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

Reports:	ECM: EPA MRID No.: 50318402. Johnson, L., and W.R. Vandaveer. 2016. Analytical Method Validation for the Determination of Orthosulfamuron and its Major Metabolites in Water 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; 198 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 50318402 & 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 50318402). 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. An updated ECM should be submitted to include the ILV modifications for improved chromatography for DOP amine. ECM performance data was not satisfactory for the LOQ analysis of DOP amine in AR water. ILV linearity was not satisfactory for orthosulfamuron, DBS amide, 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 representative chromatograms. The specificity of the method for DB amine and DOP urea was not supported by ILV representative chromatograms. ILV representative chromatograms were not provided for one of the two water matrices. ILV water matrices were the same as those of the ECM. Calculated LODs did not support the method LOD for DOP amine.
PC Code:	108209

EFED Final Reviewer:	Cheryl Sutton, Ph.D. Environmental Scientist	Signature: CHERYL SUTTON Digitally signed by CHERYL SUTTON Date: 2020.04.27 12:15:16 -04'00' Date: April 22, 2020
CDM/CSS-Dynamac JV Reviewers:	Lisa Muto, Environmental Scientist	Signature: Lisa Muto Date: 08/14/2018
	Joan Gaidos, Ph.D., Environmental Scientist	Signature: Date: 08/14/2018

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 (Water), is designed for the quantitative determination of orthosulfamuron and it metabolites O-desmethyl DOP urea, DBS acid ammonium salt, DBS amide, DOP amine, DB amine, O-desmethyl orthosulfamuron, and DOP urea in water at the LOQ of 0.17 ng/mL using LC/MS/MS. The LOQ is less than the lowest toxicological level of concern in water for all analytes. The ECM and ILV used the same two water matrices from the orthosulfamuron aquatic field dissipation test sites in Arkansas and California (MRID 50229101; Waterborne Study No. WEI 642.03). 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. For all analytes but DOP amine, the ILV validated the method after the first trial with modifications of pH adjustment and modifications to the analytical parameters. For DOP amine, the ILV validated the method after the second trial with additional modifications of a new LC/MS/MS gradient and mass transitions in order to properly separate it from the other analytes; **an updated ECM should be submitted to include the ILV modifications for improved chromatography for DOP amine**. All submitted ILV and ECM data pertaining to precision, repeatability, and reproducibility was acceptable, except for LOQ analysis of DOP amine in AR water. ILV linearity was not satisfactory for orthosulfamuron, DBS amide, 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 representative chromatograms. The specificity of the method for DB amine and DOP urea was not supported by ILV representative chromatograms. ILV representative chromatograms were not provided for one of the two water matrices. Calculated LODs did not support the method LOD for DOP amine.

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	50318402[1]	50318403[2]		Water	23/03/2016	Nichino America, Inc.	LC/MS/MS	0.17 ng/mL
O-Desmethyl DOP urea								
DBS acid ammonium salt								
DBS amide								
DOP amine								
DB amine								
O-Desmethyl orthosulfamuron								
DOP urea								

1 In the ECM, the two waters were obtained from rice paddy source waters from the orthosulfamuron aquatic field dissipation test sites in Arkansas and California (MRID 50229101; Waterborne Study No. WEI 642.03; p. 7 of MRID 50318402). The water characterization was not reported in the study report.

2 In the ILV, Arkansas and California water matrices were provided by Waterborne Environmental Inc. and were used in the study (pp. 27, 45 of MRID 50318403). The two waters were obtained from the orthosulfamuron aquatic field dissipation test sites in Arkansas and California (MRID 50229101; Waterborne Study No. WEI 642.03). The water characterization was not reported. These two waters appeared to be the same as those reported for the ECM, based on water sources.

I. Principle of the Method

Water samples were adjusted to pH 8-9 using 10 mM disodium hydrogen phosphate then a 10-mL aliquot was fortified with orthosulfamuron, DOP urea, DBS amide, DOP amine, DB amine, O-desmethyl orthosulfamuron, DBS acid ammonium salt and O-desmethyl DOP urea (p. 7; Appendix 1, Appendix 1, pp. 152-153). The sample was shaken at *ca*. 180 excursions per minute for 5 minutes. 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-20AD HPLC (Phenomenex Luna C18(2) column, 5 µm, 150 x 1.7 mm, and C18 guard column, 4 x 1.7 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, 0.00-1.70 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); DBS amide, DOP amine, 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, Appendix 1, pp. 154-156). The injection volume was 25 µL. 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* 244.100→165.000 for DBS amide, *m/z* 156.017→99.700 for DOP amine, *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. Expected retention times were *ca*. 6.6, 6.8, 7.3, 7.7, 7.5, 7.7, and 9.0 minutes for O-desmethyl DOP urea, DBS acid ammonium salt, DBS amide, DOP amine, DB amine, O-desmethyl orthosulfamuron, DOP urea, and orthosulfamuron, respectively.

