A set of 13 samples can be prepared in one 8-hour day and analyzed by overnight injection for M-3625 and M-3627, plus the next day for fluensulfone and M-3626. A LC/MS/MS analytical run containing seven levels of calibration standards and 13 samples can be completed overnight.

II. OBJECTIVE

The purpose of this study was to perform an ILV of the PTRL Method 2049W, entitled Determination of Fluensulfone and Metabolites in Soil to satisfy requirements described in 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 soil 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 2049W, for the determination of Fluensulfone and metabolites in soil. The study was carried out according to Study Protocol 11-0010 (EN-CAS Study # 11-0010), 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 1 set was planned to cover the EPA Guideline, OPPTS 850.7100 and consist of one reagent blank, one control sample not fortified with the fluensulfone combined fortification solution, five control samples fortified with the fluensulfone combined fortification solution at LOQ (10 ppb) and five at 10X the LOQ (100 ppb). Trial 2 set 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 (10 ppb) and five at 10X the LOQ (100 ppb).

The study was initiated on July 11, 2011 when the Study Director signed EN-CAS Protocol # 11-0010. Analytical standards were prepared per GLP guidelines on June 21, 2011. The experimental start date was November 9, 2011 and the experimental termination date was November 15, 2011.

IV. TEST SYSTEM

Control sandy loam and clay loam samples used in the validation study were received (ambient) on June 24, 2011 from AGVISE Laboratories, Inc., Northwood, ND. The samples were assigned unique identification ID#'s of ET5947 (sandy loam) and ET5948 (clay loam). The samples was 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.

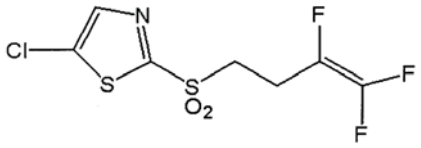
V. TEST AND REFERENCE MATERIALS

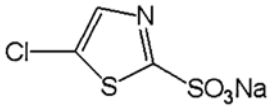
The fluensulfone (MCW-2), M-3625, M-3626 and M-3627 were received at EN-CAS from Makhteshim chemical Works Ltd. (fluensulfone) and PharmAgra Labs (M-3625, M-3626 and M-3627) and were used for preparation of stock, fortification, and calibration standards. Characterization of the test/reference materials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

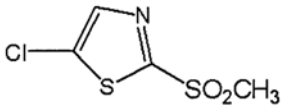
The following information accompanied the test/reference materials upon receipt at EN-CAS.

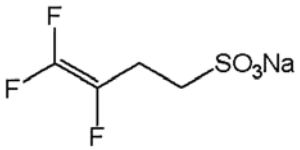
Standard Reference	EN-CAS Number	Date Received	Physical Appearance
Fluensulfone	ET5862	6/14/11	White crystalline solid
M-3625	ET5863	6/14/11	White solid
M-3626	ET5864	6/14/11	Tan solid
M-3627	ET5865	6/14/11	White solid

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	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 June 21, 2011. Fortification standard solutions and calibration standard solutions were prepared on June 22, 2011, August 2, 2011 and November 2, 2011. See Report Section VII.A.1. for further detail. Stock and fortification solutions were stored frozen at less than or equal to -10°C. 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 2049W, entitled Determination of Fluensulfone and Metabolites in Soil (attached as Appendix II), was used for this study.

As instructed by the method, a 50-gram sample was weighed into a 250-mL plastic bottle and fortified at either the LOQ (10 ppb) or 10X LOQ (100 ppb). Two unfortified samples were also prepared. An aliquot (100 mL) of ACN:HPLC H_2O, 1:1 v/v, was added and the sample was placed on an orbital shaker for one hour. The sample was centrifuged at 10,000 rpm for 10 minutes.

For fluensulfone and M-3626, an aliquot of the supernatant was transferred into a microfilterfuge tube, containing 0.45-µm filter and centrifuged at 14,000 rpm for five minutes. The filtered extract was transferred to a HPLC vial for for LC mass spectrometric (MS) analysis.

For M-3625 and M-3627, 6.0 mL of the unfiltered supernantant was transferred to a 15-mL graduated centrifuge tube and the samples was concentrated to 3 mL using an N-Evap at approximately 35°C.

