

Analytical method for orthosulfamuron and its metabolites O-desmethyl DOP urea, DBS acid ammonium salt, DB amine, O-desmethyl orthosulfamuron, and DOP urea in soil and sediment

Reports: ECM: EPA MRID No.: 50318401. Vandaveer, W.R. 2016. Analytical Method Validation for the Determination of Orthosulfamuron and its Major Metabolites in Soil and Sediment using LC-MS/MS. Study No.: 292SRLS14R01. Report prepared by SynTech Research Laboratory Services, LLC, Stilwell, Kansas, sponsored and submitted by Nichino America, Inc., Wilmington, Delaware; 201 pages. Final report issued March 23, 2016.

ILV: EPA MRID No. 50318403. Perez, S. 2017. Independent Laboratory Validation of Draft Analytical Method: For the Determination of Orthosulfamuron and its Major Metabolites in Water, Soil and Sediment Using by LC-MS/MS. ADPEN Study No.: 15H0204. Report prepared by ADPEN Laboratories, Inc., Jacksonville, Florida, and sponsored and submitted by Nichino America, Inc., Wilmington, Delaware; 709 pages. Final report issued June 23, 2017.

Document No.: MRIDs 50318401 & 50318403


Guideline: 850.6100


Statements: ECM: The study was conducted in accordance with USEPA FIFRA Good Laboratory Practice (GLP) standards (40 CFR Part 160; p. 3 of MRID 50318401). Signed and dated No Data Confidentiality, GLP, Quality Assurance, and Authenticity statements were provided (pp. 2-5).
ILV: The study was conducted in accordance with USEPA FIFRA GLP standards (p. 2 of MRID 50318403). Signed and dated No Data Confidentiality, GLP, Quality Assurance, and Authenticity statements were provided (pp. 2-5).

Classification: This analytical method is classified as unacceptable. The reproducibility of the method was not validated for DBS acid ammonium salt and DB amine at the LOQ and 10×LOQ due to unacceptable performance data in the ILV and/or ECM. The ECM should be updated to include a statement of the immediate analysis of soil extracts as a critical step, especially in the cases of DBS acid ammonium salt and DB amine. The ILV recommended that extraction employing a bead ruptor is included in the ECM. ILV linearity was not satisfactory for orthosulfamuron and DB amine. ECM linearity was not satisfactory for a majority of the calibration curves of the analytes. The specificity of the method for DBS acid ammonium salt was not supported by ECM or ILV representative chromatograms. The specificity of the method for O-desmethyl DOP urea was not supported by ILV representative chromatograms. ILV representative chromatograms were not provided for one of the two soil matrices. ILV soil matrices were the same as those of the ECM, and a sediment was not included in the ILV. Calculated LODs did not support the method LOD for DBS acid ammonium salt.

PC Code: 108209

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This Data Evaluation Record may have been altered by the Environmental Fate and Effects Division subsequent to signing by CDM/CSS-Dynamac JV personnel. The CDM/CSS-Dynamac Joint Venture role does not include establishing Agency policies.

Executive Summary

This analytical method, SynTech Research Laboratory Services Study No. 292SRLS14R01 (Soil/Sediment), is designed for the quantitative determination of orthosulfamuron and its metabolites O-desmethyl DOP urea, DBS acid ammonium salt, DB amine, O-desmethyl orthosulfamuron, and DOP urea in soil and sediment at the LOQ of 0.20 µg/kg using LC/MS/MS. The ECM and ILV used the same two soil matrices from the orthosulfamuron aquatic field dissipation test sites in Arkansas and California (MRID 50229101; Waterborne Study No. WEI 642.03). The ECM also used a Kansas pond sediment; no sediment was used in the ILV. It could not be determined if the ILV was provided with the most difficult matrices with which to validate the method. In the ECM, all analytes were identified using one ion transition; a confirmation ion transition was added in the ILV. A confirmatory method is not usually required when LC/MS and/or GC/MS are used as the primary methods to generate recovery data. The method was validated by the ILV after the second trial with modifications to the analytical parameters, including the addition of a confirmation ion transition, as well as the concentration of the combined extracts prior to analysis. The ILV recommended that extraction employing a bead ruptor is included in the ECM. However, **the reproducibility of the method was not validated for DBS acid ammonium salt and DB amine at the LOQ and 10×LOQ due to unacceptable performance data in the ILV and/or ECM.** Based on extractability data reported in the ILV, the reviewer believed that the ECM should be updated to include a statement of the immediate analysis of soil extracts as a critical step, especially in the cases of DBS acid ammonium salt and DB amine. All submitted ILV and ECM data pertaining to precision, repeatability, reproducibility, and specificity was acceptable for orthosulfamuron, O-desmethyl orthosulfamuron, and DOP urea. All submitted ILV and ECM data pertaining to precision, repeatability, and reproducibility was acceptable for O-desmethyl DOP urea, except for the ECM LOQ sediment analysis. ILV linearity was not satisfactory for orthosulfamuron and DB amine; ECM linearity was not satisfactory for a majority of the calibration curves of the analytes. The specificity of the method for DBS acid ammonium salt was not supported by ECM or ILV representative chromatograms. The specificity of the method for O-desmethyl DOP urea was not supported by ILV representative chromatograms. ILV representative chromatograms

were not provided for one of the two soil matrices. Calculated LODs did not support the method LOD for DBS Acid Ammonium Salt.

Table 1. Analytical Method Summary

Analyte(s) by Pesticide	MRID		EPA Review	Matrix	Method Date (dd/mm/yyyy)	Registrant	Analysis	Limit of Quantitation (LOQ)
	Environmental Chemistry Method	Independent Laboratory Validation						
Orthosulfamuron	50318401 ¹	50318403 ²		Soil and Sediment	23/03/2016	Nichino America, Inc.	LC/MS/MS	0.20 µg/kg
O-Desmethyl DOP urea								
DBS acid ammonium salt								
DB amine								
O-Desmethyl orthosulfamuron								
DOP urea								

1 In the ECM, the two soils were obtained from the orthosulfamuron aquatic field dissipation test sites in Arkansas and California (MRID 50229101; Waterborne Study No. WEI 642.03; p. 7 of MRID 50318401). The sediment was collected by SynTech Research from an on-site pond located in Stilwell, Kansas. The soil characterization and USDA soil texture classification were not reported in the study report.