In the ILV, the ECM was performed as written with the use of 500 mM NH_4HCO_3 to adjust the pH and modifications to the analytical parameters, including the addition of a confirmation ion transition, as well as a new gradient and mass transitions for DOP amine in order to properly separate it from the other analytes (pp. 26, 32, 44-45; Tables 58-59, pp. 105-106 of MRID 50318403). The ILV recommended that the ECM is modified to include the improved chromatography for DOP amine. For analyte identification of all analytes but DOP amine, an Agilent 1290 UPLC (Acquity HSS T3 column, 2.1 x 150 mm, 1.8 µm; 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, 0.0-3.0 min. 100:0, 5.0-6.0 min. 90:10, 10.0-12.0 min. 5:95, 12.1-15.0 min. 100:0] coupled to an AB Sciex 6500 (Instrument #27) MS using TurboSpray (temperature 550°C) in positive mode (all analytes but DBS ammonium salt) or negative mode (DBS ammonium salt) was used. The injection volume was 40 µL. Analytes were identified using two ion transitions (primary and confirmatory, respectively): *m/z* 185.223→100.1 and *m/z* 185.223→68.0 for O-desmethyl DOP urea, *m/z* 165.24→120.2 and *m/z* 165.24→92.2 for DB amine, *m/z* 244.046→120.3 and *m/z* 244.046→164.3 for DBS amide, *m/z* 243.06→80.0 and *m/z* 243.06→198.0 for DBS ammonium salt, *m/z* 411.158→227.3 and *m/z* 411.158→120.2 for O-desmethyl orthosulfamuron, *m/z* 199.211→156.3 and *m/z* 199.211→100.2 for DOP urea, and *m/z* 425.143→199.4 and *m/z* 425.143→227.3 for orthosulfamuron. Expected retention times were *ca*. 6.2, 8.1, 8.3, 8.4, 8.6, and 9.8 minutes for O-desmethyl DOP urea, DBS amide, DB amine, O-desmethyl orthosulfamuron, DOP urea, and orthosulfamuron, respectively. For analyte identification of DOP amine, an Agilent 1290 UPLC (Kinetex column, 2.1 x 100 mm, 1.7 µm; column temperature 50°C) with a mobile phase gradient of A: water with 0.1% formic acid and B: acetonitrile with 0.1% formic acid [A:B, 0.00-0.50 min. 100:0, 1.0-3.0 min. 90:10, 3.2-4.2 min. 5:95, 4.3-6.0 min. 100:0] coupled to an AB Sciex 6500 (Instrument #27) MS using TurboSpray in positive mode (temperature 550°C) was used. The injection volume was 40 µL. Analyte was identified using two ion transitions (primary and confirmatory, respectively): *m/z* 156.244→124.137 and *m/z* 156.244→68.04 for DOP amine (second trial transitions). Expected retention time was *ca*. 2.3 minutes.

The Limit of Quantification (LOQ) and Limit of Detection (LOD) for all analytes in water were 0.17 ng/mL and 0.035 ng/mL, respectively, in the ECM and ILV (p. 7 of MRID 50318402; p. 43 of MRID 50318403).

