**Analytical method for flutianil and its metabolites, OC-56635, OC-56574, OC-53276, and OC-53279, in surface and ground water**

**Reports:** ECM: EPA MRID No. MRID 49490588. MacGregor, J.A., and E.S. Bodle. 2015. VALIDATION OF A METHOD FOR THE DETERMINATION OF FLUTIANIL AND METABOLITES (OC56635, OC56574, OC53276, AND OC53279) IN SURFACE AND GROUND WATER. Unpublished study performed by Wildlife International, Evans Analytical Group, Easton, Maryland; sponsored and submitted by OAT Agrio Co., Ltd., Tokyo, Japan. Wildlife International Project No. 181C-116. OTSB-0508(71)-FR. 108 pages. Final report issued August 27, 2015.

ILV: EPA MRID No. 49490522. Marshall, M., and R. Perez. 2015. Independent Laboratory Validation of Draft Analytical Method: "Determination of Flutianil and (OC-56635, OC-56574, OC-53276 and OC-53279) Metabolites in Water 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 Unpublished study performed by ADPEN Laboratories, Inc., Jacksonville, Florida; sponsored and submitted by OAT Agrio Co., Ltd., Tokyo, Japan.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. 15H0104-001; Study No. 15H0104. OTSB-0508(55W)-FR. 161 pages. Final report issued September 26, 2015.

**Document No.:** MRIDs 49490588 & 49490522

**Guideline:** OCSPP 835.6100

**Statements:** ECM: The study was conducted in compliance with FIFRA, OECD and Japanese GLP standards, with the exception of the periodic analysis of the well water for contaminants (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 water matrices were not characterized in the ILV. In the ILV, representative chromatograms did not support the specificity of the method for OC-56574 in both matrices. The ILV part of this analytical method is classified as supplemental.

**PC Code:** 014018

**Reviewer:**

James Lin **Signature:**
Environmental Engineer **Date:** 7-26-2016

**Executive Summary:**

The analytical method, MRID 49490588 (Wildlife International Study Number 181C-116), is designed for the quantitative determination of flutianil and metabolites OC-56635, OC-56574, OC-53276 and OC-53279 in surface and ground water using LC-MS/MS (see **Table 1**). The method is quantitative for flutianil and metabolites OC-56635, OC-56574, OC-53276 and OC-53279 at the stated LOQ of 0.001 ppm. The independent laboratory validation (ILV) of the analytical draft method for flutianil and its metabolites was successfully completed for both surface and ground water during the first trial (environmental chemistry method). However, the water matrices were 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]**

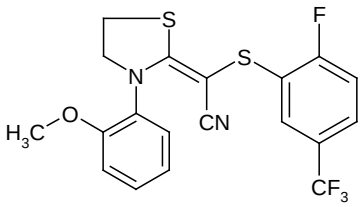
| Analyte(s) by Pesticide | MRID | | Matrix | Method Date | Registrant | Analysis | Limit of Quantitation (LOQ) |
|---|---|---|---|---|---|---|---|
| | Environmental Chemistry Method | Independent Laboratory Validation | | | | | |
| **Flutianil, OC-56635 OC-56574 OC-53276 OC-53279** | MRID 49490588 | MRID 49490522 | Surface Water Ground Water | 8/27/2015 | OAT Agrio Co., Ltd. | LC-MS/MS | 0.001 ppm |

1 Surface (lake) water (pH 7.56, hardness 76.0 mg/L $CaCO_3$, alkalinity 32.0 mg/L $CaCO_3$) and ground (well) water (pH 8.0, hardness 134 mg/L $CaCO_3$, alkalinity 176 mg/L $CaCO_3$) were used in the ECM (pp. 19-20; Appendices 7-9, pp. 104-107 of MRID 49490588).

