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Document Type	OECD Annex Point(s) Planned (Registration docs only)	PTR Number	Report / File Number(s)	Date Issued (dd-MMM-yy)
Analytical Method		10001687	110535	14-Sep-11

Document Title

Residue Method Validation for the Determination of Florasulam in Agricultural Commodities

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Materials being tested:

Active Ingredient(s) / Structural Gene(s) / Protein(s), etc.	Formulation Nos.	Product Name(s) (DAS only)
FLORASULAM		

Lab Notebook(s)	Information Release No.	GLP Substance Nos (TSN, AGR)	Other Batch/Lot Number(s)
N/A			

Key Terms including compound numbers (ex: XDE-123)

Method Validation,FLORASULAM, , LC-MS/MS, Mogi Mirim Regulatory Laboratory

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SANCO/3029/99 rev.4; SANCO/825/00 rev.8.1, EU Council Directive 91/414/EEC; USEPA OPPTS 860.1340, PMRA Dir 98-02 and OECD Principles of GLP

Global Data Matrix? ☐

State or Province of Study	Country of Study	Protocol Number(s)	Internal Study/Trial Number(s)	External Study/Trial Number(s)	Study Completion Date (dd-MMM-yy)
		110535	110535		
					GLP Status
					GLP

Analytical Methods Only:

Method Type	Analyte(s)
Residue	Florasulam

Method Technique(s)	Matrix
LC-MS/MS	Apple, Orange, Corn, Wheat, Soybean, Canola, Potato, Tomato

SUMMARY

(In accordance with 40 CFR part 152, this summary is available for public release after registration)

STUDY TITLE

Residue Method Validation for the Determination of Florasulam in Agricultural Commodities

DATA REQUIREMENTS

EPA Residue Chemistry Test Guideline OPPTS 860.1340
EU Council Directive 91/414/EEC
Section 3 of Sanco/3029/99 rev.4
Section 3 of Sanco/825/00 rev 8.1
PMRA Dir 98-02

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STUDY COMPLETED ON

14/Sep/2011

PERFORMING LABORATORY

Dow AgroSciences Ind. Ltda.
Mogi Mirim Regulatory Laboratory
Rod. SP 147, km 71.5
Mogi Mirim, SP, Brazil

LABORATORY STUDY ID

110535

Residue Method Validation for the Determination of Florasulam in Agricultural Commodities

SUMMARY

This report contains validation data for the following Dow AgroSciences Ind. Ltda. residue analytical method:

Study 110535, “Residue Method Validation for the Determination of Florasulam in Agricultural Commodities”

This method is applicable for the quantitative determination of residues of florasulam in agricultural crops (acidic crops. dry crops, oily crops and high aqueous crops). The method was validated over the concentration ranges of 0.01-1.00 µg/g with a validated limit of quantitation of 0.01 µg/g.

STUDY TITLE

Residue Method Validation for the Determination of Florasulam in Agricultural Commodities

DATA REQUIREMENTS

EPA Residue Chemistry Test Guideline OPPTS 860.1340
EU Council Directive 91/414/EEC
Section 3 of Sanco/3029/99 rev.4
Section 3 of Sanco/825/00 rev 8.1
PMRA Dir 98-02

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STUDY COMPLETED ON

14/Sep/2011

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Mogi Mirim, SP, Brazil

LABORATORY STUDY ID

110535

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

Compound: Florasulam

Title: Residue Method Validation for the Determination of Florasulam in Agricultural Commodities

No claim of confidentiality, on any basis whatsoever, is made for any information contained in this document. I acknowledge that information not designated as within the scope of FIFRA sec. 10(d)(1)(A), (B), or (C) and which pertains to a registered or previously registered pesticide is not entitled to confidential treatment and may be released to the public, subject to the provisions regarding disclosure to multinational entities under FIFRA sec. 10(g).

Company: Dow AgroSciences LLC

Company Agent: C. Niamh McMahon

Title: Regulatory Manager

Signature: Niamh McMahon

Date: Sept 6th 2011

THIS DATA MAY BE CONSIDERED CONFIDENTIAL IN COUNTRIES OUTSIDE THE UNITED STATES.

STATEMENT OF COMPLIANCE WITH GOOD LABORATORY PRACTICE STANDARDS

Title: Residue Method Validation for the Determination of Florasulam in Agricultural Commodities

Study Initiation Date:	15/July/2011		
Analytical Phase Initiation Date:	28/July/2011	Analytical Phase Completion Date:	15/August/2011

This report represents data generated after the effective date of the EPA FIFRA Good Laboratory Practice Standards.

United States Environmental Protection Agency
Title 40 Code of Federal Regulations Part 160
FEDERAL REGISTER, August 17, 1989

NIT-DICLA-035 (INMETRO) and related documents

All aspects of this study were conducted in accordance with the requirements for Good Laboratory Practice Standards, 40 CFR 160.

C. Niamh McMahon
Sponsor
Dow AgroSciences LLC

Date: Sept 6th 2011

C. Niamh McMahon
Submitter
Dow AgroSciences LLC

Date: Sept 6th 2011

Alvino Rodrigues Junior
Study Director/Author
Dow AgroSciences Ind. Ltda.

Study Completion Date: 14/SEP/2011

CERTIFICATE OF GLP COMPLIANCE

República Federativa do Brasil
Ministério do Desenvolvimento, Indústria e Comércio Exterior
Instituto Nacional de Metrologia, Normalização e Qualidade Industrial – Inmetro

Coordenação Geral de Acreditação – CGCRE/INMETRO
Certificado de Reconhecimento da Conformidade aos Princípios das Boas Práticas de Laboratório

Certificado nº BPL 0015 **Reconhecimento inicial: 11-02-2004**

MOGI MIRIM REGULATORY LABORATORY
DOW AGROSCIENCES INDUSTRIAL LTDA.
RODOVIA SP, 147 (km 71,5) – PEDERNEIRAS
MOGI MIRIM – SP

A instalação de teste foi inspecionada dentro do Programa Brasileiro de Monitoramento BPL da CGCRE/INMETRO e foi considerada estar em conformidade aos Princípios das Boas Práticas de Laboratório da OCDE, para a condução de estudos não-clínicos de segurança à saúde e ao meio-ambiente, no escopo abaixo:

Áreas de Especialidades de Estudos	***Categorias de Itens de Teste***
Estudos de Resíduos; Estudos com Organismos Geneticamente Modificados; Química Analítica e Clínica	*- Agrotóxicos, Seus Componentes e Afins* *- Organismos Geneticamente Modificados.*

Nota: As categorias de itens de teste "agrotóxicos, seus componentes e afins" e "produtos químicos industriais" estão contemplados pela adesão plena do Brasil, através da Coordenação Geral de Acreditação-Cgcre do Inmetro, aos Atos da Organização para a Cooperação e Desenvolvimento Econômico - OCDE relacionados à Aceitação Mútua de Dados (MAD) de acordo com os Princípios das Boas Práticas de Laboratório-BPL.

Aldoney Freire Costa
Emissão: 27-6-2011 Coordenador Geral de Acreditação Substituto Validade: 11-02-2012

QUALITY ASSURANCE STATEMENT

DOW AGROSCIENCES QUALITY ASSURANCE UNIT

Compound: Florasulam

Title: Residue Method Validation for the Determination of Florasulam in Agricultural Commodities

Study Initiation Date: 15/July/2011 Study Completion Date: 14/Sep/2011

GLP Quality Assurance Inspections

Date of Inspection(s)	Date reported to the Study director, Principal Field Investigator and to Test Facility Manager	Critical events inspected by the QAU
11-Jul-2011	11-Jul-2011	Protocol
29-Jul-2011	01-Aug-2011	Analytical phase: Analytical procedure of sample preparation
25, 26, 29, 30-Aug-2011	30-Aug-2011	Final report and raw data

QUALITY ASSURANCE STATEMENT:

The Quality Assurance Unit has reviewed the final report and has determined that the report reflects the raw data generated during the conduct of this study.

Bruciana Marchese
Quality Assurance Unit

14 Sep 2011
Date

SIGNATURE PAGE

Alvino Rodrigues Junior — 14/ SEP/ 2011
Alvino Rodrigues Junior
Author
Dow AgroSciences Ind. Ltda.
Date

Darcy D. Shackelford — 07- Sept- 2011
Darcy D. Shackelford
Reviewer
Dow AgroSciences LLC
Date

Brian M. Wendelburg — 07Sept 2011
Brian M. Wendelburg
Technical Leader
Dow AgroSciences LLC
Date

14. Sep. 2011
Otavio Balderrama Pinto
Test Facility Manager
Dow AgroSciences Ind. Ltda.
Date

STUDY PLAN/PROTOCOL/AMENDMENTS

This study was performed under Study Number/Protocol 110535, with one amendment and no deviations recorded during study conduction.

	Description
Amendment	
01	The Sponsor responsibilities were transferred to Mrs. C. Niamh McMahon of Dow AgroSciences, Indianapolis, United States, and the study completion date were updates from August/2011 to September/2011.

STUDY PERSONNEL

Study Director:	Alvino Rodrigues Junior
Analyst:	Alvino Rodrigues Junior
Third Part Contractor:	Luíza Helena Piccoli
Student:	Ana Tereza Colombini

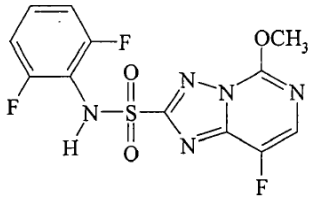
TABLE OF CONTENTS

Residue Method Validation for the Determination of Florasulam in Agricultural Commodities

INTRODUCTION

Scope

This method is applicable for the quantitative determination of residues of florasulam in agricultural commodities. The method was validated over the concentration range of 0.01-1.00 µg/g with a validated limit of quantitation of 0.01 µg/g.



Florasulam
CAS: 145701-23-1

Common and chemical name, molecular formula, and the nominal mass for the analyte are given in Table 1.

This study was conducted to fulfill data requirements outlined in the U. S. EPA Residue Chemistry Test Guidelines, OPPTS 860.1340 (1), the European Commission Guidance Document on Residue Analytical Methods, SANCO/825/00 rev.8.1 (2) and SANCO/3029/99 rev. 4 (3), and PMRA Residue Chemistry Guidelines as Regulatory Directive Dir98-02 (4).

Method Principle

Residues of florasulam are extracted from the sample matrices by homogenizing and shaking with an acetone:water: acetic acid (80:19:1) solution. A 1.0-mL aliquot of the extraction solution is diluted with 2.0 mL of deionized water. The solution is then purified using a reverse-phase polymeric solid-phase extraction (SPE) column. After elution from the SPE column with a 1-chlorobutane:ethyl acetate (70:30) solution, the eluate is dried and reconstituted with an acetonitrile:methanol:water:acetic acid (25:25:49.9:0.1) solution. The sample is analyzed by liquid chromatography with positive-ion electrospray ionization tandem mass spectrometry (LC-MS/MS).

Safety Precautions

Each analyst must be acquainted with the potential hazards of the equipment, reagents, products, solvents, and procedures used in this method before commencing laboratory work. SOURCES OF INFORMATION INCLUDE: OPERATION MANUALS, MATERIAL SAFETY DATA SHEETS, LITERATURE, AND OTHER RELATED DATA. Safety information should be obtained from the supplier. Disposal of waste materials, reagents, reactants, and solvents must be in compliance applicable governmental requirements.

Acetone, acetonitrile, l-chlorobutane, ethyl acetate, isooctane and methanol are flammable and should be used in well-ventilated areas away from ignition sources. Acetic acid is corrosive and can cause severe burns. It is imperative that proper eye and personal protection equipment be worn when handling these reagents.

Test Substance/Analytical Standard

Test Substance/ Analytical Standard	AGR/TSN Number	Percent Purity	Certification Date	Reference
Florasulam	TSN100381	99.7	25-Apr-2012	FAPC 08-163723

The above standard may be obtained from the Test Substance Coordinator, Dow AgroSciences LLC, 9330 Zionsville Road, Building 304, Indianapolis, IN 46268-1054.

Equipment, Glassware, and Materials

Equipment, glassware, materials, reagents, and chemicals considered to be equivalent to those specified may be substituted with the understanding that their performance must be confirmed by appropriate tests. Common laboratory glassware and supplies are assumed to be readily available. Unless specified otherwise, class A volumetric glassware is used to prepare analytical standards, fortification solutions, and calibration standards.

Laboratory Equipment

Balance, analytical, Model XP 205DR, Mettler-Toledo, Inc.

Balance, pan, Model XS802S, Mettler-Toledo, Inc.

Centrifuge, with rotor to accommodate 8-oz wide-mouth bottles, Model 5430R, Eppendorf GmbH.

Evaporator, SPE-Dry-96 Dual, Biotage Inc.

Grinder, Model UM 25, Stephan, Inc.

Sample homogenizer, model Autogizer Series 701 with 20-mm homogenizer probes Tomtec, Inc.

Pipetter, adjustable electronic, model Research Pro, 5-100 µL, 50-1000 µL, 100-5000 µL, Eppendorf GmbH.

Mechanical shaker, variable speed reciprocating with box carrier, model TE 240 Pendular, Tecnal Ltda.

Ultrasonic cleaner, Model 5510, Branson Cleaning Equipment Company.

Vortex mixer, Model Genie 2, Fisher Scientific, Inc.

Chromatographic Equipment

Column, analytical, Zorbax SB-C8, 4.6 x 75 mm, 3.5-µm, catalog number 866953-906, Agilent Technologies.

Liquid chromatograph autosampler, Model 1100, Agilent Technologies.

Liquid chromatograph quaternary pump, Model 1100, Agilent Technologies.

Liquid chromatograph degasser, Model 1100, Agilent Technologies.

Mass spectrometer, Model API 4000, Applied Biosystems.

