Renewal Assessment Report

under Regulation (EC) 1107/2009

Zoxamide

Volume 3

Active substance
B.5 Methods of analysis

Rapporteur Member State: Latvia
Co-Rapporteur Member State: France

Version history

Date	Subject
2001	Initial DAR Draft Assessment Report (DAR) – prepared in the context of the application for the first inclusion of the a.s. in Annex I to Council Directive 91/414/EEC. + 1st addendum Jun 2002 + 2nd addendum July 2002
2016	Initial RAR

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B.5. ANALYTICAL METHODS

B.5.1. Methods used for the Generation of Pre-Approval Data

B 5.1.1. Methods for the analysis of the active substance as manufactured

a) Determination of the pure active substance in the active substance as manufactured and specified in the dossier submitted in support of approval under Regulation (EC) No 1107/2009

Previous evaluation:	EU review (Addendum 2, Volume 4 (Rev.1), July 2002)

Reference: **CA 4.1.1/01**
Author: Kemmerer, S. C., (1998)
Title: Product Chemistry Series 830 Group A, Guideline Numbers OPPTS: 830.1550, 830.1600, 830.1620, 830.1670, 830.1700, 830.1750, 830.1800: Product Identity, Composition, and Analysis for RH – 117,281 Technical, Rohm and Haas Company, 727 Norristown Road, Spring House, PA 19477, Report No. APR-98-282
Guidelines: OPPTS Guidelines: 830.1550, 830.1600, 830.1620, 830.1670, 830.1700, 830.1750, 830.1800.
GLP: Yes

Outline of the method
Technical grade zoxamide samples are weighed into 1 oz vials (100 mg) and diluted in acetonitrile (25 mL) and dissolved completely. The samples are diluted by taking an aliquot of this solution (1 mL) and diluting with acetonitrile (10 mL). The samples are analysed by high performance liquid chromatography with ultra-violet detection (HPLC-UV) at 210 nm, using a Phenomenex Ultracarb 5 ODS (20) column (250 mm x 4.6 mm) and gradient elution with mobile phases of water with phosphoric acid and acetonitrile. Quantification is performed using external standard solutions.

Validation
All validation data were provided and deemed to be acceptable at the previous EU review (Addendum 2, Volume 4 (Rev.1), July 2002).

RMS comments and conclusion:
The method is fully validated for determination of the pure active substance in the active substance as manufactured.

b) Determination of significant and relevant impurities and additives (such as stabilisers) in the active substance as manufactured

Confidential information - refer to confidential volume 4.

B.5.1.2. Methods for risk assessment

a) Methods in soil, water, sediment, air and any additional matrices used in support of environmental fate studies (IIA 4.2.2 to 4.2.4, IIIA 5.2)

Previous evaluation:	DAR, 2001; EU review (Addendum 1), July 2002)

A summary of methods and validation is shown in Table B.5.1.2-1. Methods, including chromatograms, were acceptable unless otherwise stated.

Residues in soil (IIA 4.2.2)

a Soil samples are extracted with acetonitrile and the extract partitioned between water and dichloromethane. The organic fraction is cleaned-up using Florisil/anhydrous sodium sulphate and Alumina-B SPE columns, eluting with ethyl acetate/ hexane. Quantitation of RH-7281 is achieved using capillary GC (RTX-1 column) with ECD detection or capillary GC (RTX-5 column) with MS detection (SIM, m/z 187, 189, 257)

Guo, 1996b, DP 81847

Szuter, 1998a, DP 81852

b In a variation of the above method, quantitation of RH-7281 using capillary GC with MS detection was based on MS ion 187, 189 and 258.

Guo, 1998, DP 81851

Residues in water (IIA 4.2.3)

a Drinking water was extracted with ethyl acetate. Quantitation was by capillary GC (DB-1 column) with ECD detection. Confirmation is by capillary GC (RTX-1 column) with MS detection (MS ions m/z 187 and 258).

Volkel. 1998, (DP 81855)

b In a variation of the above method, drinking and surface water were extracted with ethyl acetate. Quantitation was by GC-ECD with confirmation by GC-MS but using MS ion m/z 258 only. Samples of surface water used to validate the method of analysis were taken from the River Wiese, 750m downstream from Mambach (Black Forrest region, Germany). This is a rural farming area with little industry. Total organic carbon content was 1.9 mg/cl, pH 6. Total residue obtained following evaporation was 60 mg/l. Validation data are summarised in the table below.

Wais, 2000, DP 97884

Residues in air (IIA 4.2.4)

RH-7281 is absorbed onto a TENAX column (flow rate of air 1 litre/minute over 6 hours) and then extracted with acetonitrile. The extract is filtered and concentrated and the residue taken up in hexane. Quantitation was by Capillary GC (DB-1 column) with ECD detection. Confirmation is by GC-MS (SIM m/z 187).

Wais, 1999b, (DP 81858)

No new environmental fate studies for which analytical methods are required are being submitted in support of the Annex I renewal of zoxamide.

b) Methods in soil, water and any additional matrices used in support of efficacy studies

No new efficacy studies for which analytical methods are required are being submitted in support of the Annex I renewal of zoxamide.

c) Methods in feed, body fluids and tissues, air and any additional matrices used in support of toxicological studies

No new toxicology studies for which analytical methods are required are being submitted in support of the Annex I renewal of zoxamide.

d) Methods in body fluids, air and any additional matrices used in support of operator, worker, resident and bystander exposure studies

No new operator, worker, resident or bystander exposure studies for which analytical methods are required are being submitted in support of the Annex I renewal of zoxamide.

e) Methods in or on plants, plant products, processed food commodities, food of plant and animal origin, feed and any additional matrices used in support of residues studies

Previous evaluation:	New data, submitted for the purposes of renewal

Reference: **CA 4.1.2/01 (CA 6.1/01)**
Report: Weber, H., (2012), Validation of an Enforcement Method ("QuEChERS") for the Determination of Residues of Zoxamide in Grapes and Potatoes and their Process Products using LC-MS/MS, Eurofins Agroscience Services, Germany, Report No. S12-03949
Guidelines: SANCO/825/00 rev. 8.1, Regulation (EC) No 1107/2009
GLP: Yes

Principle of the method
Homogenised samples of raisins, potato chips and flakes (5g) and grape berries and juice, wine and potato tubers (10g) are weighed into Sarstedt centrifuge tubes (50 mL). For raisins, 8.5 mL of water is added and for potato (chips and flakes), 9 mL of water is added. The samples are extracted with acetonitrile (10 mL) with vigorous shaking for 1 minute. A salt mixture (4g magnesium sulfate, 1g sodium chloride, 1g trisodium citrate dihydrate and 0.5g of disodium hydrogen citrate sesquihydrate) is added and the samples are shaken again for 1 minute. After shaking, the samples are centrifuged for 2 minutes at 4000 rpm. An aliquot (1.5 mL) of the supernatant is cleaned up by adding a mixture of PSA (40 mg) and magnesium sulphate (225 mg) and vortex mixing for 30 seconds before centrifuging for 2 minutes at 6000 rpm. An aliquot (0.2 mL for raisins, potato chips and flakes and 0.1 mL for grape berries and juice, wine and potato tubers) is diluted to 1 mL with methanol:0.05% acetic acid (1:1, v/v) and analysed by high performance liquid chromatography with tandem mass specific detection (LC-MS/MS) in positive ion mode, using a Supelco Ascentis Express C18 column (50 x 2.1 mm, 2.7µm) and gradient elution with mobile phases of methanol + 0.05% acetic acid and water + 0.05% acetic acid. Quantification is performed using external standards. The ion transition m/z 336 > 187 is used for quantification and the ion transition for 336 > 159 is used for confirmation.

See CA 4.2/01 below validation data.

Reference: **CA 4.1.2/02 (CA 6.1/01)**
Report: Weber, H., Giesau, A., (2013), Validation of an Analytical Method for the Determination of Residues of the Zoxamide Metabolite RH-150721 in Grapes and Processing Fractions using LC-MS/MS, Eurofins Agroscience Services, Germany, Report No. S12-03950
Guidelines: SANCO/825/00 rev. 8.1, Regulation (EC) No 1107/2009
GLP: Yes

Principle of the method
Grape berries - Samples (10g) are weighed into 150mL screw capped glass bottles, acetone (80 mL) and water (32 mL) are added and the samples are homogenised for 1 minute. The suspension is filtered over cotton wool into a round bottom flask (250 mL), the screw capped glass bottle and filter are rinsed with acetone and combined in the round bottom flask. The acetone is evaporated off using a rotary evaporator

(to a volume of approximately 40 mL). 1% potassium hydrogen carbonate solution (10 mL) is added to the extract and the sample centrifuged.

Grape juice and wine - Samples (10g) are weighed into 150mL screw capped glass bottles and 1 % aqueous potassium hydrogen carbonate solution (40 mL) is added.

All samples - The sample extracts are cleaned up by solid phase extraction using a polymeric reverse phase column (OASIS HLB, 3 cc, 60 mg), which is preconditioned with methanol (6 mL) and 1 % aqueous potassium hydrogen carbonate solution (6 mL). For grape berries, the cartridge is covered with cotton wool prior to filtration. The whole sample extract is added slowly through the cartridge and the cartridge is washed successively with 1 % aqueous potassium hydrogen carbonate solution (5 mL) and methanol/1 % aqueous potassium hydrogen carbonate solution, 1/1, v/v (5 mL). The analyte is eluted twice with methanol containing 1 % glacial acetic acid (2 mL) and the solvent is drawn through the cartridge using vacuum. The extracts are made to a volume of 10 mL with water (7 mL). The samples are analysed by high performance liquid chromatography with tandem mass specific detection (LC-MS/MS) in positive electrospray mode, using a Supelco Ascentis Express C18 column (150 x 3.0 mm, 2.7 µm particle size), with a pre-column Phenomenex Security Guard Cartridge, and gradient elution with mobile phases of acetonitrile + 0.1 % acetic acid and water + 0.1 % acetic acid. Quantification is performed using external standards of RH-150721 methanesulfonate. The ion transition m/z 318 > 159 is used for quantification and the ion transition m/z 318 > 187 is used for confirmation of grapes (berries) and the ion transition m/z 318 > 123 is used for confirmation of grapes (juice and wine).

Specificity

LC-MS/MS monitoring two mass transitions is considered to be a highly specific technique.

No interferences at >30% of the LOQ were present at the retention time of interest in control matrix samples.

Linearity

Linearity of detector response was demonstrated for RH-150721 using eight matrix matched external standard solutions across the working range of 2.5 to 500 ng/mL (calculated as free base). The results are presented in Table B.5.1.2-2 below.

Precision (Repeatability)

Repeatability data was generated from five samples of each matrix fortified at the LOQ and five samples fortified at 10 x LOQ. The relative standard deviations (RSD) obtained were within the guideline requirements and are presented in Table B.5.1.2-3 below.

Accuracy (Recovery)

Recovery data was generated from five samples of each matrix fortified at the LOQ and five samples fortified at 10 x LOQ. The mean percentage recoveries obtained were within the guideline requirements and are presented in Table B.5.1.2-3 below.

Limit of Quantification (LOQ)

The limit of quantitation (LOQ), defined as the lowest fortification level where acceptable precision and accuracy data were obtained, was determined to be 0.01 mg/kg for RH-150721.

Conclusion

The analytical procedure has been successfully validated in terms of specificity, linearity, precision, accuracy and LOQ, in accordance with all of the requirements of SANCO/825/00 rev. 8.1.

Table B.5.1.2-2: Linearity Data

Matrix	Ion Transition (m/z)	Concentration Range (ng/mL)	Coefficient of Determination (R^2)	Slope	Intercept
Grapes (Berries)	318 > 159	2.50 - 500	0.9914	6926	61778
	318 > 187	2.50 - 500	0.9927	12639	98599
Grapes (Juice)	318 > 159	2.50 - 500	0.9986	8336.8	20450
	318 > 123	2.50 - 500	0.9981	3755.7	8057.6
Grapes (Wine)	318 > 159	2.50 - 500	0.9975	7211.8	33420
	318 > 123	2.50 - 500	0.9981	3262.7	12228

Table B.5.1.2-3: Precision and Accuracy Data

Matrix	Fortification Level (mg/kg)	Recoveries (%)	Mean Recovery (%)	RSD (%)	Acceptable RSD (%)	Acceptable Recovery (%)
Grapes (Berries)	**Ion transition m/z 318 > 159 (quantification)**					
	0.01	77, 74, 71, 67, 72	72	5.1	30	60 – 120
	0.1	72, 68, 72, 75, 68	71	4.2	20	70 – 120
	Ion transition m/z 318 > 187 (confirmation)					
	0.01	70, 80, 75, 65, 72	72	7.7	30	60 – 120
	0.1	73, 68, 72, 74, 69	71	3.6	20	70 – 120
Grapes (Juice)	**Ion transition m/z 318 > 159 (quantification)**					
	0.01	73, 69, 67, 69, 77	71	5.6	30	60 – 120
	0.1	78, 73, 73, 78, 73	75	3.7	20	70 – 120
	Ion transition m/z 318 > 123 (confirmation)					
	0.01	66, 64, 68, 64, 73	67	5.6	30	60 – 120
	0.1	81, 74, 76, 81, 77	78	4.0	20	70 – 120
Grapes (Wine)	**Ion transition m/z 318 > 159 (quantification)**					
	0.01	71, 79, 84, 72, 95	80	12	30	60 – 120
	0.1	79, 84, 72, 76, 79	78	5.7	20	70 – 120
	Ion transition m/z 318 > 123 (confirmation)					
	0.01	70, 81, 82, 71, 91	79	11	30	60 – 120
	0.1	82, 90, 75, 83, 81	82	6.5	20	70 – 120

RMS comments and conclusions

The limit of quantification is 0.01 mg/kg for RH-150721 in grape (berries), and its processing products wine and juice. The mean recovery for the method are within acceptable range of limits 60-120% and <30% RSD for each fortification level at the limit of quantitation (0.01 mg/kg) and 70-120% for a mean recovery and <20% RSD at 0.1 mg/kg that comply with SANCO/825/00 rev. 8.1 requirements.

