

Analytical method for fluometuron and its metabolites des-methyl-fluometuron and CGA72903 in water

Reports: ECM: EPA MRID No.: MRID 50556602. Geschke, S. 2012. Validation of an Analytical Method for Determination of Fluometuron and its Metabolites Des-methyl-Fluometuron and CGA72903 in Surface Water and Drinking Water. Sponsor Study No.: 90014920. Study Code: S12-00099. Lab/Trial Phase Code: S12-00099-L1. Report prepared by Eurofins Agrosience Services, EcoChem GmbH, Niefern-Öschelbronn, Germany, and sponsored and submitted by Agan Chemical Manufacturers Ltd., Ashdod, Israel; 84 pages (including 2A). Final report issued July 16, 2012.

ILV: EPA MRID No.: MRID 50693101. Jutson, J.I. 2018. Independent Laboratory Validation of the Analytical Method for Determination of Fluometuron and its Metabolites (Des-methyl-Fluometuron and CGA 72903) in Surface Water and Drinking Water by LC-MS/MS. Smithers Viscient Study No.: 14090.6108. ADAMA Reference No.: 90020121. Report prepared by Smithers Viscient, Wareham, Massachusetts, and sponsored by ADAMA Agan Ltd., Ashdod, Israel, and submitted by Agan Chemical Manufacturers Ltd., Ashdod, Israel, and Pyxis Registry Consulting, Inc., Gig Harbor, Washington (p. 3; Appendix 3, p. 120); 121 pages. Final report issued September 20, 2018.

Document No.: MRIDs 50556602 & 50693101

Guideline: 850.6100

Statements: ECM: The study was conducted in compliance with German and OECD Good Laboratory Practices (GLP) standards, which are also accepted by the European community, USA (FDA and EPA) and Japan (MHW, MAFF, and METI; p. 2; Appendix E, Figure 27, p. 83 of MRID 50556602). Signed and dated Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2, 2A, 3; Appendix E, Figure 27, p. 83). The statement of authenticity was included with the Quality Assurance statement.

ILV: The study was conducted in compliance with USEPA FIFRA and OECD GLP standards, with the exception of the characterization of the test substances (p. 3 of MRID 50693101). Signed and dated Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2-3, 5). The statement of authenticity was included with the Quality Assurance statement.

Classification: This analytical method is classified as **supplemental**. The specificity of the method was acceptable, but not fully supported by ILV representative chromatograms.

PC Code: 035503

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This Data Evaluation Record may have been altered by the Environmental Fate and Effects Division subsequent to signing by CDM/CSS-Dynamac JV personnel. The CDM/CSS-Dynamac Joint Venture role does not include establishing Agency policies.

Executive Summary

The analytical method, Agan Chemical Manufacturers, Ltd., Study No. 90014920, is designed for the quantitative determination of fluometuron and its metabolites des-methyl-fluometuron and CGA72903 in water at the stated LOQ of 0.05 µg/L. The LOQ is less than the lowest toxicological level of concern of 0.03 mg/L in water for all three analytes. The ECM and ILV used two characterized waters, different drinking and surface water matrices. The ILV validated the ECM in the first trial for all three analytes in both water matrices with the minor modification of the LC mobile phase and insignificant modifications of the MS parameters and analytical equipment. All submitted ILV and ECM data pertaining to precision, repeatability, reproducibility, linearity, and specificity was acceptable at the LOQ and 10×LOQ for all three analytes in both matrices. The specificity of the method was acceptable, but not fully supported by ILV representative chromatograms since some minor contaminants were observed near the analyte peak RT for the majority of the analytes/matrices.

Table 1. Analytical Method Summary

Analyte(s) by Pesticide	MRID		EPA Review	Matrix	Method Date (dd/mm/yyyy)	Registrant	Analysis	Limit of Quantitation (LOQ)
	Environmental Chemistry Method	Independent Laboratory Validation						
Fluometuron	MRID 50556602 ¹	MRID 50693101 ²		Water	16/07/2012	Agan Chemical Manufacturers, Ltd. ³	LC/MS/MS	0.05 µg/L
Des-Methyl-Fluometuron								
CGA72903								

1 In the ECM, the drinking water [pH 7.62 (at 17.5°C), total hardness 4.1 mmol/L, 0.44 mg/L dissolved organic carbon, specific electric conductivity 754 µS/cm (at 20°C)] was collected as tap water from the test facility and surface water [pH 8.02 (at 17.8°C), total hardness 1.4 mmol/L, 4.7 mg/L dissolved organic carbon, specific electric conductivity 271 µS/cm (at 20°C)] was collected from the river Dendelbach in Spregelberg, Germany (pp. 14-15; Appendix E, Figures 25-26, pp. 81-82 of MRID 50556602). Waters were well characterized by Eurofins Institut Jäger GmbH, Tübingen.

