REGISTRATION REPORT

Part B

Section 5

Analytical Methods

Detailed summary of the risk assessment

Product code: GF-3307

Product name(s): Not yet defined

Chemical active substances:

Fenpicoxamid (XDE-777), 50 g/L

Prothioconazole, 100 g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(authorization)

Applicant: Corteva Agriscience

Submission date: July 2021, updated May 2022, August 2022

Finalisation date: October 2022 (initial Core Assessment)

January 2023 (final Core Assessment)

Version history

When	What
July 2021	New submission of GF-3307 in the Central Zone
May 2022	Austria removed from cMS, GAP table updated with 1 use = 1 crop + 1 disease Efate and ecotox updates aligned to request on GF ~~-3308~~ 3307
August 2022	Additional information on the validation of analytical methods for decomposition products
October 2022	Initial ZRMS assessment. The report in the dRR format has been prepared by the Applicant, therefore all comments, additional evaluations and conclusions of the zRMS are presented in grey commenting boxes. Minor changes are introduced directly in the text and highlighted in grey. Not agreed or not relevant information are ~~struck through and shaded for transparency~~.
January 2023	Final report (Core Assessment updated following the commenting period). No additional information or assessments after the commenting period.

Table of Contents

5 Analytical methods

5.1 Conclusion and summary of assessment

zRMS-PL conclusions:

Fenpicoxamid
EFSA in EFSA Journal 2018;16(1):5146 concluded:
"*Fenpicoxamid residues and also its metabolite X642188 can be monitored in food and feed of plant origin by liquid chromatography with tandem mass spectrometry (LC–MS/MS) with limit of quantifications (LOQs) of 0.01 mg/kg in all plant commodity groups for each analyte. Monitoring residues of fenpicoxamid and metabolite X642188 in milk, meat, liver, fat and poultry egg can be performed using LC–MS/MS with LOQs of 0.01 mg/kg all matrices for both compounds. The residue definition for monitoring in soil and water was defined as fenpicoxamid and its metabolite X642188.*
Appropriate LC–MS/MS methods exist for monitoring fenpicoxamid and metabolite X642188 in soil and water with LOQs of 0.05 mg/kg and LOQs of 0.05 lg/L, respectively, for both analytes. Fenpicoxamid residues in air can be determined by LC–MS/MS with a LOQ of 1.39 µg/m^3.
Determination of residues of fenpicoxamid in urine and blood can be done by LC–MS/MS with a LOQ of 0.05 mg/L."

List of End-point (UK, 2017):

Analytical methods for residues (Regulation (EU) N° 283/2013, Annex Part A, point 4.2 & point 7.4.2)
Residue definitions for monitoring purposes

Food of plant origin		XDE-777
Food of animal origin		No residue definition is proposed.
Soil		XDE-777 and metabolite X642188
Sediment		No data has been provided by the applicant and therefore it is not possible to set residue definition for sediment.
Water	surface	XDE-777 and metabolite X642188
	drinking/ground	XDE-777 and metabolite X642188
Air		XDE-777
Body fluids and tissues		XDE-777

Monitoring/Enforcement methods

Food/feed of plant origin (analytical technique and LOQ for methods for monitoring purposes)	LC/MS/MS (ESI+) LOQ = 0.01 mg/kg for XDE-777 and its metabolite X642188 in plants (rye, lettuce, lemon and oilseed rape). LC/MS/MS (ESI+) LOQ = 0.01 mg/kg for XDE-777 and its metabolite X642188 in plants and processed fractions (cereal grain and straw, lettuce, cabbage, orange, grapefruit, oil seed rape seed, olive, bran, flour, bread).
Food/feed of animal origin (analytical technique and LOQ for methods for monitoring purposes)	LC/MS/MS (ESI+) LOQ = 0.01 mg/kg for XDE-777 in animal (bovine milk, meat, liver and fat and poultry egg) LOQ = 0.01 mg/kg for the metabolite X642188 in animal (bovine milk, meat, liver and fat and poultry egg). LOQ = 0.01 mg/kg for the metabolite X12326349 in animal (bovine milk, liver and fat and poultry egg).
Soil (analytical technique and LOQ)	LC/MS/MS (ESI+) LOQ = 0.05 mg/kg for XDE-777 and its metabolite X642188 in the four types of soil and in one type of sediment
Water (analytical technique and LOQ)	LC/MS/MS (ESI+) LOQ = 0.05 µg/L for XDE-777 and its metabolite X642188 in surface, ground and drinking water.
Air (analytical technique and LOQ)	LC/MS/MS (ESI+) LOQ = 0.5 µg for XDE-777 equivalent to 1.39 µg/m^3 of ambient air and warm and humid air.
Body fluids and tissues (analytical technique and LOQ)	LC/MS/MS (ESI+) LOQ = 0.05 mg/L for XDE-777 in urine and blood

Applicant submitted several new methods used in support of ecotoxicology studies. An overview of these methods and their evaluations are presented in Appendix 2 of Part B5.

Sufficiently sensitive and selective analytical methods for post-authorization control and monitoring purposes are available for all analytes included in the residue definitions.
In SANTE/2020/12830, Rev.1 it is stated that analytical methods for monitoring residues in body fluids and tissues must be validated with the following matrix groups:
- Body fluids (either blood, serum, plasma or urine),
- Body tissues (either meat, liver or kidney).
For body tissues, a method for the determination of XDE-777 in bovine milk, meat, liver and fat and poultry egg with LOQ=0.01 mg/kg is available. This is acceptable.
For body fluids, a method for the determination of XDE-777 in urine and blood with LOQ = 0.05 mg/L is available. However, according to the SANTE/2020/12830, Rev.1 (24. February 2021), a lower LOQ is required for analytical methods for body fluids, the LOQ should be 0.01 mg/L instead of 0.05 mg/L (SANCO/825/00 rev. 8.1).
Information submitted by Applicant (February 2022):
"*Since SANTE/2020/12830, Rev.1 was published on 24-February-2021, Corteva did not have the opportunity to validate a new body fluids method prior to submission date for this plant protection product (June 2021). We recognize the need to update the body fluids method to lower the LOQ to 0.01 mg/L and have a study planned for 2023. The new body fluids method will be presented as part of the active substance renewal dossier in 2025*."

Additionally, the study concerning extraction efficiency, conducted with using 3 different solvent systems, was submitted in the framework of this application (Study No. S20-01536; DAS Study No. 200456; the study was evaluated in the Registration Report, Part B5 for GF-3308 on 24.08.2022).
This study has proven the satisfactory extraction efficiency of the extraction used in the analytical methods (MOR Method/ DAS #120615, MRM Method/DAS # 120998) for the quantitative determination of residues of XDE-777 when compared with the NOR Method/DAS #110334 for fenpicoxamid (XDE-777) in banana, barley grain and oilseed rape seed matrices.
The study is acceptable. Summary is presented in Appendix 2.

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 <u>for plant products</u> were proposed as 'prothioconazole-desthio (sum of isomers)' <u>for enforcement</u> and, as follows, <u>for the risk assessment</u>:
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 <u>for enforcement in animal products</u> was set as prothioconazole-desthio (sum of isomers) for all the livestock matrices (EFSA Journal 2014;12(5):3689).

<u>For risk assessment</u>, the residue was defined <u>in all commodities of animal origin</u> 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.
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 prothioconazole-desthio was submitted by Bayer and is being evaluated within the framework of the active substance renewal. The limit of quantification was established at 0.05 mg/L, expressed as prothioconazole-desthio, but according to the SANTE/2020/12830, Rev.1, 24. February 2021, the LOQ should be lower - 0.01 mg/L for body fluids and 0.01 mg/kg for body tissues.
The applicant provided the following information: "*Bayer is also planning on including prothioconazole in the method and lowering the LOQ for prothioconazole-desthio to 0.01 mg/L as part of the active substance renewal process.*"

In our opinion, it is necessary to supply the method for determining the residues of prothioconazole in body fluids with lower LOQ=0.01 mg/L at the renewal of the active substance and/or re-evaluation of plant production product.

- 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 01009 for the determination of JAU 6476-desthio, JAU 6476-3-hydroxy-desthio, JAU 6476-4-hydroxy-desthio, JAU 6476-3,4-dihydroxydesthio, and JAU 6476-4,5-dihydroxy-desthio in/on matrices of animal origin: milk, muscle, kidney, liver, fat and egg with LOQ 0.01 mg/kg. The BCS Analytical Method No. 010091 has been independently validated.

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 GF-3307.

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:

- an analytical method for the determination of prothioconazole in body fluids with lower LOQ=0.01 mg/L is required according to SANTE/2020/12830, Rev.1, 24. February 2021 and should be provided at the renewal of the active substance and/or re-evaluation of plant production product.

Commodity/crop	Supported/ Not supported
Wheat, rye, triticale, spelt	Supported
Barley	Supported

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

5.2.1 Analysis of the plant protection product (KCP 5.1.1)

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

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

Comments of zRMS:	The proposed method is acceptable and was successfully validated for the determination of the content of Fenpicoxamid and Prothioconazole in GF-3307 formulation according to the requirements laid down by SANCO3030/99 rev.4.

Reference:	KCP 5.1.1
Report	Analytical Method and Validation for the Determination of XDE-777 and Prothioconazole in GF-3307 and GF-3310 Formulations, Frank A., Jahnke, A., 2015, DAS-AM-G-14-24
Guideline(s):	Yes, U.S. EPA OPPTS Test Guideline 830.1800 EEC Guideline SANCO/3030/99 rev.4
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Internal standard composed of dibutylphthalate in acetonitrile is prepared. Standard solutions are prepared by dissolving the analytical standards with 10 mL of internal standard solution and 40 mL of acetonitrile. Samples are prepared by weighing aliquots into a glass jar and adding 10 mL of internal standard solution and 40 mL of acetonitrile. Solutions are then sonicated. The concentrations of fenpicoxamid and prothioconazole are determined using internal standard calibration using peak areas.

Validation - Results and discussions

Table 5.2-1: Methods suitable for the determination of active substances Fenpicoxamid and Prothioconazole in plant protection product GF-3307

	Fenpicoxamid	Prothioconazole	Internal Standard
Author(s), year	Frank, A., Jahnke, M., 2015		
Principle of method	Analytical method for determination of Fenpicoxamid and Prothioconazole in GF-3307 and GF-3310 formulations. A high pressure liquid chromatographic (HPLC) method was validated using an Ascentis Express C18 column, 5 cm x 3.0 mm, 2.7 micron, with an ultra-violet detector set at 240 nm. Concentrations were determined using internal standard calibration.		
Linearity (linear between mg/mL / % range of the declared content)	The detector response was shown to be linear for Fenpicoxamid over a range of 0.253 – 0.960 mg/mL	The detector response was shown to be linear for Prothioconazole over a range of 0.480 – 1.89	The detector response was shown to be linear for the internal standard from 0.403

	Fenpicoxamid	Prothioconazole	Internal Standard
(correlation coefficient, expressed as r)	(R2 = 0.9981).	mg/mL (R2 = 0.9986).	– 1.61 mg/mL (R2 = 0.9999).
Precision – Repeatability Mean n = 10 (%RSD)	The relative standard deviation was 0.34% at an average concentration of 4.61% of Fenpicoxamid	The relative standard deviation was 0.11% at an average concentration of 9.45% of Prothioconazole	-
Accuracy n = 7 (% Recovery)	Recovery data were obtained over the range of 2.43 – 9.48% Fenpicoxamid, at an average recovery of 100%	Recovery data were obtained over the range of 4.74 – 18.2% Prothioconazole, at an average recovery of 98%	-
Interference/ Specificity	No significant interferences were detected between the solvent blank, formulation blank, internal standard and technical grade active ingredient.		
Comment	No comment	No comment	No comment

Conclusion
This method has been successfully validated for Fenpicoxamid and Prothioconazole active substances in GF-3307.

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

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

Comments of zRMS:	The proposed method was successfully validated for the determination of Impurity in GF-3307 formulation according to the requirements laid down by SANCO3030/99 rev.4. This HPLC/MS method is applicable to the determination of desthio in formulation GF-3307. The method was validated over a range of 0.0019 – 0.0069 wt% (19 – 69 ppm) desthio in the end-use product, GF-3307.

Reference:	KCP 5.1.1
Report	Analytical Method and Validation for the Determination of the Desthio Impurity in GF-3307 Formulation, Moe, T., 2015, DAS-AM-G-14-38
Guideline(s):	Yes, U.S. EPA OPPTS Test Guideline 830.1800 EEC Guideline SANCO/3030/99 rev.4
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods
Standard calibration curve is prepared by dissolving the analytical standard in acetonitrile to create a 5 point standard curve from 1500-250 ppb. Samples are prepared by weighing aliquots into a 25-mL volumetric flask and making to volume with acetonitrile. Solutions are then mixed by hand. The concentrations of desthio are determined using a linear regression equation using peak areas.

Validation - Results and discussions

Table 5.2-2 Method suitable for the determination of prothioconazole-desthio in plant protection product (PPP) GF-3307

	Desthio
Author(s), year	Moe, T., (2015)
Principle of method	Validation of an analytical method for the determination of Desthio (2-(1-chlorocyclopropyl)-1-(2-chlorophenyl)-3-(1H-1,2,4-triazol-1-yl)propan-2-ol) in formulation GF-3307. A high pressure liquid chromatographic (HPLC) with Mass Spectrometry (MS) detection was validated using a Waters Xbridge C8 column and an injection volume of 5 µL. Concentrations were determined using a linear curve.
Linearity (linear between mg/L) (correlation coefficient, expressed as r)	0.00027 – 0.00162 mg/mL R2 = 0.9980
Precision – Repeatability Mean n = 10 (%RSD)	Day 1: %RSD = 3.72 at an average concentration of 0.0034wt% Day 2: %RSD = 5.66 at an average concentration of 0.0046wt%
Accuracy n = 7 (% Recovery)	0.0010 – 0.0069% at an average recovery of 89.7%
Interference/ Specificity	No interferences
LOQ	LOQ was 0.0019% at an average recovery of 83.8%
Comment	No comment

Conclusion

This method has been successfully validated for relevant impurity prothioconazole-desthio in GF-3307.

Comments of zRMS:	The proposed method was successfully validated for the determination of Impurity in GF-3307 formulation according to the requirements laid down by SANCO3030/99 rev.4.

Reference:	KCP 5.1.1
Report	Analytical Method and Validation for the Determination of Toluene in GF-3307 Formulation, Nelson, R.M., 2018, DAS-AM-G-15-44
Guideline(s):	Yes, U.S. EPA OPPTS Test Guideline 830.1800 EEC Guideline SANCO/3030/99 rev.4
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

An internal standard solution containing 50 µg/mL of ethylbenzene in dimethylsulfoxide (DMSO) is prepared. Six 210 mg aliquots of the GF-3307 sample are weighed into individual headspace vials. Samples are spiked with either 2 mL of DMSO or with 2 mL of one of five spike solutions containing 5, 10, 25, 50 or 100 µg/mL of toluene in DMSO. A 2 mL aliquot of the internal standard solution is then added to each vial, and vials are crimped tightly. The solutions are analyzed by headspace gas chromatography using a DB-624 column with flame ionization detection. Quantitation is done using standard addition quantitation.

Validation - Results and discussions

Table 5.2-3: Method suitable for the determination of Toluene in plant protection product (PPP) GF-3307

	Toluene
Author(s), year	Nelson, R. M. (2018)
Principle of method	A headspace method was validated for the determination of toluene in GF-3307. The method uses a DB-624 column with flame ionization detection and internal standard calibration using ethylbenzene. Quantitation is by standard addition.
Linearity (linear between mg/L) (correlation coefficient, expressed as r)	2.6 – 130 µg/mL concentration range for toluene with R2 = 0.9998, equivalent to 0.0025 to 0.124%; 10.2 – 50.8 µg/mL concentration range for ethylbenzene with R2 = 0.9989
Precision – Repeatability Mean n = 10 (%RSD)	For 10 samples analysed over two days, the average concentration was 0.024%, with RSD of 5.5%.
Accuracy n = 7 (% Recovery)	0.00942 to 0.0588% at an average recovery was 95.2%
Interference/ Specificity	No interferences.
LOQ	0.00033%
LOD	0.00010%
Comment	No comment

Conclusion

This method has been successfully validated for Toluene in GF-3307.

Comments of zRMS:	The proposed methods was successfully validated for the determination of potential degradates in GF-3307 formulation according to the requirements laid down by SANCO3030/99 rev.4.

Reference: KCP 5.1.1
Report: Analytical Method and Validation for the Determination of Potential Degradates in GF-3307 Formulation, Hofer, C., 2017, DAS-AM-G-170058
Guideline(s): Yes
Deviations: No
GLP: Yes
Acceptability: Yes

Materials and methods

Standard calibration curve is prepared by dissolving the analytical standard in an acidified dilution solution (9/1/0.01 acetonitrile/water/formic acid) to create a 4 point standard curve from 0.01 – 0.03 mg/mL X12314005. Samples are prepared by weighing aliquots into a 50-mL volumetric flask and making to volume with dilution solution. Solutions are then mixed by hand. The concentrations of X12314005 are determined using a linear regression equation using peak areas.

Validation - Results and discussions

Table 5.2-4 Method suitable for the determination of Inatreq Degradants in plant protection product (PPP) GF-3307

	X12314005
Author(s), year	Hofer, C. (2017)
Principle of method	A high pressure liquid chromatographic (HPLC) method was validated for the determination of X12314005 (LAC-IBU) in GF-3307. The method uses a Waters Acquity CSH C18 column with mass spectroscopy detection and external standard calibration. Quantitation is by linear regression.
Linearity (linear between mg/L) (correlation coefficient, expressed as r)	0.6 – 0.046 mg/mL concentration range for X12314005 with R2 = 0.997, equivalent to 0.05 to 0.4 wt%

Precision – Repeatability Mean n = 10 (%RSD)	For 10 samples analysed over two days, the average concentration was 0.084%, with RSD of 0.28%.
Accuracy n = 7 (% Recovery)	0.05 to 0.4% at an average recovery was 102%
Interference/ Specificity	The test system contained a small amount of X12314005. The interference peak areas were subtracted from the total peak areas to give corrected areas for X12314005. No significant interferences were observed.
LOQ	0.042%
Comment	No comment

Conclusion
This method has been successfully validated for X12314005 in GF-3307.

Reference:	KCP 5.1.1
Report	PDF titled: DATA CRD Response to GF-3307 Method Precision
Guideline(s):	SANCO/3030/99
Deviations:	No
GLP:	No
Acceptability:	Yes

The test system, TSN309553, did contain a small amount of X12314005. The amount was determined as part LOD/LOQ and had a peak area count of 80284. The peak area was corrected for in the linearity and recovery calculations, as seen in Table 4, Table 5 and Figure 3 by subtracting this amount from the peak areas obtained in the linearity and recovery samples.

With regards to the method and system precision, there was a calculation error and when corrected, the precision did not pass Horwitz. Therefore, the precision analysis was repeated, non-GLP, and had acceptable precision. The following is the description and results of this analysis. GF-3307, TSN309552, was prepared and analyzed using similar conditions that were submitted for the LC/MS method DAS-AM-G-170058. It was concluded that the method had acceptable precision at 0.20 average wt%.

Preparation of dilution solution
Combined 900 mL of acetonitrile and 100 mL Milli-Q water and 1 mL of formic acid into a 1-L glass bottle.

Preparation of Calibration Solutions
Impurity Stock Solution: Weighed approximately 51 mg of the X12314005 impurity standard (TSN306252) into a 2-oz jar and added 50 mL of dilution solution by Eppendorf Repeater pipet and mixed well until fully dissolved. Calibration Standard Solutions: Using an Eppendorf Repeater pipet, added the appropriate amount of Impurity stock solution into 20-mL volumetric flasks. Dilute to volume with dilution solution to make a 4 point standard curve from 0.01 – 0.03 mg/mL.

Preparation of sample solutions
The formulation sample (TSN309552) available at the time of this study contained an amount of X12314005 that was outside of the validated range of the method, so diluted samples were used and spiked with X12314005 to a level within the validated range in order to assess method and system precision. Five replicate samples were prepared by weighing approximately 25 mg of GF-3307 into a 1-oz jar. The sample was diluted with 25 mL of the dilution solution, added by Eppendorf Repeater pipet. Each sample was then spiked with 0.2 mL of the impurity stock solution.

LC analysis conditions:
HPLC System: HPLC System: Agilent 1290 Infinity II Quaternary HPLC
Column: Waters Acquity CSH C18 2.1 x100mm, 1.7 µm
Column Temperature: Ambient
Injection Volume: 0.5 µL

Flow: 0.2 mL/min
Detection: Agilent 6470 Triple quadrupole mass spectrometer
Eluent A: 0.1% formic acid in water
Eluent B: 0.1% formic acid in acetonitrile
Gradient elution

MS Parameters:
Source Condition Value
Interface: Electrospray
Polarity: Positive
Scan Type: MRM
Resolution: Q-1 Unit, Q-3 Unit
Gas Temperature: 300oC
Gas Flow: 5 L/min.
Nebulizer: 45 psi
Sheath Gas Temperature: 250oC
Sheath Gas Flow: 11 L/min.
Capillary Voltage: 3500 V
Nozzle Voltage: 500 V

Table I. Method Precision Data for X12314005 in GF-3307

Sample ID	**Wt% X12314005**
Precision 1	0.205
Precision 2	0.197
Precision 3	0.197
Precision 4	0.19
Precision 5	0.201
Overall Average	0.20
Std. Dev.	0.006
Overall RSD	2.8
Horwitz RSDR	5.1
Horwitz RSDr	3.4
Acceptable? (Overall RSD<Horwitz RSDr)	Acceptable

Table II System Precision Data for X12314005 in GF-3307

Sample ID	**Wt% X12314005**
Precision 5-1	0.201
Precision 5-2	0.203
Precision 5-3	0.201
Precision 5-4	0.208
Precision 5-5	0.209
Overall Average	0.20
Std. Dev.	0.004
Overall RSD	1.9
Horwitz RSDR	5.1
Horwitz RSDr	3.4
Acceptable? (Overall RSD<Horwitz RSDr)	Acceptable

Reference:	KCP 5.1.1
Report	Analytical Method and Validation for the Determination of X12335723 Impurity in GF-3307 Formulation, Frank, A 2016, DAS-AM-G-15-1
Guideline(s):	Yes

Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods
Standard solutions are prepared by dissolving the analytical standard in an acidified dilution solution (0.1% formic acid in dimethylformamide). Samples are prepared by weighing aliquots into a 50-mL volumetric flask and making to volume with dilution solution. Solutions are then mixed by hand. The concentrations of X12335723 are determined using external standard calibration using peak areas.

Validation - Results and discussions

Table 5.2-5 Method suitable for the determination of the X12335723 Impurity in plant protection product (PPP) GF-3307

	X12335723
Author(s), year	Frank, A. (2016)
Principle of method	A high pressure liquid chromatographic (HPLC) method was validated for the determination of X12335723 in GF-3307. The method uses a Waters XSelect CSH C18 column with ultra-violet detection and external standard calibration. Quantitation is by linear regression.
Linearity (linear between mg/L) (correlation coefficient, expressed as r)	0.0077 – 0.077 mg/mL concentration range for X12335723 with R2 = 0.9996, equivalent to 0.038 to 0.39 wt%
Precision – Repeatability Mean n = 10 (%RSD)	For 10 samples analysed over two days, the average concentration was 0.14%, with RSD of 1.6%.
Accuracy n = 7 (% Recovery)	0.038 to 0.39% at an average recovery was 103%
Interference/ Specificity	No significant interferences (>3%) were observed.
LOQ	0.034%
LOD	0.0015%
Comment	No comment

Conclusion
This method has been successfully validated for X12335723 in GF-3307.

Reference:	KCP 5.1.1
Report	Analytical Method and Validation for the Determination of Retro-Michael in GF-3307 Formulation, Frank, A., 2015, DAS-AM-G-14-35
Guideline(s):	Yes
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods
Standard solutions are prepared by dissolving the analytical standard in acetonitrile. Samples are prepared by weighing aliquots into a 25-mL volumetric flask and making to volume with acetonitrile. Solutions are then mixed by hand. The concentrations of X12393285 (Retro-Michael) are determined using external standard calibration using peak areas.

Validation - Results and discussions

Table 5.2-6 Method suitable for the determination of the X12393285 Impurity in plant protection product (PPP) GF-3307

	X12393285
Author(s), year	Frank, A. (2016)
Principle of method	A high pressure liquid chromatographic (HPLC) method was validated for the determination of X12393285 in GF-3307. The method uses a Ascentis Express C18 column with ultra-violet detection and external standard calibration. Quantitation is by linear regression.
Linearity (linear between mg/L) (correlation coefficient, expressed as r)	0.0069 – 0.069 mg/mL concentration range for X12393285 with R2 = 0.9998, equivalent to 0.034 to 0.34 wt%
Precision – Repeatability Mean n = 10 (%RSD)	For 10 samples analysed over two days, the average concentration was 0.088%, with RSD of 1.0%.
Accuracy n = 7 (% Recovery)	0.034 to 0.34% at an average recovery was 99%
Interference/ Specificity	No significant interferences (>3%) were observed.
LOQ	0.027%
LOD	0.0080%
Comment	No comment

Conclusion
This method has been successfully validated for X12393285 in GF-3307.

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

No methods are required as none of the co-formulants are defined as relevant for toxicity (environment, health).

5.2.1.4 Applicability of existing CIPAC methods (KCP 5.1.1)

There is currently no CIPAC method available for the determination of Fenpicoxamid and Prothioconazole in GF-3307.

5.2.2 Methods for the determination of residues, Fenpicoxamid (KCP 5.1.2)

An overview on the acceptable methods and possible data gaps for analysis of residues of Fenpicoxamid for the generation of pre-authorization data is given in the following table. These studies have already been evaluated during the EU approval process of the active substance (EFSA 2018). For the detailed evaluation of new/additional studies, refer to Appendix 2.

The residue definition for risk assessment for food of plant origin is fenpicoxamid (EFSA Journal 2018;16(1):5146). The crop method used to analyze for fenpicoxamid residues in cereal studies 150650, 140648, 140649, 150649, 180126, 170191, and 180128 (KCA 6.3.1/01 – KCA 6.3.1/06 and KCA 6.3.1/07) was the EU agreed Method No. 120615 (Watson, G., 2012). The extraction efficiency of Method No. 120615 was successfully evaluated using incurred radiolabeled wheat samples (grain, hay, straw, forage): fenpicoxamid residue levels determined using the manual extraction procedure outlined in the crop analytical method (acetonitrile/water (90/10, v/v)) were comparable (differed by no more than 30%) to residue levels determined using the accelerated solvent extraction (ASE) procedure outlined in the wheat nature of residue (NOR) study (Li, Q., Dixit, V., 2013). The crop method used to analyze for fenpicoxamid residues in cereal study 170192 (KCA 6.3.1/08) used a solvent (acetonitrile/water/phosphoric acid (90/10/0.1, v/v/v)) that differs in composition by no more than 20 vol% compared to the solvent used in analytical method 120615 (acetonitrile/water (90/10, v/v)).

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

	Component of residue definition: Fenpicoxamid				
Matrix type	**Method No.**	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
High water content, high acid content, high oil content, high protein/high starch content (dry) (Residues)	120615*	Primary	0.01 mg/kg	LC-MS/MS	Watson, G., 2012, EU agreed
High water content, high protein/high starch content (dry) (Residues)	170192	Primary	0.01 mg/kg	LC-MS/MS	Eversfield, S., 2019
Animal products (feeding study)	130949	Primary	0.01 mg/kg	LC-MS/MS	Rawle, N.W., 2013, EU agreed
~~Pollen, nectar (Residues)~~	~~200670~~	~~Primary~~	~~0.001 mg/kg~~	~~LC-MS/MS~~	~~Appeltauer, A, 2021~~
Soil (Environmental fate: TFD study)	141042	Primary	0.012 mg/kg	LC-MS/MS	Li, Q., Hastings, M., Slinkard, E.W., 2015, EU Agreed
Water (Ecotoxicology)	140479 140489	Primary	0.0217 ng/mL	LC-MS/MS	Dinehart, S., 2014, revised 2017, final report addendum 2019 Hadsell, R., 2014, revised 2018
	140491		0.120 ng/mL		Hicks, S., 2014, final report addendum 2020
	160101		0.070 ng/mL		Goudie, O., 2016a
	160102		0.066 ng/mL		Goudie, O., 2016b
	180975		0.123 ng/mL		Dinehart, S., 2018
	191366		7.05 µg/L		Goudie, O., 2020
	202284		19.7 ng/L		Goudie, O., 2021
	181382		0.025 µg/L		Bruggermann, 2020
	160125		0.050 µg/L		Hicks, S., 2017
Honey Bee (Ecotoxicology)	171043	Primary	0.0705 mg/kg (larval diet) 0.705 mg/L (water)	LC-MS/MS	Oberrauch, S., 2018
	170077		0.00235 mg/kg		Vergé, E., Kästel, A., 2018
	170673		0.001 mg/kg		Kleinhenz, M., 2018
	200660		0.001 mg/kg		Gonsoir, G., 2021
	201076		3.44 g a.i./L (sugar solution) 50.0 g a.i./L (acetone)		Cornement, M., 2022a
	201075		0.0161 g a.i./L (sugar solution)		Cornement, M., 2022b

	Component of residue definition: Fenpicoxamid				
Matrix type	**Method No.**	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
			0.341 g a.i./L (water)		

*Also used as a post-registration enforcement method.

Component of residue definition: Metabolite X642188					
Matrix type	**Method No.**	**Method Type**	**Method LOQ**	**Method Principle**	**Author(s), year / missing / EU agreed**
Water (Ecotoxicology)	180562	Primary	0.02 µg/L	LC-MS/MS	Goudie, O., 2018
	181382		0.0015 µg/L	LC-MS/MS	Bruggermann, 2020
Sediment, Water (Ecotoxicology)	180563	Primary	0.02 µg/L (overlaying water) 14 mg/L (porewater) 0.046 mg/kg (sediment)	LC-MS/MS	Beasley, J., 2018
	180639		0.33 µg/L (water) 0.046 mg/kg (sediment)		Dinehart, S., 2019
Soil (Environmental fate: TFD study)	141042	Primary	0.012 mg/kg	LC-MS/MS	Li, Q., Hastings, M., Slinkard, E.W., 2015, EU Agreed

	Component of residue definition: Metabolite X12326349				
Matrix type	**Method No.**	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Animal products (feeding study)	130949	Primary	0.01 mg/kg	LC-MS/MS	Rawle, N.W., 2013, EU agreed

Component of residue definition: Metabolite X12335723					
Matrix type	**Method No.**	**Method Type**	**Method LOQ**	**Method Principle**	**Author(s), year / missing / EU agreed**
Sediment, Water (Ecotoxicology)	180564	Primary	0.015 mg/L (water) 0.0069 mg/kg (sediment)	LC-MS/MS	Leak, T., 2018

Component of residue definition: Metabolite X12019520					
Matrix type	**Method No.**	**Method Type**	**Method LOQ**	**Method Principle**	**Author(s), year / missing / EU agreed**
Water (Ecotoxicology)	180560	Primary	4.9 mg/L	LC-MS/MS	Hughes, J., 2018a

Component of residue definition: Metabolite X12446477					
Matrix type	**Method No.**	**Method Type**	**Method LOQ**	**Method Principle**	**Author(s), year / missing / EU agreed**
Water (Ecotoxicology)	180561	Primary	0.096 mg/L	LC-MS/MS	Hughes, J., 2018b

5.2.3 Methods for the determination of residues, Prothioconazole (KCP 5.1.2)

An overview on the acceptable methods and possible data gaps for analysis of residues of prothioconazole for the generation of pre-authorization data is given in the following tables.

The residue definition for risk assessment for food of plant origin is 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) (EFSA Journal 2014;12(5):3689). The crop method used to analyze for prothioconazole-desthio residues in cereal studies 140649, 150649, 180126, 170191, and 180128 (KCA 6.3.1/03 – KCA 6.3.1/06 and KCA 6.3.1/07) was the EU agreed Method No. 00598 (Heinemann, O., 2000). The extraction efficiency of this method was evaluated using aged radioactive residues from the metabolism study following spray application of ^{14}C-prothioconazole on wheat (Haas, M., 2001). The residue method extraction (using acetonitrile/water as solvent) and the amount extracted in the metabolism studies were in good agreement. The method's extraction efficiency is also being re-revaluated as part of the active substance renewal process.

Prothioconazole is a triazole containing pesticide, so the residue definition for risk assessment for food of plant origin also includes 1,2,4-triazole (1,2,4-T), triazole alanine (TA), triazole acetic acid (TAA), and triazole lactic acid (TLA) (EFSA Journal 2018; 16(7):5376). An extensive data package on TDMs generated by the task force Triazole Derivative Metabolite Group (TDMG) was evaluated by EFSA and is under final steps of the review process within the European Commission. To ensure harmonization of assessments carried out for all triazole active substances and the plant protection products containing them, the EU Commission has agreed that Austria, in its capacity as RMS for paclobutrazole, with evaluate the additional TDMG studies (SANTE/E4/MW/df (2021)1403576). TDM data specific to prothioconazole will have been submitted by Bayer for evaluation during the active substance renewal. As such, no new studies or data on TDMs are presented for evaluation in this submission.

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

Component of residue definition: Prothioconazole (JAU6476)					
Matrix type	**Method No.**	**Method Type**	**Method LOQ**	**Method Principle**	**Author(s), year / missing / EU agreed**
~~Pollen, nectar (Residues)~~	~~200670~~	~~Primary~~	~~0.001 mg/kg~~	~~LC-MS/MS~~	~~Appeltauer, A, 2021~~
Water (Ecotoxicology)	140491	Primary	0.235 ng/mL	LC-MS/MS	Hicks, S., 2014
	180975		0.245 ng/mL		Dinehart, S., 2018
	181382		0.050 µg/L		Bruggermann, 2020
Honey Bee (Ecotoxicology)	170673	Primary	0.001 mg/kg	LC-MS/MS	Kleinhenz, M., 2018
	200660		0.001 mg/kg		Gonsoir, G., 2021
	201075		0.0333 g a.i./L (sugar solution) 0.704 g a.i./L (water)		Cornement, M., 2022b

Component of residue definition: Metabolite Prothioconazole-desthio (M04, JAU6476-desthio)					
Matrix type	**Method No.**	**Method Type**	**Method LOQ**	**Method Principle**	**Author(s), year / missing / EU agreed**
High water content, high oil content, high protein/high starch content (dry)	00598	Primary	0.05 mg/kg (wheat, barley forage and straw) 0.01 mg/kg (wheat, barley grain)	HPLC-MS/MS	Heinemann, O., 2000, EU reviewed

Component of residue definition: Metabolite Prothioconazole-desthio (M04, JAU6476-desthio)					
Matrix type	**Method No.**	**Method Type**	**Method LOQ**	**Method Principle**	**Author(s), year / missing / EU agreed**
	00598/M001		0.05 mg/kg (wheat, barley forage and straw) 0.01 mg/kg (wheat, barley grain, canola seed)		Heinemann, O., 2000b, EU reviewed
	00647		0.05 mg/kg (wheat, barley forage and straw) 0.01 mg/kg (wheat, barley grain, canola seed)		Heinemann, O., 2001, EU reviewed
Animal products (feeding study)	00655*	Primary	0.01 mg/kg (milk, meat, liver, kidney, fat)	HPLC-MS/MS	Heinemann, O, 2001b, EU reviewed
Animal products (feeding study)	00655/M001*	Primary	0.004 mg/kg (milk)	HPLC-MS/MS	Heinemann, O, 2001c, EU reviewed
~~Pollen, nectar (Residues)~~	~~200670~~	~~Primary~~	~~0.001 mg/kg~~	~~LC-MS/MS~~	~~Appeltauer, A, 2021~~
Honey Bee (Ecotoxicology)	170673	Primary	0.001 mg/kg	LC-MS/MS	Kleinhenz, M., 2018
	200660		0.001 mg/kg		Gonsoir, G., 2021

*Also used as a post-registration enforcement method.

Component of residue definition: Metabolites JAU6476-3-hydroxy-desthio and JAU6476-4-hydroxy-desthio					
Matrix type	**Method No.**	**Method Type**	**Method LOQ**	**Method Principle**	**Author(s), year / missing / EU agreed**
Animal products (cow's milk, meat, liver, kidney, fat)	00655	Primary	0.01 mg/kg	HPLC-MS/MS	Heinemann, O, 2001b, EU reviewed
Animal products (milk)	00655/M001	Primary	0.004 mg/kg	HPLC-MS/MS	Heinemann, O, 2001c, EU reviewed

Component of residue definition: Metabolites 1,2,4-T, TA, TAA, TLA					
Matrix type	**Method No.**	**Method Type**	**Method LOQ**	**Method Principle**	**Author(s), year / missing / EU agreed**
N/A*					

*Evaluation of existing TDM data available from EFSA Journal 2018; 16(7):5376. New TDMG data will be assessed by Austria per EU Commission agreement on a harmonized risk assessment and new PTZ specific data will be assessed during the ongoing active substance renewal.

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

5.3.1 Analysis of the plant protection product (KCP 5.2)

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

5.3.2 Description of analytical methods for the determination of residues of

Fenpicoxamid (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) and the EFSA Conclusion (EFSA Journal 2018;16(1):5146) 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	Fenpicoxamid	0.01 mg/kg	Reg (EU) 2019/50
Plant, high acid content		0.01 mg/kg	Reg (EU) 2019/50
Plant, high protein/high starch content (dry commodities)		0.01 mg/kg Wheat 0.6 mg/kg ~~Barley 0.8 mg/kg~~ Barley 0.01mg/kg	Reg (EU) 2019/50 Reg (EU) 2019/50 ~~Pending Assessment~~ Reg (EU) 2019/50
Plant, high oil content		0.01 mg/kg	Reg (EU) 2019/50
Plant, difficult matrices (hops, spices, tea)		0.05 mg/kg	Reg (EU) 2019/50
Muscle	X12326349 expressed as fenpicoxamid	0.01 mg/kg	Reg (EU) 2019/50
Milk		0.01 mg/kg	Reg (EU) 2019/50
Eggs		0.01 mg/kg	Reg (EU) 2019/50
Fat		0.01 mg/kg	Reg (EU) 2019/50
Liver, kidney		0.01 mg/kg 0.02 mg/kg (bovine kidney; sheep liver and kidney)	Reg (EU) 2019/50
Soil (Ecotoxicology)	Fenpicoxamid and X642188	0.05 mg/kg	Common Limit EFSA Journal 2018;16(1):5146 $NOEC_{corr}$ = 3.97 mg a.s./kg dsw, *F. candida* $NOEC_{corr}$ = 2.8 mg X642188/kg dsw, *E. fetida*
Drinking water (Human toxicology)	Fenpicoxamid and X642188	0.1 µg/L 0.05 µg/L	Common Limit, Directive 2006/118/EC EFSA Journal 2018;16(1):5146
Surface water (Ecotoxicology)	Fenpicoxamid and X642188	NOEC = 0.37 µg a.s./L, *P. promelas* EC_{50} = 0.79 µg X642188/L, *D. magna*	EFSA Journal 2018;16(1):5146 Goudie, O. 2018, Study No. 180562
Air	Fenpicoxamid	~~15 µg/m³~~ LOQ = 0.5 µg for XDE-777 equivalent to 1.39 µg/m³ of ambient air and warm and humid air	EFSA Journal 2018;16(1):5146 AOEL: 0.05 mg/kg bw/d
Body tissues (meat or liver)	Fenpicoxamid	0.1 mg/kg 0.01 mg/kg	Common Limit, SANCO/825/00 rev. 8.1, Reg (EU) 283/2013, EFSA Journal 2018;16(1):5146

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
			SANTE/2020/12830, Rev.1 24. February 2021
Body fluids (urine or blood)	Fenpicoxamid	0.05 mg/L 0.01 mg/L	Common Limit, SANCO/825/00 rev. 8.1, Reg (EU) 283/2013, EFSA Journal 2018;16(1):5146 SANTE/2020/12830, Rev.1 24. February 2021

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 Fenpicoxamid in plant matrices is given in the following tables. These studies have already been evaluated during the EU approval process of the active substance (EFSA 2018)

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: Fenpicoxamid				
Matrix type	**Method Type**	**Method No.**	**Method LOQ**	**Method Principle**	**Author(s), year / missing / EU agreed**
High water content, high acid content, high oil content, high protein/high starch content (dry)	Primary/ Confirmatory	120615	0.01 mg/kg	LC-MS/MS	Watson, G., 2012, EU agreed
	ILV		0.01 mg/kg	LC-MS/MS	Chambers, J., Jarrett, H., 2013, EU agreed
	Primary/ Confirmatory (Multi-residue)	120998	0.01 mg/kg	LC-MS/MS	Lindner, M., Giesau, A., 2013, EU agreed
	ILV (Multi-residue)		0.01 mg/kg	LC-MS/MS	Amic, S., 2013, EU agreed

Table 5.3-3: Statement on extraction efficiency

	Method for products of plant origin
Required, available from:	Li, Q., Dixit, V., 2013, EU agreed Senciuc, M., 2021

Extraction efficiency for the primary method (Watson, G., 2012) was evaluated by comparing residue levels determined using the manual extraction procedure outlined in the method (acetonitrile/water, 90/10, v/v) to residue levels determined using the accelerated solvent extraction (ASE) procedure outlined in the wheat nature of residue (NOR) study (Ma, M., Jackson, A.U., 2013). Incurred radiolabeled samples, obtained from the wheat NOR study, were used for the quantitation of fenpicoxamid in both extraction procedures. Comparable extraction efficiency was demonstrated for any fenpicoxamid residue levels above the LOQ (Li, Q., Dixit, V., 2013).

In a more recent study, extraction efficiencies for the primary method (Watson, G., 2012) and the multi-residue method (Linder, M., Giesau, A., 2013) were evaluated by comparing residue levels determined using the extraction procedures outlined in the two analytical methods (Watson: acetonitrile/water (90/10, v/v); Linder: acetonitrile/water (1/1, v/v)) to residue levels determined using the ASE extraction procedure outlined in the wheat nature of residue (NOR) study (Ma, M., Jackson, A.U., 2013). Incurred samples from banana, barley grain, and oilseed rape matrices were used for quantitation of fenpicoxamid in all three extraction procedures. Satisfactory extraction efficiency was demonstrated for both analytical methods in determining fenpicoxamid residue levels (Senciuc, M., 2021).

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 Fenpicoxamid in animal matrices is given in the following tables. These studies have already been evaluated during the EU approval process of the active substance (EFSA 2018).

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

Component of residue definition: X12326349 expressed as fenpicoxamid					
Matrix type	**Method Type**	**Method No.**	**Method LOQ**	**Method Principle**	**Author(s), year / missing / EU agreed**
Milk, eggs, muscle, fat, kidney, liver	Primary/ Confirmatory	130712	0.01 mg/kg	LC-MS/MS	Garcia-Alix, M., 2014, EU agreed
	ILV		0.01 mg/kg	LC-MS/MS	Lindner M., Grewe, D., 2014, EU agreed
	Primary/ Confirmatory (Multi-residue)	120998	0.01 mg/kg	LC-MS/MS	Lindner, M., Giesau, A., 2013, EU agreed
	ILV (Multi-residue)		0.01 mg/kg	LC-MS/MS	Amic, S., 2013, EU agreed

Table 5.3-5: Statement on extraction efficiency

	Method for products of animal origin
Required, available from:	Garcia-Alix, M., 2014, EU agreed Extraction solvent used in the analytical method is identical to that used in the animal (ruminant) metabolism study (Rotondaro, Y., Adelfinskaya, Y., 2013): acetonitrile/water/phosphoric acid (75/25/0.1, v/v/v)

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 Fenpicoxamid in soil is given in the following tables. This study has already been evaluated during the EU approval process of the active substance (EFSA 2018).

Table 5.3-6: Validated methods for soil

	Component of residue definition: Fenpicoxamid and X642188			
Method type	**Method No.**	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Primary/ Confirmatory	131045	0.05 mg/kg	LC-MS/MS	Lindner, M.; Giesau A., 2014, EU agreed

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 Fenpicoxamid in surface and drinking water is given in the following tables. These studies have already been evaluated during the EU approval process of the active substance (EFSA 2018).

Table 5.3-7: Validated methods for water

Component of residue definition: Fenpicoxamid and X642188					
Matrix Type	**Method Type**	**Method No.**	**Method LOQ**	**Method Principle**	**Author(s), year / missing / EU agreed**
Drinking water, Surface water	Primary/ Confirmatory	131046	0.05 µg/L	LC-MS/MS	Austin, R., Turner, R., 2014, EU agreed
	ILV		0.05 µg/L	LC-MS/MS	Lindner, M., Giesau, A., 2014b, EU agreed

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 Fenpicoxamid in air is given in the following tables. This study has already been evaluated during the EU approval process of the active substance (EFSA 2018).

Table 5.3-8: Validated methods for air

	Component of residue definition: Fenpicoxamid			
Method type	**Method No.**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Primary/ Confirmatory	120681	0.5 µg (1.39 µg/m^3)	LC-MS/MS	Bacher, R., 2012, EU agreed

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 Fenpicoxamid in body fluids and tissues is given in the following table. This study has already been evaluated during the EU approval process of the active substance (EFSA 2018).

