Introduction

Objective of Study

The purpose of this project is to analyze soil samples collected from Arkansas and California along with a sediment sample collected from Kansas for orthosulfamuron and its major metabolites during a method validation using a liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS) system. The target limit of quantitation (LOQ) is 0.2 ppb.

Section 1 Analytical Summary

Section 1.1 Method Summary

The residue method was developed and validated (Appendix 1, Protocol Amendment 3) for orthosulfamuron and its major metabolites in soil obtained from the orthosulfamuron aquatic field dissipation test sites located in Arkansas and California (Waterborne Study Number WEI 642.03). Along with sediment collected by SynTech Research from an on-site pond located in Stilwell, KS. A 10 g sample was weighed* into a centrifuge tube and fortified if necessary. The room temperature sample was initially extracted with 10 mL of 7:3 acetonitrile/33 mM sodium bicarbonate (v/v) by shaking vigorously for 40 minutes at 1200 rpm using a GenoGrinder homogenizer and centrifuged at 4000 rpm for 15 minutes to form a solid pellet. The supernatant was transferred into a separate centrifuge tube and the sample was extracted a second time with 10 mL of 1:1 acetonitrile/33 mM sodium bicarbonate (v/v). The sample was shaken vigorously for 40 minutes at 1200 rpm using a GenoGrinder homogenizer and centrifuged at 4000 rpm for 15 minutes to form a solid pellet. The supernatant from the second extraction was combined with the supernatant from the first extraction and mixed by vortexing. A ~ 1.5 mL aliquot was filtered through a syringe filter into an autosampler vial, capped and analyzed by LC/MS/MS.

* Note: Prior to weighing out sediment samples, centrifuge the sediment to form a solid pellet, decant off the water, and then break up the soil pellet with a spatula.

Section 1.2 Method Validation and Limit of Quantitation

The method was successfully validated by analysis of blank untreated control (UTC) soil samples fortified with 0.2 and 2.0 ppb of each analyte. These data support a method limit of quantitation (LOQ) of 0.2 ppb for each analyte.

Section 1.3 Determination of Limit of Detection

The limit of detection (LOD) (Appendix 1) is the lowest analyte concentration for a measurement statistically different from that of a blank UTC sample. The LOD for orthosulfamuron and its major metabolites in soil and sediment were calculated by multiplying **SD**, the standard deviation of the analyte recovery measurements at the target LOQ, by $\mathbf{t_{0.99}}$, the appropriate one-tailed Student's t statistic. See Section 4 for the LOD calculations for orthosulfamuron and its major metabolites. The LOD was verified by analysis of UTC soil and sediment samples fortified at the target LOQ level of 0.2 ppb each of orthosulfamuron and its major metabolites. The equation for calculating the LOD is given below.

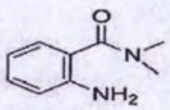
$$LOD = (t_{0.99} \times SD)$$

Section 1.4 Analytical Standards Used in this Study

Solutions of the analytical reference standards were prepared in acetonitrile, or in a mixture of acetonitrile and water with 10 mM disodium hydrogen phosphate, and were corrected for purity during initial standard preparation. Standard solutions and linearity curve solutions were stored in a laboratory freezer (BLDG2WF1) at an average temperature of ≤ –18°C. The standards for orthosulfamuron and its metabolites were obtained from ABC Laboratories, Inc.

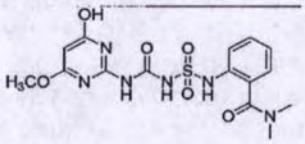
Common Name:	DB Amine
Molecular Weight:	164.20 g/mol
Appearance:	off-white solid
Lot Number:	D16M
Purity:	99.7%
Expiration Date:	2 years from date of C of A
Storage Conditions:	Room Temperature
Chemical Name (IUPAC):	2-Amino-N,N-dimethylbenzamide
CAS#:	6526-66-5

Structure:



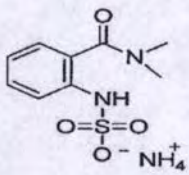
Common Name:	O-Desmethyl-Orthosulfamuron
Molecular Weight:	410.41 g/mol
Appearance:	white solid
Lot Number:	81481-65
Purity:	97.6
Expiration Date:	2 years from date of C of A
Storage Conditions:	≤-20°C
Chemical Name (IUPAC):	1-(4-hydroxy-6-methoxypyrimidin-2-yl)-3-[2-(dimethoxycarbamoyl)phenylsulfamoyl]-urea

Structure:



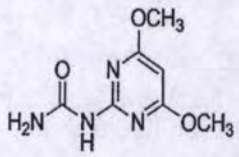
Common Name:	DBS Acid Ammonium Salt
Molecular Weight:	261.30 g/mol
Appearance:	white solid
Lot Number:	81481-1-39-2
Purity:	97.8
Expiration Date:	2 years from date of C of A
Storage Conditions:	≤-20°C
Chemical Name (IUPAC):	(2-dimethylcarbamoylphenyl)-sulfamic acid ammonium salt

Structure:

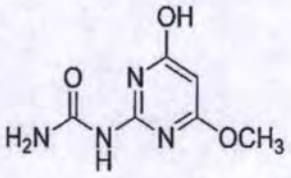


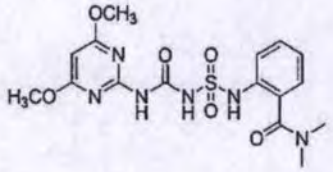
Common Name:	DOP Urea
Molecular Weight:	198.18 g/mol
Appearance:	off-white Solid
Lot Number:	81481-1-33-4
Purity:	99.8
Expiration Date:	2 years from date of C of A
Storage Conditions:	≤-20°C
Chemical Name (IUPAC):	(4,6-dimethoxy-2-pyrimidinyl)-urea
CAS#:	151331-81-6

Structure:



Common Name:	O-Desmethyl DOP urea	Common Name:	Orthosulfamuron
Molecular Weight:	184.15 g/mol	Molecular Weight:	424.43 g/mol
Appearance:	white solid	Appearance:	white solid
Lot Number:	81481-1-72-1	Lot Number:	81481-1-41-1
Purity:	97.7	Purity:	99.1
Expiration Date:	2 years from date of C of A	Expiration Date:	2 years from date of C of A
Storage Conditions:	≤-20°C	Storage Conditions:	≤-20°C
Chemical Name (IUPAC):	N-(4-hydroxy-6-methoxytpyrimidin-2-yl)-urea	Chemical Name (IUPAC)	1-(4,6-Dimethoxy-2-pyrimidinyl)-3-[2-(dimethylcarbamoyl)phenylsulfamoyl]-urea
CAS#	888225-63-6	CAS#:	213464-77-8
Structure:		Structure:	





Section 1.5 Instrument Detector Response – Range and Linearity

Calibration standards and samples were analyzed using LC/MS/MS. Calibration curves and recovery values were calculated using Analyst 1.5.1 or higher data handling software using linear regression with 1/x weighting.

The standards were fit to the linear equation: $Y = MX + B$ with 1/x weighting

where: X is the concentration of the reference standard
M is the calibration line slope
B is the calibration line intercept
Y is the native peak area

The relative response of the LC/MS/MS to orthosulfamuron and its metabolites was linear over a range of 0.098 ppb to 2.976 ppb using matrix-matched standards. The correlation coefficients (r value in Analyst file) of the linearity curves were all ≥0.99 (see worksheets in Section 5). Matrix-matched standards were necessary due to the difference in detector response for a solvent standard compared to a matrix sample. An enhancement effect was observed when analyzing matrix samples and that necessitated the use of matrix-matched standards to have comparable responses to accurately calculate the residues. See Appendix 2 for an example of the instrument conditions that were used.

Section 1.6 Reporting of Residue Recoveries, Method Accuracy, and Selectivity

Method validation consisted of a solvent blank, two UTCs and at least 7 fortified UTCs at the target LOQ (0.2 ppb) and at least 5 fortified UTCs at 10 x the target LOQ (2.0 ppb) with recoveries determined using a calibration curve. The acceptable method accuracy at each concentration must be within 70 - 120% of the spiked concentrations. Results were considered acceptable if the average recovery value (of the two or more concurrent recovery samples) falls within this range. Selectivity was evaluated by analyzing a blank soil UTC to determine if any interference was greater than 30% of the fortified LOQ concentrations. All averaged fortified recoveries fell within the criteria (except for DBS acid ammonium salt and DB amine for soil and DB amine for sediment) and any interference was less than 30% of the fortified LOQ

concentrations. Due to the method of calculations and rounding, the reported residues and calculations may not exactly match in all sections.

