ANALYTICAL METHOD FOR THE DETERMINATION OF SULFOMETURON METHYL AND METABOLITES IN WATER USING LC/MS/MS

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1.0 SUMMARY

The purpose of this study was to develop an analytical method for the detection, confirmation, and quantitative analysis of sulfometuron methyl and metabolites (IN-00581, IN-X0993, and IN-D5803) in water. Sulfometuron methyl and metabolites were extracted from water samples using a solid phase extraction (SPE) cartridge. The analytes were eluted from the SPE cartridge using a solution of acetonitrile. An aliquot of the eluate was evaporated to a volume of approximately 2-ml and a 0.5-mL aliquot of 0.01 M aqueous ammonium acetate was added. The extracts were evaporated further until only the aqueous phase remained. The extracts were diluted to 1.0 mL using 0.01 M aqueous ammonium acetate. Sulfometuron methyl and metabolites were separated from co-extracts by reversed phase liquid chromatography (LC) and detected using turbospray mass spectrometry/mass spectrometry (MS/MS). The Limit of Quantitation (LOQ) was 0.10 µg/kg (ppb). The Limit of Detection (LOD) was estimated to be 0.03 µg/kg (ppb).

2.0 INTRODUCTION

The structure and molecular weight of sulfometuron methyl and metabolites can be found in Appendix 1. The method was validated on three types of water.

Sulfometuron methyl and metabolites were extracted from water samples using a Solid Phase Extraction (SPE) cartridge. The analytes were eluted from the SPE cartridge using a solution of acetonitrile. An aliquot of the eluate was evaporated to a volume of approximately 2-ml and a 0.5-mL aliquot of 0.01 M aqueous ammonium acetate was added. The extracts were evaporated further until only the aqueous phase remained. The extracts were diluted to 1.0 mL using 0.01 M aqueous ammonium

acetate. All samples extracts were analyzed using turbospray ionization and LC/MS/MS

The Limit of Quantitation (LOQ) was 0.10 µg/kg (ppb). The Limit of Detection (LOD) was estimated to be 0.03 µg/kg. Validation data is presented for the quantitation and confirmation ion transitions.

3.0 MATERIALS

Equivalent equipment and materials may be substituted unless otherwise specified. Note any specification 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

Instrumentation

LC system, Agilent 1290 with temperature controlled autosampler (Agilent Technologies, Wilmington, DE)

Mass Spectrometer System, API 5000 triple quadrupole mass spectrometer using a Turbo Ion Spray (Applied Biosystems/ Sciex, Foster City, CA)

Biohit Proline Electronic Pipettors, Variable Volume with Tip Ejector, Vanguard, 5.0-100 µL Cat. No. 53495-200, 50-1000 µL Cat. No. 53495-205 and 0.10-5.0 mL Cat. No. 53495-290 (VWR Scientific Co., Bridgeport, NJ)

Chromatographic Supplies

HPLC Column: 2.0 mm i.d. × 100 mm, 3µ packing , Phenomenex C-18 (2) analytical column Part # 000-4251-B0 (Torrance, CA)

HPLC Vials, Target DP Amber Kit, T/S/T Septa, 100 PK, Part # 5182-0556 (Agilent Technologies, Wilmington, DE)

Labware

Pyrex Brand Single Metric Scale Graduated Cylinders, 10-mL and 100-mL capacity, Cat. No. 24709-715 and 24709-748, respectively (VWR Scientific Co., Bridgeport, NJ)

VWR brand Disposable Pasteur Pipettes, Borosilicate Glass, 9 in, Cat. No. 53283-914 equipped with 2 mL, 13 X 32 mm rubber bulbs, Cat. No. 56310-240 (VWR Scientific Co., Bridgeport, NJ)

Centrifuge tubes, Polystyrene 50-mL capacity, Cat. No. 21008-939 (VWR Scientific Co., Bridgeport, NJ)

Centrifuge tubes, Polystyrene 15-mL capacity, Cat. No. 21008-930 (VWR Scientific Co., Bridgeport, NJ)

Solid-Phase Extraction Equipment

Visiprep 12 port SPE vacuum manifold, PN 5-7030 (Supelco, Bellefonte, PA)

Waters Oasis™ HLB cartridge, 1.0g/ 20 mL, PN 186000117 (Milford, MA).
Do not substitute.

