Imazapic (CL 263,222): LC/MS Method for the Determination of CL 263,222 Residues in Water.

A. Principle

Residues of CL 263,222 are extracted from water using solid phase extraction. Measurement of the CL 263,222 is accomplished by liquid chromatography/electrospray ionization mass spectrometry (LC/ESMS). Results are calculated as CL 263,222 by the direct comparison of peak areas to those of external standards. The validated sensitivity (LOQ, Limit of Quantitation) of the method is 0.05 ppb.

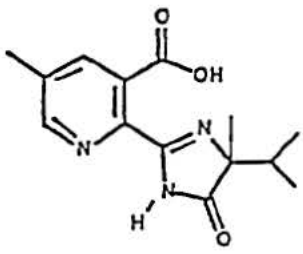
B. Reagents

(Items from manufacturers other than those listed may be used provided they are functionally equivalent.)

Precautions are to be taken to restrict exposure to all chemicals specified in this method. The proper use of appropriate safety glasses/goggles, gloves, laboratory coat, ventilation, and handling techniques are to be observed. Appropriate Material Safety Data Sheets (MSDS) are to be reviewed for all chemicals used in the method prior to starting work. The current MSDS for CL 263499 is obtainable from American Cyanamid Company, Agricultural Products Research Division, P.O. Box 400, Princeton, New Jersey 08543-0400.

1. Analytical Standard: Analytical grade, known purity, American Cyanamid Company, Agricultural Products Research Division, P.O. Box 400, Princeton, New Jersey 08543-0400.

CL 263,222: [nicotinic acid, 5-methyl-2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl)]



M.W.=275

NOTE: This method extends the LOQ of Method M 2669 to 0.05 ppb. See Section L for changes to the method.

2. Water, Deionized: Water passed through a Millipore Milli-Q Plus Ultra Pure Water System. Use this water for all steps.

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

   a. Methanol
   b. Methylene Chloride
   c. Hexane (UV Grade)
   d. Acetone

4. Chemicals:

   a. Hydrochloric Acid, Concentrated, "Baker Analyzed" Reagent
   b. Glacial Acetic Acid, "Baker Analyzed" Reagent

5. Solutions:

   a. 1N Hydrochloric Acid: Add 83 mL of concentrated hydrochloric acid (B.4.a) to 500 mL of Milli-Q water in a 1-liter volumetric flask and dilute to 1 liter with Milli-Q water. Mix well.

   b. 0.1N Hydrochloric Acid: Dilute 100 mL of reagent solution B.5.a. to 1 liter with Milli-Q water. Mix well.

   c. 0.01N Hydrochloric Acid: Dilute 100 mL of reagent solution B.5.b. to 1 liter with Milli-Q water. Mix well.

   d. 1% Acetic Acid in Water: Pipet 10 mL of glacial acetic acid (B.4.b) into 500 mL of Milli-Q water in a 1-liter volumetric flask and dilute to 1 liter with Milli-Q water. Mix well.

   e. 1% Acetic Acid in Methanol: Pipet 10 mL of glacial acetic acid (B.4.b) into 500 mL of methanol (B.3.a) in a 1-liter volumetric flask and dilute to 1 liter with methanol. Mix well.

C. Apparatus

(Items from other manufacturers may be used provided they are functionally equivalent.)

1. Liquid Chromatograph: Two (2) Shimadzu Model LC-10AD pumps controlled by a Shimadzu SCL-10A system controller.

2. LC Column: 5-cm x 4.6-mm ID TSK-GEL Super-ODS, Cat. No. 18154, TosoHaas, Montgomeryville, PA.

3. LC Injector: Rheodyne Model 7725 fitted with a 100-µL loop. (See Note 1.)

4. Mixing Tee: Cat. No. P-728, Upchurch.

5. Low-volume Static Mixer: Cat. No. CMA0110113T, Lee Scientific.

6. Mass Spectrometer: Finnigan MAT TSQ700.

7. LC/MS Interface: Finnigan MAT Atmospheric Pressure Ionization (API) System.

8. Balance, Analytical: Sartorius, Model R200D, precision ± 0.05 mg.

9. Disposable Serological Pipets: 10 mL in 1/10 mL, Falcon Model 7551, Becton-Dickinson.

10. Disposable Scintillation Vials: 20 mL, Fisher Scientific.

11. Assorted Glassware: General laboratory.

12. Sample Concentrator: Dri-Block Model DB-3D, Techne (Cambridge) Ltd, Duxford Cambridge U.K.

13. Vacuum Processing Station: IST VacMaster, Cat. No. 121-2016, equipped with PTFE stopcock/needle system, Cat. No.121-0009, International Sorbent Technology, Mid Glamorgan, U.K., Distributed by Jones Chromatography, Lakewood, CO.