Table 5.3-9: Methods for body fluids

Component of residue definition: Fenpicoxamid				
Method type	**Method No.**	**Method LOQ**	**Method Principle**	**Author(s), year / missing**
Primary/ Confirmatory	120682	0.05 mg/L	LC-MS/MS	Göcer, M., 2012, EU agreed

Table 5.3-10: Methods for body tissues

Component of residue definition: Fenpicoxamid				
Method type	**Method No.**	**Method LOQ**	**Method Principle**	**Author(s), year / missing**
Primary/ Confirmatory	130712	0.01 mg/kg	LC-MS/MS	Garcia-Alix, M., 2014, EU agreed

5.3.2.8 Other studies/ information

Not required.

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

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

legal residue definition is not identical.
The proposed residue definition for enforcement in plant and animal commodities given in the EFSA Scientific Report (2007) is summarised below.
The EFSA's recent reasoned opinion on the review of the existing MRLs for prothioconazole according to Article 12 of Regulation (EC) N° 396/2005 (EFSA Journal 2014; 12(5):3689) proposed the residue definition for enforcement in animal products as prothioconazole-desthio (sum of isomers) for all livestock matrices.

Matrices	Residue definition		Reference
Food of plant origin	Risk assessment	Sum of prothioconazole-desthio and all metabolites containing the 2-(1-chlorocyclopropyl)-3-(2-chloro-phenyl)-2-hydroxypropyl-2H-1,2,4-triazole moiety) expressed as prothioconazole-desthio.	EFSA Scientific Report (2007) 106, 1-98
	Monitoring	Prothioconazole-desthio (sum of isomers)	
Food of animal origin	Risk assessment	Sum of prothioconazole-desthio and all metabolites containing the 2-(1-chlorocyclopropyl)-3-(2-chloro-phenyl)-2-hydroxypropyl-2H-1,2,4-triazole moiety) expressed as prothioconazole-desthio.	
	Monitoring	Sum of prothioconazole-desthio and its glucuronide conjugate, expressed as prothioconazole-desthio*	

* in EFSA Journal 2014; 12(5):3689, the enforcement residue definition is proposed as prothioconazole-desthio (sum of isomers) only.

Table 5.3-11: 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	Prothioconazole-desthio (sum of isomers)	0.01 mg/kg	Reg (EU) 2019/552
Plant, high acid content		0.01 mg/kg	Reg (EU) 2019/552
Plant, high protein/high starch content (dry commodities)		0.01 mg/kg Wheat 0.1 mg/kg	Reg (EU) 2019/552
Plant, high oil content		0.01 mg/kg	Reg (EU) 2019/552
Muscle	prothioconazole-desthio (sum of isomers)	0.01 mg/kg	Reg (EU) 2019/552
Milk		0.01 mg/kg	Reg (EU) 2019/552
Egg		0.01 mg/kg	Reg (EU) 2019/552
Liver, kidney		0.5 mg/kg 0.1 mg/kg (poultry)	Reg (EU) 2019/552
Fat		0.02 mg/kg 0.01 mg/kg (poultry)	Reg (EU) 2019/552
Soil (Ecotoxicology)	Prothioconazole and prothioconazole-desthio	0.05 mg/kg	Common Limit EFSA Journal 2007;106:98 NOEC = 1.33 mg a.s./kg dsw, *E.foetida* NOEC = 1 mg p.m./kg dsw, *E. foetida*
Drinking water (Human toxicology)	Prothioconazole and prothioconazole-desthio	0.1 µg/L	Common Limit, Directive 2006/118/EC
Surface water (Ecotoxicology)	Prothioconazole and prothioconazole-desthio	NOEC = 0.308 mg a.s./L., *O.mykiss* (prothioconazole)	EFSA Journal 2007;106:98

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
		NOEC = 3.34 µg p.m./L, *O.mykiss*	
Air	Prothioconazole and prothioconazole-desthio	60 µg/m^3 (prothioconazole) 3 µg/m^3 (prothioconazole-desthio)	EFSA Scientific Report (2007) 106, 1-98 AOEL, prothioconazole: 0.2 mg/kg bw/d AOEL, Prothioconazole-desthio: 0.01 mg/kg bw/d
Body tissues (meat or liver)	prothioconazole-desthio	0.1 mg/kg	Common Limit, SANCO/825/00 rev. 8.1, Reg (EU) 283/2013
Body fluids (urine or blood)	prothioconazole-desthio	0.05 mg/L	Common Limit, SANCO/825/00 rev. 8.1, Reg (EU) 283/2013

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 in plant matrices is given in the following tables.

Table 5.3-12: 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 (sum of isomers)					
Matrix type	**Method No.**	**Method Type**	**Method LOQ**	**Method Principle**	**Author(s), year / missing / EU agreed**
High water content, high acid content, high oil content, high protein/high starch content (dry)	0086/M003	Primary (Multi-residue)	0.02 mg/kg (wheat, barley (grain), canola (seed), tomato, orange (fruit) 0.05 mg/kg (wheat, barley forage, straw)	GC-MS	Weeren, R.D., Pelz, S., 2000, EU agreed
		ILV (Multi-residue)	0.02 mg/kg (cereal grain) 0.05 mg/kg (cereal straw and forage)	GC-MS	Class, Th., 2001, EU agreed
	01300/M018*	Primary/ Confirmatory (Multi-residue)	0.01 mg/kg	LC-MS/MS (2 MRMs)	Chambers, J., Jarrett, H. 2014, dRAR 2018*
		ILV (Multi-residue)	0.01 mg/kg	LC-MS/MS (2 MRMs)	Thies, S., 2014, dRAR 2018*

*A new plant enforcement method with corresponding ILV was submitted by Bayer and is being evaluated within the framework of the active substance renewal.

Table 5.3-13: Statement on extraction efficiency

	Method for products of plant origin
Required, available from:	Haas, M. , 2001, EU agreed Desmaris, F., 2015, dRAR 2018

The extraction efficiency of the residue method in cereals and rape (Heinemann, O.) was tested using aged

radioactive residues from the metabolism study following spray application of 14C-prothioconazole on wheat (Haas, M.). 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, but will also be re-evaluated at the active substance renewal. The extraction efficiency of the new enforcement method was evaluated in Desmaris, F. 2015 and is under evaluation as part of the active substance renewal.

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

Table 5.3-14: Validated methods for food and feed of animal origin (if appropriate)

Component of residue definition: prothioconazole-desthio (Sum of isomers)					
Matrix type	**Method No.**	**Method Type**	**Method LOQ**	**Method Principle**	**Author(s), year / missing / EU agreed**
Meat, liver, kidney, fat	00655	Primary	0.01 mg/kg	HPLC-MS/MS (1 MRM)	Heinemann, O., 2001b, EU agreed
		ILV	0.01 mg/kg	HPLC-MS/MS	Dubey, L., 2001, EU agreed
	00655/M002*	Confirmatory	0.01 mg/kg	HPLC-MS/MS (2 MRMs)	Freitag, Th., 2007, amended 2013, dRAR 2018*
		ILV	0.01 mg/kg	HPLC-MS/MS	Schwarz, T., Class, T., 2007, dRAR 2018*
Milk	00655	Primary	0.01 mg/kg	HPLC-MS/MS (1 MRM)	Heinemann, O., 2001b, EU agreed
	00655/M001	Primary	0.004 mg/kg	HPLC-MS/MS	Heinemann, O., 2001c, EU agreed
		ILV	0.004 mg/kg	HPLC-MS/MS	Dubey, L., 2001, EU agreed
	00655/M002*	Confirmatory	0.004 mg/kg	HPLC-MS/MS (2 MRMs)	Freitag, Th., 2007, dRAR 2018*
		ILV	0.004 mg/kg	HPLC-MS/MS	Schwarz, T., Class, T., 2007, dRAR 2018*
Milk, meat, liver, kidney, fat, egg	01009*	Primary/ Confirmatory	0.01 mg/kg	HPLC-MS/MS	Billian, P., Wolters, A., 2006, EU agreed; amended Schulte G., Oel D., 2013, dRAR 2018*
		ILV	0.01 mg/kg	HPLC-MS/MS	Bacher, R., 2006, dRAR 2018*

*Several new animal enforcement methods with corresponding ILVs were submitted by Bayer and are being evaluated within the framework of the active substance renewal.

Table 5.3-15: Statement on extraction efficiency

	Method for products of animal origin
Required, available from:	Weber, H., 2001, EU agreed

The extraction efficiency of the residue method in animal matrices (Heinemann, O, 2001) was tested using aged radioactive residues from the goat metabolism study (Weber, H, 2001). In summary, the comparison of the residue analytical method for animal matrices with the 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. The new studies (Freitag, Th., 2007; Billian, P., 2006) also use an acetonitrile/water solvent system. Extraction efficiency will be re-evaluated during active substance renewal.

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

Table 5.3-16: Validated methods for soil

Component of residue definition: Prothioconazole and Prothioconazole-desthio				
Method Type	**Method No**	**Method LOQ**	**Method Principle**	**Author(s), year / missing / EU agreed**
Primary	00086/M038	0.01 mg/kg (Prothioconazole-desthio)	GC-MS	Steinhauer, S., 2001, EU agreed
	00610	0.006 mg/kg (Prothioconazole & prothioconazole-desthio)	HPLC-MS/MS (1 MRM)	Schramel, O., 2000, EU agreed
Confirmatory	00610/M001	0.006 mg/kg (Prothioconazole & prothioconazole-desthio)	HPLC-MS/MS (2^{nd} MRMs)	Brumhard, B., 2005, EU agreed

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 in surface and drinking water is given in the following tables. For the detailed evaluation of new/additional studies refer to Appendix 2.

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

Component of residue definition: Prothioconazole and Prothioconazole-desthio					
Matrix type	**Method No.**	**Method type**	**Method LOQ**	**Method Principle**	**Author(s), year / missing / EU agreed**
Drinking water	00684	Primary	0.1 µg/L (prothiozonazole) 0.05 µg/L (prothiozonazole-desthio)	HPLC-MS/MS (1 MRM)	Sommer, H., 2001, EU agreed
	00684/M001	Confirmatory	0.05 µg/L (Prothioconazole & prothioconazole-desthio)	HPLC-MS/MS (2 MRMs)	Brumhard, B., 2005b, EU agreed
	01387/M002*	Primary/ Confirmatory	0.05 µg/L	HPLC-MS/MS	Krebber, R., Sandau, C., 2015, dRAR 2018*
		ILV	0.05 µg/L	HPLC-MS/MS	Thies, S., 2015, dRAR 2018*
Surface Water	00684	Primary	0.1 µg/L (prothiozonazole) 0.05 µg/L (prothiozonazole-desthio)	HPLC-MS/MS (1 MRM)	Sommer, H., 2001, EU agreed
	00684/M001	Confirmatory	0.05 µg/L (Prothioconazole & prothioconazole-desthio)	HPLC-MS/MS (2 MRMs)	Brumhard, B., 2005b, EU agreed

*A new drinking water enforcement method with corresponding ILV was submitted by Bayer and is being evaluated within the framework of the active substance renewal.

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

An overview on the acceptable methods and possible data gaps for analysis of Prothioconazole and prothioconazole-desthio in air is given in the following tables. For the detailed evaluation of new/additional studies refer to Appendix 2.

Table 5.3-18: Validated methods for air

Component of residue definition: Prothioconazole and Prothioconazole-desthio				
Method Type	**Method No**	**Method LOQ**	**Method Principle**	**Author(s), year / missing / EU agreed**
Primary	00724	15 µg/m^3 (prothioconazole)	HPLC-MS/MS	Maasfeld, W., 2002, EU agreed
	00731	0.6 µg/m^3 (prothioconazole-desthio)	HPLC-MS/MS (1 MRM)	Maasfeld, W., 2002b, EU agreed
Confirmatory	00731/M001	0.3 µg/m^3 (prothioconazole-desthio)	HPLC-MS/MS (2 MRMs)	Anft, T. and Bardel, P., 2005, EU agreed

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

Table 5.3-19: Validated methods for body fluids (blood)

Component of residue definition: prothioconazole-desthio (sum of isomers)				
Method Type	**Method No.**	**Method LOQ**	**Method Principle**	**Author(s), year / missing / EU agreed**
Primary/ Confirmatory	01471*	0.05 mg/L*	LC-MS/MS (2 transition)	Hoeppner, S., 2015, dRAR 2018*

*A body fluids method for prothioconazole-desthio was submitted by Bayer and is being evaluated within the framework of the active substance renewal. Bayer is also planning on including prothioconazole in the method and lowering the LOQ for prothioconazole-desthio to 0.01 mg/L as part of the active substance renewal process.

Table 5.3-20: Validated methods for body tissues

Component of residue definition: prothioconazole-desthio (sum of isomers)				
Method Type	**Method No.**	**Method LOQ**	**Method Principle**	**Author(s), year / missing / EU agreed**
Primary/ Confirmatory	00655	0.01 mg/kg	HPLC-MS/MS	Heinemann, O., 2001b, EU agreed

5.3.3.8 Other studies/ information

N/A

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 5.1.1/1	Frank, A.	2015	Analytical Method and Validation for the Determination of XDE-777 and Prothioconazole in GF-3307 and GF-3310 Formulations DAS Report No.DAS-AM-G-14-24 Dow AgroSciences, LLC GLP/GEP (Y/N): Yes Published (Y/N): No	N	DAS	N
KCP 5.1.1/2	Moe, T	2015	Analytical Method and Validation for the Determination of the Desthio Impurity in GF-3307 Formulation DAS Report No.DAS-AM-G-14-38 Dow AgroSciences, LLC GLP/GEP (Y/N): Yes Published (Y/N): No	N	DAS	N
KCP 5.1.1/3	Nelson, R.M.	2018	Analytical Method and Validation for the Determination of Toluene in GF-3307 Formulation DAS Report No.DAS-AM-G-15-44 Dow AgroSciences, LLC GLP/GEP (Y/N): Yes Published (Y/N): No	N	DAS	N
KCP 5.1.1/4	Hofer, C.	2017	Analytical Method and Validation for the Determination of Potential Degradates in GF-3307 DAS Report No.DAS-AM-G-170058 Dow AgroSciences, LLC GLP/GEP (Y/N): Yes Published (Y/N): N	N	DAS	N
KCP 5.1.1/4		2021	Supplemental data: DATA CRD Response to GF-3307 Method Precision	N	DAS	N
KCP 5.1.1/5	Frank, A.	2016	Analytical Method and Validation for the Determination of Potential Degradates in GF-3307 DAS Report No.DAS-AM-G-15-1 Dow AgroSciences, LLC GLP/GEP (Y/N): Yes Published (Y/N): N	N	DAS	N
KCP 5.1.1/6	Frank, A.	2015	Analytical Method and Validation for the Determination of Potential Degradates in GF-3307	N	DAS	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?
			DAS Report No.DAS-AM-G-14-35 Dow AgroSciences, LLC GLP/GEP (Y/N): Yes Published (Y/N): N			
KCP 5.2.2/02 (KCA 6.3.1/02-8)	Eversfield, S.	2019	Residues of Fenpicoxamid in Barley and its Processed Commodities at Harvest Following Two Applications of GF-3307 – Europe – 2018 Report No. S18-00056, DAS Study ID 170192 Eurofins Agroscience Services, Wilson, Derbyshire, DE73 8AG, UK GLP/GEP (Y/N): Yes Published (Y/N): No	N	DAS	N
KCP 10.2.1/1	Dinehart, S.	2014, revised 2017, Final report addendum 2019	GF-3307: Acute Toxicity to the Rainbow Trout, Oncorhynchus mykiss, Determined Under Static-Renewal Test Conditions DAS Report No.140479 ABC Laboratories, Inc., 7200 E. ABC Lane Columbia, Missouri 65202, USA GLP/GEP (Y/N): Yes Published (Y/N): No	Y	DAS	N
KCP 10.2.1/2	Dinehart, S.	2018	GF-3307: Acute Toxicity to the Rainbow Trout, Oncorhynchus mykiss, Determined Under Flow-Through Test Conditions xxxxxxxxxx GLP/GEP (Y/N): Yes Published (Y/N): No	Y	DAS	N
KCP 10.2.1/3	Goudie, O.	2016a	GF-3308: Acute Toxicity to the Rainbow Trout, Oncorhynchus mykiss, Determined Under Flow-Through Test Conditions xxxxxxxxxxx GLP/GEP (Y/N): Yes Published (Y/N): No	Y	DAS	Y evaluated in the dRR for GF-3308 on 24.08.2022
KCP 10.2.1/4	Goudie, O.	2016b	GF-3308: Acute Toxicity to the Cladoceran, Daphnia magna, Determined Under Static Renewal Test Conditions DAS# 160102 ABC Laboratories, Inc., Columbia, Missouri, USA GLP/GEP (Y/N): Yes Published (Y/N): No	N	DAS	Y evaluated in the dRR for GF-3308 on 24.08.2022

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	Vertebrate study Y/N	Owner	**Previously used Y/N** If yes, for which data point?
KCP 10.2.1/5	Goudie, O.J.	2018	X1642188 (a metabolite of XDE-777): Acute Toxicity Test to Cladoceran, Daphnia magna, Determined Under Flow-Through Test Conditions DAS# 180562 ABC Laboratories, Inc., Columbia, Missouri, USA GLP/GEP (Y/N): Yes Published (Y/N): No	N	DAS	Y evaluated in the dRR for GF-3308 on 24.08.2022
KCP 10.2.1/6	Goudie, O.J	2020	GF-3307: A 48-Hour Static-Renewal Acute Toxicity Test with the Cladoceran (Daphnia magna) DAS Report No. 191366 Eurofins EAG Agroscience, LLC, Easton, Maryland, USA GLP/GEP (Y/N): Yes Published (Y/N): No	N	DAS	Y evaluated in the dRR for GF-3308 on 24.08.2022
KCP 10.2.1/7	Goudie, O.	2021	GF-2925: A Static-Renewal Acute Toxicity to the Cladoceran (Daphnia magna) DAS# 202284 Eurofins EAG Agroscience, LLC, Easton, MD, USA GLP/GEP (Y/N): Yes Published (Y/N): No	N	DAS	Y evaluated in the dRR for GF-3308 on 24.08.2022
KCP 10.2.1/8	Hadsell, R. L., Hoover, E.	2014, revised 2018	GF-3307: Acute Toxicity to the Cladoceran, Daphnia magna, Determined Under Static-Renewal Test Conditions DAS Report No.140489 ABC Laboratories, Inc., Columbia, Missouri, USA GLP/GEP (Y/N): Yes Published (Y/N): No	N	DAS	Y evaluated in the dRR for GF-3308 on 24.08.2022
KCP 10.2.1/9	Hicks, S	2014, Final report addendum 2020	GF-3307: Growth Inhibition Test with the Unicellular Green Alga, Pseudokirchneriella subcapitata DAS Report No.140491 ABC Laboratories, Inc., 7200 E. ABC Lane Columbia, Missouri 65202, USA GLP/GEP (Y/N): Yes Published (Y/N): No	N	DAS	N

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Vertebrate study Y/N**	**Owner**	**Previously used Y/N** If yes, for which data point?
KCP 10.2.1/10	Hughes, J.P.	2018a	X12019520 (a metabolite of XDE-777): Acute Toxicity to the Rainbow Trout, Oncorhynchus mykiss, Determined Under Static-Renewal Test Conditions xxxxxxxxxxxx GLP/GEP (Y/N): Yes Published (Y/N): No	Y	DAS	Y evaluated in the dRR for GF-3308 on 24.08.2022
KCP 10.2.1/11	Hughes, J.P.	2018b	X12446477 (metabolite of XDE-777): Acute Toxicity to the Rainbow Trout, Oncorhynchus mykiss, Determined Under Static-Renewal Test Conditions xxxxxxxxxxxx GLP/GEP (Y/N): Yes Published (Y/N): No	Y	DAS	Y evaluated in the dRR for GF-3308 on 24.08.2022
KCP 10.2.2/1	Beasley, J.	2018	X1642188 (a metabolite of XDE-777): Chronic Toxicity in Whole Sediment to Freshwater Midge, Chironomus riparius, Using Spiked Sediment DAS# 180563 ABC Laboratories, Inc., Columbia, Missouri, USA GLP/GEP (Y/N): Yes Published (Y/N): No	N	DAS	Y evaluated in the dRR for GF-3308 on 24.08.2022
KCP 10.2.2/2	Dinehart, S.	2019	X642188 (a metabolite of XDE-777): A Prolonged Sediment Toxicity Test with Lumbriculus variegatus Using Spiked Sediment DAS Study No. 180639 Eurofins EAG Agroscience, LLC, Columbia, Missouri, USA GLP/GEP (Y/N): Yes Published (Y/N): No	N	DAS	Y evaluated in the dRR for GF-3308 on 24.08.2022
KCP 10.2.2/3	Leak, T.	2018	X12335723 (a metabolite of XDE-777): Chronic Toxicity in Whole Sediment to Freshwater Midge, Chironomus riparius, Using Spiked Sediment DAS# 180564 ABC Laboratories, Inc., Columbia, Missouri, USA GLP/GEP (Y/N): Yes Published (Y/N): No	N	DAS	Y evaluated in the dRR for GF-3308 on 24.08.2022
KCP 10.2.3/2	Brüggemann, M., Böhmer, W., Kosak, L	2020	GF-3307: Population Effects Study in an Indoor Aquatic Microcosm with Daphnia magna DAS Study No. 181382 Fraunhofer Institute for Molecular Biology and Applied Ecology (IME), Schmallenberg, Germany GLP/GEP (Y/N): Yes Published (Y/N): No	N	DAS	N

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Vertebrate study Y/N**	**Owner**	**Previously used Y/N** If yes, for which data point?
KCP 10.2.3/3	Hicks, S.	2017	XDE-777: Population Effects Study in an Indoor Aquatic Microcosm with Daphnia magna DAS# 160125 ABC Laboratories, Inc., 7200 E. ABC Lane Columbia, Missouri 65202, USA GLP/GEP (Y/N): Yes Published (Y/N): No	N	DAS	Y evaluated in the dRR for GF-3308 on 24.08.2022
KCP 10.3.1.2/1	Oberrauch, S.	2018	GF-3307 - Honey Bee (*Apis mellifera* L.) 22 Day Larval Toxicity Test (Repeated Exposure) DAS# 171043 Institut für Biologische Analytik und Consulting IBACON GmbH, 64380 Rossdorf, Germany GLP/GEP (Y/N): Yes Published (Y/N): No	N	DAS	N
KCP 10.3.1.2/2	Verge, E., ~~Kastel, A.~~	2018	GF-3307 - Assessment of Effects on the Adult Honey Bee, *Apis mellifera* L., in a 10 Day Chronic Feeding Test under Laboratory Conditions DAS# 170077 Eurofins Agroscience Services EcoChem / Eurofins Agroscience Services Ecotox GmbH GLP/GEP (Y/N): Yes Published (Y/N): No	N	DAS	N
KCP 10.3.1.5/1	Kleinhenz, M.	2018	GF-3307 (Fenpicoxamid + Prothioconazole): Brood Development of the Honeybee (*Apis mellifera* L.) in a Semi-Field Tunnel Study in *Phacelia tanacetifolia* in Germany 2017 DAS Report No. 170673 Eurofins Agroscience Services EcoChem GmbH / Eurofins Agroscience Services Ecotox GmbH, Niefern-Öschelbronn, Germany GLP: Yes Published: No	N	DAS	N
KCP 5.3.2.2/05	Senciuc, M.	2021	Cross-Validation – Comparing Amounts of Fenpicoxamid Extracted from Samples of Barley Grain, Oil Seed Rapeseed and Banana with Incurred Residues using 3 Different Solvent Systems Lab Study No S20-01536; Sponsor Study No. 200456 EAG Laboratories GmbH, Ulm, Germany GLP/GEP (Y/N): Yes Published (Y/N): No	N	DAS	Y evaluated in the dRR for GF-3308 on 24.08.2022
KCP 5.3.3.2/03	Chambers, J., Jarrett, H.	2014	Modification M018 of the analytical method 01300 (based on QuEChERS method) for the determination of residues of prothioconazole-desthio and iprovalicarb in wheat grain, grapes, rapeseed, dry bean and cucumber Battelle UK Ltd., Chelmsford, Essex, United Kingdom Bayer CropScience,	N	BCS*	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?
			Report No.: VC/13/017, Edition Number: M-498384-01-1 Method Report No.: VC/13/017 Date: 2014-09-30 GLP/GEP (Y/N): Yes Published (Y/N): No			
KCP 5.3.3.2/04	Thies, S.	2014	Amendment no.2 to study 2014/0110/01 - Independent laboratory validation of BCS method 01300/M018 (based on "QuEChERS" method) for the determination of residues of prothioconazole-desthio and iprovalicarb in/on plant matrices by LC/MS/MS Currenta GmbH & Co. OHG, Leverkusen, Germany Bayer CropScience, Report No.: 2014/0110/01, Edition Number: M-508116-03-1 Date: 2014-12-17 GLP/GEP (Y/N): Yes Published (Y/N): No	N	BCS*	N
KCP 5.3.3.2/06	Desmaris, F.	2015	Amendment no. 1 to the final report - Cross validation of extraction methods for the determination of residues of prothioconazole-desthio in plant material by HPLC-MS/MS Bayer S.A.S., Bayer CropScience, Lyon, France Bayer CropScience, Report No.: MR-15/117, Edition Number: M-536877-02-1 Method Report No.: MR-15/117 Date: 2015-10-26 ...Amended: 2015-10-27 GLP/GEP (Y/N): Yes Published (Y/N): No	N	BCS*	N
KCP 5.3.3.3/02	Freitag, Th..	2007 amended 2013	Amendment No. 1 to report no: MR-06/199 - Analytical method 00655/M002 for the determination of residues of JAU6476-desthio, JAU6476-3-hydroxy-desthio and JAU6476-4-hydroxy-desthio in/on matrices of animal origin by HPLC-MS/MS Method no. 00655/M002, Report no. MR-06/199 Bayer CropScience GLP/GEP (Y/N): Yes Published (Y/N): No	N	BCS*	N

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Vertebrate study Y/N**	**Owner**	**Previously used Y/N** If yes, for which data point?
KCP 5.3.3.3/03	Schwarz, T., Class, T.	2007	Independent laboratory validation of Bayer CropScience method 00655/M002 for the determination and confirmation of residues of JAU6476-desthio, JAU6476-3-hydroxy-desthio and JAU6476-4-hydroxy-desthio in/on matrices of animal origin by HPLC-MS/MS Bayer CropScience Method no. 00655/M002, Report no. P/B 1226 G Bayer CropScience GLP/GEP (Y/N): Yes Published (Y/N): No	N	BCS*	N
KCP 5.3.3.3/05	Schulte, G., Oel, D.	2006, amended 2014	Analytical method 01009 for the determination of residues of JAU 6476-desthio,JAU 6476-3-hydroxy-desthio, JAU 6476-4-hydroxy-desthio, JAU 6476-3,4- dihydroxy-desthio, and JAU 6476-4,5-dihydroxy-desthio in/on matrices of animal origin by ... Bayer CropScience, Report No.: M-279725-03-1, Edition Number: M-279725-03-1 Method Report No.: MR-06/120 Date: 2006-10-26 **...**Amended: 2014-06-18 GLP/GEP (Y/N): Yes Published (Y/N): No	N	BCS*	N
KCP 5.3.3.3/06	Bacher, R.	2006	Independent laboratory validation of Bayer CropScience method No. 01009 for the determination of residues of JAU 6476-desthio, JAU 6476-3-hydroxy-desthio, JAU 6476-4-hydroxy-desthio, JAU 6476-3,4-dihydroxydesthio, and JAU 6476-4,5-dihydroxy-desthio in/on matrices of animal origin by HPLC-MS/MS report no. P/B 1111G, study no. P613060597, ASB2011-13494 GLP: Yes Published: No BVL-2283225, BVL-2295523, ASB2011-13494	N	BCS*	N
KCP 5.3.3.5/03	Krebber, R., Sandau, C.	2015	Modification M002 of analytical method 01387 for the determination of various pesticides in drinking and surface water by HPLC-MS/MS TF- BCS-Adama Agan, Report No.: MR-15/025, Edition Number: M-526061-01-1	N	TF- BCS*-Adama Agan	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?
			Date: 2015-06-01 GLP/GEP (Y/N): Yes Published (Y/N): No			
KCP 5.3.3.5/04	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, Leverkusen, Germany TF- BCS-Adama Agan, Report No.: 2015/0034/01, Edition Number: M-536990-01-1 Date: 2015-10-27 GLP/GEP (Y/N): Yes Published (Y/N): No	N	TF- BCS*-Adama Agan	N
KCP 5.3.3.7/01	Hoeppner, S.	2015	Validation of the BCS analytical method 01471 for the determination of prothiconazole-desthio in body fluid by HPLC-MS/MS Currenta GmbH & Co. OHG, Leverkusen, Germany Bayer CropScience, Report No.: M-535874-02-1, Edition Number: M-535874-02-1 Method Report No.: 2015/0047/01 Date: 2015-10-06 **...**Amended: 2015-11-11 GLP/GEP (Y/N): Yes Published (Y/N): No	N	BCS*	N
~~KCA 6.10.1/1 KCP 10.3.1.6~~	~~Appeltauer, A~~	~~2021~~	~~Determination of Residues of Fenpicoxamid and Prothioconazole in Nectar, Pollen and Plants of Winter Oilseed Rape after One Application of GF-3307 in a Semi-Field Residue Study in Central and Southern Europe in 2020. Eurofins Agroscience Services Ltd DAS Report No.: 200670 GLP/GEP (Y/N): Y Published (Y/N): N~~	~~N~~	~~Corteva Agriscience~~	not evaluated in B7 and B9; not necessary to support the uses of GF-3307
KCP 10.3.1.6/1	Gonsoir, G.	2021	Assessment of Side-Effects on the GF-3307 (Fenpicoxamid and Prothioconazole): Brood Development of the Honey Bee (Apis mellifera L.) in a Colony Feeding Test in Germany 2020 DAS Report No. 200660	N	Corteva Agriscience	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?
			Eurofins Agroscience Services EcoChem GmbH / Eurofins Agroscience Services Ecotox GmbH, Niefern-Öschelbronn, Germany GLP: Yes Published: No			
KCP 10.3.1.1.1/3	Cornement, M., Morgenthal, K.	2022a	XDE-777 TGAI - Acute Oral and Contact Toxicity to Bumble Bees (Bombus terrestris) under Laboratory Conditions Corteva Report No. 201076 IES GLP/GEP (Y/N): Yes Published (Y/N): No	N	Corteva Agriscience	N
KCP 10.3.1.1.1/4	Cornement, M., Morgenthal, K.	2022	GF-3307 - Acute Oral and Contact Toxicity to Bumble Bees (Bombus terrestris) under Laboratory Conditions Corteva Report No. 201075 IES GLP/GEP (Y/N): Yes Published (Y/N): No	N	Corteva Agriscience	N
KCA 6.3.1/01	White, T.	2016	Determination of Residues of XDE-777 And Pyraclostrobin, After Two Applications of GF-3309 To Spring And Winter Wheat, At 5 Sites In Northern Europe And 5 Sites In Southern Europe, 2015 Report No. S15-02628, DAS Study ID 150650 Eurofins AgroScience Services, Wilson, Derbyshire DE73 1AG, UK GLP/GEP (Y/N): Yes Published (Y/N): No	N	DAS/Corteva Agriscience	Y evaluated in the dRR for GF-3308 on 24.08.2022
KCA 6.3.1/02	Eversfield, S.	2016	Determination of Residues of XDE-777 And Pyraclostrobin After Two Applications of GF-3312 And After Two Applications of GF-2925 In Winter Wheat And Spring Wheat At 4 Sites In Northern Europe And 4 Sites In Southern Europe In 2014 Report No. S14-01569, DAS Study ID 140648 Eurofins Agroscience Services, Wilson, Derbyshire, DE73 8AG, UK GLP/GEP (Y/N): Yes Published (Y/N): No	N	DAS/Corteva Agriscience	Y evaluated in the dRR for GF-3308 on 24.08.2022
KCA 6.3.1/03	Eversfield, S.	2016	Determination of Residues of XDE-777 and Prothioconazole after Two Applications of GF-3307 and after Two Applications of GF-3310 in Winter Wheat and Spring Wheat at 4 sites in Northern Europe and 4 sites in Southern Europe in 2014, Report No. S14-01568, DAS Study ID 140649, Eurofins Agroscience Services Ltd	N	DAS/Corteva Agriscience	**Y for XDE-777** evaluated in the dRR for GF-3308 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?
			GLP, Unpublished			24.08.2022; **N for PTZ**
KCA 6.3.1/04	White, T.	2016	Determination of Residues of XDE-777 and Prothioconazole after Two Applications of GF-3307 to Spring and Winter Wheat, at 5 sites in Northern Europe and 5 sites in Southern Europe, 2015, Report No. S15-02629, DAS Study ID 150649, Eurofins Agroscience Services Ltd GLP, Unpublished	N	DAS/Corteva Agriscience	**Y for XDE-777** evaluated in the dRR for GF-3308 on 24.08.2022; **N for PTZ**
KCA 6.3.1/05	Semrau J, Thomas B	2019	Residues of Fenpicoxamid and Prothioconazole in Wheat at Harvest Following One Application of GF-3307 – Southern and Northern Europe – 2018. Report No.S18-01566, DAS Study ID 180126 Eurofins Agroscience Services Ltd GLP, Unpublished	N	DAS	N
KCA 6.3.1/06	Semrau, J., Thomas, B.	2019	Residues of Fenpicoxamid and Prothioconazole in Barley at Interval and at Harvest Following Two Applications of GF-3307 – Southern and Northern Europe – 2017 and 2018. Report No. S17-01904/ 170191. Eurofins AgroScience Services GmbH, Carl-Goerdeler-Weg 5 21684 Stade, Germany GLP/GEP (Y/N): Yes Published (Y/N): No	N	DAS	N
KCA 6.3.1/08	Eversfield, S.	2019	Residues of Fenpicoxamid in Barley and its Processed Commodities at Harvest Following Two Applications of GF-3307 – Europe – 2018. Report No. S18-00056/ 170192 Eurofins Agroscience Services, Wilson, Derbyshire, DE73 8AG, UK GLP/GEP (Y/N): Yes Published (Y/N): No	N	DAS	N
KCA 6.3.1/07	Semrau, J., Kühnel S.	2019	Residues of Fenpicoxamid and Prothioconazole in Barley at Harvest Following One Application of UNIVOQ – Southern and Northern Europe – 2018. Semrau, J., Kühnel S. 2019. Report no. S18-01567/ 180128. Eurofins AgroScience Services GmbH, Carl-Goerdeler-Weg 5 21684 Stade, Germany GLP/GEP (Y/N): Yes Published (Y/N): No	N	DAS	N

*Letter of Access is provided in Part A for Bayer CropScience data

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
KCA 4.1.1 (a)/1	Hamilton T	2013	Analytical Method and Validation for the Determination of Active Ingredient in XDE-777 Technical by Liquid Chromatography The Dow Chemical Company DAS Report No.: ML AL-2013-012856 GLP/GEP (Y/N): Y Published (Y/N): N	N	DAS
KCA 4.1.1 /2	Kerbleski HK Hamilton TD Birk KH Zhang L	2013	Analytical Method and Validation for the Determination of Active Ingredient and Impurities in XDE-777 Technical by Liquid Chromatography The Dow Chemical Company DAS Report No.: ML AL-2013-005479 GLP/GEP (Y/N): Y Published (Y/N): N	N	DAS
KCA 4.1.1 /3	Crispin TA Hamilton TD	2013	Analytical Method and Validation for the Determination of Residual Solvents and Process Impurities in XDE-777 Technical by Gas Chromatography The Dow Chemical Company DAS Report No.: ML AL-2013-005805 GLP/GEP (Y/N): Y Published (Y/N): N	N	DAS
KCP 5.1.1/1	Speak T	2012	Analytical Method for the Determination of XDE-777 in GF-2925 Dow AgroSciences (NZ) Ltd DAS Report No.: DAS-AM-G-12-19 GLP/GEP (Y/N): Y Published (Y/N): N	N	DAS
KCP 5.2.2/01	Watson, G.	2012	XDE-777 and its Metabolite X642188 – Validation of the Method for the Determination of Residues of XDE-777 and its Metabolite X642188 in Crops by LC-MS/MS Eurofins Agroscience Services Ltd DAS Report No.: 120615 GLP/GEP (Y/N): Yes Published (Y/N): No	N	DAS
KCP 5.2.2/03 (KCA 6.4.2/01)	Rawle NW	2013	Data generation method for XDE-777 Livestock Feeding Study: Magnitude of Residue in Milk, Muscle, Liver, Kidney and Fat of Lactating Dairy Cattle xxxxxxxxxxx	Y	DAS

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Vertebrate study Y/N**	**Owner**
			GLP/GEP (Y/N): Y Published (Y/N): N		
KCP 5.2.2/04	Li, Q., Hasting, M., Slinkard, E.W.	2015	Method Validation Study for the Determination of XDE-777 and Its Metabolites in Soil by Liquid Chromatography with Tandem Mass Spectrometry Dow AgroSciences LLC, Indianapolis, Indiana, USA DAS Report No.: 141042 GLP/GEP (Y/N): Y Published (Y/N): N	N	DAS
KCP 5.2.3/01	Heinemann, O.	2000	Analytical determination of residues of JAS 6476 and desthio-JAU 6476 in/on cereals by HPLC/MS/MS Method No. 00598; M-028457-01-1 Bayer AG, Leverkusen, Germany, Bayer CropScience GLP/GEP (Y/N): Yes Published (Y/N): No	N	BCS*
KCP 5.2.3/02	Heinemann, O.	2000b	Analytical determination of residues of JAU6476 and JAU6476-desthio in/on cereals and canola by HPLC-MA/MA (method modification 00598/M001) Method No. 00598/M001; M-047681-01-1 Bayer AG, Leverkusen, Germany, Bayer CropScience GLP/GEP (Y/N): Yes Published (Y/N): No	N	BCS*
KCP 5.2.3/03	Heinemann, O.	2001	Analytical determination of residues of JAU6476-sulfonic acid and JAU6476-desthio in/on cereals and canola by HPLC-MS/MS; Method No. 00647 Method No. 00647; M-047681-01-1 Bayer AG, Leverkusen, Germany, Bayer CropScience GLP/GEP (Y/N): Yes Published (Y/N): No	N	BCS*
KCP 5.2.3/04	Heinemann, O.	2001b	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 Method-No. 00655, Report No.: 00655 Bayer AG, Leverkusen, Germany, Bayer CropScience GLP/GEP (Y/N): Yes Published (Y/N): No	N	BCS*
KCP 5.2.3/05	Heinemann, O.	2001c	Analytical determination of residues of JAU6476-3-hydroxy-desthio, JAU6476-4-hydroxy-desthio, and JAU6476-desthio in milk by HPLC-MS/MS (00655/M001) Method-No. 00655/M001, Report No.: MR-170/01	N	BCS*

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
			Bayer AG, Leverkusen, Germany, Bayer CropScience GLP/GEP (Y/N): Yes Published (Y/N): No		
KCP 5.3.2.2/01	Chambers, J., Jarrett H.	2013	Independent Laboratory Validation: XDE-777 and X641288 Residue Determination in Crops (Revision) Battelle UK Ltd DAS Report No.: 120951 GLP/GEP (Y/N): Yes Published (Y/N): No	N	DAS
KCP 5.3.2.2/02	Lindner M Giesau A	2013	Validation of a Multi-residue Method Following the QuEChERS Sample Preparation Technique for the Determination of XDE-777 and Its Metabolite X642188 in Matrices of Plant and Animal Origin Eurofins Agroscience Services Ltd DAS Report No.: 120998 GLP/GEP (Y/N): Y Published (Y/N): N	N	DAS
KCP 5.3.2.2/03	Amic S	2013	Independent Laboratory Validation of a Multi-residue Method Following the QuEChERS Sample Preparation Technique for the Determination of XDE-777 and Its Relevant Metabolite X642188 in Matrices of Plant and Animal Origin Eurofins Agroscience Services Chem SAS DAS Report No.: 130114 GLP/GEP (Y/N): Y Published (Y/N): N	N	DAS
KCP 5.3.2.2/04	Li Q Dixit V	2013	Evaluation of the Extraction Efficiency in Analytical Method - Determination of XDE-777 and Its X642188 Metabolite in Agricultural Commodities Using Liquid Chromatography with Tandem Mass Spectrometry Detection Dow AgroSciences LLC DAS Report No.: 121023 GLP/GEP (Y/N): Y Published (Y/N): N	N	DAS
KCA 6.2.1/1	Ma, M Jackson, U	2013	A NATURE OF THE RESIDUE STUDY WITH [^{14}C]-XR-777 APPLIED TO WHEAT Dow AgroSciences LLC; Research for Hire DAS Report No.: 110334 GLP/GEP (Y/N): Y Published (Y/N): N	N	DAS
KCP 5.3.2.3/01	Garcia-Alix M	2014	Method Validation for the Determination of XDE-777 and Its Metabolite (X12326349) in Animal Matrices CEM Analytical Services	N	DAS

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
			DAS Report No.: 131027 GLP/GEP (Y/N): Y Published (Y/N): N		
KCP 5.3.2.3/02	Lindner M Grewe D	2014	Independent Laboratory Validation of an Analytical Method for the Determination of XDE-777 and its Metabolite X12326349 in Matrices of Animal Origin Eurofins Agrosciences Services DAS Report No.: 130712 GLP/GEP (Y/N): Y Published (Y/N): N	N	DAS
KCA 6.2.3	Rotondaro, S Adelfinskaya, Y	2013	A NATURE OF THE RESIDUE STUDY IN THE RUMINANT WITH [14C]-XR-777 xxxxxxxxxxxx GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KCP 5.3.2.4/01	Lindner M Giesau A	2014	Validation of an Analytical Method for the Determination of Residues of XDE-777 and its Metabolite X642188 in Soil and Sediment Eurofins Agrosciences Services DAS Report No.: 131045 GLP/GEP (Y/N): Y Published (Y/N): N	N	DAS
KCP 5.3.2.5/01	Austin R Turner R	2014	Method Validation Study for the Determination of Residues of XDE-777 and Its Metabolite X642188 in Water by LC-MS/MS Battelle UK Ltd. DAS Report No.: 131046 GLP/GEP (Y/N): Y Published (Y/N): N	N	DAS
KCP 5.3.2.5/02	Lindner M Giesau A	2014b	Independent Laboratory Validation of an Analytical Method for the Determination of XDE-777 and its Metabolite X642188 in Water Eurofins Agrosciences Services DAS Report No.: 130711 GLP/GEP (Y/N): Y Published (Y/N): N	N	DAS
KCP 5.3.2.6/01	Bacher R	2012	The Development and Validation of a Method for the Analysis of XDE-777 in Air PTRL Europe GmbH DAS Report No.: 120681	N	DAS

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			GLP/GEP (Y/N): Y Published (Y/N): N		
KCP 5.3.2.7/01	Göcer M	2012	Development and Validation of an Analytical Method for the Determination of XDE-777 in Body Fluid(s) PTRL Europe GmbH DAS Report No.: 120682 GLP/GEP (Y/N): Y Published (Y/N): N	N	DAS
KCP 5.3.3.2/01	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.: 0086/M033, Date 200-11-20	N	BCS*
KCP 5.3.3.2/02	Class, Th	2001	Independent laboratory validation of DFG method S19 (extended revision) for the determination of residuesof 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	N	BCS*
KCP 5.3.3.2/05	Haas, M.	2001	Extraction efficiency testing of the residue method (00647) for the determination of JAU 6476 residues in spring wheat using aged radioactive residues Bayer AG Report No.: MR-084/01 Date:2001-05-15	N	BCS*
KCP 5.3.3.3/01	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	N	BCS*
KCP 5.3.3.3/04	Billian, P.; Wolters, A.	2006	Analytical method 01009 for the determination of residues of JAU 6476-desthio, JAU 6476-3-hydroxy-desthio, JAU 6476-4-hydroxy-desthio, JAU 6476-3,4-dihydroxy-desthio, and JAU 6476-4,5-dihydroxy-desthio in/on matrices of animal origin by HPLCMS/MS. Method no. 01009, report no. MR-06/120, ASB2010-11620 incl. Amendment no. 1 ASB2013-9506 GLP: Yes Published: No	N	BCS*

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**
			BVL-2283223, BVL-2295522, ASB2010-11620		
KCA 6.2.2/01	Weber, H.;Spiegel, K.	2001	(Phenyl-UL-14C)JAU6476 Absorption, distribution, excretion and metabolism in the lactating goat Bayer AG, Report No.: MR-092/01	N	BCS*
KCP 5.3.3.4/01	Steinhauer, S.	2001	Enforcement method 00086/M038 for the determination of the residues of JAU 6476-desthio in soil - Validation of DFG method S 19 (extended revision) Report No.: 00086/M038 GLP: Yes Published: No BVL-2291543, MET2002-407	N	BCS*
KCP 5.3.3.4/02	Schramel, O.	2000	Residue analytical method 00610 (MR-643/99) for the determination of JAU6476 and the metabolites JAU6476-desthio and JAU6476-S-methyl in soil by HPLC-MS/MS Report Number: 00610 GLP: Yes Published: No BVL-2291544, MET2002-405	N	BCS*
KCP 5.3.3.4/03	Brumhard, B.	2005	Modification M001 of method 00610 for the determination of JAU6476 and the metabolites JAU6476-desthio and JAU6476-S-methyl in soil by HPLCMS/MS. Method no. 00610/M001, report no. MR-183/04, MET2005-358 GLP: Yes Published: No BVL-2283232, BVL-2291546, MET2005-358	N	BCS*
KCP 5.3.3.5/01	Sommer, H.	2001	Enforcement method 00684 for determination of JAU6476 and JAU6476-desthio in drinking and surface water by HPLC-MS/MS Report Number 00684 GLP: Yes Published: No BVL-2291528, MET2002-411	N	BCS*
5.3.3.5/02	Brumhard, B.	2005b	Modification M001 of method 00684 for the determination of JAU6476 and JAU6476-desthio in drinking and surface water by HPLC-MS/MS Method no. 00684/M001, report no. MR-184/04, MET2005-359 GLP: Yes	N	BCS*

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
			Published: No BVL-2283234, BVL-2291531, MET2005-359		
KCP 5.3.3.6/01	Maasfeld, W.	2002	Method for the determination of JAU 6476 in air by HPLC-MS/MS Report Number 00724 Bayer AG, Leverkusen, Germany, Bayer CropScience GLP/GEP (Y/N): Yes Published (Y/N): No	N	BCS*
KCP 5.3.3.6/02	Maasfeld, W.	2002b	Method for the determination of JAU 6476-desthio (SXX-0665) in air by HPLC-MS/MS Report Number 00731 Bayer AG, Leverkusen, Germany, Bayer CropScience GLP/GEP (Y/N): Yes Published (Y/N): No	N	BCS*
KCP 5.3.3.6/03	Anft, T.; Bardel, P.	2005	Modification M001 of method 00731 for the determination of residues of JAU 6476-desthio (SXX 0665) in air by HPLC/MS/MS MR-166/04 ! 00731/M001, P 606 041201, MO-05-001163, M-242870-01-1 GLP: Yes Published: No BVL-2283237, BVL-2291532, MET2005-360	N	BCS*

*Letter of Access is provided in Part A for Bayer CropScience data

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
KCA 6.10.1/1 KCP 10.3.1.6	Appeltauer, A	2021	Determination of Residues of Fenpicoxamid and Prothioconazole in Nectar, Pollen and Plants of Winter Oilseed Rape after One Application of GF-3307 in a Semi-Field Residue Study in Central and Southern Europe in 2020. Eurofins Agroscience Services Ltd DAS Report No.: 200670 GLP/GEP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience

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 GF-3307

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

A 2.1.1.1 Analytical method 1

A 2.1.1.1.1 Method validation

Comments of zRMS:	The study was evaluated in the Registration Report, Part B5 for GF-3308 on 24.08.2022. Summary: The analytical method (reported in Eurofins study no. S12-01537 / Dow AgroSciences study code 120615) used for the residues trials S15-02628 for the determination of fenpicoxamid (XDE-777) and its X642188 metabolite in spring and winter wheat (grains, straw and whole plants) using Liquid Chromatography Mass Spectrometry (LC-MS/MS) were validated with the following LOQ: Fenpicoxamid: 0.01 mg/kg in grain, straw and whole plants. X642188: 0.01 mg/kg in grain, straw and whole plants.

