

US EPA ARCHIVE DOCUMENT



*make*

*S. Kocake*  
*B91-21*

SOP M1981  
B. Babbitt, M. Roman and  
M. Safarpour/ct  
02/08/90

Approved By: *G. L. Picard*  
G. L. Picard  
Date *2/8/90*

AMERICAN CYANAMID COMPANY  
AGRICULTURAL RESEARCH DIVISION  
CHEMICAL DEVELOPMENT  
P. O. BOX 400  
PRINCETON, NEW JERSEY 08540

Recommended Method of Analysis

Imazethapyr Herbicide (CL 263,499): GC Method for the Determination of  
CL 263,499 and CL 288,511 Residues in  
Corn Forage, Grain and Fodder

A. Principle

Residues of CL 263,499 and CL 288,511 are extracted from the sample with acidic methanol-water. The CL 263,499 and CL 288,511 are subjected to suitable cleanup involving solvent partitioning and solid phase extraction techniques. Measurement of the CL 263,499 and CL 288,511 is accomplished by gas chromatography using an instrument equipped with a nitrogen-sensitive detector. Results are calculated as CL 263,499 or CL 288,511 by the direct comparison of peak heights to those of external standards. The validated sensitivity of the method is 0.05 ppm for each compound in each commodity.

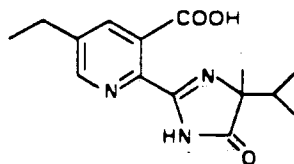
B. Reagents

(Items from manufacturers other than those listed may be used provided they are functionally equivalent.)

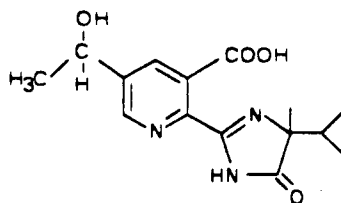
*MS*  
*MR*  
*8/5*

1. Analytical Standards: Analytical grade, known purity, American Cyanamid Company, Agricultural Research Division, P.O. Box 400, Princeton, New Jersey 08540.

- a. CL 263,499: ( $\pm$ )-2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-5-ethyl-3-pyridinecarboxylic acid



- b. CL 288,511: 2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-5-(1-hydroxyethyl)-3-pyridinecarboxylic acid



2. GC Packing: 3% OV-17 on 100/120 mesh Supelcoport, Cat. No. 1-1790, Supelco, Incorporated.
3. Water, Deionized: Millipore's Milli-Q water. Use deionized water for all steps.
4. Solvents: B & J Brand High Purity Solvents, Baxter Burdick and Jackson.
- |                       |                 |
|-----------------------|-----------------|
| a. Acetone            | d. Acetonitrile |
| b. Methanol           | e. Hexane       |
| c. Methylene Chloride |                 |
5. Methylation Reagent: Trimethylphenylammonium Hydroxide, 0.1M in methanol, Cat. No. 10943, Eastman Kodak Company (See Note 1).
6. Solutions:
- a. 1N Hydrochloric Acid: Add 83 mL of concentrated hydrochloric acid to 500 mL of deionized water in a 1 liter volumetric flask and dilute to 1 liter with deionized water.
- b. 0.05N Hydrochloric Acid: Dilute 50 mL of reagent B.6.a. to 1 liter with deionized water.

MS  
cell

Bub

- c. 0.005N Hydrochloric Acid: Dilute 100 mL of reagent B.6.b. to 1 liter with deionized water.
- d. Extraction Solvent: Mix 40 mL of 1N hydrochloric acid with 1560 mL deionized water and 2400 mL methanol.
- e. 25% Methanol in 0.005N Hydrochloric Acid: Dilute 250 mL of methanol to 1 liter with 0.005N hydrochloric acid.
- f. Saturated Potassium Chloride-Methanol: Add 50 g of potassium chloride to 1 liter of methanol, stir for 5 minutes, allow excess potassium chloride to settle.

7. Chemicals: "Baker Analyzed" Reagents, J. T. Baker Company.

- a. Potassium Chloride
- b. Hydrochloric Acid, Concentrated

8. "Darco" G-60 Charcoal, MC/B Brand or equivalent.

C. Apparatus (Items from other manufacturers may be used provided they are functionally equivalent).

1. Gas Chromatograph: Tracor Model 540 equipped with a Model 702 nitrogen-phosphorus detector.
2. Integrator: Spectra Physics Model 4290 recording integrator.
3. Balance: Analytical, Sartorius, precision  $\pm 0.05$  mg.
4. Balance: Pan, Sartorius, Model 2254, precision  $\pm 5$  mg.
5. Assorted Glassware: General laboratory.
6. Flasks: 24/40  $\nabla$  100-, 250-, and 1,000-mL round bottom.
7. Filtering Flasks: 125- and 500-mL capacity, Corning Glass Works.
8. Filtering Funnels: Buchner, porcelain, 7- and 9-cm diameter.
9. Filter Paper: 9-cm diameter, glass-fiber filter, 934-AH, Whatman, Incorporated and 7 cm Whatman #50 hardened equivalent.
10. Flash Evaporator: Buchler Instruments, equipped with a heated water bath (approximately 35°C) in which evaporator flasks can be partially submerged.

MS  
MS  
MS

11. GC Column: 182-cm x 2-mm ID glass, packed with 3% OV-17 on 100/120-mesh Supelcoport. The column was packed using a slight vacuum and silylated glass-wool plugs at each end. The packed column was then conditioned overnight at 250°C with a carrier gas flow of 30 mL/min.
12. Plastic Syringe, Disposable: Luer-Lok, 30-mL capacity, Becton Dickinson.
13. Solid Phase Extraction Cartridges:
  - a. Analytichem SCX Benzenesulfonic Acid Bond-Elut Cartridge (1000 mg): Catalog Number 617406, Analytichem, International.
  - b. Analytichem C-18 Bond-Elut Cartridge (500 mg): Catalog Number 607303, Analytichem, International.
14. Vac-Elut Processing Station or Equivalent: Catalog Number AI 6000, Analytichem, International.
15. Separatory Funnels: Squibb-type with teflon stopcocks, 250-mL capacity, Kontes Glass Company.
16. Microliter Syringes: Hamilton, Model 701, (10-mL capacity) and Hamilton Model 725, (250-mL capacity).
17. Frit Filter Reservoirs, Disposable: 75-mL capacity, Catalog Number 607520, Analytichem, International.
18. Reservoirs, Disposable: 75-mL capacity, Catalog Number 607500, Analytichem, International.
19. Bond Elut Adapters: Catalog Number 636001, Analytichem International.
20. Omni Mixer: Model 17105, OCI Instruments.

