

**Test Material:** Metsulfuron methyl

**MRID:** 49585702

**Title:** Analytical Method for the Determination of Metsulfuron methyl and Metabolites in Water Using LC/MS/MS

**MRID:** 49585703

**Title:** Independent Laboratory Validation of DuPont-28807 “Analytical Method for the Determination of Metsulfuron Methyl and Metabolites in Water Using LC/MS/MS”

**EPA PC Code:** 122010

**OCSPP Guideline:** 850.6100

**For CDM Smith**

**Primary Reviewer:** Lisa Muto

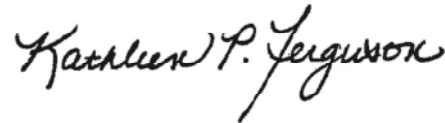
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**Date:** 9/28/15

**Secondary Reviewer:** Kathleen Ferguson

**Signature:**



**Date:** 9/28/15

**QC/QA Manager:** Joan Gaidos

**Signature:**



**Date:** 9/28/15

**Analytical method for metsulfuron methyl (DPX-T6376) and metabolites, IN-A4098, IN-D5119, IN-00581, IN-NC148, IN-B5685, IN-B5067 and IN-F5438, in water**

**Reports:** ECM: EPA MRID No. 49585702. Henze, R.M. and J.J. Stry. 2003. Analytical Method for the Determination of Metsulfuron methyl and Metabolites in Water Using LC/MS/MS. DuPont Study No.: DuPont-28807. Report prepared by E.I. du Pont de Nemours and Company, Newark, Delaware; sponsored and submitted by E.I. du Pont de Nemours and Company, Wilmington, Delaware; 159 pages. Final report issued November 15, 2010.

ILV: EPA MRID No. 49585703. Fiorito, B. 2014. Independent Laboratory Validation of DuPont-28807 "Analytical Method for the Determination of Metsulfuron Methyl and Metabolites in Water Using LC/MS/MS". DuPont Study Project ID: DuPont-39347. Alliance Pharma Project No.: 140111. Report prepared by Alliance Pharma, Malvern, Pennsylvania; sponsored and submitted by E.I. du Pont de Nemours and Company, Wilmington, Delaware; 245 pages. Final report issued May 20, 2014.

**Document No.:** MRIDs 49585702 & 49585703

**Guideline:** 850.6100

**Statements:** ECM: The study was not conducted with the restriction of compliance with USEPA FIFRA (40 CFR, Part 160) or OECD Good Laboratory Practice (GLP) standards; however, work was done in a GLP facility following Standard Operating Procedures (p. 3 of MRID 49585702). Signed and dated Data Confidentiality, No Data Confidentiality, GLP, and Certification of Authenticity statements were provided (pp. 2-4). A Quality Assurance statement was not provided.

ILV: The study was conducted in accordance with the USEPA FIFRA and OECD GLP standards (p. 3 of MRID 49585703). Signed and dated Data Confidentiality, No Data Confidentiality, GLP, Quality Assurance and Certification of Authenticity statements were provided (pp. 2-5).

**Classification:** This analytical method is classified as **unacceptable**. An updated ECM to include the procedure to minimize matrix suppression should be submitted. The determinations of the LOQ and LOD were not based on scientifically acceptable procedures. In the ILV, the mean recovery for the second ion transition was <70% for IN-00581, and the number of samples for one of the secondary ion analyses for IN-B5067 was insufficient (n = 2). In the ILV, linearity was not satisfactory for a majority of the linear regressions. The purities of the IN-NC148 and IN-B5067 standards were low (<90%). Sample recoveries were corrected in the ECM. Some of the water matrices were not characterized in the ECM and ILV.

**PC Code:** 122010

**EPA Primary Reviewer:** Kristy Crews, Chemist

**Signature:**



**Date:** 2/14/17

**EPA Secondary Reviewer:** William Eckel, Senior Scientist Advisor

**Signature:**



**Date:** 2/14/17

## Executive Summary

This analytical method, DuPont Study No. DuPont-28807, is designed for the quantitative determination of metsulfuron methyl (DPX-T6376) and metabolites, IN-A4098, IN-D5119, IN-00581, IN-NC148, IN-B5685, IN-B5067 and IN-F5438 in surface water, ground water and drinking water at the LOQ (0.050 µg/L) using LC/MS/MS. The LOQ is **less than** the lowest toxicological level of concern in water for all analytes. The method was validated by the ILV with the first trial for all analytes in tap water. The method was validated with the first trial for all analytes, except IN-B5067, in ground water. The method was validated with the first trial for all analytes, except IN-B5067, IN-NC148 and IN-F5438, in surface water. After the modification of the method to include washing the SPE column with pH 3 water instead of neutral water (to combat matrix suppression), acceptable recoveries were achieved in the second trials of IN-B5067 in ground and surface water and of IN-NC148 and IN-F5438 in surface water. An updated ECM to include the procedure to minimize matrix suppression should be submitted. Both ion transitions were quantified for all analytes in the ILV; only the primary ion transition was quantified in the ECM. One of the secondary ion recoveries for IN-00581 was <70% in the ILV; the number of samples for one of the secondary ion analyses for IN-B5067 was insufficient (n = 2) in the ILV.

**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						
Metsulfuron methyl (DPX- T6376)	49585702	49585703 <sup>1</sup>		Water <sup>2,3</sup>	15/11//2010	E.I. du Pont de Nemours and Company	LC/MS/MS	0.050 µg/L (0.050 ppb)
IN-A4098								
IN-D5119								
IN-00581								
IN-NC148								
IN-B5685								
IN-B5067								
IN-F5438								

<sup>1</sup> The method was validated by the ILV with the first or second trial for all analytes in drinking, ground and surface water (p. 28 of MRID 49585703).

