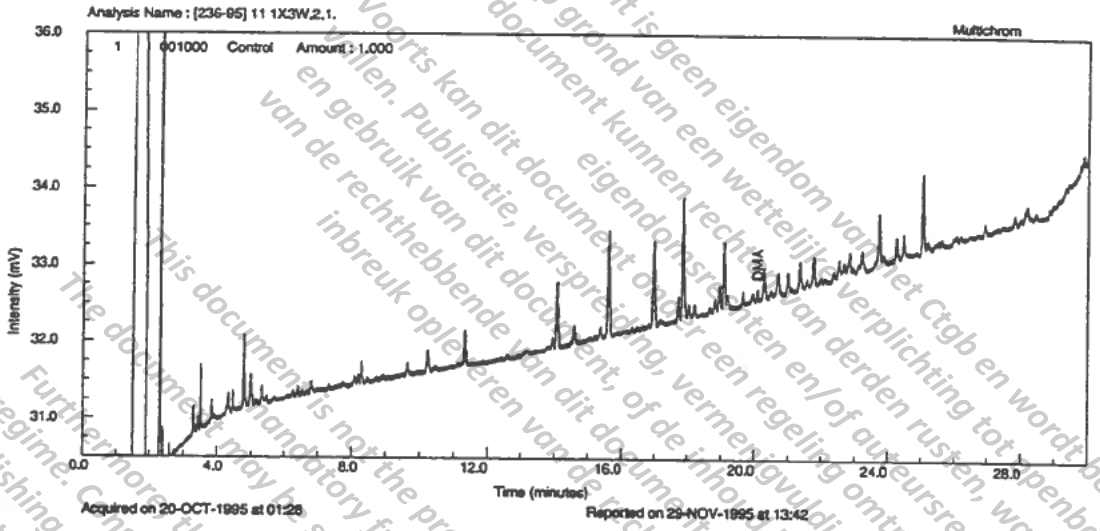
Sample 130852, ^{14}C-CGA-329351-treated sample, 0.37 mg inj, 0.12 ng found, 0.34 ppm uncorrected, 0.81 ppm CGA-329351



Sample 130852, ^{14}C-CGA-329351-treated sample, 0.28 mg inj, 0.12 ng found, 0.41 ppm uncorrected, 1.00 ppm CGA-329351

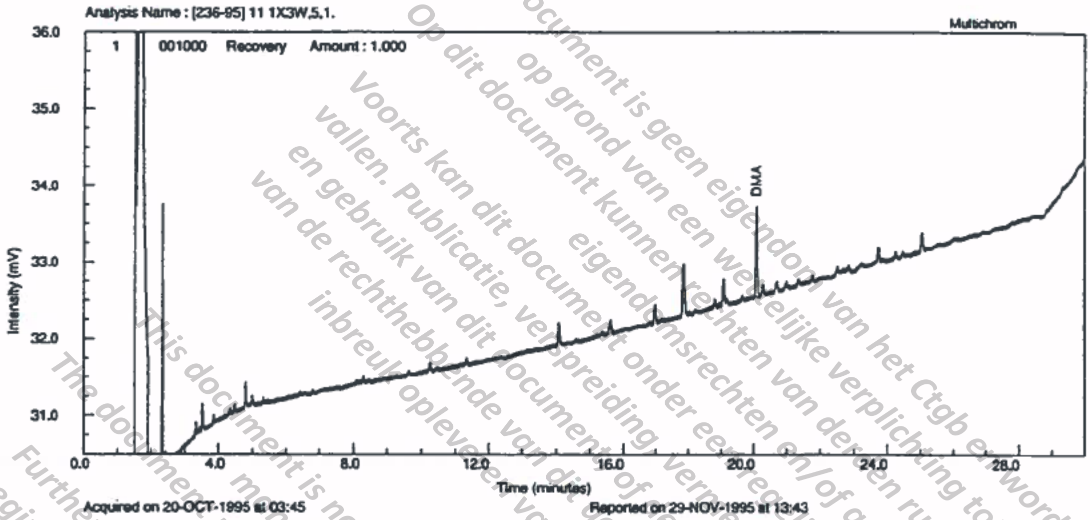
Treated samples corrected for concurrently analyzed fortified control recoveries <100% and with the MW conversion factor of 2.305.