#### D. Preparation of Standard Solutions

1. Stock Solutions (Prepare monthly, store in amber bottles in refrigerator).
  - a. CL 263,499 - Weigh accurately a known amount (approximately 10 mg) of CL 263,499 into a 100-mL volumetric flask. Dilute to the mark with acetone and mix well. Calculate and record the exact concentration of CL 263,499.

- b. CL 288,511 - Weigh accurately a known amount (approximately 10 mg) of CL 288,511 into a 100-mL volumetric flask. Dilute to the mark with acetone and mix well. Calculate and record the exact concentration of CL 288,511.

NOTE: Resulting concentrations of each standard stock solution must be corrected for standard purity.

2. Standard Fortification Solutions

- a. Pipet into a single 100-mL volumetric flask an appropriate amount of each stock solution D.1.a and D.1.b to deliver 1000 mcg of each compound (CL 263,499 and CL 288,511). Dilute to the mark with acetone and mix well. This solution contains 10 mcg/mL of each compound.
- b. Pipet into separate 100-mL volumetric flasks 20-, 10- and 5-mL aliquots of stock solution D.2.a. Dilute to the mark with acetone and mix well. These solutions contain 2, 1 and 0.5 mcg/mL, respectively, of each compound.

3. Standard Gas Chromatographic Solutions

Pipet a 1-mL aliquot of 2, 1 and 0.5 mcg/mL (Standard Fortification Solutions D.2.b) into 100-mL round-bottom flasks. Add 100 mcL of methylating reagent to each flask, 10 mL of methanol and evaporate to dryness. Re-dissolve with 1.0 mL of methanol to give concentrations of 2.0, 1.0 and 0.5 mcg/mL, respectively, of both CL 263,499 and CL 288,511. These solutions are used for the linearity check.

NOTE: The 1 mcg/mL CL 263,499 and CL 288,511 chromatographic standard solution is prepared each day and used as that day's GC standard for quantitation of CL 263,499 and CL 288,511 (See Note 2).

E. Gas Chromatographic Conditions

1. Instrument: Tracor Model 540 gas chromatograph.
2. Detector: Model 702 N-P detector. Bead setting of 600 to 800 to give a peak height of approximately 50% FSD (full-scale deflection) for a 5-ng injection of standard CL 263,499 and approximately 30% FSD for a 5-ng injection of CL 288,511.
3. Column: 182-cm x 2-mm ID glass, packed with 3% OV-17 on 100/120 mesh Supelcoport.

45  
red  
(1981)

4. Instrument Conditions:

- |                                      |               |
|--------------------------------------|---------------|
| a. Column Temperature                | 250°C         |
| b. Inlet Temperature                 | 300°C         |
| c. Detector Temperature              | 260°C         |
| d. Carrier Gas Flow Rate<br>(Helium) | 25 mL/min     |
| e. Hydrogen Flow Rate                | 2.5 mL/min    |
| f. Air Flow Rate                     | 120 mL/min    |
| g. Input Attenuation                 | 1             |
| h. Chart Speed                       | 0.5 cm/minute |

5. Sensitivity: Attenuation on recording integrator set so that 5 ng of CL 263,499 gives a peak height of approximately 50% FSD and 5 ng of CL 288,511 gives a peak height of approximately 30% FSD.

6. Retention Time: Approximately 3.0 minutes for CL 263,499 and approximately 4.0 minutes for CL 288,511.

F. Linearity Check

The gas chromatography should be checked for linearity of response whenever a new column or instrument is used.

- Adjust the GC conditions to attain a peak height of approximately 50% full-scale deflection for a 5-ng injection of CL 263,499 and 30% FSD for a 5-ng injection of CL 288,511. The GC response can be stabilized with several injections of sample extracts (containing methylation reagent).
- Inject 5-mL aliquots of solutions prepared in Section D.3.
- Plot the height for each peak versus the nanograms injected to show linearity of response. Significant departure from linearity over this range indicates instrumental difficulties which should be corrected before proceeding.

G. Sample Preparation

- Pulverize sufficient dry ice in a Hobart Model 84185-D food chopper to chill thoroughly the bowl and blade.

4/5  
 HED  
 (signature)

2. Add the prefrozen corn forage and corn fodder samples in portions of sufficiently small size to enable reduction to fine particle size. (It may be necessary to add small portions of dry ice during the chopping procedure to ensure that the samples remain in a frozen state).
3. For analysis of corn grain samples, blend the whole sample with dry ice for several minutes in a Waring Blendor to break the grain and pulverize the sample.
4. Allow the samples to stand in a freezer overnight for the dry ice to dissipate completely.
5. Keep all samples frozen until ready for analysis.

#### H. Recovery Test

The validity of the procedure should always be demonstrated by recovery tests before analysis of unknown samples is attempted. A fortified sample should also be processed with each day's batch of samples analyzed.

1. Weigh a 20-g subsample of control into a 1-quart Mason jar.
2. Add by pipet a volume of standard fortification solution appropriate to the fortification level to be tested.
3. Add the fortification solution dropwise and mix the sample well before adding the extraction solvent.
4. Continue with the extraction and cleanup steps.

#### I. Extraction and Preliminary Cleanup

1. Weigh 20 g of sample into a 1-quart Mason jar.
2. Add 200 mL of extraction solvent (Reagent B.6.d.) to corn fodder samples or 140 mL of extraction solvent to corn forage or grain samples and blend at medium speed for 5 minutes using an Omni mixer.
3. Filter through a filter paper positioned on a 9-cm Buchner funnel (See Note 3).
4. Wash the Mason jar and filter with 3 x 25-mL portions of extraction solvent.
5. Pour the filtrate into a 250-mL graduated mixing cylinder, dilute to 250 mL with methanol, mix and pour 125 mL into a 1000-mL round-bottom flask. Discard the other half of the sample.