Data Point:	KCA 6.3.1/01
Report author:	White, T
Report year:	2016
Report title:	Determination of residues of XDE-777 and pyraclostrobin, after two applications of GF-3309 to spring and winter wheat, at 5 sites in Northern Europe and 5 sites in Southern Europe, 2015
Report No.:	150650
Testing Facility Report No.:	S15-02628
Method(s) used:	S12-01537 / Dow AgroSciences study number 120615
Guidelines followed in study:	SANCO/3029/99 rev.4
Deviation from current test guidelines:	No
Analytical Performing Laboratory:	Eurofins Agroscience Services Chem Ltd Wilson, Derbyshire, UK
GLP/Officially recognised testing facilities:	Yes
Acceptability/Reliability:	Yes

MATERIALS AND METHODS

Method Principle

Residues of GF-3309, based on the analysis of XDE-777, X642188 and pyraclostrobin, were determined from wheat samples (whole plant, grain, and straw). XDE-777 and X642188 residues were extracted with acetonitrile/water (90/10, v/v) by homogenisation and shaking. Following extract dilution with acetonitrile/water/formic acid (90/10/0.1, v/v/v), the final sample was analysed for XDE-777 and X642188 by liquid chromatography coupled with positive-ion electrospray tandem mass spectrometry (LC-MS/MS). Pyraclostrobin analysis is not summarized here.

RESULTS AND DISCUSSION

For XDE-777, mean recovery values at each fortification concentration were within the acceptance range (mean recovery 70-110%; RSD ≤ 20%) with few exceptions. Mean recovery value at 0.01 mg/kg was higher than 110% but is still considered acceptable since the precision of the assay (%RSD) was less than

20%. RSD value at 5 mg/kg was higher than 20% but still considered acceptable due to only minor deviation (20.9%).
For X642188, mean recovery values at each fortification concentration were within the acceptance range (mean recovery 70-110%; RSD ≤ 20%).
The results obtained are summarised in the following tables.

Table A 1: Recovery results from method validation of XDE-777 (m/z 615/239) using the analytical method

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Wheat, Whole plant	XDE-777	0.01	98	12.9	5	79, 92, 106, 106, 109
Wheat, Whole plant	XDE-777	5.0	81	20.9	5	100, 98, 64, 72, 70
Wheat, Grain	XDE-777	0.01	101	2.1	5	99, 103, 98, 101, 102
Wheat, Grain	XDE-777	0.1	95	4.2	5	100, 90, 95, 97, 92
Wheat, Straw	XDE-777	0.01	112	14.9	6	118, 124, 84, 114, 101, 129
Wheat, Straw	XDE-777	20	103	3.7	7	102, 105, 102, 107, 96, 107, 103

Table A 2: Recovery results from method validation of X642188 (m/z 515/239) using the analytical method

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Wheat, Whole plant	X642188	0.01	98	11.3	5	82, 92, 110, 103, 104
Wheat, Whole plant	X642188	0.50	102	7.0	5	100, 96, 101, 98, 114
Wheat, Grain	X642188	0.01	101	1.1	5	102, 101, 100, 101, 99
Wheat, Grain	X642188	0.10	101	4.4	5	106, 94, 102, 102, 99
Wheat, Straw	X642188	0.01	109	11.7	6	102, 117, 116, 119, 114, 86
Wheat, Straw	X642188	0.1	102	-	1*	102
Wheat, Straw	X642188	1.0	102	4.6	5	104, 105, 103, 94, 105

* While only one fortification was done at 10x LOQ (0.1 mg/kg), five fortifications were done at the 100x LOQ (1.0 mg/kg) to encompass the maximum concentration of observed residues. This is in compliance with SANCO/3029/99 rev.4.

Table A 3: Characteristics for the analytical method used for validation of XDE-777 and X684188 residues in wheat (whole plant, grain and straw)

	XDE-777	X684188
Specificity	m/z 615/239 Quantification m/z 615/515 Confirmation blank value <30% LOQ	m/z 515/239 Quantification m/z 515/124 Confirmation blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting r≥0.99 7 data points	linear regression analysis with 1/x weighting r≥0.99 7 data points
Calibration range	Concentration range of 0.0075-1.0 ng/mL(equivalent sample concentration 0.003- 0.40 mg/kg)	Concentration range of 0.0075-1.0 ng/mL(equivalent sample concentration 0.003-0.40 mg/kg)
Limit of quantitation	LOQ=0.01 mg/kg	LOQ=0.01 mg/kg

CONCLUSION

This method was successfully validated for the determination of XDE-777 and X684188 in wheat (whole plant, grain, and straw).

A 2.1.1.2 Analytical method 2

A 2.1.1.2.1 Method validation

Comments of zRMS:	The study was evaluated in the Registration Report, Part B5 for GF-3308 on 24.08.2022. Summary: The analytical method (reported in Eurofins study no. S12-01537 / Dow AgroSciences study code 120615) used for the residues trials S14-01569 for the determination of fenpicoxamid (XDE-777) and its X642188 metabolite in spring and winter wheat (grains, straw and whole plants) using Liquid Chromatography Mass Spectrometry (LC-MS/MS) were validated with the following LOQ: Fenpicoxamid: 0.01 mg/kg in grain, straw and whole plants. X642188: 0.01 mg/kg in grain, straw and whole plants.

Data Point:	KCA 6.3.1/02
Report author:	Eversfield, S
Report year:	2017, Amended Report
Report title:	Determination of Residue of XDE-777 and Pyraclostrobin after Two Applications of GF-3312 and after Two Applications of GF-2925 in Winter Wheat and Spring Wheat at 4 sites in Northern Europe and 4 sites in Southern Europe in 2014
Report No.:	140648
Testing Facility Report No.:	S14-01569
Method(s) used:	S12-01537 / Dow AgroSciences study number 120615
Guidelines followed in study:	SANCO/3029/99 rev.4
Deviation from current test guidelines:	Yes, a minimum of 5 recoveries per fortification level was not achieved for each analyte.
Analytical Performing Laboratory:	Eurofins Agroscience Services Chem GmbH Hamburg, Germany
GLP/Officially recognised testing facilities:	Yes/Behörde für Gesundheit und Verbraucherschutz (BGV)
Acceptability/Reliability:	Yes

MATERIALS AND METHODS

Method Principle

Residues of GF-3312 and GF-2925, based on the analysis of XDE-777, X642188, and pyraclostrobin, were determined from wheat samples (whole plant, grain, and straw). XDE-777 and X642188 residues were extracted with acetonitrile/water (90/10, v/v) by homogenisation and shaking. Following extract dilution with acetonitrile/water/formic acid (90/10/0.1, v/v/v), the final sample was analysed for XDE-777 and X642188 by liquid chromatography coupled with positive-ion electrospray tandem mass spectrometry (LC-MS/MS).
Pyraclostrobin analysis is not summarized here.

RESULTS AND DISCUSSION

A reduced method verification set was run slightly prior to field sample analysis (see Tables A1-A2). Procedural recoveries were run concurrently with field samples (see Tables A3-A4)
For XDE-777, mean recovery values at each fortification concentration were within the acceptance range (mean recovery 70-110%; RSD ≤ 20%).

For X642188, mean recovery values at each fortification concentration were within the acceptance range (mean recovery 70-110%; RSD ≤ 20%).
The results obtained are summarised in the following tables.

Table A 4: Recovery results from method verification of XDE-777 (m/z 615/239) using the analytical method

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Wheat, Whole plant	XDE-777	0.01	93	5.4	3	96, 88, 92
Wheat, Whole plant	XDE-777	0.1	95	4.0	3	97, 98, 91

Table A 5: Recovery results from method verification of X642188 (m/z 515/239) using the analytical method

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Wheat, Whole plant	X642188	0.01	106	4.9	3	100, 110, 107
Wheat, Whole plant	X642188	0.1	92	3.1	3	94, 94, 89

Table A 6: Recovery results from method validation (procedural recoveries) of XDE-777 (m/z 615/239) using the analytical method

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Wheat, Whole plant	XDE-777	0.01	96	15	6	110,108,104, 98, 78,77
Wheat, Whole plant	XDE-777	0.1	101	7.5	6	103, 89, 110,108, 98, 99
Wheat, Whole plant	XDE-777	20	96	-	2	101, 91
Wheat, Grain	XDE-777	0.01	90	14	6	88, 107, 104, 87, 75, 80
Wheat, Grain	XDE-777	0.1	102	4.7	6	109, 96, 101, 100, 101, 107
Wheat, Straw	XDE-777	0.01	105	9.2	6	120, 106, 99, 100, 111, 93
Wheat, Straw	XDE-777	0.1	106	4.9	6	106, 104, 105, 104, 116, 101
Wheat, Straw	XDE-777	0.8	108	6.7	3	100, 114, 110
Wheat, Straw	XDE-777	20	109	4.7	3	113, 110, 103

Table A 7: Recovery results from method validation (procedural recoveries) of X642188 (m/z 515/239) using the analytical method

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Wheat, Whole plant	X642188	0.01	100	5.9	6	103, 93, 107, 104, 94, 96
Wheat, Whole plant	X642188	0.1	104	4.7	6	105, 95, 108, 108, 102, 103
Wheat, Whole plant	X642188	20	97	-	2	98, 95
Wheat, Grain	X642188	0.01	100	8.1	6	101, 105, 105, 110, 90, 91
Wheat, Grain	X642188	0.1	106	5.7	6	109, 94, 107, 108, 109, 110

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Wheat, Straw	X642188	0.01	107	6.0	6	119, 100, 105, 105, 109, 105
Wheat, Straw	X642188	0.1	109	5.1	6	116, 100, 108, 106, 113, 108
Wheat, Straw	X642188	0.8	104	13	3	88, 113, 110

Table A 8: Characteristics for the analytical method used for validation of XDE-777 and X642188 residues in wheat (whole plant, grain and straw)

	XDE-777	X642188
Specificity	m/z 615/239 Quantification m/z 615/515 Confirmation blank value <30% LOQ	m/z 515/239 Quantification m/z 515/124 Confirmation blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting r≥0.99 min 6 data points	linear regression analysis with 1/x weighting r≥0.99 min 6 data points
Calibration range	Concentration range of 0.0075-0.375 ng/mL(equivalent sample concentration 0.003- 0.15 mg/kg)	Concentration range of 0.0075-0.375 ng/mL(equivalent sample concentration 0.003-0.15 mg/kg)
Limit of quantitation	LOQ=0.01 mg/kg	LOQ=0.01 mg/kg

CONCLUSION

This method was considered acceptable for the determination of XDE-777 and X684188 in wheat (whole plant, grain, and straw) based on acceptable precision and accuracy demonstrated within this study.

A 2.1.1.3 Analytical method 3

A 2.1.1.3.1 Method validation

Comments of zRMS:	The study was evaluated in the Registration Report, Part B5 for GF-3308 on 24.08.2022 for XDE-777 only. The data for prothioconazole is evaluated in this document and a summary is also provided below. Summary: **XDE-777** The analytical method (reported in Eurofins study no. S12-01537 / Dow AgroSciences study code 120615) used for the residues trials S14-01568 for the determination of fenpicoxamid (XDE-777) and its X642188 metabolite in wheat (grains, straw and whole plants) using Liquid Chromatography Mass Spectrometry (LC-MS/MS) were validated with the following LOQ: Fenpicoxamid: 0.01 mg/kg in grain, straw and whole plants. X642188: 0.01 mg/kg in grain, straw and whole plants. **Prothioconazole** The analytical method 'Analytical Determination of Residues of JAU 6476 and desthio-JAU 6476 in/on Cereals by HPLC-MS/MS' has been validated for grain and straw for residues of prothioconazole-desthio. The limit of detection (LOD) and limit of quantitation (LOQ) for prothionconazole-desthio in wheat grain were 0.003 mg/kg and 0.01 mg/kg, respectively. The limit of detection (LOD) and limit of quantitation (LOQ) for prothionconazole-desthio in wheat straw were 0.015 mg/kg and 0.05 mg/kg, respectively. The validation of methods are acceptable.

Data Point:	KCA 6.3.1/03
Report author:	Eversfield, S
Report year:	2016
Report title:	Determination of Residues of XDE-777 and Prothioconazole After Two Applications of GF-3307 and After Two Applications of GF-3310 in Winter Wheat and Spring Wheat at 4 Sites in Northern Europe and 4 Sites in Southern Europe in 2014
Report No.:	140649
Testing Facility Report No.:	S14-01568
Method(s) used:	S12-01537 / Dow AgroSciences study number 120615 Bayer Method No. 00598
Guidelines followed in study:	SANCO/3029/99 rev.4
Deviation from current test guidelines:	Yes, a minimum of 5 recoveries per fortification level was not achieved for each analyte
Analytical Performing Laboratory:	Eurofins Agroscience Services Chem Ltd Wilson, Derbyshire, UK
GLP/Officially recognised testing facilities:	Yes/Department of Health (U.K.)
Acceptability/Reliability:	Yes

MATERIALS AND METHODS

Method Principle

Residues of GF-3307 and GF-3310, based on the analysis of XDE-777, X642188 and prothioconazole-desthio, were determined from wheat samples.

XDE-777 and X642188 residues were extracted from samples of wheat (whole plant, grain, and straw) with acetonitrile/water (90/10, v/v) by homogenisation and shaking. Following extract dilution with acetonitrile/water/formic acid (90/10/0.1, v/v/v), the final sample was analysed for XDE-777 and X642188 by liquid chromatography coupled with positive-ion electrospray tandem mass spectrometry (LC-MS/MS).

Prothioconazole-desthio residues were extracted from samples of wheat (grain and straw) with acetonitrile/water (80/20, v/v) by homogenisation. Before extraction, a cysteine hydrochloride solution (250 mg/mL) was added for stabilization. Following extract dilution with acetonitrile/water (50/50, v/v) + 20 g/L cysteine HCl, the final sample was analysed for prothioconazole-desthio by liquid chromatography coupled with positive-ion electrospray tandem mass spectrometry (LC-MS/MS).

RESULTS AND DISCUSSION

Mean recovery values at each fortification concentration were within the acceptance range (mean recovery 70-110%; RSD ≤ 20%). The results obtained are summarized in the following tables.

Table A 9: Recovery results from method validation of XDE-777 (*m/z* 615/239) using the analytical method

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Whole Plant	XDE-777	0.01	101.5	6.5	4	105, 109, 96, 96
Whole Plant	XDE-777	1.0	90.0	8.5	6	91, 85, 87, 80, 101, 96
Whole Plant	XDE-777	5.0	104.0	-	2	102, 106
Grain	XDE-777	0.01	109.2	1.8	6	112, 106, 109, 109, 109, 110
Grain	XDE-777	0.1	107.5	3.4	4	109, 112, 105, 104
Straw	XDE-777	0.01	111.0	2.9	6	112, 114, 107, 108, 110, 115
Straw	XDE-777	40.0	104.5	-	2	104, 105

Table A 10: Recovery results from method validation of X642188 (*m/z* 515/239) using the analytical method

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Whole Plant	X642188	0.01	101.3	15.1	4	114, 115, 87, 89
Whole Plant	X642188	1.0	86.8	5.8	4	86, 80, 90, 91
Grain	X642188	0.01	100.3	4.3	4	101, 97, 97, 106
Grain	X642188	0.1	101.5	3.6	4	97, 100, 104, 105
Straw	X642188	0.01	96.3	13.9	8	101, 90, 83, 103, 109, 72, 102, 110
Straw	X642188	5.0	101.0	8.5	6	104, 100, 107, 112, 88, 95

Table A 11: Recovery results from method validation of prothioconazole-desthio (*m/z* 312/70) using the analytical method

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Grain	Prothioconazole-desthio	0.01	107.2	2.0	5	104, 110, 107, 107, 108
Grain	Prothioconazole-desthio	0.1	105.4	2.0	5	102, 105, 107, 107, 106
Straw	Prothioconazole-desthio	0.05	101.2	6.8	9	113, 111, 99, 98, 100, 101, 100, 99, 90
Straw	Prothioconazole-desthio	5.0	103.3	5.8	7	105, 114, 107, 102, 100, 99, 96
Straw	Prothioconazole-desthio	10.0	105.0	1.0	3	105, 106, 104

Table A 12: Characteristics for the analytical method used for validation of XDE-777 and X642188 in wheat (whole plant, grain, and straw)

	XDE-777	X642188
Specificity	*m/z* 615/239 (Q) *m/z* 615/515 (C) blank value <30% LOQ	*m/z* 515/239 (Q) *m/z* 515/124 (C) blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting r≥0.995 8 data points	linear regression analysis with 1/x weighting r≥0.995 8 data points
Calibration range	Concentration range of 0.0075-1.0 ng/mL(equivalent sample concentration 0.003- 0.40 mg/kg)	Concentration range of 0.0075-1.0 ng/mL(equivalent sample concentration 0.003- 0.40 mg/kg)
Limit of determination/quantification	LOQ = 0.01 mg/kg	LOQ = 0.01 mg/kg

Table A 13: Characteristics for the analytical method used for validation of prothioconazole-desthio residues in wheat (grain and straw)

	Prothioconazole-desthio
Specificity	*m/z* 312/70 (Q) *m/z* 312/125 (C) blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting r≥0.995 7 data points
Calibration range	Grain: Concentration range of 0.025-5 ng/mL(equivalent sample concentration 0.003- 0.6 mg/kg) Straw: Concentration range of 0.075-10.0 ng/mL(equivalent sample concentration 0.016-2.1 mg/kg)
Limit of determination/quantification	LOQ = 0.01 mg/kg (wheat grain) LOQ = 0.05 mg/kg (wheat straw)

CONCLUSION

This method was considered acceptable for the determination of XDE-777 and X684188 in wheat (whole plant, grain, and straw) and for prothioconazole-desthio in wheat (grain and straw) based on acceptable precision and accuracy demonstrated within this study.

A 2.1.1.4 Analytical method 4

A 2.1.1.4.1 Method validation

Comments of zRMS:	The study was evaluated in the Registration Report, Part B5 for GF-3308 on 24.08.2022 for XDE-777 only. The data for prothioconazole is evaluated in this document and a summary is also provided below. <u>Summary:</u> **XDE-777** The analytical method (reported in Eurofins study no. S12-01537 / Dow AgroSciences study code 120615) used for the residues trials S15-02629 for the determination of fenpicoxamid (XDE-777) and its X642188 metabolite in spring and winter wheat (grains, straw and whole plants) using Liquid Chromatography Mass Spectrometry (LC-MS/MS) were validated with the following LOQ: Fenpicoxamid: 0.01 mg/kg in grain, straw and whole plants. X642188: 0.01 mg/kg in grain, straw and whole plants. **Prothioconazole** Analytical method (Bayer Ag. method number '00598') using Liquid Chromatography Mass Spectrometry (LC-MS/MS) has been validated for grain and straw for residues of prothioconazole-desthio. The limit of detection (LOD) and limit of quantitation (LOQ) for prothionconazole-desthio in wheat grain were 0.003 mg/kg and 0.01 mg/kg, respectively. The limit of detection (LOD) and limit of quantitation (LOQ) for prothioconazole-desthio in wheat straw were 0.015 mg/kg and 0.05 mg/kg, respectively. The validation of methods are acceptable.

Data Point: KCA 6.3.1/04
Report author: White, T
Report year: 2016

Report title:	Determination of residues of XDE-777 and prothioconazole, after two applications of GF-3307 to spring and winter wheat, at 5 sites in Northern Europe and 5 sites in Southern Europe, 2015
Report No.:	150649
Testing Facility Report No.:	S15-02629
Method(s) used:	S12-01537 / Dow AgroSciences study number 120615 Bayer Method No. 00598
Guidelines followed in study:	SANCO/3029/99 rev.4
Deviation from current test guidelines:	No
Analytical Performing Laboratory:	Eurofins Agroscience Services Chem Ltd Wilson, Derbyshire, UK
GLP/Officially recognised testing facilities:	Yes/Department of Health (U.K.)
Acceptability/Reliability:	Yes

Method Principle

Residues of GF-3307, based on the analysis of XDE-777, X642188, and prothioconazole-desthio, were determined from wheat samples.

XDE-777 and X642188 residues were extracted from samples of wheat (whole plant, grain, and straw) with acetonitrile/water (90/10, v/v) by homogenisation and shaking. Following extract dilution with acetonitrile/water/formic acid (90/10/0.1, v/v/v), the final sample was analysed for XDE-777 and X642188 by liquid chromatography coupled with positive-ion electrospray tandem mass spectrometry (LC-MS/MS).

Prothioconazole-desthio residues were extracted from samples of wheat (grain and straw) with acetonitrile/water (80/20, v/v) by homogenisation. Before extraction, a cysteine hydrochloride solution (250 mg/mL) was added for stabilization. Following extract dilution with acetonitrile/water (50/50, v/v) + 20 g/L cysteine HCl, the final sample was analysed for prothioconazole-desthio by liquid chromatography coupled with positive-ion electrospray tandem mass spectrometry (LC-MS/MS).

RESULTS AND DISCUSSION

For XDE-777, mean recovery values at each fortification concentration were within the acceptance range (mean recovery 70-110%; RSD ≤ 20%) with one exception. Mean recovery value at 0.01 mg/kg in straw was higher than 110% but is still considered acceptable since the precision of the assay (%RSD) was less than 20%.

For X642188, mean recovery values at each fortification concentration were within the acceptance range (mean recovery 70-110%; RSD ≤ 20%) with few exceptions. Mean recovery value at 0.01 mg/kg in grain and 5.0 mg/kg in straw was higher than 110% but is still considered acceptable since the precision of the assay (%RSD) was less than 20%.

For prothioconazole-desthio, mean recovery values at each fortification concentration were within the acceptance range (mean recovery 70-110%; RSD ≤ 20%).

The results obtained are summarised in the following tables.

Table A 14: Recovery results from method validation of XDE-777 (m/z 615/239) using the analytical method

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Wheat, Whole plant	XDE-777	0.01	102	4.5	5	105, 95, 101, 101, 107
Wheat, Whole plant	XDE-777	5.0	92	2.1	5	89, 93, 93, 92, 94
Wheat, Grain	XDE-777	0.01	107	7.0	5	119, 102, 109, 102, 102
Wheat, Grain	XDE-777	0.1	101	2.2	5	101, 100, 104, 100, 98
Wheat, Straw	XDE-777	0.01	112	2.7	5	115, 113, 109, 109, 113

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Wheat, Straw	XDE-777	20	109	3.1	5	113, 112, 106, 107, 106

Table A 15: Recovery results from method validation of X642188 (m/z 515/239) using the analytical method

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Wheat, Whole plant	X642188	0.01	94	4.4	5	97, 96, 98, 88, 92
Wheat, Whole plant	X642188	0.5	93	4.1	5	86, 95, 94, 95, 94
Wheat, Grain	X642188	0.01	112	8.5	5	124, 102, 110, 120, 105
Wheat, Grain	X642188	0.1	107	6.1	5	107, 104, 108, 116, 98
Wheat, Straw	X642188	0.01	109	2.1	5	108, 108, 112, 106, 110
Wheat, Straw	X642188	5.0	111	5.4	5	106, 103, 114, 114, 117

Table A 16: Recovery results from method validation of prothioconazole-desthio (m/z 312/70) using the analytical method

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Wheat, Grain	Prothiconazole-desthio	0.01	103	9.5	5	97, 110, 97, 117, 95
Wheat, Grain	Prothiconazole-desthio	0.10	97	5.7	5	100, 92, 101, 90, 102
Wheat, Straw	Prothiconazole-desthio	0.05	100	7.6	6	97, 92, 102, 96, 100, 114
Wheat, Straw	Prothiconazole-desthio	0.50	-	-	1	108
Wheat, Straw	Prothiconazole-desthio	10	103	5.2	5	103, 94, 107, 105, 107

Table A 17: Characteristics for the analytical method used for validation of XDE-777 and X642188 residues in wheat (whole plant, grain and straw)

	XDE-777	X642188
Specificity	m/z 615/239 Quantification m/z 615/515 Confirmation blank value <30% LOQ	m/z 515/239 Quantification m/z 515/124 Confirmation blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting r≥0.99 8 data points	linear regression analysis with 1/x weighting r≥0.99 8 data points
Calibration range	Concentration range of 0.0075-1.0 ng/mL(equivalent sample concentration 0.003- 0.40 mg/kg)	Concentration range of 0.0075-1.0 ng/mL(equivalent sample concentration 0.003- 0.40 mg/kg)
Limit of quantitation	LOQ=0.01 mg/kg	LOQ=0.01 mg/kg

Table A 18: Characteristics for the analytical method used for validation of prothioconazole-desthio residues in wheat (grain and straw)

	Prothioconazole-desthio
Specificity	m/z 312/70 Quantification m/z 312/125 Confirmation blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting r≥0.99 6 data points
Calibration range	Grain: Concentration range of 0.025-2.5 ng/mL(equivalent sample concentration 0.003- 0.27 mg/kg) Straw: Concentration range of 0.075-5.0 ng/mL(equivalent sample concentration 0.016- 1.1 mg/kg)
Limit of quantitation	LOQ = 0.01 mg/kg (wheat grain) LOQ = 0.05 mg/kg (wheat straw)

CONCLUSION

This method was successfully validated for the determination of XDE-777 and X684188 in wheat (whole plant, grain, and straw) and for prothioconazole-desthio in wheat (grain and straw).

A 2.1.1.5 Analytical method 5

A 2.1.1.5.1 Method validation

Comments of zRMS:	The methods were validated for the determination of fenpicoxamid, X642188 and prothioconazole-desthio in specimens of wheat grain following one application of GF-3307 according to the SANCO/3029/99 rev.4. The final determination of the analytes in the untreated and treated specimens was performed by single extraction and single injection with liquid chromatography and mass spectrometric detection (LC/MS/MS). The limit of detection (LOD) and limit of quantitation (LOQ) for fenpicoxamid, X642188 and prothioconazole-desthio in wheat grain were 0.003 mg/kg and 0.01 mg/kg, respectively. The maximum period of extract storage for fenpicoxamid and its metabolites in wheat grain was 3 days. No analyte residues above the analytical method LOQ where detected in any of the untreated samples. The accuracy and precision for the analysis of all wheat grain samples were considered acceptable since mean recoveries of each fortification level are in the range of 70-110% and RSDs are less than 20%. The validation of methods are acceptable.

Method Identifier No.:	120615 and P60293002
Performing Laboratory:	Eurofins Agrosciences Chem SAS Vergèze, France
Reference:	KCA 6.3.1/05
Report:	Semrau, J; ~~Kühnel, S~~ Thomas, B.; 2019 Residues of Fenpicoxamid and prothioconazole in wheat at harvest following one application of GF-3307 – Southern and Northern Europe - 2018; Eurofins Agroscience Services Chem SAS, 75B Avenue de Pascalet30310 Vergeze France; Lab Study No. S18-0156~~7~~6; DAS Study No. DAS Study No. 180126 ; 14 October 2019; Unpublished

Guideline(s):	Yes, SANCO/3029/99 rev.4
Guideline Deviations:	No
GLP:	Yes
Acceptability:	Yes
Method Alterations:	N/A

MATERIALS AND METHODS

Method Principle

Residues of fenpicoxamid and X642188 are extracted from samples of wheat grain using acetonitrile/ultra-pure water (90/10, v/v) (Method Identifier No. 120615). An aliquot is then diluted in acetonitrile/ultra-pure water/formic acid (90/10/0.1, v/v/v) and analysed by liquid chromatography coupled with positive-ion electrospray tandem mass spectrometry (LC-MS/MS). The limit of detection (LOD) and limit of quantitation (LOQ) were 0.003 mg/kg and 0.01 mg/kg, respectively, for both analytes.

Residues of prothioconazole-desthio are extracted from samples of wheat grain using acetonitrile/ultra-pure water (80/20, v/v) (Method Identifier No. P60293002). After filtration on Buchner system, a liquid-liquid partition is performed with n-hexane and dichloromethane. The organic phase is evaporated to dryness and the sample is reconstituted in acetonitrile and water. Samples are analysed by liquid chromatography coupled with positive-ion electrospray tandem mass spectrometry (LC-MS/MS). The limit of detection (LOD) and limit of quantitation (LOQ) were 0.003 mg/kg and 0.01 mg/kg, respectively.

RESULTS AND DISCUSSION

Mean recovery values for fenpicoxamid, X642188 and prothioconazole-desthio at each fortification concentration were within the acceptance range (mean recovery 70-110%; RSD ≤ 20%). The results obtained are summarised in the following tables.

Table A 19: Recovery results from method validation of fenpicoxamid (*m/z* 615.3/239.0) using the analytical method 120615

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Wheat Grain	Fenpicoxamid	0.01	102	4	5	
Wheat Grain		0.1	97	2	5	

Table A 20: Recovery results from method validation of X642188 (*m/z* 515.3/239.0) using the analytical method 120615

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Wheat Grain	X642188	0.01	101	4	5	
Wheat Grain		0.1	100	4	5	

Table A 21: Recovery results from method validation of prothioconazole-desthio (312.6/125.0) using the analytical method P60293002

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Wheat Grain	Prothioconazole-desthio	0.01	85	5	5	
Wheat Grain		0.1	82	15	5	

Table A 22: Characteristics for the analytical method (120615) used for validation of fenpicoxamid and X642188 in wheat grain

	fenpicoxamid	X642188
Specificity	*m/z* 615.3/239.0 (quantitative) *m/z* 615.3/515.2 (confirmatory) blank value <30% LOQ	*m/z* 515.3/239.0 (quantitative) *m/z* 515.2/124.0 (confirmatory) blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting $r \geq 0.9999$ 8 data points	linear regression analysis with 1/x weighting $r \geq 0.9999$ 8 data points
Calibration range	Concentration range of 0.0075-1.0 ng/mL, equivalent to 0.003 mg/kg to 0.4 mg/kg	Concentration range of 0.0075-1.0 ng/mL, equivalent to 0.003 mg/kg to 0.4 mg/kg
Limit of quantification	LOQ = 0.01 mg/kg	LOQ = 0.01 mg/kg

Table A 23: Characteristics for the analytical method (P60293002) used for validation of prothioconazole-desthio in wheat grain

	prothioconazole-desthio
Specificity	*m/z* 312.6/125.0 (quantitative) *m/z* 312.6/70.0 (confirmatory) blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting $r \geq 0.9999$ 7 data points
Calibration range	Concentration range of 0.5-100 ng/mL, equivalent to 0.0025 mg/kg to 0.5 mg/kg
Limit of quantification	LOQ = 0.01 mg/kg

CONCLUSION

The methods were successfully validated for the determination of fenpicoxamid, X642188 and prothioconazole-desthio in wheat grain in accordance with SANCO/3029/99 rev.4 (European Commission, 2000).

A 2.1.1.6 Analytical method 6

A 2.1.1.6.1 Method validation

Comments of zRMS:	The analytical methods for the determination of fenpicoxamid (XDE-777), X642188 and prothioconazole-desthio in raw agricultural commodities have been validated and demonstrated to be satisfactory in terms of accuracy, precision, linearity, and specificity. The methods were validated over the concentration range of 0.003-0.4 mg/kg with a limit of quantitation of 0.010 mg/kg for fenpicoxamid (XDE-777) and X642188 in all matrices, 0.0025-5 mg/kg with a limit of quantitation of 0.010 mg/kg for prothioconazole-desthio in barley grain and 0.1-20 mg/kg with a limit of quantitation of 0.050 mg/kg for prothioconazole-desthio in barley straw and whole plant. Samples were analysed by liquid chromatography coupled with positive-ion electrospray tandem mass spectrometry (LC-MS/MS). The mean recovery values were between 70-110% for all matrices and analytes with relative standard deviations all less than 20%. The validation of the methods are acceptable.

Method Identifier No.: 120615 and P60293002
Performing Laboratory: Eurofins Agroscience Services GmbH
Stade, Germany
Reference: KCA 6.3.1/06

Report:	Semrau, J; Thomas, B.; 2019; Residues of Fenpicoxamid and Prothioconazole in Barley at Interval and at Harvest Following Two Applications of GF-3307 – Southern and Northern Europe – 2017 and 2018; Eurofins Agroscience Services GmbH, Stade, Germany; Lab Study No. S17-01904; DAS Study No. 170191; 02 September 2019; Unpublished
Guideline(s):	Yes, SANCO/3029/99 rev. 4
Guideline Deviations:	No
GLP:	Yes
Acceptability:	Yes
Method Alterations:	No

MATERIALS AND METHODS

Method Principle

Residues of fenpicoxamid and X642188 were determined from samples of barley grain, straw and whole plant by extracting with acetonitrile/water, homogenizing, and diluting in acetonitrile/water/formic acid (Method Identifier No. 120615). All samples were analyzed by liquid chromatography coupled with positive-ion electrospray tandem mass spectrometry (LC-MS/MS). The limit of detection (LOD) and limit of quantitation (LOQ) were 0.003 mg/kg and 0.01 mg/kg, respectively, for both analytes in barley grain, straw and whole plant.

Residues of prothioconazole-desthio were determined from samples of barley grain, straw and whole plant by extracting with acetonitrile/water (Method Identifier No. P60293002). A liquid-liquid extraction was performed with hexane and two further liquid-liquid extractions were then performed with dichloromethane. The extract was taken, evaporated to dryness and then dissolved in acetonitrile/water. All samples were analyzed by liquid chromatography coupled with positive-ion electrospray tandem mass spectrometry (LC-MS/MS). The limit of detection (LOD) and limit of quantitation (LOQ) in barley straw and whole plant were 0.015 mg/kg and 0.05 mg/kg, respectively. The limit of detection (LOD) and limit of quantitation (LOQ) in barley grain were 0.003 mg/kg and 0.01 mg/kg, respectively.

RESULTS AND DISCUSSION

Mean recoveries for all fortification levels were within the 70 - 110% range and all RSD values were ≤ 20% for all analytes in all matrices. The results obtained are summarised in the following tables.

Table A 24: Recovery results from method validation of fenpicoxamid (*m/z* 615/239) in barley using the analytical method 120615

Matrix	Analyte	Fortification Level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Grain	Fenpicoxamid	0.01	85	13	9	
	Fenpicoxamid	0.10	96	2	5	
	Fenpicoxamid	1.0	89	--	2	n<5*
	Fenpicoxamid	10	73	--	2	n<5*
Straw	Fenpicoxamid	0.01	90	12	11	
	Fenpicoxamid	0.10	89	11	5	
	Fenpicoxamid	10	89	3	6	
Whole Plant	Fenpicoxamid	0.01	92	9	15	
	Fenpicoxamid	0.10	88	1	5	
	Fenpicoxamid	1.0	92	7	4	n<5*
	Fenpicoxamid	10	103	4	6	

*This is considered to have no impact on the quality of residue study as n≥5 at LOQ and n≥5 at 10xLOQ or higher. Additionally, the overall fortification number/matrix is n ≥18.

Table A 25: Recovery results from method validation of X642188 (*m/z* 515/239) in barley using the analytical method 120615

Matrix	Analyte	Fortification Level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Grain	X642188	0.01	95	7	9	
	X642188	0.10	98	3	5	
	X642188	1	93	--	2	n<5*
	X642188	10	93	--	2	n<5*
Straw	X642188	0.01	99	6	9	
	X642188	0.10	95	8	5	
	X642188	1	104	--	2	n<5*
	X642188	10	98	--	2	n<5*
Whole Plant	X642188	0.01	83	9	15	
	X642188	0.10	79	1	5	
	X642188	1	82	12	6	
	X642188	10	89	8	4	n<5*

*This is considered to have no impact on the quality of residue study as n≥5 at LOQ and n≥5 at 10xLOQ or higher. Additionally, the overall fortification number/matrix is n ≥18.

Table A 26: Recovery results from method validation of prothioconazole-desthio (*m/z* 314/127) in barley using the analytical method P60293002

Matrix	Analyte	Fortification Level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Grain	Prothioconazole-desthio	0.01	90	10	13	
	Prothioconazole-desthio	0.10	97	4	9	
	Prothioconazole-desthio	1.0	99	9	4	n<5*
Straw	Prothioconazole-desthio	0.05	89	13	13	
	Prothioconazole-desthio	0.50	86	9	11	
	Prothioconazole-desthio	50	105	1	2	n<5*
Whole Plant	Prothioconazole-desthio	0.05	94	15	21	
	Prothioconazole-desthio	0.50	93	12	15	
	Prothioconazole-desthio	5.0	93	--	2	n<5*
	Prothioconazole-desthio	50	81	3	4	n<5*

*This is considered to have no impact on the quality of residue study as n≥5 at LOQ and n≥5 at 10xLOQ or higher. Additionally, the overall fortification number/matrix is n ≥26.

Table A 27: Characteristics for the analytical method (120615) used for validation of fenpicoxamid and X642188 in barley grain, straw and whole plant

	fenpicoxamid	X642188
Specificity	*m/z* 615/239 (quantitative) *m/z* 615/515 (confirmatory) blank value <30% LOQ	*m/z* 515/239 (quantitative) *m/z* 515/124 (confirmatory) blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting r≥0.99 8 data points	linear regression analysis with 1/x weighting r≥0.99 8 data points
Calibration range	Concentration range of 0.0075-1.0 ng/mL, equivalent to 0.003-0.4 mg/kg	Concentration range of 0.0075-1.0 ng/mL, equivalent to 0.003-0.4 mg/kg
Limit of quantification	LOQ=0.01 mg/kg	LOQ=0.01 mg/kg

Table A 28: Characteristics for the analytical method (P60293002) used for validation of prothioconazole-desthio in barley grain, straw and whole plant

	prothioconazole-desthio
Specificity	*m/z* 314/127 (quantitative) *m/z* 313/70 (confirmatory) blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting r≥0.99 8 data points
Calibration range	Grain: Concentration range of 0.5-100.00 ng/mL, equivalent to 0.0025-0.5 mg/kg Straw and Whole Plant: Concentration range of 1.0-200.0 ng/mL, equivalent to 0.01-2 mg/kg
Limit of quantification	Grain: LOQ=0.01 mg/kg Straw and Whole Plant: LOQ = 0.05 mg/kg

CONCLUSION

The methods were successfully validated for the determination of fenpicoxamid, X642188, and prothioconazole-desthio in barley grain, straw and whole plant in accordance with SANCO/3029/99 rev.4 (European Commission, 2000).

A 2.1.1.7 Analytical method 7

A 2.1.1.7.1 Method validation

Comments of zRMS:	The analytical method (Reference ID 140696) was successflully validated and is suitable for determination of residues of fenpicoxamid, X642188, X12019520, X12314005, X12264475 and X12335723 in samples of barley grain (residue samples) and its processed fractions (RAC grain (grain prior to processing), cleaned grain, malt sprouts, brewing malt, spent grain, flocs, brewer's yeast, beer, pot barley, barley bran, barley flour and bread) with an LOQ of 0.01 mg/kg. The final determination of the analytes in the untreated and treated specimens was performed by single extraction and single injection with liquid chromatography and mass spectrometric detection (LC/MS/MS). No analyte residues above the analytical method LOQ where detected in any of the untreated samples, except for barley flour samples no. S18-00056-L2-011A and S18-00056-L2-059A and bread sample S18-00056-L2-060A. This indicates that untreated control plots and samples remained largely uncontaminated through the course of the analytical phase. Mean recoveries for all fortification levels were within the 70 - 110% range and all RSD values were ≤ 20% for all analytes in all matrices. The validation of the method is acceptable.

Method Identifier No.:	140696
Performing Laboratory:	Eurofins Agroscience Services Ltd Derby, UK
Reference:	KCA 6.3.1/08
Report:	Eversfield, S.; 2019; Residues of Fenpicoxamid in Barley and its Processed Commodities at Harvest Following Two Applications of GF-3307 – Europe - 2018; Eurofins Agroscience Services Ltd, Derby, UK;

	Lab Study No. S18-00056; DAS Study No. 170192; 28 August 2019; Unpublished
Guideline(s):	Yes, SANCO/3029/99 rev. 4
Guideline Deviations:	No
GLP:	Yes
Acceptability:	Yes
Method Alterations:	No

MATERIALS AND METHODS

Method Principle

Residues of fenpicoxamid, metabolite X642188 and hydrolysis degradates X12019520, X12314005, X12264475 and X12335723 were determined from samples of barley (grain) and processed commodities by extracting in acetonitrile/water/phosphoric acid (90/10/0.1 v/v/v) by homogenizing, shaking and centrifuging (Method Identifier No. 140696). Supernatants were filtered and diluted in acetonitrile/water/phosphoric acid (90/10/0.1 v/v/v). For non-polar analytes (fenpicoxamid, X642188, X12019520 and X12314005), an aliquot of the crude extract from the previous step was combined with acetonitrile/water/phosphoric acid (10/90/0.1 v/v/v) and centrifuged. Polar analytes X12264475 and X12335723 were further separated using liquid-liquid partitioning. If necessary, final extracts were diluted to be within the validated calibration range prior to analysis. Samples were analyzed using an LC/MS/MS system operating with an electrospray ionization interface (ESI) operating in the positive mode. Two parent-to-daughter ion transitions (primary/quantitative and confirmatory) were monitored during analysis for each analyte. The limit of detection (LOD) and limit of quantitation (LOQ) were 0.003 mg/kg and 0.01 mg/kg, respectively, for all analytes in all matrices.

RESULTS AND DISCUSSION

Mean recoveries for all fortification levels were within the 70 - 110% range and all RSD values were ≤ 20% for all analytes in all matrices. The results obtained are summarised in the following tables.

