TRADE SECRET

Study Title

ANALYTICAL METHOD FOR THE DETERMINATION OF RIMSULFURON IN WATERY AND DRY CROP MATRICES BY HPLC/ESI-MS/MS

Test Guidelines

EEC Directive 91/414/EEC, Annex IIA 4.2.1 as amended by EC Directive 96/46/EC; SANCO/825/00 rev.6 (20/06/00) Guidance Document on Residue Analytical Methods

U.S. EPA Residue Chemistry Test Guidelines, August 1996
OPPTS 860.1340 Residue Analytical Method

Authors

Anne Pentz
Jocelyn Bilas
Maria Elena Y. Cabusas

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Performing Laboratory

E.I. du Pont de Nemours and Company
DuPont Agricultural Products
Global Technology Division
Stine-Haskell Research Center
Newark, Delaware 19714-0030

Study Number

DuPont-15033

LIST OF ABBREVIATIONS AND SYMBOLS

aq	Aqueous solution
°C	Degree 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
HOAc	Acetic acid
HPLC	High-performance liquid chromatography
ESI-MS/MS	Electrospray mass spectrometry/ mass spectrometry
LOQ	Limit of quantitation
M	Molar
MeOH	Methanol
mL	Milliliter(s)
mM	Millimolar
min	Minute(s)
mm	Millimeter(s)
n	Total number of samples analyzed
OECD	Organization for Economic Co-operation and Development
ppm	Parts per million
rpm	Revolution per minute
RSD	Relative standard deviation
S/N	Signal-to-noise ratio
SD	Standard deviation
SPE	Solid phase extraction
t_R	Retention time
μL	Microliter(s)
μm	Micrometer(s)

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ANALYTICAL METHOD FOR THE DETERMINATION OF RIMSULFURON IN WATERY AND DRY CROP MATRICES BY HPLC/ESI-MS/MS

Anne Pentz, Jocelyn Bilas and Maria Elena Y. Cabusas

1.0 ABSTRACT

An analytical method was developed for the determination of rimsulfuron (DPX-E9636) in watery and dry crop matrices. The method quantifies rimsulfuron at a limit of quantitation (LOQ) of approximately 0.010 mg/kg (ppm) in 5.0-g fruit matrices. Rimsulfuron was extracted from each of the fruit matrices with 0.1 M potassium phosphate (pH 7)/methanol (4:1, v/v) solution using a Tekmar® homogenizer. Following centrifugation, extract aliquots were diluted with 0.5% acetic acid (1:1), then purified and concentrated using SPE Oasis™ MAX cartridges. Rimsulfuron was eluted with 0.1% formic acid in acetonitrile after the cartridges were sequentially washed with water, methanol, ethyl acetate, and acetonitrile. The eluates were evaporated to dryness at 30°C, reconstituted in 5 mM ammonium acetate/methanol (8:2, v/v) solution and filtered. The purified extracts were analyzed by reversed-phase HPLC using electrospray mass spectrometry/mass spectrometry (ESI-MS/MS) for detection.

Acceptable recoveries of rimsulfuron were obtained from various fortified watery, dry, and oily fruit matrices. The overall average recoveries ranged from 86% to 99% (with RSD <20%, n=10 per matrix) as shown below. All unfortified control of fruit matrices showed no detectable residues of rimsulfuron.

Fruit Matrix	% Recovery Rimsulfuron in Various Watery, Dry, and Oily Fruit Matrices					
	0.010 mg/kg (n[a]=5)		0.10 mg/kg (n=5)		Overall (n=10)	
	Mean (± RSD[b])	Range	Mean (± RSD)	Range	Mean (± RSD)	Range
Apple	91 (± 2.0)	89-94	98 (± 2.9)	93-100	95 (± 4.2)	89-100
Cherry	88 (± 12.1)	74-100	97 (± 4.1)	94-104	93 (± 9.8)	74-104
Grape	93 (± 6.1)	84-99	93 (± 3.9)	90-99	93 (± 4.9)	84-99
Lemon	88± 5.8	80-94	93 (± 5.9)	89-102	91 (± 6.3)	80-102
Lime	89± 6.6	83-96	85 (± 6.7)	79-91	87 (± 6.7)	79-96
Plum	100 (± 9.5)	91-116	98 (± 1.9)	96-101	99 (± 6.6)	91-116
Almond Hull[c]	89 (± 6.2)	78-93	82 (± 14.0)	70-93	86 (± 10.0)	70-93
Prune	90 (± 3.6)	85-93	92 (± 2.0)	89-94	91 (± 2.9)	85-94

[a]n = Total number of fortifications per fruit matrix [b]RSD = Relative standard deviation [c]LOQ, n = 6; 10xLOQ, n = 4

Rimsulfuron in each investigative sample was quantitatively determined using average response factor method. The relative standard deviation of response factors from the calibration standards per analytical set was below 15%. Each calibration curve showed a linear response with correlation coefficient (R) greater than 0.99.

The efficiency of the method extraction procedure was evaluated on similar type of watery, dry, and oily crop matrices. Identical extraction procedure was evaluated previously using field samples of corn plants treated with radiolabeled rimsulfuron. The extraction efficiency was estimated to range from 89% to 93%. Moreover, the extraction procedure was also used to analyze rimsulfuron in corn (forage and grain), potato, and tomato samples stored frozen for 24, 24, and 6 months, respectively. The average normalized recoveries of rimsulfuron from corn (forage and grain), potatoes, and tomatoes were 99 ± 7.9% (n=13), 105 ± 5.1% (n=7), and 79 ± 11.5% (n=8), respectively. The somewhat low recovery of rimsulfuron from tomato samples maybe attributed to its slight degradation in the acidic matrix.

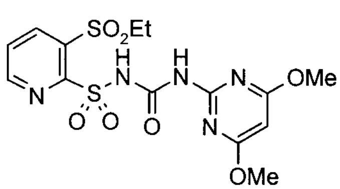
The confirmatory method was based on detection and the relative ratios of the two MS/MS parent-to-daughter ion transitions (m/z = 182 and 325) monitored during the validation.

A single analyst can extract, purify, and analyze 10-12 samples for an 8-hour working day. The total chromatographic run time for the HPLC/MS/MS analysis is about 5 min/sample.

2.0 INTRODUCTION

Rimsulfuron (DPX-E9636) is a sulfonylurea herbicide used for post-emergence control of broadleaf and grass weeds during the production of corn and other crops. The chemical structures and pertinent information of the test substances are shown below:

Structure



DuPont Code: DPX-E9636

Common Name: Rimsulfuron

IUPAC Name: 1-(4,6-dimethoxypyrimidin-2-yl)-3-(3-ethylsulfonyl-2-pyridylsulfonyl)urea

***C.A.* Name**: N-[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]-3-(ethylsulfonyl)-2-pyridinesulfonamide

***CAS* Registry No.**: 122931-48-0
Empirical Formula: $C_{14} H_{17} N_5 O_7 S_2$
Molecular Weight: 431.451 u
Monoisotopic Mass: 431.057 u
pKa: 4.0 (25°C)
Stability: On hydrolysis (25C) DT50 4.6 days (pH 5), 7.2 days (pH 7), 0.3 days (pH 9)

This analytical method for rimsulfuron in watery and dry crop matrices at an LOQ of approximately 0.010 mg/kg (ppm), was developed in accordance to EU Annex II 4.2.1 and U.S. EPA Residue Chemistry Test Guidelines. This method is intended as a regulatory method for the determination of rimsulfuron in various watery and dry crop matrices.