2 Uncharacterized surface and ground water were used in the ILV (p. 18 of MRID 49490522).

## I. Principle of the Method

**Table 2 Flutianil Nomenclature**

| | |
|---|---|
| **Flutianil** |  |
| **Common name** | **flutianil** |
| **Company experimental name** | **OK-5203** |
| **IUPAC name** | **(Z)-2-[2-fluoro-5-(trifluoromethyl)phenylthio]-2-[3-(2-methoxyphenyl)-1,3-thiazolidin-2-ylidene]acetonitrile** |
| **CAS name** | **(2Z)-2-[[2-fluoro-5-(trifluoromethyl)phenyl]thio]-2-[3-(2-methoxyphenyl)-2-thiazolidinylidene]acetonitrile** |
| **CAS #** | **958647-10-4** |
| **End-use product/EP** | **Flutianil 5% EC** |

Residues of flutianil and its metabolites, OC-56635, OC-56574 OC-53276 and OC-53279 were analyzed using an 8.00-mL spiked portion of ground and surface water samples (see Table 3). Bulk water samples were filtered using PTFE syringe filter and 8 milliliters of the filtered sample was transferred to a 15-mL volumetric flask. The sample was then mixed with 2.00 mL of 0.5% formic acid in acetonitrile to achieve a final solvent composition of acetonitrile: HPLC grade water: formic acid (20:80:1, v/v/v). The sample was mixed well by inverting and vortexing. A portion of the sample was transferred to an HPLC vial for LC-MS/MS analysis.

Concentrations of flutianil and its metabolites (OC56636, OC56574, OC53276, and OC53279) in water samples were determined using HPLC coupled with MS/MS operated in both negative and positive ion, multiple reaction monitoring (MRM) mode (see Table 3). The instrumental conditions consisted of a Phenomenex LUNA 5 C-18(2) column (150 x 2.0 mm, 5-µm; column temperature not reported), 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 25.0 µL. Two parent-daughter ion transitions were monitored per analyte.

Calibration curves were generated from analyses of combined calibration standard solutions of flutianil and its metabolites analyzed concurrently with each series of method validation samples.

**Table 3 Summary Parameters for the Analytical Method Used for the Quantitation of Flutianil and Metabolite Residues in Surface and Ground Water.[1]**

| | |
|---|---|
| **Method ID** | **No ID given in report. Method developed by Wildlife International** |
| **Analyte(s)** | **Flutianil and metabolites OC56636, OC56574, OC53276, and OC53279** |
| **Extraction solvent/technique** | **All solvents used in this study were HPLC grade or equivalent.<br>Technique: An aliquot of each aqueous sample was initially combined/diluted volumetrically in a graduated tube with an aliquot of acetonitrile: 0.5% formic acid solution to achieve a final solvent composition of acetonitrile: HPLC grade water: formic acid (20:80:1, v/v/v). Analysis was by direct injection (no extraction).** |
| **Cleanup strategies** | **None mentioned.** |
| **Instrument/Detector** | **Hewlett-Packard Series 1200 High Performance Liquid Chromatograph (HPLC) coupled with an AB SCIEX TRIPLE QUAD™ 5500 Tandem Mass Spectrometer (MS/MS) operated in both negative and positive ion, multiple reaction monitoring (MRM) modes.** |
| **Standardization method** | **None** |
| **Stability of standard solutions** | **Not mentioned in report but analytes known to be stable.** |
| **Approximate Retention times** | **Flutianil – 10.0 minutes<br>OC 53279 – 9.2 minutes<br>OC 56574 – 8.8 minutes<br>OC 53276 – 8.7 minutes<br>OC 56635 – 6.0 minutes** |
| **Monitored transitions[2]** | **Quantitation Ion Transition:<br>Flutianil – 427 → 192 amu<br>OC 53279 – 443 → 190 amu<br>OC 56574 – 443 → 136 amu<br>OC 53276 – 443 → 192 amu<br>OC 56635 – 243 → 179 amu<br>Confirmation Ion Transition:<br>Flutianil – 427 → 132 amu<br>OC 53279 – 443 → 425 amu<br>OC 56574 – 443 → 136 amu (ECM); 443 → 181 amu (ILV)<br>OC 53276 – 443 → 132 amu<br>OC 56635 – 243 → 80 amu (ECM); 243 → 143 amu (ILV)** |

1 Data provided for ECM unless otherwise noted.
2 Data obtained from Table 1, p. 30 of MRID 49490588 and Table 23, p. 52 of MRID 49490522.

