XDE-729 Methyl Ester – Independent Laboratory Validation of Analytical Method 110718 for the Determination of XDE-729 Methyl Ester and its Metabolites Residues in Water

ABSTRACT

This study was conducted to provide independent laboratory validation data for the determination of residues of XDE-729 methyl ester (X11393728) and its metabolites XDE-729 acid (X11393729), X11406790 and X11449757 in drinking water, surface water and ground water, following the analytical method, Dow AgroSciences LLC, Study Number 110718, "Method Validation Study for the Determination of Residues of XDE-729 and its metabolites in Surface Water, Ground Water and Drinking Water by liquid chromatography with Tandem Mass Spectrometry"(reference 6). The validated limit of quantification was 0.05 µg/L, for all the water samples.

For the independent validation of the method, for drinking water, groundwater and surface water, after fortification with the analytes, the following specimens were analysed by LC-MS/MS:

- 5 specimens fortified at the LOQ level of 0.05 µg/L
- 5 specimens fortified at 10 LOQ level of 0.50 µg/L
- 2 unfortified, untreated control specimens,
- 1 specimen fortified at the LOD level of 0.015 µg/L
- 1 reagent blank, taken through sample cleanup with the samples

All of the individual recovery values for XDE-729 methyl ester (X11393728), XDE-729 acid (X11393729), X11406790 and X11449757 in all of the water samples were within the EPA acceptance range of 70-120%. Average recoveries at each fortification level were within the EU acceptance range of 70-110%. The relative standard deviation (RSD) never exceeded ± 20% at any fortification level. There were no interferences present greater than 30% of the LOQ seen in the chromatograms of the untreated control samples for the quantification or confirmatory transitions in any of the blank and unfortified specimens.

This independent laboratory validation was conducted to satisfy requirements of EU Regulation (EC) No 1107/2009, and the European Commission Guidance Document on

Residue Analytical Methods, SANCO/825/00 rev. 8.1 and SANCO/3029/99 rev. 4. The study was also conducted to satisfy the requirements of U.S. EPA Guideline OPPTS 850.7100, PR Notice 96-1 and PR Notice 2011-3.

INTRODUCTION

An analytical method was developed and validated for the determination of XDE-729 methyl ester (X11393728) and its metabolites XDE-729 acid (X11393729), X11406790 and X11449757 in drinking water, surface water and ground water. The method is identified as Dow AgroSciences Study Number 110718, "Method Validation Study for the Determination of Residues of XDE-729 and its metabolites in Surface Water, Ground Water and Drinking Water by liquid chromatography with Tandem Mass Spectrometry" (reference 6). This method is referenced as AGR/MOA/XDE-1 at Eurofins Agroscience Services Chem for the independent validation study.
The method was found to be suitable for the determination of residues of XDE-729 methyl ester (X11393728) and its metabolites XDE-729 acid (X11393729), X11406790 and X11449757 in drinking water, surface water and ground water over the concentration range of 0.15 to 20 µg/L. The validated limit of quantification was confirmed to be 0.05 µg/L for all water types.

An independent laboratory validation of the analytical method was conducted on drinking water, surface water and ground water to satisfy the requirements of the European Commission Guidance Document on Residue Analytical Methods, SANCO/825/00 rev. 8.1 (Reference 1) and SANCO/3029/99 rev. 4 (Reference 2). The study was also conducted to satisfy the requirements of U.S. EPA Guideline OPPTS 850.7100 (Reference 3), PR Notice 96-1 (Reference 4) and PR Notice 2011-3 (Reference 5).

The independent laboratory, the Study Director, and the analyst chosen to conduct the ILV were unfamiliar with the method, both in its development and subsequent use in analyzing samples. The independent laboratory used all of its own equipment and supplies, so that there was no common link between Dow AgroSciences and the ILV analyst. Throughout the conduct of the study, any communications between Dow AgroSciences and the Study Director and/or the analyst were logged for inclusion in the report. No one from

Dow AgroSciences was allowed to visit the independent laboratory during the ILV trial to observe, offer help, or assist the chemists or technicians. These steps successfully maintained the integrity of the ILV study.

ANALYTICAL

Preparation and Storage of Samples

The independent laboratory validation was carried out on three water specimens; surface water, ground water and drinking water. The drinking water was obtained from a "drinking water" tap at Comps (30300 - France), the ground water was obtained from Evian and the surface water was obtained from the Tech River, Pyrénées-Orientales (66), France.

