

1.0 INTRODUCTION

1.1 Principle and Scope of the Method

JRFI Method 228-2-12-9333 was developed for the LC-MS/MS analysis of Bensulfuron-methyl in surface, ground and drinking water at JRF India (JRFI). The sources of the waters as well as the characterization results are document in the study file.

The independent laboratory validation was conducted using LOQ as per original method developed at JRFI, 0.05 µg/L and 10x LOQ (0.50µg/L) fortification levels for bensulfuron methyl in drinking, ground, and surface water. For each fortification and matrix, fives samples were examined. Matrix matched standards were prepared for each matrix. Additionally, one reagent blank and two unfortified control samples were analyzed. The method LOQ was set at 0.05 µg/L or 0.05 ppb. All standards were prepared matrix matched. A total of 10 mL of drinking, surface, and ground water were used for each sample 9.80 uL of the matrix water was added with 20 µL of the fortification standard with a final volume of 10 mL. The PR LOQ 10x samples were diluted 10x to fall within the curve. The final determination was conducted using LC/MS/MS analysis in positive ion mode.

1.2 Specificity

Bensulfuron-methyl was analyzed using a quantification ion and a confirmatory ion.

Bensulfuron-methyl quantification ion (m/z 411.26 \rightarrow 149.0)

Bensulfuron-methyl confirmation ion (m/z 411.26 \rightarrow 182.0)

2.0 MATERIALS AND METHODS

2.1 Test Systems

Source Water	JRFA ID	Collection Date & Time	Description
Surface Water	105125	Skipack Creek at Hoy Park, Collegeville PA 9/25/14 at 4:10 PM	River water taken 6 inches deep at a temperature of 22 ° C
Drinking Water	25791	11/20/13 Walmart, Audubon PA at 11:15 AM	Aquafina, Drinking Water (24) 500mL bottles
Ground Water	105123	591 West Boot Lane, West Chester, PA 19380 9/25/14 at 8:00 AM	Take from Spigot of the Bennett residence well water

2.2 Summarized Physio-Chemical Properties of Matrix

Test System	Physico-Chemical Properties	Observation
Drinking Water	pH	6.2
	Conductivity	0.04 mmhos/cm
	Total Hardness as CaCO ₃	Below detection limit of 1 mg
	TOC	1.0 ppm
Ground Water	pH	6.5
	Conductivity	0.62 mmhos/cm
	Total Hardness as CaCO ₃	198 mg
	TOC	1.4 ppm
Surface Water	pH	8.3
	Conductivity	1.92 mmhos/cm
	Total Hardness as CaCO ₃	184 mg
	TOC	4.7 ppm

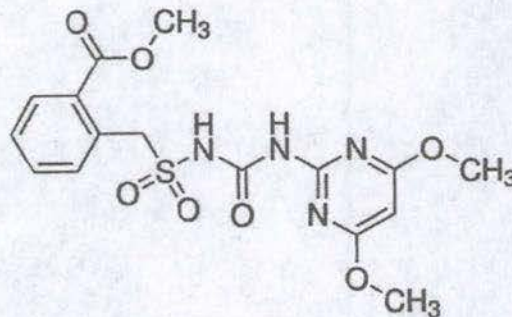
Control, drinking, ground, and surface water matrix were acquired by JRF America. The samples were logged in at JRF America and given unique laboratory identification numbers. The samples were stored refrigerated.

The characterization of the water used was performed by Agvise Laboratories and is given in the respective attached reports Appendix B Water Characterization – Surface Water, Appendix C Water Characterization – Ground Water, and Appendix D Water Characterization Drinking Water.