II. Recovery Findings

ECM (MRID 50318402): Mean recoveries and relative standard deviations (RSDs) were within guideline requirements (mean 70-120%; RSD ≤20%) for analysis of orthosulfamuron, O-desmethyl DOP urea, DBS acid ammonium salt, DBS amide, DOP amine, DB amine, O-desmethyl orthosulfamuron, and DOP urea at fortification levels of 0.17 ng/mL (LOQ) and 1.7 ng/mL (10×LOQ) in two water matrices, except for the LOQ analysis of DOP amine in AR rice paddy water (mean 122%; pp. 11-12, 34; DER Attachment 2). For the LOQ analysis of DOP amine in AR rice paddy water, statistics were reviewer-calculated using 9 recovery data values since the statistics provided by the study author excluded two outlier values (p. 34; see DER Attachment 2). 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; residues were quantified in a majority of the controls (pp. 10, 13-20). Both waters were obtained from rice paddy source waters from the orthosulfamuron aquatic field dissipation test sites in Arkansas and California (MRID 50229101; Waterborne Study No. WEI 642.03; p. 7). The water characterization was 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, DBS amide, DOP amine, DB amine, O-desmethyl orthosulfamuron, and DOP urea at fortification levels of 0.17 ng/mL (LOQ) and 1.7 ng/mL (10×LOQ) in two water matrices (pp. 15-18). Analytes were identified using two ion transitions. Performance data (recovery results) from quantitation and confirmation analyses were comparable. Arkansas and California water matrices were provided by Waterborne Environmental Inc. and were used in the study (pp. 27, 45). The two waters were obtained from the orthosulfamuron aquatic field dissipation test sites in Arkansas and California (MRID 50229101; Waterborne Study No. WEI 642.03). The water characterization was not reported. These two waters appeared to be the same as those reported for the ECM, based on water sources. For all analytes but DOP amine, the method was validated after the first trial with modifications of the use of 500 mM NH_4HCO_3 to adjust the pH and modifications to the analytical parameters, including the addition of a confirmation ion transition (pp. 26, 32, 44-45; Tables 58-59, pp. 105-106). For DOP amine, the method was validated after the second trial with additional modifications of a new LC/MS/MS gradient and mass transitions in order to properly separate it from the other analytes; the ILV recommended that the ECM is modified to include the improved chromatography for DOP amine.

Table 2. Initial Validation Method Recoveries for Orthosulfamuron and its Metabolites O-Desmethyl DOP Urea, DBS Acid Ammonium Salt, DBS amide, DOP amine, DB amine, O-Desmethyl Orthosulfamuron, and DOP Urea in Water[1,2]

Analyte	Fortification Level (ng/mL)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Arkansas Rice Paddy Water					
Orthosulfamuron	0.17	9	98-109	103	3.8	3.7
	1.7	8	98-102	100	1.7	1.7
O-Desmethyl DOP Urea	0.17	9	118-125	120	2.3	1.9
	1.7	8	102-113	108	4.0	3.7
DBS Acid Ammonium Salt	0.17	9	94-110	99	5.5	5.6
	1.7	8	105-114	109	3.8	3.5
DBS amide	0.17	9	112-125	118	4.4	3.7
	1.7	8	103-110	105	2.4	2.3
DOP amine	0.17	9[3]	106-131	122	9	7
	1.7	8	103-108	105	2.0	1.9
DB amine	0.17	9	115-126	119	3.9	3.3
	1.7	8	103-109	106	2.1	2.0
O-Desmethyl Orthosulfamuron	0.17	9	106-113	110	2.6	2.4
	1.7	8	101-109	104	2.6	2.5
DOP Urea	0.17	9	106-112	109	2.0	1.8
	1.7	8	101-107	104	2.3	2.2
	California Rice Paddy Water					
Orthosulfamuron	0.17	9	58-94	74	12.0	16.2
	1.7	8	82-110	91	8.7	9.6
O-Desmethyl DOP Urea	0.17	9	111-127	118	5.0	4.2
	1.7	8	79-109	98	10.3	10.5
DBS Acid Ammonium Salt	0.17	9	85-129	113	18.2	16.1
	1.7	8	97-127	112	11.4	10.2
DBS amide	0.17	9	69-106	86	13.2	15.3
	1.7	8	103-133	110	9.9	9.0
DOP amine	0.17	9	62-97	81	12.0	14.8
	1.7	8	96-128	106	10.0	9.4
DB amine	0.17	9	75-99	87	7.7	8.9
	1.7	8	96-114	102	5.6	5.5
O-Desmethyl Orthosulfamuron	0.17	9	72-104	86	10.4	12.1
	1.7	8	96-121	102	8.4	8.2
DOP Urea	0.17	9	63-101	81	12.5	15.4
	1.7	8	99-128	106	9.5	9.0

Data (recovery results were corrected when residues were quantified in the controls, pp. 10, 13-20) were obtained from pp. 11-12 of MRID 50318402 and DER Attachment 2 (p. 34).

1 Both waters were obtained from rice paddy source waters from the orthosulfamuron aquatic field dissipation test sites in Arkansas and California (MRID 50229101; Waterborne Study No. WEI 642.03; p. 7). The water characterization was 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* 244.100→165.000 for DBS amide, *m/z* 156.017→99.700 for DOP amine, *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 9 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 (106-130%, 120 ±8.8%, RSD 7.3%).

