CYANAMID

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Recommended Method of Analysis - M 2253.01

Herbicide (CL 263,222): HPLC Method for the Determination of CL 263,222 and Metabolite CL 263,284 Residues in Peanut Hull and Peanut Meat

A. Principle

Residues of CL 263,222 and CL 263,284 are extracted from the sample with Acetone: Methanol: Water (1: 1: 1). The CL 263,222 and CL 263,284 are subjected to suitable cleanup involving liquid-liquid partitioning and solid phase extraction techniques. Measurement of CL 263,222 and CL 263,284 is accomplished by high performance liquid chromatography (HPLC) . Results are calculated by direct comparison of the sample peak heights of the CL 263,222 or CL 263,284 to those of external standards. The validated sensitivity of the method is 0.1 ppm for each compound in each commodity.

B. Apparatus

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

1. Adapter: Bond Elute 12,20 mL adaptor (Cat. No. 1213-1003) and 1,3,6 mL adaptor (Cat. No. 1213-100)(Varian, distributed by Jones Chromatography, Lakewood, CO.)

2. Analytical Balance: Sartorius Model R200D, readability 0.01/0.1 mg, weighing range 0-42 and 0-205 g, Scientific Products, Edison, NJ.

3. Centrifuge: Beckman Model J2-2, Beckman Instruments, Palo Alto, CA.

4. Centrifuge bottles: Polypropylene with Cap Assemblies, 38 x 102 mm, Beckman Instruments Palo Alto, CA, Cat. No. 355624.

5. Cleanup Cartridges:

   a. AG 1-X8 ($HCOO^-$): 200-400 mesh, bed size 0.8 x 4 cm, BIO-RAD Laboratories, Richmond, CA, Cat. No. 731-6221.

NOTE: Supercedes M 2253 - Corrections made Section I.2.j. (page 8). Deionized water was used not pH 2 water.

b. Aromatic Sulfonic Acid (SCX): Mega Bond-Elute, 1000 mg, Varian Cat. No. 1225-6011, distributed by Jones Chromatography, Lakewood, CO.

c. C18: Mega Bond-Elute, 500 mg, Varian Cat. No. 1210-2028, distributed by Jones Chromatography, Lakewood, CO.

6. HPLC Column: Suplecosil LC 8-DB reverse phase C8, 5 micron, 15 cm x 4.6 mm deactivated for bases, Supelco, Belledonte, PA, Cat. No. 5-8347M.

7. Detector: UV photometer set at 254 nanometers, Kratos Model 783, Ramsey, NJ.

8. Disposable Syringes: Luer Slip Tip, 30-mL, and 60-mL (Cat. No. S9519-30S, and S9519-60S), Scientific Products, Edison, NJ.

9. Filter paper: Glass microfiber. Whatman 934-AH, 9 cm diameter.

10. Guard Column: Fitted with an LC 8-DB cartridge, Supelcoguard, Supelco, Bellefonte, PA, Cat. No. 5-9563M.

11. Homogenizer: Polytron Model PT3000, Brinkman Westbury, NY.

12. Integrator: Chromjet recording integrator, Spectra-Physics, Fremont, CA.

13. Liquid Chromatograph: LC pump capable of isocratic operation up to 2 mL/min, Kratos Spectroflow 400, Ramsey, NJ.

14. pH Meter: Orion Model 701A, Boston, MA.

15. Pipettes: Pipette capable of operation in range of 0-100 mcL and 100-1000 mcL, Rainin edp Battery-Operated Motorized pipettes, Woburn, MA.

16. Reservoirs, Disposable: 75-mL capacity with frits (Cat. No. 1213-1018) and 8-mL capacity (Cat. No. 600820), Analytichem International, Harbor City, CA.

17. Rotary Evaporator: Buchler Instruments Model RE-121C equipped with a heated water bath at 35-40° C, Westbury, NY.

18. Sample Injector: Equipped with a 200-mcL loop, Rheodyne Model 7125.

19. SPE Processing Station: Visprep solid phase extraction vacuum manifold, Cat. No. 5-7044M, Supelco, Bellfonte, PA.

20. Stopcock: One-Way stopcocks, Cat. No. 2406, Applied Separations, Bethlehem, PA.

21. Top-Loading Balance: Readability 0.1 g, weighing range 0-3100 g. Mettler Model PE 3000, Denver, CO.

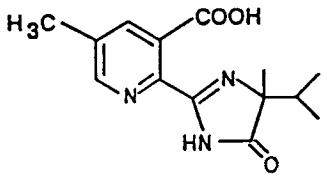
22. Vacuum Filtration Device: A 500-mL suction flask fitted with a 600-mL Buchner porcelain funnel by means of a rubber adapter.