2 In the ILV, Arkansas clay loam soil matrix [PA.AR.T.CHAR.0-6²; 20% sand 42% silt 38% clay; pH 6.6 (1:1 soil:water ratio), 1.2% organic matter Walkley-Black] and California sandy clay loam soil matrix [PA.CA.T.CHAR.0-6²; 54% sand 16% silt 30% clay; pH 7.7 (1:1 soil:water ratio), 1.1% organic matter Walkley-Black] were provided by Waterborne Environmental Inc. and were used in the study (USDA soil texture classification; pp. 27, 45; Appendix F, pp. 699-700 of MRID 50318403). The two soils were obtained from the orthosulfamuron aquatic field dissipation test sites in Arkansas and California (MRID 50229101; Waterborne Study No. WEI 642.03). The soil characterization was performed by Agvise Laboratories, Northwood, North Dakota. These two soils appeared to be the same as those reported for the ECM, based on soil sources.

I. Principle of the Method

Soil samples were fortified with orthosulfamuron, DOP urea, DB amine, O-desmethyl orthosulfamuron, DBS acid ammonium salt and O-desmethyl DOP urea (p. 7; Appendix 1, Protocol Amendment 3, pp. 180-188). Samples (10 g) were extracted for 40 minutes at 1200 rpm with 10 mL acetonitrile:33 mM ammonium bicarbonate (7:3, v:v) using a GenoGrinder homogenizer. Following centrifugation (4000 rpm for 15 minutes), the supernatant was decanted into a centrifuge tube. The extraction was repeated with 10 mL acetonitrile:33 mM ammonium bicarbonate (1:1, v:v). The extracts were combined, and a *ca.* 1.5-mL aliquot was filtered through a 0.45 µm Nylon syringe filter into an autosampler vial and analyzed by LC/MS.

Samples were for analytes using a Shimadzu LC-20ADXR HPLC (Phenomenex Luna C18(2) column, 5 µm, 150 x 2.0 mm, and C18 guard column, 4 x 2.0 mm; column temperature 35°C) with a mobile phase gradient of A: 10 mM ammonium formate in water with 0.1% formic acid and B: 10 mM ammonium formate in water:acetonitrile (10:90) with 0.1% formic acid [A:B, 0.00-2.00 min. 100:0, 7.50-10.00 min. 10:90, 10.10-15.00 min. 100:0] coupled with a Sciex Triple Quad LC/MS/MS (550°C) using TurboIonSpray ion source in positive ion mode [O-desmethyl DOP urea (Period 1); DB amine, O-desmethyl orthosulfamuron, DOP urea, and

orthosulfamuron (Period 3)] or negative ion mode [DBS acid ammonium salt (Period 2)] and multiple reaction monitoring (MRM; Appendix 1, Protocol Amendment 3, pp. 189-191). The injection volume was 25 μ L. Analytes were identified using one ion transition: m/z 185.000 \rightarrow 142.100 for O-desmethyl DOP urea, m/z 243.165 \rightarrow 80.104 for DBS acid ammonium salt, m/z 164.740 \rightarrow 119.800 for DB amine, m/z 411.074 \rightarrow 227.000 for O-desmethyl orthosulfamuron, m/z 199.200 \rightarrow 156.000 for DOP urea, and m/z 425.089 \rightarrow 199.000 for orthosulfamuron. Expected retention times were *ca.* 6.8, 6.9, 7.7, 7.6, 7.9, and 9.3 minutes for O-desmethyl DOP urea, DBS acid ammonium salt, DB amine, O-desmethyl orthosulfamuron, DOP urea, and orthosulfamuron, respectively.

In the ILV, the ECM was performed as written with modifications to the analytical parameters, including the addition of a confirmation ion transition, as well as the concentration of the combined extracts prior to analysis (a 10 mL aliquot of the combined extract was concentrated to 4.0 mL and reconstituted to 10 mL using 10 mM ammonium bicarbonate prior to analysis; pp. 26, 32, 44-45; Table 60, p. 107-108 of MRID 50318403). Additionally, a bead ruptor was used for the extraction, and the ILV recommended that the ECM is modified to include extraction employing a bead ruptor (Appendix A, pp. 248-250). For analyte identification of all analytes but DBS acid ammonium salt, an Agilent 1290 UPLC (Acquity HSS T3 column, 2.1 x 150 mm, 1.8 μ m; column temperature 50°C) with a mobile phase gradient of A: 10 mM ammonium formate in water with 0.1% formic acid and B: 10 mM ammonium formate in water:acetonitrile (10:90) with 0.1% formic acid [A:B, 0.0-0.5 min. 100:0, 5.0-6.5 min. 0:100, 6.6-9.0 min. 100:0] coupled to an Agilent 6490 Triple Quad (Instrument #26) MS using ElectroIonSpray (ESI) in positive mode [temperature 150°C (likely erroneous - should be 550°C)] was used. The injection volume was 20 μ L. Analytes were identified using two ion transitions (primary and confirmatory, respectively): m/z 185.07 \rightarrow 100.0 and m/z 185.07 \rightarrow 68.0 for O-desmethyl DOP urea, m/z 165.1 \rightarrow 119.9 and m/z 165.1 \rightarrow 92.0 for DB amine, m/z 411.11 \rightarrow 226.9 and m/z 411.11 \rightarrow 119.9 for O-desmethyl orthosulfamuron, m/z 199.09 \rightarrow 156.0 and m/z 199.09 \rightarrow 181.9 for DOP urea, and m/z 425.13 \rightarrow 198.9 and m/z 425.13 \rightarrow 226.9 for orthosulfamuron. Expected retention times were *ca.* 3.2, 4.0, 3.7, 4.1, and 5.0 minutes for O-desmethyl DOP urea, DB amine, O-desmethyl orthosulfamuron, DOP urea, and Orthosulfamuron, respectively. For analyte identification of DBS acid ammonium salt, an Agilent 1290 UPLC (Acquity HSS T3 column, 2.1 x 150 mm, 1.8 μ m; column temperature 50°C) with a mobile phase gradient of A: 10 mM ammonium formate in water with 0.1% formic acid and B: 10 mM ammonium formate in water:acetonitrile (10:90) with 0.1% formic acid [A:B, 0.00-0.50 min. 100:0, 2.00-3.00 min. 95:5, 3.10-5.00 min. 0:100] coupled to an AB Sciex 6500 (Instrument #27) MS using TurboSpray in negative mode (temperature 550°C) was used. The injection volume was 40 μ L. Analyte was identified using two ion transitions (primary and confirmatory, respectively): m/z 243.06 \rightarrow 80.0 and m/z 243.06 \rightarrow 198.0 for DBS acid ammonium salt. Expected retention time was *ca.* 6.4 minutes.

