

REGISTRATION REPORT

Part B

Section 5

Analytical Methods

Detailed summary of the risk assessment

Product code: A22773A

Product name(s): ORONDIS EVO

Chemical active substance(s):

Azoxstrobin 250.0 g/L

Oxathiapiprolin 12.0 g/L

Interzonal

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(New authorization)

Applicant: Syngenta

Submission date: November 2021, updated September 2022

MS Finalisation date: July 2022, updated October 2022
(initial Core Assessment)

June 2023 (final Core Assessment)

Version history

When	What
November 2021	Part B - Section 5 - Core Assessment - Interzone
June 2022	Addition of body fluids method for oxathiapiprolin to sections 5.3.3.4 and A 2.2.2.3
July 2022	Initial izRMS assessment The report in the dRR format has been prepared by the Applicant, therefore all comments, additional evaluations and conclusions of the zRMS are presented in grey commenting boxes. Minor changes are introduced directly in the text and highlighted in grey. Not agreed or not relevant information are struck through and shaded for transparency .
September 2022	Applicant update: 5.2.2: Addition of analytical methods for bumble bees in Table 5.2-28, Table 5.2-29 (data points also updated) Appendix 1: Reference details added for body fluids and bumble bee studies Appendix 2. New study summary added; subsequent table numbers and chapter numberings updated 5.3.3.4: Table 5.3-15 - table title error corrected
October 2022	Initial assessment by the izRMS update: The report in the dRR format has been prepared by the Applicant, therefore all comments, additional evaluations and conclusions of the zRMS are presented in grey commenting boxes. Minor changes are introduced directly in the text and highlighted in grey. Not agreed or not relevant information are struck through and shaded for transparency . Following the evaluation and before sending the document for commenting, all coloured highlighting was removed, from the parts updated by the Applicant, for better legibility.
June 2023	Final report (Core Assessment updated following the commenting period) Additional information/assessments included by the zRMS in the report in response to comments received from the cMS and the Applicant are highlighted in yellow.

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5 Analytical methods

5.1 Conclusion and summary of assessment

zRMS conclusions:

Azoxystrobin

The methods available for azoxystrobin in plant and animal matrices have been summarized in EFSA document “Review of the existing MRLs for azoxystrobin” (EFSA Journal 2013;11(12):3497):

“1. Methods for enforcement of residues in food of plant origin

During the renewal peer review under Directive 91/414/EC, the multi-residue method DFG S 19 using HPLC-MS/MS and its ILV were evaluated and validated in plant matrices for the determination of parent azoxystrobin with an LOQ of 0.01 mg/kg in dry (cereals grain), acidic (orange), high water content (lettuce) and high oil content (oilseed rape) commodities (United Kingdom, 2009a, 2009b; FAO, 2008).

Furthermore, an analytical method using HPLC-MS/MS and its ILV were evaluated and adequately validated in plant matrices for the determination of parent azoxystrobin with an LOQ of 0.01 mg/kg in dry (wheat, barley grain), acidic (grape, mandarin, orange), high water content (tomato, lettuce, cabbage, carrot, kale, potato) and high oil content (avocado, sunflower seed, oilseed rape,) commodities, and in hops (United Kingdom, 2009b; FAO, 2008).

The multi-residue QuEChERS methods in combination with HPLC-MS/MS and GC/MS, as described by CEN (2008), are also available to analyse parent azoxystrobin but validation data were not evaluated in detail because a validated analytical method is reported above.

Hence, it is concluded that parent azoxystrobin can be enforced in food of plant origin with an LOQ of 0.01 mg/kg in dry, acidic, high water content and high oil content commodities, and in hops. As the active substance does not contain a chiral center, the analytical method is considered as specific to the active substance.

2. Methods for enforcement of residues in food of animal origin

During the peer review under Directive 91/414/EEC, an analytical method using GC-NPD and its ILV were evaluated and validated for determination of parent azoxystrobin with an LOQ of 0.001 mg/kg in milk and 0.01 mg/kg in eggs, liver, fat, muscle. Nevertheless, no confirmatory method was available (United Kingdom, 2009a; FAO, 2008).

Furthermore, an analytical method using HPLC-MS/MS and its ILV were evaluated in the JMPR report and validated in food of animal origin for determination of parent azoxystrobin with an LOQ of 0.01 mg/kg in muscle, fat, milk, kidney, liver and eggs (FAO, 2008).

Hence, it is concluded, that parent azoxystrobin can be enforced in food of animal origin with an LOQ of at least 0.01 mg/kg in muscle, fat, milk, kidney, liver and eggs.”

Therefore, no further consideration of monitoring methods for plant and animal matrices is necessary.

In “Peer Review of the pesticide risk assessment of the active substance azoxystrobin” (EFSA Journal 2010; 8(4):1542) it is stated that “Monitoring of residues of azoxystrobin in groundwater, drinking water and surface water can be done by GC-MSD. Pending on the data gap identified in section 4, the residue definition for water might change and therefore further methods could be required in the future. Adequate methods are available for the determination of residues of azoxystrobin in soil and air.”

The Applicant submitted a number of methods for analysis of residues of azoxystrobin for the generation of pre-authorization data and methods for post-authorization control and monitoring purposes. The methods have been evaluated in Draft Registration Report for Orondis Evo (Central Zone, zRMS-PL, July 2022).

The details of the evaluation of new and additional studies are referred in Appendix 2.

Oxathiapiprolin

EFSA concluded in EFSA Journal 2016;14(7):4504 - “Peer review of the pesticide risk assessment of the active substance oxathiapiprolin” that: *Oxathiapiprolin residues can be monitored in food and feed of plant origin by the multi-residue method DFG S19 using LC-MS/MS in dry, high water content and acidic matrices with limits of quantification (LOQs) of 0.01 mg/kg, or by a single HPLC-MS/MS method with LOQs of 0.01 mg/kg for all plant*

commodity groups. Residues of oxathiapiprolin in food of animal origin can be monitored with the multi-residue method DFG S19 using LC-MS/MS in meat, fat, liver, milk and eggs with LOQs of 0.01 mg/kg or by a single HPLC-MS/MS method with LOQs of 0.01 mg/kg for all animal matrices.

Residues of oxathiapiprolin in soil, water and air can be monitored by LC-MS/MS with LOQs of 1 µg/kg, 0.1 µg/L and 0.05 µg/m³, respectively.

No analytical method is required for the determination of oxathiapiprolin in body fluids and tissues as oxathiapiprolin is not classified as toxic or very toxic.

The Applicant submitted a number of methods for analysis of residues of oxathiapiprolin for the generation of pre-authorization data and methods for post-authorization control and monitoring purposes. The methods have been evaluated in Draft Registration Report for Orondis Evo (Central Zone, zRMS-PL, July 2022).

The details of the evaluation of new and additional studies are referred in Appendix 2.

No additional data is required to support this application.

Sufficiently sensitive and selective analytical methods are available for the active substances and relevant impurities in the plant protection product.

Noticed data gaps are: None

Sufficiently sensitive and selective analytical methods are available for all analytes included in the residue definitions.

Noticed data gaps are: none

Commodity/crop	Supported/ Not supported
tomato, eggplant	supported
bell pepper, okra	supported
cucumber and zucchini (courgette), gherkin and cucurbits with edible peel	supported
melon, watermelon, pumpkin and squash	supported
lettuce, salad plants, endive, sweet basil and spinach	supported

5.2 Methods used for the generation of pre-authorization data (KCP 5.1)

5.2.1 Analysis of the plant protection product (KCP 5.1.1)

5.2.1.1 Determination of active substance and/or variant in the plant protection product (KCP 5.1.1)

The plant protection product A22773A has not been reviewed at EU level as a consequence of the review of Azoxystrobin and Oxathiapiprolin.

An overview on the acceptable methods and possible data gaps for analysis of Azoxystrobin and Oxathiapiprolin in plant protection product is provided as follows:

Comments of zRMS:	The analytical method was successfully validated for the determination of Azoxystrobin and Oxathiapiprolin in plant protection product according to the requirements laid down by SANCO3030/99 rev.5.
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Reference:	KCP 5.1.1
Report	SF-1060/1 – Determination of Azoxystrobin and Oxathiapiprolin in A22773A by HPLC, Bradbury L.M., 2021, Syngenta File No. VV-898893, unpublished
Guideline(s):	Not applicable
Deviations:	Not applicable
GLP:	Not applicable
Acceptability:	Yes

Reference:	KCP 5.1.1
Report	Oxathiapiprolin/Azoxystrobin A22773A – Validation of analytical method SF-1060/01, Khot S.B., 2021, Report No. SMG16623, Syngenta File No. VV-898895, unpublished
Guideline(s):	SANCO 3030/99 rev. 5 “Validation of analytical methods for active constituents and agricultural products” document dated 1 July 2014 by the Australian Pesticides and Veterinary Medicines Authority (APVMA)
Deviations:	None
GLP:	Yes
Acceptability:	Yes

Materials and methods

The analytical method SF-1060/01 determines the active ingredients Azoxystrobin and Oxathiapiprolin in formulation A22773A - Oxathiapiprolin/Azoxystrobin SC (250/12). The validation has been performed under GLP (Study No. SMG16623). Both active substances are determined in the formulation by high performance liquid chromatography using a reversed phase column, using aq. Phosphoric acid (1.0% v/v)/acetonitrile gradient and diode array detection (DAD) at 284 nm and 260 nm. The azoxystrobin and oxathiapiprolin content was determined by external standard calibration using both purified compounds as reference standards. The peak area for the active ingredients and reference standards are measured by a data handling system and used to calculate the azoxystrobin and oxathiapiprolin content of the sample.

Table 5.2-1: Materials and method of SF-1060/1 for the determination of active substances Azoxystrobin and Oxathiapiprolin in plant protection product A22773A

	Azoxystrobin and Oxathiapiprolin	
Author(s), year	Bradbury L.M., 2021	
Principle of method	HPLC	
	Chromatograph	Agilent 1200 series HPLC system
	Dwell volume	1240 µL

	Column		ACE 3 C18 phase (HiChrom)		
			Column Particle size		3 µm
			Column length		75 mm
			Column ID		4.6 mm
	Column temperature		40°C		
	Injection volume		5 µL		
	Flow rate		1.0 mL/minute		
	Duration of chromatography		Approx. 15 minutes		
	Typical Backpressure		85 bar (at start)		
	Gradient program				
	Time [min]		1.0% aq. phosphoric acid (v/v) [%]		Acetonitrile [%]
	0		55		45
	9.0		55		45
	9.1		5		95
	11.5		5		95
	11.6		55		45
	15.0		55		45
Time (min.)	Wavelength (nm)		Bandwidth (nm)	Reference Wavelength (nm)	Reference bandwidth (nm)
0	284		4	Not used	Not used
7.0	260		4	Not used	Not used
Retention time (min.)		5.4 (Oxathiapiprolin) 8.7 (Azoxystrobin)			

Validation - Results and discussions

Table 5.2-2: Methods suitable for the determination of active substances Azoxystrobin and Oxathiapiprolin in plant protection product A22773A

	Oxathiapiprolin	Azoxystrobin
Author(s), year	Khot S.B., 2021	
Principle of method	HPLC with diode array detection (DAD)	
Linearity n = 6 (two determinations each) Tested over the range of 50% to 150% of prescribed weight of active ingredients.	y = 0.142*x+0.041 r = 0.99992	Y = 0.036*x+0.922 r = 0.99997
Precision / Repeatability	1.11 % w/w (0.56 % RSD, Horrat: 0.21)	23.1 % w/w (0.58 % RSD, Horrat: 0.35)
Recovery n = 4 (two determinations each) Tested over the range of 70% to 130% of prescribed weight of active ingredients.	mean recovery = 101.3%	mean recovery = 100.2%
Interference/Specificity	no significant co-elution	
Comment	The method is acceptably validated	

Conclusion

Analytical method SF-1060/1 is suitable for the specific, accurate and precise determination of Azoxystrobin and Oxathiapiprolin in A22773A.

5.2.1.2 Description of analytical methods for the determination of relevant impurities (KCP 5.1.1)

Toluene and the Azoxystrobin Z-isomer (R230310) are relevant impurities of azoxystrobin technical material, that could be present in A22773A. Analytical methods have been used for the determination of Toluene (SD-1540/1) and the azoxystrobin Z-isomer (R230310) (SD-1464/1) in A22773A. Both methods have previously been validated for comparable formulations and have been reviewed specifically for A22773A.

Toluene

Analytical method SD-1540/1 has been used for the determination of the relevant impurity Toluene in A22773A. Toluene is an impurity which may be found in A22773A at trace levels as a result of the Azoxystrobin manufacturing process. Toluene is not formed during manufacture or storage of A22773A. The analytical method SD-1540/1 for the determination of Toluene in a comparable azoxystrobin 100 FS formulation (coded A16283D) has previously been validated (cf. supporting data for analytical method and validation on A16283D as below) and has been reviewed for A22773A as reported in the following section.

Comments of zRMS:	The analytical method SD-1540/1 using standard addition sample preparation coupled with headspace gas chromatography has been properly described and validated for the determination of toluene in a plant protection product.
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Validation - Results and discussions

Reference:	KCP 5.1.1
Report	Heintz K. (2021). Statement on Validation of the Analytical Method SD-1540/1 for the determination of Toluene in A22773A oxathiapiprolin/azoxystrobin SC (012/250). Syngenta Crop Protection, Muenchwil, Switzerland, Issued date 21.05.2021, Syngenta File No. VV-903656
Guideline(s):	None
Deviations:	None
GLP:	No
Acceptability:	Yes

The analytical method SD-1540/1 determines Toluene in formulations using standard addition sample preparation coupled with headspace gas chromatography. This method uses the standard addition procedure, which implies that calibration solutions are prepared by adding Toluene directly to formulation samples and diluting all samples to the same final volume. The resulting spiked solutions contain different known levels of Toluene, ranging from 0.05 % to 1.0 % relative to the active ingredient, and by plotting the amount of Toluene added against the instrument response (area of Toluene in comparison to area of internal standard), the calibration curve is generated. One of the samples is prepared without the addition of Toluene, as it is from this sample that the actual content of Toluene can be calculated using the calibration curve generated. Due to the fact that the analyte of interest, in this case Toluene, is directly added to the sample, all sample matrix effects with a potential influence on specificity, linearity, recovery, repeatability or the limit of quantification, can be accounted for. The fact that this method also utilises headspace chromatography allows the assumption that the volatile components, being introduced into the gas chromatograph, will be extremely clean.

This method of analyte calibration therefore provides specificity, linearity, recovery and the limit of quantification for every individual sample.

Table 5.2-3 contains the validation data for formulation A22773A and it is evident from this that the

linearity, recovery and specificity are acceptable.

Repeatability was not carried out for this formulation, as it was concluded that the method of sample preparation and analysis used provided enough confidence in the result. Repeatability could be expected to be similar to other formulations using this combination of standard addition coupled to headspace gas chromatography.

With SD-1540/1 being originally validated for a 100 FS formulation type (A16283D, cf reference VV-400661), with a multiple point linear calibration conducted for every sample analysed, combined with compensation for any formulation matrix effects, it was concluded that the repeatability data generated for A16283D provides the confidence that repeatability would also be acceptable for A22773A as the other measured parameters are identical.

Table 5.2-3: Methods suitable for the determination of Toluene in plant protection product (PPP) A22773A

Relevant impurities in A22773A	Toluene max. content in PPP ≤ 0.52 g/l ^{*)}
Author(s), year	Heintz K., 2021
Principle of method	headspace GC and FID detection
Linearity n = 5 (duplicate injections) Tested between 0.05 – 1.03 % of Toluene relative to the amount of Azoxystrobin in the formulation	r = 0.9999 y = 15.914*X – 0.004
Accuracy n = 3 (duplicate injections) Tested at 0.05 % (L ₁), 0.26 % (L ₂) and 1.03 % (L ₃) of Toluene relative to the amount of Azoxystrobin in the formulation	mean recovery L ₁ = 97.6 % mean recovery L ₂ = 101.0 % mean recovery L ₃ = 100.0 % mean recovery = 99.5 %
Interference/Specificity	no significant interference
LOQ	0.05% w/w (0.125 g/L in the formulated product)
Comment	The method is acceptably validated

^{*)} reported for a minimum purity of 96.5% azoxystrobin

Conclusion

Method SD-1540/1 is therefore valid for the determination of Toluene in formulation A22773A.

Supporting data

As supporting data the following two references for the used analytical method and the validation on a comparable formulation are given (VV-127729 and VV-400661):

Reference:	KCP 5.1.1
Report	Adolph S. (2011). Analytical Method SD-1540/1 Determination of toluene in formulation by headspace gas chromatography. Syngenta Crop Protection, Muenchwilten, Switzerland. Unpublished Report No. SD-1540/1, Issued date 30.11.2011, Syngenta File No. VV-127729
Guideline(s):	None
Deviations:	None
GLP:	No
Acceptability:	Yes

Table 5.2-4: Materials and method of SD-1540/1 for the determination of Toluene in the plant protection product A22773A

Author(s), year	Adolph S., 2011	
Principle of method	headspace GC and FID detection	
	Gas Chromatograph: Agilent 6890A	
	Detector	FID, output voltage: 1V
	Column	fused silica, 30 m length, 0.32 mm i.d. stationary phase: DB-624 film thickness: 1.8 µm
	Injector liner:	Straight quartz liner
	Column temperature:	50°C, 2 min isothermal, 50-110°C heating rate 3°C/min 110-280°C, heating rate 40°C/min, 4 min isothermal
	Detector temperature:	300°C
	Injector temperature:	220°C
	Split ratio:	20:1
	Carrier gas:	Helium, 2.0 mL/minute (constant flow)
	Make-up gas	Nitrogen, 30 mL/minute
	Duration of chromatography:	approx. 31 minutes
	Retention time (Toluene)	11.1 minutes

Reference:	KCP 5.1.1
Report	De Benedictis S. (2011). Validation of analytical method SD-1540/1 – Toluene in A16283D. Syngenta Crop Protection, Muenchwilten, Switzerland. Unpublished Report No. 123787, Issued date 24.11.2011, Syngenta File No. VV-400661
Guideline(s):	SANCO 3030/99 rev. 4
Deviations:	None
GLP:	Yes
Acceptability:	Yes

Recovery

The recovery was evaluated for all 5 spiking levels (level 1-5, 2 determinations each) of 5 independent determinations of Toluene in A16283D. The mean results for levels 1 and 3 (corresponding to the addition of 0.05 % and 0.25 % Toluene relative to the amount of Difenoconazole present in formulation) are tabulated below.

	Recovery Obtained Level 1 [%]	Recovery Obtained Level 3 [%]
Toluene	99.3	100.3

Repeatability

Due to the very low amount of Toluene present in A16283D spiking level 3 (which corresponds to a level of 0.25 % Toluene relative to Difenoconazole) was used to determine the repeatability. To assess the the

content of Toluene was checked at level 3 (2 each) of 5 independent sample determinations of Toluene in A16283D.

The mean value and the relative standard deviation obtained are shown in the table below.

	Mean value [%]	Repeatability Obtained [% RSD]
Toluene	0.2485	0.93

Acceptable repeatability has been demonstrated for Toluene in formulation A16283D.

Level	Toluene added relative to AI [%]	Added Toluene found relative to AI [%]
L _{3_1}	0.2476	0.2511
L _{3_1}	0.2476	0.2527
L _{3_2}	0.2472	0.2464
L _{3_2}	0.2472	0.2468
L _{3_3}	0.2483	0.2473
L _{3_3}	0.2483	0.2492
L _{3_4}	0.2492	0.2504
L _{3_4}	0.2492	0.2461
L _{3_5}	0.2463	0.2481
L _{3_5}	0.2463	0.2466
Mean:		0.2485
Standard deviation:		0.0023
rel. Standard deviation [%]		0.93

Given the Modified Horwitz equation:

$$\% \text{ RSDr} = [2^{(1-0.5\log C)}] * 0.67$$

The Horrat index can be calculated by:

$$\text{Horrat} = \frac{\% \text{RSD}_r \text{ (determined)}}{\% \text{RSD}_r \text{ (predicted by modified Horwitz equation)}}$$

The %RSD in VV400661 is 0.93, concentration found is 0.2485 % w/w.

The predicted %RSDr is 1.65, so Horrat is <1 and hence fulfilled

Reference:	KCP 5.1.1
Report	Heintz K. (2023). Statement on Validation of the Analytical Method SD-1540/1 for the determination of Toluene in A22773A oxathiapiprolin/azoxystrobin SC (012/250) SD-1540/1 is equivalent to CIPAC MT 198.
Guideline(s):	None
Deviations:	None
GLP:	Yes
Acceptability:	Yes

Linearity

The linearity was evaluated using 5 levels (2 determinations each) corresponding to the addition of 0.05 %, 0.10 %, 0.25 %, 0.50 % and 1.00 % of Toluene relative to the amount of Azoxystrobin in formulation A22773A. The results are illustrated both in tabular form (Table 1) and graphically (Fig.1)

Level	Toluene added (absolute) [mg]	Toluene added relative to Azoxystrobin [%]	Ratio Toluene / IS (corrected)
L _{1_1}	0.010180	0.05	0.16234
L _{1_2}	0.010180	0.05	0.16147
L _{2_1}	0.020360	0.10	0.33068
L _{2_2}	0.020360	0.10	0.32197
L _{3_1}	0.050899	0.26	0.82336
L _{3_2}	0.050899	0.26	0.82113
L _{4_1}	0.101798	0.52	1.62578
L _{4_2}	0.101798	0.52	1.62035
L _{5_1}	0.203596	1.03	3.21676
L _{5_2}	0.203596	1.03	3.26934
		Slope	15.914
		Intercept	0.004
		Correlation coefficient	0.9999

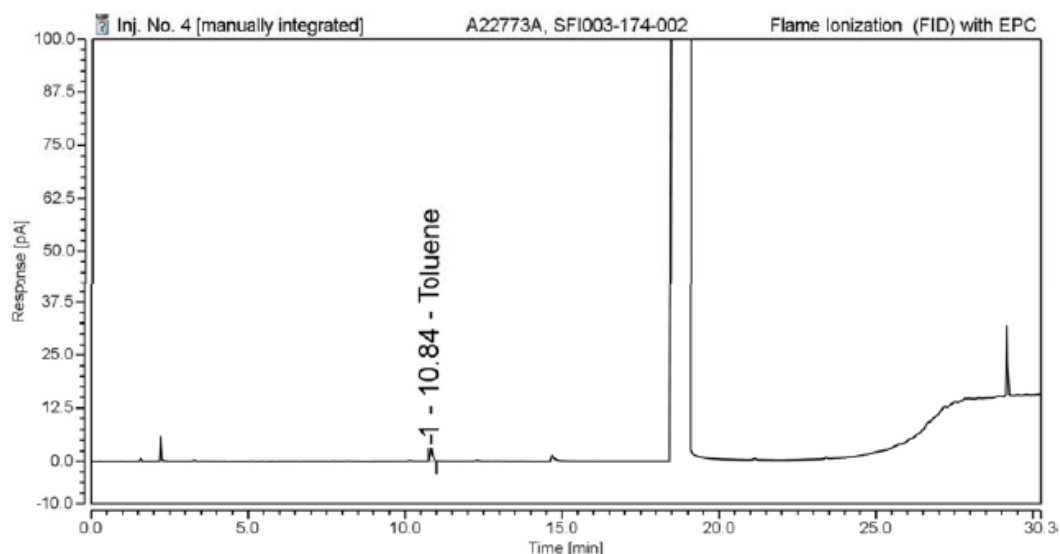
Recovery and limit of quantification

The recovery was evaluated at 3 concentration levels (2 determinations each) corresponding to the addition of 0.05 %, 0.26 %, and 1.03 % of Toluene relative to the amount of Azoxystrobin in formulation A22773A. With the recovery at the lowest concentration level being acceptable, a 0.05 % level of Toluene relative to Azoxystrobin is established as limit of quantification. The results are tabulated below.

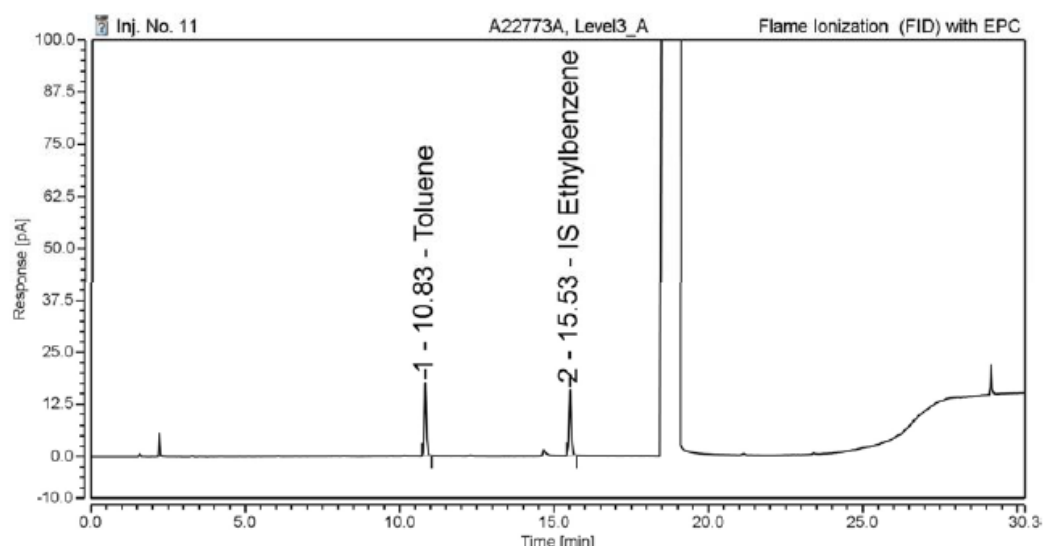
Level	Ratio Toluene / IS (corrected)	Ratio Toluene / IS calculated	Recovery [%]
L _{1_1}	0.16234	0.16600	97.8
L _{1_2}	0.16147	0.16600	97.3
Mean L₁			97.6
L _{3_1}	0.82336	0.81401	101.1
L _{3_2}	0.82113	0.81401	100.9
Mean L₃			101.0
L _{5_1}	3.21676	3.24403	99.2
L _{5_2}	3.26934	3.24403	100.8
Mean L₅			100.0

Specificity:

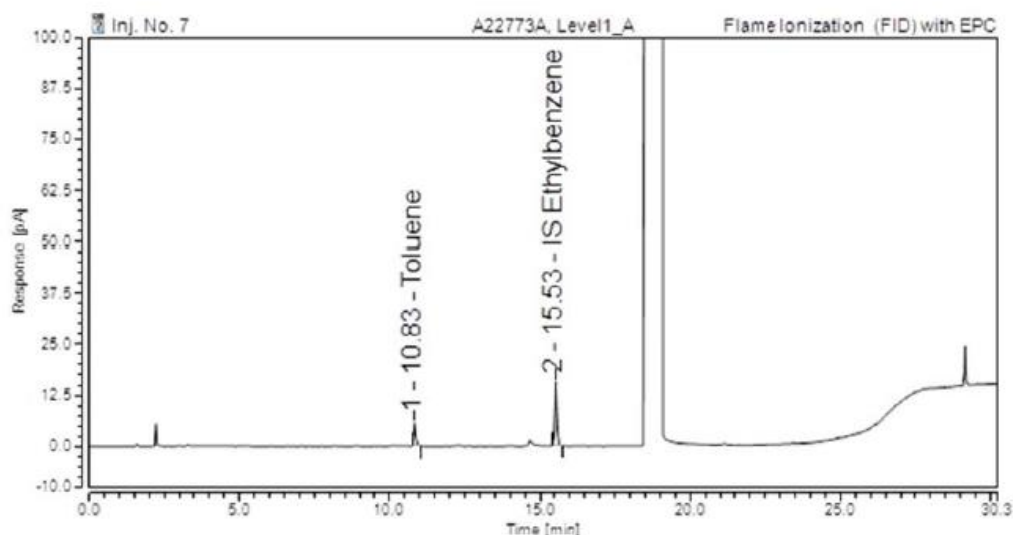
The following chromatograms show that there is no interference between Toluene, the internal standard Ethylbenzene, and any other ingredients contained within formulation A22773A. The specificity of the method has therefore been established.



Formulation dissolved in DMSO



Formulation spiked with internal standard (IS) and 0.26 % Toluene relative to the amount of Azoxystrobin present in formulation A22773A.



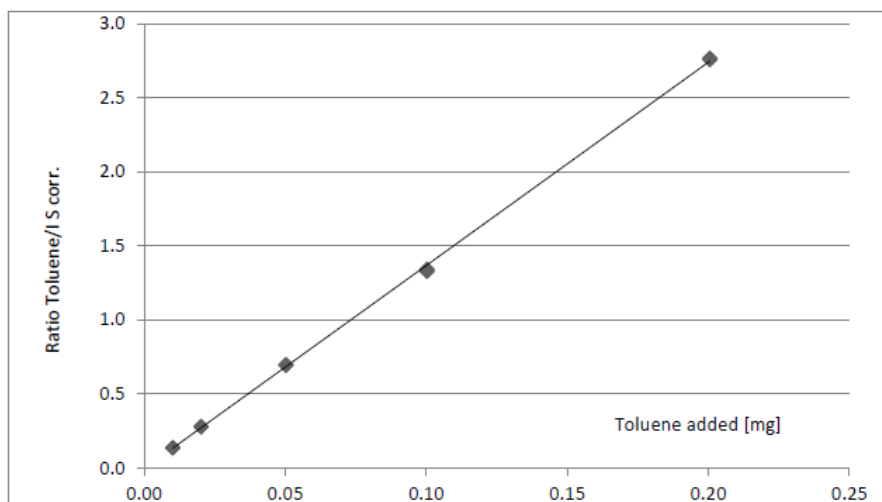
Formulation spiked with internal standard (IS) and 0.05 % Toluene relative to the amount of Azoxystrobin present in formulation A22773A.

Repeatability and related results of Toluene determination in A16283D according to SD-1540/1:

Linearity

The linearity was evaluated using 5 levels (2 determinations each) corresponding to the addition of 0.05 %, 0.10 %, 0.25 %, 0.50 % and 0.99 % of Toluene relative to the amount of difenoconazole in formulation A16283D.

Level	Toluene added (absolute) [mg]	Toluene added relative to difenoconazole [%]	Ratio Toluene / IS (corrected)
L _{1_1}	0.009970	0.05	0.13731
L _{1_2}	0.009970	0.05	0.13606
L _{2_1}	0.019940	0.10	0.28037
L _{2_2}	0.019940	0.10	0.27797
L _{3_1}	0.049850	0.25	0.69365
L _{3_2}	0.049850	0.25	0.69813
L _{4_1}	0.099700	0.50	1.34269
L _{4_2}	0.099700	0.50	1.32755
L _{5_1}	0.199400	0.99	2.76798
L _{5_2}	0.199400	0.99	2.75835
		Slope	13.803
		Intercept	-0.004
		Correlation coefficient	0.99979



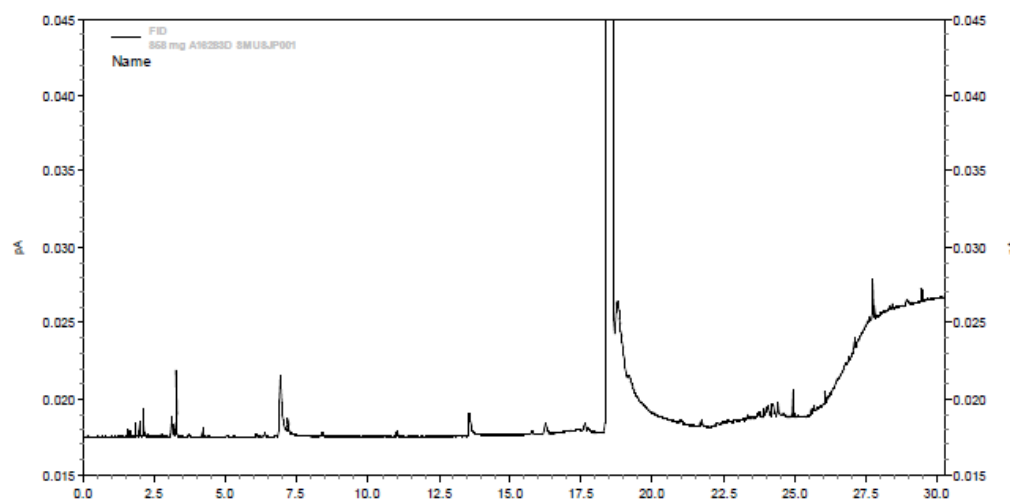
Recovery and limit of quantification

The recovery was evaluated at 3 concentration levels (2 determinations each) corresponding to the addition of 0.05 %, 0.25 %, and 1.01 % of Toluene relative to the amount of difenoconazole in formulation A16283D. With the recovery at the lowest concentration level being acceptable, a 0.05 % level of Toluene relative to difenoconazole is established as limit of quantification. The results are tabulated below.

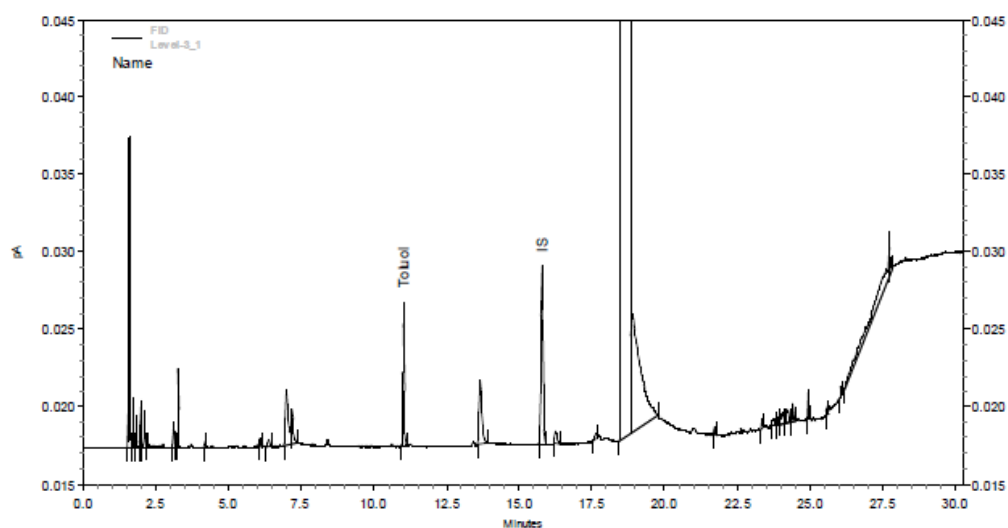
Level	Ratio Toluene / IS (corrected)	Ratio Toluene / IS calculated	Recovery [%]
L _{1_1}	0.13731	0.13362	102.8
L _{1_2}	0.13606	0.13362	101.8
Mean L₁			102.3
L _{3_1}	0.69365	0.68408	101.4
L _{3_2}	0.69813	0.68408	102.1
Mean L₃			101.8
L _{5_1}	2.76798	2.74832	100.7
L _{5_2}	2.75835	2.74832	100.4
Mean L₅			100.6

Specificity

The following chromatograms show that there is no interference between Toluene, the internal standard Ethylbenzene, and any other ingredients contained within formulation A16283D. The specificity of the method has therefore been established.



Formulation dissolved in DMSO



Formulation spiked with internal standard (IS) and 0.25 % Toluene relative to the amount of difenoconazole present in formulation A16283D

Repeatability

Due to the very low amount of Toluene present in A16283D, spiking level 3 (which corresponds to a level of 0.25 % Toluene relative to difenoconazole) was used to determine the repeatability. The repeatability was assessed using the results obtained at the level 3 Toluene addition, where five independent Toluene determinations (five complete sets including sample plus spiking at five levels, double determination each) were analysed. The mean value and the relative standard deviation obtained are shown in the table below.

Level	Toluene added relative to difenoconazole [%]	Added Toluene calculated relative to difenoconazole [%]
L _{3_1} (1)	0.2476	0.2511
L _{3_2} (1)	0.2476	0.2527
L _{3_1} (2)	0.2472	0.2464
L _{3_2} (2)	0.2472	0.2468
L _{3_1} (3)	0.2483	0.2473
L _{3_2} (3)	0.2483	0.2492
L _{3_1} (4)	0.2492	0.2504
L _{3_2} (4)	0.2492	0.2461
L _{3_1} (5)	0.2463	0.2481
L _{3_2} (5)	0.2463	0.2466
	Mean	0.2485
	Standard deviation	0.0023
	rel. Standard deviation	0.93

Correlation coefficient

The linearity was evaluated using all 5 spiking levels (level 1-5, 2 determinations each) of the determinations of Toluene in A16283D. A16283D was spiked with Toluene corresponding to the addition of 0.05 %, 0.10 %, 0.25 %, 0.50 % and 1.00 % of Toluene relative to the amount of difenoconazole present in formulation A16283D. The corresponding correlation coefficients are given below.

Determination	Correlation Coefficient
1	0.99979
2	0.99998
3	0.99999
4	0.99997
5	0.99998
Mean	0.99994

Azoxystrobin Z-isomer (R230310)

Analytical method SD-1464/1 has been used for the determination of the relevant impurity R230310 in formulation A22773A. R230310 is an impurity which may be found in A22773A at trace levels as a result of the Azoxystrobin manufacturing process. R230310 is not formed during manufacture or storage of A22773A. The analytical method for the determination of R230310 in a comparable azoxystrobin formulation (i.e. azoxystrobin/benzovindiflupyr EC 100/50, coded A17961A) has previously been validated (cf. supporting data for analytical method and validation on A17961A as below) and has been reviewed for A22773A as reported in following the section.

Comments of zRMS:	The following data shows acceptable linearity and recovery for method SD-1464/1 when used for formulation A22773A. With the recovery at the lowest concentration level being acceptable, a 0.05 % level of R230310 relative to Azoxystrobin is established as limit of
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	quantification.
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Validation - Results and discussions

Reference:	KCP 5.1.1
Report	Khot S. (Statement on Validation of the Analytical Method SD-1464/1 for the determination of R230310 in A22773A - oxathiapiprolin/azoxystrobin SC (012/250)). Syngenta Biosciences Pvt. Ltd., Goa, India, Issued date 13.10.2021, Syngenta File No. VV-911906
Guideline(s):	None
Deviations:	None
GLP:	No
Acceptability:	Yes

The analytical method SD-1464/1 for the determination of R230310 in formulation has been validated in GLP study123137.

The analytical method SD-1464/1 determines R230310 (z-isomer of azoxystrobin) in formulations by using liquid chromatography.

The linearity, recovery, repeatability and limit of quantification was performed by spiking the known concentration of R230310 reference standard with formulation blank (including azoxystrobin and oxathiapiprolin analytical standard).

The resulting spiked solutions contain different known levels of R230310, ranging from 0.05 % to 0.9 % relative to the azoxystrobin content in the formulation. One of the test solutions containing only azoxystrobin analytical standard was prepared in order to correct for by-product R230310 potentially present in the reference standard of azoxystrobin.

Appendix 1 of the statement contains the validation data for formulation A22773A, and it is evident from this that the linearity, recovery, repeatability, limit of quantification and specificity are acceptable.

Table 5.2-5: Methods suitable for the determination of R230310 in plant protection product (PPP) A22773A

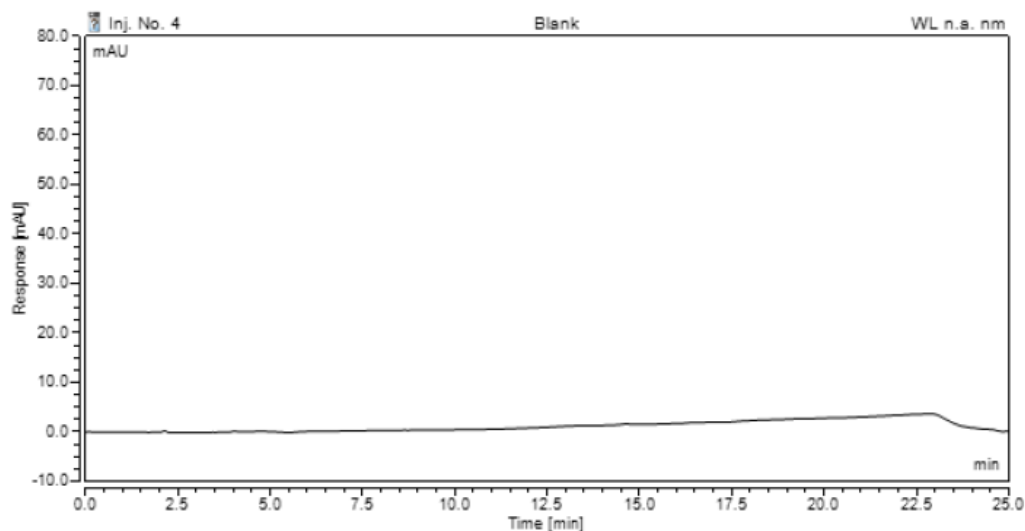
Relevant impurities in A17961A	R230310 max. content in PPP $\leq 1.8 \text{ g/L}$ 6.48 g/L		
Author(s), year	Khot S., 2021		
Principle of method	HPLC and UV detection		
Linearity n = 6 (2 determinations each) Tested between 0.05 – 0.9 % of R230310 relative to the amount of Azoxystrobin	r = 0.9999 y = 1.87*X+0.0003		
Precision as repeatability n = 12 (6 weighings, double injection each)	mean conc.=	0.047%	0.857 %
	S _{rel} (%RSD) =	1.27	0.69
	%RSDr		
	(mod. Horwitz)=	4.24%	2.74%
	Horrat =	0.30	0.25
Accuracy n = 6 concentration levels (L1-L6, 2 injections each) Tested between (L1) 0.05 – (L6) 0.9 % of R230310 relative to the amount of Azoxystrobin	mean recovery = 99.6 %		
Interference/Specificity	no significant interference		
LOQ	$\leq 0.05\% \text{ w/w}$ (0.125 g/L in the formulated product)		
Comment	The method is acceptably validated		

^{a)} reported for a minimum purity of 96.5% azoxystrobin

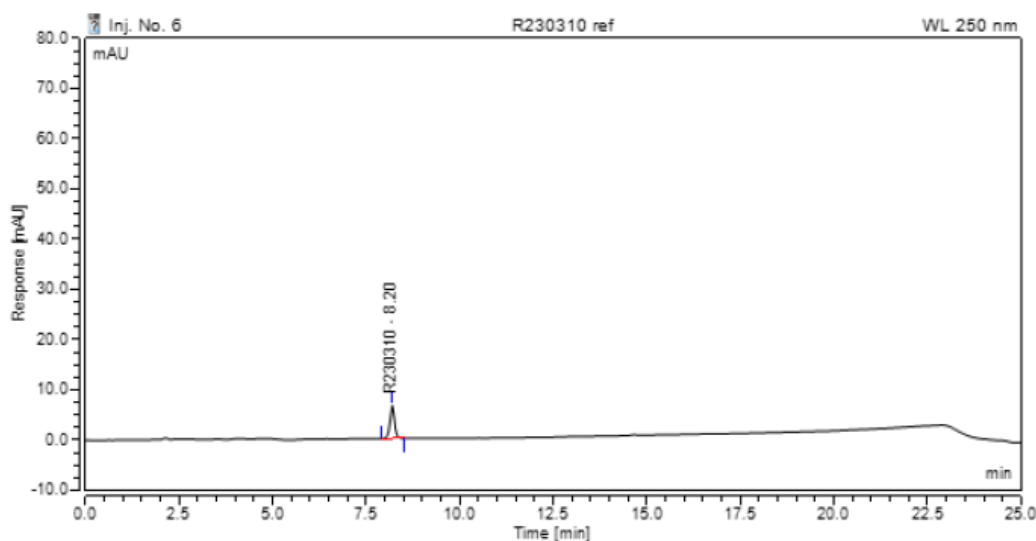
Specificity:

The following chromatograms show that there is no interference between R230310, the formulation blank, azoxystrobin and oxathiapiprolin analytical standards and any other ingredients contained within formulation A22773A. The specificity of the method has therefore been established.

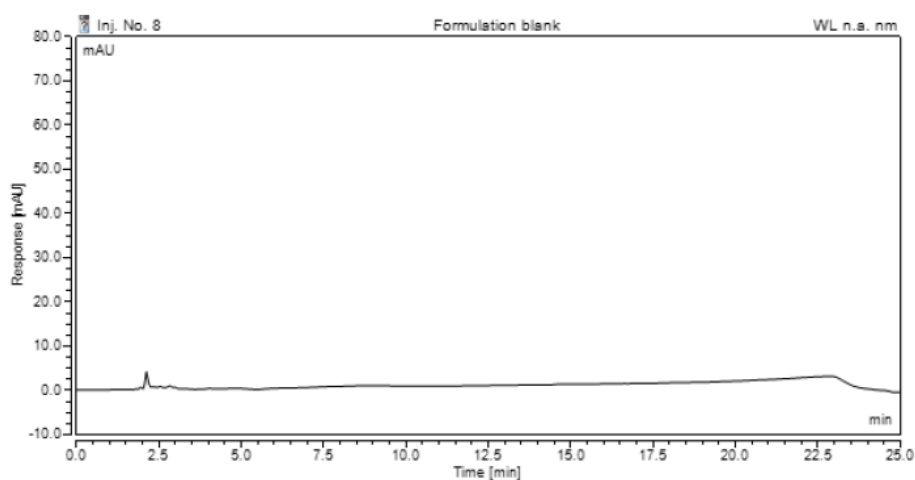
An examination of the chromatograms for A22773A, azoxystrobin technical material, oxathiapiprolin technical material and Formulation Blank of A22773A showed no significant co-elution between the R230310 and / or formulation components.



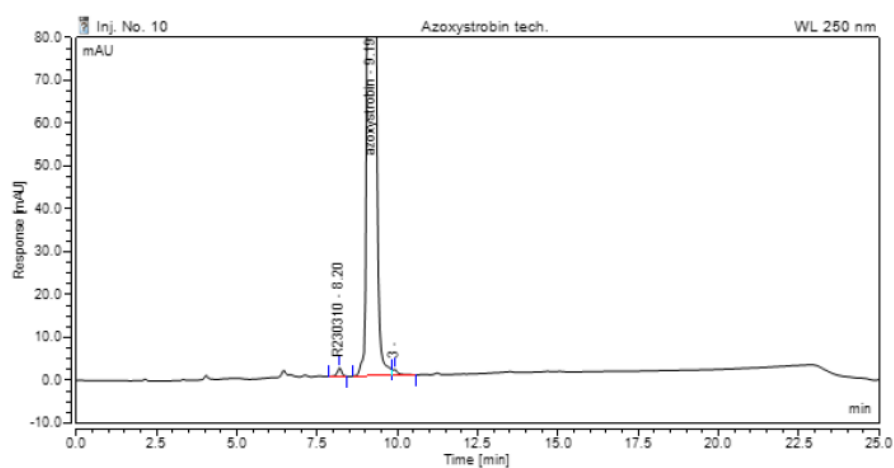
Diluent - acetonitrile



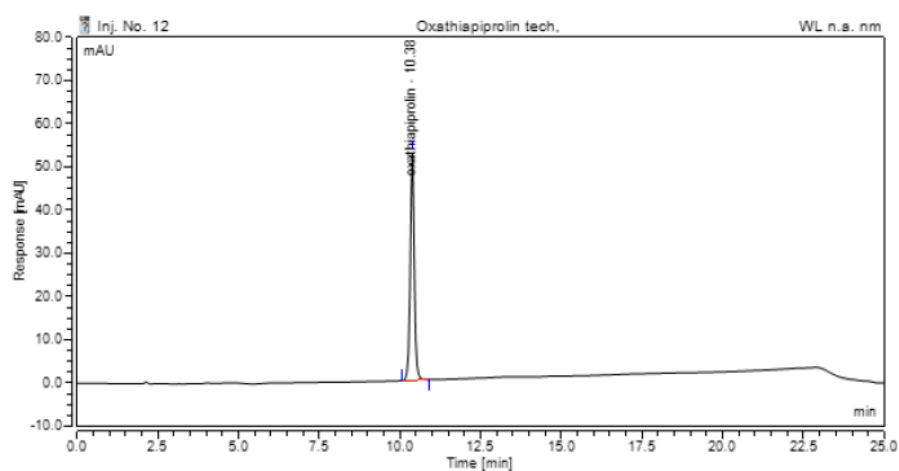
R230310 – Reference Solution



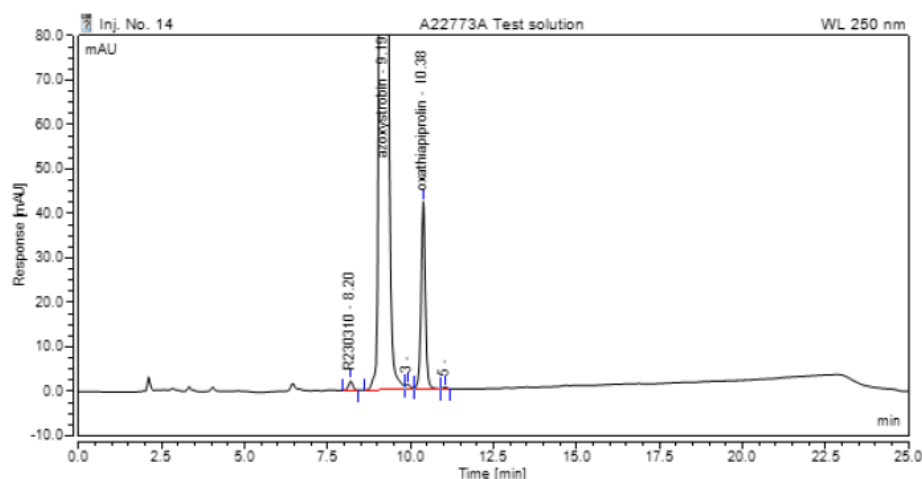
A22773A – Formulation Blank



Azoxystrobin technical



Oxathiapiprolin technical



A22773A Formulation – Test Solution

Conclusion

Analytical Method SD-1464/1 is therefore valid for the determination of R230310 in formulation A22773A.

