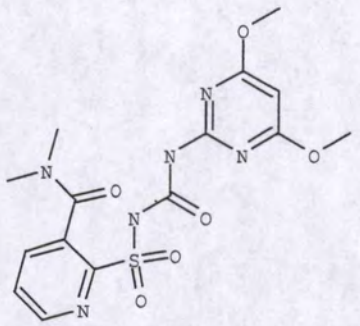
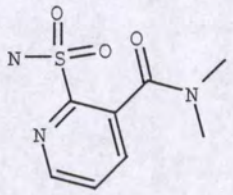
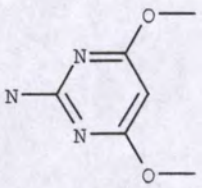


DuPont Code: DPX-V9360
Trivial Name: Nicosulfuron
IUPAC Name: 1-(4,6-Dimethoxypyrimidin-2-yl)- 3-(3-dimethylcarbamoyl-2-pyridylsulfonyl)urea
Chemical Abstracts Name: 2-[[[[(4,6-Dimethoxy-2-pyrimidinyl)amino]carbonyl] amino]= sulfonyl]-*N,N*-dimethyl-3-pyridinecarboxamide monohydrate
***CAS* Registry Number**: 111991-09-4
Molecular Formula: $C_{15}H_{18}N_6O_6S$
Molecular Weight: Average, 410.41 u; monoisotopic, 410.10 u
pKa (25°C): 4.6
Solubility (g/kg), 25°C: Buffered Water: 3.59 (pH 5); 12.2 (pH 7); 39.2 (pH 9)
Organic: 18 (acetone); 4.5 (ethanol); 23 (acetonitrile); <0.02 (hexane); 160 (dichloromethane)
Stability: Relatively stable at pH7 and pH 9. Hydrolysis DT_{50} 15 days (pH 5)



DuPont Code: IN-V9367
Chemical Abstracts Name: 2-(Aminosulfonyl)-N,N-dimethyl-3-pyridinecarboxamide
***CAS* Registry Number**: 112006-75-4
Molecular Formula: $C_8H_{11}N_3O_3S$
Molecular Weight: Average, 229.26 u; monoisotopic, 229.05 u



DuPont Code: IN-J0290
Chemical Abstracts Name: 4,6-dimethoxy-2-pyrimidinamine
***CAS* Registry Number**: 36315-01-2
Molecular Formula: $C_6H_9N_3O_2$
Molecular Weight: Average, 155.16 u; monoisotopic, 155.07 u

This analytical method for nicosulfuron, IN-V9367 and IN-J0290 in water at an LOQ of approximately 0.1 ng/mL (ppb), was developed to satisfy the requirements of the U.S. EPA Pesticide Assessment Guidelines Subdivision N and the EU Annex II 4.2.3.

Nicosulfuron and its metabolites were extracted from 20-g water samples, concentrated and purified by passing the sample through Oasis® HLB SPE cartridges. The purified extracts were analyzed by reversed-phase HPLC/ESI-MS/MS.

The confirmatory method for the HPLC/MS/MS method was based on detection and the relative ratios of the two MS/MS parent-to-daughter ion transitions monitored during the validation.

3.0 MATERIALS

Equivalent equipment and materials may be substituted unless otherwise specified; note any specifications in the following descriptions before making substitutions. Substitutions should only be made if equivalency/suitability has been verified with acceptable control and fortification recovery data.

3.1 Equipment

Balances

Mettler Analytical Balance, Model AG104, for weighing solid standards (Mettler Instrument Corporation, Hightstown, NJ)

Mettler Top-Loading Balance, Model PB602, for weighing water samples and salts (Mettler Instrument Corporation)

Beckman pH Meter – Model Φ340 pH/Temp Meter (Beckman Instruments, Fullerton, CA)

HPLC/MS/MS System

HP Series 1290 Infinity Liquid Chromatograph with G4220A binary pump, G4226A WPALS autosampler, G1330B ALS Thermo autosampler chiller, G1316C column compartment (Agilent Technologies, Little Falls, DE)

MDS Sciex API 5000 triple quadrupole mass spectrometer using an electrospray interface (ESI) and Analyst Version 1.4 software (Applied Biosystems, Framingham, MA).