Reference: **CA 4.1.2/03 (CA 6.1/01)**

Report: Weber, H., Giesau, A., (2013), Validation of an Analytical Method for the Determination of Residues of Zoxamide Metabolites RH-1452 and RH-1455 in Potatoes and Processing

Fractions using LC-MS/MS, Eurofins Agroscience Services, Germany, Report No. S12-03951
Guidelines: SANCO/825/00 rev. 8.1, Regulation (EC) No 1107/2009
GLP: Yes

Principle of the method

Samples of potato tubers (10g) and potato chips and flakes (2.5g) are weighed into 150mL screw capped glass bottles. Prior to sample preparation, the specimens of potato chips and flakes are soaked with water (8 g). Methanol: 0.01 N sodium hydroxide solution, 7:3, v:v (50 mL) is added to the samples and the samples are homogenised. The resulting suspension is then filtered into a 100 mL volumetric flask. An additional methanol: 0.01 N sodium hydroxide solution, 7:3, v:v (30 mL) is added to the samples and the samples are homogenised again, and the resulting suspension filtered into the same 100 mL volumetric flask. The filter is rinsed with additional methanol: 0.01 N sodium hydroxide solution, 7:3, v:v and the combined extracts are diluted to 100 mL with the same solution. An aliquot (10 mL) is then mixed with 0.01 N sodium hydroxide solution (25 mL). The sample extracts (35 mL) are transferred to a pre-conditioned solid phase extraction cartridge (OASIS MAX, 500 mg, 6 mL; Waters) and washed with 0.01 N sodium hydroxide solution (10 mL) followed by methanol (10 mL). The cartridge is dried for 15 minutes under vacuum. The analytes are eluted with methanol: 37% hydrochloric acid, 100:1, v:v (5 mL) and the eluate is evaporated to ~1 mL in a nitrogen stream. The extract of potato (tubers) is diluted to 10 mL with methanol: 0.2% formic acid (2:8, v:v) and the sample extracts of potato (chips and flakes) are diluted to 5 mL with methanol: 0.2% formic acid (2:8, v:v). The samples are analysed by high performance liquid chromatography with tandem mass specific detection (LC-MS/MS) in negative ion mode, using a Phenomenex Synergi 4µ Polar-RP 80A, C18 column (150 x 4.6 mm, 4.0 µm) and gradient elution with mobile phases of methanol + 0.05% acetic acid and water + 0.05% acetic acid. Quantification is performed using matrix matched external standards. For the zoxamide metabolite RH-1452 in potato tubers, the ion transition m/z 219 > 175 is used for quantification and the ion transition for 221 > 147 is used for confirmation, in potato chips the ion transition m/z 221 > 147 is used for quantification and the ion transition for 221 > 177 is used for confirmation and in potato flakes the ion transition m/z 221 > 147 is used for quantification and the ion transition for 219 > 175 is used for confirmation. For the zoxamide metabolite RH-1455 in potato tubers, the ion transition m/z 235 > 191 is used for quantification and the ion transition for 233 > 109 is used for confirmation, and in potato chips and flakes the ion transition m/z 233 > 189 is used for quantification and the ion transition for 233 > 109 is used for confirmation.

Specificity

LC-MS/MS monitoring two mass transitions is considered to be a highly specific technique.

No interferences at >30% of the LOQ were present at the retention time of interest in control matrix samples.

Linearity

Linearity of detector response was demonstrated for RH-1452 and RH-1455 using at least nine external standard solutions covering a concentration range of 0.3 to 100 ng/mL for potato tubers and at least eight external standard solutions covering a concentration range of 0.6 to 100 ng/mL for potato chips and flakes. Matrix-matched standards were used, due to observed matrix effects. The results are presented in Table Table B.5.1.2-4 below.

Precision (Repeatability)

Repeatability data was generated for each analyte from five samples of each matrix fortified at the LOQ and five samples fortified at 10 x LOQ. The relative standard deviations (RSD) obtained for each fortification level were within the guideline requirements and are presented in Table B.5.1.2-5 and Table B.5.1.2-6 below.

Accuracy (Recovery)

Recovery data was generated for each analyte from five samples of each matrix fortified at the LOQ and five samples fortified at 10 x LOQ. The mean percentage recoveries at each fortification level were within the guideline requirements and are presented in Table B.5.1.2-5 and Table B.5.1.2-6 below.

Limit of Quantification (LOQ)

The limit of quantitation (LOQ), defined as the lowest fortification level where acceptable precision and accuracy data were obtained, was determined to be 0.01 mg/kg in potato (tubers) and 0.05 mg/kg in potato (chips and flakes) for RH-1452 and RH-1455.

Conclusion

The analytical procedure has been successfully validated in terms of specificity, linearity, precision, accuracy and LOQ, in accordance with all of the requirements of SANCO/825/00 rev. 8.1.

Table B.5.1.2-4: Linearity Data

Matrix	Analyte	Ion Transition (m/z)	Concentration Range (ng/mL)	Correlation Coefficient (r)	Slope	Intercept
Potato (tuber)	RH-1452	219 > 175	0.300 - 100	0.9997	13824	226.44
		221 > 147	0.300 - 100	0.9994	3462.4	1796.8
	RH-1455	235 > 191	0.300 - 100	0.9986	12063	-4471.7
		233 > 109	0.300 - 100	0.999	4275.8	-1117.1
Potato (chips)	RH-1452	221 > 147	0.600 - 100	0.9997	2421.6	-1060.9
		221 > 177	0.600 - 100	0.9998	3238.9	-1576
	RH-1455	233 > 189	0.600 - 100	0.9991	9668.7	-10207
		233 > 109	0.600 - 100	0.9996	1988.3	-492.71
Potato (flakes)	RH-1452	221 > 147	0.600 - 100	0.9992	957.88	-130.37
		219 > 175	0.600 - 100	0.9999	6339.6	-1461.7
	RH-1455	233 > 189	0.600 - 100	0.9992	7691.5	-5220.5
		233 > 109	0.600 - 100	0.9984	1610.7	-1167.2

Table B.5.1.2-5: Precision and Accuracy Data for RH-1452

Matrix	Fortification Level (mg/kg)	Recoveries (%)	Mean Recovery (%)	RSD (%)	Acceptable RSD (%)	Acceptable Recovery (%)
Potato (tuber)	**Ion transition m/z 219 > 175 (quantification)**					
	0.01	90, 90, 86, 94, 99	92	5.4	30	60 – 120
	0.1	97, 94, 95, 94, 94	95	1.4	20	70 – 120
	Ion transition m/z 221 > 147 (confirmation)					
	0.01	96, 80, 91, 95, 87	90	7.3	30	60 – 120
	0.1	92, 93, 91, 91, 90	91	1.2	20	70 – 120
Potato (chips)	**Ion transition m/z 221 > 147 (quantification)**					
	0.05	113, 105, 104, 103, 99	105	4.9	20	70 – 120
	0.5	103, 95, 92, 96, 92	96	4.7	15	70 – 110

	Ion transition m/z 221 > 177 (confirmation)					
	0.05	92, 105, 103, 89, 86	95	9.0	20	70 – 120
	0.5	105, 100, 102, 106, 98	102	3.3	15	70 – 110
Potato (flakes)	**Ion transition m/z 221 > 147 (quantification)**					
	0.05	96, 108, 111, 107, 111	107	5.8	20	70 – 120
	0.5	103, 99, 96, 98, 96	98	2.9	15	70 – 110
	Ion transition m/z 219 > 175 (confirmation)					
	0.05	103, 94, 93, 105, 100	99	5.4	20	70 – 120
	0.5	104, 102, 101, 99, 104	102	2.1	15	70 – 110

Table B.5.1.2-6: Precision and Accuracy Data for RH-1455

Matrix	**Fortification Level (mg/kg)**	**Recoveries (%)**	**Mean Recovery (%)**	**RSD (%)**	**Acceptable RSD (%)**	**Acceptable Recovery (%)**
Potato (tuber)	**Ion transition m/z 235 > 191 (quantification)**					
	0.01	84, 88, 99, 100, 86	91	8.2	30	60 – 120
	0.1	93, 92, 93, 92, 90	92	1.3	20	70 – 120
	Ion transition m/z 233 > 109 (confirmation)					
	0.01	82, 84, 90, 86, 90	86	4.1	30	60 – 120
	0.1	94, 94, 92, 94, 88	92	2.8	20	70 – 120
Potato (chips)	**Ion transition m/z 233 > 189 (quantification)**					
	0.05	84, 86, 80, 87, 84	84	3.2	20	70 – 120
	0.5	105, 101, 97, 107, 98	102	4.3	15	70 – 110
	Ion transition m/z 233 > 109 (confirmation)					
	0.05	103, 104, 100, 102, 93	100	4.4	20	70 – 120
	0.5	95, 94, 87, 95, 89	92	4.1	15	70 – 110
Potato (flakes)	**Ion transition m/z 233 > 189 (quantification)**					
	0.05	93, 98, 94, 96, 95	95	2.0	20	70 – 120
	0.5	109, 104, 113, 104, 110	108	3.6	15	70 – 110
	Ion transition m/z 233 > 109 (confirmation)					
	0.05	100, 101, 102, 100, 94	99	3.1	20	70 – 120
	0.5	107, 104, 112, 104, 107	107	3.1	15	70 – 110

RMS comments and conclusion:

Mean recovery values in potato (tubers) obtained by LC- MS for RH-1452 and RH-1455 at fortification levels 0.01 mg/kg (LOQ) and 0.1 mg/kg (10xLOQ) comply with acceptance criteria of SANCO Guideline

825/00 rev. 8.1. The accuracy and precision for the method are within acceptable limits of 60-120% for mean recovery and <30% RSD for each fortification level at the limit of quantitation (0.010 mg/kg) and 70-120% for a mean recovery and <20% RSD at 0.10 mg/kg.
Mean recovery values in potato chips and potato flakes at fortification levels 0.05 mg/kg (LOQ) and 0.5 mg/kg (10xLOQ) are within acceptable limits indicated in SANCO Guideline 825/00 rev. 8.1.

Reference: **CA 4.1.2/04 (CA 6.3.1/01 and CA 6.3.2/01)**
Report: Luciani, G.P. (2010), Determination of Zoxamide residues after five applications of ELECTIS MZ and ZOXIUM 240 SC on potato under field conditions – Italian trial, AgriParadigma S.r.l, Italy, Report No. AGRI 012/10 GLP DEC
Guidelines: Commission Directive 96/68/EC amending Council Directive 91/414/EEC
Working document 7029/VI/95 Rev.5: Appendix B
Italian Decrees: D.L. n. 194/95, D.L. n. 50/07 and D.M. 5 Agosto 1999
GLP: Yes

Principle of the method

Homogenised samples of potatoes and grapes (10 g) are transferred into a Teflon centrifuge tube (50 mL), acetonitrile (10 mL) is added and the sample is shaken vigorously for 1 minute using a vortex mixer. Anhydrous magnesium sulfate (4 g), sodium chloride (1 g), trisodium citrate dehydrate (1 g) and disodium hydrogencitrate sesquihydrate (0.5 g) are added and the sample is vortex mixed for 5 minutes before being centrifuged at 3000 rpm for 5 minutes. The samples are analysed by high performance liquid chromatography with tandem mass specific detection (HPLC-MS/MS) in positive polarity mode, using a Waters Acquity UPLC HSS T3 column (100 x 2.1 mm, 1.8 µm particle size) and gradient elution with mobile phases of methanol/water, 10/90, v/v + 0.005M ammonium formiate and methanol + 0.005M ammonium formiate. Quantification is performed using external standards. The ion transition m/z 336.1 > 186.8 is used for quantification and the ion transition m/z 336.1 > 158.8 is used for confirmation.

Specificity

LC-MS/MS monitoring two mass transitions is considered to be a highly specific technique. No interferences at >30% of the LOQ were present at the retention time of interest in the control matrix samples.

Linearity

Linearity of detector response for zoxamide was demonstrated using five external standard solutions across the working range of 0.005 – 0.1 mg/L, with a correlation coefficient of 0.9998 (slope = 2522248, intercept = 3085).

Precision (Repeatability)

Repeatability data was generated from five samples fortified at the LOQ and five samples fortified at a level appropriate to the expected residue for each matrix. The relative standard deviations (RSD) obtained at each fortification level were within the guideline requirements and the results are presented in Table Table B.5.1.2-7 below.

Accuracy (Recovery)

Recovery data was generated from five samples fortified at the LOQ and five samples fortified at a level appropriate to the expected residue for each matrix. The mean recoveries found were within the guideline requirements and are presented in Table B.5.1.2-7 below.

Limit of quantification (LOQ)

The limit of quantification (LOQ), defined as the lowest fortification level where acceptable precision and accuracy data were obtained, was demonstrated to be 0.005 mg/kg for potato and 0.01 mg/kg for grapes.

Conclusion
The analytical procedure has been successfully validated in terms of specificity, linearity, precision, accuracy and LOQ, in accordance with all of the requirements of SANCO/3029/99 rev. 4.

Table B.5.1.2-7: Precision and Accuracy data

Matrix	Fortification Level (mg/kg)	Recoveries (%)*	Mean Recovery (%)*	Acceptable Recovery (%)	RSD (%)	Acceptable RSD (%)
Potato	0.005	72, 92, 108, 96, 104	94.4	70 - 110	14.9	20
	0.20	73.5, 77.5, 80.5, 91.5, 95	83.6	70 - 110	11.1	20
White table grape	0.010	88, 109, 107, 94, 104	100.4	70 - 110	9.0	20
	1.00	100, 107, 109, 109, 100	105	70 - 110	4.4	20
Black wine grape	0.010	104, 109, 101, 110, 107	106.2	70 - 110	3.5	20
	0.75	87.7, 86.4, 101.3, 109.1, 110	98.9	70 - 110	11.5	20

* Manually calculated from values presented in report.