Supporting data

As supporting data the following two references for the used analytical method and the validation on a comparable formulation are given (VV-127958 and VV-397754):

Reference:	KCP 5.1.1
Report	Kettner R. (2011). Analytical Method SD-1464/1 Determination of R230310 in Formulation by HPLC. Syngenta Crop Protection, Muenchwilen, Switzerland. Unpublished Report No. SD-1464/1, Issued date 08.07.2011, Syngenta File No. VV-127958
Guideline(s):	None
Deviations:	None
GLP:	No
Acceptability:	Yes

Table 5.2-6: Materials and method of SD-1464/1 for the determination of azoxystrobin Z-isomer (R230310) in the plant protection product A22773A

Author(s), year	Kettner R., 2011		
Principle of method	HPLC and UV detection		
	Chromatograph	Agilent Technologies 1200 Series	
	Column	Nucleosil C18	
		Particle size	5 µm
		Column length	250 mm
		Column i.d.	4.0 mm
	Column temperature	room temperature	
	Injection volume	5 µL	
	Flow rate	1.0 mL/minute	
	Duration of chromatography	Approx. 25 minutes	
	Gradient program		

	Time [min]	0.1 % aq. H ₃ PO ₄ [%]	acetonitrile [%]	methanol
	0	60	40	0
	20	10	80	10
	22	60	40	0
	25	60	40	0
Retention time (min.)	9.7 (R230310)			

Reference:	KCP 5.1.1
Report	Kettner R. (2011). R230310 - Validation of analytical method SD-1464/1 (A17961A), Syngenta Crop Protection, Münchwilen, Switzerland. Report No. 123137, Issued date 11.07.2011, Syngenta File No. VV-397754
Guideline(s):	SANCO 3030/99 rev. 4
Deviations:	None
GLP:	Yes
Acceptability:	Yes

5.2.1.3 Description of analytical methods for the determination of formulants (KCP 5.1.1)

Not required.

5.2.1.4 Applicability of existing CIPAC methods (KCP 5.1.1)

CIPAC method 571 exists for the determination of azoxystrobin in SC formulations, see CIPAC Handbook M, page 10.

There are no CIPAC methods for the determination of oxathiapiprolin in SC formulations.

There are no CIPAC methods for the determination of azoxystrobin and oxathiapiprolin in mixed suspension concentrate formulations.

5.2.2 Methods for the determination of residues of Azoxystrobin (KCP 5.1.2)

An overview on the acceptable methods and possible data gaps for analysis of residues of azoxystrobin for the generation of pre-authorization data is given in the following tables. For the detailed evaluation of new studies it is referred to Appendix 2.

Table 5.2-7: Validated methods for the generation of pre-authorization data for azoxystrobin in soil, water, air (KCP 5.1.2.1 in support of environmental fate studies)

Table not included;

No analytical methods were used to support the environmental fate data generated on this product.

Table 5.2-8: Validated methods for the generation of pre-authorization data azoxystrobin in soil, water (KCP 5.1.2.2 in support of efficacy studies)

Table not included;

No specific analytical methods were used to support the efficacy data generated on this product.

Table 5.2-9: Validated methods for the generation of pre-authorization data for azoxystrobin in feed, body fluids and tissues and air (KCP 5.1.2.3 in support of toxicological studies)

Table not included;

No analytical methods were used to support the toxicology data generated on this product.

Table 5.2-10: Validated methods for the generation of pre-authorization data for azoxystrobin in body fluids, air and any additional matrices used (KCP 5.1.2.4 in support of operator, worker, resident and bystander exposure studies)

Table not included;

No specific operator, worker, resident or bystander exposure studies were conducted to support this product. Consequently no analytical methods were required.

Table 5.2-11: Validated methods for the generation of pre-authorization data for azoxystrobin in plant and animal products (KCP 5.1.2.5 in support of residues studies)

Method type	Matrix type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / Report No. / EU agreed
Component of residue definition for plant products: azoxystrobin (EFSA, 2010)^(a)				
RAM 243/02	High protein/high starch content (dry) - <i>Cereal grain</i>	0.01 mg/kg	GC-NPD	Method: Clarke D.M., Sapiets A., 1994 / Report RAM 243/02 (ICI5504/2392) Validation: Clarke D.M., Sapiets A., 1994 / Report RJ1557B (ICI5504/1024) EU agreed (United Kingdom, 2009a, 2009b)
	High acid content - <i>Grape</i>	0.01 mg/kg		
	No group - <i>Cereal straw</i> - <i>Wine</i>	0.02 mg/kg 0.01 mg/kg		
RAM 243/03	High water content - <i>Cereal forage</i>	0.02 mg/kg	GC-NPD and HPLC-UV	Method: Burke S.R., Sapiets A., 1994 / Report No. RAM 243/03 (ICI5504/1021) Validation: Burke S.R., Sapiets A., 1995 / Report RJ1729B (ICI5504/0260) EU agreed (United Kingdom, 2009a, 2009b)
	High protein/high starch content (dry) - <i>Cereal grain</i>	0.01 mg/kg		
	High acid content - <i>Grape</i>	0.01 mg/kg		
	No group - <i>Cereal straw</i> - <i>Wine</i>	0.02 mg/kg 0.01 mg/kg		
RAM 243/04	No formal validation was performed on RAM 243/04 as the method was very similar to RAM 243/03. Differences were minor and limited to the incorporation of additional validation data from regulatory studies to extend the scope of the method to cover the analysis of: High water - <i>Leafy Crop</i> - <i>Cucumber</i> - <i>Tomato</i> - <i>Banana (whole fruit, skin and pulp)</i> - <i>Melon peel</i> - <i>Melon pulp</i> - <i>Apple</i> - <i>Peach</i> - <i>Chilli</i> High protein/high starch content (dry) - <i>Dried bean</i> - <i>Rice (grain)</i> - <i>Root Crop</i>	0.05 mg/kg 0.10 mg/kg 0.50 mg/kg 0.02 mg/kg 0.20 mg/kg 0.02 mg/kg 0.10 mg/kg 0.10 mg/kg 0.01 mg/kg 0.01 mg/kg	GC-NPD or HPLC-UV	Method: Burke S.R., 1996 / Report No. RAM 243/04 (ICI5504/2393) Validation: Clarke D.M., Sapiets A., 1994 / Report RJ1557B (ICI5504/1024) Burke S.R., Sapiets A., 1995 / Report RJ1729B (ICI5504/0260) EU agreed (United Kingdom, 2009a, 2009b)

Method type	Matrix type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / Report No. / EU agreed
	No group - <i>Orange Juice</i> - <i>Cereal (Middlings, Bran, Shorts, Germ, Flour)</i> - <i>Tomato paste</i> - <i>Tomato pomace</i> - <i>Rice (straw)</i> Recovery data for these additional crops have been extracted from a residue study – these data are summarised in the method.	0.10 mg/kg 0.05 mg/kg 0.01 mg/kg 0.10 mg/kg 0.10 mg/kg 0.50 mg/kg 0.50 mg/kg		
RAM 243/05	No formal validation conducted (minor update of RAM 243/04)	See above	GC-NPD or HPLC-UV	<i>Method:</i> Burke S.R., 1997 / Report RAM 243/05 (ICI5504/1389) <i>Validation:</i> Clarke D.M., Sapiets A., 1994 / Report RJ1557B (ICI5504/1024) Burke S.R., Sapiets A., 1995 / Report RJ1729B (ICI5504/0260) EU agreed (United Kingdom, 2009a, 2009b)
RAM 260/01	High oil content - <i>Peanut</i> - <i>Peacan</i>	0.01 mg/kg	GC-NPD	<i>Method</i> Burke S.R. and Gentle W., 1995 / Report SOP RAM 260/01 (ICI5504/1008) <i>Validation:</i> Burke S.R., 1995 / Report RJ1787B (ICI5504/0261) EU agreed (United Kingdom 2009a)
RAM 260/02	No formal validation was performed on RAM 260/02 as the method was very similar to RAM 260/01. Differences were minor and limited to the incorporation of additional recovery data to extend the scope of the method from peanut and peacan to: High oil content - <i>Oilseed rape seed</i> - <i>Coffee</i> - <i>Peanut oil</i> No group - <i>Citrus (peel)</i> - <i>Peanut meal</i> - <i>Peanut hay</i> There were no changes in the analytical procedures. Thus validation of RAM 260/01 is applicable to RAM 260/02. Recovery data for additional crops have been extracted from residue studies – these data are summarised in the method.	0.01 mg/kg	GC-NPD	<i>Method</i> Burke S.R., 1996a / Report SOP RAM 260/02 (ICI5504/1016) <i>Validation:</i> Burke S.R., 1995 / Report RJ1787B (ICI5504/0261) EU agreed (United Kingdom 2009a)

Method type	Matrix type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / Report No. / EU agreed
RAM 260/03	High oil content - <i>Peanut kernel</i> - <i>Pecan kernel</i>	0.01 mg/kg	GC-NPD	<i>Method</i> Burke S.R., 1997 / Report SOP RAM 260/03 (ICI5504/0250) <i>Validation:</i> Burke S.R., 1997 / Report RJ2385B (ICI5504/0278) EU agreed (United Kingdom 2009a)
	High oil content - <i>Oilseed rape seed</i> - <i>Coffee bean</i> - <i>Peanut oil</i>	0.01 mg/kg		
	No group - <i>Lemon peel</i> - <i>Orange peel</i>	0.01 mg/kg		
RAM 305/01	High protein/starch content (dry) - <i>Lentil</i> - <i>Potato</i> - <i>Sugar beet</i> - <i>Barley grain</i> - <i>Carrot</i>	0.01 mg/kg	LC-MS/MS	<i>Method</i> Robinson N.J. <i>et al.</i> , 1999 / Report RAM 305/01 (ICI5504/1022) <i>Validation</i> Lister N.J., 1999 / Report RJ2770B (ICI5504/0280) EU agreed (United Kingdom, 2009a, 2009b)
	High water content - <i>Pear</i> - <i>Plum</i> - <i>Onion</i> - <i>Cabbage</i> - <i>Lettuce</i> - <i>Leek</i> - <i>Peas (with pods)</i> - <i>Tomato</i> - <i>Melon</i> - <i>Wheat (forage)</i>	0.01 mg/kg		
	High acid content - <i>Orange (peel/pulp)</i> - <i>Grape</i> - <i>Strawberry</i>	0.01 mg/kg		
	High oil content - <i>Oilseed rape seed</i> - <i>Avocado</i>	0.01 mg/kg		
	No group - <i>Wheat (straw)</i>	0.01 mg/kg		
RAM 305/02 (method also used for post-registration purposes, Section 5.3.2.2)	No formal validation was performed on RAM 305/02 as the method was very similar to RAM 305/01. Differences were minor and limited to the incorporation of additional recovery data to extend the scope of the method to cover the analysis of: No group - <i>Hops</i> Recovery data for this additional crop has been extracted from a residue study – these data are summarised in the method.	0.01 mg/kg	LC-MS/MS	<i>Method</i> Robinson N.J. <i>et al.</i> , 1999 / Report RAM 305/02 (ICI5504/0249) <i>Validation</i> Lister N.J., 1999 / Report RJ2770B (ICI5504/0280) EU agreed (United Kingdom, 2009a, 2009b)

Method type	Matrix type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / Report No. / EU agreed
RAM 305/03 (method also used for post-registration purposes, Section 5.3.2.2)	High protein/high starch content (dry) - <i>Wheat grain</i>	0.01 mg/kg	LC-MS/MS	<i>Method:</i> Chaggar S. <i>et al.</i> , 2004 / Report RAM 305/03 (ICI5504/2686) <i>Validation:</i> Lister N.J., 1999 / Report RJ2770B (ICI5504/0280) Chaggar S., 2004 / Report RJ3552B (ICI5504/2636) EU agreed (United Kingdom, 2009a, 2009b) <i>Validation (honey):</i> Bocksch S, 2008 / Report T011298-06-REG (VV-382035) <i>New data</i>
	High water content - <i>Cabbage</i>	0.01 mg/kg		
	High acid content - <i>Mandarin</i>	0.01 mg/kg		
	High oil content - <i>Sunflower seed</i>	0.01 mg/kg		
	No group - <i>Beer</i> - <i>Wheat straw</i> - <i>Wheat flour</i> - <i>Honey</i>	0.01 mg/kg		
Component of residue definition for animal products: azoxystrobin (EFSA, 2010) ^{(a)(b)}				
RAM 255/01	Milk	0.001 mg/kg	GC-NPD	<i>Method & Validation:</i> Ryan J., Sapiets A. / 1994, Report RAM 255/01 (ICI5504/1012) EU agreed (United Kingdom, 2009a, 2009b)
	Muscle	0.01 mg/kg		
	Fat	0.01 mg/kg		
	Liver	0.01 mg/kg		
RAM 255/02	Eggs	0.01 mg/kg	GC-NPD	<i>Method & Validation:</i> Ryan J. <i>et al.</i> , 1995 / Report RAM 255/02 (ICI5504/1014) EU agreed (United Kingdom, 2009a, 2009b)
RAM 255/03	No formal validation conducted (minor update of RAM 255/02)	See above	GC-NPD	<i>Method & Validation:</i> Ryan J. <i>et al.</i> , 1996 / Report RAM 255/03 (ICI5504/1421) EU agreed (United Kingdom, 2009a, 2009b)
RAM 255/04	No formal validation conducted (minor update of RAM 255/02 and 03)	See above	GC-NPD	<i>Method & Validation:</i> Ryan J. <i>et al.</i> , 1997 / Report RAM 255/04 (ICI5504/0257) EU agreed (United Kingdom, 2009a, 2009b)
RAM 399/01 (method also used for post-registration purposes, (see Section 5.3.2.3))	Milk (bovine)	0.01 mg/kg	LC-MS/MS	<i>Method:</i> xxxxxx. 2002 / Report RAM 399/01 (VV-124385) New data <i>Validation:</i> Richards S., 2002 / Report RJ3350B (VV-331095) <i>New data</i>
	Eggs (hen)	0.01 mg/kg		
	Muscle (bovine)	0.01 mg/kg		
	Fat (bovine)	0.01 mg/kg		
	Kidney (lamb)	0.01 mg/kg		
	Liver (lamb)	0.01 mg/kg		

(a): Residue definition for risk assessment

(b): The RMS, following evaluation of the confirmatory data addressing the data gaps identified during the MRL review, concluded that ‘the group of goat metabolites referred to in the open point of the Peer Review of azoxystrobin (L1, L4 and L9) and K1, the glucuronide conjugate of L1 are not deemed to be of toxicological concern and should not be included in the

definition of the residue for risk assessment of azoxystrobin in animal products, which should remain as parent azoxystrobin only, because all of the compounds fell considerably below the toxicological threshold of concern for both chronic and acute risk when considering all the uses on commodities potentially fed to livestock as assessed in the EFSA Article 12 review of azoxystrobin MRLs (EFSA Journal 2013;11(12):3497)' (UK, 2019). However, EFSA highlighted that 'further risk management considerations should be given to decide whether the argument of the low exposure calculated for metabolites L1, L4, L9 and K1 (conjugate of L1) is acceptable to waive the need to submit data on the general toxicity of L1, L4 and L9. Meanwhile, the residue definition for risk assessment in animal commodities is still deemed tentative' (EFSA, 2020).

Statement on extraction efficiency (plant products)

Suitable methods are available for the determination of residues of azoxystrobin in plant products. The available methods support this submission.

Table 5.2-12: Statement on extraction efficiency

	Method for products of plant origin
Required, available from:	-
Not required, because:	This is a request for a new product authorisation for which no new MRL is required. In line with SANTE 2017/10632 (Rev. 3, 22 November 2017), the data requirements used for the latest renewal or approval should be considered. Since azoxystrobin is currently under old data requirements, the demonstration of extraction efficiency is not required. However, it should be noted that RAM 305/03 uses 90/10 v/v acetonitrile/water to extract azoxystrobin from plant tissues. As part of the validation of analytical method RAM 305/01, which also uses 90/10 v/v acetonitrile/water as its extraction system, radiolabelled samples of wheat straw and grapes from metabolism studies were analysed. This analysis verified the extraction efficiency of parent azoxystrobin and metabolite R230310 as >85% extracted thus validating the suitability of the extraction solvents. Lister N.J., 1999 / Report RJ2770B / EU agreed (United Kingdom, 2009a).

Statement on extraction efficiency (animal products)

Suitable methods are available for the determination of residues of azoxystrobin in animal products. The available methods support this submission.

Table 5.2-13: Statement on extraction efficiency

	Method for products of animal origin
Required, available from:	-
Not required, because:	This is a request for a new product authorisation for which no new MRL is required. In line with SANTE 2017/10632 (Rev. 3 22 November 2017), the data requirements used for the latest renewal or approval should be considered. Since azoxystrobin is currently under old data requirements, the demonstration of extraction efficiency is not required. However, it should be noted that method RAM 399/01 uses the same extraction solvent system as RAM 255/03 (previously evaluated at EU level and not summarised here). Method RAM 255/03 states that the radiolabelled liver samples from the animal metabolism study were analysed using the methodology and verified using the extraction solvent system (acetonitrile). 90% of parent and metabolite R230310 were extracted. Ryan J. <i>et al.</i> , 1996 / Report RAM 255/03 / EU agreed (United Kingdom, 2009a, 2009b)

zRMS comments:

During the commenting period Applicant submitted the following information:

Since azoxystrobin was last renewed prior to the implementation of the extraction efficiency guideline SANTE/2017/10632 (implementation date: 23 Nov 2019) and, hence, an assessment of extraction efficiency was not required during the renewal, the demonstration of extraction efficiency is not required to support this product submission.

The Applicant Syngenta confirms that, in accordance with SANTE/2017/10632, an assessment of extraction

efficiency has been provided in the ongoing renewal (AIR) submission for the active substance azoxystrobin for all analytical methods used to support the analysis of residues in residue studies. Extraction efficiency has been robustly demonstrated for relevant solvent systems in representatives from each of the relevant crop and animal matrix groups in accordance with SANTE/2020/12830.

Table 5.2-14: Methods and relationship to studies presented in Appendix 2 document Part B, Section 7

Method	Study (Part B Section 7)		
Identifier	Data Point	Report Reference	Crop/Matrix
RAM 305/03	KCA1 6.3	R B6259 S16-03752 S11-00518-REG S11-00519-REG	Tomato (fruit)
RAM 305/03	KCA1 6.3	FSGD-067-REG FSGD-066-REG T009417-07-REG T009418-07-REG	Bell pepper (fruit)
RAM 305/03	KCA1 6.3	FSGD-059-REG FSGD-060-REG T009551-07-REG T009546-07-REG T011469-06-REG	Cucumber (fruit)
RAM 305/03	KCA1 6.3	T011465-06-REG T009544-07-REG FSGD-055-REG FSGD-056-REG FSGD-003	Melon (peel, pulp)
RAM 305/03	KCA1 6.3	S12-01256 S12-01257 T001111-09-REG	Lettuce (leaf)
RAM 305/02	KCA1 6.3	CEMR-1735 CEMR-1733 CEMR-1736 CEMR-1737	Lettuce (leaf)
	KCA1 6.10	T011298-06-REG	Honey
RAM 243/05	KCA1 6.5.3	RJ2488B	Tomato (processing)

Table 5.2-15: Validated methods for the generation of pre-authorization data for azoxystrobin in water and medium (KCP 5.1.2.6 in support of ecotoxicological studies)
Refer to EFSA Conclusion 2010 for previously reviewed active substance methods.

Table 5.2-16: Validated methods for the generation of pre-authorization data in support of A22773A in water and other matrices (KCP 5.1.2.6 in support of ecotoxicological studies)

Component of residue definition: Azoxystrobin				
Method type	Matrix type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
Primary	Water	0.0625 mg A22773A/L (0.0141 mg a.i./L)	LC-MS/MS	Method & Validation: Beuter L-K, 2020 Report S20-05053 (VV-884613) New Data
Primary	Medium	0.0625 mg A22773A/L (0.0141 mg a.i./L)	LC-MS/MS	Method & Validation: Beuter L-K, 2020 Report S20-05052 (VV-884821) New Data

Component of residue definition: Azoxystrobin				
Method type	Matrix type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
Primary	Medium	0.00954 mg A22773A/L (0.00215 mg a.i./L)	LC-MS/MS	Method & Validation: Obert-Rausser P, 2020 Report S20-05054 (VV-884825) New Data
GRM057.01A (see also in 5.3 – Methods for post-authorization control and monitoring purposes (KCP 5.2.5))	Drinking and surface water	0.05 µg a.i./L ^{a)}	LC-MS/MS (2 transitions)	Method: Amic S., 2012 Report GRM057.01A (VV-128281) Validation: Amic S., 2012a Report S11-03538 (VV-401211) New Data

a) Method also validated for metabolite R234886 (LOQ 0.05 µg/L)

Table 5.2-17: Validated methods for the generation of pre-authorization data in support of A12705B in stock solution (KCP 5.1.2.6 in support of ecotoxicological studies)

Component of residue definition: Azoxystrobin				
Method type	Matrix type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
Primary	Stock solution	25 g A12705B/L (5.7 g a.i./L)	HPLC-UV	Method & Validation: Ehmke A., 2015 Report 100921032 (VV-414544) New Data

Table 5.2-18: Methods and relationship to studies presented in document Part B, Section 9

Method	Supported study (Part B Section 9)	
Identifier	Data Point	Report Reference
Water LC-MS/MS method	KCP 10.2.1	Report S20-05053 (study target organism: <i>Oncorhynchus mykiss</i>)
Medium LC-MS/MS method	KCP 10.2.1	Report S20-05052 (study target organism: <i>Daphnia magna</i>)
Medium LC-MS/MS method	KCP 10.2.1	Report S20-05054 (study target organism: <i>Raphidocelis subcapitata</i>)
Stock solution HPLC-UV method	KCP 10.3.1.2	Report 100921032 (study target organism: <i>Apis mellifera</i>)
GRM057.01A (water monitoring method)	KCP 10.6.2	Report 159471087 (study target organism: non-target plant species, i.e. <i>Brassica napus</i> , <i>Brassica oleracea</i> , <i>Glycine max</i> , <i>Beta vulgaris</i> , <i>Lactuca sativa</i> , <i>Cucumis sativus</i> , <i>Zea mays</i> , <i>Lolium perenne</i> , <i>Allium cepa</i> , <i>Avena sativa</i>)

Table 5.2-19: Validated methods for the generation of pre-authorization data for azoxystrobin in water, buffer solutions (KCP 5.1.2.7 in support of physical and chemical properties tests)

Table not included;

No specific analytical methods were used to support the physical and chemical properties generated on this product.

5.2.3 Methods for the determination of residues of oxathiapiprolin (KCP 5.1.2)

An overview on the acceptable methods and possible data gaps for analysis of residues of oxathiapiprolin

for the generation of pre-authorization data is given in the following tables. For the detailed evaluation of new studies it is referred to Appendix 2.

Table 5.2-20: Validated methods for the generation of pre-authorization data for oxathiapiprolin in soil, water, air (KCP 5.1.2.1 in support of environmental fate studies)

Table not included;

No specific analytical methods were used to support the environmental fate studies generated on this product.

Table 5.2-21: Validated methods for the generation of pre-authorization data for oxathiapiprolin in soil, water (KCP 5.1.2.2 in support of efficacy studies)

Table not included;

No specific analytical methods were used to support the efficacy data generated on this product.

Table 5.2-22: Validated methods for the generation of pre-authorization data for oxathiapiprolin in feed, body fluids and tissues and air (KCP 5.1.2.3 in support of toxicological studies)

Table not included;

No analytical methods were used to support the toxicology data generated on this product.

Table 5.2-23: Validated methods for the generation of pre-authorization data for oxathiapiprolin in body fluids, air and any additional matrices used (KCP 5.1.2.4 in support of operator, worker, resident and bystander exposure studies)

Table not included;

No specific operator, worker, resident or bystander exposure studies were conducted to support this product. Consequently no analytical methods were required.

Table 5.2-24: Validated methods for the generation of pre-authorization data for oxathiapiprolin in plant and animal products (KCP 5.1.2.5 in support of residues studies)

Method type	Matrix type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / Report No. / EU agreed
Component of residue definition for plant products: oxathiapiprolin (EFSA, 2016)^(a)				
DuPont-30422	High protein/high starch content (dry) - <i>Wheat grain</i> - <i>Potatoes</i>	0.01 mg/kg	LC-MS/MS	Method: Henze R.M. & Stry J.J., 2011a, 2013 / Report No. DuPont-30422 Validation: Brown D. & Woodmnansey L., 2012 / Report No. DuPont-31091 EU agreed (Ireland, 2015) Validation: Donald C. & Gibson R., 2020 / Report No. 231693 Syngenta File No. VV-870136 New Data Method: Lakaschus S. & Reinhardt R., 2020 / Report No. S19-02718 Syngenta File No. VV-854039 New Data
	High water content - <i>Grapes</i> - <i>Tomatoes</i> - <i>Wheat forage</i> - <i>Pepper</i> - <i>Cucumber</i> - <i>Melon</i> - <i>Leek</i> - <i>Broccoli</i> - <i>Cauliflower</i> - <i>Cabbage</i> - <i>Kale</i> - <i>Brussel sprouts</i> - <i>Lettuce</i>	0.01 mg/kg		
	High oil content - <i>Canola seed</i> - <i>Soybean, hops</i>	0.01 mg/kg		
	No group - <i>Wheat straw</i>	0.01 mg/kg		

Method type	Matrix type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / Report No. / EU agreed
	Processed commodities - <i>Grapes pomace</i> - <i>Grape juice</i> - <i>Wine</i>	0.01 mg/kg		
DuPont-33818 (Supplement 1 to DuPont-30422)	High protein/high starch content (dry) - <i>Wheat grain</i> - <i>Potatoes</i>	0.01 mg/kg	LC-MS/MS	<i>Method:</i> Chapleo S., Inns L., 2013 / Report No. Dupont-33818
	High water content - <i>Grapes</i> - <i>Tomatoes</i> - <i>Ginseng</i> - <i>Wheat forage</i>	0.01 mg/kg		<i>Validation:</i> Within in the report EU agreed (Ireland, 2015)
	High oil content - <i>Canola seed</i> - <i>Soybean</i>	0.01 mg/kg		<i>Validation (honey):</i> Ford, K., 2020 / Report No. CEMR-9533 Syngenta File No. VV-885771
	No group - <i>Wheat straw</i>	0.01 mg/kg		New Data
	High acid content - <i>Oranges</i> ^(c)	0.01 mg/kg		
	Other - <i>Honey</i>	0.01 mg/kg		
	Component of residue definition for animal products: oxathiapiprolin (EFSA, 2016)^(a)			
Please refer to enforcement methods, point 5.3.3.3.				

(a): Residue definition for risk assessment

Statement on extraction efficiency

Suitable methods are available for the generation of pre-authorisation data for oxathiapiprolin in or on food of plant origin. The available methods support this submission.

Table 5.2-25: Statement on extraction efficiency

	Method for products of plant origin
Required, available from:	Extraction efficiency was demonstrated for all EU crop groups for which incurred radiolabelled residues were generated during the radiolabelled metabolism studies (Chapleo. S., Inns, L., 2013, Dupont-33818; Inns, L., 2012, DuPont-36106). Extraction efficiency was demonstrated for the watery (foliage), acidic (grape berries), and dry (wheat grain) EU crop groups. The radiolabelled metabolism studies conducted did not generate any incurred residues samples that could be classified as an oily crop matrices. In addition, the MOR studies did not generate any samples with incurred residues greater than 0.01 mg/kg in oily crops that could be used to demonstrate extraction efficiency. Due to the lack of oily crop incurred residue samples extraction efficiency was tested on only three of the four EU crop groups. The residue method extraction procedure removed between 98-113% (for method: Henze, R.M., Stry, J.J., 2011b, DuPont-30422), 81.3-103.3% (for method Weber, H., 2012a; DuPont-31140), 77.5-81.2% (for method Schwarz, T., 2009, DuPont-28696) of the incurred residue removed by the metabolism extraction procedure. For the crop matrices tested the residue method demonstrated the ability to extract incurred residues. EU agreed (Ireland, 2015)
Not required, because:	-

zRMS comments:

During the commenting period Applicant submitted the following information:

The Applicant Syngenta would like to highlight that the Technical Guideline on the Evaluation of Extraction Efficiency, SANTE/2017/10632, states that 'for renewal of product authorisations or for new product authorisations or extension of uses for which no change of the MRL is needed, the data requirements used for the latest renewal or approval should be considered. This means that no additional proof of extraction efficiency is required if it had not been required in the renewal of approval/approval procedure itself.' Since oxathiapiprolin was last renewed prior to the implementation of the extraction efficiency guideline SANTE/2017/10632 (implementation date: 23 Nov 2019) and, hence, an assessment of extraction efficiency was not required during the renewal, the demonstration of extraction efficiency is not required to support this product submission.

Additionally, it should be noted that an ILV for hops was submitted as part of the evaluation of the MRL for oxathiapiprolin in hops (EFSA Journal 2019;17(7):5759)

EFSA concluded that *The applicant submitted validation data (including confirmatory data) and an ILV for the multi-residue DFG-19 method which was proposed as enforcement method by the peer review. Validation data were provided for different matrices that are considered as complex: coffee beans, hops (dried), black tea (leaves) and dried tobacco (Ireland, 2017a). The validation data demonstrate that DFG-19 method is acceptable to enforce residues of oxathiapiprolin in coffee beans, hops, black tea and dried tobacco at the LOQ of 0.01 mg/kg.*

No additional data are required to support this product submission.

Table 5.2-26: Methods and relationship to studies presented in Appendix 2 document Part B, Section 7

Method	Study (Part B Section 7)		
Identifier	Data Point	Report Reference	Crop/Matrix
DuPont-30422 (inc. CRL method 1846A and A.P.1846cV2.01) ⁽¹⁾	KCA2 6.3	Dupont-31737	Tomato (fruit)
	KCA2 6.3	DuPont-31731	Bell pepper (fruit)
	KCA2 6.3	DuPont-31733	Cucumber (fruit)
	KCA2 6.3	Dupont-31746	Melon (peel, pulp)
	KCA2 6.3	IF20-05334826	Lettuce (leaf)
	KCA2 6.10	CEMR-9533	Honey

(1) CRL method 1846A and A.P.1846cV2.01 are based on DuPont-30422

Table 5.2-27: Validated methods for the generation of pre-authorization data for oxathiapiprolin in test solution and other matrices (KCP 5.1.2.6 in support of ecotoxicological studies)

Refer to EFSA Conclusion 2016 for previously reviewed active substance methods.

Component of residue definition: Oxathiapiprolin				
Method type	Matrix type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
Primary	Test solution	0.6 g test item/L	HPLC-UV	Method & Validation: Tänzler V., 2015 Report 94441136 New Data
Primary	Larval diet Acetone	0.5 mg a.i./kg 150 mg a.i./L	HPLC-MS/MS	Method & Validation: Oberrauch S., 2017 Report S17-01639 New Data

Table 5.2-28: Validated methods for the generation of pre-authorization data in support of A22773A solution (KCP 5.1.2.6 in support of ecotoxicological studies)

Component of residue definition: Oxathiapiprolin				
Method type	Matrix type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
Primary ECO_052_03A	Honeybee larval diet (royal jelly)	0.009 mg a.i./kg	HPLC-MS/MS	Method & Validation: Lünsmann V., 2020

Component of residue definition: Oxathiapiprolin				
Method type	Matrix type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
	Adult honey bee feeding solution (aqueous 50% w/v sucrose solution)	0.009 mg a.i./kg		Report 20 35 CRB 0103 (VV-884296) New Data
Primary (ECO_052_03B)	Adult bumble bee contact test solution (0.5% v/v TritonX solution)	0.010 mg a.i./L	HPLC-MS/MS	Method & Validation: Lünsmann V., 2022 Report 21 35 CRB 0127 (VV-948172) New Data

Table 5.2-29: Methods and relationship to studies presented in document Part B, Section 9

Method	Supported study (Part B Section 9)	
Identifier	Data Point	Report Reference
ECO_052_03A	KCP 10.3.1.2	Report 20 48 BAC 0043 (study target organism: <i>Apis mellifera</i> , <i>Chronic Adult formulation</i>)
	KCP 10.3.1.3	Report 20 48 BLC 0043 (study target organism: <i>Apis mellifera</i> , <i>Chronic Larval formulation</i>)
ECO_052_03B	KCP 10.3.1.1.1	Report 21 48 BBA 0032 (study target organism: <i>Bombus terrestris</i> , <i>acute oral</i>)
	KCP 10.3.1.1.2	Report 21 48 BBA 0032 (study target organism: <i>Bombus terrestris</i> , <i>acute contact</i>)
Test solution HPLC-UV method	KCA 8.3.1.2	Report 94441136 (study target organism: <i>Apis mellifera</i> , <i>chronic adult</i> , <i>active substance</i>)
Larval diet & acetone HPLC-MS/MS method	KCA 8.3.1.3	Report S17-01639 (study target organism: <i>Apis mellifera</i> , <i>chronic larval</i> , <i>active substance</i>)

Table 5.2-30: Validated methods for the generation of pre-authorization data for oxathiapiprolin in water, buffer solutions (KCP 5.1.2.7 in support of physical and chemical properties tests)

Table not included;

No specific analytical methods were used to support the physical and chemical properties generated on this product.

5.3 Methods for post-authorization control and monitoring purposes (KCP 5.2)

5.3.1 Analysis of the plant protection product (KCP 5.2)

Analytical methods for the determination of the active substance and relevant impurities in the plant protection product shall be submitted, unless the applicant shows that these methods already submitted in accordance with the requirements set out in point 5.2.1 can be applied.

5.3.2 Description of analytical methods for the determination of residues of azoxystrobin (KCP 5.2)

5.3.2.1 Overview of residue definitions and levels of azoxystrobin for which compliance is required

Compared to the residue definition proposed in the Draft Assessment Report (incl. its addenda) the current legal residue definition is identical.

Table 5.3-1: Relevant residue definitions for monitoring/enforcement and levels of azoxystrobin for which compliance is required

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Plant, high water content	Azoxystrobin	0.01 mg/kg	MRL Regulation (EU) 2019/552 Reg. (EU) 2022/476 Reg. (EU) 2023/129
Plant, high acid content		0.01 mg/kg	MRL Regulation (EU) 2019/552 Reg. (EU) 2022/476 Reg. (EU) 2023/129
Plant, high protein/high starch content (dry commodities)		0.01 mg/kg	MRL Regulation (EU) 2019/552 Reg. (EU) 2022/476 Reg. (EU) 2023/129
Plant, high oil content		0.01 mg/kg	MRL Regulation (EU) 2019/552 Reg. (EU) 2022/476 Reg. (EU) 2023/129
Plant, difficult matrices (hops, spices, tea)		0.05 mg/kg	MRL Regulation (EU) 2019/552 Reg. (EU) 2022/476 Reg. (EU) 2023/129
Muscle	Azoxystrobin The definition of residues and MRLs for animal commodities should be considered as tentative. This is due to the absence of additional information on the toxicological relevance of metabolites L1, L4 and L9 (EFSA, 2010) ⁽¹⁾	0.01 mg/kg	MRL Regulation (EU) 2019/552 Reg. (EU) 2022/476 Reg. (EU) 2023/129
Milk		0.01 mg/kg	MRL Regulation (EU) 2019/552 Reg. (EU) 2022/476 Reg. (EU) 2023/129
Eggs		0.01 mg/kg	MRL Regulation (EU) 2019/552 Reg. (EU) 2022/476 Reg. (EU) 2023/129
Fat		0.01 mg/kg	MRL Regulation (EU) 2019/552 Reg. (EU) 2022/476 Reg. (EU) 2023/129
Liver, kidney		0.01 mg/kg	MRL Regulation (EU) 2019/552 Reg. (EU) 2022/476 Reg. (EU) 2023/129
Soil (Ecotoxicology)	Azoxystrobin	0.05 mg/kg	Common limit SANTE/2020/12830, Rev.1
Drinking water (Human toxicology)	Azoxystrobin	0.1 µg/L	general limit for drinking water SANTE/2020/12830, Rev.1

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Surface water (Ecotoxicology)	Azoxystrobin	9.54 µg/L	<i>Mysidopsis bahia</i> , reproduction (based on adult mortality), 28- day study (EFSA, 2010)
Air	Azoxystrobin	3 µg/m ³	AOEL sys/AOEL inhal: 0.2 mg/kg bw/d (EFSA, 2010)
Tissue (meat or liver)	Azoxystrobin	0.01 mg/kg	Requested under (EU) No 283/2013
Body fluids		0.01 mg/L	Requested under (EU) No 283/2013 SANTE/2020/12830, Rev.1

- (1) The RMS, following evaluation of the confirmatory data addressing the data gaps identified during the MRL review, concluded that ‘the group of goat metabolites referred to in the open point of the Peer Review of azoxystrobin (L1, L4 and L9) and K1, the glucuronide conjugate of L1 are not deemed to be of toxicological concern and should not be included in the definition of the residue for risk assessment of azoxystrobin in animal products, which should remain as parent azoxystrobin only, because all of the compounds fell considerably below the toxicological threshold of concern for both chronic and acute risk when considering all the uses on commodities potentially fed to livestock as assessed in the EFSA Article 12 review of azoxystrobin MRLs (EFSA Journal 2013;11(12):3497)’ (UK, 2019). However, EFSA highlighted that ‘further risk management considerations should be given to decide whether the argument of the low exposure calculated for metabolites L1, L4, L9 and K1 (conjugate of L1) is acceptable to waive the need to submit data on the general toxicity of L1, L4 and L9. Meanwhile, the residue definition for risk assessment in animal commodities is still deemed tentative’ (EFSA, 2020).

5.3.2.2 Description of analytical methods for the determination of residues of azoxystrobin in plant matrices (KCP 5.2.1)

An overview on the acceptable methods and possible data gaps for analysis of azoxystrobin in plant matrices is given in the following tables. For the detailed evaluation of new studies it is referred to Appendix 2.

Table 5.3-2: Validated methods for food and feed of plant origin

Component of residue definition: azoxystrobin				
Matrix	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / Report No. / EU agreed
Method DFG S19 (extended revision)				
High water content - Lettuce - Kohlrabi - Garlic	Primary	0.01 mg/kg	LC-MS/MS	Validation: Weeren R., Pelz S., 2001 / Report ZEN- 0002V (VV-327232) <i>New data</i> Stahl F., 2017 / Report IF-04/00192716 (VV-379800) <i>New data</i>
	ILV	0.01 mg/kg	LC-MS/MS	ILV: Lakaschus S., Gizler A., 2017 / Report SYN-0422V (VV-380727) <i>New data</i>
	Confirmatory	-	-	Not required, primary method is highly specific

Component of residue definition: azoxystrobin				
Matrix	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / Report No. / EU agreed
High acid content - <i>Orange</i>	Primary	0.01 mg/kg	LC-MS/MS	<i>Validation:</i> Weeren R., Pelz S., 2001 / Report ZEN-0002V (VV-327232) <i>New data</i> Stahl F., 2017 / Report IF-04/00192716 (VV-379800) <i>New data</i>
	ILV	-	-	Not available
	Confirmatory	-	-	Not required, primary method is highly specific
High oil content - <i>Oilseed rape</i>	Primary	0.01 mg/kg	LC-MS/MS	<i>Validation:</i> Stahl F., 2017 / Report IF-04/00192716 (VV-379800) <i>New data</i>
	ILV	-	-	Not available
	Confirmatory	-	-	Not required, primary method is highly specific
High protein/high starch content (dry) - <i>Wheat, grain</i>	Primary	0.01 mg/kg	LC-MS/MS	<i>Validation:</i> Stahl F., 2017/ Report IF-04/00192716 (VV-379800) <i>New data</i>
	ILV	0.01 mg/kg	LC-MS/MS	<i>ILV:</i> Lakaschus S., Gizler A. 2017 / Report SYN-0422V (VV-380727) <i>New data</i>
	Confirmatory	-	-	Not required, primary method is highly specific
Difficult to analyse - <i>Camomille</i> - <i>Tea</i> - <i>Fennel seed</i>	Primary	0.05 mg/kg	LC-MS/MS	<i>Validation:</i> Weeren R., Pelz S., 2001 / Report ZEN-0002V (VV-327232) <i>New data</i>
	ILV	-	-	Not available
	Confirmatory	-	-	Not required, primary method is highly specific
Method RAM 305/02 and RAM 305/03 (see also Section 5.3.2.2)				
High water content - <i>Cabbage</i> - <i>Leek</i> - <i>Lettuce</i> - <i>Melon</i> - <i>Onion</i> - <i>Pear</i> - <i>Plum</i> - <i>Tomato</i> - <i>Pea (with pods)</i> - <i>Wheat (forage)</i>	Primary	0.01 mg/kg	LC-MS/MS	<i>Method:</i> Robinson N.J. <i>et al.</i> , 1999 / Report RAM 305/02 (ICI5504/0249) Chaggar S. <i>et al.</i> , 2004 / Report RAM 305/03 (ICI5504/2686) <i>Validation:</i> Lister N.J., 1999 / Report RJ2770B (ICI5504/0280) Chaggar S., 2004 / Report RJ3552B (ICI5504/2636) EU agreed (United Kingdom, 2009a)
	ILV	0.01 mg/kg	LC-MS/MS	<i>ILV:</i> Kang J., 2003 / Report CEMR-1708 (ICI5504/1411) Croucher A., 2002 / Report 1983/029-D2149 (ICI5504/1336) EU agreed (United Kingdom, 2009a)

Component of residue definition: azoxystrobin				
Matrix	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / Report No. / EU agreed
	Confirmatory	-	-	Not required, primary method is highly specific
High acid content - Grape - Orange (peel and pulp) - Mandarin - Strawberry	Primary	0.01 mg/kg	LC-MS/MS	<i>Method:</i> Robinson N.J. <i>et al.</i> , 1999 / Report RAM 305/02 (ICI5504/0249) Chaggar S. <i>et al.</i> , 2004 / Report RAM 305/03 (ICI5504/2686) <i>Validation:</i> Lister N.J., 1999 / Report RJ2770B (ICI5504/0280) Chaggar S. <i>et al.</i> , 2004 / Report RJ3552B (ICI5504/2636) EU agreed (United Kingdom, 2009a)
	ILV	-	-	Not available
	Confirmatory	-	-	Not required, primary method is highly specific
High oil content - Avocado - Oilseed rape - Sunflower seed	Primary	0.01 mg/kg	LC-MS/MS	<i>Method:</i> Robinson N.J. <i>et al.</i> , 1999 / Report RAM 305/02 (ICI5504/0249) Chaggar S. <i>et al.</i> , 2004 / Report RAM 305/03 (ICI5504/2686) <i>Validation:</i> Lister N.J., 1999 / Report RJ2770B (ICI5504/0280) Chaggar S., 2004 / Report RJ3552B (ICI5504/2636) EU agreed (United Kingdom, 2009a)
	ILV	-	-	Not available
	Confirmatory	-	-	Not required, primary method is highly specific
High protein/high starch content (dry) - Barley, wheat grain - Lentil - Carrot - Potato - Sugarbeet - Maize kernels	Primary	0.01 mg/kg	LC-MS/MS	<i>Method:</i> Robinson N.J. <i>et al.</i> , 1999 / Report RAM 305/02 (ICI5504/0249) Chaggar S. <i>et al.</i> , 2004 / Report RAM 305/03 (ICI5504/2686) <i>Validation:</i> Lister N.J., 1999 / Report RJ2770B (ICI5504/0280) Chaggar S., 2004 / Report RJ3552B (ICI5504/2636) Croucher A., 2002 / Report 1983/029-D2419 (ICI5504/1336) EU agreed (United Kingdom, 2009a)
	ILV	0.01 mg/kg	LC-MS/MS	<i>ILV:</i> Kang J., 2003 / Report CEMR-1708 (ICI5504/1411) EU agreed (United Kingdom, 2009a)
	Confirmatory			Not required, primary method is highly specific

Component of residue definition: azoxystrobin				
Matrix	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / Report No. / EU agreed
Difficult to analyse - Dried hops	Primary	0.01 mg/kg	LC-MS/MS	<i>Method:</i> Robinson N.J. <i>et al.</i> , 1999 / Report RAM 305/02 (ICI5504/0249) Chaggar S., 2004 / Report RAM 305/03 (ICI5504/2686) <i>Validation:</i> Chaggar S., 2004 / Report RJ3552B (ICI5504/0280) EU agreed (United Kingdom, 2009a)
	ILV	0.01 mg/kg	LC-MS/MS	<i>ILV:</i> Kang J., 2003 / Report CEMR-1995 (ICI5504/1976) EU agreed (United Kingdom, 2009a)
	Confirmatory	-	-	Not required, primary method is highly specific
No group - Beer - Wheat (straw) - Wheat flour - Honey	Primary	0.01 mg/kg	LC-MS/MS	<i>Method:</i> Robinson N.J. <i>et al.</i> , 1999 / Report RAM 305/02 (ICI5504/0249) Chaggar S. <i>et al.</i> , 2004 / Report RAM 305/03 (ICI5504/2686) <i>Validation:</i> Lister N.J., 1999 / Report RJ2770B (ICI5504/0280) Chaggar S., 2004 / Report RJ3552B (ICI5504/2636) EU agreed (United Kingdom, 2009a) <i>Validation (honey):</i> Bocksch S, 2008 / Report T011298-06-REG (VV-382035) New data
	ILV	-	-	Not available
	Confirmatory	-	-	Not required, primary method is highly specific

For any special comments or remarkable points concerning the analytical methods for the determination of residues in plant matrices, please refer to Appendix 2.

Table 5.3-3: Statement on extraction efficiency

	Method for products of plant origin
Required, available from:	<p>This is a request for a new product authorisation for which no new MRL is required. In line with SANTE 2017/10632 (Rev. 3 22 November 2017), the data requirements used for the latest renewal or approval should be considered. Since azoxystrobin is currently under old data requirements, the demonstration of extraction efficiency is not required.</p> <p>However, it should be noted that RAM 305/03 uses 90/10 v/v acetonitrile/water to extract azoxystrobin from plant tissues. As part of the validation of analytical method RAM 305/01, which also uses 90/10 v/v acetonitrile/water as its extraction system, radiolabelled samples of wheat straw and grapes from metabolism studies were analysed. This analysis verified the extraction efficiency of parent azoxystrobin and metabolite R230310 as >85% extracted thus validating the suitability of the extraction solvents.</p> <p>Lister N.J., 1999 / Report RJ2770B (ICI5504/0280) / EU agreed (United Kingdom, 2009a)</p>

	Method for products of plant origin
Not required, because:	-

5.3.2.3 Description of analytical methods for the determination of residues of azoxystrobin in animal matrices (KCP 5.2.2)

An overview on the acceptable methods and possible data gaps for analysis of azoxystrobin in animal matrices is given in the following tables. For the detailed evaluation of new studies it is referred to Appendix 2.

Table 5.3-4: Validated methods for food and feed of animal origin

Component of residue definition: azoxystrobin				
Matrix	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / Report No. / EU agreed
Method DFG S19 (extended revision)				
Milk	Primary	0.02 mg/kg	GC-MSD	<i>Validation:</i> xxxxx 1997 / Report ZEN-9505V (VV-323618) <i>New data</i>
	ILV	-	-	Not required (public multi-residue method)
	Confirmatory	-	-	Not available
Muscle	Primary	0.02 mg/kg	GC-MSD	<i>Validation:</i> xxxxxx 1997 / Report ZEN-9505V (VV-323618) <i>New data</i>
	ILV	-	-	Not required (public multi-residue method)
	Confirmatory	-	-	Not available
Liver	Primary	0.2 mg/kg	GC-MSD	<i>Validation:</i> xxxxxx 1997 / Report ZEN-9505V (VV-323618) <i>New data</i>
	ILV	-	-	Not required (public multi-residue method)
	Confirmatory	-	-	Not available
RAM 399/01 (see also Section 5.2.2)				
Milk (bovine)	Primary	0.01 mg/kg	LC-MS/MS	<i>Method:</i> xxxxxx. 2002 / Report RAM 399/01 (VV-124385) New data <i>Validation:</i> xxxxxx 2002 / Report RJ3350B (VV-331095) <i>New data</i>
	ILV	0.01 mg/kg	LC-MS/MS	<i>ILV:</i> Atkinson S., 2003 / Report CEMR-1907 (VV-328461) <i>New data</i>
	Confirmatory	-	-	Not required, primary method is highly specific

Component of residue definition: azoxystrobin				
Matrix	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / Report No. / EU agreed
Eggs (hen)	Primary	0.01 mg/kg	LC-MS/MS	Method: xxxxxx. 2002 / Report RAM 399/01 (VV-124385) New data Validation: Richards S., 2002 / Report RJ3350B (VV-331095) New data
	ILV	-	-	Not available
	Confirmatory	-	-	Not required, primary method is highly specific
Muscle (bovine)	Primary	0.01 mg/kg	LC-MS/MS	Method: xxxxxx. 2002 / Report RAM 399/01 (VV-124385) New data Validation: Richards S., 2002 / Report RJ3350B (VV-331095) New data
	ILV	0.01 mg/kg	LC-MS/MS	ILV: Atkinson S., 2003 / Report CEMR-1907 (VV-328461) New data
	Confirmatory	-	-	Not required, primary method is highly specific
Fat (bovine)	Primary	0.01 mg/kg	LC-MS/MS	Method: xxxxxx. 2002 / Report RAM 399/01 (VV-124385) New data Validation: Richards S., 2002 / Report RJ3350B (VV-331095) New data
	ILV	-	-	Not available
	Confirmatory	-	-	Not required, primary method is highly specific
Kidney (lamb)	Primary	0.01 mg/kg	LC-MS/MS	Method: xxxxxx. 2002 / Report RAM 399/01 (VV-124385) New data Validation: Richards S., 2002 / Report RJ3350B (VV-331095) New data
	ILV	-	-	Not available
	Confirmatory	-	-	Not required, primary method is highly specific
Liver (lamb)	Primary	0.01 mg/kg	LC-MS/MS	Method: xxxxxx. 2002 / Report RAM 399/01 (VV-124385) New data Validation: Richards S., 2002 / Report RJ3350B (VV-331095) New data

Component of residue definition: azoxystrobin				
Matrix	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / Report No. / EU agreed
	ILV	-	-	Not available
	Confirmatory	-	-	Not required, primary method is highly specific

For any special comments or remarkable points concerning the analytical methods for the determination of residues in animal matrices, please refer to Appendix 2.

Table 5.3-5: Statement on extraction efficiency

	Method for products of animal origin
Required, available from:	<p>This is a request for a new product authorisation for which no new MRL is required. In line with SANTE 2017/10632 (Rev. 3 22 November 2017), the data requirements used for the latest renewal or approval should be considered. Since azoxystrobin is currently under old data requirements, the demonstration of extraction efficiency is not required.</p> <p>However, it should be noted that method RAM 399/01 uses the same extraction solvent system as RAM 255/03 (previously evaluated at EU level and not summarised here). Method RAM 255/03 states that the radiolabelled liver samples from the animal metabolism study were analysed using the methodology and verified using the extraction solvent system (acetonitrile). 90% of parent and metabolite R230310 were extracted.</p> <p>Ryan J. <i>et al.</i>, 1996 / Report RAM 255/03 (ICI5504/1421) / EU agreed (United Kingdom, 2009a, 2009b)</p>
Not required, because:	-

5.3.2.4 Description of methods for the analysis of azoxystrobin in body fluids and tissues (KCP 5.2.3)

An overview on the acceptable methods and possible data gaps for analysis of azoxystrobin in body fluids and tissues is given in the following tables. For the detailed evaluation of new studies it is referred to Appendix 2.