Specimen	EAS Sample Reference Number
Surface Water	163
Ground Water	215
Drinking Water	248

Upon receipt, the specimens were stored at between 0 and 9 °C.

Characterisation of Samples

The water specimens were characterized by Cereco SA – Laboratoire Sud (a non-GLP facility, however, one that is ISO certified), Zone Aéropole 30128 Garons, France, details of the characterization results are as follows:

Specimen	pH (20°C)	Total Hardness (°F)	Total Suspended Solids (mg/L)	Alkalinity (mg/L)	Dried Residues (mg/L)	Dissolved Organic Carbon (g/100g)
Method	NFT90-008	NF T90-003	NF EN872	NF EN ISO 9963-1	NF T90-029	M0046S3
Surface Water	6.80	7.8	< 2	83	92	<0.01
Ground Water	7.45	30.4	< 2	357	310	<0.01
Drinking Water	7.75	9.4	<2	287	483	<0.01

Preparation of Solutions and Standards

Reagents used were of equivalent specifications as described in the analytical method.

The following analytical test substances/analytical standards were utilized during the independent laboratory method validation:

Test Substance/ Analytical Standard:	XDE-729 Methyl Ester (X11393728)
Supplier:	Sponsor
Reference Number:	TSN031117-0002
Batch/Lot no:	XW7-38246-49
Purity:	97.4%
Expiry date:	09 Nov 2013
Storage:	20±4°C

Test Substance/ Analytical Standard:	XDE-729 Acid (X11393729)
Supplier:	Sponsor
Reference Number:	TSN030751-004
Batch/Lot no:	DC6-E2622-77
Purity:	99%
Expiry date:	2 Nov 2013
Storage:	20±4°C

Test Substance/ Analytical Standard:	X11449757
Supplier:	Sponsor
Reference Number:	TSN031413-0003
Batch/Lot no:	YB1-100780-103
Purity:	99%
Expiry date:	30 Jun 2012
Storage:	between 0 and 9°C

Test Substance/ Analytical Standard:	X11406790
Supplier:	Sponsor
Reference Number:	TSN302182
Batch/Lot no:	SYN-FS08644-062
Purity:	95%
Expiry date:	20 Nov 2014
Storage:	20±4°C

The certificates of analysis were provided by Dow AgroScience LLC. They are located in appendix B.

Standard solutions and calibration standard solutions were prepared as described in the analytical method. Details of these materials are presented in appendix C for the study along with details of the preparation of all analytical and fortification standards prepared from the primary reference items. The test/reference items and specimens will be retained until expiry and then disposed of with the approval of the Study Monitor.

Fortification of Recovery Samples

The control specimens were fortified as described below with XDE-729 methyl ester (X11393728), XDE-729 acid (X11393729) X11406790 and X11449757:

Matrix	Untreated Control Specimen Reps	Replicates at Fortification Level (LOD)*	Replicates at Fortification Level (LOQ)**	Replicates at Fortification Level
Drinking Water	2	1 at 0.015 µg/L	5 at 0.05 µg/L	5 at 0.5 µg/L
Groundwater	2	1 at 0.015 µg/L	5 at 0.05 µg/L	5 at 0.5 µg/L
Surface Water	2	1 at 0.015 µg/L	5 at 0.05 µg/L	5 at 0.5 µg/L

*LOD – Limit of determination
**LOQ – Limit of Quantification

Each sample was fortified as per the table above. One sample was fortified to achieve a fortification level of 0.015 µg/L (LOD), five samples were fortified to achieve the fortification level of 0.05 µg/L (LOQ) and five samples were fortified to achieve the upper fortification level of 0.5 µg/L for drinking water, surface water and ground water. The fortification solution was injected directly into the matrix.

Sample Extraction, Purification and Analysis

Specimens were assayed according to the analytical method Dow AgroSciences Study Number 110718, "Method Validation Study for the Determination of Residues of XDE-729 and its metabolites in Surface Water, Ground Water and Drinking Water by liquid chromatography with Tandem Mass Spectrometry" (reference 6). The method was internally referenced at Eurofins Agroscience Services Chem under the number AGR/MOA/XDE-1.
The water sample matrices were acidified with a formic acid solution, and then the entire sample was purified and residues of XDE-729 methyl ester (X11393728), XDE-729 acid (X11393729) X11406790 and X11449757 were extracted using a Strata-X reversed-phase solid-phase extraction (SPE) column.

