

REGISTRATION REPORT

Part B

Section 5

Analytical Methods

Detailed summary of the risk assessment

Product code: ADM.03502.F.1.A
(alternative codes: MCW-2091)

Product name(s): see part A

Chemical active substance:

Fenpropidin 250 g/L

Prothioconazole 175 g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(authorisation)

Applicant: Country organisation / representative
as specified in Part A

Submission date: September 2021, updated March 2022, October
2022

Finalisation date: December 2022 (initial Core Assessment)

May 2023 (final Core Assessment)

Version history

When	What
September 2021	Initial dRR – ADAMA Polska Sp. z o.o.
March 2022	Updated dRR – ADAMA Polska Sp. z o.o. The Part B Section 5 was updated, mainly to reflect the changes made in the updated Part B Section 7.
October 2022	Updated dRR – ADAMA Polska Sp. z o.o.
December 2022	<p>Initial zRMS assessment.</p> <p>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.</p> <p>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.</p>
May 2023	<p>Final report (Core Assessment updated following the commenting period)</p> <p>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.</p>

DATA PROTECTION CLAIM

In order to present a dossier fully compliant with today's requirements (Reg. 284/2013), studies have been performed on ADM.03502.F.1.A. Under Article 59, Regulation 1107/2009/EC, on behalf of the Sponsor Company the applicant claims data protection for the studies conducted with ADM.03502.F.1.A. 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The summaries and evaluations contained in this document may be based on unpublished proprietary data submitted for the purpose of the assessment undertaken by the regulatory authority that prepared it.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
5.1	Conclusion and summary of assessment	5
5.2	Methods used for the generation of pre-authorization data (KCP 5.1).....	9
5.2.1	Analysis of the plant protection product (KCP 5.1.1)	9
5.2.1.1	Determination of active substance and/or variant in the plant protection product (KCP 5.1.1).....	9
5.2.1.2	Description of analytical methods for the determination of relevant impurities (KCP 5.1.1).....	10
5.2.1.3	Description of analytical methods for the determination of formulants (KCP 5.1.1).....	12
5.2.1.4	Applicability of existing CIPAC methods (KCP 5.1.1)	12
5.2.2	Methods for the determination of residues (KCP 5.1.2).....	13
5.3	Methods for post-authorization control and monitoring purposes (KCP 5.2)	16
5.3.1	Analysis of the plant protection product (KCP 5.2)	16
5.3.2	Description of analytical methods for the determination of residues Prothioconazole (KCP 5.2).....	16
5.3.2.1	Overview of residue definitions and levels for which compliance is required.....	16
5.3.2.2	Description of analytical methods for the determination of residues in plant matrices (KCP 5.2)	17
5.3.2.3	Description of analytical methods for the determination of residues in animal matrices (KCP 5.2)	19
5.3.2.4	Description of methods for the analysis of soil (KCP 5.2).....	21
5.3.2.5	Description of methods for the analysis of water (KCP 5.2).....	21
5.3.2.6	Description of methods for the analysis of air (KCP 5.2)	21
5.3.2.7	Description of methods for the analysis of body fluids and tissues (KCP 5.2)	22
5.3.2.8	Other studies/ information	22
5.3.3	Description of analytical methods for the determination of residues of Fenpropidin (KCP 5.2)	23
5.3.3.1	Overview of residue definitions and levels for which compliance is required.....	23
5.3.3.2	Description of analytical methods for the determination of residues in plant matrices (KCP 5.2)	24
5.3.3.3	Description of analytical methods for the determination of residues in animal matrices (KCP 5.2)	25
5.3.3.4	Description of methods for the analysis of soil (KCP 5.2).....	26
5.3.3.5	Description of methods for the analysis of water (KCP 5.2).....	27
5.3.3.6	Description of methods for the analysis of air (KCP 5.2)	27
5.3.3.7	Description of methods for the analysis of body fluids and tissues (KCP 5.2)	28
5.3.3.8	Other studies/ information	28
Appendix 1	Lists of data considered in support of the evaluation.....	29
Appendix 2	Detailed evaluation of submitted analytical methods.....	36
A 2.1	Analytical methods for all active substances.....	36
A 2.1.1	Methods used for the generation of pre-authorization data (KCP 5.1).....	36
A 2.1.1.1	Residue analytical methods.....	36
A 2.1.1.2	Operator, worker, resident and bystander exposure studies - analytical methods ...	69
A 2.1.1.3	Environmental fate analytical methods	70
A 2.1.1.4	Ecotoxicology analytical methods.....	74
A 2.1.1.5	Phys-Chem analytical methods	84
A 2.2	Analytical methods for Prothioconazole	85
A 2.2.1	Methods for post-authorization control and monitoring purposes (KCP 5.2)	85
A 2.3	Analytical methods for Fenpropidin.....	106
A 2.3.1	Methods for post-authorization control and monitoring purposes (KCP 5.2)	106

5 Analytical methods

5.1 Conclusion and summary of assessment

zRMS-PL 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-prothioconazole-desthio 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 for crops 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.03502.F.1.A.

Fenpropidin

In EFSA Scientific Report (2007) 124, 1-84, “Conclusion on the peer review of Fenpropidin” it is stated that: A multi-residue method like the Dutch MM1 or the German S19 is not applicable due the nature of the residues. Residues of fenpropidin in products of plant origin are analysed by LC-MS/MS with an LOQ of 0.01 mg/kg. For products of animal origin fenpropidin and CGA 2892673 were analysed by LC-MS/MS with an LOQ of 0.005 mg/kg in milk and an LOQ of 0.01 mg/kg in muscle, kidney, liver, fat and eggs. There was also a GC-NPD method for milk, eggs and fat with an LOQ of 0.005 mg/kg in milk and 0.01 mg/kg in eggs and fat. Soil is analysed for fenpropidin by LC-MS/MS with an LOQ of 0.01 mg/kg. Drinking/groundwater can be analysed for by HPLC-UV with confirmation by GC-MS with an LOQ of 0.05 µg/L. Surface water can be analysed for fenpropidin by HPLC-UV with confirmation by GC-MS the LOQ is 0.1 µg/L. Air is analysed for fenpropidin by LC-MS/MS with an LOQ of 0.15 µg/m³.

Fenpropidin list of end points (Nov 2006):

Analytical methods for residues (Annex IIA, point 4.2)

Food/feed of plant origin (analytical technique and LOQ for methods for monitoring purposes)	GC with nitrogen phosphorus detector (NPD) LOQ: 0.02 mg/kg (cereal grain)
Food/feed of animal origin (analytical technique and LOQ for methods for monitoring purposes)	GC with nitrogen phosphorus detector (NPD) LOQ: 0.01 mg/kg (tissues, fat), 0.005 mg/kg (milk)
Soil (analytical technique and LOQ)	GC with nitrogen phosphorus detector (NPD) LOQ: 0.05 mg/kg
Water (analytical technique and LOQ)	<u>Fenpropidin</u> HPLC-UV LOQ 0.05 µg/l (drinking water), 0.1 µg/l (surface water) <u>CGA289267</u> ¹ HPLC-UV LOQ 0.05 µg/l (drinking water), 0.1 µg/l (surface water)
Air (analytical technique and LOQ)	GC with nitrogen phosphorus detector (NPD) LOQ 1 µg/m ³
Body fluids and tissues (analytical technique and LOQ)	Not required (fenpropidin is not classified as toxic or highly toxic)

The Applicant submitted a number of methods for analysis of residues of fenpropidin for the generation of pre-authorization data and methods for post-authorization control and monitoring purposes.

¹ CGA 289267: 2-methyl-2-[4-(2-methyl-3-piperidin-1-yl-propyl)-phenyl]-propionic acid.

Methods for post-authorization control and monitoring purposes

According to the Reg. 283/2013 an analytical method for the residues of fenpropidin in body fluids and tissues is required.

- A body fluids method for the determination of residues of fenpropidin, CGA289267 and CGA28926ß in blood (Cross, M., 2017, report no CEMR-8288) has been submitted by Applicant. The limit of quantification was established at 0.01 mg/kg.

Additionally:

- Applicant submitted the analytical method GRM024.03A (with its ILV) for the determination of fenpropidin and CGA289267 in surface, drinking and ground water (Richardson, M., 2007) with LOQ of 0.05 µg/L.

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.03502.F.1.A.

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

Noticed data gaps are:

- none

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

Noticed data gaps are:

- none

Commodity/crop		Supported/ Not supported
High starch	Wheat	Supported
	Barley	Supported
	Rye	Supported
	Triticale	Supported
	Oats	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 substances prothioconazole and fenpropidin in the plant protection product ADM.03502.F.1.A is provided as follows:

Comments of zRMS:	The method was successfully validated according to SANCO/3030/99 rev.5 and is acceptable for the quantification of prothioconazole and fenpropidin in ADM.03502.F.1.A.
-------------------	--

The following study has not been evaluated during the EU peer review of prothioconazole and/or fenpropidin.

Reference:	KCP 5.1.1/01 (filed in KCP 2.1/01)
Report	Determination of storage stability and physical-chemical properties of prothioconazole 175 g/L + fenpropidin 250 g/L EC (ADM.03502.F.1.A) stored at 54 °C for 14 days and at 0°C for 7 days, Tsesin, N., 2020, Report no.: 000105029.061FL, Sponsor no.: 000105029
Guideline(s):	SANCO/3030/99 rev. 5
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

For the determination of prothioconazole, the sample of the formulation was diluted with acetonitrile. For the determination of fenpropidin, the sample of the formulation was diluted with methanol. Final analysis was carried out by HPLC-DAD.

Samples for recovery measurements were prepared by addition of Prothioconazole and Fenpropidin analytical standards at appropriate quantities, into blank formulation at the appropriate quantities

Table 5.2-1: Chromatographic conditions

Detector	HPLC-DAD
Wavelength	245 nm

Validation - Results and discussions

Table 5.2-2: Suitable method for the determination of prothioconazole and fenpropidin in the plant protection product ADM.03502.F.1.A

	Prothioconazole	Fenpropidin
Author(s), year	Tsesin, N., 2020	
Principle of method	HPLC-DAD	
Linearity (linear between mg/L) (correlation coefficient, expressed as r) (number of calibration points)	External standard calibration. ~ 0.2 mg/mL - ~ 0.62 mg/mL (about 50-150% of the active ingredient content in test item solution) r = 0.9992 7 calibration points Linearity curve: $y = 66.8922x + 1.1723$	External standard calibration. ~ 0.2 mg/mL - ~ 0.62 mg/mL 0.3 mg/mL - 0.8 mg/mL (about 50-140% of the active ingredient content in test item solution) r = 0.9998 6 calibration points Linearity curve: $y = 41.4832 + 1.0788$
Precision – Repeatability Mean	Assay 1: 16.88% w/w (175.6 g/L), n=5, RSD ¹ = 0.283 %. Assay 2: 16.88% w/w (175.4 g/L), n=5, RSD ¹ =	Assay 1: 24.34% w/w (253.1 g/L), n=5, RSD ¹ = 0.330 % Assay 2: 24.37% w/w (253.4 g/L), n=5, RSD ¹

	Prothioconazole	Fenpropidin
	1.25 % Horwitz RSDr ² = 1.75 Horrat value (Hr) ³ for assay 1= 0.16 Horrat value (Hr) ³ for assay 2= 0.71	= 0.424 % Horwitz RSDr ² = 1.65 Horrat value (Hr) ³ for assay 1= 0.2 Horrat value (Hr) ³ for assay 2= 0.26
Accuracy (% Recovery)	Total recovery: High level (120%) = 99% (n=2) Medium level (100%) = 99% (n=2) Low level (80%) = 100% (n=2)	Total recovery: High level (120%) = 98% (n=2) Medium level (100%) = 98% (n=2) Low level (80%) = 101% (n=2)
Interference/ Specificity	No interference	No interference
Comment	-	-

¹RSD = Relative Standard Deviation

²RSD calculated via Horwitz equation: % RSDR = 2(1-0.5 logC)

³Horrat value (Hr) calculated as %RSD/%RSDr is considered acceptable when < 1

Conclusion

The analytical method provides a specific determination of the active ingredients prothioconazole and fenpropidin in the formulation ADM.03502.F.1.A 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 methods summarized below have been successfully validated according to SANCO/3030/99 rev.5 and are acceptable for the quantification of relevant impurities prothioconazole-desthio and toluene in ADM.03502.F.1.A.
-------------------	---

The following study has not been evaluated during the EU peer review of prothioconazole or fenpropidin.

Reference:	KCP 5.1.1/01 (filed in KCP 2.1/01)
Report	Determination of storage stability and physical-chemical properties of prothioconazole 175 g/L + fenpropidin 250 g/L EC (ADM.03502.F.1.A) stored at 54 °C for 14 days and at 0°C for 7 days, Tsesin, N., 2020, Report no.: 000105029.061FL, Sponsor no.: 000105029
Guideline(s):	SANCO/3030/99 rev. 5
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

For the determination of the relevant impurity prothioconazole-desthio, acetonitrile was added and the sample was sonicated to complete dissolution. The sample was finally analysed by HPLC-MS/MS.

Table 5.2-3: Chromatographic conditions

Detector	HPLC-MS/MS
Mass transition	312.2 -> 70.1 (quantitation)
	312.2 -> 124.9 (qualification)

Validation - Results and discussions

Table 5.2-4: Suitable method for the determination of prothioconazole-desthio in the plant protection product ADM.03502.F.1.A

	Prothioconazole-desthio
Author(s), year	Tsesin, N., 2020
Principle of method	HPLC-MS/MS
Linearity (linear between mg/L) (correlation coefficient, expressed as r)	External standard calibration. 7 calibration points ~ 0.06 – 0.2 µg/mL (0.006 – 0.02% w/w, relative to test item concentration) r = 0.9998 linearity curve: $y = 1475519.1698x + 1456.6168$
Precision – Repeatability Mean	Spiking level 60 mg/kg (w/w): RSD ¹ = 6.06 % (n=5) Horwitz RSD ² = 5.79 % Horrat value (Hr) ³ = 1.1* *Horrat value = 1.1 is acceptable for the case when the target analyte is present in the unspiked sample at about 70% of the added material
Accuracy (% Recovery)	Spiking level 150 mg/kg: Total recovery: 106 ±1.34 % (n=4) Spiking level 84 mg/kg: Total recovery: 110 ±1.39 % (n=4) Spiking level 60 mg/kg: Total recovery: 104 ±6.06 % (n=6)
LOQ	60 mg/kg (0.06 g/L)
Interference/ Specificity	No interference
Comment	-

¹RSD = Relative Standard Deviation

²RSD calculated via Howitz equation: % RSDR = 2(1-0.5 logC)

³Horrat value (Hr) calculated as %RSD/%RSDr

⁴One outlier was discarded according to the Grubbs test

Conclusion

The analytical method provides a specific determination of the relevant impurity prothioconazole-desthio in the formulation ADM.03502.F.1.A and fulfils the requirements of SANCO/3030/99 rev.5.

The following study has not been evaluated during the EU peer review of prothioconazole or fenpropidin.

Reference:	KCP 5.1.1/02
Report	Analytical method validation and quantification of toluene in Prothioconazole 175 g/L + Fenpropidin 250 g/L EC (ADM.03502.F.1.A), Tsesin, N., 2020, Report no.: 000105028.064FL, Sponsor no.: 000105028
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.

