

Analytical method for flutianil and its metabolites, OC-56635, OC-56574, and OC-53276, in soil

Reports: ECM: EPA MRID No. MRID 49490505. Hattermann, D.R., and M. Lee. 2015. Terrestrial Field Dissipation of Residues Following Application of Flutianil to Bare Soil. Unpublished study performed by Landis International, Inc., Valdosta, Georgia; sponsored and submitted by OAT Agrio Co., Ltd., Tokyo, Japan. Landis Protocol No. 47621A003. Wildlife International Project No. 181C-103. OTSB-0508(27)-FR. 554 pages. Final report issued September 29, 2015.

ILV: EPA MRID No. 49490591. Marshall, M., and R. Perez. 2015. Independent Laboratory Validation of Draft Analytical Method: "Determination of Flutianil and its Metabolites In Soil Using LC-MS/MS". Unpublished study performed by ADPEN Laboratories, Inc., Jacksonville, Florida; sponsored and submitted by OAT Agrio Co., Ltd., Tokyo, Japan. ADPEN Report No. ADPEN-2K15-1404-0204-001; Study No. 2K15-1404-0204. OTSB-0508(55S)-FR. 100 pages. Final report issued September 26, 2015.

Document No.: MRIDs 49490505 & 49490591

Guideline: OCSPP 835.6100

Statements: ECM: The study was conducted in compliance with FIFRA, OECD and Japanese GLP standards, with the exception of the pesticide history, weather data, plot establishment and maintenance activities, irrigation, maps and diagrams, field preparation and plot coordinate determinations, certificates of analysis for analytical standards and the 5% EC formulation (p. 3). Signed and dated Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2-4). A Certification of Authenticity was not provided.

ILV: The study was conducted in compliance with FIFRA GLP standards (p. 3). Signed and dated Data Confidentiality, GLP, Quality Assurance and Certification of Authenticity statements were provided (pp. 2-5).

Classification: The ECM part of this analytical method is classified as acceptable. However, due to the soil matrix not characterized in the ILV. In the ILV, representative chromatograms did not support the specificity of the method for OC-56574. Linearity was not satisfactory ($r^2 \geq 0.995$) for the quantification ion calibration curve of OC-53276. The ILV part of this analytical method is classified as supplemental.

PC Code: 014018

Reviewer:

James Lin
Environmental Engineer

Signature: 
Date: 07-26-2016

Any page numbers cited for MRID 49490505 refer to those written at the bottommost middle of the document pages.

Executive Summary:

The analytical method, MRID 49490505 (Landis International Study Number 47621A003), is designed for the quantitative determination of flutianil and metabolites OC-56635, OC-56574, and OC-53276 in soil using LC-MS/MS (see **Table 1**). The method is quantitative for flutianil and metabolites OC-56635, OC-56574, and OC-53276 at the stated LOQ of 0.01 ppm. The independent laboratory validation (ILV) of the analytical draft method for flutianil and its metabolites was successfully completed for soil during the first trial (environmental chemistry method). However, the soil matrix was not characterized in the ILV. It could not be determined if the ILV was provided with the most difficult matrix with which to validate the method. Additionally, the provided ILV chromatograms did not support the specificity of the method for OC-56574 due to significant matrix and contaminant interferences.

Table 1 Analytical Method Summary^{1,2}

Analyte(s) by Pesticide	MRID		Matrix	Method Date	Registrant	Analysis	Limit of Quantitation (LOQ)
	Environmental Chemistry Method	Independent Laboratory Validation					
Flutianil, OC-56635 OC-56574 OC-53276	MRID 49490505	MRID 49490591	Soil	9/26/2015	OAT Agrio Co., Ltd.	LC- MS/MS	0.01 ppm

¹ In the ECM, the soil for method validation was collected from the 0-3 in. horizon of the Iowa field site in Greene County (Sample No. LA12-2217; Table 8, p. 42; Appendix V, p. 203 of MRID 49490505). The 0-6 in. depth soil from Iowa was characterized as loam (46% sand, 31% silt, 23% clay; pH 6.2; total organic carbon 3.5%). No separate characterization was provided for the 0-3 in. depth soil sample.

