STUDY TITLE

Method Validation for Determination of Residues of XDE-659 and X12485649 in Soil by LC-MS/MS

DATA REQUIREMENTS

SANCO/825/00 rev 8.1
OCSPP Guideline 850.6100

AUTHOR(S)

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STUDY COMPLETED ON

29-Jan-2020

PERFORMING LABORATORY

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STUDY NUMBER

JRF America Study No: AU-2019-09
Sponsor Study ID.: 190830

PAGE COUNT

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1.0 INTRODUCTION

1.1 Scope

The objective of this study was to develop and prepare an analytical method for the determination of XDE-659 and X12485649 in soil and sediment. This rapid analytical method is applicable for the quantitative determination of residues of XDE-659 and X12485649 in a three (3) representative soils and one (1) sediment.

The method is detailed in JRFA Analytical Method AU-295R0 "Analytical Method for the Determination of XDE-659 and X12485649 in soil and sediment." See Appendix VI. The method was validated over the concentration range of 0.05-0.50 mg/kg with a validated limit of quantitation of 0.05 mg/kg. Common names, chemical names, and molecular formulas for the analyte are given in Table 1.

This study was conducted to fulfill data requirements that satisfy SANCO/825/00 rev. 8.1(1.) and OCSPP 850.6100(2.). This study was conducted following JRF America Standard Operating Procedures. There were no protocol amendments or deviations associated with this study.

1.2 Method Principle

Five (5) g of samples were fortified then extracted twice with 0.25% H_2PO_4 in 80:20 ACN:Water for 30 minutes each. Samples were centrifuged for 5 minutes at 2000 rpm. Extracts were combined and filtered, then analyzed by LC-MS/MS.

Test Substances/Reference Compounds/Analytical Standards

Test Substance	TSN	LOT No.	Percent Purity	Recertification Date
XDE-659	TSN310889	YN4-156527-070-B	99.1%	17-May-2020
X12485649	TSN313826	GZX-01-015-1	98%	21-May-2021

The test substance was received on October 17, 2019. The material was stored in ambient conditions. The certificates of analysis for the reference substance can be found in Appendix VIII. The above standard may be obtained from Dow AgroSciences LLC, 9330 Zionsville Road, Building 304, Indianapolis, IN 46268-1053.

Equipment, Glassware, and Materials

Laboratory Equipment:

Equipment	Size, Description	Manufacturer	Catalog No.
Balance	Analytical Balance	Mettler Toledeo	K51405
Balance	Top Loading Balance	Mettler PC 2000 Sartorius	A50798 70305614
Flasks, Volumetric	Various sizes	Various	----
Pipettes	Various volumes	Eppendorf	----
Wrist Action Shaker	Wrist Action Shaker	Burrell	Model 75
Bottle, Amber glass	Qorpak , 2 oz and 4 oz Boston Round, Amber with Teflon®-	VWR Scientific Products	----
Plastic Centrifuge Tubes	50 mL	CellTreat®	229421
Vortex Mixer	BenchMark, vortex	BenchMark Scientific, Inc.	13112194
Centrifuge	Allegra™ 6R	Beckman Coulter	141016-060
PTFE Filter	0.45 µm	Agilent	5190-5268
HPLC vials	2 mL	Agilent Technologies	5182-0716
HPLC vial caps	PTFE/red silicone septa	Agilent Technologies	5182-0717

UHPLC-UV/MS/MS:UHPLC-RES 10			
Module Type	Manufacturer	Model No.	Serial/ID No.
Column Oven	Aglient 1290	G1316C	DEBAC08348
Auto sampler	Aglient 1290	G2446A	DEBAP05490
Solvent Delivery System	Aglient 1290	G4220A	DEBAA04368
Mass Spectrometer	AB Sciex 6500 QTrap TripQuad	6500	BL23451404
3 x 50mm , 1.8 µm	Zorbax	Eclipse Plus Phenyl Hexyl	USDER01319