23. Vortex Mixer: S/P vortex mixer, Cat. No., S8223-1, American Scientific Products, Edison, NJ.

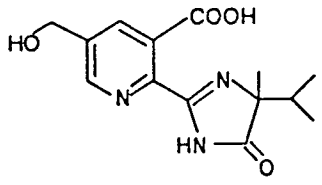
C. **Reagents**

1. Analytical Standards:

   a. CL 263,222 [nicotinic acid, 5-methyl-2-(4-isopropyl-4-methyl-oxo-2-imidazolin-2-yl)-], analytical grade, known purity, American Cyanamid Company, Agricultural Research Division, Princeton, NJ. 08543-0400.



   b. CL 263,284 [nicotinic acid, 5-methyl-2-(4-isopropyl-4-methyl-oxo-2-imidazolin-2-yl)-], analytical grade, known purity, American Cyanamid Company, Agricultural Research Division, Princeton, NJ. 08543-0400.



2. Solvents: High purity, HPLC grade (Burdick and Jackson; Baxter, Inc.)

   a. Methylene Chloride

   b. Methanol

   c. Hexane

   d. Acetone

   e. Acetonitrile

3. Water:High purity, Deionized (Milli-Q water system)

4. Chemicals:

   a. Aldrich A. C. S. Reagent

      Formic acid 96% (Cat. No. 25,136-4)

   b. "Baker Analyzed" Reagents, J. T. Baker Co.

      1. Hydrochloric Acid, concentrated (Cat. No. 9535-1)

2. Potassium Chloride (Cat. No. 3040-01)

3. Filter Aid: (Celite 545 John-Manville Co.)

c. VWR, Scientific

Lead (II) Acetate 3-hydrate, 99.5% purity (Cat. No. EM 7374-1)

5. Solutions:

a. Extraction solvent, Methanol:Water:Acetone (1: 1: 1): Measure equal volumes of methanol, high purity water and acetone. Mix well (prepare daily).

b. 10% Formic acid in Methanol: Measure 10 mL formic acid in 100 mL graduated cylinder and dilute it to 100 mL with Methanol (prepare daily).

c. pH 2 high purity Water: Adjust pH of high purity water to 2.0 with 6N HCl using pH meter.

d. Saturated Potassium Chloride-Methanol: Add 50 g of potassium chloride to 1 liter of methanol, stir, allow the excess potassium chloride to settle.

D. Preparation of Standard Solutions

1. Stock Solutions (1000 mcg/mL) (Prepare monthly, store in amber bottles in refrigerator).

Accurately weigh separately 50 mg $\pm$ 0.05 mg each of the analytical standards (CL 263,222 or CL 263,284) into a 50-mL volumetric flask, dissolve in methanol and dilute to the mark with methanol. These solutions contains 1000 mcg/mL of either standard.

Note: Minor deviation from the indicate weight must be adjusted for the volume to result 1000 mcg/mL standard solution.

2. Mixed Standard Fortification Solutions (100, 10 and 1 mcg/mL)

a. Mixed Standard Fortification Solutions (100 mcg/mL of CL 263,222 and CL 263,284 mixed standard).

Pipet into a single 100-mL volumetric flask 10 mL of each stock solution containing 1000 mcg/mL of CL 263,222 and CL 263,284. Dilute to the mark with methanol and mix well. This solution contains 100 mcg/mL of mixed standard.

b. Mixed Standard Fortification Solutions (10 mcg/mL of CL 263,222 and CL 263,284 mixed standard).

Pipet into a single 100-mL volumetric flask 10 mL of 100 mcg/mL Mixed Standard Fortification Solution. Dilute to the mark with methanol and mix well. This solution contains 10 mcg/mL mixed standard.

c. Mixed Standard Fortification Solutions (1 mcg/mL of CL 263,222 and CL 263,284 mixed standard).

