Test Material: Bensulfuron

MRID: 49588204

Title: "Analytical Method Validation for the Analysis of Bensulfuron-methyl in Soil and Sediment"

MRID: 49839204

Title: Independent Laboratory Validation of an Analytical Method for the Determination of Bensulfuron-methyl in Sediment and Soil

EPA PC Code: 128820

OCSPP Guideline: 850.6100

For CDM/CSS-Dynamac JV

Primary Reviewer: Lisa Muto   **Signature:**

Date: 11/18/16

Secondary Reviewer: Kathleen Ferguson   **Signature:**

Date: 11/18/16

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

Date: 11/18/16

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

Analytical method for bensulfuron in soil and sediment

Reports:	ECM: EPA MRID No.: 49588204. Budgeon, A., D. Rutt and J. Unrath. 2015. "Analytical Method Validation for the Analysis of Bensulfuron-methyl in Soil and Sediment". JRFA Study No.: AU-2014-07 and Method No.: AU-270R0. Report prepared by JFR America, Audubon, Pennsylvannia, and sponsored and submitted by RICECO International, Memphis, Tennessee; 124 pages. Final report issued February 6, 2015.

ILV: EPA MRID No. 49839204. Keenan, D. 2015. Independent Laboratory Validation of an Analytical Method for the Determination of Bensulfuron-methyl in Sediment and Soil. PTRL Study No.: 2785W. Report prepared by PTRL West (a division of EAG, Inc.), Hercules, California, sponsored and submitted by RICECO LLC, Memphis, Tennessee; 62 pages. Final report issued December 16, 2015.

Document No.: MRIDs 49588204 & 49839204

Guideline: 850.6100

Statements: ECM: The study was conducted in accordance with USEPA FIFRA Good Laboratory Practice (GLP) standards, with the exception that the sieving of the sediment samples was not documented (p. 3 of MRID 49588204). Signed and dated No Data Confidentiality, GLP, Quality Assurance and Authenticity statements were provided (pp. 2-5).

ILV: The study was conducted in accordance with USEPA FIFRA GLP standards (p. 3 of MRID 49839204). Signed and dated No Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2-4). A statement of the authenticity of the study report was included with the quality assurance statement (p. 4).

Classification: This analytical method is classified as **unacceptable**. The ECM method with matrices prepared in acetonitrile:water (50:50, v:v) was not validated by the ILV. For the ECM (ACN), insufficient samples were prepared for the 10×LOQ fortification. For the ECM (ACN:H_2O), performance data at LOQ (0.1 ng/g) was not reported to validate the method.

PC Code: 128820

EPA Primary Reviewer:	Kristy Crews, Chemist	Signature:	KRISTY CREWS *Digitally signed by KRISTY CREWS Date: 2017.06.06 16:45:55 -04'00'*
		Date:	
EPA Secondary Reviewer:	William Eckel, Senior Scientist Advisor	Signature:	Mark Corbin *Digitally signed by Mark Corbin Date: 2017.06.06 16:51:46 -04'00'*

Date:

Executive Summary

This analytical method, JRFA Analytical Method AU-270R0 (Study No. AU-2014-07), is designed for the quantitative determination of bensulfuron-methyl in soil and sediment using LC/MS/MS at the LOQ of 0.1 ng/g. The LOQ is **less than** the lowest toxicological level of concern in soil and sediment. Two validation sets were prepared in the ECM: one of the validation sets using characterized loam soil and sandy loam and loam sediments was prepared in acetonitrile:water (50:50, v:v); the other validation set using characterized clay loam and loam soils and sandy loam sediment was prepared in acetonitrile. The validation set prepared in acetonitrile was fortified at the method LOQ of 0.1 ng/g; the other was not. The ILV used characterized loam soil and sand sediment to validate the method at the LOQ of 0.1 ng/g. The number of trials was not specified, but the reviewer assumed that the method was validated in the first trial with insufficient modifications to the analytical method. In the ILV, the solvent with which the matrices were diluted was not reported as either acetonitrile or acetonitrile:water, 50:50 (v:v); the reviewer assumed that the solvent for diluting the matrices was acetonitrile based on the method LOQ validated. Therefore, the ECM method prepared in acetonitrile:water (50:50, v:v) [ECM (ACN:H_2O)] was not validated by the ILV. For the ECM (ACN), insufficient samples were prepared for the $10 \times$LOQ fortification (n = 2). For the ECM (ACN:H_2O), performance data at LOQ (0.1 ng/g) was not reported to validate the method. ILV correlation coefficients were unsatisfactory in sediment, and all ECM correlation coefficients were unsatisfactory ($r^2 < 0.995$).

