

**Test Material:** Nicosulfuron

**MRID:** 48790911

**Title:** Analytical method for the determination of nicosulfuron (DPX-V9360), IN-V9367 and IN-J0290 in water using HPLC/ESI-MS/MS.

**MRID:** 48790913

**Title:** Independent laboratory validation of DuPont-32132, "Analytical method for the determination of nicosulfuron (DPX-V9360), IN-V9367 and IN-J0290 in water by HPLC/ESI-MS/MS".

**EPA PC Code:** 129008

**OCSPP Guideline:** 850.6100


**For CDM Smith**

**Primary Reviewer:** Lisa Muto

**Signature:** 

**Date:** 3/20/14

**Secondary Reviewer:** Dan Hunt

**Signature:** 

**Date:** 3/20/14

**QC/QA Manager:** Joan Gaidos

**Signature:** 

**Date:** 3/20/14

**Analytical method for nicosulfuron in water**

**Reports:** ECM: EPA MRID No. 48790911. Morgan, E.A. and Cabusas, M.E.Y. 2012. Analytical method for the determination of nicosulfuron (DPX-V9360), IN-V9367 and IN-J0290 in water using HPLC/ESI-MS/MS. Laboratory Project ID: DuPont-32132. 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; 54 pages. Final report issued March 16, 2012.

ILV: EPA MRID No. 48790913. Robaugh, D.A. 2012. Independent laboratory validation of DuPont-32132, "Analytical method for the determination of nicosulfuron (DPX-V9360), IN-V9367 and IN-J0290 in water by HPLC/ESI-MS/MS". DuPont Study No: DuPont-32578. Pyxant Labs Project ID: 2388. Report prepared by Pyxant Labs Inc., Colorado Springs, Colorado; sponsored and submitted by E.I. du Pont de Nemours and Company, Newark, Delaware; 92 pages. Final report issued March 30, 2012.

**Document No.:** MRIDs 48790911 & 48790913

**Guideline:** 850.6100

**Statements:** ECM: The study was conducted in a GLP compliant facility following standard operating procedures but not with the restriction of compliance with USEPA FIFRA GLP standards, 40 CFR, Part 160 (p. 3). 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 GLP standards (40 CFR Part 160) and OECD Principles of GLP (p. 3). 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 **Acceptable**. However, the LOD and LOQ of nicosulfuron in this method for water may not be low enough to resolve nicosulfuron at toxicologically relevant concentrations for aquatic plants. There appears to be other methods available with lower LOQs for nicosulfuron in water.

**PC Code:** 129008

**Reviewer:** Gabriel Rothman  
Environmental Scientist

**Signature:**   
**Date:** May 5, 2014

**All page citations refer to MRID 48790911 (ECM) unless otherwise noted.**

**Executive Summary**

This analytical method, Laboratory Project ID: DuPont-32132, is designed for the quantitative determination of nicosulfuron, IN-V9367 and IN-J0290 in water using LC/MS/MS. The method is quantitative for nicosulfuron and its transformation products at the stated LOQ of 0.10 ng/mL.

The lowest toxicological level of concern in water was not reported. No major issues were discovered by the independent laboratory.

**Table 1. Analytical Method Summary**

Analyte(s) by Pesticide	MRID		EPA Review	Matrix	Method Date	Registrant	Analysis	Limit of Quantitation (LOQ)
	Environmental Chemistry Method	Independent Laboratory Validation						
Nicosulfuron (DPX-V9360), IN-V9367 and IN-J0290	48790911	48790913		Water	3/16/2012	E.I. du Pont de Nemours and Company	LC/MS/MS	0.10 ng/mL

## I. Principle of the Method

Samples (20 g) were acidified to a pH of 3.5 to 4.0 with 1 M formic acid then filtered through an Oasis<sup>®</sup> HLB SPE cartridge (pp. 9, 14, 18). The analytes were eluted with methanol:0.50 M ammonium hydroxide (9:1, v:v). The eluent was acidified to a pH of *ca.* 4.5 with 1 mL of 1 M formic acid and reduced to  $\leq 2$  mL under a stream of nitrogen at *ca.* 25-30°C. The volume was adjusted to 2 mL with water, if necessary, then to 5 mL with 5 mM ammonium formate:methanol (19:1, v:v). The resulting solution was filtered (13-mm, 0.45- $\mu$ m PTFE filter) prior to analysis.

