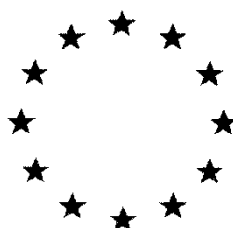


European Commission



**Draft Assessment Report prepared according to the Commission
Regulation (EU) N° 1107/2009**

XDE-729 Methyl (Halauxifen-methyl) Volume 3 – B.5

**Rapporteur Member State : United Kingdom
Co-Rapporteur Member State : France**

Version History

When	What
2013-12-19	Initial DAR

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B.5 METHODS OF ANALYSIS

Note – The parent compound is referred to in this chapter interchangeably as XDE-729 Me, XDE-ME, XDE-729 Methyl and XDE-729 Methyl Ester in order to reflect how it was referred within the original study reports. XDE-729 acid may also be identified by the code X11393729.

B.5.1 Analytical methods for formulation analysis (IIA 4.1, IIIA 5.1)**B.5.1.1 Technical active substance (IIA 4.1)****Description of analytical methods for the analysis of the active substance as manufactured**

Reference: Hamilton, T. 19-April-2012 “Analytical Method and Validation for the Determination of Active Ingredient in XDE-729 Me Technical by Liquid Chromatography, Laboratory Project ID: DECO ML AL-2012-003865

An internal standard high performance liquid chromatographic (HPLC) gradient method with UV detection was used. In the sample preparation, XDE-729 Me technical (~100 mg) and 50 mL of internal standard solution [2 mg/mL methane bis(4-hydroxyphenyl) in acetonitrile] were weighed into a jar, mixed thoroughly and then injected into a liquid chromatograph using a gradient method (Mobile Phase A: 80% (1 g/L ammonium acetate in water, pH = 5.0 w/ acetic acid) + 20% acetonitrile, Mobile Phase B: 100% Acetonitrile). An alternative gradient system was also proposed for no pre-mixing of mobile phases (Alternative Mobile Phase A: 100% (1 g/L ammonium acetate in water, pH = 5.0 w/ acetic acid, Alternative Mobile Phase B: 100% Acetonitrile). The components were separated using a Phenomenex Kinetex C18 100Å, 100 x 4.6 mm, 2.6 µm column and detected with a UV detector (270 nm). Approximate retention times were 6.9 min - ISTD and 11.8 min - XDE-729 Me. Quantitation was made by peak area measurements with internal standardization.

Specificity	The LC/UV technique is specific for the determination of the active ingredient by virtue of the chromatographic conditions. Lack of interference was demonstrated through submission of chromatograms of the acetonitrile blank, an internal standard blank, and an analytical standard blank using the method conditions. Confirmation of the presence of the active ingredient is obtained by comparative retention time analysis of the analyte in the sample and the analyte in the calibration standards.
Interference by other substances	The method has no known interferences at typical concentrations
Explanation of interferences contributing more than ±3%	None noted

Linearity and range, equation and r^2	All correlation coefficients (r^2) were above 0.99, demonstrating good linearity. Results presented in the table below Table: Linearity of the method for the determination of active ingredient in XDE-729 Me technical <table><tr><th rowspan="2">Component</th><th rowspan="2">Concentration range (mg/mL)</th><th colspan="3">Linear Regression</th></tr><tr><th>Slope</th><th>Intercept</th><th>r^2</th></tr><tr><td>XDE-729 Me</td><td>0.897 – 2.99 (single determinations at 5 concentrations).</td><td>2585.9</td><td>135.69</td><td>0.9969</td></tr><tr><td>Internal Standard</td><td>0.924 – 3.08</td><td>708.22</td><td>33.646</td><td>0.9971</td></tr></table>	Component	Concentration range (mg/mL)	Linear Regression			Slope	Intercept	r^2	XDE-729 Me	0.897 – 2.99 (single determinations at 5 concentrations).	2585.9	135.69	0.9969	Internal Standard	0.924 – 3.08	708.22	33.646	0.9971																															
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Accuracy	Not applicable																																																	
Repeatability	The precision of the method was evaluated by replicate analysis of XDE-729 Me technical sample, which had been fortified with impurities. Five samples were prepared from the same batch of technical material, and analysed on each of two days. The repeatability (precision) of the method was assessed by determination of the overall Relative Standard Deviation (RSD%) on the results of analysis of the 5 individual samples collected over two days. The RSD values confirm the repeatability of the method, and are presented in the table below. <table><tr><th colspan="11">Method Precision Results</th></tr><tr><th>Component</th><th>1</th><th>2</th><th>3</th><th>4</th><th>5</th><th>6</th><th>7</th><th>8</th><th>9</th><th>10</th></tr><tr><td>XDE-729 Me (wt. %)</td><td>93.2</td><td>93.3</td><td>93.1</td><td>93.1</td><td>93.1</td><td>92.8</td><td>93.2</td><td>93.3</td><td>93.0</td><td>93.0</td></tr></table> <table><tr><th>Component</th><th>Average</th><th>Std. Dev.</th><th>% RSD</th><th>Wt. Fraction</th><th>RSDR</th><th>RSDr</th><th>Horwitz Pass/Fail</th></tr><tr><td>XDE-729 Me (wt. %)</td><td>93.1</td><td>0.1668</td><td>0.18</td><td>0.931</td><td>2.0</td><td>1.4</td><td>Pass</td></tr></table>	Method Precision Results											Component	1	2	3	4	5	6	7	8	9	10	XDE-729 Me (wt. %)	93.2	93.3	93.1	93.1	93.1	92.8	93.2	93.3	93.0	93.0	Component	Average	Std. Dev.	% RSD	Wt. Fraction	RSDR	RSDr	Horwitz Pass/Fail	XDE-729 Me (wt. %)	93.1	0.1668	0.18	0.931	2.0	1.4	Pass
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Applicability of existing CIPAC methods	None available																																																	

This method is applicable to the determination of active ingredient in XDE-729 Me technical. The method can be considered to be acceptably validated in accordance with SANCO/3030/99 rev. 4.

B.5.1.2 Impurities (IIA 4.1)

Please refer to the Confidential Information (Annex C/Volume 4).

B.5.1.3 Plant protection product (IIIA 5.1)

Reference: Speak, T. 22nd November 2010 “Analytical Method for the Determination of XR-729 Methyl and cloquintocet-Mexyl in GF-2573 and GF-2685, Laboratory Study ID: DAS-AM-G-10-34.

Determination of XDE-729 Methyl and Cloquintocet-mexyl in ‘GF-2573’

(It should be noted that the study report refers to the active substance as XR-729; however, the notifier has confirmed that XR-729 is identical to XDE-729)

‘GF-2573’ (approx.2.0 g) was weighed into a glass bottle, and propiophenone internal standard solution and methanol were added. Samples were shaken and then passed through a 0.2µm PTFE syringe filter to remove suspended particles. The resulting solution was analysed by High Performance Liquid Chromatography (HPLC) using a Luna C8 (2) column (150 x 4.6 mm i.d., 3 µm particle size) with an acetonitrile + 0.1% phosphoric acid/water + 0.1% phosphoric acid mobile phase coupled with Ultraviolet detection (UV) at 300 nm. Quantification was achieved by peak area measurements using an internal standard calculation. Representative chromatograms were submitted and were acceptable, with no signs of interference. The method has been satisfactorily validated and a summary of validation data are given in the table below.

Component (code)	Analyte	linearity (µg/ml)	Theoretical content (%)	Actual Content (%)	Recovery (%)	Precision (%w/w)
GF-2573	XDE-729 Methyl	43-1701 (equivalent to 0.1 - 4.3 %w/w (GF- 2573)) R ² =0.99978	0.529	0.532	100.6	0.84
			0.640	0.640	100.0	0.84
			0.855	0.854	99.9	0.85
			0.848	0.849	100.0	0.85
			0.858	0.857	99.9	0.85
			1.058	1.053	99.5	0.85
			1.268	1.265	99.8	0.85
						0.85
						0.85
						0.85
Mean = 0.85 %RSD = 0.5						
GF-2573	Cloquintocet- mexyl	40 – 1597 (equivalent to 0.1 – 4.0 %w/w (GF- 2573)) R ² =0.99964	0.527			0.81
			0.637			0.81
			0.851			0.81
			0.844		101.1	0.81
			0.854		100.3	0.81
			1.053	0.532	99.9	0.81
			1.262	0.639	100.1	0.81
				0.850	100.00	0.81
				0.845	99.6	0.81
				0.854	100.1	0.81
		(248 – 9927 µg/mL Propiophenon e internal standard, R ² =0.99960)	1.049		Mean =	Mean = 0.81 %RSD = 0.2
			1.263		100.2%	
					%RSD = 0.5	

Linearity – A linear relationship between peak area and concentration was noted for XDE-729 methyl, cloquintocet-mexyl and propiophenone. Eight concentrations (with single measurement) were tested over the concentration range *ca.* 43-1701 µg/mL XDE-729 methyl (equivalent to 0.1 - 4.3 %w/w (GF-2573)), 40 – 1597 µg/mL Cloquintocet-mexyl (equivalent to 0.1 – 4.0 %w/w (GF-2573)) and 248 – 9927 µg/mL Propiophenone). A plot of peak area against concentration gave a straight line (see table above for correlation coefficients) which shows that the plot is linear with respect to XDE-729 methyl, Cloquintocet-mexyl and Propiophenone concentration.

Recovery (accuracy) – Recoveries were determined over the range of approximately 75-125% w/w active ingredient. Recovery samples were prepared by weighing varying amounts of the test substances, XDE-729 methyl (TSN031117-0004), cloquintocet-mexyl (SM04H044) into glass bottles with the formulation inert mixes (TSN032900-0002) to give a total weight of approximately 2.0 g for GF-2573. Seven synthetic samples were prepared with single measurement recovery determinations made at the seven different analyte concentrations. All recoveries for XDE-729 Methyl were in the range of *ca.* 99.5-100.6% (mean = 100 %, RSD 0.3%) and for Cloquintocet-mexyl 99.6-101.1% (mean 100.2 %, RSD 0.5%).

Precision (repeatability) – Precision samples were prepared and analysed five times each on two separate days. The individual recoveries obtained for five replicate samples (with single measurement) of one batch of test item were determined. The associated %RSD of the five replicates was 0.5% (XDE-729 Methyl) and 0.2% (Cloquintocet-mexyl) and fulfils the Horwitz criteria (Horwitz RSDr applicable to 0.85% w/w and 0.81% are 2.74 and 2.76 respectively), which is a good indication of analytical precision.

Specificity - The retention times of the sample and reference item were compared and were identical, indicating that the method is specific to the analyte of interest. Chromatograms of formulation blanks (the formulation containing all co-formulants, but no active ingredient or cloquintocet-mexyl) demonstrated no interference at the retention times of interest.

B.5.2 Analytical methods (residue) for treated plants, plant products, foodstuffs of plant and animal origin and feedingstuffs (IIA 4.2.1, IIIA 5.2)

Summary of monitoring methods

Matrix	Method	Analyte	Limit of Quantification	Annex Point and Reference
Crops XDE-729 (Multi-residue)				
<u>Wet crops</u> kale leaves <u>Dry crops</u> barley grain <u>Oily crops</u> oilseed rape seed <u>Acidic crops</u> lemon	DAS 110293 (LC/MS/MS)	XDE-729 ME XDE-729 acid	0.010 mg/kg	IIA 4.3/01 Daneva, E. Taeufer, A. (2011)
Crops XDE-729 ILV (Multi-residue)				
<u>Wet crops</u> cabbage <u>Oily crops</u> oilseed rape seed	DAS 110712 (LC/MS/MS)	XDE-729 ME XDE-729 acid	0.010 mg/kg	IIA 4.3/02 Bacher, R. (2011)

Matrix	Method	Analyte	Limit of Quantification	Annex Point and Reference
Crops XDE-729 (Enforcement)				
<u>Wet crops</u> turnip root wheat forage <u>Dry crops</u> barley grain barley hay barley straw wheat grain wheat hay wheat straw <u>Oily crops</u> canola seed soybean seed <u>Acidic crops</u> apple, whole orange, whole <u>Processed Products</u> aspirated grain bran bread flour germ gluten shorts starch	DAS 110004 (LC/MS/MS)	XDE-729 ME XDE-729 acid	0.010 mg/kg	IIA 4.3/03 Ma, M. (2012)
Crops XDE-729 ILV (Enforcement)				
<u>Wet crops</u> wheat forage <u>Oily crops</u> canola oil <u>Processed Products</u> wheat flour	DAS 110825 (LC/MS/MS)	XDE-729 ME XDE-729 acid	0.010 mg/kg	IIA 4.3/04 Robaugh, D. A. (2012)

Matrix	Method	Analyte	Limit of Quantification	Annex Point and Reference
Crops XDE-729 (Data Generation)				
<u>Wet crops</u> turnip root wheat forage <u>Dry crops</u> barley grain barley hay barley straw wheat grain wheat hay wheat straw <u>Oily crops</u> canola seed soybean seed <u>Acidic crops</u> apple, whole orange, whole <u>Processed Products</u> aspirated grain bran bread flour germ gluten shorts starch	DAS 110005 (LC/MS/MS)	XDE-729 ME XDE-729 acid	0.010 mg/kg	IIA 4.3/05 Olberding, E. L. (2011)
Crops Safener (Enforcement)				
<u>Wet crops</u> wheat forage <u>Dry crops</u> wheat grain wheat hay wheat straw	Enviro-Test M313 (LC/MS/MS)	cloquintocet- mexyl cloquintocet acid	0.010 mg/kg	IIA 4.3/06 McLean, N. (2006)

Matrix	Method	Analyte	Limit of Quantification	Annex Point and Reference
Crops Safener ILV (Enforcement)				
<u>Wet crops</u> wheat shoots <u>Dry crops</u> wheat grain wheat straw	DAS 040096 (LC/MS/MS)	cloquintocet- mexyl cloquintocet acid	0.010 mg/kg	IIA 4.3/07 Glass, T. (2005)
Animal Tissues XDE-729 (Multi-residue)				
<u>Bovine</u> muscle liver kidney whole milk <u>Poultry</u> muscle eggs	DAS 110574 (LC/MS/MS)	XDE-729 ME XDE-729 acid	0.010 mg/kg	IIA 4.3/08 Lindner, M. (2011)
<u>Bovine</u> fat	DAS 110750 (LC/MS/MS)	XDE-729 ME XDE-729 acid	0.010 mg/kg	IIA 4.3/09 Lindner, M. (2011)
Animal Tissues XDE-729 ILV (Multi-residue)				
<u>Bovine</u> muscle whole milk fat	DAS 110294 (LC/MS/MS)	XDE-729 ME XDE-729 acid	0.010 mg/kg	IIA 4.3/10 Bacher, R. (2011)

Matrix	Method	Analyte	Limit of Quantification	Annex Point and Reference
Animal Tissues XDE-729 (Enforcement)				
<u>Bovine</u> muscle liver kidney fat whole milk cream <u>Poultry</u> muscle liver fat eggs	DAS 110505 (LC/MS/MS)	XDE-729 ME XDE-729 acid	0.010 mg/kg	IIA 4.3/11 Ma M. Li. Q. (2012)
Animal Tissues XDE-729 ILV (Enforcement)				
<u>Bovine</u> muscle liver kidney fat whole milk cream <u>Poultry</u> muscle liver fat eggs	DAS 110828 (LC/MS/MS)	XDE-729 ME XDE-729 acid	0.010 mg/kg	IIA 4.3/12 Langridge, G. (2012)

B.5.3 Analytical methods (residue) in soil, water and air (IIA 4.2.2 to 4.2.4, IIIA 5.2)

Matrix	Method	Analyte	Limit of Quantification	Annex Point and Reference
Soil XDE-729				
Soil	DAS 110716 (LC/MS/MS)	XDE-729 ME XDE-729 acid X11449757	0.05 µg/kg	IIA 4.4/01 Blakeslee, B. A. (2012)
Soil XDE-729 ILV				
Soil	DAS 120894 (LC/MS/MS)	XDE-729 ME XDE-729 acid X11449757	0.05 µg/kg	IIA 4.4/02 Gemrot, F. (2012)
Water XDE-729				
<u>Water</u> drinking ground surface	DAS 110718 (LC/MS/MS)	XDE-729 ME XDE-729 acid X11449757 X11406790	0.05 µg/L	IIA 4.5/01 Rodrigues Junior, A. Souza, N. Li. Q. (2012)
Water XDE-729 ILV				
<u>Water</u> drinking ground surface	DAS 110827 (LC/MS/MS)	XDE-729 ME XDE-729 acid X11449757 X11406790	0.05 µg/L	IIA 4.5/02 Gemrot, F. (2012)
Air XDE-729				
<u>Air</u> ambient warm, humid	DAS 110028 (LC/MS/MS)	XDE-729 ME XDE-729 acid	0.8 µg/m ³	IIA 4.7/01 Class, T. (2011)
Body Fluids XDE-729				
<u>Body Fluids</u> urine whole blood	DAS 110029 (LC/MS/MS)	XDE-729 ME XDE-729 acid	0.05 mg/L	IIA 4.8/01 Senciuc, M. (2011)

B.5.2.1 Multi-residue Method Testing for the Determination of XDE 729 Methyl Ester and XDE 729 Acid in Plant Matrices

Reference: Daneva, E. and Taeufer, A. (2011) Validation of a Multi-Residue Method for Determination of a XDE 729 Methyl Ester and XDE 729 Acid on Different Matrices of Plant Origin. Dow AgroSciences LLC Study Number 110293
Eurofins Analytical Services Study Number S11-02423

Guidelines: SANCO/825/00 rev. 8.1
SANCO/3029/99 rev. 4

This method of analysis has been submitted in support of post-registration monitoring and control for components included in the residue definition for plants and animals. The proposed residue definition for Risk Assessment and Enforcement/Monitoring in plants and livestock is XDE-729 methyl and XDE-729 acid.

Matrix, limit of quantitation	kale leaves (wet crops) 0.01 mg/kg barley grain (dry crops) 0.01 mg/kg oilseed rape seed (oily crops) 0.01 mg/kg lemon (acidic crops) 0.01 mg/kg
Description	<u>Scope</u> This QuEChERS multi-residue method is applicable for the quantitative determination of residues of XDE-729 methyl ester and XDE-729 acid in agricultural commodities representative of the four European crop groupings. The method was validated over the concentration range of 0.01-0.10 mg/kg with a validated limit of quantitation of 0.01 mg/kg. <u>Principle</u> Residues of XDE-729 methyl ester and XDE-729 acid were extracted from the sample matrices (specimen weight 2.5 g for oilseed rape (seeds), 5.0 g for barley (grain) and 10 g for kale (leaves) and lemon (whole fruit)) by shaking with an acetonitrile/water solution. After addition of a salt mixture consisting of magnesium sulfate, sodium chloride, trisodium citrate dihydrate, and disodium hydrogen citrate sesquihydrate, the extract was shaken. Minor modifications to the procedure were made for acidic samples (e.g., lemons) to adjust the pH to be less acidic. After centrifugation, an aliquot of the acetonitrile extract was purified by freezing out and/or dispersive solid phase extraction using a primary secondary amine sorbent or ODS and subsequently diluted to volume with aqueous 0.1% formic acid. After preparation, the samples were analyzed by liquid chromatography using a Synergi Hydro-RP C18 50 x 2.0 mm, 2.5 μm, 100A (Phenomenex cat. No.00B-4387-BO) column. Separation was achieved by gradient elution (Mobile Phase Conditions were Solvent A: acetonitrile + 0.1% (v/v) formic acid and solvent B: water + 0.1% (v/v) formic acid), retention times were approx. 3.8 min (XDE-729 methyl ester) and 1.6 min (XDE-729 acid). The Synergi Hydro-RP C18 column was coupled to positive-ion electrospray tandem mass spectrometry (LC/MS/MS), monitoring two MS/MS transitions characteristic of each analyte. The ion transitions monitored were :

	XDE-729 ME <i>m/z</i> 345/250 (quantitation) <i>m/z</i> 345/207 (confirmation) XDE-729 acid <i>m/z</i> 331/250 (quantitation) <i>m/z</i> 331/207 (confirmation) <u>Calibration</u> For each analyte, the linearity of detector response was evaluated using solvent standard solutions as well as matrix-matched standard solutions. Calibration curves were calculated by linear regression analysis without weighting. For solvent standard solutions, calibration curves resulting from the injection of a minimum of five standards over the concentration range of 0.10-50 ng/mL (equivalent to 0.002 mg/kg-1.0 mg/kg) demonstrated linearity with coefficients of determination (r^2) of at least 0.990. For matrix-matched standard solutions, calibration curves resulting from the injection of a minimum of five standards over the concentration range of 0.10-15 ng/mL (equivalent to 0.002 mg/kg-0.33 mg/kg) demonstrated linearity with coefficients of determination (r^2) of at least 0.990. See comments below for matrix effects. <u>Validation</u> For each analyte, the method was validated over the concentration range of 0.01-0.10 mg/kg with a limit of quantitation of 0.01 mg/kg. At the 0.01-mg/kg level (LOQ), the mean recoveries were within the range of 70-102%, with relative standard deviations within the range of 1.3-29%. At the 0.10-mg/kg level (10x LOQ), the mean recoveries were within the range of 73-101%, with relative standard deviations within the range of 1.6-18%. It is noted however that although the mean recoveries for lemon (acidic crop) were acceptable, individual recoveries for the quantitation and confirmatory transitions for detection on XDE-729 acid in this matrix were low (see table below). <u>Selectivity/Confirmation</u> The LC/MS/MS method is highly selective for both the quantitation and confirmation of XDE-729 methyl ester and XDE-729 acid. Analysis of control samples resulted in no significant signals at the expected retention times of the analytes. Interference at the retention times of the analytes was below 30% of the LOQ for the control crop samples in compliance with SANCO Guideline 825/00 rev.8.1. Unambiguous identification is ensured by monitoring two MS/MS transitions characteristic of each analyte as follows: XDE-729 ME <i>m/z</i> 345/250 (quantitation) <i>m/z</i> 345/207 (confirmation) XDE-729 acid <i>m/z</i> 331/250 (quantitation) <i>m/z</i> 331/207 (confirmation) Confirmation was demonstrated by determining the recovery and precision
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for both MS/MS transitions; these data are listed in the tables below.

Stability of Solutions and Sample Extracts

All stock and calibration standard solutions were stored in a refrigerator at 3-8 °C when not in use.

Stock solutions of XDE-729 methyl ester and XDE-729 acid prepared in acetonitrile were tested after 138 days of storage and were found to be stable.

Calibration standard solutions of XDE-729 methyl ester and XDE-729 acid prepared in acetonitrile/0.10% formic acid (10:90) were tested after 13 days of storage and found to be stable.

Final sample extracts were evaluated for storage periods ranging from 6 to 12 days. In general, the mean recoveries of the second injection of sample extracts were within 20% of the mean recoveries obtained from the first injection. Exceptions were observed for the determination of XDE-729 methyl ester in barley grain at the 0.01 mg/kg level where the recovery of the second injection (after 9 days) decreased 22% and for the determination of XDE-729 methyl ester in kale leaves at the 0.01 mg/kg level where the recovery of the second injection (after 10 days) decreased 25%. It is also noted that mean recovery of the XDE-729 acid in oilseed is outside acceptable recovery levels, since the initial recovery was low, therefore a small amount of degradation has resulted in a recovery that is outside acceptable limits following storage after 7 days.

Matrix Effects

Matrix effects were determined by comparing the peak areas of solvent calibration standards to peak areas of matrix-matched standards at two concentration levels.

For XDE-729 methyl ester, matrix effects (suppression) were greater than 20% for both MS/MS transitions and thus considered significant.

Therefore, matrix-matched standards were used for quantitation of XDE-729 methyl ester in these matrices.

For XDE-729 acid, matrix effects for kale leaves and barley grain were less than 20% for both MS/MS transitions and thus considered insignificant.

Therefore, neat solvent standard solutions were used for quantitation of XDE-729 acid in these matrices. For oilseed rape seed and lemon, matrix effects (enhancement) were greater than 20% for both MS/MS transitions and thus considered significant. Therefore, matrix-matched standards were used for quantitation of XDE-729 acid in these matrices.

