REGISTRATION REPORT

Part B

Section 5

Analytical Methods

Detailed summary of the risk assessment

Product code: ADM.03503.F.1.A

Product name(s): see Part A

Chemical active substances:

Fluxapyroxad 75 g/L
Prothioconazole 150 g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(authorization)

Applicant: Country organisation / representative
as specified in Part A

Submission date: April 2022

MS Finalisation date: August 2023 (initial Core Assessment)
December 2023 (final Core Assessment)

Version history

When	What
April 2022	Version 1 Applicant
May 2023	Version 2 Applicant – updated following requests by zRMS
August 2023	Initial zRMS assessment The report in the dRR format has been prepared by the Applicant, therefore all comments, additional evaluations and conclusions of the zRMS are presented in grey commenting boxes. Minor changes are introduced directly in the text and highlighted in grey. Not agreed or not relevant information are ~~struck through~~ and shaded for transparency.
December 2023	Final report (Core Assessment updated following the commenting period) Additional information/assessments included by the zRMS in the report in response to comments received from the cMS and the Applicant are highlighted in yellow. Information no longer relevant is ~~struck through~~ and shaded.

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DATA PROTECTION CLAIM

In order to present a dossier fully compliant with today's requirements (Reg. 284/2013), studies have been performed on ADM.03503.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.03503.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The summaries and evaluations contained in this document may be based on unpublished proprietary data submitted for the purpose of the assessment undertaken by the regulatory authority that prepared it.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authorities should not grant, amend, or renew a registration on the basis of the summaries and evaluation of unpublished proprietary data contained in this document unless they have received the data on which the summaries and evaluation are based, either –

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5 Analytical methods

5.1 Conclusion and summary of assessment

zRMS summary and conclusions:

Prothioconazole

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

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

3) Triazole acetic acid (TAA)

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

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

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

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

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

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

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

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

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

Soil (principle of method and LOQ)	Schramel 2000 (HPLC-MS/MS, JAU6476, JAU6476-desthio, JAU6476-S-methyl*) * for monitoring not needed LOQ Soil: 0.006 mg/kg Add'l method: Steinhauer 2001 (GC-MS, JAU6476-desthio) LOQ Soil: 0.01 mg/kg
Water (principle of method and LOQ)	Sommer 2001b (HPLC-MS/MS, JAU6476, JAU6476-desthio) LOQ Surface and Drinking water: 0.1 µg/L for JAU6476 and 0.05 µg/L for JAU6476-desthio
Air (principle of method and LOQ)	Maasfeld 2002a (HPLC-MS/MS, JAU6476) LOQ Air: 0.015 mg/m^3 Additional method: Maasfeld 2002b (HPLC-MS/MS, JAU6476-desthio) LOQ Air: 0.0006 mg/m^3
Body fluids and tissues (principle of method and LOQ)	Open, data will be required if ECB classify the active as toxic

According to the EFSA Journal 2014;12(5):3689:

Methods for enforcement of residues in food of plant origin

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

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

Methods for enforcement of residues in food of animal origin

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

The Applicant submitted a number of methods for analysis of residues of prothioconazole for the generation of pre-authorization data and methods for post-authorization control and monitoring purposes.
Since many MRLs have been lowered to 0.01 mg/kg, the validated LOQ of the EU agreed methods by Weeren and Pelz (2000) and Class (2001) is not sufficient to monitor these lowered MRLs for food of plant origin. To cover the current residue definition and MRL limits, at the request of the evaluator, the applicant 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 and Watson, G., 2022a).
The studies of Lefresne, S., 2020 and Watson, G., 2022a were evaluated in Registration Report for ADM.03500.F.2.B on March 2023 by zRMS-PL
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 ILV (HPLC-MS/MS analytical method) of the analytical method for 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.03503.F.1.A.

Fluxapyroxad
In the EFSA Journal 2012;10(1):2522 – "Peer Review of the pesticide risk assessment of the active substance fluxapyroxad (BAS 700 F) it is stated that *Appropriate analytical methods are available for the post-registration monitoring of fluxapyroxad (BAS 700 F) in food and feed of plant origin with a LOQ of 0.01 mg/kg (dry, high water, high fat and high acid commodities). Residues of fluxapyroxad (BAS 700 F) in food and feed of animal origin can be monitored with a LOQ of 0.01 mg/kg, and with a LOQ of 0.001 mg/kg in milk, skimmed milk, cream and eggs. Residues of fluxapyroxad (BAS 700 F) (as well as its metabolites M700F001 and M700F002) in soil can be analysed by HPLC-MS/MS and UPLC-MS/MS with a LOQ of 0.001 mg/kg. Residues of fluxapyroxad (BAS 700 F) (as well as its metabolites M700F001, M700F002 and M700F007) in drinking water and surface water can be monitored by HPLC-MS/MS with a LOQ of 0.03 µg/L. Fluxapyroxad (BAS 700 F) residues in air can be determined by HPLC-MS/MS or UPLCMS/ MS with a LOQ of 0.06 µg/m^3. A method for residues in body fluids and tissues is not required as the active substance is not classified as toxic or very toxic.*

According to the SANTE/2020/12830:
- A validation of the primary monitoring method in an independent laboratory (ILV) is required for the determination of residues in drinking water. The ILV shall confirm the LOQ of the primary method, or at least cover the lowest MRL.
- 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 methods for the residues of fluxapyroxad in body fluids and tissues and ILV for drinking water are required.

The Applicant submitted information that the required studies report were included in BASF Chemical Active dossier (CA) for Fluxapyroxad active substance renewal and were submitted in May 2022 (for drinking water - Lee, M., 2021, KCP 5.2/07; for body fluids and tissues - Richter, S., Djedovic, S., 2016; KCP 5.2/08).

It should be noted that the documentation: BASF Chemical Active dossier (CA) for Fluxapyroxad active substance renewal on the DMS cannot be located at this moment and therefore the above-mentioned studies have been evaluated in this registration report by zRMS-PL (see Appendix 2).

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.03503.F.1.A.

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

- none

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

- none

Commodity/crop	Supported/ Not supported
Wheat	Supported
Barley	Supported
Rye	Supported
Triticale	Supported

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

5.2.1 Analysis of the plant protection product (KCP 5.1.1)

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

An overview on the acceptable methods for analysis of Fluxapyroxad and Prothioconazole in plant protection product is provided as follows:

Comments of zRMS:	The method is sufficiently described and validated according to SANCO/3030/99 rev. 5 (22 March 2019) and is suitable for the determination of active substance in a plant protection product.

Reference:	KCP 5.1.1/01
Report	Determination of the Content of the Active Substances and Impurities including Analytical Method Validation and Determination of Density Riedl, S. (2021) Noack Laboratorien GmbH, Germany Study SO20252/CGB19043, Report 000106478
Guideline(s):	SANCO/3030/99 rev. 5
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods (Determination of Fluxapyroxad and Prothioconazole)
A sample (corresponding to a concentration of nominal 1000 mg/L) of ADM.03503.F.1.A (0.1 mL) is transferred to a 10 mL measuring flask and acetonitrile (4.9 mL) is added. The flask is made up to volume with water containing 0.2% formic acid. The final solution has a nominal test concentration of 10 mg/L in a matrix of acetonitrile:water (50:50 v/v containing 0.1% formic acid). Analysis is carried out by high performance liquid chromatography with ultra-violet detection (UPLC-DAD) at 250 nm for Fluxapyroxad and 256 nm for Prothioconazole using a Water Acquity UPLC BEH Phenyl column (50 x 2.1 mm, 1.7 µm) and gradient elution with mobile phases of water+0.1% formic acid and acetonitriler+0.1% formic acid. Quantification of Fluxapyroxad and Prothioconazole is performed using external standards.

Specificity
No interferences at >3% of the LOQ were reported at the retention time of interest in the blank formulation. Analyte identity was confirmed by comparison of the retention time and mass spectrum of the analyte with that of a reference standard.

Linearity
The linearity of the detector response was demonstrated using seven calibration standards at concentrations across the working range 0.1 to 4 mg/L for each analyte, with a coefficient (r^2) >0.99. Data are presented in Table 5.2-1 below.

Precision (Repeatability)
Repeatability data were generated from the analyses of five sample preparations at the nominal level. The Horrat ratio was determined to be <1 for both analytes and presented in Table 5.2-1 below.

Accuracy (Recovery)
Accuracy (recovery) was assessed with the preparation of five samples of blank formulation fortified with both analytes at 80 %, 100 % and 120 % of nominal levels. The mean recovery in each case was found to be within guideline requirements (97-103%). The results are provided in Table 5.2-1 below.

Limit of Quantification
Not a requirement

Validation - Results and discussions

Table 5.2-1: Methods suitable for the determination of active substances Fluxapyroxad and Prothioconazole in plant protection product Fluxapyroxad 75 Prothioconazole 150 g/L EC (ADM.03503.F.1.A)

	Fluxapyroxad	**Prothioconazole**
Author(s), year	Riedl, S. (2021)	Riedl, S. (2021)
Principle of method	UPLC-DAD	UPLC-DAD
Linearity (linear between mg/L / % range of the declared content) (coefficient of determination, expressed as r^2)	Calibration Curve Range: 0.1 – 4 mg/L (equiv. 1.00 – 40 %w/w) 7 concentrations Coefficient of determination (r^2) = 0.999880 Slope = 811.714 Intercept = -4.04039	Calibration Curve Range: 0.1 – 4 mg/L (equiv. 1.00 – 40 %w/w) 7 concentrations Coefficient of determination (r^2) = 0.999996 Slope = 905.729 Intercept = -5.99812
Precision – Repeatability	RSD = 0.66 % Horrat value = 0.33 n=5	RSD = 0.57 % Horrat value = 0.32 n=5
Accuracy - Recovery	Fortification level: 80% of nominal Mean recovery = 103% n=5 (RSD = 0.55%) Fortification level: 100% of nominal Mean recovery = 103% n=5 (RSD = 0.37%) Fortification level: 120% of nominal Mean recovery = 102% n=5 (RSD = 0.43%)	Fortification level: 80% of nominal Mean recovery = 102% n=5 (RSD = 0.72%) Fortification level: 100% of nominal Mean recovery = 102% n=5 (RSD = 0.24%) Fortification level: 120% of nominal Mean recovery = 101% n=5 (RSD = 0.24%)
Interference/ Specificity	No signals detected at the retention time of the analytes in the blank formulation. Analyte i.d. confirmed by mass spectrometry.	No signals detected at the retention time of the analytes in the blank formulation. Analyte i.d. confirmed by mass spectrometry.

	Fluxapyroxad	Prothioconazole
Comment	The requirements of SANCO/3030/99 rev. 5 22/03/2019 are met	The requirements of SANCO/3030/99 rev. 5 22/03/2019 are met

Conclusion
The analytical procedure has been validated in terms of specificity, linearity, accuracy and precision in accordance with the requirements of SANCO/3030/99 rev. 5 22/03/2019.

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 ADM.03503.F.1.A is provided as follows:

Comments of zRMS:	The method is sufficiently described and validated according to SANCO/3030/99 rev. 5 (22 March 2019) and is suitable for the determination of relevant impurities in a plant protection product.

Reference:	KCP 5.1.1/02
Report	Determination of the Content of the Active Substances and Impurities including Analytical Method Validation and Determination of Density Riedl, S. (2021) Noack Laboratorien GmbH, Germany Study SO20252/CGB19043, Report 000106478
Guideline(s):	SANCO/3030/99 rev. 5
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods (Determination of Toluene)
A sample of ADM.03503.F.1.A (0.9 mL) is diluted with internal standard solution (1 mg Toluene-d8/L) and mixed well. The solution has a nominal test concentration of 900 mg/L. Analysis is carried out by gas chromatography with mass selective detection (GC-MS) using TG-WAXMS column (30 x 0.32 mm, 0.25 µm film thickness). Quantification of toluene is performed using external standards using an internal standard for normalization, monitoring the ion m/z 92.00 for quantification and m/z 91.00 & m/z 65.00 for confirmation. (For the internal standard, Toluene-d8, the following ions were monitored: m/z 100 for quantification, and m/z 98.00 & m/z 99.00 for confirmation).

Specificity
For the impurity Toluene, interfering signals were detected in background samples (i.e. blank formulation samples spiked with the a.s.): A signal was detected in the range of 0.0079% w/w test item at nominal 1 g/L. Since the analysis of the a.s. standards at the same concentration as used for the background of the fortified samples shows a signal of a comparable size, this was deemed to be an impurity of the analytical standards of the a.s. Hence, the specificity was deemed to be verified for Toluene. Analyte identity was confirmed by comparison of the retention time of the analyte with that of a reference standard.

Linearity
The linearity of the detector response was demonstrated using eleven calibration standards at concentrations across the working range 0.01 to 2 mg/L (equivalent to 0.001 – 0.2 %w/w), with a coefficient (r^2) >0.999354. Data are presented in Table 5.2-2 below.

Precision (Repeatability)

Repeatability data were generated from the analyses of five sample preparations. Two assay data sets were generated. The Horrat ratio in the first assay, 1.64, can be explained by ubiquitous distribution of unavoidable traces of toluene in laboratory equipment and the analytical device, as seen in the chromatograms of pure blank formulation samples as well as purge injections. The Horrat ratio in the second assay was found to be 0.46. Thus, the method precision is considered acceptable. The data are given in Table 5.2-2 below.

Accuracy (Recovery)
Recovery data were generated from the analyses of five sample preparations of the blank formulation fortified at LOQ and 22xLOQ levels. The mean recoveries obtained were within guideline requirements and are given in Table 5.2-2 below.

Limit of Quantification
The LOQ was determined to be 0.004% for toluene.

Validation - Results and discussions

Table 5.2-2: Methods suitable for the determination of the relevant impurity Toluene in plant protection product Fluxapyroxad 75 Prothioconazole 150 g/L EC (ADM.03503.F.1.A)

	Toluene **Max content 0.852 g/L (0.79%w/w) in PPP**
Author(s), year	Riedl, S. (2021)
Principle of method	GC-MS
Linearity **(linear between** **mg/L / % range of the declared content)** **(correlation coefficient, expressed as r)**	Calibration Curve Range: 0.01 – 2 mg/L (equiv. 0.001 – 0.2 %w/w) 11 concentrations Coefficient of determination (r^2) = 0.999354 Slope = 1.143825 Intercept = 0.032343
Precision – Repeatability	RSD: Assay 1: 7.93 % Assay 2: 2.22 % Horrat value: Assay 1: 1.64 Assay 2: 0.46 n=5 in each case
Accuracy - Recovery	LOQ fortification level: (0.004%w/w) Mean (marginal) recovery: 99% (RSD = 1.61%, n=4, 1 outlier) Range: 96-100% 22xLOQ fortification level (0.0879%w/w) Mean (total) recovery: 101% (RSD = 0.86%, n=4, 1 outlier) Range: 100-103%
Interference/ Specificity	Interfering signals were detected in background samples (i.e. blank formulation samples spiked with the a.s.): A signal was detected in the range of 0.0079% w/w test item at nominal 1 g/L. Since the analysis of the a.s. standards at the same concentration as used for the background of the fortified samples shows a signal of a comparable size, this was deemed to be an impurity of the analytical standards of the a.s. Hence, the specificity was deemed to be verified for Toluene. Analyte identity was confirmed by comparison of the retention time of the analyte with that of a reference standard.
LOQ	The limit of quantification was determined to be 0.004% for Toluene
Comment	The requirements of SANCO/3030/99 rev. 5 22/03/2019 are met

Materials and methods (Determination of Desthio-prothioconazole)
A sample of ADM.03503.F.1.A (0.1 mL) is transferred to a 10 mL measuring flask and acetonitrile (4.9 mL) is added. The flask is made up to volume with water containing 0.2% formic acid. The final

solution has a nominal test concentration of 10 mg/L in a matrix of acetonitrile:water (50:50 v/v containing 0.1% formic acid). Analysis is carried out by high performance liquid chromatography with tandem mass selective detection (UPLC-MS/MS) in positive ion mode using a Water Acquity UPLC BEH Phenyl column (50 x 2.1 mm, 1.7 µm) and gradient elution with mobile phases of water+0.1% formic acid and acetonitriler+0.1% formic acid. Quantification of desthio-prothioconazole is performed using external standards monitoring the ion transitions m/z 312.03>69.99 for quantification and m/z 312.03>124.96 for confirmation.

Specificity
For the impurity desthio-prothioconazole, interfering signals were detected in blank formulation samples spiked with the active substances. Since previous non-GLP measurements indicated desthio-prothioconzale as an impurity of the analytical standard Prothioconazole and no signals of this analyte were detected for the unspiked blank formulation (i.e. without the active substances), the specificity was deemed to be verified for desthio-prothioconazole. Analyte identity was confirmed by comparison of the retention time of the analyte with that of a reference standard.

Linearity
The linearity of the detector response was demonstrated using seven calibration standards at concentrations across the working range 0.002 to 0.04 mg/L (equivalent to 0.0002 – 0.004 %w/w), with a coefficient (r^2) >0.998022. Data are presented in Table 5.2-3 below.

Precision (Repeatability)
Repeatability data were generated from the analyses of five sample preparations. Two assay data sets were generated. The Horrat ratio in the first assay, 1.40 may have been due to small fluctuations of the detector response during analysis. During the second assay the Horwitz ratio was determined to be 0.46. Precision was additionally verified at the LOQ level during the determination of accuracy (see below) leading to Horwitz-ratio of 0.24. Thus, the method precision is considered acceptable. The data are given in Table 5.2-3 below.

Accuracy (Recovery)
Recovery data were generated from the analyses of five sample preparations of the blank formulation fortified at LOQ and 5xLOQ levels. The mean recoveries obtained were within guideline requirements (75-125%) and are given in Table 5.2-3 below.

Limit of Quantification
The limit of quantification was determined to be 0.0005% for desthio-prothioconazole.

Validation - Results and discussions

Table 5.2-3: Methods suitable for the determination of the relevant impurity Desthio-prothioconazole in plant protection product Fluxapyroxad 75 Prothioconazole 150 g/L EC (ADM.03503.F.1.A)

	Desthio-prothioconazole Max content 0.0773 (0.072% w/w) in PPP
Author(s), year	Riedl, S. (2021)
Principle of method	UPLC-MS/MS
Linearity (linear between mg/L / % range of the declared content) (correlation coefficient, expressed as r)	Calibration Curve Range: 0.002 – 0.04 mg/L (equiv. 0.0002 – 0.004 %w/w) 7 concentrations Coefficient of determination (r^2) = 0.998022 Slope = 2535.35 Intercept = 1313.09
Precision – Repeatability	RSD: Assay 1: 10.79 % Assay 2: 3.49 % Horrat value: Assay 1: 1.40

	Desthio-prothioconazole **Max content 0.0773 (0.072% w/w) in PPP**
	Assay 2: 0.46 n=5 in each case
Accuracy - Recovery	LOQ fortification level: (0.0005%w/w) Mean recovery: 81% (RSD = 1.83%, n=5, Horrat ratio = 0.24) Range: 79-83% 5xLOQ fortification level (0.0025%w/w) Mean recovery: 87% (RSD = 1.83%, n=5) Range: 85-89%
Interference/ Specificity	For the impurity desthio-prothioconazole, interfering signals were detected in blank formulation samples spiked with the active substances. Since previous non-GLP measurements indicated Des-thio-prothioconzale as an impurity of the analytical standard Prothioconazole and no signals of this analyte were detected for the unspiked blank formulation (i.e. without the active substances), the specificity was deemed to be verified for desthio-prothioconazole. Analyte identity was confirmed by comparison of the retention time of the analyte with that of a reference standard.
LOQ	The limit of quantification was determined to be 0.0005% for desthio-prothioconazole
Comment	The requirements of SANCO/3030/99 rev. 5 22/03/2019 are met

Conclusion
The analytical procedures have been validated in terms of specificity, linearity, accuracy, precision and LOQ in accordance with the requirements of SANCO/3030/99 rev. 5 22/03/2019.

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

Not required as the plant protection product contains no formulants of toxicological concern.

5.2.1.4 Applicability of existing CIPAC methods (KCP 5.1.1)

No CIPAC methods are available for the determination of fluxapyroxad. CIPAC methods (745) are available for the determination of prothioconazole in technical material, emulsifiable concentrates, suspension concentrates and flowable concentrates for seed treatments.

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

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

Component of residue definition: Fluxapyroxad				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Barley (Residues)	Primary	0.01 mg/kg	HPLC-MS/MS	Huaulmé, J-M., 2022, Appendix 2, KCP 5.1.2/01
Wheat (Residues)	Primary	0.01 mg/kg	HPLC-MS/MS	Le Mineur, A., 2022, Appendix 2, KCP 5.1.2/02
ISO medium	Primary	0.06515 mg/L	HPLC-MS/MS	[redacted] 2021a, Appendix 2, KCP 5.1.2/03

Component of residue definition: Fluxapyroxad				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
(Ecotoxicology)				
ISO medium (Ecotoxicology)	Primary	0.1394 mg/L	HPLC-MS/MS	Juckeland, D., 2021b, Appendix 2, KCP 5.1.2/04
OECD medium (Ecotoxicology)	Primary	0.1191 mg/L	HPLC-MS/MS	Juckeland, D., 2021c Appendix 2, KCP 5.1.2/05
50% sucrose solution (Ecotoxicology	Primary	33.5 mg/kg (18.4 µg/L in diluted sample)	HPLC-MS/MS	Dreßler, K., 2021, Appendix 2, KCP 5.1.2/06
Larval bee diet solution (Ecotoxicology	Primary	0.00815 mg/kg (0.407 µg/L in diluted sample)	HPLC-MS/MS	Hänsel, M., 2021, Appendix 2, KCP 5.1.2/07
Flowers, nectar, pollen (Ecotoxicology)	Primary	0.01 mg/kg	HPLC-MS/MS	Lindner M., Grewe D., 2021, Appendix 2, KCP 5.1.2/08
Spray solution (Ecotoxicology	Primary	230.1 mg/L	HPLC-UV	Friedemann, A., 2021a, Appendix 2, KCP 5.1.2/09
				Friedemann, A., 2021b, Appendix 2, KCP 5.1.2/10

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

Component of residue definition: M700F002				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Barley (Residues)	Primary	0.01 mg/kg	HPLC-MS/MS	Huaulmé, J-M., 2022, Appendix 2, KCP 5.1.2/01
Wheat (Residues)	Primary	0.01 mg/kg	HPLC-MS/MS	Le Mineur, A., 2022, Appendix 2, KCP 5.1.2/02

Table 5.2-6: Validated methods for the generation of pre-authorization data

Component of residue definition: M700F008				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Barley (Residues)	Primary	0.01 mg/kg	HPLC-MS/MS	Huaulmé, J-M., 2022, Appendix 2, KCP 5.1.2/01
Wheat (Residues)	Primary	0.01 mg/kg	HPLC-MS/MS	Le Mineur, A., 2022, Appendix 2, KCP 5.1.2/02

Table 5.2-7: Validated methods for the generation of pre-authorization data

Component of residue definition: M700F048				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Barley (Residues)	Primary	0.01 mg/kg	HPLC-MS/MS	Huaulmé, J-M., 2022, Appendix 2, KCP 5.1.2/01

Component of residue definition: M700F048				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Wheat (Residues)	Primary	0.01 mg/kg	HPLC-MS/MS	Le Mineur, A., 2022, Appendix 2, KCP 5.1.2/02

Statement on extraction efficiency

Note: Solvents used in the analytical method to analyse residue samples can be considered equivalent to that used in metabolism studies – see Table 5.3-3 below. The analytical method for generation of pre-registration data is the same as proposed for monitoring.

