M-1713.02
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Recommended Method of Analysis

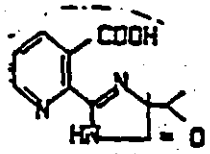
CL 243,997 imazapyr: HPLC Method for the Determination of CL 243,997 Residues in Soil

A. Principle

Residues of CL 243,997 are extracted from soil with 0.5M sodium hydroxide in water. After adjusting the pH to 2.0, further cleanup is achieved by using solid phase extraction cartridges. The solution is then partitioned with methylene chloride. The methylene chloride is evaporated to dryness and the residue is dissolved in water. Quantitation of CL 243,997 is accomplished by liquid chromatography using a UV detector (240 nm) and the external standard technique. The validated sensitivity of the method is 5 ppb.

B. Reagents

1. Analytical Standard: CL 243,997 [nicotinic acid, 2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl)-] analytical grade, known purity, obtained from American Cyanamid Company, Agricultural Research Division, P.O. Box 400, Princeton, New Jersey 08540.



NOTE: This method supercedes M-1713 for the analysis of soil. This newer method is essentially the same as the previous one except that a larger sized aromatic sulfonic acid SPE cartridge is now used. This was found to be necessary for soils with very low organic matter.

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2. Solvents, Specially Purified: B&J Brand High Purity Solvent, American Burdick and Jackson, or equivalent.

   a. Methanol
   b. Methylene Chloride
   c. Acetonitrile (UV grade)
   d. Hexane

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

   a. Acetic Acid, glacial
   b. Hydrochloric Acid, concentrated
   c. Sodium Hydroxide, pellets
   d. Potassium phosphate, dibasic

4. Solutions:

   a. Extraction Solvent, 0.5N Sodium Hydroxide in Water: Dissolve 80 g of sodium hydroxide pellets in 4 L of deionized water, and mix well.

   b. 50% Methanol in Water: Add 250 mL of methanol to 250 mL of deionized water and mix well.

   c. 6N Hydrochloric Acid: Add 250 mL of hydrochloric acid to 200 mL of deionized water in a 500-mL volumetric flask. Dilute to the mark with deionized water and mix well.

   d. pH 3.5 Phosphate Buffer: Dissolve 5 grams of potassium phosphate, dibasic, in 1 L of deionized water, adjust to pH 3.5 with 6N hydrochloric acid.

5. Solid Phase Cartridge Elution Solvent: Dissolve 50 g of potassium phosphate, dibasic, in 1 L of deionized water, and adjust to pH 6.5 with 6N hydrochloric acid.

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

7. Liquid Chromatographic Mobile Phase: Mix 700 mL of deionized water, 300 mL of methanol and 10 mL of acetic acid. Filter through a Rainin Nylon-66 (0.45 um) filter or equivalent.

C. Apparatus

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

2. Assorted Glassware: General laboratory.

3. Flash Evaporator: Buchler Instruments Model PF-10DN or equivalent equipped with a heated water bath maintained at approximately 35°C in which the evaporation flasks can be partially submerged.

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4. Microliter Syringe: 1-mL, Glenco, No. 19925-1.

5. Liquid Chromatograph:

   a. Pump: Kratos, Spectroflow Model 400
   b. Detector: Kratos, Spectroflow Model 783 UV detector
   c. Sample Injector: Reodyne valve, Model 7125, 500-mcL loop

6. Recorder: SP 4270 recording integrator, Spectra-Physics.

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

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

9. Adapters: Part #636001, Analytichem International.

10. Vac-Elut Processing Station or Equivalent: Cat. No. AI 6000, Analytichem International.

11. Filter Paper: Glass microfibre, Whatman 934-AH, 9-cm diameter.

12. Solid Phase Extraction Cartridges:

    a. Bond-Elut C18 Cartridge (1,000 mg): Catalog Number 607406, Analytichem International.

    b. Bond-Elut Aromatic Sulfonic Acid Cartridge (1,000 mg): Catalog Number 617406, Analytichem International.

13. Centrifuge: Damon Model C4-500, IEC Division, Needham, Massachusetts, with appropriate head for 500-mL bottles.

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

15. Centrifuge Bottles: 500-mL capacity, wide-mouth polypropylene, Catalog Number 05-562-24, Fisher Scientific Company.

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

17. Aquatest IV: Karl Fisher moisture titrator (or equivalent), Photovolt Corporation, Indianapolis, Indiana.

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

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D. Preparation of Standard Solution

1. Stock Solutions (Prepare Monthly)

Weigh accurately 10 ± 0.05 mg of CL 243,997 analytical standard into a 100-mL volumetric flask. Dilute to the mark with methanol and mix well. This standard solution contains 100 mcg/mL of CL 243,997.

