

FINAL REGISTRATION REPORT

Part B

Section 5

Analytical Methods

Detailed summary of the risk assessment

Product code: GLOB2021dF

Product name: PIRLANKO

Chemical active substances:

Prothioconazole, 156 g/L

Boscalid, 156 g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(authorization)

Applicant: GLOBACHEM NV

Submission date: November 2023

zRMS Assessment : 31/01/2025

Following commenting period: 15/05/2025

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Version history

When	What
July 2024	Update after comments
Sep 2024	Update after comments
January 2025	zRMS evaluation
May 2025	Following commenting period
October 2024	Update after zRMS question
November 2025	zRMS evaluation after completion by the applicant
November 2025	zRMS Auto-correction
January 2026	Following II commenting period
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Table of Contents

5	Analytical methods.....	5
5.1	Conclusion and summary of assessment.....	5
5.2	Methods used for the generation of pre-authorization data (KCP 5.1).....	6
5.2.1	Analysis of the plant protection product (KCP 5.1.1)	6
5.2.1.1	Determination of active substance and/or variant in the plant protection product (KCP 5.1.1).....	6
5.2.1.2	Description of analytical methods for the determination of relevant impurities (KCP 5.1.1).....	10
5.2.1.3	Description of analytical methods for the determination of formulants (KCP 5.1.1)	18
5.2.1.4	Applicability of existing CIPAC methods (KCP 5.1.1).....	18
5.2.2	Methods for the determination of residues (KCP 5.1.2).....	18
5.3	Methods for post-authorization control and monitoring purposes (KCP 5.2)	20
5.3.1	Analysis of the plant protection product (KCP 5.2)	20
5.3.2	Description of analytical methods for the determination of residues boscalid (KCP 5.2).....	20
5.3.2.1	Overview of residue definitions and levels for which compliance is required	20
5.3.2.2	Description of analytical methods for the determination of residues in plant matrices (KCP 5.2).....	21
5.3.2.3	Description of analytical methods for the determination of residues in animal matrices (KCP 5.2).....	23
5.3.2.4	Description of methods for the analysis of soil (KCP 5.2).....	24
5.3.2.5	Description of methods for the analysis of water (KCP 5.2).....	25
5.3.2.6	Description of methods for the analysis of air (KCP 5.2).....	25
5.3.2.7	Description of methods for the analysis of body fluids and tissues (KCP 5.2)	26
5.3.2.8	Other studies/ information	26
5.3.3	Description of analytical methods for the determination of residues prothioconazole (KCP 5.2)	26
5.3.3.1	Overview of residue definitions and levels for which compliance is required	26
5.3.3.2	Description of analytical methods for the determination of residues in plant matrices (KCP 5.2).....	28
5.3.3.3	Description of methods for the analysis of soil (KCP 5.2).....	29
5.3.3.4	Description of methods for the analysis of water (KCP 5.2).....	29
5.3.3.5	Description of methods for the analysis of air (KCP 5.2).....	30
5.3.3.6	Description of methods for the analysis of body fluids and tissues (KCP 5.2)	30
5.3.3.7	Other studies/ information	31
Appendix 1	Lists of data considered in support of the evaluation	32
Appendix 2	Detailed evaluation of submitted analytical methods	39
A 2.1	Analytical methods for boscalid and prothioconazole.....	39

5 Analytical methods

5.1 Conclusion and summary of assessment

State whether submitted data are sufficient for evaluation. Data gaps and conditions for authorization should be listed, if appropriate.

Sufficiently sensitive and selective analytical methods are available for the active substance(s) and relevant impurities in the plant protection product.

Noticed data gap:

A validated analytical method for the relevant impurities is required (according to the guideline SAN-CO/3030/99 rev.5). ~~data gap~~. Sufficiently sensitive and selective analytical methods are available for the active substance(s) and relevant impurities in the plant protection product.

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

Noticed data gaps are:

- A validated analytical method determination of residues in honey for boscalid is missing. (primary, confirmatory, ILV) – ILV method for monitoring boscalid residues in honey must be provided at the renewal of the active substance. - data gap The required data must be submitted within one year from the date of issuance of this authorization. Failure to provide the required supplements or to obtain a positive assessment of the submitted documentation may result in a modification of the authorization conditions or revocation of the authorization.
- A validated analytical method determination of residues in honey for prothioconazole is missing. (confirmatory, ILV). ILV method for monitoring boscalid residues in honey must be provided at the renewal of the active substance. - data gap The required data must be submitted within one year from the date of issuance of this authorization. Failure to provide the required supplements or to obtain a positive assessment of the submitted documentation may result in a modification of the authorization conditions or revocation of the authorization.
- ILV method for monitoring boscalid residues in egg at the lowered MRLs must be provided at the renewal of the active substance. Data gap The required data must be submitted within one year from the date of issuance of this authorization. Failure to provide the required supplements or to obtain a positive assessment of the submitted documentation may result in a modification of the authorization conditions or revocation of the authorization.
- According to COMMISSION REGULATION (EU) No Reg. No 283/2013 and Reg. No 284/2013, a fully validated analytical method for monitoring boscalid residues in body fluids muscle, fat, milk and egg with LOQ at 0.01 mg/kg is required. ILV method for monitoring boscalid residues in body fluids muscle, fat, milk and egg must be provided at the renewal of the active substance. The required data must be submitted within one year from the date of issuance of this authorization. Failure to provide the required supplements or to obtain a positive assessment of the submitted documentation may result in a modification of the authorization conditions or revocation of the authorization.

- According to Reg. No 284/2013 an ILV method for monitoring boscalid residues in drinking water is required. ILV method for monitoring boscalid residues in drinking water must be provided at the renewal of the active substance.
- According to COMMISSION REGULATION (EU) No Reg. No 283/2013 and Reg. No 284/2013, a fully validated analytical method for monitoring prothioconazole residues in body fluids is required. Methods for the analysis of body fluids and tissues must be provided at the renewal of the active substance.
- According to Reg. No 284/2013 an ILV method for monitoring prothioconazole residues in drinking water is required. ILV method for monitoring boscalid residues in drinking water must be provided at the renewal of the active substance.
- Analytical methods (primary, confirmatory, ILV) for high water content and high acid content commodities as well as ILVs for dry and high fat content commodities are missing. Methods for the analysis of high water content and high acid content commodities must be provided at the renewal of the active substance. The required data must be submitted within one year from the date of issuance of this authorization. Failure to provide the required supplements or to obtain a positive assessment of the submitted documentation may result in a modification of the authorization conditions or revocation of the authorization.

Commodity/crop	Supported/ Not supported
Winter & spring oilseed rape	supported
Cereals	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)

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

Comments of zRMS:	Validation data (Raksha V.S., 2022) are acceptable according Sanco 3030/99 rev. 5, provided for method GLOB2021dF and suitable for the determination of active substances boscalid and prothioconazole in plant protection product GLOB2021dF.
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Reference:	KCP 5.1.1
Report	Purity analysis of GLOB2021dF, Raksha V.S., 2022, AG-0.130
Guideline(s):	Yes, Sanco 3030/99 rev. 5
Deviations:	No
GLP:	Yes

Acceptability: Yes

Summary

Purity analysis of GLOB2021dF was carried out using an in-house validated high performance liquid chromatography method as per SANCO 3030/99 rev.5.

The method validation results are summarized below:

Materials and methods

Comments of zRMS:

Reference Standard

- Boscalid reference standard, Lot No.: G1266173, Purity: 98.97%, Expiry Date: 01 Jul 2026, Source: LGC DR Ehrenstorfer (Certificate of Analysis enclosed as Appendix 1).
- Prothioconazole reference standard, Batch No.: BCCB2271, Purity: 99.9%, Expiry Date: FEB 2024, Source: Sigma Aldrich (Certificate of Analysis enclosed as Appendix 2).

Chemicals

- Formic acid Grade: AR, Batch No.: L17A/0717/0812/31, Make: SDFCL.
- Acetonitrile Grade: HPLC, Lot No.: A04361119CV, Make: Finar Limited.

Reagents

Preparation of 0.1% v/v formic acid in Milli Q water: An aliquot of 2 mL of formic acid was transferred into a 2000 mL volumetric flask containing about 800 mL of Milli Q water, dissolved and the volume was made up to the mark with Milli-Q water. The contents of the flask were sonicated for 5 minutes.

Apparatus

- Analytical balance, Make: Mettler, Model: AE 240
- High-Performance Liquid Chromatograph (HPLC) equipped with PDA, auto sampler and PC based data system: Lab solutions.
- Phenomenex Luna® C18 (2) 100Å, 250 × 4.6mm, 5µm particle size.
- General laboratory glassware (like, volumetric flask, beaker, etc.,)
- Sonicator, Make: S.V Scientific

Active Ingredient analysis

Preparation of working standard solution:

Preparation of Stock Solution of Boscalid reference standard

Accurately 0.00508 g of Boscalid reference standard was weighed into a 5 mL volumetric flask, dissolved in about 2 mL of acetonitrile, and sonicated for 2 minutes. After equilibrating to room temperature solution was diluted up to the mark with acetonitrile.

Concentration – 1016.0 µg/mL

Preparation of Stock Solution of Prothioconazole reference standard

Accurately 0.00510 g of Prothioconazole reference standard was weighed into a 5 mL volumetric flask, dissolved in about 2 mL of acetonitrile, and sonicated for 2 minutes. After equilibrating to room temperature solution was diluted up to the mark with acetonitrile.

Concentration – 1020.0 µg/mL

Aliquot of Boscalid stock solution taken (mL)	Aliquot of Prothioconazole stock solution taken (mL)	Volume made up (mL)	Conc. Of Boscalid in diluted solution (µg/mL)	Conc. Of Prothioconazole in diluted solution (µg/mL)	Identification code
1.0	1.0	10	101.6	102.0	Working Standard

Preparation of test item solutions: About 0.75 g of test item was weighed in three replications into separate 100mL volumetric flask. About 40 mL of acetonitrile was added and the flasks were placed in an ultrasonic bath for 2 min. These solutions were allowed to cool to room temperature and diluted to volume with acetonitrile. Further an aliquot of 5 mL from each of the above solution was diluted to 50 mL using acetonitrile.

HPLC Conditions

The active ingredient content in test item was determined under the chromatographic conditions given below:

Instrument	: High Performance Liquid Chromatograph equipped with PDA and PC based data system
Column	: Phenomenex Luna® C18 (2) 100Å, 250 × 4.6mm, 5µm particle size.
Mobile phase	: 0.1% Formic acid in Milli-Q Water: Acetonitrile (40: 60, % v/v)
Flow Rate	: 1.0 mL/min
Wavelength	: 254 nm
Column Temperature	: 30 °C
Injection volume	: 10 µL
Run Time	: 15 minutes

- All the parameters were maintained constant throughout the analysis.
- After every 3 to 9 sample injections and after the last sample injection, an injection of the calibration (standard) solution was made.
- Boscalid and Prothioconazole peaks in the sample and in the standard was identified by comparing the retention times with those obtained through separate injections of solutions.
- Peak area of Boscalid and Prothioconazole for each injection was recorded.
- The calibration (standard) solution peak area (injections before and after the sample injections) was averaged and used for calculating the percent active ingredient (a.i.) of Boscalid and Prothioconazole in the samples.

TABLE 1. Results of Method Validation: Detector Linearity Check and Specificity

Boscalid		Prothioconazole	
Concentration (µg/mL)	Peak Area	Concentration (µg/mL)	Peak Area
49.98	1251123	50.45	980790
49.98	1249754	50.45	985626
49.98	1249439	50.45	985558
79.97	2019033	80.72	1601922
79.97	2014746	80.72	1598772
79.97	2007856	80.72	1593290
99.96	2491228	100.90	1993026
99.96	2488023	100.90	1990707
99.96	2486890	100.90	1990164
119.95	3010013	121.08	2436059
119.95	3010272	121.08	2436213
119.95	3013503	121.08	2439491
149.94	3770729	151.35	3040178
149.94	3770739	151.35	3039694
149.94	3769112	151.35	3039240
$y = 25174.573x - 9619.658$ where, y = peak area x = concentration (µg/mL) Intercept (a) = - 9619.658 Slope (b) = 25194.573 $r = 0.9999$ Range = 49.98 to 149.94 µg/mL		$y = 20431.774x - 51517.310$ where, y = peak area x = concentration (µg/mL) Intercept (a) = - 51517.310 Slope (b) = 20431.774 $r = 0.9999$ Range = 50.45 to 151.35 µg/mL	
Specificity		Specificity	
Specific for analysis of Boscalid		Specific for analysis of Prothioconazole	

Validation - Results and discussions

Table 5.2-1: Methods suitable for the determination of active substances boscalid and prothioconazole in plant protection product GLOB2021dF

	boscalid	prothioconazole
Author(s), year	Raksha, V.S.; 2022	
Principle of method	High Performance Liquid Chromatographic analytical method	
Linearity (linear between mg/L / % range of the declared content) (correlation coefficient, expressed as r)	49.98 – 149.94 µg/mL $r : 0.9999$	50.45 – 151.35 µg/mL $r : 0.9999$
Precision – Repeatability Mean n = 5 (%RSD)	Horrat value: 0.04 Mean: 13,93	Horrat value: 0.08 Mean: 14,33
Accuracy n = 3 (% Recovery)	99.93 ± 1.34	100.28 ± 1.43
Interference/ Specificity	There was no interference observed at the retention time of the analyte (a.i.) indicating the specificity of the analytical method	

	boscalid	prothioconazole
Comment	-	-

Conclusion

Instrument response was found to be linear in the concentration range of 49.98 µg/mL to 149.94 µg/mL with $r = 0.9999$ for Boscalid and 50.45 µg/mL to 151.35 µg/mL with $r = 0.9999$ for Prothioconazole.

The Horrat value established for the precision of the method, was found to be 0.04 for Boscalid and 0.08 for Prothioconazole which was found to be ≤ 1 . Hence the method was acceptable.

The Horrat value established for the intermediate precision of the method, the Horrat value was found to be 0.08 for Boscalid and 0.08 for Prothioconazole which was found to be ≤ 1 . Hence the method was acceptable.

Accuracy of the method for Boscalid, mean % recovery ranged between 98.64 and 101.58 % with an overall mean recovery of $99.93 \pm 1.34\%$, and for Prothioconazole, mean % recovery ranged between 99.15 and 102.07 % with an overall mean recovery of $100.28 \pm 1.43\%$. The method was considered acceptable, since the obtained % recovery for Boscalid was in the range of 97 to 103%. The method was specific for the analysis of GLOB2021dF.

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

An overview on the acceptable methods and possible data gaps for analysis of relevant impurities in plant protection product is provided as follows. A method developed for the analysis of impurities of the product GLOB2021dF is presented here.

Comments of zRMS:	The analytical method is fully validated in terms of specificity, linearity, accuracy and repeatability according to SANCO/3030/99 rev. 5. The methods are acceptable for the determination of Desthi-Prothioconazole and Toluene impurities in GLOB2021dF.
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Reference:	KCP 5.1.1
Report	Determination of Desthi-Prothioconazole and Toluene in GLOB2021dF. Smitha B., 2024, AG-G3562.
Guideline(s):	Yes (SANCO/3030/99 rev.5)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

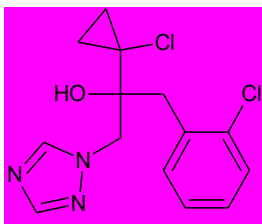
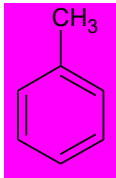
Summary

The study on “Determination of Desthi-Prothioconazole and Toluene in GLOB2021dF” was sponsored by Globachem NV Brustem Industriepark, Lichtenberglaan 2019, 3800, Sint- Truiden, Belgium.

The test item, GLOB2021dF were analyzed (in triplicate) for Impurity-1 and Impurity-2 by using in-house developed and validated method The methods used for analysis is as given below:

Constituent	Method of Analysis
Impurity-1	LC-MSMS Method
Impurity-2	HSGC-FID Method

The chemical name, CAS registry number, Molecular formula and molecular structure of the constituents of GLOB2021dF are given hereafter.

Constituent	Chemical Name	CAS Registry Number	Molecular Formula	Structural Formula
Impurity-1	2-(1-chlorocyclopropyl)-1-(2-chlorophenyl)-3-(1 <i>H</i> -1,2,4-triazol-1-yl)propan-2-ol	120983-64-4	C ₁₄ H ₁₅ Cl ₂ N ₃ O	
Impurity-2	Toluene(Methylbenzene)	108-88-3	C ₇ H ₈	

The methods used for the analysis of GLOB2021dF associated impurities, viz.: Impurity-1 and Impurity-2 of the test item were validated and the method validation results are given in Section 8.

The reference standards of Impurity-2 was characterized by using mass spectra.

The Impurity-2 in the test item batch was characterized and confirmed by the mass spectral analysis. The presence of the impurities (Impurity-1 and Impurity-2) in the test item was also confirmed by comparison of the retention times with that of the respective standards by Liquid Chromatography with mass spectrometry (LC-MSMS) and Gas Chromatographic (GC) analysis.

The results of analysis of the test item, GLOB2021dF are summarized as follows:

Constituent	Content (mean of 3 replications) (% m/m)
	Batch No.
	FOP5819-5
Impurity-1	BLOD
Impurity-2	BLOD

BLOD – Below Limit of detection

The results of the study clearly establish the following:

- The Impurity-1 in the test item GLOB2021dF was found to be below Limit of Detection (0.00000385% m/m).
- The Impurity-2 in the test item GLOB2021dF was found to be below Limit of Detection (0.000172% m/m).

Results impurity-1: Prothioconazole-Desthio

Parameter	Results obtained	Acceptance criteria
Specificity	There was no interference observed from the diluent (Acetonitrile) samples at the retention time of the Impurity-1 indicating the specificity of the analytical method. The specificity and selectivity of the method was established by injecting single preparation of diluent along with the impurity standard solution (Section 7.2.2.1) to the LC-MS/MS operated under the conditions given in the Section 7.2.3.2. The chromatograms of the diluent were checked for the absence of any interference at the retention time of Impurity-1. There was no interference observed from the diluent (Acetonitrile) samples at the retention time of the Impurity-1 indicating the specificity of the analytical method.	Specificity for the active ingredient analysis, there should not be any interference at the retention time of the active ingredient. Specificity for the analysis of impurities and additives should be addressed to the extent that the technical active substance or technical concentrate is properly characterized.
Linearity Correlation coefficient (r) Coefficient of determination (R ²)	0.99977 0.99954 $Y = a + bx$ $y = 10847701.722x + 4528.226$ Slope (b) = 10847701.722 Intercept(a) = 4528.226 R² = 0.99957 r = 0.99979 Range: 0.000760 – 0.0102 % m/m	≥ 0.99 ≥ 0.98
Precision and Intermediate Precision (% relative standard deviation)	0.00253 ± 0.00005 %m/m (fortified) 1.976	$< 2^{(1 - 0.5 \log C)} \times 0.67$

Parameter	Results obtained	Acceptance criteria				
ation) (n = 5)	$H_r = \frac{1.976 \%}{6.592 \%} = 0.30$					
Combined data of precision & intermediate precision (% relative standard deviation).	2.410	$< 2^{(1 - 0.5 \log C)}$				
Limit of Detection (LOD)	0.00150 mg/L 11.7	Signal to noise ratio ≥ 3				
Limit of Quantification (LOQ)	0.010 mg/L 56.1 <table border="1"><thead><tr><th>Name</th><th>S/N Ratio</th></tr></thead><tbody><tr><td>Impurity-1</td><td>56.1</td></tr></tbody></table>	Name	S/N Ratio	Impurity-1	56.1	Signal to noise ratio ≥ 10 The Signal to noise ratio of limit of quantification should be above 10 with acceptable recovery and precision.
Name	S/N Ratio					
Impurity-1	56.1					
Horrat Value	0.24 $H_r = \frac{2.410 \%}{9.862 \%} = 0.24$	≤ 1.0				
Accuracy (As mean % recovery) (n = 5) Impurities with content level of < 0.01%	109.158 ± 3.769 mean recovery of two levels 112,473 (n = 5) and 105,843 (n = 5)	70 to 130 %				

Comments of zRMS: Determination and preparation of 2-(1-chlorocyclopropyl)-1-(2-chlorophenyl)-3-(1H-1,2,4-triazol-1-yl)propan-2-ol as Impurity-1.

7.2.3 Determination of Impurity-1 Content

7.2.3.1 Preparation of Test Item Solutions

About 0.025 g of the test item, GLOB2021dF of each batch was weighed in triplicate in to separate 25 mL volumetric flasks and the contents of the flask were dissolved in about 10mL of diluent (Acetonitrile) and sonicated for 2.0 minutes, the volume was made up to the mark with diluent (Acetonitrile), the solution was shaken thoroughly and used for the analysis of impurity content by injecting to LC-MS/MS operated under the conditions given in Section 7.2.3.2. The content Impurity-1 was calculated using the equation given in Section 7.2.4.

7.2.3.2 Chromatographic Analysis

The content of Impurity-1 in the test item was determined under the chromatographic conditions given below:

LC Conditions

Instrument : LC-MS/MS (Shimadzu Prominence Liquid Chromatograph with Tandem Mass Spectrometer API-3200)
Column : Shim-pack C18, 250 mm long, 4.6 mm i.d., 5 µm.
Mobile Phase A : 0.1% Formic acid in Milli-Q-Water
Mobile Phase B : Methanol
Gradient :

Time (min.)	A %	B %
0.01	45	55
28.00	15	85
28.01	45	55
35.00	45	55

Temperature : 40°C
Solvent Flow Rate : 1.0 mL/min.
Injection Volume : 20 µL
Run Time : 35.00 minutes.

MS Conditions

Scan Type : MRM
Ionization Mode : ESI +ve (Polarity)
Ion Source : Turbo spray

Compound Name	Scan type (MRM)	Mass	m/z value
Desthio Prothioconazole	Quantitative Transition	Q1	312.300
		Q3	70.100
	Confirmatory Transition	Q1	312.300
		Q3	125.200

Source Parameters

Curtain Gas : 28 psi
Ion Spray Voltage : 4200.00
Temperature : 450.00°C
Ion Source Gas (GS1) : 50.00 psi
Ion Source Gas (GS2) : 60.00 psi
De-clustering Potential : 50.00
Entrance Potential : 5.00
Cell Exit Potential : 18.08

- All the parameters was maintained constant throughout the analysis.
- After every 3 to 9 sample injections, an injection of the standard solution of Impurity-1 was made.
- Peak area of the Impurity-1 for each injection was recorded.
- The standard solution peak area (injections before and after the sample injections) was averaged and used for calculating the Impurity-1 content in the test item- samples.

Comments of zRMS:

The Horrat (Horwitz ratio) value H_r was calculated using the following formula:

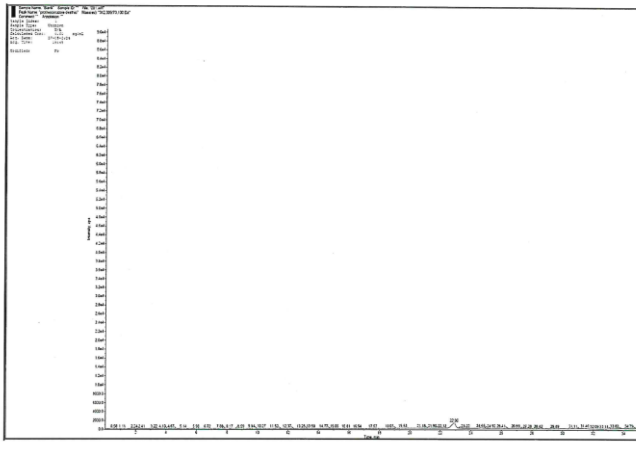
$$H_r = \frac{\%RSD}{\%RSD_r}$$

%RSD: obtained repeatability/precision

%RSD_r: expected repeatability/precision obtained with modified Horwitz equation

Chromatogram of the blank formulation of impurity 1.

FIGURE 1. Representative Chromatogram of Diluent – Specificity Test (Impurity-1)



Results impurity-1: Toluene Results impurity-2: Toluene

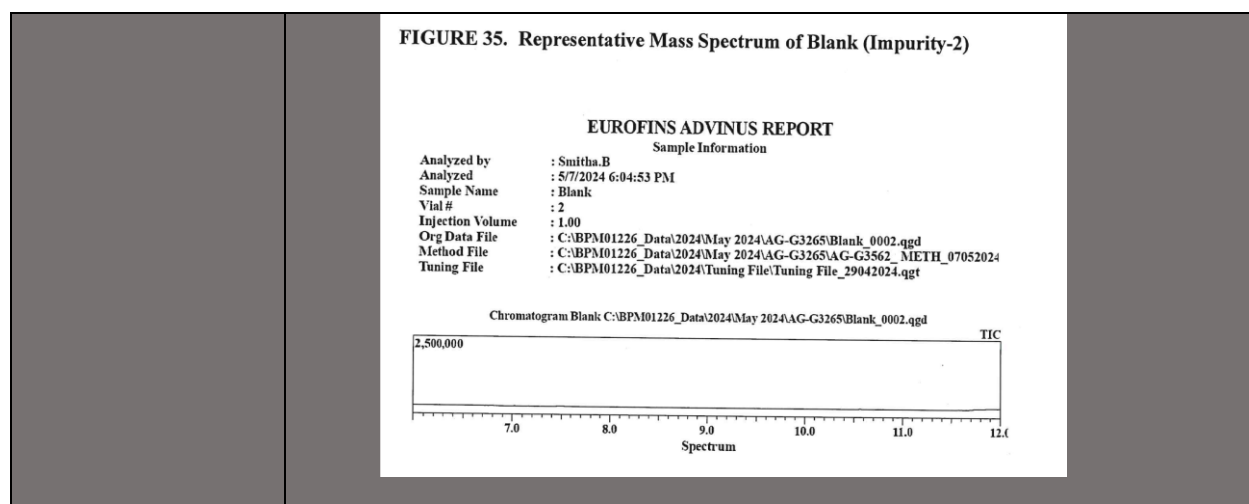
Parameter	Results obtained	Acceptance criteria
Specificity	There was no interference observed from the diluent blank at the retention time of the Impurity-2 indicating the specificity of the analytical method. The specificity and selectivity of the method was established by injecting diluent blank (N-methyl-2-pyrrolidinone) and Impurity-2 standard solution (Section 7.3.2.1) to the GC operated under the conditions given in Section 7.3.3.2. The chromatogram of the diluent blank was checked for the absence of any interference at the retention time of the Impurity-2.	Specificity for the active ingredient analysis, there should not be any interference at the retention time of the active ingredient. Specificity for the analysis of impurities and additives should be addressed to the extent that the technical active substance or technical concentrate is properly characterized.
Linearity Correlation coefficient (r) Coefficient of determination (R ²)	0.99978 0.99956 Range: 0.000201 – 0.00503 % m/m Y = a + bx y = 3250.522 – 155.699 Slope (b) = 3250.522 Intercept(a) = -155.699 R² = 0.99956 r = 0.99978	≥ 0.99 ≥ 0.98

Parameter	Results obtained	Acceptance criteria				
Precision and Intermediate Pre- cision (% relative standard deviation)	0.00154 ± 0.00002 %m/m 1.299 $H_r = \frac{1.299 \%}{7.103 \%} = 0.18$	< 2 ^(1 – 0.5 log C) × 0.67				
Combined data of precision & intermediate precision (% relative standard deviation).	2.649	< 2 ^(1 – 0.5 log C)				
Limit of Detection (LOD)	0.202 mg/L 3.531	Signal to noise ratio ≥ 3				
Limit of Quantification (LOQ)	0.656 mg/L-0.686 mg/L 22.909 <table><tr><td>Name</td><td>S/N Ratio</td></tr><tr><td>Impurity-2</td><td>22.909</td></tr></table>	Name	S/N Ratio	Impurity-2	22.909	Signal to noise ratio ≥ 10 The Signal to noise ratio of limit of quantification should be above 10 with acceptable recovery and precision.
Name	S/N Ratio					
Impurity-2	22.909					
Horrat Value	0.25 $Horrat\ value = \frac{2.649 \%}{10.633 \%} = 0.25$	≤ 1.0				
Accuracy (As mean % recovery) Impurities with content level of < 0.01%	94.604 ± 5.222 mean recovery of two levels 98,235 (n = 5) and 90,973 (n = 5)	70 to 130 %				

Comments of zRMS: Determination and preparation of Toluene(Methylbenzene) as Impurity-2

	7.3.3 Determination of Impurity-2 7.3.3.1 Preparation of Test Item Solutions About 0.1 g of the test item, GLOB2021dF (Batch No.: FOP5819-5) was weighed in triplicate in to separate into separate head space vials and 1.0 mL of diluent (N-methyl-2-pyrrolidinone) was added. The head space vials were sealed with rubber septa and aluminum cap using crimper. The vials were used for the determination of Impurity-2 by analysing on GC operated under the conditions given in Section 7.3.3.2. The impurity content was calculated using the equation given in Section 7.3.4. 7.3.3.2 Chromatographic Analysis The content of Impurity-2 in the test item was determined under the chromatographic conditions given below: Instrument : Gas Chromatograph equipped with Flame Ionization Detector and PC based data system Column : RXi-624 Sil MS, 30 m long, 0.32mm internal diameter, 1.8 µm film thickness or similar Split ratio : 10:1 <u>Temperatures</u> Detector : 260°C Column Oven : Initial: 40°C hold for 5 min. Ramp 1: 10°C/min. to 150°C, hold for 0 min. Ramp 1: 30°C/min. to 250°C, hold for 5 min. <u>Gas Flow Rates</u> Column Flow : 2.0 mL/min. Hydrogen : 32 mL/min. Air : 200 mL/min. Make up flow : 24 mL/min. <u>Condition of Head Space Sampler</u> GC Cycle Time : 32.00 min Oven Temperature : 95 °C Sample line Temperature : 105 °C Transfer line Temperature : 115°C Sample equilibration Time : 10 min. Load fill Time : 0.50 min. Inject Time : 0.50 min. Pressurization Time : 0.5 min. • All the parameters will be maintained constant throughout the analysis. • After every 3 to 9 sample injections, an injection of the standard solution of impurity will be made. • Peak area of the impurity for each injection will be recorded. • The standard solution peak area (injections before and after the sample injections) will be averaged and used for calculating the Impurity-2 content in the test item- samples.	
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Comments of zRMS:	The Horrat (Horwitz ratio) value H_r was calculated using the following formula: $H_r = \frac{\%RSD}{\%RSD_r}$ %RSD: obtained repeatability/precision %RSD_r: expected repeatability/precision obtained with modified Horwitz equation <u>Spectra of the blank formulation of impurity 2.</u>
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5.2.1.3 Description of analytical methods for the determination of formulants (KCP 5.1.1)

Under current EU legislation, methods on formulants are not required. However, if a formulant is defined as relevant for toxicity (environment, health), then a method needs to be provided. There are however no formulants in GLOB2021dF that are defined as relevant for toxicity.

