

**TRADE SECRET**

*Study Title*

**MULTIRESIDUE ANALYTICAL METHOD FOR THE DETERMINATION OF  
SULFONYLUREA HERBICIDES IN OILY, WATERY, ACIDIC AND DRY  
CROPS USING SPE PURIFICATION AND LC/MS/MS DETECTION**

*Test Guidelines*

EEC Directive 91/414/EEC, Annex IIA 4.2.1 as amended by EC Directive 96/46/EC;  
SANCO/825/00 rev.7 (17/03/2004) Guidance Document on Residue Analytical  
Methods

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

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*Date Study Completed*

Original report:	January 28, 2004
Revision No. 1:	January 14, 2005
Supplement No. 1:	April 23, 2007

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*Project Identification Numbers*

DuPont-13412, Supplement No. 1

**LIST OF ABBREVIATIONS AND SYMBOLS**

%	percent
°C	degrees centigrade
Cat. No.	catalog number
ESI	electrospray interface
HPLC	high-performance liquid chromatography
LC/MS	liquid chromatography/mass spectrometry
kg	kilogram
µg	microgram
min	minute
MS/MS	tandem mass spectrometry (2-stage mass analysis experiment), MS <sup>2</sup>
m/z	mass/charge ratio
n	number
ng	nanogram
ppb	parts per billion
ppm	parts per million
Rec	recovery
RF	response factor (analyte peak area / analyte concentration)
RSD	relative standard deviation (StDev / mean)
RSQ	r-squared; the square of the Pearson product moment correlation coefficient (determined using Excel <sup>®</sup> function RSQ)
sec	second
StDev	standard deviation (determined using Excel <sup>®</sup> function STDEV)

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# MULTIRESIDUE ANALYTICAL METHOD FOR THE DETERMINATION OF SULFONYLUREA HERBICIDES IN OILY, WATERY, ACIDIC AND DRY CROPS USING SPE PURIFICATION AND LC/MS/MS DETECTION

*Timothy J. Devine and Sergio C. Nanita*

## REASON FOR SUPPLEMENT

The European Union is beginning to require that registrants of crop protection products provide validated analytical methods for the identification and quantitation of the active ingredients in matrices of four major crop groups; i.e. dry, oily, watery, and acidic. Consequently, the study presented here expands the use of the analytical method described in DuPont-13412, Revision No. 1 (Reference 1) by validating it for selected sulfonylurea herbicides in the following representative crops: wheat grain (dry), soybean seed (oily), lettuce leaf (watery), and orange fruit (acidic).

## 1.0 SUMMARY

The analytical method DuPont-13412, Revision No. 1 (Reference 1) is validated herein for the analysis of sulfonylurea herbicides in various crops. This study is part of an ongoing effort to have validated methods readily available for the analysis of thirteen DuPont sulfonylurea herbicides in matrices from the four major crop groups: dry, oily, watery, and acidic. The method was successfully validated for selected sulfonylurea herbicides in each crop group as shown below.

<b>Wheat grain (Dry)</b>	<b>Soybean seed (Oily)</b>	<b>Lettuce (Watery)</b>	<b>Oranges (Acidic)</b>
Azimsulfuron	Azimsulfuron	Azimsulfuron	Azimsulfuron
Bensulfuron-methyl	Bensulfuron-methyl	Bensulfuron-methyl	Bensulfuron-methyl
Chlorimuron-ethyl	Chlorsulfuron	Chlorimuron-ethyl	Chlorimuron-ethyl
Ethametsulfuron-methyl	Flupyralsulfuron-methyl	Ethametsulfuron-methyl	Chlorsulfuron
Sulfometuron-methyl	Metsulfuron-methyl	Sulfometuron-methyl	Ethametsulfuron-methyl
Triflusaluron-methyl	Sulfometuron-methyl		Flupyralsulfuron-methyl
	Triflusaluron-methyl		Metsulfuron-methyl
	Nicosulfuron		Nicosulfuron
			Sulfometuron-methyl
			Thifensulfuron-methyl
			Tribenuron-methyl
			Triflusaluron-methyl

The method limit of quantitation (LOQ) was 0.010 ppm and the limit of detection (LOD) was estimated to be 0.001 ppm (for the least responsive analyte). The method was validated at 0.010 ppm and 0.10 ppm in each matrix using a LC/MS/MS system operating with an electrospray interface (ESI) in positive ion mode.

The analyses of validation sets were performed following the analytical method described in DuPont-13412, Revision No. 1 (Reference 1), "Analytical Method for the Determination of Nicosulfuron, Thifensulfuron-methyl, Rimsulfuron, Tribenuron-methyl, and Chlorimuron-ethyl in Oily Crop Matrices Using SPE Purification and LC/MS/MS Detection". Only minor modifications were made, which improved method ruggedness and shortened the LC/MS/MS analysis time.

Briefly, 10 g samples are extracted twice with 75/25 acetonitrile/aqueous potassium phosphate buffer (adjusted to pH = 7.0). The extracts are then purified by a liquid/liquid partition with hexane, followed by SPE with Varian ENV Bond-Elut<sup>®</sup> cartridges. The purified extract solution is filtered to remove minute solid particles prior to HPLC/MS/MS analysis.

Typically, a single analyst can prepare (sample extraction and purification) a set of 6 to 8 samples in an 8-hour day. Quantitative LC/MS/MS analyses were generally performed overnight (up to 3 sample sets are possible) and the results processed the next day.

The recoveries from wheat grain, soybean seed, lettuce, and orange samples fortified at 0.010 (LOQ) and 0.10 ppm support the satisfactory performance of this method. The following tables summarize the average recovery results from fortified matrix samples.

### Lettuce

		LOQ			10x LOQ		
Compound	DPX number	% Recovery (%RSD)	Range	n	% Recovery (%RSD)	Range	n
Azimsulfuron	DPX-A8947	94 (3.4)	89 - 98	5	99 (5.5)	90 - 103	5
Bensulfuron methyl	DPX-F5384	97 (4.7)	92 - 102	5	100 (2.9)	95 - 102	5
Chlorimuron ethyl	DPX-F6025	100 (2.2)	97 - 102	5	100 (4.0)	93 - 103	5
Ethametsulfuron methyl	DPX-A7881	100 (2.3)	97 - 102	5	100 (4.1)	93 - 103	5
Sulfometuron methyl	DPX-T5648	99 (2.7)	96 - 102	5	100 (3.4)	94 - 103	5
		Overall					
Compound	DPX number	% Recovery (%RSD)	Range	n			
Azimsulfuron	DPX-A8947	96 (5.3)	89 - 103	10			
Bensulfuron methyl	DPX-F5384	98 (3.9)	92 - 102	10			
Chlorimuron ethyl	DPX-F6025	100 (3.0)	93 - 103	10			
Ethametsulfuron methyl	DPX-A7881	100 (3.1)	93 - 103	10			
Sulfometuron methyl	DPX-T5648	100 (2.9)	94 - 103	10			

**Orange**

		LOQ			10x LOQ		
Compound	DPX number	% Recovery (%RSD)	Range	n	% Recovery (%RSD)	Range	n
Azimsulfuron	DPX-A8947	80 (1.1)	79 - 81	5	83 (4.5)	79 - 89	5
Bensulfuron methyl	DPX-F5384	94 (2.5)	92 - 97	5	90 (1.8)	88 - 91	5
Chlorimuron ethyl	DPX-F6025	93 (3.1)	89 - 97	5	90 (5.0)	84 - 96	5
Chlorsulfuron	DPX-W4189	92 (3.3)	89 - 96	5	92 (2.0)	90 - 95	5
Ethametsulfuron methyl	DPX-A7881	98 (4.9)	90 - 103	5	100 (6.1)	92 - 108	5
Flupyralsulfuron methyl	DPX-KE459	88 (7.4)	81 - 97	5	88 (4.5)	85 - 93	5
Metsulfuron methyl	DPX-T6376	100 (7.8)	89 - 108	5	96 (4.4)	91 - 101	5
Nicosulfuron	DPX-V9360	104 (4.2)	98 - 110	5	96 (3.6)	93 - 100	5
Sulfometuron methyl	DPX-T5648	91 (4.5)	86 - 95	5	89 (4.1)	86 - 95	5
Thifensulfuron methyl	DPX-M6316	96 (1.4)	95 - 98	5	97 (4.4)	92 - 103	5
Tribenuron methyl	DPX-L5300	76 (2.1)	74 - 78	5	72 (3.5)	70 - 76	5
Triflurosulfuron methyl	DPX-66037	90 (6.2)	87 - 100	5	89 (5.1)	84 - 95	5
		Overall					
Compound	DPX number	% Recovery (%RSD)	Range	n			
Azimsulfuron	DPX-A8947	82 (3.6)	79 - 89	10			
Bensulfuron methyl	DPX-F5384	92 (3.2)	88 - 97	10			
Chlorimuron ethyl	DPX-F6025	92 (3.5)	84 - 97	10			
Chlorsulfuron	DPX-W4189	92 (2.7)	89 - 96	10			
Ethametsulfuron methyl	DPX-A7881	99 (5.4)	90 - 108	10			
Flupyralsulfuron methyl	DPX-KE459	88 (5.8)	81 - 97	10			
Metsulfuron methyl	DPX-T6376	98 (6.7)	89 - 108	10			
Nicosulfuron	DPX-V9360	100 (4.7)	93 - 110	10			
Sulfometuron methyl	DPX-T5648	90 (4.2)	86 - 95	10			
Thifensulfuron methyl	DPX-M6316	96 (3.1)	92 - 103	10			
Tribenuron methyl	DPX-L5300	74 (3.9)	70 - 78	10			
Triflurosulfuron methyl	DPX-66037	90 (5.4)	84 - 100	10			