6. Use a flash evaporator to evaporate the methanol from the mixture of the methanol-water extract in the 1000-mL round-bottom flask. Do not evaporate to dryness. The aqueous volume after this step should be approximately 50 mL.
7. Add 250 mL of acetone to the extract in the 1000-mL round-bottom flask.
8. Evaporate the acetone mixture in the 1000-mL round-bottom flask using a flash evaporator. Do not evaporate to dryness. The aqueous volume after this step should not exceed 15 mL. (See Note 4).
9. Pour the aqueous solution from the 1000-mL round-bottom flask into a 250-mL separatory funnel. Rinse the 1000-mL flask with 1 mL of methanol and 3 mL of 0.05N hydrochloric acid and pour the resulting mixture into the separatory funnel and add 1 mL of 1N hydrochloric acid to the funnel. Rinse the 1000-mL round-bottom flask with 100 mL of methylene chloride, swirl and pour into the separatory funnel.
10. Partition vigorously for 30 seconds. Draw down the lower methylene chloride layer into a 1000-mL round-bottom flask and partition the upper aqueous layer with 3 x 100-mL additional portions of methylene chloride, combining the lower methylene chloride layers in the 1000-mL round-bottom flask.
11. Evaporate the combined methylene chloride extracts to dryness, using a flash evaporator (See Note 5).
12. Rinse the inside walls of the round-bottom flask with 2 mL of methanol followed by 50 mL of acetonitrile and pour the combined solvent mixture into a 250-mL separatory funnel. Rinse the round-bottom flask with 50 mL of hexane and combine with the methanol- acetonitrile mixture in the 250-mL separatory funnel.
13. Partition by shaking vigorously for 30 seconds. Allow the layers to separate and draw down the lower acetonitrile layer into a 250-mL round-bottom flask. Discard the upper hexane layer.
14. Evaporate the acetonitrile to dryness using a flash evaporator.
15. Dissolve the residue in 1 mL of methanol followed by 15 mL of 0.05N hydrochloric acid in preparation for solid phase extraction cleanup.

J. Solid Phase Extraction Cleanup

1. Prepare an Analytichem Bond Elut C-18 cartridge using an Analytichem Vac-Elut Processing Station by washing the cartridge with 3 mL of methanol followed by 3 mL of deionized water.

NS  
A  
12/12

2. Assemble a 75-mL disposable frit-filter reservoir onto the top of the prepared Analytichem C-18 cartridge using an adapter.
3. Pass the extract from Step I.15 through the Analytichem C-18 cartridge using the Vac-Elut Processing Station at the rate of 2-3 drops per second.
4. Wash the flask, reservoir and the cartridge with one 5-mL portion of 0.05N hydrochloric acid.
5. Remove the reservoir and adapter and wash the C-18 cartridge with 4 x 3 mL of deionized water.
6. Remove the C-18 cartridge from the Vac-Elut Processing Station.
7. Prepare two Analytichem SCX cartridges by washing each with 5 mL of hexane, 5 mL of methanol and 2 x 5 mL of 1N hydrochloric acid.
8. Assemble a 75-mL disposable non-fritted reservoir onto the top of the C-18 cartridge. Connect the C-18 cartridge onto the top of the two (in tandem) SCX cartridges and elute the three-tandem system with 50 mL of 25% methanol in 0.005N hydrochloric acid at the rate of 2 drops per second. Discard the eluate.
9. Discard the C-18 cartridge and connect a 30-mL syringe to the top SCX cartridge and wash the tandem SCX cartridges with 5 mL of methanol. Discard the eluate.
10. Remove the SCX cartridges and syringe from the Vac-Elut, and elute the SCX tandem cartridge system with 20 mL of saturated potassium chloride in methanol into a 250-mL round-bottom flask (See Note 6).
11. Evaporate the saturated potassium chloride-methanol eluate to dryness using a flash evaporator.
12. Add 1 mL of methanol to the 250-mL round-bottom flask, swirl and add 4 mL of 0.05N hydrochloric acid, swirl and pour the resulting solution into a 250-mL separatory funnel.
13. Add an additional 3 mL of 0.05N hydrochloric acid to the 250-mL round-bottom flask, swirl and combine with the hydrochloric acid in the separatory funnel. Rinse the round-bottom flask with 100 mL of methylene chloride, swirl and pour into the separatory funnel.
14. Partition vigorously for 30 seconds, draw down the lower methylene chloride layer into a 1000-mL round-bottom flask and partition the upper aqueous layer with 3 x 100-mL additional portions of methylene chloride, combining the lower methylene chloride layers in the 1000-mL round-bottom flask.

15. Evaporate the methylene chloride to dryness using a flash evaporator (See Note 5).
16. Dissolve the residue in 10 mL of methanol, pour into a 100-mL round-bottom flask, rinse the 100-mL round-bottom flask with an additional 10 mL of methanol, combine with the first 10-mL portion of methanol in the 100-mL round-bottom flask.
17. Add 100 mL of methylating reagent to the 20 mL of methanol in the 100-mL round-bottom flask and evaporate to dryness (See Note 7). Add another 20-mL portion of methanol and re-evaporate to dryness.
18. Dissolve the residue in 1.0 mL of methanol in preparation for gas chromatographic analysis (Section K).

K. Gas Chromatographic Analysis (Note 8)

1. After obtaining a stable GC response as described in Section F, inject a 5-mL (See Note 9) aliquot of sample into a GC equipped with a nitrogen-phosphorus detector.
2. Compare the peak height with that obtained from a 5-ng injection of the 1 mcg/mL GC standard solution (See Section D.3, Note 7).
3. If the sample peak goes off scale, dilute to an appropriate volume with methanol. Pipet 1.0 mL of this diluted sample into a 100-mL round-bottom flask, add enough methylating reagent so that the final solution contains the equivalent of 100 mL of methylating reagent/mL, evaporate to dryness on a flash evaporator and re-dissolve in 1.0 mL of methanol (See Note 7).
4. Make a standard injection after every sample or every other sample and use the average peak height of the standard injection before and after the sample injections for the calculation.

*Handwritten initials and signature:*  
M/S  
Cul  
D. B. S.

**L. Calculations**

For each sample calculation, use the sample peak height and the average peak height measurement of the external standard obtained before and after the sample injections as follows:

$$\text{ppm} = \frac{\text{R(SAMP)} \times (\text{V1}) \times (\text{V3}) \times \text{C(STD)} \times (\text{V5}) \times \text{D.F.}}{\text{R(STD)} \times \text{W} \times (\text{V2}) \times (\text{V4})}$$

Where:

R(SAMP) = Peak height of sample in millimeters

R(STD) = Average peak height of working standard in millimeters

W = Weight of sample taken for analysis in grams

V1 = Volume to which extraction solvent is diluted to in milliliters

V2 = Aliquot of extract taken for analysis in milliliters

V3 = Volume of methanol added to dissolve final residues for chromatographic analysis in milliliters

V4 = Volume of sample solution injected in microliters

V5 = Volume of working standard solution injected in microliters

C(STD) = Concentration of working standard solution injected in micrograms per milliliter

D.F. = Dilution factor

Typical chromatograms for corn forage, grain and fodder are shown in Figures 1 through 3.