After elution from the SPE column with methanol, the eluate was dried under a stream of nitrogen. The final sample was reconstituted with an acetonitrile/ultrapure water/formic acid solution (10/90/0.1, v/v/v). The sample was analyzed for the determination of XDE-729 methyl ester (X11393728), XDE-729 acid (X11393729) X11406790 and X11449757 by liquid chromatography with positive-ion electrospray ionisation (ESI+) tandem mass spectrometry (LC-MS/MS).

Full extraction details:

1. Forty 10-mL portions of each water sample were measured into a 15-mL centrifuge tube.

2. For preparing fortified samples, appropriate aliquots of the appropriate spiking solutions were added to untreated control water to encompass the necessary concentration range:

Concentration of Fortified Sample (µg/L)	Volume of Spiking Solution (µL)	Concentration of Spiking Solution (µg/mL)
0.015	15	0.01 µg/mL
0.05	50	0.01 µg/mL
0.5	50	0.10 µg/mL

3. One mL of 10% formic acid was added to each tube. The tubes were mixed for 30 seconds using a vortex mixer.

4. The clean up of samples on the Strata-X polymeric sorbent reversed-phase SPE cartridge used the following procedure:

   a. Place a Strata-X reversed-phase SPE column (60-mg, 3 mL) on a vacuum manifold box.

   b. The SPE cartridge was conditioned with 3 mL of methanol followed by 3 mL of ultrapure water/formic acid (100/01, v/v) discarding the condition solvents. Full vacuum was applied for about 5 seconds between solvent additions.

   c. The entire portion of each acidified sample (from Step 3) was ultimately transferred to the SPE cartridge. The samples were pulled through the SPE cartridge at approximately 1 mL/min, using vacuum if necessary. The sample void was discarded.

   d. The sample tube was rinsed with 1 mL of ultrapure water/formic acid (100/0.1, v/v), the rinse solution was then transferred to the SPE cartridge. The

rinse solution was pulled through the SPE cartridge at approximately 1 mL/min, using vacuum if necessary. The void was discarded, and the SPE column was dried under full vacuum for 5 seconds after the rinse step.

e. The analytes were eluted from the SPE cartridge at a rate of approximately 1 mL/min using three separate 500-µL aliquots of methanol for each cartridge. The three eluates from each cartridge were collected in the same centrifuge tube.

5. One hundred µL of the mixed internal standard (5 µg/L) was added to the eluate.

6. The eluate was evaporated near to dryness under a stream of nitrogen at 40ºC.

7. The final sample was reconstituted with 1.0 mL of acetonitrile/ultrapure water/formic acid solution (10/90/0.1, v/v/v). The sample was vortexed for a few seconds and sonicated for 2-3 minutes.

8. A portion of the sample was transferred to a 2-mL autosampler vial.

9. The sample was analyzed by liquid chromatography with positive-ion electrospray ionisation (ESI+) tandem mass spectrometry (LC-MS/MS).

Analytical Instrumentation and Equipment

- LC-MS/MS API 5500 QTRAP (Sciex)
- Column oven CTO-20AC (Shimadzu)
- Automatic sampler SIL20AC (Shimadzu)
- Pump LC20AD (Shimadzu)
- Column Zorbax SB-C8 75 mm x 4.6 mm – 3.5µm
- Cartridge Strata-X reversed-phase (60 mg/3 mL)
- UV spectrometer
- Precision balances
- Standard laboratory glassware (volumetric flasks, measuring cylinders)
- Ultrasonic bath
- Various pipettes
- Vortex

- Heating block with nitrogen flow

The instrumental conditions used during the ILV trial were as described in the analytical method, and are given below.

Typical HPLC Operating Conditions

Column:	Zorbax SB-C8 – 75x4.6 mm – 3.5 µm
Column Temperature:	25°C
Automatic sampler temperature:	4°C
Injection Volume:	10 µL
Injection Wash	Acetonitrile / ultrapure water / formic acid (70/30/0.1, v/v/v)
Run Time:	12.0 minutes
Mobile Phase:	A – Methanol + 0.1% formic acid B – Ultra pure water + 0.1% formic acid
Flow Rate:	0.80 mL/min.

