1 SCOPE

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

1.2 A quantitative reporting limit of 10 ppb for each analyte has been demonstrated using a concentration factor of 1g/1mL.

1.3 This revision (Revision 1) will allow small modifications to the chromatography conditions and acqusition parameters to be made as needed.

2 METHOD VALIDATION

2.1 This method has been validated (Revision 0) by performing a Method Detection Limit[11.1] (MDL) study using soil from a site in Fairmont, North Carolina. The resulting data have 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 a different soil, it is prudent to verify its applicability. No additional validation was required for this revision.

2.2 Revision 1 also incorporates the sponsor's request to change the extraction solvent from 0.1% HCl:ACN to 0.1N HCL:ACN. This change was validated by comparing the results from spikes at 10 ppb (in duplicate), 20 ppb (in duplicate) and 50 ppb extracted with 0.1% HCl:ACN and extracted with 0.1N HCl:ACN. Two sites were involved in this comparision. (See BAYER Protocol F3212401 and F3212402).

3 PRINCIPLES

3.1 Soil samples (10 g) are extracted with 20 mL of 0.1N HCl:ACN (1:1) by shaking on an orbital shaker for 1 hour. After centrifuging, a 10 mL aliquot of the extract is transferred to a conical tube. After the addition of 100 μL of an internal standard solution and ~1 mL of methanol, the solution is concentrated to ~4.9 mL using nitrogen and a water bath at 25-30 ° C. The concentrate is brought up to 5 mL with 0.1% formic acid. After syringe filtering, the extracts are analyzed by LC-ESI/MS/MS (5g/5mL).

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

4.1.5 Vials, 40-mL VOA (volatile organic analysis) (CMS-273-346 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 Pipets, 10-mL disposable (CMS 344-432 or equivalent).

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

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

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

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

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

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

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

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

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

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

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

4.1.26 Centrifuge (IEC Centra-8 or equivalent).

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

4.1.28 Mechanical shaker, orbital (Fisher 12-812 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 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 0.1N HCl (v/v), dilute 8.3 mL hydrochloric acid to 1 L with water.

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

4.2.10 Extraction solvent, 0.1N HCl:ACN (1:1 v/v).

4.2.11 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 toxicity or carcinogenicity of each reagent used in this method has not been precisely determined; however, 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

Note: Storage containers for standard solutions are amber bottles with Teflon lined screw caps. These concentrations are suggested. Different preparation and concentration schemes may be used and additional standard concentrations may be prepared and used as needed.

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. Store the standards in a freezer at -10° C or colder and protect from light when not in use. Stock solutions must be replaced or 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. Store the standards in a freezer at -10° C or colder and protect from light when not in use. Stock solutions must be replaced or 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 2 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 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 no less than 100 µL or no more than 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 40 ng/mL.

Example Calibration Curve:

200 ng/mL standard (200 ppb), dilute 4 mL of the 5 µg/mL mixed intermediate native stock (6.1.3) and 2 mL of the 2 µg/mL mixed internal standard stock (6.1.4) to 100 mL with 0.1% formic acid.

40 ng/mL standard (40 ppb), dilute 800 µL of the 5 µg/mL mixed intermediate native stock (6.1.3), 2 mL of the 2 µg/mL mixed internal standard stock (6.1.4) and 3.2 mL of methanol to 100 mL with 0.1% formic acid.

20 ng/mL standard (20 ppb), dilute 400 µL of the 5 µg/mL mixed intermediate native stock (6.1.3), 2 mL of the 2 µg/mL mixed internal standard stock (6.1.4) and 3.6 mL of methanol to 100 mL with 0.1% formic acid.

5 ng/mL standard (5 ppb), dilute 100 µL of the 5 µg/mL mixed intermediate native stock (6.1.3), 2 mL of the 2 µg/mL mixed internal standard stock (6.1.4) and 3.9 mL of methanol to 100 mL with 0.1% formic acid.

6.2 Extraction/Concentration

6.2.1 Add 20 mL of extraction solvent 0.1N HCl:ACN (1:1) to 10 g of sample in a VOA vial. Any sample fortification should be done at this step and prior to the addition of the extraction solvent (see Section 6.3).