**Notes to Method M-1981**

1. Each lot of methylating reagent must be tested for a potential impurity which could interfere with CL 288,511 determinations. Prepare a reagent blank by adding 100 mL methylating reagent to 10 mL of methanol in a 100-mL round-bottom flask, evaporate to dryness and dissolve the residue in 1.0 mL of methanol. Gas

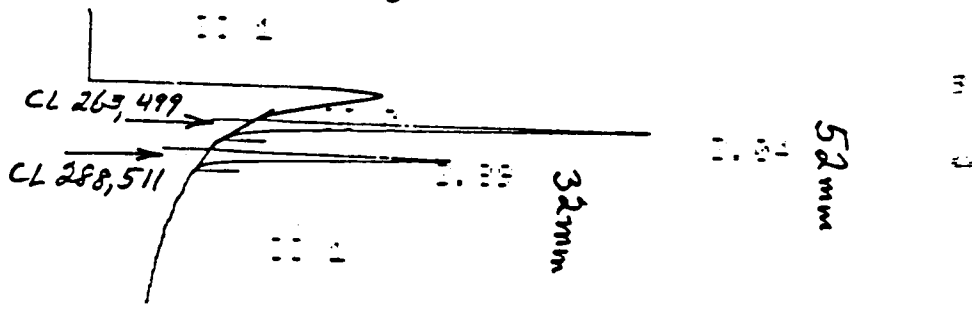
13  
2/12  
2/12

chromatography of this reagent blank as described in Section K will determine if any interfering impurities are present.

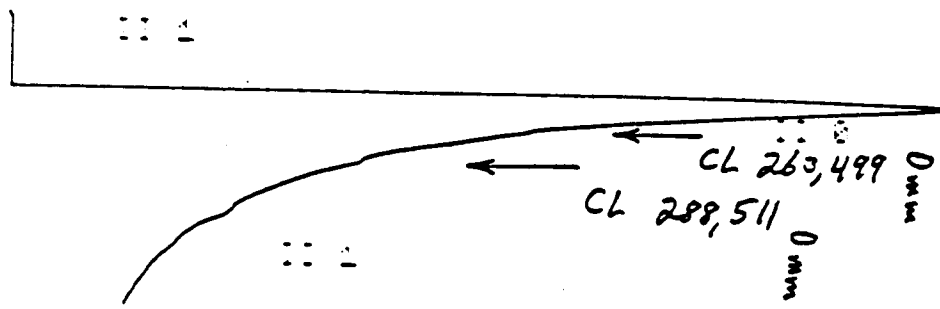
If impurity peaks are a problem, purify the methylating reagent by adding 50 mL to a 125-mL Erlenmeyer flask containing 5 g activated "Darco" G-60 charcoal (MC/B brand or equivalent). Swirl for 10 seconds and allow to stand for 5 minutes. Filter the mixture through a 7-cm Buchner funnel containing a double layer of 7-cm filter paper (Whatman #50, hardened or equivalent). Thoroughly wet the filter paper with methanol and allow to stand 60 seconds before use. Vacuum filter the charcoal mixture using a 125-mL filter flask and wash the charcoal filter cake with 10 mL of methanol, combining the wash and initial filtrate. Transfer the filtrate to a 50-mL graduated cylinder and adjust the volume to 50 mL with methanol. The purified methylating reagent should be re-tested before use.

2. The gas chromatographic standard prepared in Section D.3 is used to quantitate the CL 263,499 and CL 288,511 residues in the sample.
3. Filtration with Buchner funnel (Step I.3): Use vacuum suction to aid the filtration in these steps.
4. If the evaporation of acetone does not result in reducing the aqueous volume to 15 mL, add another 250 mL of acetone and re-evaporate. The aqueous volume should not exceed 15 mL to ensure effective partitioning of CL 288,511 into the methylene chloride in Step I.10.
5. Drying of methylene chloride extracts is not necessary (Steps I.11 and J.15). Do not use sodium sulfate to dry these extracts or losses will occur.
6. Elution of the SPE cartridges (Step J.10): Use a plunger with the syringe and apply pressure to elute the residues off the SCX cartridges.
7. There may be some variation in the concentration of methylation reagent resulting from the purification procedure outlined in Note 1. When performing any recovery test and a low recovery value is obtained, add an additional 50 mL of methylating reagent to the recovery sample and GC standard so that the final concentration of methylating reagent is 150 mL/mL solution. If 150 mL/mL reagent is needed for recovery samples, the concentration of methylating reagent must also be increased to 150 mL/mL for samples.
8. If large numbers of samples are being analyzed, the glass wool should be changed daily in the inlet section of the GC column.
9. A 5-mL or 10-mL injection aliquot may be used to achieve the desired peak height for the GC standard and samples.

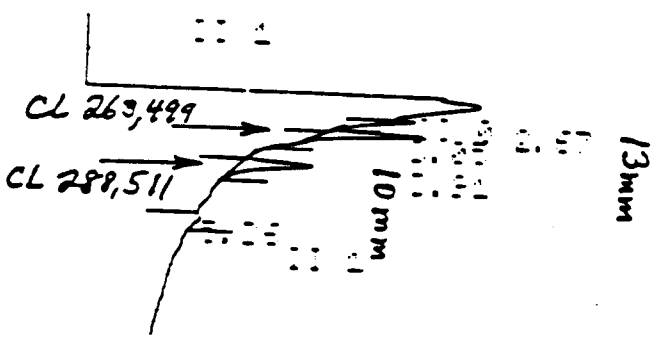
Figure 1: Typical Chromatograms for the Determination of CL 263,499 and CL 288,511 Residues in Corn Forage



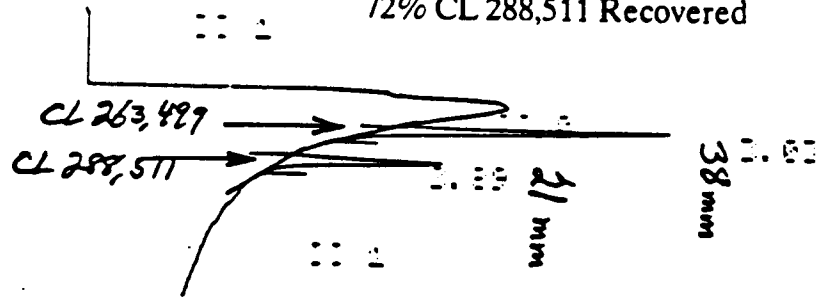
CL 263,499 and CL 288,511 Standards, 10 ng of each Injected



Control Corn Forage, Kentucky, 5421.147C, 100 mg Injected, <0.002 ppm Apparent CL 263,499 and <0.002 ppm Apparent CL 288,511 Found



Control Corn Forage, Kentucky, 5421.147C, Fortified with CL 263,499 and CL 288,511 at 0.05 ppm, 100 mg Injected, 58% CL 263,499 and 72% CL 288,511 Recovered

