M-1761
A. Khunachak/G. Picard/cb
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Approved By:

J. M. Devine

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

Recommended Method of Analysis

ASSERT® Herbicide (CL 222,293): GC Method for the Determination of Total CL 222,293 and CL 263,840 Residues, Measured as CL 263,840, in Sunflower Seed and Wheat and Barley Grain and Straw

A. Principle

Residues of CL 222,293 and CL 263,840 are extracted from the sample with boiling 0.5N sodium hydroxide. Any CL 222,293 extracted is converted to CL 263,840 by basic hydrolysis. This method measures total CL 222,293 and CL 263,840 residues, determined as CL 263,840. The sample is subjected to suitable cleanup involving solvent partitioning and solid phase extraction techniques. Measurement of the CL 263,840 is accomplished by gas chromatography using an instrument equipped with a nitrogen-sensitive detector. Results are calculated as total CL 263,840 by the direct comparison of peak heights to those of an external CL 263,840 standard. The validated sensitivity of the method is 0.1 ppm for each commodity.

NOTE: This method is a compilation of Methods M-1531 (GC) and M-1735 (HPLC) to unify the residue method for wheat, barley, and sunflowers and present one type of determinative step (GC) for regulatory purposes.

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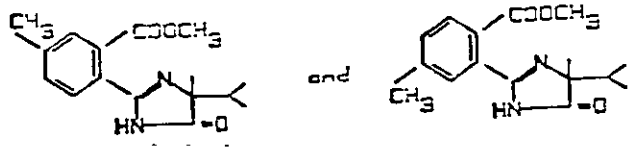
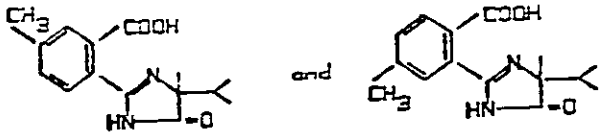
B. Reagents

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

   a. CL 222,293: [40:60 mixture of m-toluic acid, 6-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl) methyl ester and p-toluic acid, 2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl) methyl ester].



   b. CL 263,840: [40:60 mixture of m-toluic acid, 6-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl) and p-toluic acid, 2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl)].



2. GC Packing: 3% OV-25 on 100/120 mesh Supelcoport, Catalog Number 1-1790, Supelco, Incorporated.

3. Deionized Water: Millipore's Milli-Q water or equivalent.

4. Solvents: B & J Brand High Purity Solvents, American Burdick and Jackson, or equivalent.

   a. Acetone
   b. Methanol
   c. Methylene Chloride
   d. Acetonitrile
   e. Hexane

5. TMAH Methylation Reagent: Trimethylphenylammonium hydroxide, 0.1M in methanol, Catalog Number 135-6542, Eastman Kodak Company.

6. Solutions:

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

   b. Extraction Solvent, 0.5N Sodium Hydroxide: Dissolve 80 grams of sodium hydroxide in 2 liters of Milli-Q water. Add 2 liters of methanol and mix thoroughly.

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c. pH 6.5 Phosphate Buffer: Dissolve 50 grams of potassium phosphate dibasic in 1 liter of Milli-Q water, adjust to pH 6.5 with 6N hydrochloric acid.

d. pH 3.5 Phosphate Buffer: Dissolve 5 g of potassium phosphate dibasic in 1 liter of Milli-Q water, adjust to pH 3.5 with 6N hydrochloric acid.

e. 0.1N Sodium Hydroxide: Dissolve 4 grams of sodium hydroxide in 1 liter of deionized water and mix thoroughly.

f. 0.2N Hydrochloric Acid: Dilute 16.7 mL of concentrated hydrochloric acid to 1 liter with deionized water.

g. 0.05N Hydrochloric Acid: Dilute 4.2 mL of concentrated hydrochloric acid to 1 liter with Milli-Q water.

h. 1N Hydrochloric Acid: Dilute 83 mL of concentrated hydrochloric acid to 1 liter with Milli-Q water.

i. 50% Methanol in Water: Dilute 500 mL of methanol to 1 liter with Milli-Q water.