Pipet into a single 100-mL volumetric flask 10 mL of 10 mcg/mL Mixed Standard Fortification Solution. Dilute to the mark with methanol and mix well. This solution contains 1 mcg/mL mixed standard.

3. Mixed HPLC Standard Solutions
(Prepare monthly, store in amber bottles in refrigerator).

a. 0.1 mcg/mL

Pipet a 10-mL aliquot of the 1 mcg/mL Mixed Standard Fortification Solution into a 100-mL evaporation flask, dry the methanol and add accurately 100 mL high purity water.

b. 0.05 mcg/mL

Dilute 0.1 mcg/mL Mixed HPLC standard 1:2 with high purity water.

c. 0.025 mcg/mL

Dilute 0.05 mcg/mL Mixed HPLC standard 1:2 with high purity water.

Use the 0.1, 0.05, and 0.025 mcg/mL CL 263,222 and CL 263,284 Mixed Standards for quantitation of CL 263,222 and CL 263,284 residues in the samples as well as for linearity check.

E. HPLC Conditions

1.	Flow rate:	Set up the instrument for isocratic operation at a flow rate of 1.0 mL/min.
2.	Wavelength:	Set the UV detector at 254 nanometers.
3.	Chart Speed:	0.5 cm/min
4.	Mobile phase:	15% Acetonitrile, 1% formic acid, and 84% water
5.	Loop size:	200 mcL
6.	Retention Time:	About 4-6 min. for CL 263,284 and around 9-11 min for CL 263,222.

Instrument sensitivity should be set so that a 200-mcL injection of the 0.025 mcg/mL standard gives a chromatographic peak height of at least 20 mm for CL 263,284 and 15 mm for CL 263,222 or the equivalent in integrator units.

The conditions given above are not absolute and can be varied to achieve the desired response for the HPLC standard and samples.

F. Linearity Check

The liquid chromatography should be checked for linearity of response whenever is used for analysis. After stable condition of the HPLC, inject Standard solutions of 0.10, 0.05 , 0.025 mcg/mL. Calculate the unit response (response/concentration) for each concentration and average the values. Departure at any concentration of more than 15% indicates instrument malfunction, faulty standard preparation or faulty operating conditions which must be corrected before proceeding with sample analysis.

G. Sample Preparation

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

2. Add the prefrozen peanut hull or peanut meat in small portions 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. Allow the samples to stand in a freezer overnight for the dry ice to dissipate completely.

4. Keep all samples frozen until ready for analysis.

H. Recovery studies

The validity of the procedure should always be demonstrated by recovery tests before analysis of unknown samples is attempted. A fortified sample should be processed at the validated sensitivity limit with each daily set of samples analyzed.

1. Weigh a 20 g subsample of control peanut hull or peanut meal into a 500-mL beaker.

2. Add dropwise an accurately measured 2 mL volume of 1.0 mcg/mL mized standard fortification solution.

3. Mix the sample well before adding the extraction solvent.

4. Continue with the extraction and cleanup steps as described in the method.

I. Sample Analysis

1. Extraction and Preliminary Cleanup

   a. Weigh 20 $\pm$ 0.1 g sample into a 400-mL beaker.

   b. Add 200 mL of extraction solvent (Methanol: Water: Acetone) (1: 1: 1) and blend the mixture with a Polytron homogenizer at medium speed for 5 minutes.

   c. Pass it by suction through Whatman glass fiber filter paper (934-AH; 9 cm) positioned on a 9-cm Buchner funnel into a 500-mL filtration flask.

   d. Return the filter paper and cake to the same blending beaker and add an additional 200 mL of the extraction solvent. Remove the filter paper and place it on a fresh filter paper in the Buchner funnel. Blend the filter cake again for 5 more minutes and pass it by suction through the filter paper. Rinse the cake and adjust the volume to 400 mL with extraction solvent. Mix the extract thoroughly.

e. Add 5 g celite to a 75-mL fritted reservoir. Wash the celite with 20 mL of pH 2 water. Dry by vacuum completely. Pass about 20 mL of the extract through the column of celite by suction and discard the effluent. Filter the remaining extract and collect about 150 mL of the filtered extract.

f. Take a 50-mL aliquot, and evaporate off the organic solvents by rotary evaproator to about 16 mL final volume [Initially start with low vacuum until bumping stops, then gradually increase the vacuum].

g. Transfer the extract to a 100-mL centrifuge tube. Wash the flask with 5 mL water and transfer to the same centrifuge tube. Add 5 mL of 10% Lead Acetate.

h. Balance the centrifuge bottles to equal weight.

i. Centrifuge at 10,000 rpm for 15 min.

j. Transfer the extract to a 100-mL beaker. Wash the centrifuge tube with 2 x 2 mL water, and transfer to the beaker.

