Test Material: Flufenacet

MRID: 48897603

Title: Bethem, R.A., Peterson, R.G., Leimkuehler, W.A., and Mattern, G.C. Determination of FOE 5043 and the Alcohol, Oxalate, Thiadone and Sulfonic Acid Metabolites in Groundwater by High Performance Liquid Chromatography Electrospray Tandem Mass Spectrometry (LC-ESI/MS/MS).

MRID: 48897604

Title: Bruns, G. and Hoshowski, J. Independent Laboratory Validation of Analytical Method AMFOE3 for the Analysis of Groundwater for FOE 5043 and Four Metabolite Residues.

EPA PC Code: 121903

OCSPP Guideline: 850.6100

For CDM Smith

Primary Reviewer: Richard Lester **Signature:** Richard R Lester

Date: 5/14/14

Secondary Reviewer: Dan Hunt **Signature:** Dan Hunt

Date: 5/14/14

QC/QA Manager: Joan Gaidos **Signature:**

Date: 5/14/14

Analytical method for flufenacet in water

Reports: ECM: MRID 48897603. Bethem, R.A., Peterson, R.G., Leimkuehler, W.A., and Mattern, G.C. 1995. Determination of FOE 5043 and the Alcohol, Oxalate, Thiadone and Sulfonic Acid Metabolites in Groundwater by High Performance Liquid Chromatography Electrospray Tandem Mass Spectrometry (LC-ESI/MS/MS). Submitting Laboratory: Bayer Corporation, Kansas City, Missouri, Report No. 107138. Performing Laboratory: Alta Analytical, El Dorado Hills, California, Study No. AMFOE3. June 22, 1995. 22 pages.

ILV: Bruns, G. and Hoshowski, J. 1998. Independent Laboratory Validation of Analytical Method AMFOE3 for the Analysis of Groundwater for FOE 5043 and Four Metabolite Residues. Submitting Laboratory: Bayer Corporation, Kansas City, Missouri, Report No. 108265, Study No. F3112402. Performing Laboratory: Enviro-Test Laboratories, Edmonton, Alberta, Canada, Document No. 98BAY05.REP. April 30, 1998. 85 pages.

Document No.: MRIDs 48897603 and 48897604

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 the LOQ and 10 × LOQ**.** The water used in the ECM and ILV was not characterized. Chromatograms were not provided for reagent blanks, matrix blanks, or spiked samples in the ECM.

PC Code: 121903

Reviewer: Gabriel S. Rothman **Signature:** Gabe Rothman
Environmental Scientist, USEPA **Date:** September 24, 2015

All page citations refer to MRID 48897603 unless otherwise noted.

Executive Summary

This analytical method, Alta Analytical Study No. AMFOE3, 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 water using LC-ESI/MS/MS (see Table 1). The method is quantitative for the analytes at the stated LOQ of 0.10 µg/L. Lowest toxicological levels of concern in groundwater 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	48897603	48897604		Water	06/22/95	Bayer Corporation	LC-ESI//MS	0.10 µg/L

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 water (p. 6). Analytes are extracted from water by drawing 50 mL of acidified sample through a 6-mL octadecyl (C18) SPE column (p. 10). The analytes are eluted with 6 mL of methanol and concentrated to *ca*.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 and a portion of the extract is syringe filtered (≤0.45 µm).

Extracts were analyzed by LC-ESI/MS/MS using an Inertsil ODS-2 column (50 x 2 mm, 5 µ; p. 11; Tables I-II, pp. 14-15). 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. 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 ≤20%) for all analytes in water. No validation was performed at the stated LOQ or 10×LOQ fortification level in the ECM. The only validation was performed at 2×LOQ.

Table 2. Initial Validation Method Recoveries for Analytes in Water

Analyte	Fortification Level (µg/L)[1]	Number of Tests	Recovery Range (%)[2]	Mean Recovery (%)[2]	Standard Deviation (%)[3]	Relative Standard Deviation (%)
FOE 5043	0.2	7	93-98	95	1.9	2.0
FOE 5043 sulfonic acid	0.2	7	95-98	97	1.2	1.2
FOE 5043 oxalate	0.2	7	91-99	94	2.5	2.6
FOE 5043 thiadone	0.2	7	89-101	94	3.8	4.0
FOE 5043 alcohol	0.2	7	94-99	97	1.8	1.8

Data were obtained from Tables III-IV, pp. 16-17 of the ECM study report.

[1] Fortification level is not stated, but is inferred from Tables III and IV (pp. 16-17).

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

[3] 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 Water

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)[1]	Relative Standard Deviation (%)
FOE 5043	0.115 (LOQ)	5	99-108	104	3.5	3.4
	1.15	5	113-115	114	0.8	0.68
FOE 5043 sulfonic acid	0.105 (LOQ)	5	96-109	104	5.3	5.1
	1.05	5	106-109	107	1.1	1.0
FOE 5043 oxalate	0.102 (LOQ)	5	110-116	112	2.3	2.0
	1.02	5	115-119	117	1.6	1.4
FOE 5043 thiadone	0.109 (LOQ)	5	78-104	91	9.6	11
	1.09	5	108-117	113	4.0	3.5
FOE 5043 alcohol	0.104 (LOQ)	5	102-112	108	4.0	3.7
	1.04	5	105-108	106	1.4	1.3

Data were obtained from MRID 48897604, pp. 25.