Rimsulfuron was extracted from a 5-g sample of watery crops with 0.1 M potassium phosphate (pH 7)/methanol (4:1, v/v) solution using a Tekmar® homogenizer. Following centrifugation, an aliquot of the extract was diluted and mixed to homogeneity with 0.5% acetic acid (1:1). The extract was then purified and concentrated using an SPE Oasis™ MAX cartridge. The analyte was eluted with 0.1% formic acid in acetonitrile after the cartridge was sequentially washed with water, methanol, ethyl acetate, and acetonitrile. The eluate was evaporated to dryness at 30°C under a gentle stream of nitrogen, reconstituted in 5 mM ammonium acetate/methanol (8:2, v/v) solution and filtered. The purified extract was analyzed by reversed-phase HPLC using a Phenomenex Luna® phenyl-hexyl column and a mobile phase of methanol and 0.01% formic acid in 0.1 mM ammonium formate and methanol. Detection of rimsulfuron was by electrospray mass spectrometry/mass spectrometry (ESI-MS/MS) in the positive ion mode. The two parent-to-daughter ion transitions of rimsulfuron monitored during sample analysis were 182 and 325 (m/z).

The efficiency of the method extraction procedure was evaluated on similar type of watery, dry, and oily crop matrices. Identical extraction procedure was evaluated previously using field samples of corn plants treated with radiolabeled rimsulfuron (Reference 2). The extraction efficiency was estimated to be in the range of 89-93% (Reference 3). Moreover, the extraction procedure was also used in the freezer storage stability studies to analyze rimsulfuron in corn (forage and grain), potato, and tomato samples stored for 24, 24, and 6 months, respectively (References 4-6). The average normalized recoveries of rimsulfuron from corn (forage and grain), potatoes, and tomatoes were 99 ± 7.9% (n=13), 105 ± 5.1% (n=7), and 79 ± 11.5% (n=8), respectively. The somewhat low recovery of rimsulfuron from tomato samples maybe attributed to its slight degradation in the acidic matrix (pH range of tomato and its processed fractions 4-5; Reference 7).

Acceptable recoveries of rimsulfuron were obtained from the watery and dry crop samples. The overall average recoveries of rimsulfuron from fruit matrices ranged from 86% to 99% with a RSD range of 2.9% - 10.0%. There were no detectable residues in the control samples. Furthermore, there was only minimal influence of matrix effect on the recoveries of rimsulfuron from the fruit matrices.

The confirmatory 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, one post-fortified sample was analyzed for each set of analytical samples to determine if matrix effect, either suppression or enhancement, influenced the recovery of rimsulfuron. The post-fortified sample in this study was an extract of control crop sample that was purified and prepared in the same manner as with the other samples, but fortified with rimsulfuron prior to sample analysis.

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 crop samples and salts (Mettler Instrument Corporation)

Centrifuge System

- Sorvall® Centrifuge Model RC5C with Super-lite™ GSA model SLA-1500 rotor (Sorvall Instruments, Wilmington, DE)
- Sorvall® Centrifuge Model GLC-2B (Sorvall Instruments)

Filtration System

- Sample Extract Filtration: Bond Elut reservoirs with 20-µm polyethylene frits, Catalog No. 12131017 (can be prepared from plastic Reservoirs – 20-mL size, Cat. No. 12131011, and frits for 20-mL reservoirs, catalog No. 12131023) (Varian Inc., Harbor City, CA)
- HPLC Sample Filtration: Disposable syringes with Luer-Lok® Tip – 5 mL, sterilized, Cat. No. BD309585 (VWR International, Bridgeport, NJ)
- HPLC Syringe Filters – Acrodisc®, 13 mm dia, 0.2 µm pore size, PTFE (Pall Gelman Laboratory, Ann Arbor, MI)

HPLC/MS System

- HP Series 1100 Liquid Chromatograph with G1332A degasser, G1312A binary pump, G1313A chilled autosampler, G1316A column compartment (Agilent Technologies, Little Falls, DE); Luna® Phenyl-Hexyl analytical column, 4.6 mm i.d. × 50 cm with 3-µm diameter packing, Part No. 00B-4256-E0 (Phenomenex, Torrance,CA (DO NOT SUBSTITUTE ANALYTICAL COLUMN.)
- Quattro II triple quadrupole mass spectrometer using an electrospray interface (Micromass Inc., Altrincham, UK) with Valco zero-dead volume 3-port connector for 1/10 split flow to mass spectrometer.

Homogenizer

- Tissumizer® Mark II Variable Speed Motor, Item No. 10-0413-018, equipped with a Model STD-182 EN shaft & generator, Item No. 10-0104-000 (Tekmar Co., Cincinnati, OH)

Nitrogen Evaporator

- N-Evap® Model 112 (Organomation Associates, Berlin, MA); unit is attached to a nitrogen source

pH Meter

- BeckmanModel ϕ340 pH/Temperature Meter (Beckman Instruments, Inc., Fullerton, CA)

Pipettes and Tips

- Biohit Proline® Electronic Pipettors, variable volume, with tip ejector, 10-250 L and 50-1000 L, Catalog No. 53495-210 and 53496-205 (VWR International)
- Rainin Pipetman Pipettors, Variable Volume with Tip Ejector, Gilson® - Model P-200, 0-200 μL, Cat. No. P-200, Model P-1000, 0-1000 μL Cat. No. P-1000, Model P-5000, 0-5000, Cat No. P-5000 (Rainin Instrument Inc., Woburn, MA)
- Disposable Pasteur Pipets, Borosilicate glass, 9-inch length, Catalog No. 14673-043 (VWR International)
- EDP Electronic Digital Pipette, Catalog No. EP-10 ML (Rainin Instrument, Inc.)
- Sorenson™ Multifit 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 Inc.)
- Rainin Pipetman Pipette tips, Gilson® – Cat. No. RC-20 for Model P-200, Cat. No. RC-1000 for Model P-1000, Cat. No. RC-5000 for Model P-5000 (Rainin Instrument Inc.)

Sample Containers (Centrifuge Tubes, Bottles, and Vials)

- Centrifuge Bottles with Screw Caps, Polypropylene Copolymer, Nalgene®, 250-mL capacity, Cat. No. 3120-0250 (VWR International)
- Disposable Polypropylene Centrifuge Tube – Corning®, graduated, 50-mL capacity, Cat. No. 430291 (VWR International)
- Kimax® Graduated Mixing Cylinder with Stopper, 100-mL capacity, Cat. No. 24763-095 (VWR International)
- Pyrex® Borosilicate Glass Tubes – Pyrex, 40 x 130 mm, 130-mL, Cat. No. 60856-136 (VWR International)

- VWR® Bottles, HDPE , Wide Mouth - 125 mL, Cat. No. 16126-063 (VWR International)
- Kimble® Disposable 50-mL Tubes and Screw Caps (S/T closure BLPH PTFEV/R, Cat. No. 73785-50 and 73802-24400 (Kimble Glass, Inc., Vineland, NJ)
- HPLC Vials with Teflon/Silicone/Teflon Septa, 2-mL, Cat. No. 5182-0865 (Agilent Technologies)

Solid-Phase Extraction Apparatus and Accessories

- Visiprep™ SPE Manifold, Cat. No. 5-7044 (Supelco, Inc., Bellefonte, PA)
- Solid-Phase Extraction Disposable Flow Control Valve Liners - for the Visiprep™, Cat. No. 57059 (Supelco, Inc.)
- Waters Oasis® MAX 6cc/500mg LP Extraction Cartridge, Cat. No.186000865, (Waters Corp., Milford, MA). DO NOT SUBSTITUTE.
- SPE Plastic Reservoirs for Sample Loading - 25-mL size, Cat. No. 1213-1011 (Varian Inc.)