Extraction Efficiency

It is noted that extraction efficiencies have not been addressed as part of the method validation; however, residues are not expected to be found in cereal grain (the representative use). Therefore, extraction efficiency does not need to be addressed for this evaluation. This will need to be addressed in future however if new uses give rise to positive residues.

Validation Data (quantitation)	Matrix	Fortification Level (mg/kg)	Recovery Rate (%)		RSD (%)	n
			mean	range		
XDE-729 ME <i>m/z</i> 345→250	kale leaves (wet crop)	0.01	79*	73-86	7.6	5
		0.10	73	71-75	2.5	5
		0.01-0.10	76	71-86	7.1	10
XDE-729 ME <i>m/z</i> 345→250	barley grain (dry crop)	0.01	100**	99-102	1.3	5
		0.10	93	84-102	7.1	5
		0.01-0.10	97	84-102	6.2	10
XDE-729 ME <i>m/z</i> 345→250	oilseed rape seed (oily crop)	0.01	97***	87-105	7.4	5
		0.10	101	88-109	7.8	5
		0.01-0.10	99	87-109	7.5	10
XDE-729 ME <i>m/z</i> 345→250	lemon (acidic crop)	0.01	102****	78-119	16	5
		0.10	78	73-79	6.1	5
		0.01-0.10	90	73-119	19	10

* Following 10 days storage mean recovery (Kale) = 54%

** Following 10 days storage mean recovery (barley) = 78%

*** Following 13 days storage mean recovery (rape) = 95%

**** Following 13 days storage mean recovery (lemon) = 100%

Validation Data (quantitation)	Matrix	Fortification Level (mg/kg)	Recovery Rate (%)		RSD (%)	n
			mean	range		
XDE-729 acid <i>m/z</i> 331→250	kale leaves (wet crop)	0.01	70*	68-75	4.2	5
		0.10	85	65-96	14	5
		0.01-0.10	78	65-96	15	10
XDE-729 acid <i>m/z</i> 331→250	barley grain (dry crop)	0.01	78**	72-86	7.0	5
		0.10	92	86-100	6.6	5
		0.01-0.10	85	72-100	11	10
XDE-729 acid <i>m/z</i> 331→250	oilseed rape seed (oily crop)	0.01	70***	60-75	10	4
		0.10	85	83-88	2.2	5
		0.01-0.10	78	60-88	12	9
XDE-729 acid <i>m/z</i> 331→250	lemon (acidic crop)	0.01	80****	51-104	29	5
		0.10	78	71-80	5.9	5
		0.01-0.10	79	51-104	20	10

* Following 7 days storage mean recovery (kale) = 61%

** Following 10 days storage mean recovery (barley) = 83%

*** Following 7 days storage mean recovery (oilseed) = 59%

**** Following 10 days storage mean recovery (Lemon) = 66%

Validation Data (confirmation)	Matrix	Fortification Level (mg/kg)	Recovery Rate (%)		RSD (%)	n
			mean	range		
XDE-729 ME <i>m/z</i> 345→207	kale leaves (wet crop)	0.01	76	69-83	7.6	5
		0.10	73	70-75	2.5	5
		0.01-0.10	74	69-83	5.8	10
XDE-729 ME <i>m/z</i> 345→207	barley grain (dry crop)	0.01	97	95-100	2.4	5
		0.10	99	92-104	5.1	5
		0.01-0.10	98	92-104	4.0	10
XDE-729 ME <i>m/z</i> 345→207	oilseed rape seed (oily crop)	0.01	99	88-105	8.3	5
		0.10	101	98-104	2.4	5
		0.01-0.10	100	88-105	5.8	10
XDE-729 ME <i>m/z</i> 345→207	lemon (acidic crop)	0.01	91	66-103	16	5
		0.10	83	77-87	4.6	5
		0.01-0.10	87	66-103	12	10

Validation Data (confirmation)	Matrix	Fortification Level (mg/kg)	Recovery Rate (%)		RSD (%)	n
			mean	range		
XDE-729 acid <i>m/z</i> 331→207	kale leaves (wet crop)	0.01	70	66-74	5.4	5
		0.10	86	60-97	18	5
		0.01-0.10	78	60-97	17	10
XDE-729 acid <i>m/z</i> 331→207	barley grain (dry crop)	0.01	75	71-83	6.4	5
		0.10	88	81-98	7.8	5
		0.01-0.10	82	71-98	11	10
XDE-729 acid <i>m/z</i> 331→207	oilseed rape seed (oily crop)	0.01	71	65-77	7.3	4
		0.10	80	79-82	1.6	5
		0.01-0.10	76	65-82	7.7	9
XDE-729 acid <i>m/z</i> 331→207	lemon (acidic crop)	0.01	81	55-103	27	5
		0.10	74	68-80	6.1	5
		0.01-0.10	77	55-103	20	10

Conclusion

The QuEChERS method has been validated in all areas; However, it has been noted that although the mean recoveries for lemon (acidic crop) were acceptable, individual recoveries for the quantitation and confirmatory transitions for detection on XDE-729 acid in this matrix were low (see table above).

Storage stability of final sample extracts demonstrated unacceptable levels of degradation for the determination of XDE-729 methyl ester in barley grain at the 0.01 mg/kg level where the recovery of the second injection (after 9 days) decreased 22%, and for the determination of XDE-729 methyl ester in kale leaves at the 0.01 mg/kg level where the recovery of the second injection (after 10 days) decreased 25%. It is also noted that mean recovery of the XDE-729 acid in oilseed is outside acceptable recovery levels, since the initial recovery was low, therefore a small amount of degradation has resulted in a recovery that is outside acceptable limits following storage after 7 days.

B.5.2.2 Independent Laboratory Validation of the Multi-residue Method for the Determination of XDE-729 Methyl Ester and XDE-729 Acid in Plant Matrices

Reference: Bacher, R. (2011) XDE-729: Independent Laboratory Validation of a Multi-Residue Method for the Determination of XDE-729 Methyl Ester and XDE-729 Acid in Plant Matrices. Dow AgroSciences LLC Study Number 110712 PTRL Europe Study Number P 2399 G

Guidelines: SANCO/825/00 rev. 8.1
SANCO/3029/99 rev. 4

This method of analysis has been submitted in support of Post-registration monitoring and control for components included in the residue definition for plants and animals. The proposed residue definition for Risk Assessment and Enforcement/Monitoring in plants and livestock is XDE-729 methyl and XDE-729 acid.

Matrix, limit of quantitation	cabbage (wet crops) 0.01 mg/kg oilseed rape seed (oily crops) 0.01 mg/kg
Description	<u>Scope</u> The objective of this study was to independently validate Eurofins Agroservices Services multi-residue method (Study Number S11-02423), “Validation of a Multi-Residue Method for Determination of a XDE-729 Methyl Ester and XDE-729 Acid on Different Matrices of Plant Origin.” This QuEChERS multi-residue method is applicable for the quantitative determination of residues of XDE-729 methyl ester and XDE-729 acid in agricultural commodities. The method was independently validated at concentrations of 0.01 mg/kg and 0.10 mg/kg for a representative wet crop (cabbage) and oily crop (oilseed rape seed). <u>Principle</u> See description of the method above in Section B 5.2.1. <u>Calibration</u> For each analyte, the linearity of detector response was evaluated using solvent standard solutions as well as matrix-matched standard solutions. Calibration curves were calculated by linear regression analysis with 1/x weighting. For XDE-729 methyl ester solvent standard solutions, calibration curves resulting from the injection of a minimum of six standards over the concentration range of 0.02-5 ng/mL demonstrated linearity with correlation coefficients of (r) of at least 0.99. For XDE-729 acid solvent standard solutions, calibration curves resulting from the injection of a minimum of six standards over the concentration range of 0.10-50 ng/mL demonstrated linearity with correlation coefficients (r) of at least 0.99. For XDE-729 acid matrix-matched standard solutions, calibration curves resulting from the injection of a minimum of six standards over the concentration range of 0.10-10 ng/mL demonstrated linearity with correlation coefficients of (r) of at least 0.990.

Validation

For each analyte, the method was validated over the concentration range of 0.01-0.10 mg/kg with a limit of quantitation of 0.01 mg/kg.

At the 0.01-mg/kg level (LOQ), the mean recoveries were within the range of 62-114%, with relative standard deviations within the range of 2-6%.

At the 0.10-mg/kg level (10x LOQ), the mean recoveries were within the range of 72-113%, with relative standard deviations within the range of 2-14%.

It is noted that there are a couple of low individual recoveries for the determination of XDE-729 acid in cabbage (59%) for the confirmatory transition; however the mean recovery falls within the acceptable range.

The above results generally comply with the acceptance criteria of SANCO/825/00/rev. 8.1.

Selectivity/Confirmation

The LC/MS/MS method is highly selective for both the quantitation and confirmation of XDE-729 methyl ester and XDE-729 acid. Analysis of control samples resulted in no significant signals at the expected retention times of the analytes. Interference at the retention times of the analytes was below 30% of the LOQ for the control crop samples in compliance with SANCO Guideline 825/00 rev.8.1. Unambiguous identification is ensured by monitoring two MS/MS transitions characteristic of each analyte as follows:

XDE-729 ME m/z 345/250 (quantitation)
 m/z 345/207 (confirmation)

XDE-729 acid m/z 331/250 (quantitation)
 m/z 331/207 (confirmation)

Confirmation was demonstrated by determining the recovery and precision for both ion mass transitions; these data are listed in the tables below.

Stability of Solutions and Sample Extracts

All stock and calibration standard solutions were stored in a refrigerator at approximately 4 °C when not in use. The evaluation of the stability of stock, spiking, and calibration solutions is not a requirement of an independent laboratory validation. Specimen extracts however are considered to be sufficiently stable during the course of the study as the recoveries obtained were all within the required range with acceptable variations (60 –120 % for LOQ specimens, 70-120 % for 10xLOQ specimens, RSD ≤20 %).

Matrix Effects

Matrix effects were determined by comparing the peak areas of solvent calibration standards to peak areas of matrix-matched standards at two concentration levels.

In these experiments, matrix effects were demonstrated to be insignificant

	(< 20 %) with exception of the determination of XDE-729 acid in oilseed rape seed. Thus calibration solutions in neat solvent were used for the determination of residues in all matrix/analyte combinations with the exception of determination of XDE-729 acid in oilseed rape seed. Oilseed rape seed specimens were evaluated for XDE-729 acid using matrix-matched standards.
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Validation Data (quantitation)	Matrix	Fortification Level (mg/kg)	Recovery Rate (%)		RSD (%)	n
			mean	range		
XDE-729 ME <i>m/z</i> 345→250	cabbage (wet crop)	0.01	114	109-123	5	5
		0.10	113	111-116	2	5
		0.01-0.10	114	109-123	4	10
XDE-729 ME <i>m/z</i> 345→250	oilseed rape seed (oily crop)	0.01	109	103-115	5	5
		0.10	108	97-113	6	5
		0.01-0.10	108	97-115	5	10

Validation Data (quantitation)	Matrix	Fortification Level (mg/kg)	Recovery Rate (%)		RSD (%)	n
			mean	range		
XDE-729 acid <i>m/z</i> 331→250	cabbage (wet crop)	0.01	63	60-67	5	5
		0.10	75	63-84	14	5
		0.01-0.10	69	60-84	14	10
XDE-729 acid <i>m/z</i> 331→250	oilseed rape seed (oily crop)	0.01	92	88-94	2	5
		0.10	89	86-94	4	5
		0.01-0.10	90	86-94	3	10

Validation Data (confirmation)	Matrix	Fortification Level (mg/kg)	Recovery Rate (%)		RSD (%)	n
			mean	range		
XDE-729 ME <i>m/z</i> 345→207	cabbage (wet crop)	0.01	112	107-115	3	5
		0.10	109	106-112	2	5
		0.01-0.10	111	106-115	3	10
XDE-729 ME <i>m/z</i> 345→207	oilseed rape seed (oily crop)	0.01	106	98-112	6	5
		0.10	107	104-111	2	5
		0.01-0.10	106	98-112	4	10

Validation Data (confirmation)	Matrix	Fortification Level (mg/kg)	Recovery Rate (%)		RSD (%)	n
			mean	range		
XDE-729 acid <i>m/z</i> 331→207	cabbage (wet crop)	0.01	62	59-65	4	5
		0.10	72	60-81	14	5
		0.01-0.10	67	59-81	13	10
XDE-729 acid <i>m/z</i> 331→207	oilseed rape seed (oily crop)	0.01	91	88-97	4	5
		0.10	90	86-94	3	5
		0.01-0.10	91	86-97	4	10

Conclusion

According to SANCO/825/00 rev.8.1, an ILV must be conducted with samples of representative commodities of all matrix groups for which the primary method is required, with the same number of samples and fortification levels. If the primary method is identical for all matrix groups, it is sufficient to perform the ILV for commodities of two of these groups, one of them with high water content. In this instance, the primary method is not the same for all commodities; however, the ILV study has selected two matrix groups that allow demonstration of both procedures. Given that the representation use is cereal, and the method is not the same for all matrix types, it is considered that ideally a dry crop should have been considered in the ILV study. In this instance however, given that the method for a wet crop (cabbage) is the same as the method for cereal, the RMS considers the ILV study is acceptable.

B.5.2.3 Enforcement Method for the Determination of XDE-729 Methyl Ester and XDE-729 Acid in Plant Matrices

Reference: Ma, M.; Li, Q. (2012) Method Validation Study for the Determination of Residues of XDE-729 Methyl Ester and XDE-729 Acid in Agricultural Commodities and Wheat Processed Products using Offline Solid-Phase Extraction and Liquid Chromatography with Mass Spectrometry Detection, Dow AgroSciences LLC Study Number 110004

Guidelines:

SANCO/825/00 rev. 8.1

SANCO/3029/99 rev. 4

This method has been submitted in support of both pre-registration data requirements and post-registration monitoring and control for components included in the residue definition for plants and animals. The proposed residue definition for Risk Assessment and Enforcement/Monitoring in plants and livestock is XDE-729 methyl and XDE-729 acid. The enforcement method validated above (QuEChERS multi-residue method) was satisfactorily validated according to SANCO/825/00 rev.8.1, therefore this method is an additional method in terms of support of post registration monitoring. It is noted however that although mean recoveries in acidic matrices (lemon) and wet matrices (cabbage) were acceptable for the QuEChERS method, individual recoveries were occasionally low for both the quantitation and confirmatory transitions for detection of XDE-729 acid in acidic crops and for the confirmatory transition for the detection of XDE-729 acid in wet crops in the original method validation report and the ILV study report

respectively. The following method does demonstrate a more robust recovery data set. The following method has also been submitted in support of pre-registration data

Matrix, limit of quantitation	turnip root (wet crops) 0.01 mg/kg wheat forage (wet crops) 0.01 mg/kg barley grain (dry crops) 0.01 mg/kg barley hay (dry crops) 0.01 mg/kg barley straw (dry crops) 0.01 mg/kg wheat grain (dry crops) 0.01 mg/kg wheat hay (dry crops) 0.01 mg/kg wheat straw (dry crops) 0.01 mg/kg canola seed (oily crops) 0.01 mg/kg soybean seed (oily crops) 0.01 mg/kg apple, whole (acidic crops) 0.01 mg/kg orange, whole (acidic crops) 0.01 mg/kg aspirated grain 0.01 mg/kg bran 0.01 mg/kg bread 0.01 mg/kg flour 0.01 mg/kg germ 0.01 mg/kg gluten 0.01 mg/kg shorts 0.01 mg/kg starch 0.01 mg/kg
Description	<u>Scope</u> This method is applicable for the quantitative determination of residues of XDE-729 methyl ester and XDE-729 acid in agricultural commodities representative of the four European crop groupings and wheat processed products. The method was validated over the concentration range of 0.01-1.00 mg/kg with a validated limit of quantitation of 0.01 mg/kg. <u>Principle</u> Residues of XDE-729 methyl ester and XDE-729 acid were extracted from a 5.0-gram sample by homogenizing and shaking with 100.0 mL of an acetonitrile/water (80:20) solution. Fifty microliters of a glycerin/methanol solution (10:90 w/v) were added to a 1000-µL aliquot of the acetonitrile/water extraction solution and the sample was concentrated to near dryness. The sample extract was then diluted to 1.0 mL with aqueous 0.1% formic acid and purified using a Phenomenex Strata-X reversed-phase polymeric solid-phase extraction (SPE) cartridge. After elution from the SPE cartridge with a methanol/water (95:5) solution containing 0.10% formic acid, 200 µL of a mixed stable-isotope internal standard solution was added to the eluate, which was subsequently concentrated to approximately 1 mL. The solution was then adjusted to 2.0 mL with water. After preparation, the samples were analyzed by liquid chromatography using a Zorbax SB-C8 column (gradient elution: Mobile Phase A – water containing 0.10% formic acid and Mobile Phase B – methanol containing 0.10% formic acid) coupled with positive-ion electrospray tandem mass spectrometry (LC/MS/MS), monitoring two MS/MS transitions

characteristic of each analyte and one MS/MS transition characteristic of each internal standard.

Calibration

For each analyte, the linearity of detector response was evaluated using solvent standard solutions. Calibration curves were calculated by linear regression analysis with 1/x weighting. For the least squares equation which describes the detector response as a function of the standard concentration, calibration curves resulting from the injection of a minimum of eight standards over the concentration range of 0.075-12.5 ng/mL (equivalent to 0.003 mg/kg – 0.5 mg/kg) demonstrated linearity with coefficients of determination (r^2) of at least 0.999.

Validation

The method validation study was conducted to determine the recovery levels and the precision of the method for the determination of residues of XDE-729 methyl ester and XDE-729 acid in agricultural commodities. The performance of the analytical method was determined with each set of samples by fortifying aliquots of the appropriate control matrix with a mixed solution of XDE-729 methyl ester and XDE-729 acid and analyzing the set following the procedures described within the study report. Samples were fortified at the limit of detection (LOD) of 0.003 µg/g, the limit of quantitation (LOQ) of 0.010 µg/g, and at 1.00 µg/g. Samples fortified at the LOD were analyzed only to demonstrate observable peaks at the LOD level; the results were not included for average percent recovery calculations. An unfortified control matrix and reagent blank were also included in each set.

For each analyte, the method was validated over the concentration range of 0.01-1.00 mg/kg with a limit of quantitation of 0.01 mg/kg.

The individual recoveries were within the range of 76-104%.

At the 0.01-mg/kg level (LOQ), the mean recoveries were within the range of 86-95%, with relative standard deviations within the range of 1.1-7.4%.

At the 1.00-mg/kg level (100x LOQ), the mean recoveries were within the range of 91-100%, with relative standard deviations within the range of 1.1-6.5%.

It is noted that full validation data are only obtained for the primary (quantitation) MS/MS transition. According to SANCO/825/00 rev. 8.1 the additional confirmatory transition should also be validated for calibration data, recovery and precision data for samples fortified at the LOQ (n=5) and for 2 blank samples. It is a short coming of the study that full validation data has only been presented for the primary transition; however, this data has been presented for high water and high oil matrices for the ILV of this method (see below). Only two matrices were considered in the ILV, however the primary extraction method is the same

(For information, the notifier has since informed the RMS “the report will be revised to include the confirmatory transition data, including calibration, recovery and precision. The revised version will be available by June 2013”. In the absence of this report at the time of submission, for the reasons shown above, the method may still be considered fit for purpose according to SANCO/825/00 rev. 8.1).

The LC/MS/MS method is highly selective for both the quantitation and confirmation of XDE-729 methyl ester and XDE-729 acid. Analysis of control samples resulted in no significant signals at the expected retention times of the analytes. Unambiguous identification is ensured by monitoring two MS/MS transitions characteristic of each analyte and one MS/MS transition characteristic of each internal standard as follows:

Confirmation was determined according to Commission Decision (2002/657/EC). The confirmation ratios for each analyte were determined by calculating the peak area ratios of the confirmation transition to the quantitation transition. Confirmation of the presence of the analytes in the recovery samples was indicated when the retention times of the analytes in the samples matched the retention times of the analytes in the calibration standards, and the confirmation ratios of the analytes in the samples were $\pm 20\%$ of the average confirmation ratios of the standards. The confirmation ratios for each analyte in all sample matrices were within $\pm 20\%$ of the average confirmation ratios of the standards, indicating that the method is selective for the determination of XDE-729 methyl ester and XDE-729 acid in agricultural commodities and wheat processed products. This data is contained in the report.

The stability of the stock solutions and calibration standard solutions was demonstrated in the Dow AgroSciences method (Study Number 110005; IIA 4.3/05), ‘Determination of Residues of XDE-729 Methyl Ester and XDE-729 Acid in Agricultural Commodities and Wheat Processed

Products using Online Solid-Phase Extraction and Liquid Chromatography with Tandem Mass Spectrometry.’

Stock solutions of XDE-729 methyl ester and XDE-729 acid prepared in methanol were tested after 295 days of storage at ambient temperature and were found to be stable.

Calibration standard solutions of XDE-729 methyl ester and XDE-729 acid prepared in methanol/water (20:80) were tested after 131 days of storage at approximately 4 °C and were found to be stable.

The stability of the final sample extracts was evaluated as part of the current study. Final sample extracts were evaluated for a storage period of 11 days. For XDE-729 methyl ester, on Day 0 the recoveries ranged from 81-99%, while on Day 11, the recoveries ranged from 82-98%. For XDE-729 acid, on Day 0 the recoveries ranged from 76-95%, while on Day 11, the recoveries ranged from 82-97%. These results comply with the acceptance criteria of SANCO/825/00/rev.8.1.

Matrix Effects

Matrix effects were determined by comparing the peak areas of solvent calibration standards to peak areas of matrix-matched standards at a single concentration level.

For XDE-729 methyl ester, matrix effects ranged from -57% to 3% across all crops. For XDE-729 acid, matrix effects ranged from -3% to 132% across all crops. It is noted however that matrix effects are normalized through the use of stable-isotope internal standards, resulting in accurate quantitative results irrespective of the magnitude of matrix effects, therefore this is acceptable.

In this assay, the analytes and internal standards are quantitated using MS/MS transitions characteristic of each compound. When using stable-isotope labelled internal standards, there is a possibility that isotopic contributions will occur between the transitions used for quantitation of the unlabelled and labelled compounds. The degree of isotopic crossover for each analyte and its respective stable isotope internal standard was determined and expressed as the crossover factor. The study report demonstrates that the analyte → ISTD crossover contribution was minimal for the concentration range of the calibration curve and the concentration of the labelled internal standard. As a result, no significant mass spectral isotopic crossover was observed and therefore no correction of the measured quantitation ratio was performed. The study report states however that if through use of the method isotopic crossover is encountered, it should be assessed and the respective quantitation ratios corrected for accurate determination of concentrations.

Extraction Efficiency

The extraction efficiency of this method was demonstrated in the Dow AgroSciences method (Study Number 110005), ‘Determination of Residues of XDE-729 Methyl Ester and XDE-729 Acid in Agricultural Commodities and Wheat Processed Products using Online Solid-Phase Extraction and Liquid Chromatography with Tandem Mass Spectrometry.’

The extraction efficiency of the method was evaluated using representative wheat matrices from a XDE-729 methyl ester nature of residue study (Study Number 101080). Samples containing incurred ^{14}C residues were reanalyzed with the sample analysis procedure described above. Results obtained using the analytical method were equivalent to those obtained in the metabolism study, demonstrating the suitability of this analytical method for the determination of XDE-729 methyl ester and XDE-729 acid in plant matrices.

Summary of Extraction Efficiency of XDE-729 Methyl Ester and XDE-729 Acid from Wheat Samples

Sample Identification	XDE-729 Methyl Ester		XDE-729 Acid	
	110005 ($\mu\text{g/g}$)	101080 ($\mu\text{g/g}$)	110005 ($\mu\text{g/g}$)	101080 ($\mu\text{g/g}$)
R051002-48 (Grain Control)	ND ^a	NA ^b	ND	NA
R051002-55 (PH-Grain)	ND	NA	ND	NA
R051002-37 (Forage Control)	ND	NA	ND	NA
R051002-40 (PY-FOR)	0.016	0.011	ND	0.002
R051002-38 (PY-FOR-CQC)	0.011	0.012	ND	0.003
R051002-42 (Hay Control)	ND	NA	ND	NA
R051002-45 (PY-HAY)	0.013	0.014	ND	0.006
R051002-43 (PY-HAY-CQC)	0.019	0.022	ND	0.007
R051002-47 (Straw Control)	ND	NA	ND	NA
R051002-56 (PH-S)	0.012	0.009	ND	0.004
R051002-54 (PY-S)	0.023	0.019	ND	0.004

^a ND = Not detected, below 0.003 $\mu\text{g/g}$ limit of detection (LOD).