2 In the ILV, the drinking water [pH 6.9, total hardness 78 mmol/L as CaCO₃, total alkalinity 20 mmol/L as CaCO₃, conductivity 604 µS/cm] was collected as laboratory well water and surface water [Lot No. 17Oct16Wat-A-3; pH 6.9, dissolved oxygen concentration 9.3 mg/L] was collected from the Weweantic River in West Wareham, Massachusetts (p. 17 of MRID 50693101). Water characterization source information was not provided.

3 c/o Pyxis Registry Consulting, Inc., Gig Harbor, Washington (p. 3; Appendix 3, p. 119 of MRID 50693101).

I. Principle of the Method

Water (50 mL) was fortified with mixed fortification solutions (25 μ L of 0.1 or 1 μ g/mL solution) in 100-mL screw-cap glass containers (pp. 16-17 of MRID 50556602). In Vial 1 (standard addition), an aliquot (1.0 mL) of the fortified sample was transferred to a glass vial and analyzed by LC/MS/MS. In Vial 2 (direct dilution), an aliquot (1.0 mL) of the fortified sample was transferred to a glass vial, fortified with mixed fortification solutions (50 μ L of 1 or 10 μ g/mL solution), and analyzed by LC/MS/MS. One vial was used for analyte identification; the other vial was used for analyte quantification. The fortification procedure was verified by the analysis of 1.0 mL of HPLC grade water fortified with mixed fortification solutions.

Samples were analyzed for fluometuron, des-methyl-fluometuron, and CGA72903 using an Agilent HPLC coupled to a Sciex API5000 triple stage quadrupole mass spectrometer equipped with electrospray ionization (ESI) interface in the positive ion, multiple reaction monitoring (MRM) mode (pp. 16, 18-20 of MRID 50556602). The following LC conditions were used: Agilent ZORBAX Eclipse XDB-C18 column (4.6 mm x 150 mm, 5 μ m; oven temperature 40°C) + 4 mm guard column, mobile phase of (A) 0.5% formic acid in water and (B) 0.5% formic acid in acetonitrile [mobile gradient phase of percent A:B (v:v) at 0.00-0.50 min. 50:50, 5.00-7.00 min. 5:95, 7.01-9.00 min. 50:50], MS temperature 400°C, and injection volume of 84 μ L. Expected retention times were *ca.* 5.2, 4.7, and 5.5 minutes for fluometuron, des-methyl-fluometuron, and CGA72903, respectively. Two ion pair transitions were monitored (quantifier and qualifier, respectively): m/z 233.0 \rightarrow 159.9 and m/z 233.0 \rightarrow 71.9 for fluometuron, m/z 219.2 \rightarrow 162.0 and m/z 219.2 \rightarrow 142.0 for des-methyl-fluometuron, and m/z 162.0 \rightarrow 142.0 and m/z 162.0 \rightarrow 92.9 for CGA72903.

The ILV performed the ECM method as written, except for the use of 0.25% formic acid in water and 0.25% formic acid in acetonitrile for the LC mobile phase, modifications of the MS parameters, and insignificant modifications to the analytical equipment (pp. 16, 21-25 of MRID 50693101). Samples were analyzed for fluometuron, des-methyl-fluometuron, and CGA72903 using Shimadzu LC-20AD HPLC system coupled with a MDS Sciex API 5000 mass spectrometer equipped with a ESI Turbo V source. The LC/MS/MS parameters were the same as those of the ECM, except that injection volume was increased to 100 μ L and MS temperature 600°C. No guard column was specifically reported for the LC. Two ion pair transitions were monitored (quantitation and confirmation, respectively): m/z 233 \rightarrow 160 and m/z 233 \rightarrow 72 for fluometuron, m/z 219 \rightarrow 162 and m/z 219 \rightarrow 142 for des-methyl-fluometuron, and m/z 162 \rightarrow 142 and m/z 162 \rightarrow 93 for CGA72903. Expected retention times were *ca.* 5.5, 5.0, and 5.8 minutes for fluometuron, des-methyl-fluometuron, and CGA72903, respectively.