Miscellaneous

6 Port Electrically Actuated Valve, Valco Instruments Co. Inc., PN 1384 (Alltech, Deerfield, IL)

VWR brand Vortex Geni 2 Mixer, Cat. No. 58815-178 (VWR Scientific Co., Bridgeport, NJ)

3.2 Reagents and Standards

Equivalent reagents may be substituted for those listed below. To determine if impurities in substituted reagents interfere with analyses, appropriate amounts of the solvents should be taken through the entire method using the chromatographic conditions specified in this report.

Acetonitrile - EM Omni Solv®, HPLC-grade acetonitrile , #AX0142-1 (EM Science, Gibbstown, NJ)

Ammonium Acetate - #AX12201-1 (EM Science, Gibbstown, NJ)

Ammonium Hydroxide Solution - 28-30%, #AX-1303-13 (EM Science, Gibbstown, NJ)

Formic Acid - Guaranteed Reagent 98% minimum, #FX0440-5 (EM Science, Gibbstown, NJ)

Methanol - EM Omni Solv®, HPLC-grade methanol, #MX0488-1 (EM Science, Gibbstown, NJ)

Water - EM Omni Solv®, HPLC-grade water, #WX0004-1 (EM Science, Gibbstown, NJ)

IN-00581-006, Purity 99%, purchased from Aldrich Chemical and supplied by DuPont Crop Protection, Global Technology Division, E. I. du Pont de Nemours and Company

IN-X0993-007, Purity 98%, prepared by DuPont Crop Protection, Global Technology Division, E. I. du Pont de Nemours and Company

IN-D5803-007, Purity 98.1%, prepared by DuPont Crop Protection, Global Technology Division, E. I. du Pont de Nemours and Company

Sulfometuron methyl – DPX-T5648-009, Purity 98.9%, prepared by DuPont Crop Protection, Global Technology Division, E. I. du Pont de Nemours and Company

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 used. An MSDS sheet for the analytes is available from DuPont Crop Protection, Global Technology Division, E. I. du Pont de Nemours and Company.

4.0 METHOD

4.1 Principles of the Analytical Method

Sulfometuron methyl and metabolites were extracted from water samples using a SPE cartridge. The analytes were eluted from the cartridge using acetonitrile solution. An aliquot of the extracts underwent solvent exchange into 0.01 M aqueous ammonium acetate. The extracts were analyzed using turbospray ionization and LC/MS/MS

4.2 Analytical Procedure

4.2.1 Glassware and Equipment Cleaning

Glassware should be scrubbed with a brush using a laboratory soap solution, rinsed two to five times with tap water, rinsed with distilled or deionized water and finally rinsed with acetone or another suitable solvent and allowed to air dry prior to each use.

4.2.2 Preparation of Solutions

The following solutions should be prepared monthly and stored at room temperature unless stated otherwise:

Mobile Phase A : 0.01M aqueous ammonium acetate

0.01 M Aqueous Ammonium Acetate : Add 0.77g of ammonium acetate to 900 mL of HPLC grade water. Mix the resulting solution to homogeneity and dilute to 1000 mL with HPLC grade water.

pH=3 Water : - Add 10 µL of concentrated formic acid to 200 mL of the HPLC grade water and mix the resulting solution to homogeneity. This solution may be prepared weekly. The pH of the water was not adjusted.

1.0 M ammonium hydroxide : Add 6.9 mL of ammonium hydroxide solution (28-30% NH_3) to a volume of 93.1 mL of EM Science water. Mix the resulting solution to homogeneity.

Basic Acetonitrile : - Add 20 mL of 1.0 M ammonia hydroxide to 980 mL of the acetonitrile volume and mix the resulting solution to homogeneity. This solution may be prepared monthly.

4.2.3 Preparation and Stability of Stock Standard

Use Class A volumetric flasks when preparing standard solutions.

Prepare standard stock solutions for IN-00581, IN-X0993, IN-D5803 and sulfometuron methyl by accurately weighing 10 ± 0.01 mg into individual 100-mL

volumetric flask using an analytical balance. Record the accurate weight of the standard. Dissolve the standards in approximately 50 mL of HPLC-grade methanol. After dissolving, bring the solution to a volume of 100 mL using HPLC-grade methanol and invert the volumetric flask to mix the solution to homogeneity. The standard solutions are stable for approximately 6 months when stored in a freezer at approximately -20°C immediately after each use. The concentration of each analyte in solution is 100 µg/mL.