Ultrasonicator

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

Vortex mixer

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

3.2 Reagents and Standards

- Acetonitrile – EMD® #AX0142-1, HPLC grade, (EMD Chemicals, Inc., Gibbstown, NJ)
- Ammonium Acetate - "Baker Analyzed" ® HPLC Reagent, #0599-08 (J.T. Baker, Phillipsburg, NJ)
- Ethyl Acetate - chromatography grade, EMD®#EX0241-01 (EMD Chemicals, Inc.,)
- Formic Acid – Suprapur®, 98-100%, #11670-1 (EMD Chemicals, Inc.)
- Formic Acid, Ammonium Salt – A.C.S. reagent, #M530-08 (J.T. Baker)
- Glacial Acetic Acid - reagent grade, EMD®#AX0073-6 (EMD Chemicals, Inc.)
- Methanol - HPLC grade, EMD® # MX0488-1 (EMD Chemicals, Inc.)
- Potassium Phosphate, Dibasic, Powder – A.C.S. Reagent, #3252-01 (J.T. Baker)
- Ultrapure Water – deionized water passed through a Milli-Q® Water Purification System (Millipore)
- Reference standards - DPX-E9636-22 (Rimsulfuron), 99.1% Purity (DuPont Crop Protection, Newark, DE)

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 was extracted from a 5-g sample of watery and dry crops with 0.1 M potassium phosphate (pH 7)/methanol (4:1, v/v) solution using a Tekmar® homogenizer. Following centrifugation, an aliquot of the extract was diluted and mixed to homogeneity with 0.5% acetic acid (1:1). The extract was then purified and concentrated using an SPE Oasis™ MAX cartridge. The analyte was eluted with 0.1% formic acid in acetonitrile after the cartridge was sequentially washed with water, methanol, ethyl acetate, and acetonitrile. The eluate was evaporated to dryness at 30°C under a gentle stream of nitrogen, reconstituted in 5 mM ammonium acetate/methanol (8:2, v/v) solution and filtered. The purified extract was analyzed by reversed-phase HPLC using a Phenomenex Luna® phenyl-hexyl column and a mobile phase of methanol and 0.01% formic acid in 0.1 mM ammonium formate and methanol. Detection of rimsulfuron was by electrospray mass spectrometry/mass spectrometry (ESI-MS/MS) in the positive ion mode. The two parent-to-daughter ion transitions of rimsulfuron monitored during sample analysis were 182 and 325 (m/z).

The efficiency of the method extraction procedure was evaluated previously using field samples of corn plants treated with radiolabeled rimsulfuron (Reference 2). The extraction efficiency was estimated to be in the range of 89-94% (Reference 3).

The confirmatory 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, one post-fortified sample was analyzed for each set of analytical samples to determine if matrix effect, either suppression or enhancement, influenced the recovery of rimsulfuron. The post-fortified sample in this study was an extract of control crop sample that was purified and prepared in the same manner as with the other samples, but fortified with rimsulfuron prior to sample 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 Potassium Hydrogen Phosphate (aq)

Using a 1.0-L volumetric flask, dissolve 174 g of potassium hydrogen phosphate (K_2HPO_4) and dilute to the mark with HPLC grade water. Mix and store this solution capped at room temperature. This solution should be prepared monthly.

0.1 M Potassium Phosphate (pH 7) Buffer/Methano/ (4:1, v/v) (Extraction Solution)

Mix 160 mL of 1.0 M K_2HPO_4 with 1440 mL of deionized water. Adjust the pH of this solution (as measured by a calibrated pH meter) to 7.0-7.5 by gradual addition of concentrated phosphoric acid (1-1.5 mL). Add 400 mL of methanol and mix. This solution is stored capped at room temperature and should be prepared monthly.

~0.5% Acetic Acid (aq)

Dilute 2.5 mL of glacial acid with 500 mL of deionized water in a 500-mL glass bottle. This solution is stored capped at room temperature and should be prepared monthly.

~0.1% Formic Acid in Acetonitrile (SPE Eluent)

Mix 500 µL concentrated formic acid and 500 mL of acetonitrile in a capped 500-mL glass bottle. This solution is stored at room temperature and should be prepared weekly.

1.0 M Ammonium Acetate (aq)

In a 1.0-L volumetric flask, dissolve 77.08 grams of ammonium acetate (NH_4OAc) and dilute to the mark with water. This solution is stored capped at room temperature and should be prepared monthly.

5 mM Ammonium Acetate (aq)

Using a 500-mL volumetric flask, dilute 2.5 mL of 1.0 M ammonium acetate to the mark with HPLC grade water and mix. This solution is stored capped at room temperature and should be prepared monthly.

5 mM ammonium acetate/methanol (8:2, v/v) solution

Mix 160 mL of 5 mM ammonium acetate with 40 mL of methanol. This solution is stored capped at room temperature and should be prepared weekly.

1.0 M Ammonium Formate (aq)

In a 1.0-L volumetric flask, dissolve 63.06 grams of ammonium formate and dilute to the to the mark with ultrapure water. This solution is stored capped at room temperature and should be prepared monthly.

1:1 Extraction solution/~0.5% Acetic Acid (aq)

Mix 500 mL of the Extraction Solution and 500 mL of ~0.5% acetic acid (aq) in a capped 1.0-L glass bottle. This solution is stored at room temperature and should be prepared monthly.

0.01% Formic Acid in 0.1 mM Ammonium Formate (aq) (Mobile Phase)

Using a 1.0-L volumetric flask, dilute 100 µL of 1.0 M ammonium formate to the mark with ultrapure water. Add 100 µL of concentrated formic acid and mix. This solution is stored capped at room temperature and should be prepared monthly.

4.2.3 <u>*Stock and Intermediate Standards Preparation and Stability*</u>

Ten milligrams of rimsulfuron analytical standard is accurately weighed into a 100-mL volumetric flask, dissolved, and diluted to the mark using acetonitrile to make a stock standard solution of approximately 100 µg/mL.

Prepare the intermediate standard solution of 50-µg/mL by adding 5.0 mL of the 100-µg/mL stock solution into a 10-mL volumetric flask. Dilute to the mark with acetonitrile and mix.

Solutions of the stock standard and intermediate standard are stored in the freezer maintained at a temperature below 0°C and are stable for 6 months.

4.2.4 <u>*Fortification Standard Preparation and Stability*</u>

Using two 10-mL volumetric flasks, dilute 1.0 mL and 0.10 mL of the 50-µg/mL intermediate standard to the mark with acetonitrile to make fortification solutions of 5.0-µg/mL and 0.50-µg/mL. Transfer each of the fortification solutions in ten 2-mL HPLC vials. These solutions are stable for six months when stored in the freezer maintained at a temperature below 0°C.

4.2.5 <u>*Chromatographic Standard Preparation and Stability*</u>

Dilute 0.20 mL of the 5.0-µg/mL fortification standard with 5 mM ammonium acetate/methanol (8:2, v/v) solution to the mark of a 2-mL volumetric flask to prepare the intermediate calibration standard of 0.50-µg/mL of rimsulfuron.

Prepare the intermediate calibration standard of 0.050-µg/mL in a 2-mL volumetric flask by diluting 0.20 mL of the 0.50-µg/mL intermediate calibration standard to the mark with 5 mM ammonium acetate/methanol (8:2, v/v) solution.

Prepare chromatographic standards ranging from 0.50 to 20.0 ng/mL (or in concentrations expected to cover the range of rimsulfuron in the investigative samples) in 2-mL volumetric flasks using the 5 mM ammonium acetate/methanol (8:2, v/v) solution as diluent. Keep all chromatographic standards at or below 4°C right after preparation. These standards should be prepared daily. The following tables shown below describe how standards were prepared for the validation work presented in this report:

Standard Conc. (ng/mL)	μL added	Intermediate Standard Used	Final Volume (mL)
20.0	80	0.50 μg/mL (int. cal. std)	2.0
10.0	400	0.050 μg/mL (int. cal. std)	2.0
5.0	200	0.050 μg/mL (int. cal. std)	2.0
1.0	200	10.0 ng/mL (cal. std)	2.0
0.50	200	5.0 ng/mL (cal. std)	2.0

Int. = intermediate; cal. = calibration; std = standard

4.2.6 *Source of Samples*

Some samples of fruit matrices were control samples from the magnitude of residue studies. Other samples were purchased from grocery stores and preprocessed by a DuPont laboratory.

4.2.7 *Storage & Preparation of Samples*

Preprocessed fruit samples were received frozen and stored in a freezer maintained at a temperature below 0°C prior to preparation for analysis.

