Analytical method for mesosulfuron-methyl (AE F130060) and its transformation products, AE F160459, AE F160460, AE F140584, AE F147447 and AE F092944, in water

Reports: ECM: EPA MRID No.: 49829804. Miller, A. 2016. Bayer Method MM-002-W15-02: An Analytical Method for the Determination of Residues of Mesosulfuron-methyl (AE F130060) and its metabolites AE F160459, AE F160460, AE F140584, AE F147447 and AE F092944 in Water Using LC/MS/MS. Bayer Method MM-002-W15-02. Report prepared, sponsored and submitted by Bayer CropScience, Research Triangle Park, North Carolina; 34 pages. Final report completed January 27, 2016.

ILV: EPA MRID No.: 49829802. Klosi, E. 2016. Independent Laboratory Validation (ILV) of An Analytical Method for the Determination of Residues of Mesosulfuron-methyl (AE F130060) and its Metabolites AE F160459, AE F160460, AE F140584, AE F147447 and AE F092944 in Water Using LC/MS/MS. Ricerca Document No.: 034249-1. Bayer Study ID: RAMMN092. Report prepared by Ricerca Biosciences, LLC, Concord, Ohio, sponsored and submitted by Bayer CropScience, Research Triangle Park, North Carolina; 250 pages. Final report issued January 28, 2016.

Document No.: MRIDs 49829804 & 49829802

Guideline: 850.6100

Statements: ECM: The study was not conducted in compliance with USEPA Good Laboratory Practice (GLP) standards since it was not an experimental study according to 40 CFR Part 160.3 (p. 3 of MRID 49829804). Signed and dated Data Confidentiality and GLP statements were provided (pp. 2-3). Quality Assurance and Authenticity Certification statements were not provided.

ILV: The study was conducted in compliance with USEPA FIFRA GLP standards (40 CFR 160; p. 3 of MRID 49829802). Signed and dated Data Confidentiality, GLP, Certificate of Authenticity and Quality Assurance statements were provided (pp. 2-5).

Classification: This analytical method is classified as **Unacceptable**.
Only one set of performance data was submitted to support the method; the ECM MRID 49829804 was a method description only and did not report any experimental data. The LOD was not reported. The precision and accuracy of the method was not demonstrated in the ILV recovery results for AE F160460 and AE F160459. The correlation coefficients of the ILV linear regressions for mesosulfuron-methyl were unsatisfactory.

PC Code: 122009

Reviewer: Lewis Ross Brown, III
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Signature: Lewis Ross Brown, III
Date: 11/15/2016

Page citations for MRID 49829802 in this review refer to the page numbers located in the upper right corner of each page of the MRID. Page citations for MRID 49829804 in this review refer to the page numbers located in the bottom-center of each page of the MRID.

Executive Summary

The analytical method, Bayer Method MM-002-W15-02, is designed for the quantitative determination of mesosulfuron-methyl (AE F130060) and its transformation products, AE F160459, AE F160460, AE F140584, AE F147447 and AE F092944, in water at the LOQ of 0.25 ng/mL using HPLC/MS/MS. The LOQ is greater than the lowest toxicological level of concern in water for all analytes. Two sets of performance data were not submitted to support the method; the ECM MRID 49829804 was a method only and did not report any experimental data. The method contained one procedure for the analysis of mesosulfuron-methyl, AE F140584 and AE F092944 and another procedure for the analysis of AE F147447, AE F160460 and AE F160459. The ILV validated the method using characterized pond water. The method was validated with the first trial for mesosulfuron-methyl, AE F140584 and AE F092944 with no modifications; the method was validated with the third trial for AE F147447, AE F160460 and AE F160459 with minor modifications which included pre-washing glassware with 2% formic acid, increasing the concentration of the internal standard and changing the reconstitution solution to match the solvent of the standard solutions. Based on the ILV results, the reviewer believed that the ECM procedure for AE F147447, AE F160459 and AE F160460 required additional optimization to reduce analyte loss. The precision and accuracy of the method was not demonstrated in the ILV recovery results for AE F160460 and AE F160459 based on the LOQ quantitation ion results. Correlation coefficients for ECM linear regressions were unsatisfactory for AE F160459 and AE F147447; correlation coefficients for ILV linear regressions were unsatisfactory for mesosulfuron-methyl. The LOD for the method was not reported in the ECM and ILV.

