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

Recommended Method of Analysis

Imazamethabenz-methyl Herbicide (CL 222,293): HPLC Method for the Determination of CL 222,293 and CL 263,840 (acid metabolite) Residues in Soil.

A. Principle

Residues of CL 222,293 and CL 263,840 are extracted from soil with acidic methanol followed by aqueous sodium hydroxide. The cleanup of the combined extracts is achieved by using a C-18 reverse phase cartridge followed by an SCX cartridge. The CL 222,293 and CL 263,840 residues eluted from the SCX cartridge are partitioned with methylene chloride, the methylene chloride is evaporated and the residue is dissolved in a measured volume of water. Quantitation is accomplished by liquid chromatography using an instrument equipped with a UV detector (240 nm). Results are calculated as CL 222,293 and CL 263,840 by comparison of peak heights to those of external standards.

The validated sensitivity of the method is 10 ppb for each compound.

B. Reagents

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

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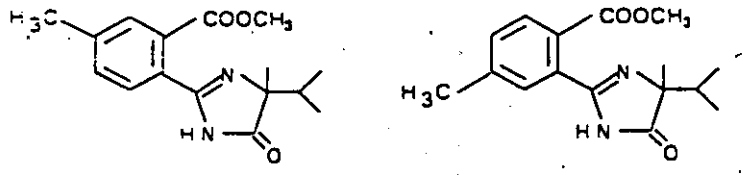
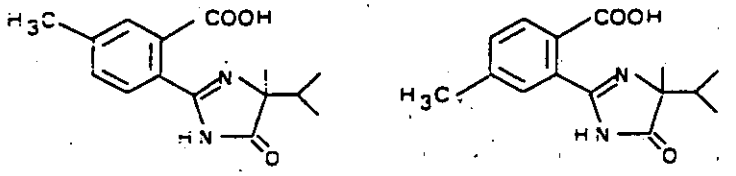
a. CL 222,293: [40:60 mixture of m-toluic acid, 6-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl) methyl ester and p-toluic acid, 2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl) methyl ester].



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. Deionized Water: Millipore's Milli-Q water. Use deionized water for all steps.

3. Solvents: B & J Brand High Purity Solvents, Baxter Burdick and Jackson.

   a. Methanol
   b. Methylene Chloride
   c. Acetonitrile, UV Grade

4. Chemicals: "Baker Analyzed" Reagents - J. T. Baker Company.

   a. Sodium Hydroxide, pellets
   b. Hydrochloric Acid, concentrated
   c. Acetic Acid, glacial
   d. Triethylamine
   e. Potassium Chloride
   f. Potassium Phosphate, Monobasic

5. Solutions

   a. Extraction Solvent, 1% Hydrochloric Acid in Methanol: Add 40 mL of concentrated hydrochloric acid to 4 L of methanol. Mix thoroughly.

   b. Extraction Solvent, 0.05N Sodium Hydroxide: Dissolve 8 g of sodium hydroxide in 4 L of deionized water. Mix thoroughly.

   c. pH 3.5 Phophate Buffer: Dissolve 50 g of potassium phosphate monobasic in 1 L of deionized water. Adjust to pH 3.5 with 6N hydrochloric acid.

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d. 6N Hydrochloric Acid: Add 250 mL of concentrated hydrochloric acid to 250 mL of deionized water and mix well.

e. 5% Sodium Hydroxide: Dissolve 50 g of sodium hydroxide in 1 L of deionized water and mix thoroughly.

f. 5% Hydrochloric Acid: Dilute 50 mL of concentrated hydrochloric acid to 1 L with deionized water and mix well.

g. 50% Methanol in Water: Dilute 500 mL of methanol to 1000 mL with deionized water and mix well.

h. Saturated Potassium Chloride-Methanol: Add 1 L of methanol to 50 g of potassium chloride, shake, allow excess potassium chloride to settle.

i. Liquid Chromatographic Mobile Phase: Mix 250 mL of acetonitrile, 750 mL of deionized water, 40 mL of acetic acid and 1.0 mL of triethylamine. Filter the mobile phase through a Rainin Nylon-66 (0.45μm) filter or equivalent.

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

1. Balance, Analytical: Mettler, precision of $\pm$ 0.05 mg.

2. Balance, Pan: Sartorius, Model 2254, precision of $\pm$ 5 mg.

3. Assorted Glassware: General laboratory.

4. Centrifuge Bottles: One pint capacity, wide-mouth polypropylene, Catalog Number 05-562-24, Fisher Scientific Company.

5. Flash Evaporator: Buchi Instruments Model 461, or equivalent, equipped with a heated water bath (approximately at 35°C) in which the evaporation flasks can be partially submerged.

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

7. pH Meter: Orion Model 701A or equivalent.

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

9. Plastic Syringe, Disposable: Lure-Lok, 30-mL capacity, Becton-Dickinson.

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

11. Reservoir Disposable: 75-mL capacity, Catalog Number 607500, Analytichem International.

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

13. Microliter Syringe: 1 mL B-D Yale Tuberculin, Reorder No. 2004, Becton-Dickinson Company.

14. Syringe Needles: Hamilton, Catalog Number 2-1744, Supelco, Incorporated.

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

16. Horizontal Reciprocating Shaker: A. H. Thomas Company, No. 8291-510.

17. 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 (1000 mg): Catalog Number 607406, Analytichem, International.

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

19. Liquid Chromatograph:

    a. Pump - Kratos Spectroflow Model 400.

    b. Detector - Kratos, Spectroflow Model 783 UV detector.

    c. Rheodyne Injector Valve - Model 7125.

    d. Sample Loop - 500 mcL.

20. Integrator: Spectra Physics Model 4290 recording integrator or equivalent.

21. HPLC Column: Supelco LC-8-DB (octyldimethylsilyl, deactivated for basic compounds), 15 cm x 4.6 mm ID, Cat. No. 5-8347.

22. HPLC Guard Column: Supelco LC-8-DB, 2 cm, Cat. No. 5-9053.

D. Preparation of Standard Solutions

1. Stock Solutions (Store in amber bottles in refrigerator. Stable for at least 2 months.)

   a. CL 222,293 - Weigh accurately a known amount (approximately 10 mg) of CL 222,293 into a 100-mL volumetric flask. Dilute to the mark with acetone and mix well. Calculate and record the exact concentration of CL 222,293.

   b. CL 263,840 - Weigh accurately a known amount (approximately 10 mg) of CL 263,840 into a 100-mL volumetric flask. Dilute to the mark with acetone and mix well. Calculate and record the exact concentration of CL 263,840.

   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 enough of each stock solution D.1.a. and D.1.b. to deliver 1000 mcg of each compound (CL 222,293 and CL 263,840). Dilute to the mark with acetone and mix well. This solution contains 10 mcg/mL respectively, of each compound.

   b. Pipet into separate 100-mL volumetric flasks 40-, 20-, 8-, and 4-mL aliquots of standard solution D.2.a. Dilute to the mark with acetone and mix well. These solutions contain 4, 2, 0.8 and 0.4 mcg/mL, respectively, of each compound.

3. Standard Liquid Chromatography Solution

   Pipet a 1.0-aliquot of the 0.4 mcg/mL (Standard Fortification Solutions D.2.b.) and 0.5- and 1.0 mL aliquots of the 2.0 mcg/mL into separate 100-mL round-bottom flasks. Evaporate the acetone and add 10-mL of deionized water to each flask using a volumetric pipet. These standard solutions contain 0.04, 0.1 and 0.2 mcg/mL, respectively, of each CL 222,293 and CL 263,840. These solutions are

used for the linearity check. Note: The 0.1 mcg/mL standard should be prepared each day and used as that day's LC standard for quantitation of CL 222,293 and CL 263,840.

E. Liquid Chromatographic Conditions

1. Instrument:

a. Pump: Kratos Spectroflow 400 or equivalent.

b. Detector: Kratos, Spectroflow 783 UV detector.

2. Column: Supelco LC-8-DB, 15 cm x 4.6 mm ID.

3. Guard Column: Supelco LC-8-DB.

4. Instrument Conditions:

a.	Column Temperature:	Room temperature (approx. 25°C)
b.	Mobile Phase:	Acetonitrile:Water:Acetic Acid: Triethylamine (25:75:4:0.1)
c.	Flow Rate:	0.5 mL/min (300 psi)
d.	Detector Wavelength:	240 nm
e.	Loop Injector:	500 mcL
f.	Chart Speed:	0.5 cm/min
g.	Retention Times (approximately)	10 minutes meta isomer of CL 263,840 11 minutes para isomer of CL 263,840 20 minutes for CL 222,293

5. Sensitivity: Attenuation on recording integrator set so that 50 ng of CL 263,840 and 50 ng of CL 222,293 gives peak heights of approximately 50% Full Scale Deflection (FSD).

F. Linearity Check

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

1. Adjust the HPLC conditions to attain peak heights of approximately 50% full-scale deflection for a 50 ng injection of CL 263,840 and CL 222,293.

2. Inject 500-mcL aliquots of solutions prepared in Section D.3.

3. Plot the combined peak heights of the isomers of CL 263,840 and the peak height of CL 222,293 versus the nanograms injected to show the linearity of response. Significant departure from linearity over the range indicates instrumental difficulties which should be corrected before proceeding.

G. Sample Preparation

1. Samples are kept frozen except for the short time they are being prepared for analysis..

2. Refer to appropriate standard operating procedures for soil sample preparation.

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 40-gram portions of the control soil into one-pint plastic centrifuge bottles fitted with a leak proof cap.

2. Add by pipet an aliquot of the respective standard solutions appropriate to the fortification level to be tested. Add the solution dropwise and spread it over the surface of the sample.

3. Mix the soil well swirling the bottle, allow most of the acetone to evaporate and continue from Step 2 of Section I.

I. Sample Extraction

1. Weigh a 40-g soil subsample for analysis into a one-pint plastic centrifuge bottle fitted with a leak proof cap.

2. Add 10 mL of deionized water and 200 mL of 1% hydrochloric acid in methanol and cap tightly. Shake on a horizontal reciprocating shaker for 30 minutes.

3. Remove from the shaker, uncap the lid, balance the centrifuge bottle with methanol and centrifuge at 1500 rpm for 10 minutes.

4. Decant the supernatant solution into a 1-L round bottom flask and concentrate the solution to within 10 mL on a flash evaporator and save this extract until Step 7.

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5. Resuspend the soil marc in the plastic centrifuge bottle with 200 mL of 0.05N sodium hydroxide and shake for 30 minutes.

6. Remove from the shaker, uncap the lid, balance the centrifuge bottle with deionized water, and centrifuge at 1500 rpm for 10 minutes.

7. Decant the supernatant solution into the round bottom flask from Step 4, combining the two extracts.

8. Transfer the solution from the round bottom flask (Step 7) into a 600-mL beaker, using 2 x 10 mL of deionized water to rinse the flask.

9. Adjust the combined solution to pH 3.5 with 5% sodium hydroxide or 5% hydrochloric acid (Note 1).

10. Transfer the mixture into a one-pint plastic centrifuge bottle, rinse the beaker with 2 x 10 mL of deionized water, add the rinses into the centrifuge bottle.

11. Balance the centrifuge bottle with deionized water, and centrifuge at 1500 rpm for 10 minutes.

12. Decant the supernatant into the 600-mL beaker and save this solution until Step 16.

13. Add 100 mL of methanol to the precipitate left in the centrifuge bottle, cap and shake vigorously for 30 seconds.

14. Balance the centrifuge bottle with methanol, and centrifuge at 1500 rpm for 10 minutes.

15. Decant the supernatant methanol solution into a 500-mL round bottom flask and evaporate to near dryness to remove the methanol.

16. Transfer the solution from the 600-mL beaker from Step 12 into the round bottom flask, swirl, and decant back into the 600-mL beaker.

17. Rinse the 500-mL round bottom flask into the 600-mL beaker using 2 x 10 mL of deionized water.

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J. Solid Phase Extraction Cleanup

1. Prepare an Analytichem Bond Elut C-18 cartridge as follows: add 5 mL of methanol to the cartridge and allow to stand for 2-3 minutes in the Vac-Elut Processing Station with the vacuum off. Then turn on the vacuum, pass the methanol through the cartridge and wash the column with 5 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.17 through the Analytichem C-18 cartridge using the Vac-Elut Processing Station at the rate of 2-3 drops per second (use 5 mm Hg of vacuum).