The Limit of Quantification (LOQ) and Limit of Detection (LOD) for all analytes in soil were 0.20 μ g/kg and 0.035 μ g/kg, respectively, in the ECM and ILV (p. 7 of MRID 50318401; p. 43 of MRID 50318403).

II. Recovery Findings

ECM (MRID 50318401): Mean recoveries and relative standard deviations (RSDs) were within guideline requirements (mean 70-120%; RSD \leq 20%) for analysis of orthosulfamuron, O-desmethyl DOP urea, O-desmethyl orthosulfamuron, and DOP urea at fortification levels of 0.20 $\mu\text{g}/\text{kg}$ (LOQ) and 2.0 $\mu\text{g}/\text{kg}$ (10 \times LOQ) in two soil matrices and one sediment matrix, except for the LOQ analysis of O-desmethyl DOP urea in sediment (RSD 21.1%; pp. 11-12). Mean recoveries and RSDs were within guideline requirements for analysis of DBS acid ammonium salt at fortification levels of 0.20 $\mu\text{g}/\text{kg}$ (LOQ) and 2.0 $\mu\text{g}/\text{kg}$ (10 \times LOQ) in one sediment matrix, but not in the two soil matrices (AR means 123%, CA means 125-137%). Mean recoveries and RSDs were not within guideline requirements for analysis of DB amine at fortification levels of 0.20 $\mu\text{g}/\text{kg}$ (LOQ) and 2.0 $\mu\text{g}/\text{kg}$ (10 \times LOQ) in two soil matrices (AR means 40.4-48.5%, CA means 40.1-41.2%, CA LOQ RSD 20.1%) and one sediment matrix (means 38.6-44.8%). All analytes were identified using one ion transition. A confirmatory method is not usually required when LC/MS and/or GC/MS are used as the primary methods to generate recovery data. Recovery results were corrected when residues were quantified in the controls; recovery residues of DOP urea in AR soil were corrected since residues were quantified in the controls (pp. 10, 13-21). Both soils were obtained from the orthosulfamuron aquatic field dissipation test sites in Arkansas and California (MRID 50229101; Waterborne Study No. WEI 642.03; p. 7). The sediment was collected by SynTech Research from an on-site pond located in Stilwell, Kansas. The soil characterization and USDA soil texture classification were not reported in the study report.

ILV (MRID 50318403): Mean recoveries and RSDs were within guideline requirements for analysis of orthosulfamuron, O-desmethyl DOP urea, DBS acid ammonium salt, DB amine, O-desmethyl orthosulfamuron, and DOP urea at fortification levels of 0.20 $\mu\text{g}/\text{kg}$ (LOQ) and 2.0 $\mu\text{g}/\text{kg}$ (10 \times LOQ) in two soil matrices, except for the LOQ quantitation analysis of DBS acid ammonium salt in both soils (AR/CA means 121%; pp. 15-18). Analytes were identified using two ion transitions. Performance data (recovery results) from quantitation and confirmation analyses were comparable, although RSDs of the confirmation analysis tended to be higher than those of the quantitation analysis. Arkansas clay loam soil matrix [PA.AR.T.CHAR.0-6"; 20% sand 42% silt 38% clay; pH 6.6 (1:1 soil:water ratio), 1.2% organic matter Walkley-Black] and California sandy clay loam soil matrix [PA.CA.T.CHAR.0-6"; 54% sand 16% silt 30% clay; pH 7.7 (1:1 soil:water ratio), 1.1% organic matter Walkley-Black] were provided by Waterborne Environmental Inc. and were used in the study (USDA soil texture classification; pp. 27, 45; Appendix F, pp. 699-700 of MRID 50318403). The two soils were obtained from the orthosulfamuron aquatic field dissipation test sites in Arkansas and California (MRID 50229101; Waterborne Study No. WEI 642.03). The soil characterization was performed by Agvise Laboratories, Northwood, North Dakota. These two soils appeared to be the same as those reported for the ECM, based on soil sources. The method was validated after the second trial with modifications to the analytical parameters, including the addition of a confirmation ion transition, as well as the concentration of the combined extracts prior to analysis (pp. 26, 32, 44-45; Table 60, p. 107-108). Additionally, a bead ruptor was used for the extraction, and the ILV recommended that the ECM is modified to include extraction employing a bead ruptor (Appendix A, pp. 248-250).