In the ILV, the ECM was performed as written, except for minor modifications to the analytical method. An Agilent 1290 LC was coupled to an AB Sciex 6490 QQQ MS. The following instrumental conditions differed from those of the ECM: column temperature 40°C, no guard column was used, injection volume (20 µL), and two different confirmation mass transitions (see Table 3). Reported retention times were 8.5, 7.4, 7.3, 7.8 and 5.4 minutes for flutianil, OC 56574, OC 53276, OC 53279 and OC 56635, respectively. None of these modifications were considered significant.

For flutianil and its four metabolites OC-56635, OC-56574, OC-53276 and OC-53279, the limit of quantitation (LOQ) was set to 0.001 ppm. For flutianil and its four metabolites OC-56635, OC-56574, OC-53276 and OC-53279, the limit of detection (LOD) was set to 0.0002 ppm.

## II. Recovery Findings

The mean recovery of flutianil and its 4 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 49490588): Mean recoveries and relative standard deviations (RSDs) were within guidelines (mean 70-120%; RSD ≤20%) for analysis of flutianil and metabolites OC-56635, OC-56574, OC-53276 and OC-53279 in surface and ground water matrices at fortification levels of 0.001 ppm (LOQ) and 0.01 ppm (10×LOQ; see Table 4 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 water matrices were well characterized. Surface water (pH 7.56, hardness 76.0 mg/L $CaCO_3$, alkalinity 32.0 mg/L $CaCO_3$) was obtained from Tuckahoe Lake in Tuckahoe State Park near Ridgely, Maryland. Ground water (pH 8.0, hardness 134 mg/L $CaCO_3$, alkalinity 176 mg/L $CaCO_3$) was obtained from a well approximately 40 meters deep located on the Wildlife International site; the ground water was characterized as moderately-hard.

ILV (MRID 49490522): Mean recoveries and relative standard deviations (RSDs) were within guidelines for analysis of flutianil and metabolites OC-56635, OC-56574, OC-53276 and OC-53279 in surface and ground water matrices at fortification levels of 0.001 ppm (LOQ) and 0.01 ppm (10×LOQ; see Table 5 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 water matrices were not characterized. Surface and ground water samples were supplied by ADPEN Laboratories, Inc. The method was validated in the first trial for all analytes in surface and ground water matrices with only minor modifications to the analytical parameters.

**Table 4 Initial Validation Method Recoveries for flutianil and its metabolites, OC-56635, OC-56574 OC-53276 and OC-53279 in surface and ground water**

| Analyte | Fortification Level (mg/L) | Number of Tests | Recovery Range (%) | Mean Recovery (%) | Standard Deviation (%) | Relative Standard Deviation (%) |
|---|---|---|---|---|---|---|
| Flutianil (Surface Water) | 0.001 | 5 | 93.8 to 101 | 97.2 | 2.78 | 2.86 |
| | 0.01 | 5 | 98.1 to 106 | 102 | 3.19 | 3.13 |
| OC56635 (Surface Water) | 0.001 | 5 | 99.1 to 108 | 104 | 3.50 | 3.37 |
| | 0.01 | 5 | 103 to 108 | 105 | 2.41 | 2.30 |
| OC56574 (Surface Water) | 0.001 | 5 | 99.5 to 104 | 101 | 2.12 | 2.10 |
| | 0.01 | 5 | 98.0 to 109 | 104 | 4.39 | 4.22 |
| OC53276 (Surface Water) | 0.001 | 5 | 101 to 108 | 104 | 3.27 | 3.14 |
| | 0.01 | 5 | 103 to 111 | 108 | 3.87 | 3.58 |
| OC53279 (Surface Water) | 0.001 | 5 | 97.5 to 106 | 102 | 3.09 | 3.03 |
| | 0.01 | 5 | 100 to 105 | 103 | 2.07 | 2.01 |
| Flutianil (Ground Water) | 0.001 | 5 | 91.9 to 99.2 | 96.6 | 2.75 | 2.85 |
| | 0.01 | 5 | 98.7 to 107 | 102 | 3.57 | 3.50 |
| OC56635 (Ground Water) | 0.001 | 5 | 102 to 105 | 103 | 1.41 | 1.37 |
| | 0.01 | 5 | 102 to 106 | 104 | 1.64 | 1.58 |
| OC56574 (Ground Water) | 0.001 | 5 | 95.4 to 102 | 99.5 | 2.74 | 2.75 |
| | 0.01 | 5 | 102 to 108 | 104 | 2.61 | 2.51 |
| OC53276 (Ground Water) | 0.001 | 5 | 102 to 107 | 104 | 1.87 | 1.80 |
| | 0.01 | 5 | 104 to 114 | 108 | 4.34 | 4.02 |
| OC53279 (Ground Water) | 0.001 | 5 | 98.5 to 101 | 100 | 1.06 | 1.06 |
| | 0.01 | 5 | 101 to 106 | 104 | 2.12 | 2.04 |

