Analysis of Sulfentrazone and Sulfentrazone-3-Carboxylic Acid in Well Water

1. Scope:

This section method documents sulfentrazone and sulfentrazone-3-carboxylic acid (SCA) in well water and is followed by all authorized EMON personnel.

2. Principle:

A Water's Oasis ® WAX cartridge mixed-mode Weak Anion-eXchange is used to retain the sulfentrazone and sulfentrazone-3-carboxylic acid from 250mL acidified well water samples. The analytes are eluted with 5% ammonium hydroxide in methanol. The eluant is concentrated to ~ 0.3 mL and then brought up to 1 mL with water. An additional 1 mL of acetonitrile is added to make the final volume 2mL with 1:1 water/acetonitrile. The extract is analyzed by Liquid Chromatography coupled to a Linear Ion Trap Quadrupole LC/MS/MS. The reporting limit is 0.01/0.1 ppb for sulfentrazone and sulfentrazone-3-carboxylic acid respectively.

3. Safety:

All general laboratory safety rules for sample preparation and analysis shall be followed.

4. Interferences:

There were no matrix interferences that caused quantitative problems during method development and validation.

5. Apparatus and Equipment:

5.1 Nitrogen Evaporator (Meyer N-EVAP Organomation Model #112 or equivalent)
5.2 Balance (Mettler PC 4400 or equivalent)
5.3 Vortex-vibrating mixer
5.4 Solid phase extraction manifold, Supelco Visiprep TM24 or equivalent
5.5 Solid phase extraction manifold accessories: vacuum source, vacuum chamber, vacuum controller, cartridge fittings (tube adapters) and connectors, sample delivery tubing with stainless steel weight, sample collection tubes and rack.
5.6 pH meter ©Mettler-Toledo or equivalent
5.7 Ultra High-Performance Liquid Chromatography (UHPLC) equipped with a linear ion trap quadrupole (MS/MS)

6. Reagents and Supplies:

6.1 Sulfentrazone CAS# 87392-12-9
6.2 Sulfentrazone-3-carboxylic acid CAS# 171118-09-5
6.3 Methanol, MS grade, Burdick & Jackson or equivalent
6.4 Water, MS grade, Burdick & Jackson or equivalent
6.5 Hydrochloric acid 3 N
6.6 Ammonium hydroxide, reagent grade or equivalent.
6.7 Elution reagent: 5% ammonium hydroxide in methanol.
6.8 Pipettes; air cushioned and positive displacement, various volumes and types, Eppendorf or equivalent
6.9 Disposable Pasteur pipettes and other laboratory ware as needed
6.10 Solid phase extraction cartridges: Waters Oasis® WAX 6 cc (150 mg), 30-micron particle size cartridge or a (500mg) cartridge.
6.11 Graduated test tube, 15 mL (calibrated at 0.5mL with methanol)
6.12 LCMS Columns:
Analytical column: ACE Excel C18 2.0 µm, 2.1 x 100mm column or equivalent
6.13 Formic acid, HPLC grade
6.14 Ammonium formate 1.0 M
6.15 Aqueous Solution: For 500 mL, mix 470 ± 2mL water, 25 ± 0.5 mL methanol, 4.50 ± 0.25 mL 1 M ammonium formate and 0.5 ± 0.05 mL formic acid.
6.16 Organic Solution: For 500mL, mix 450 ± 2mL methanol and 45 ± 0.5 mL water with 4.50 ± 0.25 mL 1 M ammonium formate and 0.5 ± 0.05 mL formic acid.

7. Standards Preparation:

7.1 Individual stock standards of 1.0mg/mL were obtained from the CDFA/CAC Standards Repository.

The standards were diluted to 10 µg/mL with acetonitrile. A combination standard of 10/1.0 µg/mL SCA/sulfentrazone was prepared from the individual mg/mL standards in acetonitrile. The combination standard was also used to dilute to the following concentrations: 0.00125/0.0125,0.005/0.05, 0.0125/0.125, 0.025/0.25 and 0.05/0.5 µg/mL in acetonitrile which were later diluted 1-2 with clean background matrix in water for instrument calibration.