A Bond-Elut 500-mg/6-cc SPE cartridge was conditioned with 5 mL of ACN followed by 5 mL of H_2O. The sample was loaded onto the cartridge and the eluate was collected in a 15-mL graduated tube. The sample tube was rinsed with 5 mL of H_2O, applied to the cartridge, and the eluate collected into the same 15-mL graduated tube. The volume was recorded and the sample was mixed by vortexing. The samples was transferred to a HPLC vial for LC/MS/MS analysis.

Analytical sample sets contained a minimum of seven 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 soil method:

1. An orbital shaker was used instead of a wrist action shaker.
2. 10-mL graduated tubes were used instead of 15-mL graduated tubes.
3. HPLC vials were used instead of snap-top GC vials.
4. For the clay loam trial, the injection volume was reduced to minimize peak splitting 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 1.00 ng/mL to 100 ng/mL. The 2.50 ng/mL standard is equivalent to a sample fortified at a level of 50% 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).

Aliquots of the parent and metabolite stocks were combined and diluted with 50:50 ACN:H_2O to prepare a 1.0 µg/mL fortification solution on 6/22/11, 8/2/11 and 11/2/11. A 10.0 µg/mL fortification solution was also prepared from the parent and metabolite stocks with 50:50 ACN:H_2O on 6/22/11. Aliquots of the metabolite stocks were combined and diluted with 50:50 ACN:H_2O to prepare a 10.0 µg/mL fortification solution on 6/22/11.

The 1.0 µg/mL fortification solutions were diluted in 50:50 MeOH:H_2O to prepare 2.5 ng/mL, 5.0 ng/mL, 10 ng/mL, 25 ng/mL, 50 ng/mL and 100 ng/mL calibration standards. The 2.5 ng/mL standard was further diluted to prepare 0.25 ng/mL, 0.5 ng/mL, 0.75 ng/mL and 1.0 ng/mL standards for injection.

Stock and fortification standards were stored under freezer conditions at less than -10^{o}C. 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. 11-0010 raw data files.

2. Calibration Curve

Standards were injected at the beginning and throughout the run at the following levels: 1.0 ng/mL, 2.5 ng/mL, 5.0 ng/mL, 10 ng/mL, 25 ng/mL, 50 ng/mL and 100 ng/mL for both soil matrices. The calibration curve used was a linear regression curve, $y = mx + b$ where m

is the slope and b is the y-intercept. A validated Excel spreadsheet was used to calculate the data. Calibration curves appear as Figures 8, 20, 32 and 43. Spreadsheets appear as Figures 47 through 54.

3. Chromatography

The control sandy loam and clay loam samples were free of interferences at the analyte retention time. Example chromatograms of standards, controls, and fortified samples are shown in Figures 1 through 46.

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 WPALS Autosampler was used. Detailed operating conditions are listed below:

Fluensulfone and M-3626

HPLC Conditions

Column:	Synergi 4u Fusion-RP 80A 2 x 75 mm, 4 µm particle size.; ID 258; S/N 546328
Injector:	Agilent: Autosampler 1100 WPALS Pump 1100 QuatPump
Mobile Phase:	Sol 1: 0.01% formic acid in ACN Sol 2: 0.01% formic acid in D.I. H_2O
Oven:	FIAtron CH50/CH30 @ 45°C
Flow Rate:	200 µL/min
Injection Volume:	20 µL
Retention Time:	Fluensulfone = 9.75 min (sandy loam) 14.2 min (clay loam) M-3626 = 10.5 min (sandy loam) ~10.5 min (clay loam)
Run Time:	Fluensulfone = 25 min M-3626 = 25 min
Standard/Sample Solvent:	50:50 MeOH:H_2O

Mass Spectrometer Conditions

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

API Source: APCI V/L 11.0/4.55 600°C

MS Mode: Tandem (MS/MS) Positive

MS Parameters: Fluensulfone = CE/CXP/CAD/DP/EP 25/15/12/40/10
M-3626 = CE/CXP/CAD/DP/EP 30/10/12/40/10