**Soybean Seed**

		LOQ			10x LOQ		
Compound	DPX number	% Recovery (%RSD)	Range	n	% Recovery (%RSD)	Range	n
Azimsulfuron	DPX-A8947	93 (8.9)	81 - 103	5	96 (3.7)	91 - 100	5
Bensulfuron methyl	DPX-F5384	101 (3.3)	96 - 105	5	100 (4.8)	95 - 106	5
Chlorsulfuron	DPX-W4189	90 (14.1)	74 - 105	5	98 (1.4)	96 - 100	5
Flupyralsulfuron methyl	DPX-KE459	99 (6.8)	90 - 107	5	102 (1.5)	100 - 104	5
Metsulfuron methyl	DPX-T6376	93 (13.8)	76 - 106	5	98 (1.7)	95 - 99	5
Nicosulfuron	DPX-V9360	100 (11.9)	85-111	5	106 (2.4)	102-109	5
Sulfometuron methyl	DPX-T5648	97 (4.6)	91 - 102	5	96.4 (1.9)	94 - 97	5
Triflurosulfuron methyl	DPX-66037	102 (2.9)	98 - 106	5	100 (2.1)	97 - 102	5
		Overall					
Compound	DPX number	% Recovery (%RSD)	Range	n			
Azimsulfuron	DPX-A8947	95 (6.5)	81 - 103	10			
Bensulfuron methyl	DPX-F5384	100 (3.9)	95 - 106	10			
Chlorsulfuron	DPX-W4189	94 (10.0)	74 - 105	10			
Flupyralsulfuron methyl	DPX-KE459	101 (4.8)	90 - 107	10			
Metsulfuron methyl	DPX-T6376	96 (9.4)	76 - 106	10			
Nicosulfuron	DPX-V9360	103 (8.6)	85-111	10			
Sulfometuron methyl	DPX-T5648	97 (3.3)	91 - 102	10			
Triflurosulfuron methyl	DPX-66037	101 (2.7)	97 - 105	10			

**Wheat Grain**

Compound	DPX number	LOQ			10x LOQ		
		% Recovery (%RSD)	Range	n	% Recovery (%RSD)	Range	n
Azimsulfuron	DPX-A8947	98 (3.1)	94 - 101	5	96 (2.3)	93 - 98	5
Bensulfuron methyl	DPX-F5384	100 (3.4)	95 - 104	5	97 (2.0)	95 - 100	5
Chlorimuron ethyl	DPX-F6025	93 (4.6)	89 - 100	5	94 (2.8)	91 - 98	5
Ethametsulfuron mehtyl	DPX-A7881	97 (4.5)	89 - 100	5	96 (2.0)	94 - 99	5
Sulfometuron methyl	DPX-T5648	100 (5.0)	93 - 105	5	98 (3.7)	93 - 102	5
Triflusulfuron methyl	DPX-66037	98 (1.2)	96 - 99	5	97 (2.7)	93 - 100	5
		Overall					
Compound	DPX number	% Recovery (%RSD)	Range	n			
Azimsulfuron	DPX-A8947	97 (2.8)	93 - 101	10			
Bensulfuron methyl	DPX-F5384	99 (3.0)	95 - 104	10			
Chlorimuron ethyl	DPX-F6025	94 (3.7)	89 - 100	10			
Ethametsulfuron mehtyl	DPX-A7881	97 (3.3)	89 - 100	10			
Sulfometuron methyl	DPX-T5648	99 (4.4)	93 - 105	10			
Triflusulfuron methyl	DPX-66037	97 (2.0)	93 - 100	10			

**2.0 BACKGROUND INFORMATION**

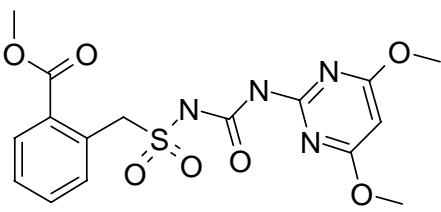
The chemical information is provided below for the twelve DuPont sulfonylurea herbicides studied.

Common Name	Azimsulfuron
Structure	
DPX Number	DPX-A8947
CAS Chemical Name	N-[[4,6-dimethoxy-2-pyrimidinyl]amino]carbonyl]-1-methyl-4-(2-methyl-2H-tetrazol-5-yl)-1H-pyrazole-5-sulfonamide
CAS Number	120162-55-2
Formula	C <sub>13</sub> H <sub>16</sub> N <sub>10</sub> O <sub>5</sub> S
Molecular Weight	424.40
Monoisotopic Weight	424.10
pKa	3.6

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Common Name	Bensulfuron-Methyl
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Structure	
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DPX Number	DPX-F5384
CAS Chemical Name	Methyl-2-[[[[(4,6-dimethoxypyrimidin-2-yl)-amino]carbonyl]amino]sulfonyl]methyl]-benzoate
CAS Number	83055-99-6
Formula	C <sub>16</sub> H <sub>18</sub> N <sub>4</sub> O <sub>7</sub> S
Molecular Weight	410.41
Monoisotopic Weight	410.09
pKa	5.2

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Common Name	Chlorimuron-ethyl
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Structure	
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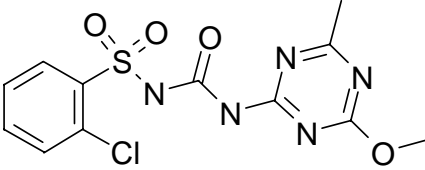
DPX Number	DPX-F6025
CAS Chemical Name	Ethyl 2-[[[[(4-chloro-6-methoxypyrimidin-2-yl)amino]carbonyl]amino]sulfonyl]benzoate
CAS Number	90982-32-4
Formula	C <sub>15</sub> H <sub>15</sub> N <sub>4</sub> O <sub>6</sub> SCl
Molecular Weight	414.83
Monoisotopic Weight	414.04
pKa	4.2

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Common Name	Chlorsulfuron
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Structure	
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DPX Number	DPX-W4189
CAS Chemical Name	2-Chloro-N-[4-methoxy-6-methyl-1,3,5-triazin-2-yl]aminocarbonyl]benzenesulfonamide
CAS Number	64902-72-3
Formula	C <sub>12</sub> H <sub>12</sub> ClN <sub>5</sub> O <sub>4</sub> S
Molecular Weight	357.78
Monoisotopic Weight	357.03
pKa	3.6

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Common Name	Ethametsulfuron-Methyl
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Structure	
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DPX Number	DPX-A7881
CAS Chemical Name	Methyl 2-[[[[[4-ethoxy-6-(methylamino)-1,3,5-triazin-2-yl]amino]carbonyl]amino]sulfonyl]benzoate
CAS Number	977080-06-8
Formula	C <sub>15</sub> H <sub>18</sub> N <sub>6</sub> O <sub>6</sub> S
Molecular Weight	410.41
Monoisotopic Weight	410.10
pKa	4.6

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Common Name	Flupyr-sulfuron-Methyl
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Structure	
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DPX Number	DPX-KE459
CAS Chemical Name	Methyl-2-[[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonylamino]sulfonyl]-6-(trifluoromethyl)-3-pyridinecarboxylate sodium salt
CAS Number	144740-54-5
Formula	C <sub>15</sub> H <sub>13</sub> N <sub>5</sub> O <sub>7</sub> SF <sub>3</sub> Na
Molecular Weight	487.35
Monoisotopic Weight	487.04
pKa	4.9

Common Name	Metsulfuron-Methyl
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Structure	
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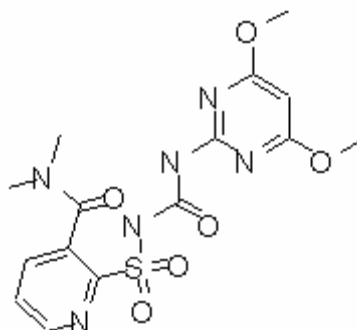
DPX Number	DPX-T6376
CAS Chemical Name	Methyl 2-[[[(4-methoxy-6-methyl-1,2,3-triazin-2-yl)amino]carbonyl]amino]sulfonyl]benzoate
CAS Number	74223-64-6
Formula	C <sub>14</sub> H <sub>15</sub> N <sub>5</sub> O <sub>6</sub> S
Molecular Weight	381.37
Monoisotopic Weight	381.07
pKa	3.3

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Common Name	Nicosulfuron
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Structure	
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DPX Number	DPX-V9360
CAS Chemical Name	2-[[[(4,6-Dimethoxy-pyrimidin-2-yl)amino]carbonyl]amino]sulfonyl]-N,N-dimethyl-3-pyridinecarboxamide
CAS Number	111991-09-4
Formula	C <sub>15</sub> H <sub>18</sub> N <sub>6</sub> O <sub>6</sub> S
Molecular Weight	410.41
Monoisotopic Weight	410.10
pKa	4.3

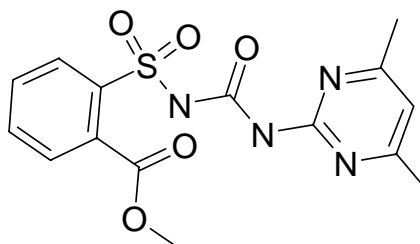
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Common Name	Sulfometuron-Methyl
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Structure	
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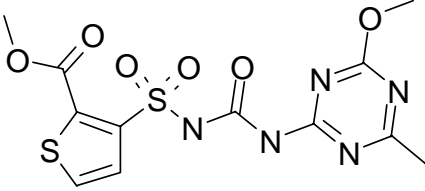
DPX Number	DPX-T5648
CAS Chemical Name	Methyl 2-[[[(4,6-dimethyl-2-pyrimidinyl)-amino]carbonyl]amino]sulfonyl]benzoate
CAS Number	74222-97-2
Formula	C <sub>15</sub> H <sub>16</sub> N <sub>4</sub> O <sub>5</sub> S
Molecular Weight	364.38
Monoisotopic Weight	364.08
pKa	5.2

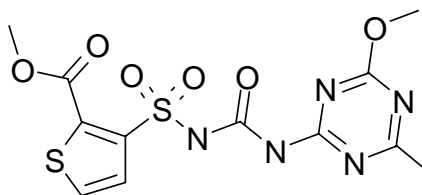
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Common Name	Thifensulfuron-Methyl
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Structure	
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DPX Number	DPX-M6316
CAS Chemical Name	Methyl 3-[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)-amino]carbonyl]amino]sulfonyl]-2-thiophenecarboxylate
CAS Number	79277-27-3
Formula	C <sub>12</sub> H <sub>13</sub> N <sub>5</sub> O <sub>6</sub> S <sub>2</sub>
Molecular Weight	387.40
Monoisotopic Weight	387.03
pKa	4.0