Table 3. Independent Validation Method Recoveries for Orthosulfamuron and its Metabolites O-Desmethyl DOP Urea, DBS Acid Ammonium Salt, DBS amide, DOP amine, DB amine, O-Desmethyl Orthosulfamuron, and DOP Urea in Water[1,2]

Analyte	Fortification Level (ng/mL)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Arkansas Water					
	Quantitation Ion Transition					
Orthosulfamuron	0.17	5	92-104	100	4.8	4.7
	1.7	5	89-106	99	6.9	6.9
O-Desmethyl DOP Urea	0.17	5	97-105	100	2.9	2.9
	1.7	5	101-103	102	0.9	0.9
DBS Acid Ammonium Salt	0.17	5	99-109	104	3.9	3.8
	1.7	5	101-107	103	2.4	2.3
DBS amide	0.17	5	96-135	112	14.7	13.1
	1.7	5	104-119	111	6.9	6.2
DOP amine	0.17	5	100-108	104	2.8	2.7
	1.7	5	98-104	101	2.2	2.2
DB amine	0.17	5	91-104	96	5.2	5.4
	1.7	5	96-99	98	1.5	1.5
O-Desmethyl Orthosulfamuron	0.17	5	78-101	86	9.3	10.8
	1.7	5	81-95	89	5.3	6.0
DOP Urea	0.17	5	90-99	96	3.6	3.8
	1.7	5	90-97	94	2.5	2.7
	Confirmation Ion Transition					
Orthosulfamuron	0.17	5	92-102	97	4.2	4.3
	1.7	5	92-106	97	5.8	5.9
O-Desmethyl DOP Urea	0.17	5	98-104	99	2.9	2.9
	1.7	5	98-103	101	1.7	1.7
DBS Acid Ammonium Salt	0.17	5	94-103	99	4.0	4.1
	1.7	5	101-104	103	1.2	1.1
DBS amide	0.17	5	97-127	107	14.8	13.8
	1.7	5	83-104	93	7.5	8.1
DOP amine	0.17	5	98-104	101	2.3	2.3
	1.7	5	100-106	103	2.6	2.5
DB amine	0.17	5	93-106	95	6.9	7.3
	1.7	5	96-102	99	3.1	3.1
O-Desmethyl Orthosulfamuron	0.17	5	77-87	83	4.3	5.2
	1.7	5	76-90	82	5.2	6.4
DOP Urea	0.17	5	92-97	95	2.2	2.3
	1.7	5	97-102	98	2.4	2.4
	California Water					
	Quantitation Ion Transition					
Orthosulfamuron	0.17	5	80-100	90	8.1	9.0
	1.7	5	80-102	91	8.0	8.8
O-Desmethyl DOP Urea	0.17	5	75-99	93	10.1	10.9
	1.7	5	98-104	100	2.1	2.1

Analyte	Fortification Level (ng/mL)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
DBS Acid Ammonium Salt	0.17	5	90-102	96	5.3	5.5
	1.7	5	95-98	97	1.2	1.2
DBS amide	0.17	5	89-111	102	8.6	8.4
	1.7	5	88-110	100	10.7	10.6
DOP amine	0.17	5	103-112	108	3.6	3.3
	1.7	5	102-105	104	1.3	1.3
DB amine	0.17	5	89-98	92	4.1	4.5
	1.7	5	98-100	99	0.5	0.5
O-Desmethyl Orthosulfamuron	0.17	5	84-95	89	4.8	5.4
	1.7	5	79-88	84	4.2	4.9
DOP Urea	0.17	5	88-95	91	3.1	3.3
	1.7	5	92-97	94	2.8	2.9
	Confirmation Ion Transition					
Orthosulfamuron	0.17	5	87-99	92	4.8	5.2
	1.7	5	82-97	92	6.1	6.6
O-Desmethyl DOP Urea	0.17	5	79-101	94	9.2	9.8
	1.7	5	99-102	101	1.3	1.3
DBS Acid Ammonium Salt	0.17	5	89-98	93	3.6	3.9
	1.7	5	95-102	98	3.0	3.0
DBS amide	0.17	5	77-106	91	10.7	11.8
	1.7	5	86-105	95	10.0	10.4
DOP amine	0.17	5	108-111	109	1.1	1.0
	1.7	5	104-107	105	1.2	1.2
DB amine	0.17	5	88-100	93	5.1	5.4
	1.7	5	98-100	99	0.6	0.6
O-Desmethyl Orthosulfamuron	0.17	5	77-87	81	4.1	5.0
	1.7	5	77-84	81	2.9	3.6
DOP Urea	0.17	5	91-99	96	3.3	3.4
	1.7	5	92-96	93	1.7	1.8

Data (uncorrected recovery results, Tables 1-32, pp. 47-78; Figure 49, p. 246) were obtained from pp. 9-14 of MRID 50318403.