Table 5.3-6: Methods for body fluids and tissues

Component of residue definition: azoxystrobin				
Matrix	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / Report No. / EU agreed
Human whole blood	Primary (Method RAM 399/01)	0.01 µg/mL	LC-MS/MS	<p>Method and validation: Gemrot F., 2011 / Report S10-03815 (VV-398250)</p> <p>New data</p>

For any special comments or remarkable points concerning the analytical methods for body fluids and tissues please refer to Appendix 2.

5.3.2.5 Description of methods for the analysis of azoxystrobin in soil (KCP 5.2.4)

An overview on the acceptable methods and possible data gaps for analysis of azoxystrobin in soil is given in the following table.

Table 5.3-7: Validated methods for soil

Component of residue definition: azoxystrobin			
Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Primary (Method RAM 269/03)	0.02 mg/kg	LC-MS/MS (HPLC-UV confirmatory)	<i>Method and validation:</i> Johnson R.I. <i>et al.</i> , 2000 / Report No. RAM 269/03 / EU agreed (VV-123986)
Primary (Method GRM057.06A)	0.02 mg/kg ^{a)}	LC-MS/MS (2 transitions)	<i>Method:</i> Link T., Poperechna N., Crook S., 2019 / Report No. GRM057.06A / New data (VV-635391) <i>Validation:</i> Link T., Kravchuk O., 2019 / Report No. IF18-04490185/ New data (VV-635374)

a) Method also validated for metabolites R230310 and R234886 (LOQ 0.02 mg/kg) and R401553 (SYN501657) and R402173 (SYN501114) (LOQ 0.01 mg/kg)

For any special comments or remarkable points concerning the analytical methods for soil please refer to Appendix 2.

5.3.2.6 Description of methods for the analysis of azoxystrobin of water (KCP 5.2.5)

An overview on the acceptable methods and possible data gaps for analysis of azoxystrobin in surface and drinking water is given in the following tables. For the detailed evaluation of new/additional studies it is referred to Appendix 2.

Table 5.3-8: Validated methods for water

Component of residue definition: azoxystrobin				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Water	Primary (Method RAM 358/01)	0.1 µg/L	GC-MSD	<i>Method and validation:</i> Robinson N.J., 2000 / Report No. RAM 358/01 / EU agreed (VV-127880)
Drinking and surface water	Primary (Method GRM057.01A)	0.05 µg/L ^{a)}	LC-MS/MS (2 transitions)	<i>Method:</i> Amic S., 2012 / Report No. GRM057.01A / New data (VV-128281) <i>Validation:</i> Amic S., 2012a / Report No. S11- 03538 / New data (VV-401211)
Drinking water	ILV (Method GRM057.01A)	0.05 µg/L ^{a)}	LC-MS/MS (2 transitions)	Brown D., 2019 / Report No. RES-00193 / New data (VV-619234)
Ground and surface water	Primary (Method GRM057.04A)	0.1 µg/L ^{b)}	LC-MS/MS (2 transitions)	<i>Method:</i> Mayer L.C., 2012 / Report No. GRM057.04A / New data (VV-185347) <i>Validation:</i> Mayer L.C., 2012a / Report No. TK0120502 / New data (VV-506623)
	ILV	0.1 µg/L ^{b)}	LC-MS/MS	Smith R.J., 2012 / Report No.

Component of residue definition: azoxystrobin				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
	(Method GRM057.04A		(2 transitions)	1781.6873 / New data (VV-507766)

a) Method also validated for metabolite R234886 (LOQ 0.05 µg/L)

b) Method also validated for Z-isomer R230310 (LOQ 0.1 µg/L)

For any special comments or remarkable points concerning the analytical methods for water please refer to Appendix 2.

5.3.2.7 Description of methods for the analysis of azoxystrobin in air (KCP 5.2.6)

An overview on the acceptable methods and possible data gaps for analysis of azoxystrobin in air is given in the following table.

Table 5.3-9: Validated methods for air

Component of residue definition: azoxystrobin			
Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Primary (Method RAM 376/01)	3 µg/m ³	GC-MSD	<i>Method and validation:</i> Crawford N., 2001 / Report No. TMJ4658B / EU agreed (VV-321041)

For any special comments or remarkable points concerning the analytical methods for air it is referred to Appendix 2.

5.3.2.8 Other studies/ information

Not relevant.

5.3.3 Description of analytical methods for the determination of residues of oxathiapiprolin (KCP 5.2)

5.3.3.1 Overview of residue definitions and levels of oxathiapiprolin for which compliance is required

Compared to the residue definition proposed in the Draft Assessment Report (incl. its addenda) the current legal residue definition is identical.

Table 5.3-10: Relevant residue definitions for monitoring/enforcement and levels of oxathiapiprolin for which compliance is required

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Plant, high water content	Oxathiapiprolin	0.01 mg/kg (LOQ)	MRL Regulation (EU) No 2017/1016 Reg. (EU) 2021/1807 Reg. (EU) 2023/163
Plant, high acid content		0.01 mg/kg (LOQ)	MRL Regulation (EU) No 2017/1016 Reg. (EU) 2021/1807 Reg. (EU) 2023/163
Plant, high protein/high		0.01 mg/kg (LOQ)	MRL Regulation (EU) No

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
starch content (dry commodities)			2017/1016 Reg. (EU) 2021/1807 Reg. (EU) 2023/163
Plant, high oil content		0.01 mg/kg (LOQ)	MRL Regulation (EU) No 2017/1016 Reg. (EU) 2021/1807 Reg. (EU) 2023/163
Plant, difficult matrices (hops, spices, tea)		0.05 mg/kg (LOQ)	MRL Regulation (EU) No 2017/1016 Reg. (EU) 2021/1807 Reg. (EU) 2023/163
Muscle	Oxathiapiprolin	0.01 mg/kg (LOQ)	MRL Regulation (EU) No 2017/1016 Reg. (EU) 2021/1807 Reg. (EU) 2023/163
Milk		0.01 mg/kg (LOQ)	MRL Regulation (EU) No 2017/1016 Reg. (EU) 2021/1807 Reg. (EU) 2023/163
Eggs		0.01 mg/kg (LOQ)	MRL Regulation (EU) No 2017/1016 Reg. (EU) 2021/1807 Reg. (EU) 2023/163
Fat		0.01 mg/kg (LOQ)	MRL Regulation (EU) No 2017/1016 Reg. (EU) 2021/1807 Reg. (EU) 2023/163
Liver, kidney		0.01 mg/kg (LOQ)	MRL Regulation (EU) No 2017/1016 Reg. (EU) 2021/1807 Reg. (EU) 2023/163
Soil (Ecotoxicology)	Oxathiapiprolin	0.05 mg/kg Method LOQ: 0.001 mg/kg	Common limit NOEC for other soil macro-organisms (<i>Hypoaspis aculeifer</i>): 6.25 mg/kg dw soil (EFSA Journal 2016;14(7):4504)
Drinking water (Human toxicology)	Oxathiapiprolin	0.1 µg/L	general limit for drinking water SANTE/2020/12830, Rev.1
Surface water (Ecotoxicology)	Oxathiapiprolin	0.058 mg/L Method LOQ: 0.1 µg/L	NOEC for aquatic invertebrate (<i>Americamysis bahia</i>), reproduction, 32-day flow-through study (EFSA Journal 2016;14(7):4504)
Air	Oxathiapiprolin	0.05 µg/m ³	AOEL sys: 0.04 mg/kg bw/d
Tissue (meat or liver)	Not required	0.01 mg/kg (LOQ)	MRL Regulation (EU) No 2017/1016 Reg. (EU) 2021/1807 Reg. (EU) 2023/163 SANTE/2020/12830, Rev.1
Body fluids		0.05 0.01 mg/L	SANTE/2020/12830, Rev.1

5.3.3.2 Description of analytical methods for the determination of residues of oxathiapiprolin in plant matrices (KCP 5.2.1)

An overview on the acceptable methods and possible data gaps for analysis of oxathiapiprolin in plant matrices is given in the following tables. For the detailed evaluation of new studies it is referred to Appendix 2.

Table 5.3-11: Validated methods for food and feed of plant origin

Component of residue definition: Oxathiapiprolin				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
Single residue method DuPont-30422				
High water content - Ginseng - Tomatoes - Carrot roots - Spinach leaves - Wheat forage - Spinach - Broccoli - Whole pepper - Peppers - Cucumbers - Melon - Leek - Broccoli - Cauliflower - Cabbage - Kale - Brussels Sprouts	Primary	0.01 mg/kg	LC-MS/MS	<i>Method:</i> Henze R.M., Stry J.J., 2011b, 2013 / Reports DuPont-30422, Supplement No. 1 and 2 <i>Validation:</i> Brown D. & Woodmansey L., 2012 / Report DuPont-31091 Cairns S., et al., 2013 / Report DuPont-31091 Supplement No.1 Vincent T., 2013 / Report DuPont-31545 EU agreed (Ireland, 2015) <i>Validation:</i> Donald C. & Gibson R., 2020 / Report No. 231693 Syngenta File No. VV-870136 New Data
	ILV	0.01 mg/kg	LC-MS/MS	<i>ILV:</i> Lissemore L., Li P., 2013 / Report DuPont-37739 EU agreed (Ireland, 2015)
	Confirmatory (if required)	-	-	Not required, primary method is highly specific

Component of residue definition: Oxathiapiprolin				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
High acid content - Oranges - Grapes	Primary	0.01 mg/kg	LC-MS/MS	<p><i>Method:</i> Henze R.M., Stry J.J., 2011b, 2013 / Reports DuPont-30422, Supplement No. 1 and 2</p> <p><i>Validation:</i> Brown D., & Woodmnansey L., 2012 / Report DuPont-31091</p> <p>Cairns S., et al., 2013 / Report DuPont-31091 Supplement No.1</p> <p>Vincent T., 2013 / Report DuPont-31545</p> <p>EU agreed (Ireland, 2015)</p>
	ILV	0.01 mg/kg	LC-MS/MS	<p><i>ILV:</i> Lissemore L., Li P., 2013 / Report DuPont-37739</p> <p>EU agreed (Ireland, 2015)</p>
	Confirmatory (if required)	-	-	Not required, primary method is highly specific
High oil content - Canola, dried - Soybean seed	Primary	0.01 mg/kg	LC-MS/MS	<p><i>Method:</i> Henze R.M., Stry J.J., 2011b, 2013 / Reports: DuPont-30422, Supplement No. 1 and 2</p> <p><i>Validation:</i> Brown D., & Woodmnansey L., 2012 / Report: DuPont-31091</p> <p>Cairns S., et al., 2013 / Report: DuPont-31091 Supplement No.1</p> <p>Vincent T., 2013 / Report: DuPont-31545</p> <p>EU agreed (Ireland,2015)</p>
	ILV	-	-	-
	Confirmatory (if required)	-	-	Not required, primary method is highly specific

Component of residue definition: Oxathiapiprolin				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
High protein/high starch content (dry) - Wheat grain - Potatoes - Dried beans - Dried tobacco leaves - Dried ginseng roots - Dried bulb onion	Primary	0.01 mg/kg	LC-MS/MS	<p><i>Method:</i> Henze R.M., Stry J.J., 2011b, 2013 / Reports: DuPont-30422, Supplement No. 1 and 2</p> <p><i>Validation:</i> Brown D., & Woodmnansey L., 2012 / Report: DuPont-31091</p> <p>Cairns S., et al., 2013 / Report: DuPont-31091 Supplement No.1</p> <p>Vincent T., 2013 / Report: DuPont-31545</p> <p>EU agreed (Ireland,2015)</p>
	ILV	0.01 mg/kg	LC-MS/MS	<p><i>ILV:</i> Lissemore L., Li P., 2013 / Report DuPont-37739</p> <p>EU agreed (Ireland, 2015)</p>
	Confirmatory (if required)	-	-	Not required, primary method is highly specific
Difficult to analyse - Hops	Primary	0.01 mg/kg	LC-MS/MS	<p><i>Method:</i> Henze R.M. & Stry J.J., 2011a, 2013 / Report No. DuPont-30422</p> <p><i>Validation:</i> Brown D. & Woodmnansey L., 2012 / Report No. DuPont-31091</p> <p>EU agreed (Ireland, 2015)</p> <p><i>Validation:</i> Donald C. & Gibson R., 2020 / Report No. 231693 Syngenta File No. VV-870136</p> <p>New Data</p>
	ILV	-	-	-
	Confirmatory (if required)	-	-	Not required, primary method is highly specific

Component of residue definition: Oxathiapiprolin				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
No group - <i>Wheat straw</i> - <i>Honey</i>	Primary	0.01 mg/kg	LC-MS/MS	<p><i>Method:</i> Henze R.M., Stry J.J., 2011b, 2013 / Reports: DuPont-30422, Supplement No. 1 and 2</p> <p><i>Validation:</i> Brown D., & Woodmnansey L., 2012 / Report: DuPont-3109</p> <p>Cairns S., et al., 2013 / Report: DuPont-31091 Supplement No.1</p> <p>Vincent T., 2013 / Report: DuPont-31545</p> <p>EU agreed (Ireland,2015)</p> <p><i>Validation (honey):</i> Ford, K., 2020 / Report No. CEMR-9533 Syngenta File No. VV-885771</p> <p>New Data</p>
	ILV	-	-	-
	Confirmatory (if required)	-	-	Not required, primary method is highly specific
Processed commodities - <i>Potato chips</i> - <i>Dry grape pomace</i> - <i>Tomato juice</i>	Primary	0.01 mg/kg	LC-MS/MS	<p><i>Method:</i> Henze R.M., Stry J.J., 2011b, 2013 / Reports: DuPont-30422, Supplement No. 1 and 2</p> <p><i>Validation:</i> Brown D., & Woodmnansey L., 2012 / Report: DuPont-3109</p> <p>Cairns S., et al., 2013 / Report: DuPont-31091 Supplement No.1</p> <p>Vincent T., 2013 / Report: DuPont-31545</p> <p>EU agreed (Ireland,2015)</p>
	ILV	-	-	-
	Confirmatory (if required)	-	-	Not required, primary method is highly specific
Multi-residue method DFG S19				
High water content - <i>Apples</i> - <i>Tomatoes</i>	Primary	0.01 mg/kg	LC-MS/MS	<p><i>Method:</i> Weber H., 2012a / Report DuPont-31140</p> <p><i>Validation:</i> Within the report DuPont-31140</p> <p>EU agreed (Ireland, 2015)</p>

Component of residue definition: Oxathiapiprolin				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
	ILV	0.01 mg/kg	LC-MS/MS	ILV: Richter S., 2013 / Report DuPont-37477 EU agreed (Ireland, 2015)
	Confirmatory (if required)	-	-	Not required, primary method is highly specific
High acid content - <i>Citrus</i>	Primary	0.01 mg/kg	LC-MS/MS	<i>Method:</i> Weber H., 2012a / Report DuPont-31140 <i>Validation:</i> Within the report DuPont-31140 EU agreed (Ireland, 2015)
	ILV	-	-	-
	Confirmatory (if required)	-	-	Not required, primary method is highly specific
High oil content - <i>Oilseed rape</i>	Primary	0.01 mg/kg	LC-MS/MS	<i>Method:</i> Weber H., 2012a / Report DuPont-31140 <i>Validation:</i> Within the report DuPont-31140 EU agreed (Ireland, 2015)
	ILV	0.01 mg/kg	LC-MS/MS	ILV: Richter S., 2013 / Report DuPont-37477 EU agreed (Ireland, 2015)
	Confirmatory (if required)	-	-	Not required, primary method is highly specific
High protein/high starch content (dry) - <i>Barley grain</i>	Primary	0.01 mg/kg	LC-MS/MS	<i>Method:</i> Weber H., 2012a / Report DuPont-31140 <i>Validation:</i> Within the report DuPont-31140 EU agreed (Ireland, 2015)
	ILV	0.01 mg/kg	LC-MS/MS	ILV: Richter S., 2013 / Report DuPont-37477 EU agreed (Ireland, 2015)
	Confirmatory (if required)	-	-	Not required, primary method is highly specific
Multi-residue method QuEChERS				

Component of residue definition: Oxathiapiprolin				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
High water content - <i>Lettuce</i>	Primary	0.01 mg/kg	LC-MS/MS	<i>Method:</i> Schwarz T., 2009 / Report DuPont-28696 <i>Validation:</i> Within the report DuPont-28696 EU agreed (Ireland, 2015)
	ILV	-	-	-
	Confirmatory (if required)	-	-	Not required, primary method is highly specific
High acid content - <i>Oranges</i>	Primary	0.01 mg/kg	LC-MS/MS	<i>Method:</i> Schwarz T., 2009 / Report DuPont-28696 <i>Validation:</i> Within the report DuPont-28696 EU agreed (Ireland, 2015)
	ILV	-	-	-
	Confirmatory (if required)	-	-	Not required, primary method is highly specific
High protein/high starch content (dry) - <i>Maize grain</i> - <i>Wheat grain</i>	Primary	0.01 mg/kg	LC-MS/MS	<i>Method:</i> Schwarz T., 2009 / Report DuPont-28696 <i>Validation:</i> Within the report DuPont-28696 EU agreed (Ireland, 2015)
	ILV	-	-	-
	Confirmatory (if required)	-	-	Not required, primary method is highly specific

For any special comments or remarkable points concerning the analytical methods for the determination of residues in plant matrices, please refer to Appendix 2.

Table 5.3-12: Statement on extraction efficiency

	Method for products of plant origin
Required, available from:	Extraction efficiency was demonstrated for all EU crop groups for which incurred radiolabelled residues were generated during the radiolabelled metabolism studies (Chapleo. S., Inns, L., 2013, Dupont-33818; Inns, L., 2012, DuPont-36106). Extraction efficiency was demonstrated for the watery (foliage), acidic (grape berries), and dry (wheat grain) EU crop groups. The radiolabelled metabolism studies conducted did not generate any incurred residues samples that could be classified as an oily crop matrices. In addition, the MOR studies did not generate any samples with incurred residues greater than 0.01 mg/kg in oily crops that could be used to demonstrate extraction efficiency. Due to the lack of oily crop incurred residue samples extraction efficiency was tested on only three of the four EU crop groups. The residue method extraction procedure removed between 98-113% (for method: Henze, R.M., Stry, J.J., 2011b, DuPont-30422), 81.3-103.3% (for method Weber, H., 2012a; DuPont-31140), 77.5-81.2% (for method Schwarz, T., 2009, DuPont-28696) of the incurred residue removed by the metabolism extraction procedure. For the

	Method for products of plant origin
	crop matrices tested the residue method demonstrated the ability to extract incurred residues. EU agreed (Ireland, 2015)
Not required, because:	-

5.3.3.3 Description of analytical methods for the determination of residues of oxathiapiprolin in animal matrices (KCP 5.2.2)

An overview on the acceptable methods and possible data gaps for analysis of oxathiapiprolin in animal matrices is given in the following tables. For the detailed evaluation of new studies it is referred to Appendix 2.

Table 5.3-13: Validated methods for food and feed of animal origin

Component of residue definition: Oxathiapiprolin				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Single residue method DuPont-31138				
Milk	Primary	0.01 mg/kg	LC-MS/MS	<i>Method:</i> Henze R.M., Stry J.J., 2012 / Report No: DuPont-31138 <i>Validation:</i> Within the report DuPont-31138 EU agreed (Ireland, 2015)
	ILV	0.01 mg/kg	LC-MS/MS	<i>ILV:</i> Harris J.A., 2012 / Report No: DuPont-32355 EU agreed (Ireland, 2015)
	Confirmatory (if required)	-	-	Not required, primary method is highly specific
Eggs	Primary	0.01 mg/kg	LC-MS/MS	<i>Method:</i> Henze R.M., Stry J.J., 2012 / Report No: DuPont-31138 <i>Validation:</i> Within the report DuPont-31138 EU agreed (Ireland, 2015)
	ILV	0.01 mg/kg	LC-MS/MS	<i>ILV:</i> Harris J.A., 2012 / Report No: DuPont-32355 EU agreed (Ireland, 2015)
	Confirmatory (if required)	-	-	Not required, primary method is highly specific

Component of residue definition: Oxathiapiprolin				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Muscle	Primary	0.01 mg/kg	LC-MS/MS	<i>Method:</i> Henze R.M., Stry J.J., 2012 / Report No: DuPont-31138 <i>Validation:</i> Within the report DuPont-31138 EU agreed (Ireland, 2015)
	ILV	0.01 mg/kg	LC-MS/MS	<i>ILV:</i> Harris J.A., 2012 / Report No: DuPont-32355 EU agreed (Ireland, 2015)
	Confirmatory (if required)	-	-	Not required, primary method is highly specific
Fat	Primary	0.01 mg/kg	LC-MS/MS	<i>Method:</i> Henze R.M., Stry J.J., 2012 / Report No: DuPont-31138 <i>Validation:</i> Within the report DuPont-31138 EU agreed (Ireland, 2015)
	ILV	-	-	-
	Confirmatory (if required)	-	-	Not required, primary method is highly specific
Kidney	Primary	0.01 mg/kg	LC-MS/MS	<i>Method:</i> Henze R.M., Stry J.J., 2012 / Report No: DuPont-31138 <i>Validation:</i> Within the report DuPont-31138 EU agreed (Ireland, 2015)
	ILV	-	-	-
	Confirmatory (if required)	-	-	Not required, primary method is highly specific

Component of residue definition: Oxathiapiprolin				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Liver	Primary	0.01 mg/kg	LC-MS/MS	<i>Method:</i> Henze R.M., Stry J.J., 2012 / Report No: DuPont-31138 <i>Validation:</i> Within the report DuPont-31138 EU agreed (Ireland, 2015)
	ILV	0.01 mg/kg	LC-MS/MS	<i>ILV:</i> Harris J.A., 2012 / Report No: DuPont-32355 EU agreed (Ireland, 2015)
	Confirmatory (if required)	-	-	Not required, primary method is highly specific
Heavy cream	Primary	0.01 mg/kg	LC-MS/MS	<i>Method:</i> Henze R.M., Stry J.J., 2012 / Report No: DuPont-31138 <i>Validation:</i> Within the report DuPont-31138 EU agreed (Ireland, 2015)
	ILV	-	-	-
	Confirmatory (if required)	-	-	Not required, primary method is highly specific
Multi-residue method DFG S19				
Milk	Primary	0.01 mg/kg	LC-MS/MS	<i>Method:</i> Weber H., 2012b / Report No: DuPont-31951 <i>Validation:</i> Within the report DuPont-31951 EU agreed (Ireland, 2015)
	ILV	0.01 mg/kg	LC-MS/MS	<i>ILV:</i> Asekunowo J., Bacher R., 2013 / Report DuPont-37476 EU agreed (Ireland, 2015)
	Confirmatory (if required)	-	-	Not required, primary method is highly specific

Component of residue definition: Oxathiapiprolin				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Eggs	Primary	0.01 mg/kg	LC-MS/MS	<i>Method:</i> Weber H., 2012b / Report No: DuPont-31951 <i>Validation:</i> Within the report DuPont-31951 EU agreed (Ireland, 2015)
	ILV	0.01 mg/kg	LC-MS/MS	<i>ILV:</i> Asekunowo J., Bacher R., 2013 / Report DuPont-37476 EU agreed (Ireland, 2015)
	Confirmatory (if required)	-	-	Not required, primary method is highly specific
Muscle	Primary	0.01 mg/kg	LC-MS/MS	<i>Method:</i> Weber H., 2012b / Report No: DuPont-31951 <i>Validation:</i> Within the report DuPont-31951 EU agreed (Ireland, 2015)
	ILV	0.01 mg/kg	LC-MS/MS	<i>ILV:</i> Asekunowo J., Bacher R., 2013 / Report DuPont-37476 EU agreed (Ireland, 2015)
	Confirmatory (if required)	-	-	Not required, primary method is highly specific
Fat	Primary	0.01 mg/kg	LC-MS/MS	<i>Method:</i> Weber H., 2012b / Report No: DuPont-31951 <i>Validation:</i> Within the report DuPont-31951 EU agreed (Ireland, 2015)
	ILV	0.01 mg/kg	LC-MS/MS	<i>ILV:</i> Asekunowo J., Bacher R., 2013 / Report DuPont-37476 EU agreed (Ireland, 2015)
	Confirmatory (if required)	-	-	Not required, primary method is highly specific

Component of residue definition: Oxathiapiprolin				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Liver	Primary	0.01 mg/kg	LC-MS/MS	<i>Method:</i> Weber H., 2012b / Report No: DuPont-31951 <i>Validation:</i> Within the report DuPont-31951 EU agreed (Ireland, 2015)
	ILV	-	-	-
	Confirmatory (if required)	-	-	Not required, primary method is highly specific

For any special comments or remarkable points concerning the analytical methods for the determination of residues in animal matrices, please refer to Appendix 2.

Table 5.3-14: Statement on extraction efficiency

	Method for products of animal origin
Required, available from:	<p>The residue analytical method (Henze, R.M., Stry, J.J., 2012 Report No: DuPont-31138 uses the same solvents (acetonitrile and hexane) as in the goat and hen metabolism studies (DuPont-28213 and DuPont-28244). The only difference is that the metabolism extraction protocol used a homogenizing probe while in the analytical residue method extraction, samples are extracted using a Genogrinder. Since the extraction protocols are similar, it can be concluded that Method DuPont-31138 provides acceptable extraction efficiency.</p> <p>The extraction efficiency of DFG S19 method (Weber, H., 2012b Report No: DuPont-31951) was examined in DuPont-36106 using samples of muscle, kidney, fat and milk containing incurred residues of radiolabelled test substance. The residue profiles obtained using DFG S19 extraction procedures were compared with those obtained for the same samples in the goat metabolism study. Both extraction procedures showed similar distribution and levels of metabolites. The levels of oxathiapiprolin and metabolites obtained using the residue method for animal matrices were within 95 to 135% of those found in the metabolism study, indicating that the residue method provides acceptable extraction efficiency.</p> <p>EU agreed (Ireland, 2015)</p>
Not required, because:	-

5.3.3.4 Description of methods for the analysis of oxathiapiprolin in body fluids and tissues (KCP 5.2.3)

An overview on the acceptable methods and possible data gaps for analysis of oxathiapiprolin in body fluids is given in the following tables. For the detailed evaluation of new/additional studies it is referred to Appendix 2.

Table 5.3-15: Methods for body fluids and tissues

Component of residue definition: Oxathiapiprolin			
Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Primary	0.01 mg/L	LC-MS/MS oxathiapiprolin	Method: xxxxxxx 2022 Report No. S22-02422; Corteva Study No. 220385 New study

5.3.3.5 Description of methods for the analysis of oxathiapiprolin in soil (KCP 5.2.4)

An overview on the acceptable methods and possible data gaps for analysis of oxathiapiprolin in soil is given in the following tables.

Table 5.3-16: Validated methods for soil

Component of residue definition: Oxathiapiprolin			
Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Primary	0.001 mg/kg	LC-MS/MS oxathiapiprolin	Method: Henze, Stry & McCorquodale, 2010 (revised 2013) Report No. DuPont-28806 & DuPont-29443, Revision 1 EU agreed (2015)
Primary	0.001 mg/kg	LC-MS/MS oxathiapiprolin	Method: Henze, R.M., Stry, J.J., & Henze, R.M., Stry, J.J., & 2010, 2013, 2013 Report No. DuPont-31005, DuPont-31005 (Supplement No. 1), & DuPont-29443, Revision No. 1 ILV: Ju, L., & McCorquodale, G, 2011, DuPont-31141 EU agreed (2015)

5.3.3.6 Description of methods for the analysis of oxathiapiprolin in water (KCP 5.2.5)

An overview on the acceptable methods and possible data gaps for analysis of oxathiapiprolin in surface and drinking water is given in the following tables.

Table 5.3-17: Validated methods for water

Component of residue definition: Oxathiapiprolin				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Drinking water/ Surface water	Primary	Potable, groundwater and surface water: 0.0001 mg/kg	LC-MS/MS Oxathiapiprolin	Method: Henze, Stry, 2011, 2013 Report No. DuPont-32124, DuPont-32124, Supplement No. 1 EU agreed (2015)

Component of residue definition: Oxathiapiprolin				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
	ILV	Potable, groundwater and surface water: 0.0001 mg/kg	LC-MS/MS Oxathiapiprolin	ILV: Xu, 2012 Report No. DuPont-32693 EU agreed (2015)
Drinking water/ Surface water	Primary	Groundwater, surface water 0.01 µg/mL	LC/UV Oxathiapiprolin	Henze, Stry, 2012 Report No. DuPont-32692 EU agreed (2015)

5.3.3.7 Description of methods for the analysis of oxathiapiprolin in air (KCP 5.2.6)

An overview on the acceptable methods and possible data gaps for analysis of oxathiapiprolin in air is given in the following tables.

Table 5.3-18: Validated methods for air

Component of residue definition: Oxathiapiprolin			
Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Primary	0.05 µg/m ³	HPLC-MS/MS oxathiapiprolin	Method: Traub, 2012 Report No. DuPont-32356 EU agreed (2015)

For any special comments or remarkable points concerning the analytical methods for air it is referred to Appendix 2.

5.3.3.8 Other studies/ information

Not relevant.

5.4 References

SANCO/3029/99 rev.4. 11/07/00. EU Commission Guidance Document: Residues: Guidance for generating and reporting methods of analysis in support of pre-registration data requirements for Annex II (part A, Section 4) and Annex III (part A, Section 5) of Directive 91/414.

SANCO/825/00 rev.8.1. 16/11/2010. EU Commission Guidance Document: Guidance document on pesticide residue analytical methods.

SANTE 2017/10632 rev. 3. 22/11/2017. EC (European Commission). Technical Guideline on the Evaluation of Extraction Efficiency of Residue Analytical Methods.

SANTE/2020/12830 rev. 1. 24/02/2021. Guidance Document on Pesticide Analytical Methods for Risk Assessment and Post-approval Control and Monitoring Purposes.

Azoxystrobin

EFSA (European Food Safety Authority), 2010. Conclusion on the peer review of the pesticide risk assessment of the active substance azoxystrobin. EFSA Journal 2010; 8(4):1542. [110 pp.]. doi:10.2903/j.efsa.2010.1542.

United Kingdom, 2009a. Draft assessment report on the active substance azoxystrobin prepared by the rapporteur Member State United Kingdom in the framework of Council Directive 91/414/EEC, May, 2009.

United Kingdom, 2009b. Final addendum to the assessment report on the active substance azoxystrobin prepared by the rapporteur Member State United Kingdom in the framework of Council Directive 91/414/EEC, compiled by EFSA, December, 2009.

Oxathiapiprolin

EFSA (European Food Safety Authority), 2016. Conclusion on the peer review of the pesticide risk assessment of the active substance oxathiapiprolin. EFSA Journal 2016;14(7):4504, [89 pp.]. doi:10.2903/j.efsa.2016.4504.

Ireland, 2015. Draft Assessment Report prepared according to the Commission Regulation (EU) N° 1107/2009, December 2015.

Appendix 1 Lists of data considered in support of the evaluation

List of data submitted by the applicant and relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
A22773A					
KCP 5.1.1	Adolph, S.	30/11/2011	Determination of Toluene in Formulation by Headspace Gas Chromatography Report No. 10476553 Document No. VV-127729 , A16283D_10108 Test Facility Syngenta Crop Protection Not GLP Unpublished	N	SYN
KCP 5.1.1	Bradbury, L.	09/04/2021	SF-1060/1- Determination of Azoxystrobin and Oxathiapiprolin in A22773A by HPLC Report No. N/A Document No. VV-898893 Test Facility Syngenta Limited Not GLP Unpublished	N	SYN
KCP 5.1.1	De Benedictis, S.	24/11/2011	A16283D - Validation of analytical method SD-1540/1 - toluene in A16283D Report No. 123787 Document No. VV-400661 , A16283D_10107 Test Facility Syngenta Crop Protection GLP Unpublished	N	SYN
KCP 5.1.1	Heintz, K.	21/05/2021	Statement on Validation of the Analytical Method SD-1540/1 for the Determination of Toluene in A22773A Oxathiapiprolin/azoxystrobin SC (012/250) SD-1540/1 is Equivalent to CIPAC MT 198 Report No. N/A Document No. VV-903656 Test Facility N/A Not GLP Unpublished	N	SYN

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 5.1.1	Heintz, K.	07/03/2023	Statement on Validation of the Analytical Method SD-1540/1 for the determination of Toluene in A22773A oxathiapiprolin/azoxystrobin SC (012/250) SD-1540/1 is equivalent to CIPAC MT 198 Report No. N/A Test Facility Syngenta Crop Protection GLP Unpublished	N	SYN
KCP 5.1.1	Kettner, R.	08/07/2011	Determination of R230310 in formulation by HPLC (A17961A) Report No. SD-1464/1 Document No. VV-127958 , A17961A_10048 Test Facility Syngenta Crop Protection Not GLP Unpublished	N	SYN
KCP 5.1.1	Kettner, R.	11/07/2011	R230310 - Validation of analytical method SD-1464/1 (A17961A) Report No. 123137 Document No. VV-397754 , A17961A_10049 Test Facility Syngenta Crop Protection GLP Unpublished	N	SYN
KCP 5.1.1	Khot, S.	05/04/2021	A22773A – Validation of Analytical Method SF-1060/1 Report No. SMG16623 Document No. VV-898895 Test Facility Syngenta Biosciences Pvt., Ltd. - GLP Testing Facility GOA GLP Unpublished	N	SYN
KCP 5.1.1	Khot, S.	13/10/2021	Statement on Validation of the Analytical Method SD-1464/1 for the Determination of R230310 in A22773A - Oxathiapiprolin/azoxystrobin SC (012/250) Report No. N/A Document No. VV-911906 Test Facility Syngenta Biosciences Pvt., Ltd. - GLP Testing Facility GOA Not GLP Unpublished	N	SYN

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 5.1.2.6	Beuter, L-K.	30/11/2020	Oxathiapiprolin/Azoxystrobin SC (A22773A) - Toxicity to the Water Flea Daphnia magna Straus under Laboratory Conditions (Acute Immobilisation Test – Static) Report No. S20-05052 Document No. VV-884821 Test Facility Eurofins Agroscience Services EcoTox GmbH GLP Unpublished	N	SYN
KCP 5.1.2.6	Beuter, L-K.	30/11/2020	Oxathiapiprolin/Azoxystrobin SC (A22773A) - Toxicity to the Rainbow Trout Oncorhynchus mykiss under Laboratory Conditions (Acute Toxicity Test –Static) Report No. S20-05053 Document No. VV-884613 Test Facility Eurofins Agroscience Services EcoTox GmbH GLP Unpublished	N	SYN
KCP 5.1.2.6	Ehmke, A.	19/11/2015	Azoxystrobin SC (A12705B) – Honey Bee (Apis mellifera L.) Larval Toxicity Test, Repeated Exposure Report No. 100921032 Document No. VV-414544 , A12705B_13717 Test Facility Ibacon GmbH GLP Unpublished	N	SYN
KCP 5.1.2.6	Lunsmann, V.	07/12/2020	Oxathiapiprolin - Analytical Method ECO_052_03A and Validation for the Determination of Oxathiapiprolin in Honey Bee Larvae Diets and Adult Honey Bee Feeding Solutions Report No. 20 35 CRB 0103 Document No. VV-884296 Test Facility BioChem agrar GmbH GLP Unpublished	N	SYN
KCP 5.1.2.6	Lunsmann, V.	25/04/2022	Oxathiapiprolin – Analytical Method ECO_052_03B and Validation for the Determination of Oxathiapiprolin in Bumble Bee Contact Test Solutions Report No. 21 35 CRB 0127 Document No. VV-948172 Test Facility BioChem agrar GmbH GLP Unpublished	N	SYN

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 5.1.2.6	Obert-Rauser, P.	04/12/2020	Oxathiapiprolin/Azoxystrobin SC (A22773A) - Toxicity to the Single Cell Green Alga Raphidocelis subcapitata Korshikov under Laboratory Conditions Report No. S20-05054 Document No. VV-884825 Test Facility Eurofins Agroscience Services EcoTox GmbH GLP Unpublished	N	SYN
KCP 5.2.2	xxxxxx	2022	Method Validation of Oxathiapiprolin in Body Fluids Report No. S22-02422 Document No. 220385 xxxxxx GLP Unpublished	N	SYN
Azoxystrobin					
KCP 5.1.2.5	Bocksch, S.	08/02/2008	Azoxystrobin (ICI5504) and Cyproconazole (SAN619) - residues in honey following exposure of bees to treated winter oil-seed rape in Germany during 2007 Report No. T011298-06-REG Document No. VV-382035 , ICI5504_10398 Test Facility GAB Biotechnologie GmbH Not GLP Unpublished	N	SYN
KCP 5.1.2.5	xxxxxx	12/12/2002	Residue Analytical Method for the Determination of Residues of Azoxystrobin and R230310 in Bovine Muscle Tissue, Fat and Milk, Lamb Liver and Kidney and Hen Egg Samples. Final Determination by HPLC-MS-MS Report No. RAM 399/01 Document No. VV-124385 , ICI5504/1651 xxxxxxx Not GLP Unpublished	N	SYN
KCP 5.1.2.5	Richards, S.	21/11/2002	Azoxystrobin and R230310 : Validation of Analytical Method RAM 399/01 for the Determination of Residues in Bovine Muscle, Fat and Milk, Lamb's Kidney and Liver and Hen's Eggs. Report No. RJ3350B Document No. VV-331095 , ICI5504/1652 Test Facility Syngenta—Jealott's Hill GLP Unpublished	N	SYN

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 5.1.2.6	Amic, S.	28/02/2012	Azoxystrobin – Residue Method for the Determination of Azoxystrobin and its Metabolite R234886 in Water Report No. GRM057.01A Document No. VV-128281 , ICI5504_11505 Test Facility Eurofins - ADME Bioanalyses Not GLP Un Published	N	SYN
KCP 5.1.2.6	Amic, S.	07/02/2012	Azoxystrobin – Validation of Analytical Method for the Determination of Azoxystrobin and its Metabolite R234886 in Water. Report No. S11-03538 Document No. VV-401211 , ICI5504_11490 Test Facility Eurofins – ADME Bioanalyses GLP Unpublished	N	SYN
KCP 5.2.1	Lakasehus, S. Gizler, A.	05/04/2017	HLV for the determination of residues of azoxystrobin in lettuce and wheat grain by multi-residue method S19 (L-00.00-34) validated by a third party laboratory Report No. SYN-0422V Document No. VV-380727 , ICI5504/2948 Test Facility Dr. Specht & Partner Chem. Laboratorien GmbH GLP Unpublished	N	SYN
KCP 5.2.1	Stahl, F.	12/04/2017	Analytical Method Development and Validation of the DFG Method S19 for the Determination of Residues of Azoxystrobin and the metabolite R230310 in Plant Matrices Report No. IF-04/00192716 Document No. VV-379800 , ICI5504/2766 Test Facility SGS Institut Fresenius GmbH GLP Unpublished	N	SYN
KCP 5.2.1	Boeksch, S.	08/02/2008	Azoxystrobin (ICI5504) and Cyproconazole (SAN619) – residues in honey following exposure of bees to treated winter oil seed rape in Germany during 2007 Report No. T011298-06-REG Document No. VV-382035 , ICI5504_10398 Test Facility GAB Biotechnologie GmbH Not GLP Unpublished	N	SYN

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 5.2.1	Weeren, R. Pelz, S.	16/07/2001	Validation of the DFG Method S 19 (extended Version) for the Determination of Residues of Azoxystrobin in Plant Materials Report No. ZEN 0002V Document No. VV 327232 , ICI5504/1368 Test Facility Dr. Specht & Partner Chem. Laboratorien GmbH GLP Unpublished	N	SYN
KCP 5.2.2	xxxxxx.	12/12/2002	Residue Analytical Method for the Determination of Residues of Azoxystrobin and R230310 in Bovine Muscle Tissue, Fat and Milk, Lamb Liver and Kidney and Hen Egg Samples. Final Determination by HPLC-MS-MS Report No. RAM 399/01 Document No. VV-124385 , ICI5504/1651 xxxxxx Not GLP Unpublished	N	SYN
KCP 5.2.2	Richards, S.	21/11/2002	Azoxystrobin and R230310 : Validation of Analytical Method RAM 399/01 for the Determination of Residues in Bovine Muscle, Fat and Milk, Lamb's Kidney and Liver and Hen's Eggs. Report No. RJ3350B Document No. VV 331095 , ICI5504/1652 Test Facility Syngenta – Jealott's Hill GLP Unpublished	N	SYN
KCP 5.2.2	Atkinson, S.	28/02/2003	Independent Laboratory Validation of a Method for the Determination of Residues of Azoxystrobin in Animal Tissue Report No. CEMR 1907 Document No. VV 328461 , ICI5504/1921 Test Facility CEMAS GLP Unpublished	N	SYN
KCP 5.2.2	xxxxxxxxx	04/04/1997	Validation of DFG Method S 19 (Modified Extraction) for the Determination of the Residues of ICIA5504 (Azoxystrobin in Milk, Muscle, Kidney, Liver and Egg Report No. ZEN 9505V Document No. VV-323618 , ICI5504/0276 Test Facility N/A GLP Unpublished	N	SYN

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 5.2.3	Gemrot, F.	28/09/2011	Azoxystrobin – Validation of analytical method RAM 399/01 for the determination of azoxystrobin, R230310 and R234886 in human whole blood. Report No. S10-03815 Document No. VV-398250 , ICI5504_11467 Test Facility Eurofins – ADME Bioanalyses GLP Unpublished	N	SYN
KCP 5.2.4	Link, T. Kravchuk, O.	08/08/2019	Azoxystrobin - Validation of Analytical Method GRM057.06A for the Determination of Azoxystrobin, R230310, R234886, R401553 and R402173 in Soil Report No. IF18-04490185 Document No. VV-635374 , ICI5504_12486 Test Facility SGS Institut Fresenius GmbH GLP Unpublished	N	SYN
KCP 5.2.4	Link, T. Poperechna, N. Crook, S.	30/08/2019	Azoxystrobin - Analytical Method GRM057.06A for the Determination of Azoxystrobin, R230310, R234886, R401553 and R402173 in Soil Report No. GRM057.06A Document No. VV-635391 , ICI5504_12487 Test Facility SGS Institut Fresenius GmbH GLP Unpublished	N	SYN
KCP 5.2.5	Amic, S.	28/02/2012	Azoxystrobin – Residue Method for the Determination of Azoxystrobin and its Metabolite R234886 in Water Report No. GRM057.01A Document No. VV-128281 , ICI5504_11505 Test Facility Eurofins - ADME Bioanalyses Not GLP Unpublished	N	SYN
KCP 5.2.5	Amic, S.	07/02/2012	Azoxystrobin – Validation of Analytical Method for the Determination of Azoxystrobin and its Metabolite R234886 in Water. Report No. S11-03538 Document No. VV-401211 , ICI5504_11490 Test Facility Eurofins – ADME Bioanalyses GLP Unpublished	N	SYN

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 5.2.5	Brown, D.	17/07/2019	Azoxystrobin – Independent Laboratory Validation of Analytical Method GRM057.01A for the Determination of Residues of Azoxystrobin and its Metabolite R234886 in Water Report No. RES-00193 Document No. VV-619234 , ICI5504_12452 Test Facility ResChem Analytical Limited GLP Unpublished	N	SYN
KCP 5.2.5	Mayer, L.	12/06/2012	Azoxystrobin - Residue Method (GRM057.04A) for the Determination of Azoxystrobin and Z-Isomer R230310 in Water by LC-MS/MS Report No. GRM057.04A Document No. VV-185347 , R230310_50005 Test Facility Syngenta Crop Protection, LLC Not GLP Unpublished	N	SYN
KCP 5.2.5	Mayer, L.	12/06/2012	Azoxystrobin - Validation of Residue Method (GRM057.04A) for the Determination of Azoxystrobin and Z-Isomer R230310 in Water by LC-MS/MS Report No. GRM057.04A TK0120502 Document No. VV-506623 , R230310_50004 Test Facility Syngenta Crop Protection, LLC GLP Unpublished	N	SYN
KCP 5.2.5	Smith, R.	26/10/2012	Azoxystrobin - Independent Laboratory Validation (ILV) of Residue Method (GRM057.04A) for the Determination of Azoxystrobin and Z-Isomer R230310 in Water by LC-MS/MS Report No. GRM057.04A 1781.6873 Document No. VV-507766 , ICI5504_51024 Test Facility Smithers GLP Unpublished	N	SYN
Oxathiapiprolin					

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 5.1.2.5	Donald, C. Gibson, R.	27/08/2020	Oxathiapiprolin (SYN546539): Validation of the Analytical Method DuPont-30422 for the Determination of Residues of Oxathiapiprolin in Crop Matrices by LC-MS/MS Report No. 231693 Document No. VV-870136 Test Facility Charles River Laboratories Edinburgh, Ltd. GLP Unpublished	N	SYN
KCP 5.1.2.5	Ford, K.	15/12/2020	Oxathiapiprolin – Honey Residue Study on Spring Oilseed Rape in Northern and Southern Europe in 2020 Report No. CEMR-9533 Document No. VV-885771 Test Facility CEM Analytical Services Limited (CEMAS) GLP Unpublished	N	SYN
KCP 5.1.2.5	Reinhardt, R. Lakaschus, S.	27/04/2020	Oxathiapiprolin - Residue Study on Protected Lettuce in Northern France, Germany, Italy, Spain and the United Kingdom in 2019 Report No. S19-02718 Document No. VV-854039 Test Facility Eurofins Agrosience Services Chem GmbH GLP Unpublished	N	SYN
KCP 5.1.2.6	Tanzler, V.	2015	PLACEHOLDER for LoA:Oxathiapiprolin (DPX-QGU42) 100 g/L OD: Chronic oral toxicity to the honey bee, Apis mellifera L. (Hymenoptera, Apidae) Report No. 94441136, DuPont-41989 Document No. VV-910995 Test Facility N/A Not GLP Unpublished	N	Corteva (SYN have access)
KCP 5.1.2.6	Oberrauch, S.	2017	PLACEHOLDER for LoA: Oxathiapiprolin (DPX-QGU42) technical: Honey bee (Apis mellifera L.) 22 day larval toxicity test (re-peated exposure) Report No. S17-01639, DuPont-48606 Document No. VV-911004 Test Facility N/A Not GLP Unpublished	N	Corteva (SYN have access)

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 5.2.1	Donald, Gibson, R.	C. 27/08/2020	Oxathiapiprolin (SYN546539): Validation of the Analytical Method DuPont-30422 for the Determination of Residues of Oxathiapiprolin in Crop Matrices by LC-MS/MS Report No. 231693 Document No. VV-870136 Test Facility Charles River Laboratories Edinburgh, Ltd. GLP Unpublished	N	SYN
KCP 5.2.1	Ford, K.	15/12/2020	Oxathiapiprolin – Honey Residue Study on Spring Oilseed Rape in Northern and Southern Europe in 2020 Report No. CEMR-9533 Document No. VV-885771 Test Facility CEM Analytical Services Limited (CEMAS) GLP Unpublished	N	SYN

List of data submitted or referred to by the applicant and relied on, but already evaluated at EU peer review

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
Azoxystrobin					
KCP 5.1.2.5	Bocksch, S.	08/02/2008	Azoxystrobin (ICI5504) and Cyproconazole (SAN619) - residues in honey following exposure of bees to treated winter oil-seed rape in Germany during 2007 Report No. T011298-06-REG Document No. VV-382035 , ICI5504_10398 Test Facility GAB Biotechnologie GmbH Not GLP Unpublished	N	SYN

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 5.1.2.5	xxxxxxx	21/11/2002	Azoxystrobin and R230310 : Validation of Analytical Method RAM 399/01 for the Determination of Residues in Bovine Muscle, Fat and Milk, Lamb's Kidney and Liver and Hen's Eggs. Report No. RJ3350B Document No. VV-331095 , ICI5504/1652 xxxxxx GLP Unpublished	N	SYN
KCP 5.1.2.6	Amic, S.	07/02/2012	Azoxystrobin – Validation of Analytical Method for the Determination of Azoxystrobin and its Metabolite R234886 in Water. Report No. S11-03538 Document No. VV-401211 , ICI5504_11490 Test Facility Eurofins - ADME Bioanalyses GLP Unpublished	N	SYN
KCP 5.2.1	Lakaschus, S. Gizler, A.	05/04/2017	ILV for the determination of residues of azoxystrobin in lettuce and wheat grain by multi-residue method S19 (L 00.00-34) validated by a third party laboratory Report No. SYN-0422V Document No. VV-380727 , ICI5504/2948 Test Facility Dr. Specht & Partner Chem. Laboratorien GmbH GLP Unpublished	N	SYN
KCP 5.2.1	Stahl, F.	12/04/2017	Analytical Method Development and Validation of the DFG Method S19 for the Determination of Residues of Azoxystrobin and the metabolite R230310 in Plant Matrices Report No. IF-04/00192716 Document No. VV-379800 , ICI5504/2766 Test Facility SGS Institut Fresenius GmbH GLP Unpublished	N	SYN
KCP 5.2.1	Bocksch, S.	08/02/2008	Azoxystrobin (ICI5504) and Cyproconazole (SAN619) - residues in honey following exposure of bees to treated winter oil-seed rape in Germany during 2007 Report No. T011298-06-REG Document No. VV-382035 , ICI5504_10398 Test Facility GAB Biotechnologie GmbH Not GLP Unpublished	N	SYN

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 5.2.1	Weeren, R. Pelz, S.	16/07/2001	Validation of the DFG Method S 19 (extended Version) for the Determination of Residues of Azoxystrobin in Plant Materials Report No. ZEN-0002V Document No. VV-327232 , ICI5504/1368 Test Facility Dr. Specht & Partner Chem. Laboratorien GmbH GLP Unpublished	N	SYN
KCP 5.2.2	xxxxxx	21/11/2002	Azoxystrobin and R230310 : Validation of Analytical Method RAM 399/01 for the Determination of Residues in Bovine Muscle, Fat and Milk, Lamb's Kidney and Liver and Hen's Eggs. Report No. RJ3350B Document No. VV-331095 , ICI5504/1652 xxxxxxx GLP Unpublished	N	SYN
KCP 5.2.2	xxxxxx	28/02/2003	Independent Laboratory Validation of a Method for the Determination of Residues of Azoxystrobin in Animal Tissue Report No. CEMR-1907 Document No. VV-328461 , ICI5504/1921 Test Facility xxxxxx GLP Unpublished	N	SYN
KCP 5.2.3	xxxxxx	28/09/2011	Azoxystrobin – Validation of analytical method RAM 399/01 for the determination of azoxystrobin, R230310 and R234886 in human whole blood. Report No. S10-03815 Document No. VV-398250 , ICI5504_11467 Test Facility xxxxxx GLP Unpublished	N	SYN
KCP 5.2.5	Amic, S.	07/02/2012	Azoxystrobin – Validation of Analytical Method for the Determination of Azoxystrobin and its Metabolite R234886 in Water. Report No. S11-03538 Document No. VV-401211 , ICI5504_11490 Test Facility Eurofins - ADME Bioanalyses GLP Unpublished	N	SYN

List of data submitted by the applicant and not relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
-	-	-	-	-	-

List of data relied on not submitted by the applicant but necessary for evaluation

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
-	-	-	-	-	-

Appendix 2 Detailed evaluation of submitted analytical methods

A 2.1 Analytical methods for azoxystrobin

A 2.1.1 Methods used for the generation of pre-authorization data (KCP 5.1)

A 2.1.1.1 Description of analytical methods for the determination of residues in support of environmental fate studies (KCP 5.1.2.1)

No new or additional studies have been submitted

A 2.1.1.2 Description of analytical methods for the determination of residues in support of efficacy studies (KCP 5.1.2.2)

No new or additional studies have been submitted

A 2.1.1.3 Description of analytical methods for the determination of residues in support of toxicological studies (KCP 5.1.2.3)

No new or additional studies have been submitted

A 2.1.1.4 Description of analytical methods for the determination of residues in support of operator, worker, resident and bystander exposure studies (KCP 5.1.2.4)

No new or additional studies have been submitted

A 2.1.1.5 Description of analytical methods for the determination of residues in support of residues studies (KCP 5.1.2.5)

A 2.1.1.5.1 Analytical method 1: RAM 305/03

A 2.1.1.5.1.1 Method validation (T011298-06-REG)

Comments of zRMS:	The study has been reviewed at EU level.
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Reference: KCP 5.1.2.5 (and KCP 5.2.1)

Report Azoxystrobin (ICI5504) and Cyproconazole (SAN619) - Residues in Honey following Exposure of Bees to Treated Winter Oil-seed Rape in Germany during 2007
Bocksch, S (2008)
Report No. T011298-06-REG, Syngenta File No. VV-382035

Guideline(s): Guidance Document for Residue Trials on Honey of the Federal Office for Consumer Protection and Food Safety, version 2, 03/10/2003.
IVA (1992), EU 91/414/EEC (1997)

Deviations: None

GLP: Yes

Acceptability: Yes

Executive summary

This study contained three trials during 2007 in Northern and Southern Germany. In each trial tunnels were placed in oil seed rape fields to maximise the exposure of the bee colonies to the treated rape plants. Each trial consisted of four tunnels (one control and three treated). To each of the three treated tunnels a single foliar application of azoxystrobin (ICI5504, A 12750B, 250 SC) and cyproconazole (SAN619, A9898A, 100 SL) as a tank mixture was made to oil-seed rape at the onset of flowering. Honey was then collected for analysis of azoxystrobin and cyproconazole.