2.3 Test and Reference Substances

Appendix E - Certificate of Analysis – Reference Material Bensulfuron-methyl. One reference substance will be evaluated: Bensulfuron-methyl. An archive sample of the test/reference substance will be retained at JRF America. The following sample information and chemical/physical properties for the test/reference substance are:

Common Name	Bensulfuron-methyl
Chemical Name	Methyl 2-[(4,6-dimethoxypyrimidin-2-yl)carbamoylsulfamoylmethyl]benzoate
CAS Registry No.	83055-99-6
Molecular Formula	C ₁₆ H ₁₈ N ₄ O ₇ S
Molecular Weight	410.40
Batch No.	SZBC166XV
ReAssay Date	July 1 2015
Source	Sigma Aldrich



2.4 Equipment

Equipment	Size, Description	Manufacturer	Catalog No.
Flasks, Volumetric	Various sizes	Various	----
Cylinder, graduated	Various sizes	Various	----
Pipettes	Various volumes	Eppendorf	----
Bottle, Amber glass	Various Sizes	Thomas Scientific	----
Plastic Centrifuge Tubes	15 mL	Thomas Scientific	6264-500
Ultrasonic bath	Model FS 7652H	Fisher Scientific	13112194
HPLC vials	2 mL	Agilent Technologies	----
HPLC vial caps	PTFE/red silicone septa	Agilent Technologies	5182-0716
Transfer Pipettes	6 mL, Extra Long	Thomas Scientific	1227W98
Liquid Chromatographic System	Waters Acquity UPLC	Waters	5182-0717
Mass Spectrometer	AB Sciex API 4000 QTrap	AB Sciex	----
HPLC Column	Waters X-Bridge, C-18 150x4.6mm, 3.5µm particle size	Waters	----

2.5 Reagents and Chemicals

Chemical	Grade	Manufacturer/Supplier	Lot Number
LC/MS Acetonitrile	LC/MS	EMD Omni Solv	54113, 54150
Formic Acid	LC/MS	Sigma-Fluka Analytical	94318
LC/MS Water	LC/MS	EMD Omni-Solv	54309

2.5.1 Solutions and Solvent Mixtures

Description	Composition
Solvent system Flow	Isocratic - Acetonitrile (60):0.1% Formic Acid (40)v/v
HPLC mobile phase A	0.1% Formic Acid in LCMS Water Add 500 mL of LCMS Water into a 2L volumetric flask. Add 2 mL of LC/MS Formic Acid, fill to the mark with LC/MS Water.
HPLC mobile phase B	100% LC/MS Grade Acetonitrile

2.6 Experimental Design & Analytical Procedures

The analytical method for the determination of residues of Bensulfuron-methyl in drinking, water, ground water and surface water was independently validated using LC-MS-MS. The validation covered the specificity, linear dynamic range (LDR), limit of detection (LOD), limit of quantification (LOQ), precision (%RSD) and accuracy (% Recovery) at LOQ and 10x LOQ level.

2.6.1 Preparation of 0.1% Formic Acid in Water

A volume of 2.0 mL of LC/MS Formic Acid was transferred into a volumetric flask of 2000mL capacity containing 500mL of LCMS Water and the volume was filled to the mark with LC/MS water. The content was mixed thoroughly.

2.7 Standard Solutions- Preparation of Stock Solution

A 0.1245 mg/mL stock solution was prepared by weighing an appropriate amount of bensulfuron mehthyl into a 100 mL volumetric flask and diluting to volume in LC/MS acetonitrile.

Analyte ID	Purity	Weight (mg)	Final Volume (mL)	Concentration (µg/mL)
Bensulfuron-methyl	99.6%	12.45	100	124.5

Corrected for purity

3.0 Preparation of Fortification & Calibration Solutions

3.1 Preparation of Drinking Water Fortification & Calibration Standards

Fortification Standard

Dil. #	Primary Conc.	Primary JRFA ID	Aliquot (mL)	Total Final Volume (mL)	Final Conc.	New JRFA ID
1	124.5 ppm	JRFA 450 4-1	0.803	20	1000 ppb	JRFA 450 4-2
2	1000 ppb	JRFA 450 4-2	10	20	500 ppb	JRFA 450 5-1
3	500 ppb	JRFA 450 5-1	10	20	250 ppb	JRFA 450 5-2
4	250 ppb	JRFA 450 5-2	2	20	25 ppb	JRFA 450 5-3

Calibration Standard

5	25 ppb	JRFA 450 5-3	0.2	10	0.5 ppb	JRFA 450 5-4
6	0.5 ppb	JRFA 450 5-4	5	10	0.25 ppb	JRFA 450 5-5
7	0.25 ppb	JRFA 450 5-5	4	10	0.1 ppb	JRFA 450 5-6
8	0.10 ppb	JRFA 450 5-6	5	10	0.05 ppb	JRFA 450 5-7
9	0.05 ppb	JRFA 450 5-7	5	10	0.025 ppb	JRFA 450 5-8

