

# REGISTRATION REPORT

## **Part B**

### **Section 5**

#### **Analytical Methods**

Detailed summary of the risk assessment

Product code: GF-3307 (S7K-3-3)

Product name(s): QUEEN

Chemical active substances:

Fenpicoxamid (XDE-777), 50 g/L

Prothioconazole, 100 g/L

Central Zone

Zonal Rapporteur Member State: Poland

#### **CORE ASSESSMENT**

(extension of use)

Applicant: Corteva Agriscience

Submission date: March 2025

Finalisation date: August 2025 (initial Core Assessment),

November 2025 (final Core Assessment)

### Version history

When	What
March 2025	Submission of GF-3307 (S7K-3-3) Sugar beet/Fodder beet Extension of Use in the Central Zone.
August 2025	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 <del>struck through</del> and shaded for transparency.
November 2025	Final report (Core Assessment updated following the commenting period)  No additional information or assessments after the commenting period.

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## 5 Analytical methods

### 5.1 Conclusion and summary of assessment

#### **zRMS-PL conclusions:**

This application concerns the extension of the use of GC-3307 on sugar and fodder beet.

In accordance with the SANTE/2020/12830, Rev.2, 14. February 2023 sugar and fodder beets (roots and tops) belong to the analytical group of commodities with high water content. Applicant provided sufficient new pre-registration analytical methods for sugar beet.

In addition, Applicant provided new analytical methods for risk assessment and monitoring purposes. All methods are acceptable. The details of the evaluation of new and additional studies are referred in Appendix 2.

Several analytical methods for risk assessment and monitoring have been evaluated in the Registration Report, Part B5 for Queen (GF-3307, zRMS-PL, January 2023) or in the Registration Report, Part B5 for GF-3308 on 24.08.2022 and are still valid in the context of the extension of use assessment. The analytical methods are not being re-assessed in this application.

#### **Summary**

##### **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 µg/L, respectively, for both analytes. Fenpicoxamid residues in air can be determined by LC–MS/MS with a LOQ of 1.39 µg/m³.*

*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 <sup>3</sup> 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.

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 new method for the determination of XDE-777 in urine with LOQ = 0.01 mg/L is available.

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 for plant products were proposed as ‘prothioconazole-desthio (sum of isomers)’ for enforcement and, as follows, for the risk assessment:

- 1) sum of prothioconazole-desthio and all metabolites containing the 2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl-2H-1,2,4-triazole moiety, expressed as prothioconazole-desthio (sum of isomers)
- 2) Triazole alanine (TA) and triazole lactic acid (TLA)
- 3) Triazole acetic acid (TAA)
- 4) 1,2,4-triazole (1,2,4-T).

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

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

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

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

During the peer review under Directive 91/414/EEC, 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 <sup>3</sup> Additional method: Maasfeld 2002b (HPLC-MS/MS, JAU6476-desthio) LOQ Air: 0.0006 mg/m <sup>3</sup>
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.2, 2023 and should be provided at the renewal of the active substance and/or re-evaluation of plant production product.

Commodity/crop	Supported/Not supported
Sugar and fodder beet	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:	<p>Study acceptable.</p> <p>The analytical method for the determination of fenpicoxamid and prothioconazole in plant protection product was fully validated according to SANCO/3030/99 rev. 5.</p> <p>The analytical method was assessed in the final version of the RR in January 2023 and remains valid in the context of the extension of use assessment. No new data were submitted.</p>
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Reference:	KCP 5.1.1/1
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.5
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) (correlation coefficient, expressed as r)	<p>n = 7</p> <p>The detector response was shown to be linear for Fenpicoxamid over a range of 0.253 – 0.960 mg/mL equivalent to 2.43-9.48 wt%</p> <p><math>y = 4503729 x + 23473</math> (R2 = 0.9981).</p>	<p>n = 7</p> <p>The detector response was shown to be linear for Prothioconazole over a range of 0.480 – 1.89 mg/mL equivalent to 4.74-18.2 wt%</p> <p><math>y = 8899368 x + 160848</math> (R2 = 0.9986).</p>	<p>n = 7</p> <p>The detector response was shown to be linear for the internal standard (dibutylphthalate) from 0.403 – 1.61 mg/mL</p> <p><math>y = 8932544 x + 64908</math> (R2 = 0.9999).</p>



	Fenpicoxamid	Prothioconazole	Internal Standard
<b>Precision – Repeatability Mean n = 10 (%RSD)</b>	The relative standard deviation was 0.34% at an average concentration of 4.61% of Fenpicoxamid Horwitz% RSD <sub>r</sub> = 2.13	The relative standard deviation was 0.11% at an average concentration of 9.45% of Prothioconazole Horwitz% RSD <sub>r</sub> = 1.9	-
<b>Accuracy n = 7 (% Recovery)</b>	Recovery data were obtained over the range of 2.43 – 9.48% Fenpicoxamid, at an average recovery of 100% Level 9.48 wt%: 99.3% Level 7.40 wt%: 99.3% Level 6.34 wt%: 99.6% Level 4.91 wt%: 99.8% Level 4.70 wt% : 99.9% Level 3.93 wt% : 100.1% Level 2.43 wt% : 100.9%	Recovery data were obtained over the range of 4.74 – 18.2% Prothioconazole, at an average recovery of 98% Level 4.74 wt%: 98.1% Level 7.09 wt%: 98.8% Level 9.45 wt%: 98.6% Level 9.72 wt%: 98.7% Level 12.1 wt% : 98.3% Level 13.4 wt% : 98.1% Level 18.2 wt% : 97.9%	-
<b>Interference/ Specificity</b>	Chromatograms of the calibration solution, the blank formulation and the formulation are available. No significant interferences were detected between the solvent blank, formulation blank, internal standard and technical grade active ingredient.		
<b>Comment</b>	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 analytical methods for the determination of relevant impurities in plant protection product was fully validated according to SANCO/3030/99 rev. 5.  All studies described below have been evaluated in the final version of the RR of January 2023 and remain valid for the purpose of extension of use. No new studies have been submitted.
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Reference:	KCP 5.1.1/2
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.5
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 R <sup>2</sup> = 0.9980
Precision – Repeatability Mean n = 10 (%RSD)	Day 1: %RSD = 3.72 at an average concentration of 0.0034wt% Horwitz% RSD <sub>r</sub> = 6.30 Day 2: %RSD = 5.66 at an average concentration of 0.0046wt% Horwitz% RSD <sub>r</sub> = 6.02
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.