**Table 5 Independent Validation Method Recoveries for flutianil and its metabolites, OC-56635, OC-56574, OC-53276 and OC-53279 in surface and ground water**

| Analyte | Fortification Level (ppm) | Number of Tests | Recovery Range (%) | Mean Recovery (%) | Standard Deviation (%) | Relative Standard Deviation (%) |
|---|---|---|---|---|---|---|
| Quantitation (*m/z* 427→ 192) | | | | | | |
| Flutianil (Surface Water) | 0.001 | 5 | 113 to 119 | 115 | 2.1 | 1.8 |
| | 0.01 | 5 | 106 to 110 | 107 | 1.4 | 1.3 |
| Confirmatory (*m/z* 427→ 132) | | | | | | |
| Flutianil (Surface Water) | 0.001 | 5 | 115 to 119 | 116 | 1.8 | 1.5 |
| | 0.01 | 5 | 104 to 112 | 108 | 3.0 | 2.8 |
| Quantitation (*m/z* 243→ 179) | | | | | | |
| OC56635 (Surface Water) | 0.001 | 5 | 96 to 113 | 104 | 6.2 | 5.9 |
| | 0.01 | 5 | 102 to 109 | 105 | 2.7 | 2.5 |
| Confirmatory (*m/z* 243→ 143) | | | | | | |
| OC56635 (Surface Water) | 0.001 | 5 | 98 to 114 | 107 | 5.6 | 5.2 |
| | 0.01 | 5 | 102 to 110 | 106 | 3.3 | 3.1 |
| Quantitation (*m/z* 443 → 136) | | | | | | |
| OC56574 (Surface Water) | 0.001 | 5 | 103 to 114 | 109 | 4.0 | 3.7 |
| | 0.01 | 5 | 104 to 107 | 105 | 1.3 | 1.2 |
| Confirmatory (*m/z* 443 → 181) | | | | | | |
| OC56574 (Surface Water) | 0.001 | 5 | 106 to 117 | 111 | 3.7 | 3.3 |
| | 0.01 | 5 | 103 to 108 | 105 | 1.8 | 1.7 |
| Quantitation (*m/z* 443 → 192) | | | | | | |
| OC53276 (Surface Water) | 0.001 | 5 | 106 to 133 | 115 | 10.6 | 9.3 |
| | 0.01 | 5 | 104 to 107 | 105 | 1.5 | 1.4 |
| Confirmatory (*m/z* 443 → 132) | | | | | | |
| OC53276 (Surface Water) | 0.001 | 5 | 105 to 129 | 112 | 9.7 | 8.7 |
| | 0.01 | 5 | 104 to 108 | 105 | 1.9 | 1.8 |
| Quantitation (*m/z* 443 → 189) | | | | | | |
| OC53279 (Surface Water) | 0.001 | 5 | 110 to 115 | 112 | 2.1 | 1.9 |
| | 0.01 | 5 | 104 to 107 | 105 | 1.2 | 1.2 |
| Confirmatory (*m/z* 443 → 425) | | | | | | |
| OC53279 (Surface Water) | 0.001 | 5 | 106 to 112 | 109 | 2.3 | 2.1 |
| | 0.01 | 5 | 101 to 110 | 105 | 3.2 | 3.1 |
| Quantitation (*m/z* 427→ 192) | | | | | | |
| Flutianil (Ground Water) | 0.001 | 5 | 113 to 119 | 116 | 2.4 | 2.1 |
| | 0.01 | 5 | 111 to 116 | 113 | 2.1 | 1.8 |
| Confirmatory (*m/z* 427→ 132) | | | | | | |
| Flutianil (Ground Water) | 0.001 | 5 | 112 to 117 | 115 | 1.9 | 1.6 |
| | 0.01 | 5 | 110 to 113 | 111 | 1.2 | 1.1 |
| Quantitation (*m/z* 243→ 179) | | | | | | |