Phenomonex® Luna analytical column, 4.6mm i.d. × 150 mm with 3.0-µm diameter packing, Part No. 00F-4256-E0 (Phenomonex, Torrance, CA)

HPLC Vials – Agilent screw cap vials, write on spot, 2-mL, Catalog No. 5182-0865 (Agilent Technologies)

Nitrogen Evaporator - N-EVAP, Model 111 (Organomation Assoc., Berlin, MA)

Pipettes

Biohit proline® variable volume pipettor, with tip ejector, 1-10 mL, Biohit Catalog No. 720-110, VWR Cat. No. 53495-286 (VWR International)

Biohit eline® variable volume pipettor, with tip ejector, model e5000, 100-5000 µL, Biohit Catalog No. 720-100, VWR Cat. No. 14220-570 (VWR International)

Biohit eline® variable volume pipettor, with tip ejector, model e1000, 50-1000 µL, Biohit Catalog No. 730-080, VWR Cat. No. 14212-004 (VWR International)

Biohit eline® variable volume pipettor, with tip ejector, model e300, 10-300 µL, Biohit Catalog No. 730-060, VWR Cat. No. 14212-002 (VWR International)

Pipet Tips

Sorenson™ Multi-fit Research Pipet Tips, 5-200 µL and 100-1000 µL, Catalog No. 53550-076 and 53503-076 (VWR International)

Rainin Certified Disposable Pipette Tip, 10 mL, Catalog No. RC-10 ML (Rainin Instrument Co.)

Biohit extended length pipet tips, 100-5000 µL, Biohit Cat No. 780-303, VWR Cat. No. 13502-312 (VWR International)

Sample Collection Vials

VWR Disposable Skirted Centrifuge Tube, 50-mL, Polypropylene, cs. of 500, Part # 21008-480 (VWR International)

Glass Centrifuge Tubes – Pyrex® Conical Centrifuge Tubes, graduated, 50-mL capacity, Catalog No. 21048-050 (VWR International)

VWR Scintillation Vials, Borosilicate Glass, with unattached White Urea Cap and Cone Shaped Liner, case of 500, Cat. No. 66022-128 (VWR International)

Solid-Phase Extraction Apparatus

Visiprep™ SPE Manifold, Catalog No. 5-7030M (Supelco, Inc., Bellefonte, PA)

Solid-Phase Extraction Disposable Flow Control Valve Liners – for the Visiprep™, Catalog No. 57059 (Supelco, Inc.)

Solid-Phase Extraction Cartridges

Oasis® HLB cartridge, 20cc/1 g, Part No. 186000177 (Waters Inc, through VWR International)

Solid Phase Extraction Plastic Reservoirs – 60-mL size, Catalog No. 1213-1012 (Varian Inc.), or 60-mL size, Catalog No. 57022 (Supelco Analytical)

Syringes – 5cc Disposable plastic syringes, Catalog No. 309603 (Becton Dickinson, Franklin Lakes, NJ)

Syringe Filters – PTFE disposable syringe filter, 0.45-µm pore size, 25-mm diameter, VWR Catalog No. 28145-497 (North America) or 514-0071 (European) (VWR International)

Bransonic® Ultrasonic Cleaner, 0.75-gallon capacity, Model 2200, Catalog No. 952-214 (Branson Ultrasonics Corp., Danbury, CT)

Vortex mixer – Fisher Vortex Genie®, Catalog No. 12-812 (Fisher Scientific Co., Pittsburgh, PA)

3.2 Reagents and Standards

Acetonitrile – OmniSolv® #AX0142-1, High Purity Solvent (EMD, Gibbstown, NJ)

Formic Acid – Suprapur®, 98-100%, #11670-1 (EM Chemicals, Inc.)