Section 1.7 Example Residue Recovery Calculation

Using the equation from Section 1.5, the residue (ppb) was determined as shown below:

(ppb) = [(Y-B) X D] / M	(where D is the dilution factor, if applicable)
Sample Set ID:	LJ151105AR Soil
Sample Name/ID:	642.02-AR.BK.SOIL.SYNTECH-001-051
Analyte:	Orthosulfamuron

a) Residue Level (measured amount)

Native Peak Area:	279000
Intercept (B):	–12900
Slope (M):	1570000
D:	1.0
Curve Equation:	ppb = [(279000 – (–12900) x 1] / 1570000
ppb:	0.186 ppb

b) Percent Recovery

Fortification Level:	0.2 ppb
Corresponding Control ID:	642.02-AR.BK.SOIL.SYNTECH-001-049 642.02-AR.BK.SOIL.SYNTECH-001-050
Average Corresponding Control Residue:	0.0
Percent Recovery:	[(0.186 – 0.0) / 0.2] x 100 = 93%

Appendix 1

ANALYTICAL METHOD VALIDATION FOR THE DETERMINATION OF ORTHOSULFAMURON AND ITS MAJOR METABOLITES IN WATER USING LC-MS/MS

Prepared by: Walter R. Vandaveer, Research Scientist
SynTech Research Laboratory Services

Date: 04/13/2015

1.0 OBJECTIVE

The purpose of this project is to analyze water samples collected from Arkansas and California for orthosulfamuron and its major metabolites during a method validation using a liquid chromatography-mass spectrometry/mass spectroscopy (LC-MS/MS) system. The target limit of quantitation (LOQ) is 0.17 ng/mL.

2.0 EXPERIMENTAL DESIGN

2.1 Test solutions from the study will be analyzed.

2.2 The analytical method will be developed and validated before the sample analysis.

2.3 O-Desmethyl DOP urea, Lot Number 81481-1-72-1, 97.7% purity, expiration date 11/26/2016; DBS acid ammonium salt, Lot Number 81481-1-39-2, 97.8% purity, expiration date 11/26/2016; DBS amide, Lot Number 81481-1-16, 91.9% purity, expiration date 11/26/2016; DOP amine, Lot Number S61826V, 99.8% purity, expiration date 11/26/2016; DBS amine, Lot Number D16M, 99.7% purity, expiration date 11/26/2016; O-Desmethyl Orthosulfamuron, Lot Number 81481-65, 97.6% purity, expiration date 11/26/2016; DOP urea, Lot Number 81481-1-33-4, 99.8% purity, expiration date 11/26/2016; Orthosulfamuron, Lot Number 81481-1-41-1, 99.1% purity, expiration date 11/26/2016 will be supplied by ABC Laboratories, Inc.

2.4 Method validation will be completed prior to the analysis of study samples. This will consist of a solvent blank, two controls, and 5 spiked controls at the target LOQ (0.17 ng/mL) and 5 spiked controls at 10 x the target LOQ (1.7 ng/mL) with recovery determined using a calibration curve. All procedures developed during the method validation phase of the project will be employed during the study sample analysis. A recovery spike for orthosulfamuron and its major metabolites will be required to monitor methodology and individual performance. The acceptable recoveries are 70 - 120%. Results will be considered acceptable if the average recovery value (of the two or more concurrent recovery samples) falls within this range. If the lab spike falls outside of this range, the results may be rejected.

2.5 All analytical results will be reported to the study director.

3.0 METHODOLOGY

3.1 Preparation of Calibration Standards

3.1.1 Weigh ~ 1 mg of each standard into a 50 mL volumetric flask, dilute to volume with the appropriate solvent, and mix well by inversion (see the following table) to create a 20 µg/mL standard stock solution.

Compound Name	Lot No.	Amount (mg)	Purity (%)	Solvent	Volume (mL)	Target Concentration (µg/mL)
O-Desmethyl DOP urea	81481-1-72-1	1.03	97.7	ACN/$H_2$0 with 10mM Na_2HPO_4 (50/50, %/%)	50	20
DBS acid ammonium salt	81481-1-39-2	1.03	97.8	ACN/$H_2$0 with 10mM Na_2HPO_4 (50/50, %/%)	50	20
DBS amide	81481-1-16	1.09	91.9	ACN	50	20
DOP amine	S61826V	1.0	99.8	ACN	50	20
DBS amine	D16M	1.0	99.7	ACN	50	20
O-Desmethyl Orthosulfamuron	81481-65	1.03	97.6	ACN/$H_2$0 with 10mM Na_2HPO_4 (50/50, %/%)	50	20
DOP urea	81481-1-33-4	1.0	99.8	ACN	50	20
Orthosulfamuron	81481-1-41-1	1.01	99.1	ACN/$H_2$0 with 10mM Na_2HPO_4 (50/50, %/%)	50	20

3.1.2 Pipette 1.0 mL of each ~ 20 µg/mL standard stock solution into a 100 mL volumetric flask, dilute to volume with the appropriate solvent, and mix well by inversion (see the following table) to create a 0.2 µg/mL standard stock solution.

Compound Name	Lot No.	Amount (mL)	Solvent	Volume (mL)	Target Concentration (µg/mL)
O-Desmethyl DOP urea	81481-1-72-1	1.0	ACN/H_2O with 10mM Na_2HPO_4 (50/50, %/%)	100	0.20
DBS acid ammonium salt	81481-1-39-2	1.0	ACN/H_2O with 10mM Na_2HPO_4 (50/50, %/%)	100	0.20
DBS amide	81481-1-16	1.0	ACN	100	0.20
DOP amine	S61826V	1.0	ACN	100	0.20
DBS amine	D16M	1.0	ACN	100	0.20
O-Desmethyl Orthosulfamuron	81481-65	1.0	ACN/H_2O with 10mM Na_2HPO_4 (50/50, %/%)	100	0.20
DOP urea	81481-1-33-4	1.0	ACN	100	0.20
Orthosulfamuron	81481-1-41-1	1.0	ACN/H_2O with 10mM Na_2HPO_4 (50/50, %/%)	100	0.20

3.1.3 Pipette ~ 3 mL of each ~ 0.2 µg/mL standard stock solution into a 25 mL volumetric flask, dilute to volume with ACN/H_2O with 10 mM Na_2HPO_4 (50/50, %/%), and mix well by inversion to create a 25 ng/mL mixed standard stock solution. The exact volume of standard stock solution will need to be calculated based on the actual standard stock concentration for each analyte.

3.1.4 Pipette 5.0 mL of the 25 ng/mL mixed standard stock solution into a 25 mL volumetric flask, dilute to volume with ACN/H_2O with 10 mM Na_2HPO_4 (50/50, %/%), and mix well by inversion to create a 5 ng/mL mixed standard stock solution.

3.1.5 Pipette 0.5 mL of the 5 ng/mL mixed standard stock solution into a 25 mL volumetric flask, dilute to volume with ACN/H_2O with 10 mM Na_2HPO_4 (50/50, %/%), and mix well by inversion to create a 0.1 ng/mL calibration standard solution.

3.1.6 Pipette 0.75 mL of the 5 ng/mL mixed standard stock solution into a 25 mL volumetric flask, dilute to volume with ACN/H_2O with 10 mM Na_2HPO_4 (50/50, %/%), and mix well by inversion to create a 0.15 ng/mL calibration standard solution.

3.1.7 Pipette 1.0 mL of the 5 ng/mL mixed standard stock solution into a 25 mL volumetric flask, dilute to volume with ACN/H_2O with 10 mM Na_2HPO_4 (50/50, %/%), and mix well by inversion to create a 0.20 ng/mL calibration standard solution.

3.1.8 Pipette 2.5 mL of the 5 ng/mL mixed standard stock solution into a 25 mL volumetric flask, dilute to volume with ACN/$H_2$0 with 10 mM Na_2HPO_4 (50/50, %/%), and mix well by inversion to create a 0.50 ng/mL calibration standard solution.

3.1.9 Pipette 1.0 mL of the 25 ng/mL mixed standard stock solution into a 25 mL volumetric flask, dilute to volume with ACN/$H_2$0 with 10 mM Na_2HPO_4 (50/50, %/%), and mix well by inversion to create a 1.0 ng/mL calibration standard solution.

3.1.10 Pipette 1.5 mL of the 25 ng/mL mixed standard stock solution into a 25 mL volumetric flask, dilute to volume with ACN/$H_2$0 with 10 mM Na_2HPO_4 (50/50, %/%), and mix well by inversion to create a 1.5 ng/mL calibration standard solution.

