

**Analytical method for imazapic and its metabolite CL 354825 in soil**

**Reports:** ECM: EPA MRID No.: 50120103. Panek, M.G. 2004. Validation of BASF Method D0307: Analytical Method for the LC/MS/MS Determination of BAS 715 H and Metabolite CL 354825 in Soil. BASF Protocol No.: 121735. BASF Registration Document No.: 2003/5000549. Report prepared, sponsored and submitted by BASF Corporation, Research Triangle Park, North Carolina; 98 pages. Final report issued January 5, 2004.

ILV: EPA MRID No. 50120104. Stewart, J. 2004. Independent Laboratory Validation of BASF Analytical Method D0307 entitled "Analytical Method for the LC/MS/MS Determination of Imazapic (BAS 715 H) and Metabolite CL 354825 in Soil". BASF Study No.: 92327. BASF Registration Document No.: 2003/5000570. Report prepared, sponsored and submitted by BASF Corporation, Research Triangle Park, North Carolina; 161 pages. Final report issued December 20, 2016.

**Document No.:** MRIDs 50120103 & 50120104

**Guideline:** 850.6100

**Statements:** ECM: The study was conducted in accordance with USEPA FIFRA Good Laboratory Practice (GLP) standards, 40 CFR, Part 160 (p. 3 of MRID 50120103). Signed and dated No Data Confidentiality, GLP, and Authenticity statements were provided (pp. 2-3, 5). A signed Quality Assurance statement was provided, but the signature was not dated (p. 4).

ILV: The study was conducted in accordance with USEPA FIFRA GLP standards (p. 3 of MRID 50120104). Signed and dated No Data Confidentiality, GLP, Quality Assurance, and Authenticity statements were provided (pp. 2-5).

**Classification:** This analytical method is classified as unacceptable. Only the C18 SPE sample processing was validated by the ILV. The ECM and ILV calibration curves did not adequately bracket the instrument response for the LOQ sample. The linearity of the method was not supported by the ECM and ILV calibration curves. The soil matrix was not characterized, and the LOD was not reported in the ILV. This analytical method validation report for soil was required to support a small-scale prospective ground-water monitoring (PGW) study MRID 47001701. The lowest toxicological level of concern in soil for imazapic (0.13 ppb) is less than the method LOQ (1 ppb). A new ILV should be submitted which is independent of the ECM.

**PC Code:** 129041

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

Signature: IWONA MAHER  
Date: 9-5-2019

Digitally signed by IWONA MAHER  
Date: 2019.09.05  
12:55:29 -04'00'

**CDM/CSS-Dynamac JV Reviewers:** 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 D0307, is designed for the quantitative determination of imazapic (BAS 715 H) and its major metabolite CL 354825 in soil at the LOQ of 0.001 mg/kg (equivalent to 1 ppb) using LC/MS/MS. The LOQ is greater than the lowest toxicological level of concern in soil for imazapic (0.13 ppb). The ECM validated the method using characterized sand and loamy sand soil matrices. The ILV validated the method for imazapic and CL 354825 in uncharacterized soil imazapic and CL 354825 in soil was validated in the second trial with insignificant modifications to the analytical method; the first trial failed due to analyte contamination which caused very high recoveries. Only the C18 SPE sample processing was validated by the ILV, not the optional C18 & SCX SPE sample processing. However, ILV was not conducted independently of the ECM due to collusion between the ILV study author and ECM study author throughout the ILV trials. A new ILV should be submitted which is independent of the ECM. All ECM and ILV data regarding repeatability, accuracy, precision, and specificity were satisfactory for imazapic and CL 354825, except for the recovery of CL 354825 in the German soil at the LOQ using C18 SPE sample processing. 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 both analytes in the ECM and ILV. The LOD was not reported 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) | 50120103 <sup>1</sup>          | 50120104 <sup>2,3</sup>           |            | Soil   | 05/01/2004               | BASF Corporation | LC/MS/MS | 0.001 mg/kg                 |
| CL 354825                       |                                |                                   |            |        |                          |                  |          |                             |

1 In the ECM, the sand soil (bulksoil031102-A; 0.0-0.5': 91% sand, 4% silt, 5% clay, pH 5.0, 1.4% organic carbon; 0.5-1.0': 91% sand, 4% silt, 5% clay, pH 5.6, 0.6% organic carbon) was obtained from the North Carolina site used for a BAS 715 H small-scale ground water monitoring study (BASF Study No. 97799); soil characterization performed by Agvise Laboratories, Northwood, North Dakota (p. 13; Appendix B, pp. 54-55 of MRID 50120103). The loamy sand soil (2.2 F2.22100; 85.8% sand, 12.9% silt, 1.2% clay, pH 6.1, 2.25% organic carbon) was obtained as a standard from Germany; soil characterization performed by the soil provider. USDA soil texture classification was employed for both soils.

2 In the ILV, the soil was obtained from BASF Agro Research Study No. 121235 which was cited as BASF Study No. 97799 (a BAS 715 H small-scale ground water monitoring study in North Carolina) in the ILV reference section (pp. 10, 14 of MRID 50120104). The soil matrix was not characterized, but the BASF study source was the same as that of the ECM.

3 Only C18 SPE sample processing was validated by the ILV, not the optional C18 & SCX SPE sample processing.

## I. Principle of the Method

Samples (10.0 g) were transferred to a centrifuge bottle and fortified, as necessary, with imazapic or CL 354825 fortification solutions, and mixed well (pp. 13-14; Figures 1-2, pp. 20-21; Appendix C, pp. 67-70 of MRID 50120103). The soil was extracted twice with 20 mL of 0.5 N NaOH by shaking on a reciprocal shaker at 250 rpm for 30 minutes at room temperature. After centrifugation at 3000 rpm for 15 minutes, the supernatant was transferred to a graduated cylinder. The volume of the combined extracts was adjusted to 40.0 mL with water (if filter was used, wash with 2-3 mL of water and adjust volume to 45.0 mL). An aliquot (5.0 mL) of the combined extract was treated with 1 mL of 5.0 M formic acid to pH 3, vortexed, and centrifuged (2000 rpm for 10 minutes) to precipitate the humic material. An aliquot (1 mL) of the acidic supernatant was purified via C18 solid phase extraction (SPE; 200 mg, 3 mL) preconditioned with one-column volume consecutively of hexane, dichloromethane, methanol, water, and 0.01 N HCl. After adding the sample aliquot, the cartridge was washed with 1% formic acid and then hexane, one-column volume of each. The analytes were eluted with dichloromethane, two-column volume. The eluate was reduced to dryness via N-evap. The residue was reconstituted in water and analyzed by LC/MS/MS. If the recovery of the analytes was low, the aliquot of the acidic supernatant should be eluted from the C18 SPE with dichloromethane. Then, the C18 SPE cartridge was connected to a SCX SPE (200 mg, 3 mL) cartridge preconditioned with one-column volume of methanol. The C18 SPE cartridge was eluted with one-column volume of methanol onto the SCX SPE cartridge. The SCX SPE was washed with methanol, one-column volume. The analytes were eluted by water:methanol (20:80, v:v), two-column volume. The combined eluates [dichloromethane and water:methanol (20:80, v:v)] were reduced to dryness via N-evap. The residue was reconstituted in water and analyzed by LC/MS/MS.

The method noted that the sample processing procedure must be carried-out to the acidification step prior to stopping otherwise loss of recoveries of the analytes was observed (Appendix C, p. 68 of MRID 50120103).

Samples were analyzed for analytes using a PE Series 200 Micro Pump system coupled to a PE Sciex API 3000 Biomolecular Mass Analyzer (p. 14; Appendix C, pp. 70-71 of MRID 50120103). The LC/MS conditions consisted of a TSK-gel Super ODS column (2.0 x 50.0 mm, 2- $\mu$ m; 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 0.0-0.5 min. 90:10, 4.0 min. 50:50, 7.0 min. 15:85, 7.5-9.5 min. 10:90, 9.6-10.6 min. 90:10] and MS/MS detection in ESI positive ion mode (ionization temperature not reported). Injection volume was 25  $\mu$ L. One ion transition was monitored per analyte:  $m/z$  276.1 $\rightarrow$ 231.0 for imazapic and  $m/z$  278.1 $\rightarrow$ 232.9 for CL 354825. Retention times were *ca.* 4.4 and 6.3 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 (p. 10 of MRID 50120104). A Sciex API 4000 LC/MS/MS was used in the second trial, due to unavailability of the API 3000. Most of the LC/MS conditions were the same, except that the collection window as extended by 30 seconds for trials one and two and by 1.5 minutes for the second injection of trial two to optimize signal intensity. One ion transition was monitored per analyte:  $m/z$  276.1 $\rightarrow$ 231.0 for imazapic and  $m/z$  278.1 $\rightarrow$ 233.0 for CL 354825; retention times were *ca.* 6-7 and 6.5 minutes for imazapic and CL 354825, respectively (Figures 7-8, pp. 24-25; Figures 16-17, pp. 33-34). Based on the results of the ILV trials using only the C18

SPE sample processing, the study author determined that the addition of SCX SPE sample processing was not required (pp. 12-13). The ILV modifications were considered insignificant.

The Limit of Quantification (LOQ) was 0.001 mg/kg for imazapic and CL 354825 in soil in the ECM and ILV (pp. 11, 14; Appendix C, p. 72 of MRID 50120103; pp. 6, 11 of MRID 50120104). The Limit of Detection (LOD) was 0.048 µg/kg for imazapic and CL 354825 in soil in the ECM; the LOD was not reported in the ILV.

## II. Recovery Findings

ECM (MRID 50120103): 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 two soil matrices at fortification levels of 0.001 mg/kg (LOQ) and 0.01 mg/kg (10×LOQ) using C18 SPE and C18 SPE & SCX SPE sample processing, except for the analysis of CL 354825 in the German soil at the LOQ using C18 SPE sample processing (RSD 22.9%; Tables 1-3, pp. 25-27). Means and relative standard deviations (RSDs) were reviewer-calculated for the analysis of the North Carolina soil extracts after C18 SPE & SCX SPE sample processing since the study author excluded the results of one sample at LOQ and 10×LOQ for both analytes; the study author reported that those sample solutions were partially spilled after SPE elution. North Carolina soil recovery results were corrected for residues found in controls (Figure 3, pp. 22-24; Table 2, p. 26). One ion transition was monitored for imazapic and CL 354825; a confirmatory method is not usually required when LC/MS and GC/MS is the primary method. The sand soil (bulksoil031102-A; 0.0-0.5': 91% sand, 4% silt, 5% clay, pH 5.0, 1.4% organic carbon; 0.5-1.0': 91% sand, 4% silt, 5% clay, pH 5.6, 0.6% organic carbon) was obtained from the North Carolina site used for a BAS 715 H small-scale ground water monitoring study (BASF Study No. 97799); soil characterization performed by Agvise Laboratories, Northwood, North Dakota (p. 13; Appendix B, pp. 54-55). The loamy sand soil (2.2 F2.22100; 85.8% sand, 12.9% silt, 1.2% clay, pH 6.1, 2.25% organic carbon) was obtained as a standard from Germany; soil characterization performed by the soil provider. USDA soil texture classification was employed for both soils.

ILV (MRID 50120104): Mean recoveries and RSDs were within guideline requirements for analysis of imazapic and its metabolite CL 354825 in one soil matrix at fortification levels of 0.001 mg/kg (LOQ) and 0.01 mg/kg (10×LOQ) using C18 SPE sample processing (Table 1, p. 11). One ion transition was monitored for imazapic and CL 354825. Based on the results of the ILV trials using only the C18 SPE sample processing, the study author determined that the addition of SCX SPE sample processing was not required (pp. 12-13). The soil was obtained from BASF Agro Research Study No. 121235 which was cited as BASF Study No. 97799 (a BAS 715 H small-scale ground water monitoring study in North Carolina, MRID 47001701) in the reference section (pp. 10, 14). The soil matrix was not characterized, but the BASF study source was the same as that of the ECM. The method for imazapic and CL 354825 in soil was validated in the second trial with insignificant modifications to the analytical method; the first trial failed due to analyte contamination which caused very high recoveries (pp. 6, 11-12).

**Table 2. Initial Validation Method Recoveries for Imazapic and CL 354825 in Soil<sup>1,2</sup>**

| Analyte  | Fortification Level (mg/kg) | Number of Tests | Recovery Range (%) | Mean Recovery (%) | Standard Deviation (%) <sup>3</sup> | Relative Standard Deviation (%) |
|--|-----------------------------|-----------------|--------------------|-------------------|-------------------------------------|---------------------------------|
| <b>North Carolina Soil - Sand</b>              |                             |                 |                    |                   |                                     |                                 |
| C18 SPE  |                             |                 |                    |                   |                                     |                                 |
| Imazapic<br>(CL 263222;<br>BAS 715 H)          | 0.001 (LOQ)                 | 5               | 95-109             | 102               | 5                                   | 4.6                             |
|  | 0.01                        | 5               | 87-99              | 92                | 5                                   | 5.3                             |
| CL 354825                                      | 0.001 (LOQ)                 | 5               | 98-118             | 111               | 8                                   | 7.5                             |
|  | 0.01                        | 5               | 84-125             | 106               | 15                                  | 13.9                            |
| C18 & SCX SPE <sup>4</sup>                     |                             |                 |                    |                   |                                     |                                 |
| Imazapic<br>(CL 263222;<br>BAS 715 H)          | 0.001 (LOQ)                 | 5               | 72-111             | 95                | 14                                  | 15                              |
|  | 0.01                        | 5               | 71-103             | 94                | 13                                  | 14                              |
| CL 354825                                      | 0.001 (LOQ)                 | 5               | 70-101             | 88                | 11                                  | 13                              |
|  | 0.01                        | 5               | 80-112             | 97                | 12                                  | 12                              |
| <b>German (2.2 F2.22100) Soil – Loamy Sand</b> |                             |                 |                    |                   |                                     |                                 |
| C18 SPE  |                             |                 |                    |                   |                                     |                                 |
| Imazapic<br>(CL 263222;<br>BAS 715 H)          | 0.001 (LOQ)                 | 5               | 87-117             | 100               | 12                                  | 11.7                            |
|  | 0.01                        | 5               | 66-100             | 89                | 14                                  | 15.3                            |
| CL 354825                                      | 0.001 (LOQ)                 | 5               | 46-88              | 73                | 17                                  | <b>22.9</b>                     |
|  | 0.01                        | 5               | 67-98              | 83                | 13                                  | 15.9                            |
| C18 & SCX SPE                                  |                             |                 |                    |                   |                                     |                                 |
| Imazapic<br>(CL 263222;<br>BAS 715 H)          | 0.001 (LOQ)                 | 5               | 89-109             | 99                | 8                                   | 8.5                             |
|  | 0.01                        | 5               | 88-104             | 96                | 6                                   | 6.2                             |
| CL 354825                                      | 0.001 (LOQ)                 | 5               | 70-94              | 81                | 10                                  | 12.1                            |
|  | 0.01                        | 5               | 83-111             | 93                | 11                                  | 12.3                            |

Data (corrected recovery results when residues found in controls (NC soil only; Figure 3, pp. 22-24; Table 2, p. 26) were obtained from Tables 1-3, pp. 25-27 of MRID 50120103 and DER Attachment 2.

1 The sand soil (bulksoil031102-A; 0.0-0.5': 91% sand, 4% silt, 5% clay, pH 5.0, 1.4% organic carbon; 0.5-1.0': 91% sand, 4% silt, 5% clay, pH 5.6, 0.6% organic carbon) was obtained from the North Carolina site used for a BAS 715 H small-scale ground water monitoring study (BASF Study No. 97799); soil characterization performed by Agvise Laboratories, Northwood, North Dakota (p. 13; Appendix B, pp. 54-55). The loamy sand soil (2.2 F2.22100; 85.8% sand, 12.9% silt, 1.2% clay, pH 6.1, 2.25% organic carbon) was obtained as a standard from Germany; soil characterization performed by the soil provider. USDA soil texture classification was employed for both soils.

2 One ion transition was monitored per analyte:  $m/z$  276.1→231.0 for imazapic and  $m/z$  278.1→232.9 for CL 354825.

3 Standard deviations (s.d.s) 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.

4 The study author excluded the results of one sample at LOQ and 10×LOQ for both analytes because the sample solutions were partially spilled after SPE elution (Table 2, p. 26).

**Table 3. Independent Validation Method Recoveries for Imazapic and CL 354825 in Soil<sup>1,2</sup>**

| Analyte                               | Fortification Level (mg/kg) | Number of Tests | Recovery Range (%) | Mean Recovery (%) | Standard Deviation (%) | Relative Standard Deviation (%) |
|---------------------------------------|-----------------------------|-----------------|--------------------|-------------------|------------------------|---------------------------------|
| Soil <sup>2</sup>                     |                             |                 |                    |                   |                        |                                 |
| C18 SPE                               |                             |                 |                    |                   |                        |                                 |
| Imazapic<br>(CL 263222;<br>BAS 715 H) | 0.001 (LOQ)                 | 5               | 89-106             | 98                | 7                      | 7                               |
|                                       | 0.01                        | 5               | 89-98              | 95                | 3                      | 4                               |
| CL 354825                             | 0.001 (LOQ)                 | 5               | 66-76              | 73                | 4                      | 6                               |
|                                       | 0.01                        | 5               | 61-78              | 70                | 7                      | 9                               |

Data (uncorrected recovery results, Table 2, p. 15) were obtained from Table 1, p. 11 of MRID 50120104.

1 The soil was obtained from BASF Agro Research Study No. 121235 which was cited as BASF Study No. 97799 (a BAS 715 H small-scale ground water monitoring study in North Carolina) in the reference section (pp. 10, 14). The soil matrix was not characterized, but the BASF study source was the same as that of the ECM.

2 One ion transition was monitored per analyte:  $m/z$  276.1→231.0 for imazapic and  $m/z$  278.1→233.0 for CL 354825.

### III. Method Characteristics

The LOQ was 0.001 mg/kg for imazapic and CL 354825 in soil in the ECM and ILV (pp. 11, 14; Appendix C, p. 72 of MRID 50120103; pp. 6, 11 of MRID 50120104). In the ECM, the LOQ was defined as the lowest fortification level successfully tested. No calculations or comparisons to background levels were reported to justify the LOQ for the method. In the ILV, the LOQ was reported from the ECM without justification. The LOD was 0.048 µg/kg for imazapic and CL 354825 in soil in the ECM; the LOD was not reported in the ILV. In the ECM, the LOD was defined by signal-to-noise ratio and set at 25% of the lowest calibration standard (0.02 ng/mL, equivalent to 0.192 µg/kg); no calculations were reported to justify the LOD for the method.

**Table 4. Method Characteristics**

| Analyte  |                      | Imazapic<br>(CL 263222; BAS 715 H)  | CL 354825  |
|--|----------------------|---|--|
| Limit of Quantitation (LOQ)  | ECM                  | 0.001 mg/kg   |  |
|  | ILV                  |   |  |
| Limit of Detection (LOD)   | ECM                  | 0.048 µg/kg   |  |
|  | ILV                  |   |  |
| Linearity (calibration curve $r^2$ and concentration range) <sup>1</sup> | ECM <sup>2</sup>     | $r^2 = \mathbf{0.9900}$   | $r^2 = \mathbf{0.9884}$  |
|  | ILV                  | $r^2 = \mathbf{0.9882}$   | $r^2 = \mathbf{0.9944}$  |
|  |                      | 0.02-0.2 ng/mL  |  |
| Repeatable   | ECM <sup>2,3,4</sup> | C18 SPE   | Yes at LOQ and 10×LOQ (characterized sand and loamy sand soil matrices)<br>Yes at LOQ and 10×LOQ (characterized sand soil matrix)<br><b>No</b> at LOQ (RSD <b>22.9%</b> ), and<br>Yes at 10×LOQ (characterized loamy sand soil matrix) |
|  |                      | C18 & SCX SPE   | Yes at LOQ and 10×LOQ (characterized sand <sup>5</sup> and loamy sand soil matrices)   |
|  | ILV <sup>6,7</sup>   | C18 SPE   | Yes at LOQ and 10×LOQ (one uncharacterized soil matrix)  |
|  |                      | C18 & SCX SPE   | <b>Not performed</b>   |
| Reproducible   | C18 SPE              | Yes at LOQ and 10×LOQ   |  |
|  | C18 & SCX SPE        | <b>Could not be determined</b>  |  |
| Specific   | ECM                  | Matrix interferences were <1% of the LOQ (based on peak area). Minor baseline noise interfered with peak integration. | Matrix interferences were <i>ca.</i> 12% and <1% of the LOQ (based on peak area) in the sand and loamy sand soils, respectively. Minor baseline noise interfered with peak integration.  |
|  | ILV                  | No matrix interferences were observed or quantified.  |  |

Data were obtained from pp.; Tables 1-3, pp. 25-27 (recovery data); Appendix A, Figures A3-A4, pp. 44-45 (calibration curves); Appendix A, Figures A5-A12, pp. 46-53 (chromatograms) of MRID 50120103; pp.; Table 1, p. 11 (recovery data); Figure 5, p. 22 and Figure 13, p. 31 (calibration curves); Figures 6-9, pp. 23-26 and Figures 15-18, pp. 32-35 (chromatograms) of MRID 50120104; and DER Attachment 2.

<sup>1</sup> Correlation coefficients ( $r^2$ ) values were reviewer-calculated from  $r$  values provided in the study report (Appendix A, Figures A3-A4, pp. 44-45 of MRID 50120103; Figure 5, p. 22 and Figure 13, p. 31 of MRID 50120104; DER Attachment 2). Solvent-based calibration standards were used.

<sup>2</sup> One ion transition was monitored for each analyte. A confirmatory method is not usually required when LC/MS and GC/MS is the primary method.

<sup>3</sup> In the ECM, the sand soil (bulksoil031102-A; 0.0-0.5': 91% sand, 4% silt, 5% clay, pH 5.0, 1.4% organic carbon; 0.5-1.0': 91% sand, 4% silt, 5% clay, pH 5.6, 0.6% organic carbon) was obtained from the North Carolina site used for a BAS 715 H small-scale ground water monitoring study (BASF Study No. 97799); soil characterization performed by Agvise Laboratories, Northwood, North Dakota (p. 13; Appendix B, pp. 54-55 of MRID 50120103). The loamy sand soil (2.2 F2.22100; 85.8% sand, 12.9% silt, 1.2% clay, pH 6.1, 2.25% organic carbon) was obtained as a standard from Germany; soil characterization performed by the soil provider. USDA soil texture classification was employed for both soils.

<sup>4</sup> North Carolina sand soil recovery results were corrected for residues found in controls (Figure 3, pp. 22-24; Table 2, p. 26 of MRID 50120103).

<sup>5</sup> Means and relative standard deviations (RSDs) were reviewer-calculated for the analysis of the North Carolina sand soil extracts after C18 SPE & SCX SPE sample processing since the study author omitted recovery values of samples which were spilled during sample processing (see DER Attachment 2).

<sup>6</sup> In the ILV, the soil was obtained from BASF Agro Research Study No. 121235 which was cited as BASF Study No. 97799 (a BAS 715 H small-scale ground water monitoring study in North Carolina) in the ILV reference section

(pp. 10, 14 of MRID 50120104). The soil matrix was not characterized, but the BASF study source was the same as that of the ECM.

7 The method for imazapic and CL 354825 in soil was validated in the second trial with insignificant modifications to the analytical method; the first trial failed due to analyte contamination which caused very high recoveries (pp. 6, 11-12 of MRID 50120104).

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

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

1. The ILV was not conducted independently of the ECM; a new ILV should be submitted which is independent of the ECM. The ILV and ECM were same laboratory and location: by BASF Corporation, 26 Davis Drive, Research Triangle Park, North Carolina (p. 1 of MRID 50120103; p. 1 of MRID 50120104). In this case, OCSPP guidelines state that if the laboratory that conducted the validation belonged to the same organization as the originating laboratory, the analysts, study director, equipment, instruments, and supplies of the two laboratories must have been distinct and operated separately and without collusion. Also, the analysts and study director of the ILV must have been unfamiliar with the method both in its development and subsequent use in field studies. In the list of laboratory personnel, no personnel of the ECM were listed in the ILV personnel list (p. 10 of MRID 50120103; p. 8 of MRID 50120104). However, communications between the ILV study author and ECM study author (referred to as the principal developer in the ILV communication documentation) occurred prior to and throughout the ILV trials (pp. 12-13 of MRID 50120104). The communication involved none technical communication and result updates, but it also contained discussions of the causes of 1) the contamination which caused the first ILV trial to fail, 2) the unacceptable calibration curve of CL 354825, and 3) the shifting retention times of imazapic. These discussions qualify as collusion between the ECM and ILV. Additionally, the ECM and ILV did not list all laboratory equipment and laboratory instrument serial numbers in order to ensure that the equipment and instruments used in the ILV were distinct from those in the ECM. The reviewer noted that the ILV report that a different LC/MS/MS instrument was used for the second trial because the API 3000 "suggested in the method" was not available (p. 10 of MRID 50120104).
2. The ECM method contained two sample processing options: a primary C18 SPE clean-up sample processing and an optional C18 & SCX SPE sample clean-up processing (pp. 13-14; Figures 1-2, pp. 20-21; Appendix C, pp. 67-70 of MRID 50120103). The C18 & SCX SPE sample processing was presented in cases where analyte recovery, especially of CL 354825, was unsatisfactory. The optional C18 & SCX SPE sample clean-up processing was not validated by the ILV because the optional clean-up was deemed unnecessary based on the results of the ILV trial results; therefore, the reproducibility of this portion of the ECM method could not be determined (pp. 12-13 of MRID 50120104).
3. The ECM and ILV calibration curves did not adequately bracket the instrument response for the LOQ sample. The calibration standards were 0.02, 0.04, 0.10, and 0.20 ng/mL in the ECM and ILV. In both studies, there was only one calibration standard above the response of the LOQ, based on peak areas or intensities. 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 3613 counts, while the two highest calibration standards yielded peak areas of 3134-3399 and 7243 counts for the 0.1 and 0.2 ng/mL calibration standards, respectively (Appendix A, Figure A1, p. 41, Figure A6, p. 47, and Figure A10, p. 51 of MRID 50120103). Based on the ILV representative chromatograms, the LOQ peak area was *ca.* 1300 cps, while the two highest calibration standards yielded approximate peak intensities of 1300 and 2600 cps for the 0.1 and 0.2 ng/mL calibration standards, respectively (Figures 3-4, pp. 20-21 and Figure 7, p. 24 of MRID 50120104).

CL 354825: Based on the ECM representative chromatograms, the LOQ peak area was 3205-3340 counts, while the two highest calibration standards yielded peak areas of 2974 and 6401 counts for the 0.1 and 0.2 ng/mL calibration standards, respectively (Appendix A, Figure A2, p. 43, Figure A8, p. 49 and Figure A12, p. 53 of MRID 50120103). Based on the ILV representative chromatograms, the LOQ peak area was *ca.* 1300 cps, while the two highest calibration standards yielded approximate peak intensities of 1400 and 3000 cps for the 0.1 and 0.2 ng/mL calibration standards, respectively (Figures 12-13, pp. 29-30 and Figure 16, p. 33 of MRID 50120104).

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 above the LOQ response in general.

4. All of the ECM and ILV analyses were insufficient for the linearity of both analytes. In the ECM,  $r^2 = 0.9900$  and  $0.9884$  for imazapic and CL 354825, respectively (Appendix A, Figures A3-A4, pp. 44-45 of MRID 50120103). In the ILV,  $r^2 = 0.9882$  and  $0.9944$  for imazapic and CL 354825, respectively (Figure 5, p. 22 and Figure 13, p. 31 of MRID 50120104). Linearity is satisfactory when  $r^2 \geq 0.995$ .
5. The ECM LOQ is greater than the lowest toxicological level of concern in soil for imazapic (0.13 ppb). Therefore, the ECM is not adequate to address the risk concern to terrestrial plants which are the most sensitive species.
6. The soil matrices were not characterized in the ILV; therefore, it could not be determined if the ILV was provided with the most difficult matrix with which to validate the method. The soil was obtained from BASF Agro Research Study No. 121235 which was cited as BASF Study No. 97799 (a BAS 715 H small-scale ground water monitoring study in North Carolina, MRID 47001701) in the ILV reference section (pp. 10, 14 of MRID 50120104). The reviewer noted that the BASF study source was the same as that of the North Carolina sand soil used in the ECM; therefore, the ILV soil should be the same North Carolina soil as the one used in the ECM. However, the North Carolina soil was collected and characterized in several half-foot depth increments (Appendix B, p. 55 of MRID 50120103). In the ECM, the study author specified that the test soil was collected from the 0-1' depth (p. 13). The soil collection depth was not specified in the ILV; therefore, the reviewer could not determine which soil classification applied to the ILV test soil.
7. The analysis of CL 354825 in the German soil at the LOQ using C18 SPE sample

- processing was not acceptable (RSD 22.9%; Tables 3, p. 27 of MRID 50120103). OCSPP Guideline 850.6100 criteria for precision and accuracy state that the mean recoveries for replicates at each spiking level are between 70% and 120% and relative standard deviations (RSD)  $\leq 20\%$  at the stated LOQ and at higher concentrations.
8. 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. 11, 14; Appendix C, p. 72 of MRID 50120103; pp. 6, 11 of MRID 50120104). In the ECM, the LOQ was defined as the lowest fortification level successfully tested. No calculations or comparisons to background levels were reported to justify the LOQ for the method. In the ILV, the LOQ was reported from the ECM without justification. In the ECM, the LOD was defined by signal-to-noise ratio and set at 25% of the lowest calibration standard (0.02 ng/mL, equivalent to 0.192  $\mu\text{g}/\text{kg}$ ); no calculations were reported to justify the LOD for the method. The LOD was not reported in the ILV.
  9. In the ILV, the retention time of imazapic shifted a great deal between *ca.* 6-7 minutes (Figures 7-8, pp. 24-25; Figures 16-17, pp. 33-34 of MRID 50120104). The ILV study author noted that extending the LC/MS/MS equilibration time after the gradient can reduce retention time shifting (p. 13). The reviewer noted that the retention time of the imazapic standard was consistently *ca.* 6 minutes (Figures 1-4, pp. 18-21).
  10. In the ECM, extract stability of the acidified soil extracts was determined to be acceptable (recoveries 74-118% for both analytes) up to 40 hours at room temperature, based on the LC/MS/MS re-analysis of the North Carolina soil extracts (p. 14; Table 2, p. 26 of MRID 50120103). The storage stability of the stock solutions was found to be acceptable up to 2 months and 10 days with refrigeration (1-10°C) in amber glass bottles; the storage stability of the calibration standards was found to be acceptable up to *ca.* 1 months with refrigeration (1-10°C) in amber glass bottles (p. 16; Table 4, p. 28).
  11. The ILV report reported a few minor suggestions for the Method D0307, including the correction of typographical errors, listing the concentrations of stock solutions, and including the suggestion of extending the instrument equilibration time to reduce analyte peak shifting (p. 13 of MRID 50120104).
  12. It was reported for the ILV that the method required *ca.* 8 hours of work for one analyst; LC/MS/MS was performed overnight (p. 13 of MRID 50120104).

## 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.

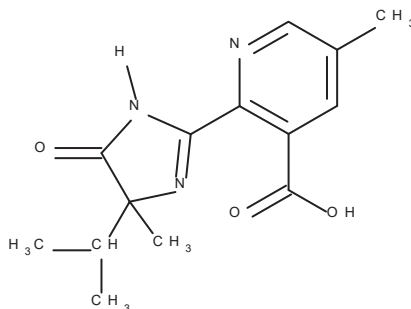
**Attachment 1: Chemical Names and Structures****Imazapic (BAS 715 H; AC 263222; CL 263,222)**

**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

**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

