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FINAL REPORT

Study Title

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

Guidelines

40 CFR Part 160
OCSPP 850.6100

MSV

ABSTRACT

The purpose of the study was to demonstrate that JRFA Analytical Method AU-270R0: "Analytical Method for the Analysis of Bensulfuron-methyl in Soil and Sediment" is suitable for the determination of Bensulfuron-methyl in Soil and Sediment. Samples of soil matrices were obtained from two different sites in Louisiana and California, whereas the sediment matrices were obtained from two different sites in North Carolina and Oklahoma, comprising a total of four matrices in which this method was validated. This study complies with the requirements of U.S. EPA Residue Chemistry Test Guidelines, OCSPP 850.6100 (1).

Principle of the Method: The recoveries of Bensulfuron-methyl are determined from fortified soil and sediment. Residues of Bensulfuron-methyl are extracted from soil and sediment samples using a solution of 1% acetic acid in acetonitrile. The sample extract is centrifuged and an aliquot of the supernatant is cleaned up using QuEChERS dispersive solid phase extraction (dSPE) and centrifuged again. The final extracts are dispensed neat into HPLC vials or diluted as needed with 50/50 (v/v) acetonitrile/water or 100% acetonitrile for analysis. Measurement is accomplished by liquid chromatography with mass spectrometric detection (LC-MS/MS). The results are calculated by direct comparison of the sample peak response to those of external standards.

Test Conditions: The method was validated at three fortification levels (0.1, 1.0 and 10 ng/g) for each soil and sediment matrix. For each matrix diluted in 50/50 (v/v) acetonitrile/water, seven replicates were analyzed at a 1 ng/g fortification and five additional replicates were analyzed at a 10 ng/g fortification level. For each matrix diluted with 100% acetonitrile, seven replicates were analyzed at a 0.1 ng/g fortification, two replicates at a 1.0 ng/g fortification, and five additional replicates were analyzed at a 10 ng/g fortification level. Additionally, two replicates of unfortified samples were examined, as well as a method blank in which all method reagents were run through the procedure without any sample matrix present.

Limit of Quantitation (LOQ) and Limit of Detection (LOD): The LOQ was defined as the lowest fortification level tested. The target LOQ as per the protocol was 1.0 ng/g of Bensulfuron-methyl in all four matrices. The LOQ of the analytical method was lowered to 0.1 ng/g based upon expected levels in an anticipated study. This lowered LOQ was used in validation sets prepared in 100% acetonitrile as per the method. The limit of detection was set at 0.3 ng/g for sets using the protocol LOQ, and 0.03 ng/g for sets using the method LOQ.

Specificity: The method determines residues of Bensulfuron-methyl in soil and sediment. No interfering peaks above 30% of the LOQ were found at the retention times of the analyte. Matrix testing determined matrix effects were less than 20% for suppression/enhancement.

Linearity: Standards were prepared covering three ranges. For the LA soil run in 50:50 acetonitrile:water, a range of 0.5 ng/mL to 5 ng/mL was used. For sediment runs in 50:50 ACN:H_2O, a range of .0025 ng/mL to 0.1 ng/mL was used. For the soil and sediment runs in 100% acetonitrile, a range of 0.0025 ng/mL to 0.05 ng/mL was used. Good linearity ($r^2 > 0.99$) was observed for all ranges.

1. PRINCIPLE AND SCOPE OF THE METHOD

JRFA Method AU-270R0 (Attachment A) was developed to determine residues of Bensulfuron-methyl in soil and sediment matrices at JRF America, Audubon, PA. The method was validated at JRF America.

The method validation was conducted using three fortification levels (0.1 ng/g, 1.0 ng/g, and 10 ng/g) for two different soil and two different sediment matrices. For each matrix diluted in 50/50 (v/v) acetonitrile, seven replicates were analyzed at a 1.0 ng/g fortification and five additional replicates were analyzed at a 10 ng/g fortification level. For each matrix diluted with 100% acetonitrile, seven replicates were analyzed at a 0.1 ng/g fortification, two replicates at a 1.0 ng/g fortification, and five additional replicates were analyzed at a 10 ng/g fortification level. Additionally, two unfortified control samples and a method blank, in which all method reagents were run through the procedure without any sample matrix present, were examined.

The residue of Bensulfuron-methyl was extracted from soil and sediment (10 g) using 10 mL of an acidic acetonitrile extraction solvent and QuEChERS dSPE clean up. The final determination was conducted using LC-MS/MS in positive ion mode.