Chemical	Grade	Manufacturer/Supplier	Lot No.
Formic Acid	LC/MS	Honeywell/Fluka Sigma-Aldrich St. Louis, MO	J1070
Phosphoric Acid	Reagent	Honeywell/Fluka	I3030
Water	LC/MS	Honeywell/Fluka Sigma-Aldrich St. Louis, MO Honeywell/Fluka Fisher Chemical	195465 196233 195466
Acetonitrile	LC/MS	Honeywell/Fluka Sigma-Aldrich St. Louis, MO OmniSolv/Millipore Billerica, MA Honeywell/Fluka Fisher Chemical	DW926-US 193278
Methanol	LC/MS	OmniSolv/Millipore Billerica, MA Honeywell/Fluka Fisher Chemical	59235 194362

Prepared Solutions:

Mobile Phase A: 0.1 % Formic Acid in Water

Measure 800 mL of LC/MS water into a 1000 mL volumetric flask and then add 1 mL of LC/MS grade formic acid. Following bring the solution to the mark by adding 200 mL of LC/MS water. Invert three times and sonicate briefly. Pour into 1L bottle using funnel if needed.

Mobile Phase B: 0.1 % Formic Acid in 80: 20 MeOH:ACN

Measure 800 mL of LC/MS Acetonitrile and 200 mL LC/MS MeOH into a 1000 mL volumetric flask, removed 1mL and then added 1 mL of reagent grade formic acid. Invert three times. Pour into 1L bottle using funnel if needed.

Dilution Solvent: 0.1% Formic Acid in Acetonitrile

Measure 1 L ACN and remove 1 mL and replace with 1 mL formic acid.

Extraction Solvent: 0.25% Phosphoric Acid in 80:20 Acetonitrile:Water

Measure 400 mL of DI water and 1600 mL of ACS ACN into a 2 L bottle. Then add 5.0 mL of phosphoric acid. Shake bottle to mix.

2.0 EXPERIMENTAL

2.1 Sample Origin, Numbering, Preparation and Storage

Soil and sediment samples were obtained from Dow AgroSciences. Complete source documentation is included in the study file. During the course of the study, the samples were stored in temperature-monitored refridgerators at approximately 4 °C, except when removed for analysis.

2.2 Instrumentation

XDE-659 and X12485649

HPLC:	Agilent 1290 Infinity
Mobile Phase A:	0.1 % Formic Acid in Water
Mobile Phase B:	0.1 % Formic Acid in 80: 20 MeOH:ACN
Flow Rate:	500µL/min
Column:	Zorbax Eclipse Plus Phenyl Hexyl 3 x 50 mm, 1.8 µm (SN: USDER01319)
Column Oven Temperature:	30°C
Injection Volume:	5 µL
Run Time:	5 min
Detector:	SciEx 6500 QTrap with Analyst™ software version 1.6.2

Retention Time:	XDE-659 :	~3.24 min
	X12485649:	~2.89 min
Pre-Equilibration before standards/samples analyzed:	~30 min	
Pre-equilibration within each Injection:	40 secs	

A gradient elution, using an increased percentage of organic solvent (methanol) in the mobile phase, is used to resolve interferences and improve separation. See the specific gradient listed below:

Time (Min)	A% 0.1% Formic Acid in Water	B% 0.1% Formic Acid in 80:20 MeOH:ACN	Flow (µL/min)
0.00	50.0	50.0	500
1.30	40.0	60.0	500
2.00	40.0	60.0	500
3.00	10.0	90.0	500
3.10	0.0	100.0	500
4.50	0.0	100.0	500
4.60	50.0	50.0	500
5.00	50.0	50.0	500

Note: Retention times may differ depending upon the flow rate, column, and gradient used.

Acquisition Ions and Compound Dependent Parameters:

Analyte	Mass Transition (*m/z*)	Dwell (msec)	DP (V)	CE (V)	CXP (V)
XDE-659 (Quantitation)	*512.960→231.100*	150.00	86.00	31.00	6.00
XDE-659 (Confirmatory)	*512.960→ 108.900*	150.00	86.00	73.00	18.00
X12485649 (Quantitation)	*470.813→230.900*	150.00	66.00	25.00	18.00
X12485649 (Confirmatory)	*470.813→ 109.000*	150.00	66.00	55.00	10.00

Typical MS/MS Conditions Used:

Sample Analysis	
Ionization Mode	ESI
Scan Type	MRM
Polarity	Positive
Curtain gas (N_2) (psi)	50
GS1 (psi)	80
GS2 (psi)	40
CAD gas (N_2)	Medium
Ion Spray (V)	5500
Temperature (°C)	600

The above conditions may need to be optimized on each instrument.

