Dow AgroSciences

STUDY TITLE

Independent Laboratory Validation of Dow AgroSciences LLC Method – Determination of Residues of Florasulam and its 5-OH Metabolite in Drinking Water, Ground Water and Surface Water by Liquid Chromatography with Tandem Mass Spectrometric Detection

DATA REQUIREMENTS
OPPTS 850.7100
SANCO/3029/99 rev.4
SANCO/825/00 rev.8.1, 16 Nov 2010

ANALYTICAL

Preparation and Storage of Samples

The independent laboratory validation was carried out on three water specimens; surface water, ground water and drinking water. The drinking water was obtained from a "drinking water" tap at the Mogi Mirim Regulatory Laboratory Test Facility, the ground water was obtained from a well in the Dow AgroSciences Field Station in Mogi-Mirim City, São Paulo State, Brazil and the surface water was obtained from the River Sapezal, in Mogi-Mirim City, São Paulo State, Brazil. All samples were collected in the same day, September 29th of 2011.

Specimen	Sample number
Ground Water	110539-001
Surface Water	110539-002
Drinking Water	110539-003

On receipt the specimens were stored at approximately -20 °C before and after analysis.

Characterisation of Samples

The water specimens were characterised at NL Laboratórios, in Mogi-Mirim City, São Paulo State, Brazil. The laboratory is capable of following Brazilian laws for water analysis, such as stated by the Ministry of Environmental. As this is not a GLP facility, the data generated are non-GLP. Details of the characterisation results are as follows:

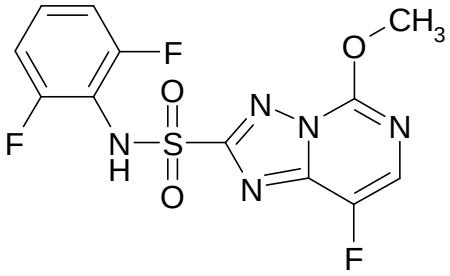
Sample Number	pH	Turbidity NTU	Total Hardness mg/L as $CaCO_3$	Total Organic Carbon (mg/L)	Chemical Oxygen Demand (mg/L)	Total Suspended Solids (mg/L)
110539-001	7.22	0.23	68.1	<2.0	<2.0	<2.0
110539-002	6.46	4.74	4.0	<1.0	13.1	<2.0
110539-003	7.29	0.34	68.1	<2.0	<2.0	<2.0

Certificates of Analysis of these specimens are included in the raw data package.

Preparation of Solutions and Standards

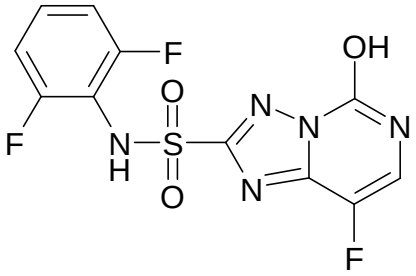
Reagents used were of equivalent specifications as described in the method. The following analytical test substances/analytical standards (obtained from the Sponsor) were utilized during the independent laboratory method validation:

Test Substance/Analytical Standard:	Florasulam
Supplier:	Sponsor
AGR/TSN Number:	TSN100381
Batch no:	DECO-293-021
Purity:	99.7%
Expiry date:	25-Apr-2012
Reference:	FA&PC 08-163723
Storage:	Ambient
CAS Number	145701-23-1
Molecular Formula:	C12H8F3N5O3S
Formula Weight	359.29



N-(2,6-difluorophenyl)-8-fluoro-5-methoxy[1,2,4]triazolo[1,5-c]pyrimidine-2-sulfonamide

Test Substance/Analytical Standard:	5-Hydroxy-Florasulam
Supplier:	Sponsor
AGR/TSN Number:	TSN101151
Batch no:	DECO-393-053
Purity:	98.1%
Expiry date:	19-Jun-2013
Reference:	FA&PC 09- 225597
Storage:	Ambient
CAS Number	N/A
Molecular Formula:	345.269
Formula Weight	C11H6F3N5O3S



N-(2,6-difluorophenyl)-8-fluoro-5-hydroxy[1,2,4]triazolo[1,5-c]pyrimidine-2-sulfonamide

A stock solution of florasulam and the 5-OH metabolite were prepared as follows:

Analyte	Amount weighed [mg]	Dilute to [mL]	Concentration of stock solution [mg/mL]
Florasulam	50.5	50 (Acetone)	1.0
5-OH Florasulam	50.1	50 (Acetonitrile)	1.0

These solutions were further diluted volumetrically into acetonitrile to obtain mixed intermediate solutions with 10 and 0.10µg/mL per analyte. These intermediate solutions were further diluted volumetrically into acetonitrile to obtain mixed fortification solutions with 5.0, 1.0 and 0.10ng/mL per analyte.

