STUDY TITLE

Independent Laboratory Validation of XDE-659 and Metabolite in Soil

DATA REQUIREMENTS

SANCO/825/00 Rev. 8.1 (2010); U.S. EPA Guidance OPPTS 860.1000, Background, OCSPP 850.6100, Independent Laboratory Validation

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STUDY COMPLETED ON

12-February-2020

PERFORMING LABORATORY

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STUDY SPONSOR

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STUDY NUMBER

Performing Laboratory Study No: SGS-19-01-13
Sponsor Study ID.: 190829

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INTRODUCTION

The purpose of this study was to demonstrate that "Method Validation for Determination of Residues of XDE-659 and X12485649 in Soil by LC-MS/MS ", AU-2019-09, could be performed successfully at an outside facility with no prior experience with the method (Reference 1).

Principle of the method. Residues of XDE-659 and X12485649 are extracted twice from soil with a mixture of acetonitrile/water/phosphoric acid. Sample supernatants are combined, then filtered and analyzed via LC-MS/MS.

Test conditions. For validation, the analytical set consisted of one reagent blank, two matrix controls, one control fortified at LOD (limit of detection), five replicates fortified at LOQ (limit of quantitation), and five replicates fortified at 10X LOQ. The mass transitions used for analysis are listed below.

	Quantitation (*m/z*)
XDE-659	*m/z* 513.1 → 231.1
XDE-659[1]	*m/z* 513.1 → 109.0
X12485649	*m/z* 471.1 → 231.1
X12485649[1]	*m/z* 471.1 → 109.0

[1]Denotes confirmatory ion.

Limit of Quantification (LOQ) and Limit of Detection (LOD). During the independent laboratory validation of the method, the limit of quantitation (LOQ) of XDE-659 and X12485649 was confirmed to be 0.05 µg/g (ppm) for soil. The LOD for XDE-659 and X12485649 was set at 30% of the defined LOQ.

EXPERIMENTAL

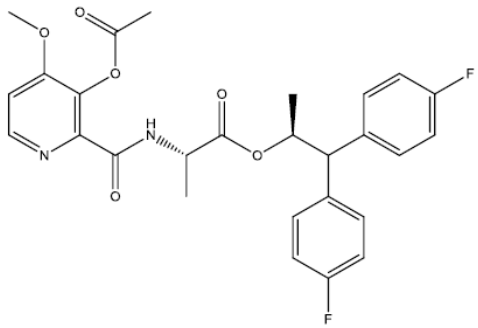
Test Systems

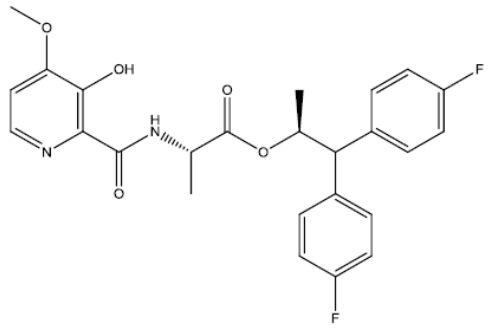
The test system considered in this study was soil. The control sample was provided by Dow AgroSciences and sent to SGS via overnight shipping on dry ice. The soil sample (M 1132) was received by SGS on October 22, 2019. The test system was received frozen and stored under frozen conditions at all times, unless necessary for laboratory analysis.

Test and Reference Substances

The XDE-659 and X12485649 reference substances were stored at room temperature. The Sponsor has retained a reserve sample of these chemicals, and has documentation specifying the location of the synthesis and characterization information available at Dow AgroSciences in Indianapolis, Indiana.

The XDE-659 and X12485649 reference substances were provided by the sponsor and received on October 24, 2019. Upon receipt, the reference substances were stored in the ambient lockbox with the temperature ranging from 19 to 21 °C. All standards were stored under locked conditions. The certificates of analysis are presented in Appendix A. A detailed summary of the reference substances is presented below.

