REGISTRATION REPORT

Part B

Section 5

Analytical Methods

Detailed summary of the risk assessment

Product code: ADM.03500.F.2.B
(alternative codes: ADM.3500.F.2.B; MCW-2075)

Product name(s): see part A

Chemical active substance:

Prothioconazole, 250 g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(authorisation)

Applicant: Country organisation / representative
as specified in Part A

Submission date: June 2021, Update September 2022

Finalisation date: November 2022 (initial Core Assessment)

April 2023 (final Core Assessment)

Version history

When	What
2021/06	Version 1 Applicant
2022/02	Version 2 Applicant. The Part B Section 5 was updated, mainly to reflect the changes made in the updated Part B Section 7.
2022/09	Version 3 Applicant. This section was amended by applicant to address zRMS questions during the evaluation phase on ILV for drinking water. In addition, monitoring methods for other matrices not included in the previous version of the Part B Section 5 have been added to this updated version of the B5.
November 2022	Initial ZRMS assessment. The report in the dRR format has been prepared by the Applicant, therefore all comments, additional evaluations and conclusions of the zRMS are presented in grey commenting boxes. Minor changes are introduced directly in the text and highlighted in grey. Not agreed or not relevant information are ~~struck through and shaded for transparency~~. Following the evaluation and before sending the document for commenting, all coloured highlighting was removed, from the parts updated by the Applicant, for better legibility.
April 2023	Final report (Core Assessment updated following the commenting period) Additional information/assessments included by the zRMS in the report in response to comments received from the cMS and the Applicant are highlighted in yellow. Information no longer relevant ~~is struck through and shaded~~.

DATA PROTECTION CLAIM

In order to present a dossier fully compliant with today's requirements (Reg. 284/2013), studies have been performed on ADM.03500.F.2.B. Under Article 59, Regulation 1107/2009/EC, on behalf of the Sponsor Company the applicant claims data protection for the studies conducted with ADM.03500.F.2.B. The data protection status and corresponding justification as valid for the respective country will be confirmed in the respective PART A.

STATEMENT FOR OWNERSHIP

The summaries and evaluations contained in this document may be based on unpublished proprietary data submitted for the purpose of the assessment undertaken by the regulatory authority that prepared it. Other registration authorities should not grant, amend, or renew a registration on the basis of the summaries and evaluation of unpublished proprietary data contained in this document unless they have received the data on which the summaries and evaluation are based, either –

- from the owner of the data, or
- from a second party that has obtained permission from the owner of the data for this purpose or,
- following expiry of any period of exclusive use, by offering – in certain jurisdictions – mandatory compensation, unless the period of protection of the proprietary data concerned has expired.

Table of Contents

5 Analytical methods

5.1 Conclusion and summary of assessment

zRMS summary and conclusions:

Prothioconazole

The endpoints reported in EFSA Scientific Report (2007) 106 are still valid for the ongoing evaluations.
However, taking into account conclusions EFSA regarding residue definitions presented in EFSA Journal 2020;18(2):5999, EFSA Journal 2014;12(5):3689 and EFSA Journal 2018;16(7):5376, based on the metabolic pattern identified in metabolism studies, hydrolysis studies, the toxicological significance of metabolites and degradation products, the residue definitions for plant products were proposed as 'prothioconazole-desthio (sum of isomers)' for enforcement and, as follows, for the risk assessment:
1) sum of prothioconazole-desthio and all metabolites containing the 2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl-2H-1,2,4-triazole moiety, expressed as prothioconazole-desthio (sum of isomers)

2) Triazole alanine (TA) and triazole lactic acid (TLA)

3) Triazole acetic acid (TAA)

4) 1,2,4-triazole (1,2,4-T).

Since all compounds included in the residue definitions are a mixture of enantiomers and since there are no enantiospecific analytical methods, the residue definitions are expressed as "sum of isomers".

Although the residue definition for risk assessment includes consideration of all metabolites containing a common moiety, it is not possible to develop a common moiety method to meet the residue definition for risk assessment. For this reason, all the analytes have to be determined separately. 6 analytes, representing the major portion of the TRR (Total Radioactive Residue) for prothioconazole in the plant metabolism studies, should be determined in residue trials. These are: prothioconazole-desthio, 3-hydroxy-prothioconazole-desthio, 4-hydroxy-prothioconazole-desthio, 5-hydroxy-prothioconazole-desthio, 6-hydroxy-prothioconazoledesthio and alpha-hydroxy-prothioconazole-desthio (including all their acid-hydrolysable conjugates).

The residue definition for enforcement in animal products was set as prothioconazole-desthio (sum of isomers) for all the livestock matrices (EFSA Journal 2014;12(5):3689).

For risk assessment, the residue was defined in all commodities of animal origin as the sum of prothioconazole-desthio and all metabolites containing the 2-(1-chlorocyclopropyl)-3-(2- chlorophenyl)-2-hydroxypropyl-2H-1,2,4-triazole moiety, expressed as prothioconazole-desthio (sum of isomers).

During the peer review under Directive 91/414/EEC, an analytical methods were evaluated and validated for the determination of prothioconazole-desthio in plant matrices and in food of animal origin. The available analytical methods are not enantioselective, hence the sum of isomers will be analyzed (EFSA Journal 2014;12(5):3689).

In EFSA Scientific Report (2007) 106, 1-98, "Conclusion on the peer review of prothioconazole" it is stated that:
„Methods are available to monitor all compounds given in the respective residue definition for food of plant origin, water, soil and air. Residues in food of plant origin can be determined with a multimethod (The German S19 method has been validated for prothioconazole-desthio). Only single methods are available to determine residues of prothioconazole-desthio, in products of animal origin and prothioconazole, prothioconazole-desthio in soil water and air. A method is not available to monitor the glucuronide conjugate in products of animal origin. Also if the active is classified as toxic then methods for body fluids and tissues would need to be considered."

EFSA Scientific Report (2007):
Analytical methods for residues (Annex IIA, point 4.2)

Food/feed of plant origin (principle of method and LOQ for methods for monitoring purposes)	Weeren, Pelz 2000 (GC-MS, JAU6476-desthio) LOQ Wheat, Barley (Forage, Straw): 0.05 mg/kg LOQ Wheat, Barley (Grain), Canola (Seed), Tomato, Orange (Fruit): 0.02 mg/kg
Food/feed of animal origin (principle of method and LOQ for methods for monitoring purposes)	Heinemann 2001b (HPLC-MS/MS, JAU6476-desthio, JAU6476-3 hydroxy-desthio, JAU6476-4-hydroxy-desthio) LOQ Milk: 0.004 mg/kg LOQ Meat, Liver, Kidney, Fat: 0.01 mg/kg Open: there is no method available for the glucuronide conjugate
Soil (principle of method and LOQ)	Schramel 2000 (HPLC-MS/MS, JAU6476, JAU6476-desthio, JAU6476-S-methyl*)

	* for monitoring not needed LOQ Soil: 0.006 mg/kg Add'l method: Steinhauer 2001 (GC-MS, JAU6476-desthio) LOQ Soil: 0.01 mg/kg
Water (principle of method and LOQ)	Sommer 2001b (HPLC-MS/MS, JAU6476, JAU6476-desthio) LOQ Surface and Drinking water: 0.1 µg/L for JAU6476 and 0.05 µg/L for JAU6476-desthio
Air (principle of method and LOQ)	Maasfeld 2002a (HPLC-MS/MS, JAU6476) LOQ Air: 0.015 mg/m^3 Additional method: Maasfeld 2002b (HPLC-MS/MS, JAU6476-desthio) LOQ Air: 0.0006 mg/m^3
Body fluids and tissues (principle of method and LOQ)	Open, data will be required if ECB classify the active as toxic

According to the EFSA Journal 2014;12(5):3689:
Methods for enforcement of residues in food of plant origin
During the peer review under Directive 91/414/EEC, an analytical method using GC-MS and its ILV were evaluated and validated for the determination of prothioconazole-desthio in plant matrices with an LOQ of 0.02 mg/kg in high water content (tomato), high oil content (rape seed), acidic (orange), dry (wheat grain) commodities and an LOQ of 0.05 mg/kg in straw. This method can be confirmed by an independent analytical method using HPLC-MS/MS fully validated for the determination of prothioconazole-desthio in high water content commodities and in straw with an LOQ of 0.05 mg/kg and in high oil content and in dry commodities with an LOQ of 0.01 mg/kg (United Kingdom, 2004). The analytical methods are not enantioselective, hence the sum of isomers will be analyzed.

The multi-residue QuEChERS method in combination with HPLC-MS/MS, as described by CEN (2008), is also available to analyse the prothioconazole-desthio in plant commodities. Nevertheless, the validation data reported are too limited to conclude on the validity of this analytical method (EURL, 2013).
Hence it is concluded that prothioconazole-desthio can be enforced in food of plant origin with an LOQ of 0.02 mg/kg in high oil content and dry commodities and an LOQ of 0.05 mg/kg in high water content commodities and in straw taking into account the highest LOQ of both methods.

Methods for enforcement of residues in food of animal origin
During the peer review under Directive 91/414/EEC, an analytical method using HPLC-MS/MS and its ILV were evaluated and validated for the determination of prothioconazole-desthio only in food of animal origin with an LOQ of 0.004 mg/kg in milk and an LOQ of 0.01 mg/kg in muscle, fat, liver and kidney (United Kingdom, 2004; EFSA, 2007b). Hence it is concluded that prothioconazole-desthio can be enforced in food of animal origin with an LOQ of 0.004 mg/kg in milk and an LOQ of 0.01 mg/kg in muscle, fat, liver and kidney. Nevertheless, prothioconazole-desthio cannot be enforced in eggs. Therefore, ***a fully validated analytical method for the determination of prothioconazole-desthio in eggs is required.***
The available analytical method is not enantioselective, hence the sum of isomers will be analyzed.

The Applicant submitted a number of methods for analysis of residues of prothioconazole for the generation of pre-authorization data and methods for post-authorization control and monitoring purposes.
Since many MRLs have been lowered to 0.01 mg/kg, the validated LOQ of the EU agreed methods by Weeren and Pelz (2000) and Class (2001) is not sufficient to monitor these lowered MRLs for food of plant origin. To cover the current residue definition and MRL limits, the applicant has provided a suitable monitoring method, including confirmation and ILV for all major matrix groups with a LOQ of 0.01 mg/kg for the determination of prothioconazole in plant commodities (Lefresne, S., 2020, KCP 5.2/02, Watson, G., 2022a, KCP 5.2/03).
The details of the evaluation of new and additional studies are referred in Appendix 2.

Note:
- According to the EFSA Scientific Report (2007) 106, 1-98, Conclusion on the peer review of Prothioconazole, the point regarding analytical methods for body fluids and tissues for prothioconazole is open, data will be required if ECB classify the active substance as toxic.
The active substance prothioconazole was evaluated at the EU level according to the old data requirements. The Commission Regulation (EU) No 284/2013 is applicable now.
In Regulation (EU) No 283/2013 it is stated that "…methods, with a full description, shall be submitted for the analysis in body fluids and tissues for the active substance and relevant metabolites" and this is a new requirement of SANTE/2020/12830. According to the SANTE/2020/12830: "*Analytical methods for monitoring residues in*

body fluids and tissues are required for detection of active substances and/or metabolites in humans and animals after possible intoxications or for biomonitoring purposes, regardless of their toxicological classification."
Therefore, an analytical method for the residues of prothioconazole in body fluids and tissues is required.

A body fluids method for the determination of residues of prothioconazole-desthio in blood has been submitted by Applicant. The limit of quantification was established at 0.01 mg/L.

- According to the conclusions presented in EFSA Journal 2014;12(5):3689, a fully validated analytical method for the determination of prothioconazole-desthio in eggs is required.
Applicant submitted the analytical method for the determination of prothioconazole-desthio in egg with LOQ 0.01 mg/kg. The analytical method of Watson, G., 2022 (Report No.: RES-00394) has been independently validated (Lindner, M., Büdel, A., 2022).

- Applicant submitted the analytical method of Lefresne, S., 2021 (Report No.: B21S-A4-P-04) for the determination of prothioconazole-desthio in honey with LOQ 0.01 mg/kg. The analytical method was independently validated (ILV; Lindner, M., 2022 Report No.: S21-06313).

- Applicant submitted the HPLC-MS/MS analytical method (with its ILV) for the determination of prothioconazole and prothioconazole-desthio in surface water. The method is also applicable for drinking water.

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

No additional data are required to support the intended uses for ADM.03500.F.2.B.

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

- none

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

- none

Commodity/crop		**Supported/ Not supported**
High starch	Wheat	Supported
	Barley	Supported
	Rye	Supported
	Triticale	Supported
	Oat	Supported
High oil	Oilseed rape	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 method and possible data gaps for analysis of the active substance prothioconazole in plant protection product ADM.03500.F.2.B is provided as follows:

Comments of zRMS:	The proposed analytical method was successfully validated for the determination of active substance prothioconazole in plant protection product ADM.03500.F.2.B according to the requirements laid down by SANCO3030/99 rev.5.

The following study has not been evaluated during the EU peer review of Prothioconazole.

Reference:	KCP 5.1.1/01 (filed in KCP 2.1/01)
Report	Determination of storage stability and physical-chemical properties of Prothioconazole 250 EC (ADM.3500.F.2.B) stored at 54°C for 14 days and at 0°C for 7 days Tsesin, N., 2019 Report no.: 000102642.035FL
Guideline(s):	SANCO/3030/99 rev. 5
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

A sample of the formulation was mixed with acetonitrile and the active substance was determined by HPLC-DAD.

Validation - Results and discussions

Table 5.2-1: Suitable method for the determination of prothioconazole in the plant protection product ADM.03500.F.2.B

	Prothioconazole
Author(s), year	Tsesin, N., 2019
Principle of method	HPLC-DAD
Linearity (linear between mg/L) (correlation coefficient, expressed as r) (number of calibration points)	External standard calibration. 0.25 – 0.8 mg/mL (about 50-170% of the nominal content of the active ingredient in the test item solution) r = 0.9999 7 calibration points
Precision – Repeatability Mean	RSD[1] = 0.3 % Horwitz RSDr[2] = 2.5 Horrat value (Hr)[3] = 0.12
Accuracy (% Recovery)	Mean total recovery at max. fortification level: 100 ±0.44 % Mean total recovery at med. fortification level: 101 ±0.3 % Mean total recovery at min. fortification level: 101 ±0.71 %
Interference/ Specificity	No interference
Comment	-

[1]RSD = Relative Standard Deviation

[2]RSD calculated via Howitz equation: % RSDR = 2(1-0.5 logC)
[3]Horrat value (Hr) calculated as %RSD/%RSDr is considered acceptable when < 1

Conclusion
The analytical method provides a specific determination of the active ingredient prothioconazole in the formulation ADM.03500.F.2.B and fulfils the requirements of SANCO/3030/99 rev.5.

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:

Comments of zRMS:	The analytical method was successfully validated for the quantification of the prothioconazole-desthio in the plant protection product according to the requirements laid down by SANCO3030/99 rev.5.

The following study has not been evaluated during the EU peer review of Prothioconazole.

Reference:	KCP 5.1.1/01 (filed in KCP 2.1/01)
Report	Determination of storage stability and physical-chemical properties of Prothioconazole 250 EC (ADM.3500.F.2.B) stored at 54°C for 14 days and at 0°C for 7 days Tsesin, N., 2019 Report no.: 000102642.035FL
Guideline(s):	SANCO/3030/99 rev. 5
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods
A sample of the formulation was mixed with acetonitrile and the relevant impurity prothioconazole-desthio was determined by HPLC-DAD.

Validation - Results and discussions

Table 5.2-2: Suitable method for the determination of prothioconazole-desthio in the plant protection product ADM.03500.F.2.B

	Prothioconazole-desthio
Author(s), year	Tsesin, N., 2019
Principle of method	HPLC-DAD
Linearity (linear between mg/L) (correlation coefficient, expressed as r) (number of calibration points)	External standard calibration. 0.0099 – 0.1984 mg/mL (corresponding to 0.01 – 0.2% w/w) r = 1.0000 6 calibration points
Precision – Repeatability Mean	RSD[1] = 3.35 % (concentration of 0.01%) Horwitz RSDr[2] = 5.36 (concentration of 0.01%) Horrat value (Hr)[3] = 0.625
Accuracy (% Recovery)	Mean marginal recovery at 0.05%: 109 ±0.67 % Mean marginal recovery at 0.012%: 112 ± 1.4 % Mean marginal recovery at 0.01%: 118 ± 3.82
LOQ	0.01 %

	Prothioconazole-desthio
Interference/ Specificity	No interference
Comment	-

[1]RSD = Relative Standard Deviation
[2]RSD calculated via Howitz equation: % RSDR = 2(1-0.5 logC)
[3]Horrat value (Hr) calculated as %RSD/%RSDr is considered acceptable when < 1

Conclusion

The analytical method provides a specific determination of the relevant impurity prothioconazole-desthio in the formulation ADM.03500.F.2.B and fulfils the requirements of SANCO/3030/99 rev.5.

Comments of zRMS:	The analytical method was successfully validated for the quantification of the Toluene in the plant protection product according to the requirements laid down by SANCO3030/99 rev.5.

Reference:	KCP 5.1.1/02
Report	Analytical Method Validation of Toluene in Prothioconazole 250 EC (ADM.3500.F.2.B) Tsesin, N., 2019 Report no.: 000102645.038FL
Guideline(s):	SANCO/3030/99 rev. 5
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

A sample of the formulation was mixed with acetonitrile and toluene was determined by GC-FID. Calibration was carried out by external standard calibration.

Validation - Results and discussions

Table 5.2-3: Methods suitable for the determination of the relevant impurity in ADM.03500.F.2.B

	Toluene
Author(s), year	Tsesin, N., 2019
Principle of method	GC-FID
Linearity (linear between mg/L) (correlation coefficient, expressed as r)	0.025 – 0.25 % of the working concentration 6 calibration points r = 0.99599
Precision – Repeatability Mean n = 5 (%RSD)	RSD[1] = 1.198 Horwitzs RSDr[2] = 4.21 % (at 0.05% w/w) Horrat H_r [3]= 0.285
Accuracy n = 5 (% Recovery (marginal recovery)	102.7% (level 0.025%) 98.7 % (level 0.05%) 104.3 % (level 0.1%)
Interference/ Specificity	Highly specific method, no interferences detected. GC-MS for confirmation
LOQ	0.025 %
Comment	-

[1]RSD = Relative Standard Deviation
[2]RSD calculated via Howitz equation: % RSDR = 2(1-0.5 logC)
[3]Horrat value (Hr) calculated as %RSD/%RSDr

Conclusion

The analytical method is suitable for the determination of the relevant impurity toluene in the formulation

ADM.03500.F.2.B and fulfils the requirements of SANCO/3030/99 rev.5.

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

Not required.

5.2.1.4 Applicability of existing CIPAC methods (KCP 5.1.1)

No CIPAC method is available for the determination of prothioconazole in EC formulations.

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

Table 5.2-4: Validated methods for the generation of pre-authorization data

Component of residue definition: prothioconazole*				
Matrix type	**Matrix**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Plants (Residues)	Wheat (whole plant, grain, straw)	0.01 mg/kg**	LC-MS/MS	Lefresne, S. 2020, KCP 5.1.2/02 (filed in KCA 6.1/02)
		0.01 mg/kg**	LC-MS/MS	Huaulmé, J.-M., 2019a, KCP 5.1.2/03 (filed in KCA 6.3.1/01)
		0.01 mg/kg**	LC-MS/MS	Amic, S., 2020a, KCP 5.1.2/04 (filed KCA 6.3.1/03)
		0.01 mg/kg**	LC-MS/MS	Amic, S., 2020b, KCP 5.1.2/05 (filed KCA 6.3.1/05)
		0.01 mg/kg**	LC-MS/MS	Lefresne, S. 2021, KCP 5.1.2/21 (method validation for: Le Mineur, A., 2022, KCA 6.3.1/07 Le Mineur, A., 2022, KCA 6.3.1/08)
	Barley (whole plant, grain, straw)	0.01 mg/kg**	LC-MS/MS	Huaulmé, J.-M., 2019b, KCP 5.1.2/06 (filed KCA 6.3.2/01)
		0.01 mg/kg**	LC-MS/MS	Amic, S., 2020c, KCP 5.1.2/07 (filed KCA 6.3.2/03)
		0.01 mg/kg**	LC-MS/MS	Amic, S., 2020d, KCP 5.1.2/08 (filed KCA 6.3.2/05)
		0.01 mg/kg**	LC-MS/MS	Huaulmé, J.-M., 2020, KCP 5.1.2/09 (filed in KCA 6.3.2/07)
		0.01 mg/kg**	LC-MS/MS	Huaulmé, J.-M., 2021, KCP 5.1.2/010 (filed in KCA 6.3.2/09)
		0.01 mg/kg**	LC-MS/MS	Lefresne, S. 2021, KCP 5.1.2/21 (method validation for: Barbier, G., 2022, KCA 6.3.2/13 Huaulmé, J.-M., 2021b, KCA 6.3.2/11
	Oilseed rape (whole plant, seed, straw)	0.01 mg/kg**	LC-MS/MS	Lefresne, S. 2020, KCP 5.1.2/02 (filed in KCA 6.1/02)

Component of residue definition: prothioconazole*				
Matrix type	**Matrix**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
		0.01 mg/kg**	LC-MS/MS	Roussel, Ch. H., 2020, KCP 5.1.2/11 (filed in KCA 6.3.3/01)
		0.01 mg/kg**	LC-MS/MS	Peterek, S., 2020, KCP 5.1.2/12 (filed in KCA 6.3.3/03)
		0.01 mg/kg**	LC-MS/MS	Grall, E., 2021, KCP 5.1.2/13 (filed in KCA 6.3.3/05)
	Oilseed rape (seed)	0.01 mg/kg**	LC-MS/MS	Lefresne, S. 2021, KCP 5.1.2/21 (method validation for: Amic, S., 2021, KCA 6.3.3/07
	Dry bean	0.01 mg/kg**	LC-MS/MS	Lefresne, S. 2020, KCP 5.1.2/02 (filed in KCA 6.1/02)
Animal products, food of animal origin (Residues)	Honey	0.01 mg/kg**	LC-MS/MS	Lefresne, S. 2021, KCP 5.1.2/21
	Nectar, pollen, flowers and honey	0.01 mg/kg	LC-MS/MS	Lindner, M.,Grewe, D. 2020, KCP 5.1.2/23 (method validation for: Persigehl, M. et al., 2021, KCA 6.10.1/01, Persigehl, M. et al., 2021, KCA 6.10.1/02, Persigehl, M. et al., 2021, KCA 6.10.1/03, Persigehl, M. et al., 2020, KCA 6.10.1/04)
Soil, water, sediment (Environmental fate)	-			
Soil, water (Efficacy)	-			
Feed, body fluids (Toxicology)	-			
Body fluids, air (Exposure)	-			
Soil, water, sucrose solution (Ecotoxicology)	Water (from the aqua toxicity test)	0.00982 mg/L	HPLC-MS/MS	Urban, V., 2020, KCP 5.1.2/14 (filed in KCP 10.2.1/01)
		0.0196 mg/L	HPLC-MS/MS	Zetzmann, M., 2020, KCP 5.1.2/15 (filed in KCP 10.2.1/02)
		0.00219 mg/L	HPLC-MS/MS	Schuler, L., 2020, KCP 5.1.2/16 (filed in KCP 10.2.1/03)
		0.000561mg/L	HPLC-MS/MS	Weber, K., 2020, KCP 5.1.2/17 (filed in KCP 10.2.1/04)
	Bee diet (50 % sucrose solution)	230 mg/L	HPLC-UV	Sekine, T., 2020, KCP 5.1.2/18 (filed in KCP 10.3.1.1/01)

Component of residue definition: prothioconazole*				
Matrix type	**Matrix**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
	Aqueous stock solution	29.0 mg/L	HPLC-DAD	Colli, M., 2020, KCP 5.1.2/19 (filed in KCP 10.3.1.3/01)
	Spray solution	500 mg/L	HPLC-DAD	Buttler, O., 2020, KCP 5.1.2/20
Phys-chem (Properties)	Active Substance in Formulation (Storage stability)	Not relevant	HPLC-DAD	Tsesin, N., 2019 KCP 5.1.1/01 (filed in KCP 2.1/01)

* Prothioconazole and its metabolites prothioconazole-desthio, 3-hydroxyprothioconazole-desthio expressed prothioconazole-desthio, 4-hydroxyprothioconazole-desthio expressed as prothioconazole-desthio, 5-hydroxyprothioconazole-desthio expressed as prothioconazole-desthio, 6-hydroxyprothioconazole-desthio expressed as prothioconazole-desthio and alpha-hydroxyprothioconazole-desthio expressed as prothioconazole-desthio
** For prothioconazole as the sum of all analytes: LOQ = 0.060 mg/kg

Table 5.2-5: Validated methods for the generation of pre-authorization data

Component of residue definition: 1,2,4-Triazole, Triazole alanine, Triazole acetic acid and Triazole lactic acid				
Matrix type	**Matrix**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Plants (Residues)	Cucumber	0.01 mg/kg*	LC-MS/MS	Klimmek, S and Gizler, A., 2017, KCP 5.1.2/01 (filed in KCA 6.1/01)
	Grapes	0.01 mg/kg*		
	Dry beans	0.01 mg/kg*		
	Wheat (whole plant, grain, staw), barley (whole plant, grain, straw), oilsee rape (seeds, crude oil, refined oil, pressed cake), sunflower (seeds)	0.01 mg/kg*	LC-MS/MS	Gustloff, C.; Wallbaum, P., 2021, KCP 5.1.2/22 (method validation for: Yozgatli, H.P., 2021b, KCA 6.3.1/02 Yozgatli, H.P., 2021c, KCA 6.3.1/04 Yozgatli, H.P., 2021d, KCA 6.3.1/06 Le Mineur, A., 2022, KCA 6.3.1/07 Yozgatli, H.P., 2021e, KCA 6.3.2/02 Yozgatli, H.P., 2021f, , KCA 6.3.2/04 Yozgatli, H.P., 2021g, , KCA 6.3.2/06 Mahlow, S., 2021, KCA 6.3.2/08 Yozgatli, H.P., 2021h, , KCA 6.3.2/10) Huaulmé, J.-M., 2021b, KCA 6.3.2/11 Huaulmé, J.-M., 2022, KCA 6.3.2/12 Gustloff, C., 2021, KCA 6.3.3/02 Ivanov, E., 2021a, KCA 6.3.3/04 Ivanov, E., 2021b, KCA 6.3.3/06 Amic, S., 2021, KCA 6.3.3/07
Animal products, food of animal origin (Residues)	-			
Soil, water, sediment (Environmental fate)	-			
Soil, water (Efficacy)	-			

Component of residue definition: 1,2,4-Triazole, Triazole alanine, Triazole acetic acid and Triazole lactic acid				
Matrix type	**Matrix**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Feed, body fluids (Toxicology)	-			
Body fluids, air (Exposure)	-			
Soil, water, sucrose solution (Ecotoxicology)	-			
Phys-chem (Properties)	-			

The LOQ of the analytical method is 0.01 mg/kg for each of the metabolites (1,2,4-Triazole, Triazole alanine, Triazole acetic acid and Triazole lactic acid).

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

5.3.1

5.3.2 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 are submitted under point 5.2.1.

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

It is referred to the following EU concluded residue definitions for risk assessment:

Matrix	Residue Definition	Reference
Plant commodities	Sum of prothioconazole-desthio and all metabolites containing the 2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl-2H-1,2,4-triazole moiety, expressed as prothioconazole-desthio (provisional)	EFSA Scientific report, 2007
Animal origin	Sum of prothioconazole-desthio and all metabolites containing the 2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl-2H-1,2,4-triazole moiety, expressed as prothioconazole-desthio (provisional)	EFSA Scientific report, 2007
Soil	Prothioconazole, prothioconazole-desthio (M04)13, prothioconazole-S-methyl (M01)	EFSA Scientific report, 2007
Sediment	Prothioconazole, prothioconazole-desthio (M04)	EFSA Scientific report, 2007
Surface water	Prothioconazole, prothioconazole-desthio (M04), 1,2,4-triazole	EFSA Scientific report, 2007
Drinking / ground water	Prothioconazole, prothioconazole-desthio (M04), 1,2,4-triazole	EFSA Scientific report, 2007
Air	Prothioconazole, prothioconazole-desthio (M04)	EFSA Scientific report, 2007
Body fluids / tissues	None allocated	EFSA Scientific report, 2007

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

Matrix	Residue definition	MRL / LOQ	Reference for MRL/level Remarks
Food of plant origin	Prothioconazole: prothioconazole-desthio (sum of isomers)	0.01 mg/kg for citrus fruits, pome fruits, stone fruits, berries and small fruits, tropical root and tuber vegerables, bulb vegetables, solanaceae and malvaceae, cucurbits, leafy brassica, kohlrabies, lettuces and salad plants, spinaches, legume vegetables, sugar plants 0.02 mg/kg for tree nuts, potatoes, sweet corn, oil fruits 0.05 mg/kg for flowering brassica 0.02 – 0.3 mg/kg for oilseeds 0.01 – 0.2 mg/kg for cereals	Commission Regulation (EU) 2019/552
Food of animal origin (Muscle, fat, liver/kidney, milk and egg)	Prothioconazole: prothioconazole-desthio (sum of isomers)	0.01 mg/kg	SANTE/2020/12830, Rev.1
Food of animal origin (Honey)	Prothioconazole: prothioconazole-desthio (sum of isomers)	0.06 mg/kg	Commission Regulation (EU) 2019/552
Soil (Ecotoxicology)	Prothioconazole, prothioconazole-desthio (M04)	0.05 mg/kg	General limit according to SANTE/2020/12830, Rev.1
Drinking water (Human toxicology)	Prothioconazole, prothioconazole-desthio (M04)	0.1 µg/L	General limit for drinking water
Surface water (Ecotoxicology)	Prothioconazole	7.1 µg/L	SANTE/2020/12830, Rev.1 RAC for aquatic Invertebrates
	Prothioconazole-desthio (M04)	0.334 µg/L	SANTE/2020/12830, Rev.1 RAC for aquatic fish
Air	Prothioconazole	60 µg/m^3	SANTE/2020/12830, Rev.1 (based on an AOEL of 0.2 mg/kg bw/day)
	Prothioconazole-desthio (M04)	3 µg/m3	SANTE/2020/12830, Rev.1 based on an AOEL of 0.01 mg/kg bw/day
Body fluids	Prothioconazole	not required	not classified as T / T+
	Prothioconazole-desthio (M04)	0.01 mg/L	General limit according to SANTE/2020/12830, Rev.1
Body tissues	Prothioconazole	not required	not classified as T / T+
	Prothioconazole-desthio (M04)	0.01 mg/kg	General limit according to SANTE/2020/12830, Rev.1

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 and possible data gaps for analysis of prothioconazole residues in plant matrices is given in the following tables. No new or additional studies were submitted.

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: Prothioconazole-desthio				
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 (tomato)	Primary	0.02 mg/kg	DFG S19 GC-MS	Weeren, Pelz (2000); DAR Prothioconazole, Volume 3, Annex B, 5, IIA 4.2.1.1/06 EU agreed (EFSA Scientific report 2007)
	ILV	0.02 mg/kg	DFG S19 GC-MS	Class (2001); DAR Prothioconazole, Volume 3, Annex B, 5, IIA 4.2.1.1/07 EU agreed (EFSA Scientific report 2007)
	Confirmatory	Not required		
High water content (wheat whole plant)	Primary	0.01 mg/kg	LC-MS/MS	Lefresne, S., 2020, KCP 5.2/02
	ILV	0.01 mg/kg	LC-MS/MS	Watson, G., 2022a, KCP 5.2/03
	Confirmatory	Not required		
High acid content (orange)	Primary	0.01 mg/kg	DFG S19 GC-MS	Weeren, Pelz (2000); DAR Prothioconazole, Volume 3, Annex B, 5, IIA 4.2.1.1/06 EU agreed (EFSA Scientific report 2007)
	ILV	0.02 mg/kg	DFG S19 GC-MS	Class (2001); DAR Prothioconazole, Volume 3, Annex B, 5, IIA 4.2.1.1/07 EU agreed (EFSA Scientific report 2007)
	Confirmatory	Not required		
High acid content (strawberry)	Primary	0.01 mg/kg	LC-MS/MS	Lefresne, S., 2020, KCP 5.2/02
	ILV	0.01 mg/kg	LC-MS/MS	Watson, G., 2022a, KCP 5.2/03
	Confirmatory	Not required		
High oil content (Rape seed)	Primary	0.02 mg/kg	DFG S19 GC-MS	Weeren, Pelz (2000); DAR Prothioconazole, Volume 3, Annex B, 5, IIA 4.2.1.1/06 EU agreed (EFSA Scientific report 2007)
	ILV	0.02 mg/kg	DFG S19 GC-MS	Class (2001); DAR Prothioconazole, Volume 3, Annex B, 5, IIA 4.2.1.1/07 EU agreed (EFSA Scientific report 2007)
	Confirmatory	Not required		
High oil content (Rape seed)	Primary	0.01 mg/kg	LC-MS/MS	Lefresne, S., 2020, KCP 5.2/02
	ILV	0.01 mg/kg	LC-MS/MS	Watson, G., 2022a, KCP 5.2/03
	Confirmatory	Not required		
Dry commodity with high protein/high starch content (wheat grain)	Primary	0.02 mg/kg	DFG S19 GC-MS	Weeren, Pelz (2000); DAR Prothioconazole, Volume 3, Annex B, 5, IIA 4.2.1.1/06 EU agreed (EFSA Scientific report 2007)
	ILV	0.02 mg/kg	DFG S19	Class (2001); DAR Prothioconazole,

Component of residue definition: Prothioconazole-desthio				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
			GC-MS	Volume 3, Annex B, 5, IIA 4.2.1.1/07 EU agreed (EFSA Scientific report 2007)
	Confirmatory	Not required		
Dry commodity with high protein/high starch content (wheat grain)	Primary	0.01 mg/kg	LC-MS/MS	Lefresne, S., 2020, KCP 5.2/02
	ILV	0.01 mg/kg	LC-MS/MS	Watson, G., 2022a, KCP 5.2/03
	Confirmatory	Not required		

Table 5.3-3: Statement on extraction efficiency

	Method for products of plant origin
Required, available from:	Draft Assessment Report DAR – PROTHIOCONAZOLE, July 2005, Volume 3, Annex B.5 and B7 extraction efficiency was demonstrated
Not required, because:	-

The extraction efficiency of the residue method in cereals and rape (Heinemann, O. (2001); DAR Prothioconazole, Volume 3, Annex B 5, IIA 4.2.1.1/01) was tested using aged radioactive residues from the metabolism study following spray application of [phenyl-UL-M-047681-01-1, please refer to DAR Prothioconazole, Volume 3, Annex B 7, IIA 6.1.1.1/01). The residue method extraction (using acetonitrile/water as solvent) and the amount extracted in the metabolism studies were in good agreement. The extraction efficiency was in excellent correspondence.
The extraction efficiency was in excellent correspondence. In the following the extraction efficiency of the monitoring methods is evaluated in accordance with SANTE 2017/10632 Rev. 3 following the decision tree for post-monitoring methods:
As prothioconazole residues in metabolism studies (using radiolabeled active substance) were determined at ≥ 0.01 mg/kg (step 1) and a common-moiety method without previous extraction is not required (Step 2), the amount of the extracted TRR needs to be assessed (Step 3). As described and displayed in DAR Prothioconazole, Volume 3, Annex B 7.1.1 and in the Draft (Renewal) Assessment Report Prothioconazole, Volume 3, Annex B 7.2.1, the TRR was > 70 % for all the of the investigated crop matrices wheat (dry matrix), peanut (matrix with high oil content) and sugar beet (matrix with high water content) (Step 3 (1)). However, components of the DoR were < 50% of TRR (Step 3 (2)). On the other hand, none of the compounds of the DoR were present in the non-extracted radioactive residue. Thus, solvents of the metabolism studies and of the monitoring methods are compared (Step 4). Since for the monitoring methods and for the metabolism studies acetonitrile/water was used as solvent system, the extraction efficiency of the monitoring methods is sufficiently demonstrated. Plant matrices with a high acid content were not part of the metabolism studies in the DAR. However, with regard to good results for the other matrix types, it cannot be assumed that the results for matrices with high acid content would be contradictive.

