

Bayer Method MM-001-S14-03

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.

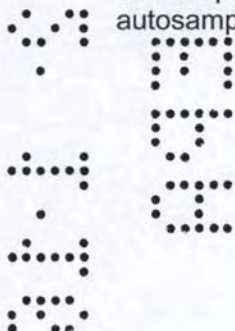
2.0 BACKGROUND

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)



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-d₆ and AE F092944-d₆ 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.

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.

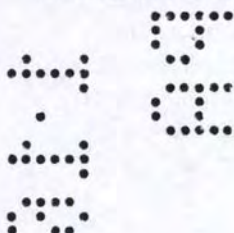
100 ng/mL mixed solution

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-d₆ and AE F092944-d₆. 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-d₆ and AE F092944-d₆ by taking an appropriate volume (~0.5 mL) of the stock internal standard solutions and diluting to 50 mL with acetonitrile.



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 ± 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.

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

Approximate retention times are listed below:

AE F154851	2.8 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	+	490.0	182.2	50	60	10	33	6
AE F154851 Confirmatory	+	489.9	83.0	50	60	10	83	6

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.

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:

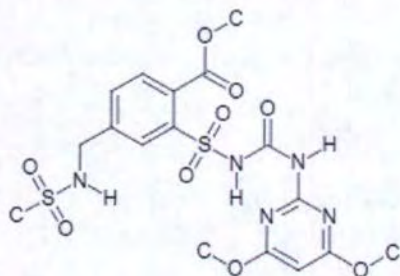
$$\text{Recovery (\%)} = \frac{(R - S)}{T} \times 100$$

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

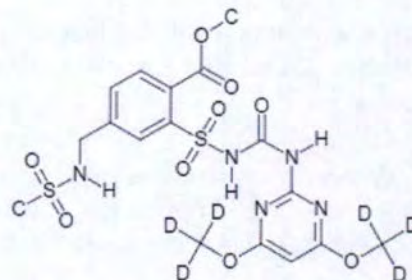
Recoveries are determined by analyzing fortified control samples alone or in conjunction with a sample set. Samples may be fortified prior to extraction at the LOQ or other appropriate level with fortification solutions. Calculate the final residue for the control (S) and fortified control (R) samples.

Appendix 1 Test and Reference Substances

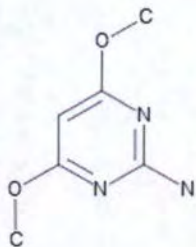
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**Mesosulfuron-methyl (AE F130060)**

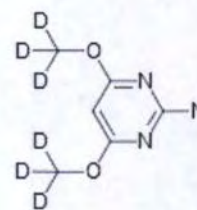
Methyl 2-[[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]-4-[[[(methylsulfonyl)amino]methyl]benzoate

**Mesosulfuron-methyl -d₆**

Methyl 2-[[[(4,6-dimethoxy-d₆-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]-4-[[[(methylsulfonyl)amino]methyl]benzoate

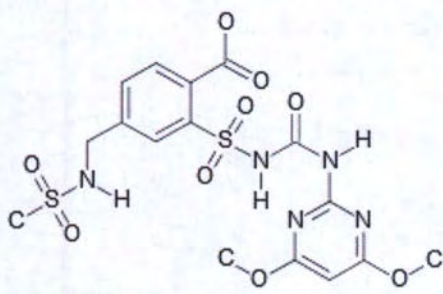
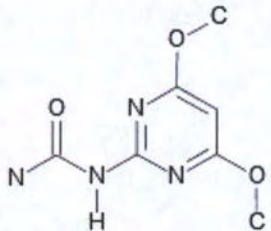
**AE F092944**

4,6-Dimethoxy-2-pyrimidinamine

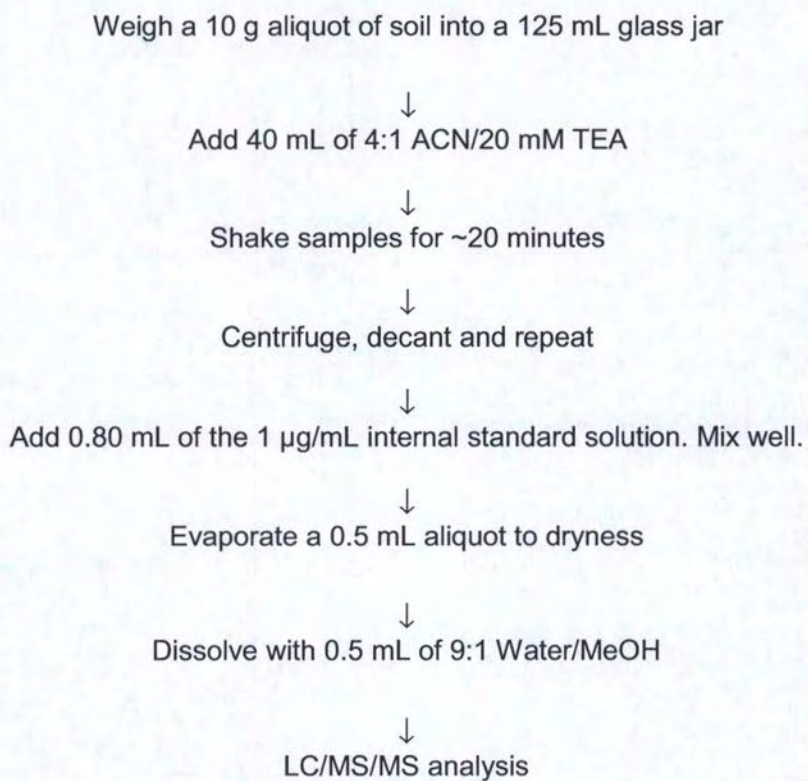
**AE F092944-d₆**

4,6-Di(methoxy-d₃)-2-pyrimidinamine

Appendix 1 Test and Reference Substances (continued)

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



Appendix 6 Revision History

Method Number	Revision	Description
MM-001-S14-01	01	Completed on development of method
MM-001-S14-02	02	Added metabolites to method
MM-001-S14-03	03	Corrected MS information for AE F154851