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

   a. Sodium Chloride
   b. Potassium Phosphate, Dibasic
   c. Hydrochloric Acid, Concentrated
   d. Sodium Hydroxide, Pellets

8. Celite 545 AW: Johns-Manville Company.

9. Darco G-60 Activated Charcoal: Catalog Number CX0645-1, MCB Manufacturing Chemists, Incorporated.

C. Apparatus

1. Gas Chromatograph: Tracor, Model 540 equipped with a Model 702 nitrogen-phosphorus detector.

2. Integrator: Spectra Physics Model 4290 recording integrator, or equivalent.

3. Balance, Analytical: Sartorius, precision of ± 0.05 mg.

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

5. Assorted Glassware: General laboratory.

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

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7. Filtering Flasks: 500-mL capacity, Corning Glass Works, Catalog Number 5340.

8. Filtering Funnels: Buchner, porcelain, 9-cm diameter.

9. Filter Paper: 9-cm diameter, glass-fiber filter, Whatman, Incorporated.

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

11. Centrifuge Tubes: 12-mL capacity, glass.

12 Centrifuge: Clay-Adams, Safety Head Model or equivalent.

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

14. GC Column: 182-cm X 2-mm ID, glass, packed with 3% OV-25 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.

15. Plastic Syringe, Disposable: Luer-Lok, 10- and 30- mL capacity, Beckton Dickinson.

16. Solid Phase Extraction Cartridges

    a. Analytichem SCX Benzenesulfonic Acid Bond-Elut Cartridge (500 mg): Catalog Number 617303, Analytichem, International.

    b. Analytichem C-18 Bond-Elut Cartridge (1,000 mg): Catalog Number 607406, Analytichem, International.

    c. Analytichem SAX Quaternary Amine Bond-Elut Cartridge (500 mg): Catalog Number 618303, Analytichem, International.

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

18. Separatory Funnels: Squibb-type with teflon stopcocks, 125-, 250- and 500-mL capacity, Kontes Glass Company.

19. Microliter Syringes: Hamilton, Model 701 (10-mcL capacity) and Model 702 (25-mcL capacity).

20. Millex Filter Unit: 0.45 Micron, Nonsterile, Catalog Number SLHV025NB, Millipore Company.

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D. Preparation of Standard Solutions

1. Stock Solutions (Prepare Monthly)

a. CL 222,293 - Weigh accurately 10.5 ± 0.05 mg of CL 222,293 into a 100-mL volumetric flask. Dilute to the mark with acetone and mix well. This solution contains 105 mcg/mL of CL 222,293, which is equivalent to 100.0 mcg/mL of CL 263,840. (Note: Molecular weight of CL 222,293 is 105% that of CL 263,840).

b. CL 263,840 - Weigh accurately 10 ± 0.05 mg of CL 263,840 into a 100-mL volumetric flask. Dilute to the mark with acetone and mix well. This solution contains 100 mcg/mL of CL 263,840.

2. Standard Fortification Solutions

Pipet into separate 100-mL volumetric flasks 10-, 5-, 2-, and 1-mL aliquots of each stock solution. Dilute to the mark with acetone and mix well. These solutions contain 10, 5, 2, and 1 mcg/mL, respectively, of each compound.

3. Standard Chromatographic Solutions

Pipet 1-mL aliquots of 10, 5, and 2 mcg/mL CL 263,840 into 250-mL round bottom flasks. Add 1,000 mcL of TMAH (for wheat and barley analysis) or 500 mcL of TMAH (for sunflower seed analysis) to each flask and evaporate to dryness. Redilute each with 5.0 mL of 10% methanol in acetone to give concentrations of 2.0, 1.0 and 0.4 mcg/mL, respectively. These solutions are used for the linearity check.

NOTE: The 2.0 mcg/mL CL 263,840 prepared this way each day is used as that day's GC standard for wheat and barley grain and straw whereas the 1.0 mcg/mL CL 263,840 is for sunflower seed.

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 30% FSD (full-scale deflection) for a 10-ng injection of standard CL 263,840.

3. Column: 182-cm x 2-mm ID, glass, packed with 3% OV-25 on 100/120 mesh Supelcoport.

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4. Instrument Conditions

a.	Column Temperature	225°C
b.	Inlet Temperature	300°C
c.	Detector Temperature	260°C
d.	Carrier Gas Flow Rate (Helium)	25 mL/minute
e.	Hydrogen Flow Rate	2.5 mL/minute
f.	Air Flow Rate	120 mL/minute
g.	Input Attenuation	1
h.	Chart Speed	0.5 cm/minute

5. Sensitivity: Attenuation on recording integrator set so that 10.0 ng of CL 263,840 gives a peak height of approximately 30% FSD.

6. Retention Time: Approximately 3.3 minutes.

F. Linearity Check

The gas chromatography 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 30% full-scale deflection for a 10.0-ng injection of CL 263,840. The GC response can be stabilized with several injections of sample extracts (containing TMAH).

