2.0 INTRODUCTION

Independent laboratory validation of enforcement methods are required by the U.S. EPA OPPTS 850.7100 (Reference 1) and EU Guidance document SANCO/825/00 rev. 8.1 (Reference 2).

The subject method is applicable for the quantitation of nicosulfuron, IN-V9367 and IN-J0290 in water, as described in DuPont-32132 (Reference 3). Pond water was chosen to validate the analytical method as a representative matrix.

Fortification levels in this study were chosen to provide method performance data at the method LOQ and 10×LOQ for the matrix examined. The stated method LOQ was 0.10 ng/mL (ppb) in water.

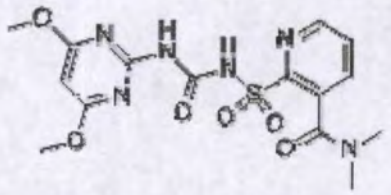
The method was successfully validated in the second of two trials. Due to significant ion suppression in Trial 1, the injection volume was decreased as suggested in the method and the method was successfully validated in Trial 2 following instrument optimization. No other significant changes were made to the method.

This independent laboratory validation study demonstrated that the analytical method DuPont-32132 is acceptable for the quantitation of nicosulfuron, IN-V9367 and IN-J0290 in water according to guidelines set forth by US EPA Ecological Effects Guidelines, OPPTS 850.7100 "Data Reporting for Environmental Chemistry Methods" (Reference 1) and EU Guidance document SANCO/825/00 rev. 8.1 (Reference 2).

3.0 MATERIALS AND METHODS

3.1 Test and Reference Substances

The *Chemical Abstract* structure and chemical name of the analyte is shown below:



Test Substance: DPX-V9360

Common Name: Nicosulfuron

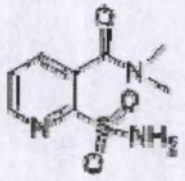
***Chemical Abstracts* Name:** 2-[[[[(4,6-Dimethoxy-2-pyrimidinyl)= amino]carbonyl] amino] sulfonyl]-*N,N*-dimethyl-3-pyridinecarboxamide monohydrate

CAS Registry No.: 111991-09-4

Lot No.: E98949-133

Purity: 97.9%

Storage: Ambient desiccator



Test Substance: IN-V9367

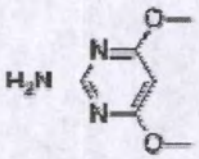
***Chemical Abstracts* Name:** 2-(Aminosulfonyl)-N,N-dimethyl-3-pyridinecarboxamide

CAS Registry No.: 112006-75-4

Lot No.: AG0132-068

Purity: 99.7%

Storage: Ambient



Test Substance: IN- J0290

***Chemical Abstracts* Name:** 4,6-dimethoxy-2-pyrimidinamine

CAS Registry No.: 36315-01-2

Lot No.: 9964-098

Purity: 99.9%

Storage: Ambient

The test substances were supplied by E. I. du Pont de Nemours and Company, DuPont Agricultural Products, Stine Haskell Research Center, Newark, DE. Information pertaining to the characterization and stability of the test substances is archived by DuPont Crop Protection, E. I. du Pont de Nemours and Company, Newark, DE. Characterization data were provided by DuPont Agricultural Products, E.I. du Pont de Nemours and Company, Wilmington, DE. Certificates of Analysis, including lot number and purity, are included with the study raw data file that will be archived by E. I. du Pont de Nemours and Company.

3.2 Test System

Pond water was chosen to validate the analytical method because it is expected to be one of the difficult water sources to analyze.

This control matrix was acquired from a local Colorado pond. The sample was stored frozen prior to being analyzed. Characterization data are included in Appendix 1.

3.3 *Equipment*

The following equipment items were used in the conduct of this independent laboratory validation.