FIGURE 10. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF CONTROL, CGA-329351-FORTIFIED AND ^{14}C-CGA-329351-TREATED MATURE LETTUCE USING AG-395

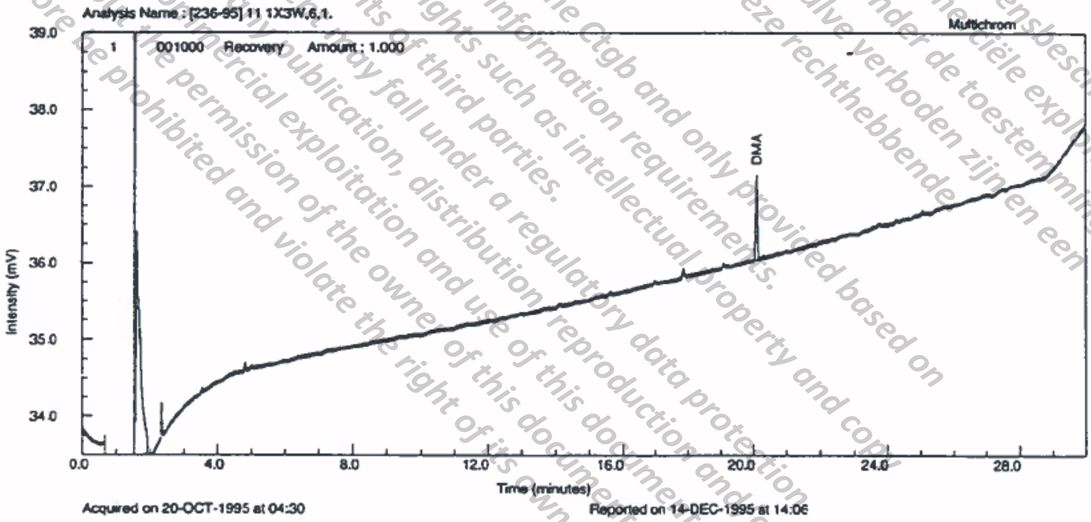


Sample 130851, Control, 1.83 mg inj, 0.027 ng found, <0.05 ppm CGA-329351

FIGURE 10. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF CONTROL, CGA-329351-FORTIFIED AND ^{14}C-CGA-329351-TREATED MATURE LETTUCE USING AG-395



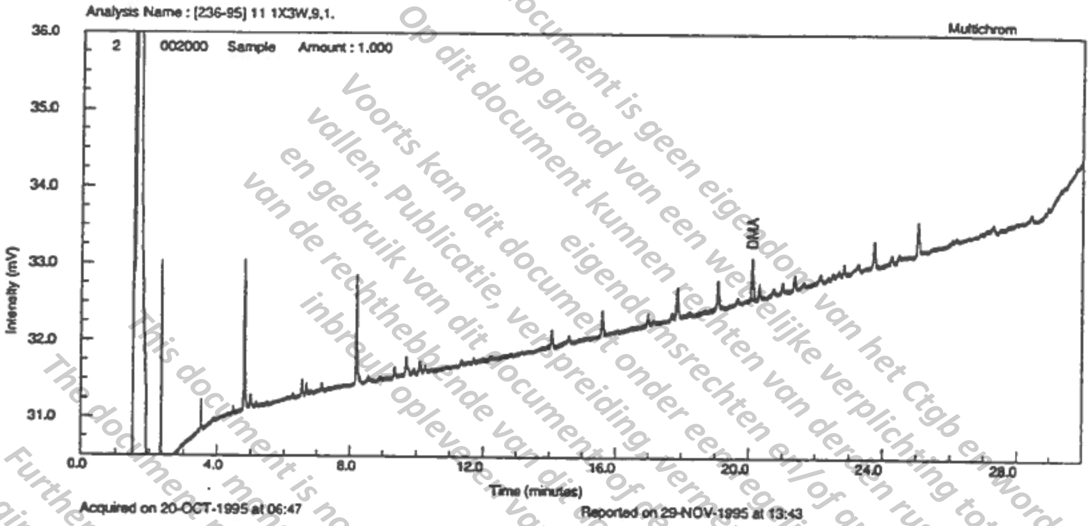
Sample 151218, 1.0 ppm CGA-329351 fortification, 0.46 mg inj, 0.18 ng found, 0.39 ppm uncorrected, 0.91 ppm CGA-329351, 87% Recovery



