1 SCOPE

1.1 This method describes the determination of FOE 5043 and four metabolites (sulfonic acid, alcohol, oxalate and thiadone) in groundwater by High Performance Liquid Chromatography Electrospray Ionization Tandem Mass Spectrometry (LC-ESI/MS/MS) following C18-Solid Phase Extraction.

1.2 A quantitative reporting limit of 0.10 ppb for all analytes has been demonstrated using a concentration factor of 25 (50 mL/2 mL).

2 METHOD VALIDATION

2.1 This method (Revision 0) has been validated by performing a Method Detection Limit (MDL) (See Reference 11.1) study using California groundwater. The resulting data has been summarized in tables III and IV. All of the associated raw data is archived at Alta Analytical Laboratory, Inc. Prior to the use of this method on water from a different source, it must be verified to demonstrate its applicability.

3 PRINCIPLES

3.1 Analytes are extracted from water by drawing 50 mL of acidified sample through a 6-mL octadecyl (C18) SPE column. The analytes are eluted with 6 mL of methanol and concentrated to ~1 mL using nitrogen and a water bath at 25-30 °C. The concentrate is brought up to 2.0 mL with 0.1% formic acid. After syringe filtering, the extracts are analyzed by LC-ESI/MS/MS.

3.2 The analytes are determined by reversed phase liquid chromatography, using 0.1% formic acid and acetonitrile. The standards and sample extracts are injected onto a base deactivated reversed phase column connected to a triple quadrupole mass spectrometer. An electrospray (ESI) atmospheric pressure ionization (API) inlet is used.

3.3 The sulfonic acid, thiadone, and oxalate metabolites are analyzed by negative ionization MS/MS and the alcohol and parent are analyzed by positive ionization MS/MS using a second injection.

3.4 Quantitation is performed using the area response factors of the native compounds relative to their stable isotope internal standards. A calibration check standard (CCS) is analyzed at the onset and completion of every analytical sample set. The response factor from each calibration check standard is compared to the average response factor from a triplicate 4 point calibration curve.