Table 5.2-2: Chromatographic conditions GC-FID

Detector	GC-FID
Temperature	150°C Isothermal
Linear velocity	38 cm/sec (Hydrogen)
Injection temperature	250°C
Determination temperature	350°C
Spit flow	100 mL/min

Table 5.2-3: Chromatographic conditions GC-MS

Detector	GC-FID
Temperature	125°C Isothermal
Linear velocity	38 cm/sec (Hydrogen)
Injection temperature	250°C
Determination temperature	320°C
Spit flow	60 mL/min

Validation - Results and discussions

Table 5.2-7: Suitable method for the determination of toluene in the plant protection product ADM.03502.F.1.A

	Toluene
Author(s), year	Tsesin, N., 2020
Principle of method	GC-FID
Linearity (linear between mg/L) (correlation coefficient, expressed as r)	0.025 – 0.25 % of the working concentration 7 calibration points r = 0.9994 linearity curve: $y = 779.9968 + 0.8189x$
Precision – Repeatability Mean n = 5 (%RSD)	RSD ¹ = 0.58 Horwitz RSDr ² = 4.67 % (at 0.025% w/w) Horrat Hr ³ = 0.12
Accuracy n = 5 (% Recovery)	<u>Spiking level 0.1%:</u> Total recovery: 102 ± 0.57% (n=4) <u>Spiking level 0.05%:</u> Marginal recovery: 102 ± 0.5% (n=4) <u>Spiking level 0.025%:</u> Marginal recovery: 101 ± 0.58 % (=5)
Interference/ Specificity	Highly specific method, no interferences detected. GC-MS for confirmation
LOQ	0.025 % (0.26 g/L)
Comment	-

¹RSD = Relative Standard Deviation

²RSD calculated via Howitz equation: % RSDr = 2(1-0.5 logC)

³Horrat value (Hr) calculated as %RSD/%RSDr

Conclusion

The analytical method provides a specific determination of the relevant impurity toluene in the formulation ADM.03502.F.1.A 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 and/or fenpropidin in SC 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 and fenpropidein 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 5.2-8: Validated methods for the generation of pre-authorization data for 1,2,4-Triazole, Triazole Alanine, Triazole Acetic Acid and Triazole Lactic Acid

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, grapes and dried beans	0.01 mg/kg*	LC-MS/MS or LC-DMS-MS/MS	Klimmek, S and Gizler, A., 2017, KCP 5.1.2/01 (filed in KCA 6.1/01)
	Wheat (whole plant, grain, straw), barley (whole plant, grain, straw), oilseed rape (seeds, crude oil, refined oil, pressed cake), sunflower (seeds)	0.01 mg/kg*	LC-MS/MS	Gustloff, C.; Wallbaum, P., 2021, KCP 5.1.2/19 (method validation for: Mahlow, S., 2021, KCA 6.3.2/02 (report no. S19-00752) Yozgatli, H.P., 2021, , KCA 6.3.2/04 (report no. S20-01302) Huauhmé, J.-M., 2021, KCA 6.3.2/05 (report no. BPL21/962/GC) Huauhmé, J.-M., 2022, KCA 6.3.2/07 (report no. BPL21/960/GC)
Animal products, food of animal origin (Residues)	-			
Soil, water, sediment (Environmental fate)	-			
Soil, water (Efficacy)	-			
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)

Table 5.2-9: Validated methods for the generation of pre-authorization data for prothioconazole

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) Huauilmé, J.-M., 2020, KCP 5.1.2/03 (filed in KCA 6.3.1/01)
		0.01 mg/kg**	LC-MS/MS	Lefresne, S. 2021, KCP 5.1.2/18 (method validation for: Le Mineur, A., 2021, KCA 6.3.1/03)
	Oilseed rape seeds, strawberry, dry bean	0.01 mg/kg**	LC-MS/MS	Lefresne, S., 2020, KCP 5.1.2/02 (filed in KCA 6.1/02)
	Barley whole plant, grain, straw	0.01 mg/kg**	LC-MS/MS	Huauilmé, J.-M., 2020, KCP 5.1.2/04 (filed in KCA 6.3.2/01) and Huauilmé, J.-M., 2021, KCP 5.1.2/05 (filed in KCA 6.3.2/03)
		0.01 mg/kg**	LC-MS/MS	Lefresne, S. 2021, KCP 5.1.2/18 (method validation for: Barbier, G., 2022, KCA 6.3.2/06 (report no. B21G-A4-P-05)
	Barley grain, straw, radish, lettuce and soil	0.01 mg/kg**	LC-MS/MS	Semrau, J. 2021, KCP 5.1.2/20 filed in KCA 6.6.2/01 (report no. S18-02513)
Animal products, food of animal origin (Residues)	-			
Body fluids, air (Exposure)	Air filters and air sampling tubes	10 ng/tube	LC-MS/MS	Anonymous, 2010, KCP 5.1.2/06 (filed in KCP 7.2.2.2/01)
Soil, water, sediment (Environmental fate)	-			
Soil, water (Efficacy)	-			
Feed, body fluids (Toxicology)	-			
Soil, water, sucrose solution (Ecotoxicology)	Water (from the aqua toxicity test)	0.3505 mg/L	HPLC-MS/MS	...
		0.1871 mg/L	HPLC-MS/MS	Renner, P., 2020, KCP 5.1.2/11 (filed in KCP 10.2.1/02)
		0.207 µg/L	LC-MS/MS	Scheerbaum, D., 2021, KCP 5.1.2/12 (filed in KCP 10.2.1/03)
		0.001561 mg/L	HPLC-MS/MS	Renner, P., 2021, KCP 5.1.2/13 (filed in KCP 10.2.1/04)
	Bee diet	76.2 mg/L	HPLC-MS/MS	Dreßler, K., 2020, KCP 5.1.2/14 (filed in KCP 10.3.1.2/01)
		0.0204 mg/L	HPLC-MS/MS	Hänsel, M., 2021, KCP 5.1.2/15 (filed in KCP 10.3.1.3/01)

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
	Spray solution	436.4 mg/L	HPLC-DAD	Kästner, K., 2020, KCP 5.1.2/16 (filed in KCP 10.6.1/01)
				Kästner, K., 2020, KCP 5.1.2/17 (filed in KCP 10.6.1/02)
Phys-chem (Properties)	Active Substance in Formulation (Storage stability)	Not relevant	HPLC-DAD	Tsesin, N., 2020 KCP 5.1.1/01

* 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 5.2-10: Validated methods for the generation of pre-authorization data for fenpropidin

Component of residue definition: Fenpropidin and its salts				
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	Huauilmé, J.-M., 2020, KCP 5.1.2/03 (filed in KCA 6.3.1/01)
	Barley whole plant, grain, straw	0.01 mg/kg	LC-MS/MS	Huauilmé, J.-M., 2020, KCP 5.1.2/04 (filed in KCA 6.3.2/01) and Huauilmé, J.-M., 2021, KCP 5.1.2/05 (filed in KCA 6.3.2/02)
Animal products, food of animal origin (Residues)	-			
Body fluids, air (Exposure)	Air filters and air sampling tubes	10 ng/tube	LC-MS/MS	Anonymous, 2010, KCP 5.1.2/06 (filed in KCP 7.2.2.2/01)
Soil, water, sediment (Environmental fate)	Soil	0.05 mg/kg	HPLC-MS/MS	Morlock, G., 2006, KCP 5.1.2/07 (filed in KCP 9.1.1.1/01)
	Soil	0.05 mg/kg	HPLC-MS/MS	Morlock, G., 2006, KCP 5.1.2/08 (filed in KCP 9.1.1.1/02)
	Soil	0.02 mg/kg*	LC-MS/MS	Flörchinger, M., 2008, KCP 5.1.2/09 (filed in KCP 9.1.1.1/03)
Soil, water (Efficacy)	-			
Feed, body fluids (Toxicology)	-			
Soil, water, sucrose solution (Ecotoxicology)	Water (from the aqua toxicity test)	0.5055 mg/L	HPLC-MS/MS	...
		0.2699 mg/L	HPLC-MS/MS	Renner, P., 2020, KCP 5.1.2/11 (filed in KCP 10.2.1/02)
		0.155 µg/L	LC-MS/MS	Scheerbaum, D., 2021, KCP 5.1.2/12 (filed in KCP 10.2.1/03)

Component of residue definition: Fenpropidin and its salts				
Matrix type	Matrix	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
		0.002252 mg/L	HPLC-MS/MS	Renner, P., 2021, KCP 5.1.2/13 (filed in KCP 10.2.1/04)
	Bee diet	110 mg/L	HPLC-MS/MS	Dreßler, K., 2020, KCP 5.1.2/14 (filed in KCP 10.3.1.2/01)
		0.0294 mg/L	HPLC-MS/MS	Hänsel, M., 2021, KCP 5.1.2/15 (filed in KCP 10.3.1.3/01)
	Spray solution	630.1 mg/L	HPLC-DAD	Kästner, K., 2020, KCP 5.1.2/16 (filed in KCP 10.6.1/01)
				Kästner, K., 2020, KCP 5.1.2/17 (filed in KCP 10.6.1/02)
Phys-chem (Properties)	Active Substance in Formulation (Storage stability)	Not relevant	HPLC-DAD	Tsesin, N., 2020 KCP 5.1.1/01

*For fenpropidin acid

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

5.3.1 Analysis of the plant protection product (KCP 5.2)

Analytical methods for the determination of the active substances and relevant impurities in the plant protection product are submitted under point 5.2.1.

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

5.3.2.1 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 / limit	Reference for MRL/level Remarks
Food of plant origin	Prothioconazole-desthio	0.05 mg/kg for wheat, barley (forage and straw) 0.02 g/kg for wheat, barley (grain), canola (seed), tomato, orange (fruit)	EFSA Scientific report 2007
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 vegetables, 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	Sum of prothioconazole-desthio and its glucuronide-conjugate, expressed as prothioconazole-desthio	0.01 mg/kg (meat, liver, kidney, fat) 0.004 mg/kg (milk)	EFSA Scientific report 2007
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
Soil (Ecotoxicology)	Prothioconazole, prothioconazole-desthio (M04)	0.006 mg/kg 0.05 mg/kg	EFSA Scientific report 2007 General limit according to SANTE/2020/12830, Rev.1
Drinking water (Human toxicology)	Prothioconazole, prothioconazole-desthio (M04)	0.1 µg/L	EFSA Scientific report 2007 General limit for drinking water
Surface water (Ecotoxicology)	Prothioconazole, prothioconazole-desthio (M04)	0.05 µg/L	
Air	Prothioconazole	0.015 mg/m ³	EFSA Scientific report 2007
	Prothioconazole-desthio (M04)	0.0006 mg/m ³	
Body fluids	None allocated	n.a.	EFSA Scientific report 2007
Body tissues			
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

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

An overview on the acceptable methods and possible data gaps for analysis of 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., 2022, 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., 2022, 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., 2022, KCP 5.2/03
	Confirmatory	Not required		
Dry commodity with high protein/high starch content	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

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
(wheat grain)				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		
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., 2022, 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. 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 radiolabelled 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 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 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.

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

An overview on the acceptable methods and possible data gaps for analysis of 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	Prothioconazole-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	Prothioconazole-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	Prothioconazole-desthio	Primary	0.01 mg/kg	HPLC-MS/MS	Watson, G., 2022, 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	Prothioconazole-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 to 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 radiolabelled 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.

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

An overview on the acceptable methods and possible data gaps for analysis of 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, 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)
Confirmatory	Not required		

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

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

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

An overview on the acceptable methods and possible data gaps for analysis of for analysis of prothioconazole residues in air is given in the following tables. 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)			
Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Primary	0.015 mg/m ³	HPLC-MS/MS	Massfeld, W. (2002); 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		

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		

5.3.2.7 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.

Table 5.3-9: 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		

5.3.2.8 Other studies/ information

No other studies were submitted.

5.3.3 Description of analytical methods for the determination of residues of Fenpropidin (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:

Matrix	Residue Definition	Reference
Plant commodities	Sum of fenpropidin and its salts	EFSA Scientific Report 2007; 124, 1-84
Animal origin	Sum of fenpropidin, its salts and CGA 289267, expressed as fenpropidin	EFSA Scientific Report 2007; 124, 1-84
Soil	Fenpropidin and its salts	EFSA Scientific Report 2007; 124, 1-84
Surface water	Fenpropidin and its salts	EFSA Scientific Report 2007; 124, 1-84
Drinking / ground water	Fenpropidin and its salts	EFSA Scientific Report 2007; 124, 1-84
Air	Fenpropidin and its salts	EFSA Scientific Report 2007; 124, 1-84
Body fluids / tissues	Fenpropidin and its salts	EFSA Scientific Report 2007; 124, 1-84

Table 5.3-10: 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 (Matrices with high water content, dry matrices, acidic matrices and matrices with high oil content)	Fenpropidin (sum of fenpropidin and its salts, expressed as fenpropidin) (R) (A)	0.01 mg/kg	Reg. (EU) No 61/2014
Food of plant origin (Dry matrices (cereals))	Fenpropidin (sum of fenpropidin and its salts, expressed as fenpropidin) (R) (A)	0.01 mg/kg	SANTE/2020/12830, Rev.1
Food of plant origin (difficult matrices)	Fenpropidin (sum of fenpropidin and its salts, expressed as fenpropidin) (R) (A)	0.02 mg/kg	Reg. (EU) No 61/2014
Food of animal origin (Muscle, fat, liver/kidney, milk and egg)	Fenpropidin (sum of fenpropidin and its salts, expressed as fenpropidin) (R) (A)	0.01 mg/kg	SANTE/2020/12830, Rev.1
Food of animal origin (Honey)	Fenpropidin (sum of fenpropidin and its salts, expressed as fenpropidin) (R) (A)	0.05 mg/kg	Reg. (EU) No 61/2014
Soil (Ecotoxicology)	Fenpropidin and its salts	0.05 mg/kg	SANTE/2020/12830, Rev.1
Drinking water (Human toxicology)		0.1 µg/L	General limit for drinking water
Surface water (Ecotoxicology)		0.8 µg/L	SANTE/2020/12830, Rev.1 (based on an NOEC for algae)
Air		6 µg/m ³	SANTE/2020/12830, Rev.1 (based on an AOEL of 0.2 mg/kg bw/day)
Body fluids		0.01 mg/L	Not classified as T / T+, however, limit specified in SANTE/2020/12830, Rev.1
Body tissues		0.01 mg/kg	Not classified as T / T+, however, limit specified in

Matrix	Residue definition	MRL / LOQ	Reference for MRL/level Remarks
			SANTE/2020/12830, Rev.1

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

For plant residue analysis, the applicant refers to the unprotected methods available on EU level (DAR Addendum 2007). An overview on the acceptable methods and possible data gaps for analysis of fenpropidin in plant matrices is given in the following table.

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

Sum of fenpropidin and its salts				
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 (sugar beet and apple))	Primary	0.01 mg/kg	LC-MS/MS	Elliot, A.J. (2004); DAR Addendum Fenpropidin, Volume 3, Annex B, 5, IIA 4.2.1 EU agreed (EFSA Scientific report 2007)
	ILV	0.01 mg/kg	LC-MS/MS	Benazeraf, L. (2005); DAR Addendum Fenpropidin, Volume 3, Annex B, 5, IIA 4.2.1 EU agreed (EFSA Scientific report 2007)
	Confirmatory	Not required		
High acid content (grapes)	Primary	0.01 mg/kg	LC-MS/MS	Elliot, A.J. (2004); DAR Addendum Fenpropidin, Volume 3, Annex B, 5, IIA 4.2.1 EU agreed (EFSA Scientific report 2007)
	ILV	0.01 mg/kg	LC-MS/MS	Benazeraf, L. (2005); DAR Addendum Fenpropidin, Volume 3, Annex B, 5, IIA 4.2.1 EU agreed (EFSA Scientific report 2007)
	Confirmatory	Not required		
High oil content (Rape seed)	Primary	0.01 mg/kg	LC-MS/MS	Elliot, A.J. (2004); DAR Addendum Fenpropidin, Volume 3, Annex B, 5, IIA 4.2.1 EU agreed (EFSA Scientific report 2007)
	ILV	0.01 mg/kg	LC-MS/MS	Benazeraf, L. (2005); DAR Addendum Fenpropidin, Volume 3, Annex B, 5, IIA 4.2.1 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	Elliot, A.J. (2004); DAR Addendum Fenpropidin, Volume 3, Annex B, 5, IIA 4.2.1 EU agreed (EFSA Scientific report 2007)
	ILV	0.01 mg/kg	LC-MS/MS	Benazeraf, L. (2005); DAR Addendum Fenpropidin, Volume 3,

Sum of fenpropidin and its salts				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
				Annex B, 5, IIA 4.2.1 EU agreed (EFSA Scientific report 2007)
	Confirmatory	Not required		

Table 5.3-12: Statement on extraction efficiency

Required, available from:	Draft Assessment Report DAR – FENPROPIDIN, June 2005, Volume 3, Annex B.5 and B7 Extraction efficiency was demonstrated
Not required, because:	-

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 fenpropidin residues in metabolism studies (using radiolabelled 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). The extraction efficiency of the analytical method used in the metabolism studies in cereals (Gross, D. 1994a and 1994b), sugar beets (Gross, D-1998a), grape vine (Gross, D. 1998b) and banana (Gentile, B., 1998) was demonstrated to be sufficient with at least 70% of the applied TTR extracted and $> 50\%$ of the TRR attributed to components of residue definition for monitoring in plant matrices. As the solvent system used in the metabolism studies (methanol/water (80:20, v/v) given above is identical to the solvent system used in the monitoring method by Elliot, A.J., 2004, the extraction of the analytical monitoring method for determination of fenpropidin in plant matrices is demonstrated to be sufficiently efficient.

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

For analysis of food and feed of animal origin, the applicant refers to the unprotected methods available on EU level (DAR Addendum 2007). An overview on the acceptable methods and possible data gaps for analysis of Fenpropidin in animal matrices is given in the following table.

Table 5.3-13: Validated methods for food and feed of animal origin (if appropriate)

Component of residue definition: Sum of fenpropidin, its salts and CGA 289267, expressed as fenpropidin				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Meat, liver, kidney, fat, eggs, milk	Primary	0.01 mg/kg for meat, liver, kidney, fat, eggs 0.005 mg/kg for milk	LC-MS/MS	Elliot, A.J. (2005); DAR Addendum Fenpropidin, Volume 3, Annex B, 5, IIA 4.2.1 EU agreed (EFSA Scientific report 2007)
Meat, fat, milk	ILV	0.01 mg/kg for meat, fat 0.005 mg/kg for milk	LC-MS/MS	Bour, D. (2006); DAR Addendum Fenpropidin, Volume 3, Annex B, 5, IIA 4.2.1 EU agreed (EFSA Scientific report 2007)
	Confirmatory	Not required		

Table 5.3-14: Statement on extraction efficiency

	Method for matrices of animal origin
Required, available from:	Draft Renewal Assessment Report RAR – Fenpropidin, May 2021,

	Method for matrices of animal origin
	Volume 3, Annex B.5 and B7 Extraction efficiency was demonstrated
Not required, because:	-

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 fenpropidin residues in metabolism studies (using radiolabelled 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). In all instances the extractability of radioactivity was very efficient ($> 85\%$ TRR) (Step 3 (1) and components of the DoR were $> 50\%$ of TRR (Step 3 (2)). Thus, solvents of the metabolism studies and of the monitoring methods are compared (Step 4). Analytical method REM 164.10 (animal) uses a solvent mixture (methanol/water, 4/1, v/v) to extract parent, CGA289267 and CGA289268 residues from liver, kidney and muscle and CGA289267 and CGA289268 from fat. Although the solvents used to extract these tissues in the supporting goat (acetonitrile and acetonitrile/water mixtures) and hen metabolism (acetonitrile and methanol) studies were not identical to that of the residue method, they had similar solvation properties. In all instances the extractability of radioactivity was very efficient ($> 85\%$ TRR) and consequently the extraction efficiency of the methanol/water, 8/2, v/v used in the residue method would be similarly efficient for these analytes. The corresponding residue method in REM 164.10 for extraction of parent fenpropidin from fat uses isohexane as an extraction solvent. Whilst the solvents used to extract fat in the goat metabolism study were not identical (hexane and hexane/diethyl ether mixture) to that of the residue method, they had similar solvation properties and was shown to extract parent fenpropidin efficiently from fat (93.9% of total identified fenpropidin in fat was present in hexane/diethyl ether extracts). Consequently, the extraction efficiency of the isohexane used in the residue method would be similarly efficient for parent.