Reference:	KCP 5.1.1/3
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.5
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**

	<b>Toluene</b>
<b>Author(s), year</b>	Nelson, R. M. (2018)
<b>Principle of method</b>	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.
<b>Linearity</b> (linear between mg/L) (correlation coefficient, expressed as r)	2.6 – 130 µg/mL concentration range for toluene with R <sup>2</sup> = 0.9998, equivalent to 0.0025 to 0.124%; 10.2 – 50.8 µg/mL concentration range for ethylbenzene with R <sup>2</sup> = 0.9989
<b>Precision – Repeatability Mean</b> <b>n = 10</b> (%RSD)	For 10 samples analysed over two days, the average concentration was 0.024%, with RSD of 5.5%. Horwitz% RSD <sub>r</sub> = 4.7
<b>Accuracy</b> <b>n = 7</b> (% Recovery)	0.00942 to 0.0588% at an average recovery was 95.2%
<b>Interference/ Specificity</b>	No interferences.
<b>LOQ</b>	0.00033%
<b>LOD</b>	0.00010%
<b>Comment</b>	No comment

## Conclusion

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

Reference:	KCP 5.1.1/4
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**

	<b>X12314005</b>
<b>Author(s), year</b>	Hofer, C. (2017)
<b>Principle of method</b>	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.
<b>Linearity</b> (linear between mg/L) (correlation coefficient, expressed as r)	0.6 – 0.046 mg/mL concentration range for X12314005 with R <sup>2</sup> = 0.997, equivalent to 0.05 to 0.4 wt%
<b>Precision – Repeatability Mean</b> <b>n = 10</b> (%RSD)	For 10 samples analysed over two days, the average concentration was 0.084%, with RSD of 0.28%.

<b>Accuracy</b> <b>n = 7</b> <b>(% Recovery)</b>	0.05 to 0.4% at an average recovery was 102%
<b>Interference/ Specificity</b>	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.
<b>LOQ</b>	0.042%
<b>Comment</b>	No comment

## Conclusion

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

Reference:	KCP 5.1.1/5
Report	PDF titled: 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
<b>Overall Average</b>	0.20
<b>Std. Dev.</b>	0.006
<b>Overall RSD</b>	2.8
<b>Horwitz RSDR</b>	5.1
<b>Horwitz RSDr</b>	3.4
<b>Acceptable? (Overall RSD&lt;Horwitz RSDr)</b>	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
<b>Overall Average</b>	0.20
<b>Std. Dev.</b>	0.004
<b>Overall RSD</b>	1.9
<b>Horwitz RSDR</b>	5.1
<b>Horwitz RSDr</b>	3.4
<b>Acceptable? (Overall RSD&lt;Horwitz RSDr)</b>	Acceptable

Reference:	KCP 5.1.1/6
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**

	<b>X12335723</b>
<b>Author(s), year</b>	Frank, A. (2016)
<b>Principle of method</b>	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.
<b>Linearity</b> (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%
<b>Precision – Repeatability Mean</b> <b>n = 10</b> (%RSD)	For 10 samples analysed over two days, the average concentration was 0.14%, with RSD of 1.6%. Horwitz% RSD <sub>r</sub> = 3.6
<b>Accuracy</b> <b>n = 7</b> (% Recovery)	0.038 to 0.39% at an average recovery was 103%
<b>Interference/ Specificity</b>	No significant interferences (>3%) were observed.
<b>LOQ</b>	0.034%
<b>LOD</b>	0.0015%
<b>Comment</b>	No comment

## Conclusion

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

Reference:	KCP 5.1.1/7
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**

	<b>X12393285</b>
<b>Author(s), year</b>	Frank, A. (2016)
<b>Principle of method</b>	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.
<b>Linearity</b> (linear between mg/L)	0.0069 – 0.069 mg/mL concentration range for X12393285 with R2 = 0.9998, equivalent to 0.034 to 0.34 wt%

<b>(correlation coefficient, expressed as r)</b>	
<b>Precision – Repeatability Mean n = 10 (%RSD)</b>	For 10 samples analysed over two days, the average concentration was 0.088%, with RSD of 1.0%. Horwitz% RSD <sub>r</sub> = 3.9
<b>Accuracy n = 7 (% Recovery)</b>	0.034 to 0.34% at an average recovery was 99%
<b>Interference/ Specificity</b>	No significant interferences (>3%) were observed.
<b>LOQ</b>	0.027%
<b>LOD</b>	0.0080%
<b>Comment</b>	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 (S7K-3-3).

