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Chlorothalonil

**Independent Laboratory Validation of Analytical Method
(GRM005.17A) for the Determination of R182281 in OVS Silica
Gel Air Sampling Tubes by LC-MS/MS**

ILV Final Report

DATA REQUIREMENT(S):

EPA 850.6100

[Redacted content]

2.0 INTRODUCTION

Described in this report is the independent laboratory validation (ILV) of Syngenta analytical method GRM005.17A, entitled "Independent Laboratory Validation of Analytical Method (GRM005.17A) for the Determination of R182281 in OVS Silica Gel Air Sampling Tubes by LC-MS/MS."

This study was designed to satisfy harmonized guideline requirements described in EPA OCSPP 850.6100 (Data Reporting for Environmental Chemistry Methods). This study was conducted in compliance with EPA FIFRA Good Laboratory Practice Standards, 40 CFR Part 160 (3).

BRIEF SUMMARY OF METHOD

R182281 trapped in OVS Silica Gel Air Sampling Tubes was extracted by acetone and then the diluted extraction solution was analyzed by high performance liquid chromatography with triple quadrupole mass spectrometric detection (LC-MS/MS). The LOQ of the method is 0.01 µg/air sample tube.

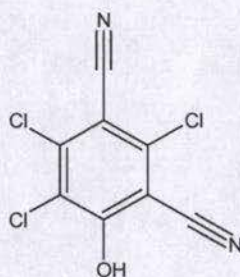
For analysis of R182281, 50 µL of 0.2 µg/mL (LOQ) and 50 µL of 2 µg/mL (10XLOQ) were spiked into the OVS tube samples and left to stand for 5 minutes. Then the contents of OVS tube were removed into extraction bottles and 10 mL extraction solution was added to them. These bottles were sonicated for 10 minutes and 0.5 mL of each extract was transferred to 1.5 mL centrifuge tube containing 0.1 mL of ultra pure water. The extracts were evaporated to near dryness and reconstituted to 1 mL with ultra pure water: acetonitrile (50:50), vortexed well and transferred to autosampler vials for analysis by LC-MS/MS.

3.0 MATERIALS AND METHODS

3.1 Test/Reference Substance

The test reference substance was obtained from Syngenta Crop Protection, LLC. The following test reference substance was used:

Compound Structure:



Syngenta Code:	R182281
CAS Name:	2,4,5-trichloro-6-hydroxyisophthalonitrile
CAS Number:	28343-61-5
Molecular Weight:	247.46
Storage Conditions:	refrigerator
Purity:	99.1%
Expiration Date:	02/28/2017

3.2 Test System

The test system evaluated for this ILV was OVS silica gel air sampling tubes. This matrix was chosen because it is a representative of the matrices the method was designed for. The control samples used in this study were provided by Syngenta Crop Protection, LLC.

3.3 Equipment and Reagents

The equipment and reagents used for this study were selected and prepared as outlined in the method. Identical or equivalent equipment and materials may have been used, as permitted by the method.

3.3.1 Equipment

Equipment	Description	Supplier
General lab glassware	General lab glassware	Thermoscientific
General lab plastic ware	General lab plastic ware	Thermoscientific
Autosampler vials	Snap cap, 2 mL size	Thermoscientific
LC-MS/MS system Including HPLC and autosampler units	Shimadzu; AB Sciex 4000 triple quadrupole mass spectrometer with Analyst TM software version 1.4.2	Shimadzu Applied Biosystems
HPLC column	ACE 3 C18, 3 x 50 mm, 3 μ m	ACE
Balance	Mettler Toledo	Mettler Toledo

3.3.2 Reagents

Reagent	Description	Supplier
Water	HPLC grade	Pharmco-Aaper
Formic acid	ACS grade	Sigma Aldrich
Acetonitrile	HPLC grade	Pharmco-Aaper
Methanol	HPLC grade	J.T. Baker
Acetone	HPLC grade	Pharmco-Aaper
R182281	GLP certified	Syngenta Crop Protection, LLC P.O Box 18300 Greensboro, NC 27419-8300

3.3.3 Preparation of Reagents

- a) Acidified Acetone: Prepared by mixing 1 mL formic acid in 100 mL acetone.
- b) Ultra pure water/:acetonitrile, 50:50 (v/v); prepared by mixing 50 mL HPLC grade methanol with 50 mL Milli-Q water.
- c) "Mobile Phase A"; 0.1% Formic acid in water prepared by mixing 4000 mL of Milli-Q water and 4.0 mL of Formic acid.
- d) "Mobile Phase B"; 0.1% Formic acid in acetonitrile prepared by mixing 4000 mL of Milli-Q water and 4.0 mL of Formic acid.