7.2 Keep all standards in the designated freezer for storage.

7.3 The expiration date of each standard is three months from the preparation date.

8. Sample Preservation and Storage:

Store all samples waiting for extraction in a separate refrigerator (4 ± 3 °C). Sample storage location and movement shall be recorded.

9. Test Sample Preparation:

9.1 Background Preparation

The Department of Pesticide Regulation (DPR) provided the well water for background to be used in method validation and QC.

9.2 Preparation of blank and spike

Matrix blank: Weigh out 250 g of background water and follow the test sample extraction procedure.

Matrix spike: Weigh out 250 g of background water. Spike a client requested amount of pesticide into the background water, mix well and let it stand for 1 minute. Follow the test sample extraction procedure.

9.3 Test Sample Extraction

9.3.1 Remove sample from refrigerator and allow them to come to ambient temperature.

9.3.2 Weigh 250 ± 0.5 g of water sample into a 600 mL beaker.

9.3.3 Adjust pH to 2.5 – 3.0 with 3 N HCL.

9.3.4 A WAX cartridge (150mg) is connected to the vacuum manifold.

9.3.5 Condition the cartridges with a total of ~6 mL of methanol at a flow rate ~ 8 mL/minutes followed by ~ 6 mL of acidified D.I. water (pH 3) by applying vacuum.

9.3.8 Turn off the vacuum when the D.I. water has just passed through the cartridges. Refill WAX cartridges with acidified D.I. water. Attach the sample delivery tubes to the cartridge and place weighted tube ends into water sample.

9.3.9 Allow the sample to pass through the conditioned cartridges by applying vacuum. Adjust the flow rate to ~ 8 mL/minute

9.3.10 After all the water sample has passed through the cartridges, increase the vacuum to ~ 20 psi for about 10 minutes. Detach the sample delivery tube from WAX cartridge. Shake out any excess water in the cartridge reservoir.

9.3.11 Place the graduated test tubes into the vacuum manifold.

9.3.12 Elute and collect all analytes with 6 ± 0.5 mL of 5% ammonium hydroxide in methanol at a flow rate of ~8 mL/minutes.

9.3.13 Concentrate the eluant to ~0.3mL in a water bath at 38 ± 2 °C under a gentle stream of nitrogen. Vortex for 10 sec. Bring to a final volume of 2.0 mL by first adjusting the volume to 1 mL with water then vortex for 30 seconds, followed by 1 mL acetontrile and vortexing for 30 seconds. Transfer the extract into an autosampler vial to be analyzed by ESI/LC/MS/MS.

10. Instrument Calibration:

10.1 A minimum of 3 levels of standards is required for linear curve.

10.2 The quadratic calibration standard curve consists of a minimum of five levels.

The recommended concentration levels of standards (Sulfentrazone/ Sulfentrazone-3-carboxylic acid) are 0.000625/0.00625, 0.00125/0.0125, 0.0025/0.025, 0.005/0.05, 0.0125/0.125, 0.025/0.25, and 0.05/0.5 µg/mL

10.3 The calibration may be achieved using linear or quadratic regression with a correlation coefficient (r) ≥ 0.995 or (r^2) ≥ 0.990.

11. Analysis:

11.1 Injection Scheme

The instrument may need to be conditioned with a few standards before running the following sequence of Standard Curve, Solvent, Matrix Blank, Matrix Spike, Test Samples and Standard Curve.

11.2 Linear Ion Trap Quadrupole LC/MS/MS Mass Spectrometer

11.1.1 LC Instrument: Shimadzu LC30
Column: ACE Excel C18 2.0 µm, 2.1 x 100 mm column
Column Temperature: 40 °C
Mobile Phase: Gradient
Solvent 1: Aqueous Solution
Solvent 2: Organic Solution

Time (min)	Flow rate (mL/min)	Solvent 1	Solvent 2
0.50	0.4	95	5
1.0	0.4	40	60
4.0	0.4	5	95
4.5	0.4	5	95
4.5	0.4	95	5

Injection Volume: 3.0 µL

11.2.2 Mass Spectrometer and Operating Parameters

Model:	ABSciex QTRAP 6500
Ion ProbeType:	Electrospray Ionization (ESI)
Ion Mode:	Positive
Curtain Gas:	35
Ion Spray Voltage:	4500
Temp:	450
Ion Source Gas 1	40
Ion Source Gas 2	60
Collision Gas:	High