Samples were analyzed for nicosulfuron, IN-V9367 and IN-J0290 by reversed-phase HPLC (Phenomenex<sup>®</sup> Luna, 4.6 mm x 150 mm, 3  $\mu$ m phenyl-hexyl column) using a mobile phase gradient of (A) 0.1 mM formic acid in 0.1 mM ammonium formate and (B) methanol [percent A:B at 0.00 min. 95:5 (v:v), 4.00 min. 50:50, 4.10 min. 35:65, 7.00 min. 10:90, 7.10-10.00 min. 5:95, 10.10-12.00 min. 95:5] with MS/MS-ESI<sup>+</sup> detection and Multiple Reaction Monitoring (MRM; pp. 9, 10, 14, 20). The ratios of two MRM parent-to-daughter ions were monitored (quantitative and confirmatory) per analyte. Injection volumes were 10-20  $\mu$ L; an injection volume of 10  $\mu$ L was indicted for pond water in order to reduce matrix effects.

The LOQ was the same in the ECM and ILV (0.10 ng/mL; p. 24; p. 11 of MRID 48790913). In the ECM, the LOD was estimated to be one-third of the LOQ, *ca.* 0.03 ng/mL. The LOD was not defined in the ILV.

## II. Recovery Findings

ECM (MRID 48790911): Mean recoveries and relative standard deviations (RSD) were within guideline requirements (mean 70-120%; RSD  $\leq 20\%$ ) for analysis of nicosulfuron, IN-V9367 and IN-J0290 in tap (drinking) water, pond water and well water (p. 24; Table 1, pp. 28-36). Confirmation of the identified peaks was based on the relative ratio of its two parent-to-daughter ion transitions; all of these ratios were accurate within the range of the equivalent ion ratios of the calibration standards (pp. 25-27).

ILV (MRID 48790913): Mean recoveries and RSDs were within guideline requirements for analysis of nicosulfuron, IN-V9367 and IN-J0290 in pond water (p. 18; Tables 4-6, pp. 24-26).

Quantitative ion and confirmatory ion results were comparable. The method was validated with the second trial (p. 11; see Comment #2).

**Table 2. Initial Validation Method Recoveries for Analytes in Water**

Analyte	Fortification Level (ng/mL)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
<b>Well Water (Kimbleville, Pennsylvania)</b>						
Nicosulfuron (DPX-V9360)	0.10 (LOQ)	5	90-94	92	1.5	1.5
	1.0	5	84-96	91	4.3	4.7
IN-V9367	0.10 (LOQ)	5	93-104	98	4.7	4.8
	1.0	5	100-112	106	4.7	4.4
IN-J0290	0.10 (LOQ)	5	86-91	88	2.1	2.4
	1.0	5	89-95	93	2.4	2.6
<b>Lums Pond Water (Lums Pond, Delaware)</b>						
Nicosulfuron (DPX-V9360)	0.10 (LOQ)	5	71-94	82	8.5	10.4
	1.0	5	91-109	98	6.9	7.1
IN-V9367	0.10 (LOQ)	5	74-92	82	8.4	10.3
	1.0	5	90-112	97	9.3	9.6
IN-J0290	0.10 (LOQ)	5	79-88	83	3.8	4.6
	1.0	5	71-82	77	5.5	7.1
<b>Tap (Drinking) Water (Newark, Delaware)</b>						
Nicosulfuron (DPX-V9360)	0.10 (LOQ)	5	82-89	85	2.5	2.9
	1.0	5	88-102	95	5.3	5.5
IN-V9367	0.10 (LOQ)	5	88-94	92	3.1	3.4
	1.0	5	98-110	106	5.2	4.9
IN-J0290	0.10 (LOQ)	5	68-83	77	6	7
	1.0	5	74-87	83	5.3	6.4

Data were obtained from p. 24 and Table 1, pp. 28-36 in the study report.

