TRADE SECRET

Study Title

ANALYTICAL METHOD FOR THE DETERMINATION OF RIMSULFURON (DPX-E9636) AND ITS METABOLITES IN SOIL AND WATER USING HPLC/ESI-MS/MS

Test Guidelines

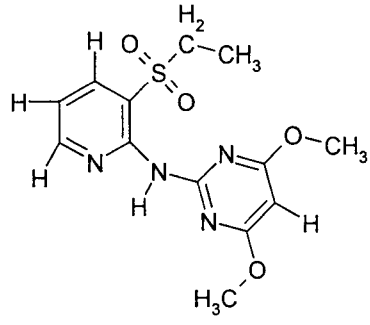
U.S. EPA Ecological Effects Test Guidelines, OPPTS 850.7100, Data Reporting for Environmental Chemistry Methods (Draft, April, 1996)

European Commission, Directorate General Health and Consumer Protection, "Guidance Document on Residue Analytical Methods", SANCO/825/00 rev. 8.1, November 16, 2010

LIST OF ABBREVIATIONS AND SYMBOLS

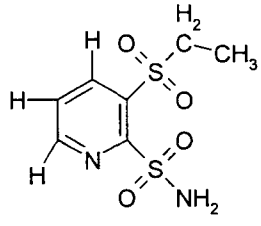
Aq	Aqueous solution
°C	Degrees Celsius
CAS	*Chemical Abstracts* Service
CFR	Code of Federal Regulations
EEC	European Economic Community
EPA	Environmental Protection Agency
g	Gram(s)
Σ	Total
GLP	Good Laboratory Practice
HPLC	High-performance liquid chromatography
ESI-MS/MS	Electrospray mass spectrometry/ mass spectrometry
LOQ	Limit of quantitation/quantification
M	Molar
MeOH	Methanol
mL	Milliliter(s)
mM	Millimolar
min	Minute(s)
mm	Millimeter(s)
n	Total number of samples analyzed
ng	Nanogram(s)
OECD	Organization for Economic Co-operation and Development
OM	Organic matter
ppb	Parts per billion
psig	Pound per square inch gauge
rpm	Revolution per minute
RSD	Relative standard deviation
s	Second(s)
S/N	Signal-to-noise ratio
SD	Standard deviation
SPE	Solid-phase extraction
t_R	Retention time
amu	Atomic mass unit
µL	Microliter(s)
µm	Micrometer(s)

DuPont Code: IN-70942
Chemical Structure:



Chemical Abstracts Name: N-((3-Ethylsulfonyl)-2-pyridinyl)-4,6-dimethoxy-2-pyrimidinamine
CAS Registry Number: 151331-80-5
Molecular Formula: $C_{13}H_{16}N_4O_4S$
Molecular Weight (g/mol): Average 324.36; monoisotopic, 324.09

DuPont Code: IN-E9260
Structure:



Chemical Abstracts Name: 3-(Ethylsulfonyl)-2-pyridinesulfonamide
CAS Registry Number: 117671-01-9
Molecular Formula: $C_7H_{10}N_2O_4S_2$
Molecular Weight (g/mol): 250.30; monoisotopic, 250.01

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*

EQUIPMENT DESCRIPTION	PRODUCT ID AND DESCRIPTION	SUPPLIER
Analytical Balances	Model AE163 Dual Range Balance for weighing solid standards	Mettler Instrument Corp. (Hightstown, N.J.)
	Model PM460 Toploading Balance for weighing samples and reagents	Mettler Instrument Corp. (Hightstown, N.J.)
Analytical Evaporator	N-Evap® Model 112 Nitrogen Evaporator with stainless steel luer fit needles	Organomation Assoc. (South Berlin, Mass.)
Centrifuge	Sorvall® Refrigerated Centrifuge, Model RT7 with a RTH750 rotor or Model RC3B Plus	Sorvall Instruments (Wilmington, Del.)
Filtration	Syringe filters, 13 mm, 0.2 µm PTFE, cat. no. 28145-491	VWR, Inc. (West Chester, Pa.)
Ultrasonicator	Branson 5210	Branson Ultrasonics Corp. (Danbury, Conn.)
Vortex Mixer	Vortex Genie® 2 or K-550-G	VWR, Inc. (West Chester, Pa.)
Solid Phase Extraction	Oasis™ HLB SPE cartridge, 1g/20 mL, Cat. No. 186000117 **(do not substitute)**	Waters Corporation (Milford, Mass.)
	Supelco Visiprep™ SPE Vacuum Manifold standard, 12-port model, Cat. No. 57030-U with valve liners Cat. No. 57059	Supelco (Bellefonte, PA)
	Falcon® 2098 (50 mL), 2097 (15 mL) Polypropylene Centrifuge Tubes ; Disposable Syringes, 3-cc Cat. No. BD301073 and 10-cc Cat. No. 301031	Becton Dickinson (Franklin Lakes, N.J.)
	KIMAX No. 45153-15 (15 mL) and No. 45153-50 (50 mL) glass centrifuge tubes, conical bottom, graduated.	Kimble / Kontes (Vineland, N.J.)
Labware	Electronic 1000-µL and 10-mL Pipettors	Rainin (Walnut Creek, Calif.)
	Mechanical, positive displacement, 25-µL, 50-µL, 250-µL and 1000-µL Pipettors	Gilson Inc. (Middletown, Wis.)
	VWR Graduated Cylinders, Class B, 1000-mL capacity, Cat. No. 89000-260; Disposable PET Transfer Pipettes	VWR, Inc. (West Chester, Pa.)
	Glas measuring measuring ipipets (25 mL, 10 mL)	VWR, Inc. (West Chester, Pa.)