Mass Calibration: Based on PPG masses; 59, 175.133, 616.464, 906.673, 1254.925, 1545.134, 2010.469, 2242.637

Masses Monitored: Fluensulfone = 292 → 166
M-3626 = 198 → 120

Dwell Time: 200 ms

Gradient Table:

Step	Time	Flow	Sol. 1	Sol. 2
0	0.0	200	95	5
1	1.0	200	95	5
2	13.5	200	10	90
3	14.0	400	0	100
4	16.0	500	0	100
5	16.3	400	95	5
6	18.0	200	95	5
7	25.0	200	95	5

M-3625 and M-3627

HPLC Conditions

Column: Synergi 2.5u Fusion-RP 100A 2 x 100 mm,
2.5 µm particle size.; ID 257; S/N 579495-7

Injector: Agilent: Autosampler 1100 WPALS
Pump 1100 QuatPump

Mobile Phase: Sol 1: 0.05% formic acid in ACN
Sol 2: 0.05% formic acid in D.I. H_2O

Oven: FIAtron CH50/CH30 @ 30°C

Flow Rate: 200 µL/min

Injection Volume: 20 µL (sandy loam), 10 µL (clay loam)

Retention Time: M-3625 = 7.0 – 7.5 min (sandy loam)
6.9 – 7.5 min (clay loam)
M-3627 = 4.8 – 5.2 min (sandy loam)
4.6 – 5.1 min (clay loam)

Run Time: M-3625 = 16 min
M-3627 = 16 min

Standard/Sample Solvent: 50:50 MeOH:H_2O

Mass Spectrometer Conditions

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

API Source: Turbo Ion Spray V/L 0.45/4.55 250°C

MS Mode: Tandem (MS/MS) Negative

MS Parameters: M-3625 = CE/CAD/DP/EP/CXP -31/12/-40/-10/-5
M-3627 = CE/CAD/DP/EP/CXP -25/12/-40/-10/-5

Mass Calibration: Based on PPG masses; 59, 175.133, 616.464, 906.673, 1254.925, 1545.134, 2010.469, 2242.637

Masses Monitored: M-3625 = 198 → 82
M-3627 = 189 → 81

Dwell Time: 150 ms

Gradient Table:

Step	Time	Flow	Sol. 1	Sol. 2
0	0.0	250	95	5
1	1.0	250	95	5
2	9.0	250	42	58
3	9.5	300	0	100
4	10.5	350	0	100
5	11.5	300	95	5
6	16.0	250	95	5

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. Quantitation of the injected nanograms of an unknown sample was accomplished by inserting the analyte peak area into the appropriate linear regression equations. From the injected nanograms, the residue ppb and percent recovery were calculated for each fortified control sample. Average percent recovery, standard deviation, and relative standard deviation were calculated for fluensulfone, M-3625, M-3626 and M-3627 at each fortification level; overall percent recovery, standard deviation, and relative standard deviation were calculated for propanil across the fortification levels. 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 Aliquot Sample-Equivalent

$$\text{Aliquot sample-equiv.} = \frac{\text{g sample extracted} \times \text{mL sample aliquot}}{\text{mL extraction volume}}$$

2. Calculation of µL-Equivalent Injected

$$\text{µL-equiv. injected} = \frac{\text{Aliquot sample-equiv (g).} \times \text{µL inj.} \times 1000\ \text{µL/g}}{\text{mL final vol.} \times \text{dilution factor} \times 1000\ \text{µL/mL}}$$

3. Calculation of pg Found

The picograms of analyte found were determined from the standard curve as follows:

$$\text{pg Found} = \frac{\text{Peak area (counts)} - \text{standard curve y-intercept (counts)}}{\text{standard curve slope (counts/pg)}}$$

4. Calculation of ppb Found

$$\text{ppb found} = \frac{\text{pg found}}{\text{mg-equivalent injected}}$$

5. Calculation of ppb Found (Corrected for Control Contribution)

ppb found (control corrected) = ppb found (fort.) - ppb found (control)

6. Calculation of Percent Recovery in Fortification Samples

$$\% \text{ Recovery} = \frac{\text{ppb found (control corrected)}}{\text{fortification level (ppb)}} \times 100$$

7. Example Calculation for a Procedural Recovery Sample

Sandy loam sample ET5947-S1, Set # 1-01-MV-(A), LC/MS/MS Run # 88803 (see Figure 11).