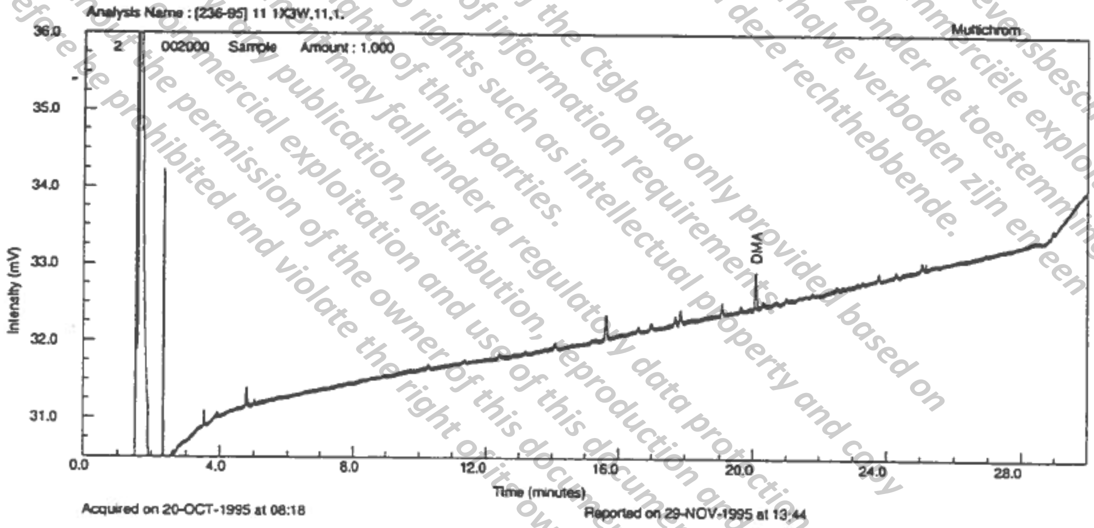
Sample 151219, 5.0 ppm CGA-329351 fortification, 0.10 mg inj, 0.17 ng found, 0.16 ppm uncorrected, 3.75 ppm CGA-329351, 74% Recovery

Recovery results corrected for control ppm values and with MW conversion factor of 2.305.

FIGURE 10. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF CONTROL, CGA-329351-FORTIFIED AND ^{14}C-CGA-329351-TREATED MATURE LETTUCE USING AG-395 (Continued)



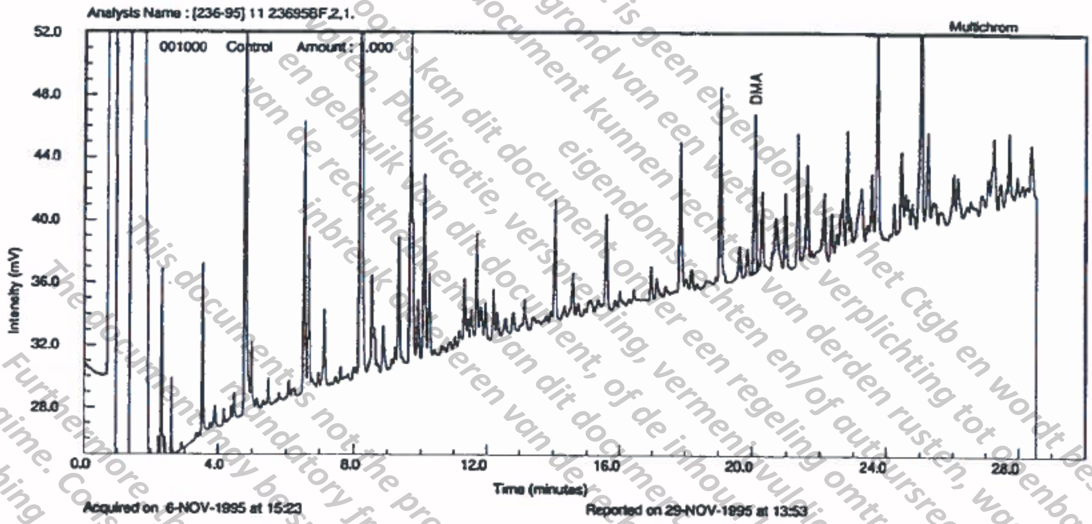
Sample 130853, ^{14}C-CGA-329351-treated sample, 0.46 mg inj, 0.084 ng found, 0.18 ppm uncorrected, 0.52 ppm CGA-329351



Sample 130853, ^{14}C-CGA-329351-treated sample, 0.46 mg inj, 0.072 ng found, 0.16 ppm uncorrected, 0.45 ppm CGA-329351