RMS comments and conclusion:
The precision and accuracy data indicate that the values acceptable accordingly SANCO/3029/99 rev. 4 requirements and suitable for total zoxamide residue analysis in potato, white table grape and black wine grape.

Reference: **CA 4.1.2/08 (**CA 6.1/04**)**
Report: Weber, H., Zetzsch, A., Giesler, W. (2016)
Storage Stability of residues of Zoxamide, RH-150721, RH-1452 and RH-1455 in Grape and Processed Products and Potato
Report no.: S12-03952 Final Report
Guidelines: Regulation (EC) no 1107/2009; Concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC
EU guidance document 7032/VI/95, rev. 5, Appendix H of EC document 1607/VI/97 rev. 2 "Storage Stability of Residue Samples", 22-Jul-1997.
OECD guideline No. 506; Stability of pesticide residues in stored commodities (16/10/2007)
GLP: Yes

Cross reference to Residues Section vol.3, B7.1.1.

Analysis for zoxamide

Grape berries, grape juice, wine, raisin and potato samples were analysed for zoxamide using the QuEChERS LC-MS/MS method, validated in EAS study S12-03949 (**Reference:CA 4.2/01**

Analysis for RH-150721

Grape berry and wine samples were analysed for the metabolite RH-150721 using an LC-MS/MS method,validated in EAS study S12-03950. The method validation data are presented **Reference: CA 4.1.2/02 (CA 6.1/01)**

Analysis for RH-141452 and RH-141455

Potato samples were analysed for the metabolites RH-141452 and RH-141455 using an LC-MS/MS method, validated in EAS study S12-03951. The method validation data are presented above **CA 4.1.2/03 (CA 6.1/01)**.

Linearity

The linearity of detector response for Zoxamide, RH-150721, RH-1452 and RH-1455 was confirmed by injecting at least 8 standard solutions covering the working range. The actual analytical concentrations were ±20 % of the calibration standards.

Specificity

The concentration of the analytes in the final extracts was determinated by high performance liquid chromatography with MS/MS detection. Significant matrix effects were observed for Zoxamide in raisins, RH-150721in grapes (juice) and wine as well as for RH-1452 and RH-1455 in potato (tubers) during the method validations. Therefore, matrix matched standards were used for quantification of these matrices.

Limit of Quantification

For all analytes in all matrices, the limit of quantification (LOQ) was 0.01 mg/kg with limit of detection (LOD) of 0.003 mg/kg.

Recovery Findings

For zoxamide (RH-7281) in grapes (berries and juice), wine, raisins and potato (tubers), the zoxamide metabolite RH-150721 in grapes (berries) and wine, and the zoxamide metabolite of RH-141452 and RH-141455 in potato (tubers), procedural recoveries were in the range between 70% and 110%.

The following recoveries were obtained for **Zoxamide**:

Storage Time (Months)	Recovery in stored samples					Mean Recovery Corrected (%)*	Recovery in freshly fortified sample (%)
	Recoveries (%)			Mean (%)	RSD (%)		
	Grapes (berries)						
0	104	99	108	104	4.3	100	-
6	99	98	-	99	-	97	102
12	99	97	-	98	-	100	98
18	82	81	-	82	-	84	98
	Grapes (juice)						
0	100	108	108	105	4.4	100	-
6	92	91	-	92	-	95	97
12	93	93	-	93	-	102	91
18	101	105	-	103	-	98	105
24	91	93	-	92	-	93	99
	Wine						
0	90	83	93	89	5.8	100	-
6	80	81	-	81	-	104	78
12	80	76	-	78	-	98	80
18	90	91	-	91	-	102	89
24	81	80	-	81	-	88	92
	Raisins						
0	103	99	102	101	2.1	100	-
6	104	104	-	104	-	100	104
12	95	94	-	95	-	94	101
18	103	101	-	102	-	97	105
24	88	93	-	91	-	101	90
	Potato (tuber)						
0	105	103	100	103	2.5	100	-
6	88	90	-	89	-	94	95
12	87	83	-	85	-	94	90
18	94	104	-	99	-	97	102
24	85	84	-	85	-	90	94

* corrected for procedural recovery of freshly fortified sample in the same analytical set

The following recoveries were obtained for **RH-150721**:

Storage Time (Months)	Recovery in stored samples					Mean Recovery Corrected (%)*	Recovery in freshly fortified sample (%)
	Recoveries (%)			Mean (%)	RSD (%)		
	Grapes (berries)						
0	76	75	75	75	0.8	100	-
6	77	70	-	74	-	99	75
12	81	84		83	-	100	83
18	71	84		78	-	92	85
	Wine						
0	89	92	97	93	4.4	100	-
6	107	107	-	107	-	109	98
12	92	96		94	-	119	79
18	101	89		95	-	102	93
24	77	74	-	76	-	84	91

* corrected for procedural recovery of freshly fortified sample(s) in the same analytical set

The following recoveries were obtained for **RH-1452** in potato (tuber):

Storage Time (Months)	Recovery in stored samples					Mean Recovery Corrected (%)*	Recovery in freshly fortified samples (%)
	Recoveries (%)			Mean (%)	RSD (%)		
	Potato (tuber)						
0	88	89	87	88	1.1	100	-
6	95	89	-	92	-	103	89
12	81	86	-	84	-	99	85
18	92	94	-	93	-	100	93
24	99	96	-	98	-	117	84

* corrected for procedural recovery of freshly fortified sample(s) in the same analytical set

The following recoveries were obtained with for **RH-1455** in potato (tuber):

Storage Time (Months)	Recovery in stored samples					Mean Recovery Corrected (%)*	Recovery in freshly fortified samples (%)
	Recoveries (%)			Mean (%)	RSD (%)		
	Potato (tuber)						
0	86	89	85	87	2.4	100	-
6	86	86	-	86	-	91	94
12	82	76	-	79	-	88	90
18	81	85	-	83	-	97	86
24	96	89	-	93	-	97	96

* corrected for procedural recovery of freshly fortified sample(s) in the same analytical set

RMS comments and conclusions

The analytical procedure has been successfully validated in terms of specificity, linearity, precision, accuracy and LOQ , in accordance with requirements of SANCO/3029/99 rev. 4.

Reference: **CA 4.1.2/09 (CA 6.3.2/21)**

Report: Luciani, G.P. (2010b)
Determination of Zoxamide residues after five application of ELECTIS MZ and ZOXIUM 240 SC on wine grape and table grape under field conditions – Italian trial, year 2010

	Report no.: AGRI 010/10 GLP DEC
Guidelines:	Commission Directive 96/68/EC amending Council Directive 91/414/EEC Working document 7029/VI/95 Rev.5: Appendix B Italian Decrees: D.L. n. 194/95, D.L. n. 50/07 and D.M. 5 Agosto 1999
Deviations:	None
GLP:	Yes (certified laboratory)

Cross reference to Residues Section vol.3, B7.3.2.

Samples were analysed for zoxamide using method Agri BPL 015 Rev.2 'Determination of zoxamide residues in tomato, potato and grape. The method was validated on grapes in this study, and the method validation data are presented above **CA 4.1.2/04** .

Samples were extracted with acetonitrile by shaking using a vortex mixer. Anhydrous magnesium sulphate, sodium chloride and buffering citrate salts (trisodium citrate dehydrate and disodium hydrogencitrate sesquihydrate) were added and the mixture vortex mixed and centrifuged to separate the phases. The final extract was analysed directly by HPLC-MS/MS. The limit of quantification (LOQ) for table and wine grapes was 0.01 mg/kg. The zoxamide residues in the treated samples were not corrected for the recovery values.

Zoxamide fortifications in table grape samples were made from 0.01 to 1.00 mg/kg. Recovery ranges from 100.5% to 105.2 %. Zoxamide fortifications in wine grape samples were made from 0.01 to 0.75 mg/kg. Recovery ranges from 98.9% to 106.0 %. The lowest fortification level corresponds to the limit of quantification (LOQ).

RMS comments and conclusions

The analytical procedure has been successfully validated accordance with requirements of SANCO/3029/99 rev. 4.

Reference:	**CA 4.1.2/10 (CA 6.5.3/02)**
Report:	Wais, A. (2001) Determination of residues of RH-117,281 and mancozeb in/on vine grapes (RAC grapes and processing products) following treatment with RH-7281/mancozeb 75WG from a field trial (semi residue decline study) in Italy; 1999 Report no.: 734580 ER Ref: R77.10
Guidelines:	Commission Directive 96/68/EC Working document 1607/VI/97 Rev.1 and 7029/VI/95 Rev.5
Deviations:	None
GLP:	Yes (certified laboratory)
Validity of the study:	Valid
Previous evaluations:	Submitted for the purpose of renewal

Cross reference to Residues Section vol.3, B7.5.3.

Description of analytical procedures

Samples of grapes, must, pomace and wine were analysed for zoxamide using validated method TR 34-98-150 (CA 4.2.1/08 (ER 15.3)). The method was validated on grapes (report 647177), must (report 676888) and pomace (report 673683). Samples of wine were analysed for zoxamide and RH-150721 using validated method TR 34-98-179 (CA4.2.1/12 (ER29.15)). The method was validated on wine (report 707310). The method validation data were presented in the DAR, Point B5.2.

Samples of grapes were extracted by homogenisation with methanol/water (80:20 v/v). The extract was filtered through celite and the filtrate concentrated by rotary evaporation. After the addition of 0.1M sodium chloride solution, the extract was partitioned twice against dichloromethane. The combined organic phases were dried with sodium sulphate, evaporated to dryness and redissolved in hexane. The extract was cleaned-up by SPE using an ENVI-Carb SPE column, followed by an LC-Alumina-B SPE column. The ethyl acetate/hexane eluate was collected, evaporated to dryness and redissolved in hexane for final determination by GC-ECD.

Grape pomace was extracted by homogenisation with methanol/water (80:20 v/v). The extract was filtered through celite and the filtrate concentrated by rotary evaporation. After the addition of 0.1M sodium chloride solution, the extract was partitioned twice against hexane and twice against dichloromethane. The combined organic phases were evaporated to dryness and redissolved in ethyl acetate/hexane (10:90 v/v). The extract was cleaned-up by SPE using a carbon cartridge, followed by a Florisil column and an LC-Alumina-B SPE column. The ethyl acetate/hexane eluate was collected, evaporated to dryness and redissolved in hexane for final determination by GC-ECD.

Must was extracted by adding 0.1M sodium chloride solution and partitioning twice against dichloromethane. The combined organic phases were evaporated to dryness and redissolved in ethyl acetate/hexane (5:95 v/v). The extract was cleaned-up by SPE using a carbon cartridge, followed by an LC-Alumina-B SPE column. The ethyl acetate/hexane eluate was collected, evaporated to dryness and redissolved in hexane for final determination by GC-ECD.

Wine was extracted by adding 1% potassium hydrogen carbonate and partitioning against ethyl acetate. The organic phase was rotary evaporated to a reduced volume and made up to a known volume with ethyl acetate. Final determination was by GC-ECD.

The limit of quantification (LOQ) for was 0.01 mg/kg for zoxamide in grapes, must, pomace and wine, and 0.01 mg/kg for RH-150721 in wine. Grape samples were stored for up 5 months prior to analysis, and pomace, must and wine samples were stored for up to 4 months prior to analysis.

Recoveries for RH-7281 ranges: Grapes: 82.9-97.3%, Pomace 76.4-109.7%, Must 71.2-78.9%, Wine: 91.5-103.3
Overall mean: 87.4%
Recoveries RH-150721: Wine: 99.3-100.8%
Mean: 100.1%

RMS comments and conclusions

The analytical procedure has been successfully validated accordance with requirements of SANCO/3029/99 rev. 4.

Reference:	**CA 4.1.2/11 (CA 6.2.1/09)** Hein, W. (2014b) Extraction Efficiency of [phenyl-UL-14C] Zoxamide from Plant Metabolism Samples (Pea) Report no.: AS362
Guideline(s):	OECD Guidance document No 72 (ENV/JM/MONO(2007)17 SANCO/825/00 rev.8.1, 16/11/2101 OECD guideline No. 501; Metabolism in Crops (08/01/2007)
Deviations:	None
GLP:	Yes
Validity of the study:	Valid
Previous evaluations:	No; Submitted for the purpose of renewal of a.s. approval

Cross reference to Residues Section vol.3, B.7.2.1.3

Executive Summary

Samples from the pea metabolism study summarised (CA 6.2.1/09, report AS290) were used to radiovalidate the extraction method used in the QuEChERS method (See above

Reference: CA 4.1.2/01 (CA 6.1/01)

Samples of immature whole plant (5-fold dose, after surface washing) and dry peas (1-fold dose) containing incurred residues of zoxamide were extracted using the QuEChERS method extraction with acetonitrile and a salt solution. The organic extracts were profiled by radio-TLC and HPLC, and the profiles compared with those obtained in the metabolism study.

The purpose of the study was the comparison of the extraction yields of the extraction method used in the pea metabolism study (extraction with methanol followed by phase separation) with the extraction yields of the validated Quechers extraction method. Test Items were two specimens from the above mentioned plant metabolism study.

Both extraction methods (i.e. Quechers method and metabolism study AS290 solvent extraction method) are in good agreement as far as extraction of zoxamide is concerned.

