

**Analytical method for imazapic in water**

**Reports:** ECM: EPA MRID No.: 50120101 (Appendix B, pp. 37-52). daCunha, A., and S.J. Stout (p. 46). 1998. Imazapic (CL 263,222): LC/MS Method for the Determination of CL 263,222 Residues in Water. American Cyanamid Method M 2669.01. Report prepared and sponsored by American Cyanamid Company, Princeton, New Jersey, and submitted by BASF Corporation, Research Triangle Park, North Carolina; 15 pages. Final report issued May 20, 1998.

ILV: EPA MRID No. 50120101. Stout, S.J., and S. Zheng. 1999. CL 263222 (Imazapic): Independent Laboratory Validation of LC/MS Determinative Method M 2669.01 for the Analysis of Residues of CL 263222 in Water. American Cyanamid Report No. RES 98-107 and Protocol # CD98PT05. Centre Analytical Laboratories Report No. 007-209. BASF Registration Document No.: IA-243-006. Report prepared by Centre Analytical Laboratories, Inc., State College, Pennsylvania, and sponsored and submitted by BASF Corporation, Research Triangle Park, North Carolina; 52 pages. Final report issued March 17, 1999.

**Document No.:** MRID 50120101

**Guideline:** 850.6100

**Statements:** ECM: No Good Laboratory Practice (GLP) standards were cited for the study. Signed and dated No Data Confidentiality, GLP, Quality Assurance, and Authenticity statements were not provided. A signed approvals page was included (Appendix B, p. 46 of MRID 50120101).

ILV: The study was conducted in accordance with USEPA FIFRA GLP standards (p. 3 of MRID 50120101). Signed and dated No Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2-4). A statement of authenticity was not included.

**Classification:** This analytical method is classified as unacceptable. ECM and ILV performance data were insufficient to support the reproducibility of the method. An insufficient number of samples was prepared in the ECM. The linearity of the method could not be determined because calibration curves and linear regression were not performed in the ECM and ILV, and the water matrices were not characterized. This analytical method validation report for water was required to support a small-scale prospective ground-water monitoring (PGW) study (MRID 47002001).

**PC Code:** 129041

**EFED Final Reviewer:** Iwona L. Maher  
Chemist

Signature: IWONA MAHER  
Date: 09/05/19

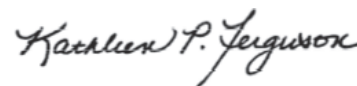
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**Reviewers:**Kathleen Ferguson, Ph.D.,  
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11/13/17

*This Data Evaluation Record may have been altered by the Environmental Fate and Effects Division subsequent to signing by CDM/CSS-Dynamac JV personnel.*

**Executive Summary**

The analytical method, American Cyanamid Method M 2669.01, is designed for the quantitative determination of imazapic (BAS 715 H; CL 263222) in water at the LOQ of 0.05 µg/L using LC/MS. The LOQ is less than the lowest toxicological level of concern in water for imazapic. The ECM validated the method using uncharacterized drinking and surface water matrices. The ILV validated the method for imazapic in uncharacterized surface (pond) water in the first trial with insignificant modifications to the analytical method. The method for imazapic in uncharacterized ground (well) water was validated by the ILV in the second trial with insignificant modifications to the analytical method and a cleaning of the reusable parts of the sample processing procedure; the failure of the first trial was due to possible contamination. One ion was quantified in the ECM and ILV; a confirmatory method is not typically required where GC/MS and/or LC/MS methods are used as the primary method(s) to generate study data. In the ECM, no samples were prepared at the LOQ or 10×LOQ; an insufficient number of samples was prepared at 2×LOQ. In the ILV, precision and accuracy was satisfactory at the LOQ; however, no samples were prepared at 10×LOQ. The linearity of the method could not be determined because calibration curves and linear regression were not performed in the ECM and ILV. Calculations of percent recovery was not performed by comparison to a calibration curve of standards. Instead, the amount recovered was calculated based on the peak response of the sample and average peak response of the working standards. The specificity of the method was satisfactory in the ECM and ILV; however, the analyte peak and representative chromatograms were small and difficult to analyze in the ILV.

**Table 1. Analytical Method Summary**

Analyte(s) by Pesticide	MRID		EPA Review	Matrix	Method Date (dd/mm/yyyy)	Registrant	Analysis	Limit of Quantitation (LOQ)
	Environmental Chemistry Method	Independent Laboratory Validation						
Imazapic (CL 263222; BAS 715 H)	50120101 (Appendix B)	50120101		Water <sup>1,2</sup>	20/05/1998	BASF Corporation	LC/MS/MS	0.05 µg/L

<sup>1</sup> In the ECM, drinking and surface water were not characterized; the specific sources of the water matrices were not reported.

