

**Test Material:** Nicosulfuron

**MRID:** 48790912

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

**MRID:** 48790914

**Title:** Independent laboratory validation of DuPont-33143, "Analytical method for the determination of nicosulfuron (DPX-V9360), IN-V9367 and IN-J0290 in soil using 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 soil**

**Reports:** ECM: EPA MRID No. 48790912. Cabusas, M.E.Y. 2012. Analytical method for the determination of nicosulfuron (DPX-V9360), IN-V9367 and IN-J0290 in soil using HPLC/ESI-MS/MS. Laboratory Project ID: DuPont-33143. 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; 50 pages. Final report issued March 16, 2012.

ILV: EPA MRID No. 48790914. Rogers, P. 2012. Independent laboratory validation of DuPont-33143, "Analytical method for the determination of nicosulfuron (DPX-V9360), IN-V9367 and IN-J0290 in soil using HPLC/ESI-MS/MS". DuPont Project ID: DuPont-33031. Alliance Pharma Project No.: 120103. Report prepared by Alliance Pharma, Inc., Malvern, Pennsylvania, sponsored and submitted by E.I. du Pont de Nemours and Company, Wilmington, Delaware; 52 pages. Final report issued March 22, 2012.

**Document No.:** MRIDs 48790912 & 48790914

**Guideline:** 850.6100

**Statements:** ECM: The study was not conducted with the restriction of compliance with USEPA FIFRA GLP standards, 40 CFR, Part 160, or OECD GLP (p. 3). Signed and dated Data Confidentiality, No Data Confidentiality, and GLP statements were provided (pp. 2-3). Certification of Authenticity and Quality Assurance statements were not provided.

ILV: The study was conducted in accordance with the USEPA FIFRA Good Laboratory Practice (GLP) standards (40 CFR Part 160) and OECD 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 soil were not low enough to resolve nicosulfuron at toxicologically relevant concentrations for plants.

**PC Code:** 129008

**Reviewer:** Gabriel Rothman  
Environmental Scientist

**Signature:** 

**Date:** May 5, 2014

**All page citations refer to MRID 48790912 (ECM) unless otherwise noted.**

**Executive Summary**

This analytical method, Laboratory Project ID: DuPont-33143, is designed for the quantitative determination of nicosulfuron, IN-V9367 and IN-J0290 in soil using LC/MS/MS. The method is quantitative for nicosulfuron and its transformation products at the stated LOQ of 1.0 µg/kg. The lowest toxicological level of concern in soil 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	48790912	48790914		Soil	3/16/2012	E.I. du Pont de Nemours and Company	LC/MS/MS	1.0 µg/kg

## I. Principle of the Method

Samples (10 g) were extracted twice by shaking for 15-20 minutes with 0.1 M ammonium carbonate:acetone (9:1, v:v) then centrifuged (pp. 8, 10, 12-13, 17-19). The supernatant was decanted and filtered. One-fourth of the combined extract was purified by passing through an Isolute<sup>®</sup> NH<sub>2</sub> solid-phase extraction (SPE) cartridge. The acetone was removed from the partially-purified extract by evaporation using nitrogen-evaporator (N<sub>2</sub>-vap) system at 30°C. The reduced extract was acidified to pH 3 with 1M formic acid then purified by passing through stacked Oasis<sup>™</sup> HLB and ENV<sup>+</sup><sup>®</sup> SPE cartridges. The analytes were eluted with methanol:1 M ammonium hydroxide (9:1, v:v) solution and methanol. The eluent was reduced to *ca.* 3 mL in an N<sub>2</sub>-vap system at 30°C. The final volume was adjusted to 5 mL with 5 mM ammonium formate:methanol (19:1, v:v). The resulting solution was filtered (25-mm, 0.45-µm PTFE filter) prior to analysis.