## 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. Many 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.

**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	Method Principle	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
Sugar beet top and roots (Residues)	220541	Primary	0.01 mg/kg	LC-MS/MS	Munro, M., 2024
High water content, high protein/high starch content (dry) (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	110213		0.400 µg/L		■■■■■, 2012, EU agreed

(Ecotoxicology)	110214		0.610 µg/L		██████████, 2012, EU agreed
	110215		0.04 µg/L		Fournier, A., 2012, EU agreed
	110216		0.075 µg/L		Fournier, A., 2012, EU agreed
	120374		2.5 µg/L		██████████, 2013, EU agreed
	120375		0.44 µg/L 0.0025 µg/L (new formulation lot)		Stadler, T., 2014, EU agreed
	120376		0.0998 mg/L		Holou, M.m 2013, EU agreed
	120383		0.05 mg/L		Rebstock, M., 2013, EU agreed
	120392		0.01 mg/L		██████████, 2012, EU agreed
	130983		0.10 mg/L (water) 5.0 µg/kg (fish tissue)		██████████, 2014, EU agreed
	140483		0.07 µg/L		Lamichhanie, K., 2014, EU agreed
	141002		0.0580 mg/L		VanHooser, A., 2015a, EU agreed
	140479		0.0217 ng/mL		██████████, 2014, revised 2017, final report addendum 2019
	140489				██████████, 2014, revised 2018
	140491		0.120 ng/mL		Hicks, S., 2014, final report addendum 2020
	160101		0.070 ng/mL		Goudie, O., 2016
	160102		0.066 ng/mL		Goudie, O., 2016
	160125		0.0500 ug/L		Hicks, S., 2017
	160126		0.000050 mg/L		Hicks, S., 2016
	160128		0.050 µg/L		██████████, 2016, EU agreed
	160129		24 µg/L		██████████, 2016, EU agreed
	160130		0.325 µg/L		██████████, 2016, EU agreed
	161022		0.650 µg/L		██████████, 2016, EU agreed
	180975		0.123 ng/mL		██████████, 2018
	181382		0.025 µg/L		Bruggermann, M., 2020
	191366		7.05 µg/L		Goudie, O., 2020
	202284		19.7 ng/L		Goudie, O., 2021
Sediment, Water (Ecotoxicology)	130984	Primary	0.05 µg/L (water) 0.15 µg/kg (sediment)	LC-MS/MS	██████████, 2014, EU agreed
Avian Diet (Ecotoxicology)	120384	Primary	100 µg/L	LC-MS/MS	██████████, 2013, EU agreed
	140424		75.0 mg/kg		██████████, 2015, EU agreed
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 in 50 % (w/v) aqueous sugar solution  50.0 g a.i./L in acetone		Cornement, M., 2022
Honey Bee (Ecotoxicology)	201075	Primary	0.0161 g a.i./L in 50 % (w/v) aqueous sugar solution  0.341 g a.i./L in water containing 0.5 % Etalfix® Pro	LC-MS/MS	Cornement, M., 2022

\*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)	120380	Primary	0.001 mg/L	LC-MS/MS	Rebstock, M., 2013, EU agreed
	120381		0.0001 mg/L		Holou, M., 2013, EU agreed
	120382		0.00050 mg/L		██████, 2012, EU agreed
	131295		2.3 µg/L		Lamichhane, K., 2015
	141003		0.045 mg/L		VanHooser, A., 2015b, EU agreed
	160126		0.0000040 mg/L		Hicks, S., 2016
	160128		0.0040 µg/L		██████, 2016, EU agreed
	180562		0.02 µg/L		Goudie, O., 2018
	181382		0.025 µg/L		Bruggermann, M., 2020
Sediment, Water (Ecotoxicology)	130984	Primary	0.05 µg/L (water)  0.15 µg/kg (sediment)	LC-MS/MS	██████, 2014, EU agreed
	180639		0.33 µg/L (water)  0.046 mg/kg (sediment)		██████, 2019
	180563		0.02 µg/L (overlying water)  14 mg/L (porewater)  0.046 mg/kg		Beasley, J., 2018

Component of residue definition: Metabolite X642188					
Matrix type	Method No.	Method Type	Method LOQ	Method Principle	Author(s), year / missing / EU agreed
			(sediment)		
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	Method Principle	Author(s), year / missing / EU agreed
Animal products (feeding study)	130949	Primary	0.01 mg/kg	LC-MS/MS	██████, 2013, EU agreed

Component of residue definition: Metabolite X696476					
Matrix type	Method No.	Method Type	Method LOQ	Method Principle	Author(s), year / missing / EU agreed
Water (Ecotoxicology)	130364	Primary	0.480 mg/L	LC-MS/MS	██████, 2014, EU agreed
	130375		0.480 mg/L		Stadler, T., 2014, EU agreed
	160128		0.302 µg/L		██████, 2014, EU agreed

Component of residue definition: Metabolite X696872					
Matrix type	Method No.	Method Type	Method LOQ	Method Principle	Author(s), year / missing / EU agreed
Water (Ecotoxicology)	130363	Primary	0.25 mg/L	LC-MS/MS	██████, 2014, EU agreed
	130374		0.25 mg/L		Stadler, T., 2014, EU agreed
Sediment, Water (Ecotoxicology)	130984	Primary	0.05 µg/L (water) 0.15 µg/kg (sediment)	LC-MS/MS	██████, 2014, EU agreed