2.3 Calculation of Standard Calibration Curve

Calculation of a standard curve begins with the injection of a series of calibration standards and acquisition of peak areas for the following analytes.

XDE-659	m/z Q1/Q3 *512.960/231.100* (quantitative)
	m/z Q1/Q3 *512.960/108.900* (confirmatory)
X12485649	m/z Q1/Q3 *470.813/230.900* (quantitative)
	m/z Q1/Q3 *470.813/109.000* (confirmatory)

For each analyte, the linearity of detector response was evaluated. A standard curve was generated by plotting the analyte concentration on the abscissa (x-axis) and the quantitation ratio on the ordinate (y-axis) in Analyst®. Using regression analysis, the equation was determined for the curve with respect to the abscissa. Refer to Figure 1 to Figure 4 for method validation data. Refer to Table 2 to Table 17 for method validation data and Table 18 and Table 33 for extract stability data.

2.4 Confirmation of Residue Identity

The method is selective for the determination of XDE-659 and X12485649, by virtue of the chromatographic separation and selective detection system used. To demonstrate further confirmation, an additional MS/MS ion transition was monitored for the analyte.

2.5 Statistical Treatment of Data

Statistical treatment of data included, but was not limited to, the calculation of regression equations, correlation coefficients (r) and coefficient 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.

3.1 Assay Time

A typical analytical set contains six calibration standards at different concentration levels. The samples for the method validation consisted of a reagent blank, two controls (a non-fortified sample), and five fortified controls for the LOQ and five for the 10x LOQ. The methodology is normally performed with a batch of 14 samples. On average, one chemist can complete the analysis of one batch of 14 samples including instrument analysis and data processing in a period of 8 working hours.

3.6 Example Calculations

Soil 204956 LOQ1 sample was analyzed for the quantitiation ion of XDE-659

$$\text{Test Item Found } \left({}^{\mu g}/_{L}\right) = \frac{\text{Peak Area} - \text{Curve Intercept}}{\text{Curve Slope}}$$

Sample weight = 5 g

Extract volume = 40 mL

Peak area in the quantitation transition was 3910000 counts

Calibration curve generated in the run was y = 609356.9336 * x + 46466.93472

$$x = \frac{y - b}{m}$$

$$x = \frac{3910000 - 46466.93472}{609356.9336}$$

$$x = 6.34\ {}^{ng}/_{mL}$$

$$= \frac{6.34\ \frac{ng}{mL} \times 40\ mL}{5.00\ g}$$

$$= 51\ \frac{ng}{g} \times \frac{1\ \mu g}{1000 ng}$$

$$= 0.051\ \frac{\mu g}{g}$$

Recovery Results

The recovery factor, expressed as a percentage (Recovery %), is calculated using the following equation:

$$\%Recovery = \frac{Measured\ concentration\ (\mu g/L) - Control\ concentration\ (\mu g/L)}{Theoretical\ concentration\ (\mu g/L)} * 100\%$$

Where the measured and control concentrations are taken from instrument outputs for the sample and untreated control sample (UTC), respectively, and the theoretical concentration is the known amount of analyte added to the sample, if applicable.

Example:

Soil 204956 sample was analyzed.

As no residues of the analyte were found in the control, the recovery was calculated as:

$$\%\text{Recovery} = \frac{[measured\ concentration]\ \frac{\mu g}{g}}{[theoretical\ concentration]\ \mu g/g} * 100\% = [recovery]\%$$

$$\%\text{Recovery} = \frac{0.051\ \frac{\mu g}{g}}{0.05\ \frac{\mu g}{g}} * 100\% = 101\%$$

3.12 Allowable Instrumental Changes to Method

Changes to the analytical method may be made to the optimization parameters and instrumental conditions.

3.13 Critical Steps of the Method

No critical method steps were identified during the course of the method validation.

4.0 CONCLUSIONS

The analytical method for the determination of XDE-659 and X12485649 in soil and sediment has been demonstrated to be satisfactory in terms of accuracy, precision, linearity, and specificity. The method was validated over the concentration range of 0.05 – 0.50 mg/kg with a validated limit of quantitation of 0.05 mg/kg.