5.2.1.4 Applicability of existing CIPAC methods (KCP 5.1.1)

There is no CIPAC method available for the determination of boscalid and prothioconazole

5.2.2 Methods for the determination of residues (KCP 5.1.2)

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

Table 5.2-3: Validated methods for the generation of pre-authorization data

Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
Component of residue definition: Prothioconazole-desthio				
Food/feed of plant origin (Residues)	Primary	0.02 mg/kg (Wheat and Barley grain, Rape seed, Tomato, Orange fruit) 0.05 mg/kg (Wheat, Barley forage and straw)	GC-MS	EFSA, 2007 EFSA, 2014

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 (Rape seed, Wheat and Barley grain) 0.05 mg/kg (Wheat and Barley straw, green material)	HPLC-MS/MS	EFSA, 2014
	Confirmatory	Not required		
Component of residue definition: Sum of Prothioconazole-desthio and its glucuronide conjugate, expressed as prothioconazole-desthio				
Food/feed animal origin (Residues)	Primary	0.004 mg/kg (milk) 0.01 mg/kg (meat, liver, kidney, fat)	HPLC-MS/MS	EFSA, 2007 EFSA, 2014
	ILV	0.004 mg/kg (milk) 0.01 mg/kg (meat)	HPLC-MS/MS	UK, 2004
	Confirmatory	Not required		
Component of residue definition: Prothioconazole, Prothioconazole-desthio				
Soil (Environmental fate, Efficacy, Ecotoxicology)	Primary	0.006 mg/kg	HPLC-MS/MS	EFSA, 2007
		0.01 mg/kg (Prothioconazole-desthio)	GC-MS	EFSA, 2007
	Confirmatory	Not required		
Surface water and drinking water (Environmental fate, Efficacy, Ecotoxicology)	Primary	0.05 µg/L (Prothioconazole-desthio) 0.1 µg/L (Prothioconazole)	HPLC-MS/MS	EFSA, 2007
	Confirmatory	Not required		
Air (Exposure)	Primary	0.015 mg/m³ (Prothioconazole)	HPLC-MS/MS	EFSA, 2007
		0.0006 mg/m³ (Prothioconazole-desthio)	HPLC-MS/MS	EFSA, 2007
	Confirmatory	Not required		
Body fluids and tissues (Exposure)	Primary	Not required		UK, 2004 EFSA, 2007
	Confirmatory	Not required		

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 boscalid (KCP 5.2)

5.3.2.1 Overview of residue definitions and levels 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 not identical.

Indeed, the previous residue definition for Boscalid was different for all the commodities under the pesticide-code 1000000 (products of animal origin-terrestrial animals), where residue definition was sum of Boscalid and its hydroxy metabolite 2-chloro-N-(4'-chloro-5-hydroxybiphenyl-2-yl)nicotinamide (free and conjugated) expressed as Boscalid, and now the residue definition is different for pesticide-code number 1000000 except 1040000, 1011010, 1011020, 1011050, 1012010, 1012020, 1012050, 1013010, 1013020, 1013050, 1014010, 1014020, 1014050, 1015010, 1015020, 1015050, 1016010, 1016020, 1017010, 1017020, 1017050, 1020000 and 1030000.

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

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Plant, high water content	Boscalid	0.01 mg/kg	Reg. (EU) 2016/156 Reg. (EU) No. 2022/1324
Plant, high acid content		0.01 mg/kg	Reg. (EU) 2016/156 Reg. (EU) No. 2022/1324
Plant, high protein/high starch content (dry commodities)		3.0 mg/kg	Reg. (EU) 2016/156 Reg. (EU) No. 2022/1324
Plant, high oil content		0.01 mg/kg	Reg. (EU) 2016/156 Reg. (EU) No. 2022/1324
Plant, difficult matrices (hops, spices, tea)		0.01 mg/kg	Reg. (EU) 2016/156 Reg. (EU) No. 2022/1324
Muscle	Sum of Boscalid and its hydroxy metabolite 2-chloro-N-(4'-chloro-5-hydroxybiphenyl-2-yl)nicotinamide (free and conjugated) expressed as Boscalid (except for the pesticide code numbers mentioned above) The residue definition differs for the following combinations pesticide-code number: code	0.01 mg/kg	Reg. (EU) 2016/156 Reg. (EU) No. 2022/1324
Milk		0.02 mg/kg	Reg. (EU) 2016/156 Reg. (EU) No. 2022/1324
Eggs		0.01 mg/kg	Reg. (EU) 2016/156 Reg. (EU) No. 2022/1324
Fat		0.07 mg/kg	Reg. (EU) 2016/156 Reg. (EU) No. 2022/1324
Liver, kidney		0.05 mg/kg	Reg. (EU) 2016/156 Reg. (EU)

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
	1000000 except 1040000, 1011010, 1011020, 1011050, 1012010, 1012020, 1012050, 1013010, 1013020, 1013050, 1014010, 1014020, 1014050, 1015010, 1015020, 1015050, 1016010, 1016020, 1017010, 1017020, 1017050, 1020000, 1030000: Sum of boscalid and its hydroxy metabolite 2-chloro-N-(4'- chloro-5-hydroxybiphenyl-2- yl)nicotinamide (free and conju- gated) expressed as boscalid		No. 2022/1324
Honey	Boscalid	0.05	Reg. (EU) No. 2022/1324
Soil (Ecotoxicology)	Boscalid	0.05 mg/kg	Common limit
Drinking water (Human toxicology)	Boscalid	0.1 µg/L	General limit for drinking water
Surface water (Ecotoxicology)	Boscalid	125 µg/L	Lowest NOEC from aquatic toxicity study on <i>O. mykiss</i>
Air	Boscalid	30 µg/m³	AOEL sys/AOEL inhal: 0.1 mg/kg bw/d
Tissue (meat or liver)	-	Not required 0.01 mg/kg	Not classified as T / T+ SANTE/2020/12830 rev.1
Body fluids		Not required 0.01 mg/kg	Not classified as T / T+ SANTE/2020/12830 rev.1

5.3.2.2 Description of analytical methods for the determination of residues in plant matrices (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of boscalid in plant matrices is given in the following tables.

Table 5.3-2: Validated methods for food and feed of plant origin (required for all matrix types, “difficult” matrix only when indicated by intended GAP)

Component of residue definition: Boscalid				
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	Primary	0.01 mg/kg 0.02 0.05 mg/kg	GC-MS HPLC-MS/MS	DAR 2002 (Weeren and Pelz, 1999) DAR 2002; FAO, 2006 (Funk and Mackenroth, 2000)
	ILV	0.01 mg/kg	GC-MS	DAR 2002 (Reichert, 2001)

Component of residue definition: Boscalid				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
	Confirmatory (if required)	0.01 mg/kg 0.05 mg/kg	GC-MS HPLC-MS/MS	DAR 2002 (Weeren and Pelz, 1999) DAR 2002; FAO, 2006 (Funk and Mackenroth, 2000)
High acid content	Primary	0.01 mg/kg	GC-MS	DAR 2002 (Reichert, 2001)
	ILV	0.02 mg/kg 0.05 mg/kg	GC-MS HPLC-MS/MS	DAR 2002 (Weeren and Pelz, 1999) DAR 2002; FAO, 2006 (Funk and Mackenroth, 2000)
	Confirmatory (if required)	0.02 mg/kg	GC-MS	DAR 2002 (Reichert, 2001)
High oil content	Primary	0.01 mg/kg	GC-MS	DAR 2002 (Weeren and Pelz, 1999)
	ILV	0.01 mg/kg	GC-MS	DAR 2002 (Reichert, 2001)
	Confirmatory (if required)	0.05 mg/kg	GC-MS *	DAR 2002 (Reichert, 2001)
High protein/high starch content (dry)	Primary	0.03 mg/kg 0.04 0.05 mg/kg	GC-MS HPLC-MS/MS	DAR 2002 (Weeren and Pelz, 1999) DAR 2002; FAO, 2006 (Funk and Mackenroth, 2000)
	ILV	0.01 mg/kg	GC-MS	DAR 2002 (Reichert, 2001)
	Confirmatory (if required)	0.02 mg/kg 0.05 mg/kg	GC-MS HPLC-MS/MS	DAR 2002 (Weeren and Pelz, 1999) DAR 2002; FAO, 2006 (Funk and Mackenroth, 2000)
Difficult (if required, depends on intended use)	Primary	0.01 mg/kg	GC-MS	DAR 2002 (Reichert, 2001)
Honey	Primary	0.010 mg/kg	LC-MS/MS	Poráčzki.K, 2023
	ILV	-	-	-
	Confirmatory	-	-	-

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

zRMS Comment:

The validated limits of quantification (LOQs) of the EU-agreed methods developed by Class (2000) and Grosshans (2000) are insufficient for monitoring the reduced maximum residue limits (MRLs) in eggs and meat. The interlaboratory validation (ILV) conducted by Kampke-Thiel (2001) has been adequately validated, but only for milk and liver/kidney matrices, where an acceptable LOQ was demonstrated. Accordingly, an ILV for eggs, as well as comprehensive analytical methods (primary, confirmatory, and ILV) for meat, remain unavailable.

The validated limits of quantification (LOQs) of the EU-agreed methods developed by Class (2000) and Grosshans (2000) are not adequate for the effective monitoring of the revised, lower maximum residue limits (MRLs) applicable to eggs and meat. The interlaboratory validation (ILV) performed by Kampke-Thiel (2001) has been demonstrated to meet the required performance criteria; however, validation with an acceptable LOQ has been achieved exclusively for milk and liver/kidney matrices. As a result, an ILV for eggs remains unavailable, and analytical methods for meat—covering primary analysis, confirmatory analysis, and ILV—are still lacking. The absence of these methods limits the ability to ensure full compliance with current regulatory requirements.

Table 5.3-3: Statement on extraction efficiency

	Method for products of plant origin
Required, available from:	Mackenroth-Lehmann, 2007a
Not required, because:	-

5.3.2.3 Description of analytical methods for the determination of residues in animal matrices (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of boscalid in animal matrices is given in the following tables.

Table 5.3-4: Validated methods for food and feed of animal origin (if appropriate)

Component of residue definition: Sum of Boscalid and its hydroxy metabolite 2-chloro-N-(4'-chloro-5-hydroxybiphenyl-2-yl)nicotinamide (free and conjugated) expressed as Boscalid				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Milk	Primary	0.01 mg/kg	GC-ECD HPLC-MS/MS	DAR 2002 (Class, 2000) DAR 2002 (Grosshans, 2000)
	ILV	0.01 mg/kg	GC-ECD	DAR 2002 (Kampke-Thiel, 2001)
	Confirmatory (if required)	0.01 mg/kg	GC-MS	DAR 2002 (Class, 2000)
Eggs	Primary	0.025 mg/kg 0.01 mg/kg	GC-ECD HPLC-MS/MS	DAR 2002 (Class, 2000) DAR 2002 (Grosshans, 2000)
	ILV	0.01 mg/kg 0.025 mg/kg	GC-ECD	DAR 2002 (Kampke-Thiel, 2001)
	Confirmatory (if required)	0.025 mg/kg	GC-MS	DAR 2002 (Class, 2000)
Muscle	Primary	0.025 mg/kg	GC-ECD HPLC-MS/MS	DAR 2002 (Class, 2000) DAR 2002 (Grosshans, 2000)
	ILV	0.025 mg/kg	GC-ECD	DAR 2002 (Kampke-Thiel, 2001)
	Confirmatory (if required)	0.025 mg/kg	GC-MS	DAR 2002 (Class, 2000)
Fat	Primary	0.025 mg/kg	GC-ECD HPLC-MS/MS	DAR 2002 (Class, 2000) DAR 2002 (Grosshans, 2000)
	ILV	0.025 mg/kg	GC-ECD	DAR 2002 (Kampke-Thiel, 2001)

Component of residue definition: Sum of Boscalid and its hydroxy metabolite 2-chloro-N-(4'-chloro-5-hydroxybiphenyl-2-yl)nicotinamide (free and conjugated) expressed as Boscalid				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
	Confirmatory (if required)	0.025 mg/kg	GC-MS	DAR 2002 (Class, 2000)
Kidney, liver	Primary	0.025 mg/kg	GC-ECD HPLC-MS/MS	DAR 2002 (Class, 2000) DAR 2002 (Grosshans, 2000)
	ILV	0.025 mg/kg	GC-ECD	DAR 2002 (Kampke-Thiel, 2001)
	Confirmatory (if required)	0.025 mg/kg	GC-MS	DAR 2002 (Class, 2000)
Honey	Primary	0.0025 mg/kg	LC-MS/MS	Poráčski, K. 2023
	ILV	-	-	-
	Confirmatory	-	-	-

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

zRMS Comment:

According to Reg. No 284/2013 an ILV method for monitoring boscalid residues in honey is required.

Table 5.3-5: Statement on extraction efficiency

	Method for products of animal origin
Required, available from:	Please refer to the DAR, Germany, 2002: “For most of the samples, the extractability with organic solvents was good. About 80% to 99% of the TRR could be extracted in case of muscle, fat, kidney and milk. For liver the extractability was not sufficient, less than 20 % were extractable with methanol. Therefore a Microwave extraction was performed, using mixtures of formic or acetic acid and acetonitrile heated at 170°C for 30 min. The extractability with organic solvents was above 90% of TRR for fat, egg and excreta samples. For liver the extractability was not sufficient, only about 12 % of TRR were extractable. Therefore a Microwave extraction was performed, using an acetonitrile formic acid mixture heated at 170°C for 30 min.”
Not required, because:	No residues > LOQ are expected

5.3.2.4 Description of methods for the analysis of soil (KCP 5.2)

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

Table 5.3-6: Validated methods for soil (if appropriate)

Component of residue definition: boscalid			
Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Primary	0.01 mg/kg	GC-MS	DAR 2002 (Keller, 1998a);

5.3.2.5 Description of methods for the analysis of water (KCP 5.2)

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

Table 5.3-7: Validated methods for water

Component of residue definition: boscalid				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Drinking water	Primary	0.05 mg/kg	GC-MS	DAR 2002 (Keller, 1998b)
Surface water	Primary	0.5 mg/kg	GC-MS	DAR 2002 (Grote, 2001)

zRMS Comment:

According to Reg. No 284/2013 an ILV method for monitoring boscalid residues in drinking water is required.

5.3.2.6 Description of methods for the analysis of air (KCP 5.2)

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

Table 5.3-8: Validated methods for air (if appropriate)

Component of residue definition: boscalid			
Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Primary	1.5 µg/m ³	GC-MS	DAR 2002 (Zangmeister, 2000)

5.3.2.7 Description of methods for the analysis of body fluids and tissues (KCP 5.2)

Table 5.3-9: Methods for body fluids and tissues (if appropriate)

Component of residue definition: Prothioconazole boscalid			
Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Primary	Missing - will be provided at the renewal of the active substance		
Confirmatory	Not required		

zRMS Comment:

According to COMMISSION REGULATION (EU) No Reg. No 283/2013 and Reg. No 284/2013, a fully validated analytical method for monitoring boscalid residues in body fluids is required. Methods for the analysis of body fluids and tissues must be provided at the renewal of the active substance.

5.3.2.8 Other studies/ information

In several residue trials in section B7 and ecotoxicological studies summarized in section B9 of the dRR, analytical methods were used for the detection of GLOB2021dF in the different test mediums. The analytical part of these studies is summarized in Appendix 2.

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

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

Table 5.3-9: Relevant residue definitions for monitoring/enforcement and levels for which compliance is required

Prothioconazole & metabolite Prothioconazole-desthio			
Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Plant, high water content	Prothioconazole-desthio (sum of isomers)	0.02 mg/kg 0.01 mg/kg (LOQ)	EFSA, 2007 Reg. (EU) 2019/552 (EU) No. 2024/1318
Plant, high acid content		0.02 mg/kg 0.01 mg/kg (LOQ)	EFSA, 2007 Reg. (EU) 2019/552 (EU) No. 2024/1318
Plant, high protein/high starch content (dry commodities)		0.02 mg/kg 0.01 mg/kg (LOQ)	EFSA, 2007 Reg. (EU) 2019/552 (EU) No. 2024/1318

Prothioconazole & metabolite Prothioconazole-desthio			
Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Plant, high oil content	Prothioconazole-desthio (sum of isomers)	0.02 mg/kg (LOQ) Oilseed rape: 0.15 mg/kg (LOQ) 0.01 mg/kg	EFSA, 2007 Reg. (EU) 2019/552 (EU) No. 2024/1318
Plant, difficult matrices (forage, straw)		0.05 mg/kg	EFSA, 2007 (EU) No. 2024/1318
Muscle		0.01 mg/kg	EFSA, 2007 (EU) No. 2024/1318
Milk		0.004 mg/kg 0.01 mg/kg (LOQ)	EFSA, 2007 Reg. (EU) 2019/552 (EU) No. 2024/1318
Fat		0.01 mg/kg	EFSA, 2007 (EU) No. 2024/1318
Liver, kidney		0.01 mg/kg 0.1 mg/kg	EFSA, 2007 Reg. (EU) 2019/552 (EU) No. 2024/1318
Honey		0.05 mg/kg	(EU) No. 2024/1318
Soil (Ecotoxicology)	Prothioconazole and prothioconazole-desthio (M04)	0.006 mg/kg	EFSA, 2007 (EU) No. 2024/1318
Drinking water (Human toxicology)	Prothioconazole and prothioconazole-desthio (M04)	0.1 µg/L (Prothioconazole) 0.05 µg/L (Prothioconazole-desthio)	EFSA, 2007
Surface water (Ecotoxicology)	Prothioconazole and prothioconazole-desthio (M04)	0.1 µg/L (Prothioconazole) 0.05 µg/L (Prothioconazole-desthio)	EFSA, 2007
Air	Prothioconazole and prothioconazole-desthio (M04)	0.015 mg/m ³ (Prothioconazole) 0.0006 mg/m ³ (Prothioconazole-desthio)	EFSA, 2007
Tissue (meat or liver)	Prothioconazole-desthio (sum of isomers)	Not required 0.01 mg/kg	Not classified as T / T+ SANTE/2020/12830 rev.1
Body fluids		Not required 0.01 mg/kg	Not classified as T / T+ SANTE/2020/12830 rev.1

An overview on the acceptable methods and possible data gaps for analysis of Prothioconazole in animal matrices is given in the following tables.

Table 5.3-10: Validated methods for food and feed of animal origin (if appropriate)

Component of residue definition: Sum of Prothioconazole-desthio and its glucuronide conjugate, expressed as prothioconazole-desthio				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Milk	Primary	0.004 mg/kg	HPLC-MS/MS	EFSA, 2007 EFSA, 2014
	ILV	0.004 mg/kg	HPLC-MS/MS	UK, 2004
	Confirmatory	/	/	/
Eggs	Primary	Missing - will be provided at the renewal of the active substance		
	ILV	/	/	/
	Confirmatory	/	/	/
Muscle	Primary	0.01 mg/kg	HPLC-MS/MS	EFSA, 2007 EFSA, 2014
	ILV	0.01 mg/kg	HPLC-MS/MS	UK, 2004
	Confirmatory			
Fat	Primary	0.01 mg/kg	HPLC-MS/MS	EFSA, 2007 EFSA, 2014
	ILV	/	/	/
	Confirmatory	/	/	/
Kidney, liver	Primary	0.01 mg/kg	HPLC-MS/MS	EFSA, 2007 EFSA, 2014
	ILV	/	/	/
	Confirmatory	/	/	/

The extraction method used in the analytical method for food and feed of animal origin is the same as the one used in the storage stability study. The extraction efficiency does not need to be proven again.

5.3.3.2 Description of analytical methods for the determination of residues in plant matrices (KCP 5.2)

An overview on the acceptable methods for the analysis of prothioconazole metabolite in plant matrices is given in the following tables.

Table 5.3-11: Validated methods for food and feed of plant origin (required for all matrix types, “difficult” matrix only when indicated by intended GAP)

Component of residue definition: prothioconazole-desthio				
Matrix type	Method type	Method LOQ	Principle of method	EU agreed
Dry commodity (wheat and barley straw)	Primary	0.05 mg/kg	GC-MS	Heinemann (2000) EFSA Scientific Report (2007) 106, 1-98

High starch commodity (wheat and barley grain)	Primary	0.02 mg/kg	GC-MS	Heinemann (2000) EFSA Scientific Report (2007) 106, 1-98
	ILV			
High oil commodity (canola seed)	Primary	0.02 mg/kg	GC-MS	Heinemann (2000) EFSA Scientific Report (2007) 106, 1-98
High water commodity (tomato)	Primary	0.02 mg/kg	GC-MS	Weeren and Pelz (2000) EFSA Scientific Report (2007) 106, 1-98
	ILV			
High acid commodity (orange fruit)	Primary	0.02 mg/kg	GC-MS	Weeren and Pelz (2000) EFSA Scientific Report (2007) 106, 1-98

5.3.3.3 Description of methods for the analysis of soil (KCP 5.2)

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

Table 5.3-12: Validated methods for soil

Component of residue definition: Prothioconazole and prothioconazole-desthio (M04)			
Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Primary	0.006 mg/kg	HPLC-MS/MS	EFSA, 2007
	0.02 mg/kg (Prothioconazole-desthio only)	GC-MS	EFSA, 2007
Confirmatory	/	/	/

5.3.3.4 Description of methods for the analysis of water (KCP 5.2)

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

Table 5.3-13: Validated methods for water (if appropriate)

Component of residue definition: Prothioconazole and prothioconazole-desthio (M04)				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Drinking water	Primary	0.05 µg/L (Prothioconazole-desthio) 0.1 µg/L (Prothioconazole)	HPLC-MS/MS	EFSA, 2007
	Confirmatory	/	/	/
Surface water	Primary	0.05 µg/L	HPLC-MS/MS	EFSA, 2007

Component of residue definition: Prothioconazole and prothioconazole-desthio (M04)				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
		(Prothioconazole-desthio) 0.1 µg/L (Prothioconazole)		
	Confirmatory	/	/	/

zRMS Comment:

According to Reg. No 284/2013 an ILV method for monitoring prothioconazole residues in drinking water is required.

5.3.3.5 Description of methods for the analysis of air (KCP 5.2)

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

Table 5.3-14: Validated methods for air (if appropriate)

Component of residue definition: Fludioxonil Prothioconazole			
Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Primary	0.015 mg/m ³ (Prothioconazole)	HPLC-MS/MS	EFSA, 2007
	0.0006 mg/m ³ (Prothioconazole-desthio)	HPLC-MS/MS	EFSA, 2007
Confirmatory	/	/	/

5.3.3.6 Description of methods for the analysis of body fluids and tissues (KCP 5.2)

Table 5.3-9: Methods for body fluids and tissues (if appropriate)

Component of residue definition: Prothioconazole			
Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Primary	Missing - will be provided at the renewal of the active substance		
Confirmatory	Not required		

zRMS Comment:

According to COMMISSION REGULATION (EU) No Reg. No 283/2013 and Reg. No 284/2013, a fully validated analytical method for monitoring Prothioconazole residues in body fluids is required. Methods for the analysis of body fluids and tissues must be provided at the renewal of the active substance.

5.3.3.7 Other studies/ information

In several residue trials in section B7 and ecotoxicological studies summarized in section B9 of the dRR, analytical methods were used for the detection of GLOB2021dF in the different test mediums. The analytical part of these studies is summarized in Appendix 2.

Appendix 1 Lists of data considered in support of the evaluation

Tables considered not relevant can be deleted as appropriate.

MS to blacken authors of vertebrate studies in the version made available to third parties/public.

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
KCP 5.1.1	Raksha, V.S.	2022	Purity Analysis of GLOB2021dF AG-0130 EUROFINS ADVINUS AGROSCIENCES SERVICES INDIA PRIVATE LIMITED GLP Unpublished	N	Globachem NV
KCP 5.1.1	Smitha B.	2024	Determination of Desthio-Prothioconazole and Toluene in GLOB2021dF AG-G3562 Eurofins Advinus Agrosciences services India Private Limited GLP Unpublished	N	Globachem NV
KCP 5.1.10 submitted as KCA 6.3	Sikorski P., Markowicz A.	2019	Analytical Phase: Magnitude of the residues of prothioconazole and its metabolites in barley (whole plant, grain and straw), following two applications of prothioconazole 300 EC in eight trials (4 DCS and 4 HS), Northern and Southern Europe (Poland, Hungary, Portugal and Greece) – 2017 Report: EGL-17-28967 Staphyt Spain S.L. GLP Unpublished	N	Globachem NV
KCP 5.1.11 submitted as KCA 6.3	Markowicz A.	2021	Prothioconazole – Residue study on barley in Northern and Southern Europe - 2020 Report: EGL-20-42539 Staphyt Spain S.L. GLP	N	Globachem NV

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
			Unpublished		
KCP 5.1.12 submitted as KCA 6.3	Bodsch J., Kravchuk O.	2022	Study on the residue behaviour of prothioconazole in barley after treatment with Prothioconazole 300 EC at two sites under field conditions in Southern Europe Report: IF21-05707589 SGS Institute Fresenius, GmbH GLP Unpublished	N	Globachem NV
KCP 5.1.13 submitted as KCA 6.3	Sikorski P., Markowicz A., Grall E.	2019	Analytical Phase: Magnitude of the residues of prothioconazole and its metabolites in wheat (whole plant, grain and straw), following two applications of prothioconazole 300 EC in eight trials (4 DCS and 4 HS), Northern and Southern Europe (Poland, Hungary, Portugal and Greece) – 2017 Report: EGL-17-28966 Staphyt Spain S.L. GLP Unpublished	N	Globachem NV
KCP 5.1.14 submitted as KCA 6.3	Markowicz A., Grall E.	2019	Analytical Phase: Magnitude of the residues of prothioconazole and its metabolites in wheat (whole plant, grain and straw), following two applications of prothioconazole 300 EC in eight trials (4 DCS and 4 HS), Northern and Southern Europe (Poland, Hungary, Portugal and Greece) – 2018 Report: EGL-17-28966 EGL-18-32944 Staphyt Spain S.L. GLP Unpublished	N	Globachem NV
KCP 5.1.15 submitted as KCA 6.3	Bodsch J., Kravchuk O., Gabriel E.	2022	Study on the residue behaviour of prothioconazole in wheat after treatment with Prothioconazole 300 EC at eight sites under field conditions in Southern Europe Report: IF21-05707610 SGS Institute Fresenius, GmbH GLP Unpublished	N	Globachem NV

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 5.1.16 submitted as KCA 6.3	Bodsch J., Meyer M.	2022	Study on the residue behaviour of prothioconazole in oilseed rape after treatment with Prothioconazole 300 EC at eight sites under field conditions in Northern Europe Report: IF21-05707333 SGS Institute Fresenius, GmbH GLP Unpublished	N	Globachem NV
KCP 5.1.17 submitted as KCA 6.3	Sikorski P., Markowicz A.	2018	Analytical Phase: Magnitude of the residues of prothioconazole and its metabolites in oilseed rape (whole plant, seeds and straw), following two applications of prothioconazole 300 EC in eight trials (4 DCS and 4 HS), Northern and Southern Europe (Poland, Hungary, Portugal and Greece) – 2017 Report: RDE-17-28968 Staphyt Spain S.L. GLP Unpublished	N	Globachem NV
KCP 5.1.18 submitted as KCA 6.3	Markowicz A.	2018	Analytical Phase: Magnitude of the residues of prothioconazole and its metabolites in oilseed rape (whole plant, seeds and straw), following two applications of HAG 610 01F Northern Europe – 2019 Report: RDE-19-38550 Staphyt Spain S.L. GLP Unpublished	N	Globachem NV
KCP 5.1.19 submitted as KCA 6.3	Bodsch J.,	2022	Study on the residue behaviour of prothioconazole in oilseed rape after treatment with Prothioconazole 300 EC at six sites under field conditions in Southern Europe - 2021 Report: IF21-05707367 SGS Institute Fresenius, GmbH GLP Unpublished	N	Globachem NV
KCP 5.1.20 submitted as KCA 6.3	Bodsch J.,	2022	Study on the residue behaviour of prothioconazole in wheat after treatment with Prothioconazole 300 EC at two sites under field conditions in Northern Europe - 2021 Report: IF22-06125006	N	Globachem NV

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
			SGS Institute Fresenius, GmbH GLP Unpublished		
KCP 5.1.1 submitted as KCA 6.3	Schlewitz P.	2023	Validation of the Analytical Method for the Analysis of Boscalid in Cereals (high water content and dry commodities) Report: C3085 GLP Unpublished	N	Globachem NV
KCP 5.2-1	Biesiada M.	2022	Validation of analytical method for the determination of active substances boscalid and prothioconazole concentrations in aqueous media solutions of the test item GLOB2021dF Report: 0064/0029/FA Sorbolab research laboratory LLC GLP Unpublished	N	Globachem NV
KCP 5.2-2	Ballai C.	2023a	Validation of Analytical Method for the Determination of GLOB2021dF from Feeding Solutions Report: FBPSTUDY-269-VAL1 Fumoprep Kft GLP Unpublished	N	Globachem NV
KCP 5.2-3	Ballai C.	2023b	Validation of Analytical Method for the Determination of GLOB2021dF from Surface-treat Solution Report: FBPSTUDY-269-VAL3 Fumoprep Kft GLP Unpublished	N	Globachem NV
KCP 5.1.29 Submitted as KCP 10.2	Arputharaj P.	2024	Analytical report: Acute toxicity study of GLOB2021dF in Fish (<i>Oncorhynchus mykiss</i>) Report: 23-600-G, Annex 1 p29-110 Vanta Bioscience Limited GLP Unpublished	N	Globachem NV

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
KCA 6.3-06	Grall E.	2022	Prothioconazole – Residue study on barley in Northern Europe – 2020 Report: EGL-20-45487 Staphyt Spain S.L. GLP Unpublished	N	Globachem NV
KCP 5.1.23 submitted as KCA 6.3-07	Bodsch, J. Kravchuk, O.	2022	Study on the Residue Behaviour of Prothioconazole in Barley after Treatment with Prothioconazole 300 EC at two Sites under Field Conditions Northern Europe, 2021 Report: IF21-05704459 SGS GLP Unpublished	N	Globachem NV HELM AG ASCENZA Agro, S.A.
KCP 5.1.24 submitted as KCA 6.3-11	Bodsch, J. Kravchuk, O.	2023	Prothioconazole – Residue Study on Wheat in Northern Europe – 2020 Report: EGL20-42538 – IF20-05411779 SGS GLP Unpublished	N	Globachem NV HELM AG ASCENZA Agro, S.A.
KCA 5.1.25 submitted as 6.3-12	Thirkell C.	2021	Study on the residue behaviour of prothioconazole in wheat after treatment with Prothioconazole 300 EC at six sites under field conditions in Northern Europe, 2021 Report: IF21-05705310-21-00226 SGS INSTITUT FRESENIUS GmbH GLP Unpublished	N	Globachem NV
KCA 5.1.26 submitted as 6.10.1-01	Walsch Metzler N. Schnetzer	2022	Analytical phase report: Tunnel study for determining the magnitude of residues of Prothioconazole 300EC in honey according to SANTE/11956/2016 rev. 9 Report: IF21-05844957 SGS INSTITUT FRESENIUS GmbH GLP Unpublished	N	Globachem NV HELM AG ASCENZA Agro, S.A.
Submitted as	Biesiada M.	2022	Validation of analytical method for the determination of active substances boscalid and prothioconazole concentrations in M7 media solutions of the test item GLOB2021dF.	N	Globachem NV

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
0064/0031/E			0064/0037/FA		

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
KCP XX	Author	YYYY	Title Company Report N Source GLP/non GLP/GEP/non GEP Published/Unpublished	Y/N	Owner

The following tables are to be completed by MS

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
KCP XX	Author	YYYY	Title Company Report N Source GLP/non GLP/GEP/non GEP Published/Unpublished	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
KCP XX	Author	YYYY	Title Company Report N Source GLP/non GLP/GEP/non GEP Published/Unpublished	Y/N	Owner

Appendix 2 Detailed evaluation of submitted analytical methods

A 2.1 Analytical methods for boscalid and prothioconazole

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

Comments of zRMS:	Analytical method (Markowicz A., 2017, EGL-17-28967) is acceptable and suitable for the determination of residues prothioconazole and its metabolites in barley (whole plant, grain and straw).
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Reference: KCP 5.1-10

Report Analytical Phase: Magnitude of the residues of prothioconazole and its metabolites in barley (whole plant, grain and straw), following two applications of prothioconazole 300 EC in eight trials (4 DCS and 4 HS), Northern and Southern Europe (Poland, Hungary, Portugal and Greece) – 2017 Sikorski P., Markowicz A.
Report No.: EGL-17-28967
Staphyt Spain S.L.
GLP
Unpublished

Guidelines: Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21-Oct-2009 concerning the placing of plant protection products on the market and repealing council Directives 79/117/EEC and 91/414/EC
Guideline 7029/VI/95 (rev. 5) to Directive 91/414/EEC and Regulations (EU) 283/2013 and 284/2013 implementing Regulation (EC) 1107/2009
EU Guidance Document SANCO/3029/99 rev. 4
EU Guidance Document SANCO/825/00 rev. 8.1

Deviations: No

GLP: Yes

Acceptability: Yes

Summary

For the purpose of this analytical phase two different analytical procedures were validated.