The health effects on the bee colonies were also monitored.

Samples were analysed for azoxystrobin, its metabolite R230310 and cyproconazole; due to low amounts of honey in trial G07N013B both ship and retain samples were sent for analysis. Only data for azoxystrobin is presented and discussed here.

The method was validated in compliance with European guidelines for residue analytical methods SANCO/825/00 Rev.7 (17/03/2004) and SANCO/3029/99 Rev.4 (11/07/2000) and complies also with the new analytical guideline for residue analytical methods SANTE/2020/12830, Rev.1 (24/02/2021).

At each fortification level, the mean recoveries for each analyte were in the range 71-103% and the relative standard deviation less than 20%. The specificity of the method was demonstrated with the two transitions described in the methods, with no significant interference being detected in any of the blank and unfortified specimens. The linearity of the detector was checked using calibration solutions. The calibration curves obtained were linear with correlation coefficients above 0.990.

In two tunnels from one of the trials (G07N011B), residues of azoxystrobin were found to be at the limit of quantification (0.01 mg/kg), all other residues were below LOQ.

Materials and Methods

Materials

Test Materials

Common Name:	Azoxystrobin
Code Name:	A12705B
Description	SC 250
Content	252 g/L
Source:	Syngenta
Standard Reference:	ASJ10008-03 (Batch)
Purity:	99.7%
Storage Conditions:	Stored at ADME Bioanalyses at 0-9 °C
Expiration Date:	Jan 2011

Test System

Crop:	Oilseed rape (Brassica napus)
Pollinator:	Honey bee (Apis mellifera)
Processed Commodities:	Honey

Test Facilities

Field Phase (Trial G07N011B):	Eurofins-GAB GmbH, Eutinger Str. 24, 75223 Niefern Oselbronn, Germany
Field Phase (Trial G07N012B):	BioChem agrar GmbH, Kupferstr. 6, D-04827 Gerichshain, Germany
Field Phase (Trial G07N013B):	LAVES Institut für Bienenkunde Celle, Herzogin-Eleonore-Allee 5, 29221 Celle, Germany
Analytical Phase:	Eurofins/ADME BIOANALYSES, 75 chemin de Sommières, 30310 Vergèze, France.

Study Design and Methods

Field phase

Three residue trials (G07N011B, G07N012B, G07N013B) were conducted with A12705B SC 250 (250 g/L, azoxystrobin nominal concentration) and A9898A SL 100 (100 g/L cyproconazole, nominal

concentration) on oilseed rape in Germany in 2007. One application, at growth stages BBCH 63, was made at 250 g ai/ha for azoxystrobin and at 100 g a.s./ha for cyproconazole. The health of the colonies was assessed prior to introduction into the tunnels and at the end of the trial when the honey had been collected. No differences were seen between the treatment and control for any parameters measured. Considering the restricted conditions in the tunnel tents and beekeeper activities, the health status of all colonies was good throughout the trial, all colonies were free of visible symptoms of diseases.

Honey was then collected at maturity, with the exception of deviations documented (water content >20%) for trials G07N011B in the samples of plot T3, and in the trial G07N013B in all samples except the samples of T3 at DAA+8

Analytical phase

Samples were stored under deep-frozen conditions for a period of about 2 months prior to analysis. Azoxystrobin has been shown to be stable in a range of crops for at least 12 months under these storage conditions.

Samples were extracted with acetonitrile/water (90/10 v/v) for azoxystrobin. Final quantitation was by HPLC-MS/MS with external standardisation.

HPLC-MS/MS Conditions

HPLC system:	Not reported			
Pumps:	Not reported			
Degasser:	Not reported			
Column Oven:	Not reported			
Detector:	Applied Biosystems API 4000 (Sciex Instruments)			
Autosampler:	Not reported			
Column:	Kromasil C18 (50 mm x 3.2 mm, 5 µm)			
Mobile phase:	A: Ultra-pure water with 0.2% acetic acid (v/v)			
	B: Acetonitrile			
	Time	%A	%B	Gradient
	Not reported	50	50	Isocratic
	Not reported	50	50	Isocratic
Flow rate:	1 mL/min			
Column oven temperature:	Not reported			
Injection volume:	20 µL			
Retention time:	- Azoxystrobin: approximately 1.4 min			
	- R230310: approximately 1.0 min			

Detector	API 4000	
Ionisation mode:	Not reported	
Source polarity:	Not reported	
Nebuliser gas (NEB):	Not reported	
Curtain gas (CUR):	Not reported	
Temperature (TEM):	Not reported	
IonSpray voltage (IS):	Not reported	
Collision gas setting (CAD):	Not reported	
Dwell time:	Not reported	
Resolution Q1 and Q2:	Not reported	
Electron multiplier (CEM):	Not reported	

Source and detection parameters for MS/MS experiments:

Compound	Parent (m/z)	CE (V)	DP (V)	CXP (V)	Fragment ions (m/z)	
Azoxystrobin and R230310	404.2	n.r.	n.r.	n.r.	372.4	Quantification
	404.2	n.r.	n.r.	n.r.	343.8	Confirmation

CE: Collision energy; CXP: Collision cell exit potential ; DP : Declustering Potential
n.r. not reported

Quantification: Peak areas of fragment ion at $m/z = 372.4$, external standards in matrix

Confirmation: Peak areas of fragment ion at $m/z = 343.8$, external standards in matrix

The method RAM 305/03 was successfully validated for azoxystrobin in honey during the course of this study.

The limit of quantification (LOQ) was 0.01 mg/kg for azoxystrobin in honey. Method validation procedural recoveries are summarised in Table A 5. Procedural recoveries from the analyses of honey samples are summarised in Table A 6.

During the validation experiments, the matrix effects of honey were also checked by comparing, for each analyte, the peak area of an analytical standard in the mobile phase and in a matrix-matched analytical standard. Matrix effect for azoxystrobin is above 10%, and therefore considered as significant. It is recommended to use matrix matched calibration standards for the quantification of azoxystrobin in honey.

Table A 1: Method validation recovery data – primary and confirmatory transitions

Azoxystrobin (Primary Transition 404.2 → 372.4 m/z)					
Matrix	Fortification Level (mg/kg)	Recovery (%)	Mean (%)	RSD (%)	Range (%)
Honey	0.01	78, 77, 75, 81, 71	76	4.8	71-81
	0.10	83, 82, 83, 89, 82	84	3.4	82-89
	Overall		80	6.3	71-89
Azoxystrobin (Secondary Transition 404.2 → 343.8 m/z)					
Matrix	Fortification Level (mg/kg)	Recovery (%)	Mean (%)	RSD (%)	Range (%)
Honey	0.01	98, 93, 95, 103, 90	96	5.2	90-103
	0.10	83, 80, 81, 90, 85	84	4.5	80-90
	Overall		90	8.4	80-103

Table A 2: Procedural recovery (sample analysis)

Substrate (Control)	Fortification Levels (mg/kg)	Azoxystrobin (%)
Honey	0.01	91
	0.1	91
Mean		91

Results and Discussion

Azoxystrobin residues in honey are presented in Table A 7.

Table A 3: Azoxystrobin residues in honey

Trial	Tunnel	Sampling Interval (Days After Application)	Matrix	Azoxystrobin Residue (mg/kg)
G07N011B	1	13 DAA	Honey	0.01
	2	13 DAA	Honey	0.01
	3	13 DAA	Honey	<0.01
G07N012B	1	14 DAA	Honey	<0.01
	2	14 DAA	Honey	<0.01
	3	14 DAA	Honey	<0.01
G07N013B	1	14 DAA	Honey	<0.01
	2	14 DAA	Honey	<0.01
	3	14 DAA	Honey	<0.01

Specificity

LC-MS/MS with two transitions is considered to be a highly specific detection technique and therefore according to EU guidance (SANTE/2020/12830, Rev.1, 24/02/2021) no further confirmatory technique is required. The method includes two MS/MS transitions, both of which have been validated. No significant interferences arising from the crop matrices, the labware, reagents or solvents have been observed at the retention times of interest.

Linearity

The linearity of the LC-MS/MS detector was tested using matrix matched standard solutions (0.00005 µg/L to 0.0015 µg/L; equivalent to 0.005 mg/kg to 0.15 mg/kg in honey) for both transitions. Standards at five different concentrations were injected (weighting not reported). The linear range is equivalent to 50% of the LOQ to at least 20% above the highest analyte concentration detected. The detector response was linear. Linear equations and correlation coefficients are reported for the following matrices:

Honey

Quantification: $y = 71696411x + 1130$ ($r = 0.9992$)

Confirmation: not reported

Accuracy

Fortified samples were analysed in quintuplet at the limit of quantification (LOQ, 0.01 mg/kg) and at ten times the LOQ (0.1 mg/kg). Acceptable mean recoveries of between 70% and 110% were found for both transitions and therefore according to EU guidance (SANTE/2020/12830, Rev.1, 24/02/2021) demonstrate the method has satisfactory accuracy.

Repeatability

The relative standard deviations (RSDs) of azoxystrobin recoveries at each fortification level and overall during method validation were < 20% and therefore according to the EU guidance (SANTE/2020/12830, Rev.1, 24/02/2021) demonstrate the method has satisfactory repeatability.

Limit of Quantification

The limit of quantification for azoxystrobin residues in honey was established at 0.01 mg/kg. No interfering peaks around the retention time of compoundname were found in any of the control samples at levels above 30% of the limit of quantification.

Matrix Effects

Matrix effects (suppression and enhancement) on the detector response of azoxystrobin were $\leq \pm 20\%$ and deemed to be insignificant. Nevertheless, matrix matched standards were used for quantification throughout the study.

Stability of Final Extracts

Final extracts were stored at 0 - 9°C for a maximum of 1 day before analysis. The recoveries of the analytes in final sample extracts were within the acceptable range of 70 - 120%, measured against freshly fortified standards, thus proving stability in accordance with SANTE/2020/12830, Rev. 1.

Stability of Standard Solutions

The stability of azoxystrobin in standard solutions was not assessed within this study.

Conclusion

Residues of azoxystrobin in the honey specimens from trial G07N011B were at 0.01 mg/kg in Tunnel 1 and Tunnel 2 at 13 DAA, and were below the limit of quantification of 0.01 mg/kg in Tunnel 3 at 13 DAA.

No residues of azoxystrobin were detected at or above the limit of quantification of 0.01 mg/kg in any of the honey specimens from trials G07N012B and G07N013B at 14 DAA.

No residues of azoxystrobin were detected at or above the limit of quantification (0.01 mg/kg) in the control honey specimens.

The method RAM 305/03 was successfully validated for azoxystrobin in honey during the course of this study.

A 2.1.1.5.1.2 Confirmatory method

No confirmatory method is required. LC-MS/MS with two transitions is considered to be a high-ly

specific detection technique. The method includes two MS/MS transitions, both of which have been validated.

A 2.1.1.5.2 Analytical method 2: RAM 399/01

Comments of zRMS:	<p>The method has been evaluated in Draft Registration Report for Orondis Evo (Central Zone, zRMS-PL, July 2022).</p> <p>Below is a copied assessment:</p> <p>The method described is suitable for the analysis of azoxystrobin and R230310 residues in bovine muscle tissue, fat and milk, lamb's liver and kidney and hen's egg samples. Only commercially available laboratory equipment and reagents are required. Untreated and fortified samples should be extracted and analysed with each set of samples to demonstrate absence of any interference and adequate recovery, if possible. The limit of quantitation has been set at 0.01 mg with nal determination by HPLC MS-MS.</p> <p>The method is acceptable.</p>
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Reference: KCP 5.1.2.5 (and KCP 5.2.2)

Report: xxxxxx., 2002.
Residue Analytical Method for the Determination of Residues of Azoxystrobin and R230310 in Bovine Muscle Tissue, Fat and Milk, Lamb Liver and Kidney and Hen Egg Samples
Report No. SOP RAM 399/01
Syngenta File No. VV-124385
unpublished

Guideline(s): None stated - compliant with SANCO/825/00 rev. 6, 20/06/2000

Deviations: No

GLP: No

Acceptability: Yes

A 2.1.1.5.2.1 Method validation (RJ3350B)

Comments of zRMS:	The study has been reviewed at EU level.
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Reference: KCP 5.1.2.5 (and KCP 5.2.2)

Report: Azoxystrobin and R230310: Validation of Analytical Method RAM 399/01 for the Determination of Residues in Bovine Muscle, Fat and Milk, Lamb's Kidney and Liver and Hen's Eggs
Richards S., (2002)
Report No. RJ3350B
Syngenta File No. VV-331095

Guideline(s): None stated - compliant with SANCO/825/00 rev. 6, 20/06/2000

Deviations: No

GLP: Yes

Acceptability: Yes

Materials and methods

Test Material 1¹	Azoxystrobin
Lot/Batch #:	ASJ10008-03
Purity (%):	99.7
IUPAC name:	methyl (E)-2-{2-[6-(2-cyanophenoxy)pyrimidin-4-yloxy]phenyl}-3-methoxyacrylate
CAS number:	131860-33-8

Test Material 2¹	R230310
Lot/Batch #:	ASJ10075-03
Purity (%):	98
IUPAC name:	methyl (Z)-2-{2-[6-(2-cyanophenoxy)pyrimidin-4-yloxy]phenyl}-3-methoxyacrylate
CAS number:	143130-94-3

¹ Certificates of analysis for test substances were not provided in the study report

Animal	Commodity	Source
Bovine	Muscle	Local supermarket
Bovine	Fat	Local supermarket
Bovine	Milk	Local supermarket
Lamb	Kidney	Local supermarket
Lamb	Liver	Local supermarket
Hen	Eggs	Local supermarket

Study Design and Methods

Test facility: Syngenta, Jealott's Hill International Research Centre, Berkshire, UK

Study start date: 21 August 2002

Study end date: 21 November 2002

Analytical phase dates: 04 October to 25 October 2002

Analytical method RAM 399/01 was validated for bovine muscle, fat and milk, lamb's kidney and liver and hen's egg. Residues of azoxystrobin (and R230310; R230310 is not discussed further as it is not a relevant animal metabolite) are extracted by maceration with acetonitrile. Extracts are centrifuged and aliquots are diluted with ultra-pure water. A C18 solid phase extraction (SPE) procedure is carried out to facilitate sample clean-up. Final determination is by high performance liquid chromatography with triple quadrupole mass spectrometric detection (LC-MS/MS).

HPLC-MS/MS Conditions

HPLC system:

Pumps: Agilent 1100 series quaternary pump model number G1311A
 Degasser: Agilent 1000 series model number G1322A
 Column Oven: Agilent 1100 series model number G1316A
 Detector: Applied Biosystems API 3000 triple quadrupole mass spectrometer
 Autosampler: CTC PAL
 Column: KR100 5C18 5 µm 50 mm x 3.2 mm i.d.
 Isocratic Mobile phase: A: Acetonitrile
 B: Ultrapure water with 0.2% acetic acid

Time	% A	% B	Gradient
0.0	50	50	Not reported
2.00	50	50	Not reported

Flow rate: 1 ml/min

Column oven temperature: 40°C

Injection volume: 10 µL

Retention time: Azoxystrobin: approximately 1.2 min

R230310: approximately 0.94 min

Detector

API 3000
Ionisation mode: TurboIonSpray
Scan type: MRM
Source polarity: Positive
Curtain gas (CUR): 12 (arbitrary units)
Gas 1 (GSI): Not reported
Gas 2 (GSI): Not reported
Temperature (TEM): 450°C
Ionspray voltage (IS): 5000 V
Collision gas setting (CAD): 4
Entrance potential (EP): 10 V
Dwell time: 400 msec
Resolution Q1: Low
Resolution Q2: High

Source and detection parameters for MS/MS experiments:

Compound	Parent (<i>m/z</i>)	CE (V)	DP (V)	CXP (V)	Fragment ions (<i>m/z</i>)	
Azoxystrobin	404.2	21	41	24	372.4	Quantification

CE: Collision energy; CXP: Collision cell exit potential; DP: Declustering Potential

Quantification: Peak areas of fragment ion at $m/z = 372.4$, external standards in solvent (acetonitrile/water, 50/50, v/v)

Results and discussions

Table A 4: Recovery results from method validation of azoxystrobin using the analytical method (quantification transition m/z 404.2 \rightarrow 372)

Matrix	Analyte	Fortification level (mg/kg)	Individual recoveries ¹ (%)	Range of recoveries (%) ($n = x$)	Mean recovery (%)	RSD (%)	Comments
Bovine muscle	Azoxystrobin	Mass transition m/z = 404.2 \rightarrow 372.4 (quantification)					
		0.01	99, 99, 99, 97, 94	94 – 99 ($n = 5$)	98	2.2	-
		0.1	94, 96, 95, 98, 95	94 – 98 ($n = 5$)	96	1.6	-
		Overall	-	94 – 99 ($n = 10$)	97	2.1	-
Bovine fat	Azoxystrobin	Mass transition m/z = 404.2 \rightarrow 372.4 (quantification)					
		0.01	95, 95, 101, 97, 95	95 – 101 ($n = 5$)	97	2.7	-
		0.1	97, 92, 95, 97, 98	92 – 98 ($n = 5$)	96	2.4	-
		Overall	-	92 – 101 ($n = 10$)	96	2.5	-
Bovine milk	Azoxystrobin	Mass transition m/z = 404.2 \rightarrow 372.4 (quantification)					
		0.01	101, 91, 96, 89, 92	89 – 101 ($n = 5$)	94	4.8	-
		0.1	95, 92, 92, 97, 95	92 – 97 ($n = 5$)	94	2.2	-
		Overall	-	89 – 101 ($n = 10$)	94	3.4	-
Lamb kidney	Azoxystrobin	Mass transition m/z = 404.2 \rightarrow 372.4 (quantification)					
		0.01	99, 92, 98, 101, 104	92 – 104 ($n = 5$)	99	4.5	-
		0.1	102, 99, 97, 94, 91	91 – 102 ($n = 5$)	97	3.1	-
		Overall	-	91 – 104 ($n = 10$)	98	4.2	-
Lamb liver	Azoxystrobin	Mass transition m/z = 404.2 \rightarrow 372.4 (quantification)					
		0.01	97, 89, 94, 90, 87	87 – 97 ($n = 5$)	91	4.5	-
		0.1	96, 94, 95, 97, 92	92 – 97 ($n = 5$)	95	2.1	-
		Overall	-	87 – 97 ($n = 10$)	93	3.8	-
Hen egg	Azoxystrobin	Mass transition m/z = 404.2 \rightarrow 372.4 (quantification)					
		0.01	91, 97, 98, 95, 94	91 – 98 ($n = 5$)	95	2.9	-
		0.1	99, 97, 93, 98, 92	92 – 98 ($n = 5$)	96	3.0	-
		Overall	-	91 – 98 ($n = 10$)	95	2.8	-

Specificity

LC-MS/MS is a highly specific detection technique and therefore a confirmatory technique is not required (SANTE/2020/12830, Rev.1 (24/02/2021)). No interfering peaks around the retention time of azoxystrobin were found in any of the control samples at levels above 30% of the limit of quantification. A confirmatory transition can be monitored (m/z 404.2 \rightarrow 344.3), and has been validated for method RAM 399/01 in blood (xxxxxx 2011, Report S10-03815).

Linearity

The linearity of the LC-MS/MS detector was tested using five standard solutions at concentrations between 0.00005 µg/L and 0.01 µg/L (0.005 to 1.0 mg/kg in animal samples) in acetonitrile:water (50:50, v/v). Standards were injected in triplicate and the response plotted against concentration. Linear correlations with coefficients ≥ 0.9996 were obtained for azoxystrobin. Linear equations and correlation coefficients are reported for azoxystrobin in acetonitrile:water (50:50, v/v):

Azoxystrobin in acetonitrile:water (50:50, v/v)

Quantification (with intercept set to 0): $y = 1 \times 10^8 x$ ($r = 0.9998$)

Quantification (with intercept not set to 0): $y = 1 \times 10^8 x + 3559.7$ ($r = 0.9998$)

Accuracy

Fortified samples were analysed in quintuplet at the limit of quantification (LOQ, 0.01 mg/kg) and at ten times the LOQ (0.1 mg/kg). Acceptable mean recoveries of between 70% and 110% were found for both transitions and therefore according to EU guidance (SANTE/2020/12830, Rev.1, 24/02/2021) demonstrate the method has satisfactory accuracy.

Repeatability

The relative standard deviations (RSDs) of azoxystrobin recoveries at each fortification level and overall during method validation were $< 20\%$ and therefore according to the EU guidance (SANTE/2020/12830, Rev.1, 24/02/2021) demonstrate the method has satisfactory repeatability.

Limit of Quantification

The limit of quantification for azoxystrobin was 0.01 mg/kg. No interfering peaks around the retention time of azoxystrobin were found in any of the control samples at levels above 30% of the limit of quantification.

Matrix Effects

Matrix effects (suppression and enhancement) on the detector response of azoxystrobin were $\leq \pm 20\%$ and deemed to be insignificant. Non-matrix-matched standards were used for quantification throughout the study.

Stability of Final Extracts

Final extracts were stored at $< 7^\circ\text{C}$ for a minimum of 7 days before analysis. The recoveries of the analytes in final sample extracts were within the acceptable range of 70 - 120%, measured against freshly fortified standards, thus proving stability in accordance with SANTE/2020/12830, Rev. 1.

Stability of Standard Solutions

The stability of azoxystrobin in standard solutions was not assessed within this study.

Conclusion

Analytical method RAM 399/01 has been successfully validated for the analysis of azoxystrobin in animal tissues, milk and eggs. Results obtained were within the guideline requirements (mean recovery 70-110%; RSD $\leq 20\%$).

A 2.1.1.5.2.2 Confirmatory method

No confirmatory method is required. LC-MS/MS with two transitions is considered to be a highly specific detection technique. The method includes two MS/MS transitions, both of which have been validated.

A 2.1.1.6 Description of analytical methods for the determination of residues in support of ecotoxicological studies (KCP 5.1.2.6)

Azoxystrobin

A 2.1.1.6.1 Analytical method 1 – azoxystrobin in water

A 2.1.1.6.1.1 GRM057.01A - Method validation

Comments of zRMS:	<p>The method has been evaluated in Draft Registration Report for Orondis Evo (Central Zone, zRMS-PL, July 2022).</p> <p>Below is a copied assessment:</p> <p>Analytical method GRM057.01A is suitable for the determination of azoxystrobin and its metabolite R234886 in water.</p> <p>In summary, residues were determined after direct injection by high performance liquid chromatography with triple quadrupole mass spectrometric detection (LC-MS/MS).</p> <p>The limit of quantification (LOQ) of the method has been established at 0.05 µg/L.</p> <p>This method satisfies EC Guidance Documents SANCO/3029/99 rev. 4 and SANCO/825/00 rev 8.1.</p> <p>The method is acceptable.</p>
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Reference:	KCP 5.1.2.6
Report	Azoxystrobin - Residue Method for the Determination of Azoxystrobin and its Metabolite R234886 in Water, Amic S., 2012, report No GRM057.01A, document No VV-128281
Guideline(s):	<p>ENV/JM/MONO(2007)17</p> <p>EPA OCSPP 860.1340</p> <p>EC SANCO/3029/99 rev 4</p> <p>EC SANCO/825/00 rev 8.1</p>
Deviations:	None
GLP:	Yes No
Acceptability:	Yes

Comments of zRMS:	The study has been reviewed at EU level.
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Reference:	KCP 5.1.2.6
Report	Azoxystrobin - Validation of Analytical Method for the Determination of Azoxystrobin and its Metabolite R234886 in Water, Amic S., 2012, report No S11-03538, document No VV-401211
Guideline(s):	<p>ENV/JM/MONO(2007)17</p> <p>EPA OCSPP 860.1340</p> <p>EC SANCO/3029/99 rev 4</p> <p>EC SANCO/825/00 rev 8.1</p>
Deviations:	None
GLP:	Yes
Acceptability:	Yes

This analytical method is also used for the generation of post-authorisation data. The method description and validation of this method (GRM057.01A) are summarised in A 2.1.2.5 (KCP 5.2.5).

A22773A

A 2.1.1.6.2 Analytical method 1 – azoxystrobin in water

A 2.1.1.6.2.1 Method validation

Comments of zRMS:	The method has been evaluated in Draft Registration Report for Orondis Evo (Central
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	<p>Zone, zRMS-PL, July 2022).</p> <p>Below is a copied assessment:</p> <p>The analytical method (Syngenta method no. GRM057.01A with minor deviation) for the determination of azoxystrobin in water was fully validated with the standard acceptance criteria of the guidance document SANCO/3029/99 rev. 4 11/07/2000.</p> <p>LOQ=0.0625 mg test item/L, corresponding to 0.0141 mg azoxystrobin/L.</p> <p>Mean recoveries and relative standard deviations per fortification level were in the range 70-110% with ≤ 20% RSD.</p> <p>The study is acceptable.</p>
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Reference:	KCP 5.1.2.6
Report	Oxathiapiprolin/Azoxystrobin SC (A22773A) - Toxicity to the Rainbow Trout <i>Oncorhynchus mykiss</i> under Laboratory Conditions (Acute Toxicity Test –Static), Beuter, L-K. (2020) Report no. S20-05053. document no. VV-884613.
Guideline(s):	SANCO/3029/99 rev 4
Deviations:	Stability of extracts and standards not investigated.
GLP:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability:	Yes

Materials

Test Material	Oxathiapiprolin/Azoxystrobin SC (A22773A)
Lot/Batch #:	SFI003-174-001
Purity (%):	1.02, 22.5
IUPAC name:	1-(4-{4-[5-(2,6-difluorophenyl)-4,5-dihydro-1,2-oxazol-3-yl]-1,3-thiazol-2-yl}piperidin-1-yl)-2-[5-methyl-3-(trifluoromethyl)-1H-pyrazol-1-yl]ethanone, Methyl (E)-2-{2-[6-(2-cyanophenoxy)pyrimidin-4-yloxy]phenyl}-3-methoxyacrylate
CAS number:	1003318-67-9, 131860-33-8

Analytical Standard	Azoxystrobin
Lot/Batch #:	ASJ10008-05
Purity (%):	99.8
IUPAC name:	Methyl (E)-2-{2-[6-(2-cyanophenoxy)pyrimidin-4-yloxy]phenyl}-3-methoxyacrylate
CAS number:	131860-33-8

Study Design and Methods

Test facility: Eurofins Agrosience Services Ecotox GmbH, Eutinger Strasse 24, 75223 Niefern-Öschelbronn, Germany

Study start date: 14th September 2020

Study end date: 30th November 2020

Analytical phase dates: 21st September to 13th October 2020

Dechlorinated water samples were fortified with standard solutions of test item in acetonitrile/water (1:1, v/v). Five samples were fortified at the limit of quantification (LOQ; 0.0625 mg test item/L, corresponding to 0.0141 mg azoxystrobin/L) and five at a higher level (13 mg test item/L, corresponding to 2.93 mg azoxystrobin/L). The fortified samples were analysed alongside untreated control samples.

Principle of the Method

Dechlorinated water samples (10 mL) are mixed with acetonitrile (10 mL) and shaken well using a Vortex mixer for 10 seconds. The samples are further diluted with acetonitrile/test medium (1:1, v/v) prior to quantification by high performance liquid chromatography with tandem mass specific detection (HPLC-MS/MS), monitoring two transitions, $m/z = 404 \rightarrow 372$ (quantification) and $m/z = 404 \rightarrow 344$

(confirmation). The limit of quantification (LOQ) of the method was 0.0625 mg test item/L (corresponding to 0.0141 mg azoxystrobin/L).

Detailed information on the analytical method can be found in Appendix 1, page 30 of the report.

LC-MS/MS Conditions

HPLC system: Thermo Vanquish Charger
Pumps: Thermo VF-P10-A
Column Oven: Thermo VH-C10-A
Detector: Thermo TSQ Altis triple quadrupole MS system
Autosampler: Thermo VF-A10-A
Column: Phenomenex Kinetex 1.7 μ C18 100A, No. 00B-4475-AN, 50 mm x 2.1 mm, 1.7 μ m + 2.1 mm C18 UHPLC guard column
Mobile phase: A: Water + 0.2% acetic acid
B: Acetonitrile

Time	%A	%B	Gradient
0.0	90	10	-
1.0	50	50	-
2.5	50	50	-
3.0	10	90	-
3.5	10	90	-
4.0	90	10	-
5.0	90	10	-

Flow rate: 0.8 mL/minute
Column oven temperature: 40°C
Injection volume: 10 μ L
Retention time: Azoxystrobin: approximately 1.7 minutes

Detector MS/MS

Ionisation mode: Heated electrospray (HESI)
Polarity: Positive
Scan type: Single reaction monitoring (SRM)
Vaporiser temperature: 250°C
Ion transfer tube temperature: 350°C
Ionspray voltage: 1500 V
Sheath gas pressure: 56 (arbitrary units)
Ion sweep gas pressure: 1 (arbitrary units)
Auxiliary gas pressure: 24 (arbitrary units)
Collision gas pressure: 1.5 mTorr argon
Cycle time: 0.1 seconds

Source and detection parameters for MS/MS experiments:

Compound	Parent m/z	CE (V)	RF lens (V)	Quad 1 width	Quad 2 width	Fragment ion m/z	
Azoxystrobin	404	14	56	0.7	1.2	372	Quantification
	404	25	56	0.7	1.2	344	Confirmation

CE: Collision energy

Quantification: Peak areas of SRM transition 404 \rightarrow 372, external standards in matrix
Confirmation: Peak areas of SRM transition 404 \rightarrow 344, external standards in matrix

Recovery data

Recovery and precision data of azoxystrobin obtained from test medium at each fortification level using the analytical method are presented in the table below.

Table A 5: Accuracy and precision results from validation of the analytical method for azoxystrobin in test medium

Matrix	Test Item Fortification Level (mg/L)	Nominal Azoxystrobin Concentration (mg/L)	Accuracy (%)	Number (n)	Mean Accuracy (%)	RSD (%)	Accuracy Range (%)
Dechlorinated Water	0.0625	0.0141*	100, 107, 101, 102, 101	5	103	3.0	100 - 107
	13.0	2.93	100, 99, 95, 103, 95	5	98	4.0	95 - 103
	Overall		-	10	100	4.0	95 - 107

*Limit of quantitation, defined by the lowest validated fortification level

Residues in control samples and reagent blanks were less than 30% of the LOQ

Table A 6: Characteristics of the analytical method used for the quantification of azoxystrobin in test medium

Analyte	Azoxystrobin
Equipment/ Chromatographic method	HPLC-MS/MS
Accuracy/ Precision (repeatability)	Fortified samples were analysed for azoxystrobin in quintuplet at the limit of quantification (LOQ) of 0.0625 mg test item/L (0.0141 mg azoxystrobin/L) and at 13.0 mg test item/L (2.93 mg azoxystrobin/L). Acceptable mean accuracy values of 103% and 98% were obtained in the test medium which fall within the acceptable EU guidance range of 70-110% and demonstrates satisfactory accuracy.
Precision (reproducibility)	The relative standard deviations (RSDs) of azoxystrobin recovery values at each fortification level and overall during method validation were <20% which according to the EU guidance demonstrates the method has satisfactory repeatability.
Specificity	No peaks were detected in controls above 30% of LOQ. LC-MS/MS with two MRM transitions provides high specificity for the analysis and detection of azoxystrobin for the purpose of ecotoxicity studies i.e. clean, well described test matrix analysing a pre-defined quantity of test item. No significant interferences from the sample matrix, the labware, reagents or solvents were detected in the LC-MS/MS chromatograms at the retention time corresponding to azoxystrobin in any of the control samples tested.
Confirmatory method	Not required.
Assessment of matrix effects is presented	Not investigated. Matrix-matched standards were used throughout the method.
Extract and standard stability	Not investigated.
Extraction efficiency	Not investigated.
Calibration/Linearity	The linearity of the HPLC-MS/MS detector was tested for azoxystrobin (m/z 404 → 372) using matrix-matched standards from 0.02 – 1.00 ng/mL. Standards with at least five different concentrations were injected and the signal area plotted against concentration for all calibration points. The equation of the line and coefficient of determination were: Quantification: $y = 4.246E+02 + 5.531E+04 x$ ($r = 0.9999$) The lower margin of detector response verification was below 30% of the LOQ and the upper margin was higher by at least 30% as the highest concentrations in the diluted samples.
Limit of detection (LOD)	0.02 ng/mL
Limit of quantification (LOQ)	The LOQ for azoxystrobin test medium was established at 0.0625 mg test item/L (corresponding to 0.0141 mg azoxystrobin/L). No interfering peaks around the retention time of azoxystrobin were found in any of the control samples at levels above 30% of the LOQ.

Conclusion

The analytical method has been demonstrated to be a reliable and accurate procedure for the determination of azoxystrobin in test medium with a limit of quantification (LOQ) of 0.0625 mg test item/L (corresponding to 0.0141 mg azoxystrobin/L) in accordance with SANTE/2020/12830 rev.1, using commercially available laboratory equipment and reagents.

(Beuter, L-K., 2020)

A 2.1.1.6.2 Confirmatory method

No confirmatory method is required.

A 2.1.1.6.3 Analytical method 2 – azoxystrobin in medium

A 2.1.1.6.3.1 Method validation

Comments of zRMS:	<p>The method has been evaluated in Draft Registration Report for Orondis Evo (Central Zone, zRMS-PL, July 2022).</p> <p>Below is a copied assessment:</p> <p>The analytical method (Syngenta method no. GRM057.01A with minor deviation) for the determination of azoxystrobin in ElenDt M4 media was fully validated with the standard acceptance criteria of the guidance document SANCO/3029/99 rev. 4 11/07/2000.</p> <p>The mean recoveries at each fortification level were in the range between 70% and 110% with relative standard deviations below 20%.</p> <p>LOQ=0.00625 mg test item/L (corresponding to 0.00141 mg azoxystrobin/L).</p> <p>The study is acceptable.</p>
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Reference: KCP 5.1.2.6

Report Oxathiapiprolin/Azoxystrobin SC (A22773A) - Toxicity to the Water Flea *Daphnia magna* Straus under Laboratory Conditions (Acute Immobilisation Test – Static), Beuter, L-K. (2020a) Report no. S20-05052. document no. VV-884821.

Guideline(s): SANCO/3029/99 rev 4

Deviations: Stability of extracts and standards not investigated.

GLP: Yes, conducted under GLP/Officially recognised testing facilities

Acceptability: Yes

Materials

Test Material	Oxathiapiprolin/Azoxystrobin SC (A22773A)
Lot/Batch #:	SFI003-174-001
Purity (%):	1.02, 22.5
IUPAC name:	1-(4-{4-[5-(2,6-difluorophenyl)-4,5-dihydro-1,2-oxazol-3-yl]-1,3-thiazol-2-yl}piperidin-1-yl)-2-[5-methyl-3-(trifluoromethyl)-1H-pyrazol-1-yl]ethanone, Methyl (E)-2-{2-[6-(2-cyanophenoxy)pyrimidin-4-yloxy]phenyl}-3-methoxyacrylate
CAS number:	1003318-67-9, 131860-33-8

Analytical Standard	Azoxystrobin
Lot/Batch #:	ASJ10008-05
Purity (%):	99.8
IUPAC name:	Methyl (E)-2-{2-[6-(2-cyanophenoxy)pyrimidin-4-yloxy]phenyl}-3-methoxyacrylate
CAS number:	131860-33-8

Study Design and Methods

Test facility: Eurofins Agroscience Services Ecotox GmbH, Eutinger Str. 24, 75223 Niefern-Öschelbronn, Germany

Study start date: 14th September 2020

Study end date: 30th November 2020

Analytical phase dates: 22nd September to 2nd October 2020

ElenDt M4 media samples were fortified with standard solutions of test item in acetonitrile/water (1:1, v/v). Five samples were fortified at the limit of quantification (LOQ; 0.00625 mg test item/L,

corresponding to 0.00141 mg azoxystrobin/L) and five at a higher level (1.30 mg test item/L, corresponding to 0.293 mg azoxystrobin/L). The fortified samples were analysed alongside untreated control samples.

Principle of the Method

Elendt M4 media samples (10 mL) are mixed with acetonitrile (10 mL) and shaken well using a Vortex mixer for 10 seconds. The samples are further diluted with acetonitrile/test medium (1:1, v/v) prior to quantification by high performance liquid chromatography with tandem mass specific detection (HPLC-MS/MS), monitoring two transitions, $m/z = 404 \rightarrow 372$ (quantification) and $m/z = 404 \rightarrow 344$ (confirmation). The limit of quantification (LOQ) of the method was 0.00625 mg test item/L (corresponding to 0.00141 mg azoxystrobin/L).

Detailed information on the analytical method can be found in Appendix 1, page 32 of the report.

LC-MS/MS Conditions

HPLC system: Thermo Vanquish Charger
Pumps: Thermo VF-P10-A
Column Oven: Thermo VH-C10-A
Detector: Thermo TSQ Altis triple quadrupole MS system
Autosampler: Thermo VF-A10-A
Column: Phenomenex Kinetex 1.7 μm C18 100A, No. 00B-4475-AN, 50 mm x 2.1 mm, 1.7 μm + 2.1 mm C18 UHPLC guard column
Mobile phase: A: Water + 0.2% acetic acid
B: Acetonitrile

Time	%A	%B	Gradient
0.0	90	10	-
1.0	50	50	-
2.5	50	50	-
3.0	10	90	-
3.5	10	90	-
4.0	90	10	-
5.0	90	10	-

Flow rate: 0.8 mL/minute
Column oven temperature: 40°C
Injection volume: 10 μL
Retention time: Azoxystrobin: approximately 1.7 minutes

Detector MS/MS
Ionisation mode: Heated electrospray (HESI)
Polarity: Positive
Scan type: Single reaction monitoring (SRM)
Vaporiser temperature: 250°C
Ion transfer tube temperature: 350°C
Ionspray voltage: 1500 V
Sheath gas pressure: 56 (arbitrary units)
Ion sweep gas pressure: 1 (arbitrary units)
Auxiliary gas pressure: 24 (arbitrary units)
Collision gas pressure: 1.5 mTorr argon
Cycle time: 0.1 seconds

Source and detection parameters for MS/MS experiments:

Compound	Parent m/z	CE (V)	RF lens (V)	Quad 1 width	Quad 2 width	Fragment ion m/z	
Azoxystrobin	404	14	56	0.7	1.2	372	Quantification
	404	25	56	0.7	1.2	344	Confirmation

CE: Collision energy

Quantification: Peak areas of SRM transition 404 → 372, external standards in matrix
Confirmation: Peak areas of SRM transition 404 → 344, external standards in matrix

Recovery data

Recovery and precision data of azoxystrobin obtained from Elendt M4 media at each fortification level using the analytical method are presented in the table below.

Table A 7: Accuracy and precision results from validation of the analytical method for azoxystrobin in Elendt M4 media

Matrix	Test Item Fortification Level (mg/L)	Nominal Azoxystrobin Concentration (mg/L)	Accuracy (%)	Number (n)	Mean Accuracy (%)	RSD (%)	Accuracy Range (%)
Elendt M4 medium	0.00625	0.00141*	100, 100, 99, 97, 99	5	99	1	97 - 100
	1.30	0.293	101, 101, 100, 101, 99	5	100	1	99 - 101
	Overall		-	10	100	1	97 - 101

*Limit of quantitation, defined by the lowest validated fortification level

Residues in control samples and reagent blanks were less than 30% of the LOQ

Table A 8: Characteristics of the analytical method used for the quantification of azoxystrobin in Elendt M4 media

Analyte	Azoxystrobin
Equipment/ Chromatographic method	HPLC-MS/MS
Accuracy/ Precision (repeatability)	Fortified samples were analysed for azoxystrobin in quintuplet at the limit of quantification (LOQ) of 0.00625 mg test item/L (0.00141 mg azoxystrobin/L) and at 1.30 mg test item/L (0.293 mg azoxystrobin/L). Acceptable mean accuracy values of 99% and 100% were obtained in Elendt M4 media which fall within the acceptable EU guidance range of 70-110% and demonstrates satisfactory accuracy.
Precision (reproducibility)	The relative standard deviations (RSDs) of azoxystrobin recovery values at each fortification level and overall during method validation were <20% which according to the EU guidance demonstrates the method has satisfactory repeatability.
Specificity	No peaks were detected in controls above 30% of LOQ. LC-MS/MS with two MRM transitions provides high specificity for the analysis and detection of azoxystrobin for the purpose of ecotoxicity studies i.e. clean, well described test matrix analysing a pre-defined quantity of test item. No significant interferences from the sample matrix, the labware, reagents or solvents were detected in the LC-MS/MS chromatograms at the retention time corresponding to azoxystrobin in any of the control samples tested.
Assessment of matrix effects is presented	Not investigated. Matrix-matched standards were used throughout the method.
Extract and standard stability	Not investigated.
Extraction efficiency	Not investigated.
Calibration/Linearity	The linearity of the HPLC-MS/MS detector was tested for azoxystrobin (m/z 404 → 372) using matrix-matched standards from 0.02 – 1.00 ng/mL. Standards with at least five different concentrations were injected and the signal area plotted against concentration for all calibration points. The equation of the line and coefficient of determination were: Quantification: $y = 2.207E+02 + 4.377E+04 x$ ($r = 1.0000$) The lower margin of detector response verification was below 30% of the LOQ and the upper margin was higher by at least 30% as the highest concentrations in the diluted samples.
Limit of detection (LOD)	0.02 ng/mL
Limit of quantification (LOQ)	The LOQ for azoxystrobin test medium was established at 0.00625 mg test item/L (corresponding to 0.00141 mg azoxystrobin/L). No interfering peaks around the retention time of azoxystrobin were found in any of the control samples at levels above 30% of the LOQ.

Conclusion

The analytical method has been demonstrated to be a reliable and accurate procedure for the determination of azoxystrobin in Elendt M4 media with a limit of quantification (LOQ) of 0.00625 mg

test item/L (corresponding to 0.00141 mg azoxystrobin/L) in accordance with SANTE/2020/12830 rev.1, using commercially available laboratory equipment and reagents.

(Beuter, L-K., 2020)

A 2.1.1.6.3.2 Confirmatory method

No confirmatory method is required.

Azoxystrobin

A 2.1.1.6.4 Analytical method 3 – azoxystrobin in stock solution

A 2.1.1.6.4.1 Method validation

Comments of zRMS:	<p>The method has been evaluated in Draft Registration Report for Orondis Evo (Central Zone, zRMS-PL, July 2022).</p> <p>Below is a copied assessment:</p> <p>The analytical method for the determination of azoxystrobin in stock solution was fully validated with the standard acceptance criteria of the guidance document SANCO/3029/99 rev. 4 11/07/2000.</p> <p>The mean recoveries at each fortification level were in the range between 70% and 110% with relative standard deviations below 20%.</p> <p>The LOQ = 25 g A12705B /L (corresponding to 5.7 g a.i./L).</p> <p>The method is acceptable.</p>
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Reference: KCP 5.1.2.6

Report Azoxystrobin SC (A12705B) - Honey Bee (*Apis mellifera* L.) Larval Toxicity Test, Repeated Exposure, Ehmke, A. (2015) Report no. 100921032. document no. VV-414544.

Guideline(s): SANCO/3029/99 rev 4

Deviations: Stability of extracts and standards not investigated.

GLP: Yes, conducted under GLP/Officially recognised testing facilities

Acceptability: Yes

Materials

Test Material	Azoxystrobin 250 g/L SC (A12705B)
Lot/Batch #:	GRA2L121D
Purity (%):	22.7
IUPAC name:	Methyl (E)-2-{2-[6-(2-cyanophenoxy)pyrimidin-4-yloxy]phenyl}-3-methoxyacrylate
CAS number:	131860-33-8

Analytical Standard	Azoxystrobin
Lot/Batch #:	30131
Purity (%):	98.5
IUPAC name:	Methyl (E)-2-{2-[6-(2-cyanophenoxy)pyrimidin-4-yloxy]phenyl}-3-methoxyacrylate
CAS number:	131860-33-8

Study Design and Methods

Test facility: ibacon GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany

Study start date: 27th March 2015

Study end date: 26th October 2015 (Amd. 1 19th November 2015)

Analytical phase dates: 20th May to 25th May 2015 (experimental phase)

Stock solution samples were fortified with standard solutions of test item in pure water (50 g/mL). Five samples were fortified at the limit of quantification (LOQ; 25 g A12705B /L, corresponding to 5.7 g a.i./L) and five at a higher level (50 g A12705B /L, corresponding to 11.4 g a.i./L). The fortified samples were analysed alongside untreated control samples.

Principle of the Method

The whole stock solution sample was diluted in pure water with a dilution factor of approximately 10. An aliquot of each diluted sample was further diluted with acetonitrile/pure water (50/50 v/v). Quantification was done by high performance liquid chromatography with ultra-violet detection (HPLC-UV), set at 220 nm. The limit of quantification (LOQ) of the method was 25 g A12705B /L (corresponding to 5.7 g a.i./L).

Detailed information on the analytical method can be found in Appendix 3, page 30 of the report.

HPLC-UV Conditions

HPLC system:	VWR Hitachi
Detector:	UV-Vis at 220 nm
Column:	US ES RP18E (250 mm × 4 mm × 5 µm)
Mobile phase:	A: acetonitrile B: pure water A/B: 60/40(isocratic)
Flow rate:	1 mL/minute
Oven temperature:	25°C
Injection volume:	10 µL
Retention time:	Azoxystrobin: approximately 5.6 minutes
Detector	UV-Vis
Wavelength:	220 nm

Recovery data

Recovery and precision data of azoxystrobin obtained from stock solution at each fortification level using the analytical method are presented in the table below.

Table A 9: Accuracy and precision results from validation of the analytical method for azoxystrobin in stock solution

Matrix	Test Item Fortification Level (g/L)	Nominal Azoxystrobin Concentration (g/L)	Accuracy (%)	Number (n)	Mean Accuracy (%)	RSD (%)	Accuracy Range (%)
Stock solution	25	5.7*	98, 98, 100, 99, 100	5	99	1	98 - 100
	50	11.4	101, 99, 97, 98, 98	5	99	2	97 - 101
	Overall		-	10	99	1	97 - 101

*Limit of quantitation, defined by the lowest validated fortification level
Residues in control samples were less than 30% of the LOQ

Table A 10: Characteristics of the analytical method used for the quantification of azoxystrobin in stock solution

Analyte	Azoxystrobin
Equipment/ Chromatographic method	HPLC-UV
Accuracy/ Precision (repeatability)	Fortified samples were analysed for azoxystrobin in quintuplet at the limit of quantification (LOQ) of 25 g A12705B /L (5.7 g a.i./L) and at 50 g A12705B/L (11.4 g a.i./L). Acceptable mean accuracy values of 99% were obtained in stock solution which fall within the acceptable EU guidance range of 70-110% and demonstrates satisfactory accuracy.
Precision (reproducibility)	The relative standard deviations (RSDs) of azoxystrobin recovery values at each fortification level and overall during method validation were <20% which according to the EU guidance demonstrates the method has satisfactory repeatability.

Specificity	No peaks were detected in controls above 30% of LOQ. No significant interference of total peak area for the target analyte was found.
Assessment of matrix effects is presented	Not applicable, stock solution does not contain matrix.
Extract and standard stability	Not investigated.
Extraction efficiency	Not investigated.
Calibration/Linearity	<p>The linearity of the HPLC-UV detector was tested for azoxystrobin using solvent standards from 1-30 mg a.i./L. Standards with seven different concentrations were injected. The linearity of the detector response was assessed by the correlation of peak area of different standard solutions with their corresponding concentrations, using a linear regression.</p> <p>The equation of the line and coefficient of determination were: Quantification: $y = 214500x - 10307$ ($r = 1.0000$)</p> <p>The lower margin of detector response verification was below 30% of the LOQ and the upper margin was higher by at least 30% as the concentration in the sample stock solution (9.1 g a.i./L nominal).</p>
Limit of detection (LOD)	0.086 mg a.i./L
Limit of quantification (LOQ)	The LOQ for azoxystrobin stock solution was established at 25 g A12705B /L (corresponding to 5.7 g a.i./L). No interfering peaks around the retention time of azoxystrobin were found in any of the control samples at levels above 30% of the LOQ.

