Analytical method for imazapic and its metabolite CL 354825 in water

Reports: ECM: EPA MRID No.: 50120105. White, M.T. 2004. Laboratory Validation of BASF Method D0304 entitled: "Method for the Determination of Residues of BAS 715 H and its Major Metabolite CL 354825 in Groundwater Utilizing LC MS/MS.". BASF Study No.: 121737. BASF Registration Document No.: 2004/5000373. Report prepared, sponsored and submitted by BASF Corporation, Research Triangle Park, North Carolina; 41 pages. Final report issued June 29, 2004 (GLP statement signed April 11, 2005).

ILV: EPA MRID No. 50182301. Perez, S., and A. Ratliff. 2016. Independent Laboratory Validation of BASF Analytical Method D0304: "Method for the Determination of Residues of BAS 715 H and its Major Metabolite CL 354825 in Groundwater Utilizing LC MS/MS". BASF Study ID No.: 779580. BASF Registration Document No.: 2016/7006669. ADPEN Study No.: 16D1404. Report prepared by ADPEN Laboratories, Inc., Jacksonville, Florida, sponsored and submitted by BASF Corporation, Research Triangle Park, North Carolina; 161 pages. Final report issued December 20, 2016.

Document No.: MRIDs 50120105 & 50182301

Guideline: 850.6100

Statements: ECM: The study was conducted in accordance with USEPA FIFRA Good Laboratory Practice (GLP) standards, 40 CFR, Part 160, except that the report and associated raw data was inadvertently not archives at the completion of the study (p. 3 of MRID 50120105). Signed and dated No Data Confidentiality, GLP, Quality Assurance, and Authenticity statements were provided (pp. 2-5).

ILV: The study was conducted in accordance with USEPA FIFRA GLP standards (p. 3 of MRID 50182301). Signed and dated No Data Confidentiality, GLP, Quality Assurance, and Authenticity statements were provided (pp. 2-5).

Classification: This analytical method is classified as supplemental for imazapic and unacceptable/upgradable for CL 354825. An updated ECM incorporating the recommendations of the ILV was not provided. The ECM and ILV calibration curves did not adequately bracket the instrument response for the LOQ sample. The linearity of the method for CL 354825 was not supported by the ECM and ILV calibration curves. The water matrices were not characterized in the ECM and ILV. This analytical method validation report for water was required to support a small-scale prospective ground-water monitoring (PGW) study MRID 47001701.

PC Code: 129041

EFED Final Reviewer: Iwona L. Maher, Chemist
Signature: IWONA MAHER Digitally signed by IWONA MAHER Date: 2019.09.05 17:21:45 -04'00'
Date: 09/05/19

CDM/CSS-Dynamac JV Lisa Muto, Environmental Scientist
Signature: Lisa Muto
Date: 11/13/17

Reviewers: Kathleen Ferguson, Ph.D., Environmental Scientist

Signature: Kathleen P. Ferguson

Date: 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, BASF Method D0304, is designed for the quantitative determination of imazapic (BAS 715 H) and its major metabolite CL 354825 in water at the LOQ of 0.05 µg/L using LC/MS/MS. The LOQ is less than the lowest toxicological level of concern in water for imazapic. The ECM validated the method using uncharacterized ground water. The ILV validated the method for imazapic in uncharacterized drinking and surface water and for CL 354825 in uncharacterized drinking water in the first trial with significant modifications to the analytical method. The method for CL 354825 in uncharacterized surface water was validated in the second trial with significant modifications to the analytical method. The ILV recommended that the improvement of the analytical method and method modifications for enhanced separation of CL 354825 in surface water be incorporated into the method. An updated ECM incorporating the recommendations of the ILV was not provided. Two or three ion transitions were quantified in the ILV, while only one ion transition was quantified in the ECM. All ECM and ILV data regarding repeatability, accuracy, precision, and specificity were satisfactory for imazapic and CL 354825, based on the quantitation ion results. The confirmation/tertiary ion analysis in the ILV was insufficient for the specificity of CL 354825 in drinking and surface water, but 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. The linearity of the method was not supported by the calibration curves because ECM and ILV calibration curves did not adequately bracket the instrument response for the LOQ sample. Also, the linearity was unsatisfactory for CL 354825 in the ECM and 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)	50120105	50182301		Water[1,2]	29/06/2004	BASF Corporation	LC/MS/MS	0.05 µg/L
CL 354825								

1 In the ECM, the ground water was obtained from trial sites of BASF Study 97799 (Prospective Groundwater Study) conducted in the US (p. 12 of MRID 50120105). The water sample was not characterized; water source was not further specified.