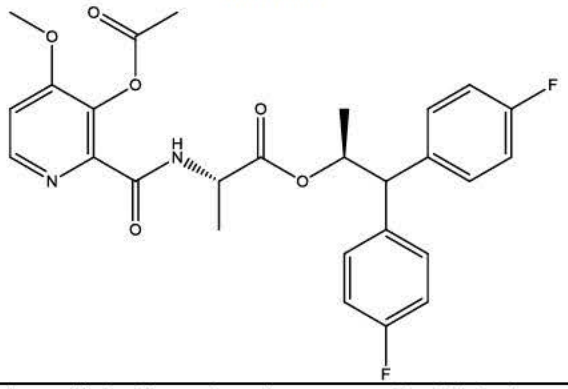
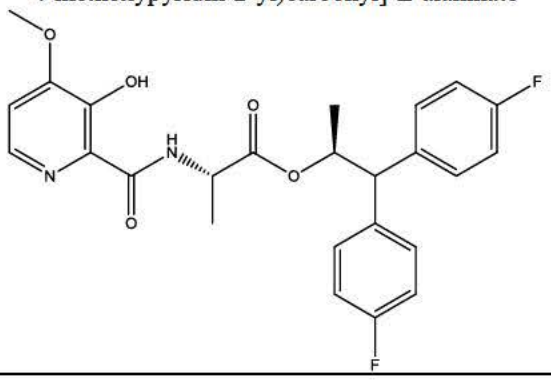
5.0 ARCHIVING

The protocol, raw data, and the original version of the final report will be filed in the Dow AgroSciences LLC archives at 9330 Zionsville Road Bldg. 306, Ab/002H in Indianapolis, Indiana 46268. JRF America will keep copies of raw data and final report for two years. Sample extracts will be retained and disposed of following the completion of study and authorization of sponsor.

7.0 APPENDICES

7.1 Appendix I: Tables

Table 1 Identity and Structure of XDE-659 and X12485649

Identifying Information	Structure and CAS Name
Common Name of Compound: XDE-659 Molecular Formula: C27H26F2N2O6 Formula Weight: 512.51 Nominal Mass: 512 CAS Number: 1961312-55-9	(2S)-1,1-bis(4-fluorophenyl)propan-2-yl N-{[3-(acetyloxy)-4-methoxypyridin-2-yl]carbonyl}-L-alaninate 
Common Name of Compound: X12485649 Molecular Formula: C25H24F2N2O5 Formula Weight: 470.47 Nominal Mass: 470 CAS Number: N/A	(2S)-1,1-bis(4-fluorophenyl)propan-2-yl N-[(3-hydroxy-4-methoxypyridin-2-yl)carbonyl]-L-alaninate 

Analytical Method – (continued)

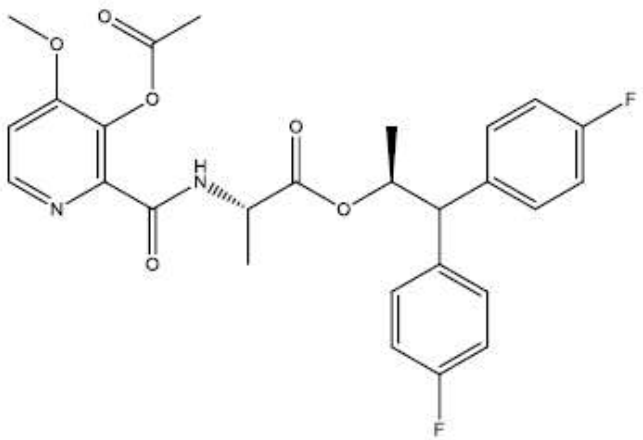
1.0 INTRODUCTION

1.1. Scope and Chemical Structures

An analytical Method AU-295R1 was developed for analysis of XDE-659 and X12485649 in three soils and one sediment. The method was developed using LC-MS/MS for detection. The limit of quantitation (LOQ) of the method has been established at 0.050 mg/kg and 10x LOQ at 0.50 mg/kg. This method satisfies US EPA guidelines OCSPP 850.6100 and SANCO/825/00 rev. 8.1.