Component of residue definition: Metabolite X763024					
Matrix type	Method No.	Method Type	Method LOQ	Method Principle	Author(s), year / missing / EU agreed
Water (Ecotoxicology)	130378	Primary	0.5 mg/L	LC-MS/MS	Romine, J., 2014, EU agreed

Component of residue definition: Metabolite X11963422					
Matrix type	Method No.	Method Type	Method LOQ	Method Principle	Author(s), year / missing / EU agreed
Water (Ecotoxicology)	130361	Primary	0.40 mg/L	LC-MS/MS	██████, 2014, EU agreed
	130372		0.20 mg/L		Romine, J., 2014, EU agreed
	130385		0.038 mg/L		Berfield, A., 2014, EU agreed
Sediment, Water (Ecotoxicology)	140860	Primary	0.1 µg/L (water) 0.5 µg/kg (sediment)	LC-MS/MS	Mueller, 2015, EU agreed

Component of residue definition: Metabolite X12019520					
Matrix type	Method No.	Method Type	Method LOQ	Method Principle	Author(s), year / missing / EU agreed
Water (Ecotoxicology)	130380	Primary	0.50 mg/L	LC-MS/MS	Romine, J., 2014, EU agreed
	160128		0.20 µg/L		██████, 2014, EU agreed
	180560		4.9 mg/L		██████, 2018a
Sediment, Water (Ecotoxicology)	140860	Primary	0.1 µg/L (water) 0.5 µg/kg (sediment)	LC-MS/MS	Mueller, 2015, EU agreed

Component of residue definition: Metabolite X12255349					
Matrix type	Method No.	Method Type	Method LOQ	Method Principle	Author(s), year / missing / EU agreed
Water (Ecotoxicology)	140484	Primary	5.0 µg/L	LC-MS/MS	Lamichhane, K., 2014, EU agreed
	141000		0.60 mg/L		██████, 2015, EU agreed
	141004		0.60 mg/L		Hadsell, R., 2015, EU agreed
	140999		1.3 µg/L		Lamichhane, K., 2015, EU agreed
	141001		0.60 mg/L		Aufderheide, J., 2015, EU agreed
	160126		0.0000090 mg/L		Hicks, S., 2016
	160128		0.0090 µg/L		██████, 2016, EU agreed

Component of residue definition: Metabolite 12264475					
Matrix type	Method No.	Method Type	Method LOQ	Method Principle	Author(s), year / missing / EU agreed
Water (Ecotoxicology)	130360	Primary	0.126 mg/L	LC-MS/MS	Dinehart, S., 2014, EU agreed
	130371		0.0250 mg/L		Huffman, 2014, EU agreed
	130384		0.0504 mg/L		Aufderheide, 2014, EU agreed
	160128		3.0 µg/L		██████, 2016, EU agreed
Sediment, Water (Ecotoxicology)	140860	Primary	0.1 µg/L (water) 0.5 µg/kg (sediment)	LC-MS/MS	Mueller, 2015, EU agreed

Component of residue definition: Metabolite X12313581					
Matrix type	Method No.	Method Type	Method LOQ	Method Principle	Author(s), year / missing / EU agreed
Water (Ecotoxicology)	130362	Primary	0.40 mg/L	LC-MS/MS	██████, 2014, EU agreed
	130373		0.40 mg/L		Romine, J., 2014, EU agreed
	160128		0.30 µg/L		██████, 2016, EU agreed
Sediment, Water (Ecotoxicology)	140860	Primary	0.1 µg/L (water) 0.5 µg/kg (sediment)	LC-MS/MS	Mueller, 2015, EU agreed

Component of residue definition: Metabolite X12314005					
Matrix type	Method No.	Method Type	Method LOQ	Method Principle	Author(s), year / missing / EU agreed
Water	130365	Primary	0.30 mg/L	LC-MS/MS	██████, 2014, EU agreed

Component of residue definition: Metabolite X12314005					
Matrix type	Method No.	Method Type	Method LOQ	Method Principle	Author(s), year / missing / EU agreed
(Ecotoxicology)	130376		1.0 mg/L		Dinehart, S., 2014, EU agreed
Sediment, Water (Ecotoxicology)	140860	Primary	0.1 µg/L (water) 0.5 µg/kg (sediment)	LC-MS/MS	Mueller, 2015, EU agreed

Component of residue definition: Metabolite X12335723					
Matrix type	Method No.	Method Type	Method LOQ	Method Principle	Author(s), year / missing / EU agreed
Water (Ecotoxicology)	130377	Primary	0.50 mg/L	LC-MS/MS	Dinehart, S., 2014, EU agreed
	160128		0.30 µg/L		■■■■■, 2014, EU agreed
Sediment, Water (Ecotoxicology)	180564	Primary	0.015 mg/L (water) 0.0069 mg/kg (sediment)	LC-MS/MS	Leak, T., 2018
	140860		0.1 µg/L (water) 0.5 µg/kg (sediment)		Mueller, 2015, EU agreed

Component of residue definition: Metabolite X12386481					
Matrix type	Method No.	Method Type	Method LOQ	Method Principle	Author(s), year / missing / EU agreed
Water (Ecotoxicology)	130379	Primary	0.4 mg/L	LC-MS/MS	Stadler, T., 2014, EU agreed
	160128		0.30 µg/L		■■■■■, 2014, EU agreed