² Uncharacterized soil was used in the ILV (p. 15 of MRID 49490591).

I. Principle of the Method

The analytical method applied to analysis of soil for flutianil, OC 53276, OC 56574, and OC 56635. The method was validated prior to use for recovery of flutianil, OC 53276, OC 56574, and OC 56635.

The method consisted of extracting 10 grams of soil with 25 mL of acetonitrile by hand shaking followed by sonication for one minute followed by centrifugation and decanting the supernatant. The extraction was repeated with 25 mL of acetonitrile:water (75:25, v/v) followed by centrifugation and decanting the supernatant to combine with the original super. Another 25 mL of acetonitrile:water (75:25, v/v) was added to the soil pellet and extracted again using a gyratory shaker table. The sample was centrifuged and the supernatant was combined with other extract portions for that same sample. After adjusting the extract to an 80 mL volume, a 5-mL aliquot was filtered. A 2.5-mL aliquot of the filtered extract was transferred to a plastic centrifuge tube and evaporated to aqueous remainder using a nitrogen evaporator, about 0.5 milliliter. A 1-mL aliquot of acetonitrile was added to each aqueous remainder, mixed well and adjusted to 10 mL final volume using a solution of 0.1% formic acid in water to achieve a final extract of acetonitrile:water:formic acid (10:90:0.1, v:v:v). After mixing well, an aliquot was analyzed by LC/MS/MS.

Each analytical sequence consisted of injection of the calibration standard solution series, followed by sample injections interspersed with standard solution injections and concluding with the same complete series of calibration standard solutions. A calibration standard solution was injected following no more than five sample extract injections.

An aliquot of each sample extract was transferred to an auto sampler vial for subsequent separation of analytes and quantitation by LC/MS/MS. The liquid chromatograph was connected to the mass spectrometer through a Valco valve that diverted only the eluate from 5.5 to 11.0 minutes post-injection to the LC/MS/MS. Flutianil, OC 53276, and OC 56574 were quantified in the positive-ion multiple reaction monitoring (MRM) mode. OC 56635 was quantified in the negative ion MRM mode.

Concentrations of flutianil and its metabolites (OC56636, OC56574, and OC53276) in soil samples were determined using HPLC coupled with MS/MS operated in both negative and positive ion, multiple reaction monitoring (MRM) mode (see Table 2). The instrumental conditions consisted of a Phenomenex LUNA 5 C-18(2) column (150 x 2.0 mm, 5- μ m; column temperature 40°C), Phenomenex Security C-18 column (4 x 3 mm), a gradient mobile phase of (A) water containing 0.2% formic acid and (B) acetonitrile containing 0.2% formic acid [percent A:B (v:v) at 0.0-2.0 min. 80.0:20.0, 9.0-10.0 min. 5.0:95.0, 10.5-15.0 min. 80.0:20.0], and injection volume 50.0 μ L. Two parent-daughter ion transitions were monitored per analyte.

Table 2 Summary Parameters for the Analytical Method Used for the Quantitation of Flutianil and Metabolite Residues in Surface and Ground Water.¹

Instrument/Detector	Hewlett-Packard Series 1200 High Performance Liquid Chromatograph (HPLC) coupled with an MDS SCIEX API 5000 Tandem Mass Spectrometer (MS/MS) with QJet Ion Guide Technology operated in both negative and positive ion, multiple reaction monitoring (MRM) modes.
Approximate Retention times²	Flutianil – 10.3 minutes OC 56574 – 9.2 minutes OC 53276 – 9.1 minutes OC 56635 – 7.2 minutes
Monitored transitions²	Quantitation Ion Transition: Flutianil – 427 → 192 amu OC 56574 – 443 → 136 amu OC 53276 – 443 → 192 amu OC 56635 – 243 → 179 amu Confirmation Ion Transition: Flutianil – 427 → 132 amu OC 56574 – 443 → 181 amu OC 53276 – 443 → 132 amu OC 56635 – 243 → 80 amu

¹ Data provided for ECM unless otherwise noted.

² Data obtained from Appendix V, Table 1, pp. 220-221 of MRID 49490505.