4.2.4 *Preparation and Stability of Intermediate and Fortification Standards*

Use Class A volumetric flasks when preparing standard solutions.

Prepare a 1.0-µg/mL IN-00581, IN-X0993, IN-D5803 and sulfometuron methyl intermediate standard in methanol by pipetting 1.00 mL of each 100.0-µg/mL stock standard into a 100-mL volumetric flask. Dilute the standard to approximately 50 mL with methanol and mix to homogeneity. Bring to volume using with methanol and mix to homogeneity.

Prepare a 0.10-µg/mL IN-00581, IN-X0993, IN-D5803 and sulfometuron methyl standard in methanol by pipetting 1.00 mL of the 1.0-µg/mL standard into a 10-mL volumetric flask. Dilute the standard to approximately 5 mL with methanol and mix to homogeneity. Bring to volume using with methanol and mix to homogeneity.

Alternate or additional solutions may be prepared as needed. All standard solutions prepared in with methanol are stable for approximately 6 months if stored in a freezer at approximately -20°C immediately after each use.

4.2.5 *Preparation and Stability of Calibration Standards*

Prepare the calibration standards as showed in the table below. (Alternative or additional standards may be prepared as needed):

Standard Used	Volume Transferred (µL)	Volume of 0.01 M Aqueous Ammonium Acetate added (µL)	Final Concentration (ng/mL)
1.0 µg/mL	25	975	25.0
1.0 µg/mL	10	990	10.0
0.10 µg/mL	50	950	5.0
25.0 ng/mL	100	900	2.5
10.0 ng/mL	100	900	1.0
5.0 ng/mL	100	900	0.50

During method validation these standard solutions were freshly prepared with each sample set and stored approximately 4°C prior to use. The standards have shown stability for 2 weeks. Each of the calibration standards was vortex mixed for 30 seconds prior to placing them in the auto-sampler.

4.2.6 *Source of Samples*

Water control samples were obtained from local supplies. The water characterizations are provided in Appendix 4.

4.2.7 *Storage and Preparation of Samples*

All samples should be stored frozen at approximately -20°C. All samples were allowed the completely thaw before subsampling. No additional purification was performed prior to sample processing.

4.2.8 *Sample Fortification Procedure*

Fortifications were made directly to the 20.0-g water sample after weighing the sample. Fortified samples were prepared using a 0.10-µg/mL and a 1.0-µg/mL standard solution.

FORTIFICATION LEVEL (µG/KG)	STANDARD USED	VOLUME OF STANDARD (ML)
0.10	0.10 µg/mL	0.020
1.0	1.0 µg/mL	0.020

4.2.9 *Analysis of Water Samples*

1. Accurately measure 20.0-g (± 1%) of water into a 50-mL plastic centrifuge tubes, add 1.0 µL of concentrated formic acid to each sample. Fortify samples if necessary, cap and shake the samples vigorously.
2. Place 1.0g/20-mL Waters Oasis HLB cartridges on a SPE vacuum manifold. Condition the cartridges with 20 mL of methanol followed by 20 mL of pH=3 water. Do not allow the cartridges to go to dryness.
3. Using gravity flow, filter the samples through the cartages allowing the eluate to go to waste. Once the sample has completely passed through the cartridges use vacuum for 10-minutes to remove any remaining water.
4. Place a 50-mL centrifuge tube under each SPE cartridge. Measure 20 mL of basic acetonitrile into each sample tube. Load the acetonitrile into the SPE cartridges collecting the eluate. Once all of the acetonitrile has passed through the cartridges use slight vacuum to empty the solution into the collection tubes.
5. Remove the collection tube from the SPE manifold and dilute the volume to 20 mL using basic acetonitrile. Transfer a 10-mL aliquot of each extract into a 15-mL centrifuge tube. Using a flow of nitrogen and a water bath set to 25-30°C evaporate the extracts to approximately 2 mL. Add 0.5 mL of 0.01 M aqueous ammonium acetate and continue evaporating the extracts until the volume is less than 0.5 mL.