2 In the ILV, the drinking and surface water matrices were provided by the Sponsor (p. 17 of MRID 50182301). The water matrices were not characterized; the specific sources of the water matrices were not reported.

I. Principle of the Method

Samples (unspecified volume) were transferred to a volumetric flask and fortified, as necessary, with 1.0 mg/mL fortification solutions of either imazapic or CL 354825, and mixed well (p. 12; Appendix B, p. 38; Figure 1, p. 16 of MRID 50120105). Samples could be filtered (Gelman 0.45 µ nylon acrodisc), if needed or indicated. An aliquot (1-2 mL) was transferred to an autosampler vial capped with a pre-slit, Teflon-lined cap and analyzed directly by LC/MS/MS.

Samples were analyzed for analytes using a PE Series 200 Micro Pump system coupled to a PE Sciex API 3000 Biomolecular Mass Analyzer (p. 12; Appendix B, p. 39 of MRID 50120105). The LC/MS conditions consisted of a TSK-gel Super ODS column (2.0 x 50.0 mm, 2-µm; column temperature not reported), 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, 3.0-4.5 min. 10:90, 4.6-7.0 min. 90:10] and MS/MS detection in ESI positive ion mode (ionization temperature 250°C). Injection volume was 10, 20, 25, 50 or 100 µL. Two ion transitions were monitored (quantitation and confirmatory, respectively) as follows: *m/z* 276.10→231.00 (±0.2) and *m/z* 276.10→163.00 (±0.2) for imazapic and *m/z* 278.10→232.90 (±0.2) and *m/z* 278.10→235.80 (±0.2) for CL 354825; however, only results from the quantitation ion transition were reported. Retention times were *ca*. 2.90 and 3.67 minutes for imazapic and CL 354825, respectively.

In the ILV, the ECM was performed as written, except for the use of a different LC/MS/MS system and methods (pp. 17, 20; Table 11, pp. 34-35; Appendix A, pp. 96-98 of MRID 50182301). The water sample volume was reported as 10 mL. An Agilent 1290 UPLC System coupled to an Agilent 6490 Triple Quad mass spectrometer was used. Most of the LC/MS conditions were the same (column temperature was reported as 40°C and injection volume 40 µL), except that the ionization temperature was 150°C and the mobile phase gradient was optimized to (A) water with 1% acetic acid and (B) methanol with 1% acetic acid [percent A:B (v:v) at 0.0 min. 99:1, 4.0-5.5 min. 0:100, 5.6-8.0 min. 99:1]. Two ion transitions were monitored (quantitation and confirmatory, respectively) as follows: *m/z* 276.14→231.00 and *m/z* 276.14→163.00 for imazapic and *m/z* 278.12→233.00 and *m/z* 278.12→236.10 for CL 354825. Retention times were *ca*. 2.90 and 3.67 minutes for imazapic and CL 354825, respectively. For the surface water analysis of CL 354825, a co-eluting interference peak was observed for the secondary transition, so a new gradient (percent A:B (v:v) at 0.0 min. 99:1, 4.0-5.5 min. 1:99, 5.6-8.0 min. 99:1) and confirmatory (tertiary) transition (*m/z* 278.12→165.00) was used for identification and quantification. The ILV recommended that the improvement of the analytical method and method modifications for enhanced separation of CL 354825 in surface water be incorporated into the method (p. 21).

The Limit of Quantification (LOQ) and Limit of Detection (LOD) were 0.05 µg/L and 0.01 µg/L, respectively, for imazapic and CL 354825 in water in the ECM and ILV (pp. 6, 9 of MRID 50120105; pp. 20-21 of MRID 50182301).