2. Cleanup

a. Condition AG 1-X8 column with 15 mL of concentrated formic acid followed by 50 mL of deionized water, using a solid phase extraction vacuum manifold and a flow rate of about 1drop/ second (2-3 Hg/mm). Add 5 mL water and cover the cartridge and shake it vigorously. Let it settle, slowly drain excess water (1-2 drops/5 sec). Do not allow the liquid level to drain below the top of the resin bed.

b. Apply the sample to the top of the column with a Pasteur pipette without disturbing the resin bed. Pull the sample through by suction (about 1-2 drop/5 sec) and discard the effluent. Do not allow the liquid level to drain below the top of the resin bed.

c. Rinse the container with 5 mL methanol and transfer it to the column (about 2 drops/5 sec). Wash the column with additional 5 mL methanol. Discard the effluent.

d. Elute with 70 mL of 10% formic acid in methanol. Collect the eluate (about 2 drops/sec) in a 100-mL beaker.

e. Transfer the eluate to a 250-mL pear-shaped concentration flask, and rinse the beaker carefully with methanol to ensure quantitative transfer. Evaporate the eluate in a rotary evaporator just to dryness. Rinse down the deposits on the wall into the tip of the concentration flask with about 10 mL of methanol, and evaporate the methanol just to dryness (do not overdry). Repeat the rinsing several times until the residue from the eluate is all contained in the tip of the flask.

f. Redissolve in 2 mL methanol plus 3 mL of pH 2 water and transfer to a 125-mL separatory funnel. Rinse the concentration flask with an additional 5 mL of pH 2 water and transfer the rinse to the same separatory funnel.

g. Rinse the evaporation flask with 100 mL of methylene chloride and add the methylene chloride to the separatory funnel. Shake the funnel vigorously for 1 minute, allow the phases to separate and draw off the lower organic layer into a 500-mL round-bottom flask. Evaporate most of the methylene chloride.

h. Repeat Step g three more times, each time adding the methylene chloride layer to the evaporation flask shake it and then transfer it to the separatory funnel.

i. Evaporate the remaining methylene chloride from the last partitioning just to dryness. Remove remaining traces of methylene chloride by adding 10 mL of methanol and evaporating the methanol. Redissolve in 10 mL of pH 2 water.

j. Condition a 1000-mg SCX cartridge by washing in sequence with 2 x 5 mL hexane, 2 x 5 mL methanol, and 2 x 5 mL high purity water. Pass the extract through this cartridge at a rate of about 2 drops/5 seconds. Rinse the 500-mL round-bottom flask with 5 mL methanol and pass each rinse through the SCX cartridge (about 2 drops/5 sec). Rinse the SCX cartridge with 5 mL deionized water followed by 5 mL methanol. Discard the eluate.

k. Using an adapter, attach a clean 30-mL syringe to the top of the SCX cartridge. Add 30 mL saturated KCl solution in methanol to the syringe. Using the syringe plunger, force the solution through the packing at a rate of about 1drop/sec and collect the effluent in a 100-mL pear-shaped evaporation flask.

l. Evaporate the methanol from the previous step to dryness and dissolve the deposit in 10 mL of high purity water and vortex in preparation for HPLC analysis.

K. HPLC Analysis

1. Condition and stabilize the HPLC system by repeatedly injecting 200-mcL aliquots of the 0.1 mcg/mL Mixed HPLC Standard Solution (and a processed sample extract if necessary) until three sequential injections of the standard agree to within 8%.

2. Inject a 200-mcL aliquot the 0.025 mcg/mL Mixed HPLC Standard, followed by up to maximum of four sample solutions, followed by another aliquot of the 0.025 mcg/mL Mixed HPLC Standard and another set of up to the maximum of four sample solutions until all of the samples have been injected. Complete the run by injecting a final aliquot of the 0.025 mcg/mL Mixed HPLC Standard (if sample responses exceed that of 0.025 mcg/mL Mixed HPLC Standard Solution then use one of the other Mixed HPLC Standard Solution which is most closely matches).

