

ABSTRACT

The purpose of the study was to demonstrate that the draft method, “Determination of Flutianil and Metabolites (OC56635, OC56574, OC53276 and OC53279) in Surface and Ground Water” could be performed successfully at an outside facility with no prior experience with the method. (Reference 1)

Principle of the method. Water samples fortified with flutianil and its metabolites, OC56635, OC56574, OC53276 and OC53279 were analyzed. Bulk water samples were filtered using PTFE syringe filter and 8 milliliters of the filtered sample was transferred to a 10-mL volumetric flask. The sample was then brought to volume with 0.5% formic acid in acetonitrile. Any dilutions necessary were made using acetonitrile:water:formic acid (20:80:0.1). A portion of the sample was transferred to an HPLC vial for LC-MS/MS analysis.

The analytical method was successfully conducted at ADPEN Laboratories, Inc. in both surface and ground water. Flutianil, OC56635, OC56574, OC53276 and OC53279 were recovered within acceptance limits.

Limit of Quantification (LOQ) and Limit of Detection (LOD). The limit of quantitation (LOQ) for flutianil and its four metabolites, OC56635, OC56574, OC53276 and OC53279 as was set at 0.001 ppm in water. The limit of detection (LOD) for flutianil and its four metabolites, OC56635, OC56574, OC53276 and OC53279 was set at 0.0002 ppm.

Selectivity. The method was highly selective 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, OC53276, OC53279 and OC56574 and negatively charged molecule ion to two typical fragment ions in MS/MS mode for OC56635 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.

	<u>Quantitation</u>	<u>Confirmatory</u>
Flutianil	427 → 192	427 → 132
OC56635	243 → 179	243 → 143 ¹
OC56574	443 → 136	443 → 181 ²
OC53276	443 → 192	443 → 132
OC53279	443 → 190	443 → 425

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

Recovery and Repeatability. 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 OCSP 850.6100 guideline requirements (<20 % RSD at each fortification level).

¹ An alternate confirmatory transition (243 → 143) was used instead of the one listed in the method (243 → 80) due to enhanced sensitivity.

² The transition ions were duplicated in the analytical method. Another transition ion with appropriate sensitivity was selected instead.

1.0 INTRODUCTION

1.1 Purpose of the Study

The purpose of the study was to demonstrate that the draft method, "Determination of Flutianil and Metabolites (OC56635, OC56574, OC53276 and OC53279) in Surface and Ground Water" could be performed successfully at an outside facility with no prior experience with the method.

1.2 Summary of the Study

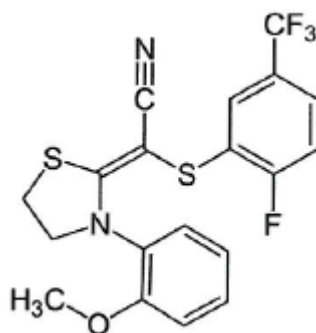
The independent laboratory validation of the analytical method was successfully completed for all analytes in the first trial.

2.0 REFERENCE SUBSTANCE AND SAMPLING HISTORY

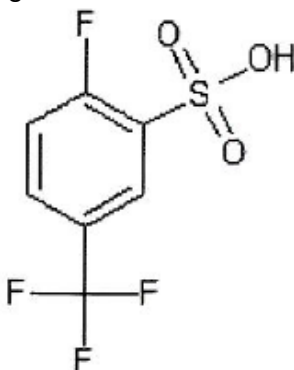
2.1 Reference Materials

Reference substances flutianil, OC56635, OC56574, OC53276 and OC53279 were combined in solutions for fortifications and LC-MS/MS calibration. Concentrated (stock), fortification, and calibration standards were prepared according to the analytical draft method. The certificates of analysis for the reference substance is presented in Appendix A. Neat standards used for this study were stored at in refrigerator E-119 which maintained an average temperature of 3°C. A brief description of the reference standard used in this study is presented below.