HPLC/MS System/Supplies		
Equipment Description	**Product ID and Description**	**Supplier**
HPLC/UHPLC	Agilent Series 1290 Infinity Ultra-High Performance Liquid Chromatograph Shimadzu HPLC: LC-20ADXR, SIL-20ACXR, CTO-20AC, DGU-20A5, CBM-20A	Agilent Technologies, Inc. (Palo Alto, Calif.) Shimadzu Scientific Instruments
HPLC/MS/MS	API 5000 triple quadrupole mass spectrometer using an electrospray interface (ESI); Analyst Version 1.5.1 software; Analyst Version 1.4.2 software.	Applied Biosystems, (Framingham, MA)
HPLC Column	Phenomenex Kinetex C18, 2.1 x 50 mm, 1.7µm, 100A, Cat. No. 00B-4498-AN-902 Zorbax Eclipse Plus C18; 2.1 × 50 mm, 1.8-µm particle size, Cat. No. 959757-902	Phenomenex (Torrance, CA) Agilent Technologies, Inc. (Palo Alto, CA)
Autosampler Vials	Screwcap Vials Cat. No. 5182-0715 and Caps, Screw Type green (Cat. No. 5182-0724) or Red (Cat. No. 5182-0725)	Agilent Technologies, Inc. (Palo Alto, Calif.)

3.2 *Reagents and Standards*

Reagents	Product Description	Product ID	Supplier
Acetonitrile	OmniSolv®, 4L	AX0142-1	EMD Science (Gibbstown, N. J.)
Ammonium Acetate	Baker Analyzed®	0599-08	EMD Science (Gibbstown, N. J.)
Ammonium Hydroxide	28-30% assay, ACS grade	AX1303-13	EMD Science (Gibbstown, N. J.)
Formic Acid	Suprapur® 98-100%	11670-1	EMD Science (Gibbstown, N. J.)
Formic Acid, Ammonium Salt	A.C.S. reagent 96%	M530-08	JT Baker (Phillipsburg, N. J.)
HPLC-grade Water	OmniSolv®, 4L	WX0004-1	EMD Science (Gibbstown, N. J.)
Methanol	OmniSolv®, 4L	MX0488-1	EMD Science (Gibbstown, N. J.)
Phosphoric Acid	Baker Analyzed®	0260-01	EMD Science (Gibbstown, N. J.)

Reference standards (DuPont Crop Protection, Newark, DE):
DPX-E9636, IN-70941, IN-70942 and IN-E9260

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

Rimsulfuron, IN-70941, IN-70942 and IN-E9260 were extracted twice from 5-g soil samples by vigorous shaking in 9:1 0.1 M aqueous ammonium acetate:methanol at ambient temperature. Each sample was centrifuged after shaking and the supernatants/extracts were pooled together. Extract aliquots were taken and purified by solid-phase extraction (SPE) on Oasis™ HLB cartridges. The analytes were retained in the cartridge and eluted sequentially with acetonitrile and 9:1 acetonitrile:0.5 M ammonium hydroxide. About 1 mL of 5 mM ammonium acetate were added to each eluate and it was then evaporated until aqueous (1.0 ± 0.1 mL) in an N_2 vap using a moderate flow of nitrogen and a water bath temperature of 30°C. Each extract was diluted with 0.5 mL of acetonitrile and HPLC-grade water to bring the final volume to 5 mL and filtered using a 0.2-µm PTFE syringe filter. The purified extracts were analyzed by reversed-phase HPLC using a Kinetex C18, (2.1 x 50 mm, 1.7-µm) or a Zorbax® Eclipse Plus C18 (2.1 x 50 mm, 1.8 µm) column and a mobile phase of 0.01 M formic acid and methanol. Detection of the analytes was by electrospray mass spectrometry/mass spectrometry (ESI-MS/MS). At least two parent-to-daughter ion transitions per analyte were monitored during analysis. In order to optimize the LC/MS/MS response per analyte, as well as minimize any interference, three MS/MS monitoring periods were used.