5.3.3.3 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 prothioconazole residues in animal matrices is given in the following tables. No new or additional studies were submitted.

Table 5.3-4: 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	**Analyte**	**Method type**	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Fat Muscle Liver, kidney	Pothioconazole-desthio, JAU6476-3-hydroxy-desthio, JAU6476-4-hydroxy-desthio	Primary	0.01 mg/kg	HPLC-MS/MS	Heinemann, O. (2001); DAR Prothioconazole, Volume 3, Annex B, 5, IIA 4.2.1.1/04 EU agreed (EFSA Scientific report 2007)
		ILV	0.01 mg/kg	HPLC-MS/MS	Dubey, L. (2001); DAR Prothioconazole, Volume 3, Annex B, 5, IIA 4.2.1.1/08 EU agreed (EFSA Scientific report 2007)
		Confirmatory	Not required		
Milk	Pothioconazole-desthio, JAU6476-3-hydroxy-desthio, JAU6476-4-hydroxy-desthio	Primary	0.004 mg/kg	HPLC-MS/MS	Heinemann, O. (2001); DAR Prothioconazole, Volume 3, Annex B, 5, IIA 4.2.1.1/05 EU agreed (EFSA Scientific report 2007)
		ILV	0.004 mg/kg	HPLC-MS/MS	Dubey, L. (2001); DAR Prothioconazole, Volume 3, Annex B, 5, IIA 4.2.1.1/08 EU agreed (EFSA Scientific report 2007)
		Confirmatory	Not required		
Egg	Pothioconazole-desthio	Primary	0.01 mg/kg	HPLC-MS/MS	Watson, G., 2022b, KCP 5.2/04
		ILV	0.01 mg/kg	HPLC-MS/MS	Lindner, M., Büdel, A., 2022, KCP 5.2/05
		Confirmatory	Not required		
Honey	Pothioconazole-desthio	Primary	0.01 mg/kg	HPLC-MS/MS	Lefresne, S., 2021, KCP 5.2/06
		ILV	0.01 mg/kg	HPLC-MS/MS	Lindner, M., 2022, KCP 5.2/07
		Confirmatory	Not required		

*The current application proposes uses on cereals for which the supervised crop residue profile demonstrates that the existing animal dietary burden considered by EFSA would not be further exceeded. Therefore since there are no additional uses on feed items proposed and the current assessment is within existing dietary burden calculations it is concluded that no further evaluation of residue definition to include conjugates of prothioconazole-desthio is warranted.

Table 5.3-5: Statement on extraction efficiency

	Method for products of animal origin
Required, available from:	Draft Assessment Report DAR – PROTHIOCONAZOLE, July 2005, Volume 3, Annex B.5 and B7 extraction efficiency was demonstrated
Not required, because:	-

The extraction efficiency of the residue method in animal matrices was previously demonstrated for the Annex I inclusion by Heinemann, O (2001).; "Analytical determination of residues of JAU6476-3-hydroxy-desthio, JAU6476-4-hydroxy-desthio, and JAU6476-desthio in/on matrices of animal origin by HPLC-MS/MS"; document M-037709-01-1, (please refer ro DAR Prothioconazole, Volume 3, Annex B, 5, IIA 4.2.1.1/04) using aged radioactive residues from the goat metabolism study (Weber, H., Weber, E. and Spiegel, K.; DAR Prothioconazole, Volume 3, Annex B 7, IIA 6.2.2.1/01). In summary, the comparison of the residue analytical method of extraction for animal matrices with the extraction method used in the metabolism study demonstrated the suitability of the analytical method (extracting with an

acetonitrile/water solvent system) for the determination of the relevant residue in animal matrices. No further consideration is necessary. In the following the extraction efficiency of the monitoring methods is evaluated in accordance with SANTE 2017/10632 Rev. 3 following the decision tree for post-monitoring methods:
As prothioconazole residues in metabolism studies (using radiolabeled active substance) were determined at ≥ 0.01 mg/kg (step 1) and a common-moiety method without previous extraction is not required (Step 2), the amount of the extracted TRR needs to be assessed (Step 3). As described and displayed in DAR Prothioconazole, Volume 3, Annex B 7.2 and in the Draft (Renewal) Assessment Report Prothioconazole, Volume 3, Annex B 7.2.2, the TRR was not > 70 % for all the of the animal matrices (Step 3 (1)) and components of the DoR were < 50% of TRR (Step 3 (2)). On the other hand, none of the compounds of the DoR was present in the non-extracted radioactive residue. Thus, solvents of the metabolism studies and of the monitoring methods are compared (Step 4). Since for the monitoring methods and for the majority of the metabolism studies acetonitrile/water was used as solvent system, the extraction efficiency of the monitoring methods is sufficiently demonstrated.

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

An overview on the acceptable methods and possible data gaps for analysis of prothioconazole residues in soil is given in the following tables. No new or additional studies were submitted.

Table 5.3-6: Validated methods for soil (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	0.006 mg/kg 0.006 mg/kg for Prothioconazole-desthio	HPLC-MS/MS	Schrammel, O. (2001); DAR Prothioconazole, Volume 3, Annex B, 5, IIA 4.2.2.1/01 EU agreed (EFSA Scientific report 2007)
Confrimatory	Not required		
Component of residue definition: 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	Schrammel, O. (2001); DAR Prothioconazole, Volume 3, Annex B, 5, IIA 4.2.2.1/01 EU agreed (EFSA Scientific report 2007)
Confrimatory	Not required		

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

An overview on the acceptable methods and possible data gaps for analysis of prothioconazole residues in surface and drinking water is given in the following tables. No new or additional studies were submitted.

Table 5.3-7: Validated methods for water (if appropriate)

Component of residue definition: Prothioconazole, 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	HPLC-MS/MS	Krebber, R., Sandau, C., 2015, KCP 5.2/08
	Confirmatory	Not required as the primary method is highly specific.		
	ILV	0.05 µg/L	HPLC-MS/MS	Thies, S., 2015, KCP 5.2/09

Component of residue definition: Prothioconazole, prothioconazole-desthio (M04)				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
	Confirmatory	Not required		
Surface water / groundwater	Primary	0.05 µg/L	HPLC-MS/MS	Sommer, H. (2001); DAR Prothioconazole, Volume 3, Annex B, 5, IIA 4.2.3.1/03 EU agreed (EFSA Scientific report 2007)
	Confirmatory	Not required as the primary method is highly specific		

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

An overview on the acceptable methods and possible data gaps for analysis of prothioconazole residues in air is given in the following table. No new or additional studies were submitted.

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

Component of residue definition: Prothioconazole, prothioconazole-desthio (M04)				
Analyte	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Prothioconazole	Primary	0.015 mg/m^3	HPLC-MS/MS	Massfeld, W. (2002a); DAR Prothioconazole, Volume 3, Annex B, 5, IIA 4.2.4.1/01 EU agreed (EFSA Scientific report 2007)
	Confirmatory	Not required as the primary method is highly specific		
Prothioconazole-desthio	Primary	0.0006 mg/m^3	HPLC-MS/MS	Massfeld, W. (2002b); DAR Prothioconazole, Volume 3, Annex B, 5, IIA 4.2.4.1/01 EU agreed (EFSA Scientific report 2007)
	Confirmatory	Not required as the primary method is highly specific		

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

An overview on the acceptable methods and possible data gaps for analysis of prothioconazole in body fluids is given in the following table. For the detailed evaluation of new study, it is referred to Appendix 2.

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

Component of residue definition: Prothioconazole-desthio (M04)			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	0.01 mg/L	LC-MS/MS	Brown, S., 2022, KCP 5.2/01
Confirmatory	Not required as the primary method is highly specific		

5.3.3.8 Other studies/ information

No other studies were submitted.

Appendix 1 Lists of data considered in support of the evaluation

List of data submitted by the applicant and relied on

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	Vertebrate study Y/N	Owner
KCP 5.1.1/01 filed in KCP 2.1/01	Tsesin, N.	2019	Determination of storage stability and physical-chemical properties of Prothioconazole 250 EC (ADM.3500.F.2.B) stored at 54°C for 14 days and at 0°C for 7 days Report no. 000102642.035FL, Sponsor no. 000102642 ADAMA Makhteshim Ltd., Beer-Sheva, Israel GLP / GEP Unpublished	N	ADM
KCP 5.1.1 02	Tsesin, N.	2019	Analytical method validation of toluene in Prothioconazole 250 EC (ADM.3500.F.2.B) Report no. 000102645.038FL, Sponsor no. 000102645 ADAMA Makhteshim Ltd., Beer-Sheva, Israel GLP / GEP Unpublished	N	ADM
KCP 5.1.2/01 (filed in KCA KCP 8/ KCA 6.1/01)	Klimmek, S. and Gizler, A.	2017	Freezing storage stability & validation of residues of 1,2,4-Triazole, Triazole Alanine, Triazole Acetic Acid and Triazole Lactic Acid in water, acid and dry matrix: cucumber, grapes and dry bean at 0, 3, 6, 12, 18, 24 and 36 months. Report No.: S12-00072, sponsor no.: R-30330 Eurofins Agroscience Services Chem GmbH, Hamburg, Germany GLP Unpublished	N	ADM
KCP 5.1.2/02 (filed in KCA KCP 8/ KCA 6.1/02)	Lefresne, S.	2020	Freezing storage stability of prothioconazole-desthio, 3-hydroxy-prothioconazole-desthio, 4-hydroxy-prothioconazole-desthio, 5-hydroxy-prothioconazole-desthio, 6-hydroxy-prothioconazole-desthio and alpha-hydroxy-prothioconazole-desthio in plant matrices at/below -18°C during 24 months (0, 1, 3, 12, 18 and 24 months): Wheat whole plant (high water content), wheat grain (high starch content), wheat straw (difficult commodity), oilseed rape grain (high oil content), strawberry (high acid content) and dry bean (high protein content). Report no. B18S-A4-P-02, Sponsor no. R-39653 POLLENIZ / GIRPA, Beaucouze Cedex, France GLP / GEP Unpublished	N	ADM
KCP 5.1.2/03 (filed in KCA 6.3.1/01)	Huaulmé, J.-M.	2019a	Residue study of azoxystrobin, prothioconazole and its metabolites in wheat whole plants and Raw Agricultural Commodity after one foliar application of MCW-2073 - 1 harvest and 2 decline trials – Northern Europe (France and Poland) - 2018, Huaulmé, J.-M. Report no. BPL18/713/GC, Sponsor no. R-39643 BIOTEK Agriculture, Saint-Pouange, France	N	ADM

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**
			GLP / GEP Unpublished		
KCP 5.1.2/04 (filed in KCA 6.3.1/03)	Amic, S.	2020a	Residue study of azoxystrobin, prothioconazole and its metabolites in wheat whole plant and Raw Agricultural Commodity after one foliar application of MCW-2073 - 3 harvest and 2 decline trials – Northern Europe (FR, HU, PL) – 2019 Report no. BPL19/757/GC, Sponsor no. 000102745 BIOTEK Agriculture, Saint-Pouange, France GLP / GEP Unpublished	N	ADM
KCP 5.1.2/05 (filed in KCA 6.3.1/05)	Amic, S.	2020b	Residue study of prothioconazole and its metabolites in wheat whole plant and RAC after one foliar application of ADM.3500.F.2.B (250 g a.s./L of prothioconazole) - 2 harvest and 2 decline trials – Northern Europe (FR, HU, PL) – 2019 Report no. BPL19/762/GC, Sponsor no. 000102751 BIOTEK Agriculture, Saint-Pouange, France GLP / GEP Unpublished	N	ADM
KCP 5.1.2/06 (filed in KCA 6.3.2/01)	Huaulmé, J.-M.	2019b	Residue study of azoxystrobin, prothioconazole and its metabolites in barley whole plants and Raw Agricultural Commodity after one foliar application of MCW-2073 - 1 harvest and 2 decline trials – Northern Europe (France) – 2018 Report no. BPL18/715/GC, Sponsor no. R-39645 BIOTEK Agriculture, Saint-Pouange, France GLP / GEP Unpublished	N	ADM
KCP 5.1.2/07 (filed in KCA 6.3.2/03)	Amic, S.	2020c	Residue study of azoxystrobin, prothioconazole and its metabolites in barley whole plant and Raw Agricultural Commodity after one foliar application of MCW-2073 - 3 harvest and 2 decline trials - Northern Europe (France, Poland and Hungary) - 2019 Report no. BPL19/759/GC, Sponsor no. 000102749 BIOTEK Agriculture, Saint-Pouange, France GLP / GEP Unpublished	N	ADM
KCP 5.1.2/08 (filed in KCA 6.3.2/05)	Amic, S.	2020d	Residue study of prothioconazole and its metabolites in barley whole plant and RAC after one foliar application of ADM.3500.F.2.B (250 g a.s./L of prothioconazole) - 2 harvest and 2 decline trials – Northern Europe (FR, HU, PL) - 2019 Report no. BPL19/764/GC, Sponsor no. 000102753 BIOTEK Agriculture, Saint-Pouange, France	N	ADM

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
			GLP / GEP Unpublished		
KCP 5.1.2/09 (filed in KCP 8/ KCA 6.3.2/07)	Huaulmé, J.-M.	2020	Residue study of prothioconazole and its metabolites, and fenpropidin in barley whole plant and RAC (grain and straw) after one foliar application of ADM.3502.F.1.A - 2 harvest and 2 decline trials - Northern Europe (France, Poland and Hungary) - 2019. Report no.: BPL19/772/GC, sponsor no.: 000102761 BIOTEK Agriculture, Saint-Pouange, France GLP Unpublished	N	ADM
KCP 5.1.2/10 (filed in KCP 8/ KCA 6.3.2/09)	Huaulmé, J.-M.	2021a	Residue study of prothioconazole and its metabolites, and fenpropidin in barley whole plant and raw agricultural commodity after one foliar application of ADM.3502.F.1.A - 2 harvest and 2 decline trials – Northern Europe (FR, PL, HU) - 2020. Report no.: BPL20/844/GC, sponsor no.: 000105350 BIOTEK Agriculture, Saint-Pouange, France GLP Unpublished	N	ADM
KCP 5.1.2/11 (filed in KCA 6.3.3/01)	Roussel, Ch. H.	2020	Magnitude of the residues of azoxystrobin + prothioconazole and metabolites in oilseed rape (RAC whole plant and seeds), following one application of MCW-2073 in 3 trials (2 DCS and 1 HS), Northern Europe (Northern France and Poland) – 2018 Report no. ChR-18-33731, Sponsor no. R-39647 Staphyt, Inchy-en-Artois, France GLP / GEP Unpublished	N	ADM
KCP 5.1.2/12 (filed in KCA 6.3.3/03)	Peterek, S.	2020	Magnitude of the residues of azoxystrobin + prothioconazole and metabolites in oilseed rape (RAC whole plant, seeds and straw), following one application of MCW-2073 in 6 trials (2 DCS, 3 HS and 1 backup HS), Northern Europe (PL, N-FR, DE) – 2019 Report no. SPK-19-38368, Sponsor no. 000102602 Staphyt, Inchy-en-Artois, France GLP / GEP Unpublished	N	ADM
KCP 5.1.2/13 KCP 8/ KCA 6.3.3/03 (filed in KCA 6.3.3/05)	Grall, E.	2021	Magnitude of the residues of prothioconazole and metabolites in oilseed rape (RAC whole plant, seeds and straw), following one application of ADM.3500.F.2.B in 4 trials (2 DCS and 2 HS), Northern Europe (Poland, Northern France and Germany) – 2019/2020. Report no.: SPK-19-38370, sponsor no.: 000102604 STAPHYT, Gines, Spain	N	ADM

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**
			GLP Unpublished		
KCP 5.1.2/14 (filed in KCP 10.2.1/01)	xxxxxxxxxxxxx	2020	ADM.3500.F.2.B: Toxicity to the rainbow trout *Oncorhynchus mykiss* under laboratory conditions (Acute toxicity test – Semi-static) Report no. S19-03475, Sponsor no. 000102732 xxxxxxxxxxxxxxxxxxx GLP Unpublished	N	ADM
KCP 5.1.2/15 (filed in KCP 10.2.1/02)	Zetzmann, M.	2020	ADM.3500.F.2.B: Toxicity to the water flea *Daphnia magna* Straus under laboratory conditions (Acute immobilisation test – Semi-static) Report no. S19-03474, Sponsor no. 000102731 Eurofins Agroscience Services Ecotox GmbH GLP Unpublished	N	ADM
KCP 5.1.2/16 (filed in KCP 10.2.1/03)	Schuler, L.	2020	ADM.3500.F.2.B: Toxicity to the single cell green alga *Pseudokirchneriella subcapitata* Hindák under laboratory conditions Report no. S19-03473, Sponsor no. 000102730 Eurofins Agroscience Services Ecotox GmbH GLP Unpublished	N	ADM
KCP 5.1.2/17 (filed in KCP 10.2.1/04)	Weber, K.	2020	ADM.3500.F.2.B: Toxicity to the duckweed *Lemna gibba* under laboratory conditions (Growth inhibition test – Semi-static) Report no. S19-03476, Sponsor no. 000102733 Eurofins Agroscience Services Ecotox GmbH GLP Unpublished	N	ADM
KCP 5.1.2/18 (filed in KCP 10.3.1.1/01)	Sekine, T.	2020	ADM.3500.F.2.B: Effects (acute contact and oral) on honey bees (*Apis mellifera* L.) in the laboratory Report no. 137191035, Sponsor no. 000101260 Ibacon GmbH GLP Unpublished	N	ADM
KCP 5.1.2/19 (filed in KCP 10.3.1.3/01)	Colli, M.	2020	Effects of ADM.3500.F.2.B on honeybees (*Apis mellifera* L.) 22-day larval toxicity test with repeated exposure Report no. BT109/19, Sponsor no. 000101262 Biotecnologie BT S.r.l.	N	ADM

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**
			GLP Unpublished		
KCP 5.1.2/20	Buttler, O.	2020	ADM.3500.F.2.B - Method Validation for the Determination of Prothioconazole in Spray Solutions for Terrestrial Plant Tests Report no. 190403AR / CMV18620, Sponsor no. 000103710 Noack Laboratorinen GmbH GLP Unpublished	N	ADM
KCP 5.1.2/21	Lefresne, S.	2021	Validation of an analytical method for the determination of prothioconazole residues in cereals, honey, oilseed rape and sugar beet. Report no. B21S-A4-P-01, EFSA-2021-00003265, Sponsor no. 000108024 GIRPA, Beaucouzé Cedex, France GLP Unpublished	N	ADM
KCP 5.1.2/22	Gustloff, C.; Wallbaum, P.	2021	Validation of an analytical method for the determination of triazole metabolites (TDMs) in crop matrices of season 2021 Report no. S21-02262, MAC-2135V, Sponsor no. 000107909 Eurofins Agroscience Services Chem GmbH, Hamburg, Germany GLP Unpublished	N	ADM
KCP 5.1.2/23	Lindner, M.; Grewe, D.	2020	Validation of an analytical method for the determination of prothioconazole, prothioconazole-desthio and azoxystrobin in nectar, pollen, flower and honey report no.: S19-20860 (MAC-1940V), sponsor no.: 000104134 Eurofins Agroscience Services Chem GmbH, Hamburg, Germany GLP, Unpublished	N	ADM
KCP 5.1.2/24 (filed in KCA 6.6.2/01)	Semrau, J.,	2021	Determination of Residues of Prothioconazole and its Metabolites after One Application of MCW-2073 on Bare Soil in Rotational Crops (Radish, Leaf lettuce and Barley) at 2 Sites in Northern Europe and 2 Sites in Southern Europe 2018/2019 Report no. S18-02513, Sponsor no.: R-39638 Eurofins Agroscience Services GmbH, Stade, Germany GLP, Unpublished	N	ADM
KCP 5.1.2/25 (filed in KCA 6.1/05)	Kalathoor, R.	2021	Residue analytical method 01602 and short term storage of 1,2,4-triazole, triazole alanine, triazole acetic acid and triazole lactic acid in/on honey HPLC DMS-MS/MS, Report Amendment No. 2 Study no.: M-680825-03-1, sponsor no.: not stated	N	TDMG

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
			Not stated GLP Unpublished		
KCP 5.2/01	xxxxxxxxxxx	2022	Development and Validation of an Analytical Method for Determination of Residues of Prothioconazole-desthio in Body Fluids (Blood) by LC-MS/MS Report no.: RES-00373, Sponsor no.: 000109608 xxxxxxxxxxxxxxxxxx GLP Unpublished	N	ADM
KCP 5.2/02	Lefresne, S.	2020	Validation of an analytical method for the determination of prothioconazole residues in wheat (whole plant, grain, straw), oilseed rape (grain), strawberry and dried bean Report no.: B18S-A4-P-01, Sponsor no.: R-39651 FREDON Pays de la Loire / GIRPA, Beaucouzé Cedex, France GLP Unpublished	N	ADM
KCP 5.2/03	Watson, G.	2022a	Independent laboratory validation of an analytical method B18S-A4-P-01 (Adama study No- R-39651) for the determination of residues of prothioconazole-desthio in crops by LC-MS/MS Report no.: RES-00393, Sponsor no.: 000110772 ResChem Analytical Limited, Derby, UK GLP Unpublished	N	ADM
KCP 5.2/04	Watson, G.	2022b	Validation of an analytical method for the determination of residues of prothioconazole-desthio in egg by LC-MS/MS Report no.: RES-00394, Sponsor no.: 000110773 ResChem Analytical Limited, Derby, UK GLP Unpublished	N	ADM
KCP 5.2/05	Lindner, M., Büdel, A.	2022	Independent Laboratory Validation of an Analytical Method for the Determination of Residues of Prothioconazole-desthio in Egg by LC-MS/MS Report no.: S22-04421 (MAC-2219V), Sponsor no.: 000111069 Eurofins Agroscience Services Chem GmbH, Hamburg, Germany GLP Unpublished	N	ADM
KCP 5.2/06	Lefresne, S.	2021	Validation of an analytical method for the determination of prothioconazole residues in honey	N	ADM

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**
			Report no.: B21S-A4-P-04, Sponsor no.: 000108774 FREDON Pays de la Loire / GIRPA, Beaucouzé Cedex, France GLP Unpublished		
KCP 5.2/07	Lindner, M.	2022	Independent Laboratory Validation of an Analytical Method for Determination of Prothiconazole Residues in Honey Report no.: S21-06313 (MAC-2144V), Sponsor no.: 000108775 Eurofins Agroscience Services Chem GmbH, Hamburg, Germany GLP Unpublished	N	ADM
KCP 5.2/08	Krebber, C., Sansau, C.	2015	Modification M002 of analytical method 01387 for the determination of various pesticides in drinking and surface water by HPLC-MS/MS Report no.: MR-15/025 Bayer CropScience AG, Monheim am Rhein, Germany GLP Unpublished	N	BCS/ADM
KCP 5.2/09	Thies, S.	2015	Independent laboratory validation of the BCS analytical method 01387/M002 for the determination of various pesticides in surface water by HPLC-MS/MS Report no.: 2015/0034/01 Currenta GmbH & Co. OHG Analytik, Leverkusen, Germany GLP Unpublished	N	BCS/ADM

ADM = Property of ADAMA Agricultural Solution and all affiliates.
BCS = Bayer Crop Science

List of data relied on and not submitted by the applicant

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

List of data 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
-	-	-	-	-	-

List of data submitted by the applicant and not relied on

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

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

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

Appendix 2 Detailed evaluation of submitted analytical methods

A 2.1 Analytical methods for Prothioconazole

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

A 2.1.1.1 Residue analytical methods

Comments of zRMS:	The analysis of the triazole metabolites was performed according to Syngenta method GRM053.01A and a reduced validation was successfully performed within this study using LC-MS/MS and LC-DMS-MS/MS. The limit of quantification (LOQ) for all triazole metabolites was 0.01 mg/kg. The limit of detection (LOD) was 0.003 mg/kg. During the validation and stability tests mean recoveries were in the range of 70 - 120% with relative standard deviation of < 20% (validation tests) for each matrix and fortification level. The method complies with EU Guidelines SANCO/825/00 rev.8.1 and SANCO/3029/99 rev.4. The method is acceptable.

Reference	KCP 5.1.2/01 (filed in KCA 6.1/01)
Report	Freezing storage stability & validation of residues of 1,2,4-Triazole, Triazole Alanine, Triazole Acetic Acid and Triazole Lactic Acid in water, acid and dry matrix : cucumber, grapes and dry bean at 0, 3, 6,12,18, 24 and 36 months; Klimmek, S and Gizler, A., 2017, Report No.: S12-00072, Sponsor no.: R-30330
Guideline(s):	For method validation: SANCO/3029/99 rev. 4
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods
Cucumber (fruit), grapes (bunches) and dried beans (seed) specimens were extracted with methanol/water (4/1, v/v). After filtration and evaporation to the aqueous remainder, the volume was adjusted with ultra-pure water. After sonication, final determination took place with LC-MS/MS (for validation samples and for storage samples up until the 18 months storage time point) or with LC-DMS-MS/MS.

Results and discussions
For an overview of recovery results obtained during the validation, please refer to tables below. Recovery results were in a range of 70 to 110 % with an RSD ≤ 20. No outliers were identified. No interference (< 30 % LOQ) of total peak area for the target analytes were found in unfortified control samples. The LOQ of 1,2,4-Triazole (1,2,4 T), Triazole alanine (TA), Triazole acetic acid (TAA) and Triazole lactic acid (TLA) was 0.01 mg/kg, for each analyte and for each matrix.

Table A 1: Recovery results from method validation of 1,2,4-Triazole (1,2,4 T), Triazole alanine (TA), Triazole acetic acid (TAA) and Triazole lactic acid (TLA) in cucumber

Fortification level [mg/kg]	Analyte	1,2,4-Triazole		Triazole alanine		Triazole acetic acid		Triazole lactic acid	
	Method	LC-MS/MS	LC-DMS-MS/MS	LC-MS/MS	LC-DMS-MS/MS	LC-MS/MS	LC-DMS-MS/MS	LC-MS/MS	LC-DMS-MS/MS
0.010	Range	90-103	90-103	87 – 103	99-114	98 - 106	91-114	91-113	92-108
	Mean ± RSD	94 ± 8.7	96 ± 6.8	94 ± 8.9	104 ± 8.0	101 ± 4.3	104 ± 11	100 ± 12	102 ± 8.8
	n	3	3	3	3	3	3	3	3
0.100	Range	100-112	108-112	93-108	102-118	101-109	98-116	101-105	106-109
	Mean ± RSD	108 ± 6.2	110 ± 1.8	99 ± 7.8	110 ± 7.3	105 ± 3.9	105 ± 9.0	103 ± 1.9	107 ± 1.4
	n	3	3	3	3	3	3	3	3
0.01 and 0.10	Overall ± RSD	101 ± 10	103 ± 6.8	97 ± 8.1	107 ± 7.5	103 ± 4.1	105 ± 9.2	101 ± 7.6	105 ± 6.1
	n	6	6	6	6	6	6	6	6

RSD = relative standard deviation, n = number of replicates

Table A 2: Recovery results from method validation of 1,2,4-Triazole (1,2,4 T), Triazole alanine (TA), Triazole acetic acid (TAA) and Triazole lactic acid (TLA) in grapes

Fortification level [mg/kg]	Analyte	1,2,4-Triazole		Triazole alanine		Triazole acetic acid		Triazole lactic acid	
	Method	LC-MS/MS	LC-DMS-MS/MS	LC-MS/MS	LC-DMS-MS/MS	LC-MS/MS	LC-DMS-MS/MS	LC-MS/MS	LC-DMS-MS/MS
0.010	Range	96-119	94-112	86-97	98-107	88-110	107-110	67-74	90-124
	Mean ± RSD	108 ± 11	104 ± 8.8	92 ± 6.0	104 ± 5.0	100 ± 11	108 ± 1.4	70 ± 5.2	105 ± 16
	n	3	3	3	3	3	3	3	3
0.100	Range	104-116	99-108	94-104	94-102	87-116	95-103	89-99	103-112
	Mean ± RSD	110 ± 5.5	103 ± 4.6	100 ± 5.1	97 ± 4.3	99 ± 15	100 ± 4.2	92 ± 6.3	108 ± 4.4
	n	3	3	3	3	3	3	3	3
0.01 and 0.10	Overall ± RSD	109 ± 7.6	103 ± 6.4	96 ± 6.8	101 ± 5.5	99 ± 12	104 ± 5.3	81 ± 16	107 ± 11
	n	6	6	6	6	6	6	6	6

RSD = relative standard deviation, n = number of replicates

Table A 3: Recovery results from method validation of 1,2,4-Triazole (1,2,4 T), Triazole alanine (TA), Triazole acetic acid (TAA) and Triazole lactic acid (TLA) in dried beans

Fortification level [mg/kg]	Analyte	1,2,4-Triazole		Triazole alanine		Triazole acetic acid		Triazole lactic acid	
	Method	LC-MS/MS	LC-DMS-MS/MS	LC-MS/MS	LC-DMS-MS/MS	LC-MS/MS	LC-DMS-MS/MS	LC-MS/MS	LC-DMS-MS/MS
0.010	Range	87-109	79-103	101-116	76-87	103-113	96-121	74-89	101-117
	Mean ± RSD	100 ± 8.4	91 ± 13	108 ± 6.9	81 ± 7.0	107 ± 4.8	110 ± 12	81 ± 9.2	107 ± 8.4
	n	5	3	3	3	3	3	3	3
0.100	Range	91-118	89-101	78-89	92-97	108-111	107-112	77-82	107-116
	Mean ± RSD	103 ± 10	96 ± 6.5	82 ± 7.8	94 ± 2.7	110 ± 1.4	110 ± 2.6	80 ± 3.3	107 ± 8.4
	n	5	3	3	3	5	3	3	3
0.01 and 0.10	Overall ± RSD	102 ± 8.9	94 ± 9.6	95 ± 14	88 ± 9.7	109 ± 3.3	110 ± 7.6	81 ± 6.3	110 ± 6.6
	n	10	6	6	6	6	6	6	6

RSD = relative standard deviation, n = number of replicates

Table A 4: Characteristics for the analytical method used for validation of triazole metabolites residues in cucumber, grapes and dried beans

	Triazole metabolites*
Specificity	Blank value < 30 % LOQ
Calibration (type, number of data points)	Individual calibration data presented r > 0.99 9 - 11 calibration points
Calibration range	0.240 - 400 ng/mL
Assessment of matrix effects is presented	Matrix effects were excluded by calibration with matrix-matched standards.
Limit of quantification	LOQ: 0.01 mg/kg

* 1,2,4-Triazole (1,2,4 T), Triazole alanine (TA), Triazole acetic acid (TAA) and Triazole lactic acid (TLA)

Conclusion
The method fulfils the requirements of SANCO/3029/99 rev. 4 and is suitable for the determination of the triazole metabolites 1,2,4-Triazole (1,2,4 T), Triazole alanine (TA), Triazole acetic acid (TAA) and Triazole lactic acid (TLA) residues in cucumber, grapes and dried beans.

Comments of zRMS:	The LC-MS/MS (QuEChERS-method) analytical method has been successfully validated for the determination of prothioconazole (sum of prothioconazole-desthio, 3-hydroxy-prothioconazole-desthio, 4-hydroxy-prothioconazole-desthio, 5-hydroxy-prothioconazole-desthio, 6-hydroxyprothioconazole-desthio and alpha-hydroxy-prothioconazole-desthio, expressed as prothioconazole-desthio) in whole plant of wheat, grain of wheat, straw of wheat, grain of oilseed rape, strawberry and dry bean. The LOQ of prothioconazole-desthio, 3-hydroxy-prothioconazoledesthio expressed as prothioconazole-desthio, 4-hydroxy-prothioconazole-desthio expressed as prothioconazole-desthio, 5-hydroxy-prothioconazole-desthio expressed as prothioconazole-desthio, 6-hydroxy-prothioconazole-desthio expressed as prothioconazole-desthio and alphahydroxy-prothioconazole-desthio expressed as prothioconazole-desthio was 0.010 mg/kg, for each reference item. The LOQ for the sum of all prothioconazole-items was 0.060 mg/kg for each matrix. The method complies with EU Guidelines SANCO/825/00 rev.8.1 and SANCO/3029/99 rev.4. The method is acceptable.

Reference:	KCP 5.1.2/02 (filed in KCA 6.1/02)
Report	Freezing storage stability of prothioconazole-desthio, 3-hydroxy-prothioconazole-desthio, 4-hydroxy-prothioconazole-desthio, 5-hydroxy-prothioconazole-desthio, 6-hydroxy-prothioconazole-desthio and alpha-hydroxy-prothioconazole-desthio in plant matrices at/below -18°C during 24 months (0, 1, 3, 12, 18 and 24 months): Wheat whole plant (high water content), wheat grain (high starch content), wheat straw (difficult commodity), oilseed rape grain (high oil content), strawberry (high acid content) and dry bean (high protein content). Lefresne, S., 2020 Report No.: B18S-A4-P-02, Sponsor no.: R-39653
Guideline(s):	For method validation: SANCO/3029/99 rev. 4
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods
The analytical method is based on European Committee for Standardization (CEN): EN 15662:2009-02,

paragraph 8 – QuEChERS-method. Residues of prothioconazole (sum of prothioconazole-desthio, 3-hydroxy-prothioconazoledesthio, 4-hydroxy-prothioconazole-desthio, 5-hydroxy-prothioconazole-desthio, 6-hydroxyprothioconazole-desthio and alpha-hydroxy-prothioconazole-desthio, expressed as prothioconazole-desthio) were extracted from homogenised matrices by maceration with acetonitrile/water. Then, extracts were purified by dispersive solid phase extraction. The quantification was performed by LC-MS/MS (QuEChERS-method) with two mass transitions. The analytical method was fully validated during the course of other studies for Wheat whole plant (high water content), wheat grain (high starch content), wheat straw (difficult commodity), oilseed rape grain (high oil content), strawberry (high acid content) and dry bean (high protein content) according to guideline SANCO/3029/99 rev. 4:
Study code: B18S-A4-P-01, Sponsor reference: R-39651.