4 MATERIALS AND REAGENTS

4.1 Apparatus

4.1.1 Balance, Analytical, capable of weighing to the nearest 0.0001 g.

4.1.2 Balance, Toploader, capable of weighing to the nearest 0.01 g.

4.1.3 Bottle, amber, appropriate size for storage of standard solutions.

4.1.4 Vials, 40-mL VOA (volatile organic analysis) (CMS-273-346 or equivalent).

4.1.5 Bottle, 250-mL polypropylene (Nalgene 3121-0250 or equivalent).

4.1.6 Graduated centrifuge tubes, 10-mL calibrated "To Contain", readable to 0.1-mL (CMS 253-819 or equivalent).

4.1.7 Eppendorf Repeater Pipette (CMS 109-967 or equivalent).

4.1.8 Eppendorf 5-mL tip (CMS 171-959 or equivalent).

4.1.9 Pipets, 0.5-mL (CMS 080-465 or equivalent).

4.1.10 Pipets, 1-mL (CMS 080-507 or equivalent).

4.1.11 Pipets, 2-mL (CMS 080-515 or equivalent).

4.1.12 Pipets, 5-mL (CMS 080-523 or equivalent).

4.1.13 Pipets, 10-mL (CMS 080-531 or equivalent).

4.1.14 Gastight syringe, 100 μL (SUPELCO 2-0688 or equivalent).

4.1.15 Gastight syringe, 250 μL (SUPELCO 2-0689 or equivalent).

4.1.16 Gastight syringe, 500 μL (SUPELCO 2-0690 or equivalent).

4.1.17 Positive displacement micropipets, 50/100 μL, 100/200 μL (WIRETROL II, Cole Parmer N-07951-20,-25 or equivalent).

4.1.18 Pasteur pipettes (CMS 355-123 or equivalent).

4.1.19 Flask, volumetric 25-mL (CMS 105-304 or equivalent).

4.1.20 Flask, volumetric 50-mL (CMS 106-138 or equivalent).

4.1.21 Flask, volumetric 100-mL (CMS 105-320 or equivalent).

4.1.22 Autosampler vials, 1-mL (Waters 78514 or equivalent).

4.1.23 Disposable syringe, 5-mL with Luer-Lok (CMS 262-273 or equivalent).

4.1.24 Syringe filter disks, Gelman Acrodisc CR 0.45-μm or smaller (CMS 141-226 or equivalent).

4.1.25 N-EVAP, 24 position (Organomation Model 112-P or equivalent).

4.1.26 BAKERBOND SPE-24G manifold system (VWR JT7208-0 or equivalent).

4.1.27 BAKERBOND SPE column reservoirs, 75-mL (VWR JT7120-3 or equivalent).

4.1.28 Bond Elut column adapters (Varian A1-121310-01 or equivalent).

4.1.29 Vacuum pump (Grainger #4Z0260 or equivalent).

4.1.30 Mega Bond Elut SPE C18 extraction columns, 1 g capacity (Varian 1225-6001 or equivalent).

4.2 Reagents

4.2.1 Water, Omnisolve, HPLC grade (CMS MWX004-1 or equivalent).

4.2.2 Hydrochloric acid, 37% (Mallinckrodt 2612-07 or equivalent).

4.2.3 Formic acid, Chempure (CMS 830-937 or equivalent).

4.2.4 Methanol, EM Omnisolve HPLC grade (CMS MX0488-1 or equivalent).

4.2.5 Acetonitrile, EM Omnisolve HPLC grade (CMS MAX 0142-1 or equivalent).

4.2.6 HPLC aqueous mobile phase, 0.1% formic acid (v/v). Add 1 mL of formic acid to 1 L of water.

4.2.7 HPLC organic modifier, ACN with 0.1% formic acid.

4.2.8 1N HCl, add 83 mL hydrochloric acid to 917 mL of water.

4.2.9 0.1% Formic acid (v/v). Add 1 mL formic acid to 1 L of water.

4.2.10 The FOE 5043, FOE 5043 alcohol, FOE 5043 oxalate, FOE 5043 sulfonic acid (sodium salt monohydrate form) and their deuterated analogs and FOE 5043 thiadone and its $^{13}C/^{15}N_2$ analog were provided by Bayer Corp., Agricultural Division, 17745 South Metcalf, Stilwell, KS 66085-9104.

5 SAFETY AND HEALTH

5.1 The in depth toxicity and potential carcinogenicity of FOE 5043 and its metabolites have not been determined. Thus, each chemical compound must be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available.

6 ANALYTICAL PROCEDURES

6.1 Preparation of Standard Solutions

These are guidance examples; the actual exact weights, volumes and concentrations may vary slightly but must be documented appropriately.

6.1.1 Stock solutions (nominally 0.1 mg/mL) of each native analyte are prepared by dissolving reference material in methanol. In the case where the FOE 5043 sulfonic acid is in the sodium salt monohydrate form (molecular weight = 315.3), a correction for the weight discrepancy must be made. If the compound purity for the native analyte is certified at 96% or greater, the weight may be used without correction to calculate the concentration

of the native stock standard. Transfer the stock solutions into amber bottles and seal with teflon lined caps only. Store the standards in a freezer at -10 °C or colder and protect from light when not in use. New stock solutions must be prepared or solutions must be re-assayed after 1 year or sooner if comparison with check standards indicates a problem. Analytes received already in solution need only be diluted appropriately prior to use.

6.1.2 Stock solutions (nominally 0.1 mg/mL) of each labeled analyte (internal standard) are prepared by dissolving reference material in methanol. No correction for purity or form is necessary. Transfer the stock solutions into amber bottles and seal with teflon lined caps only. Store the standards in a freezer at -10° C or colder and protect from light when not in use. New stock solutions must be prepared or solutions must be re-assayed after 1 year or sooner if comparison with check standards indicates a problem. Analytes received already in solution need only be diluted appropriately prior to use.

6.1.3 Using the native stock solution from 6.1.1, prepare a 5 µg/mL mixed intermediate native stock solution in methanol, either directly or via other intermediate stock solutions.

6.1.4 Using the internal standard stock solution from 6.1.2, prepare a 1 µg/mL mixed internal standard solution in methanol, either directly or via other intermediate stock solutions.

6.1.5 Using the 5 µg/mL mixed intermediate native stock solution from 6.1.3, prepare a 1 µg/mL mixed intermediate native stock solution or fortification solution in methanol.

6.1.6 If needed, additional fortification standards may be prepared by dilution of the 5 µg/mL or 1 µg/mL solutions (6.1.3, 6.1.5) with methanol. Fortification standards should be prepared such that between 100 µL and 1 mL is used for sample fortification.

6.1.7 A four point LC/MS/MS calibration curve should be prepared (in 0.1% formic acid) such that the low standard represents a concentration at 1/2 of the reporting limit. Each calibration curve standard solution will contain the internal standard at 50 ng/mL.