Formic Acid, Ammonium Salt – A.C.S. reagent, #M530-08 (J.T. Baker)

Methanol – OmniSolv® #MX0488-1, High Purity Solvent (EMD, Gibbstown, NJ)

Milli-Q Water – Milli-Q gradient UV+ water system, (Waters Millipore, Bedford, MA)

Ammonium Hydroxide – EM Science, GR, 28-30% assay, AX1303-13 (EM Science)

Reference standards (DuPont Crop Protection, Newark, DE)

DPX-V9360-170 (Nicosulfuron), 98.3% purity;
IN-V9367-005 analytical standard, 99.7% purity;
IN-J0290-003 analytical standard, 99.9% purity

3.3 Safety and Health

No unusually hazardous materials are used in this method. All appropriate material safety data sheets should be read and followed, and proper personal protective equipment should be used.

4.0 METHODS

4.1 Principle of the Analytical Method

Nicosulfuron and its metabolites IN-V9367 and IN-J0290) were extracted and concentrated by passing the sample through an Oasis® HLB SPE cartridge. After the cartridge was washed with water, the analytes were eluted with a 9:1 methanol/0.50 M ammonium hydroxide. The eluate was evaporated to ≤2 mL in a waterbath at 35°C under a stream of nitrogen, diluted to 5 mL with 5 mM ammonium formate/methanol (19:1, v/v) and filtered. The purified extract was analyzed by reversed-phase HPLC using a Phenomenex® Luna 3.0-µm phenyl-hexyl column (4.6x150 mm) and a mobile phase of 0.1 mM formic acid in 0.1 mM ammonium formate (aq) and methanol. Detection of the analytes was by electrospray mass spectrometry/mass spectrometry (ESI-MS/MS) in the positive ion mode.

In the mobile phase, 0.10 mM of formic acid was necessary to detect molecular ions ($[M-H]^+$) of nicosulfuron, IN-V9367, and IN-J0290 using ESI in the positive ion mode. The presence of ammonium formate appeared to reduce the formation of the sodium adducts and favor the formation of molecular ions. Sodium adducts were not favorable for MS/MS analysis because it could not be fragmented further into daughter ions.

The confirmatory method for the HPLC/MS/MS method was based on detection and the relative ratios of the two MS/MS parent-to-daughter ion transitions monitored during the validation.

During method validation, post-fortified samples were analyzed for each water type to determine if matrix effect, suppression or enhancement, influenced percent recovery of nicosulfuron and its metabolites. The post-fortified samples, in this study, were extracts of control water samples that were purified and prepared in the same manner as with the other samples, but fortified with a known concentration of analytes prior to HPLC/ESI-MS/MS analysis.

4.2 Analytical Procedure

4.2.1 Glassware & Equipment Cleaning Procedures

The effectiveness of any cleaning procedure used should be demonstrated by preparation and analysis of reagent blanks. In general, all reusable glassware and plasticware should be washed in hot tap water with laboratory grade, non-phosphate

detergent, rinsed several times with tap water, rinsed several times with deionized water, rinsed once with acetone, and allowed to fully dry before use. Care should be taken to avoid working with high levels of the analyte being monitored in the same laboratory where samples are being extracted and analyzed.

4.2.2 *Preparation & Stability of Reagent Solutions*

1.0 M Ammonium Formate (aq)
In a 100-mL volumetric flask, dissolve 6.3 grams of ammonium formate with about 50 mL of ultrapure water. Dilute to the mark with ultrapure water and mix to homogeneity. This solution is stored capped at room temperature and should be prepared monthly.

0.10 M Ammonium Formate (aq)
Using a 10-mL volumetric flask, dilute 1 mL of 1.0 M ammonium formate with ultrapure water to the mark and mix to homogeneity. This solution is stored capped at room temperature and should be prepared monthly.

0.5 M Ammonium Hydroxide (aq)
In a 100-mL volumetric flask, mix 50 mL of ultrapure water and 3.5 mL of concentrated (28% – 30% NH3) ammonium hydroxide. Dilute to the mark with ultrapure water and mix well. This solution is stored well capped at room temperature and should be prepared monthly.

1.0 M Formic Acid (aq)
In a 100-mL volumetric flask, mix 50 mL of ultrapure water with 3.85 mL of concentrated formic acid. Dilute to the mark with the ultrapure water and mix well. This solution is stored capped at room temperature and should be prepared monthly.