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

Table 5.2-8: Validated methods for the generation of pre-authorization data

Component of residue definition: Prothioconazole*				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Wheat (residues)	Primary	0.01 mg/kg**	LC-MS/MS	Lefresne, S. 2020, KCP 5.1.2/12 (filed in KCA 6.1/02)
Wheat (residues)	Primary	0.01 mg/kg**	LC-MS/MS	Amic, S., 2020b, KCP 5.1.2/13 (filed KCA 6.3.1/01)
Wheat (residues)	Primary	0.01 mg/kg**	LC-MS/MS	Lefresne, S. 2021, KCP 5.1.2/15, method validation for: Le Mineur, A., 2022a, KCA 6.3.1/03 Le Mineur, A., 2022b, KCA 6.3.1/04)
Barley (Residues)	Primary	0.01 mg/kg**	LC-MS/MS	Amic, S., 2020d, KCP 5.1.2/16 (filed in KCA 6.3.2/01)
Barley (Residues)	Primary	0.01 mg/kg**	LC-MS/MS	Huaulmé, J.-M., 2021a, KCP 5.1.2/17 (filed in KCA 6.3.2/03)
Barley (Residues)	Primary	0.01 mg/kg**	LC-MS/MS	Lefresne, S. 2021, KCP 5.1.2/15, method validation for: Barbier, G., 2022, KCA 6.3.2/05 Huaulmé, J.-M., 2022a, KCA 6.3.2/06
Radish, leaf lettuce, barley (Residues)	Primary	0.01 mg/kg**	LC-MS/MS	Semrau, J., 2021 KCP 5.2.1/18 (filed in KCA 6.6.2/01)

Component of residue definition: Prothioconazole*				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
ISO medium (Ecotoxicology)	Primary	0.1246 mg/L in test media	HPLC-MS/MS	Juckeland, D., 2021a, Appendix 2, KCP 5.1.2/03
ISO medium (Ecotoxicology)	Primary	0.2665 mg/L in test media	HPLC-MS/MS	Juckeland, D., 2021b, Appendix 2, KCP 5.1.2/04
OECD medium (Ecotoxicology)	Primary	0.2277 mg/L in test media	HPLC-MS/MS	Juckeland, D., 2021c Appendix 2, KCP 5.1.2/05
50% sucrose solution (Ecotoxicology	Primary	63.9 mg/kg (35.2 µg/L in diluted sample)	HPLC-MS/MS	Dreßler, K., 2021, Appendix 2, KCP 5.1.2/06
Larval bee diet solution (Ecotoxicology	Primary	0.0156 mg/kg (0.779 µg/L in diluted sample)	HPLC-MS/MS	Hänsel, M., 2021, Appendix 2, KCP 5.1.2/07
Flowers, nectar, pollen, honey (Ecotoxicology)	Primary	0.01 mg/kg	HPLC-MS/MS	Lindner M., Grewe D., 2020, Appendix 2, KCP 5.1.2/19
Spray solution (Ecotoxicology	Primary	440.0 mg/L	HPLC-UV	Friedemann, A., 2021a, Appendix 2, KCP 5.1.2/09
				Friedemann, A., 2021b, Appendix 2, KCP 5.1.2/10

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

Component of residue definition: 1,2,4-Triazole, Triazole alanine, Triazole acetic acid and Triazole lactic acid				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Cucumber		0.01 mg/kg*	LC-MS/MS	Klimmek, S and Gizler, A., 2017, KCP 5.1.2/11
Grapes		0.01 mg/kg*		
Dry beans		0.01 mg/kg*		
Wheat, barley (residues)		0.01 mg/kg*	LC-MS/MS	Gustloff, C.; Wallbaum, P., 2021, KCP 5.1.2/14, method validation for: Yozgatli, H.P., 2021d, KCA 6.3.1/02 Yozgatli, H.P., 2021g, , KCA 6.3.2/02 Yozgatli, H.P., 2021h, , KCA 6.3.2/04) Le Mineur, A., 2022a, KCA 6.3.1/03 Huaulmé, J.-M., 2022a, KCA 6.3.2/06

*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 5.2-10: Statement on extraction efficiency

	Extraction efficiency for risk assessment studies
Required, available from:	Please refer to the assessment below
Not required, because:	-

As shown under point 5.3.3.2 of Part B 5 and table 5.3-12, the extraction efficiency of the residue pre-registration studies is sufficiently demonstrated according to SANTE 2017/10632 Rev. 3. Thus, further assessment of the extraction efficiency is not considered necessary, since the same analytical method was used.
The analytical methods validated for studies conducted in 2018 were limited with respect to analysis of the hydroxy prothioconazole-desthio metabolites since the method did not include a hydrolysis step to allow for the inclusion of conjugates. This has been addressed in the residue section by acknowledging that 2 methods including the use of QuEChERS had been employed between 2018-2020 trials. Therefore in terms of extraction efficiency whilst the extraction solvents used were consistent with that used in metabolism, the overall residue for risk assessment may be underestimated since conjugates would not have been accounted for. Residue trials using a hydrolysis method were repeated in 2020 to address this concern. Since prothioconazole-desthio was not shown to produce a significant level of conjugates in plants during the metabolism studies, the direct extraction using acetonitrile/water as used for the QuEChERS analytical method was concluded as appropriate for analysis. Metabolism studies on pulses and oilseeds are suitable to support the extraction efficiency from oilseed rape for an assessment according to SANTE 2017/10632 Rev. 3.

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)

See 5.2.1 above.

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

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

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

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

Matrix	**Residue definition**	**MRL / limit**	**Reference for MRL*/level Remarks**
Plant, high water content	Fluxapyroxad	0.01 mg/kg (LOQ)	EFSA Journal 2012;10(1):2522 Reg. (EU) 2022/1324
Plant, high acid content		0.01 mg/kg (LOQ)	EFSA Journal 2012;10(1):2522 Reg. (EU) 2022/1324
Plant, high protein/high starch content (dry commodities)		0.01 mg/kg (LOQ)	EFSA Journal 2012;10(1):2522 Reg. (EU) 2022/1324
Plant, high oil content		0.01 mg/kg (LOQ)	EFSA Journal 2012;10(1):2522 Reg. (EU) 2022/1324
Muscle	Fluxapyroxad	0.01 mg/kg (LOQ) 0.015 mg/kg (MRL)	EFSA Journal 2012;10(1):2522 Reg. (EU) 2022/1324
Milk		0.01 mg/kg (LOQ) 0.02 mg/kg (MRL)	EFSA Journal 2012;10(1):2522 Reg. (EU) 2022/1324

Matrix	Residue definition	MRL / limit	Reference for MRL*/level Remarks
Eggs		0.01 mg/kg (LOQ) 0.02 mg/kg (MRL)	EFSA Journal 2012;10(1):2522 Reg. (EU) 2022/1324
Fat		0.01 mg/kg (LOQ) 0.05 mg/kg (MRL)	EFSA Journal 2012;10(1):2522 Reg. (EU) 2022/1324
Liver, kidney		0.01 mg/kg (LOQ) 0.01 mg/kg (MRL)	EFSA Journal 2012;10(1):2522 Reg. (EU) 2022/1324
Soil (Ecotoxicology)	Fluxapyroxad	0.01 mg/kg (LOQ)	EFSA Journal 2012;10(1):2522
Drinking water (Human toxicology)	Fluxapyroxad	0.03 µg/L (LOQ)	EFSA Journal 2012;10(1):2522
Surface water (Ecotoxicology)	Fluxapyroxad	0.03 µg/L (LOQ)	EFSA Journal 2012;10(1):2522
Air	Fluxapyroxad	0.06 µg/m^3 (LOQ)	EFSA Journal 2012;10(1):2522
Tissue (meat or liver)	-	~~Not required~~ 0.01 mg/kg	~~Not classified as T / T+~~ General limit according to SANTE/2020/12830, Rev.1
Body fluids		~~Not required~~ 0.01 mg/L	~~Not classified as T / T+~~ General limit according to SANTE/2020/12830, Rev.1

Evaluator comments:
* The MRLs currently in place for fluxapyroxad are published in Commission Regulation (EU) Reg. (EU) 2022/1324.

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 fluxapyroxad in plant matrices is given in the following tables. For the detailed evaluation of new/additional studies, refer to Appendix 2.

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: fluxapyroxad				
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, onion, apple)	Primary and confirmatory	0.01 mg/kg	LC-MS/MS	Lehmann, Mackenroth, 2009, EU agreed, EFSA Journal 2012;10(1):2522
	ILV	0.01 mg/kg	LC-MS/MS	Class, Jooβ, 2009, EU agreed, EFSA Journal 2012;10(1):2522
High acid content (lemon, grapes)	Primary and confirmatory	0.01 mg/kg	LC-MS/MS	Lehmann, Mackenroth, 2009, EU agreed, EFSA Journal 2012;10(1):2522
	ILV	0.01 mg/kg	LC-MS/MS	Class, Jooβ, 2009, EU agreed, EFSA Journal 2012;10(1):2522
High oil content (avocado, soybean seed)	Primary and confirmatory	0.01 mg/kg	LC-MS/MS	Lehmann, Mackenroth, 2009, EU agreed, EFSA Journal 2012;10(1):2522
	ILV	0.01 mg/kg	LC-MS/MS	Class, Jooβ, 2009, EU agreed, EFSA

Component of residue definition: fluxapyroxad				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
				Journal 2012;10(1):2522
High protein/high starch content (dry) (wheat grain, straw, pea dry seed)	Primary and confirmatory	0.01 mg/kg	LC-MS/MS	Lehmann, Mackenroth, 2009, EU agreed, EFSA Journal 2012;10(1):2522
	ILV	0.01 mg/kg	LC-MS/MS	Class, Jooβ, 2009, EU agreed, EFSA Journal 2012;10(1):2522

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

Table 5.3-3: Statement on extraction efficiency

	Method for products of plant origin
Not required, because:	The solvent system used in the metabolism study is similar to the monitoring method extraction procedure, residues in metabolism studies were <0.01 mg/kg in rice grain. No further data required according to SANTE 2017/10632 rev. 3. According to the DAR, for all metabolism studies on tomato, soybean and wheat the extraction solvent system included extraction firstly with methanol and then with water. Within each metabolism study, information was presented concerning the extraction efficiency of both solvents which were then combined to provide data on overall solvent extraction. Within the study on tomato further extraction methods were performed which demonstrated that methanol/water 1/1 v/v gave comparable extraction results. It is noted that within both studies on soybean and wheat that extraction efficiency initially performed with 100% methanol was reduced especially for oilseed and grain matrices where water was shown to improve overall extraction efficiency. The method indicated here is exactly the same method developed by the original applicant and summarized in the existing DAR and has been developed based on the observations within the metabolism studies. The method involves first adding water to the matrix and allowing the sample to stand for 30 minutes prior to adding methanol followed by extraction. This extraction promotes methanol as the primary extraction solvent but at the same time aiding extraction efficiency with presence of water. Overall, it is concluded that extraction efficiency in accordance requirements of SANTE/2017/10632 have been adequately shown and methods are valid.

For the detailed evaluation of (additional) studies on extraction efficiency, it is referred to Appendix 2.

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 fluxapyroxad in animal matrices is given in the following tables. For the detailed evaluation of new/additional studies, refer to Appendix 2.

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

Component of residue definition: fluxapyroxad				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Milk	Primary and confirmatory	0.01 mg/kg	LC-MS/MS	Class, Jooβ, 2009, EU agreed, EFSA Journal 2012;10(1):2522
	ILV	0.01 mg/kg	LC-MS/MS	Hopf, Mackenroth, 2009, EU agreed, EFSA Journal 2012;10(1):2522
Eggs	Primary and confirmatory	0.01 mg/kg	LC-MS/MS	Class, Jooβ, 2009, EU agreed, EFSA Journal 2012;10(1):2522
	ILV	0.01 mg/kg	LC-MS/MS	Hopf, Mackenroth, 2009, EU agreed, EFSA Journal 2012;10(1):2522
Muscle	Primary and confirmatory	0.01 mg/kg	LC-MS/MS	Class, Jooβ, 2009, EU agreed, EFSA Journal 2012;10(1):2522
	ILV	0.01 mg/kg	LC-MS/MS	Hopf, Mackenroth, 2009, EU agreed, EFSA Journal 2012;10(1):2522
Fat	Primary and confirmatory	0.01 mg/kg	LC-MS/MS	~~Class, Jooβ, 2009, EU agreed, EFSA Journal 2012;10(1):2522~~ Hopf, Mackenroth, 2009, EU agreed, EFSA Journal 2012;10(1):2522
	ILV	0.01 mg/kg	LC-MS/MS	Class, Jooβ, 2009, EU agreed, EFSA Journal 2012;10(1):2522
Kidney, liver	Primary and confirmatory	0.01 mg/kg	LC-MS/MS	Class, Jooβ, 2009, EU agreed, EFSA Journal 2012;10(1):2522
	ILV	0.01 mg/kg	LC-MS/MS	Hopf, Mackenroth, 2009, EU agreed, EFSA Journal 2012;10(1):2522
Honey	Primary and confirmatory	Studies not yet finalised and will be included in the Chemical Active dossier (CA) for Fluxapyroxad active substance renewal to be submitted May 2022. in case required, study summaries can be post-submitted on request via LOA.		
	ILV			

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

Table 5.3-5: Statement on extraction efficiency

	Method for products of animal origin
Not required, because:	The solvent system used in the metabolism study is similar to the monitoring method extraction procedure, no fur-ther data required according to SANTE 2017/10632 rev.3.

For the detailed evaluation of (additional) studies on extraction efficiency please refer to Appendix 2.

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 fluxapyroxad in soil is given in the following tables. For the detailed evaluation of new/additional studies it is referred to Appendix 2.

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

Component of residue definition: fluxapyroxad			
Method type	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary and confirmatory	0.001 mg/kg	LC-MS/MS	Zangmeister, 2009, EU agreed, EFSA Journal 2012;10(1):2522

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

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 fluxapyroxad in surface and drinking water is given in the following tables. For the detailed valuation of new/additional studies it is referred to Appendix 2.

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

Component of residue definition: fluxapyroxad				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Drinking and surface water	Primary and confirmatory	0.03 µg/L	LC-MS/MS	Zangmeister, 2009, EU agreed, EFSA Journal 2012;10(1):2522
	ILV	0.03 µg/L	LC-MS/MS	Lee, M., 2021 KCP 5.2/07 Study report included in BASF Chemical Active dossier (CA) for Fluxapyroxad active substance renewal submitted May 2022

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

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 fluxapyroxad in air is given in the following tables. For the detailed evaluation of new/additional studies please refer to Appendix 2.

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

Component of residue definition: fluxapyroxad			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary and confirmatory	0.06 µg/m^3	LC-MS/MS	Zangmeister, 2009, EU agreed, EFSA Journal 2012;10(1):2522

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

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 fluxapyroxad 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: None allocated			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	0.01 mg/kg	LC-MS/MS	Richter, S., Djedovic, S., 2016 KCP 5.2/08 Study report included in BASF Chemical Active dossier (CA) for Fluxapyroxad active substance renewal submitted May 2022

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 prothioconazole (KCP 5.2)

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

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

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

Table 5.3-10: 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 (sum of isomers)	~~0.05 mg/kg for wheat, barley (forage and straw)~~ ~~0.02 g/kg for wheat, barley (grain), canola (seed), tomato, orange (fruit)~~ 0.01 mg/kg for citrus fruits, pome fruits, stone fruits, berries and small fruits, tropical root and tuber	~~EFSA Scientific report 2007~~ Commission Regulation (EU) 2019/552

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
		vegerables, bulb vegetables, solanaceae and malvaceae, cucurbits, leafy brassica, kohlrabies, lettuces and salad plants, spinaches, legume vegetables, sugar plants 0.02 mg/kg for tree nuts, potatoes, sweet corn, oil fruits 0.05 mg/kg for flowering brassica 0.02 – 0.3 mg/kg for oilseeds 0.01 – 0.2 mg/kg for cereals	
Food of animal origin	~~Sum of prothioconazole-desthio and its glucuronide conjugate, expressed as prothioconazole-desthio~~ Prothioconazole: prothioconazole-desthio (sum of isomers)	~~0.01 mg/kg (meat, liver, kidney, fat)~~ ~~0.004 mg/kg (milk)~~ 0.01 mg/kg	~~EFSA Scientific report 2007~~ SANTE/2020/12830, Rev.1
Soil (Ecotoxicology)	Prothioconazole, prothioconazole-desthio (M04)	0.006 mg/kg	EFSA Scientific report 2007
Drinking water (Human toxicology)	Prothioconazole, prothioconazole-desthio (M04)	0.1 µg/L	EFSA Scientific report 2007
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/m3	
Body fluids	~~None allocated~~ Prothioconazole-desthio (M04)	~~n.a.~~ 0.01 mg/L	~~n.a.~~ General limit according to SANTE/2020/12830, Rev.1
Body tissues		0.01 mg/kg	

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

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

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

Component of residue definition: Prothioconazole-desthio				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (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		

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

Table 5.3-12: 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

	Method for products of plant origin
	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. 5 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.

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

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

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

Component of residue definition: Sum of prothioconazole-desthio and its glucuronide conjugate, expressed as prothioconazole-desthio				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Fat Muscle Liver, kidney	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	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

Component of residue definition: Sum of prothioconazole-desthio and its glucuronide conjugate, expressed as prothioconazole-desthio				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
				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	Primary	0.01 mg/kg	HPLC-MS/MS	Watson, G., 2022, KCP 5.2/05
	ILV	0.01 mg/kg	HPLC-MS/MS	Lindner, M., Büdel, A., 2022, KCP 5.2/06
	Confirmatory	Not required		
Honey	Primary and confirmatory	0.01 mg/kg	LC-MS/MS	Lefresne, S., 2021, Appendix 2, KCP 5.2/01
	ILV	0.01 mg/kg	LC-MS/MS	Lindner, M., 2022, Appendix 2, KCP 5.2/02

Table 5.3-14: 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.

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

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

Table 5.3-15: 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		

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

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

Table 5.3-16: 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	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		
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)
	ILV	0.05 µg/L	HPLC-MS/MS	Thies, S., 2015, Appendix 2, KCP 5.2/03
	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 for analysis of prothioconazole residues in air is given in the following tables. No new or additional studies were submitted.

Table 5.3-17: 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		

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

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

Table 5.3-18: 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 5.2/04

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**
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 2.6.1/02)	Riedl, S.	2021	Fluxapyroxad 75 Prothioconazole 150 g/L EC (ADM.03503.F.1.A) - Determination of the Content of the Active Substances and Impurities including Analytical Method Validation and Determination of Density Study SO20252/CGB19043, Report 000106478 Noack Laboratorien GmbH, Germany GLP Unpublished	N	ADM	N
KCP 5.1.1/02 (filed in KCP 2.6.1/02)	Riedl, S.	2021	Fluxapyroxad 75 Prothioconazole 150 g/L EC (ADM.03503.F.1.A) - Determination of the Content of the Active Substances and Impurities including Analytical Method Validation and Determination of Density Study SO20252/CGB19043, Report 000106478 Noack Laboratorien GmbH, Germany GLP Unpublished	N	ADM	N
KCP 5.1.2/01 (filed in KCP 8/ KCA 6.3.2/06)	Huaulmé, J.-M.	2022a	Residue study of fluxapyroxad and prothioconazole and their metabolites in barley raw agricultural commodities after application of ADM.03503.F.1.A under field conditions - Northern Europe - 2021 Study no.: BPL21/962/GC, sponsor no.: 000107616 SynTech Research France, La Chapelle de Guinchay, France GLP Unpublished	N	ADM	**Y for prothioconazole,** evaluated in the RR for ADM.03500.F.2.B on March 2023 **N for fluxapyroxad**
KCP 5.1.2/02 (filed in KCP 8/ KCA 6.3.1/03)	Le Mineur, A.	2022	Residue study of Prothioconazole and Fluxapyroxad and their respective metabolites in wheat Raw Agricultural Commodities after foliar application of ADM.03503.F.1.A under field conditions –Northern Europe - 2021 BIOTEK Agriculture, France, Study No.: BPL21/954/GC, EFSA ref. EFSA-2021-00000513, spnosor no.: 000107608 GLP Unpublished	N	ADM	**Y for prothioconazole**, evaluated in the RR for ADM.03500.F.2.B on March 2023 **N for fluxapyroxad**
KCP 5.1.2/03 (filed in KCP 10.2.1/01)	[redacted]	2021a	Acute ADM.03503.F.1.A to *Oncorhynchus mykiss* in a 96-hour semi-static test [redacted] GLP	Y	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?
			Unpublished			
KCP 5.1.2/04 (filed in KCP 10.2.1/02)	Juckeland, D.	2021b	Acute toxicity of ADM.03503.F.1.A to *Daphnia magna* in a 48-hour static test BioChem agrar GmbH, Germany, Study No.: 20 48 ADL 0005, ADAMA Ref No.: 000105070 GLP Unpublished	N	ADM	N
KCP 5.1.2/05 (filed in KCP 10.2.1/03)	Juckeland, D.	2021c	Effects of ADM.03503.F.1.A on *Pseudokirchneriella subcapitata* in an algal growth inhibition test BioChem agrar GmbH, Germany, Study No.: 20 48 AAL 0007, ADAMA Ref No.: 000105071 GLP Unpublished	N	ADM	N
KCP 5.1.2/06 (filed in KCP 10.3.1.2/01)	Dreßler, K.	2021	Chronic toxicity of ADM.03503.F.1.A to the honey bee *Apis mellifera* L. under laboratory conditions BioChem agrar GmbH, Germany, Study No.: 20 48 BAC 0010, ADAMA Ref No.: 000105073 GLP Unpublished	N	ADM	N
KCP 5.1.2/07 (filed in KCP 10.3.1.3/01)	Hänsel, M.	2021	ADM.03503.F.1.A – Repeated exposure of honey bee larvae (*Apis mellifera* L.) under laboratory conditions BioChem agrar GmbH, Germany, Study No.: 20 48 BLC 0012, ADAMA Ref No.: 000105074 GLP Unpublished	N	ADM	N
KCP 5.1.2/08	Lindner, M. & Grewe, D.	2021	Validation of an Analytical Method for Determination of Fluxapyroxad in Flowers, Nectar and Pollen Eurofins Agroscience Services Chem GmbH, Germany Study No.: S21-00223, ADAMA Ref No.: 000107307 GLP Unpublished	N	ADM	N
KCP 5.1.2/09 (filed in KCP 10.6.2/01)	Friedemann, A.	2021a	Effects of ADM.03503.F.1.A on seedling emergence and seedling growth of six non-target terrestrial plant species under greenhouse conditions BioChem agrar GmbH, Germany, Study No.: 20 46 PSE 0004, ADAMA Ref No.: 000105081 GLP Unpublished	N	ADM	N
KCP 5.1.2/10	Friedemann, A.	2021b	Effects of ADM.03503.F.1.A on vegetative vigour of six non-target terrestrial plant species under	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 10.6.2/02)			greenhouse conditions BioChem agrar GmbH, Germany, Study No.: 20 46 PVV 0006, ADAMA Ref No.: 000105082 GLP Unpublished			
KCP 5.1.2/11 (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.:000074067 (R30330) Eurofins Agroscience Services Chem GmbH, Hamburg, Germany GLP Unpublished	N	ADM	Y evaluated in the RR for ADM.03500.F.2.B on March 2023
KCP 5.1.2/12 (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. 000107139 (R-39653) POLLENIZ / GIRPA, Beaucouze Cedex, France GLP / GEP Unpublished	N	ADM	Y evaluated in the RR for ADM.03500.F.2.B on March 2023
KCP 5.1.2/13 (filed in KCP 8/ KCA 6.3.1/01)	Amic, S.	2020b	Residue study of prothioconazole and its metabolites in wheat whole plant and Raw Agricultural Commodity after one foliar application of ADM.3500.F.2.B (250 g a.s./L of prothioconazole) - 2 harvest and 2 decline trials – Northern Europe (France, Hungary and Poland) – 2019 Report no. BPL19/762/GC, Sponsor no. 000102751 BIOTEK Agriculture, Saint-Pouange, France GLP / GEP Unpublished	N	ADM	Y evaluated in the RR for ADM.03500.F.2.B on March 2023
KCP 5.1.2/14	Gustloff, C.; Wallbaum, P.	2021	Validation of an analytical method for the determination of triazole metabolites (TDMs) in crop matrices of season 2021 Report no. S21-02262, MAC-2135V, Sponsor no. 000107909 Eurofins Agroscience Services Chem GmbH, Hamburg, Germany GLP	N	ADM	Y evaluated in the RR for ADM.03500.F.2.B on March 2023