6.2.2 Place the sample/vials (horizontally) on an orbital shaker and shake for 1 hour at ~220 rpm.

6.2.3 Centrifuge the sample/vials for ~10 minutes at ~1000 rpm.

6.2.4 Add ~1 mL of methanol and 100 µL of the 2 µg/mL internal standard solution via Eppendorf or positive displacement pipet to a calibrated 10-mL conical tube.

6.2.5 Transfer a 10 mL aliquot of centrifuged extract to the conical tube. [Aliquot factor, Af = 0.5]. The extract aliquot may be stored in a freezer (approx. -15 °C or colder) while awaiting concentration (6.2.6).

6.2.6 Evaporate to ~4.9 mL with nitrogen and a water bath at 25-30 °C. Adjust to a final volume of 5 mL with 0.1% formic acid. Evaporation below this volume may result in the loss of thiadone due to its volatility.

6.2.7 Syringe filter a portion of the extract using a 0.45-µm or smaller filter into a HPLC autosampler vial. Store these in a freezer (approx. -15 °C or colder) until analysis.

6.3 Fortification

6.3.1 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.

6.3.2 Sample fortification should be conducted such that no less than 100 µL and no more than 1 mL is used. The preparation of the fortification standards is described in Section 6.1.

6.3.3 Add an appropriate fortification solution to the sample. (Example: add 100 µL of a 1 µg/mL fortification solution to 10 g of sample to fortify at 10 ppb.) Shake the sample bottle to mix the contents and remove the cap. Allow the vial to sit at least 10 minutes before the addition of the extraction solvent.

6.4 Dry Weight/Wet Weight Determination (If Requested)

6.4.1 Place a moisture tin on the toploader balance and press "TARE" or "ZERO" to zero the balance.

6.4.2 Transfer ~5-6 g of sample (record the actual weight as "Wet Weight") to the tin.

6.4.3 Place the tin/sample in a 107 °C oven for at least overnight.

6.4.4 Remove the tin/sample from the oven and cool to room temperature.

6.4.5 Place the tin/sample on the toploader balance and press "TARE" or "ZERO" to zero the balance.

6.4.6 Dump/wipe the residue from the tin and return the tin to the toploader (record the "negative" weight as "Dry Weight").

6.5 Sample Analysis

6.5.1 Standards and extracts are analyzed by LC-ESI/MS/MS on a base deactivated reversed phase column (50 mm x 3 mm). The analyses are performed using gradient chromatography. The liquid flow is introduced directly with a post-column split to the ESI interface which may be optimized to allow an optimum flow (50 μL to 300 μL per min.) into the ESI interface. See Table I for a description of chromatography conditions for FOE 5043 and the alcohol degradate. See Table II for a description of the chromatography conditions for the sulfonic acid, oxalate, and thiadone degradates.

6.5.2 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 of the precursors (CID) in the collision cell of the mass spectrometer (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.

6.5.3 The chromatography conditions and interface parameters used for every sample analysis set must be recorded.

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

6.5.5 An analysis set may begin with one or more system performance check injections. Usually, these injections are standard solutions which are treated as check injections, and therefore not used for quantitation.

6.5.6 See Figure 1 for an example of a positive MS/MS analysis. See Figure 2 for a 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.

7.2 The mobile phase composition and conditions may be altered if the analysis is subject to 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 resolution between the thiadone and alcohol 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 6.2.6.

8 TIME REQUIRED

8.1 Approximately 20 samples can be prepared for analysis in 8 hours.

8.2 Each analysis requires approximately 15 minutes.

9 METHOD OF QUANTITATION

9.1 Quantitation is based on the use of an initial four point calibration curve analyzed in triplicate using internal standards to adjust for instrument response.

9.2 The average response factor (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, 20 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 (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, one of the following must be performed:

9.4.1 Take a smaller aliquot of the original extract (un-concentrated and without internal standards) and process to final volume (with internal standards) and perform the appropriate instrumental analysis.