In soil and sediment matrices the method had a protocol target limit of quantification of 1.0 ng/g. For the validation sets in 100% acetonitrile, the method target limit of quantification was lowered to 0.1 ng/g. The limit of detection for the analyte was set to 0.3 ng/g for sets using the Protocol LOQ, and 0.03 ng/g for sets using the Method LOQ (ACN dilutions).

2. MATERIALS AND METHODS

2.1 Test systems

The following test systems were considered in this study. The characterization of the soil and sediment used was performed by Agvise Laboratories and is given in the respective reports located in Appendix B.

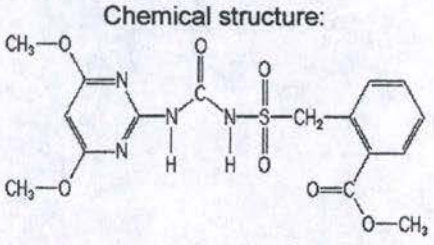
Test System 1: Louisiana soil; Washington, LA 70589 (JRFA ID #104775)
Test System 2: California soil; supplied by Research For Hire Inc., CA (JRFA ID #104945)
Test System 3: North Carolina sediment; Lucama, NC 27851 (JRFA ID #104004)
Test system 4: Oklahoma sediment; Weatherford, OK (JRFA ID #104029)

2.2 Test and Reference Substances

2.2.1 Bensulfuron-methyl

Chemical Name:	methyl α-[(4,6-dimethoxypyrimidin-2-ylcarbamoyl)sulfamoyl]-*o*-toluate
CAS Registry No.:	83055-99-6
Molecular Formula:	$C_{16}H_{18}N_4O_7S$
Molecular Weight:	410.4
Batch No.:	SZBC166XV
Re-assay Date:	July 01, 2015
Purity:	99.6%
Recommended Storage:	Ambient
Source:	Sigma-Aldrich

Chemical structure:



2.3 Equipment

Equipment	Size, Description	Manufacturer	Catalog No.
Balance	Top Loading	Mettler PC 2000	—
Balance	Analytical	Mettler Toledo AT200	—
Balance	Top Loading	Pennsylvania Model 7500	—
Cylinder, Graduated	Various sizes	Fisher	—
Grinder/Processor	Bowl Grinder/Processor	Hobart	—
Bottle, glass	1L	Pyrex	—
Volumetric, pipettes	Various sizes	Eppendorf	—
Flasks, Volumetric	Various sizes	Various	—
Plastic Centrifuge Tubes	50 mL and 15mL	VWR	—
QuEChERS dSPE Kits	2 mL tubes containing 50 mg of PSA and 150 mg of $MgSO_4$	Phenomenex	S351-012
Ultra sonic Bath	FS60	Fisher Scientific	—
Centrifuge	MD4R IEC	IEC	—
Liquid Chromatographic System	1290 Infinity	Agilent	—
Mass Spectrometer	AB Sciex API 6500 QTrap	ABSciex	—
HPLC Column	Atlantis T3 2.1mm x 100 x 3µm	Waters	186003718

2.4 Reagents

2.4.1 Chemicals

Chemical	Grade	Manufacturer/Supplier	Lot No.
Glacial Acetic Acid	HPLC	EMD	48347
Acetonitrile	LC/MS	EMD	54059
Water	LC/MS	EMD	54006
Formic Acid	LC/MS	Fluka Analytical	BCBM1192V

2.4.2 Solutions and Solvent Mixtures

Description	Composition	Preparation
Extraction solvent	1% (v/v) acetic acid in Acetonitrile.	Add 10 mL of glacial acetic acid to a 1 L volumetric flask containing 490 mL of acetonitrile and mix well to ensure a complete homogeneous solution. Fill to the mark with acetonitrile.
Final Diluent A	50/50 (v/v) Acetonitrile/Water	Add 500 mL of water to a 1 L volumetric flask. Fill to the mark with acetonitrile. Mix well to ensure a complete homogeneous solution.
Final Diluent B	100% Acetonitrile	100% Acetonitrile
HPLC Mobile Phase A	0.1% Formic Acid in Water	Add 1 mL of concentrated formic acid into a 1 L volumetric flask. Fill to the mark with water and mix well to ensure complete homogeneous solution
HPLC Mobile Phase B	0.1% Formic Acid in Acetonitrile	Add 1 mL of concentrated formic acid into a 1 L volumetric flask. Fill to the mark with acetonitrile and mix well to ensure complete homogeneous solution.

2.4.3 Standard Solutions

Preparation of Stock Solutions

A 1 mg/mL stock solution was prepared by weighing an appropriate amount of analyte into a 25 mL volumetric flask and diluting to volume. Weight in the table is adjusted for percent purity.