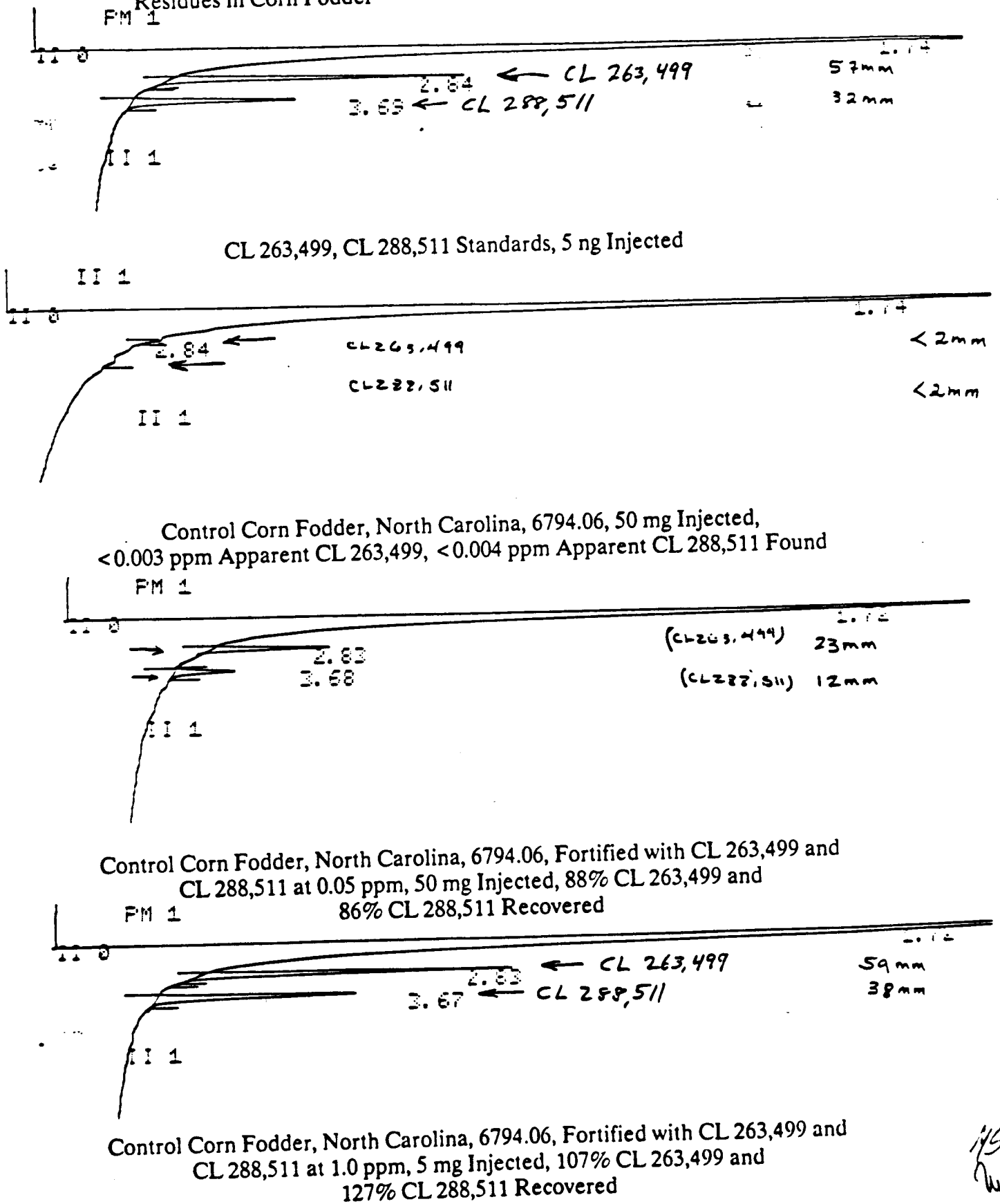


Control Corn Forage, Illinois, 5421.148D, Fortified with CL 263,499 and CL 288,511 at 10 ppm, 1.0 mg Injected, 78% CL 263,499 and 72% CL 288,511 Recovered

1.0 ppm?

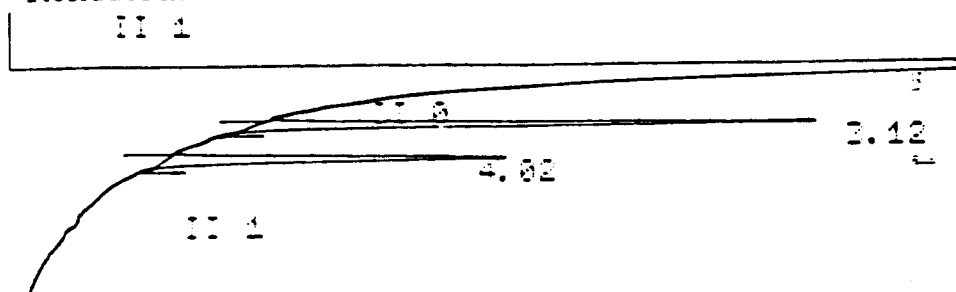
MS  
MR  
P

Figure 2: Typical Chromatograms for the Determination of CL 263,499 and CL 288,511 Residues in Corn Fodder

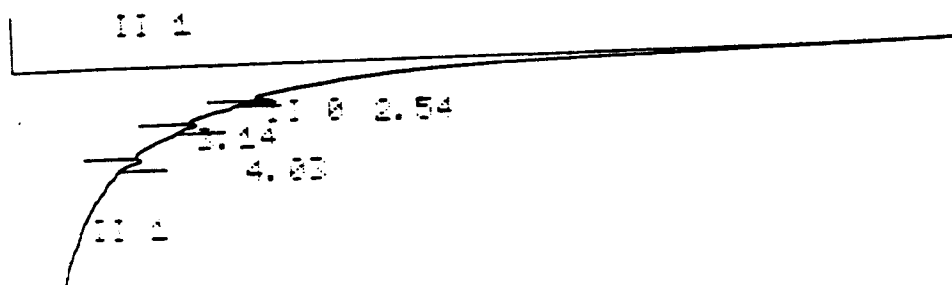


*Handwritten initials/signature*

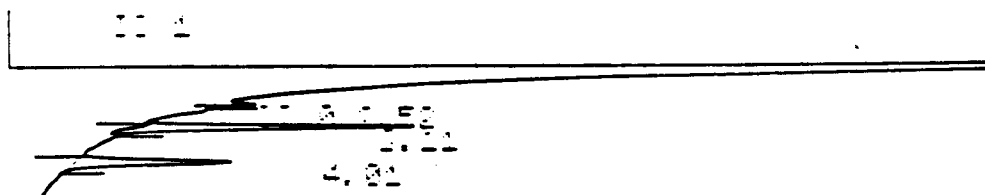
Figure 3: Typical Chromatograms for the Determination of CL 263,499 and CL 288,511 Residues in Corn Grain