Conclusion

The analytical method has been demonstrated to be a reliable and accurate procedure for the determination of azoxystrobin in stock solution with a limit of quantification (LOQ) of 25 g A12705B/L (corresponding to 5.7 g a.i./L) in accordance with SANTE/2020/12830 rev.1, using commercially available laboratory equipment and reagents.

(Ehmke, A., 2015)

A 2.1.1.6.4.2 Confirmatory method

No confirmatory method is required.

A22773A

A 2.1.1.6.5 Analytical method 4 – azoxystrobin in medium

A 2.1.1.6.5.1 Method validation

Comments of zRMS:	<p>The method has been evaluated in Draft Registration Report for Orondis Evo (Central Zone, zRMS-PL, July 2022).</p> <p>Below is a copied assessment:</p> <p>The analytical method (Syngenta method no. GRM057.01A with minor deviation) for the determination of azoxystrobin in AAP media was fully validated with the standard acceptance criteria of the guidance document SANCO/3029/99 rev. 4 11/07/2000.</p> <p>The mean recoveries at each fortification level were in the range between 70% and 110% with relative standard deviations below 20%.</p> <p>LOQ= 0.00954 mg test item/L, corresponding to 0.00215 mg azoxystrobin/L)</p> <p>The study is acceptable.</p>
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Reference: KCP 5.1.2.6

Report Oxathiapiprolin/Azoxystrobin SC (A22773A) - Toxicity to the Single Cell Green Alga *Raphidocelis subcapitata* Korshikov under Laboratory Conditions, Obert-Rausser, P. (2020) Report no. S20-05054. document no. VV-884825.

Guideline(s): SANCO/3029/99 rev 4
Deviations: Stability of extracts and standards not investigated.
GLP: Yes, conducted under GLP/Officially recognised testing facilities
Acceptability: Yes

Materials

Test Material	Oxathiapiprolin/Azoxystrobin SC (A22773A)
Lot/Batch #:	SFI003-174-001
Purity (%):	1.02, 22.5
IUPAC name:	1-(4-{4-[5-(2,6-difluorophenyl)-4,5-dihydro-1,2-oxazol-3-yl]-1,3-thiazol-2-yl}piperidin-1-yl)-2-[5-methyl-3-(trifluoromethyl)-1H-pyrazol-1-yl]ethanone, Methyl (E)-2-{2-[6-(2-cyanophenoxy)pyrimidin-4-yloxy]phenyl}-3-methoxyacrylate
CAS number:	1003318-67-9, 131860-33-8

Analytical Standard	Azoxystrobin
Lot/Batch #:	ASJ10008-05
Purity (%):	99.8
IUPAC name:	Methyl (E)-2-{2-[6-(2-cyanophenoxy)pyrimidin-4-yloxy]phenyl}-3-methoxyacrylate
CAS number:	131860-33-8

Study Design and Methods

Test facility: Eurofins Agrosience Services Ecotox GmbH, Eutinger Str. 24, 75223 Niefern-Öschelbronn, Germany

Study start date: 14th September 2020

Study end date: 4th December 2020

Analytical phase dates: 21st September to 14th October 2020

AAP media samples were fortified with standard solutions of test item in acetonitrile/water (1:1, v/v). Five samples were fortified at the limit of quantification (LOQ; 0.00954 mg test item/L, corresponding to 0.00215 mg azoxystrobin/L) and five at a higher level (13.0 mg test item/L, corresponding to 2.93 mg azoxystrobin/L). The fortified samples were analysed alongside untreated control samples.

Principle of the Method

AAP media samples (10 mL) are mixed with acetonitrile (10 mL) and shaken well using a Vortex mixer for 10 seconds. The samples are further diluted with acetonitrile/test medium (1:1, v/v) prior to quantification by high performance liquid chromatography with tandem mass specific detection (HPLC-MS/MS), monitoring two transitions, $m/z = 404 \rightarrow 372$ (quantification) and $m/z = 404 \rightarrow 344$ (confirmation). The limit of quantification (LOQ) of the method was 0.00954 mg test item/L (corresponding to 0.00215 mg azoxystrobin/L).

Detailed information on the analytical method can be found in Appendix 1, page 35 of the report.

LC-MS/MS Conditions

HPLC system: Thermo Vanquish Charger
Pumps: Thermo VF-P10-A
Column Oven: Thermo VH-C10-A
Detector: Thermo TSQ Altis triple quadrupole MS system
Autosampler: Thermo VF-A10-A
Column: Phenomenex Kinetex 1.7µm C18 100A, No. 00B-4475-AN, 50 mm x 2.1 mm, 1.7 µm + 2.1 mm C18 UHPLC guard column
Mobile phase: A: Water + 0.2% acetic acid
B: Acetonitrile

Time	%A	%B	Gradient
0.0	90	10	-
1.0	50	50	-

	2.5	50	50	-
	3.0	10	90	-
	3.5	10	90	-
	4.0	90	10	-
	5.0	90	10	-
Flow rate:	0.8 mL/minute			
Column oven temperature:	40°C			
Injection volume:	10 µL			
Retention time:	Azoxystrobin: approximately 1.7 minutes			
Detector	MS/MS			
	Ionisation mode:	Heated electrospray (HESI)		
	Polarity:	Positive		
	Scan type:	Single reaction monitoring (SRM)		
	Vaporiser temperature:	250°C		
	Ion transfer tube temperature:	350°C		
	Ionspray voltage:	1500 V		
	Sheath gas pressure:	56 (arbitrary units)		
	Ion sweep gas pressure:	1 (arbitrary units)		
	Auxiliary gas pressure:	24 (arbitrary units)		
	Collision gas pressure:	1.5 mTorr argon		
	Cycle time:	0.1 seconds		

Source and detection parameters for MS/MS experiments:

Compound	Parent m/z	CE (V)	RF lens (V)	Quad 1 width	Quad 2 width	Fragment ion m/z	
Azoxystrobin	404	14	56	0.7	1.2	372	Quantification
	404	25	56	0.7	1.2	344	Confirmation

CE: Collision energy

Quantification: Peak areas of SRM transition 404 → 372, external standards in matrix

Confirmation: Peak areas of SRM transition 404 → 344, external standards in matrix

Recovery data

Recovery and precision data of azoxystrobin obtained from AAP media at each fortification level using the analytical method are presented in the table below.

Table A 11: Accuracy and precision results from validation of the analytical method for azoxystrobin in AAP media

Matrix	Test Item Fortification Level (mg/L)	Nominal Azoxystrobin Concentration (mg/L)	Accuracy (%)	Number (n)	Mean Accuracy (%)	RSD (%)	Accuracy Range (%)
AAP media	0.00954	0.00215*	100, 100, 99, 98, 98	5	99	1	98 - 100
	13.0	2.93	101, 101, 100, 102, 99	5	101	1	99 - 102
	Overall		-	10	100	1	98 - 102

*Limit of quantitation, defined by the lowest validated fortification level

Residues in control samples and reagent blanks were less than 30% of the LOQ

Table A 12: Characteristics of the analytical method used for the quantification of azoxystrobin in AAP media

Analyte	Azoxystrobin
Equipment/ Chromatographic method	LC-MS/MS
Accuracy/ Precision (repeatability)	Fortified samples were analysed for azoxystrobin in quintuplet at the limit of quantification (LOQ) of 0.00954 mg test item/L (0.00215 mg azoxystrobin/L) and at 13.0 mg test item/L (2.93 mg azoxystrobin/L). Acceptable mean accuracy values of 99% and 101% were obtained in AAP

	medium which fall within the acceptable EU guidance range of 70-110% and demonstrates satisfactory accuracy.
Precision (reproducibility)	The relative standard deviations (RSDs) of azoxystrobin recovery values at each fortification level and overall during method validation were <20% which according to the EU guidance demonstrates the method has satisfactory repeatability.
Specificity	No peaks were detected in controls above 30% of LOQ. LC-MS/MS with two MRM transitions provides high specificity for the analysis and detection of azoxystrobin for the purpose of ecotoxicity studies i.e. clean, well described test matrix analysing a pre-defined quantity of test item. No significant interferences from the sample matrix, the labware, reagents or solvents were detected in the LC-MS/MS chromatograms at the retention time corresponding to azoxystrobin in any of the control samples tested.
Assessment of matrix effects is presented	Not investigated. Matrix-matched standards were used throughout the method.
Extract and standard stability	Not investigated.
Extraction efficiency	Not investigated.
Calibration/Linearity	The linearity of the HPLC-MS/MS detector was tested for azoxystrobin (m/z 404 → 372) using matrix-matched standards from 0.01 – 1.00 ng/mL. Standards with at least five different concentrations were injected and the signal area plotted against concentration for all calibration points. The equation of the line and coefficient of determination were: Quantification: $y = 2.371E+01 + 3.572E+04 x$ ($r = 0.9998$) The lower margin of detector response verification was below 30% of the LOQ and the upper margin was higher by at least 30% as the highest concentrations in the diluted samples.
Limit of detection (LOD)	0.01 ng/mL
Limit of quantification (LOQ)	The LOQ for azoxystrobin AAP media was established at 0.00954 mg test item/L (corresponding to 0.00215 mg azoxystrobin/L). No interfering peaks around the retention time of azoxystrobin were found in any of the control samples at levels above 30% of the LOQ.

Conclusion

The analytical method has been demonstrated to be a reliable and accurate procedure for the determination of azoxystrobin in AAP media with a limit of quantification (LOQ) of 0.00954 mg test item/L (corresponding to 0.00215 mg azoxystrobin/L) in accordance with SANTE/2020/12830 rev.1, using commercially available laboratory equipment and reagents.

(Obert-Rausser, P., 2020)

A 2.1.1.6.5.2 Confirmatory method

No confirmatory method is required.

A 2.1.1.7 Description of analytical methods for the determination of residues in support of physical and chemical properties tests (KCP 5.1.2.7)

No new or additional studies have been submitted.

A 2.1.2 Methods for post-authorization control and monitoring purposes (KCP 5.2)

A 2.1.2.1 Description of analytical methods for the determination of residues in plant matrices (KCP 5.2.1)

A 2.1.2.1.1 Analytical method 1: DFG-S19

A 2.1.2.1.1.1 Method validation (ZEN-0002V)

Comments of zRMS:	The study has been reviewed at EU level.
	The Applicant Syngenta confirms that, in accordance with SANTE/2017/10632, an assessment of extraction efficiency has been provided in the ongoing renewal (AIR) submission for the active substance azoxystrobin for all analytical methods used to support the analysis of residues in residue studies. Extraction efficiency has been robustly demonstrated for relevant solvent systems in representatives from each of the relevant crop and animal matrix groups in accordance with SANTE/2020/12830.

Reference: KCP 5.2.1

Report: Validation of the DFG Method S19 (Extended Revision) for the Determination of Residues of Azoxystrobin in Plant Materials, Weeren R.D., Pelz S. (2001)
Report No. ZEN-0002V, Syngenta File No. VV-327232

Guideline(s): Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/825/00 rev. 6, 20/06/2000)

Deviations: No

GLP: Yes

Acceptability: Yes

Materials and methods

Analytical method DFG S19 (extended revision) was validated in citrus (orange), kohlrabi, garlic, camomile, fennel (seed) and black tea. Hops could not be validated.

For citrus (orange), kohlrabi and garlic: extraction module E 1, clean-up module GPC and detection module D 4 (MSD). For camomile, fennel (seed) and black tea: extraction module E 2, clean-up modules GPC and additional C I and detection module D 4 (MSD).

Specimen material was extracted with acetone. Water was added beforehand in an amount taking full account of the natural water content of the specimen so that during extraction the acetone/water ratio remained constant at 2/1 (v/v). For liquid-liquid partition, ethyl acetate/cyclohexane (1/1, v/v) and sodium chloride were added and after repeated mixing, excess water was separated. The evaporated residue of an aliquot of the organic phase was cleaned up by gel-permeation chromatography (GPC) on Bio Beads S-X3 polystyrene gel using a mixture of ethyl acetate/cyclohexane (1/1, v/v) as eluent. For camomile, fennel (seed) and black tea a silica gel mini column chromatography was used for a further clean-up step after GPC. The residue containing fractions were concentrated and analysed for azoxystrobin by gas chromatography using mass-selective detection.

GC-MSD Conditions

GC system: Hewlett Packard 6890

Detector: MSD HP5972

Autosampler: HP7673

Column: *For citrus, garlic, kohlrabi, black tea:*
Fused silica capillary column DB-5 (J&W)
Methyl silicone with 5% phenyl groups
30 m x 0.25 mm, 0.25 µm film thickness
For camomile, fennel (seed):
Fused silica capillary column DB-1 (J&W)
Dimethylpolysiloxane
15 m x 0.25 mm, 0.25 µm film thickness

Oven temperature: *For citrus, garlic, kohlrabi, black tea:*
Initial temperature: 60°C (hold for 1 minute)
Heat rate: 30°C/minute to 250°C (hold for 17 minutes)
Heat rate: 40°C/minute to 280°C (hold for 5 minutes)

	<i>For camomile, fennel (seed):</i>
	Initial temperature: 60°C (hold for 1 minute)
	Heat rate: 40°C/minute to 250°C (hold for 0 minutes)
	Heat rate: 10°C/minute to 280°C (hold for 10 minutes)
Injector:	Injector: 250°C
	Interface: 280°C
Injection volume:	<i>For citrus, garlic, kohlrabi, black tea:</i> 2 µL, splitless
	<i>For camomile, fennel (seed):</i> 1 µL, splitless
Gas flow rates:	Helium carrier: 1.6 mL/minute
Retention time:	Azoxystrobin: approximately 22.4 minutes (<i>citrus, garlic, kohlrabi, black tea</i>); approximately 18.5 minutes (<i>camomile, fennel (seed)</i>)
Selected ions:	Quantitation: <i>m/z</i> 344
	Verification: <i>m/z</i> 345, 388
Detector	MSD HP5972
	Ionisation mode: Electron ionization (EI)
	Source polarity: Not reported
	Nebuliser gas (NEB): Not reported
	Curtain gas (CUR): Not reported
	Temperature (TEM): Not reported
	Ionisation energy: 70 eV
	Collision gas setting (CAD): Not reported
	Dwell time: Not reported
	Resolution Q1 and Q2: Not reported
	Electron multiplier (CEM): Not reported

Results and discussions

Table A 13: Recovery results from method validation of azoxystrobin using the analytical method

Matrix	Fortification Level (mg/kg)	Recovery (%)		RSD (%)	n
		Mean	Range		
Orange	0.02	89	83 - 97	6	5
	0.2	91	81 - 102	9	5
	Overall	90	81 - 102	7	10
Garlic	0.05	112	110 - 113	1	3
	0.5	111	100 - 120	9	3
	Overall	111	100 - 120	6	6
Kohlrabi	0.02	116	113 - 120	3	3
	0.2	94	89 - 98	5	3
	Overall	105	89 - 120	12	6
Camomile	0.05	118	109 - 126	7	3
	0.5	80	79 - 82	2	3
	Overall	99	79 - 126	22	6
Fennel seed	0.05	124	116 - 131	6	3
	0.5	114	109 - 119	4	3
	Overall	119	109 - 131	7	6
Tea, black	0.05	68	62 - 74	9	3
	0.5	87	74 - 94	13	3
	Overall	77	62 - 94	17	6

Specificity

GC-MSD monitoring three ions above m/z 100 is a highly specific detection technique and therefore a confirmatory technique is not required (SANTE/2020/12830, Rev.1 (24/02/2021)).

Linearity

The linearity of the GC-MSD detector response was demonstrated from 0.0167 to 2.5 µg/mL.

Azoxystrobin in ethyl acetate

Quantification: $y = -118797 + 2384356 x$ ($r = 0.9988$)

Accuracy

Recovery of azoxystrobin through the S19 method was acceptable according to SANTE/2020/12830, Rev.1 (24/02/2021), although the mean azoxystrobin recoveries overall for each crop were not all between 70% and 110% (garlic 111%, fennel seed 119%).

Repeatability

The relative standard deviation of azoxystrobin recoveries overall for each crop was < 20% except in the case of camomile (22%).

Limit of Quantification

The limit of quantification for azoxystrobin in citrus (orange) and kohlrabi was 0.02 mg/kg and in garlic, camomile, fennel (seed) and black tea was 0.05 mg/kg.

Reproducibility

An independent laboratory validation of this method was performed.

Matrix Effects

Matrix effects (suppression and enhancement) on the detector response of azoxystrobin were significant (i.e. > ±20%). Additional clean-up steps using modules GPC and C1 were introduced to remove the matrix effects and quantification was performed using external standards in ethyl acetate.

Stability of Final Extracts

The stability of azoxystrobin and R230310 in final extracts was not assessed. The recoveries of the samples were within the acceptable range of 70 – 110%, measured against freshly fortified standards, thus proving stability in accordance with SANTE/2020/12830, Rev. 1.

Stability of Standard Solutions

The stability of azoxystrobin in standard solutions was not assessed within this study.

Conclusion

Method DFG S19 is considered valid for the determination of azoxystrobin residues in crops at the LOQ (0.01 mg/kg) and over concentration ranges typical of those for which the method will be used.

A 2.1.2.1.1.2 Method validation (IF-04/00192716)

Comments of zRMS:	<p>The study has been reviewed at EU level.</p> <p>The Applicant Syngenta confirms that, in accordance with SANTE/2017/10632, an assessment of extraction efficiency has been provided in the ongoing renewal (AIR) submission for the active substance azoxystrobin for all analytical methods used to support the analysis of residues in residue studies. Extraction efficiency has been robustly demonstrated for relevant solvent systems in representatives from each of the relevant crop and animal matrix groups in accordance with SANTE/2020/12830.</p>
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Report	Analytical Method Development and Validation of the DFG Method S19 for the Determination of Residues of Azoxystrobin and the Metabolite R230310 in Plant Matrices, Stahl F. (2017) Report No. IF-04/00192716, Syngenta File No. VV-379800
Guideline(s):	Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/825/00 rev. 8.1, 16/11/2010). Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/3029/99 rev. 4, 11/07/2000). OECD Guidance Document on Pesticide Residue Analytical Methods ENV/JM/MONO(2007)17. Regulation (EC) No 1107/2009 of the European Parliament and of the council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC. Residue Chemistry Test Guidelines OPPTS 860.1340 Residue Analytical Method, EPA 712-C-96-174, August 1996
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Analytical method DFG S19 (extended revision) was validated in wheat grain, lettuce, rape seed and orange.

Materials and methods

Test Material 1	Azoxystrobin
Lot/Batch #:	ASJ10008-03
Purity (%):	99.7
IUPAC name:	methyl (E)-2-{2-[6-(2-cyanophenoxy)pyrimidin-4-yloxy]-phenyl}-3-methoxyacrylate
CAS number:	131860-33-8

Test Material 2	R230310
Lot/Batch #:	ASJ10075-04
Purity (%):	98
IUPAC name:	methyl (Z)-2-{2-[6-(2-cyanophenoxy)pyrimidin-4-yloxy]-phenyl}-3-methoxyacrylate
CAS number:	143130-94-3

Test commodities			
Crop	Commodity	Commodity type ¹	Source
Wheat	Grain	Dry ²	Originated from a field trial
Lettuce	Not reported	High water content	Local greengrocers
Oilseed rape	Seed	High oil content	Local greengrocers
Orange	Not reported	High acid content	Local greengrocers

¹ Commodity type matrix groups given as defined in SANTE/2020/12830 Rev. 1 Appendix 1

² Defined as “dry commodities (high protein/high starch content)” in SANCO/825/00 rev. 8.1 and “high starch content” in OECD ENV/JM/MONO(2007)17

Study Design and Methods

Test facility: SGS INSTITUT FRESENIUS GmbH, Germany

Study start date: 24 June 2004

Study end date: 21 September 2004 (final report) and 12 April 2017 (final report Amendment 1)

Analytical phase dates: 20 July 2004 to 28 July 2004

The residue analytical method for azoxystrobin and the metabolite R230310 in plant matrices is based on the multi residue method DFG-method S-19 (Modular Multiple Analytical Method for the Determination of Pesticide Residues in Foodstuffs, L 00.00-34 of the Collection of Official Methods according to §35 of the German Federal Food Act, extended and revised version of DFG Method S-19, November 1999). For the extraction, the modules E1 for lettuce, E2 for wheat grain, E3 for orange and E7 for oilseed rape (seed) were used.

The clean-up procedure is carried out according to module GPC (gel permeation chromatography). Azoxystrobin and the metabolite R230310 are quantified by high performance liquid chromatography with mass spectrometric detection (LC-MS/MS), monitoring for the primary transition (m/z 404-372) and the confirmatory transition (m/z 404-344).

The multi residue DFG method S19 was validated in a wide range of crops, wheat grain (high protein content), lettuce (high water content), oilseed rape seed (high oil content) and orange (high acid content). R230310 is not discussed further as it is not considered to be a relevant analyte in crops (i.e. it is not in the definition of residue for monitoring).

LC-MS/MS Conditions

HPLC system:	Agilent, No. 1100		
Pumps:	Agilent, G1311A		
Degasser:	Agilent, G1322A		
Column Oven:	Agilent G1316A		
Detector:	Applied Biosystems, API 3000		
Autosampler:	CTC Analytics, HTS Pal		
Column:	Supelco, Discovery C18, 150 mm x 2.1 mm, 5 µm		
Mobile phase:	A: Acetonitrile with 0.1% formic acid		
	B: Ultrapure water with 0.1% formic acid		
	Time	%A	%B
	0	20	80
	3	20	80
	13	60	40
	14	90	10
	17	90	10
	18	20	80
	22	20	80
			Gradient
			Not reported
			Not reported
			Not reported
			Not reported
			Not reported
			Not reported
			Not reported
Flow rate:	0.5 mL/min		
Column oven temperature:	40°C		
Injection volume:	20 µL		
Retention time:	Azoxystrobin: 12.5 min		
	R230310: 11.7 min		

Detector	API 3000	
Ionisation mode:	MRM	
Source polarity:	Positive	
Curtain gas (CUR):	12 (arbitrary units)	
Gas 1 (GSI):	Not reported	
Gas 2 (GSI):	Not reported	
Temperature (TEM):	400°C	
Interface heater (IHC):	On	
Ionspray voltage (IS):	5500 V	
Collision gas setting (CAD):	9	
Entrance potential (EP):	10 V	
Dwell time:	Not reported	
Resolution Q1 and Q2	Not reported	

Source and detection parameters for MS/MS experiments:

Compound	Parent	CE	CXP	Fragment ions	
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	(<i>m/z</i>)	(V)	(V)	(<i>m/z</i>)	
Azoxystrobin and R230310	404	21 35	22 20	372 344	Quantification Confirmation

CE: Collision energy; CXP: Collision cell exit potential

Quantification: Peak areas of fragment ion at *m/z* = 372, external standards in solvent (water/acetonitrile, 80/20, v/v)

Confirmation: Peak areas of fragment ion at *m/z* = 344, external standards in solvent (water/acetonitrile, 80/20, v/v)

Results and discussions

Table A 14: Recovery results from method validation of azoxystrobin using the analytical method (quantification Transition *m/z* 404→372)

Matrix	Fortification Level (mg/kg)*	Recovery (%)	Number of Analysis (n)	Mean Recovery(%)	RSD (%)	Recovery Range (%)
Wheat grain	0.01*	82, 79, 82, 79, 76	5	79.6	3.2	76 – 82
	0.3	87, 82, 84, 83, 92	5	85.6	4.7	82 – 92
	Overall	-	10	82.6	5.4	76 – 92
Lettuce	0.01*	86, 87, 85, 85, 92	5	87	3.4	85 – 92
	3	88, 84, 86, 88, 95	5	88.2	4.7	84 – 95
	Overall	-	10	87.6	3.9	84 – 95
Oilseed rape	0.01*	95, 89, 92, 89, 90	5	91.0	2.8	89 – 95
	0.5	79, 91, 77, 71, 95	5	82.6	12.2	71 – 95
	Overall	-	10	86.8	9.5	71 – 95
Orange	0.01*	75, 80, 80, 80, 79	5	78.8	2.8	75 – 80
	10	86, 94, 92, 84, 85	5	88.2	5.1	84 – 94
	Overall	-	10	83.5	7.1	75 – 94

*Limit of quantitation, defined by the lowest validated fortification level

Table A 15: Recovery results from validation of the DFG method S19 for azoxystrobin in crops: confirmatory transition *m/z* 404-344

Matrix	Fortification Level (mg/kg)*	Recovery (%)	Number of Analysis (n)	Mean Recovery (%)	RSD (%)	Recovery Range (%)
Wheat grain	0.01*	75, 73, 73, 73, 74	5	73.4	1.4	73 – 75
	0.3	85, 74, 77, 86, 89	5	82.0	7.5	74 – 89
	Overall	-	10	77.7	7.9	73 – 89
Lettuce	0.01*	87, 86, 83, 84, 94	5	86.9	4.7	83 – 94
	3	89, 85, 88, 88, 94	5	88.7	3.9	85 – 94
	Overall	-	10	87.8	4.2	83 – 94
Oilseed rape	0.01*	90, 81, 93, 84, 89	5	87.3	5.7	81 – 93
	0.5	75, 87, 73, 68, 94	5	79.3	13.7	68 – 94
	Overall	-	10	83.3	10.8	68 – 94
Orange	0.01*	73, 84, 83, 87, 78	5	81.0	6.5	73 – 87
	10	85, 94, 93, 86, 86	5	89.0	4.9	85 – 94
	Overall	-	10	85.0	7.3	73 – 94

*Limit of quantitation, defined by the lowest validated fortification level

Specificity

LC-MS/MS with two transitions is considered to be a highly specific detection technique and therefore according to EU guidance (SANTE/2020/12830, Rev.1 (24/02/2021)) no further confirmatory technique is required. The method includes two MS/MS transitions, both of which have been validated. No

significant interferences arising from the crop matrices, the labware, reagents or solvents have been observed at the retention times of interest.

Linearity

The linearity of the LC-MS/MS detector was tested using standard solutions (0.05 ng/ml to 0.7 ng/ml for both analytes). Linearity was tested in the solvent mixture used and for both MS/MS transitions. Standards at eight different concentrations were injected and the signal area plotted against concentration for all calibration points. Straight lines with correlation coefficients ranging from 0.9991 to 0.9994 were obtained for azoxystrobin. Linear equations and correlation coefficients are reported:

Azoxystrobin in water/acetonitrile (80/20, v/v)

Quantification: $y = 5339332.152x + 963.460$ ($r = 0.99938$)

Confirmation: $y = 1665054.779x + 479.877$ ($r = 0.99914$)

Accuracy

Fortified samples were analysed in quintuplet at the limit of quantification (LOQ, 0.01 mg/kg) for all crops and at 30xLOQ (0.3 mg/kg), 300xLOQ (3 mg/kg), 50xLOQ (0.5 mg/kg) and 1000xLOQ (10 mg/kg) for wheat grain, lettuce, oilseed rape and orange, respectively. Acceptable mean recoveries of between 70% and 110% were found for both transitions on all matrices tested and therefore according to EU guidance (SANTE/2020/12830, Rev.1 (24/02/2021)) demonstrate the method has satisfactory accuracy.

Repeatability

The relative standard deviations (RSDs) of azoxystrobin recoveries at each fortification level and overall for each crop tested during method validation were < 20% and therefore according to the EU guidance (SANTE/2020/12830, Rev.1 (24/02/2021)) demonstrate the method has satisfactory repeatability.

Limit of Quantification

The limit of quantification for azoxystrobin in crop matrices using the multi residue DFG method S19 was established at 0.01 mg/kg. No interfering peaks around the retention time of azoxystrobin or the metabolite R230310 were found in any of the control samples at levels above 30% of the limit of quantification.

Matrix Effects

Matrix effects (suppression and enhancement) on the detector response of azoxystrobin were not assessed within this study. Solvent standards (water/acetonitrile, 80/20, v/v) were used for quantification throughout the study.

Stability of Final Extracts

The stability of azoxystrobin and R230310 in final extracts was not assessed. The recoveries of the samples were within the acceptable range of 70 – 110%, measured against freshly fortified standards, thus proving stability in accordance with SANTE/2020/12830, Rev. 1.

Stability of Standard Solutions

Azoxystrobin and R230310 were found to be stable in stock solutions (water/acetonitrile, 80/50, v/v) when stored at 4 – 8°C for at least 9 days.

Reproducibility

An independent laboratory validation of this method was performed, please refer to study SYN-0422V below.

Conclusion

Method DFG S19 is considered valid for the determination of azoxystrobin residues in crops at the LOQ (0.01 mg/kg) and over concentration ranges typical of those for which the method will be used.

A 2.1.2.1.1.3 Independent laboratory validation (SYN-0422V)

Comments of zRMS:	The study has been reviewed at EU level.
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Reference: KCP 5.2.1

Report: Independent Laboratory Validation for the Determination of Residues of Azoxystrobin in Lettuce and Wheat Grain by Multi-Residue Method S19 (L 0000-34) Validated by a Third Party Laboratory, Lakaschus, S., Gizler, A. (2017)
Report No. SYN-0422V, Syngenta File No. VV-380727

Guideline(s): Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/825/00 rev. 8.1, 16/11/2010).
Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/3029/99 rev. 4, 11/07/2000).
OECD Guidance Document on Pesticide Residue Analytical Methods ENV/JM/MONO(2007)17.
Regulation (EC) No 1107/2009 of the European Parliament and of the council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC.
Residue Chemistry Test Guidelines OPPTS 860.1340 Residue Analytical Method, EPA 712-C-96-174, August 1996.

Deviations: No

GLP: Yes

Acceptability: Yes

Materials and methods

Materials	Test Material 1	Azoxystrobin
Lot/Batch #:		ASJ10008-03
Purity (%):		99.7
IUPAC name:		methyl (E)-2-{2-[6-(2-cyanophenoxy)pyrimidin-4-yloxy]-phenyl}-3-methoxyacrylate
CAS number:		131860-33-8

Test commodities			
Crop	Commodity	Commodity type ¹	Source
Wheat	Grain	Dry ²	Provided by test facility
Lettuce	Not reported	High water content	Provided by test facility

¹ Commodity type matrix groups given as defined in SANTE/2020/12830 Rev. 1 Appendix 1

² Defined as “dry commodities (high protein/high starch content)” in SANCO/825/00 rev. 8.1 and “high starch content” in OECD ENV/JM/MONO(2007)17

Study Design and Methods

Test facility: Dr. Specht & Partner Chemische Laboratorien GmbH, Hamburg, Germany

Study start date: 13 December 2004

Study end date: 02 February 2005 (final study report) and 05 April 2017 (final report amendment)

Analytical phase dates: 13 December 2004 to 17 December 2004

The residue analytical method for azoxystrobin in plant matrices is based on the multi residue method DFG-method S-19 (Modular Multiple Analytical Method for the Determination of Pesticide Residues in Foodstuffs, L 00.00-34 of the Collection of Official Methods according to §35 of the German Federal Food Act, extended and revised version of DFG Method S-19, November 1999). For the extraction, the modules E1 for lettuce and E2 for wheat grain were used.

The clean-up procedure is carried out according to module GPC (gel permeation chromatography). Azoxystrobin is quantified by high performance liquid chromatography with mass spectrometric detection

(LC-MS/MS), monitoring for the primary transition (m/z 404-372) and the confirmatory transition (m/z 404-344).

The multi residue method DFG method S19 was independently validated in wheat grain (high protein content) and lettuce (high water content).

LC-MS/MS Conditions

HPLC system: Hewlett-Packard Series 1100 HPLC (Agilent Technologies)
Pumps: Not reported
Degasser: Not reported
Column Oven: Not reported
Detector: PE-Sciex API 4000
Autosampler: Not reported
Column: Phenomenex® LUNA C18(2), 2.0 x 150 mm
Mobile phase: A: Acetonitrile with 0.1% formic acid
B: Water with 0.1% formic acid

Time	%A	%B	Gradient
4.0	20	80	Not reported
0.0	20	80	Not reported
6.0	90	10	Not reported
9.0	90	10	Not reported

Flow rate: 0.4 mL/min
Column oven temperature: 25°C
Injection volume: 20 µL
Retention time: Azoxystrobin: 7.8 min

Detector API 3000
Ionisation mode MRM
Source polarity: Positive
Curtain gas (CUR): 30 (arbitrary units)
Gas 1 (GSI): 30 (arbitrary units)
Gas 2 (GSI): 50 (arbitrary units)
Temperature (TEM): 400°C
Interface heater (IHC): On
Ionspray voltage (IS): 5500V
Collision gas setting (CAD): Not reported
Entrance potential (EP): Not reported
Dwell time: 0.5 sec
Resolution Q1 and Q2 Not reported

Source and detection parameters for MS/MS experiments:

Compound	Parent (m/z)	CE (V)	DP (V)	Fragment ions (m/z)	
Azoxystrobin	404	23	61	372	Quantification
		35	61	344	Confirmation

CE: Collision energy; DP: Declustering potential

Quantification: Peak areas of fragment ion at m/z = 372, external standards in solvent (water/acetonitrile, 80/20, v/v)

Confirmation: Peak areas of fragment ion at m/z = 344, external standards in solvent (water/acetonitrile, 80/20, v/v)

Results and discussions

Summaries of the results for azoxystrobin are presented in

Table A 16 and Table A 17.

Table A 16: Recovery results from independent laboratory validation of azoxystrobin using the analytical method (quantification transition m/z 404 \rightarrow 372)

Matrix	Fortification Level (mg/kg)	Recovery (%)	Number of Analysis (n)	Mean Recovery (%)	RSD (%)	Recovery Range (%)
Wheat grain	0.01*	107, 109, 109, 108, 106	5	108	1.2	106 – 109
	0.3	95, 102, 103, 106, 102	5	102	3.9	95 – 106
	Overall	-	10	105	4.1	95 – 109
Lettuce	0.01*	111, 109, 110, 105, 115	5	110	3.3	105 – 115
	3	97, 97, 97, 99, 91	5	96	3.1	91 – 99
	Overall	-	10	103	7.7	91 – 115

*Limit of quantitation, defined by the lowest validated fortification level

Table A 17: Recovery results from validation of the DFG method S19 for azoxystrobin in crops: confirmatory transition m/z 404-344

Matrix	Fortification Level (mg/kg)	Recovery (%)	Number of Analysis (n)	Mean Recovery (%)	RSD (%)	Recovery Range (%)
Wheat grain	0.01*	103, 108, 107, 107, 102	5	105	2.6	102 – 108
	0.3	97, 105, 104, 108, 103	5	103	3.9	97 – 108
	Overall	-	10	104	3.3	97 – 108
Lettuce	0.01*	110, 107, 104, 103, 114	5	108	4.2	103 – 114
	3	101, 101, 100, 103, 95	5	100	3.0	95 – 103
	Overall	-	10	104	5.2	95 – 114

Specificity

LC-MS/MS with two transitions is considered to be a highly specific detection technique and therefore according to EU guidance (SANTE/2020/12830, Rev.1 (24/02/2021)) no further confirmatory technique is required. The method includes two MS/MS transitions, both of which have been validated. No significant interferences arising from the crop matrices, the labware, reagents or solvents have been observed at the retention times of interest.

Linearity

The linearity of the LC-MS/MS detector was tested using standard solutions (0.2 to 20.0 ng/ml). Linearity was tested in the solvent mixture used and for both MS/MS transitions. Standards at six different concentrations were injected and the signal area plotted against concentration for all calibration points. Straight lines with correlation coefficients ranging from 0.9986 to 0.9995 were obtained for azoxystrobin. Linear equations and correlation coefficients are reported:

Azoxystrobin in water/acetonitrile (80/20, v/v) (used for wheat grain)

Quantification: $y = 503072x + 147367$ ($r = 0.9993$)

Confirmation: $y = 128998x + 22952$ ($r = 0.9997$)

Azoxystrobin in water/acetonitrile (80/20, v/v) (used for lettuce)

Quantification: $y = 503072x + 147367$ ($r = 0.9993$)

Confirmation: $y = 128998x + 22952$ ($r = 0.9997$)

Accuracy

Fortified samples were analysed in quintuplet at the limit of quantification (LOQ, 0.01 mg/kg) for both crops and at 30xLOQ (0.3 mg/kg) and 300xLOQ for wheat grain and lettuce, respectively. Acceptable mean recoveries of between 70% and 110% were found for both transitions on all matrices tested and therefore according to EU guidance (SANTE/2020/12830, Rev.1 (24/02/2021)) demonstrate the method has satisfactory accuracy.

Repeatability

The relative standard deviations (RSDs) of azoxystrobin recoveries at each fortification level and overall for each crop tested during method validation were < 20% and therefore according to the EU guidance

(SANTE/2020/12830, Rev.1 (24/02/2021)) demonstrate the method has satisfactory repeatability

Limit of Quantification

The limit of quantification for azoxystrobin residues in crop matrices using multi residue method DFG-method S-19 was established at 0.01 mg/kg. No interfering peaks around the retention time of azoxystrobin were found in any of the control samples at levels above 30% of the limit of quantification.

Matrix Effects

Matrix effects (suppression and enhancement) on the detector response of azoxystrobin were not assessed within this study. Solvent standards (water/acetonitrile, 80/20, v/v) were used for quantification throughout the study.

Stability of Final Extracts

The stability of azoxystrobin in final extract was not assessed within this study.

Stability of Standard Solutions

The stability of azoxystrobin in standard solutions was not assessed within this study.

Reproducibility

This successful validation by an independent laboratory demonstrates the reproducibility of the DFG S19 method for the determination of azoxystrobin residues in crops.

Conclusion

The DFG S19 method is, therefore, suitable for monitoring of azoxystrobin residues in crops.

A 2.1.2.1.1.4 Confirmatory method

No confirmatory method is required. LC-MS/MS with two transitions is considered to be a highly specific detection technique. The method includes two MS/MS transitions, both of which have been validated.

A 2.1.2.1.1.5 Extraction efficiency

No new or additional studies have been submitted.

A 2.1.2.1.2 Analytical method 2: RAM 305/03

A 2.1.2.1.2.1 Method validation (T011298-06-REG)

Comments of zRMS:	The study has been reviewed at EU level.
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Reference: KCP 5.2.1 and KCP 5.1.2.5

Report Azoxystrobin (ICI5504) and Cyproconazole (SAN619) - Residues in Honey following Exposure of Bees to Treated Winter Oil-seed Rape in Germany during 2007
Bocksch, S. (2008)
Report No. T011298-06-REG, Syngenta File No. VV-382035

Guideline(s): Guidance Document for Residue Trials on Honey of the Federal Office for Consumer Protection and Food Safety, version 2, 03/10/2003.
IVA (1992), EU 91/414/EEC (1997)

Deviations: None

GLP: Yes

Analytical method RAM 305/03 is also used for the generation of pre-authorisation data. The validation of this method (T011298-06-REG) is summarised in A 2.1.1.5 (KCP 5.1.2.5).

A 2.1.2.1.2.2 Independent laboratory validation

No new or additional studies have been submitted.

A 2.1.2.1.2.3 Confirmatory method

Please refer to A 2.1.1.5 (KCP 5.1.2.5).

A 2.1.2.1.2.4 Extraction efficiency

No new or additional studies have been submitted.

A 2.1.2.2 Description of analytical methods for the determination of residues in animal matrices (KCP 5.2.2)

A 2.1.2.2.1 Analytical method 1: DFG S19

A 2.1.2.2.1.1 Method validation (ZEN-9505V)

Comments of zRMS:	Analytical method DFG S19 (modified extraction) is not suitable for monitoring of azoxystrobin residues in animal matrices.
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Reference: KCP 5.2.2

Report: Validation of DFG Method S19 (Modified Extraction) for the Determination of Residues of ICIA5504 (Azoxystrobin) Milk, Muscle, Kidney, Liver and Egg
xxxxxx (1997)
Report No. ZEN-9505V, Syngenta File No. VV-323618

Guideline(s): Not stated

Deviations: No

GLP: Yes

Acceptability: No

Materials and methods

Samples were extracted with acetone. Water was added beforehand in an amount taking full account of the natural water content of the sample so that during extraction the acetone/water ratio remained constant at 2/1 (v/v). The extract was saturated with sodium chloride and mixed thoroughly. For liquid-liquid partition ethyl acetate/cyclohexane (1/1) was added and after repeated mixing excess water was separated. The evaporation residue of the organic phase was cleaned up by gel-permeation chromatography on Bio Beads S-X3 polystyrene gel, using an automated gel chromatograph. The residue-containing fraction is concentrated and directly analysed by gas chromatography using a capillary column and mass-selective detector (MSD).

GC-MSD Conditions

GC system: Hewlett Packard 5890
Detector: MSD HP5971

Autosampler: HP7673
Column: Fused silica capillary column HP-5 MS
30 m x 0.25 mm, 0.25 µm film thickness
Oven temperature: Initial temperature: 60°C (hold for 1 minute)
Heat rate: 30°C/minute to 300°C (hold for 6 minutes)
Injector: Injector: 300°C
Interface: 280°C
Injection volume: 1 µL, splitless
Gas flow rates: Helium carrier: 70 kPa
Retention time: Azoxystrobin: approximately 13.2 minutes
Selected ion: *m/z* 344

Detector: MSD HP5971
Ionisation mode: Not reported
Source polarity: Not reported
Nebuliser gas (NEB): Not reported
Curtain gas (CUR): Not reported
Temperature (TEM): Not reported
Ionisation energy: Not reported
Collision gas setting (CAD): Not reported
Dwell time: Not reported
Resolution Q1 and Q2: Not reported
Electron multiplier (CEM): Not reported

Results and discussions

Table A 18: Recovery results from method validation of azoxystrobin using the analytical method (modified extraction)

Matrix	Fortification level (mg/kg)	Recovery (%)		RSD (%)	n
		Mean	Range		
Milk	0.02	96	80 - 112	-	2
	0.2	91	90 - 92	-	2
	Overall	94	80 - 112	14	4
Muscle	0.02	109	100 - 118	-	2
	0.2	94	92 - 96	-	2
	Overall	102	92 - 118	11	4
Kidney	0.02	-	- (a)	-	2
	0.2	-	- (a)	-	2
	Overall	-	-	-	4
Liver	0.02	-	- (a)	-	2
	0.2	83	80 - 86	-	2
	Overall	83	80 - 86	-	2
Egg	0.02	-	- (a)	-	2
	0.2	-	- (a)	-	2
	Overall	-	-	-	4

Specificity

No confirmatory technique was included in this validation study.

Linearity

The linearity of the GC-MSD detector response is not indicated in the report.

Accuracy

The mean azoxystrobin recoveries overall for milk and muscle, and for liver at the 0.2 mg/kg fortification level, were between 70% and 110%. For liver at the 0.02 mg/kg fortification level, and for kidney and egg at any level, evaluation of the recoveries was not possible.

Repeatability

The relative standard deviation of azoxystrobin recoveries overall was <20% for milk and muscle. No RSDs were obtained for kidney, liver and egg.

Limit of Quantification

The limit of quantification for azoxystrobin was 0.02 mg/kg in milk and muscle, and 0.2 mg/kg in liver. No LOQ could be determined for kidney and egg.

Matrix Effects

Matrix effects (suppression and enhancement) on the detector response were not reported.

Stability of Final Extracts

The stability of azoxystrobin and R230310 in final extracts was not assessed.

Stability of Standard Solutions

The stability of azoxystrobin in standard solutions was not assessed within this study.

Reproducibility

An independent laboratory validation of this method was not performed.

Conclusion

Analytical method DFG S19 (modified extraction) has been successfully validated for the analysis of azoxystrobin in milk and muscle, but not in kidney, liver and egg. Analytical method DFG S19 (modified extraction) is therefore not suitable for monitoring of azoxystrobin residues in animal matrices.

A 2.1.2.2.1.2 Independent laboratory validation

No new or additional studies have been submitted.

A 2.1.2.2.1.3 Confirmatory method

No confirmatory method is required. LC-MS/MS with two transitions is considered to be a highly specific detection technique. The method includes two MS/MS transitions, both of which have been validated.

A 2.1.2.2.1.4 Extraction efficiency

No new or additional studies have been submitted.

A 2.1.2.2.2 Analytical method 2: RAM 399/01

Comments of zRMS:	The method has been evaluated in Draft Registration Report for Orondis Evo (Central Zone, zRMS-PL, July 2022). Below is a copied assessment: The analytical procedure described is suitable for the analysis of azoxystrobin and its Z isomer, R230310, in bovine muscle tissue, fat and milk, lamb's liver and kidney and hen's egg samples using an external standardisation procedure. The limit of quantification has
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	been set at 0.01 mg/kg for all matrices with nal determination by HPLC MS-MS. Only commercially available laboratory equipment and reagents are required. Untreated and fortified samples should be extracted and analysed with each set of samples to demonstrate absence of any interference and adequate recovery, if possible. The method is acceptable.
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Reference: KCP 5.2.2 (and KCP 5.1.2.5)

Report: Residue Analytical Method for the Determination of Residues of Azoxystrobin and R230310 in Bovine Muscle Tissue, Fat and Milk, Lamb Liver and Kidney and Hen Egg Samples
xxxxxx (2002)
Report No. SOP RAM 399/01
Syngenta File No. VV-124385
unpublished

Guideline(s): None stated - compliant with SANCO/825/00 rev. 6, 20/06/2000

Deviations: No

GLP: No

Acceptability: Yes

Analytical method RAM 399/01 is also used for the generation of pre-authorisation data. The method description and validation of this method (RJ3350B) are summarised in A 2.1.1.5 (KCP 5.1.2.5).

A 2.1.2.2.2.1 Method validation (RJ3350B)

Comments of zRMS:	The study has been reviewed at EU level.
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Reference: KCP 5.2.2 (and KCP 5.1.2.5)

Report: Azoxystrobin and R230310: Validation of Analytical Method RAM 399/01 for the Determination of Residues in Bovine Muscle, Fat and Milk, Lamb's Kidney and Liver and Hen's Eggs
Richards S. (2002)
Report No. RJ3350B
Syngenta File No. VV-331095
unpublished

Guideline(s): None stated - compliant with SANCO/825/00 rev. 6, 20/06/2000

Deviations: No

GLP: Yes

Acceptability: Yes

Analytical method RAM 399/01 is also used for the generation of pre-authorisation data. The method description and validation of this method (RJ3350B) are summarised in A 2.1.1.5 (KCP 5.1.2.5).

A 2.1.2.2.2.2 Independent laboratory validation (CEMR-1907)

Comments of zRMS:	The study has been reviewed at EU level.
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Reference: KCP 5.2.2

Report: Independent Laboratory Validation of a Method for the Determination of Residues of Azoxystrobin in Animal Tissue
xxxxxxx (2003)
Report No. CEMR-1907
Syngenta File No. VV-328461
Unpublished

Guideline(s): Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/825/00 rev. 6, 20/06/2000).

Deviations: No

GLP: Yes

Acceptability: Yes

Materials and methods

Test Material 1	Azoxystrobin
Lot/Batch #:	ASJ10008-03
Purity (%):	99.7
IUPAC name:	methyl (E)-2-{2-[6-(2-cyanophenoxy)pyrimidin-4-yloxy]phenyl}-3-methoxyacrylate
CAS number:	131860-33-8

Test Material 2	R230310
Lot/Batch #:	ASJ10075-03
Purity (%):	98
IUPAC name:	methyl (Z)-2-{2-[6-(2-cyanophenoxy)pyrimidin-4-yloxy]phenyl}-3-methoxyacrylate
CAS number:	143130-94-3

Animal	Commodity	Source
Bovine	Milk	Local supermarket
Bovine	Muscle	Local supermarket

Study Design and Methods

Test facility: CEM Analytical Services Ltd (CEMAS), North Ascot, Berkshire, United Kingdom

Study start date: 07 February 2003

Study end date: 28 February 2003

Analytical phase dates: 07 February 2003 to 25 February 2003

Homogenised sub-samples of each test commodity (5 g) were fortified with standard solutions of azoxystrobin and R230310 in acetonitrile/water. Five samples of each matrix were fortified at the limit of quantification (LOQ; 0.01 mg/kg) and five at a higher level (10x LOQ; 0.10 mg/kg). Matrices used were bovine milk and muscle.

Principle of the method

Residues of azoxystrobin and R230310 are extracted by maceration with acetonitrile. Extracts are centrifuged and aliquots are diluted with ultra-pure water. A C18 solid phase extraction (SPE) procedure is carried out to facilitate sample clean-up. Final determination is performed by high performance liquid chromatography with triple quadrupole mass spectrometric detection (LC-MS/MS).

HPLC-MS/MS Conditions

HPLC system:

Pumps: Agilent 1100 series quaternary pump model number G1311A

Degasser: Agilent 1000 series model number G1322A
Column Oven: Agilent 1100 series model number G1316A
Detector: Applied Biosystems API 3000 triple quadrupole mass spectrometer
Autosampler: CTC PAL
Column: KR100 5C18 5 µm 50 mm x 3.2 mm i.d.
Isocratic Mobile phase: A: Acetonitrile
B: Ultrapure water with 0.2% acetic acid

Time	% A	% B	Gradient
0.0	50	50	Isocratic
2.00	50	50	Isocratic

Flow rate: 1 mL/min
Column oven temperature: 40°C
Injection volume: 10 µL
Retention time: Azoxystrobin: 1.2 min
R230310: 0.94 min

Detector API 3000

Ionisation mode:	TurboIonSpray
Scan type:	MRM
Source polarity:	Positive
Curtain gas (CUR):	12 (arbitrary units)
Gas 1 (GSI):	Not reported
Gas 2 (GSI):	Not reported
Temperature (TEM):	450°C
Ionspray voltage (IS):	5000 V
Collision gas setting (CAD):	4
Entrance potential (EP):	10 V
Dwell time	400 msec
Resolution Q1	Low
Resolution Q2	High

Source and detection parameters for MS/MS experiments:

Compound	Parent (<i>m/z</i>)	CE (V)	DP (V)	CXP (V)	Fragment ions (<i>m/z</i>)	
Azoxystrobin and R230310	404.2	21	41	24	372.4	Quantification

CE: Collision energy; CXP: Collision cell exit potential; DP: Declustering Potential

Quantification: Peak areas of fragment ion at $m/z = 372.4$, external standards in solvent (acetonitrile/water, 50/50, v/v).

Results and discussions

Summaries of the results for azoxystrobin are presented in

Table A 19.

Table A 19: Recovery results from independent laboratory validation of azoxystrobin using the analytical method

Matrix	Fortification Level (mg/kg)	Recovery (%)		RSD (%)	n
		Mean	Range		
Bovine milk	0.01	95	93 - 99	2.5	5
	0.1	101	91 - 110	8.7	5
	Overall	98	91 - 110	7.0	10
Bovine muscle	0.01	95	92 - 98	3.0	5
	0.1	101	90 - 111	9.9	5

Matrix	Fortification Level (mg/kg)	Recovery (%)		RSD (%)	n
		Mean	Range		
	Overall	98	90 - 111	7.9	10

Specificity

LC-MS/MS is a highly specific detection technique and therefore a confirmatory technique is not required (SANTE/2020/12830, Rev.1 (24/02/2021)). No interfering peaks around the retention time of azoxystrobin were found in any of the control samples at levels above 30% of the limit of quantification. Confirmatory transition can be monitored (m/z 404.2→ 344.3), and has been validated for method RAM 399/01 in blood (xxxxxx 2011, Report S10-03815).