<sup>2</sup> Drinking, surface and ground water matrices were used in the ECM and ILV. In the ECM, surface water (creek) was fully characterized; ground water (well) and drinking water (tap or bottled) were not characterized (p. 15; Appendix 5, pp. 158-159 of MRID 49585702). In the ILV, surface water (river) and ground water were characterized; drinking water (tap) was not characterized (p. 22; Appendix 2, pp. 243-245 of MRID 49585703).

## I. Principle of the Method

Samples (100 ± 1% mL) were measured into 125-mL polycarbonate Erlenmeyer flasks and fortified, as necessary (pp. 10-11, 13-14, 16 of MRID 49585702; Reviewer Comment #12). The sample was shaken vigorously with 1 mL of 0.01 M ammonium formate (pH = 3.5) and 50 µL concentrated formic acid (98%). A 20-cc, 0.5-g Oasis® HLB solid phase extraction (SPE) cartridge fitted with an adaptor and 75-mL reservoir (the study authors noted that this equipment could not be

substituted) was pre-conditioned with methanol and HPLC grade water (10 mL each). The sample was loaded on the SPE cartridge under vacuum (2-10 mL/min flow rate). The cartridge was rinsed with 10 mL of hexane then vacuum-dried for 3 minutes. Analytes were eluted with 20 mL of Solution A (basic acetonitrile: 20 mL of 0.1 M ammonium hydroxide in 980 mL of acetonitrile) using gravity (low vacuum at initiation and termination of flow, as necessary). The volume of the eluate was adjusted to 20 mL using Solution A. A 10-mL aliquot of the extract was transferred to a 15-mL centrifuge tube; the solvent was evaporated to *ca.* 3.0 mL under nitrogen in an N-Evap at *ca.* 30°C. After 0.250 mL of water was added to the residue, it was further reduced via evaporation to *ca.* 0.25 mL. The residue was reconstituted to 2.5 mL using the Injection Solution (0.005 M aqueous ammonium acetate). An aliquot of the sample was filtered (0.2 µm Acrodisc filter) into a HPLC vial prior to analysis. The study authors noted that the extracts will be stable for *ca.* 24 hours at 4°C.

Samples were analyzed for metsulfuron methyl using an Agilent HP1200 HPLC coupled to an API 5000 triple quadrupole mass spectrometer using Turbo Ion Spray (pp. 10, 18-20; Appendix 4, pp. 124-156 of MRID 49585702). The reversed-phase HPLC/MS/MS conditions consisted of a Polaris C18 column (4.6 x 150 mm, 3-µm, column temperature 40°C), a mobile phase gradient of (A) 0.02 M aqueous formic acid and (B) methanol [percent A:B (v:v) at 0.0-1.0 min. 98:2, 3.5 min. 70:30, 12.0 min. 20:80, 12.5-15.0 min. 2:98, 15.2-25.0 min. 98:2], and MS/MS detection in positive (IN-A4098) or negative (all other analytes) ion mode with Multiple Reaction Monitoring (MRM). Two parent-daughter ion transitions were monitored for each analyte (quantitative and confirmatory, respectively):  $m/z$  380.0 → 138.8 and  $m/z$  380.0 → 107.1 for metsulfuron methyl;  $m/z$  141.0 → 57.3 and  $m/z$  141.0 → 43.0 for IN-A4098;  $m/z$  199.85 → 91.9 and  $m/z$  199.85 → 155.9 for IN-D5119;  $m/z$  181.9 → 42.0 and  $m/z$  182.1 → 105.7 for IN-00581;  $m/z$  341.8 → 181.9 and  $m/z$  341.8 → 42.1 for IN-NC148;  $m/z$  257.3 → 42.0 and  $m/z$  257.3 → 182.7 for IN-B5685;  $m/z$  366.1 → 125.1 and  $m/z$  366.1 → 42.1 for IN-B5067; and  $m/z$  366.1 → 155.9 and  $m/z$  366.1 → 200.3 for IN-F5438. Retention times were 12.0, 4.2, 6.8, 7.5, 8.5, 8.7, 9.8 and 10.3 minutes for metsulfuron methyl, IN-A4098, IN-D5119, IN-00581, IN-NC148, IN-B5685, IN-B5067 and IN-F5438, respectively. Injection volume was 0.025 mL.

The ECM study authors noted the following special precautions: 1) the solvent must be removed in the evaporation steps to eliminate interference in the chromatography, especially interference with the most polar compounds, IN-A4098 and IN-D5119; 2) the IN-00581 peak must be positively identified by recording the retention time of the IN-00581 standard since it is structurally similar to IN-B5685 and IN-B5067; 3) the IN-NC148 standard concentration must be corrected for the low purity of the standard compound (77.0%); and 4) standards must be refrigerated to reduce degradation of metsulfuron methyl and standards should be checked for stability routinely (p. 24 of MRID 49585702).

In the ILV, DuPont-28807 was performed as written, except that a Shimadzu HPLC coupled with an AB Sciex Triple Quad 5500 was used as the analytical instrument, and the SPE column was washed with pH 3 water instead of neutral water for the second trials of ground and surface water (pp. 23-26, 28 of MRID 49585703). In the reported parent-daughter ion transitions, a few of the parent  $m/z$  values differed slightly from the parent  $m/z$  values reported in the ECM.

In both the ECM and ILV, the LOQ for all analytes was reported as 0.050 µg/L (0.050 ppb; 0.050 µg/kg; pp. 8-9, 23 of MRID 49585702; pp. 12, 17 of MRID 49585703). The LOD for all analytes was 0.02 µg/L (0.02 ppb) in the ECM and ILV.

