CYANAMID

SOP M1908.01
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Approved By:

J.M.Devine — 02/21/90 Date

AMERICAN CYANAMID COMPANY
AGRICULTURAL RESEARCH DIVISION
CHEMICAL DEVELOPMENT
P. O. BOX 400
PRINCETON, NEW JERSEY 08543-0400

Recommended Method of Analysis

Imazethapyr Herbicide (CL 263,499):GC Method for the Determination of CL 263,499, CL 288,511 and CL 182,704 Residues in Peanut Hulls and Meat

A. Principle

Residues of CL 263,499, CL 288,511 and CL 182,704 are extracted from the sample with acidic methanol-water. The CL 263,499 and CL 288,511 are separated from the CL 182,704 (glucose conjugate of CL 288,511) by solvent partitioning and subjected to suitable cleanup involving solid phase extraction techniques. CL 182,704 is acid hydrolyzed to release CL 288,511 and this is processed separately. Measurement of the CL 263,499, CL 288,511 and CL 182,704 (hydrolyzed to CL 288,511) is accomplished by gas chromatography using an instrument equipped with a nitrogen-sensitive detector. Results are calculated as CL 263,499, CL 288,511 or CL 182,704 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.

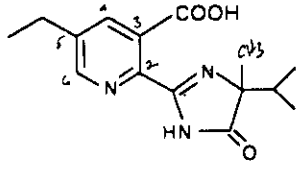
NOTE: This method supercedes M-1908 for the analysis of peanuts. This newer method is essentially the same as the previous one except that two aromatic sulfonic acid SPE cartridges are now used instead of one and the loading solution for these cartridges is modified slightly. This was found to be necessary due to variability between the aromatic sulfonic acid cartridges.

B. Reagents

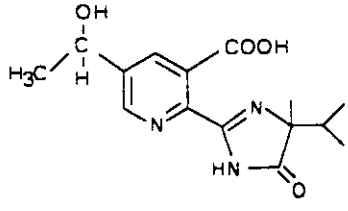
Items from manufacturers other than those listed may be used provided they are functionally equivalent.

1. Analytical Standards: Analytical grade, known purity, American Cyanamid Company, Agricultural Research Division, P.O. Box 400, Princeton, New Jersey 08543-0400.

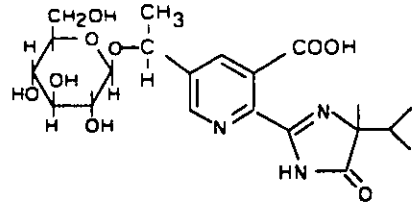
   a. CL 263,499:
   (±)-2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-5-ethyl-3-pyridinecarboxylic acid



   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



   c. CL 182,704:
   5-[1-(beta-D-glucopyranosyloxy) ethyl]-2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-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
   b. Methanol
   c. Methylene Chloride
   d. Acetonitrile
   e. Hexane

5. Methylation Reagent: Trimethylphenylammonium Hydroxide, 0.1M in methanol, Cat. No. 10943, Eastman Kodak Company (See Note 1).

6. Solutions:

   a. 6N Hydrochloric Acid: Add 250 mL of concentrated hydrochloric acid to 250 mL of deionized water.

   b. 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.

   c. 0.05N Hydrochloric Acid: Dilute 50 mL of reagent B.6.b. to 1 liter with deionized water.

   d. 0.005N Hydrochloric Acid: Dilute 100 mL of reagent B.6.c. to 1 liter with deionized water.

   e. Extraction Solvent: Mix 40 mL of 1N hydrochloric acid with 1560 mL deionized water and 2400 mL methanol.

   f. 10% Sodium Hydroxide: Dissolve 100 grams of sodium hydroxide in 1 liter of deionized water and mix thoroughly.

   g. 25% Methanol in 0.005N Hydrochloric Acid: Dilute 250 mL of methanol to 1 liter with 0.005N hydrochloric acid.

   h. Saturated Potassium Chloride Methanol: Add 50 g of potassium chloride to 1 liter of methanol, stir 5 minutes, allow excess potassium chloride to settle.

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

   a. Potassium Chloride

   b. Hydrochloric Acid, Concentrated

   c. Sodium Hydroxide, Pellets

d. Sodium Hydroxide, 50% Solution

8. Celite 545 AW: Johns-Manville Company.

9. "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 ± 0.05 mg.