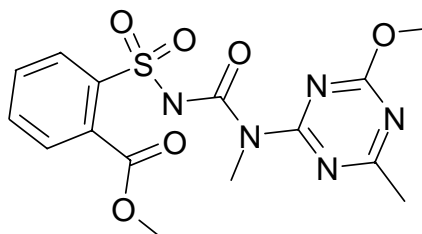
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Common Name	Tribenuron-methyl
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Structure	
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DPX Number	DPX-L5300
CAS Chemical Name	Methyl 2-[[[N-(4-methoxy-6-methyl-1,3,5-triazin-2-yl)methylamino]carbonyl]amino]sulfonyl]-benzoate
CAS Number	101200-48-0
Formula	C <sub>15</sub> H <sub>17</sub> N <sub>5</sub> O <sub>6</sub> S
Molecular Weight	395.39
Monoisotopic Weight	395.09
pKa	5.0

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Common Name	Triflurosulfuron-Methyl
Structure	
DPX Number	DPX-66037
CAS Chemical Name	Methyl-2-[[[(4-dimethylamino)-6-(2,2,2-trifluoromethoxy)-1,3,5-triazin-2-yl]amino]carbonyl]amino]sulfonyl-3-methyl
CAS Number	126535-15-7
Formula	C <sub>17</sub> H <sub>19</sub> N <sub>6</sub> O <sub>6</sub> SF <sub>3</sub>
Molecular Weight	492.44
Monoisotopic Weight	492.10
pKa	4.4

The European Union is beginning to require that registrants of crop protection products provide validated analytical methods for the identification and quantitation of the active ingredients in matrices of four major crop groups; i.e. dry, oily, watery, and acidic. The study presented here expands the use of the analytical method described in DuPont-13412 (Reference 1) by validating it for selected sulfonylurea herbicides in the following representative crops: wheat grain (dry), soybean seed (oily), lettuce leaf (watery), and orange fruit (acidic). Independent Laboratory Validations (ILVs) of the analytical method have been done by Inveresk (Tranent, Scotland), DuPont-13398 entitled, “Independent Laboratory Validation of Analytical Method DuPont-13412 for the Determination of Thifensulfuron Methyl, Ethametsulfuron Methyl, Rimsulfuron, Tribenuron Methyl, and Chlorimuron Ethyl in Olives and Soybean Seed Using SPE Purification and LC/MS/MS Detection” (Reference 2), and by Exygen Research, State College, PA, USA, DuPont-17207, Revision No. 1 entitled, “Independent Laboratory Method Validation of a Multi-residue Method for the Analysis of Sulfonylurea Herbicides in Crops”, DuPont-13412, Revision No.1” (Reference 3).

### 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*

All equipment used is contained in the equipment list found in DuPont-13412, Revision No. 1, with the exception of the LC/MS/MS system. No additional equipment was needed. The description of the LC/MS/MS system used for sample analysis is provided below.

#### LC/MS/MS SYSTEM

HPLC	HP1100: G1379A Degasser, G1312A Binary Pump, G1367A Chilled Autosampler; G1316A Column Unit	Agilent Technologies, Inc. (Palo Alto, CA)
Autosampler Vials	Target DP Amber Kit, T/S/T Septa, 100 PK, Cat. No. 5182-0556	Agilent Technologies, Inc. (Palo Alto, CA)
HPLC Column	Luna <sup>®</sup> Phenyl-Hexyl; 4.6 mm × 150 mm, 3- $\mu$ m particle size diameter	Phenomenex <sup>®</sup> (Torrance, CA)
Splitter tee	Valco zero dead-volume tee (split-flow to MS), Cat. No. ZT1C	Valco Instruments, Inc. (Houston, TX)
Switching Valve	Valco 6 Port Electrically Actuated Valve, Cat. No. 1384	Valco Instruments, Inc. (Houston, TX)
Triple Quadrupole MS	Applied Biosystems/MDS Sciex API-4000, equipped with electrospray ionization source, and Analyst 1.4.1 software	Applied Biosystems/MDS Sciex (Foster City, CA)

### 3.2 *Reagents and Standards*

#### 3.2.1 *Reagents*

No significant changes were made to the reagent list.

#### 3.2.2 *Reference Analytical Standards*

Reference analytical standards of azimsulfuron (DPX-A8947, Lot 063, 99.6% pure), bensulfuron-methyl (DPX-F5384, Lot 349, 99.6% pure), chlorimuron-ethyl (DPX-F6025, Lot 165, 98.8% pure), chlorsulfuron (DPX-W4189, Lot 735, 99.4% pure), ethametsulfuron-methyl (DPX-A7881, Lot 061, 98.9% pure), flupyr sulfuron-methyl (DPX-KE459, Lot 014, 93.4% pure), metsulfuron-methyl (DPX-T6376, Lot 265, 98.9% pure), nicosulfuron (DPX-V9360, Lot 116, 97.9% pure), tribenuron-methyl (DPX-L5300, Lot 226, 98.4% pure), sulfometuron-methyl (DPX-T5648, Lot 069, 98.9% pure), thifensulfuron-methyl (DPX-M6316, Lot 186, 99.7% pure), triflurosulfuron-methyl (DPX-66037, Lot 634, 99.4% pure) were synthesized at E. I. du Pont de Nemours and Company, DuPont Agricultural Products, Wilmington, DE. Characterization data are archived by DuPont Agricultural Products, E. I. du Pont de Nemours and Company, Wilmington, DE.

### 3.3 *Safety and Health*

Each analyst must be acquainted with the potential hazards of the reagents, products, and solvents used in this method before commencing laboratory work. All appropriate material safety data sheets should be read and followed, and proper personal protective equipment should be used.

## 4.0 METHODS

All validations were performed following the analytical method described in DuPont-13412, Revision No. 1. Only minor modifications were made, which are identified throughout this section.

### 4.1 *Principle of the Analytical Method*

This method was originally developed (DuPont-13412) for the determination of nicosulfuron, thifensulfuron methyl, ethametsulfuron methyl, rimsulfuron, tribenuron methyl, and chlorimuron ethyl residues in oily crop matrices to support regulatory studies. This report expands the use of the method to other DuPont sulfonylurea herbicides, and matrices from all four EU crop groups. The method was successfully validated at a target LOQ of 0.010 mg/kg (ppm) and 0.10 mg/kg (10×LOQ) in wheat grain, soybean seed, lettuce leaf, and orange fruit. A mostly organic (75% acetonitrile) solution containing pH 7 aqueous buffer (25% 20 mM dibasic potassium phosphate) is used to extract the sulfonylurea analytes from crop matrices. The ratio of extraction solution to sample is 9:1 (v:w) and samples are extracted twice by mechanical tissue grinding. A 5% aliquot of the extract is partitioned with hexane to remove oils and co-extracts. The hexane fraction is discarded and one-half of the remaining extract is evaporated in a stream of nitrogen to near aqueous in preparation for solid-phase extraction (SPE) purification using an ENV (a high purity styrene divinyl benzene polymer) cartridge. Extract solution is filtered through the SPE cartridges where the analytes are adsorbed onto the ENV sorbent. The sorbent is washed with hexane, and the analytes are eluted from the SPE cartridge in 25 mM ammonium hydroxide in methanol solution into a collection tube containing 0.5 mL of aqueous 50 mM ammonium acetate (keeper solution). The methanol is removed from the collected eluate by evaporation at a controlled temperature (30-35°C). The sample extract is diluted to final composition of 10% acetonitrile/90% aqueous 50 mM ammonium acetate for instrumental analysis. The analytes are resolved by HPLC and detected by electrospray MS/MS. Quantitative analysis was accomplished using the sum of the signal intensity from two molecular ion transitions for each analyte.

### 4.2 *Analytical Procedure*

#### 4.2.1 *Glassware & Equipment Cleaning Procedures*

See original report (i.e. DuPont-13412, Revision No. 1).

#### 4.2.2 *Preparation & Stability of Reagent Solutions*

For details regarding the preparation of reagent solutions, with the exception of mobile phases, refer to the original report (i.e. DuPont-13412, Revision No. 1). The mobile phases used to generate the data presented in this supplement were prepared as described below.

*Aqueous 0.01% formic acid (mobile phase A)*

Per liter volume, add 0.1 mL of concentrated formic acid to approximately 100 mL of distilled, deionized water in a 1-L graduated cylinder, and dilute to final volume with distilled, deionized water. Transfer the solution to a clean bottle for use as HPLC mobile phase. The solution and bottle should be replaced at least weekly to avoid microbial growth. *Note that the concentration of formic acid in mobile phase A is lower than the one specified in DuPont-13412, Revision No. 1.*

*Reason: An increase in sensitivity was observed.*

*0.01% formic acid in methanol (mobile phase B)*

Per liter volume, add 0.1 mL of concentrated formic acid to approximately 100 mL of methanol in a 1-L graduated cylinder and dilute to final volume with methanol. Transfer the solution to a clean bottle for use as HPLC mobile phase. The solution and bottle should be replaced at least monthly. *Note that the concentration of formic acid in mobile phase B is lower than the one specified in DuPont-13412, Revision No. 1.* *Reason: An increase in sensitivity was observed.*

#### 4.2.3 Stock Standard Preparation and Stability

If possible, use standards with purity greater than 95% and determine sample weights to 3 significant figures. The analytical balance must provide a weight precision to 3 significant figures, or the amount and volume must be adjusted to meet this condition. Clearly label as stock solutions with date prepared, analyte, and concentration. Prepare individual  $100 \pm 3$ - $\mu\text{g}/\text{mL}$  stock standards solutions for azimsulfuron, bensulfuron-methyl, chlorimuron-ethyl, chlorsulfuron, ethametsulfuron-methyl, flupyrsulfuron-methyl, metsulfuron-methyl, nicosulfuron, tribenuron-methyl, sulfometuron-methyl, thifensulfuron-methyl, and triflurosulfuron-methyl by weighing  $10.0 \pm 0.3$  mg of standard (adjusted for purity) into a tared 100-mL volumetric flask, mixing and dissolving analyte in acetonitrile, then diluting to final volume in acetonitrile. These solutions are stored at or below  $-10^\circ\text{C}$  and are stable for at least six months.

#### 4.2.4 Intermediate and Fortification Standard Preparation and Stability

*5.0  $\mu\text{g}/\text{mL}$  Fortification Solution*

Combine 5.0 mL of the stock solution for each analyte into a common 100-mL volumetric flask, dilute to volume with acetonitrile, cap, and mix well. Store in a freezer (below  $-10^\circ\text{C}$ ) and replace monthly. *Note that the concentration of each analyte in this fortification solution is  $\frac{1}{2}$  of that specified in DuPont-13412 Revision No. 1, Section 4.2.4.* *Reason: A total of twelve stock standard solutions (instead of six) are combined to make this fortification solution, thus concentration was lowered to allow preparation in a 100-mL volumetric flask.*

*1.0  $\mu\text{g}/\text{mL}$  Fortification Solution*

Transfer 20.0 mL of the 5.0  $\mu\text{g}/\text{mL}$  Fortification Solution to a 100-mL volumetric flask, dilute to volume with acetonitrile, cap, and mix well. Store in a freezer (below  $-10^\circ\text{C}$ ) and replace monthly.