## II. Recovery Findings

ECM (MRID 49585702): Mean recoveries and relative standard deviations (RSD) were within guidelines (mean 70-120%; RSD  $\leq$ 20%) for analysis of metsulfuron methyl, IN-A4098, IN-D5119, IN-00581, IN-NC148, IN-B5685, IN-B5067 and IN-F5438 in creek water, well water and drinking water at the LOQ (0.05  $\mu\text{g/L}$ ) and 10 $\times$ LOQ (0.5  $\mu\text{g/L}$ ; n =5; p. 9; Table 1, pp. 28-43). Two parent-daughter ion transitions were monitored, but only the primary ion transition was quantified. Confirmation of the method was evaluated using ion ratios (pp. 24-26; Appendix 3, pp. 117-123). Recoveries were corrected for any residues detected in the controls; only residues of IN-D5119 were detected in the controls (tap and well waters only; p. 21; Figure 5, pp. 74, 98). Surface water was obtained from White Clay Creek, Newark, Delaware, and fully characterized (p. 15; Appendix 5, pp. 158-159). Ground water was obtained from a well in Kemblesville, Pennsylvania. Drinking water was obtained as either tap water from Stine-Haskell Research Center, Newark, Delaware, or bottled water from a local grocery store (it was not clear which source of drinking water was used). No physical characterization of the ground and drinking water samples was performed.

ILV (MRID 49585703): Mean recoveries and RSDs were within guidelines for analysis of metsulfuron methyl, IN-A4098, IN-D5119, IN-00581, IN-NC148, IN-B5685, IN-B5067 and IN-F5438 in surface water, ground water and drinking (tap) water at the LOQ (0.05  $\mu\text{g/L}$ ) and 10 $\times$ LOQ (0.5  $\mu\text{g/L}$ ; based on data from both ion transitions), except for the confirmatory ion analysis of IN-00581 at 10 $\times$ LOQ in surface water (mean recovery 69%; pp. 29-32; Tables 1-24, pp. 35-82). The number of samples was sufficient for all analytes/matrices (n = 5), except for the confirmation ion analysis of IN-B5067 at the LOQ in surface water (n = 2). Two parent-daughter ion transitions were monitored. The results of both ion transitions were reported for all analytes; the results were comparable. The surface water was collected from Goose River and submitted for characterization by ABS Labs/Clark (p. 22; Appendix 2, pp. 243-245). The ground water was submitted for characterization by Morse Laboratories/Dupont (the source was not specified). The drinking (tap) water was collected from the test facility. Only the surface and ground water matrices were fully characterized (Agvise Laboratories, Northwood, North Dakota). The method was validated with the first trial for all analytes in tap water (p. 28). The method was validated with the first trial for all analytes, except IN-B5067, in ground water. The method was validated with the first trial for all analytes, except IN-B5067, IN-NC148 and IN-F5438, in surface water. After the modification of the method to include washing the SPE column with pH 3 water instead of neutral water (to combat matrix suppression), acceptable recoveries were achieved in the second trials of IN-B5067 in ground and surface water and of IN-NC148 and IN-F5438 in surface water.

**Table 2. Initial Validation Method Recoveries for Metsulfuron Methyl (DPX-T6376) and Metabolites, IN-A4098, IN-D5119, IN-00581, IN-NC148, IN-B5685, IN-B5067 and IN-F5438, in Water.<sup>1,2</sup>**

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
<b>Drinking (Tap) Water</b>						
Metsulfuron methyl (DPX-T6376)	0.05 (LOQ)	5	88-102	95	5	5.4
	0.50	5	92-99	95	3	2.7
IN-A4098	0.05 (LOQ)	5	88-98	94	4	4.6
	0.50	5	85-88	87	1	1.5
IN-D5119	0.05 (LOQ)	5	87-105	95	7	7.2
	0.50	5	85-95	90	4	4.2
IN-00581	0.05 (LOQ)	5	91-106	97	7	6.8
	0.50	5	84-91	85	3	1.3
IN-NC148	0.05 (LOQ)	5	94-120	105	11	10
	0.50	5	90-108	96	7	7.5
IN-B5685	0.05 (LOQ)	5	89-104	98	6	6.5
	0.50	5	81-86	84	2	2.3
IN-B5067	0.05 (LOQ)	5	76-90	85	6	7.0
	0.50	5	66-98	86	13	15
IN-F5438	0.05 (LOQ)	5	78-90	83	4	5.3
	0.50	5	76-84	80	3	3.8
<b>Well Water</b>						
Metsulfuron methyl (DPX-T6376)	0.05 (LOQ)	5	92-102	97	4	4.3
	0.50	5	93-102	97	4	4.2
IN-A4098	0.05 (LOQ)	5	96-112	105	6	5.8
	0.50	5	89-109	97	8	7.7
IN-D5119	0.05 (LOQ)	5	90-108	101	7	6.6
	0.50	5	94-102	99	3	3.0
IN-00581	0.05 (LOQ)	5	92-111	102	8	8.0
	0.50	5	89-102	97	6	5.9
IN-NC148	0.05 (LOQ)	5	96-104	100	4	3.9
	0.50	5	91-99	96	3	3.1
IN-B5685	0.05 (LOQ)	5	94-110	101	7	7.0
	0.50	5	84-96	91	5	5.2
IN-B5067	0.05 (LOQ)	5	80-102	91	9	9.3
	0.50	5	79-96	88	7	7.7
IN-F5438	0.05 (LOQ)	5	94-100	98	3	3.0
	0.50	5	91-99	96	3	3.2
<b>Creek Water (Surface)</b>						

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Metsulfuron methyl (DPX-T6376)	0.05 (LOQ)	5	93-101	96	4	3.9
	0.50	5	94-103	99	3	3.4
IN-A4098	0.05 (LOQ)	5	90-103	95	6	6.4
	0.50	5	86-97	90	4	4.8
IN-D5119	0.05 (LOQ)	5	99-110	103	4	4.2
	0.50	5	94-104	98	4	3.7
IN-00581	0.05 (LOQ)	5	96-107	99	5	4.8
	0.50	5	88-94	90	2	2.5
IN-NC148	0.05 (LOQ)	5	99-110	104	5	4.9
	0.50	5	94-104	99	4	4.4
IN-B5685	0.05 (LOQ)	5	87-95	91	3	3.7
	0.50	5	82-91	85	4	4.3
IN-B5067	0.05 (LOQ)	5	74-92	87	8	8.6
	0.50	5	80-107	99	11	11
IN-F5438	0.05 (LOQ)	5	92-97	94	2	2.0
	0.50	5	91-99	95	3	3.1

Data (recovery results) were obtained from p. 9; Table 1, pp. 28-43 of MRID 49585702. Standard deviations were reviewer-calculated based on individual recovery data reported in the study report (see DER Attachment 2). The study authors provided means and RSDs for all sample sets. Reported sample recoveries in the study report were corrected for any residues found in the matrix blanks; only residues of IN-D5119 were detected in the controls (tap and well waters only; p. 21; Figure 5, pp. 74, 98).