14. Solid Phase Extraction Cartridge: Varian Bond Elut C18 cartridge, 200 mg, 3-mL capacity tube, Cat. No. 1210-2025, Varian, Harbor City, CA.

15. Reservoir with Frits: 15 mL, Cat. No. 1213-1016, Varian.

16. Adapter: Cat. No. 120-1101, International Sorbent Technology.

D. Preparation of Standard Solutions

1. Stock Solution (Prepare monthly, store in amber bottles in refrigerator)

Weigh accurately a known amount (approximately 100 mg) of CL 263,222 standard and transfer to a 100-mL volumetric flask. Dilute to the mark with methanol and mix well. Calculate and record the exact concentration of CL 263,222, correcting for the standard purity.

2. Standard Fortification Solutions (Prepare monthly, store in amber bottles in refrigerator)

a. Pipet into a 100-mL volumetric flask an appropriate amount of the stock solution from D.1 to deliver 1000 mcg of CL 263,222. Evaporate the methanol under a stream of nitrogen. Dissolve the residue in Milli-Q water and dilute to the mark with Milli-Q water. Mix well. This Standard Fortification Solution contains 10 mcg/mL of CL 263,222.

b. Pipet into a 100-mL volumetric flask a 10-mL aliquot of Standard Fortification Solution D.2.a. Dilute to the mark with Milli-Q water and mix well. This Standard Fortification Solution contains 1.0 mcg/mL of CL 263,222.

c. Pipet into a 100-mL volumetric flask a 10-mL aliquot of Standard Fortification Solution D.2.b. Dilute to the mark with Milli-Q water and mix well. This Standard Fortification Solution contains 0.1 mcg/mL of CL 263,222.

3. Standard Liquid Chromatographic (LC) Solutions (Prepare monthly, store in amber bottles in refrigerator)

a. Pipet into a 100-mL volumetric flask a 10-mL aliquot of Standard Fortification Solution D.2.c. Dilute to the mark with Milli-Q water and mix well. This LC

Standard Solution contains 0.01 mcg/mL of CL 263,222.

b. Pipet into a 100-mL volumetric flask a 10-mL aliquot of the 0.01 mcg/mL Standard LC Solution D.3.a. Dilute to the mark with Milli-Q water and mix well. This Standard LC Solution contains 0.001 mcg/mL of CL 263,222 and is the working standard for LC/ESMS analysis.

c. Pipet into a 100-mL volumetric flask a 5-mL aliquot of the 0.01 mcg/mL Standard LC Solution D.3.a. Dilute to the mark with Milli-Q water and mix well. This Standard LC Solution contains 0.0005 mcg/mL of CL 263,222.

d. Pipet into a 100-mL volumetric flask a 2.5-mL aliquot of the 0.01 mcg/mL Standard LC Solution D.3.a. Dilute to the mark with Milli-Q water and mix well. This Standard LC Solution contains 0.00025 mcg/mL of CL 263222.

E. Liquid Chromatographic / Mass Spectrometric Conditions

Operating conditions described below are provided for use as a guide in establishing actual operating conditions and should be adjusted as necessary to obtain peak shapes and resolution from background peaks equivalent to or better than those shown in this method.

1. Liquid Chromatograph: Two (2) Shimadzu Model LC-10AD pumps controlled by a Shimadzu SCL-10A system controller.

2. Column: 5-cm x 4.6-mm ID TSK-GEL Super-ODS, Cat. No. 18154, TosoHaas, Montgomeryville, PA.

3. LC Solutions:

   a. Aqueous: 1% acetic acid in water (B.5.d).
   b. Organic: 1% acetic acid in methanol (B.5.e).