2. Inject aliquots of solutions prepared in Section D.3. using 5-mcL for the analysis of wheat and barley grain and straw and 10-mcL for the analysis of sunflower seed.

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 the blade.

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2. Add the prefrozen straw 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. Allow the samples to stand in a freezer overnight for the dry ice to dissipate completely.

4. For analysis of grain and seed samples, blend the whole sample with dry ice for several minutes in a Waring blender to break the seed heads and pulverize the sample.

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 10-g subsample of control into a 50-mL beaker.

2. Add by pipet a volume of standard fortification solution appropriate to the fortification level to be tested.

3. Add the solution dropwise and mix the sample well before adding sample to the boiling extraction solvent.

4. Continue with the extraction and cleanup steps.

I. Extraction and Preliminary Cleanup for Sunflower Seeds

1. Weigh 10 g of seed into a 50-mL beaker.

2. Bring 150 mL of 0.5N sodium hydroxide solution in (1:1) methanol: water to a boil in a 600-mL beaker covered with a watch glass on a stirring hot plate. Add the 10 grams of seed and stir with boiling uncovered for 10 additional minutes.

3. Remove the beaker from the stirring hot plate and allow to cool at room temperature.

4. Adjust the pH of the mixture to pH 6.0 using 6N hydrochloric acid (approximately 10-15 mL).

5. Add 5 g of Celite to the beaker and filter the mixture through a filter paper prepared on a 9-cm Buchner funnel.

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6. Wash the beaker and filter with 50 mL of methanol.

7. Measure the volume of filtrate in a 250 mL graduated cylinder and pour half the volume measured into a 1,000-mL round bottom flask. Discard the other half of the sample.

8. Use a flash evaporator to evaporate the methanol from the mixture of the methanol-water extract in the 1,000-mL round bottom flask. Do not evaporate to dryness. The aqueous volume after this step should be approximately 30-40 mL.

9. Add 300 mL of acetone to the extract in the 1,000-mL round bottom flask. Decant the solution through a 9-cm Buchner funnel fitted with a 9-cm glass fiber paper, leaving the precipitate and a small amount of acetone in the flask. Add 5 mL of Milli-Q water and dissolve the precipitate by swirling the flask or using a sonic oscillator. Add 100 mL of acetone to reprecipitate the coextractives. Decant, filter, and combine with the original filtrate. Add 25 to 50 mL more of acetone to help complete the transfer. Filter and combine with the filtrate in the flask.

10. Evaporate the acetone from the combined filtrate in a 1,000-mL round bottom flask. Do not evaporate to dryness. The aqueous volume after this step should be approximately 15-25 mL.

11. Add 10 mL of pH 6.5 phosphate buffer to the flask and decant into a 250-mL separatory funnel. Rinse the flask with 5 mL of methanol into the separatory funnel. Rinse the flask with 50 mL of methylene chloride into the separatory funnel.

12. Partition by shaking vigorously for 20 seconds. Allow the layers to separate and discard the lower methylene chloride layer.

13. Decant the aqueous phase into a 100-mL beaker and adjust to pH 3.5 with 6N hydrochloric acid, monitoring the pH with a pH meter.

14. Dissolve 8 g of sodium chloride in the pH 3.5 solution.

15. Pour the solution back into the 250-mL separatory funnel, rinse the beaker with 5 mL of Milli-Q water and partition with 2 x 100 mL and 1 x 50 mL of methylene chloride, combining the lower methylene chloride layers in a 500-mL round bottom flask.

16. Evaporate the combined methylene chloride extracts to dryness.

17. Add 2 mL of methanol using sonic oscillation to dissolve the residue, then add 1 ml of 1N hydrochloric acid and 17 mL of Milli-Q water.

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J. Solid Phase Extraction Cleanup for Sunflower Seeds

1. Prepare an Analytichem Bond Elut C-18 cartridge using an Analytichem Vac-Elut Processing Station by washing the cartridge with 5 mL of methanol followed by 5 mL of water. Use 5 mm Hg of vacuum.

2. Assemble a 30-mL disposable syringe into which a pledget of glass wool has been placed onto the top of the prepared Analytichem C-18 cartridge.

3. Pass the extract from step I.17 through the Analytichem C-18 cartridge using the Vac-Elut Processing Station (use 5 mm Hg of vacuum).