Sample Wt	= 50 g	Injection volume	= 20 µL
Extraction volume	= 100 mL	Peak area (recovery)	= 1509 counts
Aliquot volume	= 100 mL	y-intercept	= 27.84018634 counts
Final volume	= 100 mL	Slope	= 15.90940548 counts/pg
Dilution factor	= 1	Fortification level	= 10 ppb
Avg. Control	= 0.0000 ppb		

$$\text{aliquot sample-equiv.} = \frac{50 \text{ g} \times 100 \text{ mL}}{100 \text{ mL}} = 50 \text{ sample-equiv.}$$

$$\text{µL-equiv. injected} = \frac{50 \text{ smpl-equiv} \times 100 \text{ mL} \times 20 \text{ µL} \times 1000 \text{ µL/g}}{100 \text{ mL} \times 100 \text{ mL} \times 1 \times 1000 \text{ µL/mL}} = 10 \text{ µL-equiv.}$$

$$\text{pg found} = \frac{1509 \text{ counts} - 27.84018634 \text{ counts}}{15.90940548 \text{ counts/pg}} = 93.0996 \text{ pg}$$

$$\text{raw ppb found} = \frac{93.0996 \text{ pg}}{10 \text{ µL-equiv.}} = 9.3100 \text{ ppb}$$

ppb found = 9.3100 ppb (sample) - 0.0000 ppb (control) = 9.3100 ppb (corrected)

$$\% \text{ recovery} = \frac{9.3100 \text{ ppb}}{10 \text{ ppb}} \times 100 = 93\% \text{ Fluensulfone}$$

VIII. METHOD OBSERVATIONS

A. Problems Encountered

The sandy loam trial showed some peak splitting for M-3625 and M-3627. Therefore, the HPLC injection volume was reduced to minimize peak splitting in the clay loam trial.

B. Critical Steps

There are no steps that must be followed exactly as detailed in the method in order to obtain adequate recoveries.

C. Matrix or Solvent Effects

No problems were detected with matrix or solvent effects.

D. Signal Enhancement or Suppression

No problems were detected.

E. Stability of Solutions

Sandy loam sample solutions were injected immediately after sample preparation except for fluensulfone and M-3626 which were injected one day after sample extraction. Acceptable recoveries seem to indicate good stability of sample solutions for at least that amount of time. Clay loam sample solutions were injected immediately after sample preparation except M-3625 and M-3627 which were injected four days after the samples were extracted. Acceptable recoveries seem to indicate good stability of sample solutions for at least that amount of time.

IX. RECOMMENDED CHANGES TO METHOD

The speed and amount of time used for the second centrifuge step would be useful.

Less acidic soils may result in unacceptably broad peaks for M-3625 and M-3627. an option to reduce injection volume would be appropriate.

X. CONCLUSIONS

The PTRL Method 2049W, entitled Determination of Fluensulfone and Metabolites in Soil was successfully validated in sandy loam and clay loam.

XI. TIME REQUIREMENTS

A set of 13 samples can be prepared by one analyst and put on LC/MS/MS for overnight analysis in one eight-hour day.

XII. CONTACTS WITH SPONSOR

No contact with the Sponsor was needed.

XIII. CIRCUMSTANCES THAT MAY HAVE AFFECTED THE DATA

No circumstances occurred that might have affected the integrity of raw data for the study.

XIV. RETENTION OF DATA AND SAMPLES

At the completion of the study, all original paper data generated by EN-CAS Analytical Laboratories will be temporarily archived at EN-CAS Analytical Laboratories until specific instructions are received from the Sponsor for forwarding to the Sponsor's designated archive facility. Verified exact copies of all raw data, as well as a signed copy of the final report and all original facility-specific raw data, will be retained in the EN-CAS Analytical Laboratories archives for the period of time specified in 40 CFR 160.195 (b). Control matrices will be stored at EN-CAS until use in another project or disposal.