Linearity

The linearity of the LC-MS/MS detector was tested using five standard solutions at concentrations between 0.05 and 10 µg/L. Standards were injected in triplicate and the response plotted against concentration. Linear correlations with coefficients ≥ 0.995 were obtained for azoxystrobin. Linear equations and correlation coefficients are reported:

Azoxystrobin in acetonitrile/water (50/50, v/v)

Quantification: $y = 4085038 x + 225$ ($r = 0.9991$)

Accuracy

Recovery of azoxystrobin through method RAM 399/01 was acceptable, with all mean recovery values in the range 70% to 110%.

Repeatability

The relative standard deviation of azoxystrobin recoveries for each animal matrix was $< 20\%$.

Limit of Quantification

The limit of quantification for azoxystrobin in bovine milk and muscle was 0.01 mg/kg.

Matrix Effects

Matrix effects (suppression and enhancement) on the detector response of azoxystrobin were not assessed. Quantification was performed using external standards in acetonitrile/water (50/50, v/v).

Stability of Final Extracts

The stability of azoxystrobin in final extracts was not assessed within this study.

Stability of Standard Solutions

The stability of azoxystrobin in standard solutions was not assessed within this study.

Reproducibility

This successful validation by an independent laboratory demonstrates the reproducibility of method RAM 399/01 for the determination of azoxystrobin residues in animal matrices.

Conclusion

Analytical method RAM 399/01 has been successfully validated for the analysis of azoxystrobin in representative animal matrices, including bovine milk, fat and muscle, lamb's kidney and liver and hen's egg. Results obtained were within the guideline requirements (mean recovery 70 – 110%; RSD $< 20\%$). Analytical method RAM 399/01 has also been successfully validated by an independent laboratory. Method RAM 399/01 is, therefore, suitable for monitoring of azoxystrobin residues in animal matrices.

A 2.1.2.2.2.3 Confirmatory method

No confirmatory method is required. LC-MS/MS with two transitions is considered to be a highly specific detection technique. The method includes two MS/MS transitions, both of which have been validated.

A 2.1.2.2.2.4 Extraction efficiency

No new or additional studies have been submitted.

A 2.1.2.3 Description of Methods for the Analysis of Body Fluids and Tissues (KCP 5.2.3)

A 2.1.2.3.1 Analytical method 1: RAM 399/01

A 2.1.2.3.1.1 Method validation (S10-03815)

Comments of zRMS:	The study has been reviewed at EU level.
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Reference: KCP 5.2.3

Report: Azoxystrobin - Validation of Analytical Method RAM 399/01 for the Determination of azoxystrobin, R230310 and R234886 in Human Whole Blood
Gemrot F. (2011)
Report No. S10-03815, Syngenta File No. VV-398250

Guideline(s): Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/825/00 rev. 8.1, 16/11/2010)
Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/3029/99 rev. 4, 11/07/2000)
Method Validation and Quality Control Procedures for Pesticide Residue Analysis in Food and Feed (SANCO/10684/2009, 01/10/2010)
EPA Residue Chemistry Test Guidelines OPPTS 850.7100
Residue Chemistry Test Guidelines OPPTS 860.1340 Residue Analytical Method, EPA 712-C-96-174, August 1996

Deviations: No

GLP: Yes

Acceptability: Yes

Materials and methods

Test Material 1	Azoxystrobin (ICI5504)
Lot/Batch #:	ASJ10008-03
Purity (%):	99.7
IUPAC name:	Methyl (E)-2-{2-[6-(2-cyanophenoxy)pyrimidin-4-yloxy]phenyl}-3-methoxyacrylate
CAS number:	131860-33-8

Test Material 2	R230310
Lot/Batch #:	ASJ10075-04
Purity (%):	98.0
IUPAC name:	(Z)-2-{2-[6-(2-cyanophenoxy)pyrimidin-4-yloxy]phenyl}-3-methoxyacrylit acid methyl ester
CAS number:	152542-38-6

Test Material 3	R234886
Lot/Batch #:	ASJ10063-01S
Purity (%):	100.0
IUPAC name:	(E)-2-(2-[6-cyanophenoxy]-pyrimidin-4-yloxy)-phenyl-3-methoxyacrylic acid
CAS number:	N/A

Animal	Commodity	Source
Human	Lithium heparinised whole blood	Commercial source (EFS, Montpellier)

Study Design and Methods

Test facility: Eurofins ADME BIOANALYSES, 30310 Vergèze, France

Study start date: 20 May 2011

Study end date: 28 September 2011

Analytical phase dates: 26 May to 14 June 2011

Homogenised sub-samples of lithium heparinised whole blood (200 µL) were fortified with standard solutions of azoxystrobin, R230310 and R234886 in ultra-pure water/acetonitrile (50/50, v/v). Five samples were fortified at the limit of quantification (LOQ; 0.01 µg/mL) and five at a higher level (10x LOQ; 0.10 µg/mL). The matrix used was human blood. The fortified samples were analysed alongside untreated control samples.

Principle of the method (RAM 399/01)

Samples of lithium heparinised whole blood are extracted with ultra-pure water/acetonitrile (50/50, v/v) and formic acid in acetonitrile (0.1%) using a high-speed homogeniser. The extracts are centrifuged, the supernatant transferred to a clean tube, ultra-pure water/acetonitrile (50/50, v/v) are added and the tube is centrifuged again. Analyte(s) are determined by high-performance liquid chromatography with mass spectrometric detection (LC-MS/MS).

LC-MS/MS Conditions

HPLC system: LC-20AD, Shimadzu
Pumps: LC-20AD, Shimadzu
Degasser: Not reported
Column Oven: Not reported
Detector: API 4000, Sciex
Autosampler: CTC PAL
Column: Kromasil KR100 5C18, 50 mm x 3.0 mm, 5 µm
Mobile phase: A: Acetonitrile
B: Ultra-pure water with 0.2% acetic acid

Time	%A	%B	Gradient
Not reported	40	60	Not reported

Flow rate: 1.0 mL/minute
Column oven temperature: 40°C
Injection volume: 10 µL
Retention time: Azoxystrobin: approximately 3.1 minutes
R230310: approximately 2.2 minutes
R234886: approximately 1.2 minutes

Detector: API 4000

Ionisation mode:	ESI
Source polarity:	Positive
Collision gas (CAD):	4 (arbitrary units)
Curtain gas (CUR):	25 (arbitrary units)
Ion source gas 1 (GS1):	40 (arbitrary units)
Ion source gas 2 (GS2):	70 (arbitrary units)
Temperature (TEM):	600°C
Ion spray voltage (IS):	5500 V
Resolution Q1 and Q2:	unit

Source and detection parameters for MS/MS experiments:

Compound	Parent (<i>m/z</i>)	CE (V)	DP (V)	CXP (V)	Fragment ions (<i>m/z</i>)	
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Azoxystrobin	404.2	19 33	50 50	12 11	372.2 344.3	Quantification Confirmation
R230310	404.2	19 33	50 50	12 11	372.2 344.3	Quantification Confirmation
R234886	390.1	19 33	50 50	12 11	372.2 344.3	Quantification Confirmation

CE: Collision energy; DP: Declustering potential; CXP: Collision cell exit potential

Quantification: Peak areas of fragment ion at $m/z = 372.2$, external standards in solvent (ultra-pure water/acetonitrile, 90/10, v/v)

Confirmation: Peak areas of fragment ion at $m/z = 344.3$, external standards in solvent (ultra-pure water/acetonitrile, 90/10, v/v)

Results and discussions

Method RAM 399/01 was validated in human whole blood, fortified with azoxystrobin, R230310 and R234886 at the proposed limit of quantification (LOQ) of the method (0.01 µg/mL) and at 10 times the LOQ (0.1 µg/mL).

The recoveries obtained for all analytes for the primary and confirmatory mass transition are detailed in Table A 20 and

Table A 21 respectively.

Table A 20: Recovery results from method validation of azoxystrobin using the analytical method (primary transition)

Matrix	Analyte	Fortification (µg/mL)	Number of analyses (n)	Mean Recovery (%)	RSD (%)	Recovery Range (%)
Human whole blood	Azoxystrobin (m/z 404.2→372.2)	0.01	5	103	3	99 – 106
		0.1	5	98	2	96 – 101
		Overall	10	101	3	96 – 106
	R230310 (m/z 404.2→372.2)	0.01	5	105	2	102 – 108
		0.1	5	102	2	99 – 104
		Overall	10	104	3	99 – 108
	R24886 (m/z 390.1→372.2)	0.01	5	104	4	96 – 107
		0.1	5	99	3	95 – 103
		Overall	10	101	4	95 – 107

Table A 21: Recovery results from method validation of azoxystrobin using the analytical method (confirmatory transition)

Matrix	Analyte	Fortification (µg/mL)	Number of analyses (n)	Mean Recovery (%)	RSD (%)	Recovery Range (%)
Human whole blood	Azoxystrobin (m/z 404.2→344.3)	0.01	5	101	6	93 – 108
		0.1	5	98	3	94 – 102
		Overall	10	100	5	93 – 108
	R230310 (m/z 404.2→344.3)	0.01	5	107	3	104 – 110
		0.1	5	102	2	100 – 104
		Overall	10	104	3	100 – 110
	R24886 (m/z 390.1→344.3)	0.01	5	109	7	100 – 117
		0.1	5	99	3	96 – 102
		Overall	10	104	7	96 – 117

Specificity

LC-MS/MS is a highly specific detection technique, based on the specific detection of two characteristic daughter ions, and therefore a confirmatory technique is not required (SANTE/2020/12830, Rev.1 (24/02/2021)). Representative chromatograms have been provided to demonstrate specificity.

Linearity

The linearity of the LC-MS/MS detector was tested using standard solutions. For each compound, 2 ion transitions were monitored. Standards at eight different concentrations were injected and the response plotted against concentration. Straight lines with correlation coefficients (r^2) ranging from 0.9958 to 0.9989 was obtained for azoxystrobin, R230310 and R234886 in quantification mode and ranging from 0.9962 to 0.9977 for azoxystrobin, R230310 and R234886 in confirmatory mode. The lowest concentration injected was at 0.00025 ug/ml of the method. The highest concentration level injected was equivalent to 0.05 ug/mL. No interfering peaks around the retention time of azoxystrobin, R230310 and R234886 were found in any of the control samples at levels above 30% of the limit of quantification. Linear equations and correlation coefficients are reported:

Azoxystrobin in water/acetonitrile (90/10, v/v)

Quantification: $y = 6235x + 593$ ($r = 0.9982$)

Confirmation: $y = 1013x - 31$ ($r = 0.9986$)

R230310 in water/acetonitrile (90/10, v/v)

Quantification: $y = 4920x + 994$ ($r = 0.9979$)

Confirmation: $y = 789x + 137$ ($r = 0.9981$)

R234886 in water/acetonitrile (90/10, v/v)

Quantification: $y = 1557x - 189$ ($r = 0.9994$)

Confirmation: $y = 258x - 51$ ($r = 0.9988$)

Accuracy

The mean azoxystrobin, R230310 and R234886 at each validated level and overall for human whole blood were between 70% and 110%.

Repeatability

The relative standard deviation of azoxystrobin, R230310 and R234886 recoveries at each validated level and overall for human whole blood were below 20% and therefore compliant with the guidelines.

Limit of Quantification

The limit of quantification for azoxystrobin, R230310 and R234886 was 0.01 µg/mL.

Matrix Effects

Matrix effects on the detection of azoxystrobin, R230310 and R234886 were $< \pm 20\%$ and deemed to be insignificant for human blood and all analytes. Therefore, solvent standards in water/acetonitrile (90/10, v/v) were used for quantification throughout the study.

Stability of Final Extracts

Azoxystrobin, R230310 and R234886 were found to be stable in final extracts when stored at 0 – 9°C for at least 19 days.

Stability of Standard Solutions

The stability of azoxystrobin in standard solutions was not assessed within this study.

Conclusion

Analytical method RAM 399/01 has been successfully validated for the analysis of azoxystrobin, R230310 and R234886 in human whole blood. Results obtained were within the guideline requirements (mean recovery 70 – 110%; $RSD \leq 20\%$).

A 2.1.2.3.1.2 Independent laboratory validation

An independent laboratory validation is not required.

A 2.1.2.3.1.3 Confirmatory method

No confirmatory method is required. LC-MS/MS with two transitions is considered to be a highly specific detection technique. The method includes two MS/MS transitions, both of which have been validated.

A 2.1.2.3.1.4 Extraction efficiency

No new or additional studies have been submitted.

A 2.1.2.4 Description of Methods for the Analysis of Soil (KCP 5.2.4)

A 2.1.2.4.1.1 GRM057.06A - Method validation

Comments of zRMS:	<p>The method has been evaluated in Draft Registration Report for Orondis Evo (Central Zone, zRMS-PL, July 2022).</p> <p>Below is a copied assessment:</p> <p>Analytical method GRM057.06A is suitable for the determination of azoxystrobin, R230310, R234886, R401553 and R402173 in soil. The limit of quantification (LOQ) for azoxystrobin, R230310 and R234886 has been established at 0.02 mg/kg and the limit of quantitation for R401553 and R402173 has been established at 0.01 mg/kg.</p> <p>This method satisfies Guidance Documents SANCO/3029/99 rev 4 and SANCO/825/00 rev 8.1.</p> <p>The method is acceptable.</p>
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Reference: KCP1 5.2.4

Report: Link T., Poperechna N., Crook S., 2019
Azoxystrobin - Analytical Method GRM057.06A for the Determination of Azoxystrobin, R230310, R234886, R401553, and R402173 in Soil
Report No. GRM057.06A
Syngenta File No. VV-635391
unpublished

Guideline(s): Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/825/00 rev. 8.1, 16/11/2010).
Guidelines and Criteria for the Preparation and Presentation of Complete Dossiers and of Summary Dossiers for the Inclusion of Active Substances in Regulations (EU) 283/2013 and 284/2013 implementing Regulation (EC) 1107/2009.

Deviations: No change to guideline

GLP: No, not conducted under GLP/Officially recognised testing facilities (GLP was not a requirement for methods at the time the study was performed)

Acceptability: Yes

Comments of zRMS:	<p>The method has been evaluated in Draft Registration Report for Orondis Evo (Central Zone, zRMS-PL, July 2022).</p> <p>Below is a copied assessment:</p> <p>The analytical method GRM057.06A for the determination of residues of azoxystrobin, and its metabolites R230310, R234886, R401553 and R402173 in soil by LC-MS/MS has been validated during the study at a limit of quantification (LOQ) of 0.02 mg/kg for</p>
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	<p>azoxystrobin, R230310 and R234886 and 0.01 mg/kg for R401553 and R402173 according to the EU guidelines SANCO/3029/99 rev. 4 and SANCO/825/00 rev. 8.1.</p> <p>The mean recoveries at each fortification level were in the range between 70% and 110% with relative standard deviations below 20%.</p> <p>The study is acceptable.</p>
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Reference: KCP1 5.2.4

Report: Link T., Kravchuk O., 2019
Azoxystrobin - Validation of Analytical Method GRM057.06A for the Determination of Azoxystrobin, R230310, R234886, R401553 and R402173 in Soil
Report No. IF18-04490185
Syngenta File No. VV-635374
unpublished

Guideline(s): Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/825/00 rev. 8.1, 16/11/2010).
Guidelines and Criteria for the Preparation and Presentation of Complete Dossiers and of Summary Dossiers for the Inclusion of Active Substances in Regulations (EU) 283/2013 and 284/2013 implementing Regulation (EC) 1107/2009.

Deviations: No change to guideline

GLP: Yes, conducted under GLP/Officially recognised testing facilities

Acceptability: Yes

Principle of the Method

10 g sub samples of soil are extracted by a two-step extraction with methanol/1M hydrochloric acid (75/25, v/v). The extracts are combined and centrifuged. An aliquot of the centrifuged supernatant is then diluted in acetonitrile/ultra-pure water (50/50, v/v) prior to final determination by direct injection high performance liquid chromatography with triple quadrupole mass spectrometric detection (LC-MS/MS). The limit of quantification (LOQ) of the method is 0.02 mg/kg for azoxystrobin, R230310 and R234886 and 0.01 mg/kg for R401553 and R402173.

Recovery Findings

Summaries of the results for azoxystrobin, R230310, R234886, R401553 and R402173 are presented below.

Table A 22: Recovery results from validation for azoxystrobin in soil: primary transition m/z 404 → 372

Matrix	Fortification Level (mg/kg)	Recovery (%)**	Number of Analysis (n)	Mean (%)	RSD (%)	Range (%)
Soil (Germany, loam)	0.02*	105, 109, 110 ,107, 114	5	109	3	105-114
	0.20	105, 109, 108 ,107, 107	5	107	1	105-109
	Overall		10	108	3	105-114
Soil (Italy, loam)	0.02*	109, 98, 94 ,109, 116	5	105	9	94-116
	0.20	106, 106, 106 ,115, 110	5	109	4	106-115
	Overall		10	107	6	94-116

*Limit of quantitation, defined by the lowest validated fortification level

Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ

% Mean and % RSD calculated using unrounded values

Table A 23: Recovery results from validation for azoxystrobin in soil: confirmatory transition m/z 404 → 344

Matrix	Fortification Level (mg/kg)	Recovery (%)**	Number of Analysis (n)	Mean (%)	RSD (%)	Range (%)
Soil (Germany, loam)	0.02*	107, 105, 108 ,105, 108	5	107	2	105-108
	0.20	102, 110, 107 ,105, 102	5	105	3	102-110
	Overall		10	106	3	102-110
Soil (Italy, loam)	0.02*	110, 101, 96 ,107, 119	5	107	8	96-119
	0.20	106, 106, 105 ,116, 110	5	109	4	105-116
	Overall		10	108	6	96-119

*Limit of quantitation, defined by the lowest validated fortification level

Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ

% Mean and % RSD calculated using unrounded values

Table A 24: Recovery results from validation for R230310 in soil: primary transition m/z 404 → 372

Matrix	Fortification Level (mg/kg)	Recovery (%)**	Number of Analysis (n)	Mean (%)	RSD (%)	Range (%)
Soil (Germany, loam)	0.02*	101, 102, 102 ,108, 106	5	103	3	101-108
	0.20	105, 105, 108 ,104, 104	5	105	1	104-108
	Overall		10	104	2	101-108
Soil (Italy, loam)	0.02*	109, 103, 88 ,106, 114	5	104	10	88-114
	0.20	107, 108, 107 ,112, 110	5	109	2	107-112
	Overall		10	106	7	88-114

*Limit of quantitation, defined by the lowest validated fortification level

Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ

% Mean and % RSD calculated using unrounded values

Table A 25: Recovery results from validation for R230310 in soil: confirmatory transition m/z 404 → 344

Matrix	Fortification Level (mg/kg)	Recovery (%)**	Number of Analysis (n)	Mean (%)	RSD (%)	Range (%)
Soil (Germany, loam)	0.02*	107, 98, 106 ,102, 114	5	105	6	98-114
	0.20	105, 106, 106 ,107, 103	5	105	1	103-107
	Overall		10	105	4	98-114
Soil (Italy, loam)	0.02*	104, 93, 94 ,113, 104	5	102	8	93-113
	0.20	102, 110, 107 ,115, 106	5	108	5	102-115
	Overall		10	105	7	93-115

*Limit of quantitation, defined by the lowest validated fortification level

Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ

% Mean and % RSD calculated using unrounded values

Table A 26: Recovery results from validation for R234886 in soil: primary transition m/z 390 → 372

Matrix	Fortification Level (mg/kg)	Recovery (%)**	Number of Analysis (n)	Mean (%)	RSD (%)	Range (%)
Soil (Germany, loam)	0.02*	97, 102, 100 ,109, 107	5	103	5	97-109
	0.20	100, 103, 102 ,107, 105	5	103	3	100-107
	Overall		10	103	4	97-109
Soil (Italy, loam)	0.02*	109, 101, 91 ,104, 110	5	103	8	91-110
	0.20	105, 103, 106 ,104, 107	5	105	2	103-107
	Overall		10	104	5	91-110

*Limit of quantitation, defined by the lowest validated fortification level

Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ

% Mean and % RSD calculated using unrounded values

Table A 27: Recovery results from validation for R234886 in soil: confirmatory transition m/z 390 → 344

Matrix	Fortification Level (mg/kg)	Recovery (%)**	Number of Analysis (n)	Mean (%)	RSD (%)	Range (%)
Soil (Germany, loam)	0.02*	101, 93, 105 ,106, 104	5	102	5	93-106
	0.20	96, 110, 104 ,109, 105	5	105	5	96-110
	Overall		10	103	5	93-110
Soil (Italy, loam)	0.02*	112, 108, 98 ,108, 110	5	107	5	98-112
	0.20	93, 100, 100 ,112, 101	5	101	7	93-112
	Overall		10	104	6	93-112

*Limit of quantitation, defined by the lowest validated fortification level

Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ

% Mean and % RSD calculated using unrounded values

Table A 28: Recovery results from validation for R401553 in soil: primary transition m/z 214 → 187

Matrix	Fortification Level (mg/kg)	Recovery (%)**	Number of Analysis (n)	Mean (%)	RSD (%)	Range (%)
Soil (Germany, loam)	0.01*	97, 92, 101 ,92, 94	5	95	4	92-101
	0.10	87, 91, 91 ,81, 81	5	86	6	81-91
	Overall		10	91	7	81-101
Soil (Italy, loam)	0.01*	107, 97, 97 ,102, 111	5	103	6	97-111
	0.10	95, 99, 100 ,108, 101	5	100	5	95-108
	Overall		10	102	5	95-111

*Limit of quantitation, defined by the lowest validated fortification level

Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ

% Mean and % RSD calculated using unrounded values

Table A 29: Recovery results from validation for R401553 in soil: confirmatory transition m/z 214 → 120

Matrix	Fortification Level (mg/kg)	Recovery (%)**	Number of Analysis (n)	Mean (%)	RSD (%)	Range (%)
Soil (Germany, loam)	0.01*	79, 85, 83 ,80, 83	5	82	3	79-85
	0.10	89, 85, 91 ,91, 83	5	88	4	83-91
	Overall		10	85	5	79-91
Soil (Italy, loam)	0.01*	98, 90, 95 ,107, 110	5	100	8	90-110
	0.10	98, 108, 103 ,112, 107	5	106	5	98-112
	Overall		10	103	7	90-112

*Limit of quantitation, defined by the lowest validated fortification level

Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ

% Mean and % RSD calculated using unrounded values

Table A 30: Recovery results from validation for R402173 in soil: primary transition m/z 334 → 316

Matrix	Fortification Level (mg/kg)	Recovery (%)**	Number of Analysis (n)	Mean (%)	RSD (%)	Range (%)
Soil (Germany, loam)	0.01*	98, 97, 98 ,100, 107	5	100	4	97-107
	0.10	97, 99, 103 ,98, 97	5	99	2	97-103
	Overall		10	99	3	97-107
Soil (Italy, loam)	0.01*	108, 105, 100 ,106, 110	5	106	3	100-110
	0.10	106, 109, 107 ,110, 103	5	107	3	103-110
	Overall		10	106	3	100-110

*Limit of quantitation, defined by the lowest validated fortification level

Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ

% Mean and % RSD calculated using unrounded values

Table A 31: Recovery results from validation for R402173 in soil: confirmatory transition m/z 334 → 121

Matrix	Fortification Level (mg/kg)	Recovery (%)**	Number of Analysis (n)	Mean (%)	RSD (%)	Range (%)
Soil (Germany, loam)	0.01*	84, 87, 93 ,97, 97	5	92	7	84-97
	0.10	98, 98, 99 ,98, 100	5	99	1	98-100
	Overall		10	95	6	84-100
Soil (Italy, loam)	0.01*	110, 99, 90 ,98, 113	5	102	9	90-113
	0.10	103, 107, 108 ,113, 108	5	108	3	103-113
	Overall		10	105	7	90-113

*Limit of quantitation, defined by the lowest validated fortification level

Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ

% Mean and % RSD calculated using unrounded values

Accuracy (analytical recovery)

A reagent blank sample was analysed, duplicate control samples were analysed, 5 replicates fortified at the LOQ (0.02 mg/kg for azoxystrobin, R230310 and R234886 and 0.01 mg/kg for R401553 and R402173) and 5 replicates fortified at a higher level (0.20 mg/kg for azoxystrobin, R230310 and R234886

and 0.10 mg/kg for R401553 and R402173) were analysed.

Mean recoveries between 82% and 109% were found for azoxystrobin, R230310, R234886, R401553 and R402173 in soil.

Repeatability

The relative standard deviation (RSD) of azoxystrobin, R230310, R234886, R401553 and R402173 at each fortification level and overall for soil were lower than 20% for all fortifications (1 to 10% RDS). According to the EU guidance (see guidance section of this summary), these data demonstrate the method has satisfactory repeatability.

Confirmatory Analysis

LC-MS/MS with two transitions is considered a highly specific detection technique and therefore according to EU guidance (see guidance section of this summary) no further confirmatory technique is required. For each analyte, the method includes two MS/MS transitions, both of which have been validated.

Specificity

No significant interferences arising from the matrices tested, the labware, reagents or solvents have been observed at the retention times of interest.

Matrix Effects

No significant matrix effects (suppression or enhancement) were observed in either matrix tested and therefore during this study, non-matrix standards were used for calibration and quantification.

Linearity

The linearity of the LC-MS/MS detector was tested using non-matrix calibration standard solutions for azoxystrobin, R230310, R234886, R401553 and R402173 (range 0.12 to 10.00 ng/mL, equivalent to 0.006 to 0.50 mg/kg equivalent sample concentration for azoxystrobin, R230310 and R234886; range 0.06 to 5.00 ng/mL, equivalent to 0.003 to 0.25 mg/kg equivalent sample concentration for R401553 and R402173). The calibration range was therefore equivalent to 30 to 2500% of the relevant LOQ. Standards at nine different concentrations were injected and the signal area plotted against concentration for all calibration points. A straight line was obtained for azoxystrobin, R230310, R234886, R401553 and R402173 with correlation coefficients between 0.9977 and 1.0000.

Limit of Quantification

The LOQ using method GRM057.06A was established at 0.02 mg/kg for azoxystrobin, R230310 and R234886 and 0.01 mg/kg for R401553 and R402173. No interfering peaks around the retention time of the analytes were found in any of the control samples at levels above 30% of the LOQ.

Limit of Detection

The LOD values for azoxystrobin, R230310, R234886, R401553 and R402173 in soil were determined to be less than 30% of the LOQ (0.006 mg/kg for azoxystrobin, R230310 and R234886 and 0.003 mg/kg for R401553 and R402173).

Stability of Samples

The stability of azoxystrobin, R230310, R234886, R401553 and R402173 in soil when stored frozen has not been determined in this study.

Stability of Final Extracts

The stability of azoxystrobin, R230310, R234886, R401553 and R402173 in the final extracts when stored at 2-8°C has been demonstrated for up to 9 days.

Stability of Standard Solutions

Stability of standard solutions of azoxystrobin, R230310, R234886, R401553 and R402173 stored at 2-8°C was checked after a storage period of 208 days, against freshly prepared standard solutions. The

mean response values for stored and fresh solutions were within 20% of each other. The results demonstrated that azoxystrobin, R230310, R234886, R401553 and R402173 are stable in standard solution.

Conclusion

Analytical method GRM057.06A has been demonstrated to be a reliable and accurate procedure for the determination of azoxystrobin, R230310, R234886, R401553 and R402173 in soil to a limit of quantification of 0.02 mg/kg for azoxystrobin, R230310 and R234886 and to a limit of quantification of 0.01 mg/kg for R401553 and R402173, using commercially available laboratory equipment and reagents.

A 2.1.2.4.1.2 Independent laboratory validation

Not required.

A 2.1.2.4.1.3 Confirmatory method

LC-MS/MS with two transitions is considered to be a highly specific detection technique and therefore, according to EU guidance (*SANCO/825/00 rev.8.1, 16/11/2010*), no further confirmatory technique is required.

A 2.1.2.5 Description of Methods for the Analysis of Water (KCP 5.2.5)

A 2.1.2.5.1.1 GRM057.01A - Method validation

Comments of zRMS:	<p>The method has been evaluated in Draft Registration Report for Orondis Evo (Central Zone, zRMS-PL, July 2022).</p> <p>Below is a copied assessment:</p> <p>Analytical method GRM057.01A is suitable for the determination of azoxystrobin and its metabolite R234886 in water. The limit of quantification (LOQ) of the method has been established at 0.05 µg/L.</p> <p>This method satisfies Guidance Documents SANCO/3029/99 rev 4 and SANCO/825/00 rev 8.1.</p> <p>The method is acceptable.</p>
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Reference: KCP 5.2.5

Report Azoxystrobin - Residue Method for the Determination of Azoxystrobin and its Metabolite R234886 in Water, Amic S., 2012, report No GRM057.01A, document No VV-128281

Guideline(s): ENV/JM/MONO(2007)17
EPA OCSPP 860.1340
EC SANCO/3029/99 rev 4
EC SANCO/825/00 rev 8.1

Deviations: None

GLP: Yes

Acceptability: Yes

Comments of zRMS:	The study has been reviewed at EU level.
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Reference: KCP 5.2.5

Report Azoxystrobin - Validation of Analytical Method for the Determination of Azoxystrobin and its Metabolite R234886 in Water, Amic S., 2012, report No S11-03538, document No VV-401211

Guideline(s): ENV/JM/MONO(2007)17
EPA OCSPP 860.1340
EC SANCO/3029/99 rev 4
EC SANCO/825/00 rev 8.1

Deviations: None

GLP: Yes

Acceptability: Yes

Materials

Test Material	Azoxystrobin
Lot/Batch #:	ASJ10008-03
Purity (%):	99.7
IUPAC name:	Methyl (E)-2-(2-[6-(2-cyanophenoxy)pyrimidin-4-yloxy]phenyl)-3-methoxyacrylate
CAS number:	131860-33-8

Test Material	R234886
Lot/Batch #:	ASJ10063-01S
Purity (%):	100
IUPAC name:	(E)-2-{2-[6-cyano-phenoxy]-pyrimidin-4-yloxy}phenyl}-3- methoxy-acrylic acid
CAS number:	1185255-09-7

Study Design and Methods

Test facility: Eurofins|ADME BIOANALYSES

Study start date: 04 October 2011

Study end date: 07 February 2012

Analytical phase dates: 19 October 2011 – 18 November 2011

Sub-samples of each surface and drinking water were fortified with standard solutions of azoxystrobin and R234886 in acetonitrile/ultra-pure water (50/50, v/v). Five samples of each matrix were fortified at the limit of quantification (LOQ; 0.05 µg/L) and five at a higher level (10x LOQ).

Principle of the method

In summary, residues were determined after direct injection by high performance liquid chromatography with triple quadrupole mass spectrometric detection (LC-MS/MS) monitoring for two transitions:

LC-MS/MS Conditions

Pumps: LC20AD, Shimadzu
Column Oven: CTO-20AC, Shimadzu
Detector: API 4000, Sciex with Analyst™ software version 1.5.1
Autosampler: HTC PAL, CTC Analytics
Column: Kromasil KR100 5C18 – 50 mm x 3 mm, 5 µm
Mobile phase: A: Acetonitrile
B: Ultra-pure water + 0.2% acetic acid

Time (min)	%A	%B
0.0	10	90
1.0	50	50
2.5	50	50
3.0	90	10
3.5	90	10
4.0	10	90
5.0	10	90

Flow rate: 1 mL/min
Column oven temperature: 40°C

Injection volume: 40 µL
Retention time: Azoxystrobin: 2.51 min
R234886: 1.84 min

Detector: API 4000
Ionisation mode: ESI
Source polarity: Positive
Curtain gas (CUR): Nitrogen set at 25 (arbitrary units)
Gas 1 (GS1): Air set at 40 (arbitrary units)
Gas 2 (GS2): Air set at 70 (arbitrary units)
Temperature (TEM): 600°C
Interface heater (IHC): On
Ionspray voltage (IS): 5500V
Collision gas setting (CAD): Nitrogen set at 4 (arbitrary units)
Entrance potential (EP): 10 V
Dwell time: 200 msec

Source and detection parameters for MS/MS experiments:

Compound	Parent (m/z)	CE (V)	DP (V)	CXP (V)	Fragment ions (m/z)	
Azoxystrobin	404.2	19	50	12	372.2	Quantification
		33	50	11	344.3	Confirmation
R234886	390.1	19	50	12	372.2	Quantification
		33	50	11	344.3	Confirmation

CE: Collision energy; CXP: Collision cell exit potential; DP: Declustering Potential

Results

Recoveries of azoxystrobin and R234886 obtained from water samples at each fortification level using method GRM057.01A are presented in the table below.

Table A 32: Recovery results from method validation of azoxystrobin using the analytical method GRM057.01A in water

Matrix	Analyte	Fortification level (µg/L)	Individual recoveries (%)	Range of recoveries (%) (n = x)	Mean recovery (%)	RSD (%)
Drinking water	Azoxystrobin	Mass transition m/z = 404 → 372 (quantification)				
		0.05	99, 100, 103, 105, 99	99-105 (n = 5)	101	3
		0.5	96, 105, 89, 98, 100	89-105 (n = 5)	98	6
		Overall		89-105 (n = 10)	100	5
		Mass transition m/z = 404 → 344 (confirmation)				
		0.05	104, 100, 111, 111, 93	93-111 (n = 5)	104	7
		0.5	103, 116, 94, 97, 101	94-116 (n = 5)	102	8
		Overall		93-116 (n = 10)	103	7
Surface water	Azoxystrobin	Mass transition m/z = 404 → 372 (quantification)				
		0.05	86, 94, 93, 103, 91	86-103 (n = 5)	94	6
		0.5	105, 101, 98, 107, 93	93-107	101	6

Matrix	Analyte	Fortification level (µg/L)	Individual recoveries (%)	Range of recoveries (%) (n = x)	Mean recovery (%)	RSD (%)
				(n = 5)		
		Overall		86-107 (n = 10)	97	7
		Mass transition m/z = 404 → 344 (confirmation)				
		0.05	78, 93, 96 ,116, 91	78-116 (n = 5)	95	14
		0.5	107, 98, 101 ,106, 98	98-107 (n = 5)	102	4
		Overall		78-116 (n = 10)	98	10

Table A 33: Recovery results from method validation of R234886 using the analytical method GRM057.06A in water

Matrix	Analyte	Fortification level (µg/L)	Individual recoveries (%)	Range of recoveries (%) (n = x)	Mean recovery (%)	RSD (%) Comments
Drinking water	R234886	Mass transition m/z = 390 → 372 (quantification)				
		0.05	100, 96, 111 ,110, 101	96-111 (n = 5)	104	6
		0.5	101, 93, 98 ,89, 88	88-101 (n = 5)	94	6
		Overall		88-111 (n = 10)	99	8
		Mass transition m/z = 390 → 344 (confirmation)				
		0.05	109, 86, 108 ,118, 108	86-118 (n = 5)	106	11
		0.5	103, 93, 94 ,93, 85	85-103 (n = 5)	94	7
		Overall		85-118 (n = 10)	100	11
Surface water	R234886	Mass transition m/z = 390 → 372 (quantification)				
		0.05	95, 88, 88 ,84, 101	84-101 (n = 5)	91	7
		0.5	92, 94, 98 ,107, 98	92-107 (n = 5)	98	6
		Overall		84-107 (n = 10)	95	7
		Mass transition m/z = 390 → 344 (confirmation)				
		0.05	95, 104, 90, 94, 87	87-104 (n = 5)	94	7
		0.5	98, 94, 104 ,107, 104	94-107 (n = 5)	101	5
		Overall		87-107 (n = 10)	98	7

Table A 34: Characteristics of the enforcement analytical method GRM057/01A used for the quantification of azoxystrobin and R234886 residues in water

Analyte	Azoxystrobin and R234886
Equipment/ Chromatographic method	LC-MS/MS
Accuracy/ Precision (repeatability)	Acceptable mean recoveries between 70% and 110% with a relative standard deviation less than 20% were obtained for azoxystrobin and R234886 for each transition in both matrices tested.
Specificity	LC-MS/MS with two transitions is a highly specific detection technique and therefore, according to EU guidance (SANCO/825/00 rev.8.1, 16/11/2010), no further confirmatory technique is required. Residues of azoxystrobin and R234886 measured in the control samples were lower than 30 % of the LOQ in all the control and reagent blank samples used in this study.
Confirmatory method	See above
Assessment of matrix effects is presented	Yes No significant matrix effects (>20%) were observed for azoxystrobin and R234886 in both drinking water and surface water tested. Non matrix-matched standards were used for calibration and quantification/confirmation of azoxystrobin and R234886 in drinking water. Due to unavailability of matrix effect results when injecting the batch, matrix-matched standards were used for calibration and quantification/confirmation of azoxystrobin and R234886 in surface water.

Calibration/Linearity	The LC-MS/MS response was confirmed to be linear by injecting at least 6 standard solutions covering the working range.
	The response of the LC-MS/MS instrument was shown to be linear for azoxystrobin and R234886 at concentrations ranging from 0.0125 to 2.5 µg/L (equivalent to 0.5 to 100 pg of analyte injected on to the column, based on a 40 µL injection). The lower margin of the linearity test was 25% of the LOQ and the upper margin was higher by at least 20% above the highest concentrations in the final extracts.
	The detector response was linear ($r^2 = >0.99$). Azoxystrobin <u>Drinking water</u> Quantification - $y = 5629x - 513$ ($R^2 = 0.9963$) Confirmation - $y = 858x - 17$ ($R^2 = 0.9970$) <u>Surface water</u> Quantification - $y = 5992x - 477$ ($R^2 = 0.9945$) Confirmation - $y = 929x - 109$ ($R^2 = 0.9963$) R234886 <u>Drinking water</u> Quantification - $y = 2527x - 314$ ($R^2 = 0.9960$) Confirmation - $y = 415x - 82$ ($R^2 = 0.9964$) <u>Surface water</u> Quantification - $y = 2655x - 206$ ($R^2 = 0.9966$) Confirmation - $y = 426x + 4.05$ ($R^2 = 0.9980$)
Limit of quantification (LOQ)	Azoxystrobin and R234886 with a LOQ of 0.05 µg/L
Limit of detection (LOD)	Estimated LOD in µg/L in drinking water: Azoxystrobin: 0.0011 (primary transition) and 0.0081 (confirmatory transition) R234886: 0.0047 (primary transition) and 0.0080 (confirmatory transition) Estimated LOD in mg/kg in surface water: Azoxystrobin: 0.0008 (primary transition) and 0.0036 (confirmatory transition) R234886: 0.0026 (primary transition) and 0.0040 (confirmatory transition)
Extract Stability	Sample extract storage stability was determined for each matrix. The results proved that azoxystrobin and R234886 residues were stable in the final extracts for at least 30 days when stored between 0 and 9°C.
Standard Solution Stability	Stock solution of azoxystrobin was prepared in ultra-pure water/acetonitrile (50/50, v/v). This solution has been proven to be stable for 4 months when stored between 0 and 9°C and protected from light. Stock solution of R234886 was prepared in ultra-pure water/acetonitrile (50/50, v/v). This solution has been proven to be stable for 22 days when stored between 0 and 9°C and protected from light. The fortification and the calibration standards solutions were freshly prepared.
Extractability	Residues of azoxystrobin and R234886 in water are analysed by direct injection and extractability is not relevant for direct injection methods.

Conclusion

Method GRM057.01A has been successfully validated for the determination of residues of azoxystrobin, and R234886 in surface and drinking water with a limit of quantification (LOQ) of 0.05 µg/L. It fully meets the requirements of SANCO/825/00 Rev. 8.1 for analytical methods used for post-registration monitoring.

(Amic S., 2012)

A 2.1.2.5.1.2 GRM057.01A - Independent laboratory validation

Comments of zRMS:	The method has been evaluated in Draft Registration Report for Orondis Evo (Central Zone, zRMS-PL, July 2022). Below is a copied assessment: Analytical method GRM057.01A is suitable for the determination of azoxystrobin and its metabolite R234886 in water. The limit of quantification (LOQ) of the method has been established at 0.05 g/L. This method satisfies Guidance Documents SANCO/3029/99 rev 4 and SANCO/825/00 rev 8.1. The method is acceptable.
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Reference:	KCP1 5.2.5
Report:	Azoxystrobin - Residue method for the determination of azoxystrobin and its metabolite R234886 in water, Amic S., 2012, Report No. GRM057.01A, Syngenta File No. VV-128281
Guideline(s):	<ul style="list-style-type: none"> • OECD Guidance Document on Pesticide Residue Analytical Methods, ENV/JM/MONO(2007)17 • EPA Residue Chemistry Test Guidelines, OPPTS 860.1340 (1996) Residue Analytical Method • European Commission Guidance for Generating and Reporting Methods of Analysis in Support of Pre-registration Data Requirements for Annex II (Part A, Section 4) and Annex III (Part A, Section 5) of Directive 91/414, SANCO/3029/99 Rev.4 (2000) • European Commission Guidance Document on Residue Analytical Method, SANCO/825/00 Rev.7 (2004)
Deviations:	No
GLP:	No
Acceptability:	Yes

Comments of zRMS:	The study has been reviewed at EU level.
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Reference:	KCP 5.2.5
Report:	Azoxystrobin - Validation of analytical method for the determination of azoxystrobin and its metabolite R234886 in water, Amic S., 2012a, Report No. S11-03538, Syngenta File No. VV-401211
Guideline(s):	<ul style="list-style-type: none"> • OECD Guidance Document on Pesticide Residue Analytical Methods, ENV/JM/MONO(2007)17 • EPA Residue Chemistry Test Guidelines, OPPTS 860.1340 (1996) Residue Analytical Method • European Commission Guidance for Generating and Reporting Methods of Analysis in Support of Pre-registration Data Requirements for Annex II (Part A, Section 4) and Annex III (Part A, Section 5) of Directive 91/414, SANCO/3029/99 Rev.4 (2000) • European Commission Guidance Document on Residue Analytical Method, SANCO/825/00 Rev.7 (2004)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Method GRM057.01A was developed and validated to demonstrate the suitability of this method to determine residues of azoxystrobin and R234886 in water (drinking water and surface water). Environmental water sample residues are determined after direct injection by high performance liquid chromatography with triple quadrupole mass spectrometric detection (LC-MS/MS). The surface and drinking water used for method validation were supplied by Eurofins ADME BIOANALYSES as specified in the study plan. The characterisation of water control samples is provided in the following table.

Water Type	Surface water	Drinking water
Source	“Le Tech” river	Tap water
pH ^(a)	7.2	7.3
Silt content (mg/L) (Method NF T90-029)	84	531
Dissolved organic carbon (DOC) (mg/L) (method MS99999)	2.4	1.7
Total hardness as CaCO ₃ (mg/L) (Method NF EN ISO 9963-1 ^(b))	65	307

(a): measured at Eurofins ADME BIOANALYSES

(b): accredited method (NF EN 17025)

Results and discussions

Recovery

Method GRM057.01A was validated on drinking and surface water, fortified with azoxystrobin and R234889 at the proposed limit of quantification (LOQ) of the method (0.05 µg/L) and at 10 times the LOQ (0.5 µg/L).

The recoveries obtained for azoxystrobin for the primary and confirmatory mass transition are detailed in Table A 35 and

Table A 36 respectively.

Table A 35: Recovery results obtained during validation of method GRM057.01A for azoxystrobin in water (primary transition, m/z 404.2 → 372.2)

Water Matrix (type)	Analyte	Fortification (µg/L)	Number of analyses (n)	Mean Recovery (%)	RSD (%)	Recovery Range (%)
Drinking water	Azoxystrobin	0.05	5	101	3	99-105
		0.5	5	98	6	89-105
		Overall	10	100	5	89-105
Surface Water	Azoxystrobin	0.05	5	94	6	86-103
		0.5	5	101	6	93-107
		Overall	10	97	7	86-107

Table A 36: Recovery results obtained during validation of method GRM057.01A for azoxystrobin in water (confirmatory transition, m/z 404.2 → 344.3)

Water Matrix (type)	Analyte	Fortification (µg/L)	Number of analyses (n)	Mean Recovery (%)	RSD (%)	Recovery Range (%)
Drinking water	Azoxystrobin	0.05	5	104	7	93-111
		0.5	5	102	8	94-116
		Overall	10	103	7	93-116
Surface Water	Azoxystrobin	0.05	5	95	14	78-116
		0.5	5	102	4	98-107
		Overall	10	98	10	78-116

The recoveries obtained for R234886 for the primary and confirmatory mass transition are detailed in Table A 37 and

Table A 38 respectively.

Table A 37: Recovery results obtained during validation of method GRM057.01A for R234886 in water (primary transition, m/z 390.1 \rightarrow 372.2)

Water Matrix (type)	Analyte	Fortification ($\mu\text{g/L}$)	Number of analyses (n)	Mean Recovery (%)	RSD (%)	Recovery Range (%)
Drinking water	Azoxystrobin	0.05	5	104	6	96-111
		0.5	5	94	6	88-101
		Overall	10	99	8	88-111
Surface Water	Azoxystrobin	0.05	5	91	7	84-101
		0.5	5	98	6	92-107
		Overall	10	95	7	84-107

Table A 38: Recovery results obtained during validation of method GRM057.01A for R234886 in water (confirmatory transition, m/z 390.1 \rightarrow 344.3)

Water Matrix (type)	Analyte	Fortification ($\mu\text{g/L}$)	Number of analyses (n)	Mean Recovery (%)	RSD (%)	Recovery Range (%)
Drinking water	Azoxystrobin	0.05	5	106	11	86-118
		0.5	5	94	7	85-103
		Overall	10	100	11	85-118
Surface Water	Azoxystrobin	0.05	5	94	7	87-104
		0.5	5	101	5	94-107
		Overall	10	98	7	87-107

Specificity

LC-MS/MS as a detection technique with primary and confirmatory transitions is considered to be highly specific and therefore according to the guidance (see guidance section of this summary) further confirmation is not required. No significant interferences above 30% of the lower limit of quantification, arising from the water matrices, the lab ware, reagents or solvents tested have been observed at the retention times of interest for azoxystrobin and R234886.

Linearity

The linearity of the LC-MS/MS detector responses was confirmed for both the primary quantification and confirmatory transitions by generating calibration curves.

The linearity of the detector response was assessed by analysis of a minimum of 8 standard solutions covering the working range of at least 25% of the LOQ to 50 times the LOQ concentrations in the final extracts (0.0125 to 2.5 $\mu\text{g/L}$ for azoxystrobin and R234886). The correlation coefficients (r) of all calibration plots were found to be ≥ 0.9945 .

The following equations of the calibration line were obtained:

Analyte, transition	Drinking water	Surface water
Azoxystrobin, m/z 404.2 \rightarrow 372.2	$y = 5629x - 513, r^2 = 0.9963$	$y = 5992x - 477, r^2 = 0.9945$
Azoxystrobin, m/z 404.2 \rightarrow 344.3	$y = 858x - 17, r^2 = 0.9970$	$y = 929x - 109, r^2 = 0.9963$
R234886, m/z 390.1 \rightarrow 372.2	$y = 2527x - 314, r^2 = 0.9960$	$y = 2655x - 206, r^2 = 0.9966$
R234886, m/z 390.1 \rightarrow 344.3	$y = 415x - 82, r^2 = 0.99664$	$y = 426x + 4.05, r^2 = 0.9980$

Accuracy

The mean azoxystrobin and R234886 recoveries for both primary quantification and confirmatory transitions at each fortification level and overall for each water type tested during method validation were between 91-106%. These values are all between 70% and 110% and therefore according to the guidance

these results demonstrate the method has satisfactory accuracy.

Matrix Effect

The effect of each water matrix on the LC-MS/MS response was assessed by preparing standards in the presence of matrix and comparing the peak areas of azoxystrobin and R234886 against non-matrix standards at an equivalent concentration. No significant enhancement or suppression of the detector response was observed in the presence of either drinking or surface water.

Repeatability

The relative standard deviations (RSDs) of azoxystrobin and R234886 recoveries for both primary quantification and confirmatory transitions at each fortification level and overall for each water type tested during method validation were between 3-14%. These values are all below 20% and therefore according to the guidance these results demonstrate the method has satisfactory repeatability.

Final Extract Stability

The stability of the final extracts of azoxystrobin and R234886 was assessed by storing drinking water extracts between 0 and 9 °C. The samples were initially analysed after extraction and were re-analysed after 30 days of storage. The resultant mean recoveries were between 70% and 110% and therefore acceptable, demonstrating that azoxystrobin and R234886 in water extracts are stable on storage under these conditions.

Limit of Quantification

The limit of quantification (LOQ) of a method is defined as the lowest analyte concentration in a sample at which the methodology has been validated and for which a mean recovery of 70-110% with a relative standard deviation (RSD) of \leq 20% has been obtained.

The limit of quantification for azoxystrobin and R234886 residues in water using method GRM057.01A was established at 0.05 µg/L. Residues of all analytes measured in the control samples were always below 30% of the LOQ during method validation.

Conclusion

Method GRM057.01A was successfully validated for the analysis of residues of azoxystrobin and R234886 in water and an LOQ of 0.05 µg/L was established. Therefore it is proposed that method GRM057.01A is suitable to be used in support of post-registration data requirement.

A 2.1.2.5.1.3 Independent laboratory validation

Comments of zRMS:	<p>The method has been evaluated in Draft Registration Report for Orondis Evo (Central Zone, zRMS-PL, July 2022).</p> <p>Below is a copied assessment:</p> <p>The method GRM057.01A is independently validated according to SANCO/825/00 rev. 8.1 for the determination of residues of azoxystrobin and R234886 in drinking and surface water with a limit of quantification of 0.05 µg/L.</p> <p>Acceptable mean accuracy values of between 70% and 120% with RSD $<$20% for azoxystrobin and R234886 were found for both transitions in drinking water and surface water.</p> <p>A confirmatory method is included.</p> <p>The method is acceptable.</p>
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Reference: KCP1 5.2.5

Report: Brown D (2019).
Azoxystrobin – Independent Laboratory validation of Analytical Method GRM057.01A for the determination of residues of azoxystrobin and its metabolite R234886 in Water.
Report No. RES-00193
Syngenta File No. VV-619234

unpublished

Guideline(s): Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/825/00 rev. 8.1, 16/11/2010).
Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/3029/99 rev. 4, 11/07/2000).
Residue Chemistry Test Guidelines OCSPP 850.6100 Environmental Chemistry Methods and Associated Independent Laboratory Validation, EPA 712-C-001, January 2012.
Residue Chemistry Test Guidelines OCSPP 860.1340 Residue Analytical Method, EPA 712-C-96-174, August 1996.