1 Arkansas and California water matrices were provided by Waterborne Environmental Inc. and were used in the study (pp. 27, 45). The two waters were obtained from the orthosulfamuron aquatic field dissipation test sites in Arkansas and California (MRID 50229101; Waterborne Study No. WEI 642.03). The water characterization was not reported. These two waters appeared to be the same as those reported for the ECM, based on water sources.

2 Analytes were identified using two ion transitions (primary and confirmatory, respectively): *m/z* 185.223→100.1 and *m/z* 185.223→68.0 for O-desmethyl DOP urea, *m/z* 165.24→120.2 and *m/z* 165.24→92.2 for DB amine, *m/z* 244.046→120.3 and *m/z* 244.046→164.3 for DBS amide, *m/z* 243.06→80.0 and *m/z* 243.06→198.0 for DBS ammonium salt, *m/z* 411.158→227.3 and *m/z* 411.158→120.2 for O-desmethyl orthosulfamuron, *m/z* 199.211→156.3 and *m/z* 199.211→100.2 for DOP urea, and *m/z* 425.143→199.4 and *m/z* 425.143→227.3 for orthosulfamuron [first trial]; *m/z* 156.244→124.137 and *m/z* 156.244→68.04 for DOP amine [second trial].

III. Method Characteristics

The LOQ for water was 0.17 ng/mL in the ECM and ILV (pp. 7, 21-29 of MRID 50318402; 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 tested. The LOD was reported as 0.035 ng/mL in the ECM and ILV. In the ECM, the LOD was defined as the lowest analyte concentration for a measurement statistically different form the response in the control sample. The LOD was also calculated for each analyte/matrix using the following equation:

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

Where, $t_{0.99}$ is the one-tailed t statistic for n = 9 (2.896) 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 DOP amine. The calculated LODs for the AR water matrix supported the method LOD better than those of the CA water matrix. In the ILV, the LOD was set at 20% 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 4a. Method Characteristics

Analyte			**Orthosulfamuron**	**O-Desmethyl DOP Urea**	**DBS Acid Ammonium Salt**	**DBS amide**
Limit of Quantitation (LOQ)			0.17 ng/mL			
Limit of Detection (LOD)	ECM	Method	0.035 ng/mL			
		Calculated	0.0188-**0.0592** ng/mL	0.0119-0.0245 ng/mL	0.0273-**0.0899** ng/mL	0.0224-**0.0654** ng/mL
	ILV		0.035 ng/mL			
Linearity (calibration curve r^2 and concentration range)[1]	ECM	AR Water	r^2 = **0.9787**	r^2 = **0.9857**	r^2 = **0.9892**	r^2 = **0.9843**
		CA Water	r^2 = 0.9996	r^2 = 0.9992	r^2 = **0.9908**	r^2 = 0.9986
		Range	0.10-2.5 ng/mL			
	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 = **0.9932** (Q) r^2 = **0.9922** (C)
	Concentration range		0.035-10 ng/mL			
Repeatable	ECM[2,3]		Yes at LOQ and 10×LOQ (two uncharacterized waters).			
	ILV[2,4,5]					
Reproducible			Yes at LOQ and 10×LOQ.			
Specificity	ECM		Yes, matrix interferences were <1% of the LOQ (based on peak area). Some non-uniform peak integration was noted.	Yes, matrix interferences were *ca*. 15-16% of the LOQ (based on peak area). Non-uniform peak integration was noted.	**No**, matrix interferences were *ca*. 6-9% of the LOQ (based on peak area), but analyte peak was completely unresolved.[6]	Yes, matrix interferences were 1-2% of the LOQ (based on peak area).
	ILV		Yes, matrix interferences were *ca*. 3-4% of the LOQ (based on peak area).	Yes, no matrix interferences were observed.	Yes, matrix interferences were <1% of the LOQ (based on peak area). Elevated baseline noise was noted around the analyte peak.	Yes, matrix interferences were *ca*. 10-13% of the LOQ (based on peak area).
			Representative chromatograms from only **one** water matrix (AR) were provided for review.			

Data were obtained from pp. 7, 21-29; pp. 11-20, 34 (recovery data); pp. 31-46 (calibration curve); pp. 47-142 (chromatograms) of MRID 50318402; pp. 43-45; pp. 9-14 and Tables 1-32, pp. 47-78 (recovery data); Figures 1-8, pp. 110-117 (calibration curves); Figures 9-16, pp. 118-189 (calibration chromatograms); Figures 17-34, pp. 190-221 (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. 31-46 of MRID 50318402; Figures 1-8, pp. 110-117 of MRID 50318403; DER Attachment 2). Matrix-matched standards were used in the ECM (p. 9 of MRID 50318402); 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 waters were obtained from rice paddy source waters from the orthosulfamuron aquatic field dissipation test sites in Arkansas and California (MRID 50229101; Waterborne Study No. WEI 642.03; p. 7 of MRID 50318402). The water characterization was not reported in the study report.