Acidified Water (0.4 mM aqueous Formic Acid Solution; SPE Cartridge Wash)
Mix 500 mL of ultrapure water and 200 µL of 1 M formic acid in a 250-mL glass storage bottle. This solution is stored capped at room temperature and should be prepared monthly.

Elution Solution (9:1 Methano/0.50 M Ammonium Hydroxide)
Mix 450 mL of methanol and 50 mL of 0.5 M ammonium hydroxide solution in a 500-mL glass storage bottle. This solution is stored capped at room temperature and should be prepared monthly.

Sample Diluent (19:1 5 mM Ammonium Formate/Methanol)
Mix 237 mL of ultrapure water, 1.2 mL of 1.0 M ammonium formate and 12.5 mL of methanol in a 250-mL glass storage bottle. This solution is stored at room temperature and should be prepared monthly.

Mobile Phase A (0.1 mM formic acid in 0.10 mM ammonium formate (aq))
To a 1-L volumetric flask that is partially filled with ultrapure water, add 0.10mL of 1.0 M ammonium formate and 0.10 mL 1.0 M formic acid. Dilute to the mark with ultrapure water and mix to homogeneity. This solution is stored cap at room temperature and should be prepared monthly.

4.2.3 *Stock and Intermediate Standards Preparation and Stability*

Weigh 10.00 ± 0.50 mg each of the analytical standards of nicosulfuron (DPX-V9360), IN-V9367, and IN-J0290 into separate 100-mL volumetric flasks. Dissolve and dilute to the mark using acetonitrile to make stock standard solutions of approximately 100 µg/mL.

Prepare the first intermediate mix standard of 10-µg/mL (DPX-V9360, IN-V9367, and IN-J0290) by adding 1.0 mL of each of the 100-µg/mL DPX-V9360, IN-V9367, IN-GDC42, and IN-J0290 solutions into a 10-mL volumetric flask. Dilute to the mark with methanol and mix.

Solutions of the stock standards and intermediate mix standards are stored in the freezer maintained at a temperature below ≤-10 °C and are stable for six months.

4.2.4 *Fortification Standards Preparation and Stability*

To a 10-mL volumetric flask, add 2.0 mL of the 10-µg/mL intermediate mix standards to the flask, then add methanol to the mark to make an intermediate mix standards of 2.0-µg/mL (DPX-V9360, IN-V9367, and IN-J0290).

Dilute 1.0 and 0.10 mL of the 2.0-µg/mL of the intermediate mix standards with methanol to the mark of 10-mL volumetric flasks to make fortification solutions of 0.20- and 0.020-µg/mL (DPX-V9360, IN-V9367, and IN-J0290), respectively. These solutions are stable for at least 3 months when stored at ≤ -10° C.

4.2.5 *Chromatographic Standard Preparation and Stability*

Prepare a calibration standard solution by adding 15 mL of the 5mM ammonium formate/methanol (19:1, v/v) solution into a 25-mL volumetric flask. Dilute to the mark with ultrapure water and mix to homogeneity.

Prepare chromatographic standards ranging from 0.25 to 10.0 ng/mL (or in concentrations expected to cover the range of nicosulfuron and metabolites in the investigative samples). Prepare the chromatographic standards from the 200-ng/mL (DPX-V9360, IN-V9367, and IN-J0290) fortification solution using the calibration standard solution and then doing a series of serial dilutions. Keep all chromatographic standards at or below 5°C right after preparation. These standards should be prepared fresh even they are stable for 72 hours. The table shown below describes how standards were prepared for the validation work presented in this report:

Standard Conc. (ng/mL)	mL added	Intermediate Standard Used (ng/mL)	Final Volume (mL)
10	0.50	200 (0.2-ppm fortification Std)	10.0
8.0	0.40	200 (0.2-ppm fortification Std)	10.0
5.0	0.25	200 (0.2-ppm fortification Std)	10.0
1.0	1.00	10 (chromatographic std)	10.0
0.50	1.00	5.0 (chromatographic std)	10.0
0.25	0.50	5.0 (chromatographic std)	10.0

4.2.6 *Source (& Characterization) of Samples*

Water samples from three different sources were used for the method validation. The sources of the water samples and the pertinent physical characteristics are summarized in the following table. Water samples were characterized at Agvise Laboratories (Northwood, ND). Characterization records are maintained at DuPont Agricultural Products.