Results and discussions
Recovery results were in a range of 70 to 110% with an RSD ≤ 20. No outliers were identified. No interference (< 30 % LOQ) of total peak area for the target analytes were found in unfortified control samples. The LOQ of prothioconazole-desthio, 3-hydroxyprothioconazole-desthio expressed as prothioconazole-desthio, 4-hydroxyprothioconazole-desthio expressed as prothioconazole-desthio, 5-hydroxyprothioconazole-desthio expressed as prothioconazole-desthio, 6-hydroxyprothioconazole-desthio expressed as prothioconazole-desthio and alpha-hydroxyprothioconazole-desthio expressed as prothioconazole-desthio was 0.010 mg/kg for each analyte and for each matrix. The LOQ for the sum of all prothioconazole-items was 0.060 mg/kg for each matrix.

Table A 5: Recovery results from method validation of prothioconazole metabolites in whole plant of wheat

Fortification level	Analyte	Prothioconazole-desthio		3-hydroxy-prothioconazole-desthio		4-hydroxy-prothioconazole-desthio		5-hydroxy-prothioconazole-desthio		6-hydroxy-prothioconazole-desthio		alpha-hydroxy-prothioconazole-desthio	
[mg/kg]	Transition ion	70 m/z	125 m/z	70 m/z	141 m/z	141 m/z	70 m/z	70 m/z	141 m/z	70 m/z	141 m/z	70 m/z	141 m/z
0.010	Range	100-102	99-103	100-102	99-105	100-105	101-108	101-105	98-109	104-108	105-110	104-107	99-102
	Mean ± RSD	101 ± 1	101 ± 2	101 ± 1	102 ± 2	103 ± 2	105 ± 2	103 ± 2	105 ± 4	106 ± 1	108 ± 2	106 ± 1	100 ± 1
	n	5	5	5	5	5	5	5	5	5	5	5	5
0.100	Range	100-108	99-106	103-112	103-111	103-114	105-118	101-113	100-113	108-114	106-115	105-114	99-110
	Mean ± RSD	103 ± 3	101 ± 3	107 ± 4	107 ± 3	108 ± 5	110 ± 5	107 ± 5	108 ± 5	110 ± 2	110 ± 3	110 ± 3	106 ± 4
	n	5	5	5	5	5	5	5	5	5	5	5	5
0.01 and 0.10	Overall ± RSD	102 ± 2	101 ± 2	104 ± 4	104 ± 3	106 ± 4	107 ± 4	105 ± 4	106 ± 5	108 ± 2	109 ± 2	108 ± 3	103 ± 4
	n	10	10	10	10	10	10	10	10	10	10	10	10

RSD = relative standard deviation, n = number of replicates

Table A 6: Recovery results from method validation of prothioconazole metabolites in grain of wheat

Fortification level	Analyte	Prothioconazole-desthio		3-hydroxy-prothioconazole-desthio		4-hydroxy-prothioconazole-desthio		5-hydroxy-prothioconazole-desthio		6-hydroxy-prothioconazole-desthio		alpha-hydroxy-prothioconazole-desthio	
[mg/kg]	Transition ion	70 m/z	125 m/z	70 m/z	141 m/z	141 m/z	70 m/z	70 m/z	141 m/z	70 m/z	141 m/z	70 m/z	141 m/z
0.010	Range	95-99	98-101	98-102	97-98	95-98	94-102	95-101	96-105	109-111	105-111	99-105	93-102
	Mean ± RSD	97 ± 2	100 ± 2	99 ± 2	98 ± 1	96 ± 1	98 ± 3	97 ± 3	99 ± 4	110 ± 1	109 ± 2	101 ± 2	97 ± 4
	n	5	5	5	5	5	5	5	5	5	5	5	5
0.100	Range	92-101	89-102	94-102	91-102	90-102	88-99	91-100	90-104	104-113	105-112	93-102	94-102
	Mean ± RSD	97 ± 4	98 ± 5	98 ± 4	97 ± 5	96 ± 4	95 ± 4	96 ± 4	97 ± 7	109 ± 3	108 ± 3	98 ± 4	98 ± 3
	n	5	5	5	5	5	5	5	5	5	5	5	5
0.01 and 0.10	Overall ± RSD	97 ± 3	99 ± 4	99 ± 3	97 ± 3	96 ± 3	96 ± 4	93 ± 3	98 ± 5	110 ± 2	108 ± 3	100 ± 3	98 ± 3
	n	10	10	10	10	10	10	10	10	10	10	10	10

RSD = relative standard deviation, n = number of replicates

Table A 7: Recovery results from method validation of prothioconazole metabolites in straw of wheat

Fortification level	Analyte	Prothioconazole-desthio		3-hydroxy-prothioconazole-desthio		4-hydroxy-prothioconazole-desthio		5-hydroxy-prothioconazole-desthio		6-hydroxy-prothioconazole-desthio		alpha-hydroxy-prothioconazole-desthio	
[mg/kg]	Transition ion	70 m/z	125 m/z	70 m/z	141 m/z	141 m/z	70 m/z	70 m/z	141 m/z	70 m/z	141 m/z	70 m/z	141 m/z
0.010	Range	94-98	93-99	97-102	101-108	94-99	93-100	95-98	93-100	103-107	102-107	105-110	99-101
	Mean ± RSD	97 ± 2	96 ± 2	99 ± 2	105 ± 3	97 ± 2	97 ± 3	96 ± 1	96 ± 3	106 ± 2	104 ± 2	108 ± 2	100 ± 1
	n	5	5	5	5	5	5	5	5	5	5	5	5
0.100	Range	86-101	87-100	87-104	93-109	85-99	86-100	85-107	82-99	98-109	94-113	97-111	85-109
	Mean ± RSD	93 ± 6	93 ± 6	96 ± 7	101 ± 6	93 ± 6	96 ± 6	95 ± 8	91 ± 8	104 ± 4	103 ± 7	106 ± 5	98 ± 10
	n	5	5	5	5	5	5	5	5	5	5	5	5
	Overall ± RSD	95 ± 4	95 ± 5	98 ± 5	103 ± 5	95 ± 5	96 ± 4	95 ± 6	93 ± 6	105 ± 3	104 ± 5	107 ± 4	99 ± 6

0.01 and 0.10	n	10	10	10	10	10	10	10	10	10	10	10	10

RSD = relative standard deviation, n = number of replicates

Table A 8: Recovery results from method validation of prothioconazole metabolites in oilseed rape seeds

Fortification level	**Analyte**	**Prothioconazole-desthio**		**3-hydroxy-prothioconazole-desthio**		**4-hydroxy-prothioconazole-desthio**		**5-hydroxy-prothioconazole-desthio**		**6-hydroxy-prothioconazole-desthio**		**alpha-hydroxy-prothioconazole-desthio**	
[mg/kg]	Transition ion	70 m/z	125 m/z	70 m/z	141 m/z	141 m/z	70 m/z	70 m/z	141 m/z	70 m/z	141 m/z	70 m/z	141 m/z
0.010	Range	72-111	71-111	80-116	78-120	77-120	79-120	74-118	81-117	69-105	66-103	83-123	81-126
	Mean ± RSD	83 ± 19	82 ± 20	90 ± 16	92 ± 18	90 ± 19	90 ± 18	89 ± 19	91 ± 16	79 ± 19	78 ± 19	95 ±17	95 ± 19
	n	5	5	5	5	5	5	5	5	5	5	5	5
0.100	Range	74-80	73-80	79-87	82-89	79-88	80-88	79 - 88	81-86	72-77	72-78	82-91	86-90
	Mean ± RSD	77 ± 3	77 ± 4	84 ± 4	85 ± 3	85 ± 4	85 ± 3	84 ± 5	84 ± 3	75 ± 3	75 ± 3	88 ± 4	88 ± 2
	n	5	5	5	5	5	5	5	5	5	5	5	5
0.01 and 0.10	Overall ± RSD	80 ± 14	80 ± 14	87 ± 12	89 ± 13	88 ± 14	88 ± 13	87 ± 14	88 ± 12	77 ± 14	76 ± 13	91 ± 13	91 ± 14
	n	10	10	10	10	10	10	10	10	10	10	10	10

RSD = relative standard deviation, n = number of replicates

Table A 9: Recovery results from method validation of prothioconazole metabolites in strawberry

Fortification level	**Analyte**	**Prothioconazole-desthio**		**3-hydroxy-prothioconazole-desthio**		**4-hydroxy-prothioconazole-desthio**		**5-hydroxy-prothioconazole-desthio**		**6-hydroxy-prothioconazole-desthio**		**alpha-hydroxy-prothioconazole-desthio**	
[mg/kg]	Transition ion	70 m/z	125 m/z	70 m/z	141 m/z	141 m/z	70 m/z	70 m/z	141 m/z	70 m/z	141 m/z	70 m/z	141 m/z
0.010	Range	73-112	75-108	96-113	97-110	101-109	100-111	93-113	93-119	108-117	106-116	96-112	104-115
	Mean ± RSD	98 ± 15	97 ± 13	103 ± 6	103 ± 5	106 ± 3	106 ± 4	104 ± 7	106 ± 9	110 ± 4	109 ± 4	103 ±6	109 ± 4
	n	5	5	5	5	5	5	5	5	5	5	5	5
0.100	Range	99-105	100-105	104-106	103-105	94-105	86-107	94 – 106	97-109	96-107	95-104	105-108	105-108
	Mean ± RSD	103 ± 2	103 ± 2	105 ± 1	104 ±1	99 ± 5	99 ± 8	101 ± 4	103 ± 4	103 ± 4	101 ± 3	106 ± 1	106 ± 1
	n	5	5	5	5	5	5	5	5	5	5	5	5
0.01 and 0.10	Overall ± RSD	101 ± 10	100 ± 9	104 ± 4	103 ± 3	103 ± 5	102 ± 7	103 ± 6	105 ± 7	107 ± 5	105 ± 5	104 ± 4	107 ± 3
	n	10	10	10	10	10	10	10	10	10	10	10	10

RSD = relative standard deviation, n = number of replicates

Table A 10: Recovery results from method validation of prothioconazole metabolites in dry bean

Fortification level	**Analyte**	**Prothioconazole-desthio**		**3-hydroxy-prothioconazole-desthio**		**4-hydroxy-prothioconazole-desthio**		**5-hydroxy-prothioconazole-desthio**		**6-hydroxy-prothioconazole-desthio**		**alpha-hydroxy-prothioconazole-desthio**	
[mg/kg]	Transition ion	70 m/z	125 m/z	70 m/z	141 m/z	141 m/z	70 m/z	70 m/z	141 m/z	70 m/z	141 m/z	70 m/z	141 m/z
0.010	Range	87-120	89-121	85-119	83-118	85-120	88-116	85-118	88-117	91-122	83-114	90-126	90-119

	Mean ± RSD n	100 ± 13 5	101 ± 13 5	99 ± 13 5	100 ± 13 5	99 ± 13 5	99 ± 11 5	99 ± 14 5	97 ± 13 5	102 ± 12 5	97 ± 13 5	102 ±14 5	101 ± 11 5
0.100	Range Mean ± RSD n	87-102 93 ± 6 5	88-103 93 ± 7 5	86-102 92 ± 7 5	85-104 91 ± 8 5	88-105 93 ± 7 5	87-103 93 ± 7 5	87 - 104 93 ± 7 5	84-101 90 ± 7 5	90-108 96 ± 7 5	91-106 95 ± 7 5	90-107 97 ± 7 5	89-107 95 ± 7 5
0.01 and 0.10	Overall ± RSD n	97 ± 10 10	97 ± 11 10	85 ± 119 10	95 ± 11 10	96 ± 11 10	96 ± 10 10	96 ± 11 10	94 ± 11 10	99 ± 10 10	96 ± 10 10	99 ± 11 10	98 ± 10 10

RSD = relative standard deviation, n = number of replicates

Table A 11: Characteristics for the analytical method used for validation of prothioconazole metabolites residues in wheat whole plant, wheat grain, wheat straw, oilseed rape grain, strawberry and dry bean

	Prothioconazole*
Specificity	Blank value < 30 % LOQ
Calibration (type, number of data points)	Individual calibration data presented r > 0.99 7 calibration points
Calibration range	0.6 - 20 µg/L 3 to 150 (only for strawberry)
Assessment of matrix effects is presented	Not rquired, since calibration was carried out with matrix-matched standards
Limit of quantification	For each analyte separately: LOQ: 0.010 mg/kg For prothioconazole as the sum of all analytes: LOQ: 0.060 mg/kg

* Including: prothioconazole-desthio, 3-hydroxyprothioconazole-desthio, 4-hydroxyprothioconazole-desthio, 5-hydroxyprothioconazole-desthio, 6-hydroxyprothioconazole-desthio and alpha-hydroxyprothioconazole-desthio

Conclusion

The method fulfils the requirements of SANCO/3029/99 rev. 4 and is suitable for the determination of prothioconazole (including: prothioconazole-desthio, 3-hydroxyprothioconazole-desthio, 4-hydroxyprothioconazole-desthio, 5-hydroxyprothioconazole-desthio, 6-hydroxyprothioconazole-desthio and alpha-hydroxyprothioconazole-desthio) in wheat whole plant, wheat grain, wheat straw, oilseed rape grain, strawberry and dry bean.

Comments of zRMS:	The analytical method fulfils the requirements of SANCO/3029/99 rev. 4 and is considered acceptable for the determination of 1,2,4-triazole, triazole alanine, triazole acetic acid and triazole lactic acid in honey. The analytical method is acceptable.

Reference:	KCP 5.1.2/25 (filed in KCA 6.1/05)
Report	Residue analytical method 01602 and short-term storage of 1,2,4-triazole, triazole alanine, triazole acetic acid and triazole lactic acid in/on honey HPLC DMS-MS/MS, Report Amendment No. 2 Kalathoor, R., 2021 Study no.: M-680825-03-1, sponsor no.: S19-01126
Guideline(s):	Yes, EC Guideline 7032/VI/95, Appendix H; OECD Guideline 506
Deviations:	n.a.
GLP:	Yes
Acceptability:	n.a.

Study owner: Triazole Derivative Metabolite Group. Access via Letter of Access.
Note: The summary of the validation data is not presented in the dRR since the applicant is not the owner of the study, and do not have access to the study report. Access to the study is via a Letter of Access.

Comments of zRMS:	The LC-MS/MS analytical method based on QuEChERS-method fulfils the requirements of SANCO/3029/99 rev. 4 and is considered acceptable for the determination prothioconazole and its metabolites in wheat whole plant, grain and straw.

	All the analytes were determined by HPLC-MS/MS using a quantitation and confirmation ion. The LOQ of each analyte was at 0.01 mg/kg for each matrix. For prothioconazole as the sum of all analytes LOQ was at 0.060 mg/kg. For method validation, the specimens were fortified at LOQ and at 10 times the LOQ for each matrix. For results validation, fortified samples (at LOQ for each matrix and an upper level) were processed concurrently with the field specimens and examined for procedural recoveries by HPLC-MS/MS. Acceptance criteria for method and results validation were fully met with average recoveries ranging from 70% to 110% and relative standard deviations ≤ 20%. Analysis (extraction) of the specimens took place 44 - 203 days after sample collection. Sufficient stability data are available to support the residue data presented in this study. The method is acceptable.

Reference:	KCP 5.1.2/03 (filed in KCA 6.3.1/01)
Report:	Residue study of azoxystrobin, prothioconazole and its metabolites in wheat whole plants and Raw Agricultural Commodity after one foliar application of MCW-2073 - 1 harvest and 2 decline trials – Northern Europe (France and Poland) - 2018, Huaulmé, J.-M., 2019a, report no.: BPL18/713/GC, sponsor no.: R-39643
Guideline(s):	For method validation: SANCO/3029/99 rev. 4
Deviations:	None
GLP:	Yes (certified laboratory)
Acceptability/Reliability:	Yes
Duplication (if vertebrate study)	Not applicable

Materials and methods

The analytical method is based on European Committee for Standardization (CEN): EN 15662:2018-05, paragraph 8 – QuEChERS-method.

Residues were extracted from homogenised matrices by maceration with acetonitrile/water. Then, extracts were purified by dispersive solid phase extraction. The quantification was performed by LC-MS/MS with two mass transitions.

The analytical method was fully validated during the course of other studies for wheat whole plant w/o roots, grain and straw according to guideline SANCO/825/00 rev.8.1 and SANCO/3029/99 rev. 4:
Study code: B18G-A4-A-01, Sponsor reference: R-39652 and
Study code: B18S-A4-P-01, Sponsor reference: R-39651.

Results and discussions

For an overview of recovery results obtained during the validation studies mentioned above, please refer to Table A 12 - Table A 14. Recovery results were in a range of 70 to 110 % with an RSD ≤ 20. No outliers were identified. No interference (< 30 % LOQ) of total peak area for the target analytes were found in unfortified control samples. The LOQ of prothioconazole-desthio, 3-hydroxyprothioconazole-desthio, 4-hydroxyprothioconazole-desthio expressed as prothioconazole-desthio, 5-hydroxyprothioconazole-desthio expressed as prothioconazole-desthio, 6-hydroxyprothioconazole-desthio expressed as prothioconazole-desthio and alpha-hydroxyprothioconazole-desthio expressed as prothioconazole-desthio was 0.010 mg/kg, for each analyte and for each matrix.

Table A 12: Recovery results from method validation of prothioconazole in whole plant of wheat (data obtained from study B18G-A4-A-01 and B18S-A4-P-01)

Fortification level	Analyte	Prothioconazole-desthio		3-hydroxy-prothioconazole-desthio		4-hydroxy-prothioconazole-desthio		5-hydroxy-prothioconazole-desthio		6-hydroxy-prothioconazole-desthio		alpha-hydroxy-prothioconazole-desthio	
[mg/kg]	Transition ion	70 m/z	125 m/z	70 m/z	141 m/z	141 m/z	70 m/z	70 m/z	141 m/z	70 m/z	141 m/z	70 m/z	141 m/z
0.010	Range	100-102	99-103	100-102	99-105	100-105	101-108	101-105	98-109	104-108	105-110	104-107	99-102
	Mean ± RSD	101 ± 1	101 ± 2	101 ± 1	102 ± 2	103 ± 2	105 ± 2	103 ± 2	105 ± 4	106 ± 1	108 ± 2	106 ± 1	100 ± 1
	n	5	5	5	5	5	5	5	5	5	5	5	5
0.100	Range	100-108	99-106	103-112	103-111	103-114	105-118	101-113	100-113	108-114	106-115	105-114	99-110
	Mean ± RSD	103 ± 3	101 ± 3	107 ± 4	107 ± 3	108 ± 5	110 ± 5	107 ± 5	108 ± 5	110 ± 2	110 ± 3	110 ± 3	106 ± 4
	n	5	5	5	5	5	5	5	5	5	5	5	5
0.01 and 0.10	Overall ± RSD	102 ± 2	101 ± 2	104 ± 4	104 ± 3	106 ± 4	107 ± 4	105 ± 4	106 ± 5	108 ± 2	109 ± 2	108 ± 3	103 ± 4
	n	10	10	10	10	10	10	10	10	10	10	10	10

RSD = relative standard deviation, n = number of replicates

Table A 13: Recovery results from method validation of prothioconazole in grain of wheat (B18G-A4-A-01, B18S-A4-P-01)

Fortification level	Analyte	Prothioconazole-desthio		3-hydroxy-prothioconazole-desthio		4-hydroxy-prothioconazole-desthio		5-hydroxy-prothioconazole-desthio		6-hydroxy-prothioconazole-desthio		alpha-hydroxy-prothioconazole-desthio	
[mg/kg]	Transition ion	70 m/z	125 m/z	70 m/z	141 m/z	141 m/z	70 m/z	70 m/z	141 m/z	70 m/z	141 m/z	70 m/z	141 m/z
0.010	Range	95-99	98-101	98-102	97-98	95-98	94-102	95-101	96-105	109-111	105-111	99-105	93-102
	Mean ± RSD	97 ± 2	100 ± 2	99 ± 2	98 ± 1	96 ± 1	98 ± 3	97 ± 3	99 ± 4	110 ± 1	109 ± 2	101 ± 2	97 ± 4
	n	5	5	5	5	5	5	5	5	5	5	5	5
0.100	Range	92-101	89-102	94-102	91-102	90-102	88-99	91-100	90-104	104-113	105-112	93-102	94-102
	Mean ± RSD	97 ± 4	98 ± 5	98 ± 4	97 ± 5	96 ± 4	95 ± 4	96 ± 4	97 ± 7	109 ± 3	108 ± 3	98 ± 4	98 ± 3
	n	5	5	5	5	5	5	5	5	5	5	5	5
0.01 and 0.10	Overall ± RSD	97 ± 3	99 ± 4	99 ± 3	97 ± 3	96 ± 3	96 ± 4	93 ± 3	98 ± 5	110 ± 2	108 ± 3	100 ± 3	98 ± 3
	n	10	10	10	10	10	10	10	10	10	10	10	10

RSD = relative standard deviation, n = number of replicates

Table A 14: Recovery results from method validation of prothioconazole in straw of wheat (B18G-A4-A-01, B18S-A4-P-01)

Fortification level	Analyte	Prothioconazole-desthio		3-hydroxy-prothioconazole-desthio		4-hydroxy-prothioconazole-desthio		5-hydroxy-prothioconazole-desthio		6-hydroxy-prothioconazole-desthio		alpha-hydroxy-prothioconazole-desthio	
[mg/kg]	Transition ion	70 m/z	125 m/z	70 m/z	141 m/z	141 m/z	70 m/z	70 m/z	141 m/z	70 m/z	141 m/z	70 m/z	141 m/z
0.010	Range	94-98	93-99	97-102	101-108	94-99	93-100	95-98	93-100	103-107	102-107	105-110	99-101
	Mean ± RSD	97 ± 2	96 ± 2	99 ± 2	105 ± 3	97 ± 2	97 ± 3	96 ± 1	96 ± 3	106 ± 2	104 ± 2	108 ± 2	100 ± 1
	n	5	5	5	5	5	5	5	5	5	5	5	5
0.100	Range	86-101	87-100	87-104	93-109	85-99	86-100	85-107	82-99	98-109	94-113	97-111	85-109
	Mean ± RSD	93 ± 6	93 ± 6	96 ± 7	101 ± 6	93 ± 6	96 ± 6	95 ± 8	91 ± 8	104 ± 4	103 ± 7	106 ± 5	98 ± 10
	n	5	5	5	5	5	5	5	5	5	5	5	5

0.01 and 0.10	Overall ± RSD n	95 ± 4 10	95 ± 5 10	98 ± 5 10	103 ± 5 10	95 ± 5 10	96 ± 4 10	95 ± 6 10	93 ± 6 10	105 ± 3 10	104 ± 5 10	107 ± 4 10	99 ± 6 10

RSD = relative standard deviation, n = number of replicates

Table A 15: Characteristics for the analytical method used for validation of prothioconazole residues in wheat

	Prothioconazole*
Specificity	Blank value < 30 % LOQ
Calibration (type, number of data points)	Individual calibration data presented r > 0.99 7 calibration points
Calibration range	0.6 - 20 µg/mL
Assessment of matrix effects is presented	Matrix effects were excluded by calibration with matrix-matched standards.
Limit of quantification	For each analyte separately: LOQ: 0.010 mg/kg For prothioconazole as the sum of all analytes: LOQ: 0.060 mg/kg

* Including: prothioconazole-desthio, 3-hydroxyprothioconazole-desthio, 4-hydroxyprothioconazole-desthio, 5-hydroxyprothioconazole-desthio, 6-hydroxyprothioconazole-desthio and alpha-hydroxyprothioconazole-desthio

Conclusion
The method fulfils the requirements of SANCO/3029/99 rev. 4 and is suitable for the determination of prothioconazole in wheat.

The following validation summary applies to the following two residue studies.

Comments of zRMS:	The analytical method was fully validated for wheat whole plant without roots, grain and straw according to guideline SANCO/3029/99 rev. 4. All the analytes were determined by LC-MS/MS using a quantitation and confirmation ion. The LOQ of each analyte was at 0.01 mg/kg for each matrix. The storage duration (interval between sampling and extraction date) was 258 days for the determination of prothioconazole and its metabolites. Sufficient stability data are available to support the residue data presented in this study. The method ~~study~~ is acceptable.

Reference:	KCP 5.1.2/04 (filed in KCA 6.3.1/03)
Report:	Residue study of azoxystrobin, prothioconazole and its metabolites in wheat whole plant and Raw Agricultural Commodity after one foliar application of MCW-2073 - 3 harvest and 2 decline trials – Northern Europe (FR, HU, PL) - 2019, Amic, S., 2020a, report no.: BPL19/757/GC, sponsor no.: 000102745
Guideline(s):	For method validation: SANCO/3029/99 rev. 4
Deviations:	None
GLP:	Yes (certified laboratory)
Acceptability/Reliability:	Yes
Duplication (if vertebrate study)	Not applicable

Comments of zRMS:	The analytical method was validated for wheat whole plant without roots, grain and straw according to guideline SANCO/3029/99 rev. 4. All the analytes were determined by LC-MS/MS using a quantitation and confirmation ion. The LOQ of each analyte was at 0.01 mg/kg for each matrix. The mean recovery was between 70% and 110% at each level of fortification, for each reference item and for each matrix. The storage duration (interval between sampling and extraction date) was 149 days for the determination of prothioconazole and its metabolites. Sufficient stability data are available to support the residue data presented in this study. The method ~~study~~ is acceptable.

Reference:	KCP 5.1.2/05 (filed in KCA 6.3.1/05)
Report:	Residue study of prothioconazole and its metabolites in wheat whole plant and RAC after one foliar application of ADM.3500.F.2.B (250 g a.s./L of prothioconazole) - 2 harvest and 2 decline trials – Northern Europe (FR, HU, PL) - 2019, Amic, S., 2020b, report no.: BPL19/762/GC, sponsor no.: 000102751
Guideline(s):	For method validation: SANCO/3029/99 rev. 4
Deviations:	None
GLP:	Yes (certified laboratory)
Acceptability/Reliability:	Yes
Duplication (if vertebrate study)	Not applicable

Materials and methods

The analytical method is based on European Committee for Standardization (CEN): EN 15662:2018-05, paragraph 8 – QuEChERS-method. Residues were extracted from homogenised matrices by maceration with acetonitrile/water. Then, extracts were purified by dispersive solid phase extraction. The quantification was performed by LC-MS/MS with two mass transitions.

The analytical method was fully validated during the course of other studies for wheat whole plant w/o roots, grain and straw according to guideline SANCO/825/00 rev.8.1 and SANCO/3029/99 rev. 4:
Study code: B19S-A4-P-01, Sponsor reference: 000102920.

Results and discussions

Recovery results were in a range of 70 to 110% with an RSD $\leq$ 20. No outliers were identified. No interference (< 30% LOQ) of total peak area for the target analytes were found in unfortified control samples. The LOQ of prothioconazole-desthio, 3-hydroxyprothioconazole-desthio expressed as prothioconazole-desthio, 4-hydroxyprothioconazole-desthio expressed as prothioconazole-desthio, 5-hydroxyprothioconazole-desthio expressed as prothioconazole-desthio, 6-hydroxyprothioconazole-desthio expressed as prothioconazole-desthio and alpha-hydroxyprothioconazole-desthio expressed as prothioconazole-desthio was 0.010 mg/kg, for each analyte and for each matrix. A LOQ of 0.06 mg/kg was set for prothioconazole expressed as the sum of all analytes.

Table A 16: Recovery results from method validation of prothioconazole in whole plant of wheat (data obtained from study B19S-A4-P-01)

Fortification level	Analyte	Prothioconazole-desthio		3-hydroxy-prothioconazole-desthio		4-hydroxy-prothioconazole-desthio		5-hydroxy-prothioconazole-desthio		6-hydroxy-prothioconazole-desthio		alpha-hydroxy-prothioconazole-desthio	
[mg/kg]	Transition ion	70 m/z	125 m/z	70 m/z	141 m/z	141 m/z	70 m/z	70 m/z	141 m/z	70 m/z	141 m/z	70 m/z	141 m/z
0.010	Range	78-89	77-89	79-89	80-89	76-88	76-87	77-88	77-89	78-90	74-89	80-87	78-89
	Mean ± RSD	83 ± 5	82 ± 5	82 ± 5	83 ± 5	81 ± 6	80 ± 5	81 ± 5	82 ± 5	82 ± 6	80 ± 8	82 ± 3	81 ± 6
	n	5	5	5	5	5	5	5	5	5	5	5	5
0.100	Range	84-91	85-90	75-84	76-85	81-90	77-89	79-89	80-92	82-90	80-92	81-88	82-88
	Mean ± RSD	87 ± 3	87 ± 2	80 ± 4	82 ± 4	84 ± 4	82 ± 5	84 ± 5	85 ± 5	86 ± 4	85 ± 6	86 ± 4	85 ± 3
	n	5	5	5	5	5	5	5	5	5	5	5	5
0.01 and 0.10	Overall ± RSD	85 ± 5	85 ± 5	81 ± 4	83 ± 4	83 ± 5	81 ± 5	83 ± 5	84 ± 5	84 ± 5	82 ± 7	84 ± 4	83 ± 5
	n	10	10	10	10	10	10	10	10	10	10	10	10

RSD = relative standard deviation, n = number of replicates

Table A 17: Recovery results from method validation of prothioconazole in grain of wheat (B19S-A4-P-01)

Fortification level	Analyte	Prothioconazole-desthio		3-hydroxy-prothioconazole-desthio		4-hydroxy-prothioconazole-desthio		5-hydroxy-prothioconazole-desthio		6-hydroxy-prothioconazole-desthio		alpha-hydroxy-prothioconazole-desthio	
[mg/kg]	Transition ion	70 m/z	125 m/z	70 m/z	141 m/z	141 m/z	70 m/z	70 m/z	141 m/z	70 m/z	141 m/z	70 m/z	141 m/z
0.010	Range	82-87	84-88	85-90	82-86	82-85	81-86	84-88	82-87	85-88	85-90	86-90	83-92
	Mean ± RSD	84 ± 2	85 ± 2	86 ± 2	84 ± 2	83 ± 1	84 ± 2	85 ± 2	85 ± 2	86 ± 1	87 ± 2	89 ± 2	88 ± 4
	n	5	5	5	5	5	5	5	5	5	5	5	5
0.100	Range	84-90	84-89	85-93	84-93	83-89	82-90	86-90	84-91	85-96	88-97	88-95	88-99
	Mean ± RSD	88 ± 3	88 ± 3	90 ± 4	89 ± 4	88 ± 3	87 ± 4	89 ± 2	89 ± 3	93 ± 5	93 ± 4	93 ± 3	94 ± 4
	n	5	5	5	5	5	5	5	5	5	5	5	5
0.01 and 0.10	Overall ± RSD	86 ± 3	86 ± 3	88 ± 4	87 ± 4	86 ± 3	85 ± 3	87 ± 3	87 ± 3	89 ± 5	90 ± 5	91 ± 4	91 ± 5
	n	10	10	10	10	10	10	10	10	10	10	10	10

Table A 18: Recovery results from method validation of prothioconazole in straw of wheat (B19S-A4-P-01)

Fortification level	Analyte	Prothioconazole-desthio		3-hydroxy-prothioconazole-desthio		4-hydroxy-prothioconazole-desthio		5-hydroxy-prothioconazole-desthio		6-hydroxy-prothioconazole-desthio		alpha-hydroxy-prothioconazole-desthio	
[mg/kg]	Transition ion	70 m/z	125 m/z	70 m/z	141 m/z	141 m/z	70 m/z	70 m/z	141 m/z	70 m/z	141 m/z	70 m/z	141 m/z
0.010	Range	89-96	93-98	88-98	88-98	90-100	90-96	89-97	88-99	89-101	90-95	89-95	90-98
	Mean ± RSD	92 ± 3	95 ± 2	94 ± 4	94 ± 4	96 ± 4	94 ± 2	94 ± 3	93 ± 4	94 ± 5	94 ± 3	93 ± 3	95 ± 4
	n	5	5	5	5	5	5	5	5	5	5	5	5
0.100	Range	77-96	81-97	81-99	78-101	82-101	81-99	82-98	80-99	83-102	83-101	81-97	84-107
	Mean ± RSD	90 ± 8	93 ± 7	92 ± 8	93 ± 10	94 ± 8	93 ± 7	92 ± 7	93 ± 8	96 ± 8	94 ± 7	92 ± 7	95 ± 9
	n	5	5	5	5	5	5	5	5	5	5	5	5
0.01 and 0.10	Overall ± RSD	91 ± 6	94 ± 5	93 ± 6	94 ± 7	95 ± 6	93 ± 5	93 ± 5	93 ± 6	95 ± 6	94 ± 5	92 ± 5	95 ± 6
	n	10	10	10	10	10	10	10	10	10	10	10	10

RSD = relative standard deviation, n = number of replicates

Table A 19: Characteristics for the analytical method used for validation of prothioconazole residues in wheat

	Prothioconazole*
Specificity	Blank value < 30 % LOQ
Calibration (type, number of data points)	Individual calibration data presented r > 0.99 7 calibration points
Calibration range	0.6 - 20 µg/L
Assessment of matrix effects is presented	Not rquired, since calibration was carried out with matrix-matched standards
Limit of quantification	For each analyte separately: LOQ: 0.010 mg/kg For prothioconazole as the sum of all analytes: LOQ: 0.060 mg/kg

* Including: prothioconazole-desthio, 3-hydroxyprothioconazole-desthio, 4-hydroxyprothioconazole-desthio, 5-hydroxyprothioconazole-desthio, 6-hydroxyprothioconazole-desthio and alpha-hydroxyprothioconazole-desthio

Conclusion
The method fulfils the requirements of SANCO/3029/99 rev. 4 and is suitable for the determination of prothioconazole in wheat.

Comments of zRMS:	The analytical method was validated for barley whole plant without roots, grain and straw according to guideline SANCO/3029/99 rev. 4. All the analytes were determined by HPLC-MS/MS using a quantitation and confirmation ion. The LOQ of each analyte was at 0.01 mg/kg for each matrix. The limit of detection (LOD) was set at 0.003 mg/kg for each analyte and each matrix. Acceptance criteria for method and results validation were fully met with average recoveries ranging from 70% to 110% and relative standard deviations ≤ 20%. Analysis (extraction) of the specimens took place 45 - 151 days after sample collection. Sufficient stability data are available to support the residue data presented in this study. The method ~~study~~ is acceptable.