*100 ng/mL Intermediate Solution*

Transfer 10.0 mL of the 1.0 µg/mL Fortification Solution to a 100-mL volumetric flask, dilute to volume with acetonitrile, cap, and mix well. Store in a freezer and replace weekly.

Alternative or additional standard concentrations may be prepared if required.

4.2.5 *Chromatographic Standard Preparation and Stability*

The 100 ng/mL Intermediate Solution is used to prepare 10.0 and 5.0 ng/mL Calibration Solutions. The remaining calibration standard concentrations are prepared from the 10 ng/mL Calibration Standard. The final solution composition for each calibration standard was approximately 10% acetonitrile and 90% aqueous 50 mM ammonium acetate. Calibration standards were generally prepared the day of analysis and were stable for at least 2 days if maintained refrigerated. The following table provides guidance for the preparation of calibration standards.

DILUTING STANDARD (NG/ML, ACN)	DILUTING STANDARD ALIQUOT (ML)	CAN (ML)	DILUTE TO FINAL VOLUME IN AQUEOUS 50 MM AMMONIUM ACETATE	FINAL CONCENTRATION (NG/ML)
100.0	1.000	0.000	10.0	<b>10.00</b>
100.0	0.500	0.500	10.0	<b>5.00</b>
10.0	1.000	0.900	10.0	<b>1.00</b>
10.0	0.500	0.950	10.0	<b>0.50</b>
10.0	0.200	0.980	10.0	<b>0.20</b>

Alternative or additional calibration standard concentrations may be prepared if required.

4.2.6 *Source of Samples*

The test samples of untreated wheat grain were collected from Cimarron, Kansas field site in DuPont field study AMR-2570-93 and identified by barcode number S00085122. This sample material was homogenized and maintained in freezer storage.

The test samples of untreated soybean seed were collected from DuPont field study ABC-49106 and identified by barcode number S00226288a. This sample material was homogenized and maintained in freezer storage.

The test samples of untreated lettuce leaf were collected from DuPont field study VEL-4336-97 and identified by barcode number S00182880. This sample material was homogenized and maintained in freezer storage.

Whole oranges were obtained from a local supermarket, homogenized in a Hobart processor, and stored in a freezer.

4.2.7 *Storage & Preparation of Samples*

Test samples are stored frozen prior to analysis. Subsamples (10.0 ± 0.02 g) of preprocessed, homogenous test sample are weighed into individual, tared

polypropylene 250-mL centrifuge bottles using a calibrated top-loading balance. Record sample weights.

#### 4.2.8 Sample Fortification Procedure

The 5.0 and 1.0 µg/mL Fortification Standards (Section 4.2.4) containing a mixture of all analytes were used to fortify test samples according to the following table.

SAMPLE IDENTIFICATION	AMOUNT (G)	FORTIFICATION SOLUTION		FORTIFICATION (MG/KG)
		µG/ML	ML	
0.010 mg/kg Fort (LOQ)	10.0	1.0	0.10	0.010
0.10 mg/kg Fort (10 x LOQ)	10.0	5.0	0.20	0.10

#### 4.2.9 Analyte Extraction Procedure

1. Accurately weigh  $10.0 \pm 0.1$  g of sample into a 250-mL centrifuge bottle. Fortify sample, if necessary, and allow at least 15 minutes for the fortification solution to evaporate in fume hood prior to extraction.
2. Add  $90 \pm 1$  mL of Extraction Solution (acetonitrile/pH7  $K_2HPO_4$ , 75/25, v/v) to each sample, cap, and shake by hand briefly.
3. Uncap and homogenize samples using a Tissumizer<sup>®</sup> for 2 minutes at 40–50% of total motor speed or at a speed that efficiently homogenizes the samples without overheating or foaming.  
*Samples should be kept cold on bed of dry ice or ice/water bath during homogenization.*
4. Centrifuge bottles for 15 minutes at 0–5°C at 13,000 rpm to achieve sufficient clarification of supernatant.  
*If high speed centrifugation is not available, samples may be centrifuged at lower speed and supernatant filtered through glass fiber or paper filter. Use care to retain as much solids as possible in centrifuge bottle while decanting supernatant.*
5. Carefully decant the supernatants into clean, labeled 250-mL measuring cylinders.
6. Add  $90 \pm 1$  mL of Extraction Solution to each sample, cap, shake, or tap vigorously to completely redisperse the pellet in the buffer solution.
7. Uncap and homogenize samples using a Tissumizer<sup>®</sup> for 2 minutes at 40–50% of total motor speed or at a speed that efficiently homogenizes the samples without overheating or foaming.  
*Samples should be kept cold on bed of dry ice or ice/water bath during homogenization.*
8. Centrifuge bottles for 15 minutes at 0–5°C at 13,000 rpm to achieve sufficient clarification of supernatant.

*If high speed centrifugation is not available, samples may be centrifuged at lower speed and supernatant filtered through glass fiber or paper filter (same filter used in step 4).*

9. Decant and combine supernatants to the respective measuring cylinders. Bring the final volume of supernatant to the 200-mL mark on the measuring cylinders with **acetonitrile**.  
*If mixing cylinders are used, cap and homogenize. In that case, step 10 is only required if extracts are going to be stored.*
10. Transfer the supernatants to clean 250-mL polypropylene laboratory bottles. Cap and shake.  
*Note that extracts may be stored in a freezer at this point. Sample extracts were shown to be stable for at least 6 days when stored frozen (target temperature  $-20^{\circ}\text{C}$ ).*

#### 4.2.10 Analyte Purification Procedure

1. Transfer 10.0 mL of extract to a 50-mL polypropylene centrifuge tube.
2. Add 5 mL of hexane, cap, and shake well (e.g. vortex for 5-10 seconds). Centrifuge 5 min at 3000–4000 rpm.
3. Remove and discard hexane layer.
4. Transfer 5 mL of extract to a clean 15-mL polypropylene centrifuge tube.
5. Evaporate extract solution to near aqueous ( $\sim 1.0$  mL) in a stream of  $\text{N}_2$  on N-Evap at  $30\text{--}35^{\circ}\text{C}$ .
6. Dilute sample to 10 mL with distilled, deionized water.
7. Place an ENV cartridge (6 cc, 500 mg) on a SPE manifold. Use a Bond Elut<sup>®</sup> adaptor to connect an empty 20-mL reservoir to the top of each ENV cartridge.
8. Precondition the ENV cartridge with 10 mL of methanol followed by 10 mL of the 10-mM ammonium acetate. Discard the eluate.  
***Do not let the cartridge go to dryness.***
9. Transfer the 10-mL dilute extract to the 20-mL reservoir above ENV cartridge. Complete quantitative transfer of sample to reservoir with 10 mM ammonium acetate solution rinses ( $<10$  mL total, e.g.  $3 \times 3$  mL). Pass this solution through the cartridge at a flow rate of  $2\text{--}5$  mL/min. Apply light vacuum if necessary. After elution, apply high vacuum for  $\sim 5$  minutes to dry the sorbent.
10. Wash the cartridge with 10 mL of hexane. Apply vacuum to pull the hexane through the cartridge. Discard the eluate. *A 10-minute vacuum drying period may be applied to the cartridge following the hexane elution (ILV Study DuPont-13398, Reference 2).*

11. Elute the analytes from the SPE cartridge with 15 mL of 25 mM ammonium hydroxide in methanol elution solution. Collect the eluate at a flow rate of 2-3 mL/minute in a 50-mL polypropylene centrifuge tube containing 0.5 mL of 50 mM ammonium acetate solution.
12. Evaporate methanol from extract solution using a RapidVap evaporator (55 rpm and 35°C under aspirator vacuum) or in a stream of N<sub>2</sub> on N-Evap at 30-35°C, to volume of ~0.75 mL.<sup>1</sup>
13. Transfer concentrated extract to clean 15-mL polypropylene centrifuge tube. Complete quantitative transfer with 0.5-mL ACN rinse of starting tube followed by 50-mM ammonium acetate rinses to bring final volume to 5.0 mL.<sup>2</sup>
14. Cap and mix well using vortex mixer.
15. Filter final sample extract through 0.45-µm (or smaller pore size) nylon Acrodisc<sup>®</sup> prior to LC/MS/MS analysis.

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<sup>1</sup> The evaporation system (DB3A Dri-Block) used in the ILV Study DuPont-13398 applied heat to entire chamber and yielded lower recoveries for tribenuron methyl. Therefore, this step was modified such that the extract was evaporated to 2.0 mL instead of 0.75 mL to improve the recovery of tribenuron methyl within the acceptable range (70-110%). The 2 mL final evaporation volume was transferred to a clean 15-mL centrifuge tube. The transfer was completed by rinsing the starting tube with 0.5 mL of ACN, followed by rinses of 50 mM ammonium acetate (water) to bring the final volume to 5 mL (organic % of the final extract approximately 40%).

<sup>2</sup> The quantitative transfer of the concentrated extract to a 15-mL polypropylene centrifuge tube is only required if a small final volume (e.g. 5.0 mL) is used. Final volume may be adjusted to match sensitivity of MS instrument. For example, if a 25.0 mL final volume is used, add 2.5 mL of acetonitrile to the 50-mL polypropylene centrifuge tubes and bringing the final volume to 25.0 mL with ammonium acetate. If sample final volume is changed, calibration standard concentrations need to be adjusted as appropriate. Dilution of final extract can increase method ruggedness.

### 4.3 *Instrumentation*

#### 4.3.1 *Description*

An Agilent 1100 Series HPLC connected to an Applied Biosystems API-4000 triple quadrupole mass spectrometer using an electrospray interface (ESI). HPLC components were: G1379A vacuum degasser, G1312A binary pump, G1316A column compartment, and G1367A refrigerated autosampler. Data acquisition and system control was by Analyst 1.4.1 software. The Applied Biosystems API-4000 was operated in LC/MS/MS positive ion mode with MRM detector output for quantitative and confirmatory analysis.