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?
			Unpublished			
KCP 5.1.2/15	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 RR for ADM.03500.F.2.B on March 2023
KCP 5.1.2/16 (filed in KCP 8/ KCA 6.3.2/01)	Amic, S.	2020d	Residue study of prothioconazole and its metabolites in barley whole plant and Raw Agricultural Commodity after one foliar application of ADM.3500.F.2.B (250 g a.s./L of prothioconazole) - 2 harvest and 2 decline trials – Northern Europe (France, Hungary and Poland) - 2019 Report no. BPL19/764/GC, Sponsor no. 000102753 BIOTEK Agriculture, Saint-Pouange, France GLP / GEP Unpublished	N	ADM	Y evaluated in the RR for ADM.03500.F.2.B on March 2023
KCP 5.1.2/17 (filed in KCP 8/ KCA 6.3.2/03)	Huaulmé, J.-M.	2021a	Residue study of prothioconazole and its metabolites, and fenpropidin in barley whole plant and raw agricultural commodity after one foliar application of ADM.3502.F.1.A (175 g a.s./L of prothioconazole and 250 g a.s./L of fenpropidin) - 2 harvest and 2 decline trials – Northern Europe (France, Poland and Hungary) - 2020. Report no.: BPL20/844/GC, sponsor no.: 000105350 BIOTEK Agriculture, Saint-Pouange, France GLP Unpublished	N	ADM	Y evaluated in the RR for ADM.03500.F.2.B on March 2023
KCP 5.1.2/18 (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.: 000109154 (R-39638) Eurofins Agroscience Services GmbH, Stade, Germany GLP, Unpublished	N	ADM	Y evaluated in the RR for ADM.03500.F.2.B on March 2023
KCP 5.1.2/19	Lindner, M. & Grewe, D.	2020	Validation of an Analytical Method for Determination of Prothioconazole, Prothioconazole-desthio and Azoxystrobin in Nectar, Pollen, Flower and Honey Eurofins Agroscience Services Chem GmbH, Germany Study No.: S19-20860 (MAC-1940V), Sponsor no.: 000104134 GLP Unpublished	N	ADM	Y evaluated in the RR for ADM.03500.F.2.B on March 2023

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 5.2/01	Lefresne, S.	2021	Validation of an analytical method for the determination of prothioconazole residues in honey Report no. B21S-A4-P-04, EFSA-2021-00004881, Sponsor no. 000108774 GIRPA, Beaucouzé Cedex, France GLP Unpublished	N	ADM	Y evaluated in the RR for ADM.03500.F.2.B on March 2023
KCP 5.2/02	Lindner, M.	2022	Independent Laboratory Validation of an Analytical Method for Determination of Prothioconazole Residues in Honey Eurofins Agroscience Services Chem GmbH, Germany Study No.: S21-06313, EFSA-2021-00006378, ADAMA Ref No.: 000108775 GLP Unpublished	N	ADM	Y evaluated in the RR for ADM.03500.F.2.B on March 2023
KCP 5.2/03	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 51368 Leverkusen Germany, Report no.: 2015/0034/01, Adama reference no. 000110077 GLP Unpublished	N	ADM	Y evaluated in the RR for ADM.03500.F.2.B on March 2023
KCP 5.2/04	Brown, S.	2022	Development and Validation of an Analytical Method for Determination of Residues of Prothioconazole-desthio in Body Fluids (Blood) by LC-MS/MS Report no.: RES-00373, EFSA-2021-00006377, Sponsor no.: 000109608 ResChem Analytical Limited, Derby, UK GLP Unpublished	N	ADM	Y evaluated in the RR for ADM.03500.F.2.B on March 2023
KCP 5.2/05	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 RR for ADM.03500.F.2.B on March 2023
KCP 5.2/06	Lindner, M., Büdel, A.	2022	Independent Laboratory Validation of an Analytical Method for the Determination of Residues of Prothioconazole-desthio in Egg by LC-MS/MS Report no.: S22-04421 (MAC-2219V), Sponsor no.: 000111069 Eurofins Agroscience Services Chem GmbH, Hamburg, Germany GLP Unpublished	N	ADM	Y evaluated in the RR for ADM.03500.F.2.B on March 2023

List of data referred to by the applicant and relied on, provided by Letter of Access

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner	Previously used Y/N If yes, for which data point?
KCP 5.2/07	Lee, M.	2021	Independent Laboratory Validation of BASF method L0143/01: Method for the Determination of BAS 700 F and its Metabolites M700F001, M700F002, and M700F007 in Water by LC-MS/MS BASF Report No 864050, AU-2020-24 2020/2108667 GLP Unpublished	N	BASF	N
KCP 5.2/08	Richter, S., Djedovic, S.	2016	Validation of BASF analytical method L0352/01 for the determination of BAS 700 F (Fluxapyroxad) in body fluids BASF Report No EU-819457, P 4055 G 2016/1217548 GLP Unpublished	N	BASF	N

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

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 5.2	EFSA	2012	EFSA Journal 2012;10(1):2522, Conclusion on the peer review of the pesticide risk assessment of the active substance fluxapyroxad (BAS 700 F)	N	EFSA
KCP 5.2	Lehmann A. and Mackenroth C.	2009	Validation of BASF Method No. L0137/01 in plant matrices GLP, not published	N	BASF
KCP 5.2	Class T. and Jooss S.	2009	BAS 700 F: Independent Laboratory Validation (ILV) of BASF Method Numbers L0137/01 and L140/02 for the determination of BAS 700 F in Plant Materials and Animal Matrices by LC/MS/MS GLP, not published	N	BASF
KCP 5.2	Hopf B. and Mackenroth C.	2009	Validation of the analytical method L0140/02: Method for the determination of BAS 700 F (Reg. No. 5094351) and its metabolites M700F002 (Reg. No. 5435595), M700F008 (Reg. No. 5566402) and M700F048 (Reg. No. 5570265) in Animal Matrices	N	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			GLP, not published		
KCP 5.2	Zangmeister W.	2009	Validation of Analytical Method L0092: Determination of Reg no. 5094351 and its metabolites Reg. No. 5069089, reg. No. 5410775 and Reg. No. 5435595 in soil by HPLC/MS-MS GLP, not published	N	BASF
KCP 5.2	Zangmeister W.	2009	Validation of Analytical Method L0143/01 determination of BAS 700 F and its metabolites M700F001, M700F002 and M700F007 in water by HPLC/MS-MS GLP, not published	N	BASF
KCP 5.2	Zangmeister W.	2009	Validation of BASF Method L0142/01: determination of BAS 700 F in Air GLP, not published	N	BASF
KCP 5.2	EFSA	2007	EFSA Scientific Report (2007) 106, 1-98, Conclusion on the peer review of prothioconazole	N	EFSA
KCP 5.2	Weeren, R. D.; Pelz, S.	2000	Modification M033 of method 00086: Validation of DFG method S 19 (extended revision) for the determination of residues of JAU 6476-desthio in materials of plant and animal origin. Dr. Specht & Partner, Chemische Laboratorien GmbH, Hamburg, Germany Bayer AG, Report No.: 00086/M033, Date:2000-11-20 GLP, not published	N	Bayer
KCP 5.2	Class, Th.	2001	Independent laboratory validation of DFG method S19 (extended revision) for the determination of residues of JAU 6476-desthio (BAYER method 00086/M033) in plant materials PTRL Europe, Ulm, Germany Bayer AG, Report No.: P/B 484 G, Date:2001-05-15 GLP, not published	N	Bayer
KCP 5.2	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 Bayer AG, Report No.: 00655, Date:2001-02-27 GLP, not published	N	Bayer
KCP 5.2	Dubey, L.	2001	Independent laboratory validation of bayer methods 00655 and 00655/M001 for the determination of residues of JAU6476-3-hydroxy- desthio, JAU6476-4-hydroxy-desthio, and JAU6476-desthio in/on matreces of animal origin by HPLC-MS/MS Battelle, Geneva Research Centres, Carouge/Geneva, Switzerland Bayer AG, Report No.: A-14-01-01, Date:2001-10-16 GLP, not published	N	Bayer
KCP 5.2	Heinemann, O.	2001	Analytical determination of residues of JAU6476-3-hydroxy-desthio, JAU6476-4-hydroxy-desthio, and JAU6476-desthio in milk by HPLC- MS/MS (00655/M001) Bayer AG, Report No.: 00655/M001, Date:2001-05-04 GLP, not published	N	Bayer
KCP 5.2	Schramel, O.	2000	Residue analytical method 00610 (MR-643/99) for the determination of JAU 6476 and the metabolites JAU6476-	N	Bayer

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
			desthio and JAU6476-S- methyl in soil by HPLC-MS/MS Bayer AG, Report No.: 00610, Date:2000-07-13 GLP, not published		
KCP 5.2	Sommer, H.	2001	Enforcement method 00684 for determination of JAU 6476 and JAU 6476-desthio in drinking and surface water by HPLC-MS/MS Bayer AG, Report No.: 00684, Date:2001-10-23 GLP, not published	N	Bayer
KCP 5.2	Maasfeld, W.	2002	Method for the determination of JAU 6476 in air by HPLC-MS/MS Bayer AG, Report No.: 00724, Date:2002-01-22 GLP, not published	N	Bayer

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 Fluxapyroxad

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

A 2.1.1.1 Analytical Method 1

Comments of zRMS:	The analytical method has been demostrated to be reliable and accurate procedure for the determination of fluxapyroxad, M700F002, M700F008 and M700F048 in barley (grain and straw). The method complies withe the guideline SANTE/2020/12830, Rev.1. All the analytes were determined by LC-MS/MS using a quantitation and confirmation ion. The LOQ of each analyte was at 0.01 mg/kg for each matrix. The method is acceptable.

Reference:	KCP 5.1.2/01 (filed in KCA 6.3.2/06)
Reports:	Huaulmé, J-M, 2022, Residue study of fluxapyroxad and prothioconazole and their metabolites in barley raw agricultural commodities after application of ADM.03503.F.1.A under field conditions - Northern Europe - 2021 Study no.: BPL21/962/GC, sponsor no.: 000107616 SynTech Research France, La Chapelle de Guinchay, France
Guideline(s):	SANTE/2020/12830 rev. 1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

The method was used to determine the residue level of Fluxapyroxad and its metabolites in specimens of barley Raw Agricultural Commodity (grain & straw) following one application of ADM.03503.F.1.A (150 g a.s./L of prothioconazole and 75 g a.s./L of Fluxapyroxad).

(i) Determination of Fluxapyroxad and its metabolites 3-(Difluoromethyl)-1H-pyrazole-4-carboxylic acid (M700F002), 3-(Difluoromethyl)-N-(3',4',5'-trifluorobiphenyl)-2-yl)-1H-pyrazole-4-carboxamide (M700F008) & 3-(Difluoromethyl)-1-(ß-D-glucopyranosyl)-N-(3',4',5'-trifluoro biphenyl-2-yl)-1H-pyrazole-4-carboxamide (M700F048)

Materials and methods
A ground sample of barley (grain, straw, 5 g) is accurately weighed into a macerator jar. Following the addition of water (50 mL), the sample is allowed to stand for 30 minutes before methanol (50 mL) is added. The sample is homogenised for approximately 2 minutes using an Ultra-Turrax macerator at about 4000 rpm. An aliquot of the extract (25 mL) is transferred to a 50 mL tube and centrifuged for about 5 minutes at about 4000 rpm. The supernatant is filtered through a 0.45 µm PTFE filter and an aliquot is transferred to a 2 mL vial. Samples were analyzed by high performance liquid chromatography with tandem mass selective detection (HPLC-MS/MS) using a C18 Hydro RP column (100 mm x 3 mm, 2.5 µm) and gradient elution with mobile phases of water +0.1% formic acid and acetonitrile +0.1% formic acid. Quantitation is by external standards using the following ion transitions:

Analyte	Instrument Ion Mode	Mass transition (m/z)	
		Quantification	Confirmation
Fluxapyroxad	Positive	382 > 362	382 > 342
M700F002	Negative	161 > 141	161 > 66
M700F008	Positive	368 > 348	368 > 328
M700F048	Negative	528 > 326	528 > 346

Table A 1: Recovery results from method validation of Fluxapyroxad using the analytical method

Matrix	Fortification Level (mg/kg)	Recovery Range (%)	n	Mean Recovery (%)	RSD (%)	Acceptable RSD (%)	Acceptable Recovery (%)
Barley (grain)	**Ion transition m/z 382 > 362 (quantification)**						
	0.01	87 - 90	5	89	1	30	60 – 120
	0.10	93 - 97	5	95	1	20	70 – 120
	Ion transition m/z 382 > 342 (confirmation)						
	0.01	84 - 89	5	86	2	30	60 – 120
	0.10	94 - 102	5	97	4	20	70 – 120
Barley (straw)	**Ion transition m/z 382 > 362 (quantification)**						
	0.01	80 - 89	5	86	4	30	60 – 120
	0.10	94 - 100	5	98	3	20	70 – 120
	Ion transition m/z 382 > 342 (confirmation)						
	0.01	80 - 88	5	85	3	30	60 – 120
	0.10	90 - 101	5	97	4	20	70 – 120

Table A 2: Characteristics for the analytical method used for validation of Fluxapyroxad residues in barley (grain/straw)

Analyte: Fluxapyroxad	**Barley (grain)**	**Barley (straw)**
Specificity	HPLC-MS/MS monitoring multiple ion transitions is considered to be a highly specific technique. No significant interferences (> 30% of the LOQ) were observed at the retention time of interest in the control matrix. Analyte identity was confirmed by comparing the retention time of the sample peak with that of standard reference material.	
Calibration (type, number of data points)	m/z 382 > 362 R^2 =0.9986 Slope = 4034508.9252 Intercept = 43719.0857 n = 9	m/z 382 > 362 R^2 = 0.9992 Slope = 2922919.2947 Intercept = 8082.8538 n = 8
Calibration range	0.15 - 10 µg/L (corresponding to 0.003 to 0.20 mg/kg as sample)	
Assessment of matrix effects is presented	Matrix effects were found to be > ±20% and thus significant. Matrix matched standards were used for quantification.	
Solution Stability	Diluted standard and fortification solutions of Fluxapyroxad were found to be stable for at least 32 days when stored in amber glassware at -18 ºC. Matrix matched standard solutions were prepared on each day of analysis. When not analysed within 24 hours final sample extracts were stored at approx -18 ºC. As the recoveries of fortified samples were within the guideline range of 70-120%, adequate stability of the solutions was demonstrated.	
Limit of determination/quantification	LOD: 0.003 mg/kg LOQ: 0.01 mg/kg	

Table A 3: Recovery results from method validation of M700F002 using the analytical method

Matrix	Fortification Level (mg/kg)	Recovery Range (%)	n	Mean Recovery (%)	RSD (%)	Acceptable RSD (%)	Acceptable Recovery (%)
Barley (grain)	**Ion transition m/z 161 > 141 (quantification)**						
	0.01	106 - 114	5	111	3	30	60 – 120
	0.10	103 - 109	5	106	2	20	70 – 120
	Ion transition m/z 161 > 66 (confirmation)						

Matrix	Fortification Level (mg/kg)	Recovery Range (%)	n	Mean Recovery (%)	RSD (%)	Acceptable RSD (%)	Acceptable Recovery (%)
	0.01	109 - 117	5	113	2	30	60 – 120
	0.10	106 - 110	5	108	2	20	70 – 120
Barley (straw)	**Ion transition m/z 161 > 141 (quantification)**						
	0.01	95 - 108	5	101	5	30	60 – 120
	0.10	100 - 121	5	109	6	20	70 – 120
	Ion transition m/z 161 > 66 (confirmation)						
	0.01	88 - 106	5	96	7	30	60 – 120
	0.10	94 - 115	5	104	7	20	70 – 120

Table A 4: Characteristics for the analytical method used for validation of M700F002 residues in barley (grain/straw)

Analyte: M700F002	Barley (grain)	Barley (straw)
Specificity	HPLC-MS/MS monitoring multiple ion transitions is considered to be a highly specific technique. No significant interferences (> 30% of the LOQ) were observed at the retention time of interest in the control matrix. Analyte identity was confirmed by comparing the retention time of the sample peak with that of standard reference material.	
Calibration (type, number of data points)	m/z 161 > 141 R^2 =0.9976 Slope = 83110.7359 Intercept = 981.4016 n = 9	m/z 161 > 141 R^2 = 0.9984 Slope = 33284.5889 Intercept = 567.6845 n = 8
Calibration range	0.15 - 10 µg/L (corresponding to 0.003 to 0.20 mg/kg as sample)	
Assessment of matrix effects is presented	Matrix effects were found to be > ±20% and thus significant. Matrix matched standards were used for quantification.	
Solution Stability	Diluted standard and fortification solutions of M700F002 were found to be stable for at least 65 days when stored in amber glassware at -18 ºC. Matrix matched standard solutions were prepared on each day of analysis. When not analysed within 24 hours final sample extracts were stored at approx -18 ºC. As the recoveries of fortified samples were within the guideline range of 70-120%, adequate stability of the solutions was demonstrated.	
Limit of determination/quantification	LOD: 0.003 mg/kg LOQ: 0.01 mg/kg	

Table A 5: Recovery results from method validation of M700F008 using the analytical method

Matrix	Fortification Level (mg/kg)	Recovery Range (%)	n	Mean Recovery (%)	RSD (%)	Acceptable RSD (%)	Acceptable Recovery (%)
Barley (grain)	**Ion transition m/z 368 > 348 (quantification)**						
	0.01	84 - 90	5	88	3	30	60 – 120
	0.10	89 - 97	5	93	3	20	70 – 120
	Ion transition m/z 368 > 328 (confirmation)						
	0.01	81 - 91	5	85	5	30	60 – 120
	0.10	88 - 97	5	92	3	20	70 – 120
Barley (straw)	**Ion transition m/z 368 > 348 (quantification)**						
	0.01	76 - 87	5	83	5	30	60 – 120
	0.10	86 - 93	5	90	2	20	70 – 120

Matrix	Fortification Level (mg/kg)	Recovery Range (%)	n	Mean Recovery (%)	RSD (%)	Acceptable RSD (%)	Acceptable Recovery (%)
	Ion transition m/z 368 > 328 (confirmation)						
	0.01	83 - 88	5	86	2	30	60 – 120
	0.10	88 - 97	5	92	4	20	70 – 120

Table A 6: Characteristics for the analytical method used for validation of M700F008 residues in barley (grain/straw)

Analyte: M700F008	Barley (grain)	Barley (straw)
Specificity	HPLC-MS/MS monitoring multiple ion transitions is considered to be a highly specific technique. No significant interferences (> 30% of the LOQ) were observed at the retention time of interest in the control matrix. Analyte identity was confirmed by comparing the retention time of the sample peak with that of standard reference material.	
Calibration (type, number of data points)	m/z 368 > 348 R^2 =0.9972 Slope = 2371498.4985 Intercept = 41276.8435 n = 9	m/z 368 > 348 R^2 = 0.9994 Slope = 1668285.4205 Intercept = 33722.4092 n = 8
Calibration range	0.15 - 10 µg/L (corresponding to 0.003 to 0.20 mg/kg as sample)	
Assessment of matrix effects is presented	Matrix effects were found to be > ±20% and thus significant. Matrix matched standards were used for quantification.	
Solution Stability	Diluted standard and fortification solutions of M700F008 were found to be stable for at least 32 days when stored in amber glassware at -18 ºC. Matrix matched standard solutions were prepared on each day of analysis. When not analysed within 24 hours final sample extracts were stored at approx -18 ºC. As the recoveries of fortified samples were within the guideline range of 70-120%, adequate stability of the solutions was demonstrated.	
Limit of determination/quantification	LOD: 0.003 mg/kg LOQ: 0.01 mg/kg	

Table A 7: Recovery results from method validation of M700F048 using the analytical method

Matrix	Fortification Level (mg/kg)	Recovery Range (%)	n	Mean Recovery (%)	RSD (%)	Acceptable RSD (%)	Acceptable Recovery (%)
Barley (grain)	**Ion transition m/z 528 > 326 (quantification)**						
	0.01	92 - 122	5	108	9	30	60 – 120
	0.10	100 - 116	5	107	5	20	70 – 120
	Ion transition m/z 528 > 346 (confirmation)						
	0.01	98 - 116	5	108	6	30	60 – 120
	0.10	101 - 121	5	107	7	20	70 – 120
Barley (straw)	**Ion transition m/z 528 > 326 (quantification)**						
	0.01	101 - 117	5	108	5	30	60 – 120
	0.10	106 - 119	5	110	4	20	70 – 120
	Ion transition m/z 528 > 346 (confirmation)						
	0.01	105 - 115	5	111	3	30	60 – 120
	0.10	109 - 121	5	116	4	20	70 – 120

Table A 8: Characteristics for the analytical method used for validation of M700F048 residues in barley (grain/straw)

Analyte: M700F048	**Barley (grain)**	**Barley (straw)**
Specificity	HPLC-MS/MS monitoring multiple ion transitions is considered to be a highly specific technique. No significant interferences (> 30% of the LOQ) were observed at the retention time of interest in the control matrix. Analyte identity was confirmed by comparing the retention time of the sample peak with that of standard reference material.	
Calibration (type, number of data points)	m/z 528 > 326 R^2 =0.9994 Slope = 1140592.268 Intercept = -4531.1683 n = 9	m/z 528 > 326 R^2 = 0.9988 Slope = 635167.4724 Intercept = 23552.264 n = 8
Calibration range	0.15 - 10 µg/L (corresponding to 0.003 to 0.20 mg/kg as sample)	
Assessment of matrix effects is presented	Matrix effects were found to be > ±20% and thus significant. Matrix matched standards were used for quantification.	
Solution Stability	Diluted standard and fortification solutions of M700F048 were found to be stable for at least 65 days when stored in amber glassware at -18 ºC. Matrix matched standard solutions were prepared on each day of analysis. When not analysed within 24 hours final sample extracts were stored at approx -18 ºC. As the recoveries of fortified samples were within the guideline range of 70-120%, adequate stability of the solutions was demonstrated.	
Limit of determination/quantification	LOD: 0.003 mg/kg LOQ: 0.01 mg/kg	

A 2.1.1.2 Analytical Method 2

Comments of zRMS:	The analytical method has been demostrated to be reliable and accurate procedure for the determination of fluxapyroxad, M700F002, M700F008 and M700F048 in wheat (grain and straw). The method complies withe the guideline SANTE/2020/12830, Rev.1. All the analytes were determined by LC-MS/MS using a quantitation and confirmation ion. The LOQ of each analyte was at 0.01 mg/kg for each matrix. The method is acceptable.

Reference: KCP 5.1.2/02 (filed in KCA 6.3.1/03)

Reports: Le Mineur, A., 2022, Residue study of Prothioconazole and Fluxapyroxad and their respective metabolites in wheat Raw Agricultural Commodities after foliar application of ADM.03503.F.1.A under field conditions – Northern Europe – 2021 BIOTEK Agriculture, France, Study No.: BPL21/954/GC, EFSA ref. EFSA-2021-00000513, ADAMA Ref No.: 000107608

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

Deviations: No

GLP: Yes

Acceptability: Yes

The method was used to determine the residue level of Fluxapyroxad and its metabolites in specimens of wheat Raw Agricultural Commodity (grain & straw) following one application of ADM.03503.F.1.A (150 g a.s./L of prothioconazole and 75 g a.s./L of Fluxapyroxad).

A reduced validation set is presented as the methods were validated for barley (grain/straw) in Huaulmé, J-M. (2022). Please refer to KCP 5.1.2/01 above.

