Analytical method for L-glufosinate (Reg. No. 6113987) and D-glufosinate (Reg. No. 6113988) [enantiomers of glufosinate (BAS 1000 H)] in water and soil

Reports: ECM: EPA MRID No.: 51693101. Gordon, B. 2021. Validation of BASF Analytical Method R0085/01: "Method for the separate determination of the D- (Reg. No. 6113988) and L- (Reg. No. 6113987) enantiomers of glufosinate (BAS 1000 H) in soil and water by chiral LC-MS/MS". Report prepared by BASF Corporation, Research Triangle Park, North Carolina, and Agvise Laboratories, Northwood, North Dakota, and sponsored and submitted by BASF Corporation, Research Triangle Park, North Carolina; 187 pages. BASF Study No.: 919195. BASF Registration Document No.: 2021/2032207. Final report issued September 10, 2021.

ILV: EPA MRID No.: 51693102. Perez, R. 2021. Independent Laboratory Validation of the Analytical Method for the Determination of D- (Reg. No. 6113988) and L- (Reg. No. 6113987) Enantiomers of Glufosinate (BAS 1000 H) in Soil and water by Chiral LC-MS/MS. Report prepared by ADPEN Laboratories, Inc., Jacksonville, Florida, and sponsored and submitted by BASF Corporation, Research Triangle Park, North Carolina; 256 pages. ADPEN Study No.: 21G0404. BASF Study No.: 919195_1. BASF Registration Document No.: 2021/2034535. Final report issued September 17, 2021.

Document No.: MRIDs 51693101 & 51693102

Guideline: 850.6100

Statements: ECM 1: The report was conducted in compliance with USEPA FIFRA Good Laboratory (GLP) standards (40 CFR Part 160; p. 3 of MRID 51693101). Signed and dated Data Confidentiality, GLP, Quality Assurance, and Authenticity statements were provided (pp. 2-5).
ILV: The study was conducted in compliance with USEPA FIFRA GLP standards (40 CFR Part 160; p. 3 of MRID 51693102). Signed and dated Data Confidentiality, GLP, Quality Assurance, and Authenticity statements were provided (pp. 2-5).

Classification: This analytical method is classified as **acceptable**. Since the reported method LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported limit of quantification (LOQ) is the lowest level of method validation (LLMV) rather than LOQ. The specificity of the method for L-glufosinate in ground water was not supported by ILV representative chromatograms. The submitted ECM study report included the ILV recommendations regarding chromatography. It could not be determined if the ILV validation was conducted independently of the internal validation (ECM). The ILV water and soil matrices were the same as those used in the ECM validation. Control soil matrix chromatograms were not provided/integrated for L-glufosinate in the ILV.

PC Code: 128300

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This Data Evaluation Record may have been altered by the Environmental Fate and Effects Division subsequent to signing by CDM/CSS-Dynamac JV personnel. The CDM/CSS-Dynamac Joint Venture role does not include establishing Agency policies.

Executive Summary

The analytical method, BASF Analytical Method R0085/01, is designed for the quantitative determination of L-glufosinate (Reg. No. 6113987) and D-glufosinate (Reg. No. 6113988) [enantiomers of glufosinate (BAS 1000 H)] in water at the stated LOQ of 0.0025 mg/kg and in soil at the stated LOQ of 0.0020 mg/kg using LC-MS/MS. The LOQs are less than the lowest toxicological level of concern in water and soil for L-glufosinate and D-glufosinate, based on the most sensitive toxicity endpoint of 0.018 mg/L in water and 0.013 mg/kg in soil[1](MRIDs 41396112, 41396113, 51036697). Since the reported method LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV), the lowest concentration tested with sufficiently accurate and precise recoveries, rather than an LOQ. Based on the performance data submitted by the ILV and ECM, the LLMV for water analysis was equivalent to the ECM reported method LOQ for L-glufosinate and D-glufosinate in the tested surface water and soil matrices.

Both ECM and ILV validations used two characterized water matrices (ground and surface) and two characterized soil matrices (loamy sand and sandy loam). The ILV water and soil matrices were the same as those used in the ECM validation. The tests soils include a lower organic matter, lower clay content loamy sand soil and a higher organic matter, higher clay content sandy loam soil. The soil selection did not cover the range of textural classes found in the terrestrial field dissipation studies for racemic (50:50 mixture of D- and L-isomers) glufosinate.

The ILV validated method (BASF Analytical Method R0085/01) for L-glufosinate and D-glufosinate with minor modifications to the analytical parameters, the use of matrix-matched calibration standards for the water analysis, and the use of a different filter vial. For soil analysis, optimization of tubing from injection to HPLC column and from HPLC column to the mass spectrometer was required to get a good separation and peak shape of the enantiomers. Performance data from the first and second ILV trials were acceptable; however, modifications were incorporated into the second ILV trial to address chromatographic issues which were present in the first ILV trial. The submitted ECM study report included the ILV recommendations regarding

[1] Based on a 6-inch soil depth and soil density of 1.5 g/cm^3

chromatography. It could not be determined if the ILV was conducted independently of the internal validation (ECM) since the email correspondence involving technical issues between the ILV Study Director and the ILV Study Monitor was only summarized, not included in the ILV study report.

All ILV and ECM data regarding repeatability, accuracy, precision, linearity, and specificity at the LOQ (0.0025 mg/kg) were satisfactory for L-glufosinate and D-glufosinate in the tested water matrices; however, the specificity of the method for L-glufosinate in ground water was not supported by ILV representative chromatograms due to the fact that the LOQ analyte peak eluted as a broad multi-peaked signal. Additionally, control ground water matrix chromatograms were not provided/integrated for L-glufosinate in the ILV.

All ILV and ECM data regarding repeatability, accuracy, precision, linearity, and specificity at the LOQ (0.0020 mg/kg) were satisfactory for L-glufosinate and D-glufosinate in the tested soil matrices; however, control soil matrix chromatograms were not provided/integrated for L-glufosinate in the ILV.

All provided ILV chromatograms were reported from the second ILV trial.

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						
L-glufosinate	51693101[1]	51693102[2]		Water	10/09/2021	BASF Corporation	LC-MS/MS	0.0025 mg/kg (2.5 µg/L)
D-glufosinate								
L-glufosinate				Soil				0.0020 mg/kg (2.0 µg/kg)
D-glufosinate								

1 In the ECM, two water and two soil matrices were used in the study (p. 22; Appendix B, pp. 39-42 of MRID 51693101). Characterization reports were provided for all matrices: ground water (Sample ID: R21G0350002R02-R04; pH 7.9, hardness 715 mg equiv. $CaCO_3$/L, conductivity 1.20 mmhos/cm, total dissolved solids 1018 ppm), surface water (Sample ID: R21G0350001R02-R04; pH 8.2, hardness 690 mg equiv. $CaCO_3$/L, conductivity 1.26 mmhos/cm, total dissolved solids 1188 ppm), Washington loamy sand soil (Sample ID: DSC-037; Sample depth: 0-3"; 87% sand, 8% silt, 5% clay; pH 8.2 in saturated paste; 0.35% organic matter – Walkley Black; cation exchange capacity 7.7 meq/100 g), and MSL-PF sandy loam soil [Sample ID: MSL-PF (2017-24/SDBN496); 65% sand, 18% silt, 17% clay; pH 6.8 in 1:1 soil:water ratio; 3.9% organic matter – Walkley Black; cation exchange capacity 15.9 meq/100 g; USDA soil texture classification]. The characterization laboratory was Agvise Laboratories, Northwood, North Dakota. The soil textures were verified by the reviewer using USDA-NRCS technical support tools.