**Table 3. Independent Validation Method Recoveries for Analytes in Pond Water**

Analyte	Fortification Level (ng/mL)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%) <sup>1</sup>
<b>Water Recoveries – Quantitative ion</b>						
Nicosulfuron (DPX-V9360)	0.10 (LOQ)	5	82-94	89	4.6	5
	1.0	5	84-92	88	3.2	4
IN-V9367	0.10 (LOQ)	5	85-92	90	3.1	3
	1.0	5	94-99	97	2.2	2
IN-J0290	0.10 (LOQ)	5	79-95	87	6.0	7
	1.0	5	75-80	78	1.9	2
<b>Confirmation ion</b>						
Nicosulfuron (DPX-V9360)	0.10 (LOQ)	5	86-100	94	5.6	--
	1.0	5	81-91	86	4.0	--
IN-V9367	0.10 (LOQ)	5	90-93	92	1.1	--
	1.0	5	94-103	99	3.2	--
IN-J0290	0.10 (LOQ)	5	72-84	77	4.4	--
	1.0	5	75-82	77	2.9	--

Data were obtained from p. 18; Tables 4-6, pp. 24-26 of MRID 48790913.

1 Reviewer-calculated for quantitative ion; confirmation ion raw data not supplied in ILV study report (see DER Attachment 2).

### III. Method Characteristics

The LOQ was the same in the ECM and ILV (0.10 ng/mL). In the ECM, the LOQ was defined as the lowest fortification level which obtained average recoveries of 70-110% and a RSD <20% (p. 24). The LOD was not determined experimentally; however, it was estimated as one-third of the LOQ (*ca.* 0.03 ng/mL). In the ILV, the LOQ was reported from the ECM, and no justification was provided (p. 11 of MRID 48790913). The LOD was not defined.

**Table 4. Method Characteristics**

	<b>Nicosulfuron</b>	<b>IN-V9367</b>	<b>IN-J0290</b>
Limit of Quantitation (LOQ)	0.1 ng/mL	0.1 ng/mL	0.1 ng/mL
Limit of Detection (LOD)	<i>ca.</i> 0.03 ng/mL	<i>ca.</i> 0.03 ng/mL	<i>ca.</i> 0.03 ng/mL
Linearity (calibration curve $r^2$ and concentration range)	$r^2 = 0.9996^1$ (0.25-10 ng/mL)	$r^2 = 0.9951-0.9999^1$ (0.25-10 ng/mL)	$r^2 = 0.9929-0.9993^1$ (0.25-10 ng/mL)
Repeatable	Yes	Yes	Yes
Reproducible	Yes <sup>2</sup>	Yes <sup>2</sup>	Yes <sup>2</sup>
Specific	Yes	Yes	Yes

Data were obtained from p. 24; Figure 1, p. 37; Appendices 1-2, pp. 46-54.

1 ILV calibration curves yielded similar linearity,  $r^2 = 0.9974-0.9993$ , for concentration range of 0.25-10 ng/mL (see p. 17 and Figures 1-3, pp. 27-29 of MRID 48790913).

2 The ILV successfully validated the method at the LOQ in the second trial, after decreasing the injection volume to 10  $\mu$ L as specified in the ECM for pond water (see Comment #2).

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

1. It may be possible to obtain lower LOCs and LOQs for nicosulfuron in water using other methods. The reviewer found references to other LOQs determined for nicosulfuron of 0.01 ppb, approximately one order of one magnitude lower than this evaluated analytical method (Battaglin et al., 2001).
2. In general, toxicologically relevant concentrations of nicosulfuron are > 1.0 ppm range for aquatic animals. However, there is uncertainty with the toxicity of aquatic plants as this study has not been submitted to the Agency, but has been requested as part of the Registration Review program. Given nicosulfuron's phytotoxic mode of action, it is likely that aquatic plants will be far more sensitive to nicosulfuron exposure than aquatic animals. Therefore, the analytical method should possess the ability to measure the lowest concentrations possible such as indicated by Battaglin et al., 2001 to account for nicosulfuron's potential toxicity to aquatic plants.
3. In the ILV, Method Validation Trial 1 was unsuccessful (pp. 10, 17-18 and Appendix 3, p. 92 of MRID 48790913). After consultation with the Sponsor, the ILV decreased the HPLC injection volume to 10  $\mu$ L, as suggested in the ECM method for pond water (p. 20), and successfully validated the method with Trial 2.