Deviations: No

GLP: Yes

Acceptability: Yes

Principle of the Method

Water samples (20 mL) were vialled up for direct injection. Final determination was by high performance liquid chromatography with triple quadrupole mass spectrometric detection (LC-MS/MS). The limit of quantification of the method was 0.05 µg/L.

Recovery Findings

Summaries of the results for Azoxystrobin and R234886 are presented below.

Table A 39: Accuracy and precision results from independent laboratory validation of GRM057.01A for azoxystrobin in drinking water and surface water for primary transition m/z 404 → 372 and confirmatory transition 404 > 344.

Compound	Fort. Level (µg/L)	Recoveries Single Values (%)					No. of Analyses	Overall Recovery		
								Mean (%)	Rel. Std. Dev. (%)	Range (%)
Drinking Water Transition 404 > 372 (m/z) (Primary)										
azoxystrobin	0.05*	102	103	101	101	102	5	102	0.6	101 - 103
	0.5	99	100	100	101	101	5	100	1.0	99 - 101
	Overall							101	1.1	99 - 103
Drinking Water Transition 404 > 344 (m/z) (Confirmatory)										
azoxystrobin	0.05*	103	102	100	101	103	5	102	1.2	100 - 103
	0.5	100	100	100	102	102	5	101	0.8	100 - 102
	Overall							101	1.0	100 - 103
Surface Water Transition 404 > 372 (m/z) (Primary)										
azoxystrobin	0.05*	100	102	101	101	100	5	101	1.0	100 - 102
	0.5	96	96	95	96	97	5	96	1.1	95 – 97
	Overall							98	2.6	95 - 102
Surface Water Transition 404 > 344 (m/z) (Confirmatory)										
azoxystrobin	0.05*	101	103	99	100	101	5	101	1.4	99 - 103
	0.5	95	97	96	96	98	5	96	0.9	95 - 98
	Overall							98	2.7	95 - 103

*Limit of quantitation, defined by the lowest validated fortification level

Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ

Table A 40: Accuracy and precision results from independent laboratory validation of GRM057.01A for R234886 in drinking water and surface water for primary transition m/z 390 → 372 and confirmatory transition 390 > 344.

Compound	Fort. Level (µg/L)	Recoveries Single Values (%)					No. of Analyses	Overall Recovery		
								Mean (%)	Rel. Std. Dev. (%)	Range (%)
Drinking Water Transition 390 > 372 (m/z) (Primary)										
R234886	0.05*	98	99	101	102	97	5	99	1.9	97 - 102

	0.5	98	98	97	98	95	5	97	1.4	95 – 98
	Overall							98	1.9	95 - 102
Drinking Water Transition 390 > 344 (m/z) (Confirmatory)										
R234886	0.05*	99	103	100	101	94	5	99	3.5	94 - 103
	0.5	96	95	98	95	95	5	96	1.5	95 - 98
	Overall							98	3.1	94 - 103
Surface Water Transition 390 > 372 (m/z) (Primary)										
R234886	0.05*	95	95	95	99	93	5	96	2.4	93 - 99
	0.5	97	92	93	95	96	5	94	2.0	92 - 97
	Overall							95	2.2	92 - 99
Surface Water Transition 390 > 344 (m/z) (Confirmatory)										
R234886	0.05*	95	96	94	94	94	5	95	1.3	94 - 96
	0.5	94	92	94	94	95	5	94	1.1	92 - 95
	Overall							94	1.2	92 - 96

*Limit of quantitation, defined by the lowest validated fortification level

Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ

Deviations from test guidelines

None.

Specificity

LC-MS/MS with two transitions is considered to be a highly specific detection technique and therefore according to EU guidance (see guidance section of this summary) no further confirmatory technique is required. The method includes two MS/MS transitions, both of which have been validated. No significant interferences arising from the water matrices, the labware, reagents or solvents have been observed at the retention times of interest.

Linearity

The linearity of the LC-MS/MS detector was tested for azoxystrobin and R234886 using matrix matched calibration standard solutions from 0.0000125 to 0.0025 µg/mL (equivalent to 0.0125 to 2.5 µg/L considering the final sample concentration of 0.001 L/mL). Standards at eight different concentrations were injected and the signal area plotted against concentration for all calibration points. Straight lines with correlation coefficients of 0.9995 (primary transition m/z 404 → 372) and 0.9999 (confirmatory transition m/z 404 → 344) were obtained for azoxystrobin in drinking water. Straight lines with correlation coefficients of 0.9998 (primary transition m/z 404 → 372) and 1.0000 (confirmatory transition m/z 404 → 344) were obtained for azoxystrobin in surface water. For R234886, straight lines with correlation coefficients of 1.0000 (primary transition m/z 390 → 372) and 1.0000 (confirmatory transition m/z 390 → 344) were obtained for drinking water. Straight lines with correlation coefficients of 0.9999 (primary transition m/z 390 → 372) and 0.9999 (confirmatory transition m/z 390 → 344) were obtained for R234886 in surface water.

Accuracy

Fortified samples were analysed in quintuplet at the limit of quantification (LOQ) of 0.05 µg/L and at ten times the LOQ (0.5 µg/L). Acceptable mean accuracy values of between 70 % and 120 % were found for both transitions in drinking water and surface water and therefore according to EU guidance (see guidance section of this summary) demonstrate the method has satisfactory accuracy.

Repeatability

The relative standard deviations (RSDs) of azoxystrobin and R234886 accuracy values at each fortification level and overall for the drinking water and surface water samples tested during method validation were <20 % and therefore according to the EU guidance (see guidance section of this summary) demonstrate the method has satisfactory repeatability.

Limit of Quantification

The LOQ for azoxystrobin and R234886 in drinking water and surface water using method GRM057.01A was established at 0.05 µg/L. No interfering peaks around the retention time of azoxystrobin or R234886 were found in any of the control samples at levels above 30% of the LOQ.

Matrix Effects

No significant matrix effects were observed for azoxystrobin or R234886 in drinking water and surface water during the method validation.

Stability of Final Extracts

The stability of the sample extracts fortified with azoxystrobin and R234886 in drinking water was demonstrated after a storage period of 10 days at 2-8 oC against freshly prepared calibration standards. The results showed that azoxystrobin and R234886 residues in the stored fortified water samples were stable (the mean accuracy values were between 70 – 120 % with an RSD \leq 20%). The stability of the sample extracts fortified with azoxystrobin and R234886 in surface water was demonstrated after a storage period of 8 days at 2-8 oC against freshly prepared calibration standards. The results showed that azoxystrobin and R234886 residues in the stored fortified water samples were stable (the mean accuracy values were between 70 – 120 % with an RSD \leq 20%).

Stability of Standard Solutions

The stability of the stored working standard solutions of azoxystrobin and R234886 were checked after a storage period of 14 days at 2-8 oC against freshly prepared calibration standards. The mean response values for stored and fresh solutions were within 20 % of each other and the results therefore demonstrated that azoxystrobin and R234886 residues were stable in the standard solutions.

Conclusion

Analytical method GRM057.01A has been demonstrated to be a reliable and accurate procedure for the determination of azoxystrobin and R234886 in drinking water and surface water to a limit of quantification of 0.05 µg/L, using commercially available laboratory equipment and reagents.

A 2.1.2.5.1.4 Confirmatory method

No confirmatory method is required.

A 2.1.2.5.1.5 GRM057.04A - Method validation

Comments of zRMS:	<p>The method has been evaluated in Draft Registration Report for Orondis Evo (Central Zone, zRMS-PL, July 2022).</p> <p>Below is a copied assessment:</p> <p>Analytical method GRM057.04A is suitable for the determination of Azoxystrobin and Z-isomer R230310 in surface and ground water. The limit of quantitation (LOQ) of the method has been established at a 0.1 µg/L. Analytical method GRM057.04A supersedes method RAM 235/01. This method was developed to include the Z-isomer R230310 at a LOQ of 0.1 µg/L which was not present in previous methodology.</p> <p>This method satisfies Guidance Documents SANCO/3029/99 rev 4 (2000) and SANCO/825/00 rev 8.1 (2010).</p> <p>The method is acceptable</p>
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Reference: KCP 5.2.5

Report Azoxystrobin – Residue Method (GRM057.04A) for the Determination of Azoxystrobin and Z-Isomer R230310 in Water by LC-MS/MS, Mayer L.C., 2012, report No GRM057.04A, document No VV-185347

Guideline(s): OECD ENV/JM/MONO(2007)17
EPA OPPTS 850.7100 (1996)
EC SANCO/3029/99 rev 4 (2000)
EC SANCO/825/00 rev 8.1 (2010)

Deviations: No

GLP: Yes

Acceptability: Yes

Comments of zRMS:	<p>The method has been evaluated in Draft Registration Report for Orondis Evo (Central Zone, zRMS-PL, July 2022). Below is a copied assessment: Analytical Method GRM057.04A was successfully validated for the determination of residues of Azoxystrobin and its Z-isomer R2301310 in surface and ground water samples using high performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS). The LOQ of the analytical method was established at 0.1 µg/L for azoxystrobin and R2301310 in water. overall mean recovery: 95%, relative standard deviation: 9.4%. This method satisfies Guidance Documents SANCO/3029/99 rev 4 (2000) and SANCO/825/00 rev 8.1 (2010). The study is acceptable.</p>
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Reference: KCP 5.2.5

Report Azoxystrobin – Validation of Residue Method (GRM057.04A) for the Determination of Azoxystrobin and Z-Isomer R2301310 in Water by LC-MS/MS, Mayer L.C., 2012, report No TK0120502, document No VV-506623

Guideline(s): OECD ENV/JM/MONO(2007)17
EPA OPPTS 850.7100 (1996)
EC SANCO/3029/99 rev 4 (2000)
EC SANCO/825/00 rev 8.1 (2010)

Deviations: Deviations from SANCO/825/00 rev. 8.1:
- Linearity range not from 30% LOQ but 50% LOQ
The validation was performed under GLP and according to EU guideline SANCO/3029/99 Rev. 4. Whilst the validation is not fully compliant to the new guidance SANTE/2020/12830 Rev. 1, it exceeds the minimum validation requirements set out therein.

GLP: Yes

Acceptability: Yes

Materials

Test Material	Azoxystrobin
Lot/Batch #:	600191 / ASJ10008-03
Purity (%):	99.7%
IUPAC name:	(E)-methyl 2-(2-[6-(2-cyanophenoxy)pyrimidin-4-yloxy]phenyl)-3-methoxyacrylate
CAS number:	131860-33-8

Test Material	R2301310
Lot/Batch #:	601321
Purity (%):	94.0%
IUPAC name:	Methyl (Z)-2-(2-[6-(2-cyanophenoxy)pyrimidin-4-yloxy]phenyl)-3-methoxyacrylate
CAS number:	143130-64-3

Study Design and Methods

Test facility: Syngenta Crop Protection, LLC, 410 Swing Road Greensboro, NC 27409 USA

Study start date: April 23, 2012

Study end date: June 12, 2012

Analytical phase date: April 30, 2012

Sub-samples of each surface and groundwater were fortified with standard solutions of azoxystrobin and R230310. Samples of each matrix were fortified at the limit of quantification 0.10 µg/L, 0.01 µg/L and 1.0 µg/L. The fortified samples were analysed alongside untreated control samples.

Principle of the method

Surface and ground water samples are analysed directly by LC-MS/MS after dilution with acetonitrile/ultra-pure water (50/50 v/v) using non-matrix matched external calibration, monitoring for two transitions.

LC-MS/MS Conditions

HPLC system: UFLC XR, Shimadzu
Detector: Applied BioSystem API 5500 triple QTRAP with Analyst™ software (version 1.5.1)
Column: ACE 5 C18 - 50 mm x 3.0 mm
Mobile phase: A: OPTIMA grade water in 0.1% acetic acid
B: OPTIMA grade acetonitrile in 0.1% acetic acid

Time (min)	%A	%B
0.0	50	50
3.0	50	50

Flow rate: 0.8 mL/min
Column oven temperature 40°C
Injection volume: 30 µL
Retention time: Azoxystrobin: 1.28 min
R230310: 1.02 min

Detector API 5500
Ionisation mode: ESI
Source polarity: Positive
Scan type: MRM
Curtain gas (CUR): 30 (arbitrary units)
Gas 1 (GS1): Air set at 50 (arbitrary units)
Gas 2 (GS2): Air set at 50 (arbitrary units)
Temperature (TEM): 500°C
Interface heater (IHE): On
Ionspray voltage (IS): 2500V
Collision gas setting (CAD): Nitrogen set at Medium (arbitrary units)
Entrance potential (EP): 10 V
Dwell time 50 msec

Source and detection parameters for MS/MS experiments:

Compound	Parent (m/z)	CE (V)	DP (V)	CXP (V)	Fragment ions (m/z)	
Azoxystrobin	404	18	50	10	372	Quantification
		42	50	10	329	Confirmation
R230310	404	18	50	10	372	Quantification
		42	50	10	329	Confirmation

CE: Collision energy; CXP: Collision cell exit potential; DP: Declustering Potential

Results

Recoveries of azoxystrobin and R230310 obtained from water at each fortification level using method GRM057.04A are presented in the table below.

Table A 41: Recovery results from method validation of azoxystrobin and R230310 using the analytical method GRM057.04A in water

Matrix	Analyte	Fortification level (µg/L)	Individual recoveries (%)**	Range of recoveries (%) (n = x)	Mean recovery (%)	RSD (%)
Surface water	Azoxystrobin	Mass transition m/z = 404 → 372 (quantification)				
		0.1*	79, 82, 77, 92, 78	77 – 92 (n = 5)	82	7.9
		1.0	97, 100, 94, 96, 94	94 – 100 (n = 5)	96	2.4
		Overall		77 – 100 (n = 10)	89	10.1
		Mass transition m/z = 404 → 329 (confirmation)				
		0.1*	75, 72, 82, 105, 88	72 – 105 (n = 5)	84	15.8
		1.0	102, 107, 99, 95, 100	95 – 107 (n = 5)	101	4.4
		Overall		72 – 107 (n = 10)	92	13.7
Surface water	R230310	Mass transition m/z = 404 → 372 (quantification)				
		0.1*	88, 81, 83, 96, 95	81 – 96 (n = 5)	89	7.5
		1.0	99, 104, 97, 95, 93	93 – 104 (n = 5)	98	4.3
		Overall		81 – 104 (n = 10)	93	7.5
		Mass transition m/z = 404 → 329 (confirmation)				
		0.1*	87, 84, 73, 100, 91	73 – 100 (n = 5)	87	11.3
		1.0	100, 107, 98, 101, 99	98 – 107 (n = 5)	101	3.7
		Overall		73 – 107 (n = 10)	94	10.8
Ground water	Azoxystrobin	Mass transition m/z = 404 → 372 (quantification)				
		0.1*	91, 97, 100, 100, 94	91 – 100 (n = 5)	96	3.9
		1.0	98, 92, 90, 90, 93	90 – 98 (n = 5)	93	3.7
		Overall		90 – 100 (n = 10)	95	4.2
		Mass transition m/z = 404 → 329 (confirmation)				
		0.1*	103, 93, 99, 91, 74	74 – 103 (n = 5)	92	12.3
		1.0	97, 90, 87, 88, 97	87 – 97 (n = 5)	92	5.2
		Overall		74 – 103 (n = 10)	92	8.9
Ground water	R230310	Mass transition m/z = 404 → 372 (quantification)				
		0.1*	114, 109, 103, 111, 99	99 – 114 (n = 5)	107	5.4

Matrix	Analyte	Fortification level (µg/L)	Individual recoveries (%)**	Range of recoveries (%) (n = x)	Mean recovery (%)	RSD (%)
		1.0	109, 97, 87, 94, 105	87 – 109 (n = 5)	98	9.1
		Overall		87 – 114 (n = 10)	103	8.3
		Mass transition m/z = 404 → 329 (confirmation)				
		0.1*	83, 104, 87, 105, 104	83 – 105 (n = 5)	97	11.2
		1.0	96, 94, 91, 92, 103	91 – 103 (n = 5)	95	4.9
		Overall		83 – 105 (n = 10)	96	8.2

*Limit of quantification, defined by the lowest validated fortification level

**Residues in control samples and reagent blanks were less than 30% of the LOQ.

Table A 42: Characteristics of the enforcement analytical method used for the quantification of azoxystrobin and R230310 residues in water

Analyte	Azoxystrobin, R230310
Equipment/ Chromatographic method	LC-MS/MS
Accuracy/ Precision (repeatability)	Acceptable mean recoveries between 70% and 110% with a relative standard deviation less than 20% were obtained for azoxystrobin and R230310 for each transition in both matrices tested.
Specificity	LC-MS/MS with two transitions is a highly specific detection technique and therefore, according to EU guidance (SANCO/825/00 rev.8.1, 16/11/2010), no further confirmatory technique is required. No peaks in control samples above 30% of LOQ were detected.
Confirmatory method	See above
Assessment of matrix effects is presented	Yes No significant matrix effects (maximum 15% suppression) were observed in the water types tested during method validation and non-matrix standards should generally be used for quantification.
Calibration/Linearity	Calibration was performed by measuring 5 single level of the respective analyte covering the working range.
	The linearity of the LC-MS/MS detector response for azoxystrobin and R230310 were tested in the range from 0.15 pg to 60 pg injected on column using a 30.0 µL injection volume (equivalent to 0.05 µg/L to 2 µg/L in standards (corresponding to 50% of the LOQ (0.1 µg/L) and 2x the highest level tested)) and was found to be linear.
	The detector response was linear with coefficients of determination always above 0.998. Azoxystrobin <u>Ground water</u> Quantification - $y=2.25e+004x + 216$ ($r = 0.9993$) Confirmation - $y=6.49e+003x + 95.7$ ($r = 0.9981$) R230310 <u>Ground water</u> Quantification - $y=1.44e+004x + -175$ ($r = 0.9988$) Confirmation - $y=4.14e+003x + 12$ ($r = 0.9984$)
Limit of quantification (LOQ)	The limit of quantitation (LOQ) of the method has been established at a 0.1 µg/L (0.1 ppb).
Limit of detection (LOD)	The limit of detection (LOD) of the method is defined as the lowest analyte amount injected on column detectable above the mean amplitude of the background noise at the corresponding retention time. The limit of detection for analytical method GRM057.04A was determined as 0.15 pg injected on column corresponding to the lowest standard concentration.
Extract Stability	Final fraction samples from the analysis of ground and surface water in acetonitrile/ultra-pure water (50/50 v/v) at the method LOQ of 0.10 ppb was found to be stable for at least 7 days upon storage at a temperature of approximately 4°C during method validation.
Standard Solution Stability	An expiration date of six months for azoxystrobin and R230310 is recommended unless additional data are generated to support a longer expiration date.
Extractability	Residues of azoxystrobin and R230310 in water are analysed by direct injection and extractability is not relevant for direct injection methods.

Conclusion

Method GRM057.04A has been successfully validated for the determination of residues of azoxystrobin and R230310 in water with a limit of quantification (LOQ) of 0.1 µg/L. It fully meets the requirements of SANCO/825/00 Rev. 8.1 for analytical methods used for post-registration monitoring.

(Mayer L.C., 2012)

A 2.1.2.5.1.6 GRM057.04A - Independent laboratory validation

Comments of zRMS:	The method has been evaluated in Draft Registration Report for Orondis Evo (Central Zone, zRMS-PL, July 2022). Below is a copied assessment: The analytical method GRM057.04A has been successfully validated in an independent laboratory and demonstrated to be suitable for the determination of Azoxystrobin and Z-Isomer 230310 in surface and ground water at a LOQ of 0.1 µg/L.
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	Mean recoveries were between 70% and 110% with a relative standard deviation less than 20%.
	Taking into account the deviations of this study, the validation is still acceptable.

Reference:	KCP 5.2.5
Report	Azoxystrobin-Independent Laboratory Validation (ILV) of Residue Method (GRM057.04A) for the Determination of Azoxystrobin and Z-Isomer R230310 in Water by LC-MS/MS, Smith R.J., 2012, report No 1781.6873, document No VV-507766
Guideline(s):	EPA OCSPP 850.6100, Data Reporting for Environmental Chemistry Methods EPA FIFRA
Deviations:	Deviations from SANCO/825/00 rev. 8.1: - Linearity range not from 30% LOQ but 50% LOQ The validation was performed under GLP and according to EU guideline SANCO/3029/99 Rev. 4. Whilst the validation is not fully compliant to the new guidance SANTE/2020/12830 Rev. 1, it exceeds the minimum validation requirements set out therein.
GLP:	Yes
Acceptability:	Yes

Materials

Test Material	Azoxystrobin
Lot/Batch #:	ASJ10008-04
Purity (%):	99.7% ± 0.3%
IUPAC name:	Methyl (E)-2-(2-[6-(2-cyanophenoxy)pyrimidin-4-yloxy]phenyl)-3-methoxyacrylate
CAS number:	131860-33-8

Test Material	Z-Isomer R230310
Lot/Batch #:	601321
Purity (%):	94% ± 5% (wt/wt)
IUPAC name:	Methyl (Z)-2-(2-[6-(2-cyanophenoxy)pyrimidin-4-yloxy]phenyl)-3-methoxyacrylate
CAS number:	143130-94-3

Study Design and Methods

Test facility: Smithers Viscient, 790 Main Street Wareham, MA 02571-1037 USA

Study start date: 13 August 2012

Study end date: 26 October 2012

Analytical phase dates: 30 August - 31 August 2012

Sub-samples of each surface and groundwater were fortified with standard solutions of azoxystrobin and R230310. Samples of each matrix were fortified at the limit of quantification (0.1 µg/L) and 1.0 µg/L. The fortified samples were analysed alongside untreated control samples.

Principle of the method

Surface and ground water samples are analysed directly by LC-MS/MS after dilution with acetonitrile/ultra-pure water (50/50 v/v) using non-matrix matched external calibration, monitoring for two transitions.

LC-MS/MS Conditions

LC-MS/MS system:	Shimadzu 20AD HPLC with MDS Sciex 5000
Pumps:	LC-20AD binary pump, Shimadzu
Injector:	SIL-20ACHT autoinjector
Degasser:	20AD vacuum degasser, Shimadzu
Column Oven:	CTO-20A

Detector: AB MDS Sciex 5000 Turbo V ESI with Analyst™ software version 1.4.2
Column: ACE 5 C18 - 50 mm x 3.0 mm
Mobile phase: A: 0.1% acetic acid in ultra-pure reagent water
B: 0.1% acetic acid in ultra-pure acetonitrile

Time (min)	%A	%B
0.0	50	50
3.0	50	50

Flow rate: 0.8 mL/min
Column oven temperature 40°C
Injection volume: 30 µL
Retention time: Azoxystrobin: 1.47 min
R230310: 1.18 min

Detector MDS Sciex 5000
Ionisation mode: ESI
Source polarity: Positive
Scan type: MRM
Curtain gas (CUR): 25.00
Gas 1 (GS1): 35.00
Gas 2 (GS2): 35.00
Temperature (TEM): 450°C
Interface heater (IHE): On
Ionspray voltage (IS): 3000V
Collision gas setting (CAD): 8.00
Entrance potential (EP): 10 V
Dwell time 150 msec

Source and detection parameters for MS/MS experiments:

Compound	Parent (m/z)	CE (V)	DP (V)	CXP (V)	Fragment ions (m/z)	
Azoxystrobin	404.38	21.00	91.00	44.00	372.10	Quantification
		42.00	91.00	26.00	329.00	Confirmation
		35.00	91.00	26.00	344.10	Confirmation
R230310	404.39	17.00	126.00	42.00	372.00	Quantification
		42.00	126.00	40.00	329.00	Confirmation
		35.00	126.00	40.00	344.10	Confirmation

CE: Collision energy; CXP: Collision cell exit potential; DP: Declustering Potential

Exceptions to the method GRM057.04A performed at Smithers Viscient are as follows:

- An additional confirmation transition of 404.4/344.1 was analysed for both azoxystrobin & Z-isomer.
- The instrument used for analysis was a LC-MS/MS 5000, as opposed to a LC-MS/MS 5500.

Results

Recoveries of azoxystrobin and R230310 obtained from water at each fortification level using method GRM057.04A are presented in the table below.

Table A 43: Recovery results from the independent laboratory method validation of azoxystrobin and R230310 using the analytical method GRM057.04A in water

Matrix	Analyte	Fortification level (µg/L)	Individual recoveries (%)	Range of recoveries (%) (n = x)	Mean recovery (%)	RSD (%)
Surface water	Azoxystrobin	Mass transition m/z = 404 → 372 (quantification)				
		0.1	105; 105; 102; 99; 99	99 – 105 (n = 5)	102	3.0

Matrix	Analyte	Fortification level (µg/L)	Individual recoveries (%)	Range of recoveries (%) (n = x)	Mean recovery (%)	RSD (%)
		1.0	96; 91; 95; 96; 95	91 – 96 (n = 5)	95	2.0
		Overall		91 – 105 (n = 10)	98	4.6
		Mass transition m/z = 404 → 329 (confirmation)				
		0.1	96; 109; 101; 101; 110	96 – 110 (n = 5)	104	5.7
		1.0	97; 97; 96; 95; 96	95 – 97 (n = 5)	96	1.0
		Overall		95 – 110 (n = 10)	100	5.6
		Mass transition m/z = 404 → 344 (confirmation)				
		0.1	103; 103; 97; 95; 107	95 – 107 (n = 5)	101	5.1
		1.0	98; 96; 95; 97; 100	95 – 100 (n = 5)	97	2.0
		Overall		95 – 107 (n = 10)	99	4.2
Surface water	R230310	Mass transition m/z = 404 → 372 (quantification)				
		0.1	95 99; 97; 98; 95	95 – 99 (n = 5)	97	1.7
		1.0	96; 96; 95; 97; 94	94 – 97 (n = 5)	96	1.0
		Overall		94 – 99 (n = 10)	96	1.4
		Mass transition m/z = 404 → 329 (confirmation)				
		0.1	108; 109; 105; 103; 96	96 – 109 (n = 5)	104	5.0
		1.0	95; 93; 96; 97; 93	93 – 97 (n = 5)	95	1.9
		Overall		93 – 109 (n = 10)	99	6.1
		Mass transition m/z = 404 → 344 (confirmation)				
		0.1	110; 92; 84; 100; 105	84 – 110 (n = 5)	98	10.5
		1.0	97; 97; 96; 99; 96	96 – 99 (n = 5)	97	1.3
		Overall		84 – 110 (n = 10)	98	7.1
Ground water	Azoxystrobin	Mass transition m/z = 404 → 372 (quantification)				
		0.1	100; 102; 104; 101; 102	100 – 104 (n = 5)	102	1.5
		1.0	96; 94; 97; 93; 94	93 – 97 (n = 5)	95	1.7
		Overall		93 – 104 (n = 10)	98	4.1
		Mass transition m/z = 404 → 329 (confirmation)				

Matrix	Analyte	Fortification level (µg/L)	Individual recoveries (%)	Range of recoveries (%) (n = x)	Mean recovery (%)	RSD (%)
		0.1	106; 108; 101; 95; 104	95 – 108 (n = 5)	103	4.9
		1.0	99; 93; 98; 98; 100	93 – 100 (n = 5)	97	2.4
		Overall		93 – 108 (n = 10)	100	4.7
		Mass transition m/z = 404 → 344 (confirmation)				
		0.1	104; 93; 100; 106; 93	93 – 106 (n = 5)	99	6.1
		1.0	94; 91; 94; 94; 97	91 – 97 (n = 5)	94	2.2
		Overall		91 – 106 (n = 10)	97	5.2
Ground water	R230310	Mass transition m/z = 404 → 372 (quantification)				
		0.1	100; 102; 99; 95; 97	95 – 102 (n = 5)	98	3.2
		1.0	94; 95; 93; 97; 94	93 – 97 (n = 5)	95	1.7
		Overall		93 – 102 (n = 10)	97	3.1
		Mass transition m/z = 404 → 329 (confirmation)				
		0.1	105; 108; 108; 96; 100	96 – 108 (n = 5)	103	5.2
		1.0	95; 99; 95; 98; 95	95 – 99 (n = 5)	97	1.8
		Overall		95 – 108 (n = 10)	100	5.2
		Mass transition m/z = 404 → 344 (confirmation)				
		0.1	94; 96; 97; 90; 98	90 – 98 (n = 5)	95	3.5
		1.0	98; 99; 92; 95; 94	92 – 99 (n = 5)	96	3.1
		Overall		90 – 99 (n = 10)	95	3.2

Table A 44: Characteristics of the enforcement analytical method used for the quantification of azoxystrobin and R230310 residues in water

Analyte	Azoxystrobin and Z isomer R230310
Equipment/ Chromatographic method	LC-MS/MS
Accuracy/ Precision (repeatability)	Acceptable mean recoveries between 70% and 110% with a relative standard deviation less than 20% were obtained for azoxystrobin and R230310 for each transition in both matrices tested.
Precision (reproducibility)	This independent laboratory validation [ILV] study was conducted to verify the reliability of method No. GRM057.04A for the determination of azoxystrobin and Z-Isomer R230310 residues in water. The results indicate that the method is reproducible.
Specificity	LC-MS/MS with two transitions is a highly specific detection technique and therefore, according to EU guidance (SANCO/825/00 rev.8.1, 16/11/2010), no further confirmatory technique is required. Untreated control and reagent blank samples analysed with each set contained ≤50% of the LOD in the matrices tested and are considered negligible.
Confirmatory method	See above

Assessment of matrix effects is presented	Not investigated.
Calibration/Linearity	Calibration was performed by measuring 5 single level of the respective analyte covering the working range.
	The linearity of the LC-MS/MS detector response for azoxystrobin and R230310 were tested in the range from 0.15 pg to 60 pg injected on column (equivalent to 0.05 µg/L to 2 µg/L standards (corresponding to 50% of the LOQ (0.1 µg/L) and 2x the highest level tested) when using a 30.0 µL injection volume) and was found to be linear.
	The detector response was linear with coefficients of determination always above 0.99.
	Azoxystrobin <u>Ground water</u> Quantification - $y = -1.3916E+05x^2 + 2.3070E+06x - 7.3272E+01$ ($R^2 = 0.99846$) Confirmation - $y = -3.1907E+04x^2 + 5.6844E+05x - 5.1763E+01$ ($R^2 = 0.99916$) Confirmation - $y = -3.9475E+04x^2 + 7.0655E+05x - 3.9885E+02$ ($R^2 = 0.99894$) R230310 <u>Ground water</u> Quantification - $y = -1.3742E+05x^2 + 1.8227E+06x + 2.2032E+02$ ($R^2 = 0.99857$) Confirmation - $y = -2.8900E+04x^2 + 4.6298E+05x - 2.4998E+02$ ($R^2 = 0.99815$) Confirmation - $y = -3.6065E+04x^2 + 5.5990E+05x + 1.0380E+02$ ($R^2 = 0.99837$)
Limit of quantification (LOQ)	The stated LOQ of the method is 0.100µg/L for ground and surface water and the lowest validated level with acceptable recovery and precision.
Limit of detection (LOD)	0.05 µg/L
Extract Stability	Please refer to method validation
Standard Solution Stability	Please refer to method validation
Extractability	Residues of azoxystrobin and R230310 in water are analysed by direct injection and extractability is not relevant for direct injection methods.

Conclusion

Method GRM057.04A has been successfully validated in an independent laboratory for the determination of residues of azoxystrobin and R230310 in water with a limit of quantification (LOQ) of 0.1 µg/L. It fully meets the requirements of SANCO/825/00 rev. 8.1 for post-registration monitoring of residues in drinking water.

(Smith R.J., 2012)

A 2.1.2.5.1.7 Confirmatory method

No confirmatory method is required.

A 2.1.2.6 Description of Methods for the Analysis of Air (KCP 5.2.6)

No new or additional studies have been submitted.

A 2.1.2.7 Other Studies/ Information

No new or additional studies have been submitted.

A 2.2 Analytical methods for the oxathiapiprolin

A 2.2.1 Methods used for the generation of pre-authorization data (KCP 5.1)

A 2.2.1.1 Description of analytical methods for the determination of residues in support of environmental fate studies (KCP 5.1.2.1)

No new or additional studies have been submitted.

A 2.2.1.2 Description of analytical methods for the determination of residues in support of efficacy studies (KCP 5.1.2.2)

No new or additional studies have been submitted.

A 2.2.1.3 Description of analytical methods for the determination of residues in support of toxicological studies (KCP 5.1.2.3)

No new or additional studies have been submitted.

A 2.2.1.4 Description of analytical methods for the determination of residues in support of operator, worker, resident and bystander exposure studies (KCP 5.1.2.4)

No new or additional studies have been submitted.

A 2.2.1.5 Description of analytical methods for the determination of residues in support of residues studies (KCP 5.1.2.5)

A 2.2.1.5.1 Analytical method 1: DuPont-30422

A 2.2.1.5.1.1 Method validation (231693)

Comments of zRMS:	<p>The method has been evaluated in Draft Registration Report for Orondis Evo (Central Zone, zRMS-PL, July 2022).</p> <p>Below is a copied assessment:</p> <p>The analytical residue method DuPont-30422 was successfully validated for the determination of residues of oxathiapiprolin in crop matrices (peppers, cucumbers, melon, leek, broccoli, cauliflower, cabbage, kale, brussel sprouts and hops) at a limit of quantification (LOQ) of 0.01 mg/kg.</p> <p>For all fortification levels (0.01 mg/kg, 0.10 mg/kg), acceptable mean recoveries in the range of 70 - 110 % with a relative standard deviation (RSD) of ≤ 20 % were found for oxathiapiprolin for both the quantification and confirmation mass transitions, in all matrices.</p> <p>The method has therefore been successfully validated according to the EU guidelines SANCO/3029/99 rev.4.</p> <p>The study is acceptable.</p>
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Reference: KCP 5.1.2.5 (and KCP 5.2.1)

Report Oxathiapiprolin (SYN546539) – Validation of the Analytical method DuPont-30422 for the Determination of Residues of Oxathiapiprolin in Crop Matrices by LC-MS/MS
Donald C., Gibson R. (2020)
Report No. 231693, Syngenta File No. VV-870136

Guideline(s): Yes
Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/3029/99 rev. 4, 11/07/2000).
OECD Guidance Document on Pesticide Residue Analytical Methods ENV/JM/MONO(2007)17.
Regulation (EC) No 1107/2009 of the European Parliament and of the council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC.
Residue Chemistry Test Guidelines OPPTS 860.1340 Residue Analytical Method, EPA 712-C-96-174, August 1996.

Deviations: No

GLP: Yes

Acceptability: Yes

Materials and methods

Five gram sample of high water content crop (2.5 g for hops) was extracted three times by homogenising in a genogrinder using a solution of formic acid/water/acetonitrile. For all matrices except hops, an aliquot of the extract was diluted and analysed by LC-MS/MS. For hops, a solid phase extraction (SPE) clean-up using graphitized carbon cartridges giving a final sample concentration of 0.0071 g/mL for high water content crops and 0.01 g/mL for hops.

Final extracts were analysed for oxathiapiprolin (SYN546539) by high performance liquid chromatography with mass spectrometric detection (LC-MS/MS), monitoring for the primary transition (m/z 539.8-499.9) and the confirmatory transition (m/z 539.8-163.0).

HPLC-MS/MS Conditions

HPLC system: Shimadzu Nexera X2 UPLC
Detector: AB Sciex API 6500 equipped with a TurboIonSpray source
Column: ACE 3 C18-PFP, 15 cm x 3.0 mm
Mobile phase (High water content samples): A: 0.05% aqueous Formic Acid
B: 0.01% Formic Acid in Methanol

Time	%A	%B	Gradient
0.00	90	10	
11.00	1	99	
13.00	1	99	
13.50	90	10	
21.00	90	10	

Mobile (Hops): A: 0.05% aqueous Formic Acid
B: 0.01% Formic Acid in Methanol

Time	%A	%B	Gradient
0.00	40	60	
0.50	40	60	
4.50	1	99	
5.50	1	99	
6.00	40	60	
8.00	40	60	

Flow rate: 0.5 ml/min
Column oven temperature 40°C
Injection volume: 20 µL
Retention time: High water samples: Oxathiapiprolin (SYN546539): approx. 10.7 min
Hops: Oxathiapiprolin (SYN546539): approx. 4.7 min
Detector AB Sciex API 6500 equipped with a TurboIonSpray source

Ionisation mode: TurboIonSpray
Source polarity: Positive
Dwell time: 200 msec

Source and detection parameters for MS/MS experiments:

Compound	Parent m/z	CE (V)	DP (V)	CXP (V)	
Oxathiapirolin (SYN546539)	539.8 → 499.9	33	82	40	Quantification
	539.8 → 163.0	49	82	19	Confirmation

CE: Collision energy; CXP: Collision cell exit potential ; DP : Declustering Potential

The analytical method, DuPont-30422 was validated in peppers, cucumbers, melon, leek, broccoli, cauliflower, cabbage, kale, brussel sprouts and hops.

Results and discussions

The analytical method was validated in peppers, cucumber, melons, leek, broccoli, cauliflower, cabbage, kale, Brussels sprouts and hops, fortified with oxathiapirolin.

The recoveries obtained for oxathiapirolin are detailed in the table below.

Table A 45: Recovery results from method validation of oxathiapirolin using the analytical method

Matrix	Analyte	Fortification (mg/kg)	Number of analyses (n)	Mean Recovery (%)	RSD (%)	Recovery Range (%)
Primary transition m/z 539.8 – 499.9						
Peppers	Oxathiapirolin	0.01	5	99	4	93 – 103
		0.10	5	95	8	83 – 102
		Overall	10	97	6	83 – 103
Cucumbers	Oxathiapirolin	0.01	5	94	7	83 – 99
		0.10	5	96	6	88 – 103
		Overall	10	95	6	83 – 103
Melon	Oxathiapirolin	0.01	5	86	14	67 – 95
		0.10	5	95	13	73 – 105
		Overall	10	90	14	67 – 105
Leek	Oxathiapirolin	0.01	5	93	6	84 – 97
		0.10	5	97	3	94 – 100
		Overall	10	95	5	84 – 100
Broccoli	Oxathiapirolin	0.01	5	91	6	86 – 97
		0.10	5	91	2	88 – 92
		Overall	10	91	4	86 – 97
Cauliflower	Oxathiapirolin	0.01	5	95	8	87 – 103
		0.10	5	98	8	85 – 107
		Overall	10	97	8	85 – 107
Cabbage	Oxathiapirolin	0.01	5	91	2	88 – 92
		0.10	5	100	2	98 – 102
		Overall	10	95	5	88 – 102
Kale	Oxathiapirolin	0.01	5	85	3	83 – 89
		0.10	5	96	5	87 – 100
		Overall	10	90	7	83 – 100

Matrix	Analyte	Fortification (mg/kg)	Number of analyses (n)	Mean Recovery (%)	RSD (%)	Recovery Range (%)
Brussels Sprouts	Oxathiapiprolin	0.01	5	78	7	72 – 85
		0.10	5	84	4	80 – 88
		Overall	10	81	6	72 – 88
Hops	Oxathiapiprolin	0.01	5	90	12	80 – 108
		0.10	5	85	5	81 – 92
		Overall	10	88	9	80 – 108
Confirmatory transition m/z 539.8 – 163.0						
Peppers	Oxathiapiprolin	0.01	5	95	6	88 – 101
		0.10	5	95	8	82 – 102
		Overall	10	95	7	82 – 102
Cucumbers	Oxathiapiprolin	0.01	5	97	9	84 – 110
		0.10	5	98	6	90 – 105
		Overall	10	97	7	84 – 110
Melon	Oxathiapiprolin	0.01	5	87	14	67 – 100
		0.10	5	98	14	74 – 110
		Overall	10	93	15	67 – 110
Leek	Oxathiapiprolin	0.01	5	92	7	81 – 98
		0.10	5	97	4	92 – 101
		Overall	10	94	6	81 – 101
Broccoli	Oxathiapiprolin	0.01	5	87	7	78 – 94
		0.10	5	91	2	89 – 94
		Overall	10	89	6	78 – 94
Cauliflower	Oxathiapiprolin	0.01	5	103	9	92 – 113
		0.10	5	99	8	86 – 108
		Overall	10	101	8	86 – 113
Cabbage	Oxathiapiprolin	0.01	5	91	6	85 – 98
		0.10	5	101	1	99 – 102
		Overall	10	96	7	85 – 102
Kale	Oxathiapiprolin	0.01	5	82	7	75 – 88
		0.10	5	97	5	88 – 102
		Overall	10	89	10	75 – 102
Brussels Sprouts	Oxathiapiprolin	0.01	5	79	6	74 – 85
		0.10	5	79	4	76 – 82
		Overall	10	79	4	74 – 85
Hops	Oxathiapiprolin	0.01	5	93	8	85 – 102
		0.10	5	84	8	79 – 96
		Overall	10	89	9	79 – 102

Specificity

LC-MS/MS with two transitions is considered to be a highly specific detection technique and therefore according to EU guidance (SANTE/2020/12830, Rev.1, 24/02/2021) no further confirmatory technique is

required. The method includes two MS/MS transitions, both of which have been validated. No significant interferences arising from the crop matrices, the labware, reagents or solvents have been observed at the retention times of interest.

Linearity

The linearity of the LC-MS/MS detector was tested using matrix matched standard solutions (0.021 ng/mL to 1.0 ng/mL for high water content crops and 0.03 ng/mL to 2 ng/mL for hops) for both transitions. Standards at eight different concentrations were injected and the signal area plotted against concentration for all calibration points. The response factor was calculated for each standard in the calibration line and the mean response factor for each calibration line was calculated. Lines with a coefficient of variation $\leq 20\%$ for all calibration lines were obtained for oxathiapiprolin (SYN546539).

The linear range is equivalent to 30% LOQ to 1400% LOQ (0.003 mg/kg to 0.14 mg/kg).

The detector response was linear. Linear equations and correlation coefficients are reported for the following matrices:

Pepper

Quantification – $y = 4.33e+005 x$

Confirmation – $y = 8.73e+004 x$

Cucumber

Quantification – $y = 5.35e+005 x$

Confirmation – $y = 1.02e+005 x$

Melon

Quantification – $y = 5.73e+005 x$

Confirmation – $y = 1.05e+005 x$

Leek

Quantification – $y = 5.09e+005 x$

Confirmation – $y = 1e+005 x$

Broccoli

Quantification – $y = 5.17e+005 x$

Confirmation – $y = 1.01e+005 x$

Cauliflower

Quantification – $y = 5.07e+005 x$

Confirmation – $y = 9.7e+004 x$

Cabbage

Quantification – $y = 5.36e+005 x$

Confirmation – $y = 1.03e+005 x$

Kale

Quantification – $y = 4.26e+005 x$

Confirmation – $y = 8.3e+004 x$

Brussel sprouts

Quantification – $y = 4.08e+005 x$

Confirmation – $y = 8.48e+004 x$

Hops

Quantification – $y = 1.92e+005 x$

Confirmation – $y = 3.71e+004 x$

Accuracy

Fortified samples were analysed in quintuplet at the limit of quantification (LOQ, 0.01 mg/kg) and at ten times the LOQ (0.1 mg/kg). Acceptable mean recoveries of between 70% and 110% were found for both transitions on all matrices tested and therefore according to EU guidance (SANTE/2020/12830, Rev.1, 24/02/2021) demonstrate the method has satisfactory accuracy.

Repeatability

The relative standard deviations (RSDs) of oxathiapiprolin (SYN546539) recoveries at each fortification level and overall for each crop tested during method validation were <20% and therefore according to the EU guidance (SANTE/2020/12830, Rev.1, 24/02/2021) demonstrate the method has satisfactory repeatability.

Limit of Quantification

The limit of quantification for oxathiapiprolin (SYN546539) residues in crop matrices using method DuPont-30422 was established at 0.01 mg/kg. No interfering peaks around the retention time of compoundname were found in any of the control samples at levels above 30% of the limit of quantification.

Matrix Effects

Significant matrix effects (suppression and enhancement) were observed in some of the crop matrices tested during method validation, therefore matrix matched linearity standards were used for all matrices.

Stability of Final Extracts

The stability of sample extracts fortified with oxathiapiprolin (SYN546539) at the LOQ level was checked after a storage period of at least 7 days in a refrigerator set to 4 °C against freshly prepared calibration standards. The results proved that the oxathiapiprolin (SYN546539) residues in the stored fortified samples were stable except for brussel sprouts and hops, where it is recommended these are analysed immediately after extraction. The mean recovery values at the LOQ level were between 70% and 110%, with a RSD of $\leq 20\%$ and within $\pm 20\%$ of the initial values when re-analysed.

Stability of Standard Solutions

The stability of the stored working standard solutions of oxathiapiprolin (SYN546539) in acetonitrile at 100 µg/mL and working solutions of oxathiapiprolin (SYN546539) in 70/30 acetonitrile/water ranging from 10-1000 ng/mL were checked after a storage period of 82 days in a freezer at -20°C against freshly prepared standard solutions. All standard solutions were diluted to 1 ng/mL prior to analysis. The results demonstrated that oxathiapiprolin (SYN546539) residues in the stored working standard solutions were stable. The concentrations were within $\pm 10\%$ of the initial values. The standard solutions can thus be considered as stable.

The stability of calibration solutions of oxathiapiprolin prepared in 70/30 acetonitrile/water was assessed by comparing a batch standard made from freshly prepared calibration solutions to a batch standard made from stored calibration solutions after a storage period of 82 days in a freezer set to -20°C. The concentrations were within $\pm 10\%$ of the initial values. The calibration solutions can thus be considered as stable.

Analytical method DuPont-30422 (Report No. 231693) was used to generate residue data in Report No. S19-02718 Report No. CEMR-9533. The standard stability data presented in Report No. 231693 supports the use of this method in these studies.

Conclusion

Analytical method DuPont-30422 has been demonstrated to be a reliable and accurate procedure for the determination of oxathiapiprolin (SYN546539) in crops to a limit of quantification of 0.01 mg/kg, using commercially available laboratory equipment and reagents.

A 2.2.1.5.1.2 Confirmatory method

No confirmatory method is required. LC-MS/MS with two transitions is considered to be a highly specific detection technique. The method includes two MS/MS transitions, both of which have been validated.

A 2.2.1.5.1.3 Method & Validation (S19-02718)

Comments of zRMS:	<p>The method has been evaluated in Draft Registration Report for Orondis Evo (Central Zone, zRMS-PL, July 2022).</p> <p>Below is a copied assessment:</p> <p>The method DuPont-30422 has been successfully validated for determination of oxathiapiprolin residues in lettuce.</p> <p>The specificity, linearity, accuracy, precision and repeatability has been demonstrated for both the quantification and confirmatory transitions by taking one reagent blank, two control samples, five samples fortified at the LOQ and five at 10 x LOQ through the method using LC-MS/MS detection.</p> <p>Recovery values at the LOQ were in the range 103 – 106% with a mean recovery of 105% and 1.1% relative standard deviation (RSD) for the primary transition (m/z 540/500) and were in the range 97 – 108% with a mean recovery of 104% and 4.1% relative standard deviation (RSD) for the confirmatory mass transition (m/z 500/163).</p> <p>No interferences were present at or above 30% of the LOQ in any of the control samples and reagent blanks. The LOD and the matrix effect were assessed. LOQ = 0.01 mg/kg</p> <p>The method DuPont-30422 has been validated according to the EU guidelines SANCO/3029/99 rev.4.</p> <p>The method is acceptable.</p>
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Reference: KCP 5.1.2.5

Report Oxathiapiprolin - Residue Study on Protected Lettuce in Northern France, Germany, Italy, Spain and the United Kingdom in 2019
Lakaschus S., Reinhardt, R. (2020)
Report No. S19-02718, Syngenta File No. VV-854039

Guideline(s): SANCO/3029/99 rev. 4 (2000)
ENV/JM/MONO(2007)17
EPA OPPTS 860.1340 (1996)
Regulation (EC) No 1107/2009

Deviations: No

GLP: Yes

Acceptability: Yes

Principle of the method

5 g sample of lettuce was extracted three times by homogenising in a genogrinder using a solution of formic acid/water/acetonitrile. An aliquot of the extract was diluted and analysed by LC-MS/MS giving a final sample concentration of 0.0071 g/mL for high water content crops.

Final extracts were analysed for oxathiapiprolin (SYN546539) by high performance liquid chromatography with mass spectrometric detection (LC-MS/MS), monitoring for the primary transition (m/z 540→500) and the confirmatory transition (m/z 540→163).

HPLC-MS/MS Conditions

HPLC system: 1260 Infinity Binary LC System, Agilent Technologies (HPLC, ≤ 600 bar)

Detector: TripleQuad 5500 System, SCIEX (Triple quadrupole mass spectrometer)

Column: Ascentis Express C18 (100 mm x 2.1 mm, 2.7 µm, Supelco, Art. No. 53823-U)

Mobile phase (High water content samples): A: Methanol + 0.1 % formic acid + 5 mM ammonium formate
B: Water + 0.1 % formic acid + 5 mM ammonium formate

Time	% A	% B	Gradient
0.00	40	60	
3.5	95	5	
4.50	95	5	
4.60	40	60	
6.00	40	60	

Flow rate: 0.5 ml/min

Column oven temperature 40°C

Injection volume: 15 µL

Retention time: Oxathiapiprolin (SYN546539): approx. 3.6 min

Detector AB Sciex API 6500 equipped with a TurboIonSpray source

Ionisation mode: Electrospray (ESI, TurboIonSpray)
Source polarity: Positive
Dwell time 0.1 s

Source and detection parameters for MS/MS experiments:

Compound	Parent m/z	CE (V)	DP (V)	CXP (V)	
Oxathiapiprolin (SYN546539)	540 → 500	45	140	10	Quantification
	540 → 163	35	180	12	Confirmation

CE: Collision energy; CXP: Collision cell exit potential ; DP : Declustering Potential

The analytical method, DuPont-30422 was validated in lettuce.

Recovery Findings

Summaries of the results for oxathiapiprolin (SYN546539) are presented in the tables below.

Table A 46: Recovery results from validation for oxathiapiprolin (SYN546539) in crops: Primary transition *m/z* 539.8-499.9

Matrix	Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)	Range (%)
Lettuce	0.01*	106, 105, 105, 103, 104	5	105	1.2	103 - 106
	0.1	101, 99, 103, 109, 107	5	104	4.0	99 - 109
	Overall		10	104	2.8	99 - 109

*Limit of quantitation, defined by the lowest validated fortification level

Table A 47: Recovery results from validation for oxathiapiprolin (SYN546539) in crops: Confirmatory transition *m/z* 539.8-163.0

Matrix	Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)	Range (%)
Lettuce	0.01*	97, 106, 103, 108, 105	5	104	4.1	97 - 108
	0.1	102, 100, 102, 105, 105	5	103	2.1	100 - 105
	Overall		10	103	3.1	97 - 108

*Limit of quantitation, defined by the lowest validated fortification level

Specificity

LC-MS/MS with two transitions is considered to be a highly specific detection technique and therefore according to EU guidance (*SANCO/825/00 rev.8.1, 16/11/2010*) no further confirmatory technique is required. The method includes two MS/MS transitions, both of which have been validated. No significant interferences arising from the crop matrices, the labware, reagents or solvents have been observed at the retention times of interest.