The results with respect to zoxamide can be summarised as follows:

Recovery of [^{14}C]-zoxamide following extraction

Matrix	**Extraction method**	**Recovery of [^{14}C]-zoxamide[1]**	
		mg/kg fresh weight	**% of initial TRR**
DAT 7 Immature whole plant (5-fold dose)	QuEChERS extraction	0.442	36.5
	AS290 Metabolism study extraction	0.449	37.0
DAT 30 Dry peas (1-fold dose)	QuEChERS extraction	0.013	7.9
	AS290 Metabolism study extraction	0.019	11.8

[1] Recoveries based on TLC analysis

The recovery of zoxamide using the QuEChERS method was 98.4% for immature whole plant and 68.4% for dry peas, compared to the amount extracted using the metabolism study extraction method. The lower recovery obtained for the dry pea sample is attributed to the low absolute concentration of zoxamide in the sample.

RMS comments and conclusions

The results of this study indicate that both extraction methods are comparable.

f) Methods in soil, water, sediment, feed and any additional matrices used in support of ecotoxicology studies

Reference: **CA 4.1.2/05 (CA 8.2.4.2/01)**

Report: Nixon, W.B. and Sulaiman, M.W. (1997), RH-117,281 Technical: A 96-Hour-Flow Through Acute Toxicity Test with the Saltwater Mysid (*Mysidopsis bahia*), Wildlife International Ltd., Report No. 129A-136

Guidelines: Not stated

GLP: Yes

Principle of the method

Samples (100 mL) are transferred to separatory funnels and extracted with, ethyl ether/cyclohexane, 25/75, v/v (100 mL) by shaking for one minute. The phases are allowed to separate and the aqueous layer is run off to waste. The organic layer is filtered through a bed of anhydrous sodium sulphate and collected in an evaporating flask. The sodium sulphate bed is rinsed with ethyl ether/cyclohexane, 25/75, v/v (20 mL) and the combined extracts are evaporated to near dryness using a rotary evaporator and a bath temperature not exceeding 45ºC. The sample is reduced to dryness under a gentle stream of nitrogen and reconstituted in hexane (10 mL). An aliquot (5 mL) of the extract is loaded onto a pre-conditioned Superclean LC-CN SPE column and allowed to run to waste. The column is rinsed with hexane (5 mL) and the analyte is eluted with acetone/hexane, 2/98, v/v (25 mL). The extract is transferred to a round bottom flask and evaporated to near dryness using a rotary evaporator with the bath temperature not exceeding 42ºC. The residue is reconstituted in acetonitrile/water, 60/40, v/v (5 mL) and diluted, if necessary, with mobile phase. The samples are analysed by high performance liquid chromatography with ultra-violet detection (HPLC-UV) at 212 nm using a Inertsil C8 column (250 x 2.0 mm, 5µm particle size) and isocratic elution with a mobile phase of 45/55, v/v, Solvent A (50/50, v/v, acetonitrile/water) / Solvent B (90/10, v/v, acetonitrile/water). Quantification is performed using external standards.

Specificity

No interferences at >30% of the LOQ were present at the retention time of interest in the control matrix samples.

Linearity

Linearity of detector response was demonstrated using external standard solutions across the working range of 0.010 µg/mL to 0.30 µg/mL, with a correlation coefficient of > 0.999 (slope = 1764087, intercept = 2128.98).

Precision (Repeatability)

Repeatability data was generated from five different sample concentrations ranging from 0.016 mg/L to 0.12 mg/L. The relative standard deviations (RSD) obtained at each fortification level are within the guideline requirements and are presented in Table B.5.1.2-8 below.

Accuracy (Recovery)

Recovery data was generated from five different sample concentrations ranging from 0.016 mg/L to 0.12 mg/L. The mean percentage recoveries for each fortification level were within the guideline requirements and are presented in Table B.5.1.2-8 below.

Limit of quantification (LOQ)

The limit of quantification (LOQ), defined as the lowest fortification level where acceptable precision and accuracy data were obtained, was demonstrated to be 0.016 mg/L.

Conclusion

The analytical procedure has been successfully validated in terms of specificity, linearity, precision, accuracy and LOQ.

Table B.5.1.2-8: Precision and Accuracy Data

Matrix	Fortification Level (mg/L)	Recovery (%)	Mean Recovery (%)	Acceptable Recovery (%)	RSD (%)	Acceptable RSD (%)
Saltwater	0.016	95.6, 108	101.8	70 - 110	8.77	20
	0.026	106, 103	104.5	70 – 110	2.12	20
	0.043	100, 93.0	96.5	70 - 110	4.95	20

	0.072	106, 101	103.5	70 – 110	3.54	20
	0.12	105, 100	102.5	70 – 110	3.54	20
	Overall	-	102	70 - 110	4.72	20

RMS comments and conclusion:
The method is suitable for analysis of zoxamide in saltwater with LOQ = 0.016 mg/L. The validation data indicate acceptable accuracy (overall mean recovery between 70 and 110 %). However it be noted that 5 samples needed for each fortification levels.

Reference: **CA 4.1.2/06 (CA 8.2.5.2/01)**
Report: Kendall, T. Z. (1998), RH-117,281 Technical: A Flow-Through Life-Cycle Toxicity Test with the Saltwater Mysid (*Mysidopsis bahia*), Wildlife International Ltd., Report No. 129A-142
Guidelines: Not stated
GLP: Yes

Principle of the method
Saltwater samples are analysed by high performance liquid chromatography with ultra-violet detection (HPLC-UV) at 212 nm using a Phenomenex Inertsil ODS–2 column (250 x 4.6 mm, 5µm particle size) and gradient elution with mobile phases of water and acetonitrile. Quantification is performed using external standards.

Specificity
No interferences at >50% of the LOQ were present at the retention time of interest in the control matrix samples.

Linearity
Linearity of detector response was demonstrated using external standard solutions across the working range of 10.0 µg/L to 50.0 µg/L, with a coefficient of determination (R^2) of 0.9984 (slope = 1.898, intercept = 0.341).

Precision (Repeatability)
Repeatability data was generated from three fortifications concentrations of 20.0, 25.0 and 30.0 µg/L. The results are presented in Table B.5.1.2-9 below.

Accuracy (Recovery)
Recovery data was generated from three fortification concentrations of 20.0, 25.0 and 30.0 µg/L. The percentage recovery at each fortification level was within the guideline requirement and the results are presented in Table B.5.1.2-9 below.

Limit of quantification (LOQ)
The limit of quantification (LOQ), defined as the lowest fortification level where acceptable precision and accuracy data were obtained, was demonstrated to be 20.0 µg/L.

Conclusion
The analytical procedure has been successfully validated in terms of specificity, linearity, precision, accuracy and LOQ

Table B.5.1.2-9: Precision and Accuracy Data

Matrix	Fortification Level (µg/L)	Recoveries (%)	Acceptable Recovery (%)	RSD (%)	Acceptable RSD (%)
Saltwater	20.0	99.0	70 - 110	1.26	20
	25.0	101	70 – 110		
	30.0	98.7	70 - 110		

RMS comments and conclusion:
The method is suitable for analysis of zoxamide in saltwater with LOQ = 20 µg /L. The validation data indicate acceptable accuracy (overall mean recovery between 70 and 110 %). However it be noted that 5 samples recommended for each fortification levels.

Reference: **CA 4.1.2/07 (CP 10.2.1.1)**
Report: Aversa, S., (2010), Validation of an Analytical Method for the Determination of Zoxamide in Solutions of Aquatic Toxicity with GOW 008, Biotecnologie BT Srl, Italy, Report No. BT102/10
Guidelines: SANCO/825/00 rev. 7 and SANCO/3029/99 rev. 4
GLP: Yes

Principle of the method
Samples (25.5 mg) are weighed into graduated flasks (250 mL) and diluted to volume with test medium (daphnia/fish medium prepared according to OECD 202 & 203). An aliquot (0.8 mL) of this stock solution is transferred to a graduated flask (25 mL) and diluted to volume with test media. The samples are analysed by high performance liquid chromatography with ultra-violet detection (HPLC-UV) at 240 nm, using a Zorbax Eclipse Plus C18 column (100 x 2.1 mm, 1.8 µm particle size) and isocratic elution with a mobile phase of acetonitrile/water with 0.1 % formic acid, 70/30, v/v. Quantification is performed using external standard solutions.

Specificity
No interferences were observed at the retention time of interest in any sample solutions, demonstrating the specificity of the method. Analyte identity was confirmed by comparison of the UV spectra of a sample and a reference standard.

Linearity
The linearity of detector response was demonstrated using three external standard solutions (in duplicate) across the working range of 0.12 – 1.21 mg/L, with a coefficient of determination (R^2) of 0.9989 (slope = 38.379, intercept = - 0.2512).

Precision (Repeatability)
Repeatability data was generated form five samples of test media fortified at the LOQ and five samples fortified at 10 x LOQ. The relative standard deviations (RSD) obtained at each fortification level were within the guideline requirements and the results are presented in Table B.5.1.2-10 below.

Accuracy (Recovery)
Recovery data was generated from five samples of test media fortified at the LOQ and five samples fortified at 10 x LOQ. The mean recovery values obtained at each fortification level were within the guideline requirements of 70 – 120 % and the results are presented in Table B.5.1.2-10 below.

Limit of quantification (LOQ)

The limit of quantification (LOQ), defined as the lowest fortification level where acceptable accuracy and precision data were obtained, was demonstrated to be 0.12 mg/L.

Conclusion

The analytical procedure has been successfully validated in terms of specificity, linearity, precision, accuracy and LOQ in accordance with guideline requirements of SANCO/825/00 rev. 7 and SANCO/3029/99 rev.4.

Table B.5.1.2-10: Precision and Accuracy Data

Matrix	Fortification Level (mg/L)	Recoveries (%)	Mean Recovery (%)	Acceptable Recovery (%)	RSD (%)	Acceptable RSD (%)
Daphnia/fish medium	0.12	95.7, 95.9, 96.3, 96.3, 95.0	95.9	70 - 110	0.56	20
	1.21	92.7, 95.3, 94.6, 94.2, 94.7	94.3	70 - 110	1.02	20

RMS comments and conclusion:

The analytical method is suitable for determination of zoxamide. The limit of quantification is 0.12 mg/L in Daphnia/fish medium. Obtained results comply with guidelines requirements of SANCO/825/00 rev. 8.1 and SANCO/3029/99 rev.4.

g) Methods in water, buffer solutions, organic solvents and any additional matrices resulting from the physical and chemical properties tests

No new physical and chemical properties studies for which analytical methods are required are being submitted in support of the Annex I renewal of zoxamide.

Table B.5.1.2-1: Summary of method description and validation (treated plants, plant products, foodstuffs, feeding stuffs, environmental samples)

Substrate	Analyte	Dissolution/ extraction	Partition, clean-up	Quantification	Limit of quantif-ication (mg/kg)	Recovery fortifica-tion level (mg/kg)	Recoveries % range (mean)	Precision Repeatability RSD (%) (n)	Linearity demon-strated	Ref. DP No.
Potato	RH-7281	ACN/ $KHCO_3$	Partition into ethylacetate. Clean up with Florisil & Alumina B columns	GC (Rtx-5 (0.25 mm ID x 30 m; 0.25 µm film))/ECD	0.02	0.02 0.05 0.1 0.15	64.6 – 106 (89) 77 – 108 (96) 58.8 – 120 86 73 – 120 95	15.7 (26) 10.4 (14) 22.1 (14) 15.8 (21)	Yes	Meyer, 1998 (DP 81812)
Independent laboratory validation of above method						0.02 0.05	84, 79 (81.5) 79, 111 (95)			Bruns 1998 (DP 81816)
Potato	RH-141455	ACN/ $KHCO_3$	Partition into $KHCO_3$. Clean up with Florisil column	GC (Rtx 225(0.32 ID x 30 m; 0.25 µm film))/ECD	0.02	0.02 0.05 0.1 0.15	42 – 82 (64) 48 – 103 (73) 49 – 101 (71) 43 – 91.9 (72)	21.9 (19) 19.7 (9) 21.4 (9) 20.8 (17)	Yes	Meyer, 1998 (DP 81812)
Potato	RH 141452	Independent laboratory validation of above method				0.02 0.05	109, 111 (110) 83, 80 (81.5)			Bruns 1998 (DP 81816)

Substrate	Analyte	Dissolution/ extraction	Partition, clean-up	Quantification	Limit of quantif-ication (mg/kg)	Recovery fortifica-tion level (mg/kg)	Recoveries % range (mean)	Precision Repeatability RSD (%) (n)	Linearity demon-strated	Ref. DP No.
Potato	RH-141452	As above	As above	As above	0.02	0.02 0.05 0.1 0.15	53 – 103 (81) 54 – 91.6 (74) 50 – 89 (72) 50 – 108 (76)	17.3 (19) 20.3 (9) 19.4 (9) 18.4 (16)	Yes	Meyer, 1998 (DP 81812)
Potato	RH 141455	Independent laboratory validation of above method				0.02 0.05	108, 109 (108.5) 91, 86 (88.5)			Bruns 1998 (DP 81816)
Grape	RH-7281	Methanol/ water	Partition between dichloromethane and aqueous sodium chloride. Clean up with carbon and Alumina B columns	GC (Rtx-1 column)/ ECD	0.01 mg/kg	0.01 0.05 0.1 0.15 3.0	74.9 – 111 (106) 80 – 123 (96.1) 83 – 109 (97.9) 80.2 – 109 (98) 95.8 (95.8)	13.8 (15) 15.71 (8) 11.95 (7) 12.24 (7) (1)	Yes	Burdge 1998 (DP 81828)
Grape	RH-7281	Independent laboratory validation of above method				0.01 5.0	119, 91 (105) 77, 90 (84)	18.9 (2) 11 (2)		Szuter 1998b (DP 81832)

