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Date

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AGRICULTURAL RESEARCH DIVISION
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RECOMMENDED METHOD OF ANALYSIS

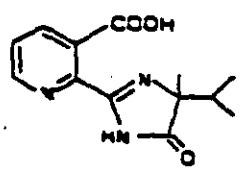
Imazapyr (CL 243,997): HPLC Method for the Determination of CL 243,997 Residues in Water

A. Principle

Residues of CL 243,997 are extracted from water by using a C18 solid phase extraction (SPE) cartridge. Additional cleanup and specificity are achieved by passing through an aromatic sulfonic acid SPE column. The eluant is then partitioned with methylene chloride. The methylene chloride is evaporated to dryness and the residue 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 1.0 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, P.O. Box 400, Princeton, New Jersey 08540.



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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 Chloride

   d. Potassium Phosphate, dibasic

4. Solutions:

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

   b. 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.

   c. pH 2.0 Deionized Water: Adjust the pH of 1 L of deionized water to 2.0 with 6N hydrochloric acid and a pH meter.

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 and a pH meter.

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

7. Liquid Chromatographic Mobile Phase: Mix 45 mL of acetonitrile, 60 mL methanol, 390 mL of deionized water and 5 mL of acetic acid. Filter through a Rainin Nylon-66 (0.45 um) filter or equivalent.

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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.

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 Incorporated, Cat. No. 5-8347.

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

9. Adapters: Part No. 636001, Analytichem International.

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

11. Solid Phase Extraction Cartridges:

    a. Bond-Elut C18 Cartridge (1,000 mg): Cat. No. 607406, Analytichem International.

    b. Bond-Elut Aromatic Sulfonic Acid Cartridge (1,000 mg): Cat. No. 617406, Analytichem International.

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

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13. Reservoir: 75-mL capacity, Cat. No. 607500, Analytichem International.

14. Filter Paper: Glass microfibre filters, 9.0 cm diameter: Cat. No. 1827-090, Whatman International Limited.

15. Funnel, Buchner: Coors porcelain funnels, 10 cm plate diameter: Cat. No. 10-356D, Fisher Scientific.

D. Preparation of Standard Solutions (Keep in Dark Bottle and Refrigerated)

1. Stock Solution: (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 25-mL aliquot of the standard stock solution prepared in D.1 into a 50-mL volumetric flask. Dilute to the mark with deionized water and mix well. This standard solution contains 50 mcg/mL of CL 243,997.

b. Pipet a 10-mL aliquot of the standard stock solution prepared in D.2.a into a 50-mL volumetric flask. Dilute to the mark with deionized water and mix well. This standard solution contains 10 mcg/mL of CL 243,997.

c. Pipet a 5-mL aliquot of the standard solution prepared in D.2.b into a 50-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.

d. Pipet a 5-mL aliquot of the standard stock solution prepared in D.2.c into a 50-mL volumetric flask. Dilute to the mark with deionized water and mix well. This standard solution contains 0.1 mcg/mL of CL 243,997.

3. HPLC Standard Solutions: (Prepare the Day of Use)

Pipet 0.25-, 0.5- and 1-mL aliquots of the 1.0 mcg/mL standard solution D.2.c into a 10-mL volumetric flask. Dilute to the mark with deionized water. These standard solutions contain 0.025, 0.05 and 0.1 mcg/mL of CL 243,997.

Note: The 0.05 mcg/mL CL 243,997 standard is used as a working standard for quantitation while the others are used for the linearity check.

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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, Incorporated, Cat. No. 5-8347.

3. Instrument Conditions:

a.	Column Temperature:	Room Temperature (approx. 23°C)
b.	Mobile Phase:	Acetonitrile:Methanol:Water:Acetic Acid (9:12:78:1)
c.	Flow Rate:	1.00 mL/min (approx. 900 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 7.5 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 25-ng injection of CL 243,997.

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

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.

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G. 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. Measure a 100-mL subsample of control water into a 150-mL beaker.
2. Add by pipet a volume of standard fortification solution appropriate to the fortification level to be tested.
3. Continue with the extraction and cleanup steps as described in the following sections.

H. Solid Phase Extraction and Cleanup

1. Mix the water sample well and filter an aliquot through glass-fibre filter paper using a Buchner funnel and vacuum.
2. Transfer 100 mL of water into a 150-mL beaker and adjust the solution to pH 2.0 using 6N hydrochloric acid and a pH meter.
3. Add 20 g of sodium chloride to the solution and stir until dissolved.
4. 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.
5. Connect a 75-mL reservoir onto the top of the C18 cartridge using an adapter. Using vacuum, pull the sample from step H.3 through the C18 cartridge (rate of 5-10 mL per minute) and continue adding into the reservoir until all the sample is added.
6. When the water level reaches the top of the packing in the C18 cartridge, remove the reservoir and the adapter and wash the cartridge by adding pH 2.0 water to the top of the cartridge (about 5 mL). Pull the water through the C18 cartridge and discard this wash. Repeat this wash two more times to remove all salt.
7. 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.
8. Connect the C18 cartridge onto the top of the Aromatic Sulfonic Acid cartridge using an adapter.

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

10. Remove the 30-mL syringe and discard the C18 cartridge. Connect only a 30-mL syringe onto the top of the Aromatic Sulfonic Acid cartridge using an adapter. Elute the CL 243,997 from the Aromatic Sulfonic Acid cartridge with 20 mL of pH 6.5 elution solvent (B.5) into a 25-mL beaker, using the syringe plunger to apply pressure.

I. Partitioning Cleanup

1. Adjust the pH of the solution from step H.10 to 2.0 using 6N hydrochloric acid and a pH meter.

2. Pour the pH 2 solution into a 250-mL separatory funnel. Partition the aqueous phase with 50 mL of methylene chloride, shaking for 30 seconds. Repeat the partitioning with another 50 mL of methylene chloride and evaporate the combined methylene chloride phases in a 250-mL flask.

3. Dissolve the residue in 4.0 mL of deionized water. (If necessary, the sample may be left overnight and injected the next day.)

J. 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 (0.05 mcg/mL), 500-mcL aliquots of two samples and another 500-mcL of 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 then included in the calculations (See Section K).

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

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K. 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 = Volume of sample taken for analysis in mL.

V1 = Volume in mL of extracting solvent (use 1 for calculations).

V2 = Volume in mL of extract taken for analysis (use 1 for calculations).

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.

DF = Dilution Factor

Figure 1 shows typical chromatograms for the analysis of CL 243,997 in water.

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