3.1.11 Pipette 2.0 mL of the 25 ng/mL mixed standard stock solution into a 25 mL volumetric flask, dilute to volume with ACN/$H_2$0 with 10 mM Na_2HPO_4 (50/50, %/%), and mix well by inversion to create a 2.0 ng/mL calibration standard solution.

3.1.12 Pipette 2.5 mL of the 25 ng/mL mixed standard stock solution into a 25 mL volumetric flask, dilute to volume with ACN/$H_2$0 with 10 mM Na_2HPO_4 (50/50, %/%), and mix well by inversion to create a 2.5 ng/mL calibration standard solution.

3.2 Preparation of Test Solutions

3.2.1 If necessary, thaw the water sample(s) thoroughly. The day before the analysis, remove the sample from the freezer and place it in a refrigerator overnight. The day of analysis, remove the sample from the refrigerator and place it on a benchtop and allow to warm to room temperature.

3.2.2 Determine the pH of the water sample using a calibrated pH probe.

3.2.3 Desired pH range is 8 to 9 to assist with stabilizing the analytes in the water sample.

3.2.4 If necessary, buffer the water sample to above pH 8 using 10 mM disodium hydrogen phosphate: for example add approximately 1.42 g into 1 L of water and shake vigorously, this will yield a buffered water sample with 10 mM disodium hydrogen phosphate.
Observation: water sample goes from clear to a bit cloudy after the addition of disodium hydrogen phosphate

3.2.5 Verify the pH using a calibrated pH probe.

3.2.6 Label 12 x 50 mL plastic centrifuge tubes: 2 controls, 5 for LOQ, and 5 for 10 x LOQ

3.2.7 Shake the water sample and then add 10 mL of buffered water sample into each of the 12 labeled centrifuge tubes.

3.2.8 The 2 tubes labeled as control are left unfortified.

3.2.9 To prepare 7 fortified recovery QC samples at the target LOQ (0.17 ng/mL), pipette 352 µL of the 5 ng/mL mixed standard stock solution into each labeled centrifuge tube containing 10 mL of buffered water sample, for a total volume of 10.352 mL, and mix well by inversion.

3.2.10 To prepare 5 fortified recovery QC samples at 10 x the target LOQ (1.7 ng/mL), pipette 730 µL of the 25 ng/mL mixed standard stock solution into the labeled centrifuge tube containing 10 mL of buffered water sample, for a total volume of 10.730 mL, and mix well by inversion.

3.2.11 Shake all samples (12 tubes) at ~ 180 excursions per minute for 5 minutes.

3.2.12 Approximately 1.5 mL of each sample will be aliquoted and filtered through a 0.45 µm syringe filter into an autosampler vial, cap vial and proceed to LC-MS/MS analysis.

3.2.13 Analyze all samples using the parameters shown in Section 3.4.

3.3 Calculation of Orthosulfamuron and its Major Metabolites Concentrations and Recoveries

Generate a calibration curve with each set of samples analyzed by plotting the average response of the peak areas versus the concentration of each standard solution.

Calibration curve equation: $y = mx + b$, where b = intercept, m = slope of the curve, x = sample concentration, y = response of each analytes peak area.

$$\text{Calculated Conc. (ng/mL)} = \frac{[(\text{Peak Area Response}) - (\text{intercept})]}{\text{Slope of Calibration Curve}} \times \text{DF}$$

Where: DF = Dilution Factor

$$\text{Percent Recovery} = \frac{\text{Analyte Conc., ng/mL}}{\text{Nominal Conc., ng/mL}} \times 100$$

3.4 Instrument Conditions

Note: The parameters provided below are suggested parameters for the method. These parameters may be adjusted to compensate for column-to-column and system-to-system variability.

LC-MS/MS System Conditions for Analysis of Orthosulfamuron and Metabolites

Liquid Chromatograph (LC): Shimadzu LC-20AD HPLC Pumps and CBM 20A Controller
Autosampler: Shimadzu SIL-20A/HT
Valco 2-position switching valve (optional)
Shimadzu CTO-20A Column Oven
Column: Phenomenex Luna C18(2), 150 mm x 2.0 mm ID, 5 µm particle size
Guard Column: C18, 4 mm x 2.0 mm ID
Mass Spectrometer (MS): Sciex Triple Quad LC/MS/MS System
Software: Analyst Software (Version 1.6.1)

LC Conditions

Oven Temperature:	35°C
Initial Flow:	0.250 mL/min
Injection Volume:	25 µL
Mobile Phase:	Organic = 10 mM Ammonium Formate in water/ACN with 0.1% formic acid (10:90, v/v) Aqueous = 10 mM Ammonium Formate in water with 0.1% formic acid Rinse = ACN/Water (1:1, v/v)

Gradient Method:

Time (min)	%Aqueous	%Organic	Flow (mL/min)
0.00	100	0	0.250
2.00	100	0	0.250
7.50	10	90	0.250
10.00	10	90	0.250
10.10	100	0	0.250
15.00	100	0	0.250

Approx. Retention Times (min):

O-Desmethyl DOP urea	–	6.6
DBS acid ammonium salt	–	6.8
DBS amide	–	7.3
DOP amine	–	7.7
DBS amine	–	7.5
O-Desmethyl Orthosulfamuron	–	7.5
DOP urea	–	7.7
Orthosulfamuron	–	9.0

Autosampler Conditions

Rinsing Volume:	1000 µL
Needle Stroke:	52 mm
Rinsing Speed:	35 µL/sec
Sampling Speed:	2.0 µL/sec
Purge Time:	25.0 min
Rinse Dip Time:	0 sec
Rinse Mode:	Before and after aspiration
Control Vial Needle Stroke:	52 mm
Rinse Method:	Rinse Port Only

Rinse Time:	1 sec
Mass Spectrometer Conditions for LC-MS/MS Analysis	
Period 1: Analyte:	O-Desmethyl DOP urea
Scan Type:	MRM
Ion Source:	Turbospray
Polarity:	Positive
Q1 Mass (amu):	185.000
Q3 Mass (amu):	142.100
Dwell (msec):	20.00
Period Time (min):	0.0 to 6.60
Declustering Potential (DP):	94.0 V
Entrance Potential (EP):	7.0 V
Collision Energy (CE):	23.0 V
Collision Cell Exit Potential (CXP):	6.0 V
Curtain Gas (CUR):	35.0
Temperature (TEM):	550°C
Ion Source Gas 1 (GS1):	35.0
Ion Source Gas 2 (GS2):	55.0
Collision Gas (CAD):	7.0
Ionspray Voltage (IS):	3000 V
Period 2: Analyte:	DBS acid ammonium salt
Scan Type:	MRM
Ion Source:	Turbospray
Polarity:	Negative
Q1 Mass (amu):	243.165
Q3 Mass (amu):	80.104
Dwell (msec):	20.00
Period Time (min):	6.60 to 6.85
Declustering Potential (DP):	–40.0 V
Entrance Potential (EP):	–5.0 V
Collision Energy (CE):	–21.0 V
Collision Cell Exit Potential (CXP):	–12.0 V
Curtain Gas (CUR):	35.0
Temperature (TEM):	550°C
Ion Source Gas 1 (GS1):	35.0
Ion Source Gas 2 (GS2):	55.0
Collision Gas (CAD):	7.0
Ionspray Voltage (IS):	–4400 V

Period 3:

Analyte	DBS amide	DOP amine	DBS amine	O-Desmethyl Orthosulfamuron	DOP urea	Orthosulfamuron
Scan Type	MRM	MRM	MRM	MRM	MRM	MRM
Ion Source	Turbospray	Turbospray	Turbospray	Turbospray	Turbospray	Turbospray
Polarity	Positive	Positive	Positive	Positive	Positive	Positive
Q1 Mass (amu)	244.100	156.017	164.740	411.074	199.200	425.089
Q3 Mass (amu)	165.000	99.700	119.800	227.000	156.000	199.000
Dwell (msec)	20.00	20.00	20.00	20.00	20.00	20.00
Period Time (min)	6.85 to 15.0	6.85 to 15.0	6.85 to 15.0	6.85 to 15.0	6.85 to 15.0	6.85 to 15.0
Declustering Potential (DP)	39.0 V	50.0 V	55.0 V	51.0 V	60.0 V	56.0 V
Entrance Potential (EP)	6.0 V	6.0 V	7.0 V	3.0 V	5.0 V	4.0 V
Collision Energy (CE)	13.0 V	21.0 V	13.0 V	17.0 V	19.0 V	15.0 V
Collision Cell Exit Potential (CXP)	6.0 V	50.0 V	4.0 V	28.0 V	10.0 V	22.0 V
Curtain Gas (CUR)	35.0	35.0	35.0	35.0	35.0	35.0
Temperature (TEM)	550.0°C	550.0°C	550.0°C	550.0°C	550.0°C	550.0°C
Ion Source Gas 1 (GS1)	35.0	35.0	35.0	35.0	35.0	35.0
Ion Source Gas 2 (GS2)	55.0	55.0	55.0	55.0	55.0	55.0
Collision Gas (CAD)	7.0	7.0	7.0	7.0	7.0	7.0
Ionspray Voltage (IS)	3000 V	3000 V	3000 V	3000 V	3000 V	3000 V

Divert Valve Program: 0.0 to 5.5 min – To Waste
5.5 to 10.5 min – To Instrument
10.5 to 15.0 min – To Waste

Note: The indicated LC-MS/MS parameters are guidelines and should be optimized for the instrument and column actually used. Instrument parameters and mobile phase compositions may be adjusted to improve separation from interfering peaks.