Drinking, ground and surface water samples were added with 5 mM ammonium acetate and acetonitrile and analyzed for residues of rimsulfuron and its metabolites by reversed-phase HPLC/ESI-MS/MS using solvent standards.

Extraction efficiency of the method was evaluated by analyzing laboratory-aged soil samples, which were fortified with rimsulfuron, IN-70941, IN-70942 and IN-E9260 and then aged for three-four days. The fortification level of each analyte was at 300-µg/kg. Freshly fortified samples were extracted and analyzed simultaneously with the laboratory-aged samples. Recoveries from aged fortified soil samples were compared to those from freshly fortified samples in order to obtain normalized recoveries.

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

DuPont-38604 soil method is a modified version of DuPont-4048 (Reference 4). The sample extraction is the same in DuPont-4048 except that the sample weight and extraction solution volume is reduced four-fold in order to be cost-effective. The sample cleanup still uses 1-g/20cc Oasis™ HLB cartridges, but slight changes were made in order to simplify and obtain acceptable recoveries for all analytes with the analysis time reduced from 21 to 7 minutes.

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 plastic ware 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*

The following solutions are stable for at least three months when stored capped in a glass storage container at room temperature unless stated otherwise:

0.1 M aqueous ammonium acetate, pH 6.5

Dissolve 7.7 g of ammonium acetate with 900 mL of HPLC-grade water. Dilute to 1000 mL with HPLC-grade water. Adjust the pH of the solution to 6.5 using the 1:10 (v/v) phosphoric acid solution (approximately 8-15 drops required) and mix well.

9:1 0.1 M aqueous ammonium acetate (pH 6.5):methanol (Extraction Solvent)

Combine 900 mL of 0.1 M aqueous ammonium acetate (pH 6.5) with 100 mL of methanol in a 1-L glass storage bottle. Mix the resulting solution to homogeneity

10 mM aqueous ammonium acetate (SPE Wash)

Dissolve 0.770 g of ammonium acetate with 900 mL of HPLC-grade water. Dilute to 1000-mL with HPLC-grade water and mix well.

5 mM aqueous ammonium acetate

Dissolve 0.385 g of ammonium acetate with about 900 mL of HPLC grade water in a 1-L volumetric flask. Dilute to 1000-mL with HPLC grade water. Mix well. Transfer the solution into a 1-L glass storage bottle.

9:1 5 mM aqueous ammonium acetate:acetonitrile (standard diluent)

Mix 90 mL of 5 mM aqueous ammonium acetate with 10 mL of acetonitrile to homogeneity in a 125-mL glass storage container. This solution should be prepared fresh.

1.0 M Ammonium Hydroxide (aq)

To a 100-mL volumetric flask that is partially filled with HPLC-grade water, add 7 mL of concentrated ammonium hydroxide (28% - 30% NH_3) and mix. Bring to the mark with HPLC-grade water and mix to homogeneity. This solution is stored capped at room temperature and this stable for at least a month.

9:1 Acetonitrile:0.5 M Ammonium Hydroxide (SPE Eluent)

Add 25 mL of 1.0 M ammonium hydroxide solution, 25 mL of ultrapure water and 450 mL of acetonitrile in a 500-mL glass storage bottle and mix well. This solution is stored capped at room temperature and should be prepared weekly.

0.010 M Formic Acid (aqueous mobile phase)

Add 420 µL of formic acid to 950-mL of HPLC-grade water in a 1-liter volumetric flask. Dilute to 1000 mL with HPLC-grade water and mix well.

1:10 (v/v) Phosphoric Acid solution

Add 10.0 mL of phosphoric acid to 90.0 mL of HPLC-grade water in a 125-mL glass storage bottle. Mix the resulting solution to homogeneity.

4.2.3 *Stock and Intermediate Standards Preparation and Stability*

Use Class A volumetric flasks when preparing standard solutions.

Prepare standard stock solutions by accurately weighing 10.10 ± 0.10 mg of each analyte into a 100-mL volumetric flask using an analytical balance. Record the accurate weight of the standard. Dissolve the standard in approximately 50 mL of HPLC-grade acetonitrile. After dissolving, bring the solution to a volume of 100 mL using HPLC-grade acetonitrile, cap, and mix the solution to homogeneity. These standard solutions are stable for approximately 6 months when stored frozen at a temperature of ≤-10°C immediately after each use. The concentration of the each solution is 100-µg/mL in acetonitrile.