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						
Bensulfuron-methyl	49588204[1]	49839204[2]		Soil & Sediment	06/02/2015	RICECO International	LC/MS/MS	0.1 ng/g

1 In the ECM, Louisiana loam soil (JRFA ID# 104775; LA-003; 41% sand 46% silt 13% clay; 1.4% organic matter; pH 5.8 in 1:1, soil:water) from Washington, Louisiana, California clay loam soil (JRFA ID# 104945; UTC SOIL 0-3"; 44% sand 21% silt 35% clay; 0.87% organic matter; pH 7.8 in 1:1, soil:water) from Research For Hire, Inc., California, North Carolina loam sediment (JRFA ID# 104004; SEDIMENT 104004; 43% sand 34% silt 23% clay; 3.7% organic matter; pH 5.5 in 1:1, soil:water) from Lucama, North Carolina and Oklahoma sandy loam sediment (JRFA ID# 104029; SEDIMENT 104029; 63% sand 22% silt 15% clay; 2.2% organic matter; pH 7.8 in 1:1, soil:water) from Weatherford, Oklahoma were well characterized (USDA soil texture classification; p. 13; Appendix B, pp. 73-76 of MRID 49839204).

2 In the ILV, loam soil (2705W-025; 45% sand 44% silt 11% clay; 0.97% organic matter; pH 6.8 in 1:1, soil:water) and sand sediment (2706W-018; 91% sand 5% silt 4% clay; 2.2% organic matter; pH 5.8 in 1:1, soil:water) matrices were well characterized (USDA soil texture classification; p. 14; Appendix C, pp. 61-62 of MRID 49839204). The soil/sediment matrices were provided by PTRL West.

I. Principle of the Method

Soil/sediment (10 g) was fortified (0.100 mL) with the appropriate fortification solution, as necessary, in a 50-mL plastic centrifuge tube (pp. 14, 17, 24 of MRID 49588204). The sample was extracted with 10 mL of 1% (v/v) acetic acid in acetonitrile via vigorous hand shaking for *ca*. 1 minute. For LA soil and NC and OK sediment, samples were sonicated for 5 minutes. For CA soil, samples were sonicated for *ca*. 1 minute, followed by vortexing for 30 seconds; this step was repeated four times. After centrifugation at *ca*. 3500 rpm for *ca*. 5 minutes, 2 mL of the supernatant was transferred to a 15-mL plastic centrifuge tube with the contents of two QuEChERS dSPE kit tubes (2 mL tubes containing 50 mg PSA and 150 mg $MgSO_4$. The centrifuge tubes were vortexed for *ca*. 30 seconds, then centrifuged at *ca*. 3500 rpm for *ca*. 2 minutes. For the controls and LOQ samples, the sample was directly analyzed by LC/MS/MS. For samples with fortifications above the LOQ, the samples were diluted with acetonitrile:water (50:50, v:v) or acetonitrile, as necessary. One of the validation sets was prepared in acetonitrile:water (50:50, v:v); the other validation set was prepared in acetonitrile.

Samples were analyzed for bensulfuron-methyl by Agilent 1290 Infinity UPLC system coupled with an ABSciex API 6500 Q Trap MS (Atlantis T3 C_{18}, 2.1 mm x 100 mm, 3 µm column; column temperature ambient) using a mobile phase of (A) water:formic acid (1000:1, v:v) and (B) acetonitrile:formic acid (1000:1, v:v) [percent A:B at 0.00-0.10 min. 80:20, 5.00-7.00 min. 5:95, 7.20-10.00 min. 80:20) with MS/MS-ESI (electrospray ionization) detection in positive ion mode and multiple reaction monitoring (MRM; pp. 17-18 of MRID 49588204). Injection volume was 5-10 µL. Bensulfuron-methyl was identified using two ion transitions; one for quantitation (Q) and one for confirmation (C). Ion transitions monitored were *m/z* 410.997→148.9 (Q) and *m/z* 410.997→182.1 (C). Approximate retention time was 3.82 minutes.