Table 1. Analytical Method Summary

Analyte(s) by Pesticide[1]	**MRID**		**EPA Review**	**Matrix**	**Method Date (dd/mm/yyyy)**	**Registrant**	**Analysis**	**Limit of Quantitation (LOQ)**
	Environmental Chemistry Method	**Independent Laboratory Validation**						
Mesosulfuron-methyl (AE F130060)	49829804[2]	49829802[3]		Water	28/01/2016	Bayer CropScience	LC/MS/MS	0.25 ng/mL
AE F160459								
AE F160460								
AE F140584								
AE F147447								
AE F092944								

[1] Mesosulfuron-methyl (AE F130060) = Methyl 2-[(4,6-dimethoxypyrimidin-2-ylcarbamoyl)sulfamoyl]-α-(methanesulfonamido)-p-toluate (IUPAC); Methyl 2-[[[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]-4-[[(methylsulfonyl)amino]methyl]benzoate (CAS). AE F160459 = Methyl 2-[[[[(4-hydroxy-6-methoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]-4-[[(methylsulfonyl)amino]methyl]benzoate (CAS). AE F160460 = 2-[[[[(4-Hydroxy-6-methoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]-4-[[(methylsulfonyl)amino]methyl]benzoic acid (CAS). AE F140584 = Methyl 2-(aminosulfonyl)-4-[[(methylsulfonyl)amino]methyl]benzoate (CAS). AE F147447 = N-[(2,3-Dihydro-1,1-dioxido-3-oxo-1,2-benzisothiazol-6-yl)methyl]methanesulfonamide (CAS). AE F092944 = 4,6-Dimethoxy-2-pyrimidinamine (CAS).

[2] The ECM was a method only; no experimental data was provided. The method contained one procedure for the analysis of mesosulfuron-methyl, AE F140584 and AE F092944 and another procedure for the analysis of AE F147447, AE F160460 and AE F160459.

3 In the ILV, the pond water sample (pH 7.8, dissolved organic carbon 4.6 ppm) was provided by Bayer and fully characterized by Agvise Laboratories, Northwood, North Dakota (Sample ID: 031115-W POND WATER; p. 15; Appendix B, pp. 56-57 of MRID 49829802).

I. Principle of the Method

Mesosulfuron-methyl, AE F140584 and AE F092944: Samples (10 mL) were placed in 20-mL glass vials and fortified, as necessary (p. 10; Appendix 2A, p. 20 of MRID 49829804). After 0.1 mL of the 1 µg/mL internal standard solution was added to each vial, the sample was mixed well then analyzed by LC/MS/MS.

Samples are analyzed using an AB Sciex API 6500 equipped with electrospray ionization interface (pp. 6, 12-15 of MRID 49829804). The following LC conditions were used: Thermo Scientific Aquasil C18 column (3.0 mm x 100 mm, 3 µm; column temperature 50°C), mobile phase of (A) water:methanol (9:1, v:v) containing 10 mM ammonium formate and 120 µL/L formic acid and (B) water:methanol (1:9, v:v) containing 10 mM ammonium formate and 120 µL/L formic acid [percent A:B (v:v) at 0.0-0.5 min. 95:5, 4.0-5.0 min. 0:100, 5.1 min. 95:5], and injection volume of 50 µL (the injection volume was noted as adjustable based on LC/MS/MS system being used). The following MS/MS conditions were used: TEM source temperature, 400°C; positive ion polarity for mesosulfuron-methyl and AE F092944; negative ion polarity for AE F140584; and multiple reaction monitoring (MRM). Two ion pair transitions were monitored for each analyte (quantitative and confirmatory, respectively): *m/z* 504.2 → 181.9 and *m/z* 504.2 → 83.0 for mesosulfuron-methyl, *m/z* 320.9 → 289.0 and *m/z* 320.9 → 209.0 for AE F140584, and *m/z* 156.2 → 100.0 and *m/z* 156.2 → 124.0 for AE F092944. Expected retention times were *ca*. 3.8, 2.3, and 3.1 minutes for mesosulfuron-methyl, AE F140584, and AE F092944, respectively. Internal standards of mesosulfuron-methyl and AE F092944, as well as 2-carbomethoxy benzenesulfonamide, were used to quantify the analytes; 2-carbomethoxy benzenesulfonamide was used to quantify AE F140584.

AE F147447, AE F160459 and AE F160460: Samples (10 mL) were placed in 20-mL glass vials and fortified, as necessary (pp. 7, 11; Appendix 2B, p. 21 of MRID 49829804). After 0.05 mL of the 1 µg/mL internal standard solution and 0.2 mL of formic acid were added to each vial, the sample was mixed well. The sample was purified using a Strata-XL C18 SPE cartridge (200 mg, 6 mL; pre-conditioned with one cartridge volume of methanol then one cartridge volume of 2% formic acid in water). The sample was applied to the cartridge without vacuum. The sample vial was rinsed with 1 mL of 2% formic acid in water, and the rinse was applied to the cartridge without vacuum. The sample was eluted with 2 mL of acetonitrile:water (9:1, v:v) without vacuum. After elution, the cartridge was dried with vacuum, collecting the remainder of the solvent. The purified sample was concentrated to dryness using evaporation (Turbo Vap) at *ca*. 50°C. The residue was reconstituted using 0.5 mL of water:methanol (9:1, v:v) with vortex mixing then analyzed by LC/MS/MS.