Measurement	Lums Pond Water [A]	Newark Drinking Water [B]	Kemblesville Well Water [C]
pH	6.8	7.6	7.4
Calcium (ppm)	8.1	7.8	10
Magnesium (ppm)	5.2	1.9	7.5
Sodium (ppm)	14	2.6	11
Hardness (mg equivalent $CaCO_3$/L)	42	27	57
Conductivity (mmhos/cm)	0.17	0.08	0.17
Sodium Adsorption Ratio (SAR)	0.93	0.21	0.62
Total Dissolved Solids (ppm)	340	58	210
Turbidity (NTU)	6.80	0.48	2.98

[A] Pond water source, Lums Pond , Newark, DE, USA

[B] Tap water source, Stine-Haskell Research Center, Newark, DE, USA

[C] Well water source, Kemblesville, PA, USA

4.2.7 *Storage & Preparation of Samples*

Water samples were stored capped and refrigerated at 4°C after collection. Samples that were cloudy or turbid in appearance were filtered through a 0.45-µm filter prior to analysis. Samples with a high degree of turbidity (e.g., pond water samples containing algae) were centrifuged, instead of filtered, to remove particulate matter.

4.2.8 *Sample Fortification Procedure*

Exactly 100 µL of the 0.20- and 0.020-µg/mL of DPX-V9360, IN-V9367, and IN-J0290 fortification solutions in methanol were added to 20.0-g water samples for the 1.0- and 0.10-ng/ml fortification levels, respectively.

4.2.9 *Sample Concentration and Purification Procedure*

Weigh 20.00g ± 0.10 g of water samples in 50-mL disposable polypropylene centrifuge tubes.

Fortify samples, if necessary. Cap each tube, shake and vortex mix the sample to homogeneity.

Adjust the pH of each water sample to 3.5-4.0 with 1 M formic acid. This may require different amounts of acid for different water sources (tap, well, etc). A 20-g sample of control water may be adjusted first using 5-µL aliquots of 1 M formic acid. When the proper pH is reached, the total amount of 1 M formic acid used can be used to adjust the pH of other samples. Usually this is somewhere between 20 - 40 µL of 1 M formic acid.

Tap the top frit down of each Oasis® HLB (1-g/20 cc) cartridge three times using a 5-mL disposable syringe plunger to pack the sorbent particles firmly. Attach the cartridges to an SPE vacuum manifold.

Condition each cartridge with 6 mL of methanol followed by 10 mL of acidified water (0.4 mM formic acid) by gravity flow. Do not let the cartridges to go to dryness.

Pass the acidified water samples through the cartridges by gravity flow. Use vacuum as needed to achieve a flow rate of 1-2 drops/second. Discard eluates.

After each entire sample has passed through, rinse each sample tube with 10 mL of acidified water and pass this rinse through the corresponding cartridge by gravity flow or by vacuum at 1-2 drops/second. Pass 10 mL of water through each of the cartridges by gravity flow or by vacuum at 1-2 drops/second. Vacuum dry (fully opened) the cartridges for 2 minutes in order to remove remaining liquid. Discard eluates.

Elute the analytes from each cartridge with 20 mL of methanol/0.50 M ammonium hydroxide (9:1, v/v) solution by gravity flow. Use light vacuum, if needed, to start the flow. Collect each eluate in a 50-mL graduated glass centrifuge tube. Turn off vacuum once flow starts and use gravity flow. After all elution solution has passed through, open vacuum for 10-15 seconds to dry each cartridge and collect the remaining liquid into the same centrifuge tube.

Add 1mL of 1 M formic acid to each eluate, cap and vortex mix to homogeneity. The final pH of the sample should be ~4.5.

Evaporate each eluate to ≤ 2 mL in an N2-vap using a moderate stream of nitrogen and the waterbath set at 25°C - 30°C (~2.0 hrs). Add water, if necessary, to bring back the volume to 2 mL. Bring the final volume to 5 mL with 19/1 5 mM ammonium formate/methanol solution.