[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 × the standard deviation, citing 40 CFR Part 136, Appendix B (p. 16). The LOQ was calculated as 10 × 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)	0.04 µg/L	0.02 µg/L	0.05 µg/L	0.08 µg/L	0.04 µg/L
Limit of Detection (LOD)	0.012 µg/L	0.008 µg/L	0.015 µg/L	0.024 µg/L	0.011 µg/L
Linearity (calibration curve r^2 and concentration range)	$r^2 = 1.00$ 1.25-100 µg/L	$r^2 = 1.00$ 1.25-100 µg/L	$r^2 = 1.00$ 1.25-100 µg/L	$r^2 = 0.999$ 1.25-100 µg/L	$r^2 = 1.00$ 1.25-100 µg/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. 16-17).
Linearity data are those reported in the ILV (pp. 19, 23).

IV. Method Deficiencies and Reviewer's Comments

1. The ECM did not provide performance data at either the LOQ or 10 × LOQ. The only data provided were at 2 × LOQ.

2. LODs and LOQs were not calculated in the ILV.

3. The water used in both the ECM and the ILV was not characterized. Only the source of the groundwater used in the ILV was reported (Janesville, Iowa; MRID 48897604, p. 14).

4. The ECM only provided representative chromatograms for standards, and calibration curves were not provided (Figures 3-4, pp. 20-22). 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 water was not reported. The established LOQ must be less than toxicological levels of concern.

6. The ECM provided calculation steps (pp. 12-13), but did not provide example calculations showing how the raw data were converted to a final concentration.

7. A communication log between the submitting laboratory (Bayer) and the ILV (Enviro-Test Laboratories) was provided in Appendix III of MRID 48897604 (p. 61).

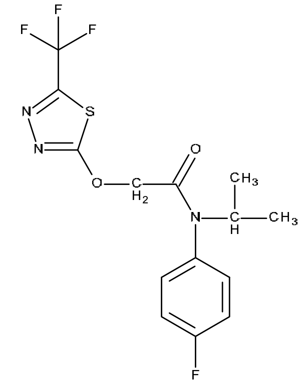
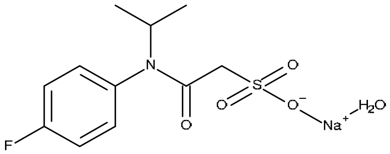
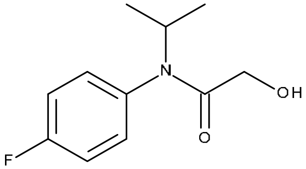
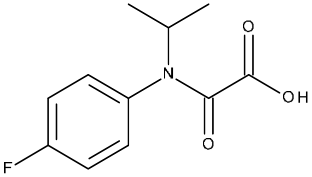
8. It was noted in the ILV that the LC/MS/MS system sensitivity was significantly greater than what appeared in the analytical method, and in order to obtain similar sensitivities to

those in the method, the injection volume was changed from 50 to 20 µL for the negative ion analysis and from 50 to 40 µL for the positive ion analysis (MRID 48897604, pp. 22-23). Also, it was stated in the ILV that the HPLC conditions were changed for both the positive and negative MS/MS analysis to a gradient common to both analyses in order to obtain retention similar to that in the original method (MRID 48897604, pp. 21, 23).

9. The initial QC calibration curve for thiadone produced a r^2 of 0.994 (MRID 48897604, pp. 23, 26). Following communication with the Study Monitor, a new standard for thiadone was provided and a newly prepared calibration standard solution was prepared for the method validation trial and gave a $r^2 = 0.999$ for thiadone (MRID 48897604, p. 13; Appendix III, p. 61).

V. References

Attachment 1: Chemical Names and Structures

Code Name/ Synonym	Chemical Name	Chemical Structure
Flufenacet **(FOE 5043)**	**IUPAC: 4′-Fluoro-N-isopropyl-2-[5-(trifluoromethyl)-1,3,4-thiadiazol-2-yloxy]acetanilide** **CAS: N-(4-fluorophenyl)-N-(1-methylethyl)-2-[[5-(trifluoromethyl)-1,3,4-thiadiazol-2-yl]oxy]acetamide** **CAS #: 142459-58-3** **Formula: $C_{14}H_{13}F_4N_3O_2S$** **MW: 363.3 g/mol** **SMILES: CC(C)N(c1ccc(cc1)F)C(=O)COc2nnc(s2)C(F)(F)F**	
FOE 5043 Sulfonic Acid, Sodium Salt, Monohydrate	**IUPAC:** 4-Fluoro-N-methylethylanilinesulfoacetamide,sodiumsalt, monohydrate **Formula: $C_{11}H_{15}FNO_5SNa$** **MW: 315.29 g/mol** **SMILES: [H]O([H])[Na+][O-]S(=O)(=O)CC(=O)N(c1ccc(cc1)F)C(C)C**	
FOE 5043 Alcohol	**IUPAC:** N-(4-fluorophenyl)-2-hydroxy-N-(1-methylethyl)acetamide **Formula: $C_{11}H_{14}FNO_2$** **MW: 211.2 g/mol** **SMILES: CC(C)N(c1ccc(cc1)F)C(=O)CO**	
FOE 5043 Oxalate	**IUPAC:** N-(4-fluorophenyl)-N-methylethyl-aminooxoacetic acid **Formula: $C_{11}H_{12}FNO_3$** **MW: 225.2 g/mol** **SMILES: CC(C)N(c1ccc(cc1)F)C(=O)C(=O)O**	
FOE 5043 Thiadone	**CAS:** 5-Trifluoromethyl-1,3,4-thiadiazol-2(3H)ol **CAS #: 84352-75-0** **Formula: $C_3HF_3N_2OS$** **MW: 170.1 g/mol** **SMILES: c1(nnc(s1)O)C(F)(F)F**