Mass spectrometer data system, Analyst 1.5, Applied Biosystems.

Glassware and Materials

High Density Polyethylene Flasks, 125-mL and 250-mL, Nalgene.

Graduated cylinders with cap, 50, 100, 500, 1000 and 2000-mL, Corning Brasil Vidros Especiais Ltda.

Collection plate, 96-well, 2-mL, Masterblocks.

High Density Polyethylene 15-mL conical vial with cap, Nunc.

Flask, volumetric, 50 and 100-mL, Corning Brasil - Vidros Especiais Ltda.

SPE 96-well plate, Phenomenex Strata X 33 µm, 30-mg packing, catalog number 8E-Sl00-TGB, Phenomenex.

Pipette tips, 200-µL, 1000-µL and 5000-µL capacity, Eppendorf.

Vial, autosampler, 2-mL, Agilent Technologies.

Vial cap, for autosampler vial, Agilent Technologies.

Reagents

Acetic acid, glacial, ChromAr grade, catalog number V155-06, Mallinckrodt.

Acetone, UltimAr grade, catalog number H451-10 Mallinckrodt.

Acetonitrile, HPLC grade, catalog number 9012-03, J. T. Baker.

l-Chlorobutane, 99 % purity, catalog number 125008, Sigma.

Ethyl acetate, UltimAr grade, catalog number V553-10, Mallinckrodt.

Isooctane (2,2,4-trimethylpentane), ChromAr grade, catalog number 6043-10, Mallinckrodt.

Methanol, HPLC grade, catalog number 9093-03, J. T. Baker.

Standard

Florasulam (XDE-570): N-(2,6-difluorophenyl)-8-fluoro-5-methoxy[1,2,4]triazolo-[1,5-c]pyrimidine-2-sulfonamide.

Obtain standard from the Test Substance Coordinator, Dow AgroSciences LLC, 9330

Zionsville Road, Building 304, Indianapolis, IN 46268-1054.

Prepared Solutions

Acetone/water/acetic acid (80: 19: 1) (extraction solution)

Measure 150 mL of deionized water to using a 500-mL graduated cylinder and transfer to 1000-mL volumetric flask. Pipet 10.0 mL of acetic acid into the same volumetric flask and swirl the flask. Add 800 mL of acetone using a 1000-mL graduated cylinder and transfer to the flask. Stopper the flask and invert several times to mix well. Allow the solution to equilibrate to room temperature, and dilute to volume with water before use.

Acetonitrile/methanol (50:50) + 0.1% acetic acid (mobile phase A)

Measure 500 mL of acetonitrile using a 500-mL graduated cylinder and transfer to a l-L media bottle. Next measure 500 mL of methanol using a 500-mL graduated cylinder and transfer to the same media bottle. Pipet 1.0 mL of acetic acid into the media bottle. Cap the bottle and shake to mix well. Allow the solution to equilibrate to room temperature before use.

l-Chlorobutane/ethyl acetate (70:30) (SPE elution solution)

Pipet 30.0 mL of ethyl acetate into a 100-mL volumetric flask. Dilute to volume with l-chlorobutane. Stopper the flask and invert several times to mix well before use.

Acetonitrile/methanol/water/acetic acid (25:25:49.9:0.1) (sample diluent)

Measure 250 mL of acetonitrile using a 500-mL graduated cylinder, and transfer to a 1000-mL volumetric flask. Next measure 250 mL of methanol using a 500-mL graduated cylinder, and transfer to the same flask. Pipet 1.0 mL of acetic acid into the flask and swirl the flask to mix. Add approximately 499 mL of deionized water to the flask. Stopper the flask and invert several times to mix well. Allow the solution to equilibrate to room temperature, and dilute to volume with water before use.

Water + 0.1% acetic acid (mobile phase B)

Measure 1000 mL of HPLC grade water using a 1000-mL graduated cylinder, and transfer to a 1-L media bottle. Pipet 1.0 mL of acetic acid into the media bottle. Cap the bottle and shake to mix well. Allow the solution to equilibrate to room temperature before use.

EXPERIMENTAL

Instrumental Conditions

Typical HPLC Operating Conditions (Supplemental Note 1)

Instrumentation:	Agilent Model 1100 autosampler Agilent Model 1100 quaternary pump Agilent Model 1100 degasser MDS/Sciex API 4000 LC/MS/MS System MDS/Sciex Analyst 1.5 data system
Column:	Zorbax SB-C8 4.6 x 75 mm, 3.5-µm
Column Temperature:	35 °C
Injection Volume:	25 µL
Run Time:	10.0 minutes
Mobile Phase:	A – acetonitrile/methanol (50:50) + 0.1% acetic acid B – water+ 0.1% acetic acid
Flow Rate:	900 µL/min (approx 200 µL/min split to source)

Gradient:

Time, min	Solvent A, %	Solvent B, %
0.0	10	90
5.0	100	0
7.0	100	0
7.1	10	90
10.0	10	90

Flow Diverter Program:
1) 0.0 to 3.0 min: flow to waste
2) 3.0 to 7.0 min: flow to source
3) 7.0 to 10.0 min: flow to waste

Typical Mass Spectrometry Operating Conditions

Interface:	Electrospray
Polarity:	Positive
Scan Type:	MRM
Resolution:	Q1 – unit, Q3 – unit
Curtain Gas (CUR):	15
Collision Gas (CAD):	6
Temperature (TEM):	400 °C
Ion Source Gas 1 (GS1):	50
Ion Source Gas 2 (GS2):	40

Period 1	
Pre-acquisition Delay:	0.0 min
Acquisition Time	10.0 min
IonSpray Voltage (IS):	5500 volts
Entrance Potential (EP):	10 volts
Declustering Potential (DP):	71 volts

Analytes:	Precursor Ion Q1	Product Ion Q3	Collision Time	Collision Energy	Cell Exit Potential
Florasulam *m/z* 360/129 (quantitation)	359.9	129.1	150 ms	31 V	12 V
Florasulam *m/z* 360/109 (confirmation)	359.9	109.2	150 ms	79 V	8 V

Certificate of analysis, representative spectra and calibration curve shown in Figures 1-3. Typical chromatograms for the determination of florasulam in agricultural commodities are illustrated in Figures 4-7.

Preparation of Standard Solutions

Preparation of Florasulam Stock Solution (Supplemental Note 2)

Weigh 0.0500 g of florasulam analytical standard and quantitatively transfer to a 50-mL volumetric flask with acetone. Dilute to volume with acetone to obtain a 1000-µg/mL stock solution.

Preparation of Florasulam Spiking Solutions

Pipet 2.5 mL of the 1000-µg/mL stock solution into a 50-mL volumetric flask and dilute to volume with methanol to obtain a 50.0-µg/mL stock solution.

Prepare fortification solutions in methanol by diluting the 50.0-µg/mL stock solution or the 5.00-µg/mL fortification solution prepared below as follows:

Initial Stock Solution Concentration µg/mL	Aliquot Of Stock Solution mL	Final Solution Volume mL	Resulting Stock Solution Concentration µg/mL	Equivalent Sample Concentration[a] µg/g
50	5.0	50	5.0	1.00
50	0.5	50	0.5	0.100
5.0	0.5	50	0.05	0.010
0.5	1.5	50	0.015	0.003

[a] The equivalent sample concentration is based on fortifying a 5.0-g crop sample with 1.0 mL of the appropriate spiking solution.

Preparation of Florasulam Calibration Standards for Quantitation

Pipet 1.0 mL of the 1000.0-µg/mL florasulam stock solution prepared into a 100-mL volumetric flask and adjust to volume with acetonitrile/methanol/water/acetic acid (25:25:49.9:0.1) to obtain a 10.0-µg/mL stock solution.

Pipet 10.0 mL of the 10.0-µg/mL florasulam stock solution prepared into a 100-mL volumetric flask and adjust to volume with acetonitrile/methanol/water/acetic acid (25:25:49.9:0.1) to obtain a 1.0-µg/mL stock solution.

Prepare calibration standards for florasulam by following the table below. Use a volumetric pipette to dispense the appropriate amount of florasulam stock solution into a volumetric flask and dilute to volume with an acetonitrile/methanol/water/acetic acid (25:25:49.9:0.1) solution.

Concentration of Stock Solution µg/mL	Aliquot Of Stock Solution mL	Final Solution Volume mL	Calibration Solution Final Concentration µg/mL	Equivalent Sample Concentration[a] µg/g
1	2.5	50	0.050	2.0
1	1.75	50	0.035	1.4
1	1.0	50	0.020	0.8
1	0.5	50	0.010	0.4
0.05	5	50	0.005	0.2
0.05	1	50	0.001	0.04
0.05	0.5	50	0.0005	0.02
0.001	5.0	50	0.0001	0.004
0.001	3.75	50	0.00008	0.003

[a] The equivalent sample concentration relative to the calibration standard concentration is based on taking a 0.5-mL equivalent crop sample extract through the cleanup process and diluting it to a final sample volume of 1.0 mL, which is equivalent to a 0.025-g crop sample.

Sample Origin, Numbering, Preparation and Storage

Untreated control samples of the crops were obtained from the Mogi Mirim Regulatory Laboratory sample inventory. All samples were tracked in the Mogi Mirim Regulatory Laboratory Regulatory Labs Information Management System (RLIMS) database. Unique sample numbers were assigned to the samples to track them during receipt, storage, and analysis. Complete source documentation is included in the study file.

During the course of the study, the samples were stored in temperature-monitored freezers at approximately -20 °C, except when removed for analysis.

Method Validation

The analytical procedure described in GRM 04.13 (5) was validated by analyzing the following:

At least one reagent blank.

At least one unfortified different untreated control sample for each matrix/fraction per sample set.

At least one replicate at the proposed limit of detection (0.003 µg/g).

At least five replicates at the proposed limit of quantitation (0.01 µg/g).

At least five replicates at the maximum concentration of the validation range (1 µg/g).

Analysis Procedure

1. Weigh 5.0 ± 0.05 g of the prepared sample into 125-mL HDPE bottles.

2. For preparation of recovery samples, add 1.0-mL aliquots of the appropriate spiking solutions to untreated control matrices to encompass the desired concentration range.

3. Add 100.0 mL of the acetone/water/acetic acid (80:19:1) extraction solution to the sample bottle.

4. Blend the sample for approximately 1 minute in the sample homogeneizer.

5. Shake the sample for 60 minutes on a reciprocating shaker at approximately 130 excursions/minute.

6. Centrifuge the sample bottle for about 10 minutes at approximately 2000 rpm.

7. Pipet 1.0 mL of the sample extract into 15-mL High Density Polyethylene conical vial.

8. Add 2.0 mL of deionized water to aliquot extract, cap and vortex mix for 5 seconds.

9. Purify the sample using the following SPE procedure:

    a) Place a Phenomenex Strata-X 30-mg SPE 96-well plate on the vacuum manifold.

    b) Condition the SPE column with 1 mL of methanol followed by 1 mL of deionized water. Dry the column under full vacuum (Supplemental Note 3) for 10 seconds between solvents.

    c) Transfer 1.5 mL of the sample solution (step 8) to the conditioned SPE plate. Allow the sample to flow through the plate at a rate of about 1 mL/minute, using vacuum if necessary. After the sample has eluted, apply full vacuum for about 10 minutes. Discard the eluate. (Critical step: Achieving adequate vacuum is necessary for

efficient drying of the SPE. Vacuum can be increased for more efficient drying by covering the unused SPE openings with clear packaging tape.)

d) Rinse the SPE column with two 1-mL aliquots of isooctane. Discard the eluate. Dry the SPE column under full vacuum for 10 seconds between aliquots.

e) Elute the florasulam from the SPE column with two 750-µL aliquots of an l-chlorobutane/ethyl acetate (70:30) solution, with vacuum if necessary. Collect the eluate in a 2-mL deep 96-well collection plate.

10. Evaporate the eluate to complete dryness using a 96-well evaporator set at 40 °C using an air flow of approximately 500 mL/minute.

11. Reconstitute the sample by adding 1.0 mL of acetonitrile/methanol/water/acetic acid (25:25:49.9:0.1) to the vial.

12. Cap the 96-well collection plate and vortex mix, sonicate, and vortex mix the collection plate again for 4 minutes for each step. (Critical step: It is imperative that adequate sample mixing be achieved for uniformity of the final sample).

13. Transfer a portion of the sample to a 2-mL autosampler vial and seal the vial with a cap.

14. Analyze the calibration standards and samples by liquid chromatography with positive-ion electrospray ionization mass spectrometry as described in the Instrumental Conditions section. Determine the suitability of the chromatographic system using the following performance criteria:

a) Standard curve linearity: determine that the correlation coefficient equals or exceeds 0.995 for the least squares equation which describes the detector response as a function of standard curve concentration (Supplemental Note 4).

b) Peak resolution: Visually determine that sufficient resolution (higher or equals 1) has been achieved for the analyte relative to background interferences.

c) Appearance of chromatograms: Visually determine that the chromatograms resemble those shown in Figure 4-7 with respect to peak response, baseline noise, and background interference. Visually determine that a minimum signal-to-noise ratio of 20:1 has been attained for each analyte in the 0.0005-µg/mL calibration standard.

15. Dilute any sample which contain florasulam concentrations that are greater than 2.00 µg/g (equivalent to a 0.05-µg/mL calibration solution) with acetonitrile/methanol/water/acetic acid (25:25:49.9:0.1) to give a concentration within the middle range of the calibration curve.

Calculations

Inject the series of calibration standards described in the standard preparation section using the conditions listed in the instrument section and determine the peak areas for the analyte as follow:

Florasulam Quantitation ion	Q1/Q3 *m/z* 359.9/129.1
Florasulam Confirmation ion	Q1/Q3 *m/z* 359.9/109.2

For each sample and standard calculate the confirmation ratio:

$$\text{Confirmation Ratio} = \left(\frac{\text{peak area m/z 359.9/109.2}}{\text{peak area m/z 359.9/129.1}} \right)$$

Confirmation of the presence of the analyte is indicated when the retention time of the samples matches that of the standards and the confirmation ratio is in the range of ±20% of the average found for the standards.

