INDEPENDENT LABORATORY VALIDATION OF DUPONT-39340 "ANALYTICAL METHOD FOR THE DETERMINATION OF SULFOMETURON METHYL AND METABOLITES IN WATER USING LC/ MS/MS"

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1.0 SUMMARY

The objective of this study was to conduct an independent laboratory validation of analytical method DuPont-39340, entitled "Analytical Method for the Determination of Sulfometuron Methyl and Metabolites in Water Using LC/MS/MS", as written. This study was designed to fulfill the requirements of U.S. EPA guidelines found in the Ecological Effects Test Guidelines: OCSPP 850.6100: Environmental Chemistry Methods and Associated Independent Laboratory Validation and the EC Directive 96/46/EC; SANCO/825/00 rev.8.1 (16/11/2010) - Guidance Document on Residue Analytical Methods.

The method under evaluation has a stated Limit of Quantitation (LOQ) of 0.10 µg/kg (ppb). In this study, the method was validated at the LOQ and 10×LOQ in water.

A single analyst completed two sample sets (one set consisting of 1 control and 5 LOQs, one set consisting of 1 control and 5 10xLOQs) in the course of an eight-hour workday (8 hours), with LC-MS/MS analysis performed unattended that same day.

During the study, strong background interference was found in metabolite IN-D5803 MRM transition state 233.2→77.1 m/z. Communication between the study monitor and the study director was therefore required before the laboratory trial to request permission to use the transition state 233.2→135.1 m/z instead of 233.2→77.1 m/z.

2.0 INTRODUCTION

To satisfy US regulatory ILV requirements, a residue analytical method must be validated at an independent laboratory prior to its submission to the appropriate regulatory authority. This study was conducted to fulfill those requirements.

The analytical method DuPont-39340 entitled "Analytical Method for the Determination of Sulfometuron Methyl and Metabolites in Water Using LC/MS/MS" is applicable for the quantitation of sulfometuron methyl (DPX-T5648) and its metabolites (IN-00581, IN-X0993 and IN-D5803) in water.

Sulfometuron methyl and its metabolites were extracted from water fortified with the analytes at LOQ (0.10 µg/kg (ppb)) and 10x LOQ by solid phase extraction. The analytes were eluted from the SPE cartridges using a basic acetonitrile (ACN) solution. An aliquot of the eluate was evaporated down to a volume of ~2 mL and 0.5 mL of 0.01 M aqueous ammonium acetate was added. The extracts were evaporated down to a volume of ~0.5 mL, and then a volume of 50 µL methanol was added. The extracts were then diluted to 1.0 mL with 0.01 M ammonium acetate. The purified extract was analyzed by reversed phase LC/MS/MS. Two transitions were monitored for each analyte. Both transition states of sulfometuron methyl and its metabolites, IN-X0993 and IN-D5803, were detected by positive ion MS/MS. Both transition states of the metabolite IN-00581 were detected by negative ion MS/MS.

The analytical method was designed to achieve an LOQ of 0.10 µg/kg and the Limit of Detection (LOD) was estimated to be 0.03 µg/kg. The independent validation thus evaluated recoveries of sulfometuron methyl and its metabolites, IN-00581, IN-D5803, and IN-X0993, in samples fortified at 1x and 10x the LOQ level. The method was used as written.

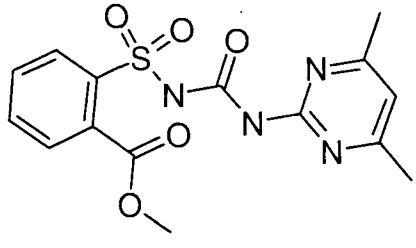
3.0 MATERIALS AND METHODS

3.1 Test Substance

The reference analytical standards (test substances) used for this study were:

DuPont code: DPX-T5648 (Sulfometuron Methyl)

Chemical Structure:



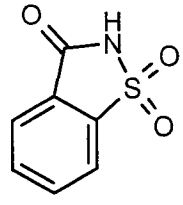
DPX-T5648

CAS Name:	Methyl 2-[[[[(4,6-dimethyl-2-pyrimidinyl)-amino]-carbonyl]amino]sulfonyl]benzoate
Molecular weight:	364.38 amu
Formula:	$C_{15}H_{16}N_4O_5S$
Source:	Du Pont
CAS Number:	74222-97-2
Batch/Lot Number:	E76835-143
Purity:	98.9%