Component of residue definition: Metabolite X12393285					
Matrix type	Method No.	Method Type	Method LOQ	Method Principle	Author(s), year / missing / EU agreed
Water (Ecotoxicology)	130383	Primary	0.5 mg/L	LC-MS/MS	Romine, J., 2014, EU agreed

Component of residue definition: Metabolite 12442397					
Matrix type	Method No.	Method Type	Method LOQ	Method Principle	Author(s), year / missing / EU agreed
Water (Ecotoxicology)	130382	Primary	0.5 mg/L	LC-MS/MS	Romine, J., 2014, EU agreed

Component of residue definition: Metabolite 12442403					
Matrix type	Method No.	Method Type	Method LOQ	Method Principle	Author(s), year / missing / EU agreed
Water (Ecotoxicology)	140486	Primary	0.40 mg/L	LC-MS/MS	Dinehart, S., 2015, EU agreed

Component of residue definition: Metabolite X12446477					
Matrix type	Method No.	Method Type	Method LOQ	Method Principle	Author(s), year / missing / EU agreed
Water (Ecotoxicology)	140485	Primary	0.095 mg/L	LC-MS/MS	Lamichhane, K., 2014, EU agreed
	180561		0.096 mg/L		■■■■■, 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. For the detailed evaluation of new/additional studies, refer to Appendix 2.

Prothioconazole is a triazole containing pesticide, so the residue definition for risk assessment for food of plant origin 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, only one new honey study on TDMs is presented 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	Appeltaufer, A., 2021
Honey	01600	Primary	0.01 mg/kg	LC-MS/MS	Kalathoor, R., 2020
Water (Ecotoxicology)	140491	Primary	0.235 ng/mL	LC-MS/MS	Hicks, S., 2014
	180975		0.245 ng/mL		██████, 2018
	181382		0.050 µg/L		Bruggermann, M., 2020
	191366		1.46 µg/L		Goudie, O., 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 in 50 % (w/v) aqueous sugar solution  0.704 g a.i./L in water containing 0.5 % Etalfix® Pro		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) (Residues)	00598	Primary	0.01 mg/kg	HPLC-MS/MS	Heinemann, O., 2000, EU reviewed
	00598/M001		0.05 mg/kg (canola plant) 0.01 mg/kg (canola seed)		Heinemann, O., 2000b, EU reviewed
Pollen, nectar (Residues)	200670	Primary	0.001 mg/kg	LC-MS/MS	Appeltaufer, A., 2021

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
Honey	01600	Primary	0.01 mg/kg	LC-MS/MS	Kalathoor, R., 2020
Animal products (feeding study)	00655*	Primary	0.01 mg/kg (milk, meat, liver, kidney, fat)	HPLC-MS/MS	Heinemann, O, 2001, EU reviewed
Animal products (feeding study)	00655/M001*	Primary	0.004 mg/kg (milk)	HPLC-MS/MS	Heinemann, O, 2001b, EU reviewed
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 JAU 6476- $\alpha$ -hydroxy-desthio, JAU 6476-3-hydroxy-desthio, JAU 6476-4-hydroxy-desthio, JAU 6476-5-hydroxy-desthio, JAU 6476-6-hydroxy-desthio					
Matrix type	Method No.	Method Type	Method LOQ	Method Principle	Author(s), year / missing / EU agreed
High water content, high oil content (Residues)	00979/M001	Primary	0.01 mg/kg	HPLC-MS/MS	Freitag, T., 2009, EU agreed
Honey	01601	Primary	0.01 mg/kg	HPLC-MS/MS	Kalathoor, R., 2020b

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
Honey	01602	Primary	0.01 mg/kg	HPLC-MS/MS	Kalathoor, R., 2020e

\*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 5.31: 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	<del>Reg (EU) 2019/50</del> Reg (EU) 2023/1069
Plant, high acid content		0.01 mg/kg	<del>Reg (EU) 2019/50</del> Reg (EU) 2023/1069
Plant, high protein/high starch content (dry commodities)		0.01 mg/kg	<del>Reg (EU) 2019/50</del> Reg (EU) 2023/1069
Plant, high oil content		0.01 mg/kg	<del>Reg (EU) 2019/50</del> Reg (EU) 2023/1069
Plant, difficult matrices (hops, spices, tea)		0.05 mg/kg	<del>Reg (EU) 2019/50</del> Reg (EU) 2023/1069
Muscle	X12326349 expressed as fenpicoxamid	0.01 mg/kg	<del>Reg (EU) 2019/50</del> Reg (EU) 2023/1069
Milk		0.01 mg/kg	<del>Reg (EU) 2019/50</del> Reg (EU) 2023/1069
Eggs		0.01 mg/kg	<del>Reg (EU) 2019/50</del> Reg (EU) 2023/1069
Fat		0.01 mg/kg	<del>Reg (EU) 2019/50</del> Reg (EU) 2023/1069
Liver, kidney		0.01 mg/kg 0.02 mg/kg (bovine kidney; sheep liver and kidney)	<del>Reg (EU) 2019/50</del> Reg (EU) 2023/1069
Honey	Fenpicoxamid	0.5 mg/kg	Pending Assessment
Soil (Ecotoxicology)	Fenpicoxamid and X642188	0.05 mg/kg	Common Limit EFSA Journal 2018;16(1):5146 NOEC <sub>corr</sub> = 3.97 mg a.s./kg dsw, <i>F. candida</i> NOEC <sub>corr</sub> = 2.8 mg X642188/kg dsw, <i>E. fetida</i>
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, <i>P. promelas</i> EC <sub>50</sub> = 0.79 µg X642188/L, <i>D. magna</i>	EFSA Journal 2018;16(1):5146 Goudie, O. 2018, Study No. 180562
Air	Fenpicoxamid	15 µg/m <sup>3</sup> LOQ = 0.5 µg for XDE-777 equivalent to 1.39 µg/m <sup>3</sup> of ambient air and	EFSA Journal 2018;16(1):5146 AOEL: 0.05 mg/kg bw/d