In the ECM and ILV, the Limit of Quantification (LOQ) was 0.05 μ g/L for fluometuron, des-methyl-fluometuron and CGA72903 in water (p. 25 of MRID 50556602; pp. 26-27, 29-31 of MRID 50693101). In the ECM, the Limit of Detection (LOD) was defined as 0.015 μ g/L (30% of the LOQ). In the ILV, the LOD was calculated to be 0.0105 μ g/L (method, direct dilution procedure) and 0.0100 μ g/L (instrument, standard addition procedure) for all three analytes.

II. Recovery Findings

ECM (MRID 50556602): Mean recoveries and RSDs were within guidelines (mean 70-120%; RSD \leq 20%) for analysis of fluometuron, des-methyl-fluometuron, and CGA72903 at fortification levels of 0.05 $\mu\text{g/L}$ (LOQ) and 0.50 $\mu\text{g/L}$ (10 \times LOQ) in one water matrix (Tables 4-6, pp. 22-23). Two ion pair transitions were monitored for fluometuron, des-methyl-fluometuron and CGA72903 using LC/MS/MS in positive mode; the quantification and confirmation ion data was comparable for all analytes/matrices. The drinking water [pH 7.62 (at 17.5 $^{\circ}\text{C}$), total hardness 4.1 mmol/L, 0.44 mg/L dissolved organic carbon, specific electric conductivity 754 $\mu\text{S/cm}$ (at 20 $^{\circ}\text{C}$)] was collected as tap water from the test facility and surface water [pH 8.02 (at 17.8 $^{\circ}\text{C}$), total hardness 1.4 mmol/L, 4.7 mg/L dissolved organic carbon, specific electric conductivity 271 $\mu\text{S/cm}$ (at 20 $^{\circ}\text{C}$)] was collected from the river Dendelbach in Spregelberg, Germany (pp. 14-15; Appendix E, Figures 25-26, pp. 81-82). Waters were well characterized by Eurofins Institut Jäger GmbH, Tübingen.

ILV (MRID 50693101): Mean recoveries and RSDs were within guidelines (mean 70-120%; RSD \leq 20%) for analysis of fluometuron, des-methyl-fluometuron, and CGA72903 at fortification levels of 0.05 $\mu\text{g/L}$ (LOQ) and 0.50 $\mu\text{g/L}$ (10 \times LOQ) in two water matrices (Tables 1-12, pp. 35-46). Two ion pair transitions were monitored for fluometuron, des-methyl-fluometuron and CGA72903 using LC/MS/MS in positive mode; the quantification and confirmation ion data was comparable. The drinking water [pH 6.9, total hardness 78 mmol/L as CaCO_3 , total alkalinity 20 mmol/L as CaCO_3 , conductivity 604 $\mu\text{S/cm}$] was collected as laboratory well water and surface water [Lot No. 17Oct16Wat-A-3; pH 6.9, dissolved oxygen concentration 9.3 mg/L] was collected from the Weweantic River in West Wareham, Massachusetts (p. 17). Water characterization source information was not provided. The ILV validated the ECM in the first trial for all three analytes in both water matrices with the substitution of 0.25% formic acid in water and 0.25% formic acid in acetonitrile for the LC mobile phase, modifications of the MS parameters, and insignificant modifications to the analytical equipment (pp. 16, 21-25, 30).

Table 2. Initial Validation Method Recoveries for Fluometuron, Des-Methyl-Fluometuron and CGA72903 in Water