3.4 Preparation of Standard Solutions

3.4.1 Stock Standard Solutions

Prepared a 100 µg/mL stock solution for R182281 following method preparation instructions:

Weighed approximately (using a five figure balance) 5.08mg of R182281 analytical standard into an amber "Class A" volumetric flask (50-mL). Diluted to the mark with methanol and mixed well to give a 102 µg/mL stock solution concentration.

3.4.2 Fortification Standards

Sample fortification solutions should be prepared by serial dilution with methanol. The following solutions were prepared: 10 µg/mL, 2.0 µg/mL and 0.20 µg/mL for fortification purposes.

Example Fortification Standard Solution Preparation

Stock Solution			Intermediate Fortification Solution				
Standard Solution ID	Compound	Conc. (µg/mL)	Aliquot Taken (mL)	Diluted To (mL)	Solvent	Conc. (µg/mL)	Intermediate Calibration Solution ID
S20160303-1	R182281	102	1.0	10	MeOH	10	F20160303-1
F20160303-1	R182281	10	2.0	10	MeOH	2.0	F20160303-2
F20160303-2	R182281	2	1.0	10	MeOH	0.2	F20160303-3

Note: All solutions were transferred to polypropylene tubes, covered in foil to protect from white light, and stored under refrigeration.

2. Extracted R182281 residue from the sample contents using 10 mL of acidified acetone (1% formic acid).
3. Sonicated sample for 10 minutes.
4. Transferred 0.5 mL of extract to 1.5 mL centrifuge tube along with 0.1 mL ultra-pure water to act as a keeper, evaporated to near dryness.
5. Reconstituted sample to 1 mL final volume using ultra-pure water: acetonitrile (50:50 v/v), vortexed well.
6. Analyzed by LC-MS/MS (ESI Negative Mode).

Instrumentation

3.5.1 LC-MS/MS Instrument Description for Analysis of R182281

HPLC System:	Shimadzu system
MS Detector:	API 4000 with Analyst™ software version 1.4.2

Chromatography Conditions

Column	: ACE 3 C18 3 x 50mm, 3μ
Column Oven Temperature	: 30°C
Injection volume	: 10 μL
Stop Time	: 5.0 minutes
Injection protocol	: Analyze calibration standard after 5 sample injections
Mobile phase	: Solvent 1 = 0.1% Formic Acid in Optima Water Solvent 2 = 0.1% Formic Acid in Optima Acetonitrile

Isocratic/Gradient Flow:

Time (min)	0.1% Formic Acid in Water	0.1% Formic Acid in ACN	Flow rate, mL/min
0.0	70	30	1.0
0.5	70	30	1.0
2.5	10	90	1.0
3.5	10	90	1.0
4.0	70	30	1.0
5.0	70	30	1.0

Under these conditions the retention time of R182281 is 1.5 minutes.

Mass Spectrometer Conditions

Interface : ESI
Ionization mode : Negative
Curtain gas (CUR) : Nitrogen set at 20 (arbitrary units)
Source temperature (TEM) : 650 °C
Ionspray Voltage (IS) : -4200
Collision gas setting (CAD) : Nitrogen set at 6 (arbitrary units)
Gas 1 (GS1) : Air set at 50 (arbitrary units)
Gas 2 (GS2) : Air set at 50 (arbitrary units)
Interface heater (ihe) : On
Scan type : MRM

	R182281 Primary	R182281 Confirmatory
MRM Conditions		
Q1 <i>m/z</i>	: 244.8	244.8
Q3 <i>m/z</i>	: 181.8	174.8
Dwell time	: 100 ms	100 ms
Resolution Q1	: Unit	Unit
Resolution Q3	: Unit	Unit
Declustering potential (DP)	: -60 V	-60 V
Entrance potential (EP)	: -10 V	-10 V
Collision energy (CE)	: -40 V	-40 V
Collision cell exit potential (CXP)	: -10 V	-10 V

TABLE 1 OVS Silica Gel Air Sampling Tubes Fortification Levels

Soil Type	Fortification Level	Number of Recovery Samples
OVS silica gel air sampling tubes	Control	2
	LOQ (0.01 ug/tube)	5
	10×LOQ (0.1 ug/tube)	5

FIGURE 1 R182281

Compound Code Number : R182281
Alternative : CSCA105253
CAS Number : 28343-61-5
IUPAC Name : 2,4,5-Trichloro-6-hydroxy-isophthalonitrile
Molecular Formula : C₈ H Cl₃ N₂ O
Molecular Weight : 247.46