4. Balance: Pan, Sartorius, Model 2254, precision ± 5 mg.

5. Assorted Glassware: General laboratory.

6. Flasks: 24/40 ℧ 100-, 250-, 500- and 1,000-mL round bottom.

7. Filtering Flasks: 125-, 250- 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 or equivalent.

10. Flash Evaporator: Buchler Instruments, equipped with a heated water bath (approximately 35°C) in which evaporator flasks can be partially submerged.

11. pH Meter: Orion, Model 701A or equivalent.

12. 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.

13. Plastic Syringe, Disposable: Luer-Lok, 30-mL capacity, Becton Dickinson.

14. 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.

15. Vac-Elut Processing Station or Equivalent: Catalog Number AI 6000, Analytichem, International.

16. Separatory Funnels: Squibb-type with teflon stopcocks, 250-mL capacity, Kontes Glass Company.

17. Microliter Syringes: Hamilton, Model 701, (10-mcL capacity) and Hamilton Model 725, (250-mcL capacity).

18. Frit Filter Reservoirs, Disposable: 75-mL capacity, Catalog Number 607520, Analytichem, International.

19. Reservoirs, Disposable: 75-mL capacity, Catalog Number 607500, Analytichem, International.

20. Bond Elut Adapters: Catalog Number 636001, Analytichem International.

21. 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.

c. CL 182,704 - Weigh accurately a known amount (approximately 10 mg) of CL 182,704 into a 100-mL volumetric flask. Dilute to the mark with deionized water and mix well. Calculate and record the exact concentration of CL 182,704.

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.

c. Pipet into a 100-mL volumetric flask an appropriate amount of stock solution D.1.c to deliver 1000 mcg of CL 182,704. Dilute to the mark with deionized water and mix well. This solution contains 10 mcg/mL of CL 182,704.

d. Pipet into separate 100-mL volumetric flasks, 20- and 10-mL aliquots of solution D.2.c. Dilute to the mark with deionized water and mix well. These solutions contain 2 and 1 mcg/mL, respectively, of CL 182,704.

3. Standard Gas Chromatographic Solutions

a. 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.

b. A 10-mL aliquot of the aqueous 1 mcg/mL CL 182,704 (See Step D.2.d.) is mixed with 19 mL deionized water and 6 mL of concentrated hydrochloric acid in a 250-mL round-bottom flask. This standard should be processed with the samples as described in Steps K.4 to K.14. After the evaporation of the methylene chloride in Step K.14, add 10.0 mL of methanol to the 1000-mL round-bottom flask, swirl, mix 0.5-, 1- and 2-mL aliquots of this solution with 10 mL of methanol and 100 mcL of methylating agent in separate 100-mL round-bottom flasks. Evaporate to dryness and re-dissolve with 1.0 mL of methanol. These solutions contain 0.5, 1 and 2 mcg/mL of CL 182,704, respectively. These solutions are used for the linearity check.

NOTE: The 1 mcg/mL CL 182,704 chromatographic standard solution is prepared each day and used as that day's GC standard for quantitation of CL 182,704.

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 10-ng injection of standard CL 263,499 and approximately 30% FSD for a 10-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.

4. Instrument Conditions:

a. Column Temperature	250°C
b. Inlet Temperature	300°C
c. Detector Temperature	260°C
d. Carrier Gas Flow Rate (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 10 ng of CL 263,499 gives a peak height of approximately 50% FSD and 10 ng of CL 288,511 gives a peak height of approximately 30% FSD.

6. Retention Time: Approximately 4.0 minutes for CL 263,499 and approximately 5.0 minutes for CL 288,511.

F. Linearity Check

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

1. Adjust the GC conditions to attain a peak height of approximately 50% full-scale deflection for a 10-ng injection of CL 263,499 and 30% FSD for a 10-ng injection of CL 288,511. The GC response can be stabilized with several injections of sample extracts (containing methylation reagent).

2. Inject 10-mcL aliquots of solutions prepared in Section D.3.

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

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

2. Add the prefrozen peanut hull 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 peanut meat samples, blend the whole sample with dry ice for several minutes in a Waring Blendor to break the peanuts 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.e.) to peanut hull samples or 140 mL of extraction solvent to peanut meat samples and blend at medium speed for 5 minutes using an Omni mixer.