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%) ¹	Relative Standard Deviation (%)
Drinking Water²						
Quantitation ion ³						
Fluometuron	0.05 (LOQ)	5	78-101	87	11	12
	0.50	5	95-108	102	5	5
Des-Methyl-Fluometuron	0.05 (LOQ)	5	79-91	84	5	6
	0.50	5	88-105	99	7	7
CGA72903	0.05 (LOQ)	5	79-105	87	11	12
	0.50	5	90-97	93	3	3
Confirmation (Qualifier) ion ³						
Fluometuron	0.05 (LOQ)	5	84-91	86	3	3
	0.50	5	94-104	99	4	4
Des-Methyl-Fluometuron	0.05 (LOQ)	5	75-106	87	12	14
	0.50	5	93-104	99	4	4
CGA72903	0.05 (LOQ)	5	77-91	83	6	7
	0.50	5	86-101	95	5	6
Surface Water²						
Quantitation ion ³						
Fluometuron	0.05 (LOQ)	5	78-98	89	9	10
	0.50	5	86-106	96	7	7
Des-Methyl-Fluometuron	0.05 (LOQ)	5	84-95	90	4	5
	0.50	5	89-104	99	6	6
CGA72903	0.05 (LOQ)	5	74-89	82	6	8
	0.50	5	84-107	96	8	9
Confirmation (Qualifier) ion ³						
Fluometuron	0.05 (LOQ)	5	82-93	87	5	5
	0.50	5	90-104	97	5	5
Des-Methyl-Fluometuron	0.05 (LOQ)	5	84-94	88	4	4
	0.50	5	93-105	99	6	6
CGA72903	0.05 (LOQ)	5	73-94	85	8	9
	0.50	5	92-107	99	5	5

Data (uncorrected recovery results; pp. 21-22) were obtained from Tables 6-8, pp. 23-24 of MRID 50556602 and DER Attachment 2.

1 Reported values for standard deviation were reviewer-calculated because the study author did not provide these values (see DER Attachment 2). Rules of significant figures were followed.

2 The drinking water [pH 7.62 (at 17.5°C), total hardness 4.1 mmol/L, 0.44 mg/L dissolved organic carbon, specific electric conductivity 754 µS/cm (at 20°C)] was collected as tap water from the test facility and surface water [pH 8.02 (at 17.8°C), total hardness 1.4 mmol/L, 4.7 mg/L dissolved organic carbon, specific electric conductivity 271 µS/cm (at 20°C)] was collected from the river Dendelbach in Spregelberg, Germany (pp. 14-15; Appendix E, Figures 25-26, pp. 81-82). Waters were well characterized by Eurofins Institut Jäger GmbH, Tübingen.

3 Two ion pair transitions were monitored (quantifier and qualifier, respectively): m/z 233.0→159.9 and m/z 233.0→71.9 for fluometuron, m/z 219.2→162.0 and m/z 219.2→142.0 for des-methyl-fluometuron, and m/z 162.0→142.0 and m/z 162.0→92.9 for CGA72903.

Table 3. Independent Validation Method Recoveries for Fluometuron, Des-Methyl-Fluometuron, and CGA72903 in Water

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Drinking Water¹						
Quantitation ion ²						
Fluometuron	0.05 (LOQ)	5 ³	80.6-132	100	19	20
	0.50	5	96.1-110	104	6.20	5.96
Des-Methyl-Fluometuron	0.05 (LOQ)	5	95.3-102	97.4	2.62	2.69
	0.50	5	93.7-108	102	5.35	5.27
CGA72903	0.05 (LOQ)	5	94.0-115	102	7.92	7.74
	0.50	5	94.9-98.8	97.4	1.58	1.62
Confirmation ion ²						
Fluometuron	0.05 (LOQ)	5	89.3-105	97.8	5.69	5.82
	0.50	5	102-109	105	2.54	2.42
Des-Methyl-Fluometuron	0.05 (LOQ)	5	90.9-97.4	94.9	2.75	2.90
	0.50	5	95.5-114	102	7.08	6.97
CGA72903	0.05 (LOQ)	5	79.8-96.0	87.8	6.49	7.40
	0.50	5	103-111	108	3.39	3.15
Surface Water¹						
Quantitation ion ²						
Fluometuron	0.05 (LOQ)	5	88.1-112	103	9.62	9.35
	0.50	5	93.0-104	97.6	4.58	4.70
Des-Methyl-Fluometuron	0.05 (LOQ)	5	94.8-111	104	7.44	7.15
	0.50	5	85.8-00.2	93.6	5.70	6.09
CGA72903	0.05 (LOQ)	5	75.5-86.5	81.4	5.58	6.86
	0.50	5	80.2-86.8	85.9	3.41	3.97
Confirmation ion ²						
Fluometuron	0.05 (LOQ)	5	96.9-110	109	9.17	8.41
	0.50	5	97.3-115	107	7.38	6.92
Des-Methyl-Fluometuron	0.05 (LOQ)	5	91.7-106	98.5	5.26	5.34
	0.50	5	95.8-109	101	5.68	5.62
CGA72903	0.05 (LOQ)	5	79.1-85.0	82.5	2.13	2.59
	0.50	5	87.5-97.0	93.5	3.84	4.11

Data (uncorrected recovery results; pp. 28-29) were obtained from Tables 1-12, pp. 36-47 of MRID 50693101 and DER Attachment 2.