4. Wash the flask and the cartridge with 5 mL of 0.05N hydrochloric acid.

5. Remove the syringe and adapter and wash the C-18 cartridge with an additional 5 mL of 0.05N hydrochloric acid followed by 5 mL of Milli-Q water.

6. Remove the C-18 cartridge from the Vac-Elut Processing Station.

7. Use the Vac-Elut system to prepare an Analytichem Bond-Elut SCX cartridge by washing with 3 mL hexane, 3 mL methanol and 3 mL of water. Connect the C-18 onto the top of the SCX cartridge using an adapter.

8. Connect a 10-mL syringe onto the top of the C-18 cartridge.

9. Elute the tandem cartridge system with 10 mL of 50% methanol-water (use 10 mm Hg vacuum). Discard the eluate.

10. Discard the upper C-18 cartridge, connect a 30-mL syringe to the top of the SCX cartridge and successively wash the SCX cartridge with 5 mL methanol, 5 mL water and 5 mL of pH 3.5 phosphate buffer. Discard the eluants.

11. Remove the SCX cartridge from the vac-elut system and, using the 30-mL syringe, elute with 25 mL of pH 6.5 phosphate buffer directly into a 250-mL separatory funnel.

12. Partition the phosphate buffer eluate for 15 seconds with 50 mL of methylene chloride. Allow the layers to separate and discard the lower methylene chloride layer.

13. Pour the phosphate buffer from the separatory funnel into a 50-mL beaker and adjust to pH 3.5 with 6N hydrochloric acid and a pH meter.

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14. Pour the solution from the beaker back into the 250-mL separatory funnel. Rinse the beaker with 5 mL of Milli-Q water and add this rinse to the separatory funnel.

15. Partition the solution with 100 mL of methylene chloride, shaking vigorously for 20 seconds. Evaporate the methylene chloride to dryness in a 250-mL round bottom flask.

16. Repartition the solution in the separatory funnel twice more with 100 mL and then 50 mL of methylene chloride. Evaporate the combined methylene chloride extracts to dryness in the same 250-mL round bottom flask used in step J.15.

17. Add 100 mcL of TMAH and 25 mL of methanol to the flask and evaporate to dryness.

18. Repeat with a second 25-mL portion of methanol to ensure complete removal of methylene chloride.

19. Dissolve the residue in 1.0 mL of 10% methanol in acetone for gas chromatographic analysis (Section M).

K. Extraction and Preliminary Cleanup for Wheat and Barley

1. Weigh accurately 10 grams of grain or straw onto a 6 inch by 6 inch piece of glassine paper.

2. Bring 150 mL of 0.5N sodium hydroxide solution in (1:1) methanol: water to a boil in a 600-mL beaker covered with a watch glass on a stirring hot plate. Add the 10 grams of grain or straw and stir with boiling uncovered for 5 additional minutes.

3. Remove the beaker from the stirring hot plate, add 20 mL of methanol and 15 grams of Celite.

4. Filter the mixture through a 15-gram Celite pad prepared on a 9-cm Buchner funnel using 9-cm glass-fiber filter paper.

5. Wash the beaker and filter cake with 2 x 50 mL of methanol.

6. Partition the filtrate with 200 mL of hexane vigorously for 10 seconds. Draw off the lower aqueous methanolic layer into a 400-mL beaker and discard the upper hexane layer.

7. For grain samples, continue with Step K.11.

8. For straw samples, add 10 grams of Darco G-60 to the aqueous-methanol solution and stir for 5 minutes using a stirring bar and a magnetic stirrer.

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9. Filter the mixture through a double layer of glass-fiber filter paper fitted onto a 9-cm Buchner funnel.

10. Wash the beaker and filter cake with 30 mL of methanol.

11. For both grain and straw samples, adjust the extract to pH 3.5 using 6N hydrochloric acid.

12. Add 25 grams of sodium chloride and stir for 5 minutes.

13. Decant the solution into a 500-mL separatory funnel.

14. Wash the excess sodium chloride with 5 mL of distilled water plus 5 mL of methanol.

15. Combine this wash with the solution in the 500-mL separatory funnel, discarding the excess sodium chloride.

16. Partition the solution with 3 x 200 mL of methylene chloride, shaking vigorously for 15 seconds each time. Difficult to handle emulsions can be broken by adding 20 mL of methanol to the separatory funnel between methylene chloride partitionings.