Table 2. Initial Validation Method Recoveries for Orthosulfamuron and its Metabolites O-Desmethyl DOP Urea, DBS Acid Ammonium Salt, DB Amine, O-Desmethyl Orthosulfamuron, and DOP Urea in Soil and Sediment^{1,2}

Analyte	Fortification Level (µg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Arkansas Soil						
Orthosulfamuron	0.20	8	84.0-98.0	89.9	4.3	4.8
	2.0	6	77.0-83.5	81.9	2.6	3.2
O-Desmethyl DOP Urea	0.20	8	72.0-107	92.3	10.5	11.4
	2.0	6	77.0-91.0	82.7	5.2	6.3
DBS Acid Ammonium Salt	0.20	8	85.5-147	123	18.5	15.1
	2.0	6	120-128	123	3.5	2.8
DB Amine	0.20	8	42.7-54.0	48.5	4.2	8.7
	2.0	6	33.5-45.5	40.4	4.3	10.6
O-Desmethyl Orthosulfamuron	0.20	8	78.0-106	89.3	8.2	9.2
	2.0	6	76.0-89.0	83.6	5.2	6.2
DOP Urea	0.20	8	94.7-107	99.4	4.2	4.2
	2.0	6	89.4-97.9	94.1	3.4	3.6
California Soil						
Orthosulfamuron	0.20	8	65.5-84.0	77.4	6.0	7.8
	2.0	6	65.5-82.5	76.7	6.2	8.1
O-Desmethyl DOP Urea	0.20	8	63.5-80.0	71.8	5.2	7.2
	2.0	6	68.5-77.0	74.1	3.2	4.3
DBS Acid Ammonium Salt	0.20	8	122-145	137	8.4	6.1
	2.0	6	117-132	125	4.9	3.9
DB Amine	0.20	8	31.6-54.5	41.2	8.3	20.1
	2.0	6	33.8-46.8	40.2	5.3	13.2
O-Desmethyl Orthosulfamuron	0.20	8	71.0-90.5	81.9	7.1	8.7
	2.0	6	68.0-92.0	81.9	8.2	10.0
DOP Urea	0.20	8	77.5-117	97.0	13.1	13.5
	2.0	6	76.5-98.0	89.0	7.4	8.3
Kansas Pond Sediment						
Orthosulfamuron	0.20	8	77.0-82.5	80.7	2.0	2.5
	2.0	6	73.5-77.5	75.6	1.9	2.5
O-Desmethyl DOP Urea	0.20	8	61.5-101	77.9	16.4	21.1
	2.0	6	68.5-74.0	71.4	2.2	3.1
DBS Acid Ammonium Salt	0.20	8 ³	48-87.0	71	12	17
	2.0	6	71.0-82.0	75.8	3.6	4.7
DB Amine	0.20	8	41.9-49.1	44.8	2.7	6.0
	2.0	6	33.2-44.7	38.6	4.3	11.1
O-Desmethyl Orthosulfamuron	0.20	8	72.0-87.5	78.7	4.8	6.1
	2.0	6	69.5-79.5	73.3	3.8	5.2
DOP Urea	0.20	8	79.0-89.0	84.4	3.9	4.6
	2.0	6	73.0-80.5	77.0	3.1	4.0

Data (recovery results were corrected when residues were quantified in the controls, pp. 10, 13-21) were obtained from pp. 11-12 of MRID 50318401 and DER Attachment 2 (p. 45).

¹ Both soils were obtained from the orthosulfamuron aquatic field dissipation test sites in Arkansas and California (MRID 50229101; Waterborne Study No. WEI 642.03; p. 7). The sediment was collected by SynTech Research

from an on-site pond located in Stilwell, Kansas. The soil characterization and USDA soil texture classification were not reported in the study report.

- 2 Analytes were identified using one ion transition: m/z 185.000→142.100 for O-desmethyl DOP urea, m/z 243.165→80.104 for DBS acid ammonium salt, m/z 164.740→119.800 for DB amine, m/z 411.074→227.000 for O-desmethyl orthosulfamuron, m/z 199.200→156.000 for DOP urea, and m/z 425.089→199.000 for orthosulfamuron.
- 3 Reported statistics were reviewer-calculated using 8 recovery data values; rules of significant figures were followed (see DER Attachment 2). Statistics provided in the study report used only 7 recovery data values (62.0-87.0%, $74.4 \pm 8.7\%$, RSD 11.7%).

Table 3. Independent Validation Method Recoveries for Orthosulfamuron and its Metabolites O-Desmethyl DOP Urea, DBS Acid Ammonium Salt, DB Amine, O-Desmethyl Orthosulfamuron, and DOP Urea in Soil^{1,2}

Analyte	Fortification Level (µg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Arkansas Clay Loam Soil						
Quantitation Ion Transition						
Orthosulfamuron	0.20	5	93-112	104	7.0	6.7
	2.0	5	92-99	97	2.6	2.7
O-Desmethyl DOP Urea	0.20	5	96-144	118	17.9	15.2
	2.0	5	86-107	100	8.6	8.6
DBS Acid Ammonium Salt	0.20	5	109-134	121	9.6	8.0
	2.0	5	104-119	113	5.9	5.2
DB Amine	0.20	5	92-106	98	6.5	6.6
	2.0	5	99-104	101	1.9	1.9
O-Desmethyl Orthosulfamuron	0.20	5	108-116	112	4.3	3.9
	2.0	5	98-119	105	4.7	4.5
DOP Urea	0.20	5	82-92	87	4.6	5.3
	2.0	5	99-111	106	4.8	4.5
Confirmation Ion Transition						
Orthosulfamuron	0.20	5	94-112	107	7.6	7.1
	2.0	5	87-95	92	2.8	3.1
O-Desmethyl DOP Urea	0.20	5	83-105	94	9.6	10.2
	2.0	5	82-115	98	12.8	13.0
DBS Acid Ammonium Salt	0.20	5	107-123	117	6.4	5.5
	2.0	5	107-111	110	3.6	3.3
DB Amine	0.20	5	101-124	111	9.5	8.6
	2.0	5	99-115	110	6.3	5.8
O-Desmethyl Orthosulfamuron	0.20	5	103-122	110	7.5	6.9
	2.0	5	100-114	108	5.4	5.0
DOP Urea	0.20	5	94-112	105	7.1	6.8
	2.0	5	105-113	110	3.4	3.1
California Sandy Clay Loam Soil						
Quantitation Ion Transition						
Orthosulfamuron	0.20	5	87-111	96	9.1	9.4
	2.0	5	81-96	89	6.1	6.9
O-Desmethyl DOP Urea	0.20	5	81-91	88	3.8	4.4
	2.0	5	79-88	85	3.5	4.1
DBS Acid Ammonium Salt	0.20	5	113-134	121	8.0	6.6
	2.0	5	103-115	109	4.4	4.0
DB Amine	0.20	5	109-117	111	3.4	3.1
	2.0	5	99-105	102	2.2	2.2
O-Desmethyl Orthosulfamuron	0.20	5	112-119	116	3.2	2.8
	2.0	5	101-118	113	6.9	6.1
DOP Urea	0.20	5	84-108	98	9.1	9.3
	2.0	5	93-105	98	5.5	5.6