The corresponding residue method in REM 164.10 for extraction of parent, CGA289267 and CGA289268 residues from milk uses extraction with acetonitrile (produces an aqueous acetonitrile fraction on addition to milk). Whilst the solvent used to extract milk in the goat metabolism study was not identical (acetone, produces an aqueous acetone fraction on addition to milk) to that of the residue method, it had similar solvation properties and was shown to extract 86% of the total radioactive in milk into this fraction. The extraction efficiency of the acetonitrile used in the residue method would therefore be similarly efficient for these analytes.

The corresponding residue method in REM 164.10 for analysis of parent, CGA289267 and CGA289268 residues in eggs uses extraction with acetonitrile. In the hen metabolism studies, egg white and egg yolk samples were analysed separately. Egg white radioactive residues were efficiently extracted ($> 94\%$ of the total radioactive residue) using the same solvent as the residue method. The solvent extraction method used to extract egg yolk in the hen metabolism study was more exhaustive (acetonitrile, methanol and methanol/water; 4/1; v/v) than that of the residue method but showed high overall extractability of the radioactive residue ($> 81\%$ of the total radioactive residue). The reports do not indicate what proportion of this residue was extracted into acetonitrile only, so it is not possible to determine the extraction efficiency of egg yolk using the residue method in this instance but the weight of evidence from egg white and all other animal tissues suggests fenpropidin and its metabolites are generally very efficiently extracted from animal matrices using organic solvents such as acetonitrile, methanol or aqueous/mixtures of either of these solvents. It is therefore likely that the extraction efficiency of acetonitrile on whole egg residues will be high.

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 residues of fenpropidin is given in the following table.

Table 5.3-15: Validated methods for soil (if appropriate)

Component of residue definition: Fenpropidin and its salts				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Soil	Primary	0.01 mg/kg	LC-MS/MS	Hargreaves, S. L. (2007); DAR Addendum Fenpropidin, Volume 3, Annex B, 5, IIA 4.2.2/02 EU agreed (EFSA Scientific report 2007)
	Confirmatory	Not required as the primary method is highly specific		

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

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 fenpropidin in surface and drinking water is given in the following tables.

Table 5.3-16: Validated methods for water (if appropriate)

Component of residue definition: Fenpropidin and its salts				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Drinking water	Primary	0.1 µg/L	LC-MS/MS	Royer, A. (2007); DAR Addendum Fenpropidin, Volume 3, Annex B, 5, IIA 4.2.3 EU agreed (EFSA Scientific report 2007)
	Confirmatory	Not required as the primary method is highly specific		
	Primary	0.05 µg/L	LC-MS/MS	Richardson M., 2007, KCP 5.2/10
	ILV	0.05 µg/L	LC-MS/MS	Devine, T., 2017, KCP 5.2/11
	Confirmatory	Not required as the primary method is highly specific		
Surface water	Primary	0.1 µg/L	LC-MS/MS	Royer, A. (2007); DAR Addendum Fenpropidin, Volume 3, Annex B, 5, IIA 4.2.3 EU agreed (EFSA Scientific report 2007)
	Primary	0.05 µg/L	LC-MS/MS	Richardson M., 2007, KCP 5.2/10
	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 Fenpropidin in air is given in the following tables.

Table 5.3-17: Validated methods for air (if appropriate)

Component of residue definition: Fenpropidin and its salts			
Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Primary	0.15 µg/m ³	LC-MS/MS	Evans, P.G. (2006); DAR

Component of residue definition: Fenpropidin and its salts			
Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
			Addendum Fenpropidin, Volume 3, Annex B, 5, IIA 4.2.4 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 fenpropidin in body fluids is given in the following table.

Table 5.3-18: Methods for body fluids and tissues (if appropriate)

Component of residue definition: None allocated (EFSA Scientific report, 2007)			
Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Primary	0.01 mg/kg for blood	LC-MS/MS	Cross, M., 2017, KCP 5.2/11
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	Previously used Y/N If yes, for which data point?
KCP 5.1.1/01 filed in KCP 2.1/01	Tsesin, N.	2020	Determination of storage stability and physical-chemical properties of prothioconazole 175 g/L + fenpropidin 250 g/L EC (ADM.03502.F.1.A) stored at 54 °C for 14 days and at 0°C for 7 days Report no. 000105029.061FL, Sponsor no. 000105029 ADAMA Makhteshim Ltd., Beer-Sheva, Israel GLP / GEP Unpublished	N	ADM	
KCP 5.1.1/02	Tsesin, N.	2020	Analytical method validation and quantification of toluene in Prothioconazole 175 g/L + Fenpropidin 250 g/L EC (ADM.03502.F.1.A) Report no. 000105028.064FL, Sponsor no.: 000105028 ADAMA Makhteshim Ltd., Beer-Sheva, Israel GLP / GEP Unpublished	N	ADM	
KCP 5.1.2/01 (filed in 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	Y evaluated in the dRR for ADM.03500.F.2.B (Soratel) on 11.2022
KCP 5.1.2/02 (filed in 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, Beaucauzé Cedex, France GLP Unpublished	N	ADM	Y evaluated in the dRR for ADM.03500.F.2.B (Soratel) on 11.2022

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	Previously used Y/N If yes, for which data point?
KCP 5.1.2/03 (filed in KCA 6.3.1/01)	Huaultmé, J.-M.	2020	Residue study of prothioconazole and its metabolites, and fenpropidin in wheat whole plant and RAC (grain and straw) after one foliar application of ADM.3502.F.1.A - 2 HS and 2 DCS - Northern Europe (France, Poland and Hungary) - 2019 Report no.: BPL19/770/GC, sponsor no.: 000102759 BIOTEK Agriculture, Saint-Pouange, France GLP Unpublished	N	ADM	N
KCP 5.1.2/04 (filed in KCA 6.3.2/01)	Huaultmé, 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	Y for prothioconazole evaluated in the dRR for ADM.03500.F.2.B (Soratel) on 11.2022 N for fenpropidin
KCP 5.1.2/05 (filed in KCA 6.3.2/02)	Huaultmé, J.-M.	2021	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	Y for prothioconazole evaluated in the dRR for ADM.03500.F.2.B (Soratel) on 11.2022 N for fenpropidin
KCP 5.1.2/06 (filed in KCP 7.2.2.2/01)	Anonymous	2010	Development of air sampling methodology in support of determining risk of bystander and resident exposure to pesticides SID 5 (Rev. 07/10), DEFRA Project PS2023 Non-GLP Published	N	n.a.	N
KCP 5.1.2/07 (filed in KCP 9.1.1.1/01)	Morlock, G.	2006a	Degradation of Fenpropidin in 3 different soils under aerobic conditions at 20° C in the dark Report No 20051244/01-CABJ, sponsor no. 00012949 GLP Unpublished	N	IRVITA*	N
KCP 5.1.2/08 (filed in	Morlock, G.	2006b	Degradation of Fenpropidin in one soil under aerobic conditions at 20° C in the dark Report No 20051244/02-CABJ, sponsor no. 00012950 GLP	N	IRVITA*	N

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	Previously used Y/N If yes, for which data point?
KCP 9.1.1.1/02)			Unpublished			
KCP 5.1.2/09 (filed in KCP 9.1.1.1/03)	Flörchinger M.	2008	Degradation of Fenpropidin Acid in 3 Different Soils under Aerobic Conditions at 20°C in the Dark Eurofins-GAB GmbH Report No.S08-01156, sponsor no. 00016350 GLP Unpublished	N	IRVITA*	N
KCP 5.1.2/10 (filed in KCP 10.2.1/01)	...	2020a	Acute toxicity of ADM.03502.F.1.A to <i>Oncorhynchus mykiss</i> in a 96-hour semi-static test Report no ..., Sponsor no.: GLP Unpublished	Y	ADM	N
KCP 5.1.2/11 (filed in KCP 10.2.1/02)	Renner, P.	2020b	Acute toxicity of ADM.03502.F.1.A to <i>Daphnia magna</i> in a 48-hour semi-static test Report no 2048ADL0008, Sponsor no.: 000104840 BioChem agrar, Machern/Gerichshain, Germany GLP Unpublished	N	ADM	N
KCP 5.1.2/12 (filed in KCP KCP 10.2.1/03)	Scheerbaum, D.	2021	ADM.03502.F.1.A - Alga, Growth Inhibition Test with <i>Desmodesmus subspicatus</i> , 72 hours Report no. SO21519 / SSO19707, Sponsor no.: 000108687 Noack Laboratorien GmbH, Sarstedt, Germany GLP Unpublished	N	ADM	N
KCP 5.1.2/13 (filed in KCP 10.2.1/04)	Renner, P.	2021	Effects of ADM.03502.F.1.A on <i>Lemna gibba</i> in a growth inhibition test under semi-static test conditions Report no 2048ALE0006, Sponsor no.: 000104842 BioChem agrar, Machern/Gerichshain, Germany GLP Unpublished	N	ADM	N
KCP 5.1.2/14 (filed in KCP 10.3.1.2/01)	Dreßler, K.	2020	Chronic oral toxicity of ADM.03502.F.1.A to the honey bee <i>Apis mellifera</i> L. under laboratory conditions Report no.: 2048BAC0011, Sponsor no.: 000104844 BioChem agrar, Machern/Gerichshain, Germany GLP Unpublished	N	ADM	N
KCP 5.1.2/15	Hänsel, M.	2021	ADM.03502.F.1.A – Repeated exposure of honey bee larvae (<i>Apis mellifera</i> L.) under laboratory conditions Report no.: 2048BLC0013, Sponsor no.: 000104845	N	ADM	N

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	Previously used Y/N If yes, for which data point?
(filed in KCP 10.3.1.3/01)			BioChem agrar, Machern/Gerichshain, Germany GLP Unpublished			
KCP 5.1.2/16 (filed in KCP 10.6.1/01)	Kästner, K.	2020a	Effects of ADM.03502.F.1.A on seedling emergence and seedling growth of six non-target terrestrial plant species under greenhouse conditions Report no.: 2046PSE0007, Sponsor no.: 000104852 BioChem agrar, Machern/Gerichshain, Germany GLP Unpublished	N	ADM	N
KCP 5.1.2/17 (filed in KCP 10.6.1/02)	Kästner, K.	2020b	Effects of ADM.03502.F.1.A on vegetative vigour of six non-target terrestrial plant species under greenhouse conditions Report no.: 2035CRX0012, Sponsor no.: 000104853 BioChem agrar, Machern/Gerichshain, Germany GLP Unpublished	N	ADM	N
KCP 5.1.2/18	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	Y evaluated in the dRR for ADM.03500.F.2.B (Soratel) on 11.2022
KCP 5.1.2/19	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 Agrosience Services Chem GmbH, Hamburg, Germany GLP Unpublished	N	ADM	Y evaluated in the dRR for ADM.03500.F.2.B (Soratel) on 11.2022
KCP 5.1.2/20 (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 Agrosience Services GmbH, Stade, Germany GLP, Unpublished	N	ADM	Y evaluated in the dRR for ADM.03500.F.2.B (Soratel) on 11.2022
KCP 5.2/01	Brown, S.	2022	Development and Validation of an Analytical Method for Determination of Residues of Prothioconazole-desthio in	N	ADM	Y

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	Previously used Y/N If yes, for which data point?
			Body Fluids (Blood) by LC-MS/MS Report no.: RES-00373, Sponsor no.: 000109608 ResChem Analytical Limited, Derby, UK GLP Unpublished			evaluated in the dRR for ADM.03500.F.2.B (Soratel) on 11.2022
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, Israel GLP Unpublished	N	ADM	Y evaluated in the dRR for ADM.03500.F.2.B (Soratel) on 11.2022
KCP 5.2/03	Watson, G.	2022	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	Y evaluated in the dRR for ADM.03500.F.2.B (Soratel) on 11.2022
KCP 5.2/04	Watson, G.	2022	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	Y evaluated in the dRR for ADM.03500.F.2.B (Soratel) on 11.2022
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 Agrosience Services Chem GmbH, Hamburg, Germany GLP Unpublished	N	ADM	Y evaluated in the dRR for ADM.03500.F.2.B (Soratel) on 11.2022
KCP 5.2/06	Lefresne, S.	2021	Validation of an analytical method for the determination of prothioconazole residues in honey Report no.: B21S-A4-P-04, Sponsor no.: 000108774 FREDON Pays de la Loire / GIRPA, Beaucouzé Cedex, Israel GLP Unpublished	N	ADM	Y evaluated in the dRR for ADM.03500.F.2.B (Soratel) on 11.2022

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	Previously used Y/N If yes, for which data point?
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	Y evaluated in the dRR for ADM.03500.F.2.B (Soratel) on 11.2022
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	Y evaluated in the dRR for ADM.03500.F.2.B (Soratel) on 11.2022
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 Currenta GmbH & Co. OHG Analytik, Leverkusen, Germany GLP Unpublished	N	BCS/ADM	Y evaluated in the dRR for ADM.03500.F.2.B (Soratel) on 11.2022
KCP 5.2/10	Richardson, M.	2007	Fenpropidin (CGA114900) – Residue method for the determination of Fenpropidin and metabolite CGA289267 in water. Final determination by LC-MS/MS Report no.: GRM024.03A, Sponsor no.: - Syngenta, Jealott's Hill International Research Centre, Bracknell, Berkshire, UK Not GLP Unpublished	N	SYN/ADM	N
KCP 5.2/11	Devine, T.	2016	Fenpropidin (CGA114900) - Independent Laboratory Validation of an Analytical Method GRM024.03A for the Determination of Residues of Fenpropidin (CGA114900) and its Metabolite CGA289267 in Water by LC-MS/MS Report no.: RES-00373, Sponsor no.: - CEM Analytical Services Limited (CEMAS), Wokingham, UK GLP Unpublished	N	SYN/ADM	N
KCP 5.2/12	Cross, M.	2017	Fenpropidin: Validation of Analytical Method REM 164.10 for the Determination of Residues of Fenpropidin and its Metabolites CGA289267 and CGA289268 in Blood by LC-MS/MS Report no.: CEMR-8288, Sponsor no.: - CEM Analytical Services Limited (CEMAS), Wokingham, UK GLP Unpublished	N	SYN/ADM	N

* IRVITA, now ADAMA Irvita N.V.

ADM is ADAMA Makhteshim Ltd. All ADAMA affiliates are member of ADAMA Agricultural Solutions Ltd.

BCS/ADM = Study is co-owned by Bayer Crop Science and ADAMA Agricultural Solution and all affiliates

SYN/ADM = Study is co-owned by Syngenta Ltd and ADAMA Agricultural Solution and all affiliates

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

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 all active substances

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

A 2.1.1.1 Residue analytical methods

The following study provides also the method validation for Le Mineur, A. 2021 (KCA 6.3.1/02, report no.: BPL21/956/GC, sponsor no.: 000107610).

Comments of zRMS:	<p>The study of Klimmek, S. and Gizler, A., 2017 (Report No.: S12-00072) on 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 during 36 months has been evaluated in Registration Report for ADM.03500.F.2.B (Soratel) on November 2022 by zRMS-PL and the summary is presented below.</p> <p>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.</p> <p>The limit of quantification (LOQ) for all triazole metabolites was 0.01 mg/kg. The limit of detection (LOD) was 0.003 mg/kg.</p> <p>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.</p> <p>The method complies with EU Guidelines SANCO/825/00 rev.8.1 and SANCO/3029/99 rev.4.</p> <p>The method is acceptable.</p>
-------------------	--

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 n	103 ± 10 5	96 ± 6.5 3	82 ± 7.8 3	94 ± 2.7 3	110 ± 1.4 5	110 ± 2.6 3	80 ± 3.3 3	107 ± 8.4 3
0.01 and 0.10	Overall ± RSD n	102 ± 8.9 10	94 ± 9.6 6	95 ± 14 6	88 ± 9.7 6	109 ± 3.3 6	110 ± 7.6 6	81 ± 6.3 6	110 ± 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:	<p>The study of Lefresne, S., 2020 (Report No.: B18S-A4-P-02) on freezing storage stability of prothioconazole-desthio and hydroxy metabolites in plant matrices at/below -18°C during 24 months has been evaluated in Registration Report for ADM.03500.F.2.B (Soratel) on November 2022 by zRMS-PL and the summary is presented below.</p> <p>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.</p> <p>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.</p> <p>The LOQ for the sum of all prothioconazole-items was 0.060 mg/kg for each matrix.</p> <p>The method complies with EU Guidelines SANCO/825/00 rev.8.1 and SANCO/3029/99 rev.4.</p> <p>The method is acceptable.</p>
-------------------	---

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-prothioconazole-desthio, 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 $RSD \leq 20$. 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. The LOQ for the sum of all prothioconazole-items was 0.060 mg/kg for each matrix.