In the ILV, the ECM was performed as written with the specifications of the centrifuge speed (3500 rpm) and filter (0.2 μ m PURASDISK 25 TF syringe filter). Also, a mechanical shaker (15 minutes at approximately 250 rpm) was used instead of a gyratory shaker table for mixing. The ILV employed a few minor modifications to the analytical method. An Agilent 1290 LC was

coupled to an AB Sciex Qtrap 5500 MS (AB Sciex 5500 QT MS). The following instrumental conditions differed from those of the ECM: no guard column was used and injection volume, 60 μ L. Reported retention times were 9.0, 7.69, 7.73 and 7.59 minutes for flutianil, OC 53276, OC 56574 and OC 56635, respectively. None of these modifications were considered significant.

The LOQ in soil was the same for flutianil and the metabolites OC 53276, OC 56574 and OC 56635 at 10 μ g/Kg. The LOQ in water (field irrigation sampling) was 0.100 ng/mL. The LOD in soil was 0.002 ppm for all analytes.

II. Recovery Findings

The mean recovery of flutianil and its 3 metabolites in soil was within 70-120% and the relative standard deviation (%RSD) at the quantitation and confirmation ion transitions was within the OCSPP 850.6100 guideline requirements (<20% RSD at each fortification level).

ECM (MRID 49490505): Mean recoveries and relative standard deviations (RSDs) were within guidelines (mean 70-120%; RSD \leq 20%) for analysis of flutianil and metabolites OC-56635, OC-56574 and OC-53276 in soil at fortification levels of 0.01 ppm (LOQ) and 0.1 ppm (10 \times LOQ; see Table 3 below). For all analytes, two ion transitions were monitored using LC/MS/MS; however, performance data (recovery results) were only evaluated for the quantitative ion transition. The soil matrix was well characterized. The soil for method validation was collected from the 0-3 in. horizon of the Iowa field site in Greene County (Sample No. LA12-2217). The 0-6 in. depth soil from Iowa was characterized as loam (46% sand, 31% silt, 23% clay; pH 6.2; total organic carbon 3.5%). No separate characterization was provided for the 0-3 in. depth soil sample.

ILV (MRID 49490591): Mean recoveries and relative standard deviations (RSDs) were within guidelines for analysis of flutianil and metabolites OC-56635, OC-56574 and OC-53276 in soil at fortification levels of 0.01 ppm (LOQ) and 0.1 ppm (10 \times LOQ; see Table 4 below). For all analytes, two ion transitions were monitored using LC/MS/MS; performance data (recovery results) of the quantitative and confirmatory results were comparable. The soil matrix was not characterized or described. Soil samples were supplied by ADPEN Laboratories, Inc. The method was validated in the first trial for all analytes in the soil matrix with only minor modifications to the analytical parameters.

Table 3 Initial Validation Method Recoveries for flutianil and its metabolites, OC-56635, OC-56574 and OC-53276 in soil

Analyte	Fortification Level (µg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Flutianil	10	7	89.1 to 93.8	91.3	1.8	1.9
	100	3	91.4 to 93.9	92.6	1.3	1.4
OC56635	10	7	96.8 to 101	99.5	1.3	1.3
	100	3	102 to 103	103	0.2	0.2
OC56574	10	7	93.8 to 98.2	96.3	1.6	1.6
	100	3	106 to 107	107	0.7	0.6
OC53276	10	7	99.9 to 106	102	1.9	1.9
	100	3	101 to 103	102	0.8	0.8

Table 4 Independent Validation Method Recoveries for flutianil and its metabolites, OC-56635, OC-56574, and OC-53276 in soil