Samples were acidified (10 µL of 1M formic acid added to 990 µL of sample) then analyzed for nicosulfuron, IN-V9367 and IN-J0290 by reversed-phase HPLC (Phenomenex<sup>®</sup> Luna, 4.6 mm x 150 mm, 3 µm phenyl-hexyl column) using a mobile phase gradient of (A) 0.1 mM formic acid in 0.01 mM ammonium formate and (B) methanol [percent A:B at 0.0 min. 95.0:5.0 (v:v), 10-13 min. 5.0:95.0, 13.1-17 min. 95.0:5.0] with MS/MS-ESI<sup>+</sup> detection and Multiple Reaction Monitoring (MRM; pp. 8, 10, 13, 19-21). The ratios of two MRM parent-to-daughter ions were monitored (quantitative and confirmatory) per analyte. Injection volumes were 75 µL.

The LOQ was the same in the ECM and ILV (1.0 µg/kg; p. 24; p. 11 of MRID 48790914). In the ECM, the LOD was not experimentally determined (p. 24); however, was reported as one-third of the LOQ or 0.33 µg/kg for the Drummer clay loam soil (Appendix 4, pp. 47-49). In the ILV, the LOD was estimated to be 0.3 µg/kg (p. 11 of MRID 48790914).

## II. Recovery Findings

ECM (MRID 48790912): Mean recoveries and relative standard deviations (RSD) were within guideline requirements (mean 70-120%; RSD ≤20%) for analysis of nicosulfuron, IN-V9367 and IN-J0290 in clay loam, loam, silty loam and sandy loam soils (p. 23; Table 1, pp. 28-39). 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. 25-26).

ILV (MRID 48790914): Mean recoveries and RSDs were within guideline requirements for analysis of nicosulfuron, IN-V9367 and IN-J0290 in one soil (pp. 18-19; Tables 1-6, pp. 22-27). Quantitative ion and confirmatory ion results were comparable. The relative ratios of the two parent-to-daughter ion transitions were similar between the LOQ and 10xLOQ sample sets (Tables 7-9, pp. 28-30). The method was validated with the first trial (p. 10).

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

Analyte	Fortification Level (µg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
<b>Clay loam (Drummer #7 from Illinois)</b>						
Nicosulfuron (DPX-V9360)	1.0 (LOQ)	5	99-115	104	6.1	5.8
	10.0	5	95-106	101	4.3	4.2
IN-V9367	1.0 (LOQ)	5	85-100	90	7.1	7.9
	10.0	5	82-93	88	4.8	5.5
IN-J0290	1.0 (LOQ)	5	70-85	77	5.7	7.4
	10.0	5	71-78	75	3.0	4.0
<b>Loam (Mattapex #25 from Maryland)</b>						
Nicosulfuron (DPX-V9360)	1.0 (LOQ)	5	94-107	100	5.8	5.8
	10.0	5	96-110	101	5.9	5.9
IN-V9367	1.0 (LOQ)	5	78-94	87	6.5	7.6
	10.0	5	82-94	87	5.1	5.9
IN-J0290	1.0 (LOQ)	5	72-89	80	6.4	8.0
	10.0	5	76-85	81	3.8	4.8
<b>Silty loam (Namsheim from France)</b>						
Nicosulfuron (DPX-V9360)	1.0 (LOQ)	5	92-104	99	4.5	4.6
	10.0	5	96-105	99	3.7	3.7
IN-V9367	1.0 (LOQ)	5	85-105	97	7.8	8.0
	10.0	5	98-100	98	0.9	0.9
IN-J0290	1.0 (LOQ)	5	73-86	82	5.5	6.7
	10.0	5	83-85	84	1.0	1.2
<b>Sandy loam (Speyer 2.2 from Germany)</b>						
Nicosulfuron (DPX-V9360)	1.0 (LOQ)	5	94-113	106	8.3	7.8
	10.0	5	97-120	104	9.1	8.7
IN-V9367	1.0 (LOQ)	5	82-94	89	4.8	5.3
	10.0	5	82-96	88	5.8	6.6
IN-J0290	1.0 (LOQ)	5	78-92	88	5.8	6.6
	10.0	5	74-95	82	8.1	9.9

Data were obtained from p. 23; Table 1, pp. 28-39; and Appendix 4, pp. 47-49 in the study report.