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	100-102 101 ± 1 5	99-103 101 ± 2 5	100-102 101 ± 1 5	99-105 102 ± 2 5	100-105 103 ± 2 5	101-108 105 ± 2 5	101-105 103 ± 2 5	98-109 105 ± 4 5	104-108 106 ± 1 5	105-110 108 ± 2 5	104-107 106 ± 1 5	99-102 100 ± 1 5
0.100	Range Mean ± RSD n	100-108 103 ± 3 5	99-106 101 ± 3 5	103-112 107 ± 4 5	103-111 107 ± 3 5	103-114 108 ± 5 5	105-118 110 ± 5 5	101-113 107 ± 5 5	100-113 108 ± 5 5	108-114 110 ± 2 5	106-115 110 ± 3 5	105-114 110 ± 3 5	99-110 106 ± 4 5
0.01 and 0.10	Overall ± RSD n	102 ± 2 10	101 ± 2 10	104 ± 4 10	104 ± 3 10	106 ± 4 10	107 ± 4 10	105 ± 4 10	106 ± 5 10	108 ± 2 10	109 ± 2 10	108 ± 3 10	103 ± 4 10

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

RSD = relative standard deviation, n = number of replicates

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 [mg/kg]	Analyte	Prothioconazole-desthio		3-hydroxy-prothioconazole-desthio		4-hydroxy-prothioconazole-desthio		5-hydroxy-prothioconazole-desthio		6-hydroxy-prothioconazole-desthio		alpha-hydroxy-prothioconazole-desthio	
		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 [mg/kg]	Analyte	Prothioconazole-desthio		3-hydroxy-prothioconazole-desthio		4-hydroxy-prothioconazole-desthio		5-hydroxy-prothioconazole-desthio		6-hydroxy-prothioconazole-desthio		alpha-hydroxy-prothioconazole-desthio	
		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 [mg/kg]	Analyte	Prothioconazole-desthio		3-hydroxy-prothioconazole-desthio		4-hydroxy-prothioconazole-desthio		5-hydroxy-prothioconazole-desthio		6-hydroxy-prothioconazole-desthio		alpha-hydroxy-prothioconazole-desthio	
		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
Assessment of matrix effects is presented	Not required, 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:	<p>For fenpropidin and prothioconazole and its metabolites, the analytical methods were validated on wheat (whole plant, grain and straw), following the guideline SANCO/3029/99.</p> <p>All the analytes were determined by LC-MS/MS using a quantitation and confirmation ion. LOQ: 0.01 mg/kg for each analyte,</p> <p>The mean recoveries 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.</p> <p>The storage duration (interval between sampling and extraction date) was 108 days for the determination of prothioconazole and its metabolites and fenpropidin.</p> <p>Sufficient stability data are available to support the residue data presented in this study.</p> <p>The study is acceptable.</p>
-------------------	---

Reference:	KCP 5.1.2/03 (filed in KCA 6.3.1/01)
Report	Residue study of prothioconazole and its metabolites, and fenpropidin in wheat whole plant and RAC (grain and straw) after one foliar application of ADM.3502.F.1.A - 2 HS and 2 DCS - Northern Europe (France, Poland and Hungary) – 2019, Huauilmé, J.-M., 2020, Report no.: BPL19/770/GC, sponsor no.: 000102759
Guideline(s):	For method validation: SANCO/3029/99 rev. 4
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Prothioconazole

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 another study 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.

Fenpropidin

Residues of fenpropidin 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.

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. Detailed recovery results for prothioconazole and fenpropidin are provided in the following.

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 n	85 ± 5 10	85 ± 5 10	81 ± 4 10	83 ± 4 10	83 ± 5 10	81 ± 5 10	83 ± 5 10	84 ± 5 10	84 ± 5 10	82 ± 7 10	84 ± 4 10	83 ± 5 10

Table A 13: Recovery results from method validation of prothioconazole in grain of wheat (B19S-A4-P-01)

[illegible]

Table A 14: 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
	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

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 15: Recovery results from method validation of fenpropidin in whole plant of wheat, wheat grain and wheat straw (data obtained from study B19S-B5-FP-01)

Fortification level [mg/kg]	Matrix	Wheat whole plant		Wheat grain		Wheat straw	
	Transition ion	147 m/z	117 m/z	147 m/z	117 m/z	147 m/z	117 m/z
0.010	Range	93-98	95-103	96-103	96-100	89-92	89-93
	Mean ± RSD	96 ± 2	99 ± 3	98 ± 3	98 ± 2	90 ± 1	91 ± 2
	n	5	5	5	5	5	5
0.100	Range	95-101	97-101	85-93	83-92	85-91	85-90
	Mean ± RSD	99 ± 5	99 ± 2	89 ± 3	88 ± 4	87 ± 6	87 ± 2
	n	5	5	5	5	5	5
0.01 and 0.10	Overall ± RSD	97 ± 3	99 ± 2	94 ± 6	93 ± 7	94 ± 6	89 ± 3
	n	10	10	10	10	10	10

RSD = relative standard deviation, n = number of replicates

Table A 16: Characteristics for the analytical method used for validation of prothioconazole and fenpropidin residues in wheat

Analyte	Prothioconazole*	Fenpropidin
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	Individual calibration data presented $r > 0.99$ 7 calibration points
Calibration range	0.6 - 20 µg/L	0.6 - 40 µg/L
Assessment of matrix effects is presented	Not required, since calibration was carried out with matrix-matched standards	Not required, 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	LOQ: 0.010 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 and fenpropidin in wheat.

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

Comments of zRMS:	<p>The study of Huauilmé, J.-M., 2020 (Report No.: BPL19/772/GC) on determination of residue of prothioconazole and their metabolites in barley whole plant and RAC (grain and straw) after one foliar application of ADM.3502.F.1.A has been evaluated in Registration Report for ADM.03500.F.2.B (Soratel) on November 2022 by zRMS-PL and the summary is presented below.</p> <p>The data for fenpropidin is evaluated in this document and a summary is also provided below.</p> <p><u>Prothioconazole</u></p> <p>The analytical method was validated for barley whole plant without roots, grain and straw according to guideline SANCO/3029/99 rev. 4.</p> <p>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.</p> <p>The mean recovery was between 70% and 110% at each level of fortification, for each reference item and for each matrix.</p> <p><u>Fenpropidin</u></p> <p>The analytical method was fully validated for each matrix (barley whole plant without roots, grain and straw) in compliance with the guideline SANCO/3029/99 rev.4 of 11/07/2000. LOQ: 0.01 mg/kg.</p> <p>The mean recovery was between 70% and 110% at each level of fortification, for each reference item and for each matrix.</p> <p>The storage duration (interval between sampling and extraction date) was 114 days for the determination of prothioconazole and its metabolites and fenpropidin.</p> <p>Sufficient stability data are available to support the residue data presented in this study.</p> <p>The study is acceptable.</p>
-------------------	--

Reference:

KCP 5.1.2/04 (filed in KCA 6.3.2/01)

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
Huauilmé, J.-M., 2020
Report no.: BPL19/772/GC, sponsor no.: 000102761
For method validation: SANCO/3029/99 rev. 4

Guideline(s):
Deviations: None
GLP: Yes
Acceptability: Yes

Comments of zRMS:	<p>The study of Huauilmé, J.-M., 2021 (Report no.: BPL20/844/GC) on determination of residue of prothioconazole and their metabolites in barley whole plant and RAC (grain and straw) after one foliar application of ADM.3502.F.1.A has been evaluated in Registration Report for ADM.03500.F.2.B (Soratel) on November 2022 by zRMS-PL and the summary is presented below.</p> <p>The data for fenpropidin is evaluated in this document and a summary is also provided below.</p> <p><u>Prothioconazole</u></p> <p>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.</p> <p><u>Fenpropidin</u></p> <p>The analytical method was previously fully validated in barley (whole plants without roots, grain, straw), in compliance with Guideline SANCO/3029/99 rev.4 of 11/07/2000 during another study or analytical phase performed at GIRPA in 2019-2020 (study code: B19S-A4-P-01 and analytical phase code: B19G-B5-FP-03). 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. The mean recovery was between 70% and 110% at each level of fortification, for each matrix. The storage duration (interval between sampling and extraction date) was 147 days for the determination of prothioconazole and its metabolites. Sufficient stability data are available to support the residue data presented in this study.</p> <p>The study is acceptable.</p>
-------------------	---

Reference: KCP 5.1.2/05 (filed in KCA 6.3.2/03)
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
Huauilmé, J.-M., 2021
Report no.: BPL20/844/GC, sponsor no.: 000105350
For method validation: SANCO/3029/99 rev. 4

Guideline(s):
Deviations: None
GLP: Yes
Acceptability: Yes

Materials and methods

Prothioconazole

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-prothioconazole-desthio, 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.

Fenpropidin

Residues of fenpropidin were extracted from ground specimen by maceration with acetonitrile/water. Then, extracts were purified by dispersive solid phase extraction. The quantification was performed by LC-MS/MS.

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. Detailed recovery results for prothioconazole (including: prothioconazole-desthio, 3-hydroxyprothioconazole-desthio, 4-hydroxyprothioconazole-desthio, 5-hydroxyprothioconazole-desthio, 6-hydroxyprothioconazole-desthio and alpha-hydroxyprothioconazole-desthio) and fenpropidin are provided in the following.

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 n	85 ± 3 10	85 ± 3 10	85 ± 3 10	86 ± 3 10	85 ± 2 10	84 ± 3 10	84 ± 3 10	85 ± 3 10	87 ± 3 10	87 ± 3 10	85 ± 3 10	85 ± 4 10

Table A 18: Recovery results from method validation of prothioconazole metabolites in grain of barley

[illegible]

Table A 19: 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 20: Recovery results from method validation of fenpropidin in whole plant of barley, barley grain and barley straw (data obtained from study B19G-B5-FP-03)

Fortification level [mg/kg]	Matrix	Barley whole plant		Barley grain		Barley straw	
	Transition ion	147 m/z	117 m/z	147 m/z	117 m/z	147 m/z	117 m/z
0.010	Range	71-100	72-97	78-108	82-110	88-108	88-104
	Mean ± RSD	81 ± 14	78 ± 13	94 ± 12	98 ± 12	94 ± 8	93 ± 7
	n	5	5	5	5	5	5
0.100	Range	66-85	65-84	87-106	87-105	90-100	87-100
	Mean ± RSD	78 ± 10	77 ± 10	98 ± 7	98 ± 6	95 ± 5	94 ± 6
	n	5	5	5	5	5	5
0.01 and 0.10	Overall ± RSD	80 ± 12	78 ± 11	96 ± 9	98 ± 9	95 ± 6	94 ± 6
	n	10	10	10	10	10	10

RSD = relative standard deviation, n = number of replicates

Table A 21: Characteristics for the analytical method used for validation of prothioconazole metabolite and fenpropidin residues in barley whole plant, barley grain and barley straw

	Prothioconazole*	Fenpropidin
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	Individual calibration data presented $r > 0.99$ 7 calibration points
Calibration range	0.6 - 40 µg/L	0.6 - 100 µg/L
Assessment of matrix effects is presented	Not required, since calibration was carried out with matrix-matched standards	Not required, 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	LOQ: 0.010 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) and fenpropidin in barley whole plant, barley grain and barley straw.

The following study also provides the method validation for Le Mineur, A. 2021 (KCA 6.3.1/03, report no.: BPL21/958/GC, sponsor no.: 000107612 and Barbier, G., 2022 (KCA 6.3.2/06, report no. B21G-A4-P-05).

Comments of zRMS:	<p>The study of Lefresne, S., 2021 (Report no.: B21S-A4-P-01) on validation of an analytical method for the determination of prothioconazole residues in cereals, honey, oilseed rape and sugar beet has been evaluated in Registration Report for ADM.03500.F.2.B (Soral) on November 2022 by zRMS-PL and the summary is presented below.</p> <p>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.</p> <p>LOQ for each analyte separately: 0.010 mg/kg.</p> <p>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)).</p> <p>Acceptance criteria for method validations were met, with average recoveries ranging from 70% to 110% and relative standard deviations $\leq 20\%$.</p> <p>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).</p>
-------------------	---

Reference:	KCP 5.1.2/18
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 $RSD \leq 20$. 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. The LOQ for the sum of all prothioconazole-items was 0.060 mg/kg for each matrix.

Table A 22: 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 23: 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 24: 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 25: 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 26: 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 27: 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

A 28: 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

A 29: 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

A 30: 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

A 31: Recovery results from method validation of prothioconazole metabolites in wheat straw

Fortification level [mg/kg]	Analyte	Prothioconazole-desthio		3-hydroxy-prothioconazole-desthio		4-hydroxy-prothioconazole-desthio		5-hydroxy-prothioconazole-desthio		6-hydroxy-prothioconazole-desthio		alpha-hydroxy-prothioconazole-desthio	
		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

A 32: Recovery results from method validation of prothioconazole metabolites in wheat whole plant

Fortification level [mg/kg]	Analyte	Prothioconazole-desthio		3-hydroxy-prothioconazole-desthio		4-hydroxy-prothioconazole-desthio		5-hydroxy-prothioconazole-desthio		6-hydroxy-prothioconazole-desthio		alpha-hydroxy-prothioconazole-desthio	
		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 33: 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:

- Mahlow, S., 2021, KCA 6.3.2/02 (report no. S19-00752)
- Yozgatli, H.P., 2021, , KCA 6.3.2/04 (report no. S20-01302)
- Huaulmé, J.-M., 2021, KCA 6.3.2/05 (report no. BPL21/962/GC)
- Huaulmé, J.-M., 2022, KCA 6.3.2/07 (report no. BPL21/960/GC)

Comments of zRMS:	<p>The study of Gustloff, C.; Wallbaum, P., 2021 (Report no.: S21-02262) on validation of an analytical method for the determination of triazole metabolites (TDMs) in crop matrices has been evaluated in Registration Report for ADM.03500.F.2.B (Soratel) on November 2022 by zRMS-PL and the summary is presented below.</p> <p>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).</p> <p>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).</p> <p>Acceptance criteria for method validations were met, with average recoveries ranging from 70% to 110% and relative standard deviations $\leq 20\%$.</p> <p>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.</p>
-------------------	---

Reference: KCP 5.1.2/19
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 (GRM053.01A2). 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 \leq 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 34: 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 \pm RSD	109 \pm 7.2	97 \pm 9	103 \pm 1.5	113 \pm 4	88 \pm 6.4	113 \pm 4.1	87 \pm 13	116 \pm 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 \pm RSD	107 \pm 13	99 \pm 10	105 \pm 6.9	104 \pm 7	90 \pm 6.7	111 \pm 2.8	93 \pm 0.7	94 \pm 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 35: 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
0.010	Range	102-113	113-118	93-98	78-98	77-88	89-104	72-75	78-103
	Mean \pm RSD	107 \pm 4.9	116 \pm 2.3	96 \pm 2.3	87 \pm 12	84 \pm 7.2	98 \pm 8.1	73 \pm 2.1	92 \pm 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 \pm RSD	103 \pm 2.2	94 \pm 17	93 \pm 2.0	88 \pm 6.6	70 \pm 12	87 \pm 13	79 \pm 8.6	85 \pm 13
	n	3	3	3	3	3	3	3	3

RSD = relative standard deviation, n = number of replicates

² 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

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

Table A 36: 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 37: 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 38: 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 39: Recovery results from method validation of triazole metabolites in barley 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	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 40: 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 ±	89 ± 7.9	106 ±	96 ± 15	96 ± 11	88 ± 8.6	99 ±	86 ± 16	87 ±
	RSD		9.4				4.2		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 ±	104 ± 5.2	88 ± 17	87 ± 8.6	84 ± 12	94 ± 4.0	99 ±	87 ± 2.8	95 ±
	RSD						7.0		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

A Table A 41: 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 ±	88 ± 9.7	86 ±	95 ± 12	105 ±	93 ± 2.4	87 ±	92 ± 8.0	89 ±
	RSD		2.2		15		1.8		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 ±	103 ± 4.5	91 ± 12	96 ± 4.29	95 ±	94 ± 2.2	95 ±	95 ± 5.5	98 ±
	RSD				4.9		2.0		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 42: 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 ±	95 ± 8.3	81 ±	86 ± 6.5	87 ±	102 ± 6.8	94 ±	103 ± 4.0	99 ±
	RSD		4.5		5.2		7.8		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 ±	93 ± 7.4	90 ±	85 ± 2.3	85 ±	90 ± 6.3	81 ±	92 ± 1.1	90 ±
	RSD		8.4		0.7		3.5		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 43: 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 ±	111 ± 6.8	110 ±	101 ± 0.9	116 ±	100 ± 8.2	94 ±	84 ± 27	82 ± 16
	RSD		4.8		3.7		5.0		
	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 44: 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 45: 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 46: 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 47: 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 also the method validation for Semrau, J. 2022 (KCA 6.6.2/02, report no.: S21-00408, sponsor no.: 000107470).

Comments of zRMS:	<p>The study of Semrau, J., 2021 (Report no.: S18-02513) on 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) has been evaluated in Registration Report for ADM.03500.F.2.B (Soratel) on November 2022 by zRMS-PL and the summary is presented below.</p> <p>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.</p> <p>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.</p> <p>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.</p> <p>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</p> <p>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.</p> <p>The method is acceptable for the determination of prothioconazole radish, lettuce, barley, and soil.</p>
-------------------	--

Reference:

KCP 5.1.2/20 (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-prothioconazole-desthio, 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 $RSD \leq 20$. 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. The LOQ for the sum of all prothioconazole-items was 0.060 mg/kg for each matrix.