4.2.8 *Sample Fortification Procedure*

Fortifications were made using the 0.50- and 5.0-μg/mL intermediate-standard solutions in acetonitrile. 100 μL of the 0.50- and 5.0-μg/mL standard solutions were added to 5.0-g samples of sample for the 0.010- and 0.10-mg/kg fortification levels, respectively. All fortifications were made prior to addition of extracting solution.

4.2.9 *Analyte Extraction Procedure*

1. Weigh 5.00 g ± 0.10 g of sample into each of the 250-mL polypropylene centrifuge bottles, fortify as needed and allow to sit in the hood for 10 minutes. (Do not leave the samples in the hood for more than 10 minutes to minimize degradation of rimsulfuron.)
2. Add 50 mL of extraction solution (0.1 M potassium phosphate (pH 7)/methanol (4:1, v/v)) to each of the watery crop samples. Cap and let the sample soak for 10-15 minutes under the hood. For dry crop matrices, add 70 mL of extraction solution, cap sample bottles, and let samples soak for at least 60 minutes in the hood.
3. Homogenize each sample for 1 minute using Tissumizer® at about 60% of maximum output (at higher output mixture foams excessively).

4. Let samples sit for 5 minutes, then repeat the homogenization-rest procedure for two more times.
5. When homogenization is completed, cap sample bottles and centrifuge at 13,000 rpm for 15 minutes at 2°C. If using a slower speed, centrifuge until most particulates have separated from the extraction solution.
6. Attach Bond Elut reservoirs with 20-µm frits onto an SPE vacuum manifold port. Place a 130-mL glass collection tubes under the filter assembly to catch the extracts.
7. After centrifugation, carefully transfer the entire extract (supernatant), with the aid of a Pasteur pipet, into the filter assembly. Apply light vacuum to start filtration of the extract.
8. After the entire extract is filtered, add 10 mL of extraction solution and gently swirl around the pellet. Using a Pasteur pipet, transfer the entire rinse to the filter assembly and filter. Collect the filtrate into the same tube used for the sample extract.
9. Carefully transfer the entire extract to a 100-mL graduated mixing cylinder. Rinse collection tube twice with 5-mL of the extraction solution and transfer rinse to the graduated cylinder. Bring the extracts of watery crop matrices to a common volume of 70 mL with the extraction solution. For the dry crop extracts, bring to a common volume of 90 mL. Cap the mixing tube and shake to homogeneity.
10. Proceed to the next section (Sample Cleanup Documentation) to purify and concentrate the sample extracts right away. If purification has to be done at a later time, the sample extracts may be transferred to 125-mL centrifuge tubes and stored frozen at ≤-10°C. (Sample extracts are relatively stable for at least 3 days when immediately stored frozen at ≤-10°C.)

4.2.10 *Analyte Purification/Concentration Procedure*

1. Transfer 1/10 of extract (7 mL and 9 mL of the watery and dry crop extracts, respectively) into a 50-mL disposable graduated polypropylene centrifuge tube.
2. Attach a 500-mg/6-cc Waters Oasis® MAX cartridge to an SPE manifold. Using an adapter, stack a 25-mL SPE reservoir to top of the cartridge. Condition the cartridge with 5-6 mL of methanol followed by 5-6 mL of 1:1 extraction solution /0.5% acetic acid (aq) solution by gravity flow. (Cartridge will not go to dryness when vacuum is not applied.)

   Note: A Pasteur pipet can be used to transfer initially the liquid from reservoir to the cartridge. This is done by letting the pipet's narrow end pass through the hole of the reservoir to deliver the liquid to the cartridge. An alternative is to slightly open the adapter on top of the cartridge to allow liquid transfer to the cartridge and close it when the cartridge is half-filled with liquid. Be careful not to spill any liquid from the reservoir.

3. Dilute sample extract with 0.5% acetic acid (aq) solution in a 1:1 ratio (i.e., 7 mL and 9 mL of extracts with 7 mL and 9 mL of acid, respectively.) Cap the tube and vortex the extract for 5-10 seconds. (*Note*: Rimsulfuron hydrolyzes fast at pH below 5. Do not let the sample stay in acidic conditions for a long time.)
4. Carefully transfer the entire extract to the reservoir and pass through the conditioned cartridge by gravity flow. Discard the eluate.
5. Rinse the sample tube twice with 5 mL of ultrapure water (a total of 10 mL). Pass rinsates through the cartridge by gravity flow. Discard the eluate.
6. Wash the cartridge sequentially, by gravity flow, with 10 mL of ultrapure water, 10 mL of methanol, 10 mL of ethyl acetate, and 5 mL of acetonitrile. Allow each wash solvent to pass entirely through the cartridge before adding another solvent. Additionally, control flow so that liquids (specifically, the ethyl acetate and acetonitrile wash) are coming out in a drop-wise fashion. Discard eluates.

   Note: This is to minimize the presence of water in the later eluate, which hastens the degradation or rimsulfuron in the presence of acid. Water droplets adhering to the inner walls of the cartridge should also be washed off by the organic solvents added.)
7. Place a 50-mL disposable glass centrifuge tube under the cartridge to collect the next eluate.
8. Elute the cartridge with 30 mL of 0.1% formic acid in acetonitrile by gravity flow and in drop-wise fashion. After the entire sample is passed through the cartridge, apply vacuum to remove all liquid.
9. Evaporate the eluate immediately to dryness under a gentle stream of nitrogen gas in a 30°C water-bath. (With the N_2-vap properly set up, drying should take only an hour.)
10. Reconstitute the sample in 5 mL of 5 mM ammonium acetate/methanol (8:2, v/v) solution. Cap the sample vial, vortex for 5-10 seconds, sonicate for 5 minutes, and vortex again for 5-10 seconds.
11. Transfer the sample into a 5-mL disposable syringe fitted with a disposable 13-mm, 0.2-μm PFTE syringe filter. Filter the sample back into the 50-mL glass tube or into a 20-mL disposable, glass scintillation vial. Cap the tube or vial and vortex for 5 seconds.
12. Transfer about 1 mL of the filtered sample extract into a 2-mL HPLC sample vial and analyze by HPLC/ESI-MS/MS.
13. Prepare the post-extraction fortified sample by transferring 950 μL of the filtered control sample (from Step 11) into an HPLC vial, then add 50 μL of the 20.0-ng/mL calibration standard, cap and vortex. If there is a delay in the analysis, the samples may be stored frozen at ≤–10°C and they are relatively stable for 2 days. When refrigerated at 4°C, the samples are relatively stable for 24 hours.

4.3 *Instrumentation*

4.3.1 Description

Method validation data in this study were generated using an Agilent HP Series 1100 HPLC coupled to Micromass Quattro II MS/MS (a triple quadrupole MS) with an electrospray ion source.

4.3.2 Operating Conditions

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

HPLC Conditions

System:	Agilent HP1100 HPLC				
Analytical Column:	Phenomenex Luna® Phenyl-hexyl, 4.6 × 50 mm, 3.0-μm dp (Alternate: TSK Gel Super ODS, 4.6 × 50 mm, 2.0-μm dp)				
Column Temperature:	40°C				
Injection Volume:	10-20 μL				
Autosampler Temperature:	4°C				
Conditions:	Time	%A	%B	Flow	A: 0.01% Formic acid in
	0.00	35 (55)	65 (45)	1.0	0.1mM Ammonium formate
	3.00	20 (40)	80 (60)	1.0	B: Methanol
	3.01	5	95	1.5	Flow in mL/min
	4.00	5	95	1.5	
	4.01	35 (55)	65 (45)	1.0	
	5.00	35 (55)	65 (45)	1.0	
Approximate Retention Time:	(Minutes)				
Rimsulfuron (DPX-V9360)	~2.6 (~2.2)				
Total Run Time:	5.0 minutes				

MS Conditions (Micromass Quattro II Triple Quadrupole MS):

Analyte Monitored	Ions Monitored	Collision Energy	Dwell Time (Seconds)	Acquisition Timing (Min.)
DPX-V9360	431.1 → 182.0 ± 0.2 amu	19 V	0.20	1.0-4.0
	431.1 → 325.0 ± 0.2 amu	15 V	0.20	1.0-4.0
Ion Source/Ion Mode:		ESI/ Positive ion - selected reaction monitoring (MRM)		
Capillary Voltage:		3.5 kV		
Source Temperature:		80°C		
Cone Voltage:		40 V		
Detector Voltage		650 V		
Gas Cell Pressure		2.9e-3 mBar		
Nebulising Gas Flow		18 L/h		
Drying Gas Flow:		350 L/h		
MS Flow Rate (Post-column split):		100-µL/min (approximately 10:1 split)		

A triple quadrupole MS instrument with an electrospray ionization (ESI) source was used for the detection of rimsulfuron. The response of rimsulfuron 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. The positive or negative 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-to-daughter ion transitions were monitored for nicosufuron (m/z = 182 and 325).