Analyte	Fortification Level (µg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Confirmation Ion Transition						
Orthosulfamuron	0.20	5	83-112	97	10.8	11.2
	2.0	5	81-97	87	6.3	7.2
O-Desmethyl DOP Urea	0.20	5	63-105	91	16.0	17.7
	2.0	5	77-109	88	13.0	14.8
DBS Acid Ammonium Salt	0.20	5	111-122	118	4.4	3.7
	2.0	5	105-113	108	3.2	2.9
DB Amine	0.20	5	100-115	108	7.2	6.7
	2.0	5	102-111	105	3.2	3.0
O-Desmethyl Orthosulfamuron	0.20	5	101-124	114	9.3	8.2
	2.0	5	85-116	107	12.9	12.1
DOP Urea	0.20	5	102-119	113	6.7	6.0
	2.0	5	104-107	106	1.3	1.2

Data (uncorrected recovery results, Tables 33-56, pp. 79-102; Figure 49, p. 246) were obtained from pp. 15-18 of MRID 50318403.

- 1 Arkansas clay loam soil matrix [PA.AR.T.CHAR.0-6"; 20% sand 42% silt 38% clay; pH 6.6 (1:1 soil:water ratio), 1.2% organic matter Walkley-Black] and California sandy clay loam soil matrix [PA.CA.T.CHAR.0-6"; 54% sand 16% silt 30% clay; pH 7.7 (1:1 soil:water ratio), 1.1% organic matter Walkley-Black] were provided by Waterborne Environmental Inc. and were used in the study (USDA soil texture classification; pp. 27, 45; Appendix F, pp. 699-700 of MRID 50318403). The two soils were obtained from the orthosulfamuron aquatic field dissipation test sites in Arkansas and California (MRID 50229101; Waterborne Study No. WEI 642.03). The soil characterization was performed by Agvise Laboratories, Northwood, North Dakota. These two soils appeared to be the same as those reported for the ECM, based on soil sources.
- 2 Analytes were identified using two ion transitions (primary and confirmatory, respectively): m/z 185.07→100.0 and m/z 185.07→68.0 for O-desmethyl DOP urea, m/z 165.1→119.9 and m/z 165.1→92.0 for DB amine, m/z 411.11→226.9 and m/z 411.11→119.9 for O-desmethyl orthosulfamuron, m/z 199.09→156.0 and m/z 199.09→181.9 for DOP urea, m/z 425.13→198.9 and m/z 425.13→226.9 for Orthosulfamuron, and m/z 243.06→80.0 and m/z 243.06→198.0 for DBS acid ammonium salt.

III. Method Characteristics

The LOQ for soil was 0.20 µg/kg in the ECM and ILV (pp. 7, 22-31 of MRID 50318401; pp. 43-44 of MRID 50318403). No justification of the LOQ was reported in the ECM; in the ILV, the LOQ was defined as the lowest fortification level. The LOD was reported as 0.035 µg/kg in the ECM and ILV. In the ECM, the LOD was defined as the lowest analyte concentration for a measurement statistically different from the response in the control sample. The LOD was also calculated for each analyte/matrix using the following equation:

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

Where, $t_{0.99}$ is the one-tailed t statistic for $n = 8$ (2.998) and SD is the standard deviation of the analyte recovery measurements at the target LOQ. The calculated LODs are reported in the table below and supported the method LOD for all analytes in at least one matrix, except DBS Acid Ammonium Salt. In the ILV, the LOD was set at 18% of the LOQ and defined as the absolute amount of analyte (0.00140 ng) injected into the LC/MS/MS using the lowest calibration standard; no calculations for the LOD were provided in the ILV.