II. Recovery Findings

ECM (MRID 50120105): Mean recoveries and relative standard deviations (RSDs) were within guideline requirements (mean 70-120%; RSD ≤20%) for analysis of imazapic (CL 263222; BAS 715 H) and its metabolite CL 354825 in one water matrix at fortification levels of 0.05 µg/L (LOQ) and 0.5 µg/L (10×LOQ; Tables 1-2, p. 13). Relative standard deviations (RSDs) were reviewer-calculated since these values were not reported by the study author. Two ion transitions were monitored for imazapic and CL 354825; however, only results from the quantitation ion transition were reported. A confirmatory method is not usually required when LC/MS and GC/MS is the primary method. The ground water was obtained from trial sites of BASF Study 97799 (Prospective Groundwater Study) conducted in the US (p. 12). The water sample was not characterized; water source was not further specified.

ILV (MRID 50182301): Mean recoveries and RSDs were within guideline requirements for analysis of imazapic and its metabolite CL 354825 in two water matrices at fortification levels of 0.05 µg/L (LOQ) and 0.5 µg/L (10×LOQ; Tables 2-17, pp. 26-41). Imazapic was identified using two ion transitions; CL 354825 was identified using two (drinking water) or three (surface water) ion transitions. Performance data (recovery results) from primary and confirmatory analyses were comparable for imazapic and fairly comparable for CL 354825. The drinking and surface water matrices were provided by the Sponsor (p. 17). The water matrices were not characterized; the specific sources of the water matrices were not reported. The method for imazapic in drinking and surface water and for CL 354825 in drinking water was validated in the first trial with significant modifications to the analytical method (p. 21; Appendix A, p. 96). The method for CL 354825 in surface water was validated in the second trial with significant modifications to the analytical method. The ILV recommended that the improvement of the analytical method and method modifications for enhanced separation of CL 354825 in surface water be incorporated into the method (p. 21).

Table 2. Initial Validation Method Recoveries for Imazapic and CL 354825 in Water[1]

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)[2]
	Ground Water					
	Quantitation ion[3]					
Imazapic (CL 263222; BAS 715 H)	0.05 (LOQ)	10	89.8-104.5	96.4	4.9	5.1
	0.5	10	84.3-112.0	95.7	9.3	9.7
CL 354825	0.05 (LOQ)	10	80.3-117.5	96.4	10.9	11.3
	0.5	10	102.0-120.0	109.6	5.0	4.6

Data (uncorrected recovery results, p. 17) were obtained from Tables 1-2, p. 13 of MRID 50120105 and DER Attachment 2.

[1] The ground water was obtained from trial sites of BASF Study 97799 (Prospective Groundwater Study; final study MRID 47001701) conducted in the US (p. 12). The water sample was not characterized in this report; water source was not further specified.

[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] Two ion transitions were monitored (quantitation and confirmatory, respectively) as follows: *m/z* 276.10→231.00 (±0.2) and *m/z* 276.10→163.00 (±0.2) for imazapic and *m/z* 278.10→232.90 (±0.2) and *m/z* 278.10→235.80 (±0.2) for CL 354825; however, only results from the quantitation ion transition were reported.

Table 3. Independent Validation Method Recoveries for Imazapic and CL 354825 in Water[1]

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Surface Water[2]					
	Quantitation ion					
Imazapic (CL 263222; BAS 715 H)	0.05 (LOQ)	5	91-101	96	5.1	5.3
	0.5	5	96-99	97	1.2	1.2
CL 354825	0.05 (LOQ)	5	88-101	95	4.4	4.7
	0.5	5	112-120	117	3.7	3.2
	Confirmatory ion					
Imazapic (CL 263222; BAS 715 H)	0.05 (LOQ)	5	88-101	95	5.9	6.2
	0.5	5	97-104	101	3.0	3.0
CL 354825	0.05 (LOQ)	5	83-110	96	9.4	9.8
	0.5	5	116-132	122	6.0	5.0
	Tertiary ion					
CL 354825	0.05 (LOQ)	5	94-120	109	9.8	9.0
	0.5	5	106-126	115	8.4	7.3
	Drinking Water[3]					
	Quantitation ion					
Imazapic (CL 263222; BAS 715 H)	0.05 (LOQ)	5	93-100	96	2.6	2.7
	0.5	5	105-110	108	2.1	1.9
CL 354825	0.05 (LOQ)	5	75-106	90	13.7	15.2
	0.5	5	80-118	105	17.7	16.9
	Confirmatory ion					
Imazapic (CL 263222; BAS 715 H)	0.05 (LOQ)	5	94-104	98	4.1	4.1
	0.5	5	106-110	107	1.8	1.6
CL 354825	0.05 (LOQ)	5	86-116	103	11.8	11.4
	0.5	5	80-114	98	14.0	14.3

Data (uncorrected recovery results, Tables 1-9, pp. 24-32) were obtained from Tables 1-9, pp. 24-32 of MRID 50182301.