Rimsulfuron was identified in the investigative sample by its retention time, the presence of two parent-to-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 the analyte was integrated and the peak area was used for quantitation. Quantitation was performed using the total ion current (TIC).

A six-port electronically activated switching valve was used to direct the HPLC column effluent to waste prior to and following the elution of rimsulfuron. The retention time of the analyte was about 4 minutes. The chromatographic run time is 5 minutes, but the MS sample collection time is 1-4 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 100-µL/min actually passed through the interface (approximately 10:1 split), the remainder going to waste.

4.3.3 *Calibration Procedures*

Prepare chromatographic standards that bracket the levels of rimsulfuron found in the different fruit 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 treated/fortified samples. In addition, one post-extraction fortified sample will be analyzed for every sample set to verify if matrix effect influences the recovery of samples, if any. A post-extraction fortified sample is prepared from the extract of control sample that is purified in the same manner as the other samples and fortified at the LOQ level.

A solvent blank (5.0 mM ammonium acetate/MeOH (8:2, v/v)) 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 and/or fortified/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:

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 in the fortified samples (mg/kg found) was then calculated using the equation below:

mg/kg found of rimsulfuron =

$$\frac{A \times Rfave\,(ng/mL/area\ counts) \times Extract\ Volume\,(mL) \times Final\ Volume\,(mL) \times Dilution\ Factor}{1000\,(ng/\mu g) \times Sample\ Weight\,(g) \times Aliquot\ Volume\,(mL)}$$

where:

A = Corrected Peak Area Counts

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

Rfave = Average Response Factor

Percent Recovery was calculated as:

% Recovery

$$= \frac{Analyte\ Found\ (mg/kg)}{Fortification\ Level\ (mg/kg)} \times 100$$

4.4.2 *Examples*

Calculation for the percent recovery of rimsulfuron (DPX-E9636) from a cherry sample fortified at 0.010 mg/kg (Cherry_Validation_2, Appendix _) was prepared and analyzed on June 30, 2004 is shown below.

Rf_{avg} of five DPX-E9636 standards = 4.71 x 10-3 ng/mL/area counts

Peak Area Counts (ac) for DPX-E9636, fortified sample = 194

Peak Area Counts (ac) for DPX-E9636, control = 0

Sample Weight = 5.00 grams

Final Volume = 5.0 mL

Fortification Level = 0.010 mg/kg

$$\text{DPX-E9636 Found} = \frac{194\ ac \times 4.71 \times 10\text{-}3\ ng/mL/ac \times 70\ mL \times 5.0\ mL \times 1}{1000\ ng/\mu g \times 5.0\ g \times 7\ mL}$$

$$= 0.0091\ \mu g/g = 0.0091\ mg/kg$$

$$\text{DPX-E9636 \%Recovery} = \frac{0.0091\ mg/kg}{0.010\ mg/kg} \times 100$$

5.0 RESULTS AND DISCUSSION

5.1 Method Validation Results

5.1.1 Detector Response

Representative chromatograms of 0.5 and 20 ng/mL standard solutions of rimsulfuron are shown in Appendix 2. All calibration curves (peak area versus concentration) for rimsulfuron showed good linearity, i.e., the correlation coefficient R was >0.99. Calibration curves generated from a set of standards for the analysis of rimsulfuron in cherry is shown in Fig.1 and Appendix 4. This is a typical calibration curve with good linearity (R^2 >0.99).

The demonstration of good linearity of response from calibration standards (0.50 - 20.0 ng/mL range) and the precision of the response factors (RSD less than 15%, Appendix 4) indicate that the use of response factors is a valid method for the calculation of results.

5.1.2 Controls

Chromatograms for control samples were generally free from matrix interference in the elution region of rimsulfuron (Appendix 3). If interference peak was present at the elution regions of rimsulfuron, the peak was less than 5% of the LOQ. Furthermore, using the confirmatory method, the interference peak was not confirmed to be rimsulfuron. The confirmation method is shown to be specific and quantitative.

5.1.3 Recoveries (Accuracy & Precision)

Percent recoveries of rimsulfuron from various fruit samples fortified at 0.010- and 0.10-mg/kg (LOQ) are summarized in the table below. The overall average recovery for rimsulfuron from the different fruit matrices ranged from 86% to 99% with relative standard deviations ranging from 2.9% to 10.0%. At the 0.010-mg/kg fortification (LOQ) level, the average recoveries of rimsulfuron in 5-6 fortified samples per matrix ranged from 90% to 100% with RSD ranging from 2.0% to 12.1%. At the 0.10- mg/kg fortification level, the range of average recoveries and relative standard deviations were 82%-98% and 2.0%-14.0% (n=4-5 per fruit matrix), respectively.

Fruit Matrix	% Recovery Rimsulfuron in Various Watery, Dry, and Oily Fruit Matrices					
	0.010 mg/kg (n[a]=5)		0.10 mg/kg (n=5)		Overall (n=10)	
	Mean (± RSD[b])	Range	Mean (± RSD)	Range	Mean (± RSD)	Range
Apple	91 (± 2.0)	89-94	98 (± 2.9)	93-100	95 (± 4.2)	89-100
Cherry	88 (± 12.1)	74-100	97 (± 2.9)	94-101	92 (± 9.4)	74-101
Grape	93 (± 6.1)	84-99	93 (± 3.9)	90-99	93 (± 4.9)	84-99
Lemon	88± 5.8	80-94	93 (± 5.9)	89-102	91 (± 6.3)	80-102
Lime	89± 6.6	83-96	85 (± 6.7)	79-91	87 (± 6.7)	79-96
Plum	100 (± 9.5)	91-116	98 (± 1.9)	96-101	99 (± 6.6)	91-116
Almond Hull[c]	89 (± 6.2)	78-93	82 (± 14.0)	70-93	86 (± 10.0)	70-93
Prune	90 (± 3.6)	85-93	92 (± 2.0)	89-94	91 (± 2.9)	85-94

[a]n = Total number of fortifications per fruit matrix [b]RSD = Relative standard deviation [c]LOQ, n = 6; 10xLOQ, n = 4

There was only minimal influence of matrix effect to the recoveries of rimsulfuron. The average recoveries of the post-fortified samples per matrix ranged from 92% to 108%. Percent recoveries for all post-fortifications ranged from 90% to 110%.

Further information on recoveries, standard deviations, and relative standard deviations by fortification levels is found in Appendix 4. The 0.010- and 0.10-mg/kg fortified samples are approximately equivalent to 1.0 and 10.0 ng/mL standard concentrations, respectively.

5.1.4 Extraction Efficiency

5.1.5 Limit of Quantitation

Overall, the limit of quantitation of this method was determined to be 0.010 mg/kg (ppm) for rimsulfuron in various fruit matrices. The LOQ is defined as the lowest fortification level at which average recoveries of 70-110% and a RSD ≤20% are achieved. In addition, at this fortification level the analyte peak consistently represents a signal-to-noise ratio of approximately 5-20 in the chromatograms (Appendix 6).