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
		warm and humid air	
Body tissues (meat or liver)	Fenpicoxamid	0.1 mg/kg 0.01 mg/kg	Common Limit, Reg (EU) 283/2013, EFSA Journal 2018;16(1):5146 SANTE/2020/12830, Rev.1 24. February 2021
Body fluids (urine or blood)	Fenpicoxamid	0.05 mg/L 0.01 mg/kg L	Common Limit, 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. Many 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.

**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	120615 (Study No. 120951)	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)	120998 (Study No. 130114)	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. Many 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.

**Table 5.3-4: Validated methods for food and feed of animal origin**

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	131027	0.01 mg/kg	LC-MS/MS	Garcia-Alix, M., 2014, EU agreed
	ILV	131027 (Study No. 130712)	0.01 mg/kg	LC-MS/MS	Lindner M., Grewe, D., 2014, EU agreed
	Primary/Confirmatory	220575	0.01 mg/kg	LC-MS/MS	Senciuc, M., Przybylek, A. 2022
	ILV	230145	0.01 mg/kg	LC-MS/MS	Moore, S., Shepherd, J. 2023

**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 (██████, 2013): acetonitrile/water/phosphoric acid (75/25/0.1, v/v/v)

**Table 5.3-6: Validated methods for food and feed of animal origin (honey)**

Component of residue definition: Fenpicoxamid					
Matrix type	Method Type	Method No.	Method LOQ	Method Principle	Author(s), year / missing / EU agreed
Honey	Primary/Confirmatory	200660	0.001 mg/kg	LC-MS/MS	Gonsoir, G., 2021
	ILV	200660 (Study No. 210700)	0.001 mg/kg	LC-MS/MS	Skaggs, C., 2021
	Primary/Confirmatory	220576	0.05 mg/kg	LC-MS/MS	Senciuc, M., Przybylek, A.; 2022
	ILV	230146	0.05 mg/kg	LC-MS/MS	Moore, S., Shepherd, J.; 2023

**Table 5.3-7: Statement on extraction efficiency**

	Method for products of animal origin (honey)
Required, available from:	Satisfactory extraction efficiency of fenpicoxamid has been demonstrated in dry commodities, commodities with high water content, and commodities with high oil content, using incurred residue samples of barley grain, banana, and oilseed rape (Senciuc, M., 2021). It can therefore be assumed that fenpicoxamid residues in honey are satisfactorily

	<b>Method for products of animal origin (honey)</b>
	quantitated through dilution with a similar (varies by no more than 20% vol) extraction solvent.

### 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 5.3-8: Validated methods for soil**

Component of residue definition: Fenpicoxamid and X642188				
Method type	Method No.	Method LOQ	Method Principle	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 5.3-9: 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	131046 (Study No. 130711)	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 5.3-10: 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 <sup>3</sup> )	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 5.3-11: 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
Primary/ Confirmatory	221208	0.01 mg/L	LC-MS/MS	Senciuc, M. , 2023

**Table 5.3-12: Methods for body tissues**

Component of residue definition: Fenpicoxamid				
Method type	Method No.	Method LOQ	Method Principle	Author(s), year / missing
Primary/ Confirmatory	131027	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	Monitoring	Prothioconazole-desthio (sum of isomers)	EFSA Scientific Report (2007) 106, 1-98
Food of animal origin	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 5.3-13: 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) 2024/1318
Plant, high acid content		0.01 mg/kg	Reg (EU) 2024/1318
Plant, high protein/high starch content (dry commodities)		0.01 mg/kg canola seed 0.15 mg/kg	Reg (EU) 2024/1318
Plant, high oil content		0.01 mg/kg	Reg (EU) 2024/1318
Muscle	Prothioconazole-desthio (sum of isomers*)	0.01 mg/kg	Reg (EU) 2024/1318
Milk		0.01 mg/kg	Reg (EU) 2024/1318
Egg		0.01 mg/kg	Reg (EU) 2024/1318
Liver, kidney		0.5 mg/kg 0.1 mg/kg (poultry)	Reg (EU) 2024/1318
Fat		0.02 mg/kg 0.01 mg/kg (poultry)	Reg (EU) 2024/1318
Honey		0.05 mg/kg	Reg (EU) 2024/1318
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, <i>E.foetida</i> NOEC = 1 mg p.m./kg dsw, <i>E.foetida</i>
Drinking water (Human toxicology)	Prothioconazole and prothioconazole-desthio	0.1 µg/L	Common Limit, Directive 2006/118/EC

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Surface water (Ecotoxicology)	Prothioconazole and prothioconazole-desthio	NOEC = 0.308 mg a.s./L., <i>O.mykiss</i> (prothioconazole)  NOEC = 3.34 mg p.m./L., <i>O.mykiss</i>	EFSA Journal 2007;106:98
Air	Prothioconazole and prothioconazole-desthio	60 µg/m <sup>3</sup> (prothioconazole)  3 µg/m <sup>3</sup> (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  0.01 mg/kg	Common Limit, Reg (EU) 283/2013 SANTE/2020/12830, Rev.2
Body fluids (urine or blood)	Prothioconazole-desthio	0.05 mg/L  0.01 mg/L	Common Limit, Reg (EU) 283/2013 SANTE/2020/12830, Rev.2

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

### 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. For the detailed evaluation of new/additional studies it is referred to Appendix 2.