[1] The drinking and surface water matrices were provided by the Sponsor (p. 17). The water matrices were not characterized; the specific sources of the water matrices were not reported.

[2] Two ion transitions were monitored (quantitation and confirmatory, respectively) as follows: *m/z* 276.14→231.00 and *m/z* 276.14→163.00 for imazapic and *m/z* 278.12→233.00 and *m/z* 278.12→236.10 for CL 354825. For the surface water analysis of CL 354825, a co-eluting interference peak was observed for the secondary transition, so a new confirmatory (tertiary) transition (*m/z* 278.12→165.00) was used for identification and quantification. Matrix-matched standards were used.

[3] Two ion transitions were monitored (quantitation and confirmatory, respectively) as follows: *m/z* 276.14→231.00 and *m/z* 276.14→163.00 for imazapic and *m/z* 278.12→233.00 and *m/z* 278.12→236.10 for CL 354825. Solvent-based standards were used.

III. Method Characteristics

The LOQ and LOD were 0.05 µg/L and 0.01 µg/L, respectively, for imazapic and CL 354825 in water in the ECM and ILV (pp. 6, 9, 14 of MRID 50120105; pp. 20-21 of MRID 50182301). In the ECM and ILV, the LOQ was defined as the lowest fortification level successfully tested. The LOD was set at 20% of the LOQ in the ECM and ILV 2 and was justified by the fact that the lowest standard for each analyte in the calibration curve had a signal-to-noise ratio > 3:1. No calculations or comparisons to background levels were reported to justify the LOQ for the method in the ECM and ILV; no calculations were reported to justify the LOD for the method in the ECM and ILV.

Table 4. Method Characteristics

Analyte			**Imazapic (CL 263222; BAS 715 H)**	**CL 354825**
Limit of Quantitation (LOQ)	ECM		0.05 µg/L	
	ILV			
Limit of Detection (LOD)	ECM		0.01 µg/L	
	ILV			
Linearity (calibration curve r^2 and concentration range)[1]	ECM[2]		r^2 = 0.9976 (Q)	r^2 = **0.9926** (Q)
			0.02-0.2 ng/mL	
	ILV	Drinking Water	r^2 = 0.9982 (Q) r^2 = 0.9968 (C)	r^2 = 0.9960 (Q) r^2 = 0.9986 (C)
		Surface Water	r^2 = 0.9980 (Q) r^2 = 0.9984 (C)	r^2 = **0.9831** (Q) r^2 = 0.9964 (C) r^2 = **0.9932** (T)
			0.02-2 ng/mL	
Repeatable	ECM[2,3,4]		Yes at LOQ and 10×LOQ (uncharacterized ground water matrix)	
	ILV[5,6]		Yes at LOQ and 10×LOQ (uncharacterized drinking and surface water matrices)	
Reproducible			Yes at LOQ and 10×LOQ	
Specific	ECM		No matrix interferences were observed or quantified; however, minor baseline noise interfered with peak integration.	
	ILV	Drinking Water	No matrix interferences were observed or quantified.	Q: No matrix interferences were observed or quantified; however, minor baseline noise interfered with peak integration. C: No matrix interferences were observed or quantified; however, peak was very small at LOQ.[7,8]
		Surface Water		Q: No matrix interferences were observed or quantified. C: Analyte peak co-eluted with a peak which was *ca*. 6×LOQ peak area.[8,9] T: Matrix interferences were *ca*. 50% of the LOQ (based on peak area).[8,9]

Data were obtained from pp. 6, 9, 11, 14; Tables 1-2, p. 13 (recovery data); Appendix A, Figure A5, p. 25 and Figure A13, p. 29 (calibration curves); Appendix A, Figures A6-A8, pp. 25-26 and Figures A14-A16, pp. 29-30 (chromatograms) of MRID 50120105; pp. 20-21; Tables 1-9, pp. 24-32 (recovery data); Figures 1-4, pp. 37-41 (calibration curves); Figures 9-20, pp. 74-93 (chromatograms) of MRID 50182301; and DER Attachment 2. Q = Quantitation ion transition; C = Confirmation ion transition; T = Tertiary ion transition.