3.5 Revisions

None

Method Amendment

Modification Number: 1
Study Number: 292SRLS14R01

Method Title: Analytical Method Validation for the Determination of Orthosulfamuron and its Major Metabolites in Water using LC-MS/MS

Reason for the Change/addition and Impact on the Method:
The method modifications address typographical errors, using matrix matched standards, and different spiking concentrations for recovery QC samples. No adverse impact on the method.

Location and Description of Changes:

2.0 EXPERIMENTAL DESIGN

2.4 Clarify that there shall be at least 7 spiked controls at the target LOQ and at least 5 spiked controls at 10 x the target LOQ

3.0 METHODOLOGY

3.1 Preparation of Calibration Standards

3.1.2 Pipette 1.0 mL of each ~ 20 µg/mL standard stock solution into one 10 mL volumetric flask, dilute to volume with ACN/H_2O with 10mM Na_2HPO_4 (50/50, %/%), and mix well by inversion to create a 2.0 µg/mL (2000 ng/mL) mixed standard stock solution.

3.1.3 Pipette 0.1 mL of the 2000 ng/mL mixed standard stock solution into a 50 mL volumetric flask, dilute to volume with ACN/H_2O with 10 mM Na_2HPO_4 (50/50, %/%), and mix well by inversion to create a 4.0 ng/mL spiking matrix matched calibration standard solution.

3.1.4 Pipette 0.15 mL of the 2000 ng/mL mixed standard stock solution into a 50 mL volumetric flask, dilute to volume with ACN/H_2O with 10 mM Na_2HPO_4 (50/50, %/%), and mix well by inversion to create a 6.0 ng/mL spiking matrix matched calibration standard solution.

3.1.5 Pipette 0.2 mL of the 2000 ng/mL mixed standard stock solution into a 50 mL volumetric flask, dilute to volume with ACN/H_2O with 10 mM Na_2HPO_4 (50/50, %/%), and mix well by inversion to create a 8.0 ng/mL spiking matrix matched calibration standard solution.

3.1.6 Pipette 0.5 mL of the 2000 ng/mL mixed standard stock solution into a 50 mL volumetric flask, dilute to volume with ACN/H_2O with 10 mM Na_2HPO_4 (50/50, %/%), and mix well by inversion to create a 20 ng/mL spiking matrix matched calibration standard solution.

3.1.7 Pipette 1.0 mL of the 2000 ng/mL mixed standard stock solution into a 50 mL volumetric flask, dilute to volume with ACN/H_2O with 10 mM Na_2HPO_4 (50/50, %/%), and

Method Amendment

mix well by inversion to create a 40 ng/mL spiking matrix matched calibration standard solution.

3.1.8 Pipette 1.5 mL of the 2000 ng/mL mixed standard stock solution into a 50 mL volumetric flask, dilute to volume with ACN/H_2O with 10 mM Na_2HPO_4 (50/50, %/%), and mix well by inversion to create a 60 ng/mL spiking matrix matched calibration standard solution.

3.1.9 Pipette 2.0 mL of the 2000 ng/mL mixed standard stock solution into a 50 mL volumetric flask, dilute to volume with ACN/H_2O with 10 mM Na_2HPO_4 (50/50, %/%), and mix well by inversion to create a 80 ng/mL spiking matrix matched calibration standard solution.

3.1.10 Pipette 2.5 mL of the 2000 ng/mL mixed standard stock solution into a 50 mL volumetric flask, dilute to volume with ACN/H_2O with 10 mM Na_2HPO_4 (50/50, %/%), and mix well by inversion to create a 100 ng/mL spiking matrix matched calibration standard solution.

3.1.11 Pipette 25 µL of the 4.0 ng/mL spiking matrix matched calibration standard solution into an HPLC vial that contains 1 mL of buffered and filtered matrix. Mix solution well by inversion to create a 0.1 ng/mL matrix matched calibration standard solution.

3.1.12 Pipette 25 µL of the 6.0 ng/mL spiking matrix matched calibration standard solution into an HPLC vial that contains 1 mL of buffered and filtered matrix. Mix solution well by inversion to create a 0.15 ng/mL matrix matched calibration standard solution.

3.1.13 Pipette 25 µL of the 8.0 ng/mL spiking matrix matched calibration standard solution into an HPLC vial that contains 1 mL of buffered and filtered matrix. Mix solution well by inversion to create a 0.20 ng/mL matrix matched calibration standard solution.

3.1.14 Pipette 25 µL of the 20 ng/mL spiking matrix matched calibration standard solution into an HPLC vial that contains 1 mL of buffered and filtered matrix. Mix solution well by inversion to create a 0.50 ng/mL matrix matched calibration standard solution.

3.1.15 Pipette 25 µL of the 40 ng/mL spiking matrix matched calibration standard solution into an HPLC vial that contains 1 mL of buffered and filtered matrix. Mix solution well by inversion to create a 1.0 ng/mL matrix matched calibration standard solution.

3.1.16 Pipette 25 µL of the 60 ng/mL spiking matrix matched calibration standard solution into an HPLC vial that contains 1 mL of buffered and filtered matrix. Mix solution well by inversion to create a 1.5 ng/mL matrix matched calibration standard solution.

3.1.17 Pipette 25 µL of the 80 ng/mL spiking matrix matched calibration standard solution into an HPLC vial that contains 1 mL of buffered and filtered matrix. Mix solution well by inversion to create a 2.0 ng/mL matrix matched calibration standard solution.

3.1.18 Pipette 25 µL of the 100 ng/mL spiking matrix matched calibration standard solution into an HPLC vial that contains 1 mL of buffered and filtered matrix. Mix solution well by inversion to create a 2.5 ng/mL matrix matched calibration standard solution.

Method Amendment

3.2 Preparation of Test Solutions

3.2.6 Label the appropriate number of 15 mL plastic centrifuge tubes: 2 controls, at least 7 for target LOQ, and at least 5 for 10 x target LOQ

3.2.7 Shake the water sample and then add 10 mL of buffered water sample into each of the labeled centrifuge tubes.

3.2.9 To prepare at least 7 fortified recovery QC samples at the target LOQ (0.17 ng/mL), pipette 170 µL of the 10 ng/mL mixed standard solution into each labeled centrifuge tube containing 10 mL of buffered water sample and mix well by inversion.

3.2.10 To prepare at least 5 fortified recovery QC samples at 10 x the target LOQ (1.7 ng/mL), pipette 170 µL of the 100 ng/mL mixed standard solution into each labeled centrifuge tube containing 10 mL of buffered water sample and mix well by inversion.

3.2.11 Shake all samples at ~ 180 excursions per minute for 5 minutes.

3.5 Revisions

See Method Amendment, Modification Number 1

Appendix 2

ANALYTICAL METHOD VALIDATION FOR THE DETERMINATION OF ORTHOSULFAMURON AND ITS MAJOR METABOLITES IN SOIL USING LC-MS/MS

Prepared by: Walter R. Vandaveer, Research Scientist
SynTech Research Laboratory Services

Date: 08/10/2015

1.0 OBJECTIVE

The purpose of this project is to analyze soil samples collected from Arkansas and California for orthosulfamuron and its major metabolites during a method validation using a liquid chromatography-mass spectrometry/mass spectroscopy (LC-MS/MS) system. The target limit of quantitation (LOQ) is 0.04 ppb.