1 The drinking water [pH 6.9, total hardness 78 mmol/L as CaCO₃, total alkalinity 20 mmol/L as CaCO₃, conductivity 604 µS/cm] was collected as laboratory well water and surface water [Lot No. 17Oct16Wat-A-3; pH 6.9, dissolved oxygen concentration 9.3 mg/L] was collected from the Weweantic River in West Wareham, Massachusetts (p. 17). Water characterization source information was not provided.

2 Two ion pair transitions were monitored (quantitation and confirmation, respectively): *m/z* 233→160 and *m/z* 233→72 for fluometuron, *m/z* 219→162 and *m/z* 219→142 for des-methyl-fluometuron, and *m/z* 162→142 and *m/z* 162→93 for CGA72903; these were similar to those of the ECM.

3 Mean, standard deviation, and relative standard deviation were reviewer-calculated using all five recovery values since the study author omitted the 132% value from the statistics since it was outside of the acceptance criteria (see DER Attachment 2). Rules of significant figures were followed.

III. Method Characteristics

In the ECM and ILV, the LOQ was 0.05 µg/L for fluometuron, des-methyl-fluometuron and CGA72903 in water (p. 25 of MRID 50556602; pp. 26-27, 29-31 of MRID 50693101). In the ECM and ILV, the LOQ was defined as the lowest fortification level for which mean recoveries were 70-120%, RSD was ≤20%, and blanks did not exceed 30%. No calculations were provided for the LOQ in the ECM or ILV. In the ECM, the LOD was defined as 0.015 µg/L (30% of the LOQ). In the ILV, the LOD was also defined as the lowest concentration in test samples which can be detected based on the concentration of the low calibration standard and the dilution factor of the control solutions using the following equation:

$$\text{LOD} = \text{LOD}_{\text{LCAL}} \times \text{DF}_{\text{CTRL}}$$

Where, LOD is the limit of detection of the analysis (0.0100 µg/L × 1.00 = 0.0100 µg/L for direct dilution procedure; 0.0100 µg/L × 1.05 = 0.0105 µg/L for standard addition procedure), LOD_{LCAL} is the lowest concentration calibration standard (0.0100 µg/L), and DF_{CTRL} is the dilution factor of the control samples (smallest dilution factor used, i.e., 1.00). In the ILV, the LOD was calculated to be 0.0105 µg/L (method, direct dilution procedure) and 0.0100 µg/L (instrument, standard addition procedure) for all three analytes.

Table 4. Method Characteristics for Fluometuron, Des-Methyl-Fluometuron and CGA72903 in Water

Parameter		Fluometuron	Des-Methyl-Fluometuron	CGA72903
Limit of Quantitation (LOQ)	ECM	0.05 µg/L		
	ILV			
Limit of Detection (LOD)	ECM (Method)	0.015 µg/L (30% of the LOQ)		
	ILV (Calculated)	0.0105 µg/L (method, direct dilution procedure) 0.0100 µg/L (instrument, standard addition procedure)		
Linearity (calibration curve r^2 and concentration range) ¹	ECM	$r^2 = 0.9984$ (Q) $r^2 = 0.9972$ (C)	$r^2 = 0.9990$ (Q) $r^2 = 0.9984$ (C)	$r^2 = 0.9986$ (Q) $r^2 = 0.9988$ (C)
		0.1-10 µg/L		
	ILV	$r^2 = 1.0000$ (Q) $r^2 = 0.9980$ (C)	$r^2 = 0.9980$ (Q & C)	$r^2 = 1.0000$ (Q) $r^2 = 0.9980-1.0000$ (C)
		0.01-10 µg/L		
Repeatable	ECM ²	Yes at LOQ and 10×LOQ. (two characterized water matrices used)		
	ILV ^{3,4}	Yes at LOQ and 10×LOQ. (two characterized water matrices used)		
Reproducible		Yes at LOQ and 10×LOQ.		
Specific	ECM	Yes, no matrix interferences were observed; elevated baseline noted.	Yes, no matrix interferences were observed.	Yes, no matrix interferences were observed; elevated baseline noted.
	ILV	Yes, no matrix interferences were observed, but some minor contaminants were observed near the analyte peak RT. Some non-uniform peak integration noted in the C ion.		

