Analysis of Sulfentrazone and Sulfentrazone-3-Carboxylic Acid in Soil and Turf

1. Scope:

   This section method (SM) documents sulfentrazone and its metabolite sulfentrazone-3-carboxylic acid (SCA) analysis in soil and turf are followed by all authorized EMON personnel.

2. Principle:

   The SM describes the method for determination of sulfentrazone and its metabolite sulfentrazone-3-carboxylic acid in soil and turf. The sample is homogenized and a 10g subsample is extracted with 1:1 acetonitrile/water for 2 minutes. The extract is then allowed to set for 30 minutes, before centrifuged at 3500rpm for 6 minutes to facilitates the partition of solid and solvent phases. The sample extract is then filtered and analyzed by Liquid Chromatography coupled to a Linear Ion Trap Quadrupole LC/MS/MS. The reporting limit is 0.01/0.1 ppm for sulfentrazone and sulfentrazone-3-carboxylic acid.

3. Safety:

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

   3.2 Acetonitrile is a flammable and toxic solvent; It should be handled with care in a ventilated area.

4. Interferences:

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

5. Apparatus and Equipment:

   5.1 Centrifuge, (IEC/Centra/Gp8 or equivalent)
   5.2 Balance, (Mettler PC 4400 or equivalent)
   5.3 Cuisinart™ food processor (Model DLC7) or equivalent
   5.3 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 Acetonitrile, nanograde or equivalent pesticide grade
6.4 Water, MS grade, Burdick & Jackson or equivalent
6.5 50 mL polypropylene centrifuge tube
6.6 3/8" Stainless steel grinding balls, (Spex-Certiprep)
6.7 13 mm, 0.2µm hydrophilic PTFE membrane syringe filter, Advantec
6.8 1 mL polypropylene syringe
6.9 Formic acid, HPLC grade
6.10 Ammonium formate 1.0 M
6.11 Borosilicate disposable glass tube 12x75 mm or equivalent
6.12 Disposable Pasteur pipettes and other laboratory ware as needed
6.13 Pipettes; air cushioned and positive displacement, various volumes and types, Eppendorf or equivalent
6.14 Recommended UHPLC analytical column:
ACE Excel C18 2.0 µm, 2.1 x 100 mm column or equivalent
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 1.0 mg/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 with clean background matrix to the following concentrations: 0.0125/0.00125, 0.025/0.0025, 0.05/0.005, 0.125/0.0125, 0.25/0.025, 0.5/0.05 and 1.0/0.01 µg/mL 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 freezer. If soil samples are to be extracted the next day, they may be stored in the refrigerator. Sample extracts shall be stored in the 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 soil and turf for background to be used in method validation. The background turf was chopped and then ground with dry ice to homogenize it. The homogenize background turf was stored in the freezer with lid lose to allow the dry ice to sublime overnight. The lid was tightened the next day. The background soil was passed through a # 8 sieve (2.36 mm) and mixed well to homogenize it. The soil used for background was dry and sandy.

9.1.1 Blank

Mix background sample well before weighing out 10 ± 0.1 g. Proceed to step 9.2.2 of section 9.2.

9.1.2 Spike

Mix background sample well before weighing out 10 ± 0.1 g. Fortify at the level requested by client and mix well to ensure that the pesticides are well distributed. The spiked background was allowed to sit for 30 minutes before proceeding to step 9.2.2 of section 9.2.

9.1.3 Moistures

9.1.3.1 Thaw soil and homogenized thoroughly.

9.1.3.2 Weigh out a 15 – 20 g sub-sample into a pre-weighed aluminum weighing pan.

9.1.3.3 Dry the pan with soil for at least 6 hours in a ~ 105°C oven.

9.1.3.4 Weigh soil after cooling in a dessicator.

9.1.3.5 Report the wet and dry weights on Chain of Custody sample sheets.

9.2 Sample Preparation

9.2.1 Turf Sample

Turf sample was chopped and then ground in a Cuisinart with dry ice to homogenize it. The homogenize sample was transfer back to the original container, covered with aluminum foil and capped with lid loosely. The sample was stored in the freezer overnight to allow the dry ice to sublime. The lid was tightened the next day.

9.2.2 Soil Sample

Soil sample was homogenized by hand mixing and any large debris (e.g., gravel, sticks) was removed.

9.3 Test Sample Extraction

9.3.1 Soil sample -Take sample out of freezer and allow it to come to ambient temperature. Weigh out a 10 ± 0.1 grams sub-sample of homogenized soil into a 50mL polypropylene centrifuge tube.

Turf sample-Take homogenized sample out of freezer and weigh out immediately 10 ± 0.1 grams sub-sample into a 50mL polypropylene centrifuge tube. Return remaining sample to freezer immediately to avoid additional thawing.