In the ILV, the ECM was performed as written, following the sonication method for LA soil and NC and OK sediment (pp. 14-17, 24; Figure 1, p. 27 of MRID 49839204). An Agilent 1200 HPLC System (Atlantis T3 C_{18}, 2.1 mm x 100 mm, 3 µm column; Atlantis T3 guard column, 2.1 x 10 mm, 3 µm; column temperature 25°C) was coupled to an Applied Biosystems API 5500 Tandem MS for analyte identification. Injection volume was 10 µL. Bensulfuron-methyl was identified using two ion transitions; one for quantitation (Q) and one for confirmation (C). Ion transitions monitored were *m/z* 411.7→149.1 (Q) and *m/z* 411.7→182.2 (C). The ILV study author noted that the MS instrument of the ILV was less sensitive than the MS instrument of the ECM; no other modifications of the ECM were reported; the calibration standards were modified for the lower sensitivity of the analytical instrument (p. 24). The solvent with which the matrices were diluted was not reported as either acetonitrile or acetonitrile:water, 50:50 (v:v; p. 17). The reviewer assumed that the solvent for diluting the matrices was acetonitrile based on the LOQ validated.

The Limit of Quantitation (LOQ) for soil and sediment was 0.1 ng/g in the ECM and ILV (pp. 6, 24-25; Attachment A, p. 110 of MRID 49588204; pp. 11, 24 of MRID 49839204). The Limit of Detection (LOD) for soil and sediment was reported as 0.03 ng/g in the ECM and 0.02 ng/g in the ILV.

II. Recovery Findings

ECM (MRID 49588204): For matrices diluted with acetonitrile (ACN), mean recoveries and relative standard deviations (RSDs) were within guideline requirements (mean 70-120%; RSD ≤20%) for analysis of bensulfuron-methyl in CA clay loam and LA loam soils and OK sandy loam sediment at fortification levels of 0.1 ng/g (LOQ), 1.0 ng/g (10×LOQ) and 10 ng/g (100×LOQ; quantitation and confirmatory ions; pp. 7-9, 20-23; Tables 11.1-11.12, pp. 27-38; Attachment A, Appendix 1, Tables 1-4, p. 113). Insufficient samples were prepared for the 10×LOQ fortification (n = 2). Performance data (recovery results) from quantitation ion analyses and confirmation ion analyses were comparable. For matrices diluted with water:acetonitrile (50:50, v:v), mean recoveries and relative standard deviations (RSDs) were within guideline requirements (mean 70-120%; RSD ≤20%) for analysis of bensulfuron-methyl in LA loam soil and OK sandy loam and NC loam sediments at fortification levels of 1.0 ng/g (10×LOQ) and 10 ng/g (100×LOQ; quantitation and confirmatory ions). No samples were prepared at the LOQ. Performance data (recovery results) from quantitation ion analyses and confirmation ion analyses were comparable. The data for CA soil and OK sediment diluted with ACN was reported in JRF Method AU-270R0 (Attachment A) and JRF Method AU-270R0 / JRF America Study AU-2014-07 (main study report). The Louisiana loam soil (JRFA ID# 104775; LA-003; 41% sand 46% silt 13% clay; 1.4% organic matter; pH 5.8 in 1:1, soil:water) from Washington, Louisiana, California clay loam soil (JRFA ID# 104945; UTC SOIL 0-3"; 44% sand 21% silt 35% clay; 0.87% organic matter; pH 7.8 in 1:1, soil:water) from Research For Hire, Inc., California, North Carolina loam sediment (JRFA ID# 104004; SEDIMENT 104004; 43% sand 34% silt 23% clay; 3.7% organic matter; pH 5.5 in 1:1, soil:water) from Lucama, North Carolina and Oklahoma sandy loam sediment (JRFA ID# 104029; SEDIMENT 104029; 63% sand 22% silt 15% clay; 2.2% organic matter; pH 7.8 in 1:1, soil:water) from Weatherford, Oklahoma were well characterized (USDA soil texture classification; p. 13; Appendix B, pp. 73-76 of MRID 49839204). All soil/sediment characterization was performed by Agvise Laboratories, Northwood, North Dakota.