<sup>2</sup> In the ILV, the surface (pond; AC 10079.39) and ground (well; AC 10079.40) water matrices were provided by the Sponsor (American Cyanamid Company; p. 7 of MRID 50120101). The water matrices were not characterized; the specific sources of the water matrices were not reported.

## I. Principle of the Method

Samples (10 mL) were transferred to a test flask and fortified, as necessary, with imazapic fortification solution (p. 7; Appendix B, pp. 42-43 of MRID 50120101). A Varian Bond Elut C18 cartridge was pre-conditioned sequentially with *ca.* 3 mL each of hexane, methylene chloride, methanol, Milli-Q water then 0.01 N HCl. After filling the cartridge barrel *ca.* halfway with 0.01 N HCl, a 15-mL reservoir was attached to the cartridge. After the water sample (10 mL) was added to the reservoir, 0.1 N HCl was added to the reservoir. The sample was loaded onto the column with minimal vacuum. The cartridge was washed with one column volume of Milli-Q water then one column volume of hexane. The analyte was eluted with one column volume of methylene chloride at a rate of *ca.* 1-2 drops per second. The solvent was evaporated under low heat (*ca.* 60C) and a stream of nitrogen. If any water remains, a small amount (*ca.* 1 mL) of UV grade methanol can be added to aid in the evaporation of the water. The residue was dissolved in 1 mL of Milli-Q water for analysis. CL 354825 was not included as an analyte.

Samples were analyzed for imazapic using Shimadzu Model LC-10AD Pump systems coupled to a Finnigan MAT TSQ700 (Appendix B, pp. 39-42 of MRID 50120101). The LC/MS conditions consisted of a TSK-gel Super ODS column (4.6 x 50.0 mm; column temperature ambient), a mobile phase of (A) water with 1% acetic acid and (B) methanol with 1% acetic acid [percent A:B (v:v) at 0.0 min. 90:10, 8.0-9.0 min. 10:90, 9.0-10.0 min. 90:10], flow rate 0.5 mL/min. and atmospheric pressure ionization (API; ionization temperature 300°C). Injection volume was 100 µL. One ion was monitored: *m/z* 276 ( $\pm 0.2$ ); retention time was *ca.* 5.0 minutes for imazapic.

In the ILV, the ECM was performed as written for imazapic, except for the use of a different LC/MS/MS system and methods (pp. 8-9 of MRID 50120101). Hewlett Packard Series 1100 HPLC System coupled to a PE SCIEX API 365 mass spectrometer was used. The LC/MS conditions consisted of the same LC column (column temperature 35°C), a mobile phase of (A) Type I water with 0.2% acetic acid and (B) methanol with 0.2% acetic acid [percent A:B (v:v) at 0.0-2.0 min. 75:25, 10.0 min. 10:90, 11.0 min. 0:100, 12.0-15.0 min. 75:25], and flow rate 0.6 mL/min. One ion was monitored: *m/z* 276; retention time was *ca.* 6 minutes for imazapic.

The Limit of Quantification (LOQ) and Limit of Detection (LOD) for imazapic in water was 0.05 µg/L in the ECM and ILV (pp. 5, 7; Appendix B, p. 38 of MRID 50120101). The LOD for imazapic in water was 0.005 µg/L in the ILV; the LOD was not reported in ECM.

## II. Recovery Findings

ECM (Appendix B, pp. 37-52 of MRID 50120101): Mean recoveries and RSDs were within guideline requirements for analysis of imazapic in two water matrices at the fortification level of 0.1 µg/L (2×LOQ; Appendix B, Figure 4, p. 50; Appendix B, Figure 6, p. 52). The number of samples was insufficient for all analyses (n = 1). No samples were prepared at the LOQ or 10×LOQ. Imazapic was identified using one ion; a confirmatory method is not usually required when LC/MS and GC/MS is the primary method. The drinking and surface water were not characterized; the specific sources of the water matrices were not reported.

ILV (MRID 50120101): Mean recoveries and RSDs were within guideline requirements for analysis of imazapic in two water matrices at the fortification level of 0.05 µg/L (LOQ; Tables III-IV, p. 19). No samples were prepared at 10×LOQ. Relative standard deviations (RSDs) were reviewer-calculated since these values were not reported by the study author. Imazapic was identified using one ion; a confirmatory method is not usually required when LC/MS and GC/MS is the primary method. The surface (pond; AC 10079.39) and ground (well; AC 10079.40) water matrices were provided by the Sponsor (American Cyanamid Company; p. 7). The water matrices were not characterized; the specific sources of the water matrices were not reported. The method for imazapic in surface (pond) water was validated in the first trial with insignificant modifications to the analytical method (pp. 5, 10). The method for imazapic in ground (well) water was validated in the second trial with insignificant modifications to the analytical method and a cleaning of the reusable parts of the sample processing procedure; the failure of the first trial was due to possible contamination.