Prepare a standard curve using linear regression analysis with 1/x weighting by plotting the equivalent analyte concentration on the abscissa (x-axis) and the respective peak area on the ordinate (y-axis) as shown in Figure 3.

For example, using linear regression with the florasulam data from Figure 3:

$$\text{equivalent concentration in sample (µg/mL)} = \left(\frac{\text{florasulam peak area} - \text{intercept}}{\text{slope}} \right) \times \text{dilution factor}$$

Convert the concentration in µg/mL, of florasulam found in the final sample prepared for analysis to µg/g of florasulam accounting for the original sample aliquot as follows:

$$\text{florasulam (gross µg/g)} = \text{µg/mL} \times \left(\frac{(\text{Ext vol, mL}) \times (\text{Final vol, mL})}{(\text{Init Aliq, mL}) \times (\text{SPE, mL/Dil, mL}) \times (\text{Initial wt, g})} \right) \times \text{Dil}$$

$$\text{florasulam (gross µg/g)} = \text{µg/mL} \times \left(\frac{(100\,\text{mL}) \times (1\,\text{mL})}{(1\,\text{mL}) \times (1.5\text{mL}/3\,\text{mL}) \times (5\,\text{g})} \right) \times 1$$

where:

Extraction volume (initial) = 100 mL
Final volume of sample = 1.0 mL
Initial sample aliquot volume taken = 1.0 mL
Final volume of initial sample aliquot after dilution = 3.0 mL
Aliquot volume taken through SPE = 1.5 mL

Sample weight (initial) = 5.0 grams (always input 5.0 grams as the nominal amount for recovery samples)
Dilution factor = 1 (unless further dilution is needed)

Determine the percent recovery by dividing the net concentration of each recovery sample by the theoretical concentration added.

$$\text{Recovery (\%)} = \left(\frac{\text{Concentration found}}{\text{Concentration added}}\right) \times 100$$

An example calculation, using reagent blank, a control aliquot and a LOQ fortified aliquot is shown in Figure 8.

Confirmation of Residue Identity

The GRM 04.13 method is selective for the determination of florasulam by virtue of the chromatographic separation and selective detection system used. Confirmation of the presence of florasulam in agricultural commodities is made by comparison of retention times (liquid chromatography) of recovery samples with the retention times of the calibration standards as well as by monitoring two characteristic MS/MS transition ions by tandem mass spectrometry.

$$\text{Confirmation Ratio} = \frac{\text{peak area m/z 359.9/109.2}}{\text{peak area m/z 359.9/129.1}}$$

According to published guidelines(6), when detection is performed using tandem mass spectrometry methods, confirmation of the presence of the analyte requires the observation of a precursor ion representing the intact molecule plus two structurally significant product ions observed at the same retention time. The confirmation peak area ratios of the two characteristic MS/MS transitions for each sample should fall within the range of ± 20% of the average confirmation peak area ratios found for the standards within each sample set.

$$\text{Percent Difference} = 1 - \left(\text{sample confirmation ratio} \div \text{standards mean confirmation ratio}\right) \times 100$$

Examples of confirmation ratio calculation and its percent difference evaluation are showed in Figures 9-12.

Stability of Sample Extracts

Following initial cleanup and analysis of sample extracts, the resulting samples prepared for analysis were stored in a refrigerator at 3-8°C for at least 7 days. After this period, the samples were reanalyzed by single injection and compared to the original analytical recovery values in order to confirm their stability. Samples are said to be stable if the mean recoveries are still

within the required range (70-120 %, RSD ≤ 20%). The recovery results for extracts after 7 days of extraction are showed in Table 7.

Determination of Matrix Effect

The matrix effects that the sample extracts have upon the analytes during analysis were determined. Matrix effects were evaluated by preparation of matrix-matched standards (for each matrix type) to be used for comparison with standards prepared in a neat solvents. Matrix matched standards are prepared by taking untreated control sample matrix for each matrix type through the cleanup procedure, then fortifying the resulting samples with a known amount of the analyte just prior to injection. Experiments should demonstrate that matrix effects are not significant (i.e. < 20% enhancement or suppression). The matrix effect results for all crop groups are presented in Table 8.

Statistical Treatment of Data

Statistical treatment of data included the calculation of regression equations, coefficients of determination (r^2) for describing the linearity of calibration curves, and means, standard deviations, and relative standard deviations of the results for the fortified recovery samples.

RESULTS AND DISCUSSION

Assay Time

A typical analytical set would consist of a minimum of nine calibration standards encompassing the expected range of sample concentrations, a reagent blank, a control (a non-fortified sample), a minimum of three fortified controls (one of which must be at the LOQ), and 56 samples. This typical analytical run requires approximately 7 hours to prepare a sample set, followed by the chromatographic analysis.

Acceptable stopping points to allow for the continuation of a set the following day are upon the completion of:

a. Step 1
b. Step 7
c. Step 8
d. Step 9e
e. Step 10

If analysis is suspended overnight, refrigerate sample.

Extraction Efficiency

For this protocol, no new extraction efficiency studies were conducted. Extraction was performed as previously reported in the two most recent methods developed for extraction of florasulam which both used a solution of acetone/water/acetic acid (80:19:1) for extraction (7,8).

Stock and Calibration Standard Stability

The stability of the working solutions is determined to at least cover the length of time over which these solutions were used for sample analysis during the validation study. Based on 041016 report (8), florasulam working solutions are stable for at least 8 months, which is an adequate period for this purposed study, and this topic was not conducted during the course of this study.

Standard Curve Linearity

For the linear regression equations describing the detector response as a function of the standard calibration curve concentrations, the coefficients of determination (r^2) were at least 0.999 for all of the quantitative calibration curve determinations during the method validation. These results indicate linearity of the detector response as a function of the standard concentration.

Analytical Recovery Data

The method validation study was conducted to determine the recovery levels and the precision of the method for the determination of residues of florasulam in agricultural commodities. The efficiency of the analytical method was determined at the time of analysis of each set of samples by fortifying aliquots of the appropriate control matrix with florasulam and analyzing the samples. An unfortified control matrix and reagent blank were included in each set. The LOD recovery samples were analyzed only to demonstrate observable peaks at the LOD level; therefore, any results calculated were used only for verification and not included for average percent recovery calculations.

The following matrices were used in the method validation:

acidic crops (orange whole fruit and apple whole fruit);

dry crops (corn grain and wheat grain);

oily crops (soybean grain and canola seed);

wet crops (whole tomato and potato tuber).

Fortified recoveries were analyzed over a sample concentration range of 0.01-1.00 µg/g. The recovery results are summarized below and results are listed individually in Tables 2-5 and summarized at each recovery level for the matrices in Table 6.

Matrix	Validation Range (µg/g)	Average Recovery (%)	Recovery Range (%)	SD (%)	RSD (%)	Number of Samples (n)
Acidic Crops	0.01 – 1.00	94	78 – 115	9.1	9.8	23
Dry Crops	0.01 – 1.00	97	88 – 114	6.7	7.0	24
Oily Crops	0.01 – 1.00	99	84 – 120	7.3	7.3	24
Wet Crops	0.01 – 1.00	90	71– 118	8.7	9.7	24

The individual recoveries for all samples were between 70 and 120%. The average recoveries for all samples were between 70 and 110% at each fortification level. Standard deviations at each fortification level were all less than 20%.

Calculated Limits of Quantitation and Detection

The proposed limits of detection (LOD) and quantitation (LOQ) were targeted at the initiation of the study at 0.003 µg/g and 0.01 µg/g, respectively. Following established guidelines (9), the LOQ and LOD for the determination of residues of florasulam in agricultural commodities were calculated using the standard deviation from the 0.01-µg/g recovery results. The LOQ was calculated as ten times the standard deviation (10*s*), and the LOD was calculated as three times the standard deviation (3*s*) of the results. The results are summarized below for florasulam.

Matrix	Average Recovery (µg/g)	Standard Deviation (s)	Limit of Detection (3s)	Limit of Quantitation (10s)	Number of Samples (n)
Acidic Crops	0.00995	0.00071	0.00212	0.00706	12
Dry Crops	0.01008	0.00070	0.00209	0.00695	12
Oily Crops	0.01037	0.00072	0.00216	0.00721	12
Wet Crops	0.00944	0.00086	0.00259	0.00862	12

The calculated LOQ for florasulam supports the validated LOQ of 0.01 µg/g for all matrices. Since the lowest level of fortification for recovery samples was 0.01 µg/g, the method LOQ is considered to be 0.01 µg/g for all matrices. The calculated LOD of 0.0021-0.0026 µg/g for florasulam supports a LOD of 0.003 ug/g.

In actual residue samples, numerical results should be reported as less than the LOQ for residues that are equal to or above the LOD, but less than the validated LOQ, indicating that the results are being reported at a lower confidence level. For residues less than the LOD, results should be reported as ND.

Stability of Sample Extracts

Following initial cleanup and analysis of sample extracts of one matrix for each four crop grouping (apple for acidic, corn for dry, canola for oily and potato for aqueous), the resulting samples prepared for analysis were stored in a refrigerator at 3-8°C for 7 days. After this period, the samples were reanalyzed by single injection and compared to the original analytical recovery values in order to confirm their stability. All samples for all matrices are considered stable due the mean recoveries are still within the required range (70-120 %, RSD ≤ 20%). These recovery values are showed in Table 7.

Determination of Matrix Effect

During the validation of the method, the effects that each matrix type had upon the LC-MS/MS response of the analyte, and thus the reported recoveries, were determined at one fortification level (0.020 µg/mL final concentration—in the mid range of the calibration curve). This was done by carrying an unfortified control sample of each matrix all the way through the extraction and cleanup procedure as if for preparation for analysis by LC-MS/MS. The resulting samples were then fortified with a known amount of the analyte (essentially, matrix-matched standards were prepared). Each of the matrix-matched standards prepared were injected in triplicate, and the resulting peak areas were averaged. The same was done for the corresponding 0.020 µg/mL standard prepared in neat solvent. The matrix effects of the matrix-matched standards were calculated as follows:

(((Ave peak area of matrix-matched standard ÷ Ave peak area of neat solvent standard) -1) * 100)

Enhancement is expressed as a positive value while suppression is expressed as a negative value. The results obtained for florasulam are summarized in the table below.

Sample Description	Matrix Effect
Control Apple	-2%
Control Orange	-10%
Control Corn	-14%
Control Wheat	-10%
Control Soybean	-17%
Control Canola	-12%
Control Tomato	-17%
Control Potato	-11%

The data indicate that there are no significant matrix effect (< 20% enhancement or suppression) for florasulam analysis in agricultural commodities. The florasulam area values for all injections and the standards used for comparison are listed in Table 8.

Standardization of SPE Elution Profile

There is a possibility that variation in the Phenomenex Strata-X SPE plates may influence the elution profile of florasulam. If it is necessary to obtain an elution profile for each lot of SPE columns used to ensure optimum recovery and clean-up efficiency, the following procedure can be used:

1. To an 15-mL vial containing 0.985 mL of acetone/water/acetic acid (80:19:1) to extraction solution, add 15.0 µL of the 5.00-µg/mL stock solution and 2.0 mL of deionized water and vortex mix.

2. Place a Phenomenex Strata-X 30-mg SPE column on the vacuum manifold.

3. Condition the SPE column with 1 mL of methanol followed by 1 mL of deionized water. Dry the column under full vacuum for 10 seconds between solvents.

4. Transfer two 1.5 mL aliquot of the sample from Step 1 to the SPE column. Draw the sample through the column at a flow rate of approximately 1 mL/min, discarding the eluate. Dry the SPE column under full vacuum for 10 minutes.

5. Rinse the SPE column with two 1-mL aliquots of isooctane. Draw the solvent through the column at a flow rate of approximately 1 mL/min, discarding the eluate. Dry the SPE column under full vacuum for 10 seconds between aliquots.

6. Elute the florasulam from the SPE column with eight 250-µL aliquots of an l-chlorobutane/ethyl acetate (70:30) solution. Collect each fraction in the same well of a 2-mL deep well collection plate, stopping to quantitatively transfer each fraction to a separate

96-well plate between solvent additions using a disposable polyethylene pipet.

7. Evaporate the eluate to complete dryness using a 96-well evaporator set at 40 °C using an air flow of approximately 500 mL/minute.

8. Reconstitute the sample by adding 1.0 mL of acetonitrile/methanol/water/acetic acid (25 :25:49.9:0.1) solution to the vial. Vortex mix and sonicate the culture tubes for approximately for 30 seconds.

9. Transfer a portion of the sample to a 2-mL autosampler vial and seal the vial with a cap.

10. Analyze the samples by liquid chromatography with positive-ion electrospray ionization mass spectrometry as described in the instrumental conditions section.

11. Calculate the percent recovery as described in the Calculations section.

12. A typical elution profile is illustrated in Figure 13. If the elution profile differs from that shown, adjust the volume of the 1-chlorobutane/ethyl acetate (70:30) solution to be collected in Step 9.e of the Analysis Procedure section.

Supplemental Notes

1. The instrumental conditions for HPLC and mass spectrometer may be modified to obtain optimal chromatographic separation and sensitivity.

2. Aliquots and final volumes may be adjusted accordingly sample throughput.

3. Full house vacuum was equivalent to approximately -380 mm of Hg.

4. The type of regression model can be chosen to give the best fit (coefficient of determination) for the data.

CONCLUSIONS

Dow Agro Sciences Ind. Ltda. analytical method 110535 has been demonstrated to be suitable for its intended purpose. The method was validated over the concentration range of 0.01-1.0 µg/g with a validated limit of quantitation of 0.01 µg/g.