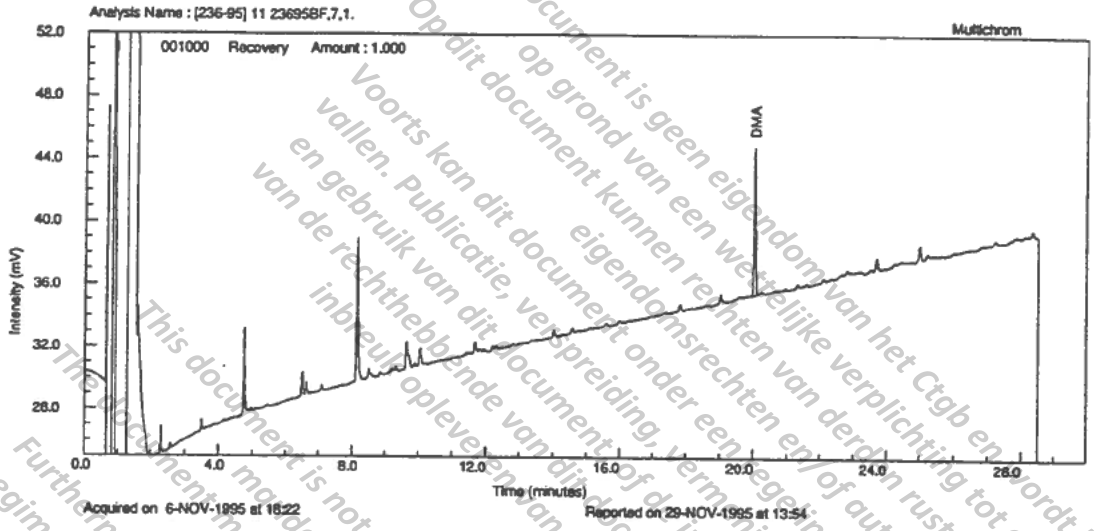
Treated samples corrected for concurrently analyzed fortified control recoveries <100% and with the MW conversion factor of 2.305.

FIGURE 11. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF CONTROL, CGA-48988-FORTIFIED AND ^{14}C-CGA-48988-TREATED IMMATURE LETTUCE USING AG-395

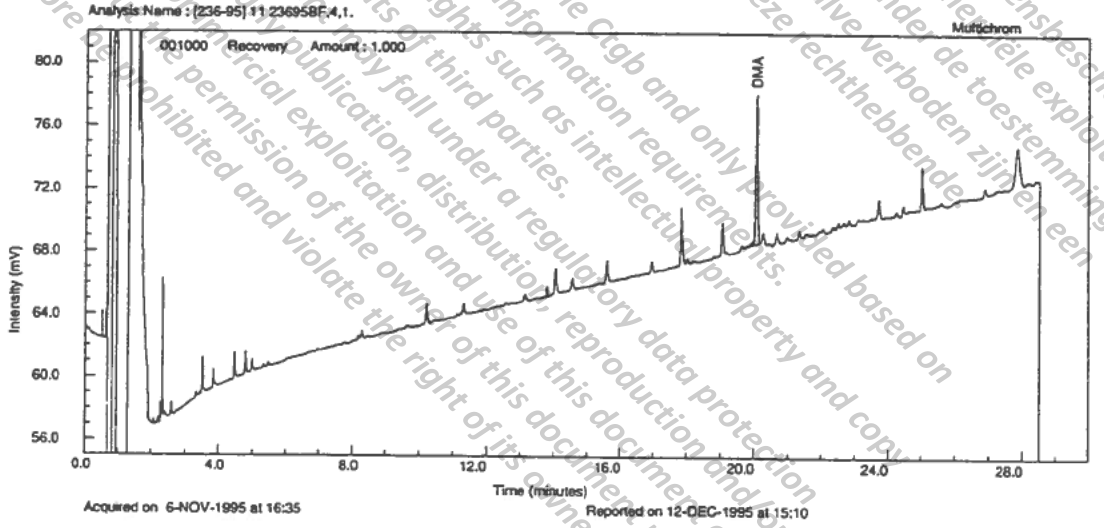


Sample 130850, Control, 1.83 mg inj, 0.21 ng found, 0.12 ppm uncorrected, 0.27 ppm CGA-48988