**Table 2. Initial Validation Method Recoveries for Imazapic in Water<sup>1,2</sup>**

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
<b>Drinking (Tap) Water</b>						
Imazapic (CL 263222; BAS 715 H)	0.05 (LOQ)	<b>Not reported</b>				
	0.1	<b>1</b>	96	-- <sup>3</sup>	--	--
<b>Surface (Lake) Water</b>						
Imazapic (CL 263222; BAS 715 H)	0.05 (LOQ)	<b>Not reported</b>				
	0.1	<b>1</b>	93	--	--	--

Data (uncorrected recovery results, Appendix B, p. 44) were obtained from Appendix B, Figure 4, p. 50 and Appendix B, Figure 6, p. 52 of MRID 50120101.

1 The drinking and surface water were not characterized; the specific sources of the water matrices were not reported.

2 One ion was monitored: *m/z* 276.

3 Could not be calculated because n = 1.

**Table 3. Independent Validation Method Recoveries for Imazapic in Water<sup>1</sup>**

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%) <sup>2</sup>
<b>Surface (Pond) Water<sup>3</sup></b>						
Imazapic (CL 263222; BAS 715 H)	0.05 (LOQ)	5	84-95	91	4.9	5
<b>Ground (Well) Water<sup>3</sup></b>						
Imazapic (CL 263222; BAS 715 H)	0.05 (LOQ)	5	87-103	94	6.5	7

Data (uncorrected recovery results, Tables I-II, pp. 17-18) were obtained from Tables III-IV, p. 19 of MRID 50120101 and DER Attachment 2.

1 The surface (pond; AC 10079.39) and ground (well; AC 10079.40) water matrices were provided by the Sponsor (American Cyanamid Company; p. 7). The water matrices were not characterized; the specific sources of the water matrices were not reported.

2 Relative standard deviations (RSDs) were reviewer-calculated since these values were not reported by the study author (see DER Attachment 2). Rules of significant figures were followed when reporting results.

3 One ion was monitored: *m/z* 276.

### III. Method Characteristics

The LOQ for imazapic in water was 0.05 µg/L in the ECM without justification or calculations; the LOD was not reported (Appendix B, p. 38 of MRID 50120101). The LOQ and LOD for imazapic in water were 0.05 µg/L and 0.005 µg/L, respectively, in ILV (pp. 5, 7 of MRID 50120101). No calculations or comparisons to background levels were reported to justify the LOQ and LOD for the method in ILV.

**Table 4. Method Characteristics**

Analyte		Imazapic (CL 263222; BAS 715 H)
Limit of Quantitation (LOQ)	ECM	0.05 µg/L
	ILV	
Limit of Detection (LOD)	ECM	0.005 µg/L
	ILV	
Linearity (calibration curve $r^2$ and concentration range) <sup>1</sup>	ECM	Calibration curves not used. <sup>2</sup>
	ILV	
	Concentration Range	
Repeatable	ECM <sup>3</sup>	<b>Could not be determined</b> at 2×LOQ because n = 1. <b>No</b> samples were prepared at LOQ or 10×LOQ. (uncharacterized drinking and surface water matrices)
	ILV <sup>4,5,6</sup>	Yes at LOQ. <b>No</b> samples were prepared at 10×LOQ. (uncharacterized ground and surface water matrices)
Reproducible		<b>Could not be determined</b> at LOQ. <b>No</b> samples were prepared at 10×LOQ.
Specific	ECM	Matrix interferences were <12% of the LOQ (based on amount quantified) at the analyte retention time. Minor matrix contaminants near the analyte retention time interfered with peak integration.
	ILV	No matrix interferences were observed or quantified; however, the analyte peak and representative chromatogram were small and difficult to analyze.

Data were obtained from Appendix B, p. 38; Appendix B, Figure 4, p. 50 and Figure 6, p. 52 (recovery data); Appendix B, Figures 2-6, pp. 48-52 (chromatograms) of MRID 50120105; pp. 5, 7, 16; Tables III-IV, p. 19 (recovery data); Figures 1-6, pp. 20-23 (chromatograms) of MRID 50120101; and DER Attachment 2.

1 One ion was monitored:  $m/z$  276. A confirmatory method is not usually required when LC/MS and GC/MS is the primary method.

2 The calculation of percent recovery was not performed by comparison to a calibration curve of standards. Instead, the amount recovered was calculated based on the peak response of the sample and working standards (see Reviewer's Comment #3).

3 In the ECM, drinking and surface water were not characterized; the specific sources of the water matrices were not reported.