Table A 48: 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 49: 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 50: 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 51: Recovery results from method validation of prothioconazole metabolites in barley straw

Fortification level [mg/kg]	Analyte	Prothioconazole-desthio		3-hydroxy-prothioconazole-desthio		4-hydroxy-prothioconazole-desthio		5-hydroxy-prothioconazole-desthio		6-hydroxy-prothioconazole-desthio		alpha-hydroxy-prothioconazole-desthio	
		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 52: Recovery results from method validation of prothioconazole-desthio metabolites in soil

Fortification level [mg/kg]	Analyte	Prothioconazole-desthio	
		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 53: 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 Operator, worker, resident and bystander exposure studies - analytical methods

Comments of zRMS:	<p><u>Trial 1</u> Residues of prothioconazole present in sample extracts were quantified using liquid chromatography tandem quadrupole mass spectrometry (LC-MS/MS). Chromatographic separation of the analytes was achieved by reversed phase chromatography.</p> <p><u>Trial 2</u> Residues of fenpropidin present in sample extracts were quantified using gas chromatography tandem quadrupole mass spectrometry (GC-MS/MS). Chromatographic separation of the analytes was achieved by capillary chromatography. Two MS/MS transitions were optimised for each analyte to meet analyte identification criteria set out in the EU method Validation Guidelines (currently Document N° SANCO/12495/2011) in place at the time of analysis. Analytes were quantified against multi-level calibration solvent standards, using internal standard calibration with TPP as a volumetric internal standard.</p> <p>Results for method validation and stability are presented in Tables A54 and A55. It should be noted that it is difficult to validate these methods because the performance of each individual sorbent tube can be variable (hence all analyses were conducted in triplicate), the low target concentrations required and the fact that it is not possible to prepare matrix-matched standards. Another issue is the lack of availability of labelled internal standards for the analytes of interest.</p>
-------------------	--

Reference:	KCP 5.1.2/06 (filed in KCP 7.2.2.2/01)
Report	Development of air sampling methodology in support of determining risk of bystander and resident exposure to pesticides., Anonymous., 2010, report no DEFRA Project PS202, 2010, sponsor no.: -
Guideline(s):	SANCO/3029/99 rev. 4
Deviations:	No calibration data presented
GLP:	No
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Air sampling filters and sorbent tubes were sonicated in acetonitrile for prothioconazole or ethyl acetate for fenpropidin. The solvent extract was analysed by LC-MS/MS for prothioconazole and by GC-MS/MS for fenpropidin. An internal standard was used for calibration (triphenyl phosphate (TPP) for prothioconazole and tetraphenylethylene (TPE) for fenpropidin).

Results and discussions

Recovery results were in a range of 110 – 129 % with an RSD \leq 13 % for prothioconazole and in a range of 83 – 157 % with an RSD \leq 14 % for fenpropidin. No outliers were identified. The LOQ was set at 10 ng/tube for prothioconazole and for fenpropidin, corresponding to the lowest fortification level with satisfying validation results.

Table A 54: Recovery results from method validation of prothioconazole using the analytical method

Matrix	Analyte	Fortification level (ng/tube) (n = 7)	Mean recovery (%)	RSD (%)	Comments
Air sampling Tubes with no air aspiration post spiking	Prothioconazole	1	129	8	-
		10	110	13	-
Air sampling Tubes with 24 hour air aspiration post spiking		1	113	5	-
		10	114	7	-

Table A 55: Recovery results from method validation of fenpropidin using the analytical method

Matrix	Analyte	Fortification level (ng/tube) (n = 7)	Mean recovery (%)	RSD (%)	Comments
Air sampling Tubes with no air aspiration post spiking	Fenpropidin	10	91	14	-
		100	157	11	-
Air sampling Tubes with 24 hour air aspiration post spiking		10	83	10	-
		100	90	11	-

Table A 56: Characteristics for the analytical method used for validation of prothioconazole and fenpropidin in air filters and air sampling tubes

	Prothioconazole	Fenpropidin
Specificity	Highly specific method	Highly specific method
Calibration (type, number of data points)	No calibration data presented	No calibration data presented
Calibration range	No data presented	No data presented
Assessment of matrix effects is presented	No	No
Limit of quantification	10 ng/tube	10 ng/tube

Conclusion

The method is fit for purpose for the determination of prothioconazole and fenpropidin in air filters and sampling tubes.

A 2.1.1.3 Environmental fate analytical methods

Comments of zRMS:	<p>The validation of the analytical method for the determination of fenpropidin in soil samples is acceptable and fulfils the requirements of guideline SANCO/3029/99 rev, 4. LOQ = 0.05 mg/kg.</p> <p>The mean recoveries at each fortification level were in the range of 70 – 110% with relative standard deviation(s) below 20%.</p> <p>The method is acceptable for the determination of fenpropidin in soil.</p>
-------------------	--

Reference:	KCP 5.1.2/07 (filed in KCP 9.1.1.1/01)
Report	Degradation of Fenpropidin in 3 different soils under aerobic conditions at 20° C in the dark. Morlock, G., 2006a, Report No 20051244/01-CABJ, sponsor no. 00012949
Guideline(s):	SANCO/3029/99 rev. 4
Deviations:	None
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Soil samples were extracted with methanol and addition of aqueous ammonia and analysed for fenpropidin by HPLC-MS/MS detection.

Results and discussions

Recovery results were in a range of 87 – 102% with an RSD \leq 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 0.05 mg/kg for fenpropidin.

Table A 57: Recovery results from method validation of fenpropidin using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (n = 5)	Mean recovery (%)	RSD (%)	Comments
LUFA soil 3A	Fenpropidin	0.05	87	4	-
		1.125	102	4	-
LUFA soil 5M	Fenpropidin	0.05	91	3	-
		1.125	100	3	-
LUFA soil 6S	Fenpropidin	0.05	89	3	-
		1.125	96	4	-

Table A 58: Characteristics for the analytical method used for validation of fenpropidin in soil

	Fenpropidin
Specificity	blank value $< 30\%$ LOQ
Calibration (type, number of data points)	individual calibration data presented calibration line equation presented
Calibration range	0.3 – 50 ng/L r = 1.0000 8 calibration points
Assessment of matrix effects is presented	Not significant
Limit of quantification	0.05 mg/kg

Conclusion

The method fulfils the requirements of SANCO/3029/99 rev. 4 and is suitable for the determination of fenpropidin in soil.

Comments of zRMS:	<p>The validation of the analytical method for the determination of fenpropidin in soil samples is acceptable and fulfils the requirements of guideline SANCO/3029/99 rev, 4.</p> <p>LOQ = 0.05 mg/kg.</p> <p>The mean recoveries at each fortification level were in the range of 70 – 110% with relative standard deviation below 20%.</p> <p>The method is acceptable for the determination of fenpropidin in soil.</p>
-------------------	--

Reference:	KCP 5.1.2/08 (filed in KCP 9.1.1.1/02)
Report	Degradation of Fenpropidin in one soil under aerobic conditions at 20° C in the dark. Morlock, G., 2006b, Report No 20051244/02-CABJ, sponsor no. 00012950
Guideline(s):	SANCO/3029/99 rev. 4
Deviations:	Matrix effects were not assessed
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Soil samples were extracted with methanol and addition of aqueous ammonia and analysed for fenpropidin by HPLC-MS/MS detection.

Results and discussions

Recovery results were in a range of 99 – 101% with an RSD \leq 2%. 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 mg/kg for fenpropidin.

Table A 59: Recovery results from method validation of fenpropidin using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (<i>n</i> = 5)	Mean recovery (%)	RSD (%)	Comments
LUFA soil 2.3	Fenpropidin	0.05	101	1	-
		1.125	99	2	-

Table A 60: Characteristics for the analytical method used for validation of fenpropidin in soil

	Fenpropidin
Specificity	blank value $< 30\%$ LOQ
Calibration (type, number of data points)	individual calibration data presented calibration line equation presented
Calibration range	0.3 – 50 ng/L <i>r</i> = 1.0000 8 calibration points
Assessment of matrix effects is presented	No
Limit of quantification	0.05 mg/kg

Conclusion

The method fulfils the requirements of SANCO/3029/99 rev. 4 and is suitable for the determination of fenpropidin in soil.

Comments of zRMS:	<p>The validation of the analytical method for the determination of fenpropidin acid in 3 different soil samples is acceptable and fulfils the requirements of guideline SANCO/3029/99 rev. 4.</p> <p>LOQ = 0.02 mg/kg.</p> <p>Mean recoveries ranged from 86% to 100% with an RSD \leq 12%.</p> <p>The method is acceptable.</p>
-------------------	--

Reference:	KCP 5.1.2/09 (filed in KCP 9.1.1.1/03)
Report	Degradation of Fenpropidin Acid in 3 Different Soils under Aerobic Conditions at 20°C in the Dark, Flörchinger M., 2008, Report No.S08-01156, sponsor no. 00016350
Guideline(s):	SANCO/3029/99 rev. 4
Deviations:	Matrix effects were not assessed
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Soil samples were extracted with acetonitrile/water 1:1 (v/v) and analysed for fenpropidin acid by HPLC-MS/MS detection.

Results and discussions

Recovery results were in a range of 86 – 100% with an RSD \leq 12%. 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 0.02 mg/kg for fenpropidin acid.

Table A 61: Recovery results from method validation of fenpropidin acid using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (<i>n</i> = 5)	Mean recovery (%)	RSD (%)	Comments
LUFA soil 2.2	Fenpropidin acid	0.02	96	8	-
		0.4	100	6	-
LUFA soil 2.3	Fenpropidin acid	0.02	86	8	-
		0.4	95	2	-
LUFA soil 5M	Fenpropidin acid	0.02	95	6	-
		0.4	88	12	-

Table A 62: Characteristics for the analytical method used for validation of fenpropidin acid in soil

	Fenpropidin acid
Specificity	blank value $< 30\%$ LOQ
Calibration (type, number of data points)	individual calibration data presented calibration line equation presented
Calibration range	1 – 50 ng/L <i>r</i> = 0.9989 10 calibration points
Assessment of matrix effects is presented	No
Limit of quantification	0.02 mg/kg

Conclusion

The method fulfils the requirements of SANCO/3029/99 rev. 4 and is suitable for the determination of fenpropidin acid in soil.

A 2.1.1.4 Ecotoxicology analytical methods

Comments of zRMS:	<p>The validation of the analytical method for the determination of prothioconazole and fenpropidin in water used in an aqua toxicity test is acceptable and fulfils the requirements of guideline SANCO/3029/99 rev. 4.</p> <p>LOQ for prothioconazole was 0.3505 mg/L.</p> <p>LOQ for fenpropidin was 0.5055 mg/L.</p> <p>The mean recoveries at each fortification level were in the range between 70% and 110% with a relative standard deviation below 20%.</p> <p>The method is acceptable.</p>
-------------------	---

Reference:	KCP 5.1.2/10 (filed in KCP 10.2.1/01)
Report	Acute toxicity of ADM.03502.F.1.A to <i>Oncorhynchus mykiss</i> in a 96-hour semi-static test,, 2020, report no ..., sponsor no.: ...
Guideline(s):	SANCO/3029/99 rev. 4
Deviations:	None
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Water samples were stabilised with an equal amount of methanol after sampling and thawed at room temperature and homogenised. After dilution with methanol, samples were analysed for prothioconazole and fenpropidin by HPLC-MS/MS detection.

Results and discussions

Recovery results were in a range of 91.7. – 92.7 % with an RSD \leq 2.4 % for prothioconazole and in a range of 95.9 – 97.2 % with an RSD \leq 0.9 % for fenpropidin. 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 0.3505 mg/L for prothioconazole and at 0.5055 mg/L for fenpropidin.

Table A 63: Recovery results from method validation of prothioconazole using the analytical method

Matrix	Analyte	Fortification level (mg/L) (n = 5)	Mean recovery (%)	RSD (%)	Comments
Water	Prothioconazole	0.3505	91.7	2.4	-
		2.65	92.7	1.6	-

Table A 64: Recovery results from method validation of fenpropidin using the analytical method

Matrix	Analyte	Fortification level (mg/L) (n = 5)	Mean recovery (%)	RSD (%)	Comments
Water	Fenpropidin	0.5055	95.9	0.5	-
		3.822	97.2	0.9	-

Table A 65: Characteristics for the analytical method used for validation of prothioconazole and fenpropidin in water (from aqua toxicity test)

	Prothioconazole	Fenpropidin
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	0.1036 –3.187mg/L r = 0.9997 7 calibration points	0.01494 – 4.597 mg/L r = 0.9999 7 calibration points
Assessment of matrix effects is presented	Not required, since calibration was carried out with matrix-matched standards	Not required, since calibration was carried out with matrix-matched standards
Limit of quantification	0.3505 mg/L	0.5055 mg/L

Conclusion

The method fulfils the requirements of SANCO/3029/99 rev. 4 and is suitable for the determination of prothioconazole and fenpropidin in water from aqua toxicity tests.

Comments of zRMS:	<p>The validation of the analytical method for the determination of prothioconazole and fenpropidin in water used in an aqua toxicity test is acceptable and fulfils the requirements of guideline SANCO/3029/99 rev, 4.</p> <p>LOQ for prothioconazole was 0.1871 mg/L.</p> <p>LOQ for fenpropidin was 0.2699 mg/L.</p> <p>The mean recoveries at each fortification level were in the range between 70% and 110% with a relative standard deviation below 20%.</p> <p>The method is acceptable.</p>
-------------------	---

Reference:	KCP 5.1.2/11 (filed in KCP 10.2.1/02)
Report	Acute toxicity of ADM.03502.F.1.A to <i>Daphnia magna</i> in a 48-hour semi-static test, Renner, P., 2020, report no 2048ADL0008, sponsor no.: 000104840
Guideline(s):	SANCO/3029/99 rev. 4
Deviations:	None
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Water samples were stabilised with an equal amount of methanol after sampling and thawed at room temperature and homogenised. After dilution with methanol, samples were analysed for prothioconazole and fenpropidin by HPLC-MS/MS detection.

Results and discussions

Recovery results were in a range of 92.7. – 95.5 % with an RSD \leq 9.7 % for prothioconazole and in a range of 90.1 – 97.9 % with an RSD \leq 0.8 % for fenpropidin. 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 0.1871 mg/L for prothioconazole and at 0.2699 mg/L for fenpropidin.

Table A 66: Recovery results from method validation of prothioconazole using the analytical method

Matrix	Analyte	Fortification level (mg/L) (n = 5)	Mean recovery (%)	RSD (%)	Comments
Water	Prothioconazole	0.1871	92.7	9.2	-
		1.497	95.5	4.2	-

Table A 67: Recovery results from method validation of fenpropidin using the analytical method

Matrix	Analyte	Fortification level (mg/L) (n = 5)	Mean recovery (%)	RSD (%)	Comments
Water	Fenpropidin	0.2699	90.1	0.5	-
		2.159	97.9	0.8	-

Table A 68: Characteristics for the analytical method used for validation of prothioconazole and fenpropidin in water (from aqua toxicity test)

	Prothioconazole	Fenpropidin
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	6.911 –113.3 µg/L r = 0.9988 8 calibration points	0.07975 – 2.615 mg/L r = 0.9999 8 calibration points
Assessment of matrix effects is presented	Not required, since calibration was carried out with matrix-matched standards	Not required, since calibration was carried out with matrix-matched standards
Limit of quantification	0.1871 mg/L	0.2699 mg/L

Conclusion

The method fulfils the requirements of SANCO/3029/99 rev. 4 and is suitable for the determination of prothioconazole and fenpropidin in water from aqua toxicity tests.

Comments of zRMS:	<p>The validation of the analytical method for the determination of prothioconazole and fenpropidin in water used in an aqua toxicity test is acceptable and fulfils the requirements of guideline SANCO/3029/99 rev, 4.</p> <p>LOQ for prothioconazole was 0.207 µg/L.</p> <p>LOQ for fenpropidin was 0.155 µg/L.</p> <p>The mean recoveries at each fortification level were in the range between 70% and 120% with a relative standard deviation below 20%.</p> <p>The method is acceptable.</p>
-------------------	---

Reference:	KCP 5.1.2/12 (filed in KCP 10.2.1/03)
Report	ADM.03502.F.1.A - Alga, Growth Inhibition Test with <i>Desmodesmus subspicatus</i> , 72 hours, Scheerbaum, D., 2021, report no.: SO21519 / SSO19707, sponsor no.: 000108687
Guideline(s):	SANCO/3029/99 rev. 4
Deviations:	None
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Water samples were diluted factor 2 with acetonitrile containing 0.2% formic acid and analysed for prothioconazole and fenpropidin by LC-MS/MS detection.

Results and discussions

Recovery results were in a range of 86 – 100% with an RSD \leq 13% for prothioconazole and in a range of 97 – 111% with an RSD \leq 3.4% for fenpropidin. 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 0.207 $\mu\text{g/L}$ for prothioconazole and at 0.155 $\mu\text{g/L}$ for fenpropidin.

Table A 69: Recovery results from method validation of prothioconazole using the analytical method

Matrix	Analyte	Fortification level ($\mu\text{g/L}$) ($n = 5$)	Mean recovery (%)	RSD (%)	Comments
Water	Prothioconazole	0.207	86	8.6	-
		0.326	86	13	-
		25.9	100	5.3	-

Table A 70: Recovery results from method validation of fenpropidin using the analytical method

Matrix	Analyte	Fortification level (mg/L) ($n = 5$)	Mean recovery (%)	RSD (%)	Comments
Water	Fenpropidin	0.155	97	3.4	-
		25.9	111	2.7	-

Table A 71: Characteristics for the analytical method used for validation of prothioconazole and fenpropidin in water (from aqua toxicity test)

	Prothioconazole	Fenpropidin
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	10 – 1000 ng/L $r = 0.9991$ 8 calibration points	5 – 500 ng/L $r = 0.9995$ 8 calibration points
Assessment of matrix effects is presented	Not required, since calibration was carried out with matrix-matched standards	Not required, since calibration was carried out with matrix-matched standards
Limit of quantification	0.207 $\mu\text{g/L}$	0.155 $\mu\text{g/L}$

Conclusion

The method fulfils the requirements of SANCO/3029/99 rev. 4 and is suitable for the determination of prothioconazole and fenpropidin in water from aqua toxicity tests.