(i) Determination of Fluxapyroxad and its metabolites 3-(Difluoromethyl)-1H-pyrazole-4-carboxylic

acid (M700F002), 3-(Difluoromethyl)-N-(3',4',5'-trifluorobiphenyl)-2-yl)-1H-pyrazole-4-carboxamide (M700F008) & 3-(Difluoromethyl)-1-(ß-D-glucopyranosyl)-N-(3',4',5'-trifluoro biphenyl-2-yl)-1H-pyrazole-4-carboxamide (M700F048)

Materials and methods

A ground sample of wheat (grain, straw, 5 g) is accurately weighed into a macerator jar. Following the addition of water (50 mL), the sample is allowed to stand for 30 minutes before methanol (50 mL) is added. The sample is homogenised for approximately 2 minutes using an Ultra-Turrax macerator at about 4000 rpm. An aliquot of the extract (25 mL) is transferred to a 50 mL tube and centrifuged for about 5 minutes at about 4000 rpm. The supernatant is filtered through a 0.45 µm PTFE filter and an aliquot is transferred to a 2 mL vial. Samples were analyzed by high performance liquid chromatography with tandem mass selective detection (HPLC-MS/MS) using a C18 Hydro RP column (100 mm x 3 mm, 2.5 µm) and gradient elution with mobile phases of water +0.1% formic acid and acetonitrile +0.1% formic acid. Quantitation is by external standards using the following ion transitions:

Analyte	Instrument Ion Mode	Mass transition (m/z)	
		Quantification	Confirmation
Fluxapyroxad	Positive	382 > 362	382 > 342
M700F002	Negative	161 > 141	161 > 66
M700F008	Positive	368 > 348	368 > 328
M700F048	Negative	528 > 326	528 > 346

Table A 9: Recovery results from method validation of Fluxapyroxad using the analytical method

Matrix	Fortification Level (mg/kg)	Recovery Range (%)	n	Mean Recovery (%)	RSD (%)	Acceptable RSD (%)	Acceptable Recovery (%)
Wheat (grain)	**Ion transition m/z 382 > 362 (quantification)**						
	0.01	95 – 96	3	95	1	30	60 – 120
	0.10	94 – 101	3	98	3	20	70 – 120
	Ion transition m/z 382 > 342 (confirmation)						
	0.01	95 - 97	3	96	1	30	60 – 120
	0.10	93 - 100	3	97	3	20	70 – 120
Wheat (straw)	**Ion transition m/z 382 > 362 (quantification)**						
	0.01	80 - 86	3	83	3	30	60 – 120
	0.10	83 - 86	3	85	2	20	70 – 120
	Ion transition m/z 382 > 342 (confirmation)						
	0.01	73 - 78	3	75	3	30	60 – 120
	0.10	75 - 84	3	79	5	20	70 – 120

Table A 10: Characteristics for the analytical method used for validation of Fluxapyroxad residues in wheat (grain/straw)

Analyte: Fluxapyroxad	Wheat (grain)	Wheat (straw)
Specificity	HPLC-MS/MS monitoring multiple ion transitions is considered to be a highly specific technique. No significant interferences (> 30% of the LOQ) were observed at the retention time of interest in the control matrix. Analyte identity was confirmed by comparing the retention time of the sample peak with that of standard reference material.	
Calibration (type, number of data points)	m/z 382 > 362 R^2 =0.9992 Slope = 3334650.3421 Intercept = 20936.5912 n = 7 m/z 382 > 342	m/z 382 > 362 R^2 = 0.9912 Slope = 3629747.0452 Intercept = 11672.3624 n = 5 m/z 382 > 342

Analyte: Fluxapyroxad	**Wheat (grain)**	**Wheat (straw)**
Specificity	HPLC-MS/MS monitoring multiple ion transitions is considered to be a highly specific technique. No significant interferences (> 30% of the LOQ) were observed at the retention time of interest in the control matrix. Analyte identity was confirmed by comparing the retention time of the sample peak with that of standard reference material.	
	R^2 =0.9990 Slope = 2850478.6099 Intercept = 21514.8578 n = 7	R^2 =0.9950 Slope = 3316283.5571 Intercept = 48113.7076 n = 5
Calibration range	0.15 - 10 µg/L (corresponding to 0.003 to 0.20 mg/kg as sample)	
Assessment of matrix effects is presented	Matrix effects were found to be < ±20% and thus not significant for wheat (grain). Matrix effects were found to be > ±20% and thus significant for wheat (straw). Matrix matched standards were used for quantification throughout nevertheless.	
Solution Stability	Diluted standard and fortification solutions of Fluxapyroxad & M700F008 were found to be stable for at least 32 days when stored in amber glassware at -18 ºC. Similarly, diluted standard and fortification solutions of M700F002 & M700F048 were found to be stable for at least 65 days when stored in amber glassware at -18 ºC. Matrix matched standard solutions were prepared on each day of analysis. When not analysed within 24 hours final sample extracts were stored at approx -18 ºC. As the recoveries of fortified samples were within the guideline range of 70-120%, adequate stability of the solutions was demonstrated.	
Limit of determination/quantification	LOD: 0.003 mg/kg LOQ: 0.01 mg/kg	

Table A 11: Recovery results from method validation of M700F002 using the analytical method

Matrix	**Fortification Level (mg/kg)**	**Recovery Range (%)**	**n**	**Mean Recovery (%)**	**RSD (%)**	**Acceptable RSD (%)**	**Acceptable Recovery (%)**
Wheat (grain)	**Ion transition m/z 161 > 141 (quantification)**						
	0.01	94 - 99	3	96	3	30	60 – 120
	0.10	92 - 102	3	98	5	20	70 – 120
	Ion transition m/z 161 > 66 (confirmation)						
	0.01	97- 99	3	98	1	30	60 – 120
	0.10	93 - 106	3	100	5	20	70 – 120
Wheat (straw)	**Ion transition m/z 161 > 141 (quantification)**						
	0.01	92 - 100	3	96	3	30	60 – 120
	0.10	107 - 116	3	110	4	20	70 – 120
	Ion transition m/z 161 > 66 (confirmation)						
	0.01	97 - 102	3	99	2	30	60 – 120
	0.10	103 - 109	3	106	2	20	70 – 120

Table A 12: Characteristics for the analytical method used for validation of M700F002 residues in wheat (grain/straw)

Analyte: M700F002	**Wheat (grain)**	**Wheat (straw)**
Specificity	HPLC-MS/MS monitoring multiple ion transitions is considered to be a highly specific technique. No significant interferences (> 30% of the LOQ) were observed at the retention time of interest in the control matrix. Analyte identity was confirmed by comparing the retention time of the sample peak with that of standard reference material.	

Calibration (type, number of data points)	m/z 161 > 141 R^2 =0.9988 Slope = 79352.5856 Intercept = 1601.9308 n = 8 m/z 161 > 66 R^2 =0.9992 Slope = 47329.9279 Intercept = 1079.3673 n = 8	m/z 161 > 141 R^2 = 0.9996 Slope = 46571.7263 Intercept = 2016.3204 n = 8 m/z 161 > 66 R^2 =0.9998 Slope = 26805.3795 Intercept = 2406.9115 n = 8
Calibration range	0.15 - 10 µg/L (corresponding to 0.003 to 0.20 mg/kg as sample)	
Assessment of matrix effects is presented	Matrix effects were found to be < ±20% and thus not significant for wheat (grain, primary transition). Matrix effects were found to be > ±20% and thus significant for wheat (straw) & wheat (grain, confirmatory transition). Matrix matched standards were used for quantification throughout nevertheless.	
Solution Stability	Diluted standard and fortification solutions of Fluxapyroxad & M700F008 were found to be stable for at least 32 days when stored in amber glassware at -18 ºC. Similarly, diluted standard and fortification solutions of M700F002 & M700F048 were found to be stable for at least 65 days when stored in amber glassware at -18 ºC. Matrix matched standard solutions were prepared on each day of analysis. When not analysed within 24 hours final sample extracts were stored at approx -18 ºC. As the recoveries of fortified samples were within the guideline range of 70-120%, adequate stability of the solutions was demonstrated.	
Limit of determination/quantification	LOD: 0.003 mg/kg LOQ: 0.01 mg/kg	

Table A 13: Recovery results from method validation of M700F008 using the analytical method

Matrix	Fortification Level (mg/kg)	Recovery Range (%)	n	Mean Recovery (%)	RSD (%)	Acceptable RSD (%)	Acceptable Recovery (%)
Wheat (grain)	**Ion transition m/z 368 > 348 (quantification)**						
	0.01	93 - 94	3	94	0.5	30	60 – 120
	0.10	90 - 106	3	98	6	20	70 – 120
	Ion transition m/z 368 > 328 (confirmation)						
	0.01	92 - 95	3	94	1	30	60 – 120
	0.10	92 - 102	3	98	4	20	70 – 120
Wheat (straw)	**Ion transition m/z 368 > 348 (quantification)**						
	0.01	68 - 72	3	69	3	30	60 – 120
	0.10	87 - 97	3	93	5	20	70 – 120
	Ion transition m/z 368 > 328 (confirmation)						
	0.01	60 - 68	3	64	5	30	60 – 120
	0.10	91 - 97	3	93	3	20	70 – 120

Table A 14: Characteristics for the analytical method used for validation of M700F008 residues in wheat (grain/straw)

Analyte: M700F008	**Wheat (grain)**	**Wheat (straw)**
Specificity	HPLC-MS/MS monitoring multiple ion transitions is considered to be a highly specific technique. No significant interferences (> 30% of the LOQ) were observed at the retention time of interest in the control matrix. Analyte identity was confirmed by comparing the retention time of the sample peak with that of standard reference material.	

Calibration (type, number of data points)	m/z 368 > 348 R^2 =0.9970 Slope = 2059816.883 Intercept = 90630.7308 n = 8 m/z 368 > 328 R^2 =0.9976 Slope = 1999831.0875 Intercept = 65402.9684 n = 8	m/z 368 > 348 R^2 = 0.9968 Slope = 1234103.3006 Intercept = 95389.8538 n = 5 m/z 368 > 328 R^2 =0.9994 Slope = 1280823.5545 Intercept = 147439.3194 n = 5
Calibration range	0.15 - 10 µg/L (corresponding to 0.003 to 0.20 mg/kg as sample)	
Assessment of matrix effects is presented	Matrix effects were found to be < ±20% and thus not significant for wheat (grain). Matrix effects were found to be > ±20% and thus significant for wheat (straw). Matrix matched standards were used for quantification throughout nevertheless.	
Solution Stability	Diluted standard and fortification solutions of Fluxapyroxad & M700F008 were found to be stable for at least 32 days when stored in amber glassware at -18 ºC. Similarly, diluted standard and fortification solutions of M700F002 & M700F048 were found to be stable for at least 65 days when stored in amber glassware at -18 ºC. Matrix matched standard solutions were prepared on each day of analysis. When not analysed within 24 hours final sample extracts were stored at approx -18 ºC. As the recoveries of fortified samples were within the guideline range of 70-120%, adequate stability of the solutions was demonstrated.	
Limit of determination/quantification	LOD: 0.003 mg/kg LOQ: 0.01 mg/kg	

Table A 15: Recovery results from method validation of M700F048 using the analytical method

Matrix	Fortification Level (mg/kg)	Recovery Range (%)	n	Mean Recovery (%)	RSD (%)	Acceptable RSD (%)	Acceptable Recovery (%)
Wheat (grain)	**Ion transition m/z 528 > 326 (quantification)**						
	0.01	101 - 105	3	104	2	30	60 – 120
	0.10	92 - 123	3	112	9	20	70 – 120
	Ion transition m/z 528 > 346 (confirmation)						
	0.01	88 - 103	3	96	7	30	60 – 120
	0.10	99 - 111	3	104	5	20	70 – 120
Wheat (straw)	**Ion transition m/z 528 > 326 (quantification)**						
	0.01	99 - 105	3	101	2	30	60 – 120
	0.10	98 - 102	3	100	2	20	70 – 120
	Ion transition m/z 528 > 346 (confirmation)						
	0.01	89 - 107	3	99	8	30	60 – 120
	0.10	101 - 114	3	108	5	20	70 – 120

Table A 16: Characteristics for the analytical method used for validation of M700F048 residues in wheat (grain/straw)

Analyte: M700F048	**Wheat (grain)**	**Wheat (straw)**
Specificity	HPLC-MS/MS monitoring multiple ion transitions is considered to be a highly specific technique. No significant interferences (> 30% of the LOQ) were observed at the retention time of interest in the control matrix. Analyte identity was confirmed by comparing the retention time of the sample peak with that of standard reference material.	

Calibration (type, number of data points)	m/z 528 > 326 R^2 =0.9948 Slope = 998059.663 Intercept = -8562.5428 n = 5 m/z 528 > 346 R^2 =0.9992 Slope = 882361.3684 Intercept = 1992.0958 n = 5	m/z 528 > 326 R^2 = 0.9982 Slope = 557168.117 Intercept = -22063.647 n = 7 m/z 528 > 346 R^2 =0.9961 Slope = 441366.6285 Intercept = -5470.8438 n = 7
Calibration range	0.15 - 10 µg/L (corresponding to 0.003 to 0.20 mg/kg as sample)	
Assessment of matrix effects is presented	Matrix effects were found to be > ±20% and thus significant. Matrix matched standards were used for quantification throughout.	
Solution Stability	Diluted standard and fortification solutions of Fluxapyroxad & M700F008 were found to be stable for at least 32 days when stored in amber glassware at -18 ºC. Similarly, diluted standard and fortification solutions of M700F002 & M700F048 were found to be stable for at least 65 days when stored in amber glassware at -18 ºC. Matrix matched standard solutions were prepared on each day of analysis. When not analysed within 24 hours final sample extracts were stored at approx -18 ºC. As the recoveries of fortified samples were within the guideline range of 70-120%, adequate stability of the solutions was demonstrated.	
Limit of determination/quantification	LOD: 0.003 mg/kg LOQ: 0.01 mg/kg	

A 2.1.1.3 Analytical Method 3

Comments of zRMS:	The method was sufficiently validated for the quantification of fluxapyroxad and prothioconazole in test matrix with LOQ of 0.1246 mg/L for prothioconazole and 0.06515 mg/L for fluxapyroxad. The method is acceptable for risk assessment.

Reference: KCP 5.1.2/03 (filed in KCP 10.2.1/01)

Reports: [redacted] 2021a, Acute ADM.03503.F.1.A to *Oncorhynchus mykiss* in a 96-hour semi-static test, [redacted]

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

Deviations: No

GLP: Yes

Acceptability: Yes

Materials and methods

ISO medium samples stabilised with an equal volume of methanol after sampling are thawed to room temperature and homogenised by shaking and further diluted as appropriate in equal volumes of methanol and test matrix. The diluted samples are analysed directly by high performance liquid chromatography with tandem mass specific detection (HPLC-MS/MS) in positive ionisation mode using an ACE Excel 3 C18-AR column (100 mm x 2.1 mm, 3 µm) and gradient elution, with mobile phases of 0.1% formic acid & 5 mM ammonium formate in water and 0.1% formic acid & 5 mM ammonium formate in methanol. Quantification is performed using external standards. For Fluxapyroxad, the ion transition m/z 382.00 > 342.10 is used for quantification and the ion transitions m/z 382.00 > 270.80 & m/z 382.00 > 313.90 is used for confirmation. For Prothioconazole, the ion transition m/z 344.10 > 325.95 is used for quantification and the ion transitions m/z 344.10 > 124.95 & m/z 344.10 > 153.95 is used for confirmation.

Table A 17: Recovery results from method validation of Fluxapyroxad & Prothioconazole using the analytical method

Matrix	Analyte	Nominal Fortification Level (mg/L)	Recoveries (%)	Mean Recovery (%)	RSD (%)
ISO medium	Prothioconazole	0.1246	94.7, 95.8, 96.8, 98.1, 95.0	96.1	1.4
		1.253	84.8, 95.7, 88.8, 92.8, 85.1	89.5	5.3
	Fluxapyroxad	0.06515	98.4, 97.3, 98.0, 96.6, 94.8	97.0	1.5
		0.6554	100.4, 102.5, 98.7, 99.9, 98.7	100.0	1.6

Table A 18: Characteristics for the analytical method used for validation of Fluxapyroxad & Prothioconazole using the analytical method

<table>
<tr><th></th><th>Fluxapyroxad & Prothioconazole</th></tr>
<tr><td>Specificity</td><td>HPLC-MS/MS monitoring two mass transitions is considered to be a highly specific technique. No interferences at >30% of the LOQ were present at the retention time of interest in the control matrix samples. Analyte identity was confirmed by comparison of the retention time of the analyte with that of a reference standard.</td></tr>
<tr><td>Calibration (type, number of data points)</td><td>Prothioconazole
1.864 µg/L – 56.49 µg/L (n =8) (corresponding to sample concentration of 0.03729 mg/L – 1.57 mg/L)
<table>
<tr><th>m/z</th><th>Coeff. of detn. (r^2)</th><th>Slope</th><th>Intercept</th></tr>
<tr><td>344.10>325.95</td><td>0.99966</td><td>211523</td><td>-52493</td></tr>
</table>
Fluxapyroxad
0.9765 µg/L – 29.59 µg/L (n =8) (corresponding to sample concentration of 0.01953 mg/L – 0.7891 mg/L)
<table>
<tr><th>m/z</th><th>Coeff. of detn. (r^2)</th><th>Slope</th><th>Intercept</th></tr>
<tr><td>382.00>342.10</td><td>0.99998</td><td>2787040</td><td>128943</td></tr>
</table></td></tr>
<tr><td>Assessment of matrix effects is presented</td><td>No (matrix matched standards employed)</td></tr>
<tr><td>Solution stability</td><td>Not assessed</td></tr>
<tr><td>Limit of determination/quantification</td><td>The limit of quantification, defined as the lowest fortification level where acceptable precision and accuracy data were obtained, has been determined to be 0.1246 mg/L for prothioconazole and 0.06515 mg/L for fluxapyroxad in ISO medium.</td></tr>
</table>

Conclusion
The analytical procedure has been successfully validated in terms of specificity, linearity, precision, accuracy and LOQ in accordance with the requirements of SANTE/2020/12830 rev.1.

A 2.1.1.4 Analytical Method 4

Comments of zRMS:	The method was sufficiently validated for the quantification of fluxapyroxad and prothioconazole in ISO medium with LOQ of 0.2665 mg/L for prothioconazole and 0.1394 mg/L for fluxapyroxad. The method is acceptable for risk assessment.

Reference: KCP 5.1.2/04 (filed in KCP 10.2.1/02)

Reports: Juckeland, D., 2021b, Acute toxicity of ADM.03503.F.1.A to *Daphnia*

magna in a 48-hour static test, BioChem agrar GmbH, Germany, Study No.: 20 48 ADL 0005, ADAMA Ref No.: 000105070

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

Deviations: No

GLP: Yes

Acceptability: Yes

Materials and methods

ISO medium samples stabilised with an equal volume of methanol after sampling are thawed to room temperature and homogenised by shaking and further diluted as appropriate in equal volumes of methanol and test matrix. The diluted samples are analysed directly by high performance liquid chromatography with tandem mass specific detection (HPLC-MS/MS) in positive ionisation mode using an ACE Excel 3 C18-AR column (100 mm x 2.1 mm, 3 µm) and gradient elution, with mobile phases of 0.1% formic acid & 5 mM ammonium formate in water and 0.1% formic acid & 5 mM ammonium formate in methanol. Quantification is performed using external standards. For Fluxapyroxad, the ion transition m/z 382.00 > 342.10 is used for quantification and the ion transitions m/z 382.00 > 270.80 & m/z 382.00 > 313.90 is used for confirmation. For Prothioconazole, the ion transition m/z 344.10 > 325.95 is used for quantification and the ion transitions m/z 344.10 > 124.95 & m/z 344.10 > 153.95 is used for confirmation.

Table A 19: Recovery results from method validation of Fluxapyroxad & Prothioconazole using the analytical method

Matrix	Analyte	Fortification Level (mg/L)	Recoveries (%)	Mean Recovery (%)	RSD (%)
ISO medium	Prothioconazole	0.2665	101.5, 99.3, 100.5, 101.2, 95.7	99.6	2.4
		2.688	95.1, 96.6, 95.1, 94.7, 98.4	96.0	1.6
	Fluxapyroxad	0.1394	104.0, 102.4, 102.6, 102.2, 98.8	102.0	1.9
		1.406	101.3, 101.4, 100.8, 100.3, 101.2	101.0	0.4

Table A 20: Characteristics for the analytical method used for validation of Fluxapyroxad & Prothioconazole using the analytical method

<table>
<tr><th></th><th>Fluxapyroxad & Prothioconazole</th></tr>
<tr><td>Specificity</td><td>HPLC-MS/MS monitoring two mass transitions is considered to be a highly specific technique. No interferences at >30% of the LOQ were present at the retention time of interest in the control matrix samples. Analyte identity was confirmed by comparison of the retention time of the analyte with that of a reference standard.</td></tr>
<tr><td>Calibration (type, number of data points)</td><td>Prothioconazole
3.968 µg/L – 121.4 µg/L (n =8) (corresponding to sample concentration of 0.07937 mg/L – 3.236 mg/L)
<table>
<tr><th>m/z</th><th>Coeff. of detn. (r^2)</th><th>Slope</th><th>Intercept</th></tr>
<tr><td>344.10>325.95</td><td>0.99990</td><td>102052</td><td>-27557.6</td></tr>
</table>
Fluxapyroxad
2.079 µg/L – 63.57 µg/L (n =8) (corresponding to sample concentration of 0.04157 mg/L – 1.695 mg/L)
<table>
<tr><th>m/z</th><th>Coeff. of detn. (r^2)</th><th>Slope</th><th>Intercept</th></tr>
<tr><td>382.00>342.10</td><td>0.99998</td><td>1412230</td><td>126956</td></tr>
</table></td></tr>
</table>

Assessment of matrix effects is presented	No (matrix matched standards employed)
Solution stability	Not assessed
Limit of determination/quantification	The limit of quantification, defined as the lowest fortification level where acceptable precision and accuracy data were obtained, has been determined to be 0.2665 mg/L for prothioconazole and 0.1394 mg/L for fluxapyroxad in ISO medium.

Conclusion
The analytical procedure has been successfully validated in terms of specificity, linearity, precision, accuracy and LOQ in accordance with the requirements of SANTE/2020/12830 rev.1.

A 2.1.1.5 Analytical Method 5

Comments of zRMS:	The method was sufficiently validated for the quantification of fluxapyroxad and prothioconazole in OECD medium with LOQ of 0.2277 mg/L for prothioconazole and 0.1191 mg/L for fluxapyroxad. The method is acceptable for risk assessment.

Reference: KCP 5.1.2/05 (filed in KCP 10.2.1/03)

Reports: Juckeland, D., 2021c, Effects of ADM.03503.F.1.A on *Pseudokirchneriella subcapitata* in an algal growth inhibition test, BioChem agrar GmbH, Germany, Study No.: 20 48 AAL 0007, ADAMA Ref No.: 000105071

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

Deviations: No

GLP: Yes

Acceptability: Yes

Materials and methods
OECD medium samples stabilised with an equal volume of methanol after sampling are thawed to room temperature and homogenised by shaking and further diluted as appropriate in equal volumes of methanol and test matrix. The diluted samples are analysed directly by high performance liquid chromatography with tandem mass specific detection (HPLC-MS/MS) in positive ionisation mode using an ACE Excel 3 C18-AR column (100 mm x 2.1 mm, 3 µm) and gradient elution, with mobile phases of 0.1% formic acid & 5 mM ammonium formate in water and 0.1% formic acid & 5 mM ammonium formate in methanol. Quantification is performed using external standards. For Fluxapyroxad, the ion transition m/z 382.00 > 342.10 is used for quantification and the ion transitions m/z 382.00 > 270.80 & m/z 382.00 > 313.90 is used for confirmation. For Prothioconazole, the ion transition m/z 344.10 > 325.95 is used for quantification and the ion transitions m/z 344.10 > 124.95 & m/z 344.10 > 153.95 is used for confirmation.