17. Evaporate the combined methylene chloride portions to near dryness in a 1,000-mL round bottom flask.

18. Add 25 mL of acetonitrile and evaporate the remaining few milliliters of water to dryness.

19. Add 2 mL of deionized water to the round bottom flask.

20. Then add 25 mL of acetone to the 1,000-mL round bottom flask and use sonic oscillation to dislodge all the salt from the flask walls.

21. Filter the mixture through a Millex filter using a 30-mL disposable syringe into which a pledget of glass wool has been placed.

22. Wash the 1,000-mL round bottom flask and filter system with an additional 25 mL of acetone.

23. Evaporate the combined acetone filtrates to dryness in a 250-mL round bottom flask. Evaporate any traces of water with 10 mL of acetonitrile.

L. Solid Phase Extraction Cleanup for Wheat and Barley

1. Prepare an Analytichem SCX benzenesulfonic acid Bond-Elute cartridge using an Analytichem Vac-Elute Processing Station by washing with 3 mL of hexane, 3 mL of methanol and 3 mL of 0.02N hydrochloric acid.

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2. Assemble a 30-mL disposable syringe (without the plunger) on top of the prepared Analytichem SCX cartridge.

3. Place a pledget of glass wool inside the barrel of the syringe.

4. Dissolve the residue from Step K.23 in 1 mL of methanol and then add 9 mL of 0.02N hydrochloric acid.

5. Transfer the contents of the flask to a 12-mL centrifuge tube, saving the 250-mL round bottom flask.

6. Centrifuge the sample at 5,000 rpm for 5 minutes. Decant the centrifugate into the 30-mL syringe-sulfonic acid column assembly prepared in Step L.3 and apply enough vacuum to pass the sample through the column at a rate of 10 mL/minute.

7. Wash the 250-mL round bottom flask saved in Step L.5 with 5 mL of 0.1N sodium hydroxide and transfer the contents of this flask to the precipitate in the 12-mL centrifuge tube using a disposable Pasteur capillary pipette to help disperse most of the precipitate in the tube.

8. Reprecipitate the sample in the 12-mL centrifuge tube with 5 mL of 0.2N hydrochloric acid and centrifuge once again at 5,000 rpm for 5 minutes.

9. Decant the centrifugate into the 30-mL syringe-sulfonic acid column assembly used in Step L.6 and again apply enough vacuum to pass the sample through the column at a rate of 10 mL/minute.

10. Remove the 30-mL disposable syringe barrel from the sulfonic-acid column and wash the column with 3 mL of methanol, 3 mL of acetone, and 3 mL of deionized water. Discard these washes.

11. Remove the sulfonic-acid column from the Analytichem Vac-Elute Processing Station.

12. Prepare an Analytichem SAX quaternary amine Bond-Elute cartridge using an Analytichem Vac-Elute Processing Station by washing with 3 mL of hexane, 3 mL of methanol, and 3 mL of pH 6.5 phosphate buffer.

13. Remove the quaternary amine cartridge from the Analytichem Vac-Elute Processing Station and connect the sulfonic-acid column saved in Step L.11 onto the top of the quaternary amine cartridge by means of an adaptor.

14. Elute the tandem columns with 10 mL of pH 6.5 phosphate buffer by means of a 10-mL disposable syringe directly into a 25-mL beaker.

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15. Add 5 mL of methanol to the eluate in the 25-mL beaker and adjust the solution to pH 3.5 with 0.2N hydrochloric acid.

16. Partition the pH 3.5 solution with 4 x 35 mL of methylene chloride, shaking 15 seconds each time and combine the methylene chloride portions in a 250-mL round bottom flask.

17. Evaporate the combined methylene chloride extracts to dryness on a rotary evaporator.

18. Add 200 mcL of TMAH and 25 mL of methanol to the flask and evaporate to dryness.

19. Repeat with a second 25-mL portion of methanol to ensure complete removal of methylene chloride.

20. Dissolve the residue in 1.0 mL of 10% methanol in acetone in preparation for gas chromatographic analysis.

M. Gas Chromatographic Analysis

1. After obtaining a stable GC response as described in Section F, inject a 5-mcL aliquot (for wheat and barley analysis) or a 10-mcL aliquot (for sunflower analysis) of sample into a GC equipped with a nitrogen-phosphorus detector. Note: If sample chromatographic fronts become too large, change the glass wool in the inlet section of the GC column.

2. Compare the peak height with that obtained from a 10-ng injection of the appropriate GC standard solution (see Section D.3).