6. Add 0.050 mL of methanol to each extract and dilute to 1.0 mL using 0.01 M aqueous ammonium acetate. Thoroughly mix the extracts using a vortex mixer. Using a syringe filter transfer an aliquot of each extract into an auto-sampler vial and analyze using LC/MS/MS.

Extracts will be stable for approximately 2 days if stored at 8°C.

4.3 Instrumentation for the Method

4.3.1 Chromatography

Reversed-phase chromatography was used to separate sulfometuron methyl and its metabolites from co-extracts. A Phenomenex C18 (2) column was selected. Alternative chromatographic conditions can be used, provided the analytical method is validated and provides acceptable recoveries as defined by regulatory method guidelines.

SYSTEM:	Agilent 1290 HPLC			
COLUMN:	2.0 mm i.d. × 100 mm, Phenomenex C18 (2)			
COLUMN TEMPERATURE:	40°C			
SAMPLE TEMPERATURE	4°C			
INJECTION VOLUME:	0.010 mL			
FLOW RATE:	0.500 mL/min			
CONDITIONS:	A: 0.01M aqueous ammonium acetate			
	B: Methanol			
	Time	%A	%B	Flow (mL/Min.)
	0.0	90	10	0.50
	0.3	90	10	0.50
	7.0	60	40	0.50
	7.1	1	99	0.50
	8.5	1	99	0.50
	8.6	90	10	0.50
IN-00581 RETENTION TIME:	1.7 minutes			
IN-X0993 RETENTION TIME:	4.0 minutes			
IN-D5803 RETENTION TIME:	4.2 minutes			
DPX-T5648 RETENTION TIME:	6.0 minutes			
TOTAL RUN TIME:	11.0 min			

A six-port electronically activated switching valve was used to direct the flow to waste prior to and following the elution of the compounds of interest. The use of this valve reduces source contamination and enables additional samples to be analyzed prior to source cleaning. The valve switching times are given in the following table.

TIME (MINUTES)	COLUMN ELUATE FLOW
0.0-1.0	Waste
1.0-7.0	MS source
7.0-End	Waste

4.3.2 *LC/MS/MS Analysis*

The quantitative analysis of sulfometuron methyl and metabolites was performed using an Applied Biosystem API 5000 LC/MS/MS system. Quantitative analysis was based on the integration of a single ion transition. A summary of the experimental conditions is provided in the following table:

PERIOD 1 ANALYTES	IONS MONITORED	DECLUSTERING POTENTIAL (DP)	COLLISION ENERGY (CE)	EXIT POTENTIAL (CXP)
IN-00581	**182.0→ 105.9 AMU**	**-90**	**-26**	**-9**
	182.0→ 61.9 AMU	-90	-28	-9
Time:	0-2.5 minutes			
Ion Mode:	Negative			
Turbospray Voltage:	-4500 V			
Source Temperatures:	600°C			
CUR:	30			
CAD:	4			
GS1:	40			
GS2:	50			
Dwell	0.15 Seconds			
PERIOD 2 ANALYTE	**IONS MONITORED**	**DECLUSTERING POTENTIAL (DP)**	**COLLISION ENERGY (CE)**	**EXIT POTENTIAL (CXP**
IN-X0993	**124.1→ 67.0 AMU**	**76**	**39**	**12**
	124.1→ 107.0 AMU	76	27	18
IN-D5803	**233.2→ 199.0 AMU**	**56**	**17**	**14**
	233.2→ 77.1 AMU	56	61	12
Sulfometuron methyl	**365.0→ 150.1 AMU**	**36**	**25**	**26**
	365.0→ 67.0 AMU	36	95	12
	365.0→ 77.0 AMU	36	77	14
Time:	2.5-11 minutes			
Ion Mode:	Positive			
Turbospray Voltage:	5500 V			
Source Temperatures:	600°C			
CUR:	30			
CAD:	4			
GS1:	40			
GS2:	50			
Dwell	0.15 Seconds			

A complete list of the experimental parameters is given in Appendix 3. A typical LC/MS and LC/MS/MS full scan spectrum of IN-00581, IN-X099, IN-D5803 and sulfometuron methyl is shown in Figure 1. Peak area was used for quantitation. **Quantitation was performed using the ion transition displayed in bold face print.**

For the analysis of IN-D5803 ammonia adducts were used instead of the protonated compound. In the electrospray ion source IN-D5803 did not readily add a proton, however it did readily add NH_4^+. To aid in the addition of NH_4^+ to IN-D5803, 0.01 M aqueous ammonia acetate was used as the mobile phase. The other transitions were used to confirm any detected residues.