ARCHIVING

The protocol, raw data, and the original version of the final report are all filed in the Dow AgroSciences Ind. Ltda. archives at Rod. SP 147, km 71,5, Mogi Mirim, SP – Brasil – 13800-970.

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Table 1. Identity and Structure of Florasulam

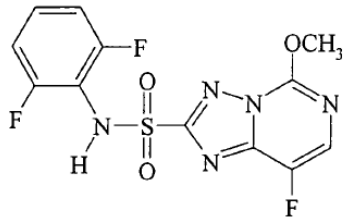
Common Name of Compound	Structure and CAS Name
Florasulam Molecular Formula: $C_{12}H_8F_3N_5O_3S$ Nominal Mass: 359 Formula Weight: 359.286 CAS Number: 145701-23-1 (10)	 N-[2-6-difluorophenyl]-8-fluoro-5-methoxyl[1,2,4]triazolo[1,5-c]pyrimidine-2-sulfonamide

Table 2. Recovery of Florasulam from Acidic Crops

Sample Number[a]	Matrix	Analysis Date[b]	Added (µg/g)	Found (µg/g)	Percent Recovery	Conf. Ratio Deviation (%)
001-0001A15	Apple Whole Fruit	29-Jul-11	0	ND[c]	NA[d]	NA
005-0001A17	Orange Whole Fruit	12-Aug-11	0	ND	NA	NA
001-0001A2	Apple Whole Fruit	29-Jul-11	0.003	0.0028	NA	2.7
005-0001A18	Orange Whole Fruit	12-Aug-11	0.003	0.0027	NA	8.8
001-0001A3	Apple Whole Fruit	29-Jul-11	0.01	0.0096	96	1.8
001-0001A4	Apple Whole Fruit	29-Jul-11	0.01	0.0096	96	7.1
001-0001A5	Apple Whole Fruit	29-Jul-11	0.01	0.0094	94	6.3
001-0001A6	Apple Whole Fruit	29-Jul-11	0.01	0.0088	88	5.9
001-0001A7	Apple Whole Fruit	29-Jul-11	0.01	0.0102	102	6.3
001-0001A8	Apple Whole Fruit	29-Jul-11	0.01	0.0102	102	3.4
005-0001A19	Orange Whole Fruit	12-Aug-11	0.01	0.0099	99	0.6
005-0001A20	Orange Whole Fruit	12-Aug-11	0.01	0.0094	94	1.6
005-0001A21	Orange Whole Fruit	12-Aug-11	0.01	0.0101	101	8.8
005-0001A22	Orange Whole Fruit	12-Aug-11	0.01	0.0099	99	3.7
005-0001A23	Orange Whole Fruit	12-Aug-11	0.01	0.0108	108	2.5
005-0001A24	Orange Whole Fruit	12-Aug-11	0.01	0.0115	115	2.7
001-0001A9	Apple Whole Fruit	29-Jul-11	1.00	0.8000	80	4.4
001-0001A11	Apple Whole Fruit	29-Jul-11	1.00	0.8320	83	3.7
001-0001A12	Apple Whole Fruit	29-Jul-11	1.00	0.8520	85	1.7
001-0001A13	Apple Whole Fruit	29-Jul-11	1.00	0.8560	86	1.4
001-0001A14	Apple Whole Fruit	29-Jul-11	1.00	0.8440	84	2.7
005-0001A25	Orange Whole Fruit	12-Aug-11	1.00	0.8720	87	3.3
005-0001A26	Orange Whole Fruit	12-Aug-11	1.00	0.7800	78	0.5
005-0001A27	Orange Whole Fruit	12-Aug-11	1.00	0.9640	96	1.8
005-0001A28	Orange Whole Fruit	12-Aug-11	1.00	0.8760	88	0.4
005-0001A29	Orange Whole Fruit	12-Aug-11	1.00	0.9920	99	0.3
005-0001A30	Orange Whole Fruit	12-Aug-11	1.00	0.9200	92	1.5

[a] Full Sample ID number is preceded by 110535.
[b] The 'Date of Analysis' indicates the date that the samples were extracted.
[c] ND = not detected. The residue was below the 0.003-µg/g limit of detection.
[d] NA = not applicable. The residue was below the 0.01-µg/g limit of quantitation.

Table 3. Recovery of Florasulam from Dry Crops

Sample Number[a]	Matrix	Analysis Date[b]	Added (µg/g)	Found (µg/g)	Percent Recovery	Conf. Ratio Deviation[e] (%)
002-0001A15	Corn Grain	29-Jul-11	0	ND[c]	NA[d]	NA
006-0001A17	Wheat Grain	12-Aug-11	0	ND	NA	NA
002-0001A20	Corn Grain	29-Jul-11	0.003	0.0034	NA	15.0
006-0001A18	Wheat Grain	12-Aug-11	0.003	0.0035	NA	4.1
002-0001A3	Corn Grain	29-Jul-11	0.01	0.0094	94	8.9
002-0001A4	Corn Grain	29-Jul-11	0.01	0.0107	107	18.0
002-0001A5	Corn Grain	29-Jul-11	0.01	0.0096	96	4.3
002-0001A6	Corn Grain	29-Jul-11	0.01	0.0099	99	9.6
002-0001A7	Corn Grain	29-Jul-11	0.01	0.0114	114	0.9
002-0001A8	Corn Grain	29-Jul-11	0.01	0.0102	102	0.6
006-0001A19	Wheat Grain	12-Aug-11	0.01	0.0090	90	0.1
006-0001A20	Wheat Grain	12-Aug-11	0.01	0.0108	108	3.4
006-0001A21	Wheat Grain	12-Aug-11	0.01	0.0104	104	5.5
006-0001A22	Wheat Grain	12-Aug-11	0.01	0.0101	101	17.2
006-0001A23	Wheat Grain	12-Aug-11	0.01	0.0093	93	1.5
006-0001A24	Wheat Grain	12-Aug-11	0.01	0.0104	104	0.2
002-0001A9	Corn Grain	29-Jul-11	1.00	0.9200	92	2.1
002-0001A10	Corn Grain	29-Jul-11	1.00	0.9040	90	2.8
002-0001A11	Corn Grain	29-Jul-11	1.00	0.8920	89	3.6
002-0001A12	Corn Grain	29-Jul-11	1.00	0.9040	90	3.5
002-0001A13	Corn Grain	29-Jul-11	1.00	0.8800	88	2.8
002-0001A14	Corn Grain	29-Jul-11	1.00	0.8920	89	1.8
006-0001A25	Wheat Grain	12-Aug-11	1.00	0.9520	95	3.2
006-0001A26	Wheat Grain	12-Aug-11	1.00	0.9560	96	1.5
006-0001A27	Wheat Grain	12-Aug-11	1.00	0.9760	98	3.1
006-0001A28	Wheat Grain	12-Aug-11	1.00	0.9520	95	3.7
006-0001A29	Wheat Grain	12-Aug-11	1.00	0.9240	92	4.5
006-0001A30	Wheat Grain	12-Aug-11	1.00	0.9600	96	2.3

[a] Full Sample ID number is preceded by 110535.
[b] The 'Date of Analysis' indicates the date that the samples were extracted.
[c] ND = not detected. The residue was below the 0.003-µg/g limit of detection.
[d] NA = not applicable. The residue was below the 0.01-µg/g limit of quantitation.
[e] The 'Conf. Ratio Deviation' is regarded with mean standard confirmation ratio from analysis set.

Table 4. Recovery of Florasulam from Oily Crops

Sample Number[a]	Matrix	Analysis Date[b]	Added (µg/g)	Found (µg/g)	Percent Recovery	Conf. Ratio Deviation[e] (%)
003-0001A1	Canola Seed	29-Jul-11	0	ND[c]	NA[d]	NA
007-0001A17	Soybean Grain	12-Aug-11	0	ND	NA	NA
003-0001A2	Canola Seed	29-Jul-11	0.003	0.0038	NA	14.0
007-0001A18	Soybean Grain	12-Aug-11	0.003	0.0034	NA	12.4
003-0001A3	Canola Seed	29-Jul-11	0.01	0.0106	106	3.2
003-0001A4	Canola Seed	29-Jul-11	0.01	0.0104	104	4.8
003-0001A5	Canola Seed	29-Jul-11	0.01	0.0108	108	11.7
003-0001A6	Canola Seed	29-Jul-11	0.01	0.0120	120	3.4
003-0001A7	Canola Seed	29-Jul-11	0.01	0.0102	102	12.1
003-0001A8	Canola Seed	29-Jul-11	0.01	0.0095	95	8.7
007-0001A19	Soybean Grain	12-Aug-11	0.01	0.0101	101	3.4
007-0001A20	Soybean Grain	12-Aug-11	0.01	0.0111	111	0.9
007-0001A21	Soybean Grain	12-Aug-11	0.01	0.0096	96	1.1
007-0001A22	Soybean Grain	12-Aug-11	0.01	0.0097	97	11.2
007-0001A23	Soybean Grain	12-Aug-11	0.01	0.0097	97	11.4
007-0001A24	Soybean Grain	12-Aug-11	0.01	0.0106	106	6.1
003-0001A9	Canola Seed	29-Jul-11	1.00	0.9280	93	4.6
003-0001A10	Canola Seed	29-Jul-11	1.00	0.9440	94	1.1
003-0001A11	Canola Seed	29-Jul-11	1.00	0.9480	95	3.0
003-0001A12	Canola Seed	29-Jul-11	1.00	0.9320	93	1.4
003-0001A13	Canola Seed	29-Jul-11	1.00	0.9360	94	2.0
003-0001A14	Canola Seed	29-Jul-11	1.00	0.9480	95	2.6
007-0001A25	Soybean Grain	12-Aug-11	1.00	0.9960	100	3.0
007-0001A26	Soybean Grain	12-Aug-11	1.00	0.9840	98	4.3
007-0001A27	Soybean Grain	12-Aug-11	1.00	0.9640	96	3.9
007-0001A28	Soybean Grain	12-Aug-11	1.00	0.9760	98	4.3
007-0001A29	Soybean Grain	12-Aug-11	1.00	0.9640	96	4.1
007-0001A30	Soybean Grain	12-Aug-11	1.00	0.8440	84	2.8

[a] Full Sample ID number is preceded by 110535.
[b] The 'Date of Analysis' indicates the date that the samples were extracted.
[c] ND = not detected. The residue was below the 0.003-µg/g limit of detection.
[d] NA = not applicable. The residue was below the 0.01-µg/g limit of quantitation
[e] The 'Conf. Ratio Deviation' is regarded with mean standard confirmation ratio from analysis set.

Table 5. Recovery of Florasulam from Wet Crops

Sample Number[a]	Matrix	Analysis Date[b]	Added (µg/g)	Found (µg/g)	Percent Recovery	Conf. Ratio Deviation[e] (%)
004-0001A1	Potato Tuber	29-Jul-11	0	ND[c]	NA[d]	NA
008-0001A17	Tomato Whole Fruit	12-Aug-11	0	ND	NA	NA
004-0001A2	Potato Tuber	29-Jul-11	0.003	0.0044	NA	12.0
008-0001A18	Tomato Whole Fruit	12-Aug-11	0.003	0.0027	NA	8.6
004-0001A3	Potato Tuber	29-Jul-11	0.01	0.0104	104	5.7
004-0001A4	Potato Tuber	29-Jul-11	0.01	0.0094	94	3.5
004-0001A5	Potato Tuber	29-Jul-11	0.01	0.0096	96	10.3
004-0001A6	Potato Tuber	29-Jul-11	0.01	0.0089	89	14.5
004-0001A7	Potato Tuber	29-Jul-11	0.01	0.0090	90	12.7
004-0001A8	Potato Tuber	29-Jul-11	0.01	0.0088	88	9.7
008-0001A19	Tomato Whole Fruit	12-Aug-11	0.01	0.0118	118	0.5
008-0001A20	Tomato Whole Fruit	12-Aug-11	0.01	0.0090	90	7.8
008-0001A21	Tomato Whole Fruit	12-Aug-11	0.01	0.0091	91	1.6
008-0001A22	Tomato Whole Fruit	12-Aug-11	0.01	0.0092	92	5.6
008-0001A23	Tomato Whole Fruit	12-Aug-11	0.01	0.0091	91	1.6
008-0001A24	Tomato Whole Fruit	12-Aug-11	0.01	0.0090	90	1.2
004-0001A9	Potato Tuber	29-Jul-11	1.00	0.8880	89	2.3
004-0001A10	Potato Tuber	29-Jul-11	1.00	0.8800	88	3.8
004-0001A11	Potato Tuber	29-Jul-11	1.00	0.8720	87	4.4
004-0001A12	Potato Tuber	29-Jul-11	1.00	0.8880	89	2.8
004-0001A13	Potato Tuber	29-Jul-11	1.00	0.9120	91	2.9
004-0001A14	Potato Tuber	29-Jul-11	1.00	0.8480	85	5.7
008-0001A25	Tomato Whole Fruit	12-Aug-11	1.00	0.9160	92	1.4
008-0001A26	Tomato Whole Fruit	12-Aug-11	1.00	0.8840	88	5.4
008-0001A27	Tomato Whole Fruit	12-Aug-11	1.00	0.7120	71	5.2
008-0001A28	Tomato Whole Fruit	12-Aug-11	1.00	0.8520	85	5.7
008-0001A29	Tomato Whole Fruit	12-Aug-11	1.00	0.7400	74	5.9
008-0001A30	Tomato Whole Fruit	12-Aug-11	1.00	0.8680	87	3.0

[a] Full Sample ID number is preceded by 110535.
[b] The 'Date of Analysis' indicates the date that the samples were extracted.
[c] ND = not detected. The residue was below the 0.003-µg/g limit of detection.
[d] NA = not applicable. The residue was below the 0.01-µg/g limit of quantitation.
[e] The 'Conf. Ratio Deviation' is regarded with mean standard confirmation ratio from analysis set.