4.2.4 *Fortification Standards Preparation and Stability*

Use Class A volumetric flasks when preparing standard solutions.

Prepare a 2.0-µg/mL multianalyte intermediate standard/fortification solution in acetonitrile by pipetting 2.0 mL of each 100-µg/mL stock standard into a 100-mL volumetric flask. Bring to volume using HPLC-grade acetonitrile and mix to homogeneity.

Prepare a 0.10-µg/mL multianalyte fortification standard solution in acetonitrile by pipetting 500-µL of the 2.0-µg/mL multianalyte intermediate standard into a 10-mL volumetric flask. Bring to volume using HPLC-grade acetonitrile and mix to homogeneity.

Prepare a 0.010-µg/mL multianalyte fortification standard solution in acetonitrile by pipetting 1000-µL of the 0.10-µg/mL multianalyte intermediate standard into a 10-mL volumetric flask. Bring to volume using HPLC-grade acetonitrile and mix to homogeneity.

Alternate or additional solutions may be prepared as needed. All standard solutions prepared in acetonitrile are stable for approximately 6 months when stored frozen at a temperature of ≤-10°C immediately after each use. Standard solutions prepared in the standard diluent are stable for 24 hours when stored at 4°C.

4.2.5 *Chromatographic Standard Preparation and Stability*

Use class A Volumetric Flasks

Prepare the intermediate calibration standard of 20.0 ng/mL by diluting 100 µL of the 2.0-µg/mL multianalyte fortification standard solution to the mark with the standard diluent (9:1 5 mM aqueous ammonium acetate:acetonitrile) of a 10.0-mL Class A volumetric flask. Cap and shake or vortex mix until homogeneous.

The calibration standards are then prepared by pipetting accurate volumes of the 20.0 intermediate calibration standard and diluting it with the standard diluent as shown below. All standard solutions should be mixed well prior to its use. These standards are stable for 24 and 48 hours when stored at 4°C and ≤-10°C, respectively.

DESIRED STANDARD CONCENTRATION (NG/ML)	VOLUME OF INTERMEDIATE SOLUTION (ML)	FINAL VOLUME (ML)*
20.0**	0.10 (2.0 µg/mL Fortification solution)	10
5. 0	2.50 (20.0 ng/mL)	10
1.0	0.50 (20.0 ng/mL)	10
0.50	0.25 (20.0 ng/mL)	10
0.20	0.10(20.0 ng/mL)	10
0.10	0.050 (20.0 ng/mL)	10
0.050	0.025 (20.0 ng/mL)	10

* Diluent: 9:1 5 mM aqueous ammonium acetate: acetonitrile

** Intermediate Standard

DESIRED STANDARD CONCENTRATION (NG/ML)	VOLUME OF INTERMEDIATE SOLUTION (µL)	DILUENT VOLUME (µL)	FINAL VOLUME (ML)*
20.0**	0.10 (2.0 µg/mL Fortification solution)	-	10.0
5. 0	250 (20.0 ng/mL)	750	1.0
1.0	50 (20.0 ng/mL)	950	1.0
0.50	25 (20.0 ng/mL)	975	1.0
0.20	40 (5.0 ng/mL)	960	1.0
0.10	20 (5.0 ng/mL)	980	1.0
0.050	50 (1.0 ng/mL)	950	1.0

* Diluent: 9:1 5 mM aqueous ammonium acetate: acetonitrile

** Intermediate Standard; prepared in a Class A volumetric flask

4.2.6 *Source (& Characterization) of Samples*

The source of the soil and water samples and pertinent physical characteristics are summarized in the following table. Soil and water samples were characterized at Harris Environmental Technologies (Lincoln, NE) and Agvise Laboratories (Northwood, ND), respectively. Characterization records are maintained at DuPont Crop Protection.

Soil Name (Field Soil Dissipation Study*)	Soil Type	pH_w	Sand (%)	Silt (%)	Clay (%)	OM_{ash} (%)
North France (DuPont-34236)	Silt Loam	6.5	25	56	19	2.6
Italy (DuPont-34237)	Loam	5.8	46	38	16	1.6

*(Reference 6 and Reference 7)

Measurement	Lums Pond Surface 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)	6.8	0.48	2.98

[A] Pond water, Lums Pond State Park, Bear, DE, USA

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

[C] Well water, Kemblesville, PA, USA

4.2.7 *Storage & Preparation of Samples*

Soil samples should be stored frozen at approximately -20°C. Allow the sample to thaw, remove sticks, rocks, and leaves by hand. The soil should be mixed extensively to ensure homogeneity. The procedure used to homogenize the samples is briefly described below (Reference 5).