4.3.3 *Calibration Procedure and Sample Analysis*

A 0.50-ng/mL chromatographic standard should be analyzed for sulfometuron methyl prior to the start of analyses to establish that the instrument is working properly. If a signal-to-noise ratio of approximately 5-10 to 1 is not attained, the instrument must be tuned or cleaned prior to sample analysis. Operating parameters must be tailored to the particular instrument used, especially if it is to be an alternate vendor's instrument, and should be checked daily. Note that some ion channels other than those used for development of this method may need to be added or eliminated when utilizing this method on other instrumentation. Each ion channel used for sample analysis/quantitation must be checked to insure it is free of interference. The control will be used to demonstrate that baseline interference is less than signal-to-noise 3:1. Begin each sample set by injecting a minimum of 2 calibration standards. The first injection should always be disregarded.

4.4 Calculations

4.4.1 *Methods*

Average Response Factor (RF_{Avg}) was calculated as follows:

$$RF_{Ave} = \frac{(\text{Conc. A} \div \text{Area A}) + (\text{Conc. B} \div \text{Area B}) + (\text{Conc. C} \div \text{Area C}) + (\text{Conc. D} \div \text{Area D}) + (\text{Conc. E} \div \text{Area E})}{\text{Total Number of Standards Injected}}$$

µg/g (ppm) found was calculated as follows:

$$\text{ppm Found} = \frac{(\text{Peak Area}) \times (RF_{Ave}) \times (\textit{Aliquot}\ \text{Factor}) \times (\text{Final Volume})}{(\text{Sample Weight})}$$

In the event a peak was detected in the control, a corrected peak area was used to calculate ppm found for freshly fortified samples. The corrected peak area is the area of the fortified sample minus the area of the control sample.

The percent recovery found was calculated as follows:

$$\%\ \text{Recovery} = \frac{\mu\text{g/kg Found}}{\mu\text{g/kg Fortified}} \times \frac{100}{1}$$

4.4.2 *Example*

For a surface water sample fortified with sulfometuron methyl at 0.00010 ppm [Date analyzed 15-Oct-13, LOQ 1], the concentration found was calculated as follows:

Average Response Factor was calculated as follows:

$$RF_{Ave} = \frac{(0.50\ ng/mL \div 553000AC) + (1.0\ ng/mL \div 1170000\ AC) + (2.5\ ng/mL \div 2670000AC) + (5.0\ ng/mL \div 5100000\ AC) + (10.0\ ng/mL \div 10400000\ AC) + (25.0\ ng/mL \div 20200000\ AC)}{6}$$

(AC ≡ Area Counts)

$RF_{Avg} = 9.79124\ e^{-7}$ ng/mL/AC

ng/g (ppb) found was calculated as follows:

$$\text{ppb Found} = \frac{(1050000\ AC - 5480\ AC) \times (9.79124e-7\ ng/mL/AC) \times 2 \times 1.0\ mL)}{(20\ g)}$$

ng/g Found = 0.102 = 0.102 µg/kg

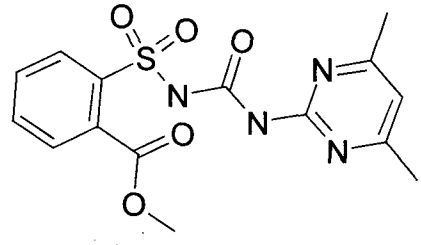
The percent recovery found was calculated as follows:

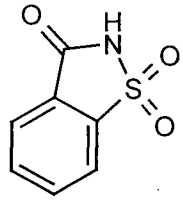
$$\%\ \text{Recovery} = \frac{0.102\ \mu g/kg}{0.10\ \mu g/kg} \times \frac{100}{1}$$

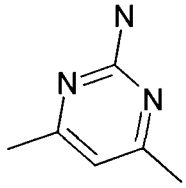
Recovery = 102%

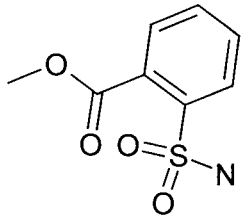
(percent recoveries are rounded to the nearest whole number in Table, without rounding the concentration found)