Reference:	KCP 5.1.2/06 (filed in KCA 6.3.2/01)
Report:	Residue study of azoxystrobin, prothioconazole and its metabolites in barley whole plants and Raw Agricultural Commodity after one foliar application of MCW-2073 - 1 harvest and 2 decline trials – Northern Europe (France) - 2018, Huaulmé, J.-M., 2019b, report no.: BPL18/715/GC, sponsor no.: R-39645
Guideline(s):	For method validation: SANCO/3029/99 rev. 4
Deviations:	None
GLP:	Yes (certified laboratory)
Acceptability/Reliability:	Yes
Duplication (if vertebrate study)	Not applicable

Materials and methods
The analytical method is based on European Committee for Standardization (CEN): paragraph 8 – QuEChERS-method. Residues were extracted from homogenised matrices by maceration with acetonitrile/water. Then, extracts were purified by dispersive solid phase extraction. The quantification was performed by LC-MS/MS with two mass transitions.
The analytical method was fully validated during the course of another study for barley whole plant w/o roots, grain and straw according to guideline SANCO/3029/99 rev. 4:
Study code: B18G-B5-AP-03, Sponsor reference: R-39645 A.

Results and discussions

For an overview of recovery results obtained during the validation study mentioned above, please refer to Table A 20 - Table A 22. Recovery results were in a range of 70 to 110 % with an RSD ≤ 20. No outliers were identified. No interference (< 30 % LOQ) of total peak area for the target analytes were found in unfortified control samples. The LOQ of prothioconazole-desthio, 3-hydroxyprothioconazole-desthio, 4-hydroxyprothioconazole-desthio expressed as prothioconazole-desthio, 5-hydroxyprothioconazole-desthio expressed as prothioconazole-desthio, 6-hydroxyprothioconazole-desthio expressed as prothioconazole-desthio and alpha-hydroxyprothioconazole-desthio expressed as prothioconazole-desthio was 0.010 mg/kg, for each analyte and for each matrix.

Table A 20: Recovery results from method validation of prothioconazole in whole plant of barley (data obtained from study B18G-B5-AP-03)

Fortification level	Analyte	Prothioconazole-desthio		3-hydroxy-prothioconazole-desthio		4-hydroxy-prothioconazole-desthio		5-hydroxy-prothioconazole-desthio		6-hydroxy-prothioconazole-desthio		alpha-hydroxy-prothioconazole-desthio	
[mg/kg]	Transition ion	70 m/z	125 m/z	70 m/z	141 m/z	141 m/z	70 m/z	70 m/z	141 m/z	70 m/z	141 m/z	70 m/z	141 m/z
0.010	Range	95-100	92-99	93-100	94-100	95-99	92-99	94-99	97-106	73-101	68-76	96-100	97-104
	Mean ± RSD	98 ± 2	96 ± 3	97 ± 3	97 ± 3	97 ± 2	96 ± 3	97 ± 2	103 ± 4	98 ± 3	72 ± 4	98 ± 2	100 ± 3
	n	5	5	5	5	5	5	5	5	5	5	5	5
0.100	Range	81-88	81-88	79-86	80-87	80-86	80-87	83-92	88-95	84-92	71-79	76-83	78-84
	Mean ± RSD	85 ± 4	85 ± 3	83 ± 3	83 ± 3	83 ± 3	84 ± 4	89 ± 4	92 ± 3	88 ± 4	76 ± 5	80 ± 4	81 ± 3
	n	5	5	5	5	5	5	5	5	5	5	5	5
0.01 and 0.10	Overall ± RSD	91 ± 8	91 ± 7	90 ± 9	90 ± 9	90 ± 8	90 ± 8	93 ± 6	98 ± 7	93 ± 6	74 ± 5	89 ± 11	90 ± 11
	n	10	10	10	10	10	10	10	10	10	10	10	10

RSD = relative standard deviation, n = number of replicates

Table A 21: Recovery results from method validation of prothioconazole in barley grain (data obtained from study B18G-B5-AP-03)

Fortification level	Analyte	Prothioconazole-desthio		3-hydroxy-prothioconazole-desthio		4-hydroxy-prothioconazole-desthio		5-hydroxy-prothioconazole-desthio		6-hydroxy-prothioconazole-desthio		alpha-hydroxy-prothioconazole-desthio	
[mg/kg]	Transition ion	70 m/z	125 m/z	70 m/z	141 m/z	141 m/z	70 m/z	70 m/z	141 m/z	70 m/z	141 m/z	70 m/z	141 m/z
0.010	Range	87-95	89-91	88-90	83-94	86-93	87-94	88-92	88-92	89-97	88-93	91-93	88-91
	Mean ± RSD	90 ± 3	90 ± 1	89 ± 1	89 ± 5	90 ± 3	90 ± 3	90 ± 2	90 ± 2	92 ± 3	90 ± 2	93 ± 1	90 ± 1
	n	5	5	5	5	5	5	5	5	5	5	5	5
0.100	Range	78-85	81-86	78-83	76-81	78-83	78-84	85-91	85-91	83-90	84-91	76-81	74-78
	Mean ± RSD	81 ± 3	82 ± 3	80 ± 2	79 ± 2	81 ± 2	81 ± 3	87 ± 3	87 ± 3	86 ± 3	86 ± 3	78 ± 3	76 ± 2
	n	5	5	5	5	5	5	5	5	5	5	5	5
0.01 and 0.10	Overall ± RSD	86 ± 6	86 ± 5	85 ± 6	84 ± 8	85 ± 6	85 ± 6	88 ± 3	89 ± 3	89 ± 4	88 ± 4	85 ± 9	83 ± 9
	n	10	10	10	10	10	10	10	10	10	10	10	10

RSD = relative standard deviation, n = number of replicates

Table A 22: Recovery results from method validation of prothioconazole in barley straw (data obtained from study B18G-B5-AP-03)

Fortification level	Analyte	Prothioconazole-desthio		3-hydroxy-prothioconazole-desthio		4-hydroxy-prothioconazole-desthio		5-hydroxy-prothioconazole-desthio		6-hydroxy-prothioconazole-desthio		alpha-hydroxy-prothioconazole-desthio	
[mg/kg]	Transition ion	70 m/z	125 m/z	70 m/z	141 m/z	141 m/z	70 m/z	70 m/z	141 m/z	70 m/z	141 m/z	70 m/z	141 m/z
0.010	Range	71-87	70-88	73-89	75-87	73-89	70-89	71 ± 89	74-92	72-89	73-90	75-92	80-91
	Mean ± RSD	82 ± 9	81 ± 9	83 ± 9	81 ± 7	82 ± 8	81 ± 10	83 ± 10	85 ± 9	82 ± 8	84 ± 9	85 ± 9	87 ± 5
	n	5	5	5	5	5	5	5	5	5	5	5	5
0.100	Range	73-81	74-81	71-77	70-76	73-89	72-79	76-83	76-85	75-82	76-83	69-77	70-75
	Mean ± RSD	77 ± 4	77 ± 3	74 ± 3	73 ± 3	75 ± 3	75 ± 3	79 ± 3	80 ± 4	79 ± 4	80 ± 4	73 ± 4	73 ± 3
	n	5	5	5	5	5	5	5	5	5	5	5	5
	Overall ± RSD	79 ± 7	79 ± 7	78 ± 9	77 ± 8	79 ± 8	78 ± 8	81 ± 7	83 ± 7	80 ± 6	82 ± 7	79 ± 11	80 ± 10

0.01 and 0.10	n	10	10	10	10	10	10	10	10	10	10	10	10

RSD = relative standard deviation, n = number of replicates

Table A 23: Characteristics for the analytical method used for validation of prothioconazole residues in barley

	Prothioconazole*
Specificity	Blank value < 30 % LOQ
Calibration (type, number of data points)	Individual calibration data presented r > 0.99 8 calibration points
Calibration range	0.6 - 40 µg/L
Assessment of matrix effects is presented	No
Limit of quantification	For each analyte separately: LOQ: 0.010 mg/kg For prothioconazole as the sum of all analytes: LOQ: 0.060 mg/kg

* Including: prothioconazole-desthio, 3-hydroxyprothioconazole-desthio, 4-hydroxyprothioconazole-desthio, 5-hydroxyprothioconazole-desthio, 6-hydroxyprothioconazole-desthio and alpha-hydroxyprothioconazole-desthio

Conclusion
The method fulfils the requirements of SANCO/3029/99 rev. 4 and is suitable for the determination of prothioconazole in barley.

Comments of zRMS:	The analytical method was validated for barley whole plant without roots, grain and straw according to guideline SANCO/3029/99 rev. 4. All the analytes were determined by LC-MS/MS using a quantitation and confirmation ion. The LOQ of each analyte was at 0.01 mg/kg for each matrix. The mean recovery was between 70% and 110% with a RSD less than or equal to 20% at each level of fortification, for each reference item and for each matrix. The storage duration (interval between sampling and extraction date) was 217 days for the determination of prothioconazole and its metabolites. Sufficient stability data are available to support the residue data presented in this study. The method ~~study~~ is acceptable.

Reference:	KCP 5.1.2/07 (filed in KCA 6.3.2/03)
Report:	Residue study of azoxystrobin, prothioconazole and its metabolites in barley whole plant and Raw Agricultural Commodity after one foliar application of MCW-2073 - 3 harvest and 2 decline trials - Northern Europe (France, Poland and Hungary) - 2019, Amic, S., 2020c, report no.: BPL19/759/GC, sponsor no.: 000102749
Guideline(s):	For method validation: SANCO/3029/99 rev. 4
Deviations:	None
GLP:	Yes (certified laboratory)
Acceptability/Reliability:	Yes
Duplication (if vertebrate study)	Not applicable

Materials and methods
The analytical method is based on European Committee for Standardization (CEN): (CEN): EN 15662:2018-05, paragraph 8 – QuEChERS-method. Residues were extracted from homogenised matrices by maceration with acetonitrile/water. Then, extracts were purified by dispersive solid phase extraction. The quantification was performed by LC-MS/MS with two mass transitions.
The analytical method was fully validated during the course of another study for barley whole plant w/o roots, grain and straw according to guideline SANCO/3029/99 rev. 4:
Study code: B18G-B5-AP-03, Sponsor reference: R-39645 A.

Results and discussions
For an overview of recovery results obtained during the validation study mentioned above, please refer to

Table A 20 - Table A 22. Recovery results were in a range of 70 to 110% with an RSD ≤ 20. No outliers were identified. No interference (< 30% LOQ) of total peak area for the target analytes were found in unfortified control samples. The LOQ of prothioconazole-desthio, 3-hydroxyprothioconazole-desthio, 4-hydroxyprothioconazole-desthio expressed as prothioconazole-desthio, 5-hydroxyprothioconazole-desthio expressed as prothioconazole-desthio, 6-hydroxyprothioconazole-desthio expressed as prothioconazole-desthio and alpha-hydroxyprothioconazole-desthio expressed as prothioconazole-desthio was 0.010 mg/kg, for each analyte and for each matrix. The LOQ for the sum of all prothioconazole-items was 0.060 mg/kg for each matrix.

Table A 24: Characteristics for the analytical method used for validation of prothioconazole residues in barley

	Prothioconazole*
Specificity	Blank value < 30 % LOQ
Calibration (type, number of data points)	Individual calibration data presented r > 0.99 8 calibration points
Calibration range	0.6 - 40 µg/L
Assessment of matrix effects is presented	No
Limit of quantification	For each analyte separately: LOQ: 0.010 mg/kg For prothioconazole as the sum of all analytes: LOQ: 0.060 mg/kg

* Including: prothioconazole-desthio, 3-hydroxyprothioconazole-desthio, 4-hydroxyprothioconazole-desthio, 5-hydroxyprothioconazole-desthio, 6-hydroxyprothioconazole-desthio and alpha-hydroxyprothioconazole-desthio

Conclusion
The method fulfils the requirements of SANCO/3029/99 rev. 4 and is suitable for the determination of prothioconazole in barley.

Comments of zRMS:	The analytical method was validated for barley whole plant without roots, grain and straw according to guideline SANCO/3029/99 rev. 4. All the analytes were determined by LC-MS/MS using a quantitation and confirmation ion. The LOQ of each analyte was at 0.01 mg/kg for each matrix. The mean recovery was between 70% and 110% at each level of fortification, for each reference item and for each matrix. The storage duration (interval between sampling and extraction date) was 158 days for the determination of prothioconazole and its metabolites. Sufficient stability data are available to support the residue data presented in this study. The method ~~study~~ is acceptable.

Reference:	KCP 5.1.2/08 (filed in KCA 6.3.2/05)
Report:	Residue study of prothioconazole and its metabolites in barley whole plant and RAC after one foliar application of ADM.3500.F.2.B (250 g a.s./L of prothioconazole) - 2 harvest and 2 decline trials – Northern Europe (FR, HU, PL) - 2019, Amic, S., 2020d, report no.: BPL19/764/GC, sponsor no.: 000102753
Guideline(s):	For method validation: SANCO/3029/99 rev. 4
Deviations:	None
GLP:	Yes (certified laboratory)
Acceptability/Reliability:	Yes
Duplication (if vertebrate study)	Not applicable

Materials and methods
The analytical method is based on European Committee for Standardization (CEN): EN 15662:2009-02,

paragraph 8 – QuEChERS-method. Residues were extracted from homogenised matrices by maceration with acetonitrile/water. Then, extracts were purified by dispersive solid phase extraction. The quantification was performed by LC-MS/MS with two mass transitions.
The analytical method was fully validated during the course of another study for barley whole plant w/o roots, grain and straw according to guideline SANCO/3029/99 rev. 4:
Study code: B19S-A4-P-01, Sponsor reference: 000102920.

Results and discussions
The method validation according to guideline SANCO/3029/99 rev. 4 obtained during the validation study mentioned above is presented in table Table A 16 -
Table A 18. Recovery results were in a range of 70 to 110% with an RSD ≤ 20. No outliers were identified. No interference (< 30% LOQ) of total peak area for the target analytes were found in unfortified control samples. The LOQ of prothioconazole-desthio, 3-hydroxyprothioconazole-desthio, 4-hydroxyprothioconazole-desthio expressed as prothioconazole-desthio, 5-hydroxyprothioconazole-desthio expressed as prothioconazole-desthio, 6-hydroxyprothioconazole-desthio expressed as prothioconazole-desthio and alpha-hydroxyprothioconazole-desthio expressed as prothioconazole-desthio was 0.010 mg/kg, for each analyte and for each matrix. The LOQ for the sum of all prothioconazole-items was 0.060 mg/kg for each matrix.

Table A 25: Characteristics for the analytical method used for validation of prothioconazole residues in barley

	Prothioconazole*
Specificity	Blank value < 30% LOQ
Calibration (type, number of data points)	Individual calibration data presented r > 0.99 8 calibration points
Calibration range	0.6 - 40 µg/L
Assessment of matrix effects is presented	No
Limit of quantification	For each analyte separately: LOQ: 0.010 mg/kg For prothioconazole as the sum of all analytes: LOQ: 0.060 mg/kg

* Including: prothioconazole-desthio, 3-hydroxyprothioconazole-desthio, 4-hydroxyprothioconazole-desthio, 5-hydroxyprothioconazole-desthio, 6-hydroxyprothioconazole-desthio and alpha-hydroxyprothioconazole-desthio

Conclusion
The method fulfils the requirements of SANCO/3029/99 rev. 4 and is suitable for the determination of prothioconazole in barley.

The following validation summary applies to the following two residue studies:

Comments of zRMS:	The analytical method was validated for barley whole plant without roots, grain and straw according to guideline SANCO/3029/99 rev. 4. All the analytes were determined by LC-MS/MS using a quantitation and confirmation ion. LOQ = 0.06 mg/kg for prothioconazole expressed as prothioconazole-desthio as a sum of metabolites. The mean recovery was between 70% and 110% at each level of fortification, for each reference item and for each matrix. The storage duration (interval between sampling and extraction date) was 114 days for the determination of prothioconazole and its metabolites. Sufficient stability data are available to support the residue data presented in this study. The method ~~study~~ is acceptable.

Reference:	KCP 5.1.2/09 (filed in KCA 6.3.2/07)
Report:	Residue study of prothioconazole and its metabolites, and fenpropidin in barley whole plant and RAC (grain and straw) after one foliar application of ADM.3502.F.1.A - 2 harvest and 2 decline trials - Northern Europe (France, Poland and Hungary) - 2019 Huaulmé, J.-M., 2020 Report no.: BPL19/772/GC, sponsor no.: 000102761
Guideline(s):	For method validation: SANCO/3029/99 rev. 4
Deviations:	None
GLP:	Yes
Acceptability:	Yes

Comments of zRMS:	The analytical method was validated for barley whole plant without roots, grain and straw according to guideline SANCO/3029/99 rev. 4 (reduced validation). LOQ: 0.01 mg/kg for each analyte, 0.06 mg/kg for prothioconazole expressed as prothioconazole-desthio as a sum of metabolites. The mean recovery was between 70% and 110% at each level of fortification, for each reference item and for each matrix. The storage duration (interval between sampling and extraction date) was 70 days for the determination of prothioconazole and its metabolites. Sufficient stability data are available to support the residue data presented in this study. The method ~~study~~ is acceptable.

Reference:	KCP 5.1.2/10 (filed in KCA 6.3.2/09)
Report:	Residue study of prothioconazole and its metabolites, and fenpropidin in barley whole plant and raw agricultural commodity after one foliar application of ADM.3502.F.1.A - 2 harvest and 2 decline trials – Northern Europe (FR, PL, HU) - 2020 Huaulmé, J.-M., 2021a Report no.: BPL20/844/GC, sponsor no.: 000105350
Guideline(s):	For method validation: SANCO/3029/99 rev. 4
Deviations:	None
GLP:	Yes
Acceptability:	Yes

Materials and methods

The analytical method is based on European Committee for Standardization (CEN): EN 15662:2009-02, paragraph 8 – QuEChERS-method. Residues of prothioconazole (sum of prothioconazole-desthio, 3-hydroxy-prothioconazoledesthio, 4-hydroxy-prothioconazole-desthio, 5-hydroxy-prothioconazole-desthio, 6-hydroxyprothioconazole-desthio and alpha-hydroxy-prothioconazole-desthio, expressed as prothioconazole-desthio) were extracted from homogenised matrices by maceration with acetonitrile/water. Then, extracts were purified by dispersive solid phase extraction. The quantification was performed by LC-MS/MS (QuEChERS-method) with two mass transitions. The analytical method was fully validated during

the course of other studies for barley whole plant, barley grain and barley straw according to guideline SANCO/3029/99 rev. 4:
Study code: B19S-A4-P-01, Sponsor reference: 000102920.

Results and discussions
Recovery results were in a range of 70 to 110% with an RSD ≤ 20. No outliers were identified. No interference (< 30% LOQ) of total peak area for the target analytes were found in unfortified control samples. The LOQ of prothioconazole-desthio, 3-hydroxyprothioconazole-desthio expressed as prothioconazole-desthio, 4-hydroxyprothioconazole-desthio expressed as prothioconazole-desthio, 5-hydroxyprothioconazole-desthio expressed as prothioconazole-desthio, 6-hydroxyprothioconazole-desthio expressed as prothioconazole-desthio and alpha-hydroxyprothioconazole-desthio expressed as prothioconazole-desthio was 0.010 mg/kg for each analyte and for each matrix. The LOQ for the sum of all prothioconazole-items was 0.060 mg/kg for each matrix.

Table A 26: Recovery results from method validation of prothioconazole metabolites in whole plant of barley

Fortification level	Analyte	Prothioconazole-desthio		3-hydroxy-prothioconazole-desthio		4-hydroxy-prothioconazole-desthio		5-hydroxy-prothioconazole-desthio		6-hydroxy-prothioconazole-desthio		alpha-hydroxy-prothioconazole-desthio	
[mg/kg]	Transition ion	70 m/z	125 m/z	70 m/z	141 m/z	141 m/z	70 m/z	70 m/z	141 m/z	70 m/z	141 m/z	70 m/z	141 m/z
0.010	Range	80-88	81-89	81-88	82-90	82-86	80-86	79-87	81-87	84-89	83-89	81-89	80-88
	Mean ± RSD	84 ± 4	85 ± 4	84 ± 3	85 ± 4	84 ± 2	82 ± 3	82 ± 4	84 ± 3	86 ± 3	85 ± 3	84 ± 4	84 ± 5
	n	5	5	5	5	5	5	5	5	5	5	5	5
0.100	Range	81-88	82-88	82-89	83-88	83-87	82-88	82-88	82-87	85-90	85-92	83-90	84-90
	Mean ± RSD	86 ± 3	86 ± 3	85 ± 3	86 ± 3	85 ± 2	85 ± 3	85 ± 2	85 ± 2	88 ± 2	89 ± 3	87 ± 3	87 ± 2
	n	5	5	5	5	5	5	5	5	5	5	5	5
0.01 and 0.10	Overall ± RSD	85 ± 3	85 ± 3	85 ± 3	86 ± 3	85 ± 2	84 ± 3	84 ± 3	85 ± 3	87 ± 3	87 ± 3	85 ± 3	85 ± 4
	n	10	10	10	10	10	10	10	10	10	10	10	10

RSD = relative standard deviation, n = number of replicates

Table A 27: Recovery results from method validation of prothioconazole metabolites in grain of barley

Fortification level	Analyte	Prothioconazole-desthio		3-hydroxy-prothioconazole-desthio		4-hydroxy-prothioconazole-desthio		5-hydroxy-prothioconazole-desthio		6-hydroxy-prothioconazole-desthio		alpha-hydroxy-prothioconazole-desthio	
[mg/kg]	Transition ion	70 m/z	125 m/z	70 m/z	141 m/z	141 m/z	70 m/z	70 m/z	141 m/z	70 m/z	141 m/z	70 m/z	141 m/z
0.010	Range	83-88	84-90	81-88	82-90	82-86	80-86	79-87	81-87	84-89	83-89	81-89	80-88
	Mean ± RSD	86 ± 2	87 ± 3	84 ± 3	85 ± 4	84 ± 2	82 ± 3	82 ± 4	84 ± 3	86 ± 3	85 ± 3	84 ± 4	84 ± 5
	n	5	5	5	5	5	5	5	5	5	5	5	5
0.100	Range	73-88	73-87	82-89	83-88	83-87	82-88	82-88	82-87	85-90	85-92	83-90	84-90
	Mean ± RSD	83 ± 7	83 ± 7	85 ± 3	86 ± 3	85 ± 2	85 ± 3	85 ± 2	85 ± 2	88 ± 2	89 ± 3	87 ± 3	87 ± 2
	n	5	5	5	5	5	5	5	5	5	5	5	5
0.01 and 0.10	Overall ± RSD	84 ± 5	85 ± 5	85 ± 3	86 ± 3	85 ± 2	84 ± 3	84 ± 3	85 ± 3	87 ± 3	87 ± 3	85 ± 3	85 ± 4
	n	10	10	10	10	10	10	10	10	10	10	10	10

RSD = relative standard deviation, n = number of replicates

Table A 28: Recovery results from method validation of prothioconazole metabolites in straw of barley

Fortification level	Analyte	Prothioconazole-desthio		3-hydroxy-prothioconazole-desthio		4-hydroxy-prothioconazole-desthio		5-hydroxy-prothioconazole-desthio		6-hydroxy-prothioconazole-desthio		alpha-hydroxy-prothioconazole-desthio	
[mg/kg]	Transition ion	70 m/z	125 m/z	70 m/z	141 m/z	141 m/z	70 m/z	70 m/z	141 m/z	70 m/z	141 m/z	70 m/z	141 m/z
0.010	Range	85-87	81-84	85-87	85-89	83-86	82-86	83-86	84-85	86-91	83-88	83-87	79-86
	Mean ± RSD	86 ± 1	83 ± 2	86 ± 1	86 ± 2	85 ± 2	84 ± 2	85 ± 1	85 ± 1	88 ± 2	86 ± 2	84 ± 2	84 ± 3
	n	5	5	5	5	5	5	5	5	5	5	5	5
0.100	Range	84-85	83-85	82-84	83-85	83-84	81-82	81-83	83-83	84-87	86-88	83-84	83-85
	Mean ± RSD	84 ± 1	84 ± 1	83 ± 1	84 ± 1	83 ± 0.5	83 ± 1	82 ± 1	83 ± 0.5	86 ± 1	87 ± 1	84 ± 1	84 ± 1
	n	5	5	5	5	5	5	5	5	5	5	5	5
	Overall ± RSD	85 ± 1	83 ± 1	85 ± 2	85 ± 2	84 ± 2	83 ± 2	83 ± 2	84 ± 1	87 ± 2	87 ± 2	84 ± 1	84 ± 2

0.01 and 0.10	n	10	10	10	10	10	10	10	10	10	10	10	10

RSD = relative standard deviation, n = number of replicates

Table A 29: Characteristics for the analytical method used for validation of prothioconazole metabolites residues in barley whole plant, barley grain and barley straw

	Prothioconazole*
Specificity	Blank value < 30 % LOQ
Calibration (type, number of data points)	Individual calibration data presented r > 0.99 7 calibration points
Calibration range	0.6 - 40 µg/L
Assessment of matrix effects is presented	Not rquired, since calibration was carried out with matrix-matched standards
Limit of quantification	For each analyte separately: LOQ: 0.010 mg/kg For prothioconazole as the sum of all analytes: LOQ: 0.060 mg/kg

* Including: prothioconazole-desthio, 3-hydroxyprothioconazole-desthio, 4-hydroxyprothioconazole-desthio, 5-hydroxyprothioconazole-desthio, 6-hydroxyprothioconazole-desthio and alpha-hydroxyprothioconazole-desthio

Conclusion

The method fulfils the requirements of SANCO/3029/99 rev. 4 and is suitable for the determination of prothioconazole (including: prothioconazole-desthio, 3-hydroxyprothioconazole-desthio, 4-hydroxyprothioconazole-desthio, 5-hydroxyprothioconazole-desthio, 6-hydroxyprothioconazole-desthio and alpha-hydroxyprothioconazole-desthio) in barley whole plant, barley grain and barley straw.

Comments of zRMS:	The analytical method for prothioconazole and its metabolites was validated within this study, according to SANCO/3029/99 rev.4. The LOQ (Limit of quantification) of prothioconazole (sum of prothioconazole-desthio, 3-hydroxyprothioconazole-desthio, 4-hydroxy-prothioconazole-desthio, 5-hydroxy-prothioconazole-desthio, 6-hydroxy-prothioconazole-desthio and alpha-hydroxy-prothioconazole-desthio, expressed as prothioconazole-desthio) was 0.060 mg/kg, for each matrix (0.01 mg/kg per analyte). The quantification was performed by liquid chromatography with tandem mass spectrometry detection (LC-MS/MS). (QuEChERS) Acceptance criteria for method validations were met, with average recoveries ranging from 70% to 110% and relative standard deviations ≤20%. The storage duration (interval between sampling and extraction date) was 525 days for the determination of prothioconazole and its metabolites. Sufficient stability data are available to support the residue data presented in this study. The method ~~study~~ is acceptable

Reference:	KCP 5.1.2/11 (filed in KCA 6.3.3/01)
Report:	Magnitude of the residues of azoxystrobin + prothioconazole and metabolites in oilseed rape (RAC whole plant and seeds), following one application of MCW-2073 in 3 trials (2 DCS and 1 HS), Northern Europe (Northern France and Poland) – 2018, Roussel, Ch. H., 2020, report no.: ChR-18-33731, sponsor no.: R-39647
Guideline(s):	For method validation: SANCO/3029/99 rev. 4
Deviations:	None
GLP:	Yes (certified laboratory)
Acceptability/Reliability:	Yes
Duplication (if vertebrate study)	Not applicable

Materials and methods

The analytical method is based on European Committee for Standardization (CEN): EN 15662:2018-05, paragraph 8 – QuEChERS-method. Residues were extracted from homogenised matrices by maceration

with acetonitrile/water. Then, extracts were purified by dispersive solid phase extraction. The quantification was performed by LC-MS/MS with two mass transitions.

Results and discussions

Recovery results were in a range of 70 to 110% with an RSD ≤ 20. No outliers were identified. No interference (< 30% LOQ) of total peak area for the target analytes were found in unfortified control samples. The LOQ of prothioconazole (sum of prothioconazole-desthio, 3-hydroxyprothioconazole-desthio, 4-hydroxyprothioconazole-desthio, 5-hydroxyprothioconazole-desthio, 6-hydroxyprothioconazole-desthio and alpha-hydroxyprothioconazole-desthio, expressed as prothioconazole-desthio) was 0.060 mg/kg (0.01 mg/kg for each analyte) and for each matrix.

Table A 30: Recovery results from method validation of prothioconazole in whole plant of oilseed rape

Fortification level	Analyte	Prothioconazole-desthio		3-hydroxy-prothioconazole-desthio		4-hydroxy-prothioconazole-desthio		5-hydroxy-prothioconazole-desthio		6-hydroxy-prothioconazole-desthio		alpha-hydroxy-prothioconazole-desthio	
[mg/kg]	Transition ion	70 m/z	125 m/z	70 m/z	141 m/z	141 m/z	70 m/z	70 m/z	141 m/z	70 m/z	141 m/z	70 m/z	141 m/z
0.010	Range	99-102	98-104	99-104	98-102	98-104	99-103	102-104	105-111	103-104	97-101	105-108	104-107
	Mean ± RSD	101 ± 1	101 ± 2	102 ± 2	100 ± 1	101 ± 2	101 ± 1	103 ± 1	107 ± 2	103 ± 1	100 ± 2	107 ± 1	105 ± 1
	n	5	5	5	5	5	5	5	5	5	5	5	5
0.100	Range	93-100	95-101	93-103	95-101	94-100	95-102	96-103	101-106	96-105	94-102	100-107	99-107
	Mean ± RSD	97 ± 3	97 ± 2	99 ± 4	98 ± 3	99 ± 3	99 ± 2	100 ± 3	103 ± 2	101 ± 4	98 ± 3	103 ± 3	103 ± 3
	n	5	5	5	5	5	5	5	5	5	5	5	5
0.01 and 0.10	Overall ± RSD	99 ± 3	99 ± 3	100 ± 3	99 ± 3	100 ± 3	100 ± 2	102 ± 3	105 ± 3	102 ± 3	99 ± 3	105 ± 3	104 ± 2
	n	10	10	10	10	10	10	10	10	10	10	10	10

RSD = relative standard deviation, n = number of replicates

Table A 31: Recovery results from method validation of prothioconazole in oilseed rape seeds

Fortification level	Analyte	Prothioconazole-desthio		3-hydroxy-prothioconazole-desthio		4-hydroxy-prothioconazole-desthio		5-hydroxy-prothioconazole-desthio		6-hydroxy-prothioconazole-desthio		alpha-hydroxy-prothioconazole-desthio	
[mg/kg]	Transition ion	70 m/z	125 m/z	70 m/z	141 m/z	141 m/z	70 m/z	70 m/z	141 m/z	70 m/z	141 m/z	70 m/z	141 m/z
0.010	Range	72-111	71-111	80-116	78-120	77-120	79-120	74-118	81-117	69-105	66-103	83-123	81-126
	Mean ± RSD	83 ± 19	82 ± 20	90 ± 16	92 ± 18	90 ± 19	90 ± 18	89 ±19	91 ± 16	79 ± 19	78 ± 19	95 ± 17	95 ± 19
	n	5	5	5	5	5	5	5	5	5	5	5	5
0.100	Range	74-80	73-80	79-87	82-89	79-88	80-88	79-88	81-86	72-77	72-78	82-91	86-90
	Mean ± RSD	77 ± 3	77 ± 4	84 ± 4	85 ± 3	85 ± 4	85 ± 3	84 ± 5	84 ± 3	75 ± 3	75 ± 3	88 ± 4	88 ± 2
	n	5	5	5	5	5	5	5	5	5	5	5	5
0.01 and 0.10	Overall ± RSD	80 ± 14	80 ± 14	87 ± 12	89 ± 13	88 ± 14	88 ± 13	87 ± 14	88 ± 12	77 ± 14	76 ± 13	91 ± 13	91 ± 14
	n	10	10	10	10	10	10	10	10	10	10	10	10

RSD = relative standard deviation, n = number of replicates

Table A 32: Recovery results from method validation of prothioconazole in oilseed rape straw

Fortification level	Analyte	Prothioconazole-desthio		3-hydroxy-prothioconazole-desthio		4-hydroxy-prothioconazole-desthio		5-hydroxy-prothioconazole-desthio		6-hydroxy-prothioconazole-desthio		alpha-hydroxy-prothioconazole-desthio	
[mg/kg]	Transition ion	70 m/z	125 m/z	70 m/z	141 m/z	141 m/z	70 m/z	70 m/z	141 m/z	70 m/z	141 m/z	70 m/z	141 m/z
0.010	Range	89-94	86-92	90-96	85-96	93-96	90-94	93-100	74-95	92-98	97-105	91-101	93-102
	Mean ± RSD	91 ± 2	89 ± 3	92 ± 3	91 ± 4	95 ± 2	92 ± 2	96 ± 3	85 ± 9	95 ± 2	103 ± 3	97 ± 3	98 ± 4
	n	5	5	5	5	5	5	5	5	5	5	5	5
0.100	Range	87-96	86-96	88-97	87-95	89-97	87-95	89-99	79-94	88-98	91-102	89-98	90-98
	Mean ± RSD	91 ± 4	90 ± 4	92 ± 4	91 ± 4	93 ± 4	91 ± 4	94 ± 5	85 ± 7	93 ± 4	98 ± 5	93 ± 4	93 ± 4
	n	5	5	5	5	5	5	5	5	5	5	5	5
	Overall ± RSD	91 ± 3	90 ± 3	92 ± 3	91 ± 4	94 ± 3	91 ± 3	95 ± 4	85 ± 8	94 ± 3	100 ± 4	95 ± 4	95 ± 4

0.01 and 0.10	n	10	10	10	10	10	10	10	10	10	10	10	10

RSD = relative standard deviation, n = number of replicates

Table A 33: Characteristics for the analytical method used for validation of prothioconazole residues in oilseed rape

	Prothioconazole*
Specificity	Blank value < 30 % LOQ
Calibration (type, number of data points)	Individual calibration data presented r > 0.99 7 calibration points
Calibration range	0.6 - 20 µg/mL
Assessment of matrix effects is presented	No
Limit of quantification	For each analyte separately: LOQ: 0.010 mg/kg For prothioconazole as the sum of all analytes: LOQ: 0.060 mg/kg

* Including: prothioconazole-desthio, 3-hydroxyprothioconazole-desthio, 4-hydroxyprothioconazole-desthio, 5-hydroxyprothioconazole-desthio, 6-hydroxyprothioconazole-desthio and alpha-hydroxyprothioconazole-desthio

Conclusion
The method fulfils the requirements of SANCO/3029/99 rev. 4 and is suitable for the determination of prothioconazole in oilseed rape.

The following validation summary applies to the following two residue studies.

Comments of zRMS:	The analytical method for prothioconazole and its metabolites was validated within this study, according to SANCO/3029/99 rev.4. The LOQ (Limit of quantification) of prothioconazole (sum of prothioconazole-desthio, 3-hydroxyprothioconazole-desthio, 4-hydroxy-prothioconazole-desthio, 5-hydroxy-prothioconazole-desthio, 6-hydroxy-prothioconazole-desthio and alpha-hydroxy-prothioconazole-desthio, expressed as prothioconazole-desthio) was 0.060 mg/kg, for each matrix (0.01 mg/kg per analyte). The quantification was performed by liquid chromatography with tandem mass spectrometry detection (LC-MS/MS). (QuEChERS) Acceptance criteria for method validations were met, with average recoveries ranging from 70% to 110% and relative standard deviations ≤20%. The storage duration (interval between sampling and extraction date) was 248 days for the determination of prothioconazole and its metabolites. Sufficient stability data are available to support the residue data presented in this study. The method ~~study~~ is acceptable.