The general principles of the analytical procedure for determination of prothioconazole and prothioconazole-desthio in barley whole plant were based on the sample preparation method described in EN 15662:2008 [3] with some modifications. In brief, target samples undergo shake extraction with mixture of acetonitrile/water containing 2 mg/mL L-Cysteine HCl 4:1 (v:v).

Prothioconazole is quite unstable and might react with oxygen in air to form other products thus the addition of L-Cysteine HCl is necessary to minimize the oxidation process. After addition of a buffer-salt mixture containing magnesium sulfate and sodium chloride the sample was shaken again. Follow-

ing centrifugation, the supernatant was further cleaned and the excess of water removed with a single d-SPE step by mixing with magnesium sulphate and graphitized carbon black (GCB). The sample was vortex and centrifuged.

The general principles of the analytical procedure (reflux method) for determination of JAU-6476-desthio (M04), JAU-6476-3-hydroxy-desthio (M14), JAU-6476-4-hydroxy-desthio (M15), JAU-6476-5-hydroxy-desthio (M16), JAU-6476-6-hydroxy-desthio (M17) and JAU-6476- α -hydroxy-desthio (M18) in barley whole plant, straw and grain were derived from Bayer CropScience analytical method 00979/M001 [4]. In brief, target analytes undergo shake-extraction with a mixture of acetonitrile/water 4:1 (v:v) followed by a liquid-liquid partitioning step and phase separation by adding salt mixture containing magnesium sulphate and sodium chloride. Following centrifugation, an aliquot of the acetonitrile phase was evaporated almost to dryness. The remainder was diluted and acidified with 5 N hydrochloric acid and heated at reflux for 2 hours. This hydrolysis step was performed to convert glycoside-bound analogues into the respective hydroxy analytes. Subsequently the samples were neutralized with sodium hydroxide and undergo second shake-extraction with acetonitrile.

After addition of salt mixture containing magnesium sulfate and sodium chloride extract was shaken again. Following centrifugation an aliquot of the acetonitrile phase was decanted into Eppendorf tube containing magnesium sulphate for additional dehydration. The sample was vortex and centrifuged.

For quantitative analysis in both extraction methods the final extract was diluted with acidified water and subjected to LC-ESI-MS/MS. All analytes were detected using electrospray ionization in the positive ion mode (ESI+)

Selectivity and Confirmation of Residue Identity

Quantification was performed by use of highly selective liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). Two selected ion mass transitions were evaluated in order to demonstrate that the method achieves a high level of selectivity. The retention times of target analyte in extracts corresponds to that of the calibration standards with a tolerance of $< \pm 0.15$ min. Confirmation ion ratio for all analytes in all samples were within ± 30 % of the average found for the standards.

No significant interference above 30 % of LOQ was detected in any of the reagent blanks or control specimen extracts for barley whole plant, straw and grain matrices, so that a highly level of selectivity was demonstrated and an additional confirmatory method is not necessary.

Matrix Effects

Determination of prothioconazole (JAU-6476), JAU-6476-desthio (M04), JAU-6476-3-hydroxy-desthio (M14), JAU-6476-4-hydroxy-desthio (M15), JAU-6476-5-hydroxy-desthio (M16), JAU-6476-6 h droxydesthio (M17) and JAU-6476- α -hydroxy-desthio (M18) was performed using matrix-matched calibration standards.

Linearity

The linearity of the detector response for JAU-6476 and M04 was demonstrated by single determination of matrix-matched calibration standards at nine concentration levels (dilution factor x10) ranging from 0.0003 $\mu\text{g/mL}$ to 0.1 $\mu\text{g/mL}$ and at seven concentrations levels (dilution factor x100) ranging from 0.001 $\mu\text{g/mL}$ to 0.1 $\mu\text{g/mL}$ for barley whole plant. These ranges correspond from 0.003 mg/kg to 1 mg/kg (dilution factor x10) and from 0.1 mg/kg to 10 mg/kg (dilution factor x100) for barley whole

plant thus covering the range from no more than 30% of the LOQ and at least + 20 % of the highest analyte concentration level detected in samples.

The linearity of the detector response for M04 and M14, M15, M16, M17, M18 (all barley matrices) was demonstrated by single determination of matrix-matched calibration standards at seven concentration levels (dilution factor x20) ranging from 0.0001 µg/mL to 0.05 µg/ml for barley whole plant, grain and straw. These ranges correspond from 0.002 mg/kg to 1 mg/kg thus covering the range from no more than 30% of the LOQ and at least + 20 % of the highest analyte concentration level detected in samples.

The calibration curves were not forced through zero (y-intercept was calculated) and were derived from a weighted (1/x) linear regression for barley. For JAU-6476, M04, M14, M15, M16, M17 and M18 both ion mass transitions calibration curves (quantification, confirmation) were linear with the coefficients of correlation (R) greater than 0.99.

Stability of Sample Extracts

The stability of final sample extracts (barley whole plant, grain and straw) was evaluated by comparison of initially acquired results from analysis of fortification samples at LOQ (0.01 mg/kg) and results obtained from analysis of sample extracts stored refrigerated at 5°C ±4°C. The obtained results presented in Table 28 confirm stability extracts when stored under refrigerated conditions for tested period of time.

Stability of Analyte in Final Dilution

The stability of target analytes in final dilution was not tested specifically. Recoveries of the fortified samples within the acceptable range of 70-110% obtained with calibration solutions and the use of bracketing standards to insure integrity of the analytical sequence sufficiently demonstrate the stability.

Quantification

Quantification was performed by using weighted (1/x) linear regression as described in the section “Linearity”.

Accuracy and Precision

Accuracy was determined by fortification of control samples with known amounts of the reference items and subsequent determination of the recoveries when applying the extraction procedures.

Precision was determined by repeatability (relative standard deviation – RSD).

The mean recovery values at the fortification levels of 0.01 mg/kg, 0.5 mg/kg and 7.5 mg/kg for both ion mass transitions were all in the range 70 – 110 % and thus comply with the standard acceptance criteria of the guidance document SANCO/825/00, rev. 8.1. [1] and SANCO/3029/99, rev. 4 [2].

All precision values at the fortification levels of 0.01 mg/kg, 0.5 mg/kg and 7.5 mg/kg for both ion mass transitions were < 20%.

Table 1: Summary of recovery results

Analyte	Crop/ Matrix	Fortification Level	Mean Recovery	RSD	n	Overall	
		[mg/kg]	(%)	(%)		Mean [%]	RSD [%]
Prothioconazole	Quantification Ion Mass Transition m/z 344.0→154.0						
	Barley whole plant	0.01*	90	6.0	5	96	7.8
		0.5*	102	1.5	5		
		0.5**	93	5.3	5	97	5.9
		7.5**	100	4.9	5		
	Confirmation Ion Mass Transition m/z 344.0→125.0						
	Barley whole plant	0.01*	91	2.8	5	96	5.9
		0.5*	101	1.0	5		
		0.5**	94	4.9	5	98	6.0
		7.5**	102	4.7	5		
JAU-6476- desthio	Quantification Ion Mass Transition m/z 312.0→70.1						
	Barley whole plant	0.01*	99	3.0	5	101	3.8
		0.5*	104	1.9	5		
		0.5**	107	3.2	5	104	5.2
		7.5**	100	4.0	5		
	Confirmation Ion Mass Transition m/z 312.0→125.0						
	Barley whole plant	0.01*	97	2.7	5	101	4.4
		0.5*	105	1.9	5		
		0.5**	107	3.8	5	103	4.7
		7.5**	100	3.5	5		

* - dilution factor x10

** - dilution factor x100

Comments of zRMS:	Analytical method (Markowicz A., 2017, EGL-20-42539) is acceptable and suitable for the determination of residues prothioconazole in barley (whole plant, grain and straw).
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Reference: KCP 5.1-11

Report Prothioconazole – Residue study on barley in Northern and Southern Europe – 2020
Markowicz A. 2022
Report No.: EGL-20-42539
Staphyt Spain S.L.
GLP
Unpublished

Guidelines:	Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21-Oct-2009 concerning the placing of plant protection products on the market and repealing council Directives 79/117/EEC and 91/414/EC Guideline 7029/VI/95 (rev. 5) to Directive 91/414/EEC and Regulations (EU) 283/2013 and 284/2013 implementing Regulation (EC) 1107/2009 EU Guidance Document SANCO/3029/99 rev. 4 EU Guidance Document SANCO/825/00 rev. 8.1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Summary

For the purpose of this analytical phase two different analytical procedures were used.

The general principles of the analytical procedure (online-SPE method) for the determination of JAU-6476 and JAU-6476-desthio (M04) in barley whole plant were based on the sample preparation method described in EN 15662:2018 [4] with some modifications. In brief, target samples undergo shake extraction with mixture of acetonitrile/water containing 2 mg/mL L-Cysteine HCl 4:1 (v:v). JAU-6476 is quite unstable and might react with oxygen in air to form other products thus the addition of L-Cystine HCl is necessary to minimize the oxidation process. After addition of a buffer-salt mixture containing magnesium sulfate and sodium chloride, the sample was shaken again. Following centrifugation, the supernatant was further cleaned and the excess of water removed with a single d-SPE step by mixing with magnesium sulphate and graphitized carbon black (GCB). The sample was vortex and centrifuged.

The general principles of the analytical procedure (reflux method) for determination of JAU-6476-desthio (M04), JAU-6476-3-hydroxy-desthio (M14), JAU-6476-4-hydroxy-desthio (M15), JAU-6476-5-hydroxy-desthio (M16), JAU-6476-6-hydroxy-desthio (M17) and JAU-6476- α -hydroxy-desthio (M18) in barley whole plant, straw and grain were derived from Bayer CropScience analytical method 00979/M001 [5]. In brief, target analytes undergo shake-extraction with a mixture of acetonitrile/water 4:1 (v:v) followed by a liquid-liquid partitioning step and phase separation by adding salt mixture containing magnesium sulphate and sodium chloride. Following centrifugation, an aliquot of the acetonitrile phase was evaporated almost to dryness. The remainder was diluted and acidified with 5 N hydrochloric acid and heated at reflux for 2 hours. This hydrolysis step was performed to convert glycoside-bound analogues into the respective hydroxy analytes. Subsequently, the samples were neutralized with sodium hydroxide and undergo second shake-extraction with acetonitrile. After addition of salt mixture containing magnesium sulfate and sodium chloride extract was shaken again. Following centrifugation, an aliquot of the acetonitrile phase was decanted into an Eppendorf tube containing magnesium sulphate for additional dehydration. The sample was vortex and centrifuged.

For quantitative analysis in both extraction methods, the final extract was diluted with acidified water and subjected to LC-ESI-MS/MS. All analytes were detected using electrospray ionization in the positive ion mode (ESI+).

Selectivity and Confirmation of Residue Identity

Quantification was performed by use of highly selective liquid chromatography coupled with tandem

mass spectrometry (LC-MS/MS). Two selected ion mass transitions were evaluated in order to demonstrate that the method achieves a high level of selectivity. The retention times of target analytes in extracts correspond to that of the calibration standards with a tolerance of $< \pm 0.1$ min. Confirmation ion ratio for all analytes in all samples was within ± 30 % of the average found for the standards.

No significant interference above 30 % of LOQ was detected in any of the reagent blanks or control specimen extracts for barley (whole plant, grain and straw) matrices so that a highly level of selectivity was demonstrated and an additional confirmatory method is not necessary.

Matrix Effects

Determination of prothioconazole (JAU-6476), JAU-6476-desthio (M04), JAU-6476-3-hydroxy-desthio (M14), JAU-6476-4-hydroxy-desthio (M15), JAU-6476-5-hydroxy-desthio (M16), JAU-6476-6-hydroxydesthio (M17) and JAU-6476- α -hydroxy-desthio (M18) was performed using matrix-matched calibration standards.

Linearity

The linearity of the detector response for JAU-6476 and M04 (online-SPE method) was demonstrated by single determination of matrix-matched calibration standards at nine concentration levels (dilution factor x10) ranging from 0.0003 $\mu\text{g/mL}$ to 0.1 $\mu\text{g/mL}$ and at seven concentrations levels (dilution factor x100) ranging from 0.001 $\mu\text{g/mL}$ to 0.1 $\mu\text{g/mL}$ for barley whole plant. These ranges correspond from 0.003 mg/kg to 1 mg/kg (dilution factor x10) and from 0.1 mg/kg to 10 mg/kg (dilution factor x100) for barley whole plant thus covering the range from no more than 30% of the LOQ and at least + 20 % of the highest analyte concentration level detected in samples.

The linearity of the detector response for M04, M14, M15, M16, M17 and M18 (reflux method)) was demonstrated by single determination of matrix-matched calibration standards at seven concentration levels (dilution factor x20) ranging from 0.0001 $\mu\text{g/mL}$ to 0.05 $\mu\text{g/mL}$ for barley (whole plants, grain and straw). These ranges correspond from 0.002 mg/kg to 1 mg/kg thus covering the range from no more than 30% of the LOQ and at least + 20 % of the highest analyte concentration level detected in samples.

The calibration curves were not forced through zero (y-intercept was calculated) and were derived from a weighted (1/x) linear regression for barley. For JAU-6476, M04, M14, M15, M16, M17 and M18 both ion mass transitions calibration curves (quantification, confirmation) were linear with the coefficients of correlation (R) greater than 0.99.

Stability of Analytes in Final Dilution

The stability of target analytes in final dilution was not tested specifically. Recoveries of the fortified samples within the acceptable range of 70-110% obtained with calibration solutions and the use of bracketing standards to ensure integrity of the analytical sequence sufficiently demonstrate the stability.

Quantification

Quantification was performed by using weighted (1/x) linear regression as described in the section "Linearity".

Accuracy and Precision

Accuracy was determined by fortification of control samples with known amounts of the reference

items and subsequent determination of the recoveries when applying the extraction procedures.

Precision was determined by repeatability (relative standard deviation – RSD).

The mean recovery values at the fortification levels of 0.01 mg/kg and 0.1 mg/kg for both ion mass transitions were all in the range 70 – 110 % and thus comply with the standard acceptance criteria of the guidance documents SANCO/825/00, rev. 8.1. [1] and SANCO/3029/99, rev. 4 [2]. All precision values at the fortification levels of 0.01 mg/kg and 0.1 mg/kg for both ion mass transitions were < 20%.

Mean recovery and precision results for both ion mass transitions of prothioconazole (JAU-6476), JAU-6476-desthio (M04).

Comments of zRMS:	Analytical method (Meyer M., 2017, IF21-05707589) is acceptable and suitable for the determination of residues prothioconazole in barley (whole plant, grain and straw).
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Reference: KCP 5.1-12

Report Study on the residue behaviour of prothioconazole in barley after treatment with Prothioconazole 300 EC at two sites under field conditions in Southern Europe
~~Bodsch J., Kravchuk O.~~ Meyer M.
Report No.: IF21-05707589
SGS Institute Fresenius, GmbH
GLP
Unpublished

Guidelines: SANTE/2020/12830, Rev.1 (24. February 2021)
OECD ENV/JM/MONO(2007) 17 - Guidance Document on Pesticide Residue Analytical Methods Good Laboratory Practice Regulations: Appendix 1 to § 19a, Section 1, of the German Chemikaliengesetz (Chemicals Act)
OECD Principles of Good Laboratory Practice ENV/MC/CHEM(98)17, Paris 1998
The application of the OECD Principles of GLP to the Organisation and Management of Multi-Site Studies, ENV/JM/MONO(2002)9, 25/06/2002.

Deviations: No

GLP: Yes

Acceptability: Yes

Summary

During the growing season of 2021 two field trials with barley were conducted in Southern Europe (Trial 21-00229-01 –France, Trial 21-00229-02 – Italy). The magnitude of residues in barley specimens was determined for the triazole metabolites 1,2,4-Triazole (1,2,4-T), Triazole alanine (TA), Triazole acetic acid (TAA) and Triazole lactic acid (TLA) after spray application with Prothioconazole 300 EC.

Prothioconazole 300 EC (300 g Prothioconazole/L) was applied two times with nominal content 195 g

prothioconazole/ha and a nominal water volume of 200-400 L/ha.

Specimens of barley were collected at a nominal sampling timing 0 (S1), 7±1 (S2) and 14±1 (S3) DALA as whole plant, 35 ± 3 DALA (S4) as grain and straw and at normal commercial harvest (growth stage BBCH 89) as grain and straw (S5).

The barley specimens were analysed at SGS INSTITUT FRESENIUS GmbH, c/o SGS Germany GmbH, Hamburg, Germany for residues of the triazol metabolites following the analytical method 01062/M004[1]. The method was validated at the analytical test site in study IF21-05707367[3] for the matrix groups relevant in this study.

Comments of zRMS:	Principle of the method: 1,2,4-T, TA, TAA and TLA were extracted with a mixture of methanol and water. An aliquot was filtered, concentrated and cleaned-up by dispersive C18-SPE step. The analytes were determined by LC-DMS/MS/MS, using two different HPLC stationary phases and a LC-MS/MS instrument equipped with SelexION ion mobility technology which is based on planar differential spectrometry (DMS). Residues were quantified using stable isotopically labelled internal standards to compensate matrix effects.
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The Limit of Quantification (LOQ) of the analytical method was defined as 0.010 mg/kg for each analyte. The Limit of Detection (LOD) was defined to be 0.003 mg/kg for each analyte (lowest calibration standard used).

Linearity was shown over a range of 1.25 to 100 ng/mL for the analytes with correlation coefficients of ≥ 0.998 and no visible trend in the residuals. Therefore, the calibration model was defined to be suitable. The accuracy of the method was demonstrated by recovery experiments, control specimens were fortified prior to extraction with 1,2,4-T, TA, TAA and TLA.

The mean recovery rate values, the standard deviations (SD) and the coefficients of variation (CV) were as follows:

Crop	Matrix	TA		
		Mean (%)	RSD	n
Barley	Overall	92.6	6.9	21
Barley	Overall	86.8	11	21
Barley	Overall	88.8	9.7	21
Barley	Overall	88.0	8.0	21

Crop	Matrix	Fortification Level (nominal) [mg/kg]	1,2,4-T		
			Mean [%]	RSD [±]	n
Barley	Grain	0.010, 0.10 and 2.0	92.9	6.3	7
	Straw	0.010, 0.10 and 2.0	93.9	6.6	7
	Whole Plant no Roots	0.010, 0.10 and 2.0	91.1	8.4	7
Overall			92.6	6.9	21

RSD: *Relative* Standard deviation; n: Number of specimens

Crop	Matrix	Fortification Level (nominal) [mg/kg]	TA		
			Mean [%]	RSD [±]	n
Barley	Grain	0.010, 0.10 and 2.0	80.2	13	7
	Straw	0.010, 0.10 and 2.0	84.1	5.7	7
	Whole Plant no Roots	0.010, 0.10 and 2.0	96.1	6.4	7
Overall			86.8	11	21

RSD: *Relative* Standard deviation; n: Number of specimens

Crop	Matrix	Fortification Level (nominal) [mg/kg]	TAA		
			Mean [%]	RSD [±]	n
Barley	Grain	0.010, 0.10 and 2.0	79.5	4.1	7
	Straw	0.010, 0.10 and 2.0	90.8	6.5	7
	Whole Plant no Roots	0.010, 0.10 and 2.0	96.1	6.0	7
Overall			88.8	9.7	21

RSD: *Relative* Standard deviation; n: Number of specimens

Crop	Matrix	Fortification Level (nominal) [mg/kg]	TLA		
			Mean [%]	RSD [±]	n
Barley	Grain	0.010, 0.10 and 2.0	80.8	5.3	7
	Straw	0.010, 0.10 and 2.0	88.7	5.2	7
	Whole Plant no Roots	0.010, 0.10 and 2.0	94.5	4.5	7
Overall			88.0	8.0	21

RSD: *Relative* Standard deviation; n: Number of specimens

Comments of zRMS:	Analytical method (Grall E., 2017, EGL-17-28966) is acceptable and suitable for the determination of residues prothioconazole and its metabolites in wheat (whole plant, grain and straw).
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Reference: KCP 5.1-13

Report Analytical Phase: Magnitude of the residues of prothioconazole and its metabolites in wheat (whole plant, grain and straw), following two applications of prothioconazole 300 EC in eight trials (4 DCS and 4 HS), Northern and Southern Europe (Poland, Hungary, Portugal and Greece) – 2017
~~Sikorski P., Markowicz A.~~ Grall E.
Report No.: ~~EGL-17-28967~~ EGL-17-28966
Staphyt Spain S.L.
GLP
Unpublished

Guidelines: Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21-Oct-2009 concerning the placing of plant protection products on the market and repealing council Directives 79/117/EEC and 91/414/EC Guideline 7029/VI/95 (rev. 5) to Directive 91/414/EEC and Regulations (EU) 283/2013 and 284/2013 implementing Regulation (EC) 1107/2009
EU Guidance Document SANCO/3029/99 rev. 4
EU Guidance Document SANCO/825/00 rev. 8.1

Deviations: No

GLP: Yes

Acceptability: Yes

Summary

For the purpose of this analytical phase two different analytical procedures were validated.

The general principles of the analytical procedure for determination of prothioconazole and prothioconazole-desthio in wheat whole plant were based on the sample preparation method described in EN 15662:2008 [3] with some modifications. In brief, target samples undergo shakeextraction with mixture of acetonitrile/water containing 2 mg/mL L-Cysteine HCl 4:1 (v:v).

Prothioconazole is quite unstable and might react with oxygen in air to form other products thus the addition of L-Cystine HCl is necessary to minimize the oxidation process. After addition of a buffer-salt mixture containing magnesium sulfate and sodium chloride the sample was shaken again. Following centrifugation, the supernatant was further cleaned and the excess of water removed with a single d-SPE step by mixing with magnesium sulphate and graphitized carbon black (GCB). The sample was vortex and centrifuged.

The general principles of the analytical procedure (reflux method) for determination of JAU-6476-desthio (M04), JAU-6476-3-hydroxy-desthio (M14), JAU-6476-4-hydroxy-desthio (M15), JAU-6476-5-hydroxy-desthio (M16), JAU-6476-6-hydroxy-desthio (M17) and JAU-6476- α -hydroxy-desthio (M18) in wheat whole plant, straw and grain were derived from Bayer CropScience analytical method 00979/M001 [4]. In brief, target analytes undergo shake-extraction with a mixture of acetonitrile/water

4:1 (v:v) followed by a liquid-liquid partitioning step and phase separation by adding salt mixture containing magnesium sulphate and sodium chloride. Following centrifugation, an aliquot of the acetonitrile phase was evaporated almost to dryness. The remainder was diluted and acidified with 5 N hydrochloric acid and heated at reflux for 2 hours. This hydrolysis step was performed to convert glycoside-bound analogues into the respective hydroxy analytes. Subsequently the samples were neutralized with sodium hydroxide and undergo second shake-extraction with acetonitrile.

After addition of salt mixture containing magnesium sulfate and sodium chloride extract was shaken again. Following centrifugation an aliquot of the acetonitrile phase was decanted into Eppendorf tube containing magnesium sulphate for additional dehydration. The sample was vortex and centrifuged.

For quantitative analysis in both extraction methods the final extract was diluted with acidified water and subjected to LC-ESI-MS/MS. All analytes were detected using electrospray ionization in the positive ion mode (ESI+)

Selectivity and Confirmation of Residue Identity

Quantification was performed by use of highly selective liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). Two selected ion mass transitions were evaluated in order to demonstrate that the method achieves a high level of selectivity. The retention times of target analyte in extracts corresponds to that of the calibration standards with a tolerance of $< \pm 0.15$ min. Confirmation ion ratio for all analytes in all samples were within ± 30 % of the average found for the standards.

No significant interference above 30 % of LOQ was detected in any of the reagent blanks or control specimen extracts for wheat whole plant, straw and grain matrices, so that a highly level of selectivity was demonstrated and an additional confirmatory method is not necessary.

Matrix Effects

Determination of prothioconazole (JAU-6476), JAU-6476-desthio (M04), JAU-6476-3-hydroxy desthio (M14), JAU-6476-4-hydroxy-desthio (M15), JAU-6476-5-hydroxy-desthio (M16), JAU-6476-6 hydroxydesthio (M17) and JAU-6476- α -hydroxy-desthio (M18) was performed using matrix-matched calibration standards.

Linearity

The linearity of the detector response for JAU-6476 and M04 was demonstrated by single determination of matrix-matched calibration standards at nine concentration levels (dilution factor x10) ranging from 0.0003 $\mu\text{g/mL}$ to 0.1 $\mu\text{g/mL}$ and at seven concentrations levels (dilution factor x100) ranging from 0.001 $\mu\text{g/mL}$ to 0.1 $\mu\text{g/mL}$ for wheat whole plant. These ranges correspond from 0.003 mg/kg to 1 mg/kg (dilution factor x10) and from 0.1 mg/kg to 10 mg/kg (dilution factor x100) for wheat whole plant thus covering the range from no more than 30% of the LOQ and at least + 20 % of the highest analyte concentration level detected in samples.

The linearity of the detector response for M04 and M14, M15, M16, M17, M18 (all wheat matrices) was demonstrated by single determination of matrix-matched calibration standards at seven concentration levels (dilution factor x20) ranging from 0.0001 $\mu\text{g/mL}$ to 0.05 $\mu\text{g/mL}$ for whole plant and grain and at six concentration levels (dilution factor x20) ranging from 0.0001 $\mu\text{g/mL}$ to 0.05 $\mu\text{g/mL}$ for straw. These ranges correspond from 0.002 mg/kg to 1 mg/kg thus covering the range from no more than 30% of the LOQ and at least + 20 % of the highest analyte concentration level detected in samples.

The calibration curves were not forced through zero (y-intercept was calculated) and were derived from a weighted (1/x) linear regression for wheat. For JAU-6476, M04, M14, M15, M16, M17 and M18 both ion mass transitions calibration curves (quantification, confirmation) were linear with the coefficients of correlation (R) greater than 0.99.

Stability of Sample Extracts

The stability of final sample extracts (wheat whole plant, grain and straw) was evaluated by comparison of initially acquired results from analysis of fortification samples at LOQ (0.01 mg/kg) and results obtained from analysis of sample extracts stored refrigerated at 5°C ±4°C. ~~The obtained results presented in Table 28 confirm stability extracts when stored under refrigerated conditions for tested period of time.~~ The stability of final sample extracts as prepared for analysis from representative matrices was evaluated over a period of 2-10 days from extraction.

Stability of Analyte in Final Dilution

The stability of target analytes in final dilution was not tested specifically. Recoveries of the fortified samples within the acceptable range of 70-110% obtained with calibration solutions and the use of bracketing standards to insure integrity of the analytical sequence sufficiently demonstrate the stability.

Quantification

Quantification was performed by using weighted (1/x) linear regression as described in the section “Linearity”.

Accuracy and Precision

Accuracy was determined by fortification of control samples with known amounts of the reference items and subsequent determination of the recoveries when applying the extraction procedures.

Precision was determined by repeatability (relative standard deviation – RSD).

The mean recovery values at the fortification levels of 0.01 mg/kg, 0.5 mg/kg and 5 mg/kg for both ion mass transitions were all in the range 70 – 110 % and thus comply with the standard acceptance criteria of the guidance document SANCO/825/00, rev. 8.1. [1] and SANCO/3029/99, rev. 4 [2].

All precision values at the fortification levels of 0.01 mg/kg, 0.5 mg/kg and 5 mg/kg for both ion mass transitions were < 20%.

Mean recovery and precision results for both ion mass transitions of prothioconazole (JAU-6476), JAU-6476-desthio (M04), JAU-6476-3-hydroxy-desthio (M14), JAU-6476-4-hydroxy-desthio (M15), JAU-6476-5-hydroxy-desthio (M16), JAU-6476-6-hydroxy-desthio (M17) and JAU-6476- α -hydroxy-desthio (M18) are shown in Table 1.

Table 1: Summary of recovery results

Analyte	Crop/ Matrix	Fortification Level	Mean Recovery	RSD	n	Overall	
		[mg/kg]	(%)	(%)		Mean [%]	RSD [%]
Prothioconazole	Quantification Ion Mass Transition m/z 344.0→154.0						
	Wheat whole plant	0.01*	88	7.8	5	88	5.3
		0.5*	89	1.5	5		
		0.5**	93	1.3	5	98	5.4
		5**	102	2.4	5		
	Confirmation Ion Mass Transition m/z 344.0→125.0						
	Wheat whole plant	0.01*	91	5.1	5	91	3.7
		0.5*	90	1.9	5		
		0.5**	92	3.1	5	96	6.0
		5**	101	2.2	5		
JAU-6476- desthio	Quantification Ion Mass Transition m/z 312.0→70.1						
	Wheat whole plant	0.01*	93	2.4	5	94	2.1
		0.5*	95	0.9	5		
		0.5**	108	1.2	5	106	2.5
		5**	104	2.6	5		
	Confirmation Ion Mass Transition m/z 312.0→125.0						
	Wheat whole plant	0.01*	92	2.7	5	94	2.8
		0.5*	95	0.9	5		
		0.5**	107	1.4	5	106	2.0
		5**	105	2.4	5		

* - dilution factor x10

** - dilution factor x100

Table 1: Summary of recovery results (cont.)