#### 4.3.2 *Operating Conditions*

##### *HPLC Operating Conditions:*

Injection Volume:	10-100 $\mu$ L (15 $\mu$ L injections used for validation)
Column:	Luna Phenyl-Hexyl, 4.6 mm $\times$ 150 mm, 3- $\mu$ m diameter particulate
Column Temperature:	40°C
Solvent A:	0.01% v/v formic acid in water
Solvent B:	0.01% v/v formic acid in methanol
Post Run Time:	0.0 min [ <i>re-equilibration to initial conditions</i> ]

TIME	FLOWRATE (ML/MIN)	%A	%B	COMMENTS
0.00	1.000	50	50	
20.00	1.000	20	80	
20.10	1.000	10	90	
23.00	1.000	10	90	
23.10	1.000	50	50	
28.00	1.000	50	50	End Run

##### Approximate Analyte Retention Times:

Nicosulfuron	(DPX-V9360)	= 9.2 min
Sulfometuron-methyl	(DPX-T5648)	= 9.8 min
Thifensulfuron-methyl	(DPX-M6316)	= 9.9 min
Metsulfuron-methyl	(DPX-T6376)	= 10.2 min
Ethametsulfuron-methyl	(DPX-A7881)	= 11.1 min
Chlorsulfuron	(DPX-W4189)	= 11.7 min
Azimsulfuron	(DPX-A8947)	= 15.1 min
Bensulfuron-methyl	(DPX-F5384)	= 15.4 min
Flupyrsulfuron-methyl	(DPX-KE459)	= 15.7 min
Tribenuron-methyl	(DPX-L5300)	= 15.7 min
Chlorimuron-ethyl	(DPX-F6025)	= 18.0 min
Triflusulfuron-methyl	(DPX-66037)	= 18.1 min

**Post-column Split:** ~100  $\mu\text{L}/\text{min}$  to MS and ~900  $\mu\text{L}/\text{min}$  to waste

**Triple Quadrupole MS operating conditions**

Interface: electrospray (ESI)  
 Mode: MRM  
 Resolution Q1: Unit  
 Resolution Q3: Unit  
 ESI Source Voltage: 4.5 kV, positive for all analytes  
 Divert Valve: 0.0–7.0 min to waste  
 7.0–20.0 min to source  
 20.0–28.0 min to waste

AB Sciex API-4000 Acquisition Parameters (ESI Interface, MRM mode)															
Period (min)	Analyte	Q1 (m/z)	Q3 (m/z)	Dwell (msec)	CUR (psi)	GS1 (psi)	GS2 (psi)	TEM ( $^{\circ}\text{C}$ )	ihe	IS (V)	CAD (psi)	DP (V)	EP (V)	CE (V)	CXP (V)
7.5 to 13.5	DPX-V9360	411	182	100	10	50	50	325	on	4500	8	60	10	25	10
		411	213												
	DPX-T5648	365	150												
		365	199												
	DPX-M6316	388	167												
		388	205												
	DPX-T6376	382	167												
		382	199												
DPX-A7881	411	196													
	411	168													
DPX-W4189	358	167													
	358	141													
13.5 to 20	DPX-A8947	425	182	100	10	50	50	325	off	4500	8	60	10	25	10
		425	244												
	DPX-F5384	411	149												
		411	182												
	DPX-L5300	396	155												
		396	181												
	DPX-KE459	466	156												
		466	182												
DPX-F6025	415	186													
	415	213													
DPX-66037	493	238													
	493	264													

### 4.3.3 Calibration Procedures

Use standard mass spectrometer tuning and calibration techniques. If confidence in the mass calibration needs to be established (modern mass spectrometers under digital control generally do not need frequent mass calibration, especially for quantitative modes), use vendor recommended calibrating solution. Optimization tuning of MS system may be accomplished by infusion of one or more of the test analytes. This method uses external standards, prepared as described in Section 4.2.5.

Instrument calibration was based on the average response factor ( $\text{RF}_{\text{avg}}$ ; defined here as analyte concentration/peak area response) obtained for external calibration standards. Two ion transitions were monitored for each analyte (as show in the table in section 4.3.2); instrument calibration was performed using the sum of both transitions for each compound, and using the Excel<sup>®</sup> functions AVERAGE, STDEV,

and RSD. Acceptance criteria for valid quantitation are: (1) a %RSD  $\leq 20\%$  for the individual calibration standard response factors and (2) a RSQ ( $r^2$ ) value  $> 0.99$  for linear regression analysis of the calibration standards.

The calibrated range of instrument response was 0.20 ng/mL to 10.0 ng/mL. This range of calibration is equivalent to expected final extract concentrations from  $0.4 \times 0.010$  mg/kg (LOQ) to  $2 \times 0.10$  mg/kg. Typically, 5 calibration solutions were interspersed with samples extracts for quantitative LC/MS/MS analysis.

#### 4.3.4 Sample Analysis

Preliminary runs of at least 1 solvent blank (demonstrate no interference related to LC/MS/MS system) and 1 low-end calibration standard (demonstrate instrument sensitivity) are routinely made to insure the LC/MS system is equilibrated and performing adequately. If multiple sets are analyzed, a blank injection should be made between the last and first injections of the sets to minimize risk of carryover from high concentration sample to a low concentration calibration standard. Calibration standard analyses should precede the first sample analysis and follow the last sample analysis. Typically, the injection sequence was organized from lowest to highest expected analyte concentrations. Calibration standard runs were intermixed with the test samples and should be analyzed before and after every 1–3 samples in each analytical set.

Calibration standards prepared the day of analysis from acetonitrile intermediate standards should be used with each sample set.

### 4.4 Calculations

#### 4.4.1 Methods

Azimsulfuron, bensulfuron-methyl, chlorimuron-ethyl, chlorsulfuron, ethametsulfuron-methyl, flupyr-sulfuron-methyl, metsulfuron-methyl, nicosulfuron, tribenuron-methyl, sulfometuron-methyl, thifensulfuron-methyl, and triflurosulfuron-methyl residues found at or above the LOQ are reported to 2 significant figures. Detected residues equal to or above the limit of detection (LOD), but below the LOQ, are reported to 1 significant figure. Recoveries for fortified samples are reported to the nearest whole number percentage (%).

The calculation to determine ng/g (ppb) found in residue samples by average response analysis follows:

$$\text{Analyte found (ppb, ng/g)} = \frac{PA \times ARF \times FV \times AF}{SW}$$

where,

PA is Analyte **P**eak **A**rea,  
 FV is **F**inal extract **V**olume (5 mL),  
 XV is e**X**tract **V**olume (200 mL),

ARF is Average Response Factor  $\left( \frac{\text{concentration (ng/mL)}}{\text{peak area}} \right)$ ,

AF is Aliquot Factor (XV/volume of aliquot processed to FV), for example, during method validation the aliquot factor is 40 for all samples,

SW is Sample Weight (g) of sample aliquot extracted, and

Percent recoveries (reported to the nearest whole number) from fortified samples were calculated as follows:

$$\% \text{ Recovery} = \frac{\text{ng/g analyte found}}{\text{ng/g analyte fortified}} \times 100$$

#### 4.4.2 Examples

##### **Sulfometuron methyl**

Sample ID: SCN-2 SB (LOQ). The sample was extracted on July-07-06, and analyzed on July-07-06, and the data appears Appendix 1, "Representative Validation Recovery Results", set number SBMV1\_070706scn.

Analyte found (ppb, ng/g) =

$$\frac{46070 \text{ area} \times (1.0669 \times 10^{-5} \frac{\text{ng/mL}}{\text{area}}) \times 5.0 \text{ mL} \times 40.0}{10.0 \text{ g}} = 9.83 \text{ ng/g}$$

= 9.8 ng/g found

$$\% \text{ Recovery} = \frac{9.8 \text{ ng/g analyte found}}{10 \text{ ng/g analyte fortified}} \times 100 = 98\%$$

##### **Metsulfuron methyl**

Sample ID: SCN-5 SB (10xLOQ). The sample was extracted on July-07-06, and analyzed on July-07-06, and the data appears Appendix 1, "Representative Validation Recovery Results", set number SBMV1\_070706scn.

Analyte found (ppb, ng/g) =

$$\frac{224000 \text{ area} \times (2.12030 \times 10^{-5} \frac{\text{ng/mL}}{\text{area}}) \times 5.0 \text{ mL} \times 40.0}{10.0 \text{ g}} = 94.99 \text{ ng/g}$$

= 95 ng/g found

$$\% \text{ Recovery} = \frac{95 \text{ ng/g analyte found}}{100 \text{ ng/g analyte fortified}} \times 100 = 95\%$$

## 5.0 RESULTS AND DISCUSSION

### 5.1 *Method Validation Results*

#### 5.1.1 Detector Response

A triple quadrupole mass spectrometer using positive ion ESI and tandem mass spectrometry detection were used for instrumental analysis. Full-Scan LC/MS spectra for azimsulfuron, bensulfuron-methyl, chlorsulfuron, flupyr-sulfuron-methyl, metsulfuron-methyl, sulfometuron-methyl, and triflusulfuron-methyl from analysis of mixed standard solutions are provided in **Error! Reference source not found.** In addition, representative LC/MS/MS spectra for the respective protonated sulfonylureas also appear in **Error! Reference source not found.** Note that full-scan spectra for nicosulfuron, thifensulfuron methyl, ethametsulfuron methyl, rimsulfuron, tribenuron, and chlorimuron are provided in the original DuPont-13412, Revision No. 1 report (Reference 1).

For each analyte, calibration standards typically yielded a linear response (r-squared >0.99) with %RSD <20% for calibration standard response factors over the range of 0.2–10 ng/mL. Representative calibration curves for each analyte were constructed using calibration standards from a validation set (Orange, Set # 2) and are presented in Figure 1. Representative ion chromatograms of calibration standards are provided in Figure 2. Representative ion chromatograms of extracts from a control sample, LOQ and 10×LOQ fortification samples for lettuce, orange, soybean seed, and wheat grain are provided in **Error! Reference source not found.** through **Error! Reference source not found.**, respectively.

#### 5.1.2 Controls

No significant matrix interference was observed in the regions of azimsulfuron, bensulfuron-methyl, chlorimuron-ethyl, chlorsulfuron, ethametsulfuron-methyl, flupyr-sulfuron-methyl, metsulfuron-methyl, nicosulfuron, tribenuron-methyl, sulfometuron-methyl, thifensulfuron-methyl, and triflusulfuron-methyl elution in chromatograms of control extracts from wheat grain, soybean seed, lettuce, and oranges samples.

