

Test Material: Flufenacet

MRID: 48897601

Title: Bethem, R.A., Peterson, R.G., and Leimkuehler, W.M. Determination of FOE 5043 and Four Metabolites in Soil By High Performance Liquid Chromatography Electrospray Tandem Mass Spectrometry (LC-ESI/MS/MS).

MRID: 48897602

Title: Bruns, G. and Hoshowski, J. Independent Laboratory Validation of Analytical Method AMFOE2 for the Analysis of Soil for FOE 5043 and Four Metabolite Residues

EPA PC Code: 121903

OCSPP Guideline: 850.6100

For CDM Smith

Primary Reviewer: Richard Lester

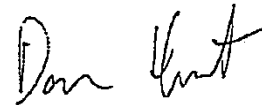
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Date: 5/14/14

Secondary Reviewer: Dan Hunt

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Date: 5/14/14

QC/QA Manager: Joan Gaidos

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Date: 5/14/14

Analytical method for flufenacet in soil

Reports: ECM: MRID 48897601. Bethem, R.A., Peterson, R.G., and Leimkuehler, W.M. 1995. Determination of FOE 5043 and Four Metabolites in Soil By High Performance Liquid Chromatography Electrospray Tandem Mass Spectrometry (LC-ESI/MS/MS). Submitting Laboratory: Bayer Corporation, Kansas City, Missouri, Report No. 106964. Performing Laboratory: Alta Analytical, El Dorado Hills, California, Study No. AMFOE2. September 6, 1995. 20 pages.
ILV: Bruns, G. and Hoshowski, J. 1998. Independent Laboratory Validation of Analytical Method AMFOE2 for the Analysis of Soil for FOE 5043 and Four Metabolite Residues. Submitting Laboratory: Bayer Corporation, Kansas City, Missouri, Report No. 108145, Study No. F3112106. Performing Laboratory: Enviro-Test Laboratories, Edmonton, Alberta, Canada, Document No. 98BAY04.REP. March 5, 1998. 90 pages.

Document No.: MRIDs 48897601 and 48897602


Guideline: 850.6100

Statements: ECM: The study was not conducted in compliance with FIFRA GLP standards (p. 3). A signed and dated Data Confidentiality statement was provided (p. 2). Quality Assurance and Certificate of Authenticity statements were not provided from the performing laboratory.
ILV: The study was conducted in accordance with FIFRA GLP standards (p. 3). Signed and dated Data Confidentiality, GLP Compliance, and Quality Assurance statements were provided (pp. 2-4, 8-9). A Certificate of Authenticity statement was not provided from the performing laboratory.

Classification: This analytical method is classified as **Supplemental**. The ECM did not provide performance data at $10 \times$ LOQ. The soil used in the ECM and ILV was not characterized. Representative chromatograms were not provided for reagent blanks, matrix blanks or spiked samples in the ECM.

PC Code: 121903

Reviewer: Gabriel Rothman
Environmental Scientist, USEPA

Signature: 
Date: 9/24/2015

All page citations refer to MRID 48897601 unless otherwise noted.

Executive Summary

This analytical method, Alta Analytical Study No. AMFOE2, is designed for the quantitative determination of FOE 5043 (flufenacet) and metabolites FOE 5043 sulfonic acid, FOE 5043 alcohol, FOE 5043 oxalate, and FOE 5043 thiadone in soil using LC-ESI/MS/MS (see Table 1). The method is quantitative for the analytes at the stated LOQ of 10 µg/kg. Lowest toxicological levels of concern in soil were not reported. No major issues were discovered by the independent laboratory.