**Table 5.3-14: 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
	0086/M003 (Study ID P/B 484 G)	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*
	01300/M018 (Study ID 2014/0110/01)	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 5.3-15: 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 <sup>14</sup>C-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 5.3-16: 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., 2001, EU agreed
	00655 (Study No. A-14-01-01)	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*
	00655/M002 (Study No. P/B 1226 G)	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., 2001, EU agreed
	00655/M001	Primary	0.004 mg/kg	HPLC-MS/MS	Heinemann, O., 2001c, EU agreed
	00655/M001 (Study No. A-14-01-01)	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*
	00655/M002 (Study No. P/B 1226 G)	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*
	01009 (Study No. P/B 1111G)	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.

Component of residue definition: prothioconazole-desthio					
Matrix type	Method No.	Method Type	Method LOQ	Method Principle	Author(s), year / missing / EU agreed
Honey	01600	Primary	0.01 mg/kg	HPLC-MS/MS (2 MRMs)	Kalathoor, R., 2020 M-680823-02-1 Report S19-01124
	01600 (Study No. M-684857-01-1)	ILV	0.01 mg/kg	HPLC-MS/MS (2 MRMs)	Fritzsche, S., 2020 M-684857-01-1 Report S19-22668

**Table 5.3-17: Statement on extraction efficiency**

	Method for products of animal origin
Required, available from:	Heinemann, O., 2001, EU agreed ; 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 5.3-18: 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 <sup>nd</sup> MRMs)	Brumhard, B., 2005, EU agreed

An analytical methods Steinhauer, S., 2001 (00086/M038) and Schramel, O., 2000 (0610) using GC-MS and HPLC-MS/MS respectively for the determination of Prothioconazole residues (prothioconazole-desthio and prothioconazole) in soil has already been provided in the monograph and were considered validated with LOQ = 0.006 mg/kg. In addition, the highly specific method Brumhard, B., 2005 (00610/M001) using HPLC-MS/MS for the determination of prothioconazole and prothioconazole desthio in soil was provided at national level in Bayer dossiers and is considered as validated with an LOQ of 0.006 mg/kg.

#### 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 5.3-19: 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 (prothioconazole)	HPLC-MS/MS (1 MRM)	Sommer, H., 2001, EU agreed



Component of residue definition: Prothioconazole and Prothioconazole-desthio					
Matrix type	Method No.	Method type	Method LOQ	Method Principle	Author(s), year / missing / EU agreed
			0.05 µg/L (prothioconazole-desthio)		
	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*
	01387/M002 (Study No. 2015/0034/01)	ILV	0.05 µg/L	HPLC-MS/MS	Thies, S., 2015, dRAR 2018*
Surface Water	00684	Primary	0.1 µg/L (prothioconazole)  0.05 µg/L (prothioconazole-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.

An analytical method Sommer, H., 2001 (684) using HPLC-MS/MS for the determination of prothioconazole desthio in surface and drinking water has already been provided in the monograph and were considered validated with LOQ = 0.1 µg/L for prothioconazole and LOQ = 0.05 µg/L for prothioconazole-desthio. An analytical method for the determination of prothioconazole and prothioconazole-desthio in drinking and surface water is currently under assessment for the renewal of the active substance with its corresponding ILV.

### 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 5.3-20: 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 <sup>3</sup> (prothioconazole)	HPLC-MS/MS	Maasfeld, W., 2002, EU agreed
	00731	0.6 µg/m <sup>3</sup>	HPLC-MS/MS	Maasfeld, W., 2002b, EU

Component of residue definition: Prothioconazole and Prothioconazole-desthio				
Method Type	Method No	Method LOQ	Method Principle	Author(s), year / missing / EU agreed
		(prothioconazole-desthio)	(1 MRM)	agreed
Confirmatory	00731/M001	0.3 µg/m <sup>3</sup> (prothioconazole-desthio)	HPLC-MS/MS (2 MRMs)	Anft, T. and Bardel, P., 2005, EU agreed

An analytical method Maasfeld, W., 2002a (724) using HPLC-MS/MS for the determination of prothioconazole and prothioconazole desthio in air has already been provided in the monograph and were considered validated with LOQ = 15 µg/m<sup>3</sup> for prothioconazole and with an LOQ of 0.6 µg/m<sup>3</sup> for prothioconazole desthio. In addition, the highly specific method Anft, T. and Bardel, P., 2005 (731/M001) using HPLC-MS/MS for the determination of prothioconazole desthio in air was provided at national level in Bayer dossiers and is considered as validated with an LOQ of 0.3 µg/m<sup>3</sup>.

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

**Table 5.3-21: 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)	Hoepfner, 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 5.3-22: 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., 2001, EU agreed

An analytical method Heinemann, O., 2001b (655) using HPLC-MS/MS for the determination of prothioconazole desthio in body tissue (meat) has already been provided in the monograph and were considered validated with LOQ = 0.01 mg/kg. Additionally an ILV was provided in the DAR (Dubey 2001). An analytical method for the determination of prothioconazole-desthio in body fluids is currently under assessment for the renewal of the active substance.