3. If the preceding and following standards in a bracket do not agree within 15% of each other, re-inject the following standard again. If the second injection of the following standard do not agree within 15% of the preceding stadnard, then re-inject the entire set.

4. Dilute with water any sample solution which produces peak sizes greater than those given by the 0.10 mcg/mL Mixed HPLC Standard Solution to bring its concentration to within the demonstrated linear range of the HPLC system. Reinject the diluted samples in groups bracketed by the Mixed HPLC Standard Solution which it most closely matches their new concentration.

L. Calculations

For purposes of calculation, use the peak height or peak area of each analyte for a given sample injection and the average of the corresponding peak measurements for the standard injections preceding and following the group which includes the that sample. Calculate the apparent residues as follows:

$$\text{Apparent residues (ppm)} = \frac{R(SAMP) \times (V1) \times (V3) \times (V5) \times C(STD) \times (DF)}{R(STD) \times (W) \times (V2) \times (V4)}$$

Where:

R(SAMP) = Average sample response (integrator units)

R(STD) = Average standard response (integrator units)

C(STD) = Concentration of working standard

V1 = Initial volume of sample extract (400 mL)

V2 = Volume of equivalent sample aliquot used for analysis (50 mL)

V3 = Final volume of sample solution for HPLC (10 mL)

V4 = Volume of sample solution injected (200 mcL)

V5 = Volume of standard solution injected (200 mcL)

W = Sample weight (20 g)

DF = Dilution Factor = 1 unless the chromatographic response exceed the most concentrated HPLC Mixed Standard. If it is necessary to make dilution, include the dilution factor in the equation.

% Recovery = (ppm found-ppm found in control*) x 100/ppm added

* Recovery values corrected for control values when the corresponding control sample contains an apparent residue which exceeded 20% of the fortification level.

Typical chromatograms for peanut hull and peanut meal are shown in Figures 1 and 2.

Notes to the Method

1. If the processing of the samples interruped for the next day, they must be stored in either methanol or methylene chloride solution.

2. During sample analysis, do not let any of the cartridges dry out.

3. Due to batch to batch differences among solid phase cartridges from same vender or variation of these cartridges among different venders, the volumes and/or concentrations of the eluation solvents may need to be varied to get satisfactory results.

4. If interference peaks appear in samples from some locations, it may be possible to achieve satisfactory cleanup by adding an extra cleanup step as follows:

   a. In Step k, dissolve the deposit in pH 2 water instead of high purity water.

   b. Prepare a 3-cc C18 cartridge (0.5 g) by washing the cartridge with 2 column volumes of methanol and 2 column volumes of high purity water. Use vacuum to pull the sample solution through the cartridge at a rate of 1-2 drops/5 seconds. Discard the effluent. Rinse the 100-mL flask with 2 x 5 mL more pH 2 water and pass it through C18 cartridge at the same rate. Discard the rinses.

   c. Rinse the C18 cartridge with 5 mL pH 2 water and 2 x 5 mL hexane. Discard the rinses.

   d. Connect a 60-mL disposable syringe barrel onto the top of the C18 cartridge using an adapter. Add 50 mL of 5% methanol in methylene chloride to the syringe barrel and use the syringe plunger to force the solution through the cartridge at a rate of about 1 drop/second. Collect the effluent in a 100-mL pear-shaped evaporation flask.

   e. Evaporate the organic solvent from the previous step to dryness. Rinse down the deposits on the wall into the tip of the concentration flask with about 10 mL of methanol, and evaporate the methanol just to dryness (do not overdry). Add 10 mL water and vortex in preparation for HPLC analysis.

5. In some instances, it may be possible to resolve interfering peaks satisfactorily by minor changes in the composition of the mobile phase or by using a column oven operated at a temperature up to about 40° C.

6. If the approaches described in either Note 4 or Note 5 are employed, the validity of the modified method for the matrix at hand must be demonstrated by running recovery tests at a range of concentrations sufficient to include the concentrations found in actual samples.