4. LC/ESMS Conditions

a.	LC Column Temperature:	Ambient
b.	LC Flow Rate:	0.5 mL/min.
c.	LC Gradient:	10% organic to 90% organic over 8 min.; hold at 90% organic for 1 min.; reset to 10% organic over 1 min.
d.	Injection Volume:	100 µL
e.	Electrospray Voltage:	5 kV
f.	Capillary Temperature:	300°C
g.	Capillary Voltage:	30 V
h.	Tube Lens Voltage:	70 V
i.	Octapole Offset:	-2.0 V
j.	Nitrogen Sheath Gas:	80 psi
k.	Auxiliary Gas Rotometer:	30
l.	MS Mode of Operation:	Q1MS
m.	Conversion Dynode Voltage:	-15 kV
n.	Electron Multiplier Voltage:	1350 V
o.	Preamplifier Gain:	1 E-08 amps/volt
p.	Scan Rate:	0.5 sec./scan
q.	Scan Range (Full Scan):	m/z 250 to 350
r.	Scan Range (SIM):	m/z 276 (nominal) ± 0.2 dalton

s. Retention Time of Analyte: approx. 5.0 min.

F. Linearity Check

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

1. Inject 100-mcL aliquots of Standard LC Solutions D.3.a, D.3.b, D.3.c and D.3.d.

2. For each standard, determine the response factor (ratio) by dividing the peak response by the amount (picograms) injected. Calculate the average response ratio. A deviation by any standard response factor by more than 20% from the average factor indicates instrumental difficulties or incorrect standard preparations which should be corrected before proceeding.

3. Linearity checks should be performed weekly during the analysis of samples from every field residue study and when the chromatographic system has been serviced.

G. Sample Preparation

1. Allow the frozen or refrigerated water samples to thaw completely in an air-tight container just prior to extraction.

2. Thoroughly mix each thawed sample to obtain a homogeneous sample.

H. Recovery Test

The validity of the procedure should always be demonstrated by recovery tests before analysis of unknown samples is attempted. As a quality control measure, at least one concurrent recovery should be run with each set of samples.

1. By pipet (C.9), place 10 mL of control water in the empty reservoir (step I.2 below).

2. By pipet, add a volume of the Standard Fortification Solution to the reservoir appropriate to the fortification level to be tested.

3. Continue with the cleanup steps in I.2 with the addition of the 0.1 N HCl to the water in the reservoir.

I. Solid Phase Extraction Cleanup

1. Prepare a Varian Bond Elut C18 cartridge (C.14) using an IST VacMaster vacuum processing station (C.13) by washing the cartridge with 1 column volume (approx. 3 mL) of each of the following solvents in the exact order listed. Allow the liquid level to drain just below the top of the frit above the sorbent bed after each wash. Leave a shallow layer of 0.01N HCl over the top frit of the cartridge. The washes should pass through the sorbent easily by gravity. Use vacuum only if necessary.

Wash Solvents: hexane, methylene chloride, methanol, Milli-Q water then 0.01 N HCl (Reagent solution B.5.c).

Close the stopcock on the vacuum processing station after the final wash.

2. Fill the cartridge barrel approximately halfway with 0.01 N HCl. Attach a 15-mL reservoir

(C.15) to the cartridge using an adapter (C.16). By pipet (C.9), transfer 10 mL of the water sample to the reservoir. By pipet, add 1 mL of 0.1 N HCl (B.5.b) to the water in the reservoir.

3. Open the stopcock on the vacuum processing station and load the water onto the sorbent bed at a rate of 1 to 2 drops per second. The extract should load satisfactorily with only a slight vacuum (< -1 in. Hg). Close the stopcock when the liquid level passes just below the top of the frit above the sorbent bed. Discard the eluate and remove the reservoir and adapter from the cartridge.

4. Wash the cartridge with 1 column volume of Milli-Q water followed by 1 column volume of hexane at the rate of approximately 1 drop per second. A vacuum of approximately -3 to -4 in. Hg will need to be applied to pull the hexane through following the water wash. Close the stopcock when the hexane wash passes just below the top of the frit above the sorbent bed.

5. Using vacuum (approximately -3 to -4 in. Hg) elute the cartridge directly into a clean 20-mL disposable scintillation vial (C.10) with 1 column volume of methylene chloride at a rate of approximately 1 to 2 drops per second.