Reference:	KCP 5.1.2/12 (filed in KCA 6.3.3/03)
Report:	Magnitude of the residues of azoxystrobin + prothioconazole and metabolites in oilseed rape (RAC whole plant, seeds and straw), following one application of MCW-2073 in 6 trials (2 DCS, 3 HS and 1 backup HS), Northern Europe (PL, N-FR, DE) – 2019, Peterek, S., 2020, report no.: SPK-19-38368, sponsor no.: 000102602
Guideline(s):	For method validation: SANCO/3029/99 rev. 4
Deviations:	None
GLP:	Yes (certified laboratory)
Acceptability/Reliability:	Yes
Duplication (if vertebrate study)	Not applicable

Comments of zRMS:	The analytical method for analysis of residues of prothioconazole and its metabolites was fully validated for the matrices whole plant seed and straw according to SANCO/3029/99 rev.4 in previous studies in 2018 and in 2019. Therefore, only daily recoveries were performed during this study. The LOQ (Limit of quantification) of prothioconazole (sum of prothioconazole-desthio, 3-hydroxyprothioconazole-desthio, 4-hydroxy-prothioconazole-desthio, 5-hydroxy-prothioconazole-desthio, 6-hydroxy-prothioconazole-desthio and alpha-hydroxy-prothioconazole-desthio, expressed as prothioconazole-desthio) was 0.060 mg/kg, for each matrix (0.01 mg/kg per analyte). The quantification was performed by liquid chromatography with tandem mass spectrometry detection (LC-MS/MS). (QuEChERS) Acceptance criteria for method validations were met, with average recoveries ranging from 70% to 110% and relative standard deviations ≤20%. The storage duration (interval between sampling and extraction date) was 252 days for the determination of prothioconazole and its metabolites. Sufficient stability data are available to support the residue data presented in this study. The method ~~study~~ is acceptable.

Reference:	KCP 5.1.2/13 (filed in KCA 6.3.3/05)
Report:	Magnitude of the residues of prothioconazole and metabolites in oilseed rape (RAC whole plant, seeds and straw), following one application of ADM.3500.F.2.B in 4 trials (2 DCS and 2 HS), Northern Europe (Poland, Northern France and Germany) – 2019/2020 Grall, E., 2021 Report no.: SPK-19-38370, sponsor no.: 000102604
Guideline(s):	For method validation: SANCO/3029/99 rev. 4
Deviations:	None
GLP:	Yes
Acceptability:	Yes

Materials and methods

The analytical method is based on European Committee for Standardization (CEN): EN 15662:2009-02, paragraph 8 – QuEChERS-method. Residues of prothioconazole (sum of prothioconazole-desthio, 3-hydroxy-prothioconazoledesthio, 4-hydroxy-prothioconazole-desthio, 5-hydroxy-prothioconazole-desthio, 6-hydroxyprothioconazole-desthio and alpha-hydroxy-prothioconazole-desthio, expressed as prothioconazole-desthio) were extracted from homogenised matrices by maceration with acetonitrile/water. Then, extracts were purified by dispersive solid phase extraction. The quantification was performed by LC-MS/MS (QuEChERS-method) with two mass transitions. The analytical method was fully validated during the course of other studies for oilseed rape (whole plants, seeds and straw) according to guideline SANCO/3029/99 rev. 4:
Study code: B18G-A4-A-01, Sponsor reference: R-39652
Study code: B18S-A4-P-01, Sponsor reference: R-39651
Study code: B18S-S2-AP-01, Sponsor reference: R-39647A
Study code: B19S-A4-P-02, Sponsor reference: 000102921

Results and discussions

The method validation according to guideline SANCO/3029/99 rev. 4 obtained during Sponsor reference: R-39651, R-39647A and 000102921 is presented in the tables below. Recovery results were in a range of 70 to 110 % with an RSD ≤ 20. No outliers were identified. No interference (< 30 % LOQ) of total peak area for the target analytes were found in unfortified control samples. The LOQ of prothioconazole-desthio, 3-hydroxyprothioconazole-desthio expressed as prothioconazole-desthio, 4-hydroxyprothioconazole-desthio expressed as prothioconazole-desthio, 5-hydroxyprothioconazole-desthio expressed as prothioconazole-desthio, 6-hydroxyprothioconazole-desthio expressed as prothioconazole-desthio and alpha-hydroxyprothioconazole-desthio expressed as prothioconazole-desthio was 0.010 mg/kg for each analyte and for each matrix. The LOQ for the sum of all prothioconazole-items was 0.060 mg/kg for each matrix.

Table A 34: Recovery results from method validation of prothioconazole metabolites in oilseed rape seeds (Study code: B18G-A4-A-01, Sponsor reference: R-39652)

Fortification level	Analyte	Prothioconazole-desthio		3-hydroxy-prothioconazole-desthio		4-hydroxy-prothioconazole-desthio		5-hydroxy-prothioconazole-desthio		6-hydroxy-prothioconazole-desthio		alpha-hydroxy-prothioconazole-desthio	
[mg/kg]	Transition ion	70 m/z	125 m/z	70 m/z	141 m/z	141 m/z	70 m/z	70 m/z	141 m/z	70 m/z	141 m/z	70 m/z	141 m/z
0.010	Range	72-111	71-111	80-116	78-120	77-120	79-120	74-118	81-117	69-105	66-103	83-123	81-126
	Mean ± RSD	83 ± 19	82 ± 20	90 ± 16	92 ± 18	90 ± 19	90 ± 18	89 ± 19	91 ± 16	79 ± 19	78 ± 19	95 ±17	95 ± 19
	n	5	5	5	5	5	5	5	5	5	5	5	5
0.100	Range	74-80	73-80	79-87	82-89	79-88	80-88	79 - 88	81-86	72-77	72-78	82-91	86-90
	Mean ± RSD	77 ± 3	77 ± 4	84 ± 4	85 ± 3	85 ± 4	85 ± 3	84 ± 5	84 ± 3	75 ± 3	75 ± 3	88 ± 4	88 ± 2
	n	5	5	5	5	5	5	5	5	5	5	5	5
0.01 and 0.10	Overall ± RSD	80 ± 14	80 ± 14	87 ± 12	89 ± 13	88 ± 14	88 ± 13	87 ± 14	88 ± 12	77 ± 14	76 ± 13	91 ± 13	91 ± 14
	n	10	10	10	10	10	10	10	10	10	10	10	10

RSD = relative standard deviation, n = number of replicates

Table A 35: Recovery results from method validation of prothioconazole metabolites in oilseed rape whole plant (Study code: B18S-S2-AP-01, Sponsor reference: R-39647A)

Fortification level	Analyte	Prothioconazole-desthio		3-hydroxy-prothioconazole-desthio		4-hydroxy-prothioconazole-desthio		5-hydroxy-prothioconazole-desthio		6-hydroxy-prothioconazole-desthio		alpha-hydroxy-prothioconazole-desthio	
[mg/kg]	Transition ion	70 m/z	125 m/z	70 m/z	141 m/z	141 m/z	70 m/z	70 m/z	141 m/z	70 m/z	141 m/z	70 m/z	141 m/z
0.010	Range	99-102	98-104	99-104	98-102	98-104	99-103	102-104	105-111	103-104	97-101	105-108	104-107
	Mean ± RSD	101 ± 1	101 ± 2	102 ± 2	100 ± 1	101 ± 2	101 ± 1	103 ± 1	107 ± 2	103 ± 1	100 ± 2	107 ±1	105 ± 1
	n	5	5	5	5	5	5	5	5	5	5	5	5
0.100	Range	93-100	95-101	93-103	95-101	94-100	95-102	96 – 103	101-106	96-105	94-102	100-107	99-107
	Mean ± RSD	97 ± 3	97 ± 2	99 ± 4	98 ± 3	99 ± 3	99 ± 2	100 ± 3	103 ± 2	101 ± 4	98 ± 3	103 ± 3	103 ± 3
	n	5	5	5	5	5	5	5	5	5	5	5	5
0.01 and 0.10	Overall ± RSD	99 ± 3	99 ± 3	100 ± 3	99 ± 3	100 ± 3	100 ± 2	102 ± 3	105 ± 3	102 ± 3	99 ± 3	105 ± 3	104 ± 2
	n	10	10	10	10	10	10	10	10	10	10	10	10

RSD = relative standard deviation, n = number of replicates

Table A 36: Recovery results from method validation of prothioconazole metabolites in oilseed rape straw (Study code: B18S-S2-AP-01, Sponsor reference: R-39647A)

Fortification level	Analyte	Prothioconazole-desthio		3-hydroxy-prothioconazole-desthio		4-hydroxy-prothioconazole-desthio		5-hydroxy-prothioconazole-desthio		6-hydroxy-prothioconazole-desthio		alpha-hydroxy-prothioconazole-desthio	
[mg/kg]	Transition ion	70 m/z	125 m/z	70 m/z	141 m/z	141 m/z	70 m/z	70 m/z	141 m/z	70 m/z	141 m/z	70 m/z	141 m/z
0.010	Range	89-94	86-92	90-96	85-96	93-96	90-94	93-100	74-95	92-98	97-105	92-101	93-102
	Mean ± RSD	91 ± 2	89 ± 3	92 ± 3	91 ± 4	95 ± 2	92 ± 2	96 ± 3	85 ± 9	95 ± 2	103 ± 3	97 ±3	98 ± 4
	n	5	5	5	5	5	5	5	5	5	5	5	5
0.100	Range	87-96	86-96	88-97	87-95	89-97	87-95	89 - 99	79-94	88-98	91-102	89-98	90-98
	Mean ± RSD	91 ± 4	90 ± 4	92 ± 4	91 ± 4	93 ± 4	91 ± 4	94 ± 5	85 ± 7	93 ± 4	98 ± 5	93 ± 4	93 ± 4
	n	5	5	5	5	5	5	5	5	5	5	5	5
0.01 and 0.10	Overall ± RSD	91 ± 3	90 ± 3	92 ± 3	91 ± 4	94 ± 3	91 ± 3	95 ± 4	85 ± 8	94 ± 3	100 ± 4	95 ± 4	95 ± 4
	n	10	10	10	10	10	10	10	10	10	10	10	10

RSD = relative standard deviation, n = number of replicates

Table A 37: Recovery results from method validation of prothioconazole in whole plant of oilseed rape (Study code: B18S-A4-P-01, Sponsor reference: R-39651)

Fortification level	Analyte	Prothioconazole-desthio		3-hydroxy-prothioconazole-desthio		4-hydroxy-prothioconazole-desthio		5-hydroxy-prothioconazole-desthio		6-hydroxy-prothioconazole-desthio		alpha-hydroxy-prothioconazole-desthio	
[mg/kg]	Transition ion	70 m/z	125 m/z	70 m/z	141 m/z	141 m/z	70 m/z	70 m/z	141 m/z	70 m/z	141 m/z	70 m/z	141 m/z
0.010	Range	70-74	70-73	74-80	75-81	72-78	70-77	70-77	71-80	70-79	70-74	72-79	72-82
	Mean ± RSD	72 ± 2	71 ± 1	77 ± 3	78 ± 3	75 ± 4	73 ± 4	74 ± 4	75 ± 5	72 ± 5	71 ± 2	76 ± 4	78 ± 5
	n	5	5	5	5	5	5	5	5	5	5	5	5
0.100	Range	70-73	71-71	70-78	72-81	70-79	71-77	70-75	70-81	71-75	70-71	71-80	73-83
	Mean ± RSD	71 ± 1	71 ± 0.5	76 ± 4	78 ± 5	75 ± 5	74 ± 3	73 ± 3	76 ± 5	72 ± 3	71 ± 1	76 ± 5	79 ± 5
	n	5	5	5	5	5	5	5	5	5	5	5	5
0.01 and 0.10	Overall ± RSD	71 ± 2	71 ± 1	76 ± 4	78 ± 4	75 ± 4	73 ± 4	73 ± 3	75 ± 5	72 ± 4	71 ± 2	76 ± 4	79 ± 5
	n	10	10	10	10	10	10	10	10	10	10	10	10

RSD = relative standard deviation, n = number of replicates

Table A 38: Recovery results from method validation of prothioconazole in oilseed rape seeds (Study code: B18S-A4-P-01, Sponsor reference: R-39651)

Fortification level	Analyte	Prothioconazole-desthio		3-hydroxy-prothioconazole-desthio		4-hydroxy-prothioconazole-desthio		5-hydroxy-prothioconazole-desthio		6-hydroxy-prothioconazole-desthio		alpha-hydroxy-prothioconazole-desthio	
[mg/kg]	Transition ion	70 m/z	125 m/z	70 m/z	141 m/z	141 m/z	70 m/z	70 m/z	141 m/z	70 m/z	141 m/z	70 m/z	141 m/z
0.010	Range	78-82	76-81	76-86	76-84	76-86	73-85	78-87	79-89	81-86	81-84	74-84	72-83
	Mean ± RSD	81 ± 4	79 ± 2	82 ± 4	81 ± 4	82 ± 5	80 ± 5	83 ± 4	85 ± 4	84 ± 2	83 ± 2	81 ± 5	80 ± 5
	n	5	5	5	5	5	5	5	5	5	5	5	5
0.100	Range	71-88	70-87	70-86	70-86	70-85	70-81	71 ± 87	75-89	74-91	73-91	72-86	72-82

	Mean ± RSD n	78 ± 9 5	77 ± 9 5	76 ± 9 5	77 ± 8 5	76 ± 8 5	74 ± 6 5	78 ± 8 5	80 ± 7 5	81 ± 9 5	80 ± 10 5	77 ± 8 5	75 ± 5 5
0.01 and 0.10	Overall ± RSD n	79 ± 7 10	78 ± 7 10	79 ± 8 10	79 ± 7 10	79 ± 8 10	77 ± 7 10	81 ± 7 10	83 ± 6 10	82 ± 6 10	81 ± 7 10	79 ± 7 10	78 ± 6 10

RSD = relative standard deviation, n = number of replicates

Table A 39: Recovery results from method validation of prothioconazole in oilseed rape straw (Study code: B18S-A4-P-01, Sponsor reference: R-39651)

Fortification level	**Analyte**	**Prothioconazole-desthio**		**3-hydroxy-prothioconazole-desthio**		**4-hydroxy-prothioconazole-desthio**		**5-hydroxy-prothioconazole-desthio**		**6-hydroxy-prothioconazole-desthio**		**alpha-hydroxy-prothioconazole-desthio**	
[mg/kg]	Transition ion	70 m/z	125 m/z	70 m/z	141 m/z	141 m/z	70 m/z	70 m/z	141 m/z	70 m/z	141 m/z	70 m/z	141 m/z
0.010	Range Mean ± RSD n	76-85 81 ± 4 5	76-85 81 ± 4 5	76-84 79 ± 4 5	76-84 81 ± 4 5	73-83 77 ± 5 5	73-83 77 ± 5 5	76-85 79 ± 5 5	74-83 78 ± 4 5	78-85 81 ± 3 5	77-84 80 ± 4 5	75-83 79 ± 4 5	73-82 78 ± 5 5
0.100	Range Mean ± RSD n	79-93 87 ± 6 5	80-95 88 ± 6 5	76-92 85 ± 7 5	79-91 86 ± 6 5	74-91 84 ± 8 5	77-91 84 ± 7 5	78-96 86 ± 8 5	76-95 85 ± 8 5	77-93 86 ± 7 5	76-91 85 ± 7 5	79-94 87 ± 7 5	77-93 86 ± 7 5
0.01 and 0.10	Overall ± RSD n	84 ± 6 10	85 ± 7 10	82 ± 7 10	84 ± 6 10	81 ± 7 10	81 ± 7 10	82 ± 8 10	81 ± 8 10	83 ± 7 10	82 ± 6 10	83 ± 8 10	82 ± 8 10

RSD = relative standard deviation, n = number of replicates

Table A 40: Characteristics for the analytical method used for validation of prothioconazole residues in oilseed rape

	Prothioconazole*
Specificity	Blank value < 30 % LOQ
Calibration (type, number of data points)	Individual calibration data presented r > 0.99 7 calibration points
Calibration range	0.6 - 20 µg/L
Assessment of matrix effects is presented	No
Limit of quantification	For each analyte separately: LOQ: 0.010 mg/kg For prothioconazole as the sum of all analytes: LOQ: 0.060 mg/kg

* Including: prothioconazole-desthio, 3-hydroxyprothioconazole-desthio, 4-hydroxyprothioconazole-desthio, 5-hydroxyprothioconazole-desthio, 6-hydroxyprothioconazole-desthio and alpha-hydroxyprothioconazole-desthio

Conclusion
The method fulfils the requirements of SANCO/3029/99 rev. 4 and is suitable for the determination of prothioconazole in oilseed rape whole plant, seeds and straw.

The following study also provides the method validation for the following studies:

- Le Mineur, A. 2022 (KCA 6.3.1/07, report no BPL21/954/GC, sponsor no.: 000107608)
- Le Mineur, A., 2022 (KCA 6.3.1/08 report no.: BPL21/958/GC, sponsor no.: 000107612)
- Barbier, G., 2022 (KCA 6.3.2/12, report no.: B21G-A4-P-05, sponsor no.: 000108763)
- Huaulmé, J.-M., 2022 (KCA 6.3.2/11, report no.: BPL21/962/GC, sponsor no.: 000107616)
- Amic, S., 2021, KCA 6.3.3/07
- Bougrier, M.-A., 2022 (KCA 6.10.1/05, report no.: 555-2021, sponsor no.: 000108776).

Comments of zRMS:	The analytical method based on the method 00979/M001 was validated for the determination of prothioconazole (sum of prothioconazole-desthio and all metabolites containing the 2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl-2H-1,2,4-triazole moiety, expressed as prothioconazole-desthio (sum of isomers)) residues in barley (whole plant, grain, straw), in honey, in oilseed rape (seed), in sugar beet (leaves with top, root, whole plant) and in wheat (whole plant, grain, straw) in compliance with Guideline SANTE/2020/12830, Rev.1. LOQ for each analyte separately: 0.010 mg/kg. These LOQ correspond to a sum of 0.060 mg/kg expressed as prothioconazole-desthio and all metabolites containing the 2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl-2H- 1,2,4-triazole moiety, expressed as prothioconazole-desthio (sum of isomers)). Acceptance criteria for method validations were met, with average recoveries ranging from 70% to 110% and relative standard deviations ≤20%. The method is acceptable for the determination of prothioconazole in barley (grain, whole plant, straw), honey, oilseed rape seed, sugar beet (root, leaves with top, whole plant).

Reference:	KCP 5.1.2/21
Report:	Validation of an analytical method for the determination of prothioconazole residues in cereals, honey, oilseed rape and sugar beet. Lefresne, S., 2021 Report no.: B21S-A4-P-01, EFSA-2021-00003265, sponsor no.: 000108024
Guideline(s):	SANTE/2020/12830, Rev.1
Deviations:	None

GLP:	Yes
Acceptability:	Yes

Materials and methods

Residues of prothioconazole-desthio, 3-hydroxy-prothioconazole-desthio, 4-hydroxy-prothioconazole-desthio, 5-hydroxy-prothioconazole-desthio, 6-hydroxy-prothioconazole-desthio, alpha-hydroxy-prothioconazole-desthio, all expressed as prothioconazole-desthio (sum of isomers) were extracted from homogenised matrices by maceration with a mixture of acetonitrile/water (80:20, v/v).

An hydrolysis step was performed to convert glycoside-bound analogues into the respective hydroxy analytes. Then, extracts were purified by dispersive solid phase extraction. The quantification was performed by liquid chromatography with tandem mass spectrometry detection (LC-MS/MS).

Results and discussions

Recovery results were in a range of 70 to 110% with an RSD ≤ 20. No outliers were identified. No interference (< 30% LOQ) of total peak area for the target analytes were found in unfortified control samples. The LOQ of prothioconazole-desthio, 3-hydroxyprothioconazole-desthio expressed as prothioconazole-desthio, 4-hydroxyprothioconazole-desthio expressed as prothioconazole-desthio, 5-hydroxyprothioconazole-desthio expressed as prothioconazole-desthio, 6-hydroxyprothioconazole-desthio expressed as prothioconazole-desthio and alpha-hydroxyprothioconazole-desthio expressed as prothioconazole-desthio was 0.010 mg/kg for each analyte and for each matrix. The LOQ for the sum of all prothioconazole-items was 0.060 mg/kg for each matrix.

Table A 41: Recovery results from method validation of prothioconazole metabolites in barley grain

Fortification level	Analyte	Prothioconazole-desthio		3-hydroxy-prothioconazole-desthio		4-hydroxy-prothioconazole-desthio		5-hydroxy-prothioconazole-desthio		6-hydroxy-prothioconazole-desthio		alpha-hydroxy-prothioconazole-desthio	
[mg/kg]	Transition ion	70 m/z	125 m/z	70 m/z	141 m/z	141 m/z	70 m/z	70 m/z	141 m/z	70 m/z	141 m/z	70 m/z	141 m/z
0.010	Range	78-92	81-93	87-100	87-100	85-95	87-100	86-97	83-95	82-95	82-94	88-101	87-97
	Mean ± RSD	83 ± 6	86 ± 6	93 ± 5	91 ± 5	90 ± 4	91 ± 6	91 ± 4	89 ± 5	88 ± 5	86 ± 5	92 ±5	93 ± 4
	n	5	5	5	5	5	5	5	5	5	5	5	5
0.100	Range	86-92	84-90	89-93	86-90	88-91	85-90	89 – 94	85-89	82-89	81-87	89-94	86-92
	Mean ± RSD	89 ± 2	87 ± 2	90 ± 2	89 ± 2	89 ± 1	87 ± 2	91 ± 2	87 ± 2	86 ± 3	85 ± 3	91 ± 2	89 ± 2
	n	5	5	5	5	5	5	5	5	5	5	5	5

RSD = relative standard deviation, n = number of replicates

Table A 42: Recovery results from method validation of prothioconazole metabolites in barley straw

Fortification level	Analyte	Prothioconazole-desthio		3-hydroxy-prothioconazole-desthio		4-hydroxy-prothioconazole-desthio		5-hydroxy-prothioconazole-desthio		6-hydroxy-prothioconazole-desthio		alpha-hydroxy-prothioconazole-desthio	
[mg/kg]	Transition ion	70 m/z	125 m/z	70 m/z	141 m/z	141 m/z	70 m/z	70 m/z	141 m/z	70 m/z	141 m/z	70 m/z	141 m/z
0.010	Range	70-84	71-82	73-84	72-84	72-81	72-83	72-82	72-85	71-77	74-88	74-86	74-85
	Mean ± RSD	76± 6	76 ± 6	78 ± 5	77 ± 5	75 ± 4	76 ± 5	76 ± 5	76 ± 6	74 ± 3	79 ± 6	78 ± 5	78 ± 5
	n	5	5	5	5	5	5	5	5	5	5	5	5
0.100	Range	81-83	81-87	79-86	82-87	79-86	79-89	79 – 87	78-87	75-85	78-84	81-90	82-88
	Mean ± RSD	82 ± 1	83 ± 2	84 ± 3	85 ± 2	82 ± 4	83 ± 4	83 ± 3	82 ± 4	79 ± 5	81 ± 3	85 ± 3	84 ± 3
	n	5	5	5	5	5	5	5	5	5	5	5	5

RSD = relative standard deviation, n = number of replicates

Table A 43: Recovery results from method validation of prothioconazole metabolites in barley whole plant

Fortification level	Analyte	Prothioconazole-desthio		3-hydroxy-prothioconazole-desthio		4-hydroxy-prothioconazole-desthio		5-hydroxy-prothioconazole-desthio		6-hydroxy-prothioconazole-desthio		alpha-hydroxy-prothioconazole-desthio	
[mg/kg]	Transition ion	70 m/z	125 m/z	70 m/z	141 m/z	141 m/z	70 m/z	70 m/z	141 m/z	70 m/z	141 m/z	70 m/z	141 m/z
0.010	Range	84-99	81-101	88-97	90-97	89-95	88-102	89-98	89-99	88-98	88-95	92-102	85-98
	Mean ± RSD	90 ± 8	89 ± 8	93 ± 5	93 ± 3	92 ± 2	94 ± 5	93 ± 4	92 ± 4	93 ± 4	92 ± 3	96 ± 4	92 ± 6
	n	5	5	5	5	5	5	5	5	5	5	5	5
0.100	Range	80-93	80-95	83-94	84-96	81-95	82-97	84 – 96	82-95	82-93	84-92	84-98	84-98
	Mean ± RSD	87 ± 5	88 ± 6	90 ± 5	91 ± 5	89 ± 6	92 ± 6	90 ± 5	90 ± 6	88 ± 4	89 ± 3	92 ± 6	91 ± 5
	n	5	5	5	5	5	5	5	5	5	5	5	5

RSD = relative standard deviation, n = number of replicates

Table A 44: Recovery results from method validation of prothioconazole metabolites in honey

Fortification level	Analyte	Prothioconazole-desthio		3-hydroxy-prothioconazole-desthio		4-hydroxy-prothioconazole-desthio		5-hydroxy-prothioconazole-desthio		6-hydroxy-prothioconazole-desthio		alpha-hydroxy-prothioconazole-desthio	
[mg/kg]	Transition ion	70 m/z	125 m/z	70 m/z	141 m/z	141 m/z	70 m/z	70 m/z	141 m/z	70 m/z	141 m/z	70 m/z	141 m/z
0.010	Range	98-115	97-118	96-115	93-111	93-113	97-118	95-118	94-107	88-114	95-116	94-112	94-110
	Mean ± RSD	106 ± 6	105 ± 7	102 ± 7	99 ± 8	100 ± 7	102 ± 8	103 ± 8	99 ± 5	99 ± 9	104 ± 7	101 ± 6	101 ± 6
	n	5	5	5	5	5	5	5	5	5	5	5	5
0.100	Range	100-109	97-109	94-105	93-106	94-102	93-106	94-104	94-106	90-104	97-107	92-106	96-107
	Mean ± RSD	105 ± 4	105 ± 4	100 ± 4	99 ± 5	98 ± 3	99 ± 5	99 ± 4	100 ± 5	99 ± 5	103 ± 4	101 ± 5	102 ± 4
	n	5	5	5	5	5	5	5	5	5	5	5	5

RSD = relative standard deviation, n = number of replicates

Table A 45: Recovery results from method validation of prothioconazole metabolites in oilseed rape seeds

Fortification level	Analyte	Prothioconazole-desthio		3-hydroxy-prothioconazole-desthio		4-hydroxy-prothioconazole-desthio		5-hydroxy-prothioconazole-desthio		6-hydroxy-prothioconazole-desthio		alpha-hydroxy-prothioconazole-desthio	
[mg/kg]	Transition ion	70 m/z	125 m/z	70 m/z	141 m/z	141 m/z	70 m/z	70 m/z	141 m/z	70 m/z	141 m/z	70 m/z	141 m/z
0.010	Range	77-93	76-99	85-102	84-102	81-98	84-101	83-100	84-100	80-99	85-96	84-104	84-108
	Mean ± RSD	85 ± 8	88 ± 9	93 ± 6	92 ± 7	89 ± 7	91 ± 7	91 ± 6	92 ± 6	90 ± 7	91 ± 5	93 ± 7	92 ± 9
	n	5	5	5	5	5	5	5	5	5	5	5	5
0.100	Range	80-92	76-94	81-96	80-93	79-91	80-95	79 ± 95	79-94	78-93	77-93	81-94	82-92
	Mean ± RSD	85 ± 5	85 ± 7	87 ± 6	87 ± 5	86 ± 5	87 ± 6	87 ± 6	87 ± 6	85 ± 6	84 ± 6	88 ± 5	87 ± 4
	n	5	5	5	5	5	5	5	5	5	5	5	5

RSD = relative standard deviation, n = number of replicates

Table A 46: Recovery results from method validation of prothioconazole metabolites in sugar beet root

Fortification level	Analyte	Prothioconazole-desthio		3-hydroxy-prothioconazole-desthio		4-hydroxy-prothioconazole-desthio		5-hydroxy-prothioconazole-desthio		6-hydroxy-prothioconazole-desthio		alpha-hydroxy-prothioconazole-desthio	
[mg/kg]	Transition ion	70 m/z	125 m/z	70 m/z	141 m/z	141 m/z	70 m/z	70 m/z	141 m/z	70 m/z	141 m/z	70 m/z	141 m/z
0.010	Range	93-101	92-104	90-100	87-99	92-98	91-97	91-99	91-99	98-108	97-107	94-100	100-105
	Mean ± RSD	96 ± 3	99 ± 4	96 ± 3	95 ± 5	94 ± 2	95 ± 3	95 ± 3	96 ± 4	103 ± 3	104 ± 4	98 ± 2	103 ± 2
	n	5	5	5	5	5	5	5	3	5	5	5	3
0.100	Range	90-97	92-98	87-94	87-95	86-92	84-91	86-92	85-94	91-99	92-99	88-96	90-97
	Mean ± RSD	94 ± 3	95 ± 2	91 ± 3	90 ± 3	90 ± 3	88 ± 3	90 ± 3	95 ± 4	95 ± 3	95 ± 3	92 ± 3	94 ± 3
	n	5	5	5	5	5	5	5	3	5	5	5	3

RSD = relative standard deviation, n = number of replicates

Table A 47: Recovery results from method validation of prothioconazole metabolites in sugar beet leaves with top

Fortification level	Analyte	Prothioconazole-desthio		3-hydroxy-prothioconazole-desthio		4-hydroxy-prothioconazole-desthio		5-hydroxy-prothioconazole-desthio		6-hydroxy-prothioconazole-desthio		alpha-hydroxy-prothioconazole-desthio	
[mg/kg]	Transition ion	70 m/z	125 m/z	70 m/z	141 m/z	141 m/z	70 m/z	70 m/z	141 m/z	70 m/z	141 m/z	70 m/z	141 m/z
0.010	Range	91-98	89-101	92-101	92-100	90-104	88-103	89-106	89-104	99-114	97-112	97-104	94-104
	Mean ± RSD	95 ± 3	97 ± 5	97 ± 4	97 ± 4	97 ± 6	96 ± 6	99 ± 7	97 ± 6	105 ± 6	105 ± 6	101 ± 3	100 ± 5
	n	3	3	3	3	3	3	3	3	3	3	3	3
0.100	Range	87-92	85-89	84-88	83-86	82-88	82-88	85-88	82-88	85-92	85-96	87-90	85-88
	Mean ± RSD	89 ± 3	87 ± 2	85 ± 2	85 ± 2	86 ± 3	86 ± 3	87 ± 1	86 ± 3	89 ± 3	90 ± 5	88 ± 2	86 ± 1
	n	3	3	3	3	3	3	3	3	3	3	3	3

RSD = relative standard deviation, n = number of replicates

Table A 48: Recovery results from method validation of prothioconazole metabolites in sugar beet whole plant

Fortification level	Analyte	Prothioconazole-desthio		3-hydroxy-prothioconazole-desthio		4-hydroxy-prothioconazole-desthio		5-hydroxy-prothioconazole-desthio		6-hydroxy-prothioconazole-desthio		alpha-hydroxy-prothioconazole-desthio	
[mg/kg]	Transition ion	70 m/z	125 m/z	70 m/z	141 m/z	141 m/z	70 m/z	70 m/z	141 m/z	70 m/z	141 m/z	70 m/z	141 m/z
0.010	Range	83-92	81-89	82-91	81-89	79-86	82-86	85-89	82-89	95-104	93-101	83-90	83-91
	Mean ± RSD	87 ± 4	86 ± 4	87 ± 5	86 ± 4	82 ± 3	84 ± 2	87 ± 2	86 ± 3	101 ± 4	98 ± 4	87 ± 3	88 ± 4
	n	3	3	3	3	3	3	3	3	3	3	3	3
0.100	Range	92-99	91-94	84-90	86-89	84-88	83-89	86-91	84-90	90-96	88-94	85-91	85-92
	Mean ± RSD	95 ± 3	92 ± 1	87 ± 3	88 ± 2	86 ± 2	87 ± 3	89 ± 3	87 ± 3	93 ± 2	91 ± 2	88 ± 3	89 ± 3
	n	3	3	3	3	3	3	3	3	3	3	3	3

RSD = relative standard deviation, n = number of replicates

Table A 49: Recovery results from method validation of prothioconazole metabolites in wheat grain

Fortification level	Analyte	Prothioconazole-desthio		3-hydroxy-prothioconazole-desthio		4-hydroxy-prothioconazole-desthio		5-hydroxy-prothioconazole-desthio		6-hydroxy-prothioconazole-desthio		alpha-hydroxy-prothioconazole-desthio	
[mg/kg]	Transition ion	70 m/z	125 m/z	70 m/z	141 m/z	141 m/z	70 m/z	70 m/z	141 m/z	70 m/z	141 m/z	70 m/z	141 m/z
0.010	Range	79-89	79-85	93-103	90-100	91-102	90-101	89-100	93-99	85-93	83-94	91-101	88-98
	Mean ± RSD	85 ± 5	82 ± 3	99 ± 4	96 ± 1	96 ± 5	96 ± 5	96 ± 5	96 ± 2	90 ± 4	90 ± 5	98 ± 4	85 ± 5
	n	3	3	3	3	3	3	3	3	3	3	3	3
0.100	Range	86-92	84-87	95-101	95-97	93-95	93-95	91-97	96-98	91-92	89-92	98-101	92-100
	Mean ± RSD	88 ± 3	85 ± 2	99 ± 2	96 ± 1	94 ± 1	95 ± 1	95 ± 3	97 ± 1	92 ± 0.2	90 ± 2	100 ± 1	95 ± 4
	n	3	3	3	3	3	3	3	3	3	3	3	3

RSD = relative standard deviation, n = number of replicates

Table A 50: Recovery results from method validation of prothioconazole metabolites in wheat straw

Fortification level	Analyte	Prothioconazole-desthio		3-hydroxy-prothioconazole-desthio		4-hydroxy-prothioconazole-desthio		5-hydroxy-prothioconazole-desthio		6-hydroxy-prothioconazole-desthio		alpha-hydroxy-prothioconazole-desthio	
[mg/kg]	Transition ion	70 m/z	125 m/z	70 m/z	141 m/z	141 m/z	70 m/z	70 m/z	141 m/z	70 m/z	141 m/z	70 m/z	141 m/z
0.010	Range	69-75	66-71	78-83	75-84	74-75	74-76	74-78	75-82	74-90	74-93	78-87	75-89
	Mean ± RSD	71 ± 5	69 ± 4	80 ± 3	80 ± 5	74 ± 1	75 ± 2	77 ± 3	80 ± 5	82 ± 10	85 ± 12	83 ± 6	84 ± 9
	n	3	3	3	3	3	3	3	3	3	3	3	3
0.100	Range	83-84	81-83	83-85	79-85	75-84	75-87	78-86	78-88	80-85	81-83	81-90	82-87
	Mean ± RSD	84 ± 1	82 ± 1	84 ± 1	83 ± 3	81 ± 5	83 ± 6	83 ± 4	84 ± 5	81 ± 3	82 ± 1	85 ± 4	85 ± 3
	n	3	3	3	3	3	3	3	3	3	3	3	3

RSD = relative standard deviation, n = number of replicates

Table A 51: Recovery results from method validation of prothioconazole metabolites in wheat whole plant

Fortification level	Analyte	Prothioconazole-desthio		3-hydroxy-prothioconazole-desthio		4-hydroxy-prothioconazole-desthio		5-hydroxy-prothioconazole-desthio		6-hydroxy-prothioconazole-desthio		alpha-hydroxy-prothioconazole-desthio	
[mg/kg]	Transition ion	70 m/z	125 m/z	70 m/z	141 m/z	141 m/z	70 m/z	70 m/z	141 m/z	70 m/z	141 m/z	70 m/z	141 m/z
0.010	Range	81-86	76-86	80-88	81-89	80-87	78-89	80-90	77-86	80-89	80-87	81-86	79-89
	Mean ± RSD	83 ± 3	82 ± 5	84 ± 4	85 ± 4	83 ± 4	82 ± 6	84 ± 5	81 ± 5	83 ± 5	83 ± 4	83 ± 3	83 ± 5
	n	3	3	3	3	3	3	3	3	3	3	3	3
0.100	Range	97-100	94-103	96-108	97-107	94-106	95-102	94-105	95-106	94-106	90-100	97-106	96-106
	Mean ± RSD	98 ± 1	97 ± 4	100 ± 5	101 ± 5	99 ± 5	98 ± 3	99 ± 5	99 ± 5	99 ± 5	94 ± 4	100 ± 4	100 ± 4
	n	3	3	3	3	3	3	3	3	3	3	3	3

RSD = relative standard deviation, n = number of replicates

Table A 52: Characteristics for the analytical method used for validation of prothioconazole residues in cereals, oilseed rape, honey and sugar beet

	Prothioconazole*
Specificity	Blank value < 30% LOQ
Calibration (type, number of data points)	Individual calibration data presented r > 0.99 7 calibration points
Calibration range	0.3 to 20 µg/L for barley (straw, whole plant), honey, oilseed rape (seed) and wheat (straw, whole plant) 0.75 to 50 µg/L for barley (grain), sugar beet (root, leaves with top, whole plant) and wheat (grain)
Assessment of matrix effects is presented	Yes, however, matrix-matched standard solutions were used for calibration.
Limit of quantification	For each analyte separately: LOQ: 0.010 mg/kg For prothioconazole as the sum of all analytes: LOQ: 0.060 mg/kg

* Including: prothioconazole-desthio, 3-hydroxyprothioconazole-desthio, 4-hydroxyprothioconazole-desthio, 5-hydroxyprothioconazole-desthio, 6-hydroxyprothioconazole-desthio and alpha-hydroxyprothioconazole-desthio

Conclusion

The method fulfils the requirements of SANTE/2020/12830, Rev.1 and is suitable for the determination of prothioconazole in barley (grain, whole plant, straw), honey, oilseed rape seed, sugar beet (root, leaves with top, whole plant).