Table 6. Summary of Recovery of Florasulam in Agricultural Commodities

Matrix	Fortification Level (µg/g)	Number of Samples	Recovery Range (%)	Mean (%)	SD (%)	RSD (%)
Acidic Crops	0.01	12	88 - 115	100	7.1	7.1
Acidic Crops	1.00	11	78 - 99	87	6.5	7.4
Acidic Crops	0.01 - 1.00	23	78 - 115	94	9.1	9.8
Dry Crops	0.01	12	90 - 114	101	7.0	6.9
Dry Crops	1.00	12	88 - 98	93	3.2	3.5
Dry Crops	0.01 - 1.00	24	88 - 114	97	6.7	7.0
Oily Crops	0.01	12	95 - 120	104	7.2	6.9
Oily Crops	1.00	12	84 - 100	95	3.9	4.1
Oily Crops	0.01 - 1.00	24	84 - 120	99	7.3	7.3
Wet Crops	0.01	12	88 - 118	94	8.6	9.1
Wet Crops	1.00	12	71 - 92	86	6.4	7.5
Wet Crops	0.01 - 1.00	24	71 - 118	90	8.7	9.7

Table 7. Summary of Florasulam Calculated Recoveries in Agricultural Commodities after 7 Days of Sample Extraction

Sample Number[a]	Matrix	Analysis Date[b]	Injection Date	Added (µg/g)	Found (µg/g)	Percent Recovery	Conf.Ratio Deviation[c] (%)
001-0001A3	Apple Whole Fruit	29-Jul-11	04-Aug-11	0.01	0.0079	79	0.9
001-0001A4	Apple Whole Fruit	29-Jul-11	04-Aug-11	0.01	0.0086	86	0.9
001-0001A5	Apple Whole Fruit	29-Jul-11	04-Aug-11	0.01	0.0078	78	7.5
001-0001A6	Apple Whole Fruit	29-Jul-11	04-Aug-11	0.01	0.0081	81	11.0
001-0001A7	Apple Whole Fruit	29-Jul-11	04-Aug-11	0.01	0.0081	81	8.0
001-0001A8	Apple Whole Fruit	29-Jul-11	04-Aug-11	0.01	0.0082	82	11.0
001-0001A9	Apple Whole Fruit	29-Jul-11	04-Aug-11	1.00	0.7967	80	2.9
001-0001A11	Apple Whole Fruit	29-Jul-11	04-Aug-11	1.00	0.8286	83	4.3
001-0001A12	Apple Whole Fruit	29-Jul-11	04-Aug-11	1.00	0.8545	85	2.6
001-0001A13	Apple Whole Fruit	29-Jul-11	04-Aug-11	1.00	0.8168	82	2.5
001-0001A14	Apple Whole Fruit	29-Jul-11	04-Aug-11	1.00	0.8285	83	4.9
002-0001A3	Corn Grain	29-Jul-11	04-Aug-11	0.01	0.0084	84	2.2
002-0001A4	Corn Grain	29-Jul-11	04-Aug-11	0.01	0.0093	93	2.4
002-0001A5	Corn Grain	29-Jul-11	04-Aug-11	0.01	0.0088	88	6.8
002-0001A6	Corn Grain	29-Jul-11	04-Aug-11	0.01	0.0081	81	13.1
002-0001A7	Corn Grain	29-Jul-11	04-Aug-11	0.01	0.0086	86	1.8
002-0001A8	Corn Grain	29-Jul-11	04-Aug-11	0.01	0.0084	84	7.2
002-0001A9	Corn Grain	29-Jul-11	04-Aug-11	1.00	0.8970	90	6.7
002-0001A10	Corn Grain	29-Jul-11	04-Aug-11	1.00	0.8674	87	2.6
002-0001A11	Corn Grain	29-Jul-11	04-Aug-11	1.00	0.8748	87	2.2
002-0001A12	Corn Grain	29-Jul-11	04-Aug-11	1.00	0.9014	90	2.1
002-0001A13	Corn Grain	29-Jul-11	04-Aug-11	1.00	0.8833	88	0.5
002-0001A14	Corn Grain	29-Jul-11	04-Aug-11	1.00	0.8785	88	4.3
003-0001A3	Canola Seed	29-Jul-11	04-Aug-11	0.01	0.0095	95	4.6
003-0001A4	Canola Seed	29-Jul-11	04-Aug-11	0.01	0.0095	95	2.5
003-0001A5	Canola Seed	29-Jul-11	04-Aug-11	0.01	0.0089	89	4.5
003-0001A6	Canola Seed	29-Jul-11	04-Aug-11	0.01	0.0091	91	4.6
003-0001A7	Canola Seed	29-Jul-11	04-Aug-11	0.01	0.0092	92	9.5
003-0001A8	Canola Seed	29-Jul-11	04-Aug-11	0.01	0.0093	93	6.1
003-0001A9	Canola Seed	29-Jul-11	04-Aug-11	1.00	0.8870	89	4.1
003-0001A10	Canola Seed	29-Jul-11	04-Aug-11	1.00	0.9734	97	1.2
003-0001A11	Canola Seed	29-Jul-11	04-Aug-11	1.00	0.9174	92	2.6
003-0001A12	Canola Seed	29-Jul-11	04-Aug-11	1.00	0.8945	89	2.2
003-0001A13	Canola Seed	29-Jul-11	04-Aug-11	1.00	0.8432	84	1.2
003-0001A14	Canola Seed	29-Jul-11	04-Aug-11	1.00	0.8637	86	2.2
004-0001A3	Potato Tuber	29-Jul-11	04-Aug-11	0.01	0.0083	83	11.2
004-0001A4	Potato Tuber	29-Jul-11	04-Aug-11	0.01	0.0084	84	2.7
004-0001A5	Potato Tuber	29-Jul-11	04-Aug-11	0.01	0.0090	90	18.6
004-0001A6	Potato Tuber	29-Jul-11	04-Aug-11	0.01	0.0089	89	2.6

Table 7 (Cont.). Summary of Florasulam Calculated Recoveries in Agricultural Commodities after 7 Days of Sample Extraction

Sample Number[a]	Matrix	Analysis Date[b]	Injection Date	Added (µg/g)	Found (µg/g)[c]	Percent Recovery	Conf.Ratio Deviation[c] (%)
004-0001A7	Potato Tuber	29-Jul-11	04-Aug-11	0.01	0.0087	87	12.0
004-0001A8	Potato Tuber	29-Jul-11	04-Aug-11	0.01	0.0091	91	12.3
004-0001A9	Potato Tuber	29-Jul-11	04-Aug-11	1.00	0.8117	81	2.0
004-0001A10	Potato Tuber	29-Jul-11	04-Aug-11	1.00	0.7873	79	4.7
004-0001A11	Potato Tuber	29-Jul-11	04-Aug-11	1.00	0.8537	85	0.7
004-0001A12	Potato Tuber	29-Jul-11	04-Aug-11	1.00	0.8380	84	4.1
004-0001A13	Potato Tuber	29-Jul-11	04-Aug-11	1.00	0.8328	83	0.6
004-0001A14	Potato Tuber	29-Jul-11	04-Aug-11	1.00	0.8167	82	1.1

[a] Full Sample ID number is preceded by 110535.
[b] The 'Date of Analysis' indicates the date that the samples were extracted.
[c] The 'Conf. Ratio Deviation' is regarded with mean standard confirmation ratio from analysis set.

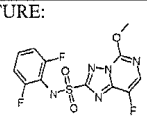
Table 8. Summary of Florasulam Peak Area Values in Matrix Effect Evaluation

Sample Description[a]	Date of Injection	Florasulam (360/129) Peak Area	Average Peak Area	Matrix Effect
002-0001A16(Control Corn) + 20 ng/mL #1	10-Aug-11	749820	726420	-14%
002-0001A16(Control Corn) + 20 ng/mL #2	10-Aug-11	723610		
002-0001A16(Control Corn) + 20 ng/mL #3	10-Aug-11	705830		
003-0001A16(Control Canola) + 20 ng/mL #1	10-Aug-11	770640	743650	-12%
003-0001A16(Control Canola) + 20 ng/mL #2	10-Aug-11	727640		
003-0001A16(Control Canola) + 20 ng/mL #3	10-Aug-11	732670		
004-0001A16(Control Potato) + 20 ng/mL #1	10-Aug-11	778500	748227	-11%
004-0001A16(Control Potato) + 20 ng/mL #2	10-Aug-11	747590		
004-0001A16(Control Potato) + 20 ng/mL #3	10-Aug-11	718590		
005-0001A16(Control Orange) + 20 ng/mL #1	10-Aug-11	783800	758213	-10%
005-0001A16(Control Orange) + 20 ng/mL #2	10-Aug-11	756420		
005-0001A16(Control Orange) + 20 ng/mL #3	10-Aug-11	734420		
006-0001A16(Control Wheat) + 20 ng/mL #1	10-Aug-11	771460	755403	-10%
006-0001A16(Control Wheat) + 20 ng/mL #2	10-Aug-11	758920		
006-0001A16(Control Wheat) + 20 ng/mL #3	10-Aug-11	735830		
007-0001A16(Control Soybean) + 20 ng/mL #1	10-Aug-11	722760	703000	-17%
007-0001A16(Control Soybean) + 20 ng/mL #2	10-Aug-11	702360		
007-0001A16(Control Soybean) + 20 ng/mL #3	10-Aug-11	683880		
008-0001A16(Control Tomato) + 20 ng/mL #1	10-Aug-11	715310	702410	-17%
008-0001A16(Control Tomato) + 20 ng/mL #2	10-Aug-11	701100		
008-0001A16(Control Tomato) + 20 ng/mL #3	10-Aug-11	690820		
Neat + 20 ng/mL #1	10-Aug-11	858110	843080	NA
Neat + 20 ng/mL #2	10-Aug-11	845450		
Neat + 20 ng/mL #3	10-Aug-11	825680		
001-0001A16(Control Apple) + 20 ng/mL #1	12-Aug-11	972110	977140	-3%
001-0001A16(Control Apple) + 20 ng/mL #2	12-Aug-11	962110		
001-0001A16(Control Apple) + 20 ng/mL #3	12-Aug-11	997200		
Neat + 20 ng/mL #1	12-Aug-11	1002600	1009000	NA
Neat + 20 ng/mL #2	12-Aug-11	1004700		
Neat + 20 ng/mL #3	12-Aug-11	1019700		

REPORT **FA&PC NUMBER** **08-163723**

CERTIFICATE OF ANALYSIS FOR TEST/REFERENCE/CONTROL SUBSTANCES

TITLE OBJECTIVE: Determination of purity and/or identity of the following test/reference/control substance for use in a study.

TEST/REFERENCE/CONTROL SUBSTANCE:			
TEST SUBSTANCE NO.:	TSN100381		
LOT NO.:	DECO-293-021		
DESCRIPTION:	Florasulam, Analytical Standard		
STRUCTURE: 	CHEMICAL NAME:	N-[2,6-difluorophenyl]-8-fluoro-5-methoxy[1,2,4]triazolo[1,5-c]pyrimidine-2-sulfonamide	
	MOLECULAR FORMULA:	$C_{12}H_8F_3N_5O_3S$	
	MOLECULAR WEIGHT:	359.2885	
REFERENCE SUBSTANCE USED:	N.A.	PURITY:	N.A.

INITIATION DATE: January 31, 2008

METHODS USED:

PURITY:	IDENTIFICATION:
HPLC	IR

RESULTS and CONCLUSIONS:

X **RECERTIFICATION: UNCHANGED**
Current value of 99.7% is within experimental variation of previously established purity of 99.7%. The purity is unchanged and remains 99.7%.

X **IDENTITY:**
Spectroscopic identity was consistent with the proposed structure.

N.A. **OTHER:**
N.A.

RE-CERTIFICATION DATE: April 25, 2012

CALCULATIONS:

Area Normalized: X Internal Standard: N.A. External Standard: N.A.
Other (explain): N.A.