Samples are analyzed using an AB Sciex API 6500 equipped with electrospray ionization interface (pp. 6, 12-15 of MRID 49829804). The following LC conditions were used: Imtakt Scherzo SM-C18 column (4.6 mm x 100 mm, 3 µm; column temperature 50°C), mobile phase of (A) 5 mM ammonium formate in water and (B) 0.2 M ammonium formate in water:methanol (1:1, v:v) [percent A:B (v:v) at 0.0-0.5 min. 50:50, 4.5-5.5 min. 0:100, 5.6 min. 50:50], and injection volume of 50 µL (the injection volume was noted as adjustable based on LC/MS/MS system being used).

The following MS/MS conditions were used: TEM source temperature, 300°C; positive ion polarity for AE F160460 and AE F160459; negative ion polarity for AE F147447; and multiple reaction monitoring (MRM). Two ion pair transitions were monitored for each analyte (quantitative and confirmatory, respectively): *m/z* 288.8 → 209.0 and *m/z* 288.8 → 181.0 for AE F147447, *m/z* 490.0 → 168.0 and *m/z* 490.0 → 100.0 for AE F160459, and *m/z* 476.0 → 168.0 and *m/z* 476.0 → 142.1 for AE F160460. Expected retention times were *ca*. 2.0, 4.0, and 4.2 minutes for AE F147447, AE F160459 and AE F160460, respectively. The internal standard of AE F147447 was used to quantify the analytes.

ILV: The extraction procedures for mesosulfuron-methyl, AE F140584 and AE F092944 were performed exactly as written in the ECM (pp. 19-20 of MRID 49829802). For AE F147447, AE F160459 and AE F160460, the ILV extraction procedure was performed exactly as written in the ECM, except that all glass vials and culture tubes were pre-rinsed with 2% formic acid and allowed to dry prior to use, the concentration of the internal standard was increased ten-fold and the reconstitution solvent was changed to water:methanol (9:1, v:v) +10% acetonitrile (pp. 21-23, 29). The samples are analyzed for analytes by HPLC/MS/MS using a Shimadzu HPLC system and a SCIEX API 6500 MS (pp. 23-27, 29). The LC and MS parameters were the same for each of the analytes as those of the ECM; however, for the third attempt analysis of AE F147447, AE F160459 and AE F160460, the resolution of the LC/MS/MS in the first period was changed from Q3-High to Q3-Low. Two ion pair transitions were monitored for all analytes (quantitative and confirmatory, respectively): *m/z* 504.035 → 182.062 and *m/z* 504.080 → 83.070 for mesosulfuron-methyl, *m/z* 320.9 → 289.0 and *m/z* 320.9 → 209.0 for AE F140584, *m/z* 156.08 → 100.093 and *m/z* 156.08 → 124.062 for AE F092944, *m/z* 288.957 → 208.961 and *m/z* 288.957 → 181.049 for AE F147447, *m/z* 490.0 → 168.0 and *m/z* 490.0 → 100.0 for AE F160459, and *m/z* 476.0 → 168.0 and *m/z* 476.0 → 142.1 for AE F160460 (these were similar to those reported in the ECM). Expected retention times were *ca*. 4.10, 2.6, and 3.41 minutes for mesosulfuron-methyl, AE F140584, and AE F092944, respectively, and *ca*. 2.51, 4.76, and 5.05 minutes for AE F147447, AE F160459 and AE F160460, respectively. The same internal standards, as in the ECM, were used to quantify the analytes.

LOQ and LOD: In the ECM and ILV, Limit of Quantification (LOQ) was 0.25 ng/mL for all analytes (p. 6 of MRID 49829804; p. 20 of MRID 49829802). In the ECM and ILV, the Limit of Detection (LOD) was not reported.

II. Recovery Findings

ECM (MRID 49829804): MRID 49829804 was not an experimental study; it was a method only. No recovery data was reported or determined.

ILV (MRID 49829802): Mean recoveries and relative standard deviations (RSDs) were within guidelines (mean 70-120%; RSD ≤20%) for analysis of mesosulfuron-methyl (AE F130060) and its transformation products, AE F140584, AE F092944, AE F147447 and AE F160460, at fortification levels of 0.25 ng/mL (LOQ) and 2.5 ng/mL (10×LOQ) in one water matrix, except for the LOQ confirmation analyses for AE F140584 (RSD 29.2%) and AE F147447 (RSD 36.6%) and the LOQ analyses of AE F160460 (mean recovery, 63.7% quantitation ion and 62.7% confirmation ion; uncorrected recovery results; pp. 28-29; Tables 1-3, pp. 30-35). Mean recoveries and RSDs were not within guidelines for AE F160459 analysis in the water matrix: LOQ quantitation ion, mean 60.9% RSD 25.7%, and confirmation ion, mean 59.1% RSD 31.1%; 10×LOQ quantitation ion, mean 62.9% RSD 23.4%, and confirmation ion, mean 63.1% RSD 23.9%. Two ion transitions were monitored for each analyte. Results of the quantitation and confirmation ion transitions were generally comparable. The pond water sample (pH 7.8, dissolved organic carbon 4.6 ppm) was provided by Bayer and fully characterized by Agvise Laboratories, Northwood, North Dakota (Sample ID: 031115-W POND WATER; p. 15; Appendix B, pp. 56-57). The method was validated with the first trial for mesosulfuron-methyl, AE F140584 and AE F092944 with no modifications; the method was validated with the third trial for AE F147447, AE F160460 and AE F160459 with minor modifications which included pre-washing glassware with 2% formic acid, increasing the concentration of the internal standard and changing the reconstitution solution to match the solvent of the standard solutions (pp. 21-23, 29). The method contained one procedure for the analysis of mesosulfuron-methyl, AE F140584 and AE F092944 and another procedure for the analysis of AE F147447, AE F160460 and AE F160459.

Table 2. Initial Validation Method Recoveries for Mesosulfuron-methyl (AE F130060) and Its Transformation Products, AE F160459, AE F160460, AE F140584, AE F147447 and AE F092944, in Water[1]

Analyte	Fortification Level (ng/mL)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Water						
	Quantitation ion transition					
Mesosulfuron-methyl (AE F130060)	0.25 (LOQ)	No data reported or determined				
	2.5					
AE F140584	0.25 (LOQ)					
	2.5					
AE F092944	0.25 (LOQ)					
	2.5					
AE F147447	0.25 (LOQ)					
	2.5					
AE F160460	0.25 (LOQ)					
	2.5					
AE F160459	0.25 (LOQ)					
	2.5					
	Confirmation ion transition					
Mesosulfuron-methyl (AE F130060)	0.25 (LOQ)	No data reported or determined				
	2.5					
AE F140584	0.25 (LOQ)					
	2.5					
AE F092944	0.25 (LOQ)					
	2.5					
AE F147447	0.25 (LOQ)					
	2.5					
AE F160460	0.25 (LOQ)					
	2.5					
AE F160459	0.25 (LOQ)					
	2.5					

1 Mesosulfuron-methyl (AE F130060) = Methyl 2-[(4,6-dimethoxypyrimidin-2-ylcarbamoyl)sulfamoyl]-α-(methanesulfonamido)-p-toluate (IUPAC); Methyl 2-[[[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]-4-[[(methylsulfonyl)amino]methyl]benzoate (CAS). AE F160459 = Methyl 2-[[[[(4-hydroxy-6-methoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]-4-[[(methylsulfonyl)amino]methyl]benzoate (CAS). AE F160460 = 2-[[[[(4-Hydroxy-6-methoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]-4-[[(methylsulfonyl)amino]methyl]benzoic acid (CAS). AE F140584 = Methyl 2-(aminosulfonyl)-4-[[(methylsulfonyl)amino]methyl]benzoate (CAS). AE F147447 = N-[(2,3-Dihydro-1,1-dioxido-3-oxo-1,2-benzisothiazol-6-yl)methyl]methanesulfonamide (CAS). AE F092944 = 4,6-Dimethoxy-2-pyrimidinamine (CAS).

Table 3. Independent Validation Method Recoveries for Mesosulfuron-methyl (AE F130060) and Its Transformation Products, AE F160459, AE F160460, AE F140584, AE F147447 and AE F092944, in Water[1,2,3,4]

Analyte	Fortification Level (ng/mL)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Water						
	Quantitation ion transition					
Mesosulfuron-methyl (AE F130060)	0.25 (LOQ)	5	95.3-99.9	98	2.1	2.14
	2.5	5	102-107	104	2	2.19
AE F140584	0.25 (LOQ)	5	95.7-109	99.5	5.5	5.6
	2.5	5	91.4-102	97.5	3.8	3.9
AE F092944	0.25 (LOQ)	5	92.0-100	95.7	3.7	3.89
	2.5	5	100-109	103.4	4	3.39
AE F147447	0.25 (LOQ)	5	93.0-140	108	19.6	18.2
	2.5	5	74.8-117	101	17.2	17.1
AE F160460	0.25 (LOQ)	5	52.4-76.5	**63.7**	10.5	16.4
	2.5	5	65.5-74.9	70.7	3.4	4.8
AE F160459	0.25 (LOQ)	5	44.5-79.9	**60.9**	15.7	**25.7**
	2.5	5	37.4-74.7	**62.9**	14.7	**23.4**
	Confirmation ion transition					
Mesosulfuron-methyl (AE F130060)	0.25 (LOQ)	5	99.1-108	103	3.5	3.44
	2.5	5	99.7-107	102	2.9	2.86
AE F140584	0.25 (LOQ)	5	61.2-128	94.4	27.6	**29.2**
	2.5	5	93.3-104	100.7	4.2	4.2
AE F092944	0.25 (LOQ)	5	79.2-93.5	85.0	6.6	7.8
	2.5	5	99.0-107	103.1	3.6	3.53
AE F147447	0.25 (LOQ)	5	58.2-144	98	35.7	**36.6**
	2.5	5	70.8-120	100	19.0	19.1
AE F160460	0.25 (LOQ)	5	51.1-76.9	**62.7**	11.1	17.8
	2.5	5	65.6-75.2	71.1	3.5	4.9
AE F160459	0.25 (LOQ)	5	38.0-80.8	**59.1**	18.4	**31.1**
	2.5	5	37.2-76.0	**63.1**	15.1	**23.9**

Data (uncorrected recovery results; p. 28) were obtained from Tables 1-3, pp. 30-35 of MRID 49829802 and DER Attachment 2.

1 The pond water sample (pH 7.8, dissolved organic carbon 4.6 ppm) was provided by Bayer and fully characterized by Agvise Laboratories, Northwood, North Dakota (Sample ID: 031115-W POND WATER; p. 15; Appendix B, pp. 56-57).

2 Mesosulfuron-methyl (AE F130060) = Methyl 2-[(4,6-dimethoxypyrimidin-2-ylcarbamoyl)sulfamoyl]-α-(methanesulfonamido)-p-toluate (IUPAC); Methyl 2-[[[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]-4-[[(methylsulfonyl)amino]methyl]benzoate (CAS). AE F160459 = Methyl 2-[[[[(4-hydroxy-6-methoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]-4-[[(methylsulfonyl)amino]methyl]benzoate (CAS). AE F160460 = 2-[[[[(4-Hydroxy-6-methoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]-4-[[(methylsulfonyl)amino]methyl]benzoic acid (CAS). AE F140584 = Methyl 2-(aminosulfonyl)-4-[[(methylsulfonyl)amino]methyl]benzoate (CAS). AE F147447 = N-[(2,3-Dihydro-1,1-dioxido-3-oxo-1,2-benzisothiazol-6-yl)methyl]methanesulfonamide (CAS). AE F092944 = 4,6-Dimethoxy-2-pyrimidinamine (CAS).

3 Two ion pair transitions were monitored for all analytes (quantitative and confirmatory, respectively):): *m/z* 504.035 → 182.062 and *m/z* 504.080 → 83.070 for mesosulfuron-methyl, *m/z* 320.9 → 289.0 and *m/z* 320.9 → 209.0 for AE F140584, *m/z* 156.08 → 100.093 and *m/z* 156.08 → 124.062 for AE F092944, *m/z* 288.957 → 208.961 and *m/z* 288.957 → 181.049 for AE F147447, *m/z* 490.0 → 168.0 and *m/z* 490.0 → 100.0 for AE F160459, and *m/z* 476.0 → 168.0 and *m/z* 476.0 → 142.1 for AE F160460; p. 27).

4 Data provided for mesosulfuron-methyl, AE F140584 and AE F092944 were from the first trial with no modifications; data provided for AE F147447, AE F160460 and AE F160459 were from the third trial with several modifications (p. 29).

III. Method Characteristics

In the ECM and ILV, the LOQ was 0.25 ng/mL for all analytes (p. 6 of MRID 49829804; p. 20 of MRID 49829802). No justifications, calculations or discussions were provided to support the method LOQ. In the ECM and ILV, the LOD was not reported.

Table 4. Method Characteristics

Analyte[1]		**Mesosulfuron-methyl (AE F130060)**	**AE F140584**	**AE F092944**	**AE F147447**	**AE F160460**	**AE F160459**
Limit of Quantitation (LOQ)		0.25 ng/mL					
Limit of Detection (LOD)		Not reported					
Linearity (calibration curve r^2 and concentration range)	ECM[2]	$r^2 = 0.9954$	$r^2 = 0.9990$	$r^2 = 0.9994$	$r^2 =$ **0.9801**[3]	$r^2 = 0.9998$	$r^2 =$ **0.9801**[3]
		0.1-100 ng/mL			1-100 ng/mL		
	ILV[4]	$r^2 =$ **0.9912** (Q & C)	$r^2 = 0.9986$ (Q & C)	$r^2 = 0.9980$ (Q & C)	$r^2 = 0.9998$ (Q & C)	$r^2 = 0.9980$ (Q & C)	$r^2 = 0.9980$ (Q & C)
		0.1-100 ng/mL			1-100 ng/mL		
Repeatable	ECM	**No** data reported or determined; method only.					
	ILV[5,6]	First trial with no modifications			Third trial with minor modifications		
		Yes at LOQ and 10×LOQ.	Q: Yes at LOQ and 10×LOQ. C: Yes at 10×LOQ; **No** at LOQ (RSD **29.2%**).	Yes at LOQ and 10×LOQ.	Q: Yes at LOQ and 10×LOQ. C: Yes at 10×LOQ; **No** at LOQ (RSD **36.6%**).	Q: Yes at 10×LOQ; **No** at LOQ (mean **63.7%**). C: Yes at 10×LOQ; **No** at LOQ (mean **62.7%**).	**No** at LOQ (Q/C mean **59.1-60.9%**, RSD **25.7-31.1%**) and 10×LOQ (Q/C mean **62.9-63.1%**, RSD **23.4-23.9%**).
		One characterized pond water matrix was used.					
Reproducible		Could not be determined; only one set of performance data was provided.					
Specific	ECM	Could not be determined. Only chromatograms of standards were provided.					
	ILV	Yes. Matrix interferences were <10% of LOQ, based on peak area, at analyte retention times. Some minor peak tailing and non-uniform integration was noted in most of the LOQ and 10×LOQ chromatograms.					

Data were obtained from pp. 6, 9-10; Appendix 3, pp. 22-24 (calibration curves); Appendix 4, pp. 25-30 (chromatograms of standards) of MRID 49829804; pp. 20, 29; Tables 1-3, pp. 30-35 (data tables and correlation coefficients); Appendix C, Figures 1-70, pp. 58-134 (AE F147447, AE F160460 and AE F160459 chromatograms of standards, controls, LOQ and 10×LOQ); Appendix C, Figures 71-169, pp. 135-233 (mesosulfuron-methyl, AE F140584 and AE F092944 chromatograms of standards, controls, LOQ and 10×LOQ) of MRID 49829802 and DER Attachment 2. Q = quantitation ion transition; C = confirmation ion transition.

[1] Mesosulfuron-methyl (AE F130060) = Methyl 2-[(4,6-dimethoxypyrimidin-2-ylcarbamoyl)sulfamoyl]-α-(methanesulfonamido)-p-toluate (IUPAC); Methyl 2-[[[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]-4-[[(methylsulfonyl)amino]methyl]benzoate (CAS). AE F160459 = Methyl 2-[[[[(4-hydroxy-6-methoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]-4-[[(methylsulfonyl)amino]methyl]benzoate (CAS). AE F160460 = 2-[[[[(4-Hydroxy-6-methoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]-4-[[(methylsulfonyl)amino]methyl]benzoic acid (CAS). AE F140584 = Methyl 2-(aminosulfonyl)-4-[[(methylsulfonyl)amino]methyl]benzoate (CAS). AE F147447 = N-[(2,3-Dihydro-1,1-dioxido-3-oxo-1,2-benzisothiazol-6-yl)methyl]methanesulfonamide (CAS). AE F092944 = 4,6-Dimethoxy-2-pyrimidinamine (CAS).

2 ECM correlation coefficients for all analytes were reviewer-calculated from r values of 0.99-0.9999 (analytes combined; only one set of calibration curves was provided; Appendix 3, pp. 22-24 of MRID 49829804; DER Attachment 2).

3 The value reported in the ECM was incomplete: "0.99…" (Appendix 3, pp. 22-24 of MRID 49829804).

4 ILV correlation coefficients for all analytes were reviewer-calculated from r values of 0.9956-0.9999 (Q & C; analytes combined; Tables 1-3, pp. 30-35 of MRID 49829802; DER Attachment 2).

5 The ILV pond water sample (pH 7.8, dissolved organic carbon 4.6 ppm) was provided by Bayer and fully characterized by Agvise Laboratories, Northwood, North Dakota (Sample ID: 031115-W POND WATER; p. 15; Appendix B, pp. 56-57 of MRID 49829802).

6 The method contained one procedure for the analysis of mesosulfuron-methyl, AE F140584 and AE F092944 and another procedure for the analysis of AE F147447, AE F160460 and AE F160459 (p. 29 of MRID 49829802).

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

IV. Method Deficiencies and Reviewer's Comments

1. Two sets of performance data were not submitted to support the method; the ECM MRID 49829804 was a method only and did not report any experimental data. According to the OCSPP guidelines, two sets of performance data should be submitted, one for the initial or other internal validation and one for the ILV, with the following exception. If the initial validation was performed by a governmental agency, a reference to the agency's documentation of the ECM will serve as the ECM report.

2. The following ILV procedural recoveries did not meet OCSPP guidelines for precision and accuracy (mean 70-120%; RSD ≤20%): the LOQ confirmation analyses for AE F140584 (RSD 29.2%) and AE F147447 (RSD 36.6%); the LOQ analyses of AE F160460 (mean recovery, 63.7% quantitation ion and 62.7% confirmation ion); and all AE F160459 analyses (LOQ quantitation ion, mean 60.9% RSD 25.7%, and confirmation ion, mean 59.1% RSD 31.1%; 10×LOQ quantitation ion, mean 62.9% RSD 23.4%, and confirmation ion, mean 63.1% RSD 23.9%; uncorrected recovery results; pp. 28-29; Tables 1-3, pp. 30-35 of MRID 49829804). The reviewer noted that a confirmatory method is not usually required when LC/MS and GC/MS is the primary method.

   The reviewer noted that the precision (RSD values) of the ILV recovery data for AE F160459 were better and within OCSPP guidelines in the second trial: LOQ quantitation ion, mean 62.2% RSD 3.3%, and confirmation ion, mean 63.6% RSD 6.4%; 10×LOQ quantitation ion, mean 61.0% RSD 3.1%, and confirmation ion, mean 61.0% RSD 2.8%; uncorrected recovery results; pp. 28-29; Tables 2-3, pp. 33-35 of MRID 49829804).

3. The modifications to the ECM procedure for AE F147447, AE F160459 and AE F160460 included 1) all glass vials and culture tubes were pre-rinsed with 2% formic acid and allowed to dry prior to use; 2) the concentration of the internal standard was increased ten-fold; and 3) the reconstitution solvent was changed to water:methanol (9:1, v:v) +10% acetonitrile (pp. 21-23, 29 of MRID 49829802). The reviewer did not consider these modifications to be significant; however, satisfactory quantitation ion results were only produced for the LOQ and 10×LOQ analyses of AE F147447 and the 10×LOQ analysis of AE F160460 (Tables 1-3, pp. 30-35). No LOQ confirmation ion analyses produced satisfactory results. Based on the ILV results, the reviewer believed that the ECM procedure for AE F147447, AE F160459 and AE F160460 required additional optimization to reduce analyte loss.

4. The estimations of the LOQ in ECM and ILV were not based on scientifically acceptable procedures as defined in 40 CFR Part 136 (p. 6 of MRID 49829804; p. 20 of MRID 49829802). No calculations were reported in ECM or ILV to support the method LOQ. The LOD was not reported in the ECM and ILV.

5. The correlation coefficients of the ILV linear regressions for mesosulfuron-methyl were unsatisfactory (Tables 1-3, pp. 30-35 of MRID 49829802). The correlation coefficients of the ECM linear regressions for AE F147447 and AE F160459 were unsatisfactory, as well; however, the values in the study report were incomplete, written as "0.99…" (Appendix 3, pp. 22-24 of MRID 49829804). Linearity is satisfactory when $r^2 \geq 0.995$.

6. In the ECM, only chromatograms for standards were provided (Appendix 4, pp. 25-30 of

MRID 49829804). OCSPP guidelines states that representative chromatograms should be provided for reagent blanks, matrix blanks, standard curves, and spiked samples at the LOQ and 10× LOQ for all analytes in each matrix. Additionally, chromatograms of the reagent blanks were not included.

7. In the ILV MRID 49829802, the reviewer noted the following significant typographical errors in metabolite codes: AE F1474**47** was incorrectly written as AE F1474**77** in the study report text, tables and figures (p. 29; Table 2, p. 32; Table 3, p. 34; Appendix C, pp. 58-60; Appendix C, Figures 1-66, pp. 65-130) and AE F1405**84** was incorrectly written as AE F1405**94** (Appendix C, p. 63; Appendix C, Figures 153-154, pp. 217-218). The following minor typographical error was noted in the ILV: the second "CV (RSD) LOQ" in Table 1 for AE F140584 results should be written as "CV (RSD) 10x LOQ" (Table 1, p. 31).

8. ECM MRID 49829804 was an amended report. The changes made to the amended final report were listed in Appendix 6, p. 34 of MRID 49829804. The changes involved adding the metabolite AE F147447 internal standard to the method.

9. In the ILV, the total time required to perform the extraction was *ca.* 6 hours for a set of 13 water samples (p. 29 of MRID 49829802). The time for LC/MS/MS analysis was not reported.

10. Communications between the ILV and Study Monitor were reported as the discussion of the successful completion or failures of the first, second and third trials, as well the ILV modifications to the method (Appendix D, pp. 234-236 of MRID 49829802).

V. References

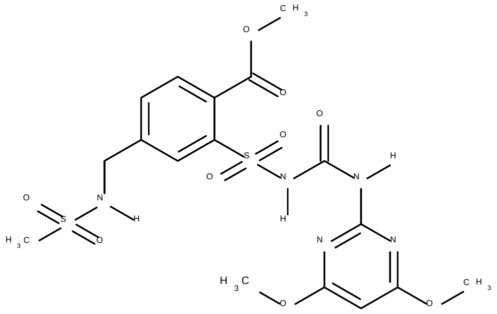
U.S. Environmental Protection Agency. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.

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 (

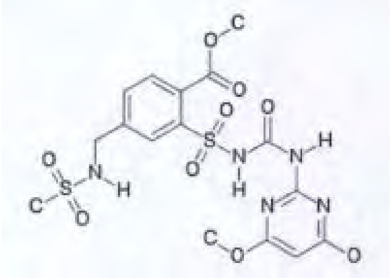
Mesosulfuron-methyl (AE F130060)

IUPAC Name:	Methyl 2-[(4,6-dimethoxypyrimidin-2-ylcarbamoyl)sulfamoyl]-α-(methanesulfonamido)-p-toluate
CAS Name:	Methyl 2-[[[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]-4-[[(methylsulfonyl)amino]methyl]benzoate
CAS Number:	208465-21-8
SMILES String:	[H]N(Cc1ccc(c(c1)S(=O)(=O)N([H])C(=O)N([H])c2nc(cc(n2)OC)OC)C(=O)OC)S(=O)(=O)C



AE F160459 (Des-O-Methyl Mesosulfuron-methyl)

IUPAC Name:	Not reported
CAS Name:	Methyl 2-[[[[(4-hydroxy-6-methoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]-4-[[(methylsulfonyl)amino]methyl]benzoate
CAS Number:	Not provided
SMILES String:	Not found



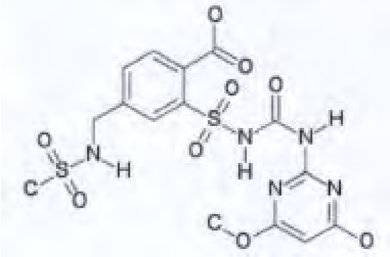
AE F160460 (Des-O-Methyl DesMethyl Ester Mesosulfuron)

IUPAC Name: Not reported

CAS Name: 2-[[[[(4-Hydroxy-6-methoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]-4-[[(methylsulfonyl)amino]methyl]benzoic acid

CAS Number: Not provided

SMILES String: Not found



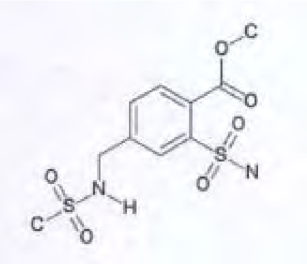
AE F140584 (Mesosulfuron Sulfonamide)

IUPAC Name: Not reported

CAS Name: Methyl 2-(aminosulfonyl)-4-[[(methylsulfonyl)amino]methyl]benzoate

CAS Number: 393509-80-3

SMILES String: Not found



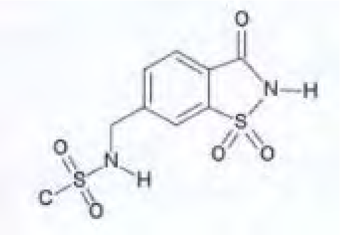
AE F147447 (Saccharin methanesulfonamide)

IUPAC Name: Not reported

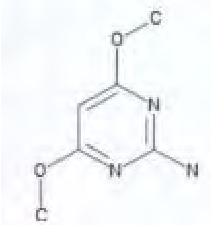
CAS Name: N-[(2,3-Dihydro-1,1-dioxido-3-oxo-1,2-benzisothiazol-6-yl)methyl]methanesulfonamide

CAS Number: Not provided

SMILES String: Not found



AE F092944 (ADMP)
IUPAC Name: Not reported
CAS Name: 4,6-Dimethoxy-2-pyrimidinamine
CAS Number: 36315-01-2
SMILES String: Not found



Test Material: Mesosulfuron-methyl (AE F130060)

MRID: 49829804

Title: Bayer Method MM-002-W15-02: An Analytical Method for the Determination of Residues of Mesosulfuron-methyl (AE F130060) and its metabolites AE F160459, AE F160460, AE F140584, AE F147447 and AE F092944 in Water Using LC/MS/MS

MRID: 49829802

Title: Independent Laboratory Validation (ILV) of An Analytical Method for the Determination of Residues of Mesosulfuron-methyl (AE F130060) and its Metabolites AE F160459, AE F160460, AE F140584, AE F147447 and AE F092944 in Water Using LC/MS/MS

EPA PC Code: 122009

OCSPP Guideline: 850.6100

For CDM/CSS-Dynamac JV

Primary Reviewer: Lisa Muto **Signature:** Lisa Muto

Date: 9/29/16

Secondary Reviewer: Kathleen Ferguson **Signature:** Kathleen P. Ferguson

Date: 9/29/16

Quality Assurance Manager: Joan Gaidos **Signature:**

Date: 9/29/16

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