Data were obtained from p. 25 (LOQ/LOD); Tables 6-8, pp. 23-24 (recovery results); p. 25; Appendix B, Figures 1-6, pp. 34-39 (calibration data & curves); Appendix D, Figures 10-21, pp. 42-77 (chromatograms) of MRID 50556602; pp. 26-27, 29-31 (LOQ/LOD); Tables 1-12, pp. 36-47 (recovery results); pp. 31-32 (calibration data); Figures 1-42, pp. 60-101 (calibration curves & chromatograms) of MRID 50693101; DER Attachment 2. Q = quantitation ion; C = confirmation ion. All results reported for Q and C ions unless specified otherwise. All results reported for both test matrices in each study unless specified otherwise.

1 Reported r^2 values were reviewer-calculated from r values provided in the study report (Appendix B, Figures 1-6, pp. 34-39 of MRID 50556602; p. 32 of MRID 50693101; DER Attachment 2). Reported ILV values include sample set correlation data from both waters.

2 In the ECM, the drinking water [pH 7.62 (at 17.5°C), total hardness 4.1 mmol/L, 0.44 mg/L dissolved organic carbon, specific electric conductivity 754 µS/cm (at 20°C)] was collected as tap water from the test facility and surface water [pH 8.02 (at 17.8°C), total hardness 1.4 mmol/L, 4.7 mg/L dissolved organic carbon, specific electric conductivity 271 µS/cm (at 20°C)] was collected from the river Dendelbach in Spregelberg, Germany (pp. 14-15; Appendix E, Figures 25-26, pp. 81-82 of MRID 50556602). Waters were well characterized by Eurofins Institut Jäger GmbH, Tübingen.

3 In the ILV, the drinking water [pH 6.9, total hardness 78 mmol/L as CaCO₃, total alkalinity 20 mmol/L as CaCO₃, conductivity 604 µS/cm] was collected as laboratory well water and surface water [Lot No. 17Oct16Wat-A-3; pH 6.9, dissolved oxygen concentration 9.3 mg/L] was collected from the Weweantic River in West Wareham, Massachusetts (p. 17 of MRID 50693101). Water characterization source information was not provided.

4 The ILV validated the ECM in the first trial for all three analytes in both water matrices with the substitution of 0.25% formic acid in water and 0.25% formic acid in acetonitrile for the LC mobile phase, modifications of the MS

parameters, and insignificant modifications to the analytical equipment (pp. 16, 21-25, 30 of MRID 50693101).

IV. Method Deficiencies and Reviewer's Comments

1. The specificity of the method was acceptable, but not fully supported by ILV representative chromatograms since some minor contaminants were observed near the analyte peak RT for the majority of the analytes/matrices (Figures 1-42, pp. 60-101 of MRID 50693101). This suggests that some water matrices could require additional sample clean-up prior to analysis for accurate quantitation of the analyte.
2. The necessity and use of two different sample processing methods (direct injection and sample addition) in the ECM was not clear, although the standard/sample addition method was reportedly used to compensate for possible matrix effects in the ECM (p. 25 of MRID 50556602). For direct injection, the samples were fortified with a mixed fortification solution at the LOQ and 10×LOQ then directly analyzed by LC/MS/MS (p. 16 of MRID 50556602). For the sample addition, the fortified samples were dosed again prior to LC/MS/MS. Representative chromatograms from both processing methods were shown in the ECM (Appendix D, Figures 10-21, pp. 42-77); however, only recovery data from direct injection samples was used for method validation (Appendix A, Tables 9-14, pp. 28-33). The ILV performed both sample processing methods and, also, only used recovery data from direct injection samples for method validation (pp. 21-23; Tables 1-12, pp. 36-47 of MRID 50693101); however, no representative chromatograms from the sample addition method were shown in the ILV (Figures 1-42, pp. 60-101).
3. The communications between the ILV and Study Monitor (Janelle Kay of Pyxis Registry Consulting, Inc., Gig Harbor, Washington, as an agent for ADAMA Agan Ltd.) were reportedly limited to approval of the protocol and method, timing updates, and the results of the first attempt of the ILV (p. 27; Appendix 3, pp. 116-122 of MRID 50693101). A complete summary list of communication was provided.
4. The reviewer considered all ILV modifications to be optimization of the LC/MS/MS parameters, which is usually considered to be adjusted from laboratory to laboratory, unless the ILV stated that the original LC/MS/MS parameters were defective. The reviewer determined that an updated ECM was not required.
5. The determinations of the LOD and LOQ in the ECM and ILV were not based on scientifically acceptable procedures as defined in 40 CFR Part 136 p. 25 of MRID 50556602; pp. 26-27, 29-31 of MRID 50693101). In the ECM and ILV, the LOQ was defined as the lowest fortification level for which mean recoveries were 70-120%, RSD was ≤20%, and blanks did not exceed 30%. No calculations were provided for the LOQ in the ECM or ILV. In the ECM, the LOD was defined as 0.015 µg/L (30% of the LOQ). In the ILV, the LOD was also defined as the lowest concentration in test samples which can be detected based on the concentration of the low calibration standard and the dilution factor of the control solutions using the following equation: $LOD = LOD_{LCAL} \times DF_{CTRL}$, where, LOD is the limit of detection of the analysis ($0.0100 \mu\text{g/L} \times 1.00 = 0.0100 \mu\text{g/L}$ for direct dilution procedure; $0.0100 \mu\text{g/L} \times 1.05 = 0.0105 \mu\text{g/L}$ for standard addition procedure), LOD_{LCAL} is the lowest concentration calibration standard (0.0100 µg/L), and DF_{CTRL} is the dilution factor of the control samples (smallest dilution factor used, i.e., 1.00). In the ILV, the LOD