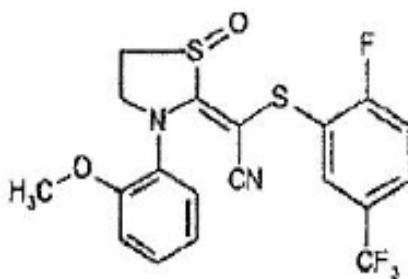
Name:	OK-5203
Common Name:	Flutianil
Batch No.:	05DF2
CAS Number:	958647-10-4
IUPAC Name:	(Z)-2-[2-fluoro-5-(trifluoromethyl)phenylthio]-2-[3-(2-methoxyphenyl)-1,3-thiazolidin-2-ylidene]acetonitrile
Molecular Formula:	C ₁₉ H ₁₄ F ₄ N ₂ OS ₂
Molecular Weight:	426.5 g/mol
Purity:	99.54%
Expiration Date: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



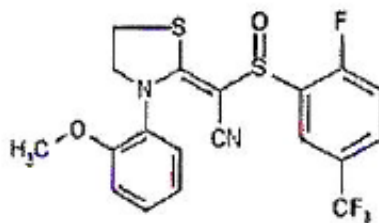
Common Name: OC56635
Batch No.: 81010
IUPAC Name: (2-fluoro-5-trifluoromethyl)benzenesulfonic acid
Molecular Formula: $C_7H_4F_4O_3S$
Molecular Weight: 244.16 g/mol
Purity: 97.3%
Expiration Date: 04/22/18
Storage: Refrigerated in darkness
Chemical Structure:



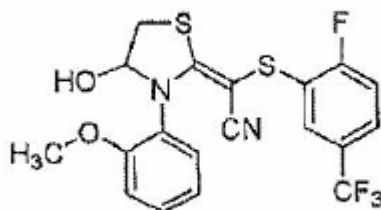
Common Name: OC56574
Batch No.: TT0908011
IUPAC Name: (Z)-2-[2-fluoro-5-trifluoromethyl)phenylthio]-2-[3-(2-methoxyphenyl)-1-oxo-2-thiazolidinyldene]acetonitrile
Molecular Formula: $C_{19}H_{14}F_4N_2O_2S_2$
Molecular Weight: 442.45 g/mol
Purity: 98.7%
Expiration Date: July 06, 2018
Storage: Refrigerated in darkness
Chemical Structure:



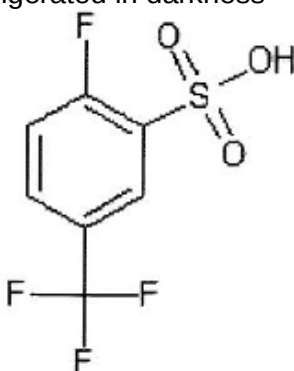
Common Name: OC53276
Batch No.: TT1005019
IUPAC Name: (Z)-2-[2-fluoro-5-(trifluoromethyl)phenylsulfanyl]-2-[3-(2-methoxyphenyl)thiazolidinylidene]acetonitrile
Molecular Formula: C₁₉H₁₄F₄N₂O₂S₂
Molecular Weight: 442.45 g/mol
Purity: 96.54%
Expiration Date: July 06, 2018
Storage: Refrigerated in darkness
Chemical Structure:



Common Name: OC53279
Batch No.: TT1506013
IUPAC Name: (Z)-2-[2-fluoro-5-(trifluoromethyl)phenylthio]-2-[4-hydroxy-3-(2-methoxyphenyl)thiazolidinylidene]acetonitrile
Molecular Formula: C₁₉H₁₄F₄N₂O₂S₂
Molecular Weight: 442.45 g/mol
Purity: 97.94%
Expiration Date: July 06, 2018
Storage: Refrigerated in darkness
Chemical Structure:



Common Name: OC56635
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:



2.2 Test System

The analysis of flutianil and its four metabolites included both surface and ground water. An untreated sample of each water type was supplied by ADPEN Laboratories, Inc. Prior to analysis, the control samples were stored in refrigerator E122 which operated at an average temperature of 5°C during the course of this study.

The Laboratory Information Management System (LIMS) provided a unique laboratory analysis code (i.e. 150914001-001) for the control sample, which is cross-referenced on detailed analytical data reports to the sample identification number.

3.0 ANALYTICAL METHOD

The analytical method, "Determination of Flutianil and its Metabolites (OC56635, OC56574, OC53276 and OC53279) in Surface and Ground Water" was used for the analysis.

3.1 Preparation of Solutions

All solutions used for the conduct of the ILV were prepared as described in the analytical method. Below are the solutions and solvent mixtures used for this study and their composition:

Description	Composition
Sample dilution solvent	0.5% formic acid in acetonitrile Measure 900 mL of acetonitrile to a 1-L volumetric flask. Add 5 mL formic acid. Bring to volume with acetonitrile. Mix well.
Final diluting solvent	20:80 acetonitrile: water + 0.1% formic acid Add 800 mL of water and 200 mL of acetonitrile in a 1-L glass storage bottle. Mix well and then add 1 mL of formic acid.
HPLC mobile phase A	0.2% formic acid in HPLC water Add 500 mL of water and 2 mL of concentrated formic acid into a 1L volumetric flask, bring up to volume, mix well and sonicate.
HPLC mobile phase B	0.2% formic acid in acetonitrile Add 500 mL of acetonitrile and 2 mL of concentrated formic acid into a 1L volumetric flask, bring up to volume, mix well and sonicate.