9.3.2 Add 30 mL mixture of acetonitrile/water 1:1 and two stainless steel balls to the centrifuge tube. Wipe any particle from rim of tubes before capping and keep tubes in vertical position to minimize leaking. Cap tubes securely and shake vigorously for 2 minutes.

9.3.3 Allowed the sample to sit for 30 min. Vortex the sample for 15 seconds before centrifuge for 6 minutes at 3500rpm.

9.3.4 Pour ~5 mL of extract into pre-labeled 12x75 mm disposable tubes taking care not to disturb the solid layer.

9.3.5 Using a 1 mL polypropylene syringe remove ~ 1 mL of extract and filter through 0.2µm hydrophilic PTFE membrane syringe filter into autosampler vial. Analyze using 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/ Sulfontrazone-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

11.2.1 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:	AB Sciex QTRAP 6500
Ionization method:	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	Precusor 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 soil and turf samples were spiked at 0.02/0.2 ppm 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 ppm 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 ppm) 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 $\pm$ 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 ± 0.1minute.

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 external standard (ESTD) curve calculation using either the peak area or height. Comparison of sample response to a standard response curve within the validated range Alternatively, at chemist discretion, concentrations may be calculated using the response factor for the standard whose value is closest to the level in the sample.

$$ppm = \frac{(\text{sample peak area or ht}) \times (\text{std conc}) \times (\text{std vol. Injected}) \times (\text{final vol of sample})}{(\text{std.peak area or ht}) \times (\text{sample vol injected}) \times (\text{sample wt (g)})}$$

14. Reporting Procedure:

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

15. Discussion:

15.1 The QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) extraction procedure was tried for turf sample since the approach helps to minimize solvent use and sample manipulation. Sulfentrazone had recoveries of 70-80% while the metabolite SCA had no significant recovery. Acidifying the extraction solvent, helped a little, sulfentrazone had a recovery of ~90% while the recovery for SCA was ~ 30%. Reducing the sample size with same amount extracting solvent gave similar low SCA recovery results.

When the salting out (magnesium Sulfate/Sodium Acetate) and PSA (primary and secondary amine) steps were skipped in the QuECHERS method, the recovery improved 60% for SCA. Therefore,1:1 Water/Acetonitrile was tried as the extraction solvent with two steel grinding beads added to help with extraction efficiency.
Sulfentrazone-3-carboxylic acid is very water soluble making water the better extraction solvent but adding acetonitrile improved recoveries for sulfentrazone. Recoveries range in the 80-90% for both compounds. The samples extract was filter through the 0.2 µm hydrophilic PTFE syringe filter and experienced no sample recovery loss. Two kinds of Nylon Acrodisc ® 0.2 µm and 0.45 µm syringe filter, the SCA had < 50% recovery and Sulfentrazone kept the same recovery as not filtered.

15.2 Decreased sensitivity in calibration standards during the run became another issue which had to be addressed. Various SPE cartridges were tried to clean up the sample extract but didn't solve the matrix suppression issues and lead to recovery losses. The matrix suppression issue was minimized by using matrix-matched standards.

15.3 CDFA/CAC standard Respository 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. Standards will be prepared in acetonitrile and stored in the freezer.

15.4 A storage stability study was done with this project. The storage stability study consisted of a 0.25/2.5 ppm spike level for sulfentrazone/SCA and 3 replicates over a 28-day period. Twenty-four 50 mL polypropylene centrifuge tubes contain 10g of background soil and twenty-four 50 mL polypropylene centrifuge tube containing 10g of background turf were spiked and then capped and stored in the freezer. The spiked samples were then analyzed on 0,1, 2, 4, 7, 14, 22 and 28 days by LCMSMS. Along with the storage spikes a blank and method control spike were also extracted.
Results for the storage study are shown in Appendix 3.

16. References:

16.1 FMC Corporation 162E6698E2 *Conventional Analytical Method for Sulfentrazone and Sulfentrazone-3-Carboxylic Acid in/on Soil*

16.2 Ana Beatriz R.J. Passos, Marco Antonio M. Freitas, Valdinei A. Goncalves, Gustavo S. Silva, Antonio Alberto da Silva, Maria Eliana L. R. Queiroz, Claudio F. Lima, Daniel V. Silva, *Leaching of Sulfentrazone in soils of reforestation in Brazil,* Environ Earth Sci. DOI 10.1007/S1265-015-4110-7

16.3 RES-SM-4, Rev.4; 2018 California Department of Food and Agriculture, Center for Analytical Chemistry, Pesticide Residue Laboratory, Section Method.