The limit of detection (LOD) 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 sample types and from day to day as would be common for all trace analyses. The estimated LOD is approximately 1/3 of the LOQ, ~0.003 mg/kg.

5.2 *Timing*

A single analyst can prepare and analyze 10-12 samples for an 8-hour working day. Extraction by homogenization and SPE purification for HPLC/ESI-MS/MS analysis can be performed within one 8-hour working day. Chromatographic run time per sample takes 5 minutes and a set of analytical samples can be run unattended overnight. Data processing is completed using an Excel spreadsheet template on the following day for about 10 minutes.

5.3 *Modifications or Special Precautions*

Since analysis is done at low levels of quantitation 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 of rimsulfuron are all stable under ambient conditions for at least 5 hours. When stored at 4°C, they are stable for at least 24 hours. However, it is recommended to keep them stored in a freezer maintained below -10°C when not in use. When stored frozen, aqueous solutions of standards and samples are relatively stable for about 2 days.

Rimsulfuron hydrolyzes at pH ≤5 and ≥9, but is relatively stable at pH 7. During sample purification and concentration, it is necessary that the sample be processed immediately. Do not let the sample stay in acidic conditions for a long time.

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/plasticware 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*

5.4.3.1 *Example of Calculation*

The values presented here are from data sheet number Cherry_Validation_2_Confirmatory (Appendix 5).

Sample	Area (431.9 →182.0)	Area (431.9 → 325.0)	Ratio (182.0/325.0)
0.50 ng/mL std	68	41	1.7
Cherry LOQ 2	131	65	2.0
1.0 ng/mL std	144	61	2.4
Cherry 10xLOQ4	1354	703	1.9
5.0 ng/mL std	677	368	1.8
10.0 ng/mL std	1420	772	1.8
20.0 ng/mL std	2863	1527	1.9

Average Ratio of Calibration Standards: 1.9
RSD: 13.8

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) = 1.3

The ion ratios for the LOQ sample (Cherry LOQ 2) and the 10X LOQ sample (Cherry 10xLOQ 4) 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 as an enforcement method for the quantitation of rimsulfuron in watery/dry crop matrices at approximately 0.010-mg/kg. It meets the U.S. EPA and EU guidelines.

- The overall average recoveries (±RSD) for rimsulfuron in 10 fortified samples of apples, cherries, grapes, limes, plums, almond hulls and prunes were 95% (±4.2%), 93% (±9.8%), 93% (±4.9%), 87% (±6.7%), 99% (±6.6%), 86% (±10.0%), and 91% (±2.9%), respectively.
- There were no detectable residues in the control samples.
- Assessment of the method extraction efficiency using field corn samples treated with radiolabeled rimsulfuron showed acceptable recoveries 89-93%. Storage stability studies of rimsulfuron in corn (forage and grain), potato, and tomato samples stored frozen for 24, 24, and 6 months showed average normalized recoveries of 99 ± 7.9% (n=13), 105± 5.1% (n=7), and 79± 11.5% (n=8). The

somewhat low recovery of rimsulfuron from tomato samples maybe attributed to its slight degradation in the acidic matrix.

- Residues of rimsulfuron are confirmed based on detection and the relative ratios of the two MS/MS parent-to-daughter ion transitions monitored during sample analysis.

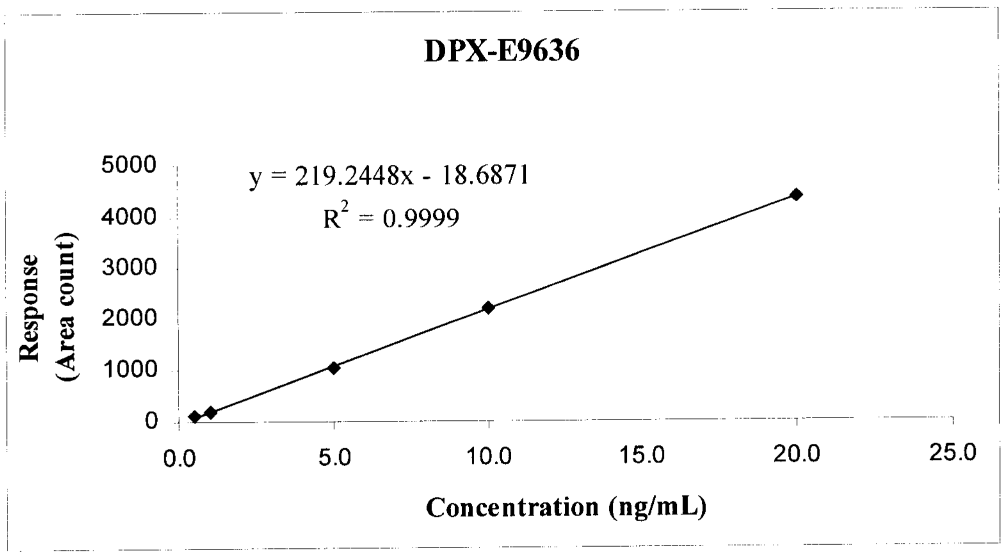
7.0 RETENTION OF RECORDS

8.0 REFERENCES

1. The Pesticide Manual, 11th Edition, C.D.S. Tomlin, Editor, British Crop Protection Council, 1997, pp. 304-305.
2. J.H. La Rochelle, L.J. Major, and P.G. Rossi, "Analytical Method for the Quantitation of DPX-E9636 in Corn (Forage and Grain)", DuPont Report No. AMR 1241-88 RV1, 1989.
3. A.M. Brown, "Extraction Efficiency Experiments Using C^{14}-Treated Corn", E.I. du Pont de Nemours & Company, Inc. Laboratory Notebook No. E58268, pp. 123-140.
4. P. Devine and D. Tomic, "Freezer Storage Stability of DPX-E9636 Herbicide on Corn", DuPont Report No. AMR 1243-88, 1991.
5. J.S. Amoo, "Freezer Storage Stability of DPX-E9636 Herbicide on Potatoes", DuPont Report No. AMR 1886-90, 1992.
6. J.S. Amoo, "Storage Stability of DPX-E9636 in Frozen Analytical Samples of Tomato Fruit", DuPont Report No. AMR 3443-95, 1996.
7. "Approximate pH of Foods and food Products", US Food and Drug Administration, Center for Food Safety and Applied Nutrition website http://vm.cfsan.fda.gov/~comm/lacf-phs.html, October 2003.

9.0 ACKNOWLEDGMENT

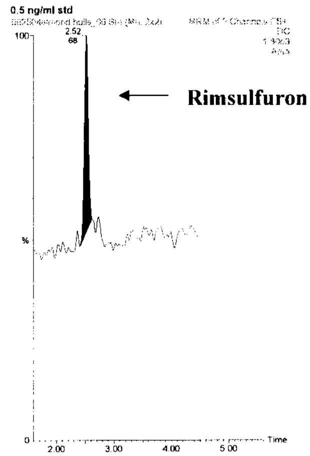
FIGURE 1. REPRESENTATIVE CALIBRATION CURVE FOR RIMSULFURON (DPX-E9636) STANDARDS