3.2 Preparation of Standard Solutions

All stock, fortification and calibration standard solutions were stored at refrigerated temperatures in amber bottles. An example of the standard preparation solutions is presented in Table 22.

3.2.1 Stock Standard Solutions

The individual stock standard solutions were prepared as described in the method in a 10-mL volumetric flask. The standards were dissolved, using sonication and/or vortexing and then diluted to the mark with methanol and having a concentration of approximately 1.0 mg/mL.

3.2.2 Fortification Standard Solutions

Five mix-analyte fortification solutions were prepared at 0.2 and 0.02 µg/mL. The two volumetric flasks containing the fortification standards were diluted volumetrically with methanol as described in the method and complete homogeneous solutions were accomplished by sonication and/or vortexing.

3.2.3 Calibration Standard Solutions

Calibration standard solutions for LC-MS/MS analysis were prepared using intermediate solutions prepared separately from the fortification standards as described in the method. Dilutions were prepared volumetric flasks using acetonitrile: water (20:80, v/v) + 0.1% formic acid. A description of the standard preparation for calibration is shown below.

Parent Standard (ng/mL)	Volume (mL)	Final volume (mL)	Concentration (ng/mL)
1000	0.250	25.0	10.0
1000	0.125		5.0
1000	0.050		2.0
100	0.250		1.0
100	0.125		0.50
100	0.050		0.20

3.3 Extraction and Analysis

Extraction: Water samples fortified with flutianil and its metabolites, OC56635, OC56574, OC53276 and OC53279 were analyzed. Bulk water samples were filtered using PTFE syringe filter and 8 milliliters of the filtered sample was transferred to a 10-mL volumetric flask. The sample was then brought to volume with 0.5% formic acid in acetonitrile. Any necessary dilutions were made using acetonitrile:water:formic acid (20:80:0.1). A portion of the sample was transferred to an HPLC vial for LC-MS/MS analysis.

Final Sample Preparation: A portion of the sample was transferred to an HPLC vial for LC-MS/MS analysis.

A method flow diagram of the method used is presented in Table 1.

3.4 LC-MS/MS Instrumentation and Conditions

The LC-MS/MS instrumentation and conditions including the both the quantitation and confirmatory transitions (m/z) monitored during this validation study are presented in Table 23.

4.0 LIMITS OF QUANTITATION AND DETECTION

For flutianil and its four metabolites OC56635, OC56574, OC53276 and OC53279, the limit of quantitation (LOQ) was set to 0.001 ppm. For flutianil and its four metabolites OC56635, OC56574, OC53276 and OC53279, the limit of detection (LOD) was set to 0.0002 ppm.

5.0 CALIBRATION, CALCULATIONS, AND STATISTICS

Quantitation of residues in all samples was achieved using an external calibration curve calculated by linear regression of instrument responses for the reference substance at multiple concentrations.

A calibration standard curve was prepared using all four analytes in a mix standard solution by injecting standard solutions prepared as described in Section 3.2.3 of this report; calibration standard concentrations ranged from 0.20 to 10 ng/mL. The calibration standards were interspersed with sample injections. Agilent Technologies MassHunter software was used to create the standard curve based on linear regression using "1/x" weighting as well as peak integration and quantitation as obtained from the instrument used. The regression functions were used to calculate the best-fit line by plotting the analyte found (ng) on the x-axis versus the detector's peak response (peak area) on the y-axis. The calibration curve equation was used to determine the amount of analyte found (ng) during sample analysis. Typical quantitation and confirmatory calibration curves for flutianil, OC56635, OC56574, OC53276 and OC53279 are presented in Figures 1 and 2, Figures 13 and 14, Figures 25 and 26, Figures 37 and 38 and Figures 49 and 50, respectively. Representative chromatograms of calibration standards for both mass transitions (m/z) for flutianil, OC56635, OC56574, OC53276 and OC53279 are presented in Figures 3 and 4, Figures 15 and 16, Figures 27 and 28, Figures 39 and 40 and Figures 51 and 52, respectively. Recovery results and additional sample concentrations were calculated for each set of samples within the LIMS and reported in Microsoft® Excel spreadsheet as detailed analytical data reports, which are presented in Appendix B.