Comments of zRMS:	<p>The validation of the analytical method for the determination of prothioconazole and fenpropidin in the aquatic test medium is acceptable and fulfils the requirements of guideline SANCO/3029/99 rev, 4.</p> <p>LOQ for prothioconazole was 0.001561 mg/L.</p> <p>LOQ for fenpropidin was 0.002252 mg/L.</p> <p>The mean recoveries at each fortification level were in the range between 70% and 110% with a relative standard deviation below 20%.</p> <p>The method is acceptable.</p>
-------------------	---

Reference:	KCP 5.1.2/13 (filed in KCP 10.2.1/04)
Report	Effects of ADM.03502.F.1.A on <i>Lemna gibba</i> in a growth inhibition test under semi-static test conditions, Renner, P., 2021, report no.: 2048ALE0006, sponsor no.: 000104842
Guideline(s):	SANCO/3029/99 rev. 4
Deviations:	None
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Analytical evaluation of the active substances of test item were carried out via HPLC-MS/MS on a reversed phase column. An electrospray tandem mass spectrometer operating in positive ion mode was used as detector. Prothioconazole and Fenpropidin were used as external standards for matrix-matched calibration.

Results and discussions

Recovery results were in a range of 86 – 100% with an RSD \leq 5.3% for prothioconazole and in a range of 97 – 111% with an RSD \leq 3.4% for fenpropidin. 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 0.2 2.252 $\mu\text{g/L}$ for prothioconazole and at 0.15 1.561 $\mu\text{g/L}$ for fenpropidin.

Table A 72: Recovery results from method validation of prothioconazole using the analytical method

Matrix	Analyte	Fortification level (mg/L) (n = 5)	Mean recovery (%)	RSD (%)	Comments
Water	Prothioconazole	0.001561	87.1	2.3	-
		4.423	96.5	1.6	-

Table A 73: Recovery results from method validation of fenpropidin using the analytical method

Matrix	Analyte	Fortification level (mg/L) (n = 5)	Mean recovery (%)	RSD (%)	Comments
Water	Fenpropidin	0.002252	97.1	2.6	-
		6.379	102.5	1.4.	-

Table A 74: Characteristics for the analytical method used for validation of prothioconazole and Fenpropidin in water (from aqua toxicity test)

	Prothioconazole	Fenpropidin
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	0.2308 –26.83 µg/L r ≥ 0.99 8 calibration points	0.3329 – 38.7 µg/L r ≥ 0.99 7 calibration points
Assessment of matrix effects is presented	Not required, since calibration was carried out with matrix-matched standards	Not required, since calibration was carried out with matrix-matched standards
Limit of quantification	0.001561 mg/L	0.002252 mg/L

Conclusion

The method fulfils the requirements of SANCO/3029/99 rev. 4 and is suitable for the determination of prothioconazole and fenpropidin in water from aqua toxicity tests.

Comments of zRMS:	<p>The validation of the analytical method for the determination of prothioconazole and fenpropidin in feeding solutions is acceptable and fulfils the requirements of guideline SANCO/3029/99 rev. 4.</p> <p>LOQ for prothioconazole was 76.2 mg/kg.</p> <p>LOQ for fenpropidin was 110 mg/kg.</p> <p>The mean recoveries at each fortification level were in the range between 70% and 110% with a relative standard deviation below 20%.</p> <p>The method is acceptable.</p>
-------------------	--

Reference:	KCP 5.1.2/14 (filed in KCP 10.3.1.2/01)
Report	Chronic oral toxicity of ADM.03502.F.1.A to the honey bee <i>Apis mellifera</i> L. under laboratory conditions, Dreßler, K., 2020, report no.: 2048BAC0011, sponsor no.: 000104844
Guideline(s):	SANCO/3029/99 rev. 4
Deviations:	None
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	Not applicable

Materials and methods

The bee diet samples were extracted by QuEChERS procedure. Acetonitrile /water (50/50, v/v) as well as a QuEChERS salt mix was added. After shaking, and dilution into calibration range with acetonitrile, the samples were analysed for prothioconazole and fenpropidin by HPLC-MS/MS.

Results and discussions

Recovery results were in a range of 94.6 – 95.5% with an RSD ≤ 2.08% for prothioconazole and in a range of 90.4 – 93.2% with an RSD ≤ 3.12% for fenpropidin. 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 76.2 mg/kg for prothioconazole and at 110 mg/kg for fenpropidin.

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

Matrix	Analyte	Fortification level (mg/kg) (n = 5)	Mean recovery (%)	RSD (%)	Comments
Bee diet	Prothioconazole	76.2	94.6	2.08	m/z 344 → 189
			95.5	1.02	m/z 344 → 125
		1385	95.0	1.29	m/z 344 → 189
			94.6	1.20	m/z 344 → 125

Recovery results from method validation of fenpropidin using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (n = 5)	Mean recovery (%)	RSD (%)	Comments
Bee diet	Fenpropidin	110	90.4	2.96	m/z 274 → 147
			90.5	3.12	m/z 274 → 132
		2000	93.0	1.56	m/z 274 → 147
			93.2	1.28	m/z 274 → 132

Table A 76: Characteristics for the analytical method used for validation of prothioconazole and fenpropidin in bee diet

	Prothioconazole	Fenpropidin
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	9.44 –189 µg/L r > 0.99 > 5 calibration points	13.3 –269 µg/L r > 0.99 > 5 calibration points
Assessment of matrix effects is presented	Not required, since calibration was carried out with matrix-matched standards	Not required, since calibration was carried out with matrix-matched standards
Limit of quantification	76.2 mg/kg	110 mg/kg

Conclusion

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

Comments of zRMS:	<p>The validation of the analytical method for the determination of prothioconazole and fenpropidin in test item stock solutions in a honey bee larve chronic toxicity test is acceptable and fulfils the requirements of guideline SANCO/3029/99 rev, 4.</p> <p>LOQ for prothioconazole was 0.0204 mg/kg.</p> <p>LOQ for fenpropidin was 0.0294 mg/kg.</p> <p>The mean recoveries at each fortification level were in the range between 70% and 110% with a relative standard deviation below 20%.</p> <p>The method is acceptable.</p>
-------------------	--

Reference:	KCP 5.1.2/15 (filed in KCP 10.3.1.3/01)
Report	ADM.03502.F.1.A – Repeated exposure of honey bee larvae (Apis mellifera L.) under laboratory conditions, Hänsel, M., 2021, report no.: 2048BLC0013, sponsor no.: 000104845
Guideline(s):	SANCO/3029/99 rev. 4
Deviations:	None

GLP: Yes
Acceptability: Yes
Duplication (if vertebrate study) Not applicable

Materials and methods

The bee diet samples were extracted by QuEChERS procedure. Acetonitrile /water (50/50, v/v) as well as a QuEChERS salt mix was added. After shaking, and dilution into calibration range with acetonitrile, the samples were analysed for prothioconazole and fenpropidin by HPLC-MS/MS.

Results and discussions

Recovery results were in a range of 74.9 – 109% with an RSD \leq 6.64% for prothioconazole and in a range of 81.3 – 84.5% with an RSD \leq 4.62% for fenpropidin. 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 0.0204 mg/kg for prothioconazole and at 0.0294 mg/kg for fenpropidin.

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

Matrix	Analyte	Fortification level (mg/kg) (n = 5)	Mean recovery (%)	RSD (%)	Comments
Bee diet	Prothioconazole	0.0204	74.9	6.13	m/z 344 → 189
			75.5	6.64	m/z 344 → 125
		4.59	109	2.79	m/z 344 → 189
			108	1.95	m/z 344 → 125

Recovery results from method validation of fenpropidin using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (n = 5)	Mean recovery (%)	RSD (%)	Comments
Bee diet	Fenpropidin	0.0294	81.3	4.62	m/z 274 → 147
			82.9	4.24	m/z 274 → 132
		6.62	84.4	1.87	m/z 274 → 147
			84.5	1.62	m/z 274 → 132

Table A 78: Characteristics for the analytical method used for validation of prothioconazole and fenpropidin in bee diet

	Prothioconazole	Fenpropidin
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	0.435 – 8.71 $\mu\text{g/L}$ $r > 0.99$ > 5 calibration points	0.628 – 12.6 $\mu\text{g/L}$ $r > 0.99$ > 5 calibration points
Assessment of matrix effects is presented	Not required, since calibration was carried out with matrix-matched standards	Not required, since calibration was carried out with matrix-matched standards
Limit of quantification	0.0204 mg/kg	0.0294 mg/kg

Conclusion

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

Comments of zRMS:	<p>The validation of the analytical method for the determination of prothioconazole and fenpropidin in test solutions seedling emergence and seedling growth test is acceptable and fulfils the requirements of guideline SANCO/3029/99 rev, 4.</p> <p>LOQ for prothioconazole was 436.4 mg/L.</p> <p>LOQ for fenpropidin was 630.1 mg/L.</p> <p>The mean recoveries at each fortification level were in the range between 70% and 110% with a relative standard deviation below 20%.</p> <p>The method is acceptable.</p>
-------------------	--

Reference:	KCP 5.1.2/16 (filed in KCP 10.6.1/01)
Report	Effects of ADM.03502.F.1.A on seedling emergence and seedling growth of six non-target terrestrial plant species under greenhouse conditions, Kästner, K., 2020, report no.: 2046PSE0007, sponsor no.: 000104852
Guideline(s):	SANCO/3029/99 rev. 4
Deviations:	None
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	Not applicable

Materials and methods

Spray solution samples were thawed at room temperature and homogenised by shaking. After dilution of the samples with acetonitrile, the samples were analysed for prothioconazole and fenpropidin by HPLC-DAD.

Results and discussions

Recovery results were in a range of 102.9 – 103.2% with an RSD \leq 1.6% for prothioconazole and in a range of 103.3 – 103.9% with an RSD \leq 1.7% for fenpropidin. 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 436.4 mg/L for prothioconazole and at 630.1 mg/L for fenpropidin.

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
Spray solution	Prothioconazole	436.4	103.2	1.6	-
		1145	102.9	0.2	-

Table A 80: Recovery results from method validation of fenpropidin using the analytical method

Matrix	Analyte	Fortification level (mg/L) (n = 5)	Mean recovery (%)	RSD (%)	Comments
Spray solution	Fenpropidin	630.1	103.9	1.7	-
		1654	103.3	0.3	-

Table A 81: Characteristics for the analytical method used for validation of prothioconazole and fenpropidin in spray solution

	Prothioconazole	Fenpropidin
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	1.307–13.76 mg/L r = 1.0000 6 calibration points	1.888–19.87 mg/L r = 0.9999 6 calibration points
Assessment of matrix effects is presented	Not required, since calibration was carried out with matrix-matched standards	Not required, since calibration was carried out with matrix-matched standards
Limit of quantification	436.4 mg/L	630.1 mg/L

Conclusion

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

Comments of zRMS:	The validation of the analytical method for the determination of prothioconazole and fenpropidin in test solutions of a vegetative vigour test is acceptable and fulfils the requirements of guideline SANCO/3029/99 rev. 4. LOQ for prothioconazole was 436.4 mg/L. LOQ for fenpropidin was 630.1 mg/L. The mean recoveries at each fortification level were in the range between 70% and 110% with a relative standard deviation below 20%. The method is acceptable.
-------------------	---

Reference:	KCP 5.1.2/17 (filed in KCP 10.6.1/02)
Report	Effects of ADM.03502.F.1.A on vegetative vigour of six non-target terrestrial plant species under greenhouse conditions, Kästner, K., 2020, report no.: 2035CRX0012, sponsor no.: 000104853
Guideline(s):	SANCO/3029/99 rev. 4
Deviations:	None
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	Not applicable

Materials and methods

Spray solution samples were thawed at room temperature and homogenised by shaking. After dilution of the samples with acetonitrile, the samples were analysed for prothioconazole and fenpropidin by HPLC-DAD.

Results and discussions

Recovery results were in a range of 102.4 – 103.5 % with an RSD \leq 1.5 % for prothioconazole and in a range of 103.4 – 103.9 % with an RSD \leq 1.6 % for fenpropidin. 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 436.4 mg/L for prothioconazole and at 630.1 mg/L for fenpropidin.

Table A 82: 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	436.4	103.5	1.5	-
		1145	102.4	0.1	-

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

Matrix	Analyte	Fortification level (mg/L) (n = 5)	Mean recovery (%)	RSD (%)	Comments
Spray solution	Fenpropidin	630.1	103.9	1.6	-
		1654	103.4	0.3	-

Table A 84: Characteristics for the analytical method used for validation of prothioconazole and fenpropidin in spray solution

	Prothioconazole	Fenpropidin
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	1.307–13.76 mg/L r = 1.0000 6 calibration points	1.888–19.87 mg/L r = 0.9999 6 calibration points
Assessment of matrix effects is presented	Not required, since calibration was carried out with matrix-matched standards	Not required, since calibration was carried out with matrix-matched standards
Limit of quantification	436.4 mg/L	630.1 mg/L

Conclusion

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

A 2.1.1.5 Phys-Chem analytical methods

The analytical method used in the phys-chem study Tsesin, N., 2020, Report no 000105029.061FL [filed in KCP 2.1/01] (which was used for the determination of the active substances in the phys-chem part, where relevant) 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:	<p>The study of Lefresne, S., 2020 (Report no.: B18S-A4-P-01) on validation of an analytical method for the determination of prothioconazole residues in wheat (whole plant, grain, straw), oilseed rape (grain), strawberry and dried bean has been evaluated in Registration Report for ADM.03500.F.2.B (Soratel) on November 2022 by zRMS-PL and the summary is presented below.</p> <p>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.</p> <p>The quantification was performed by liquid chromatography with tandem mass spectrometry detection (LC-MS/MS), two mass transitions were monitored for each reference item.</p> <p>LOQ (Limit of quantification): 0.010 mg/kg for each metabolites.</p> <p>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.</p> <p>The mean recoveries at each fortification level were in the range between 70% and 110% with relative standard deviation below 20%.</p> <p>The method is acceptable.</p>
-------------------	--

Reference:	KCP 5.2/02
Report	<p>Validation of an analytical method for the determination of prothioconazole residues in wheat (whole plant, grain, straw), oilseed rape (grain), strawberry and dried bean</p> <p>Lefresne, S., 2020</p> <p>Report No.: B18S-A4-P-01, Sponsor no.: R-39651</p>
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-prothioconazole-desthio, 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 85: 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-desthio-1 used for quantification or confirmation	328 → 70	14	53	81	150
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.

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	100-102 101 ± 1 5	99-103 101 ± 2 5	100-102 101 ± 1 5	99-105 102 ± 2 5	100-105 103 ± 2 5	101-108 105 ± 2 5	101-105 103 ± 2 5	98-109 105 ± 4 5	104-108 106 ± 1 5	105-110 108 ± 2 5	104-107 106 ± 1 5	99-102 100 ± 1 5
0.100	Range Mean ± RSD n	100-108 103 ± 3 5	99-106 101 ± 3 5	103-112 107 ± 4 5	103-111 107 ± 3 5	103-114 108 ± 5 5	105-118 110 ± 5 5	101-113 107 ± 5 5	100-113 108 ± 5 5	108-114 110 ± 2 5	106-115 110 ± 3 5	105-114 110 ± 3 5	99-110 106 ± 4 5
0.01 and 0.10	Overall ± RSD n	102 ± 2 10	101 ± 2 10	104 ± 4 10	104 ± 3 10	106 ± 4 10	107 ± 4 10	105 ± 4 10	106 ± 5 10	108 ± 2 10	109 ± 2 10	108 ± 3 10	103 ± 4 10

Table A 87: Recovery results from method validation of prothioconazole metabolites in grain of wheat

[illegible]

Table A 88: 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 89: Recovery results from method validation of prothioconazole metabolites in oilseed rape seeds

Fortification level [mg/kg]	Analyte	Prothioconazole-desthio		3-hydroxy-prothioconazole-desthio		4-hydroxy-prothioconazole-desthio		5-hydroxy-prothioconazole-desthio		6-hydroxy-prothioconazole-desthio		alpha-hydroxy-prothioconazole-desthio	
		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 90: Recovery results from method validation of prothioconazole metabolites in strawberry

Fortification level [mg/kg]	Analyte	Prothioconazole-desthio		3-hydroxy-prothioconazole-desthio		4-hydroxy-prothioconazole-desthio		5-hydroxy-prothioconazole-desthio		6-hydroxy-prothioconazole-desthio		alpha-hydroxy-prothioconazole-desthio	
		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 91: Recovery results from method validation of prothioconazole metabolites in dry bean

Fortification level [mg/kg]	Analyte	Prothioconazole-desthio		3-hydroxy-prothioconazole-desthio		4-hydroxy-prothioconazole-desthio		5-hydroxy-prothioconazole-desthio		6-hydroxy-prothioconazole-desthio		alpha-hydroxy-prothioconazole-desthio	
		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 92: 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 equation: $y = 118131.63x + 6877.72$
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
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 $y = 77547.41x + 15265.52$ OSR 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 $y = 91887.09x + 30786.62$
Assessment of matrix effects is presented	Not required, 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. Standard stability in solvent MeCN was shown for 10 days when stored refrigerated.
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 study of Watson, G., 2022 (Report no.: RES-00393) on 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 has been
-------------------	--

	<p>evaluated in Registration Report for ADM.03500.F.2.B (Soratel) on November 2022 by zRMS-PL and the summary is presented below.</p> <p>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 independently 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.</p> <p>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 $\leq 20\%$ for all matrices.</p> <p>The independent laboratory validation met the criteria detailed in SANTE/2020/12830, Rev.1</p>
--	--

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., 2022 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 93: 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 94: Recovery results from method validation of prothioconazole-desthio in crop matrices

Fortification level [mg/kg]	Crop matrix	Wheat grain		Dried broad bean		Wheat whole plant		Oilseed rape seed		Strawberry	
		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 95: Characteristics for the analytical method used for validation of prothioconazole-desthio residues in wheat grain, dried broad beans, oilseed rape seed and strawberry

	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: $y = 4.59e^4x + 375$
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)
Equations	Wheat whole plant Quantification $y = 4.69e+004x - 215$ Confirmation $y = 2.64e+004x + 77.2$ Wheat Grain Quantification $y = 4.59e+004x + 375$ Confirmation $y = 2.59e+004x + 1.1e+003$ OSR seed Quantification $y = 4.65e+004x + 2.84e+003$ Confirmation $y = 2.63e+004x + 1.23e+003$ Dry bean Quantification $y = 4.72e+004x + 1.23e+003$ Confirmation $y = 2.65e+004x + 1.25e+003$ Strawberry Quantification $y = 3.01e+004x + 5.59e+003$ Confirmation $y = 1.51e+004x + 2.11e+003$
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 7 days. Standard stability in solvent MeCN was shown for 10 days 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 and strawberry.