APPENDIX 1 STRUCTURE OF SULFOMETURON METHYL AND METABOLITES

Common Name	Sulfometuron methyl
Structure	
DPX Number	DPX-T5648
Trade Name	Oust
CAS Chemical Name	Methyl 2-[[[[(4,6-dimethyl-2-pyrimidinyl)-amino]-carbonyl]amino]sulfonyl]benzoate
CAS Number	74222-97-2
Formula	$C_{15}H_{16}N_4O_5S$
Molecular Weight	364.38
Monoisotopic Weight	364.08
pKa	5.2

Common Name	Saccharin
Structure	
DPX Number	IN-00581
CAS Chemical Name	1,2-benzisothiazol-3(2H)-one, 1,1-dioxide
CAS Number	81-07-2
Formula	$C_7H_5NO_3S$
Molecular Weight	183.1845
Monoisotopic Weight	182.9990

Common Name	None
Structure	
DPX Number	IN-X0993
Formula	$C_6H_9N_3$
Molecular Weight	123.1558
Monoisotopic Weight	123.0796

Common Name	None
Structure	
DPX Number	IN-D5803
Formula	$C_8H_9NO_4S$
Molecular Weight	215.2264
Monoisotopic Weight	215.2052

APPENDIX 3 EXPERIMENTAL CONDITIONS

File Information for Sample 7 (LOQ 1Water) of 10152013T5648andMetsWhiteClayCreekWaterVal1Plastic.wiff

File Name: 10152013T5648andMetsWhiteClayCreekWaterVal1Plastic.wiff

File Path: D:\Analyst Data\Projects\02282013DpxT5648andMetabolites\2013_02_28\Data\

Original Name: 10152013T5648andMetsWhiteClayCreekWaterVal1Plastic.wiff

Software Version: Analyst 1.5.2

Log Information from Devices at Start of acquisition:

Pump		Agilent 1290 G4220A	
Firmware Version	B.06.53		
Serial Number	DEBAA03467		
Time from start =0.0000 min	AutoSampler	Agilent 1290 G4226A	
Firmware Version	A.06.50		
Serial Number		DEBAP04093	
Linked Pump	G4220A	DEBAA03467	
Injection Volume used	10.00 µl		
Time from start =0.0000 min	Column Oven	Agilent 1290 G1316C	
Firmware Version	A.06.53		
Serial Number		DEBAC06281	
Switching Valve	CSV	SN#	0030969185
Time from start =0.0000 min	AutoSampler	Agilent 1290 G4226A	
Start of Run -		Temperature	

Tray Temperature	5.00 C	
Time from start =0.0000 min	Mass Spectrometer	API 5000
Config Table Version	01	
Firmware Version	M401402 B4T0301 M3L1417 B3T0300	
Component Name	Triple Quadrupole LC/MS/MS Mass Spectrometer	
Component ID		API 5000
Manufacturer		AB Sciex Instruments
Model		API 5000
Serial Number	AG13130610	
Time from start =0.0000 min	Mass Spectrometer	API 5000
Start of Run -		Detailed Status
Vacuum Status	At Pressure	
Vacuum Gauge (10e-5 Torr)	1.9	
Backing Pump		Ok
Interface Turbo Pump	Normal	
Analyzer Turbo Pump	Normal	
Sample Introduction Status	Ready	
Source/Ion Path Electronics	On	
Source Type		Turbo Spray
Source Temperature (at setpoint)		600.0 C
Source Exhaust Pump	Ok	
Interface Heater	Ready	

Acquisition Info

Acquisition Method:	\10082013T5648SoilMethodKinetex.dam
Acquisition Path:	D:\Analyst Data\Projects\02282013DpxT5648andMetabolites\2013_02_28\Acquisition Methods\
First Sample Started:	Tuesday, October 15, 2013 1:10:47 PM
Last Sample Finished:	Tuesday, October 15, 2013 5:40:24 PM
Sample Acq Time:	Tuesday, October 15, 2013 2:21:07 PM
Sample Acq Duration:	11min0sec
Number of Scans:	0
Periods in File:	2
Batch Name:	\10152013T5648andMetsWhiteClayCreekWaterVal1Plastic.dab
Batch Path:	D:\Analyst Data\Projects\02282013DpxT5648andMetabolites\2013_02_28\Batch\
Logged-on User:	S3151244LCMS1@dupontnet.net
Synchronization Mode:	LC Sync
Auto-Equilibration:	Off
Software Version:	Analyst 1.5.2
Set Name:	10152013T5648andMetsWhiteClayCreekWaterVal1Plastic
Sample Name	LOQ 1Water
Autosampler Vial:	22
Rack Code:	10 By 10
Rack Position:	1
Plate Code:	N/A
Plate Position	0

