1. INTRODUCTION

1.1 Scope of the Method

The validation of the analytical methods were developed to determine the residues of orthosulfamuron and its metabolites DOP urea, DBS amide, DB amine, DOP amine, DBS acid ammonium salt, O-desmethyl DOP urea, and O-desmethyl orthosulfamuron in soil/sediment and water matrices using LC-MS/MS at SynTech Research Laboratory Services (SRLS) in Stilwell, KS. This method was independently validated at ADPEN Laboratories, Inc (ADPEN).

The independent lab validation was conducted using two fortification levels limit of quantitation (0.170 and 0.200 ppb for water and soil) and ten times of limit of quantitation (1.7 and 2.0 ppb for water and soil). For each fortification level and matrix, five replicates were analyzed. Additionally, one reagent blank and two replicates of unfortified samples were examined.

1.2 Principle of the Method

The soil/sediment and water samples (10 g and 10 mL) were fortified and extracted with acetonitrile:33 mM ammonium bicarbonate (7:3, v/v) and thoroughly mixed. An aliquot of resulting solution was analyzed to determine the residues of orthosulfamuron and its metabolites using LC-MS/MS. The transitions for orthosulfamuron and its metabolites were monitored in positive mode for primary and secondary quantification.

1.3 Specificity

To demonstrate the specificity of the analytical method, an additional confirmatory mass transition (secondary) was monitored simultaneous to the primary quantitation transition for analysis of all analytes. Primary and secondary transitions for each analyte are listed below:

Analyte	Transition (*m/z*)	
	Primary	Secondary
Orthosulfamuron	425.14 → 199.40	425.14 → 227.30
DOP urea	199.21 → 100.20	199.21 → 156.30 (water) 199.21 → 181.90 (soil)
DBS amide	244.05 → 120.30	244.05 → 165.30
DB amine	165.24 → 120.20	165.24 → 92.20
DOP amine (First Trial)	156.24 → 82.10	156.24 → 100.00
DOP amine (Second Trial)	156.24 → 124.14	156.24 → 68.04
DBS acid ammonium salt	243.05 → 80.00	243.05 → 198.00
O-desmethyl DOP urea	185.22 → 100.10	185.22 → 68.00
O-desmethyl Orthosulfamuron	411.16 → 227.30	411.16 → 120.20

The method was able to accurately determine residues of orthosulfamuron and its metabolites and no interference was observed at the retention time of the analyte peaks. No matrix suppression or enhancement was found to affect the analytes in soil and water.

2. REFERENCE SUBSTANCE

2.1 Test Systems

The test systems considered in this study were soil/sediment and water.

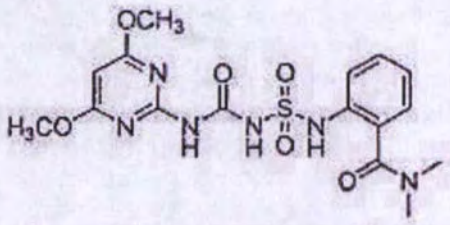
The control soil/sediment and water samples were provided by Waterborne Environmental Inc. The soil/sediment and water samples from the Arkansas test site were sent from Waterborne on October 13, 2014 and received at ADPEN on November 12, 2014. The soil/sediment and water samples from the California test site were sent from Waterborne on November 7, 2014 and received at ADPEN on December 8, 2014. The soil/sediment control samples were collected from the orthosulfamuron California and Arkansas aquatic field (rice paddy) dissipation test sites (Ref. 3). The soil test systems are considered a soil/sediment since the rice paddy cycled from soil to sediment depending on flood stage of the paddy. Upon arrival at the laboratory, the sample was opened, inspected, and checked against enclosed shipping forms. The test systems were received frozen in good condition and stored under frozen conditions at all times, unless necessary for laboratory analysis. The test systems were characterized at AGVISE Laboratories (604 Highway 15 West, Northwood, ND 58267). A copy of the characterization data for the sample is provided in Appendix F.

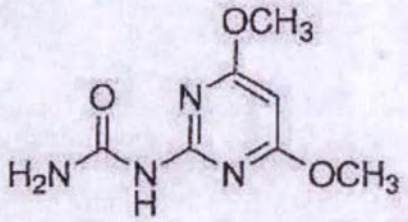
The Laboratory Information Management System (LIMS) provided a unique laboratory analysis code (e.g., 151207004-001) for the soil and water samples, which is cross-referenced on the detailed analytical data reports to the assigned unique sample number.

2.2 Test and Reference Substances

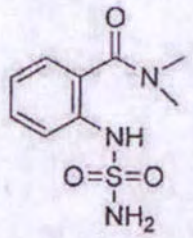
The standard substances were stored in a freezer (≤ −5°C) until use, except DB amine and DOP amine which were stored at room temperature in a storage cabinet. ABC Laboratories, Inc. has retained a reserve sample of this chemical, and has documentation specifying the location of the synthesis and characterization information available at ABC Laboratories, Inc., Columbia, Missouri.

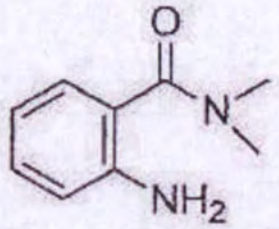
The reference substances of orthosulfamuron, DOP urea, DBS amide, DB amine, DOP amine, DBS acid ammonium salt, O-desmethyl DOP urea, and O-desmethyl orthosulfamuron were provided by ABC Laboratories, Inc. and received on June 6, 2015. The certificates of analysis for all substances are presented in Appendix B. A summary of the reference substances are presented below.

Common Name:	Orthosulfamuron
Lot Number:	81481-1-41-1
CAS Number:	213464-77-8
IUPAC Name:	1-(4,6-Dimethoxy-2-pyrimidinyl)-3-[2-(dimethylcarbamoyl)phenylsulfamoyl]-urea
Molecular Formula:	$C_{16}H_{20}N_6O_6S$
Molecular Weight:	424.43 g/mol
Purity:	99.1%
Expiration Date:	January 20, 2019
Chemical Structure:	

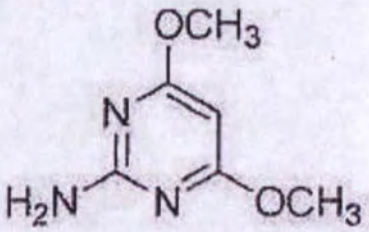
Common Name:	DOP urea (IR 7825)
Lot Number:	81481-1-33-4
CAS Number:	151331-81-6
IUPAC Name:	(4,6-dimethoxy-2-pyrimidinyl)-urea
Molecular Formula:	$C_7H_{10}N_4O_3$
Molecular Weight:	198.18 g/mol
Purity:	99.8%
Expiration Date:	January 20, 2019
Chemical Structure:	

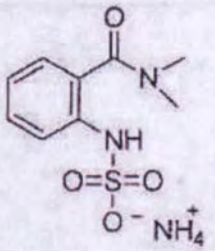
Reference Substances (continued):

Common Name:	DBS amide
Lot Number:	81481-1-16
CAS Number:	N/A
IUPAC Name:	(2-dimethylcarbamoylphenyl)-sulfanamide
Molecular Formula:	$C_9H_{13}N_3O_3S$
Molecular Weight:	243.28 g/mol
Purity:	91.9%
Expiration Date:	January 20, 2019
Chemical Structure:	

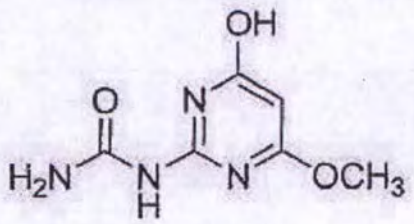
Common Name:	DB amine
Lot Number:	D16M
CAS Number:	6526-66-5
IUPAC Name:	2-Amino-N,N-dimethylbenzamide
Molecular Formula:	$C_9H_{12}N_2O$
Molecular Weight:	164.20 g/mol
Purity:	99.7%
Expiration Date:	January 20, 2019
Chemical Structure:	

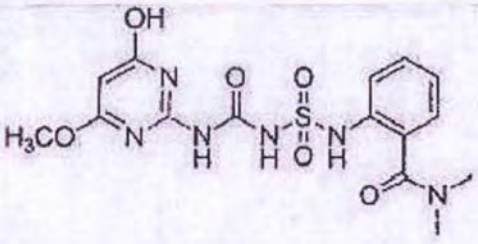
Reference Substances (continued):

Common Name:	DOP amine
Lot Number:	S61826V
CAS Number:	36315-01-2
IUPAC Name:	(2-amino-4,6-dimethoxy)-pyrimidine
Molecular Formula:	$C_6H_9N_3O_2$
Molecular Weight:	155.15 g/mol
Purity:	99.8%
Expiration Date:	January 20, 2019
Chemical Structure:	

Common Name:	DBS acid ammonium salt (IR 7863 ammonium salt)
Lot Number:	81481-1-39-2
CAS Number:	N/A
IUPAC Name:	(2-dimethylcarbamoylphenyl)-sulfamic acid ammonium salt
Molecular Formula:	$C_9H_{15}N_3O_4S$
Molecular Weight:	261.30 g/mol
Purity:	97.8%
Expiration Date:	January 20, 2019
Chemical Structure:	

Reference Substances (continued):

Common Name:	O-desmethyl DOP urea (IR 9512)
Lot Number:	81481-1-72-1
CAS Number:	888225-63-6
IUPAC Name:	N-(4-hydroxy-6-methoxytpyrimidin-2-yl)-urea
Molecular Formula:	$C_6H_8N_4O_3$
Molecular Weight:	184.15 g/mol
Purity:	97.7%
Expiration Date:	January 20, 2019
Chemical Structure:	

Common Name:	O-desmethyl Orthosulfamuron (IR 8181); O-desmethyl IR5878
Lot Number:	81481-65-1
CAS Number:	N/A
IUPAC Name:	1-(4-hydroxy-6-methoxypyrimidin-2-yl)-3-[2-(dimethoxycarbamoyl)phenylsulfamoyl]-urea
Molecular Formula:	$C_{15}H_{18}N_6O_6S$
Molecular Weight:	410.41 g/mol
Purity:	97.6%
Expiration Date:	January 20, 2019
Chemical Structure:	

3. ANALYTICAL METHOD

The analytical methods for water, soil and sediment represented by the method validations, "Analytical Method Validation for the Determination of Orthosulfamuron and its Major Metabolites in Water using LC-MS/MS" and "Analytical Method Validation for the Determination of Orthosulfamuron and its Major Metabolites in Soil and Sediment using LC-MS/MS" were used for the analysis of the samples.

The residues of orthosulfamuron and its metabolites DOP urea, DBS amide, DB amine, DOP amine, DBS acid ammonium salt, O-desmethyl DOP urea, and O-desmethyl orthosulfamuron are extracted from 10 grams of soil by adding 10 mL of 7:3 acetonitrile:33 mM ammonium bicarbonate (v/v) and then 10 mL of 1:1 acetonitrile:33 mM ammonium bicarbonate (v/v). An aliquot (10 mL) is taken and concentrated down to 4.0 mL. The samples are reconstituted to 10 mL with 10mM ammonium bicarbonate and diluted. Diluted samples were transferred to a 2 mL-vial for LC-MS/MS determination. The residues of orthosulfamuron and its metabolites are extracted from 10 mL of water by adding 0.1 mL of 500 mM NH4HCO3 to adjust the pH and mixed by vortex after fortification. An aliquot (0.5 mL) is taken and filtered through a syringe filter into a vial for LC-MS/MS determination. Instrument parameters for the methods are described in Tables 58–59.

The primary (quantitative) and secondary (confirmatory) transition ions, which were monitored in water and soil are presented below:

Analyte	Matrix	Transition (*m/z*)		Ionization Mode	Retention Time (min)
		Primary	Secondary		
Orthosulfamuron	Water	425.14 → 199.4	425.14 → 227.3	Positive	9.8
DOP urea		199.21 → 156.3	199.21 → 100.2		8.6
DBS amide		244.05 → 120.3	244.05 → 165.3		8.0
DB amine		165.24 → 120.2	165.24 → 92.2		8.3
DOP amine (First Trial)		156.24 → 82.1	156.24 → 100.0		8.6
DOP amine (Second Trial)		156.24 → 124.14	156.24 → 68.04		2.3
DBS acid ammonium salt		243.05 → 80.0	243.05 → 198.0	Negative	6.6
O-desmethyl DOP urea		185.22 → 100.1	185.22 → 68.0	Positive	6.3
O-desmethyl Orthosulfamuron		411.16 → 227.3	411.16 → 120.2		8.4

Analyte	Matrix	Transition (*m/z*)		Ionization Mode	Retention Time (min)
		Primary	Secondary		
Orthosulfamuron	Soil	425.1 → 198.9	425.1 → 226.9	Positive	9.8
DOP urea		199.1 → 156.0	199.1 → 181.9		8.6
DB amine		165.1 → 119.9	165.1 → 92.0		8.3
DBS acid ammonium salt		243.05 → 80.0	243.05 → 198.0	Negative	6.6
O-desmethyl DOP urea		185.1 → 100.0	185.1 → 68.0	Positive	6.3
O-desmethyl Orthosulfamuron		411.1 → 226.9	411.1 → 119.9		8.4

7. RECOMMENDATIONS/CONCLUSIONS FROM ILV

This independent laboratory validation was successfully completed on the first trial for all analytes in water, except DOP amine. It was completed on the second trial for all analytes in soil and DOP amine in water; however some changes were made during the ILV. Using the instrument conditions as described in the method did not allow for proper separation of DOP amine from other analytes in water because the compounds share the similar transition ions. A second trial was conducted which used a new gradient along with new mass transitions for DOP amine in water. Therefore, improved chromatography was necessary to resolve the two peaks. Recovery results and statistical data demonstrate the methods can be performed successfully for quantitation of orthosulfamuron, DOP urea, DBS amide, DB amine, DOP amine, DBS acid ammonium salt, O-desmethyl DOP urea and O-desmethyl orthosulfamuron in soil and water.

The method was modified to enhance the separation of DOP amine in water only and recommendations for improvement of the analytical method are presented in Appendix A and it is recommended that they be incorporated into the method.

8. PROTOCOL, AMENDMENTS, AND DEVIATIONS

Three protocol amendments were documented for the independent laboratory validation study.

Amendment 1 addressed six changes:

1. Matrix effects testing will be performed.
2. Standard preparation scheme is suggested, but may be modified as needed.
3. Extract stability within at least 7 days after extraction will be performed.
4. Reference to the method validation report for water was added.
5. Limit of detection (LOD) determination is not necessary.
6. Standard solution (stock, fortification, and calibration) expiration dates will be determined.

Amendment 2 addressed one change:

1. Reference to the method validation report for soil was added.

Amendment 3 addressed one change:

1. Study Monitor signature removal from any changes to the protocol.

Amendment 4 addressed one change:

1. EPA Guideline OPPTS 850.7100 was removed from the protocol guideline requirements.

Amendment 5 addressed one change:

1. The validation sets for soil were fortified at 0.2 and 2.0 ppb instead of 0.17 and 1.7 ppb.

Amendment 6 addressed one change:

1. Corrected the matrix from soil and sediment to soil/sediment.

Amendment 7 addressed one change:

1. Addition of reference standard information for DOP amine from the recertification study.

The changes in all amendments had no negative impact on the study. These changes occurred during the experimental analysis of the first trial.

Figure 49 Residue Calculations for Soil and Water Matrices

Peak integration and quantitation were performed within Analyst® 1.6.2 software; using the calibration curve equation to determine amount of analyte found (ng) during sample analysis. Recovery results and concentration found (ppb) were calculated for each set of samples within LIMS and reported in Microsoft® Office Excel spreadsheet data reports, which are presented in Appendix C.

For the validation recoveries, the exact sample weight was used in calculating the final residues (ppb).

The following equations are used for residue and recovery calculations for orthosulfamuron and its metabolites in water.

a) Calibration curve: $y = mx + b$ Solving for x: $x = \frac{y-bm}{}$

Where, m = slope
b = y-intercept
x = Amount found (ng)
y = Peak area

b) Amount of sample injected (mL)= $\frac{\text{(injection size (mL) × sample wt. (mL))final sample volume (mL)}}{}$

c) Residue found (ppb) = $\frac{\text{Amount found (ng)}}{\text{Amount of sample injected (mL)}}$

d) Recovery (%) = $\frac{\text{Residue in sample (ppb)}}{\text{Amount fortified (ppb)}} \times 100$

As an example, calculations to obtain orthosulfamuron (primary transition) recovery results using 15120903-Recovery1-1 from work order WO-15120903 (water) are shown below:

a) Calibration curve: $y = (8.98e+006)x + 2.33e+003$

Solving for x: $x = \frac{58745 - 2.33e+003}{8.98e+006} = 0.00628311$ ng

b) Amount of sample injected (mL) = $\frac{10.00 \text{ mL} \times 0.04 \text{ mL}}{10.0 \text{ mL}} = 0.04000$ mL

c) Residue found (ppb) = $\frac{0.00628311 \text{ ng}}{0.04000 \text{ mL}} = 0.15708$ ppb

d) Recovery (%) = $\frac{0.15708 \text{ ppb}}{0.170 \text{ ppb}} \times 100 = 92\%$

Statistical treatment of the data included calculation of means, standard deviations (SD), and percent relative standard deviations (%RSD). These calculations were performed using Microsoft® Excel and LIMS software. Results were rounded only for reporting purposes. No calculations were made with rounded numbers.

Appendix A. Recommendations for the Analytical Methodology

The following recommendations should be incorporated into the technical procedure:

1. Section 1.1: Method Summary

   The method was modified to include extraction by employing a bead ruptor. It is recommended that the method be modified to follow these extraction steps shown below.

   i. Thaw soil sample and weigh 10 g + 0.1 g into a PPP centrifuge tube (suitable for BeadRuptor extraction).

   ii. Add 10 mL of (7:3) ACN / 33mM Ammonium Bicarbonate (NH4HCO3) to each sample and tightly cap centrifuge tubes.

   iii. Load sample tubes into BeadRuptor rack and run 1 cycle for 1 minute at 5 m/s.

   iv. Centrifuge sample at 4000 rpm for 15 min or until a solid pellet is formed.

   v. Transfer supernatant into a clean 50 mL graduated centrifuge tube.

   vi. Mesure 10 mL of (1:1) ACN / 33mM Ammonium Bicarbonate (NH4HCO3). Add ~8 mL of (1:1) ACN / 33mM Ammonium Bicarbonate (NH4HCO3) to each sample and vortex to break the pellet. If vortexing doesn't break the pellet, use a spatula to break it, rinsing the spatula into the centrifuge tube with the remaining 2 mL of (1:1) ACN / 33mM Ammonium Bicarbonate (NH4HCO3).

   vii. Load sample tubes back into the BeadRuptor and repeat extraction from Step 4.

   viii. Centrifuge sample at 4000 rpm for 15 min or until a solid pellet is formed.

   ix. Transfer supernatant into graduated centrifuge tube from step 6 and vortex to mix the two extracts.

   x. After sample is homogeneous take a 2 mL aliquot and filter through a 0.45 mm, 25mm Nylon syringe filter discarding ~0.5ml to waste and the remaining 1.5mL filter into an autosample vial for LC-MS/MS analysis.

2. Appendix 2: Example Instrument Conditions

Below is the original gradient:

HPLC Conditions			
Chromatographic System:	Agilent 1290 UPLC		
Column:	Acquity UPLC HSS T3; 1.8 µm, 2.1 × 150 mm; S/N 01693517516076		
Temperature:	50 °C		
Flow rate (µL/min)	600		
Gradient:	Time (min)	Mobile Phase A (%)	Mobile Phase B (%)
	0.00	100.0	0.0
	3.00	100.0	0.0
	5.00	90.0	10.0
	6.00	90.0	10.0
	10.00	5.0	95.0
	12.00	5.0	95.0
	12.10	100.0	0.0
	15.00	100.0	0.0
Mobile Phase A:	10 mM Ammonium Formate in Water wih 0.1% Formic Acid		
Mobile Phase B:	(10:90) 10 mM Ammonium Formate in Water: Acetonitrile with 0.1% Formic Acid		
Injection Volume:	40 µL		

MS/MS Conditions						
Detection System:	AB SCIEX 6500 (Instrument #27)					
Ionization:	Turbo Spray					
Polarity:	Positive					
Curtain gas (CUR):	20.00					
Temperature (TEM):	550 °C					
Collision gas setting (CAD):	9.00					
GS1:	55.00					
GS2:	55.00					
Entrance potential (EP):	10.00					
Scan type:	MRM					
MRM Conditions	Transition (m/z)	Dwell (msec)	DP	CE	CXP	Retention Time (min)
DOP Amine Reg. No. 5824382	156.244 → 100.000	25.00	136.00	23.00	8.00	8.6
	156.244 → 82.100			31.00	14.00	

It was seen during method adaptation that the above gradient produced poor peak shape for DOP Amine. Modifications were made to give better peak shape. The below gradient was used for the analysis of DOP Amine in water (primary and secondary transitions) in trial 2. In order to achieve better separation of DOP Amine from any interference peaks, new transitions for DOP Amine in water was added and a new gradient created. This new gradient and transition is below. A second trial was conducted for the new transition in water only. It is suggested that the gradient and transitions be modified to the following for DOP Amine in water:

HPLC Conditions			
Chromatographic System:	Agilent 1290 UPLC		
Column:	Kinetex PFP; 1.7 µm, 2.1 × 100 mm; S/N 681573-3		
Temperature:	50 °C		
Flow rate (µL/min)	600		
Gradient:	Time (min)	Mobile Phase A (%)	Mobile Phase B (%)
	0.00	100.0	0.0
	0.50	100.0	0.0
	1.00	90.0	10.0
	3.00	90.0	10.0
	3.20	5.0	95.0
	4.20	5.0	95.0
	4.30	100.0	0.0
	6.00	100.0	0.0
Mobile Phase A:	0.1% Formic Acid in Water		
Mobile Phase B:	0.1% Formic Acid in Acetonitrile		
Injection Volume:	40 µL		

MS/MS Conditions						
Detection System:	AB SCIEX 6500 (Instrument #27)					
Ionization:	Turbo Spray					
Polarity:	Positive					
Curtain gas (CUR):	20.00					
Temperature (TEM):	550 °C					
Collision gas setting (CAD):	9.00					
GS1:	55.00					
GS2:	55.00					
Entrance potential (EP):	10.00					
Scan type:	MRM					
MRM Conditions	Transition (m/z)	Dwell (msec)	DP	CE	CXP	Retention Time (min)
DOP Amine Reg. No. 5824382	156.244 → 124.137	25.00	66.00	23.00	14.00	2.3
	156.244 → 68.040			31.00	8.00	