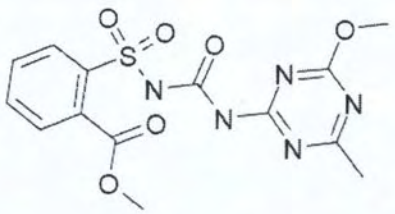
DuPont code: DPX-T6376 (Metsulfuron Methyl)

Chemical Structure:

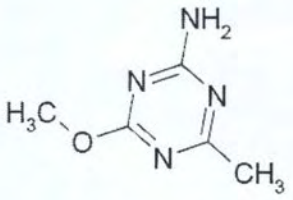


DPX-T6376

CAS Name:	Methyl 2-[[[[(4-methoxy-6-methyl-1,2,3-triazin-2-yl)amino]carbonyl]amino]sulfonyl]benzoate
Molecular weight:	381.37 amu
Formula:	$C_{14}H_{15}N_5O_6S$
Source:	Du Pont
CAS Number:	74223-64-6
Batch/Lot Number:	OCT06MA027
Purity:	99.0%
Receipt date:	24 January, 2014
Expiration date:	29 May, 2014
Storage:	Ambient

DuPont code: IN-A4098

Chemical Structure:



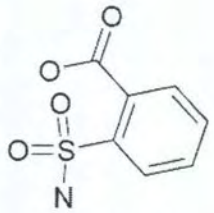
IN-A4098

Molecular weight:	140.15 amu
Formula:	$C_5H_8N_4O$
Source:	Du Pont

Batch/Lot Number:	050942-015
Purity:	98.7%
Receipt date:	24 January, 2014
Expiration date:	02 September, 2019
Storage:	Ambient

DuPont code: IN-D5119

Chemical Structure:

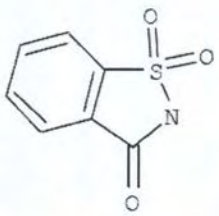


IN-D5119

Molecular weight:	201.20 amu
Formula:	$C_7H_5NO_4S$
Source:	Du Pont
Batch/Lot Number:	E10035-086
Purity:	99.5%
Receipt date:	24 January, 2014
Expiration date:	29 March, 2021
Storage:	-20°C

DuPont code: IN-00581

Chemical Structure:



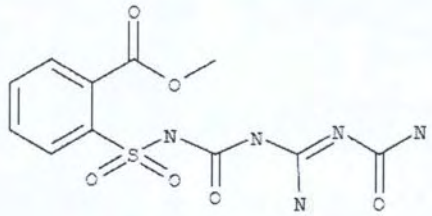
IN-00581

Molecular weight:	183.19 amu

Formula:	$C_7H_5NO_3S$
Source:	Du Pont
Batch/Lot Number:	07028EU
Purity:	99.8%
Receipt date:	24 January, 2014
Expiration date:	25 September, 2023
Storage:	Ambient

DuPont code: IN-NC148

Chemical Structure:

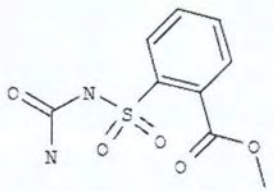


IN-NC148

Molecular weight:	343.32 amu
Formula:	$C_{11}H_{13}N_5O_6S$
Source:	Du Pont
Batch/Lot Number:	D101698-81
Purity:	82.7%
Receipt date:	24 January, 2014
Expiration date:	11 June, 2016
Storage:	Ambient

DuPont code: IN-B5685

Chemical Structure:

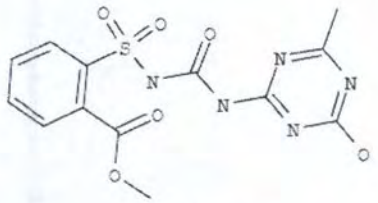


IN-B5685

Molecular weight:	258.25 amu
Formula:	$C_9H_{10}N_2O_5S$
Source:	Du Pont
Batch/Lot Number:	GVK-DU-P593-1
Purity:	97.0%
Receipt date:	24 January, 2014
Expiration date:	11 December, 2015
Storage:	Ambient