Substrate	Analyte	Dissolution/ extraction	Partition, clean-up	Quantification	Limit of quantif-ication (mg/kg)	Recovery fortifica-tion level (mg/kg)	Recoveries % range (mean)	Precision Repeatability RSD (%) (n)	Linearity demon-strated	Ref. DP No.
Grape juice	RH-7281	Ethyl acetate	Clean up on Alumina B column	GC (Rtx-1 column)/ ECD	0.01 mg/kg	0.01 0.02 0.05 0.1 0.15	80.5 - 125 (101) 69.9 – 101 (86.2) 69.7 – 97.5 (85.2) 78.5 – 91.5 (84.2) 71.4 – 98.1 (85.8)	13.2 (11)	Yes	Burdge 1998 DP 81828
Raisin	RH-7281	Grind sample with Bondesil ODS silica packing.	Elute with hexane/ ethylacetate. Clean up on Florosil column	GC (Rtx-1 column)/ ECD	0.01 mg/kg	0.01 0.05 0.10 0.50 1.0	87.2 – 143 (108) 75.3 – 92.5 (85.9) 76.3 – 103 (86.5) 62.5 – 96.3 (76.7) 74.0 – 106 (86.1)	19.1 (6)		Burdge 1998 DP 81828
Wine	RH-7281	Partition between ethyl acetate and $KHCO_3$ soln.		GC (Rtx-5 column)/ ECD.	0.01 mg/kg	0.01 0.02 0.05 0.10 0.15	90 - 125 (107) 81.1 115 (99.3) 90.9 – 114 (101) 97.9 – 131 (113) 70.3 - 105 (93.8)	12.8 (31)	Yes	Brudge 1999 (DP 81841)

Substrate	Analyte	Dissolution/ extraction	Partition, clean-up	Quantification	Limit of quantif-ication (mg/kg)	Recovery fortifica-tion level (mg/kg)	Recoveries % range (mean)	Precision Repeatability RSD (%) (n)	Linearity demon-strated	Ref. DP No.
Wine	RH-150721	Partition between ethyl acetate and $KHCO_3$ soln.		GC (Rtx-5 column)/ ECD.	0.01 mg/kg	0.01 0.02 0.05 0.10 0.15	62.6 – 99.5 (89) 85.1 – 101 (93.8) 62.4 – 102 (91.2) 84.3 – 115 (100) 89 - 111 (96.6)	12.5 (31)	Yes	Brudge 1999 (DP 81841)
Soil	RH-7281	Acetonitrile	Partition with water/ dichloromethane. Further clean-up through Florisil/anhydrous sodium sulphate and Alumina-B SPE, eluting with ethyl acetate/ hexane.	Capillary GC (RTX-1 column) with ECD detection. Capillary GC (RTX-5 column) with MS detection (SIM, m/z 187, 189, 257)	0.01 0.01	0.01 0.01-0.1 0.01 0.03-0.23	86-105 (93) 76-122 (98) 100-145 (127) 78-122 (99)	8.3 (4) 16.2 (10) 17.4 (4) 15.2 (12)	Yes Yes	4.2.2/01 Guo, 1996b (DP 81847)
Soil	RH-7281	As above	As above	Capillary GC-ECD as above. Capillary GC-MS as above, (except MS ions 187, 189, 258)	0.01 0.01	0.01 0.02-0.5 0.01 0.02-0.5	63-111 (92) 63-100 (87) 55-127 (99) 63-134 (89)	12.5 (33) 13.0 (43) 18.4 (26) 16.3 (45)	Yes Yes	4.2.2/02 Guo, 1998 (DP 81851)
Soil (ILV of the above method)	RH-7281	As above	As above	Capillary GC-ECD as above.	0.01	0.01 0.1	88-108 (97) 94-99 (97)	8.2 (5) 2.8 (5)	-	4.2.2/03 Szuter, 1998a (DP 81853)

Substrate	Analyte	Dissolution/ extraction	Partition, clean-up	Quantification	Limit of quantif-ication (mg/kg)	Recovery fortifica-tion level (mg/kg)	Recoveries % range (mean)	Precision Repeatability RSD (%) (n)	Linearity demon-strated	Ref. DP No.
Drinking water	RH-7281	Ethyl acetate	-	Capillary GC (DB-1 column) with ECD detection. Capillary GC (RTX-1 column) with MS detection (SIM, m/z 187, 258), used for confirmation only.	0.05 µg/l	0.05 µg/l 0.1 µg/l 0.5 µg/l 1.0 µg/l overall 91.4 0.05 µg/l 0.1 µg/l 0.5 µg/l 1.0 µg/l	96.5-98 (97) 67-97.6 (80) 88-93 (91) 87-107 (97) 91-98.5 (95) 98-106 (105) 94-107 (103) 90-91.2 (91) overall 98.5	0.6 (3) 24.3 (3) 3.1 (3) 10.7 (3) overall 12.8 4.7 (3) 5.4 (3) 6.7 (3) 0.4 (3) overall 7.5	Yes	4.2.3/01 Volkel, 1998 (DP 81855)
Drinking water Surface water	RH-7281	As above	-	GC-ECD as above. GC-MS as above except only m/z 258.	0.05 µg/l 0.05 µg/l	0.05 µg/l 0.5 µg/l 0.05 µg/l 0.5 µg/l	84.2-104 (95) 76-101 (94.4) overall 94.5 86-99 (90.4) 95-111.6 (105) overall 97.7	7.7 (5) 11.1 (5) overall 9.0 6.1 (5) 7.6 (5) overall 10.6	Yes	4.2.3/02 Wais 2000 DP 97884
Air 20°C, rH 40% 35°C, rH 80%	RH-7281	Flow of air through TENAX 1litre/min, for 6 hours. Extraction of TENAX by ultrasonicating with acetonitrile.	Filtering, evaporation and dissolution in hexane.	Capillary GC (DB-1 column) with ECD detection. Confirmation by GC-MS as above (SIM m/z 187)	0.003 mg/m^3	0.0027 0.027 mg/m^3 0.0027 0.027 mg/m^3	81-97 (88) 80-108 (96.2) overall 92.1 71.5-89 (80.5) 77.6-109 (91) overall 85.0	7.8 (8) 9.9 (8) overall 9.8 9.0 (8) 10.4 (8) overall 11.3 Overall 9.9 (32)		4.2.4 Wais, 1999b DP 81858

B.5.2. Methods for Post-Approval Control and Monitoring Purposes

a) Methods for the determination of all components included in the monitoring residue definition as submitted in accordance with the provision of point 6.7.1 in order to enable Member States to determine compliance with established maximum residue levels (MRLs); they shall cover residues in or on food and feed of plant and animal origin

The residue definition for Zoxamide in commodities of plant origin has been agreed as parent Zoxamide for both risk assessment and monitoring. Based on proposed uses, no residues definition should be set for residues in foodstuff of animal origin. According to Reg. (EU) No 520/2011, MRLs have not been stated for the animal commodities. So, analytical methods are not required.

MRLs are set at European Level and the review is performed by EFSA. The EU MRLs of Zoxamide, for the crops are fixed by Reg. (EU) No. 520/2011. EU MRLs are currently set for the intended uses 0.02* mg/kg for potatoes and 5 mg/kg for grapes (*indicates lower limit of analytical determination).

Residues in plants

Reference:	**CA 4.2/01**
Report:	Weber, H., (2012), Validation of an Enforcement Method ("QuEChERS") for the Determination of Residues of Zoxamide in Grapes and Potatoes and their Process Products using LC-MS/MS, Eurofins Agroscience Services, Germany, Report No. S12-03949
Guidelines:	SANCO/825/00 rev. 8.1, Regulation (EC) No 1107/2009
GLP:	Yes

Principle of the method

Homogenised samples of raisins, potato chips and flakes (5g) and grape berries and juice, wine and potato tubers (10g) are weighed into Sarstedt centrifuge tubes (50 mL). For raisins, 8.5 mL of water is added and for potato (chips and flakes), 9 mL of water is added. The samples are extracted with acetonitrile (10 mL) with vigorous shaking for 1 minute. A salt mixture (4g magnesium sulfate, 1g sodium chloride, 1g trisodium citrate dihydrate and 0.5g of disodium hydrogen citrate sesquihydrate) is added and the samples are shaken again for 1 minute. After shaking, the samples are centrifuged for 2 minutes at 4000 rpm. An aliquot (1.5 mL) of the supernatant is cleaned up by adding a mixture of PSA (40 mg) and magnesium sulphate (225 mg) and vortex mixing for 30 seconds before centrifuging for 2 minutes at 6000 rpm. An aliquot (0.2 mL for raisins, potato chips and flakes and 0.1 mL for grape berries and juice, wine and potato tubers) is diluted to 1 mL with methanol:0.05% acetic acid (1:1, v/v) and analysed by high performance liquid chromatography with tandem mass specific detection (LC-MS/MS) in positive ion mode, using a Supelco Ascentis Express C18 column (50 x 2.1 mm, 2.7µm) and gradient elution with mobile phases of methanol + 0.05% acetic acid and water + 0.05% acetic acid. Quantification is performed using external standards. The ion transition m/z 336 > 187 is used for quantification and the ion transition for 336 > 159 is used for confirmation.

Specificity

LC-MS/MS monitoring two mass transitions is considered to be a highly specific technique.

No interferences at >30% of the LOQ were present at the retention time of interest in control matrix samples.

Linearity

Linearity of detector response was demonstrated using at least eight external standard solutions of zoxamide covering a concentration range of 0.3 to 100 ng/mL. Solvent based standards were used for the quantification of grapes (berries, juice), wine and potato (tuber, flakes), whereas matrix-matched standards were used for the quantification of raisins and potato chips, due to observed matrix effects. The results are presented in Table B.5.2-1 below.

Precision (Repeatability)

Repeatability data was generated from five samples of each matrix fortified at the LOQ and five samples fortified at 10 x LOQ. The relative standard deviations (RSD) obtained for each fortification level were within the guideline requirements and are presented in Table B.5.2-2 below.

Accuracy (Recovery)

Recovery data was generated from five samples fortified at the LOQ and five samples fortified at 10 x LOQ for each matrix. The mean percentage recoveries at each fortification level were within the guideline requirements and are presented in Table B.5.2-2 below.

Limit of Quantification (LOQ)

The limit of quantitation (LOQ), defined as the lowest fortification level where acceptable precision and accuracy data were obtained, was determined to be 0.01 mg/kg in all matrices.

Conclusion

The analytical procedure has been successfully validated in terms of specificity, linearity, precision, accuracy and LOQ, in accordance with all of the requirements of SANCO/825/00 rev. 8.1.

Table B.5.2-1: Linearity Data

Matrix	Ion Transition (m/z)	Concentration Range (ng/mL)	Correlation Coefficient (r)	Slope	Intercept
Grapes (Berries)	336 > 187	0.300 - 100	0.9986	41910	32399
	336 > 159	0.300 - 100	0.9991	9150.9	5469.8
Grape (Juice)	336 > 187	0.300 - 100	0.9992	23679	10655
	336 > 159	0.300 - 100	1.0000	11613	1386.5
Grape (Wine)	336 > 187	0.300 - 100	0.9992	23679	10655
	336 > 159	0.300 - 100	1.0000	11613	1386.5
Raisins	336 > 187	0.300 - 100	0.9999	20389	5812.1
	336 > 159	0.300 - 100	1.0000	9981.1	1009.7
Potato (Tuber)	336 > 187	0.300 - 100	0.9998	24052	8200.8
	336 > 159	0.300 - 100	0.9997	11447	4072
Potato (Chips)	336 > 187	0.300 - 100	0.9998	19798	2756.3
	336 > 159	0.300 - 100	0.9996	9486	- 477.88
Potato (Flakes)	336 > 187	0.300 - 100	0.9985	41197	35887
	336 > 159	0.300 - 100	0.9991	9025.1	5659.2

Table B.5.2-2: Precision and Accuracy Data

Matrix	Fortification Level (mg/kg)	Recoveries (%)	Mean Recovery (%)	RSD (%)	Acceptable RSD (%)	Acceptable Recovery (%)
Grape (Berries)	**Zoxamide Ion transition m/z 336 > 187 (quantification)**					
	0.01	96, 96, 93, 93, 98	95	2.3	20	60 – 120
	0.1	99, 98, 99, 99, 99	99	0.5	20	70 – 110
	Zoxamide Ion transition m/z 336 > 159 (confirmation)					
	0.01	94, 94, 94, 89, 94	93	2.4	20	60 – 120
	0.1	99, 97, 97, 96, 96	97	1.3	20	70 – 110
Grape (Juice)	**Zoxamide Ion transition m/z 336 > 187 (quantification)**					
	0.01	100, 102, 103, 96, 94	99	3.9	20	60 – 120
	0.1	99, 96, 94, 97, 96	96	1.9	20	70 – 110
	Zoxamide Ion transition m/z 336 > 159 (confirmation)					
	0.01	96, 102, 99, 96, 95	98	3.0	20	60 – 120
	0.1	101, 96, 97, 97, 99	98	2.0	20	70 – 110
Wine	**Zoxamide Ion transition m/z 336 > 187 (quantification)**					
	0.01	86, 82, 85, 85, 83	84	2.0	20	60 – 120
	0.1	81, 85, 84, 83, 81	83	2.2	20	70 – 110
	Zoxamide Ion transition m/z 336 > 159 (confirmation)					
	0.01	86, 86, 84, 85, 90	86	2.6	20	60 – 120
	0.1	85, 83, 85, 83, 83	84	1.3	20	70 – 110
Raisins	**Zoxamide Ion transition m/z 336 > 187 (quantification)**					
	0.01	109, 103, 109, 101, 107	106	3.4	20	60 – 120
	0.1	110, 114, 117, 109, 113	113	2.9	20	70 – 110
	Zoxamide Ion transition m/z 336 > 159 (confirmation)					
	0.01	105, 103, 110, 103, 110	106	3.4	20	60 – 120
	0.1	111, 114, 117, 108, 111	112	3.0	20	70 – 110
Potato (Tuber)	**Zoxamide Ion transition m/z 336 > 187 (quantification)**					
	0.01	90, 94, 92, 90, 94	92	2.2	20	60 – 120
	0.1	94, 94, 91, 90, 92	92	1.9	20	70 – 110
	Zoxamide Ion transition m/z 336 > 159 (confirmation)					
	0.01	96, 90, 92, 96, 94	94	2.8	20	60 – 120
	0.1	97, 94, 95, 90, 92	94	2.9	20	70 – 110

Table B.5.2-2: Precision and Accuracy Data continued

Matrix	Fortification Level (mg/kg)	Recoveries (%)	Mean Recovery (%)	RSD (%)	Acceptable RSD (%)	Acceptable Recovery (%)
Potato (Chips)	**Zoxamide Ion transition m/z 336 > 187 (quantification)**					
	0.01	101, 105, 101, 100, 98	101	2.5	20	60 – 120
	0.1	102, 103, 101, 108, 104	104	2.6	20	70 – 110
	Zoxamide Ion transition m/z 336 > 159 (confirmation)					
	0.01	102, 104, 99, 98, 96	100	3.2	20	60 – 120
	0.1	101, 103, 102, 105, 104	103	1.5	20	70 – 110
Potato (Flakes)	**Zoxamide Ion transition m/z 336 > 187 (quantification)**					
	0.01	94, 89, 92, 97, 97	94	3.6	20	60 – 120
	0.1	97, 101, 97, 99, 96	98	2.0	20	70 – 110
	Zoxamide Ion transition m/z 336 > 159 (confirmation)					
	0.01	91, 82, 87, 97, 92	90	6.3	20	60 – 120
	0.1	98, 100, 94, 100, 95	97	2.9	20	70 – 110

RMS comments and conclusion:

The analytical method is suitable for determination of zoxamide in grape and potato and their processing products. Obtained results comply with guideline requirements of SANCO/825/00 rev. 8.1.