^b NA = Not applicable

Study number 110005 represents the enforcement method and study number 101080 represents the nature of residue metabolism study.

Validation Data (quantitation)	Matrix	Fortification Level (mg/kg)	Recovery Rate (%)		RSD (%)	n
			mean	range		
XDE-729 ME <i>m/z</i> 345→250	<u>wet crops</u>	0.01	90	86-92	2.5	12
	turnip root	1.00	93	92-95	1.1	12
	wheat forage	0.01-1.00	91	86-95	3.1	24
XDE-729 ME <i>m/z</i> 345→250	<u>dry crops</u>	0.01	90	79-97	5.3	36
	barley grain	1.00	95	85-101	3.4	35
	barley hay	0.01-1.00	92	79-101	5.3	71
	barley straw					
	wheat grain					
	wheat hay					
	wheat straw					
XDE-729 ME <i>m/z</i> 345→250	<u>oily crops</u>	0.01	92	76-99	7.0	12
	canola seed	1.00	100	97-104	1.9	12
	soybean seed	0.01-1.00	96	76-104	6.3	24
XDE-729 ME <i>m/z</i> 345→250	<u>acidic crops</u>	0.01	89	88-91	1.1	12
	apple, whole	1.00	91	89-93	1.3	12
	orange, whole	0.01-1.00	90	88-93	1.8	24
XDE-729 ME <i>m/z</i> 345→250	<u>processed</u>	0.01	95	80-101	5.9	24
	<u>products</u>	1.00	99	96-101	1.5	24
	aspirated grain	0.01-1.00	97	80-101	4.7	48
	bran					
	bread					
	flour					
	germ					
	gluten					
	shorts					
	starch					

Validation Data (quantitation)	Matrix	Fortification Level (mg/kg)	Recovery Rate (%)		RSD (%)	n
			mean	range		
XDE-729 acid <i>m/z</i> 331→250	<u>wet crops</u>	0.01	86	79-94	5.7	12
	turnip root	1.00	93	87-97	2.7	12
	wheat forage	0.01-1.00	90	79-97	5.8	24
XDE-729 acid <i>m/z</i> 331→250	<u>dry crops</u>	0.01	88	76-95	6.3	36
	barley grain	1.00	94	84-100	3.5	35
	barley hay	0.01-1.00	91	76-100	5.8	71
	barley straw					
	wheat grain					
	wheat hay					
	wheat straw					
XDE-729 acid <i>m/z</i> 331→250	<u>oily crops</u>	0.01	90	82-96	6.2	12
	canola seed	1.00	94	87-97	3.4	12
	soybean seed	0.01-1.00	92	82-97	5.2	24
XDE-729 acid <i>m/z</i> 331→250	<u>acidic crops</u>	0.01	87	81-96	5.9	12
	apple, whole	1.00	92	89-96	1.8	12
	orange, whole	0.01-1.00	90	81-96	5.1	24
XDE-729 acid <i>m/z</i> 331→250	<u>processed</u>	0.01	93	76-100	7.4	24
	<u>products</u>	1.00	94	78-99	6.5	24
	aspirated grain	0.01-1.00	93	76-100	6.9	48
	bran					
	bread					
	flour					
	germ					
	gluten					
	shorts					
	starch					

Conclusion

It is noted that full validation data are only obtained for the primary (quantitation) MS/MS transition. According to SANCO/825/00 rev. 8.1 the additional confirmatory transition should also be validated for calibration data, recovery and precision data for samples fortified at the LOQ (n=5) and for 2 blank samples. It is a short coming of the study that full validation data has only been presented for the primary transition; however, this data has been presented for high water and high oil matrices for the ILV of this method (see below). Only two matrices were considered in the ILV, however the primary extraction method is the same for all matrices therefore this is acceptable. Given that the ILV study report demonstrates that both the quantitative and confirmatory MS/MS transitions are acceptably validated, in this instance the RMS proposes that consideration of the complete data set from the validation report Study Number 110004 and the ILV report (see below, Study Number 2386) indicates that the method is fit for purpose according to SANCO/825/00 rev. 8.1. (For information, the notifier has since informed the RMS "the report will be revised to include the confirmatory transition data, including calibration, recovery and precision. The revised version will be available by June

2013". In the absence of this report at the time of submission, for the reasons shown above, the method may still be considered fit for purpose according to SANCO/825/00 rev. 8.1).

The method however does rely on stable isotope labelled internal standards. According to SANCO/825/00 rev. 8.1, all analytical standard materials used in an analytical method must be commonly available. This applies to stable isotope labelled compounds or other internal standards. If a standard is not commercially available the standard should be made generally available by the applicant and the contact details be provided. The notifier was contacted during this evaluation however, and confirmed that the stable isotope labelled internal standards would be made available in line with guidance.

B.5.2.4 Independent Laboratory Validation of the Enforcement Method for the Determination of XDE 729 Methyl Ester and XDE 729 Acid in Plant Matrices

Reference: Robaugh, D. A. (2012) Independent Laboratory Validation of Method for the Determination of Residues of XDE-729 Methyl Ester and XDE-729 Acid in Agricultural Commodities and Wheat Processed Products using Offline Solid-Phase Extraction and Liquid Chromatography with Mass Spectrometry Detection, Dow AgroSciences LLC Study Number 110825
Pyxant Labs Study Number 2386

Guidelines:

SANCO/825/00 rev. 8.1

This method has been submitted in support of both pre-registration data requirements and post-registration monitoring and control for components included in the residue definition for plants and animals. The proposed residue definition for Risk Assessment and Enforcement/Monitoring in plants and livestock is XDE-729 methyl and XDE-729 acid.

Matrix, limit of quantitation	wheat forage (wet crops) 0.01 mg/kg canola oil (oily crops) 0.01 mg/kg wheat flour 0.01 mg/kg
Description	<u>Scope</u> The objective of this study was to independently validate Dow AgroSciences method (Study Number 110004), "Method Validation Study for the Determination of Residues of XDE-729 Methyl Ester and XDE-729 Acid in Agricultural Commodities and Wheat Processed Products using Offline Solid-Phase Extraction and Liquid Chromatography with Mass Spectrometry Detection." This method is applicable for the quantitative determination of residues of XDE-729 methyl ester and XDE-729 acid in agricultural commodities representative of the four European crop groupings and wheat processed products. The method was validated over the concentration range of 0.01-1.00 mg/kg with a validated limit of quantitation of 0.01 mg/kg. <u>Principle</u> The method was repeated as described above for study ID 110004 without deviation. A summary is provided below for information: Residues of XDE-729 methyl ester and XDE-729 acid were extracted from

a 5.0-gram sample by homogenizing and shaking with 100.0 mL of an acetonitrile/water (80:20) solution. Fifty microliters of a glycerin/methanol solution (10:90 w/v) were added to a 1000- μ L aliquot of the acetonitrile/water extraction solution and the sample was concentrated to near dryness. The sample extract was then diluted to 1.0 mL with aqueous 0.1% formic acid and purified using a Phenomenex Strata-X reversed-phase polymeric solid-phase extraction (SPE) cartridge. After elution from the SPE cartridge with a methanol/water (95:5) solution containing 0.10% formic acid, 200 μ L of a mixed stable-isotope internal standard solution was added to the eluate, which was subsequently concentrated to approximately 1 mL. The solution was then adjusted to 2.0 mL with water. After preparation, the samples were analyzed by liquid chromatography using a Zorbax SB-C8 column coupled with positive-ion electrospray tandem mass spectrometry (LC/MS/MS), monitoring two MS/MS transitions characteristic of each analyte and one MS/MS transition characteristic of each internal standard.

Calibration

For each analyte, the linearity of detector response was evaluated using solvent standard solutions. Calibration curves were calculated by linear regression analysis with 1/x weighting. For the least squares equation which describes the detector response as a function of the standard concentration, calibration curves resulting from the injection of a minimum of eight standards over the concentration range of 0.075-12.5 ng/mL (equivalent to 0.003 mg/kg - 0.5 mg/kg) demonstrated linearity with correlation coefficients (r) of at least 0.999 and coefficients of determination (r^2) of at least 0.998.

Validation

For each analyte, the method was validated over the concentration range of 0.01-1.00 mg/kg with a limit of quantitation of 0.01 mg/kg.

The individual recoveries were within the range of 65-106%. While there were five recoveries from a total of 120 measurements that were between 65-69%, the mean recoveries were at least 70% with relative standard deviations $\leq 20\%$. At the 0.01-mg/kg level (LOQ), the mean recoveries were within the range of 70-92%, with relative standard deviations within the range of 3-18%.

At the 1.00-mg/kg level (100x LOQ), the mean recoveries were within the range of 75-91%, with relative standard deviations within the range of 3-6%.

The above results comply with the acceptance criteria of SANCO/825/00/rev. 8.1.

Selectivity/Confirmation

The LC/MS/MS method is highly selective for both the quantitation and confirmation of XDE-729 methyl ester and XDE-729 acid. Analysis of control samples resulted in no significant signals at the expected retention times of the analytes. Unambiguous identification is ensured by monitoring two MS/MS transitions characteristic of each analyte and one MS/MS transition characteristic of each internal standard as follows:

XDE-729 ME	m/z 345/250 (quantitation)
	m/z 345/235 (confirmation)
(M+6 ISTD)	m/z 351/256 (quantitation)
XDE-729 acid	m/z 331/250 (quantitation)
	m/z 331/235 (confirmation)
(M+6 ISTD)	m/z 337/256 (quantitation)

Confirmation was demonstrated using one of two techniques. In the first, recovery and precision data were determined for both ion mass transitions; these are listed in the tables below. In the second, confirmation was determined according to Commission Decision (2002/657/EC). The confirmation ratios for each analyte were determined by calculating the peak area ratios of the confirmation transition to the quantitation transition. Confirmation of the presence of the analytes in the recovery samples was indicated when the retention times of the analytes in the samples matched the retention times of the analytes in the calibration standards, and the confirmation ratios of the analytes in the samples were $\pm 20\%$ of the average confirmation ratios of the standards. The confirmation ratios for each analyte in all sample matrices were within $\pm 20\%$ of the average confirmation ratios of the standards, indicating that the method is selective for the determination of XDE-729 methyl ester and XDE-729 acid in agricultural commodities and wheat processed products. This data is contained in the report.

Stability of Solutions and Sample Extracts

The evaluation of the stability of stock solutions, calibration solutions, and sample extracts is not a requirement of an independent laboratory validation.

All standard and calibration solutions were stored in a refrigerator at approximately $2-8^{\circ}\text{C}$ when not in use. The storage period between extraction and analysis was within the 11 days that the storage stability was proven for the original validation of the method (Study Number 110004). Specimen extracts therefore are considered to be sufficiently stable during the course of the study, furthermore recoveries obtained were all within the required range with acceptable variations (60 –120 % for LOQ specimens, 70-120 % for 10xLOQ specimens, $\text{RSD} \leq 20\%$).

Matrix Effects

Matrix effects are normalized through the use of stable-isotope internal standards, resulting in accurate quantitative results irrespective of the magnitude of matrix effects.

Validation Data (quantitation)	Matrix	Fortification Level (mg/kg)	Recovery Rate (%)		RSD (%)	n
			mean	range		
XDE-729 ME <i>m/z</i> 345→250	<u>wet crops</u> wheat forage	0.01	82	68-100	15	5
		1.00	83	81-87	3	5
		0.01-1.00	83	68-100	10	10
XDE-729 ME <i>m/z</i> 345→250	<u>oily crops</u> canola oil	0.01	79	72-90	9	5
		1.00	75	70-83	6	5
		0.01-1.00	77	70-90	8	10
XDE-729 ME <i>m/z</i> 345→250	<u>processed products</u> wheat flour	0.01	70	66-76	6	5
		1.00	79	76-81	3	5
		0.01-1.00	75	66-81	8	10

Validation Data (quantitation)	Matrix	Fortification Level (mg/kg)	Recovery Rate (%)		RSD (%)	n
			mean	range		
XDE-729 acid <i>m/z</i> 331→250	<u>wet crops</u> wheat forage	0.01	90	83-97	6	5
		1.00	91	86-94	4	5
		0.01-1.00	91	83-97	5	10
XDE-729 acid <i>m/z</i> 331→250	<u>oily crops</u> canola oil	0.01	92	85-106	9	5
		1.00	87	84-91	3	5
		0.01-1.00	90	84-106	7	10
XDE-729 acid <i>m/z</i> 331→250	<u>processed products</u> wheat flour	0.01	90	86-93	3	5
		1.00	86	82-90	4	5
		0.01-1.00	88	82-93	4	10

Validation Data (confirmation)	Matrix	Fortification Level (mg/kg)	Recovery Rate (%)		RSD (%)	n
			mean	range		
XDE-729 ME <i>m/z</i> 345→235	<u>wet crops</u> wheat forage	0.01	79	65-101	18	5
		1.00	83	80-86	3	5
		0.01-1.00	81	65-101	12	10
XDE-729 ME <i>m/z</i> 345→235	<u>oily crops</u> canola oil	0.01	77	69-88	9	5
		1.00	75	70-82	6	5
		0.01-1.00	76	69-88	7	10
XDE-729 ME <i>m/z</i> 345→235	<u>processed products</u> wheat flour	0.01	71	68-76	4	5
		1.00	81	77-83	3	5
		0.01-1.00	76	68-83	8	10

Validation Data (confirmation)	Matrix	Fortification Level (mg/kg)	Recovery Rate (%)		RSD (%)	n
			mean	range		
XDE-729 acid <i>m/z</i> 331→235	<u>wet crops</u> wheat forage	0.01	91	80-99	9	5
		1.00	88	85-91	3	5
		0.01-1.00	89	80-99	6	10
XDE-729 acid <i>m/z</i> 331→235	<u>oily crops</u> canola oil	0.01	85	70-94	12	5
		1.00	89	85-94	4	5
		0.01-1.00	87	70-94	8	10
XDE-729 acid <i>m/z</i> 331→235	<u>processed products</u> wheat flour	0.01	87	78-98	9	5
		1.00	87	83-90	4	5
		0.01-1.00	87	78-98	6	10

Conclusion

The primary method is identical for all matrix groups, therefore it was considered to be sufficient to perform the ILV on commodities from two of the crop groupings. Wheat forage and canola (rapeseed) oil were chosen as the representative commodities on which to perform the ILV study. In order to meet guidelines, wheat forage was chosen because of its high water content. Wheat flour was also taken through the ILV because the method for preparing it for analysis (as well as the other processed products which are finely ground to a powder-like consistency) uses a ten-fold lower (reduced) sample size and volumes of fortification and extraction solutions are reduced proportionally. In line with the conclusions made under B.5.2.3, the RMS proposes that the method is fit for purpose according to SANCO/825/00 rev. 8.1. The method however does rely on stable isotope labelled internal standards. According to SANCO/825/00 rev. 8.1, all analytical standard materials used in an analytical method must be commonly available. This applies to stable isotope labelled compounds or other internal standards. If a standard is not commercially available the standard should be made generally available by the applicant and the contact details be provided. The notifier was contacted during this evaluation however, and confirmed that the stable isotope labelled internal standards would be made available in line with guidance.

B.5.2.5 Multi-residue Method Testing for the Determination of XDE-729 Methyl Ester and XDE-729 Acid in Animal Matrices

Reference: Lindner, M. (2011) Validation of a Multi-Residue Method for the Determination of XDE-729 Methyl Ester and XDE-729 Acid in Animal Matrices, Dow AgroSciences LLC Study Number 110574
Eurofins Analytical Services Study Number S11-02424

This method has been submitted in support of post-registration monitoring and control for components included in the residue definition for plants and animals. The proposed residue definition for Risk Assessment and Enforcement/Monitoring in plants and livestock is XDE-729 methyl and XDE-729 acid.

Guidelines:**SANCO/825/00 rev. 8.1****SANCO/3029/99 rev. 4**

Matrix, limit of quantitation	bovine muscle 0.01 mg/kg bovine kidney 0.01 mg/kg bovine liver 0.01 mg/kg bovine whole milk 0.01 mg/kg poultry muscle 0.01 mg/kg poultry eggs 0.01 mg/kg
Description	<u>Scope</u> This QuEChERS multi-residue method is applicable for the quantitative determination of residues of XDE-729 methyl ester and XDE-729 acid in animal matrices including bovine muscle, bovine kidney, bovine liver, bovine whole milk, poultry muscle, and poultry eggs. The method was validated over the concentration range of 0.01-0.10 mg/kg with a validated limit of quantitation of 0.01 mg/kg. <u>Principle</u> Residues of XDE-729 methyl ester and XDE-729 acid were extracted from the sample matrices by shaking with an acetonitrile/water solution. The specimen weight taken for analysis was 5g for all matrices. After addition of a salt mixture consisting of magnesium sulfate, sodium chloride, trisodium citrate dihydrate, and disodium hydrogen citrate sesquihydrate, the extract was shaken. After centrifugation, an aliquot of the acetonitrile extract was purified by freezing out the fats and subsequently diluted to volume with aqueous 0.1% formic acid. After preparation, the samples were analyzed by liquid chromatography using a Synergi Hydro-RP C18 column with gradient elution (50 x 2.0 mm, 2.5µm, 100A, Mobile Phase A: acetonitrile + 0.1% (v/v) formic acid and Mobile Phase B: water + 0.1% (v/v) formic acid) coupled with positive-ion electrospray tandem mass spectrometry (LC/MS/MS), monitoring two MS/MS transitions characteristic of each analyte. <u>Calibration</u>

For each analyte, the linearity of detector response was evaluated using solvent standard solutions as well as matrix-matched standard solutions. Calibration curves were calculated by linear regression analysis without weighting. Calibration curves resulting from the injection of a minimum of five standards over the concentration range of 0.10-10 ng/mL (0.002 mg/kg - 0.3 mg/kg) demonstrated linearity with coefficients of determination (r^2) of at least 0.993.

The calibration standards covered the range of at least $\leq 30\%$ of the LOQ up to 20 % above the highest analysed sample extract concentration level as outlined in SANCO Guideline 825100 rev. 8.1.

Validation

For each analyte, the method was validated over the concentration range of 0.01-0.10 mg/kg with a limit of quantitation of 0.01 mg/kg.

At the 0.01-mg/kg level (LOQ), the mean recoveries were within the range of 69-104%, with relative standard deviations within the range of 0.7-8.4%.

At the 0.10-mg/kg level (10x LOQ), the mean recoveries were within the range of 71-100%, with relative standard deviations within the range of 1.3-9.4%.

The above results comply with the acceptance criteria of SANCO/825/00/rev. 8.1.

Selectivity/Confirmation

The LC/MS/MS method is highly selective for both the quantitation and confirmation of XDE-729 methyl ester and XDE-729 acid. Analysis of control samples resulted in no significant signals (less than 30% LOQ) at the expected retention times of the analytes. Unambiguous identification is ensured by monitoring two MS/MS transitions characteristic of each analyte as follows:

XDE-729 ME	m/z 345/250 (quantitation)
	m/z 345/207 (confirmation)
XDE-729 acid	m/z 331/250 (quantitation)
	m/z 331/207 (confirmation)

Confirmation was demonstrated by determining the recovery and precision for both MS/MS transitions; these data are listed in the tables below.

Stability of Solutions and Sample Extracts

All stock and calibration standard solutions were stored in a refrigerator at 3-8 °C when not in use.

Stock solutions of XDE-729 methyl ester and XDE-729 acid prepared in acetonitrile were tested after 138 days of storage and were found to be stable.

Calibration standard solutions of XDE-729 methyl ester and XDE-729 acid prepared in acetonitrile/0.10% formic acid (10:90) were tested after 24 days of storage and found to be stable.

Final sample extracts were evaluated for storage periods ranging from 8 to 12 days. At the 0.01-mg/kg level (LOQ), the mean recoveries of the second injection of the extracts were within the range of 66-101%,

	indicating that the sample extracts were stable. These results comply with the acceptance criteria of SANCO/825/00/rev. 8.1. <u>Matrix Effects</u> Matrix effects were determined by comparing the peak areas of solvent calibration standards to peak areas of matrix-matched standards at two concentration levels. For both analytes, matrix effects were less than 10% for both MS/MS transitions and thus considered insignificant. Therefore, solvent standard solutions were used for quantitation throughout the study.
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Validation Data (quantitation)	Matrix	Fortification Level (mg/kg)	Recovery Rate (%)		RSD (%)	n
			mean	range		
XDE-729 ME <i>m/z</i> 345→250	bovine muscle	0.01	87	82-90	3.9	4*
		0.10	80	77-84	3.5	5
		0.01-0.10	83	77-90	5.4	9
XDE-729 ME <i>m/z</i> 345→250	bovine kidney	0.01	87	83-89	2.8	5
		0.10	88	85-91	2.8	5
		0.01-0.10	87	83-91	2.7	10
XDE-729 ME <i>m/z</i> 345→250	bovine liver	0.01	76	73-78	2.7	5
		0.10	76	73-79	2.9	5
		0.01-0.10	76	73-79	2.7	10
XDE-729 ME <i>m/z</i> 345→250	bovine whole milk	0.01	102	94-107	5.4	5
		0.10	96	93-100	3.0	5
		0.01-0.10	99	93-107	5.3	10
XDE-729 ME <i>m/z</i> 345→250	poultry muscle	0.01	101	96-106	3.8	5
		0.10	100	96-104	3.6	5
		0.01-0.10	100	96-106	3.5	10
XDE-729 ME <i>m/z</i> 345→250	poultry eggs	0.01	104	102-108	2.6	5
		0.10	97	93-99	2.6	5
		0.01-0.10	101	93-108	4.4	10

*see comment in conclusions below

Validation Data (quantitation)	Matrix	Fortification Level (mg/kg)	Recovery Rate (%)		RSD (%)	n
			mean	range		
XDE-729 acid <i>m/z</i> 331→250	bovine muscle	0.01	70	68-73	3.3	5
		0.10	75	72-80	4.2	5
		0.01-0.10	73	68-80	4.7	10
XDE-729 acid <i>m/z</i> 331→250	bovine kidney	0.01	69	65-73	5.3	5
		0.10	73	70-77	3.9	5
		0.01-0.10	71	65-77	5.4	10
XDE-729 acid <i>m/z</i> 331→250	bovine liver	0.01	70	68-71	1.6	5
		0.10	72	71-73	1.4	5
		0.01-0.10	71	68-73	2.3	10
XDE-729 acid <i>m/z</i> 331→250	bovine whole milk	0.01	82	76-87	5.1	5
		0.10	85	84-88	2.3	5
		0.01-0.10	84	76-88	4.3	10
XDE-729 acid <i>m/z</i> 331→250	poultry muscle	0.01	70	69-70	0.6	5
		0.10	72	68-75	4.3	5
		0.01-0.10	71	68-75	3.2	10
XDE-729 acid <i>m/z</i> 331→250	poultry eggs	0.01	70	65-72	4.0	5
		0.10	77	71-89	9.4	5
		0.01-0.10	73	65-89	8.6	10

Validation Data (confirmation)	Matrix	Fortification Level (mg/kg)	Recovery Rate (%)		RSD (%)	n
			mean	range		
XDE-729 ME <i>m/z</i> 345→207	bovine muscle	0.01	87	82-93	5.5	4*
		0.10	80	77-82	3.2	5
		0.01-0.10	83	77-93	6.0	9
XDE-729 ME <i>m/z</i> 345→207	bovine kidney	0.01	85	81-88	4.0	5
		0.10	86	83-88	2.7	5
		0.01-0.10	85	81-88	3.3	10
XDE-729 ME <i>m/z</i> 345→207	bovine liver	0.01	74	72-75	1.8	5
		0.10	74	72-78	3.1	5
		0.01-0.10	74	72-78	2.4	10
XDE-729 ME <i>m/z</i> 345→207	bovine whole milk	0.01	100	98-104	2.4	5
		0.10	97	95-100	1.9	5
		0.01-0.10	99	95-104	2.7	10
XDE-729 ME <i>m/z</i> 345→207	poultry muscle	0.01	100	98-106	3.2	5
		0.10	99	92-104	5.2	5
		0.01-0.10	100	92-106	4.2	10
XDE-729 ME <i>m/z</i> 345→207	poultry eggs	0.01	101	99-105	2.4	5
		0.10	96	91-100	3.6	5
		0.01-0.10	99	91-105	4.0	10

*see comment in conclusions below

Validation Data (confirmation)	Matrix	Fortification Level (mg/kg)	Recovery Rate (%)		RSD (%)	n
			mean	range		
XDE-729 acid <i>m/z</i> 331→207	bovine muscle	0.01	70	65-74	4.8	5
		0.10	75	72-79	3.6	5
		0.01-0.10	72	65-79	5.4	10
XDE-729 acid <i>m/z</i> 331→207	bovine kidney	0.01	69	63-77	8.4	5
		0.10	75	71-80	4.5	5
		0.01-0.10	72	63-80	7.5	10
XDE-729 acid <i>m/z</i> 331→207	bovine liver	0.01	70	65-72	4.0	5
		0.10	71	69-73	2.6	5
		0.01-0.10	70	65-73	3.2	10
XDE-729 acid <i>m/z</i> 331→207	bovine whole milk	0.01	81	78-83	2.4	5
		0.10	86	85-87	1.3	5
		0.01-0.10	83	78-87	3.6	10
XDE-729 acid <i>m/z</i> 331→207	poultry muscle	0.01	71	69-73	2.6	5
		0.10	74	71-79	4.8	5
		0.01-0.10	73	69-79	4.6	10
XDE-729 acid <i>m/z</i> 331→207	poultry eggs	0.01	70	67-72	1.8	5
		0.10	73	69-82	5.4	5
		0.01-0.10	71	67-82	5.8	10

Conclusion

The QuEChERS method has been validated in all areas according to SANCO/825/00 rev. 8.1, and the study report was well presented.