Calibration solutions were prepared by volumetric dilution to obtain concentrations of 1.0 µg/mL and 0.010µg/mL (intermediate), and further to obtain concentrations of 1.0, 0.50, 0.10, 0.050, 0.025 and 0.010ng/mL in acetonitrile/water; (10/90)(v/v) used for calibrations.

Full details of these materials are included in the raw data package for the study along with the preparation of all analytical and fortification standards prepared from the primary reference items. The test/reference items will be retained until expiry and then disposed of. A retained sample of each reference item used in this study is kept at Mogi Mirim Regulatory Laboratory, Rodovia SP 147, km 71.5, Mogi Mirim, São Paulo, Brazil.

Fortification of Recovery Samples

The control specimens were fortified as described below:

Matrix	Reference Items	Untreated Replicates	Replicates at Fortification Level (LOD)	Replicates at Fortification Level (LOQ)	Replicates at Fortification Level (10 x LOQ)
Surface Water	Florasulam	2	1 at 0.015 µg/L	6 at 0.05 µg/L	6 at 0.5 µg/L
	5-OH Florasulam	2	1 at 0.015 µg/L	6 at 0.05 µg/L	6 at 0.5 µg/L
Ground Water	Florasulam	2	1 at 0.015 µg/L	6 at 0.05 µg/L	6 at 0.5 µg/L
	5-OH Florasulam	2	1 at 0.015 µg/L	6 at 0.05 µg/L	6 at 0.5 µg/L
Drinking Water	Florasulam	2	1 at 0.015 µg/L	6 at 0.05 µg/L	6 at 0.5 µg/L
	5-OH Florasulam	2	1 at 0.015 µg/L	6 at 0.05 µg/L	6 at 0.5 µg/L

Sample Extraction, Purification and Analysis

Specimens were assayed following the analytical method exactly as written in the Dow AgroSciences report 110538, “Method Validation Study for the Determination of Residues of Florasulam and its 5-OH Metabolite in Surface Water, Ground Water and Drinking Water by Liquid Chromatography with Tandem Mass Spectrometry”.

Residues of florasulam and the 5-OH metabolite were determined from the water sample matrices by adding 0.1 mL aliquot of correspondent fortification solutions into an autosampler vial containing 1.0 mL of the sample. The same amount of acentonitrile was added to the reagent blank and unfortified control samples. Sample vials were mixed by shaking and vortex mixing before being analyzed by liquid chromatography with negative-ion electrospray ionization (ESI) tandem mass spectrometry (LC-MS/MS).

Analytical Instrumentation and Equipment

The instrumental conditions used during the ILV trial were as described in the method, with minor adaptations. The instrumental conditions used were as follows:

Typical LC-MS/MS Operating Conditions for Florasulam and Florasulam 5-OH Metabolite

Instrumentation:	Agilent Model 1100 autosampler		
	Agilent Model 1100 Quaternary pump		
	Agilent Model 1100 degasser		
	MDS/Sciex API 4000 MS/MS System		
	MDS/Sciex Analyst 1.5 data system		
Column:	Phenomenex Onyx Monolithic C18 4.6 × 100 mm, 3.0 µm		
Column Temperature:	40 °C		
Injection Volume:	100 µL		
Run Time:	10.0 minutes		
Mobile Phase:	A – water with 0.1% acetic acid		
	B – acetonitrile/methanol (1/1, v/v) with 0.1% acetic acid		
Flow Rate:	1000 µL/min (aprox. 300 µL/mL to split source)		
Gradient:	Time, min	Solvent A, %	Solvent B, %
	0.01	10	90
	1.00	10	90
	5.00	100	0
	8.00	100	0
	8.10	10	90
	10.00	10	90
Flow Diverter Program:	1) 0.0 to 1.0 min: flow to waste 2) 1.0 to 7.0 min: flow to source 3) 7.0 min: flow to waste		