Agilent LC Pump Method Properties

Pump Model:		Agilent 1290 Binary Pump
Minimum Pressure (psi):	0.0	
Maximum Pressure (psi):	17404.0	
Dead Volume (µl):	40.0	
Stroke Volume (µl):	-1.0	
Maximum Flow Ramp (ml/min²):		100.0
Maximum Pressure Ramp (psi/sec):		290.0
Max Flow Ramp Up (ml/min²):		100.0
Max Flow Ramp Dn (ml/min²):		100.0

Step Table:

Step	Total Time(min)	Flow Rate(µl/min)	A (%)	B (%)
0	0.00	500	90.0	10.0
1	0.30	500	90.0	10.0
2	7.00	500	60.0	40.0
3	7.10	500	1.0	99.0
4	8.50	500	1.0	99.0
5	8.60	500	90.0	10.0
6	11.00	500	90.0	10.0

Left Stroke Volume (µl):	-1.0
Right Stroke Volume (µl):	-1.0
Left Solvent:	A1
Right Solvent:	B1

Agilent Autosampler Properties

Autosampler Model:	Agilent 1290 Infinity Autosampler
Syringe Size (µl):	20
Injection Volume (µl):	10.00
Draw Speed (µl/min):	100.0
Eject Speed (µl/min):	200.0
Needle Level (mm):	0.00
Temperature Control	Enabled
Setpoint (4 - 40 C):	5

Wash is not used

Automatic Delay Volume Reduction	Not Used	
Equilibration Time (sec): 2		
Enable Vial/Well Bottom Sensing	No	
Use Custom Injector Program	Yes	

Contents of Custom Injector Program

1: DRAW def. amount from sample	def. speed	def. offset
2: INJECT		
3: WAIT 1.00 min.		
4: CONTACT A CLOSED		
5: WAIT 0.10 min.		
6: CONTACT A OPEN		
7: WAIT 6.00 min.		
8: CONTACT B CLOSED		

9: WAIT 0.10 min.

10: CONTACT B OPEN

Agilent Column Oven Properties

Left Temperature (°C):	40.00	
Right Temperature (°C):	40.00	
Temperature Tolerance +/- (°C):		1.00
Start Acquisition Tolerance +/- (°C):		0.50
Time Table		(Not Used)
Column Switching Valve	Installed CSV	SN#: 0030969185
Position for first sample in the batch:		Right

Use same position for all samples in the batch

Quantitation Information:

Sample Type: Unknown

Dilution Factor:1.000000

Period 1:

Scans in Period: 324

Relative Start Time: 0.00 msec

Experiments in Period: 1

Period 1 Experiment 1:

Scan Type: MRM (MRM)

Scheduled MRM: No

Polarity:	Negative	
Scan Mode:	N/A	
Ion Source:	Turbo Spray	
Resolution Q1:	Unit	
Resolution Q3:	Unit	
Intensity Thres.:	0.00 cps	
Settling Time:	0.0000 msec	
MR Pause:	5.0070 msec	
MCA:		No
Step Size:	0.00 Da	

Q1 Mass (Da)	Q3 Mass (Da)	Dwell(msec)	Param	Start	Stop	ID
182.001	105.916	150.00	CE	-26.00	-26.00	IN-00581
			CXP	-9.00	-9.00	

Q1 Mass (Da)	Q3 Mass (Da)	Dwell(msec)	Param	Start	Stop	ID
182.001	42.047	150.00	CE	-42.00	-42.00	IN-00581
			CXP	-7.00	-7.00	

Q1 Mass (Da)	Q3 Mass (Da)	Dwell(msec)	Param	Start	Stop	ID
182.001	61.995	150.00	CE	-28.00	-28.00	IN-00581
			CXP	-9.00	-9.00	