Table A 29: Recovery results from method validation of fenpicoxamid (*m/z* 615/239) using the analytical method

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
grain	fenpicoxamid	0.01	99	4.1	5	
	fenpicoxamid	0.10	91	4.9	5	
	fenpicoxamid	5.00	89	3.5	5	
cleaned grain	fenpicoxamid	0.01	96	7.7	5	
	fenpicoxamid	0.10	95	7.9	5	
	fenpicoxamid	5.00	81	2.3	5	
malt sprouts	fenpicoxamid	0.01	84	4.6	5	
	fenpicoxamid	0.10	86	2.8	8	
	fenpicoxamid	5.00	87	5.9	5	
brewing malt	fenpicoxamid	0.01	110	5.2	5	
	fenpicoxamid	0.10	108	4.2	5	
	fenpicoxamid	1.00	87	2.8	5	
spent grain	fenpicoxamid	0.01	87	8.6	5	
	fenpicoxamid	0.10	80	3.0	5	
flocs	fenpicoxamid	0.01	95	2.9	5	
	fenpicoxamid	0.10	95	5.9	5	
brewer's	fenpicoxamid	0.01	95	5.6	5	

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
yeast	fenpicoxamid	0.10	97	5.5	5	
beer	fenpicoxamid	0.01	85	3.4	5	
	fenpicoxamid	0.10	81	3.6	5	
pot barley	fenpicoxamid	0.01	98	8.8	5	
	fenpicoxamid	0.10	104	3.3	5	
	fenpicoxamid	5.00	94	5.1	5	
barley bran	fenpicoxamid	0.01	77	7.1	5	
	fenpicoxamid	0.10	92	2.3	5	
	fenpicoxamid	5.00	82	2.3	5	
barley flour	fenpicoxamid	0.01	70	3.9	5	
	fenpicoxamid	0.10	78	3.0	5	
	fenpicoxamid	5.00	71	2.8	5	
bread	fenpicoxamid	0.01	80	17	7	
	fenpicoxamid	0.10	91	4.1	7	
	fenpicoxamid	1.00	86	3.7	5	

Table A 30: Recovery results from method validation of X642188 (*m/z* 515/239) using the analytical method

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
grain	X642188	0.01	89	3.1	5	
	X642188	0.10	78	3.4	5	
cleaned grain	X642188	0.01	104	8.1	5	
	X642188	0.10	104	8.3	5	
malt sprouts	X642188	0.01	105	6.7	5	
	X642188	0.10	96	8.7	8	
	X642188	5.00	91	2.7	5	
brewing malt	X642188	0.01	100	9.2	5	
	X642188	0.10	96	5.1	5	
	X642188	1.00	87	2.6	5	
spent grain	X642188	0.01	96	11	5	
	X642188	0.10	87	13	5	
flocs	X642188	0.01	108	8.6	5	
	X642188	0.10	99	15	5	
brewer's yeast	X642188	0.01	106	2.8	5	
	X642188	0.10	103	8.9	5	
beer	X642188	0.01	97	4.8	5	
	X642188	0.10	93	2.7	5	
pot barley	X642188	0.01	97	8.6	5	
	X642188	0.10	103	2.1	5	
barley bran	X642188	0.01	89	4.5	5	
	X642188	0.10	93	3.8	5	

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
barley flour	X642188	0.01	73	6.4	5	
	X642188	0.10	78	4.5	5	
bread	X642188	0.01	90	15	7	
	X642188	0.10	92	9.6	7	

Table A 31: Recovery results from method validation of X12019520 (*m/z* 189/143) using the analytical method

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
grain	X12019520	0.01	99	2.2	5	
	X12019520	0.10	98	1.8	5	
cleaned grain	X12019520	0.01	96	9.7	5	
	X12019520	0.10	93	3.3	5	
malt sprouts	X12019520	0.01	84	12	5	
	X12019520	0.10	81	4.6	8	
brewing malt	X12019520	0.01	105	5.2	5	
	X12019520	0.10	110	5.7	5	
spent grain	X12019520	0.01	97	9.9	5	
	X12019520	0.10	84	5.4	5	
flocs	X12019520	0.01	100	5.0	5	
	X12019520	0.10	104	3.4	5	
brewer's yeast	X12019520	0.01	105	8.0	5	
	X12019520	0.10	104	6.4	5	
beer	X12019520	0.01	88	6.2	5	
	X12019520	0.10	73	3.9	5	
pot barley	X12019520	0.01	96	11	5	
	X12019520	0.10	105	2.0	5	
barley bran	X12019520	0.01	99	4.7	5	
	X12019520	0.10	97	1.9	5	
barley flour	X12019520	0.01	73	4.4	5	
	X12019520	0.10	75	2.7	5	
bread	X12019520	0.01	94	8.5	7	
	X12019520	0.10	83	3.9	7	

Table A 32: Recovery results from method validation of X12314005 (*m/z* 277/189) using the analytical method

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
grain	X12314005	0.01	98	5.0	5	
	X12314005	0.10	98	5.8	5	
cleaned grain	X12314005	0.01	102	7.0	5	
	X12314005	0.10	91	4.1	5	

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
malt sprouts	X12314005	0.01	85	7.7	5	
	X12314005	0.10	80	2.9	8	
brewing malt	X12314005	0.01	102	3.7	5	
	X12314005	0.10	106	11	5	
spent grain	X12314005	0.01	96	3.8	5	
	X12314005	0.10	84	5.0	5	
flocs	X12314005	0.01	105	2.0	5	
	X12314005	0.10	106	4.7	5	
brewer's yeast	X12314005	0.01	103	3.9	5	
	X12314005	0.10	102	9.7	5	
beer	X12314005	0.01	90	11	5	
	X12314005	0.10	77	3.5	5	
pot barley	X12314005	0.01	95	6.6	5	
	X12314005	0.10	98	3.6	5	
barley bran	X12314005	0.01	88	2.3	5	
	X12314005	0.10	91	3.9	5	
barley flour	X12314005	0.01	76	9.9	5	
	X12314005	0.10	79	7.2	5	
bread	X12314005	0.01	90	5.9	7	
	X12314005	0.10	81	9.7	7	

Table A 33: Recovery results from method validation of X12264475 using the analytical method

Matrix	Analyte	Mass Transition	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
grain	X12264475	*m/z* 257/152	0.01	97	5.0	5	
			0.10	105	6.8	5	
cleaned grain	X12264475	*m/z* 257/152	0.01	103	11	5	
			0.10	102	14	5	
malt sprouts	X12264475	*m/z* 257/124	0.01	87	12	5	
			0.10	75	2.3	5	
			1.00	72	5.5	5	
brewing malt	X12264475	*m/z* 257/152	0.01	100	13	5	
			0.10	77	12	5	
spent grain	X12264475	*m/z* 257/142	0.01	96	3.8	5	
			0.10	103	11	5	
flocs	X12264475	*m/z* 257/142	0.01	107	5.8	5	
			0.10	102	4.2	5	
brewer's yeast	X12264475	*m/z* 257/142	0.01	85	5.9	5	
			0.10	75	7.0	5	
beer	X12264475	*m/z* 257/142	0.01	76	11	5	
			0.10	76	7.5	5	

Matrix	Analyte	Mass Transition	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
pot barley	X12264475	*m/z* 257/152	0.01	110	13	5	
			0.10	100	7.1	5	
barley bran	X12264475	*m/z* 257/152	0.01	95	18	5	
			0.10	99	9.2	5	
barley flour	X12264475	*m/z* 257/152	0.01	80	3.1	5	
			0.10	70	4.9	5	
bread	X12264475	*m/z* 257/152	0.01	77	7.5	5	
			0.10	82	2.5	5	

Table A 34: Recovery results from method validation of X12335723 using the analytical method

Matrix	Analyte	Mass Transition	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
grain	X12335723	*m/z* 357/257	0.01	92	11	5	
			0.10	92	18	5	
cleaned grain	X12335723	*m/z* 357/257	0.01	80	19	8	
			0.10	95	18	5	
malt sprouts	X12335723	*m/z* 257/152	0.01	80	7.4	5	
			0.10	76	7.2	5	
brewing malt	X12335723	*m/z* 357/257	0.01	79	17	5	
			0.10	78	20	5	
spent grain	X12335723	*m/z* 257/152	0.01	85	2.9	5	
			0.10	87	7.0	5	
flocs	X12335723	*m/z* 257/152	0.01	89	7.8	5	
			0.10	86	14	5	
brewer's yeast	X12335723	*m/z* 257/152	0.01	100	7.5	5	
			0.10	102	3.7	5	
beer	X12335723	*m/z* 257/152	0.01	80	7.8	5	
			0.10	73	3.6	5	
pot barley	X12335723	*m/z* 357/257	0.01	108	14	5	
			0.10	102	4.5	5	
barley bran	X12335723	*m/z* 257/152	0.01	78	4.2	5	
			0.10	88	7.0	5	
			0.50	85	7.0	5	
barley flour	X12335723	*m/z* 257/152	0.01	96	3.1	5	
			0.10	98	8.1	5	
bread	X12335723	*m/z* 257/152	0.01	96	9.6	5	
			0.10	79	5.8	5	

Table A 35: Characteristics for the analytical method used for validation of fenpicoxamid, X642188, X12019520, X12314005, X12264475 and X12335723 residues in barley (grain) and processed products

	fenpicoxamid	**X642188**	**X12019520**	**X12314005**	**X12264475**	**X12335723**
Specificity	*m/z* 615/239 (quantitative) *m/z* 615/515 (confirmatory) blank value <30% LOQ	*m/z* 515/239 (quantitative) *m/z* 515/124 (confirmatory) blank value <30% LOQ	*m/z* 189/143 (quantitative) *m/z* 189/128 (confirmatory) blank value <30% LOQ	*m/z* 277/189 (quantitative) *m/z* 277/143 (confirmatory) blank value <30% LOQ	**m/z* 257/152 (quantitative) *m/z* 257/124 (confirmatory) blank value <30% LOQ	***m/z* 357/257 (quantitative) *m/z* 257/124 (confirmatory) blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting r≥0.99 8 data points	linear regression analysis with 1/x weighting r≥0.99 8 data points	linear regression analysis with 1/x weighting r≥0.99 8 data points	linear regression analysis with 1/x weighting r≥0.99 8 data points	linear regression analysis with 1/x weighting r≥0.99 6-8 data points	linear regression analysis with 1/x weighting r≥0.99 8 data points
Calibration range	Concentration range of 0.075-5.0 ng/mL, equivalent to 0.003-0.2 mg/kg	Concentration range of 0.075-5.0 ng/mL, equivalent to 0.003-0.2 mg/kg	Concentration range of 0.075-5.0 ng/mL, equivalent to 0.003-0.2 mg/kg	Concentration range of 0.075-5.0 ng/mL, equivalent to 0.003-0.2 mg/kg	Concentration range of 0.15-10 ng/mL, equivalent to 0.003-0.2 mg/kg	Concentration range of 0.15-10 ng/mL, equivalent to 0.003-0.2 mg/kg
Limit of quantification	LOQ=0.01 mg/kg	LOQ=0.01 mg/kg	LOQ=0.01 mg/kg	LOQ=0.01 mg/kg	LOQ=0.01 mg/kg	LOQ=0.01 mg/kg

* For X12264475 in spent grain, flocs, brewer's yeast and beer, mass transitions were 257/142 (quantitative) and 257/170 (confirmatory) and in malt sprouts, mass transitions were 257/124 (quantitative) and 257/170 (confirmatory).
** For X12335723 in malt sprouts, spent grain, flocs, brewer's yeast, beer, barley bran, flour and bread, mass transitions were 257/152 (quantitative) and 257/170 (confirmatory).

CONCLUSION

This method was successfully validated for the determination of fenpicoxamid, X642188, X12019520, X12314005, X12264475 and X12335723 residues in samples of barley (grain) and processed commodities in accordance with SANCO/3029/99 rev.4 (European Commission, 2000).

A 2.1.1.8 Analytical method 8

A 2.1.1.8.1 Method validation

Comments of zRMS:	The analytical methods were succesfully validated for the determination of fenpicoxamid (XDE-777) and its metabolites and prothioconazole-desthio in barley grain according to SANCO/3029/99, rev. 4. The final determination of the analytes in the untreated and treated specimens was performer by single extraction and single injection with liquid chromatography and mass spectrometric detection (LC/MS/MS). The limit of detection (LOD) and limit of quantitation (LOQ) for all analytes were 0.003 mg/kg and 0.01 mg/kg respectively. No analyte residues above the analytical method LOQ where detected in any of the untreated samples. Mean recoveries for all fortification levels were within the 70 - 110% range and all RSD values were ≤ 20% for all analytes in grain matrices. The validation of the method is acceptable.

Method Identifier No.: 120615 and P60293002
Performing Laboratory: Eurofins Agrosciences Chem SAS

	Vergèze, France
Reference:	KCA 6.3.1/07
Report:	Semrau, J; Kühnel, S; 2019 Residues of Fenpicoxamid and Prothioconazole in Barley at Harvest Following One Application of GF-3307 – Southern and Northern Europe - 2018; Eurofins Agroscience Services Chem SAS, 75B Avenue de Pascalet30310 Vergeze France; Lab Study No. S18-01567; DAS Study No. DAS Study No. 180128 ; 14 October 2019; Unpublished
Guideline(s):	Yes, SANCO/3029/99 rev.4
Guideline Deviations:	No
GLP:	Yes
Acceptability:	Yes
Method Alterations:	N/A

MATERIALS AND METHODS

Method Principle

Residues of fenpicoxamid and X642188 are extracted from samples of barley grain using acetonitrile/ultra-pure water (90/10, v/v) (Method Identifier No. 120615). An aliquot is then diluted in acetonitrile/ultra-pure water/formic acid (90/10/0.1, v/v/v) and analysed by liquid chromatography coupled with positive-ion electrospray tandem mass spectrometry (LC-MS/MS). The limit of detection (LOD) and limit of quantitation (LOQ) were 0.003 mg/kg and 0.01 mg/kg, respectively, for both analytes.

Residues of prothioconazole-desthio are extracted from samples of barley grain using acetonitrile/ultra-pure water (80/20, v/v) (Method Identifier No. P60293002). After filtration on Buchner system, a liquid-liquid partition is performed with n-hexane and dichloromethane. The organic phase is evaporated to dryness and the sample is reconstituted in acetonitrile and water. Samples are analysed by liquid chromatography coupled with positive-ion electrospray tandem mass spectrometry (LC-MS/MS). The limit of detection (LOD) and limit of quantitation (LOQ) were 0.003 mg/kg and 0.01 mg/kg, respectively.

RESULTS AND DISCUSSION

Mean recovery values for fenpicoxamid, X642188 and prothioconazole-desthio at each fortification concentration were within the acceptance range (mean recovery 70-110%; RSD ≤ 20%). The results obtained are summarised in the following tables.

Table A 36: Recovery results from method validation of fenpicoxamid (*m/z* 615.3/239.0) in barley using the analytical method 120615

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Barley grain	Fenpicoxamid	0.01	99	5	5	
Barley grain		0.1	99	3	5	

Table A 37: Recovery results from method validation of X642188 (*m/z* 515.3/239.0) in barley using the analytical method 120615

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Barley grain	X642188	0.01	100	4	5	
Barley grain		0.1	101	2	5	

Table A 38: Recovery results from method validation of prothioconazole-desthio (312.6/125.0) in barley using the analytical method P60293002

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Barley grain	Prothioconazole-desthio	0.01	78	10	5	
Barley grain		0.1	81	10	5	

Table A 39: Characteristics for the analytical method (120615) used for validation of fenpicoxamid and X642188 in barley grain

	fenpicoxamid	X642188
Specificity	*m/z* 615.3/239.0 (quantitative) *m/z* 615.3/515.2 (confirmatory) blank value <30% LOQ	*m/z* 515.3/239.0 (quantitative) *m/z* 515.2/124.0 (confirmatory) blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting r ≥ 0.9999 8 data points	linear regression analysis with 1/x weighting r = 1.0000 8 data points
Calibration range	Concentration range of 0.0075-1.0 ng/mL, equivalent to 0.003 mg/kg to 0.4 mg/kg	Concentration range of 0.0075-1.0 ng/mL, equivalent to 0.003 mg/kg to 0.4 mg/kg
Limit of quantification	LOQ = 0.01 mg/kg	LOQ = 0.01 mg/kg

Table A 40: Characteristics for the analytical method (P60293002) used for validation of prothioconazole-desthio in barley grain

	prothioconazole-desthio
Specificity	*m/z* 312.6/125.0 (quantitative) *m/z* 312.6/70.0 (confirmatory) blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting r ≥ 0.9999 8 data points
Calibration range	Concentration range of 0.5-100 ng/mL, equivalent to 0.0025 mg/kg to 0.5 mg/kg
Limit of quantification	LOQ = 0.01 mg/kg

CONCLUSION

The methods were successfully validated for the determination of fenpicoxamid, X642188 and prothioconazole-desthio in barley grain in accordance with SANCO/3029/99 rev.4 (European Commission, 2000).

A 2.1.1.9 Analytical method 9

A 2.1.1.9.1 Method validation

Comments of zRMS:	Test solutions were analyzed for the concentrations of XDE-777, one of the active substances in GF-3307, using a liquid chromatography equipped with tandem mass spectrometry (LC-MS/MS) system. LOQ = 0.009 mg T.P./L, equivalent to 0.0217 ng a.i./mL The validation of method is acceptable.

Method Identifier No.: 140479 Amendment 1

Performing Laboratory: ABC Laboratories, Inc. (now EAG, Inc.)
Columbia, Missouri, USA

Reference: KCP 10.2.1/1

Report:	Dinehart, S.; 2014; GF-3307: Acute Toxicity to the Rainbow Trout, *Oncorhynchus mykiss*, Determined Under Static-Renewal Test Conditions; ABC Laboratories, Inc. (now EAG, Inc.), Columbia, Missouri, USA; Lab Study No. 81071; DAS Study No. 140479 ; 08 December 2014, Revised 2017, Final report addendum 2019; Unpublished
Guideline(s):	Yes, SANCO/3029/99 rev.4
Guideline Deviations:	No
GLP:	Yes
Acceptability:	Yes
Method Alterations:	None

MATERIALS AND METHODS

Method Principle

Residues of GF-3307, based on analysis of XDE-777, were determined from samples of freshwater by diluting with 0.2% formic acid in acetonitrile (ACN) and, if necessary, further diluting with 0.1:50:50 acid:ACN:water. The final sample was analysed for GF-3307 by liquid chromatography coupled with positive-ion electrospray tandem mass spectrometry (LC-MS/MS).

RESULTS AND DISCUSSION

Mean recovery values at each fortification concentration were within the acceptance range (mean recovery 70-110%; RSD ≤ 20%). The results obtained are summarised in the following tables.

Table A 41: Recovery results from method validation of GF-3307, based on analysis of XDE-777, (*m/z* 615.0/239.2) using the analytical method

Matrix	Analyte	Fortification level (mg T.P./L)	Mean Recovery (%)	RSD (%)	n	Comments
Freshwater	XDE-777	0.00603	102	NA	1	
Freshwater	XDE-777	0.00900	98	2	3	
Freshwater	XDE-777	0.0560	94	3	3	
Freshwater	XDE-777	0.140	96	NA	1	
Freshwater	XDE-777	0.560	102	2	3	
Freshwater	XDE-777	1.40	97	2	3	

Table A 42: Characteristics for the analytical method used for validation of GF-3307, based on analysis of XDE-777, residues in freshwater

	GF-3307, based on analysis of XDE-777
Specificity	*m/z* 615.0/239.2 blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting r≥0.995 7 data points
Calibration range	Concentration range of 0.0200-0.750 ng/ XDE-777mL Sample equivalent range of 0.00833-0.313 mg GF-3307/L
Limit of determination/quantification	LOQ = 0.009 mg T.P./L, equivalent to 0.0217 ng a.i./mL

CONCLUSION

This method was successfully validated for the determination of GF-3307, based on the analysis of XDE-777, in freshwater and is suitable to generate data in support of ecotoxicology studies.

A 2.1.1.10 Analytical method 10

A 2.1.1.10.1 Method validation

Comments of zRMS:	Test solutions were analyzed for the concentration of XDE-777 and prothioconazole, the active ingredients in GF-3307, using a liquid chromatography system with tandem mass spectrometry (LC-MS/MS). The limit of quantification (LOQ) for prothioconazole in freshwater was 5.00 µg GF 3307/L, equivalent to 0.245 ng a.i./mL, for XDE-777 LOQ=5.00 µg GF 3307/L, equivalent to 0.123 ng a.i./mL. Mean recoveries were in the range of 88 – 97% with relative standard deviations of ≤20% for XDE-777 at each level. Mean recoveries were in the range of 64 – 110% with relative standard deviations of ≤20% for prothioconazole at each level. Mean recovery value at 0.00500 mg GF 3307/L level was lower than 70% and cannot be considered acceptable according to the SANCO/3029/99 rev.4 . The following argumentation: "*The ecotoxicology study did not use the recoveries of prothioconazole to represent recoveries of GF-3307, therefore the lack of a validated fortification level at 0.00500 mg GF-3307/L is mitigated*" is acceptable. The study is acceptable.

Method Identifier No.:	180975 Protocol
Performing Laboratory:	Analytical Bio-Chemistry Laboratories, Inc. (EAG, Inc.) Columbia, Missouri, USA
Reference:	KCP 10.2.1/2
Report:	Dinehart, S.; 2018; GF-3307: Acute Toxicity to the Rainbow Trout, *Oncorhynchus mykiss*, Determined Under Flow-Through Test Conditions; Analytical Bio-Chemistry Laboratories, Inc. (EAG, Inc.), Columbia, Missouri, USA; Lab Study No. 87719; DAS Study No. 180975; 23 October 2018; Unpublished
Guideline(s):	Yes, SANCO/3029/99 rev.4
Guideline Deviations:	Yes. Mean recovery values at 0.00500 mg GF-3307/L fortification concentration for GF-3307, based on prothioconazole analysis, were lower than 70% and cannot be considered acceptable. The ecotoxicology study did not use the recoveries of prothioconazole to represent recoveries of GF-3307, therefore the lack of a validated fortification level at 0.00500 mg GF-3307/L is mitigated.
GLP:	Yes
Acceptability:	Yes
Method Alterations:	None

MATERIALS AND METHODS

Method Principle

Residues of GF-3307, based on XDE-777 active ingredient analysis, are determined from samples of freshwater by diluting with 0.2% formic acid in acetonitrile (ACN), and further diluting, if necessary, with 0.1:50:50 formic acid:ACN:water. The final sample is analysed for XDE-777 by liquid chromatography coupled with positive-ion electrospray tandem mass spectrometry (LC-MS/MS).

Residues of GF-3307, based on prothioconazole active ingredient analysis, are determined from samples of freshwater by diluting with ACN, and further diluting, if necessary, 50:50 ACN:freshwater. The final sample is analysed for prothioconazole by liquid chromatography coupled with negative-ion electrospray tandem mass spectrometry (LC-MS/MS).

RESULTS AND DISCUSSION

Mean recovery values at each fortification concentration for GF-3307, based on XDE-777 analysis, and mean recovery values at 0.01, 1.00, and 6.00 mg GF-3307/L fortification concentration for GF-3307, based on prothioconazole analysis, were within the acceptance range (mean recovery 70-110%; RSD ≤ 20%). Mean recovery values at 0.00500 mg GF-3307/L fortification concentration for GF-3307, based on prothioconazole analysis, were lower than 70% and cannot be considered acceptable. The ecotoxicology study did not use the recoveries of prothioconazole to represent recoveries of GF-3307, therefore the lack of a validated fortification level at 0.00500 mg GF-3307/L is mitigated. The results obtained are summarised in the following tables.

Table A 43: Recovery results from method validation of GF-3307, based on XDE-777 analysis, (*m/z* 615.0/239.0) using the analytical method

Matrix	Analyte	Fortification level (mg GF-3307/L)	Mean Recovery (%)	RSD (%)	n	Comments
Freshwater	GF-3307, based on XDE-777 analysis	0.00500	88	12	11	5 method validation samples + 6 QC samples from definitive test analyses, ranging from 72 to 100%
Freshwater	GF-3307, based on XDE-777 analysis	0.0100	97	NA	1	1 QC sample from definitive test analyses, ranging from 97%
Freshwater	GF-3307, based on XDE-777 analysis	1.00	83	6	5	5 method validation samples, ranging from 79 to 91%
Freshwater	GF-3307, based on XDE-777 analysis	6.00	97	3	7	7 QC samples from definitive test analyses, ranging from 91 to 102%

Table A 44: Recovery results from method validation of GF-3307, based on prothioconazole analysis, (*m/z* 342.0/100.0) using the analytical method

Matrix	Analyte	Fortification level (mg GF-3307/L)	Mean Recovery (%)	RSD (%)	n	Comments
Freshwater	GF-3307, based on prothioconazole analysis	0.00500	64	11	5	5 method validation samples, ranging from 57 to 74%
Freshwater	GF-3307, based on prothioconazole analysis	0.0100	83	28	12	5 method validation samples + 7 QC samples from definitive test analyses, ranging from 43 to 116%
Freshwater	GF-3307, based on prothioconazole analysis	1.00	100	4	5	5 method validation samples, ranging from 95 to 105%
Freshwater	GF-3307, based on prothioconazole analysis	6.00	110	13	7	7 QC samples from definitive test analyses, ranging from 96 to 129%

Table A 45: Characteristics for the analytical method used for validation of GF-3307, based on XDE-777 and prothioconazole active ingredients analysis, residues in freshwater

	GF-3307, based on XDE-777 active ingredient analysis	**GF-3307, based on prothioconazole active ingredients analysis**
Specificity	*m/z* 615.0/239.0 *m/z* 615.0/515.0 *m/z* 615.0/124.0 blank value <30% LOQ	*m/z* 342.0/100.0 *m/z* 342.0/125.0 *m/z* 342.0/180.0 *m/z* 342.0/264.0 blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting r≥0.997 6 data points	linear regression analysis with 1/x weighting r≥0.996 6 data points
Calibration range	Concentration range of 0.0500 – 1.60 ng a.i./mL Sample equivalent range of 2.04 – 65.3 µg GF-3307/L	Concentration range of 0.0500 – 1.20 ng a.i./mL Sample equivalent range of 1.02 – 24.5 µg GF-3307/L
Limit of determination/quantification	LOQ=5.00 µg GF-3307/L, equivalent to 0.123 ng a.i./mL	LOQ=5.00 µg GF-3307/L, equivalent to 0.245 ng a.i./mL

CONCLUSION

This method was successfully validated for the determination of GF-3307, based on XDE-777 (from 0.00500 – 6.00 mg GF-3307/L) and prothioconazole (from 0.0100 – 6.00 mg GF-3307/L) active ingredients analysis in freshwater.

A 2.1.1.11 Analytical method 11

A 2.1.1.11.1 Method validation

Comments of zRMS:	The study was evaluated in the Registration Report, Part B5 for GF-3308 on 24.08.2022. Summary: The validation of the analytical method for the determination of XDE-777 in samples of freshwater using LC-MS/MS was successfully performed following the EU guideline SANCO/3029/99 rev.4, except numer of replicates recoveries. The number of replicate recoveries (N = 4) assessed at each fortifica-tion level was less than described in the guideline (N = 5). The mean recovery of each fortification level and the overall mean recovery value was between 70 – 110% with RSD < 20%. LOQ = 0.0140 mg GF-3308/L, equivalent to 0.070 ng XDE-777/mL The validation parameters are acceptable. The method is considered fit for purpose.

Method Identifier No.:	160101
Performing Laboratory:	ABC Laboratories, Inc. (now EAG Laboratories) Columbia, Missouri, USA
Reference:	KCP 10.2.1/3
Report:	Goudie, O.; 2016; GF-3308: Acute Toxicity to the Rainbow Trout, *Oncorhynchus mykiss*, Determined Under Flow-Through Test Conditions; ABC Laboratories, Inc. (now EAG Laboratories), Columbia, Missouri, USA; Lab Study No. 83494; DAS Study No. 160101 ; 08 July 2016; Unpublished
Guideline(s):	Yes, SANCO/3029/99 rev.4
Guideline Deviations:	Yes

	1.The number of replicate recoveries (N = 4) assessed at each fortification level was less than described in the guideline (N = 5)
GLP:	Yes
Acceptability:	Yes
Method Alterations:	160101 Amendment 1 was based on 160103 Amendment 1. The original method was performed in freshwater algal nutrient medium (FWAM) instead of freshwater as used in this study. The original method included centrifugation, rinsing the culture tube, and adding the resulting rinse to the sample, none of which occurred in this study. The original method had MQLs of 0.20, 0.41, 0.020, and 0.00041 mg GF-3308/L and this study had an MQL of 0.0020 mg GF-3308/L. The original method used fortification levels of 0.985, 2.25, 45.9, and 65.6 mg GF-3308/L while this study used fortification levels of 0.0140 and 0.299 mg GF-3308/L.

MATERIALS AND METHODS

Method Principle

Residues of GF-3308, based upon the analysis of XDE-777, were determined from samples of freshwater by diluting with 0.2% formic acid in acetonitrile (ACN). Further dilutions were conducted, if necessary to dilute within the range of the calibration curve, using formic acid:ACN:water (0.1:50:50). The final sample was analysed for GF-3308, based upon the analysis of XDE-777, by liquid chromatography coupled with positive-ion electrospray tandem mass spectrometry (LC-MS/MS).

RESULTS AND DISCUSSION

Mean recovery values at each fortification concentration were within the acceptance range (mean recovery 70-110%; RSD ≤ 20%). The results obtained are summarised in the following tables.

Table A 46: Recovery results from method validation of GF-3308, based upon the analysis of XDE-777, (*m/z* 615.0/239.2) using the analytical method

Matrix	Analyte	Fortification level (mg GF-3308/L)	Mean Recovery (%)	RSD (%)	n	Comments
Freshwater	XDE-777	0.0140	107	0	4	
Freshwater	XDE-777	0.299	104	5	4	

Table A 47: Characteristics for the analytical method used for validation of GF-3308, based upon the analysis of XDE-777, residues in freshwater

	GF-3308, based upon the analysis of XDE-777
Specificity	*m/z* 615.0/239.2 *m/z* 615.0/515.4 blank value <30% LOQ
Calibration (type, number of data points)	Linear regression analysis with 1/x weighting r≥0.995 5 data points
Calibration range	Concentration range of 0.0100-0.160 ng/mL Sample equivalent range of 0.0020-0.033 mg GF-3308/L
Limit of determination/quantification	LOQ = 0.0140 mg GF-3308/L, equivalent to 0.070 ng XDE-777/mL

CONCLUSION

The method was considered acceptable for the determination of GF-3308, based upon the analysis of XDE-777, in freshwater due to acceptable precision and accuracy demonstrated within this study.

A 2.1.1.12 Analytical method 12

A 2.1.1.12.1 Method validation

Comments of zRMS:	The study was evaluated in the Registration Report, Part B5 for GF-3308 on 24.08.2022. Summary: The analytical method of Goudie (2016) for the determination of XDE-777 in samples of freshwater by LC-MS/MS has been successfully validated in accordance with the EU guidance document SANCO/3029/99 rev. 4 (11/07/00), except numer of replicates recoveries. The number of replicate recoveries (N = 4) assessed at the highest fortification level was less than described in the guideline (N = 5). LOQ = 0.0279 mg GF-3308/L, equivalent to 0.066 ng XDE-777/mL The mean recovery of each fortification level and the overall mean recovery value was 70 – 120% with an RSD < 20%. This is acceptable according to SANTE/2020/12830, Rev.1. The validation parameters are acceptable. The method is considered fit for purpose.

Method Identifier No.:	160102
Performing Laboratory:	ABC Laboratories, Inc. (now EAG Laboratories) Columbia, Missouri, USA
Reference:	KCP 10.2.1/4
Report:	Goudie, O.; 2016; GF-3308: Acute Toxicity to the Cladoceran, *Daphnia magna*, Determined Under Static-Renewal Test Conditions; ABC Laboratories, Inc. (now EAG Laboratories), Columbia, Missouri, USA; Lab Study No. 83495; DAS Study No. 160102; 01 December 2016; Unpublished
Guideline(s):	Yes, SANCO/3029/99 rev.4
Guideline Deviations:	Yes The number of replicate recoveries (N = 4) assessed at the highest fortification level was less than described in the guideline (N = 5)
GLP:	Yes
Acceptability:	Yes
Method Alterations:	160102 Amendment 1 was based on 160103 Amendment 1. The original method was performed in freshwater algal nutrient medium (FWAM) instead of freshwater as used in this study. The original method included centrifugation, rinsing the culture tube, and adding the resulting rinse to the sample, none of which occurred in this study. The original method had MQLs of 0.20, 0.41, 0.020, and 0.00041 mg GF-3308/L and this study had an MQL of 0.0042 mg GF-3308/L. The original method used fortification levels of 0.985, 2.25, 45.9, and 65.6 mg GF-3308/L while this study used fortification levels of 0.0279 and 0.572 mg GF-3308/L.

MATERIALS AND METHODS

Method Principle

Residues of GF-3308, based upon the analysis of XDE-777, were determined from samples of freshwater by diluting with 0.2% formic acid in acetonitrile (ACN). Further dilutions were performed using formic acid:ACN:water (0.1:50:50) to dilute within the range of the calibration curve, if necessary. The final sample was analysed for GF-3308, based upon the analysis of XDE-777, by liquid chromatography coupled with positive-ion electrospray tandem mass spectrometry (LC-MS/MS).

RESULTS AND DISCUSSION

Mean recovery values at each fortification concentration were within the acceptance range (mean recovery 70-110%; RSD ≤ 20%). The results obtained are summarised in the following tables.

Table A 48: Recovery results from method validation of GF-3308, based upon the analysis of XDE-777, (*m/z* 615.0/239.2) using the analytical method

Matrix	Analyte	Fortification level (mg GF-3308/L)	Mean Recovery (%)	RSD (%)	n	Comments
Freshwater	XDE-777	0.0279	113	8	6	
Freshwater	XDE-777	0.572	109	5	4	

Table A 49: Characteristics for the analytical method used for validation of GF-3308, based upon the analysis of XDE-777, residues in freshwater

	GF-3308, based upon the analysis of XDE-777
Specificity	*m/z* 615.0/239.2 *m/z* 615.0/515.4 blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting r≥0.995 5 data points
Calibration range	Concentration range of 0.0100 to 0.160 ng/mL Sample equivalent range of 0.0042-0.067 mg GF-3308/L
Limit of determination/quantification	LOQ = 0.0279 mg GF-3308/L, equivalent to 0.066 ng XDE-777/mL

CONCLUSION

The method was considered acceptable for the determination of GF-3308, based upon the analysis of XDE-777, in freshwater due to acceptable precision and accuracy demonstrated within this study.

A 2.1.1.13 Analytical method 13

A 2.1.1.13.1 Method validation

Comments of zRMS:	The study was evaluated in the Registration Report, Part B5 for GF-3308 on 24.08.2022. Summary: The analytical method of Goudie (2018) for the determination of X642188 (a metabolite of XDE-777) in samples of moderately hard freshwater by LC-MS/MS has been successfully validated in accordance with the EU guidance document SANCO/3029/99 rev. 4. LOQ = 0.02 µg/L. The mean recovery of each fortification level and the overall mean recovery value was 70 – 110% with an RSD < 20%. The validation parameters are acceptable. The method is considered fit for purpose.

Reference:	KCP 10.2.1/5
Report:	Goudie, O; 2018; X642188 (a metabolite of XDE-777): Acute Tox-icity to the Cladoceran, Daphnia magna, Determined Under Flow-Through Test Conditions; ABC Laboratories, Inc. (now EAG, Inc.), Columbia, Missouri, USA; Lab Study No. 87148; DAS Study No. 180562 ; 30 August 2018; Unpublished
Guideline(s):	Yes, SANCO/3029/99 rev.4
Guideline Deviations:	No
GLP:	Yes
Acceptability:	Yes

Method Alterations: 180562 Protocol was based on 180563 Amendment 1, except that the matrix in 180562 Protocol was freshwater and the applicable matrix in 180563 Amendment 1 was freshwater (overlying water).

MATERIALS AND METHODS

Method Principle

Residues of X642188 were determined from samples of moderately hard freshwater by diluting with 0.2% formic acid in acetonitrile (ACN) and, if necessary, further diluting with 0.1:50:50 formic acid:ACN:water. The final sample is analysed for X642188 by liquid chromatography coupled with positive-ion electrospray tandem mass spectrometry (LC-MS/MS).

RESULTS AND DISCUSSION

Mean recovery values at each fortification concentration were within the acceptance range (mean recovery 70-110%; RSD ≤ 20%). The results obtained are summarised in the following tables.

Table A 50: Recovery results from method validation of freshwater (*m/z* 515.00/124.00) using the analytical method

Matrix	Analyte	Fortification level (µg X642188/L)	Mean Recovery (%)	RSD (%)	n	Comments
Freshwater	X642188	0.020	106	9	10	5 QC samples from definitive test analyses, ranging from 90 to 115%
Freshwater	X642188	30	99	5	10	5 QC samples from definitive test analyses, ranging from 93 to 107%

Table A 51: Characteristics for the analytical method used for validation of X642188 residues in freshwater

	X642188
Specificity	*m/z* 515.000/124.00 *m/z* 515.000/152.00 *m/z* 515.000/239.00 blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting r≥0.994 6 data points
Calibration range	Concentration range of 0.0050 – 0.16 ng/mL freshwater. Sample equivalent range of 0.010 – 0.32 mg X642188/L in freshwater
Limit of determination/quantification	LOQ = 0.02 µg/L

CONCLUSION

This method was successfully validated for the determination of X642188 in freshwater.

A 2.1.1.14 Analytical method 14

A 2.1.1.14.1 Method validation

Comments of zRMS:	The study was evaluated in the Registration Report, Part B5 for GF-3308 on 24.08.2022. Summary: The analytical method of Goudie, O.J., Schneider, S.Z., Zhang, L, and. Martin, K.H. (2020) for the determination of fenpicoxamid in samples of freshwater by LC-MS/MS has been

	successfully validated in accordance with the EU guidance document SANCO/3029/99 rev. 4. LOQ=15.0 µg GF-3307/L (0.705 µg fenpicoxamid/L) The mean recovery of each fortification level and the overall mean recovery value was 70 – 110% with an RSD < 20%. The validation parameters are acceptable. The method is considered fit for purpose.

Method Identifier No.:	191366
Performing Laboratory:	Eurofins EAG Agroscience, LLC, Easton, Maryland, USA
Reference:	KCP 10.2.1/6
Report:	Goudie, O.J., Schneider, S.Z., Zhang, L, and. Martin, K.H.; 2020; GF-3307: A 48-Hour Static-Renewal Acute Toxicity Test with the Cladoceran (*Daphnia magna*); Eurofins EAG Agroscience, LLC, 8598 Commerce Drive, Easton, MD 21601, USA; Lab Study No. 379A-305; DAS Study No. 191366 ; 20 February 2020; Unpublished
Guideline(s):	Yes, SANCO/3029/99 rev.4
Guideline Deviations:	No
GLP:	Yes
Acceptability:	Yes
Method Alterations:	None

MATERIALS AND METHODS

Method Principle

Residues of GF-3307, analyzed for fenpicoxamid and prothioconazole, are determined from samples of freshwater by diluting the samples into calibration curve range using 50:50: 0.1 (v/v/v) acetonitrile:freshwater:formic acid. The final sample is analysed for fenpicoxamid and prothioconazole by liquid chromatography coupled with positive-ion electrospray tandem mass spectrometry (LC-MS/MS).

RESULTS AND DISCUSSION

Mean recovery values at each fortification concentration were within the acceptance range (mean recovery 70-110%; RSD ≤ 20%). The results obtained are summarised in the following tables.

Table A 52: Recovery results from matrix fortification samples of GF-3307 analyzed for fenpicoxamid (*m/z* 615.200/239.000) using the analytical method

Matrix	Analyte	Fortification level (µg GF-3307/L)	Mean Recovery (%)	RSD (%)	n	Comments
Freshwater	fenpicoxamid	15.0	94.5	1.71	5	5 QC samples from definitive test analyses, ranging from 92.6 to 97.5%
Freshwater	fenpicoxamid	520	99.6	9.27	5	5 QC samples from definitive test analyses, ranging from 93.7 to 116%

Table A 53: Recovery results from matrix fortification samples of GF-3307 analyzed for prothioconazole (m/z 334.100/326.000) using the analytical method

Matrix	Analyte	Fortification level (µg GF-3307/L)	Mean Recovery (%)	RSD (%)	n	Comments
freshwater	prothioconazole	15.0	96.9	4.62	5	5 QC samples from definitive test analyses, ranging from 90.9 to 103%

Matrix	Analyte	Fortification level (µg GF-3307/L)	Mean Recovery (%)	RSD (%)	n	Comments
freshwater	prothioconazole	520	102	18.2	5	5 QC samples from definitive test analyses, ranging from 90.0 to 134%

Table A 54: Characteristics for the analytical method used for determination of GF-3307, analyzed for fenpicoxamid and prothioconazole, residues in freshwater

	fenpicoxamid	prothioconazole
Specificity	*m/z* 615.200/239.000 blank value <30% LOQ	m/z 334.100/326.000 blank value <30% LOQ
Calibration (type, number of data points)	Linear regression analysis with 1/x weighting r≥0.998 5 data points	Linear regression analysis with 1/x weighting r≥0.999 5 data points
Calibration range	Concentration range of 0.240 – 4.00 µg a.i./L Sample equivalent range of 0.511-85.1 ug GF-3307/L	Concentration range of 0.240 – 4.00 µg a.i./L Sample equivalent range of 2.47 – 41.2 ug GF-3307/L
Limit of determination/quantification	LOQ=15.0 µg GF-3307/L (~~7.05~~ 0.705 µg fenpicoxamid/L) LOD = 4.50 µg GF-3307/L (~~2.12~~ 0.212 µg fenpicoxamid/L)	LOQ=15.0 µg GF-3307/L (1.46 µg prothioconazole/L) LOD = 4.50 µg GF-3307/L (0.437 µg prothioconazole/L)

CONCLUSION

The method was considered acceptable for the determination of GF-3307, analyzed for fenpicoxamid and prothioconazole, in freshwater.

A 2.1.1.15 Analytical method 15

A 2.1.1.15.1 Method validation

Comments of zRMS:	The study was evaluated in the Registration Report, Part B5 for GF-3308 on 24.08.2022. Summary: The analytical method of Goudie, O.J., Schneider, S.Z., Sneckenberger, G., and Zhang, L. (2021) for the determination of fenpicoxamid in samples of freshwater by LC-MS/MS has been successfully validated in accordance with the EU guidance document SANCO/3029/99 rev. 4. LOQ=0.160 µg GF 2925/L (19.7 ng a.i./L) The mean recovery of each fortification level and the overall mean recovery value was 70 – 120% with an RSD < 20%. This is acceptable according to SANTE/2020/12830, Rev.1. The validation parameters are acceptable. The method is considered fit for purpose.

Method Identifier No.:	202284 Appendix 6
Performing Laboratory:	Eurofins EAG Agroscience, LLC Easton, Maryland, U.S.A.
Reference:	KCP 10.2.1/7
Report:	Goudie, O.J., Schneider, S.Z., Sneckenberger, G., and Zhang, L.; 2021; GF-2925: A Static-Renewal Acute Toxicity Test with the Cladoceran (*Daphnia magna*); Eurofins EAG Agroscience, LLC, 8598 Commerce Drive, Easton, MD 21601, USA; Lab Study No. 379A-343; DAS Study No. 202284 ; 05 March 2021; Unpublished

Guideline(s):	SANCO/3029/99 rev.4
Guideline Deviations:	No
GLP:	Yes
Acceptability:	Yes
Method Alterations:	None

MATERIALS AND METHODS

Method Principle

Residues of GF-2925 (analysed for active ingredient fenpicoxamid) are determined from samples of freshwater. The samples were diluted initially with 0.2% formic acid in acetonitrile to achieve a solvent composition of 50 : 50 : 0.1 (v/v/v) acetonitrile : freshwater : formic acid. Additional dilutions were performed, as necessary to bring all samples into the range of the calibration curve, using 50 : 50 : 0.1 (v/v/v) acetonitrile : freshwater : formic acid. The final samples are analysed for fenpicoxamid by liquid chromatography coupled with positive-ion electrospray tandem mass spectrometry (LC-MS/MS).

RESULTS AND DISCUSSION

Mean recovery values at each fortification concentration were within the acceptance range, or slightly exceeded the acceptance range (mean recovery 70-110%; RSD ≤ 20%). The results obtained are summarised in the following tables.

Table A 55: Method validation results for fenpicoxamid (*m/z* 615.200/239.000) using the analytical method

Matrix	Analyte	Fortification level (ng a.i./L)	Mean Recovery (%)	RSD (%)	n	Comments
freshwater	fenpicoxamid	19.7	111	8.8	5	
freshwater	fenpicoxamid	6150	108	14	5	

Table A 56: Characteristics for the analytical method used for analysis of GF-2925 (analysed for active ingredient fenpicoxamid) residues in freshwater

	GF-2925 (analysed for fenpicoxamid)
Specificity	*m/z* 615.2/239.0 (Q) *m/z* 615.2/515.1 (C) blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting r≥0.999 6 data points
Calibration range	Concentration range of 3.00-40.0 ng a.i./L (equivalent to 0.024-0.33 µg GF-2925/L)
Limit of determination/quantification	LOD=0.0480 µg GF-2925/L (5.90 ng a.i./L) LOQ=0.160 µg GF-2925/L (19.7 ng a.i./L)

CONCLUSION

The method was considered acceptable for the determination of GF-2925 (analysed for active ingredient fenpicoxamid) in freshwater because the precision of all matrix fortification samples and mean of the high-level matrix fortification samples and overall mean met acceptance criteria. The mean of the low-level matrix fortification samples slightly exceeded the acceptance criteria of 110% (111%).

A 2.1.1.16 Analytical method 16

A 2.1.1.16.1 Method validation

Comments of zRMS:	The study was evaluated in the Registration Report, Part B5 for GF-3308 on 24.08.2022. Summary: The analytical method of Hadsell, R. (2014) for the determination of fenpicoxamid in samples of freshwater by LC-MS/MS has been successfully validated in accordance with the EU guidance document SANCO/3029/99 rev. 4. LOQ = 0.009 mg GF-3307/L, equivalent to 0.0217 ng a.i./mL. The mean recovery of each fortification level and the overall mean recovery value was 70 – 110% with an RSD < 20%. The validation parameters are acceptable. The method is considered fit for purpose.