Typical Mass Spectrometry Operating Conditions for Florasulam and Florasulam 5-OH Metabolite

Interface:	ESI
Polarity:	Negative
Scan Type:	MRM
Resolution:	Q1 - unit, Q3 - unit
Curtain Gas (CUR):	30
Collision Gas (CAD):	6
Temperature (TEM):	450
Ion Source Gas 1 (GS1):	40
Ion Source Gas 1 (GS1):	60
Acquisition Time:	10 min
IonSpray Voltage (IS):	-4500
Entrance Potential (EP):	-10

Analytes:	Precursor Ion Q1 *(m/z)*	Product Ion Q3 *(m/z)*	Dwell Time (ms)	Collision Energy (CE), V	Declustering Potential (DP), V	Cell Exit Potential (CXP),V
Florasulam (quantification)	358.2	167.0	150	-24	-55	-9
Florasulam (confirmation)	358.2	152.1	150	-44	-55	-9
Florasulam 5-OH (quantification)	344.1	324.1	150	-24	-55	-5
Florasulam 5-OH (confirmation)	344.1	104.0	150	-40	-55	-5

Calculations

Quantification of the analytes was accomplished by the external standard method. Recovery results derived from LC-MS/MS analysis and calculations are shown in detail in Table 1 through Table 3. The values reported in the tables are calculated with full precision, but are displayed with rounding.

The concentrations C_{End} (ng/mL) of the analytes found in the final dilution prepared for analysis (V_{End} = 1.1 mL) were converted to the residues R (µg/L) of the analytes in the original water sample aliquot (V_{Water} = 1.0 mL) as follows:

R = C_{End} x (V_{End} / V_{Water})

= C_{End} x 1.10

Recoveries (Rec.) were calculated for the fortified specimens as follows:

Rec. = (R / $R_{fortified}$) x 100 %

Example for Calculation

The florasulam calculation is exemplified with the tap water specimen 110539-003-0001A9 + 0.5 ug/L.

One mL (V_{Water}) of water were fortified at 0.5 µg/L (10xLOQ) with florasulam and the 5-OH metabolite by dosing 0.10 mL of the 5.0 ng/mL fortification solution to obtain a final volume V_{End} = 1.1 mL.

The final extract was examined by LC/MS/MS in run file 110539_S1_rep1, resulting in a florasulam concentration C_{End} of 0.464 ng/mL (358 m/z -> 167 m/z), and a residue result (R) of 0.5104 µg/L.

Recoveries (Rec.) were calculated for the fortified specimens as follows:

Rec. = (R / $R_{fortified}$) x 100 %

= (0.5104 µg/L / 0.50 µg/L) x 100 %

= 102 %

Statistical Treatment of Data

The mean recoveries for the fortified samples were calculated using the “AVERAGE” function of the Microsoft Excel spreadsheet computer program, which divides the sum of the selected cells by the number of determinations. The standard deviation of the recoveries for a fortification level of one matrix type was calculated using the “STDEV” function of the same spreadsheet program, which sums the squares of the individual deviations from the mean, divides by the number of degrees of freedom, and extracts the square root of the quotient. Percent relative standard deviation, % RSD, was calculated by dividing the standard deviation by the mean, and then multiplying by 100.

Appendix A: Excerpts from Dow AgroSciences Report for Study 110538

INTRODUCTION

Analytical method GRM 07.23, "Determination of Residues of Florasulam and its 5-OH Florasulam Metabolite in Waters by Liquid Chromatography with Tandem Mass Spectrometry Detection", was developed and validated at Dow AgroSciences LLC.

In the present study the method was revised for direct injection of water samples into the LC/MS/MS system and found to be suitable for the determination of residues of florasulam and its 5-OH florasulam metabolite in waters over the concentration range of 0.05-0.5 µg/L. The validated limit of quantitation of the method was 0.05 µg/L.