ILV (MRID 49839204): Mean recoveries and RSDs were within guideline requirements for analysis of bensulfuron-methyl in loam soil and sand sediment at fortification levels of 0.1 ng/g (LOQ) and 1.0 ng/g (10×LOQ; quantitation and confirmatory ions; Tables I-II, p. 26). Performance data (recovery results) from quantitation ion analyses and confirmation ion analyses were comparable. The loam soil (2705W-025; 45% sand 44% silt 11% clay; 0.97% organic matter; pH 6.8 in 1:1, soil:water) and sand sediment (2706W-018; 91% sand 5% silt 4% clay; 2.2% organic matter; pH 5.8 in 1:1, soil:water) matrices were well characterized (USDA soil texture classification; p. 14; Appendix C, pp. 61-62 of MRID 49839204). All soil/sediment characterization was performed by Agvise Laboratories, Northwood, North Dakota. The soil/sediment matrices were provided by PTRL West. The number of trials was not specified, but the reviewer assumed that the method was validated in the first trial. The reviewer assumed that the solvent for diluting the matrices was acetonitrile based on the LOQ validated; therefore, the ECM method prepared in acetonitrile:water (50:50, v:v) was not validated by the ILV.

Table 2. Initial Validation Method Recoveries for Bensulfuron-methyl in Soil/Sediment[1,2]

Analyte	Fortification Level (ng/g)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
JRF Method AU-270R0						
CA Clay Loam Soil (ACN)[3,4]						
Quantitation ion						
Bensulfuron-methyl	0.1 (LOQ)	7	109-123	116	4.3	3.7
	1.0	**2**	107-108	108	0.7	0.7
	10	5	105-120	112	5.9	5.2
Confirmatory ion						
Bensulfuron-methyl	0.1 (LOQ)	7	103-120	114	5.8	5.1
	1.0	**2**	101-104	103	2.1	2.1
	10	5	103-110	106	2.9	2.7
OK Sandy Loam Sediment (ACN)[3,4]						
Quantitation ion						
Bensulfuron-methyl	0.1 (LOQ)	7	83.1-102	96.1	6.4	6.7
	1.0	**2**	83.7-97.7	90.7	9.9	10.9
	10	5	93.2-99.8	97.3	2.7	2.8
Confirmatory ion						
Bensulfuron-methyl	0.1 (LOQ)	7	92.6-108	100	5.1	5.1
	1.0	**2**	84.3-93.1	88.7	6.2	7.0
	10	5	89.5-102	97.2	5.8	6.0
JRF Method AU-270R0 / JRF America Study AU-2014-07						
LA Loam Soil (ACN)[3]						
Quantitation ion						
Bensulfuron-methyl	0.1 (LOQ)	7	110-123	116	5.4	4.6
	1.0	**2**	113-116	115	2.1	1.9
	10	5	100-118	108	7.0	6.5
Confirmatory ion						
Bensulfuron-methyl	0.1 (LOQ)	7	114-124	118	3.4	2.9
	1.0	**2**	111-114	113	2.1	1.9
	10	5	97.6-122	108	9.0	8.4
LA Loam Soil (50:50, ACN:Water)[3]						
Quantitation ion						
Bensulfuron-methyl	1.0	7	107-111	109	1.3	1.2
	10	5	107-117	113	3.6	3.2
Confirmatory ion						
Bensulfuron-methyl	1.0	7	106-111	109	1.7	1.6
	10	5	110-117	114	2.9	2.5
OK Sandy Loam Sediment (50:50, ACN:Water)[3]						
Quantitation ion						
Bensulfuron-methyl	1.0	7	81.9-98.8	89.1	6.1	6.8
	10	5	83.3-90.2	86.9	3.2	3.7
Bensulfuron-methyl	1.0	7	76-102	90.3	9.1	10.0
	10	5	79-109	95.2	10.8	11.4

Analyte	Fortification Level (ng/g)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	NC Loam Sediment (50:50, ACN:Water)[3]					
	Quantitation ion					
Bensulfuron-methyl	1.0	7	92.4-115	105	7.6	7.3
	10	5	107-115	112	3.2	2.8
	Confirmatory ion					
Bensulfuron-methyl	1.0	7	91.4-119	112	9.8	8.8
	10	5	105-116	109	4.2	3.9

Data (uncorrected recovery results, pp. 18-19) were obtained from pp. 7-9, 20-23; Tables 11.1-11.12, pp. 27-38 and Attachment A, Appendix 1, Tables 1-4, p. 113 of MRID 49588204.