**Table 3. Independent Validation Method Recoveries for Analytes in Soil**

Analyte	Fortification Level (µg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
<b>Soil Recoveries – Quantitative ion</b>						
Nicosulfuron (DPX-V9360)	1.0 (LOQ)	5	105-114	109	3.8	3
	10.0	5	97-108	103	4.8	5
IN-V9367	1.0 (LOQ)	5	72-84	77	5.4	7
	10.0	5	74-82	78	3.1	4
IN-J0290	1.0 (LOQ)	5	84-91	89	3.1	4
	10.0	5	72-83	80	4.5	6
<b>Confirmation ion</b>						
Nicosulfuron (DPX-V9360)	1.0 (LOQ)	5	100-120	106	8.8	8
	10.0	5	93-102	98	4.2	4
IN-V9367	1.0 (LOQ)	5	58-86	71	10.1	14
	10.0	5	70-80	75	3.7	5
IN-J0290	1.0 (LOQ)	5	82-84	83	0.8	1
	10.0	5	77-88	83	4.2	5

Data were obtained from pp. 11, 19; and Tables 1-6, pp. 22-27 of MRID 48790914.

### III. Method Characteristics

The LOQ was the same in the ECM and ILV (1.0 µg/kg). 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 reported as one-third of the LOQ (0.33 ng/g) for the Drummer clay loam soil (Appendix 4, pp. 47-49). In the ILV, the LOQ was reported from the ECM, and no justification was provided (p. 11 of MRID 48790914). The LOD was estimated to be 0.3 µg/kg; no justification was provided.

**Table 4. Method Characteristics**

	Nicosulfuron	IN-V9367	IN-J0290
Limit of Quantitation (LOQ)	1.0 µg/kg	1.0 µg/kg	1.0 µg/kg
Limit of Detection (LOD)	0.33 µg/kg <sup>1</sup>	0.33 µg/kg <sup>1</sup>	0.33 µg/kg <sup>1</sup>
Linearity (calibration curve r <sup>2</sup> and concentration range)	r <sup>2</sup> = 1.0000 <sup>2</sup> (0.25-20 ng/mL)	r <sup>2</sup> = 0.9996 <sup>2</sup> (0.25-20 ng/mL)	r <sup>2</sup> = 0.9999 <sup>2</sup> (0.25-20 ng/mL)
Repeatable	Yes	Yes	Yes
Reproducible	Yes	Yes	Yes
Specific	Yes	Yes	Yes

Data were obtained from pp. 22, 24; Figure 1, p. 40; and Appendix 4, pp. 47-79.

<sup>1</sup> Reported for Drummer clay loam soil. The LOD was reported as 0.3 µg/kg in the ILV (p. 11 of MRID 48790914).

<sup>2</sup> ILV calibration curves were linear, r<sup>2</sup> = 0.9940-0.9952, for concentration range of 0.25-20 ng/mL (see p. 19 and Figure 4, pp. 40-42 of MRID 48790914).