Receipt date:	24 January, 2014
Expiration date:	21 March, 2014
Storage:	Ambient

DuPont code: IN-00581 (Saccharin)

Chemical Structure:

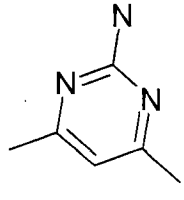


IN-00581

CAS Name:	1,2-benzisothiazol-3(2H)-one, 1,1-dioxide
Molecular weight:	183.18 amu
Formula:	$C_7H_5NO_3S$
Source:	Du Pont
CAS Number:	81-07-2
Batch/Lot Number:	07028EU
Purity:	99.8%
Receipt date:	24 January, 2014
Expiration date:	25 September, 2023
Storage:	Ambient

DuPont code: IN-X0993

Chemical Structure:



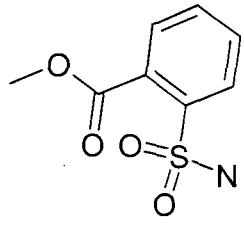
IN-X0993

Molecular weight:	123.16 amu

Formula:	$C_6H_9N_3$
Source:	Du Pont
Batch/Lot Number:	1000734
Purity:	98.0%
Receipt date:	24 January, 2014
Expiration date:	28 November, 2023
Storage:	Ambient

DuPont code: IN-D5803

Chemical Structure:



IN-D5803

Molecular weight:	215.23 amu
Formula:	$C_8H_9NO_4S$
Source:	Du Pont
Batch/Lot Number:	06807TS
Purity:	98.0%
Receipt date:	24 January, 2014
Expiration date:	30 March, 2015
Storage:	Ambient

Sulfometuron methyl, IN-00581, IN-D5803, and IN-X0993 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 water analyzed is presented in Appendix 2.

Fortifications of the samples were made using 20.0 g (±1%) of water spiked with 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 Symmetry C18 100 x 2.1 mm 3-µm column was used instead of a Phenomenex Luna C18 100 x 2.0 mm 3-µm column. A Shimadzu LC-20AD HPLC was used instead of an Agilent 1290 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 sulfometuron methyl and metabolites, as described in the method DuPont-39340. 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-39340).

Sulfometuron methyl and metabolites were extracted from 20.0 g (± 1%) of water sample. An aliquot of 10 µL concentrated formic acid was added to each sample and those requiring fortification were fortified with the appropriate standard solution and mixed thoroughly. On an SPE vacuum manifold, 1.0g/20-mL Waters Oasis HLB cartridges were conditioned with 20 mL of methanol followed by 20 mL of pH=3 water. The samples were filtered via gravity through the cartridges. The cartridges were dried using vacuum for approximately 10 minutes, and the eluate was disposed of. A volume of 20 mL of basic acetonitrile (ACN) was measured into each sample tube, and then was loaded into the SPE cartridges and placed under slight vacuum. The eluate was collected in 50 mL centrifuge tubes.

The eluate was diluted to 20 mL with basic ACN and a 10 mL aliquot was transferred to a 15 mL centrifuge tube. The extract was then evaporated under nitrogen flow in a water bath set to approximately 30°C. When the extract volume was approximately 2 mL, 0.5 mL of 0.01 M aqueous ammonium acetate was added and the extract was allowed to continue evaporating until the volume was less than 0.5 mL. The extracts were removed from the water bath and 50 µL of methanol were added to each tube. The extracts were then diluted to 1.0 mL with 0.01 M aqueous ammonium acetate.

Tubes were vortexed and all samples were transferred to HPLC injection vials via a syringe filter.

