STUDY TITLE

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

Test Guidelines:
US EPA OCSPP 850.6100
US EPA FIFRA Good Laboratory Practice Standards, 40 CFR §160

SUMMARY

An analytical method for the quantitation of Bensulfuron-methyl in a sediment and soil test systems were independently validated. The method is described in "Analytical Method Validation for the Analysis of Bensulfuron-methyl in Soil and Sediment", JRF America, Study Number AU-2014-07.

The test substance, Bensulfuron-methyl was analyzed using external standardization by Liquid Chromatography with Tandem Mass Spectrometry Detection (LC-MS/MS). Sediment and soil samples were fortified with 0.1 ng/g (LOQ) and 1.0 ng/g (10X LOQ). The limit of detection was defined as approximately 20% of LOQ using the current methodology.

The experiments for the soil and sediment matrices were conducted with one reagent blank, two untreated controls, and five control samples spiked for each fortification level: one at the LOQ level and another at 10X LOQ level, and analyzed samples by LC-MS/MS.

Bensulfuron-methyl content was quantitated against a 1/x weighted linear curves of the reference substance Bensulfuron-methyl for both the quantitation and confirmation ions, with concentrations ranging from 0.006 ng/mL to 0.04 ng/mL for sediment and 0.01 ng/mL to 0.4 ng/mL for soil. The calibration for Bensulfuron-methyl for both analyses yielded acceptable linearity (correlation coefficients $r > 0.99$) over the range examined. The quantitation of Bensulfuron-methyl was based on the peak area response and concentrations of the calibration standards. The amount of Bensulfuron-methyl was determined with the quantitation MS/MS ion transition from m/z 411 to m/z 148.9 and the confirmation MS/MS ion transition from m/z 411 to m/z 182.1. Method recovery from fortified samples was determined by calculating the found concentration of Bensulfuron-methyl and dividing the concentration by the relevant fortification level.

The LOD in soil is estimated to be 0.02 ng/g for Bensulfuron-methyl using either MS/MS transition.

The LOD in sediment is estimated to be 0.02 ng/g for Bensulfuron-methyl using either MS/MS transition.

No interferences or residues were detected in control soil or sediment with the MS/MS transitions selected for quantitation and confirmation.

INTRODUCTION

The purpose of this study was to conduct an independent laboratory validation (ILV) for the determination of Bensulfuron-methyl in soil and sediment. The analysis of the test substance was performed by Liquid Chromatography with Tandem Mass Spectrometry Detection (LC-MS/MS) based on the method described in " Analytical Method Validation for the Analysis of Bensulfuron-methyl in Soil and Sediment", JRF America, Study Number AU-2014-07 [1].

This study was designed to satisfy US EPA Guideline requirements described in OCSPP 850.6100. The study was initiated on August 4, 2015. The experimental work was conducted from September 10, 2015 through October 30, 2015 at PTRL West, 625-B Alfred Nobel Drive, Hercules, CA 94547 under an approved protocol and protocol amendment (Appendix A) according to the US EPA FIFRA Good Laboratory Practice Standards, 40 CFR §160.

MATERIAL AND METHODS

Test and Reference Substance

Common Name:	**Bensulfuron-methyl**
Chemical Name:	Methtyl α-[(4'6-dimethoxypyrimidin-2-ylcarbamoyl)sulfamoyl]-o-toluate
CAS Registry No.:	83055-99-6
Chemical Structure:	
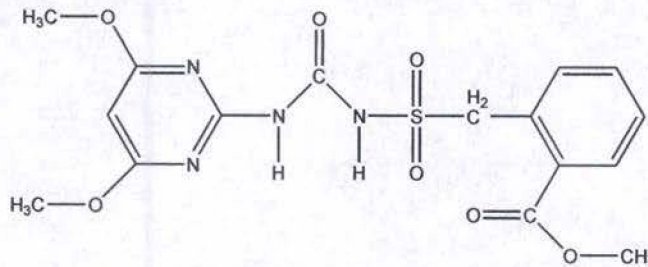
Molecular Formula:	$C_{16}H_{18}N_4O_7S$
Molecular Mass:	410.4 grams/mole
Supplier:	Sigma Aldrich
Lot No.:	SZBC166XV
PTRL Inventory No.	2785W-001
Purity:	99.6%
Date of Expiry:	September 10, 2017
Storage Conditions:	Ambient Temperature

Certificate of Analysis for the test/reference substance is provided in Appendix B.