Analyte	Fortification Level (ppm)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Quantitation (<i>m/z</i> 427 → 192)						
Flutianil	0.01	5	77 to 84	79	2.7	3.3
	0.1	5	71 to 82	77	3.6	4.7
Confirmatory (<i>m/z</i> 427 → 132)						
Flutianil	0.01	5	78 to 84	80	2.3	2.9
	0.1	5	68 to 80	73	4.4	6.0
Quantitation (<i>m/z</i> 243 → 179)						
OC56635	0.01	5	91 to 95	93	1.9	2.1
	0.1	5	86 to 92	89	2.0	2.2
Confirmatory (<i>m/z</i> 243 → 80)						
OC56635	0.01	5	82 to 96	90	5.5	6.1
	0.1	5	87 to 91	89	1.3	1.5
Quantitation (<i>m/z</i> 443 → 136)						
OC56574	0.01	5	72 to 99	89	12.0	13.4
	0.1	5	79 to 85	82	2.7	3.3
Confirmatory (<i>m/z</i> 443 → 181)						
OC56574	0.01	5	72 to 101	88	11.9	13.6
	0.1	5	79 to 85	82	2.3	2.8
Quantitation (<i>m/z</i> 443 → 192)						
OC53276	0.01	5	97 to 104	102	3.0	2.9
	0.1	5	98 to 101	99	1.3	1.3
Confirmatory (<i>m/z</i> 443 → 132)						
OC53276	0.01	5	92 to 104	100	5.0	5.0
	0.1	5	93 to 96	94	0.9	0.9

III. Method Characteristics

For flutianil and its three metabolites OC-56635, OC-56574, and OC-53276, the limit of quantitation (LOQ) was set to 0.01 ppm (10 µg/kg). For flutianil and its three metabolites OC-56635, OC-56574, and OC-53276, the limit of detection (LOD) was set to 0.002 ppm (2 µg/kg). In the ECM, the LOQ and LOD were calculated using the following equations:

$$\text{LOD} = (t_{0.99})(S)$$
$$\text{LOQ} = 3 \times \text{LOD}$$

Where t = the one-tailed t-statistic at the 99% confidence level for $n-1$ replicates, and
 S = the standard deviation of n samples fortified at the defined/target LOQ.

Using these equations, the calculated LODs for flutianil, OC 53276, OC56574 and OC 56635 were 0.55, 0.60, 0.50 and 0.42 µg/kg, respectively. The calculated LOQs for flutianil, OC 53276, OC56574 and OC 56635 were 1.7, 1.8, 1.5 and 1.3 µg/kg, respectively. The calculated LODs and LOQs supported those of the study since they were <20% of the target LOQ/LOD for all analytes. In the ILV, the LOQ and LOD values were reported from the ECM without justification or calculation.

The method was highly selective/specific for analysis of the test item (mass transitions from the positively charged molecule ion to two typical fragment ions in MS/MS mode for flutianil, OC 53276 and OC 56574 and negatively charged molecule ion to two typical fragment ions in MS/MS mode for OC 56635 as listed below). The two MRM transitions used to flutianil and its metabolites were defined in the draft method provided. The retention time of the test item in matrix matched the retention times in fortified samples. No peak interferences occurred at the retention times of the test item.

For analysis of the test item by LC-MS/MS, the detector response was linear ($r^2 > 0.99$) within the range from 0.05 ng/mL to 10.0 ng/mL for both transitions of each analyte.

The mean recovery of flutianil and its 3 metabolites in soil was within 70–120% and the relative standard deviation (%RSD) at the quantitation and confirmation ion transitions was within the OCSPP 850.6100 guideline requirements (<20 % RSD at each fortification level).

Table 5 Method Characteristics

Characteristic		Flutianil	OC-56635	OC-56574	OC-53276
Limit of Quantitation (LOQ)		0.01 ppm	0.01 ppm	0.01 ppm	0.01 ppm
Limit of Detection (LOD) ¹		0.002 ppm	0.002 ppm	0.002 ppm	0.002 ppm
Linearity (calibration curve r^2 and concentration range)		$r^2 > 0.99$ 0.05 ng/mL to 10.0 ng/mL	$r^2 > 0.99$ 0.05 ng/mL to 10.0 ng/mL	$r^2 > 0.99$ 0.05 ng/mL to 10.0 ng/mL	$r^2 > 0.99$ 0.05 ng/mL to 10.0 ng/mL
	ECM ²	$r^2 = 0.9996$	$r^2 = 0.9998$	$r^2 = 0.9994$	$r^2 = 0.9996$
	ILV ³	$r^2 = 0.9994$ (Q) $r^2 = 0.9982$ (C)	$r^2 = 1.0000$ (Q) $r^2 = 0.9996$ (C)	$r^2 = 0.9968$ (Q) $r^2 = 0.9974$ (C)	$r^2 = 0.9916$ (Q) $r^2 = 0.9986$ (C)
Repeatable ⁴		Yes	Yes	Yes	Yes
Reproducible ⁴		Yes	Yes	Yes	Yes
Specific		Yes	Yes	Yes	Yes
	ECM ⁵	No matrix interferences.			
	ILV ⁶	Matrix interference was <5% of the LOQ at retention time of analyte.	No matrix interferences.	Control soil samples contained significant (<i>ca.</i> 70% of LOQ) of the analyte. ⁷ Minor baseline interference observed due to a contaminant occurring at RT <i>ca.</i> 8 min. Analyte peak integration was affected by the contaminant peak.	Minor baseline interference observed due to a contaminant occurring at RT <i>ca.</i> 8 min. Analyte peak integration was slightly affected by the contaminant peak.