4. The calibration standards in the ECM and ILV included only samples in the range of 0.25 ng/mL to 10 ng/mL when the LOQ for method validation was a 0.10 ng/mL spiking level; however, the linear regressions of the peak area versus concentration of the calibration standards were not used to calculate the analyte found (p. 22). The calibration standards were used to calculate Response Factors (concentration of standard/peak area counts) which were used to calculate the analyte found. Also, the peak areas of the samples were within range of the peak areas of the calibration standards, most likely due to the concentration procedures used during the method (Appendices 1-2, pp. 46-54).
5. Matrix characterization of the pond, well and drinking water in the ECM (p. 17) and pond water in the ILV was reported (Appendix 1, p. 84 of MRID 48790913).
6. The linear regression equations of the reviewer-generated calibration curves did not exactly match those reported in the ECM and ILV; however, the equations and  $r^2$  values were similar, in general.
7. The reviewer was unable to verify the recoveries for the ECM from data and equations provided in Appendices 1-2, pp. 46-54 of the study report. The equation [% recovery = (analyte found \* 100)/(fortification level)] was provided by the study authors; however, all of the significant figures of the “analyte found” data were not reported. For example, in the recoveries of IN-V9367 in the well water samples dosed at the LOQ, the test samples LOQ 1 and LOQ 3 had the same “analyte found” (0.10 ng/mL) and sample weight (20.00 g), but differing percent recoveries of 93% and 95%, respectively (Appendix 2, p. 53). The reviewer did not calculate “analyte found” from the raw data.
8. It was reported for the ILV that a single analyst completed a sample set consisting of 15 samples in *ca.* 8-10 hours or 2 calendar days (p. 19 of MRID 48790913).

## V. References

- Battaglin W.A., Furlong E.T., and M.R. Burkhardt., 2001. Concentration of Selected Sulfonylurea Sulfonamide, and Imidazolinone Herbicides, Other Pesticides, and Nutrients in 71 Streams, 5 Reservoir Outflows, and 25 Wells in the Midwestern United States, 1998. U.S. Geological Survey, Denver, CO.
- 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.

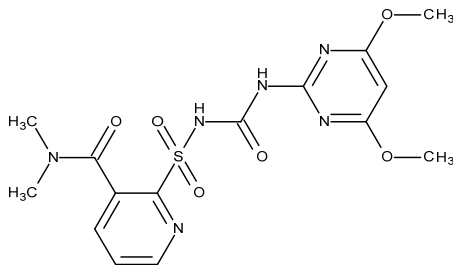
**Attachment 1: Chemical Names and Structures****Nicosulfuron; DPX-V9360**

**IUPAC Name:** 1-(4,6-Dimethoxypyrimidin-2-yl)-3-(3-dimethylcarbamoyl-2-pyridylsulfonyl)urea.  
2-[(4,6-Dimethoxypyrimidin-2-ylcarbamoyl)sulfamoyl]-N,N-dimethylnicotinamide.

**CAS Name:** 2-[[[(4,6-Dimethoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]-N,N-dimethyl-3-pyridinecarboxamide monohydrate.

**CAS Number:** 111991-09-4.

**SMILES String:** c1(c(nccc1)S(=O)(=O)NC(=O)Nc2nc(cc(n2)OC)OC)C(=O)N(C)C

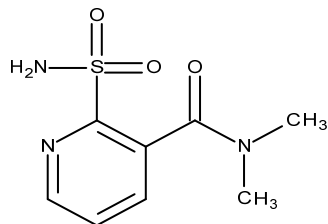
**IN-V9367**

**IUPAC Name:** Not reported.

**CAS Name:** 2-(Aminosulfonyl)-N,N-dimethyl-3-pyridinecarboxamide.

**CAS Number:** 112006-75-4.

**SMILES String:** c1(ccnc1S(=O)(=O)N)C(=O)N(C)C

**IN-J0290**

**IUPAC Name:** Not reported.

**CAS Name:** 4,6-Dimethoxy-2-pyrimidinamine.

**CAS Number:** 36315-01-2.

**SMILES String:** Nc1nc(cc(n1)OC)OC