Compound	RT	Precursor Ion	Product Ion	Dwell (msec)	Declustering Potential	Collision Energy	Entrance Potential	Exit Potential
SCA	2.75	416.8	**279.8**	120	141	31	10	18
		416.8	348.8	120	141	23	10	16

Sulfentrazone	3.51	403.9	**306.9**	120	41	33	10	38
		403.9	272.9	120	41	45	10	34

12. Quality Control:

12.1 Method Detection Limits (MDL)

Method Detection Limit (MDL) refers to the lowest concentration of the analyte that a method can detect reliably. To determine the MDL, 7 well water samples were spiked at 0.02/0.2 ppb for sulfentrazone/SCA and processed through the entire method along with a blank. The standard deviation derived from the spiked sample recoveries was used to calculate the MDL for SCA and sulfentrazone using the following equation:

MDL = t

Where t is the Student single tailed t test value for the 99% confidence level with n-1 degrees of freedom and S denotes the standard deviation obtained from n replicate analyses. For the n=7 replicates used to determine the MDL, t=3.143.

The results for the standard deviations and MDL are in Appendix 1.

12.2 Reporting Limit (RL)

Reporting limit (RL) refers to a level at which reliable quantitative results may be obtained. The MDL is used as a guide to determine the RL. The RL is chosen in a range 1-5 times the MDL, as per client agreement. The reporting limits for sulfentrazone and its metabolite SCA is 0.01/0.1 ppb respectively.

12.3 Method Validation

The method validation consisted of 5 sample sets for each matrix. Each set included five levels of fortification for sulfentrazone/SCA (0.02/0.2, 0.05/0.5, 0.1/1.0, 0.25/2.5 and 0.5/5.0 ppb) respectively and a method blank. All spikes and method blanks are processed through the entire analytical method. Recoveries for sulfentrazone and SCA are tabulated in Appendix 2.

12.4 Control Charts and Limits

Control charts were generated using the data from the method validation. The upper and lower warning and control limits are set at ± 2 and 3 standard deviations of the average percent recovery, respectively, shown in Appendix 2.

12.5 Acceptance Criteria

12.5.1 Each set of samples will have a matrix blank and a spiked matrix sample.

12.5.2 For positive results the retention time shall not vary from standards more than ± 0.1 minute.

12.5.3 Presence of both Qual and Quan ion.

12.5.4 The recoveries of the matrix spikes shall be within the control limits.

12.5.5 The sample shall be diluted if result exceed 10% of the highest calibration standard on the curve.

13. Calculations:

Quantitation is based on an external standard (ESTD) calculation using either the peak area or height. The software uses linear or quadratic curve fit. Alternatively, at the chemist's discretion, concentrations may be calculated using the response factor for the standard whose value is < 30% to the level in the sample.

$$ppb = \frac{(\text{sample peak area or ht}) \times (\text{std conc}) \times (\text{std vol. Injected}) \times (\text{final vol of sample}) \ (1000\ \mu L/mL)}{(\text{std.peak area or ht}) \times (\text{sample vol injected}) \times (\text{sample wt (g)})}$$

14. Reporting Procedure:

Sample results are reported out according to the client's analytical laboratory specification sheets.

Discussion:

15.1 Attempting liquid/liquid extraction for sulfentrazone and sulfentrazone- 3-carboxylic acid (SCA) resulted in ~ 80% recovery for sulfentrazone and no recovery for SCA. Acidifying the water did not improve recovery.

15.2 Experimented with several different solid phase cartridges, (HLB, MAX and C18) resulted in no successful recovery of sulfentrazone or SCA. The WAX mixed-mode Weak Anion-eXchange cartridge worked to trap both sulfentrazone and its metabolite when the pH of the water sample was adjusted to 3.

15.3 CDFA/CAC Standard Repository found that sulfentrazone-3-carboxylic acid broke down in methanol after 3 months. The standards were prepared in acetonitrile and will be monitored closely for breakdown.