APPROVALS:

Authors:

H. Nejad — 2-24-93 — T. Cavalier — 2-24-93

H. Nejad — Date — T. Cavalier — Date

B. Relifford — 2-24-93

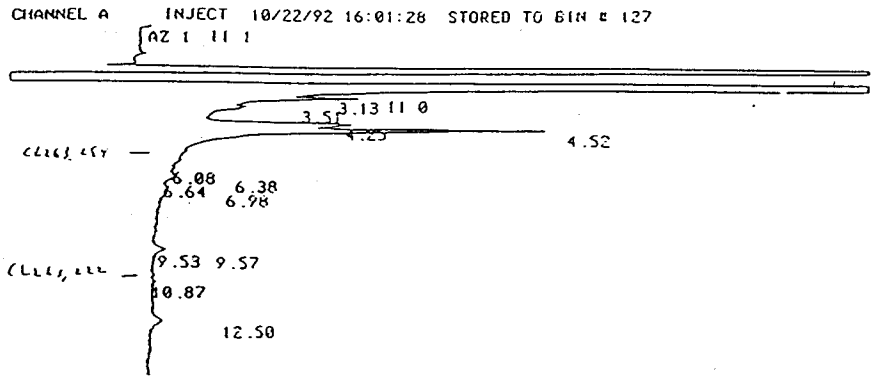
B. Relifford — Date

Group Leader:

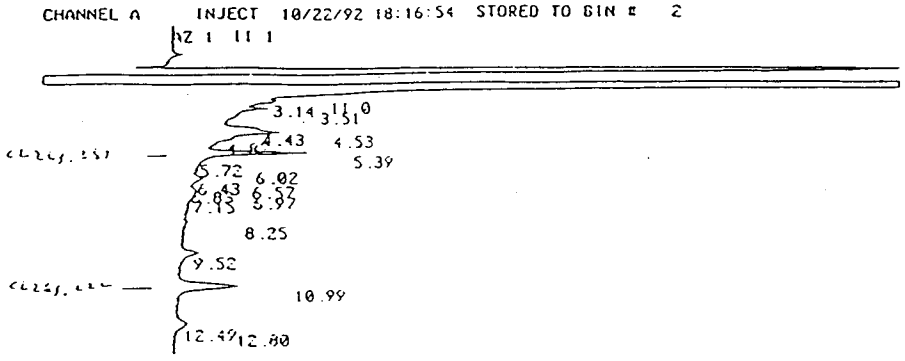
J. E. Boyd — 930224

J. E. Boyd — Date

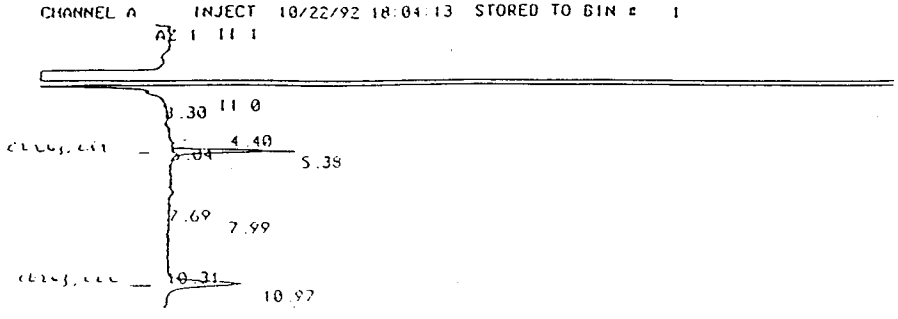
Figure 1: Typical Chromatograms for the Analysis of CL 263,284 and CL 263,222 Residues in Peanut Meat



Control Peanut Meat 7085.0106 (Georgia), 800 mg injected; Peak Area Units=0, No Apparent CL 263,284 or CL 263,222 Residue found.