Parameter Table(Period 1 Experiment 1)

CAD:	4.00
CUR:	30.00
GS1:	40.00
GS2:	50.00
ihe:	ON
IS:	-4500.00
TEM:	600.00
DP	-90.00
EP	-10.00

Period 2:

Scans in Period:	219
Relative Start Time:	2.51 min
Experiments in Period:	1

Period 2 Experiment 1:

Scan Type:	MRM (MRM)
Scheduled MRM:	No
Polarity:	Positive
Scan Mode:	N/A
Ion Source:	Turbo Spray
Resolution Q1:	Unit
Resolution Q3:	Unit
Intensity Thres.:	0.00 cps
Settling Time:	700.0000 msec

MR Pause:	5.0070 msec	
MCA:		No
Step Size:	0.00 Da	

Q1 Mass (Da)	Q3 Mass (Da)	Dwell(msec)	Param	Start	Stop	ID
124.100	67.014	150.00	DP	76.00	76.00	IN-X0993
			CE	39.00	39.00	
			CXP	12.00	12.00	

Q1 Mass (Da)	Q3 Mass (Da)	Dwell(msec)	Param	Start	Stop	ID
124.100	107.028	150.00	DP	76.00	76.00	IN-X0993
			CE	27.00	27.00	
			CXP	18.00	18.00	

Q1 Mass (Da)	Q3 Mass (Da)	Dwell(msec)	Param	Start	Stop	ID
238.128	23.000	150.00	DP	76.00	76.00	IN-D5803
			CE	39.00	39.00	
			CXP	10.00	10.00	

Q1 Mass (Da)	Q3 Mass (Da)	Dwell(msec)	Param	Start	Stop	ID
233.222	199.000	150.00	DP	56.00	56.00	In-D5803
			CE	17.00	17.00	
			CXP	14.00	14.00	

Q1 Mass (Da)	Q3 Mass (Da)	Dwell(msec)	Param	Start	Stop	ID
233.222	77.152	150.00	DP	56.00	56.00	IN-D5803
				CE	61.00	61.00
				CXP	12.00	12.00
Q1 Mass (Da)	Q3 Mass (Da)	Dwell(msec)	Param	Start	Stop	ID
365.104	150.086	150.00	DP	36.00	36.00	DPX-T5648
				CE	25.00	25.00
				CXP	26.00	26.00
Q1 Mass (Da)	Q3 Mass (Da)	Dwell(msec)	Param	Start	Stop	ID
365.104	67.097	150.00	DP	36.00	36.00	DPX-T5648
				CE	95.00	95.00
				CXP	12.00	12.00
Q1 Mass (Da)	Q3 Mass (Da)	Dwell(msec)	Param	Start	Stop	ID
365.104	77.161	150.00	DP	36.00	36.00	DPX-T5648
				CE	77.00	77.00
				CXP	14.00	14.00

Parameter Table(Period 2 Experiment 1)

CAD:	4.00
CUR:	30.00
GS1:	40.00
GS2:	50.00
ihe:	ON
IS:	5500.00
TEM:	600.00
EP	10.00

Resolution tables

Quad 1	Positive	Unit
Last Modification Date Time: August 19, 2013 16:51:28		
IE1	0.500	
Quad 1	Negative	Unit
Last Modification Date Time: October 22, 2012 10:40:33		
IE1	-0.600	
Quad 3	Positive	Unit
Last Modification Date Time: August 19, 2013 16:51:28		
Quad 3	Negative	Unit
Last Modification Date Time: January 24, 2012 09:46:46		
IE3	0.000	

Calibration tables

Quad 1 Positive Unit Resolution

Last Modification Date Time: October 22, 2012 10:27:30

Quad 1 Negative Unit Resolution

Last Modification Date Time: October 22, 2012 10:39:46

Quad 3 Positive Unit Resolution

Last Modification Date Time: October 22, 2012 10:30:18

Quad 3 Negative Unit Resolution

Last Modification Date Time: October 22, 2012 10:42:30

Instrument Parameters:

Detector Parameters (Positive):

CEM	2300.0
DF	-400.0

Detector Parameters (Negative):

CEM	2200.0
DF	400.0

Keyed Text:

File was created with the software version: Analyst 1.5.2