Method Identifier No.:	140489 Amendment 1
Performing Laboratory:	ABC Laboratories, Inc. (now EAG, Inc.) Columbia, Missouri, USA
Reference:	KCP 10.2.1/8
Report:	Hadsell, R.; Erin Hoover; 2014; GF-3307: Acute Toxicity to the Cladoceran, *Daphnia magna*, Determined Under Static-Renewal Test Conditions; ABC Laboratories, Inc. (now EAG, Inc.), Columbia, Missouri, USA; Lab Study No. 81070; DAS Study No. 140489 ; 28 August 2014, Revised 2018; Unpublished
Guideline(s):	Yes, SANCO/3029/99 rev.4
Guideline Deviations:	No
GLP:	Yes
Acceptability:	Yes
Method Alterations:	N/A

MATERIALS AND METHODS

Method Principle

Residues of GF-3307, based on analysis of XDE-777, were determined from samples of freshwater by diluting with 0.2% formic acid in acetonitrile and, if necessary, further diluting with 0.1:50:50 acid: acetonitrile:water The final sample was analysed for XDE-777 by liquid chromatography coupled with positive-ion electrospray tandem mass spectrometry (LC-MS/MS).

RESULTS AND DISCUSSION

Mean recovery values at each fortification concentration were within the acceptance range (mean recovery 70-110%; RSD ≤ 20%). The results obtained are summarised in the following tables.

Table A 57: Recovery results from method validation of GF-3307, based on analysis of XDE-777, (*m/z* 615.0/239.2) using the analytical method

Matrix	Analyte	Fortification level (mg GF-3307/L)	Mean Recovery (%)	RSD (%)	n	Comments
Freshwater	XDE-777	0.00900	98	4	4	
Freshwater	XDE-777	0.560	100	3	4	

Table A 58: Characteristics for the analytical method used for validation of GF-3307, based on analysis of XDE-777, residues in freshwater

	GF-3307, based on analysis of XDE-777
Specificity	*m/z* 615.0/239.2 blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting r≥0.995 7 data points
Calibration range	Concentration range of 0.0200-0.750 ng/ XDE-777mL Sample equivalent range of 0.00833-0.313 mg GF-3307/L
Limit of determination/quantification	LOQ = 0.009 mg GF-3307/L, equivalent to 0.0217 ng a.i./mL

CONCLUSION

The method was considered acceptable for the determination of GF-3307 based on XDE-777 in freshwater.

A 2.1.1.17 Analytical method 17

A 2.1.1.17.1 Method validation

Comments of zRMS:	A method has been validated for the determination of XDE-777 and prothioconazole in freshwater algal nutrient medium (FWAM) according to the SANCO/3029/99 rev.4. The samples were analyzed using LC-MS/MS. LOQ for XDE-777= 0.050 mg T.P./L, equivalent to 0.120 ng a.i./mL LOQ for prothioconazole= 0.050 mg T.P./L, equivalent to 0.235 ng a.i./mL Mean recoveries were in the range of 70 – 110% with relative standard deviations of ≤20% for XDE-777 and prothioconazole at each level. The study is acceptable.

Method Identifier No.:	140491 Amendment 1
Performing Laboratory:	ABC Laboratories, Inc. (now EAG, Inc.) Columbia, Missouri, USA
Reference:	KCP 10.2.1/9
Report:	Hicks, S.; 2014; GF-3307: Growth Inhibition Test with the Unicellular Green Alga, *Pseudokirchneriella subcapitata*; ABC Laboratories, Inc. (now EAG, Inc.), Columbia, Missouri, USA; Lab Study No. 81069; DAS Study No. 140491 ; 24 December 2014, Final report addendum 2020; Unpublished
Guideline(s):	Yes, SANCO/3029/99 rev.4
Guideline Deviations:	No
GLP:	Yes
Acceptability:	Yes
Method Alterations:	In the original method, both XDE-777 and prothioconazole use positive-ion polarity. In the study, prothioconazole used negative-ion polarity and XDE-777 used positive-ion polarity.

MATERIALS AND METHODS

Method Principle

Residues of GF-3307, based on analysis of XDE-777 and prothioconazole, were determined from samples of freshwater algal nutrient medium (FWAM) by diluting with 0.2% formic acid in acetonitrile (ACN),

centrifuging the sample, and, if necessary, further diluting the supernatant with 0.1:50:50 acid:ACN:water. The final sample was analysed for GF-3307 by liquid chromatography coupled with negative-ion (for prothioconazole) and positive-ion (for XDE-777) electrospray tandem mass spectrometry (LC-MS/MS).

RESULTS AND DISCUSSION

Mean recovery values at each fortification concentration were within the acceptance range (mean recovery 70-110%; RSD ≤ 20%). The results obtained are summarised in the following tables.

Table A 59: Recovery results from method validation of GF-3307, based on analysis of XDE-777 (*m/z* 615.0/239.2) using the analytical method

Matrix	Analyte	Fortification level (mg T.P./L)	Mean Recovery (%)	RSD (%)	n	Comments
FWAM	XDE-777	0.050	99	5	6	
FWAM	XDE-777	70.8	102	3	6	

Table A 60: Recovery results from method validation of GF-3307, based on analysis of prothioconazole, (*m/z* 342.0/100.0) using the analytical method

Matrix	Analyte	Fortification level (mg T.P./L)	Mean Recovery (%)	RSD (%)	n	Comments
FWAM	prothioconazole	0.050	97	3	3	
FWAM	prothioconazole	70.8	103	1	3	

Table A 61: Characteristics for the analytical method used for validation of GF-3307, based on analysis of XDE-777 and prothioconazole residues in FWAM

	GF-3307, based on analysis of XDE-777	GF-3307, based on analysis of prothioconazole
Specificity	*m/z* 615.0/239.2 *m/z* 615.0/515.4 blank value <LOQ	*m/z* 342.0/100.0 *m/z* 342.0/306.0 and *m/z* 342.0/180.0 blank value < LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting r≥0.995 7 data points	linear regression analysis with 1/x weighting r≥0.995 7 data points
Calibration range	Concentration range of 0.0200-1.00 ng/mL Sample equivalent range of 0.00833-0.417 mg GF-3307/L	Concentration range of 0.0200-1.00 ng/mL Sample equivalent range of 0.00426-0.213 mg GF-3307/L
Limit of determination/quantification	LOQ = 0.050 mg T.P./L, equivalent to 0.120 ng a.i./mL	LOQ = 0.050 mg T.P./L, equivalent to 0.235 ng a.i./mL

CONCLUSION

The method was considered acceptable for the determination of GF-3307 based on analysis of XDE-777 and prothioconazole.

A 2.1.1.18 Analytical method 18

A 2.1.1.18.1 Method validation

Comments of zRMS:	The study was evaluated in the Registration Report, Part B5 for GF-3308 on 24.08.2022. Summary:

	The analytical method of Hughes, J. (2018) for the determination of X12019520 (a metabolite of XDE-777) in samples of moderately hard freshwater by LC-MS/MS has been successfully validated in accordance with the EU guidance document SANCO/3029/99 rev. 4. LOQ = 4.9 mg/L. The mean recovery of each fortification level and the overall mean recovery value was 70 – 110% with an RSD < 20%. The number of replicate recoveries (N = 4) assessed at the lowest fortification level was less than described in the guideline (N = 5). The validation parameters are acceptable. The method is suitable for fit for purpose.

Reference:	KCP 10.2.1/10
Report:	Hughes, J.; 2018; X12019520 (a metabolite of XDE-777): Acute Toxicity to the Rainbow Trout, Oncorhynchus mykiss, Determined Under Static-Renewal Test Conditions; xxxxxxxxxxxxxxx ; 07 August 2018; Unpublished
Guideline(s):	Yes, SANCO/3029/99 rev.4
Guideline Deviations:	No
GLP:	Yes
Acceptability:	Yes
Method Alterations:	180560 Protocol was based on 160128 Amendment 2.

MATERIALS AND METHODS

Method Principle

Residues of X12019520 (a metabolite of XDE-777) were determined from samples of moderately hard freshwater by diluting with 0.2% formic acid in acetonitrile, and, if necessary, further diluted with 0.1:50:50 formic acid:acetonitrile:water. The final sample was analysed for X12019520 by liquid chromatography system with tandem mass spectrometry (LC-MS/MS).

RESULTS AND DISCUSSION

Mean recovery values at each fortification concentration were within the acceptance range (mean recovery 70-110%; RSD ≤ 20%). The results obtained are summarised in the following tables.

Table A 62: Recovery results from method validation of X12019520 (*m/z* 189.00/143.00) using the analytical method

Matrix	Analyte	Fortification level (mg X12019520/L)	Mean Recovery (%)	RSD (%)	n	Comments
Freshwater	X12019520	4.9	106	7	4	
Freshwater	X12019520	14	110	5	9	

Table A 63: Characteristics for the analytical method used for validation of X12019520 residues in freshwater

	X12019520
Specificity	*m/z* 189.00/143.00 *m/z* 189.00/128.00 blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting r≥0.999 6 data points
Calibration range	Concentration range of 0.010-0.52 ng/mL Sample equivalent range of 0.80-42 mg X12019520/L

Limit of determination/quantification	LOQ = 4.9 mg/L

CONCLUSION

This method was successfully validated for the determination of X12019520 in freshwater.

A 2.1.1.19 Analytical method 19

A 2.1.1.19.1 Method validation

Comments of zRMS:	The study was evaluated in the Registration Report, Part B5 for GF-3308 on 24.08.2022. Summary: The analytical method of Hughes, J. (2018) for the determination of X12019520 (a metabolite of XDE-777) in samples of moderately hard freshwater by HPLC-UV has been successfully validated in accordance with the EU guidance document SANCO/3029/99 rev. 4. LOQ = 0.096 mg/L The mean recovery of each fortification level and the overall mean recovery value was 70 – 110% with an RSD < 20%. The number of replicate recoveries (N = 4) assessed at the lowest fortification level was less than described in the guideline (N = 5). The validation parameters are acceptable. The method is suitable for fit for purpose.

Reference:	KCP 10.2.1/11
Report:	Hughes, J.; 2018; X12446477 (a metabolite of XDE-777): Acute Tox-icity to the Rainbow Trout, Oncorhynchus mykiss, Determined Un-der Static-Renewal Test Conditions; xxxxxxxxxxxxxx ; 18 July 2018; Unpublished
Guideline(s):	Yes, SANCO/3029/99 rev.4
Guideline Deviations:	No
GLP:	Yes
Acceptability:	Yes
Method Alterations:	180561 Protocol was based on 140485 Amendment 1.

MATERIALS AND METHODS

Method Principle

Residues of X12446477 (a metabolite of XDE-777) were determined from samples of moderately hard freshwater by diluting, if necessary, with HPLC water. The final sample was analysed for X12446477 by high performance liquid chromatography with ultraviolet detection (HPLC-UV).

RESULTS AND DISCUSSION

Mean recovery values at each fortification concentration were within the acceptance range (mean recovery 70-110%; RSD ≤ 20%). The results obtained are summarised in the following tables.

Table A 64: Recovery results from method validation of X12446477 using the analytical method

Matrix	Analyte	Fortification level (mg X12446477/L)	Mean Recovery (%)	RSD (%)	n	Comments
Freshwater	X12446477	0.096	101	1	4	
Freshwater	X12446477	17	106	1	9	

Table A 65: Characteristics for the analytical method used for validation of X12446477 residues in

freshwater

	X12446477
Specificity	blank value <30% MQL
Calibration (type, number of data points)	linear regression analysis without weighting r≥0.999 6 data points
Calibration range	Concentration range of 0.050-1.6 mg/L
Limit of determination/quantification	LOQ = 0.096 mg/L

CONCLUSION

This method was successfully validated for the determination of X12446477 in freshwater.

A 2.1.1.20 Analytical method 20

A 2.1.1.20.1 Method validation

Comments of zRMS:	The study was evaluated in the Registration Report, Part B5 for GF-3308 on 24.08.2022. Summary: The analytical method of Beasley, J. (2018) for the determination of X642188 (a metabolite of XDE-777) in samples of sediment, freshwater and porewater by LC-MS/MS has been validated in accordance with the EU guidance document SANCO/3029/99 rev. 4. The lowest fortification level for X642188 in porewater was 0.000020 mg/L, however precision and accuracy were unacceptable. LOQ = 0.000020 mg/L (overlaying water) LOQ = 14 mg/L (porewater) LOQ = 0.046 mg/kg (sediment) The mean recovery of each fortification level and the overall mean recovery value was 70 – 120% with an RSD < 20% (without freshwater (pore water) at 0.000020 mg/L level). The validation parameters are acceptable. The method is suitable for fit for purpose.

Reference:	KCP 10.2.2/1
Report:	Beasley, J.; 2018; X642188 (a metabolite of XDE-777): Chronic Tox-icity in Whole Sediment to Freshwater Midge, Chironomus riparius, Using Spiked Sediment; ABC Laboratories, Inc. (now EAG, Inc.), Columbia, Missouri, USA; Lab Study No. 87149; DAS Study No. 180563; 30-Aug-2018; Unpublished
Guideline(s):	Yes, SANCO/3029/99 rev.4
Guideline Deviations:	Yes, method recoveries for X642188 were outside the acceptable range of 70-110%, and RSD values exceeded 20% at the 0.000020 mg/L concentration level in pore water. Although the method was not sufficiently demonstrated in pore water at the 0.000020 mg/L level, the analytical methods used to support this study were otherwise acceptable and authenticate the values driving the study endpoints. The overall scope and purpose of this study is unaffected by this guideline deviation.
GLP:	Yes
Acceptability:	Yes
Method Alterations:	None

MATERIALS AND METHODS

Method Principle

Residues of X642188 were determined from samples of sediment by centrifuging the sample to remove pore water (retained for subsequent analysis), then diluting with 0.1:50:50 formic acid:ACN:water, followed by shaking and centrifugation, and transferring the liquid layer to a Falcon tube. The shaking and centrifugation process was repeated two additional times with the resulting transferred liquid layers to the 50-mL Falcon tube, then the liquid was extracted by diluting with 0.1:50:50 formic acid:ACN:water, and, if necessary, further diluting with 0.1:50:50 formic acid:ACN:water. Residues of X642188 were determined from samples of moderately hard freshwater (pore water) by centrifuging to utilize the supernatant, then diluting the supernatant with 0.2% formic acid in acetonitrile (ACN) and, if necessary, further diluting with 0.1:50:50 formic acid:ACN:water. Residues of X642188 were determined from samples of moderately hard freshwater (overlaying water) by diluting with 0.2% formic acid in acetonitrile (ACN) and, if necessary, further diluting with 0.1:50:50 formic acid:ACN:water. The final sample was analysed for X642188 by liquid chromatography coupled with positive-ion electrospray tandem mass spectrometry (LC-MS/MS).

RESULTS AND DISCUSSION

Mean recovery values at each fortification concentration in sediment and freshwater (overlaying water), and at the 14 mg X642188/L in overlying and pore water were within the acceptance range (mean recovery 70-110%; RSD ≤ 20%). Mean recovery values at 0.000020 mg X642188/L in overlying water were higher than 110%, but the precision of the assay (%RSD) was < 20%, therefore were considered acceptable. Mean recovery values at 0.000020 mg X642188/L in freshwater (pore water) were higher than 110% and the precision of the assay (%RSD) was greater than 20%. Increased low spike (0.000020 mg X642188/L) recoveries in pore water may have been the result of matrix enhancement. The results obtained are summarised in the following tables.

Table A 66: Recovery results from method validation of X642188 (*m/z* 515.000/124.000) in sediment using the analytical method

Matrix	Analyte	Fortification level (mg X642188/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Sediment	X642188	0.046	86	12	10	5 method validation samples + 5 QC samples from definitive test analyses, ranging from 70 to 121%
Sediment	X642188	16	89	11	10	5 method validation samples + 5 QC samples from definitive test analyses, ranging from 71 to 115%

Table A 67: Recovery results from method validation of X642188 (*m/z* 515.000/124.000) in freshwater (pore water) using the analytical method

Matrix	Analyte	Fortification level (mg X642188/L)	Mean Recovery (%)	RSD (%)	n	Comments
Freshwater (pore water)	X642188	0.000020	122	47	10	5 method validation samples + 5 QC samples from definitive test analyses, ranging from 83 to 263%
Freshwater (pore water)	X642188	14	98	11	10	5 method validation samples + 5 QC samples from definitive test analyses, ranging from 75 to 111%

Table A 68: Recovery results from method validation of X642188 (*m/z* 515.000/124.000) in freshwater (overlaying water) using the analytical method

Matrix	Analyte	Fortification level (mg X642188/L)	Mean Recovery (%)	RSD (%)	n	Comments
Freshwater (overlaying water)	X642188	0.000020	114	8	5	5 QC samples from definitive test analyses, ranging from 99 to 121%
Freshwater (overlaying water)	X642188	14	99	15	5	5 QC samples from definitive test analyses, ranging from 77 to 115%

Table A 69: Characteristics for the analytical method used for validation of X642188 residues in sediment and freshwater (pore and overlaying water)

	X642188
Specificity	*m/z* 515.000/124.000 *m/z* 515.000/152.000 *m/z* 515.000/239.000 blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting r≥0.997 6 data points
Calibration range	Concentration range of 0.0050 – 0.16 ng/mL in sediment and freshwater (pore and overlaying water). Sample equivalent range of 0.0038 – 0.12 mg X642188/kg in sediment and 0.000010 – 0.0032 mg X642188/L in freshwater (pore and overlaying water)
Limit of determination/quantification	LOQ = 0.000020 mg/L (overlaying water) LOQ = 14 mg/L (porewater) LOQ = 0.046 mg/kg (sediment)

CONCLUSION

This method was successfully validated for the determination of X642188 in sediment, freshwater and porewater (at the 14 mg/L concentration level). Although the method was unable to be validated in porewater at the 0.000020 mg/L level due to unacceptable precision and accuracy, the overall analytical supporting data has been demonstrated to be effective for supporting the purpose of this study.

A 2.1.1.21 Analytical method 21

A 2.1.1.21.1 Method validation

Comments of zRMS:	The study was evaluated in the Registration Report, Part B5 for GF-3308 on 24.08.2022. Summary: The analytical method of Dinehart, S. (2019) for the determination of X642188 (a metabolite of XDE-777) in samples of sediment and freshwater by LC-MS/MS has been successfully validated in accordance with the EU guidance document SANCO/3029/99 rev. 4. LOQ=0.046 mg/kg in sediment LOQ=0.00033 mg/L in water The mean recovery of each fortification level and the overall mean recovery value was 70 – 110% with an RSD < 20%. The validation parameters are acceptable. The method is suitable for fit for purpose.

Method Identifier No.: 180563 Amendment 1

Performing Laboratory:	Eurofins EAG Agroscience, LLC Columbia, Missouri
Reference:	KCP 10.2.2/2
Report:	Dinehart, S.; 2019; X642188 (a metabolite of XDE-777): A Prolonged Sediment Toxicity Test with Lumbriculus variegatus Using Spiked Sediment; Eurofins EAG Agroscience, LLC, Columbia, Missouri; Lab Study No. 87169; DAS Study No. 180639; 23 October 2019; Unpublished
Guideline(s):	Yes, SANCO/3029/99 rev.4
Guideline Deviations:	No
GLP:	Yes
Acceptability:	Yes
Method Alterations:	180639 Amendment No. 2 was based on 180563 Amendment 1 with no modification.

MATERIALS AND METHODS

Method Principle

Residues of X642188 were determined from samples of sediment by centrifuging the sample to remove pore water (retained for subsequent analysis), then diluting with 0.1:50:50 formic acid:ACN:water, followed by shaking and centrifugation, and transferring the liquid layer to a Falcon tube. The shaking and centrifugation process was repeated two additional times with the resulting liquid layers transferred to the 50-mL Falcon tube, then the liquid was extracted by diluting with 0.1:50:50 formic acid:ACN:water, and, if necessary, further diluting with 0.1:50:50 formic acid:ACN:water. Residues of X642188 were determined from samples of moderately hard freshwater (pore water) by centrifuging to utilize the supernatant, then diluting the supernatant with 0.2% formic acid in acetonitrile (ACN) and, if necessary, further diluting with 0.1:50:50 formic acid:ACN:water. Residues of X642188 were determined from samples of moderately hard freshwater (overlaying water) by diluting with 0.2% formic acid in acetonitrile (ACN) and, if necessary, further diluting with 0.1:50:50 formic acid:ACN:water. The final sample was analysed for X642188 by liquid chromatography coupled with positive-ion electrospray tandem mass spectrometry (LC-MS/MS).

RESULTS AND DISCUSSION

Mean recovery values at each fortification concentration were within the acceptance range (mean recovery 70-110%; RSD ≤ 20%). The results obtained are summarised in the following tables.

Table A 70: Recovery results from method validation of X642188 (*m/z* 515.000/124.000) using the analytical method

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Sediment	X642188	0.046	85	4	5	5 QC samples from definitive test analyses, ranging from 81 to 89%
Sediment	X642188	98	91	3	5	5 QC samples from definitive test analyses, ranging from 86 to 94%

Table A 71: Recovery results from method validation of X642188 (*m/z* 515.000/124.000) using the analytical method

Matrix	Analyte	Fortification level (mg/L)	Mean Recovery (%)	RSD (%)	n	Comments
Freshwater (overlying water)	X642188	0.00033	97	7	5	5 QC samples from definitive test analyses, ranging from 87 to 105%
Freshwater (overlying water)	X642188	96	106	3	5	5 QC samples from definitive test analyses, ranging from 103 to 111%

Table A 72: Recovery results from method validation of X642188 (*m/z* 515.000/124.000) using the analytical method

Matrix	Analyte	Fortification level (mg/L)	Mean Recovery (%)	RSD (%)	n	Comments
Freshwater (pore water)	X642188	0.00033	95	5	5	5 QC samples from definitive test analyses, ranging from 89 to 100%
Freshwater (pore water)	X642188	96	102	4	5	5 QC samples from definitive test analyses, ranging from 97 to 107%

Table A 73: Characteristics for the analytical method used for validation of X642188 residues in sediment and freshwater

	X642188
Specificity	*m/z* 515.000/124.000 *m/z* 515.000/152.000 blank value <LOD
Calibration (type, number of data points)	linear regression analysis with 1/x weighting r≥0.996 6 data points
Calibration range	Concentration range of 0.0050 – 0.16 ng/mL in sediment and freshwater (pore and overlaying water). Sample equivalent range of 0.0038 – 0.123 mg/kg in sediment and 0.00010 – 0.0032 mg/L in freshwater (pore and overlaying water)
Limit of quantification	LOQ=0.046 mg/kg in sediment LOQ=0.00033 mg/L in water

CONCLUSION

This method was successfully validated for the determination of X642188 in sediment and freshwater.

A 2.1.1.22 Analytical method 22

A 2.1.1.22.1 Method validation

Comments of zRMS:	The study was evaluated in the Registration Report, Part B5 for GF-3308 on 24.08.2022. Summary: The analytical method of Leak, T. (2018) for the determination of X12335723 (a metabolite of XDE-777) in samples of overlying water, pore water, and sediment.by LC-MS/MS has been successfully validated in accordance with the EU guidance document SANCO/3029/99 rev. 4. LOQ = 0.015 mg/L (water) LOQ = 0.0069 mg/kg (sediment) The mean recovery of each fortification level and the overall mean recovery value was 70 – 110% with an RSD < 20%. The validation parameters are acceptable. The method is suitable for fit for purpose.

Reference:	KCP 10.2.2/3
Report:	Leak, T.; 2018; X12335723 (a metabolite of XDE-777): Chronic Toxicity in Whole Sediment to Freshwater Midge, Chironomus ri-parius, Using Spiked Sediment; ABC Laboratories, Inc. (now EAG, Inc.), Columbia, Missouri, USA; Lab Study No. 87150; DAS Study No. 180564 ; 31 August 2018; Unpublished
Guideline(s):	Yes, SANCO/3029/99 rev.4
Guideline Deviations:	No
GLP:	Yes
Acceptability:	Yes
Method Alterations:	None

MATERIALS AND METHODS

Method Principle

Residues of X12335723 were determined from samples of sediment by centrifuging the sample to remove pore water (retained for subsequent analysis), then diluting with 0.1:50:50 formic acid:acetonitrile (ACN):water, followed by shaking and centrifugation, and transferring the liquid layer to a Falcon tube. The shaking and centrifugation process was repeated two additional times with the resulting transferred liquid layers to the 50-mL Falcon tube, then the liquid was extracted by diluting with 0.1:50:50 formic acid:ACN:water, and, if necessary, further diluting with 0.1:25:75 formic acid:ACN:water. Residues of X12335723 were determined from samples of moderately hard freshwater (pore water) by centrifuging to utilize the supernatant, then diluting the supernatant with 0.2% formic acid in acetonitrile (ACN) and, if necessary, further diluting with 0.1:50:50 formic acid:ACN:water. Residues of X12335723 were determined from samples of moderately hard freshwater (overlying water) by diluting with 0.2% formic acid in acetonitrile (ACN) and, if necessary, further diluting with 0.1:50:50 formic acid:ACN:water. The final sample is analysed for X12335723 by liquid chromatography coupled with positive-ion electrospray tandem mass spectrometry (LC-MS/MS).

RESULTS AND DISCUSSION

Mean recovery values at each fortification concentration were within the acceptance range (mean recovery 70-110%; RSD ≤ 20%). The results obtained are summarised in the following tables.

Table A 74: Recovery results from method validation of X12335723 (*m/z* 357.300/257.000) in sediment using the analytical method

Matrix	Analyte	Fortification level (mg X12335723/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Sediment	X12335723	0.0069	95	13	10	5 method validation samples + 5 QC samples from definitive test analyses, ranging from 77 to 117%
Sediment	X12335723	17	92	9	10	5 method validation samples + 5 QC samples from definitive test analyses, ranging from 72 to 103%

Table A 75: Recovery results from method validation of X12335723 (m/z 357.300/257.000) in freshwater (pore water) using the analytical method

Matrix	Analyte	Fortification level (mg X12335723/L)	Mean Recovery (%)	RSD (%)	n	Comments
Freshwater (pore water)	X12335723	0.015	103	2	10	5 method validation samples + 5 QC samples from definitive test analyses, ranging from 99 to 106%
Freshwater (pore water)	X12335723	14	110	7	10	5 method validation samples + 5 QC samples from definitive test analyses, ranging from 92 to 118%

Table A 76: Recovery results from method validation of X12335723 (m/z 357.300/257.000) in freshwater (overlying water) using the analytical method

Matrix	Analyte	Fortification level (mg X12335723/L)	Mean Recovery (%)	RSD (%)	n	Comments
Freshwater (overlying water)	X12335723	0.015	102	2	5	5 QC samples from definitive test analyses, ranging from 100 to 106%
Freshwater (overlying water)	X12335723	14	110	5	5	5 QC samples from definitive test analyses, ranging from 101 to 115%

Table A 77: Characteristics for the analytical method used for validation of X12335723 residues in sediment and freshwater (pore and overlying water)

	X12335723
Specificity	*m/z* 357.300/257.000 *m/z* 357.300/239.000 *m/z* 357.300/211.000 blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting / r≥0.998 6 data points
Calibration range	Concentration range of 0.0050 – 0.16 ng/mL in sediment and freshwater (pore and overlying water). Sample equivalent range of 0.0038 – 0.12 mg X12335723/kg in sediment and 0.0040 – 0.13 mg X12335723/L in freshwater (pore and overlying water)
Limit of determination/quantification	LOQ = 0.015 mg/L (water) LOQ = 0.0069 mg/kg (sediment)

CONCLUSION

This method was successfully validated for the determination of X12335723 in overlying water, pore water, and sediment.

A 2.1.1.23 Analytical method 23

A 2.1.1.23.1 Method validation

Comments of zRMS:	The analytical method for the determination of fenpicoxamid (XDE-777), X642188 (metabolite of XDE-777) and prothioconazole in holding- and dilution water has been successfully validated with regards to specificity, linearity, accuracy and precision in accordance with guideline SANCO/3029/99, rev. 4.

	Mean recovery values at each fortification concentration were within the acceptance range, mean recovery 70 - 110% with RSD ≤ 20%. The limit of quantification (LOQ) for prothioconazole was 0.050 µg/L. The limit of quantification (LOQ) for XDE-777 was 0.025 µg/L and for metabolite X642188 was 0.0015 µg/L. The method is acceptable.

Method Identifier No.: 181382

Performing Laboratory: Fraunhofer Institute for Molecular Biology and Applied Ecology (IME), Auf dem Aberg 1, 57392 Schmallenberg, Germany

Reference: KCP 10.2.3/2

Report: Brüggemann, M., Böhmer, W., Kosak, L.; 2020; GF-3307: Population Effects Study in an Indoor Aquatic Microcosm with Daphnia magna; Fraunhofer Institute for Molecular Biology and Applied Ecology (IME), Auf dem Aberg 1, 57392 Schmallenberg, Germany; Lab Study No. DOW-051/7-50/G; Sponsor Study No. 181382; February 19, 2020; Unpublished

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

Guideline Deviations: No

GLP: Yes

Acceptability: Yes

Method Alterations: None

MATERIALS AND METHODS

Method Principle

Residues of the analytes Fenpicoxamid (XDE-777), X642188 (metabolite of XDE-777) and Prothioconazole are determined from samples of holding- and dilution water by diluting the samples with equal volumes of aqueous test media (holding- and dilution water) and acidified acetonitrile (Fenpicoxamid and X642188) or pure acetonitrile (Prothioconazole). The final diluted sample is analysed by liquid chromatography coupled with positive electrospray tandem mass spectrometry (LC-MS/MS).

RESULTS AND DISCUSSION

Mean recovery values at each fortification concentration were within the acceptance range (mean recovery 70-110%; RSD ≤ 20%). The results obtained are summarised in the following tables.

Table A 78: Recovery results from method validation of Fenpicoxamid (m/z 615.34 → m/z 239.00) using the analytical method

Matrix	Fortification level (µg/L)	Mean Recovery (%)	RSD (%)	n	Comments
Holding- and dilution water	0.0250	96.8	1.45	5	
	0.250	102.7	0.97	5	
	0.300	99.8	0.78	5	
	3.00	100.2	0.38	5	
	30.0	104.0	0.86	5	

Table A 79: Recovery results from method validation of X642188 (m/z 515.26 → m/z 124.01) using the analytical method

Matrix	Fortification level (µg/L)	Mean Recovery (%)	RSD (%)	n	Comments
Holding- and	0.0015	94.5	5.90	5	

Matrix	Fortification level (µg/L)	Mean Recovery (%)	RSD (%)	n	Comments
dilution water	0.0070	100.3	2.12	5	
	0.0150	94.0	3.14	5	
	0.0700	101.2	1.73	5	
	0.700	102.3	1.23	5	

Table A 80: Recovery results from method validation of Prothioconazole (*m/z 344.08 → m/z 125.02*) using the analytical method

Matrix	Fortification level (µg/L)	Mean Recovery (%)	RSD (%)	n	Comments
Holding- and dilution water	0.050	106.3	3.98	5	
	0.500	104.2	0.80	5	
	0.600	92.0	2.19	5	
	6.00	97.7	1.81	5	
	60.0	108.7	0.70	5	

Table A 81: Characteristics for the analytical method used for validation of XDE-777, X642188 and Prothioconazole residues in holding- and dilution water

Characteristic	Fenpicoxamid	X642188	Prothioconazole
Specificity	m/z 615.34 → 239.00 Q m/z 615.34 → 124.01 C1 m/z 615.34 → 515.16 C2 Blank value <30% LOQ	m/z 515.26 → 124.01 Q m/z 515.26→ 151.95 C1 m/z 515.26→ 239.03 C2 Blank value <30% LOQ	m/z 344.08 → 125.02 Q m/z 344.08 → 188.96 C Blank value <30% LOQ
Calibration (type, number of data points)	Linear regression analysis with 1/x weighting r = 0.9997 9 data points	Linear regression analysis with 1/x weighting r = 0.9999 9 data points	Linear regression analysis with 1/x weighting r = 0.9998 9 data points
Calibration range	Concentration range of 0.005 to 2.50 µg/L	Concentration range of 0.0005 to 0.25 µg/L	Concentration range of 0.0125 to 6.25 µg/L
Limit of determination/quantification	LOQ = 0.025 µg/L	LOQ = 0.0015 µg/L	LOQ = 0.050 µg/L

CONCLUSION

This method was successfully validated for the determination of the analytes Fenpicoxamid (XDE-777), X642188 (metabolite of XDE-777) and Prothioconazole in holding- and dilution water.

A 2.1.1.24 Analytical method 24

A 2.1.1.24.1 Method validation

Comments of zRMS:	The study was evaluated in the Registration Report, Part B5 for GF-3308 on 24.08.2022. Summary: The analytical method of Hicks, S (2016) for the determination of fenpicoxamid and its metabolites in samples of natural surface water (freshwater) by LC-MS/MS has been successfully validated in accordance with the EU guidance document SANCO/3029/99 rev. 4. Method validation results are presented for XDE-777 only. LOQ = 0.0500 µg/L for XDE-777 The mean recovery of each fortification level and the overall mean recovery value was 70 – 110% with an RSD < 20%. The validation parameters are acceptable. The method is suitable for fit for purpose.

Method Identifier No.:	160125
Performing Laboratory:	ABC Laboratories, Inc. (now EAG Laboratories) Columbia, Missouri, USA
Reference:	KCP 10.2.3/3
Report:	Hicks, S.; 2016; XDE-777: Population Effects Study in an Indoor Aquatic Microcosm with *Daphnia magna*; ABC Laboratories, Inc. (now EAG Laboratories), Columbia, Missouri, USA; Lab Study No. 83491; DAS Study No. 160125; 14 August 2017; Unpublished
Guideline(s):	Yes, SANCO/3029/99 rev.4
Guideline Deviations:	No
GLP:	Yes
Acceptability:	Yes
Method Alterations:	No

MATERIALS AND METHODS

Method Principle

Residues of fenpicoxamid and its metabolites were determined from samples of natural surface water (freshwater) by diluting with 0.2% formic acid in acetonitrile (ACN), centrifuging at 3,600 rpm for 10 minutes, and further diluting within the range of the calibration curve, as needed, with 0.1:50:50 formic acid:ACN:water. The final sample was analysed for fenpicoxamid and its metabolites by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS).

RESULTS AND DISCUSSION

For fenpicoxamid, all mean recoveries for all fortification levels were within the 70 - 110% range and all RSD values were ≤ 20%. For all metabolites, data from this study was not used to derive any ecotox risk assessment conclusions, so method validation results are negligible and not presented here. The results obtained for fenpicoxamid are summarised in the following table.

Table A 82: Recovery results from method validation of fenpicoxamid (*m/z* 615.0/239.2) using the analytical method

Matrix	Analyte	Fortification level (µg a.i./L)	Mean Recovery (%)	RSD (%)	n	Comments
Freshwater	fenpicoxamid	0.0500	102	9	11	
Freshwater	fenpicoxamid	120	102	10	11	

Table A 83: Characteristics for the analytical method used for validation of fenpicoxamid residues in freshwater

	Fenpicoxamid
Specificity	*m/z* 615.0/239.2 blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting r≥0.995 6 data points
Calibration range	Concentration range of 0.0100-0.500 ng/mL
Limit of determination/quantification	LOQ = 0.0500 µg/L

CONCLUSION

The method was considered acceptable for the determination of fenpicoxamid in natural surface water (freshwater) based on acceptable precision and accuracy demonstrated within this study.

A 2.1.1.25 Analytical method 25

A 2.1.1.25.1 Method validation

Comments of zRMS:	An analytical method for the determination of fenpicoxamid in larval diet was succesfully validated with regard to recovery, linearity of detector response, repeatability, specificity, limit of quantification and limit of detection. The analytical method fulfils the requirements of guideline SANCO/3029/99 rev. 4, 11/07/2000. The calibration functions were linear within the range from 0.030 ng/mL to 10 ng/mL with R≥ 0.997. The limit of quantification (LOQ) of the analytical method was 1.5 mg/kg of test item (0.0705 mg/kg of fenpicoxamid) in larval diet and 15 mg/L of test item (0.705 mg/L of fenpicoxamid). The mean recoveries at each fortification level were in the range between 70% and 110% with relative standard deviations below 20%. The method is acceptable.

Method Identifier No.:	171043
Performing Laboratory:	Eurofins Agroscience Services EcoChem GmbH (EAS EcoChem GmbH) / Eurofins Agroscience Services Ecotox GmbH (EAS Ecotox GmbH), 75223 Niefern-Öschelbronn, Germany
Reference:	KCP 10.3.1.2/1
Report:	Sophia Oberrauch; 2018; GF-3307 - Honey Bee (Apis mellifera L.) 22 Day Larval Toxicity Test (Repeated Exposure); Eurofins Agroscience Services EcoChem GmbH (EAS EcoChem GmbH) / Eurofins Agroscience Services Ecotox GmbH, D-75223 Niefern-Öschelbronn, Germany; Lab Study No. S17-04700; DAS Study No. 171043; 15 January 2018; Unpublished
Guideline(s):	Yes, SANCO/3029/99 rev.4
Guideline Deviations:	No
GLP:	Yes
Acceptability:	Yes
Method Alterations:	No

MATERIALS AND METHODS

Method Principle

Concentrations of GF-3307, based on fenpicoxamid analysis, are determined from larval diet samples and water samples by extraction with acetonitrile/water (1:1, v/v) + 0.1 % formic. The final sample is analysed for fenpicoxamid by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS).

RESULTS AND DISCUSSION

Mean recovery values at each fortification concentration were within the acceptance range (mean recovery 70-110%; RSD ≤ 20%). The results obtained are summarised in the following tables.

Table A 84: Recovery results from method validation of GF-3307, based on fenpicoxamid (*m/z* 615/239) analysis, using the analytical method

Matrix	Analyte	Fortification level (mg T.P./kg)	Mean Recovery (%)	RSD (%)	n	Comments
Larval diet (Diet C)	fenpicoxamid	1.5 mg T.P./kg, equivalent to 0.0705 mg a.i./kg	76	5	5	Individual recoveries: 74, 75, 73, 78, 82

Matrix	Analyte	Fortification level (mg T.P./kg)	Mean Recovery (%)	RSD (%)	n	Comments
		880 mg T.P./kg, equivalent to 41.4 mg a.i./kg	99	3	5	Individual recoveries: 96, 96, 98, 100, 104

Table A 85: Recovery results from method validation of GF-3307, based on fenpicoxamid (*m/z* 615/239) analysis, using the analytical method

Matrix	Analyte	Fortification level (mg T.P./L)	Mean Recovery (%)	RSD (%)	n	Comments
water	fenpicoxamid	15 mg T.P./L, equivalent to 0.705 mg a.i./L	80	2	5	Individual recoveries: 81, 81, 80, 80, 77
		9700 mg T.P./L, equivalent to 456 mg a.i./L	88	9	5	Individual recoveries: 87, 86, 78, 88, 101

Table A 86: Characteristics for the analytical method used for validation of GF-3307 residues, based on fenpicoxamid analysis, in larval diet (Diet C)

	Fenpicoxamid
Specificity	*m/z* 615/239 (Q) *m/z* 615/515 (C) blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting $r \geq 0.997$ 9 data points
Calibration range	Concentration range of 0.03 - 10 ng/mL
Limit of determination/quantification	LOQ=0.0705 mg a.i./kg, equivalent to 1.5 mg T.P./kg LOD= 0.0212 mg a.i./kg

Table A 87: Characteristics for the analytical method used for validation of GF-3307 residues, based on fenpicoxamid residues, in water

	Fenpicoxamid
Specificity	*m/z* 615/239 (Q) *m/z* 615/515 (C) blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting $r \geq 0.997$ 9 data points
Calibration range	Concentration range of 0.03 - 10 ng/mL
Limit of determination/quantification	LOQ=0.705 mg a.i./L, equivalent to 15 mg T.P./L LOD= 0.212 mg a.i./L

CONCLUSION

This method was successfully validated for the determination of GF-3307, based on fenpicoxamid analysis, in larval diet (Diet C) and water.

A 2.1.1.26 Analytical method 26

A 2.1.1.26.1 Method validation

Comments of zRMS:	An analytical method for the determination of fenpicoxamid in 50% (w/v) aqueous sucrose solution was succesfully validated with regard to recovery, linearity of detector response, repeatability, specificity, limit of quantification and limit of detection. The analytical method fulfils the requirements of guideline SANCO/3029/99 rev. 4, 11/07/2000. The calibration functions were linear within the range from 0.030 ng/mL to 10 ng/mL with R≥ 0.999. The limit of quantification (LOQ) of the analytical method was 0.05 mg/kg of test item (0.00235 mg/kg of fenpicoxamid). The mean recoveries at each fortification level were in the range between 70% and 110% with relative standard deviations below 20%. The method is acceptable.

Method Identifier No.:	170077
Performing Laboratory:	Eurofins Agroscience Services EcoChem GmbH (EAS EcoChem GmbH) / Eurofins Agroscience Services Ecotox GmbH (EAS Ecotox GmbH), 75223 Niefern-Öschelbronn, Germany
Reference:	KCP 10.3.1.2/2
Report:	Emmanuelle Vergé; 2018; GF-3307 - Assessment of Effects on the Adult Honey Bee, *Apis mellifera* L., in a 10 Day Chronic Feeding Test under Laboratory Conditions; Eurofins Agroscience Services EcoChem GmbH (EAS EcoChem GmbH) / Eurofins Agroscience Services Ecotox GmbH, , D-75223 Niefern-Öschelbronn, Germany; Lab Study No. S17-00198; DAS Study No. 170077; 10 January 2018; Unpublished
Guideline(s):	Yes, SANCO/3029/99 rev.4
Guideline Deviations:	No
GLP:	Yes
Acceptability:	Yes
Method Alterations:	No

MATERIALS AND METHODS

Method Principle

Concentrations of GF-3307, based on fenpicoxamid analysis, are determined from 50 % (w/v) aqueous sucrose solution samples by dilution with acetonitrile/water (1:1, v/v) + 0.1 % formic acid. The final sample is analysed for fenpicoxamid by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS).

RESULTS AND DISCUSSION

Mean recovery values at each fortification concentration were within the acceptance range (mean recovery 70-110%; RSD ≤ 20%). The results obtained are summarised in the following tables.

Table A 88: Recovery results from method validation of GF-3307, based on fenpicoxamid (*m/z* 615/239) analysis, using the analytical method

Matrix	Analyte	Fortification level (mg T.P./kg)	Mean Recovery (%)	RSD (%)	n	Comments
50 % (w/v) aqueous sucrose solution	fenpicoxamid	0.05 mg T.P./kg, equivalent to 0.00235 mg a.i./kg	70	2	5	Individual recoveries: 70, 71, 69, 69, 72

Matrix	Analyte	Fortification level (mg T.P./kg)	Mean Recovery (%)	RSD (%)	n	Comments
		20 mg T.P./kg, equivalent to 0.940 mg a.i./L	74	2	5	Individual recoveries: 73, 73, 76, 75, 75

Table A 89: Characteristics for the analytical method used for validation of GF-3307 residues, based on fenpicoxamid analysis, in 50 % (w/v) aqueous sucrose solution

	Fenpicoxamid
Specificity	*m/z* 615/239 (Q) *m/z* 615/515 (C) blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting r ≥ 0.999 9 data points
Calibration range	Concentration range of 0.03 - 10 ng/mL
Limit of determination/quantification	LOQ=0.00235 mg a.i./kg, equivalent to 0.05 mg T.P./kg LOD= 0.000705 mg a.i./kg

CONCLUSION

This method was successfully validated for the determination of GF-3307, based on fenpicoxamid analysis, in 50 % (w/v) aqueous sucrose solution.

A 2.1.1.27 Analytical method 27

A 2.1.1.27.1 Method validation

Comments of zRMS:	An analytical method for the determination of fenpicoxamid, prothioconazole-desthio and prothioconazole in pollen, nectar and plants was succesfully validated with regard to recovery, linearity of detector response, repeatability, specificity, limit of quantification and limit of detection. The analytical method fulfils the requirements of guideline SANCO/3029/99 rev. 4, 11/07/2000. The calibration functions were linear within the range from 0.06 ng/mL to 5 ng/mL (corresponds to a frotification level of 0.0003 to 0.025 mg/kg) with R≥ 0.995. The limit of quantification (LOQ) of the analytical method was 0.001 mg/kg for all analytes. The mean recoveries at each fortification level were in the range between 70% and 110% with relative standard deviations below 20%. The method is acceptable.

Method Identifier No.:	170673
Performing Laboratory:	Eurofins Agroscience Services EcoChem GmbH (EAS EcoChem GmbH) / Eurofins Agroscience Services Ecotox GmbH (EAS Ecotox GmbH), 75223 Niefern-Öschelbronn, Germany
Reference:	KCP 10.3.1.5/01
Report:	Marco Kleinhenz; 2018; GF-3307 (Fenpicoxamid + Prothioconazole): Brood Development of the Honeybee (Apis mellifera L.) in a Semi-Field Tunnel Study in Phacelia tanacetifolia in Germany 2017; Eurofins Agroscience Services EcoChem GmbH (EAS EcoChem GmbH) / Eurofins Agroscience Services Ecotox GmbH, D-75223 Niefern-Öschelbronn, Germany; Lab Study No. S17-02707; DAS Study No. 170673; 24 May 2018; Unpublished

Guideline(s):	SANCO/3029/99, rev. 4
Guideline Deviations:	No
GLP:	Yes
Acceptability:	Yes
Method Alterations:	No

MATERIALS AND METHODS

Method Principle

Residues of GF-3307, based on fenpicoxamid, prothioconazole and prothioconazole-desthio analysis, are determined from samples of pollen, nectar, and whole plant by extraction in cysteine hydrochloride (250 ng/mL) and acetonitrile/water (80/20, v/v) + 0.1 % formic acid solutions. After clean-up, a liquid-liquid extraction is performed. The final sample extract is analysed by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS).