1 Surface water was obtained from White Clay Creek, Newark, Delaware, and fully characterized (p. 15; Appendix 5, pp. 158-159 of MRID 49585702). Ground water was obtained from a well in Kemblesville, Pennsylvania. Drinking water was obtained as either tap water from Stine-Haskell Research Center, Newark, Delaware, or bottled water from a local grocery store (it was not clear which source of drinking water was used). No physical characterization of the ground and drinking water samples was performed.

2 Two parent-daughter ion transitions monitored for each analyte (quantitative and confirmatory, respectively):  $m/z$  380.0  $\rightarrow$  138.8 and  $m/z$  380.0  $\rightarrow$  107.1 for metsulfuron methyl;  $m/z$  141.0  $\rightarrow$  57.3 and  $m/z$  141.0  $\rightarrow$  43.0 for IN-A4098;  $m/z$  199.85  $\rightarrow$  91.9 and  $m/z$  199.85  $\rightarrow$  155.9 for IN-D5119;  $m/z$  181.9  $\rightarrow$  42.0 and  $m/z$  182.1  $\rightarrow$  105.7 for IN-00581;  $m/z$  341.8  $\rightarrow$  181.9 and  $m/z$  341.8  $\rightarrow$  42.1 for IN-NC148;  $m/z$  257.3  $\rightarrow$  42.0 and  $m/z$  257.3  $\rightarrow$  182.7 for IN-B5685;  $m/z$  366.1  $\rightarrow$  125.1 and  $m/z$  366.1  $\rightarrow$  42.1 for IN-B5067; and  $m/z$  366.1  $\rightarrow$  155.9 and  $m/z$  366.1  $\rightarrow$  200.3 for IN-F5438 (pp. 18-20; Appendix 4, pp. 124-156 of MRID 49585702). No confirmation ion recovery data was reported; confirmation of analytes was performed by calculating the ratios of the quantitative and confirmation ion intensities (pp. 24-25).

**Table 3. Independent Validation Method Recoveries for Metsulfuron Methyl (DPX-T6376) and Metabolites, IN-A4098, IN-D5119, IN-00581, IN-NC148, IN-B5685, IN-B5067 and IN-F5438, in Water.<sup>1,2</sup>**

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
<b>Tap Water</b>						
Quantitative Transition						
Metsulfuron methyl (DPX-T6376)	0.05 (LOQ)	5	88-108	101	7.6	8
	0.50	5	86-88	87	0.9	1
IN-A4098	0.05 (LOQ)	5	92-100	95	3.6	4
	0.50	5	98-104	101	2.3	2
IN-D5119	0.05 (LOQ)	5	100-119	110	7.7	7
	0.50	5	103-110	106	2.8	3
IN-00581	0.05 (LOQ)	5	118-125	120	2.7	2
	0.50	5	109-115	111	2.4	2
IN-NC148	0.05 (LOQ)	5	109-127	118	6.5	6
	0.50	5	102-111	104	3.4	3
IN-B5685	0.05 (LOQ)	5	104-114	109	3.8	3
	0.50	5	100-112	106	4.3	4
IN-B5067	0.05 (LOQ)	5	87-120	109	13.1	12
	0.50	5	70-85	74	6.5	9
IN-F5438	0.05 (LOQ)	5	93-108	99	6.6	7
	0.50	5	81-90	86	3.2	4
Confirmatory Ion Transition						
Metsulfuron methyl (DPX-T6376)	0.05 (LOQ)	5	95-114	106	7.1	7
	0.50	5	87-95	90	3.2	4
IN-A4098	0.05 (LOQ)	5	82-100	92	4.2	4
	0.50	5	100-105	102	2.1	2
IN-D5119	0.05 (LOQ)	5	96-113	104	6.7	6
	0.50	5	99-108	104	3.3	3
IN-00581	0.05 (LOQ)	5	112-125	119	4.8	4
	0.50	5	108-119	113	4.3	4
IN-NC148	0.05 (LOQ)	5	107-124	115	7.1	6
	0.50	5	101-104	103	1.2	1
IN-B5685	0.05 (LOQ)	5	98-116	109	7.3	7
	0.50	5	105-110	106	2.1	2
IN-B5067	0.05 (LOQ)	5	95-120	111	9.8	9
	0.50	5	74-81	76	3.0	4
IN-F5438	0.05 (LOQ)	5	93-108	97	6.3	6
	0.50	5	83-99	89	5.9	7
<b>Ground Water</b>						



Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
<b>Quantitative Ion Transition</b>						
Metsulfuron methyl (DPX-T6376)	0.05 (LOQ)	5	87-102	93	6.2	7
	0.50	5	89-102	94	5.4	6
IN-A4098	0.05 (LOQ)	5	83-103	94	10.6	11
	0.50	5	82-108	100	11.1	11
IN-D5119	0.05 (LOQ)	5	106-119	115	5.0	4
	0.50	5	86-114	104	10.9	10
IN-00581	0.05 (LOQ)	5	109-116	113	2.7	2
	0.50	5	82-119	110	16.0	15
IN-NC148	0.05 (LOQ)	5	114-120	118	2.6	2
	0.50	5	105-110	108	2.0	2
IN-B5685	0.05 (LOQ)	5	112-120	116	3.2	3
	0.50	5	101-115	111	6.0	5
IN-B5067 <sup>3</sup>	0.05 (LOQ)	5	71-84	75	5.2	7
	0.50	5	70-102	83	16.3	20
IN-F5438	0.05 (LOQ)	5	82-101	91	7.3	8
	0.50	5	79-104	92	10.0	11
<b>Confirmatory Ion Transition</b>						
Metsulfuron methyl (DPX-T6376)	0.05 (LOQ)	5	92-101	96	3.4	4
	0.50	5	91-105	96	5.7	6
IN-A4098	0.05 (LOQ)	5	84-111	98	10.9	11
	0.50	5	80-108	100	11.7	12
IN-D5119	0.05 (LOQ)	5	113-120	116	2.8	2
	0.50	5	83-117	106	13.5	13
IN-00581	0.05 (LOQ)	5	114-121	118	3.2	3
	0.50	5	81-119	111	16.9	15
IN-NC148	0.05 (LOQ)	5	114-120	118	2.5	2
	0.50	5	102-109	105	2.8	3
IN-B5685	0.05 (LOQ)	5	116-120	118	2.0	2
	0.50	5	110-120	113	4.0	4
IN-B5067 <sup>3</sup>	0.05 (LOQ)	5	71-86	77	6.1	8
	0.50	5	72-104	84	16.0	19
IN-F5438	0.05 (LOQ)	5	86-103	93	6.6	7
	0.50	5	78-101	93	8.6	9
<b>Surface Water</b>						
<b>Quantitative Ion Transition</b>						
Metsulfuron methyl (DPX-T6376)	0.05 (LOQ)	5	94-113	102	7.8	8
	0.50	5	87-100	95	5.2	6
IN-A4098	0.05 (LOQ)	5	115-120	118	1.7	1
	0.50	5	109-116	112	2.8	3

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
IN-D5119	0.05 (LOQ)	5	89-103	95	5.3	6
	0.50	5	79-88	83	4.6	6
IN-00581	0.05 (LOQ)	5	87-112	100	11.7	12
	0.50	5	65-75	70	4.4	6
IN-NC148 <sup>3</sup>	0.05 (LOQ)	5	69-80	73	4.2	6
	0.50	5	93-125	114	12.8	11
IN-B5685	0.05 (LOQ)	5	70-89	80	8.6	11
	0.50	5	70-79	74	4.4	6
IN-B5067 <sup>3</sup>	0.05 (LOQ)	5	106-120	116	6.0	5
	0.50	5	66-98	88	12.6	14
IN-F5438 <sup>3</sup>	0.05 (LOQ)	5	96-104	101	3.3	3
	0.50	5	86-115	106	12.8	12
Confirmatory Ion Transition						
Metsulfuron methyl (DPX-T6376)	0.05 (LOQ)	5	101-120	109	8.4	8
	0.50	5	85-101	95	6.3	7
IN-A4098	0.05 (LOQ)	5	114-119	117	1.9	2
	0.50	5	110-119	114	3.8	3
IN-D5119	0.05 (LOQ)	5	87-103	97	6.0	6
	0.50	5	78-89	82	5.1	6
IN-00581	0.05 (LOQ)	5	73-98	90	9.8	11
	0.50	5	64-76	69	4.9	7
IN-NC148 <sup>3</sup>	0.05 (LOQ)	5	77-115	98	14.1	14
	0.50	5	102-123	116	8.1	7
IN-B5685	0.05 (LOQ)	5	71-84	78	5.8	7
	0.50	5	67-77	72	4.7	6
IN-B5067 <sup>3</sup>	0.05 (LOQ)	2 <sup>4</sup>	114 116	115	1.2	1
	0.50	5	72-102	90	13.1	15
IN-F5438 <sup>3</sup>	0.05 (LOQ)	5	91-108	101	6.2	6
	0.50	5	83-1169	103	13.1	13

Data (uncorrected recovery results; pp. 27-28) were obtained from pp. 29-32; Tables 1-24, pp. 35-82 of MRID 49585703. All results were from the first trial, except where noted.

1 The surface water was collected from Goose River and submitted for characterization by ABS Labs/Clark (p. 22; Appendix 2, pp. 243-245). The ground water was submitted for characterization by Morse Laboratories/Dupont (the source was not specified). The drinking (tap) water was collected from the test facility. Only the surface and ground water matrices were fully characterized (Agvise Laboratories, Northwood, North Dakota).

2 Two parent-daughter ion transitions monitored for each analyte (quantitative and confirmatory, respectively):  $m/z$  380.0  $\rightarrow$  138.8 and  $m/z$  380.0  $\rightarrow$  107.1 for metsulfuron methyl;  $m/z$  141.0  $\rightarrow$  57.3 and  $m/z$  141.0  $\rightarrow$  43.0 for IN-A4098;  $m/z$  199.85  $\rightarrow$  91.9 and  $m/z$  199.85  $\rightarrow$  155.9 for IN-D5119;  $m/z$  181.9  $\rightarrow$  42.0 and  $m/z$  182.1  $\rightarrow$  105.7 for IN-00581;  $m/z$  341.8  $\rightarrow$  181.9 and  $m/z$  341.8  $\rightarrow$  42.1 for IN-NC148;  $m/z$  257.3  $\rightarrow$  42.0 and  $m/z$  257.3  $\rightarrow$  182.7 for IN-B5685;  $m/z$  366.1  $\rightarrow$  125.1 and  $m/z$  366.1  $\rightarrow$  42.1 for IN-B5067; and  $m/z$  366.1  $\rightarrow$  155.9 and  $m/z$  366.1  $\rightarrow$  200.3 for IN-F5438 (pp. 25-26 of MRID 49585703).

3 Second trial (see Reviewer Comment #1).

4 Recoveries from three of the five samples were not reported because the samples showed a signal to noise ratio of  $<5$  and were not reported in the final validation (Table 21, p. 76 of MRID 49585703).