The chemical structure ofXDE-659 is summarized as follows:

Common Name:	XDE-659
Chemical Name (IUPAC):	(2S)-1,1-bis(4-fluorophenyl)propan-2-yl N-{[3-(acetyloxy)-4-methoxypyridin-2-yl]carbonyl}-L-alaninate
CAS Registry No.:	1961312-55-9
Molecular Formula:	$C_{27}H_{26}F_2N_2O_6$
Molecular Weight:	512.51 $g \cdot mol^{-1}$
Lot No.:	YN4-156527-070-B
Reassay Date:	17-May-2020
Purity:	99.1%
Storage Condition:	Ambient
Source:	DOW AgroSciences LLC
Structure:	

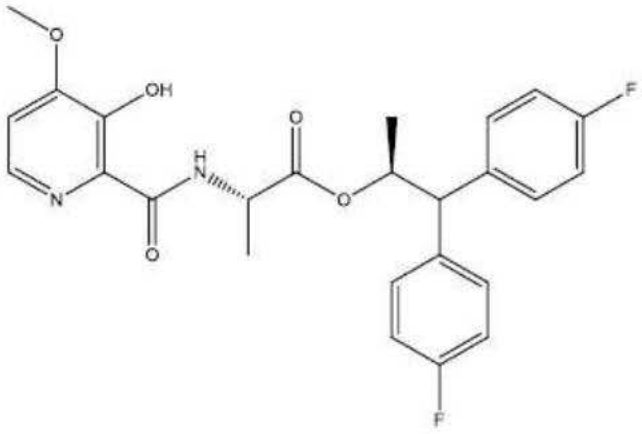


The chemical structure ofXDE-659 is summarized as follows:

Common Name:	X12485649
Chemical Name (IUPAC):	(2S)-1,1-bis(4-fluorophenyl)propan-2-yl N-[(3-

Analytical Method – (continued)

	hydroxy-4-methoxypyridin-2-yl)carbonyl]-L-alaninate
CAS Registry No.:	1961312-55-9
Molecular Formula:	$C_{25}H_{24}F_2N_2O_5$
Molecular Weight:	470.47 $g \cdot mol^{-1}$
Lot No.:	GZX-01-015-1
Reassay Date:	21-May-2021
Purity:	98%
Storage Condition:	Ambient
Source:	DOW AgroSciences LLC
Structure:	



1.2. Method Summary

Five (5) g of samples were fortified then extracted twice with 0.25% H_3PO_4 in 80:20 ACN:Water by shaking for 30 minutes each. Samples were centrifuged for 5 minutes at 2000 rpm. Extracts were combined and filtered, then analyzed by LC/MS/MS.

Analytical Method – (continued)

2.0 MATERIALS AND APPARATUS

2.1. Apparatus

The recommended equipment and apparatus are listed in Appendix I.

2.2. Reagents

All solvents and other reagents are to be of high purity, e.g., glass distilled/HPLC grade solvents and analytical grade reagents. Water must be deionized prior to use or purchased HPLC grade water utilized. Particular care must be taken to avoid contamination of the reagents used. Reagents of comparable purity may be substituted as long as acceptable performance is demonstrated. A list of reagents used in this method along with details of preparation of solutions is included in Appendix II.

2.3. Preparation of Analytical Standard Solutions

It is recommended that the following precautions should be taken when weighing the analytical materials.

1. Ensure good ventilation.
2. Wear gloves, protective eyewear and lab coat.
3. Prevent inhalation and contact with mouth.
4. Wash any contaminated areas.

2.3.1. Stock Solutions

XDE-659 stock standard solution is prepared in a solution of 0.1% formic acid in LC/MS acetonitrile. A stock standard concentration of approximately 1000 µg/L was used. The following is an example for preparing 10 mL of a 994 µg/L stock standard.

1. A mass of 0.010034 g of reference standard is weighed (adjusted for purity of 99.1 %) and transferred to a 10 mL class A volumetric flask.
2. Fill the volumetric flask halfway with 0.1% formic acid in LC/MS ACN and agitate gently (sonicate if necessary) until standard is completely dissolved.
3. Dilute to volume with 0.1% formic acid in LC/MS ACN and mix by inverting several times.
4. Calculate the exact concentration using the exact weight and purity, for example:

Analytical Method – (continued)

$$\left(\frac{0.010034\ \text{g}*0.991}{0.01\ \text{L}}\right)*\left(\frac{10^6\mu\text{g}}{\text{g}}\right) = 994\ \mu\text{g/L}$$

The same procedure was used for X12485649 stock standard.

2.3.2. Preparation of Fortification Solutions

Sample fortification solutions should be prepared by serial dilutions of the stock standard in 0.1% formic acid in ACN for XDE-659 and X12485649. It is recommended that the following concentrations are prepared for fortification standards: 1.00 µg/L, and 10.0 µg/L.

2.3.3. Preparation of Calibration Standards for LC-MS/MS

Calibration solutions suitable for LC-MS/MS analysis should be prepared in matrix (extractions from blank matrix). At least five levels of external calibration standards should be prepared to develop calibration curves for calculation of sample residues. The following dilution schemes were used to prepare the LC-MS/MS calibration solutions are as follows:

Starting Concentration (µg/L)	Volume Used (mL)	Final Volume (mL)	Final Concentration (µg/L)
800	0.100	1.00	80.0
500	0.100	1.00	50.0
100	0.100	1.00	10.0
50.0	0.100	1.00	5.00
10.0	0.100	1.00	1.00
1.00	0.100	1.00	0.100

2.3.4. Standard Solution Storage and Expiration

XDE-659 and X12485649 stock and fortification solutions should be stored in a refrigerator (~ 4 ° C) when not in use to prevent decomposition and/or concentration of the standard. Standard solutions should be allowed to equilibrate to room temperature prior to use.

An expiration date of six (6) months is recommended for the XDE-659 and X12485649 mixed stock standard solution, and one (1) month for dilutions from stock solutions for calibration standards and fortification solutions, based on company standard operating procedure and available data.

2.4. Safety Precautions and Hazards

All caution should be exercised when handling pure material or concentrated stock solutions. Avoid skin contact, swallowing and inhalation. See Safety Data Sheet (SDS) documentation accompanying standard shipment. All personnel should be familiar with all solvents and equipment precautions and hazards prior to use.

Analytical Method – (continued)

3.0 ANALYTICAL PROCEDURE

3.1. Sample Preparation

Samples should be prepared using an approved method for sample preparation for residue analysis. Soil and sediment samples should be kept refrigerated.

1. Weigh 5.0 ±0.05 g of soil of sediment into a 50 mL centfuge tube.
2. Fortify samples with the proper amount (if necessary). To fortify the LOQ samples, 250 µL of a 1.00 µg/L fortification solution was used. To fortify the 10x LOQ samples, 250 µL of 10.0 µg/L fortication solution.
3. Add 20 mL of 0.25% H_3PO_4 in ACN:water (80:20 v/v) to sample and shake for 30 minutes.
4. Centrifuge sample for 5 minutes at 2000 RPM
5. Decant supernatant into a clean 50 mL centrifuge tube.
6. Add 20 mL of 0.25% H_3PO_4 in ACN:water (80:20 v/v) to sample and shake for 30 minutes.
7. Centrifuge sample for 5 minutes at 2000 RPM
8. Decant and combine supernatants
9. Vortex sample and filter into HPLC vial using 0.45 µm PTFE filter.

3.2. Time Required for Analysis

The methodology is normally performed with a batch of 14 samples. In average, one chemist can complete the analysis of one batch of 14 samples including instrument analysis and data processing in a period of 8 working hours.

3.3. Modifications and Potential Problems

Samples should be analyzed within a week after extraction. An expiration date of six months is recommended for the XDE-659 and X12485649 stock standard solution, and one month for XDE-659 and X12485649 calibration standards and fortification solutions based on company standard operating procedure and available data.

4.0 FINAL DETERMINATION

The method has been developed for use on a SciEx 6500 QTrap and Agilent 1290 Infinity system. The following instrumentation and conditions can be used as a general guidance. Other instrumentation, column and mobile phases can also be used, though optimization may be required to achieve the desired separation and sensitivity.