Example of a Calibration Curve:

100 ng/mL standard (equivalent to a 4.0 ppb sample): dilute 2 mL of the 5 µg/mL mixed intermediate native stock (6.1.3), 5 mL of the 1 µg/mL mixed internal standard stock (6.1.4) and 43 mL of methanol to 100 mL with 0.1% formic acid.

20 ng/mL standard (equivalent to a 0.80 ppb sample): dilute 400 µL of the 5 µg/mL mixed intermediate native stock (6.1.3), 5 mL of the 1 µg/mL mixed internal standard stock (6.1.4) and 44.6 mL of methanol to 100 mL with 0.1% formic acid.

5 ng/mL standard (equivalent to a 0.20 ppb sample): dilute 500 µL of the 1 µg/mL mixed intermediate native stock (6.1.5), 5 mL of the 1 µg/mL mixed internal standard stock (6.1.4) and 44.5 mL of methanol to 100 mL with 0.1% formic acid.

1.25 ng/mL standard (equivalent to a 0.05 ppb sample): dilute 125 µL of the 1 µg/mL mixed intermediate native stock (6.1.5), 5 mL of the 1 µg/mL mixed internal standard stock (6.1.4) and 44.8 mL of methanol to 100 mL with 0.1% formic acid.

6.2 Fortification

6.2.1 The method is validated for the matrix by analyzing a control sample and one or more control samples fortified prior to the extraction procedure at or above the reporting limit.

6.2.2 Sample fortification should be conducted such that between 100 µL and 1 mL of fortification solution is used. The preparation of the fortification standards is described in Section 6.1.

6.2.3 Add an appropriate fortification solution to the sample. (Example: add 100 µL of a 1 µg/mL fortification solution to 50 mL of sample to fortify at 2 ppb.) Swirl the sample bottle to mix the contents.

6.3 Extraction/Concentration

6.3.1 Shake the sample vigorously, transfer 50 mL of sample (by weight difference or the use of a graduated cylinder) to an appropriate container. Any sample fortification should be done at this step (see Section 6.2).

6.3.2 Add 100 µL of internal standard solution (1 µg/mL).

6.3.3 Add ~10 mL of 1N HCl and mix well.

6.3.4 Attach the vacuum line to the manifold to draw the conditioning solvent through the SPE column.

6.3.5 Condition the SPE column with 6 mL of methanol followed by 12 mL of HPLC water. Do not allow the column to go dry.

6.3.6 Attach a 75 mL reservoir to the column and draw the sample through the column at ~3" Hg.

6.3.7 Rinse the sample container with ~5 mL of HPLC water and add to the reservoir.

6.3.8 Draw air through the column for <15 seconds.

6.3.9 Elute the column with 6 mL of methanol.

6.3.10 Collect the eluate in a 10 mL calibrated conical tube or collect in a test tube and transfer to a calibrated conical tube with methanol. Unless performed immediately, the eluate should be stored in a freezer while awaiting concentration (6.3.11).

6.3.11 Evaporate the eluate to ~1 mL with nitrogen using a water bath at ~30 °C. Evaporation below this ~1 mL may result in the loss of FOE 5043 thiadone due to its volatility.

6.3.12 Dilute the extract to 2.0 mL using 0.1% formic acid.

6.3.13 Filter a portion of the extract using a 0.45-µm or smaller syringe filter into an HPLC autoinjector vial. Store these samples in a freezer until analysis.

6.4 LC-ESI/MS/MS

6.4.1 Standards and extracts are analyzed by LC-ESI/MS/MS on an Inertsil (MetaChem Technology, Torrence, CA) base deactivated reversed phase column (50 mm x 2 mm). The analyses are performed using gradient chromatography with 0.1% formic acid and acetonitrile as the organic modifier. The liquid flow of 0.4 mL/min is introduced directly with a post-column split (~1:10) to the ESI interface resulting in a flow to the ESI interface of ~40 µL/min. See Table I for a description of chromatography conditions for FOE 5043 and the alcohol metabolite. See Table II for a description of the chromatography conditions for the sulfonic acid, oxalate, and thiadone metabolites.

6.4.2 The analyses are by either negative ion MS/MS using the molecular anions as precursors or positive ion MS/MS using the protonated molecular ions as precursors. Product ions are formed by collisionally induced dissociation (CID) of the precursors in the collision cell (MS/MS). The predominant product ions are mass analyzed in the third quadrupole filter. See Table I for a description of positive ion instrument conditions. See Table II for a description of the negative ion instrument conditions.