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

1. The LOD and LOQ arrived at with the analytical method used is not sufficient to address the concern for nicosulfuron's potential to adversely impact plants at very low exposure levels. The EC25 determined for the most sensitive endpoint for plant effects is very low at 0.00045 lbs./A (seedling emergence endpoint for mustard seeds, MRID No. 42220001). This endpoint can be triggered with a concentration of < 0.1 ppb of nicosulfuron in soil. The method evaluated established the LOD an order of magnitude higher at 0.33 ppb and LOQ two order of magnitude higher at 1.0 ppb. While these are low concentrations, an analytical method for nicosulfuron extractions from soil should be precise enough to resolve nicosulfuron residues at toxicologically relevant concentrations for plants.
2. The reviewer is aware of one other previously developed analytical method for nicosulfuron. This method has demonstrated the ability to measure nicosulfuron and other sulfonylurea pesticide residues in soils at very low concentrations. This analytical method utilizing LC-MS possessed LOQs as low as 6 ppt in soil (Marek and Koskinen, 1997), approximately 2 orders of magnitude lower than this reviewed analytical method.
3. In the ECM, the extraction efficiency of the method was evaluated in an aged soil (clay loam from Japan) fortified at 50 µg/kg (pp. 13, 16, 26; Appendix 5, p. 50). The original method was modified to eliminate the purification and concentration with SPE. Recoveries in the aged soil were 94-99%, 93-96% and 89-90% of the applied for nicosulfuron, IN-V0367 and IN-J0290, respectively. These recoveries were normalized using fresh soil recoveries; normalized recoveries were 101%, 97% and 102% for nicosulfuron, IN-V0367 and IN-J0290, respectively.
4. A reagent blank was not included in the ILV (p. 20; Tables 1-6, pp. 22-27 of MRID 48790914).
5. The linear regression equations of the reviewer-generated calibration curves matched those reported in the ECM; however, the linear regression equations of the reviewer-generated calibration curves indicated greater linearity than those reported in the ILV.
6. The reviewer was unable to verify the recoveries for the ECM from data provided in Tables 1-6, pp. 28-39 of the study report. The equation [% recovery = (analyte found \*100)/(fortification level)] was provided by the study authors on pp. 20-21; however, the study author noted that only two significant figures of the "analyte found" data were reported. For example, in the recoveries of nicosulfuron in the clay loam soil samples dosed at the LOQ, the test samples LOQ A and LOQ B had the same "analyte found" of 1.0 µg/kg, but differing percent recoveries of 101% and 103%, respectively (Table 1, p. 28). The reviewer did not calculate "analyte found" from the raw data; raw data was only provided for one soil, the clay loam.
7. It was reported for the ILV that a single analyst completed two sample sets consisting of 6 samples per set in 1.5 working days (p. 20 of MRID 48790914).

8. For the ILV, a Phenomenex® Luna Phenyl-Hexyl 3.0 x 150 mm, 3- $\mu$ m column was used at a flow rate of 0.425 mL/min, instead of a Phenomenex® Luna Phenyl-Hexyl 4.6 x 150 mm, 3- $\mu$ m column at a flow rate of 1.0 mL/min as described in the ECM (MRID 48790914, pp. 14-15). The study author stated that the study monitor approved of the substitution and that the substitution was demonstrated to be equivalent to that specified in the method.
9. Characterization of the test soils should have included information regarding organic carbon content, cation exchange capacity, and soil taxonomic classification as these properties may influence recoveries pesticide residues at different spiking levels in the analytical method evaluation.

## V. References

- Marek L.J. and Koskinen W.C., 1997. LC-MS Analysis of Polar Pesticides in Soil. RJ Reynolds Industry Publication, Richmond, VA.
- 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.

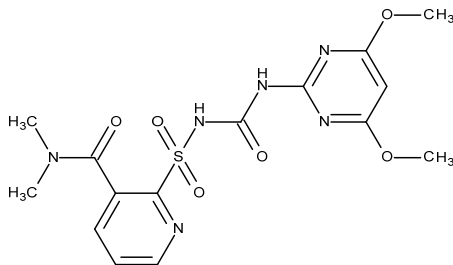
**Attachment 1: Chemical Names and Structures****Nicosulfuron; DPX-V9360**

**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

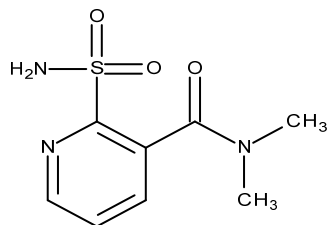
**IN-V9367**

**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