STUDY DIRECTOR SIGNATURE: **STUDY COMPLETION DATE:**

David Heim 2 MAY 2008

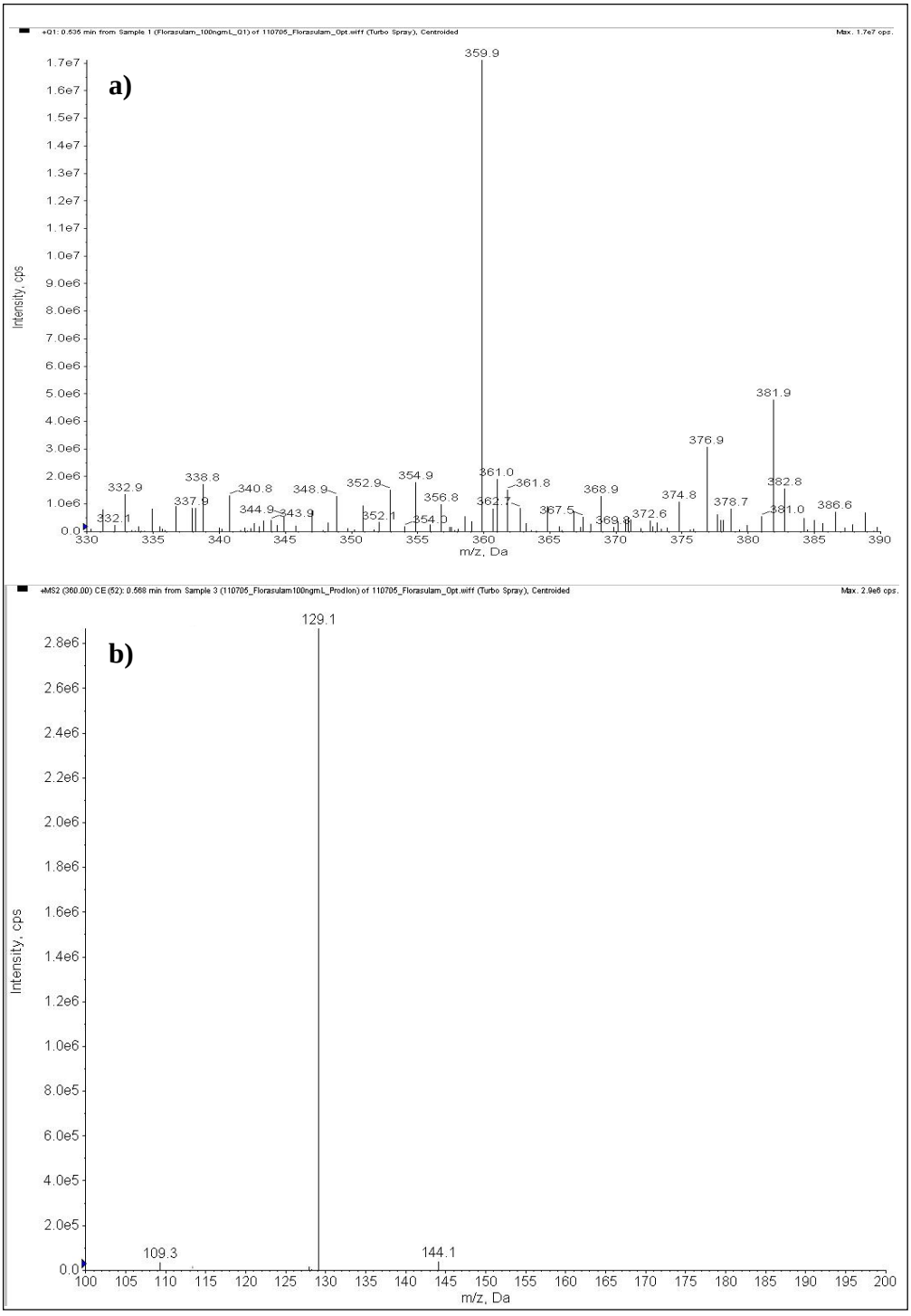
PEER REVIEWER SIGNATURE: **DATE:**

Melinda Stock 5/2/08

TESTING FACILITY ADDRESS:
Dow AgroSciences LLC
Crop Protection R&D Analytical/Product Chemistry Center of Expertise
9330 Zionsville Road
Indianapolis, Indiana 46268

All raw data and retainer samples associated with this study will be archived in the testing facility archive. Only descriptive statistics were used unless otherwise noted in the results. This study was conducted in accordance with the Good Laboratory Practice Standard, 40 CFR Part 160.135 (b).

Figure 1. Certificate of Analysis of Florasulam TSN100381



a) Q1 scan using positive-ion electrospray ionization showing $(M-H)^+$ at *m/z* 360
b) Product-ion mass spectrum of triclopyr showing $(M-H)^+$ fragment ion at *m/z* 129 and *m/z* 109

Figure 2. Positive-Ion Electrospray Mass Spectra for Florasulam

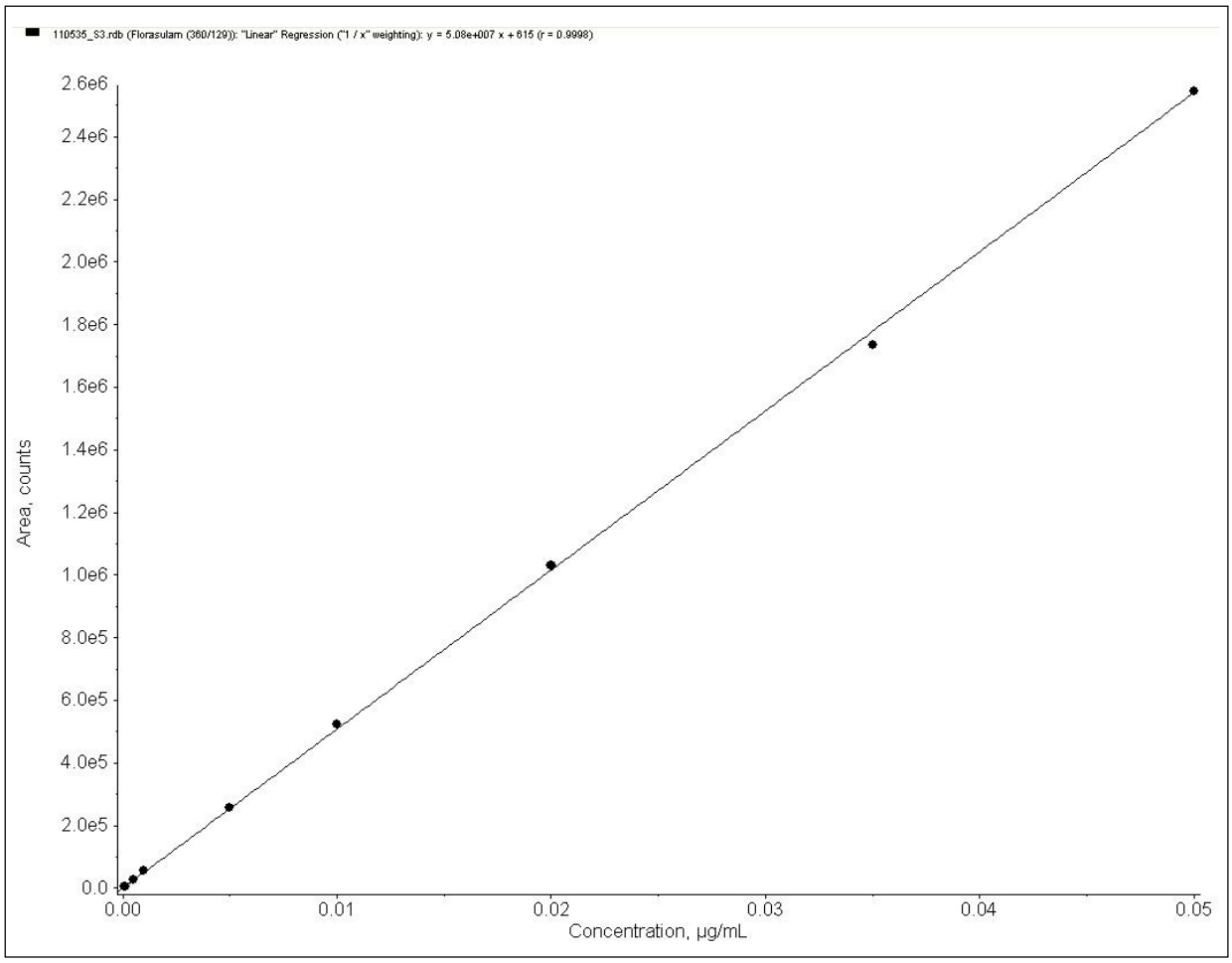
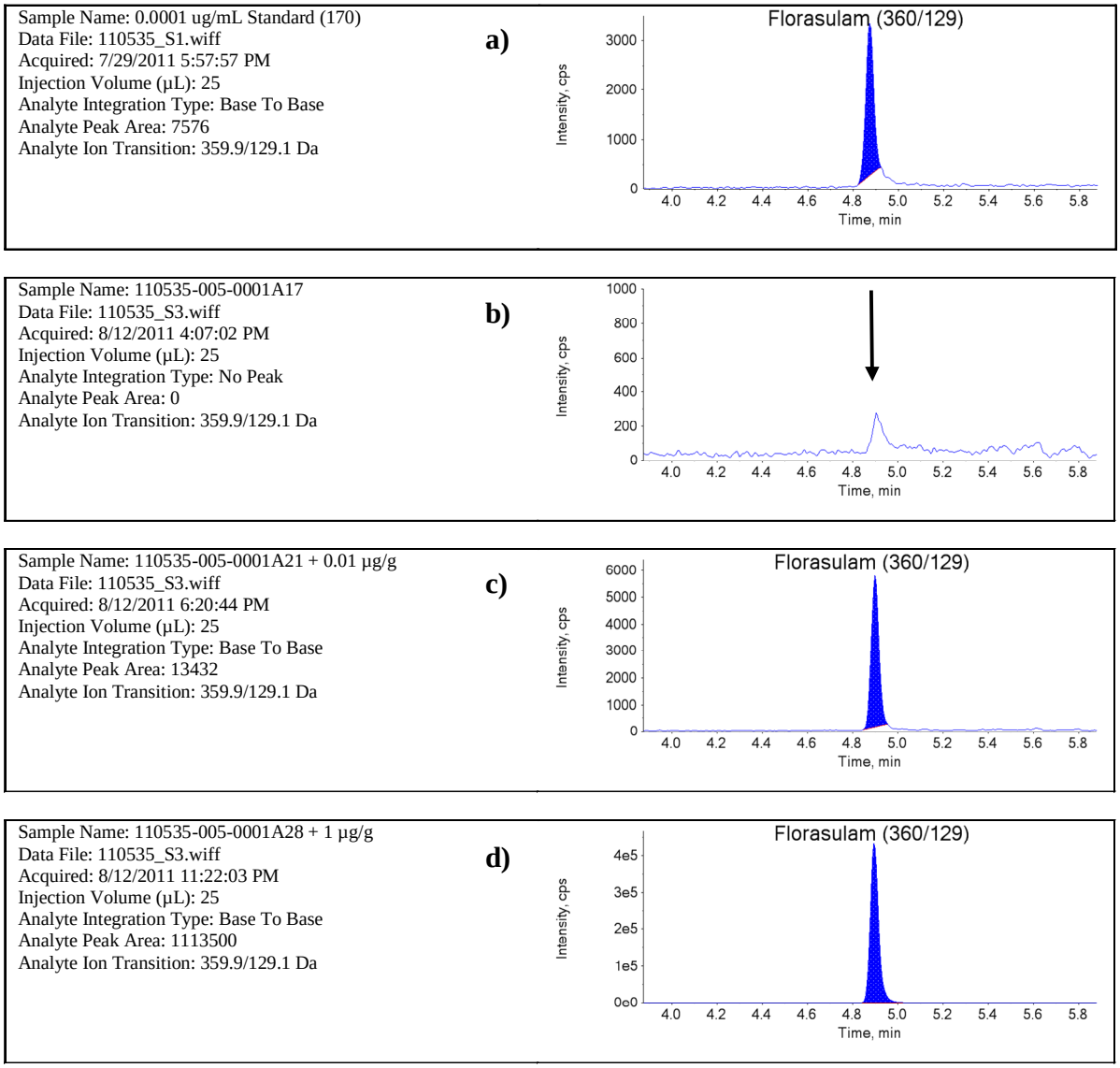
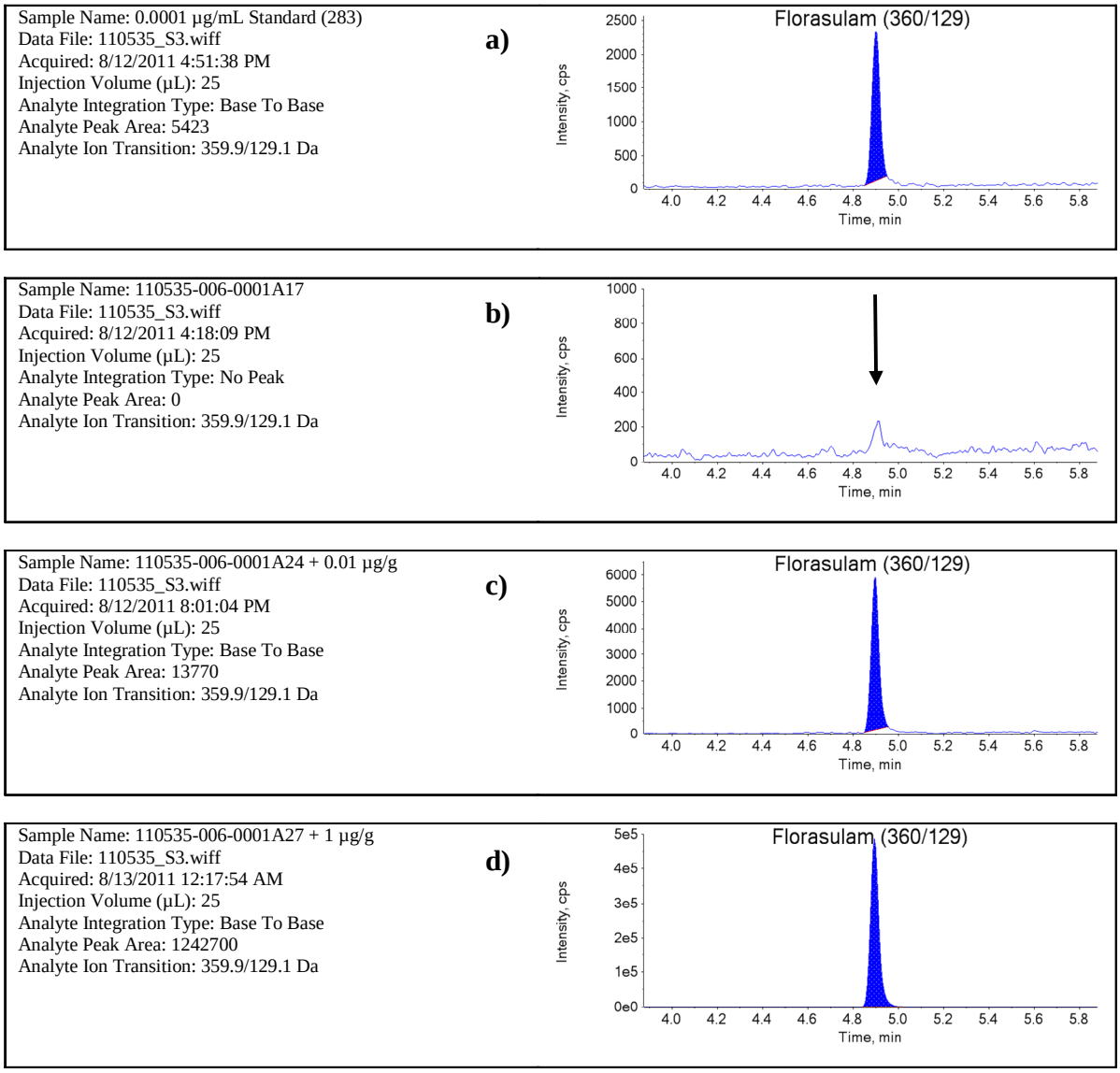