Reference: **CA 4.2/02**

Report: Richter, S., (2014a), Validation of the QuEChERs Multi-Residue Method for the Determination of Zoxamide in Various Crop Types, PTRL Europe, Report No. P3114G

Guidelines: SANCO/825/00 rev. 8.1

GLP: Yes

Principle of the method

Matrix samples (10 g for lettuce, grape vine and potato tuber, 5 g for dry bean and oilseed rape seed) are weighed into 50 mL screw capped centrifuge tubes. Water (9 mL for dry bean and oilseed rape seed, 2 mL for potato tuber, no addition of water for lettuce and grape vine) and acetonitrile (10 mL) are added and the samples are shaken vigorously for one minute manually and using a vortex mixer. $MgSO_4$ (4 g), NaCl (1 g), trisodium citrate dihydrate (1 g) and disodium hydrogencitrate sesquihydrate (0.5 g) are added. The samples are shaken vigorously for one minute manually and using a vortex mixer and centrifuged at 4000 rpm for 5 minutes. The oilseed rape seed sample is transferred to a freezer for at least 2 hours and centrifuged at 4000rpm for 1 minute. An aliquot of the raw extract (6 mL) is transferred to a Dispersive SPE Clean Up Tube 1 (Supelco 55228-U). C_{18} sorbent (0.15 g) is added to the Dispersive SPE tube (oilseed rape seed only), the tube is shaken for 30 seconds and centrifuged for 5 minutes at 4000 rpm. An aliquot (1.0 mL) of each extract is transferred to autosampler vials and acidified with acetonitrile containing 5 % formic acid (10 µL). The samples are diluted appropriately and analysed using high performance liquid chromatography with tandem mass specific detection (HPLC-MS/MS) in positive ionspray mode, using a Phenomenex Aqua C_{18} column (50 x 2.0 mm, 5 µm particle size) and gradient elution with mobile phases of water containing 0.1 % formic acid and methanol containing 0.1 % formic acid. Quantification is performed using external standards. The mass transition m/z 336 > 187 is used for quantification and the mass transition m/z 338 > 189 is used for confirmation.

Specificity

LC-MS/MS monitoring two mass transitions is considered to be a highly specific technique. No interferences at >30% of the LOQ were present at the retention time of interest in control matrix samples.

Linearity

Linearity of detector response was demonstrated using seven external standard solutions across the working range of 0.10 ng/mL to 10 ng/mL. No significant matrix effect was observed for lettuce, grape vine and potato tuber. However a significant matrix effect was observed for dry bean and oilseed rape seed therefore matrix matched standards were prepared for these matrices. The results are presented in Table B.5.2-3 below.

Precision (Repeatability)

Repeatability data was generated from five samples of each matrix fortified at the LOQ and five samples of each matrix fortified at 10 x LOQ. The relative standard deviations (RSD) obtained for each matrix and fortification level were within the guideline requirements and are presented in Table B.5.2-4 below.

Accuracy (Recovery)

Recovery data was generated from five samples of each matrix fortified at the LOQ and five samples of each matrix fortified at 10 x LOQ. The mean percentage recovery obtained for each matrix and fortification level were within the guideline requirements and are presented in Table B.5.2-4 below.

Limit of quantification (LOQ)

The limit of quantification, defined as the lowest fortification level where acceptable precision and accuracy data were obtained, has been demonstrated to be 0.01 mg/kg in all matrices.

Conclusion

The analytical procedure has been successfully validated in terms of specificity, linearity, precision, accuracy and LOQ, in accordance with the requirements of SANCO/825/00 rev. 8.1.

Table B.5.2-3: Linearity Data

Matrix	Ion Transition (m/z)	Concentration Range (ng/mL)	Correlation Coefficient (r)	Slope	Intercept
Solvent	336 > 187	0.10 – 10	0.9997	38400	263
	338 > 189	0.10 – 10	0.9998	26300	322
Dry Bean	336 > 187	0.10 – 10	0.9996	36200	622
	338 > 189	0.10 – 10	0.9995	24400	949
Oilseed Rape Seed	336 > 187	0.10 – 10	0.9993	22600	697
	338 > 189	0.10 – 10	0.9994	15400	932

Table B.5.2-4: Precision and Accuracy Data

Matrix	Fortification Level (mg/kg)	Recoveries (%)	Mean Recovery (%)	Acceptable Recovery (%)	RSD (%)	Acceptable RSD (%)
Lettuce	**Ion Transition 336 > 187 (Quantification)**					
	0.01	104, 102, 98, 101, 98	101	60 – 120	3	30
	0.10	100, 98, 102, 100, 98	100	70 – 120	1	20
	Ion Transition 338 > 189 (Confirmation)					
	0.01	102, 100, 98, 97, 100	99	60 – 120	2	30
	0.10	98, 95, 102, 99, 98	98	70 – 120	2	20
Grape Vine	**Ion Transition 336 > 187 (Quantification)**					
	0.01	105, 109, 110, 107,	108	60 – 120	2	30
	0.10	107, 103, 97, 102,	102	70 – 120	4	20
	Ion Transition 338 > 189 (Confirmation)					
	0.01	102, 107, 108, 109,	107	60 – 120	3	30
	0.10	107, 102, 97, 102,	102	70 – 120	4	20
Potato Tuber	**Ion Transition 336 > 187 (Quantification)**					
	0.01	93, 100, 98, 95, 99	97	60 – 120	3	30
	0.10	88, 93, 90, 88, 101	92	70 – 120	6	20
	Ion Transition 338 > 189 (Confirmation)					
	0.01	89, 95, 99, 98, 98	96	60 – 120	4	30
	0.10	88, 95, 86, 87, 103	92	70 – 120	8	20
Dry Bean	**Ion Transition 336 > 187 (Quantification)**					
	0.01	97, 94, 89, 91, 86	91	60 – 120	5	30
	0.10	99, 85, 85, 85, 103	91	70 – 120	10	20
	Ion Transition 338 > 189 (Confirmation)					
	0.01	101, 95, 92, 91, 88	93	60 – 120	5	30
	0.10	102, 85, 89, 87, 104	93	70 – 120	10	20
Oilseed Rape Seed	**Ion Transition 336 > 187 (Quantification)**					
	0.01	106, 96, 98, 98, 94	98	60 – 120	5	30
	0.10	91, 83, 84, 80, 81	84	70 – 120	5	20
	Ion Transition 338 > 189 (Confirmation)					
	0.01	108, 99, 95, 97, 96	99	60 – 120	5	30
	0.10	93, 83, 85, 80, 82	85	70 – 120	6	20

RMS comments and conclusion:
Multi-residue method for the determination of zoxamide in various crop types has been validated.
Obtained results comply with current guidelines requirements of SANCO/825/00 rev. 8.1 and SANCO/3029/99 rev.4.

Reference: **CA 4.2/03**
Report: Schlewitz, P., (2014), Independent Lab Validation of the Analytical Method for the Determination of Zoxamide Residues in Lettuce, Anadiag, France, Report No. R B4023
Guidelines: Regulation (EC) No. 1107/2009, SANCO/3029/99 rev.4 and SANCO/825/00 rev. 8.1
GLP: Yes

Principle of the method
Samples (10 g) are weighed into centrifuge tubes (50 mL). Acetonitrile (10 mL) is added and the samples are shaken manually for 1 minute. Magnesium sulphate (4 g), sodium chloride (1 g), sodium citrate tribasic dehydrate (1 g) and sodium citrate dibasic sesquihydrate (0.5 g) are added, the samples are shaken manually for one minute and centrifuged for 5 minutes at 4000 rpm. An aliquot of the supernatant (6 mL) is transferred to a centrifuge funnel. Magnesium sulphate (900 mg) and PSA (150 mg) are added, the samples are shaken manually for 30 seconds and centrifuged for 5 minutes at 4000 rpm. An aliquot of supernatant (1 mL) is transferred to a vial containing acetonitrile/5.0 % formic acid (10 µL) and shaken manually. An aliquot (50 µL) of this solution is transferred to a vial containing acetonitrile/ water (2/8, v/v) + 0.1 % formic acid (950 µL). The sample is homogenised and analysed by high performance liquid chromatography with tandem mass specific detection (HPLC-MS/MS) in negative ionisation mode, using a Supelco Ascentis Express C18 column (50 x 2.1 mm, 1.7 µm particle size) and gradient elution with mobile phases of water containing 0.1 % formic acid and methanol containing 0.1 % formic acid. Quantification is performed using matrix matched external standards. The mass transition m/z 336 > 187 is used for quantification and the mass transition m/z 338 > 189 is used for confirmation.

Specificity
LC-MS/MS monitoring two mass transitions is considered to be a highly specific technique. No interferences at >30% of the LOQ were present at the retention time of interest in control matrix samples.

Linearity
Linearity of detector response was demonstrated using seven matrix matched external standard solutions across the working range of 0.10 ng/mL to 10.01 ng/mL. The results are presented in Table B.5.2-4.

Precision (Repeatability)
Repeatability data was generated from five samples fortified at the LOQ and five samples fortified at 10 x LOQ. The relative standard deviation (RSD) obtained at each fortification level was within the guideline requirements and are presented in Table B.5.2-5.

Accuracy (Recovery)
Recovery data was generated from five samples fortified at the LOQ and five samples fortified at 10 x LOQ. The mean percentage recovery obtained at each fortification level was within the guideline requirements and are presented in Table B.5.2-5.

Limit of quantification (LOQ)
The limit of quantification, defined as the lowest fortification level where acceptable precision and accuracy data were obtained, has been demonstrated to be 0.01 mg/kg in lettuce.

Conclusion
The analytical procedure has been successfully validated in terms of specificity, linearity, precision, accuracy and LOQ, in accordance with the requirements of SANCO/825/00 rev. 8.1.

Table 5.2-4: Linearity Data

Matrix	Ion Transition (m/z)	Concentration Range (ng/mL)	Correlation Coefficient (r)	Slope	Intercept
Lettuce	336 > 187	0.10 – 10.01	0.99880	7.5518×10^{-4}	-0.05
	338 > 189	0.10 – 10.01	0.99864	1.1907×10^{-3}	-0.06

Table 5.2-5: Precision and Accuracy Data

Matrix	Fortification Level (mg/kg)	Recoveries (%)	Mean Recovery (%)	Acceptable Recovery (%)	RSD (%)	Acceptable RSD (%)
Lettuce	**Ion Transition 336 > 187 (Quantification)**					
	0.01	81.0, 102.4, 97.6, 89.0, 108.4	95.7	60 – 120	11.3	30
	0.10	112.5, 87.8, 103.2, 102.1, 99.5	101.0	70 – 120	8.8	20
	Ion Transition 338 > 189 (Confirmation)					
	0.01	90.8, 104.1, 108.3, 88.3, 117.6	101.8	60 – 120	12.0	30

Extraction Efficiency (CA 6.2.1/09)

The efficiency of the extraction procedure (QuEChERS) used in the monitoring methods detailed above was demonstrated using radio-labelled samples from the pea metabolism study (CA 6.2.1/08).

Samples of immature whole plant and dry peas containing incurred residues of zoxamide were extracted with acetonitrile and a salt solution in accordance with the QuEChERS method. The organic extracts were profiled by radio-TLC and HPLC, and the profiles compared with those obtained in the metabolism study.

The amount of available zoxamide in the samples, determined in the pea metabolism study, was 0.449 mg/kg for immature whole plant and 0.019 mg/kg for dry peas.
The amount of zoxamide extracted using the QuEChERS extraction method was 0.0442 mg/kg for immature whole plant and 0.019 mg/kg for dry peas, giving recoveries of 98.4% and 68.4% for immature whole plant and dry peas respectively.