Table A 21: Recovery results from method validation of Fluxapyroxad & Prothioconazole using the analytical method

Matrix	Analyte	Nominal Fortification Level (mg/L)	Recoveries (%)	Mean Recovery (%)	RSD (%)
OECD medium	Prothioconazole	0.2277	103.1, 102.0, 104.1, 102.9, 103.0	103.0	0.7
		6.250	98.2, 90.5, 103.1, 97.7, 98.8	97.6	4.6
	Fluxapyroxad	0.1191	102.8, 101.8, 102.6, 101.7, 97.5	101.3	2.2
		3.269	104.1, 102.7, 104.0, 103.4, 103.1	103.5	0.6

Table A 22: Characteristics for the analytical method used for validation of Fluxapyroxad & Prothioconazole using the analytical method

<table>
<tr><th></th><th>Fluxapyroxad & Prothioconazole</th></tr>
<tr><td>Specificity</td><td>HPLC-MS/MS monitoring two mass transitions is considered to be a highly specific technique. No interferences at >30% of the LOQ were present at the retention time of interest in the control matrix samples. Analyte identity was confirmed by comparison of the retention time of the analyte with that of a reference standard.</td></tr>
<tr><td>Calibration (type, number of data points)</td><td>Prothioconazole
3.403 µg/L – 113.4 µg/L (n =8) (corresponding to sample concentration of 0.06805 mg/L – 3.781 mg/L)
<table>
<tr><th>m/z</th><th>Coeff. of detn. (r^2)</th><th>Slope</th><th>Intercept</th></tr>
<tr><td>344.10>325.95</td><td>0.99951</td><td>94507.9</td><td>-48589.8</td></tr>
</table>
Fluxapyroxad
1.776 µg/L – 59.18 µg/L (n =8) (corresponding to sample concentration of 0.03551 mg/L – 1.973 mg/L)
<table>
<tr><th>m/z</th><th>Coeff. of detn. (r^2)</th><th>Slope</th><th>Intercept</th></tr>
<tr><td>382.00>342.10</td><td>0.99991</td><td>1355380</td><td>4762.82</td></tr>
</table></td></tr>
<tr><td>Assessment of matrix effects is presented</td><td>No (matrix matched standards employed)</td></tr>
<tr><td>Solution stability</td><td>Not assessed</td></tr>
<tr><td>Limit of determination/quantification</td><td>The limit of quantification, defined as the lowest fortification level where acceptable precision and accuracy data were obtained, has been determined to be 0.2277 mg/L for prothioconazole and 0.1191 mg/L for fluxapyroxad in OECD medium.</td></tr>
</table>

Conclusion
The analytical procedure has been successfully validated in terms of specificity, linearity, precision, accuracy and LOQ in accordance with the requirements of SANTE/2020/12830 rev.1.

A 2.1.1.6 Analytical Method 6

Comments of zRMS:	The method was sufficiently validated for the quantification of fluxapyroxad and prothioconazole in feeding solution (sample matrix sucrose solution containing 50% (w/v) sucrose and 0.1% (w/v) xanthan) with LOQ of 63.9 mg/kg for prothioconazole and 33.5 mg/kg for fluxapyroxad. The method is acceptable for risk assessment.

Reference: KCP 5.1.2/06 (filed in KCP 10.3.1.2/01)

Reports: Dreßler, K., 2021, Chronic toxicity of ADM.03503.F.1.A to the honey bee *Apis mellifera* L. under laboratory conditions, BioChem agrar GmbH, Germany, Study No.: 20 48 BAC 0010, ADAMA Ref No.: 000105073

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

Deviations: No

GLP: Yes

Acceptability: Yes

Materials and methods
An aliquot (0.5 g) of honey bee feeding solution (sucrose solution containing 50 %w/v sucrose and 0.1

%w/v xanthan) is extracted in acetonitrile:water (50:50 v/v, 10 mL) and a QuEChERS salt mix (containing 0.5 g magnesium sulfate, 0.12 g sodium chloride, 0.06 g disodium hydrogencitrate sesquihydrate and 0.12 g trisodium citrate dihydrate). The mixture is shaken vigorously and centrifuged at 3000 rpm for 2 minutes. The samples are diluted with acetonitrile and water (and blank extract as appropriate) prior to analysis by high performance liquid chromatography with tandem mass specific detection (HPLC-MS/MS) in positive ionisation mode using an ACE Excel 3 C18 column (100 mm x 2.1 mm, 3 µm) and gradient elution, with mobile phases of 0.1% formic acid & 5 mM ammonium formate in water and 0.1% formic acid in methanol. Quantification is performed using external standards. For Fluxapyroxad, the ion transitions m/z 382 > 362 and m/z 382 > 342 are used for quantification and confirmation respectively. For Prothioconazole, the ion transitions m/z 344 > 189 and m/z 344 > 125 are used for quantification and confirmation respectively.

Table A 23: Recovery results from method validation of Fluxapyroxad & Prothioconazole using the analytical method

Matrix	Analyte	Nominal Fortification Level (mg/kg)	Recoveries (%)	Mean Recovery (%)	RSD (%)
50% sucrose solution	Prothioconazole	63.9	94.7, 96.8, 101, 90.7, 90.4	94.6	4.57
		4262	81.3, 86.4, 85.3, 85.0, 87.6	85.1	2.78
	Fluxapyroxad	33.5	105, 106, 110, 98.4, 97.0	103	5.38
		2231	93.6, 98.6, 97.4, 96.8, 101	97.5	2.77

Table A 24: Characteristics for the analytical method used for validation of Fluxapyroxad & Prothioconazole using the analytical method

	Fluxapyroxad & Prothioconazole
Specificity	HPLC-MS/MS monitoring two mass transitions is considered to be a highly specific technique. No interferences at >30% of the LOQ were present at the retention time of interest in the control matrix samples. Analyte identity was confirmed by comparison of the retention time of the analyte with that of a reference standard.
Calibration (type, number of data points)	**Fluxapyroxad** 3.62 µg/L – 72.5 µg/L (n =7) (covering 20% of the lowest measuring concentration to 130% of the highest validation measuring concentration) **m/z** | **Coeff. of detn. (r^2)** | **Slope** | **Intercept** 382>362 | 0.99844582 | 34301.222860 | -5800.0853892 **Prothioconazole** 6.92 µg/L – 138.3 µg/L (n =7) (covering 20% of the lowest measuring concentration to 130% of the highest validation measuring concentration) **m/z** | **Coeff. of detn. (r^2)** | **Slope** | **Intercept** 344>189 | 0.99738786 | 440.994699 | -414.896512
Assessment of matrix effects is presented	No (matrix matched standards employed)
Solution stability	Not assessed
Limit of determination/quantification	The limit of quantification, defined as the lowest fortification level where acceptable precision and accuracy data were obtained, has been determined to be 63.9 mg/kg (35.2 µg/L in diluted sample) for prothioconazole and 33.5 mg/kg (18.4 µg/L in diluted sample) for fluxapyroxad, in 50% sucrose solution.

Conclusion

The analytical procedure has been successfully validated in terms of specificity, linearity, precision,

accuracy and LOQ in accordance with the requirements of SANTE/2020/12830 rev.1.

A 2.1.1.7 Analytical Method 7

Comments of zRMS:	The method was sufficiently validated for the quantification of fluxapyroxad and prothioconazole in test item stock solutions (Larval bee diet solution) with LOQ of 0.0156 mg/kg for prothioconazole and 0.00815 mg/kg for fluxapyroxad. The method is acceptable for risk assessment.

Reference: KCP 5.1.2/07 (filed in KCP 10.3.1.3/01)

Reports: Hänsel, M., 2021, ADM.03503.F.1.A – Repeated exposure of honey bee larvae (*Apis mellifera* L.) under laboratory conditions, BioChem agrar GmbH, Germany, Study No.: 20 48 BLC 0012, ADAMA Ref No.: 000105074

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

Deviations: No

GLP: Yes

Acceptability: Yes

Materials and methods

An aliquot (0.5 g) of larval bee diet solution (containing 15 %w/v glucose, 15 %w/v fructose & 3 %w/v yeast extract or 18 %w/v glucose, 18 %w/v fructose & 4 %w/v yeast extract) is extracted in acetonitrile:water (50:50 v/v, 10 mL) and a QuEChERS salt mix (containing 0.5 g magnesium sulfate, 0.12 g sodium chloride, 0.06 g disodium hydrogencitrate sesquihydrate and 0.12 g trisodium citrate dihydrate). The mixture is shaken vigorously for 5 minutes. Following centrifugation, the samples are diluted water (and blank extract as appropriate) and further with blank extract:water (50:50 v/v) prior to analysis by high performance liquid chromatography with tandem mass specific detection (HPLC-MS/MS) in positive ionisation mode using an ACE Excel 3 C18 column (100 mm x 2.1 mm, 3 µm) and gradient elution, with mobile phases of 0.1% formic acid & 5 mM ammonium formate in water and 0.1% formic acid in methanol. Quantification is performed using external standards. For Fluxapyroxad, the ion transitions m/z 382 > 362 and m/z 382 > 342 are used for quantification and confirmation respectively. For Prothioconazole, the ion transitions m/z 344 > 189 and m/z 344 > 125 are used for quantification and confirmation respectively.

Table A 25: Recovery results from method validation of Fluxapyroxad & Prothioconazole using the analytical method

Matrix	Analyte	Fortification Level (mg/kg)	Recoveries (%)	Mean Recovery (%)	RSD (%)
Larval bee diet solution	Prothioconazole	0.0156	100.0, 92.2, 95.0, 96.3, 93.2	95.3	3.18
		10.4	92.5, 94.5, 91.2, 91.6, 94.4	92.8	1.65
	Fluxapyroxad	0.00815	103, 102, 103, 109, 102	104	2.81
		5.43	90.3, 96.3, 96.5, 97.5, 97.8	95.7	3.20

Table A 26: Characteristics for the analytical method used for validation of Fluxapyroxad & Prothioconazole using the analytical method

	Fluxapyroxad & Prothioconazole
Specificity	HPLC-MS/MS monitoring two mass transitions is considered to be a highly specific technique. No interferences at >30% of the LOQ were present at the retention time of interest in the control matrix samples. Analyte identity was confirmed by comparison of the retention time of the analyte with that of a reference standard.
Calibration (type, number of data points)	**Fluxapyroxad**

<table>
<tr><td></td><td>0.0803 µg/L – 1.61 µg/L (n =7) (covering at least ±20% of the final extract concentrations)
<table>
<tr><th>m/z</th><th>Coeff. of detn. (r^2)</th><th>Slope</th><th>Intercept</th></tr>
<tr><td>382>362</td><td>0.99767615</td><td>48892.491756</td><td>1682.154398</td></tr>
</table>
Prothioconazole
0.161 µg/L – 3.22 µg/L (n =7) (covering at least ±20% of the final extract concentrations)
<table>
<tr><th>m/z</th><th>Coeff. of detn. (r^2)</th><th>Slope</th><th>Intercept</th></tr>
<tr><td>344>189</td><td>0.99886718</td><td>504.486994</td><td>20.927147</td></tr>
</table></td></tr>
<tr><td>Assessment of matrix effects is presented</td><td>No (matrix matched standards employed)</td></tr>
<tr><td>Solution stability</td><td>Not assessed</td></tr>
<tr><td>Limit of determination/quantification</td><td>The limit of quantification, defined as the lowest fortification level where acceptable precision and accuracy data were obtained, has been determined to be 0.0156 mg/kg (0.779 µg/L in diluted sample) for prothioconazole and 0.00815 mg/kg (0.407 µg/L in diluted sample) for fluxapyroxad, in larval bee diet solution.</td></tr>
</table>

Conclusion
The analytical procedure has been successfully validated in terms of specificity, linearity, precision, accuracy and LOQ in accordance with the requirements of SANTE/2020/12830 rev.1.

A 2.1.1.8 Analytical Method 8

Comments of zRMS:	The analytical method for the determination of fluxapyroxad in/on flowers, nectar and pollen was validated in accordance to guidance document SANTE/2020/12830, rev. 1 with the limit of quantification (LOQ) of 0.01 mg/kg. The method is acceptable.

Reference:	KCP 5.1.2/08 (Cross reference to KCP 10.3.1.5/01 and KCP 10.3.1.5/02)
Reports:	Lindner M., Grewe D., 2021, Validation of an Analytical Method for Determination of Fluxapyroxad in Flowers, Nectar and Pollen, Eurofins Agroscience Services Chem GmbH Study No S21-00223 (MAC-2110V), ADAMA Ref No.: 000107307
Guideline(s):	SANTE/2020/12830 rev. 1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods
Flowers and nectar: A sample (100 mg ± 10 mg) is extracted with methanol/L-Cysteine-solution/formic acid (50:50:0.5, v/v/v, 10mL) and shaken by hand for one minute and then for 15 minutes on a shaker. The sample is centrifuged for 5 minutes at about 3200 g and an aliquot is filtered through cotton (for flowers only) into a HPLC vial and kept at 1˚C -10˚C in the dark.

Pollen: A sample (100 mg ± 10 mg) is extracted with methanol/L-Cysteine-solution/formic acid (50:50:0.5, v/v/v, 10mL) and homogenised by FastPrep at 4.0 m/second for 2 x 1 minutes. The sample is shaken for 15 minutes on a shaker, followed by centrifugation for 5 minutes at 3200 g.

Samples are analysed by high performance liquid chromatography with tandem mass specific detection (HPLC-MS/MS) in positive ionisation mode using an Nucleoshell PFP column (50 mm x 2.1mm, 2.7 µm) and gradient elution with mobile phases of 0.1% formic acid in acetonitrile (v/v) and 0.1% formic acid in water (v/v). Quantification is performed using external standards. The ion transitions m/z 382 > 362 and m/z 382 > 342 are used for quantification and confirmation respectively.

Table A 27: Recovery results from method validation of Fluxapyroxad using the analytical method

Matrix	Analyte	Ion Transition (m/z)	Fortification Level (mg/kg)	Recoveries (%)	Mean Recovery (%)	RSD (%)
Flowers	Fluxapyroxad	382 > 362	0.01	111, 96, 94, 94, 100	99	7.2
			0.1	92, 95, 94, 99, 97	95	2.8
			Overall	92 – 111	97	5.6
		382 > 342	0.01	106, 93, 97, 97, 99	98	4.9
			0.1	95, 94, 92, 95, 95	94	1.4
			Overall	92 – 106	96	4.1
Nectar Surrogate		382 > 362	0.01	86, 107, 95, 92, 111	98	11
			0.1	87, 87, 91, 80, 87	86	4.6
			Overall	80 – 111	92	11
		382 > 342	0.01	84, 112, 99, 93, 100	98	11
			0.1	89, 87, 91, 78, 85	86	5.8
			Overall	78 – 112	92	11
Pollen		382 > 362	0.01	109, 99, 107, 108, 105	106	3.8
			0.1	97, 98, 91, 96, 94	95	2.9
			Overall	91 – 109	100	6.3
		382 > 342	0.01	108, 110, 105, 110, 111	109	2.2
			0.1	102, 102, 94, 97, 99	99	3.5
			Overall	94 – 111	104	5.7

Table A 28: Characteristics for the analytical method used for validation of Fluxapyroxad using the analytical method

	Fluxapyroxad
Specificity	HPLC-MS/MS monitoring two mass transitions is considered to be a highly specific technique. No interferences at >30% of the LOQ were present at the retention time of interest in the control matrix samples. Analyte identity was confirmed by comparison of the retention time of the analyte with that of a reference standard.
Calibration (type, number of data points)	0.025 ng/mL to 2.5 ng/mL (0.0025 to 0.25 mg/kg) (n =7) (covering at least no more than 25% of the LOQ and at least 20% of the highest analyte concentration detected in a sample extract)

Matrix	m/z	Coeff. of detn. (R^2)	Slope	Intercept
Flowers	382>362	0.9980	680247.0061	526.6233
	382>342	0.9989	506905.5400	-446.0118
Nectar Surrogate	382>362	0.9991	618640.4538	-1417.7155
	382>342	0.9991	473931.5491	-310.2292
Pollen	382>362	0.9972	397891.4803	-1181.4000
	382>342	0.9977	280525.6239	-786.5551

Assessment of matrix effects is presented	Matrix effects on the detection of fluxapyroxad in extracts of pollen were found to be significant (≥ 20 %). Therefore, matrix-matched standards were used for quantification. Matrix effects on the detection of fluxapyroxad in extracts of flowers and nectar were found to be insignificant (< 20 %). However, matrix-matched standards were used for quantification.
Solution stability	A stock solution of fluxapyroxad in methanol was found to be stable for 235 days stored at typically 1°C to 10°C in the dark. Calibration solutions of fluxapyroxad in methanol/L-cystein-solution (50 mg/L)/formic acid (50+50+0.5, v+v+v) were found to be stable for 11 days stored at typically 1°C to 10°C in the dark Flower extracts were found to be stable for 7 days, nectar surrogate extracts were found to be stable for 18 days and pollen extracts were found to be stable for 13 days, all stored at typically 1 °C to 10 °C in the dark.
Limit of determination/quantification	The limit of quantification, defined as the lowest fortification level where acceptable precision and accuracy data were obtained, has been determined to be 0.01 mg/kg. The limit of determination, defined as the lowest detectable amount of analyte and was taken to be the lowest calibration solution and determined to be 0.025 ng/mL

Conclusion
The analytical procedure has been successfully validated in terms of specificity, linearity, precision, accuracy and LOQ in accordance with the requirements of SANTE/2020/12830 rev.1.

A 2.1.1.9 Analytical Method 9

Comments of zRMS:	The method was sufficiently validated for the quantification of fluxapyroxad and prothioconazole in test solutions of a seedling emergence and growth test with LOQ of 440.0 mg/L for prothioconazole and 230.1 mg/L for fluxapyroxad. The method is acceptable for risk assessment.

Reference:	KCP 5.1.2/09 (filed in KCP 10.6.2/01)
Reports:	Friedemann, A., 2021a, Effects of ADM.03503.F.1.A on seedling emergence and seedling growth of six non-target terrestrial plant species under greenhouse conditions, BioChem agrar GmbH, Germany, Study No.: 20 46 PSE 0004, ADAMA Ref No.: 000105081
Guideline(s):	SANCO/3029/99 rev. 4
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Reference:	KCP 5.1.2/10 (Filed in KCP 10.6.2/02)
Reports:	Friedemann, A., 2021b, Effects of ADM.03503.F.1.A on vegetative vigour of six non-target terrestrial plant species under greenhouse conditions, BioChem agrar GmbH, Germany, Study No.: 20 46 PVV 0006, ADAMA Ref No.: 000105082
Guideline(s):	SANCO/3029/99 rev. 4
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

An aliquot (25 µL) of spray solution is diluted with an equal volume of acetonitrile together with 950 µL diluent (50:50 v/v acetonitrile: test matrix) in an autosampler vial. Samples are analysed by high performance liquid chromatography with ultra-violet detection (HPLC-DAD) at 254 nm for prothioconazole and 228 nm for fluxapyroxad using an ACE Excel 5 C18-AR column (150 mm x 2.1 mm, 5 µm) and gradient elution, with mobile phases of 0.1 %v/v phosphoric acid in water and 0.1 %v/v phosphoric acid in acetonitrile. Quantification is performed using external standards.

Table A 29: Recovery results from method validation of Fluxapyroxad & Prothioconazole using the analytical method

Matrix	Report	Analyte	Fortification Level (mg/L)	Recoveries (%)	Mean Recovery (%)	RSD (%)
Spray solution	000105081	Prothioconazole	440.0	93.0, 93.7, 93.2, 99.3, 91.5	94.1	3.2
			1150	94.1, 91.3, 92.3, 99.7, 92.2	93.9	3.6
		Fluxapyroxad	230.1	93.2, 93.6, 93.1, 99.4, 91.3	94.1	3.2
			601.2	94.3, 91.3, 92.3, 99.8, 92.1	94.0	3.6
	000105082	Prothioconazole	440.0	93.5, 94.1, 93.9, 100.1, 92.2	94.7	3.2
			1150	94.7, 92.1, 92.9, 100.1, 92.6	94.5	3.5
		Fluxapyroxad	230.1	93.3, 93.9, 93.4, 99.5, 91.9	94.4	3.1
			601.2	94.8, 91.6, 92.8, 100.3, 92.5	94.4	3.7

Table A 30: Characteristics for the analytical method used for validation of Fluxapyroxad & Prothioconazole using the analytical method

	Fluxapyroxad & Prothioconazole
Specificity	No interferences at >30% of the LOQ were present at the retention time of interest in the control matrix samples. Analyte identity was confirmed by comparison of the retention time and UV-spectrum of the analyte with that of a reference standard.
Calibration (type, number of data points)	**Fluxapyroxad** 1.719 mg/L – 18.10 mg/L (n =6) **Coeff. of detn. (r^2)** / **Slope** / **Intercept** 0.99995 / 72925.9 / -475.984 **Prothioconazole** 3.287 mg/L – 34.60 mg/L (n =6) **Coeff. of detn. (r^2)** / **Slope** / **Intercept** 0.99995 / 42050.2 / -2992.19
Assessment of matrix effects is presented	No (matrix matched standards employed)
Solution stability	Not assessed
Limit of determination/quantification	The limit of quantification, defined as the lowest fortification level where acceptable precision and accuracy data were obtained, has been determined to be 440.0 mg/L for prothioconazole and 230.1 mg/L for fluxapyroxad, in spray solution.

Conclusion

The analytical procedure has been successfully validated in terms of specificity, linearity, precision, accuracy and LOQ in accordance with the requirements of SANTE/2020/12830 rev.1.

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

A 2.1.2.1 Description of analytical methods for the determination of residues in plant matrices (KCP 5.2)

No new or additional studies have been submitted.

A 2.1.2.2 Description of analytical methods for the determination of residues in animal matrices (KCP 5.2)

Method for the determination of fluxapyroxad in honey:

Study not yet finalised and will be included in the Chemical Active dossier (CA) for Fluxapyroxad active substance renewal to be submitted May 2022. In case required, study summaries can be post-submitted on request via LOA.

ILV for the determination of fluxapyroxad in honey:

Study not yet finalised and will be included in the Chemical Active dossier (CA) for Fluxapyroxad active substance renewal to be submitted May 2022. In case required, study summaries can be post-submitted on request via LOA.

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

No new or additional studies have been submitted.

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

The following study summary has been provided by BASF.
Study DocID 2020/2108667 contains data for both parent Fluxapyroxad and metabolites. For this application only data for fluxapyroxad are relevant.

Analytical Method 3

Comments of zRMS:	An Independent Laboratory Validation of the analytical method L0143/01 for the determination of fluxapyroxad and its metabolites (M700F001, M700F002 and M700F007 in surface- and drinking water by LC-MS/MS was conducted. The limit of quantification (LOQ) was set at 0.03 µg/L for all analytes in drinking and surface water. The mean recovery results were all between 70% and 110% with RSD< 20%. The method is acceptable.

Reference:	CP 5.2/07
Report:	Independent Laboratory Validation of BASF method L0143/01: Method for the Determination of BAS 700 F and its Metabolites M700F001, M700F002, and M700F007 in Water by LC-MS/MS, Lee, M., 2021 Report No 864050, AU-2020-24 2020/2108667
Guideline(s):	EPA 850.6100, SANCO/825/00 rev. 8.1 (16 November 2010)
Deviations :	No
GLP :	Yes (certified by United States Environmental Protection Agency)
Acceptability:	Yes

Study summary

An Independent Laboratory Validation of the analytical method L0143/01 for the determination of FLUXAPYROXAD (Batch-No. L80-170, purity 99.9%) and its metabolites, M700F001 (Batch-No. L76-66, purity 99.3%), M700F002 (Batch-No. L80-26, purity 98.5%) and M700F007 (Batch-No. L81-108, purity 99.4%) in surface- and drinking water by LC-MS/MS was conducted with a limit of quantification (LOQ) of 0.03 µg/L.

Materials and methods

Principle of the Method

For analysis of FLUXAPYROXAD and its metabolites 0.6% formic acid is added to the water samples and samples are shaken. Samples are concentrated on a preconditioned Strata-X-AW SPE column. After elution with methanol/formic acid (90/10, v/v) the solvent is removed and the residue is reconstituted in methanol/water (50/50, v/v). The analytes are determined by HPLC-MS/MS. Analysis is accomplished by LC-MS/MS using a Waters Atlantis T3 column (150 mm x 3.0 mm, 3 µm) and a gradient of water/formic acid (1000/1, v/v) and methanol/acetic acid (1000/1, v/v) at a flow rate of 0.5 mL/min. For detection, the following mass transitions were monitored.

Analyte	Quantification Transition	Confirmatory Transition
FLUXAPYROXAD	382 → 362	382 → 342
M700F001	175 → 91	175 → 111
M700F002	161 → 141	161 → 66[1]
M700F007	176 → 156	176 → 136

[1] Original BASF Analytical Method No. L0143/01 used 161 → 97. Due to low sensitivity with this transition, the alternate confirmatory transition was used for this ILV

Recovery Findings

Drinking and surface water were fortified with the analytes at LOQ and at 10x LOQ. Mean recovery values for FLUXAPYROXAD and its metabolites in all matrices were between 70% and 110%. The detailed results are given in the table below.