Table 4. Method Characteristics

Analyte		Orthosulfamuron	O-Desmethyl DOP Urea	DBS Acid Ammonium Salt	DB Amine	O-Desmethyl Orthosulfamuron	DOP Urea	
Limit of Quantitation (LOQ)		0.20 µg/kg						
Limit of Detection (LOD)	ECM	Method	0.035 µg/kg					
		Calculated	0.0119- 0.0360 µg/kg	0.0311- 0.0982 µg/kg	0.0509-0.111 µg/kg	0.0162- 0.0496 µg/kg	0.0287- 0.0481 µg/kg	0.0232- 0.0783 µg/kg
	ILV	0.035 µg/kg						
Linearity (calibration curve r ² and concentration range) ¹	ECM	AR Soil	r ² = 0.9936	r ² = 0.9912	r ² = 0.9926	r ² = 0.9960	r ² = 0.9932	r ² = 0.9930
		CA Soil	r ² = 0.9930	r ² = 0.9978	r ² = 0.9958	r ² = 0.9924	r ² = 0.9912	r ² = 0.9948
		Sediment	r ² = 0.9980	r ² = 0.9972	r ² = 0.9932	r ² = 0.9960	r ² = 0.9976	r ² = 0.9980
		Range	0.098-2.976 µg/kg					
	ILV	r ² = 0.9924 (Q) r ² = 0.9976 (C)	r ² = 0.9992 (Q) r ² = 0.9998 (C)	r ² = 0.9990 (Q & C)	r ² = 0.9918 (Q) r ² = 0.9956 (C)	r ² = 0.9984 (Q) r ² = 0.9974 (C)	r ² = 0.9976 (Q) r ² = 0.9980 (C)	
Concentration range		0.035-10 ng/mL						
Repeatable	ECM ^{2,3}	Yes at LOQ and 10×LOQ (soils and sediment).	Yes at LOQ and 10×LOQ (soils). No at LOQ (RSD 21.1%); Yes at 10×LOQ (sediment).	Yes at LOQ and 10×LOQ (sediment). No at LOQ and 10×LOQ (soils; means 123-137%).	No at LOQ and 10×LOQ [soils (means 40.1- 48.5% ; CA LOQ RSD 20.1%) and sediment (means 38.6-44.8%)].	Yes at LOQ and 10×LOQ (soils and sediment).		
	ILV ^{2,4,5}	Yes at LOQ and 10×LOQ (soils).		No at LOQ (means 121%); Yes at 10×LOQ (soils).	Yes at LOQ and 10×LOQ (soils).			
Reproducible ⁶		Yes at LOQ and 10×LOQ.		No at LOQ; Yes at 10×LOQ.	No at LOQ and 10×LOQ.	Yes at LOQ and 10×LOQ.		
Specificity	ECM	Yes, matrix interferences were <i>ca.</i> 0-2% of the LOQ (based on peak area).	Yes, matrix interferences were <i>ca.</i> 0-4% of the LOQ (based on peak area). Analyte peak was small compared to baseline noise and not well-resolved.	No , no matrix interferences were observed, but analyte peak was completely unresolved. ⁷	Yes, no matrix interferences were observed. Some minor peak splitting was observed.	Yes, matrix interferences were <i>ca.</i> 0-3% of the LOQ (based on peak area). Some minor peak splitting was observed.	Yes, matrix interferences were <i>ca.</i> 0-3% of the LOQ (based on peak area).	

	ILV	Yes, no matrix interferences were observed.	No , matrix interferences were <i>ca.</i> 34% of the LOQ (based on peak area). ⁸ Major baseline noise interference with analyte peak was observed. Significant non-uniform peak integration was noted.	No , matrix interferences were <i>ca.</i> 3-4% of the LOQ (based on peak area), but major interference with analyte peak (RT 6.33-6.36 min.) was observed by a nearby contaminant (RT 6.40 min.). ⁹ Significant non-uniform peak integration was noted.	Yes, matrix interferences were <i>ca.</i> 16% (Q) and 33% (C) of the LOQ (based on peak area).	Yes, matrix interferences were <i>ca.</i> 5% of the LOQ (based on peak area).	Yes, matrix interferences were <i>ca.</i> 12-13% of the LOQ (based on peak area).
Representative chromatograms from only one soil matrix (AR) were provided for review.							

Data were obtained from pp. 7, 22-31; pp. 10-21 (recovery data); pp. 32-49 (calibration curve); pp. 50-145 (chromatograms) of MRID 50318401; pp. 43-45; pp. 15-18 and Tables 33-56, pp. 79-102 (recovery data); Figures 1-8, pp. 110-117 (calibration curves); Figures 9-16, pp. 118-189 (calibration chromatograms); Figures 35-48, pp. 222-245 (sample chromatograms) of MRID 50318403; DER Attachment 2. AR = Arkansas; CA = California. Q = Quantitation ion transition; C = Confirmatory ion transition.

- 1 Reported correlation coefficients were reviewer-calculated from r values reported in the study report (pp. 32-49 of MRID 50318401; Figures 1-8, pp. 110-117 of MRID 50318403; DER Attachment 2). Matrix-matched standards were used in the ECM (p. 9 of MRID 50318401); solvent standards were used in the ILV (p. 40 of MRID 50318403).
- 2 In the ECM, analytes were identified using one ion transition; a confirmatory method is not usually required when LC/MS and/or GC/MS are used as the primary methods to generate recovery data. In the ILV, analytes were identified using two ion transitions (quantitation and confirmation).
- 3 In the ECM, the two soils were obtained from the orthosulfamuron aquatic field dissipation test sites in Arkansas and California (MRID 50229101; Waterborne Study No. WEI 642.03; p. 7 of MRID 50318401). The sediment was collected by SynTech Research from an on-site pond located in Stilwell, Kansas. The soil characterization and USDA soil texture classification were not reported in the study report.
- 4 In the ILV, Arkansas clay loam soil matrix [PA.AR.T.CHAR.0-6"; 20% sand 42% silt 38% clay; pH 6.6 (1:1 soil:water ratio), 1.2% organic matter Walkley-Black] and California sandy clay loam soil matrix [PA.CA.T.CHAR.0-6"; 54% sand 16% silt 30% clay; pH 7.7 (1:1 soil:water ratio), 1.1% organic matter Walkley-Black] were provided by Waterborne Environmental Inc. and were used in the study (USDA soil texture classification; pp. 27, 45; Appendix F, pp. 699-700 of MRID 50318403). The two soils were obtained from the orthosulfamuron aquatic field dissipation test sites in Arkansas and California (MRID 50229101; Waterborne Study No. WEI 642.03). The soil characterization was performed by Agvise Laboratories, Northwood, North Dakota. These two soils appeared to be the same as those reported for the ECM, based on soil sources.
- 5 The ILV validated the ECM method after the second trial with modifications to the analytical parameters, including the addition of a confirmation ion transition, as well as the concentration of the combined extracts prior to analysis (pp. 26, 32, 44-45; Table 60, p. 107-108 of MRID 50318403). Additionally, a bead ruptor was used for the extraction, and the ILV recommended that the ECM is modified to include extraction employing a bead ruptor (Appendix A, pp. 248-250).

6 Reviewer-determined based on the results of the 3 matrices of the ECM and 2 matrices of the ILV.

7 Based on pp. 64-65, 104-105 of MRID 50318401.

8 Based on Figure 35, p. 226, Figure 36, p. 232, and Figures 45-46, pp. 242-243 of MRID 50318403.

9 Based on Figure 35, p. 225, Figure 36, p. 231, and Figures 43-44, pp. 240-241 of MRID 50318403.

Linearity is satisfactory when $r^2 \geq 0.995$.