RMS comments and conclusion:

The method for determination of zoxamide residues in matrix with high water content (lettuce) has been validated and provided in ILV. The accuracy for the method (repeatability and precision) are within acceptable limits of 60-120% for mean recovery and <30% RSD for each fortification level at the limit of quantitation (0.01 mg/kg) and 70-120% for a mean recovery and <20% RSD at 0.1 mg/kg.

b) Methods for the determination of all components included for monitoring purposes in the residue definitions for soil and water as submitted in accordance with the provisions of point 7.4.2

Residues in soil

Reference: **CA 4.2/04**
Report: Jooß, S., (2013a), Development and Validation of a Residue Method for the Determination of Zoxamide in Soil, PTRL Europe, Report No. P3051G
Guidelines: SANCO/825/00 rev. 8.1 and SANCO/3029/99 rev. 4
GLP: Yes

Principle of the method

Samples (10g) are added to a 250ml extraction bottle. Water (2.0mL) and acetonitrile (100mL) are added and the samples are shaken for 45 minutes. Sodium chloride (2.0g) is added, the samples are shaken for 10 minutes and centrifuged at 4000rpm for 3 minutes. An aliquot of supernatant (200µL) is transferred to an autosampler vial and diluted with water (790µL) and 10% formic acid (10µL). The samples are analysed by high performance liquid chromatography with tandem mass specific detection (LC-MS/MS) in positive polarity mode, using a Phenomenex Aqua C_{18} column (50 x 2 mm, 5µm) and gradient elution with mobile phases of water containing 0.1% formic acid and acetonitrile containing 0.1% formic acid. Quantification is performed using external standards. The ion transition m/z 336 > 187 is used for quantification and the ion transition m/z 338 > 189 is used for confirmation.

Specificity

LC-MS/MS monitoring two mass transitions is considered to be a highly specific technique.

No interferences at >30% of the LOQ were present at the retention time of interest in control matrix samples.

Linearity

Linearity of detector response was demonstrated for zoxamide using six matrix matched external standards across the concentration range of 0.018 ng/mL to 5.0 ng/mL.The results are presented in Table B.5.2-6 below.

Precision (Repeatability)

Repeatability data was generated from five samples of soil fortified at the LOQ and five samples fortified at 10 x LOQ. The relative standard deviations (RSD) obtained for each fortification level were within the guideline requirements of less than 20% and are presented in Table B.5.2-7 below.

Accuracy (Recovery)

Recovery data was generated from five samples of soil fortified at the LOQ and five samples fortified at 10 x LOQ. The mean percentage recoveries at each fortification level were within the guideline requirements of 70 - 120% and are presented in Table B.5.2-7 below.

Limit of Quantification (LOQ)

The limit of quantitation (LOQ), defined as the lowest fortification level at which acceptable precision and accuracy data were obtained, was determined to be 0.05 mg/kg.

The limit of detection was determined to be 0.01 mg/kg.

Conclusion

The analytical procedure has been successfully validated in terms of specificity, linearity, precision, accuracy and LOQ, in accordance with all of the requirements of SANCO/825/00 rev. 8.1. 16/11/2010 and SANCO/3029/99 rev. 4, 11/07/2000.

Table B.5.2-6: Linearity Data

Matrix	Ion Transition (m/z)	Concentration Range (ng/mL)	Correlation Coefficient (r)	Slope	Intercept
Soil	336 > 187	0.018 – 5.0	0.9996	2.39×10^5	3.27×10^3
	338 > 189		0.9998	1.68×10^5	2.41×10^3

Table B.5.2-7: Precision and Accuracy Data

Matrix	Fortification Level (mg/kg)	Recoveries (%)	Mean Recovery (%)	RSD (%)
Soil	**Zoxamide Ion transition m/z 336 > 187 (quantification)**			
	0.05	95, 94, 95, 96, 95	95	1
	0.5	108, 107, 107, 106, 109	107	1
	Zoxamide Ion transition m/z 338 > 189 (confirmation)			
	0.05	92, 94, 94, 94, 96	94	1
	0.5	109, 109, 108, 109, 110	109	1

RMS comments and conclusion:

The limit of fortification is 0.05 mg/kg for zoxamide monitoring in soil. Validation data has been provided for two transitions. Consequently, method is considered as highly specific and confirmatory methods for method is not required. The method for determination of zoxamide in soil comply requirements of SANCO/825/00 rev. 8.1 and acceptable for monitoring purposes.

Residues in water

Reference: **CA 4.2/05**

Report: Jooß, S., (2013b), Development and Validation of a Residue Method for the Determination of Zoxamide in Drinking and in Surface Water, PTRL Europe, Report No. P3050G

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

GLP: Yes

Principle of the method

Samples (1.0mL) of acidified drinking or surface water (0.1% formic acid) are transferred to an autosampler vial and analysed by high performance liquid chromatography with tandem mass specific detection (LC-MS/MS) in positive polarity mode, using a Phenomenex Aqua C_{18} column (50 x 2 mm, 5µm) and gradient elution with mobile phases of water containing 0.1% formic acid and acetonitrile containing 0.1% formic acid. Quantification is performed using matrix matched external standards. The ion transition m/z 336 > 187 is used for quantification and the ion transition m/z 338 > 189 is used for confirmation.

Specificity

LC-MS/MS monitoring two mass transitions is considered to be a highly specific technique.

No interferences at >30% of the LOQ were present at the retention time of interest in control matrix samples.

Linearity

Linearity of detector response was demonstrated for zoxamide using six external standards across the concentration range of 0.02 ng/mL to 1.20 ng/mL. The results are presented in Table B.5.2-8 below.

Precision (Repeatability)

Repeatability data was generated from five samples of drinking and surface water fortified at the LOQ and five samples fortified at 10 x LOQ. The relative standard deviations (RSD) obtained for each fortification level and matrix were within the guideline requirements of less than 20% and are presented in Table B.5.2-9 and Table B.5.2-10 below.

Accuracy (Recovery)

Recovery data was generated from five samples of drinking water and surface water fortified at the LOQ and five samples fortified at 10 x LOQ. The mean percentage recoveries at each fortification level and matrix were within the guideline requirements of 70 - 120% and are presented in Table B.5.2-9 and Table B.5.2-10.

Limit of Quantification (LOQ)

The limit of quantitation (LOQ), defined as the lowest fortification level at which acceptable precision and accuracy data were obtained, was determined to be 0.1 µg/L in both drinking and surface water. The limit of detection was determined to be 0.02 µg/L in both drinking and surface water.

Conclusion

The analytical procedure has been successfully validated in terms of specificity, linearity, precision, accuracy and LOQ, in accordance with all of the requirements of SANCO/825/00 rev. 8.1. 16/11/2010 and SANCO/3029/99 rev. 4, 11/07/2000.

Table B.5.2-8: Linearity Data

Matrix	Ion Transition (m/z)	Concentration Range (ng/mL)	Correlation Coefficient (r)	Slope	Intercept
Water	336 > 187	0.02 – 1.20	0.9998	1.77×10^5	-744
	338 > 189		0.9988	1.22×10^5	-424

Table B.5.2-9: Precision and Accuracy Data (Drinking Water)

Matrix	Fortification Level (µg/kg)	Recoveries (%)	Mean Recovery (%)	RSD (%)
Drinking Water	**Zoxamide Ion transition m/z 336 > 187 (quantification)**			
	0.1	89, 91, 84, 89, 88	88	3
	1.0	106, 108, 108, 109, 108	108	1
	Zoxamide Ion transition m/z 338 > 189 (confirmation)			
	0.1	89, 91, 85, 89, 89	89	2
	1.0	106, 109, 108, 108, 108	108	1

Table B.5.2-10: Precision and Accuracy Data (Surface Water)

Matrix	Fortification Level (µg/kg)	Recoveries (%)	Mean Recovery (%)	RSD (%)
Surface Water	**Zoxamide Ion transition m/z 336 > 187 (quantification)**			
	0.1	85, 84, 82, 86, 82	84	2
	1.0	109, 106, 110, 109, 110	109	2
	Zoxamide Ion transition m/z 338 > 189 (confirmation)			
	0.1	86, 88, 84, 88, 83	86	2
	1.0	110, 105, 110, 111, 111	109	2

RMS comments and conclusion:

Zoxamide was determined in surface and drinking water by high performance liquid chromatography with tandem mass specific detection. The limit of quantification is 0.1 mg/L in both drinking and surface water Method validation meets EU requirements in all respects and the method considered acceptable for monitoring.

Reference: **CA 4.2/06**
Report: Schlewitz, P., (2014), Independent Lab Validation of the Analytical Method for the Determination of Zoxamide Residues in Drinking Water, Anadiag, France, Report No. R B4049
Guidelines: SANCO/825/00 rev. 8.1 and SANCO/3029/99 rev. 4
GLP: Yes

Principle of the method
Samples (10 mL) are added to a centrifuge tube, formic acid (10µL) is added and the samples are shaken vigorously. An aliquot (1.0 mL) of the acidified sample is analysed by high performance liquid chromatography with tandem mass specific detection (LC-MS/MS) in negative polarity mode, using an Ascentis Express C18 column (2.1 mm x 50 mm, 1.7µm particle size) and gradient elution with mobile phases of water containing 0.1% formic acid and acetonitrile containing 0.1% formic acid. Quantitation is performed using external standards. The ion transition m/z 336.0 > 187.0 is used for quantification and the ion transition m/z 338.0 > 189.0 is used for confirmation.

Specificity
LC-MS/MS monitoring two mass transitions is considered to be a highly specific technique. No interference at >30% of the LOQ was present at the retention time of interest in control matrix samples.

Linearity
The linearity of detector response was determined using six matrix matched external standard solutions across the working range of 0.02 ng/mL to 1.21 ng/mL. The results are presented in Table B.5.2-11 below.

Precision (Repeatability)
Repeatability data was generated from five samples fortified at the LOQ and five samples fortified at 10 x LOQ. The relative standard deviation (RSD) obtained for each fortification level were within the guideline requirements of less than 20% and are presented in Table B.5.2-12 below.

Accuracy (Recovery)
Recovery data was generated from five samples fortified at the LOQ and five samples fortified at 10 x LOQ. The mean percentage recoveries were within the guideline requirements of 70-120% and are presented in Table B.5.2-12 below.

Limit of quantification (LOQ)
The limit of quantification, defined as the lowest fortification level at which acceptable accuracy and precision data were obtained, was determined to be 0.1µg/L for drinking water.
The limit of detection (LOD) was estimated to be 0.01 µg/L.

Conclusion
The analytical procedure has been successfully validated in terms of specificity, linearity, precision, accuracy and LOQ, in accordance with all the requirements of SANCO/825/00 rev. 8.1 and SANCO/3029/99 rev. 4.

Table B.5.2-11: Linearity Data

Matrix	Ion Transition (m/z)	Concentration Range (ng/mL)	Correlation Coefficient (r)	Slope	Intercept
Drinking Water	336 > 187	0.02 – 1.21	0.99616	1.4467×10^{-4}	0.00
	338 > 189		0.99278	2.3066×10^{-4}	0.00

Table B.5.2-12: Precision and Accuracy Data

Matrix	Fortification Level (µg/kg)	Recoveries (%)	Mean Recovery (%)	RSD (%)
Drinking Water	**Zoxamide Ion transition m/z 336 > 187 (quantification)**			
	0.1	91.1, 82.2, 84.2, 88.1, 77.2	84.6	6.3
	1.0	100.8, 104.0, 100.1, 100.0, 99.4	100.9	1.8
	Zoxamide Ion transition m/z 338 > 189 (confirmation)			
	0.1	99.0, 84.2, 84.2, 100.0, 88.1	91.1	8.6

RMS comments and conclusion:

Independent laboratory validations were also conducted and have fulfilled the requirement guideline SANCO/825/00 rev. 8.1.

c) Methods for the analysis in air of the active substance and relevant breakdown products formed during or after application, unless the applicant shows that exposure of operators, workers, residents or bystanders is negligible

Reference: **CA 4.2/07**
Report: Miller, C., (2013), Zoxamide: Validation of Methodology for the Determination of Residues in Air, Huntingdon Life Sciences, UK, Project No. FRK0048
Guidelines: SANCO/825/00 rev. 8.1
GLP: Yes

Principle of the method

Tenax adsorbent silica tubes fortified with zoxamide are flushed with air (35°C, 80% relative humidity) at 1 mL/min for 6 hours. The analyte is extracted with acetone (10mL) by ultra-sonicating the samples for approximately 30 minutes then vortex mixing for approximately 30 seconds. The samples are then diluted with acetonitrile to an appropriate concentration prior to analysis by high performance liquid chromatography with tandem mass specific detection (HPLC-MS/MS) in positive ionspray mode, using a Acquity UPLC® BEH C_{18} column (2.1 x 50 mm, 1.7µm) and isocratic elution with a mobile phase of 20/80, v/v, water:methanol:formic acid (90:10:0.1 v:v:v) + 0.001 M ammonium formate / methanol:formic acid (100:0.1 v:v). Quantification was performed using external standards. The ion transition m/z 338 > 189 was used for quantification.

Specificity

LC-MS/MS monitoring mass transitions of parent and daughter ions is considered to be a highly specific technique.

No interferences at >30% of the LOQ were present at the retention time of interest in control matrix samples.

Breakthrough

No significant breakthrough of zoxamide was observed on the back sections of the air cartridges.

Linearity

Linearity of detector response was demonstrated for zoxamide using nine standard solutions across the concentration range of 0.01 to 0.5 ng/mL, with a correlation coefficient (r) of 0.9998 (slope = 41946, intercept = 32.7028).

Precision (Repeatability)

Repeatability data was generated from five samples of air fortified at the LOQ (90µg/m^3) and at 10 x LOQ (900µg/m^3). The relative standard deviations (RSD) obtained for each fortification level were within the guideline requirements of less than 20% and are presented in Table B.5.2-13 below.

Accuracy (Recovery)

Recovery data was generated from five samples air fortified at the LOQ (90µg/m^3) and at 10 x LOQ (900µg/m^3). The mean percentage recoveries at each fortification level were within the guideline requirements of 70 - 120% and are presented in Table B.5.2-13 below.

Limit of Quantification (LOQ)

The limit of quantitation (LOQ), defined as the lowest fortification level at which acceptable precision and accuracy data were obtained, was determined to be 90 µg/m^3 for zoxamide in air.

The limit of detection was determined to be 27.8 µg/m^3.

Conclusion

The analytical procedure has been successfully validated in terms of specificity, linearity, precision, accuracy and LOQ, in accordance with all of the requirements of SANCO/825/00 rev. 8.1.