Table A 31: Results of the Method Validation for the Determination of FLUXAPYROXAD and its Metabolites in Drinking- and Surface Water by LC-MS/MS

Analyte	Matrix	m/z	Fortification Level [µg/L]	Number of Replicates	Mean Recovery [%]	RSD [%]	Overall Recovery [%]	RSD [%]
FLUXAPYROXAD	Drinking water	382 → 362	0.03	5	93.6	1.6	88.1	7.3
			0.3	5	82.7	4.9		
		382 → 342	0.03	5	92.9	1.5	87.7	7.1
			0.3	5	82.5	5.1		
	Drinking water[1]	382 → 362	0.03	5	88.5	1.7	84.4	6.0
			0.3	5	80.2	4.5		
		382 → 342	0.03	5	85.5	2.7	82.6	4.9
			0.3	5	79.7	4.1		
	Surface water	382 → 362	0.03	5	92.6	4.2	91.1	3.5
			0.3	5	89.6	1.2		
		382 → 342	0.03	5	90.8	2.8	90.1	2.1
			0.3	5	89.4	0.5		
M700F001	Drinking water	175 → 91	0.03	5	89.1	5.9	85.8	6.3
			0.3	5	82.5	7.2		
		175 → 111	0.03	5	89.8	6.1	85.8	7.6
			0.3	5	83.2	7.1		
	Drinking water[1]	175 → 91	0.03	5	82.9	4.7	84.0	5.2
			0.3	5	85.0	5.9		
		175 → 111	0.03	5	89.3	5.2	87.3	7.4
			0.3	5	85.3	9.2		
	Surface water	175 → 91	0.03	5	85.0	2.1	87.9	4.0
			0.3	5	90.8	1.9		
		175 → 111	0.03	5	75.2	5.7	82.6	10.3
			0.3	5	90.0	3.1		
M700F002	Drinking	161 → 141	0.03	5	95.8	3.6	91.2	7.1

Table A 31: Results of the Method Validation for the Determination of FLUXAPYROXAD and its Metabolites in Drinking- and Surface Water by LC-MS/MS

Analyte	Matrix	m/z	Fortification Level [µg/L]	Number of Replicates	Mean Recovery [%]	RSD [%]	Overall Recovery [%]	RSD [%]
	water		0.3	5	86.6	6.2		
		161 → 97[2]	0.03 0.3	5 5	- -	- -	-	-
	Drinking water[1]	161 → 141	0.03 0.3	5 5	91.0 88.2	4.3 4.2	89.6	4.4
		161 → 66[2]	0.03 0.3	5 5	91.4 87.7	7.0 7.2	89.5	7.1
	Surface water	161 → 141	0.03 0.3	5 5	75.5 86.3	6.3 3.5	80.9	8.5
		161 → 66[2]	0.03 0.3	5 5	80.6 87.3	6.5 4.1	83.9	6.5
M700F007	Drinking water	176 → 156	0.03 0.3	5 5	89.7 85.0	5.5 6.0	87.4	6.1
		176 → 136	0.03 0.3	5 5	90.7 85.1	7.7 6.0	87.9	7.4
	Drinking water[1]	176 → 156	0.03 0.3	5 5	84.1 77.3	4.5 4.4	80.7	6.1
		176 → 136	0.03 0.3	5 5	83.4 77.5	5.7 5.3	80.4	6.8
	Surface water	176 → 156	0.03 0.3	5 5	92.8 91.5	3.6 3.3	92.2	3.4
		176 → 136	0.03 0.3	5 5	94.0 92.2	6.2 3.3	93.1	4.8

[1] Reanalysis
[2] Recoveries were not acceptable for the intended mass transition therefore, samples were re-analyzed using the mass transition m/z 161 → 66 for confirmation of M700F002.

Linearity Linearity of detector response was tested using seven calibration standard concentrations in the range of 0.2 ng/mL to 20 ng/mL (0.006 µg/L to 0.6 µg/L sample residue level) with coefficients of determination of $r^2 \geq 0.98$. The calibration standards were prepared in matrix- matched solution.

Specificity The highly selective, self-confirmatory LC-MS/MS method was used for determination of FLUXAPYROXAD and its metabolites monitoring two parent-daughter transitions for quantification and confirmation, therefore no additional confirmatory method is required.

Matrix Effects Matrix effects were determined to be significant for surface water (>20% suppression) and not significant for drinking water (<20% suppression or enhancement). Matrix-matched calibration standards were used for all analytes and matrices.

Interference Significant interferences (> 30% of LOQ) were not observed at the retention time and mass transitions considered.

Limit of Quantification The method achieves a limit of quantification (LOQ) of 0.03 µg/L for all analytes (corresponding to a concentration in the final extract of 1 ng/mL) in drinking and surface water, corresponding to the lowest fortification level successfully tested.

Limit of Detection The method has a limit of detection (LOD) of 0.006 µg/L at test sample level (0.2 ng/mL at measurement sample level), corresponding to the lowest calibration level.

Stability in Working Solutions	Stability was determined during method validation study (BASF Doc ID 2009/1069396). FLUXAPYROXAD and its metabolites M700F001 and M700F002 were stable in stock solutions, prepared in methanol for 28 days, when stored refrigerated. Stability of M700F007 in stock solutions was investigated during the ILV and was confirmed to be stable for 48 days, when stored refrigerated.
Extract Stability	Stability was shown for extract solutions for 11 days for surface water and for 8 days for drinking water when stored refrigerated at approximately +4°C in the dark.
Repeatability	The relative standard deviations (RSD, %) for all analytes, matrices and fortification levels were <20%.
Reproducibility	Reproducibility of the method was confirmed during this independent laboratory validation study.

Results
The results show that BASF method No L0143/01 is suitable to determine residues of FLUXAPYROXAD and its metabolites M700F001, M700F002 and M700F007 in drinking and surface water. Samples were jointly fortified with FLUXAPYROXAD and its metabolites M700F001, M700F002, and M700F007 at LOQ (0.03 µg/L) and 10x LOQ (0.3 µg/L) level. Respective mean recovery results were all between 70% and 110%. Matrix effects were found to be significant for surface water and insignificant for drinking water. Calibration was performed with matrix-matched standards for all analytes and matrices. Good linearity ($r^2 \geq 0.98$) was observed for all analytes.

Conclusion
The independent laboratory validation demonstrated that method L0143/01 for analysis of FLUXAPYROXAD and its metabolites M700F001, M700F002, and M700F007 in water using LC-MS/MS for final determination, which is a highly specific technique, fulfils the requirements with regard to linearity, specificity, repeatability, limit of quantification and recoveries with a limit of quantification of 0.03 µg/L for drinking and surface water. Therefore, the method is considered valid to quantify FLUXAPYROXAD and its metabolites M700F001, M700F002, and M700F007 in in surface water and drinking water.

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

No new or additional studies have been submitted.

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

The following study summary has been provided by BASF.

Analytical Method 4

Comments of zRMS:	The analytical method L0352/01 was successfully validated for the determination of BAS 700 F (Fluxapyroxad) in body fluids at a limit of quantitation (LOQ) of 0.01 mg/kg. The mean recoveries for fluxapyroxad at each fortification level, and overall, for each of the matrices tested (urine and blood) were within the acceptable range of 70-110% with the relative standard deviation (RSD) within the acceptable range of ≤ 20%. The method is acceptable.

Reference:	CP 5.2/08
Report:	Validation of BASF analytical method L0352/01 for the determination of BAS 700 F (Fluxapyroxad) in body fluids, Richter, S., Djedovic, S., 2016 Report No EU-819457, P 4055 G

	2016/1217548
Guideline(s):	EPA 860.1340 (1996), OECD-ENV/JM/MONO/(2007)17, SANCO/3029/99 rev. 4 (11 July 2000), SANCO/825/00 rev. 8.1 (16 November 2010)
Deviations :	No
GLP :	Yes (certified by Landesanstalt fuer Umwelt, Messungen und Naturschutz Baden-Wuerttemberg, Karlsruhe, Germany)
Acceptability:	Yes

Study summary
BASF method L0352/01 for the determination of residues of FLUXAPYROXAD (Batch-No. L80-170, purity 99.9%) in blood and urine by LC-MS/MS was validated with a limit of quantification (LOQ) of 0.010 mg/kg.

Materials and methods
Principle of the Method
FLUXAPYROXAD is extracted from samples using acetonitrile. After addition of $MgSO_4$, NaCl and buffering citrate salts, the mixture is shaken intensively and centrifuged. For urine samples, an extract aliquot is diluted with acetonitrile/water (20/80, v/v), containing 0.1% formic acid prior to final determination. For blood samples, an aliquot of the organic extract is cleaned-up by addition of PSA and $MgSO_4$. After centrifugation an aliquot is diluted with acetonitrile/water (20/80, v/v), containing 0.1% formic acid prior to final determination. Analysis is accomplished by LC-MS/MS using a Thermo Betasil column (100 mm x 2.1 mm, 5 µm) and a gradient of water/formic acid (1000/1, v/v) and acetonitrile/formic acid (1000/1, v/v) at a flow rate of 0.6 mL/min. For detection, two mass transitions are monitored: m/z 382 → 362 for quantification and m/z 382 → 342 for confirmation.

Recovery Findings
Urine and blood specimens were fortified with the analytes at LOQ and at 10x LOQ. Mean recovery values for FLUXAPYROXAD in blood and urine were between 70% and 110%. The detailed results are given in the table below.

Table …: Results of the Method Validation for the Determination of FLUXAPYROXAD in Urine and Blood by LC-MS/MS

Analyte	Matrix	m/z	Fortification Level [mg/kg]	Number of Replicates	Mean Recovery [%]	RSD [%]	Overall Recovery [%]	RSD [%]
FLUXAPYROXAD	Urine	382 → 362	0.01	5	93.9	4.5	95.2	3.4
			0.10	5	96.6	1.2		
		382 → 342	0.01	5	94.0	5.0	94.8	3.5
			0.10	5	95.7	0.8		
	Blood	382 → 362	0.01	5	103	1.4	104	1.5
			0.10	5	105	0.7		
		382 → 342	0.01	5	103	1.6	104	1.4
			0.10	5	105	0.8		

RSD = relative standard deviation

Linearity Linearity of detector response was tested using at least five calibration standard concentrations in the range of 0.01 ng/mL to 1 ng/mL (equivalent to 0.002 mg/kg to 0.20 mg/kg) with correlation coefficients of $r \geq 0.99$. The calibration standards were prepared in acetonitrile/water (20/80, v/v), containing 0.1% formic acid.

Specificity The highly selective, self-confirmatory LC-MS/MS method was used for determination of FLUXAPYROXAD monitoring two characteristic mass transitions for quantification and qualification. Consequently, no further confirmatory method is required.

Matrix Effects The matrix effect was tested for each matrix. No significant matrix effects (i.e. > 20% suppression or enhancement) on LC-MS/MS response were

observed for the matrices. The calibration standards in solvent were used for the evaluation of the results.

Interference Significant interferences (> 30% of LOQ) were not observed at the retention time and mass transitions considered.

Limit of Quantification The method has a limit of quantification (LOQ) of 0.01 mg/kg (0.05 ng/mL at measurement level), corresponding to the lowest fortification level successfully tested.

Limit of Detection The limit of detection (LOD) is defined as the lowest calibration level corresponding to 0.002 mg/kg at test sample level (0.01 ng/mL at measurement level).

Stability in Working Solutions Stability was shown for FLUXAPYROXAD in stock and fortification solutions, prepared in methanol and calibration solutions, prepared in acetonitrile/water (20/80, v/v), containing 0.1% formic acid for a maximum duration of 30 days, when stored refrigerated at approximately ≤8°C in the dark.

Extract Stability The experiments demonstrate that FLUXAPYROXAD was stable in raw extracts in acetonitrile and final sample volumes, prepared in acetonitrile/water (20/80, v/v) containing 0.1% formic acid, over a time period of 8 days, when stored refrigerated at approximately ≤8°C in the dark.

Repeatability The relative standard deviations (RSD, %) for all fortification levels and matrices were < 20%.

Reproducibility Reproducibility of the method was not determined within the validation study.

Results
The results show that BASF method No. L0352/01 is suitable to determine residues of FLUXAPYROXAD in body fluid matrices. Urine and blood samples were fortified with FLUXAPYROXAD at LOQ (0.01 mg/kg) and 10x LOQ (0.1 mg/kg) level. Respective recovery results were all between 70% and 110%. Matrix effects were investigated and found to be not significant. Hence, calibration was performed with solvent standards for both matrices. Good linearity ($r \geq 0.99$) was observed.

Conclusion
Method L0352/01 for analysis of FLUXAPYROXAD in body fluid matrices using LC-MS/MS for final determination, which is a highly specific technique, fulfills the requirements with regard to linearity, specificity, repeatability, limit of quantification and recoveries with a limit of quantification of 0.01 mg/kg. Therefore, the method is considered valid to quantify FLUXAPYROXAD in body fluid matrices.

A 2.2 Analytical methods for Prothioconazole

A 2.2.1 Methods used for the generation of pre-authorization data (KCP 5.1)

See 5.1.2/03 to 5.1.2/10 above and 5.1.2/11 to 5.1.2/19 below.

A 2.2.1.1 Analytical Method 1

Comments of zRMS:	The study of Klimmek, S. and and Gizler, A., 2017 (Report No.: S12-00072) has been evaluated in Registration Report for ADM.03500.F.2.B on March 2023 by zRMS-PL and the summary is presented below. The analysis of the triazole metabolites was performed according to Syngenta method GRM053.01A and a reduced validation was successfully performed within this study

	using LC-MS/MS and LC-DMS-MS/MS. The limit of quantification (LOQ) for all triazole metabolites was 0.01 mg/kg. The limit of detection (LOD) was 0.003 mg/kg. During the validation and stability tests mean recoveries were in the range of 70 - 120% with relative standard deviation of < 20% (validation tests) for each matrix and fortification level. The method complies with EU Guidelines SANCO/825/00 rev.8.1 and SANCO/3029/99 rev.4. The method is acceptable.

Reference	KCP 5.1.2/11 (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.: 000074067
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 32: 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 Mean ± RSD n	90-103 94 ± 8.7 3	90-103 96 ± 6.8 3	87 – 103 94 ± 8.9 3	99-114 104 ± 8.0 3	98 - 106 101 ± 4.3 3	91-114 104 ± 11 3	91-113 100 ± 12 3	92-108 102 ± 8.8 3
0.100	Range Mean ± RSD n	100-112 108 ± 6.2 3	108-112 110 ± 1.8 3	93-108 99 ± 7.8 3	102-118 110 ± 7.3 3	101-109 105 ± 3.9 3	98-116 105 ± 9.0 3	101-105 103 ± 1.9 3	106-109 107 ± 1.4 3
0.01 and 0.10	Overall ± RSD n	101 ± 10 6	103 ± 6.8 6	97 ± 8.1 6	107 ± 7.5 6	103 ± 4.1 6	105 ± 9.2 6	101 ± 7.6 6	105 ± 6.1 6

RSD = relative standard deviation, n = number of replicates

Table A 33: 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 Mean ± RSD n	96-119 108 ± 11 3	94-112 104 ± 8.8 3	86-97 92 ± 6.0 3	98-107 104 ± 5.0 3	88-110 100 ± 11 3	107-110 108 ± 1.4 3	67-74 70 ± 5.2 3	90-124 105 ± 16 3
0.100	Range Mean ± RSD n	104-116 110 ± 5.5 3	99-108 103 ± 4.6 3	94-104 100 ± 5.1 3	94-102 97 ± 4.3 3	87-116 99 ± 15 3	95-103 100 ± 4.2 3	89-99 92 ± 6.3 3	103-112 108 ± 4.4 3
0.01 and 0.10	Overall ± RSD n	109 ± 7.6 6	103 ± 6.4 6	96 ± 6.8 6	101 ± 5.5 6	99 ± 12 6	104 ± 5.3 6	81 ± 16 6	107 ± 11 6

RSD = relative standard deviation, n = number of replicates

Table A 34: 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 Mean ± RSD n	87-109 100 ± 8.4 5	79-103 91 ± 13 3	101-116 108 ± 6.9 3	76-87 81 ± 7.0 3	103-113 107 ± 4.8 3	96-121 110 ± 12 3	74-89 81 ± 9.2 3	101-117 107 ± 8.4 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 35: 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.

A 2.2.1.2 Analytical Method 2

Comments of zRMS:	The study of Lefresne, S., 2020 has been evaluated in Registration Report for ADM.03500.F.2.B on March 2023 by zRMS-PL and the summary is presented below. The LC-MS/MS (QuEChERS-method) analytical method has been successfully validated for the determination of prothioconazole (sum of prothioconazole-desthio, 3-hydroxy-prothioconazole-desthio, 4-hydroxy-prothioconazole-desthio, 5-hydroxy-prothioconazole-desthio, 6-hydroxyprothioconazole-desthio and alpha-hydroxy-prothioconazole-desthio, expressed as prothioconazole-desthio) in whole plant of wheat, grain of wheat, straw of wheat, grain of oilseed rape, strawberry and dry bean. The LOQ of prothioconazole-desthio, 3-hydroxy-prothioconazoledesthio expressed as prothioconazole-desthio, 4-hydroxy-prothioconazole-desthio expressed as prothioconazole-desthio, 5-hydroxy-prothioconazole-desthio expressed as prothioconazole-desthio, 6-hydroxy-prothioconazole-desthio expressed as prothioconazole-desthio and alphahydroxy-prothioconazole-desthio expressed as prothioconazole-desthio was 0.010 mg/kg, for each reference item. The LOQ for the sum of all prothioconazole-items was 0.060 mg/kg for each matrix. The method complies with EU Guidelines SANCO/825/00 rev.8.1 and SANCO/3029/99 rev.4. The method is acceptable.

Reference:	KCP 5.1.2/12 (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.: 000107139
Guideline(s):	For method validation: SANCO/3029/99 rev. 4
Deviations:	No
GLP:	Yes

Acceptability: Yes

Materials and methods

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

Results and discussions

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

Table A 36: Recovery results from method validation of prothioconazole metabolites in whole plant of wheat

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

RSD = relative standard deviation, n = number of replicates

Table A 37: Recovery results from method validation of prothioconazole metabolites in grain of wheat

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

RSD = relative standard deviation, n = number of replicates

Table A 38: 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
0.01 and	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

0.10	n	10	10	10	10	10	10	10	10	10	10	10	10

RSD = relative standard deviation, n = number of replicates

Table A 39: 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 Mean ± RSD n	72-111 83 ± 19 5	71-111 82 ± 20 5	80-116 90 ± 16 5	78-120 92 ± 18 5	77-120 90 ± 19 5	79-120 90 ± 18 5	74-118 89 ± 19 5	81-117 91 ± 16 5	69-105 79 ± 19 5	66-103 78 ± 19 5	83-123 95 ±17 5	81-126 95 ± 19 5
0.100	Range Mean ± RSD n	74-80 77 ± 3 5	73-80 77 ± 4 5	79-87 84 ± 4 5	82-89 85 ± 3 5	79-88 85 ± 4 5	80-88 85 ± 3 5	79 - 88 84 ± 5 5	81-86 84 ± 3 5	72-77 75 ± 3 5	72-78 75 ± 3 5	82-91 88 ± 4 5	86-90 88 ± 2 5
0.01 and 0.10	Overall ± RSD n	80 ± 14 10	80 ± 14 10	87 ± 12 10	89 ± 13 10	88 ± 14 10	88 ± 13 10	87 ± 14 10	88 ± 12 10	77 ± 14 10	76 ± 13 10	91 ± 13 10	91 ± 14 10

RSD = relative standard deviation, n = number of replicates

Table A 40: Recovery results from method validation of prothioconazole metabolites in strawberry

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

RSD = relative standard deviation, n = number of replicates

Table A 41: Recovery results from method validation of prothioconazole metabolites in dry bean

Fortification level	**Analyte**	**Prothioconazole-desthio**		**3-hydroxy-prothioconazole-desthio**		**4-hydroxy-prothioconazole-desthio**		**5-hydroxy-prothioconazole-desthio**		**6-hydroxy-prothioconazole-desthio**		**alpha-hydroxy-prothioconazole-desthio**	
[mg/kg]	Transition ion	70 m/z	125 m/z	70 m/z	141 m/z	141 m/z	70 m/z	70 m/z	141 m/z	70 m/z	141 m/z	70 m/z	141 m/z
0.010	Range Mean ± RSD	87-120 100 ± 13	89-121 101 ± 13	85-119 99 ± 13	83-118 100 ± 13	85-120 99 ± 13	88-116 99 ± 11	85-118 99 ± 14	88-117 97 ± 13	91-122 102 ± 12	83-114 97 ± 13	90-126 102 ±14	90-119 101 ± 11

	n	5	5	5	5	5	5	5	5	5	5	5	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 42: Characteristics for the analytical method used for validation of prothioconazole metabolites residues in wheat whole plant, wheat grain, wheat straw, oilseed rape grain, strawberry and dry bean

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

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

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

A 2.2.1.3 Analytical Method 3

Comments of zRMS:	The study of Amic, S., 2020b has been evaluated in Registration Report for ADM.03500.F.2.B on March 2023 by zRMS-PL and the summary is presented below. The analytical method was validated for wheat whole plant without roots, grain and straw according to guideline SANCO/3029/99 rev. 4. All the analytes were determined by LC-MS/MS using a quantitation and confirmation ion. The LOQ of each analyte was at 0.01 mg/kg for each matrix. The mean recovery was between 70% and 110% at each level of fortification, for each reference item and for each matrix. The storage duration (interval between sampling and extraction date) was 149 days for the determination of prothioconazole and its metabolites. Sufficient stability data are available to support the residue data presented in this study. The method is acceptable.

Reference:	KCP 5.1.2/13 (filed in KCA 6.3.1/01)
Report:	Residue study of prothioconazole and its metabolites in wheat whole plant and RAC after one foliar application of ADM.3500.F.2.B (250 g a.s./L of prothioconazole) - 2 harvest and 2 decline trials – Northern Europe (FR, HU, PL) - 2019, Amic, S., 2020b, report no.: BPL19/762/GC, sponsor no.: 000102751
Guideline(s):	For method validation: SANCO/3029/99 rev. 4
Deviations:	None
GLP:	Yes (certified laboratory)
Acceptability/Reliability:	Yes
Duplication (if vertebrate study)	Not applicable

Materials and methods

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

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. A LOQ of 0.06 mg/kg was set for prothioconazole expressed as the sum of all analytes.

Table A 43: Recovery results from method validation of prothioconazole in whole plant of wheat (data obtained from study B19S-A4-P-01)

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

RSD = relative standard deviation, n = number of replicates

Table A 44: Recovery results from method validation of prothioconazole in grain of wheat (B19S-A4-P-01)

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

RSD = relative standard deviation, n = number of replicates

Table A 45: Recovery results from method validation of prothioconazole in straw of wheat (B19S-A4-P-01)

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

0.10	n	10	10	10	10	10	10	10	10	10	10	10	10

RSD = relative standard deviation, n = number of replicates

Table A 46: Characteristics for the analytical method used for validation of prothioconazole residues in wheat

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

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

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

A 2.2.1.4 Analytical Method 4

Comments of zRMS:	The study of Gustloff, C.; Wallbaum, P., 2021 has been evaluated in Registration Report for ADM.03500.F.2.B on March 2023 by zRMS-PL and the summary is presented below. The analytical method based on the method GRM053.01A was validated for the determination of of triazole metabolites (TDMs) 1,2,4-Triazole (1,2,4-T), Triazole alanine (TA), Triazole acetic acid (TAA) and Triazole lactic acid (TLA) in/on wheat (whole plant, grain and straw), barley (whole plant, grain and straw), oilseed rape (seeds, crude oil, refined oil and pressed cake), sunflower (seeds) and sugar beet (leaves with top and roots). The limit of quantification (LOQ) of the analytical method was 0.01 mg/kg for each analyte and each matrix with a limit of detection (LOD) set at 0.003 mg/kg (30% of the LOQ). Acceptance criteria for method validations were met, with average recoveries ranging from 70% to 110% and relative standard deviations ≤20%. In accordance with SANTE/2020/12830, Rev.1, there should be 5 recoveries at each level (LOQ and 10x LOQ), in the performed study only 3 recovery are presented, However, the analytical method is acceptable and suitable for determination of residues of triazole and metabolites, in wheat, barley, oilseed rape, sunflower and sugar beet.