1 The Louisiana loam soil (JRFA ID# 104775; LA-003; 41% sand 46% silt 13% clay; 1.4% organic matter; pH 5.8 in 1:1, soil:water) from Washington, Louisiana, California clay loam soil (JRFA ID# 104945; UTC SOIL 0-3"; 44% sand 21% silt 35% clay; 0.87% organic matter; pH 7.8 in 1:1, soil:water) from Research For Hire, Inc., California, North Carolina loam sediment (JRFA ID# 104004; SEDIMENT 104004; 43% sand 34% silt 23% clay; 3.7% organic matter; pH 5.5 in 1:1, soil:water) from Lucama, North Carolina and Oklahoma sandy loam sediment (JRFA ID# 104029; SEDIMENT 104029; 63% sand 22% silt 15% clay; 2.2% organic matter; pH 7.8 in 1:1, soil:water) from Weatherford, Oklahoma were well characterized (USDA soil texture classification; p. 13; Appendix B, pp. 73-76 of MRID 49839204). All soil/sediment characterization was performed by Agvise Laboratories, Northwood, North Dakota.

2 Ion transitions monitored were as follows: *m/z* 410.997→148.9 Q) and *m/z* 410.997→182.1 (C .

3 The solvent with which the matrix was diluted prior to LC/MS/MS analysis.

4 The data for CA soil and OK sediment diluted in ACN was reported in JRF Method AU-270R0 and JRF Method AU-270R0 / JRF America Study AU-2014-07.

Table 3. Independent Validation Method Recoveries for Bensulfuron-methyl in Soil/sediment[1,2,3]

Analyte	Fortification Level (ng/g)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
			Loam Soil			
			Quantitation ion			
Bensulfuron-methyl	0.1 (LOQ)	5	72.3-78.5	75	2.6	3.4
	1.0	5	80.5-101	88	8.6	9.8
			Confirmatory ion			
Bensulfuron-methyl	0.1 (LOQ)	5	71.4-86.2	80	5.5	6.8
	1.0	5	86.1-95.9	91	3.5	3.9
			Sand Sediment			
			Quantitation ion			
Bensulfuron-methyl	0.1 (LOQ)	5	100-116	106	6.4	5.8
	1.0	5	74.4-103	85	10.6	12.4
			Confirmatory ion			
Bensulfuron-methyl	0.1 (LOQ)	5	96.5-111	103	5.3	5.2
	1.0	5	75.4-104	84	11.4	13.6

Data (uncorrected recovery results, pp. 19-21; Figures 22-23, pp. 48-49) were obtained from Tables I-II, p. 26 of MRID 49839204.

1 The loam soil (2705W-025; 45% sand 44% silt 11% clay; 0.97% organic matter; pH 6.8 in 1:1, soil:water) and sand sediment (2706W-018; 91% sand 5% silt 4% clay; 2.2% organic matter; pH 5.8 in 1:1, soil:water) matrices were well characterized (USDA soil texture classification; p. 14; Appendix C, pp. 61-62 of MRID 49839204). All soil/sediment characterization was performed by Agvise Laboratories, Northwood, North Dakota. The soil/sediment matrices were provided by PTRL West.

2 Ion transitions monitored were as follows: m/z 411.7→149.1 (Q) and m/z 411.7→182.2 (C).

3 In the ILV, the solvent with which the matrices were diluted was not reported as either acetonitrile or acetonitrile:water, 50:50 (v:v; p. 17 of MRID 49839204). The reviewer assumed that the solvent for diluting the matrices was acetonitrile based on the LOQ validated.

III. Method Characteristics

In the ECM and ILV, the LOQ for soil and sediment was 0.1 ng/g (pp. 6, 24-25; Table 11.13, p. 39; Attachment A, p. 110 of MRID 49588204; pp. 11, 24 of MRID 49839204). In the ECM, the LOQ was defined by the lowest fortification level successfully tested, and calculated as three times the calculated LOD (see below). The calculated LOQs supported the method LOQ. No justification of the LOQ was reported in the ILV. The LOD for soil and sediment was reported as 0.03 ng/g in the ECM and 0.02 ng/g in the ILV. In the ECM, the LOD was defined as the lowest analyte concentration detectable above the mean amplitude of the background noise in an untreated sample at the corresponding retention time. The LOD was also estimated as three times the background noise, as well as the lowest standard for each analyte in the calibration curve which has good detectability (signal to noise ratio greater than 3:1). The LOD was calculated as the standard deviation at the LOQ multiplied by $t_{0.99}$. The calculated LODs supported the method LOD. In the ILV, the LOD was defined as 20% of the LOQ.