3.3.1 *Instrumentation/Chromatography*

MDS Sciex API 5000 LC-MS/MS System, comprised of:

MDS Sciex API 5000 MS/MS, serial no. AG22340805 (Applied Biosystems Group, Foster City, CA), equipped with a TurboIonSpray interface and Analyst software version 1.4.2

HPLC Column: 4.6 mm i.d. × 150 mm, Phenomonex Luna® 3.0 µm dp, serial no. 5700403, part no. 00F-4256-E0

Shimadzu LC-20 AD VP HPLC pumps, serial no. L201046 52734 and L201046 52735 (Shimadzu US Manufacturing Inc., Columbia, MD)

Shimadzu SIL-20AC-HT Autosampler, serial no. L203546 55317 (Shimadzu US Manufacturing Inc., Columbia, MD)

Shimadzu CTO-20AC Column Oven, serial no. L202146 50331 (Shimadzu US Manufacturing Inc., Columbia, MD)

Shimadzu DGU-20A3 Degasser, serial no. SSI-3-0911 (Shimadzu US Manufacturing Inc., Columbia, MD)

3.3.2 *General Lab Equipment/Devices*

Cahn Microbalance, model no. C-34/35, serial no. C1066/C2251 (SY2) (Orion Research Inc., Beverly, MA 01915)

Sartorius Top-Loading Balance, model no. BA2100S, serial no. 20303446 (EQ#36) (Brinkmann Instruments Co., Westbury, NY 11590)

Vortexer, Genie-2, model no. G-560, serial no. 2-177776

Microman Positive Displacement Pipettes, various sizes (Gilson, Middleton, WI)

Elmasonic Ultrasonic Cleaner, model no. E120H, serial no. 56873078 (EQ#142) (Elma)

N-Evap Water Bath, model no. 112, serial no. 3454 (EQ#18)

SPE Vacuum Manifold, model no. 112, serial no. 3454 (EQ#18)

3.3.3 *Labware*

50 mL polypropylene centrifuge tubes, part no. 89004-364 (VWR, USA)

50 mL graduated glass centrifuge tubes, part no. 048784 (Dionex)

Nylon membrane filter, 0.45 µm, part no. H718740400413 (Whatman International, England)

Nunc U96 Deepwell plate and cover, 2.0 mL polypropylene (Nalge Nunc International, Rochester, NY)

Glass Volumetric Flasks, various volumes, Class A (VWR, USA)

Pipettes, various volumes, Class A (VWR, USA)

Oasis® HLB (1-g/20 cc) cartridges, part no. 092B31280A (VWR, USA)

5-mL disposable syringes, part no. 16001-176 (VWR, USA)

13-mm, 0.45-mm PTFE filters, part no. 28145-493 (VWR, USA)

2-mL HPLC vials (VWR, USA)

3.4 Reagents

Acetonitrile, HPLC-Grade, lot no. DF268 (Honeywell Burdick and Jackson, Muskegon, NI 49442)

Ammonium Formate, 99.99+% pure, lot no. MKBD2004V (Sigma-Aldrich, St. Louis, MO 63103)

Ammonium Hydroxide, 99.99+% pure, lot no. SHBB0317V (Sigma-Aldrich, St. Louis, MO 63103)

Formic Acid, 99.0 % pure, lot no. K4135364 (Honeywell Burdick and Jackson, Muskegon, NI 49442)

Methanol, HPLC-Grade, lot nos. DE572, DF131 (Honeywell Burdick and Jackson, Muskegon, NI 49442)

Water, HPLC-Grade, lot no. DF583-C (Honeywell Burdick and Jackson, Muskegon, NI 49442)

3.5 Principles of the Analytical Method

The pH of a 20 gram sample of pond water is adjusted to approximately 3.5 to 4 with formic acid. The sample is then cleaned up using an Oasis HLB solid phase extraction cartridge. Formic acid is added to the resulting eluate and the sample is evaporated to < 2 mL using an N-Evap with nitrogen. The sample is adjusted back to 2 mL using water and a final volume adjusted to 5 mL with 19/1 5 mM ammonium formate/methanol. The sample is vortexed and sonicated to mix. The sample is then filtered using a PTFE syringe filter and a 1 mL aliquot added to an autosampler block. Just before analysis by HPLC/MS-MS, formic acid is added to standards, samples and controls.