16.4 University pf Rochester, Environmental Health and Safety, Dry Ice Handling Procedures, https://www.safety.rochester.edu/ih/dryicehandle.html

Appendix 1

Turf -The determination of Method Detection Limit (MDL) and Reporting Limit (RL)

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

Soil -The determination of Method Detection Limit (MDL) and Reporting Limit (RL)

Spk\Analyte	SCA ppm	Sulfentrazone ppm
blk	nd	nd
0.2/0.02ppm spk 1	0.221	0.0217
0.2/0.02ppm spk 2	0.215	0.0209
0.2/0.02ppm spk 3	0.199	0.0201
0.20.02ppm spk 4	0.213	0.0208
0.2/0.02ppm spk 5	0.209	0.0206
0.2/0.02ppm spk 6	0.209	0.0206
0.2/0.02ppm spk 7	0.216	0.0211
SD	**0.007**	**0.0005**
MDL	**0.022**	**0.002**
RL	**0.1**	**0.01**

Appendix 2

Summary of Method Validation for SCA/Sulfentrazone in Turf

Analyte	Spike ppm	Recovery (%) Set1	Set2	Set3	Set4	Set5		%
SCA	0.2	88.5	94.0	98.5	82.5	95.5	Mean:	91.1
	0.5	88.0	86.2	98.0	88.6	90.2	SD:	7.22
	1	86.9	92.2	110	75.4	90.5	UCL:	113
	2.5	84.8	99.2	100	91.6	86.4	UWL:	106
	5	85.2	93.6	100	87.2	84.6	LWL:	76.6
							LCL:	69.4
Sulfentrazone	0.02	87.0	91.0	105	83.0	94.5	Mean:	87.4
	0.05	85.4	90.6	105	85.8	91.6	SD:	9.73
	0.1	79.2	85.5	99.4	61.8	92.1	UCL:	117
	0.25	81.2	84.4	94.8	84.8	76.0	UWL:	107
	0.5	81.0	89.0	102	75.4	81.2	LWL:	67.9
							LCL:	58.2

Summary of Method Validation for SCA/Sulfentrazone in Soil

Analyte	Spike ppm	Recovery (%) Set1	Set2	Set3	Set4	Set5		%
SCA	0.2	103	116	107	107	90.5	Mean:	104
	0.5	105	107	95.8	98.2	93.6	SD:	7.55
	1	109	116	97.0	102	97.8	UCL:	126
	2.5	109	115	98.4	110	97.6	UWL:	119
	5	105	111	91.2	108	99.6	LWL:	88.7
							LCL:	81.1
Sulfentrazone	0.02	107.0	112	103	109	91.0	Mean:	97.4
	0.05	108	111	96.4	96.8	94.6	SD:	10.2
	0.1	110	107	88.5	91.7	74.0	UCL:	128
	0.25	101	103	82.8	100	84.8	UWL:	118
	0.5	97.6	98.2	80.4	98.0	88.8	LWL:	76.9
							LCL:	66.7

Appendix 3

Storage Study for sulfentrazone/SCA in soil/turf at 0.1 and 1.0 ppb

Turf

Analyte	Spike ppm	Recovery (%) Day 0	Day 1	Day 2	Day 4	Day 7	Day 14	Day 21	Day 28
SCA	blk								
	QC Spk		118%	109%	96.4%	112%	106%	123%	119%
	Spk 1	102%	104%	103%	116%	101%	107%	97.2%	109%
	Spk 2	84.8%	102%	103%	115%	99.6%	98.4%	105%	115%
	Spk 3	94.8%	105%	98.8%	91.2%	108%	104%	110%	112%
Sulfentrazone	blk								
	QC Spk		105%	92.8%	92.8%	97.6%	93.2%	94.8%	98.8%
	Spk 1	105%	94.0%	85.6%	106%	98.0%	94.4%	86.4%	84.4%
	Spk 2	82.4%	86.4%	89.6%	100%	94.4%	88.8%	93.2%	92.8%
	Spk 3	82.4%	88.0%	85.2%	84.4%	94.8%	92.8%	88.4%	91.6%

Soil

Analyte	Spike ppm	Recovery (%) Day 0	Day 1	Day 2	Day 4	Day 7	Day 14	Day 21	Day 28
SCA	blk								
	QC Spk		127%	116%	120%	128%	121%	122%	124%
	Spk 1	119%	128%	115%	123%	124%	118%	119%	136%
	Spk 2	127%	128%	110%	125%	119%	128%	118%	135%
	Spk 3	131%	123%	117%	112%	108%	126%	128%	128%
Sulfentrazone	blk								
	QC Spk		108%	103%	88.0%	109%	111%	103%	110%
	Spk 1	104%	108%	106%	96.0%	102%	102%	94.4%	110%
	Spk 2	105%	113%	108%	90.8%	99.2%	110%	98.4%	102%
	Spk 3	116%	105%	101%	87.6%	95.2%	106%	100%	99.6%

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Revision Log:

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