Table 4. Method Characteristics*

Analyte			Bensulfuron-methyl	
Limit of Quantitation (LOQ)			0.1 ng/g	
Limit of Detection (LOD)	ECM		0.03 ng/g	
	ILV		0.02 ng/g	
Linearity (calibration curve r^2 and concentration range)	ECM[1,2]	ACN:H$_2$O	r^2 = **0.9904** (Q)	r^2 = **0.9908** (C)
			(0.0025-0.100 ng/mL)	
		ACN	r^2 = **0.9906** (Q)	r^2 = **0.9924** (C)
			r^2 = **0.9910** (Q)	r^2 = **0.9902** (C)
			(0.0025-0.05 ng/mL)	
	ILV[1]	Soil	r^2 = 0.9972 (Q)	r^2 = 0.9982 (C)
		Sediment	r^2 = **0.9928** (Q)	r^2 = **0.9926** (C)
			(0.004-0.4 ng/mL)	
Repeatable	ECM[3]	ACN:H$_2$O	Yes at 10×LOQ and 100×LOQ, but **no** samples were prepared at LOQ	
		ACN	Yes at LOQ, 10×LOQ and 100×LOQ, but n = **2** at 10×LOQ	
	ILV[4,5]	ACN:H$_2$O	**Not performed**	
		ACN	Yes at LOQ and 10×LOQ	
Reproducible			Yes at LOQ and 10×LOQ (ACN)	
Specific	ECM[6]		Significant baseline noise in the LOQ Q chromatogram which interfered with peak integration in some matrices. Matrix interferences were <5% of the LOQ for soil and sediment (Q & C; based on peak area), except for OK sediment (C), <16% of the LOQ (based on peak area).	
	ILV		Yes, no matrix interferences were noted in the soil (Q & C) and sediment (C) chromatograms; matrix interferences in the sediment (Q) were <10% of the LOQ (based on peak area). Significant baseline noise was observed in some LOQ chromatograms.	

Data were obtained from pp. 6-9, 16, 20-25; Tables 11.1-11.12, pp. 27-38 (recovery data); Table 11.13, p. 39 (LOQ/LOD calculations); Figures 12.2-12.3, pp. 42-43 (calibration curves); Figures 12.15-12.30, pp. 55-70 (chromatograms); Attachment A, p. 104 (calibration range); Attachment A, Appendix 1, Tables 1-4, p. 113 (recovery data); Attachment A, Appendix 2, p. 114 (calibration curves); Attachment A, Figures 4-8, pp. 118-122

(chromatograms) of MRID 49588204; pp. 11, 21, 24; Tables I-II, p. 26 (recovery data); Figures 2-13, pp. 28-39 (control and standard chromatograms); Figures 14-17, pp. 40-43 (calibration curves); Figures 18-21, pp. 44-47 (chromatograms) of MRID 49839204; DER Attachment 2. Q = quantitation ion; C = confirmatory ion.

* The solvent with which the matrices were diluted: ACN = 100% acetonitrile; ACN:H2O = acetonitrile:water, 50:50 (v:v).

1 Reported correlation coefficients were reviewer-calculated from r values reported in the study report (Figures 12.2-12.3, pp. 42-43; Attachment A, Appendix 2, p. 114 of MRID 49588204; Figures 14-17, pp. 40-43 of MRID 49839204; DER Attachment 2).

2 The ECM MRID 49588204 was comprised of two reports: the main report, JRF Method AU-270R0 / JRF America Study AU-2014-07, and the initial validation report in Attachment A, JRF Method AU-270R0. Linearity data for matrices diluted in acetonitrile was from both reports. Non-matrix matched calibration standards were used (pp. 16, 25 of MRID 49588204).

3 In the ECM, Louisiana loam soil (JRFA ID# 104775; LA-003; 41% sand 46% silt 13% clay; 1.4% organic matter; pH 5.8 in 1:1, soil:water) from Washington, Louisiana, California clay loam soil (JRFA ID# 104945; UTC SOIL 0-3"; 44% sand 21% silt 35% clay; 0.87% organic matter; pH 7.8 in 1:1, soil:water) from Research For Hire, Inc., California, North Carolina loam sediment (JRFA ID# 104004; SEDIMENT 104004; 43% sand 34% silt 23% clay; 3.7% organic matter; pH 5.5 in 1:1, soil:water) from Lucama, North Carolina and Oklahoma sandy loam sediment (JRFA ID# 104029; SEDIMENT 104029; 63% sand 22% silt 15% clay; 2.2% organic matter; pH 7.8 in 1:1, soil:water) from Weatherford, Oklahoma were well characterized (USDA soil texture classification; p. 13; Appendix B, pp. 73-76 of MRID 49839204).