Analyte	Crop/ Matrix	Fortification Level	Mean Recovery	RSD	n	Overall	
		[mg/kg]	(%)	(%)		Mean [%]	RSD [%]
JAU-6476- desthio (M04)	Quantification Ion Mass Transition m/z 312.0→70.1						
	Wheat whole plant	0.01	88	2.7	5	89	2.8
		0.5	90	2.9	5		
	Wheat straw	0.01	97	3.7	4	102	5.4
		0.5	106	2.4	5		
	Wheat grain	0.01	96	1.1	5	95	3.3
		0.5	94	4.7	5		
JAU-6476- desthio (M04)	Confirmation Ion Mass Transition m/z 314.0→70.1						
	Wheat whole plant	0.01	90	5.6	5	90	4.2
		0.5	90	2.9	5		
	Wheat straw	0.01	96	3.9	4	101	5.4
		0.5	105	2.5	5		
	Wheat grain	0.01	97	2.1	5	95	4.0
		0.5	94	4.9	5		
JAU-6476-3- hydroxy-desthio (M14)	Quantification Ion Mass Transition m/z 328.1→70.2						
	Wheat whole plant	0.01	89	2.6	5	90	2.9
		0.5	91	2.9	5		
	Wheat straw	0.01	99	1.6	4	101	3.3
		0.5	104	2.1	5		
	Wheat grain	0.01	100	3.1	5	99	4.5
		0.5	98	5.7	5		
JAU-6476-3- hydroxy-desthio (M14)	Confirmation Ion Mass Transition m/z 330.1→70.2						
	Wheat whole plant	0.01	91	3.7	5	91	3.0
		0.5	91	2.7	5		
	Wheat straw	0.01	98	4.1	4	102	4.3
		0.5	104	2.5	5		
	Wheat grain	0.01	102	2.0	5	100	4.5
		0.5	97	5.4	5		

Table 1: Summary of recovery results (cont.)

Analyte	Crop/ Matrix	Fortification Level	Mean Recovery	RSD	n	Overall	
		[mg/kg]	(%)	(%)		Mean [%]	RSD [%]
JAU-6476-4- hydroxy-desthio (M15)	Quantification Ion Mass Transition m/z 328.1→70.2						
	Wheat whole plant	0.01	89	4.0	5	88	3.5
		0.5	88	3.1	5		
	Wheat straw	0.01	93	4.7	4	97	6.0
		0.5	101	4.7	5		
	Wheat grain	0.01	97	2.2	5	96	4.6
		0.5	94	6.3	5		
JAU-6476-4- hydroxy-desthio (M15)	Confirmation Ion Mass Transition m/z 330.1→70.2						
	Wheat whole plant	0.01	90	4.6	5	89	3.9
		0.5	88	3.1	5		
	Wheat straw	0.01	94	5.1	4	98	6.2
		0.5	102	5.0	5		
	Wheat grain	0.01	97	1.8	5	96	4.5
		0.5	94	6.2	5		
JAU-6476-5- hydroxy-desthio (M16)	Quantification Ion Mass Transition m/z 328.1→70.2						
	Wheat whole plant	0.01	88	3.3	5	89	2.7
		0.5	90	2.0	5		
	Wheat straw	0.01	95	5.6	4	100	6.2
		0.5	105	2.4	5		
	Wheat grain	0.01	98	2.6	5	96	5.0
		0.5	94	6.4	5		
JAU-6476-5- hydroxy-desthio (M16)	Confirmation Ion Mass Transition m/z 330.1→70.2						
	Wheat whole plant	0.01	89	3.8	5	90	2.9
		0.5	90	2.0	5		
	Wheat straw	0.01	94	4.7	4	101	7.0
		0.5	106	2.5	5		
	Wheat grain	0.01	97	4.0	5	95	5.4
		0.5	94	6.6	5		

Table 1: Summary of recovery results (cont.)

Analyte	Crop/ Matrix	Fortification Level	Mean Recovery	RSD	n	Overall	
		[mg/kg]	(%)	(%)		Mean [%]	RSD [%]
JAU-6476-6- hydroxy-desthio (M17)	Quantification Ion Mass Transition m/z 328.1→70.2						
	Wheat whole plant	0.01	81	3.6	5	81	3.5
		0.5	80	3.5	5		
	Wheat straw	0.01	90	4.1	4	95	5.7
		0.5	99	3.2	5		
	Wheat grain	0.01	90	2.3	5	88	4.6
		0.5	87	6.2	5		
JAU-6476-6- desthio (M17)	Confirmation Ion Mass Transition m/z 330.1→70.3						
	Wheat whole plant	0.01	81	4.7	5	80	4.3
		0.5	79	3.7	5		
	Wheat straw	0.01	90	7.4	4	95	6.6
		0.5	98	3.1	5		
	Wheat grain	0.01	90	2.7	5	88	4.8
		0.5	86	6.1	5		
JAU-6476-α- hydroxy-desthio (M18)	Quantification Ion Mass Transition m/z 328.1→70.2						
	Wheat whole plant	0.01	95	2.6	5	96	2.4
		0.5	98	1.6	5		
	Wheat straw	0.01	99	3.6	4	105	5.6
		0.5	109	2.4	5		
	Wheat grain	0.01	102	2.5	5	102	4.1
		0.5	102	5.6	5		
JAU-6476-α- hydroxy-desthio (M18)	Confirmation Ion Mass Transition m/z 330.1→70.2						
	Wheat whole plant	0.01	94	2.6	5	95	2.7
		0.5	97	2.0	5		
	Wheat straw	0.01	97	5.6	4	104	6.9
		0.5	109	1.7	5		
	Wheat grain	0.01	102	1.3	5	102	3.9
		0.5	102	5.7	5		

Comments of zRMS:	Analytical method (Grall E. 2019, EGL-18-32944) is acceptable and suitable for the determination of residues prothioconazole and its metabolites in wheat (whole plant, grain and straw).
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Reference: KCP 5.1-14

Report Analytical Phase: Magnitude of the residues of prothioconazole and its metabolites in wheat (whole plant, grain and straw), following two applications of prothioconazole 300 EC in eight trials (4 DCS and 4 HS), Northern and Southern Europe (Poland, Hungary, Portugal and Greece) – 2018
~~Sikorski P., Markowicz A.~~ Grall E. 2019
Report No.: EGL-18-32944
Staphyt Spain S.L.
GLP
Unpublished

Guidelines: Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21-Oct-2009 concerning the placing of plant protection products on the market and repealing council Directives 79/117/EEC and 91/414/EC Guideline 7029/VI/95 (rev. 5) to Directive 91/414/EEC and Regulations (EU) 283/2013 and 284/2013 implementing Regulation (EC) 1107/2009
EU Guidance Document SANCO/3029/99 rev. 4
EU Guidance Document SANCO/825/00 rev. 8.1

Deviations: No

GLP: Yes

Acceptability: Yes

Summary

For the purpose of this analytical phase two different analytical procedures were used.

The general principles of the analytical procedure for determination of prothioconazole and prothioconazole-desthio in wheat whole plant were based on the sample preparation method described in EN 15662:2008 [4] with some modifications. In brief, target samples undergo shakeextraction with mixture of acetonitrile/water containing 2 mg/mL L-Cysteine HCl 4:1 (v:v).

Prothioconazole is quite unstable and might react with oxygen in air to form other products thus the addition of L-Cystine HCl is necessary to minimize the oxidation process. After addition of a buffer-salt mixture containing magnesium sulfate and sodium chloride the sample was shaken again. Following centrifugation, the supernatant was further cleaned and the excess of water removed with a single d-SPE step by mixing with magnesium sulphate and graphitized carbon black (GCB). The sample was vortex and centrifuged.

The general principles of the analytical procedure (reflux method) for determination of JAU-6476-desthio (M04), JAU-6476-3-hydroxy-desthio (M14), JAU-6476-4-hydroxy-desthio (M15), JAU-6476-5-hydroxy-desthio (M16), JAU-6476-6-hydroxy-desthio (M17) and JAU-6476- α -hydroxy-desthio (M18) in wheat whole plant, straw and grain were derived from Bayer CropScience analytical method 00979/M001 [5]. In brief, target analytes undergo shake-extraction with a mixture of acetonitrile/water

4:1 (v:v) followed by a liquid-liquid partitioning step and phase separation by adding salt mixture containing magnesium sulphate and sodium chloride. Following centrifugation, an aliquot of the acetonitrile phase was evaporated almost to dryness. The remainder was diluted and acidified with 5 N hydrochloric acid and heated at reflux for 2 hours. This hydrolysis step was performed to convert glycoside-bound analogues into the respective hydroxy analytes. Subsequently the samples were neutralized with sodium hydroxide and undergo second shake-extraction with acetonitrile.

After addition of salt mixture containing magnesium sulfate and sodium chloride extract was shaken again. Following centrifugation an aliquot of the acetonitrile phase was decanted into Eppendorf tube containing magnesium sulphate for additional dehydration. The sample was vortex and centrifuged.

For quantitative analysis in both extraction methods the final extract was diluted with acidified water and subjected to LC-ESI-MS/MS. All analytes were detected using electrospray ionization in the positive ion mode (ESI+)

Selectivity and Confirmation of Residue Identity

Quantification was performed by use of highly selective liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). Two selected ion mass transitions were evaluated in order to demonstrate that the method achieves a high level of selectivity. The retention times of target analyte in extracts corresponds to that of the calibration standards with a tolerance of ± 0.15 min.

Confirmation ion ratio for all analytes in all samples were within ± 30 % of the average found for the standards.

No significant interference above 30 % of LOQ was detected in any of the reagent blanks or control specimen extracts for wheat (whole plants, grain and straw) matrices, so that a highly level of selectivity was demonstrated and an additional confirmatory method is not necessary.

Matrix Effects

Determination of prothioconazole (JAU-6476), JAU-6476-desthio (M04), JAU-6476-3-hydroxy-desthio (M14), JAU-6476-4-hydroxy-desthio (M15), JAU-6476-5-hydroxy-desthio (M16), JAU-6476-6-hydroxydesthio (M17) and JAU-6476- α -hydroxy-desthio (M18) was performed using matrix-matched calibration standards.

Linearity

The linearity of the detector response for JAU-6476 and M04 was demonstrated by single determination of matrix-matched calibration standards at nine concentration levels (dilution factor x10) ranging from 0.0003 $\mu\text{g/mL}$ to 0.1 $\mu\text{g/mL}$ and at seven concentrations levels (dilution factor x100) ranging from 0.001 $\mu\text{g/mL}$ to 0.1 $\mu\text{g/mL}$ for wheat whole plant. These ranges correspond from 0.003 mg/kg to 1 mg/kg (dilution factor x10) and from 0.1 mg/kg to 10 mg/kg (dilution factor x100) for wheat whole plant thus covering the range from no more than 30% of the LOQ and at least + 20 % of the highest analyte concentration level detected in samples.

The linearity of the detector response for M04 and M14, M15, M16, M17, M18 (all wheat matrices) was demonstrated by single determination of matrix-matched calibration standards at seven concentration levels (dilution factor x20) ranging from 0.0001 $\mu\text{g/mL}$ to 0.05 $\mu\text{g/mL}$ for wheat (whole plant, grain and straw). These ranges correspond from 0.002 mg/kg to 1 mg/kg thus covering the range from no more than 30% of the LOQ and at least + 20 % of the highest analyte concentration level detected in samples.

The calibration curves were not forced through zero (y-intercept was calculated) and were derived from a weighted (1/x) linear regression for wheat. For JAU-6476, M04, M14, M15, M16, M17 and M18 both ion mass transitions calibration curves (quantification, confirmation) were linear with the coefficients of correlation (R) greater than 0.99.

Stability of Analyte in Final Dilution

The stability of target analytes in final dilution was not tested specifically. Recoveries of the fortified samples within the acceptable range of 70-110% obtained with calibration solutions and the use of bracketing standards to insure integrity of the analytical sequence sufficiently demonstrate the stability.

Quantification

Quantification was performed by using weighted (1/x) linear regression as described in the section “Linearity”.

Accuracy and Precision

Accuracy was determined by fortification of control samples with known amounts of the reference items and subsequent determination of the recoveries when applying the extraction procedures.

Precision was determined by repeatability (relative standard deviation – RSD).

The mean recovery values at the fortification level of 0.01 mg/kg for both ion mass transitions were all in the range 70 – 110 % and thus comply with the standard acceptance criteria of the guidance document SANCO/825/00, rev. 8.1. [1] and SANCO/3029/99, rev. 4 [2]. All precision values at the fortification level of 0.01 mg/kg for both ion mass transitions were < 20%.

Comments of zRMS:	Analytical method (Bodsch J., 2021; IF21-05707610) is acceptable and suitable for the determination of residues prothioconazole and its metabolites in wheat (whole plant, grain and straw).
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Reference: KCP 5.1-15

Report Study on the residue behaviour of prothioconazole in wheat after treatment with Prothioconazole 300 EC at eight sites under field conditions in Southern Europe 2021
~~Bodsch J., Kravchuk O.~~ Gabriel E.
Report No.: IF21-05707610
SGS Institute Fresenius, GmbH
GLP
Unpublished

Guidelines: SANTE/2020/12830, Rev.1 (24. February 2021)
OECD ENV/JM/MONO(2007) 17 - Guidance Document on Pesticide Residue Analytical Methods
Good Laboratory Practice Regulations: Appendix 1 to § 19a, Section 1, of the German Chemikaliengesetz (Chemicals Act)
OECD Principles of Good Laboratory Practice ENV/MC/CHEM(98)17, Paris 1998
The application of the OECD Principles of GLP to the Organisation and Management of Multi-Site Studies, ENV/JM/MONO(2002)9, 25/06/2002.

Deviations: No

GLP: Yes

Acceptability: Yes

Abstract

During the growing season of 2021, eight field trials with wheat were conducted in southern Europe (trial 21-00227-01 – Southern France, trial 21-00227-02 – Southern France, trial 21- 00227-03 – Spain, trial 21-00227-04 – Greece, trial 21-00227-05 – Spain, trial 21-00227-06 – Greece, trial 21-00227-07 – Italy, trial 21-00227-08 – Italy). The magnitude of residues in wheat specimens was determined for the triazole metabolites 1,2,4-Triazole (1,2,4-T), Triazole alanine (TA), Triazole acetic acid (TAA) and Triazole lactic acid (TLA) after spray application with Prothioconazole 300 EC.

Prothioconazole 300 EC (300 g Prothioconazole/L) was applied two times with nominal content 195 g prothioconazole/ha and a nominal water volume of 200-400 L/ha.

Specimens of wheat were collected at a nominal sampling timing 0 (S1), 7±1 (S2) and 14±1 (S3) D LA as whole plant, 35 ± 3 DALA (S4) as ears and rest of plant or grain and straw (depending on growth stage) and at normal commercial harvest (growth stage BBCH 89) as grain and straw (S5). If 35 ± 3 DALA and NCH (BBCH 89) sampling occurred at the same time, only the NCH was done using specimen numbers of NCH.

The wheat specimens were analysed at SGS INSTITUT FRESENIUS GmbH, c/o SGS Germany GmbH, Hamburg, Germany for residues of the triazole metabolites following the analytical method 01062/M004[1].

The Limit of Quantification (LOQ) of the analytical method was defined as 0.010 mg/kg for each analyte. The Limit of Detection (LOD) was defined to be 0.003 mg/kg for each analyte (lowest calibration standard used).

Linearity was shown over a range of 1.0 to 100 ng/mL for the analytes with correlation coefficients of ≥ 0.998 and no visible trend in the residuals. Therefore, the calibration model was defined to be suitable. The accuracy of the method was demonstrated by recovery experiments, control specimens were fortified prior to extraction with 1,2,4-T, TA, TAA and TLA.

The mean recovery rate values, the standard deviations (SD) and the coefficients of variation (CV) were as follows:

Crop	Matrix	TA		
		Mean (%)	RSD	n
Wheat	Overall	97.4	8.6	38
Wheat	Overall	87.2	9.7	38
Wheat	Overall	89.4	10	38
Wheat	Overall	87.2	9.0	38

Summary of procedural recoveries for 1,2,4-Triazole (1,2,4-T), Triazole alanine (TA), Triazole acetic acid (TAA) and Triazole lactic acid (TLA)

Crop	Matrix	Fortification Level (nominal) [mg/kg]	1,2,4-T		
			Mean [%]	RSD [±]	n
Wheat	Grain	0.010, 0.10 and 2.0	96.9	11	9
	Straw	0.010, 0.10 and 2.0	89.2	8.8	7
	Ears	0.010, 0.10 and 2.0	102	5.1	7
	Rest of Plant without Roots	0.010, 0.10 and 2.0	102	6.9	7
	Whole Plant no Roots	0.010, 0.10 and 2.0	97.1	5.1	8
Overall			97.4	8.6	38
Crop	Matrix	Fortification Level (nominal) [mg/kg]	TA		
			Mean [%]	RSD [±]	n
Wheat	Grain	0.010, 0.10 and 2.0	81.7	10	9
	Straw	0.010, 0.10 and 2.0	89.7	14	7
	Ears	0.010, 0.10 and 2.0	81.1	3.3	7
	Rest of Plant without Roots	0.010, 0.10 and 2.0	91.9	3.7	7
	Whole Plant no Roots	0.010, 0.10 and 2.0	92.2	5.7	8
Overall			87.2	9.7	38
Crop	Matrix	Fortification Level (nominal) [mg/kg]	TAA		
			Mean [%]	RSD [±]	n
Wheat	Grain	0.010, 0.10 and 2.0	77.4	7.0	9
	Straw	0.010, 0.10 and 2.0	88.0	5.6	7
	Ears	0.010, 0.10 and 2.0	88.4	5.2	7
	Rest of Plant without Roots	0.010, 0.10 and 2.0	97.3	4.2	7
	Whole Plant no Roots	0.010, 0.10 and 2.0	98.7	4.1	8
Overall			89.4	10	38
Crop	Matrix	Fortification Level (nominal) [mg/kg]	TLA		
			Mean [%]	RSD [±]	n
Wheat	Grain	0.010, 0.10 and 2.0	80.2	8.7	9
	Straw	0.010, 0.10 and 2.0	86.3	8.0	7
	Ears	0.010, 0.10 and 2.0	82.5	4.9	7
	Rest of Plant without Roots	0.010, 0.10 and 2.0	94.6	3.8	7
	Whole Plant no Roots	0.010, 0.10 and 2.0	93.7	3.6	8
Overall			87.2	9.0	38

SD: Standard deviation; CV: Coefficient of variation; n: Number of specimens

Comments of zRMS:	Analytical method (Meyer M., 2022, IF21-05707333) is acceptable and suitable for the determination of residues prothioconazole and its metabolites in oilseed rape (whole plant, grain and straw).
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Reference:	KCP 5.1-15
Report	Study on the residue behaviour of prothioconazole in oilseed rape after treatment with Prothioconazole 300 EC at eight sites under field conditions in Northern Europe Meyer M., Bodesch J. Report No.: IF21-05707333 SGS Institute Fresenius, GmbH GLP Unpublished
Guidelines:	SANTE/2020/12830, Rev.1 (24. February 2021) OECD ENV/JM/MONO(2007) 17 - Guidance Document on Pesticide Residue Analytical Methods Good Laboratory Practice Regulations: Appendix 1 to § 19a, Section 1, of the German Chemikaliengesetz (Chemicals Act) OECD Principles of Good Laboratory Practice ENV/MC/CHEM(98)17, Paris 1998 The application of the OECD Principles of GLP to the Organisation and Management of Multi-Site Studies, ENV/JM/MONO(2002)9, 25/06/2002.
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Summary

During the growing season of 2021, eight field trials with wheat were conducted in Northern Europe (Trial 21-00230-01 – Germany, Trial 21-00230-02 – Germany, Trial 21-00230-03 – Northern France, Trial 21-00230-04 – Poland, Trial 21-00230-05 – Germany, Trial 21-00230-06 – Denmark, Trial 21-00230-07 – Poland, Trial 21-00230-08 – Hungary). The magnitude of residues in oilseed rape specimens was determined for the triazole metabolites 1,2,4-Triazole, Triazole alanine, Triazole acetic acid and Triazole lactic acid after spray application with Prothioconazole 300 EC.

Prothioconazole 300 EC (300 g Prothioconazole/L) was applied two times with nominal content 180 g prothioconazole/ha and a nominal water volume of 200-400 L/ha.

For decline trials specimens of oilseed rape were collected at a nominal sampling timing 0 (S1), 7±1 (S2) and 14±1 (S3) DALA as whole plant, 35 ± 3 DALA (S4) as pods and rest of plant and at normal commercial harvest (growth stage BBCH 89) as seed and straw (S5). For harvest trials specimens of oilseed rape were collected at normal commercial harvest (growth stage BBCH 89) as seed and straw only.

The oilseed rape specimens were analysed at SGS INSTITUT FRESENIUS GmbH, c/o SGS Germany GmbH, Hamburg, Germany for residues of the triazole metabolites following the analytical method 01062/M004[1]. The method was validated at the analytical test site in study IF21-05707367[4] for the matrix groups relevant in this study.

The Limit of Quantification (LOQ) of the analytical method was defined as 0.010 mg/kg for each analyte. The Limit of Detection (LOD) was defined to be 0.003 mg/kg for each analyte (lowest calibration standard used).

Linearity was shown over a range of 1.25 to 100 ng/mL for the analytes with correlation coefficients of ≥ 0.998 and no visible trend in the residuals. Therefore, the calibration model was defined to be suitable. The accuracy of the method was demonstrated by recovery experiments, control specimens were fortified prior to extraction with 1,2,4-T, TA, TAA and TLA.

The mean recovery rate values and relative standard deviations (RSD) were as follows:

Crop	Matrix	TA		
		Mean (%)	RSD	n
Oilseed rape	Overall	91.6	11	43
Oilseed rape	Overall	85.2	14	41
Oilseed rape	Overall	91.3	8.8	43
Oilseed rape	Overall	87.2	12	43

Crop	Matrix	Fortification Level (nominal) [mg/kg]	1,2,4-T		
			Mean [%]	RSD [±]	n
Oilseed Rape	Whole Plant	0.010, 0.10 and 1.0	86.1	8.3	7
	Pods	0.010, 0.10 and 1.0	98.9	7.0	7
	Rest of Plant	0.010, 0.10 and 1.0	93.1	10	7
	Straw	0.010, 0.10 and 1.0	92.9	12	12
	Seed	0.010, 0.10, 1.0 and 10	87.5	14	10
Overall			91.6	11	43
Crop	Matrix	Fortification Level (nominal) [mg/kg]	TA		
			Mean [%]	RSD [±]	n
Oilseed Rape	Whole Plant	0.010, 0.10 and 1.0	90.6	11	7
	Pods	0.010, 0.10 and 1.0	88.7	12	7
	Rest of Plant	0.010, 0.10 and 1.0	91.2	9.8	7
	Straw	0.010, 0.10 and 1.0	79.7	18	12
	Seed	0.010, 0.10, 1.0 and 10	80.6	13	8
Overall			85.2	14	41
Crop	Matrix	Fortification Level (nominal) [mg/kg]	TAA		
			Mean [%]	RSD [±]	n
Oilseed Rape	Whole Plant	0.010, 0.10 and 1.0	96.5	6.5	7
	Pods	0.010, 0.10 and 1.0	97.8	2.2	7
	Rest of Plant	0.010, 0.10 and 1.0	94.4	5.2	7
	Straw	0.010, 0.10 and 1.0	83.1	8.3	12
	Seed	0.010, 0.10, 1.0 and 10	90.6	7.2	10
Overall			91.3	8.8	43
Crop	Matrix	Fortification Level (nominal) [mg/kg]	TLA		
			Mean [%]	RSD [±]	n
Oilseed Rape	Whole Plant	0.010, 0.10 and 1.0	98.5	6.1	7
	Pods	0.010, 0.10 and 1.0	91.6	7.1	7
	Rest of Plant	0.010, 0.10 and 1.0	90.5	5.5	7
	Straw	0.010, 0.10 and 1.0	76.4	13	12
	Seed	0.010, 0.10, 1.0 and 10	86.7	6.1	10

	Overall	87.2	12	43
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RSD: Relative Standard Deviation; n: Number of specimens

Comments of zRMS:	Analytical method (Delmotte R., 2019, RDE-17-28968) is acceptable and suitable for the determination of residues prothioconazole and its metabolites in oilseed rape (whole plant, grain and straw).
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Reference:	KCP 5.1-17
Report	Analytical Phase: Magnitude of the residues of prothioconazole and its metabolites in oilseed rape (whole plant, seeds and straw), following two applications of prothioconazole 300 EC in eight trials (4 DCS and 4 HS), Northern and Southern Europe (Poland, Hungary, Portugal and Greece) – 2017 Sikorski P., Markowicz A. Delmotte R. 2019 Report No.: RDE-17-28968 Staphyt Spain S.L. GLP Unpublished
Guidelines:	Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21-Oct-2009 concerning the placing of plant protection products on the market and repealing council Directives 79/117/EEC and 91/414/EC Guideline 7029/VI/95 (rev. 5) to Directive 91/414/EEC and Regulations (EU) 283/2013 and 284/2013 implementing Regulation (EC) 1107/2009 EU Guidance Document SANCO/3029/99 rev. 4 EU Guidance Document SANCO/825/00 rev. 8.1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Summary

For the purpose of this analytical phase two different analytical procedures were validated. The general principles of the analytical procedure for determination of prothioconazole and prothioconazole-desthio in oilseed rape (OSR) whole plants and whole plants without pods were based on the sample preparation method described in EN 15662:2008 [3] with some modifications.

In brief, target samples undergo shake-extraction with mixture of acetonitrile/water containing 2 mg/mL L-Cysteine HCl 4:1 (v:v). Prothioconazole is quite unstable and might react with oxygen in air to form other products thus the addition of L-Cystine HCl is necessary to minimize the oxidation process. After addition of a buffer-salt mixture containing magnesium sulfate and sodium chloride the sample was shaken again. Following centrifugation, the supernatant was further cleaned and the excess of water removed with a single d-SPE step by mixing with magnesium sulphate and graphitized carbon black (GCB). The sample was vortex and centrifuged.

The general principles of the analytical procedure (reflux method) for determination of JAU-6476 desthio (M04), JAU-6476-3-hydroxy-desthio (M14), JAU-6476-4-hydroxy-desthio (M15), JAU-6476-

5-hydroxy-desthio (M16), JAU-6476-6-hydroxy-desthio (M17) and JAU-6476- α -hydroxy-desthio (M18) in oilseed rape pods, straw and seeds were derived from Bayer CropScience analytical method 00979/M001 [4]. In brief, target analytes undergo shake-extraction with a mixture of acetonitrile/water 4:1 (v:v) followed by a liquid-liquid partitioning step and phase separation by adding salt mixture containing magnesium sulphate and sodium chloride. Following centrifugation, an aliquot of the acetonitrile phase was evaporated almost to dryness. The remainder was diluted and acidified with 5 N hydrochloric acid and heated at reflux for 2 hours. This hydrolysis step was performed to convert glycoside-bound analogues into the respective hydroxy analytes. Subsequently the samples were neutralized with sodium hydroxide and undergo second shake-extraction with acetonitrile.

After addition of salt mixture containing magnesium sulfate and sodium chloride extract was shaken again. Following centrifugation an aliquot of the acetonitrile phase was decanted into Eppendorf tube containing magnesium sulphate for additional dehydration. The sample was vortex and centrifuged. For quantitative analysis in both extraction methods the final extract was diluted with acidified water and subjected to LC-ESI-MS/MS. All analytes were detected using electrospray ionization in the positive ion mode (ESI+)

Selectivity and Confirmation of Residue Identity

Quantification was performed by use of highly selective liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). Two selected ion mass transitions were evaluated in order to demonstrate that the method achieves a high level of selectivity. The retention times of target analyte in extracts corresponds to that of the calibration standards with a tolerance of $< \pm 0.15$ min. Confirmation ion ratio for all analytes in all samples were within ± 30 % of the average found for the standards. No significant interference above 30 % of LOQ was detected in any of the reagent blanks or control specimen extracts for oilseed rape whole plants, whole plants without pods, pods, straw and seeds matrices, so that a highly level of selectivity was demonstrated and an additional confirmatory method is not necessary.

Matrix Effects

Determination of prothioconazole (JAU-6476), JAU-6476-desthio (M04), JAU-6476-3-hydroxy desthio (M14), JAU-6476-4-hydroxy-desthio (M15), JAU-6476-5-hydroxy-desthio (M16), JAU-6476-6-hydroxydesthio (M17) and JAU-6476- α -hydroxy-desthio (M18) was performed using matrix-matched calibration standards.

Linearity

The linearity of the detector response for JAU-6476 and M04 was demonstrated by single determination of matrix-matched calibration standards at nine concentration levels (dilution factor x10) ranging from 0.0003 $\mu\text{g/mL}$ to 0.1 $\mu\text{g/mL}$ and at seven concentrations levels (dilution factor x100) ranging from 0.001 $\mu\text{g/mL}$ to 0.1 $\mu\text{g/mL}$ for OSR whole plants. These ranges correspond from 0.003 mg/kg to 1 mg/kg (dilution factor x10) and from 0.1 mg/kg to 10 mg/kg (dilution factor x100) for OSR whole plants thus covering the range from no more than 30% of the LOQ and at least + 20 % of the highest analyte concentration level detected in samples.

The linearity of the detector response for M04 and M14, M15, M16, M17, M18 (all OSR matrices) was demonstrated by single determination of matrix-matched calibration standards at seven concentration levels (dilution factor x20) ranging from 0.0001 $\mu\text{g/mL}$ to 0.05 $\mu\text{g/mL}$. These ranges correspond from 0.002 mg/kg to 1 mg/kg thus covering the range from no more than 30% of the LOQ and at least + 20 % of the highest analyte concentration level detected in samples.

The calibration curves were not forced through zero (y-intercept was calculated) and were derived from a weighted (1/x) linear regression for OSR. For JAU-6476, M04, M14, M15, M16, M17 and M18 both ion mass transitions calibration curves (quantification, confirmation) were linear with the coefficients of correlation (R) greater than 0.99.

Stability of Sample Extracts

The stability of final sample extracts (OSR whole plants, seeds and straw) was evaluated by comparison of initially acquired results from analysis of fortification samples at LOQ (0.01 mg/kg) and results obtained from analysis of sample extracts stored refrigerated at 5°C ±4°C. The obtained results presented in Table 27 confirm stability extracts when stored under refrigerated conditions for tested period of time.

Stability of Analyte in Final Dilution

The stability of target analytes in final dilution was not tested specifically. Recoveries of the fortified samples within the acceptable range of 70-110% obtained with calibration solutions and the use of bracketing standards to insure integrity of the analytical sequence sufficiently demonstrate the stability.