5.1 Residue Calculations for Flutianil

The following equations are for residue and recovery calculations for water samples:

a) Calibration curve: $y=mx+b$ Solving for x: $x= \frac{y-b}{m}$

Where, m = slope
 b = y-intercept
 x = Amount found (ng)
 y = Peak area

b) Amount of sample injected (μL) = $\frac{(\text{injection size (mL)} \times \text{sample volume (mL)})}{\text{final sample volume (mL)}} \times \frac{1000 \mu\text{L}}{1 \text{ mL}}$

c) ppm found = $\frac{\text{Amount found (ng)}}{\text{Amount of sample injected (}\mu\text{L)}}$

d) Recovery (%) = $\frac{\text{ppm found}}{\text{ppm added}} \times 100$

As an example, to calculate recovery results for flutianil (quantitation transition, 427 $\rightarrow$ 192 m/z) using sample 15091602-Recovery1-1 from work order WO-15091602, where the peak area counts were 29570, are shown below:

a) Calibration curve: $y = 1649879.906568x - 870.357853$

Solving for x: $x = \frac{29570 + 870.357853}{1649879.906568} = 0.018450 \text{ ng}$

b) Amount of sample injected (μL) = $\frac{0.02 \text{ mL} \times 8.0 \text{ mL}}{10 \text{ mL}} \times \frac{1000 \mu\text{L}}{1 \text{ mL}} = 16.0 \mu\text{L}$

c) ppm found = $\frac{0.018450 \text{ ng}}{16.0 \mu\text{L}} = 0.00115 \text{ ppm}$

d) Recovery (%) = $\frac{0.00115 \text{ ppm}}{0.0010 \text{ ppm}} \times 100\% = 115\%$

5.2 Statistical Analysis

Statistical treatment of the data included calculation of means, standard deviations (SD), and percent relative standard deviations (%RSD). These calculations were performed using Microsoft® Excel and LIMS software. Results were rounded only for reporting purposes. No calculations were made with rounded numbers.

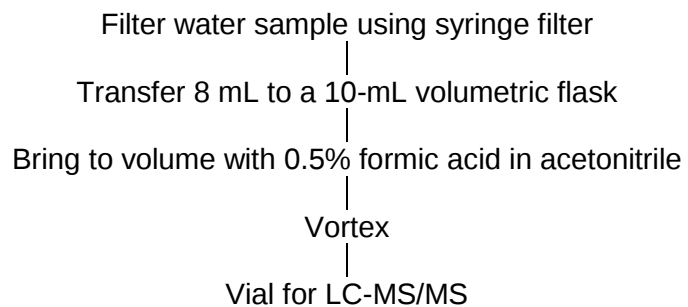
Table 1 Method Flow Diagram

Table 23 Instrument Conditions and Parameters

HPLC Conditions			
Chromatographic System:	Agilent 1290 Liquid Chromatograph		
Column:	Luna 5 C18; 5.0 µm, 2.0 x 150 mm		
Temperature:	40 °C		
Flow rate (mL/min)	0.500		
Gradient:	Time (min)	Mobile Phase A (%)	Mobile Phase B (%)
	0.0	80	20
	2.0	80	20
	9.0	5	95
	10.0	5	95
	10.5	80	20
	15.0	80	20
Mobile Phase A:	Water with 0.2% formic acid		
Mobile Phase B:	Acetonitrile with 0.2% formic acid		
Injection Volume:	20 µL		

MS/MS Conditions							
Detection System:	AB SCIEX 6490 QQQ						
Ionization:	ESI						
Gas Temperature:	150°C						
Gas Flow:	14 L/min						
Sheath Gas Heater:	300						
Sheath Gas Flow:	12						
Capillary:	3000 V						
V Charging	1600						
Scan type:	MRM						
MRM Conditions	Transition (m/z)	Dwell (msec)	Frag (V)	CE (V)	Cell Acc (V)	Polarity	Retention Time (min)
Flutianil	427 → 192	50	380	24	7	Positive	8.5
	427 → 132			56			
OC56635	243 → 179	200	380	24	5	Negative	5.4
	243 → 143 ¹			32			
OC56574	443 → 136	50	380	20	7	Positive	7.4
	443 → 181 ²			16			
OC53276	443 → 192	50	380	20	7	Positive	7.3
	443 → 132			56			
OC53279	443 → 190	50	380	32	7	Positive	7.8
	443 → 425			8			

¹ An alternate confirmatory transition (243 → 143) was used instead of the one listed in the method (243 → 80) due to enhanced sensitivity.

² The transition ions were duplicated in the analytical method. Another transition ion with appropriate sensitivity was selected instead.