FIGURE 11. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF CONTROL, CGA-48988-FORTIFIED AND ^{14}C-CGA-48988-TREATED IMMATURE LETTUCE USING AG-395 (Continued)



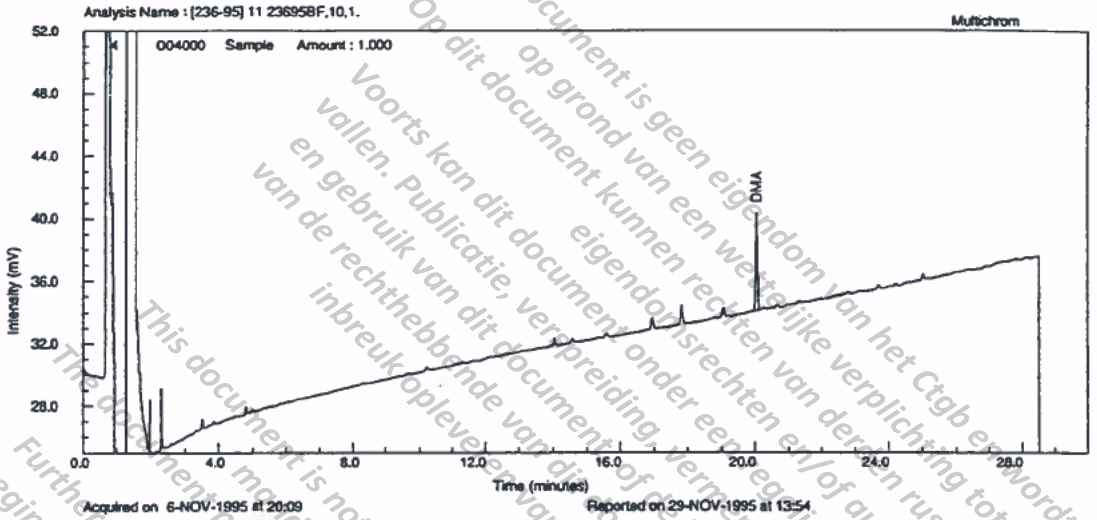
Sample 152056, 5.0 ppm CGA-48988 fortification, 0.10 mg inj, 0.20 ng found, 1.90 ppm uncorrected, 4.37 ppm CGA-48988, 82% Recovery



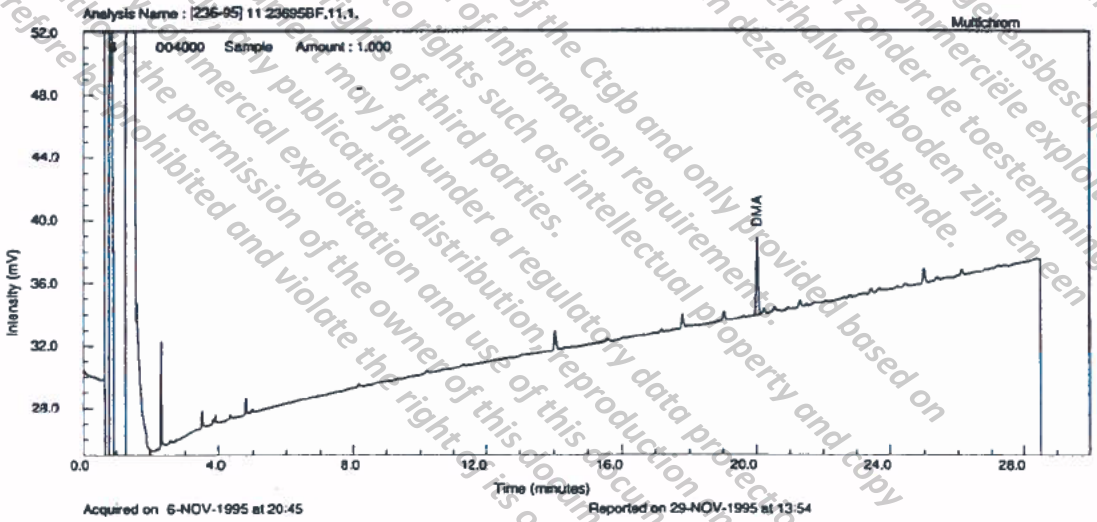
Sample 152054, 1.0 ppm CGA-48988 fortification, 0.46 mg inj, 0.29 ng found, 0.44 ppm uncorrected, 1.00 ppm CGA-48988, 74% Recovery

Recovery results corrected for control ppm values and with MW conversion factor of 2.305.