1 See text above for ECM calculated LOQs and LODs for soil.

2 Reported r^2 values were reviewer-calculated from r values of 0.9997-0.9999 (analytes combined; quantitation ion only). Data (r values) obtained from Appendix V, Figures 2-5, pp. 251-254 of MRID 49490505.

3 Reported r^2 values were reviewer-calculated from r values of 0.9958-1.0000 (analytes/ions combined). Data (r values) obtained from Figures 1-2, pp. 38-39, Figures 9-10, pp. 50-51, Figures 17-18, pp. 62-63, and Figures 25-26, pp. 74-75 of MRID 49490591.

4 At the LOQ and 10×LOQ. The soil for method validation was collected from the 0-3 in. horizon of the Iowa field site in Greene County (Sample No. LA12-2217; Table 8, p. 42; Appendix V, p. 203 of MRID 49490505). The 0-6 in. depth soil from Iowa was characterized as loam (46% sand, 31% silt, 23% clay; pH 6.2; total organic carbon 3.5%). No separate characterization was provided for the 0-3 in. depth soil sample. Uncharacterized soil was used in the ILV (p. 15 of MRID 49490591).

5 Data obtained from Appendix V, Figures 10-15, pp. 259-264 of MRID 49490505.

6 Data obtained from Figures 3-32, pp. 40-85 of MRID 49490591.

7 Figure 22 (p. 71) of the ILV MRID 49490591 showed OC-56574 in the control soil at a height of *ca.* 1.0×10^5 ; however, the study authors did not report the integration of this peak. Figure 23 (p. 72) of the ILV showed OC-56574 fortified at the LOQ at a height of *ca.* 1.3×10^5 (peak integration 606106 units).

Linearity is satisfactory when $r^2 \geq 0.995$.

IV. Method Deficiencies and Reviewer's Comments

1. The analytical portion of the ECM was contained in Appendix V of MRID 49490505 (pp. 184-512; MacGregor, J.A., and W.B. Nixon. 2015. TERRESTRIAL FIELD DISSIPATION OF RESIDUES FOLLOWING APPLICATION OF FLUTIANIL TO BARE SOIL. Unpublished study performed by Wildlife International, Evans Analytical Group, Easton, Maryland; sponsored and submitted by OAT Agrico Co., Ltd., Tokyo, Japan. Wildlife International Project No. 181C-103. Protocol (Study) No. 47621A003. 328 pages. Final report issued September 28, 2015.).
2. In the ILV, the soil matrix was not characterized (p. 15 of MRID 49490591). It could not be determined if the ILV was provided with the most difficult matrix with which to validate the method.
3. In the ILV, chromatograms for OC-56574 (RT *ca.* 7.73 min.) showed a significant amount (*ca.* 70% of LOQ) of the analyte in the control soil samples (Figure 22, p. 71 of MRID 49490591). OC-56574 in the control soil was observed at a height of *ca.* 1.0×10^5 ; however, the study authors did not report the integration of this peak. The height of OC-56574 fortified at the LOQ was *ca.* 1.3×10^5 (peak integration 606106 units; Figure 23, p. 72). Additionally, minor baseline interference observed due to a contaminant occurring at RT *ca.* 8 min.; analyte peak integration was affected by the contaminant peak (Figures 21-24, pp. 70-73).

