SUMMARY

A method for quantitation of Fluensulfone (MCW-2) and its metabolites in tap water, well water and surface water was successfully validated. Control matrices were fortified at 0.05 µg/kg (ppb, LOQ) and 0.50 µg/kg (10x LOQ). MCW-2 and its metabolites methyl sulfone (M-3626), dechloro MCW-2, butene sulfonic acid (M-3627) and thiazole sulfonic acid (M-3625) were analyzed by direct injection with LC-MS/MS analysis from fortified water samples. The limit of quantitation for all five analytes in all matrices is 0.05 ppb. The limit of detection was 0.03 ng/mL in solution.

INTRODUCTION

Makhteshim-Agan of North America, Inc. contracted with PTRL West, Inc. to conduct method development and validation of an analytical method for determination of Fluensulfone and its metabolites commonly named methyl sulfone, deschloro fluensulfone, butene sulfonic acid and thiazole sulfonic acid in pond water, well water and tap water.

The study is designed to comply with OPPTS 850.7100 and OPPTS 860.1340 guidelines. The study was initiated on July 12, 2010. This study was conducted from July 26, 2010 to August 4, 2011. The study was conducted at PTRL West, Inc., 625-B Alfred Nobel Drive, Hercules, CA 94547 under an approved protocol according to the US EPA FIFRA Good Laboratory Practice Standards, 40 CFR §160 (Appendix A).

MATERIALS AND METHODS

Reference Substances

The reference substance of MCW-2 (lot number 326-160-01, PTRL Sample No. 2088W-001B) was supplied by Makhteshim/Donna Landis Labservices with a stated purity of 99.58%. The expiration date on the certificate of analysis is December 31, 2011 and it was stored refrigerated upon receipt. The reference standard methyl sulfone (M-3626, lot number 381-173-00, PTRL Sample No. 1788W-002) was supplied by Makhteshim with a stated purity of 97%. The expiration date on the certificate of analysis was December 31, 2011 and it was stored refrigerated upon receipt. The reference substance deschloro MCW-2 (lot number 426-133-01, PTRL Sample no. 1857W-003) was supplied by Donna Landis Labservices with a stated purity of 94.8%. the expiration date on the certificate of analysis is November 5, 2012 and it was stored refrigerated upon receipt. The reference standard of thiazole sulfonic acid (M-3625, lot number 381-174-00, PTRL Sample No. 1788W-003) was supplied by Makhteshim Chemical Works with a stated purity of 90.9%. The expiration date on the certificate of analysis is December 31, 2011 and it was stored refrigerated upon receipt. The reference standard of butene sulfonic acid (M-3627, lot number 215PAL44, PTRL Sample No. 2088W-003) was supplied by Donna Landis Labservices with a stated purity of 99.5%. The expiration date on the certificate of analysis is December 12, 2012 and it was stored at room temperature upon receipt. See Appendix B for the certificates of analysis.

All stock and working solutions were stored refrigerated in amber bottles with Teflon®-lined screw-top caps. All reference substances were concluded to be stable in solution throughout the study period based on comparison of chromatograms generated over the study period.

Reagents and Solvents

All solvents were HPLC grade unless noted:

Acetonitrile (ACN)
Formic Acid
Water

Glassware and Miscellaneous Equipment

Balance

Flasks, volumetric (10 mL)

Microliter syringe, Hamilton (25 µL, 50 µL and 250 µL)

pH meter

Pipettemen, assorted sizes with disposable tips

Vials, glass with Teflon®-lined cap

ANALYTICAL PROCEDURES

Water Samples

Tap water was collected at PTRL West, Inc. (Hercules, CA) on July 22, 2011 and was given the PTRL West identifier of 1870W-007. Well water was provided by Wildlife International, Ltd. and received at PTRL West, Inc. on November 7, 2008. The well water was identified as 1870W-002. Surface water was collected the pond at Hercules Park (Hercules, CA) on July 26, 2010. The surface (pond) number was identified as 1870W 005. The pH of these waters was determined on the day of validation to be pH 8.5 for tap water, pH 8.6 for well water and pH 8.6 for surface water.