The following study provides the method validation for:

- Yozgatli, H.P., 2021b, KCA 6.3.1/02,
- Yozgatli, H.P., 2021c, KCA 6.3.1/04,
- Yozgatli, H.P., 2021d, KCA 6.3.1/06,
- Le Mineur, A., 2022, KCA 6.3.1/07,
- Yozgatli, H.P., 2021e, KCA 6.3.2/02,
- Yozgatli, H.P., 2021f, , KCA 6.3.2/04,
- Yozgatli, H.P., 2021g, , KCA 6.3.2/06,
- Mahlow, S., 2021, KCA 6.3.2/08,
- Yozgatli, H.P., 2021h, , KCA 6.3.2/10,
- Huaulmé, J.-M., 2021b, KCA 6.3.2/11,
- Huaulmé, J.-M., 2022, KCA 6.3.2/12,
- Gustloff, C., 2021, KCA 6.3.3/02,
- Ivanov, E., 2021a, KCA 6.3.3/04,
- Ivanov, E., 2021b, KCA 6.3.3/06,
- Amic, S., 2021, KCA 6.3.3/07.

Comments of zRMS:	The analytical method based on the method GRM053.01A was validated for the determination of of triazole metabolites (TDMs) 1,2,4-Triazole (1,2,4-T), Triazole alanine (TA), Triazole acetic acid (TAA) and Triazole lactic acid (TLA) in/on wheat (whole plant, grain and straw), barley (whole plant, grain and straw), oilseed rape (seeds, crude oil, refined oil and pressed cake), sunflower (seeds) and sugar beet (leaves with top and roots). The limit of quantification (LOQ) of the analytical method was 0.01 mg/kg for each analyte and each matrix with a limit of detection (LOD) set at 0.003 mg/kg (30 % of the LOQ). Acceptance criteria for method validations were met, with average recoveries ranging from 70% to 110% and relative standard deviations ≤20%. In accordance with SANTE/2020/12830, Rev.1, there should be 5 recoveries at each level (LOQ and 10x LOQ), in the performed study only 3 recovery are presented, However, the analytical method is acceptable and suitable for determination of residues of triazole and metabolites, in wheat, barley, oilseed rape, sunflower and sugar beet.

Reference:	KCP 5.1.2/22
Report:	Validation of an analytical method for the determination of triazole metabolites (TDMs) in crop matrices of season 2021 Gustloff, C.; Wallbaum, P., 2021 Report no.: S21-02262, sponsor no.: 000107909
Guideline(s):	SANTE/2020/12830, Rev.1
Deviations:	A reduced recovery sample set was conducted. For a full validation, reference is made to the peer review of the triazole derivative metabolites (TDMs) in the light of confirmatory data submitted (UK, 2018; EFSA, 2018, amended 2019).
GLP:	Yes
Acceptability:	Yes

Materials and methods

Specimens were extracted with methanol/water (4/1, v/v). After filtration and evaporation to the aqueous remainder, the volume was adjusted with ultra-pure water. After sonication, final determination of triazole metabolites took place with LC-MS/MS (for validation samples and for storage samples up until the 18 months storage time point) or with LC-DMS-MS/MS.

The present validation is a top up reduced validation to ensure continued performance of the method. The analytical method was fully validated in a separated study (GRM053.01A1). In Appendix A-B of the peer review of the triazole derivative metabolites (TDMs) in the light of confirmatory data submitted (UK, 2018; EFSA, 2018, amended 2019), the study was summarised. However, the study can be provided upon request.

Results and discussions

Recovery results were in a range of 70 to 110 % with an RSD ≤ 20 (except for the determination of triazole acetic acid in oilseed rape pressed cake at 0.01 mg/kg, which is regarded as not relevant for the validity of this study). No outliers were identified. No interference (< 30 % LOQ) of total peak area for the target analytes were found in unfortified control samples. The LOQ of triazole metabolites was 0.010 mg/kg for each analyte and for each matrix. The LOQ for the sum of all triazole metabolite items was 0.04 mg/kg for each matrix.

Table A 53: Recovery results from method validation of triazole metabolites in wheat whole plant

Fortification level [mg/kg]	Analyte	1,2,4-Triazole		Triazole alanine		Triazole acetic acid		Triazole lactic acid	
	Column type*	Hypercarb column	Synergi column	Hypercarb column	Synergi column	Hypercarb column	Synergi column	Hypercarb column	Synergi column
0.010	Range	106-118	87-103	102-105	108-117	82-92	108-117	75-95	102-124
	Mean ± RSD	109 ± 7.2	97 ± 9	103 ± 1.5	113 ± 4	88 ± 6.4	113 ± 4.1	87 ± 13	116 ± 10
	n	3	3	3	3	3	3	3	3
0.100	Range	92-119	87-106	99-113	98-112	84-96	108-115	92 – 94	80-110
	Mean ± RSD	107 ± 13	99 ± 10	105 ± 6.9	104 ± 7	90 ± 6.7	111 ± 2.8	93 ± 0.7	94 ± 16
	n	3	3	3	3	3	3	3	3

RSD = relative standard deviation, n = number of replicates

*Hypercarb column is used for quantification and Synergi column for confirmation

Table A 54: Recovery results from method validation of triazole metabolites in wheat grain

Fortification level [mg/kg]	Analyte	1,2,4-Triazole		Triazole alanine		Triazole acetic acid		Triazole lactic acid	
	Column type*	Hypercarb column	Synergi column	Hypercarb column	Synergi column	Hypercarb column	Synergi column	Hypercarb column	Synergi column

[1] Gemrot F. Triazole Metabolites: Residue Method for the Determination of 1,2,4-Triazole, Triazole alanine, Triazole Acetic Acid and Triazole Lactic Acid in Crops, GRM053.01A

0.010	Range	102-113	113-118	93-98	78-98	77-88	89-104	72-75	78-103
	Mean ± RSD	107 ± 4.9	116 ± 2.3	96 ± 2.3	87 ± 12	84 ± 7.2	98 ± 8.1	73 ± 2.1	92 ± 14
	n	3	3	3	3	3	3	3	3
0.100	Range	101-115	75-104	91-95	82-93	60-75	80-100	72 – 86	75-97
	Mean ± RSD	103 ± 2.2	94 ± 17	93 ± 2.0	88 ± 6.6	70 ± 12	87 ± 13	79 ± 8.6	85 ± 13
	n	3	3	3	3	3	3	3	3

RSD = relative standard deviation, n = number of replicates

*Hypercarb column is used for quantification and Synergi column for confirmation

Table A 55: Recovery results from method validation of triazole metabolites in wheat straw

Fortification level [mg/kg]	**Analyte**	**1,2,4-Triazole**		**Triazole alanine**		**Triazole acetic acid**		**Triazole lactic acid**	
	Column type*	Hypercarb column	Synergi column	Hypercarb column	Synergi column	Hypercarb column	Synergi column	Hypercarb column	Synergi column
0.010	Range	83-106	78-105	88-114	73-102	74-84	78-92	74-89	84-114
	Mean ± RSD	95 ± 12	90 ± 16	99 ± 14	86 ± 16	78 ± 67	86 ± 8.6	82 ± 9.3	96 ± 16
	n	3	3	3	3	3	3	3	3
0.100	Range	110-111	86-112	93-96	94-97	80-82	71-101	82 – 85	76-88
	Mean ± RSD	110 ± 0.8	101 ± 13	95 ± 1.9	95 ± 1.8	82 ± 1.4	90 ± 19	84 ± 1.7	82 ± 7.0
	n	3	3	3	3	3	3	3	3

RSD = relative standard deviation, n = number of replicates

*Hypercarb column is used for quantification and Synergi column for confirmation

Table A 56: Recovery results from method validation of triazole metabolites in barley whole plant

Fortification level [mg/kg]	**Analyte**	**1,2,4-Triazole**		**Triazole alanine**		**Triazole acetic acid**		**Triazole lactic acid**	
	Column type*	Hypercarb column	Synergi column	Hypercarb column	Synergi column	Hypercarb column	Synergi column	Hypercarb column	Synergi column
0.010	Range	84-95	73-74	95-108	97-129	92-109	85-109	98-104	91-104
	Mean ± RSD	90 ± 6.4	74 ± 0.7	104 ± 6.9	109 ± 16	99 ± 9.6	99 ± 12	100 ± 3.4	96 ± 6.9
	n	3	3	3	3	3	3	3	3
0.100	Range	92-115	103-119	94-110	93-107	86-88	104-119	98 – 102	87-103
	Mean ± RSD	102 ± 11	109 ± 8.1	102 ± 7.7	101 ± 7.6	87 ± 1.5	113 ± 7.0	99 ± 1.2	95 ± 8.0
	n	3	3	3	3	3	3	3	3

RSD = relative standard deviation, n = number of replicates

*Hypercarb column is used for quantification and Synergi column for confirmation

Table A 57: Recovery results from method validation of triazole metabolites in barley grain

Fortification level [mg/kg]	**Analyte**	**1,2,4-Triazole**		**Triazole alanine**		**Triazole acetic acid**		**Triazole lactic acid**	
	Column type*	Hypercarb column	Synergi column	Hypercarb column	Synergi column	Hypercarb column	Synergi column	Hypercarb column	Synergi column
0.010	Range	92-110	80-112	80-113	87-112	79-90	91-103	81-83	80-96
	Mean ± RSD	99 ± 10	101 ± 18	95 ± 17	100 ± 12	85 ± 6.5	95 ± 7.1	82 ± 1.5	86 ± 11
	n	3	3	3	3	3	3	3	3
0.100	Range	85-118	92-115	86-97	78-94	83-97	89-104	81 – 92	83-90
	Mean ± RSD	101 ± 16	106 ± 12	90 ± 6.4	84 ± 9.8	89 ± 8.0	96 ± 7.8	86 ± 6.8	87 ± 4.1
	n	3	3	3	3	3	3	3	3

RSD = relative standard deviation, n = number of replicates

*Hypercarb column is used for quantification and Synergi column for confirmation

Table A 58: Recovery results from method validation of triazole metabolites in barley straw

Fortification level	**Analyte**	**1,2,4-Triazole**	**Triazole alanine**	**Triazole acetic acid**	**Triazole lactic acid**

[mg/kg]	Column type*	Hypercarb column	Synergi column	Hypercarb column	Synergi column	Hypercarb column	Synergi column	Hypercarb column	Synergi column
0.010	Range	76-88	90-116	89-106	107-113	97-119	91-98	93-102	77-82
	Mean ± RSD	82 ± 7.6	101 ± 14	96 ± 9.2	110 ± 3.0	109 ± 11	94 ± 4.1	97 ± 4.6	80 ± 3.6
	n	3	3	3	3	3	3	3	3
0.100	Range	98-110	96-110	85-100	81-98	97-112	96-121	97 – 107	86-91
	Mean ± RSD	137 ± 5.9	102 ± 6.9	94 ± 8.9	89 ± 9.6	104 ± 7.1	109 ± 11	102 ± 5.1	88 ± 2.9
	n	3	3	3	3	3	3	3	3

RSD = relative standard deviation, n = number of replicates

*Hypercarb column is used for quantification and Synergi column for confirmation

Table A 59: Recovery results from method validation of triazole metabolites in oilseed rape seeds

Fortification level [mg/kg]	Analyte	1,2,4-Triazole		Triazole alanine		Triazole acetic acid		Triazole lactic acid	
	Column type*	Hypercarb column	Synergi column	Hypercarb column	Synergi column	Hypercarb column	Synergi column	Hypercarb column	Synergi column
0.010	Range	82-96	99-118	87-113	84-103	81-96	96-104	71-97	85-89
	Mean ± RSD	89 ± 7.9	106 ± 9.4	96 ± 15	96 ± 11	88 ± 8.6	99 ± 4.2	86 ± 16	87 ± 2.8
	n	3	3	3	3	3	3	3	3
0.100	Range	99-109	71-101	78-91	74-95	92-99	95-107	88 – 91	89-103
	Mean ± RSD	104 ± 5.2	88 ± 17	87 ± 8.6	84 ± 12	94 ± 4.0	99 ± 7.0	87 ± 2.8	95 ± 7.3
	n	3	3	3	3	3	3	3	3

RSD = relative standard deviation, n = number of replicates

*Hypercarb column is used for quantification and Synergi column for confirmation

Table A 60: Recovery results from method validation of triazole metabolites in oilseed rape crude oil

Fortification level [mg/kg]	Analyte	1,2,4-Triazole		Triazole alanine		Triazole acetic acid		Triazole lactic acid	
	Column type*	Hypercarb column	Synergi column	Hypercarb column	Synergi column	Hypercarb column	Synergi column	Hypercarb column	Synergi column
0.010	Range	83-98	84-88	82-102	88-118	91-95	86-89	84-98	88-90
	Mean ± RSD	88 ± 9.7	86 ± 2.2	95 ± 12	105 ± 15	93 ± 2.4	87 ± 1.8	92 ± 8.0	89 ± 1.0
	n	3	3	3	3	3	3	3	3
0.100	Range	100-108	78-97	91-99	90-99	93-97	93-97	89 – 99	93-101
	Mean ± RSD	103 ± 4.5	91 ± 12	96 ± 4.29	95 ± 4.9	94 ± 2.2	95 ± 2.0	95 ± 5.5	98 ± 4.3
	n	3	3	3	3	3	3	3	3

RSD = relative standard deviation, n = number of replicates

*Hypercarb column is used for quantification and Synergi column for confirmation

Table A 61: Recovery results from method validation of triazole metabolites in oilseed rape refined oil

Fortification level [mg/kg]	Analyte	1,2,4-Triazole		Triazole alanine		Triazole acetic acid		Triazole lactic acid	
	Column type*	Hypercarb column	Synergi column	Hypercarb column	Synergi column	Hypercarb column	Synergi column	Hypercarb column	Synergi column
0.010	Range	90-104	77-85	81-92	82-90	97-110	88-102	100-108	98-103
	Mean ± RSD	95 ± 8.3	81 ± 4.5	86 ± 6.5	87 ± 5.2	102 ± 6.8	94 ± 7.8	103 ± 4.0	99 ± 3.0
	n	3	3	3	3	3	3	3	3
0.100	Range	86-100	84-99	83-87	85-86	83-94	78-83	91 – 93	86-98
	Mean ± RSD	93 ± 7.4	90 ± 8.4	85 ± 2.3	85 ± 0.7	90 ± 6.3	81 ± 3.5	92 ± 1.1	90 ± 7.5
	n	3	3	3	3	3	3	3	3

RSD = relative standard deviation, n = number of replicates

*Hypercarb column is used for quantification and Synergi column for confirmation

Table A 62: Recovery results from method validation of triazole metabolites in oilseed rape pressed cake

Fortification level [mg/kg]	Analyte	1,2,4-Triazole		Triazole alanine		Triazole acetic acid		Triazole lactic acid	
	Column type*	Hypercarb column	Synergi column	Hypercarb column	Synergi column	Hypercarb column	Synergi column	Hypercarb column	Synergi column
0.010	Range	103-118	105-116	100-101	111-118	91-108	89-97	61-106	70-96
	Mean ± RSD	111 ± 6.8	110 ± 4.8	101 ± 0.9	116 ± 3.7	100 ± 8.2	94 ± 5.0	84 ± 27	82 ± 16
	n	3	3	3	3	3	3	3	3
0.100	Range	81-94	78-106	103-108	101-113	78-104	78-106	78 – 106	99-103
	Mean ± RSD	89 ± 7.8	90 ± 16	106 ± 2.5	107 ± 5.6	94 ± 14	94 ± 19	96 ± 16	101 ±1.8
	n	3	3	3	3	3	3	3	3

RSD = relative standard deviation, n = number of replicates

*Hypercarb column is used for quantification and Synergi column for confirmation

Table A 63: Recovery results from method validation of triazole metabolites in sunflower seeds

Fortification level [mg/kg]	Analyte	1,2,4-Triazole		Triazole alanine		Triazole acetic acid		Triazole lactic acid	
	Column type*	Hypercarb column	Synergi column	Hypercarb column	Synergi column	Hypercarb column	Synergi column	Hypercarb column	Synergi column
0.010	Range	103-116	83-99	94-104	84-105	83-100	99-117	95-106	78-101
	Mean ± RSD	110 ± 5.7	92 ± 9.4	100 ± 5.1	98 ± 12	94 ± 10	109 ± 8.3	100 ± 6.1	87 ± 15
	n	3	3	3	3	3	3	3	3
0.100	Range	94-97	81-120	81-96	80-100	80-100	86-94	89 – 96	86-108
	Mean ± RSD	95 ± 1.8	102 ± 19	87 ± 9.7	88 ± 12	88 ± 12	86 ± 9.1	92 ± 3.9	95 ± 12
	n	3	3	3	3	3	3	3	3

RSD = relative standard deviation, n = number of replicates

*Hypercarb column is used for quantification and Synergi column for confirmation

Table A 64: Recovery results from method validation of triazole metabolites in sugar beet leaves with tops

Fortification level [mg/kg]	Analyte	1,2,4-Triazole		Triazole alanine		Triazole acetic acid		Triazole lactic acid	
	Column type*	Hypercarb column	Synergi column	Hypercarb column	Synergi column	Hypercarb column	Synergi column	Hypercarb column	Synergi column
0.010	Range	92-105	89-104	107-120	117-120	105-106	85-109	107-113	82-100
	Mean ± RSD	99 ± 6.7	97 ± 7.8	115 ± 6.1	119 ± 1.1	104 ± 3.2	96 ± 13	110 ± 2.8	92 ± 10
	N	3	3	3	3	3	3	3	3
0.100	Range	88-102	95-113	106-112	105-116	86-95	97-110	99 – 106	83-103
	Mean ± RSD	93 ± 8.6	103 ± 9.0	108 ± 3.1	110 ± 5.0	91 ± 5.1	101 ± 7.1	102 ± 3.9	94 ±11
	n	3	3	3	3	3	3	3	3

RSD = relative standard deviation, n = number of replicates

*Hypercarb column is used for quantification and Synergi column for confirmation

Table A 65: Recovery results from method validation of triazole metabolites in sugar beet roots

Fortification level [mg/kg]	Analyte	1,2,4-Triazole		Triazole alanine		Triazole acetic acid		Triazole lactic acid	
	Column type*	Hypercarb column	Synergi column	Hypercarb column	Synergi column	Hypercarb column	Synergi column	Hypercarb column	Synergi column
0.010	Range	95-114	81-97	100-119	106-112	96-103	92-105	101-109	87-99
	Mean ± RSD	104 ± 8.7	87 ± 9.5	108 ± 9.0	110 ± 3.0	99 ± 3.3	100 ± 7.3	105 ± 3.9	93 ± 6.4
	n	3	3	3	3	3	3	3	3

0.100	Range	84-103	97-115	99-116	101-112	89-109	94-102	99 – 104	90-109
	Mean ± RSD	94 ± 10	104 ± 9.3	108 ± 7.9	106 ± 5.7	97 ± 11	99 ± 4.0	102 ± 6.4	100 ±9.5
	n	3	3	3	3	3	3	3	3

RSD = relative standard deviation, n = number of replicates

*Hypercarb column is used for quantification and Synergi column for confirmation

Table A 66: Characteristics for the analytical method used for validation of Triazole metabolites residues in wheat, barley, oilseed rape, sunflower and sugar beet

	Triazole metabolites *
Specificity	Blank value < 30% LOQ
Calibration (type, number of data points)	Individual calibration data presented r > 0.99 > 5 calibration points
Calibration range	0.3 to 30 µg/L corresponding to 0.003 to 0.3 mg/kg
Assessment of matrix effects is presented	Isotopically labelled internal standards were used for quantification so that possible matrix effects on determination are automatically accounted for when using the response ratio of analyte and internal standard for quantification. Therefore, matrix effects on detection were not determined within this study.
Limit of quantification	For each analyte separately: LOQ: 0.010 mg/kg For triazole metabolites as the sum of all analytes: LOQ: 0.040 mg/kg

* Including: 1,2,4-Triazole, Triazole alanine, Triazole acetic acid, Triazole lactic acid

Conclusion

The method fulfils the requirements of SANTE/2020/12830, Rev.1 and is suitable for the determination of Triazole metabolites in wheat, barley, oilseed rape, sunflower and sugar beet.

The following study provides the method validation for KCA 6.10.1/01 (Persigehl, M. et al., 2021, report no.: P19010-3, sponsor no.: 000102470), KCA 6.10.1/02 (Persigehl, M. et al., 2021, report no.: P19010-4, sponsor no.: 000102471), KCA 6.10.1/03 (Persigehl, M. et al., 2020, report no.: P19010-1, Sponsor no.: 000102468) and KCA 6.10.1/04 (Persigehl, M. et al., 2021, report no.: P19010-2, Sponsor no.: 000102469).

Comments of zRMS:	The analytical method was validated for the determination of prothioconazole and prothioconazole-desthio in nectar, pollen, flowers and honey according to the guidance documents SANCO/825/00, rev 8.1 and SANCO/3029/99/00, rev. 4. The LOQ was established at 0.01 mg/kg in nectar, pollen, flower and honey for the two mass transitions. Acceptance criteria for method validations were met, with average recoveries ranging from 70% to 110% and relative standard deviations ≤20%. The method is acceptable for the determination of prothioconazole and prothioconazole-desthio in nectar, pollen and flowers and honey.

Reference:	KCP 5.1.2/23
Report:	Validation of an Analytical Method for the Determination of Prothioconazole, Prothioconazole-desthio and Azoxystrobin in Nectar, Pollen, Flower and Honey, Lindner, M.,Grewe, D. 2020, report no.: S19-20860 (MAC-1940V), sponsor no.: 000104134
Guideline(s):	SANCO/3029/99 rev. 4
Deviations:	None relevant (for details see point E. “Study plan deviations”)
GLP:	Yes (certified laboratory)
Acceptability/Reliability:	Yes

Duplication (if vertebrate study)	Not applicable

Materials and methods

The test matrix (phacelia flowers, pollen from pollen traps and nectar from forager bee) was diluted with methanol/L-cystein-solution/formic acid (50+50+0.5, v+v+v). After shaking and centrifugation for 15 min the sample solution was analysed by LC-MS/MS.

Results and discussions

Recovery results were in a range of 70 – 85% with an RSD ≤ 8.4% for prothioconazole and, in a range of 80 – 110% with an RSD ≤ 9.6% for prothioconazole-desthio. No outliers were identified. No interference (< 30% LOQ) of total peak area for the target analytes were found in unfortified control samples. The LOQ was set at 0.01 mg/kg for prothioconazole and prothioconazole-desthio.

Table A 67: Recovery results from method validation of prothioconazole, prothioconazole-desthio ~~and azoxystrobin~~ using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
Nectar	Prothioconazole	0.01	76	8.3	-
		0.1	82	6.9	-
Pollen		0.01	76	8.4	-
		0.1	72	3.8	-
Flowers		0.01	70	4.5	-
		0.1	78	3.7	-
Honey		0.01	75	5.8	-
		0.1	85	2.4	-
Nectar	Prothioconazole-desthio	0.01	82	2.4	-
		0.1	88	2.6	-
Pollen		0.01	110	8.1	-
		0.1	97	2.9	-
Flowers		0.01	103	2.8	-
		0.1	100	2.3	-
Honey		0.01	82	9.6	-
		0.1	80	2.9	-

Table A 68: Characteristics for the analytical method used for validation of prothioconazole and prothioconazole-desthio in nectar, pollen and flowers and honey

	prothioconazole	prothioconazole-desthio
Specificity	blank value < 30 % LOQ	blank value < 30 % LOQ
Calibration (type, number of data points)	individual calibration data presented| calibration line equation presented	individual calibration data presented| calibration line equation presented
Calibration range	0.025–2.0 ng /mL (0.0025 mg/kg-0.20 mg/kg) r > 0.999 ≤ 7 calibration points	0.025–2.0 ng /mL r > 0.999 ≤ 7 calibration points
Assessment of matrix effects is presented	Yes	Yes
Limit of quantification	0.01 mg/kg	0.01 mg/kg
Limit of detection	0.003 mg/kg	0.003 mg/kg

Conclusion
The method fulfils the requirements of SANCO/3029/99 rev. 4 and is suitable for the determination of prothioconazole and prothioconazole-desthio in nectar, pollen and flowers and honey.

The following study provides also the method validation for Semrau, J. 2022 (KCA 6.6.2/02, report no.: S21-00408, sponsor no.: 000107470.

Comments of zRMS:	In the analytical phase S18-02513-L2 of this study samples of radish (leaves and roots), leaf lettuce (leaves) and barley (whole plant, grain and straw) were analysed for residues of prothioconazole-desthio (sum of isomers of PTZ-desthio, PTZ-3-; -4-; -5-; and -6-hydroxy desthio and alpha-hydroxy-PTZ-desthio, each expressed as PTZ-desthio). In addition, samples of soil were analysed for residues of prothioconazole-desthio. Sample extraction and determination of residues in the matrices radish (leaves and roots), barley (grain, straw and whole plant) and lettuce (leaves) were performed according to the GIRPA Method R-3965 based on the multi-residue method QuEChERS that was validated within this analytical phase for the matrices radish (roots), barley (grain and straw) and lettuce (leaves) according to SANCO/3029/99, rev. 4. For the analysis of soil, sample extraction and determination of residues were performed according to the multi-residue method QuEChERS that was also validated within this analytical phase according to SANCO/3029/99, rev. 4. Quantification was performed by use of LC-MS/MS detection for all analytes and matrices. The limit of quantification (LOQ) of both analytical methods was 0.01 mg/kg (expressed as prothioconazoledesthio) for each analyte and each matrix The mean recoveries at each fortification level were in the range of 70 – 110% with relative standard deviation(s) below 20% for all combinations of matrices and analytes. The method is acceptable for the determination of prothioconazole radish, lettuce, barley, and soil.

Reference:	KCP 5.1.2/24 (filed in KCA 6.6.2/01)
Report:	Determination of Residues of Prothioconazole and its Metabolites after One Application of MCW-2073 on Bare Soil in Rotational Crops (Radish, Leaf lettuce and Barley) at 2 Sites in Northern Europe and 2 Sites in Southern Europe 2018/2019, Semrau, J., 2021 Report no.: S18-02513, sponsor no.: R-39638
Guideline(s):	For method validation: SANTE/2020/12830, Rev.1
Deviations:	None
GLP:	Yes
Acceptability:	Yes

Materials and methods
The analytical method is based on European Committee for Standardization (CEN): EN 15662:2009-02, paragraph 8 – QuEChERS-method. Residues of prothioconazole (sum of prothioconazole-desthio, 3-hydroxy-prothioconazoledesthio, 4-hydroxy-prothioconazole-desthio, 5-hydroxy-prothioconazole-desthio, 6-hydroxyprothioconazole-desthio and alpha-hydroxy-prothioconazole-desthio, expressed as prothioconazole-desthio) were extracted from homogenised matrices by maceration with acetonitrile/water. Then, extracts were purified by dispersive solid phase extraction. The quantification was performed by LC-MS/MS (QuEChERS-method) with two mass transitions. The analytical method was fully validated during the course of other studies for oilseed rape (whole plants, seeds and straw) according to guideline SANCO/3029/99 rev. 4:

Results and discussions
Recovery results were in a range of 70 to 110% with an RSD ≤ 20. No outliers were identified. No interference (< 30% LOQ) of total peak area for the target analytes were found in unfortified control samples. The LOQ of prothioconazole-desthio, 3-hydroxyprothioconazole-desthio expressed as prothioconazole-desthio, 4-hydroxyprothioconazole-desthio expressed as prothioconazole-desthio, 5-

hydroxyprothioconazole-desthio expressed as prothioconazole-desthio, 6-hydroxyprothioconazole-desthio expressed as prothioconazole-desthio and alpha-hydroxyprothioconazole-desthio expressed as prothioconazole-desthio was 0.010 mg/kg for each analyte and for each matrix. The LOQ for the sum of all prothioconazole-items was 0.060 mg/kg for each matrix.