DuPont code: IN-B5067

Chemical Structure:

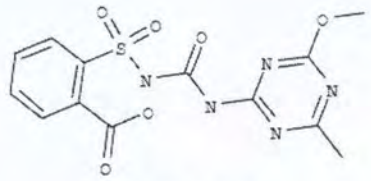


IN-B5067

Molecular weight:	367.34 amu
Formula:	$C_{13}H_{13}N_5O_6S$
Source:	Du Pont
Batch/Lot Number:	E117273-47
Purity:	94.9%
Receipt date:	24 January, 2014
Expiration date:	19 August, 2014
Storage:	Ambient

DuPont code: IN-F5438

Chemical Structure:



IN-F5438

Molecular weight:	367.34 amu
Formula:	$C_{13}H_{13}N_5O_6S$
Source:	Du Pont
Batch/Lot Number:	GF916965
Purity:	94.5%
Receipt date:	24 January, 2014
Expiration date:	11 December, 2015
Storage:	Ambient

Metsulfuron methyl and it metabolites (IN-A4098, IN-D5119, IN-00581, IN-NC148, IN-B5685, IN-B5067, and IN-F5438) were supplied by E. I. du Pont de Nemours and Company, DuPont Crop Protection, Newark, DE. Information pertaining to the characterization and stability of the test substances is archived by DuPont Crop Protection, E. I. du Pont de Nemours and Company, Newark, Delaware.

3.2 Test System

In this study, the analytical method was validated in water. Samples of surface water, and ground water were sent via FedEx from ABC Labs (Columbia, MO) to the testing facility (17 Lee Boulevard, Malvern, PA 19355). Samples of tap water were collected at the test facility. The characterization data for the surface and ground water analyzed are presented in Appendix 2.

Fortifications of the samples were made using 100.0 (±1%) mL of water spiked with 50 µL of 0.10 µg/mL or 1.0 µg/mL standard solutions. The samples were assigned unique identification by the laboratory, an alpha-numeric sample ID along with additional designations such as "control" and "LOQ", as appropriate.

3.3 Equipment

Equipment used was either the same as that specified in the analytical method or the equivalent. A Shimadzu LC-20AD HPLC was used instead of an Agilent 1200 HPLC system. An AB SCIEX Triple Quad 5500 was used instead of an API 5000 triple quad. The changes were demonstrated as equivalent to that specified in the method.

3.4 *Reagents*

Reagents used were either the same as those specified in the analytical method or equivalent grade of quality.

3.5 *Principles of the Analytical Method*

The analyses in this study followed the analytical method for metsulfuron methyl and metabolites, as described in the method for DuPont-28807. The following is a summary of the method conducted at Alliance Pharma. The complete description of the method is described in the original method (DuPont-28807).

Briefly, ground, surface and drinking water samples 100 (±1%) mL were fortified with the appropriate standard solution. One milliliter of 0.01 M ammonium formate (pH=3.5) and 50 µL of concentrated formic acid was added to each sample. The samples were mixed thoroughly. On an SPE vacuum manifold, 0.5g/20-mL Waters Oasis HLB cartridges were conditioned with 10 mL of methanol followed by 10 mL of HPLC grade water. Samples were loaded on the SPE manifold and vacuum was used to establish flow. The vacuum was then removed and the sample was allowed to flow via gravity through the cartridges. The cartridges were dried for 1 minute using vacuum, and the eluate was disposed of. The cartridges were washed with 10 mL of hexane and vacuum dried for 3 minutes. A volume of 20 mL of basic ACN was measured into each sample tube, and then was loaded into the SPE cartridges. The eluate was allowed to pass through the cartridges via gravity flow and was collected in 50 mL centrifuge tubes. Throughout the extraction, care was taken to not allow the cartridge to go to dryness.

The eluate was diluted to 20 mL with basic ACN and a 10 mL aliquot was transferred to 15 mL centrifuge tubes. The extracts were then evaporated under nitrogen flow in a water bath set to approximately 30°C. When the extract volume was approximately 3 mL, 0.25 mL of water was added and the extracts were allowed to continue evaporating until the volume was approximately 0.25 mL. The extracts were removed from the water bath and the extracts were diluted to 2.5 mL with 0.005 M aqueous ammonium acetate. Tubes were vortex mixed and all sample extracts were transferred to HPLC injection vials via a syringe filter.