ID	Purity	Weight (mg)	Concentration (µg/mL)	JRFA ID
Bensulfuron-methyl	99.6 %	25.2	1008	JRFA-442/1-1

Preparation of Fortification Solutions

A fortification solution was prepared by serial dilution of the stock solution with acetonitrile. Volumetric flasks were used for all dilutions.

Take Solution (µg/mL)	Volume (mL)	Final Volume (mL)	Bensulfuron-methyl Concentration (µg/mL)	JRFA ID
Stock (1008 µg/mL)	2.480	25	100	JRFA-442/1-2
1008	0.248	25	10	JRFA-442/1-3
1008	0.025	25	1.0	JRFA-442/1-4
1	2.5	25	0.1	JRFA-442/3-4

Preparation of Calibration Standard Solutions- 50:50 (v/v) Acetonitrile:Water

Calibration solutions were prepared by dilution of the fortification solutions with 50/50 (v/v) acetonitrile/water in volumetric flasks.

Solution Used (ng/mL)	Volume Taken (mL)	Final Volume (mL)	Bensulfuron-methyl Concentration (ng/mL)	JRFA ID
1000	0.050	10	5	JRFA-442/4-3
1000	0.040	10	4	JRFA-442/4-4
1000	0.025	10	2.5	JRFA-442/4-5
5	2.000	10	1	JRFA-442/4-6
5	1.000	10	0.5	JRFA-442/4-7

Preparation of Calibration Standard Solutions- 50:50 (v/v) Acetonitrile:Water

Calibration solutions were prepared by dilution of the fortification solutions with 50/50 (v/v) acetonitrile/water in volumetric flasks.

Solution Used (ng/mL)	Volume Taken (mL)	Final Volume (mL)	Bensulfuron-methyl Concentration (ng/mL)	JRFA ID
0.15	3.333	5	0.100	JRFA-442/5-4
0.1	2.500	5	0.050	JRFA-442/5-5
0.05	2.500	5	0.025	JRFA-442/5-6
0.025	2.000	5	0.010	JRFA-442/5-7
0.01	2.500	5	0.005	JRFA-442/5-8
0.005	2.500	5	0.0025	JRFA-442/5-9

Preparation Calibration Standard Solutions- Acetonitrile

Calibration solutions were also prepared by dilution of the intermediate solutions with acetonitrile in volumetric flasks.

Solution Used (ng/mL)	Volume Taken (mL)	Final Volume (mL)	Bensulfuron-methyl Concentration (ng/mL)	JRFA ID
100	0.25	25	1.0	JRFA-442/7-1
1	1.25	25	0.05	JRFA-442/7-2
1	0.625	25	0.025	JRFA-442/7-3
1	0.25	25	0.01	JRFA-442/7-4
1	0.125	25	0.005	JRFA-442/7-5
1	0.0625	25	0.0025	JRFA-442/7-6

All standard solutions were stored refrigerated in screw top amber bottles when not in use.

3. ANALYTICAL PROCEDURE

3.1 Weighing and Fortification

For validation sets prepared in 50:50 acetonitrile/water, fifteen samples were prepared for the matrices (LA soil, NC sediment, and OK sediment): one method blank, two control samples, seven samples treated at 1.0 ng/g, and five samples treated at 10 ng/g. For validation sets prepared in 100% acetonitrile, seventeen samples were prepared for the matrices (LA soil, CA, soil, OK sediment): one method blank, two control samples, seven samples treated at 0.1 ng/g, two samples treated at 1.0 ng/g, and five samples treated at 10 ng/g. For each sample, 10 g of material were weighed into a 50 mL plastic centrifuge tube and spiked with the appropriate fortification solution.

The following scheme was used:

Sample Type	Sample Weight (g)	Concentration of Spiking Solution (ng/mL)	Volume of Spiking Solution (mL)	Level of Fortification (ng/g)
Method Blank	0.0	N/A	N/A	0.0
Control	10	N/A	N/A	0.0
Fortification (0.1 ng/g)	10	10	0.100	0.1
Fortification (1.0 ng/g)	10	100	0.100	1.0
Fortification (10 ng/g)	10	1,000	0.100	10.0

3.2 Extraction of Sample Material

10 mL of 1% (v/v) acetic acid in acetonitrile were added to the sample, shaken vigorously by hand for approximately 1 minute. For LA Soil, NC and OK Sediment, samples were sonicated for 5 minutes. For CA Soil, samples were sonicated for approximately 1 minute, followed by vortexing for 30 seconds; this step was repeated four times (this Method Modification has been noted with AU-270R0). Samples were then centrifuged at approximately 3500 rpm for approximately 5 minutes. The contents of two QuEChERS kit tubes were emptied into one 15 mL centrifuge tube for each sample. 2 mL of the extract supernatant from the original 50 mL centrifuge tube were transfered to the 15 mL plastic centrifuge tube with the QuEChERS salts. Samples were then vortexed for approximately 30 seconds, followed by then centrifuged at approximately 3500 rpm for approximately 2 minutes.