1 Correlation coefficients (r^2) values were reviewer-calculated from r values provided in the study report (Appendix A, Figure A5, p. 25 and Figure A13, p. 29 of MRID 50120105; Figures 1-4, pp. 37-41 of MRID 50182301; DER Attachment 2). In the ILV analysis, solvent-based calibration standards were used for the drinking water experiment and matrix-based calibration standards were used for the surface water experiment.

2 Two ion transitions were monitored; however, only results from the quantitation ion transition were reported.

3 In the ECM, the ground water was obtained from trial sites of BASF Study 97799 (Prospective Groundwater Study) conducted in the US (p. 12 of MRID 50120105). The water sample was not characterized; water source was not further specified.

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

5 In the ILV, the drinking and surface water matrices were provided by the Sponsor (p. 17 of MRID 50182301). The water matrices were not characterized; the specific sources of the water matrices were not reported.

6 The method for imazapic in drinking and surface water and for CL 354825 in drinking water was validated in the first trial with significant modifications to the analytical method (p. 21; Appendix A, p. 96 of MRID 50182301). The method for CL 354825 in surface water was validated in the second trial with significant modifications to the analytical method. The ILV recommended that the improvement of the analytical method and method modifications for enhanced separation of CL 354825 in surface water be incorporated into the method (p. 21).

7 Based on Figure 20, p. 92 of MRID 50182301.

8 A confirmatory method is not usually required when LC/MS and GC/MS is the primary method.

9 Based on Figures 12-14, pp. 78-85 of MRID 50182301.

Linearity is satisfactory when $r^2 \geq 0.995$.

IV. Method Deficiencies and Reviewer's Comments

1. The ILV recommended that the improvement of the analytical method and method modifications for enhanced separation of CL 354825 in surface water be incorporated into the method (p. 21; Appendix A, p. 96 of MRID 50182301). An updated ECM incorporating the recommendations of the ILV was not provided.

2. The ECM and ILV calibration curves did not adequately bracket the instrument response for the LOQ sample. The calibration standards were 0.02, 0.05, 0.10, and 0.20 ng/mL in the ECM and 0.02, 0.05, 0.10, 0.20, 0.50, 1.0, and 2.0 ng/mL in the ILV. In both studies, there was only one calibration standard below the response of the LOQ, since 0.05 ng/mL was equivalent to the LOQ (0.05 μg/L). Typically, two calibration standards above and below the LOQ instrument response is desirable for dependable results.

   Imazapic: Based on the ECM representative chromatograms, the LOQ peak area was 3386 counts, while the two lowest calibration standards yielded peak areas of 2107 and 4055 counts for the 0.02 and 0.05 ng/mL calibration standards, respectively (Appendix A, Figures A1-A2, p. 23 and Figure A7, p. 26 of MRID 50120105). Based on the ILV drinking water representative chromatograms, the LOQ peak area was 419 (Q; 575, C) counts, while the two lowest calibration standards yielded peak areas of 166 (Q; 215, C) and 441 (Q; 553, C) counts for the 0.02 and 0.05 ng/mL calibration standards, respectively (Figures 5-20, pp. 42-93 of MRID 50182301). Based on the ILV surface water representative chromatograms, the LOQ peak area was 315 (Q; 494, C) counts, while the two lowest calibration standards yielded peak areas of 140 (Q; 171, C) and 359 (Q; 495, C) counts for the 0.02 and 0.05 ng/mL calibration standards, respectively.