3. Add approximately 10 g of Celite 545 AW to the mixture in the Mason jar and filter through a filter paper prepared 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 500 mL of acetone to the extract in the 1000-mL round-bottom flask. Add 5 g of Celite 545 AW to the mixture, swirl and filter through a 9-cm Buchner funnel fitted

with a double layer of 9-cm glass-fiber filter paper onto which a 5-g Celite pad has been formed from an acetone slurry. Wash the 1000-mL round-bottom flask and filter pad with 25 mL of acetone (See Note 3).

8. Evaporate the combined acetone filtrates in a 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. Save the upper aqueous laver for CL 182,704 determination - See Section K. (This aqueous layer may be left at room temperature for up to 72 hours if it cannot be processed immediately.)

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.

2. Assemble a 75-mL disposable fritted 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 methanol and 2 x 5 mL 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-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 50 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 500-mL round-bottom flask and partition the upper aqueous layer with 3 x 50-mL additional portions of methylene chloride, combining the lower methylene chloride layers in the 500-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 500-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 mcL of methylating reagent to the 20 mL of methanol in the 100-mL round-bottom flask and evaporate to dryness. Add another 20-mL portion of methanol and re-evaporate to dryness (See Note 7).

18. Dissolve the residue in 1.0 mL of methanol in preparation for gas chromatographic analysis (Section M).

K. Sample Preparation and Preliminary Cleanup for Determination of CL 182,704 (Conjugated CL 288,511)

1. Pour the aqueous phase from the 250-mL separatory funnel saved in Step I.10 into a 50-mL graduated cylinder.

2. Rinse the 250-mL separatory funnel with 6 mL of concentrated hydrochloric acid and pour into the graduated cylinder.

3. Dilute the contents of the graduated cylinder to 35 mL with deionized water and pour into a 250-mL round-bottom flask.

4. Add two boiling chips to both the sample from Step K.3 and the standard from Step D.3.b. in the 250-mL round-bottom flasks, bring the solution to a boil using a heating mantle and water condenser.

5. Continue boiling for 30 minutes, remove the 250-mL flask from the heating mantle and cool in an ice bath.

6. Filter the solution through a 75-mL frit-filter reservoir into a 250-mL side-arm filter flask using vacuum. Rinse the round-bottom flask and frit-filter reservoir with 3 mL of deionized water (See Note 3). Transfer the filtrate to a 100-mL beaker. Rinse the filter flask with 3 mL of water and add the rinse to the 100-mL beaker. Add 3.5 mL of 50% sodium hydroxide to the solution in the 100-mL beaker.

7. Using a pH meter, a magnetic stirrer and stirring bar, add enough 10% sodium hydroxide solution to raise the pH to 8.5-9.0.

8. Pour the contents of the beaker into a 250-mL separatory funnel, rinse the beaker with approximately 2 mL of deionized water and combine with the solution in the separatory funnel.

9. Add 100 mL of methylene chloride to the 250-mL separatory funnel and partition vigorously for 20 seconds.

10. Allow the layers to separate and discard the lower methylene chloride layer.

11. Pour the upper aqueous layer back into the 100-mL beaker.

12. Using a pH meter, a magnetic stirrer and stirring bar, use 6N and 1N hydrochloric acid solutions to lower the pH to 2.0 ± 0.1.

13. Pour the pH 2.0 solution back into the 250-mL separatory funnel and partition the solution vigorously with 4 x 100-mL portions of methylene chloride, combining the lower methylene chloride layers in a 1000-mL round-bottom flask.

14. Evaporate the methylene chloride to dryness. (Dissolve the standard as described in Step D.3.b.) See Notes 2,5.

15. Dissolve the sample in the round-bottom flask with 2 mL of methanol followed by 50 mL of acetonitrile and pour the solution 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.

16. Partition by shaking vigorously for 30 seconds. Allow the layers to separate and draw the lower acetonitrile layer into a 250-mL round-bottom flask. Discard the upper hexane layer.

17. Evaporate the acetonitrile to dryness using a flash evaporator.

18. Dissolve the residue in 1 mL of methanol followed by 15 mL of 0.05N hydrochloric acid in preparation for solid phase extraction cleanup.

L. Solid Phase Extraction Cleanup for Determination of CL 288,511 Released From CL 182,704

1. Use the same solid phase extraction cleanup described in Section J.

M. Gas Chromatographic Analysis

1. After obtaining a stable GC response as described in Section F, inject a 10-mcL aliquot of sample into a GC equipped with a nitrogen-phosphorus detector (See Note 8). Note: Change the glass wool in the inlet section of the GC column on a daily basis.