16. References:

16.1 FMC Corporation 162E6698E2 *Appendix 9 Analytical Method for Sulfentrazone and Sulfentrazone-3-Carboxylic Acid in Water (limit of quantitation = 0.1ppb; limit of detection 0.02ppb)*

16.2 Hsu, J. and Hernandez, J. *Determination of Organophosphate Pesticides in Surface Water using Gas Chromatography*, 1997, Environmental Monitoring Method, Center for Analytical Chemistry, CDFA.

16.3 Fitch, P., Tran, D., updated by Lee, P., Hsu, J., White, J. *Determination of Atrazine, Bromacil, Cyanazine, Diuron, Hexazinone, Metribuzin, Norflurazon, Prometon, Prometryn, Simazine, Deethyl Atrazine (DEA), Deisopropyl Atrazine (ACET), Diamino Chlorotraizine (DACT), Tebuthiuron and the metabolites Tebuthiuron-104, Tebuthiuron-106, Tebuthiuron-107 and Tebuthiuron-108 in Well and River Water By Liquid Chromatography- triple quadrupole mass spectrometry (LC/MS/MS).* 2014*,* Environmental Monitoring Method, Center for Analytical Chemistry, CDFA.

Appendix 1

The Determination of Method Detection Limit (MDL) and Reporting Limit (RL) in Well Water

Spk\Analyte	SCA ppb	Sulfentrazone ppb
blk	nd	nd
0.2/0.02ppb spk 1	0.200	0.0193
0.2/0.02ppb spk 2	0.198	0.0192
0.2/0.02ppb spk 3	0.187	0.0176
0.20.02ppb spk 4	0.185	0.0181
0.2/0.02ppb spk 5	0.188	0.0179
0.2/0.02ppb spk 6	0.185	0.0180
0.2/0.02ppb spk 7	0.178	0.0167
SD	**0.008**	**0.001**
MDL	**0.024**	**0.003**
RL	**0.1**	**0.01**

Appendix 2

Summary of Method validation for Sulfentrazone & SCA in Well Water

Analyte	Spike ppb	Recovery (%) Set1	Set2	Set3	Set4	Set5		%
Sulfentrazone	0.02	75.5	66.0	83.8	81.3	90.0	Mean:	81.0
	0.05	86.2	91.4	82.1	81.9	70.9	SD:	7.7
	0.1	98.8	90.4	92.5	79.1	75.3	UCL:	104
	0.25	76.0	80.2	74.3	76.8	74.3	UWL:	96.4
	0.5	89.7	75.3	77.4	78.5	77.1	LWL:	65.5
							LCL:	57.8
SCA	0.2	79.8	73.3	81.8	86.5	90.5	Mean:	83.1
	0.5	83.9	86.9	76.2	79.8	74.0	SD:	7.14
	1	96.7	86.9	90.2	80.6	79.1	UCL:	105
	2.5	80.0	90.4	70.1	89.0	69.8	UWL:	97.0
	5	94.9	87.6	84.4	85.4	80.0	LWL:	68.8
							LCL:	61.7

Appendix 3

Storage Study Well Water 0.5 ppb for sulfentrazone and SCA

Analyte	Spike ppb	Recovery (%) Day 0	Day 1	Day 2	Day 4	Day 7	Day 14	Day 21	Day 28
SCA	blk								
	QC Spk		114%	85.6%	104%	89.2%	88.8%	76.0%	92.4%
	Spk 1	117%	109%	84.8%	98.8%	92.4%	90.8%	83.6%	118%
	Spk 2	108%	115%	85.6%	102%	88.4%	84.8%	84.4%	120%
	Spk 3	117%	112%	84.4%	105%	91.6%	90.4%	85.2%	111%
Sulfentrazone	blk								
	QC Spk		94.8%	101%	106%	105%	94.8%	105%	106%
	Spk 1	106%	100%	96.4%	102%	119%	123%	132%	118%
	Spk 2	102%	105%	98.8%	102%	114%	116%	130%	119%
	Spk 3	114%	100%	100%	108%	116%	125%	134%	116%

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Revision Log:

Date	What was revised? Why?
11/5/2019	Section 10.2 added compound names for the different standard levels
	Section 12.1 added single tailed t test
	Appendix 3 added spike level