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

Comments of zRMS:	<p>The study of Watson, G., 2022 (Report no.: RES-00394) on Validation of an analytical method for the determination of residues of prothioconazole-desthio in egg by LC-MS/MS has been evaluated in Registration Report for ADM.03500.F.2.B (Soratel) on November 2022 by zRMS-PL and the summary is presented below.</p> <p>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).</p> <p>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).</p> <p>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 $\leq 20\%$.</p> <p>The method is acceptable.</p>
-------------------	---

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., 2022 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 96: 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 97: 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	82-86	82-86

	Mean \pm RSD n	83 \pm 1.7 5	83 \pm 1.7 5
0.100	Range Mean \pm RSD n	80-84 82 \pm 1.7 5	80-83 81 \pm 1.3 5

RSD = relative standard deviation, n = number of replicates

Table A 98: 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 μ g/L (equivalent to 0.003 – 0.2 mg/kg)
Equations	Quantification $y = 4.87e+004x + 1.08+003$ Confirmation $y = 2.75e+004x + 855$
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 7 days. Standard stability in solvent MeCN was shown for 9 days when stored refrigerated.
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:	<p>The study of Lindner, M., Büdel, A., 2022 (Report no.: S22-04421 (MAC-2219V)) on independent laboratory validation of an analytical method for the determination of residues of prothioconazole-desthio in egg by LC-MS/MS has been evaluated in Registration Report for ADM.03500.F.2.B (Soratel) on November 2022 by zRMS-PL and the summary is presented below.</p> <p>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.</p>
-------------------	---

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 99: 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 units)	Gas flow 1 (GS1)		Zero-grade air set at 50 (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 1 (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 100: 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	92-96	95-98
	Mean ± RSD	95 ± 2.0	96 ± 1.4
	n	5	5
0.100	Range	90-100	91-97
	Mean ± RSD	95 ± 4.0	95 ± 3.3
	n	5	5

RSD = relative standard deviation, n = number of replicates

Table A 101: 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)
Equations:	Quantification y = 73094.7843 x + 1716.8898 Confirmation y = 34951.1978 x + 48.5101
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	The final sample extracts were analysed within 24 hours after initial extraction thus no stability study was performed stock solutions in acetone were stable when stored at 1 °C to 10 °C in the dark for 234 days..

	Prothioconazole-desthio
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:	<p>The study of Lefresne, S., 2021 (Report no.: B21S-A4-P-04) on validation of an analytical method for the determination of prothioconazole residues in honey has been evaluated in Registration Report for ADM.03500.F.2.B (Soratel) on November 2022 by zRMS-PL and the summary is presented below.</p> <p>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.</p> <p>LOQ (Limit of quantification) of prothioconazole expressed as prothioconazole-desthio (sum of isomers): 0.010 mg/kg.</p> <p>The mean recoveries at each fortification level were in the range between 70% and 110% with relative standard deviation below 20%.</p> <p>The method complies with the guideline SANTE/2020/12830, Rev.1 of 24/02/2021.</p>
-------------------	---

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 102: 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 \leq 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 103: 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	106-112	107-111
	Mean \pm RSD	108 \pm 2	108 \pm 1
	n	5	5
0.100	Range	109-112	106-109
	Mean \pm RSD	110 \pm 1	108 \pm 1
	n	5	5

RSD = relative standard deviation, n = number of replicates

Table A 104: 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)
Equation	$y = -1205.5669 x^2 + 869245.8004 x + 749671.8949$ (312 \rightarrow 70 m/z) $y = -1263.3872 x^2 + 897807.189 x + 509005.172$ (312 \rightarrow 125 m/z)
Calibration range	3 – 200 μ g/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
Extract and standard stability	<u>Extract stability:</u> The final sample extracts were analysed within 24 hours after initial extraction thus no stability study was performed. <u>Standard Stability:</u> The stability of the stock standard solution has been demonstrated over a period of at least 392 days.
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:	<p>The study of Lindner, M., 2022 (Report no.: S21-06313 (MAC-2144V)) on independent laboratory validation of an analytical method for determination of prothioconazole residues in honey has been evaluated in Registration Report for ADM.03500.F.2.B (Soratel) on November 2022 by zRMS-PL and the summary is presented below.</p> <p>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.</p> <p>LC-MS/MS determination was conducted by monitoring two (2) mass transitions (m/z 312→70 and m/z 312→125).</p> <p>The limit of quantification is 0.01 mg/kg.</p> <p>Recovery results were in a range of 70 to 120% with an RSD ≤ 20.</p> <p>The method is acceptable.</p>
-------------------	---

Reference:	KCP 5.2/07
Report	Independent Laboratory Validation of an Analytical Method for Determination of Prothioconazole 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 105: 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 106: 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	94-97	95-100
	Mean ± RSD	96 ± 1.3	97 ± 2.0
	n	5	5
0.100	Range	109-113	109-113
	Mean ± RSD	111 ± 1.5	111 ± 1.6
	n	5	5

RSD = relative standard deviation, n = number of replicates

Table A 107: 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)
Equation	$y = 372120.70 x + 652.1902$ (312 → 70 m/z) $y = 203704.87x + 509.0032$ (312 → 125 m/z)
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
Extract and standard stability	<u>Extract stability:</u> Prothioconazole-desthio was found to be stable in final extracts of honey for 8 days when stored at typically 1 °C to 10 °C in the dark. <u>Standard Stability:</u> Prothioconazole-desthio was found to be stable for 203 days when prepared in acetone and stored at typically 1 °C to 10 °C in the dark.
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:	<p>The study of Krebber, R., Sandau, C., 2015 (Report no.: MR-15/025) on modification M002 of analytical method 01387 for the determination of various pesticides in drinking and surface water by HPLC-MS/MS has been evaluated in Registration Report for ADM.03500.F.2.B (Soratel) on November 2022 by zRMS-PL and the summary is presented below.</p> <p>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.</p> <p>The limit of quantitation (LOQ) is 0.05 µg/L for all analytes in surface water.</p> <p>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.</p>
-------------------	--

	The method meets all guideline criteria to determine concentrations in drinking and surface water of prothioconazole and prothioconazole-desthio at 0.05 µg/L.
	<u>Remark:</u> 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 108: 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 109: 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 = 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

Matrix	Analyte	Ion Transition (m/z)	Fortification level (µg/L) (n = 10)	Mean Area Counts	RSD (%)
	Prothioconazole-desthio	312 > 70	0.05	151037	1.9
			0.5	1522200	1.2
		312 > 125	0.05	93164	1.6
			0.5	932259	1.6

Table A 110: 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)	Individual calibration data presented r > 0.99 5 calibration points (double determination)
Equation	y = 1.7994e ⁵ x - 225.59 (m/z 344 → m/z 189) y = 1.428e ⁵ x - 659.32 (m/z 344 → m/z 154)	y = 2.9741e ⁶ x + 5603 (m/z 312 → m/z 70) y = 1.7841e ⁶ + 5882.5 (m/z 312 → m/z 125)
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
Extract and standard stability	Prothioconazole is not stable in pure water but can be stabilized by addition of cysteine hydrochloride (for more details, please report)	
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:	<p>The study of Thies, S., 2015 (Report no.: 2015/0034/01) on independent laboratory validation of the BCS analytical method 01387/M002 for the determination of various pesticides in surface water by HPLC-MS/MS has been evaluated in Registration Report for ADM.03500.F.2.B (Soratel) on November 2022 by zRMS-PL and the summary is presented below.</p> <p>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 transitions has been independently validated.</p> <p>The limit of quantitation (LOQ) for all analytes is 0.05 µg/L in surface water.</p> <p>The relative standard deviations for the peak areas were ≤ 20% for all MRM transitions of all analytes.</p> <p>The method meets all guideline criteria to determine concentrations in surface water of the described analytes at 0.05 µg/L.</p>
-------------------	--

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 111: 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 112: 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 113: 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: $y = 1.66 \times 10^5 x - 994$	individual calibration data presented Individual calibration data presented $r > 0.99$ ≥ 5 calibration points (single determination) Representative equation: $y = 6.9 \times 10^5 x + 11700$
Equation	$y = 1.66 \times 10^5 x - 994$ (m/z 344 \rightarrow m/z 189) $y = 1.39 \times 10^5 x - 1.56 \times 10^3$ (m/z 344 \rightarrow m/z 154)	$y = 1.17 \times 10^6 x + 2.54 \times 10^4$ (m/z 312 \rightarrow m/z 70) $y = 6.9 \times 10^5 x + 1.17 \times 10^5$ (m/z 312 \rightarrow m/z 125)
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
Extract and standard stability	Prothioconazole is not stable in pure water but can be stabilized by addition of cysteine hydrochloride (for more details, please report)	
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 body fluids and tissues

Comments of zRMS:	<p>The study of Brown, S., 2022 (Report no.: RES-00373) on development and validation of an analytical method for determination of residues of prothioconazole-desthio in body fluids (blood) by LC-MS/MS has been evaluated in Registration Report for ADM.03500.F.2.B (Soratel) on November 2022 by zRMS-PL and the summary is presented below.</p> <p>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.</p> <p>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 $\leq 20\%$.</p> <p><u>Remark:</u> 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.</p>
-------------------	---

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, Brown, S., 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.

Table A 114: 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	100	60 V	10 V	Primary
	312.0 → 125.0	100	45 V	10 V	Confirmatory

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 115: 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 116: 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
Representative equation	y= 3.48105e ⁸ x + 1119.06 (312 → 70 m/z) y = 2.02884e ⁸ x + 439.556 (373 → 160 m/z)
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
Extract and standard stability	Extract stability after 8 days refrigerator storage was shown not to be stable – analysis should occur within 24 hours. Standard stability in solvent MeCN was shown for 23 days when stored refrigerated
Limit of quantification	0.01 mg/L Note: Concentration levels are given as mg prothioconazole-desthio/L sample
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.

A 2.3 Analytical methods for Fenpropidin

A 2.3.1 Methods for post-authorization control and monitoring purposes (KCP 5.2)

A 2.3.1.1.1 Analytical method for the determination of fenpropidin residues water

Comments of zRMS:	<p>The analytical method GRM024.03A for the determination of residues of fenpropidin and metabolite CGA289267 in water has been validated with an LOQ of 0.05 µg/L.</p> <p>The accuracy and precision of the method for fenpropidin and metabolite CGA289267 was successfully demonstrated as the mean recovery value was between 70 – 110% with a relative standard deviation of ≤ 20%.</p> <p>This procedure has been demonstrated to be a reliable and accurate procedure for the determination of fenpropidin and CGA289267 residues in water. This method satisfies EU guidelines SANCO/3029/99 rev. 4, SANCO/825/00 rev. 7.</p>
-------------------	---

Reference:	KCP 5.2/10
Report:	Fenpropidin (CGA114900) – Residue method for the determination of Fenpropidin and metabolite CGA289267 in water. Final determination by LC-MS/MS, Richardson M., 2007, report no.: GRM024.03A
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:	No
Acceptability/Reliability:	Yes

Materials and methods

In summary, groundwater, surface water and tap water samples were diluted with acetonitrile and then analysed directly by high performance liquid chromatography with triple quadrupole mass spectrometric detection (LC-MS/MS) for fenpropidin (CGA114900) and its metabolite CGA289267. The limit of quantification (LOQ) of the method is 0.05 µg/L.

Results and discussions

Recovery results were in a range of 84 – 96 % with an RSD ≤ 7 % for fenpropidin and a range of 73 – 100 % for CGA289267. 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 0.05 µg/L.

Table A 117: Recovery results from method validation of fenpropidin using the analytical method (primary transition m/z 274 → 147)

Matrix	Fortification (µg/L)	Recovery (%)	n	Mean (%)	RSD (%)	Range (%)
Surface water	0.05	88, 84, 89, 88, 87	5	87	2	4
	0.5	98, 95, 96, 91, 94	5	96	3	7
	Overall		10	91	5	14
Ground water	0.05	90, 90, 83, 89, 89	5	88	3	7
	0.5	98, 95, 95, 96, 96	5	96	1	3
	Overall		10	92	5	15
Drinking water	0.05	85, 87, 83, 82, 84	5	84	2	5
	0.5	99, 94, 96, 97, 93	5	96	2	6

	Overall	10	90	7	17
--	---------	----	----	---	----

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

Table A 118: Recovery results from method validation of fenpropidin using the analytical method (confirmatory transition m/z 274 \rightarrow 117)

Matrix	Fortification ($\mu\text{g/L}$)	Recovery (%)	n	Mean (%)	RSD (%)	Range (%)
Surface water	0.05	90, 87, 96, 95, 86	5	91	5	10
	0.5	98, 97, 95, 93, 96	5	96	2	5
	Overall		10	93	4	12
Ground water	0.05	104, 86, 94, 90, 90	5	92	7	18
	0.5	101, 97, 96, 95, 92	5	96	3	9
	Overall		10	94	6	18
Drinking water	0.05	82, 88, 85, 84, 87	5	87	4	8
	0.5	100, 93, 91, 94, 97	5	95	4	9
	Overall		10	91	6	16

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

Table A 119: Recovery results from method validation of CGA289267 using the analytical method (primary transition m/z 304.2 \rightarrow 107.2)

Matrix	Fortification ($\mu\text{g/L}$)	Recovery (%)	n	Mean (%)	RSD (%)	Range (%)
Surface water	0.05	97, 88, 92, 93, 93	5	93	4	9
	0.5	102, 93, 97, 93, 98	5	97	4	9
	Overall		10	95	4	14
Ground water	0.05	95, 88, 97, 103, 88	5	94	7	15
	0.5	98, 92, 90, 88, 92	5	92	4	10
	Overall		10	93	5	15
Drinking water	0.05	85, 65, 63, 68, 82	5	73	5	13
	0.5	99, 94, 99, 100, 94	5	97	2	5
	Overall		10	95	5	16

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

Table A 120: Recovery results from method validation of CGA289267 using the analytical method (confirmatory transition m/z 304.2 \rightarrow 86.1)

Matrix	Fortification ($\mu\text{g/L}$)	Recovery (%)	n	Mean (%)	RSD (%)	Range (%)
Surface water	0.05	89, 97, 97, 92, 89	5	93	4	8
	0.5	96, 95, 93, 91, 99	5	95	3	8

	Overall		10	94	4	8
Ground water	0.05	100, 104, 105,96, 95	5	100	5	10
	0.5	100, 91, 90, 92, 93	5	93	4	10
	Overall		10	97	6	15
Drinking water	0.05	94, 84, 92, 90, 97	5	92	5	13
	0.5	100, 95, 97, 98, 98	5	98	2	5
	Overall		10	95	5	16

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

Table A 121: Characteristics for the analytical method used for validation of fenpropidin and CGA289267 in water

	Fenpropidin	CGA289267
Specificity	blank value < 30 % LOQ	
Calibration (type, number of data points)	Individual calibration data presented r > 0.99 6 calibration points	
Equation	<u>Surface water</u> $y = 458734x + 2053$ (274.2 → 147.2 m/z) $y = 104791x + 448$ (274.2 → 117.1 m/z) <u>Ground water</u> $y = 457787x + 2446$ (274.2 → 147.2 m/z) $y = 455164x + 1952$ (274.2 → 117.1 m/z) <u>Drinking water</u> $y = 455164x + 1952$ (274.2 → 147.2 m/z) $y = 103986x + 459$ (274.2 → 117.1 m/z)	<u>Surface water</u> $y = 54045x + 151$ (304.2 → 107.2 m/z) $y = 64266x + 192$ (304.2 → 86.1 m/z) <u>Ground water</u> $y = 57386x + 126$ (304.2 → 107.2 m/z) $y = 66804x + 73$ (304.2 → 86.1 m/z) <u>Drinking water</u> $y = 50817x + 415$ (304.2 → 107.2 m/z) $y = 61227x + 255$ (304.2 → 86.1 m/z)
Calibration range	0.0125 µg/mL to 0.5 µg/mL (equivalent to 0.5 pg to 20 pg on-column when using a 40 µL injection volume)	
Assessment of matrix effects is presented	The effect of each water matrix on the LC-MS/MS response of fenpropidin and CGA289267 was assessed by comparing the peak areas of a series of single injections of matrix-matched calibration standards covering the range 0.0075 to 0.50 ng/mL with the peak areas of a series of single injections of non-matrix-matched calibration standards prepared with the same nominal concentrations. The matrix effects observed resulted in enhancement (+) ≥89% on the instrument response for fenpropidin. Thus, matrix effects were considered significant and matrix matched standards were used for calibration	
Extract and standard stability	Information on the stability of extracts and standards is not available in the study report. However, this is not considered to be relevant for the validity of the study and the stability of extracts and standards was confirmed in the ILV filed as KCP 5.2/10 (Devine, T., 2016, report no.: 7706).	
Limit of quantification	0.05 µg/L	

Conclusion

The method fulfils the requirements of SANTE/2020/12830, Rev.1 and is suitable for the determination of fenpropidin and CGA289267 in ground, surface and drinking water.