**Table 5 Independent Validation Method Recoveries for flutianil and its metabolites, OC-56635, OC-56574, OC-53276 and OC-53279 in surface and ground water**

| Analyte | Fortification Level (ppm) | Number of Tests | Recovery Range (%) | Mean Recovery (%) | Standard Deviation (%) | Relative Standard Deviation (%) |
|---|---|---|---|---|---|---|
| OC56635 (Ground Water) | 0.001 | 5 | 108 to 129 | 113 | 9.3 | 8.2 |
| | 0.01 | 5 | 107 to 111 | 110 | 1.9 | 1.7 |
| Confirmatory (*m/z* 243 → 143) | | | | | | |
| OC56635 (Ground Water) | 0.001 | 5 | 102 to 125 | 112 | 10.9 | 9.7 |
| | 0.01 | 5 | 106 to 110 | 108 | 1.6 | 1.5 |
| Quantitation (*m/z* 443 → 136) | | | | | | |
| OC56574 (Ground Water) | 0.001 | 5 | 107 to 119 | 113 | 4.3 | 3.8 |
| | 0.01 | 5 | 105 to 109 | 107 | 1.8 | 1.7 |
| Confirmatory (*m/z* 443 → 181) | | | | | | |
| OC56574 (Ground Water) | 0.001 | 5 | 104 to 119 | 110 | 5.4 | 4.9 |
| | 0.01 | 5 | 101 to 106 | 103 | 2.0 | 1.9 |
| Quantitation (*m/z* 443 → 192) | | | | | | |
| OC53276 (Ground Water) | 0.001 | 5 | 113 to 117 | 115 | 1.6 | 1.4 |
| | 0.01 | 5 | 106 to 111 | 109 | 2.3 | 2.1 |
| Confirmatory (*m/z* 443 → 132) | | | | | | |
| OC53276 (Ground Water) | 0.001 | 5 | 109 to 116 | 112 | 2.7 | 2.5 |
| | 0.01 | 5 | 104 to 107 | 106 | 1.5 | 1.5 |
| Quantitation (*m/z* 443 → 425) | | | | | | |
| OC53279 (Ground Water) | 0.001 | 5 | 111 to 117 | 115 | 2.3 | 2.0 |
| | 0.01 | 5 | 108 to 110 | 109 | 0.9 | 0.8 |
| Confirmatory (*m/z* 443 → 136) | | | | | | |
| OC53279 (Ground Water) | 0.001 | 5 | 109 to 115 | 112 | 2.2 | 2.0 |
| | 0.01 | 5 | 107 to 112 | 110 | 1.8 | 1.7 |

## III. Method Characteristics

The limit of quantitation (LOQ) for the surface and ground water method validation was set at 0.00100 mg/L and the theoretical LOQ was 0.000250 mg/L. In the ECM, the theoretical LOQ was calculated as the product of the lowest calibration standard (0.000200 µg/mL) and the dilution factor of the matric blank samples (1.25). The LOQ was justified as the lowest level fortified and analyzed during the validation sets. For flutianil and its four metabolites OC-56635, OC-56574, OC-53276 and OC-53279, the limit of detection (LOD) was set to 0.0002 ppm. In the ECM, the instrumental limit of detection (LOD) in surface water for Flutianil and metabolites OC56635, OC56574, OC53276, and OC53279 were determined to be 0.00000528 mg/L, 0.000369 mg/L, 0.00000286 mg/L, 0.00000286 mg/L, and 0.00000838 mg/L, respectfully. The instrumental limit of detection (LOD) in ground water for Flutianil and metabolites OC56635, OC56574, OC53276, and OC53279 were determined to be 0.00000479 mg/L, 0.0000244 mg/L, 0.00000350 mg/L, 0.00000350 mg/L, and 0.00000665 mg/L, respectfully. The instrumental limit of detection (LOD) values were calculated as the products of the lowest calibration standard/(average signal to noise ratio) x 3 x the dilution factor of the standard (1.0). 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 4 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 6 Method Characteristics**