6.4.3 The chromatography conditions and interface parameters used for every sample analysis set must be recorded. See Figure 1 for the method parameters log used for positive ion MS/MS analyses. See Figure 2 for the method parameters log for negative ion MS/MS analyses.

6.4.4 Sample quantitation must begin and end with the injection of a continuing calibration standard. See Section 9 for a description of the quantitation.

6.4.5 An analysis set may begin with one or more system performance check injections. These injections are standard solutions which are not used for quantitation.

6.4.6 See Figure 3 for an example of a positive MS/MS analysis. See Figure 4 for an example of a negative MS/MS analysis.

7 METHOD NOTES

7.1 There are no known interferences originating from the sample preparation, extraction, cleanup or concentration procedure using the reagents and procedures specified.

7.2 The mobile phase composition and conditions may be altered if there are matrix interferences. If a change is made, document the change in the data packet.

7.3 The analysis is performed with two injections because the chromatographic resolution between the thiadone and alcohol metabolites is not sufficient to allow switching from negative to positive ion detection in a single run.

7.4 Due to the volatility of FOE 5043 thiadone, extracts must be concentrated carefully. See 6.3.11.

8 TIME REQUIREMENTS

8.1 Approximately 24 samples can be prepared for analysis in 8 hours.

8.2 Each MS/MS analysis requires approximately 15 minutes.

9 METHOD OF QUANTITATION

9.1 Quantitation is based on a four point calibration curve analyzed in triplicate using internal standards to adjust for instrument response.

9.2 The average response factor (See Section 10.2) from the initial calibration curve is used for all subsequent analyses, after verification that the instrument is still in calibration. Verification is accomplished by the analysis of a calibration check standard (CCS, 5 ng/mL) before and after each group of up to 30 extract injections (samples, controls, blanks and matrix spikes).

9.3 If the response factor (See Section 10.1) from the CCS is within ± 20% of the average response factor from the initial calibration curve, the instrument is considered to be in calibration and analyses may continue. If the response factor for the CCS is outside of this value, it may be immediately reinjected. If the repeat response factor is acceptable analyses may continue. In the case where the repeat response factor also fails, a new initial calibration curve must be analyzed before sample analyses may begin again.

9.4 If an analyte response exceeds or is expected to exceed that of the highest calibration standard, a smaller sample aliquot must be extracted and analyzed.

9.5 Deviations from the above guidelines must be noted in the raw data.

10 CALCULATIONS

10.1 Calculate the response factor (RF) from the following equation:

$$RF = \frac{(Area_{NAT})\,(Conc_{IS})}{(Area_{IS})\,(Conc_{NAT})}$$

Where:
- $Area_{NAT}$ = the area of response for the product ion from the native standard,
- $Area_{IS}$ = the area of response for the product ion from the internal standard,
- $Conc_{NAT}$ = concentration of the native standard (ng/mL),
- $Conc_{IS}$ = concentration of the internal standard (ng/mL),

10.2 Quantitations will be performed using the average response from an initial four point calibration curve analyzed in triplicate:

$$RF_{AVG} = (\Sigma\ RF_i)/12;\ i = 1 \text{ to } 12$$

10.3 The relative standard deviation (RSD) is derived from the coefficient of variation (CV):

$$CV = sd/RF_{AVG}$$

$$RSD = CV \times 100 \text{ (percent)}$$

Where: CV = coefficient of variation,
RF_{AVG} = Average RF (See Section 10.2),
sd = standard deviation (sample) of the RF_{AVG},

10.4 Sample calculations are done according to the following formula:

$$\text{Calc Amt} = \frac{(\text{Area}_{NAT})\,(\text{Conc}_{IS})\,(Df)}{(RF_{AVG})\,(\text{Area}_{IS})}$$

Where: Calc Amt = calculated amount (ng/mL), uploaded from mass spectrometer,
Area_{NAT} = the area of response for the native product ion from the extract,
Area_{IS} = the area of response for the internal standard product ion from the extract,
Conc_{IS} = concentration of the internal standard (ng/mL)
RF_{AVG} = average response factor,
Df = dilution factor ($\text{Vol}_{fin}/\text{Vol}_{init}$),

$$\text{Amt SAMP} = \frac{(\text{Calc Amt})\,(FV)}{(Q_{SAMP})\,(Af)}$$

Where: Amt_{SAMP} = final sample amount of native analyte (ppb),
FV = final volume (mL),
Q_{SAMP} = quantity of sample extracted (g or mL),
Af = aliquot factor ($\text{Vol}_{alq}/\text{Vol}_{total}$),

10.5 If Amt_{SAMP} is less than the reporting limit, then the results are reported as ND (not detected). Field sample results are reported to two significant figures.