A 2.3.1.1.2 Analytical method for the determination of fenpropidin residues in drinking water (Independent laboratory validation)

Comments of zRMS:	The analytical method GRM024.03A (Richardson M., 2007) for the determination of residue levels of fenpropidin (CGA114900) and its metabolite CGA289267 in three types of water: groundwater, surface water and tap water with LOQ of 0.05 µg/L by LC-MS/MS has been independently validated.
-------------------	--

	Acceptable mean recoveries within 70 – 120% and RSDs \leq 20% were found for fenpropidin and CGA289267 for both primary and confirmatory transitions in all three water matrices tested: groundwater, surface water and tap water. This ILV study was conducted in compliance with SANCO/3029/99 Rev. 4 and SANCO/825/00 Rev. 8.1.
--	---

Reference:	KCP 5.2/11
Report:	Fenpropidin (CGA114900) - Independent Laboratory Validation of an Analytical Method GRM024.03A for the Determination of Residues of Fenpropidin (CGA114900) and its Metabolite CGA289267 in Water by LC-MS/MS, Devine, T., 2016, report no.: 7706
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

In summary, groundwater, surface water and tap water samples were diluted and then analysed directly by high performance liquid chromatography with triple quadrupole mass spectrometric detection (LC-MS/MS) for fenpropidin (CGA114900) and its metabolite CGA289267. The limit of quantification (LOQ) of the method is 0.05 µg/L for each analyte.

Results and discussions

Recovery results were in a range of 83 – 103 % with an RSD \leq 9.4 % for fenpropidin and a range of 88 – 104 % for CGA289267. 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 0.05 µg/L.

Table A 122: Recovery results from method validation of fenpropidin using the analytical method (primary transition m/z 274.2 \rightarrow 147.0)

Matrix	Fortification Level (µg/L) *	Accuracy (%)	Number of Analysis (n)	Mean Accuracy (%)	RSD (%)	Accuracy Range (%)	95% Confidence Intervals (Mean \pm)
Groundwater	0.05	87, 87, 92, 85, 81	5	86	4.6	81 – 92	3.5
	0.5	83, 86, 84, 88, 88	5	86	2.7	83 – 88	2.0
	Overall	-	10	86	3.6	81 – 92	1.9
Surface Water	0.05	87, 81, 87, 82, 77	5	83	5.2	77 – 87	3.7
	0.5	88, 89, 89, 94, 92	5	90	2.8	88 – 94	2.2
	Overall	-	10	87	6.0	77 – 94	3.2
Tap (Drinking) Water	0.05	89, 92, 85, 92, 87	5	89	3.5	85 – 92	2.7
	0.5	92, 91, 89, 91, 90	5	91	1.3	89 – 92	1.0
	Overall	-	10	90	2.6	85 – 92	1.5

* 0.05 µg/L = limit of quantification, defined by the lowest validated fortification level.

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

% Mean recovery and % RSD calculated using rounded values.

Table A 123: Recovery results from method validation of fenpropidin using the analytical method (confirmatory transition m/z 274.2 \rightarrow 117.0)

Matrix	Fortification Level (µg/L) *	Accuracy (%)	Number of Analysis (n)	Mean Accuracy (%)	RSD (%)	Accuracy Range (%)	95% Confidence Intervals (mean \pm)
Groundwater	0.05	92, 100, 98, 95, 98	5	97	3.2	92 – 100	2.7
	0.5	100, 103, 101, 98, 98	5	100	2.1	98 – 103	1.9
	Overall	-	10	98	3.1	92 – 103	1.9
Surface Water	0.05	85, 88, 82, 92, 93	5	88	5.3	82 – 93	4.1
	0.5	100, 104, 99, 102, 110	5	103	4.2	99 – 110	3.8
	Overall	-	10	96	9.4	82 – 110	5.6

Tap (Drinking) Water	0.05	97, 99, 98, 98, 89	5	96	4.2	89 – 99	3.6
	0.5	102, 108, 102, 99, 103	5	103	3.2	99 – 108	2.9
	Overall	-	10	100	5.0	89 - 108	3.1

* 0.05 µg/L = limit of quantification, defined by the lowest validated fortification level.

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

% Mean recovery and % RSD calculated using rounded values.

Table A 124: Recovery results from method validation of CGA289267 using the analytical method (primary transition m/z 304.2 → 107.2)

Matrix	Fortification Level (µg/L) *	Accuracy (%)	Number of Analysis (n)	Mean Accuracy (%)	RSD (%)	Accuracy Range (%)	95% Confidence Intervals (mean ±)
Groundwater	0.05	96, 100, 94, 95, 91	5	95	3.4	91 – 100	2.9
	0.5	100, 104, 103, 100, 98	5	101	2.4	98 – 104	2.1
	Overall	-	10	98	4.2	91 – 104	2.5
Surface Water	0.05	89, 92, 88, 83, 106	5	92	9.5	83 – 106	7.6
	0.5	100, 102, 104, 103, 106	5	103	2.2	100 – 106	2.0
	Overall	-	10	97	8.7	83 – 106	5.3
Tap (Drinking) Water	0.05	93, 91, 96, 96, 93	5	94	2.3	91 – 96	1.9
	0.5	100, 110, 103, 100, 106	5	104	4.1	100 – 110	3.7
	Overall	-	10	99	6.2	91 – 110	3.8

* 0.05 µg/L = limit of quantification, defined by the lowest validated fortification level.

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

% Mean recovery and % RSD calculated using rounded values.

Table A 125: Recovery results from method validation of CGA289267 using the analytical method (confirmatory transition m/z 304.2 → 86.2)

Matrix	Fortification Level (µg/L) *	Accuracy (%)	Number of Analysis (n)	Mean Accuracy (%)	RSD (%)	Accuracy Range (%)	95% Confidence Intervals (mean ±)
Groundwater	0.05	92, 100, 98, 95, 98	5	97	3.2	92 – 100	2.7
	0.5	100, 103, 101, 98, 98	5	100	2.1	98 – 103	1.9
	Overall	-	10	98	3.1	92 – 103	1.9
Surface Water	0.05	85, 88, 82, 92, 93	5	88	5.3	82 – 93	4.1
	0.5	100, 104, 99, 102, 110	5	103	4.2	99 – 110	3.8
	Overall	-	10	96	9.4	82 – 110	5.6
Tap (Drinking) Water	0.05	97, 99, 98, 98, 89	5	96	4.2	89 – 99	3.6
	0.5	102, 108, 102, 99, 103	5	103	3.2	99 – 108	2.9
	Overall	-	10	100	5.0	89 – 108	3.1

* 0.05 µg/L = limit of quantification, defined by the lowest validated fortification level.

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

% Mean recovery and % RSD calculated using rounded values.

Table A 126: Characteristics for the analytical method used for validation of fenpropidin and CGA289267 in water

	Fenpropidin	CGA289267
Specificity	blank value < 30 % LOQ	blank value < 30 % LOQ
Calibration (type, number of data points)	Individual calibration data presented $r > 0.99$ 6 calibration points	Individual calibration data presented $r > 0.99$ 6 calibration points
Equation	$y = 675241.79x + 3498.495$ (274.2 → 147.0 m/z) $y = 61930.476x + 3158.063$ (274.2 → 117.0 m/z)	$y = 234613.695x - 282.612$ (304.2 → 107.2 m/z) $y = 225073.335x - 270.448$ (304.2 → 86.2 m/z)
Calibration range	0.0075 to 0.50 ng/mL corresponding to 0.015 – 1 µg/L in the sample	0.0075 to 0.50 ng/mL corresponding to 0.015 – 1 µg/L in the sample
Assessment of matrix effects is presented	The effect of each water matrix on the LC-MS/MS response of fenpropidin and CGA289267 was assessed by comparing the peak areas of a series of single injections of matrix-matched calibration standards covering the range 0.0075 to 0.50 ng/mL with the peak areas of a series of single injections of non-matrix-matched calibration standards prepared with the same nominal concentrations. The matrix effects observed resulted in enhancement (+) ≤ 10% or suppression (-) ≥ 15.1 on the instrument response). Thus, matrix effects were not considered significant according to the SANCO/825/00 rev.8.1 guideline (not significant if < 20%).	
Extract and standard stability	<p><u>Extract stability:</u> Final sample extracts fortified at the LOQ level (0.05 µg/L) for each matrix were re-analysed for fenpropidin and CGA289267 after storage between 2 and 8 °C. Final sample extracts of groundwater and tap water were found to be stable with respect to both fenpropidin and CGA289267 for at least 7 days (groundwater) and 8 days (tap water) when stored between 2 and 8 °C. Final extracts of surface water samples were found not to be stable for fenpropidin when tested after 7 days storage between 2 and 8 °C, but were stable for CGA289267 when tested after 7 days storage between 2 and 8 °C.</p> <p><u>Standard stability:</u> The stability of fenpropidin and CGA289267 in a 5 ng/mL mixed standard solution prepared in methanol was assessed. The results indicate that fenpropidin and CGA289267 in mixed standard solutions prepared in methanol are stable for at least 20 days when stored between 2 and 8 °C (Please refer to page 38-39 of the report).</p>	
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 fenpropidin and CGA289267 in surface water and drinking water and as ILV for Richardson, M., 2007, (report no.: GRM024.3A), KCP 5.2/10.

A 2.3.1.1.3 Analytical method for the determination of fenpropidin residues in blood

Comments of zRMS:	Analytical method REM 164.10 for the determination of residues of fenpropidin and its metabolites (CGA289267 and CGA289268) in blood was successfully validated at a limit of quantification (LOQ) of 0.01 mg/kg according to the EU guidelines SANCO/3029/99 rev.4 and SANCO/825/00 rev. 8.1. Acceptable mean recoveries in the range 70 – 110% and a relative standard deviation (RSD) of less than 20% were found for fenpropidin and its metabolites (CGA289267 and CGA289268) for both primary and confirmatory transitions in blood.
-------------------	---

Reference:	KCP 5.2/1412
Report	Fenpropidin: Validation of Analytical Method REM 164.10 for the Determination of Residues of Fenpropidin and its Metabolites CGA289267 and CGA289268 in Blood by LC-MS/MS, Cross, M., 2017, report no CEMR-8288, sponsor no.: -
Guideline(s):	SANTE/2020/12830, Rev.1
Deviations:	None
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	Not applicable

Materials and methods

A 5 g of sample was extracted with methanol:ultra-pure water (80:20, v/v), mixed and diluted with mobile phase; methanol:(HPLC water + 0.2% formic acid), 25:75 (v/v). The final determination was by high performance liquid chromatography with triple quadrupole mass spectrometric detection (LC-MS/MS), monitoring the primary transitions and confirmatory transitions for fenpropidin and its metabolites (CGA289267 and CGA289268). The limit of quantification of the method was 0.01 mg/kg.

Analytical method REM 164.10 for the determination of residues of fenpropidin and its metabolites (CGA289267 and CGA289268) in blood was successfully validated at a limit of quantification (LOQ) of 0.01 mg/kg.

Results and discussions

Recovery results were in a range of 78 – 91% with an RSD ≤ 6.1% for fenpropidin, in a range of 95 – 121% with an RSD ≤ 8.2% for CGA289267 and a range of 89 – 103% with an RSD ≤ 4.8% for CGA289268. 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 127: Recovery results from method validation of fenpropidin using the analytical method

Matrix	Fortification Level (mg/kg)	Recovery (%)	Number of Analysis (n)	Mean (%)	RSD (%)	Range (%)
Primary Transition m/z 274.2 → 147.0						
Blood	0.01*	78, 78, 82, 90, 85	5	83	6.1	78 – 90
	0.05	85, 86, 87, 88, 88	5	87	1.5	85 – 88
	Overall	-	10	85	4.9	78 – 90
Confirmatory Transition m/z 274.2 → 117.0						
Blood	0.01*	79, 91, 86, 83, 91	5	86	6.0	79 – 91
	0.05	90, 84, 88, 91, 88	5	88	3.0	84 – 91
	Overall	-	10	87	4.7	79 – 91

0.01 mg/kg = limit of quantification, defined by the lowest validated fortification level.

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

Recoveries were not corrected for residues in corresponding control samples.

% Mean and % RSD calculated using rounded values

Table A 128: Recovery results from method validation of CGA289267 using the analytical method

Matrix	Fortification Level (mg/kg)	Recovery (%)	Number of Analysis	Mean (%)	RSD (%)	Range (%)
Primary Transition m/z 304.2 \rightarrow 107.2						
Blood	0.01*	110, 110, 95, 112, 107	5	107	6.4	95 – 112
	0.05	105, 107, 108, 114, 104	5	108	3.6	104 – 114
	Overall	-	10	107	4.9	95 – 114
Confirmatory Transition m/z 304.2 \rightarrow 86.2						
Blood	0.01*	101, 111, 106, 121, 99	5	108	8.2	99 – 121
	0.05	110, 104, 106, 109, 106	5	107	2.3	104 – 110
	Overall	-	10	107	5.7	99 – 121

0.01 mg/kg = limit of quantification, defined by the lowest validated fortification level.

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

Recoveries were not corrected for residues in corresponding control samples.

% Mean and % RSD calculated using rounded values

Table A 129: Recovery results from method validation of CGA289268 using the analytical method

Matrix	Fortification Level (mg/kg)	Recovery (%)	Number of Analysis (n)	Mean (%)	RSD (%)	Range (%)
Primary Transition m/z 290.2 \rightarrow 133.2						
Blood	0.01*	89, 96, 97, 96, 99	5	95	4.0	89 – 99
	0.05	99, 96, 97, 96, 98	5	97	1.3	96 – 99
	Overall	-	10	96	2.9	89 – 99
Confirmatory Transition m/z 290.2 \rightarrow 105.2						
Blood	0.01*	90, 98, 97, 101, 102	5	98	4.8	90 – 102
	0.05	103, 96, 101, 102, 101	5	101	2.7	96 – 103
	Overall	-	10	99	4.0	90 – 103

0.01 mg/kg = limit of quantification, defined by the lowest validated fortification level.

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

Recoveries were corrected for residues in corresponding control samples.

% Mean and % RSD calculated using rounded values

Table A 130: Characteristics for the analytical method used for validation of fenpropidin, CGA289267 and CGA289268 in blood

	Fenpropidin	CGA289267	CGA289268
Specificity	blank value < 30% LOQ	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	individual calibration data presented calibration line equation presented
Calibration range	0.03 – 5 ng/mL corresponding to 0.003 to 0.5 mg/kg $r \geq 0.995$ 7 calibration points	0.03 – 5 ng/mL corresponding to 0.003 to 0.5 mg/kg $r \geq 0.995$ 7 calibration points	0.03 – 5 ng/mL corresponding to 0.003 to 0.5 mg/kg $r \geq 0.995$ 7 calibration points
Assessment of matrix effects is presented	A comparison was made of the response obtained from the matrix-matched standards against the response obtained from the standards in methanol:(HPLC water + 0.2% formic acid), (25:75, v/v). The matrix effects observed resulted in enhancement (+) $\leq 3.6\%$ or suppression (-) ≥ 9.2 on the instrument response). Thus, matrix effects were not considered significant according to the SANCO/825/00 rev.8.1 guideline (not significant if < 20%).		
Extract and standard stability	<u>Extract Stability:</u> The stability of fenpropidin and its metabolites (CGA289267 and CGA289268) in fortified final extracts stored between 2 – 8°C was assessed. Sample extracts were re-analysed after 8 days of storage against freshly prepared calibration standards. Mean recoveries and RSDs were within the acceptable ranges (70 – 110% with RSD $\leq 20\%$). The % difference in mean recoveries between the initial analysis of the samples and re-analysis after 8 days of storage was less than 20% for fenpropidin and its metabolites (CGA289267 and CGA289268) in blood. Final extracts are therefore considered stable. <u>Standard Stability:</u> The assessment of standard solution stability indicated that fenpropidin and its metabolites (CGA289267 and CGA289268) in working standard solutions (prepared in methanol) were stable		

	Fenpropidin	CGA289267	CGA289268
Specificity	blank value < 30% LOQ	blank value < 30% LOQ	blank value < 30% LOQ
	when stored at between 2 – 8°C for up to 8 days. In addition, the stock standards solutions proved to be stable for up to 22 days when stored at between 2 – 8°C.		
Limit of quantification	0.01 mg/kg	0.01 mg/kg	0.01 mg/kg

Conclusion

The method fulfils the requirements of SANTE/2020/12830, Rev.1 and is suitable for the determination of fenpropidin, CGA289267 and CGA289268 in blood.