3. If the sample peak goes off scale, dilute to an appropriate volume with methanol. Pipette 1 mL of this diluted sample into another 250-mL round bottom flask, add 200 mcL TMAH (for wheat and barley analysis) or 100 mcL TMAH (for sunflower seed analysis). Evaporate to dryness on a flash evaporator, and redilute to 1 mL with 10% methanol-acetone.

4. Make a standard injection after every sample and use the average peak height of the standard injection before and after the sample for the calculation.

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 injection as follows:

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

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Where:

R(SAMP) = Peak height of sample.

R(STD) = Average peak height of working standard.

W = Weight of sample taken for analysis in grams.

V1 = Volume of extraction solvent added to sample in milliliters.

V2 = Aliquot of extract taken for analysis in milliliters.

V3 = Volume of 10% methanol/acetone added to dissolve final residue for chromatographic analysis in millimeters.

V4 = Volume of sample solution injected in microliters.

V5 = Volume of working standard solution injected in microliters.

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

D.F. = Dilution factor.

Typical chromatograms for sunflower seeds and wheat and barley grain are shown in Figures M-1761.A, M-1761.B, and M-1761.C, respectively. Additional chromatograms for analysis of wheat and barley samples were previously presented in Method M-1531.

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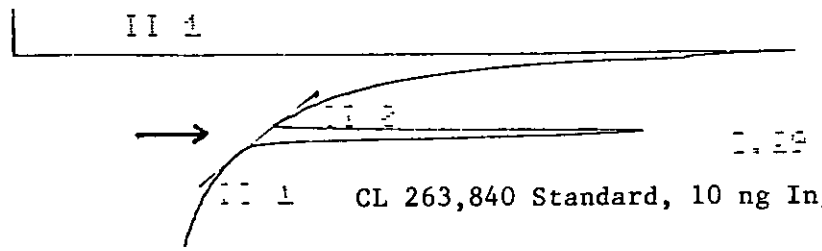
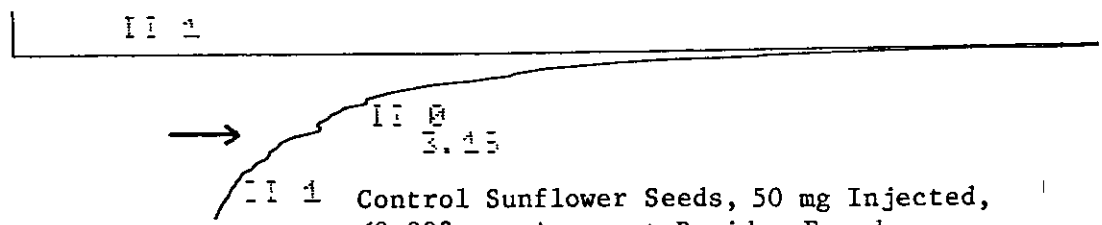
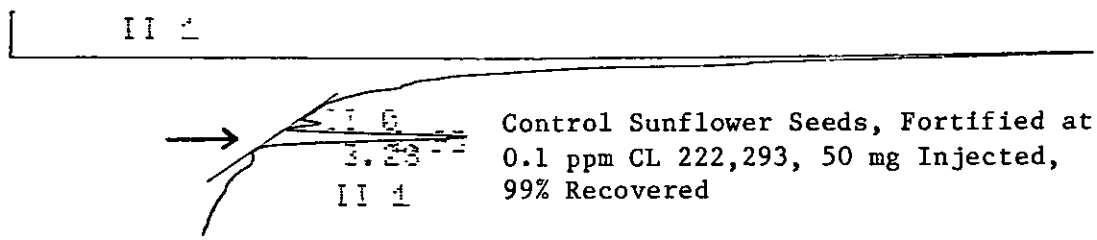
Figure M-1761.A: Typical Chromatograms for Analysis of Total CL 222,293 and CL 263,840 Residues in Sunflower Seed



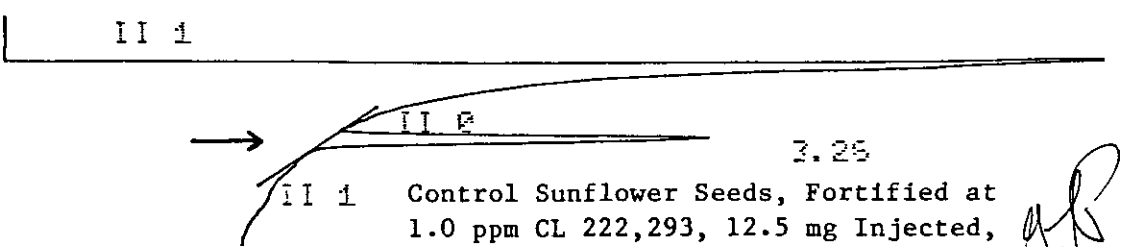
CL 263,840 Standard, 10 ng Injected



Control Sunflower Seeds, 50 mg Injected,
<0.003 ppm Apparent Residue Found



Control Sunflower Seeds, Fortified at
0.1 ppm CL 222,293, 50 mg Injected,
99% Recovered