Preparation of Stock Solutions

A stock standard of Fluensulfone (MCW-2) was prepared by dissolving 10.17 mg MCW-2 in 10 mL of acetonitrile. An additional aliquot of 0.07 mL acetonitrile was added to yield a 1.0 mg/mL stock solution. A stock solution of methyl sulfone (M-3626) was prepared by dissolving 10.73 mg in 10 mL of acetonitrile:water (1:1, v/v). An additional aliquot of 0.408 mL acetonitrile:water (1:1, v/v) was added to yield a 1.0 mg/mL stock solution of M-3626. A stock solution of deschloro MCW-2 was prepared by dissolving 11.58 mg in 10 mL acetonitrile. An additional aliquot of 0.98 mL acetonitrile was added to yield a 1.0 mg/mL stock solution of deschloro MCW-2. A stock standard of butene sulfonic acid sodium salt (M-3627) was prepared by dissolving 10.58 mg in 10 mL of acetonitrile:water (1:1, v/v). An additional aliquot of 0.53 mL acetonitrile:water (1:1, v/v) was added to yield a 1.0 mg/mL stock solution of M-3627. A stock standard of thiazole sulfonic acid sodium salt (M-3625) was prepared by dissolving 10.71 mg in 10 mL of acetonitrile:water (1:1, v/v). An additional aliquot of 0.17 mL acetonitrile:water (1:1, v/v)

was added to yield a 1.0 mg/mL stock solution of M-3625. The stock solutions were stored refrigerated when not in use.

Preparation of Fortification Standards

Mixed intermediate working solutions were prepared by dilution of the stock solutions. A 10 µg/mL mixed Fluensulfone (MCW-2), methyl sulfone (M-3626), butene sulfonic acid (M-3627) and thiazole sulfonic acid (M-3625) intermediate working solution was prepared by diluting 0.1 mL each of the respective 1.0 mg/mL stock solutions to 10 mL in a 10 mL volumetric flask, where the solution was brought to the mark with acetonitrile:water (1:1, v/v). A pipetteman with disposable tips was used to deliver the 0.1 mL of the stock solutions to avoid any contamination from glassware. A 10 µg/mL solution of deschloro MCW-2 was prepared by dilution of 0.10 mL of the 1.0 mg/mL stock solution to 10 mL with acetonitrile. A 1.0 µg/mL mixed intermediate working solution containing Fluensulfone (MCW-2), methyl sulfone (M-3626), deschloro MCW-2, butene sulfonic acid (M-3627) and thiazole sulfonic acid (M-3625) was prepared by diluting 1.0 mL each of the respective 10 µg/mL intermediate working solutions to 10 mL in a 10 mL volumetric flask, where the solution was brought to the mark with acetonitrile:water (1:1). These intermediate working solutions were stored refrigerated when not in use.

The final fortification solutions were prepared by dilution of 2.5 mL of the mixed 1.0 µg/mL intermediate mixed working solution to 10 mL in a 10 mL volumetric flask and diluting to the mark with acetonitrile:water (1:1), yielding a 250 ng/mL fortification solution. A 10-fold dilution of the 250 ng/mL fortification solution was prepared by dilution with acetonitrile:water (1:1) to yield a 25 ng/mL fortification solution. All solutions were mixed well and transferred to amber vials with Teflon-lined screw caps. The fortification solutions were stored refrigerated when not in use.

Sample Fortification

Portions of control water (10 g) were fortified with MCW-2, M-3626, deschloro MCW-2, butene sulfonic acid sodium salt (M-3627) and thiazole sulfonic acid sodium salt (M-3625) in replicates (5), as shown below, for determination of method recovery.

Fortification Level (ppb)	Volume of Mixed Fortification Solution
0.05	0.020 mL of 25 ng/mL Mixed Fort. Soln.
0.50	0.020 mL of 250 ng/mL Mixed Fort. Soln.

Preparation of Calibrants

All calibrant dilutions were prepared in 10 mL glass volumetric flasks and transferred to amber glass vials. Appropriate Hamilton microliter syringes were used to dispense mixed standard solutions. The calibrants were prepared by dilution to the mark with Burdick and Jackson High Purity Solvent Water. Matrix-based calibrants were prepared with dilution to the mark with the respective test waters.

Calibrant Number	Calibrant Conc. (ng/mL)	Conc. Stock Std. (ng/mL)	Volume (µL)	Final Volume (mL)
1	0.03	25	12	10
2	0.04	25	16	10
3	0.05	25	20	10
4	0.06	25	24	10
5	0.07	25	28	10
6	0.10	25	40	10
7	0.25	25	100	10
7	0.25	250	10	10
8	0.40	250	16	10
9	0.50	250	20	10
10	0.60	250	24	10
11	0.70	250	28	10

All calibrants were prepared within 11 days of use and were stored refrigerated when not in use.