10.6 The results for fortified samples are reported to three significant figures.

10.7 Percent recoveries are reported to two significant figures if the result is less than 100% and three significant figures if the result is greater than 100%.

11 REFERENCES

11.1 40 CFR Ch. 1, Part 136, App. B, 7-1-94 Edition.

11.2 Keith, L. H., et al. 1983. "Principles of Environmental Analysis," Anal. Chem., 55, 2210-2218.

TABLE 1 - Instrument Parameters for the Determination of FOE 5043 and FOE 5043 Alcohol.

Instrumentation:

Shimadzu LC-10AD HPLC pumps (2) with SCL-10A Controller and SIL-10A Autoinjector (or equivalent)
SCIEX API III Plus triple quadrupole mass spectrometer equiped with ESI (or equivalent)

HPLC Operating Conditions:

Column: Inertsil ODS-2 (5μ), 50 x 2 mm, (MetaChem, Torrence, CA) P/N 0296-050X020
Injection Vol. 50 μL (10-100 μL)
Flow Rate: 0.4 mL/min (split ~1:10)

Time (min.)	Gradient	0.1% Formic acid in water	Acetonitrile (0.1% formic acid)
Initial	-	90	10
6	Linear	10	90
7.1[(A)]	Step	90	10

(A) - This step may be moved out in time, as appropriate, to allow for increased rinsing of the chromatographic column between injections.

Mass Spectrometer Operating Parameters:

Curtain Gas: 1 L/min (±0.3L/min)
ISV Voltage: 4.5 kV
Orafice: 40 V (±20V)
Nebulizer Gas: 80 psi (±15 psi)
Aux. Gas 2.5 L/min (±0.5 L/min)
Collision Pressure: Argon @ 280 cgt (±25 cgt)
Collision Offset: -15 V (±3 eV)

Analyte	MW	Precursor Ion (m/z±0.1)	Product Ion (m/z±0.1)	Dwell (sec)	Retention Time (approx. min:sec)
FOE 5043	363	364	194	0.4	6:15
d7-FOE 5043	370	371	201	0.4	6:15
FOE 5043 alcohol	211	212	170	0.4	4:30
d7-FOE 5043 alcohol	218	219	171	0.4	4:30

TABLE II - Instrument Parameters for the Determination of FOE 5043 Oxalate, FOE 5043 Sulfonic Acid and FOE 5043 Thiadone.

Instrumentation:

Shimadzu LC-10AD HPLC pumps (2) with SCL-10A Controller and SIL-10A Autoinjector (or equivalent)
SCIEX API III Plus triple quadrupole mass spectrometer equiped with ESI (or equivalent)

HPLC Operating Conditions:

Column: Inertsil ODS-2 (5μ), 50 x 2 mm, (MetaChem, Torrence, CA) P/N 0296-050X020
Injection Vol. 50 μL (10-100 μL)
Flow Rate: 0.4 mL/min (split ~1:10)

Time (min.)	Gradient	0.1% Formic acid in water	Acetonitrile (0.1% formic acid)
Initial	-	90	10
1	-	90	10
6	Linear	30	70
6.1[(A)]	Step	90	10

(A) - This step may be moved out in time, as appropriate, to allow for increased rinsing of the chromatographic column between injections. Alternatively, a rinse cycle may be added if column cleaning is needed.

Mass Spectrometer Operating Parameters:

Curtain Gas: 1 L/min (± 0.3L/min)
ISV Voltage: -3.5 kV
Orafice: -60 V (± 20V)
Nebulizer Gas: 80 psi (± 15 psi)
Aux. Gas 2.5 L/min (± 0.5 L/min)
Collision Pressure: Argon @ 280 cgt (± 25 cgt)
Collision Offset: 15 V (± 3 eV)

Analyte	MW	Precursor Ion (m/z ± 0.1)	Product Ion (m/z ± 0.1)	Dwell (sec)	Retention Time (approx. min:sec)
FOE 5043 oxalate	225	224	152	0.3	5:00
d7-FOE 5043 oxalate	232	231	159	0.3	5:00
FOE 5043 sulfonic acid	275	274	121	0.3	5:45
d7-FOE 5043 sulfonic acid	282	281	121	0.3	5:45
FOE 5043 thiadone	170	169	113	0.3	4:55
FOE 5043 thiadone ($^{13}C/^{15}N_2$)	173	172	113	0.3	4:55