Control Sunflower Seeds, Fortified at
1.0 ppm CL 222,293, 12.5 mg Injected,
75% Recovered

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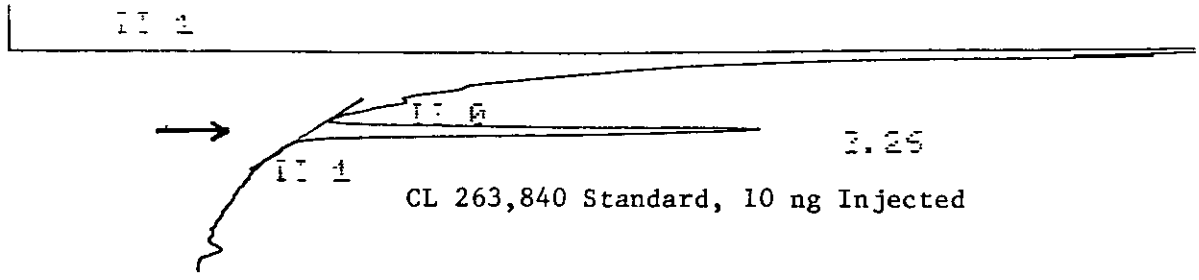
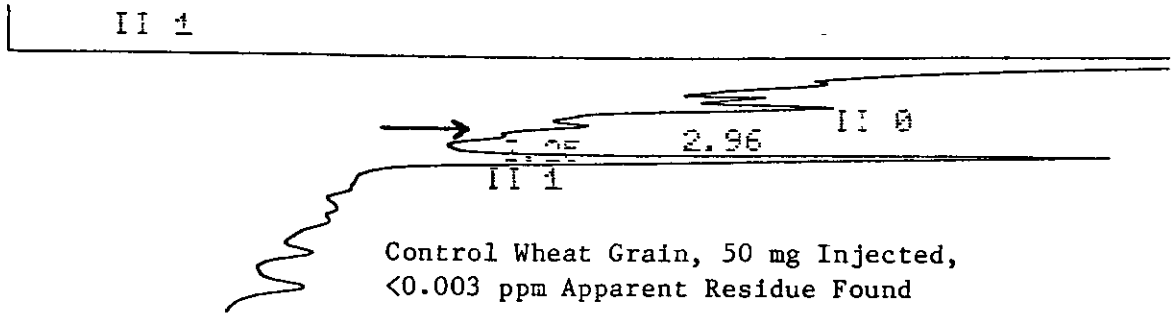
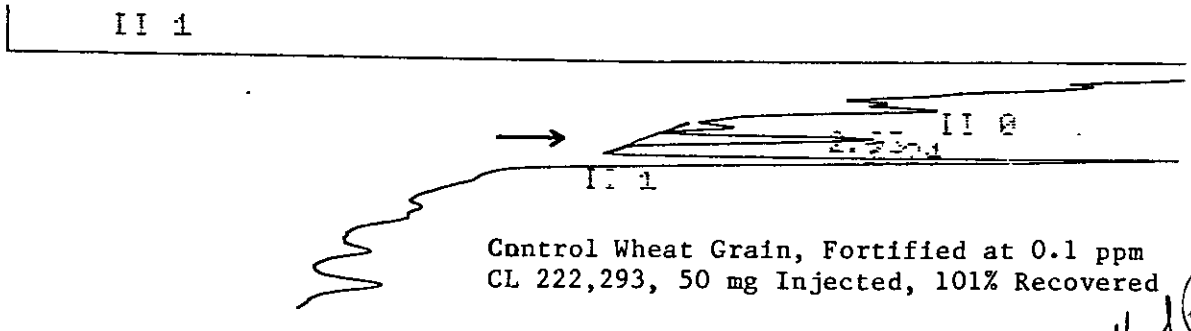
Figure M-1761.B: Typical Chromatograms for Analysis of Total CL 222,293 and CL 263,840 Residues in Wheat Grain