#### 5.1.3 Recoveries (Accuracy & Precision)

Recovery results are provided in **Error! Reference source not found.** through **Error! Reference source not found.** for lettuce, orange, soybean seed, and wheat grain respectively. The average results at the LOQ (0.010 ppm) and 10×LOQ (0.10 ppm) fortification levels with overall results in each matrix are provided in the tables below.

## Lettuce

		LOQ			10x LOQ		
Compound	DPX number	% Recovery (%RSD)	Range	n	% Recovery (%RSD)	Range	n
Azimsulfuron	DPX-A8947	94 (3.4)	89 - 98	5	99 (5.5)	90 - 103	5
Bensulfuron methyl	DPX-F5384	97 (4.7)	92 - 102	5	100 (2.9)	95 - 102	5
Chlorimuron ethyl	DPX-F6025	100 (2.2)	97 - 102	5	100 (4.0)	93 - 103	5
Ethametsulfuron methyl	DPX-A7881	100 (2.3)	97 - 102	5	100 (4.1)	93 - 103	5
Sulfometuron methyl	DPX-T5648	99 (2.7)	96 - 102	5	100 (3.4)	94 - 103	5
		Overall					
Compound	DPX number	% Recovery (%RSD)	Range	n			
Azimsulfuron	DPX-A8947	96 (5.3)	89 - 103	10			
Bensulfuron methyl	DPX-F5384	98 (3.9)	92 - 102	10			
Chlorimuron ethyl	DPX-F6025	100 (3.0)	93 - 103	10			
Ethametsulfuron methyl	DPX-A7881	100 (3.1)	93 - 103	10			
Sulfometuron methyl	DPX-T5648	100 (2.9)	94 - 103	10			

## Orange

		LOQ			10x LOQ		
Compound	DPX number	% Recovery (%RSD)	Range	n	% Recovery (%RSD)	Range	n
Azimsulfuron	DPX-A8947	80 (1.1)	79 - 81	5	83 (4.5)	79 - 89	5
Bensulfuron methyl	DPX-F5384	94 (2.5)	92 - 97	5	90 (1.8)	88 - 91	5
Chlorimuron ethyl	DPX-F6025	93 (3.1)	89 - 97	5	90 (5.0)	84 - 96	5
Chlorsulfuron	DPX-W4189	92 (3.3)	89 - 96	5	92 (2.0)	90 - 95	5
Ethametsulfuron methyl	DPX-A7881	98 (4.9)	90 - 103	5	100 (6.1)	92 - 108	5
Flupyrsulfuron methyl	DPX-KE459	88 (7.4)	81 - 97	5	88 (4.5)	85 - 93	5
Metsulfuron methyl	DPX-T6376	100 (7.8)	89 - 108	5	96 (4.4)	91 - 101	5
Nicosulfuron	DPX-V9360	104 (4.2)	98 - 110	5	96 (3.6)	93 - 100	5
Sulfometuron methyl	DPX-T5648	91 (4.5)	86 - 95	5	89 (4.1)	86 - 95	5
Thifensulfuron methyl	DPX-M6316	96 (1.4)	95 - 98	5	97 (4.4)	92 - 103	5
Tribenuron methyl	DPX-L5300	76 (2.1)	74 - 78	5	72 (3.5)	70 - 76	5
Triflusulfuron methyl	DPX-66037	90 (6.2)	87 - 100	5	89 (5.1)	84 - 95	5
		Overall					
Compound	DPX number	% Recovery (%RSD)	Range	n			
Azimsulfuron	DPX-A8947	82 (3.6)	79 - 89	10			
Bensulfuron methyl	DPX-F5384	92 (3.2)	88 - 97	10			
Chlorimuron ethyl	DPX-F6025	92 (3.5)	84 - 97	10			
Chlorsulfuron	DPX-W4189	92 (2.7)	89 - 96	10			
Ethametsulfuron methyl	DPX-A7881	99 (5.4)	90 - 108	10			
Flupyrsulfuron methyl	DPX-KE459	88 (5.8)	81 - 97	10			
Metsulfuron methyl	DPX-T6376	98 (6.7)	89 - 108	10			
Nicosulfuron	DPX-V9360	100 (4.7)	93 - 110	10			
Sulfometuron methyl	DPX-T5648	90 (4.2)	86 - 95	10			
Thifensulfuron methyl	DPX-M6316	96 (3.1)	92 - 103	10			
Tribenuron methyl	DPX-L5300	74 (3.9)	70 - 78	10			
Triflusulfuron methyl	DPX-66037	90 (5.4)	84 - 100	10			

**Soybean Seed**

		LOQ			10x LOQ		
Compound	DPX number	% Recovery (%RSD)	Range	n	% Recovery (%RSD)	Range	n
Azimsulfuron	DPX-A8947	93 (8.9)	81 - 103	5	96 (3.7)	91 - 100	5
Bensulfuron methyl	DPX-F5384	101 (3.3)	96 - 105	5	100 (4.8)	95 - 106	5
Chlorsulfuron	DPX-W4189	90 (14.1)	74 - 105	5	98 (1.4)	96 - 100	5
Flupyr sulfuron methyl	DPX-KE459	99 (6.8)	90 - 107	5	102 (1.5)	100 - 104	5
Metsulfuron methyl	DPX-T6376	93 (13.8)	76 - 106	5	98 (1.7)	95 - 99	5
Nicosulfuron	DPX-V9360	100 (11.9)	85-111	5	106 (2.4)	102-109	5
Sulfometuron methyl	DPX-T5648	97 (4.6)	91 - 102	5	96.4 (1.9)	94 - 97	5
Triflurosulfuron methyl	DPX-66037	102 (2.9)	98 - 106	5	100 (2.1)	97 - 102	5
		Overall					
Compound	DPX number	% Recovery (%RSD)	Range	n			
Azimsulfuron	DPX-A8947	95 (6.5)	81 - 103	10			
Bensulfuron methyl	DPX-F5384	100 (3.9)	95 - 106	10			
Chlorsulfuron	DPX-W4189	94 (10.0)	74 - 105	10			
Flupyr sulfuron methyl	DPX-KE459	101 (4.8)	90 - 107	10			
Metsulfuron methyl	DPX-T6376	96 (9.4)	76 - 106	10			
Nicosulfuron	DPX-V9360	103 (8.6)	85-111	10			
Sulfometuron methyl	DPX-T5648	97 (3.3)	91 - 102	10			
Triflurosulfuron methyl	DPX-66037	101 (2.7)	97 - 105	10			

**Wheat Grain**

		LOQ			10x LOQ		
Compound	DPX number	% Recovery (%RSD)	Range	n	% Recovery (%RSD)	Range	n
Azimsulfuron	DPX-A8947	98 (3.1)	94 - 101	5	96 (2.3)	93 - 98	5
Bensulfuron methyl	DPX-F5384	100 (3.4)	95 - 104	5	97 (2.0)	95 - 100	5
Chlorimuron ethyl	DPX-F6025	93 (4.6)	89 - 100	5	94 (2.8)	91 - 98	5
Ethametsulfuron methyl	DPX-A7881	97 (4.5)	89 - 100	5	96 (2.0)	94 - 99	5
Sulfometuron methyl	DPX-T5648	100 (5.0)	93 - 105	5	98 (3.7)	93 - 102	5
Triflurosulfuron methyl	DPX-66037	98 (1.2)	96 - 99	5	97 (2.7)	93 - 100	5
		Overall					
Compound	DPX number	% Recovery (%RSD)	Range	n			
Azimsulfuron	DPX-A8947	97 (2.8)	93 - 101	10			
Bensulfuron methyl	DPX-F5384	99 (3.0)	95 - 104	10			
Chlorimuron ethyl	DPX-F6025	94 (3.7)	89 - 100	10			
Ethametsulfuron methyl	DPX-A7881	97 (3.3)	89 - 100	10			
Sulfometuron methyl	DPX-T5648	99 (4.4)	93 - 105	10			
Triflurosulfuron methyl	DPX-66037	97 (2.0)	93 - 100	10			

Representative raw data spreadsheets from the analysis of individual sample sets are provided in Appendix 1.

#### 5.1.4 *Limit of Quantitation (LOQ)*

The LOQ determined in this method was 0.010 ppm for crops including wheat grain, soybean seed, lettuce, and oranges. 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 to 1 for the least responsive analyte.

#### 5.1.4.1 *Background Evaluation*

Background levels experienced in tandem mass spectrometry analyses are minimal. Generally, the chromatographic profiles of a sample extract solution and a calibration standard solution appear the same.

#### 5.1.4.2 *Limit of Detection (LOD)*

A limit of detection (LOD) was estimated for the analytes added to the analytical method in this supplement: azimsulfuron (0.0006 mg/kg), bensulfuron methyl (0.001 mg/kg), chlorsulfuron (0.001 mg/kg), flupyralsulfuron methyl (0.0006 mg/kg), metsulfuron methyl (0.0007 mg/kg), sulfometuron methyl (0.0006 mg/kg), and triflurosulfuron methyl (0.0004 mg/kg). The LOD is defined as the analyte concentration in matrix with an observed response that has a signal-to-noise ratio of approximately 3-to-1. Variation in the LOD was observed and each lab using this method should estimate an LOD value. Examples of the LOQ calculations are included in **Error! Reference source not found.**

#### 5.2 *Timing*

Typically six to eight samples can be prepared during the course of an eight-hour day. LC/MS/MS analyses were run unattended overnight.

#### 5.3 *Modifications or Special Precautions*

Because of the low levels achieved by tandem mass spectrometry detection, thoroughly clean glassware, homogenizer probes, and bench space are essential to avoid sample cross-contamination.

Changing equipment, reagents, supplies, or techniques specified in this method requires a revalidation for each analyte in the relevant matrices, unless it is known or has been shown that the change involves an equivalent item. A revalidation is clearly required for a change in a) composition of extraction solution, or b) composition of final dilution solvent (prior to instrumental analysis).