A laboratory sample will be prepared by combining the corresponding cores of each subplot. The entire soil sample of the cores will be blended mechanically using a food chopper (ADE cutter). Dry ice will be added to keep the soil frozen during blending. After approximately 10 minutes, the blending procedure will be stopped and the entire soil portion will be sieved (2 - 3 mm). Thereafter the mechanical blending procedure will be continued until a homogeneous soil mixture is obtained. The soil samples will be placed into a freezer or on dry ice as soon as possible after blending, but within 3 hours. Allow most of the dry ice to sublime before returning the soil to the freezer.

Before extraction, soil is allowed to thaw and a 5.0-g representative soil sample is removed from the homogeneously mixed sample.

Control/unfortified water samples were received frozen and stored in a freezer maintained at ≤-10°C. Water samples were allowed to thaw and stored at approximately 4°C prior to sample preparation and analysis. The water samples were shaken vigorously by hand prior to subsampling to ensure homogeneity. For surface water samples (e.g., from pond), about 150 mL of the sample was transferred into a 250-mL polypropylene centrifuge tube and then centrifuged at 1200 rpm for 10 minutes. Clear water sample aliquots were taken for analysis.

4.2.8 *Sample Fortification Procedure*

One hundred microliters of the 0.010- and 0.10-µg/mL multianalyte fortification solutions in acetonitrile (DPX-E9636, IN-70941, IN-70942 and IN-E9260) were spiked to 5.00 ± 0.50 g soil samples for 0.20- and 2.0-µg/kg fortification levels, respectively.

Fortification Level (ng/g)	Soil Weight (g)	Fortification Solution (µg/mL)	Fortification Solution Volume (mL)
0.20	5.0 ± 0.5	0.010	0.10
2.00	5.0 ± 0.5	0.10	0.10

Fifty microliters of the 0.010- and 0.10-µg/mL multianalyte fortification solutions in acetonitrile (DPX-E9636, IN-70941, IN-70942 and IN-E9260) were spiked to 5.00 ± 0.50 g water samples for 0.10- and 1.0-µg/mL fortification levels, respectively.

Fortification Level (ng/g)	Water Sample (g or mL)	Fortification Solution (µg/mL)	Fortification Solution Volume (mL)
0.10	5.0 ± 0.5	0.010	0.050
1.00	5.0 ± 0.5	0.10	0.050

4.2.9 *Soil Extraction Procedure*

1. Weigh 5.0 ±0.5 grams of soil into a 50-mL disposable centrifuge tube. Fortify the sample if necessary. Let the sample sit in the fume hood for 10 minutes to allow the fortification solution to evaporate.
2. Add 25 mL of extraction solution (9:1 0.1 M aqueous ammonium acetate:methanol). Cap and shake the sample vigorously.
3. Place on a wrist action shaker set to maximum deflection and shake the sample for 30 minutes.

   Alternatively, if a Geno-Grinder is available, add a 5/16 metal ball to the sample and shake it for 10 minutes at a setting of 1100 strokes per minute.

4. Centrifuge the sample at 4°C for 5-10 minutes to drive the particulates to the bottom of the bottle (at a rate of 3000-4000 rpm).
5. Decant the supernatant into a clean 50-mL disposable graduated centrifuge tube.
6. Add 25 mL of extraction solution to the sample, cap, and vortex mix for at least a minute or more until the soil pellet is broken completely. Repeat the shaking and centrifugation steps (Steps 2 and 3).
7. Decant the supernatant into the same extract collection tube. Adjust the final volume to 50 mL using the extraction solution (9:1 0.1 M aqueous ammonium acetate:methanol). Cap and vigorously mix the extract.

4.2.10 *Soil Extract Purification/Concentration Procedure*

Use the procedures described below to purify soil extracts. During SPE cleanup gravity flow should be used; vacuum should be avoided. Vacuum is only required to start elution flow and during the final drying step.