Cap the sample tubes, vortex mix for 5-10 seconds, sonicate for 2 minutes, and again vortex mix the samples for 5-10 seconds.

Transfer each purified sample to a 5-mL disposable syringe fitted with a 13-mm, 0.45-µm PTFE filters and filter it back into the tube. Using a pipet, transfer exactly 1.0 mL of each sample to an HPLC autosampler vial for LC/MS/MS analysis. (Samples are stable for 24 and 72 hours when kept at 5°C and –10°C, respectively.)

Prepare the following post-fortified samples (optional):

LOQ equivalent (0.4 ng/mL): In a 2-mL HPLC vial, add 960 µL of the filtered control extract from Step 12 and 40 µL of the 10-ng/mL calibration standard. Cap the vial and vortex mix the sample.

10xLOQ equivalent (4.0 ng/mL): In a 2-mL HPLC vial, add 980 µL of the filtered control extract from Step 12 and 20 µL of the 200-ng/mL fortification solution. Cap the vial and vortex mix the sample.

14. Just before LC/MS/MS analysis, add 10 µL of 1 M formic acid to each of the standards, samples and post-fortified samples. Cap and vortex mix each sample to homogeneity.

4.3 Instrumentation

4.3.1 Description

Method validation data in this study were generated using an Agilent Technologies 1290 Infinity HPLC coupled to Applied Biosystems MDS SCIEX API5000 LC/MS/MS (a triple quadrupole mass spectrometer).

4.3.2 Operating Conditions

The HPLC and MS/MS operating conditions used during method validations are summarized in the following tables:

HPLC Conditions:

System:	Agilent Technologies 1290 Infinity					
Columns:	Phenomonex® Luna, 4.6 x 150 mm, 3.0-µm dp					
Column Temperature:	40°C					
Autosampler Temperature	5°C					
Injection Volume:	10 - 20 µL (Pond water may require 10 µL to reduce matrix effects.)					
Mobile Phase Conditions:			Time	%A	%B	Flow (mL/min)
Solvent A: 0.10 mM formic acid in 0.10mM ammonium formate (aq)	0.00	95	5	1.0		
	4.00	50	50	1.0		
			4.1035	65	1.0	
			7.0010	90	1.0	
			7.105	95	1.0	
Solvent B: Methanol			10.00	5	95	1.0
			10.10	95	5	1.0
			12.00	95	5	1.0
Retention Time:	(min)					
IN-V9367	~ 4.3					
IN-J0290	~ 6.3					
DPX-V9360	~ 7.5					

Mass Spectrometer Conditions:

MS System:	Applied Biosystems MDS SCIEX API5000						
Analyte Monitored	Ions Monitored (amu)	DP[a] (V)	CE[b] (V)	CXP[c] (V)	EP[d] (V)	Dwell Time (ms)	Acquisition Timing (min)
IN-V9367	230.0 → 78.0 ± 0.1	60	50	15	10	100	2.0 – 10.0
	230.0 → 106.0 ± 0.1		30	5		100	
IN-J0290	156.0 → 57.0 ± 0.1	46	37	4	10	100	2.0 – 10.0
	156.0 → 100.0 ± 0.1		25	8		100	
DPX-V9360	411.0 → 182.0 ± 0.1	70	30	10	10	100	2.0 – 10.0
	411.0 → 213.0 ± 0.1		25	10		100	
Scan type/Polarity:	Multiple Reaction Monitoring/Positive						
Ion Source Voltage:	ESI+, 4500 V						
Collision Gas (CAD):	5 psig						
Curtain Gas (CUR):	10 psig						
Nebulizer Gas (GS1):	35 psig						
Heater Gas (GS2):	35 psig						
Source Heater (TEM):	350°C						
Interface Heater (ihe):	ON						
MS Flow Rate:	(Post-column Split): 100-µL/min (approximately 10:1 split)						

[a]Declustering Potential [b]Collision Energy [c]Collision Exit Potential [d]Entrance Potential

A triple quadrupole MS instrument with an electrospray ionization (ESI) source was used for the detection of nicosulfuron and its metabolites. The response of each analyte was optimized initially by infusing the analyte into the ionization source. The flow rate and mobile phase were adjusted to the expected elution conditions of the analyte from the HPLC column. The molecular ion detected was fragmented in the MS/MS collision cell. The tune file created was adjusted to maximize the response of the fragmented ions detected. Two parent-daughter ion transitions were monitored for each analyte (Figure 2).