Table 1. Analytical Method Summary

| Analyte(s) by Pesticide | MRID | | EPA Review | Matrix | Method Date | Registrant | Analysis | Limit of Quantitation (LOQ) |
|--|--------------------------------|-----------------------------------|------------|--------|-------------|-------------------|-----------|-----------------------------|
| | Environmental Chemistry Method | Independent Laboratory Validation | | | | | | |
| FOE 5043 (flufenacet), FOE 5043 sulfonic acid, FOE 5043 alcohol, FOE 5043 oxalate, FOE 5043 thiadone | 48897601 | 48897602 | | Soil | 09/06/95 | Bayer Corporation | LC-ESI/MS | 10 µg/kg |

I. Principle of the Method

The method was developed for the analysis of FOE 5043 and four metabolites (FOE 5043 sulfonic acid, FOE 5043 alcohol, FOE 5043 oxalate, and FOE 5043 thiadone) in soil (p. 6). Soil samples (10 g) are extracted with 20 mL of 0.1N HC1:ACN (1:1) by shaking on an orbital shaker for 1 hour (*ca.* 220 rpm; pp. 10-11). After centrifuging for 10 minutes, 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 *ca.* 1 mL of methanol, the solution is concentrated to *ca.* 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 and a portion of the extract is syringe filtered (≤ 0.45 µm).

Extracts were analyzed by LC-ESI/MS/MS (5g/5mL) using an Inertsil ODS-2 column (50 x 3 mm, 5 µ; Tables I-II, pp. 15-16). The analytes are determined by reversed phase liquid chromatography, using 0.1% formic acid and acetonitrile with 0.1% formic acid. 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. 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.

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.

II. Recovery Findings

Mean recoveries and relative standard deviations (RSD) were within guideline requirements (mean 70-120%; RSD \leq 20%) for all analytes in soil. No validation was performed at the 10 \times LOQ fortification level in the ECM.

Table 2. Initial Validation Method Recoveries for Analytes in Soil

| Analyte | Fortification Level ($\mu\text{g}/\text{kg}$) | Number of Tests | Recovery Range (%) ¹ | Mean Recovery (%) ¹ | Standard Deviation (%) ² | Relative Standard Deviation (%) |
|------------------------|---|-----------------|---------------------------------|--------------------------------|-------------------------------------|---------------------------------|
| FOE 5043 | 10 (LOQ) | 7 | 90-109 | 101 | 5.8 | 5.7 |
| FOE 5043 sulfonic acid | 10 (LOQ) | 7 | 89-110 | 99 | 8.1 | 8.2 |
| FOE 5043 oxalate | 10 (LOQ) | 7 | 83-101 | 91 | 5.5 | 6.0 |
| FOE 5043 thiadone | 10 (LOQ) | 7 | 98-109 | 104 | 4.1 | 3.9 |
| FOE 5043 alcohol | 10 (LOQ) | 7 | 97-112 | 104 | 5.4 | 5.2 |

Data were obtained from Tables III-IV, pp. 17-18 of the ECM study report.

¹ Recoveries are based on internal standard quantitation as specified in the method (p. 12). The ECM separately reports recoveries based on external standard quantitation.

² Standard deviations of measured concentrations are reported in the ECM. See reviewer Excel file for standard deviations of percent recoveries.

Table 3. Independent Validation Method Recoveries for Analytes in Soil

| Analyte | Fortification Level ($\mu\text{g}/\text{kg}$) | Number of Tests | Recovery Range (%) | Mean Recovery (%) | Standard Deviation (%) ¹ | Relative Standard Deviation (%) |
|------------------------|---|-----------------|--------------------|-------------------|-------------------------------------|---------------------------------|
| FOE 5043 | 11.5 (LOQ) | 5 | 90-97 | 93 | 2.3 | 2.5 |
| | 115 | 5 | 83-91 | 87 | 3.9 | 4.4 |
| FOE 5043 sulfonic acid | 10.5 (LOQ) | 5 | 96-105 | 99 | 3.7 | 3.7 |
| | 105 | 5 | 81-86 | 84 | 2.0 | 2.4 |
| FOE 5043 oxalate | 10.2 (LOQ) | 5 | 78-90 | 85 | 4.7 | 5.6 |
| | 102 | 5 | 74-79 | 77 | 2.6 | 3.4 |
| FOE 5043 thiadone | 10.9 (LOQ) | 5 | 94-103 | 96 | 3.8 | 4.0 |
| | 109 | 5 | 80-90 | 87 | 3.9 | 4.5 |
| FOE 5043 alcohol | 10.4 (LOQ) | 5 | 82-95 | 90 | 5.0 | 5.6 |
| | 104 | 5 | 82-86 | 84 | 2.2 | 2.6 |

Data were obtained from MRID 48897602, pp. 27.