Table B.5.2-13: Precision and Accuracy Data

Matrix	Fortification Level (µg/m^3)	Recoveries (%)	Mean Recovery (%)	RSD (%)
Air	90.0	89, 94, 91, 118, 94	97	12.2
	900	92, 88, 85, 88, 96	90	4.8

RMS comments and conclusion:

The limit of quantification is 90 µg/m^3 for zoxamide in air. The results from the method validation are acceptable and the validation process has fulfilled the guideline requirements SANCO/825/00 rev. 8.1.

d) Methods for the analysis in body fluids and tissues for active substances and relevant metabolites

Methods are required where the active substance and/or relevant metabolite is classed as toxic or highly toxic accordingly SANCO/3029/99 rev.4 11/07/00.

Not required, as zoxamide is not classified as toxic or very toxic, and is not classified according to GHS as acute toxicity (cat. 1-3), CMR (cat. 1) or STOT (cat. 1).

B.5.3. References relied on

Annex point	Author	Date	Title and Company reference	GLP	Pub.	DPDB Ref.
IIA, 4.1.1/01	Kemmerer, S.C	1998	Product Chemistry Series 830 Group A, Guideline Numbers OPPTS: 830.1550, 830.1600, 830.1620, 830.1670, 830.1700, 830.1750, 830.1800: Product Identity, Composition, and Analysis for RH-117,281 Technical, APR-98-282, ER Ref. No. 32.7	Y	N	81754
IIA, 4.2.1/02	Meyer, A, Desai, T, Guo, I	1998	Method for Parent RH-117281 and its Two Acid Metabolites, RH-141452 and RH-141455, in Potatoes and Processed Fractions, Rohm and Haas Technical Report No. 34-98-142, October 30, 1998, ER ref. no. 31.1	Y	N	81812
IIA, 4.2.1/03	Bruns, G Nelson, S	1998	Independent Laboratory Method Validation Trial of the Tolerance Enforcement Method for RH-7281 in Potato and Processed Fractions, Enviro-Test Laboratories, Rohm and Haas Technical Report No. 34-98-180, November 16, 1998, ER ref. no. 31.6	Y	N	81816
IIA, 4.2.1/04	Desai, T, Guo, I	1998b	Radiovalication of Preliminary Residue Analytical Method for Parent RH-7281 and its Two Acid Metabolites RH-1452 and RH-1455 in Potato, Rohm and Haas Technical Report No. 34-98-181, November 2, 1998, ER ref. no. 30.3	Y	N	81819
IIA, 4.2.1/08	Burdge, E, Kendi, M, Guo, I	1998	Tolerance Enforcement Method for RH-117281 in Grapes and Processed Fractions, Rohm and Haas Technical Report No. 34-98-150, September 24, 1998, ER ref. no. 15.3	Y	N	81828
IIA, 4.2.1/09	Szuter, S	1998b	Independent Laboraory Method Validation Trials of Tolerance Enforcement Method for RH-117281 in Grapes and Processed Fractions, McKenzie laboratories, Inc., Rohm and Haas Technical Report No. 34-98-177, December 17, 1998, ER ref. no. 31.7	Y	N	81832
IIA, 4.2.1/12	Burdge, E, Kendi, M, Guo, I, Meyer, A	1999	Residue Analytical Method for Parent RH-117281 and Metabolite RH-150721 in Wine, Rohm and Haas Technical Report No. 34-98-179, February 1999, ER ref. no. 29.15	Y	N	81841
IIA, 4.2.1/13 wine ILV	Wais, Andreas	1999a	Validation of a Residue Analytical Method for Parent RH-7281 and its Metabolite RH-0721 in Wine (Rohm and Haas Technical Report No. 34-98-148), RCC Ltd., Rohm and Haas Technical Report No.	Y	N	81846

Annex point	Author	Date	Title and Company reference	GLP	Pub.	DPDB Ref.
			34-99-23, January 25, 1999, ER ref. no. 22.5			
IIA, 4.2.2/01	Guo, I, Zhang, Q, Martin, D	1996b	Preliminary Residue Analytical Method for Parent RH-7281 in Soils, Rohm and Haas Technical Report No. 34-96-91, May 31, 1996, ER ref. no. 6.12	N	N	81847
IIA, 4.2.2/02	Guo, I, Martin, D, Zhang, Q	1998	RH-117281 Soil Analytical Method, Rohm and Haas Technical Report No. 34-98-126, September 3, 1998, ER ref. no. 31.2	Y	N	81851
IIA, 4.2.2/03	Szuter, Samantha	1998a	Independent laboratory Method Validation Trials of RH-117281 Analytical Method for Soil (TR 34-98-126), McKenzie Laboratories, Inc., Rohm and Haas Technical Report No. 34-98-160, September 28, 1998, ER ref. no. 31.8	Y	N	81853
IIA, 4.2.3	Volkel, Wolfgang	1998	Analytical Method for the Determination of RH-117281 in Drinking Water, RCC Ltd., Rohm and Haas Technical Report No. 34-98-52, November 10, 1998, ER ref. no. 31.5	Y	N	81855
IIA, 4.2.3/02	Wais, Andreas	2000	Validation of the residue analytical method for RH-117281 in ground- and surface water, RCC Ltd, Rohm and Haas Technical Report No. 34-99-201, January 06, 2000, ER ref. no. 40.4	Y	N	97884
IIA, 4.2.4 Air	Wais, Andreas	1999b	Validation of the Residue Analytical Method for RH-117281 in Air, RCC Ltd., Rohm and Haas Technical Report No. 34-98-51, January 25, 1999, ER ref. no. 29.17	Y	N	81858

New studies

Data point	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Vertebrate study Y/N	Data protection claimed (Y/N)	Justification if data protection claimed	Owner
CA 4.1.2/01	Weber, H.	2012	Validation of an enforcement method ("QuEChERS") for the determination of residues of zoxamide in grapes and potatoes and their processed products using LC-MS/MS. Eurofins Agrosience Services Chem GmbH Grossmoorbehogen 25, D-21079 Hamburg. Report: S12-03949 GLP, Not published.	N	Y	To ensure that the validation data comply with current requirements.	Gowan
CA 4.1.2/02	Weber, H., and Giesau, A.	2013a	Validation of analytical method for the determination of residues of the zoxamide metabolite RH-150721 in grapes and processing fractions using LC-MS/MS. Eurofins Agrosience Services Chem GmbH Grossmoorbehogen 25, D-21079 Hamburg. Report: S12-03950 GLP, Not published.	N	Y	To ensure that the validation data comply with current requirements.	Gowan
CA 4.1.2/03	Weber, H., and Giesau, A.	2013b	Validation of analytical method for the determination of residues of the zoxamide metabolites RH-1452 and RH-1455 in potatoes and processing fractions using LC-MS/MS. Eurofins Agrosience Services Chem GmbH Grossmoorbehogen 25, D-21079 Hamburg. Report: S12-03951 GLP, Not published.	N	Y	To ensure that the validation data comply with current requirements.	Gowan

Data point	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Vertebrate study Y/N	Data protection claimed (Y/N)	Justification if data protection claimed	Owner
CA 4.1.2/04	Luciani, G.P.	2010	Deterrmination of zoxamide residues after five applications of ELECTIS MZ and ZOXIUM 240 SC on potato under field conditions – Italian trial AgriParadigma S.r.l., Via Faentina, 224-48100 Ravenna, Italy Report Number AGRI 012/10 GLP DEC GLP, Not published	N	Y	To ensure that the validation data comply with current requirements.	Gowan
CA, 4.1.2/05	Nixon, W.B. and Sulairman, M.W.	1997	The analysis of RH-117,281 technical in filtered saltwater in support of Wildlife International Ltd Project No.: 129A-136 Appendix III to RH-117,281 Technical: A 96-Hour-Flow Through Acute Toxicity Test with the Saltwater Mysid (Mysidopsis bahia) Wildlife International Ltd 898 Commerce Drive, Eastern Maryland 21601, USA. Report Number: 95RC-0275 GLP, Not published	N	Y	To ensure that the validation data comply with current requirements.	Gowan
CA, 4.1.2/06	Kendall, T. Z.	1998	The analysis of RH-117,281 technical in filtered saltwater in support of Wildlife International Ltd Project No.: 129A-142 Appendix III to RH-117,281 Technical: A flow-through life-cycle toxicity test with the saltwater mysid (Mysidopsis bahia) Wildlife International Ltd 898 Commerce Drive, Eastern Maryland 21601, USA. Report Number: 97RC-0077 GLP, Not published	N	Y	To ensure that the validation data comply with current requirements.	Gowan

Data point	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Vertebrate study Y/N	Data protection claimed (Y/N)	Justification if data protection claimed	Owner
CA, 4.1.2/07	Aversa, S	2010	Validation of an analytical method for the determination of zoxamide in solutions of aquatic toxicity test with GOW 008 Biotechnologie BT Srl c/o Parco Tecnologico Agroalimentare dell'Umbria Frazione Pantalla, 06050 Todi (PG), Italy. Study BT102/10 GLP, Not published	N	Y	To ensure that the validation data comply with current requirements.	Gowan
CA, 6.1/04 (4.1.2/08)	Weber, H., Zetzsch, A., Giesler, W.	2016	Weber, H., Zetzsch, A., Giesler, W. (2016) Storage Stability of residues of Zoxamide, RH-150721, RH-1452 and RH-1455 in Grape and Processed Products and Potato Eurofins Agroscience Services Chem GmbH (EAS Chem), Großmoorbogen 25, D-21079 Hamburg, Germany Report Number: S12-03952 Final Report GLP, Not Published	N	Y	Potential data gap	Gowan
CA, 6.3.2/2 (4.1.2/09)	Luciani, G.P.	2010b	Determination of Zoxamide residues after five application of ELECTIS MZ and ZOXIUM 240 SC on Wine grape and Table grape – Italian trial, year 2010. Research Centre "Agriparadigma Srl" Via Facentina, 224 Ravenna, Italy Study Identification Code: AGRI 010/10 GLP DEC GLP, Not published	N	Y	Data not available for first Annex I inclusion and bridge to support use of new formulation and modified GAP	Gowan

Data point	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Vertebrate study Y/N	Data protection claimed (Y/N)	Justification if data protection claimed	Owner
CA, 6.5.3/02 (4.1.2/10)	Wais, A.	2001	Determination of residues of RH-117,281 and mancozeb in/on vine grapes (RAC grapes and processing products) following treatment with RH-7281/mancozeb 75WG from a field trial (semi residue decline study) in Italy; 1999 RCCLtd., CH-4452 Intigen, Switzerland Report Number: 734580, ER ref R 77.10 GLP, Not published	N	Y	Data not available for first Annex I inclusion	Gowan
CA, 6.2.1/09 (4.1.2/11)	Hein, W.	2014b	Extraction Efficiency of [phenyl-UL-14C] Zoxamide from Plant Metabolism Samples (Pea) RLP AgroScience GmbH, Breitenweg 71 67435 Neustadt / Germany. Report Number: AS362 GLP, Not published	N	Y	Data not available for first Annex I inclusion	Gowan
CA, 4.2/01	Weber, H.	2012	Validation of an enforcement method (“QuEChERS”) for the determination of residues of zoxamide in grapes and potatoes and their processed products using LC-MS/MS. Eurofins Agrosience Services Chem GmbH Grossmoorbehogen 25, D-21079 Hamburg. Report: S12-03949 GLP, Not published.	N	Y	To ensure that the validation data comply with current requirements.	Gowan
CA, 4.2/02	Richter, S.	2014	Validation of the QuEChERs Multi-Residue Method for the Determination of Zoxamide in Various Crop Types PTRL Europe, Helmholtzstr. 22, Science Park I D-89081 Ulm, Germany Report ID: P 3114G GLP, Not published.	N	Y	To ensure that the validation data comply with current requirements.	Gowan

Data point	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Vertebrate study Y/N	Data protection claimed (Y/N)	Justification if data protection claimed	Owner
CA, 4.2/03	Schlewitz, P.	2014	Independent Laboratory Validation of the Analytical Method for the Determination of Zoxamide Residues in Lettuce Aandiag, 16, rue Ampère, 67500 Haguenau, France Report ID: R B4023 GLP, Not published	N	Y	To ensure that the validation data comply with current requirements.	Gowan
CA, 4.2/04	Jooß, S.	2013a	Development and validation of a residue method for the determination of zoxamide in soil PTRL Europe, Helmholtzstr. 22, Science Park I D-89081 Ulm, Germany Report ID: P 3051 G GLP, Not published	N	Y	To ensure that the validation data comply with current requirements.	Gowan
CA, 4.2/05	Jooß, S.	2013b	Development and Validation of a Residue Method for the Determination of Zoxamide in Drinking and in Surface Water. PTRL Europe, Helmholtzstr. 22, Science Park I D-89081 Ulm, Germany Report ID: P 3050 G GLP, Not published	N	Y	To ensure that the validation data comply with current requirements.	Gowan
CA, 4.2/06	Schlewitz, P.	2014	Independent Laboratory Validation of a Residue Method for the Determination of Zoxamide in Drinking Water Anadiag 16, rue Ampère, 67500 Haguenau, France Report No. R B4049 GLP, Not published.	N	Y	To ensure that the validation data comply with current requirements.	Gowan

Data point	**Author(s)**	**Year**	**Title** **Source (where different from company)** **Company, Report No** **GLP or GEP status (where relevant),** **Published or not**	**Vertebrate study Y/N**	**Data protection claimed (Y/N)**	**Justification if data protection claimed**	**Owner**
CA, 4.2/07	Miller, C.	2014	Zoxamide: Validation of Methodology for the Determination of Residues in Air Huntingdon Life Sciences Eye Research Centre, Suffolk, IP23 7PX, UK Report ID: FRK0048 GLP, Not published	N	Y	To ensure that the validation data comply with current requirements.	Gowan