3.2 Preparation of Surface Water Fortification & Calibration Standards

Fortification Standard

Dil. #	Primary Conc.	Primary JRFA ID	Aliquot (mL)	Total Final Volume (mL)	Final Conc.	New JRFA ID
1	124.5 ppm	JRFA 450 4-1	0.803	20	1000 ppb	JRFA 450 7-1
2	1000 ppb	JRFA 450 7-1	10	20	500 ppb	JRFA 450 7-2
3	500 ppb	JRFA 450 7-2	10	20	250 ppb	JRFA 450 7-3
4	250 ppb	JRFA 450 7-3	2	20	25 ppb	JRFA 450 7-4

Calibration Standard

5	25 ppb	JRFA 450 7-4	0.2	10	0.5 ppb	JRFA 450 7-5
6	0.5 ppb	JRFA 450 7-5	5	10	0.25 ppb	JRFA 450 7-6
7	0.25 ppb	JRFA 450 7-6	4	10	0.10 ppb	JRFA 450 7-7
8	0.10 ppb	JRFA 450 7-7	5	10	0.05 ppb	JRFA 450 7-8
9	0.05 ppb	JRFA 450 7-8	5	10	0.025 ppb	JRFA 450 7-9

3.3 Preparation of Ground Water Fortification & Calibration Standards

Fortification Standard

Dil. #	Primary Conc.	Primary JRFA ID	Aliquot (mL)	Total Final Volume (mL)	Final Conc.	New JRFA ID
1	124.5 ppm	JRFA 450 4-1	0.803	20	1000 ppb	JRFA 450 6-1
2	1000 ppb	JRFA 450 6-1	10	20	500 ppb	JRFA 450 6-2
3	500 ppb	JRFA 450 6-2	10	20	250 ppb	JRFA 450 6-3
4	250 ppb	JRFA 450 6-3	2	20	25 ppb	JRFA 450 6-4

Calibration Standard

5	25 ppb	JRFA 450 6-4	0.2	10	0.5 ppb	JRFA 450 6-5
6	0.5 ppb	JRFA 450 6-5	5	10	0.25 ppb	JRFA 450 6-6
7	0.25 ppb	JRFA 450 6-6	4	10	0.1 ppb	JRFA 450 6-7
8	0.10 ppb	JRFA 450 6-7	5	10	0.05 ppb	JRFA 450 6-8
9	0.05 ppb	JRFA 450 6-8	5	10	0.025 ppb	JRFA 450 6-9

4.0 Analytical Procedure

4.1 Weighing and Fortification

Thirteen samples were prepared for each matrix (surface, ground, and drinking water): One reagent blank, two control samples, five samples treated at the LOQ, and five treated at 10xLOQ. For each fortification, 9.98 mL of water was measured into a 15 mL centrifuge tube and spiked with the appropriate fortification solution, resulting to a final volume of 10mL.

The following scheme was used:

Sample Type	Sample Volume	Concentration of Spiking Solution [µg/mL]	Volume of Spiking Solution [µL]	Level of Fortification [µg/L]
Control	10 mL	-	-	0.00
PR LOQ	10 mL	25	20	0.5
PR 10x LOQ	10 mL	250	20	5.0

5.0 Instrumentation and Conditions

5.1 UPLC/MS/MS Conditions for the Analysis of Bensulfuron-methyl

Shimadzu UPLC Conditions			
Analytical Column	Waters X-Bridge, C-18 150x4.6mm,3.5µm particle size		
Column Temperature	N/A		
Flow Rate	0.8000 mL/min		
Gradient:	Binary	Pump A: 40%	Pump B: 60%
Mobile Phase A:	0.1% Formic Acid in LC/MS Water		
Mobile Phase B:	100% LCMS Acetonitrile		
Injection Volume (µL):	15		