Quantification

Quantification was performed by using weighted (1/x) linear regression as described in the section “Linearity”.

Accuracy and Precision

Accuracy was determined by fortification of control samples with known amounts of the reference items and subsequent determination of the recoveries when applying the extraction procedures.

Precision was determined by repeatability (relative standard deviation – RSD).

The mean recovery values at the fortification levels of 0.01 mg/kg, 0.5 mg/kg and 5 mg/kg for both ion mass transitions were all in the range 70 – 110 % and thus comply with the standard acceptance criteria of the guidance document SANCO/825/00, rev. 8.1. [1] and SANCO/3029/99, rev. 4 [2].

All precision values at the fortification levels of 0.01 mg/kg, 0.5 mg/kg and 5 mg/kg for both ion mass transitions were < 20%.

Mean recovery and precision results for both ion mass transitions of prothioconazole (JAU-6476), JAU-6476-desthio (M04), JAU-6476-3-hydroxy-desthio (M14), JAU-6476-4-hydroxy-desthio (M15), JAU-6476-5-hydroxy-desthio (M16), JAU-6476-6-hydroxy-desthio (M17) and JAU-6476- α -hydroxydesthio (M18) are shown in Table 1.

Table 1: Summary of recovery results

Analyte	Crop/ Matrix	Fortification Level	Mean Recovery	RSD	n	Overall	
		[mg/kg]	(%)	(%)		Mean [%]	RSD [%]
Prothioconazole	Quantification Ion Mass Transition m/z 344.0→154.0						
	OSR Whole Plant	0.01*	87	5.7	5	90	4.9
		0.5*	92	1.9	5		
		0.5**	90	4.7	5	94	6.2
		5**	99	2.1	5		
	Confirmation Ion Mass Transition m/z 344.0→125.0						
	OSR Whole Plant	0.01*	90	5.5	5	91	4.2
		0.5*	93	1.3	5		
		0.5**	89	2.2	5	95	6.2
		5**	100	1.9	5		
JAU-6476-desthio	Quantification Ion Mass Transition m/z 312.0→70.1						
	OSR Whole Plant	0.01*	92	2.0	5	94	3.1
		0.5*	97	1.4	5		
		0.5**	103	2.3	5	103	1.8
		5**	103	1.5	5		
	Confirmation Ion Mass Transition m/z 312.0→125.0						
	OSR Whole Plant	0.01*	89	1.1	5	93	4.7
		0.5*	97	1.1	5		
		0.5**	103	2.1	5	103	1.7
		5**	104	1.3	5		

* - dilution factor x10

** - dilution factor x100

Analyte	Crop/ Matrix	Fortification Level	Mean Recovery	RSD	n	Overall	
		[mg/kg]	(%)	(%)		Mean [%]	RSD [%]
JAU-6476-desthio (M04)	Quantification Ion Mass Transition m/z 312.0→70.1						
	OSR whole plant	0.01	90	8.7	5	91	7.0
		0.5	92	5.8	5		
	OSR pods	0.01	101	16.2	5	103	11.0
		0.5	105	2.9	5		
	OSR straw	0.01	76	3.6	5	78	7.3
		0.5	80	9.7	5		
	OSR seeds	0.01	90	1.7	5	94	4.5
		0.5	98	1.5	5		
	JAU-6476-desthio (M04)	Confirmation Ion Mass Transition m/z 314.0→70.1					
OSR whole plant		0.01	90	8.2	5	91	6.9
		0.5	93	5.9	5		
OSR pods		0.01	100	18.0	5	103	12.1
		0.5	105	2.9	5		
OSR straw		0.01	76	3.1	5	78	7.4
		0.5	79	9.9	5		
OSR seeds		0.01	90	3.0	5	94	5.4
		0.5	98	1.3	5		
JAU-6476-3- hydroxy-desthio (M14)		Quantification Ion Mass Transition m/z 328.1→70.2					
	OSR whole plant	0.01	88	7.2	5	90	6.8
		0.5	91	6.6	5		
	OSR pods	0.01	93	6.0	5	99	7.9
		0.5	106	2.7	5		
	OSR straw	0.01	83	5.6	5	82	6.1
		0.5	82	7.1	5		
	OSR seeds	0.01	92	6.0	5	94	4.8
		0.5	97	1.5	5		
	JAU-6476-3- hydroxy-desthio (M14)	Confirmation Ion Mass Transition m/z 330.01→70.2					
OSR whole plant		0.01	88	8.1	5	90	7.5
		0.5	92	7.1	5		
OSR pods		0.01	92	6.6	5	99	9.1
		0.5	107	3.0	5		
OSR straw		0.01	83	2.8	5	83	5.4
		0.5	82	7.5	5		
OSR seeds		0.01	94	5.5	5	95	4.0
		0.5	96	1.9	5		

Analyte	Crop/ Matrix	Fortification Level	Mean Recovery	RSD	n	Overall	
		[mg/kg]	(%)	(%)		Mean [%]	RSD [%]
JAU-6476-4- hydroxy-desthio (M15)	Quantification Ion Mass Transition m/z 328.1→70.2						
	OSR whole plant	0.01	81	6.8	5	84	8.4
		0.5	87	8.6	5		
	OSR pods	0.01	89	5.4	5	96	8.3
		0.5	103	2.8	5		
	OSR straw	0.01	76	3.0	5	78	6.3
		0.5	80	7.9	5		
	OSR seeds	0.01	90	1.9	5	93	3.7
		0.5	96	1.8	5		
	JAU-6476-4- hydroxy-desthio (M15)	Confirmation Ion Mass Transition m/z 330.01→70.2					
OSR whole plant		0.01	82	7.5	5	85	8.7
		0.5	88	8.8	5		
OSR pods		0.01	89	6.2	5	97	9.2
		0.5	104	2.8	5		
OSR straw		0.01	76	5.5	5	78	7.2
		0.5	81	7.9	5		
OSR seeds		0.01	91	2.1	5	94	3.3
		0.5	96	1.6	5		
JAU-6476-5- hydroxy-desthio (M16)		Quantification Ion Mass Transition m/z 328.1→70.2					
	OSR whole plant	0.01	81	7.7	5	84	9.6
		0.5	87	10.6	5		
	OSR pods	0.01	92	10.2	5	101	11.2
		0.5	109	2.9	5		
	OSR straw	0.01	79	5.4	5	81	6.5
		0.5	82	7.5	5		
	OSR seeds	0.01	92	8.4	5	92	6.4
		0.5	92	4.5	5		
	JAU-6476-5- hydroxy-desthio (M16)	Confirmation Ion Mass Transition m/z 330.01→70.2					
OSR whole plant		0.01	80	8.9	5	84	10.4
		0.5	87	10.7	5		
OSR pods		0.01	90	10.0	5	100	12.3
		0.5	110	3.3	5		
OSR straw		0.01	79	4.8	5	80	6.6
		0.5	82	7.6	5		
OSR seeds		0.01	96	8.6	5	94	7.0
		0.5	92	4.8	5		

Analyte	Crop/ Matrix	Fortification Level	Mean Recovery	RSD	n	Overall	
		[mg/kg]	(%)	(%)		Mean [%]	RSD [%]
JAU-6476-6- hydroxy-desthio (M17)	Quantification Ion Mass Transition m/z 328.1→70.2						
	OSR whole plant	0.01	78	5.6	5	80	8.7
		0.5	81	11.1	5		
	OSR pods	0.01	90	9.8	5	95	8.6
		0.5	100	3.0	5		
	OSR straw	0.01	73	4.1	5	76	11.2
		0.5	80	13.9	5		
	OSR seeds	0.01	88	3.5	5	90	3.5
		0.5	92	2.0	5		
	JAU-6476-6- desthio (M17)	Confirmation Ion Mass Transition m/z 330.01→70.3					
OSR whole plant		0.01	80	5.7	5	80	8.4
		0.5	81	11.1	5		
OSR pods		0.01	89	8.9	5	95	8.8
		0.5	101	2.6	5		
OSR straw		0.01	73	5.8	5	76	11.3
		0.5	79	14.3	5		
OSR seeds		0.01	89	2.4	5	91	2.3
		0.5	92	1.5	5		
JAU-6476-α- hydroxy-desthio (M18)		Quantification Ion Mass Transition m/z 328.1→70.2					
	OSR whole plant	0.01	89	7.6	5	93	8.5
		0.5	98	6.9	5		
	OSR pods	0.01	97	1.9	5	103	6.1
		0.5	108	2.9	5		
	OSR straw	0.01	92	4.2	5	88	8.2
		0.5	84	9.3	5		
	OSR seeds	0.01	96	4.0	5	100	5.3
		0.5	104	2.3	5		
	JAU-6476-α- hydroxy-desthio (M18)	Confirmation Ion Mass Transition m/z 330.01→70.2					
OSR whole plant		0.01	90	6.6	5	94	7.8
		0.5	98	7.2	5		
OSR pods		0.01	98	1.8	5	103	5.5
		0.5	108	3.4	5		
OSR straw		0.01	90	4.8	5	87	8.1
		0.5	83	9.3	5		
OSR seeds		0.01	98	3.0	5	101	4.5
		0.5	105	1.6	5		

Comments of zRMS:	Analytical method (Delmotte R., 2019, RDE-18-32946) is acceptable and suitable for the determination of residues prothioconazole and its metabolites in oilseed rape (whole plant, grain and straw).
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Reference: KCP 5.1-18

Report Analytical Phase: Magnitude of the residues of prothioconazole and its metabolites in oilseed rape (whole plant, seeds and straw), following two applications of HAG 610 01F in six trials (3 DCS and 3 HS), Northern and Southern Europe (Poland, Hungary, Portugal and Greece) – 2018
~~Markowicz A.~~ Delmotte R.
Report No.: RDE-18-32946
Staphyt Spain S.L.
GLP
Unpublished

Guidelines: Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21-Oct-2009 concerning the placing of plant protection products on the market and repealing council Directives 79/117/EEC and 91/414/EC
Guideline 7029/VI/95 (rev. 5) to Directive 91/414/EEC and Regulations (EU) 283/2013 and 284/2013 implementing Regulation (EC) 1107/2009
EU Guidance Document SANCO/3029/99 rev. 4
EU Guidance Document SANCO/825/00 rev. 8.1

Deviations: No

GLP: Yes

Acceptability: Yes

Summary

For the purpose of this analytical phase two different analytical procedures were used.

The general principles of the analytical procedure for determination of prothioconazole and prothioconazole-desthio in oilseed rape (OSR) whole plant were based on the sample preparation method described in EN 15662:2008 [4] with some modifications. In brief, target samples undergo shake-extraction with mixture of acetonitrile/water containing 2 mg/mL L-Cysteine HCl 4:1 (v:v).

Prothioconazole is quite unstable and might react with oxygen in air to form other products thus the addition of L-Cystine HCl is necessary to minimize the oxidation process. After addition of a buffer-salt mixture containing magnesium sulfate and sodium chloride the sample was shaken again. Following centrifugation, the supernatant was further cleaned and the excess of water removed with a single d-SPE step by mixing with magnesium sulphate and graphitized carbon black (GCB). The sample was vortex and centrifuged.

The general principles of the analytical procedure (reflux method) for determination of JAU-6476-desthio (M04), JAU-6476-3-hydroxy-desthio (M14), JAU-6476-4-hydroxy-desthio (M15), JAU-6476-5-hydroxy-desthio (M16), JAU-6476-6-hydroxy-desthio (M17) and JAU-6476- α -hydroxy-desthio (M18) in oilseed rape whole plant, pods, straw and seed were derived from Bayer CropScience analytical method 00979/M001 [5]. In brief, target analytes undergo shake-extraction with a mixture of acetonitrile/water 4:1 (v:v) followed by a liquid-liquid partitioning step and phase separation by adding

salt mixture containing magnesium sulphate and sodium chloride. Following centrifugation, an aliquot of the acetonitrile phase was evaporated almost to dryness. The remainder was diluted and acidified with 5 N hydrochloric acid and heated at reflux for 2 hours. This hydrolysis step was performed to convert glycoside-bound analogues into the respective hydroxy analytes. Subsequently the samples were neutralized with sodium hydroxide and undergo second shake-extraction with acetonitrile. After addition of salt mixture containing magnesium sulfate and sodium chloride extract was shaken again. Following centrifugation an aliquot of the acetonitrile phase was decanted into Eppendorf tube containing magnesium sulphate for additional dehydration. The sample was vortex and centrifuged.

For quantitative analysis in both extraction methods the final extract was diluted with acidified water and subjected to LC-ESI-MS/MS. All analytes were detected using electrospray ionization in the positive ion mode (ESI+)

Selectivity and Confirmation of Residue Identity

Quantification was performed by use of highly selective liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). Two selected ion mass transitions were evaluated in order to demonstrate that the method achieves a high level of selectivity. The retention times of target analyte in extracts corresponds to that of the calibration standards with a tolerance of $< \pm 0.15$ min.

Confirmation ion ratio for all analytes in all samples were within ± 30 % of the average found for the standards.

No significant interference above 30 % of LOQ was detected in any of the reagent blanks or control-specimen extracts for oilseed rape (whole plants, pods, seeds and straw) matrices, so that a highly level of selectivity was demonstrated and an additional confirmatory method is not necessary.

Matrix Effects

Determination of prothioconazole (JAU-6476), JAU-6476-desthio (M04), JAU-6476-3-hydroxy-desthio (M14), JAU-6476-4-hydroxy-desthio (M15), JAU-6476-5-hydroxy-desthio (M16), JAU-6476-6-hydroxydesthio (M17) and JAU-6476- α -hydroxy-desthio (M18) was performed using matrix-matched calibration standards.

Linearity

The linearity of the detector response for JAU-6476 and M04 was demonstrated by single determination of matrix-matched calibration standards at nine concentration levels (dilution factor x10) ranging from 0.0003 $\mu\text{g/mL}$ to 0.1 $\mu\text{g/mL}$ and at seven concentrations levels (dilution factor x100) ranging from 0.001 $\mu\text{g/mL}$ to 0.1 $\mu\text{g/mL}$ for oilseed rape whole plant. These ranges correspond from 0.003 mg/kg to 1 mg/kg (dilution factor x10) and from 0.1 mg/kg to 10 mg/kg (dilution factor x100) for oilseed rape whole plant thus covering the range from no more than 30% of the LOQ and at least + 20 % of the highest analyte concentration level detected in samples.

The linearity of the detector response for M04 and M14, M15, M16, M17, M18 (all oilseed rape matrices) was demonstrated by single determination of matrix-matched calibration standards at seven concentration levels (dilution factor x20) ranging from 0.0001 $\mu\text{g/mL}$ to 0.05 $\mu\text{g/mL}$ for oilseed rape (whole plants, pods, seeds and straw). These ranges correspond from 0.002 mg/kg to 1 mg/kg thus covering the range from no more than 30% of the LOQ and at least + 20 % of the highest analyte concentration level detected in samples.

The calibration curves were not forced through zero (y-intercept was calculated) and were derived from a weighted ($1/x$) linear regression for oilseed rape. For JAU-6476, M04, M14, M15, M16, M17 and M18 both ion mass transitions calibration curves (quantification, confirmation) were linear with the coefficients of correlation (R) greater than 0.99.

Stability of Analyte in Final Dilution

The stability of target analytes in final dilution was not tested specifically. Recoveries of the fortified samples within the acceptable range of 70-110% obtained with calibration solutions and the use of bracketing standards to insure integrity of the analytical sequence sufficiently demonstrate the stability.

Quantification

Quantification was performed by using weighted ($1/x$) linear regression as described in the section “Linearity”.

Accuracy and Precision

Accuracy was determined by fortification of control samples with known amounts of the reference items and subsequent determination of the recoveries when applying the extraction procedures.

Precision was determined by repeatability (relative standard deviation – RSD).

The mean recovery values at the fortification level of 0.01 mg/kg for both ion mass transitions were all in the range 70 – 110 % and thus comply with the standard acceptance criteria of the guidance document SANCO/825/00, rev. 8.1. [1] and SANCO/3029/99, rev. 4 [2]. All precision values at the fortification level of 0.01 mg/kg for both ion mass transitions were < 20%.

Mean recovery and precision results for both ion mass transitions of prothioconazole (JAU-6476), JAU-6476-desthio (M04), JAU-6476-3-hydroxy-desthio (M14), JAU-6476-4-hydroxy-desthio (M15), JAU-6476-5-hydroxy-desthio (M16), JAU-6476-6-hydroxy-desthio (M17) and JAU-6476- α hydroxydesthio (M18) are shown in Table 1.

Table 1: Summary of recovery results

Analyte	Crop/ Matrix	Fortification Level [mg/kg]	Mean Recovery (%)	RSD (%)	n
Prothioconazole	Quantification Ion Mass Transition m/z 344.0→154.0				
	Oilseed rape whole plant	0.01*	92	0.5	3
	Confirmation Ion Mass Transition m/z 344.0→125.0				
	Oilseed rape whole plant	0.01*	96	1.7	3
JAU-6476-desthio (M04)	Quantification Ion Mass Transition m/z 312.0→70.1				
	Oilseed rape whole plant	0.01*	101	2.1	3
	Confirmation Ion Mass Transition m/z 312.0→125.0				
	Oilseed rape whole plant	0.01*	101	4.2	3

* - dilution factor x10

JAU-6476-desthio (M04) and JAU-6476-3-hydroxy-desthio (M14) recovery results performed during the study

Analyte	Crop/ Matrix	Fortification Level [mg/kg]	Mean Recovery (%)	RSD (%)	n
JAU-6476- desthio (M04)	Quantification Ion Mass Transition m/z 312.0→70.1				
	Oilseed rape whole plant	0.01	81	2.6	3
	Oilseed rape pods	0.01	101	1.0	3
	Oilseed rape straw	0.01	79	11.9	3
	Oilseed rape seed	0.01	86	5.7	3
JAU-6476- desthio (M04)	Confirmation Ion Mass Transition m/z 314.0→70.1				
	Oilseed rape whole plant	0.01	81	1.7	3
	Oilseed rape pods	0.01	101	0.8	3
	Oilseed rape straw	0.01	80	13.4	3
	Oilseed rape seed	0.01	87	3.2	3
JAU-6476-3- hydroxy- desthio (M14)	Quantification Ion Mass Transition m/z 328.1→70.2				
	Oilseed rape whole plant	0.01	86	1.8	3
	Oilseed rape pods	0.01	100	8.4	3
	Oilseed rape straw	0.01	81	5.6	3
	Oilseed rape seed	0.01	87	4.3	3
JAU-6476-3- hydroxy- desthio (M14)	Confirmation Ion Mass Transition m/z 330.1→70.2				
	Oilseed rape whole plant	0.01	86	1.1	3
	Oilseed rape pods	0.01	100	4.9	3
	Oilseed rape straw	0.01	79	4.2	3
	Oilseed rape seed	0.01	86	6.8	3

JAU-6476-4-hydroxy-desthio (M15) and JAU-6476-5-hydroxy-desthio (M16) recovery results performed during the study

Analyte	Crop/ Matrix	Fortification Level [mg/kg]	Mean Recovery (%)	RSD (%)	n
JAU-6476-4- hydroxy- desthio (M15)	Quantification Ion Mass Transition m/z 328.1→70.2				
	Oilseed rape whole plant	0.01	82	0.7	3
	Oilseed rape pods	0.01	95	9.9	3
	Oilseed rape straw	0.01	78	4.7	3
	Oilseed rape seed	0.01	83	4.1	3
JAU-6476-4- hydroxy- desthio (M15)	Confirmation Ion Mass Transition m/z 330.1→70.2				
	Oilseed rape whole plant	0.01	83	0.4	3
	Oilseed rape pods	0.01	95	10.6	3
	Oilseed rape straw	0.01	77	7.2	3
	Oilseed rape seed	0.01	84	4.2	3
JAU-6476-5- hydroxy- desthio (M16)	Quantification Ion Mass Transition m/z 328.1→70.2				
	Oilseed rape whole plant	0.01	84	1.9	3
	Oilseed rape pods	0.01	101	10.8	3
	Oilseed rape straw	0.01	80	4.6	3
	Oilseed rape seed	0.01	91	2.6	3
JAU-6476-5- hydroxy- desthio (M16)	Confirmation Ion Mass Transition m/z 330.1→70.2				
	Oilseed rape whole plant	0.01	83	2.7	3
	Oilseed rape pods	0.01	103	10.7	3
	Oilseed rape straw	0.01	80	4.5	3
	Oilseed rape seed	0.01	93	2.6	3

JAU-6476-6-hydroxy-desthio (M17) and JAU-6476- α -hydroxy-desthio (M18) recovery results performed during the study

Analyte	Crop/ Matrix	Fortification Level [mg/kg]	Mean Recovery (%)	RSD (%)	n
JAU-6476-6- hydroxy- desthio (M17)	Quantification Ion Mass Transition m/z 328.1→70.2				
	Oilseed rape whole plant	0.01	73	3.4	3
	Oilseed rape pods	0.01	90	3.4	3
	Oilseed rape straw	0.01	71	8.0	3
	Oilseed rape seed	0.01	82	3.7	3
JAU-6476-6- hydroxy- desthio (M17)	Confirmation Ion Mass Transition m/z 330.1→70.3				
	Oilseed rape whole plant	0.01	72	0.5	3
	Oilseed rape pods	0.01	91	2.8	3
	Oilseed rape straw	0.01	73	9.7	3
	Oilseed rape seed	0.01	84	4.6	3
JAU-6476- α - hydroxy- desthio (M18)	Quantification Ion Mass Transition m/z 328.1→70.2				
	Oilseed rape whole plant	0.01	93	1.6	3
	Oilseed rape pods	0.01	105	6.8	3
	Oilseed rape straw	0.01	83	12.5	3
	Oilseed rape seed	0.01	88	5.9	3
JAU-6476- α - hydroxy- desthio (M18)	Confirmation Ion Mass Transition m/z 330.1→70.2				
	Oilseed rape whole plant	0.01	93	2.5	3
	Oilseed rape pods	0.01	108	6.4	3
	Oilseed rape straw	0.01	84	11.3	3
	Oilseed rape seed	0.01	88	6.3	3

Comments of zRMS:	Analytical method (Delmotte R., 2019, RDE-17-28968) is acceptable and suitable for the determination of residues prothioconazole and its metabolites in oilseed rape (whole plant, grain and straw).
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Reference:	KCP 5.1-18
Report	Analytical Phase: Magnitude of the residues of prothioconazole and its metabolites in oilseed rape (whole plant, seeds and straw), following two applications of prothioconazole 300 EC in eight trials (4 DCS and 4 HS), Northern and Southern Europe (Poland, Hungary, Portugal and Greece) – 2017 Sikorski P., Markowicz A. Delmotte R. Report No.: RDE-17-28968 Staphyt Spain S.L. GLP Unpublished
Guidelines:	Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21-Oct-2009 concerning the placing of plant protection products on the market and repealing council Directives 79/117/EEC and 91/414/EC Guideline 7029/VI/95 (rev. 5) to Directive 91/414/EEC and Regulations (EU) 283/2013 and 284/2013 implementing Regulation (EC) 1107/2009 EU Guidance Document SANCO/3029/99 rev. 4 EU Guidance Document SANCO/825/00 rev. 8.1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Summary

For the purpose of this analytical phase two different analytical procedures were used.

The general principles of the analytical procedure for determination of prothioconazole and prothioconazole-desthio in oilseed rape (OSR) whole plant were based on the sample preparation method described in EN 15662:2018 [4] with some modifications. In brief, target samples undergo shake-extraction with mixture of acetonitrile/water containing 2 mg/mL L-Cysteine HCl 4:1 (v:v).

Prothioconazole is quite unstable and might react with oxygen in air to form other products thus the addition of L-Cystine HCl is necessary to minimize the oxidation process. After addition of a buffer-salt mixture containing magnesium sulfate and sodium chloride the sample was shaken again. Following centrifugation, the supernatant was further cleaned and the excess of water removed with a single d-SPE step by mixing with magnesium sulphate and graphitized carbon black (GCB). The sample was vortex and centrifuged.

The general principles of the analytical procedure (reflux method) for determination of JAU-6476-desthio (M04), JAU-6476-3-hydroxy-desthio (M14), JAU-6476-4-hydroxy-desthio (M15), JAU-6476-5-hydroxy-desthio (M16), JAU-6476-6-hydroxy-desthio (M17) and JAU-6476- α -hydroxy-desthio (M18) in oilseed rape whole plant, pods, straw and seed were derived from Bayer CropScience analytical method 00979/M001 [5]. In brief, target analytes undergo shake-extraction with a mixture of acetonitrile/water 4:1 (v:v) followed by a liquid-liquid partitioning step and phase separation by adding salt mixture containing magnesium sulphate and sodium chloride. Following centrifugation, an aliquot

of the acetonitrile phase was evaporated almost to dryness. The remainder was diluted and acidified with 5 N hydrochloric acid and heated at reflux for 2 hours. This hydrolysis step was performed to convert glycoside-bound analogues into the respective hydroxy analytes. Subsequently the samples were neutralized with sodium hydroxide and undergo second shake-extraction with acetonitrile.

After addition of salt mixture containing magnesium sulfate and sodium chloride extract was shaken again. Following centrifugation an aliquot of the acetonitrile phase was decanted into Eppendorf tube containing magnesium sulphate for additional dehydration. The sample was vortex and centrifuged.

For quantitative analysis in both extraction methods the final extract was diluted with acidified water and subjected to LC-ESI-MS/MS. All analytes were detected using electrospray ionization in the positive ion mode (ESI+)

Selectivity and Confirmation of Residue Identity

Quantification was performed by use of highly selective liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). Two selected ion mass transitions were evaluated in order to demonstrate that the method achieves a high level of selectivity. The retention times of target analyte in extracts corresponds to that of the calibration standards with a tolerance of $< \pm 0.15$ min. Confirmation ion ratio for all analytes in all samples were within ± 30 % of the average found for the standards.

No significant interference above 30 % of LOQ was detected in any of the reagent blanks or control specimen extracts for oilseed rape (whole plants, pods, seeds and straw) matrices, so that a highly level of selectivity was demonstrated and an additional confirmatory method is not necessary.

Matrix Effects

Determination of prothioconazole (JAU-6476), JAU-6476-desthio (M04), JAU-6476-3-hydroxy desthio (M14), JAU-6476-4-hydroxy-desthio (M15), JAU-6476-5-hydroxy-desthio (M16), JAU-6476-6-hydroxydesthio (M17) and JAU-6476- α -hydroxy-desthio (M18) was performed using matrix matched calibration standards.

Linearity

The linearity of the detector response for JAU-6476 and M04 was demonstrated by single determination of matrix-matched calibration standards at nine concentration levels (dilution factor x10) ranging from 0.0003 $\mu\text{g/mL}$ to 0.1 $\mu\text{g/mL}$ and at seven concentrations levels (dilution factor x100) ranging from 0.001 $\mu\text{g/mL}$ to 0.1 $\mu\text{g/mL}$ for oilseed rape whole plant. These ranges correspond from 0.003 mg/kg to 1 mg/kg (dilution factor x10) and from 0.1 mg/kg to 10 mg/kg (dilution factor x100) for oilseed rape whole plant thus covering the range from no more than 30% of the LOQ and at least + 20 % of the highest analyte concentration level detected in samples.

The linearity of the detector response for M04 and M14, M15, M16, M17, M18 (all oilseed rape matrices) was demonstrated by single determination of matrix-matched calibration standards at seven concentration levels (dilution factor x20) ranging from 0.0001 $\mu\text{g/mL}$ to 0.05 $\mu\text{g/mL}$ for oilseed rape (whole plants, pods, seeds and straw). These ranges correspond from 0.002 mg/kg to 1 mg/kg thus covering the range from no more than 30% of the LOQ and at least + 20 % of the highest analyte concentration level detected in samples.

The calibration curves were not forced through zero (y-intercept was calculated) and were derived from a weighted ($1/x$) linear regression for oilseed rape. For JAU-6476, M04, M14, M15, M16, M17 and M18 both ion mass transitions calibration curves (quantification, confirmation) were linear with the coefficients of correlation (R) greater than 0.99.

Stability of Analyte in Final Dilution

The stability of target analytes in final dilution was not tested specifically. Recoveries of the fortified samples within the acceptable range of 70-110% obtained with calibration solutions and the use of bracketing standards to insure integrity of the analytical sequence sufficiently demonstrate the stability.

Quantification

Quantification was performed by using weighted (1/x) linear regression as described in the section “Linearity”.

Accuracy and Precision

Accuracy was determined by fortification of control samples with known amounts of the reference items and subsequent determination of the recoveries when applying the extraction procedures.

Precision was determined by repeatability (relative standard deviation – RSD).

The mean recovery values at the fortification levels of 0.01 mg/kg and 0.1 mg/kg for both ion mass transitions were all in the range 70 – 110 % and thus comply with the standard acceptance criteria of the guidance document SANCO/825/00, rev. 8.1. [1] and SANCO/3029/99, rev. 4 [2]. All precision values at the fortification levels of 0.01 mg/kg and 0.1 mg/kg for both ion mass transitions were < 20%.

Mean recovery and precision results for both ion mass transitions of prothioconazole (JAU-6476), JAU-6476-desthio (M04), JAU-6476-3-hydroxy-desthio (M14), JAU-6476-4-hydroxy-desthio (M15), JAU-6476-5-hydroxy-desthio (M16), JAU-6476-6-hydroxy-desthio (M17) and JAU-6476- α hydroxydesthio (M18) are shown in Table 1.

Table 1: Summary of recovery results					
Analyte	Crop/ Matrix	Fortification Level [mg/kg]	Mean Recovery (%)	RSD (%)	n
Prothioconazole	Quantification Ion Mass Transition m/z 344.0→154.0				
	Oilseed rape whole plant	0.01*	92	0.5	3
	Confirmation Ion Mass Transition m/z 344.0→125.0				
	Oilseed rape whole plant	0.01*	96	1.7	3
JAU-6476-desthio (M04)	Quantification Ion Mass Transition m/z 312.0→70.1				
	Oilseed rape whole plant	0.01*	101	2.1	3
	Confirmation Ion Mass Transition m/z 312.0→125.0				
	Oilseed rape whole plant	0.01*	101	4.2	3
* - dilution factor x10					

Table 1: Summary of recovery results (cont.)