FIGURE 11. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF CONTROL, CGA-48988-FORTIFIED AND ^{14}C-CGA-48988-TREATED IMMATURE LETTUCE USING AG-395 (Continued)



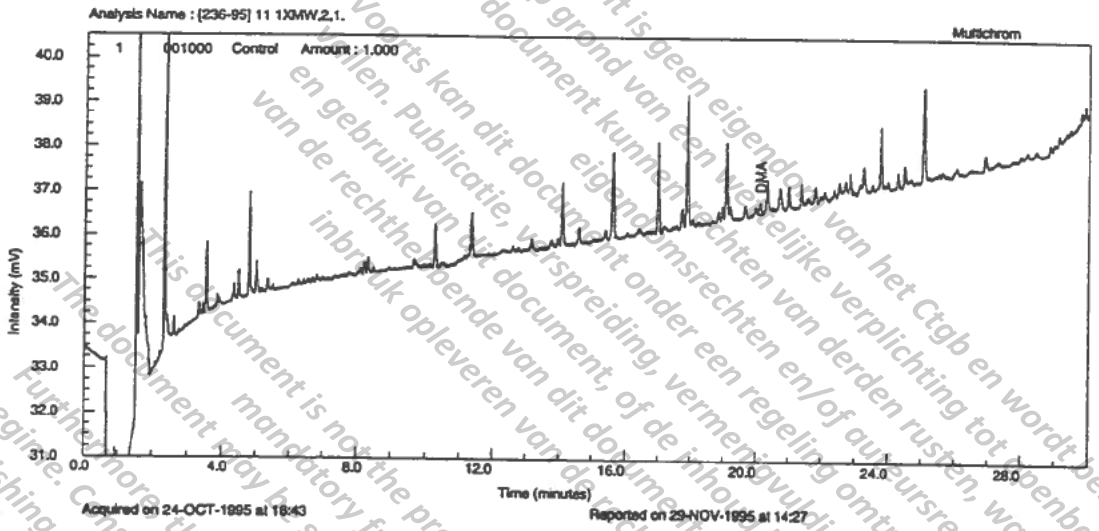
Sample 130856, ^{14}C-CGA-48988-treated sample, 0.15 mg inj, 0.14 ng found, 0.92 ppm uncorrected, 2.84 ppm CGA-48988



Sample 130856, ^{14}C-CGA-48988-treated sample, 0.15 mg inj, 0.11 ng found, 0.75 ppm uncorrected, 2.29 ppm CGA-48988

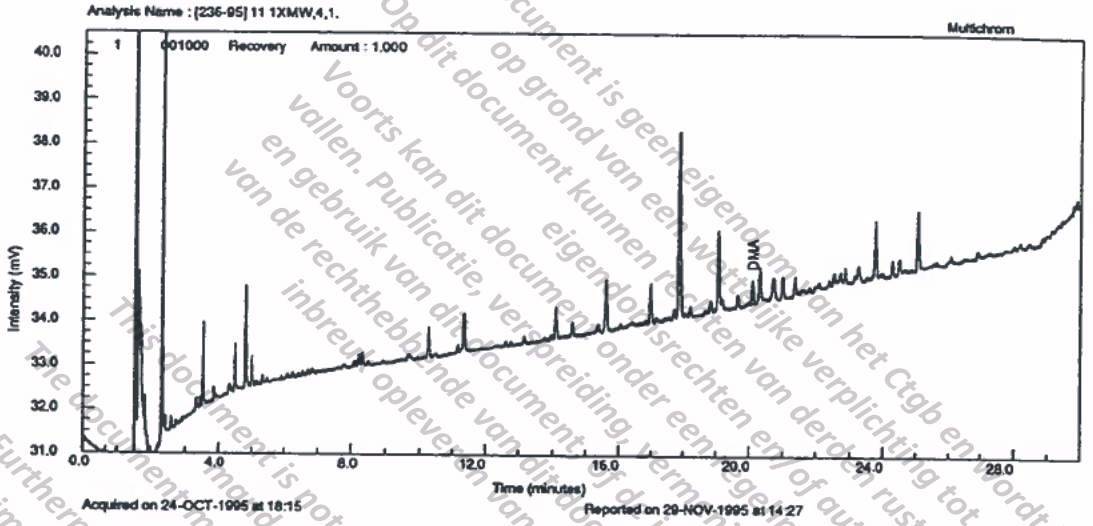
Treated samples corrected for concurrently analyzed fortified control recoveries <100% and with the MW conversion factor of 2.305.