Reference: KCP 5.1.2/14
Report: Validation of an analytical method for the determination of triazole metabolites (TDMs) in crop matrices of season 2021
Gustloff, C.; Wallbaum, P., 2021
Report no.: S21-02262, sponsor no.: 000107909
Guideline(s): SANTE/2020/12830, Rev.1
Deviations: A reduced recovery sample set was conducted. For a full validation, reference is made to the peer review of the triazole derivative metabolites (TDMs) in the light of confirmatory data submitted (UK, 2018; EFSA, 2018, amended 2019).

GLP: Yes
Acceptability: Yes

Materials and methods

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

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

Results and discussions

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

Table A 47: Recovery results from method validation of triazole metabolites in wheat whole plant

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

RSD = relative standard deviation, n = number of replicates

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

Table A 48: 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 ± RSD	107 ± 4.9	116 ± 2.3	96 ± 2.3	87 ± 12	84 ± 7.2	98 ± 8.1	73 ± 2.1	92 ± 14
	n	3	3	3	3	3	3	3	3
0.100	Range	101-115	75-104	91-95	82-93	60-75	80-100	72 – 86	75-97
	Mean ± RSD	103 ± 2.2	94 ± 17	93 ± 2.0	88 ± 6.6	70 ± 12	87 ± 13	79 ± 8.6	85 ± 13
	n	3	3	3	3	3	3	3	3

RSD = relative standard deviation, n = number of replicates

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

Table A 49: 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

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

	n	3	3	3	3	3	3	3	3
0.100	Range Mean ± RSD n	110-111 110 ± 0.8 3	86-112 101 ± 13 3	93-96 95 ± 1.9 3	94-97 95 ± 1.8 3	80-82 82 ± 1.4 3	71-101 90 ± 19 3	82 – 85 84 ± 1.7 3	76-88 82 ± 7.0 3

RSD = relative standard deviation, n = number of replicates

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

Table A 50: 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 Mean ± RSD n	84-95 90 ± 6.4 3	73-74 74 ± 0.7 3	95-108 104 ± 6.9 3	97-129 109 ± 16 3	92-109 99 ± 9.6 3	85-109 99 ± 12 3	98-104 100 ± 3.4 3	91-104 96 ± 6.9 3
0.100	Range Mean ± RSD n	92-115 102 ± 11 3	103-119 109 ± 8.1 3	94-110 102 ± 7.7 3	93-107 101 ± 7.6 3	86-88 87 ± 1.5 3	104-119 113 ± 7.0 3	98 – 102 99 ± 1.2 3	87-103 95 ± 8.0 3

RSD = relative standard deviation, n = number of replicates

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

Table A 51: 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 Mean ± RSD n	92-110 99 ± 10 3	80-112 101 ± 18 3	80-113 95 ± 17 3	87-112 100 ± 12 3	79-90 85 ± 6.5 3	91-103 95 ± 7.1 3	81-83 82 ± 1.5 3	80-96 86 ± 11 3
0.100	Range Mean ± RSD n	85-118 101 ± 16 3	92-115 106 ± 12 3	86-97 90 ± 6.4 3	78-94 84 ± 9.8 3	83-97 89 ± 8.0 3	89-104 96 ± 7.8 3	81 – 92 86 ± 6.8 3	83-90 87 ± 4.1 3

RSD = relative standard deviation, n = number of replicates

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

Table A 52: 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 Mean ± RSD n	76-88 82 ± 7.6 3	90-116 101 ± 14 3	89-106 96 ± 9.2 3	107-113 110 ± 3.0 3	97-119 109 ± 11 3	91-98 94 ± 4.1 3	93-102 97 ± 4.6 3	77-82 80 ± 3.6 3
0.100	Range Mean ± RSD n	98-110 137 ± 5.9 3	96-110 102 ± 6.9 3	85-100 94 ± 8.9 3	81-98 89 ± 9.6 3	97-112 104 ± 7.1 3	96-121 109 ± 11 3	97 – 107 102 ± 5.1 3	86-91 88 ± 2.9 3

RSD = relative standard deviation, n = number of replicates

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

Table A 53: 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 Mean ± RSD n	82-96 89 ± 7.9 3	99-118 106 ± 9.4 3	87-113 96 ± 15 3	84-103 96 ± 11 3	81-96 88 ± 8.6 3	96-104 99 ± 4.2 3	71-97 86 ± 16 3	85-89 87 ± 2.8 3

0.100	Range Mean ± RSD n	99-109 104 ± 5.2 3	71-101 88 ± 17 3	78-91 87 ± 8.6 3	74-95 84 ± 12 3	92-99 94 ± 4.0 3	95-107 99 ± 7.0 3	88 – 91 87 ± 2.8 3	89-103 95 ± 7.3 3

RSD = relative standard deviation, n = number of replicates

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

Table A 54: Recovery results from method validation of triazole metabolites in 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 Mean ± RSD n	83-98 88 ± 9.7 3	84-88 86 ± 2.2 3	82-102 95 ± 12 3	88-118 105 ± 15 3	91-95 93 ± 2.4 3	86-89 87 ± 1.8 3	84-98 92 ± 8.0 3	88-90 89 ± 1.0 3
0.100	Range Mean ± RSD n	100-108 103 ± 4.5 3	78-97 91 ± 12 3	91-99 96 ± 4.29 3	90-99 95 ± 4.9 3	93-97 94 ± 2.2 3	93-97 95 ± 2.0 3	89 – 99 95 ± 5.5 3	93-101 98 ± 4.3 3

RSD = relative standard deviation, n = number of replicates

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

Table A 55: Recovery results from method validation of triazole metabolites in 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 Mean ± RSD n	90-104 95 ± 8.3 3	77-85 81 ± 4.5 3	81-92 86 ± 6.5 3	82-90 87 ± 5.2 3	97-110 102 ± 6.8 3	88-102 94 ± 7.8 3	100-108 103 ± 4.0 3	98-103 99 ± 3.0 3
0.100	Range Mean ± RSD n	86-100 93 ± 7.4 3	84-99 90 ± 8.4 3	83-87 85 ± 2.3 3	85-86 85 ± 0.7 3	83-94 90 ± 6.3 3	78-83 81 ± 3.5 3	91 – 93 92 ± 1.1 3	86-98 90 ± 7.5 3

RSD = relative standard deviation, n = number of replicates

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

Table A 56: Recovery results from method validation of triazole metabolites in 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 Mean ± RSD n	103-118 111 ± 6.8 3	105-116 110 ± 4.8 3	100-101 101 ± 0.9 3	111-118 116 ± 3.7 3	91-108 100 ± 8.2 3	89-97 94 ± 5.0 3	61-106 84 ± 27 3	70-96 82 ± 16 3
0.100	Range Mean ± RSD n	81-94 89 ± 7.8 3	78-106 90 ± 16 3	103-108 106 ± 2.5 3	101-113 107 ± 5.6 3	78-104 94 ± 14 3	78-106 94 ± 19 3	78 – 106 96 ± 16 3	99-103 101 ±1.8 3

RSD = relative standard deviation, n = number of replicates

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

Table A 57: Recovery results from method validation of triazole metabolites in 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 Mean ± RSD n	103-116 110 ± 5.7 3	83-99 92 ± 9.4 3	94-104 100 ± 5.1 3	84-105 98 ± 12 3	83-100 94 ± 10 3	99-117 109 ± 8.3 3	95-106 100 ± 6.1 3	78-101 87 ± 15 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 58: 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 59: 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 60: 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.

A 2.2.1.5 Analytical Method 5

Comments of zRMS:	The study of Lefresne, S., 2021 has been evaluated in Registration Report for ADM.03500.F.2.B on March 2023 by zRMS-PL and the summary is presented below. The analytical method based on the method 00979/M001 was validated for the determination of prothioconazole (sum of prothioconazole-desthio and all metabolites containing the 2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl-2H-1,2,4-triazole moiety, expressed as prothioconazole-desthio (sum of isomers)) residues in barley (whole plant, grain, straw), in honey, in oilseed rape (seed), in sugar beet (leaves with top, root, whole plant) and in wheat (whole plant, grain, straw) in compliance with Guideline SANTE/2020/12830, Rev.1. LOQ for each analyte separately: 0.010 mg/kg. These LOQ correspond to a sum of 0.060 mg/kg expressed as prothioconazole-desthio and all metabolites containing the 2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl-2H- 1,2,4-triazole moiety, expressed as prothioconazole-desthio (sum of isomers)). Acceptance criteria for method validations were met, with average recoveries ranging from 70% to 110% and relative standard deviations ≤20%. The method is acceptable for the determination of prothioconazole in barley (grain, whole plant, straw), honey, oilseed rape seed, sugar beet (root, leaves with top, whole plant).

Reference: KCP 5.1.2/15
Report: Validation of an analytical method for the determination of prothioconazole residues in cereals, honey, oilseed rape and sugar beet. Lefresne, S., 2021
Report no.: B21S-A4-P-01, EFSA-2021-00003265, sponsor no.: 000108024
Guideline(s): SANTE/2020/12830, Rev.1
Deviations: None
GLP: Yes
Acceptability: Yes

Materials and methods

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

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

Results and discussions

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

Table A 61: 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 62: 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 63: 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 64: 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 65: 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 66: Recovery results from method validation of prothioconazole metabolites in sugar beet root

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

RSD = relative standard deviation, n = number of replicates

Table A 67: Recovery results from method validation of prothioconazole metabolites in sugar beet leaves with top

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

RSD = relative standard deviation, n = number of replicates

Table A 68: Recovery results from method validation of prothioconazole metabolites in sugar beet whole plant

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

RSD = relative standard deviation, n = number of replicates

Table A 69: Recovery results from method validation of prothioconazole metabolites in wheat grain

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

RSD = relative standard deviation, n = number of replicates

Table A 70: Recovery results from method validation of prothioconazole metabolites in wheat straw

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

RSD = relative standard deviation, n = number of replicates

Table A 71: Recovery results from method validation of prothioconazole metabolites in wheat whole plant

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

RSD = relative standard deviation, n = number of replicates

Table A 72: 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

Applicant's statement:
"As can be seen from the report, the potential for prothioconazole-desthio to be lost or degraded during analysis was checked via full validation of the method to ensure the hydrolysis step did not affect residues of prothioconazole-desthio. Comprehensive validation across representative matrices confirmed acceptable accuracy and precision showing no losses of the compound when subjected to hydrolysis. This is consistent with the nature of residues processing study which also demonstrated the hydrolytic stability of prothioconazole-desthio including under acidic process conditions (although not as rigorous as this analytical method – 20 mins at 90°C and pH4). In addition, for 2020 crop residue samples of barley grain and straw (Reports 000105350 and 000108763 presented in the residue section) re-analysed using the hydrolysis method showed very good correlation between analysis using the QuEChERS (non-hydrolysis) method indicating either method would be suitable for determination of prothioconazole-desthio residues. This new method permitted the simultaneous extraction and analysis of prothioconazole-desthio and the free and conjugated hydroxy-prothioconazole-desthio metabolites."

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).

A 2.2.1.6 Analytical Method 6

Comments of zRMS:	The study of Amic, S., 2020d has been evaluated in Registration Report for ADM.03500.F.2.B on March 2023 by zRMS-PL and the summary is presented below. The analytical method was validated for barley whole plant without roots, grain and straw according to guideline SANCO/3029/99 rev. 4. All the analytes were determined by LC-MS/MS using a quantitation and confirmation ion. The LOQ of each analyte was at 0.01 mg/kg for each matrix. The mean recovery was between 70% and 110% at each level of fortification, for each reference item and for each matrix. The storage duration (interval between sampling and extraction date) was 158 days for the determination of prothioconazole and its metabolites. Sufficient stability data are available to support the residue data presented in this study. The method is acceptable.

Reference: KCP 5.1.2/16 (filed in KCA 6.3.2/01)
Report: Residue study of prothioconazole and its metabolites in barley whole plant

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

Materials and methods

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

Results and discussions

The method validation according to guideline SANCO/3029/99 rev. 4 obtained during the validation study mentioned above is presented in Tables A73 – A75. Recovery results were in a range of 70 to 110 % with an RSD ≤ 20. No outliers were identified. No interference (< 30 % LOQ) of total peak area for the target analytes were found in unfortified control samples. The LOQ of prothioconazole-desthio, 3-hydroxyprothioconazole-desthio, 4-hydroxyprothioconazole-desthio expressed as prothioconazole-desthio, 5-hydroxyprothioconazole-desthio expressed as prothioconazole-desthio, 6-hydroxyprothioconazole-desthio expressed as prothioconazole-desthio and alpha-hydroxyprothioconazole-desthio expressed as prothioconazole-desthio was 0.010 mg/kg, for each analyte and for each matrix. The LOQ for the sum of all prothioconazole-items was 0.060 mg/kg for each matrix.

Table A 73: Characteristics for the analytical method used for validation of prothioconazole residues in barley

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

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

Conclusion

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

A 2.2.1.7 Analytical Method 7

Comments of zRMS:	The study of Huaulmé, J.-M., 2021a has been evaluated in Registration Report for ADM.03500.F.2.B on March 2023 by zRMS-PL and the summary is presented below. The analytical method was validated for barley whole plant without roots, grain and straw according to guideline SANCO/3029/99 rev. 4 (reduced validation).

	LOQ: 0.01 mg/kg for each analyte, 0.06 mg/kg for prothioconazole expressed as prothioconazole-desthio as a sum of metabolites. The mean recovery was between 70% and 110% at each level of fortification, for each reference item and for each matrix. The storage duration (interval between sampling and extraction date) was 70 days for the determination of prothioconazole and its metabolites. Sufficient stability data are available to support the residue data presented in this study. The method is acceptable.

Reference:	KCP 5.1.2/17 (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 Huaulmé, J.-M., 2021a Report no.: BPL20/844/GC, sponsor no.: 000105350
Guideline(s):	For method validation: SANCO/3029/99 rev. 4
Deviations:	None
GLP:	Yes
Acceptability:	Yes

Materials and methods

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

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 74: Recovery results from method validation of prothioconazole metabolites in whole plant of barley

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

RSD = relative standard deviation, n = number of replicates

Table A 75: Recovery results from method validation of prothioconazole metabolites in grain of barley

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

RSD = relative standard deviation, n = number of replicates

Table A 76: 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
0.01 and	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

0.10	n	10	10	10	10	10	10	10	10	10	10	10	10

RSD = relative standard deviation, n = number of replicates

Table A 77: Characteristics for the analytical method used for validation of prothioconazole metabolites residues in barley whole plant, barley grain and barley straw

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

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

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

A 2.2.1.8 Analytical Method 8

Comments of zRMS:	The study of Semrau, J., 2021 has been evaluated in Registration Report for ADM.03500.F.2.B on March 2023 by zRMS-PL and the summary is presented below. In the analytical phase S18-02513-L2 of this study samples of radish (leaves and roots), leaf lettuce (leaves) and barley (whole plant, grain and straw) were analysed for residues of prothioconazole-desthio (sum of isomers of PTZ-desthio, PTZ-3-; -4-; -5-; and -6-hydroxy desthio and alpha-hydroxy-PTZ-desthio, each expressed as PTZ-desthio). In addition, samples of soil were analysed for residues of prothioconazole-desthio. Sample extraction and determination of residues in the matrices radish (leaves and roots), barley (grain, straw and whole plant) and lettuce (leaves) were performed according to the GIRPA Method R-3965 based on the multi-residue method QuEChERS that was validated within this analytical phase for the matrices radish (roots), barley (grain and straw) and lettuce (leaves) according to SANCO/3029/99, rev. 4. For the analysis of soil, sample extraction and determination of residues were performed according to the multi-residue method QuEChERS that was also validated within this analytical phase according to SANCO/3029/99, rev. 4. Quantification was performed by use of LC-MS/MS detection for all analytes and matrices. The limit of quantification (LOQ) of both analytical methods was 0.01 mg/kg (expressed as prothioconazoledesthio) for each analyte and each matrix The mean recoveries at each fortification level were in the range of 70 – 110% with relative standard deviation(s) below 20% for all combinations of matrices and analytes. The method is acceptable for the determination of prothioconazole radish, lettuce, barley, and soil.

Reference: KCP 5.1.2/18 (filed in KCA 6.6.2/01, also supports KCA 6.6.2/02)
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.: 000109154
Guideline(s):	For method validation: SANTE/2020/12830, Rev.1
Deviations:	None
GLP:	Yes
Acceptability:	Yes

Materials and methods

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

Results and discussions

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

Table A 78: 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 Mean ± RSD n	85-107 96 ± 9.8 5	88-107 100 ± 8.0 5	80-86 83 ± 3.1 5	82-85 83 ± 1.5 5	79-89 84 ± 5.1 5	80-93 85 ± 6.5 5	74-83 78 ± 5.0 5	72-79 77 ± 3.7 5	79-89 86 ± 4.6 5	80-96 88 ± 7.4 5	72-78 76 ±3.3 5	74-79 77 ± 2.4 5
0.100	Range Mean ± RSD n	95-101 98 ± 2.6 5	99-104 102 ± 1.9 5	86-91 88 ± 2.5 5	82-89 86 ± 3.6 5	79-89 85 ± 4.8 5	79-87 83 ± 4.1 5	82 – 90 87 ± 3.5 5	80-86 84 ± 3.0 5	88-92 90 ± 1.8 5	84-90 87 ± 2.7 5	77-82 79 ± 2.6 5	76-80 77 ± 3.1 5

RSD = relative standard deviation, n = number of replicates

Table A 79: 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 Mean ± RSD n	97-105 99± 3.4 5	98-104 100 ± 4.0 5	70 m/z 77-87 80 ± 4.9	83-90 87 ± 3.4 5	73-90 81 ± 8.2 5	77-88 83 ± 5.5 5	75-79 76 ± 2.2 5	76-84 81 ± 4.3 5	86-100 92 ± 5.6 5	91-97 94 ± 3.0 5	78-87 82 ± 4.4 5	78-90 86 ± 6.0 5
0.100	Range Mean ± RSD n	99-112 106 ± 5.1 5	103-114 108 ± 4.0 5	5 92-98 94 ± 2.7	92-95 94 ± 1.2 5	80-83 81 ± 1.6 5	79-85 82 ± 3.2 5	86 – 92 88 ± 2.6 5	87-91 89 ± 2.6 5	102-111 107 ± 3.3 5	93-101 97 ± 3.3 5	83-87 86 ± 1.8 5	87-92 90 ± 2.4 5

RSD = relative standard deviation, n = number of replicates

Table A 80: 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 Mean ± RSD n	84-91 87 ± 5.4 5	70-83 76 ± 8.2 5	72-84 79 ± 7.2 5	73-89 80 ± 8.1 5	76-85 81 ± 5.0 5	75-82 78 ± 3.8 5	70-86 79 ± 8.9 5	76-88 83 ± 5.5 5	78-96 87 ± 9.5 5	79-89 85 ± 4.5 5	71-86 75 ± 8.3 5	74-76 75 ± 1.2 5
0.100	Range Mean ± RSD n	78-85 81 ± 4.2 4*	72-84 79 ± 6.4 4*	81-87 83 ± 3.2 4*	72-86 79 ± 7.4 4*	78-87 82 ± 5.0 4*	74-81 77 ± 4.4 4*	78 – 80 80 ± 1.3 4*	80-84 82 ± 2.3 4*	75-82 79 ± 3.9 4*	78-84 80 ± 3.8 4*	79-88 84 ± 4.4 4	77-88 82 ± 5.9 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 81: 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 Mean ± RSD n	96-111 102 ± 5.4 5	82-106 93 ± 11 5	83-97 90 ± 6.6 5	95-104 99 ± 3.6 5	85-99 94 ± 5.7 5	91-99 96 ± 3.8 5	84-101 93 ± 7.3 5	73-108 90 ± 15 5	78-100 89 ± 9.7 5	86-99 91 ± 6.0 5	83-107 93 ± 12 5	96-123 108 ± 9.4 5
0.100	Range Mean ± RSD n	99-112 104 ± 5.0 5	98-108 103 ± 4.2 5	95-104 99 ± 3.2 5	88-105 97 ± 6.5 5	86-104 95 ± 7.2 5	88-97 93 ± 4.9 5	81-97 92 ± 6.8 5	84-97 92 ± 5.9 5	85-93 89 ± 3.3 5	90-96 94 ± 2.8 5	87-100 97 ± 6.0 5	86-99 92 ± 5.2 5

RSD = relative standard deviation, n = number of replicates

Table A 82: Recovery results from method validation of prothioconazole-desthio metabolites in soil

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

RSD = relative standard deviation, n = number of replicates

Table A 83: 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 2.2.1.9 Analytical Method 9

Comments of zRMS:	The study of Lindner M., Grewe D., 2020 has been evaluated in Registration Report for ADM.03500.F.2.B on March 2023 by zRMS-PL and the summary is presented below. The analytical method was validated for the determination of prothioconazole and prothioconazole-desthio in nectar, pollen, flowers and honey according to the guidance documents SANCO/825/00, rev 8.1 and SANCO/3029/99/00, rev. 4. The LOQ was established at 0.01 mg/kg in nectar, pollen, flower and honey for the two mass transitions. Acceptance criteria for method validations were met, with average recoveries ranging from 70% to 110% and relative standard deviations ≤20%. The method is acceptable for the determination of prothioconazole and prothioconazole-desthio in nectar, pollen and flowers and honey.

Reference: KCP 5.1.2/19 (Cross reference to KCP 10.3.1.5/01 and KCP 10.3.1.5/02)

Reports: Lindner M., Grewe D., 2020, Validation of an Analytical Method for the Determination of Prothioconazole, Prothioconazole-desthio and Azoxystrobin in Nectar, Pollen, Flower and Honey, Eurofins Agroscience Services Chem GmbH Study No S19-20860 (MAC-1940V), ADAMA Ref No.: 000104134

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

Deviations: No

GLP: Yes

Acceptability: Yes

Materials and methods
Flowers and nectar: A sample (100 mg ± 10 mg) is extracted with methanol/L-Cysteine-solution/formic

acid (50:50:0.5, v/v/v, 10mL) and shaken by hand for one minute and then for 15 minutes on a shaker. The sample is centrifuged for 5 minutes at about 3200 g and kept at 1˚C -10˚C in the dark.

Pollen: A sample (100 mg ± 10 mg) is extracted with methanol/L-Cysteine-solution/formic acid (50:50:0.5, v/v/v, 10mL) and homogenised by FastPrep at 4.0 m/second for 2 x 1 minutes. The sample is shaken for 15 minutes on a shaker, followed by centrifugation for 5 minutes at 3200 g.

Samples are analysed by high performance liquid chromatography with tandem mass specific detection (HPLC-MS/MS) in negative polarity mode (positive for desthio) using a Kinetex F5 column (50 mm x 2.1mm, 2.6 µm) and gradient elution with mobile phases of 0.1% formic acid in acetonitrile (v/v) and 0.1% formic acid in water (v/v). Quantification is performed using external standards. The prothioconazole ion transitions m/z 342 > 100 and m/z 342 > 58, and the prothioconazole-desthio ion transitions 312 > 70 and 312 > 125, are used for quantification and confirmation respectively.