### III. Method Characteristics

In both the ECM and ILV, the LOQ for all analytes was reported as 0.050 µg/L (0.050 ppb; pp. 8-9, 23 of MRID 49585702; pp. 12, 17 of MRID 49585703). In the ECM, the LOQ was defined as the lowest fortification level which obtained average recoveries of 70-120% and a RSD <20%. The LOQ also corresponded to the fortification in which analyte peak heights were consistently *ca.* 10-20 times the signal in the control at the retention time of the analyte for the lowest responding analyte. The LOD was estimated for each analyte based on signal-to-noise. The LOD was defined as the concentration of IN-A4098, the least responsive analyte, at which analyte peaks were approximately three times the chromatographic baseline noise observed near the retention time or approximately 1/3 the concentration of the LOQ. The LOD for all analytes was estimated as 0.02 µg/L (0.02 ppb). In the ILV, the LOQ and LOD were reported from the ECM, and no justification was provided.

**Table 4. Method Characteristics**

		Metsulfuron methyl (DPX-T6376)	IN-A4098	IN-D5119	IN-00581	IN-NC148	IN-B5685	IN-B5067	IN-F5438	
Limit of Quantitation (LOQ)	0.05 µg/L									
Limit of Detection (LOD)	0.02 µg/L									
Linearity (calibration curve $r^2$ and concentration range)	ECM <sup>1</sup>	$r^2 = 0.9994$	$r^2 = 0.9989$	$r^2 = 0.9999$	$r^2 = 0.9974$	$r^2 = 0.9975$	$r^2 = 0.9991$	$r^2 = 0.9997$	$r^2 = 0.9996$	
	ILV <sup>2</sup>	$r^2 = \mathbf{0.9902}$ (Q) $r^2 = \mathbf{0.9912}$ (C)	$r^2 = \mathbf{0.9940}$ (Q) $r^2 = \mathbf{0.9898}$ (C)	$r^2 = 0.9970$ (Q) $r^2 = \mathbf{0.9938}$ (C)	$r^2 = 0.9970$ (Q) $r^2 = \mathbf{0.9936}$ (C)	$r^2 = 0.9962$ (Q) $r^2 = 0.9956$ (C)	$r^2 = 0.9976$ (Q) $r^2 = 0.9972$ (C)	$r^2 = \mathbf{0.9857}$ (Q) $r^2 = \mathbf{0.9803}$ (C)	$r^2 = \mathbf{0.9890}$ (Q) $r^2 = \mathbf{0.9912}$ (C)	
	Conc. Range	0.25-15 ng/mL								
Repeatable	ECM	Yes at the LOQ and 10×LOQ (n=5) in drinking, ground and surface water matrices.								
	ILV	Yes at the LOQ and 10×LOQ (n=5) in drinking, ground and surface water matrices.			Yes at the LOQ and 10×LOQ (n=5) in drinking, ground and surface water matrices, except confirmation ion analysis at 10×LOQ in surface water (mean recovery <b>69%</b> ).	Yes at the LOQ and 10×LOQ (n=5) in drinking, ground and surface water matrices.		Yes at the LOQ and 10×LOQ (n=5) in drinking, ground and surface water matrices, except n=2 for confirmation ion analysis at the LOQ in surface water.	Yes at the LOQ and 10×LOQ (n=5) in drinking, ground and surface water matrices.	
Reproducible	Tap water	Yes (first trial)	Yes (first trial)	Yes (first trial)	Yes (first trial)	Yes (first trial)	Yes (first trial)	Yes (first trial)	Yes (first trial)	
	Ground water					Yes		Yes (second trial)		
	Surface water					Yes (second trial)		Yes (second trial)		
Specific	ECM	Only the quantitative ion results and chromatograms were provided. Confirmation of results was based on the acceptable ratios of ion intensity (±30%).								
		Yes, no matrix interferences were observed.	Yes, no matrix interferences were observed in surface water, and matrix interferences	Yes, no matrix interferences were observed; however, multiple compounds were seen in the	Yes, no matrix interferences were observed. Low purity of the IN-NC148 standard was	Yes, no matrix interferences were observed; however, baseline noise was significant at the LOQ	Yes, no matrix interferences were observed.			

			were <i>ca.</i> 5% and 30% of the LOQ in drinking and ground water, respectively.	chromatograms, one of which eluted at a retention time close to the analyte (all matrices)	noted (77%).	(drinking and surface water).	
	ILV	Quantitative and confirmatory ion results and chromatograms were provided.					
		Yes, matrix interferences were <1% of the LOQ.	Yes, matrix interferences were <5% of the LOQ in drinking and surface water, and matrix interferences were <i>ca.</i> 10% of the LOQ in ground water.	Yes, matrix interferences were <5% of the LOQ in drinking and ground water, and matrix interferences were <10% of the LOQ in surface water.	Yes, matrix interferences were <1% of the LOQ in drinking water, and matrix interferences were <i>ca.</i> 30% of the LOQ in ground and surface water.	Yes, matrix interferences were <1% of the LOQ in drinking and surface water, and matrix interferences were <i>ca.</i> 30% of the LOQ in ground water  Low purity of the IN-NC148 standard was noted (82.7%).	Yes, matrix interferences were <1% of the LOQ.

Data were obtained from pp. 8-9, 15, 23-26; Table 1, pp. 28-43; Figure 3, pp. 59-61; Figure 5, pp. 74-109 of MRID 49585702; pp. 12, 17, 29-32; Tables 1-24, pp. 35-82; Figures 9-33, pp. 131-234; Appendix 1, p. 239 of MRID 49585703. Q = quantitative ion; C = confirmatory ion.

1 In the ECM, standard curves and linearity equations and coefficients were provided for the quantitative ion transition only (Figure 3, pp. 59-61 of MRID 49585702).

2 In the ILV, standard curves were weighted 1/x. ILV  $r^2$  values are reviewer-generated for all analytes from reported  $r$  values of 0.9928-0.9988 (Q) and 0.9901-0.9986 (C; calculated from data in Figure 9, pp. 131-138 of MRID 49585703; see DER Attachment 2).

