A set of 13 samples can be prepared in one 8-hour day. An LC/MS/MS analytical run, as described in the method, containing 11 calibration standards in HPLC-grade water, 11 calibration standards in matrix, and 13 samples, all injected in duplicate, can be completed in 40 hours.

II. OBJECTIVE

The purpose of this study was to perform an ILV of the PTRL Method 1870W, entitled Determination of Fluensulfone and its Metabolites in Water to satisfy guideline requirements described in the United States Environmental Protection Agency (US EPA) Ecological Effects Test Guidelines, OPPTS 850.7100, Data Reporting for Environmental Chemistry Methods and the European Commission (EC) Guidance Document on Residue Analytical Methods, SANCO/825/00 – rev. 7, dated March 20, 2004.

III. INTRODUCTION

The EPA Guideline, OPPTS 850.7100, includes a requirement for registrants to validate analytical methods for the determination of residues in water at an independent laboratory prior to submission to the EPA. The EC Guidance Document includes a requirement for registrants to provide an independent laboratory validation of methods submitted to the EC. This report details the results of the independent laboratory validation of the PTRL Method 1870W, for the determination of Fluensulfone and its metabolites in water. The study was carried out according to Study Protocol 11-0028 (EN-CAS Study # 11-0028), included as Appendix I to this report.

The independent validation trials were successful*. As described in the protocol, the validation trials consisted of separate analysis sets for each matrix. Trial 2 set, 1-02-MV (A), was planned to cover the EPA Guideline, OPPTS 850.7100 and consist of one reagent blank, one control sample not fortified with the

* 1-01-MV (A), surface water, was prepared and fortified, but not injected due both to scheduling issues and to difficulties achieving sufficent sensitivity on the LC/MS/MS system.

fluensulfone combined fortification solution, five control samples fortified with the fluensulfone combined fortification solution at LOQ (0.05 ppb) and five at 10X the LOQ (0.50 ppb). Trial 2 set, 1-01-MV, was planned to cover EC guidelines and consist of two control samples not fortified with the fluensulfone combined fortification solution, five control samples fortified with the fluensulfone combined fortification solution at LOQ (0.05 ppb) and five at 10X the LOQ (0.50 ppb).

The study was initiated on December 14, 2011 when the Study Director signed EN-CAS Protocol # 11-0028. Analytical standards were prepared per GLP guidelines from December 19, 2011 to July 16, 2012. The experimental start date was June 21, 2011 and the experimental termination date was July 18, 2012.

IV. TEST SYSTEM

Control surface water and ground water samples used in the validation study are from a previous study. The chilled control surface water sample was collected locally on March 2, 2011. The ambient control ground water sample was collected locally on April 4, 2011. The samples were assigned unique identification ID#'s of ET4699 (surface water) and ET5019 (ground water). The samples were stored at room temperature. Sample log-in information can be found in the raw data package associated with this study. Sample storage records are on file at EN-CAS Analytical Laboratories.

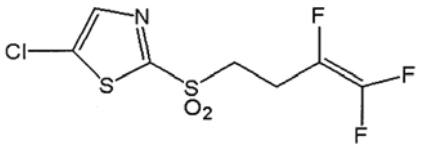
V. TEST AND REFERENCE MATERIALS

The fluensulfone (MCW-2), deschloro MCW-2, M-3625, M-3626 and M-3627 were received at EN-CAS from Makhteshim Chemical Works Ltd. (fluensulfone and deschloro MCW-2) and PharmAgra Labs (M-3625, M-3626 and M-3627) and were used for preparation of stock, fortification, and calibration standards. Characterization of the test/reference materials was performed by Makhteshim Chemical Works Ltd. and ODOM Industries. The fluensulfone and metabolites were stored at ambient temperature.

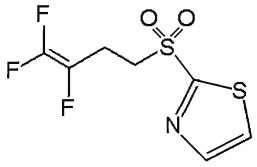
The following information accompanied the test/reference materials upon receipt at EN-CAS.