IV. Method Deficiencies and Reviewer's Comments

1. The reproducibility of the method was not validated for DBS acid ammonium salt and DB amine at the LOQ and 10×LOQ. For DBS acid ammonium salt, satisfactory recoveries were only observed in the ECM sediment and the 10×LOQ analyses in the ILV soils. For DB amine, satisfactory recoveries were only observed in the ILV soils.
2. ILV performance data was not satisfactory for the LOQ quantitation analysis of DBS acid ammonium salt in both soils (AR/CA means 121%; pp. 15-18 of MRID 50318403).

ECM performance data was not satisfactory for the LOQ analysis of O-desmethyl DOP urea in sediment (RSD 21.1%), for the LOQ and 10×LOQ analyses of DBS acid ammonium salt in the two soil matrices (AR means 123%, CA means 125-137%), and for the LOQ and 10×LOQ analyses of DB amine in two soil matrices (AR means 40.4-48.5%, CA means 40.1-41.2%, CA LOQ RSD 20.1%) and one sediment matrix (means 38.6-44.8%; pp. 11-12 of MRID 50318401).

OCSPP guideline requirements state that the mean recovery is 70-120% and the RSD is $\leq 20\%$.

The reviewer noted that, in the ILV, the extract stability was reported as 8 days of refrigeration for the soil extracts, except for DBS acid ammonium salt and DB amine (p. 41 of MRID 50318403). DBS acid ammonium salt showed increases in recovery by *ca.* 31% at 10×LOQ, while DB amine showed decreases in recovery by *ca.* 39% at the LOQ. These instability findings could explain the high recoveries (>120%) of DBS acid ammonium salt and the low recoveries (<50%) of DB amine which occurred in the ECM if the soil extracts were not analyzed immediately. In the ILV, in which the instability was noted, DB amine recoveries were acceptable, and DBS acid ammonium salt recoveries were just out-of-range (mean, 121%). Therefore, the reviewer believed that the **ECM should be updated to include a statement of the immediate analysis of soil extracts as a critical step, especially in the cases of DBS acid ammonium salt and DB amine.**

ILV recommendations for the ECM were reported in Appendix A, pp. 248-250 of MRID 50318403. The ILV recommended that extraction employing a bead ruptor is included in the ECM.

3. In the ILV, the linearity was not satisfactory for the quantitation ion analysis of orthosulfamuron, $r^2 = 0.9924$, and DB amine, $r^2 = 0.9918$ (Figures 9-16, pp. 118-189 of MRID 50318403; DER Attachment 2).

In the ECM, the linearity was not satisfactory for the Arkansas soil analysis of all analytes, but DB amine: $r^2 = 0.9912-0.9936$ (pp. 32-49 of MRID 50318401; DER Attachment 2). The linearity was not satisfactory for the California soil analysis of all analytes, but O-desmethyl DOP urea and DBS acid ammonium salt: $r^2 = 0.9912-0.9948$.

The linearity was not satisfactory for the sediment analysis of DBS acid ammonium salt: $r^2 = 0.9932$.

Linearity is satisfactory when $r^2 \geq 0.995$.

4. The specificity of the method for DBS acid ammonium salt was not supported by ECM or ILV representative chromatograms. In the ILV chromatograms, major interference with the analyte peak (RT 6.33-6.36 min.) was observed by a nearby contaminant (RT 6.40 min.; Figure 35, p. 225, Figure 36, p. 231, and Figures 43-44, pp. 240-241 of MRID 50318403). Additionally, significant non-uniform peak integration was noted. In the ECM chromatograms, the analyte peak was completely unresolved (pp. 64-65, 104-105 of MRID 50318401).

The specificity of the method for O-desmethyl DOP urea was not supported by ILV representative chromatograms. In the ILV chromatograms, matrix interferences were *ca.* 34% of the LOQ (based on peak area; Figure 35, p. 226, Figure 36, p. 232, and Figures 45-46, pp. 242-243 of MRID 50318403). Major baseline noise interference with analyte peak was observed, and significant non-uniform peak integration was noted. In the ECM chromatograms, matrix interferences were insignificant, but it was noted that the analyte peak was small compared to baseline noise and not well-resolved (pp. 50-145 of MRID 50318401).

5. ILV representative chromatograms of the California soil were not provided. Chromatograms from all fortifications and matrices should be provided for review to assess the specificity of the method.
6. The ECM and ILV soil matrices were the same, based on soil sources. The two ECM uncharacterized soils were reportedly obtained from the orthosulfamuron aquatic field dissipation test sites in Arkansas and California (MRID 50229101; Waterborne Study No. WEI 642.03; p. 7 of MRID 50318401). The two ILV characterized soils were also obtained from the orthosulfamuron aquatic field dissipation test sites in Arkansas and California (MRID 50229101; Waterborne Study No. WEI 642.03; pp. 27, 45; Appendix F, pp. 699-700 of MRID 50318403). The soils were representative of those used in field dissipation studies, but it could not be determined if the ILV was provided with the most difficult matrix with which to validate the method. Also, the ECM included a local sediment while no sediment was included in the ILV.
7. The estimation of LOQ and LOD in ECM and ILV was not based on scientifically acceptable procedures as defined in 40 CFR Part 136 (pp. 7, 22-31 of MRID 50318401; pp. 43-44 of MRID 50318403). No justification of the LOQ was reported in the ECM; in the ILV, the LOQ was defined as the lowest fortification level. The LOD was reported as 0.035 $\mu\text{g}/\text{kg}$ in the ECM and ILV. In the ECM, the LOD was defined as the lowest analyte concentration for a measurement statistically different from the response in the control sample. The LOD was also calculated for each analyte/matrix using the following equation: $\text{LOD} = (t_{0.99} \times \text{SD})$, where, $t_{0.99}$ is the one-tailed t statistic for $n = 8$ (2.998) and SD is the standard deviation of the analyte recovery measurements at the target LOQ.