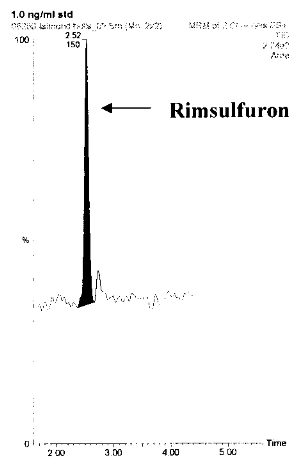
Cherry Analysis
Validation No. 2

APPENDIX 2. REPRESENTATIVE CHROMATOGRAMS OF RIMSULFURON (DPX-E9636) STANDARDS

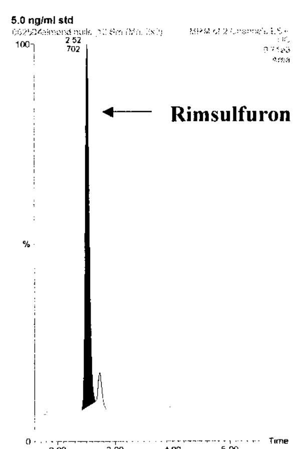
0.50 ng/mL DPX-E9636 Standard



1.0 ng/mL DPX-E9636 Standard

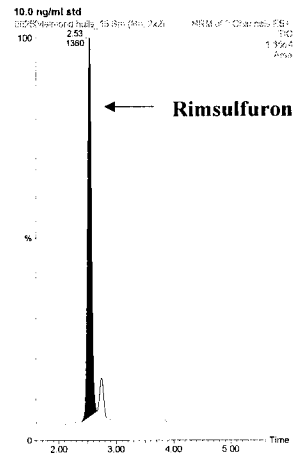


5.0 ng/mL DPX-E9636 Standard

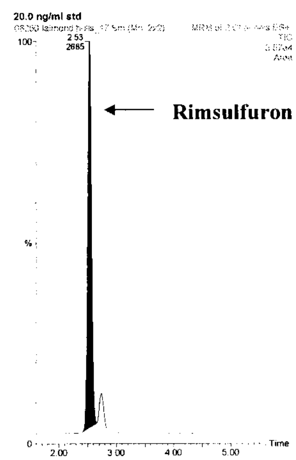


APPENDIX 2. REPRESENTATIVE CHROMATOGRAMS OF RIMSULFURON (DPX-E9636) STANDARDS (CONTINUED)

10.0 ng/mL DPX-E9636 Standard

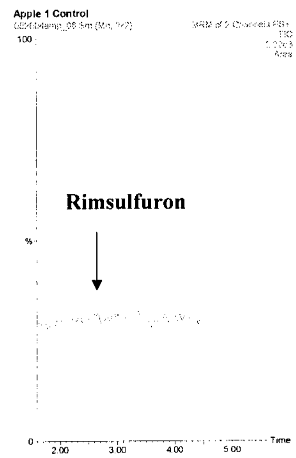


20.0 ng/mL DPX-E9636 Standard

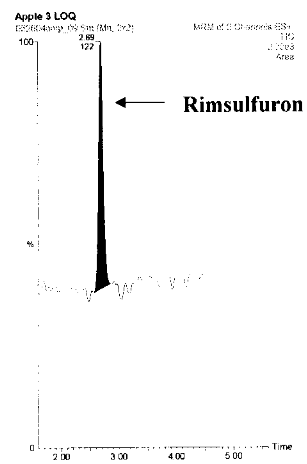


APPENDIX 3. REPRESENTATIVE CHROMATOGRAMS OF RIMSULFURON (DPX-E9636) IN CONTROL AND FORTIFIED FRUIT MATRICES

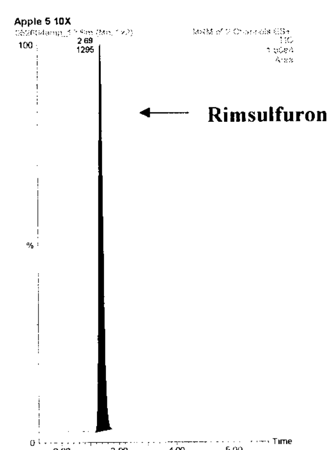
Apple Control



Apple 0.010 mg/kg Fortification

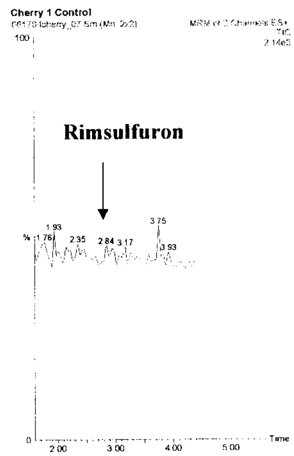


Apple 0.10 mg/kg Fortification

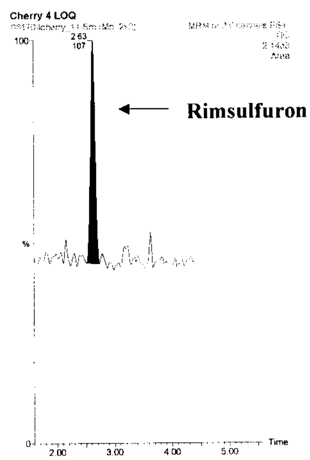


APPENDIX 3. REPRESENTATIVE CHROMATOGRAMS OF RIMSULFURON (DPX-E9636) IN CONTROL AND FORTIFIED FRUIT MATRICES (CONTINUED)

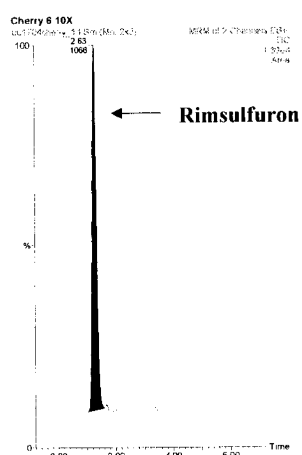
Cherry Control



Cherry 0.010 mg/kg Fortification

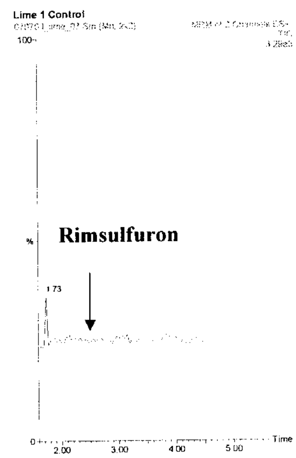


Cherry 0.10 mg/kg Fortification

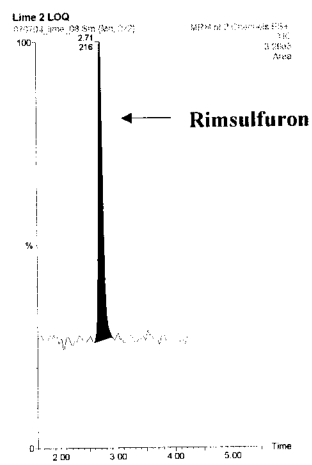


APPENDIX 3. REPRESENTATIVE CHROMATOGRAMS OF RIMSULFURON (DPX-E9636) IN CONTROL AND FORTIFIED FRUIT MATRICES (CONTINUED)

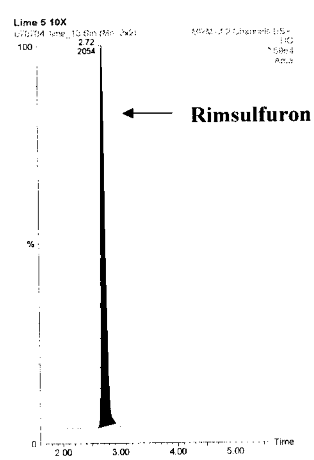
Lime Control



Lime 0.010 mg/kg Fortification

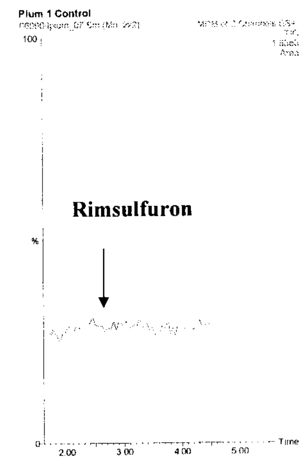


Lime 0.10 mg/kg Fortification

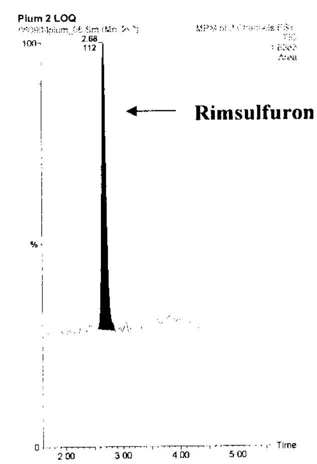


APPENDIX 3. REPRESENTATIVE CHROMATOGRAMS OF RIMSULFURON (DPX-E9636) IN CONTROL AND FORTIFIED FRUIT MATRICES (CONTINUED)