RESULTS AND DISCUSSION

Mean recovery values at each fortification concentration were within the acceptance range (mean recovery 70-110%; RSD ≤ 20%). The results obtained are summarised in the following tables.

Table A 90: Recovery results from method validation of fenpicoxamid (*m/z* 615/515), using the analytical method

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Pollen	fenpicoxamid	0.001 (LOQ)	94	7	5	Individual recoveries: 100, 98, 87, 99, 88
		0.01	107	6	5	Individual recoveries: 110, 113, 96, 108, 110
		50	109	2	5	Individual recoveries: 108, 108, 109, 112, 106
Nectar	fenpicoxamid	0.001 (LOQ)	98	13	5	Individual recoveries: 112, 101, 90, 81, 108
		0.01	102	10	5	Individual recoveries: 104, 85, 105, 100, 114
		1	81	6	5	Individual recoveries: 80, 76, 80, 79, 88
Plant	fenpicoxamid	0.001 (LOQ)	108	3	5	Individual recoveries: 108, 110, 111, 108, 102
		0.01	99	2	5	Individual recoveries: 101, 100, 99, 95, 100
		5	82	3	5	Individual recoveries: 90, 90, 92, 91, 97

Table A 91: Recovery results from method validation of prothioconazole (*m/z* 344/100) using the analytical method

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Pollen	prothioconazole	0.001 (LOQ)	97	5	5	Individual recoveries: 103, 91, 96, 101, 93
		0.01	87	8	5	Individual recoveries: 89, 97, 78, 89, 83
		50	104	6	5	Individual recoveries: 99, 105, 107, 114, 97
Nectar	prothioconazole	0.001 (LOQ)	86	8	5	Individual recoveries: 78, 79, 79, 90, 93
		0.01	87	12	5	Individual recoveries: 93, 96, 85, 90, 70
		1	76	8	5	Individual recoveries: 83, 72, 75, 68, 82
Plant	prothioconazole	0.001 (LOQ)	88	4	5	Individual recoveries: 83, 88, 92, 92, 87
		0.01	88	4	5	Individual recoveries: 93, 87, 84, 84, 90
		5	76	4	5	Individual recoveries: 74, 74, 76, 76, 82

Table A 92: Recovery results from method validation of prothioconazole-desthio (*m/z* 312/70) using the analytical method

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Pollen	prothioconazole-desthio	0.001 (LOQ)	95	9	5	Individual recoveries: 101, 83, 105, 96, 91
		0.01	105	5	5	Individual recoveries: 110, 103, 97, 110, 105
		50	106	4	5	Individual recoveries: 109, 102, 108, 109, 101
Nectar	prothioconazole-desthio	0.001 (LOQ)	100	10	5	Individual recoveries: 100, 109, 98, 84, 108
		0.01	93	13	5	Individual recoveries: 93, 79, 94, 87, 112
		1	73	3	5	Individual recoveries: 74, 71, 72, 70, 76
Plant	prothioconazole-desthio	0.001 (LOQ)	108	5	5	Individual recoveries: 106, 107, 114, 113, 102

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
		0.01	103	3	5	Individual recoveries: 105, 104, 100, 100, 105
		5	87	2	5	Individual recoveries: 87, 88, 87, 84, 90

Table A 93: Characteristics for the analytical method used for validation of fenpicoxamid, prothioconazole, and prothioconazole-desthio residues in pollen, nectar, and plant

	Fenpicoxamid	Prothioconazole	Prothioconazole-desthio
Specificity	*m/z* 615/515 (Q) *m/z* 615/239 (C) blank value <30% LOQ	*m/z* 344/100 (Q) *m/z* 344/58 (C) blank value <30% LOQ	*m/z* 312/70 (Q) *m/z* 312/125 (C) blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting r ≥ 0.995 6 data points	linear regression analysis with 1/x weighting r ≥ 0.995 6 data points	linear regression analysis with 1/x weighting r ≥ 0.995 6 data points
Calibration range	Concentration range of 0.06 - 5 ng/mL, equivalent to 0.0003 – 0.025 mg/kg	Concentration range of 0.06 - 5 ng/mL, equivalent to 0.0003 – 0.025 mg/kg	Concentration range of 0.06 - 5 ng/mL, equivalent to 0.0003 – 0.025 mg/kg
Limit of quantification	LOQ = 0.001 mg/kg	LOQ = 0.001 mg/kg	LOQ = 0.001 mg/kg

CONCLUSION

The method was successfully validated for determination of fenpicoxamid, prothioconazole and prothioconazole-desthio in pollen, nectar and plant and is suitable to generate data in support of ecotoxicology studies.

A 2.1.1.28 Analytical method 28

A 2.1.1.28.1 Method validation

Comments of zRMS:	The study of Appeltauer, A (2021, Report No. 200670) was not used in the ecotoxicology assessment and therefore an analytical method is not necessary in the assessment to support this application.

Report author: Appeltauer, A.
Report year: 2021
Report title: Determination of Residues of Fenpicoxamid and Prothioconazole in Nectar, Pollen and Plants of Winter Oilseed Rape after One Application of GF-3307 in a Semi-Field Residue Study in Central and Southern Europe in 2020
Report No.: 200670
Testing Facility Report No.: S20-01926
Method(s) used: 200670
Guidelines followed in study: SANCO/3029/99 rev. 4
Deviation from current test guidelines: No
Analytical Performing Laboratory: Eurofins Agroscience Services EcoTox GmbH Niefern- Öschelbronn , Germany
GLP/Officially recognised testing facilities: Yes

MATERIAL AND METHODS

Method Principle

Residues of fenpicoxamid, prothioconazole and prothioconazole-desthio were extracted/determined from samples of pollen from forager bees, nectar from forager bees and whole plants (winter oil seed rape) by extraction (pollen, whole plant) or dilution (nectar) with acetonitrile/water (50/50,v/v) + 0.1 % formic acid and no liquid/liquid partition for nectar or liquid/liquid partition by addition of magnesium sulphate, sodium chloride and sodium citrate followed by subsequent centrifugation for pollen and whole plant samples. No clean-up / purification was performed for nectar and purification of an aliquot of the acetonitrile extract by dispersive SPE with primary/secondary amine (PSA) and graphitized carbon black (GCB) for pollen and whole plant samples. The final sample was analysed for fenpicoxamid, prothioconazole and prothioconaole-desthio by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS).

RESULTS AND DISCUSSION

Mean recovery values at each fortification concentration were within the acceptance range (mean recovery 70-110%; RSD ≤ 20%). The results obtained are summarised in the following tables.

Table A 94: Recovery results from method validation of Fenpicoxamid (m/z 615/239Q) using the analytical method

Matrix	Fortification level mg/kg	Mean Recovery (%)	RSD (%)	n	Comments
Nectar	0.001	99	2	5	
Nectar	0.01	101	6	5	
Pollen	0.001	99	14	5	
Pollen	0.01	97	5	5	
Whole Plant	0.001	96	4	5	
Whole Plant	0.01	97	3	5	

Table A 95: Recovery results from method validation of Prothioconazole (m/z 342/58Q*) using the analytical method

Matrix	Fortification level mg/kg	Mean Recovery (%)	RSD (%)	n	Comments
Nectar	0.001	87	7	5	
Nectar	0.01	92	6	5	
Pollen	0.001	102	14	5	
Pollen	0.01	93	5	5	
Whole Plant	0.001	77	9	5	
Whole Plant	0.01	84	7	5	

*Only used for method verification, transition was changed for sample analysis due to response fluctuations when using bipolar mode for longer sequences.

Table A 96: Recovery results from method validation of Prothioconazole-desthio (m/z 312/70Q) using the analytical method

Matrix	Fortification level mg/kg	Mean Recovery (%)	RSD (%)	n	Comments
Nectar	0.001	98	2	5	
Nectar	0.01	98	3	5	
Pollen	0.001	107	11	5	
Pollen	0.01	93	2	5	
Whole Plant	0.001	98	3	5	
Whole Plant	0.01	98	2	5	

Table A 97: Procedural recovery results of Fenpicoxamid (m/z 615/239Q) using the analytical method

Matrix	Fortification level mg/kg	Mean Recovery (%)	RSD (%)	n	Comments
Nectar	0.001	90	2	5	
Nectar	0.01	109	5	5	
Nectar	10	99	3	5	
Pollen	0.001	91	19	8	
Pollen	0.01	100	7	5	
Pollen	50	92	3	5	
Whole Plant	0.001	88	4	5	
Whole Plant	0.01	106	3	8	
Whole Plant	4	104	3	5	

Table A 98: Procedural recovery results of Prothioconazole (m/z 344/189Q) (m/z 344/154Q*) using the analytical method

Matrix	Fortification level mg/kg	Mean Recovery (%)	RSD (%)	n	Comments
Nectar	0.001	102	8	5	
Nectar	0.01	98	3	5	
Nectar	10	100	3	5	
Pollen	0.001	97	12	8	
Pollen	0.01	94	8	5	
Pollen	50	93	2	5	
Whole Plant	0.001	77	4	5	
Whole Plant	0.01	89	5	8	
Whole Plant	4	91	4	5	

*Mass transition 344/154 m/z for whole plant only

Table A 99: Procedural recovery results of Prothioconazole-desthio (m/z 312/70Q) using the analytical method

Matrix	Fortification level mg/kg	Mean Recovery (%)	RSD (%)	n	Comments
Nectar	0.001	110	1	5	
Nectar	0.01	93	5	5	
Nectar	10	91	3	5	
Pollen	0.001	94	13	7	
Pollen	0.01	95	4	5	
Pollen	50	85	3	5	
Whole Plant	0.001	83	7	5	
Whole Plant	0.01	99	1	8	
Whole Plant	4	99	6	5	

Table A 100: Characteristics for the analytical method used for determination of residues of Fenpicoxamid, Prothioconazole and Prothioconazole-desthio in Pollen

Analyte	Fenpicoxamid	Prothioconazole	Prothioconazole-desthio
Matrix	Pollen	Pollen	Pollen
Technique	LC-MS/MS	LC-MS/MS	LC-MS/MS
Specificity	*m/z* 615/239Q *m/z* 615/515C blank value <30% LOQ	*m/z* 344/189Q *m/z* 344/154C blank value <30% LOQ	*m/z* 312/70Q *m/z* 312/125C blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting r≥0.9984 8 data points	linear regression analysis with 1/x weighting r≥0.9994 8 data points	linear regression analysis with 1/x weighting r≥0.9998 8 data points
Calibration range	Concentration range of 0.01-5 ng/mL(equivalent sample concentration 0.0003 – 0.15 mg/kg)	Concentration range of 0.01-5 ng/mL(equivalent sample concentration 0.0003 – 0.15 mg/kg)	Concentration range of 0.01-5 ng/mL(equivalent sample concentration 0.0003 – 0.15 mg/kg)
Limit of quantitation	0.001 mg/kg	0.001 mg/kg	0.001 mg/kg
Validation Range	0.001 – 50 mg/kg	0.001 – 50 mg/kg	0.001 – 50 mg/kg

Table A 101: **Characteristics for the analytical method used for determination of residues of Fenpicoxamid, Prothioconazole and Prothioconazole-desthio in Nectar**

Analyte	Fenpicoxamid	Prothioconazole	Prothioconzole-desthio
Matrix	Nectar	Nectar	Nectar
Technique	LC-MS/MS	LC-MS/MS	LC-MS/MS
Specificity	*m/z* 615/239Q *m/z* 615/515C blank value <30% LOQ	*m/z* 344/189Q *m/z* 344/154C blank value <30% LOQ	*m/z* 312/70Q *m/z* 312/125C blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting r≥0.9998 8 data points	linear regression analysis with 1/x weighting r≥0.9998 8 data points	linear regression analysis with 1/x weighting r≥0.9997 8 data points
Calibration range	Concentration range of 0.01-5 ng/mL(equivalent sample concentration 0.0003 – 0.15 mg/kg)	Concentration range of 0.01-5 ng/mL(equivalent sample concentration 0.0003 – 0.15 mg/kg)	Concentration range of 0.01-5 ng/mL(equivalent sample concentration 0.0003 – 0.15 mg/kg)
Limit of quantitation	0.001 mg/kg	0.001 mg/kg	0.001 mg/kg
Validation Range	0.001 – 10 mg/kg	0.001 – 10 mg/kg	0.001 – 10 mg/kg

Table A 102: **Characteristics for the analytical method used for determination of residues of Fenpicoxamid, Prothioconazole and Prothioconazole-desthio in Whole Plant**

Analyte	Fenpicoxamid	Prothioconazole	Prothioconazole-desthio
Matrix	Whole Plant	Whole Plant	Whole Plant
Technique	LC-MS/MS	LC-MS/MS	LC-MS/MS
Specificity	*m/z* 615/239Q *m/z* 615/515C blank value <30% LOQ	*m/z* 344/154Q *m/z* 344/189C blank value <30% LOQ	*m/z* 312/70Q *m/z* 312/125C blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting r≥0.9991 8 data points	linear regression analysis with 1/x weighting r≥0.9990 8 data points	linear regression analysis with 1/x weighting r≥0.9999 8 data points
Calibration range	Concentration range of 0.03-5 ng/mL(equivalent sample concentration 0.0003 – 0.05 mg/kg)	Concentration range of 0.03-5 ng/mL(equivalent sample concentration 0.0003 – 0.05 mg/kg)	Concentration range of 0.03-5 ng/mL(equivalent sample concentration 0.0003 – 0.05 mg/kg)
Limit of quantitation	0.001 mg/kg	0.001 mg/kg	0.001 mg/kg
Validation Range	0.001 – 4 mg/kg	0.001 – 4 mg/kg	0.001 – 4 mg/kg

CONCLUSION

This method was successfully validated for the determination of fenpicoxamid, prothioconazole and prothioconazole-desthio in nectar, pollen and whole plants from winter oilseed rape.

A 2.1.1.29 Analytical method 29

A 2.1.1.29.1 Method validation

Comments of zRMS:	The analytical method for the determination of fenpicoxamid, prothioconazole-desthio and prothioconazole in pollen, nectar, honey, pupae, larvae, worker jelly and feeding solution was succesfully validated according to SANCO/3029/99 rev. 4, 11/07/2000 (and SANCO/825/00 rev. 8.1 for matrix honey only).

	The limit of quantification (LOQ) of the analytical method was 0.001 mg/kg for all analytes. The mean recoveries at each fortification level were in the range between 70% and 110% with relative standard deviations below 20%. The method is acceptable.

Reference:	KCP 10.3.1.6/1
Report author:	Gonsoir, G.
Report year:	2021
Report title:	GF-3307 (Fenpicoxamid and Prothioconazole) Brood Development of the Honey Bee (*Apis mellifera* L.) in a Colony Feeding Test in Germany 2020
Report No.:	200660
Testing Facility Report No.:	S20-02058
Method(s) used:	200660
Guidelines followed in study:	SANCO/3029/99 rev. 4 OR SANCO/825/00 rev. 8.1 (for matrix honey only)
Deviation from current test guidelines:	No
Analytical Performing Laboratory:	Eurofins Agroscience Services EcoChem GmbH 75223 Niefern-Öschelbronn, Eutinger Str. 24 Germany
GLP/Officially recognised testing facilities:	Yes

Method Principle

For honey, nectar and feeding solutions, residues of fenpicoxamid, prothioconazole and prothioconazole-desthio were extracted by homogenizing and shaking with the mixture of cysteine hydrochloride solution (250 mg/mL) and acetonitrile/water (50/50, v/v) containing 0.1 % formic acid until the material is completely dissolved. After adjustment to the final volume, the final extract was analysed by liquid chromatography with electrospray ionization tandem
mass spectrometry (LC-MS/MS). The limit of detection (LOD) and limit of quantitation
(LOQ) were 0.0003 mg/kg and 0.001 mg/kg, respectively, for all analytes.

For pollen, pupae and larvae, residues of fenpicoxamid, prothioconazole and prothioconazole-desthio were extracted with the mixture of cysteine hydrochloride solution (250 mg/mL), ascorbic acid solution (100 mg/mL) and acetonitrile/water (50/50, v/v) containing 0.1 % formic acid using a FastPrep homogenizer. After addition of a mixture of 1.35 g anhydrous magnesium sulphate, 0.34 g sodium chloride, 0.34 g trisodium citrate and 0.17 g disodium citrate sesquihydrate (Citrate Kit 1/3), the sample was shaken and centrifuged. After the phase separation, an aliquot of the upper layer was purified by dispersive solid phase extraction with primary-secondary amino phase / GCB (PSA Kit-05). After centrifugation the cleaned extract was diluted with 0.5 mL of methanol/water (4/6 v/v) containing 50 mg/mL cysteine hydrochloride and analysed by liquid chromatography with electrospray ionization tandem mass spectrometry (LC-MS/MS). The limit of detection (LOD) and limit of quantitation (LOQ) were 0.0003 mg/kg and 0.001 mg/kg, respectively, for all analytes.

For worker jelly, residues of fenpicoxamid, prothioconazole and prothioconazole-desthio were extracted by homogenizing and shaking with the mixture of cysteine hydrochloride solution (250 mg/mL), ascorbic acid solution (100 mg/mL) and acetonitrile/water (50/50, v/v) containing 0.1 % formic acid. After addition of a mixture of 1.35 g anhydrous magnesium sulphate, 0.34 g sodium chloride, 0.34 g trisodium citrate and 0.17 g disodium citrate sesquihydrate (Citrate Kit 1/3), the sample was shaken and centrifuged. After the phase separation, an aliquot of the upper layer was purified by dispersive solid phase extraction with primary-secondary amino phase / GCB (PSA Kit-05). After centrifugation the cleaned extract was diluted with 0.5 mL of methanol/water (4/6, v/v) containing 50 mg/mL cysteine hydrochloride and analysed by liquid chromatography with electrospray ionization tandem mass spectrometry (LC-MS/MS). The limit of detection (LOD) and limit of quantitation (LOQ) were 0.0003 mg/kg and 0.001 mg/kg, respectively, for all analytes.

For feeding solutions, residues of dimethoate and fenoxycarb were extracted by homogenizing and shaking with an acetonitrile/water (80/20, v/v). After dilution with water/acetonitrile (95/5, v/v) the final extract was analysed by liquid chromatography with electrospray ionization tandem mass spectrometry (LC-MS/MS). The limit of detection (LOD) and limit of quantitation (LOQ) were 0.003 mg/kg and 0.01 mg/kg, respectively, for all analytes.

RESULTS AND DISCUSSION

Mean recovery values at each fortification concentration were within the acceptance range (mean recovery 70-110%; RSD ≤ 20%). The results obtained are summarised in the following tables.

Table A 103: Recovery results from method validation of Fenpicoxamid (m/z 615/239Q) using the analytical method

Matrix	Fortification level mg/kg	Mean Recovery (%)	RSD (%)	n	Comments
Honey	0.001	96	7	5	
Honey	0.01	104	2	5	
Pupae	0.001	99	3	5	
Pupae	0.01	103	4	5	
Larvae	0.001	96	3	5	
Larvae	0.01	103	4	5	
Worker Jelly	0.001	99	1	5	
Worker Jelly	0.01	103	1	5	
Feeding Solution	0.001	96	4	5	
Feeding Solution	0.01	99	3	5	

Table A 104: Recovery results from method validation of Prothioconazole (m/z 342/58Q and m/z 344/89Q (for worker jelly only)) using the analytical method

Matrix	Fortification level mg/kg	Mean Recovery (%)	RSD (%)	n	Comments
Honey	0.001	100	9	5	
Honey	0.01	103	9	5	
Pupae	0.001	109	7	5	
Pupae	0.01	101	4	5	
Larvae	0.001	106	3	5	
Larvae	0.01	110	1	5	
Feeding Solution	0.001	100	5	5	
Feeding Solution	0.01	109	4	5	
Worker Jelly	0.001	93	10	5	
Worker Jelly	0.01	103	3	5	

Table A 105: Recovery results from method validation of Prothioconazole-desthio (m/z 312/70Q) using the analytical method

Matrix	Fortification level mg/kg	Mean Recovery (%)	RSD (%)	n	Comments
Honey	0.001	99	4	5	
Honey	0.01	92	2	5	
Pupae	0.001	106	1	5	
Pupae	0.01	97	3	5	
Larvae	0.001	100	9	5	
Larvae	0.01	99	1	5	
Worker Jelly	0.001	94	13	5	
Worker Jelly	0.01	107	4	5	
Feeding Solution	0.001	99	2	5	
Feeding Solution	0.01	96	2	5	

Table A 106: Recovery results from method validation of Dimethoate (m/z 230/199Q) using the analytical method

Matrix	Fortification level mg/kg	Mean Recovery (%)	RSD (%)	n	Comments
Feeding Solution	0.01	97	9	3	
Feeding Solution	0.1	101	7	3	

Table A 107: Recovery results from method validation of Fenoxycarb (m/z 302/88Q) using the analytical method

Matrix	Fortification level mg/kg	Mean Recovery (%)	RSD (%)	n	Comments
Feeding Solution	0.01	103	2	3	
Feeding Solution	0.1	82	7	3	

Table A 108: Procedural recovery results of Fenpicoxamid (m/z 615/239Q) using the analytical method

Matrix	Fortification level mg/kg	Mean Recovery (%)	RSD (%)	n	Comments
Pollen	0.001	93	14	9	
Pollen	0.01	96	10	7	
Pollen	50	92	3	5	
Nectar	0.001	85	2	5	
Nectar	0.01	97	4	5	
Nectar	10	99	3	5	
Honey	0.001	101	5	5	
Honey	0.01	109	5	5	
Honey	7	105	3	5	
Pupae	0.001	103	3	5	
Pupae	0.01	103	7	5	
Larvae	0.001	99	9	5	
Larvae	0.01	108	2	5	
Larvae	0.2	106	2	4	
Worker Jelly	0.001	81	4	5	
Worker Jelly	0.01	87	3	5	
Worker Jelly	4	99	3	5	
Feeding Solution	0.001	93	13	5	
Feeding Solution	0.01	95	11	5	
Feeding Solution	50	99	2	5	
Feeding Solution	70	108	6	5	

Table A 109: Procedural recovery results of Fenpicoxamid (m/z 615/515C) using the analytical method

Matrix	Fortification level mg/kg	Mean Recovery (%)	RSD (%)	n	Comments
Honey	0.001	98	5	5	
Honey	0.01	106	5	5	
Honey	7	106	2	5	

Table A 110: Procedural recovery results of Prothioconazole (m/z 344/154Q) using the analytical method

Matrix	Fortification level mg/kg	Mean Recovery (%)	RSD (%)	n	Comments
Pollen	0.001	88	12	9	
Pollen	0.01	90	6	7	
Pollen	50	91	3	5	

Table A 111: Procedural recovery results of Prothioconazole (m/z 342/58Q) using the analytical method

Matrix	Fortification level mg/kg	Mean Recovery (%)	RSD (%)	n	Comments
Honey	0.001	95	13	5	
Honey	0.01	96	16	5	
Honey	7	91	10	5	

Table A 112: Procedural recovery results of Prothioconazole (m/z 344/58C) using the analytical method

Matrix	Fortification level mg/kg	Mean Recovery (%)	RSD (%)	n	Comments
Honey	0.001	89	17	5	
Honey	0.01	99	14	5	
Honey	7	92	8	5	

Table A 113: Procedural recovery results of Prothioconazole (m/z 344/189Q) using the analytical method

Matrix	Fortification level mg/kg	Mean Recovery (%)	RSD (%)	n	Comments
Nectar	0.001	84	6	5	
Nectar	0.01	102	5	5	
Nectar	10	103	3	5	
Pupae	0.001	104	4	5	
Pupae	0.01	104	5	5	
Larvae	0.001	85	9	5	
Larvae	0.01	108	3	5	
Larvae	0.2	109	4	4	
Worker Jelly	0.001	77	6	5	
Worker Jelly	0.01	87	5	5	
Worker Jelly	4	100	2	5	
Feeding Solution	0.001	90	19	5	
Feeding Solution	0.01	93	12	5	
Feeding Solution	50	99	3	5	
Feeding Solution	70	99	3	5	

Table A 114: Procedural recovery results of Prothioconazole-desthio (m/z 312/70Q) using the analytical method

Matrix	Fortification level mg/kg	Mean Recovery (%)	RSD (%)	n	Comments
Pollen	0.001	98	16	9	
Pollen	0.01	95	3	7	
Pollen	50	85	3	5	
Nectar	0.001	109	1	5	
Nectar	0.01	94	5	5	
Nectar	10	91	3	5	
Honey	0.001	96	2	5	
Honey	0.01	102	5	5	
Honey	7	91	4	5	
Pupae	0.001	110	5	5	
Pupae	0.01	102	4	5	
Larvae	0.001	106	10	5	
Larvae	0.01	101	2	5	
Larvae	0.20	95	1	4	
Worker Jelly	0.001	99	13	5	
Worker Jelly	0.01	85	5	5	
Worker Jelly	4	92	1	5	
Feeding Solution	0.001	103	7	5	
Feeding Solution	0.01	98	3	5	
Feeding Solution	50	98	2	5	
Feeding Solution	70	97	3	5	

Table A 115: Procedural recovery results of Prothioconazole-desthio (m/z 312/125C) using the analytical method

Matrix	Fortification level mg/kg	Mean Recovery (%)	RSD (%)	n	Comments
Honey	0.001	106	5	5	
Honey	0.01	102	4	5	
Honey	7	93	2	5	

Table A 116: Procedural recovery results of Dimethoate (m/z 230/199Q) using the analytical method

Matrix	Fortification level mg/kg	Mean Recovery (%)	RSD (%)	n	Comments
Feeding Solution	0.01	104	1	5	
Feeding Solution	0.1	110	3	5	
Feeding Solution	100	110	1	5	

Table A 117: Procedural recovery results of Fenoxycarb (m/z 302/88Q) using the analytical method

Matrix	Fortification level mg/kg	Mean Recovery (%)	RSD (%)	n	Comments
Feeding Solution	0.01	108	4	5	
Feeding Solution	0.1	110	4	5	
Feeding Solution	100	92	5	5	

Table A 118:Characteristics for the analytical method used for determination of residues of Fenpicoxamid in pollen, nectar, honey and pupae

Analyte	Fenpicoxamid	Fenpicoxamid	Fenpicoxamid	Fenpicoxamid
Matrix	Pollen	Nectar	Honey	Pupae
Technique	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
Specificity	*m/z* 615/239Q *m/z* 615/515C blank value <30% LOQ	*m/z* 615/239Q *m/z* 615/515C blank value <30% LOQ	*m/z* 615/239Q *m/z* 615/515C blank value <30% LOQ	*m/z* 615/239Q *m/z* 615/515C blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting r≥0.995 ≥5 data points	linear regression analysis with 1/x weighting r≥0.995 ≥5 data points	linear regression analysis with 1/x weighting r≥0.995 ≥5 data points	linear regression analysis with 1/x weighting r≥0.995 ≥5 data points
Calibration range	Concentration range of 0.01-5.0 ng/mL (equivalent sample concentration 0.0003 - 0.15 mg/kg)	Concentration range of 0.01-5.0 ng/mL (equivalent sample concentration 0.0003 - 0.15 mg/kg)	Concentration range of 0.01-5.0 ng/mL (equivalent sample concentration 0.0003 - 0.15 mg/kg)	Concentration range of 0.01-5.0 ng/mL (equivalent sample concentration 0.0003 - 0.15 mg/kg)
Limit of quantitation	0.001 mg/kg	0.001 mg/kg	0.001 mg/kg	0.001 mg/kg
Validation Range	0.001-50 mg/kg	0.001-10 mg/kg	0.001-7 mg/kg	0.001-0.01 mg/kg

Table A 119: Characteristics for the analytical method used for determination of residues of Fenpicoxamid in larvae, worker jelly and feeding solution

Analyte	Fenpicoxamid	Fenpicoxamid	Fenpicoxamid
Matrix	Larvae	Worker Jelly	Feeding Solution
Technique	LC-MS/MS	LC-MS/MS	LC-MS/MS
Specificity	*m/z* 615/239Q *m/z* 615/515C blank value <30% LOQ	*m/z* 615/239Q *m/z* 615/515C blank value <30% LOQ	*m/z* 615/239Q *m/z* 615/515C blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting r≥0.995 ≥5 data points	linear regression analysis with 1/x weighting r≥0.995 ≥5 data points	linear regression analysis with 1/x weighting r≥0.995 ≥5 data points
Calibration range	Concentration range of 0.01-5.0 ng/mL (equivalent sample concentration 0.0003 - 0.15 mg/kg)	Concentration range of 0.01-5.0 ng/mL (equivalent sample concentration 0.0003 - 0.15 mg/kg)	Concentration range of 0.01-5.0 ng/mL (equivalent sample concentration 0.0003 - 0.15 mg/kg)
Limit of quantitation	0.001 mg/kg	0.001 mg/kg	0.001 mg/kg
Validation Range	0.001-0.20 mg/kg	0.001-4 mg/kg	0.001-70 mg/kg

Table A 120: Characteristics for the analytical method used for determination of residues of Prothioconazole in pollen, nectar, honey and pupae

Analyte	**Prothioconazole**	**Prothioconazole**	**Prothioconazole**	**Prothioconazole**
Matrix	Pollen	Nectar	Honey	Pupae
Technique	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
Specificity	*m/z* 344/154Q *m/z* 344/189C blank value <30% LOQ	*m/z* 344/189Q *m/z* 344/154C blank value <30% LOQ	*m/z* 342/58Q *m/z* 344/58C blank value <30% LOQ	*m/z* 344/189Q *m/z* 344/189C blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting r≥0.995 ≥5 data points	linear regression analysis with 1/x weighting r≥0.995 ≥5 data points	linear regression analysis with 1/x weighting r≥0.995 ≥5 data points	linear regression analysis with 1/x weighting r≥0.995 ≥5 data points
Calibration range	Concentration range of 0.01-5.0 ng/mL (equivalent sample concentration 0.0003 - 0.15 mg/kg)	Concentration range of 0.01-5.0 ng/mL (equivalent sample concentration 0.0003 - 0.15 mg/kg)	Concentration range of 0.01-5.0 ng/mL (equivalent sample concentration 0.0003 - 0.15 mg/kg)	Concentration range of 0.01-5.0 ng/mL (equivalent sample concentration 0.0003 - 0.15 mg/kg)
Limit of quantitation	0.001 mg/kg	0.001 mg/kg	0.001 mg/kg	0.001 mg/kg
Validation Range	0.001-50 mg/kg	0.001-10 mg/kg	0.001-7 mg/kg	0.001-0.01 mg/kg

Table A 121: Characteristics for the analytical method used for determination of residues of Prothioconazole in larvae, worker jelly and feeding solution

Analyte	**Prothioconazole**	**Prothioconazole**	**Prothioconazole**
Matrix	Larvae	Worker jelly	Feeding Solution
Technique	LC-MS/MS	LC-MS/MS	LC-MS/MS
Specificity	*m/z* 344/189Q *m/z* 344/154C blank value <30% LOQ	*m/z* 344/189Q *m/z* 344/154C blank value <30% LOQ	*m/z* 344/189Q *m/z* 344/154C blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting r≥0.995 ≥5 data points	linear regression analysis with 1/x weighting r≥0.995 ≥5 data points	linear regression analysis with 1/x weighting r≥0.995 ≥5 data points
Calibration range	Concentration range of 0.01-5.0 ng/mL (equivalent sample concentration 0.0003 - 0.15 mg/kg)	Concentration range of 0.01-5.0 ng/mL (equivalent sample concentration 0.0003 - 0.15 mg/kg)	Concentration range of 0.01-5.0 ng/mL (equivalent sample concentration 0.0003 - 0.15 mg/kg)
Limit of quantitation	0.001 mg/kg	0.001 mg/kg	0.001 mg/kg
Validation Range	0.001-0.2 mg/kg	0.001-4 mg/kg	0.001-70 mg/kg

Table A 122: Characteristics for the analytical method used for determination of residues of Prothioconazole-desthio in pollen, nectar, honey and pupae

Analyte	**Prothioconazole-desthio**	**Prothioconazole-desthio**	**Prothioconazole-desthio**	**Prothioconazole-desthio**
Matrix	Pollen	Nectar	Honey	Pupae
Technique	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
Specificity	*m/z* 312/70Q *m/z* 312/125C blank value <30% LOQ	*m/z* 312/70Q *m/z* 312/125C blank value <30% LOQ	*m/z* 312/70Q *m/z* 312/125C blank value <30% LOQ	*m/z* 312/70Q *m/z* 312/125C blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting r≥0.995 ≥5 data points	linear regression analysis with 1/x weighting r≥0.995 ≥5 data points	linear regression analysis with 1/x weighting r≥0.995 ≥5 data points	linear regression analysis with 1/x weighting r≥0.995 ≥5 data points
Calibration range	Concentration range of 0.01-5.0 ng/mL (equivalent sample concentration 0.0003 - 0.15 mg/kg)	Concentration range of 0.01-5.0 ng/mL (equivalent sample concentration 0.0003 - 0.15 mg/kg)	Concentration range of 0.01-5.0 ng/mL (equivalent sample concentration 0.0003 - 0.15 mg/kg)	Concentration range of 0.01-5.0 ng/mL (equivalent sample concentration 0.0003 - 0.15 mg/kg)
Limit of quantitation	0.001 mg/kg	0.001 mg/kg	0.001 mg/kg	0.001 mg/kg
Validation Range	0.001-50 mg/kg	0.001-10 mg/kg	0.001-7 mg/kg	0.001-0.01 mg/kg

Table A 123: Characteristics for the analytical method used for determination of residues of Prothioconazole-desthio in larvae, worker jelly and feeding solution

Analyte	**Prothioconazole-desthio**	**Prothioconazole-desthio**	**Prothioconazole-desthio**
Matrix	Larvae	Worker jelly	Feeding solution
Technique	LC-MS/MS	LC-MS/MS	LC-MS/MS
Specificity	*m/z* 312/70Q *m/z* 312/125C blank value <30% LOQ	*m/z* 312/70Q *m/z* 312/125C blank value <30% LOQ	*m/z* 312/70Q *m/z* 312/125C blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting r≥0.995 ≥5 data points	linear regression analysis with 1/x weighting r≥0.995 ≥5 data points	linear regression analysis with 1/x weighting r≥0.995 ≥5 data points
Calibration range	Concentration range of 0.01-5.0 ng/mL (equivalent sample concentration 0.0003 - 0.15 mg/kg)	Concentration range of 0.01-5.0 ng/mL (equivalent sample concentration 0.0003 - 0.15 mg/kg)	Concentration range of 0.01-5.0 ng/mL (equivalent sample concentration 0.0003 - 0.15 mg/kg)
Limit of quantitation	0.001 mg/kg	0.001 mg/kg	0.001 mg/kg
Validation Range	0.001-0.2 mg/kg	0.001-4 mg/kg	0.001-70 mg/kg

Table A 124: Characteristics for the analytical method used for determination of residues of Dimethoate and Fenoxycarb in feeding solution

Analyte	Dimethoate	Fenoxycarb
Matrix	Feeding solution	Feeding solution
Technique	LC-MS/MS	LC-MS/MS
Specificity	*m/z* 230/199Q *m/z* 230/125C blank value <30% LOQ	*m/z* 302/88Q *m/z* 302/116C blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting r≥0.995 ≥5 data points	linear regression analysis with 1/x weighting r≥0.995 ≥5 data points
Calibration range	Concentration range of 0.024-100 ng/mL (equivalent sample concentration 0.003 – 1.25 mg/kg)	Concentration range of 0.024-100 ng/mL (equivalent sample concentration 0.003 – 1.25 mg/kg)
Limit of quantitation	0.01 mg/kg	0.01 mg/kg
Validation Range	0.01-100 mg/kg	0.01-100 mg/kg

CONCLUSION

The method was successfully conducted for determination of fenpicoxamid, prothioconazole and prothioconazole-desthio in pollen, nectar, honey, pupae, larvae, worker jelly and feeding solution with an LOQ of 0.001 mg/kg and up to 50 mg/kg for pollen, 10 mg/kg for nectar, 7 mg/kg for honey, 0.2 mg/kg for larvae, 4 mg/kg for worker jelly and 70 mg/kg for feeding solution as well as for determination of dimethoate and fenoxycarb in feeding solution with an LOQ of 0.01 mg/kg and up to 100 mg/kg according to the guidance document SANCO/3029/99, rev. 4 (and SANCO/825/00 rev. 8.1 (for honey only)).

A 2.1.1.30 Analytical method 30

A 2.1.1.30.1 Method validation

Comments of zRMS:	The analytical method for the determination of fenpicoxamid in aqueous sugar solution (50% w/v) and in acetone was succesfully validated according to SANCO/3029/99 rev. 4, 11/07/2000. The concentrations of fenpicoxamid was determined by liquid chromatography coupled with positive-ion electrospray tandem mass spectrometry (LC-MS/MS) using external standard calibration. The limits of quantification were derived from the lowest spike level at which acceptable accuracy and precision (repeatability) data were obtained. The LOQ for the determination of fenpicoxamid in 50% (w/v) aqueous sugar solution (oral administration) is thus at 3.42 g a.i./L and the LOQ for the determination of fenpicoxamid in acetone is at 49.7 g a.i./L. For fenpicoxamid, the actual confirmed linear working range was from 0.00710 mg a.i./L to 0.0265 mg a.i./L. The mean recoveries at each fortification level were in the range between 70% and 110% with relative standard deviations below 20%. The method is acceptable.

Reference:	KCP 10.3.1.1.1/3
Report author:	Cornement, M.; Morgenthal, K.
Report year:	2022
Report title:	XDE-777 TGAI - Acute Oral and Contact Toxicity to Bumble Bees (*Bombus terrestris*) under Laboratory Conditions
Report No.:	201076
Testing Facility Report No.:	20200224
Method(s) used:	201076
Guidelines followed in study:	SANCO/3029/99 rev. 4
Deviation from current test guidelines:	No
Analytical Performing Laboratory:	Innovative Environmental Services (IES) Ltd Witterswil Switzerland
GLP/Officially recognised testing facilities:	Yes

MATERIAL AND METHODS

Method Principle

Residues of fenpicoxamid were determined from 50% (w/v) aqueous sugar solution samples (oral toxicity test) and from samples of acetone (contact toxicity test) by dilution with acetone/methanol (50/50; v/v). The final sample was diluted into the calibration range with acetone/methanol/water (25/25/50; v/v/v) and analysed for fenpicoxamid by liquid chromatography coupled with positive-ion electrospray tandem mass spectrometry (LC-MS/MS).

RESULTS AND DISCUSSION

Mean recovery values at each fortification concentration were within the acceptance range (mean recovery 70-110%; RSD ≤ 20%). The results obtained are summarised in the following tables.

Table:1 Recovery results from method validation of fenpicoxamid (m/z615/239Q) using the analytical method

Matrix	Fortification level g/L	Mean Recovery (%)	RSD (%)	n	Comments
50 % (w/v) aqueous sugar solution (for oral administration)	3.42 g a.i./L	100	5.9	5	107, 107, 102, 91, 99
50 % (w/v) aqueous sugar solution (for oral administration)	6.21 g a.i./L	100	2.3	5	99, 96, 101, 101, 100
acetone (for contract administration)	49.7 g a.i./L	96	0.6	5	95, 96, 96, 95, 96
acetone (for contract administration)	124 g a.i./L	97	7.0	5	90, 96, 96, 93, 108

Table:2 Procedural recovery results of fenpicoxamid (m/z 615/239Q) using the analytical method

Matrix	Fortification level g/L	Mean Recovery (%)	RSD (%)	n	Comments
50 % (w/v) aqueous sugar solution (for oral administration)	3.44 g a.i./L	104	7.8	5	97, 105, 95, 114, 109
50 % (w/v) aqueous sugar solution (for oral administration)	6.25 g a.i./L	100	3.7	5	95, 102, 102, 104, 98
acetone (for contract administration)	50.0 g a.i./L	89	6.1	5	80, 92, 88, 94, 89
acetone (for contract administration)	122 g a.i./L	104	3.0	5	106, 106, 103, 104, 99

Table:3 Characteristics for the analytical method used for determination of residues of fenpicoxamid in 50% (w/v) aqueous sugar solution (oral toxicity test) and in acetone (contact toxicity test)

Analyte	fenpicoxamid	fenpicoxamid
Matrix	50 % (w/v) aqueous sugar solution	acetone
Technique	LC-MS/MS	LC-MS/MS
Specificity	*m/z* 615/239Q blank value <30% LOQ	*m/z* 615/239Q blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis without weighting r≥0.99 8 data points	linear regression analysis without weighting r≥0.99 8 data points
Calibration range	Concentration range of 0.000710-0.0265 mg a.i./L	Concentration range of 0.000710-0.0265 mg a.i./L
Limit of quantitation	3.44 g a.i./L	50.0 g a.i./L
Validation Range	3.42 – 6.21 g a.i./L	49.7 – 124 g a.i./L

CONCLUSION

This method was successfully validated for the determination of fenpicoxamid in aqueous sugar solution (50% w/v) and in acetone according to the requirements set forth in SANCO/3029/99 rev. 4.

A 2.1.1.31 Analytical method 31

A 2.1.1.31.1 Method validation

Comments of zRMS:	The analytical method for the determination of fenpicoxamid and prothioconazole as the active ingredients of the test item GF-3307 in feeding solution samples was succesfully validated according to SANCO/3029/99 rev. 4, 11/07/2000. The concentrations of fenpicoxamid and prothioconazole was determined by liquid chromatography coupled with positive-ion electrospray tandem mass spectrometry (LC-MS/MS) using external standard calibration. The LOQ for the determination of prothioconazole in 50% (w/v) aqueous sugar solution (oral administration) is thus at 0.0333 g a.i./L, whereas the LOQ for the determination of fenpicoxamid in 50% (w/v) aqueous sugar solution is at 0.0161 g a.i./L. The LOQ for the determination of prothioconazole in water containing 0.5 % Etalfix® Pro (contact administration) is thus at 0.704 g a.i./L whereas the LOQ for the determination of Fenpicoxamid in water containing 0.5 % Etalfix® Pro is at 0.341 g a.i./L. For Prothioconazole, the actual confirmed linear working range was from 0.0558 mg a.i./L to 1.06 mg a.i./L. The R^2 fits of the constructed calibration lines were 0.9969 for the quantitative transition and 0.9972 for the confirmatory transition. For Fenpicoxamid, the actual confirmed linear working range was from 0.0290 mg a.i./L to 0.566 mg a.i./L. The R^2 fits of the constructed calibration lines were 0.9997 for the quantitative transition and 0.9996 for the confirmatory transition. The mean recoveries at each fortification level were in the range between 70% and 110% with relative standard deviations below 20%. The method is acceptable.

Reference:	KCP 10.3.1.1.1/4
Report author:	Cornement, M. and Dr. Morgenthal, K.
Report year:	2022
Report title:	GF-3307 - Acute Oral and Contact Toxicity to Bumble Bees (*Bombus terrestris*) under Laboratory Conditions
Report No.:	201075
Testing Facility Report No.:	20200222
Method(s) used:	HPLC/MS/MS
Guidelines followed in study:	SANTE/2020/12830/Rev.1
Deviation from current test guidelines:	No
Analytical Performing Laboratory:	Innovative Environmental Services (IES) Ltd Benkenstrasse, Witterswil, Switzerland
GLP/Officially recognised testing facilities:	Yes

MATERIAL AND METHODS

Method Principle

The concentrations of fenpicoxamid and prothioconazole as the active ingredients of the test item in appilcation solution samples were determined by liquid chromatography coupled with positive-ion electrospray tandem mass spectrometry (LC-MS/MS) using external standard calibration with calibration standards prepared in solvent.

An inertsil ODS-3 column (50 x 2.1 mm) was used. Gradient elution was applied using 0.1 % formic acid in water and methanol as mobile phases.

Application solutions were worked up by serial dilution of defined aliquots with a mixture of acetone/methanol/water (25/25/50; v/v/v) was used.

LC/MS/MS detection was carried out in ESI positive mode using the following mass transitions:

RESULTS AND DISCUSSION

Mean recovery values at each fortification concentration were within the acceptance range (mean recovery 70-120%; RSD ≤ 20%). The results obtained are summarised in the following tables.