4 In the ILV, the surface (pond; AC 10079.39) and ground (well; AC 10079.40) water matrices were provided by the Sponsor (American Cyanamid Company; p. 7 of MRID 50120101). The water matrices were not characterized; the specific sources of the water matrices were not reported.

5 Relative standard deviations (RSDs) were reviewer-calculated since these values were not reported by the study author (see DER Attachment 2).

6 The method for imazapic in surface (pond) water was validated in the first trial with insignificant modifications to the analytical method (pp. 5, 10 of MRID 50120101). The method for imazapic in ground (well) water was validated in the second trial with insignificant modifications to the analytical method and a cleaning of the reusable parts of the sample processing procedure; the failure of the first trial was due to possible contamination.

#### IV. Method Deficiencies and Reviewer's Comments

1. The reproducibility of the method was not supported by the ECM and ILV performance data. In the ECM, no samples were prepared at the LOQ or 10×LOQ; in the ILV, no samples were prepared at 10×LOQ.



2. The repeatability of the method was not supported by the ECM performance data because an insufficient number of samples was prepared at 2×LOQ (n = 2). A OCSPP Guideline 850.6100 criteria state that a minimum of five samples should be prepared at the stated LOQ and at higher concentrations.
3. The linearity of the method could not be determined because calibration curves and linear regression were not performed in the ECM and ILV. Calculations of percent recovery was not performed by comparison to a calibration curve of standards. Instead, the amount recovered was calculated based on the peak response of the sample and average peak response of the working standards (100 pg; pp. 10, 16; Figure 1, p. 20 of MRID 50120101). The following equations were used:

$$\text{PPB} = [\text{R}(\text{SAMP}) \times (\text{V1}) \times (\text{V3}) \times (\text{V5}) \times \text{C}(\text{STD}) \times \text{DF}] / [\text{R}(\text{STD}) \times (\text{V2}) \times (\text{V4}) \times (\text{W})]$$

Where,

- R(SAMP) = peak response of sample
- R(STD) = average peak response of working standards
- W = weight of sample (g)
- V1 = volume of extracting solvent (mL)
- V2 = volume of aliquot for analysis (mL)
- V3 = volume of final solution used for analysis (mL)
- V4 = volume of sample solution injected (μL or nL)
- C(STD) = concentration of standard solution (μg/mL)
- V5 = volume of standard solution injected
- DF = dilution factor of final solution, if needed

$$\% \text{ Recovery} = [\text{PPB found} \times 100] / [\text{FV} \times \text{FC} \times (1000/\text{W})] \times 100$$

Where,

- FV = fortification volume (mL)
- FC = fortification concentration of standard solution added (μg/mL)

4. The water matrices were not characterized in the ECM and ILV. In the ECM, no information about the drinking and surface water matrices was provided. In the ILV, the surface (pond; AC 10079.39) and ground (well; AC 10079.40) water matrices were provided by the Sponsor (American Cyanamid Company; p. 7 of MRID 50120101). The water matrices were not characterized; the specific sources of the water matrices were not reported.
5. The analyte peak and representative chromatograms were small and difficult to analyze in the ILV (Figures 1-6, pp. 20-23 of MRID 50120101).
6. The estimations of LOQ and LOD in ECM and ILV were not based on scientifically acceptable procedures as defined in 40 CFR Part 136. (Appendix B, p. 38; pp. 5, 7 of MRID 50120101). The LOQ was reported in the ECM without justification or calculations; the LOD was not reported. No calculations or comparisons to background levels were reported to justify the LOQ and LOD for the method in ILV.

7. The ILV study author reported that no technical communications between the ILV and ECM personnel occurred prior to the validation (p. 5 of MRID 50120101).
8. It was reported for the ILV that one sample set of seven samples required *ca.* four hours for one person (p. 7 of MRID 50120101). LC/MS/MS analysis time for the seven samples and three standards was *ca.* 2.5 hours.

## V. References

- U.S. Environmental Protection Agency. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.
- 40 CFR Part 136. Appendix B. Definition and Procedure for the Determination of the Method Detection Limit-Revision 1.11, pp. 317-319.



**Attachment 1: Chemical Names and Structures****Imazapic (BAS 715 H; AC 263222; CL 263,222)**

**IUPAC Name:** 2-[(RS)-4-Isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl]-5-methylnicotinic acid

**CAS Name:** 2-[4-Isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl]-5-methylnicotinic acid  
2-[4,5-Dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-5-methyl-3-pyridinecarboxylic acid

**CAS Number:** 81334-60-03 & 104098-48-8

**SMILES String:** [H]N1C(=NC(C1=O)(C)C(C)C)c2c(cc(cn2)C)C(=O)O