1. Place a 20 cc, 1-g Oasis HLB cartridge on an SPE manifold. Precondition the cartridge with 6 - 10 mL of methanol, discard the conditioning solution. **Do not let the cartridge go to dryness**. Condition the Oasis cartridge with 6 - 10 mL of Extraction Solution (9:1 0.1 M aqueous ammonium acetate:methanol). **Do not let the cartridge go to dryness**.
2. Measure exactly 25 mL of the extract using a measuring pipet. Pass extract through the conditioned Oasis cartridge at a flow rate of 2-5 mL/min. Rinse the cartridge with 10 mL of HPLC-grade water just before all of the extraction solution would have passed through. Discard eluates.
3. Pass 10 mL of 10 mM aqueous ammonium acetate through the cartridge and discard the eluate.
4. Pass 10 mL of ultrapure water through the cartridge and discard the eluate. Using full vacuum pull the cartridge to dryness for at least 2 minutes. Discard the eluate.
5. Rinse the sample tube (Step 2) with acetone and discard rinse.
6. Place the rinsed 50-mL graduated glass centrifuge tube under the cartridge. Elute the analytes using the following solvents by gravity flow: 10 mL of acetonitrile (use only low vacuum as needed to start the elution at a flow rate of 1-2 drops/second and then turn it off) followed by 5 mL of 9:1 acetonitrile:0.5 M ammonium hydroxide. After the entire elution solution has passed through, the cartridge, apply a full vacuum for 10-15 seconds to remove all liquid. Collect all eluates in the same 50-mL glass centrifuge tube.
7. Add immediately 1 mL of 5 mM aqueous ammonium acetate to the eluate (alternatively this can be added to the eluent collection tubes prior to elution) and evaporate until 1.0 ± 0.1 mL (aqueous) using a N_2 evaporator with a moderate nitrogen flow and the water bath set at 30°C (this takes ~1hr). Do not let the extract to go to dryness!

8. Add 0.5 mL of acetonitrile to the eluate, cap and vortex mix. Bring the final volume to 5 mL using 5 mM aqueous ammonium acetate. Vortex to mix the sample, sonicate for 5 minutes, and again vortex mix.
9. Using a disposable syringe, filter about 1.5 mL of the purified extract from Step 7 through a 0.2-µm Acrodisc PTFE disk into an HPLC vial. For the control extract, filter about 3 mL through a 0.2-µm Acrodisc PTFE disk into a 20-mL glass scintillation vial. Some will be used to prepare post-fortified samples in Step 9.

   Analyze the sample solution by LC/MS/MS as described in the following section. Extracts are stable for approximately 24 hours and 72 hours if stored at 4°C and ≤ -10°C, respectively.
10. Prepare the following post-fortified samples (optional):

    LOQ equivalent (0.1 ng/mL): In a 2-mL HPLC vial, add 980 µL of the control extract from Step 8 and 20 µL of the 5.0-ng/mL calibration standard. Cap the vial and vortex mix the sample.

    10xLOQ equivalent (1.0 ng/mL): In a 2-mL HPLC vial, add 990 µL of the control extract from Step 8 and 10 µL of the 0.10-µg/mL fortification solution. Cap the vial and vortex mix the sample.

4.2.13 *Water Sample Preparation*

1. Measure **accurately** 5.00 ± 0.10 g or 5.0 ± 0.1 mL of each water sample into a 15-ml polypropylene centrifuge tube.
2. Fortify the sample if necessary.
3. Add 500 µL of acetonitrile and 25 µL of 1 M ammonium formate into each sample. Cap and shake the sample vigorously.

   Water samples are stable for approximately 24 hours and 72 hours if stored at 4°C and ≤ -10°C, respectively.
4. Transfer about 2 mL of the sample into a 2-mL HPLC vial and analyze by LC/MS/MS as described in the following section.

4.3 Instrumentation

4.3.1 *Description*

Method validation data in this study were generated from an Agilent Infinity 1290 UHPLC or Shimadzu UHPLC coupled to Applied Biosystems MDS SCIEX API 5000 (a triple quadrupole MS) with an electrospray ion source.

4.3.2 *Operating Conditions*

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

HPLC Conditions					
System:	Agilent Series 1290 Infinity UHPLC Shimadzu UHPLC				
Columns:	Kinetex C18 column, 2.1x50 mm, 1.7-µm dp; OR Zorbax Eclipse Plus C18, 2.1 × 50 mm, 1.8-µm dp				
Column Temperature:	40°C or 50°C				
Autosampler Temperature:	4-7°C				
Injection Volume:	20-25 µL				
Mobile Phase Conditions: Solvent A: 0.01 M Formic acid Solvent B: Methanol	Time 0.00 0.30 0.40 4.90 5.00 6.00 6.10 7.00*	%A 95.0 95.0 85.0 45.0 5.0 5.0 95.0 95.0	%B 5.0 5.0 15.0 55.0 95.0 95.0 5.0 5.0	Flow (mL/min) 0.6 0.6 0.6 0.6 1.0 1.0 0.6 0.6	
Retention Time,, min: IN-E9260,, ~1.1; IN-70941, ~3.1; IN-70942, ~3.8; DPX-E9636, ~4.5 Note: The run time can be extended to 8 min. in order to extend the column flushing.					