Linearity

The linearity of the LC-MS/MS detector was tested using matrix matched standard solutions (0.02 ng/mL to 2.5 ng/mL for lettuce) for both transitions. Standards at eight different concentrations were injected and the signal area plotted against concentration for all calibration points. The response factor was calculated for each standard in the calibration line and the mean response factor for each calibration line was calculated. Lines with a coefficient of variation $\leq 20\%$ for all calibration lines were obtained for oxathiapiprolin (SYN546539).

The detector response was linear.

Lettuce

Quantification – $y = 338445.6981x - 103.5261$, $R^2 = 0.9998$

Confirmation – $y = 70060.3627x - 8.5290$, $R^2 = 0.9990$

Accuracy

Fortified samples were analysed in quintuplet at the limit of quantification (LOQ, 0.01 mg/kg) and at ten times the LOQ (0.1 mg/kg). Acceptable mean recoveries of between 70% and 110% were found for both transitions on all matrices tested and therefore according to EU guidance (*SANCO 3029/99 rev.4 11/7/00*) demonstrate the method has satisfactory accuracy.

Repeatability

The relative standard deviations (RSDs) of oxathiapiprolin (SYN546539) recoveries at each fortification level and overall for each crop tested during method validation were $<20\%$ and therefore according to the EU guidance (*SANCO 3029/99 rev.4 11/7/00*) demonstrate the method has satisfactory repeatability.

Limit of Quantification

The limit of quantification for oxathiapiprolin (SYN546539) residues in crop matrices using method DuPont-30422 was established at 0.01 mg/kg. No interfering peaks around the retention time of oxathiapiprolin (SYN546539) were found in any of the control samples at levels above 30% of the limit of quantification.

Matrix Effects

No significant matrix effects (suppression and enhancement) were observed in lettuce matrix during method validation, therefore matrix matched linearity standards were used for all matrices.

Stability of Final Extracts

The stability of sample extracts fortified with oxathiapiprolin (SYN546539) at the LOQ level was checked after a storage period of at least 9 days in a refrigerator set to 5 °C against freshly prepared calibration standards. The results proved that the oxathiapiprolin (SYN546539) residues in the stored

fortified samples were stable. The mean recovery values at the LOQ level were between 70 % and 110 %, with a RSD of ≤ 20 % and within $\pm 20\%$ of the initial values when re-analysed.

Conclusion

Analytical method DuPont-30422 has been demonstrated to be a reliable and accurate procedure for the determination of oxathiapiprolin (SYN546539) in lettuce to a limit of quantification of 0.01 mg/kg, using commercially available laboratory equipment and reagents.

A 2.2.1.5.1.4 Confirmatory method

No confirmatory method is required. LC-MS/MS with two transitions is considered to be a highly specific detection technique. The method includes two MS/MS transitions, both of which have been validated.

A 2.2.1.5.2 Analytical method 2: DuPont-30422 (Supplement 1)

A 2.2.1.5.2.1 Method validation (CEMR-9533)

Comments of zRMS:	<p>The method has been evaluated in Draft Registration Report for Orondis Evo (Central Zone, zRMS-PL, July 2022).</p> <p>Below is a copied assessment:</p> <p>The method DuPont-30422 – Supplement No.1 has been successfully validated for determination of oxathiapiprolin residues in honey.</p> <p>The specificity, linearity, accuracy, precision and repeatability was demonstrated for both the primary and confirmatory transitions by taking one reagent blank, two control samples, five samples fortified at the LOQ and five at $10 \times$ LOQ through the method using LC-MS/MS detection. No interferences were present at or above 30% of the LOQ in any of the control samples and reagent blanks.</p> <p>LOQ=0.01 mg/kg</p> <p>The mean recovery values at the LOQ level were between 70% and 110%, with a RSD of $\leq 20\%$.</p> <p>The analytical method DuPont-30422 Supplement No.1 was successfully validated for the determination of oxathiapiprolin in honey according to the EU guideline SANCO/3029/99 rev.4.</p> <p>The sugar content of the honey samples was assessed by performing BRIX analysis.</p> <p>The method is acceptable.</p>
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Reference: KCP 5.1.2.5

Report Oxathiapiprolin – Honey Residue Study on Spring Oilseed Rape in Northern and Southern Europe in 2020
Ford K. (2020)
Report No. CEMR-9533, Syngenta File No. VV-885771

Guideline(s): SANCO/825/00 rev. 8.1 (2010)SANCO/3029/99 rev. 4 (2000)
ENV/JM/MONO(2007)17
EPA OPPTS 860.1340 (1996)
SANTE/11956/2016 rev. 9

Deviations: No

GLP: Yes

Acceptability: Yes

Materials and methods

DuPont-30422 Supplement No. 1:

Residues of oxathiapiprolin are extracted by homogenizing in a genogrinder with acetonitrile/water and formic acid three times. Extracts are combined and mixed and 0.5 mL aliquots are diluted with 2 mL of methanol and 4.5 mL of 1% formic acid in water. Final determination is by high performance liquid

chromatography with triple quadrupole mass spectrometric detection (LC-MS/MS), monitoring for the primary transition (m/z 540.2 – 500.2) and the confirmatory transition (m/z 540.2 – 163.2).

Analytical method DuPont-30422 Supplement No. 1 was validated in honey.

HPLC-MS/MS Conditions

HPLC system: Agilent 1100 LC system
Detector: AB Sciex 5000 Triple Quad Mass Spectrometer
Column: ACE 3 C18-PFP, 150 mm x 3.0 mm
Mobile phase: A: 0.05% aqueous Formic Acid
B: 0.01% Formic Acid in Methanol

Time	% A	% B	Gradient
0.00	40	60	
0.50	40	60	
4.50	1	99	
5.50	1	99	
6.00	40	60	
8.00	40	60	

Flow rate: 500 µL/minute
Column oven temperature 40°C
Injection volume: 20 µL
Retention time: Oxathiapirolin (SYN546539): approx. 5 min

Ionisation mode: Positive
Ionspray voltage: 4500 V

Source and detection parameters for MS/MS experiments:

Compound	Parent m/z	CE (V)	DP (V)	CXP (V)	
Oxathiapirolin (SYN546539)	540.2 → 500.2 540.2 → 163.2	34 60	81 81	36 16	Quantification Confirmation

CE: Collision energy; CXP: Collision cell exit potential ; DP : Declustering Potential

Results and discussions

The analytical method was validated in honey, fortified with oxathiapirolin.
The recoveries obtained for oxathiapirolin are detailed in the table below.

Table A 48: Recovery results from method validation of oxathiapirolin using the analytical method

Matrix	Analyte	Fortification (mg/kg)	Number of analyses (n)	Mean Recovery (%)	RSD (%)	Recovery Range (%)
Primary transition m/z 540.2 – 500.2						
Honey	Oxathiapirolin	0.01	5	102	5.1	95 – 108
		0.1	5	102	2.5	98 – 105
		Overall	10	102	3.8	95 – 108
Confirmatory transition m/z 540.2 – 163.2						
Honey	Oxathiapirolin	0.01	5	104	3.6	99 – 108

Matrix	Analyte	Fortification (mg/kg)	Number of analyses (n)	Mean Recovery (%)	RSD (%)	Recovery Range (%)
		0.1	5	104	3.4	99 – 108
		Overall	10	104	3.3	99 – 108

Specificity

LC-MS/MS with two transitions is considered to be a highly specific detection technique and therefore according to EU guidance (SANTE/2020/12830, Rev.1, 24/02/2021) no further confirmatory technique is required. The method includes two MS/MS transitions, both of which have been validated. No significant interferences arising from the honey matrix, the labware, reagents or solvents have been observed at the retention times of interest.

Linearity

The linearity of the LC-MS/MS detector was tested using standard solutions (0.02 ng/mL to 2 ng/mL). Matrix-matched linearity was used for both MS/MS transitions. Standards at seven different concentrations were injected and the signal area plotted against concentration for all calibration points. Straight lines with correlation coefficients all ≥ 0.995 were obtained for oxathiapiprolin.

The linear range is equivalent to 30% LOQ to 2800% LOQ (0.003 mg/kg to 0.28 mg/kg).

The detector response was linear. Linear equations and correlation coefficients are reported for the following matrices:

Honey

Quantification – $y = 47346x - 119$ ($r^2 = 0.9984$)

Confirmation – $y = 18599.3x - 57.8$ ($r^2 = 0.9981$)

Accuracy

Fortified samples were analysed in quintuplet at the limit of quantification (LOQ, 0.01 mg/kg) and at ten times the LOQ (0.1 mg/kg). Acceptable mean recoveries of between 70% and 110% were found for both transitions on the honey matrix tested and therefore according to EU guidance (SANTE/2020/12830, Rev.1, 24/02/2021) demonstrate the method has satisfactory accuracy.

Repeatability

The relative standard deviations (RSDs) of oxathiapiprolin recoveries at each fortification level and overall during method validation were $<20\%$ and therefore according to the EU guidance (SANTE/2020/12830, Rev.1, 24/02/2021) demonstrate the method has satisfactory repeatability.

Limit of Quantification

The limit of quantification for oxathiapiprolin residues in honey matrix using method DuPont-30422 Supplement No. 1 was established at 0.01 mg/kg. No interfering peaks around the retention time of oxathiapiprolin were found in any of the control samples at levels above 30% of the limit of quantification.

Matrix Effects

No significant matrix effects were observed in the honey matrix tested during method validation however matrix-matched linearity standards were used for quantification.

Stability of Final Extracts

The stability of sample extracts fortified with oxathiapiprolin at the LOQ level was checked after a storage period of 6 days in a refrigerator at 2-8°C against freshly prepared calibration standards. The results proved that the oxathiapiprolin residues in the stored fortified samples were stable. The mean recovery values at the LOQ level were between 70% and 110%, with a RSD of $\leq 20\%$ and within $\pm 20\%$ of the initial values when re-analysed.

Conclusion

Analytical method DuPont-30422 Supplement No. 1 has been demonstrated to be a reliable and accurate procedure for the determination of oxathiapiprolin in honey to a limit of quantification of 0.01 mg/kg, using commercially available laboratory equipment and reagents.

A 2.2.1.5.2.2 Confirmatory method

No confirmatory method is required. LC-MS/MS with two transitions is considered to be a highly specific detection technique. The method includes two MS/MS transitions, both of which have been validated.

A 2.2.1.6 Description of analytical methods for the determination of residues in support of ecotoxicological studies (KCP 5.1.2.6)

A22773A

A 2.2.1.6.1 Analytical method 1 – oxathiapiprolin in bee diets and feeding solutions

A 2.2.1.6.1.1 Method validation

Comments of zRMS:	<p>The method has been evaluated in Draft Registration Report for Orondis Evo (Central Zone, zRMS-PL, July 2022).</p> <p>Below is a copied assessment:</p> <p>The method ECO_052_03A has been successfully validated for determination of oxathiapiprolin in honey bee larvae diets (royal jelly/ASS (50/50 w/w)) and adult honey bee feeding solutions (50% w/v sucrose containing 0.1% xanthan).</p> <p>The limit of quantification has been set at 0.009 mg/kg.</p> <p>The mean recovery values at the LOQ level were between 70% and 110%, with a RSD of ≤ 20%.</p> <p>This method satisfies the EC Guidance Document SANCO/3029/99 rev. 4.</p> <p>The method is acceptable.</p>
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Reference: KCP 5.1.2.6

Report: Oxathiapiprolin - Analytical Method ECO_052_03A and Validation for the Determination of Oxathiapiprolin in Honey Bee Larvae Diets and Adult Honey Bee Feeding Solutions, Lünsmann V., 2020, Report No. 20 35 CRB 0103, Syngenta File No. VV-884296

Guideline(s): EPA OCSPP 850.6100
EC SANCO/3029/99 rev 4

Deviations: No

GLP: Yes

Acceptability: Yes

Materials

Test Material	Oxathiapiprolin
Lot/Batch #:	953157
Purity (%):	96.7% w/w
IUPAC name:	1-(4-(4-((5 <i>RS</i>)-5-(2,6-difluorophenyl)-4,5-dihydro-1,2-oxazol-3-yl)-1,3-thiazol-2-yl)-1-piperidyl)-2-(5-methyl-3-(trifluoromethyl)-1 <i>H</i> -pyrazol-1-yl)ethanone
CAS number:	1003318-67-9

Study Design and Methods

Test facility: BioChem agrar, Labor für biologische und chemische Analytik GmbH, Kupferstr. 6, 04827 Machern OT Gerichshain, Germany
Study start date: 15 June 2020

Study end date: 18 June 2020

Homogenised sub-samples of royal jelly/ASS and 50% sucrose samples were fortified with standard solutions of oxathiapiprolin. Five samples of each matrix were fortified at the limit of quantification (LOQ; 0.0090 mg/kg) and five at the highest fortification level (100.3 mg/kg). The fortified samples were analysed alongside untreated control samples.

Principle of the Method

Royal jelly/ASS and 50% sucrose solution samples with concentrations are extracted by solid phase extraction (SPE) prior to quantification by LC-MS/MS, monitoring for two transitions (primary transition $m/z = 540.2 \rightarrow 500.1$ and confirmatory transition $m/z = 540.2 \rightarrow 163$). The limit of quantification (LOQ) of the method was 0.0090 mg/kg.

HPLC-MS/MS Conditions

HPLC-System: Agilent 1200

Pump: G1312B

Degasser: G4225A

Column compartment: G1316C

Multisampler: G7167A

Mass spectrometer: Agilent 6470 Triple Quadropole

Vacuum pump: Edwards, GI969-80230

Gas supply: Nitrogen, in-house; Parker Balston N2-22

Column: Ace Excel Super C₁₈ (100 x 2.1mm, 3µm; Article No.: EXL-1111-1002U)

Column oven temperature: 35 °C

Injection volume: 2 µL

Stop time: 6.5 min (+ 3 min post time)

Injection protocol: Re-analyse calibration standards after 5 -10 sample injections

Mobile phase: A: Ultrapure water containing 0.1 % formic acid and 5 mM ammonium formate

B: Methanol containing 0.1% formic acid

Mobile Phase Composition

Time (min)	% solvent A	% solvent B	Flow rate (mL/min)
0.0	60	40	0.35
5.0	0	100	
6.5	0	100	
6.51	60	40	
9.51	60	40	

Mass Spectrometer Conditions for Oxathiapiprolin

Interface: Agilent Jet Stream

Polarity: Positive

Gas flow (L/min): Nitrogen set at 8

Gas temperature: 320 °C

Capillary voltage: 3500 V

Collision gas setting (CAD): Nitrogen

Scan type: MRM

MRM Conditions

	Oxathiapiprolin primary transition	Oxathiapiprolin confirmatory transition
Precursor ion Q1 m/z	540.2	540.2
Product ion Q3 m/z	500.1	163
Dwell time (ms)	200	200
Resolution Q1	Unit	Unit

Resolution Q3	Unit	Unit
Fragmentor (V)	134	134
Cell accelerator voltage (V)	4	4
Collision energy (V)	29	50

Recovery data

Recovery and precision data of oxathiapiprolin obtained from honey bee larvae diets and adult honey bee feeding solutions at each fortification level using method ECO_052_03A are presented in the table below.

Table A 49: Accuracy and precision results from validation of ECO_052_03A for oxathiapiprolin in Royal jelly/ASS

Matrix	Fortification Level (mg/kg)	Accuracy (%)**	Number of Analysis (n)	Mean Accuracy (%)	RSD (%)	Accuracy Range (%)
Royal jelly/ASS	Primary Transition $m/z = 540.2 \rightarrow 500.1$					
	0.0090*	93, 91, 88, 90, 93	5	91	2.3	88-93
	100.3	94, 99, 99, 103, 101	5	99	3.3	94-103
	Overall	-	10	95	5	88-103
Royal jelly/ASS	Confirmatory Transition $m/z = 540.2 \rightarrow 163$					
	0.0090*	98, 95, 89, 95, 87	5	93	4.9	87-98
	100.3	95, 100, 100, 103, 102	5	100	3.2	95-103
	Overall	-	10	96	5	87-103

Table A 50: Accuracy and precision results from validation of ECO_052_03A for oxathiapiprolin in 50% w/v sucrose solution

Matrix	Fortification Level (mg/kg)	Accuracy (%)	Number of Analysis (n)	Mean Accuracy (%)	RSD (%)	Accuracy Range (%)
50% w/v sucrose	Primary Transition $m/z = 540.2 \rightarrow 500.1$					
	0.0090*	89, 87, 89, 84, 83	5	86	3.2	83-89
	100.3	95, 97, 98, 99, 98	5	98	1.5	95-99
	Overall	-	10	92	7	83-99
50% w/v sucrose	Confirmatory Transition $m/z = 540.2 \rightarrow 163$					
	0.0090*	83, 86, 87, 83, 81	5	84	2.7	81-87
	100.3	95, 97, 99, 99, 98	5	98	1.5	95-99
	Overall	-	10	91	8	81-99

*Limit of quantitation, defined by the lowest validated fortification level

Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ

Table A 51: Characteristics of the analytical method used for the quantification of oxathiapiprolin in royal jelly/ASS and 50% w/v sucrose solution

Analyte	Oxathiapiprolin
Equipment/ Chromatographic method	HPLC-MS/MS
Accuracy/ Precision (repeatability)	Fortified samples were analysed in quintuplet at the limit of quantification (LOQ) of 0.0090 mg/kg and 100.3 mg/kg fortification level. Acceptable mean accuracy values of between 70 % and 110 % were found in water and therefore according to EU guidance demonstrate the method has satisfactory accuracy. The overall recoveries were calculated at 95% (primary transition) and 96% (confirmatory transition) for the royal jelly/ASS samples and at 92% (primary transition) and 91% (confirmatory transition) for 50% w/v sucrose solution.
Precision (reproducibility)	The relative standard deviations (RSDs) of oxathiapiprolin recovery values at each fortification level and overall during method validation were <20 % and therefore according to the EU guidance demonstrate the method has satisfactory repeatability.
Specificity	LC-MS/MS provides high specificity for the analysis and detection of oxathiapiprolin for the purpose of ecotoxicity studies i.e. clean, well described test matrix analysing a pre-defined quantity of test item. No significant interferences from the sample matrix, the labware, reagents or solvents were detected in the LC-MS/MS chromatograms at the retention time corresponding to oxathiapiprolin in any of the control samples tested.
Assessment of matrix effects is presented	No significant matrix effects were observed for oxathiapiprolin in royal jelly/ASS and 50% w/v sucrose solution. Matrix-matched standards were used throughout the method.
Calibration/Linearity	The linearity of the LC-MS/MS detector was tested for oxathiapiprolin using standard solutions

	from to 0.054 µg/L to 77.75 µg/L. Standards at 8 different concentrations were injected and the signal area plotted against concentration for all calibration points. The equation of the line for the royal jelly/ASS samples is $y=1260.547124x + 24.098173$ (primary transition) and $y=945.987521x + 23.012326$ (confirmatory transition). For the 50% w/v sucrose samples, this was calculated at $y=1048.976068x + 29.075644$ (primary transition) and $y=785.561616x + 23584345$ (confirmatory transition). The correlation coefficient for both matrices is >0.999 .
Limit of quantification (LOQ)	The LOQ for oxathiapiprolin in royal jelly/ASS and 50% w/v sucrose solution using method ECO_052_03A was established at 0.009 mg/L. No interfering peaks around the retention time of oxathiapiprolin were found in any of the control samples at levels above 30% of the LOQ.

Conclusion

Analytical method ECO_052_03A has been demonstrated to be a reliable and accurate procedure for the determination of oxathiapiprolin in water to a limit of quantification of 0.0090 mg/kg in accordance with SANTE/2020/12830 rev.1, using commercially available laboratory equipment and reagents.

(Lünsmann V., 2020)

A 2.2.1.6.2 Analytical method 2 – oxathiapiprolin in contact and feeding solution

A 2.2.1.6.2.1 Method validation

Comments of zRMS:	An analytical method ECO_052_03B for the determination of oxathiapiprolin in bumble bee contact test solutions (0.5% v/v tritonX solution) has been successfully validated in accordance with to SANTE/2020/12830, Rev. 1. Final determination by LC-MS/MS with two transitions is considered to be highly specific. The limit of quantification (LOQ) was set to 0.010 mg/L. The mean recovery values were between 70% and 110%, with a RSD of $\leq 20\%$. This method satisfies the EC Guidance Document SANTE/2020/12830, Rev. 1, so it is acceptable.
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Reference:	KCP 5.1.2.6
Report	Oxathiapiprolin – Analytical Method ECO_052_03B and Validation for the Determination of Oxathiapiprolin in Bumble Bee Contact Test Solutions, Lünsmann, V., 2022, 21 35 CRB 0127, document No VV-948172, Authority registration No.
Guideline(s):	EPA OCSPP 850.6100 SANTE/2020/12830 Rev. 1 (2021)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials

Test Material	Oxathiapiprolin
Lot/Batch #:	953157
Purity (%):	96.7
IUPAC name:	1-(4-(4-((5RS)-5-(2,6-difluorophenyl)-4,5-dihydro-1,2-oxazol-3-yl)-1,3-thiazol-2-yl)-1-piperidyl)-2-(5-methyl-3-(trifluoromethyl)-1H-pyrazol-1-yl)ethanone
CAS number:	1003318-67-9

Study Design and Methods

Test facility: BioChem agrar GmbH/ Kupferstr. 6, 04827 Machern OT Gerichshain, Germany
Study start date: 28 October 2021
Study end date: 27 January 2022
Experimental start date: 02 November 2021

Experimental end date: 02 November 2021

Five replicates of homogenised sub-samples of matrix (0.5% v/v TritonX solution) were prepared with stock solutions of Oxathiapiprolin in acetone at the limit of quantification (LOQ; 0.01 mg/L) and five samples at a higher level (about 8033 mg/L). The fortified samples were analysed alongside untreated control samples.

Principle of the Method

Bumble bee contact solution samples (0.5% v/v TritonX solution; each 0.5 mL) were extracted with 10 mL of acetonitrile/water (50/50 v/v) prior to quantification by LC-MS/MS, monitoring for two transitions ($m/z = 540.2 \rightarrow 500.1$ and $m/z = 540.2 \rightarrow 163$). The limit of quantification (LOQ) of the method was 0.01 mg/L.

Detailed information on the analytical method can be found in appendix 3, page 54 of the report.

HPLC-MS/MS Conditions

HPLC system: Agilent 1200
Pumps: G1312B
Degasser: G4225A
Column Oven: G1316C.,
Detector: Agilent 6470 Triple Quadropole with Software Agilent Mass Hunter Version B.06.00
Multisampler: G7167A
Column: ACE Excel Super C₁₈ (75 x 2.1 mm, 3 µm; Article No.: EXL-111-7502U)
Mobile phase: A: HPLC grade water with 0.1 % formic acid and 5 mM ammonium formate
B: HPLC grade methanol with 0.1 % formic acid

Time	%A	%B	Gradient
0.0	60	40	-
5.0	0	100	Linear
6.5	0	100	-
6.51	60	40	Linear
9.51	60	40	-

Flow rate: 0.35 ml/min
Column oven temperature: 35 °C
Injection volume: 2 µL
Retention time: Oxathiapiprolin: 5.0 min
Detector: Agilent 6470

Ionisation mode: ESI
Source polarity: Positive
Gas flow (L/min): 8
Gas temperature (°C): 320
Nebulizer (psi): 60
Sheath Gas Heater (°C): 250
Capillary voltage (V): 3500
Collision gas setting (CAD): Nitrogen
Resolution Q1 and Q2: Unit
Scan Type: MRM

Source and detection parameters for MS/MS experiments:

Compound	Parent m/z	Collision energy (V)	Fragmentor	Cell accelerator voltage (V)	Fragment ions (m/z)	
Oxathiapiprolin	540.1	29 50	134 134	4 4	500.1 163	Quantification Confirmation

Quantification: Peak areas of fragment ion at $m/z = 500.1$, external standards in matrix

Confirmation: Peak areas of fragment ion at $m/z = 163$, external standards in matrix

Recovery data

Recovery and precision data of **Błąd! Nie można odnaleźć źródła odwołania.** obtained from bumble bee contact solutions at each fortification level using method ECO_052_03B are presented in the table below

Table A 52: Accuracy and precision results from validation of ECO_052_03B for Oxathiapiprolin in bumble bee contact solutions.

In Sample See Contact Solutions:						
Matrix	Fortification Level	Recovery (%)	n	Mean recovery	RSD	Range
	(mg/L)			(%)	(%)	(%)
Mass transition 540.2 → 500.1 m/z (Primary)						
0.5% v/v TritonX solution	0.0103	87, 84, 86, 88, 87	5	86	1.9	84-88
	8032.5	96, 98, 98, 98, 99	5	98	0.9	96-99
	0.000	-	2	-	-	-
	Overall		12	92	6.8	84-99
Mass transition 540.2 → 163 m/z (Confirmatory)						
0.5% v/v TritonX solution	0.0103	86, 84, 89, 90, 86	5	87	2.6	84-90
	8032.52	98, 97, 97, 98, 99	5	98	1.0	97-99
	0.000	-	2	-	-	-
	Overall		12	92	6.3	84-99

* Limit of quantitation, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ

Table A 53: Characteristics of the analytical method used for the quantification of Oxathiapiprolin in bumble bee contact solutions

Analyte	Oxathiapiprolin
Equipment/ Chromatographic method	HPLC-MS/MS
Accuracy/ Precision (repeatability)	84-99% recovery across all matrices and both transitions. Fortified samples were analysed in quintuplet at the limit of quantification (LOQ) of 0.010 mg/L, and at 8033 mg/L. Acceptable mean accuracy values of between 70 % and 120 % were found in all matrices and therefore according to EU guidance demonstrate the method has satisfactory accuracy.
Precision (reproducibility)	The relative standard deviations (RSDs) of Oxathiapiprolin recovery values at each fortification level and overall during method validation were <20% and therefore according to the EU guidance (see guidance section of this summary) demonstrate the method has satisfactory repeatability.
Specificity	No peaks in controls above 30% of LOQ. LC-MS/MS provides high specificity for the analysis and detection of Oxathiapiprolin for the purpose of ecotoxicity studies i.e. clean, well described test matrices analysing a pre-defined quantity of test item. No significant interferences from the sample matrix, the labware, reagents or solvents were detected in the LC-MS/MS chromatograms at the retention time corresponding to Oxathiapiprolin in any of the control samples tested.
Confirmatory method	Two LC-MS/MS mass transitions were used to monitor Oxathiapiprolin, and therefore the method achieves a high level of specificity.
Assessment of matrix effects is presented	Matrix effects were not significant (< ±20%) and were 6.13% for the quantifier and 4.59% for the qualifier transition. Matrix-matched standards are routinely used. No significant matrix effects were observed for Oxathiapiprolin in bumble bee contact test solutions during the method validation. Matrix-matched standards were used throughout the method.

Calibration /Linearity	<p>Calibration was performed with 8 levels in duplicate.</p> <p>The calibration range was from 0.062 to 6.19 µg/L, corresponding to 0.0026 to 10834 mg/L (applying the total dilution factor of the LOQ (=42) for the lower calibration level and the total dilution factor of the high validation level (=1 750 000) for the upper calibration end)</p> <p>The linear range was from 25% of the LOQ to 135% above the highest fortification level measured, or similar.</p> <p>The linearity of the LC-MS/MS detector was tested for Oxathiapiprolin using matrix-matched standard solutions from 0.062 to 6.19 µg/L. This range is equivalent to 0.0026 to 10834 mg/L in samples. The linearity of the given calibration functions was confirmed by a residual analysis (no trend visible). Standards at eight different concentrations were injected in duplicate and the signal area plotted against concentration for all calibration points. A correlation coefficient of >0.999 was obtained for Oxathiapiprolin (primary transition:540.2 → 500.1, confirmatory transition:540.2 → 163)</p> <p>Matrix: 0.5% v/v TritonX solution</p> <p>Quantification - $y = 2248.27145 x + 10.286$ ($r^2 = 0.9999$)</p> <p>Confirmation - $y = 1322.2854 x + 58.9615$ ($r^2 = 0.9999$)</p>
Limit of quantification (LOQ)	<p>Limit of quantification representing the lowest validated level with acceptable recovery and precision</p> <p>The LOQ for Oxathiapiprolin in honey bee matrices using method ECO_052_03B was established at 0.010 mg/L. No interfering peaks around the retention time of Oxathiapiprolin were found in any of the control samples at levels above 30% of the LOQ.</p>
Limit of detection (LOD)	The LOD for Oxathiapiprolin in bumble bee contact test solutions using method ECO_052_03B was established at 0.0026 mg/L
Stability of extracts	Stability of final extracts was shown in method ECO_052_03A for a period of three days for extracts of Oxathiapiprolin in acetonitrile/water 50/50 v/v stored in the refrigerator.
Stability of analytical standard	Analytical standards were prepared freshly for each measurement. Due to the same composition, matrix-matched standards will have the same stability as the final extracts (seven days when stored in the refrigerator).
Extractability	Since no incurred residues were analysed, the extraction efficiency corresponds to the recovery of fortified samples and was 84-99%.

Conclusion:

Analytical method ECO_052_03B has been demonstrated to be a reliable and accurate procedure for the determination of Oxathiapiprolin in honey bee matrices with a limit of quantification (LOQ) of 0.010 mg/L in accordance with to SANTE/2020/12830, Rev. 1, using commercially available laboratory equipment and reagents.

(V. Lünsmann, 2022)

A 2.2.1.6.2.2 Confirmatory method

No confirmatory method is required.

Oxathiapiprolin

A 2.2.1.6.3 Analytical method 2 – oxathiapiprolin in test solution

A 2.2.1.6.3.1 Method validation

Comments of zRMS:	<p>The method has been evaluated in Draft Registration Report for Orondis Evo (Central Zone, zRMS-PL, July 2022).</p> <p>Below is a copied assessment:</p> <p>This method was successfully validated for the determination of oxathiapiprolin in test solution in accordance with SANCO/3029/99 rev.4.</p> <p>LOQ = 0.6 g/L</p> <p>The mean recovery values were between 70% and 110%, with a RSD of ≤ 20%.</p> <p>The study is acceptable.</p>
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Reference: KCP 5.1.2.6

Report Oxathiapiprolin (DPX-QGU42) 100 g/L OD: Chronic oral toxicity to the honey bee, *Apis mellifera* L. (Hymenoptera, Apidae), Tänzler V., 2015, Report No. 94441136, Corteva Study No. DuPont-41989 (Syngenta have

access)

Document No. VV-910995

Guideline(s): SANCO/3029/99 rev 4

Deviations: No

GLP: Yes

Acceptability: Yes

Materials and methods

Test samples were analysed based upon the analytical methodology provided by DuPont and validated as part of the current study. An aliquot of each sample was diluted with methanol/pure water (50/50 v/v) while solutions were stirring. The final samples were analysed using high performance liquid chromatography with ultraviolet detection (HPLC/UV).

Results and discussions

Summaries of the results for oxathiapiprolin (DPX-QGU42) are presented in the tables below.

Recovery values at each fortification concentration were within the acceptance range (recovery 70-110%; RSD /C.V. ≤ 20%).

Table A 54: Recovery results from method validation of oxathiapiprolin using the analytical method

Matrix	Analyte	Fortification level (g test item/L)	Mean Recovery (%)	RSD (%)	Mean Recovery (%)	RSD (%)	n	Comments
Test Solution	DPX-QGU42	0.6	108	8	94	17	5	N/A
		1.7	80	8			5	N/A

Specificity of method: The HPLC-UV method is specific in the retention time of known standards (figure 2) and fortified samples (figure 4). As shown in the matrix blank (figure 3, sucrose solution) there is a small peak in the area of the retention time but as demonstrated in the Analytical Results (Table 16) this peak (0.349 mg a.i./L undiluted) is less than 11% of the LOQ (3.266 mg a.i./L 20X diluted). The combination of all above demonstrates the specificity of the method. Final extract stability can be leveraged from the CEMR-9533 honey study as both extracts are methanol and water. Study CEMR-9533 showed final extract stability for 6 days when refrigerated.

Table A 55: Characteristics for the analytical method used for validation of oxathiapiprolin residues in test solution

	Oxathiapiprolin
Specificity	250 nm Retention time: 6.2 minutes
Calibration (type, number of data points)	linear $r \geq 1.000$ 9 data points
Calibration range	Concentration range of 0.482 to 24.089 mg a.s./L
Limit of quantification	LOQ = 0.6 g/L LOD = 0.2 mg a.s./L

Conclusion

This method was successfully validated for the determination of DPX-QGU42 in test solution in accordance with SANCO/3029/99 rev.4 (European Commission, 2000).

(Tänzler V., 2015)

A 2.2.1.6.3.2 Confirmatory method

No confirmatory method is required.

Oxathiapiprolin

A 2.2.1.6.4 Analytical method 3 – oxathiapiprolin in larval diet and acetone

A 2.2.1.6.4.1 Method validation

Comments of zRMS:	<p>The method has been evaluated in Draft Registration Report for Orondis Evo (Central Zone, zRMS-PL, July 2022).</p> <p>Below is a copied assessment:</p> <p>This method was successfully validated for the determination of oxathiapiprolin in larval diet sample in accordance with SANCO/3029/99 rev.4.</p> <p>LOQ = 0.5 mg/kg for larval diet (Diet C)</p> <p>LOQ =150 mg/L for acetone</p> <p>The mean recovery values were between 70% and 110%, with a RSD of $\leq 20\%$.</p> <p>The study is acceptable.</p>
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Reference: KCP 5.1.2.6

Report Oxathiapiprolin (DPX-QGU42) technical: Honey bee (*Apis mellifera* L.) 22 day larval toxicity test (repeated exposure), Oberrauch S., 2017, Report No. S17-01639, Corteva Study No. DuPont-48606 (Syngenta have access)
Document No. VV-911004

Guideline(s): SANCO/3029/99 rev 4

Deviations: No

GLP: Yes

Acceptability: Yes

Materials and methods

Matrix blank extract:

Matrix blank extract was prepared by fortification of untreated larval diet (Diet C was used). About 500 mg untreated larval diet were weighed into a 15 mL plastic tube. About 10 mL acetonitrile/water (8:2, v/v) were added and the samples were homogenized. A constant ratio of sample weight and extraction solvent of 500 mg / 10 mL was used for extraction. The samples were homogenized for 10 min on a flatbed shaker. The samples were then centrifuged at 4000 rpm for 5 min. After phase separation 1 mL respectively 5 mL of the organic phase was diluted with 9 mL respectively 45 mL acetonitrile/water (8:2, v/v) by a factor f1.

Analysis of larval diet samples

About 500 mg larval diet sample were weighed into a 15 mL plastic tube. About 10 mL acetonitrile/water (8:2, v/v) were added and the samples were homogenized. A constant ratio of sample amount and extraction solvent of 500 mg / 10 mL was used for extraction. The samples were homogenized for 10 min on a flatbed shaker. The samples were then centrifuged at 4000 rpm for 5 min. After phase separation 1 mL of the organic phase was diluted with 9 mL acetonitrile/water (8:2, v/v) (f1). If necessary the samples were further diluted (f2) with larval diet blank extract prior to analysis by HPLC-MS/MS.

For a few R-samples the complete original sample vessel was used for sample preparation: The sample vessels were weighed and the rest of the sample were each transferred into 50 mL plastic tubs. The original sample vessels were rinsed with 4 x 2 mL water, 2 x 2.5 mL acetonitrile/water (8:2, v/v) and 2 x 2 mL acetonitrile. The rinsing solvents were combined in the new sample vessel and 28 mL acetonitrile were added. After drying the original empty vessels were weighed again.

The samples were homogenized on a Vortex-Mixer and shaken for 10 minutes on a flatbed shaker. Samples were centrifuged at 4000 rpm for 5 minutes. The samples were further diluted with acetonitrile/water (8:2, v/v) by a factor f1. If necessary, the further dilution steps f2 were performed with larval diet blank extract before analysis.

Analysis of larval diet recovery samples:

Recovery samples were prepared by fortification of larval diet (Diet C was used) with the test item. About 500 mg untreated larval diet were weighed into a 15 mL plastic tube. The necessary spiking volume, adjusted to the sample weight of about 500 mg, was added and about 10 mL acetonitrile/water (8:2, v/v) were added and the recovery samples were homogenized. A constant ratio of sample weight and extraction solvent of 500 mg / 10 mL was used for extraction. The recovery samples were homogenized for 10 min on a flatbed shaker and centrifuged at 4000 rpm for 5 min. After phase separation 1 mL of the organic phase was diluted with 9 mL acetonitrile/water (8:2, v/v) by a factor f1. If necessary the recovery samples were further diluted (f2) with larval diet blank extract prior to analysis by HPLC-MS/MS.

Analysis of acetone samples:

After sampling, the acetone samples were stored deep-frozen (≤ -18 °C) until analysis. At the analytical laboratory, the samples were thawed to ambient temperature shaken well using a Vortex-Mixer and ultrasonicated for 30 seconds. The samples were then diluted with acetonitrile/water (8:2, v/v). If necessary the samples were further diluted (f2) with acetonitrile/water (8:2, v/v) prior to analysis by HPLC-MS/MS.

Analysis of solvent recovery samples:

Recovery samples were prepared by fortification of untreated samples of acetone with the test item. The samples were then diluted with acetonitrile/water (8:2, v/v). If necessary the samples were further diluted (f2) with acetonitrile/water (8:2, v/v) prior to analysis by HPLC-MS/MS.

Final extract stability can be leveraged from study 220385 as both the final extracts are in acetonitrile/water. Study 220385 has shown stability for 9 days when refrigerated.

Results and discussions

Mean recovery values at each fortification concentration were within the acceptance range (mean recovery 70-110%; RSD \leq 20%). The results obtained are summarised in the following tables.

Table A 56: Recovery results from method validation of oxathiapiprolin (*m/z* 540/522) using the analytical method

Matrix	Analyte	Fortification level (mg/kg)	Mean recovery (%)	RSD (%)	n
Larval diet (diet C)	Oxathiapiprolin	0.5	87	3	5
		400	103	6	5
Matrix	Analyte	Fortification level (mg/L)	Mean recovery (%)	RSD (%)	n
Acetone	Oxathiapiprolin	150	101	2	5
		82000	93	2	5

Table A 57: Characteristics for the analytical method used for validation of oxathiapiprolin residues in larval diet (Diet C) and acetone

	Oxathiapiprolin	
	Larval diet (Diet C)	Acetone
Specificity	<i>m/z</i> 540/522 <i>m/z</i> 540/500 <i>m/z</i> 540/350 blank value < 30% LOQ	<i>m/z</i> 540/522 <i>m/z</i> 540/500 <i>m/z</i> 540/350 blank value < 30% LOQ

	Oxathiapiprolin	
	Larval diet (Diet C)	Acetone
Calibration (type, number of data points)	linear regression analysis with 1/x weighting $r \geq 0.997$ 7 data points	linear regression analysis with 1/x weighting $r \geq 0.997$ 7 data points
Calibration range	Concentration range of 0.5-15 ng/mL	Concentration range of 0.5-15 ng/mL
Limit of determination/quantification	LOQ = 0.5 mg/kg	LOQ =150 mg/L

Conclusion

This method was successfully validated for the determination of oxathiapiprolin in larval diet (Diet C) and acetone.

(Oberrauch S., 2017)

A 2.2.1.6.4.2 Confirmatory method

No confirmatory method is required.

A 2.2.1.7 Description of analytical methods for the determination of residues in support of physical and chemical properties tests (KCP 5.1.2.7)

No new or additional studies have been submitted.

A 2.2.2 Methods for post-authorization control and monitoring purposes (KCP 5.2)

A 2.2.2.1 Description of analytical methods for the determination of residues in plant matrices (KCP 5.2.1)

A 2.2.2.1.1 Analytical method 1: DuPont-30422

A 2.2.2.1.1.1 Method validation (231693)

Comments of zRMS:	<p>See point A 2.2.1.5.1.1.</p> <p>The method has been evaluated in Draft Registration Report for Orondis Evo (Central Zone, zRMS-PL, July 2022).</p> <p>Below is a copied assessment:</p> <p>The analytical residue method DuPont-30422 was successfully validated for the determination of residues of oxathiapiprolin in crop matrices (peppers, cucumbers, melon, leek, broccoli, cauliflower, cabbage, kale, brussel sprouts and hops) at a limit of quantification (LOQ) of 0.01 mg/kg.</p> <p>For all fortification levels (0.01 mg/kg, 0.10 mg/kg), acceptable mean recoveries in the range of 70 - 110 % with a relative standard deviation (RSD) of ≤ 20 % were found for oxathiapiprolin for both the quantification and confirmation mass transitions, in all matrices.</p> <p>The method has therefore been successfully validated according to the EU guidelines SANCO/3029/99 rev.4.</p> <p>The study is acceptable.</p>
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Reference: KCP 5.2.1 (and KCP 5.1.2.5)

Report Oxathiapiprolin (SYN546539) – Validation of the Analytical method DuPont-30422 for the Determination of Residues of Oxathiapiprolin in Crop Matrices by LC-MS/MS

Donald C., Gibson R. (2020)
Report No. 231693, Syngenta File No. VV-870136

Guideline(s):

Yes

Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/3029/99 rev. 4, 11/07/2000).

OECD Guidance Document on Pesticide Residue Analytical Methods ENV/JM/MONO(2007)17.

Regulation (EC) No 1107/2009 of the European Parliament and of the council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC.

Residue Chemistry Test Guidelines OPPTS 860.1340 Residue Analytical Method, EPA 712-C-96-174, August 1996.

Deviations:

No

GLP:

Yes

Acceptability:

Yes

Analytical method DuPont-30422 is also used for the generation of pre-authorisation data. The validation of this method (231693) is summarised in A 2.1.1.5 (KCP 5.1.2.5).

A 2.2.2.1.1.2 Independent laboratory validation

No new or additional studies have been submitted.

A 2.2.2.1.1.3 Confirmatory method

Please refer to A 2.2.1.5.1 (KCP 5.1.2.5).

A 2.2.2.1.1.4 Extraction efficiency

No new or additional studies have been submitted.

A 2.2.2.1.2 Analytical method 2: DuPont-30422 (Supplement 1)

A 2.2.2.1.2.1 Method validation (CEMR-9533)

Comments of zRMS:	<p>See point A 2.2.1.5.2.1.</p> <p>The method has been evaluated in Draft Registration Report for Orondis Evo (Central Zone, zRMS-PL, July 2022).</p> <p>Below is a copied assessment:</p> <p>The method DuPont-30422 – Supplement No.1 has been successfully validated for determination of oxathiapiprolin residues in honey.</p> <p>The specificity, linearity, accuracy, precision and repeatability was demonstrated for both the primary and confirmatory transitions by taking one reagent blank, two control samples, five samples fortified at the LOQ and five at 10 × LOQ through the method using LC-MS/MS detection. No interferences were present at or above 30% of the LOQ in any of the control samples and reagent blanks.</p> <p>LOQ=0.01 mg/kg</p> <p>The mean recovery values at the LOQ level were between 70% and 110%, with a RSD of ≤ 20%.</p> <p>The analytical method DuPont-30422 Supplement No.1 was successfully validated for the determination of oxathiapiprolin in honey according to the EU guideline SANCO/3029/99 rev.4.</p> <p>The sugar content of the honey samples was assessed by performing BRIX analysis.</p> <p>The method is acceptable.</p>
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Reference: KCP 5.2.1 (and KCP 5.1.2.5)

Report Oxathiapiprolin – Honey Residue Study on Spring Oilseed Rape in Northern and Southern Europe in 2020
Ford K. (2020)
Report No. CEMR-9533, Syngenta File No. VV-885771

Guideline(s): SANCO/825/00 rev. 8.1 (2010)SANCO/3029/99 rev. 4 (2000)
ENV/JM/MONO(2007)17
EPA OPPTS 860.1340 (1996)
SANTE/11956/2016 rev. 9

Deviations: No

GLP: Yes

Acceptability: Yes

Analytical method DuPont-30422 (Supplement 1) is also used for the generation of pre-authorisation data. The validation of this method for honey (CEMR-9533) is summarised in A 2.1.1.5 (KCP 5.1.2.5).

A 2.2.2.1.2.2 Independent laboratory validation

No new or additional studies have been submitted.

A 2.2.2.1.2.3 Confirmatory method

Please refer to A 2.1.1.5 (KCP 5.1.2.5).

A 2.2.2.1.2.4 Extraction efficiency

No new or additional studies have been submitted.

A 2.2.2.2 Description of analytical methods for the determination of residues in animal matrices (KCP 5.2.2)

No new or additional studies have been submitted.

A 2.2.2.3 Description of Methods for the Analysis of Body Fluids and Tissues (KCP

5.2.3)

A 2.2.2.3.1.1 Method validation

Comments of zRMS:	<p>The method has been evaluated in Draft Registration Report for Orondis Evo (Central Zone, zRMS-PL, July 2022).</p> <p>Below is a copied assessment:</p> <p>The method has been validated for the determination of oxathiapiprolin in body fluids (in urine) in accordance to guidance document SANTE/2020/12830, rev.1 for risk assessment and/or monitoring.</p> <p>The limit of quantification is 0.01 mg/L.</p> <p>All mean recovery values at fortification levels of 0.01 mg/L for two mass transitions are within 70% - 120% with relative standard deviations \leq 20% and thereby comply with the standard acceptance criteria of the guidance document SANTE/2020/12830, rev. 1.</p> <p>The method is acceptable.</p>
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Reference:	KCP 5.2.1
Report	<p>Method Validation of Oxathiapiprolin in Body Fluids</p> <p>xxxxxx 2022</p> <p>Report No. S22-02422, Corteva Study No. 220385</p>
Guideline(s):	SANTE/2020/12830 rev. 1
Deviations:	No, SANTE/2020/12830 rev.1
GLP:	Yes
Acceptability:	Yes

Materials and Methods

Test Item(s)

Test item (common name):	Oxathiapiprolin
Purity:	98.9 %
Description (physical state):	powder
Lot/batch no.:	E105317-115 (TSN315458)

Method Scope

This method is applicable for the quantitative determination of residues of Oxathiapiprolin in body fluids. The method was validated in urine at LOQ level of 0.01 mg/L with a validated limit of quantitation of 0.01 mg/L.

Method Principle

Residues of Oxathiapiprolin are extracted from samples by shaking with acetonitrile. An aliquot of the supernatant is diluted with water. The final sample is analyzed for Oxathiapiprolin by liquid chromatography coupled with positive-ion electrospray tandem mass spectrometry (LC-MS/MS).

Linearity

For each analyte, the linearity of detector response was evaluated using solvent standard solutions. Calibration curves were calculated by linear regression analysis with 1/x weighting, coefficients of determination (R^2) obtained for both transitions were \geq 0.999. Eight non-matrix matched calibration standards were injected over a concentration range of 0.09 – 9.0 ng/mL (equivalent to 0.003 – 0.3 mg/L). The lowest calibration standard was equivalent to 30% of the LOQ and the highest standard was 30% above the highest fortification level in final sample extracts. The calibration range covered a maximum of two orders of magnitude, as required by SANTE/2020/12830 rev. 1.

Selectivity

The LC-MS/MS method is highly selective for both the quantitation and confirmation of Oxathiapiprolin. Significant peak response ($>30\%$ of the LOQ peak area) is not observed in reagent blank and extracts of

untreated blank control samples at the expected retention times of the analyte. Unambiguous identification is ensured by monitoring two MS/MS transitions characteristic of each analyte as follows in the table below.

Oxathiapiprolin	m/z 540/500 Q (quantitative)
Oxathiapiprolin	m/z 540/522 C (confirmatory)

Confirmation

Confirmation of the presence of Oxathiapiprolin was by comparison of retention times liquid chromatography of recovery samples with the retention times of the calibration standards as well as by monitoring two structurally characteristic MS/MS transitions for each analyte by tandem mass spectrometry. Validation data obtained using the confirmatory MS/MS transition met the same acceptance criteria as the validation data generated using the quantitative MS/MS transition, therefore demonstrating that the analyte signal of the quantitative MS/MS transition is correct and not affected by any other compound.

Limits of Detection and Quantitation

The limit of quantitation, defined as the lowest concentration of an analyte tested at which an unambiguous identification of the analyte can be proven and at which an acceptable mean recovery with an acceptable relative standard deviation is obtained, is 0.01 mg/L.

The limit of detection, defined as 30% of the LOQ, is 0.003 mg/L.

Results and Discussion

Summary of Recovery

Results obtained were within guideline requirements (mean recovery 70-120%; RSD ≤ 20%). The two ion mass transitions could be used interchangeably for quantification and confirmation. The results obtained are summarized in the following tables.

Table A 58: Summary of quantitative recovery of Oxathiapiprolin (m/z 540/500)

Matrix group	Matrix	Fortification level	Recovery (%)		SD	RSD	n
		mg/L	mean	range	(%)	(%)	
Body fluids	Urine	0.01	98	94 – 103	3.4	3.5	5

Table A 59: Summary of confirmatory recovery of Oxathiapiprolin (m/z 500/522)

Matrix group	Matrix	Fortification level	Recovery (%)		SD	RSD	n
		mg/L	mean	range	(%)	(%)	
Body fluids	Urine	0.01	99	95 – 104	3.2	3.2	5

Working Solution Stability

Stock solutions of Oxathiapiprolin prepared in acetonitrile were tested after 361 days of storage at cooled (typically 1 °C-10 °C) and were found to be stable.

Calibration standard solutions of Oxathiapiprolin prepared in acetonitrile/water (1:5, v/v) were tested after 12 days of storage at typically 1 °C-10 °C and were found to be stable.

Sample Extract Stability

Sample extracts of Oxathiapiprolin in acetonitrile/water were tested after 9 days of storage at cooled (typically 1 °C-10 °C) and were found to be stable.

Matrix Effects

Matrix effects were evaluated by comparing the response of the analyte fortified in a control extract after processing to the response of the analyte fortified in neat solvent. The results demonstrate that matrix effects are within ±20%.

Matrix matched standards were used for quantification for this study.

Conclusion

Method is acceptable based on current guidelines: EPA Residue Chemistry Test Guidelines OPPTS 860.1340 and SANTE/2020/12830 Rev.1, as well as PMRA Regulatory Directive Dir98 02.

A 2.2.2.4 Description of Methods for the Analysis of Soil (KCP 5.2.4)

No new or additional studies have been submitted.

A 2.2.2.5 Description of Methods for the Analysis of Water (KCP 5.2.5)

No new or additional studies have been submitted.

A 2.2.2.6 Description of Methods for the Analysis of Air (KCP 5.2.6)

No new or additional studies have been submitted.

A 2.2.2.7 Other Studies/ Information

No new or additional studies have been submitted.