#### 5.4 *Method Ruggedness*

##### 5.4.1 *Confirmatory Procedure*

Two MS/MS ion transitions were monitored and recorded for each analyte during analysis in this method. An average ratio of fragment ion responses (base peak/minor peak) for each analyte was determined from calibration standards at 0.20, 0.50, 1.0, 5.0, and 10 ng/mL concentration within an analytical set. The ion ratios can vary with individual instrument conditions and should be established for each sample set. The injection volume may be increased so the minor fragment ion response can be integrated at the LOQ equivalent concentration. As an example and to demonstrate the performance of the confirmatory procedure for the analytes added to the analytical method (azimsulfuron, bensulfuron-methyl, chlorsulfuron, flupyralsulfuron-methyl, metsulfuron-methyl, sulfometuron-methyl, and triflurosulfuron-methyl), a validation set (Orange, #1) was re-analyzed by LC/MS/MS using a larger injection

volume (25 microliters). The results are included in **Error! Reference source not found.**. The following table provides a summary of representative LC/MS/MS confirmatory responses for azimsulfuron, bensulfuron-methyl, chlorsulfuron, flupyrsulfuron-methyl, metsulfuron-methyl, sulfometuron-methyl, and triflusulfuron-methyl.

ANALYTE	MOLECULAR ION (M/Z)	MS/MS FRAGMENT ION (M/Z)	APPROX. ION RATIO
Azimsulfuron	425	182 <b>244</b>	15.0 : 1
Bensulfuron methyl	411	149 <b>182</b>	1.9 : 1
Chlorsulfuron	358	141 <b>167</b>	1.1 : 1
Flupyrsulfuron methyl	466	182 <b>156</b>	8.5 : 1
Metsulfuron methyl	382	167 <b>199</b>	13.7 : 1
Sulfometuron methyl	365	150 <b>199</b>	16.1 : 1
Triflusulfuron methyl	493	264 <b>238</b>	32.8 : 1

The acceptance criteria for confirmation are: (1) the %RSD for the ion ratio determined from individual standards is  $\leq 20\%$ , (2) the ion ratio for an analyte response in a sample extract is within  $\pm 30\%$  of the ion ratio determined from standards, and (3) HPLC analyte retention time is within  $\pm 0.2$  min of the respective average retention time in calibration standards. An example of analyte confirmation from the LC/MS/MS analysis of a validation set (Orange, #1, extracted on 9/8/06 and analyzed on 9/14/06) is included in **Error! Reference source not found.**

## 6.0 CONCLUSIONS

- In summary, the ILVs reports DuPont-13398 (Reference 2) and DuPont-17207, Revision No. 1 (Reference 3), together with analytical method and validation data included in DuPont-13412, Revision No. 1 (Reference 1), and DuPont-13412, Revision No. 1, Supplement No. 1 demonstrate that the method can be successfully employed to analyze for (at least) the sulfonylurea herbicides shown below in matrices representative of all four EU crop groups:

<b>Dry</b>	<b>Oily</b>	<b>Watery</b>	<b>Acidic</b>
Azimsulfuron	Azimsulfuron	Azimsulfuron	Azimsulfuron
Bensulfuron-methyl	Bensulfuron-methyl	Bensulfuron-methyl	Chlorimuron-ethyl
Chlorimuron-ethyl	Chlorimuron-ethyl	Chlorimuron-ethyl	Chlorsulfuron
Ethametsulfuron-methyl	Chlorsulfuron	Ethametsulfuron-methyl	Ethametsulfuron-methyl
Sulfometuron-methyl	Ethametsulfuron-methyl	Sulfometuron-methyl	Flupyr sulfuron-methyl
Triflusaluron-methyl	Flupyr sulfuron-methyl		Metsulfuron-methyl
	Metsulfuron-methyl		Nicosulfuron
	Nicosulfuron		Sulfometuron-methyl
	Rimsulfuron		Thifensulfuron-methyl
	Sulfometuron-methyl		Triflusaluron-methyl
	Thifensulfuron-methyl		
	Tribenuron-methyl		
	Triflusaluron-methyl		

- The LOQ for the analytical multiresidue method is 0.010-mg/kg.
- The overall average recoveries for each analyte and matrix in the validation trials ranged from 74% (tribenuron methyl in orange) to 101% (Flupyr sulfuron methyl and triflusaluron methyl) with maximum RSD of 10%.
- There were no detectable residues or matrix interference peaks in the control samples of lettuce, soybean seed, wheat grain, and orange.
- Residue confirmation for each analyte was demonstrated at 0.010 mg/kg (LOQ) and 0.10 mg/kg fortification levels based on retention time and the relative ratios of two MS/MS parent-to-fragment ion transitions detected during sample analysis.
- The method meets EEC Directive 91/414/EEC, Annex IIA 4.2.1 as amended by EC Directive 96/46/EC and the U.S. EPA Residue Chemistry Test Guidelines, August 1996, OPPTS 860.1340 Residue Analytical Method guidelines.

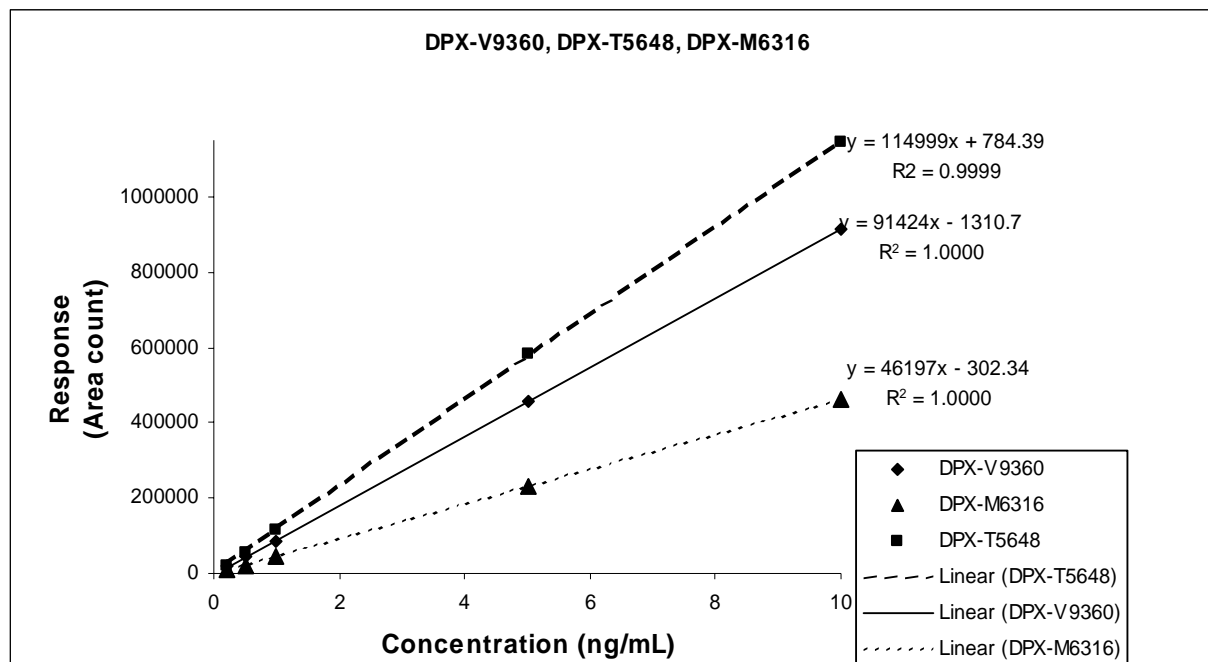
## **7.0 RETENTION OF RECORDS**

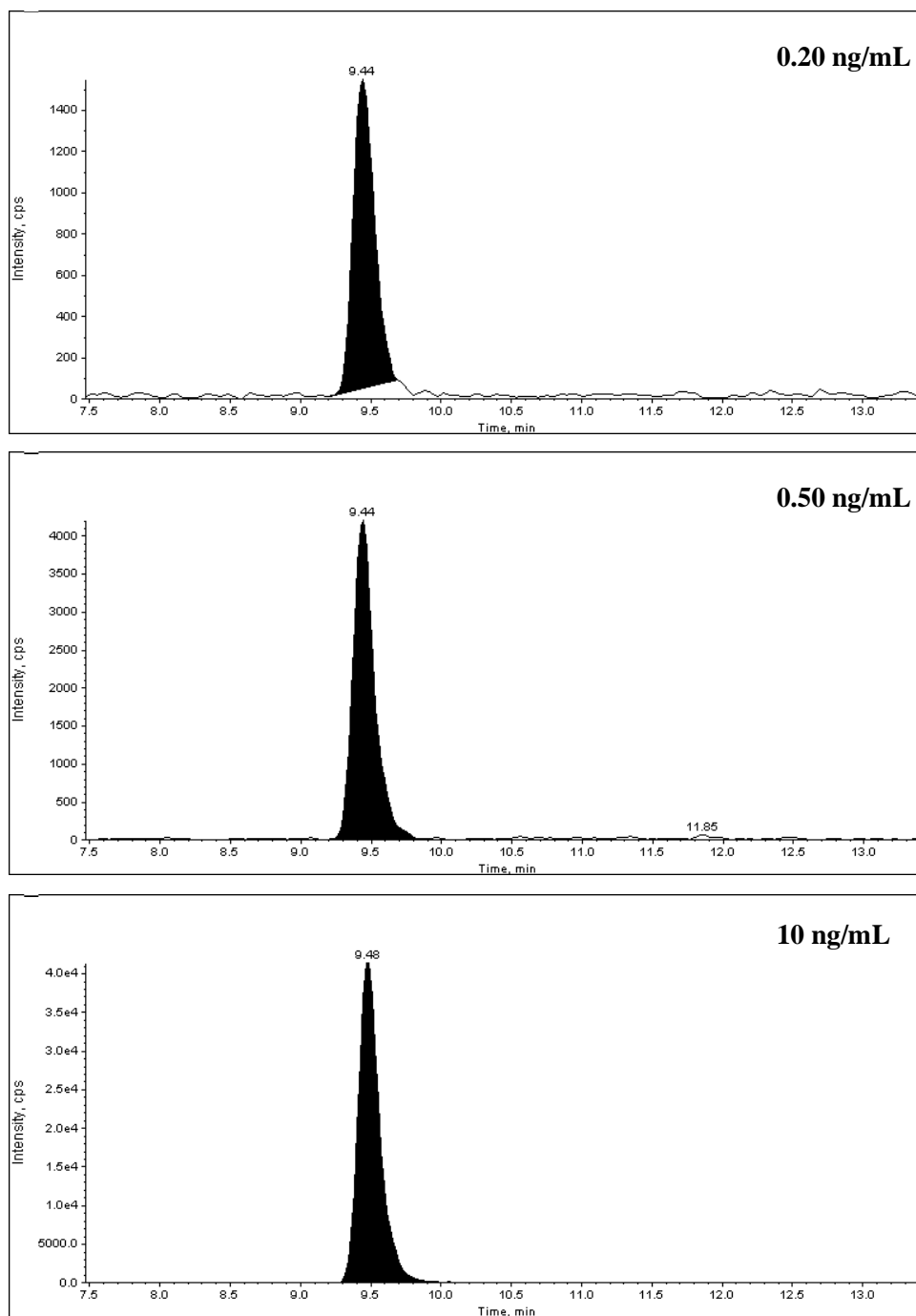
Originals or exact copies of all raw data and pertinent information, and the final report will be retained at:

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

## **8.0 REFERENCES**

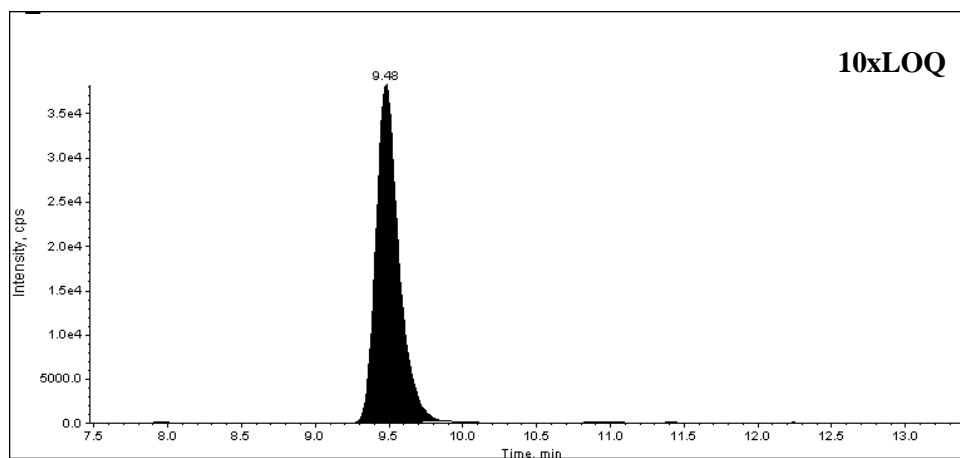
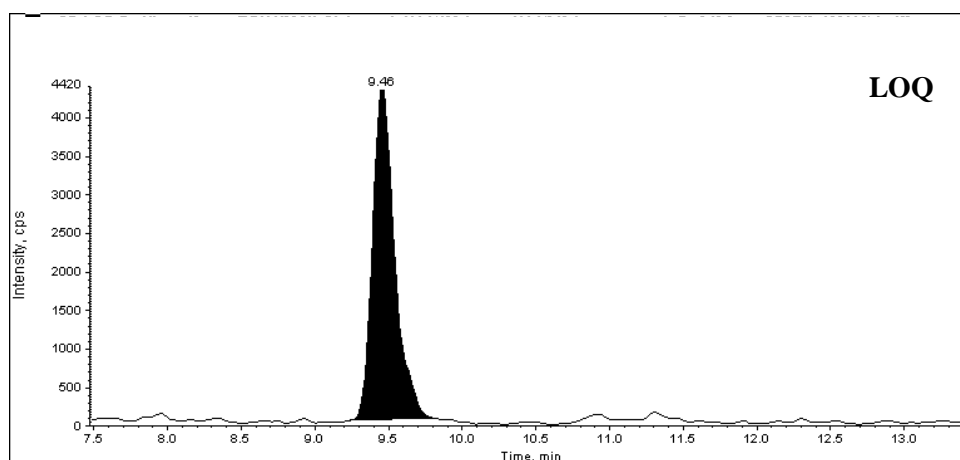
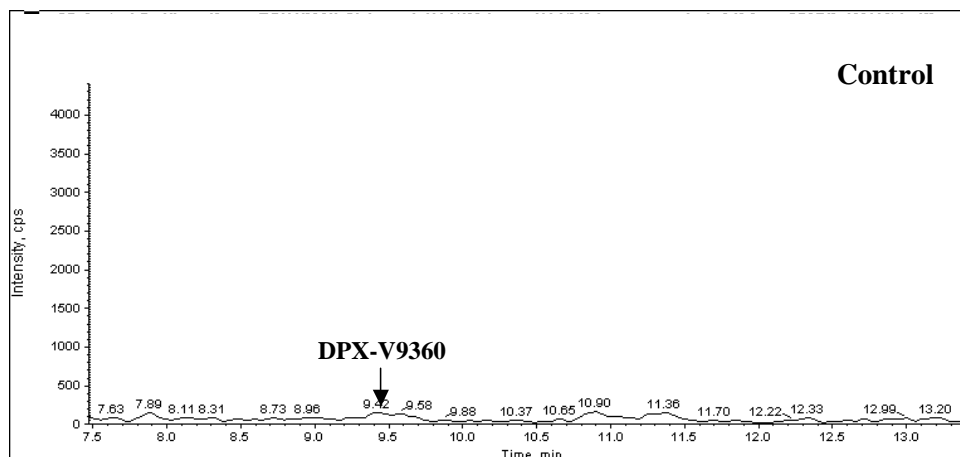
1. Bramble, F.Q., Pentz, A. “Analytical Method for the Determination of Nicosulfuron, Thifensulfuron Methyl, Ethametsulfuron Methyl, Rimsulfuron, Tribenuron Methyl, and Chlorimuron Ethyl in Oily Crop Matrices Using SPE Purification and LC/MS/MS Detection”, Analytical Method Report DuPont-13412, Revision No. 1. E. I. du Pont de Nemours and Company, Wilmington, DE.
2. Charles, E., Doran, A. M. “Independent Laboratory Validation of Analytical Method DuPont-13412 for the Determination of Thifensulfuron Methyl, Ethametsulfuron Methyl, Rimsulfuron, Tribenuron Methyl and Chlorimuron Ethyl in Olives and Soybean Seed Using SPE Purification and LC/MS/MS Detection”, ILV Report DuPont-13398. E. I. du Pont de Nemours and Company, Wilmington, DE.
3. Connolly, P. “Independent Laboratory Method Validation of a Multi-residue Method for the Analysis of Sulfonylurea Herbicides in Crops”, ILV Report DuPont-17207, Revision No. 1. E. I. du Pont de Nemours and Company, Wilmington, DE.

**FIGURE 1 REPRESENTATIVE CALIBRATION CURVES***Nicosulfuron, Sulfometuron methyl, Thifensulfuron methyl*

**FIGURE 2** REPRESENTATIVE CALIBRATION STANDARDS CHROMATOGRAMS  
(CONTINUED)*Nicosulfuron*

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*Nicosulfuron*



# APPENDIX 1 REPRESENTATIVE VALIDATION RECOVERY RESULTS

## RAW DATA REPORT

Analysts: Sergio Nanita

Study No.: DuPont-13412  
Instrument: Sciex API4000  
Analyte: DPX-T5648

Standard Prep. Dates:  
Calibration: 07/07/06  
Fortification: 07/07/06  
Fortification Standard:  
5.0 and 1.0 µg/mL DPX-T5648

Set No.: SBMV1\_070706scn  
Injection Vol.: 15 µL  
Limit of Quantification: 10 ng/g  
Limit of Detection: 3 ng/g  
Masses Monitored: 365.0 -> 150.0; 365.0 -> 199.0  
TIC used for quantitation.

Extraction date : 07/07/06  
Analyzed on : 07/07/06

Sample ID	Matrix (Soybean)	Sample Type	Std. Concentration (ng/mL)	Sample Weight (g)	Extract Volume (mL)	Aliquot Volume (mL)	Final Volume (mL)	HPLC Dilution Factor	DPX-T5648 Peak Area TIC	Response Factor	Fort. Level (ng/g)	Analyte Found (ng/g)	Rounded Analyte (ng/g)	Recovery %
Solvent Blank	-	O	-	-	-	-	-	-	-	-	-	-	-	-
0.20 ng/mL DPX-T5648	-	Std	0.20	-	-	-	-	-	18260	1.09529E-05	-	-	-	-
0.50 ng/mL DPX-T5648	-	Std	0.50	-	-	-	-	-	46670	1.07135E-05	-	-	-	-
SCN-1 SB (Control)	(Seed)	C	-	10.0	200.0	5.0	5.0	1	0	-	-	-	-	-
SCN-7 SB (Control Fort.)	(Seed)	F	-	10.0	200.0	5.0	5.0	1	472200	-	100.00	100.7584	100.76	101
1.0 ng/mL DPX-T5648	-	Std	1.00	-	-	-	-	-	95030	1.05230E-05	-	-	-	-
SCN-2 SB (LOQ)	(Seed)	F	-	10.0	200.0	5.0	5.0	1	46070	-	10.00	9.8305	9.83	98
SCN-3 SB (LOQ)	(Seed)	F	-	10.0	200.0	5.0	5.0	1	47970	-	10.00	10.2359	10.24	102
SCN-4 SB (LOQ)	(Seed)	F	-	10.0	200.0	5.0	5.0	1	44010	-	10.00	9.3909	9.39	94
5.0 ng/mL DPX-T5648	-	Std	5.00	-	-	-	-	-	465400	1.07434E-05	-	-	-	-
SCN-5 SB (10xLOQ)	(Seed)	F	-	10.0	200.0	5.0	5.0	1	441700	-	100.00	94.2503	94.25	94
SCN-6 SB (10xLOQ)	(Seed)	F	-	10.0	200.0	5.0	5.0	1	462500	-	100.00	98.6886	98.69	99
10 ng/mL DPX-T5648	-	Std	10.00	-	-	-	-	-	960400	1.04123E-05	-	-	-	-
Average Response Factor =										1.06690E-05				
RSD =										2.0				

**Sample Type Key**

Std = Standard  
C = Field Control  
F = Fortification  
O = Other  
PSC = Post Spike Control

Fortification Level = Amount Fortified/ Sample Weight

Analyte Found (ng/g) =  $\frac{\text{Peak Area} \times \text{Av. Response Factor} \times \text{Extract Vol.} \times \text{Final Vol} \times \text{HPLC Dilution Factor}}{\text{Sample Weight} \times \text{Aliquot Vol.}}$

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