Table A 69: Recovery results from method validation of prothioconazole metabolites in radish roots

Fortification level	Analyte	Prothioconazole-desthio		3-hydroxy-prothioconazole-desthio		4-hydroxy-prothioconazole-desthio		5-hydroxy-prothioconazole-desthio		6-hydroxy-prothioconazole-desthio		alpha-hydroxy-prothioconazole-desthio	
[mg/kg]	Transition ion	70 m/z	125 m/z	70 m/z	141 m/z	141 m/z	70 m/z	70 m/z	141 m/z	70 m/z	141 m/z	70 m/z	141 m/z
0.010	Range	85-107	88-107	80-86	82-85	79-89	80-93	74-83	72-79	79-89	80-96	72-78	74-79
	Mean ± RSD	96 ± 9.8	100 ± 8.0	83 ± 3.1	83 ± 1.5	84 ± 5.1	85 ± 6.5	78 ± 5.0	77 ± 3.7	86 ± 4.6	88 ± 7.4	76 ±3.3	77 ± 2.4
	n	5	5	5	5	5	5	5	5	5	5	5	5
0.100	Range	95-101	99-104	86-91	82-89	79-89	79-87	82 – 90	80-86	88-92	84-90	77-82	76-80
	Mean ± RSD	98 ± 2.6	102 ± 1.9	88 ± 2.5	86 ± 3.6	85 ± 4.8	83 ± 4.1	87 ± 3.5	84 ± 3.0	90 ± 1.8	87 ± 2.7	79 ± 2.6	77 ± 3.1
	n	5	5	5	5	5	5	5	5	5	5	5	5

RSD = relative standard deviation, n = number of replicates

Table A 70: Recovery results from method validation of prothioconazole metabolites in lettuce leaves

Fortification level	Analyte	Prothioconazole-desthio		3-hydroxy-prothioconazole-desthio		4-hydroxy-prothioconazole-desthio		5-hydroxy-prothioconazole-desthio		6-hydroxy-prothioconazole-desthio		alpha-hydroxy-prothioconazole-desthio	
[mg/kg]	Transition ion	70 m/z	125 m/z	70 m/z	141 m/z	141 m/z	70 m/z	70 m/z	141 m/z	70 m/z	141 m/z	70 m/z	141 m/z
0.010	Range	97-105	98-104	70 m/z	83-90	73-90	77-88	75-79	76-84	86-100	91-97	78-87	78-90
	Mean ± RSD	99± 3.4	100 ± 4.0	77-87	87 ± 3.4	81 ± 8.2	83 ± 5.5	76 ± 2.2	81 ± 4.3	92 ± 5.6	94 ± 3.0	82 ± 4.4	86 ± 6.0
	n	5	5	80 ± 4.9	5	5	5	5	5	5	5	5	5
0.100	Range	99-112	103-114	5	92-95	80-83	79-85	86 – 92	87-91	102-111	93-101	83-87	87-92
	Mean ± RSD	106 ± 5.1	108 ± 4.0	92-98	94 ± 1.2	81 ± 1.6	82 ± 3.2	88 ± 2.6	89 ± 2.6	107 ± 3.3	97 ± 3.3	86 ± 1.8	90 ± 2.4
	n	5	5	94 ± 2.7	5	5	5	5	5	5	5	5	5

RSD = relative standard deviation, n = number of replicates

Table A 71: Recovery results from method validation of prothioconazole metabolites in barley grain

Fortification level	Analyte	Prothioconazole-desthio		3-hydroxy-prothioconazole-desthio		4-hydroxy-prothioconazole-desthio		5-hydroxy-prothioconazole-desthio		6-hydroxy-prothioconazole-desthio		alpha-hydroxy-prothioconazole-desthio	
[mg/kg]	Transition ion	70 m/z	125 m/z	70 m/z	141 m/z	141 m/z	70 m/z	70 m/z	141 m/z	70 m/z	141 m/z	70 m/z	141 m/z
0.010	Range	84-91	70-83	72-84	73-89	76-85	75-82	70-86	76-88	78-96	79-89	71-86	74-76
	Mean ± RSD	87 ± 5.4	76 ± 8.2	79 ± 7.2	80 ± 8.1	81 ± 5.0	78 ± 3.8	79 ± 8.9	83 ± 5.5	87 ± 9.5	85 ± 4.5	75 ± 8.3	75 ± 1.2
	n	5	5	5	5	5	5	5	5	5	5	5	5
0.100	Range	78-85	72-84	81-87	72-86	78-87	74-81	78 – 80	80-84	75-82	78-84	79-88	77-88
	Mean ± RSD	81 ± 4.2	79 ± 6.4	83 ± 3.2	79 ± 7.4	82 ± 5.0	77 ± 4.4	80 ± 1.3	82 ± 2.3	79 ± 3.9	80 ± 3.8	84 ± 4.4	82 ± 5.9
	n	4*	4*	4*	4*	4*	4*	4*	4*	4*	4*	4	4

RSD = relative standard deviation, n = number of replicates
*there were only four replicate results for barley (grain) instead of five for the fortification level 0.1 mg/kg due to a sample lost during sample work up

Table A 72: Recovery results from method validation of prothioconazole metabolites in barley straw

Fortification level	Analyte	Prothioconazole-desthio		3-hydroxy-prothioconazole-desthio		4-hydroxy-prothioconazole-desthio		5-hydroxy-prothioconazole-desthio		6-hydroxy-prothioconazole-desthio		alpha-hydroxy-prothioconazole-desthio	
[mg/kg]	Transition ion	70 m/z	125 m/z	70 m/z	141 m/z	141 m/z	70 m/z	70 m/z	141 m/z	70 m/z	141 m/z	70 m/z	141 m/z
0.010	Range	96-111	82-106	83-97	95-104	85-99	91-99	84-101	73-108	78-100	86-99	83-107	96-123
	Mean ± RSD	102 ± 5.4	93 ± 11	90 ± 6.6	99 ± 3.6	94 ± 5.7	96 ± 3.8	93 ± 7.3	90 ± 15	89 ± 9.7	91 ± 6.0	93 ± 12	108 ± 9.4
	n	5	5	5	5	5	5	5	5	5	5	5	5
0.100	Range	99-112	98-108	95-104	88-105	86-104	88-97	81-97	84-97	85-93	90-96	87-100	86-99
	Mean ± RSD	104 ± 5.0	103 ± 4.2	99 ± 3.2	97 ± 6.5	95 ± 7.2	93 ± 4.9	92 ± 6.8	92 ± 5.9	89 ± 3.3	94 ± 2.8	97 ± 6.0	92 ± 5.2
	n	5	5	5	5	5	5	5	5	5	5	5	5

RSD = relative standard deviation, n = number of replicates

Table A 73: Recovery results from method validation of prothioconazole-desthio ~~metabolites~~ in soil

Fortification level	Analyte	Prothioconazole-desthio	
[mg/kg]	Transition ion	70 m/z	125 m/z
0.010	Range	91-100	92-104
	Mean ± RSD	95 ± 3.6	97 ± 4.5
	n	5	5
0.100	Range	95-100	97-105
	Mean ± RSD	98 ± 3.7	99 ± 3.2
	n	5	5

RSD = relative standard deviation, n = number of replicates

Table A 74: Characteristics for the analytical method used for validation of prothioconazole residues in radish, lettuce, barley, and soil

	Prothioconazole*
Specificity	Blank value < 30 % LOQ
Calibration (type, number of data points)	Individual calibration data presented r > 0.99 ≥ 7 calibration points
Calibration range	1.0 – 100 ng/mL corresponding to 0.002 to 0.2 mg/kg for radish an lettuce leaves 0.3 – 50 ng/mL corresponding to 0.003 to 0.5 mg/kg for barley grain, straw and whole plant 0.5 – 50 ng/mL corresponding to 0.002 to 0.2 mg/kg for soil
Assessment of matrix effects is presented	Yes, however, matrix-matched standard solutions were used for calibration.
Limit of quantification	For each analyte separately: LOQ: 0.010 mg/kg For prothioconazole as the sum of all analytes: LOQ: 0.060 mg/kg

* Including: prothioconazole-desthio, 3-hydroxyprothioconazole-desthio, 4-hydroxyprothioconazole-desthio, 5-hydroxyprothioconazole-desthio, 6-hydroxyprothioconazole-desthio and alpha-hydroxyprothioconazole-desthio

Conclusion
The method fulfils the requirements of SANTE/2020/12830, Rev.1 and is suitable for the determination of prothioconazole radish, lettuce, barley, and soil.

A 2.1.1.2 Ecotoxicology analytical methods

Comments of zRMS:	The validation of the analytical method for the determination of prothioconazole in water used in an aqua toxicity test is acceptable and fulfils the requirements of guideline SANCO/3029/99 rev, 4. LOQ was 0.00982 mg/L.

Reference:	KCP 5.1.2/14 (filed in KCP 10.2.1/01)
Report	ADM.3500.F.2.B: Toxicity to the rainbow trout *Oncorhynchus mykiss* under laboratory conditions (Acute toxicity test – Semi-static), xxxxxxxxxxxx., 2020, report no.: S19-03475, sponsor no.: 000102732
Guideline(s):	For method validation: SANCO/3029/99 rev. 4
Deviations:	None
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods
Samples of the test matrix (water from the aqua toxicity test) were diluted with test medium/formic acid (1000:0.5, v/v) + 38.4 mg/L L-Cysteine and finally analysed HPLC-MS/MS.

Results and discussions
Recovery results were at 96% with an RSD ≤ 5%. No outliers were identified. No interference (< 30% LOQ) of total peak area for the target analytes were found in unfortified control samples. The LOQ was set at 0.00982 mg/L for prothioconazole.

Table A 75: Recovery results from method validation of prothioconazole using the analytical method

Matrix	Analyte	Fortification level (mg/L) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
Water (from aqua toxicity test)	Prothioconazole	0.00982	96	3	m/z 344→ 189 (quant.)
		2.99	96	3	m/z 344→ 189 (quant.)
		0.00982	96	5	m/z 344→ 154 (conf.)
		2.99	96	3	m/z 344→ 154 (conf.)

Table A 76: Characteristics for the analytical method used for validation of prothioconazole in water (from aqua toxicity test)

	prothioconazole
Specificity	blank value < 30% LOQ
Calibration (type, number of data points)	individual calibration data presented| calibration line equation presented
Calibration range	2.5 – 40 ng reference item/mL r = 1.0000 6 calibration points
Assessment of matrix effects is presented	Yes
Limit of quantification	0.00982 mg/L

Conclusion
The method fulfils the requirements of SANCO/3029/99 rev. 4 and is suitable for the determination of prothioconazole in water used in an aqua toxicity test.

Comments of zRMS:	An analytical method for the determination of prothioconazole in test medium was validated with regards to recovery, linearity of detector response, repeatability, specificity, limit of quantification and limit of detection. The limit of quantification (LOQ) of the analytical method was 0.0854 mg/L of test item (0.0196 mg/L of prothioconazole). The mean recoveries at each fortification level were in the range between 70% and 110% with a relative standard deviation below 20%. The analytical method fulfils the requirements of guideline SANCO/3029/99 rev. 4, 11/07/2000.

Reference:	KCP 5.1.2/15 (filed in KCP 10.2.1/02)
Report	ADM.3500.F.2.B: Toxicity to the water flea *Daphnia magna* Straus under laboratory conditions (Acute immobilisation test – Semi-static), Zetzmann, M., 2020, report no.: S19-03474, sponsor no.: 000102731
Guideline(s):	For method validation: SANCO/3029/99 rev. 4
Deviations:	None
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods
Samples of the test matrix (water from the aqua toxicity test) were diluted with test medium/formic acid (1000:0.5, v/v) + 38.4 mg/L L-Cysteine and finally analysed HPLC-MS/MS.

Results and discussions

Recovery results were in a range of 85 - 94% with an RSD ≤ 3%. No outliers were identified. No interference (< 30 % LOQ) of total peak area for the target analytes were found in unfortified control samples. The LOQ was set at 0.0196 mg/L.

Table A 77: Recovery results from method validation of prothioconazole using the analytical method

Matrix	Analyte	Fortification level (mg/L) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
Water (from aqua toxicity test)	Prothioconazole	0.0196	94	3	m/z 344→ 189 (quant.)
		5.98	85	3	m/z 344→ 189 (quant.)
		0.0196	94	2	m/z 344→ 154 (conf.)
		5.98	85	3	m/z 344→ 154 (conf.)

Table A 78: Characteristics for the analytical method used for validation of prothioconazole in water (from aqua toxicity test)

	prothioconazole
Specificity	blank value < 30 % LOQ
Calibration (type, number of data points)	individual calibration data presented| calibration line equation presented
Calibration range	2.5 – 50 ng reference item/mL r = 0.9999 8 calibration points
Assessment of matrix effects is presented	Yes
Limit of quantification	0.0196 mg/L

Conclusion

The method fulfils the requirements of SANCO/3029/99 rev. 4 and is suitable for the determination of prothioconazole in water used in an aqua toxicity test.

Comments of zRMS:	An analytical method for the determination of prothioconazole in test medium was validated with regards to recovery, linearity of detector response, repeatability, specificity, limit of quantification and limit of detection. The limit of quantification (LOQ) of the analytical method was 0.00954 mg/L of test item (0.00219 mg/L of prothioconazole). The mean recoveries at each fortification level were in the range between 70% and 110% with relative standard deviation below 20%. The analytical method fulfils the requirements of guideline SANCO/3029/99 rev. 4, 11/07/2000.

Reference:	KCP 5.1.2/16 (filed in KCP 10.2.1/03)
Report	ADM.3500.F.2.B: Toxicity to the single cell green alga *Pseudokirchneriella subcapitata* Hindák under laboratory conditions, Schuler, L., 2020, report no.: S19-03473, sponsor no.: 000102730
Guideline(s):	For method validation: SANCO/3029/99 rev. 4
Deviations:	None
GLP:	Yes
Acceptability:	Yes

Duplication (if vertebrate study)	No

Materials and methods
Samples of the test matrix (water from the aqua toxicity test) were diluted with test medium/formic acid (1000:0.5, v/v) + 38.4 mg/L L-Cysteine and finally analysed HPLC-MS/MS.

Results and discussions
Recovery results were in a range of 96 - 107 % with an RSD ≤ 4 %. No outliers were identified. No interference (< 30 % LOQ) of total peak area for the target analytes were found in unfortified control samples. The LOQ was set at 0.00219 mg/L.

Table A 79: Recovery results from method validation of prothioconazole using the analytical method

Matrix	Analyte	Fortification level (mg/L) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
Water (from aqua toxicity test)	Prothioconazole	0.00219	106	4	m/z 344→ 189 (quant.)
		2.99	96	3	m/z 344→ 189 (quant.)
		0.00219	107	2	m/z 344→ 154 (conf.)
		2.99	97	2	m/z 344→ 154 (conf.)

Table A 80: Characteristics for the analytical method used for validation of prothioconazole in water (from aqua toxicity test)

	prothioconazole
Specificity	blank value < 30 % LOQ
Calibration (type, number of data points)	individual calibration data presented| calibration line equation presented
Calibration range	0.6 – 12.0 ng reference item/mL r = 0.9999 7 calibration points
Assessment of matrix effects is presented	Yes
Limit of quantification	0.00219 mg/L

Conclusion
The method fulfils the requirements of SANCO/3029/99 rev. 4 and is suitable for the determination of prothioconazole in water used in an aqua toxicity test.

Comments of zRMS:	An analytical method for the determination of prothioconazole in test medium was validated with regards to recovery, linearity of detector response, repeatability, specificity, limit of quantification and limit of detection. The limit of quantification (LOQ) of the analytical method was 0.00244 mg/L of test item (0.000561 mg/L of Prothioconazole). The mean recoveries at each fortification level were in the range between 70% and 110% with relative standard deviation below 20%. The analytical method fulfils the requirements of guideline SANCO/3029/99 rev. 4, 11/07/2000.

Reference:	KCP 5.1.2/17 (filed in KCP 10.2.1/04)
Report	ADM.3500.F.2.B: Toxicity to the duckweed *Lemna gibba* under laboratory conditions (Growth inhibition test – Semi-static), Weber, K., 2020, report no.: S19-03476, sponsor no.: 000102733

Guideline(s):	For method validation: SANCO/3029/99 rev. 4
Deviations:	None
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods
Samples of the test matrix (water from the aqua toxicity test) were diluted with test medium/formic acid (1000:0.5, v/v) + 38.4 mg/L L-Cysteine and finally analysed HPLC-MS/MS.

Results and discussions
Recovery results were in a range of 88 - 98 % with an RSD ≤ 5 %. No outliers were identified. No interference (< 30 % LOQ) of total peak area for the target analytes were found in unfortified control samples. The LOQ was set at 0.000561mg/L.

Table A 81: Recovery results from method validation of prothioconazole using the analytical method

Matrix	Analyte	Fortification level (mg/L) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
Water (from aqua toxicity test)	Prothioconazole	0.000561	97	3	m/z 344→ 189 (quant.)
		29.9	88	5	m/z 344→ 189 (quant.)
		0.000561	98	2	m/z 344→ 154 (conf.)
		29.9	88	5	m/z 344→ 154 (conf.)

Table A 82: Characteristics for the analytical method used for validation of prothioconazole in water (from aqua toxicity test)

	prothioconazole
Specificity	blank value < 30 % LOQ
Calibration (type, number of data points)	individual calibration data presented| calibration line equation presented
Calibration range	0.8 – 10.0 ng reference item/mL r = 0.995 7 calibration points
Assessment of matrix effects is presented	Yes
Limit of quantification	0.000561 mg/L

Conclusion
The method fulfils the requirements of SANCO/3029/99 rev. 4 and is suitable for the determination of prothioconazole in water used in an aqua toxicity test.

Comments of zRMS:	The analytical method is suitable for the determination of prothioconazole in bee diet (50 % sucrose solution).

Reference:	KCP 5.1.2/18 (filed in KCP 10.3.1.1/01)
Report	ADM.3500.F.2.B: Effects (acute contact and oral) on honey bees (*Apis mellifera* L.) in the laboratory, Sekine, T., 2020, report no.: 137191035, sponsor no.: 000101260

Guideline(s):	For method validation: SANCO/3029/99 rev. 4
Deviations:	None
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	Not applicable

Materials and methods
The test matrix (50 % sucrose solution) was analysed HPLC-UV.

Results and discussions
Recovery results were in a range of 94 – 96% with an RSD ≤ 4%. No outliers were identified. No interference (< 30% LOQ) of total peak area for the target analytes were found in unfortified control samples. The LOQ was set at 230 mg/L.

Table A 83: Recovery results from method validation of prothioconazole using the analytical method

Matrix	Analyte	Fortification level (mg/L) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
50% sucrose solution	Prothioconazole	230	96	4	-
		5750	94	3	-

Table A 84: Characteristics for the analytical method used for validation of prothioconazole in 50 % sucrose solution

	prothioconazole
Specificity	blank value < 30 % LOQ
Calibration (type, number of data points)	individual calibration data presented| calibration line equation presented
Calibration range	2.5 – 30 mg reference item/L r = 0.9997 6 calibration points
Assessment of matrix effects is presented	No
Limit of quantification	230 mg/L

Conclusion
The method fulfils the requirements of SANCO/3029/99 rev. 4 and is suitable for the determination of prothioconazole in bee diet (50 % sucrose solution).

Comments of zRMS:	An analytical method for the determination of prothioconazole in aqueous stock solutions of test item ADM.3500.F.2.B was validated with regards to recovery, linearity of detector response, repeatability, specificity, limit of quantification and limit of detection. The limit of quantification (LOQ) of the analytical method was 29 mg/L. The mean recoveries at each fortification level were in the range between 70% and 110% with relative standard deviation below 20%. The analytical method fulfils the requirements of guideline SANCO/3029/99 rev. 4, 11/07/2000.

Reference:	KCP 5.1.2/19 (filed in KCP 10.3.1.3/01)
Report	Effects of ADM.3500.F.2.B on honeybees (*Apis mellifera* L.) 22-day larval toxicity test with repeated exposure, Colli, M., 2020, report no.: BT109/19,

	sponsor no.: 000101262
Guideline(s):	For method validation: SANCO/3029/99 rev. 4
Deviations:	None
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	Not applicable

Materials and methods
The test matrix (aqueous stock solution) was analysed HPLC-DAD.

Results and discussions
Recovery results were in a range of 98.68 – 102.34 % with an RSD ≤ 1.71 %. No outliers were identified. No interference (< 30 % LOQ) of total peak area for the target analytes were found in unfortified control samples. The LOQ was set at 29.0 mg/L.

Table A 85: Recovery results from method validation of prothioconazole using the analytical method

Matrix	Analyte	Fortification level (mg/L) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
Aqueous stock solution	Prothioconazole	29.0	102.34	1.71	-
		1100	98.68	1.44	-

Table A 86: Characteristics for the analytical method used for validation of prothioconazole in aqueous stock solution

	prothioconazole
Specificity	blank value < 30 % LOQ
Calibration (type, number of data points)	individual calibration data presented| calibration line equation presented
Calibration range	1.006 – 10.0596 mg reference item/L r = 0.9998 5 calibration points
Assessment of matrix effects is presented	Yes
Limit of quantification	29 mg/L

Conclusion
The method fulfils the requirements of SANCO/3029/99 rev. 4 and is suitable for the determination of prothioconazole in aqueous stock solution.

The following study provides the validation for the analytical method used in the study reports KCP 10.6.1/01 and 10.6.1/02.

Comments of zRMS:	The analytical method for the determination of prothioconazole in spray solutions for terrestrial plant tests of the test item ADM.3500.F.2.B was validated according to SANCO/3029/99 rev. 4 (2000) with satisfactory results regarding linearity, limit of quantification (LOQ), accuracy, precision and specificity. The limit of quantification (LOQ) was assigned at 500 mg test item/L (corresponding to 0.0926 L test item/ha) according to the spray solutions from the terrestrial plant tests.

Reference: KCP 5.1.2/20

Report	ADM.3500.F.2.B - Method Validation for the Determination of Prothioconazole in Spray Solutions for Terrestrial Plant Tests; Buttler, O., 2020, report no.: 190403AR / CMV18620, sponsor no.: 000103710
Guideline(s):	For method validation: SANCO/3029/99 rev. 4
Deviations:	None
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	Not applicable

Materials and methods
The test matrix (spray solution) was analysed LC-MS/MS.

Results and discussions
Recovery results were in a range of 96 – 98% with an RSD ≤ 3.8%. No outliers were identified. No interference (< 30% LOQ) of total peak area for the target analytes were found in unfortified control samples. The LOQ was set at 500 mg/L.

Table A 87: Recovery results from method validation of prothioconazole using the analytical method

Matrix	Analyte	Fortification level (mg/L) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
Spray solution	Prothioconazole	500	97	3.4	-
		5000	98	2.3	-

Table A 88: Characteristics for the analytical method used for validation of prothioconazole in spray solution

	prothioconazole
Specificity	blank value < 30% LOQ
Calibration (type, number of data points)	individual calibration data presented| calibration line equation presented
Calibration range	20 –200 µg/L r = 0.995 ~~5~~ 7 calibration points
Assessment of matrix effects is presented	No
Limit of quantification	~~29.0 mg/L~~ 500 mg test item/L

Conclusion
The method fulfils the requirements of SANCO/3029/99 rev. 4 and is suitable for the determination of prothioconazole in spray solution.

A 2.1.1.3 Phys-Chem analytical methods

The analytical method used in the phys-chem study Tsesin, N., 2019, Report no 000102642.035FL [filed in KCP 2.1/01] is summarised under 5.1.1/01.

A 2.2 Analytical methods for Prothioconazole

A 2.2.1 Methods for post-authorization control and monitoring purposes (KCP 5.2)

A 2.2.1.1.1 Analytical method for the determination of prothioconazole residues in crops

Comments of zRMS:	The analytical method for the determination of prothioconazole (sum of prothioconazole-desthio, 3-hydroxy-prothioconazole-desthio, 4-hydroxy-prothioconazole-desthio, 5-hydroxy-prothioconazole-desthio, 6-hydroxy-prothioconazole-desthio and alpha-hydroxy-prothioconazole-desthio, expressed as prothioconazole-desthio) residues in whole plant of wheat (commodity with high water content), grain of wheat (dried commodity with high starch content), straw of wheat (difficult commodity), grain of oilseed rape (commodity with high oil content), strawberry (commodity with high acid content) and dried bean (dried commodity with high protein content) has been successfully validated according to the SANTE/2020/12830, Rev.1. The quantification was performed by liquid chromatography with tandem mass spectrometry detection (LC-MS/MS), two mass transitions were monitored for each reference item. LOQ (Limit of quantification): 0.010 mg/kg for each metabolites. The LOQ of prothioconazole (sum of prothioconazole-desthio, 3-hydroxy-prothioconazole-desthio, 4-hydroxy-prothioconazole-desthio, 5-hydroxy-prothioconazole-desthio, 6-hydroxy-prothioconazole-desthio and alpha-hydroxy-prothioconazole-desthio, expressed as prothioconazole-desthio) was 0.060 mg/kg corresponding to a LOD of 0.018 mg/kg. The mean recoveries at each fortification level were in the range between 70% and 110% with relative standard deviation below 20%. The method is acceptable.

Reference:	KCP 5.2/02
Report	Validation of an analytical method for the determination of prothioconazole residues in wheat (whole plant, grain, straw), oilseed rape (grain), strawberry and dried bean Lefresne, S., 2020 Report No.: B18S-A4-P-01, Sponsor no.: R-39651
Guideline(s):	For method validation: SANTE/2020/12830, Rev.1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The analytical method is based on European Committee for Standardization (CEN): EN 15662:2009-02, paragraph 8 – QuEChERS-method. Residues of prothioconazole (sum of prothioconazole-desthio, 3-hydroxy-prothioconazoledesthio, 4-hydroxy-prothioconazole-desthio, 5-hydroxy-prothioconazole-desthio, 6-hydroxyprothioconazole-desthio and alpha-hydroxy-prothioconazole-desthio, expressed as prothioconazole-desthio) were extracted from homogenised matrices by maceration with acetonitrile/water. Then, extracts were purified by dispersive solid phase extraction. The quantification was performed by LC-MS/MS (QuEChERS-method) with two mass transitions.

Table A 89: Chromatographic conditions

MS System	**PE-Sciex 6500+QTRAP tandem mass Spectrometer**				
Analyte monitored	**Mass transitions (m/z)**	**Collision cell eXit Potential (V)**	**Collision Energy (V)**	**Declustering Potential (V)**	**Dwell Time (ms)**
Prothioconazole-desthio-1 used for quantification	312 → 70	8	51	56	150
Prothioconazole-desthio-2 used for confirmation	312 → 125	6	51	56	150
X-hydroxy-prothioconazole-	328 → 70	14	53	81	150

desthio-1 used for quantification or confirmation					
X-hydroxy-prothioconazole-desthio-2 used for quantification or confirmation	328 → 141	14	35	46	150
Ion Mode :	Positive Multiple reaction Monitoring (MRM)				
Entrance Potential (V)	10				
IonSpray voltage (V)	5500				
Ionspray Turbo Heater (°C)	300				
Collision gas (CAD) (psi)	8				
Curtain Gas Flow (psi)	40				
Gas Flow 1 (psi)	60				
Gas Flow 2 (psi)	70				

Results and discussions

Recovery results were in a range of 70 to 110 % with an RSD ≤ 20. No outliers were identified. No interference (< 30 % LOQ) of total peak area for the target analytes were found in unfortified control samples. The LOQ of prothioconazole-desthio, 3-hydroxyprothioconazole-desthio expressed as prothioconazole-desthio, 4-hydroxyprothioconazole-desthio expressed as prothioconazole-desthio, 5-hydroxyprothioconazole-desthio expressed as prothioconazole-desthio, 6-hydroxyprothioconazole-desthio expressed as prothioconazole-desthio and alpha-hydroxyprothioconazole-desthio expressed as prothioconazole-desthio was 0.010 mg/kg for each analyte and for each matrix. The LOQ for the sum of all prothioconazole-items was 0.060 mg/kg for each matrix.

Table A 90: Recovery results from method validation of prothioconazole metabolites in whole plant of wheat

Fortification level	Analyte	Prothioconazole-desthio		3-hydroxy-prothioconazole-desthio		4-hydroxy-prothioconazole-desthio		5-hydroxy-prothioconazole-desthio		6-hydroxy-prothioconazole-desthio		alpha-hydroxy-prothioconazole-desthio	
[mg/kg]	Transition ion	70 m/z	125 m/z	70 m/z	141 m/z	141 m/z	70 m/z	70 m/z	141 m/z	70 m/z	141 m/z	70 m/z	141 m/z
0.010	Range	100-102	99-103	100-102	99-105	100-105	101-108	101-105	98-109	104-108	105-110	104-107	99-102
	Mean ± RSD	101 ± 1	101 ± 2	101 ± 1	102 ± 2	103 ± 2	105 ± 2	103 ± 2	105 ± 4	106 ± 1	108 ± 2	106 ± 1	100 ± 1
	n	5	5	5	5	5	5	5	5	5	5	5	5
0.100	Range	100-108	99-106	103-112	103-111	103-114	105-118	101-113	100-113	108-114	106-115	105-114	99-110
	Mean ± RSD	103 ± 3	101 ± 3	107 ± 4	107 ± 3	108 ± 5	110 ± 5	107 ± 5	108 ± 5	110 ± 2	110 ± 3	110 ± 3	106 ± 4
	n	5	5	5	5	5	5	5	5	5	5	5	5
0.01 and 0.10	Overall ± RSD	102 ± 2	101 ± 2	104 ± 4	104 ± 3	106 ± 4	107 ± 4	105 ± 4	106 ± 5	108 ± 2	109 ± 2	108 ± 3	103 ± 4
	n	10	10	10	10	10	10	10	10	10	10	10	10

RSD = relative standard deviation, n = number of replicates

Table A 91: Recovery results from method validation of prothioconazole metabolites in grain of wheat

Fortification level	Analyte	Prothioconazole-desthio		3-hydroxy-prothioconazole-desthio		4-hydroxy-prothioconazole-desthio		5-hydroxy-prothioconazole-desthio		6-hydroxy-prothioconazole-desthio		alpha-hydroxy-prothioconazole-desthio	
[mg/kg]	Transition ion	70 m/z	125 m/z	70 m/z	141 m/z	141 m/z	70 m/z	70 m/z	141 m/z	70 m/z	141 m/z	70 m/z	141 m/z
0.010	Range	95-99	98-101	98-102	97-98	95-98	94-102	95-101	96-105	109-111	105-111	99-105	93-102
	Mean ± RSD	97 ± 2	100 ± 2	99 ± 2	98 ± 1	96 ± 1	98 ± 3	97 ± 3	99 ± 4	110 ± 1	109 ± 2	101 ± 2	97 ± 4
	n	5	5	5	5	5	5	5	5	5	5	5	5
0.100	Range	92-101	89-102	94-102	91-102	90-102	88-99	91-100	90-104	104-113	105-112	93-102	94-102
	Mean ± RSD	97 ± 4	98 ± 5	98 ± 4	97 ± 5	96 ± 4	95 ± 4	96 ± 4	97 ± 7	109 ± 3	108 ± 3	98 ± 4	98 ± 3
	n	5	5	5	5	5	5	5	5	5	5	5	5
0.01 and 0.10	Overall ± RSD	97 ± 3	99 ± 4	99 ± 3	97 ± 3	96 ± 3	96 ± 4	93 ± 3	98 ± 5	110 ± 2	108 ± 3	100 ± 3	98 ± 3
	n	10	10	10	10	10	10	10	10	10	10	10	10

RSD = relative standard deviation, n = number of replicates

Table A 92: Recovery results from method validation of prothioconazole metabolites in straw of wheat

Fortification level	Analyte	Prothioconazole-desthio		3-hydroxy-prothioconazole-desthio		4-hydroxy-prothioconazole-desthio		5-hydroxy-prothioconazole-desthio		6-hydroxy-prothioconazole-desthio		alpha-hydroxy-prothioconazole-desthio	
[mg/kg]	Transition ion	70 m/z	125 m/z	70 m/z	141 m/z	141 m/z	70 m/z	70 m/z	141 m/z	70 m/z	141 m/z	70 m/z	141 m/z
0.010	Range	94-98	93-99	97-102	101-108	94-99	93-100	95-98	93-100	103-107	102-107	105-110	99-101
	Mean ± RSD	97 ± 2	96 ± 2	99 ± 2	105 ± 3	97 ± 2	97 ± 3	96 ± 1	96 ± 3	106 ± 2	104 ± 2	108 ± 2	100 ± 1
	n	5	5	5	5	5	5	5	5	5	5	5	5
0.100	Range	86-101	87-100	87-104	93-109	85-99	86-100	85-107	82-99	98-109	94-113	97-111	85-109
	Mean ± RSD	93 ± 6	93 ± 6	96 ± 7	101 ± 6	93 ± 6	96 ± 6	95 ± 8	91 ± 8	104 ± 4	103 ± 7	106 ± 5	98 ± 10
	n	5	5	5	5	5	5	5	5	5	5	5	5
	Overall ± RSD	95 ± 4	95 ± 5	98 ± 5	103 ± 5	95 ± 5	96 ± 4	95 ± 6	93 ± 6	105 ± 3	104 ± 5	107 ± 4	99 ± 6

0.01 and 0.10	n	10	10	10	10	10	10	10	10	10	10	10	10

RSD = relative standard deviation, n = number of replicates

Table A 93: Recovery results from method validation of prothioconazole metabolites in oilseed rape seeds

Fortification level	**Analyte**	**Prothioconazole-desthio**		**3-hydroxy-prothioconazole-desthio**		**4-hydroxy-prothioconazole-desthio**		**5-hydroxy-prothioconazole-desthio**		**6-hydroxy-prothioconazole-desthio**		**alpha-hydroxy-prothioconazole-desthio**	
[mg/kg]	Transition ion	70 m/z	125 m/z	70 m/z	141 m/z	141 m/z	70 m/z	70 m/z	141 m/z	70 m/z	141 m/z	70 m/z	141 m/z
0.010	Range	72-111	71-111	80-116	78-120	77-120	79-120	74-118	81-117	69-105	66-103	83-123	81-126
	Mean ± RSD	83 ± 19	82 ± 20	90 ± 16	92 ± 18	90 ± 19	90 ± 18	89 ± 19	91 ± 16	79 ± 19	78 ± 19	95 ±17	95 ± 19
	n	5	5	5	5	5	5	5	5	5	5	5	5
0.100	Range	74-80	73-80	79-87	82-89	79-88	80-88	79 - 88	81-86	72-77	72-78	82-91	86-90
	Mean ± RSD	77 ± 3	77 ± 4	84 ± 4	85 ± 3	85 ± 4	85 ± 3	84 ± 5	84 ± 3	75 ± 3	75 ± 3	88 ± 4	88 ± 2
	n	5	5	5	5	5	5	5	5	5	5	5	5
0.01 and 0.10	Overall ± RSD	80 ± 14	80 ± 14	87 ± 12	89 ± 13	88 ± 14	88 ± 13	87 ± 14	88 ± 12	77 ± 14	76 ± 13	91 ± 13	91 ± 14
	n	10	10	10	10	10	10	10	10	10	10	10	10

RSD = relative standard deviation, n = number of replicates

Table A 94: Recovery results from method validation of prothioconazole metabolites in strawberry

Fortification level	**Analyte**	**Prothioconazole-desthio**		**3-hydroxy-prothioconazole-desthio**		**4-hydroxy-prothioconazole-desthio**		**5-hydroxy-prothioconazole-desthio**		**6-hydroxy-prothioconazole-desthio**		**alpha-hydroxy-prothioconazole-desthio**	
[mg/kg]	Transition ion	70 m/z	125 m/z	70 m/z	141 m/z	141 m/z	70 m/z	70 m/z	141 m/z	70 m/z	141 m/z	70 m/z	141 m/z
0.010	Range	73-112	75-108	96-113	97-110	101-109	100-111	93-113	93-119	108-117	106-116	96-112	104-115
	Mean ± RSD	98 ± 15	97 ± 13	103 ± 6	103 ± 5	106 ± 3	106 ± 4	104 ± 7	106 ± 9	110 ± 4	109 ± 4	103 ±6	109 ± 4
	n	5	5	5	5	5	5	5	5	5	5	5	5
0.100	Range	99-105	100-105	104-106	103-105	94-105	86-107	94 – 106	97-109	96-107	95-104	105-108	105-108
	Mean ± RSD	103 ± 2	103 ± 2	105 ± 1	104 ±1	99 ± 5	99 ± 8	101 ± 4	103 ± 4	103 ± 4	101 ± 3	106 ± 1	106 ± 1
	n	5	5	5	5	5	5	5	5	5	5	5	5
0.01 and 0.10	Overall ± RSD	101 ± 10	100 ± 9	104 ± 4	103 ± 3	103 ± 5	102 ± 7	103 ± 6	105 ± 7	107 ± 5	105 ± 5	104 ± 4	107 ± 3
	n	10	10	10	10	10	10	10	10	10	10	10	10

RSD = relative standard deviation, n = number of replicates

Table A 95: Recovery results from method validation of prothioconazole metabolites in dry bean

Fortification level	**Analyte**	**Prothioconazole-desthio**		**3-hydroxy-prothioconazole-desthio**		**4-hydroxy-prothioconazole-desthio**		**5-hydroxy-prothioconazole-desthio**		**6-hydroxy-prothioconazole-desthio**		**alpha-hydroxy-prothioconazole-desthio**	
[mg/kg]	Transition ion	70 m/z	125 m/z	70 m/z	141 m/z	141 m/z	70 m/z	70 m/z	141 m/z	70 m/z	141 m/z	70 m/z	141 m/z
0.010	Range	87-120	89-121	85-119	83-118	85-120	88-116	85-118	88-117	91-122	83-114	90-126	90-119

	Mean ± RSD n	100 ± 13 5	101 ± 13 5	99 ± 13 5	100 ± 13 5	99 ± 13 5	99 ± 11 5	99 ± 14 5	97 ± 13 5	102 ± 12 5	97 ± 13 5	102 ±14 5	101 ± 11 5
0.100	Range Mean ± RSD n	87-102 93 ± 6 5	88-103 93 ± 7 5	86-102 92 ± 7 5	85-104 91 ± 8 5	88-105 93 ± 7 5	87-103 93 ± 7 5	87 - 104 93 ± 7 5	84-101 90 ± 7 5	90-108 96 ± 7 5	91-106 95 ± 7 5	90-107 97 ± 7 5	89-107 95 ± 7 5
0.01 and 0.10	Overall ± RSD n	97 ± 10 10	97 ± 11 10	85 ± 119 10	95 ± 11 10	96 ± 11 10	96 ± 10 10	96 ± 11 10	94 ± 11 10	99 ± 10 10	96 ± 10 10	99 ± 11 10	98 ± 10 10

RSD = relative standard deviation, n = number of replicates

Table A 96: Characteristics for the analytical method used for validation of prothioconazole metabolites residues in wheat whole plant, wheat grain, wheat straw, oilseed rape grain, strawberry and dry bean

	Prothioconazole*
Specificity	Blank value < 30 % LOQ
Calibration (type, number of data points)	Individual calibration data presented r > 0.99 7 calibration points (single determination)
Representative equations	**Wheat whole plant** Quantification y=118131.63x + 6877.72 Confirmation y = 95161.80x + 14046.69 **Wheat Grain** Quantification y = 110732.52x + 5648.09 Confirmation y = 87873.65x + 11781.69 **Wheat Straw** Quantification y = 94709.63x + 11609.09 Confirmation y = 77547.41x + 15265.52 **Oilseed rape seed** Quantification y = 146110.37x + 6826.23 Confirmation y = 117766.59x + 13144.31 **Dry bean** Quantification y = 146618.17x -2519.25 Confirmation y = 118610.60x -318.41 **Strawberry** Quantification y = 113641.65x + 30316.94 Confirmation y = 91887.09x + 30786.62
Calibration range	0.6 - 200 µg/L corresponding to 0.003 – 0.1 mg/kg for wheat whole plant, wheat grain, wheat straw, oilseed rape grain and dry bean 0.6 - 200 µg/L corresponding to 0.003 – 0.15 mg/kg for strawberry
Assessment of matrix effects is presented	Not rquired, since calibration was carried out with matrix-matched standards
Extract and standard stability	The final sample extracts were analysed within 24 hours after initial extraction thus no stability study was performed.
Limit of quantification	For each analyte separately: LOQ: 0.010 mg/kg For prothioconazole as the sum of all analytes: LOQ: 0.060 mg/kg Note: Concentration levels are given as mg substance/kg sample

* Including: prothioconazole-desthio, 3-hydroxyprothioconazole-desthio, 4-hydroxyprothioconazole-desthio, 5-hydroxyprothioconazole-desthio, 6-hydroxyprothioconazole-desthio and alpha-hydroxyprothioconazole-desthio

Conclusion

The method fulfils the requirements of SANTE/2020/12830, Rev.1 and is suitable for the determination of prothioconazole (including: prothioconazole-desthio, 3-hydroxyprothioconazole-desthio, 4-hydroxyprothioconazole-desthio, 5-hydroxyprothioconazole-desthio, 6-hydroxyprothioconazole-desthio and alpha-hydroxyprothioconazole-desthio) in wheat whole plant, wheat grain, wheat straw, oilseed rape grain, strawberry and dry bean.