The calculated LODs are reported in the table below and supported the method LOD for all analytes in at least one matrix, except DBS Acid Ammonium Salt. In the ILV, the LOD was set at 18% of the LOQ and defined as the absolute amount of analyte (0.00140 ng) injected into the LC/MS/MS using the lowest calibration standard; no calculations for the LOD were provided in the ILV. Detection limits should not be based on arbitrary values.

8. In the ECM, recovery results were corrected when residues were quantified in the controls; recovery residues of DOP urea in AR soil were corrected since residues were quantified in the controls (pp. 10, 13-21 of MRID 50318401).
9. The communication between the ILV testing facility and the method developer or ECM was not detailed (p. 45 of MRID 50318403).
10. The ILV trials required to validate the method was reported as the “first” and the “second” in the study report (pp. 37-40, 44-45 of MRID 50318403). The reviewer reported the validation as the second trial since it was repeated.
11. The ECMs for soil and water (MRIDs 50318401 & 50318402) were both designated as SynTech Research Laboratory Services Study No. 292SRLS14R01 (p. 1 of MRID 50318401; p. 1 of MRID 50318402).
12. In the ECM, matrix-matched standards were used (p. 9 of MRID 50318401). Solvent standards were used in the ILV (pp. 40, 45 of MRID 50318403). The ILV reported that the matrix effects were evaluated and found to be insignificant, but cited the ECMs for water and soil (MRIDs 50318401 & 50318402; p. 45).
13. In the ILV, the extract stability was reported as 8 days of refrigeration for the soil extracts, except for DBS acid ammonium salt and DB amine (p. 41 of MRID 50318403).
14. In the ECM, the time required to complete the extraction of one set of 13 samples required *ca.* 8 hours of work, including calculation of results (p. 43 of MRID 50318403).

V. References

- U.S. Environmental Protection Agency. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.
- 40 CFR Part 136. Appendix B. Definition and Procedure for the Determination of the Method Detection Limit-Revision 1.11, pp. 317-319.

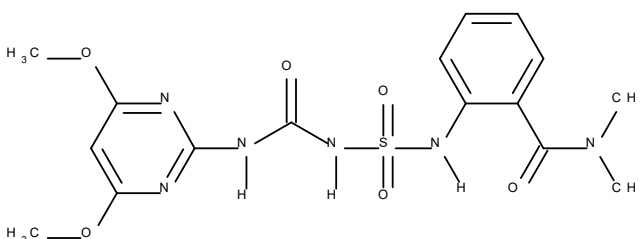
Attachment 1: Chemical Names and Structures**Orthosulfamuron (IR5878)**

IUPAC Name: 1-(4,6-Dimethoxypyrimidin-2-yl)-3-[2-(dimethylcarbamoyl)phenyl sulfamoyl]urea

CAS Name: 2-[[[[(4,6-Dimethoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]amino]-N,N-dimethylbenzamide

CAS Number: 213464-77-8

SMILES String: [H]N(c1cccc1C(=O)N(C)C)S(=O)(=O)N([H])C(=O)N([H])c2nc(cc(n2)OC)OC

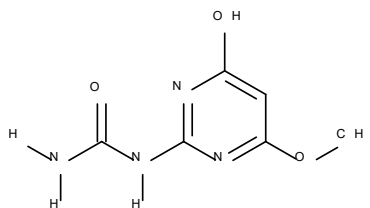
**O-Desmethyl DOP Urea**

IUPAC Name: N-(4-hydroxy-6-methoxypyrimidin-2-yl)-urea

CAS Name: Not reported

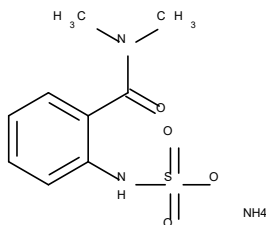
CAS Number: 888225-63-6

SMILES String: COc1cc(O)nc(NC(=O)N)n1

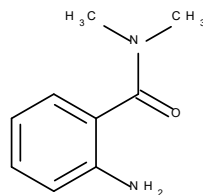


DBS Acid Ammonium Salt (IR7863 Ammonium Salt)

IUPAC Name: (2-Dimethylcarbamoylphenyl)-sulfamic acid ammonium salt
CAS Name: Not reported
CAS Number: Not reported
SMILES String: N.CN(C)C(=O)c1ccccc1NS(=O)(=O)O

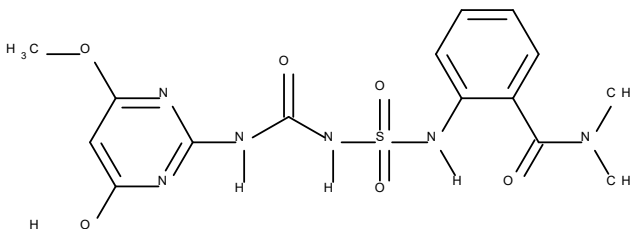
**DB Amine**

IUPAC Name: 2-Amino-N,N-dimethyl-benzamide
CAS Name: Not reported
CAS Number: 6526-66-5
SMILES String: CN(C)C(=O)c1ccccc1N



O-Desmethyl Orthosulfamuron (IR8181)

IUPAC Name: 1-(4-hydroxy-6-methoxypyrimidin-2-yl)-3-[2-(dimethylcarbamoyl)phenylsulfamoyl]urea
CAS Name: Not reported
CAS Number: Not reported
SMILES String: [H]N(c1cccc1C(=O)N(C)C)S(=O)(=O)N([H])C(=O)N([H])c2nc(cc(n2)OC)O[H]

**DOP Urea (IR7825)**

IUPAC Name: N-(4,6-Dimethoxypyrimidin-2-yl)urea
CAS Name: (4,6-Dimethoxy-2-pyrimidinyl)urea
CAS Number: 151331-81-6
SMILES String: [H]N([H])C(=O)N([H])c1nc(cc(n1)OC)OC