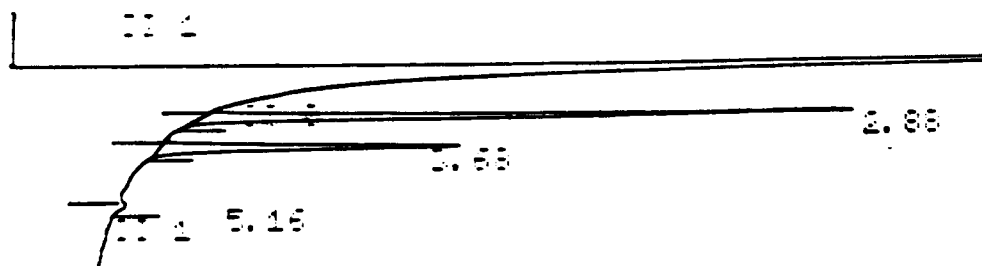
CL 263,499 and CL 288,511 Standard, 5 ng of each Injected



Control Corn Grain, North Carolina, 6794.07, 50 mg Injected, <0.005 ppm Apparent CL 263,499 and 0.007 ppm Apparent CL 288,511 Found



Control Corn Grain, North Carolina, 6794.07, Fortified with CL 263,499 and CL 288,511 at 0.05 ppm, 50 mg Injected, 99% CL 263,499 and 93% CL 288,511 Recovered



Control Corn Grain, North Carolina, 6794.07, Fortified with CL 263,499 and CL 288,511 at 1.0 ppm, 5 mg Injected, 91% CL 263,499 and 86% CL 288,511 Recovered

*Handwritten initials:* H/S, ML, and a signature.



TABLE 1

TYPE OF EXPERIMENT: RECOVERY STUDY-PU89PT12  
 SAMPLE TYPE: CORN PLANT  
 ANALYZED FOR: CL 263,499  
 METHOD USED: SOP M1981  
 VALIDATED SENSITIVITY: 0.050 PPM  
 RESPONSE MEASURED AS: PEAK HEIGHT IN MILLIMETERS  
 V4 = V5 = : 10

$$\text{APP. PPM FOUND} = \frac{R(\text{SAMP}) * V1 * V3 * V5 * C(\text{STD}) * D.F.}{R(\text{STD}) * W * V2 * V4}$$

$$\% \text{ RECOVERY} = \frac{\text{PPM FOUND} * 100}{\text{PPM ADDED}} = \frac{\text{PPM FOUND} * 100}{FV * FC / W}$$

SAMPLE NUMBER	FORTIFICATION			SAMPLE PREPARATION					STAND. SOLN.	RESPONSES		APPARENT	PERCENT RECOVERY
	FV (mls)	FC (mcg/ml)	PPM added	W (gms)	V1 (mls)	V2 (mls)	V3 (mls)	DIL. fac.	C(Std) mcg/ml	R(SAMP) units	R(STD) units	PPM FOUND	
5421.148D	0	0	0	20	250	125	1.00	1	1.00	-2	52	<0.004	
5421.147C	0	0	0	20	250	125	1.00	1	1.00	N.M.	49.5	<0.004	
AVERAGES												0.004	
5421.148D	1.00	1.00	0.050	20	250	125	1.00	1	1.00	22	52.5	0.042	83.8
5421.147C	1.00	1.00	0.050	20	250	125	1.00	1	1.00	13	44.5	0.029	58.4
AVERAGES												0.036	71.1
5421.148D	2.00	1.00	0.100	20	250	125	1.00	1	1.00	42	52.5	0.080	80.0
5421.147C	2.00	1.00	0.100	20	250	125	1.00	1	1.00	28	44	0.064	63.6
AVERAGES												0.072	71.8
5421.148D	2.00	10.0	1.000	20	250	125	10.0	1	1.00	40	50	0.800	80.0
5421.147C	2.00	10.0	1.000	20	250	125	10.0	1	1.00	50	74.5	0.671	67.1
AVERAGES												0.736	73.6
5421.148D	1.78	112.5	10	20	250	125	100	1	1.00	38	49	7.755	77.5
5421.147C	1.78	112.5	10	20	250	125	100	1	1.00	67	76	8.816	88.0
AVERAGES												8.286	82.8

N.M. = not measurable (less than 2 units), corresponding, for control samples, to the limits given above in the APPARENT-residue column. These limits are calculated by insertion of the number 2 into the calculation as the value for R(SAMP) wherever N.M. is indicated. For treated samples, N.M. is expressed as less than the validated sensitivity of the method.

Control samples are indicated by a minus sign before the R(SAMP) value.

*Handwritten signature and initials*

TABLE II

TYPE OF EXPERIMENT: RECOVERY STUDY-PUB9PT12  
 SAMPLE TYPE: CORN PLANT  
 ANALYZED FOR: CL 288,511  
 METHOD USED: SOP M1981  
 VALIDATED SENSITIVITY: 0.050 PPM  
 RESPONSE MEASURED AS: PEAK HEIGHT IN MILLIMETERS  
 V4 = V5 = : 10

$$\text{APP. PPM FOUND} = \frac{R(\text{SAMP}) * V1 * V3 * V5 * C(\text{STD}) * D.F.}{R(\text{STD}) * W * V2 * V4}$$

$$\% \text{ RECOVERY} = \frac{\text{PPM FOUND} * 100}{\text{PPM ADDED}} = \frac{\text{PPM FOUND} * 100}{FV * FC / W}$$