9.4.2 Re-extract the sample, using a smaller aliquot, and perform the appropriate instrumental analysis.

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

10 CALCULATIONS

10.1 Calculate the response factor 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):

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

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

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

10.4 Sample calculations are done according to the following formula:

$$Calc\ Amt = \frac{(Area_{NAT})\ (Conc_{IS})}{(RF_{AVG})\ (Area_{IS})\ (Df)}$$

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 (Vol_{init}/Vol_{fin}),

Where: Amt_{SAMP} = final sample amount of native analyte (ppb),
FV = final volume (mL),
Q_{SAMP} = quantity of sample extracted (g),

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

Af = aliquot factor (Vol_{aliq}/Vol_{total}),

10.5 Residue values are corrected (if requested) for percent moisture:

$$Amt_{DryWt} = \frac{Amt_{SAMP}\ (ppb)}{(M)}$$

Where: Amt_{DryWt} = final sample amount percent moisture corrected
M = moisture content correction factor (DryWt/WetWt).

10.6 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.

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

10.8 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 I - Instrument Parameters
(FOE 5043 and FOE 5043 alcohol)

Instrumentation:

Waters 600-MS HPLC gradient pump (or equivalent), Waters WISP 717 autosampler (or equivalent), Finnigan MAT TSQ-700 equipped with heated capillary API (or equivalent)

HPLC Operating Conditions:

Column: Inertsil ODS-2 (5μ), 50 x 3 mm, (MetaChem, Torrence, CA) P/N 0296-050X030
Injection Vol. 50 μL
Flow Rate: 0.8 mL/min

Time (min.)	Gradient	0.1% formic acid	Acetonitrile (0.1% formic acid)
Initial	-	90	10
6	Linear	10	90
7.1	Step	90	10

Mass Spectrometer Operating Parameters:

Capillary Temperature: 280° C
Spray Voltage: 4 kV
Sheath Gas: 80 psi
Aux. Gas 30 mL/min
Collision Pressure: Argon @ approximately 1.8 mtorr

Analyte	MW	Precursor Ion	Product Ion	Collision Offset	Dwell (sec)	Retention Time (approx.)
FOE 5043	363	364	194	-15 eV	0.2	5:50
d7-FOE 5043	370	371	201	-15 eV	0.2	5:50
FOE 5043 alcohol	211	212	170	-15 eV	0.2	3:50
d7-FOE 5043 alcohol	218	219	171	-15 eV	0.2	3:50

Note: The preceding specifications are suggested and may be altered as needed. Actual conditions used and any changes must be documented with the raw data.

TABLE II - Instrument Parameters
(FOE 5043 oxalate, FOE 5043 sulfonic acid and FOE 5043 thiadone)

Instrumentation:

Waters 600-MS HPLC gradient pump (or equivalent), Waters WISP 717 autosampler (or equivalent) Finnigan MAT TSQ-700 equipped with heated capillary API (or equivalent)

HPLC Operating Conditions:

Column: Inertsil ODS-2 (5μ), 50 x 3 mm, (MetaChem, Torrence, CA) P/N 0296-050X030
Injection Vol. 50 μL
Flow Rate: 0.8 mL/min

Time (min.)	Gradient	0.1% formic acid	Acetonitrile (0.1% formic acid)
Initial	-	90	10
1	-	90	10
8	Linear	30	70
8.1	Step	90	10

Mass Spectrometer Operating Parameters:

Capillary Temperature: 280° C
Spray Voltage: 3 kV
Sheath Gas: 80 psi
Aux. Gas 30 mL/min
Collision Pressure: Argon @ approximately 1.8 mtorr

Analyte	MW	Precursor Ion	Product Ion	Collision Offset	Dwell (sec)	Retention Time (approx.)
FOE 5043 oxalate	225	224	152	13 eV	0.2	5:00
d7-FOE 5043 oxalate	232	231	159	13 eV	0.2	5:00
FOE 5043 sulfonic acid	275	274	121	20 eV	0.2	5:45
d7-FOE 5043 sulfonic acid	282	281	121	20 eV	0.2	5:45
FOE 5043 thiadone	170	169	113	20 eV	0.2	4:55
FOE 5043 thiadone ($^{13}C/^{15}N_2$)	173	172	113	20 eV	0.2	4:55

Note: The preceding specifications are suggested and may be altered as needed. Actual conditions used and any changes must be documented with the raw data.