Table 1: Recovery results from up-front in-study method validation of fenpicoxamid (m/z 615/239Q) using the analytical method

Matrix	Fortification level mg/L	Mean Recovery (%)	RSD (%)	n	Individual Recoveries (%)
50 % (w/v) aqueous sugar solution (for oral administration)	16.1 mg a.i./L	93	0.7	5	93, 92, 93, 93, 93
50 % (w/v) aqueous sugar solution (for oral administration)	717 mg a.i./L	90	2.3	5	89, 93, 88, 89, 89
water containing 0.5 % Etalfix® Pro (for contract administration)	341 mg a.i./L	90	0.7	5	90, 91, 90, 90, 91
water containing 0.5 % Etalfix® Pro (for contract administration)	14200 mg a.i./L	89	1.1	5	89, 90, 88, 90, 91

Table 2: Recovery results from up-front in-study method validation of fenpicoxamid (m/z 615/515C) using the analytical method

Matrix	Fortification level mg/L	Mean Recovery (%)	RSD (%)	n	Individual Recoveries (%)
50 % (w/v) aqueous sugar solution (for oral administration)	16.1 mg a.i./L	91	1.1	5	93, 91, 90, 90, 91
50 % (w/v) aqueous sugar solution (for oral administration)	717 mg a.i./L	89	2.5	5	87, 93, 88, 89, 89
water containing 0.5 % Etalfix® Pro (for contract administration)	341 mg a.i./L	90	0.7	5	90, 90, 89, 90, 90
water containing 0.5 % Etalfix® Pro (for contract administration)	14200 mg a.i./L	88	1.9	5	88, 87, 87, 88, 91

Table 3: Recovery results from up-front in-study method validation of prothioconazole (m/z 344/153Q) using the analytical method

Matrix	Fortification level mg/L	Mean Recovery (%)	RSD (%)	n	Individual Recoveries (%)
50 % (w/v) aqueous sugar solution (for oral administration)	33.3 mg a.i./L	110	1.2	5	109, 110, 108, 111, 109
50 % (w/v) aqueous sugar solution (for oral administration)	1480 mg a.i./L	109	1.9	5	108, 113, 108, 109, 108
water containing 0.5 % Etalfix® Pro (for contract administration)	704 mg a.i./L	102	0.8	5	101, 102, 102, 103, 102
water containing 0.5 % Etalfix® Pro (for contract administration)	29300 mg a.i./L	101	1.2	5	101, 102, 99, 100, 101

Table 4: Recovery results from up-front in-study method validation of prothioconazole (m/z 344/125C) using the analytical method

Matrix	Fortification level mg/L	Mean Recovery (%)	RSD (%)	n	Individual Recoveries (%)
50 % (w/v) aqueous sugar solution (for oral administration)	33.3 mg a.i./L	109	1.3	5	108, 111, 107, 111, 110
50 % (w/v) aqueous sugar solution (for oral administration)	1480 mg a.i./L	108	2.2	5	108, 112, 107, 108, 107
water containing 0.5 % Etalfix® Pro (for contract administration)	704 mg a.i./L	102	0.7	5	100, 102, 101, 102., 102
water containing 0.5 % Etalfix® Pro (for contract administration)	29300 mg a.i./L	101	1.0	5	100, 102, 100, 100, 101

Table 5: Concurrent recovery testing results of fenpicoxamid (m/z 615/239Q) using the analytical method

Matrix	Fortification level mg/L	Mean Recovery (%)	RSD (%)	n	Individual Recoveries (%)
50 % (w/v) aqueous sugar solution (for oral administration)	16.1 mg a.i./L	80	1.2	5	80, 78, 81, 81, 80
50 % (w/v) aqueous sugar solution (for oral administration)	717 mg a.i./L	81	3.7	5	76, 82, 81, 83, 82
water containing 0.5 % Etalfix® Pro (for contract administration)	341 mg a.i./L	91	1.9	5	91, 89, 94, 90, 91
water containing 0.5 % Etalfix® Pro (for contract administration)	14200 mg a.i./L	83	5.3	5	89, 78, 81, 84, 80

Table 6: Concurrent recovery testing results of prothioconazole (m/z 344/153Q) using the analytical method

Matrix	Fortification level mg/L	Mean Recovery (%)	RSD (%)	n	Individual Recoveries (%)
50 % (w/v) aqueous sugar solution (for oral administration)	33.3 mg a.i./L	95	2.2	5	94, 97, 92, 96, 96
50 % (w/v) aqueous sugar solution (for oral administration)	1480 mg a.i./L	96	4.5	5	88, 98, 100, 97, 97
water containing 0.5 % Etalfix® Pro (for contract administration)	704 mg a.i./L	103	2.9	5	99, 106, 106, 103, 100
water containing 0.5 % Etalfix® Pro (for contract administration)	29300 mg a.i./L	89	5.8	5	98, 84, 86, 89, 88

Table 7: Characteristics for the analytical method used for determination of residues of fenpicoxamid in application solutions

Analyte	Fenpicoxamid	Fenpicoxamid
Matrix	50 % (w/v) aqueous sugar solution	water containing 0.5 % Etalfix® Pro
Technique	LC-MS/MS	LC-MS/MS
Specificity	m/z 615/239Q m/z 615/515C blank value < 30% LOQ	m/z 615/239Q m/z 615/515C blank value < 30% LOQ
Calibration (type, number of data points)	linear regression analysis, no weighting r≥0.99 12 data points	linear regression analysis, no weighting r≥0.99 12 data points
Calibration range	Concentration range of 0.0290 – 0.566 mg a.i./L (equivalent sample concentration 0.0029 – 2.3 g a.i./L)	Concentration range of 0.0290 – 0.566 mg a.i./L (equivalent sample concentration 0.062 – 49 g a.i./L)
Limit of quantitation	0.0161 g a.i./L	0.341 g a.i./L
Validation Range	0.0161 – 0.717 g a.i./L in 50 % (w/v) aqueous sugar solution	0.341 – 14.2 g a.i./L in water containing 0.5 % Etalfix® Pro

Table: 8 Characteristics for the analytical method used for determination of residues of prothioconazole in application solutions

Analyte	Prothioconazole	Prothioconazole
Matrix	50 % (w/v) aqueous sugar solution	water containing 0.5 % Etalfix® Pro
Technique	LC-MS/MS	LC-MS/MS
Specificity	m/z 344/153Q m/z 344/125C blank value < 30% LOQ	m/z 344/153Q m/z 344/125C blank value < 30% LOQ
Calibration (type, number of data points)	linear regression analysis, no weighting r≥0.99 14 data points	linear regression analysis, no weighting r≥0.99 14 data points
Calibration range	Concentration range of 0.0558 – 1.06 mg a.i./L (equivalent sample concentration 0.0056 – 4.2 g a.i./L)	Concentration range of 0.0588 – 1.06 mg a.i./L (equivalent sample concentration 0.12 – 92 g a.i./L)
Limit of quantitation	0.0333 g a.i./L	0.704 g a.i./L
Validation Range	0.0333 – 1.48 g a.i./L in 50 % (w/v) aqueous sugar solution	0.704 – 29.3 g/L in water containing 0.5 % Etalfix® Pro

The matrix effects (tested for 50% (w/v) aqueous sugar solution) were found to be < 20% for both, fenpicoxamid and prothioconazole, and thus negligible.

CONCLUSION

This method was successfully validated for the determination of fenpicoxamid and prothioconazole in application solutions.

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)

A 2.1.2.1.1 Method validation/Extraction efficiency

Comments of zRMS:	The study was evaluated in the Registration Report, Part B5 for GF-3308 on 24.08.2022.

	Summary: The objective of this cross-validation study was to compare residue amounts of fenpicoxamid extracted from samples of barley grain, oil seed rapeseed and banana with incurred residues when extracting with solvent systems as used in method DAS#120615, and QuEChERS method (DAS#120998) and when extracting with solvent systems as were used in metabolism study DAS #110334, in accordance to the technical guideline on the evaluation of extraction efficiency of residue analytical methods, SANTE 2017/10632, rev. 3. For XDE-777, the average residue values from the Method 1 (MOR Method, DAS #120615) and Method 2 (MRM Method, DAS # 120998) are similar to the residue values obtained from the ASE extraction, Method 3 (NOR Method, DAS #110334) for all three matrices. The extraction efficiency results obtained by MOR Method (DAS #120615) and MRM Method (DAS # 120998) were higher than 70% when compared with the results obtained for the method NOR Method (DAS #110334). The average of % extracted ranged from 107-118%. The %RSDs were calculated to be less than 20%. This study has proven the satisfactory extraction efficiency of the extraction used in the analytical methods (MOR Method/ DAS #120615, MRM Method/DAS # 120998) for the quantitative determination of residues of XDE-777 when compared with the NOR Method/DAS #110334 for fenpicoxamid (XDE-777) in banana, barley grain and oilseed rape seed matrices. The study is acceptable.

Reference:	KCP 5.3.2.2/05
Report:	Senciuc, M.; 2021; Summary of Cross-Validation - Comparing Amounts of Fenpicoxamid Extracted from Samples of Barley Grain, Oil Seed Rapeseed and Banana with Incurred Residues using 3 Different Solvent Systems; EAG Laboratories GmbH; Ulm, Germany; Lab Study No. Study No. S20-01536; DAS Study No. 200456; 28 January 2021; Unpublished
Guideline(s):	Yes, OPPTS 860.1340, SANCO/825/00 rev. 8.1, SANCO/3029/99 rev. 4, SANTE 2017/10632 rev.3, Dir98-02
Guideline Deviations:	No
GLP:	Yes
Acceptability:	Yes
Method Alterations:	None

STUDY SUMMARY

This study was conducted to evaluate the extraction efficiency of Dow AgroSciences residue analytical method DAS#120615 "XDE-777 and its Metabolite X642188 – Validation of the Method for the Determination of Residues of XDE-777 and its Metabolite X642188 in Crops by LC-MS/MS" and Dow AgroSciences residue analytical method DAS#120998, "Validation of a Multi-residue Method Following the QuEChERS Sample Preparation Technique for the Determination of XDE-777 and Its Metabolite X642188 in Matrices of Plant and Animal Origin" with respect to NOR Study DAS# 110334 "A Nature of the Residue Study with [14C]-XR-777 Applied to Wheat". This method is applicable for the quantitative determination of residues Fenpicoxamid (XDE-777), in agricultural commodities (wet crops, dry crops, and oily crops).

Incurred residues are extracted from banana fruit, barley grain and oilseed rape seeds using acetonitrile/water, 90/10 v/v (analytical method 120998) and acetonitrile/water, 50/50 v/v followed by cleaned up using PSA/magnesium sulfate (analytical method 120998). Extracted residue levels are determined by LC-MS/MS. The method limit of quantitation (LOQ) is 0.01 mg/kg (ppm). The methods are considered suitable for enforcement purposes based on current guidelines: EPA Residue Chemistry Test Guidelines OPPTS 860.1340, SANCO/3029/99 rev.4 and SANCO/825/00 rev.8.1, as well as PMRA Regulatory Directive Dir98-02.

Results obtained by Method 1 (MOR Method, DAS #120615) and Method 2 (MRM Method, DAS # 120998) are similar to the residue values obtained from the ASE extraction, Method 3 (NOR Method, DAS #110334) for all three matrices. The % RSDs were calculated to be less than 20%. The average of % extracted ranged from 107%-118%, if considering that the residue extracted by NOR Method, DAS #110334 is 100%.
The extraction efficiency results obtained by MOR Method (DAS #120615) and MRM Method (DAS # 120998) were higher than 70% when compared with the results obtained for the method NOR Method (DAS #110334).

Extraction efficiency results obtained when compared with NOR Method: DAS #110334	Banana	Barley Grain	Oilseed Rape Seeds
MOR Method: DAS #120615	115%	115%	118%
MRM Method: DAS # 120998	118%	111%	107%

This study has proven the satisfactory extraction efficiency of the extraction used in the analytical methods (MOR Method/ DAS #120615, MRM Method/DAS # 120998) for the quantitative determination of residues of XDE-777 when compared with the NOR Method/DAS #110334 for fenpicoxamid (XDE-777) in banana, barley grain and oilseed rape seed matrices.
Extraction efficiency is acceptable based on current guidelines: EPA Residue Chemistry Test Guidelines OPPTS 860.1340, SANCO/3029/99 rev.4 and SANCO/825/00 rev.8.1, as well as PMRA Regulatory Directive Dir98-02.

MATERIALS AND METHODS

Test Item(s)

Test item (Common name):	Fenpicoxamid (XDE-777)
Purity:	98.7%
Description (physical state):	White powder
Lot/batch no.:	SYN-FS08251-080 / TSN 302306

Method Scope

This method is applicable for the quantitative determination of residues Fenpicoxamid (XDE- 777) in agricultural commodities (banana, barley grain, oilseed rapeseed). The method was concurrently validated over the concentration range of 0.01-0.1 mg/kg, except barley grain with a range of 0.01 to 2.0 mg/kg, always with a validated limit of quantitation of 0.01 mg/kg.

Method Principle

Residues of Fenpicoxamid (XDE- 777) are extracted from incurred samples with acetonitrile/water, 90/10 v/v for analytical method 120615 and respectively with acetonitrile/water, 50/50 v/v for analytical method 120998. The final sample is analysed for Fenpicoxamid (XDE- 777), by liquid chromatography coupled with positive-ion electrospray tandem mass spectrometry (LC- MS/MS).
Within the nature of residue study, residues of Fenpicoxamid (XDE- 777), are extracted from samples by using acetonitrile containing 0.1% phosphoric acid following by acetonitrile/water/phosphoric acid 50/50/0.1 v/v/v. The final sample is analysed for Fenpicoxamid (XDE- 777), by liquid chromatography coupled with positive-ion electrospray tandem mass spectrometry (LC-MS/MS).

Linearity

For analyte, the linearity of detector response was evaluated using matrix-matched standards, except for banana extracted using the analytical method from DAS study 120615. Calibration curves were calculated by linear regression analysis with 1/x weighting. For analytical method from DAS study 120615 and DAS study 110334, calibration curves resulting from the injection of at least 5 standards over the concentration range of 0.0075-1.0 ng/mL (or the sample equivalent range of 0.003-0.4 mg/kg) demonstrated linearity with correlation coefficients (r) of at least 0.999. For analytical method listed in DAS study 120998, calibration curves resulting from the injection of at least 5 standards over the concentration range of 0.075-5.0 ng/mL (or the sample equivalent range of 0.003-0.20 mg/kg) demonstrated linearity with correlation coefficients (r) of at least 0.999.

Selectivity

Table A 125: Transitions monitored

Fenpicoxamid (XDE- 777)	m/z Q1/Q3 615/239 (quantitative)
Fenpicoxamid (XDE- 777)	m/z Q1/Q3 615/515 (confirmatory)*

* this transition was only monitored, but not reported.

RESULTS AND DISCUSSION

Extraction Efficiency

Extraction efficiency is sufficiently proven because the residue amount obtained for the incurred samples extracted using the method listed in the studies DAS 120615 and DAS 120998 differs by no more than 30% compared to the results obtained with the solvent from the DAS study 110334. The results obtained are summarised in the following tables.

Table A 126: Extraction efficiency data for Fenpicoxamid (XDE-777) (m/z 615/239Q) using analytical method 120615

Matrix	Residue Analytical Method	NOR Method	%NOR Findings	n Method 120615 / NOR 110334
	mean (mg/kg)	mean (mg/kg)	(%)	
Banana	0.0242	0.0210	115%	3/4
Barley Grain	1.017	0.886	115%	4/4
Oilseed Rape Seed	0.0160	0.0135	118%	3/3

Table A 127: Extraction efficiency data for Fenpicoxamid (XDE-777) (m/z 615/239Q) using analytical method 120998

Matrix	Residue Analytical Method	NOR Method	%NOR Findings	n Method 120998 / NOR 110334
	mean (mg/kg)	mean (mg/kg)	(%)	
Banana	0.0246	0.0210	118%	4/4
Barley Grain	0.980	0.886	111%	4/4
Oilseed Rape Seed	0.0144	0.0135	107%	4/3

CONCLUSION

Extraction efficiency is acceptable based on current guidelines: EPA Residue Chemistry Test Guidelines OPPTS 860.1340, SANCO/3029/99 rev.4 and SANCO/825/00 rev.8.1, as well as SANTE 2017/10632 rev.3 and PMRA Regulatory Directive Dir98-02.

A 2.1.2.1.2 Method validation (Report 1) and Extraction efficiency (Report 2)

Comments of zRMS:	**1. Document No. M-498384-01-1** The analytical method 01300/M018 based on "QuEChERS" method was validated for prothioconazole-desthio in/on wheat grain, grapes, rapeseed, dry bean and cucumber. The limit of quantitation (LOQ) was 0.01 mg/kg for prothioconazoledesthio in all tested plant matrices. The mean recoveries at each fortification level were in the range between 70% and 110% with relative standard deviations below 20%. The method meets all guideline criteria to determine residues of prothioconazoledesthio in plant matrices with the LOQ of 0.01 mg/kg. The modification of the method is accepted. **2. Document No. M-536877-02-1** The analytical methods 01013 and 01300/M018 were developed to determine prothioconazole-desthio (JAU6476-desthio) in plant matrices. The objective of this study was to investigate the extraction efficiency of these two methods in comparison to the methods used in the corresponding metabolism studies (cross validation). Results:

Each sample was analysed three times using each extraction procedure. The average recoveries (concurrent and validation) were within the acceptable range of 70 – 110% (with minor exceptions). RSD values were below 20%.
The extraction efficiency of methods 01013 and 01300/M018 was calculated as the ratio (expressed as percentage) between the average residues measured after extracting the samples according to these procedures and the average residues measured using the corresponding procedure of the corresponding metabolism study (criteria at least 70% of residues extracted compared to metabolism method corresponding to 100%).
Either study M1730851-5 for barley grain, wheat straw and wheat green material samples, or M1731145-2 for rape seed samples.

Table 1: JAU6476-desthio: Residue Levels determined by Method 01013, 01300/M018, M1730851-5 and M1731145-2.

Sample material	Examination Sample No.	Method used	Mean value(1) (mg/kg)	RSD (%)	Ratio* (%)
Barley Grain	12-2132-08-0019R	01013	0.022	18.1	96
		01300/M018	0.023	29.4	97
		M1730851-5	0.023	6.5	100
Wheat Green Material	12-2131-01-0023R	01013	0.30	6.9	88
		01300/M018	0.33	3.0	96
		M1730851-5	0.34	4.4	100
Wheat Straw	13-2138-03-0050R	01013	1.1	5.1	105
		01300/M018	0.84	3.7	80
		M1730851-5	1.0	8.8	100
Rape Seed	13-3402-02-0011R	01013	0.35	7.3	155
		01300/M018	0.31	3.7	140
		M1731145-2	0.22	9.3	100

R = Reserve sample, RSD = Relative standard deviation.

(1) Mean value of 3 independent analyses of each sample.

*Note: The extraction efficiency of the method 01013 and 01300/M018 is calculated as the ratio (expressed as percentage) between the average residues measured using these extraction procedures and the average residues measured using the extraction procedure of either studies M1730851-5 for barley grain, wheat straw and wheat green material samples, or M1731145-2 for rape seed.

For barley grain, wheat green material and wheat straw, the extraction efficiency of the methods 01013 and 01300/M018 is similar to the metabolism method M1730851-5 as the ratios ranged from 80 to 105%.

For rape seed, the methods 01013 and 01300/M018 showed higher results compared to the method M1731145-2. This could be attributed to the non-optimized extraction conditions of the metabolism M1731145-2 study originally performed on peanuts and adapted on rape seed for the purpose of this study (loss of analyte during the extraction steps may occur). The extraction efficiency of the methods 01013 and 01300/M018 on rape seed is at least equivalent to the metabolism study M1731145-2.

The methods 01013 and 01300/M018 meet all necessary criteria to sufficiently extract and determine the residues of prothioconazole-desthio in plant matrices (barley grain, wheat green material, wheat straw and rape seed). The study is acceptable.

Reference 1: **KCP 5.3.3.2/03 (method validation)**

Report 1: Chambers, J., Jarrett, H.; 2014; Modification M018 of the analytical method 01300 (based on "QuEChERS" method) for the determination of residues of prothioconazole-desthio and Document No. M-498384-01-1iprovalicarb in wheat grain, grapes, rapeseed, dry bean and cucumber; Battelle UK Ltd., Essex, UK; Report No. VC/13/017; Document No. M-498384-01-1; 30 September 2014; Unpublished

Reference 2: **KCP 5.3.3.2/06 (extraction efficiency)**

Report 2: Desmaris, F.; 2015; Amendment no. 1 to the final report – Cross-validation of extraction methods for the determination of residues of

	prothioconazole-desthio in plant material by HPLC-MS/MS; Bayer CropScience, Lyon, France; Report No. MR-15/117; Document No. M-536877-02-1; 26 October 2015; Unpublished
Guideline(s):	Yes, Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC, European Commission Guidance Document for Generating and Reporting Methods of Analysis in Support of Pre-Registration data Requirements for Annex II (part A, Section 4) and Annex III (part A, section 5) of directive 91/414, SANCO/3029/99 rev. 4, Guidance document on residue analytical methods, SANCO/825/00 rev. 8.1, European Commission, Directorate General Health and Consumer Protection 16/11/2010, US EPA Residue Chemistry Test Guideline OPPTS 860.1340: Residue Analytical Method
GLP:	Yes
Acceptability:	Yes

The objective of this study is to validate an established multi-residue monitoring method (QuEChERS) for the determination of residues of prothioconazole-desthio in wheat grain, grapes (whole bunches), rapeseed (seeds), dry bean (cannellini) and cucumber (whole fruits) to fulfil the requirements according to guidance document SANCO 825/00/ rev. 8.1.

Principle of the method
The method for the determination of prothioconazole-desthio is based on the "QuEChERS" procedures which involves extraction of residues with acetonitrile/water (1/1 v/v) after addition of water only for matrices with low water content (water was added for wheat grain, rapeseed and dry bean, no addition of water to grape or cucumber), addition of buffer salts to facilitate phase separation, clean-up of an aliquot by solid-phase dispersion and determination by LC-MS/MS using a Luna 100 5 C18, 150 mm length, 4.6 mm diameter column. The MS/MS instrument was operated in the Multiple Reaction Monitoring mode (MRM).

The initial extraction procedure deviates from the referenced method and involves shaking for an extended period of 15 minutes, because one minute shaking as foreseen in the original QuEChERS method was shown to be in many cases not sufficient to quantitatively extract incurred residues.

The mass transition m/z 312 → 7 0 was selected for all matrices tested for quantitation. For confirmation the mass transition m/z 312 → 125 was monitored for all matrices.

Table A 128: Recovery results from method 01300/M018 for the determination of prothioconazole-desthio in various plant matrices

Analyte	Matrix	LOQ (mg/kg)	Recovery fortification (mg/kg)	Recoveries % range (mean)	Repeatability RSD (%) (n)
Prothioconazole - desthio m/z 312 → 70 quantitation	Wheat (grain)	0.01	0.01	104-109 (107)	2.4 (5)
			0.10	100-106 (103)	2.6 (5)
	Grapes		0.01	98/103 (101)	1.9 (5)
			0.10	98-101 (100)	1.3 (5)
	Rapeseed		0.01	65-74 (70)	5.6 (5)
			0.10	68/74 (70)	2.0 (5)

	Dry Bean		0.01	83-95 (90)	5.0 (5)
			0.10	91-96 (94)	2.2 (5)
	Cucumber		0.01	92-96 (94)	1.7 (5)
			0.10	84-114 (95)	12 (5)
Prothioconazole - desthio m/z 312 → 125 confirmation	Wheat (grain)	0.01	0.01	104-110 (107)	2.4 (5)
			0.10	99-106 (103)	3.1 (5)
	Grapes		0.01	102-105 (103)	1.3 (5)
			0.10	100-102 (101)	1.1 (5)
	Rapeseed		0.01	68-75 (71)	4.1 (5)
			0.10	68-72 (70)	2.2 (5)
	Dry Bean		0.01	83-95 (91)	5.4 (5)
			0.10	91-96 (93)	2.1 (5)
	Cucumber		0.01	93-98 (95)	2.1 (5)
			0.10	84-114 (95)	12 (5)

Specificity
Confirmation of identity was demonstrated by determining the recovery and precision for both MS/MS transitions. HPLC-MS/MS method is highly specific and an additional confirmatory method is not necessary. Apparent residues in control samples of prothioconazole-desthio were all below 30% x LOQ. The recoveries were not corrected for interferences.

Limit of Quantification
The LOQ is 0.01 mg/kg for prothioconazole-desthio in all matrices tested.

Linearity
The linearity of the detector response was confirmed by solvent standard solutions with a range between 0.25 ng/mL to 15 ng/mL corresponding to 0.0025 mg/kg to 0.15 mg/kg. The correlation coefficient of the regression line was always > 0.99 (weighted 1/x). Matrix effects were tested for both mass transitions by comparing the peak areas of matrix-matched standards with solvent standards. In all cases the matrix effects were below or equal 20%, hence solvent standards were used for all determinations.

Accuracy (recovery)
Recovery rates were determined for five replicate samples of the matrices spiked with prothioconazole-desthio at 0.01 mg/kg (LOQ) and 0.1 mg/kg (10 x LOQ). Results were within guideline requirements (mean recovery 70-120%; RSD ≤ 20%). The mean recoveries at each fortification for the matrices were between 70-107%.

Repeatability (precision)
The repeatability of the method was determined for all matrices by running five recoveries at concentrations of 0.01 mg/kg (LOQ) and 0.1 mg/kg (10 x LOQ). The RSDs of the repeatability for each recovery set ranged from 1.1-12%. The results show good repeatability as all relative standard deviations were below 20%.

Stability of Sample Extracts
The stability in final extracts of samples fortified at the 10xLOQ was checked for the tested sample materials over a period of seventeen days. The stored extracts were quantified against fresh solvent standard

solutions. Prothioconazole-desthio is considered stable in matrix matched extract solutions of wheat grain, grapes, rapeseed, dry bean and cucumber for at least fifteen days when stored at about 4°C under dark conditions.

Reproducibility (ILV)
An ILV was conducted; see study report no. 2014/0110/01 below.

Extraction Efficiency
The extraction efficiency was demonstrated by method 01300/M018 in KCP 5.3.3.2/06; Desmaris, F.; 2015; M-536877-02-1 (Study Report Number MR-15/117), 'Amendment no. 1 to the final report - Cross validation of extraction methods for the determination of residues of prothioconazole-desthio in plant material by HPLC-MS/MS'. The extraction efficiency of the method was evaluated using barley grain, wheat green material, wheat straw and rape seed matrices from nature of residue metabolism studies (M-041657-01-1 and M-103268-01-2). Samples containing incurred prothioconazole-desthio residues were reanalysed with the sample analysis procedure described above. Results obtained using the analytical method were equivalent to those obtained in the metabolism study, demonstrating the suitability of this analytical method for the determination of prothioconazole in plant matrices.

The extraction efficiency was calculated as the ratio (expressed as percentage) between the average residues measured after extracting the samples according to the procedure and the average residues measured using the procedure of the corresponding metabolism study. Summary of results are shown below:

Analyte	Matrix	Mean value (mg/kg)	RSD (%)	Ratio (%)
Prothioconazole - desthio	Barley Grain	0.023	29.4	97
	Wheat Green Material	0.33	3.0	96
	Wheat Straw	0.84	3.7	80
	Rape Seed	0.31	3.7	140

Method 01300/M018 meet all necessary criteria (at least 70% of residues extracted compared to metabolism method corresponding to 100%) to sufficiently extract and determine the residues of prothioconazole in plant matrices.

Conclusion
The method has been fully validated in accordance with SANCO/825/00 rev. 8.1.

A 2.1.2.1.2.1 Method ILV

Comments of zRMS:	The analytical method 01300/M018 (based on QuEChERS) was independently validated for the determination of residues of prothioconazole-desthio in/on wheat grain, grapes (whole bunches), rapeseed (weeds), dry bean (cannellini) and cucumber (whole fruits). The limit of quantitation (LOQ) was 0.01 mg/kg for prothioconazole-desthio in wheat grain, grapes, rapeseed, dry bean and cucumber. Mean recoveries for each fortification level and the overall mean recovery were within the 70 - 110% range for prothioconazole-desthio with a RSD < 20%. All method validation data are in compliance with the guideline requirements for residue data generation and enforcement. The study is acceptable.

Reference: KCP 5.3.3.2/04

Report: Thies, S.; 2014; Amendment no.2 to study 2014/0110/01 - Independent laboratory validation of BCS method 01300/M018 (based on "QuEChERS" method) for the determination of residues of prothioconazole-desthio;

	Currenta GmbH & Co. OHG, Leverkusen, Germany; Report No. 2014/0110/01; Document No. M-508116-03-1; 17 December 2014; Unpublished
Guideline(s):	Yes, Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC, European Commission Guidance Document for Generating and Reporting Methods of Analysis in Support of Pre-Registration data Requirements for Annex II (part A, Section 4) and Annex III (part A, section 5) of directive 91/414, SANCO/3029/99, Guidance document on residue analytical methods; SANCO/825/00 rev. 8.1, European Commission, Directorate General Health and Consumer Protection; 2010-11-16, OECD Guidance Document on Pesticide Residue analytical Methods, ENV/JM/Mono (2007); 2007-08-13
GLP:	Yes
Acceptability:	Yes

The objective of this study was to independently validate the analytical BCS method 01300/M18 (based on "QuEChERS") for the determination of prothioconazole-desthio residues in/on wheat (grain), grapes, rapeseed, dry bean and cucumber.

Principle of the method
The analytical method 01300/M018 (based on QuEChERS) was independently validated for the determination of residues of prothioconazole-desthio in/on wheat grain, grapes (whole bunches), rapeseed (seeds), dry bean (cannellini) and cucumber (whole fruits). Prothioconazole-desthio residues were extracted using acetonitrile. For matrices with low water content (< 80%) water was added to the samples prior to extraction. After the samples were shaken for about 15 min, magnesium sulphate, sodium chloride and buffering citrate salts were added to the extracts which were shaken manually for 2 minutes and then centrifuged. An aliquot of the supernatant was transferred to a dispersive SPE clean up tube containing magnesium sulphate and PSA sorbent. After homogenisation and centrifugation, an aliquot was diluted for measurement by reversed phase HPLC-MS/MS using a Phenomenex Luna 100 C18, 150 mm length, 4.6 mm diameter, 5 µm particle size column in positive ion mode without further clean-up. Residues were quantified using solvent standards.

Table A 129: Recovery results for the independent validation of the analytical method 01300/M018 for the determination of prothioconazole-desthio in various plant matrices

Analyte	Matrix	LOQ (mg/kg)	Recovery fortification (mg/kg)	Recoveries % range (mean)	Repeatability RSD (%) (n)
Prothioconazole - desthio m/z 312 → 70 quantitation	Wheat (grain)	0.01	0.01	98-109 (102)	4.4 (5)
			0.10	83-97 (90)	6.3 (5)
	Grapes		0.01	95-101 (97)	2.6 (5)
			0.10	85-94 (90)	4.4 (5)
	Rapeseed		0.01	69-80 (75)	6.9 (5)
			0.10	70-74 (71)	2.4 (5)
	Dry Bean		0.01	92-103 (95)	4.7 (5)
			0.10	81-90 (87)	3.8 (5)
	Cucumber		0.01	94-101 (98)	2.6 (5)

			0.10	87-96 (91)	3.7 (5)
Prothioconazole - desthio m/z 312 → 125 confirmation	Wheat (grain)	0.01	0.01	99-111 (105)	5.6 (5)
			0.10	92-102 (98)	3.8 (5)
	Grapes		0.01	96-104 (100)	3.3 (5)
			0.10	85-95 (90)	4.2 (5)
	Rapeseed		0.01	71-84 (76)	7.0 (5)
			0.10	70-74 (71)	2.0 (5)
	Dry Bean		0.01	94-106 (98)	4.9 (5)
			0.10	85-96 (90)	4.9 (5)
	Cucumber		0.01	93-104 (99)	4.6 (5)
			0.10	82-94 (90)	5.6 (5)

Specificity
Confirmation of identity was demonstrated by determining the recovery and precision for both MS/MS transitions. HPLC-MS/MS method is highly specific and an additional confirmatory method is not necessary. Apparent residues in control samples of prothioconazole-desthio were all below 30% x LOQ. The recoveries were not corrected for interferences.

Limit of Quantification
The limit of quantitation (LOQ) for prothioconazole-desthio was 0.01 mg/kg in wheat grain, grapes, rapeseed, dry bean and cucumber.

Linearity
The linearity of the detector response for prothioconazole-desthio was confirmed by solvent standard solutions in the working range of 0.25 ng/mL to 20 ng/mL (corresponding to 0.0025 mg/kg - 0.20 mg/kg). The coefficients of determination (R2) were always > 0.99 (weighted 1/x). Matrix effects were not tested but this not necessary since the primary method has already demonstrated that there was negligible matrix effect.

Accuracy (recovery)
Recovery rates were determined for five replicate samples of the matrices spiked with prothioconazole-desthio at 0.01 mg/kg (LOQ) and 0.1 mg/kg (10 x LOQ). Results were within guideline requirements (mean recove1y 70-120 %). The mean recoveries at each fortification for the matrices of wheat, grape, rapeseed, dry bean and cucumber were between 71-105%.

Repeatability (precision)
The repeatability of the method was determined for all matrices by running five recoveries at concentrations of 0.01 mg/kg (LOQ-level) and 0.1 mg/kg (tenfold LOQ-level). The RSDs of the repeatability for each recovery set ranged from 2.0-7.0%. The results show good repeatability as all relative standard deviations were below 20%.

Conclusion
The ILV confirms the LOQ for prothioconazole-desthio is 0.01 mg/kg in each matrix tested.

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

A 2.1.2.2.1 Method validation

Comments of zRMS:	The analytical method modification 00655/M002 presented was performed to provide additional validation data for confirmatory purpose. The analytical method 00655/M002 for the determination of residues of JAU6476-desthio, JAU6476-3-hydroxy-desthio, and JAU6476-4-hydroxy-desthio in milk, meat, fat, kidney, liver by HPLC-MS/MS using matrix matched standards has been succesfully validated. The limit of quantitation (LOQ) for each single analyte is 0.004 mg/kg in milk and 0.01 mg/kg in all other matrices tested. Mean recoveries for each fortification level (LOQ and tenfold LOQ) and the overall mean recovery were within the 70 - 110% range with relative standard deviations below 20% for all analytes and all matrices. All method validation data are in compliance with the guideline requirements for residue data generation and enforcement. The study is acceptable.

Reference:	KCP 5.3.3.3/02
Report:	Freitag, T.; 2013; Amendment No. 1 to report no: MR-06/199; Analytical method 00655/M002 for the determination of residues of JAU6476-desthio, JAU6476-3-hydroxy-desthio and JAU6476-4- hydroxy-desthio in/on matrices of animal origin by HPLC-MS/MS; Bayer CropScience; Report No. MR-06/199; Document No. M-284607-02-1; 15 January 2013; Unpublished
Guideline(s):	Yes, EU Council Directive 91/414/EEC amended by Commission Directive 96/68/EC European Commission Guidance Document for Generating and Reporting Methods of Analysis in Support of Pre-Registration data Requirements for Annex II (part A, Section 4) and Annex III (part A, section 5) of directive 91/414, SANCO/3029/99 Guidance document on residue analytical methods; SANCO/825/00 rev. 7, European Commission, Directorate General Health and Consumer Protection, 2004-03-17
GLP:	Yes
Acceptability:	Yes

The purpose of this study was to provide a confirmatory detection for the HPLC-MS/MS method 00655/M00l for the determination of prothioconazole residues (JAU6476-3-hydroxy-desthio, JAU6476-4-hydroxy-dsethio and JAU6476-desthio) in/on matrices of animal origin. In addition, the method modification M00l to Bayer method 00655 was performed to provide additional validation data for milk samples, analysed at the lower LOQ of 0.004 mg/kg (formerly: 0.01 mg/kg in method no. 00655).

Principle of the method
Homogenized sample materials were extracted with solvent [acetonitrile/water (4/1, v/v) for meat, liver and kidney samples; water for milk samples; and acetonitrile/water (4/1, v/v), n-hexane for fat samples] by high-speed blending and centrifuged. The combined supernatants are evaporated to the aqueous remainder. The aqueous remainder is diluted with water, acidified with 5 N HCl solution and refluxed for 2 h. This hydrolysis step is performed to convert non-aromatic precursor compounds and glycosidic bound analogues into the analytes JAU6476-3-hydroxy-desthio and JAU6476-4-hydroxy- desthio. An aliquot is neutralized and purified on a ChemElut 1020 cartridge. The analytes are eluted with cyclohexane/ethyl acetate (85/15, v/v). The eluate is evaporated to dryness and the remainder is resolved in acetonitrile/water (1/1, v/v) for determination.
The analytes were chromatographed by reversed-phase HPLC on a silica-based C18- column using a

gradient acetonitrile/water eluent containing acetic acid. A triple-stage mass spectrometer with an electrospray interface (ESI: TurboIonSpray) operated in the positive ion mode with respect to all analytes under multiple-reaction monitoring (MRM) conditions was coupled to the outlet of the HPLC column to obtain highly sensitive and selective detection (RP-HPLC-ESI-MS/MS). In this mode the protonated molecular ions were separated and impulsed immediately with nitrogen to its characteristic product ions. The product ions were used for quantification. Calibration was performed against external bracketing standards in solvent.

MRM mass transitions for quantification and confirmation of JAU6476-desthio, JAU6476-3-hydroxy-desthio and JAU6476-4-hydroxy-desthio:

Analyte	Transition mass	
	For quantitification	For confirmation
JAU6476-desthio	m/z 312 → 70	m/z 312 → 125
JAU6476-3-hydroxy-desthio	m/z 328 → 70	m/z 328 → 141
JAU6476-4-hydroxy-desthio	m/z 328 → 70	m/z 328 → 141

The analytes were fortified, determined and expressed as themselves.

Table A 130: Recovery results from method validation of method 00655/M002 - Quantification

Analyte	Matrix	LOQ (mg/kg)	Recovery fortification (mg/kg)	Recoveries % range (mean)	Repeatability RSD (%) (n)
JAU6476-desthio m/z 312 → 70 quantitation	Meat	0.01	0.01	89-97 (91)	3.5 (5)
			0.10	87-91 (89)	1.7 (5)
	Liver		0.01	83-91 (87)	3.5 (5)
			0.10	85-90 (88)	2.4 (5)
	Kidney		0.01	70-93 (81)	12.1 (5)
			0.10	85-95 (90)	5.2 (5)
	Fat		0.01	89-90 (89)	0.5 (5)
			0.10	82-96 (88)	7.8 (5)
	Milk	0.004	0.004	72-88 (80)	7.7 (5)
			0.04	89-91 (90)	1.1 (5)
JAU6476-3-hydroxy-desthio m/z 328 → 70 quantitation	Meat	0.01	0.01	93-97 (96)	1.8 (5)
			0.10	90-92 (91)	1.0 (5)
	Liver		0.01	90-93 (92)	1.5 (5)
			0.10	89-91 (90)	1.1 (5)
	Kidney		0.01	90-92 (91)	0.9 (5)
			0.10	89-91 (90)	0.9 (5)
	Fat		0.01	90-93 (91)	1.2 (5)
			0.10	82-100 (91)	8.9 (5)
	Milk	0.004	0.004	88-97 (94)	4.4 (5)

			0.04	99-96 (90)	2.0 (5)
JAU6476-4-hydroxy-desthio m/z 328 → 70 quantitation	Meat	0.01	0.01	90-96 (92)	2.5 (5)
			0.10	89-91 (90)	1.0 (5)
	Liver		0.01	88-92 (90)	2.3 (4)
			0.10	89-91 (91)	1.0 (5)
	Kidney		0.01	91-94 (93)	1.2 (5)
			0.10	88-90 (89)	1.1 (5)
	Fat		0.01	91-94 (93)	1.3 (5)
			0.10	84-98 (89)	7.1 (5)
	Milk	0.004	0.004	90-97 (93)	3.5 (5)
			0.04	89-94 (92)	2.2 (5)

Table A 131: Recovery results from method validation of method 00655/M002 - Confirmation

Analyte	Matrix	LOQ (mg/kg)	Recovery fortification (mg/kg)	Recoveries % range (mean)	Repeatability RSD (%) (n)
JAU6476-desthio m/z 312 → 125 confirmation	Meat	0.01	0.01	89-97 (92)	3.1 (5)
			0.10	87-91 (91)	1.6 (5)
	Liver		0.01	83-91 (86)	3.0 (5)
			0.10	85-90 (88)	3.3 (5)
	Kidney		0.01	70-93 (80)	11.1 (5)
			0.10	85-95 (89)	6.6 (5)
	Fat		0.01	89-90 (89)	1.7 (5)
			0.10	82-96 (88)	7.0 (5)
	Milk	0.004	0.004	72-88 (82)	7.6 (5)
			0.04	89-91 (91)	1.8 (5)
JAU6476-3-hydroxy-desthio m/z 328 → 141 confirmation	Meat	0.01	0.01	93-97 (93)	4.1 (5)
			0.10	90-92 (91)	0.5 (5)
	Liver		0.01	90-93 (90)	4.2 (5)
			0.10	89-91 (90)	1.6 (5)
	Kidney		0.01	90-92 (93)	2.2 (5)
			0.10	89-91 (91)	1.2 (5)
	Fat		0.01	90-93 (93)	3.2 (5)
			0.10	82-100 (91)	8.0 (5)

	Milk	0.004	0.004	88-97 (88)	3.7 (5)
			0.04	99-96 (92)	2.1 (5)
JAU6476-4-hydroxy-desthio m/z 328 → 141 confirmation	Meat	0.01	0.01	90-96 (95)	1.7 (5)
			0.10	89-91 (91)	1.4 (5)
	Liver		0.01	88-92 (92)	1.0 (4)
			0.10	89-91 (91)	1.4 (5)
	Kidney		0.01	91-94 (92)	2.0 (5)
			0.10	88-90 (89)	1.6 (5)
	Fat		0.01	91-94 (95)	0.5 (5)
			0.10	84-98 (90)	6.6 (5)
	Milk	0.004	0.004	90-97 (89)	3.6 (5)
			0.04	89-94 (92)	1.2 (5)

Specificity
Confirmation of identity was demonstrated by determining the recovery and precision for both MS/MS transitions. HPLC-MS/MS method is highly specific and an additional confirmatory method is not necessary. Apparent residues in control samples of prothioconazole-desthio were all below 30% x LOQ. The recoveries were not corrected for interferences.

Limit of Quantification
The limits of quantification for JAU6476-desthio, JAU6476-3-hydroxy-desthio and JAU6476-4- hydroxy-desthio were established and validated at 0.01 mg/kg in cattle meat (muscle), liver, fat and kidney, and at 0.004 mg/kg in milk.

Linearity
Injection of matrix matched standard solutions at 5 concentration levels ranging from 0.04 ng/L to 8 ng/L for milk (corresponding to 0.0000004 mg/kg - 0.08 mg/kg) and from 0.1 ng/L to 20 µg/L (corresponding to 0.001 mg/kg - 0.20 mg/kg) for all other matrices resulted in good linear correlations between injected amount of the analytes and detector response. Correlation coefficients of the 1/x weighted linear regressions were always >0.9902 for all matrices.

Accuracy (recovery)
Mean recoveries for all analytes (JAU6476-desthio, JAU6476-3-hydroxy-desthio and JAU6476-4-hydroxy-desthio) in all four matrices (cattle meat (muscle), liver, kidney, fat and milk) at all fortification levels (LOQ and 10-fold LOQ) were well within the 70–120% range. The mean recoveries at each fortification for the matrices were between 80-96%.

Repeatability (precision)
The repeatability of the method was determined for all matrices by running five recoveries at concentrations at LOQ and 10xLOQ apart for JAU6476-4-hydroxy-desthio in liver at 0.01 fortification, which only had 4 recoveries but this was still considered to be acceptable given the low RSD value. The RSDs of the repeatability for each recovery set ranged from 0.5-11.1%. The results show good repeatability as all relative standard deviations were below 20%.

Reproducibility (ILV)
An ILV was conducted; see study no. P/B 1226 G below.

Extraction Efficiency

The extraction efficiency of the residue method in animal matrices was previously demonstrated for the Annex I inclusion by Heinemann, O.; "ANALYTICAL DETERMINATION OF RESIDUES OF JAU6476-3-HYDROXYDESTHIO, 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 KIIA 4.2.1.1 from original Annex I inclusion) using aged radioactive residues from the goat metabolism study (Weber, H., Weber, E. and Spiegel, K.; document M-042103-01-1, please refer to KIIA 6.2.2.2. from original Annex I inclusion). 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.

Conclusion
The Bayer method 00655/M002 was validated for the determination of JAU6476-desthio, JAU6476-3-hydroxy-desthio and JAU6476-4-hydroxy-desthio in/on cattle meat (muscle), liver, kidney, fat and milk. The results of the method validation were confirmed using a second MRM transition.

Quantification limits of 0.004 mg/kg (for milk) and 0.01 mg/kg (for all other matrices) were achieved for the determination of JAU6476-desthio, JAU6476-3-hydroxy-desthio and JAU6476-4-hydroxy- desthio. The method has been fully validated in accordance with SANCO/825/00 rev. 8.1.

A 2.1.2.2.1.1 Method ILV

Comments of zRMS:	BCS analytical method No. 00655/M0021 for the determination of residues of JAU 6476-desthio, JAU 6476-3-hydroxydesthio and JAU 6476-4-hydroxy-desthio in/on animal matrices, exemplified for cow´s milk (limit of quantification LOQ 0.004 mg/kg per each individual analyte), bovine meat, liver and fat (limit of quantification LOQ 0.01 mg/kg per each individual analyte) has been independently validated. Mean recoveries for each fortification level (LOQ and tenfold LOQ) and the overall mean recovery were within the 70 - 110% range with relative standard deviations below 20% for all analytes and all matrices. The study is acceptable.