Analyte	Crop/ Matrix	Fortification Level [mg/kg]	Mean Recovery (%)	RSD (%)	n
JAU-6476-desthio (M04)	Quantification Ion Mass Transition m/z 312.0→70.1				
	Oilseed rape whole plant	0.01	81	2.6	3
	Oilseed rape pods	0.01	101	1.0	3
	Oilseed rape straw	0.01	79	11.9	3
	Oilseed rape seed	0.01	86	5.7	3
JAU-6476-desthio (M04)	Confirmation Ion Mass Transition m/z 314.0→70.1				
	Oilseed rape whole plant	0.01	81	1.7	3
	Oilseed rape pods	0.01	101	0.8	3
	Oilseed rape straw	0.01	80	13.4	3
	Oilseed rape seed	0.01	87	3.2	3
JAU-6476-3- hydroxy-desthio (M14)	Quantification Ion Mass Transition m/z 328.1→70.2				
	Oilseed rape whole plant	0.01	86	1.8	3
	Oilseed rape pods	0.01	100	8.4	3
	Oilseed rape straw	0.01	81	5.6	3
	Oilseed rape seed	0.01	87	4.3	3
JAU-6476-3- hydroxy-desthio (M14)	Confirmation Ion Mass Transition m/z 330.1→70.2				
	Oilseed rape whole plant	0.01	86	1.1	3
	Oilseed rape pods	0.01	100	4.9	3
	Oilseed rape straw	0.01	79	4.2	3
	Oilseed rape seed	0.01	86	6.8	3

Table 1: Summary of recovery results (cont.)

Analyte	Crop/ Matrix	Fortification Level [mg/kg]	Mean Recovery (%)	RSD (%)	n
JAU-6476-4- hydroxy-desthio (M15)	Quantification Ion Mass Transition m/z 328.1→70.2				
	Oilseed rape whole plant	0.01	82	0.7	3
	Oilseed rape pods	0.01	95	9.9	3
	Oilseed rape straw	0.01	78	4.7	3
	Oilseed rape seed	0.01	83	4.1	3
JAU-6476-4- hydroxy-desthio (M15)	Confirmation Ion Mass Transition m/z 330.1→70.2				
	Oilseed rape whole plant	0.01	83	0.4	3
	Oilseed rape pods	0.01	95	10.6	3
	Oilseed rape straw	0.01	77	7.2	3
	Oilseed rape seed	0.01	84	4.2	3
JAU-6476-5- hydroxy-desthio (M16)	Quantification Ion Mass Transition m/z 328.1→70.2				
	Oilseed rape whole plant	0.01	84	1.9	3
	Oilseed rape pods	0.01	101	10.8	3
	Oilseed rape straw	0.01	80	4.6	3
	Oilseed rape seed	0.01	91	2.6	3
JAU-6476-5- hydroxy-desthio (M16)	Confirmation Ion Mass Transition m/z 330.1→70.2				
	Oilseed rape whole plant	0.01	83	2.7	3
	Oilseed rape pods	0.01	103	10.7	3
	Oilseed rape straw	0.01	80	4.5	3
	Oilseed rape seed	0.01	93	2.6	3

Table 1: Summary of recovery results (cont.)

Analyte	Crop/ Matrix	Fortification Level [mg/kg]	Mean Recovery (%)	RSD (%)	n
JAU-6476-6- hydroxy-desthio (M17)	Quantification Ion Mass Transition m/z 328.1→70.2				
	Oilseed rape whole plant	0.01	73	3.4	3
	Oilseed rape pods	0.01	90	3.4	3
	Oilseed rape straw	0.01	71	8.0	3
	Oilseed rape seed	0.01	82	3.7	3
JAU-6476-6- hydroxy-desthio (M17)	Confirmation Ion Mass Transition m/z 330.1→70.3				
	Oilseed rape whole plant	0.01	72	0.5	3
	Oilseed rape pods	0.01	91	2.8	3
	Oilseed rape straw	0.01	73	9.7	3
	Oilseed rape seed	0.01	84	4.6	3
JAU-6476-α- hydroxy-desthio (M18)	Quantification Ion Mass Transition m/z 328.1→70.2				
	Oilseed rape whole plant	0.01	93	1.6	3
	Oilseed rape pods	0.01	105	6.8	3
	Oilseed rape straw	0.01	83	12.5	3
	Oilseed rape seed	0.01	88	5.9	3
JAU-6476-α- hydroxy-desthio (M18)	Confirmation Ion Mass Transition m/z 330.1→70.2				
	Oilseed rape whole plant	0.01	93	2.5	3
	Oilseed rape pods	0.01	108	6.4	3
	Oilseed rape straw	0.01	84	11.3	3
	Oilseed rape seed	0.01	88	6.3	3

Comments of zRMS:	Analytical method (Stolze J., 2022, IF21-05707367) is acceptable and suitable for the determination of residues prothioconazole and its metabolites in oilseed rape (whole plant, grain and straw).
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Reference: KCP 5.1-19

Report Study on the residue behaviour of prothioconazole in oilseed rape after treatment with Prothioconazole 300 EC at six sites under field conditions in Southern Europe - 2021
~~Bodsch J.~~ Stolze J.
Report No.: IF21-05707367
SGS Institute Fresenius, GmbH
GLP
Unpublished

Guidelines: SANTE/2020/12830, Rev.1 (24. February 2021)
OECD ENV/JM/MONO(2007) 17 - Guidance Document on Pesticide Residue Analytical Methods
Good Laboratory Practice Regulations: Appendix 1 to § 19a, Section 1, of the German Chemikaliengesetz (Chemicals Act)
OECD Principles of Good Laboratory Practice ENV/MC/CHEM(98)17, Paris 1998
The application of the OECD Principles of GLP to the Organisation and Management of Multi-Site Studies, ENV/JM/MONO(2002)9, 25/06/2002.

Deviations: No

GLP: Yes

Acceptability: Yes

Summary

During the growing season of 2021, six field trials with oilseed rape were conducted in Southern Europe (Trial 21-00231-01 – Southern France, Trial 21-00231-02 – Italy, Trial 21-00231-04 – Greece, Trial 21-00231-05 – Southern France, Trial 21-00231-06 – Italy, Trial 21-00231-08 – Greece). The magnitude of residues in oilseed rape specimens were determined for the triazole metabolites 1,2,4-Triazole, Triazole alanine, Triazole acetic acid and Triazole lactic acid after spray application with Prothioconazole 300 EC.

Prothioconazole 300 EC (300 g Prothioconazole/L) was applied two times with nominal content 180 g prothioconazole/ha and a nominal water volume of 200-400 L/ha.

Specimens of oilseed rape were collected at a nominal sampling timing 0 (S1), 7±1 (S2) and 14±1 (S3) DALA as whole plant, 35 ± 3 DALA (S4) as pods and rest of plant and at normal commercial harvest (growth stage BBCH 89) as seed and straw (S5).

The oilseed rape specimens were analysed at SGS INSTITUT FRESENIUS GmbH, c/o SGS Germany GmbH, Hamburg, Germany for residues of the triazol metabolites following the analytical method 01062/M004[1]. For the matrix groups high water content (whole plant), high oil content (seed) and dry matrix (straw) method validations according to SANTE/2020/12830 rev.1 were carried out by using procedural recovery samples which were analysed in parallel to the field samples. At least 5xLOQ and 5x10LOQ fortified samples were evaluated on both mass traces with regard to recovery, repeatability and selectivity/specificity.

Linearity was proven and a statement for matrix effect tests given.

The Limit of Quantification (LOQ) of the analytical method was defined as 0.010 mg/kg for each analyte. The

Limit of Detection (LOD) was defined to be 0.003 mg/kg for each analyte (lowest calibration standard used). Linearity was shown over a range of 1.0 to 100 ng/mL for the analytes with correlation coefficients of ≥ 0.998 and no visible trend in the residuals. Therefore, the calibration model was defined to be suitable.

The accuracy of the method was demonstrated by recovery experiments, control specimens were fortified prior to extraction with 1,2,4-T, TA, TAA and TLA.

The mean recovery rate values and the relative standard deviations (RSD) were as follows:

Crop	Matrix	TA		
		Mean (%)	RSD	n
Oilseed rape	Overall	91.5	5.7	51
Oilseed rape	Overall	87.7	11	51
Oilseed rape	Overall	94.2	5.7	51
Oilseed rape	Overall	92.1	7.5	51

Crop	Matrix	Fortification Level (nominal) [mg/kg]	1,2,4-T		
			Mean [%]	RSD [±]	n
Oilseed rape	Whole Plant	0.010, 0.10 and 1.0	90.5	5.8	11
	Pods	0.010, 0.10 and 1.0	93.6	5.3	10
	Rest of Plant	0.010 and 0.10	89.3	3.9	6
	Straw	0.010, 0.10 and 1.0	88.9	4.9	11
	Seed	0.010, 0.10, 1.0 and 10	93.9	6.0	13
Overall:			91.5	5.7	51
Crop	Matrix	Fortification Level (nominal) [mg/kg]	TA		
			Mean [%]	RSD [±]	n
Oilseed rape	Whole Plant	0.010, 0.10 and 1.0	93.8	7.5	11
	Pods	0.010, 0.10 and 1.0	83.8	8.7	11
	Rest of Plant	0.010 and 0.10	91.7	15	6
	Straw	0.010, 0.10, and 1.0	83.1	6.1	11
	Seed	0.010, 0.10, 1.0 and 10	88.1	14	12
Overall:			87.7	11	51
Crop	Matrix	Fortification Level (nominal) [mg/kg]	TAA		
			Mean [%]	RSD [±]	n
Oilseed rape	Whole Plant	0.010, 0.10 and 1.0	99.0	4.3	11
	Pods	0.010, 0.10 and 1.0	93.9	7.3	10
	Rest of Plant	0.010 and 0.10	95.3	4.0	6
	Straw	0.010, 0.10 and 1.0	94.6	3.9	11
	Seed	0.010, 0.10, 1.0 and 10	90.6	4.9	13
Overall:			94.2	5.7	51
Crop	Matrix	Fortification Level (nominal) [mg/kg]	TLA		
			Mean [%]	RSD [±]	n
Oilseed rape	Whole Plant	0.010, 0.10 and 1.0	93.6	4.2	11
	Pods	0.010, 0.10 and 1.0	86.9	10.4	10
	Rest of Plant	0.010 and 0.10	90.6	3.4	6
	Straw	0.010, 0.10 and 1.0	92.6	6.6	11
	Seed	0.010, 0.10, 1.0 and 10	95.0	7.8	13
Overall:			92.1	7.5	51

RSD: Relative standard deviation; n: Number of specimens

Comments of zRMS:	Analytical method (Thirkell C., 2022, IF22-06125006) is acceptable and suitable for the determination of residues prothioconazole and its metabolites in oilseed rape (whole plant, grain and straw).
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Reference: KCP 5.1-19

Report Study on the residue behaviour of prothioconazole in wheat after treatment with Prothioconazole 300 EC at two sites under field conditions in Northern Europe – 2021-2022
Bodsch J., Thirkell C.
Report No.: IF22-06125006
SGS Institute Fresenius, GmbH
GLP
Unpublished

Guidelines: SANTE/2020/12830, Rev.1 (24. February 2021)
OECD ENV/JM/MONO(2007) 17 - Guidance Document on Pesticide Residue Analytical Methods
Good Laboratory Practice Regulations: Appendix 1 to § 19a, Section 1, of the German Chemikaliengesetz (Chemicals Act)
OECD Principles of Good Laboratory Practice ENV/MC/CHEM(98)17, Paris 1998
The application of the OECD Principles of GLP to the Organisation and Management of Multi-Site Studies, ENV/JM/MONO(2002)9, 25/06/2002.

Deviations: No

GLP: Yes

Acceptability: Yes

Summary

During the growing season of 2022, one decline (trial 22-00143-01 – Auménancourt, Northern France) and one harvest field trial (trial 22-00143-02 – Aabenraa, Denmark) with wheat (whole plant, ears, rest of plant, grain and straw) were conducted in Northern Europe. The magnitude of residues in wheat specimens was determined for the triazole metabolites 1,2,4-Triazole (1,2,4-T), Triazole alanine (TA), Triazole acetic acid (TAA) and Triazole lactic acid (TLA) after two foliar applications with Prothioconazole 300 EC.

Prothioconazole 300 EC (300 g Prothioconazole/L) was applied two times with nominal content 195 g prothioconazole/ha and a nominal water volume of 200-400 L/ha.

The decline trial was sampled four times at 0 DALA (S1), 8 DALA (S2) and 14 DALA (S3) as whole plant and 35 DALA (normal commercial harvest (NCH) BBCH 89 (S5))¹ as grain and straw. The harvest trial was sampled once at NCH (BBCH 89) (S1) as grain and straw.

The wheat specimens were analysed at SGS INSTITUT FRESENIUS GmbH, c/o SGS Germany GmbH, Hamburg, Germany for residues of the triazole metabolites following the analytical method 01062/M004^[1]. The method was validated at the analytical test site in study IF21-05707367^[3] for the matrix groups relevant in this study.

¹ The S4 sampling event was not conducted because BBCH 89 coincided with 35 DALA.

The Limit of Quantification (LOQ) of the analytical method was defined as 0.010 mg/kg for each analyte. The Limit of Detection (LOD) was defined to be 0.003 mg/kg for each analyte (lowest calibration standard used).

Linearity was shown over a range of 1.25 to 100 ng/mL for the analytes with correlation coefficients of ≥ 0.998 and no visible trend in the residuals. Therefore, the calibration model was defined to be suitable. The accuracy of the method was demonstrated by recovery experiments, control specimens were fortified prior to extraction with 1,2,4-T, TA, TAA and TLA.

The mean recovery rate values and the relative standard deviations (RSD) were as follows:

Crop	Matrix	Fortification Level (nominal) [mg/kg]	1,2,4-T		
			Mean [%]	RSD [±]	n
Wheat	Grain	0.010 and 0.10	86.9	14	6
	Straw	0.010 and 0.10	92.3	8.4	6
	Whole Plant no Roots	0.010 and 0.10	97.0	3.9	6
Overall			92.1	9.9	18

RSD: relative standard deviation; n: number of specimens

Crop	Matrix	Fortification Level (nominal) [mg/kg]	TA		
			Mean [%]	RSD [±]	n
Wheat	Grain	0.010 and 0.10	84.9	13	6
	Straw	0.010 and 0.10	74.4	4.8	6
	Whole Plant no Roots	0.010 and 0.10	96.8	3.7	6
Overall			85.4	14	18

RSD: relative standard deviation; n: number of specimens

Crop	Matrix	Fortification Level (nominal) [mg/kg]	TAA		
			Mean [%]	RSD [±]	n
Wheat	Grain	0.010 and 0.10	82.6	6.2	6
	Straw	0.010 and 0.10	86.6	3.6	6
	Whole Plant no Roots	0.010 and 0.10	94.9	5.7	6
Overall			88.0	7.8	18

RSD: relative standard deviation; n: number of specimens

Crop	Matrix	Fortification Level (nominal) [mg/kg]	TLA		
			Mean [%]	RSD [±]	n
Wheat	Grain	0.010 and 0.10	76.8	3.1	6
	Straw	0.010 and 0.10	83.4	3.1	6
	Whole Plant no Roots	0.010 and 0.10	96.8	2.6	6
Overall			85.7	10	18

RSD: relative standard deviation; n: number of specimens

Summary of residues in/on the untreated/treated wheat specimens for 1,2,4-T, TA, TAA and TLA

Sam- pling No.	Portion analysed	Plot	DALA	BBCH	n	Residues [mg/kg]	
Trial: 22-00143-						01	02
						1,2,4-Triazole (1,2,4-T)	
5	Grain	U	35 - 42	89	2	< 0.010	< 0.010
	Straw				2	< 0.010	< 0.010
1	Whole Plant	T	0	69	1	< 0.010	-
2	Whole Plant		8	75	1	< 0.010	-
3	Whole Plant		14	77	1	< 0.010	-
5	Grain		35 - 42	89	2	< 0.010	< 0.010
	Straw			89	2	< 0.010	< 0.010
						Triazole alanine (TA)	
5	Grain	U	35 - 42	89	2	0.010	0.099
	Straw				2	< 0.010	0.020
1	Whole Plant	T	0	69	1	0.071	-
2	Whole Plant		8	75	1	0.13	-
3	Whole Plant		14	77	1	0.13	-
5	Grain		35 - 42	89	2	0.28	0.61
	Straw			89	2	0.020	0.094
						Triazole acetic acid (TAA)	
5	Grain	U	35 - 42	89	2	< 0.010	0.057
	Straw				2	< 0.010	0.043
1	Whole Plant	T	0	69	1	0.011	-
2	Whole Plant		8	75	1	0.013	-
3	Whole Plant		14	77	1	0.015	-
5	Grain		35 - 42	89	2	0.057	0.15
	Straw			89	2	0.013	0.088
						Triazole lactic acid (TLA)	
5	Grain	U	35 - 42	89	2	< 0.010	< 0.010
	Straw				2	< 0.010	0.045
1	Whole Plant	T	0	69	1	0.015	-
2	Whole Plant		8	75	1	0.020	-
3	Whole Plant		14	77	1	0.019	-
5	Grain		35 - 42	89	2	< 0.010	< 0.010
	Straw			89	2	0.020	0.10

DALA: Days after last application; U: Untreated plot; T: Treated plot

Comments of zRMS:	Analytical method (Thirkell C., 2024, IF22-06085351) is acceptable and suitable for the determination of residues prothioconazole and its metabolites in oilseed rape (whole plant, grain and straw).
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Reference: KCP 5.1-20

Report Study on the Residue Behaviour of Prothioconazole in Oilseed Rape after Treatment with Prothioconazole 300 EC at two Sites under Field Conditions in Southern Europe, 2023, Analytical Phase
Thirkell C. Bodsch J.
Report No.: IF22-06085351
SGS Institute Fresenius, GmbH
GLP
Unpublished

Guidelines: SANTE/2020/12830, Rev.1 (24. February 2021)
OECD ENV/JM/MONO(2007) 17 - Guidance Document on Pesticide Residue Analytical Methods
Good Laboratory Practice Regulations: Appendix 1 to § 19a, Section 1, of the German Chemikaliengesetz (Chemicals Act)
OECD Principles of Good Laboratory Practice ENV/MC/CHEM(98)17, Paris 1998
The application of the OECD Principles of GLP to the Organisation and Management of Multi-Site Studies, ENV/JM/MONO(2002)9, 25/06/2002.

Deviations: No

GLP: Yes

Acceptability: Yes

Abstract

During the growing season of 2022/23, two field trials with oilseed rape were conducted in Southern Europe (decline trial 22-00037-001 – Greece, harvest trial 22-00037-02 – Italy). The magnitude of residues in oilseed rape specimens was determined for the triazole metabolites 1,2,4-Triazole, Triazole alanine, Triazole acetic acid and Triazole lactic acid after two applications with Prothioconazole 300 EC at two sites.

Prothioconazole 300 EC (300 g Prothioconazole/L) was applied two times with nominal content 180 g prothioconazole/ha and a nominal water volume of 200-400 L/ha.

For decline trial specimens of oilseed rape were collected at a nominal sampling timing 0 (S1), 7±1 (S2) and 14±1 (S3) DALA as whole plant, 35 ± 3 DALA (S4) as pods and rest of plant and at normal commercial harvest (growth stage BBCH 89) as seed and straw (S5). For harvest trials specimens of oilseed rape were collected at normal commercial harvest (growth stage BBCH 89) as seed and straw only.

The oilseed rape specimens were analysed at SGS INSTITUT FRESENIUS GmbH, c/o SGS Germany GmbH, Hamburg, Germany for residues of the triazole metabolites following the analytical method

01062/M004^[1]. The method was validated at the analytical test site in study IF21-05707367^[4] for the matrix groups relevant in this study.

The Limit of Quantification (LOQ) of the analytical method was defined as 0.010 mg/kg for each analyte. The Limit of Detection (LOD) was defined to be 0.003 mg/kg for each analyte (lowest calibration standard used).

Linearity was shown over a range of 1.50 to 100 ng/mL for the analytes with correlation coefficients of ≥ 0.998 and no visible trend in the residuals. Therefore, the calibration model was defined to be suitable. The accuracy of the method was demonstrated by recovery experiments, control specimens were fortified prior to extraction with 1,2,4-T, TA, TAA and TLA. The mean recovery rate values and relative standard deviations (RSD) were as follows:

Crop	Matrix	Fortification Level (nominal) [mg/kg]	1,2,4-T		
			Mean [%]	RSD [±]	n
Oilseed Rape	Whole plant	0.010, 0.10 and 1.0	91.9	4.5	7
	Pods	0.010, 0.10 and 1.0	90.8	4.5	7
	Rest of Plant	0.010, 0.10 and 1.0	85.1	5.5	7
	Seed	0.010, 0.10 and 1.0	85.2	7.4	7
	Straw	0.010, 0.10, 1.0 and 10	92.1	4.2	7
Overall			89.0	6.1	35

RSD: Relative Standard Deviation; n: Number of specimens

Crop	Matrix	Fortification Level (nominal) [mg/kg]	TA		
			Mean [%]	RSD [±]	n
Oilseed Rape	Whole plant	0.010, 0.10 and 1.0	93.3	6.1	7
	Pods	0.010, 0.10 and 1.0	94.5	7.3	7
	Rest of Plant	0.010, 0.10 and 1.0	86.5	11	7
	Seed	0.010, 0.10 and 1.0	88.7	12	7
	Straw	0.010, 0.10, 1.0 and 10	72.9	4.2	7
Overall			87.2	12	35

RSD: Relative Standard Deviation; n: Number of specimens

Crop	Matrix	Fortification Level (nominal) [mg/kg]	TAA		
			Mean [%]	RSD [±]	n
Oilseed Rape	Whole plant	0.010, 0.10 and 1.0	94.7	2.8	7
	Pods	0.010, 0.10 and 1.0	87.1	5.9	7
	Rest of Plant	0.010, 0.10 and 1.0	93.5	5.2	7
	Seed	0.010, 0.10 and 1.0	91.1	2.4	7
	Straw	0.010, 0.10 and 1.0	86.6	7.9	7
Overall			90.6	6.1	35

RSD: Relative Standard Deviation; n: Number of specimens

Crop	Matrix	Fortification Level (nominal) [mg/kg]	TLA		
			Mean [%]	RSD [±]	n
Oilseed Rape	Whole plant	0.010, 0.10 and 1.0	93.8	3.8	7
	Pods	0.010, 0.10 and 1.0	87.0	7.9	7
	Rest of Plant	0.010, 0.10 and 1.0	91.6	4.4	7
	Seed	0.010, 0.10 and 1.0	87.4	4.4	7
	Straw	0.010, 0.10 and 1.0	81.6	7.1	7
Overall			88.3	7.2	35

RSD: Relative Standard Deviation; n: Number of specimens

Summary of residues in/on the untreated/treated Oilseed rape specimens for 1,2,4-T

Sam- ling No.	Portion analysed	Plot	DALA	BBCH	n	Residues [mg/kg]	
Trial: 22-00037-						01	02
1,2,4-Triazole (1,2,4-T)							
1	Whole Plant	U	0 DBLA	69	1	< 0.010	---
4	Pods		33	82	1	< 0.010	---
	Rest of Plant				1	< 0.010	---
5	Seed		55/62	89	2	< 0.010	< 0.010
	Straw				2	< 0.010	< 0.010
1	Whole Plant	T	0	69	1	< 0.010	---
2	Whole Plant		6	75	1	< 0.010	---
3	Whole Plant		13	78	1	< 0.010	---
4	Pods		33	82	1	< 0.010	---
	Rest of Plant				1	< 0.010	---
5	Seed		55/62	89	2	< 0.010	< 0.010

	Straw			2	< 0.010	< 0.010
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DALA: Days after last application;DBLA: Days before last application; U: Untreated plot; T: Treated plot

Summary of residues in/on the untreated/treated Oilseed rape specimens for TA

Samp-ling No.	Portion analysed	Plot	DALA	BBCH	n	Residues [mg/kg]	
Trial: 22-00037-						01	02
Triazole alanine (TA)							
1	Whole Plant	U	0 DBLA	69	1	0.038	---
4	Pods		33	82	1	0.36	---
	Rest of Plant				1	0.036	---
5	Seed		55/62	89	2	0.83	0.84
	Straw				2	0.019	0.010
1	Whole Plant	T	0	69	1	0.15	---
2	Whole Plant		6	75	1	0.14	---
3	Whole Plant		13	78	1	0.12	---
4	Pods		33	82	1	0.39	---
	Rest of Plant				1	0.073	---
5	Seed		55/62	89	2	0.77	0.40
	Straw				2	0.034	0.065

DALA: Days after last application;DBLA: Days before last application; U: Untreated plot; T: Treated plot

Summary of residues in/on the untreated/treated Oilseed rape specimens for TAA

Samp-ling No.	Portion analysed	Plot	DALA	BBCH	n	Residues [mg/kg]	
Trial: 22-00037-						01	02
Triazole acetic acid (TAA)							
1	Whole Plant	U	0 DBLA	69	1	< 0.010	---
4	Pods		33	82	1	0.017	---
	Rest of Plant				1	< 0.010	---
5	Seed		55/62	89	2	0.016	0.011
	Straw				2	0.051	< 0.010
1	Whole Plant	T	0	69	1	< 0.010	---
2	Whole Plant		6	75	1	< 0.010	---
3	Whole Plant		13	78	1	< 0.010	---
4	Pods		33	82	1	0.013	---
	Rest of Plant				1	< 0.010	---

5	Seed	55/62	89	2	0.015	<0.010 (0.005)
	Straw			2	0.062	<0.010 (0.003)

DALA: Days after last application; DBLA: Days before last application; U: Untreated plot; T: Treated plot

Summary of residues in/on the untreated/treated Oilseed rape specimens for TLA

Samp- ling No.	Portion analysed	Plot	DALA	BBCH	n	Residues [mg/kg]	
Trial: 22-00037-						01	02
Triazole lactic acid (TLA)							
1	Whole Plant	U	0 DBLA	69	1	< 0.010	---
4	Pods		33	82	1	0.036	---
	Rest of Plant				1	< 0.010	---
5	Seed		55/62	89	2	0.038	0.028
	Straw				2	0.027	< 0.010
1	Whole Plant	T	0	69	1	<0.010 (0.003)	---
2	Whole Plant		6	75	1	<0.010 (0.003)	---
3	Whole Plant		13	78	1	<0.010 (0.003)	---
4	Pods		33	82	1	0.029	---
	Rest of Plant				1	< 0.010	---
5	Seed		55/62	89	2	0.033	0.011
	Straw				2	0.044	<0.010 (0.003)

DALA: Days after last application; DBLA: Days before last application; U: Untreated plot; T: Treated plot

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)

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)

No new or additional studies have been submitted

A 2.1.2.3 Description of Methods for the Analysis of Soil (KCP 5.2)

No new or additional studies have been submitted

A 2.1.2.4 Description of Methods for the Analysis of Water (KCP 5.2)

No new or additional studies have been submitted

A 2.1.2.5 Description of Methods for the Analysis of Air (KCP 5.2)

No new or additional studies have been submitted

A 2.1.2.6 Description of Methods for the Analysis of Body Fluids and Tissues (KCP 5.2)

No new or additional studies have been submitted

A 2.1.2.7 A.2.A.9 Other Studies/ Information

Comments of zRMS:	Validation data (Schlewitz P., 2023, 3085) are acceptable according SAN-TE/2020/12830, Rev.2 and suitable for the determination of active substances boscalid in Cereals (high water content and dry commodities).
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Reference:	KCP 5.1.1
Report	Validation of the Analytical Method for the Analysis of Boscalid in Cereals (high water content and dry commodities), Schlewitz P., 2023, C3085
Guideline(s):	Yes, Regulation (EC) No. 1107/2009 SANTE/2020/12830, Rev.2 ENV/JM/MONO(2007)17
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Summary

The objective of the study was to validate the analytical method for the analysis of boscalid in cereals (high water content and dry commodities).

Samples were analysed using an Anadiag method based on QuEChERS method (NF EN 15662-May 2018) entitled “Foods of plant origin- Multimethod for the determination of pesticide residues using GC- and LC-based analysis following acetonitrile extraction/partitioning and clean-up by dispersive SPE - Modular QuEChERS-method”.

(Anadiag reference MC 641)

Outline of the method:

Residues are extracted from cereals (high water content and dry commodities) with water and acetonitrile/acetic acid 99.9/0.1 % mixture. After addition of magnesium sulphate and sodium chloride, the mixture is shaken intensively and centrifuged for phase separation. An aliquot of the organic phase is filtered through PET filter, transferred into a vial for LC-MS/MS analysis.

ANADIAG references for the application of the method were:

- **MP 745** (for extraction and purification)
- **MA 1916** (for analytical determination)

The method under discussion describes the determination of boscalid in cereals high water content (whole plant) and dry commodities (grain). The method was validated at 0.01 mg/kg for each matrix.

The analytical method was validated according to SANTE/2020/12830, Rev.2.

The following points were examined during the study:

Matrix effects

Matrix effects, expressed in % enhancement or suppression, were assessed for each matrix and analyte, for both primary and confirmatory methods. They were considered significant if they exceeded $\pm 20\%$.

Matrix effects (enhancement or suppression) on the instrument response were considered significant. Consequently, matrix-matched calibration solutions were used for calibration.

Calibration

The analytical calibration consisted of matrix-matched calibration solutions of boscalid, at least at 5 concentration levels, ranged from 0.76 ng/mL to 30 ng/mL (corresponding to 0.003 to 0.12 mg/kg).

Linear weighted calibration (1/x weighting) curves were run for each analysis sequence for both primary and confirmatory methods.

The linear correlation coefficients were > 0.990 , showing good linearity with regression residuals randomly distributed for both primary and confirmatory methods.

Limit of detection

The limit of detection (LOD) was expressed as lowest calibration standard.

The LOD was 0.76 ng/mL for boscalid in cereals high water content (whole plant) and dry commodities (grain) (corresponding to 0.003 mg/kg).

Limit of quantification

The limit of quantification (LOQ) was the lowest validated level with sufficient recovery and precision.

The LOQ was 0.01 mg/kg for boscalid in cereals high water content (whole plant) and dry commodities (grain).

Recovery and repeatability

For samples fortified at 0.01 mg/kg, mean recoveries were within the acceptable range 60-120% with RSD less than 30% for both primary and confirmatory methods.

For samples fortified at 0.10 mg/kg, mean recoveries were within the acceptable range 70-120% with RSD less than 20% for primary method.

Selectivity and specificity

Representative, clearly labelled chromatograms of standard at the lowest calibrated level, untreated sample and sample fortified at the lowest fortification level for each analyte/matrix combination and for both primary and confirmatory methods were provided to prove selectivity of the method.

Mass spectra were provided to justify the selection of ions used for determination.

Untreated samples (non-fortified samples) were determined from the matrices used in fortification experiments and were not higher than 30% of the LOQ for both primary and confirmatory methods.

Confirmation

The confirmatory method was required to confirm that the primary method detected the correct analyte (analyte identity) and that the analyte signal of the primary method was quantitatively correct and not affected by any other compound.

Confirmation simultaneously to primary detection:

The confirmatory method was achieved by monitoring 1 additional transition for each matrix.

	Primary transition	Confirmatory transition
Boscalid	m/z 343.1 > 307.0	m/z 343.1 > 271.0

Stability results for extracts

The stability of extracts during frozen storage was investigated.

Boscalid residues were stable for at least 21 days of frozen storage in cereals high water content (whole plant) extracts and for at least 20 days of frozen storage dry commodities (grain) extracts.