Figure 3. Typical Calibration Curve for the Determination of Florasulam in Agricultural Commodities



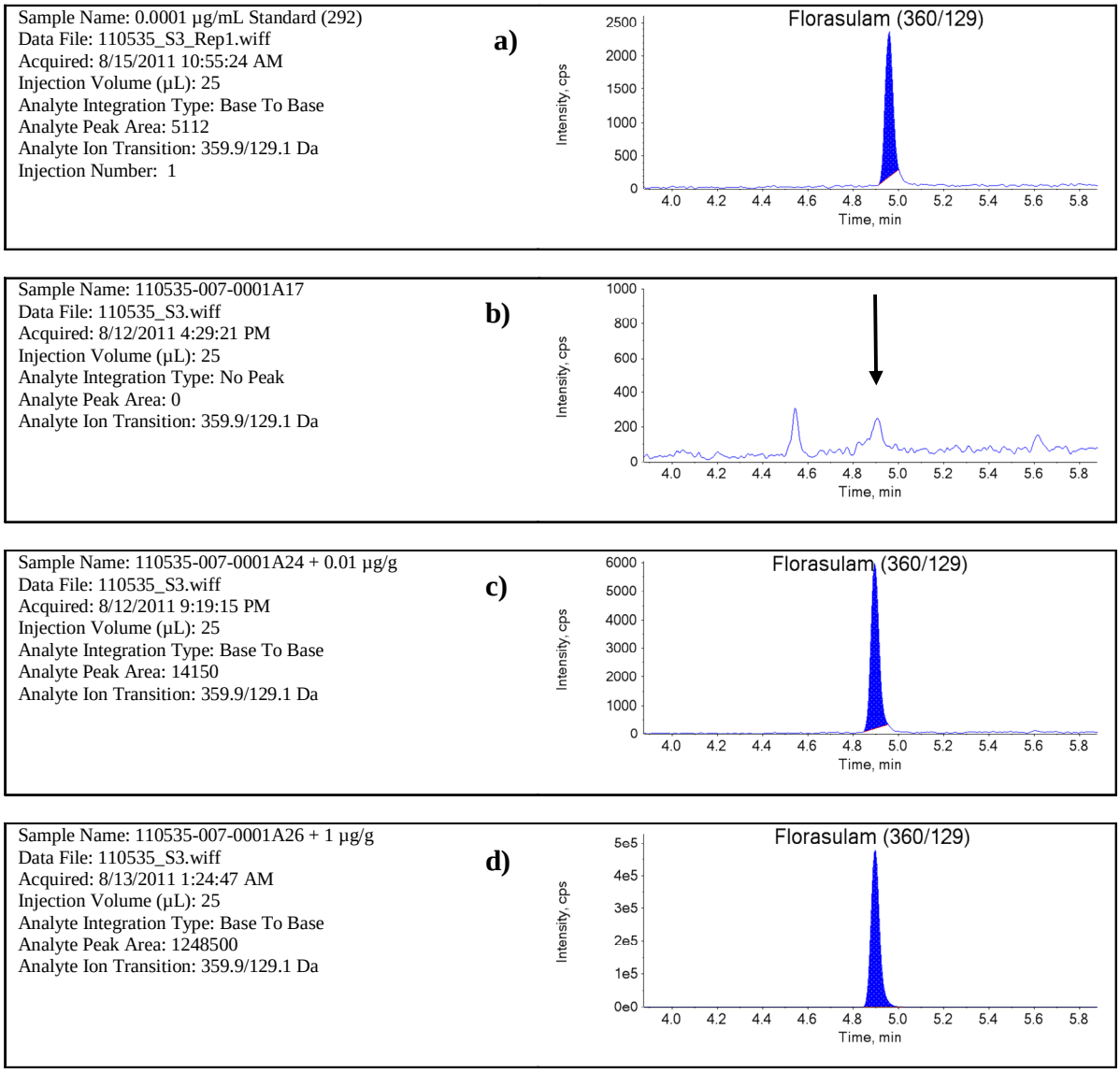
a) Florasulam 0.0001 µg/mL calibration standard, set 110535_S1
b) Orange Whole Fruit control 005-0001A17, set 110535_S3
c) Orange Whole Fruit control 005-0001A21 fortified with 0.01 µg/g of florasulam, set 110535_S3
d) Orange Whole Fruit control 005-0001A28 fortified with 1.00 µg/g of florasulam, set 110535_S3

Figure 4. Typical Chromatograms for the Quantitation of Florasulam in Agricultural Commodities (Acidic Crops)



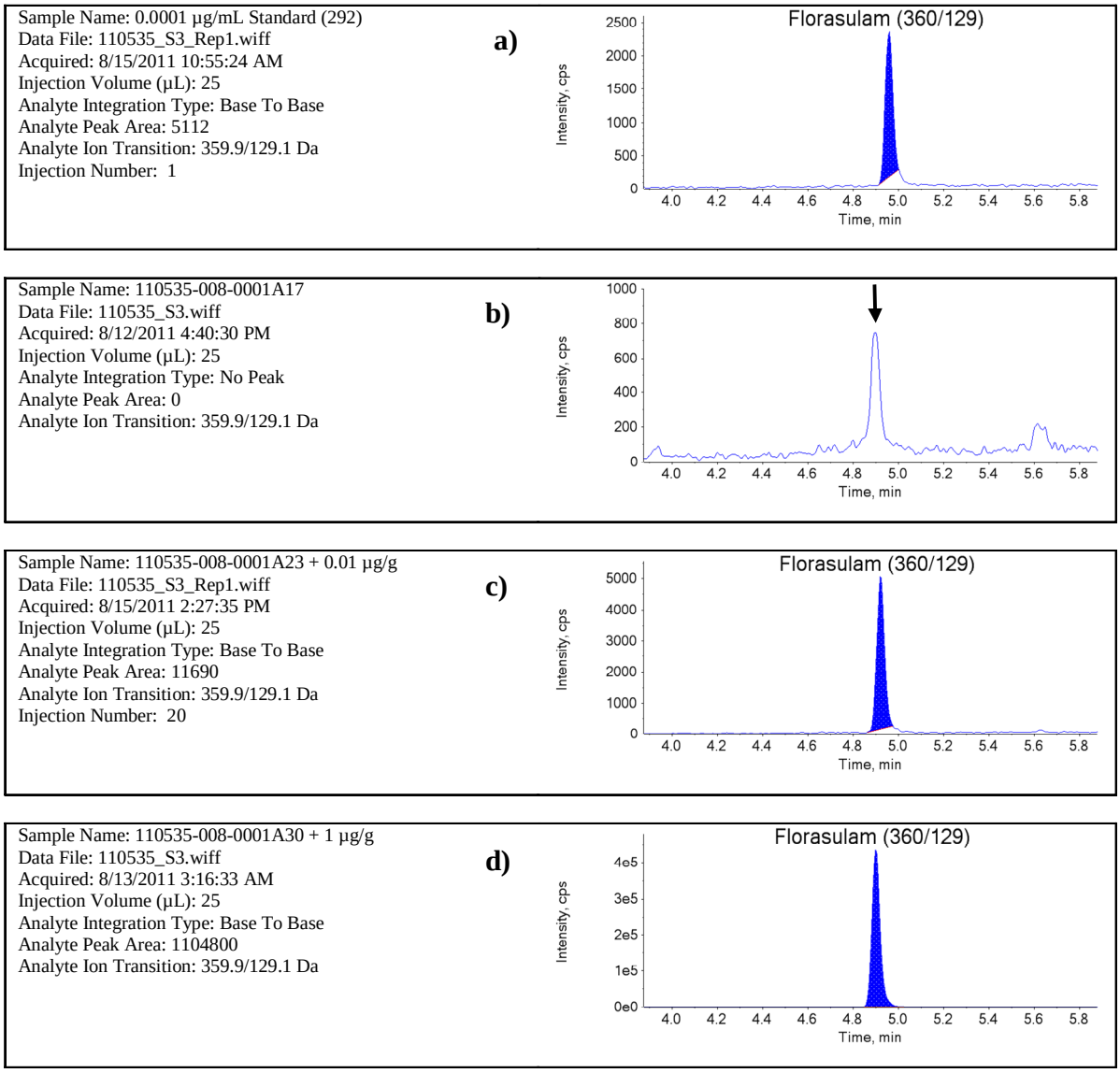
a) Florasulam 0.0001 µg/mL calibration standard, set 110535_S3
b) Wheat grain control 006-0001A17, set 110535_S3
c) Wheat grain control 006-0001A24 fortified with 0.01 µg/g of florasulam, set 110535_S3
d) Wheat grain control 006-0001A27 fortified with 1.00 µg/g of florasulam, set 110535_S3

Figure 5. Typical Chromatograms for the Quantitation of Florasulam in Agricultural Commodities (Dry Crops)



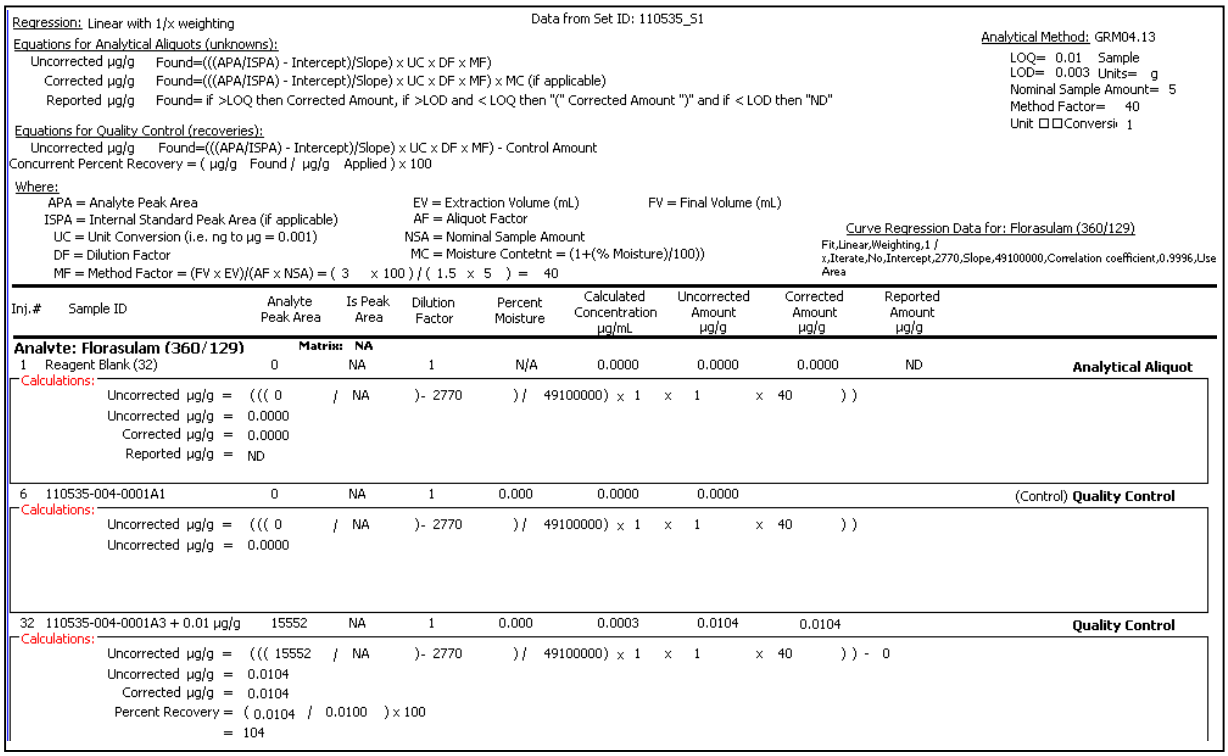
a) Florasulam 0.0001 µg/mL calibration standard, set 110535_S3_Rep1
b) Soybean grain control 007-0001A17, set 110535_S3
c) Soybean grain control 007-0001A24 fortified with 0.01 µg/g of florasulam, set 110535_S3
d) Soybean grain control 007-0001A26 fortified with 1.00 µg/g of florasulam, set 110535_S3

Figure 6. Typical Chromatograms for the Quantitation of Florasulam in Agricultural Commodities (Oily Crops)



a) Florasulam 0.0001 µg/mL calibration standard, set 110535_S3_Rep1
b) Tomato whole fruit control 008-0001A17, set 110535_S3
c) Tomato whole fruit control 008-0001A23 fortified with 0.01 µg/g of florasulam, set 110535_S3_Rep1
d) Tomato whole fruit control 008-0001A30 fortified with 1.00 µg/g of florasulam, set 110535_S3

Figure 7. Typical Chromatograms for the Quantitation of Florasulam in Agricultural Commodities (Wet Crops)



Regression: Linear with 1/x weighting

Data from Set ID: 110535_S1

Equations for Analytical Aliquots (unknowns):

Uncorrected µg/g Found=(((APA/ISPA) - Intercept)/Slope) x UC x DF x MF)