6. Place the vial on the sample concentrator (C.12) at low heat (approximately 60°C) and carefully evaporate the methylene chloride under a stream of nitrogen. If any water droplets remain after evaporation of the methylene chloride, a small amount (approximately 1 mL) of UV grade methanol (B.3.a) may be added to help evaporate the water.

7. Dissolve the residue in 1 mL of Milli-Q water for analysis.

J. LC/ESMS Analysis

1. Using the parameters detailed in Section E, a 1000-pg on-column injection of CL 263,222 (100 mcL of Standard LC Solution D.3.a) should give a background-subtracted mass spectrum similar to that shown in Figure 1. Determine the mass centroid of the ion at m/z 276 and set the mass spectrometer for selected ion monitoring of this ion with a ±0.2 dalton scan window and dwell time of 500 msec/ion (0.5 sec/scan).

2. Inject 100-mcL aliquots of the working standard (0.001 mcg/mL, Standard LC Solution D.3.b) until a reasonably constant response is obtained (Figure 2).

3. Establish the linearity of the responses following the procedure described in Section F.

4. An injection of the working standard is to be made after at least every four sample injections.

5. The peak response variation between two adjacent bracketing standards must not exceed 20%. If the variation exceeds 20%, instrumental parameters should be adjusted to restore adequate sensitivity. If such adjustments are made, inject duplicate aliquots of the working standard to determine the new response values of the standard. Then, the samples bracketed when this >20% variation occurred must be reinjected.

6. If an analyte response exceeds the linear range, dilute an appropriate volume of the sample with additional Milli-Q water (B.2) in a separate 20-mL scintillation vial.

K. Calculations

For each sample calculation, use the sample peak area and the average peak area of the external standards injected before and after the sample injections as follows:

$$ppb = \frac{R(SAMP) \times (V1) \times (V3) \times C(STD) \times (V5) \times D.F.}{R\ (STD) \times W \times (V2) \times (V4)} \times 1000$$

$$\%\ Recovery = \frac{ppb\ Found \times 100}{FV \times FC \times 1000 / W} = \frac{ppb\ Found}{ppb\ Added} \times 100$$

Where:

R(SAMP) = Peak area of sample

R(STD) = Average peak area of bracketing standards

W = Weight of sample taken for analysis in grams (10 g)

V1 = Volume of extraction solvent in milliliters (10 mL)

V2 = Aliquot of extract taken for analysis in milliliters (10 mL)

V3 = Final volume of sample solution for LC/MS analysis in milliliters (1 mL)

V4 = Volume of sample solution injected in microliters (100 mcL)

V5 = Volume of working standard solution injected in microliters (100 mcL)

C(STD) = Concentration of working standard solution injected in micrograms per milliliter (0.001 mcg/mL)

D.F. = Dilution factor

FV = Fortification volume in milliliters

FC = Fortification concentration (of standard solution added) in mcg/mL

Typical chromatograms from LC/ESMS are shown in Figures 2-6 .

L. Notes to Method M 2669.01

1. To minimize chromatographic dead volumes and concomitant peak broadening, the LC column (C.2) can be readily connected between the Rheodyne injector provided with the Finnigan MAT API System (located on the front of the instrument) and the electrospray ionization interface. Chromatographic connections between the LC pumps, mixing tee, static mixer, and injector should be kept as short as possible.

2. The following modifications have been made to method M 2669:

   a. The method number has been changed to M 2669.01 and is indicated in the header of page 1 and in the footer of each page thereafter.

b. The date, located in the footer of page 1, has been changed to 1998.

c. The generic name has been changed to imazapic.

d. The Limit of Quantitation (LOQ) of the method has been extended to 0.05 ppb.

e. Safety precautions have been added under Reagents.

f. Step D.3.d has been added to give a 0.00025 mcg/mL standard for the lower LOQ of the method.

g. Step F.1: Standard LC Solution D.3.d has been included in the linearity check.

h. Step F.2: The allowable deviation in the standard response factors from the average factor has been changed to 20%.

i. Step J.5: The allowable peak response variation between bracketing standards has been changed to 20%.

j. There are now 15 total pages (instead of 14) for the method because of the required modifications.