4 In the ILV, Arkansas and California water matrices were provided by Waterborne Environmental Inc. and were used in the study (pp. 27, 45 of MRID 50318403). The two waters were obtained from the orthosulfamuron aquatic field dissipation test sites in Arkansas and California (MRID 50229101; Waterborne Study No. WEI 642.03). The water characterization was not reported. These two waters appeared to be the same as those reported for the ECM, based on water sources.

5 For all analytes but DOP amine, the ILV validated the method after the first trial with modifications of the use of 500 mM NH_4HCO_3 to adjust the pH and modifications to the analytical parameters, including the addition of a confirmation ion transition (pp. 26, 32, 44-45; Tables 58-59, pp. 105-106 of MRID 50318403). For DOP amine, the ILV validated the method after the second trial with additional modifications of a new LC/MS/MS gradient and mass transitions in order to properly separate it from the other analytes; the ILV recommended that the ECM is modified to include the improved chromatography for DOP amine.

6 Based on pp. 52-54, 112-114 of MRID 50318402.

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

Table 4b. Method Characteristics (con't)

Analyte			**DOP amine**	**DB amine**	**O-Desmethyl Orthosulfamuron**	**DOP Urea**
Limit of Quantitation (LOQ)			0.17 ng/mL			
Limit of Detection (LOD)	ECM	Method	0.035 ng/mL			
		Calculated	**0.0470-0.0587** ng/mL	0.0190-**0.0380** ng/mL	0.0134-**0.0507** ng/mL	0.0099-**0.0612** ng/mL
	ILV		0.035 ng/mL			
Linearity (calibration curve r^2 and concentration range)[1]	ECM	AR Water	r^2 = **0.9825**	r^2 = **0.9851**	r^2 = **0.9894**	r^2 = **0.9896**
		CA Water	r^2 = 0.9972	r^2 = 0.9992	r^2 = 0.9996	r^2 = 0.9992
		Range	0.10-2.5 ng/mL			
	ILV		r^2 = 0.9982 (Q) r^2 = 0.9994 (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 (CA water). **No** at LOQ (mean **122**%); Yes at 10×LOQ (AR water).	Yes at LOQ and 10×LOQ (two uncharacterized waters).		
	ILV[2,4,5]		Yes at LOQ and 10×LOQ (two uncharacterized waters).			
Reproducible			Yes at LOQ and 10×LOQ.			
Specificity	ECM		Yes, matrix interferences were *ca*. 0-2% of the LOQ (based on peak area). A significant nearby contaminant (RT 7.43/7.86 min.) interfered with analyte peak (RT 7.59/8.01 min.) attenuation and integration.[6]	Yes, matrix interferences were <1% of the LOQ (based on peak area).	Yes, matrix interferences were <1% of the LOQ (based on peak area).	Yes, matrix interferences were <1% of the LOQ (based on peak area).
	ILV		Yes, matrix interferences were <1% of the LOQ (based on peak area). Some non-uniform peak integration was noted	**No**, matrix interferences were *ca*. 27% of the LOQ (based on peak area).[7] Baseline noise interfered with analyte peak attenuation and integration.	Yes, matrix interferences were *ca*. 6-7% of the LOQ (based on peak area).	**No**, matrix interferences were *ca*. 49% of the LOQ (based on peak area).[8] Significant non-uniform peak integration was noted.
			Representative chromatograms from only **one** water matrix (AR) were provided for review.			

Data were obtained from pp. 7, 21-29; pp. 11-20, 34 (recovery data); pp. 31-46 (calibration curve); pp. 47-142 (chromatograms) of MRID 50318402; pp. 43-45; pp. 9-14 and Tables 1-32, pp. 47-78 (recovery data); Figures 1-8, pp. 110-117 (calibration curves); Figures 9-16, pp. 118-189 (calibration chromatograms);

Figures 17-34, pp. 190-221 (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. 31-46 of MRID 50318402; Figures 1-8, pp. 110-117 of MRID 50318403; DER Attachment 2). Matrix-matched standards were used in the ECM (p. 9 of MRID 50318402); 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 waters were obtained from rice paddy source waters from the orthosulfamuron aquatic field dissipation test sites in Arkansas and California (MRID 50229101; Waterborne Study No. WEI 642.03; p. 7 of MRID 50318402). The water characterization was not reported in the study report.