The purified final extracts were analyzed by reversed-phase HPLC using a Waters Symmetry C18 100 x 2.1 mm column with mobile phases of 0.01 M aqueous ammonium acetate and methanol. The analytes sulfometuron methyl, IN-X0993, and IN-D5803 were detected by mass spectrometry/mass spectrometry (MS/MS) in the positive ion mode and IN-00581 was detected using negative ion mode. Two parent-to-daughter ion transitions of each analyte were monitored as follows: sulfometuron methyl using 365.0→150.1 and 365.0→67.0 m/z, IN-00581 using 182.0→105.9 and 182.0→61.9 m/z, IN-D5803 using 233.2→199.0 and 233.2→135.1 m/z, and IN-X0993 using 124.1→67.0 and 124.1→107.0 m/z. The confirmatory method was based on the recovery of secondary MS/MS ion transitions.

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 Waters Symmetry C18 100 x 2.1 mm 3-µm column was used instead of a Phenomenex Luna C18 100 x 2.0 mm 3-µm column.

2. A Shimadzu HPLC was used instead of Agilent HPLC. An AB SCIEX Triple Quad 5500 was used instead of an API 5000 triple quad.

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:	Symmetry C18 2.1x100 mm
Column Temperature:	4°C
Injection Volume:	10 µL
Autosampler Temperature:	4°C
Conditions:	A: 0.01 M ammonium acetate in H_2O
	B: Methanol
	Flow in mL/minute
	Time %A %B Flow 0.0 90 10 0.500 0.3 90 10 0.500 7.0 60 40 0.500 7.1 1 99 0.500 8.5 1 99 0.500 8.6 90 99 0.500 11.0 STOP
Analyte Retention Times (minutes)	
IN-00581	~1.7
IN-X0993	~4.0
IN-D5803	~4.2
DPX-T5648	~6.0

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 sulfometuron methyl and metabolites are shown below:

SYSTEM:	AB SCIEX TRIPLE QUAD 5500			
ANALYTES	IONS MONITORED	DECLUSTERING POTENTIAL (DP)	COLLISION ENERGY (CE)	EXIT POTENTIAL (CXP)
Sulfometuron Methyl (DPX-T5648)	365.0 → 150.1 AMU	60	60	14
	365.0 → 67.0 AMU	60	60	14
IN-X0993	124.1 → 67.0 AMU	50	50	14
	124.1 → 107.0 AMU	50	50	14
IN-D5803	233.2 → 199.0 AMU	40	40	14
	233.2 → 135.1 AMU	40	40	14
Ion Mode:	Positive			
Turbo Spray Voltage:	5500 V			
Source Temperatures:	500 °C			
CUR:	30 psig			
CAD:	5			
GS1:	50 psig			
GS2:	50 psig			
Dwell:	50 ms			

SYSTEM:	AB SCIEX TRIPLE QUAD 5500			
ANALYTES	IONS MONITORED	DECLUSTERING POTENTIAL (DP)	COLLISION ENERGY (CE)	EXIT POTENTIAL (CXP)
IN-00581	182.0 → 105.9 AMU	-90	-26	-14
	182.0 → 61.9 AMU	-90	-25	-14
Ion Mode:	Negative			
Turbo Spray Voltage:	-4500 V			
Source Temperatures:	500 °C			
CUR:	30 psig			
CAD:	5			
GS1:	50 psig			
GS2:	50 psig			
Dwell:	150 ms			

The instrument was operated in the MS/MS (MRM) positive ion mode for quantitative analysis of sulfometuron methyl, IN-X0993, and IN-D5803 and was operated in the negative mode for the quantitative analysis of IN-00581. 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 sulfometuron 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 0.5 ng/mL to 25 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 Applied Biosystems' 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.

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

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
W:	Water sample weight: 20 g

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

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

Example: Table 1, Sample LOQ-1, Sulfometuron Methyl, Tap Water, Fortified @ 0. 1 ppb, transition ions 365.0→150.1:

Calibration curve calculated by Analyst software:

$y = 1.19e+006\ x - 2.75e+003$

Where:

y: Peak area

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

$C_{End} = x = (1268929 + 2.75e+003) / 1.19e+006$

= 1.073 ng/mL

$R_{fortified} = 0.1$ ppb

$V_F = 1.0$ mL

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

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

$= 1.073\ (ng/mL) * (1.0\ mL) * (2) / 20\ g = 0.1073\ ng/g =$ **0.1073 µg/kg (ppb)**

$Rec. = (R / R_{fortified}) \times 100\ \% = (0.1073 / 0.1) \times 100\% =$ **107%**

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.