Storage stability of final sample extracts demonstrated an acceptable level of degradation for the determination of both analytes in all matrices, with the exception of XDE-729 acid in extracts of poultry eggs. The mean recovery for the re-injected LOQ sample extracts was 66 % and thus below 70 %. However since the recovery was 70 % upon first injection, the deviation is insignificant and the stability of XDE-729 acid in the final extract was proven. It is accepted that the storage stability is acceptable; however, the recovery is low but is within the guideline acceptable limits (SANCO/825/00 rev. 8.1) with the range of the mean recovery at 0.01mg/kg 60-120%).

*For recoveries of XDE-729 methyl ester in bovine meat, sample 17 demonstrated high recoveries outside acceptable limits, therefore only four recovery determinations have been carried out instead of five, since this sample was removed as an outlier. Although this is a short coming of the validation of the method, given that overall the data in all other areas of the validation are well within guideline acceptable limits, and the other samples (with the exception of sample 17) for recovery of XDE-729 methyl ester in bovine meat give consistent values within acceptable limits, it is reasonable in this instance to accept that the data set as a whole is adequately validated for recovery and precision.

It is noted that extraction efficiencies have not been addressed as part of the method validation; however, residues are not expected to be found in products of animal origin for the proposed use. Therefore, extraction efficiency does not need to be addressed for this evaluation. This will need to be addressed in future however if new uses give rise to positive residues.

Overall The study demonstrated that the multiple analytical method for the determination of pesticide residues in foodstuffs QuEChERS, without the use of PSA but with clean-up by freezing out, in combination with LC-MS/MS detection allows reliable quantification of residues of XDE-729 methyl ester and XDE-729 acid in matrices of animal origin with an LOQ of 0.01 mg/kg up to 0.1 mg/kg according to SANCO Guideline 825/00, rev. 8.1.

B.5.2.6 Multi-residue Method Testing for the Determination of XDE 729 Methyl Ester and XDE 729 Acid in Bovine Fat

Reference: Lindner, M. (2011) Validation of a Multi-Residue Method for the Determination of XDE-729 Methyl Ester and XDE-729 Acid in Bovine Fat, Dow AgroSciences LLC Study Number 110750, Eurofins Analytical Services Study Number S11-03369

This method has been submitted in support of post-registration monitoring and control for components included in the residue definition for plants and animals. The proposed residue definition for Risk Assessment and Enforcement/Monitoring in plants and livestock is XDE-729 methyl and XDE-729 acid.

Guidelines:

SANCO/825/00 rev. 8.1

SANCO/3029/99 rev. 4

Matrix, limit of quantitation	bovine fat 0.01 mg/kg
Description	<u>Scope</u> This QuEChERS multi-residue method is applicable for the quantitative determination of residues of XDE-729 methyl ester and XDE-729 acid in bovine fat. The method was validated over the concentration range of 0.01-0.10 mg/kg with a validated limit of quantitation of 0.01 mg/kg. <u>Principle</u> Residues of XDE-729 methyl ester and XDE-729 acid were extracted from the bovine fat sample by shaking with an acetonitrile/water solution. After addition of a salt mixture consisting of magnesium sulfate, sodium chloride, trisodium citrate dihydrate, and disodium hydrogen citrate sesquihydrate, the extract was shaken. After centrifugation, an aliquot of the acetonitrile extract was purified by freezing out the fats and subsequently diluted to volume with aqueous 0.1% formic acid. After preparation, the samples were analyzed by liquid chromatography using a Synergi Hydro-RP C18 column with gradient elution (50 x 2.0 mm, 2.5µm, 100A, Mobile Phase A: acetonitrile + 0.1% (v/v) formic acid and Mobile Phase B: water + 0.1% (v/v) formic acid) coupled with positive-ion electrospray tandem mass spectrometry (LC/MS/MS), monitoring two

	MS/MS transitions characteristic of each analyte. <u>Calibration</u> For each analyte, the linearity of detector response was evaluated using solvent standard solutions. Calibration curves were calculated by linear regression analysis without weighting. Calibration curves resulting from the injection of a minimum of five standards over the concentration range of 0.10-10 ng/mL (0.002 mg/kg - 0.2 mg/kg) demonstrated linearity with coefficients of determination (r^2) of at least 0.992. The calibration standards always covered the range of at least ≤ 30 % of the LOQ up to 20 % above the highest analysed sample extract concentration level as demanded in SANCO Guideline 825/00 rev. 8.1. <u>Validation</u> For each analyte, the method was validated over the concentration range of 0.01-0.10 mg/kg with a limit of quantitation of 0.01 mg/kg. At the 0.01-mg/kg level (LOQ), the mean recoveries were within the range of 93-99%, with relative standard deviations within the range of 1.7-6.5%. At the 0.10-mg/kg level (10x LOQ), the mean recoveries were within the range of 92-105%, with relative standard deviations within the range of 2.0-4.2%. The above results comply with the acceptance criteria of SANCO/825/00/rev. 8.1. <u>Selectivity/Confirmation</u> The LC/MS/MS method is highly selective for both the quantitation and confirmation of XDE-729 methyl ester and XDE-729 acid. Analysis of control samples resulted in no significant signals (below 30% of the LOQ) at the expected retention times of the analytes. Unambiguous identification is ensured by monitoring two MS/MS transitions characteristic of each analyte as follows: <table border="0"> <tr> <td>XDE-729 ME</td><td>m/z 345/250 (quantitation)</td></tr> <tr> <td></td><td>m/z 345/207 (confirmation)</td></tr> <tr> <td>XDE-729 acid</td><td>m/z 331/250 (quantitation)</td></tr> <tr> <td></td><td>m/z 331/207 (confirmation)</td></tr> </table> Confirmation was demonstrated by determining the recovery and precision for both ion mass transitions; these data are listed in the tables below. <u>Stability of Solutions and Sample Extracts</u> Stability of stock solutions and solvent calibration solutions over the herein required ranges was previously verified in EAS Chem validation study S 11-02424 (see above, Dow AgroSciences No. 110574) All stock and calibration standard solutions were stored in a refrigerator at 3-8 °C when not in use.	XDE-729 ME	m/z 345/250 (quantitation)		m/z 345/207 (confirmation)	XDE-729 acid	m/z 331/250 (quantitation)		m/z 331/207 (confirmation)
XDE-729 ME	m/z 345/250 (quantitation)								
	m/z 345/207 (confirmation)								
XDE-729 acid	m/z 331/250 (quantitation)								
	m/z 331/207 (confirmation)								

	Stock solutions of XDE-729 methyl ester and XDE-729 acid prepared in acetonitrile were tested after 138 days of storage and were found to be stable. Calibration standard solutions of XDE-729 methyl ester and XDE-729 acid prepared in acetonitrile/0.10% formic acid (10:90) were tested after 24 days of storage and found to be stable. Final sample extracts were evaluated for a storage period of 11 days. At the 0.01-mg/kg level (LOQ), the mean recoveries of the second injection of the extracts were within the range of 92-94%, indicating that the sample extracts were stable. These results comply with the acceptance criteria of SANCO/825/00/rev. 8.1. <u>Matrix Effects</u> Matrix effects were determined by comparing the peak areas of solvent calibration standards to peak areas of matrix-matched standards at two concentration levels. For both analytes, matrix effects were less than 15% for both MS/MS transitions and thus considered insignificant. Therefore, solvent standard solutions were used for quantitation throughout the study. <u>Extraction Efficiency</u> It is noted that extraction efficiencies have not been addressed as part of the method validation; however, residues are not expected to be found in products of animal origin for the proposed use. Therefore, extraction efficiency does not need to be addressed for this evaluation. This will need to be addressed in future however if new uses give rise to positive residues.
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Validation Data (quantitation)	Matrix	Fortification Level (mg/kg)	Recovery Rate (%)		RSD (%)	n
			mean	range		
XDE-729 ME m/z 345→250	bovine fat	0.01	99	90-106	6.5	5
		0.10	92	89-98	4.2	5
		0.01-0.10	96	89-106	6.3	10

Validation Data (quantitation)	Matrix	Fortification Level (mg/kg)	Recovery Rate (%)		RSD (%)	n
			mean	range		
XDE-729 acid m/z 331→250	bovine fat	0.01	99	97-101	1.7	5
		0.10	101	96-105	4.0	5
		0.01-0.10	100	96-105	3.2	10

Validation Data (confirmation)	Matrix	Fortification Level (mg/kg)	Recovery Rate (%)		RSD (%)	n
			mean	range		
XDE-729 ME <i>m/z</i> 345→207	bovine fat	0.01	93	86-98	5.8	5
		0.10	92	89-97	3.7	5
		0.01-0.10	93	86-97	4.7	10

Validation Data (confirmation)	Matrix	Fortification Level (mg/kg)	Recovery Rate (%)		RSD (%)	n
			mean	range		
XDE-729 acid <i>m/z</i> 331→207	bovine fat	0.01	98	93-102	3.3	5
		0.10	105	103-108	2.0	5
		0.01-0.10	101	93-108	4.5	10

Conclusion

The QuEChERS method has been validated in all areas according to SANCO/825/00 rev. 8.1, and the study report was well presented.

Overall, the study demonstrated that the multiple analyte method for the determination of pesticide residues in foodstuffs QuEChERS, without the use of PSA but with clean-up by freezing out, in combination with LC-MS/MS detection allows reliable quantitation of residues of XDE-729 methyl ester and XDE-729 acid in bovine fat with an LOQ of 0.01 mg/kg up to 0.1 mg/kg according to SANCO Guideline 825/00, rev. 8.1.

B.5.2.7 Independent Laboratory Validation of the Multi-residue Method for the Determination of XDE 729 Methyl Ester and XDE 729 Acid in Animal Matrices

Reference: Bacher, R. (2011) XDE-729: Independent Laboratory Validation of a Multi-Residue Method for the Determination of XDE-729 Methyl Ester and XDE-729 Acid in Animal Matrices, Dow AgroSciences LLC Study Number 110294 PTRL Europe Study Number P 2172 G

This method has been submitted in support of post-registration monitoring and control for components included in the residue definition for plants and animals. The proposed residue definition for Risk Assessment and Enforcement/Monitoring in plants and livestock is XDE-729 methyl and XDE-729 acid.

Guidelines:

SANCO/825/00 rev. 8.1

SANCO/3029/99 rev. 4

Matrix, limit of quantitation	bovine muscle 0.01 mg/kg bovine whole milk 0.01 mg/kg bovine fat 0.01 mg/kg
Description	<u>Scope</u> The objective of this study was to independently validate the multi-residue method for the determination of XDE-729 methyl ester and XDE-729 acid in animal matrices. The method was Eurofins Agrosiences Services multi-residue method (Study Number S11-02424), “Validation of a Multi-Residue Method for the Determination of XDE-729 Methyl Ester and XDE-729 Acid in Animal Matrices” and Eurofins Agrosiences Services multi-residue method (Study Number S11-03369), “Validation of a Multi-Residue Method for the Determination of XDE-729 Methyl Ester and XDE-729 Acid in Bovine Fat.” The QuEChERS multi-residue method is applicable for the quantitative determination of residues of XDE-729 methyl ester and XDE-729 acid in animal matrices including bovine muscle, bovine kidney, bovine liver, bovine whole milk, bovine fat, poultry muscle, and poultry eggs. The method was validated over the concentration range of 0.01-0.10 mg/kg with a validated limit of quantitation of 0.01 mg/kg. <u>Principle</u> The method was repeated as described above for studies S11-02424 and S11-03369 without deviation. A summary is provided below for information (see above evaluation of studies S11-02424 and S11-03369 for more detailed description of chromatography solvent system and column): Residues of XDE-729 methyl ester and XDE-729 acid were extracted from the sample matrices by shaking with an acetonitrile/water solution. After addition of a salt mixture consisting of magnesium sulfate, sodium chloride, trisodium citrate dihydrate, and disodium hydrogen citrate sesquihydrate, the extract was shaken. After centrifugation, an aliquot of the acetonitrile extract was purified by freezing out the fats and subsequently diluted to volume with aqueous 0.1% formic acid. After preparation, the samples were analyzed by liquid chromatography using a Synergi Hydro-RP C18 column coupled with positive-ion electrospray tandem mass spectrometry (LC/MS/MS), monitoring two MS/MS transitions characteristic of each analyte. <u>Calibration</u> For each analyte, the linearity of detector response was evaluated using solvent standard solutions. Calibration curves were calculated by linear regression analysis with 1/x weighting. For XDE-729 methyl ester, calibration curves resulting from the injection of a minimum of six standards over the concentration range of 0.02-5 ng/mL (equivalent to 0.002 mg/kg – 0.5 mg/kg) demonstrated linearity with correlation coefficients of (r) of at least 0.99. For XDE-729 acid, calibration curves resulting from the injection of a minimum of six standards over the concentration range of 0.10-10 ng/mL (equivalent to 0.003 mg/kg – 0.3 mg/kg) demonstrated linearity with correlation coefficients (r) of at least 0.99.

No significant matrix effect was observed for any of the animal foodstuff matrices investigated (<20%), therefore linearity of response was evaluated in all cases using solvent standard solutions.

Validation

For each analyte, the method was validated over the concentration range of 0.01-0.10 mg/kg with a limit of quantitation of 0.01 mg/kg.

At the 0.01-mg/kg level (LOQ), the mean recoveries for both MS/MS transitions were within the range of 72-98%, with relative standard deviations within the range of 2-8%.

At the 0.10-mg/kg level (10x LOQ), the mean recoveries for both MS/MS transitions were within the range of 76-99%, with relative standard deviations within the range of 1-9%.

The above results comply with the acceptance criteria of SANCO/825/00/rev. 8.1.

Selectivity/Confirmation

The LC/MS/MS method is highly selective for both the quantitation and confirmation of XDE-729 methyl ester and XDE-729 acid. Analysis of control samples resulted in no significant signals at the expected retention times of the analytes. Unambiguous identification is ensured by monitoring two MS/MS transitions characteristic of each analyte as follows:

XDE-729 ME m/z 345/250 (quantitation)
 m/z 345/207 (confirmation)

XDE-729 acid m/z 331/250 (quantitation)
 m/z 331/207 (confirmation)

Confirmation was demonstrated by determining the recovery and precision for both ion mass transitions; these data are listed in the tables below.

Stability of Solutions and Sample Extracts

All stock and calibration standard solutions were stored in a refrigerator at approximately 4 °C when not in use. The evaluation of the stability of stock, spiking, and calibration solutions is not a requirement of an independent laboratory validation.

Final sample extracts were evaluated for a storage period of at least 7 days of refrigerated storage. At the 0.01-mg/kg (LOQ) and 0.10-mg/kg (10x LOQ) levels, the mean recoveries of the second injection of the extracts were within the range of 65-101% (at fortification level 0.01 mg/kg) and 86%-99% (at fortification level 0.1 mg/kg) indicating that the sample extracts were stable. These results comply with the acceptance criteria of SANCO/825/00/rev. 8.1.

Matrix Effects

Matrix effects were determined by comparing the peak areas of solvent calibration standards to peak areas of matrix-matched standards at two

	concentration levels. For both analytes, matrix effects were less than 20% for both MS/MS transitions and thus considered insignificant. Therefore, solvent standard solutions were used for quantitation throughout the study.
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Validation Data (quantitation)	Matrix	Fortification Level (mg/kg)	Recovery Rate (%)		RSD (%)	n
			mean	range		
XDE-729 ME <i>m/z</i> 345→250	bovine muscle	0.01	98	92-103	4	5
		0.10	99	90-105	6	5
		0.01-0.10	98	90-105	5	10
XDE-729 ME <i>m/z</i> 345→250	bovine whole milk	0.01	90	82-94	6	5
		0.10	83	76-93	9	5
		0.01-0.10	86	76-94	8	10
XDE-729 ME <i>m/z</i> 345→250	bovine fat	0.01	93	89-97	4	5
		0.10	96	92-103	5	5
		0.01-0.10	95	92-103	5	10

Validation Data (quantitation)	Matrix	Fortification Level (mg/kg)	Recovery Rate (%)		RSD (%)	n
			mean	range		
XDE-729 acid <i>m/z</i> 331→250	bovine muscle	0.01	73	65-77	8	5
		0.10	76	74-79	3	5
		0.01-0.10	75	65-79	6	10
XDE-729 acid <i>m/z</i> 331→250	bovine whole milk	0.01	87	84-90	3	5
		0.10	86	83-88	3	5
		0.01-0.10	86	83-90	3	10
XDE-729 acid <i>m/z</i> 331→250	bovine fat	0.01	95	94-98	2	5
		0.10	99	95-102	3	5
		0.01-0.10	97	94-102	3	10

Validation Data (confirmation)	Matrix	Fortification Level (mg/kg)	Recovery Rate (%)		RSD (%)	n
			mean	range		
XDE-729 ME <i>m/z</i> 345→207	bovine muscle	0.01	97	92-103	5	5
		0.10	97	91-101	4	5
		0.01-0.10	97	91-103	4	10
XDE-729 ME <i>m/z</i> 345→207	bovine whole milk	0.01	87	78-91	6	5
		0.10	86	79-97	9	5
		0.01-0.10	87	78-97	7	10
XDE-729 ME <i>m/z</i> 345→207	bovine fat	0.01	95	88-101	5	5
		0.10	97	91-103	4	5
		0.01-0.10	96	91-103	4	10

Validation Data (confirmation)	Matrix	Fortification Level (mg/kg)	Recovery Rate (%)		RSD (%)	n
			mean	range		
XDE-729 acid <i>m/z</i> 331→207	bovine muscle	0.01	72	67-78	7	5
		0.10	78	75-81	4	5
		0.01-0.10	75	67-81	7	10
XDE-729 acid <i>m/z</i> 331→207	bovine whole milk	0.01	86	82-89	3	5
		0.10	86	84-87	1	5
		0.01-0.10	86	82-89	2	10
XDE-729 acid <i>m/z</i> 331→207	bovine fat	0.01	92	90-95	2	5
		0.10	97	94-101	3	5
		0.01-0.10	95	90-101	4	10

Conclusion

The analytical method was independently validated for the determination of XDE-729 methyl ester and its metabolite XDE-729 acid in foodstuffs of animal origin using LC-MS/MS with a limit of quantitation (LOQ) of 0.01 mg/kg and a limit of detection (LOD) of 0.003 mg/kg. The method fulfils the EC Guidance documents on residue analytical methods SANCO/825/00 rev. 8.1.

The ILV study noted that “The API 5500 LC-MS/MS system showed a very high sensitivity especially for the determination of XDE-729 methyl ester. Thus, it was necessary to further dilute extracts fortified with this analyte (additional dilution factor: 5: 0.20 mL of extract was diluted with 0.80 mL of acetonitrile/0.1 % formic acid (1/9, v/v)) to be within the linear range of the LC-MS/MS calibration curves used”. This is considered to be a minor modification that does not impact on the validity of the study.

B.5.2.8 Enforcement Method for the Determination of XDE 729 Methyl Ester and XDE 729 Acid in Animal Matrices

Reference: Ma, M. and Li, Q. (2012) Method Validation Study for the Determination of Residues of XDE-729 Methyl Ester and XDE-729 Acid in Bovine and Poultry Tissues using Offline Solid-Phase Extraction and Liquid Chromatography with Mass Spectrometry Detection, Dow AgroSciences LLC Study Number 110505

This method has been submitted in support of both pre-registration and post-registration monitoring and control for components included in the residue definition for plants and animals. The proposed residue definition for Risk Assessment and Enforcement/Monitoring in plants and livestock is XDE-729 methyl and XDE-729 acid.

Guidelines:**SANCO/825/00 rev. 8.1****SANCO/3029/99 rev. 4**

Matrix, limit of quantitation	bovine muscle 0.01 mg/kg bovine liver 0.01 mg/kg bovine kidney 0.01 mg/kg bovine fat 0.01 mg/kg bovine whole milk 0.01 mg/kg bovine cream 0.01 mg/kg poultry muscle 0.01 mg/kg poultry liver 0.01 mg/kg poultry fat 0.01 mg/kg poultry eggs 0.01 mg/kg
Description	<u>Scope</u> This method is applicable for the quantitative determination of residues of XDE-729 methyl ester and XDE-729 acid in bovine and poultry tissues. The method was validated over the concentration range of 0.01-1.00 mg/kg with a validated limit of quantitation of 0.01 mg/kg. <u>Principle</u> Residues of XDE-729 methyl ester and XDE-729 acid were extracted from a 1.0-gram bovine or poultry sample by shaking with 20 mL of an acetonitrile/water (80:20) solution and partitioning with 20 mL of n-hexanes. Fifty microliters of a glycerin/methanol solution (10:90 w/v) were added to a 1000-µL aliquot of the acetonitrile/water extraction solution and the sample was concentrated to near dryness. The sample extract was then diluted to 1.0 mL with aqueous 0.1% formic acid and purified using a Phenomenex Strata-X reversed-phase polymeric solid-phase extraction (SPE) cartridge. After elution from the SPE cartridge with a methanol/water (95:5) solution containing 0.10% formic acid, 200 µL of a mixed stable-isotope internal standard solution was added to the eluate, which was subsequently concentrated to approximately 1 mL.

The solution was then adjusted to 2.0 mL with water. After preparation, the samples were analyzed by liquid chromatography using a Zorbax SB-C8 column (4.6 mm x 75 mm, 3.5- μ m particle size, gradient elution, mobile phase A – water containing 0.10% formic acid, mobile phase B – methanol containing 0.1% formic acid) coupled with positive-ion electrospray tandem mass spectrometry (LC/MS/MS), monitoring two MS/MS transitions characteristic of each analyte and one MS/MS transition characteristic of each internal standard.

Calibration

For each analyte, the linearity of detector response was evaluated using solvent standard solutions. Calibration curves were calculated by linear regression analysis with 1/x weighting. For the least squares equation which describes the detector response as a function of the standard concentration, calibration curves resulting from the injection of a minimum of eight standards over the concentration range of 0.075-12.5 ng/mL (equivalent to 0.003 mg/kg – 0.5 mg/kg) demonstrated linearity with coefficients of determination (r^2) of at least 0.999.

No significant matrix effect was observed for any of the animal foodstuff matrices investigated (<20%), therefore linearity of response was evaluated in all cases using solvent standard solutions.

Validation

For each analyte, the method was validated over the concentration range of 0.01-1.00 mg/kg with a limit of quantitation of 0.01 mg/kg.

At the 0.01-mg/kg level (LOQ), the mean recoveries were within the range of 89-100%, with relative standard deviations within the range of 1.3-4.0%.