4 In the ILV, loam soil (2705W-025; 45% sand 44% silt 11% clay; 0.97% organic matter; pH 6.8 in 1:1, soil:water) and sand sediment (2706W-018; 91% sand 5% silt 4% clay; 2.2% organic matter; pH 5.8 in 1:1, soil:water) matrices were well characterized (USDA soil texture classification; p. 14; Appendix C, pp. 61-62 of MRID 49839204). The soil/sediment matrices were provided by PTRL West.

5 In the ILV, the solvent with which the matrices were diluted was not reported as either acetonitrile or acetonitrile:water, 50:50 (v:v; p. 17 of MRID 49839204). The reviewer assumed that the solvent for diluting the matrices was acetonitrile based on the LOQ validated.

6 Only one set of chromatograms was provided for each matrix; chromatograms were not provided for each matrix/dilution solvent. The reviewer assumed that chromatograms provided for LA loam soil and OK sandy loam sediment were for those diluted with acetonitrile since LOQ chromatograms were included, and no LOQ fortifications were prepared for matrices diluted with acetonitrile:water, 50:50 (v:v). Therefore, chromatograms were not provided for LA loam soil and OK sandy loam sediment diluted with acetonitrile:water, 50:50 (v:v).

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

IV. Method Deficiencies and Reviewer's Comments

1. ECM MRID 49588204 reported two LOQs and two LODs, one "per the protocol" and one "per the method" (p. 24 of MRID 49588204). The ECM study authors and ILV study author cited the method LOQ (0.1 ng/g) as the LOQ for the method validation (pp. 6, 24-25; Attachment A, p. 110 of MRID 49588204; pp. 11, 24 of MRID 49839204). Similarly, the reviewer reported the method LOD of the ECM. The ECM study authors noted that the use of 100% acetonitrile allowed the method target LOQ to be lowered to 0.1 ng/g and the LOD to be lowered to 0.03 ng/g (p. 13 of MRID 49588204).

2. Two validation sets were prepared in the ECM: one of the validation sets was prepared in acetonitrile:water (50:50, v:v); the other validation set was prepared in acetonitrile (pp. 6-9 of MRID 49588204). The validation set prepared in acetonitrile was fortified at the method LOQ of 0.1 ng/g; the other was not. In the ILV, the solvent with which the matrices were diluted was not reported as either acetonitrile or acetonitrile:water, 50:50 (v:v; p. 17 of MRID 49839204). The reviewer assumed that the solvent for diluting the

matrices was acetonitrile based on the LOQ validated; therefore, the ECM method prepared in acetonitrile:water (50:50, v:v) was not validated by the ILV.

3. For the ECM matrices diluted with acetonitrile:water, 50:50 (v:v), performance data at LOQ (0.1 ng/g) was not reported to validate the method. A validation sample set should consist of, at a minimum, a reagent blank, two unspiked matrix control samples, five matrix control samples spike at the LOQ, and five matrix control samples spiked at 10×LOQ for each analyte and matrix.

    For the ECM matrices diluted with acetonitrile, insufficient samples were prepared for the 10×LOQ fortification (1.0 ng/g; n = 2). A validation sample set should consist of, at a minimum, a reagent blank, two unspiked matrix control samples, five matrix control samples spike at the LOQ, and five matrix control samples spiked at 10×LOQ for each analyte and matrix.

4. In the ILV, the solvent with which the matrices were diluted was not reported as either acetonitrile or acetonitrile:water, 50:50 (v:v; p. 17 of MRID 49839204). The reviewer assumed that the solvent for diluting the matrices was acetonitrile.

5. The coefficient of determination of the calibration curves of the ILV for bensulfuron-methyl in sediment were unsatisfactory ($r^2 < 0.995$): $r^2 = 0.9928$ (Q) and $r^2 = 0.9926$ (C; Figures 14-17, pp. 40-43 of MRID 49839204; DER Attachment 2).

    All of the coefficient of determination of the calibration curves of the ECM for bensulfuron-methyl were unsatisfactory ($r^2 < 0.995$): $r^2 = 0.9904$-0.9910 (Q) and $r^2 = 0.9902$-0.9924 (C) for matrices diluted in acetonitrile or acetonitrile:water, 50:50 (v:v; Figures 12.2-12.3, pp. 42-43; Attachment A, Appendix 2, p. 114 of MRID 49588204; DER Attachment 2).