MS Conditions								
MS System:	Applied Biosystems MDS SCIEX API5000							
Analyte Monitored	Ion Transitions Monitored	DP (V) [a]	EP (V) [b]	CE (V) [c]	CXP (V) [d]	Dwell Time (ms)	Period Timing (min)	Acquisition Timing (min)
DPX-E9636	432.0 → 325.0 ± 0.1 [f]	100	2	20	25	150	3.7 – 5.4	0.5 – 5.5
	432.0 → 182.0 ± 0.1 [e]	75	14	30	25			
IN-70941	368.0 → 325.0 ± 0.1 [e]	75	4	20	35	150	2.3 – 3.7	0.5 – 5.5
	368.0 → 231.0 ± 0.1 [f]	75	4	50	35			
IN-70942	325.0 → 279.0 ± 0.1 [f]	150	10	30	40	150	3.7 – 5.4	0.5 – 5.5
	325.0 → 231.0 ± 0.1 [e]	150	10	40	25			
IN-E2960	251.0 → 234.0 ± 0.1 [f]	100	2	20	25	150	0.5 – 2.3	0.5 – 5.5
	251.0 → 106.0 ± 0.1 [e]	50	8	30	15			

Scan type/Polarity:	Multiple Reaction Monitoring/Positive		
Ion Source Voltage:	ESI+, 3000 V		
Collision Gas (CAD):	12 psig	Heater Gas (GS2):	60 psig
Curtain Gas (CUR):	10 psig	Source Heater (TEM):	700°C
Nebulizer Gas (GS1):	60 psig	Interface Heater (ihe):	ON
MS Flow Rate:	(Post-column split) 300-µL/min to the MS		

[a] Entrance Potential [b] Declustering Potential [c] Collision Energy [d] Collision Exit Potential
[e] Quantification Transition [f] Confirmatory Transition

A triple quadrupole MS instrument with an electrospray ionization (ESI) source was used for the detection of rimsulfuron 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 elution conditions of the analyte from the HPLC column. Each of the positive or negative molecular ions 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.

Rimsulfuron and its metabolites were each identified in soil 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.

A six-port electronically activated switching valve was used to direct the HPLC column effluent to waste prior to and following the elution of analytes. For example, in the 7-minute analysis, the retention times of the analytes were within 2-5 minutes. thus the MS sample collection time is 0.50 to 5.5 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.

Since the electrospray interface is optimal at low flow rates, the column effluent flow was split such that only a flow of about 300-µL/min actually passed through the interface the remainder going to waste. This process also 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 rimsulfuron and its metabolites found in the soil and 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 rimsulfuron 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.

To minimize carry over, standards and samples MUST be injected in the order from low to high concentrations of calibration standards (e.g., solvent blank first, then 0.050-ng/mL standard, control, LOQ fortifications, 0.10-ng/mL standard, LOQ fortifications, 0.50-ng/mL standard, 10 × LOQ fortifications, 1.0-ng/mL standard, 10 × LOQ fortifications, 2.5-ng/mL and 5.0-ng/mL standard last followed by three blank injections).

It is recommended to inject a solvent blank (standard/sample diluent) at the beginning of any analytical set in order to verify that the system is clean, as well as check if the diluent is also clean (no contamination). It is also recommended that at the end of any analytical set calibration solvent should be injected for at least three times to ensure that the column is flushed completely.

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 rimsulfuron and its metabolites in soil and water samples (ppb found) was then calculated using the equation below:

µg/kg or µg/L (ppb) found =

$$\frac{A \times Rf_{ave}\,(ng/mL/\text{area counts}) \times \text{Extract Volume (mL)} \times \text{Final Volume (mL)} \times \text{Dilution Factor}}{\text{Sample Weight (g)} \times \text{Aliquot Volume(mL)}}$$

where:

A = Corrected Peak Area Counts
= Peak Area Counts in sample – Peak Area Counts in control
Rf_{ave} = Average Response Factor

Note: 1 µg = 1000 ng; 1 kg = 1000 g; 1 L = 1000 mL; therefore, 1 ng/g = 1 µg/kg and 1 ng/mL = 1 µg/L

Density of water = 1 g/mL

Percent Recovery was calculated as:

$$\%\ \text{Recovery} = \frac{\text{Analyte Found } (\mu g/kg \text{ or } \mu g/L)}{\text{Fortification Level } (\mu g/kg \text{ or } \mu g/L)} \times 100$$

5.1.5 *Limit of Quantitation*

The limit of quantification for rimsulfuron, IN-70941, IN-70942 and IN-E9260 in soil and water methods was determined to be 0.20 µg/kg, and 0.1 µg/L, respectively. The LOQ is defined as the lowest fortification level at which average recoveries of 70-120% and a RSD ≤20% was achieved (Appendix 2).

The limit of detection was not determined experimentally during the course of this work. The limit of detection is considered to be a level of approximately three times the background around the retention time of the peak of interest. It must be recognized that the limit of detection will vary between soil types and from day to day as would be common for all trace analyses.