ANALYTICAL

Preparation and Storage of Samples

Drinking (Tap) Water

Water was collected from a PTRL Europe laboratory tap located in Ulm, in Southern Germany. The appearance of the water was clear and without any odor. The water was characterized for physical and chemical properties as follows: pH 8.36, total water hardness: 2.40 mmol/L (Deutsche Härtegrade, 13.5°d). The water was characterized by accredited Institute Alpha (Ulm, Germany following common DIN or EN guidelines and methods, non-GLP).

Surface (River) Water

Water was collected on 23-Aug-10 from the Brenz River in Herbrechtingen, located in Southern Germany. The water was characterized for physical and chemical properties as follows: pH 8.43. The river water was characterized by accredited Institute Alpha (Ulm, Germany following common DIN or EN guidelines and methods), resulting in the following (non-GLP):

total water hardness:	2.98 mmol/L (Deutsche Härtegrade, 16.7°d)	
TOC (total organic carbon, EN 1484:1997):	0.84	mg/L
DOC (diluted organic carbon, EN 1484: 1997):	0.64	mg/L
Silt content (EN 872 Whatman GF 6):	<5.0	mg/L
Turbidity (EN 7027:1999)	0.64	NTU

Ground (Well) Water

Water was collected on 23-Aug-10 from Herbrechtingen, in Southern Germany. The water was characterized for physical and chemical properties as follows: pH 8.28. The ground water was

characterized by accredited Institute Alpha (Ulm, Germany following common DIN or EN guidelines and methods), resulting in the following (non-GLP):

total water hardness:	3.37 mmol/L (Deutsche Härtegrade, 18.9°d)	
TOC (total organic carbon, EN 1484:1997):	1.1	mg/L
DOC (diluted organic carbon, EN 1484: 1997):	1.0	mg/L
Silt content (EN 872 Whatman GF 6):	<5.0	mg/L
Turbidity (EN 7027:1999)	5.80	NTU

Water was stored at room temperature in the dark when not used.

Apparatus

Analytical balance: Sartorius RC210D (used for analytical standard).

Ultrasonic bath: Transsonic 460 (Elma).

Vortex mixer Assistent Reamix.

Typical glassware and laboratory equipment.

All the glassware was cleaned in a laboratory dishwasher and air-dried before use.

Reagents, Chemicals and Miscellaneous

Acetonitrile, acetone, methanol, all HPLC or residue grade (Promochem).

Millipore water (supply at PTRL).

Glacial acetic acid (100 %, Merck).

LC-MS/MS System

Agilent 1200 Series HPLC system (vacuum solvent degasser, binary HPLC pump, column oven), and CTC Analytics HTC-Pal Autosampler.

Supelco Ascentis Express 100 x 3 mm, 2.7-µm particle size with Phenomenex C_{18}, 4 x 3 mm, pre-column.

Applied Biosystems MDS Sciex API 5500 triple quadrupole LC-MS/MS system with TurboIonSpray ESI source. Analyst 1.5 Instrument control and data acquisition software.

Preparation of Solutions and Standards

A stock solution of florasulam and its 5-OH metabolite were prepared as follows:

Analyte	Purity [%]	Amount weighed [mg]	Dilute to [mL]	Concentration of stock solution [mg/mL]
Florasulam	99.7	20.06	20 (Acetone)	1.0
5-OH Florasulam	98.1	20.36	20 (Acetonitrile)	1.0

These solutions were further diluted volumetrically into acetonitrile to obtain mixed intermediate solutions with 10 and 0.10 µg/mL per analyte. These intermediate solutions were further diluted volumetrically into acetonitrile to obtain mixed fortification solutions with 5.0 and 0.50 ng/mL per analyte.

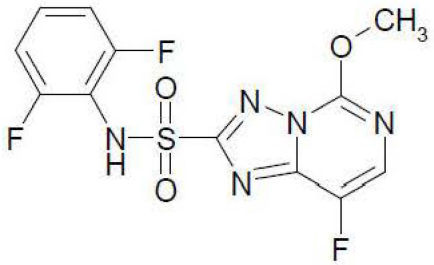
Calibration solutions were prepared by volumetric dilution to obtain concentrations of 1.0 µg/mL and 0.010 µg/mL (intermediate), and further to obtain concentrations of 1.0, 0.50, 0.20, 0.10, 0.050, 0.025 and 0.010 ng/mL in acetonitrile/water; 10/90; v/v used for calibrations.