Gradient:

Time, min	A, %	B, %
0.0	10	90
7.0	100	0
9.0	100	0
9.1	10	90
12.0	10	90

Flow Diverter Program:

1) 0.0→3.5 min: flow to waste
2) 3.5→9.0 min: flow to source
3) 9.0→end of run: flow to waste

Retention times:

Approx. 6.8 min for XDE-729 methyl ester
Approx. 5.0 min for XDE-729 acid
Approx. 6.2 min for X11406790
Approx. 4.1 min for X11449757

Typical Mass Spectrometry Operating Conditions

Instrumentation:	MDS SCIEX API 5500 LC-MS/MS System
	MDS SCIEX Analyst 1.5.1 data system
Polarity:	Positive
Interface	Electrospray
Scan Type:	MRM
Resolution:	Q1 – unit, Q3 – unit
Curtain Gas (CUR):	20 psi
Collision Gas	Medium
Temperature (TEM):	550°C
Ion Source Gas 1	55 psi
Ion Source Gas 2	40 psi
Dwell Time:	200 ms

Analyte	Parent ion (*m/z*)	Daughter ion (*m/z*)	DP (V)	EP (V)	CXP (V)	CE (V)
XDE-729 methyl ester	345.0	285.1 (quantification)	71	10	8	31
		250.1 (confirmatory)	71	10	14	45
XDE-729 acid	330.8	250.0 (quantification)	80	10	16	31
		235.0 (confirmatory)	80	10	16	43
X11406790	331.0	236.0 (quantification)	80	10	14	33
		271.0 (confirmatory)	80	10	3	45
X11449757	317.0	236.0 (quantification)	80	10	14	31
		270.9 (confirmatory)	80	10	14	31

DP: Declustering Potential, CE: Collision Energy, CXP Cell Exit Potential

Internal standards

Analyte	Parent ion (*m/z*)	Daughter ion (*m/z*)	DP (V)	EP (V)	CXP (V)	CE (V)
XDE-729 methyl ester M+6	351	256	80	10	35	41
XDE-729 acid M+6	336.8	256	60	10	35	41
X11406790 M+6	337	242	80	10	32	49
X11449757 M+6	323	242	80	10	18	45

DP: Declustering Potential, CE: Collision Energy, CXP Cell Exit Potential

Calculation of Results

For each analytical batch, a range of 7 calibration standards was injected over the range 0.15 ng/mL to 20 ng/mL. A calibration curve was prepared for each analyte by plotting the quantification peak area ratio obtained versus the analyte concentration ratio.

Example: XDE-729 methyl ester recovery at 0.05 ng/mL

A linear calibration curve was calculated using the method of least squares (1/x weighting):

$$Y = A \times C + B$$

Y = detector response (as peak area ratio) for XDE-729 methyl ester = 0.067

A = slope of the linear least squares fit of the calibration curve = 0.543

C = Concentration ratio determined from standard curve $C = \frac{C_a}{IS}$

C_a = Analyte concentration

IS = internal standard concentration = 5 ng/mL

B = Y-intercept of the linear least squares fit of the calibration curve = 0.0104

The concentration determined from standard curve is $C_a = \frac{(Y - B)}{A} \times IS$ = 0.52 µg/L

The residue of XDE-729 methyl ester in each test specimen is calculated as follows:

$$\text{Residue (µg/L)} = \frac{V_f}{V_1} \times \text{extract concentration (µg/L)}$$

Where:

V_1 (mL)	=	total extraction volume (10 mL)
V_f (mL)	=	final volume (1 mL)
Extract concentration	=	0.52 µg/L
Residue (µg/L)	=	0.052 µg/L

Procedural recovery data from fortified samples are calculated via the following equation:

$$\text{Recovery } (\%) = \frac{A}{S} \times 100$$

Where:

A = concentration of XDE-729 methyl ester found in spiked sample = 0.052 µg/L.

S = concentration of XDE-729 methyl ester added in spiked sample = 0.052 µg/L.

Recovery = 105% (calculation performed on unrounded values)

Statistical Treatment of Data

The mean recoveries for the fortified samples were calculated using the "AVERAGE" function of the Microsoft Excel spreadsheet computer program, which divides the sum of the selected cells by the number of determinations. The standard deviation of the recoveries for each fortification level for each matrix type was calculated using the "STDEV" function of the same spreadsheet program, which sums the squares of the individual deviations from the mean, divides by the number of degrees of freedom (n-1), and extracts the square root of the quotient. Percent relative standard deviation, % RSD, was calculated by dividing the standard deviation by the mean, and then multiplying by 100.

Confirmation of Residue Identity

The LC-MS/MS method is highly selective for the determination of residues of XDE-729 methyl ester (X11393728) and its metabolites XDE-729 acid (X11393729), X11406790 and X11449757 in drinking water, surface water and ground water by virtue of the chromatographic separation and selective detection system used. To demonstrate further confirmation, a second MS/MS ion transition was monitored for each analyte. Calculations of %Recovery and %RSD were carried out on the confirmatory ions data (Tables 1, 2 and 3).