| Characteristic | | Flutianil | OC-56635 | OC-56574 | OC-53276 | OC-53279 |
|---|---|---|---|---|---|---|
| Limit of Quantitation (LOQ) | | 0.001 ppm | 0.001 ppm | 0.001 ppm | 0.001 ppm | 0.001 ppm |
| Limit of Detection (LOD)[1] | | 0.0002 ppm | 0.0002 ppm | 0.0002 ppm | 0.0002 ppm | 0.0002 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 | $r^2 > 0.99$ 0.05 ng/mL to 10.0 ng/mL |
| | ECM[2] | $r^2 = 0.9996$ | $r^2 = 0.9989$ | $r^2 = 0.9992$ | $r^2 = 0.9998$ | $r^2 = 0.9995$ |
| | ILV[3] | $r^2 = 0.9966$ (Q) $r^2 = 0.9972$ (C) | $r^2 = 0.9938$ (Q) $r^2 = 0.9948$ (C) | $r^2 = 0.9952$ (Q) $r^2 = 0.9976$ (C) | $r^2 = 0.9974$ (Q) $r^2 = 0.9962$ (C) | $r^2 = 0.9976$ (Q) $r^2 = 0.9956$ (C) |
| Repeatable[4] | | Yes | Yes | Yes | Yes | Yes |
| Reproducible[4] | | Yes | Yes | Yes | Yes | Yes |
| Specific | | Yes | Yes | Yes | Yes | Yes |
| | ECM[5] | No matrix interferences. | | | | |
| | ILV[6] | No matrix interferences. | | Significant matrix interference from residues in the controls (*ca.* 25% of the LOQ) and a nearby contaminant (RT 7.5 min.; peak area *ca.* 25% of the LOQ). | No matrix interferences. | |

1 See text above for ECM calculated instrumental LODs for ground and surface water matrices.
2 Reported $r^2$ values were reviewer-calculated from r values of 0.9994265-0.9998760 (analytes combined; quantitation ion only). Data (r values) obtained from Figure 2, p. 42, Figure 11, p. 51, Figure 20, p. 60, Figure 29, p. 69, and Figure 38, p. 78 of MRID 49490588.
3 Reported $r^2$ values were reviewer-calculated from r values of 0.9969-0.9988 (analytes/ions combined). Data (r values) obtained from Figures 1-2, pp. 54-55, Figures 13-14, pp. 70-71, Figures 25-26, pp. 86-87, Figures 37-38, pp. 102-103, and Figures 49-50, pp. 118-119 of MRID 49490522.
4 At the LOQ and 10×LOQ. Surface (lake) water (pH 7.56, hardness 76.0 mg/L $CaCO_3$, alkalinity 32.0 mg/L $CaCO_3$) and ground (well) water (pH 8.0, hardness 134 mg/L $CaCO_3$, alkalinity 176 mg/L $CaCO_3$) were used in the ECM (pp. 19-20; Appendices 7-9, pp. 104-107 of MRID 49490588). Uncharacterized surface and ground water were used in the ILV (p. 18 of MRID 49490522).
5 Data obtained from Figures 3-46, pp. 43-86 of MRID 49490588.
6 Data obtained from Figures 3-60, pp. 56-133 of MRID 49490522.

## IV. Method Deficiencies and Reviewer's Comments

1. The estimations of the LOQ and LOD in the ECM and ILV were not based on scientifically acceptable procedures as defined in 40 CFR Part 136 (pp. 22-23 of MRID 49490588; p. 20 of MRID 49490522). In the ECM, no justification or calculation was provided to support the LOQ; the LOQ was justified as the lowest level fortified and analyzed during the validation sets. The LOD was supported by calculations in the ECM; however, the calculations were based on the lowest calibration standard, not the standard deviation. In the ILV, the LOQ and LOD were reported from the ECM without justification or calculation. Detection limits should not be based on the arbitrarily selected lowest concentration in the spiked samples. Additionally, 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.

2. In the ILV, the water matrices were not characterized (p. 18 of MRID 49490522). 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*. 7.35 min.) showed significant matrix interference from residues in the controls (*ca*. 25% of the LOQ; Figures 27-36, pp. 88-101 of MRID 49490522). Additionally, a nearby contaminant (RT 7.5 min.; peak area *ca*. 25% of the LOQ) greatly disrupted peak attenuation and the baseline around the analyte.

   In the ECM, representative chromatograms were not complete; a chromatogram of the reagent blank was not included in the validation.