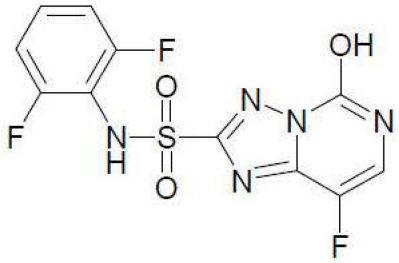
Stability of Solutions and Extracts

All solutions were stored in a refrigerator (at approximately ≤ 8 °C) when not in use.

Final sample dilutions ready for analysis were stable up to nine days as shown by acceptable recovery results, when re-analyzing selected extracts after they had been stored refrigerated for 5 to 9 days.

The following analytical standards (obtained from the Sponsor) were utilized during the method validation:

Common Name of Compound	Structure and CAS Name
Florasulam Molecular Formula: $C_{12}H_8F_3N_5O_3S$ Formula Weight 359.29 Nominal Mass: 359 CAS Number: 145701-23-1	 *N*-(2,6-difluorophenyl)-8-fluoro-5-methoxy[1,2,4]triazolo[1,5-*c*]pyrimidine-2-sulfonamide

5-OH Florasulam Molecular Formula: $C_{11}H_6F_3N_5O_3S$ Formula Weight 345.269 Nominal Mass: 345 CAS Number: N/A	 *N*-(2,6-difluorophenyl)-8-fluoro-5-hydroxy[1,2,4]triazolo[1,5-*c*]pyrimidine-2-sulfonamide

Test Substance/ Analytical Standard	AGR/TSN Number	Percent Purity	Certification Date	Reference
Florasulam	TSN100381	99.7%	02-May-2008 Recertification: 25-Apr-2012	FAPC08-163723
5-OH Florasulam	TSN101151	98.1%	30-Jun-2009 Recertification: 19-Jun-2013	FAPC 09-225597

Preparation of Water Samples for LC/MS/MS Injection

1. An aliquot of 1.0 mL (V_{Sample}) water was dosed into an autosampler vial.
2. 100 µL of the corresponding fortification solution were added, if necessary.
3. 100 µL of acetonitrile were added to blank control samples.
4. Shake with vortexer.
5. Determination of the analytes was done by using LC/MS/MS.

Analytical Instrumentation and Equipment

For Q1 and product ion scan of florasulam and its 5-OH metabolite see Figure 18 and Figure 19. The following LC/MS/MS method was used:

Liquid Chromatography Operating Conditions

Instrumentation:	CTC Analytics HTC PAL Autosampler Agilent Model 1200 binary pump Agilent Model 1200 degasser
Column:	Supelco Ascentis Express 100 x 3 mm, 2.7-µm particle size (C18-type stationary phase) Securityguard: Phenomenex, C18, 4 x 3 mm,
Column Temperature:	40°C
Injection Volume:	60 µL
Mobile Phase:	A – water with 0.1% acetic acid B – acetonitrile/methanol (1/1, v/v) with 0.1% acetic acid
Flow Rate:	500 µL/min

Gradient:

Time, min	A, %	B, %
0.00	90	10
1.00	90	10
5.00	0	100
9.00	0	100
9.10	90	10
13.00	90	10

Mass Spectrometry Operating Conditions

Instrumentation:	Applied Biosystems API 5500 LC/MS/MS System Applied Biosystems Analyst 1.5 data system
Interface:	TurboIonSpray
Scan Type:	MRM
Resolution:	Q1 – Unit, Q3 – Unit
Curtain Gas (CUR):	30
Collision Gas (CAD):	Medium
GS1:	40
GS2:	60
Temperature (TEM):	450°C
Polarity:	Negative
IonSpray Voltage (IS):	-4500 V
Declustering Potential (DP):	-95
Entrance Potential (EP):	-10

Analytes:	Ion, m/z		Dwell Time, ms	CE/CXP, V
	Q1	Q3		
Florasulam (quantitation)	358.2	167.0	200	-24/-5
Florasulam (confirmation)	358.2	152.1	300	-44/-5
5-OH Florasulam (quantitation)	344.1	324.1	200	-24/-5
5-OH Florasulam (confirmation)	344.1	104.0	300	-40/-5

Calculations

Quantification of the analytes were accomplished by the external standard method. Results derived from LC-MS/MS analysis and calculations are shown in detail in Table 1 to Table 3. The values reported in the tables are calculated with full precision, but are displayed with rounding.