2. Compare the peak height with that obtained from a 10-ng injection of the 1 mcg/mL GC standard solution (See Sections D.3).

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 mcL 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 two sample injections for the calculation. Also, inject a reagent blank after every GC injection. The reagent blank is prepared by evaporating a mixture of 10 mL of methanol and 100 mcL of methylating reagent to dryness and diluting to 1.0 mL with methanol. (This prevents carryover from a standard or sample containing positive residues.)

N. 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:

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

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 peanut meat and peanut hulls are shown in Figures 1 through 4.

Notes to Method M-1908

1. Each lot of methylating reagent must be tested for a potential impurity which could interfere with CL 288,511 and CL 182,704 determinations. Prepare a reagent blank by adding 100 mcL 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 chromatography of this reagent blank as described in Section M 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.a. is used to quantitate the CL 263,499 and CL 288,511 residues in the sample. The gas chromatographic standard prepared in Section D.3.b. by processing 10 mcg of CL 182,704 from steps K.4 through K.14 is used to quantitate the CL 182,704 residue in the sample.

3. Filtration with Buchner funnels (Steps I.3, I.7, K.6): 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.

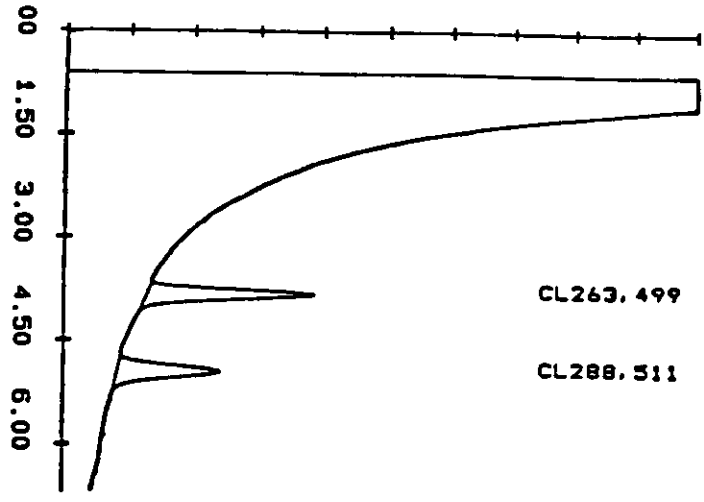
5. Drying of methylene chloride extracts is not necessary (Steps I.11 and K.14). Do not use sodium sulfate to dry these extracts or losses will occur.

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.

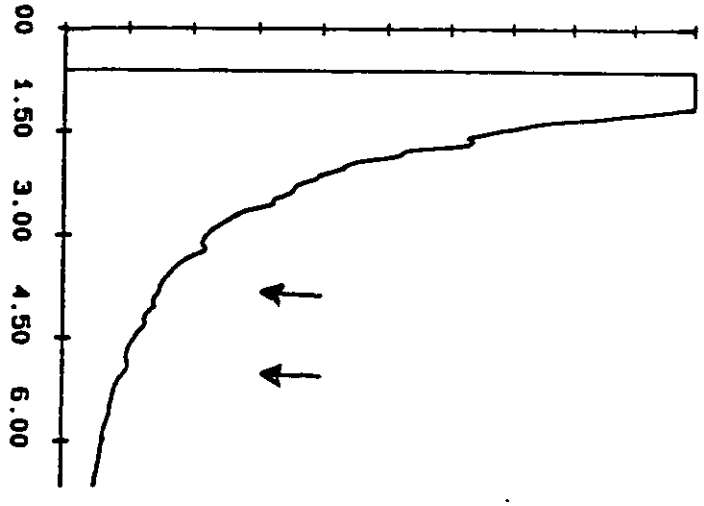
7. There may be some variation in the concentration of methylation reagent resulting from the purification procedure outlined in Note 1. When performing any recovery test and a low recovery value is obtained, add an additional 50 mcL of methylating reagent to the recovery sample and GC standard so that the final concentration of methylating reagent is 150 mcL/mL solution. If 150 mcL/mL reagent is needed for recovery samples, the concentration of methylating reagent must also be increased to 150 mcL/mL for samples.