FIGURE 12. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF CONTROL, CGA-48988-FORTIFIED AND ^{14}C-CGA-48988-TREATED MATURE LETTUCE USING AG-395

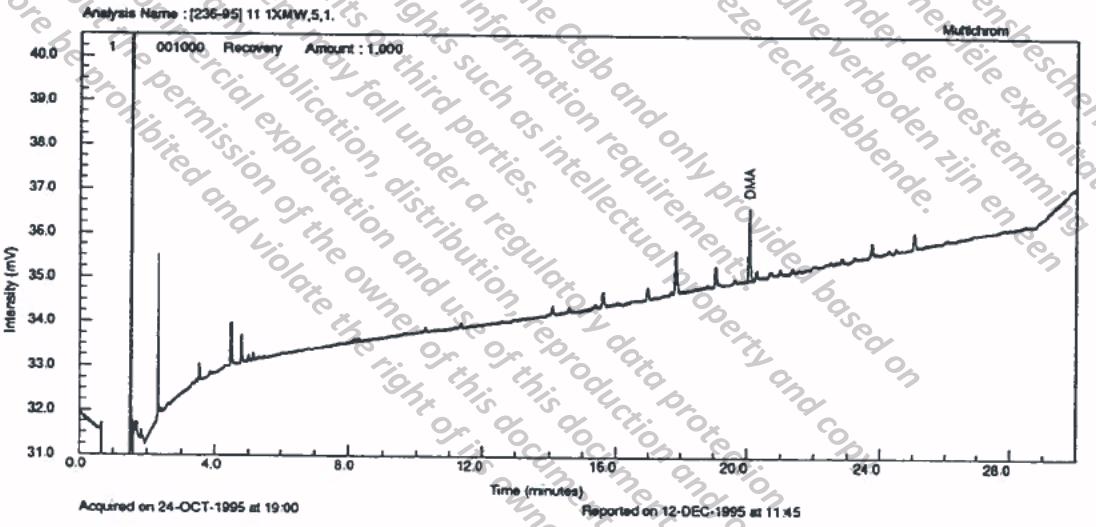


Sample 130851, Control, 1.83 mg inj, 0.034 ng found, 0.019 ppm uncorrected, 0.043 ppm CGA-48988

FIGURE 12. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF CONTROL, CGA-48988-FORTIFIED AND ^{14}C-CGA-48988-TREATED MATURE LETTUCE USING AG-395



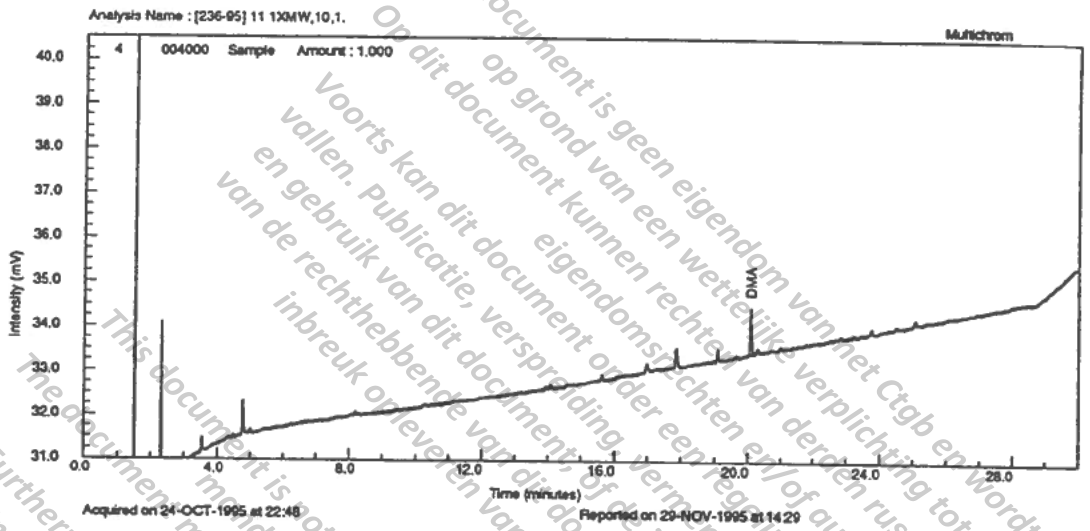
Sample 151614, 0.05 ppm CGA-48988 fortification, 1.83 mg inj, 0.065 ng found, 0.036 ppm uncorrected, 0.082 ppm CGA-48988, 76% Recovery