¹ Standard deviations of measured concentrations are reported in the ILV. See reviewer Excel file for standard deviations of percent recoveries.

III. Method Characteristics

The LOD was calculated in the ECM as the students' t-value \times the standard deviation, citing 40 CFR Part 136, Appendix B (p. 17). The LOQ was calculated as $10 \times$ the standard deviation, using scientifically accepted calculation procedures. Reproducibility is not satisfactory because no LODs or LOQs were calculated in the ILV. Observed standard deviations in the ILV, however, were similar to those in the ECM.

Table 4. Method Characteristics

| | FOE 5043 | FOE 5043 sulfonic acid | FOE 5043 oxalate | FOE 5043 thiadone | FOE 5043 alcohol |
|---|--|---|--|---|--|
| Limit of Quantitation (LOQ) | 5.8 $\mu\text{g}/\text{kg}$ | 8.1 $\mu\text{g}/\text{kg}$ | 5.5 $\mu\text{g}/\text{kg}$ | 4.1 $\mu\text{g}/\text{kg}$ | 5.4 $\mu\text{g}/\text{kg}$ |
| Limit of Detection (LOD) | 1.8 $\mu\text{g}/\text{kg}$ | 2.5 $\mu\text{g}/\text{kg}$ | 1.7 $\mu\text{g}/\text{kg}$ | 1.3 $\mu\text{g}/\text{kg}$ | 1.7 $\mu\text{g}/\text{kg}$ |
| Linearity (calibration curve r^2 and concentration range) | $r^2 = 1.00$ 5-200 $\mu\text{g}/\text{L}$ | $r^2 = 0.998$ 5-200 $\mu\text{g}/\text{L}$ | $r^2 = 1.00$ 5-200 $\mu\text{g}/\text{L}$ | $r^2 = 0.999$ 5-200 $\mu\text{g}/\text{L}$ | $r^2 = 1.00$ 5-200 $\mu\text{g}/\text{L}$ |
| Repeatable | Yes | Yes | Yes | Yes | Yes |
| Reproducible | No | No | No | No | No |
| Specific | Yes | Yes | Yes | Yes | Yes |

The LOQ and LOD are those reported in the ECM (Tables III and IV, pp. 17-18). Linearity data are those reported in the ILV (Trial II, pp. 19, 26).

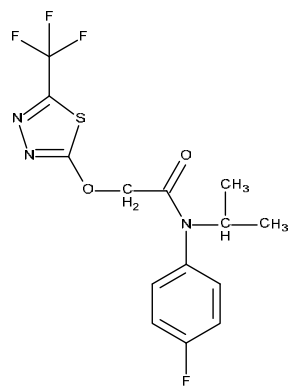
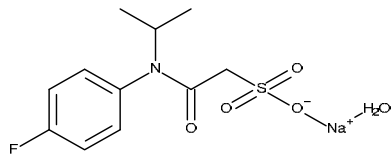
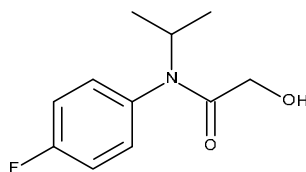
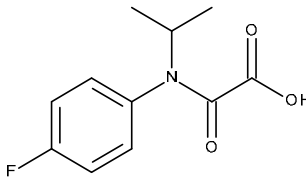
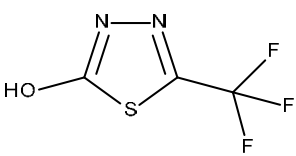
IV. Method Deficiencies and Reviewer's Comments