Nicosulfuron and its metabolites were each identified in water by its retention time, the presence of two parent-daughter ion transitions with a signal-to-noise ratio greater than 5, and the ratio of the two ion transitions within an acceptable range as determined during the method validation. For quantitation, the ion chromatogram for each analyte was integrated and the peak area was used for quantitation. Quantitation was performed using the total ion current (TIC).

A ten-port electronically activated switching valve was used to direct the HPLC column effluent to waste prior to and following the elution of analytes. The retention times of the analytes were within 4 –8 minutes. The chromatographic run time is 12 minutes, but the MS sample collection time is 2.0 to 10.0 minutes. Outside of this sample collection time, the column effluent was directed to waste. This process reduced the ionization source contamination and allowed more samples to be analyzed prior to source cleaning.

4.3.3 *Calibration Procedures*

Prepare chromatographic standards that bracket the levels of nicosulfuron and its metabolites found in the water samples to be analyzed. Preparation of standards is described in Section *4.2.5* of this report.

4.3.4 *Sample Analysis*

Each set of analytical samples should consist of calibration standards, at least one control (a sample without the analyte of interest and matches the analytical samples as closely as possible), and the investigative (treated/fortified) samples. In addition, at least one post-fortified sample of the control with nicosulfuron and its metabolites at a known level should be included to assess if matrix effect, if any, influence the residue levels found or percent recovery.

A calibration solvent blank should be injected prior to the chromatographic runs of standards and samples in an analytical set. Then a standard can be analyzed, followed by a maximum of 4 samples (controls, fortified controls, or treated samples), followed by another standard, etc. The last injection should be a standard.

4.4 ***Calculations***

4.4.1 *Methods*

The average response factor was calculated as follows:

$$\text{Response} = \frac{\text{Concentration (ng/mL) of Standard}}{\text{Peak Area Counts}}$$

$$Rf_{avg} = \frac{\sum \text{Standard Response}}{n}$$

where:

Rf_{ave} = Average Response Factor

n = total number of standards analyzed in a sample set

Concentration of nicosulfuron and its metabolites in the fortified samples (µg/kg found) was then calculated using the equation below:

$$\text{ng/mL found} = \frac{A \times Rf_{ave}\,(\text{ng/mL/area counts}) \times \text{Final Volume (mL)} \times \text{Dilution Factor}}{\text{Sample Volume (mL)}}$$

= ng/mL = ppb

where:

A = Corrected Peak Area Counts

= Peak Area Counts in sample – Peak Area Counts in control

Rf_{ave} = Average Response Factor

Percent Recovery was calculated as:

$$\%\ \text{Recovery} = \frac{\text{Analyte Found (ppb)}}{\text{Fortification Level (ppb)}} \times 100$$

4.4.2 *Examples*

Calculation for the percent recovery of nicosulfuron (DPX-V9360) in Well Water, Kimblesville PA, USA water sample fortified at 0.10 ng/mL (sample LOQ1, Appendix 1) which was prepared and analyzed on September 29, 2010 is shown below:

Rf_{avg} of six DPX- V9360 standards = 8.8251×10^{-6} ng/mL/area counts

Peak Area Counts (ac) for DPX- V9360, fortified sample = 42900

Peak Area Counts (ac) for DPX- V9360, Control 1 and Control 2 = average (1370, 0) = 685

Sample Volume = 20.0 mL

Final Volume = 5.0 mL

Fortification Level = 0.0101 = 0.010 ng/mL

$$\text{DPX-V9360 Found} = \frac{(42900 - 685)\ \text{ac} \times 8.8251 \times 10^{-6}\ \text{ng/mL/ac} \times 5.0\ \text{mL} \times 1}{20.0\ \text{mL}}$$

= 0.0931 ng/mL = 0.093 ng/mL