8. A 5-mcL or 10-mcL injection aliquot may be used to achieve the desired peak height for the GC standard and samples.

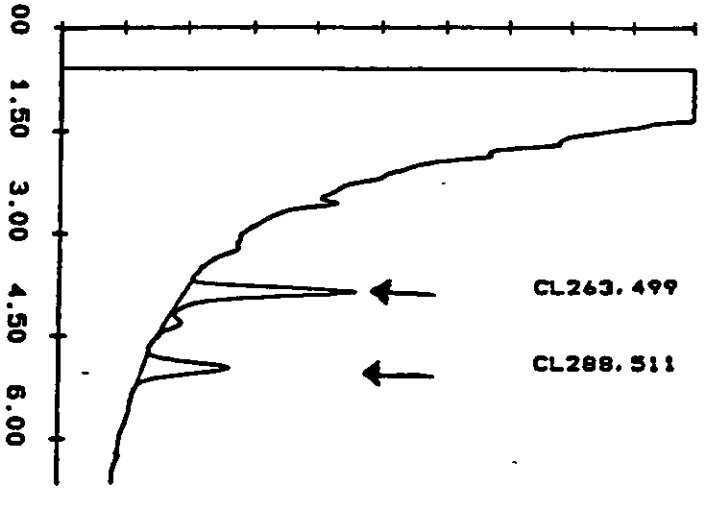
Figure 1: Typical Chromatograms for the Determination of CL 263,499 and CL 288,511 Residues in Peanut Meat



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

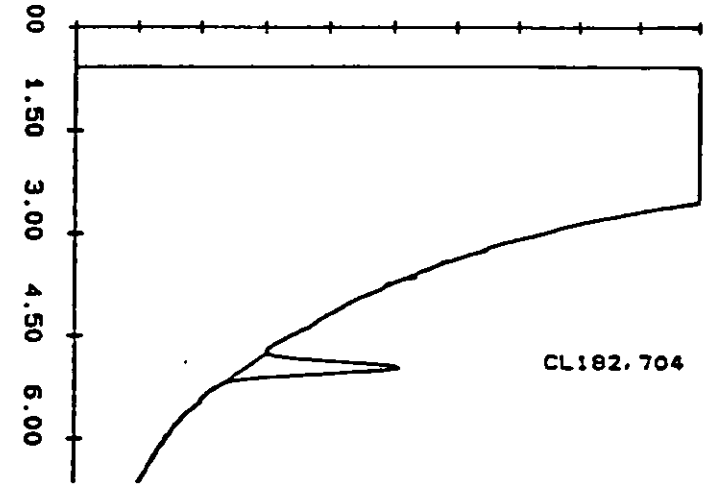


Control Peanut Meat (AC5421.136), 100 mg Injected, <0.002 ppm Apparent CL 263,499 and <0.004 ppm Apparent CL 288,511

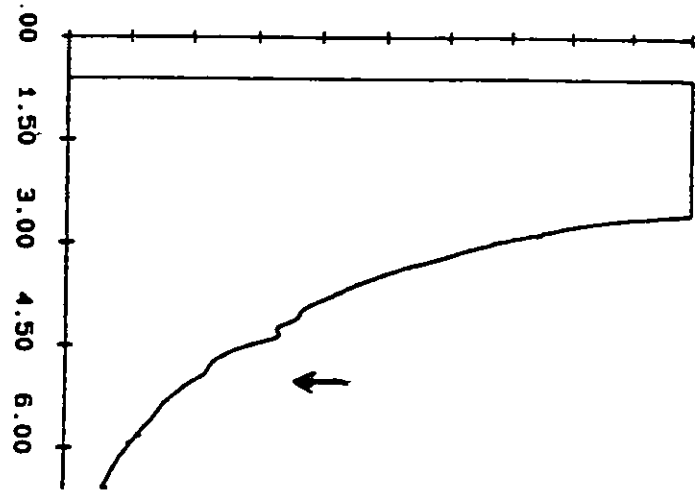


Control Peanut Meat (AC5421.136), Fortified with CL 263,499 and CL 288,511 at 0.10 ppm, 100 mg Injected, 95.0% CL 263,499 and 78.0% CL 288,511 Recovered