Other Chemicals

HPLC grade water and acetonitrile were obtained from Burdick & Jackson; acetic acid was obtained from Fisher Scientific.

Equipment List

Laboratory Balances
Beakers
Pipetmen with plastic disposable tips
Vortex mixer
Centrifuge
Ultrasonic bath
Plastic Bottles
Wrist Action Shaker

AB Sciex API 5500 Series Tandem Mass Spectrometer with Agilent 1200 HPLC system (LC-MS/MS)

Test System

Source of Test System

The sediment and soil test systems that were used in the validation study were provided by the PTRL West. The bulk soil/sediment systems were stored under refrigerated conditions when not in use (Inventory Nos. 2705W-025, soil and 2706W-014, sediment). Characterization details are provided in Appendix C.

Test Method

The analytical method for the analysis of Bensulfuron-methyl validated at PTRL West by Liquid Chromatography with Tandem Mass Spectrometry Detection (LC-MS/MS) was described in the method validation report [1]

The soil and sediment samples were spiked with known concentrations of Bensulfuron-methyl. An aliquot of the final sample solution was injected onto the high performance liquid chromatography and subjected to reversed phase chromatography coupled with tandem mass spectrometry (MS/MS) with electro spray ionization. The percent method

recovery was determined using external standardization where linear curve for calibration standards was generated along with the samples.

Preparation of Stock Solution

Duplicate stock solutions were prepared by weighing an aliquot of the reference substance into a volumetric flask. The stock solutions were dissolved and diluted with acetonitrile to yield nominal concentrations of 1 mg/mL. The concentration of stock solutions were corrected for the purity of the reference substance as shown in the following table. The stock solutions were transferred into amber bottles and stored in the freezer (typically < -10°C) when not in use.

Ptrl West No.	Stock ID	Standard Name	Weight (mg)	Final volume (mL)	Purity (%)	Theoretical Conc. (µg/mL)
2785W-001	Stock A	Bensulfuron-methyl	25.10	25.100	99.6	1000
*2785W-001	Stock C	Bensulfuron-methyl	10.15	10.109	99.6	1000

*Stock C prepared for accuracy check only

Preparation of Bensulfuron-methyl Intermediate and Fortification Solutions (Stock Solution A)

A 100 µg/mL intermediate solution was prepared by measuring 2.5 mL of stock solution "A" using a calibrated automatic pipet into a 25 mL volumetric flask. Final solution was diluted to the mark with acetonitrile.

A 10 µg/mL intermediate solution was prepared by measuring 0.25 mL of stock solution "A" using a calibrated automatic pipet into a 25 mL volumetric flask. Final solution was diluted to the mark with acetonitrile.

A 1.0 µg/mL intermediate solution was prepared by measuring 0.025 mL of stock solution "A" using a calibrated automatic pipet into a 25 mL volumetric flask. Final solution was diluted to the mark with acetonitrile.

A 100 ng/mL fortification solution was prepared by measuring 2.5 mL of the 1 µg/mL intermediate solution using a calibrated automatic pipet into a 25 mL volumetric flask. Final solution was diluted to the mark with acetonitrile.

A 10 ng/mL fortification solution was prepared by measuring 1.0 mL of the 100 ng/mL fortification solution using a calibrated automatic pipet into a 10 mL volumetric flask. Final solution was diluted to the mark with acetonitrile.

A 1 ng/mL intermediate solution was prepared by measuring 0.1 mL of the 100 ng/mL fortification solution using a calibrated automatic pipet into a 10 mL volumetric flask. Final solution was diluted to the mark with acetonitrile.

The intermediate and fortification solutions were mixed well, transferred into amber bottles and stored in the freezer (typically < -10°C) when not in use.

