M-1854
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Recommended Method of Analysis

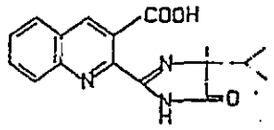
CL 252,214 (imazaquin): HPLC Method for the Determination of CL 252,214 Residues in Soil (5 ppb sensitivity)

A. Principle

Residues of CL 252,214 are extracted from soil with 0.5N sodium hydroxide in 30% methanol-water. Cleanup is achieved using solid phase extraction cartridges followed by solvent partitioning. Quantitation of CL 252,214 is accomplished by liquid chromatography with an instrument equipped with a UV detector (240 nm). Results are calculated by the direct comparisor of peak heights to those of external CL 252,214 standards. The validated sensitivity of the method is 5 ppb.

B. Reagents

1. Analytical Standard: CL 252,214 [3-quinolinecarboxylic acid, 2-[4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl)-] analytical grade, known purity, American Cyanamid Company, Agricultural Research Division, P.O. Box 400, Princeton, New Jersey 08540.



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

   a. Methylene Chloride

   b. Methanol

   c. Hexane

NOTE: This Method supercedes M-1631. It was developed to provide a more rapid method for analyzing soil samples.

3. Chemicals: Reagent Grade, J. T. Baker Chemical Company.

   a. Hydrochloric acid, concentrated

   b. Sodium hydroxide, pellets

   c. Potassium phosphate, dibasic

   d. Acetic acid, glacial

4. Solutions

   a. Extraction Solvent, 0.5N Sodium Hydroxide in 30% Methanol-Water: Dissolve 80 g of sodium hydroxide pellets in 2,800 mL of deionized water, add 1,200 mL of methanol, and mix well.

   b. 1N Hydrochloric Acid: Add 83 mL of concentrated hydrochloric acid to 700 mL of deionized water in a 1-L volumetric flask. Dilute to the mark with 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.

5. pH 6.5 Phosphate Buffer: Dissolve 50 g of potassium phosphate, dibasic, in 900 mL of deionized water in a 1-L volumetric flask and adjust to pH 6.5 with 6N hydrochloric acid. Dilute to the mark with deionized water and mix well.

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

7. Liquid Chromatographic Mobile Phase: Mix 500 mL of deionized water, 500 mL of methanol and 40 mL of acetic acid. Filter the mobile phase through a Rainin Nylon-66 (0.45 um) filter or equivalent.

8. Celite 545 AW: Johns Manville Company.

C. Apparatus

1. Liquid Chromatograph:

   a. Pump: Kratos Spectroflow 400 or equivalent.

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

   c. Sample Loop: 500-mcL capacity.

   d. Injector: Rheodyne Model 7125.

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

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

4. Assorted Glassware: General laboratory.

5. Microliter Syringe: 1 mL, B-D Yale Tuberculin Syringe (Becton - Dickinson Cat. No. 2004) equipped with Hamilton Needles (Supelco Cat. No. 2-1744).

6. Recording Integrator: Spectra-Physics 4290 or equivalent.

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

8. HPLC Guard Column: Supelguard Kit with 2 cm x 4.6 mm Supelcosil LC-8-DB cartridge, Cat. No. 5-9553.

9. Plastic Syringes, Disposable: Luer-Lok 30-mL capacity, Becton - Dickinson.

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

11. Solid Phase Extraction Cartridges:

    a. Analytichem Bond Elut Octadecyl (C-18) Cartridge (1000 mg): Cat. No. 607406, Analytichem International.

    b. Analytichem Bond Elut Benzenesulfonic Acid (SCX) Cartridge (500 mg): Cat. No. 617303, Analytichem International.

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

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

14. Flash Evaporator: Buchler Instruments, equipped with a heated water bath (approximately 35° - 40°C) in which evaporation flasks can be partially submerged.

15. Weighing Boat: Size 100-mL, Cat. No. B2045-10, American Scientific Products.

16. Bond Elut Reservoir: 75-mL capacity, Cat. No. 607500, Analytichem International.

17. Bond Elut Adaptor: Cat. No. 636001, Analytichem International.

18. Vac Elut Processing Station: Cat. No. AI6000 or equivalent.

D. Preparation of Standard Solutions (Prepare monthly - Store under refrigeration in amber bottles)

1. Stock Solution

   Weigh accurately (approximately 10 mg) of CL 252,214 into a 100-mL volumetric flask. Dilute to the mark with methanol and mix well. Record the accurate concentration of CL 252,214 in this solution.

2. Standard CL 252,214 Fortification Solutions

   a. Pipet an appropriate aliquot of the CL 252,214 standard solution prepared in D.1 to deliver 1,000 mcg of CL 252,214 into a 100-mL volumetric flask. This solution will contain 10 mcg/mL of CL 252,214 when diluted to the mark with deionized water and mixed well.

   b. Pipet a 10-mL aliquot of the standard prepared in D.2.a into a 100-mL flask. Dilute to the mark with deionized water and mix well. This solution contains 1 mcg/mL CL 252,214.

c. Pipet a 10-mL aliquot of the standard prepared in D.2.b into a 100-mL flask. Dilute to the mark with deionized water and mix well. This solution contains 0.1 mcg/mL of CL 252,214.