At the 0.10-mg/kg level (10x LOQ), the mean recoveries were within the range of 89-98%, with relative standard deviations within the range of 1.1-2.6%.

The above results comply with the acceptance criteria of SANCO/825/00/rev. 8.1 for validation of the primary (quantitation) MS/MS transition. According to SANCO/825/00 rev. 8.1 however, the additional confirmatory transition should also be validated for calibration data, recovery and precision data for samples fortified at the LOQ (n=5) and for 2 blank samples. See comment below in the conclusions section.

Selectivity/Confirmation

The LC/MS/MS method is highly selective for both the quantitation and confirmation of XDE-729 methyl ester and XDE-729 acid. Analysis of control samples resulted in no significant signals at the expected retention times of the analytes. Unambiguous identification is ensured by monitoring two MS/MS transitions characteristic of each analyte and one

	MS/MS transition characteristic of each internal standard as follows: XDE-729 ME m/z 345/250 (quantitation) m/z 345/235 (confirmation) (M+6 ISTD) m/z 351/256 (quantitation) XDE-729 acid m/z 331/250 (quantitation) m/z 331/235 (confirmation) (M+6 ISTD) m/z 337/256 (quantitation) Confirmation was determined according to Commission Decision (2002/657/EC). The confirmation ratios for each analyte were determined by calculating the peak area ratios of the confirmation transition to the quantitation transition. Confirmation of the presence of the analytes in the recovery samples was indicated when the retention times of the analytes in the samples matched the retention times of the analytes in the calibration standards, and the confirmation ratios of the analytes in the samples were $\pm 20\%$ of the average confirmation ratios of the standards. The confirmation ratios for each analyte in all sample matrices were within $\pm 20\%$ of the average confirmation ratios of the standards, indicating that the method is selective for the determination of XDE-729 methyl ester and XDE-729 acid in bovine and poultry tissue matrices. This data is contained in the report. <u>Stability of Solutions and Sample Extracts</u> The stability of the stock solutions and calibration standard solutions was demonstrated in the Dow AgroSciences method (Study Number 110005; IIA 4.3/05), "Determination of Residues of XDE-729 Methyl Ester and XDE-729 Acid in Agricultural Commodities and Wheat Processed Products using Online Solid-Phase Extraction and Liquid Chromatography with Tandem Mass Spectrometry." Stock solutions of XDE-729 methyl ester and XDE-729 acid prepared in methanol were tested after 295 days of storage at ambient temperature and were found to be stable. Calibration standard solutions of XDE-729 methyl ester and XDE-729 acid prepared in methanol/water (20:80) were tested after 131 days of storage at approximately 4 °C and were found to be stable. The stability of the final sample extracts was evaluated as part of the current study. Final sample extracts were evaluated for a storage period of 9 days. For XDE-729 methyl ester, on Day 0 the recoveries ranged from 92-95%, while on Day 9, the recoveries ranged from 92-94%. For XDE-729 acid, on Day 0 the recoveries ranged from 92-97%, while on Day 9, the recoveries ranged from 94-98%. These results comply with the acceptance criteria of SANCO/825/00/rev. 8.1. <u>Matrix Effects</u> Matrix effects were determined by comparing the peak areas of solvent calibration standards to peak areas of matrix-matched standards at a single concentration level. For both analytes, matrix effects were less than 15% for both MS/MS
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	transitions and thus considered insignificant. Furthermore, matrix effects are normalized through the use of stable-isotope internal standards, resulting in accurate quantitative results irrespective of the magnitude of matrix effects. In this assay, the analytes and internal standards are quantitated using MS/MS transitions characteristic of each compound. When using stable-isotope labelled internal standards, there is a possibility that isotopic contributions will occur between the transitions used for quantitation of the unlabelled and labelled compounds. The degree of isotopic crossover for each analyte and its respective stable isotope internal standard was determined and expressed as the crossover factor. The study report demonstrates that the analyte → ISTD crossover contribution was minimal for the concentration range of the calibration curve and the concentration of the labelled internal standard. As a result, no significant mass spectral isotopic crossover was observed and therefore no correction of the measured quantitation ratio was performed. The study report states however, that if through use of the method isotopic crossover is encountered, it should be assessed and the respective quantitation ratios corrected for accurate determination of concentrations. <u>Extraction Efficiency</u> The extraction efficiency of this method was evaluated using representative poultry and ruminant tissues from XDE-729 methyl ester nature of residue studies (IIA 6.2.2 and IIA 6.2.3). Samples containing incurred ¹⁴C residues were reanalyzed with the sample analysis procedure described above. Results obtained using the analytical method were equivalent to those obtained in the metabolism study, demonstrating the suitability of this analytical method for the determination of XDE-729 methyl ester and XDE-729 acid in animal tissues.
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Validation Data (quantitation)	Matrix	Fortification Level (mg/kg)	Recovery Rate (%)		RSD (%)	n
			mean	range		
XDE-729 ME <i>m/z</i> 345→250	bovine muscle	0.01	91	88-92	2.2	5
		1.00	89	86-90	1.9	6
		0.01-1.00	89	86-92	2.2	11
XDE-729 ME <i>m/z</i> 345→250	bovine liver	0.01	89	87-92	2.1	6
		1.00	91	89-94	1.7	6
		0.01-1.00	90	87-94	2.1	12
XDE-729 ME <i>m/z</i> 345→250	bovine kidney	0.01	90	87-95	3.5	5
		1.00	93	91-94	1.2	6
		0.01-1.00	92	87-95	2.8	11
XDE-729 ME <i>m/z</i> 345→250	bovine fat	0.01	91	88-94	2.2	6
		1.00	92	89-94	2.5	6
		0.01-1.00	91	88-94	2.3	12
XDE-729 ME <i>m/z</i> 345→250	bovine whole milk	0.01	90	88-92	2.1	6
		1.00	89	86-91	2.1	6
		0.01-1.00	89	86-92	2.0	12
XDE-729 ME <i>m/z</i> 345→250	bovine cream	0.01	100	96-103	2.4	6
		1.00	94	92-98	2.4	6
		0.01-1.00	97	92-103	3.5	12
XDE-729 ME <i>m/z</i> 345→250	poultry muscle	0.01	92	90-95	2.3	6
		1.00	92	90-94	1.8	6
		0.01-1.00	92	90-95	2.0	12
XDE-729 ME <i>m/z</i> 345→250	poultry liver	0.01	96	91-101	3.4	6
		1.00	94	91-97	2.6	6
		0.01-1.00	95	91-101	3.0	12
XDE-729 ME <i>m/z</i> 345→250	poultry fat	0.01	95	92-98	2.4	6
		1.00	94	92-96	1.3	6
		0.01-1.00	94	92-98	1.9	12
XDE-729 ME <i>m/z</i> 345→250	poultry eggs	0.01	96	93-103	3.8	6
		1.00	94	93-97	1.7	6
		0.01-1.00	95	93-103	3.0	12

Validation Data (quantitation)	Matrix	Fortification Level (mg/kg)	Recovery Rate (%)		RSD (%)	n
			mean	range		
XDE-729 acid <i>m/z</i> 331→250	bovine muscle	0.01	89	87-90	1.3	5
		1.00	90	89-92	1.5	6
		0.01-1.00	89	87-92	1.6	11
XDE-729 acid <i>m/z</i> 331→250	bovine liver	0.01	92	89-95	2.7	6
		1.00	90	89-93	2.0	6
		0.01-1.00	91	89-95	2.4	12
XDE-729 acid <i>m/z</i> 331→250	bovine kidney	0.01	92	87-96	4.0	5
		1.00	93	91-94	1.4	6
		0.01-1.00	92	87-96	2.8	11
XDE-729 acid <i>m/z</i> 331→250	bovine fat	0.01	93	90-95	2.3	6
		1.00	95	93-96	1.2	6
		0.01-1.00	94	90-96	2.1	12
XDE-729 acid <i>m/z</i> 331→250	bovine whole milk	0.01	91	88-96	3.2	6
		1.00	93	91-94	1.3	6
		0.01-1.00	92	88-96	2.7	12
XDE-729 acid <i>m/z</i> 331→250	bovine cream	0.01	95	92-97	2.2	6
		1.00	97	94-99	2.3	6
		0.01-1.00	96	92-99	2.5	12
XDE-729 acid <i>m/z</i> 331→250	poultry muscle	0.01	89	84-91	2.8	6
		1.00	90	89-93	1.7	6
		0.01-1.00	90	84-93	2.3	12
XDE-729 acid <i>m/z</i> 331→250	poultry liver	0.01	92	88-95	3.2	6
		1.00	94	92-96	2.0	6
		0.01-1.00	93	88-96	2.8	12
XDE-729 acid <i>m/z</i> 331→250	poultry fat	0.01	94	92-96	1.5	6
		1.00	98	97-100	1.1	6
		0.01-1.00	96	92-100	2.4	12
XDE-729 acid <i>m/z</i> 331→250	poultry eggs	0.01	93	89-97	2.8	6
		1.00	95	94-98	1.9	6
		0.01-1.00	94	89-98	2.6	12

Conclusion

It is noted that full validation data are only obtained for the primary (quantitation) MS/MS transition. According to SANCO/825/00 rev. 8.1 the additional confirmatory transition should also be validated for calibration data, recovery and precision data for samples fortified at the LOQ (n=5) and for 2 blank samples. It is a short coming of the study that full validation data has only been presented for the primary transition; however, this data has been presented for bovine fat, bovine liver and poultry eggs for the ILV of this method (see below). Only three matrices were considered in the ILV, however the primary extraction method is the same for all matrices therefore this is acceptable. Given that the ILV study report demonstrates that both the quantitative and confirmatory MS/MS transitions are acceptably validated, in this instance the RMS proposes that consideration of the complete data set from the validation report Study

Number 110505 and the ILV report (see below, Study Number 110828) indicates that the method is fit for purpose according to SANCO/825/00 rev. 8.1.

The method however does rely on stable isotope labelled internal standards. According to SANCO/825/00 rev. 8.1, all analytical standard materials used in an analytical method must be commonly available. This applies to stable isotope labelled compounds or other internal standards. If a standard is not commercially available the standard should be made generally available by the applicant and the contact details be provided. The notifier was contacted during this evaluation however, and confirmed that the stable isotope labelled internal standards would be made available in line with guidance.

B.5.2.9 Independent Laboratory Validation of the Enforcement Method for the Determination of XDE 729 Methyl Ester and XDE 729 Acid in Animal Matrices

Reference: Langridge, G. (2012) Independent Laboratory Validation of an Analytical Method for the Determination of XDE-729 Methyl Ester and XDE-729 Acid in Animal Matrices, Dow AgroSciences LLC Study Number 110828, CEMAS Study Number CEMS-5325

This method has been submitted in support of both pre-registration and post-registration monitoring and control for components included in the residue definition for plants and animals. The proposed residue definition for Risk Assessment and Enforcement/Monitoring in plants and livestock is XDE-729 methyl and XDE-729 acid.

Guidelines:

SANCO/825/00 rev. 8.1

SANCO/3029/99 rev. 4

Matrix, limit of quantitation	bovine liver 0.01 mg/kg bovine fat 0.01 mg/kg poultry eggs 0.01 mg/kg
Description	<u>Scope</u> The objective of this study was to independently validate Dow AgroSciences method (Study Number 110505), “Method Validation Study for the Determination of Residues of XDE-729 Methyl Ester and XDE-729 Acid in Bovine and Poultry Tissues using Offline Solid-Phase Extraction and Liquid Chromatography with Mass Spectrometry Detection.” This method is applicable for the quantitative determination of residues of XDE-729 methyl ester and XDE-729 acid in animal tissues. The method was independently validated at concentrations of 0.01 mg/kg, 0.10 mg/kg, and 0.10 mg/kg for representative ruminant tissues (bovine liver and bovine fat) and poultry tissues (eggs) with a validated limit of quantitation of 0.01 mg/kg. Since the primary method was the same for all matrices, it is sufficient to perform the ILV on the three matrices listed above. <u>Principle</u> Residues of XDE-729 methyl ester and XDE-729 acid were extracted from a 1.0-gram bovine or poultry sample by shaking with 20 mL of an acetonitrile/water (80:20) solution and partitioning with 20 mL of

n-hexane. Fifty microliters of a glycerin/methanol solution (10:90 w/v) were added to a 1000- μ L aliquot of the acetonitrile/water extraction solution and the sample was concentrated to near dryness. The sample extract was then diluted to 1.0 mL with aqueous 0.1% formic acid and purified using a Phenomenex Strata-X reversed-phase polymeric solid-phase extraction (SPE) cartridge. After elution from the SPE cartridge with a methanol/water (95:5) solution containing 0.10% formic acid, 200 μ L of a mixed stable-isotope internal standard solution was added to the eluate, which was subsequently concentrated to approximately 1 mL. The solution was then adjusted to 2.0 mL with water. After preparation, the samples were analyzed by liquid chromatography using a Zorbax SB-C8 column coupled with positive-ion electrospray tandem mass spectrometry (LC/MS/MS), monitoring two MS/MS transitions characteristic of each analyte and one MS/MS transition characteristic of each internal standard.

Calibration

For each analyte, the linearity of detector response was evaluated using solvent standard solutions. Calibration curves were calculated by linear regression analysis without weighting. For the least squares equation which describes the detector response as a function of the standard concentration, calibration curves resulting from the injection of a minimum of eight standards over the concentration range of 0.075-12.5 ng/mL demonstrated linearity with coefficients of determination (r^2) of at least 0.995. This range covers from 30% of the LOQ to at least 20% above the highest measured level in the samples.

Validation

For each analyte, the method was validated over the concentration range of 0.01-1.00 mg/kg with a limit of quantitation of 0.01 mg/kg.

At the 0.01-mg/kg level (LOQ), the mean recoveries were within the range of 92-103%, with relative standard deviations within the range of 2.2-11.3%.

At the 0.10-mg/kg level (10x LOQ), the mean recoveries were within the range of 79-103%, with relative standard deviations within the range of 2.3-9.5%.

At the 1.00-mg/kg level (100x LOQ), the mean recoveries were within the range of 88-101%, with relative standard deviations within the range of 0.9-5.3%.

The above results comply with the acceptance criteria of SANCO/825/00/rev. 8.1.

Selectivity/Confirmation

The LC/MS/MS method is highly selective for both the quantitation and confirmation of XDE-729 methyl ester and XDE-729 acid. Analysis of

	control samples resulted in no significant signals at the expected retention times of the analytes. Unambiguous identification is ensured by monitoring two MS/MS transitions characteristic of each analyte and one MS/MS transition characteristic of each internal standard as follows: XDE-729 ME m/z 345/250 (quantitation) m/z 345/235 (confirmation) (M+6 ISTD) m/z 351/256 (quantitation) XDE-729 acid m/z 331/250 (quantitation) m/z 331/285 (confirmation) (M+6 ISTD) m/z 337/256 (quantitation) Confirmation was demonstrated using one of two techniques. In the first, recovery and precision data were determined for both ion mass transitions; these are listed in the tables below. In the second, the confirmation ratios for each analyte were determined by calculating the peak area ratios of the confirmation transition to the quantitation transition according to Commission Decision (2002/657/EC). Confirmation of the presence of the analytes in the recovery samples was indicated when the retention times of the analytes in the samples matched the retention times of the analytes in the calibration standards, and the confirmation ratios of the analytes in the samples were $\pm 20\%$ of the average confirmation ratios of the standards. This data is contained in the report. <u>Stability of Solutions and Sample Extracts</u> The evaluation of the stability of stock solutions, calibration solutions, and sample extracts is not a requirement of an independent laboratory validation. <u>Matrix Effects</u> The evaluation of matrix effects is not a requirement of an independent laboratory validation. However, it is noted that matrix effects are normalized through the use of stable-isotope internal standards, resulting in accurate quantitative results irrespective of the magnitude of matrix effects.
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Validation Data (quantitation)	Matrix	Fortification Level (mg/kg)	Recovery Rate (%)		RSD (%)	n
			mean	range		
XDE-729 ME <i>m/z</i> 345→250	bovine liver	0.01	92	91-96	2.4	5
		0.10	90	88-94	3.0	5
		1.00	89	85-96	5.3	5
		0.01-0.10	90	85-96	3.9	15
XDE-729 ME <i>m/z</i> 345→250	bovine fat	0.01	95	93-98	2.2	5
		0.10	83	81-88	3.4	5
		1.00	88	86-93	3.3	5
		0.01-0.10	89	81-98	6.0	15
XDE-729 ME <i>m/z</i> 345→250	poultry eggs	0.01	95	89-98	3.6	5
		0.10	79	70-86	8.3	5
		1.00	89	88-90	0.9	5
		0.01-0.10	88	70-98	8.8	15

Validation Data (quantitation)	Matrix	Fortification Level (mg/kg)	Recovery Rate (%)		RSD (%)	n
			mean	range		
XDE-729 acid <i>m/z</i> 331→250	bovine liver	0.01	99	92-102	3.9	5
		0.10	95	92-99	3.1	5
		1.00	97	94-100	2.5	5
		0.01-0.10	97	92-102	3.3	15
XDE-729 acid <i>m/z</i> 331→250	bovine fat	0.01	101	90-110	8.1	5
		0.10	103	98-108	3.6	5
		1.00	101	99-103	1.5	5
		0.01-0.10	102	90-110	4.9	15
XDE-729 acid <i>m/z</i> 331→250	poultry eggs	0.01	98	94-102	3.4	5
		0.10	94	86-103	8.2	5
		1.00	99	95-102	3.1	5
		0.01-0.10	97	86-103	5.5	15

Validation Data (confirmation)	Matrix	Fortification Level (mg/kg)	Recovery Rate (%)		RSD (%)	n
			mean	range		
XDE-729 ME <i>m/z</i> 345→207	bovine liver	0.01	96	92-98	2.4	5
		0.10	86	84-89	2.3	5
		1.00	91	88-94	2.9	5
		0.01-0.10	91	84-98	5.1	15
XDE-729 ME <i>m/z</i> 345→207	bovine fat	0.01	96	89-102	5.1	5
		0.10	85	82-87	2.3	5
		1.00	90	87-95	3.6	5
		0.01-0.10	90	82-102	6.1	15
XDE-729 ME <i>m/z</i> 345→207	poultry eggs	0.01	95	92-98	2.4	5
		0.10	81	71-86	7.6	5
		1.00	92	90-93	1.5	5
		0.01-0.10	90	71-98	7.8	15

Validation Data (confirmation)	Matrix	Fortification Level (mg/kg)	Recovery Rate (%)		RSD (%)	n
			mean	range		
XDE-729 acid <i>m/z</i> 331→207	bovine liver	0.01	102	87-114	11.3	5
		0.10	90	85-96	4.8	5
		1.00	99	96-104	3.3	5
		0.01-0.10	97	85-114	8.9	15
XDE-729 acid <i>m/z</i> 331→207	bovine fat	0.01	95	80-107	11.0	5
		0.10	98	90-107	7.4	5
		1.00	99	97-104	3.3	5
		0.01-0.10	97	80-107	7.4	15
XDE-729 acid <i>m/z</i> 331→207	poultry eggs	0.01	103	96-114	7.0	5
		0.10	96	86-109	9.5	5
		1.00	100	97-102	2.0	5
		0.01-0.10	99	86-114	6.9	15

Conclusion

The primary method is identical for all matrix groups, therefore it was considered to be sufficient to perform the ILV bovine fat, bovine liver and poultry eggs. In line with the conclusions made under B.5.2.12, the RMS proposes that the method is fit for purpose according to SANCO/825/00 rev. 8.1. The method however does rely on stable isotope labelled internal standards. According to SANCO/825/00 rev. 8.1, all analytical standard materials used in an analytical method must be commonly available. This applies to stable isotope labelled compounds or other internal standards. If a standard is not commercially available the standard should be made generally available by the applicant and the contact details be provided. The notifier was contacted during this evaluation however, and confirmed that the stable isotope labelled internal standards would be made available in line with guidance.

B.5.3.1 Residues in soil**B.5.3.1.1 Description of methods for analysis of soil for parent compound and metabolites of toxicological, ecotoxicological or environmental concern.**

Reference: Blakeslee, B. A. (2012) Method Validation Study for the Determination of Residues of X11393728 (XDE-729 Methyl), X119393729 (XDE-729 Acid), and X11449757 (des-Methyl XDE-729 Acid) in Soil using High Performance Liquid Chromatography with Positive-Ion Electrospray Ionization Mass Spectrometry Detection, Dow AgroSciences LLC Study Number 110716.

Guidelines:**SANCO/825/00 rev. 8.1****SANCO/3029/99 rev. 4**

This method has been submitted in support for both pre-registration data requirements and post-registration monitoring and control for components included in the residue definition for soil (XDE-729 methyl ester and its metabolites, XDE-729 acid and X11449757).

Matrix, limit of quantitation	soil 0.05 µg/kg
Description	<u>Scope</u> This method is applicable for the quantitative determination of residues of XDE-729 methyl ester and its metabolites, XDE-729 acid and X11449757, in 4 soil types. The method was validated over the concentration range of 0.05-8.0 µg/kg with a validated limit of quantitation of 0.05 µg/kg. <u>Principle</u> Residues of XDE-729 methyl ester and its metabolites were extracted from a 5.0-gram soil sample with 25 mL of a methanol/water (50:50) solution containing 0.1% phosphoric acid using two cycles of accelerated solvent extraction (ASE) at 90 °C and 1500-2500 psi. After extraction, 40 µL of a mixed stable-isotope internal standard solution were added to a 5.0-mL aliquot of the methanol/water extraction solution, which was subsequently concentrated to approximately 1 mL. One-hundred microliters of acetonitrile were added to the sample extract and the solution was then filtered prior to analysis. The samples were analyzed by liquid chromatography using a Synergi Hydro-RP C18 (75x4.6mm id) column coupled with positive-ion electrospray tandem mass spectrometry (LC/MS/MS), monitoring two MS/MS transitions characteristic of each analyte and one MS/MS transition characteristic of each internal standard with gradient elution of water containing 0.1% formic acid and acetonitrile containing 0.1% formic acid. <u>Calibration</u> For each analyte, the linearity of detector response was evaluated using solvent standard solutions. Calibration curves were calculated by linear regression analysis with 1/x weighting. For the least squares equation which describes the detector response as a function of the standard

concentration, calibration curves resulting from the injection of a minimum of eight standards over the concentration range of 0.02-10.0 ng/mL (equivalent to 0.02 ng/g – 10 ng/g) demonstrated linearity with coefficients of determination (r^2) of at least 0.998.

The calibration standards always covered the range of at least $\leq 30\%$ of the LOQ up to 20 % above the highest analysed sample extract concentration level as demanded in SANCO Guideline 825/00 rev. 8.1.

Validation

The method was validated using four soils obtained from the Dow AgroSciences Soils Database; a sandy soil, a clay loam soil, a silt loam soil, and a clay soil. Full characterization of the soil samples was contained in the method validation report.

For each analyte, the method was validated over the concentration range of 0.05-8.0 µg/kg with a limit of quantitation of 0.05 µg/kg.

At the 0.05-µg/kg level (LOQ), the mean recoveries were within the range of 63-103%, with relative standard deviations within the range of 4-26%.

At the 0.10-µg/kg level (2x LOQ), the mean recoveries were within the range of 71-109%, with relative standard deviations within the range of 2-14%.

At the 8.0-µg/kg level (160x LOQ), the mean recoveries were within the range of 62-116%, with relative standard deviations within the range of 1-8%.

The mean recovery in all cases, apart from X11449757 in all soil types (sand, clay loam, silt loam and clay) and XDE-729 acid in sand, are above the acceptable recovery level of 60%. The precision were all acceptable.

The notifier stated that considering that the LOQ of 0.05 µg/kg of this method is approximately 200-fold lower than the typical method LOQ of 10 µg/kg, the above results generally comply with the acceptance criteria of SANCO/825/00/rev. 8.1.

Selectivity/Confirmation

The LC/MS/MS method is highly selective for both the quantitation and confirmation of XDE-729 methyl ester, XDE-729 acid, and X11449757.