4. 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. 27 of MRID 49490522).

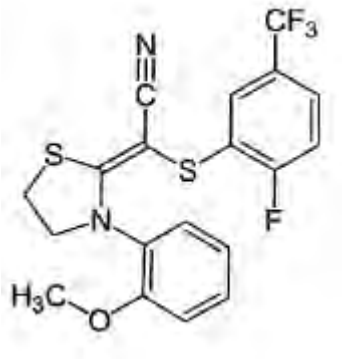
5. It was reported for the ILV that the analytical procedure for two sets of 13 samples required approximately four hours for laboratory preparation (p. 27 of MRID 49490522). The time required for LC/MS/MS was not reported. The overall time was not reported.

## 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.

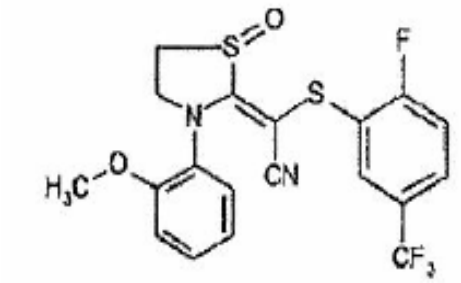
## Attachment 1: Chemical Names and Structures

| | |
|---|---|
| **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:** | $C_{19}H_{14}F_4N_2OS_2$ |
| **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)**

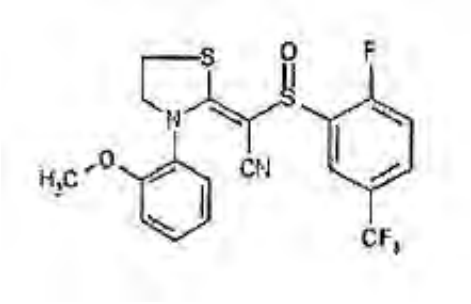
| | |
|---|---|
| **Common Name:** | OC 56635 |
| **Batch No.:** | 81010 |
| **IUPAC Name:** | (2-fluoro-5-trifluoromethyl)benzenesulfonic acid |
| **Molecular Formula:** | $C_7H_4F_4O_3S$ |
| **Molecular Weight:** | 244.16 g/mol |
| **Purity:** | 97.3% |
| **Expiration Date:** | 04/22/18 |
| **Storage:** | 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:** | $C_{19}H_{14}F_4N_2O_2S_2$ |
| **Molecular Weight:** | 442.45 g/mol |
| **Purity:** | 98.7% |
| **Expiration Date:** | July 06, 2018 |
| **Storage:** | Refrigerated in darkness |
| **Chemical Structure:** |  |

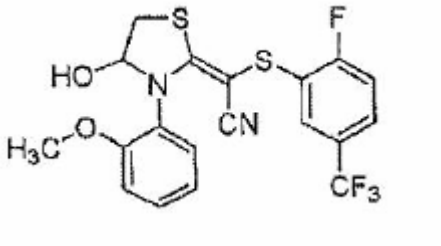
**Attachment 1: Chemical Names and Structures for the Reference Materials (Cont'd)**

| | |
|---|---|
| **Common Name:** | OC 53276 |

| | |
|---|---|
| **Batch No.:** | TT1005019 |
| **IUPAC Name:** | (Z)-2-[2-fluoro-5-(trifluoromethyl)phenylsulfinyl]-2-[3-(2methoxyphenyl)thiazolidinylidene]acetonitrile |
| **Molecular Formula:** | $C_{19}H_{14}F_4N_2O_2S_2$ |
| **Molecular Weight:** | 442.45 g/mol |
| **Purity:** | 96.54% |
| **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 53279 |
| **Batch No.:** | TT1506013 |
| **IUPAC Name:** | (Z)-2-[2-fluoro-5-(trifluoromethyl)phenylthio]-2-[4-hydroxy-3-(2-methoxyphenyl)thiazolidinylidene]acetonitrile |
| **Molecular Formula:** | $C_{19}H_{14}F_4N_2O_2S_2$ |
| **Molecular Weight:** | 442.45 g/mol |
| **Purity:** | 97.94% |
| **Expiration Date:** | July 06, 2018 |
| **Storage:** | Refrigerated in darkness |
| **Chemical Structure:** |  |