SAMPLE NUMBER	FORTIFICATION			SAMPLE PREPARATION					DIL. fac.	STAND. SOLN. C(Std) mcg/ml	RESPONSES		APPARENT PPM FOUND	PERCENT RECOVERY
	FV (mls)	FC mcg/ml	PPM added	W (gms)	V1 (mls)	V2 (mls)	V3 (mls)	R(SAMP) units			R(STD) units			
5421.148D	0	0	0	20	250	125	1.00	1	1.00	-2	35.5	<0.006		
5421.147C	0	0	0	20	250	125	1.00	1	1.00	-N.M.	31	<0.006		
AVERAGES												0.006		
5421.148D	1.00	1.00	0.050	20	250	125	1.00	1	1.00	14	34.5	0.041	81.2	
5421.147C	1.00	1.00	0.050	20	250	125	1.00	1	1.00	10	28	0.036	71.4	
AVERAGES												0.039	76.3	
5421.148D	2.00	1.00	0.100	20	250	125	1.00	1	1.00	25	34.5	0.072	72.5	
5421.147C	2.00	1.00	0.100	20	250	125	1.00	1	1.00	14	27	0.052	51.8	
AVERAGES												0.062	62.2	
5421.148D	2.00	10.0	1.000	20	250	125	10.0	1	1.00	25	33	0.758	75.8	
5421.147C	2.00	10.0	1.000	20	250	125	10.0	1	1.00	28	43	0.651	65.1	
AVERAGES												0.705	70.5	
5421.148D	1.75	114.8	10	20	250	125	100	1	1.00	21	29	7.241	72.1	
5421.147C	1.75	114.8	10	20	250	125	100	1	1.00	37	44.5	8.315	82.8	
AVERAGES												7.778	77.5	

N.M. = not measurable (less than 2 units), corresponding, for control samples, to the limits given above in the APPARENT-residue column. These limits are calculated by insertion of the number 2 into the calculation as the value for R(SAMP) wherever N.M. is indicated. For treated samples, N.M. is expressed as less than the validated sensitivity of the method.

Control samples are indicated by a minus sign before the R(SAMP) value.

M.S.  
 [Signature]  
 [Signature]

TABLE III

TYPE OF EXPERIMENT: RECOVERY STUDY PUB9PT12  
 SAMPLE TYPE: CORN GRAIN  
 ANALYZED FOR: CL 263,499  
 METHOD USED: M-1981  
 VALIDATED SENSITIVITY: 0.050 PPM  
 RESPONSE MEASURED AS: PEAK HEIGHT IN MM  
 V4 = V5 = : 5

$$\text{APP. PPM FOUND} = \frac{R(\text{SAMP}) * V1 * V3 * V5 * C(\text{STD}) * D.F.}{R(\text{STD}) * W * V2 * V4}$$

$$\% \text{ RECOVERY} = \frac{\text{PPM FOUND} * 100}{\text{PPM ADDED}} = \frac{\text{PPM FOUND} * 100}{FV * FC / W}$$

SAMPLE NUMBER	FORTIFICATION			SAMPLE PREPARATION					STAND.	RESPONSES		APPARENT	
	FV (mls)	FC (mcg/ml)	PPM added	W (gms)	V1 (mls)	V2 (mls)	V3 (mls)	DIL. fac.	C(Std) mcg/ml	R(SAMP) units	R(STD) units	PPM FOUND	PERCENT RECOVERY
6794.07	0	0	0	20	250	125	1	1	1.00	-N.M.	44	<0.005	
5421.147D	0	0	0	20	250	125	1	1	1.00	-N.M.	32	<0.006	
AVERAGES												0.006	
6794.07	1	1	0.050	20	250	125	1	1	1.00	35	71	0.049	98.6
5421.147D	1	1	0.050	20	250	125	1	1	1.00	19	41	0.046	92.7
AVERAGES												0.047	95.7
6794.07	1	2	0.100	20	250	125	2	1	1.00	29	65	0.089	89.2
5421.147D	1	2	0.100	20	250	125	1	1	1.00	35	38	0.092	92.1
AVERAGES												0.091	90.7
6794.07	2	2	0.200	20	250	125	4	1	1.00	28	64	0.175	87.5
5421.147D	2	2	0.200	20	250	125	2	1	1.00	40	43	0.186	93.0
AVERAGES												0.181	90.3
6794.07	2	10	1.000	20	250	125	10	1	1.00	83	91	0.912	91.2
5421.147D	2	10	1.000	20	250	125	10	1	1.00	88	88	1.000	100.0
AVERAGES												0.956	95.6

N.M. = not measurable (less than 2 units), corresponding, for control samples, to the limits given above in the APPARENT-residue column. These limits are calculated by insertion of the number 2 into the calculation as the value for R(SAMP) wherever N.M. is indicated. For treated samples, N.M. is expressed as less than the validated sensitivity of the method.

Control samples are indicated by a minus sign before the R(SAMP) value.

4.5  
*Handwritten signature*  
 BAC

TABLE IV

TYPE OF EXPERIMENT: RECOVERY STUDY PU89PT12  
 SAMPLE TYPE: CORN GRAIN  
 ANALYZED FOR: CL 288,511  
 METHOD USED: M-1981  
 VALIDATED SENSITIVITY: 0.050 PPM  
 RESPONSE MEASURED AS: PEAK HEIGHT IN MM  
 V4 = V5 = : 5

$$\text{APP. PPM FOUND} = \frac{R(\text{SAMP}) * V1 * V3 * V5 * C(\text{STD}) * D.F.}{R(\text{STD}) * W * V2 * V4}$$

$$\% \text{ RECOVERY} = \frac{\text{PPM FOUND} * 100}{\text{PPM ADDED}} = \frac{\text{PPM FOUND} * 100}{FV * FC / W}$$

SAMPLE NUMBER	FORTIFICATION			SAMPLE PREPARATION					STAND. SOLN.	RESPONSES		APPARENT	PERCENT RECOVERY
	FV (mls)	FC (mcg/ml)	PPM added	W (gms)	V1 (mls)	V2 (mls)	V3 (mls)	DIL. fac.	C(Std) mcg/ml	R(SAMP) units	R(STD) units	PPM FOUND	
6794.07	0	0	0	20	250	125	1	1	1.00	-N.M.	28	<0.007	*
5421.147D	0	0	0	20	250	125	1	1	1.00	-N.M.	18	<0.011	
AVERAGES												0.009	
6794.07	1	1	0.050	20	250	125	1	1	1.00	20	43	0.047	93.0
5421.147D	1	1	0.050	20	250	125	1	1	1.00	11	26	0.042	84.6*
AVERAGES												0.045	88.8
6794.07	1	2	0.100	20	250	125	2	1	1.00	18	38	0.095	94.7
5421.147D	1	2	0.100	20	250	125	1	1	1.00	18	21	0.086	85.7
AVERAGES												0.091	90.2
6794.07	2	2	0.200	20	250	125	4	1	1.00	18	36	0.200	100.0
5421.147D	2	2	0.200	20	250	125	2	1	1.00	23	24	0.192	95.8
AVERAGES												0.196	97.9
6794.07	2	10	1.000	20	250	125	10	1	1.00	38	44	0.864	86.4
5421.147D	2	10	1.000	20	250	125	10	1	1.00	43	43	1.000	100.0
AVERAGES												0.932	93.2

N.M. = not measurable (less than 2 units), corresponding, for control samples, to the limits given above in the APPARENT-residue column. These limits are calculated by insertion of the number 2 into the calculation as the value for R(SAMP) wherever N.M. is indicated. For treated samples, N.M. is expressed as less than the validated sensitivity of the method.