Preparation of Bensulfuron-methyl Intermediate Solutions (Stock Solution C)

A 10 µg/mL intermediate solution was prepared by measuring 0.1 mL of stock solution "C" using a calibrated automatic pipet into a 10 mL volumetric flask. Final solution was diluted to the mark with acetonitrile.

A 100 ng/mL intermediate solution was prepared by measuring 0.1 mL of the 10 µg/mL intermediate solution using a calibrated automatic pipet into a 10 mL volumetric flask. Final solution was diluted to the mark with acetonitrile.

A 1 ng/mL intermediate solution was prepared by measuring 0.1 mL of the 100 ng/mL intermediate solution using a calibrated automatic pipet into a 10 mL volumetric flask. Final solution was diluted to the mark with acetonitrile.

The intermediate solutions were mixed well, transferred into amber bottles and stored in the freezer (typically < -10°C) when not in use.

Preparation of Bensulfuron-methyl Calibration Standard Solutions

Nine calibration standard solutions were prepared by mixing appropriate volumes of intermediate/fortification solutions via calibrated automatic pipets with appropriate volumes of acetonitrile:water solution (1:1, v:v) into the volumetric flasks described below. Final calibrants were transferred into glass amber bottles and stored in the freezer (typically < -10°C) when not in use. The standard solutions ranged from 0.004 ng/mL to 0.4 ng/mL were prepared as shown below:

Theoretical Conc. (ng/mL)	Solution Used (ng/mL)	Volume of Solution (mL)	Final Volume (mL)
0.4	10	0.4	10
0.2	10	0.2	10
0.1	10	0.1	10
0.04	1.0	0.4	10
0.02	1.0	0.2	10
0.01	1.0	0.1	10
0.008	1.0	0.08	10

0.006	1.0	0.06	10
0.004	1.0	0.04	10
*0.04	1.0	0.4	

*prepared from Stock Solution C

Fortification Procedure

Fortification of untreated soil and sediment was conducted at the following two fortification levels as shown below:

Fortification Level (ppb or ng/g)	Fortification Solution
0.1	0.1 mL of 10 ng/mL in 10 g of soil
1.0	0.1 mL of 100 ng/mL in 10 g of soil

Fortification was conducted to determine the percent recovery, and accuracy within the method validation. This procedure was performed in quintuplicate during method validation at each fortification level.

Extraction Method for Bensulfuron-methyl in Soil/Sediment

1. Transfer 10 g of soil into a 50 mL plastic centrifuge tube.
2. Fortify the sample as necessary.
3. Add 10 mL of 1% acetic acid acidified acetonitrile (extraction solvent) to each sample
4. Place samples on wrist action shaker for 5 minutes
5. Sonicate samples for 5 minutes.
6. Centrifuge samples for 5 minutes at ~3500 rpm.
7. Transfer 2.0 mL to a 15 mL plastic centrifuge tube containing 100 mg PSA and 300 mg $MgSO_4$ (combine 2-2 mL Q-sep QuEChERS dSPE tubes, Restek #26124).
8. Vortex samples for approximately 30 seconds
9. Centrifuge at ~3500 rpm for 2 minutes
10. Aliquot in autosampler vials for analysis by LC-MS/MS.

A schematic diagram of the soil extraction method is presented in Figure 1.

Liquid Chromatography with Tandem Mass Spectrometry Analytical Method (LC-MS/MS)

LC conditions

Agilent 1200 HPLC system (LC-MS/MS)
Column: Waters Corp. Atlantis T3 3 μ, 100 x 2.1 mm
Guard Cartridge: Waters Corp. Atlantis T3 3μ, 10 mm x 2.1 mm
Injection volume: 10 μL
Flow rate: 0.5 mL/min
Run time: 13 minutes
Column Temperature: 25 °C

Mobile Phase:
- A: 0.1% Formic acid in water
- B: 0.01% Formic acid acetonitrile

Gradient Program:

Time (minutes)	%A	%B	Flow rate (mL/min)
0	80.0	20.0	0.5
0.1	80.0	20.0	0.5
5.0	5.0	95.0	0.5
7.0	5.0	95.0	0.5
7.2	80.0	20.0	0.5
10.0	80.0	20.0	0.5

MS conditions

An Applied Biosystems API 5500 tandem mass spectrometer was used with electrospray ionization (ESI) in positive polarity mode to acquire data by Multiple Reaction Monitoring (MRM).