A 2.2.1.1.2 Analytical method for the determination of prothioconazole residues in crops (Independent laboratory validation)

Comments of zRMS:	The analytical method B18S-A4-P-01 (Adama study No- R-39651, Reference 1) for the determination of residues of prothioconazole-desthio only in wheat grain (high starch content), dried broad bean (high protein content), wheat whole plant (high water content), oilseed rape seed (high oil content) and strawberry (high acid content) with an LOQ of 0.01 mg/kg by LC-MS/MS has been independly validated. Analysis of 3-hydroxy-prothioconazole-desthio, 4-hydroxyprothioconazole- desthio, 5-hydroxy-prothioconazole-desthio, 6-hydroxy-prothioconazole-desthio and alpha-hydroxy-prothioconazole-desthio was not included in this study. The mean recovery value for prothioconazole-desthio at the LOQ fortification level (0.01 mg/kg) and at the higher fortification level (0.1 mg/kg) was between 70 – 120% with a relative standard deviation of ≤ 20% for all matrices. The independent laboratory validation met the criteria detailed in SANTE/2020/12830, Rev.1

Reference:	KCP 5.2/03
Report	Independent laboratory validation of an analytical method B18S-A4-P-01 (Adama study No- R-39651) for the determination of residues of prothioconazole-desthio in crops by LC-MS/MS, Watson, G., 2022a Report No.: RES-00393, Sponsor no.: 000110772
Guideline(s):	For method validation: SANTE/2020/12830, Rev.1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The analytical method is based on European Committee for Standardization (CEN): EN 15662:2009-02, paragraph 8 – QuEChERS-method. Residues prothioconazole-desthio were extracted from homogenised matrices by maceration with acetonitrile/water. Then, extracts were purified by dispersive solid phase extraction. The quantification was performed by LC-MS/MS (QuEChERS-method) with two mass transitions.

Table A 97: Chromatographic conditions

Parameter	**Description**				
Ionisation Mode	Turbo Ion Spray (Electrospray)				
Polarity	Positive				
Curtain Gas 45	45 (arbitrary units)				
CAD Gas	8				
Gas 1	50 (arbitrary units)				
Gas 2	50 (arbitrary units)				
Source Temperature	550 °C				
Spray Voltage	5500 V				
Entrance Potential	10 eV				
Declustering Potential	70 eV				
Mass Transitions	Ions monitored (m/z)	Dwell time (msec)	Collision Energy	Cell Exit Potential	Primary/Confirmatory
Prothioconazoledesthio	312.0 → 70.0	50	60 V	10 V	Primary
	312.0 → 125.0	50	45 V	10 V	Confirmatory

Results and discussions

Recovery results were in a range of 70 to 110 % with an RSD ≤ 20. No outliers were identified. No interference (< 30 % LOQ) of total peak area for the target analytes were found in unfortified control samples. The LOQ of prothioconazole-desthio was 0.010 mg/kg for for each matrix.

Table A 98: Recovery results from method validation of prothioconazole-desthio in crop matrices

Fortification level	Crop matrix	Wheat grain		Dried broad bean		Wheat whole plant		Oilseed rape seed		Strawberry	
[mg/kg]	Transition ion	70 m/z	125 m/z	70 m/z	125 m/z	70 m/z	125 m/z	70 m/z	125 m/z	70 m/z	125 m/z
0.010	Range	91-97	90-97	87-92	86-88	87-90	88-90	82-83	81-85	93-96	94-96
	Mean ±	93 ±	93 ±	89 ±	87 ±	88 ±	89 ±	82 ±	84 ±	94 ±	95 ±
	RSD	2.5	2.9	2.4	1.0	1.3	0.9	0.6	1.8	1.0	1.1
	n	5	5	5	5	5	5	5	5	5	5
0.100	Range	89-91	90-93	87-91	88-90	89-91	89-91	87-89	86-89	92-95	92-94
	Mean ±	90 ±	92 ±	89 ±	89 ±	90 ±	90 ±	88 ±	88 ±	94 ±	93 ±
	RSD	1.0	1.4	1.6	0.9	0.9	0.6	0.8	1.3	1.1	1.0
	n	5	5	5	5	5	5	5	5	5	5

RSD = relative standard deviation, n = number of replicates

Table A 99: Characteristics for the analytical method used for validation of prothioconazole-desthio residues in wheat grain, dried broad beans, oilseed rape seed andstrawberry

	Prothioconazole-desthio
Specificity	Blank value < 30 % LOQ
Calibration (type, number of data points)	Individual calibration data presented r > 0.99 7 calibration points (single determination)
Representative equations	**Wheat whole plant** Quantification y= $4.69e^4x$ -215 Confirmation y= $2.64e^4x$ + 77.2 **Wheat grain** Quantification y=$4.59e^4x$ + 375 Confirmation y= $2.59e^4x$ + $1.1e^3$ **Oilseed rape seed** Quantification y= $4.65e^4x$ + $2.84e^3$ Confirmation y= $2.63e^4x$ + $1.23e^3$ **Dry bean** Quantification y=$4.72e^4x$ + $1.23e^3$ Confirmation y=$2.65e^4x$ + $1.25e^3$ **Strawberry** Quantification y= $3.01e^4x$ +$5.59e^3$ Confirmation y= $1.51e^4x$ + $2.11e^3$
Calibration range	0.6 - 30 µg/L for wheat grain, dried broad beans and oilseed rape seed (equivalent to 0.003 – 0.15 mg/kg) 3.0 - 150 µg/L for strawberry (equivalent to 0.003 – 0.15 mg/kg)
Assessment of matrix effects is presented	Matrix effects were observed to be < 20%. However, calibration was carried out with matrix-matched standards
Extract and standard stability	Extract stability was proven for 7d. Standard stability in solvent MeCN was shown for 10 d when stored refrigerated.
Limit of quantification	LOQ: 0.010 mg/kg Note: Concentration levels are given as mg substance/kg sample

Conclusion

The method fulfils the requirements of SANTE/2020/12830, Rev.1 and is suitable for the determination of prothioconazole-desthio in wheat grain, dried broad beans, oilseed rape seed andstrawberry.

A 2.2.1.1.3 Analytical method for the determination of prothioconazole residues in animal matrices (egg)

Comments of zRMS:	The analytical method was found to be valid for the determination of residues of prothioconazole-desthio in egg, with an LOQ of 0.01 mg/kg. The validation of the method met the criteria detailed in SANTE/2020/12830, Rev.1 (2021). Final determination of prothioconazole-desthio was conducted by LC-MS/MS monitoring transitions 312.0 → 70.0 m/z (primary) and 312.0 → 125.0 m/z (confirmatory). The accuracy and precision of the method was successfully demonstrated as the mean recovery value for prothioconazole-desthio at the LOQ fortification level (0.01 mg/kg) and at the higher fortification level (0.1 mg/kg) was between 70 – 120% with a relative standard deviation of ≤ 20%. The method is acceptable.

Reference:	KCP 5.2/04
Report	Validation of an analytical method for the determination of residues of prothioconazole-desthio in egg by LC-MS/MS, Watson, G., 2022b Report No.: RES-00394, Sponsor no.: 000110773
Guideline(s):	For method validation: SANTE/2020/12830, Rev.1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The analytical method involved extraction with acetonitrile/water (80/20, v/v) using an automated tissue homogeniser. After centrifugation, an aliquot of the extract was transferred to an autosampler vial prior to quantification by LC-MS/MS.

Table A 100: Chromatographic conditions

Parameter	**Description**				
Ionisation Mode	Turbo Ion Spray (Electrospray)				
Polarity	Positive				
Curtain Gas 45	45 (arbitrary units)				
CAD Gas	8				
Gas 1	50 (arbitrary units)				
Gas 2	50 (arbitrary units)				
Source Temperature	550 °C				
Spray Voltage	5500 V				
Entrance Potential	10 eV				
Declustering Potential	70 eV				
Mass Transitions	Ions monitored (m/z)	Dwell time (msec)	Collision Energy	Cell Exit Potential	Primary/Confirmatory
Prothioconazoledesthio	312.0 → 70.0	50	60 V	10 V	Primary
	312.0 → 125.0	50	45 V	10 V	Confirmatory

Results and discussions

Recovery results were in a range of 70 to 110 % with an RSD ≤ 20. No outliers were identified. No interference (< 30 % LOQ) of total peak area for the target analyte was found in unfortified control samples. The LOQ of prothioconazole-desthio was 0.010 mg/kg for egg.

Table A 101: Recovery results from method validation of prothioconazole-desthio in egg

Fortification level [mg/kg]	**Crop matrix**	**Egg**	
	Transition ion	70 m/z	125 m/z
0.010	Range Mean ± RSD n	82-86 83 ± 1.7 5	82-86 83 ± 1.7 5
0.100	Range	80-84	80-83

	Mean ± RSD n	82 ± 1.7 5	81 ± 1.3 5

RSD = relative standard deviation, n = number of replicates

Table A 102: Characteristics for the analytical method used for validation of prothioconazole-desthio in egg

	Prothioconazole-desthio
Specificity	Blank value < 30 % LOQ
Calibration (type, number of data points)	Individual calibration data presented r > 0.99 7 calibration points (single determination) Representative equation: $y = 4.87e^{4}\ x + 1.08e^{3}$
Calibration range	0.6 - 40 µg/L (equivalent to 0.003 – 0.2 mg/kg)
Assessment of matrix effects is presented	Matrix effects were observed to be < 20%. However, calibration was carried out with matrix-matched standards
Limit of quantification	LOQ: 0.010 mg/kg Note: Concentration levels are given as mg substance/kg sample
Limit of detection	LOD: 0.003 mg/kg

Conclusion
The method fulfils the requirements of SANTE/2020/12830, Rev.1 and is suitable for the determination of prothioconazole-desthio in egg.

A 2.2.1.1.4 Analytical method for the determination of prothioconazole residues in animal matrices (egg) (Independent laboratory validation)

Comments of zRMS:	The independent laboratory method validation was found to be valid according to the guidance document SANTE/2020/12830, rev.1 for the determination of prothioconazole-desthio in egg with an LOQ of 0.01 mg/kg following the procedure listed in analytical method RES-00394 with no major modifications.

Reference:	KCP 5.2/05
Report	Independent Laboratory Validation of an Analytical Method for the Determination of Residues of Prothioconazole-desthio in Egg by LC-MS/MS, Lindner, M., Büdel, A., 2022 Report No.: S22-04421 (MAC-2219V), Sponsor no.: 000111069
Guideline(s):	For method validation: SANTE/2020/12830, Rev.1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods
The analytical method involved extraction with acetonitrile/water (80/20, v/v) using an automated tissue homogeniser. After centrifugation, an aliquot of the extract was transferred to an autosampler vial prior to quantification by LC-MS/MS.

Table A 103: Chromatographic conditions

Parameter	**Description**		
MS system	API 5000 System, SCIEX (Triple quadrupole mass spectrometer)		
Ionisation type	Electrospray ionisation (ESI, TurboIonSpray)		
Polarity	Positive ion mode		
Scan type	MS/MS, Multiple Reaction Monitoring (MRM)		
Capillary voltage (IS)	5500 V	Ionspray turbo heater (TEM)	550 °C
Curtain gas (CUR)	Nitrogen set at 45 (arbitrary	Gas flow 1 (GS1)	Zero-grade air set at 50

	units)				(arbitrary units)	
Collision gas (CAD)	Nitrogen set at 8 (arbitrary units)		Gas flow 2 (GS2)		Zero-grade air set at 50 (arbitrary units)	
Analyte monitored	Mass transitions monitored (m/z)	Declustering potential (DP) [V]	Entrance potential (EP) [V]	Collision energy (CE) [eV]	Cell exit potential (CXP) [V]	Dwell time [ms]
Prothioconazole-desthio	312.0 → 70.0	70	10	60	10	50
	312.0 → 125.0	70	10	45	10	50

Results and discussions

Recovery results were in a range of 70 to 110 % with an RSD ≤ 20. No outliers were identified. No interference (< 30 % LOQ) of total peak area for the target analyte was found in unfortified control samples. The LOQ of prothioconazole-desthio was 0.010 mg/kg for egg.

Table A 104: Recovery results from method validation of prothioconazole-desthio in egg

Fortification level [mg/kg]	**Crop matrix**	**Egg**	
	Transition ion	70 m/z	125 m/z
0.010	Range Mean ± RSD n	92-96 95 ± 2.0 5	95-98 96 ± 1.4 5
0.100	Range Mean ± RSD n	90-100 95 ± 4.0 5	91-97 95 ± 3.3 5

RSD = relative standard deviation, n = number of replicates

Table A 105: Characteristics for the analytical method used for validation of prothioconazole-desthio in egg

	Prothioconazole-desthio
Specificity	Blank value < 30 % LOQ
Calibration (type, number of data points)	Individual calibration data presented r > 0.99 8 calibration points (single determination) Representative equation: y = 73094.7843 x + 1716.8898
Calibration range	0.6 - 40 µg/L (equivalent to 0.003 – 0.2 mg/kg)
Assessment of matrix effects is presented	Matrix effects were observed to be < 20%. However, calibration was carried out with matrix-matched standards
Limit of quantification	LOQ: 0.010 mg/kg Note: Concentration levels are given as mg substance/kg sample
Limit of detection	LOD: 0.003 mg/kg

Conclusion

The method fulfils the requirements of SANTE/2020/12830, Rev.1 and is suitable for the determination of prothioconazole-desthio in egg and as ILV for Watson, G., 2022 (Report No.: RES-00394, Sponsor no.: 000110773).

A 2.2.1.1.5 Analytical method for the determination of prothioconazole residues in animal matrices (honey)

Comments of zRMS:	The analytical method has been demonstrated to be a reliable and accurate procedure for the determination of prothioconazole expressed as prothioconazole-desthio (sum of isomers) in honey. LOQ (Limit of quantification) of prothioconazole expressed as prothioconazole-desthio (sum of isomers): 0.010 mg/kg. The mean recoveries at each fortification level were in the range between 70% and 110% with relative standard deviation below 20%.

	The method complies with the guideline SANTE/2020/12830, Rev.1 of 24/02/2021.

Reference:	KCP 5.2/06
Report	Validation of an analytical method for the determination of prothioconazole residues in honey, Lefresne, S., 2021 Report No.: B21S-A4-P-04, Sponsor no.: 000108774
Guideline(s):	For method validation: SANTE/2020/12830, Rev.1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Residues of prothioconazole expressed as prothioconazole-desthio (sum of isomers) were extracted from laboratory sample of honey by maceration with acetonitrile and water. Then, extracts were purified by dispersive solid phase extraction. The quantification was performed by liquid chromatography with tandem mass spectrometry detection (LC-MS/MS).

Table A 106: Chromatographic conditions

Parameter	**Description**				
Mass Transitions	Ions monitored (m/z)	Dwell time (msec)	Collision Energy	Cell Exit Potential	Primary/Confirmatory
Prothioconazoledesthio	312.0 → 70.0	150	51 V	8 V	Primary
	312.0 → 125.0	150	51 V	6 V	Confirmatory
Ion Mode	Positive Multiple reaction Monitoring (MRM)				
Entrance Potential (V)	10				
IonSpray voltage (V)	2000				
Ionspray Turbo Heater (°C)	500				
Collision gas (CAD)	9				
Curtain Gas Flow (psi)	45				
Gas Flow 1 (psi)	35				
Gas Flow 2 (psi)	70				

Results and discussions

Recovery results were in a range of 70 to 110 % with an RSD ≤ 20. No outliers were identified. No interference (< 30 % LOQ) of total peak area for the target analyte was found in unfortified control samples. The LOQ of prothioconazole-desthio was 0.010 mg/kg for honey.

Table A 107: Recovery results from method validation of prothioconazole-desthio in honey

Fortification level [mg/kg]	**Crop matrix**	**Honey**	
	Transition ion	70 m/z	125 m/z
0.010	Range Mean ± RSD n	106-112 108 ± 2 5	107-111 108 ± 1 5
0.100	Range Mean ± RSD n	109-112 110 ± 1 5	106-109 108 ± 1 5

RSD = relative standard deviation, n = number of replicates

Table A 108: Characteristics for the analytical method used for validation of prothioconazole-desthio in honey

	Prothioconazole-desthio
Specificity	Blank value < 30 % LOQ
Calibration (type, number of data points)	Individual calibration data presented r > 0.99 8 calibration points (single determination)

	Prothioconazole-desthio
	Representative equation: y = -1205.5669 x² + 869245.8004 x + 749671.8949
Calibration range	3 – 200 µg/L (equivalent to 0.003 – 0.2 mg/kg)
Assessment of matrix effects is presented	Matrix effects were observed to be < 20%. However, calibration was carried out with matrix-matched standards
Limit of quantification	LOQ: 0.010 mg/kg Note: Concentration levels are given as mg substance/kg sample
Limit of detection	LOD: 0.003 mg/kg

Conclusion
The method fulfils the requirements of SANTE/2020/12830, Rev.1 and is suitable for the determination of prothioconazole-desthio in honey.

A 2.2.1.1.6 Analytical method for the determination of prothioconazole residues in animal matrices (honey) (Independent laboratory validation)

Comments of zRMS:	An analytical method Lefresne, S., 2021 (Report No.: B21S-A4-P-04) for the determination of prothioconazole-desthio in honey was independently validated (ILV) in accordance to guidance document SANTE/2020/12830, rev.1. LC-MS/MS determination was conducted by monitoring two (2) mass transitions (m/z 312→70 and m/z 312→125). The limit of quantification is 0.01 mg/kg. Recovery results were in a range of 70 to 120% with an RSD ≤ 20. The method is acceptable.

Reference:	KCP 5.2/07
Report	Independent Laboratory Validation of an Analytical Method for Determination of Prothiconazole Residues in Honey, Lindner, M., 2022 Report No.: S21-06313 (MAC-2144V), Sponsor no.: 000108775
Guideline(s):	For method validation: SANTE/2020/12830, Rev.1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods
After addition of water samples of honey were extracted with acetonitrile. Phase separation was achieved by addition of a citrate salt mixture. An aliquot of the acetonitrile phase was prepared for the injection to LC-MS/MS.
In contrast to the original method final extracts were diluted in water/methanol (9+1, v+v) by a factor of 100 in order to operate the MS/MS detector within its linear range. Also, a C18-type LC column was used but not exactly the particular one as is given in the original method

Table A 109: Chromatographic conditions

Parameter	**Description**		
MS system	TripleQuad 5500 System, SCIEX* (Triple quadrupole mass spectrometer)		
Ionisation type	Electrospray ionisation (ESI, TurboIonSpray)		
Polarity	Positive ion mode		
Scan type	MS/MS, Multiple Reaction Monitoring (MRM)		
Capillary voltage (IS)	3000 V	Ionspray turbo heater (TEM)	550 °C
Curtain gas (CUR)	Nitrogen set at 45 (arbitrary units)	Gas flow 1 (GS1)	Zero-grade air set at 40 (arbitrary units)
Collision gas (CAD)	Nitrogen set at 9 (arbitrary units)	Gas flow 2 (GS2)	Zero-grade air set at 60 (arbitrary units)

Analyte monitored	Mass transitions monitored (m/z)	Declustering potential (DP) [V]	Entrance potential (EP) [V]	Collision energy (CE) [eV]	Cell exit potential (CXP) [V]	Dwell time [ms]
Prothioconazole-desthio	312.0 → 70.0	60	10	50	12	150
	312.0 → 125.0	60	10	50	12	150

Results and discussions

Recovery results were in a range of 70 to 110 % with an RSD ≤ 20. No outliers were identified. No interference (< 30 % LOQ) of total peak area for the target analyte was found in unfortified control samples. The LOQ of prothioconazole-desthio was 0.010 mg/kg for honey.

Table A 110: Recovery results from method validation of prothioconazole-desthio in honey

Fortification level [mg/kg]	Crop matrix	Honey	
	Transition ion	70 m/z	125 m/z
0.010	Range Mean ± RSD n	94-97 96 ± 1.3 5	95-100 97 ± 2.0 5
0.100	Range Mean ± RSD n	109-113 111 ± 1.5 5	109-113 111 ± 1.6 5

RSD = relative standard deviation, n = number of replicates

Table A 111: Characteristics for the analytical method used for validation of prothioconazole-desthio in honey

	Prothioconazole-desthio
Specificity	Blank value < 30 % LOQ
Calibration (type, number of data points)	Individual calibration data presented r > 0.99 8 calibration points (single determination) Representative equation: y = 372120.70 x + 652.1902
Calibration range	0.03 - 3 ng/L (equivalent to 0.003 – 0.3 mg/kg)
Assessment of matrix effects is presented	Matrix effects were observed to be < 20%. However, calibration was carried out with matrix-matched standards
Limit of quantification	LOQ: 0.010 mg/kg Note: Concentration levels are given as mg substance/kg sample
Limit of detection	LOD: 0.003 mg/kg

Conclusion

The method fulfils the requirements of SANTE/2020/12830, Rev.1 and is suitable for the determination of prothioconazole-desthio in honey and as ILV for Lefresne, S., 2021 (Report No.: B21S-A4-P-04, Sponsor no.: 000108774).

A 2.2.1.1.7 Analytical method for the determination of prothioconazole residues in drinking water

Comments of zRMS:	The analytical method 01387/M002 for the determination of concentrations of prothioconazole and prothioconazole-desthio in surface water by HPLC-MS/MS using two MRM transitions has been validated. The limit of quantitation (LOQ) is 0.05 µg/L for all analytes in surface water. Because of the direct measurement of the samples recovery rates cannot be calculated. The relative standard deviations for the peak areas were ≤ 20% for all analytes and MRM transitions. The method meets all guideline criteria to determine concentrations in drinking and surface water of prothioconazole and prothioconazole-desthio at 0.05 µg/L.

	Remark: A validated method for drinking water is not necessary since the limit of quantitation for surface water is equal or below the drinking water limit of 0.1 µg/L.

Reference:	KCP 5.2/08
Report:	Modification M002 of analytical method 01387 for the determination of various pesticides in drinking and surface water by HPLC-MS/MS, Krebber, R., Sandau, C., 2015, report no.: MR-15/025
Guideline(s):	SANCO/3029/99 rev. 4, SANCO/825/00 rev 8.1, OECD Guidance Document on Pesticide Residue analytical Methods; ENV/JM/Mono (2007)
Deviations:	No
GLP:	Yes (certified laboratory)
Acceptability/Reliability:	Yes

Materials and methods

Surface water samples are analysed directly for content of prothioconazole and prothioconazole-desthio by high performance liquid chromatography with tandem mass specific detection (LC-MS/MS), using gradient elution with mobile phases of water / formic acid (1000/0.120, v/v) + 10 mM ammonium formate and methanol / formic acid (1000/0.120, v/v) + 10 mM ammonium formate. The prothioconazole ion transitions m/z 344 > 189 and 344 > 154 were used for quantification and confirmation respectively. The prothioconazole-desthio ion transitions m/z 312 > 70 and 312 > 125 were used for quantification and confirmation respectively.

Table A 112: Chromatographic conditions

Parameter	**Description**		
MS system	Triple Quadrupole Tandem Mass Spectrometer, AB Sciex API 5500		
Ionisation type	Electrospray ionisation (ESI, TurboIonSpray)		
Polarity	Positive ion mode		
Scan type	MS/MS, Multiple Reaction Monitoring (MRM)		
Analyte monitored	Mass transitions monitored (m/z)	Collision energy (CE) [eV]	Dwell time [ms]
Prothioconazole	344 → 189	29	10
	344 → 154	39	10
Prothioconazole-desthio	312 → 70	25	10
	312 → 125	35	10

Results and discussions

Recovery was not determined as the samples were analysed by direct injection. Precision (% RSD) results were in a range of 2.3 – 9.5% for prothioconazole and 1.2 – 1.9% for prothioconazole-desthio. No outliers were identified. No interference (< 30 % LOQ) of total peak area for the target analytes were found in unfortified control samples. The LOQ was set at 0.05 µg/L for prothioconazole and prothioconazole-desthio.

Table A 113: Recovery results from method validation of prothioconazole and prothioconazole-desthio ~~and azoxystrobin~~ using the analytical method

Matrix	**Analyte**	**Ion Transition (m/z)**	**Fortification level (µg/L) (*n* = 10)**	**Mean Area Counts**	**RSD (%)**
Surface water	Prothioconazole	344 > 189	0.05	8680	2.3
			0.5	87797	2.3
		344 > 154	0.05	6299	9.5
			0.5	69808	3.8
	Prothioconazole-	312 > 70	0.05	151037	1.9

Matrix	Analyte	Ion Transition (m/z)	Fortification level (µg/L) (*n* = 10)	Mean Area Counts	RSD (%)
	desthio		0.5	1522200	1.2
		312 > 125	0.05	93164	1.6
			0.5	932259	1.6

Table A 114: Characteristics for the analytical method used for validation of prothioconazole and prothioconazole-desthio in surface water

	prothioconazole	prothioconazole-desthio
Specificity	blank value < 30 % LOQ	blank value < 30 % LOQ
Calibration (type, number of data points)	Individual calibration data presented r > 0.99 7 calibration points (single determination) Representative equation: y = 1.7994 e^5 x – 225.59	Individual calibration data presented r > 0.99 5 calibration points (double determination) Representative equation: y = - 2.9741 e^6 + 5603
Calibration range	0.015–10 µg/L	0.015–5 µg/L
Assessment of matrix effects is presented	Matrix effects are not relevant, since calibration was carried out with matrix-matched standards	Matrix effects are not relevant, since calibration was carried out with matrix-matched standards
Limit of quantification	0.05 µg/L	0.05 µg/L

Conclusion
The method fulfils the requirements of SANTE/2020/12830, Rev.1 and is suitable for the determination of prothioconazole and prothioconazole-desthio in surface water and drinking water.

A 2.2.1.1.8 Analytical method for the determination of prothioconazole residues in drinking water (Independent laboratory validation)

Comments of zRMS:	The analytical BCS method 01387/M002 for the determination of concentrations of prothioconazole and prothioconazole-desthio in surface water by HPLC-MS/MS using two MRM trasitions has been independly validated. The limit of quantitation (LOQ) for all analytes is 0.05 µg/L in surface water. The relative standard deviations for the peak areas were ≤ 20% for all MRM transitions of all analytes. The method meets all guideline criteria to determine concentrations in surface water of the described analytes at 0.05 µg/L.

Reference:	KCP 5.2/09
Report:	Independent laboratory validation of the BCS analytical method 01387/M002 for the determination of various pesticides in surface water by HPLC-MS/MS, Thies, S., 2015, report no.: 2015/0034/01
Guideline(s):	SANCO/3029/99 rev. 4, SANCO/825/00 rev 8.1, OECD Guidance Document on Pesticide Residue analytical Methods; ENV/JM/Mono (2007)
Deviations:	No
GLP:	Yes (certified laboratory)
Acceptability/Reliability:	Yes

Materials and methods
Surface water samples are analysed directly for content of prothioconazole and prothioconazole-desthio by high performance liquid chromatography with tandem mass specific detection (LC-MS/MS), using an ACE UltraCore Super C18 column (100 x 2.1 mm, 2.5 µm) and gradient elution with mobile phases of water / formic acid (1000/0.120, v/v) + 10 mM ammonium formate and methanol / formic acid (1000/0.120, v/v) + 10 mM ammonium formate. The prothioconazole ion transitions m/z 344 > 189 and 344 > 154 were used

for quantification and confirmation respectively. The prothioconazole-desthio ion transitions m/z 312 > 70 and 312 > 125 were used for quantification and confirmation respectively.

Table A 115: Chromatographic conditions

Parameter	**Description**		
MS system	Triple Quadrupole Tandem Mass Spectrometer, AB Sciex API 5500		
Ionisation type	Electrospray ionisation (ESI, TurboIonSpray)		
Polarity	Positive ion mode		
Scan type	MS/MS, Multiple Reaction Monitoring (MRM)		
Analyte monitored	Mass transitions monitored (m/z)	Collision energy (CE) [eV]	Dwell time [ms]
Prothioconazole	344 → 189	29	80
	344 → 154	39	80
Prothioconazole-desthio	312 → 70	25	30
	312 → 125	35	30

Results and discussions

Recovery was not determined as the samples were analysed by direct injection. Precision (% RSD) results were in a range of 2.8 – 9.5% for prothioconazole and 0.9 – 1.7% for prothioconazole-desthio. No outliers were identified. No interference (< 30 % LOQ) of total peak area for the target analytes were found in unfortified control samples. The LOQ was set at 0.05 µg/L for prothioconazole and prothioconazole-desthio.

Table A 116: Recovery results from method validation of prothioconazole, prothioconazole-desthio and azoxystrobin using the analytical method

Matrix	**Analyte**	**Ion Transition (m/z)**	**Fortification level (µg/L) (*n* = 5)**	**Mean Area Counts**	**RSD (%)**
Surface water	Prothioconazole	344 > 189	0.05	7130	7.9
			0.5	72280	8.4
		344 > 154	0.05	4658	9.5
			0.5	54760	2.8
	Prothioconazole-desthio	312 > 70	0.05	86600	1.3
			0.5	618000	1.4
		312 > 125	0.05	47920	1.7
			0.5	353800	0.9

Table A 117: Characteristics for the analytical method used for validation of prothioconazole and prothioconazole-desthio in surface water

	prothioconazole	**prothioconazole-desthio**
Specificity	blank value < 30 % LOQ	blank value < 30 % LOQ
Calibration (type, number of data points)	individual calibration data presented| Individual calibration data presented r > 0.99 ≥ 5 calibration points (single determination) Representative equation: y = 1.66 x 10^5 x - 994	individual calibration data presented| Individual calibration data presented r > 0.99 ≥ 5 calibration points (single determination) Representative equation: ~~y = 6.9 x 10^5 x + 11700~~ 1.17e+006 x + 2.54e+004
Calibration range	0.015–10 µg/L	0.015–10 µg/L
Assessment of matrix effects is presented	Matrix effects were observed to be < 20%. However, calibration was carried out with matrix-matched standards	Matrix effects were observed to be < 20%. However, calibration was carried out with matrix-matched standards
Limit of quantification	0.05 µg/L	0.05 µg/L

Conclusion
The method fulfils the requirements of SANTE/2020/12830, Rev.1 and is suitable for the determination of prothioconazole and prothioconazole-desthio in surface water and as ILV for Sommer, H. (2001); DAR Prothioconazole, Volume 3, Annex B, 5, IIA 4.2.3.1/03.

A 2.2.1.1.9 Analytical method for the determination of prothioconazole residues in ~~drinking water (Independent laboratory validation)~~ body fluids and tissues

Comments of zRMS:	The analytical method for the determination of residues of prothioconazole-desthio in pig's blood has been validated with an LOQ of 0.01 mg/L. The accuracy and precision of the method was successfully demonstrated as the mean recovery value for prothioconazole-desthio at the LOQ fortification level (0.01 mg/L) was between 70 – 120% with a relative standard deviation of ≤ 20%. Remark: According to SANTE/2020/12830, Rev.1, recovery should be done with 5 samples at LOQ and 5 samples at 10 x LOQ. In this study recoveries was only done at LOQ level.

Reference:	KCP 5.2/01
Report	Development and Validation of an Analytical Method for Determination of Residues of Prothioconazole-desthio in Body Fluids (Blood) by LC-MS/MS, xxxxxxxxxxx, 2022, report no.: RES-00373, sponsor no.: 000109608
Guideline(s):	SANTE/2020/12830, Rev.1
Deviations:	None
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	Not applicable

Materials and methods
Samples of body fluids and tissues were extracted by mixing with acetonitrile. After centrifugation, an aliquot of the extract was diluted with deionised water prior to quantification by LC-MS/MS.

Results and discussions
Recovery results were in a range of 98.68 – 102.34 % with an RSD ≤ 1.71 %. No outliers were identified. No interference (< 30 % LOQ) of total peak area for the target analyte was found in unfortified control samples. The LOQ was set at 0.01 mg/L.

Table A 118: Recovery results from method validation of prothioconazole-desthio in pig blood using the analytical method

Matrix	Analyte	Fortification level (mg/L) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
Pig's blood	Prothioconazole-desthio	0.01	92	10.9	*m/z* 312 → 70
		0.01	97	11.1	*m/z* 312 → 125

Table A 119: Characteristics for the analytical method used for validation of prothioconazole-desthio in body fluids ~~and tissues~~

	Prothioconazole-desthio
Specificity	blank value < 30 % LOQ
Calibration (type, number of data points)	individual calibration data presented| calibration line equation presented

	Prothioconazole-desthio
Specificity	blank value < 30 % LOQ
Calibration range	0.0075 – 0.375 ng/mL corresponding to 0.003 to 0.15 mg/L r ≥ 0.995 6 calibration points
Assessment of matrix effects is presented	Yes
Limit of quantification	0.01 mg/L
Limit of detection	0.003 mg/L

Conclusion

The method fulfils the requirements of SANTE/2020/12830, Rev.1 and is suitable for the determination of prothioconazole-desthio in body fluids and tissues.