    Though these are listed as deficiencies, they are not grounds for a new study.

6. In the ECM, one set of chromatograms was provided for each matrix; chromatograms were not provided for each matrix/dilution solvent. The reviewer assumed that chromatograms provided for LA loam soil and OK sandy loam sediment were for those diluted with acetonitrile since LOQ chromatograms were included, and no LOQ fortifications were prepared for matrices diluted with acetonitrile:water, 50:50 (v:v; Figures 12.15-12.30, pp. 55-70; Attachment A, Figures 4-8, pp. 118-122 of MRID 49588204). Therefore, chromatograms were not provided for LA loam soil and OK sandy loam sediment diluted with acetonitrile:water, 50:50 (v:v). Representative chromatograms from all fortification levels should be provided for review of method specificity.

7. The estimations of LOQ and LOD in ECM and ILV were not based on scientifically acceptable procedures as defined in 40 CFR Part 136 (pp. 6, 24-25; Table 11.13, p. 39; Attachment A, p. 110 of MRID 49588204; pp. 11, 24 of MRID 49839204). In the ECM, the LOQ was defined by the lowest fortification level successfully tested, and calculated

as three times the calculated LOD. The calculated LOQs supported the method LOQ. The LOD was calculated as the standard deviation at the LOQ multiplied by $t_{0.99}$. The calculated LODs supported the method LOD. No justification of the LOQ was reported in the ILV. In the ILV, the LOD was defined as 20% of the LOQ.

Additionally, the lowest toxicological level of concern in soil/sediment for bensulfuron-methyl was not reported in the ECM and ILV. An LOQ above toxicological levels of concern results in an unacceptable method classification.

8.   ECM MRID 49588204 was comprised of two reports: the main report, JRF Method AU-270R0 / JRF America Study AU-2014-07, and the initial validation report in Attachment A, JRF Method AU-270R0. The reviewer noted that Attachment A contained recovery data for the CA soil (ACN) and OK sediment (ACN), as well as calibration curves and CA soil chromatograms (Attachment A, Appendix 1, Tables 1-4, p. 113; Attachment A, Appendix 2, p. 114; Attachment A, Figures 4-8, pp. 118-122 of MRID 49588204). OK sediment chromatograms were not included in Attachment A, but were included in the main report. The CA soil and OK sediment recovery data and CA soil chromatograms of Attachment A matched that in the main report (Tables 11.1-11.12, pp. 27-38; Figures 12.15-12.30, pp. 55-70). However, the calibration curves/correlation coefficients presented in Attachment A did not match those in the main report; therefore, the reviewer reported these separately (Figures 12.2-12.3, pp. 42-43).

9.   The number of trials was not specified, but the reviewer assumed that the method was validated in the first trial.

10.   The reviewer noted that "p. 50" and "p. 51" of the ECM were duplicates, both containing Figure 12.10; however, p. 50 seemed to be generated on a very large scale compared to the rest of the document (pp. 50-51 of MRID 49588204).

11.   In the ILV, it was reported that no communications between the ILV and method developers occurred during the conduct of the study (p. 24 of MRID 49839204).

12.   In the ECM, no significant matrix effects were observed, and non-matrix-matched calibration standards were used (pp. 23, 25 of MRID 49588204).

13.   In the ECM, bensulfuron-methyl in fortified samples of NC sediment was found to be stable after 19 and 32 days of refrigerated storage (pp. 22-23 of MRID 49588204).

14.   It was reported for the ILV that one batch of thirteen samples (one reagent blank, two controls, and five samples fortified at the LOQ and 10×LOQ) required approximately eleven person hours for preparation and sample processing (pp. 21-22 of MRID 49839204). Instrument analysis and data processing of soil/sediment samples was approximately five hours. Overall, the data set required approximately sixteen hours to complete.

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
   Bensulfuron-methyl
   IUPAC Name: Methyl α-[(4,6-dimethoxypyrimidin-2-ylcarbamoyl)sulfamoyl]-o-toluate
   CAS Name: Methyl 2-[[[[[(4,6-dimethoxy-2-
                         pyrimidinyl)amino]carbonyl]amino]sulfonyl]methyl]benzoate
   CAS Number: 83055-99-6
   SMILES String: n1c(OC)cc(OC)nc1NC(=O)NS(=O)(=O)Cc2ccccc2C(=O)OC