Common Name	XDE-659
Chemical Formula	$C_{27}H_{26}F_2N_2O_6$
Test Substance Structure	
CAS Number	1961312-55-9
Supplier	Dow AgroSciences
Lot / Batch #	XZ7-169400-18A
Purity	99%
Expiration	May 17, 2020

Common Name	X12485649
Chemical Formula	$C_{25}H_{24}F_2N_2O_5$
Test Substance Structure	
CAS Number	1961312-07-1
Supplier	Dow AgroSciences
Lot / Batch #	YN4-148985-060
Purity	95.0%
Expiration	May 25, 2023

Analytical Method

"Method Validation for Determination of Residues of XDE-659 and X12485649 in Soil by LC-MS/MS ", AU-2019-09, was used for the analysis of the samples.

Residues of XDE-659 and X12485649 are extracted twice from soil with a mixture of acetonitrile/water/phosphoric acid. Sample supernatants are combined, then filtered and analyzed via LC-MS/MS. The primary (quantitative) and secondary (confirmatory) transition ions monitored are presented below:

Analyte	Transition (*m/z*)		Ionization Mode	Retention Time (min)
	Primary	Secondary		
XDE-659	513.1 → 231.1	513.1 → 109.0	Positive	~4.0
X12485649	471.1 → 231.1	471.1 → 109.0		~3.6

Calibration Standard Solutions

Target Conc. (µg/L)	**Initial Solution**	**Conc. of Initial Solution (µg/L)**	**Aliquot (mL)**	**Final volume (mL)[1]**	**Final Solvent Concentration (µg/L)**	**Final Matrix-Matched Concentration (µg/L)[2]**
800	10 µg/mL Int. Std.	10000	0.8	10	800	80
500	10 µg/mL Int. Std.	10000	0.5	10	500	50
100	1000 µg/L Cal. Std.	1000	1.0	10	100	10
50.0	500 µg/L Cal. Std.	500	1.0	10	50.0	5.0
10.0	100 µg/L Cal. Std.	100	1.0	10	10.0	1.0
1.0	10 µg/L Cal. Std.	10	1.0	10	1.0	0.1

[1] Prepared in acetonitrile with 0.1% formic acid.

[2] Matrix-matched standards were used for calibration. To prepare these, 100 µL of the appropriate calibration standard solution (above) is diluted with 900 µL of blank extract solution.

$$Matrix\ Effect = \left[\frac{Peak\ Area_{control\ sample\ fortified\ after\ processing}}{Peak\ Area_{fortified\ neat\ solvent}} - 1\right] x\ 100\%$$

XDE-659 Matrix effect = [(1135704991) / (1135594998) – 1] x 100% = 0.01%.

X12485649 Matrix effect = [(1606555476) / (1773939952) – 1] x 100% = -9.44%.

Table 3 Instrument Conditions and Parameters

HPLC Conditions			
Chromatographic System:	Shimadzu Nexera XR		
Column:	Zorbax Eclipse Plus Phenyl-Hexyl, 3.0 50 mm, 1.8µ S/N: USDER01331		
Temperature:	30 °C		
Flow rate (µL/min)	500		
Gradient:	Time (min)	Mobile Phase A (%)	Mobile Phase B (%)
	0.0	50	50
	1.3	40	60
	2.0	40	60
	3.0	10	90
	3.1	0	100
	5.5	0	100
	5.6	50	50
	8.0	50	50
Mobile Phase A:	0.1% formic acid in water		
Mobile Phase B:	1.0% formic acid in acetonitrile:methanol (8:2, v:v)		
Injection Volume:	10 µL		

MS/MS Conditions						
Detection System:	AB BioSystems/MDS Sciex API 6500+ LC/MS/MS					
Ionization:	Turbo Ion Spray					
Polarity:	Positive					
Curtain gas (CUR):	20.00					
Temperature (TEM):	600 °C					
Collision gas setting (CAD):	8.0					
GS1:	70.00					
GS2:	50.00					
IS:	5500					
Entrance potential (EP):	10.00					
Scan type:	MRM					
MRM Conditions	Transition (m/z)	Dwell (msec)	DP	CE	CXP	Retention Time (min)
XDE-659	513.1 → 231.1	150	76	27	18	4.0
	513.1 → 109.0			59	16	
X12485649	471.1 → 231.1	150	96	25	16	3.6
	471.1 → 109.0			57	16	