4 In the ILV, Arkansas and California water matrices were provided by Waterborne Environmental Inc. and were used in the study (pp. 27, 45 of MRID 50318403). The two waters were obtained from the orthosulfamuron aquatic field dissipation test sites in Arkansas and California (MRID 50229101; Waterborne Study No. WEI 642.03). The water characterization was not reported. These two waters appeared to be the same as those reported for the ECM, based on water sources.

5 For all analytes but DOP amine, the ILV validated the method after the first trial with modifications of the use of 500 mM NH_4HCO_3 to adjust the pH and modifications to the analytical parameters, including the addition of a confirmation ion transition (pp. 26, 32, 44-45; Tables 58-59, pp. 105-106 of MRID 50318403). For DOP amine, the ILV validated the method after the second trial with additional modifications of a new LC/MS/MS gradient and mass transitions in order to properly separate it from the other analytes; the ILV recommended that the ECM is modified to include the improved chromatography for DOP amine.

6 Based on pp. 77-78, 125-126 of MRID 50318402.

7 Based on Figure 17, p. 193, Figure 18, p. 201 and Figures 25-26, pp. 212-213 of MRID 50318403.

8 Based on Figure 17, p. 191, Figure 18, p. 199 and Figures 21-22, pp. 208-209 of MRID 50318403.

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

IV. Method Deficiencies and Reviewer's Comments

1. An updated ECM should be submitted to include the ILV modifications for improved chromatography for DOP amine. For DOP amine, the ILV validated the method after the second trial with modifications of the use of 500 mM NH_4HCO_3 to adjust the pH and modifications to the analytical parameters, including a new LC/MS/MS gradient and mass transitions in order to properly separate it from the other analytes (pp. 26, 32, 44-45; Tables 58-59, pp. 105-106 of MRID 50318403). These ILV modifications were necessary for the successful validation of the ECM.

   ILV recommendations for the ECM were reported in Appendix A, pp. 248-250 of MRID 50318403. The ILV included the improved chromatography parameters.

2. ECM performance data was not satisfactory for the LOQ analysis of DOP amine in AR water (means 122%; pp. 11-20, 34 of MRID 50318403; DER Attachment 2). DER-reported statistics were reviewer-calculated using 9 recovery data values; rules of significant figures were followed. Statistics provided in the study report used only 7 recovery data values (106-130%, 120 ±8.8%, RSD 7.3%). OCSPP guideline requirements state that the mean recovery is 70-120% and the RSD is ≤20%. The reviewer determined that this repeatability deviation did not affect the reproducibility of the method for DOP amine since ECM performance data in CA water was satisfactory.

3. In the ILV, the linearity was not satisfactory for the quantitation ion analysis of orthosulfamuron ($r^2 = 0.9924$), DBS amide ($r^2 = 0.9932$), and DB amine ($r^2 = 0.9918$), as well as the confirmation ion analysis of DBS amide ($r^2 = 0.9922$; Figures 9-16, pp. 118-189 of MRID 50318403; DER Attachment 2).

   In the ECM, the linearity was not satisfactory for the Arkansas water analysis of all analytes: $r^2 = 0.9787$-0.9896 (pp. 31-46 of MRID 50318402; DER Attachment 2). The linearity was not satisfactory for the California water analysis of DBS acid ammonium salt: $r^2 = 0.9908$.

   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 representative chromatograms because the analyte peak was completely unresolved (pp. 52-54, 112-114 of MRID 50318402).

   The specificity of the method for DB amine and DOP urea was not supported by ILV representative chromatograms due to matrix interferences of *ca*. 27% and 49%, respectively, of the LOQ (based on peak area; Figure 17, pp. 191, 193; Figure 18, pp. 199, 201; Figures 21-22, pp. 208-209; and Figures 25-26, pp. 212-213 of MRID 50318403). For DB amine, baseline noise also interfered with analyte peak attenuation and integration. For DOP urea, significant non-uniform peak integration was also noted.

5. ILV representative chromatograms of the California water 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 water matrices were the same, based on water sources. The two ECM uncharacterized waters were reportedly obtained from rice paddy source waters from the orthosulfamuron aquatic field dissipation test sites in Arkansas and California (MRID 50229101; Waterborne Study No. WEI 642.03; p. 7 of MRID 50318402). The two ILV uncharacterized waters 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 of MRID 50318403). It could not be determined if the ILV was provided with the most difficult matrix with which to validate the method.