The purified final extracts were analyzed by reversed-phase HPLC using a Polaris C18-A 3µm 2 x 150 mm column with mobile phases of 0.02 M aqueous formic acid solution and methanol. The analyte IN-A4098 was detected using the positive ion mode and metsulfuron methyl, IN-D5119, IN-00581, IN-NC148, IN-B5685, IN-B5067, and IN-F5438 were detected using negative ion mode. Two parent-to-daughter ion transitions of each analyte were monitored as listed in the table below.

Method validation was accomplished by analyzing the analytes in validation sets consisting of 2 blank control specimens, 5 replicate specimens fortified at the LOQ, and 5 replicate specimens fortified at 10xLOQ.

3.6 *Modifications, Interpretations, and Critical Steps*

The analytical method was run exactly as written except for the following:

1. A Shimadzu HPLC was used instead of Agilent HPLC. An AB SCIEX Triple Quad 5500 was used instead of an API 5000 triple quad.
2. In the additional trial of ground water, the SPE column was conditioned with pH 3 water instead of neutral water in order to minimize matrix suppression for the analyte IN-B5067. In the additional trial of surface water, the SPE extraction was subsequently modified by adding a wash step with pH 3 water in order to minimize matrix suppression for the analytes IN-B5067 IN-NC148, and IN-F5438.

The substitutions were demonstrated to be equivalent to the equipment specified in the method and did not impact the analytical results.

3.7 Instrumentation

HPLC Conditions

System:	Shimadzu LC-20AD / Sil-20AC Autosampler			
Column:	Polaris C18-A 3 µm 2 x150 mm			
Column Temperature:	8°C			
Injection Volume:	25 µL			
Autosampler Temperature:	4°C			
Conditions:	A: 0.02 M FA in H_2O			
	B: Methanol			
	Flow in mL/minute			
	Time	%A	%B	Flow
	0.0	98	2	0.400
	1.0	98	2	0.400
	3.5	70	30	0.400
	12.0	20	80	0.400
	12.5	2	98	0.400
	15.0	2	98	0.400
	15.2	98	2	0.400
	25.0	STOP		

Analyte Retention Times (minutes)	
IN-A4098	~4.2
IN-D5119	~6.8
IN-00581	~7.5
IN-NC148	~8.5
IN-B5685	~8.7
IN-B5067	~9.8
IN-F5438	~10.3
DPX-T6376	~12.0

The detection method utilized was LC-MS/MS employing atmospheric pressure electrospray ionization interface in both the positive and negative mode on a triple quadrupole instrument. The acquisition method was adjusted to maximize the response of the fragment ions detected. The ion transitions for metsulfuron methyl and metabolites are shown below:

SYSTEM:	AB SCIEX TRIPLE QUAD 5500			
ANALYTES	**IONS MONITORED**	**DECLUSTERING POTENTIAL (DP)**	**COLLISION ENERGY (CE)**	**EXIT POTENTIAL (CXP)**
IN-A4098	141.0 → 57.3 AMU	90	25	14
	141.0 → 43.0 AMU	90	25	14
Ion Mode:	Positive			
Turbo Spray Voltage:	4500 V			
Source Temperatures:	500 ^{0}C			
CUR:	30 psig			
CAD:	8			
GS1:	50 psig			
GS2:	50 psig			
Dwell:	150 ms			