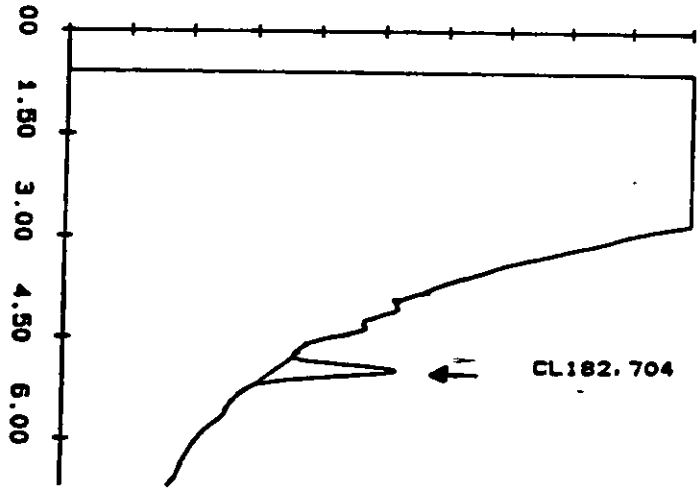
Figure 2: Typical Chromatograms for the Determination of CL 182,704 Residues in Peanut Meat



CL 182,704 Standard, 10 ng Injected

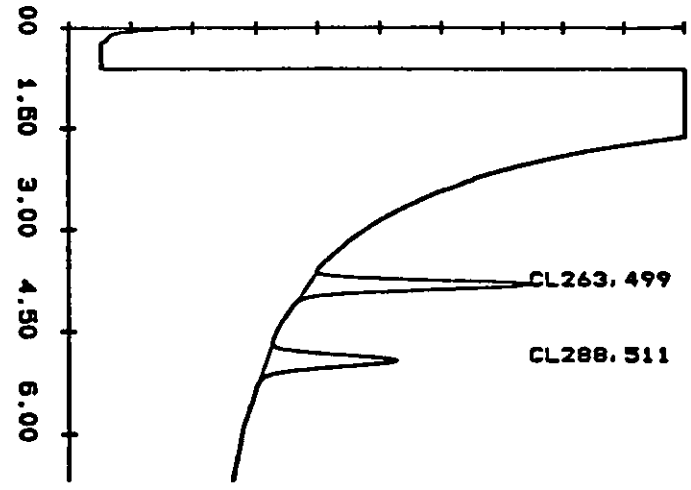


Control Peanut Meat (AC5421.136), 100 mg Injected, <0.003 ppm Apparent CL 182,704

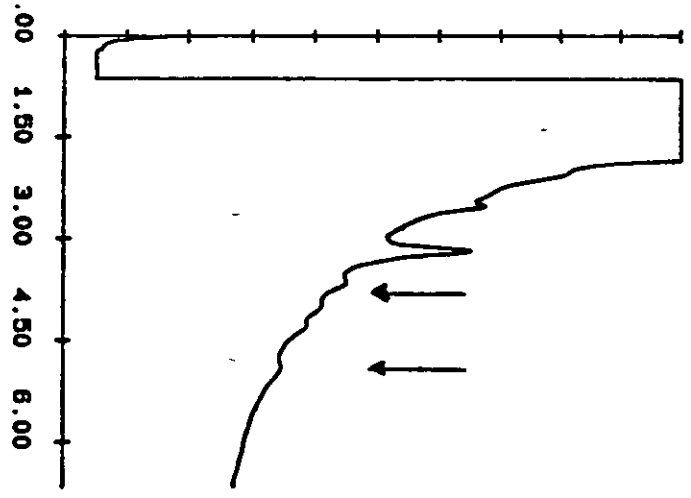


Control Peanut Meat (AC5421.136), Fortified with CL 182,704 at 0.10 ppm, 100 mg Injected, 94.6% Recovered

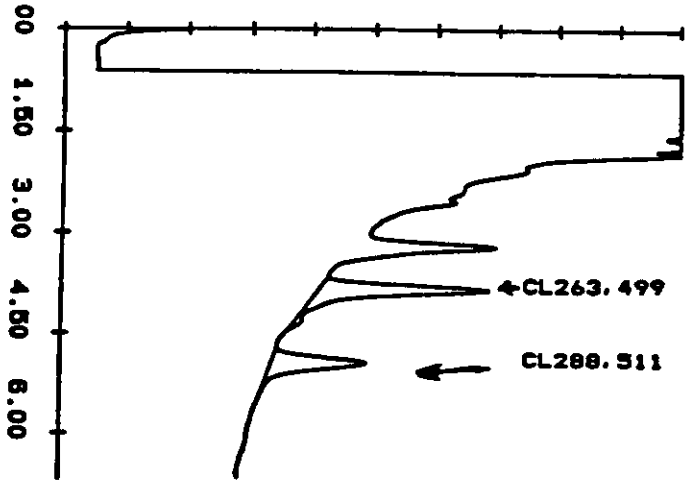
Figure 3: Typical Chromatograms for the Determination of CL 263,499 and CL 288,511 Residues in Peanut Hulls