API 5500:

Q1 Mass (amu)	Q3 Mass (amu)	Dwell (msec)	CE	CXP
411.7	149.1	200	32.0	19.3
411.7	182.2	200	26.0	10.2

CE = Collision Energy
CXP = Collision Cell Exit Potential

Source Dependent Settings:

Temperature (TEM):	600.0°C
Nebulizer Gas (GS1):	40.0
IonSpray Gas (GS2):	40.0
Curtain Gas (CUR):	25.0
Collision Activated Dissociation Gas (CAD):	6.0
Ionization Spray (IS)	1500
Entrance Potential (EP)	10.0
Declustering Potential (DP)	45.0

Post-column effluent was diverted into the mass spectrometer between 4.3 and 5.5 minutes.

LC-MS/MS Analysis

Samples were analyzed in a set consisting of a solvent blank, reagent blank, two control extracts, five 0.1 ppb fortified controls, and five 1.0 ppb fortified controls interspersed between the calibrants. In addition, to ensure accuracy was maintained over the course of the analytical set, quality control (QC) calibrants were added to the sequence. The final QC calibrant's response was within 10% when compared to its initial response. Calibrants and samples were analyzed in a single sequence of injections.

Methods of Calculation

Quantitation

Separation of Bensulfuron-methyl was achieved by LC-MS/MS. The compound was identified by the coincidence of the retention time with the respective reference standard and MS characteristics. The quantitation of Bensulfuron-methyl was conducted using peak area relative to the theoretical concentrations of the calibrants. The content of

Bensulfuron-methyl in sample was quantitated against 1/x weighted linear curve of Bensulfuron-methyl calibrants where:

$$\text{ng/mL analyte} = \frac{y - b}{m}$$

y = peak area
x = ng/mL compound injected
m = slope
b = intercept

Weighting of the calibration curve was applied to provide better curve fit at the lower concentration levels of Bensulfuron-methyl.

The calculation of weighted curve equations (linear regression) and concentrations (ng/mL) present in samples and calibrants was conducted using Analyst® software.

The residue of the analyte in the sample is determined as follows:

$$\text{Residue (ng/g)} = \frac{\text{ng/mL analyte x Initial Extract Volume (mL) x Dil. Factor}}{\text{sample weight (g)}}$$

where

Initial Extract Volume = 10 mL (volume of extraction solvent added)

The Percent Recovery of a fortified sample is determined as follows:

$$\frac{\text{Residue }(ng/g) - \text{Average Residue of Controls}\,(ng/g)}{\text{Fortification Level }(ng/g)} \times 100$$

Calibration Range

The calibration curve, ranging from 0.004 ng/mL to 0.4 ng/mL, was generated by Analyst® software for the soil method validation.

Limit of Quantitation

The limit of quantitation (LOQ) was set at 0.1 ng/g or ppb for both soil sediment which was equivalent to 0.1 ng/mL Bensulfuron-methyl, when compared to the calibration standard curve as validated in this study.

Limit of Detection

The limit of detection (LOD) was defined as approximately 20% of LOQ which was equivalent to 0.02 ng/mL of Bensulfuron-methyl in the calibration standard curve. The LOD for soil and sediment was estimated to be 0.01 ng/g or ppb for Bensulfuron-methyl.

Time Required for Completion of a Sample Set

A sample set consisted of a reagent blank (extraction solvent), two controls (untreated soil/sediment samples), and five fortified soil/sediment samples (at each level i.e. LOQ and 10X LOQ). Time required for one set from preparation of standard solutions,

initiation of extraction, until the completion of instrumental analysis and data evaluation is as follows:

- Preparation of standard solutions takes approximately 6 hours

- Sample preparation takes approximately 5 hours

- LC-MS/MS analysis and data processing (two MS/MS transitions) take approximately 5 hours

TOTAL = approximately 16 hours for one analyst to complete a set to satisfy the validation requirements.

Statistical Methods

Means, standard deviation, relative standard deviation, and 1/x linear regression fit were the only statistical methods employed in this study.