Analysis of control samples resulted in no significant signals at the expected retention times of the analytes. Unambiguous identification is ensured by monitoring two MS/MS transitions characteristic of each analyte and one MS/MS transition characteristic of each internal standard as follows:

XDE-729 ME	<i>m/z</i> 345/250 (quantitation)
	<i>m/z</i> 345/285 (confirmation)
(M+6 ISTD)	<i>m/z</i> 351/256 (quantitation)
XDE-729 acid	<i>m/z</i> 331/250 (quantitation)
	<i>m/z</i> 331/285 (confirmation)

	(M+6 ISTD) m/z 337/256 (quantitation) X11449757 m/z 317/236 (quantitation) m/z 319/238 (confirmation) (M+6 ISTD) m/z 323/242 (quantitation) Confirmation was demonstrated by determining the recovery and precision for both MS/MS transitions; these data are listed in the tables below. <u>Stability of Solutions and Sample Extracts</u> Stock solutions of XDE-729 methyl ester, XDE-729 acid, and X11449757 were tested after 90 days and 335 days of storage at approximately 4 °C and were found to be stable. Spiking solutions of XDE-729 methyl ester, XDE-729 acid, and X11449757 prepared in acetonitrile containing 0.10% formic acid were tested after 19, 90, 203, and 292 days of storage at approximately 4 °C. It was determined that the spiking solutions were stable up to 90 days. Calibration standard solutions of XDE-729 methyl ester, XDE-729 acid, and X11449757 prepared in acetonitrile/water (10:90) containing 0.10% formic acid were tested after 19, 90, and 292 days of storage at approximately 4 °C and were found to be stable. The stability of the final sample extracts was evaluated as part of the current study. Final sample extracts were evaluated for a storage period of 10 days. For XDE-729 methyl ester, on Day 0 the recoveries ranged from 92-107%, while on Day 10, the recoveries ranged from 93-106%. For XDE-729 acid, on Day 0 the recoveries ranged from 68-85%, while on Day 10, the recoveries ranged from 74-87%. For 11449757, on Day 0 the recoveries ranged from 56-73%, while on Day 10, the recoveries ranged from 55-74%. These results comply with the acceptance criteria of SANCO/825/00/rev. 8.1. <u>Matrix Effects</u> Matrix effects were determined by comparing the peak areas of solvent calibration standards to peak areas of matrix-matched standards at a single concentration level. For XDE-729 methyl ester, matrix effects ranged from -76% to -44% across all soil types. For XDE-729 acid, matrix effects ranged from -22% to 5% across all soil types. For X11449757, matrix effects ranged from -32% to 34% across all soil types. Although matrix effects are significant (i.e > 20 %), it is noted that the matrix effects are normalized through the use of stable-isotope internal standards, resulting in accurate quantitative results irrespective of the magnitude of matrix effects. In this assay, the analytes and internal standards are quantitated using MS/MS transitions characteristic of each compound. When using stable-
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	isotope labelled internal standards, there is a possibility that isotopic contributions will occur between the transitions used for quantitation of the unlabelled and labelled compounds. The degree of isotopic crossover for each analyte and its respective stable isotope internal standard was determined and expressed as the crossover factor. The study report demonstrates that the analyte → ISTD crossover contribution was minimal for the concentration range of the calibration curve and the concentration of the labelled internal standard. As a result, no significant mass spectral isotopic crossover was observed and therefore no correction of the measured quantitation ratio was performed. The study report states however that if through use of the method isotopic crossover is encountered, it should be assessed and the respective quantitation ratios corrected for accurate determination of concentrations. <u>Extraction Efficiency</u> The extraction efficiency of this method for the suitable removal of XDE-729 methyl and its metabolites from soil was demonstrated in the Dow AgroSciences aerobic soil metabolism study number 090121, 'Aerobic Soil Degradation of XDE-729 Methyl on Four Global Soils'. Extraction from sterile soils was demonstrated in the Dow AgroSciences anaerobic soil degradation study number 101654, 'Soil Degradation of XDE-729 Methyl under Anaerobic, Low Temperature and Sterile Conditions'. The non-extractable residues accounted for typically less than 10% of applied radioactivity, indicating that the extraction procedure adequately extracted aged XDE-729 methyl residues, and its metabolites, from soil.
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Validation Data (quantitation)	Matrix	Fortification Level (µg/kg)	Recovery Rate (%)		RSD (%)	n
			mean	range		
XDE-729 ME m/z 345→250	sand	0.05	96	85-112	9	8
		0.10	96	95-98	2	3
		8.00	105	102-110	3	5
		0.05-8.00	99	85-112	8	16
XDE-729 ME m/z 345→250	clay loam	0.05	100	94-106	4	8
		0.10	105	103-107	2	3
		8.00	95	92-99	3	5
		0.05-8.00	100	92-107	5	16
XDE-729 ME m/z 345→250	silt loam	0.05	101	93-109	5	12
		0.10	101	99-104	3	4
		8.00	102	97-109	4	6
		0.05-8.00	101	93-109	4	22
XDE-729 ME m/z 345→250	clay	0.05	103	94-111	5	8
		0.10	102	97-107	5	3
		8.00	95	92-97	2	5
		0.05-8.00	100	92-111	6	16

Validation Data (quantitation)	Matrix	Fortification Level (µg/kg)	Recovery Rate (%)		RSD (%)	n
			mean	range		
XDE-729 acid <i>m/z</i> 331→250	sand	0.05	77	72-83	6	8
		0.10	79	75-85	7	3
		8.00	75	70-78	4	5
		0.05-8.00	77	70-85	5	16
XDE-729 acid <i>m/z</i> 331→250	clay loam	0.05	75	64-89	11	9
		0.10	79	76-82	4	3
		8.00	70	68-74	4	5
		0.05-8.00	75	64-89	9	17
XDE-729 acid <i>m/z</i> 331→250	silt loam	0.05	79	70-92	8	16
		0.10	76	72-78	4	4
		8.00	76	73-79	3	6
		0.05-8.00	78	70-92	7	26
XDE-729 acid <i>m/z</i> 331→250	clay	0.05	84	78-93	6	8
		0.10	81	80-84	3	3
		8.00	78	76-80	2	5
		0.05-8.00	82	76-93	5	16

Validation Data (quantitation)	Matrix	Fortification Level (µg/kg)	Recovery Rate (%)		RSD (%)	n
			mean	range		
X11449757 <i>m/z</i> 317→236	sand	0.05	73	59-74	10	8
		0.10	80	76-84	5	3
		8.00	83	78-85	3	5
		0.05-8.00	77	59-85	9	16
X11449757 <i>m/z</i> 317→236	clay loam	0.05	63	56-76	11	9
		0.10	71	68-73	4	3
		8.00	62	58-68	4	5
		0.05-8.00	64	56-76	9	17
X11449757 <i>m/z</i> 317→236	silt loam	0.05	66	56-85	11	16
		0.10	74	72-75	2	4
		8.00	71	64-76	7	6
		0.05-8.00	68	56-85	10	26
X11449757 <i>m/z</i> 317→236	clay	0.05	69	55-78	13	8
		0.10	78	73-84	7	3
		8.00	73	73-74	1	5
		0.05-8.00	72	55-84	10	16

Validation Data (confirmation)	Matrix	Fortification Level (µg/kg)	Recovery Rate (%)		RSD (%)	n
			mean	range		
XDE-729 ME <i>m/z</i> 345→285	sand	0.05	87	68-106	15	8
		0.10	87	83-89	4	3
		8.00	104	100-107	2	5
		0.05-8.00	92	68-107	13	16
XDE-729 ME <i>m/z</i> 345→285	clay loam	0.05	93	84-116	11	9
		0.10	99	97-101	2	3
		8.00	94	91-98	3	5
		0.05-8.00	95	84-116	8	17
XDE-729 ME <i>m/z</i> 345→285	silt loam	0.05	93	81-108	9	12
		0.10	98	91-105	6	4
		8.00	101	97-107	4	6
		0.05-8.00	96	81-108	8	22
XDE-729 ME <i>m/z</i> 345→285	clay	0.05	99	93-104	4	8
		0.10	96	94-100	3	3
		8.00	94	91-94	1	5
		0.05-8.00	97	91-104	4	16

Validation Data (confirmation)	Matrix	Fortification Level (µg/kg)	Recovery Rate (%)		RSD (%)	n
			mean	range		
XDE-729 acid <i>m/z</i> 331→285	sand	0.05	78	57-107	26	8
		0.10	85	77-99	14	3
		8.00	75	72-79	4	5
		0.05-8.00	78	57-107	19	16
XDE-729 acid <i>m/z</i> 331→285	clay loam	0.05	78	68-90	10	9
		0.10	74	70-76	4	3
		8.00	71	68-75	4	5
		0.05-8.00	75	68-90	9	17
XDE-729 acid <i>m/z</i> 331→285	silt loam	0.05	88	74-100	9	15
		0.10	78	74-82	4	4
		8.00	75	72-77	2	6
		0.05-8.00	83	72-100	10	25
XDE-729 acid <i>m/z</i> 331→285	clay	0.05	77	66-95	12	8
		0.10	79	69-87	11	3
		8.00	80	77-82	3	5
		0.05-8.00	78	66-95	9	16

Validation Data (confirmation)	Matrix	Fortification Level (µg/kg)	Recovery Rate (%)		RSD (%)	n
			mean	range		
X11449757 <i>m/z</i> 319→238	sand	0.05	86	74-100	10	6
		0.10	109	105-117	6	3
		8.00	116	110-118	3	5
		0.05-8.00	100	74-118	16	16
X11449757 <i>m/z</i> 319→238	clay loam	0.05	79	68-90	11	9
		0.10	80	73-92	13	3
		8.00	75	70-83	8	5
		0.05-8.00	78	68-92	10	17
X11449757 <i>m/z</i> 319→238	silt loam	0.05	72	54-90	14	16
		0.10	85	75-93	10	4
		8.00	92	87-100	6	6
		0.05-8.00	79	54-100	16	26
X11449757 <i>m/z</i> 319→238	clay	0.05	71	56-83	12	8
		0.10	87	80-98	11	3
		8.00	97	96-99	1	5
		0.05-8.00	82	56-99	17	16

Conclusion

The method has been validated in all areas according to SANCO/825/00 rev. 8.1.

The mean recovery of X11449757 at 0.05 µg/kg (LOQ) in clay loam, silt loam and clay, and at 8.0 µg/kg in clay loam were below the acceptable mean recovery level of 70 %. The lowest mean recovery at LOQ was 63 %. The mean recovery for all other samples and precision were all acceptable. Overall, given that the data in all other areas of the validation are well within guideline acceptable limits, and recovery of the other samples gave consistent values within acceptable limits, it is considered in this instance to accept that the data-set, as a whole, is adequately validated for recovery and precision.

The method however does rely on stable isotope labelled internal standards. According to SANCO/825/00 rev. 8.1, all analytical standard materials used in an analytical method must be commonly available. This applies to stable isotope labelled compounds or other internal standards. If a standard is not commercially available the standard should be made generally available by the applicant and the contact details be provided. The notifier was contacted during this evaluation however, and confirmed that the stable isotope labelled internal standards would be made available in line with guidance.

B.5.3.1.2 Independent Laboratory Validation of the Method for the Determination of XDE-729 Methyl Ester and Metabolites in Soil.

Reference: Gemrot, F. (2012) Independent Laboratory Validation of Analytical Method Study Number 110716 for the Determination of XDE-729 Methyl Ester and its Metabolite Residues in Soil, Dow AgroSciences LLC Study Number 120894.

Guidelines:**SANCO/825/00 rev. 8.1****SANCO/3029/99 rev. 4**

This method has been submitted in support of both pre-registration data requirements and post-registration monitoring and control for components included in the residue definition for soil (XDE-729 methyl ester and its metabolites, XDE-729 acid and X11449757).

Matrix, limit of quantitation	soil 0.05 µg/kg
Description	<u>Scope</u> The objective of this study was to independently validate Dow AgroSciences method (Study Number 110716), “Method Validation Study for the Determination of Residues of X11393728 (XDE-729 Methyl), X119393729 (XDE-729 Acid), and X11449757 (des-Methyl XDE-729 Acid) in Soil using High Performance Liquid Chromatography with Positive-Ion Electrospray Ionization Mass Spectrometry Detection.” This method is applicable for the quantitative determination of residues of XDE-729 methyl ester and its metabolites, XDE-729 acid and X11449757, in 4 soil types – two soil groups have been independently validated as the primary method is identical for all matrix group. The method was validated over the concentration range of 0.05-0.50 µg/kg with a validated limit of quantitation of 0.05 µg/kg. <u>Principle</u> Residues of XDE-729 methyl ester and its metabolites were extracted from a 5.0-gram soil sample with 25 mL of a methanol/water (50:50) solution containing 0.1% phosphoric acid using two cycles of accelerated solvent extraction (ASE) at 90 °C and 1500 psi. After extraction, 40 µL of a mixed stable-isotope internal standard solution were added to a 5.0-mL aliquot of the methanol/water extraction solution, which was subsequently concentrated to approximately 1 mL. One-hundred microliters of acetonitrile were added to the sample extract and the solution was then filtered prior to analysis. The samples were analyzed by liquid chromatography using a Synergi Hydro-RP 80A, 4.6 x 75 mm, 4 µm, C18 column coupled with positive-ion electrospray tandem mass spectrometry (LC/MS/MS), monitoring two MS/MS transitions characteristic of each analyte and one MS/MS transition characteristic of each internal standard with gradient elution of water containing 0.1% formic acid and acetonitrile

containing 0.1% formic acid.

Calibration

For each analyte, the linearity of detector response was evaluated using solvent standard solutions. Calibration curves were calculated by linear regression analysis without weighting. For the least squares equation which describes the detector response as a function of the standard concentration, calibration curves resulting from the injection of a minimum of eight standards over the concentration range of 0.01-6.0 ng/mL (equivalent to 0.01 µg/kg to 6.0 µg/kg) demonstrated linearity with coefficients of determination (r^2) of at least 0.995.

Validation

The method was validated using a sandy soil and a clay soil, both obtained from Lufa Speyer. Full characterization of the soil samples was contained in the method validation report.

For each analyte, the method was validated over the concentration range of 0.05-0.50 µg/kg with a limit of quantitation of 0.05 µg/kg.

At the 0.05-µg/kg level (LOQ), the mean recoveries were within the range of 78-102%, with relative standard deviations within the range of 4-17%.

At the 0.50-µg/kg level (10x LOQ), the mean recoveries were within the range of 98-107%, with relative standard deviations within the range of 2-8%.

The reference stock solutions have been prepared in methanol instead of in acetonitrile. The notifier considered that this modification had no impact on the study, because stability data are already available for these solutions (unpublished Dow AgroSciences Study Number 110718, Souza, N.(2011), "Method Validation Study for the Determination of Residues of XDE-729 methyl ester and its metabolites in Surface Water, Ground Water and Drinking Water by liquid chromatography with Tandem Mass Spectrometry").

The above results comply with the acceptance criteria of SANCO/825/00/rev. 8.1.

Selectivity/Confirmation

The LC/MS/MS method is highly selective for both the quantitation and confirmation of XDE-729 methyl ester, XDE-729 acid, and X11449757.

Analysis of control samples resulted in no significant signals at the expected retention times of the analytes. Unambiguous identification is ensured by monitoring two MS/MS transitions characteristic of each analyte and one MS/MS transition characteristic of each internal standard as follows:

XDE-729 ME	m/z 345/250 (quantitation)
	m/z 345/285 (confirmation)
(M+6 ISTD)	m/z 351/256 (quantitation)
XDE-729 acid	m/z 331/250 (quantitation)
	m/z 331/285 (confirmation)

	(M+6 ISTD) m/z 337/256 (quantitation) X11449757 m/z 317/236 (quantitation) m/z 319/238 (confirmation) (M+6 ISTD) m/z 323/242 (quantitation) Confirmation was demonstrated by determining the recovery and precision for both MS/MS transitions; these data are listed in the tables below. <u>Stability of Solutions and Sample Extracts</u> The stability of the stock solutions, calibration standard solutions, and sample extracts was not evaluated as part of this study as it is not a requirement of an independent laboratory validation. <u>Matrix Effects</u> Matrix effects were not evaluated as part of this study as they are not a requirement of an independent laboratory validation. However, it is important to note that matrix effects are normalized through the use of stable-isotope internal standards, resulting in accurate quantitative results irrespective of the magnitude of matrix effects.
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Validation Data (quantitation)	Matrix	Fortification Level ($\mu\text{g/kg}$)	Recovery Rate (%)		RSD (%)	n
			mean	range		
XDE-729 ME m/z 345→250	clay soil	0.05	97	89-103	6	5
		0.50	106	97-116	6	5
		0.05-0.50	102	89-116	8	10
XDE-729 ME m/z 345→250	sandy soil	0.05	98	89-104	6	5
		0.50	106	103-109	2	5
		0.05-0.50	102	89-109	6	10

Validation Data (quantitation)	Matrix	Fortification Level ($\mu\text{g/kg}$)	Recovery Rate (%)		RSD (%)	n
			mean	range		
XDE-729 acid m/z 331→250	clay soil	0.05	90	84-98	6	5
		0.50	100	94-111	6	5
		0.05-0.50	95	84-111	8	10
XDE-729 acid m/z 331→250	sandy soil	0.05	97	87-104	7	5
		0.50	106	103-112	3	5
		0.05-0.50	101	87-112	7	10

Validation Data (quantitation)	Matrix	Fortification Level (µg/kg)	Recovery Rate (%)		RSD (%)	n
			mean	range		
X11449757 <i>m/z</i> 317→236	clay soil	0.05	102	89-114	11	5
		0.50	100	92-108	6	5
		0.05-0.50	101	89-114	8	10
X11449757 <i>m/z</i> 317→236	sandy soil	0.05	95	79-109	13	5
		0.50	99	92-102	4	5
		0.05-0.50	97	79-109	9	10

Validation Data (confirmation)	Matrix	Fortification Level (µg/kg)	Recovery Rate (%)		RSD (%)	n
			mean	range		
XDE-729 ME <i>m/z</i> 345→285	clay soil	0.05	95	89-98	4	5
		0.50	106	101-111	4	5
		0.05-0.50	101	89-111	7	10
XDE-729 ME <i>m/z</i> 345→285	sandy soil	0.05	102	94-108	5	5
		0.50	105	100-111	4	5
		0.05-0.50	104	94-111	5	10

Validation Data (confirmation)	Matrix	Fortification Level (µg/kg)	Recovery Rate (%)		RSD (%)	n
			mean	range		
XDE-729 acid <i>m/z</i> 331→285	clay soil	0.05	94	82-117	15	5
		0.50	101	95-106	5	5
		0.05-0.50	97	82-117	11	10
XDE-729 acid <i>m/z</i> 331→285	sandy soil	0.05	96	85-100	7	5
		0.50	107	102-115	5	5
		0.05-0.50	101	85-115	8	10

Validation Data (confirmation)	Matrix	Fortification Level (µg/kg)	Recovery Rate (%)		RSD (%)	n
			mean	range		
X11449757 <i>m/z</i> 319→238	clay soil	0.05	95	71-109	17	5
		0.50	98	93-104	4	5
		0.05-0.50	96	71-109	11	10
X11449757 <i>m/z</i> 319→238	sandy soil	0.05	78	60-90	17	5
		0.50	103	93-112	8	5
		0.05-0.50	91	60-112	19	10

Conclusion

The primary method is identical for all matrix groups, therefore it was considered to be sufficient to perform the ILV on 2 soil types (sand and clay). The method has been validated in all areas according to SANCO/825/00 rev. 8.1. In line with the conclusions made under B.5.3.1.1 the RMS proposes that the method is fit for purpose according to SANCO/825/00 rev. 8.1. The method however does rely on stable isotope labelled internal standards. According to SANCO/825/00 rev. 8.1, all analytical standard materials used in an analytical method must be

commonly available. This applies to stable isotope labelled compounds or other internal standards. If a standard is not commercially available the standard should be made generally available by the applicant and the contact details be provided. The notifier was contacted during this evaluation however, and confirmed that the stable isotope labelled internal standards would be made available in line with guidance.

B.5.3.2 Residues in water (IIA 4.2.3)

B.5.3.2.1 Description of methods for analysis of water (drinking water, ground water and surface water) for parent compound and metabolites of toxicological, ecotoxicological or environmental concern.

Reference: Rodrigues Junior, A., Souza, N. and Li, Q. (2012), 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, Dow AgroSciences LLC Study Number 110718.

Guidelines:

SANCO/825/00 rev. 8.1

SANCO/3029/99 rev. 4

This method has been submitted in support of both pre-registration data requirements and post-registration monitoring and control for components included in the residue definition for water (XDE-729 methyl ester and its metabolites, XDE-729 acid, X11449757, and X11406790, in drinking water, ground water, and surface water). It is noted that the fate and behaviour evaluation identified 3 further degradation metabolites in the aqueous photolysis study that exceeded 10% AR, that were identified as Deg 10, Deg 11 and Deg 14 (see Section B.8.4.6, for structures and identification of these degradation metabolites). The fate and behaviour evaluation recommends that these aqueous photolysis metabolites should be subject to aquatic exposure and risk assessment; however given that Deg 10, Deg 11 and Deg 14 had all fallen below 10% AR within 4 hours of the start of the study, and thus appear to be transient, it is highly unlikely that they will be included in the final monitoring residue definition for water, and therefore it is acceptable that these degradation products have not been included in the methods of analysis given below for water.

Matrix, limit of quantitation	drinking water 0.05 µg/L ground water 0.05 µg/L surface water 0.05 µg/L
Description	<u>Scope</u> This method is applicable for the quantitative determination of residues of XDE-729 methyl ester and its metabolites, XDE-729 acid, X11449757, and X11406790, in drinking water, ground water, and surface water. The method was validated over the concentration range of 0.05-0.50 µg/L with a validated limit of quantitation of 0.05 µg/L. <u>Principle</u> Residues of XDE-729 methyl ester and its metabolites were determined in a 10.0-mL water sample following the addition of 1.0 mL of an aqueous

10% formic acid solution. The solution was then purified using a Phenomenex Strata-X reversed-phase polymeric solid-phase extraction (SPE) cartridge. After elution from the SPE cartridge with methanol, 100 µL of a mixed stable-isotope internal standard solution was added to the eluate, which was subsequently evaporated to dryness. The residue was then reconstituted with an acetonitrile/water (10:90) solution containing 0.10% formic acid. After preparation, the samples were analyzed by liquid chromatography using a Zorbax SB-C8 column, 4.6 x 75 mm, 3.5-µm, coupled with positive-ion electrospray tandem mass spectrometry (LC/MS/MS), monitoring two MS/MS transitions characteristic of each analyte and one MS/MS transition characteristic of each internal standard with gradient elution of methanol containing 0.1% formic acid and water containing 0.1% formic acid.

Calibration

For each analyte, the linearity of detector response was evaluated using solvent standard solutions. Calibration curves were calculated by linear regression analysis using 1/x weighting. For the least squares equation which describes the detector response as a function of the standard concentration, calibration curves resulting from the injection of a minimum of six standards over the concentration range of 0.15-10 ng/mL (equivalent to 0.015 µg/L to 1.0 µg/L) demonstrated linearity with coefficients of determination (r^2) of at least 0.995.

Validation

The method was validated using drinking (tap) water, ground (well) water, and surface (river) water from the Mogi Mirim City, São Paulo State, Brazil area. Full characterization of the water samples is contained in the method validation report.

For each analyte, the method was validated over the concentration range of 0.05-0.50 µg/L with a limit of quantitation of 0.05 µg/L.

At the 0.05-µg/L level (LOQ), the mean recoveries were within the range of 76-107%, with relative standard deviations within the range of 2-22%. For the determination of X11406790 in ground water at 0.05 µg/L, the relative standard deviation was 22%, slightly above the normal acceptance criteria of 20%.

At the 0.50-µg/L level (10x LOQ), the mean recoveries were within the range of 95-109%, with relative standard deviations within the range of 2-10%.

The above results generally comply with the acceptance criteria of SANCO/825/00/rev. 8.1, however, the additional confirmatory transition should also be validated for calibration data, recovery and precision data for samples fortified at the LOQ (n=5) and for 2 blank samples. See comment below in the conclusions section.