Control samples are indicated by a minus sign before the R(SAMP) value.

\*Corn grain sample number 5421.147D fortified at 0.05 ppm CL 288,511 resulted in 0.031 ppm (62% recovery) after correction for <0.011 apparent CL 288,511 ppm found in control.

*Handwritten signature and initials*

TABLE V

TYPE OF EXPERIMENT: RECOVERY STUDY-PU89PT12  
 SAMPLE TYPE: CORN FODDER  
 ANALYZED FOR: CL 263,499  
 METHOD USED: SOP M1981  
 VALIDATED SENSITIVITY: 0.050 PPM  
 RESPONSE MEASURED AS: PEAK HEIGHT IN MILLIMETERS  
 V4 = V5 = : 5.0

$$\text{APP. PPM FOUND} = \frac{R(\text{SAMP}) * V1 * V3 * V5 * C(\text{STD}) * D.F.}{R(\text{STD}) * W * V2 * V4}$$

$$\% \text{ RECOVERY} = \frac{\text{PPM FOUND} * 100}{\text{PPM ADDED}} = \frac{\text{PPM FOUND} * 100}{FV * FC / W}$$

SAMPLE NUMBER	FORTIFICATION			SAMPLE PREPARATION					DIL. fac.	STAND. SOLN. C(Std) mcg/ml	RESPONSES		APPARENT PPM FOUND	PERCENT RECOVERY
	FV (mls)	FC (mcg/ml)	PPM added	W (gms)	V1 (mls)	V2 (mls)	V3 (mls)	R(SAMP) units			R(STD) units			
6794.06	0	0	0	20.0	250	125	1.00	1.0	1.00	-N.M.	69	<0.003		
5421.147A	0	0	0	20.0	250	125	1.00	1.0	1.00	-2	83	<0.002		
AVERAGES												0.003		
6794.06	1.00	1.00	0.050	20.0	250	125	1.00	1.0	1.00	23	52	0.044	88.5	
5421.147A	1.00	1.00	0.050	20.0	250	125	1.00	1.0	1.00	36	83	0.043	86.7	
AVERAGES												0.044	87.6	
6794.06	2.00	1.00	0.100	20.0	250	125	1.00	1.0	1.00	60	67	0.090	89.6	
5421.147A	2.00	1.00	0.100	20.0	250	125	1.00	1.0	1.00	58	74	0.078	78.4	
AVERAGES												0.084	84.0	
6794.06	2.00	10.0	1.000	20.0	250	125	1.00	10	1.00	59	55	1.073	107.3	
5421.147A	2.00	10.0	1.000	20.0	250	125	1.00	10	1.00	60	81	0.741	74.1	
AVERAGES												0.907	90.7	
6794.06	20.0	10.0	10	20.0	250	125	1.00	100	1.00	56	53	10.6	105.7	
5421.147A	20.0	10.0	10	20.0	250	125	1.00	100	1.00	58	74	7.838	78.4	
AVERAGES												9.219	92.1	

N.M. = not measurable (less than 2.00 units), corresponding, for control samples, to the limits given above in the APPARENT-residue column. These limits are calculated by insertion of the number 2.00 into the calculation as the value for R(SAMP) wherever N.M. is indicated. For treated samples, N.M. is expressed as less than the validated sensitivity of the method.

Control samples are indicated by a minus sign before the R(SAMP) value.

000011

CY 42

*Handwritten signature and initials*

TABLE VI

TYPE OF EXPERIMENT: RECOVERY STUDY-PUB9PT12  
 SAMPLE TYPE: CORN FODDER  
 ANALYZED FOR: CL 288,511  
 METHOD USED: SOP M1981  
 VALIDATED SENSITIVITY: 0.050 PPM  
 RESPONSE MEASURED AS: PEAK HEIGHT IN MILLIMETERS  
 V4 = V5 = : 5.0

$R(SAMP) * V1 * V3 * V5 * C(STD) * D.F.$   
 APP. PPM FOUND =  $\frac{R(STD) * W * V2 * V4}{PPM FOUND * 100}$   
 $\% RECOVERY = \frac{PPM FOUND * 100}{PPM ADDED} = \frac{PPM FOUND * 100}{FV * FC / W}$

SAMPLE NUMBER	FORTIFICATION			SAMPLE PREPARATION					DIL. fac.	STAND. SOLN. C(Std) mcg/ml	RESPONSES		APPARENT	
	FV (mls)	FC (mcg/ml)	PPM added	W (gms)	V1 (mls)	V2 (mls)	V3 (mls)	R(SAMP) units			R(STD) units	PPM FOUND	PERCENT RECOVERY	
6794.06	0	0	0	20.0	250	125	1.00	1.0	1.00	-N.M.	46	<0.004		
5421.147A	0	0	0	20.0	250	125	1.00	1.0	1.00	-N.M.	51	<0.004		
AVERAGES											0.004			
6794.06	1.00	1.00	0.050	20.0	250	125	1.00	1.0	1.00	12	28	0.043	85.7	
5421.147A	1.00	1.00	0.050	20.0	250	125	1.00	1.0	1.00	39	51	0.076	152.9	
AVERAGES											0.060	119.3		
6794.06	2.00	1.00	0.100	20.0	250	125	1.00	1.0	1.00	38	45	0.084	84.4	
5421.147A	2.00	1.00	0.100	20.0	250	125	1.00	1.0	1.00	57	44	0.130	129.5	
AVERAGES											0.107	107.0		
6794.06	2.00	10.0	1.000	20.0	250	125	1.00	10	1.00	38	30	1.267	126.7	
5421.147A	2.00	10.0	1.000	20.0	250	125	1.00	10	1.00	27	49	0.551	55.1	
AVERAGES											0.909	90.9		
6794.06	20.0	10.0	10	20.0	250	125	1.00	100	1.00	36	28	12.9	128.6	
5421.147A	20.0	10.0	10	20.0	250	125	1.00	100	1.00	59	44	13.4	134.1	
AVERAGES											13.1	131.4		

N.M. = not measurable (less than 2.00 units), corresponding, for control samples, to the limits given above in the APPARENT-residue column. These limits are calculated by insertion of the number 2.00 into the calculation as the value for R(SAMP) wherever N.M. is indicated. For treated samples, N.M. is expressed as less than the validated sensitivity of the method.

Control samples are indicated by a minus sign before the R(SAMP) value.

*Handwritten signature and initials*