3.3 Preparation for Measurement

For the controls and LOQ samples, the samples are ready for LC-MS/MS determination. These can be dispensed into HPLC vials neat.

Dilute the samples appropriately with 50/50 (v/v) acetonitrile/water or acetonitrile as needed to fit into the calibration curve.

4. INSTRUMENTATION AND CONDITIONS

	Parameter		
Chromatographic System	Agilent 1290 Infinity UPLC System		
Analytical-column	Atlantis T3 C_{18} 3 µm, 100 x 2.1 mm		
Column Temperature	Ambient		
Injection Volume	5 -10 µL		
Mobile Phase A Mobile Phase B	Water / formic acid, 1000/1, v/v Acetonitrile / formic acid, 1000/1, v/v		
Flow Rate	400 µL/min		
Gradient (including wash and equilibration)	Time (min)	Phase A	Phase B
	0.00	80	20
	0.10	80	20
	5.00	5	95
	7.00	5	95
	7.20	80	20
	10.00	80	20
Detection System	ABSciex API 6500 QTrap Mass Spectrometer		
Ionization	Electrospray (ESI)		
Ionization Temperature	600 °C		
Analyte	**Transitions**	**Polarity**	**Expected Retention Time**
Bensulfuron-methyl	410.997 → 148.9* 410.997 → 182.1	Positive	Approx. 3.82 min.

* Primary quantification transition. Either transition could be used for quantitation in case interference is observed at the same retention time. See Figure 12.1 for Product Ion Spectra.

4.1 Calibration Procedures

Calculation of results is based on peak area measurements using a calibration curve. The calibration curve is obtained by direct injection of the calibration standards containing known amounts of Bensulfuron-methyl in the following ranges: 0.5 ng/mL to 5 ng/mL, 0.0025 ng/mL to 0.1 ng/g, and 0.0025 ng/g to 0.05 ng/g. Linear calibration functions (1/x weighting) were used for evaluation.

4.2 Calculation of Recoveries

Calculation of results was based on area measurements. The recovery of the analyte was calculated relative to the linear curve generated with each set.

I. **Calculated Concentration** $\left(\frac{\mathbf{ng}}{\mathbf{mL}}\right) = \frac{\mathbf{y}-\mathbf{b}}{\mathbf{m}} * \mathbf{d_f}$

Where

y = measured analyte peak area counts
b = y-intercept of calibration curve (as in y = m * x + b)
x = slope of calibration curve (as in y = m * x + b)
$\mathbf{d_f}$ = Sample dilution factor

II. $$\%\ \textbf{Recovery} = \frac{\text{Calculated Concentration (ng/mL)} * \text{Control Concentration (ng/mL)}}{\text{Theoretical Concentration (ng/mL)}} * 100\%$$

5. RESULTS

5.1 Validation Data

According to the method AU-270R0, soil and sediment samples were fortified with a solution containing Bensulfuron-methyl to obtain a concentration of 0.1 ng/g (LOQ as per the method, used with acetonitrile sets), 1.0 ng/g (LOQ as per the protocol, used with 50:50 acetonitrile/water sets), and 10 ng/g respectively. To test the repeatability of the method in 50:50 acetonitrile/water, the sample replicates were divided into the following sets of analysis: one method blank, two control samples, seven fortifications at 1.0 ng/g, and five fortifications at 10 ng/g for the matrices LA soil, NC sediment, and OK sediment. To test the repeatability of the method in 100% acetonitrile, the sample replicates were divided into the following sets of analysis: one method blank, two control samples, seven fortifications at 0.1 ng/g, two fortifications at 1.0 ng/g, and five fortifications at 10 ng/g for the matrices LA soil, CA soil, and OK sediment.

Control samples were treated in exactly the same way as fortified samples. All results obtained from measurements of control samples were below the limit of detection (0.3 ng/g for sets using the protocol LOQ, and 0.03 ng/g for sets using the method LOQ) for Bensulfuron-methyl. Therefore, no blank correction in the recovery data was needed.

10. REFERENCES

1. OCSPP 850.6100, Ecological Effects Test Guidelines, U.S. Environmental Protection Agency. U.S. Government Printing Office: Washington, DC, 2012; EPA-712-C-001.