2.0 EXPERIMENTAL DESIGN

2.1 Test solutions from the study will be analyzed.

2.2 The analytical method will be developed and validated before the sample analysis.

2.3 O-Desmethyl DOP urea, Lot Number 81481-1-72-1, 97.7% purity, expiration date 11/26/2016; DBS acid ammonium salt, Lot Number 81481-1-39-2, 97.8% purity, expiration date 11/26/2016; DBS amide, Lot Number 81481-1-16, 91.9% purity, expiration date 11/26/2016; DOP amine, Lot Number S61826V, 99.8% purity, expiration date 11/26/2016; DBS amine, Lot Number D16M, 99.7% purity, expiration date 11/26/2016; O-Desmethyl Orthosulfamuron, Lot Number 81481-65, 97.6% purity, expiration date 11/26/2016; DOP urea, Lot Number 81481-1-33-4, 99.8% purity, expiration date 11/26/2016; Orthosulfamuron, Lot Number 81481-1-41-1, 99.1% purity, expiration date 11/26/2016 will be supplied by ABC Laboratories, Inc.

2.4 Method validation will be completed prior to the analysis of study samples. This will consist of a solvent blank, two controls, and at least 7 spiked controls at the target LOQ (0.04 ppb) and at least 5 spiked controls at 10 x the target LOQ (0.4 ppb) with recovery determined using a calibration curve. The concentration range of standards will be between ~ 0.02 and 0.5 ppb. All procedures developed during the method validation phase of the project will be employed during the study sample analysis. A recovery spike for orthosulfamuron and its major metabolites will be required to monitor methodology and individual performance. The acceptable recoveries are 70 - 120%. Results will be considered acceptable if the average recovery value (of the two or more concurrent recovery samples) falls within this range. If the lab spike falls outside of this range, the results may be rejected.

2.5 All analytical results will be reported to the study director.

3.0 METHODOLOGY

3.1 Preparation of Calibration Standards

3.1.1 Weigh ~ 1 mg of each standard into a 50 mL volumetric flask, dilute to volume with the appropriate solvent, and mix well by inversion (see the following table) to create a 20 µg/mL standard stock solution.

Compound Name	Lot No.	Amount (mg)	Purity (%)	Solvent	Volume (mL)	Target Concentration (µg/mL)
O-Desmethyl DOP urea	81481-1-72-1	1.03	97.7	ACN/H_2O with 10mM Na_2HPO_4 (50/50, %/%)	50	20
DBS acid ammonium salt	81481-1-39-2	1.03	97.8	ACN/H_2O with 10mM Na_2HPO_4 (50/50, %/%)	50	20
DBS amide	81481-1-16	1.09	91.9	ACN	50	20
DOP amine	S61826V	1.0	99.8	ACN	50	20
DBS amine	D16M	1.0	99.7	ACN	50	20
O-Desmethyl Orthosulfamuron	81481-65	1.03	97.6	ACN/H_2O with 10mM Na_2HPO_4 (50/50, %/%)	50	20
DOP urea	81481-1-33-4	1.0	99.8	ACN	50	20
Orthosulfamuron	81481-1-41-1	1.01	99.1	ACN/H_2O with 10mM Na_2HPO_4 (50/50, %/%)	50	20

3.1.2 Pipette 1.0 mL of each ~ 20 µg/mL standard stock solution into one 10 mL volumetric flask, dilute to volume with ACN/H_2O with 10 mM Na_2HPO_4 (50/50, %/%), and mix well by inversion to create a 2.0 µg/mL (2000 ng/mL) mixed standard stock solution.

3.1.3 Pipette 0.1 mL of the 2000 ng/mL mixed standard stock solution into a 50 mL volumetric flask, dilute to volume with ACN/H_2O with 10 mM Na_2HPO_4 (50/50, %/%), and mix well by inversion to create a 4.0 ng/mL spiking matrix matched calibration standard solution.

3.1.4 Pipette 0.15 mL of the 2000 ng/mL mixed standard stock solution into a 50 mL volumetric flask, dilute to volume with ACN/H_2O with 10 mM Na_2HPO_4 (50/50, %/%), and mix well by inversion to create a 6.0 ng/mL spiking matrix matched calibration standard solution.

3.1.5 Pipette 0.2 mL of the 2000 ng/mL mixed standard stock solution into a 50 mL volumetric flask, dilute to volume with ACN/H_2O with 10 mM Na_2HPO_4 (50/50, %/%), and mix well by inversion to create a 8.0 ng/mL spiking matrix matched calibration standard solution.

3.1.6 Pipette 0.5 mL of the 2000 ng/mL mixed standard stock solution into a 50 mL volumetric flask, dilute to volume with ACN/H_2O with 10 mM Na_2HPO_4 (50/50, %/%), and mix well by inversion to create a 20 ng/mL spiking matrix matched calibration standard solution.

3.1.7 Pipette 1.0 mL of the 2000 ng/mL mixed standard stock solution into a 50 mL volumetric flask, dilute to volume with ACN/H_2O with 10 mM Na_2HPO_4 (50/50, %/%), and mix well by inversion to create a 40 ng/mL spiking matrix matched calibration standard solution.

3.1.8 Pipette 1.5 mL of the 2000 ng/mL mixed standard stock solution into a 50 mL volumetric flask, dilute to volume with ACN/H_2O with 10 mM Na_2HPO_4 (50/50, %/%), and mix well by inversion to create a 60 ng/mL spiking matrix matched calibration standard solution.

3.1.9 Pipette 2.0 mL of the 2000 ng/mL mixed standard stock solution into a 50 mL volumetric flask, dilute to volume with ACN/H_2O with 10 mM Na_2HPO_4 (50/50, %/%), and mix well by inversion to create a 80 ng/mL spiking matrix matched calibration standard solution.

3.1.10 Pipette 2.5 mL of the 2000 ng/mL mixed standard stock solution into a 50 mL volumetric flask, dilute to volume with ACN/H_2O with 10 mM Na_2HPO_4 (50/50, %/%), and mix well by inversion to create a 100 ng/mL spiking matrix matched calibration standard solution.

3.1.11 Pipette 25 µL of the 4.0 ng/mL spiking matrix matched calibration standard solution into an HPLC vial that contains 1 mL of filtered matrix. Mix solution well by vortexing to create a ~ 0.02 ppb matrix matched calibration standard solution.

3.1.12 Pipette 25 µL of the 6.0 ng/mL spiking matrix matched calibration standard solution into an HPLC vial that contains 1 mL of filtered matrix. Mix solution well by vortexing to create a ~ 0.03 ppb matrix matched calibration standard solution.

3.1.13 Pipette 25 µL of the 8.0 ng/mL spiking matrix matched calibration standard solution into an HPLC vial that contains 1 mL of filtered matrix. Mix solution well by vortexing to create a ~ 0.04 ppb matrix matched calibration standard solution.

3.1.14 Pipette 25 µL of the 20 ng/mL spiking matrix matched calibration standard solution into an HPLC vial that contains 1 mL of filtered matrix. Mix solution well by vortexing to create a ~ 0.10 ppb matrix matched calibration standard solution.

3.1.15 Pipette 25 µL of the 40 ng/mL spiking matrix matched calibration standard solution into an HPLC vial that contains 1 mL of filtered matrix. Mix solution well by vortexing to create a ~ 0.20 ppb matrix matched calibration standard solution.

3.1.16 Pipette 25 µL of the 60 ng/mL spiking matrix matched calibration standard solution into an HPLC vial that contains 1 mL of filtered matrix. Mix solution well by vortexing to create a ~ 0.30 ppb matrix matched calibration standard solution.

3.1.17 Pipette 25 µL of the 80 ng/mL spiking matrix matched calibration standard solution into an HPLC vial that contains 1 mL of filtered matrix. Mix solution well by vortexing to create a ~ 0.40 ppb matrix matched calibration standard solution.

3.1.18 Pipette 25 µL of the 100 ng/mL spiking matrix matched calibration standard solution into an HPLC vial that contains 1 mL of filtered matrix. Mix solution well by vortexing to create a ~ 0.5 ppb matrix matched calibration standard solution.

3.2 Preparation of Test Solutions

3.2.1 Label the appropriate number of 50 mL centrifuge tubes.

3.2.2 Weigh approximately 10 g of soil into each 50 mL centrifuge tube.

3.2.3 Fortify recovery samples as necessary.

3.2.3.1 To prepare at least 7 fortified recovery QC samples at the target LOQ (0.04 ppb), pipette 20 µL of the 20 ng/mL spiking matrix matched calibration standard into each labeled centrifuge tube containing 10 g of soil.

3.2.3.2 To prepare at least 5 fortified recovery QC samples at 10 x the target LOQ (0.4 ppb), pipette 40 µL of the 100 ng/mL spiking matrix matched calibration standard into each labeled centrifuge tube containing 10 g of soil.