The concentrations C_{End} (ng/mL) of the analytes found in the final dilution prepared for analysis (V_{End} = 1.1 mL) were converted to the residues R (µg/L) of the analytes in the original water sample aliquot (V_{Water} = 1.0 mL) as follows:

$$R = C_{End} \times (V_{End} / V_{Sample})$$
$$= C_{End} \times \text{Multiplier M}$$
$$= C_{End} \times 1.10$$

Recoveries (Rec.) were calculated for the fortified specimens as follows:

$$\text{Rec.} = (R / R_{fortified}) \times 100\ \%$$

Example for Calculation

The calculation is exemplified with the tap water specimen PTRL-ID P2292-34.

1.0 mL (V_{Water}) of water were fortified at 0.5 µg/L (10xLOQ) with florasulam by dosing 0.10 mL of the 5.0 ng/mL fortification solution to obtain a final volume V_{End} = 1.1 mL.

The final extract was examined by LC/MS/MS in run file P2292-051, resulting in a florasulam concentrations C_{End} of 0.462 ng/mL (358 m/z -> 167 m/z), and a residue result R of 0.508 µg/L.

Recoveries (Rec.) were calculated for the fortified specimens as follows:

Rec. = $(R / R_{fortified})$ x 100 %

= (0.508 µg/L / 0.50 µg/L) x 100 %

= 102 %

Statistical Treatment of Data

The mean recoveries for the fortified samples were calculated using the "AVERAGE" function of the Microsoft Excel spreadsheet computer program, which divides the sum of the selected cells by the number of determinations. The standard deviation of the recoveries for a fortification level of one matrix type was calculated using the "STDEV" function of the same spreadsheet program, which sums the squares of the individual deviations from the mean, divides by the number of degrees of freedom, and extracts the square root of the quotient. Percent relative standard deviation, % RSD, was calculated by dividing the standard deviation by the mean, and then multiplying by 100.

Confirmatory Evaluation

The presence of each analyte is confirmed by comparing the liquid chromatography retention times of the analytes in the calibration standards with those found in the samples when monitoring two characteristic MS/MS transitions.

A. Introduction:

The U.S. EPA (under FIFRA) requires petitioners to provide accurate and precise data on the performance of soil and water chemistry methods that are used to develop laboratory and/or field residue data to support exposure, environmental fate, and ecological effects studies for registration and re-registration.

A residue analytical method has been developed for the determination of residues of Florasulam (N-(2,6-difluorophenyl)-8-fluoro-5-methoxy[1,2,4]triazolo[1,5-c]pyrimidine-2-sulfonamide) and its 5-OH Florasulam Metabolite (N-(2,6-difluorophenyl)-8-fluoro-5-hydroxy[1,2,4]triazolo[1,5-c]pyrimidine-2-sulfonamide) in drinking water, ground water, and surface water. The validated limit of quantitation (LOQ) is 0.05 µg/L, and the method was validated over the concentration range of 0.05-0.50 µg/L.

B. Objective:

The method must be validated at an independent laboratory prior to its submission to the U.S. EPA. The purpose of this study is to conduct an independent laboratory validation of Dow AgroSciences LLC residue analytical method validation study number 110538, "Method Validation Study for the Determination of Residues of Florasulam and its 5-OH Metabolite in Surface Water, Ground Water and Drinking Water by Liquid Chromatography with Tandem Mass Spectrometry", as written.

The independent laboratory validation (ILV) must follow the U.S. EPA guidelines found in Subdivision N (Environmental Fate), Series 164-1; Publication of Addenda for Data Reporting E, K, and N Requirements for Pesticide Assessment Guidelines; EPA Ecological Effects Test Guideline OPPTS 850.7100 "Public Draft" (1,2,3) and PR Notice 96-1 (4).