Standard Reference	EN-CAS Number	Date Received	Physical Appearance
Fluensulfone	ET5862	6/14/11	White crystalline solid
deschloro MCW-2	ET7320	12/1/11	Liquid
M-3625	ET5863	6/14/11	White solid
M-3626	ET5864	6/14/11	Tan solid
M-3627	ET5865	6/14/11	White solid

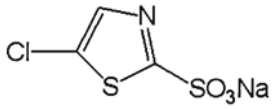
Report Name	Fluensulfone
Trade Name	MCW-2
CAS Nomenclature	5-Chloro-2-[3,4,4-trifluor—3-butene-1-y1)sulfonyl]-thiazole
CAS Number	133-07-3
Assay:	99
Expiration Date:	1/19/13
Reference Substance Lot:	326-160-01



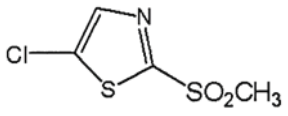
Report Name	Deschloro MCW-2
CAS Nomenclature	2-(3,4,4-trifluoro-3-buteny sulfonyl) thiazole
CAS Number	NA
Molecular Formula	$C_7H_6F_3NO_2S_2$
Molecular Weight	257.27
Assay	94.8
Expiration Date	11/5/2012
Reference Substance Lot	426-133-01
Storage Conditions	Room Temperature

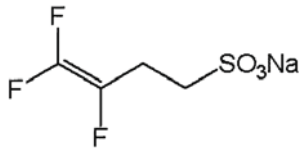


Report Name	M-3625
CAS Nomenclature	5-chlor-thiazole-2-sulfonic acid sodium salt
CAS Number	NA
Molecular Formula	$C_3HClNO_3S_2Na$
Molecular Weight	221.62 (199.64 as the acid)
Assay:	96.1 (excluding H_2O) 94.7 (including H_2O)
Expiration Date:	3/25/13
Reference Substance Lot:	213PAL080



Report Name	M-3626
CAS Nomenclature	5-chloro-2-methyl sulfonyl thiazole
CAS Number	NA
Molecular Formula	$C_4H_4ClNO_2S_2$
Molecular Weight	197.66
Assay:	98.7
Expiration Date:	2/2013
Reference Substance Lot:	231PAL052



Report Name	M-3627	
CAS Nomenclature	3,4,4-trifluoro-but-3-ene-1-sulfonic acid, sodium salt	
CAS Number	NA	
Molecular Formula	$C_4H_4F_3O_3S.Na$	
Molecular Weight	190.14 + 22.99 (Na)	
Assay:	99.5	
Expiration Date:	12/12/12	
Reference Substance Lot:	215PAL44	

The stock standard solutions were prepared on December 19, 2011. Fortification standard solutions and calibration standard solutions were prepared on December 19, 2011, December 20, 2011, December 29, 2011, December 30, 2011, May 30, 2012, June 4, 2012, June 6, 2012, June 7, 2012, July 13, 2012 and July 16, 2012. See Report Section VII.A.1. for further detail. Stock, fortification and calibration solutions were stored refrigerated at approximately 3°C. Documentation of standard preparation can be found in the raw data associated with this report.

VI. DESCRIPTION OF ANALYTICAL METHOD

The analytical method from PTRL Method 1870W, entitled Determination of Fluensulfone and its Metabolites in Water (attached as Appendix II), was used for this study.

As instructed by the method, a 10-gram sample was weighed into a 20-mL scintillation vial and fortified at either the LOQ (0.05 ppb) or 10X LOQ (0.50 ppb). Two unfortified samples were also prepared. The sample was mixed by vortexing and an aliquot was transferred to a HPLC vial for LC mass spectrometric (MS) analysis.

Analytical sample sets contained 11 calibration standards that bracketed the final sample concentrations as submitted for analysis. Analyses of these calibration standards were used to generate a linear regression curve. See Section VII.2. below for further details.

The following minor adjustments were made to the water method:

1. Pipetman type pipettors were used instead of Hamilton syringes for small volume measurements.
2. HPLC vials were used instead of snap-top GC vials.
3. For the surface water trial, the injection volume was reduced to minimize enhancement in samples for M-3625 and M-3627.

VII. EXPERIMENTAL DESIGN

A. Establish Method Chromatography and Performance Criteria

Prior to performing the ILV, EN-CAS determined approximate analyte retention times and instrument detection limits using appropriate dilutions of the standard. The linearity of instrument responses to the calibration standards and the lack of interferences in the unfortified control matrix at the analyte retention times were also checked. A calibration curve was established by injecting standards at seven levels ranging from 0.03 ng/mL to 0.70 ng/mL. The 0.03 ng/mL standard is equivalent to a sample fortified at a level of 60% of the LOQ.