3.2.4 Pipette 10 mL of acetonitrile into each tube and shake vigorously for 20 minutes at 1200 rpm using a GenoGrinder homogenizer.

3.2.5 Pipette 5 mL of UltraPure water into each tube.

3.2.6 Add a pre-mixed Quechers buffer-salt mixture to each tube. [4 g Magnesium Sulfate (MgSO4), 1 g Sodium chloride (NaCl), 0.5 g Sodium citrate dibasic sesquihydrate (Na2Cit), 1 g Sodium Citrate tribasic (Na3Cit)]

3.2.7 Shake vigorously for 1 minute at 1200 rpm using a GenoGrinder homogenizer and centrifuge at 3000 rpm for 5 minutes.

3.2.8 Pipette a 5 mL aliquot of the cleaned extract (top ACN layer) into a graduated test tube.

3.2.9 Reduce the concentration to 0.25 mL with heat (~ 35°C) and nitrogen blow down using a Turbovap.

3.2.10 Make up to the 1 mL graduation with 10 mM Ammonium Formate in water and vortex to mix.

3.2.11 Precondition each 0.45 µm Nylon syringe filter with 1 to 2 mL of 10 mM Ammonium Formate in water.

3.2.12 Filter all samples into individual HPLC vials with the conditioned 0.45 µm Nylon syringe filter, cap vial, and proceed to LC-MS/MS analysis.

3.2.13 Analyze all samples using the parameters shown in Section 3.4.

3.3 Calculation of Orthosulfamuron and its Major Metabolites Concentrations and Recoveries

Generate a calibration curve with each set of samples analyzed by plotting the average response of the peak areas versus the concentration of each standard solution.

Calibration curve equation: $y = mx + b$, where b = intercept, m = slope of the curve, x = sample concentration, y = response of each analytes peak area.

$$\text{Calculated Conc. (ppb)} = \frac{[(\text{Peak Area Response}) - (\text{intercept})]}{\text{Slope of Calibration Curve}} \times DF$$

Where: DF = Dilution Factor

$$\text{Percent Recovery} = \frac{\text{Analyte Conc., ppb}}{\text{Nominal Conc., ppb}} \times 100$$

3.4 Instrument Conditions

Note: The parameters provided below are suggested parameters for the method. These parameters may be adjusted to compensate for column-to-column and system-to-system variability.

LC-MS/MS System Conditions for Analysis of Orthosulfamuron and Metabolites

Liquid Chromatograph (LC): Shimadzu LC-20AD HPLC Pumps and CBM 20A Controller
Autosampler: Shimadzu SIL-20AC/HT
Valco 2-position switching valve (optional)
Shimadzu CTO-20A Column Oven
Column: Phenomenex Luna C18(2), 150 mm x 2.0 mm ID, 5 µm particle size
Guard Column: C18, 4 mm x 2.0 mm ID
Mass Spectrometer (MS): Sciex Triple Quad LC/MS/MS System
Software: Analyst Software (Version 1.6.1)

LC Conditions

Oven Temperature:	35°C
Initial Flow:	0.250 mL/min
Injection Volume:	25 µL
Mobile Phase:	Organic = 10 mM Ammonium Formate in water/ACN with 0.1% formic acid (10:90, v/v) Aqueous = 10 mM Ammonium Formate in water with 0.1% formic acid Rinse = ACN/Water (1:1, v/v)

Gradient Method:

Time (min)	%Aqueous	%Organic	Flow (mL/min)
0.00	100	0	0.250
2.00	100	0	0.250
7.50	10	90	0.250
10.00	10	90	0.250
10.10	100	0	0.250
15.00	100	0	0.250

Approx. Retention Times (min):

O-Desmethyl DOP urea	–	6.8
DBS acid ammonium salt	–	7.1
DBS amide	–	7.6
DOP amine	–	8.0
DBS amine	–	7.8
O-Desmethyl Orthosulfamuron	–	7.7
DOP urea	–	8.1
Orthosulfamuron	–	9.3

Autosampler Conditions

Rinsing Volume:	500 µL
Needle Stroke:	52 mm
Rinsing Speed:	35 µL/sec
Sampling Speed:	2.0 µL/sec
Purge Time:	5.0 min
Rinse Dip Time:	5 sec
Rinse Mode:	Before and after aspiration
Control Vial Needle Stroke:	52 mm
Rinse Method:	Rinse Port Only
Rinse Time:	1 sec

Mass Spectrometer Conditions for LC-MS/MS Analysis	
Period 1: Analyte:	O-Desmethyl DOP urea
Scan Type:	MRM
Ion Source:	Turbospray
Polarity:	Positive
Q1 Mass (amu):	185.000
Q3 Mass (amu):	142.100
Dwell (msec):	20.00
Period Time (min):	0.0 to 6.95
Declustering Potential (DP):	94.0 V
Entrance Potential (EP):	7.0 V
Collision Energy (CE):	23.0 V
Collision Cell Exit Potential (CXP):	6.0 V
Curtain Gas (CUR):	35.0
Temperature (TEM):	550°C
Ion Source Gas 1 (GS1):	35.0
Ion Source Gas 2 (GS2):	55.0
Collision Gas (CAD):	7.0
Ionspray Voltage (IS):	3000 V
Period 2: Analyte:	DBS acid ammonium salt
Scan Type:	MRM
Ion Source:	Turbospray
Polarity:	Negative
Q1 Mass (amu):	243.165
Q3 Mass (amu):	80.104
Dwell (msec):	20.00
Period Time (min):	6.95 to 7.20
Declustering Potential (DP):	–40.0 V
Entrance Potential (EP):	–5.0 V
Collision Energy (CE):	–21.0 V
Collision Cell Exit Potential (CXP):	–12.0 V
Curtain Gas (CUR):	35.0
Temperature (TEM):	550°C
Ion Source Gas 1 (GS1):	35.0
Ion Source Gas 2 (GS2):	55.0
Collision Gas (CAD):	7.0
Ionspray Voltage (IS):	–4400 V

Period 3:

Analyte	DBS amide	DOP amine	DBS amine	O-Desmethyl Orthosulfamuron	DOP urea	Orthosulfamuron
Scan Type	MRM	MRM	MRM	MRM	MRM	MRM
Ion Source	Turbospray	Turbospray	Turbospray	Turbospray	Turbospray	Turbospray
Polarity	Positive	Positive	Positive	Positive	Positive	Positive
Q1 Mass (amu)	244.100	156.017	164.740	411.074	199.200	425.089
Q3 Mass (amu)	165.000	99.700	119.800	227.000	156.000	199.000
Dwell (msec)	20.00	20.00	20.00	20.00	20.00	20.00
Period Time (min)	7.20 to 15.0	7.20 to 15.0	7.20 to 15.0	7.20 to 15.0	7.20 to 15.0	7.20 to 15.0
Declustering Potential (DP)	39.0 V	50.0 V	55.0 V	51.0 V	60.0 V	56.0 V
Entrance Potential (EP)	6.0 V	6.0 V	7.0 V	3.0 V	5.0 V	4.0 V
Collision Energy (CE)	13.0 V	21.0 V	13.0 V	17.0 V	19.0 V	15.0 V
Collision Cell Exit Potential (CXP)	6.0 V	50.0 V	4.0 V	28.0 V	10.0 V	22.0 V
Curtain Gas (CUR)	35.0	35.0	35.0	35.0	35.0	35.0
Temperature (TEM)	550.0°C	550.0°C	550.0°C	550.0°C	550.0°C	550.0°C
Ion Source Gas 1 (GS1)	35.0	35.0	35.0	35.0	35.0	35.0
Ion Source Gas 2 (GS2)	55.0	55.0	55.0	55.0	55.0	55.0
Collision Gas (CAD)	7.0	7.0	7.0	7.0	7.0	7.0
Ionspray Voltage (IS)	3000 V	3000 V	3000 V	3000 V	3000 V	3000 V

Divert Valve Program: 0.0 to 5.5 min – To Waste
5.5 to 10.5 min – To Instrument
10.5 to 15.0 min – To Waste

Note: The indicated LC-MS/MS parameters are guidelines and should be optimized for the instrument and column actually used. Instrument parameters and mobile phase compositions may be adjusted to improve separation from interfering peaks.

3.5 Revisions

None

Appendix 2

ANALYTICAL METHOD VALIDATION FOR THE DETERMINATION OF ORTHOSULFAMURON AND ITS MAJOR METABOLITES IN SOIL AND SEDIMENT USING LC-MS/MS

Prepared by: Walter R. Vandaveer, Research Scientist
SynTech Research Laboratory Services

Date: 11/04/2015

1.0 OBJECTIVE

The purpose of this project is to analyze soil and sediment samples for orthosulfamuron and its major metabolites during a method validation using a liquid chromatography-mass spectrometry/mass spectroscopy (LC-MS/MS) system. The target limit of quantitation (LOQ) is 0.2 ppb.

2.0 EXPERIMENTAL DESIGN

2.1 Test solutions from the study will be analyzed.

2.2 The analytical method will be developed and validated before the sample analysis.

2.3 O-Desmethyl DOP urea, Lot Number 81481-1-72-1, 97.7% purity, expiration date 11/26/2016; DBS acid ammonium salt, Lot Number 81481-1-39-2, 97.8% purity, expiration date 11/26/2016; DB amine, Lot Number D16M, 99.7% purity, expiration date 11/26/2016; O-Desmethyl Orthosulfamuron, Lot Number 81481-65, 97.6% purity, expiration date 11/26/2016; DOP urea, Lot Number 81481-1-33-4, 99.8% purity, expiration date 11/26/2016; Orthosulfamuron, Lot Number 81481-1-41-1, 99.1% purity, expiration date 11/26/2016 will be supplied by ABC Laboratories, Inc.

2.4 Method validation will be completed prior to the analysis of study samples. This will consist of a solvent blank, two controls, and at least 7 spiked controls at the target LOQ (0.2 ppb) and at least 5 spiked controls at 10 x the target LOQ (2.0 ppb) with recovery determined using a calibration curve. The concentration range of standards will be between ~ 0.1 and 3.0 ppb. All procedures developed during the method validation phase of the project will be employed during the study sample analysis. A recovery spike for orthosulfamuron and its major metabolites will be required to monitor methodology and individual performance. The acceptable recoveries are 70 - 120%. Results will be considered acceptable if the average recovery value (of the two or more concurrent recovery samples) falls within this range. If the lab spike falls outside of this range, the results may be rejected.

2.5 All analytical results will be reported to the study director.

3.0 METHODOLOGY

3.1 Preparation of Calibration Standards

3.1.1 Weigh ~ 1 mg of each standard into a 50 mL volumetric flask, dilute to volume with the appropriate solvent, and mix well by inversion (see the following table) to create a 20 µg/mL standard stock solution.

Compound Name	Lot No.	Amount (mg)	Purity (%)	Solvent	Volume (mL)	Target Concentration (µg/mL)
O-Desmethyl DOP urea	81481-1-72-1	1.0	97.7	ACN/H_2O with 10mM Na_2HPO_4 (1:1, v/v)	50	20
DBS acid ammonium salt	81481-1-39-2	1.0	97.8	ACN/H_2O with 10mM Na_2HPO_4 (1:1, v/v)	50	20
DB amine	D16M	1.0	99.7	ACN	50	20
O-Desmethyl Orthosulfamuron	81481-65	1.0	97.6	ACN/H_2O with 10mM Na_2HPO_4 (1:1, v/v)	50	20
DOP urea	81481-1-33-4	1.0	99.8	ACN	50	20
Orthosulfamuron	81481-1-41-1	1.0	99.1	ACN/H_2O with 10mM Na_2HPO_4 (1:1, v/v)	50	20

3.1.2 Pipette ~ 1.0 mL of each ~ 20 µg/mL standard stock solution into one 10 mL volumetric flask, dilute to volume with ACN/H_2O with 10 mM Na_2HPO_4 (1:1, v/v), and mix well by inversion to create a 2.0 µg/mL (2000 ng/mL) mixed standard stock solution. **Note:** pipette the appropriate volume of stock solution to result in a 2.0 µg/mL concentration.

3.1.3 Pipette 0.01 mL of the 2000 ng/mL mixed standard stock solution into a 10 mL volumetric flask, dilute to volume with ACN/H_2O with 10 mM Na_2HPO_4 (1:1, v/v), and mix well by inversion to create a 2.0 ng/mL spiking matrix matched calibration standard solution.

3.1.4 Pipette 0.015 mL of the 2000 ng/mL mixed standard stock solution into a 10 mL volumetric flask, dilute to volume with ACN/H_2O with 10 mM Na_2HPO_4 (1:1, v/v), and mix well by inversion to create a 3.0 ng/mL spiking matrix matched calibration standard solution.

3.1.5 Pipette 0.020 mL of the 2000 ng/mL mixed standard stock solution into a 10 mL volumetric flask, dilute to volume with ACN/H_2O with 10 mM Na_2HPO_4 (1:1, v/v), and mix well by inversion to create a 4.0 ng/mL spiking matrix matched calibration standard solution.

3.1.6 Pipette 0.050 mL of the 2000 ng/mL mixed standard stock solution into a 10 mL volumetric flask, dilute to volume with ACN/H_2O with 10 mM Na_2HPO_4 (1:1, v/v), and mix well by inversion to create a 10 ng/mL spiking matrix matched calibration standard solution.

3.1.7 Pipette 0.10 mL of the 2000 ng/mL mixed standard stock solution into a 10 mL volumetric flask, dilute to volume with ACN/H_2O with 10 mM Na_2HPO_4 (1:1, v/v), and mix well by inversion to create a 20 ng/mL spiking matrix matched calibration standard solution.

3.1.8 Pipette 0.205 mL of the 2000 ng/mL mixed standard stock solution into a 10 mL volumetric flask, dilute to volume with ACN/H_2O with 10 mM Na_2HPO_4 (1:1, v/v), and mix well by inversion to create a 41 ng/mL spiking matrix matched calibration standard solution.

3.1.9 Pipette 0.255 mL of the 2000 ng/mL mixed standard stock solution into a 10 mL volumetric flask, dilute to volume with ACN/$H_2$0 with 10 mM Na_2HPO_4 (1:1, v/v), and mix well by inversion to create a 51 ng/mL spiking matrix matched calibration standard solution.

3.1.10 Pipette 0.305 mL of the 2000 ng/mL mixed standard stock solution into a 10 mL volumetric flask, dilute to volume with ACN/$H_2$0 with 10 mM Na_2HPO_4 (1:1, v/v), and mix well by inversion to create a 61 ng/mL spiking matrix matched calibration standard solution.

3.1.11 Pipette 25 µL of the 2.0 ng/mL spiking matrix matched calibration standard solution into an HPLC vial that contains 1 mL of filtered control matrix. Mix solution well by vortexing to create a ~ 0.098 ppb matrix matched calibration standard solution.

3.1.12 Pipette 25 µL of the 3.0 ng/mL spiking matrix matched calibration standard solution into an HPLC vial that contains 1 mL of filtered control matrix. Mix solution well by vortexing to create a ~ 0.146 ppb matrix matched calibration standard solution.

3.1.13 Pipette 25 µL of the 4.0 ng/mL spiking matrix matched calibration standard solution into an HPLC vial that contains 1 mL of filtered control matrix. Mix solution well by vortexing to create a ~ 0.195 ppb matrix matched calibration standard solution.

3.1.14 Pipette 25 µL of the 10 ng/mL spiking matrix matched calibration standard solution into an HPLC vial that contains 1 mL of filtered control matrix. Mix solution well by vortexing to create a ~ 0.488 ppb matrix matched calibration standard solution.

3.1.15 Pipette 25 µL of the 20 ng/mL spiking matrix matched calibration standard solution into an HPLC vial that contains 1 mL of filtered control matrix. Mix solution well by vortexing to create a ~ 0.976 ppb matrix matched calibration standard solution.

3.1.16 Pipette 25 µL of the 41 ng/mL spiking matrix matched calibration standard solution into an HPLC vial that contains 1 mL of filtered control matrix. Mix solution well by vortexing to create a ~ 2.0 ppb matrix matched calibration standard solution.

3.1.17 Pipette 25 µL of the 51 ng/mL spiking matrix matched calibration standard solution into an HPLC vial that contains 1 mL of filtered control matrix. Mix solution well by vortexing to create a ~ 2.488 ppb matrix matched calibration standard solution.

3.1.18 Pipette 25 µL of the 61 ng/mL spiking matrix matched calibration standard solution into an HPLC vial that contains 1 mL of filtered control matrix. Mix solution well by vortexing to create a ~ 2.976 ppb matrix matched calibration standard solution.

3.2 Preparation of Test Solutions

3.2.1 Label the appropriate number of 50 mL centrifuge tubes.

3.2.2 Weigh approximately 10 g of sample into each 50 mL centrifuge tube.

3.2.2.1 Prior to weighing out sediment samples, centrifuge the sediment at 4000 rpm for 15 min and decant off the water.

3.2.3 Fortify recovery samples as necessary.

3.2.3.1 To prepare at least 7 fortified recovery QC samples at the target LOQ (0.2 ppb), pipette 32.8 µL of the 61 ng/mL spiking matrix matched calibration standard into each labeled centrifuge tube containing 10 g of sample.

3.2.3.2 To prepare at least 5 fortified recovery QC samples at 10 x the target LOQ (2.0 ppb), pipette 327.9 µL of the 61 ng/mL spiking matrix matched calibration standard into each labeled centrifuge tube containing 10 g of sample.

3.2.4 Pipette 10 mL of 7:3 acetonitrile/33 mM sodium bicarbonate (v/v) into each tube.

3.2.5 Shake vigorously for 40 minutes at 1200 rpm using a GenoGrinder homogenizer and centrifuge at 4000 rpm for 15 min to form a solid sample pellet.

3.2.6 Transfer supernatant (extract) into a separate 50 mL graduated centrifuge tube.

3.2.7 Using individual spatulas, break up sample pellet and scrape off any remaining sample from spatulas back into tube.

3.2.8 Using part of the below 10 mL of 1:1 acetonitrile/33 mM sodium bicarbonate (v/v) rinse each spatula into their respective tubes to remove any remaining sample.

3.2.9 Pipette the remainder of the 10 mL of 1:1 acetonitrile/33 mM sodium bicarbonate (v/v) into the original tubes containing matrix.

3.2.10 Shake vigorously for 40 minutes at 1200 rpm using a GenoGrinder homogenizer and centrifuge at 4000 rpm for 15 min to form a solid sample pellet.

3.2.11 Transfer supernatant into the original 50 mL graduated centrifuge tube containing the initial liquid extract and vortex to mix the sample.

3.2.12 Precondition 0.45 µm Nylon syringe filter with 1 to 2 mL of 10 mM Ammonium Formate in water.

3.2.13 Filter all samples into individual HPLC vials with the conditioned 0.45 µm Nylon syringe filter, cap vial, and proceed to LC-MS/MS analysis.

3.2.14 Analyze all samples using the parameters shown in Section 3.4.

3.3 Calculation of Orthosulfamuron and its Major Metabolites Concentrations and Recoveries

Generate a calibration curve with each set of samples analyzed by plotting the average response of the peak areas versus the concentration of each standard solution.

Calibration curve equation: $y = mx + b$, where b = intercept, m = slope of the curve, x = sample concentration, y = response of each analytes peak area.

$$\text{Calculated Conc. (ppb)} = \frac{[(\text{Peak Area Response}) - (\text{intercept})]}{\text{Slope of Calibration Curve}} \times DF$$

Where: DF = Dilution Factor

$$\text{Percent Recovery} = \frac{\text{Analyte Conc., ppb}}{\text{Nominal Conc., ppb}} \times 100$$

3.4 Instrument Conditions

Note: The parameters provided below are suggested parameters for the method. These parameters may be adjusted to compensate for column-to-column and system-to-system variability.

LC-MS/MS System Conditions for Analysis of Orthosulfamuron and Metabolites
Liquid Chromatograph (LC): Shimadzu LC-20ADXR HPLC Pumps and CBM-20A Controller
Autosampler: CTC PAL
Valco 2-position switching valve (optional)
Shimadzu CTO-20A Column Oven
Column: Phenomenex Luna C18(2), 150 mm x 2.0 mm ID, 5 µm particle size
Guard Column: C18, 4 mm x 2.0 mm ID
Mass Spectrometer (MS): Sciex Triple Quad LC/MS/MS System
Software: Analyst Software (Version 1.6.1)

LC Conditions

Oven Temperature:	35°C
Initial Flow:	0.250 mL/min
Injection Volume:	25 µL
Mobile Phase:	Organic = 10 mM Ammonium Formate in Water with Acetonitrile (10:90 v/v) containing 0.1% formic Acid Aqueous = 10 mM Ammonium Formate in water with 0.1% formic acid Rinse 1 – Methanol Rinse 2 – Methanol/Water (1:4, v/v)

Gradient Method:

Time (min)	%Aqueous	%Organic	Flow (mL/min)
0.00	100	0	0.250
2.00	100	0	0.250
7.50	10	90	0.250
10.00	10	90	0.250
10.10	100	0	0.250
15.00	100	0	0.250

Approx. Retention Times (min):

O-Desmethyl DOP urea	–	6.8
DBS acid ammonium salt	–	6.9
DB amine	–	7.7
O-Desmethyl Orthosulfamuron	–	7.6
DOP urea	–	7.9
Orthosulfamuron	–	9.3

Autosampler Conditions

Air Volume (µl)	3
Pre Clean with Solvent 1	0
Pre Clean with Solvent 2	0
Pre Clean with Sample	0
Filling Speed (µl/s)	5
Filling Strokes	0
Inject to	LC Vlv1
Injection Speed (µl/s)	5
Pre Inject Delay (ms)	500
Post Inject Delay (ms)	500
Post Clean with Solvent 1	1
Post Clean with Solvent 2	1

Valve Clean with Solvent 1	1
Valve Clean with Solvent 2	1
Replicate Count	1
Analysis Time	15

Mass Spectrometer Conditions for LC-MS/MS Analysis	
Period 1: Analyte:	**O-Desmethyl DOP urea**
Scan Type:	MRM
Ion Source:	Turbospray
Polarity:	Positive
Q1 Mass (amu):	185.000
Q3 Mass (amu):	142.100
Dwell (msec):	20.00
Period Time (min):	0.0 to 6.85
Declustering Potential (DP):	94.0 V
Entrance Potential (EP):	7.0 V
Collision Energy (CE):	23.0 V
Collision Cell Exit Potential (CXP):	6.0 V
Curtain Gas (CUR):	35.0
Temperature (TEM):	550°C
Ion Source Gas 1 (GS1):	35.0
Ion Source Gas 2 (GS2):	55.0
Collision Gas (CAD):	7.0
Ionspray Voltage (IS):	3000 V
Period 2: Analyte:	**DBS acid ammonium salt**
Scan Type:	MRM
Ion Source:	Turbospray
Polarity:	Negative
Q1 Mass (amu):	243.165
Q3 Mass (amu):	80.104
Dwell (msec):	20.00
Period Time (min):	6.85 to 7.10
Declustering Potential (DP):	–40.0 V
Entrance Potential (EP):	–5.0 V
Collision Energy (CE):	–21.0 V
Collision Cell Exit Potential (CXP):	–12.0 V
Curtain Gas (CUR):	35.0
Temperature (TEM):	550°C
Ion Source Gas 1 (GS1):	35.0
Ion Source Gas 2 (GS2):	55.0
Collision Gas (CAD):	7.0
Ionspray Voltage (IS):	–4400 V

Period 3: Analyte:	DB amine	O-Desmethyl Orthosulfamuron	DOP urea	Orthosulfamuron
Scan Type	MRM	MRM	MRM	MRM
Ion Source	Turbospray	Turbospray	Turbospray	Turbospray
Polarity	Positive	Positive	Positive	Positive
Q1 Mass (amu)	164.740	411.074	199.200	425.089
Q3 Mass (amu)	119.800	227.000	156.000	199.000
Dwell (msec)	20.00	20.00	20.00	20.00
Period Time (min)	7.10 to 15.0	7.10 to 15.0	7.10 to 15.0	7.10 to 15.0
Declustering Potential (DP)	55.0 V	51.0 V	60.0 V	56.0 V
Entrance Potential (EP)	7.0 V	3.0 V	5.0 V	4.0 V
Collision Energy (CE)	13.0 V	17.0 V	19.0 V	15.0 V
Collision Cell Exit Potential (CXP)	4.0 V	28.0 V	10.0 V	22.0 V
Curtain Gas (CUR)	35.0	35.0	35.0	35.0
Temperature (TEM)	550.0°C	550.0°C	550.0°C	550.0°C
Ion Source Gas 1 (GS1)	35.0	35.0	35.0	35.0
Ion Source Gas 2 (GS2)	55.0	55.0	55.0	55.0
Collision Gas (CAD)	7.0	7.0	7.0	7.0
Ionspray Voltage (IS)	3000 V	3000 V	3000 V	3000 V

Divert Valve Program: 0.0 to 5.5 min – To Waste
5.5 to 10.5 min – To Instrument
10.5 to 15.0 min – To Waste

Note: The indicated LC-MS/MS parameters are guidelines and should be optimized for the instrument and column actually used. Instrument parameters and mobile phase compositions may be adjusted to improve separation from interfering peaks.

3.5 Revisions

None