   CL 354825: Based on the ECM representative chromatograms, the LOQ peak area was 3737 counts, while the two lowest calibration standards yielded peak areas of 2281 and 5662 counts for the 0.02 and 0.05 ng/mL calibration standards, respectively (Appendix A, Figures A9-A10, p. 27 and Figure A15, p. 30 of MRID 50120105). Based on the ILV drinking water representative chromatograms, the LOQ peak area was 1042 (Q; 296, C) counts, while the two lowest calibration standards yielded peak areas of 413 (Q; 48, C) and 1036 (Q; 268, C) counts for the 0.02 and 0.05 ng/mL calibration standards, respectively (Figures 5-20, pp. 42-93 of MRID 50182301). Based on the ILV surface water representative chromatograms, the LOQ peak area was 1253 (Q; 363, C; 560, T) counts, while the two lowest calibration standards yielded peak areas of 700 (Q; 176, C; 387, T) and 1296 (Q; 383, C; 555, T) counts for the 0.02 and 0.05 ng/mL calibration standards, respectively.

This information seemed to indicate that the calibration range was inadequate for providing an accurate LOQ response assessment for both imazapic and CL 354825 in the ECM and ILV since only one calibration standard was intentionally below the LOQ response.

3. The ECM analysis and several ILV surface water analyses were insufficient for the linearity of CL 354825. In the ECM, $r^2 = 0.9926$ (Appendix A, Figure A13, p. 29 of MRID 50120105). In the ILV, $r^2 = 0.9831$ and 0.9932 for the quantitation and tertiary ion transition, respectively (Figures 1-4, pp. 37-41 of MRID 50182301). Linearity is satisfactory when $r^2 \geq 0.995$. However, in the case of the ILV tertiary ion analysis, the reviewer noted that 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.

4. The water matrices were not characterized in the ECM and ILV. the ECM, the ground water was obtained from trial sites of BASF Study 97799 (Prospective Groundwater Study) conducted in the US (p. 12 of MRID 50120105). In the ILV, the drinking and surface water matrices were provided by the Sponsor (p. 17 of MRID 50182301). The ECM and ILV water matrices were not characterized; the specific sources of the water matrices were not reported.

5. The ILV representative chromatograms of the confirmation and tertiary ion analyses did not support the specificity of the method for CL 354825 in drinking or surface water (Figures 9-20, pp. 74-93 of MRID 50182301). In the drinking water analysis, the confirmation ion peak was very small at LOQ. In the surface water analysis, the confirmation ion peak co-eluted with a peak which was *ca*. 6×LOQ peak area, and matrix interferences were *ca*. 50% of the LOQ (based on peak area) in the tertiary ion control chromatogram. However, the reviewer noted that 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. Therefore, the deficiencies of the LOQ confirmation ion analysis of these enantiomers of imazapic did not affect the acceptability of the method.

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 (pp. 6, 9, 14 of MRID 50120105; pp. 20-21 of MRID 50182301). In the ECM and ILV, the LOQ was defined as the lowest fortification level successfully tested. The LOD was set at 20% of the LOQ in the ECM and ILV 2 and was justified by the fact that the lowest standard for each analyte in the calibration curve had a signal-to-noise ratio > 3:1. No calculations or comparisons to background levels were reported to justify the LOQ for the method in the ECM and ILV; no calculations were reported to justify the LOD for the method in the ECM and ILV.

7. The communications between the ILV and BASF Study Monitor and personnel were documented in Appendix A (p. 21; Appendix A, pp. 96-98 of MRID 50182301). The communication involved the some directives from the BASF Study Monitor (Robert Gooding) and updates and explanations from the ILV study author (Steven Perez).

8. It was reported for the ILV that one sample set of 13 samples required *ca*. 13 hours of work (p. 20 of MRID 50182301).

V. References

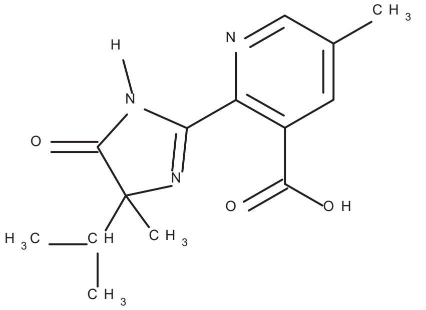
U.S. Environmental Protection Agency. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.

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
2-[4-Isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl]-5-methylnicotinic acid
CAS Name: 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



CL 354825 (5-Carboxy-3-hydroxy pyridine imidazolinone)

IUPAC Name: 5-Hydroxy-6-(isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl)nicotinic acid
CAS Name: Not reported
CAS Number: None
SMILES String: Not found