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 50318402; 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 ng/mL in the ECM and ILV. In the ECM, the LOD was defined as the lowest analyte concentration for a measurement statistically different form the response in the control sample. The LOD was also calculated for each analyte/matrix using the following equation: LOD = ($t_{0.99}$ x SD), where, $t_{0.99}$ is the one-tailed t statistic for n = 9 (2.896) 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 DOP amine. The calculated LODs for the AR water matrix supported the method LOD better than those of the CA water matrix. In the ILV, the LOD was set at 20% 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; residues were quantified in a majority of the controls (pp. 10, 13-20 of MRID 50318402).

9. The communication between the ILV testing facility and the method developer or ECM was not detailed (p. 45 of MRID 50318403).

10. 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).

11. The reviewer performed calculations for the ILV LOQ analysis in AR water for DBS amide in order to verify that the study report statistics included the out-of-range recovery values (see DER Attachment 2). The reviewer reported the statistics from the study report.

12. In the ECM, matrix-matched standards were used (p. 9 of MRID 50318402). 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 10 days of refrigeration for the water extracts (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

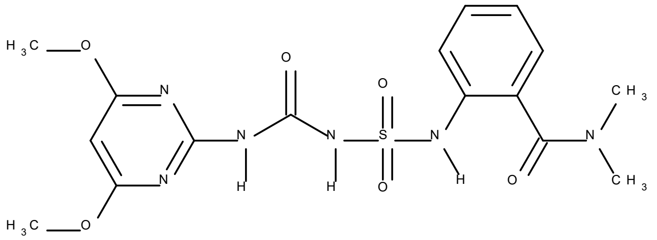
U.S. Environmental Protection Agency. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.

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

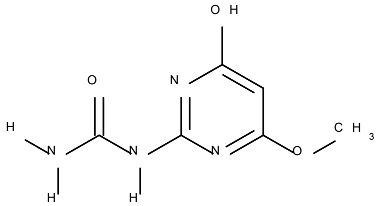
Orthosulfamuron (IR5878)

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(c1ccccc1C(=O)N(C)C)S(=O)(=O)N([H])C(=O)N([H])c2nc(cc(n2)OC)OC



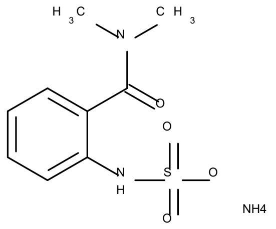
O-Desmethyl DOP Urea

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



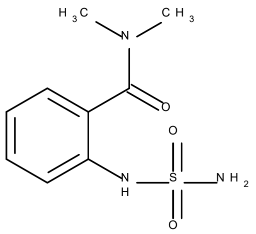
DBS Acid Ammonium Salt (IR7863 Ammonium Salt)

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



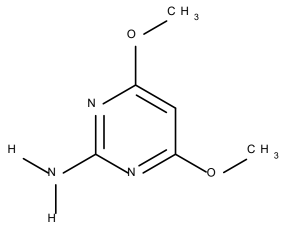
DBS amide

IUPAC Name:	(2-Dimethylcarbamoylphenyl)-sulfanamide
CAS Name:	Not reported
CAS Number:	Not reported
SMILES String:	CN(C)C(=O)c1ccccc1NS(=O)(=O)N



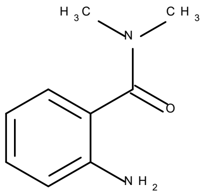
DOP amine

IUPAC Name: (2-Amino-4,6-dimethoxy)-pyrimidine
CAS Name: Not reported
CAS Number: 36315-01-2
SMILES String: [H]N([H])c1nc(cc(n1)OC)OC



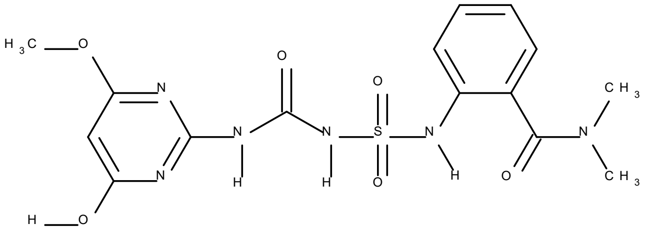
DB amine

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:	l-(4-hydroxy-6-mehoxypyrimidin-2-yl)-3-[2-(dimethylcarbamoyl) phenylsulfamoyl]urea
CAS Name:	Not reported
CAS Number:	Not reported
SMILES String:	[H]N(c1ccccc1C(=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