2. Standard Fortification Solutions (Prepare Weekly)

a. Pipet a 7.5-mL aliquot of the standard stock solution prepared in D.1 into a 10-mL volumetric flask. Dilute to the mark with deionized water and mix well. This standard solution contains 75 mcg/mL of CL 243,997.

b. Pipet a 2.5-mL aliquot of the standard stock solution prepared in D.1 into a 10-mL volumetric flask. Dilute to the mark with deionized water and mix well. This standard solution contains 25 mcg/mL of CL 243,997.

c. Pipet a 1-mL aliquot of the standard solution prepared in D.2.b into a 10-mL volumetric flask. Dilute to the mark with deionized water and mix well. This standard solution contains 2.5 mcg/mL of CL 243,997.

d. Pipet a 1-mL aliquot of the standard stock solution prepared in D.1 into a 100-mL volumetric flask. Dilute to the mark with deionized water and mix well. This standard solution contains 1 mcg/mL of CL 243,997.

e. Pipet a 5-mL aliquot of the standard solution prepared in D.2.d into a 10-mL volumetric flask. Dilute to the mark with deionized water and mix well. This standard solution contains 0.5 mcg/mL of CL 243,997.

f. Pipet a 1-mL aliquot of the standard solution prepared in D.2.b into a 100-mL volumetric flask. Dilute to the mark with deionized water and mix well. This standard solution contains 0.25 mcg/mL of CL 243,997.

3. HPLC Standard Solutions

a. Pipet a 1-mL aliquot of the standard stock solution prepared in D.1 into a 100-mL volumetric flask. Dilute to the mark with deionized water and mix well. This standard solution contains 1.0 mcg/mL of CL 243,997.

b. Pipet 0.5, 1 and 2 mL of the 1.0 mcg/mL standard solution (D.3.a) into 10-mL volumetric flasks. Dilute to the mark with deionized water. These standard solutions contain 0.05, 0.1 and 0.2 mcg/mL of CL 243,997, respectively.

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Note: The 0.1 mcg/mL CL 243,997 standard should be prepared daily and is used as the working standard for quantitation while the others are used for the linearity check.

E. Liquid Chromatographic Conditions

1. Instrument

a. Pump: Kratos, Spectroflow Model 400.
b. Detector: Kratos, Spectroflow Model 783 UV detector

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

3. Instrument Conditions

a. Column Temperature:	Room temperature (approx. 23°C)
b. Mobile Phase:	Methanol: Water: Acetic Acid (30:70:1)
c. Flow Rate:	1.0 mL/min (approx. 1500 psi)
d. Detector Wavelength:	240 nm
e. Detector Range:	0.005 AFS
f. Loop Injector:	500 mcL
g. Recorder:	0.5 cm/min chart speed, 10 mV
h. Retention Time:	approximately 7 minutes

F. Linearity Check: (The chromatograph should be checked for linearity of response whenever a new column or instrument is used.)

1. Adjust the HPLC conditions to attain a peak height of 30-50% full-scale deflection for a 50-ng injection of CL 243,997.

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

3. Plot the height of 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 (Keep soil frozen at all times except while mixing and compositing)

1. Mix the soil thoroughly removing large stones and vegetation to obtain a homogeneous sample.

2. Prior to analysis the moisture content of the soil should be determined by using an Aquatest Karl Fisher titrator or by oven drying.

H. Determination of Moisture

Before using the Aquatest unit, it should be checked for accuracy using a standard water solution.

1. Inject three 50 mcL aliquots of methanol into the Aquatest unit to determine the water content of the methanol. For a good bottle of methanol, the water content should be less than 2 mcg/mcL.

2. Using a pan balance, weigh out 1.0 g of soil from a well-mixed sample onto a piece of weighing paper.

3. Transfer the sample to a liquid scintillation vial and add 10.0 mL of methanol.

4. Cap the vial and shake vigorously for 1 minute.

5. Allow the sample to settle for at least 10 minutes.

6. Inject duplicate 50-mcL aliquots of the methanol extract into the Aquatest unit. If duplicate values are not within ± 10% of each other make two more injections.

7. Inject smaller aliquots if the titration time is greater than two minutes.

8. Calculate the percent water in the sample by using the following equation.

$$\% \text{ water} = [\text{Average mcg}_s - \text{Average mcg}_b] \times 0.02 \times \frac{50}{A}$$

Where:

Average mcg_s = Average micrograms titrated for sample.

Average mcg_b = Average micrograms titrated for methanol blank (same volume of sample).

A = Microliters of sample injected.

9. If an Aquatest unit is not available, dry a 50-g subsample of soil in a hood or oven to constant weight to determine the moisture content.

