The Chemical Company

MRID: 50120101

STUDY TITLE

CL 263222 (Imazapic): Independent Laboratory Validation of LC/MS Determinative Method M 2669.01 for the Analysis of Residues of CL 263222 in Water

EPA Guideline(s)

OPPTS 850.6100

TITLE

CL 263222 (imazapic): Independent Laboratory Validation of LC/MS Determinative Method M 2669.01 for the Analysis of Residues of CL 263222 in Water

PURPOSE

To have Centre Analytical Laboratories, Inc. conduct an independent laboratory validation of Method M 2669.01 for the determination of CL 263222 residues in water at an LOQ of 0.05 ppb.

SUMMARY

The study was conducted according to American Cyanamid Company Protocol CD98PT05 in accordance with EPA PR Notice 96-1 . Method M 2669.01 was found to be satisfactory for the determination of imazapic (CL 263222) residues in water. The validated sensitivity (Limit of Quantitation) of the method is 0.05 ppb.

Each matrix was analyzed in separate extraction sets consisting of 2 matrix control samples and five matrix control samples fortified at 0.05 ppb with CL 263222, for a total of seven samples per extraction set.

STANDARD REFERENCE MATERIAL

American Cyanamid Company supplied the standard reference material. The standard reference material was labeled upon log-in at Centre Analytical Laboratories, Inc. as follows:

Compound	Lot Number	CAL #	Purity	Expir. Date	Date Received
CL 263222	AC10606-119	98-07-235	99.3	05/22/01	03/24/98

The reference standard material, a white powder, was kept in a refrigerator at a temperature of 4°C ± 2°C. American Cyanamid Company performed characterization, solubility, and stability of the reference materials and maintains the documentation. Stock solutions, fortification solutions, and calibration solutions were prepared as documented in the raw data for the study. These solutions were kept refrigerated at 4°C ± 2°C when not in use.

TEST SYSTEM

American Cyanamid Company supplied the samples used for this study. They were identified as follows:

Control Pond Water	AC 10079.39
Control Well Water	AC 10079.40

The samples were received at Centre Analytical Laboratories, Inc. on 01/27/98. They arrived frozen, packed in dry ice. The samples were kept in a freezer at a temperature of ≤-10°C until used. They were placed back into a freezer immediately after use.

METHOD OF ANALYSIS

The method of analysis is described in American Cyanamid Method M 2669.01. Each matrix was analyzed in separate sets consisting of 2 matrix control samples and five matrix control samples fortified at 0.05 ppb with CL 263222. Each set of seven samples was put through the procedure described in the method, which involves extracting residues of CL 263222 from water using solid phase extraction. Measurement of CL 263222 was accomplished by liquid chromatography/electrospray ionization mass spectrometry (LC/ESMS). The amount of CL 263222 present in the samples was calculated by direct comparison of the resultant peak areas to those of external standards. The validated sensitivity (LOQ, Limit of Quantitation) of this method is 0.05 ppb in water, and the LOD (limit of detection) was approximately 0.005 ppb in water. The extraction time for one set of seven samples was approximately four hours for one person. The LC analysis time for one set of seven samples and three standards was approximately 2.5 hours.

The following minor modifications to method M 2669.01 were made for this study:

Section E.3. LC solutions used were:
a. Aqueous: 0.2% Acetic Acid in Type I Water
b. Organic: 0.2% Acetic Acid in Methanol

Section E.4.b. LC flow rate was changed to 0.6 mL/min.

Section E.4.c. The following LC gradient was used:

Time	%A	%B
0.0	75	25
2.0	75	25
10.0	10	90
11.0	0	100
12.0	75	25
15.0	STOP	STOP

These minor modifications had no negative impact of the validity of the study.