was calculated to be 0.0105 µg/L (method, direct dilution procedure) and 0.0100 µg/L (instrument, standard addition procedure) for all three analytes. Detection limits should not be based on arbitrary values.

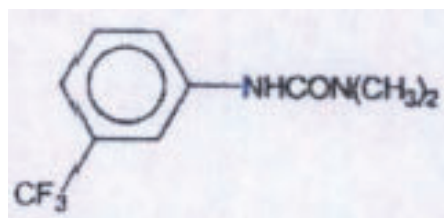
6. In the ILV, it was reported that matrix effects were insignificant (< 20% difference from non-matrix standards) for fluometuron, CGA72903 and des-methyl-fluometuron for each mass transition for the waters tested (p. 31; Tables 13-24, pp. 48-59 of MRID 50693101). Solvent-based calibration standards were used. Solvent-based calibration standards were also used in the ECM, although the standard/sample addition method was reportedly used to compensate for possible matrix effects in the ECM (pp. 13-14, 25 of MRID 50556602).
7. In the ECM, it was stated that storage stability of the extract solutions was determined to be up to 2 days in the dark (p. 26 of MRID 50556602).
8. The time required to complete the method for a set of 45 samples (20 fortified samples, four unfortified samples, two reagent blank, nine matrix effects standards, and ten calibration standards) was reported as one working day (eight hours) with LC/MS/MS performed overnight (*ca.* 12 hours) in the ILV (p. 27 of MRID 50693101).

V. References

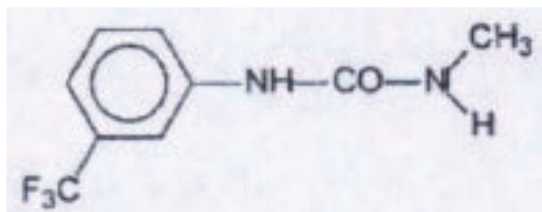
- U.S. Environmental Protection Agency. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.
- 40 CFR Part 136. Appendix B. Definition and Procedure for the Determination of the Method Detection Limit-Revision 1.11, pp. 317-319.

Attachment 1: Chemical Names and Structures**Fluometuron**

IUPAC Name: Not reported
CAS Name: N,N-dimethyl-N'-[3-(trifluoromethyl)phenyl]urea
CAS Number: 2164-17-2
SMILES String: Not found

**Des-Methyl-Fluometuron**

IUPAC Name: Not reported
CAS Name: 1-Methyl-1-hydrogen-3-(a,a,a-trifluoro-m-tolyl)urea
CAS Number: 3032-404
SMILES String: Not found

**CGA72903**

IUPAC Name: Not reported
CAS Name: 3-Trifluoromethyl aniline (TFMA)
CAS Number: 98-16-8
SMILES String: Not found