Corrected µg/g Found=(((APA/ISPA) - Intercept)/Slope) x UC x DF x MF) x MC (if applicable)

Reported µg/g Found= if >LOQ then Corrected Amount, if >LOD and < LOQ then "(" Corrected Amount ")" and if < LOD then "ND"

Analytical Method: GRM04.13
LOQ= 0.01 Sample
LOD= 0.003 Units= g
Nominal Sample Amount= 5
Method Factor= 40
Unit ☐☐Conversi 1

Equations for Quality Control (recoveries):

Uncorrected µg/g Found=(((APA/ISPA) - Intercept)/Slope) x UC x DF x MF) - Control Amount

Concurrent Percent Recovery = (µg/g Found / µg/g Applied) x 100

Where:

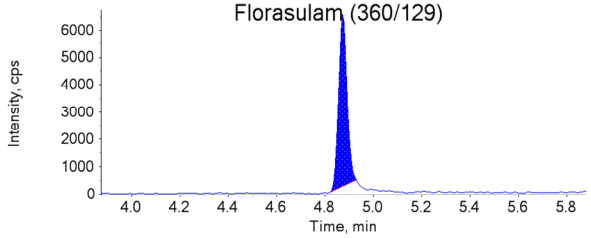
APA = Analyte Peak Area
ISPA = Internal Standard Peak Area (if applicable)
UC = Unit Conversion (i.e. ng to µg = 0.001)
DF = Dilution Factor
MF = Method Factor = (FV x EV)/(AF x NSA) = (3 x 100) / (1.5 x 5) = 40
EV = Extraction Volume (mL)
AF = Aliquot Factor
NSA = Nominal Sample Amount
MC = Moisture Contetnt = (1+(% Moisture)/100))
FV = Final Volume (mL)

Curve Regression Data for: Florasulam (360/129)
Fit,Linear,Weighting,1 / x,Iterate,No,Intercept,2770,Slope,49100000,Correlation coefficient,0.9996,Use Area

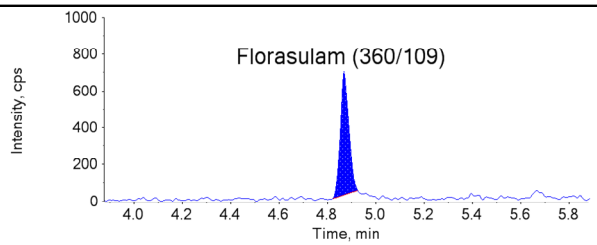
Inj.#	Sample ID	Analyte Peak Area	Is Peak Area	Dilution Factor	Percent Moisture	Calculated Concentration µg/mL	Uncorrected Amount µg/g	Corrected Amount µg/g	Reported Amount µg/g	
Analyte: Florasulam (360/129)			**Matrix: NA**							
1	Reagent Blank (32)	0	NA	1	N/A	0.0000	0.0000	0.0000	ND	**Analytical Aliquot**
Calculations:	Uncorrected µg/g = (((0 / NA)- 2770) / 49100000) x 1 x 1 x 40)) Uncorrected µg/g = 0.0000 Corrected µg/g = 0.0000 Reported µg/g = ND									
6	110535-004-0001A1	0	NA	1	0.000	0.0000	0.0000			(Control) **Quality Control**
Calculations:	Uncorrected µg/g = (((0 / NA)- 2770) / 49100000) x 1 x 1 x 40)) Uncorrected µg/g = 0.0000									
32	110535-004-0001A3 + 0.01 µg/g	15552	NA	1	0.000	0.0003	0.0104	0.0104		**Quality Control**
Calculations:	Uncorrected µg/g = (((15552 / NA)- 2770) / 49100000) x 1 x 1 x 40)) - 0 Uncorrected µg/g = 0.0104 Corrected µg/g = 0.0104 Percent Recovery = (0.0104 / 0.0100) x 100 = 104									

Figure 8. Example Calculations for the Quantitative Determination of Florasulam in Potato Tuber from Set 110535_S1

Sample Name: 110535-001-0001A7 + 0.01 µg/g
Data File: 110535_S1.wiff
Acquired: 7/29/2011 8:11:56 PM
Injection Volume (µL): 25
Analyte Integration Type: Base To Base
Analyte Peak Area: 15326
Analyte Ion Transition: 359.9/129.1 Da

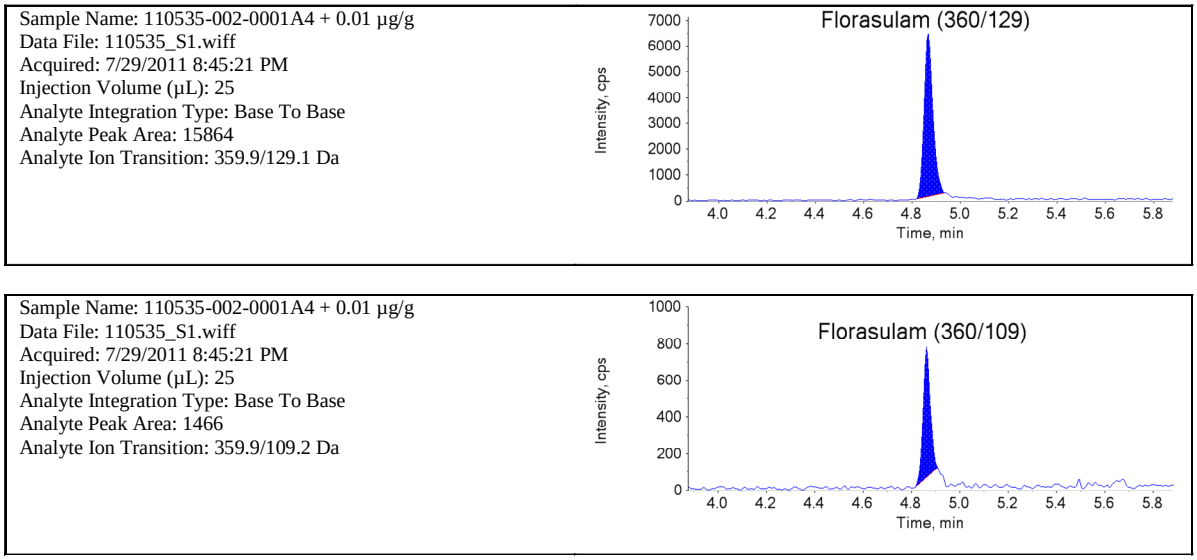


Sample Name: 110535-001-0001A7 + 0.01 µg/g
Data File: 110535_S1.wiff
Acquired: 7/29/2011 8:11:56 PM
Injection Volume (µL): 25
Analyte Integration Type: Base To Base
Analyte Peak Area: 1618
Analyte Ion Transition: 359.9/109.2 Da



Control Sample apple whole fruit 110535-001-0001A7 fortified with 0.01 µg/g of florasulam, set 110535_S1	
Confirmation (*m/z* 109.2/*m/z* 129.1)	
Peak Area Ratio	0.1055
Standard Peak Area Ratio Mean	0.1126
Percent Difference	6.3%

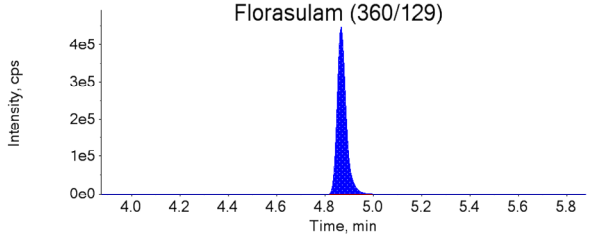
Figure 9. Typical Chromatograms for Florasulam Confirmation in Acidic Crops



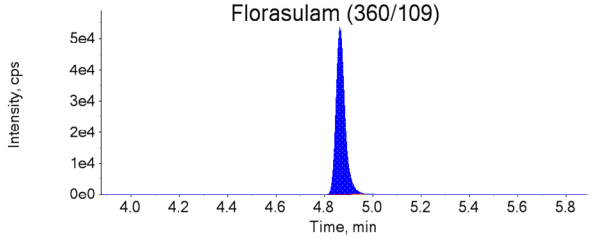
Control sample corn grain 110535-002-0001A4 fortified with 0.01 µg/g of florasulam, set 110535_S1	
Confirmation (*m/z* 109.2/*m/z* 129.1)	
Peak Area Ratio	0.0924
Standard Peak Area Ratio Mean	0.1126
Percent Difference	18.0%

Figure 10. Typical Chromatograms for Florasulam Confirmation in Dry Crops

Sample Name: 110535-003-0001A9 + 1 µg/g
Data File: 110535_S1.wiff
Acquired: 7/30/2011 3:26:58 AM
Injection Volume (µL): 25
Analyte Integration Type: Base To Base
Analyte Peak Area: 1143300
Analyte Ion Transition: 359.9/129.1 Da



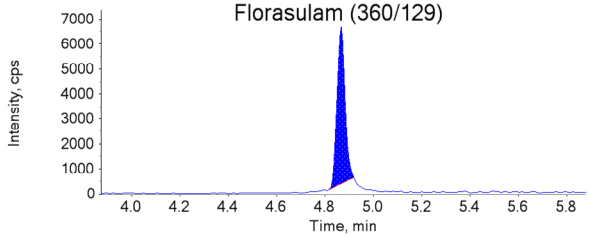
Sample Name: 110535-003-0001A9 + 1 µg/g
Data File: 110535_S1.wiff
Acquired: 7/30/2011 3:26:58 AM
Injection Volume (µL): 25
Analyte Integration Type: Base To Base
Analyte Peak Area: 134650
Analyte Ion Transition: 359.9/109.2 Da



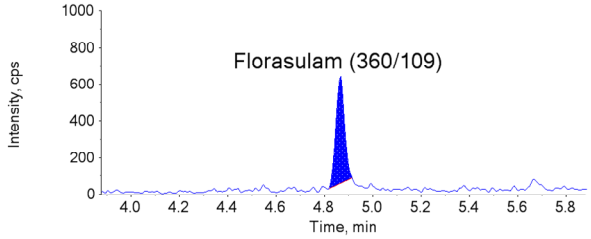
Control sample canola seed 110535-003-0001A9 fortified with 1.00 µg/g of florasulam, set 110535_S1	
Confirmation (*m/z* 109.2/*m/z* 129.1)	
Peak Area Ratio	0.1178
Standard Peak Area Ratio Mean	0.1126
Percent Difference	4.6%

Figure 11. Typical Chromatograms for Florasulam Confirmation in Oily Crops

Sample Name: 110535-004-0001A5 + 0.01 µg/g
Data File: 110535_S1.wiff
Acquired: 7/29/2011 11:43:40 PM
Injection Volume (µL): 25
Analyte Integration Type: Base To Base
Analyte Peak Area: 14516
Analyte Ion Transition: 359.9/129.1 Da



Sample Name: 110535-004-0001A5 + 0.01 µg/g
Data File: 110535_S1.wiff
Acquired: 7/29/2011 11:43:40 PM
Injection Volume (µL): 25
Analyte Integration Type: Base To Base
Analyte Peak Area: 1466
Analyte Ion Transition: 359.9/109.2 Da



Control sample potato tuber 110535-004-0001A5 fortified with 0.01 µg/g of Florasulam, set 110535_S1	
Confirmation (*m/z* 109.2/*m/z* 129.1)	
Peak Area Ratio	0.1028
Standard Peak Area Ratio Mean	0.1126
Percent Difference	5.7%

Figure 12. Typical Chromatograms for Florasulam Confirmation in Aqueous Crops

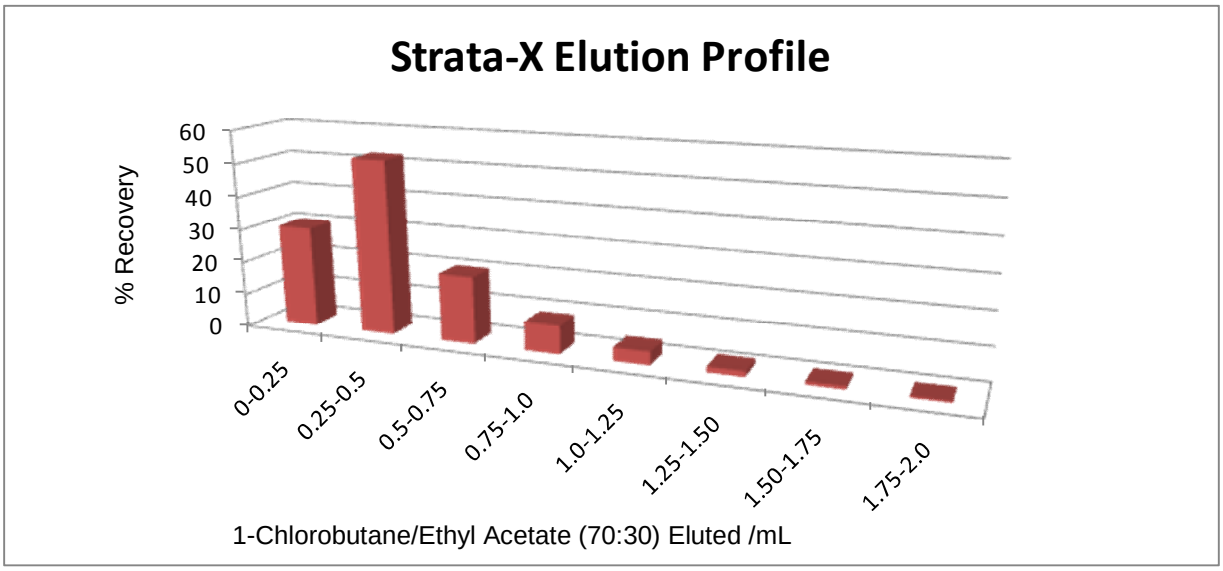


Figure 13. Typical Strata-X SPE Profile for the Determination of Florasulam in Agricultural Commodities