Stability results for matrix-matched standard solutions

The stability of matrix-matched standard solutions during frozen storage was investigated.

Boscalid, in matrix-matched calibration solutions, was stable for at least 21 days of frozen storage in cereals high water content (whole plant) and for at least 20 days of frozen storage dry commodities (grain).

Comments of zRMS:	Validation data (Biesiada M., 2022, 0064/0029/FA) are acceptable according SANTE/2020/12830, Rev.1 and suitable for the determination of active substances boscalid and prothioconazole concentrations in aqueous media solutions of the test item GLOB2021dF.
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Reference: KCP 5.1.1

Report Validation of analytical method for the determination of active substances

boscalid and prothioconazole concentrations in aqueous media solutions of the test item GLOB2021dF, Biesiada M., 2022, 0064/0029/FA

Guideline(s): Yes, SANTE/2020/12830 rev.1

Deviations: No

GLP: Yes

Acceptability: Yes

Summary

The analytical method validation for the determination of active substances – boscalid and prothioconazole concentrations in aqueous media (deionized water, AAP medium, M7 medium) solutions of the test item GLOB2021dF was performed.

At the analytical method validation the following parameters: matrix effects, selectivity, linearity, accuracy, precision, limit of detection and limit of quantification were determined.

The analytical method validation was carried out based on the experimental procedure SPB-FA/11 and SANTE/2020/12830 rev.1 guideline.

Analytical method validation parameters are presented in Table 1.

Table 1. Analytical method validation parameters

Deionized water								
Parameter	Required criterion		Result					
	Boscalid	Prothioconazole	Boscalid			Prothioconazole		
Selectivity	in place of the active substance peak, no signals from other substances with an area exceeding 30% of the LOQ area		in place of the active substance peak, no signals from other substances with an area exceeding 30% of the LOQ area					
	the comparison of the UV spectra allows for the identification of active substances		the comparison of the UV spectra allows for the identification of active substances					
Linearity	r ≥0.99		r = 0.999 (0.034 mg/L – 3.569 mg/L)			r = 0.999 (0.035 mg/L – 3.855 mg/L)		
	random regression residuals (d _i) distribution		random regression residuals (d _i) distribution			random regression residuals (d _i) distribution		
Matrix effects [%]	not exceed ±20		2.3			-4.2		
Accuracy	70-120		level I*	101.2	100	level I*	78.3	89

[%]		level II*	98.6	level II*	99.8
Precision [% RSD]	≤20	level I*	3.3	level I*	15.2
		level II*	1.8	level II*	5.6
Limit of detection (LOD) [mg/L]	<0.036	<0.038	0.034	0.035	
Limit of quantification (LOQ) [mg/L]	-		0.119	0.126	

* Nominal concentration for boscalid at level I: 0.119 mg/L, and at level II: 1.190 mg/L
for prothioconazole at level I: 0.126 mg/L, and at level II: 1.263 mg/L

** Average determined concentration for boscalid at level I: 0.120 mg/L, and at level II: 1.173 mg/L
for prothioconazole at level I: 0.099 mg/L, and at level II: 1.261 mg/L

Table 1. Analytical method validation parameters (cont.)

AAP medium				
Parameter	Required criterion		Result	
	Boscalid	Prothioconazole	Boscalid	Prothioconazole
Selectivity	in place of the active substance peak, no signals from other substances with an area exceeding 30% of the LOQ area the comparison of the UV spectra allows for the identification of active substance		in place of the active substance peak, no signals from other substances with an area exceeding 30% of the LOQ area the comparison of the UV spectra allows for the identification of active substance	
Linearity	$r \geq 0.99$ random regression residuals (d_i) distribution		$r = 0.999$ (0.034 mg/L – 3.569 mg/L) random regression residuals (d_i) distribution	$r = 0.999$ (0.035 mg/L – 3.855 mg/L) random regression residuals (d_i) distribution
Matrix effects [%]	not exceed ±20		-3.6	-2.0

Accuracy [%]	70-120		level I*	98.3	97	level I*	83.7	94
			level II*	95.6		level II*	104.8	
Precision [% RSD]	≤20		level I*	5.1		level I*	5.7	
			level II*	1.6		level II*	3.8	
Limit of detection (LOD) [mg/L]	<0.036	<0.038	0.034			0.035		
Limit of quantification (LOQ) [mg/L]	-		0.119			0.126		

* Nominal concentration for boscalid at level I: 0.119 mg/L, and at level II: 1.190 mg/L
for prothioconazole at level I: 0.126 mg/L, and at level II: 1.263 mg/L

** Average determined concentration for boscalid at level I: 0.117 mg/L, and at level II: 1.137 mg/L
for prothioconazole at level I: 0.105 mg/L, and at level II: 1.323 mg/L

Table 1. Analytical method validation parameters (cont.)

M7 medium				
Parameter	Required criterion		Result	
	Boscalid	Prothioconazole	Boscalid	Prothioconazole
Selectivity	in place of the active substance peak, no signals from other substances with an area exceeding 30% of the LOQ area the comparison of the UV spectra allows for the identification of active substance		in place of the active substance peak, no signals from other substances with an area exceeding 30% of the LOQ area the comparison of the UV spectra allows for the identification of active substances	
Linearity	r ≥ 0.99 random regression residuals (d _i) distribution		r = 0.999 (0.034 mg/L – 3.569 mg/L) random regression residuals (d _i) distribution	r = 0.999 (0.035 mg/L – 3.855 mg/L) random regression residuals (d _i) distribution
Matrix effects [%]	not exceed ±20		2.5	7.3

Accuracy [%]	70-120		level I*	97.1	98	level I*	90.5	102
			level II*	98.5		level II*	114.0	
Precision [% RSD]	≤20		level I*	0.9		level I*	1.8	
			level II*	1.8		level II*	3.6	
Limit of detection (LOD) [mg/L]	<0.036	<0.038	0.034			0.035		
Limit of quantification (LOQ) [mg/L]	-		0.119			0.126		

* Nominal concentration for boscalid at level I: 0.119 mg/L, and at level II: 1.190 mg/L
for prothioconazole at level I: 0.126 mg/L, and at level II: 1.263 mg/L

** Average determined concentration for boscalid at level I: 0.116 mg/L, and at level II: 1.172 mg/L
for prothioconazole at level I: 0.114 mg/L, and at level II: 1.440 mg/L

The results of analytical method validation confirm the method is appropriate for the determination of active substances boscalid and prothioconazole concentrations in aqueous media (deionized water, AAP medium, M7 medium) solutions of the test item GLOB2021dF.

Comments of zRMS:	Validation data (Ballai C., 2023, FPBSTUDY-269-VAL1) are acceptable according SANTE/2020/12830 rev.1 and suitable for the determination of GLOB2021dF from Feeding Solutions.
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Reference: KCP 5.1.1

Report Validation of Analytical Method for the Determination of GLOB2021dF from Feeding Solutions, Ballai C., 2023, FPBSTUDY-269-VAL1

Guideline(s): Yes, SANTE/2020/12830 rev.1

Deviations: No

GLP: Yes

Acceptability: Yes

Summary

The purpose of this study was to validate the developed analytical method under the study code: FPBSTUDY-269-MD1 (CRL-VES study code 22/136-901ANE) for the quantitative determination of *GLOB2021dF* from feeding solutions.

The determination of the *Test Item* concentration based on the area of the compounds of interest (*Prothioconazole* and *Boscalid*).

The procedure was found to be suitable for the analysis of the *Test Item* from feeding solutions.

The validation parameters met the requirements.

Summary of the validated method parameters is presented in Table 1.

Table 1. Results of the method validation

Validated parameter	Acceptance criteria	Measured values	
		<i>Boscalid</i>	<i>Prothioconazole</i>
Linearity	The correlation coefficient (R^2) $\geq$ 0.99	$R^2 \geq 0.9993$	$R^2 \geq 0.9979$
	The back calculated values for the standards are within $\pm 20\%$ of their nominal concentrations	91.7 – 107.6	93.9 – 118.3
Limit of Detection	Lowest point of the calibration; back calculated values for the standards are within $\pm 20\%$ of their nominal concentrations	100.9 – 106.4% (RSD = 3.06%; n=3)	111.5 – 113.5% (RSD = 1.39%; n=3)
Limit of Quantification	The lowest experimentally confirmed test item concentration from vehicle that can be measured with sufficient recovery and precision with the validated method.	10 $\mu\text{g/g}$	10 $\mu\text{g/g}$
Instrument Precision (Injection Repeatability)	The areas and retention times of the seven reinjected samples should be ≤ 5 RSD%	RSD% $\leq 0.16\%$ for t_R RSD% $\leq 3.01\%$ for Area	RSD% $\leq 0.10\%$ for t_R RSD% $\leq 3.79\%$ for Area
Matrix Effects	Matrix effects are considered significant if they exceed $\pm 20\%$.	8.2% No significant matrix effect	7.3% No significant matrix effect
Selectivity / Specificity	Interference in blank samples should be $\leq 30\%$ of the LOD.	Below 30%	Below 30%

Validated parameter	Acceptance criteria	Measured values				
Recovery (Accuracy)		<i>Boscalid</i>				
	<i>Concentration (µg/g)</i>	<i>10</i>	<i>31</i>	<i>98</i>	<i>313</i>	<i>1000</i>
	The measured concentration of the test item compared to the nominal concentration should be $100 \pm 20\%$ in case of suspensions.	92.5%	84.7%	86.3%	89.1%	93.7%
Method precision	The individual precision of the replicate determinations of within a prepared sample (repeatability) should be ≤ 15 RSD%.	NMT 1.85%	NMT 2.95%	NMT 8.23%	NMT 5.69%	NMT 9.81%
	The overall precision of the replicate sample preparations per concentration should be ≤ 15 RSD%.	1.81	14.16	7.51	6.53	5.90
Recovery (Accuracy)		<i>Prothioconazole</i>				
	<i>Concentration (µg/g)</i>	<i>10</i>	<i>31</i>	<i>98</i>	<i>313</i>	<i>1000</i>
	The measured concentration of the test item compared to the nominal concentration should be $100 \pm 20\%$ in case of suspensions.	101.1%	94.1%	98.7%	101.2%	98.4%
Method precision	The individual precision of the replicate determinations of within a prepared sample (repeatability) should be ≤ 15 RSD%.	NMT 2.00%	NMT 1.65%	NMT 5.82%	NMT 6.47%	NMT 9.04%
	The overall precision of the replicate sample preparations per concentration should be ≤ 15 RSD%.	2.04	6.73	2.22	4.03	4.54
Stability		<i>Boscalid</i>		<i>Prothioconazole</i>		
Autosampler Stability of the Test Item (Reference standard) in Solvent – 29 hours 43 min	The area of the stored samples in the autosampler compared to the freshly prepared calibration samples after the correction of the appropriate nominal concentrations should be $100 \pm 20\%$.	99.2		99.3		
Analytical Stock	The mean area of five replicate meas-	<i>Boscalid</i>		<i>Prothioconazole</i>		

Validated parameter	Acceptance criteria	Measured values				
Solution Stability	Measurements of dilutions from the stored stock solution compared to the area of dilutions prepared from the fresh stock solution after the correction of the appropriate nominal concentrations should be $100 \pm 20\%$.	101.9		103.1		
Boscalid						
Stability of the Test Item Formulations 15 – 25°C for 1 day	Concentration ($\mu\text{g/g}$)	10	31	98	313	1000
	The concentration of the dilutions from the stored formulation after the resuspension and dilution compared to the freshly measured concentrations should be $100 \pm 20\%$.	98.7	100.7	89.8	81.0	89.0
Stability of the Test Item Formulations under transportation condition-below -15 °C for 40 days	The concentration of the dilutions from the stored formulation after the resuspension and dilution compared to the freshly measured concentrations should be $100 \pm 20\%$.	101.8	102.8	91.1	82.2	90.5
Prothioconazole						
Stability of the Test Item Formulations 15 – 25°C for 1 day	Concentration ($\mu\text{g/g}$)	10	31	98	313	1000
	The concentration of the dilutions from the stored formulation after the resuspension and dilution compared to the freshly measured concentrations should be $100 \pm 20\%$.	95.3	99.5	94.2	82.8	87.5
Stability of the Test Item Formulations under transportation condition-below -15 °C for 40 days	The concentration of the dilutions from the stored formulation after the resuspension and dilution compared to the freshly measured concentrations should be $100 \pm 20\%$.	100.0	102.0	100.1	88.0	93.1

NMT = Not more than

TBD = to be determined

The measured values of the **Linearity** include the results of both validations (22 March 2023–03 May 2023 and 07 July 2023–16 August 2023).

The measured values of **Matrix Effects, Selectivity, Autosampler Stability of the Test Item and Analytical Stock Solution Stability** include the results of the 22 March 2023–03 May 2023 validation.

The measured values of **Limit of Detection, Limit of Quantification, Instrument Precision, Recovery, Method precision, Stability of the Test Item Formulations 15 – 25°C for 1 day, Stability of the Test Item Formulations under transportation condition below -15 °C for 40 days** include the results of the 07 July 2023–16 August 2023 validation.

Comments of zRMS:	Validation data (Ballai C., 2023, FPBSTUDY-269-VAL3) are acceptable according SANTE/2020/12830 rev.1 and suitable for the determination of GLOB2021dF from Surface-treat Solution.
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Reference: KCP 5.1.1

Report Validation of Analytical Method for the Determination of GLOB2021dF from Surface-treat Solution, Ballai C., 2023, FPBSTUDY-269-VAL3

Guideline(s): Yes, SANTE/2020/12830 rev.1

Deviations: No

GLP: Yes

Acceptability: **Yes**

Summary

The purpose of this study was to validate the developed analytical method under the study code: FPBSTUDY-269-MD1 (CRL-VES study code 22/136-901ANE) for the quantitative determination of *GLOB2021dF* from Surface-treat Solution.

The determination of the *Test Item* concentration based on the area of the compounds of interest (*Prothioconazole* and *Boscalid*).

The procedure was found to be suitable for the analysis of the *Test Item* from Surface-treat Solution.

The validation parameters met the requirements.

Summary of the validated method parameters is presented in Table 1.

Table 1. Results of the method validation

Validated parameter	Acceptance criteria	Measured values	
		<i>Boscalid</i>	<i>Prothioconazole</i>
Linearity	The correlation coefficient (R^2) ≥ 0.99	$R^2 \geq 0.9993$	$R^2 \geq 0.9979$
	The back calculated values for the standards are within $\pm 20\%$ of their nominal concentrations	84.3– 107.6	90.8 – 118.3
Limit of Detection	Lowest point of the calibration; back calculated values for the standards are within $\pm 20\%$ of their nominal concentrations	100.9 – 106.4% (RSD = 3.06%; n=3)	111.5 – 113.5% (RSD = 1.39%; n=3)
Limit of Quantification	The lowest experimentally confirmed test item concentration from vehicle that can be measured with sufficient recovery and precision with the validated method.	1 mg/mL	1 mg/mL
Instrument Precision (Injection Repeatability)	The areas and retention times of the seven reinjected samples should be ≤ 5 RSD%	RSD% $\leq 0.16\%$ for t_R RSD% $\leq 3.01\%$ for Area	RSD% $\leq 0.10\%$ for t_R RSD% $\leq 3.79\%$ for Area
Matrix Effects	Matrix effects are considered significant if they exceed $\pm 20\%$.	3.1% No significant matrix effect	3.3% No significant matrix effect
Selectivity / Specificity	Interference in blank samples should	Below 30%	Below 30%

	be $\leq$ 30% of the LOD.		
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Validated parameter	Acceptance criteria	Measured values			
Recovery (Accuracy)		<i>Boscalid</i>		<i>Prothioconazole</i>	
	<i>Concentration (mg/mL)</i>	<i>1</i>	<i>60</i>	<i>1</i>	<i>60</i>
	The measured concentration of the test item compared to the nominal concentration should be $100 \pm 20\%$ in case of suspensions.	94.1 %	96.1 %	102.3 %	103.3 %
Method precision	The individual precision of the replicate determinations of within a prepared sample (repeatability) should be ≤ 15 RSD%.	NMT 2.05 %	NMT 1.88 %	NMT 2.33 %	NMT 2.32 %
	The overall precision of the replicate sample preparations per concentration should be ≤ 15 RSD%.	1.18 %	1.57 %	1.40 %	0.86 %
Stability		<i>Boscalid</i>		<i>Prothioconazole</i>	
Autosampler Stability of the Test Item (Reference standard) in Solvent – 29 hours 43 min	The area of the stored samples in the autosampler compared to the freshly prepared calibration samples after the correction of the appropriate nominal concentrations should be $100 \pm 20\%$.	99.2		99.3	
Analytical Stock Solution Stability	The mean area of five replicate measurements of dilutions from the stored stock solution compared to the area of dilutions prepared from the fresh stock solution after the correction of the appropriate nominal concentrations should be $100 \pm 20\%$.	<i>Boscalid</i>		<i>Prothioconazole</i>	
		101.9		103.1	

Validated parameter	Acceptance criteria	Measured values
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		<i>Boscalid</i>		<i>Prothioconazole</i>	
	<i>Concentration (mg/mL)</i>	1	60	1	60
Stability of the Test Item Formulations 15 – 25°C for 1 day	The concentration of the dilutions from the stored formulation after the resuspension and dilution compared to the freshly measured concentrations should be 100 ± 20%.	99.2	100.5	91.3	92.0
Stability of the Test Item Formulations 15 – 25°C for 35 days	The concentration of the dilutions from the stored formulation after the resuspension and dilution compared to the freshly measured concentrations should be 100 ± 20%.	107.4	103.3	97.5	94.5
Stability of the Test Item Formulations under transportation condition ≤ -15°C for 35 days	The concentration of the dilutions from the stored formulation after the resuspension and dilution compared to the freshly measured concentrations should be 100 ± 20%.	107.5	105.7	96.8	97.5

NMT = Not more than

The measured values of the **Linearity** include the results of FPBDOK-STUDY-269-VAL1-REPORT-01-AM-01 and FPBDOK-STUDY-269-VAL3 validation.

The measured values of **Limit of Quantification, Matrix Effects, Selectivity, Recovery, Method precision, Stability of the Test Item Formulations 15 – 25°C for 1 day, Stability of the Test Item Formulations 15 – 25°C for 35 days and Stability of the Test Item Formulations under transportation condition** include the results of FPBDOK-STUDY-269-VAL3 validation.

The measured values of **Limit of Detection, Instrument Precision, Autosampler Stability of the Test Item and Analytical Stock Solution Stability** include the results of FPBDOK-STUDY-269-VAL1-REPORT-01-AM-01 validation.

Comments of zRMS:	Method for GLOB2021dF (Ballai C., 2023, FPBSTUDY-269-MEAS1) is acceptable according SANTE/2020/12830 rev.1 and suitable for the test item content and homogeneity of spraying solutions.
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Reference: KCP 5.1.1

Report Phase Report: GLOB2021dF: A GLP Vegetative Vigour Test, Ballai C., 2023, FPBSTUDY-269-MEAS1

Guideline(s): Yes, SANTE/2020/12830 rev.1

Deviations: No

GLP: Yes

Acceptability: Yes

Summary

Test item content and homogeneity of spraying solutions were determined in this phase study with the developed HPLC-UV method.

Validated parameter	Acceptance criteria	Measured values			
		<i>Boscalid</i>		<i>Prothioconazole</i>	
Recovery (Accuracy)	<i>Concentration (mg/mL)</i>	0.6	6	0.6	6
	The measured concentration of the test item compared to the nominal concentration should be $100 \pm 15\%$ in case of suspensions.	102.8%	99.2%	103.1%	100.5%
	The individual precision of the replicate determinations of within a prepared sample (repeatability) should be ≤ 15 RSD%.	NMT 1.57%	NMT 2.41%	NMT 1.24%	NMT 2.31%
	The overall precision of the replicate sample preparations per concentration should be ≤ 15 RSD%.	0.54%	0.86%	0.57%	0.82%
Stability					
Autosampler Stability of the Test Item in Solvent -	The area of the stored samples in the autosampler compared to the freshly prepared calibration samples after the correction of the appropriate nominal concentrations should be $100 \pm 10\%$.	98.6%		97.5%	
Analytical Stock Solution Stability	The mean area of five replicate measurements of dilutions from the stored stock solution compared to the area of dilutions prepared from the fresh stock solution after the correction of the appropriate nominal concentrations should be $100 \pm 10\%$.	105.8%		108.2%	
Stability of the Test Item Formulations 15 – 25°C for 33 days	The concentration of the dilutions from the stored formulation after the resuspension and dilution compared to the freshly measured concentrations should be $100 \pm 10\%$.	100.5%	97.0%	99.1%	96.5%

Comments of zRMS:	Method for GLOB2021dF (Arputharaj P., 2023, 23-600-G) is acceptable according SANTE/2020/12830 rev.2 and suitable for the test item content.
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Reference: KCP 5.2-29

Report Analytical report: Acute Toxicity Study of GLOB2021dF in Fish (*Oncorhynchus mykiss*), Arputharaj P., 2024, 23-600-G

Guideline(s): Yes, SANTE/2020/12830 rev.2

Deviations: No

GLP: Yes

Acceptability: Yes

Summary

The objective of this study was to validate an analytical method for GLOB2021dF using HPLC-PDA instrument and verification of Active Ingredient concentrations in Limit Dose Test samples.

The methodology was evaluated for specificity, matrix effect, LOQ, LOD, final extract stability, standard stability, system suitability, linearity, assay accuracy and precision.

Results are summarized below.

Sr. No.	Parameters		Obtained results
GLOB2021dF			
1	Specificity		No interference
2	Matrix Effect for Prothioconazole	Replication 1	-0.1376%
		Replication 2	6.6706%
	Matrix Effect for Boscalid	Replication 1	-0.5442
		Replication 2	1.1593
	Acceptable Limit for Matrix Effect %		< ± 20
3	LOQ (% Recovery) Mean recovery ± Standard Deviation	LOQ for Prothioconazole	96.78 ± 1.45
		LOQ for Boscalid	95.23 ± 2.89
4	LOD (% Recovery) Mean recovery ± Standard Deviation	LOD for Prothioconazole	92.21± 0.67
		LOD for Boscalid	99.94 ± 2.11
	Acceptable (% Recovery)		70-120

5	System Suitability	Area (%RSD)	1.7156%
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	(Method Validation & Stability 0 Hour) for Prothioconazole	Retention Time (%RSD)	0.0967%
	System Suitability (Method Validation & Stability 0 Hour) for Boscalid	Area (%RSD)	1.3463%
		Retention Time (%RSD)	0.1255%
	System Suitability (Stability 96 Hour) for Prothioconazole	Area (%RSD)	1.5378%
		Retention Time (%RSD)	0.1326%
	System Suitability (Stability 96 Hour) for Boscalid	Area (%RSD)	0.7175%
		Retention Time (%RSD)	0.1803%
	System Suitability (Dose verification 0 Hour) for Prothioconazole	Area (%RSD)	0.4183%
		Retention Time (%RSD)	0.0689%
	System Suitability (Dose verification 0 Hour) for Boscalid	Area (%RSD)	1.0174%
		Retention Time (%RSD)	0.2732%
	System Suitability (Dose verification 96 Hour) for Prothioconazole	Area (%RSD)	0.5732%
		Retention Time (%RSD)	0.0765%
	System Suitability (Dose verification 96 Hour) for Boscalid	Area (%RSD)	0.8703%
		Retention Time (%RSD)	0.1260%
	Acceptable Limit for System Suitability	Area (%RSD)	< =2.00%
		Retention Time (%RSD)	< =2.00%
		Theoretical Plates (Mean)	>= 2000

6	Linearity for Prothioconazole	Concentration range (mg/L)	0.4981 to 9.9620 mg/L
		Correlation coefficient (r)	0.9997
	Linearity for Boscalid	Concentration range (mg/L)	0.5038 to 10.0758 mg/L
		Correlation coefficient (r)	0.9998
	Acceptable Limit for Linearity		Correlation coefficient (r) ≥0.99
7	Accuracy (% Recovery) Mean recovery ± Standard Deviation	High Range for Prothioconazole	94.48 ± 2.07
		High Range for Boscalid	97.87 ± 0.71
		Low range for Prothioconazole	94.57 ± 1.16
		Low range for Boscalid	95.05 ± 3.65
	Acceptable (% Recovery)		70-120
8	Precision (%RSD)	High Range for Prothioconazole	2.19
		High Range for Boscalid	0.73
		Low range for Prothioconazole	1.23
		Low range for Boscalid	3.84
	Acceptable (% Recovery)		≤ 20
	Acceptable (% Recovery)		≤ 20

For Dose verification samples results are summarized below.

Dose Type	Dose Levels	Mean Recovery (%) ± Standard Deviation	%RSD
GLOB2021dF			
Stability 0 Hour for Prothioconazole	Control	NA	NA
	T1 (100 mg/L)	98.21± 1.61	1.64
Stability 0 Hour for Boscalid	Control	NA	NA
	T1 (100 mg/L)	97.27 ± 0.39	0.40
Stability 96 Hour for Prothioconazole	Control	NA	NA
	T1 (100 mg/L)	98.16 ± 1.47	1.50
Stability 96 Hour for Boscalid	Control	NA	NA
	T1 (100 mg/L)	99.07 ± 0.75	0.76
Dose verification 0 Hour for Prothioconazole	Control	NA	NA
	T1(9.88 mg/L)	94.10 ± 0.29	0.31
	T3(22.22 mg/L)	98.58 ± 0.16	0.16
	T5(50.00 mg/L)	101.02 ± 2.91	2.88
Dose verification 0	Control	NA	NA

Hour for Boscalid	T1(9.88 mg/L)	93.53 ± 0.20	0.21
	T3(22.22 mg/L)	97.74 ± 0.92	0.94
	T5(50.00 mg/L)	92.44 ± 0.15	0.16
Dose verification 96 Hour for Prothioconazole	Control	NA	NA
	T1(9.88 mg/L)	90.55 ± 0.17	0.19
	T3(22.22 mg/L)	96.23 ± 0.28	0.29
	T5(50.00 mg/L)	96.86 ± 2.48	2.56
Dose verification 96 Hour for Boscalid	Control	NA	NA
	T1(9.88 mg/L)	97.44 ± 0.42	0.43
	T3(22.22 mg/L)	102.36 ± 0.97	0.95
	T5(50.00 mg/L)	101.10 ± 0.29	0.29

The method was found to be accurate and precise for determination of Prothioconazole and Boscalid in the evaluated samples from the results of assay accuracy and precision at both high and low ranges. The mean recovery percentage of Prothioconazole and Boscalid was found to be in the range of 70-120 % of its nominal concentration or theoretical concentration, in the analyzed dose formulation samples with percentage of relative standard deviation (%RSD) less than 20%.

Hence, concentration of active ingredient claimed in dose verification samples were found to meet the acceptance criteria.

Based on the above results and experimental condition used in the study, the method validation parameters were found to meet the acceptance criteria. The dose samples analyzed were found to meet the acceptance criteria

Comments of zRMS:	Analytical method (Grall E., 2022; EGL-20-45487) is acceptable and suitable for the determination of residues prothioconazole and its metabolites in barley (whole plant, grain and straw).
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Reference: KCP 5.1.22

Report Prothioconazole – Residue Study on Barley in Northern and Southern Europe – 2020, Walsch-Metzler, M. Grall E., 2022, IF20-05419733/ EGL-20-45487

Guideline(s): Yes, SANCO 3029/99 rev.4 (11/07/2000)

Deviations: No

GLP: Yes

Acceptability: Yes

Summary

During the growing season of 2020, a field trial with barley was conducted in Poland and Hungary. The magnitude of residues in barley specimens was determined for the triazole metabolites 1,2,4-Triazole, Triazole alanine, Triazole acetic acid and Triazole lactic acid after spray application with PROTHIOCONAZOLE 300 EC (HAG 610 01 F).

PROTHIOCONAZOLE 300 EC (293 g prothioconazole/L) was applied two times with nominal 195 g prothioconazole /ha with a water volume of 200-400 L/ha.

Specimens of barley were collected at a nominal sampling timing 35 ± 3 DALA as ears and straw (S1) and at normal commercial harvest (BBCH 89) as grain and straw (S2).

The barley specimens were analysed at SGS INSTITUT FRESENIUS GmbH, c/o SGS Germany GmbH, Hamburg, Germany for residues of the triazol metabolites following the analytical method 01062/M004^[1].

The Limit of Quantification (LOQ) of the analytical method was defined as 0.010 mg/kg for all analytes. The Limit of Detection (LOD) was defined as 0.003 mg/kg.

Linearity was shown over a range of 1.0 to 100 ng/mL for the analytes with correlation coefficients of ≥ 0.998 . The accuracy of the method was demonstrated by recovery experiments.

To ensure the validity of the analytical method, control specimens were fortified prior to extraction with 1,2,4-T, TA, TAA and TLA.