4. Wash the flask, reservoir and the C-18 cartridge with 2 x 10 mL of deionized water.

5. Remove the C-18 cartridge, the adapter and the reservoir from the Vac-Elut Processing Station.

6. Prepare an Analytichem Bond Elut SCX Cartridge as described in Step J.1.

7. Connect the C-18 cartridge onto the top of the Aromatic Sulfonic Acid cartridge (SCX) using an adapter.

8. Connect a 75-mL reservoir onto the top of the tandem cartridge system using an adapter. Add 50 mL of 50% methanol in water into the reservior and pass the solution using the Vac-Elut station through the tandem cartridge at the rate of 2 drops per second. Discard the solution.

9. Remove the syringe, the C-18 cartridge and the adapter from the SCX cartridge. Wash the remaining SCX cartridge with 5 mL of methanol.

10. Remove the SCX cartridge from the Vac-Elut system, and connect a 30 -mL. disposable syringe onto the top of SCX cartridge using an adaptor.

11. Add 10 mL of saturated potassium chloride-methanol into the syringe and using the plunger apply pressure to elute the residues off the SCX cartridge into a 100-mL round-bottom flask.

12. Evaporate the saturated potassium chloride-methanol eluate to dryness using a flash evaporator.

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K. Methylene Chloride Partitioning

1. Add 10 mL of pH 3.5 phosphate buffer solution to the 100-mL round- bottom flask, swirl and transfer to a 250-mL separatory funnel. Rinse the round-bottom flask with 100 mL of methylene chloride into the separatory funnel.

2. Partition the buffer solution with 2 x 100 mL of methylene chloride. Shake vigorously 30 seconds each time and allow the phases to separate. Draw off the lower layer into a 500-mL round-bottom flask each time (Note 2).

3. Evaporate the combined methylene chloride to dryness using a flash evaporator.

4. Repartition the buffer solution in the separatory funnel with two additional 100 mL portions of methylene chloride and draw off the lower layer each time into the 500-mL round bottom flask (Step K.2).

5. Evaporate the combined methylene chloride to dryness, using a flash evaporator.

6. Dissolve the residue in 8 mL of deionized water for HPLC analysis.

L. Liquid Chromatographic Analysis

1. After obtaining the proper chromatography and response, inject, in sequence, a 500-mcL aliquot of the working standard (0.1 mcg/mL), 500-mcL aliquots of two samples and another 500-mcL of the working standard.

2. If the sample peaks go off-scale, dilute to an appropriate volume with deionized water and reinject. The dilution factor (D.F.) is then included in the calculation.

3. Use the average combined peak heights of the standards bracketing the samples for the quantitation.

Note: If the chromatography deteriorates, change the guard column.

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M. Calculations

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

$$ppb = \frac{R(SAMP) \times (V1) \times (V3) \times C(STD) \times (V5) \times (D.F.)}{R(STD) \times (W) \times (V2) \times (V4)} \times 1{,}000$$

Where:

R(SAMP) = Peak height of sample in millimeters (Note 3).

R(STD) = Average peak height of working standard in millimeters (Note 3).

W = Weight of sample taken for analysis in grams.

V1 = Volume of extracting solvent in milliliters.

V2 = Volume of extract taken for analysis in milliliters.

V3 = Volume of water added to dissolve final residues for HPLC analysis in milliliters.

V4 = Volume of sample solution injected in microliters.

V5 = Volume of standard solution injected in microliters.

C(STD) = Concentration of standard solution in mcg/mL.

D.F. = Dilution Factor

Typical chromatograms for the determination of CL 222,293 and CL 263,840 are shown in Figures 1, 2 and 3.

Notes to Method M-1917

1. pH Adjustments (Step I.9): All pH adjustments should be monitored with a pH meter.

2. Drying of methylene chloride extracts is not necessary (Step K.3,5). Do not use sodium sulfate to dry these extracts or losses will occur.

3. Peak height for quantitation of CL 263,840 is a total of the peak heights of the meta and para isomers of CL 263,840.

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