1. Preparation of Stock, Fortification and Calibration Standards

Stock standards (1000 µg/mL) of fluensulfone, M-3625, M-3626 and M-3627 were prepared in ACN on 6/21/11 (notebook reference NZS # 635/180). A deschloro MCW-2 Stock standard (1000 µg/mL) was prepared in ACN on 12/19/11 (notebook reference NZS # 635/279).

Aliquots of the parent and metabolite stocks were combined and diluted with 50:50 ACN:H_2O to prepare a 10.0 µg/mL fortification solution on 12/19/11 and 6/4/12. The 10 µg/mL fortification solution was further diluted in 50:50 MeOH:H_2O to prepare 1.0 µg/mL and 250 ng/mL fortification standards. The 1.0 µg/mL fortification solution was further diluted in 50:50 MeOH:H_2O to prepare a 25 ng/mL fortification standard.

The 25 ng/mL fortification solutions were diluted in HPLC-grade H_2O to prepare 0.03 ng/mL, 0.04 ng/mL, 0.05 ng/mL, 0.06 ng/mL, 0.07 ng/mL, 0.10 ng/mL, 0.25 ng/mL and 0.40 ng/mL calibration standards. The 250 ng/mL standard was further diluted to prepare 0.25 ng/mL, 0.40 ng/mL, 0.50 ng/mL, 0.60 ng/mL and 0.70 ng/mL standards for injection.

Stock, fortification and calibration standards were stored refrigerated at approximately 3°C. Further information regarding the preparation of fortification standards and LC calibration standards is located in EN-CAS Project No. 11-0028 raw data files.

2. Calibration Curve

Standards were injected at the beginning and throughout the run at the following levels: 0.03 ng/mL, 0.04 ng/mL, 0.05 ng/mL, 0.06 ng/mL, 0.07 ng/mL, 0.10 ng/mL, 0.25 ng/mL, 0.40 ng/mL, 0.50 ng/mL, 0.60 ng/mL and 0.70 ng/mL for both water matrices. The calibration curve used was a linear regression curve, $y = mx + b$, where m is the slope and

b is the y-intercept. Calibration curves appear as Figures 11, 12, 27, 42, 43, 59, 60, 75, 90 and 91.

3. Chromatography

The control surface water and ground water samples were free of interferences at the analyte retention time. Example chromatograms of standards, controls, and fortified samples are shown in Figures 1 through 94.

4. Description of Instrument and Operating Conditions

For all sample analyses, a PE Sciex API 4000 Tandem Mass Spectrometer with a MS detector tandem mode and an Agilent 1100 HPLC and Agilent WPALS Autosampler was used. Detailed operating conditions are listed below:

HPLC Conditions

Column:	Synergi 4u Fusion-RP 80A 2 x 250 mm, 4 µm particle size.; ID 260; S/N 586051-5	
Injector:	Agilent: Autosampler 1100 WPALS Pump 1100 QuatPump	
Mobile Phase:	Sol 1: 0.1% formic acid in H_2O Sol 2: 0.1% formic acid in ACN	
Oven:	FIAtron TC50/CH30 @ 30°C	
Flow Rate:	200-600 µL/min	
Injection Volume:	100 µL (50 µL M-3625 and M-3627 surface water)	
Retention Time:	Fluensulfone	= 23.6 min (surface water) 23.4 min (ground water)
	Deschloro MCW-2	= 20.6 min (surface water) 20.5 min (ground water)
	M-3626	= 17.1 min (surface water) 17.0 min (ground water)
	M-3625	= 11.3 – 11.5 min (surface water) 11.6 min (ground water)
	M-3627	= 9.01 – 9.45 min (surface water) 9.3 min (ground water)

Run Time: 30 min

Standard/Sample Solvent: Standard = HPLC grade H_2O
Sample = surface or ground water

Gradient Table:

Step	Time	Flow	Sol. 1	Sol. 2
0	0.0	200	90	10
1	24.0	200	5	95
2	24.5	600	0	100
3	25.0	600	0	100
4	26.0	400	90	10
5	29.0	200	90	10
6	30.0	200	90	10

Mass Spectrometer Conditions

LC/MS Instrument: AB-Sciex API4000 Tandem Mass Spectrometer

API Source: APCI V/L 14/6.0 500°C

MS Mode: Fluensulfone, Deschloro MCW-2 and M-3626: Tandem (MS/MS) Positive
M-3625 and M-3627: Tandem (MS/MS) Negative

