Independent Laboratory Validation of an Analytical Method for the Determination of XDE-729 Methyl and XDE-729 Acid in Soil using LC-MS/MS

INTRODUCTION

Scope

This method is applicable for the quantitative determination of residues of XDE-729 methyl and XDE-729 acid (quantified as XDE-729 butyl ester) in soil. The method is detailed in the report for Study ID 150877 'Method Validation Study for the Determination of Residues of XDE-729 Methyl and Its Acid Metabolite in Soil by Liquid Chromatography with Tandem Mass Spectrometry – Hill, R., 2015' (*1*) and was included in the appendix to the study plan. The method was independently validated over the concentration range of 0.0015-0.1 µg/kg (ng/g) with a validated limit of quantitation of 0.0015 µg/kg (ng/g). Common names, chemical names, and structural formulas for the analytes are given in Table 1.

This study was conducted to fulfil data requirements outlined in the EPA Ecological Effects Test Guidelines, OCSPP 850.6100 (*2*). The validation also complies with the requirements of SANCO/825/00 rev.8.1 (*3*). A maximum of three sample set attempts were allowed in order to validate the method for the independent laboratory validation (ILV).

Method Principle

Residues of XDE-729 methyl and XDE-729 acid are extracted from 5 g sub-samples by adding 15 mL of acetonitrile/water (80/20, v/v) containing 0.1% H_3PO_4 then shaking and centrifuging. The supernatant is transferred to a 60 mL glass vial. A further 15 mL of acetonitrile/water (80/20, v/v) containing 0.1% H_3PO_4 is added and then vortexed until soil is mixed from the bottom of the tube which then shaken and centrifuged. The supernatant is combined in the 60 mL glass vial. The sample is evaporated (evaporating the organic solvent from the extract) until less than 6 mL (approx. 5 mL) remains, 3 mL of water is added, vortex mixed and sonicated. The extract is then passed through a pre-conditioned 200 mg (3 mL) Strata SCX solid phase extraction (SPE) cartridge with a 3 mm layer of filter aid. The sample tube is rinsed with water containing 0.1% H_3PO_4 and the rinse is passed through the SPE cartridge. The SPE cartridge is washed with methanol containing 0.1% H_3PO_4. The extract is eluted from the SPE cartridge using acetonitrile/methanol (90/10, v/v) containing 1.0% NH_4OH. 15 µL of 2.5 ng/mL mixed internal standard is added to each sample. The sample is evaporated to dryness. 100 µL of coupling reagent is added and vortex mixed. 100 µL of acetonitrile/butyl chloroformate (90/10, v/v) reagent is added to derivatize XDE-729 acid to XDE-729 butyl ester and immediately vortex mixed. The samples sit at room temperature for 5 min and are then evaporated to dryness. The sample is reconstituted with 500 µL of acetonitrile/water (70/30, v/v) containing 0.1% H_3PO_4 and vortex mixed. The extract is analysed by liquid chromatography coupled with positive ion electrospray ionisation tandem mass spectrometry (LC-MS/MS).

Reference Compounds/Analytical Standards

Test Substance	TSN	Percent Purity	Recertification Date	Lot No.
XDE-729 methyl	TSN031117-0005	99.1	21 Nov 2016	201001134-26
XDE-729 acid	TSN030751-0004	99.0	07 Nov 2017	DC6-E2622-77
X11278577 (XDE-729 methyl internal standard)	TSN305882	100	16 Nov 2016	YC2-134955-71
X12278779 (XDE-729 acid internal standard)	TSN305883	100	10 Nov 2020	YC2-134955-77

The Certificates of Analysis for the reference substances can be found in Figures 1-4. The above standards may be obtained free of charge from Dow AgroSciences LLC, 9330 Zionsville Road, Building 304, Indianapolis, IN 46268-1054.

EXPERIMENTAL

Sample Origin, Numbering, Preparation, Storage, and Characterisation

Prepared sample of soil (CCON/073/016) was supplied by the Sponsor. The soil sample was characterised by Agvise Laboratories, 604 Highway 15 West, P.O. Box 510, Northwood, ND 58267.

During the course of the study, the sample was stored in temperature-monitored freezers set to maintain a sample temperature of -18 °C or below, except when removed for analysis.