3. Standard Working Solutions

Pipet 2-, 5-, and 10-mL aliquots of the 1 mcg/mL CL 252,214 standard fortification solution prepared in D.2.b into separate 100-mL volumetric flasks. Dilute to the mark with pH 6.5 phosphate buffer and mix well. These solutions contain 0.02, 0.05, and 0.1 mcg/mL CL 252,214, respectively. These solutions are used for the linearity check (see section F).

NOTE: The working standard contains 0.05 mcg/mL of CL 252,214 and should be prepared daily.

E. Liquid Chromatographic Conditions

1. Instrument:

a. Pump: Kratos Spectroflow 400 or equivalent.

b. Detector: Kratos Spectroflow 783 UV detector.

c. Sample Loop: 500-mcL capacity.

d. Injector: Rheodyne Model 7125.

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

3. Guard Column: Supelcosil LC-8-DB Cartridge, 2 cm x 4.6 mm ID.

4. Instrument Conditions

a.	Flow Rate	0.75 mL/min (80 BAR)
b.	Wavelength	240 nm
c.	Range (detector)	0.001 AFS
d.	Loop Injector	500 mcL
e.	Mobile Phase	Water:Methanol:Acetic Acid (50:50:4)
f.	Integrator	0.5 cm/min chart speed, 10 mV, Attenuation x 64
g.	Retention Time	Approximately 7 minutes

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 a peak height of approximately 30% full-scale deflection for a 25-ng injection of CL 252,214.

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

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. Before compositing, allow frozen soil samples to thaw slightly in an air tight container to prevent loss of moisture.

2. Mix the thawed soil thoroughly removing large stones and vegetation to obtain a homogeneous sample. Samples should then be kept frozen until 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 daily set of samples analyzed.

1. Weigh a subsample of control into a 32-oz, narrow-mouth bottle.

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 the extraction solvent.

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

I. Analysis of Soil

1. Weigh accurately 50 g of soil into a 32-oz, narrow-mouth bottle.

2. Shake the soil with 150 mL of 0.5N sodium hydroxide (30% methanol-70% water) for one hour on a reciprocating shaker.

3. Add 15 g of Celite and filter the mixture through a single layer of Whatman 934-AH paper fitted onto a 9-cm Buchner funnel.

4. Wash the bottle and the filter cake with 50 mL of extraction solution.

5. Dilute the combined extract and wash to 200 mL with extraction solvent and mix well.

6. Adjust the pH of an 80-m aliquot (equivalent to 20 g of soil) to pH 2 with 6N hydrochloric acid using a pH meter to monitor the pH change.

7. Add 20 g of sodium chloride to the pH 2 solution and mix thoroughly.

8. Prepare an Analytichem C-18 cartridge by washing with 3 mL of methanol followed by 3 mL of water.

9. Prepare an Analytichem SCX cartridge by washing the cartridge with 3 mL of hexane, 3 mL of methanol and 3 mL of water.

10. Connect a 75-mL reservoir (containing a pledget of glass wool on the bottom) onto the top of a C-18 cartridge and decant the solution from step 7 into the reservoir. Vacuum filter the solution through the C-18 cartridge using an Analytichem Vac-Elut processing station at the rate of approximately 3 drops per second.

11. Disconnect the reservoir and adapter from the C-18 cartridge and wash the cartridge twice by filling it with deionized water and vacuum filtering.

12. Connect the C-18 cartridge onto the top of the SCX cartridge and fit a 30-mL disposable syringe onto the top of the C-18 cartridge.

13. Vacuum filter 20 mL of a 50-50 methanol-water solution through the tandem cartridge system at a rate of approximately 2 drops per second, discarding the eluate.

14. Disconnect the C-18 cartridge from the SCX cartridge and elute the CL 252,214 from the SCX cartridge with 20 mL of pH 6.5 phosphate buffer directly into a 250-mL separatory funnel.

15. Add 5 mL of 1N hydrochloric acid to the separatory funnel, check the pH with a pH meter to make sure the pH is pH 2 $\pm$ 0.2 and partition the solution with 2 x 25 mL of methylene chloride, shaking for 15 seconds each time.

16. Evaporate the combined methylene chloride extracts to dryness in a 250-mL round bottom flask using a flash evaporator. Dissolve the residue in 4.0 mL of pH phosphate buffer in preparation for liquid chromatographic analysis. (Samples may be left overnight before analysis.)

J. Liquid Chromatographic Analysis

1. After obtaining the proper chromatography and response, inject, in sequence, a 500-mcL aliquot of the CL 252,214 working standard (0.05 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 the pH 6.5 phosphate buffer 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.

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 = Weight of sample taken for analysis in grams.

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

V2 = Aliquot of extract taken for analysis in milliliters.

V3 = Volume of pH 6.5 phosphate buffer used to dissolve the final residue (in milliliters).

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 if necessary.

Figure M-1854.A shows typical chromatograms for determining CL 252,214 residues in soil.