3.6 Modifications, Interpretations, Critical Steps, and Deviations

No significant modifications were made to the method.

3.7 *Instrumentation*

3.7.1 *Chromatography*

HPLC Conditions

System:	MDS Sciex API 5000 LC/MS/MS
Column:	HPLC Column: 4.6 mm i.d. × 150 mm, Phenomonex Luna® 3.0 µm dp, Serial No. 5700403, Part No. 00F-4256-E0
Column Temperature:	40°C
Injection Volume:	10-20 µL
Flow Rate:	1.00 mL/min
Split Flow:	Split to waste (post-column split, 100 µL /min into MS source)
Conditions:	A: 0.1 mM formic acid in 0.1 mM ammonium formate (aq) B: methanol
Approximate Retention Times:	Nicosulfuron (DPX-V9360): ~7.5 min IN-V9367: ~4.3 min IN-J0290: ~ 6.3 min
Total Run Time:	12 min

Gradient:

Time	%A	%B
0.00	95	5
4.00	50	50
4.10	35	65
7.00	10	90
7.10	5	95
10.00	5	95
10.10	95	5
12.00	95	5

3.7.2 *LC/MS/MS Analysis*

Analysis was performed using a MDS Sciex API 5000 LC/MS/MS, equipped with a TurboIonSpray source, and operated in MRM, positive ion mode. Linearity was proven prior to each analytical run over the concentration range of 0.250 to 10.0 ng/mL. Quantitation was based on a mean response factor using peak areas supplied by Analyst software version 1.4.2. A summary of representative experimental conditions is provided in the following table:

MDS Sciex API 5000 MS/MS Mass Spectrometer Conditions Positive Ion Mode

Analytes	Ions Monitored	CXP (Collision Cell Exit Potential)	DP (Declustering Potential)	Dwell Time (msec)	Collision Energy
Nicosulfuron	411 → 182.3 AMU 411 → 213 AMU	20V	80V	150	41 26.5
IN-V9367	230.2 → 80.4 AMU 230.2 → 107.5 AMU	20V	50V	150	47.9 27.5
IN-J0290	156.1 → 59.9 AMU 156.1 → 101.9 AMU	20V	80V	150	35.6 27

3.7.3 *Calibration Procedure*

Calibration standards were analyzed throughout the batch. The response factor of each calibration standard was calculated by dividing the analyte peak area of each standard by the analyte concentration for that standard. The average response was calculated for calibration standards injected with each batch.

3.8 ***Calculations***

Calculations for mean response factor and percent recovery from Section 4.4.1 of the method were used as written.

The calculated concentration of nicosulfuron and metabolites in the fortified samples was calculated using analyte response (peak area) and the mean response factor (MRF). The final concentration was calculated using the final volume and sample weight:

$$C = \left(\frac{PA}{MRF}\right) \quad \text{and} \quad C_f = \left(\frac{PA}{MRF}\right) \times \left(\frac{V_f}{W}\right)$$

Where:

C	=	Analyte concentration in ng/mL
PA	=	Analyte peak area
MRF	=	Mean response factor
C_f	=	Final analyte concentration in ng/mL
V_f	=	Volume of final extract (5 mL)
W	=	Sample weight (20 g; equivalent to 20 mL)

For example, the mean response factor used in the analysis of nicosulfuron residues in water samples from Trial 2 (Batch 2, Table 4) was determined to be 9.35E+04 (Figure 1). For fortified water sample LOQ-1 (P2386B02-004), the analyte peak area was 3.51E+04; therefore the final concentration of nicosulfuron was calculated as follows:

$$C = \left(\frac{3.51E+04}{9.35E+04}\right) = 0.375 \text{ ng/mL}$$

$$C_f = 0.375 \text{ ng/mL} \times \left(\frac{5 \text{ mL}}{20 \text{ g}}\right) = 0.0937 \text{ ng/g (equivalent to ng/mL)}$$