MS Parameters: Fluensulfone = CE/CXP/CAD/CUR/DP/EP 25/12/9/40/65/10
Deschloro MCW-2 = CE/CXP/CAD/CUR/DP/EP 25/10.7/9/40/65/10
M-3626 = CE/CXP/CAD/CUR/DP/EP 25/10/9/40/65/10

M-3625 = CE/CXP/CAD/CUR/DP/EP -33/-5/12/20/-55/-10
M-3627 = CE/CXP/CAD/CUR/DP/EP -27/-13/12/20/-55/-10

Mass Calibration: Positive Mode Based on PPG masses; 59.050, 175.133, 616.464, 906.673, 1254.925, 1545.134, 2010.469, 2242.637
Negative Mode Based on PPG masses; 44.998, 585.385, 933.636, 1223.845 1572.097, 1863.306, 2037.431, 2211.557

Masses Monitored:	Fluensulfone	= 291.9 → 166
	Deschloro MCW-2	= 257.9 → 132
	M-3626	= 197.9 → 134.9
	M-3625	= 197.8 → 81.8
	M-3627	= 188.9 → 80.9
Dwell Time:	Fluensulfone	= 200 ms
	Deschloro MCW-2	= 80 ms
	M-3626	= 160 ms
	M-3625	= 400 ms
	M-3627	= 400 ms

B. Quantitation and Example Calculation

Standards were injected at the beginning and after approximately every two samples throughout the run to generate a linear regression calibration curve. Average percent recovery, standard deviation, and relative standard deviation were calculated for fluensulfone, deschloro MCW-2, M-3625, M-3626 and M-3627 at each fortification level. No control contribution above 30% of the LOQ was detected for any of the matrices. The residue ppb was determined from the following equations:

1. Calculation of ppb Found

$$\text{ppb Found} = \frac{Y-B}{M}$$

Where:

Y = Peak Area
M = Slope
B = y intercept

2. Calculation of Percent Recovery in Fortification Samples

$$\%\ \text{Recovery} = \frac{R-S}{FL} \times 100$$

Where:

R = ppb of target analyte found in fortified sample
S = ppb of target analyte found in control sample, real or apparent
FL = Fortification Level (0.05 ppb or 0.50 ppb)

3. Example Calculation for a Procedural Recovery Sample

For ET4699-S14 (Low-level Fluensulfone procedural recovery from Set 1-02-MV (A), fortified at 0.05 ppb) (see Figure 15)

Where:

$$\text{ppb Found} = \frac{909 - 198}{15900} = 0.0447 \text{ ppb}$$

$$\%\ \text{Recovery} = \frac{0.0447 \text{ ppb} - 0}{0.05 \text{ ppb}} \times 100 = 89.4\%$$

VIII. METHOD OBSERVATIONS

A. Problems Encountered

The surface water trial showed a degree of enhancement for M-3625 and M-3627 that was both unacceptably high and unacceptably variable. Reducing the injection volume from 100 µL to 50 µL solved this problem.

B. Critical Steps

Adjusting the APCI probe position and all applicable mass spectrometer settings to maximize signal intensities for these analytes was critical for achieving the needed sensitivity.

C. Matrix or Solvent Effects

See Section VIII. A. above.

D. Signal Enhancement or Suppression

See Section VIII. A. above.

E. Stability of Solutions

Surface water sample solutions were injected immediately after sample preparation except M-3625 and M-3627 which were injected 33 days after the samples were extracted. Ground water sample solutions were injected immediately after sample preparation.

The sample injection run for each matrix was 40 hours. Acceptable recoveries seem to indicate good stability of sample solutions for at least that amount of time.

IX. RECOMMENDED CHANGES TO METHOD

The example run sequence given in the method specifies duplicate injections of each sample and standard, but the method does not state what should be done if duplicates do not agree. We recommend that samples for which the results of duplicate injections do not agree within a predetermined margin, for example 20%, should be reinjected in duplicate.

The method states that a run requires approximately 23 hours of instrument time, but the example run sequence on page 20 takes approximately 40 hours to complete. The time estimate should be updated to reflect the example run sequence.

As noted above in Method Observations, it was necessary to adjust the injection volume for surface water samples in order to overcome a matrix effect. Including the option in the method may be helpful for users.