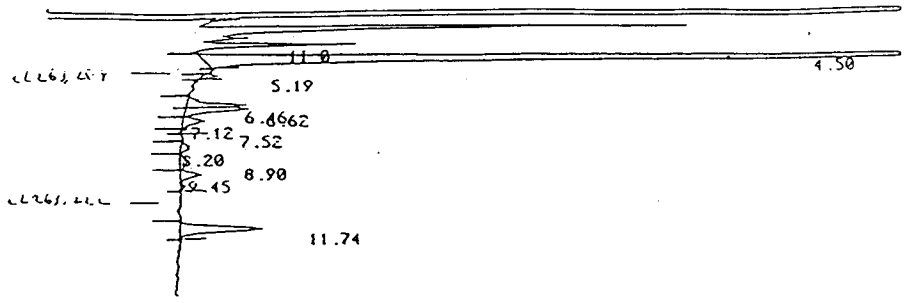


Control Peanut Meat 7085.0106 fortified at 0.1 ppm with CL 263,284 and CL 263,222 Mixed Standard, 50 mg injected; Peak Area Units for CL 263,284=134458, 0.0814 ppm Apparent Residue found; Peak Area Units for CL 263,222=136556, 0.0795 ppm Apparent Residue found; % Recoveries were 81% and 79% for CL 263,284 and CL 263,222 respectively.

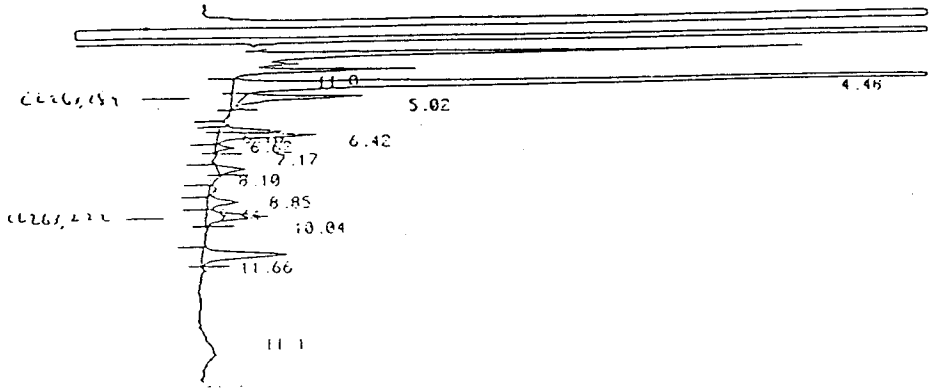


CL 263,284 and CL 263,222 Mixed Standard, 5 ng injected (0.025 mcg/mL), Peak Area Units for CL 263,282=161935, Peak Area Units for CL 263,222=178807, Equivalent to 0.1 ppm fortified sample.

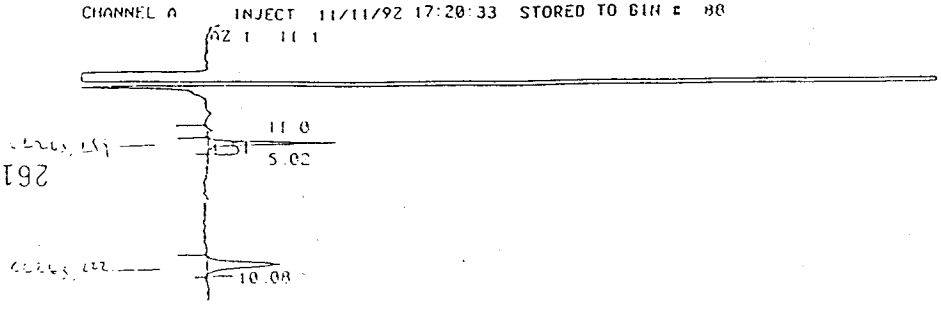
Figure 2: Typical Chromatograms for the Analysis of CL 263,284 and CL 263,222 Residues in Peanut Hull



Control Peanut Hull 7085.0105 (Georgia), 50 mg injected; Peak Area Units=0, No Apparent CL263,284 and CL 263,222 Residue found.



Control Peanut Hull 7085.0105 fortified at 0.1 ppm with CL 263,284 and CL 263,222 Mixed Standard, 50 mg injected; Peak Area Units for CL 263,284=182782, 0.113 ppm Apparent Residue found; Peak Area Units for CL 263,222=138088, 0.0824 ppm Apparent Residue found; % Recoveries were 114% and 79% for CL 263,284 and CL 263,222 respectively.



CL 263,284 and CL 263,222 Mixed Standard, 5 ng injected (0.025 mcg/mL), Peak Area Units for CL 263,284=165060, Peak Area Units for CL 263,222=174847; Equivalent to 0.1 ppm fortified sample.