4.1. Instrument Description

HPLC System:	Agilent 1290 Infinity System
Detector:	SciEx 6500 QTrap with Analyst™ software version 1.6.2

Analytical Method – (continued)

4.2. Chromatography Conditions forXDE-659 and X12485649 Analysis

Mobile Phase A:	0.1% formic acid in LC-MS H_2O
Mobile Phase B:	0.1% formic acid in LC-MS grade 80:20 MeOH:ACN
Flow Rate:	500 µL/min
Column:	Zorbax Eclipse Plus Phenyl Hexyl 3 x 50 mm, 1.8 µm (SN: USDER01319)
Column Oven Temp:	30°C
Injection Vol.:	5 µL
Run Time:	5 min
Detector:	SciEx 6500 QTrap with Analyst™ software version 1.6.2
Retention Time:	XDE-659 ~3.24 min X12485649 ~ 2.89 min

Mobile Phase Composition (linear gradient changes):

A gradient elution, using an increased percentage of organic solvent (methanol) in the mobile phase, is used to resolve interferences and improve separation. See the specific gradient listed below:

Time (Min)	A% 0.1% Formic Acid in Water	B% 0.1% Formic Acid in 80:20 MeOH:ACN	Flow (µL/min)
0.00	50.0	50.0	500
1.30	40.0	60.0	500
2.00	40.0	60.0	500
3.00	10.0	90.0	500
3.10	0.0	100.0	500
4.50	0.0	100.0	500
4.60	50.0	50.0	500
5.00	50.0	50.0	500

Note: Retention times may differ depending upon the flow rate, column, and gradient used.

Acquisition Ions and Compound Dependent Parameters:

Analyte	Mass Transition (*m/z*)	Dwell (msec)	DP (V)	CE (V)	CXP (V)
XDE-659 (Quantitation)	*512.960→231.100*	150.00	86.00	31.00	6.00
XDE-659 (Confirmatory)	*512.960→ 108.900*	150.00	86.00	73.00	18.00
X12485649 (Quantitation)	*470.813→230.900*	150.00	66.00	25.00	18.00
X12485649 (Confirmatory)	*470.813→ 109.000*	150.00	66.00	55.00	10.00

Analytical Method – (continued)

Typical MS/MS Voltage Conditions Used:

Ionization Mode	ESI
Scan Type	MRM
Polarity	Positive
Resolution Q1	unit
Resolution Q3	unit
Curtain gas (N_2, psi)	50.00
GS1 (psi)	80.00
GS2 (psi)	40.00
CAD gas (N_2)	Medium
Ion Spray (V)	5500
Temperature (°C)	600.00
EP (V)	10.00
ihe	ON

Initial and Final Q1 and Product Scans can be found in Appendix VI:

Note: The MS settings as provided above should be used as guidelines only. For optimal results, compound and source optimization should be performed by the analyst.

5.0 CALCULATION OF RESULTS

5.1. Multi Point Calibration Procedure

XDE-659 and X12485649, may be calculated in µg/L using a multi-point calibration procedure as follows.

1. Prepare standard solutions over a concentration range appropriate to the expected residues in the samples. An appropriate number of different concentrations within this range should be prepared (at least five).

2. Make an injection of each sample solution and measure the areas of the peaks corresponding to XDE-659 and X12485649. Calibration standard solutions should be interspersed throughout the analysis, after approximately four injections of sample solutions.

3. Calibration standards and samples were analyzed using HPLC/MS-MS. Calibration curves and residue values were calculated using Analyst 1.6.2 data handling software using linear regression (1/x weighting is recommended).

   The standards were fit to the linear equation $y = mx + b$

   Where: x is the concentration of sample in final extract
   m is the calibration line slope
   b is the calibration line intercept
   y is the peak area

4. The following equation can be rearranged and used to calculate residues as follows:

Analytical Method – (continued)

$$\text{Test Item Found } (\mu g/L) = \frac{\text{Peak Area} - \text{Curve Intercept}}{\text{Curve Slope}}$$