1. The ECM did not provide performance data at $10 \times$ LOQ.
2. LODs and LOQs were not calculated in the ILV.
3. The soil used in both the ECM and the ILV was not characterized. Only the source of the soil used in the ILV was reported (Wisconsin; MRID 48897602, p. 14).
4. The ECM only provided representative chromatograms for standards and calibration curves were not provided (Figures 1-2, pp. 19-20). Representative chromatograms should be provided for reagent blanks, matrix blanks, standard curves, and spiked samples at all spiking levels for all analytes.
5. The lowest toxicological level of concern in soil was not reported. The established LOQ must be less than toxicological levels of concern.
6. The ILV was validated with the second trial. Low response of the thiadone internal standard from the initial QC check calibration curve used for Trial 1 resulted in low recoveries for the 100 ng/g fortification (mean of 66%, with a RSD of 29%; MRID 48897602; pp. 13, 25). Following communication with the Study Monitor, a new standard for thiadone was provided and new standard solutions were prepared for use in the second trial.
7. The ECM provided calculation steps (pp. 13-14), but did not provide example calculations showing how the raw data were converted to a final concentration.

8. A communication log between the submitting laboratory (Bayer) and the ILV (Enviro-Test Laboratories) was provided in Appendix III of MRID 48897602 (p. 61). A brief summary of the discussion of the phone conversations following the Trial 1 results was provided on page 13 of the ILV.
9. It was noted in the ILV that the LC/MS/MS system sensitivity was significantly greater than what appeared in the analytical method and in order to obtain similar sensitivities to those in the method, the injection volume was changed from 50 to 20 μ L for the negative ion analysis (MRID 48897602, p. 23). Also a conversion in the mass spectrometry and interface parameters was required due to the use of a Turbo Ion Spray – API interfaced with a PE Sciex LC/MS/MS system versus a heated capillary – API interfaced with a Finnigan LC/MS/MS which was employed in the original method.

V. References

Attachment 1: Chemical Names and Structures

| Code Name/ Synonym | Chemical Name | Chemical Structure |
|---|--|---|
| Flufenacet (FOE 5043) | <p>IUPAC: 4'-Fluoro-N-isopropyl-2-[5-(trifluoromethyl)-1,3,4-thiadiazol-2-yloxy]acetanilide</p> <p>CAS: N-(4-fluorophenyl)-N-(1-methylethyl)-2-[[5-(trifluoromethyl)-1,3,4-thiadiazol-2-yl]oxy]acetamide</p> <p>CAS #: 142459-58-3</p> <p>Formula: C₁₄H₁₃F₄N₃O₂S</p> <p>MW: 363.3 g/mol</p> <p>SMILES: CC(C)N(c1ccc(cc1)F)C(=O)COc2nc(s2)C(F)(F)F</p> |  |
| FOE 5043 Sulfonic Acid, Sodium Salt, Monohydrate | <p>IUPAC: 4-Fluoro-N-methylethylanilinesulfoacetamide,sodiumsalt, monohydrate</p> <p>Formula: C₁₁H₁₅FNO₅SNa</p> <p>MW: 315.29 g/mol</p> <p>SMILES: [H]O([H])[Na+][O-]S(=O)(=O)CC(=O)N(c1ccc(cc1)F)C(C)C</p> |  |
| FOE 5043 Alcohol | <p>IUPAC: N-(4-fluorophenyl)-2-hydroxy-N-(1-methylethyl)acetamide</p> <p>Formula: C₁₁H₁₄FNO₂</p> <p>MW: 211.2 g/mol</p> <p>SMILES: CC(C)N(c1ccc(cc1)F)C(=O)CO</p> |  |
| FOE 5043 Oxalate | <p>IUPAC: N-(4-fluorophenyl)-N-methylethyl-aminooxoacetic acid</p> <p>Formula: C₁₁H₁₂FNO₃</p> <p>MW: 225.2 g/mol</p> <p>SMILES: CC(C)N(c1ccc(cc1)F)C(=O)C(=O)O</p> |  |
| FOE 5043 Thiadone | <p>CAS: 5-Trifluoromethyl-1,3,4-thiadiazol-2(3H)ol</p> <p>CAS #: 84352-75-0</p> <p>Formula: C₃HF₃N₂OS</p> <p>MW: 170.1 g/mol</p> <p>SMILES: c1(nnc(s1)O)C(F)(F)F</p> |  |