SYSTEM:	AB SCIEX TRIPLE QUAD 5500			
ANALYTES	IONS MONITORED	DECLUSTERING POTENTIAL (DP)	COLLISION ENERGY (CE)	EXIT POTENTIAL (CXP)
IN-D5119	199.9 → 91.9 AMU	-60	-30	-14
	199.9 → 155.9 AMU	-60	-15	-14
IN-00581	182.1 → 42.0 AMU	-120	-56	-14
	182.1 → 105.7 AMU	-120	-28	-14
IN-NC148	342.0 → 181.9 AMU	-70	-30	-14
	342.0 → 42.1 AMU	-70	-90	-14
IN-B5685	257.3 → 42.0 AMU	-55	-70	-14
	257.3 → 182.0 AMU	-55	-30	-14
IN-B5067	366.1 → 125.1 AMU	-70	-20	-14
	366.1 → 42.1 AMU	-70	-70	-14
IN-F5438	366.1 → 155.9 AMU	-70	-28	-14
	366.1 → 200.3 AMU	-70	-20	-14
DPX-T6376	380.6 → 138.8 AMU	-80	-20	-14
	380.6 → 107.1 AMU	-80	-50	-14
Ion Mode:	Negative			
Turbo Spray Voltage:	-4500 V			
Source Temperatures:	500 ^{0}C			
CUR:	30 psig			
CAD:	8			
GS1:	50 psig			
GS2:	50 psig			
Dwell:	150 ms (50 ms for IN-D5119)			

The instrument was operated in the MS/MS (MRM) positive ion mode for quantitative analysis of IN-A4098 and was operated in the negative mode for the quantitative analysis of metsulfuron methyl, IN-D5119, IN-00581, IN-NC148, IN-B5685, IN-B5067, and IN-F5438. The ion chromatograms were integrated and the peak areas were used for quantitation.

For each analytical run, a six-point standard curve was prepared by injecting constant volumes of mixed standard solutions composed of each analyte of interest. Constant volume injections were used for sample extracts, as well.

3.8 Calculations

Residue metsulfuron methyl and metabolites were quantitated by external standards. A calibration curve for each analyte was generated by plotting the detector's response in peak area versus the concentration (ng/mL) of standard injected. The data system derived an equation for the fit of the standard curve with a weighted [$(1/x^2)$ where x = concentration] linear regression, and this equation was used to calculate intercept and slope of the linear regression curve.

The calibration curve was obtained by direct injection of 10 µL of standard (ranging from 0.25 ng/mL to 15 ng/mL) into the LC-MS/MS for each analyte. In a given injection run, the same injection volume was used for all samples and standards.

Peak integration and quantitation were performed using Analyst software version 1.6. Calculations of recovery results were computed for each set of samples in a Microsoft Excel® spreadsheet. The equations used for quantitation are shown below.

$$R = (C_{End} * V_F * AF) / V_s$$

Where:

R:	Analyte residue in µg/kg (ppb)
$R_{fortified}$:	Amount of analyte residue fortified in µg/kg (ppb)
C_{End}:	Final concentration of analyte derived from calibration curve in ng/mL
AF:	Aliquot factor = Total extraction volume ($V_{Total\ Ex}$) / Aliquot extraction volume ($V_{aliq\ Ex}$)
V_F:	Final volume
V_s:	Sample volume

Recoveries (Rec.) were calculated for the fortified specimens as follows:

$$Rec. = (R / R_{fortified}) \times 100\ \%$$

Example: Table 1, Sample LOQ-1, Metsulfuron Methyl, Tap Water, Fortified @ 0.05 ppb, transition ions 380.6→138.8:

Calibration curve calculated by Analyst software:

$$y = (3.47e+005)\ x + (5.39e+003)$$

Where:

y:	Peak area
x:	C_{End}, final concentration of analyte derived from calibration curve

$$C_{End} = x = (311092 - (5.39e+003)) / (3.47e+005)$$

= 0.881 ng/mL

$R_{fortified}$ = 0.05 ppb

V_F = 1.0 mL

AF = $V_{Total\ Ex}$ / $V_{aliq\ Ex}$ = 20 mL / 10 mL = 2

R = (C_{End} * V_F * AF) / V_s

= 0.881(ng/mL) * (2.5 mL) * (2) / 100 mL = 0.06315 ng/g = **0.0441 µg/kg (ppb)**

Rec. = (R / $R_{fortified}$) x 100 % = (0.0441 / 0.05) x 100% = **88%**

NOTE: Slight rounding differences may be noted when using a hand calculator. Full computer/calculator precision was used in any intermediate calculations. Only the final value was rounded.