5.2. Example Calculation

1. Soil 204956 LOQ1 sample was analyzed.

   Sample weight = 5 g

   Extract volume = 0.040 L

   Peak area in the quantitation transition was 3910000 counts

   Calibration curve generated in the run was y = 609356.9336 * x + 46466.93472

$$x = \frac{y - b}{m}$$

$$x = \frac{3910000 - 46466.93472}{609356.9336}$$

$$x = 6.34\ \mu g/L$$

$$= \frac{6.34\ \frac{\mu g}{L} \times 0.04\ L}{5.00\ g}$$

$$= 0.051\ \frac{\mu g}{g}$$

2. Recovery Results

   The recovery factor, expressed as a percentage (Recovery %), is calculated using the following equation:

$$\%\text{Recovery} = \frac{Measured\ concentration\ (\mu g/L) - UTC\ concentration\ (\mu g/L)}{Theoretical\ concentration\ (\mu g/L)} * 100\%$$

Where the measured and control concentrations are taken from instrument outputs for the sample and UTC, respectively, and the theoretical concentration is the known amount of analyte added to the sample, if applicable.

Example:

Soil 204956 LOQ 1sample was analyzed.

As no residues of the analyte were found in the control, the recovery was calculated as:

$$\%\text{Recovery} = \frac{[measured\ concentration]\mu\frac{g}{L}}{[theoretical\ concentration]\ \mu g/L} * 100\% = [recovery]\%$$

$$\%\text{Recovery} = \frac{0.051\mu\frac{g}{L}}{0.05\ \mu g/L} * 100\% = 101\%$$

Analytical Method – (continued)

5.3. Limit of Detection (LOD)

The limits of detection (LOD) for the matrix is set at a target level 0.015 mg/kg, 30% of the LOQ.

6.0 UNTREATED CONTROL AND RECOVERY SAMPLES

If untreated control samples are available, untreated control samples should be analyzed for each set of samples analyzed to verify that samples are free from analyte contamination. A minimum of two control should be analyzed with each batch of samples.

With field sample sets, a total of two recovery samples, which are untreated samples accurately fortified with a known amount of XDE-659 and X12485649, should also be analyzed in each analytical set. The recovery levels should be run at the LOQ and a higher level to encompass the treated sample results.

7.0 SPECIFICITY

7.1. Labware Interference

All reusable glassware is suggested to be detergent washed in hot water and then rinsed with deionized water and acetone prior to use.

7.2. Reagent and Solvent Interference

None.

Analytical Method – (continued)

12.0 APPENDICES

12.1. Appendix I: Apparatus with Recommended Suppliers

A. HPLC/MS-MS System

1. Agilent 1290 Infinity HPLC System

B. SciEx 6500 QTrap MS with Analyst™ software version 1.6.2.

C. Column: Zorbax Eclipse Plus Phenyl Hexyl 3 x 50 mm, 1.8 µm SN: USDER01319

D. Eppendorf adjustable pipettes, assorted sizes

E. Balance, top-loading, Mettler or equivalent

F. Analytical Balance, for standard prep

G. Vortex, Benchmark Scientific

H. Centrifuge, Beckman Coulter

I. Wrist Action Shaker, Burrell

J. Glassware

Class A volumetric flasks, assorted sizes

Beakers, various sizes

Analytical Method – (continued)

12.2. Appendix II: Reagents

A. Solvents and Reagents.

1. LC/MS grade solvents or better should be utilized. Other brands and grades of solvents may be substituted as long as they do not produce interferences with the chromatography.
   a. Acetonitrile, Honeywell, Lot: DW926-US/ OmniSolv, Lot: 193278
   b. Water, Omni Solv, Lot: 195465, 196233, 195466
   c. Methanol, Omni Solv, Lot: 59235, 194362
   d. Formic Acid, Honeywell, Lot: J1070
   e. Phosphoric Acid, Honeywell/Fluka, Lot: I3030
2. Working Solutions
   a. Mobile phase A: 0.1% formic acid in water: 1.0 mL of formic acid to 1.0 L with water
   b. Mobile phase B: 0.1% formic acid in 80: 20 ACN: MeOH : dilute 1.0 mL of formic acid to 1.0 L with 80: 20 ACN: MeOH.
   c. Extraction Solution: 0.25% phosphoric acid in 80:20 ACN:Water: dilute 5.0 mL of phosphoric acid in 400 mL of water and 1600mL of ACN.