5.2 MS/MS Parameters

MS/MS Conditions	
Interface	AB SCIEX 4000 Qtrap® with Turbo Ion Spray®
Polarity	Positive
Ionization	Electrospray (ESI) Positive
Curtain gas (CUR)	25
Temperature (TEM)	600 °C
Ion transfer voltage (IS)	5500
Collision gas setting (CAD)	7
GS1	60.00
GS2	40.00
Scan type	MRM

5.3 Transitions

Analyte	Transitions (m/z)		Polarity	Approximate Retention Time (min.)
	Quantification	Confirmatory		
Bensulfuron-methyl	411.216 → 149.00	411.216 → 182.100	Positive	3.45

5.4 Calibration Procedures

Calculation of results is based on peak area measurements using a calibration curve. The calibration curve is obtained by injection of the calibration standards containing known amounts of Bensulfuron methyl at appropriate concentrations (0.025 to 0.50 ng/mL). Example calibration curves are presented in Section 12,13, and 14 - Representative chromatograms containing, untreated control, fortified PR LOQ, PR 10x LOQ, and standard curve are presented in Section 15, 16 and 17 - Typical Chromatogram for Drinking Water Standards

5.5 Calculation of Residues and Recoveries

Calculations of results were based on peak area measurements. Analyst[®] 1.5.1 software created the standard curve based on linear regression analysis using 1/x weighting. The recoveries of the analyte were calculated relative to the linearity curve generated with each set.

Source Water	Ion	Equation	Slope	Intercept	R ²
Drinking Water	Quantification	Y = 9.55e004x-53.9	9.55E+04	-53.9	0.9994
	Confirmation	Y = 5E004x-105	5.00E+04	-105.0	0.9990
Ground Water	Quantification	Y = 8.24E004x+111	8.24E+04	111.0	0.9994
	Confirmation	Y = 4.62E004x - 15.4	4.62E+04	-15.4	0.9997
Surface Water	Quantification	Y = 5.71E004x + 336	5.71E+04	336.0	0.9961
	Confirmation	Y = 3.64E004x + 246	3.64E+04	246.0	0.9964

$$\text{I. Concentration (ng/mL)} = \frac{\text{Response} - \text{Intercept}}{\text{Slope}} = C_A$$

The recoveries of spiked compounds are calculated according to equation above:

$$\text{II. Recovery \%} = \frac{\text{Conc. of Fortified Sample (ng/mL)} - \text{Conc. of Control (ng/mL)}}{\text{Conc. of Sample as Prepared}} \times 100$$

7.0 Summary of Method

Type of method: LC/MS/MS

Test systems: Surface (Creek) Water
Ground (Well) Water
Drinking (Bottled) Water

Bensulfuron-methyl selected mass transitions (m/z):

<u>Analyte</u>	<u>Primary</u>	<u>Secondary</u>
Bensulfuron-methyl	411.216 → 149.00	411.216 → 182.100

Analytical procedure: 10 mL of test water was fortified with Bensulfuron. The samples were analyzed using LC/MS/MS electrospray ionization in positive ion mode.

Confirmatory technique: The quantification transition ion was 411.216 → 149.00. The confirmatory transition was 411.216 → 182.100.

Limit of quantification (LOQ): The limit of quantification is 0.05 µg/L for Bensulfuron-methyl

Levels of Fortification: 0.5 ng and 5.0 ng were the levels of fortification on mass basis to achieve a final concentration of 0.05 µg/L and 0.50 µg/L.

Time Required: The analysis of 13 samples per matrix requires about 6-8 hours of work including the calculation and reporting of all data under GLP.

10.1 Method Deviation:

METHOD DEVIATION RECORD

STUDY N°	AU-2014-08	DEVIATION N°	1	EFFECTIVE DATE	03 March 2015
STUDY TITLE	Independent Laboratory Validation of JRFI Analytical Method for the Determination of Bensulfuron-methyl in Surface Water, Ground Water and Drinking Water				

ORIGINAL DETAILS*	DETAILS FOR DEVIATION	REASON FOR DEVIATION
1. The injection volume for the JRFI Analytical method (JRFI Study 228-2-12-9333) was 10 µL injections. * Page 18 of the Protocol under Instrument Parameters	1. The injection volume used for the ILV (JRFA Study AU-2014-08) was 15 µL.	1. Due to acquiring better sensitivity and reproducibility 15 µL was used instead of 10µL.