Table A 84: Recovery results from method validation of prothioconazole using the analytical method

Matrix	Analyte	Ion Transition (m/z)	Fortification Level (mg/kg)	Recoveries (%)	Mean Recovery (%)	RSD (%)
Nectar	Prothioconazole	342 > 100	0.01	67, 83, 80, 78, 73	76	8.3
			0.1	76, 77, 90, 82, 83	82	6.9
			Overall	67 – 90	79	8.0
		342 > 58	0.01	77, 84, 86, 98, 82	85	9.1
			0.1	77, 80, 89, 80, 90	83	7.1
			Overall	77 – 98	84	7.8
Pollen		342 > 100	0.01	86, 78, 73, 71, 71	76	8.4
			0.1	75, 70, 74, 70, 69	72	3.8
			Overall	69 – 86	74	6.9
		342 > 58	0.01	78, 75, 72, 77, 70	74	4.5
			0.1	74, 71, 73, 70, 70	72	2.5
			Overall	70 – 78	73	4.0
Flowers		342 > 100	0.01	72, 66, 74, 68, 70	70	4.5
			0.1	82, 80, 75, 76, 78	78	3.7
			Overall	66 – 82	74	7.0
		342 > 58	0.01	66, 78, 93, 89, 81	81	13
			0.1	83, 78, 76, 78, 80	79	3.3
			Overall	66 – 93	80	9.1
Honey		342 > 100	0.01	74, 78, 73, 81, 70	75	5.8
			0.1	82, 85, 87, 85, 87	85	2.4
			Overall	70 – 87	80	7.7
		342 > 58	0.01	75, 78, 70, 83, 86	78	8.1
			0.1	83, 85, 89, 88, 87	86	2.8
			Overall	70 – 89	82	7.5

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

Matrix	Analyte	Ion Transition (m/z)	Fortification Level (mg/kg)	Recoveries (%)	Mean Recovery (%)	RSD (%)
Nectar	Prothioconazole-desthio	312 > 70	0.01	81, 82, 80, 81, 85	82	2.4
			0.1	89, 86, 87, 92, 88	88	2.6
			Overall	80 – 92	85	4.7
		312 > 125	0.01	86, 77, 81, 77, 82	81	4.7
			0.1	91, 85, 87, 90, 88	88	2.7
			Overall	77 – 91	84	5.9
Pollen		312 > 70	0.01	118, 122, 104, 103, 105	110	8.1
			0.1	101, 98, 98, 95, 94	97	2.9
			Overall	94 – 122	104	9.0
		312 > 125	0.01	107, 98, 110, 98, 98	102	5.7
			0.1	102, 99, 100, 99, 95	99	2.6
			Overall	95 – 107	101	4.5
Flowers		312 > 70	0.01	99, 102, 105, 101, 106	103	2.8
			0.1	103, 98, 102, 99, 98	100	2.3
			Overall	98 – 106	101	2.8
		312 > 125	0.01	107, 97, 104, 96, 92	99	6.2
			0.1	103, 98, 101, 99, 98	100	2.2
			Overall	92 – 107	100	4.4
Honey		312 > 70	0.01	80, 76, 76, 84, 95	82	9.6
			0.1	80, 76, 80, 81, 82	80	2.9
			Overall	76 – 95	81	6.9
		312 > 125	0.01	79, 79, 73, 79, 92	80	8.7
			0.1	79, 78, 79, 78, 80	79	1.1
			Overall	73 – 92	80	6.0

Table A 86: Characteristics for the analytical method used for validation of priothioconazole using the analytical method

	Prothioconazole
Specificity	HPLC-MS/MS monitoring two mass transitions is considered to be a highly specific technique. No interferences at >30% of the LOQ were present at the retention time of interest in the control matrix samples. Analyte identity was confirmed by comparison of the retention time of the analyte with that of a reference standard.
Calibration (type, number of data points)	0.025 ng/mL to 2.5 ng/mL (0.0025 to 0.2 mg/kg) (n =7 minimum) (covering at least no more than 30% of the LOQ and at least 20% of the highest analyte concentration detected in a sample extract)

Matrix	m/z	Coeff. of Det. (R^2)	Slope	Intercept
Nectar Surrogate	342>100	0.9997	96555.8667	253.5695
	34 >58	0.9992	48875.1419	-60.3483
Pollen	342>100	0.9990	230473.8673	64.2959
	34 >58	0.9993	107809.9437	1449.4244

	<table><tr><td rowspan="2">Flowers</td><td>342>100</td><td>0.9995</td><td>142042.9752</td><td>1386.7355</td></tr><tr><td>34 >58</td><td>0.9982</td><td>62957.3467</td><td>242.8810</td></tr><tr><td rowspan="2">Honey</td><td>342>100</td><td>0.9997</td><td>93383.5707</td><td>-59.9302</td></tr><tr><td>34 >58</td><td>0.9995</td><td>46621.8335</td><td>81.8399</td></tr></table>
Assessment of matrix effects is presented	Matrix effects were ≥ ± 20 % and deemed to be significant for prothioconazole in pollen. Therefore, matrix-matched standards were used for quantification throughout the study. Matrix suppression or enhancement was < 20 % for prothioconazole in nectar, flowers and honey and thus deemed to be insignificant. However, matrix-matched standards were used for quantification throughout the study.
Solution stability	A stock solution of prothioconazole in acetone was found to be stable for 168 stored at typically 1 °C to 10 °C in the dark. Fortification solutions of prothioconazole in methanol were found to be stable for 8 days stored at typically 1 °C to 10 °C in the dark. Calibration solutions of prothioconazole in methanol/L-cystein-solution/formic acid (50/50/0.5, v+v+v) were found to be stable for 8 days stored at typically 1 °C to 10 °C in the dark. Prothioconazole extracts of all matrices were found to be stable for at least 7 days when stored at typically 1 °C to 10 °C in the dark.
Limit of determination/quantification	The limit of quantification, defined as the lowest fortification level where acceptable precision and accuracy data were obtained, has been determined to be 0.01 mg/kg. The limit of determination, defined as the lowest detectable amount of analyte and was taken to be the lowest calibration solution and determined to be 0.025 ng/mL

Table A 87: Characteristics for the analytical method used for validation of priothioconazole-desthio using the analytical method

	Prothioconazole-desthio
Specificity	HPLC-MS/MS monitoring two mass transitions is considered to be a highly specific technique. No interferences at >30% of the LOQ were present at the retention time of interest in the control matrix samples. Analyte identity was confirmed by comparison of the retention time of the analyte with that of a reference standard.
Calibration (type, number of data points)	0.025 ng/mL to 2.5 ng/mL (0.0025 to 0.2 mg/kg) (n =7 minimum) (covering at least no more than 30% of the LOQ and at least 20% of the highest analyte concentration detected in a sample extract) <table><tr><th>Matrix</th><th>m/z</th><th>Coeff. of Det. (R^2)</th><th>Slope</th><th>Intercept</th></tr><tr><td rowspan="2">Nectar Surrogate</td><td>312>70</td><td>0.9985</td><td>778905.9065</td><td>-1646.3062</td></tr><tr><td>312 >125</td><td>0.9985</td><td>532459.5339</td><td>-1356.5632</td></tr><tr><td rowspan="2">Pollen</td><td>312>70</td><td>0.9997</td><td>604435.1815</td><td>-2049.5052</td></tr><tr><td>312 >125</td><td>0.9993</td><td>384735.1764</td><td>3022.0615</td></tr><tr><td rowspan="2">Flowers</td><td>312>70</td><td>0.9996</td><td>810151.5094</td><td>-2765.4310</td></tr><tr><td>312 >125</td><td>0.9990</td><td>525869.7273</td><td>7060.8752</td></tr><tr><td rowspan="2">Honey</td><td>312>70</td><td>0.9999</td><td>657688.1268</td><td>-455.7768</td></tr><tr><td>312 >125</td><td>0.9998</td><td>445824.6422</td><td>-302.9158</td></tr></table>
Assessment of matrix effects is presented	Matrix effects were ≥ ± 20 % and deemed to be significant for prothioconazole-desthio in pollen. Therefore, matrix-matched standards were used for quantification throughout the study. Matrix suppression or enhancement was < 20 % for prothioconazole-desthio in nectar, flowers and honey and thus deemed to be insignificant. However, matrix-matched standards were used for quantification throughout the study.
Solution stability	A stock solution of prothioconazole-desthio in acetone was found to be stable for 182 stored at typically 1 °C to 10 °C in the dark. Fortification solutions of prothioconazole-desthio in methanol were found to be stable for 8 days stored at typically 1 °C to 10 °C in the dark. Calibration solutions of prothioconazole-desthio in methanol/L-cystein-solution/formic

	acid (50/50/0.5, v+v+v) were found to be stable for 7 days stored at typically 1 °C to 10 °C in the dark. Prothioconazole-desthio extracts of all matrices were found to be stable for at least 7 days when stored at typically 1 °C to 10 °C in the dark.
Limit of determination/quantification	The limit of quantification, defined as the lowest fortification level where acceptable precision and accuracy data were obtained, has been determined to be 0.01 mg/kg. The limit of determination, defined as the lowest detectable amount of analyte and was taken to be the lowest calibration solution and determined to be 0.025 ng/mL

Conclusion
The analytical procedure has been successfully validated in terms of specificity, linearity, precision, accuracy and LOQ in accordance with the requirements of SANTE/2020/12830 rev.1.

A 2.2.2 Methods for post-authorization control and monitoring purposes (KCP 5.2)

A 2.2.2.1 Description of analytical methods for the determination of residues in plant matrices (KCP 5.2)

No new or additional studies have been submitted.

A 2.2.2.2 Description of analytical methods for the determination of residues in animal matrices (KCP 5.2)

A 2.2.2.2.1 Analytical method 1

Comments of zRMS:	The study of Lefresne, S., 2021 has been evaluated in Registration Report for ADM.03500.F.2.B on March 2023 by zRMS-PL and the summary is presented below. The analytical method has been demonstrated to be a reliable and accurate procedure for the determination of prothioconazole expressed as prothioconazole-desthio (sum of isomers) in honey. LOQ (Limit of quantification) of prothioconazole expressed as prothioconazole-desthio (sum of isomers): 0.010 mg/kg. The mean recoveries at each fortification level were in the range between 70% and 110% with relative standard deviation below 20%. The method complies with the guideline SANTE/2020/12830, Rev.1 of 24/02/2021.

Reference:	KCP 5.2/01
Reports:	Lefresne, S., 2021, Validation of an Analytical Method for the Determination of Prothioconazole Residues in Honey, GIRPA Study No B21S-A4-P-04 (MAC-1940V), ADAMA Ref No.: 000108774
Guideline(s):	SANTE/2020/12830 rev. 1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods
A sample (10 g ± 0.1 g) is extracted with ultra-pure water/acetonitrile (50/50, v/v, 20 mL) by mechanically and horizontally shaking for 25 minutes at about 300 counts/minute. A QuEChERS extraction salt pack containing magnesium sulfate (4 g), sodium chloride (1 g), sodium citrate (1 g) and disodium citrate sesquihydrate (0.5 g) is added and the sample is shaken vigorously by hand for about 1 minute. This is then centrifuged for about 5 minutes at 4000 rpm and filtered through a Nylon® filter

(0.45 µm). Samples are analysed by high performance liquid chromatography with tandem mass specific detection (HPLC-MS/MS) in positive polarity mode using a Kinetix C_{18} column (100 mm x 4.6 mm, 2.6 µm) and gradient elution with mobile phases of ultra-pure water + 0.1% formic acid and methanol. Quantification is performed using external standards. The prothioconazole-desthio ion transitions 312 > 70 and 312 > 125 are used for quantification and confirmation respectively.

Results and discussions

The method validation according to guideline SANTE/2020/12830 rev. 1 obtained during the study mentioned above is presented in Tables A87 – A88. Recovery results were in a range of 70% to 110% with an RSD < 20%. No outliers were identified. No interferences (< 30% LOQ) were found in unfortified control samples. The LOQ of prothioconazole-desthio was 0.01 mg/kg.

Table A 88: Recovery results from method validation of prothioconazole-desthio (sum of isomers) using the analytical method

Matrix	Analyte	Ion Transition (m/z)	Fortification Level (mg/kg)	Recoveries (%)	Mean Recovery (%)	RSD (%)
Honey	Prothioconazole-desthio (sum of isomers)	312 > 70	0.01	109, 112, 108, 106, 106	108	2.3
			0.1	109, 109, 112, 111, 110	110	1.2
			Overall	-	109	2.0
		312 > 125	0.01	108, 107, 107, 111, 108	108	1.5
			0.1	109, 106, 108, 108, 107	108	1.1
			Overall	-	108	1.3

Table A 89: Characteristics for the analytical method used for validation of priothioconazole-desthio using the analytical method

	Prothioconazole-desthio
Specificity	HPLC-MS/MS monitoring two mass transitions is considered to be a highly specific technique. No interferences at >30% of the LOQ were present at the retention time of interest in the control matrix samples. Analyte identity was confirmed by comparison of the retention time of the analyte with that of a reference standard.
Calibration (type, number of data points)	3 µg/L to 200 µg/L (n = 8 minimum) (covering at least no more than 30% of the LOQ and at least 20% of the highest analyte concentration detected in a sample extract). Matrix-matched calibration standards were used. **Matrix** / **Ion Transition (m/z)** / **r^2** / **A** / **B** / **C** Honey / 312 > 70 / 0.9982 / -1205.5669 / 869245.8004 / 749671.8949 Honey / 312 > 125 / 0.9992 / -1263.3872 / 897807.1890 / 509005.1720 For the calibration line with equation $y = Ax^2 + Bx + C$ The calculation of the residuals was performed and the deviation of the back-calculated concentrations of the calibration standards from the true concentration, using the calibration curve in the relevant region was not more than ± 20%.
Assessment of matrix effects is presented	Matrix suppression or enhancement was < 20% for prothioconazole-desthio in honey and thus deemed to be insignificant. However, matrix-matched standards were used for quantification throughout the study.
Stability of Stock Solutions	Stability of a stock standard solution has been demonstrated over a period of at least 392 days of frozen storage. The standard solutions and the matrix-matched standard solutions were prepared the day of analysis.
Stability of final extracts	The final sample extracts were analysed within 24 hours after initial extraction thus no stability study was performed.
Limit of determination/quantification	The limit of quantification, defined as the lowest fortification level where acceptable precision and accuracy data were obtained, has been determined to be 0.01 mg/kg. The limit of determination, defined as the lowest detectable amount of analyte and was taken to be the lowest calibration solution and determined to be 3 µg/L.

Conclusion
The analytical procedure has been successfully validated in terms of specificity, linearity, precision, accuracy and LOQ in accordance with the requirements of SANTE/2020/12830 rev.1.

A 2.2.2.2.2 Analytical method 2

Comments of zRMS:	The study of Lindner, M., 2022 has been evaluated in Registration Report for ADM.03500.F.2.B on March 2023 by zRMS-PL and the summary is presented below. An analytical method Lefresne, S., 2021 (Report No.: B21S-A4-P-04) for the determination of prothioconazole-desthio in honey was independently validated (ILV) in accordance to guidance document SANTE/2020/12830, rev.1. LC-MS/MS determination was conducted by monitoring two (2) mass transitions (m/z 312→70 and m/z 312→125). The limit of quantification is 0.01 mg/kg. Recovery results were in a range of 70 to 120% with an RSD ≤ 20. The method is acceptable.

Reference: KCP 5.1.2/02
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): SANTE/2020/12830, Rev.1
Deviations: None
GLP: Yes
Acceptability: Yes

Materials and methods
A sample (10 g ± 0.1 g) is extracted with ultra-pure water/acetonitrile (50/50, v/v, 20 mL) by vigorous manual shaking followed by mechanically shaking for 25 minutes on a platform shaker. A QuEChERS extraction salt pack containing magnesium sulfate (4 g), sodium chloride (1 g), trisodium citrate dihydrate (1 g) and disodium hydrogen citrate sesquihydrate (0.5 g) is added and the sample is shaken vigorously by hand for 1 minute. This is then centrifuged for about 5 minutes at about 3200 x *g*. A portion (0.1 mL) is added to water/methanol (9/1, v/v, 5 mL) and made up to volume (10 mL) with water/methanol (9/1, v/v). This is vortex mixed and then analysed by high performance liquid chromatography with tandem mass specific detection (HPLC-MS/MS) in positive polarity mode using a Kinetix XB-C18 column (100 mm x 4.6 mm, 2.6 µm) and gradient elution with mobile phases of water + 0.1% formic acid and methanol. Quantification is performed using external standards. The prothioconazole-desthio ion transitions 312 > 70 and 312 > 125 are used for quantification and confirmation respectively.

Results and discussions
The method validation according to guideline SANTE/2020/12830 rev. 1 obtained during the study mentioned above is presented in Tables A89 – A90. Recovery results were in a range of 70% to 110% with an RSD < 20%. No outliers were identified. No interferences (< 30% LOQ) were found in unfortified control samples. The LOQ of prothioconazole-desthio was 0.01 mg/kg.

Table A 89: Recovery results from method validation of prothioconazole-desthio in honey

Matrix	Analyte	Ion Transition (m/z)	Fortification Level (mg/kg)	Recoveries (%)	Mean Recovery (%)	RSD (%)
Honey	Prothioconazole-desthio (sum of isomers)	312 > 70	0.01	97, 94, 97, 97, 96	96	1.3
			0.1	113, 111, 110, 109, 110	111	1.5
			Overall	-	103	7.5
		312 > 125	0.01	96, 100, 98, 95, 96	97	2.0
			0.1	113, 110, 109, 111, 110	111	1.6
			Overall	-	104	7.0

Table A 90: Characteristics for the analytical method used for validation of prothioconazole-desthio in honey

	Prothioconazole-desthio
Specificity	HPLC-MS/MS monitoring two mass transitions is considered to be a highly specific technique. No interferences at >30% of the LOQ were present at the retention time of interest in the control matrix samples. Analyte identity was confirmed by comparison of the retention time of the analyte with that of a reference standard.
Calibration (type, number of data points)	0.030 ng/mL to 3.0 ng/mL (equivalent to 0.003 mg/kg to 0.30 mg/kg) (n = 8 minimum) (covering at least no more than 30% of the LOQ and at least 20% of the highest analyte concentration detected in a sample extract). Matrix-matched calibration standards were used. **Matrix** / **Ion Transition (m/z)** / **R^2** / **Slope** / **Intercept** Honey / 312 > 70 / 0.9989 / 372120.70 / 652.1902 Honey / 312 > 125 / 0.9989 / 203704.87 / 509.0032 The calibration curves obtained for both mass transitions were linear since the regression residuals were randomly distributed.
Assessment of matrix effects is presented	Matrix suppression or enhancement was < 20% and thus deemed to be insignificant. However, matrix-matched standards were used for quantification throughout the study.
Limit of determination/quantification	The limit of quantification, defined as the lowest fortification level where acceptable precision and accuracy data were obtained, has been determined to be 0.01 mg/kg. The limit of determination, defined as the lowest detectable amount of analyte and was taken to be the lowest calibration solution and determined to be 0.03 ng/mL (equivalent to 0.003 mg/kg).
Stability in working solutions	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. Prothioconazole-desthio was found to be stable for 4 days when prepared in acetonitrile and stored at typically 1°C to 10°C in the dark. Prothioconazole-desthio was found to be stable for 9 days when prepared in water/methanol (9/1, v/v) and stored at typically 1°C to 10°C in the dark.
Stability in sample extracts	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.

Conclusion
The analytical procedure has been successfully validated in terms of specificity, linearity, precision, accuracy and LOQ in accordance with the requirements of SANTE/2020/12830 rev.1.

A 2.2.2.2.3 Analytical method 3

Comments of zRMS:	The study of Watson, G., 2022 has been evaluated in Registration Report for ADM.03500.F.2.B on March 2023 by zRMS-PL and the summary is presented below. The analytical method was found to be valid for the determination of residues of prothioconazole-desthio in egg, with an LOQ of 0.01 mg/kg. The validation of the method met the criteria detailed in SANTE/2020/12830, Rev.1 (2021). Final determination of prothioconazole-desthio was conducted by LC-MS/MS monitoring transitions 312.0 → 70.0 m/z (primary) and 312.0 → 125.0 m/z (confirmatory). The accuracy and precision of the method was successfully demonstrated as the mean recovery value for prothioconazole-desthio at the LOQ fortification level (0.01 mg/kg) and at the higher fortification level (0.1 mg/kg) was between 70 – 120% with a relative standard deviation of ≤ 20%. The method is acceptable.

Reference: KCP 5.2/05

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 90: 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 91: Recovery results from method validation of prothioconazole-desthio in egg

Fortification level [mg/kg]	**Crop matrix**	**Egg**	
	Transition ion	70 m/z	125 m/z
0.010	Range Mean ± RSD n	82-86 83 ± 1.7 5	82-86 83 ± 1.7 5
0.100	Range Mean ± RSD n	80-84 82 ± 1.7 5	80-83 81 ± 1.3 5

RSD = relative standard deviation, n = number of replicates

Table A 92: Characteristics for the analytical method used for validation of prothioconazole-desthio in egg

	Prothioconazole-desthio
Specificity	Blank value < 30 % LOQ
Calibration (type, number of data points)	Individual calibration data presented r > 0.99 7 calibration points (single determination) Representative equation: $y = 4.87e^{4}\ x + 1.08e^{3}$
Calibration range	0.6 - 40 µg/L (equivalent to 0.003 – 0.2 mg/kg)
Assessment of matrix effects is presented	Matrix effects were observed to be < 20%. However,

	Prothioconazole-desthio
	calibration was carried out with matrix-matched standards
Limit of quantification	LOQ: 0.010 mg/kg Note: Concentration levels are given as mg substance/kg sample
Limit of detection	LOD: 0.003 mg/kg

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

A 2.2.2.2.4 Analytical method 4

Comments of zRMS:	The study of Lindner, M., Büdel, A., 2022 has been evaluated in Registration Report for ADM.03500.F.2.B on March 2023 by zRMS-PL and the summary is presented below. The independent laboratory method validation was found to be valid according to the guidance document SANTE/2020/12830, rev.1 for the determination of prothioconazole-desthio in egg with an LOQ of 0.01 mg/kg following the procedure listed in analytical method RES-00394 with no major modifications.

Reference: KCP 5.2/06

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 93: 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 (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 94: Recovery results from method validation of prothioconazole-desthio in egg

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

RSD = relative standard deviation, n = number of replicates

Table A 95: Characteristics for the analytical method used for validation of prothioconazole-desthio in egg

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

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

A 2.2.2.3 Description of Methods for the Analysis of Soil (KCP 5.2)

No new or additional studies have been submitted.

A 2.2.2.4 Description of Methods for the Analysis of Water (KCP 5.2)

A 2.2.2.4.1 Analytical method 1

Comments of zRMS:	The study of Thies, S., 2015 has been evaluated in Registration Report for ADM.03500.F.2.B on March 2023 by zRMS-PL and the summary is presented below. The analytical BCS method 01387/M002 for the determination of concentrations of prothioconazole and prothioconazole-desthio in surface water by HPLC-MS/MS using two MRM trasitions has been independly validated. The limit of quantitation (LOQ) for all analytes is 0.05 µg/L in surface water. The relative standard deviations for the peak areas were ≤ 20% for all MRM transitions of all analytes. The method meets all guideline criteria to determine concentrations in surface water of the described analytes at 0.05 µg/L.

Reference: KCP 5.2/03

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 assessment of matrix effects No assessment of stability of calibration solutions No residual plot for linearity
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.

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 96: 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 97: 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| calibration line equation presented	individual calibration data presented| calibration line equation presented

Calibration range	344 > 189: 0.015–1.0 µg/L r =0.9961 slope = 1.66 x 10^5, intercept = -994 ≥ 5 calibration points 344 > 154: 0.015–1.0 µg/L r =0.9971 slope = 1.39 x 10^5, intercept = -1560 ≥ 5 calibration points	312 > 70: 0.015–10 µg/L r =0.9987 slope = 1.17 x 10^6, intercept = 25400 ≥ 5 calibration points 312 > 125: 0.015–10 µg/L r =0.9992 slope = 6.9 x 10^5, intercept = 11700 ≥ 5 calibration points
Assessment of matrix effects is presented	Yes	Yes
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.

A 2.2.2.5 Description of Methods for the Analysis of Air (KCP 5.2)

No new or additional studies have been submitted.

A 2.2.2.6 Description of Methods for the Analysis of Body Fluids and Tissues (KCP 5.2)

A 2.2.2.6.1 Analytical method 1

Comments of zRMS:	The study of Brown, S., 2022 has been evaluated in Registration Report for ADM.03500.F.2.B on March 2023 by zRMS-PL and the summary is presented below. The analytical method for the determination of residues of prothioconazole-desthio in pig's blood has been validated with an LOQ of 0.01 mg/L. The accuracy and precision of the method was successfully demonstrated as the mean recovery value for prothioconazole-desthio at the LOQ fortification level (0.01 mg/L) was between 70 – 120% with a relative standard deviation of ≤ 20%. <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.

Reference:	KCP 5.2/04
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.

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 98: Recovery results from method validation of prothioconazole-desthio 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 99: 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
Calibration range	0.0075 – 0.375 ng/mL corresponding to 0.003 to 0.15 mg/L r ≥ 0.995 6 calibration points
Assessment of matrix effects is presented	Yes
Limit of quantification	0.01 mg/L
Limit of detection	0.003 mg/L

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