The mean recovery rate values, the standard deviations (SD) and the coefficients of variation (CV) were as follows:

Crop	Matrix	Fortification Level (nominal) [mg/kg]	1,2,4-T			
			Mean [%]	SD [±]	CV [%]	n
Barley	Ears	0.010, 0.10, 10	102	3.9	3.8	3
	Straw	0.010, 0.10, 10	88.4	3.8	4.3	3
	Grain	0.010, 0.10, 10	100	4.0	4.0	3
Overall:			96.9	3.9	4.0	9

SD: Standard deviation; CV: Coefficient of variation; n: Number of specimens

Crop	Matrix	Fortification Level (nominal) [mg/kg]	TA			
			Mean [%]	SD [±]	CV [%]	n
Barley	Ears	0.010, 0.10, 10	72.2	2.9	4.0	3
	Straw	0.010, 0.10, 10	81.7	4.0	4.8	3
	Grain	0.010, 0.10, 10	74.5	9.8	13	3
Overall:			76.1	5.6	7.4	9

SD: Standard deviation; CV: Coefficient of variation; n: Number of specimens

Crop	Matrix	Fortification Level (nominal) [mg/kg]	TAA			
			Mean [%]	SD [±]	CV [%]	n
Barley	Ears	0.010, 0.10, 10	78.2	8.7	11	3
	Straw	0.010, 0.10, 10	85.2	7.7	9.0	3
	Grain	0.010, 0.10, 10	90.1	5.8	6.4	3
Overall:			84.5	7.4	8.9	9

SD: Standard deviation; CV: Coefficient of variation; n: Number of specimens

Crop	Matrix	Fortification Level (nominal) [mg/kg]	TLA			
			Mean [%]	SD [±]	CV [%]	n
Barley	Ears	0.010, 0.10, 10	79.3	7.6	9.5	3
	Straw	0.010, 0.10, 10	94.4	10	11	3
	Grain	0.010, 0.10, 10	84.7	4.1	4.8	3
Overall:			86.1	7.3	8.4	9

SD: Standard deviation; CV: Coefficient of variation; n: Number of specimens

Summary of residues in/on the untreated/treated barley specimens for 1,2,4-T, TA, TAA and TLA

Matrix	Sampling No.	Plot	Nominal Sampling Timing	No. of Spec.	Range of Residues [mg/kg]			
					1,2,4-T	TA	TAA	TLA
Ears	S1	U	35 ± 3 DALA	2	< 0.010	< 0.010 – 0.115	< 0.010 – 0.136	< 0.010 – 0.018
Straw	S1		35 ± 3 DALA	2	< 0.010	< 0.010 – 0.013	< 0.010 – 0.031	< 0.010 – 0.061
Ears	S1	T	35 ± 3 DALA	2	< 0.010	0.119 – 0.200	0.050 – 0.193	< 0.010 – 0.029
Straw	S1		35 ± 3 DALA	2	< 0.010	< 0.010 – 0.027	< 0.010 – 0.067	0.021 – 0.157
Grain	S2	U	NCH (BBCH 89)	2	< 0.010	0.014 – 0.139	< 0.010 – 0.100	< 0.010
Straw	S2		NCH (BBCH 89)	2	< 0.010	< 0.010	< 0.010 – 0.067	< 0.010 – 0.036
Grain	S2	T	NCH (BBCH 89)	2	< 0.010	0.122 – 0.316	0.050 – 0.260	< 0.010
Straw	S2		NCH (BBCH 89)	2	< 0.010	< 0.010 – 0.032	< 0.010 – 0.152	< 0.010 – 0.145

DALA: Days after last application; NCH: Nominal commercial harvest; U: Untreated plot; T: Treated plot

Comments of zRMS:	Analytical method (Thirkell C., 2022, IF21-05704459) is acceptable and suitable for the determination of residues prothioconazole and its metabolites in Barley (whole plant, grain and straw).
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Reference: KCP 5.1.23

Report Study on the Residue Behaviour of Prothioconazole in Barley after Treatment with Prothioconazole 300 EC at two Sites under Field Conditions Northern Europe, 2021, Bodsch, J.; Kravchuk, O., 2022, IF21-05704459

Guideline(s): Yes, SANTE/2020/12830, Rev.1

Deviations: No

GLP: Yes

Acceptability: Yes

During the growing season of 2021, two field trials with barley were conducted in northern Europe (trial 21-00228-01 – Germany, trial 21-00228-02 – France). The magnitude of residues in barley specimens was determined for the triazole metabolites 1,2,4-Triazole (1,2,4-T), Triazole alanine (TA), Triazole acetic acid (TAA) and Triazole lactic acid (TLA) after spray application with Prothioconazole 300 EC. Prothioconazole 300 EC (300 g Prothioconazole/L nominal) was applied two times with nominal content 195 g prothioconazole/ha and a nominal water volume of 200-400 L/ha. Specimens of barley were collected at a nominal sampling timing 35 ± 3 DALA as ears, rest of plant, grain and straw (S1) and at normal commercial harvest (growth stage BBCH 89) as grain and straw (S2). The barley specimens were analysed at SGS INSTITUT FRESENIUS GmbH, c/o SGS Germany GmbH, Hamburg, Germany for residues of the triazol metabolites following the analytical method 01062/M004[1]. The method was validated at the analytical test site in study IF21-05707367[4] for the matrix groups relevant in this study. The Limit of Quantification (LOQ) of the analytical method was defined as 0.010 mg/kg for each analyte. The Limit of Detection (LOD) was defined to be 0.003 mg/kg for each analyte (lowest calibration standard used). Linearity was shown over a range of 1.25 to 100 ng/mL for the analytes with correlation coefficients of ≥ 0.998 and no visbel trend in the residuals. Therefore, the calibration model was defined to be suitable. The accuracy of the method was demonstrated by recovery experiments, control specimens were fortified prior to extraction with 1,2,4-T, TA, TAA and TLA. The mean recovery rate values and relative standard deviations (RSD) were as follows:

Crop	Matrix	Fortification Level (nominal) [mg/kg]	1,2,4-T		
			Mean [%]	RSD [±]	n
Barley	Grain	0.010, 0.10 and 1.0	93.6	3.4	6
	Straw	0.010, 0.10 and 1.0	91.6	8.6	6
	Ears	0.010, 0.10 and 1.0	97.9	6.3	6
	Rest of Plant	0.010, 0.10 and 1.0	94.3	4.7	6
Overall			94.3	6.2	24

RSD: relative standard deviation; n: number of specimens

Crop	Matrix	Fortification Level (nominal) [mg/kg]	TA		
			Mean [%]	RSD [±]	n
Barley	Grain	0.010, 0.10 and 1.0	84.8	12	9
	Straw	0.010, 0.10 and 1.0	85.9	12	6
	Ears	0.010, 0.10 and 1.0	86.5	4.2	6
	Rest of Plant	0.010, 0.10 and 1.0	96.1	6.9	6
Overall			87.9	10	27

RSD: relative standard deviation; n: number of specimens

Crop	Matrix	Fortification Level (nominal) [mg/kg]	TAA		
			Mean [%]	RSD [±]	n
Barley	Grain	0.010, 0.10 and 1.0	85.5	8.1	6
	Straw	0.010, 0.10 and 1.0	82.8	4.5	6
	Ears	0.010, 0.10 and 1.0	85.9	4.7	6
	Rest of Plant	0.010, 0.10 and 1.0	96.8	4.4	6
Overall			87.7	8.1	24

RSD: relative standard deviation; n: number of specimens

Crop	Matrix	Fortification Level (nominal) [mg/kg]	TLA		
			Mean [%]	RSD [±]	n
Barley	Grain	0.010, 0.10 and 1.0	78.1	5.3	6
	Straw	0.010, 0.10 and 1.0	78.0	5.3	6
	Ears	0.010, 0.10 and 1.0	81.3	2.6	6
	Rest of Plant	0.010, 0.10 and 1.0	95.9	1.5	6
Overall			83.3	9.8	24

RSD: relative standard deviation; n: number of specimens

Summary of residues in/on the untreated/treated barley specimens for 1,2,4-T, TA, TAA and TLA

Sampling No.	Portion analysed	Plot	DALA	BBCH	n	Residues [mg/kg]							
						1,2,4-T		TA		TAA		TLA	
Trial: 21-00228-						01	02	01	02	01	02	01	02
2	Grain	U	37 - 47	89	2	< 0.010	< 0.010	0.069	0.016	0.041	0.016	< 0.010	< 0.010
	Straw				2	< 0.010	< 0.010	< 0.010	< 0.010	0.013	< 0.010	0.014	< 0.010
1	Ears	T	40	83	1	---	< 0.010	---	0.13	---	0.072	---	0.014
	Rest of Plant				1	---	< 0.010	---	0.016	---	0.014	---	0.028
2	Grain		37 - 47	89	2	< 0.010	< 0.010	0.23	0.13	0.089	0.066	< 0.010	< 0.010
	Straw				2	< 0.010	< 0.010	0.029	0.013	0.026	0.025	0.018	0.017

DALA: Days after last application; U: Untreated plot; T: treated plot

Comments of zRMS:	Analytical method (Grall E., 2022, EGL-20-42538) is acceptable and suitable for the determination of residues prothioconazole and its metabolites in wheat (whole plant, grain and straw).
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Reference:	KCP 5.1.24
Report	Prothioconazole – Residue Study on Wheat in Northern Europe – 2020 Bodsch, J.; Kravchuk, O-Grall E., 2022, EGL-20-42538
Guideline(s):	Yes, SANCO 3029/99 rev.4 (11/07/2000)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

During the growing season of 2020, a field trial with wheat (triticum aestivum) was conducted in Northern Europe (trial EGL-20-42538, PL01 Poland). The magnitude of residues in wheat specimens was determined for the triazole metabolites 1,2,4-Triazole (1,2,4-T), Triazole alanine (TA), Triazole acetic acid (TAA) and Triazole lactic acid (TLA) after spray application with Prothioconazole 300 EC.

Prothioconazole 300 EC (293 g prothioconazole/L) was applied two times with nominal content 195 g prothioconazole/ha with a water volume of 200-400 L/ha.

Specimens of wheat were collected at a nominal sampling timing 0 (S1), 7±1 (S2) and 14±1 (S3) DALA as Whole plant, 35 ± 3 DALA (S4) as ears and straw and at normal commercial harvest (BBCH 89) as grain and straw (S5).

The wheat specimens were analysed at SGS INSTITUT FRESENIUS GmbH, c/o SGS Germany GmbH, Hamburg, Germany for residues of the triazole metabolites following the analytical method 01062/M004^[1].

The Limit of Quantification (LOQ) of the analytical method was defined as 0.010 mg/kg for each analyte. The Limit of Detection (LOD) was defined to be 0.003 mg/kg for each analyte (lowest calibration standard used).

Linearity was shown over a range of 1.0 to 100 ng/mL for the analytes with correlation coefficients of ≥ 0.998. The accuracy of the method was demonstrated by recovery experiments. To ensure the validity of the analytical method, control specimens were fortified prior to extraction with 1,2,4-T, TA, TAA and TLA. The mean recovery rate values, the standard deviations (SD) and the coefficients of variation (CV) were as follows:

Crop	Matrix	Fortification Level (nominal) [mg/kg]	1,2,4-T			
			Mean [%]	SD [±]	CV [%]	n
Wheat	Whole plant	0.010, 0.10, 10	88.3	7.6	8.6	3
	Ears	0.010, 0.10	87.6	n.a.	n.a.	2
	Straw	0.010, 0.10	95.1	n.a.	n.a.	2
	Grain	0.010, 0.10, 10	103	6.6	6.4	3
Overall:			94.1	11	12	10

SD: Standard deviation; CV: Coefficient of variation; n: Number of specimens; n.a.: SD and CV for less than three values are not applicable

Crop	Matrix	Fortification Level (nominal) [mg/kg]	TA			
			Mean [%]	SD [±]	CV [%]	n
Wheat	Whole plant	0.010, 0.10, 10	80.4	9.6	12	3
	Ears	0.010, 0.10, 10	91.4	17	20	3
	Straw	0.010, 0.10	82.1	--	--	2
	Grain	0.010, 0.10, 10	93.6	10	11	3
Overall:			86.4	11	13	11

SD: Standard deviation; CV: Coefficient of variation; n: Number of specimens; n.a.: SD and CV for less than three values are not applicable

Crop	Matrix	Fortification Level (nominal) [mg/kg]	TAA			
			Mean [%]	SD [±]	CV [%]	n
Wheat	Whole plant	0.010, 0.10, 10	89.0	14	15	3
	Ears	0.010, 0.10	89.2	--	--	2
	Straw	0.010, 0.10	90.6	--	--	2
	Grain	0.010, 0.10, 10	89.9	1.5	1.7	3
Overall:			89.6	7.3	8.1	10

SD: Standard deviation; CV: Coefficient of variation; n: Number of specimens

Crop	Matrix	Fortification Level (nominal) [mg/kg]	TLA			
			Mean [%]	SD [±]	CV [%]	n
Wheat	Whole plant	0.010, 0.10, 10	95.3	9.5	9.9	3
	Ears	0.010, 0.10	83.9	--	--	2
	Straw	0.010, 0.10	95.8	--	--	2
	Grain	0.010, 0.10, 10	88.1	2.4	2.7	3
Overall:			91.0	6.9	7.6	10

SD: Standard deviation; CV: Coefficient of variation; n: Number of specimens

Summary of residues in/on the untreated/treated wheat specimens for 1,2,4-T, TA, TAA and TLA

Sampling No.	Portion analysed	Plot	DALA nominal	n	Residues [mg/kg]			
					1,2,4-T	TA	TAA	TLA
Trial: EGL-20-42538, PL01								
S1	Whole plant	U	0	1	< 0.010	0.0100	< 0.010	0.0122
S4	Ears		35 ± 3	1	< 0.010	0.0361	0.0142	< 0.010
	Straw			1	< 0.010	< 0.010	0.0110	0.0102
S5	Grain		NCH (BBCH 89)	1	< 0.010	0.0609	0.0141	< 0.010
	Straw			1	< 0.010	< 0.010	0.0180	< 0.010
S1	Whole plant	T	0	1	< 0.010	0.0339	0.0163	0.0131
S2	Whole plant		7 ± 1	1	< 0.010	0.159	0.0173	0.0284
S3	Whole plant		14 ± 1	1	< 0.010	0.191	0.0194	0.0274
S4	Ears		35 ± 3	1	< 0.010	0.389	0.0583	< 0.010
	Straw			1	< 0.010	< 0.010	0.0174	0.0368
S5	Grain		NCH (BBCH 89)	1	< 0.010	0.467	0.0568	< 0.010
	Straw			1	< 0.010	0.0352	0.0767	0.0741

DALA: Days after last application; NCH: Nominal commercial harvest; U: Untreated plot; T: Treated plot

Comments of zRMS:	Analytical method (Thirkell C., 2022, IF21-05705310-21-00226) is acceptable and suitable for the determination of residues prothioconazole and its metabolites in wheat (whole plant, grain and straw).
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Reference: KCP 5.1-33

Report: Study on the residue behaviour of prothioconazole in wheat after treatment with Prothioconazole 300 EC at six sites under field conditions in Northern Europe, 2021
Report: IF21-05705310-21-00226 Thirkell C., 2022
SGS INSTITUT FRESENIUS GmbH
GLP
Unpublished

Guidelines: SANTE/2020/12830, Rev.1

Deviations: No

GLP: Yes

Acceptability: Yes/No/Supplementary

Summary

For the analysis of 1,2,4-T, TA, TAA and TLA the analytical method 01062/M004[1] was used. Which determined the analysis by LC-DMS/MS/MS. The method was validated at the analytical site in study IF21-05707367 for the matrix groups relevant in this study, summarized below.

Summary

During the growing season of 2021, six field trials with oilseed rape were conducted in Southern Europe (Trial 21-00231-01 – Southern France, Trial 21-00231-02 – Italy, Trial 21-00231-04 – Greece, Trial 21-00231-05 – Southern France, Trial 21-00231-06 – Italy, Trial 21-00231-08 – Greece). The magnitude of residues in oilseed rape specimens were determined for the triazole metabolites 1,2,4-Triazole, Triazole alanine, Triazole acetic acid and Triazole lactic acid after spray application with Prothioconazole 300 EC.

Prothioconazole 300 EC (300 g Prothioconazole/L) was applied two times with nominal content 180 g prothioconazole/ha and a nominal water volume of 200-400 L/ha.

Specimens of oilseed rape were collected at a nominal sampling timing 0 (S1), 7±1 (S2) and 14±1 (S3) DALA as whole plant, 35 ± 3 DALA (S4) as pods and rest of plant and at normal commercial harvest (growth stage BBCH 89) as seed and straw (S5).

The oilseed rape specimens were analysed at SGS INSTITUT FRESENIUS GmbH, c/o SGS Germany GmbH, Hamburg, Germany for residues of the triazol metabolites following the analytical method 01062/M004[1]. For the matrix groups high water content (whole plant), high oil content (seed) and dry matrix (straw) method validations according to SANTE/2020/12830 rev.1 were carried out by using procedural recovery samples which were analysed in parallel to the field samples. At least 5xLOQ and 5x10LOQ fortified samples were evaluated on both mass traces with regard to recovery, repeatability and selectivity/specificity.

Linearity was proven and a statement for matrix effect tests given.

The Limit of Quantification (LOQ) of the analytical method was defined as 0.010 mg/kg for each analyte. The Limit of Detection (LOD) was defined to be 0.003 mg/kg for each analyte (lowest calibration standard used).

Linearity was shown over a range of 1.0 to 100 ng/mL for the analytes with correlation coefficients of ≥ 0.998 and no visible trend in the residuals. Therefore, the calibration model was defined to be suitable.

Linearity was shown over a range of 1.25 to 100 ng/mL for the analytes with correlation coefficients of ≥ 0.998 and no visible trend in the residuals. Therefore, the calibration model was defined to be suitable.

The accuracy of the method was demonstrated by recovery experiments, control specimens were fortified prior to extraction with 1,2,4-T, TA, TAA and TLA.

The mean recovery rate values and the relative standard deviations (RSD) were as follows:

Crop	Matrix	TA		
		Mean (%)	RSD	n
Oilseed rape	Overall	91.5	5.7	51
Oilseed rape	Overall	87.7	11	51

Oilseed rape	Overall	94.2	5.7	51
Oilseed rape	Overall	92.1	7.5	51

Crop	Matrix	Fortification Level (nominal) [mg/kg]	1,2,4-T		
			Mean [%]	RSD [±]	n
Wheat	Grain	0.010, 0.10 and 2.0	97.4	6.7	7
	Straw	0.010, 0.10 and 2.0	90.8	15	9
	Ears	0.010, 0.10 and 2.0	89.9	5.8	7
	Rest of Plant with- out Roots	0.010, 0.10 and 2.0	92.3	5.7	7
	Whole Plant no Roots	0.010, 0.10 and 2.0	93.2	8.4	9
Overall			92.7	9.4	39
Crop	Matrix	Fortification Level (nominal) [mg/kg]	TA		
			Mean [%]	RSD [±]	n
Wheat	Grain	0.010, 0.10 and 2.0	86.8	16	7
	Straw	0.010, 0.10 and 2.0	80.1	14	9
	Ears	0.010, 0.10 and 2.0	86.1	8.0	7
	Rest of Plant with- out Roots	0.010, 0.10 and 2.0	89.9	5.0	7
	Whole Plant no Roots	0.010, 0.10 and 2.0	93.3	6.4	9
Overall			87.2	11	39
Crop	Matrix	Fortification Level (nominal) [mg/kg]	TAA		
			Mean [%]	RSD [±]	n
Wheat	Grain	0.010, 0.10 and 2.0	88.3	14	7
	Straw	0.010, 0.10 and 2.0	77.6	7.8	9
	Ears	0.010, 0.10 and 2.0	87.1	3.5	7
	Rest of Plant with- out Roots	0.010, 0.10 and 2.0	90.2	4.5	7
	Whole Plant no Roots	0.010, 0.10 and 2.0	93.0	6.4	9
Overall			87.1	10	39
Crop	Matrix	Fortification Level (nominal) [mg/kg]	TLA		
			Mean [%]	RSD [±]	n
Wheat	Grain	0.010, 0.10 and 2.0	72.6	3.4	7
	Straw	0.010, 0.10 and 2.0	83.4	15	9
	Ears	0.010, 0.10 and 2.0	84.5	4.4	7
	Rest of Plant with- out Roots	0.010, 0.10 and 2.0	89.4	1.6	7
	Whole Plant no Roots	0.010, 0.10 and 2.0	89.1	4.4	9
Overall			84.1	10	39

Analytical Method

The determination of the analytes based on the analytical method 01062/M004. The method was validated at the analytical test site in study IF21-05707367 for the matrix groups relevant in this study.

Principle of the method:

1,2,4-T, TA, TAA and TLA were extracted with a mixture of methanol and water. An aliquot was filtered, concentrated and cleaned-up by dispersive C18-SPE step. The analytes were determined by LC-DMS/MS/MS, using two different HPLC stationary phases and a LC-MS/MS instrument equipped with SelexION ion mobility technology which is based on planar differential spectrometry (DMS). Residues were quantified using stable isotopically labelled internal standards to compensate matrix effects.

Comments of zRMS:	Analytical method (Schnetser N., 2022, study no. 558) is acceptable and suitable for the determination of residues prothioconazole and its metabolites in honey.
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Reference: KCP 5.1-33

Report Analytical phase report: Tunnel study for determining the magnitude of residues of Prothioconazole 300EC in honey according to SANTE/11956/2016 rev. 9
Report: 558 (IF21-05844957) Schnetser N.
SGS INSTITUT FRESENIUS GmbH
GLP
Unpublished

Guidelines: SANTE/2020/12830 rev.1
SANTE/2020/12830, Rev.1

Deviations: No

GLP: Yes

Acceptability: Yes

Abstract

The objective of the analytical phase of this study was to determine the magnitude of residues of Prothioconazole and its metabolite Prothioconazole-desthio and the triazole derivate metabolites (TDMs) 1,2,4-Triazole, Triazole alanine, Triazole lactic acid and Triazole acetic acid in honey obtained in the field phase of the study after application of Prothioconazole 300 EC. The field phase was conducted at four locations: Limburgerhof (L1), Stutensee (L2), Kuhardt (L3) and Georgenhausen (L4).

The analytical methods for the determination of residues of prothioconazole, prothioconazole-desthio and the TDMs in honey samples were developed and validated according to SANTE/2020/12830 rev.1 at SGS INSTITUT FRESENIUS GmbH, c/o SGS Germany GmbH, Hamburg, Germany.

The Limit of Quantification (LOQ) of the analytical method was defined as 0.010 mg/kg for each analyte. The Limit of Detection (LOD) was defined to be 0.003 mg/kg for each analyte (lowest calibration standard used).

Linearity was shown over a range of 0.06 to 3.0 ng/mL for Prothioconazole and Prothioconazole-desthio and 1.25 to 100 ng/mL for the TDMs with no visible trend in the residuals. Therefore, the calibration model was defined to be suitable.

Matrix	Fortification Level [mg/kg]	Prothioconazole		
		Mean [%]	RSD [%]	N
Honey	0.010, 0.10 and 1.0	78.4	17	5

Matrix	Fortification Level [mg/kg]	Prothioconazole-dethio		
		Mean [%]	RSD [%]	N
Honey	0.010, 0.10 and 1.0	92.5	2.6	5

Matrix	Fortification Level [mg/kg]	1,2,4-T		
		Mean [%]	RSD [%]	N
Honey	0.010, 0.10 and 1.0	100	10	3

Matrix	Fortification Level [mg/kg]	TA		
		Mean [%]	RSD [%]	N
Honey	0.010, 0.10 and 1.0	104	7.1	3

Matrix	Fortification Level [mg/kg]	TAA		
		Mean [%]	RSD [%]	N
Honey	0.010, 0.10 and 1.0	98.7	3.3	3

Matrix	Fortification Level [mg/kg]	TLA		
		Mean [%]	RSD [%]	N
Honey	0.010, 0.10 and 1.0	97.0	7.2	3

Summary of residues in/on the untreated/treated Honey samples

Portion analysed	Plot	n	Residues [mg/kg]			
Location Code			L1	L2	L3	L4
Prothioconazole						
Honey	U	4	< LOD	< LOD	< LOD	< LOD
	T	4	< LOD	< LOD	< LOD	< LOD
Prothioconazole-desthio						
Honey	U	4	< LOD	< LOD	< LOD	< LOD
	T	4	< LOD	< LOD	< LOD	< LOD
1,2,4-T						
Honey	U	4	< LOD	< LOD	< LOD	< LOD
	T	4	< LOD	< LOD	< LOD	< LOD
TA						

Honey	U	4	< LOD	< LOD	< LOD	< LOD
	T	4	< LOD	< LOD	< LOD	< LOD
TAA						
Honey	U	4	< LOD	< LOD	< LOD	< LOD
	T	4	< LOD	< LOD	< LOD	< LOD
TLA						
Honey	U	4	< LOD	< LOD	< LOD	< LOD
	T	4	< LOD	< LOD	< LOD	< LOD

U: Untreated plot; T: Treated plot

No residues for Prothioconazole, its metabolite Prothioconazole-desthio and for the triazole derivate metabolites 1,2,4-Triazole, Triazole alanine, Triazole lactic acid and Triazole acetic acid above the LOQ of 0.01 mg/kg were found in any untreated or treated honey sample.

Comments of zRMS:	Validation of analytical method (Biesiada M., 2022, 0064/0037/FA) are acceptable according SANTE/2020/12830 rev.1 and suitable for the determination of active substances boscalid and prothioconazole of the test item GLOB2021dF concentrations in M7 media solutions.
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Reference: KCP 5.1.1

Report Validation of analytical method for the determination of active substances boscalid and prothioconazole concentrations in M7 media solutions of the test item GLOB2021dF, Biesiada M., 2022, 0064/0037/FA

Guideline(s): Yes, SANTE/2020/12830 rev.1

Deviations: No

GLP: Yes

Acceptability: Yes

Summary

The analytical method validation for the determination of active substances boscalid and prothioconazole concentrations in M7 medium solutions of the test item GLOB2021dF was performed. At the analytical method validation the following parameters: stability of the standards in solvent, matrix effects, selectivity, linearity, accuracy, precision, limit of detection and limit of quantification were determined. The analytical method validation was carried out based on the experimental procedure SPB-FA/11 and SANTE/2020/12830 rev.1 guideline. Analytical method validation parameters are presented in Table 1.

Table 1. Analytical method validation parameters

Parameter	Required criterion		Result					
	Boscalid	Prothioconazole	Boscalid			Prothioconazole		
Selectivity	in place of the active substance peak, no signals from other substances with an area exceeding 30% of the LOQ area the comparison of the UV spectra allows for the identification of active substances		in place of the active substance peak, no signals from other substances with an area exceeding 30% of the LOQ area the comparison of the UV spectra allows for the identification of active substances					
Linearity	$r \geq 0.99$ random regression residuals (d_i) distribution		$r = 0.999$ (0.010 mg/L – 1.027 mg/L) random regression residuals (d_i) distribution			$r = 0.999$ (0.011 mg/L – 1.093 mg/L) random regression residuals (d_i) distribution		
Matrix effects [%]	not exceed ± 20		18.9			13.7		
Accuracy [%]	70-120		level I*	87.7	94	level I*	101.0	102
			level II*	100.7		level II*	103.3	
Precision [% RSD]	≤ 20		level I*	8.8		level I*	4.8	
			level II*	6.3		level II*	1.2	
Limit of detection (LOD) [mg/L]	< 0.012	< 0.013	0.010			0.011		
Limit of quantification (LOQ) [mg/L]	-		0.039			0.042		
Standard stability (%)	≤ 10		3.9			4.8		
* Nominal concentration	for boscalid for prothioconazole		at level I: 0.039 mg/L, and at level II: 0.391 mg/L at level I: 0.042 mg/L, and at level II: 0.415 mg/L					
** Average determined concentration	for boscalid for prothioconazole		at level I: 0.034 mg/L, and at level II: 0.394 mg/L at level I: 0.042 mg/L, and at level II: 0.429 mg/L					

The results of analytical method validation confirm the method is appropriate for the determination of active substances boscalid and prothioconazole concentrations in M7 medium solutions of the test item GLOB2021dF.

Comments of zRMS: Analytical method (Poráčki.K, 2023, 22 48 BTR 0003) is acceptable and suitable for the determination of residues boscalid in Buckwheat (*Fagopyrum esculentum*)

MOENCH).

Reference:	KCP 5.1-33
Report	Magnitude of residues of Boscalid in Buckwheat (<i>Fagopyrum esculentum</i> MOENCH) honey after two applications of GLOB1811F under semi-field conditions in Central and Southern Europe 2023 Report: 22 48 BTR 0003 Poráčski.K, 2023 BioChem agrar Labor für biologische und chemische Analytik GmbH GLP Unpublished
Guidelines:	SANTE/11956/2016 rev. 9 (2018)
Deviations:	No
GLP:	Yes
Acceptability:	Yes/No/Supplementary
Summary	

Analytical Method

Principle of the method:

1 g of the honey sample was weight out into a 50 mL centrifuge tube. Reference item was added at this point, if necessary. Fortifications were done by using a reference item of boscalid with equivalent amounts. 9 mL Milli-Q-Water were added and the mixture was shaken for 1 minute at 280 rpm. Then 10 mL acetonitrile was added and the mixture was shaken for 1 minute at 280 rpm again. For Clean-up the Quechers- Mix I was added and the mixture was extensively shaken for 1 minute, thereafter centrifuged for 2 minutes at 5000rpm. After centrifugation an aliquot of 0.5 mL was diluted with Milli-Q-Water.

Quantification of boscalid was achieved by liquid chromatography using tandem mass spectrometric detection (LC-MS/MS), monitoring two mass transitions of analyte, one proposed for quantification and one for qualification. Therefore, no further confirmatory method was required.

7.3.1 Limit of Quantification (LOQ)

The Limit of Quantification, defined as the lowest quantification level, was set to 0.0025 mg/kg for boscalid. At this level an acceptable mean recovery between 60 – 120% and a relative standard deviation less than 30% was achieved.

7.3.2 Limit of Detection (LOD)

The Limit of Detection (LOD) was defined to be 30 % of the LOQ for Boscalid. A signal to noise ration of at least three was guaranteed for the LOD.

7.3.3 Residue In Control Sample

The detector signals for boscalid in the control sample which was used for validation purposes were below 30% of the detector signals of the specimens fortified at the LOQ.

Determined residues in Control Sample 22BTR0003_01-C-A

Analyte	Transition m/z	Blank value (% LOQ)
		Honey
Boscalid	343 → 307	28
	343 → 271	28

LOQ = 0.0025 mg/kg

7.3.4 Reagent Blank Value

Detector signals for boscalid in the reagent blank sample were not detectable.

Determined reagent blank values

Analyte	Transition m/z	Blank value (% LOQ)
		Honey
Boscalid	343 → 307	n.d.
	343 → 271	n.d.

LOQ = 0.0025 mg/kg; n.d.: not detectable

7.3.5 Matrix effects

Matrix effects were determined by comparison of the peak areas of matrix-matched calibration standard with solvent calibration standard at the same concentration level. Matrix effects were not significant ($\leq 20\%$), therefore solvent standards were used for validation.

Table 6: Determined Matrix Effects of Boscalid in Honey

Matrix	m/z	Type	Boscalid		
			Conc. [ng/mL]	Quotient [ng/mL] ²⁾	Matrix effect [%]
Honey	343 > 303	Solvent	1.250	129935	9.66
		MMS ¹⁾	1.250	142488	
	343 > 270	Solvent	1.250	42392	7.66
		MMS ¹⁾	1.250	45640	

¹⁾ MMS = matrix matched standard

²⁾ Mean peak area of three injections / standard concentration ng/mL

7.3.6 Selectivity

The high selectivity of the method resulted from the HPLC separation in combination with MS/MS detection. For Boscalid two mass transitions were validated.

7.3.7 Linearity

The chromatographic system for the analysis was calibrated using external calibration of the reference items in solvent.

Calibration solutions were prepared with at least seven different concentrations. Using regression analysis, a calibration curve was calculated taking the area into account. A weighting of $1/x$ was used for all calibration curves. The calibration standards were interspersed between the samples.

The linearity of the detector response was confirmed by solvent standard solutions with the nominal working range 0.0375 to 3.00 ng/mL (corresponding to 0.000375 - 0.030 mg/kg) for boscalid. The correlation coefficients (r) were always > 0.998 .

No trend was visible in the residuals and therefore the calibration model was defined to be suitable.

7.3.8 Recovery and Repeatability

An acceptable mean recovery between 60 – 120% and an acceptable repeatability in terms of the relative standard deviation of less than 30% was achieved for boscalid in Honey matrix. An overview is given below.

Boscalid.					
Matrix	Transition m/z	Fortification Level [mg/kg]	Number of fortified Samples	R_{mean} [%]	RSD [%]
Honey	343 → 303	0.0025	5	108	5.2
		0.025	5	99.6	3.7
		overall	10	104	6.0
	343 → 271	0.0025	5	106	3.5
		0.025	5	97.9	2.3
		overall	10	102	5.1