2 In the ILV, two water and two soil matrices were used in the study (p. 19; Appendix E, pp. 232-235 of MRID 51693102). Characterization reports were provided for all matrices: well water (Sample ID: R21G0350002R05and R21G0350002R02-R04; pH 7.9, hardness 715 mg equiv. $CaCO_3$/L, conductivity 1.20 mmhos/cm, total dissolved solids 1018 ppm), surface water (Sample ID: R21G0350001R05 and R21G0350001R02-R04; pH 8.2, hardness 690 mg equiv. $CaCO_3$/L, conductivity 1.26 mmhos/cm, total dissolved solids 1188 ppm), Washington loamy sand soil (Sample ID: R21G0560001 and DSC-037; Sample depth: 0-3"; 87% sand, 8% silt, 5% clay; pH 8.2 in saturated paste; 0.35% organic matter – Walkley Black; cation exchange capacity 7.7 meq/100 g), and MSL-PF sandy loam soil [Sample ID: R21G0550001 and MSL-PF (2017-24/SDBN496); 65% sand, 18% silt, 17% clay; pH 6.8 in 1:1 soil:water ratio; 3.9% organic matter – Walkley Black; cation exchange capacity 15.9 meq/100 g; USDA soil texture classification]. The characterization laboratory was Agvise Laboratories, Northwood, North Dakota. The soil textures were verified by the reviewer using USDA-NRCS technical support tools. The reviewer noted that the ILV used the same matrices as the ECM for the validation.

I. Principle of the Method

BASF Analytical Method R0085/01 – Water Matrices

Water samples (10 mL) were transferred to a centrifuge tube (15 mL) and fortified (0.5 mL of 0.05 or 0.5 µg/mL fortification solution; p. 25; Appendix C, pp. 46, 48-53 of MRID 51693101). The stable isotope (glufosinate hydrochloride-methyl-d3; 0.1 mL of 1 µg/mL solution) was added then the samples were mixed. An aliquot of the sample was transferred to a filter vial (0.45 µm PTFE); the filter piston was plunged slowly. The sample was analyzed by Chiral HPLC/MS/MS.

BASF Analytical Method R0085/01 – Soil Matrices

Soil samples (5.0 ± 0.05 g) were transferred to a centrifuge tube (50 mL) and fortified (0.2 µmL of 0.05 or 0.5 µg/mL fortification solution; p. 25; Appendix C, pp. 46, 48-53 of MRID 51693101). The

sample was extracted with 25 mL of water via shaking (shaker on high for 30 minutes). The stable isotope (glufosinate hydrochloride-methyl-d3; 0.05 mL of 1 µg/mL solution) was added then the samples were mixed. After centrifugation (*ca*. 3700 g for 5 minutes), the samples were decanted into new 50-mL centrifuge tubes then centrifuged (12000 g for 10 minutes) again prior to solid phase extraction (SPE) clean-up. The Oasis MAX SPE column (150 mg, 6 mL) was pre-conditioned with a column of methanol then a column of 5% ammonium hydroxide in water then the sample was loaded. The column was washed with a column of 5% ammonium hydroxide in water then a column of methanol then a column of 2% formic acid in methanol. The analytes were eluted with 6 mL of 2% formic acid in water. The eluate was reduced to dryness in a Turbo-Vap at 60°C. The residue was reconstituted with 1 mL of water via sonication and vortexing. An aliquot of the sample was transferred to a filter vial (0.45 µm PTFE); the filter piston was plunged slowly. The sample was analyzed by Chiral HPLC/MS/MS.

Chiral LC/MS/MS

Samples were analyzed for L-glufosinate and D-glufosinate using a Waters Acquity with FTN coupled with an API 6500+ LC/MS/MS with SelexION DMS operated in positive ESI ionization mode with multiple reaction monitoring (MRM; p. 25; Appendix C, pp. 48, 55 of MRID 51693101). The following LC conditions were used for analysis of L-glufosinate and D-glufosinate: Daicel Crownpak CR(+) column (4 x 150 mm, 5 µm; column temperature not reported), mobile phase of (A) 4mM ammonium formate in water with 2% formic acid and (B) 0.5% formic acid in methanol [mobile gradient phase of percent A:B (v:v) at 0.00-3.75 min. 100:0, 4.00 min. 99:1, 8.00 min. 95:5, 8.01-10.00 min. 100:0] and injection volume of 25 µL. MS temperature was 650°C. Two ion pair transitions were monitored for each analyte (quantitation and confirmation, respectively): *m/z* 182→136 and *m/z* 182→119 for L-glufosinate and D-glufosinate and *m/z* 185→139 and *m/z* 185→122 for L-glufosinate IS and D-glufosinate IS. Expected retention times were *ca*. 3.5 minutes for D-glufosinate and D-glufosinate IS and *ca*. 4 minutes for L-glufosinate and L-glufosinate IS.

ILV

The ILV performed the ECM method (BASF Analytical Method R0085/01) with minor modifications to the analytical parameters, the use of matrix-matched calibration standards for the water analysis, and the use of a Thompson filter vial (0.2 µm PTFE) for final filtration prior to analysis (pp. 20-22, 28; Tables 34-35, pp. 66-69; Appendix D, pp. 230-231 of MRID 51693102). For soil analysis, optimization of tubing from injection to HPLC column and from HPLC column to the mass spectrometer was required to get a good separation and peak shape of the enantiomers. Samples were analyzed for L-glufosinate and D-glufosinate using Agilent 1290 Infinity LC coupled with a Sciex 6500 Triple Quad Mass Spectrometer with SelexION DMS. The other LC-MS/MS parameters were the same as those of the ECM, with the exception of minor MS parameter modifications and injection volumes of 40 µL for soil samples and 100 µL for water samples. Column temperature was also specified as 6°C, and DMS parameters were reported. Two ion pair transitions were monitored for each analyte (quantitation and confirmation, respectively): *m/z* 182.0→136.0 and *m/z* 182.0→119.0 for L-glufosinate and D-glufosinate and *m/z* 185.0→139.0 and *m/z* 185.0→122.0 for L-glufosinate IS and D-glufosinate IS. These ion transitions were the same as those of the ECM. Expected retention times for soil analysis were *ca*. 3.6 minutes for D-glufosinate and D-glufosinate IS and *ca*. 4 minutes for L-glufosinate and L-glufosinate IS. Expected retention times for water analysis were *ca*. 4.2 minutes for D-glufosinate and D-glufosinate IS and *ca*. 4.6 minutes for L-glufosinate and L-glufosinate IS. The ILV reported the following recommendations

for the ECM: 1) a short sample path from the HPLC injection port to the mass spectrometer inlet should be noted as important in the ECM since the HPLC column poorly retains the analytes during the initial aqueous elution and caused wide peak shape and poor separation of the enantiomers; and 2) sample chromatograms should be included with the ECM to provide expected results for other analysts (p. 29).

LOQ/LOD

The Limit of Quantification (LOQ) for L-glufosinate and D-glufosinate was reported as 0.0025 mg/kg for water and 0.0020 mg/kg for soil (pp. 6, 28 of MRID 51693101; pp. 7, 28 of MRID 51693102). In the ECM and ILV, the Limit of Detection (LOD) for L-glufosinate and D-glufosinate was reported as 0.0005 mg/kg for water and 0.0004 mg/kg for soil. Since the LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ.

II. Recovery Findings

ECM (MRID 51693101): Mean recoveries and relative standard deviations (RSDs) were within guidelines (mean 70-120%; RSD ≤20%) for analysis of L-glufosinate and D-glufosinate [enantiomers of glufosinate (BAS 1000 H)] at fortification levels of 0.0025 mg/kg (LOQ) and 0.025 mg/kg (10×LOQ) in two water matrices and at fortification levels of 0.0020 mg/kg (LOQ) and 0.020 mg/kg (10×LOQ) in two soil matrices (Tables 1-2, pp. 30-31). Two ion pair transitions were monitored; performance data was comparable between the quantitation and confirmation analyses. Two water and two soil matrices were used in the study (p. 22; Appendix B, pp. 39-42). Characterization reports were provided for all matrices: ground water (Sample ID: R21G0350002R02-R04; pH 7.9, hardness 715 mg equiv. $CaCO_3$/L, conductivity 1.20 mmhos/cm, total dissolved solids 1018 ppm), surface water (Sample ID: R21G0350001R02-R04; pH 8.2, hardness 690 mg equiv. $CaCO_3$/L, conductivity 1.26 mmhos/cm, total dissolved solids 1188 ppm), Washington loamy sand soil (Sample ID: DSC-037; Sample depth: 0-3”; 87% sand, 8% silt, 5% clay; pH 8.2 in saturated paste; 0.35% organic matter – Walkley Black; cation exchange capacity 7.7 meq/100 g), and MSL-PF sandy loam soil [Sample ID: MSL-PF (2017-24/SDBN496); 65% sand, 18% silt, 17% clay; pH 6.8 in 1:1 soil:water ratio; 3.9% organic matter – Walkley Black; cation exchange capacity 15.9 meq/100 g; USDA soil texture classification]. The characterization laboratory was Agvise Laboratories, Northwood, North Dakota. The soil textures were verified by the reviewer using USDA-NRCS technical support tools.

ILV (MRID 51693102): Mean recoveries and relative standard deviations (RSDs) were within guidelines (mean 70-120%; RSD ≤20%) for analysis of L-glufosinate and D-glufosinate [enantiomers of glufosinate (BAS 1000 H)] at fortification levels of 0.0025 mg/kg (LOQ) and 0.025 mg/kg (10×LOQ) in two water matrices and at fortification levels of 0.0020 mg/kg (LOQ) and 0.020 mg/kg (10×LOQ) in two soil matrices in the first and second ILV trials (pp. 8-12, 22-25; Tables 1-32, pp. 32-63). Two ion pair transitions were monitored. For the water analysis, performance data was comparable between the quantitation and confirmation analyses, except for the 0.025 mg/kg fortification of L-glufosinate in ground (well) water (second trial). For the Washington soil analysis, performance data was comparable between the quantitation and confirmation analyses, except for the 0.0020 mg/kg fortification of D-glufosinate (second trial). For

the MSL-PF soil analysis, performance data was generally not comparable between the quantitation and confirmation analyses, *e.g.*, the 0.0020 mg/kg fortification of D-glufosinate (first and second trial), the 0.0020 mg/kg fortification of L-glufosinate (second trial), and the 0.020 mg/kg fortification of D-glufosinate (first trial). Two water and two soil matrices were used in the study (p. 19; Appendix E, pp. 232-235). Characterization reports were provided for all matrices: well water (Sample ID: R21G0350002R05 and R21G0350002R02-R04; pH 7.9, hardness 715 mg equiv. $CaCO_3$/L, conductivity 1.20 mmhos/cm, total dissolved solids 1018 ppm), surface water (Sample ID: R21G0350001R05 and R21G0350001R02-R04; pH 8.2, hardness 690 mg equiv. $CaCO_3$/L, conductivity 1.26 mmhos/cm, total dissolved solids 1188 ppm), Washington loamy sand soil (Sample ID: R21G0560001 and DSC-037; Sample depth: 0-3"; 87% sand, 8% silt, 5% clay; pH 8.2 in saturated paste; 0.35% organic matter – Walkley Black; cation exchange capacity 7.7 meq/100 g), and MSL-PF sandy loam soil [Sample ID: R21G0550001 and MSL-PF (2017-24/SDBN496); 65% sand, 18% silt, 17% clay; pH 6.8 in 1:1 soil:water ratio; 3.9% organic matter – Walkley Black; cation exchange capacity 15.9 meq/100 g; USDA soil texture classification]. The characterization laboratory was Agvise Laboratories, Northwood, North Dakota. The soil textures were verified by the reviewer using USDA-NRCS technical support tools. The reviewer noted that the ILV used the same matrices as the ECM for the validation

The method (BASF Analytical Method R0085/01) for L-glufosinate and D-glufosinate was validated by the ILV with minor modifications to the analytical parameters, the use of matrix-matched calibration standards for the water analysis, and the use of a Thompson filter vial (0.2 µm PTFE) for final filtration prior to analysis (pp. 20-22, 28; Tables 34-35, pp. 66-69; Appendix D, pp. 230-231 of MRID 51693102). For soil analysis, optimization of tubing from injection to HPLC column and from HPLC column to the mass spectrometer was required to get a good separation and peak shape of the enantiomers. Performance data from the first and second trial were acceptable; however, modifications were incorporated into the second trial to address chromatographic issues which were present in the first trial. The ILV reported the following recommendations for the ECM: 1) a short sample path from the HPLC injection port to the mass spectrometer inlet should be noted as important in the ECM since the HPLC column poorly retains the analytes during the initial aqueous elution and caused wide peak shape and poor separation of the enantiomers; and 2) sample chromatograms should be included with the ECM to provide expected results for other analysts (p. 29). The submitted ECM study report included the ILV recommendations (p. 27 of MRID 51693101).

Table 2a. Initial Validation Method Recoveries for D-glufosinate and L-glufosinate [Enantiomers of Glufosinate (BAS 1000 H)] in Water[1,2]

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Ground Water					
	Quantitation ion transition					
D-glufosinate	0.0025 (LOQ)	5	70.8-96.8	85.2	10.1	11.9
	0.025	5	78.0-89.2	83.7	4.5	5.4
L-glufosinate	0.0025 (LOQ)	5	77.6-106	94.4	10.9	11.5
	0.025	5	83.6-101	90.0	7.3	8.1
	Confirmation ion transition					
D-glufosinate	0.0025 (LOQ)	5	71.2-94.0	84.5	9.2	10.9
	0.025	5	76.8-88.0	82.6	4.7	5.7
L-glufosinate	0.0025 (LOQ)	5	88.4-100	91.4	7.9	8.6
	0.025	5	80.0-94.8	85.2	5.9	6.9
	Surface Water					
	Quantitation ion transition					
D-glufosinate	0.0025 (LOQ)	5	76.8-88.8	81.8	4.8	5.9
	0.025	5	76.8-89.6	83.8	4.6	5.5
L-glufosinate	0.0025 (LOQ)	5	75.6-90.4	81.9	6.5	7.9
	0.025	5	80.4-97.6	90.5	7.6	8.5
	Confirmation ion transition					
D-glufosinate	0.0025 (LOQ)	5	78.8-88.8	82.2	3.9	4.7
	0.025	5	76.4-90.8	83.5	5.1	6.1
L-glufosinate	0.0025 (LOQ)	5	82.4-86.4	84.0	1.7	2.0
	0.025	5	78.4-98.4	87.5	7.4	8.5

Data (uncorrected recovery results; Appendices D-E, pp. 61-78) were obtained from Tables 1-2, pp. 30-31 of MRID 51693101.

1 In the ECM, two water matrices were used in the study (p. 22; Appendix B, pp. 39-42 of MRID 51693101). Characterization reports were provided for both matrices: ground water (Sample ID: R21G0350002R02-R04; pH 7.9, hardness 715 mg equiv. $CaCO_3$/L, conductivity 1.20 mmhos/cm, total dissolved solids 1018 ppm), and surface water (Sample ID: R21G0350001R02-R04; pH 8.2, hardness 690 mg equiv. $CaCO_3$/L, conductivity 1.26 mmhos/cm, total dissolved solids 1188 ppm). The characterization laboratory was Agvise Laboratories, Northwood, North Dakota.

2 Two ion pair transitions were monitored for each analyte (quantitation and confirmation, respectively): *m/z* 182→136 and *m/z* 182→119 for L-glufosinate and D-glufosinate.

Table 2b. Initial Validation Method Recoveries for D-glufosinate and L-glufosinate [Enantiomers of Glufosinate (BAS 1000 H)] in Soil[1,2]

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Loamy Sand Soil (Washington)					
	Quantitation ion transition					
D-glufosinate	0.0020 (LOQ)	5	97.0-108	102	3.8	3.7
	0.020	5	97.5-102	101	2.0	2.0
L-glufosinate	0.0020 (LOQ)	5	97.5-103	99.2	2.0	2.0
	0.020	5	86.5-96.5	91.4	3.8	4.2
	Confirmation ion transition					
D-glufosinate	0.0020 (LOQ)	5	96.5-101	98.9	1.9	1.9
	0.020	5	96.5-102	99.2	2.1	2.2
L-glufosinate	0.0020 (LOQ)	5	87.0-98.5	91.5	4.7	5.1
	0.020	5	90.5-100	94.3	3.7	3.9
	Sandy Loam Soil (MSL-PF)					
	Quantitation ion transition					
D-glufosinate	0.0020 (LOQ)	5	101-111	104	4.1	3.9
	0.020	5	91.5-96.5	95.1	2.1	2.2
L-glufosinate	0.0020 (LOQ)	5	92.5-106	100	5.3	5.3
	0.020	5	84.0-95.5	91.5	4.7	5.1
	Confirmation ion transition					
D-glufosinate	0.0020 (LOQ)	5	93.5-105	97.6	4.1	4.2
	0.020	5	93.5-98.5	96.0	1.9	1.9
L-glufosinate	0.0020 (LOQ)	5	87.0-102	92.8	6.0	6.5
	0.020	5	82.5-97.0	93.2	6.1	6.5

Data (uncorrected recovery results; Appendices D-E, pp. 61-78) were obtained from Tables 1-2, pp. 30-31 of MRID 51693101.

1 In the ECM, two soil matrices were used in the study (p. 22; Appendix B, pp. 39-42 of MRID 51693101). Characterization reports were provided for both matrices: Washington loamy sand soil (Sample ID: DSC-037; Sample depth: 0-3"; 87% sand, 8% silt, 5% clay; pH 8.2 in saturated paste; 0.35% organic matter – Walkley Black; cation exchange capacity 7.7 meq/100 g), and MSL-PF sandy loam soil [Sample ID: MSL-PF (2017-24/SDBN496); 65% sand, 18% silt, 17% clay; pH 6.8 in 1:1 soil:water ratio; 3.9% organic matter – Walkley Black; cation exchange capacity 15.9 meq/100 g; USDA soil texture classification]. The characterization laboratory was Agvise Laboratories, Northwood, North Dakota. The soil textures were verified by the reviewer using USDA-NRCS technical support tools.

2 Two ion pair transitions were monitored for each analyte (quantitation and confirmation, respectively): *m/z* 182→136 and *m/z* 182→119 for L-glufosinate and D-glufosinate.

Table 3a. Independent Validation Method Recoveries for D-glufosinate and L-glufosinate [Enantiomers of Glufosinate (BAS 1000 H)] in Water[1,2,3]

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Ground Water (Well)					
	First Trial Results					
	Quantitation ion transition					
D-glufosinate	0.0025 (LOQ)	5	82-104	93	7.7	8.3
	0.025	5	81-100	89	7.8	8.8
L-glufosinate	0.0025 (LOQ)	5	98-115	109	6.6	6.1
	0.025	5	80-92	85	5.0	5.9
	Confirmation ion transition					
D-glufosinate	0.0025 (LOQ)	5	81-106	95	10.9	11.4
	0.025	5	87-100	93	6.3	6.7
L-glufosinate	0.0025 (LOQ)	5	96-118	109	9.2	8.5
	0.025	5	85-94	91	4.0	4.4
	Second Trial Results					
	Quantitation ion transition					
D-glufosinate	0.0025 (LOQ)	5	88-107	93	8.1	8.7
	0.025	5	90-111	99	8.8	8.9
L-glufosinate	0.0025 (LOQ)	5	92-121	104	12.6	12.1
	0.025	5	97-117	104	8.2	7.8
	Confirmation ion transition					
D-glufosinate	0.0025 (LOQ)	5	80-106	93	10.7	11.5
	0.025	5	83-110	94	11.5	12.2
L-glufosinate	0.0025 (LOQ)	5	112-118	115	2.9	2.5
	0.025	5	79-97	85	7.4	8.6
	Surface Water					
	First Trial Results					
	Quantitation ion transition					
D-glufosinate	0.0025 (LOQ)	5	96-105	100	3.7	3.7
	0.025	5	92-99	95	2.9	3.1
L-glufosinate	0.0025 (LOQ)	5	101-108	104	2.9	2.8
	0.025	5	90-93	92	1.5	1.7
	Confirmation ion transition					
D-glufosinate	0.0025 (LOQ)	5	91-92	92	0.5	0.6
	0.025	5	89-99	94	3.7	4.0
L-glufosinate	0.0025 (LOQ)	5	100-105	102	2.0	1.9
	0.025	5	87-95	91	3.0	3.3
	Second Trial Results					
	Quantitation ion transition					
D-glufosinate	0.0025 (LOQ)	5	88-98	92	3.9	4.2
	0.025	5	89-96	92	2.8	3.1
L-glufosinate	0.0025 (LOQ)	5	88-106	94	7.3[4]	7.8
	0.025	5	85-96	91	4.1	4.5
	Confirmation ion transition					
D-glufosinate	0.0025 (LOQ)	5	87-96	92	3.8	4.1
	0.025	5	93-97	95	1.6	1.7
L-glufosinate	0.0025 (LOQ)	5	89-96	92	2.7	2.9

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	0.025	5	85-95	90	3.5	3.9

Data (uncorrected recovery results; Table 36, pp. 70-71) were obtained from pp. 8-12, 23-26; Tables 9-16, pp. 40-47; Tables 25-32, pp. 56-63 of MRID 51693102.

1 Two ILV trials were performed. Performance data from the first and second trial were acceptable; however, modifications were incorporated into the second trial to address chromatographic issues which were present in the first trial.

2 In the ILV, two water matrices were used in the study (p. 19; Appendix E, pp. 232-235 of MRID 51693102). Characterization reports were provided for both matrices: well water (Sample ID: R21G0350002R05and R21G0350002R02-R04; pH 7.9, hardness 715 mg equiv. $CaCO_3$/L, conductivity 1.20 mmhos/cm, total dissolved solids 1018 ppm), and surface water (Sample ID: R21G0350001R05 and R21G0350001R02-R04; pH 8.2, hardness 690 mg equiv. $CaCO_3$/L, conductivity 1.26 mmhos/cm, total dissolved solids 1188 ppm). The characterization laboratory was Agvise Laboratories, Northwood, North Dakota. The reviewer noted that the ILV used the same matrices as the ECM for the validation.

3 Two ion pair transitions were monitored for each analyte (quantitation and confirmation, respectively): *m/z* 182.0→136.0 and *m/z* 182.0→119.0 for L-glufosinate and D-glufosinate. These ion transitions were the same as those of the ECM.

4 Value was erroneously reported as “73” on pp. 11, 25 of MRID 51693102, but correctly reported as “7.3” in Table 27, p. 58 of MRID 51693102.

Table 3b. Independent Validation Method Recoveries for D-glufosinate and L-glufosinate [Enantiomers of Glufosinate (BAS 1000 H)] in Soil[1,2,3]

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Loamy Sand Soil (Washington)					
	First Trial Results					
	Quantitation ion transition					
D-glufosinate	0.0020 (LOQ)	5	84-113	101	11.1	10.9
	0.020	5	88-103	97	7.0	7.2
L-glufosinate	0.0020 (LOQ)	5	83-107	97	10.0	10.3
	0.020	5	91-106	99	5.9	6.0
	Confirmation ion transition					
D-glufosinate	0.0020 (LOQ)	5	91-116	106	10.0	9.5
	0.020	5	83-108	90	11.3	12.6
L-glufosinate	0.0020 (LOQ)	5	83-99	91	7.1	7.8
	0.020	5	92-108	99	6.3	6.3
	Second Trial Results					
	Quantitation ion transition					
D-glufosinate	0.0020 (LOQ)	5	97-109	103	4.8	4.6
	0.020	5	96-103	99	2.7	2.7
L-glufosinate	0.0020 (LOQ)	5	89-106	96	7.4	7.8
	0.020	5	94-101	98	3.1	3.1
	Confirmation ion transition					
D-glufosinate	0.0020 (LOQ)	5	85-97	92	5.1	5.6
	0.020	5	93-98	95	1.9	1.9
L-glufosinate	0.0020 (LOQ)	5	84-107	97	11.5	11.5
	0.020	5	85-98	93	4.8	5.1
	Sandy Loam Soil (MSL-PF)					
	First Trial Results					
	Quantitation ion transition					
D-glufosinate	0.0020 (LOQ)	5	83-109	92	9.7	10.6
	0.020	5	79-101	88	8.5	9.6
L-glufosinate	0.0020 (LOQ)	5	85-99	94	6.3	6.7
	0.020	5	84-94	89	3.6	4.0
	Confirmation ion transition					
D-glufosinate	0.0020 (LOQ)	5	95-117	105	8.7	8.3
	0.020	5	88-105	99	7.8	7.9
L-glufosinate	0.0020 (LOQ)	5	82-98	92	6.1	6.7
	0.020	5	84-96	92	5.3	5.7
	Second Trial Results					
	Quantitation ion transition					
D-glufosinate[4]	0.0020 (LOQ)	5	109-126	118	7.8	6.6
	0.020	5	95-98	96	1.2	5.1
L-glufosinate	0.0020 (LOQ)	5	108-122	113	5.3	4.7
	0.020	5	95-103	99	3.1	3.2
	Confirmation ion transition					
D-glufosinate	0.0020 (LOQ)	5	89-96	92	3.2	3.5
	0.020	5	86-93	89	2.6	2.9
L-glufosinate	0.0020 (LOQ)	5	82-105	94	9.2	9.8

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	0.020	5	86-98	90	4.8	5.4

Data (uncorrected recovery results; Table 36, pp. 70-71) were obtained from pp. 8-12, 22-25; Tables 1-8, pp. 32-39; Tables 17-24, pp. 48-55 of MRID 51693102.

1 Two ILV trials were performed. Performance data from the first and second trial were acceptable; however, modifications were incorporated into the second trial to address chromatographic issues which were present in the first trial.

2 In the ILV, two soil matrices were used in the study (p. 19; Appendix E, pp. 232-235 of MRID 51693102). Characterization reports were provided for both matrices: Washington loamy sand soil (Sample ID: R21G0560001 and DSC-037; Sample depth: 0-3"; 87% sand, 8% silt, 5% clay; pH 8.2 in saturated paste; 0.35% organic matter – Walkley Black; cation exchange capacity 7.7 meq/100 g), and MSL-PF sandy loam soil [Sample ID: R21G0550001 and MSL-PF (2017-24/SDBN496); 65% sand, 18% silt, 17% clay; pH 6.8 in 1:1 soil:water ratio; 3.9% organic matter – Walkley Black; cation exchange capacity 15.9 meq/100 g; USDA soil texture classification]. The characterization laboratory was Agvise Laboratories, Northwood, North Dakota. The soil textures were verified by the reviewer using USDA-NRCS technical support tools. The reviewer noted that the ILV used the same matrices as the ECM for the validation.

3 Two ion pair transitions were monitored for each analyte (quantitation and confirmation, respectively): *m/z* 182.0→136.0 and *m/z* 182.0→119.0 for L-glufosinate and D-glufosinate. These ion transitions were the same as those of the ECM.

4 The mean, standard deviation, and RSD for the 0.020 mg/kg fortification were reported from Table 21, p. 52 of MRID 51693102 since those values reported on pp. 11, 25 of MRID 51693102 were erroneous.

III. Method Characteristics

The LOQ for L-glufosinate and D-glufosinate [enantiomers of glufosinate (BAS 1000 H)] was reported as 0.0025 mg/kg for water and 0.0020 mg/kg for soil (pp. 6, 28 of MRID 51693101; pp. 7, 28 of MRID 51693102). In the ECM and ILV, the LOQ was defined as the lowest fortification level tested. In the ECM and ILV, the LOD for L-glufosinate and D-glufosinate was reported as 0.0005 mg/kg for water and 0.0004 mg/kg for soil. In the ECM and ILV, the LOD was defined as the lowest calibration standard, 20% of the LOQ. The ILV also defined the lowest calibration standard as the lowest concentration analyzed with an acceptable signal-to-noise ratio (S/N > 3:1). No calculations were reported for the LOQ or LOD in the ECM and ILV.

Since the LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ.

Table 4a. Method Characteristics in Water[1]

		L-glufosinate	D-glufosinate
Limit of Quantitation (LOQ)*	ECM	0.0025 mg/kg	
	ILV		
Limit of Detection (LOD)	ECM	0.0005 mg/kg (20% of the LOQ)	
	ILV		
Linearity (calibration curve r and concentration range)	ECM[2]	r = 0.9994-0.9998 (Q) r = 0.9991-0.9996 (C)	r = 0.9998-0.9999 (Q) r = 0.9906-0.9998 (C)
	ILV	First Trial (solvent-based)	
		r = 0.9993 (Q, SW) r = 0.9991 (C, SW) r = 0.9998 (Q, GW) r = 0.9995 (C, GW)	r = 0.9989 (Q, SW) r = 0.9968 (C, SW) r = 0.9993 (Q, GW) r = 0.9978 (C, GW)
		Second Trial (matrix-matched)	
		r = 0.9990 (Q & C, SW) r = 0.9975 (Q, GW) r = 0.9977 (C, GW)	r = 0.9996 (Q, SW) r = 0.9990 (C, SW) r = 0.9977 (Q, GW) r = **0.9939** (C, GW)[3]
	Range	0.5-50 ng/mL (equivalent to 0.05 ng to 5 ng injected on column)	
Repeatable	ECM[4]	Yes at LOQ (0.0025 mg/kg) and 10×LOQ (0.025 mg/kg) (two characterized water matrices – surface and ground)	
	ILV[5,6,7]		
Reproducible		Yes for 0.0025 mg/kg (LLMV)* and 0.025 mg/kg in tested water matrices	
Specific	ECM	Yes, no matrix interferences were observed.	Yes, matrix interferences were <5% of the LOQ (based on quantified residues).
	ILV	Second Trial Results	
		Yes in surface water, matrix interferences were observed but quantified as not detected. However, baseline noise/contamination and peak shouldering interfered with analyte integration and attenuation at both fortifications.[8] **No** in ground water. LOQ analyte peak was highly irregular and eluted as a multi-peaked signal.[9] Analyte integration and attenuation at the LOQ was not uniform. Control ground water matrix chromatograms were **not** provided/integrated.	Yes, matrix interferences were observed but quantified as not detected. Some peak broadening/splitting was observed in the confirmation ion analysis.

Data were obtained from pp. 6, 28 (LOQ/LOD); Tables 1-2, pp. 30-31; Appendix E, Tables A 1-A 8, pp. 63-70 (recovery results); Table 4, p. 33; Appendix C, p. 51; Appendix I, pp. 165-167 (calibration curves); Appendix F, Figures A 8-A 87, pp. 80-159 (chromatograms) of MRID 51693101; pp. 7, 28 (LOQ/LOD); pp. 8-12, 23-26; Tables 9-16, pp. 40-47; Tables 25-32, pp. 56-63 (recovery results); p. 28; Figures 17-18, pp. 117-118; Appendix B, pp. 195-202, 219-226 (calibration curves); Figures 3-33, pp. 75-180 (chromatograms) of MRID 51693102. Q = quantitation ion transition; C = confirmation ion transition. SW = Surface water; GW = Ground water.

* Since the LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ. The lowest concentration tested with sufficiently accurate and precise recoveries is the LLMV.

1 Two ILV trials were performed. Performance data from the first and second trial were acceptable; however,

modifications were incorporated into the second trial to address chromatographic issues which were present in the first trial. All ILV data was included in the Table above.

2 In the ECM, solvent-based calibration standards were used for all analyses (p. 28; Table 4, p. 33; Appendix C, p. 51 of MRID 51693101). Matrix effects were not investigated in the ECM since isotopically labeled internal standards were used to compensate for any matrix effects.

3 Deviations in the confirmation ion analysis do not affect the validity of the method since a confirmatory method is not usually required when LC/MS or GC/MS is the primary method used to generate study data.

4 In the ECM, two water matrices were used in the study (p. 22; Appendix B, pp. 39-42 of MRID 51693101). Characterization reports were provided for both matrices: ground water (Sample ID: R21G0350002R02-R04; pH 7.9, hardness 715 mg equiv. $CaCO_3$/L, conductivity 1.20 mmhos/cm, total dissolved solids 1018 ppm), and surface water (Sample ID: R21G0350001R02-R04; pH 8.2, hardness 690 mg equiv. $CaCO_3$/L, conductivity 1.26 mmhos/cm, total dissolved solids 1188 ppm). The characterization laboratory was Agvise Laboratories, Northwood, North Dakota.

5 Performance data from the first and second ILV trial.

6 In the ILV, two water matrices were used in the study (p. 19; Appendix E, pp. 232-235 of MRID 51693102). Characterization reports were provided for both matrices: well water (Sample ID: R21G0350002R05 and R21G0350002R02-R04; pH 7.9, hardness 715 mg equiv. $CaCO_3$/L, conductivity 1.20 mmhos/cm, total dissolved solids 1018 ppm), and surface water (Sample ID: R21G0350001R05 and R21G0350001R02-R04; pH 8.2, hardness 690 mg equiv. $CaCO_3$/L, conductivity 1.26 mmhos/cm, total dissolved solids 1188 ppm). The characterization laboratory was Agvise Laboratories, Northwood, North Dakota. The reviewer noted that the ILV used the same matrices as the ECM for the validation.

7 The ILV validated method (BASF Analytical Method R0085/01) for L-glufosinate and D-glufosinate with minor modifications to the analytical parameters, the use of matrix-matched calibration standards for the water analysis, and the use of a Thompson filter vial (0.2 µm PTFE) for final filtration prior to analysis (pp. 20-22, 28; Tables 34-35, pp. 66-69; Appendix D, pp. 230-231 of MRID 51693102). For soil analysis, optimization of tubing from injection to HPLC column and from HPLC column to the mass spectrometer was required to get a good separation and peak shape of the enantiomers. Performance data from the first and second ILV trials were acceptable; however, modifications were incorporated into the second ILV trial to address chromatographic issues which were present in the first ILV trial. The ILV reported the following recommendations for the ECM: 1) a short sample path from the HPLC injection port to the mass spectrometer inlet should be noted as important in the ECM since the HPLC column poorly retains the analytes during the initial aqueous elution and caused wide peak shape and poor separation of the enantiomers; and 2) sample chromatograms should be included with the ECM to provide expected results for other analysts (p. 29). The submitted ECM study report included the ILV recommendations (p. 27 of MRID 51693101).

8 Based on Figures 26-27, pp. 165-168 of MRID 51693102.

9 Based on Figures 32-33, pp. 177-180 of MRID 51693102.

Table 4b. Method Characteristics in Soil[1]

		L-glufosinate	D-glufosinate
Limit of Quantitation (LOQ)*	ECM	0.0020 mg/kg	
	ILV		
Limit of Detection (LOD)	ECM	0.0004 mg/kg (20% of the LOQ)	
	ILV		
Linearity (calibration curve r and concentration range)	ECM[2]	r = 0.9994-0.9998 (Q) r = 0.9991-0.9996 (C)	r = 0.9998-0.9999 (Q) r = 0.9906-0.9998 (C)
	ILV	First Trial (solvent-based)	
		r = 0.9986 (Q, LS) r = 0.9991 (C, LS) r = 0.9996 (Q, SL) r = 0.9993 (C, SL)	r = 0.9977 (Q, LS) r = 0.9995 (C, LS) r = 0.9951 (Q, SL) r = 0.9997 (C, SL)
		Second Trial (solvent-based)	
		r = 0.9997 (Q, LS) r = 0.9985 (C, LS) r = 0.9983 (Q, SL) r = 0.9967 (C, SL)	r = 1.0000 (Q, LS) r = 0.9988 (C, LS) r = 0.9996 (Q, SL) r = 0.9987 (C, SL)
	Range	0.4-50 ng/mL (equivalent to 0.04 ng to 5 ng injected on column)	
Repeatable	ECM[3]	Yes at LOQ (0.0025 mg/kg) and 10×LOQ (0.025 mg/kg) (two characterized soil matrices – loamy sand and sandy loam)	
	ILV[4,5,6]		
Reproducible		Yes for 0.0020 mg/kg (LLMV)* and 0.020 mg/kg in tested water matrices	
Specific	ECM	Yes, matrix interferences were <17% of the LOQ (based on quantified residues). Minor baseline noise which eluted after the analyte peak was noted at the LOQ.	Yes, matrix interferences were <10% of the LOQ (based on quantified residues).
	ILV	Second Trial Results	
		Yes, matrix interferences were <20% of the LOQ (based on quantified residues). Some peak broadening/splitting was observed. Minor baseline noise was elevated around the analyte peak. Control matrix chromatograms were **not** provided/integrated.	Yes, matrix interferences were <10% (LS) and <22% (SL) of the LOQ (based on quantified residues).

Data were obtained from pp. 6, 28 (LOQ/LOD); Tables 1-2, pp. 30-31; Appendix E, Tables A 9-A 16, pp. 71-78 (recovery results); Table 4, p. 33; Appendix C, p. 51; Appendix I, pp. 165-167 (calibration curves); Appendix F, Figures A 8-A 87, pp. 80-159 (chromatograms) of MRID 51693101; pp. 7, 28 (LOQ/LOD); pp. 8-12, 22-25; Tables 1-8, pp. 32-39; Tables 17-24, pp. 48-55 (recovery results); Figures 1-2, pp. 73-74; Appendix B, pp. 187-194, 203-218 (calibration curves); Figures 3-33, pp. 75-180 (chromatograms) of MRID 51693102. Q = quantitation ion transition; C = confirmation ion transition. LS = Loamy sand soil; SL = Sandy loam soil.

* Since the LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ. The lowest concentration tested with sufficiently accurate and precise recoveries is the LLMV.

1 Two ILV trials were performed. Performance data from the first and second trial were acceptable; however, modifications were incorporated into the second trial to address chromatographic issues which were present in the first trial. All ILV data was included in the Table above

2 In the ECM, solvent-based calibration standards were used for all analyses (p. 28; Table 4, p. 33; Appendix C, p. 51 of MRID 51693101). Matrix effects were not investigated in the ECM since isotopically labeled internal standards were

used to compensate for any matrix effects.

3 In the ECM, two soil matrices were used in the study (p. 22; Appendix B, pp. 39-42 of MRID 51693101). Characterization reports were provided for both matrices: Washington loamy sand soil (Sample ID: DSC-037; Sample depth: 0-3"; 87% sand, 8% silt, 5% clay; pH 8.2 in saturated paste; 0.35% organic matter – Walkley Black; cation exchange capacity 7.7 meq/100 g), and MSL-PF sandy loam soil [Sample ID: MSL-PF (2017-24/SDBN496); 65% sand, 18% silt, 17% clay; pH 6.8 in 1:1 soil:water ratio; 3.9% organic matter – Walkley Black; cation exchange capacity 15.9 meq/100 g; USDA soil texture classification]. The characterization laboratory was Agvise Laboratories, Northwood, North Dakota. The soil textures were verified by the reviewer using USDA-NRCS technical support tools.

4 Performance data from the first and second ILV trial.

5 In the ILV, two water matrices were used in the study (p. 19; Appendix E, pp. 232-235 of MRID 51693102). Characterization reports were provided for both matrices: well water (Sample ID: R21G0350002R05 and R21G0350002R02-R04; pH 7.9, hardness 715 mg equiv. $CaCO_3$/L, conductivity 1.20 mmhos/cm, total dissolved solids 1018 ppm), and surface water (Sample ID: R21G0350001R05 and R21G0350001R02-R04; pH 8.2, hardness 690 mg equiv. $CaCO_3$/L, conductivity 1.26 mmhos/cm, total dissolved solids 1188 ppm). The characterization laboratory was Agvise Laboratories, Northwood, North Dakota. The reviewer noted that the ILV used the same matrices as the ECM for the validation.

6 The ILV validated method (BASF Analytical Method R0085/01) for L-glufosinate and D-glufosinate with minor modifications to the analytical parameters, the use of matrix-matched calibration standards for the water analysis, and the use of a Thompson filter vial (0.2 µm PTFE) for final filtration prior to analysis (pp. 20-22, 28; Tables 34-35, pp. 66-69; Appendix D, pp. 230-231 of MRID 51693102). For soil analysis, optimization of tubing from injection to HPLC column and from HPLC column to the mass spectrometer was required to get a good separation and peak shape of the enantiomers. Performance data from the first and second ILV trials were acceptable; however, modifications were incorporated into the second ILV trial to address chromatographic issues which were present in the first ILV trial. The ILV reported the following recommendations for the ECM: 1) a short sample path from the HPLC injection port to the mass spectrometer inlet should be noted as important in the ECM since the HPLC column poorly retains the analytes during the initial aqueous elution and caused wide peak shape and poor separation of the enantiomers; and 2) sample chromatograms should be included with the ECM to provide expected results for other analysts (p. 29). The submitted ECM study report included the ILV recommendations (p. 27 of MRID 51693101).

IV. Method Deficiencies and Reviewer's Comments

1. Since the reported method LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ (pp. 6, 28 of MRID 51693101; pp. 7, 28 of MRID 51693102). The lowest concentration tested with sufficiently accurate and precise recoveries is the LLMV. Based on the performance data submitted by the ILV and ECM, the LLMV for water analysis was equivalent to the ECM reported method LOQ for L-glufosinate and D-glufosinate in the tested surface water matrices (0.0025 mg/kg). Based on the performance data submitted by the ILV and ECM, the LLMV for soil analysis was equivalent to the ECM reported method LOQ for L-glufosinate and D-glufosinate in the tested soil matrices (0.0020 mg/kg).

2. The specificity of the method for L-glufosinate in ground water was not supported by ILV representative chromatograms due to the fact that the LOQ analyte peak was highly irregular (Figures 32-33, pp. 177-180 of MRID 51693102). Analyte peak was split into 2+ peaks and not eluted like a single compound, and analyte integration and attenuation at the LOQ was not uniform. Additionally, control ground water matrix chromatograms were not provided/integrated for L-glufosinate.

   Additionally, control soil matrix chromatograms were not provided/integrated for L-glufosinate in the ILV.

3. Two ILV trials were performed. Performance data from the first and second ILV trials were acceptable; however, modifications were incorporated into the second ILV trial to address chromatographic issues which were present in the first ILV trial (p. 22, 28 of MRID 51693102). For the second trial of the water analysis, matrix-matched calibration standards were used. For the second trial of the soil analysis, optimization of tubing from injection to HPLC column and from HPLC column to the mass spectrometer was required to get a good separation and peak shape of the enantiomers. The ILV reported the following recommendations for the ECM: 1) a short sample path from the HPLC injection port to the mass spectrometer inlet should be noted as important in the ECM since the HPLC column poorly retains the analytes during the initial aqueous elution and caused wide peak shape and poor separation of the enantiomers; and 2) sample chromatograms should be included with the ECM to provide expected results for other analysts (p. 29). The submitted ECM study report included the ILV recommendations (p. 27 of MRID 51693101).

4. It could not be determined if the ILV (MRID 51693102) was conducted independently of the internal validation (ECM MRID 51693101) since the email and other correspondence between the ILV Study Director (Rolando Perez, ADPEN Laboratories, Inc.) and the ILV Study Monitor (Matthew Horowitz, BASF Corporation) was only summarized, not included in the ILV study report (pp. 1, 18, 29 of MRID 51693102). The communication involved exchange of ILV first and second trial results. The ILV Study Monitor expressed concern regarding analyte peak separation and shape and advised a new analytical column and modified mobile phase gradient. The source of the final resolution of the chromatographic issues was not reported as originating with the ILV Study Director or ILV Study Monitor. The reviewer noted that the ILV Study Monitor (Matthew Horowitz, BASF Corporation) was not reported as laboratory personnel involved with the ECM validation (pp. 1-5, 20 of

MRID 51693101).

5. It could not be determined if the ILV was provided with the most difficult matrices with which to validate the method or if the ILV soil matrices [loamy sand soil (5% clay; 0.35% organic matter – Walkley Black) and sandy loam soil (17% clay; 3.9% organic matter – Walkley Black)] covered the range of soils used in the terrestrial field dissipation studies. The ILV water and soil matrices were the same as those used in the ECM validation (p. 22; Appendix B, pp. 39-42 of MRID 51693101; p. 19; Appendix E, pp. 232-235 of MRID 51693102). While no L-glufosinate and D-glufosinate terrestrial field dissipation (TFD) studies or metabolism studies were provided to CDM/CSS-Dynamac JV personnel to assess soil range adequacy, the terrestrial field dissipation studies for racemic glufosinate cover a wider range of soil textural classes than the soils used in the ECM/ILV

   OCSPP 850.6100 guidance suggests for a given sample matrix, the registrant should select the most difficult analytical sample condition from the study (*e.g.*, high organic content versus low organic content in a soil matrix) to analyze from the study to demonstrate how well the method performs.

   No OCSPP 850.6100 guidance is found to address the use of the same matrices in the ECM and ILV validations.

6. For the water analysis, ILV performance data was comparable between the quantitation and confirmation analyses, except for the 0.025 mg/kg fortification of L-glufosinate in ground (well) water (second trial; pp. 8-12, 22-25; Tables 1-32, pp. 32-63 of MRID 51693102). For the Washington soil analysis, ILV performance data was comparable between the quantitation and confirmation analyses, except for the 0.0020 mg/kg fortification of D-glufosinate (second trial). For the MSL-PF soil analysis, ILV performance data was generally not comparable between the quantitation and confirmation analyses, *e.g.*, the 0.0020 mg/kg fortification of D-glufosinate (first and second trial), the 0.0020 mg/kg fortification of L-glufosinate (second trial), and the 0.020 mg/kg fortification of D-glufosinate (first trial).

7. The reviewer noted the following significant typographical errors in ILV performance data reporting: 1) the standard deviation for the LOQ fortification of L-glufosinate (Q) in the second trial of surface water was erroneously reported as “73” on pp. 11, 25 of MRID 51693102, but correctly reported as “7.3” in Table 27, p. 58 of MRID 51693102; and 2) the mean, standard deviation, and RSD for the 10×LOQ fortification of D-glufosinate (Q) in the second trial of sandy loam soil (MSL-PF) were reported in the DER from Table 21, p. 52 of MRID 51693102 since those values reported on pp. 11, 25 of MRID 51693102 were erroneous.

8. The stabilities of the final sample extract solutions were not studied in the ECM or ILV. The ECM study report noted that the stability of the stock, fortification, and calibration standard solutions of glufosinate in water had been determined as 30 days when refrigerated in previous studies (p. 29; Table 3, p. 32 of MRID 51693101). Matrix effects were reported as insignificant in the ECM and ILV; however, the ILV noted that analyte peak shape improved with matrix-matched calibration standards were introduced for the surface water but not for other matrices (p. 28 of MRID 51693102). The stable isotope was added to the

fortification samples prior to analysis in order to counter any matrix effects.

9. The determinations of the LOQ and LOD in the ECM and ILV were not based on scientifically acceptable procedures as defined in 40 CFR Part 136 (pp. 6, 28 of MRID 51693101; pp. 7, 28 of MRID 51693102). In the ECM and ILV, the LOQ was defined as the lowest fortification level tested. In the ECM and ILV, the LOD was defined as the lowest calibration standard, 20% of the LOQ. The ILV also defined the lowest calibration standard as the lowest concentration analyzed with an acceptable signal-to-noise ratio (S/N > 3:1). No calculations were reported for the LOQ or LOD in the ECM and ILV. Detection limits should not be based on the arbitrarily selected lowest concentration in the spiked samples.

   Since the LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ.

10. The total time required to complete one sample set of 13 samples was reported in the ILV as *ca*. 4 hours of work for water and *ca*. 8 hours of work for soil (analysis time and calculation of the results excluded; preparation of matrix-matched calibration standards required additional time; p. 27 of MRID 51693102). The total time required to complete one set of 13 samples was reported in the ECM as *ca*. 8 hours of work for each analyte, mass transition, and matrix (Table 4, p. 33 of MRID 51693101).

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.

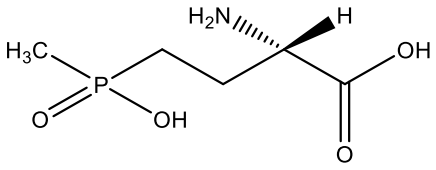
USEPA. 2012. *Environmental Chemistry Method Guidance*. Memorandum From D. Brady to Environmental Fate and Effects Division. December 20, 2012. Environmental Fate and Effects Division. Office of Pesticide Programs. Office of Chemical Safety and Pollution Prevention. U.S. Environmental Protection Agency. Available at: https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/environmental-chemistry-methods-guidance-pesticides.

40 CFR Part 136. Appendix B. Definition and Procedure for the Determination of the Method Detection Limit-Revision 1.11, pp. 344-347, and Revision 2; 2015 and 2016.

Attachment 1: Chemical Names and Structures

L-glufosinate

IUPAC Name: (2S)-2-amino-4-[hydroxy(methyl)phosphinoyl]butyric acid
CAS Name: (2S)-2-amino-4-(hydroxymethylphosphinyl)butanoic acid
CAS Number: 35597-44-5
SMILES String: OC([C@@]([H])(N)CCP(C)(O)=O)=O



D-glufosinate

IUPAC Name: (2R)-2-amino-4-(hydroxy(methyl)phosphoryl)butanoic acid
CAS Name: Not found
CAS Number: Not found
SMILES String: OC([C@](N)([H])CCP(C)(O)=O)=O