LIQUID CHROMATOGRAPHIC/MASS SPECTROMETRIC CONDITIONS

The following Liquid Chromatograph/Mass Spectrometric Conditions, which are described in section E of Method M 2669.01, were used:

1) **Instrument:** LC/MS/MS#3
PE SCIEX API 365, serial no. 196711
TurboIonSpray Liquid Introduction Interface
Temperature: 350°C Nitrogen Flow Rate: 7 L/min.
Harvard Infusion Pump (Model 22)

2) **Computer:** Power Macintosh 7300/180

3) **Software:** Macintosh system 7.5
PE Sciex LC2Tune 1.3
PE Sciex Sample Control 1.3
MacQuan 1.5

4) **HPLC Equipment:** Hewlett Packard (HP) Series 1100
HP Quat Pump
HP Vacuum Degasser
HP Autosampler
HP Column Oven

5) **HPLC Column:** TosoHaas TSK Super ODS (5 cm x 4.6 mm)

6) **Detector:** Mass Spectrometer

7) **LC Solutions:** A) Aqueous: 0.2% Acetic Acid in Type I Water
B) Organic: 0.2% Acetic Acid in Methanol

8) **LC/MS Conditions:**

a. LC Column Temperature: 35°C

b. LC Flow Rate: 0.6 mL/min.

c. LC Gradient:

Time	%A	%B
0.0	75	25
2.0	75	25
10.0	10	90
11.0	0	100
12.0	75	25
15.0	STOP	STOP

d. Injection Volume: 100 µL

e. Retention Time: ~ 6 min.

f. Ions Monitored:

Analyte	Monitored Ion
CL 263222	276

CALCULATIONS AND RESULTS

An example of a calculation using an actual sample of AC 10079.39 pond water fortified with 0.05 ppb of CL 263222 (Spike D) is presented as follows:

R(SAMP)	56130
R(STD)	(133457 + 133874)/2 = 133666
W	10 g
V1	10 mL
V2	10 mL
V3	1 mL
V4	100 µL
C(STD)	0.001 µg/mL
V5	100 µL
DF	1.0
FV	0.5 mL
FC	0.001 µg/mL

If the above data are substituted into the formula on page 16,

$$\text{PPB FOUND} = \frac{56130 \times 10 \times 1 \times 100 \times 0.001 \times 1}{133666 \times 10 \times 100 \times 10} \times 1000$$

$$= 0.042$$

$$\%\ \text{RECOVERY} = \frac{0.042 \times 100}{0.5 \times 0.001 \times 1000 / 10}$$

$$= 84\%$$

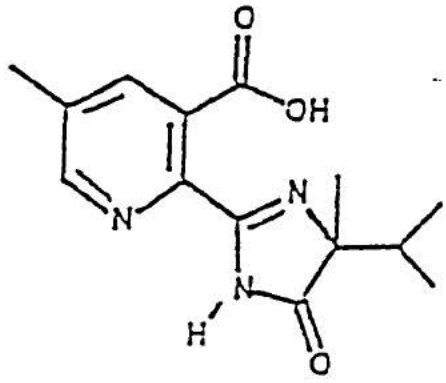
Method M 2669.01 was found to be satisfactory for the determination of CL 263222 residues in water.

Table of Compounds

Compound CL 263222

Chemical Name [nicotinic acid, 5-methyl-2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl)]

Structure



CALCULATION FORMULA AND NOTES FOR DETAILED ANALYTICAL DATA TABLES

$$PPB = \frac{R(SAMP) \times (V1) \times (V3) \times (V5) \times C(STD) \times DF}{R(STD) \times (V2) \times (V4) \times (W)} \times 1000$$

$$\%\ RECOVERY = \frac{PPB\ FOUND \times 100}{FV \times FC \times 1000 / W} = \frac{PPB\ FOUND}{PPB\ ADDED} \times 100$$

WHERE:

R(SAMP) = Peak response of sample.
R(STD) = Average peak response of working standards, R(Std)#1, and R(Std)#2.
W = Weight of sample taken for analysis in grams.
V1 = Volume in mL of extracting solvent.
V2 = Volume in mL of aliquot taken for analysis.
V3 = Volume in mL of final solution used for analysis.
V4 = Volume in mcL or nL of sample solution injected.
C(STD) = Concentration in mcg/mL of standard solution.
V5 = Volume in mcL or nL of standard solution injected.
D.F. = Dilution factor, if needed, of final solution.

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

NOTES:

(1) Control and recovery samples are indicated by a minus sign before the R (Samp) value.
(2) N.M. or -N.M. = Non-measurable peak for treated or control samples, respectively, or the minimum meaningful measurement.
(3) For Control Sample, an apparent residue value is calculated using actual peak response. Even though the calculated residue value may be lower than the validated sensitivity of the method (LOQ, limit of quantitation), the value is shown to give an indication of the detection limit of the metho
(4) For Treated Samples, if the peak response is N.M., the apparent residue is expressed as less than the validated sensitivity of the method (LOQ, limit of quantitation).
(5) Scientific notation is used for final results (i.e. 1E-1= 0.10; 1E-2= 0.01).
(6) Results are not corrected for recoveries.