5.2 ***Timing***

A single analyst can extract, purify, and analyze 12-18 soil samples for about 1.2 working days. A single analyst can prepare and analyze 20 or more water samples for about 1.2 working days. Sample analysis by HPLC/ESI-MS/MS is 7-8 minutes and can be run unattended overnight. Data processing is completed using an Excel spreadsheet template on the following day for about 30-min.

5.3 ***Modifications or Special Precautions***

Since analysis is done at low levels of quantifications using MS for detection, special precautions must be taken to avoid contamination common to all trace level analyses.

5.4 ***Method Ruggedness***

5.4.1 *Stability*

Standards and samples are all stable under ambient conditions for at least 24 hours provided solvents are not allowed to evaporate. However, it is recommended to keep them stored in the freezer maintained at 0°C when not in use.

5.4.2 *Specificity/Potential Interference*

5.4.2.1 *Interference from Glassware & Reagents, Matrices*

The level of interference in control samples from three different sources evaluated was minimal.

There was no interference from reagents.

A potential interference from non-disposable glassware/plastic ware could occur when they are not cleaned properly. All glassware was rinsed with reagent grade acetone after being washed with a non-phosphate detergent and rinsed well with tap water, followed by distilled water.

The SPE flow control valve liners could also be a potential source of interference. A new set of SPE flow control valve liners is used for every set of samples to be purified.

Another potential source of interference would be contaminated drying tubes of the N_2-vap. Prior to placing the sample tubes on the N_2 vap, the drying tubes were wiped with a paper towel soaked with acetone.

5.4.3 *Confirmatory Method*

Residues of nicosulfuron and metabolites were confirmed based on acceptable calibration and fortification recovery data generated from the second/confirmatory ion transition monitored during analysis, i.e., linear calibration curves with $R^2 > 0.99$ and average fortification recovery fortification level of 70 – 120 % with RSD <20%

Residues of nicosulfuron and metabolites were also confirmed based on the relative ratio of the intensities of two ion transitions collected for each analyte during method validations. For every sample set, the average of the ion ratios for all standards that made up the calibration curve was calculated. The observed ion ratio of each of the fortified samples was then compared to the average ion ratio of calibration standards.

In order for a sample set to be valid, the relative standard deviation of the ion ratios calculated from the calibration standards analyzed must be ≤ 20%. For the confirmation of a possible residue of nicosulfuron and metabolites in investigative samples, each ion ratio must fall within ±30% of the average ratio for all calibration standards for a specific sample set. If the ion ratio is outside the ±30% range, the signal was most likely generated from a compound, which is unrelated to the analyte. The unknown compound also has the same ion by LC/MS and a similar fragmentation pattern.

For selected sample sets analyzed during method validations, the ion ratios of the calibration standards satisfied the ≤ 20% RSD criteria. For calibration standards and fortified samples, relative standard deviations of ion ratios were < 10%. Therefore, all fortified samples analyzed meet the accuracy criteria of ±30% of the average ratio for all calibration standards in a sample set. Confirmation analyses of rimsulfuron and metabolites in water samples are shown in Appendix 2.

5.4.4 *Example of Calculation*

SAMPLE	AREA (432 → 182)	AREA (432 → 325)	RATIO (182 / 325)
0.050 ng/mL std	31200	17500	1.8
0.10 ng/mL std	64000	32000	2.0
Pond LOQ 1	**56600**	**27900**	**2.0**
0.20 ng/mL std	114000	59500	1.9
0.50 ng/mL std	283000	139000	2.0
Pond 10xLOQ 1	**432000**	**232000**	**1.9**
1.0 ng/mL std	534000	285000	0.9
5.0 ng/mL std	481000	252000	1.9
		Average Ratio of Calibration Standards:	1.9
		RSD:	4.9

The ± 30% range was calculated as:

Upper limit was 1.9 + (0.3 x 1.9) = 2.5

Lower limit was 1.9 - (0.3 x 1.9) = 1.4

The ion ratios for the LOQ sample (Pond LOQ 1) and the 10X LOQ sample (Pond 10XLOQ 1) fall within the upper and lower limits calculated. Based on the criteria outlined, the levels of rimsulfuron found in the above-fortified samples would be confirmed as a rimsulfuron residue.

6.0 CONCLUSIONS

This analytical method is suitable for the quantifications of residues of rimsulfuron and its metabolites IN-70941, IN-70942 and IN-E9260 in soil and water at a LOQ of approximately 0.20 µg/kg and 0.10 µg/L, respectively.

FIGURE 2 PROPOSED METABOLIC PATHWAY OF RIMSULFURON (DPX-E9636) IN SOILS