I. 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 daily set of samples analyzed.

1. Weigh a 50-g subsample of control soil into a centrifuge bottle (500-mL capacity).

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

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

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

J. Extraction of Soil

1. Weigh accurately 50 g of soil into a 500-mL centrifuge bottle, add 350 mL of 0.5N sodium hydroxide in water and cap the bottle tightly with the lid.

2. Shake for 30 minutes on a reciprocal shaker.

3. Uncap the lid, balance the centrifuge bottles with deionized water and centrifuge at 1500 rpm for 10 minutes.

4. Decant the solution into a 500-mL graduated, mixing cylinder. Add 50 mL of extracting solution to the centrifuge bottle. Resuspend the solids using a metal spatula.

5. Balance the bottles with deionized water and centrifuge the resuspended soil sample at 1500 rpm for 5 minutes.

6. Combine the wash solution with the first extract in the 500-mL mixing cylinder, add enough extraction solution to the cylinder so that the total volume is 400 mL. Cap the cylinder with the stopper and mix thoroughly.

7. Measure 160 mL of the extract and transfer to a 400-mL beaker. Adjust the solution to pH 2.0 using 6N hydrochloric acid and a pH meter.

8. Vacuum filter the pH 2.0 solution through a Buchner funnel fitted with 2 layers of glass fiber filter paper to remove the humic acid.

K. Solid Phase Extraction Cleanup

1. Prepare a Bond-Elut C18 cartridge using an Analytichem Vac-Elut processing station by washing the cartridge with 5 mL of methanol and 5 mL of water.

2. Connect a 75-mL reservoir in which a pledget of glass wool has been placed onto the top of the C18 cartridge using an adapter. Pull the solution from step J.8 through the C-18 cartridge (rate of 2 drops per second) and add the solution into the reservoir until all the solution is added.

3. Remove the reservoir and the adapter and wash the remaining C18 cartridge by adding deionized water to the top of the cartridge (about 5 mL). Pull the water through the C18 cartridge and discard this wash.

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4. Prepare a Bond-Elut Aromatic Sulfonic Acid cartridge by washing the cartridge with 5 mL of hexane, 5 mL of methanol and 5 mL of deionized water.

5. Connect the C18 cartridge onto the top of the Aromatic Sulfonic Acid cartridge using an adapter.

6. Connect a 30-mL disposable syringe onto the the top of the tandem cartridge system using an adapter. Add 20 mL of 50% methanol in water into the syringe and push the solution through the tandem cartridge system (rate of 1 drop per second).

7. Remove the top part (the 30-mL syringe and the C18 cartridge) and wash the remaining Aromatic Sulfonic Acid cartridge with 5 mL of methanol, 5 mL of deionized water and 5 mL of pH 3.5 phosphate buffer solution.

8. Elute the CL 243,997 from the Aromatic Sulfonic Acid cartridge with 10 mL of pH 6.5 elution solvent (B.5) into a 25-mL beaker.

L. Partitioning Cleanup

1. Adjust the pH of the solution from step K.8 to 2.0 using 6N hydrochloric acid and a pH meter.

2. Pour the pH 2 solution into a 125-mL separatory funnel. Partition the aqueous phase with 2 x 20 mL of methylene chloride, shaking for 30 seconds each time.

3. Combine the lower methylene chloride layers in a 100-mL pear-shaped flask and evaporate to dryness.

4. Dissolve the residue in 2 mL of water. (If necessary, sample may be left overnight and injected the next day.)

M. Liquid Chromatographic Analysis

1. After obtaining the proper chromatography and response, inject, in sequence, a 500-mcL aliquot of the CL 243,997 working standard (0.1 mcg/mL), 500-mcL aliquots of two samples and another 500 mcL of the working standard.

2. If a sample peak goes off-scale, dilute an aliquot to an appropriate volume with deionized water and reinject. The dilution factor (D.F.) is the included in the calculations (see Section N).

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

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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:

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

Where:

R(SAMP) = Peak height of sample.

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

W = Weight of sample taken for analysis in grams on a dry basis*.

V1 = Volume in mL of extracting solvent (400 mL).

V2 = Volume in mL of extract taken for analysis (160 mL).

V3 = Volume in mL of final solution used for HPLC analysis.

V4 = Volume in mcL of sample solution injected.

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

V5 = Volume in mcL of standard solution injected.

D.F. = Dilution factor.

Figures M-1713.02.A and M-1713.02.B show typical chromatograms for the analysis of CL 243,997 residues in soil.

*To determine the dry weight of soil used for analysis, calculate as follows:

$$\text{Corrected Sample Weight (W)} = \text{Initial Sample Weight (50g)} \times \frac{100 - \%\ \text{moisture}}{100}$$

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