RESIDUE SUPPORT STUDY PROTOCOL

Distribution: ES File
Quality Assurance Unit
Those Signing Protocol
Laboratory Personnel

PROTOCOL NUMBER: CD98PT05

PROJECT NUMBER: 575

TITLE: CL 263222 (imazapic): Independent Laboratory Validation of LC/MS Determinative Method M 2669.01 for the Analysis of Residues of CL 263222 in Water.

PURPOSE: To have Centre Analytical Laboratories, Inc. conduct an independent laboratory validation of Method M 2669.01 for the determination of CL 263222 residues in water at an LOQ of 0.05 ppb. The study will be conducted in accordance with US EPA Residue Chemistry Test Guidelines (8/96), OPPTS 860.1340 (Residue Analytical Method) and PR Notice 96-1 (Attachment I).

TEST MATERIAL:

Analytical standard of the following:

CL 263222, Lot No. AC 10606-119, purity of 99.3%, expiration date 5/22/01

1. Characterization data for the test material are on file with the Analytical, Physical and Biochemical Research (APBR) section of the American Cyanamid Company, Agricultural Products Research Division, Princeton, New Jersey.

2. The test material was shown to be soluble under the conditions of the study as described in the analytical method. The raw data is stored in the archives of the Cyanamid Agricultural Research Center, Princeton, New Jersey (American Cyanamid Company Report No. RES 93-016).

3. The test material solutions were shown to be stable under the conditions of the study. The raw data is stored in the archives of the Cyanamid Agricultural Research Center, Princeton, New Jersey (American Cyanamid Company Notebook No. AC 8996/37-55).

TEST SYSTEM:

Sample Type:	Well Water and Pond Water
Source:	Control Well Water (Sample # AC 10079.40) Control Pond Water (Sample # AC 10079.39)
Justification:	Analysis may be required to assess the potential for residues of CL 263222 in water in areas where the formulated product is used. A successful validation of the method, by an independent laboratory, must occur prior to its recommended use for routine residue analysis.
Identification:	Each sample will be identified by a unique sample number.
Sample Preparation:	Water samples were collected from the pond at American Cyanamid Company, Princeton, NJ, and from a well in Hopewell, NJ. The samples were logged-in by American Cyanamid Company's Sample Preparation Laboratory personnel and kept frozen until analysis.

METHOD OF ANALYSIS:

American Cyanamid Company Method M 2669.01, entitled "Imazapic (CL 263222): LC/MS Method for the Determination of CL 263222 Residues in Water."

EXPERIMENTAL DESIGN:

1. Analytical standard solutions will be prepared following American Cyanamid Company Method M 2669.01.

2. Using an appropriate volumetric pipet or syringe, add 0.5 mL of the 0.001 µg/mL standard solution of CL 263222 to 10 mL of the control samples to give a fortification level of 0.05 ppb. Run the 0.05 ppb fortification level five times for each water sample type. Run the control in duplicate for each water sample type.

3. Linearity of response must be checked in accordance with the procedure specified in the method prior to the beginning of the analysis of the validation samples.

4. All samples are to be analyzed according to the procedure described in American Cyanamid Company Method M 2669.01 with no significant modifications to the method except as approved by the Study Director. The percent recovery from the fortified controls will be calculated by dividing the apparent residue found (in ppb) by the amount of standard added (in ppb) and multiplying by 100.

5. If the majority of the recoveries do not fall in the range of 70-120%, the Study Director should be notified to determine the cause of the unacceptable recovery values using the following guidelines:

   a. The laboratory may contact the Study Director, developers or previous users of the method prior to the analysis of the first set of samples; however, all communications must be documented in the final report. The laboratory conducting the validation trial will not contact the sponsor during the analysis of the first set of samples (see Attachment I for a copy of PR Notice 96-1).

   b. If this set, or subsequent sample sets, are unsuccessful, the laboratory may contact the developer of the method and/or Study Director of the method validation. This communication is to be documented in the final report. Any modifications or additions to the method will be incorporated into the method write-up that is sent to the EPA for validation.

   c. If, after three attempts, the validation trial has failed the established criteria, a new method must be submitted for another independent laboratory validation trial.