A confirmation method is not necessarily required for methods which employ LC/MS as the primary identification method.

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

#### IV. Method Deficiencies and Reviewer's Comments

1. In the ILV, the modification of the ECM method to include washing the SPE column with pH 3 water instead of neutral water was necessary in order to achieve acceptable recoveries of IN-B5067 in ground and surface water and of IN-NC148 and IN-F5438 in surface water (pp. 16, 23-24, 28, 33 of MRID 49585703). This modification was made to minimize matrix suppression and was approved by the Study Monitor. The extraction was repeated for these analytes. The number of trials required to validate the method for these analytes in the affected matrices was not reported; however, the reviewer reported the number as the second trial. An updated ECM to include the procedure to minimize matrix suppression should be submitted.

Insignificant modifications to the ECM were listed in the ILV, including substitutions of instrumentation and slight differences in the  $m/z$  values of the parent-daughter ion transitions (pp. 23-26, 28 of MRID 49585703).

2. The estimations of the LOQ and LOD in the ECM were not based on scientifically acceptable procedures as defined in 40 CFR Part 136. The LOQ was defined as the lowest fortification level which obtained average recoveries of 70-120% and a RSD <20% (pp. 8-9, 23 of MRID 49585702). The LOQ also corresponded to the fortification in which analyte peak heights were consistently *ca.* 10-20 times the signal in the control at the retention time of the analyte for the lowest responding analyte. The LOD was estimated for each analyte based on signal-to-noise. The LOD was defined as the concentration of IN-A4098, the least responsive analyte, at which analyte peaks were approximately three times the chromatographic baseline noise observed near the retention time or approximately 1/3 the concentration of the LOQ. The LOQ and LOD were not adequately supported by calculations.

Detection limits should not be based on the arbitrarily selected lowest concentration in the spiked samples. Additionally, the lowest toxicological levels of concern in water were not reported. An LOQ above toxicological level of concern results in an unacceptable method classification.

3. In the ILV, the mean recovery was <70% for the second ion transition of IN-00581 at 10×LOQ in surface water (mean recovery, 69%; pp. 29-32; Tables 1-24, pp. 35-82 of MRID 49585703). OCSPP guidelines recommend that mean recoveries are 70-120% for each analyte/fortification/matrix.
4. In the ILV, the number of samples was insufficient for the second ion transition of IN-B5067 at the LOQ in surface water ( $n = 2$ ; pp. 29-32; Tables 1-24, pp. 35-82 of MRID 49585703). OCSPP guidelines recommend a minimum of five spiked replicates to be analyzed at each concentration (*i.e.*, minimally, the LOQ and 10× LOQ) for each analyte.
5. In the ILV, linearity was not satisfactory for a majority of the linear regressions (see above). Linearity is satisfactory when  $r^2 \geq 0.995$ . Linear regressions were not matrix-matched.
6. The purity of the IN-NC148 standard was low in the ECM (77.0%) and ILV (82.7%; pp. 12, 24 of MRID 49585702; Appendix 1, p. 239 of MRID 49585703). The purity of the IN-

B5067 standard was also low in the ECM (89.4%). In the ECM, the method noted that the concentrations of the standards prepared with IN-NC148 needed to be adjusted for purity.

7. In the ECM, calculations allowed for recoveries to be corrected for any residues detected in the controls; only residues of IN-D5119 were detected in the controls (tap and well waters only; p. 21; Figure 5, pp. 74, 98 of MRID 49585702). In the ILV, calculations did not contain correction for residues in the controls (pp. 27-28 of MRID 49585703).
8. Some of the water matrices were not characterized in the ECM (ground and drinking) and ILV (drinking; p. 15; Appendix 5, pp. 158-159 of MRID 49585702; p. 22; Appendix 2, pp. 243-245 of MRID 49585703). The source of the ground water was not specified in the ILV.

It was not clear which source of drinking water was used in the ECM. The drinking water was obtained as either tap water from Stine-Haskell Research Center, Newark, Delaware, or bottled water from a local grocery store (p. 15 of MRID 49585702). In the results text, tables and chromatograms of ECM, the drinking water was referred to a tap water.

9. ILV representative chromatograms showed matrix interferences in the control and reagent blanks for almost all of the analyte/ion/matrix (Figures 10-33, pp. 139-234 of MRID 49585703). These interferences were reviewer-estimated at <1% of the LOQ for metsulfuron methyl, IN-B5685, IN-B5067 and IN-F5438 in all matrices/ions, 5-10% of the LOQ for IN-A4098 and IN-D5119 in all matrices/ions, <1% of the LOQ for IN-00581 (drinking) and IN-NC148 (drinking and surface), and *ca.* 30% of the LOQ for IN-00581 (ground). Percent recovery values for residues in the controls were also reported in the ILV report tables; this data also showed that matrix interferences were *ca.* 30% of the LOQ for IN-00581 in surface water and IN-NC148 in ground water (Tables 1-24, pp. 35-82). Interferences >20% of the LOQ were generally >50% of the LOD.

ECM representative chromatograms only showed matrix interferences in the controls for IN-D5119, *ca.* 5 and 30% of the LOQ in drinking and ground water, respectively (Figure 5, pp. 74-109 of MRID 49585702). However, the reviewer noted that the baseline noise was significant in the LOQ chromatograms of IN-B5067 in ground and surface water, and three additional compounds/peaks were observed in the chromatograms of IN-00581, one of which eluted close of the retention time of the analyte. Reagent blanks were not included in the ECM.

10. The ion ratios were calculated in the ECM as confirmation method (pp. 24-26; Appendix 3, pp. 117-123 of MRID 49585702). All fortified samples yielded ion ratios of  $\pm 30\%$  of the average ratio for all calibration standards.
11. The reviewer noted two typographical errors in the ECM MRID 49585702: the SPE column dimension was reported as "0.5g/12cc" in the Equipment Section (p. 10) and "20-cc. 0.5-g" in the Analytical Procedure (p. 16); and the analyte IN-D5119 was reported as "IN-D51129" in the well water control chromatogram (p. 98). The typographical error involving the SPE column should be corrected since this equipment was noted as "Do not substitute" (p. 10). The reviewer reported the SPE column as 0.5g/20cc based on the equipment used by the ILV (p. 23 of MRID 49585703).