EXTRACTION METHOD

1. Allow water to equilibrate to room temperature. Weigh out 10 g portions of water into 20 mL clear glass vials.

2. Fortify as necessary, using a Hamilton microliter syringe (25 µL).

3. Mix well by vortexing.

4. Transfer aliquot of the sample to a snap-top GC vial for analysis.

5. Determine pH on an aliquot of the water.

LC-MS/MS CHROMATOGRAPHY

Atmospheric Chemical Ionization Mass Spectrometry

An Applied Biosystems MDS/SCIEX API 4000 tandem mass spectrometer was used with atmospheric chemical ionization (APCI) in both negative and positive polarity to acquire data by Multiple Reaction Monitoring (MRM).

Pump: Agilent 1100 series, model G1312A

Autosampler: Agilent 1100 series, model G1329A

Micro-Degasser: Agilent 1100 series, model G1379A

Column Compartment: Agilent 1100 series, model G1316A

Column: Phenomenex Synergi Fusion-RP, C18 4 µm particle size, 80A (250 mm x 2.0 mm I.D) fitted with a Phenomenex Fusion-RP 4.0 x 2.0 mm Security Guard Cartridge

Column Temperature: 30°C

Injection Volume: 100 µL

Mobile Phase System: A) 0.1% Formic Acid in HPLC grade Water
B) 0.1% Formic Acid in HPLC grade Acetonitrile

Time (min.)	Flow Rate (µL/min.)	% A	% B
0.0	200	90	10
24.0	200	5	95
24.5	600	0	100
25.0	600	0	100
26.0	400	90	10
29.0	200	90	10
30.0	200	90	10

Compound Dependent Settings:

Q1 Mass (amu)	Q3 Mass (amu)	Ioniz. Polarity	Dwell Time (msec)	Nebulizer Current	Entrance Potential	Declustering Potential	Collision Energy	Collision Cell Exit Potential
189.2	80.2	Neg.	125	-3	-10	-67	-27	-15.0
189.2	81.2	Neg.	150	-3	-10	-67	-27	-12.0
198.0	81.9	Neg.	100	-3	-10	-55	-33	-13.0
198.0	117.9	Neg.	250	-3	-10	-55	-20	-7.0
198.0	161.8	Neg.	250	-3	-10	-55	-20	-12.0
292.1	89.2	Pos.	200	5	10	65	43	6.5
292.1	166.2	Pos.	200	5	10	65	25	12.0
292.1	109.1	Pos.	200	5	10	65	27	7.1
198.2	120.4	Pos.	80	5	10	65	30	10.0
198.2	135.3	Pos.	160	5	10	65	25	10.0
198.2	93.3	Pos.	160	5	10	65	45	15.0
258.2	132.0	Pos.	80	5	10	65	25	10.7
258.2	109.2	Pos.	160	5	10	65	28	9.0

Period 1 Experiment 1 (Negative Polarity)	Period 2 Experiment 1 (Positive Polarity)
Nebulizer Temperature (TEM): 500°C	Nebulizer Temperature (TEM): 500°C
Nebulizer Gas (GS1): 30	Nebulizer Gas (GS1): 70
Curtain Gas (CUR): 20	Curtain Gas (CUR): 40
Collision Activated Dissociation Gas (CAD): 8	Collision Activated Dissociation Gas (CAD): 9
Interface Heater (ihe): on	Interface Heater (ihe): on

Source Dependent Settings:

Post-column effluent is diverted into the mass spectrometer during the chromatographic elution of the five compounds of interest for an approximate 16 minute duration from 8 to 24 minutes.

Compound	**Mass Transitions Monitored**	**Approximate Retention Times (min.)**
Butane sulfonic acid (M-3627)	189.2/80.2, 189.2/81.2	~11.2
Thiazole sulfonic acid (M-3625)	198.0/81.9, 198.0/117.9, 198.0/161.8	~12.9
Methyl sulfone (M-3626)	198.2/120.4, 198.2/135.3, 198.2/93.3	~16.5
Des-chloro-MCW-2	258.3/132.0, 258.2/109.2	~19.6
Fluensulfone (MCW-2)	292.1/89.2, 292.1/166.2, 292.1/109.1	~22.3

Separation of the analytes was achieved by HPLC. The analytes were identified by the coincidence of their retention times with the reference standards and MS characteristics, and quantitated by integration of the peak areas.