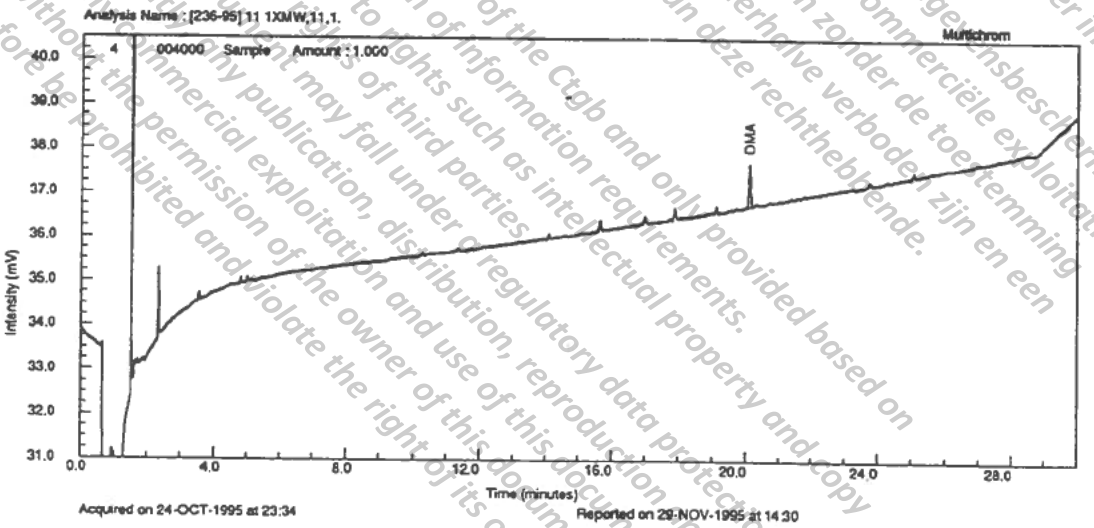
Sample 151615, 1.0 ppm CGA-48988 fortification, 0.46 mg inj, 0.19 ng found, 0.42 ppm uncorrected, 0.97 ppm CGA-48988, 93% Recovery

Recovery results corrected for control ppm values and with MW conversion factor of 2.305.

FIGURE 12. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF CONTROL, CGA-48988-FORTIFIED AND ^{14}C-CGA-48988-TREATED MATURE LETTUCE USING AG-395 (Continued)



Sample 130857, ^{14}C-CGA-48988-treated sample, 0.24 mg inj, 0.13 ng found, 0.51 ppm uncorrected, 1.40 ppm CGA-48988



Sample 130857, ^{14}C-CGA-48988-treated sample, 0.24 mg inj, 0.12 ng found, 0.48 ppm uncorrected, 1.30 ppm CGA-48988

Treated samples corrected for concurrently analyzed fortified control recoveries <100% and with the MW conversion factor of 2.305.

XII. REFERENCES

1. Ciba Protocol 236-95, "Validation of Analytical Methodology for CGA-329351 with Lettuce Samples Treated with ^{14}C-CGA-329351."

2. [redacted], [redacted]; [redacted], [redacted]. Analytical Method AG-395, "Improved Method for the Determination of Total Residues of Metalaxyl in Crop as 2,6-Dimethylaniline," December, 1982.

3. [redacted], [redacted]. ABR-83033, "Validation of Analytical Method AG-395 Using ^{14}C-Metalaxyl Treated Lettuce," May, 1983.

4. [redacted], [redacted]. BIOL-95010, "Biological Phase Report: Validation of Analytical Methodology for CGA-329351 with Lettuce Samples Treated with ^{14}C-CGA-329351."

5. [redacted], [redacted]; [redacted], [redacted]; [redacted], [redacted]; [redacted], [redacted]; [redacted], [redacted]. [redacted]-91084, "Uptake and Metabolism of Metalaxyl in Greenhouse Rotational Crops following Target Tobacco Grown in Soil Treated with [Phenyl-^{14}C]-Metalaxyl," January, 1992.

6. [redacted], [redacted]. [redacted], "Analytical Phase I Report: Validation of Analytical Methodology for CGA-329351 with Lettuce Samples Treated with ^{14}C-CGA-329351."