Selectivity/Confirmation

The LC/MS/MS method is highly selective for both the quantitation and confirmation of XDE-729 methyl ester, XDE-729 acid, X11449757, and

	X11406790. Analysis of control samples resulted in no significant signals at the expected retention times of the analytes. Unambiguous identification is ensured by monitoring two MS/MS transitions characteristic of each analyte and one MS/MS transition characteristic of each internal standard as follows: XDE-729 ME <i>m/z</i> 345/285 (quantitation) <i>m/z</i> 345/250 (confirmation) (M+6 ISTD) <i>m/z</i> 351/256 (quantitation) XDE-729 acid <i>m/z</i> 331/250 (quantitation) <i>m/z</i> 331/235 (confirmation) (M+6 ISTD) <i>m/z</i> 337/256 (quantitation) X11449757 <i>m/z</i> 317/236 (quantitation) <i>m/z</i> 317/271 (confirmation) (M+6 ISTD) <i>m/z</i> 323/242 (quantitation) X11406790 <i>m/z</i> 331/236 (quantitation) <i>m/z</i> 331/271 (confirmation) (M+6 ISTD) <i>m/z</i> 337/242 (quantitation) Confirmation was determined according to Commission Decision (2002/657/EC). The confirmation ratios for each analyte were determined by calculating the peak area ratios of the confirmation transition to the quantitation transition. Confirmation of the presence of the analytes in the recovery samples was indicated when the retention times of the analytes in the samples matched the retention times of the analytes in the calibration standards, and the confirmation ratios of the analytes in the samples were $\pm 20\%$ of the average confirmation ratios of the standards. The confirmation ratios for each analyte in all sample matrices were within $\pm 20\%$ of the average confirmation ratios of the standards, indicating that the method is selective for the determination of XDE-729 methyl ester and its metabolites in water. This data is contained in the report. <u>Stability of Solutions and Sample Extracts</u> All stock, spiking, and calibration solutions were stored in a refrigerator when not in use. The stability of XDE-729 methyl ester, XDE-729 acid, X11449757, and X11406790 in prepared sample extracts was investigated for a period of six days and was found to be stable. <u>Matrix Effects</u> Matrix effects were determined by comparing the peak areas of solvent calibration standards to peak areas of matrix-matched standards at a single concentration level. Matrix effects for each analyte were less than 10% and thus considered insignificant. Furthermore, it is important to note that matrix effects are normalized through the use of stable-isotope internal standards, resulting in accurate quantitative results irrespective of the magnitude of matrix effects.
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Validation Data (quantitation)	Matrix	Fortification Level (µg/L)	Recovery Rate (%)		RSD (%)	N
			mean	range		
XDE-729 ME <i>m/z</i> 345→285	drinking water	0.05	76	74-78	2	5
		0.50	108	104-110	2	5
		0.05-0.50	91	74-110	19	10
XDE-729 ME <i>m/z</i> 345→285	ground water	0.05	92	72-109	16	6
		0.50	95	86-111	10	6
		0.05-0.50	93	72-111	13	12
XDE-729 ME <i>m/z</i> 345→285	surface water	0.05	86	72-105	17	6
		0.50	108	100-115	5	6
		0.05-0.50	97	72-115	16	12

Validation Data (quantitation)	Matrix	Fortification Level (µg/L)	Recovery Rate (%)		RSD (%)	N
			mean	range		
XDE-729 acid <i>m/z</i> 331→250	drinking water	0.05	107	92-119	9	5
		0.50	106	95-114	6	6
		0.05-0.50	107	92-119	7	11
XDE-729 acid <i>m/z</i> 331→250	ground water	0.05	100	78-117	17	6
		0.50	98	90-105	6	6
		0.05-0.50	99	78-117	12	12
XDE-729 acid <i>m/z</i> 331→250	surface water	0.05	98	76-115	18	5
		0.50	107	97-116	6	6
		0.05-0.50	103	76-116	13	11

Validation Data (quantitation)	Matrix	Fortification Level (µg/L)	Recovery Rate (%)		RSD (%)	n
			mean	Range		
X11449757 <i>m/z</i> 317→236	drinking water	0.05	79	71-88	8	6
		0.50	103	98-115	6	6
		0.05-0.50	92	71-115	16	12
X11449757 <i>m/z</i> 317→236	ground water	0.05	103	78-119	15	6
		0.50	109	90-116	9	6
		0.05-0.50	106	78-119	12	12
X11449757 <i>m/z</i> 317→236	surface water	0.05	96	78-119	19	5
		0.50	106	101-118	6	6
		0.05-0.50	102	78-119	13	11

Validation Data (quantitation)	Matrix	Fortification Level (µg/L)	Recovery Rate (%)		RSD (%)	N
			mean	range		
X11406790 <i>m/z</i> 331→236	drinking water	0.05	78	70-82	6	5
		0.50	108	102-116	5	6
		0.05-0.50	94	70-116	18	11
X11406790 <i>m/z</i> 331→236	ground water	0.05	102	75-119	22	5
		0.50	96	87-98	5	6
		0.05-0.50	98	75-119	15	11
X11406790 <i>m/z</i> 331→236	surface water	0.05	104	81-120	19	5
		0.50	103	94-109	6	6
		0.05-0.50	104	81-120	13	11

Conclusion

The relative standard deviation of X11406790 in ground water at 0.05 µg/L (LOQ), was 22%, which is slightly above the normal acceptance criteria of 20%.

The LOQ's are adequately validated to accommodate the endpoints for the aquatic compartment, and are low enough for the critical values in surface and ground water. The LOQ meets the EU drinking water requirement of 0.1 µg/L.

It is noted that full validation data are only obtained for the primary (quantitation) MS/MS transition. According to SANCO/825/00 rev. 8.1 the additional confirmatory transition should also be validated for calibration data, recovery and precision data for samples fortified at the LOQ (n=5) and for 2 blank samples. It is a short coming of the study that full validation data has only been presented for the primary transition; however, this data has been presented in the ILV of this method (see below). Given that the ILV study report demonstrates that both the quantitative and confirmatory MS/MS transitions are acceptably validated, in this instance the RMS proposes that consideration of the complete data set from the validation report Study Number 110718 and the ILV report (see below, Study Number 110827) indicates that the method is fit for purpose according to SANCO/825/00 rev. 8.1. The method however does rely on stable isotope labelled internal standards. According to SANCO/825/00 rev. 8.1, all analytical standard materials used in an analytical method must be commonly available. This applies to stable isotope labelled compounds or other internal standards. If a standard is not commercially available the standard should be made generally available by the applicant and the contact details be provided. The notifier was contacted during this evaluation however, and confirmed that the stable isotope labelled internal standards would be made available in line with guidance.

B.5.3.2.2 Independent Laboratory Validation of the Method for the Determination of XDE-729 Methyl Ester and Metabolites in Water

Reference: Gemrot, F. (2012), 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, Dow AgroSciences LLC Study Number 110827.

Guidelines:**SANCO/825/00 rev. 8.1****SANCO/3029/99 rev. 4**

This method has been submitted in support of both pre-registration data requirements and post-registration monitoring and control for components included in the residue definition for water (XDE-729 methyl ester and its metabolites, XDE-729 acid, X11449757, and X11406790, in drinking water, ground water, and surface water).

Matrix, limit of quantitation	drinking water 0.05 µg/L ground water 0.05 µg/L surface water 0.05 µg/L
Description	<u>Scope</u> The objective of this study was to independently validate Dow AgroSciences method (Study 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.” This method is applicable for the quantitative determination of residues of XDE-729 methyl ester and its metabolites, XDE-729 acid, X11449757, and X11406790, in drinking water, ground water, and surface water. The method was independently validated over the concentration of 0.05-0.50 µg/L with a validated limit of quantitation of 0.05 µg/L. <u>Principle</u> Residues of XDE-729 methyl ester and its metabolites were determined in a 10.0-mL water sample following the addition of 1.0 mL of an aqueous 10% formic acid solution. The solution was then purified using a Phenomenex Strata-X reversed-phase polymeric solid-phase extraction (SPE) cartridge. After elution from the SPE cartridge with methanol, 100 µL of a mixed stable-isotope internal standard solution was added to the eluate, which was subsequently evaporated to dryness. The residue was then reconstituted with an acetonitrile/water (10:90) solution containing 0.10% formic acid. After preparation, the samples were analyzed by liquid chromatography using a Zorbax SB-C8 column, 4.6 x 75 mm, 3.5-µm, coupled with positive-ion electrospray tandem mass spectrometry (LC/MS/MS), monitoring two MS/MS transitions characteristic of each analyte and one MS/MS transition characteristic of each internal standard with gradient elution of methanol containing 0.1%

formic acid and water containing 0.1% formic acid.

Calibration

For each analyte, the linearity of detector response was evaluated using solvent standard solutions. Calibration curves were calculated by linear regression analysis without weighting. For the least squares equation which describes the detector response as a function of the standard concentration, calibration curves resulting from the injection of a minimum of seven standards over the concentration range of 0.15-20 µg/mL demonstrated linearity with coefficients of determination (r^2) of at least 0.9934.

Validation

The method was validated using drinking (tap) water from Comps, France; ground (well) water from Evian; and surface (river) water from the Tech River at Pyrénées-Orientales, France. Full characterization of the water samples is contained in the method validation report.

For each analyte, the method was validated over the concentration range of 0.05-0.50 µg/L with a limit of quantitation of 0.05 µg/L.

At the 0.05-µg/L level (LOQ), the mean recoveries were within the range of 90-100%, with relative standard deviations within the range of 1-8%.

At the 0.50-µg/L level (10x LOQ), the mean recoveries were within the range of 88-109%, with relative standard deviations within the range of 1-5%.

The above results comply with the acceptance criteria of SANCO/825/00/rev. 8.1

Selectivity/Confirmation

The LC/MS/MS method is highly selective for both the quantitation and confirmation of XDE-729 methyl ester, XDE-729 acid, X11449757, and X11406790. Analysis of control samples resulted in no significant signals at the expected retention times of the analytes. Unambiguous identification is ensured by monitoring two MS/MS transitions characteristic of each analyte and one MS/MS transition characteristic of each internal standard as follows:

XDE-729 ME	m/z 345/285 (quantitation)
(M+6 ISTD)	m/z 345/250 (confirmation)
XDE-729 acid	m/z 351/256 (quantitation)
(M+6 ISTD)	m/z 331/250 (quantitation)
	m/z 331/235 (confirmation)
X11449757	m/z 337/256 (quantitation)
(M+6 ISTD)	m/z 317/236 (quantitation)
	m/z 317/271 (confirmation)
X11406790	m/z 323/242 (quantitation)
(M+6 ISTD)	m/z 331/236 (quantitation)
	m/z 331/271 (confirmation)
	m/z 337/242 (quantitation)

Confirmation was demonstrated for each analyte by determining the

	recovery and precision for both MS/MS transitions; these data are listed in the tables below. <u>Stability of Solutions and Sample Extracts</u> The stability of the stock solutions, calibration standard solutions, and sample extracts was not evaluated as part of this study as it is not a requirement of an independent laboratory validation. <u>Matrix Effects</u> Matrix effects were not evaluated as part of this study as they are not a requirement of an independent laboratory validation. However, it is important to note that matrix effects are normalized through the use of stable-isotope internal standards, resulting in accurate quantitative results irrespective of the magnitude of matrix effects.
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Validation Data (quantitation)	Matrix	Fortification Level (µg/L)	Recovery Rate (%)		RSD (%)	n
			mean	range		
XDE-729 ME m/z 345→285	drinking water	0.05	98	95-101	2	5
		0.50	103	99-107	3	5
		0.05-0.50	100	95-107	4	10
XDE-729 ME m/z 345→285	ground water	0.05	99	95-105	4	5
		0.50	105	104-106	1	5
		0.05-0.50	102	95-106	4	10
XDE-729 ME m/z 345→285	surface water	0.05	95	93-97	2	5
		0.50	103	100-107	3	5
		0.05-0.50	99	93-107	4	10

Validation Data (quantitation)	Matrix	Fortification Level (µg/L)	Recovery Rate (%)		RSD (%)	n
			mean	range		
XDE-729 acid m/z 331→250	drinking water	0.05	94	93-95	1	5
		0.50	91	85-94	4	5
		0.05-0.50	92	85-95	3	10
XDE-729 acid m/z 331→250	ground water	0.05	96	94-97	1	5
		0.50	90	85-95	4	5
		0.05-0.50	93	85-97	4	10
XDE-729 acid m/z 331→250	surface water	0.05	96	94-98	2	5
		0.50	103	100-106	2	5
		0.05-0.50	99	94-106	4	10

Validation Data (quantitation)	Matrix	Fortification Level (µg/L)	Recovery Rate (%)		RSD (%)	n
			mean	range		
X11449757 <i>m/z</i> 317→236	drinking water	0.05	94	91-98	3	5
		0.50	95	88-99	5	5
		0.05-0.50	95	88-99	4	10
X11449757 <i>m/z</i> 317→236	ground water	0.05	96	92-111	8	5
		0.50	92	87-93	3	5
		0.05-0.50	94	87-111	6	10
X11449757 <i>m/z</i> 317→236	surface water	0.05	96	90-99	4	5
		0.50	102	97-106	4	5
		0.05-0.50	99	96-106	5	10

Validation Data (quantitation)	Matrix	Fortification Level (µg/L)	Recovery Rate (%)		RSD (%)	n
			mean	range		
X11406790 <i>m/z</i> 331→236	drinking water	0.05	97	93-100	3	5
		0.50	92	90-94	2	5
		0.05-0.50	95	90-100	4	10
X11406790 <i>m/z</i> 331→236	ground water	0.05	94	92-98	3	5
		0.50	97	95-98	2	5
		0.05-0.50	95	92-98	3	10
X11406790 <i>m/z</i> 331→236	surface water	0.05	98	93-103	4	5
		0.50	109	103-114	4	5
		0.05-0.50	104	93-114	7	10

Validation Data (confirmation)	Matrix	Fortification Level (µg/L)	Recovery Rate (%)		RSD (%)	n
			mean	range		
XDE-729 ME <i>m/z</i> 345→250	drinking water	0.05	100	98-104	2	5
		0.50	105	103-107	1	5
		0.05-0.50	103	98-107	3	10
XDE-729 ME <i>m/z</i> 345→250	ground water	0.05	94	89-97	3	5
		0.50	93	92-96	2	5
		0.05-0.50	94	89-97	2	10
XDE-729 ME <i>m/z</i> 345→250	surface water	0.05	94	93-96	1	5
		0.50	102	98-106	3	5
		0.05-0.50	98	93-106	5	10

Validation Data (confirmation)	Matrix	Fortification Level (µg/L)	Recovery Rate (%)		RSD (%)	n
			mean	range		
XDE-729 acid <i>m/z</i> 331→235	drinking water	0.05	91	87-96	4	5
		0.50	88	82-92	5	5
		0.05-0.50	90	82-96	4	10
XDE-729 acid <i>m/z</i> 331→235	ground water	0.05	100	97-110	5	5
		0.50	90	87-96	4	5
		0.05-0.50	95	87-110	7	10
XDE-729 acid <i>m/z</i> 331→235	surface water	0.05	95	89-100	5	5
		0.50	104	101-108	3	5
		0.05-0.50	100	89-108	6	10

Validation Data (confirmation)	Matrix	Fortification Level (µg/L)	Recovery Rate (%)		RSD (%)	n
			mean	range		
X11449757 <i>m/z</i> 317→271	drinking water	0.05	92	83-101	7	5
		0.50	95	90-99	4	5
		0.05-0.50	93	83-101	6	10
X11449757 <i>m/z</i> 317→271	ground water	0.05	96	87-106	8	5
		0.50	92	88-95	3	5
		0.05-0.50	94	87-106	6	10
X11449757 <i>m/z</i> 317→271	surface water	0.05	96	91-99	4	5
		0.50	107	105-108	1	5
		0.05-0.50	101	91-108	6	10

Validation Data (confirmation)	Matrix	Fortification Level (µg/L)	Recovery Rate (%)		RSD (%)	n
			mean	range		
X11406790 <i>m/z</i> 331→271	drinking water	0.05	96	92-99	3	5
		0.50	92	89-97	3	5
		0.05-0.50	94	89-99	4	10
X11406790 <i>m/z</i> 331→271	ground water	0.05	90	86-96	4	5
		0.50	90	88-92	2	5
		0.05-0.50	90	86-96	3	10
X11406790 <i>m/z</i> 331→271	surface water	0.05	93	89-95	3	5
		0.50	105	89-110	4	5
		0.05-0.50	99	89-110	7	10

Conclusion

The method is satisfactorily validated. The LOQ's are adequately validated to accommodate the endpoints for the aquatic compartment, and are low enough for the critical values in surface and ground water. The LOQ meets the EU drinking water requirement of 0.1 µg/L.

In line with the conclusions made under B.5.3.2.1, the RMS proposes that the method is fit for purpose according to SANCO/825/00 rev. 8.1. The method however does rely on stable isotope labelled internal standards. According to SANCO/825/00 rev. 8.1, all analytical standard materials used in an analytical method must be commonly available. This applies to stable

isotope labelled compounds or other internal standards. If a standard is not commercially available the standard should be made generally available by the applicant and the contact details be provided. The notifier was contacted during this evaluation however, and confirmed that the stable isotope labelled internal standards would be made available in line with guidance.

B.5.3.3 Residues in air (IIA 4.2.4)

B.5.3.3.1 Description of methods for analysis of air for active substance and metabolites, formed during or shortly after application, of toxicological concern

Reference: Class, T (2011), The Development and Validation of a Method for the Analysis of XDE-729 Methyl Ester and XDE-729 Acid in Air, Dow AgroSciences LLC Study Number 110028, PTRL Europe Study Number P 2169 G.

Guidelines:

SANCO/825/00 rev. 8.1

SANCO/3029/99 rev. 4

This method has been submitted in support of post-registration monitoring and control for components included in the residue definition for air (XDE-729 methyl ester and XDE-729 acid).

Matrix, limit of quantitation	air 0.8 µg/m ³
Description	<u>Scope</u> This method is applicable for the quantitative determination of residues of XDE-729 methyl ester and XDE-729 acid in ambient and humid warm air. The method was validated over the concentration range of 0.82-84 µg/m³ with a validated limit of quantitation of 0.82 µg/m³. <u>Principle</u> Air sampling tubes were fortified separately with XDE-729 methyl ester and XDE-729 acid. Residues of XDE-729 methyl ester and XDE-729 acid were extracted from air sampling tubes containing XAD resin with three 3-mL portions of methanol. The three extracts from the front portion of the air sampling tubes were combined and diluted to 10.0 mL with methanol. Aliquots of the extracts from blank samples and samples fortified at the 0.82 µg/m³ level (LOQ) were then diluted ten-fold with an acetonitrile/water (50:50) solution containing 0.1% formic acid. Extracts from recoveries fortified at the 8.2 µg/m³ level (10X LOQ) were diluted twenty-fold with an acetonitrile/water (50:50) solution containing 0.1% formic acid. Extracts from recoveries fortified at the 84 µg/m³ level (100X LOQ) were diluted one-hundred-fold with an acetonitrile/water (50:50) solution containing 0.1% formic acid. After preparation, the samples were analyzed by liquid chromatography using a Phenomenex Luna PFP column, 150 x 3.0 mm, 3.0 µm coupled with positive-ion electrospray tandem mass spectrometry (LC/MS/MS), monitoring two MS/MS transitions characteristic of each analyte with gradient elution of water with 0.1% formic acid and acetonitrile with 0.1%

formic acid.

Calibration

For each analyte, the linearity of detector response was evaluated using solvent standard solutions. Calibration curves were calculated by linear regression analysis using 1/x weighting. For the least squares equation which describes the detector response as a function of the standard concentration, calibration curves resulting from the injection of a minimum of six standards over the concentration range of 0.50-25 ng/mL demonstrated linearity with correlation coefficients (r) of at least 0.999.

Validation

The method was validated over the concentration range of 0.82-84 $\mu\text{g}/\text{m}^3$ with a validated limit of quantitation was 0.82 $\mu\text{g}/\text{m}^3$.

Front and back portions of the air sampling tubes were removed and extracted separately to demonstrate retention of XDE-729 methyl ester and XDE-729 acid under air sampling conditions. No breakthrough was observed in the back portions of the adsorption tubes (< 1%) after 0.36-0.37 m^3 (360-370 L) of air.

With air at 35 °C and 80 % relative humidity the following results were obtained:-

At the 0.82- $\mu\text{g}/\text{m}^3$ level (LOQ), the mean recoveries were within the range of 96-109% with relative standard deviations within the range of 2-4%.

At the 8.2- $\mu\text{g}/\text{m}^3$ level (10x LOQ), the mean recoveries were within the range of 97-101% with relative standard deviations within the range of 3-4%.

At the 84- $\mu\text{g}/\text{m}^3$ level (100x LOQ), the mean recoveries were within the range of 90-91% with relative standard deviations within the range of 8-13%.

The above results comply with the acceptance criteria of SANCO/825/00/rev. 8.1.

Selectivity/Confirmation

The LC/MS/MS method is highly selective for both the quantitation and confirmation of XDE-729 methyl ester and XDE-729 acid. Analysis of control samples resulted in no significant signals at the expected retention times of the analytes. Unambiguous identification is ensured by monitoring two MS/MS transitions characteristic each analyte as follows:

XDE-729 ME	m/z 345/285 (quantitation)
	m/z 345/250 (confirmation)

XDE-729 acid	m/z 331/285 (quantitation)
	m/z 331/250 (confirmation)

Confirmation was demonstrated by determining the recovery and precision for both MS/MS transitions; these data are listed in the tables below.

Stability of Solutions, Extracts, and Air Sampling Cartridges

All stock, spiking, and calibration solutions were stored in a refrigerator when not in use.

Final extracts were stable up to six days when stored at room temperature

	or when refrigerated. The mean recoveries were within the range of 105-111% with relative standard deviations within the range of 2-4%. Air sampling cartridges were stable up to seven days when stored refrigerated or frozen. The mean recoveries were within the range of 90-99% with relative standard deviations within the range of 4-6%.
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Validation Data (quantitation)	Matrix	Fortification Level ($\mu\text{g}/\text{m}^3$)	Recovery Rate (%)		RSD (%)	n
			mean	range		
XDE-729 ME m/z 345→285	air, ambient 20 °C 29% R.H.	0.83	108	105-111	3	5
		8.3	100	98-102	2	5
		84	105	105-107	0.8	5
		0.83-84	105	98-111	4	15
XDE-729 ME m/z 345→285	air, warm 35 °C 80%-82% R.H.	0.82	109	106-110	2	5
		8.2	101	96-104	3	5
		83	91	73-105	13	5
		0.82-83	100	73-110	10	15

Validation Data (quantitation)	Matrix	Fortification Level ($\mu\text{g}/\text{m}^3$)	Recovery Rate (%)		RSD (%)	n
			mean	range		
XDE-729 acid m/z 331→285	air, ambient 21 °C 27% R.H.	0.82	106	102-108	2	5
		8.2	99	96-102	3	5
		83	100	84-107	9	5
		0.82-83	101	84-108	6	15
XDE-729 acid m/z 331→285	air, warm 35 °C 80%-93% R.H.	0.82	96	93-101	4	5
		8.2	97	91-101	4	5
		82	90	77-95	8	5
		0.82-82	94	77-101	6	15

Validation Data (confirmation)	Matrix	Fortification Level ($\mu\text{g}/\text{m}^3$)	Recovery Rate (%)		RSD (%)	n
			mean	range		
XDE-729 ME m/z 345→250	air, ambient 20 °C 29% R.H.	0.83	107	105-110	2	5
		8.3	99	97-102	2	5
		84	104	103-104	0.7	5
		0.83-84	103	97-110	4	15
XDE-729 ME m/z 345→250	air, warm 35 °C 80%-82% R.H.	0.82	108	105-111	2	5
		8.2	100	96-102	3	5
		83	91	73-104	13	5
		0.82-83	100	73-111	10	15

Validation Data (confirmation)	Matrix	Fortification Level ($\mu\text{g}/\text{m}^3$)	Recovery Rate (%)		RSD (%)	n
			mean	range		
XDE-729 acid m/z 331→250	air, ambient 21 °C 27% R.H.	0.82	105	101-107	2	5
		8.2	98	95-101	3	5
		83	100	85-106	9	5
		0.82-83	101	85-107	6	15
XDE-729 acid m/z 331→250	air, warm 35 °C 80%-93% R.H.	0.82	96	92-101	4	5
		8.2	98	91-101	4	5
		82	91	78-95	8	5
		0.82-82	95	78-101	6	15

Conclusion

The method has been validated in all areas according to SANCO/825/00 rev. 8.1. The LOQ complies with the concentration calculated from the AOEL (systemic in the absence of an inhalation AOEL) as given in SANCO/825/00 rev. 8.1.