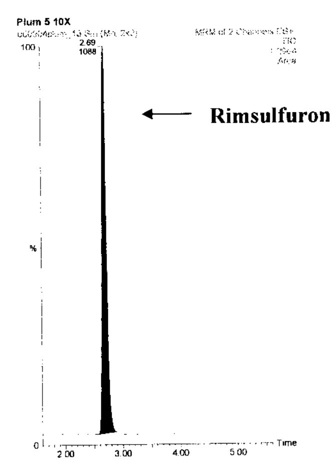
Plum Control



Plum 0.010 mg/kg Fortification

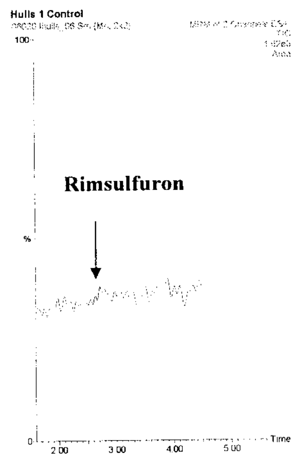


Plum 0.10 mg/kg Fortification

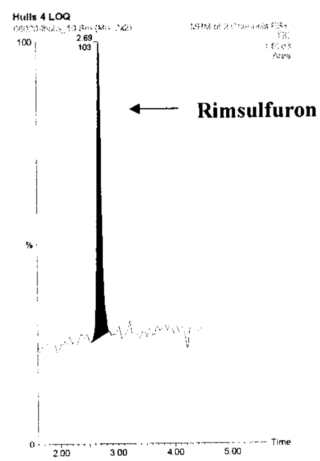


APPENDIX 3. REPRESENTATIVE CHROMATOGRAMS OF RIMSULFURON (DPX-E9636) IN CONTROL AND FORTIFIED FRUIT MATRICES (CONTINUED)

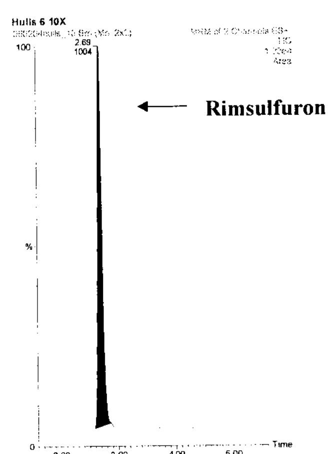
Almond Hull Control



Almond Hull 0.010 mg/kg Fortification

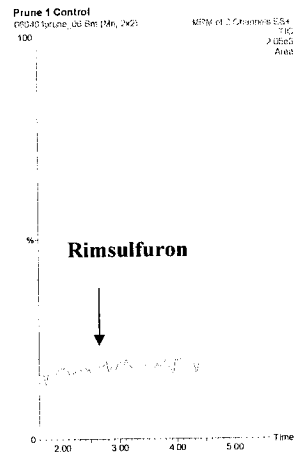


Almond Hull 0.10 mg/kg Fortification

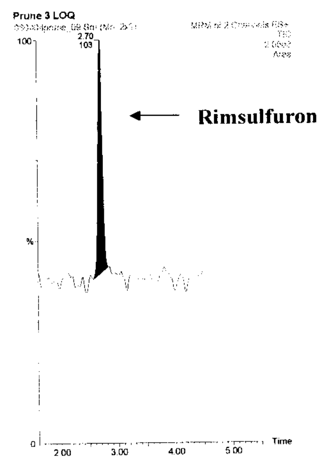


APPENDIX 3. REPRESENTATIVE CHROMATOGRAMS OF RIMSULFURON (DPX-E9636) IN CONTROL AND FORTIFIED FRUIT MATRICES (CONTINUED)

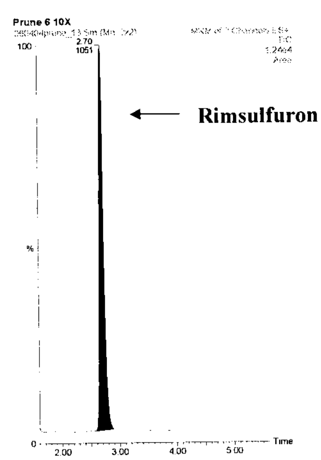
Prune Control



Prune 0.010 mg/kg Fortification



Prune 0.10 mg/kg Fortification



APPENDIX 4. EXAMPLES OF RAW DATA SHEET FOR CALCULATION OF RESULTS QUANTITATION OF RIMSULFURON

Apple

RAW DATA REPORT

Study No.: DuPont-15033
Instrument: Quattro II MS
Analytes: DPX-E9636

Standard Prep. Dates:
Calibration: 05/26/04
Fort. Soln: 05/26/04
Fortification Standard:
0.5 and 5.0 ppm DPX-E9636 in Acetonitrile

Set No.: APPLE_VALIDATION_I
Injection Vol.: 10 uL
Limit of Quantification: 0.010 mg/kg
Limit of Detection: 0.003 mg/kg
Mass Monitored (m/z): 431.9 → 182.0; 431.9 → 325.0

Analyst: Anne Pentz
M. Elena Y. Cabusas
Extraction date: 05/26/04
Analyzed on: 05/26/04

Sample ID	Matrix	Sample Type	Std. Concentration (ng/mL)	Sample Weight (g)	Total Extract Volume (mL)	Aliquot Volume (mL)	Final Volume (mL)	HPLC Dilution Factor	DPX-E9636 Peak Area TIC	Response Factor	Fort. Level (mg/kg)	Analyte Found (mg/g)	Rounded Analyte Found (mg/kg)	Recovery %	Comments
Solvent		O							-		-	-	-	-	
0.5 ng/mL DPX-E9636		Std	0.5						69	7.2464E-03	-	-	-	-	
Apple Control 1	Apple	C		5.0	70	7.0	5.0	1	0		-	0.0000	0.0000	-	
Apple LOQ 2	Apple	F		5.0	70	7.0	5.0	1	119		0.010	0.0089	0.0089	89	
1.0 ng/mL DPX-E9636		Std	1.0						129	7.7519E-03	-	-	-	-	
Apple LOQ 3	Apple	F		5.0	70	7.0	5.0	1	122		0.010	0.0091	0.0091	91	
Apple LOQ 4	Apple	F		5.0	70	7.0	5.0	1	126		0.010	0.0094	0.0094	94	
5.0 ng/mL DPX-E9636		Std	5.0						662	7.5529E-03	-	-	-	-	
Apple 10xLOQ 5	Apple	F		5.0	70	7.0	5.0	1	1295		0.100	0.0968	0.097	97	
10.0 ng/mL DPX-E9636		Std	10.0						1359	7.3584E-03	-	-	-	-	
Apple 10xLOQ 6	Apple	F		5.0	70	7.0	5.0	1	1245		0.100	0.0933	0.093	93	
Apple Control Fort	Apple	PS		5.0	70	7.0	5.0	1	127		0.010	0.0096	0.010	96	
20.0 ng/mL DPX-E9636		Std	20.0						2678	7.4683E-03		-	-	-	
									Average Response Factor =	7.4756E-03					
									RSD =	2.6					

Sample Type Key
Std = Standard
C = Field Control
F = Fortification
T = Field Treated Sample
PS = Post-spiked Control
O = Other

Fortification Level = Amount Fortified/Sample Weight

$$\text{Analyte Found (mg/kg)} = \frac{\text{(Peak Area)*(Av. Response Factor)*(Total Extract Vol)*(Final Vol)*(HPLC Dilution Factor)}}{\text{1000* (Sample Weight)*(Aliquot Volume)}}$$

$$\text{\%Recovery} = \frac{\text{Analyte Found * 100}}{\text{Fortification Level}}$$