Reference:	KCP 5.3.3.3/03
Report:	Schwarz, T., Class, T.; 2007; Independent laboratory validation of Bayer CropScience method 00655/M002 for the determination and confirmation of residues of JAU6476-desthio, JAU6476-3-hydroxydesthio and JAU6476-4-hydroxy-desthio in/on matrices of animal origin by HPLC-MS/MS; PTRL Europe GmbH, Ulm, Germany; Report No. P/B 1226 G; Document No. M-286824-01-1; 10 April 2007; Unpublished
Guideline(s):	Yes, Council Directive 91/414/EEC Annex II (Part A, section 4.2.), Annex III (Part A, section 5.2).EC Guidance document on residue analytical methods, SANCO/825/00 rev. 7 17/03/04.
GLP:	Yes
Acceptability:	Yes

The objective of this study was to independently validate the HPLC-MS/MS method 00655/M002 for the determination of prothioconazole residues (JAU6476-3-hydroxy-desthio, JAU6476-4-hydroxy- desthio and JAU6476-desthio) in/on matrices of animal origin.

Principle of the method
Residues were extracted from the specimen matrices, except milk, using acetonitrile/water (4/1; v/v). Subsequently the solutions were refluxed for 2 hours using 5 N HCl. After dilution with water and a further

clean-up by silica gel, residues of all analytes were determined using LC/MS/MS. This method is according to Bayer Crop Science residue analytical method 00655/M002 with minor modifications. These modifications were necessary for the adaptation of the method to the instrumentation of the performing laboratory.

Table A 132: Independent laboratory validation results of analytical method 006556/M002 - Quantification

Analyte	Matrix	LOQ (mg/kg)	Recovery fortification (mg/kg)	Recoveries % range (mean)	Repeatability RSD (%) (n)
JAU6476-desthio m/z 312 → 70 quantitation	Meat	0.01	0.01	82-84 (83)	1 (5)
			0.10	83-92 (89)	4 (5)
	Liver		0.01	86-90 (89)	2 (5)
			0.10	84-90 (88)	3 (5)
	Fat		0.01	68-78 (73)	5 (5)
			0.10	70-73 (71)	2 (5)
	Milk	0.004	0.004	80-85 (83)	2 (5)
			0.04	88-93 (90)	3 (5)
JAU6476-3-hydroxy-desthio m/z 328 → 70 quantitation	Meat	0.01	0.01	83-86 (84)	1 (5)
			0.10	83-95 (91)	5 (5)
	Liver		0.01	88-91 (89)	2 (5)
			0.10	83-91 (89)	4 (5)
	Fat		0.01	85-95 (90)	4 (5)
			0.10	88-92 (90)	2 (5)
	Milk	0.004	0.004	80-85 (82)	3 (5)
			0.04	88-95 (90)	3 (5)
JAU6476-4-hydroxy-desthio m/z 328 → 70 quantitation	Meat	0.01	0.01	81-87 (84)	3 (5)
			0.10	84-94 (90)	4 (5)
	Liver		0.01	84-88 (86)	2 (5)
			0.10	81-91 (89)	5 (5)
	Fat		0.01	86-97 (91)	5 (5)
			0.10	88-91 (90)	1 (5)
	Milk	0.004	0.004	80-85 (82)	3 (5)

Table A 133: Independent laboratory validation results of analytical method 006556/M002 - Confirmation

Analyte	Matrix	LOQ (mg/kg)	Recovery fortification (mg/kg)	Recoveries % range (mean)	Repeatability RSD (%) (n)

JAU6476-desthio m/z 312 → 125 confirmation	Meat	0.01	0.01	81-84 (82)	2 (5)
			0.10	83-92 (89)	4 (5)
	Liver		0.01	87-91 (89)	2 (5)
			0.10	85-91 (89)	3 (5)
	Fat		0.01	70-78 (73)	4 (5)
			0.10	70-73 (71)	2 (5)
	Milk	0.004	0.004	80-85 (82)	3 (5)
			0.04	88-93 (90)	3 (5)
JAU6476-3- hydroxy-desthio m/z 328 → 141 confirmation	Meat	0.01	0.01	83-88 (85)	3 (5)
			0.10	83-94 (90)	5 (5)
	Liver		0.01	83-90 (88)	3 (5)
			0.10	83-92 (90)	4 (5)
	Fat		0.01	84-93 (88)	5 (5)
			0.10	88-91 (90)	1 (5)
	Milk	0.004	0.004	78-88 (84)	5 (5)
			0.04	88-93 (89)	3 (5)
JAU6476-4- hydroxy-desthio m/z 328 → 141 confirmation	Meat	0.01	0.01	83-87 (85)	2 (5)
			0.10	83-94 (90)	5 (5)
	Liver		0.01	83-90 (86)	3 (5)
			0.10	82-91 (88)	4 (5)
	Fat		0.01	84-96 (90)	5 (5)
			0.10	88-91 (90)	1 (5)
	Milk	0.004	0.004	78-90 (83)	5 (5)
			0.04	88-95 (90)	3 (5)

Specificity
Confirmation of identity was demonstrated by determining the recovery and precision for both MS/MS transitions. HPLC-MS/MS method is highly specific and an additional confirmatory method is not necessary. Apparent residues in control samples of prothioconazole-desthio were all below 30% x LOQ. The recoveries were not corrected for interferences.

Limit of Quantification
The limits of quantification for JAU6476-desthio, JAU6476-3-hydroxy-desthio and JAU6476-4- hydroxy-desthio were established and validated at 0.01 mg/kg in cattle meat (muscle), liver and fat, and at 0.004 mg/kg in milk.

Linearity
Injection of matrix matched standard solutions at 6 concentration levels ranging from 0.10 ng/ml to 10

ng/ml (corresponding to 0.001 mg/kg - 0.10 mg/kg) for milk and from 0.2 ng/mL to 20 ng/mL (corresponding to 0.002 mg/kg - 0.2 mg/kg) for all other matrices resulted in good linear correlations between injected amount of the analytes and detector response. Correlation coefficients of the 1/x weighted linear regressions were always >0.997 for all matrices.

Accuracy (recovery)
Mean recoveries for all analytes (JAU6476-desthio, JAU6476-3-hydroxy-desthio and JAU6476-4-hydroxy-desthio) in all four matrices (cattle meat (muscle), liver, fat and milk) at all fortification levels (LOQ and 10-fold LOQ) were well within the 70–120% range. The mean recoveries at each fortification for the matrices were between 71-91%.

Repeatability (precision)
The repeatability of the method was determined for all matrices by running five recoveries at concentrations at LOQ and 10xLOQ apart for all analytes. The RSDs of the repeatability for each recovery set ranged from 1-5%. The results show good repeatability as all relative standard deviations were below 20%.

Conclusion
Bayer CropScience residue analytical method 00655/M002 was successfully independently validated for the determination of residues of prothioconazole (JAU 6476-desthio, JAU 6476-3-hydroxy-desthio and JAU 6476-4-hydroxy-desthio) in/on animal matrices. The ILV confirms the LOQ for all analytes tested as 0.01 mg/kg in cattle meat (muscle), liver, fat and kidney, and at 0.004 mg/kg in milk.

A 2.1.2.2.2 Method validation

Comments of zRMS:	The analytical method 01009 was successfully validated for the determination of JAU 6476-desthio, JAU 6476-3-hydroxy-desthio, JAU 6476-4-hydroxy-desthio, JAU 6476-3,4-dihydroxydesthio, and JAU 6476-4,5-dihydroxy-desthio in/on matrices of animal origin: milk, muscle, kidney, liver, fat and egg. Residues of all analytes were determined using HPLC-MS/MS. The Limit of Quantification (LOQ) for each analyte is 0.01 mg/kg (expressed as JAU 6476-desthio equivalents) in all matrices tested. Mean recoveries for all matrices per fortification level were between 70 and 103% for all mass transitions. The overall mean recoveries per matrix were between 75% and 101% with RSDs of up to 13.5% (n = 10). Relative standard deviations per analyte, fortification level, and matrix were below 20% (n = 5) for both transitions. All method validation data are in compliance with the guideline requirements for European enforcement methods (SANCO/825/00 rev. 8.1.). The study is acceptable.

Reference:	KCP 5.3.3.3/05
Report:	Schulte, G., Oel, D.; 2014; Analytical method 01009 for the determination of residues of JAU 6476-desthio,JAU 6476-3-hydroxy-desthio, JAU 6476-4-hydroxy-desthio, JAU 6476-3,4- dihydroxy-desthio, and JAU 6476-4,5-dihydroxy-desthio in/on matrices of animal origin by HPLC-MS/MS; Bayer CropScience; Report No. MR-06/120; Document No. M-279725-03-1; 26 October 2006, Amended 18 June 2014; Unpublished
Guideline(s):	Yes, EU Council Directive 91/414/EEC amended by Commission Directive 96/68/EC Guidance document on residue analytical methods; SANCO/825/00 rev. 7, European Commission, Directorate General Health and Consumer Protection
GLP:	Yes
Acceptability:	Yes

Bayer method 01009 (Billian, Wolters; 2006) is a monitoring method for the determination of residues of prothioconazole (JAU 6476-desthio, JAU 6476-3-hydroxy-desthio, JAU 6476-4-hydroxy-desthio, JAU 6476-3,4-dihydroxy-desthio, and JAU 6476-4,5-dihydroxy-desthio) in/on matrices of animal origin - cattle (milk, muscle, kidney, liver, fat) and poultry (egg).

Principle of the method
Residues were extracted from cattle (milk, muscle, kidney, liver, fat) and poultry (egg) with acetonitrile / water (4/1, v/v) using a high-speed blender. Subsequently, the solutions were refluxed for 2 hours with 5 N HCl. This hydrolysis step cleaves conjugates to agylcones and converts the metabolites with diene structure back to aromatic compounds. Residues of all analytes were determined using HPLC-MS/MS. Residues were quantified against matrix-matched standards.

MRM mass transitions for quantification and confirmation:

Analyte	Transition mass	
	For quantitification	For confirmation
JAU6476-desthio	m/z 312 → 70	m/z 312 → 125
JAU6476-3-hydroxy-desthio	m/z 328 → 70	m/z 328 → 141
JAU6476-4-hydroxy-desthio	m/z 328 → 70	m/z 328 → 141
JAU 6476-3,4-dihydroxy-desthio	m/z 344 → 70	m/z 344 → 157
JAU 6476-4,5-dihydroxy-desthio	m/z 344 → 70	m/z 344 → 157

Table A 134: Validation of method 01009 - Quantification

Analyte	Matrix	LOQ (mg/kg)	Recovery fortification (mg/kg)	Recoveries % range (mean)	Repeatability RSD (%) (n)
JAU6476-desthio m/z 312 → 70 quantitation	Milk	0.01	0.01	86-98 (92)	6.3 (5)
			0.10	84-105 (97)	9.2 (5)
	Muscle		0.01	82-98 (92)	7.4 (5)
			0.10	83-97 (91)	7.0 (5)
	Kidney		0.01	87-97 (93)	4.3 (5)
			0.10	80-92 (86)	5.6 (5)
	Liver		0.01	93-98 (95)	2.1 (5)
			0.10	99-101 (99)	0.9 (5)
	Fat		0.01	84-94 (90)	4.1 (5)
			0.10	83-88 (86)	2.2 (5)
	Egg		0.01	90-94 (92)	1.9 (5)
			0.10	86-91 (88)	2.3 (5)
JAU6476-3- hydroxy-desthio m/z 328 → 70 quantitation	Milk	0.01	0.01	86-104 (95)	8.4 (5)
			0.10	80-104 (94)	10.6 (5)
	Muscle		0.01	84-99 (93)	7.3 (5)
			0.10	82-96 (90)	6.7 (5)
	Kidney		0.01	82-109 (94)	10.9 (5)

			0.10	84-95 (90)	5.1 (5)
	Liver		0.01	88-103 (96)	5.6 (5)
			0.10	97-105 (102)	3.3 (5)
	Fat		0.01	93-97 (95)	2.0 (5)
			0.10	87-94 (91)	3.1 (5)
	Egg		0.01	94-99 (97)	2.1 (5)
			0.10	88-94 (90)	2.7 (5)
JAU6476-4- hydroxy-desthio m/z 328 → 70 quantitation	Milk	0.01	0.01	76-101 (89)	12.4 (5)
			0.10	81-103 (96)	9.4 (5)
	Muscle		0.01	83-101 (93)	8.2 (5)
			0.10	83-98 (91)	7.0 (5)
	Kidney		0.01	80-105 (90)	10.3 (5)
			0.10	85-95 (89)	4.7 (5)
	Liver		0.01	91-103 (96)	6.2 (5)
			0.10	98-105 (103)	2.7 (5)
	Fat		0.01	90-100 (96)	3.7 (5)
			0.10	91-96 (94)	2.1 (5)
	Egg		0.01	85-99 (94)	5.9 (5)
			0.10	87-94 (89)	3.0 (5)
JAU 6476-3,4-dihydroxy-desthio *m/z* 344 → 70 quantitation	Milk	0.01	0.01	82-107 (95)	11.3 (5)
			0.10	78-105 (94)	11.2 (5)
	Muscle		0.01	74-89 (82)	7.1 (5)
			0.10	66-75 (70)	5.9 (5)
	Kidney		0.01	88-104 (94)	6.3 (5)
			0.10	86-96 (91)	4.4 (5)
	Liver		0.01	82-94 (86)	6.0 (5)
			0.10	95-102 (98)	2.6 (5)
	Fat		0.01	87-98 (94)	4.8 (5)
			0.10	78-117 (94)	18.5 (5)
	Egg		0.01	89-103 (95)	6.4 (5)
			0.10	87-91 (90)	2.1 (5)

JAU 6476-4,5-dihydroxy-desthio m/z 344 → 70 quantitation	Milk	0.01	0.01	77-102 (90)	10.5 (5)
			0.10	83-111 (99)	11.3 (5)
	Muscle		0.01	83-97 (89)	6.9 (5)
			0.10	77-87 (82)	5.4 (5)
	Kidney		0.01	85-103 (94)	7.5 (5)
			0.10	82-94 (90)	5.6 (5)
	Liver		0.01	88-107 (97)	7.3 (5)
			0.10	94-97 (96)	1.8 (5)
	Fat		0.01	86-104 (93)	7.1 (5)
			0.10	84-124 (102)	17.0 (5)
	Egg		0.01	85-100 (92)	6.4 (5)
			0.10	83-89 (86)	2.9 (5)

Table A 135: Validation of method 01009 - Confirmation

Analyte	Matrix	LOQ (mg/kg)	Recovery fortification (mg/kg)	Recoveries % range (mean)	Repeatability RSD (%) (n)
JAU 6476-desthio m/z 312 → 125 confirmation	Milk	0.01	0.01	85-96 (91)	5.1 (5)
			0.10	86-104 (95)	8.0 (5)
	Muscle		0.01	84-99 (93)	6.8 (5)
			0.10	83-97 (91)	6.9 (5)
	Kidney		0.01	86-100 (92)	6.4 (5)
			0.10	82-91 (87)	4.5 (5)
	Liver		0.01	88-95 (93)	3.0 (5)
			0.10	96-99 (97)	1.7 (5)
	Fat		0.01	84-97 (91)	6.0 (5)
			0.10	84-89 (87)	2.1 (5)
	Egg		0.01	84-93 (88)	3.9 (5)
			0.10	86-91 (88)	2.1 (5)
JAU6476-3-hydroxy-desthiom/z 328 → 141 confirmation	Milk	0.01	0.01	83-101 (91)	8.8 (5)
			0.10	79-106 (96)	11.2 (5)
	Muscle		0.01	84-101 (93)	7.6 (5)
			0.10	82-97 (90)	7.1 (5)
	Kidney		0.01	90-105 (97)	7.3 (5)

			0.10	85-95 (91)	4.6 (5)
	Liver		0.01	94-104 (99)	3.9 (5)
			0.10	99-105 (103)	2.6 (5)
	Fat		0.01	83-102 (92)	8.5 (5)
			0.10	86-94 (91)	3.6 (5)
	Egg		0.01	94-99 (96)	2.6 (5)
			0.10	87-92 (89)	2.0 (5)
JAU6476-4-hydroxy-desthio m/z 328 → 141 confirmation	Milk	0.01	0.01	78-95 (87)	8.7 (5)
			0.10	79-102 (94)	10.0 (5)
	Muscle		0.01	83-101 (93)	8.2 (5)
			0.10	82-96 (90)	6.9 (5)
	Kidney		0.01	91-104 (97)	5.8 (5)
			0.10	86-94 (90)	4.0 (5)
	Liver		0.01	90-100 (95)	4.2 (5)
			0.10	96-107 (102)	4.1 (5)
	Fat		0.01	86-103 (94)	6.8 (5)
			0.10	91-97 (93)	2.3 (5)
	Egg		0.01	89-94 (92)	2.5 (5)
			0.10	88-92 (90)	1.9 (5)
JAU 6476-3,4-dihydroxy-desthiom/z *344 → 157* confirmation	Milk	0.01	0.01	75-105 (90)	14.0 (5)
			0.10	82-107 (96)	10.4 (5)
	Muscle		0.01	71-88 (79)	8.3 (5)
			0.10	66-76 (71)	6.7 (5)
	Kidney		0.01	85-101 (96)	7.1 (5)
			0.10	83-94 (90)	5.1 (5)
	Liver		0.01	90-104 (97)	6.2 (5)
			0.10	92-98 (94)	3.1 (5)
	Fat		0.01	82-91 (86)	4.9 (5)
			0.10	79-115 (94)	18.0 (5)
	Egg		0.01	92-100 (97)	2.9 (5)
			0.10	87-90 (88)	1.3 (5)
	Milk		0.01	81-98 (89)	7.6 (5)

JAU 6476-4,5-dihydroxy-desthiom/z 344 → 157 confirmation		0.01	0.10	82-107 (95)	10.6 (5)
	Muscle		0.01	82-97 (88)	6.8 (5)
			0.10	76-86 (81)	5.7 (5)
	Kidney		0.01	77-104 (94)	10.9 (5)
			0.10	84-94 (90)	4.1 (5)
	Liver		0.01	89-101 (93)	4.8 (5)
			0.10	93-99 (96)	2.2 (5)
	Fat		0.01	89-105 (95)	6.7 (5)
			0.10	85-123 (101)	16.6 (5)
	Egg		0.01	84-93 (90)	4.5 (5)
			0.10	83-87 (86)	2.2 (5)

Specificity
Confirmation of identity was demonstrated by determining the recovery and precision for both MS/MS transitions. HPLC-MS/MS method is highly specific and an additional confirmatory method is not necessary. Apparent residues in control samples of each analyte desthio were all below 30% x LOQ. The recoveries were not corrected for interferences.

Limit of Quantification
The Limit of Quantification (LOQ) for each analyte is 0.01 mg/kg (expressed as JAU 6476-desthio equivalents) in all matrices tested.

Linearity
The correlation between the injected amount of substance and the detector response at 5 concentration levels was linear for matrix-matched standard solutions in the range from 0.25 µg/L to 10 µg/L (corresponding to 0.005 mg/kg – 0.2 mg/kg). The correlation coefficients of the 1/x weighted linear regression ranged from 0.9974 to 0.9999 for both mass transitions.

Accuracy (recovery)
Mean recoveries for all analytes in all matrices (milk, muscle, kidney, liver, fat and egg) at all fortification levels (LOQ and 10-fold LOQ) were well within the 70–120% range. The mean recoveries at each fortification for the matrices were between 70-103%.

Repeatability (precision)
The repeatability of the method was determined for all matrices by running five recoveries at concentrations at LOQ and 10xLOQ The RSDs of the repeatability for each recovery set ranged from 0.9-18.5%. The results show good repeatability as all relative standard deviations were below 20%.

Reproducibility (ILV)
An ILV was conducted, see study no. P/B 1111 G below

Extraction Efficiency
The extraction efficiency of the residue method in animal matrices was previously demonstrated for the Annex I inclusion by Heinemann, O.; “ANALYTICAL DETERMINATION OF RESIDUES OF JAU6476-3-HYDROXYDESTHIO, 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 KIIA 4.2.1.1 from original Annex I inclusion) using aged radioactive resides from the goat metabolism study (document M-042103-01-1, please refer to KIIA 6.2.2.2. from original Annex I inclusion). 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

Conclusion
Method 01009 was successfully validated for the determination of JAU 6476-desthio, JAU 6476-3-hydroxy-desthio, JAU 6476-4-hydroxy-desthio, JAU 6476-3,4-dihydroxy-desthio, and JAU 6476-4,5-dihydroxy-desthio in/on matrices of from cattle (milk, muscle, kidney, liver, fat) and poultry (egg). The results of the method validation were confirmed using a second MRM transition. Quantification limit of 0.01 mg/kg (expressed as JAU 6476-desthio equivalents) was achieved for the determination of each analyte and in all matrices tested. The method has been fully validated in accordance with SANCO/825/00 rev. 8.1.

A 2.1.2.2.2.1 Method ILV

Comments of zRMS:	The BCS Analytical Method No. 010091 for the determination of residues of JAU 6476-desthio, JAU 6476-3-hydroxy-desthio, JAU 6476-4-hydroxy-desthio, JAU 6476-3,4-dihydroxy-desthio, and JAU 6476-4,5- dihydroxy-desthio in/on animal matrices, exemplified for bovine meat, cow´s milk, and whole egg (limit of quantification LOQ 0.01 mg/kg per analyte, expressed as JAU 6476-desthio equivalents) has been independently validated. For all specimen matrices, for all analytes, for each fortification level, and for both MS/MS transitions monitored, the overall recoveries per matrix and analyte were in the range between 87% and 103%, and the relative standard deviations (RSD) were ≤ 6%. The limit of quantification for the LC/MS/MS method was established at 0.01 mg/kg per analyte (expressed as JAU 6476-desthio equivalents). It is concluded that Bayer CropScience Method 01009 fulfils the reproducibility requirements as defined in EC Guidance document on residue analytical methods (SANCO/825/00 rev. 8.1) and is, therefore, applicable as enforcement method. <u>Remark:</u> Residue analysis of bovine meat, cow´s milk, and whole egg was performed according to BCS Method 01009 with minor modifications due to slightly different laboratory procedures. These modifications were necessary for adaptation of the method to the instrumentation used in the present study and do not query the quality of the original method. No major impact on the method was expected.

Reference:	KCP 5.3.3.3/06
Report:	Bacher, R.; 2006; Independent Laboratory Validation of Bayer CropScience Method No. 01009 for the Determination of Residues of JAU 6476-desthio, JAU 6476-3-hydroxy-desthio, JAU 6476-4-hydroxy-desthio, JAU 6476- 3,4-dihydroxy-desthio, and JAU 6476-4,5-dihydroxy-desthio in/on Matrices of Animal Origin by HPLC-MS/MS; PTRL Europe GmbH, Ullm, Germany; Report No. P/B 1111G; Document No. M-279818-01-1; 02 November 2006; Unpublished
Guideline(s):	Yes, Council Directive 91/414/EEC Annex II (Part A, Section 4.2, and section 5.2, Part A of Annex III) EC Guidance document on residue analytical methods, SANCO/825/00 rev. 7, 17/03/04
GLP:	Yes
Acceptability:	Yes

The purpose of this study was to independently validate the HPLC-MS/MS method 01009 for the determination of relevant residues of prothioconazole (JAU 6476-desthio, JAU 6476-3-hydroxy- desthio, JAU 6476-4-hydroxy-desthio, JAU 6476-3,4-dihydroxy-desthio, and JAU 6476-4,5- dihydroxy-desthio) in/on matrices of animal origin – (meat, milk and egg).

Principle of the method

Residues were extracted from bovine meat, cow´s milk, and whole egg with acetonitrile / water (4/1, v/v) using a high-speed blender. Subsequently, the solutions were refluxed for 2 hours with 5 N HCl. Residues of all analytes were determined using HPLC-MS/MS. The extracts were processed according to residue analytical method 01009 with minor modifications in extraction procedure. These modifications were necessary for the adaptation of the method to the instrumentation of the performing laboratory and do not query the quality of the original method.

MRM mass transitions for quantification and confirmation:

Analyte	Transition mass	
	For quantification	For confirmation
JAU6476-desthio	m/z 312 → 70	m/z 312 → 125
JAU6476-3-hydroxy-desthio	m/z 328 → 70	m/z 328 → 141
JAU6476-4-hydroxy-desthio	m/z 328 → 70	m/z 328 → 141
JAU 6476-3,4-dihydroxy-desthio	m/z 344 → 70	m/z 344 → 157
JAU 6476-4,5-dihydroxy-desthio	m/z 344 → 70	m/z 344 → 157

Table A 136: Independent laboratory validation results of analytical method 01009 – Quantification

Analyte	Matrix	LOQ (mg/kg)	Recovery fortification (mg/kg)	Recoveries % range (mean)	Repeatability RSD (%) (n)
JAU6476-desthio m/z 312 → 70 quantitation	Meat	0.01	0.01	97-100 (99)	1 (5)
			0.10	96-97 (97)	1 (5)
	Milk		0.01	100-105 (101)	2 (5)
			0.10	98-105 (101)	3 (5)
	Egg		0.01	88-91 (90)	1 (5)
			0.10	84-91 (87)	4 (5)
JAU6476-3-hydroxy-desthio m/z 328 → 70 quantitation	Meat	0.01	0.01	95-101 (98)	2 (5)
			0.10	96-102 (99)	2 (5)
	Milk		0.01	100-106 (102)	2 (5)
			0.10	96-106 (99)	4 (5)
	Egg		0.01	87-96 (91)	4 (5)
			0.10	84-89 (87)	2 (5)
JAU6476-4-hydroxy-desthio m/z 328 → 70 quantitation	Meat	0.01	0.01	95-104 (99)	4 (5)
			0.10	93-108 (99)	6 (5)
	Milk		0.01	96-109 (102)	5 (5)
			0.10	94-107 (100)	5 (5)
	Egg		0.01	87-101 (92)	6 (5)

			0.10	85-88 (87)	1 (5)
JAU 6476-3,4-dihydroxy-desthio m/z 344 → 70 quantitation	Meat	0.01	0.01	86-98 (92)	5 (5)
			0.10	86-90 (88)	2 (5)
	Milk		0.01	93-103 (97)	4 (5)
			0.10	98-109 (101)	4 (5)
	Egg		0.01	94-102 (98)	3 (5)
			0.10	89-97 (94)	3 (5)
JAU 6476-4,5-dihydroxy-desthio m/z 344 → 70 quantitation	Meat	0.01	0.01	84-92 (87)	4 (5)
			0.10	89-93 (91)	2 (5)
	Milk		0.01	96-100 (97)	2 (5)
			0.10	94-102 (97)	3 (5)
	Egg		0.01	89-96 (94)	3 (5)
			0.10	85-89 (87)	2 (5)

Table A 137: **Independent laboratory validation results of analytical method 01009 – Confirmation**

Analyte	**Matrix**	**LOQ (mg/kg)**	**Recovery fortification (mg/kg)**	**Recoveries % range (mean)**	**Repeatability RSD (%) (n)**
JAU6476-desthio m/z 312 → 125 quantitation	Meat	0.01	0.01	95-99 (97)	2 (5)
			0.10	95-97 (96)	1 (5)
	Milk		0.01	99-103 (101)	2 (5)
			0.10	98-106 (101)	3 (5)
	Egg		0.01	85-92 (89)	3 (5)
			0.10	82-89 (86)	3 (5)
JAU6476-3-hydroxy-desthio m/z 328 → 141 quantitation	Meat	0.01	0.01	99-104 (102)	2 (5)
			0.10	96-100 (98)	2 (5)
	Milk		0.01	102-108 (105)	3 (5)
			0.10	98-106 (101)	3 (5)
	Egg		0.01	93-98 (94)	2 (5)
			0.10	87-89 (88)	1 (5)
JAU6476-4-hydroxy-desthio m/z 328 → 141 quantitation	Meat	0.01	0.01	95-108 (101)	5 (5)
			0.10	93-107 (99)	6 (5)
	Milk		0.01	94-107 (101)	5 (5)
			0.10	96-106 (100)	4 (5)

	Egg		0.01	86-100 (91)	6 (5)
			0.10	85-89 (87)	2 (5)
JAU 6476-3,4-dihydroxy-desthio m/z 344 → 157	Meat	0.01	0.01	81-93 (88)	6 (5)
			0.10	85-91 (89)	3 (5)
	Milk		0.01	89-107 (95)	8 (5)
			0.10	96-104 (100)	3 (5)
	Egg		0.01	94-101 (98)	3 (5)
			0.10	91-98 (94)	3 (5)
JAU 6476-4,5-dihydroxy-desthio m/z 344 → 157	Meat	0.01	0.01	84-92 (88)	4 (5)
			0.10	89-92 (91)	2 (5)
	Milk		0.01	94-105 (98)	5 (5)
			0.10	94-101 (96)	3 (5)
	Egg		0.01	87-99 (95)	5 (5)
			0.10	87-92 (90)	2 (5)

Specificity
Confirmation of identity was demonstrated by determining the recovery and precision for both MS/MS transitions. HPLC-MS/MS method is highly specific and an additional confirmatory method is not necessary. Apparent residues in control samples of each analyte desthio were all below 30% x LOQ. The recoveries were not corrected for interferences.

Limit of Quantification
The Limit of Quantification (LOQ) for each analyte is 0.01 mg/kg (expressed as JAU 6476-desthio equivalents) in bovine meat, milk and poultry egg.

Linearity
The correlation between the injected amount of substance and the detector response at 5 concentration levels was linear for matrix-matched standard solutions in the range from 0.10 ng/ml to 10 ng/ml (corresponding to 0.002 mg/kg – 0.2 mg/kg). Correlation coefficients of the 1/x weighted linear regressions were always ≥0.997 for all matrices.

Accuracy (recovery)
Mean recoveries for all analytes in all matrices (bovine meat, milk and egg) at all fortification levels (LOQ and 10-fold LOQ) were well within the 70–120% range. The mean recoveries at each fortification for the matrices were between 86-105%.

Repeatability (precision)
The repeatability of the method was determined for all matrices by running five recoveries at concentrations at LOQ and 10xLOQ The RSDs of the repeatability for each recovery set ranged from 1-8%. The results show good repeatability as all relative standard deviations were below 20%.

Conclusion
Since the primary method is identical for all matrices, it is sufficient to perform the ILV with at least two of these matrices. In this case 3 matrices have been conducted. Method 01009 was successfully independently validated for the determination of relevant residues of prothioconazole (JAU 6476- desthio,

JAU 6476-3-hydroxy-desthio, JAU 6476-4-hydroxy-desthio, JAU 6476-3,4-dihydroxy- desthio, and JAU 6476-4,5-dihydroxy-desthio) in/on animal matrices exemplified for bovine meat, cow's milk, and whole egg.. The LOQ is confirmed to be 0.01 mg/kg for all matrices tested. The method has been fully validated in accordance with SANCO/825/00 rev. 8.1.

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)

A 2.1.2.4.1 Method validation

Comments of zRMS:	Method 01387/M002 has been sufficiently validated for the determination of prothioconazole and JAU 6476-desthio (M04) in drinking and surface water with a LOQ of 0.05 µg/L. The limit of quantitation (LOQ) is 0.05 µg/L for all analytes in surface water. Because of the direct measurement of the samples recovery rates cannot be calculated. The relative standard deviations for the peak areas were ≤ 20% for all analytes and MRM transitions. Provided that a method has been successfully validated for surface water at the LOQ required for drinking water (≤0.1 µg/L), no separate validation in drinking water is required. The method meets all guideline criteria of document SANCO/3029/99 rev. 4 and SANCO/825/00 rev. 8.1.

Reference:	KCP 5.3.3.5/03
Report:	Krebber, R., Sandau, C.; 2015; Modification M002 of analytical method 01387 for the determination of various pesticides in drinking and surface water by HPLC-MS/MS; Report No. MR-15/025; Document No. M-526061-01-1; 01 June 2015; Unpublished
Guideline(s):	Yes, Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC EC Guidance Document on Residue Analytical Methods, SANCO/825/00 rev. 8.1 of November 16, 2010 European Commission Guidance Document for Generating and Reporting Methods of Analysis in Support of Pre-Registration data Requirements for Annex II (part A, Section 4) and Annex III (part A, section 5) of directive 91/414, SANCO/3029/99 rev. 4, July 11, 2000
GLP:	Yes
Acceptability:	Yes

The objective of the study was to validate the analytical method 01387/M002 for the determination of concentrations of various pesticides, incl. prothioconazole and JAU 6476-desthio *(M04)* in drinking and surface water by HPLC-MS/MS using two MRM transitions.

Principle of the method
Water samples were determined by direct injection into the HPLC-MS/MS instrument using the positive ion mode for all analytes without further clean-up. Because of the direct measurement of the samples, recovery rates cannot be calculated hence the corresponding peak areas are presented below for completeness.

Two MRM transitions were monitored for each analyte.

MS/MS Parameters for the determination of prothioconazole and JAU 6476-desthio

Compound		Precursor Ion Q1 Mass (amu)	Product Ion Q3 Mass (amu)
Prothioconazole	quantitation	344	189
	confirmation	344	154
JAU 6476-desthio *(M04)*	quantitation	312	70
	confirmation	312	125

Table A 138: Method validation for prothioconazole for the quantitation ion (m/z 344 → m/z 189)

Sample material	Fortification level (FL) [µg/L]	Peak area (single values) [area counts]					Mean [area counts]	RSD [%]
Surface water	0.05	8645	8204	8566	8859	8738	8680	2.3
		8741	8859	8691	8636	8859		
	0.5	89774	85561	85395	85405	89321	87797	2.3
		85820	89712	88393	89082	89505		

Table A 139: Method validation for prothioconazole for the confirmatory ion (m/z 344 → m/z 154)

Sample material	Fortification level (FL) [µg/L]	Peak area (single values) [area counts]					Mean [area counts]	RSD [%]
Surface water	0.05	6790	6771	6958	6364	6920	6299	9.5
		6207	6413	5472	5755	5336		
	0.5	68113	67347	70861	76320	68686	69808	3.8
		67232	69030	69063	70477	70946		

Table A 140: Method validation for JAU 6476-desthio for the quantitation ion (m/z 312 → m/z 70)

Sample material	Fortification level (FL) [µg/L]	Peak area (single values) [area counts]					Mean [area counts]	RSD [%]
Surface water	0.05	155867	151051	152289	148150	145810	151037	1.9
		153369	151896	148989	151847	151105		
	0.5	1511351	1514428	1556334	1524425	1533506	1522200	1.2
		1500634	1523083	1542504	1506524	1509210		

Table A 141: Method validation for JAU 6476-desthio for the confirmation ion (m/z 312 → m/z 125)

Sample material	Fortification level [µg/L]	Peak area (single values) [area counts]					Mean [area counts]	RSD [%]
Surface water	0.05	94174	93527	92626	92165	91693	93164	1.6
		92026	96571	93143	93830	91886		
	0.5	950877	938876	949687	943186	921905	932259	1.6
		916213	935352	938690	912477	915328		

Specificity

No signals/peaks interfering with the detection of the analytes were observed in solutions of untreated control specimens. The blank values of all control samples were below 0.05 µg/L (<30% of LOQ). Two MRM transitions were monitored for all analytes. HPLC-MS/MS method is highly specific and an additional confirmatory method is not necessary.

Limit of Quantification

The limit of quantitation (LOQ) is 0.05 µg/L for all analytes in surface water.

Linearity

Concentrations were quantified using external matrix-matched standard solutions. The correlation between the injected amount of substance and the detector response was linear (1/x weighted) for standard solutions

in surface water (+ cysteine hydrochloride 50 mg/L) / formic acid / (1000 / 0.1, v/v) over at least 6 concentrations ranging from 0.015 µg/L to at least 1 µg/L for prothioconazole and ranging from 0.015 µg/L to 5 µg/L for JAU 6476-desthio. The correlation coefficients were ≥ 0.9990 and ≥ 0.9991 for these MRM transitions, respectively.

Accuracy (recovery)
Because of the direct measurement of the samples, recovery rates cannot be calculated and the corresponding peak areas are presented for completeness only.

Repeatability (precision)
The repeatability of the method was determined by running five surface water recoveries at concentrations at LOQ and 10-fold LOQ. The RSDs of the repeatability for each recovery set ranged from 1.2-9.5%. The results show good repeatability as all relative standard deviations were below 20%.

Storage stability of the analytes
JAU 6476-desthio was stable in surface water when stored in a freezer at ≤ -18°C for a period of 7 days. Prothioconazole can be stabilised by addition of cysteine hydrochloride.

Reproducibility (ILV)
An acceptable ILV was conducted; see Thies, S.; 2015; M-536990-01-1 below.

Conclusion
A validation for drinking water was not necessary because the limit of quantitation for surface water is equal or below the drinking water limit of 0.1 µg/L. Method 01387/M002 has been sufficiently validated for the determination of prothioconazole and JAU 6476-desthio (M04) in drinking and surface water with a LOQ of 0.05 µg/L.

A 2.1.2.4.1.1 Method ILV

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

Reference:	KCP 5.3.3.5/04
Report:	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, Leverkusen, Germany; Report No. 2015/0034/01; Document No. M-536990-01-1; 27 October 2015; Unpublished
Guideline(s):	Yes, REGULATION (EC) No 1107/2009 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC. European Commission Guidance Document for Generating and Reporting Methods of Analysis in Support of Pre-Registration data Requirements for Annex II (part A, Section 4) and Annex III (part A, section 5) of directive 91/414, SANCO/3029/99. Guidance document on residue analytical methods; SANCO/825/00 rev. 8.1,

European Commission, Directorate General Health and Consumer Protection; 2010-11-16.
OECD Guidance Document on Pesticide Residue analytical Methods; ENV/JM/Mono (2007); 2007-08-13

GLP: Yes

Acceptability: Yes

The objective of the study was the independent lab validation (ILV) of the analytical method 01387/M002 for the determination concentrations of various pesticides, incl. prothioconazole and JAU 6476-desthio *(M04)* in surface water by HPLC-MS/MS using two MRM transitions.

Principle of the method
Water samples were determined by direct injection into the HPLC-MS/MS instrument using the positive ion mode for all analytes without further clean-up. Concentrations were quantified using external matrix-matched standard solutions. Because of the direct measurement of the samples, recovery rates cannot be calculated and the peak areas are presented below for completeness only.

Table A 142: Method validation for prothioconazole for the quantitation ion (m/z 344 → m/z 189)

Sample material	Fortification level (FL) [µg/L]	Peak area (single values) [area counts]					Mean [area counts]	RSD [%]
Surface water	0.05	7510	6130	7360	7310	7340	7130	7.9
	0.5	74700	62000	77300	75600	71800	72280	8.4

Table A 143: Method validation for prothioconazole for the confirmation ion (m/z 344 → m/z 154)

Sample material	Fortification level (FL) [µg/L]	Peak area (single values) [area counts]					Mean [area counts]	RSD [%]
Surface water	0.05	4010	5080	4750	5020	4430	4658	9.5
	0.5	56600	53400	56200	53800	53800	54760	2.8

Table A 144: Method validation for JAU 6476-desthio for the quantitation ion (m/z 312 → m/z 70)

Sample material	Fortification level (FL) [µg/L]	Peak area (single values) [area counts]					Mean [area counts]	RSD [%]
Surface water	0.05	71900	70300	59600	71700	73100	69320	8.0
	0.5	682000	691000	694000	690000	694000	690200	0.7

Table A 145: Method validation for JAU 6476-desthio for the confirmation ion (m/z 312 → m/z 125)

Sample material	Fortification level (FL) [µg/L]	Peak area (single values) [area counts]					Mean [area counts]	RSD [%]
Surface water	0.05	49600	53400	48500	53100	52300	51380	4.3
	0.5	606000	462000	523000	514000	481000	517200	11

Specificity
Confirmation of identity was demonstrated by determining the recovery and precision for both MS/MS transitions. HPLC-MS/MS method is highly specific and an additional confirmatory method is not necessary. The blank values of air control samples were below 0.05µg/L (<30% of LOQ).

Limit of Quantification
The limit of quantitation of the method is 0.05 µg/L for prothioconazole and the metabolite JAU 6476-desthio in surface water.

Linearity
Concentrations were quantified using extremal matrix-matched standard solutions. The con elation between

the injected amount of substance and the detector response was linear (1/x weighted) for standard solutions in surface water (+ cysteine hydrochloride for stabilisation of prothioconazole) over at least 5 concentration levels ranging from 0.015µg/L to at least 1.0 µg/L for all analytes. Determined correlation coefficients for all analytes were > 0.99 for both MRM transitions.

Accuracy (recovery)
Because of the direct measurement of the samples, recovery rates cannot be calculated and the peak area values are presented for completeness only.

Repeatability (precision)
The repeatability of the method was determined for all matrices by running five recoveries at concentrations at LOQ and 10-fold LOQ. The RSDs of the repeatability for each recove1yset ranged from 0.7-9.5%. The results show good repeatability as all relative standard deviations were below 20%.

Conclusion
A validation for drinking water was not necessa1y because the limit of quantitation for surface water is equal or below the drinking water limit of 0.1 µg/L. The ILV confirms the LOQ for prothioconazole and JAU 6476-desthio is 0.05µg/L in surface 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)

A 2.1.2.6.1 Method validation

Comments of zRMS:	The analytical method 01471 for the determination of prothioconazole-desthio in cattle blood by HPLC-MS/MS has been validated. Blood samples were diluted with acetonitrile and analyzed by HPLC-MS/MS using electrospray ionization in the positive mode. The limit of quantitation (LOQ) in blood samples for prothiconazole-desthio was 0.05 mg/L. Mean recoveries at all fortification levels (LOQ and 10-fold LOQ) were well within the 70–120% range. The relative standard deviations for the peak areas were ≤ 20% for all MRM transitions. The method meets all criteria of guidelines SANCO/825/00 rev. 8.1 to determine concentrations of prothiconazole-desthio in body fluid at the LOQ level of 0.05 mg/L, but according to the SANTE/2020/12830, Rev.1, 24. February 2021, the LOQ should be lower - 0.01 mg/L for body fluids and 0.01 mg/kg for body tissues.

Reference:	KCP 5.3.3.7/01
Report	Hoeppner, S.; 2015; Validation of the BCS analytical method 01471 for the determination of prothioconazole-desthio in body fluid by HPLC-MS/MS; Currenta GmbH & Co. OHG, Leverkusen, Germany; Report No 2015/0047/01; Document No. M-535874-02-1; 06 October 2015; Unpublished
Guideline(s):	Yes, REGULATION (EC) No 1107/2009 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC.

	European Commission Guidance Document for Generating and Reporting Methods of Analysis in Suppo1t of Pre-Registration data Requirements for Annex II (part A, Section 4) and Annex III (part A, section 5) of directive 91/414, SANCO/3029/99. Guidance document on residue analytical methods; SANCO/825/00 rev. 8.1, European Commission, Directorate General Health and Consumer Protection; 2010-11-16. OECD Guidance Document on Pesticide Residue analytical Methods; ENV/JM/Mono (2007); 2007-08-13.
Deviations:	Not specified
GLP:	Yes
Acceptability:	Yes

The method 01471 was developed as a post-registration method for the determination of prothioconazole-desthio in blood (e.g. in case of intoxication). The method was validated using a sample of cattle blood.

Principle of the method
Prothioconazole-desthio is extracted and proteins are precipitated with acetonitrile. After centrifugation the supernatant is diluted with water and analysed by liquid chromatography with tandem mass spectrometric detection (LC-MS/MS). The triple-quadrupole is operated in the positive electrospray ionisation mode. Prothioconazole-desthio is monitored by means of the MS/MS transitions m/z 312 → 70 (quantitation) and m/z 312 → 125 (confirmation). Full validation data were generated for two MS/MS transitions. The first transition is recommended for quantification and the second transition may be used for confirmatory analyses.

Table A 146: Validation of the method 01471 for the determination of prothioconazole-desthio in blood

Substrate	Fortification level (µg/L)	Number of replicates	m/z 312 → 70		m/z 312 → 125	
			Mean (%)	RSD (%)	Mean (%)	RSD (%)
Cattle blood	50	5	85	4.2	91	7.1
	500	5	104	1.8	101	2.8
	Overall	10	94	11.0	96	7.7

Note : All the fortification levels are expressed as prothioconazole-desthio.

Specificity
Confirmation of identity was demonstrated by determining the recovery and precision for both MS/MS transitions. HPLC-MS/MS method is highly specific and an additional confirmatory method is not necessary. Apparent residues in control samples of prothioconazole desthio were all below 30% x LOQ.

Limit of quantification
The limit of quantification for prothioconazole-desthio in blood was established at 50 µg/L, expressed as itself.

Linearity
The correlation between the injected amount of substance and the detector response at 7 concentration levels was linear (1/x weighted) for standard solutions in blood ranging from 0.1 µg/L to 10.0 µg/L (0.01 $\times 10^{-6}$ to 1×10^{-6} % w/w) for both MRM transitions. Correlation coefficients were ≥0.9997 for both MRM transitions.

Accuracy (recovery)
Mean recoveries at all fortification levels (LOQ and 10-fold LOQ) were well within the 70–120% range. The mean recoveries at each fortification for the matrices were between 89-104%.

Repeatability (precision)
The repeatability of the method was determined by running five recoveries at concentrations at LOQ and

10xLOQ The RSDs of the repeatability for each recovery set ranged from 1.8-11%. The results show good repeatability as all relative standard deviations were below 20%.

Conclusion
The method 01471 was developed for the determination of prothioconazole-desthio in blood. Quantification by means of LC-MS/MS with two MS/MS transitions ensures a high level of specificity. The results obtained during validation demonstrate accuracy and repeatability of the residue determination. The limit of quantification was established at 50 µg/L, expressed as prothioconazole-desthio. Validation data were provided on two mass transitions, so a confirmatory method is not necessary. The method has been fully validated in accordance with SANCO/825/00 rev. 8.1.

An Independent laboratory validation is not required for body fluid methods of analysis.

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

No new or additional studies have been submitted