A typical injection sequence for MCW-2 in a method validation set as analyzed by LC-MS/MS was: solvent blank, solvent blank, calibrant 1, calibrant 1, calibrant 2 matrix-based, calibrant 2 matrix-based, calibrant 2, calibrant 2, calibrant 2 matrix-based, calibrant 2 matrix-based, solvent blank, solvent blank, control, control, control , control, calibrant 3, calibrant 3, calibrant 3 matrix-based, calibrant 3 matrix-based, fortified sample, fortified sample, fortified sample, fortified sample, fortified sample, fortified sample, calibrant 4, calibrant 4, calibrant 4 matrix-based, calibrant 4 matrix-based, fortified sample, fortified sample, fortified sample, fortified sample, calibrant 5, calibrant 5, calibrant 5 matrix-based, calibrant 5 matrix-based, calibrant 6, calibrant 6, calibrant 6 matrix-based, calibrant 6 matrix-based, calibrant 7, calibrant 7, calibrant 7 matrix-based, calibrant 7 matrix-based, calibrant 8, calibrant 8, calibrant 8 matrix-based, calibrant 8 matrix-based, fortified sample, fortified sample, fortified sample, fortified sample, fortified sample, fortified sample, calibrant 9, calibrant 9, calibrant 9 matrix-based, calibrant 9 matrix-based, fortified sample, fortified sample, fortified sample, fortified sample, calibrant 10, calibrant 10, calibrant 10 matrix-based, calibrant 10 matrix-based, calibrant 11, calibrant 11, calibrant 11 matrix-based, calibrant 11 matrix-based, solvent blank, solvent blank, QC standard, QC standard, QC standard matrix-based, QC standard matrix-based, etc.

Statistical Methods

The residue data included the following statistical calculations: means, standard deviations, relative standard deviations and linear regression analysis.

Limit of Quantitation

The limit of quantitation was assigned as the lowest fortification level of analyte validated by the residue method. The LOQ for MCW-2, methyl sulfone (M-3626), deschloro fluensulfone, butene sulfonic acid (M-3627) and thiazole sulfonic acid (M-3625) in water was 0.05 ppb. The limit of detection was assigned as the lowest calibrant used in the analysis or 0.03 ng/mL for all analytes.

METHODS OF CALCULATION

Preparation of Stock Standards

$$\text{Volume of solvent (mL)} = \frac{(W) \times (P)}{(FC)}$$

where W = Milligrams of neat standard
P = Chemical purity of neat standard
FC = Final Concentration (mg/mL)

Residue in Water

The MCW-2, methyl sulfone (M-3626), deschloro MCW-2, butene sulfonic acid (M-3627) and thiazole sulfonic acid (M-3625) quantitation was conducted by peak area relative to an external calibration curve. Note that M-3627 and M-3625 were used as their sodium salts.

Linear regression formula for analyte peak area, calibration curve $y = mx + b$

where y = peak area
x = ng/mL analyte injected
m = Slope
b = Calibration intercept

The linear regression calculated by the LCMS Analyst software was used to calculate the concentration (ng/mL) present in the sample..

The residue in water was calculated as follows:

$$\text{ppb MCW-2 } (\mu g/kg) = \frac{\text{ng/mL MCW - 2} \times \text{Sample vol. (mL)} \times \text{dilution factor}}{\text{Sample mass (g)}}$$

Percent recovery for each analyte was calculated as follows:

$$\% \text{ Recovery} = \frac{\text{Residue Detected (ppm) - Average ppm Control}}{\text{Fortification Level (ppm)}} \times 100$$

Validity of the MCW-2, butene sulfonic acid and thiazole sulfonic acid residue analytical methods was established by acceptable average recovery (70-110%) from fortified untreated control samples.

An example calculation for the MCW-2 recovery in the well water method validation (0.05 ppb) is shown below for the quantitation ion:

$$\text{MCW-2 ppb} = \frac{0.0395\ \text{ng/mL x}\ 10\ \text{mL x}\ 1}{10\ \text{g}} = 0.04\ \text{ppb}$$

$$\text{Percent Recovery MCW-2} = \frac{0.040 - 0.000\ \text{ppb}}{0.05\ \text{ppb}}\ \text{x}\ 100 = 80\%$$

Similar calculations were conducted for each analyte.

Time Required for Analysis

Time required per sample set, where a sample set consists of twelve (12) water samples, 11 standards:

Sample and calibrant preparation take approximately 5 hours for one analyst

LC-MS/MS analysis and data processing takes approximately 23 hours

TOTAL = approximately 28 hours for one analysis (2 calendar days)