Calculation of Standard Calibration Curve

Calculation of a standard curve began with the injection of a series of calibration standards (eight standards within the concentration range of 0.0045-2.5 ng/mL) as described in the method from Study ID 150877 'Method Validation Study for the Determination of Residues of XDE-729 Methyl and Its Acid Metabolite in Soil by Liquid Chromatography with Tandem Mass Spectrometry – Hill, R., 2015' (*1*) with the instrument conditions as described in Appendix 2, and acquisition of peak areas for the following analytes.

XDE-729 methyl	*m/z* Q1/Q3 345/250 (quantitative)
	m/z Q1/Q3 345/235 (confirmatory)
	m/z Q1/Q3 351/256 (internal standard)
XDE-729 butyl ester	*m/z* Q1/Q3 387/250 (quantitative)
	m/z Q1/Q3 387/207 (confirmatory)
	m/z Q1/Q3 393/256 (internal standard)

For each analyte, the linearity of detector response was evaluated using standard solutions in solvent (non-matrix-matched standards). In order to generate a standard curve, the analyte concentration was plotted on the abscissa (x-axis) and the respective peak area ratio (analyte peak area divided by internal standard peak area) on the ordinate (y-axis) in Excel. Using regression analysis the equation for the curve with respect to the abscissa was determined. From this standard curve the correlation coefficient (r) and the coefficient of determination (r^2) were determined. Linear regression analysis with 1/x weighting was then used to generate a standard calibration equation for determination of analyte concentrations.

Confirmation of Residue Identity

The method is specific for the determination of XDE-729 methyl and XDE-729 acid (quantified as XDE-729 butyl ester) by virtue of the chromatographic separation and selective detection system used. To demonstrate further confirmation, an additional MS/MS transition was monitored for each analyte.

XDE-729 methyl	*m/z* Q1/Q3 345/250 (quantitative)
	m/z Q1/Q3 345/235 (confirmatory)
XDE-729 butyl ester	*m/z* Q1/Q3 387/250 (quantitative)
	m/z Q1/Q3 387/207 (confirmatory)

Statistical Treatment of Data

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

LC-MS/MS Instrument Description

Instrument	:	Agilent 1260/1290 HPLC system
	:	AB Sciex Triple Quad 5500 Q-Trap MS/MS system
	:	Analyst 1.6.2 data system

Typical Chromatography Conditions

Column	:	Waters Acquity UPLC HSS T3, 50 × 2.1 mm, 1.8 µm particle size
Column Temperature	:	35 °C
Injection Volume	:	10 µL
Run Time	:	10 minutes
Mobile Phase	:	A – Water + 0.1% formic acid B – Acetonitrile + 0.1% formic acid
Flow Rate	:	450 µL/min

Mobile Phase Composition

Time (min)	Solvent A (%)	Solvent B (%)
0.00	70	30
0.50	70	30
5.50	20	80
6.00	0	100
7.00	0	100
7.10	70	30
10.00	70	30

Under these conditions the retention times of the XDE-729 methyl and XDE-729 butyl ester are approximately 5.3 minutes and 7.0 minutes, respectively.

Note: the flow rate was reduced from 550 to 450 µL/min and 3 min of equilibration time was added to the end of the gradient program, this is an allowable change in accordance with the analytical method.

Typical Mass Spectrometer Conditions

Ionisation Mode	:	Turbo Spray
Polarity	:	Positive
Scan Type	:	MRM
Resolution	:	Q1 – unit, Q3 – unit
Curtain Gas (CUR)	:	30
Collision Gas (CAD)	:	Medium
IonSpray Voltage (IS)	:	5500 V
Temperature (TEM)	:	600 °C
Ion Source Gas 1 (GS1)	:	50
Ion Source Gas 2 (GS2)	:	50
Entrance Potential (EP)	:	10 V

Compound:	Q1 Mass (amu)	Q3 Mass (amu)	Dwell (msec)	Declustering Potential (DP)	Collision Energy (CE)	Collision exit potential (CXP)
XDE-729 methyl Quantitation	344.9	250.1	100	8	43	20
XDE-729 methyl Confirmation	344.9	235.0	100	8	56	20
XDE-729 methyl internal standard	351.1	256.0	100	8	43	20
XDE-729 butyl ester Quantitation	387.0	250.1	100	8	43	20
XDE-729 butyl ester Confirmation	387.0	207.1	100	8	69	20
XDE-729 butyl ester internal standard	393.3	256.0	100	8	50	20