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

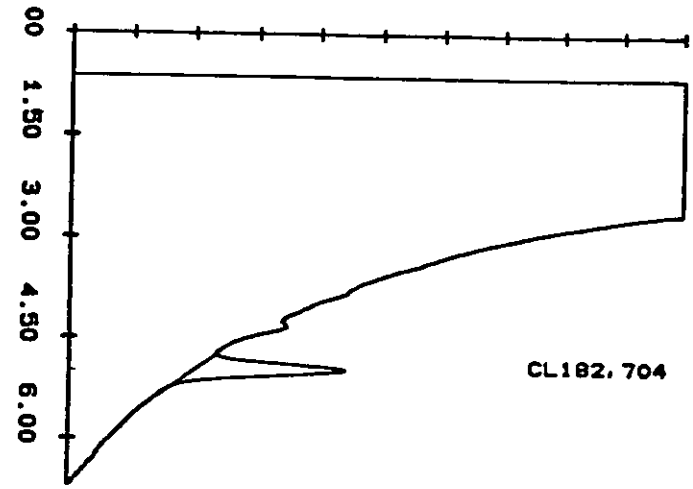


Control Peanut Hulls (AC5421.146), 100 mg Injected, <0.001 ppm Apparent CL 263,499 and <0.002 ppm Apparent CL 288,511

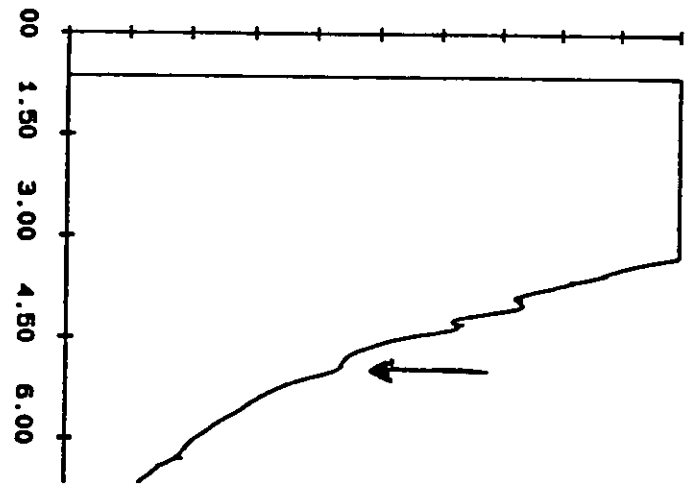


Control Peanut Hulls (AC5421.146), Fortified with CL 263,499 and CL 288,511 at 0.10 ppm, 100 mg Injected, 73.5% CL 263,499 and 72.7% CL 288,511 Recovered

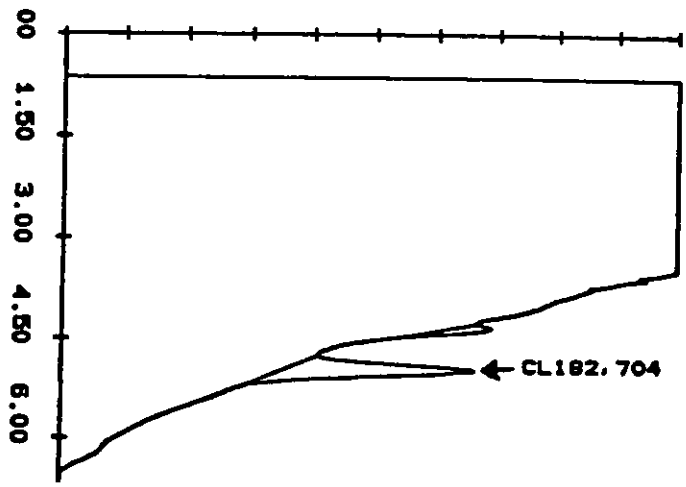
Figure 4: Typical Chromatograms for the Determination of CL 182,704 Residues in Peanut Hulls



CL 182,704 Standard, 10 ng Injected



Control Peanut Hulls (AC5421.146), 100 mg Injected, <0.002 ppm Apparent CL 182,704



Control Peanut Hulls (AC5421.146), Fortified with CL 182,704 at 0.10 ppm, 100 mg Injected, 106% Recovered