12. It was reported for the ILV that a single analyst completed two sample sets consisting of 12 samples per set in an 8-hour workday (p. 33 of MRID 49585703). The LC/MS/MS analysis was performed unattended the next day.
13. Communications between the ILV study author and ECM study monitor were limited to approval of each validation set, discussion of the failure of the trials for IN-B5067 in ground and surface water and of IN-NC148 and IN-F5438 in surface water, and the approval of the modification of the ECM method to include washing the SPE column with pH 3 water instead of neutral water (p. 33 of MRID 49585703).

## **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.



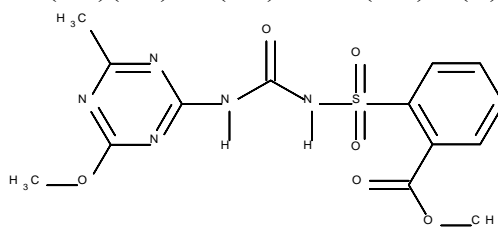
**Attachment 1: Chemical Names and Structures****Metsulfuron methyl (DPX-T6376, DPX-T6376-283)**

**IUPAC Name:** Methyl 2-(4-methoxy-6-methyl-1,3,5-triazin-2-ylcarbamoylsulfamoyl)benzoate

**CAS Name:** Methyl 2-[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]amino]sulfonyl]benzoate

**CAS Number:** 74223-64-6

**SMILES String:** COC(=O)c1ccccc1S(=O)(=O)NC(=O)Nc2nc(OC)nc(C)n2

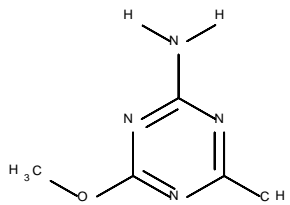
**IN-A4098 (IN-A4098-005)**

**IUPAC Name:** 4-Methoxy-6-methyl-1,3,5-triazin-2-amine

**CAS Name:** Not reported

**CAS Number:** 1668-54-8

**SMILES String:** [H]N([H])c1nc(nc(n1)OC)C

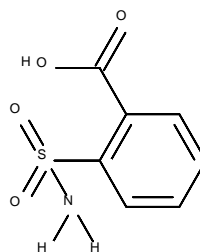
**IN-D5119 (IN-D5119-001)**

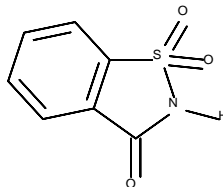
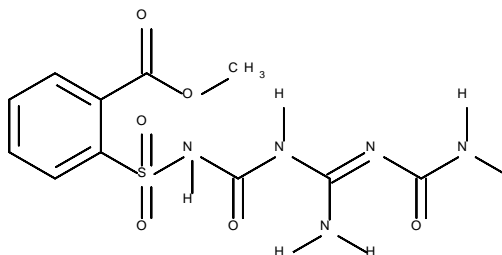
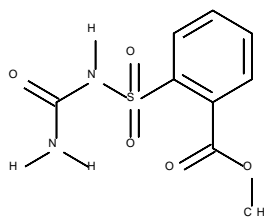
**IUPAC Name:** 2-(Aminosulfonyl)benzoic acid

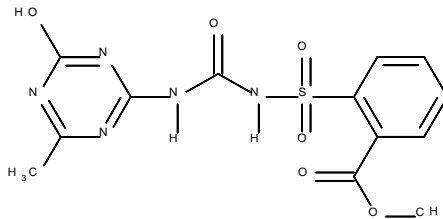
**CAS Name:** Not reported

**CAS Number:** 632-24-6

**SMILES String:** [H]N([H])S(=O)(=O)c1ccccc1C(=O)O



**IN-00581 (IN-00581-006)****IUPAC Name:** 1,1-Dioxide-1,2-benzisothiazol-3(2H)-one**CAS Name:** Not reported**CAS Number:** 81-07-2**SMILES String:** [H]N1C(=O)c2ccccc2S1(=O)=O**IN-NC148 (IN-NC148-001)****IUPAC Name:** Not reported**CAS Name:** Benzoic acid, 2-[[[[[(aminocarbonyl)amino]iminomethyl]amino]carbonyl]amino]sulfonyl]-, methyl ester**CAS Number:** 223907-38-8**SMILES String:** [H]N([H])/C(=N\C(=O)N([H])[H])/N([H])C(=O)N([H])S(=O)(=O)c1cccc1C(=O)OC**IN-B5685 (IN-B5685-017)****IUPAC Name:** Methyl 2-[[[(aminocarbonyl)amino]sulfonyl]benzoate**CAS Name:** Not reported**CAS Number:** 95473-30-6**SMILES String:** [H]N([H])C(=O)N([H])S(=O)(=O)c1cccc1C(=O)OC

**IN-B5067 (IN-B5067-005)****IUPAC Name:** Not reported**CAS Name:** Methyl 2-[[[(1,4-dihydro-6-methyl-4-oxo-1,3,5-triazin-2-yl)amino]carbonyl]amino]sulfonyl]benzoate**CAS Number:** 126312-31-0**SMILES String:** [H]N(c1nc(nc(n1)O)C)C(=O)N([H])S(=O)(=O)c2ccccc2C(=O)OC**IN-F5438 (IN-F5438-002)****IUPAC Name:** Not reported**CAS Name:** Benzoic acid, 3-[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]amino]sulfonyl]-**CAS Number:** 137224-60-3**SMILES String:** [H]N(c1nc(nc(n1)OC)C)C(=O)N([H])S(=O)(=O)c2ccccc2C(=O)O