The study will provide validation data to confirm that an independent laboratory that has no prior experience with the method can achieve results meeting the requirements of the U.S. EPA.

E. Matrix:

The independent validation will be conducted using untreated samples of drinking (tap), surface (river or pond), and ground (well) water. The untreated control samples will be obtained by the independent laboratory.

F. Sample History and Identification:

The control water samples must be characterized for physical and chemical properties, including at least the following: pH, turbidity, dissolved organic carbon, and total hardness. The source and type of control water will be provided (e.g., pond water, etc.) and the characterization will not be performed under GLP.

Upon receipt, the control matrices will be stored frozen at all times in a temperature-monitored of -20 °C or below, except when removed for analysis. Sample receipt, condition upon receipt, and storage conditions during the ILV trial must be documented by the independent lab.

All samples and standards must be identified with a unique label or number assigned to the sample which identifies the test system, study, nature of study, and the date of collection. Such identification should be either on the sample or cross-referenced to the sample and readily available.

G. Procedures:

The independent laboratory may contact the Study Monitor prior to analyzing the first set of samples for the purpose of requesting standards or samples or for clarification of any protocol issues, but not for the purpose of discussing the methodology (other than to clarify any minor method deficiencies or issues relating to differing equipment).

Using standards, the independent laboratory will establish suitable LC/MS/MS parameters prior to initiation of the first sample trial. The independent laboratory will determine that it can obtain results similar to those shown in the chromatograms in method validation study number 110538 in terms of sensitivity, retention times of the analytes, and baseline noise. The independent laboratory will additionally verify that matrix control samples are either free of interferences or that interferences are negligible at the appropriate retention times or detector settings. (If control matrix samples used are already known to be free of interferences, that verification step may be waived.) The Study Monitor will be consulted if problems arise during these preliminary phases or if the independent lab believes it is necessary to make any minor changes in the method as written.

The independent laboratory will be allowed to analyze up to three complete sample sets to obtain successful recoveries. (The acceptability of the recovery data is described in Section H.).

Once the Study Director and the Study Monitor have judged that a sample set has been successfully validated for a specific water type, it will not be necessary to conduct any additional analyses for that matrix. If the Study Director decides that the first sample set is not successful, the Study Monitor will be contacted for assistance. If the Study Director decides that a second sample set is also unsuccessful, the Study Monitor will again be contacted for assistance before attempting a third set. If the Study Director and the Study Monitor decide that a third sample set is unsuccessful, no additional sample sets for that method may be conducted by the independent lab. The method author will review the method, and, if necessary, the method will be revised. Then a new confirmatory trial will be conducted at a different laboratory.

The independent lab's QA unit will conduct at least one in-progress audit during the laboratory phase of the study.

All communications between the independent lab and the sponsoring lab must be logged and included in the ILV report. The communication log must include the reasons for the contact, any changes to the method that resulted, and the timing of the contact with respect to the progress of the validation trial (i.e., after the first sample set, during the second sample set, etc.). At the discretion of the method's author, any recommended changes will be incorporated into the method.

A minimum of 12 samples plus one reagent blank will be analyzed for a complete sample set of each water type. The method will be independently validated using both mass transitions (MRMs) as described in Section named Mass Spectrometry Operating Conditions of the method.

A complete sample set per water type will include as a minimum:

1 reagent blank (containing no matrix or analyte)
2 unfortified control samples
5 control samples fortified at 0.05 µg/L with Florasulam and its 5-OH Metabolite (the LOQ of the method).
5 control samples fortified at 0.5 µg/L with Florasulam and its 5-OH Metabolite (ten times the LOQ).

Other control, reagent blank, and recovery samples may be analyzed as needed at the discretion of the Study Director and after consultation with the Study Monitor.

H. Acceptability of Recovery Data:

The validation is successful if the results of the study satisfy the requirements of the U.S. EPA guidelines found in Subdivision N (Environmental Fate), Series 164-1; Publication of Addenda for Data Reporting E, K, and N Requirements for Pesticide Assessment Guidelines; EPA Ecological Effects Test Guideline OPPTS 850.7100 "Public Draft" (1,2,3).