CL 263,840 Standard, 10 ng Injected



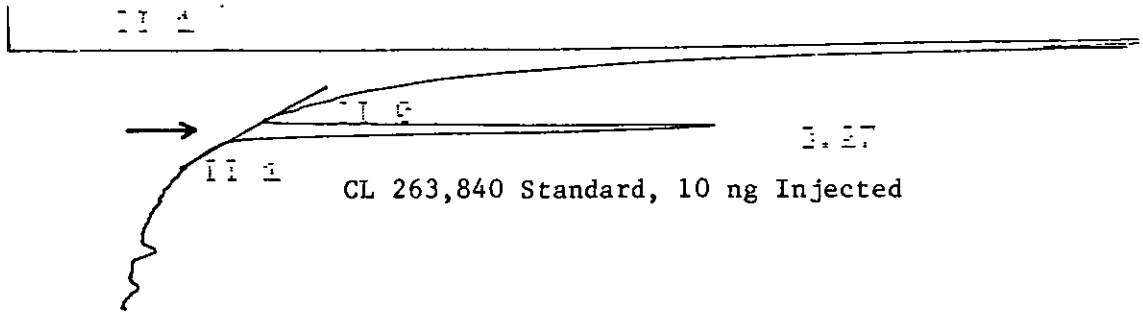
Control Wheat Grain, 50 mg Injected,
<0.003 ppm Apparent Residue Found



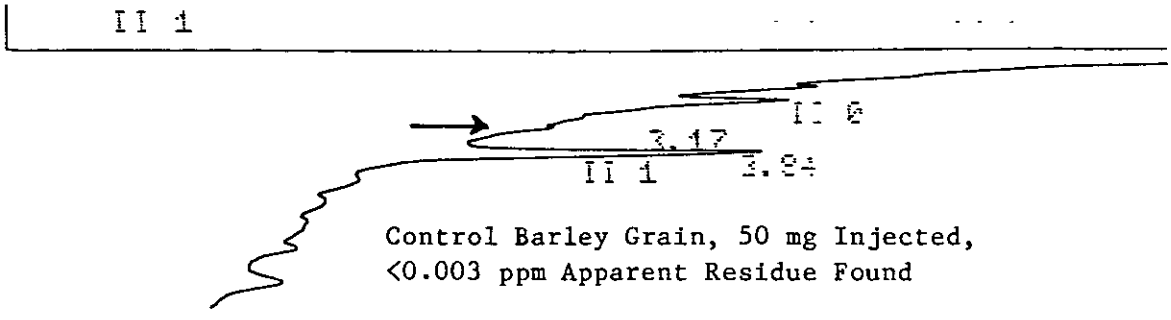
Control Wheat Grain, Fortified at 0.1 ppm
CL 222,293, 50 mg Injected, 101% Recovered

A.K.

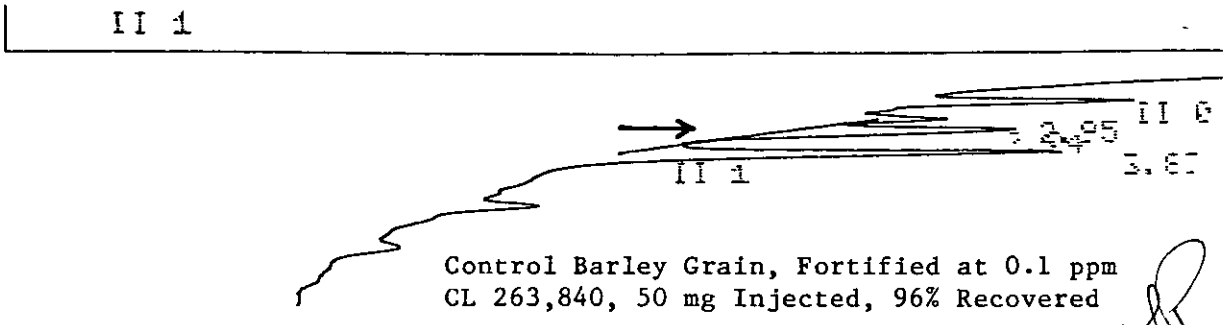
Figure M-1761.C: Typical Chromatograms for Analysis of Total CL 222,293 and CL 263,840 Residues in Barley Grain



CL 263,840 Standard, 10 ng Injected



Control Barley Grain, 50 mg Injected,
<0.003 ppm Apparent Residue Found



Control Barley Grain, Fortified at 0.1 ppm
CL 263,840, 50 mg Injected, 96% Recovered

A.K.