The mean recovery of XDE-729 methyl ester and XDE-729 acid at LOQ and 10 times LOQ in air at 35 °C and 80 % relative humidity are within acceptable mean recovery level of 70-120% and relative standard deviation of $\leq 20\%$.

B.5.4 Analytical methods for parent compound and toxicologically, ecotoxicologically or environmentally significant metabolites in body fluids and tissues

Reference: Senciuc, M. (2011), XDE-729: Development and Validation of an Analytical Method for the Determination of XDE-729 Methyl Ester and Acid in Body Fluid(s), Dow AgroSciences LLC Study Number 110029, PTRL Europe Study Number P 2171 G.

The above study was submitted; however, it has not been relied upon since XDE-Me is not classified as toxic or highly toxic; therefore monitoring methods for human tissues and body fluids are not required.

B.5.5 Summary

Satisfactory methods are available for the determination of XDE-729 in the technical material and in the formulated product 'GF-2573'. These methods were satisfactorily validated in accordance with SANCO 3030/99 rev. 4.

Analytical methods for analysis of the active substance as manufactured (Laboratory Project ID: DECO ML AL-2012-003865)

An analytical method was validated for the determination of XDE-729 in technical grade XDE-729. An internal standard high performance liquid chromatographic (HPLC) gradient method with UV detection (270 nm) was used. Quantitation was made by peak area measurements with internal standardization.

The method was satisfactorily validated in terms of precision, accuracy, specificity and linearity in accordance with SANCO 3030/99 rev. 4.

Please refer to the Confidential Information (Annex C/Volume 4) for methods of analysis for impurities in the technical material.

Analytical methods for formulation analysis (Laboratory Study ID: DAS-AM-G-10-34)

An analytical method was validated for the determination of XDE-729 and cloquintocet-Mexyl in GF-2573 and GF-2685. An internal standard high performance liquid chromatographic (HPLC) method with UV detection (300 nm) was used. Quantitation was made by peak area measurements with internal standardization.

The method was satisfactorily validated in terms of precision, accuracy, specificity and linearity in accordance with SANCO 3030/99 rev. 4.

Satisfactory methods of analysis have been submitted in support of post-registration monitoring and control for components included in the residue definition for plants and animals. The proposed residue definition for Risk Assessment and Enforcement/Monitoring in plants and livestock is XDE-729 methyl and XDE-729 acid.

Multi-residue Method Testing for the Determination of XDE 729 Methyl Ester and XDE 729 Acid in Plant Matrices (Dow AgroSciences LLC Study Number 110293, Eurofins Analytical Services Study Number S11-02423)

Independent Laboratory Validation of the Multi-residue Method for the Determination of XDE-729 Methyl Ester and XDE-729 Acid in Plant Matrices (Dow AgroSciences LLC Study Number 110712, PTRL Europe Study Number P 2399 G)

A QuEChERS multi-residue method was proposed in support of post-registration monitoring for the quantitative determination of residues of XDE-729 methyl ester and XDE-729 acid in agricultural commodities representative of the four European crop groupings. The validated limit of quantitation was 0.01 mg/kg.

Samples were analysed by LC/MS/MS, monitoring two MS/MS transitions characteristic of each analyte.

The QuEChERS method was validated in all areas; however, it was noted that although the mean recoveries for the acidic crop were acceptable, individual recoveries for the quantitation and confirmatory transitions for detection on XDE-729 acid in this matrix were low.

Storage stability of final sample extracts demonstrated unacceptable levels of degradation for the determination of XDE-729 methyl ester in barley grain (after 9 days) and in kale leaves (after 10 days). It is also noted that mean recovery of the XDE-729 acid in oilseed is outside acceptable recovery levels, since the initial recovery was low; therefore a small amount of degradation resulted in a recovery that is outside acceptable limits following storage after 7 days.

A corresponding ILV study was submitted as part of the validation of the method. According to SANCO/825/00 rev.8.1, an ILV must be conducted with samples of representative commodities of all matrix groups for which the primary method is required, with the same number of samples and fortification levels. If the primary method is identical for all matrix groups, it is sufficient to perform the ILV for commodities of two of these groups, one of them with high water content. In this instance, the primary method is not the same for all commodities; however, the ILV study has selected two matrix groups that allow demonstration of both procedures. Given that the representation use is cereal, and the method is not the same for all matrix types, it is considered that ideally a dry crop should have been considered in the ILV study. In this instance however, given that the method for a wet crop (cabbage) is the same as the method for cereal, the RMS considers the ILV study is acceptable.

Enforcement Method for the Determination of XDE-729 Methyl Ester and XDE- 729 Acid in Plant Matrices (Dow AgroSciences LLC Study Number 110004)

Independent Laboratory Validation of the Enforcement Method for the Determination of XDE 729 Methyl Ester and XDE 729 Acid in Plant Matrices (Dow AgroSciences LLC Study Number 110825, Pyxant Labs Study Number 2386)

This method was submitted in support of both pre-registration data requirements and post-registration monitoring, and is applicable for the quantitative determination of residues of

XDE-729 methyl ester and XDE-729 acid in agricultural commodities representative of the four European crop groupings and wheat processed products. The validated limit of quantitation was 0.01 mg/kg.

Samples were analyzed by liquid chromatography coupled with positive-ion electrospray tandem mass spectrometry (LC/MS/MS), monitoring two MS/MS transitions characteristic of each analyte and one MS/MS transition characteristic of each internal standard (the method employs a mixed stable-isotope internal standard).

It is noted that full validation data are only obtained for the primary (quantitation) MS/MS transition. According to SANCO/825/00 rev. 8.1 the additional confirmatory transition should also be validated for calibration data, recovery and precision data for samples fortified at the LOQ (n=5) and for 2 blank samples. It is a short coming of the study that full validation data has only been presented for the primary transition; however, this data has been presented for high water and high oil matrices for the ILV of this method. Only two matrices were considered in the ILV, however the primary extraction method is the same for all matrices therefore this is acceptable. Given that the ILV study report demonstrates that both the quantitative and confirmatory MS/MS transitions are acceptably validated, in this instance the RMS proposes that consideration of the complete data set from the validation report (Study Number 110004) and the ILV report (Study Number 2386) indicates that the method is fit for purpose according to SANCO/825/00 rev. 8.1. (For information, the notifier has since informed the RMS "the report will be revised to include the confirmatory transition data, including calibration, recovery and precision. The revised version will be available by June 2013". In the absence of this report at the time of submission, for the reasons shown above, the method may still be considered fit for purpose according to SANCO/825/00 rev. 8.1).

The method however does rely on stable isotope labelled internal standards. According to SANCO/825/00 rev. 8.1, all analytical standard materials used in an analytical method must be commonly available. This applies to stable isotope labelled compounds or other internal standards. If a standard is not commercially available the standard should be made generally available by the applicant and the contact details be provided. The notifier was contacted during this evaluation however, and confirmed that the stable isotope labelled internal standards would be made available in line with guidance.

As discussed above, acceptable ILV data has been submitted.

Multi-residue Method Testing for the Determination of XDE-729 Methyl Ester and XDE-729 Acid in Animal Matrices (Dow AgroSciences LLC Study Number 110574, Eurofins Analytical Services Study Number S11-02424)

This QuEChERS multi-residue method was submitted in support of post-registration monitoring, and is applicable for the quantitative determination of residues of XDE-729 methyl ester and XDE-729 acid in animal matrices including bovine muscle, bovine kidney, bovine liver, bovine whole milk, poultry muscle, and poultry eggs. The validated limit of quantitation of 0.01 mg/kg. Samples were analyzed by liquid chromatography coupled with positive-ion electrospray tandem mass spectrometry (LC/MS/MS), monitoring two MS/MS transitions characteristic of each analyte.

The QuEChERS method has been validated in all areas according to SANCO/825/00 rev. 8.1. It is noted that extraction efficiencies have not been addressed as part of the method validation; however, residues are not expected to be found in products of animal origin for the proposed use. Therefore, extraction efficiency does not need to be addressed for this evaluation. This will need to be addressed in future however if new uses give rise to positive residues.

Multi-residue Method Testing for the Determination of XDE 729 Methyl Ester and XDE 729 Acid in Bovine Fat (Dow AgroSciences LLC Study Number 110750, Eurofins Analytical Services Study Number S11-03369)

This QuEChERS multi-residue method was submitted in support of post-registration monitoring, and is applicable for the quantitative determination of residues of XDE-729 methyl ester and XDE-729 acid in bovine fat. The validated limit of quantitation was 0.01 mg/kg.

Samples were analyzed by liquid chromatography coupled with positive-ion electrospray tandem mass spectrometry (LC/MS/MS), monitoring two MS/MS transitions characteristic of each analyte.

The QuEChERS method has been validated in all areas according to SANCO/825/00 rev. 8.1, and the study report was well presented.

Independent Laboratory Validation of the Multi-residue Method for the Determination of XDE 729 Methyl Ester and XDE 729 Acid in Animal Matrices (Dow AgroSciences LLC Study Number 110294, PTRL Europe Study Number P 2172 G)

The objective of the ILV study was to independently validate the multi-residue method for the determination of XDE-729 methyl ester and XDE-729 acid in animal matrices. The method was Eurofins Agrosciences Services multi-residue method (Study Number S11-02424), "Validation of a Multi-Residue Method for the Determination of XDE-729 Methyl Ester and XDE-729 Acid in Animal Matrices" and Eurofins Agrosciences Services multi-residue method (Study Number S11-03369), "Validation of a Multi-Residue Method for the Determination of XDE-729 Methyl Ester and XDE-729 Acid in Bovine Fat."

The ILV study was acceptable. For information, it was noted that "The API 5500 LC-MS/MS system showed a very high sensitivity especially for the determination of XDE-729 methyl ester. Thus, it was necessary to further dilute extracts fortified with this analyte (additional dilution factor: 5: 0.20 mL of extract was diluted with 0.80 mL of acetonitrile/0.1 % formic acid (1/9, v/v)) to be within the linear range of the LC-MS/MS calibration curves used". This is considered to be a minor modification that does not impact on the validity of the study.

Enforcement Method for the Determination of XDE 729 Methyl Ester and XDE 729 Acid in Animal Matrices (Dow AgroSciences LLC Study Number 110505)

Independent Laboratory Validation of the Enforcement Method for the Determination of XDE 729 Methyl Ester and XDE 729 Acid in Animal Matrices (Dow AgroSciences LLC Study Number 110828, CEMAS Study Number CEMS-5325)

This method was submitted in support of both pre-registration and post-registration monitoring, and is applicable for the quantitative determination of residues of XDE-729 methyl ester and XDE-729 acid in bovine and poultry tissues. The validated limit of quantitation was 0.01 mg/kg. Samples were analyzed by liquid chromatography coupled with positive-ion electrospray tandem mass spectrometry (LC/MS/MS), monitoring two MS/MS transitions characteristic of each analyte and one MS/MS transition characteristic of each internal standard (the method employs a mixed stable-isotope internal standard).

It is noted that full validation data are only obtained for the primary (quantitation) MS/MS transition. According to SANCO/825/00 rev. 8.1 the additional confirmatory transition should also be validated for calibration data, recovery and precision data for samples fortified at the LOQ (n=5) and for 2 blank samples. It is a short coming of the study that full validation data has only been presented for the primary transition; however, this data has been presented for bovine fat, bovine liver and poultry eggs for the ILV of this method (Study Number 110828). Only three matrices were considered in the ILV, however the primary extraction method is the same for all matrices therefore this is acceptable. Given that the ILV study report demonstrates that both the quantitative and confirmatory MS/MS transitions are acceptably validated, in this instance the RMS proposes that consideration of the complete data set from the validation report Study Number 110505 and the ILV report (Study Number 110828) indicates that the method is fit for purpose according to SANCO/825/00 rev. 8.1.

The method however does rely on stable isotope labelled internal standards. According to SANCO/825/00 rev. 8.1, all analytical standard materials used in an analytical method must be commonly available. This applies to stable isotope labelled compounds or other internal standards. If a standard is not commercially available the standard should be made generally available by the applicant and the contact details be provided. The notifier was contacted during this evaluation however, and confirmed that the stable isotope labelled internal standards would be made available in line with guidance.

As discussed above, an ILV study was submitted, and all data contained therein are acceptable.

Description of methods for analysis of soil for parent compound and metabolites of toxicological, ecotoxicological or environmental concern (Dow AgroSciences LLC Study Number 110716)

Independent Laboratory Validation of the Method for the Determination of XDE-729 Methyl Ester and Metabolites in Soil (Dow AgroSciences LLC Study Number 120894)

This method was submitted in support for both pre-registration data requirements and post-registration monitoring, and is applicable for the quantitative determination of residues of XDE-729 methyl ester and its metabolites, XDE-729 acid and X11449757 in accordance with the residue definition in soil, in 4 soil types. The validated limit of quantitation was 0.05 µg/kg. Samples were analyzed by liquid chromatography coupled with positive-ion electrospray tandem mass spectrometry (LC/MS/MS), monitoring two MS/MS transitions characteristic of each analyte and one MS/MS transition characteristic of each internal standard (the method employs a mixed stable-isotope internal standard).

The method has been validated in all areas according to SANCO/825/00 rev. 8.1.

The mean recovery of X11449757 at 0.05 µg/kg (LOQ) in clay loam, silt loam and clay, and at 8.0 µg/kg in clay loam were below the acceptable mean recovery level of 70 %. The lowest mean recovery at LOQ was 63 %. The mean recovery for all other samples and precision were all acceptable. Overall, given that the data in all other areas of the validation are well within guideline acceptable limits, and recovery of the other samples gave consistent values within acceptable limits, it is considered in this instance to accept that the data-set, as a whole, is adequately validated for recovery and precision.

The method however does rely on stable isotope labelled internal standards. According to SANCO/825/00 rev. 8.1, all analytical standard materials used in an analytical method must be

commonly available. This applies to stable isotope labelled compounds or other internal standards. If a standard is not commercially available the standard should be made generally available by the applicant and the contact details be provided. The notifier was contacted during this evaluation however, and confirmed that the stable isotope labelled internal standards would be made available in line with guidance.

An acceptable ILV study was provided for the method. The primary method is identical for all matrix groups, therefore it was considered to be sufficient to perform the ILV on 2 soil types (sand and clay). The method has been validated in all areas according to SANCO/825/00 rev. 8.1.

Description of methods for analysis of water (drinking water, ground water and surface water) for parent compound and metabolites of toxicological, ecotoxicological or environmental concern (Dow AgroSciences LLC Study Number 110718).

Independent Laboratory Validation of the Method for the Determination of XDE-729 Methyl Ester and Metabolites in Water (Dow AgroSciences LLC Study Number 110827)

This method is applicable for the quantitative determination of residues of XDE-729 methyl ester and its metabolites, XDE-729 acid, X11449757, and X11406790, in line with the residue definition in water, in drinking water, ground water, and surface water. The validated limit of quantitation was 0.05 µg/L.

Samples were analyzed by liquid chromatography coupled with positive-ion electrospray tandem mass spectrometry (LC/MS/MS), monitoring two MS/MS transitions characteristic of each analyte and one MS/MS transition characteristic of each internal standard (the method employs a mixed stable-isotope internal standard).

The relative standard deviation of X11406790 in ground water at 0.05 µg/L (LOQ), was 22%, which is slightly above the normal acceptance criteria of 20%.

It is noted that full validation data are only obtained for the primary (quantitation) MS/MS transition. According to SANCO/825/00 rev. 8.1 the additional confirmatory transition should also be validated for calibration data, recovery and precision data for samples fortified at the LOQ (n=5) and for 2 blank samples. It is a short coming of the study that full validation data has only been presented for the primary transition; however, this data has been presented in the ILV of this method (Study Number 110827). Given that the ILV study report demonstrates that both the quantitative and confirmatory MS/MS transitions are acceptably validated, in this instance the RMS proposes that consideration of the complete data set from the validation report Study Number 110718 and the ILV report (Study Number 110827) indicates that the method is fit for purpose according to SANCO/825/00 rev. 8.1. The method however does rely on stable isotope labelled internal standards. According to SANCO/825/00 rev. 8.1, all analytical standard materials used in an analytical method must be commonly available. This applies to stable isotope labelled compounds or other internal standards. If a standard is not commercially available the standard should be made generally available by the applicant and the contact details be provided. The notifier was contacted during this evaluation however, and confirmed that the stable isotope labelled internal standards would be made available in line with guidance.

Description of methods for analysis of air for active substance and metabolites, formed during or shortly after application, of toxicological concern (Dow AgroSciences LLC Study Number 110028, PTRL Europe Study Number P 2169 G)

This method has been submitted in support of post-registration monitoring, and is applicable for the quantitative determination of residues of XDE-729 methyl ester and XDE-729 acid in line with the residue definition of air, in ambient and humid warm air. The validated limit of quantitation was 0.82 µg/m³.

Samples were analyzed by liquid chromatography coupled with positive-ion electrospray tandem mass spectrometry (LC/MS/MS), monitoring two MS/MS transitions characteristic of each analyte.

The method has been validated in all areas according to SANCO/825/00 rev. 8.1.

Analytical methods for parent compound and toxicologically, ecotoxicologically or environmentally significant metabolites in body fluids and tissues

A method was submitted for body fluids and tissues; however, it has not been relied upon since XDE-Me is not classified as toxic or highly toxic; therefore monitoring methods for human tissues and body fluids are not required.

B.5.6 References relied on

Annex Point/ Reference Number	Author(s)	Year	Title Source (where different from the Company), Company, Report Number, GLP or GEP status (where relevant), Published or not	Data Protection claimed (Y/N)	Owner
B.5.1.1	Hamilton, T. D.	2012	Analytical Method and Validation for the Determination of Active Ingredient in XDE-729 Me Technical by Liquid Chromatography The Dow Chemical Company DAS Report No.: DECO ML AL-2012- 003865 (Accession Number) 2012347 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
B.5.2.1	Daneva, E., Taeufer, A.	2011	Validation of a Multi-residue Method for Determination of a XDE-729 methyl ester and XDE-729 acid on Different Matrices of Plant Origin Eurofins AgroScience Services Chem GmbH DAS Report No.: 110293, S11-02423 (Accession Number) 2010224 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS

Annex Point/ Reference Number	Author(s)	Year	Title Source (where different from the Company), Company, Report Number, GLP or GEP status (where relevant), Published or not	Data Protection claimed (Y/N)	Owner
B.5.2.2	Bacher, R.	2011a	XDE-729: Independent Laboratory Validation of an Multi-Residue Method for the Determination of XDE-729 Methyl Ester and XDE-729 Acid in Plant Matrices PTRL Europe GmbH DAS Report No.: 110712 (Accession Number) 2011754 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
B.5.2.3	Ma, M.	2012	Method Validation Study for the Determination of Residues of XDE 729 Methyl Ester and XDE 729 Acid in Agricultural Commodities and Wheat Processed Products using Offline Solid-Phase Extraction and Liquid Chromatography with Tandem Mass Spectrometry Detection Dow AgroSciences LLC DAS Report No.: 110004 (Accession Number) 2011974 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
B.5.2.4	Robaugh, D.A.	2012	Independent Laboratory Validation of Method for the Determination of Residues of XDE-729 Methyl Ester and XDE-729 Acid in Agricultural Commodities and Wheat Processed Products using Offline Solid-Phase Extraction and Liquid Chromatography with Tandem Mass Spectrometry Detection Pyxant Labs Inc. DAS Report No.: 110825, 2386 (Accession Number) 2013610 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
B.5.2.5	Olberding, E.L.	2011	Determination of Residues of XDE-729 Methyl Ester and XDE-729 Acid in Agricultural Commodities and Wheat Processed Products using Online Solid-Phase Extraction and Liquid Chromatography with Tandem Mass Spectrometry Dow AgroSciences LLC DAS Report No.: 110005 (Accession Number) 2010805 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
B.5.2.6	McLean, N.	2006	Method Validation Report for the Determination of Cloquintocet-mexyl and its Acid Metabolite in Wheat Using Enviro-Test Laboratories Method M313	Y	DAS

Annex Point/ Reference Number	Author(s)	Year	Title Source (where different from the Company), Company, Report Number, GLP or GEP status (where relevant), Published or not	Data Protection claimed (Y/N)	Owner
			Enviro-Test Laboratories DAS Report No.: ETL04DOW05 (Accession Number) 222112 GLP/GEP (Y/N): Y Published (Y/N): N		
B.5.2.7	Class, T.	2005	Cloquintocet-mexyl: Independent Laboratory Validation of an Analytical Method for the Determination of Cloquintocet-mexyl and its Acid Metabolite in Cereal PTRL Europe GmbH DAS Report No.: 040096 (Accession Number) 206789 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
B.5.2.8	Lindner, M.	2011a	Validation of a multi-residue method for the determination of XDE-729 methyl ester and XDE- 729 acid in animal matrices Eurofins Agrosience Services Chem GmbH DAS Report No.: 110574, DOW-1103V (Accession Number) 2010067 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
B.5.2.9	Lindner, M.	2011b	Validation of a multi-residue method for the determination of XDE-729 methyl ester and XDE- 729 acid in bovine fat Eurofins Agrosience Services Chem GmbH DAS Report No.: 110750, DOW-1111V (Accession Number) 2010226 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
B.5.2.10	Bacher, R.	2011b	XDE-729: Independent Laboratory Validation of an Multi-Residue Method for the Determination of XDE-729 Methyl Ester and XDE-729 Acid in Animal Matrices PTRL Europe GmbH DAS Report No.: 110294, P 2172 G (Accession Number) 2010764 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS

Annex Point/ Reference Number	Author(s)	Year	Title Source (where different from the Company), Company, Report Number, GLP or GEP status (where relevant), Published or not	Data Protection claimed (Y/N)	Owner
B.5.2.11	Ma. M.and Li, Q.	2012	Method Validation Study for the Determination of Residues of XDE-729 Methyl Ester and XDE-729 Acid in Bovine and Poultry Tissues using Offline Solid-Phase Extraction and Liquid Chromatography with Tandem Mass Spectrometry Detection. Dow AgroSciences LLC DAS Report No.: 110505 (Accession Number) 2011973 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
B.5.2.12	Langridge, G.	2012	Independent Laboratory Validation of an Analytical Method for the Determination of XDE-729 methyl ester and XDE-729 acid in Animal Matrices CEM Analytical Services (CEMAS) DAS Report No.: 110828, CEMS-5325 (Accession Number) 2012645 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
B.5.3.1.1	Blakeslee, B. A	2012	Method Validation Study for the Determination of Residues of X11393728 (XDE 729 Methyl), X119393729 (XDE 729 Acid), and X11449757 (des-Methyl XDE 729 Acid) in Soil using High Performance Liquid Chromatography with Positive-Ion Electrospray Ionization Mass Spectrometry Detection Dow AgroSciences LLC DAS Report No.: 110716 (Accession Number) 2014176 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS

Annex Point/ Reference Number	Author(s)	Year	Title Source (where different from the Company), Company, Report Number, GLP or GEP status (where relevant), Published or not	Data Protection claimed (Y/N)	Owner
B.5.3.1.2	Gemrot, F.	2012a	Independent Laboratory Validation of Analytical Method Study Number 110716 for the Determination of XDE 729 Methyl Ester and its Metabolite Residues in Soil Eurofins Agroscience Services Chem GmbH DAS Report No.: 120894, S12-03363 (Accession Number) 2014137 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
B.5.3.2.1	Rodrigues, A., Qian, L.	2011 (Amendment 2012)	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 Dow AgroSciences Ind. Ltda. DAS Report No.: 110718S2 (Accession Number) 2010922 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
B.5.3.2.2	Gemrot, F.	2012b	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 Eurofins Agroscience Services Chem DAS Report No.: 110827, S11-04023 (Accession Number) 2012360 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
B.5.3.3.1	Class, T.	2011	The Development and Validation of a Method for the Analysis of XDE-729 Methyl Ester and XDE-729 Acid in Air PTRL Europe GmbH DAS Report No.: 110028, P 2169 G (Accession Number) 2008550 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS