

## 2.0 INTRODUCTION

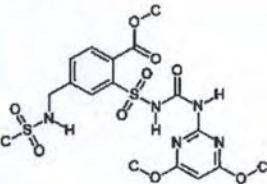
This report describes the independent laboratory validation (ILV) of Bayer Analytical Method MM-001-S14-02, "An Analytical Method for the Determination of Residues of Mesosulfuron-methyl (AE F130060) and its metabolites AE F154851, AE F099095, and AE F092944 in Soil Using LC/MS/MS" performed by ADPEN Laboratories, Inc. The analytical method submitted by Bayer is presented in Appendix 2.

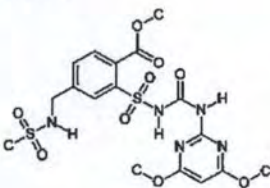
This study was designed to satisfy harmonized guideline requirements described in US EPA Test Guidelines OCSP 850.6100: Environmental Chemistry Methods and Associated Independent Laboratory Validation (Reference 2). This study was conducted in compliance with EPA FIFRA Good Laboratory Practice Standards, 40 CFR Part 160.

## 3.0 MATERIALS AND METHODS

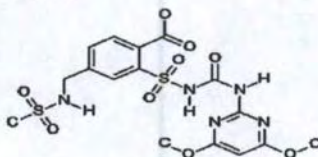
### 3.1 Reference Substances

The following reference substances were obtained from Bayer CropScience:

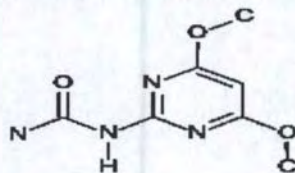
<b>Standard name:</b>	<b>Mesosulfuron-methyl (AE F130060)</b>
Standard Number:	K-2141
CAS name:	Methyl 2-[[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]-4-[[[(methylsulfonyl)amino]methyl]benzoate
CAS number:	208465-21-8
Lot Number:	0311200801
Assay:	98.5%
Expiration date:	03/21/2016
Storage conditions:	Freezer
Structure:	



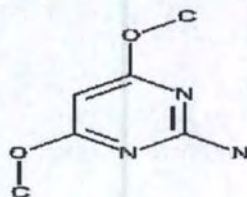
**Standard name:** AE F154851  
**Standard Number:** K-2168  
**CAS name:** 2-[[[(4,6-Dimethoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]-4-[[[(methylsulfonyl)amino]methyl]benzoic acid  
**CAS number:** 400852-66-6  
**Lot Number:** 1215200601  
**Assay:** 93.9%  
**Expiration date:** 05/15/2017  
**Storage conditions:** Freezer  
**Structure:**



**Standard name:** AE F099095  
**Standard Number:** K-2175  
**CAS name:** Urea, N-(4,6-dimethoxy-2-pyrimidinyl)-  
**CAS number:** 151331-81-6  
**Lot Number:** K-2175  
**Assay:** 97.7 %  
**Expiration date:** 02/05/2020  
**Storage conditions:** Freezer  
**Structure:**

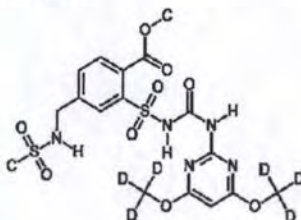


**Standard name:** AE F092944  
**Standard Number:** K-1782  
**CAS name:** 4,6-Dimethoxy-2-pyrimidinamine  
**CAS number:** 36315-01-2  
**Lot Number:** 1001200318  
**Assay:** 99.6 %  
**Expiration date:** 03/18/2020  
**Storage conditions:** Freezer  
**Structure:**

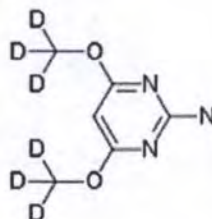


Two internal standards were also used in this study and are detailed below:

**Standard name:** Mesosulfuron-methyl -d<sub>6</sub>  
**Standard Number:** K-1395  
**CAS name:** Methyl 2-[[[(4,6-di(methoxy-d<sub>3</sub>)-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]-4-[[[(methylsulfonyl)amino]methyl]benzoate  
**CAS number:** N/A  
**Lot Number:** 1110200402  
**Assay:** 98.8 %  
**Expiration date:** 09/12/2024  
**Storage conditions:** Freezer  
**Structure:**



**Standard name:** AE F092944-d<sub>6</sub>  
**Standard Number:** K-1706  
**CAS name:** 4,6-Di(methoxy-d<sub>3</sub>)-2-pyrimidinamine  
**CAS number:** N/A  
**Common name:** 2-amino-4,6-dimethoxypyrimidine-dimethyl-d<sub>6</sub>  
**Lot Number:** 2005BRP030-192  
**Assay:** 93.1 %  
**Expiration date:** 10/16/2017  
**Storage conditions:** Freezer  
**Structure:**



The reference substances used in this study were obtained from Bayer and received at ADPEN on December 10, 2015. Reference standards were stored frozen as directed on their individual certificates of analysis. Upon receipt, the reference standards were stored in freezer E-119 which typically operates at  $-23 \pm 1$  °C.

The certificates of analysis are presented in Appendix 3. All stock, fortification and calibration solutions made from the reference substances were stored according to the method.

### 3.2 Test System

The test system used in this study was soil. The control sample was provided by Bayer and stored frozen prior to analysis. The following table is a description of the samples:

SAMPLE ID	SAMPLE DESCRIPTION
MEFVP115FL-S001	1 kg

The sample was logged into the Laboratory Information Management System (LIMS) and assigned a unique laboratory code, which is cross-referenced to the sample number on raw data and detailed residue reports. During the course of this study, the sample was stored in freezer E-16, which typically operates at  $-21 \pm 1$  °C.

### 3.3 Analytical Procedures

Analytical Method MM-001-S14-02 was independently validated, as written and submitted by Bayer (Appendix 2). The apparatus and reagents were used as outlined in the analytical method with equivalent apparatus or reagents substituted as necessary.

#### 3.3.1 Fortifications

Untreated control soil samples were fortified at LOQ (10 ng/g) and 10× LOQ (100 ng/g) using the appropriate fortification standard concentrations as per the method. Soil samples were spiked with mixed-analyte standard solutions as described below.

Matrix	Concentration (ng/μL)	Aliquot Volume (mL)	Final Concentration (ng/g)	Replicates
Soil -10 g	0.10	1.0	10.0	5
	1.00	1.0	100.0	5

#### 3.3.2 Extraction Procedure for Soil

1. A 10-g soil sample was transferred into a 125-mL glass jar.
2. Recovery samples were fortified at the appropriate level and leave undisturbed for 5 minutes.
3. 40 mL of ACN/20 mM trimethylamine in water (4:1) was added to the sample and shaken for approximately 20 minutes.
4. Sample was centrifuged at 2500 rpm for 5 minutes and the aqueous layer was decanted into a clean 125-mL glass jar.
5. Extraction steps 3 and 4 were repeated, combining the extracts.
6. A 0.80 mL aliquot of the internal standard mix (1 μg/mL) was added to the sample and it was mixed well.
7. An aliquot of 0.5 mL of the sample was transferred to a test tube.

8. The sample aliquot was evaporated to dryness using a TurboVap set at 50°C.
9. A 0.5mL aliquot of water/methanol (9:1) was added to the sample and it was sonicated and vortexed to dissolve the analytes.
10. The sample was then vialled for LC-MS/MS analysis.

### 3.3.3 Modifications

No modifications were made to the analytical method.

### 3.4 Instrument Operating Parameters

Chromatography System:	Agilent 1290 UPLC			
Analytical Column:	Aquasil C18, 100 mm x 3.0 mm, 3 µm			
Column Temperature:	50 °C			
Injection Volume:	10.0 µL			
Run Time:	5 minutes			
Mobile Phase A:	10 mM Ammonium formate in (9:1) water:methanol + 0.012% Formic Acid			
Mobile Phase B:	10 mM Ammonium formate in (1:9) water:methanol + 0.012% Formic Acid			
Gradient:	Time (min.)	Flow Rate (mL/min)	A (%)	B (%)
	0.0	0.80	95	5
	0.5	0.80	95	5
	4.0	0.80	0	100
	4.5	0.80	0	100
	4.6	0.80	95	5
	5.0	0.80	95	5

Detection System:	Agilent 6500 Triple Quad							
CUR:	25							
CAD:	10							
GS1:	30							
GS2:	30							
Temperature:	300 °C							
IS:	5500 V							
<b>MRM Conditions</b>								
Analyte (Retention time)	Polarity	Q1	Q3	Dwell (msec)	DP	EP	CE	CXP
Mesosulfuron-methyl (4.02 min)	Positive	504.1	182.1	50	70	10	29	11
Mesosulfuron-methyl (4.02 min) Confirmatory		504.1	83.1				75	
Mesosulfuron-methyl IS		510.1	188.0				80	
AE F154851 (2.87 min)	Positive	490.0	182.1	50	71	10	29	8
AE F154851 (2.87 min) Confirmatory		490.0	82.9				77	10
AE F099095 (3.44 min)	Positive	199.2	156.0	50	55	10	18	8
AE F099095 (3.44 min) Confirmatory		199.2	181.9				17	9
AE F092944 (3.22 min)	Positive	156.2	100.1	50	80	10	29	11
AE F092944 (3.22 min) Confirmatory		156.2	123.9					14
AE F092944 IS		162.1	103.0					56

### 3.5 Data Acquisition

Peak integration and peak area count quantitation were performed by Analyst® version 1.6.2 data handling software. A best-fit, linear regression equation was derived and used in conjunction with the analyte response in each sample to calculate the concentration of the analyte. The square of correlation coefficients ( $R^2$ ) for the calibration curves for each analytical set was greater than 0.99.

Statistical treatment of the data including the calculation of percent recovery, means, and standard deviations were calculated within LIMS and reported using Microsoft® Office Excel spreadsheets. Example calculations are presented in Appendix 5.

### 4.1 Method Establishment/Pre-Validation Evaluation

Prior to conducting the ILV, control of the method was established by optimizing instrument parameters for the mass spectrometer detector. This was performed by infusing a high concentration standard solution of the target analytes. Additional injections of a series of calibration standards further optimized instrument parameters, analyte retention times, instrument detection limits, and linearity. Prior to analysis of validation samples, the control soil samples were analyzed to check for target analyte contamination and interferences. No significant residues of mesosulfuron-methyl and its three metabolites were found in the test control soil sample.

Optimization was performed on an Agilent 6500 LC-MS/MS system. The Agilent 6500 mass spectrometer is coupled to an Agilent 1290 UPLC.

## 7.0 REFERENCES

1. Miller, Audry. Bayer Method MM-001-S14-01, "An Analytical Method for the Determination of Residues of Mesosulfuron-methyl (AE F130060) and its metabolites AE F154851, AE F099095, and AE F092944 in Soil Using LC/MS/MS". Bayer CropScience. December 1, 2015.
2. United States Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, 712-C-001 (OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation), January, 2012.
3. FIFRA Good Laboratory Practice Standards. *Code of Federal Regulations*, Section 160, Title 40, 1982; *Fed. Regist.* 1989, 48, 53946 ff.
4. Dean, R.B. and W.J. Dixon, 1951. *Analytical Chemistry* 23:636. American Chemical Society, Washington DC.
5. Engineering Statistics Handbook.  
<http://www.itl.nist.gov/div898/handbook/eda/section3/eda35h1.htm>. (Date accessed March 5, 2014). Section 1.3.5.17.1. Grubb's Test for Outliers.



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MM-001-S14-02

**Bayer Method MM-001-S14-02**

An Analytical Method for the Determination of Residues of Mesosulfuron-methyl (AE F130060) and its metabolites AE F154851, AE F099095, and AE F092944 in Soil Using LC/MS/MS

**1.0 SUMMARY**

An analytical method was developed to determine the residues of mesosulfuron-methyl (AE F130060) and its metabolites AE F154851, AE F099095, and AE F092944 in soil.

Residues of mesosulfuron-methyl are extracted from soil by shaking with acetonitrile:aqueous 20 mM triethylamine (4:1, v:v) followed by addition of an isotopic internal standard. The samples were analyzed by LC/MS/MS with quantification based on a comparison of peak areas with those of known standards.

The method limit of quantitation (LOQ) in soil is 10 ng/g.

**2.0 BACKGROUND**

The analytical method presented in this report is designed to measure residues of mesosulfuron-methyl and its metabolites AE F154851, AE F099095, and AE F092944 using isotopically labeled internal standards and LC/MS/MS detection.

**3.0 APPARATUS**

(Functional equivalents may be substituted)

- Various general laboratory glassware and utensils
- MicroMan pipettors and tips
- Shaker
- Centrifuge
- TurboVap
- Thermo Scientific Aquasil C18 column, 100 mm X 3.0 mm, 3 µm particle size, (Part No: 77503-103030)
- ABSciex API 6500 chromatograph/mass spectrometer (LC/MS/MS) equipped with electrospray ionization (ESI) interface, Shimadzu HPLC pumps and a CTC PAL autosampler, and Analyst 1.6.2 data collection software (ABSciex)

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**4.0 REAGENTS AND CONSUMABLES**

(Functional equivalents may be substituted)

- Water (HPLC grade)
- Acetonitrile (ACN, HPLC grade)
- Methanol (MeOH, HPLC grade)
- Triethylamine (TEA, HPLC grade, Fisher No. O4884-100)
- 9:1 Water/MeOH; prepare by adding 100 mL of MeOH to 900 mL of water and mixing well.
- 20 mM TEA; prepare by adding 1.0 mL of TEA to 500 mL of water and mixing well.
- ACN/20 mM TEA (4:1, v:v); prepare by adding 800 mL of ACN and 200 mL of 20 mM TEA to a 1 L graduated cylinder and mixing well.
- Ammonium formate (Fisher No. A639-500)
- Formic acid 99% (Acros, Part No. 14793-0010)
- Water/methanol (9:1, v:v) containing 10 mM ammonium formate and 120 µL/L formic acid; prepare by adding 900 mL water, 100 mL MeOH, 0.63 g ammonium formate and 0.120 mL of formic acid to a 1 L graduated cylinder and mixing well.
- Water/methanol (1:9, v:v) containing 10 mM ammonium formate and 120 µL/L formic acid; prepare by adding 100 mL water, 900 mL MeOH, 0.63 g ammonium formate and 0.120 mL of formic acid to a 1 L graduated cylinder and mixing well.
- Fisherbrand 125 mL 4oz glass jars (Part No. 02-911-455)
- Culture tubes (20 x 150 mm, Fisher Part No. 14-961-33)
- HPLC vials and caps (2-mL, National Scientific, Part Nos. C4011-5W and C4011-55)

**5.0 PREPARATION OF STANDARD SOLUTIONS**

Analytical standards of mesosulfuron-methyl, AE F154851, AE F099095, and AE F092944 and the isotopic internal standards mesosulfuron-methyl-d<sub>6</sub> and AE F092944-d<sub>6</sub> are needed. These standards may be obtained from Bayer CropScience, 2 T.W. Alexander Drive, Research Triangle Park, NC 27709. Additional details about these chemicals are given in [Appendix 1](#).

The toxicities of these chemicals have not been precisely determined. Thus, each chemical must be treated as a potential health hazard. Exposure to these chemicals should be reduced to the lowest reasonable level.

**NOTE:** The following procedure is an example description of how these standard solutions may be prepared. Alternate or additional standards of appropriate weight and volume may be prepared as needed.

Class "A" volumetric glassware or calibrated pipets should be used in the preparation of all analytical standards. All standard solutions should be stored in a refrigerator in amber glass bottles when not in use. Solutions should be allowed to warm to room temperature prior to use. Corrections for standard purities should be applied when expressing standard concentrations.

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### 5.1 Primary Stock Standard Solution

Prepare individual ~100 µg/mL stock solutions of mesosulfuron-methyl, AE F154851, AE F099095, and AE F092944. Standards used to prepare initial stock solutions should be weighed on an analytical balance capable of accurately weighing samples to ± 0.01 mg. Standards are typically provided in 5.0 mg aliquots. The standards are quantitatively transferred to a 50 mL volumetric flask using acetonitrile, and diluted to volume with acetonitrile.

Prepare a mixed solution containing 10 µg/mL by taking an appropriate volume (~5 mL) of each primary stock solution and diluting to 50 mL with acetonitrile.

NOTE: Corrections for standard purities should be applied when expressing standard concentrations.

### 5.2 Fortification Standard Solutions

#### 1 µg/mL mixed solution

Transfer 5 mL of the 10 µg/mL mixed standard solution into a 50 mL volumetric flask. Dilute to volume with acetonitrile. Mix well.

#### 100 ng/mL mixed solution

Transfer 5 mL of the 1 µg/mL mixed standard solution into a 50 mL volumetric flask. Dilute to volume with acetonitrile. Mix well.

### 5.3 Isotopic Internal Standard Solutions

Prepare individual ~100 µg/mL stock solutions of mesosulfuron-methyl-d<sub>6</sub> and AE F092944-d<sub>6</sub>. Standards used to prepare initial stock solutions should be weighed on an analytical balance capable of accurately weighing samples to ± 0.01 mg. Standards are typically provided in 2.5 mg aliquots. The standards are quantitatively transferred to a 25 mL volumetric flask using acetonitrile, and diluted to volume with acetonitrile.

Prepare a mixed 1 µg/mL internal standard solution containing mesosulfuron-methyl-d<sub>6</sub> and AE F092944-d<sub>6</sub> by taking an appropriate volume (~0.5 mL) of the stock internal standard solutions and diluting to 50 mL with acetonitrile.

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#### 5.4 Calibration Standard Solutions

Prepare working calibration solutions consisting of 0.5, 1, 5, 10, 50, and 100 ppb of mesosulfuron-methyl, AE F154851, AE F099095, and AE F092944 by diluting to 25 mL with 9:1 Water/MeOH. Before bringing the calibration solutions to volume, add by pipet 0.25 mL of the 1 µg/mL internal standard solution to each of the calibration solutions. (see Section 5.3 Isotopic Internal Standard Solutions)

Concentration of Standard Solution used for dilution (µg/mL)	Concentration of Internal Standard Solution used for dilution (µg/mL)	Aliquot Native mix Taken (mL)	Aliquot Internal Standard Taken (mL)	Concentration of Calibration Solution (ppb)	Concentration of Internal Standard (ppb)
10	1	0.250	0.25	100	10
10	1	0.125	0.25	50	10
1	1	0.250	0.25	10	10
1	1	0.125	0.25	5	10
0.1	1	0.250	0.25	1	10
0.1	1	0.125	0.25	0.5	10

Further calibration solutions may be prepared as needed, depending on the analytical range for the samples. At least six calibration standards are needed.

#### 6.0 PROCEDURE

##### 6.1 Soil extraction

Appendix 2 shows the analytical scheme for the extraction of mesosulfuron-methyl residues in soil. The detailed stepwise procedure is as follows:

1. Weigh  $10 \pm 0.05$  grams of soil into a 125 mL glass jar.
2. Fortify the recovery samples at the desired fortification level with the appropriate standard solution (see Section 5.2 Fortification Stock Solutions). Let the fortified samples sit for a minimum of 5 minutes.
3. Add 40 mL of 4:1 ACN/20 mM TEA to each sample. Place samples on shaker for ~20 minutes.
4. Centrifuge at ~2500 rpm for 5 minutes. Decant the supernatant into a 125 mL jar or other suitable container.
5. Repeat steps 3 and 4 collecting the supernatant into the same jar.
6. Add 0.8 mL of the 1 µg/mL internal standard solution to the combined extract and mix well.

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7. Transfer 0.5 mL of the extract into a vial or culture tube.
8. Evaporate sample to dryness using a TurboVap set at ~50 °C.
9. Add 0.5 mL of 9:1 Water/MeOH to each sample and vortex to dissolve.
10. If needed, transfer sample to a vial, and analyze by LC/MS/MS.

## 7.0 ANALYSIS BY LC/MS/MS

### 7.1 Analytical Procedure

- Step 1. Using the recommended procedures listed below, analyze an aliquot of each of the calibration standard solutions (if necessary, additional standard solutions).
- Step 2. Analyze an aliquot of each of the analytical samples.  
**Note:** Up to 20 sample analyses can be made after the analysis of the standard solutions. In the case of over 20 samples, extra standard solutions could be added between sample analyses.
- Step 3. Again, analyze an aliquot of each of the calibration standard solutions (and, if necessary, additional standard solutions).
- Step 4. When necessary, analyze additional samples and standard solutions. Always finish the procedure with the analysis of a set of standard solutions.

### 7.2 HPLC Conditions

**Note:** The analyst must optimize chromatographic conditions to obtain satisfactory chromatography. The following recommended conditions were used on an AB Sciex 6500 instrument equipped with Shimadzu HPLC pumps.

Mobile Phase A: Water/methanol (9:1, v:v) containing 10 mM Ammonium formate and 120 µL/L formic acid

Mobile Phase B: Water/methanol (1:9, v:v) containing 10 mM Ammonium formate and 120 µL/L formic acid

HPLC column: Thermo Scientific Aquasil C18, 100 mm X 3.0 mm, 3 µm

Column Temp: 50 °C

Injection volume: 10 µL (Adjust for LC/MS/MS system being used)

Time (min)	Mobile Phase A	Mobile Phase B	Flow rate µL/min
0.0	95	5	800
0.5	95	5	800
4.0	0	100	800
4.5	0	100	800
4.6	95	5	800

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Approximate retention times are listed below:

AE F154851	2.6 min
AE F092944	3.2 min
AE F099095	3.5 min
Mesosulfuron-methyl	3.9 min

### 7.3 Mass Spectrometer Conditions

Note: The analyst must optimize the mass spectrometer conditions to obtain satisfactory system response. The following conditions were used on an AB Sciex 6500 instrument.

#### Positive ion mode

CUR: Curtain Gas	50
CAD: Collision Gas	6
GS1: Ion Source Gas 1	50
GS2: Ion Source Gas 2	50
TEM: Source Temp.	300°C
IS: Ion Transfer Voltage	5500

### 7.4 Mass Spectrometer Data Collection

The daughter ions used in this method were chosen due to their optimum sensitivity on the instrument used for this study. **The analyst must optimize the mass spectrometer data collection to obtain satisfactory system response.** The following recommended ion transitions and conditions were example conditions used on an AB Sciex 6500 instrument:

Analyte Name	Polarity	Q1 Mass (amu)	Q3 Mass (amu)	Dwell (msec)	DP	EP	CE	CXP
Mesosulfuron-methyl	+	504.2	181.9	50	60	10	31	6
Mesosulfuron-methyl Confirmatory	+	504.2	83.0	50	60	10	83	6
Mesosulfuron-methyl IS	+	510.2	187.9	50	60	10	31	6
AE F092944	+	156.2	100.0	50	60	10	23	6
AE F092944 Confirmatory	+	156.2	124.0	50	60	10	25	6
AE F092944 IS	+	162.2	103.0	50	60	10	23	6
AE F154851	+	489.9	168.2	50	60	10	31	6
AE F154851 Confirmatory	+	489.9	306.1	50	60	10	37	6

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Analyte Name	Polarity	Q1 Mass (amu)	Q3 Mass (amu)	Dwell (msec)	DP	EP	CE	CXP
AE F099095	+	198.9	156.0	50	60	10	15	6
AE F099095 Confirmatory	+	198.9	182.0	50	60	10	21	6

As there is no internal standard available for AE F154851 and AE F099095, the mesosulfuron-methyl internal standard is used for quantifying these compounds.

### 8.0 CALCULATION OF RESULTS

The example calculation displayed below was used by the laboratory developing this method. Alternate calculation procedures appropriate to the reporting requirements may be substituted.

Residue concentrations were determined using calibration curves which were generated after each analysis using Analyst software (Version 1.6.2) using linear regression with 1/x weighting.

The standards were fit to the linear equation:

$$Y = MX + B \text{ with } 1/x \text{ weighting.}$$

where: X is the concentration of the reference standard in ng/mL  
M is the calibration line slope  
B is the calibration line intercept  
Y is the native peak area: isotopic peak area ratio

After regression coefficients were calculated, the residue in ng/g was determined using the following equation,

$$\text{Residue found (ng/g)} = \frac{(Y-B) \times D}{M}$$

$$\text{Where Dilution Factor (D)} = \frac{\text{Initial volume (V}_1\text{)}}{\text{Initial sample wt. (W)}} \times \frac{\text{Final dilution volume (V}_3\text{)}}{\text{Aliquot taken (V}_2\text{)}}$$

For soil samples:

$$\begin{aligned} W &= 10 \text{ g} \\ V_1 &= 80 \text{ mL} \\ V_2 &= 0.5 \text{ mL} \\ V_3 &= 0.5 \text{ mL} \\ D &= 8 \end{aligned}$$

Analyst software was used to calculate the amount in ng/g for each sample and the percent recovery for the spiked samples.

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### 8.1 Fortification Experiments

Note: Fortification experiments may be performed as needed to monitor method efficiency and reproducibility, but are not required when analysis of samples is performed for tolerance enforcement. Fortification experiments are intended to be used for data collection methods or establishing and validating method efficiency.

With each sample set, analyze an untreated control sample and one or more fortified control samples. Calculate recoveries using the following equation:

$$\text{Recovery (\%)} = \frac{(R - S)}{T} \times 100$$

Where: R = ppb of target analyte found in fortified sample  
S = ppb of target analyte found in control sample, real or apparent  
T = theoretical ppb in fortified sample

Recoveries are determined by analyzing fortified control samples alone or in conjunction with a sample set. Samples may be fortified prior to extraction at the LOQ or other appropriate level with fortification solutions. Calculate the final residue for the control (S) and fortified control (R) samples.

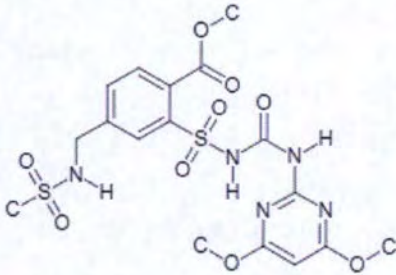
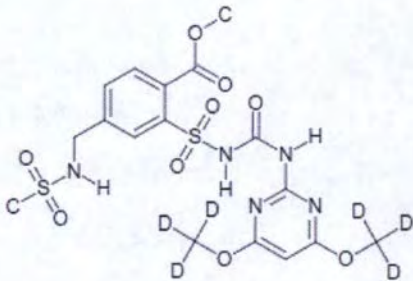
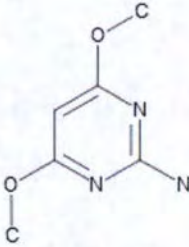
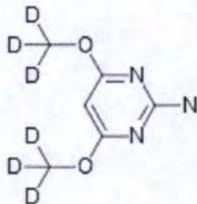


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**Appendix 1 Test and Reference Substances**

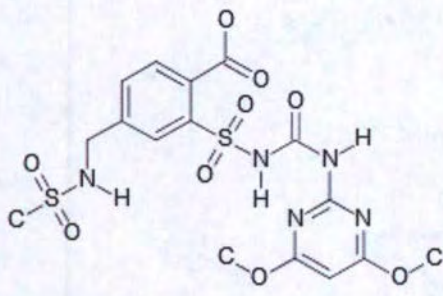
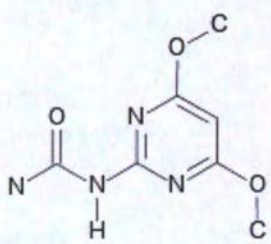
The toxicities of these chemicals have not been precisely determined. Thus, each chemical must be treated as a potential health hazard. Exposure to these chemicals should be reduced to the lowest reasonable level.

 <p><b>Mesosulfuron-methyl (AE F130060)</b></p> <p>Methyl 2-[[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]-4-[[[(methylsulfonyl)amino]methyl]benzoate</p>	 <p><b>Mesosulfuron-methyl -d<sub>6</sub></b></p> <p>Methyl 2-[[[(4,6-dimethoxy-d<sub>6</sub>-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]-4-[[[(methylsulfonyl)amino]methyl]benzoate</p>
 <p><b>AE F092944</b></p> <p>4,6-Dimethoxy-2-pyrimidinamine</p>	 <p><b>AE F092944-d<sub>6</sub></b></p> <p>4,6-Di(methoxy-d<sub>3</sub>)-2-pyrimidinamine</p>

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## Appendix 1 Test and Reference Substances (continued)

 <p><b>AE F154851</b> 2-[[[(4,6-Dimethoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]-4-[[[(methylsulfonyl)amino]methyl]benzoic acid</p>	 <p><b>AE F099095</b> Urea, N-(4,6-dimethoxy-2-pyrimidinyl)-</p>
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**Appendix 2 Extraction Scheme for Soil Samples**

Weigh a 10 g aliquot of soil into a 125 mL glass jar



Add 40 mL of 4:1 ACN/20 mM TEA



Shake samples for ~20 minutes



Centrifuge, decant and repeat



Add 0.80 mL of the 1 µg/mL internal standard solution. Mix well.



Evaporate a 0.5 mL aliquot to dryness



Dissolve with 0.5 mL of 9:1 Water/MeOH



LC/MS/MS analysis

## APPENDIX 5. Example Calculations

Residue results are calculated by comparison to the standard curves obtained from a linear regression analysis of the data found by the data system. The equation for the fit of the standard curve was used to calculate intercept and slope of the linear regression curve. The intercept and the slope were used in the equation used for quantitation. LIMS was used to calculate the ppb, percent recovery, and all other data and reported in Microsoft® Excel spread sheets. The following equations were used for quantitation:

The following equations were used for the quantitation of residues of mesosulfuron-methyl and its three metabolites:

The calculation below was performed within Analyst 1.6.2:

1. Calibration curve:  $y = mx + b$  Solving for x:  $x = \text{Error! Bookmark not$

defined.  $\frac{y - b}{m}$

- Where,
- m = slope
  - b = y intercept
  - x = Analyte Concentrations Ratio
  - y = Peak Area Ratio

The calculations below were performed within Microsoft Excel:

- b) amount of sample injected =  $\frac{\text{injection size (mL)}}{\text{final volume (mL)}} \times \text{sample weight (g)}$
- c)  $\text{ppb} = \frac{\text{ng found}}{\text{g injected}}$
- d) Percent recovery =  $\frac{(\text{ppb in the sample} - \text{ppb in the control})}{\text{ppb added}} \times 100$

As an example, calculations to obtain the percent recovery in a control soil sample fortified with Mesosulfuron-methyl in sample number 15121811-Recovery1-1 (WO-15121809). Where peak area counts for the analyte was 736391 and for the internal standard having 0.1 ng concentration was 4275054, which results in a peak area ratio of 0.17225. The calculations are shown below:

- a)  $\text{ng found} = \frac{0.17225 - 0.0407}{(0.908 \div 0.1 \text{ ng})} = 0.01449 \text{ ng}$
- b) amount of sample injected =  $\frac{0.01 \text{ mL}}{80.0 \text{ mL}} \times 9.99 \text{ grams} = 0.00125 \text{ g}$
- c)  $\text{ppb} = \frac{0.01449 \text{ ng}}{0.00125 \text{ g}} = 11.6036 \text{ ppb}$
- d) Percent recovery =  $\frac{11.6036 \text{ ppb}}{10.0 \text{ ppb}} \times 100 \% = 116.0$

## APPENDIX 7. Communications

The following is documentation of all contacts between the performing laboratory and the method developers or others familiar with the method. Included are the reasons for the contact, any changes in the method that resulted, and the date and mode of the communication.

1. 12/11/15 – Email communication
  - a. Melisa Marshall (project coordinator) consulted with Audry Miller (Study Monitor) concerning a missing lot number for the AE F099095 reference standard. It was decided to use the standard number as the lot number.
2. 01/06/16 – Email communication
  - a. Steven Perez (Laboratory Manager) notified Audry Miller (Study Monitor) that during the optimization, the analysts were unable to locate the Q3 masses for AE F154851 as recorded in the method. On 01/07/16, it was confirmed that the masses that were found are indeed correct and what was documented in the method was incorrect.
3. 01/12/16 – Email communication
  - a. Rolando Perez (Analytical Principal Investigator) notified Audry Miller (Study Monitor) the first trial for the soil ILV data passed for mesosulfuron-methyl and AE F092944. Furthermore, in this same communication, a second trial was recommended for AE F154851 at the LOQ level and for AE F099095. This recommendation was accepted by the Study Monitor on January 13, 2016, allowing a second trial.
4. 01/22/16 – Email communication
  - a. Steven Perez (Laboratory Manager) notified Audry Miller (Study Monitor) the second trial for the soil ILV data passed for the two remaining analytes (AE F154851 and AE F099095) at the LOQ fortification.