In the ILV, chromatograms for OC-53276 (RT *ca.* 7.69 min.) a contaminant occurring at RT *ca.* 8 min. slightly affected peak integration of the analyte (Figures 14-15, pp. 59-60 of MRID 49490591).

4. In the ILV, linearity was not satisfactory ($r^2 \geq 0.995$) for the quantification ion calibration curve of OC-53276 ($r^2 = 0.9916$; see Table 5).
5. The toxicological level of concern was not reported for the analytes in water. A LOQ above toxicological levels of concern results in an unacceptable method classification.
6. The ILV reported that no communications occurred between the ILV laboratory and the study director other than notification of the success of the ILV (p. 20 of MRID 49490591).
7. It was reported for the ILV that the analytical procedure for two sets of 13 samples required approximately eight hours for laboratory preparation (p. 21 of MRID 49490591). The time required for LC/MS/MS was not reported. The overall time was not reported.

VI. 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.

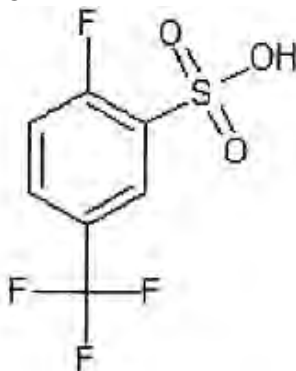
Attachment 1: Chemical Names and Structures for the Reference Materials

Name:	OK-5203
Common Name:	Flutianil
Batch No.:	05DF2
CAS Number:	958647-10-4
IUPAC Name:	(Z)-2-[2-fluoro-5-(trifluoromethyl)phenylthio]-2-[3-(2-methoxyphenyl)-1,3-thiazolidin-2-ylidene]acetonitrile
Molecular Formula:	C ₁₉ H ₁₄ F ₄ N ₂ OS ₂
Molecular Weight:	426.5 g/mol
Purity:	99.54%
Expiration Date:	November 26, 2016
Storage:	Refrigerated in darkness
Chemical Structure:	



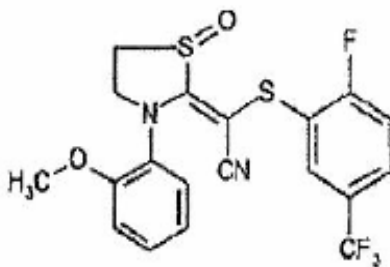
Attachment 1: Chemical Names and Structures for the Reference Materials (Cont'd)

Common Name: OC 56635
Batch No.: 81010
IUPAC Name: (2-fluoro-5-trifluoromethyl)benzenesulfonic acid
Molecular Formula: C₇H₄F₄O₃S
Molecular Weight: 244.16 g/mol
Purity: 97.3%
Expiration Date: 04/22/18
Storage: Refrigerated in darkness
Chemical Structure:



Attachment 1: Chemical Names and Structures for the Reference Materials (Cont'd)

Common Name:	OC 56574
Batch No.:	TT0908011
IUPAC Name:	(Z)-2-[(2-fluoro-5-trifluoromethyl)phenylthio]-2-[3-(2-methoxyphenyl)-1-oxo-2-thiazolidinylidene]acetonitrile
Molecular Formula:	C ₁₉ H ₁₄ F ₄ N ₂ O ₂ S ₂
Molecular Weight:	442.45 g/mol
Purity:	98.7%
Expiration Date:	July 06, 2018
Storage:	Refrigerated in darkness
Chemical Structure:	



Attachment 1: Chemical Names and Structures for the Reference Materials (Cont'd)

Common Name:	OC 53276
Batch No.:	TT1005019
IUPAC Name:	(Z)-2-[2-fluoro-5-(trifluoromethyl)phenylsulfinyl]-2-[3-(2methoxyphenyl)thiazolidinylidene]acetonitrile
Molecular Formula:	C ₁₉ H ₁₄ F ₄ N ₂ O ₂ S ₂
Molecular Weight:	442.45 g/mol
Purity:	96.54%
Expiration Date:	July 06, 2018
Storage:	Refrigerated in darkness
Chemical Structure:	