### 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
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	Corteva Agriscience
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	Corteva Agriscience
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	Corteva Agriscience
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	Corteva Agriscience
KCP 5.1.1/4	Megregian, J.	2021	GF-3307 Method Precision- Supplemental data Report No. DAS-AM-G-170058- Supplemental Corteva Agriscience GLP/GEP (Y/N): No Published (Y/N): N	N	Corteva Agriscience
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	Corteva Agriscience

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 5.1.1/6	Frank, A.	2015	Analytical Method and Validation for the Determination of Potential Degradates in GF-3307 DAS Report No.DAS-AM-G-14-35 Dow AgroSciences, LLC GLP/GEP (Y/N): Yes Published (Y/N): N	N	Corteva Agriscience
KCA 6.3.1/1	Matthew Munro	2024	Fenpicoxamid Residues in Sugar Beet Including Processed Fractions Following Two Applications of GF-3307, Europe, 2022. DAS Report No.: 220541/ Study Number: 685066 Charles River Laboratories Edinburgh Limited GLP/GEP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience
<del>KCA 6.10.1/1</del>  <del>KCP 10.3.1.6/1</del>	<del>Appeltauer, A</del>	<del>2021</del>	<del>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. DAS Report No.: 200670 Eurofins Agroscience Services Ltd GLP/GEP (Y/N): Y Published (Y/N): N</del>	<del>N</del>	<del>Corteva Agriscience</del>
<del>KCA 6.10.1/2</del>	<del>Appeltauer, A</del>	<del>2020</del>	<del>Determination of residues of prothioconazole and its metabolites in honey after two applications of PTZ-EC 250 in winter oilseed rape at 5 sites in Northern and Southern Europe in 2019. Bayer Report No.: M-682401-01-1/ Study Number: S19-00902 Eurofins Agroscience Services Ltd GLP/GEP (Y/N): Y Published (Y/N): N</del>	<del>N</del>	<del>BCS</del>
KCP 10.2.1/1	████	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 ████ GLP/GEP (Y/N): Yes Published (Y/N): No	Y	Corteva Agriscience
KCP 10.2.1/8	████	2018	GF-3307: Acute Toxicity to the Rainbow Trout, Oncorhynchus mykiss, Determined Under Flow-Through Test Conditions DAS Report No.180975 ████ GLP/GEP (Y/N): Yes Published (Y/N): No	Y	Corteva Agriscience

<b>Data point</b>	<b>Author(s)</b>	<b>Year</b>	<b>Title Company Report No. Source (where different from company) GLP or GEP status Published or not</b>	<b>Vertebrate study Y/N</b>	<b>Owner</b>
KCP 10.2.1/7	Goudie, O.	2016	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	Corteva Agriscience
KCP 10.2.1/4	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	Corteva Agriscience
KCP 10.2.1/9	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 Agrosience, LLC, Easton, Maryland, USA GLP/GEP (Y/N): Yes Published (Y/N): No	N	Corteva Agriscience
KCP 10.2.1/10	Goudie, O.	2021	GF-2925: A Static-Renewal Acute Toxicity to the Cladoceran (Daphnia magna) DAS# 202284 Eurofins EAG Agrosience, LLC, Easton, MD, USA GLP/GEP (Y/N): Yes Published (Y/N): No	N	Corteva Agriscience
KCP 10.2.1/2	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	Corteva Agriscience
KCP 10.2.1/3	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	Corteva Agriscience

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.2.1/5	████	2018a	X12019520 (a metabolite of XDE-777): Acute Toxicity to the Rainbow Trout, <i>Oncorhynchus mykiss</i> , Determined Under Static-Renewal Test Conditions DAS# 180560 ████ GLP/GEP (Y/N): Yes Published (Y/N): No	Y	Corteva Agriscience
KCP 10.2.1/6	████	2018b	X12446477 (metabolite of XDE-777): Acute Toxicity to the Rainbow Trout, <i>Oncorhynchus mykiss</i> , Determined Under Static-Renewal Test Conditions DAS# 180561 ████ GLP/GEP (Y/N): Yes Published (Y/N): No	Y	Corteva Agriscience
KCP 10.2.2/1	Beasley, J.	2018	X1642188 (a metabolite of XDE-777): Chronic Toxicity in Whole Sediment to Freshwater Midge, <i>Chironomus riparius</i> , Using Spiked Sediment DAS# 180563 ABC Laboratories, Inc., Columbia, Missouri, USA GLP/GEP (Y/N): Yes Published (Y/N): No	N	Corteva Agriscience
KCP 10.2.2/2	Leak, T.	2018	X12335723 (a metabolite of XDE-777): Chronic Toxicity in Whole Sediment to Freshwater Midge, <i>Chironomus riparius</i> , Using Spiked Sediment DAS# 180564 ABC Laboratories, Inc., Columbia, Missouri, USA GLP/GEP (Y/N): Yes Published (Y/N): No	N	Corteva Agriscience
KCP 10.3.1.2/1	Oberrauch, S.	2018	GF-3307 - Honey Bee ( <i>Apis mellifera</i> 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	Corteva Agriscience
KCP 10.3.1.2/2	Verge, E., Kastel, A.	2018	GF-3307 - Assessment of Effects on the Adult Honey Bee, <i>Apis mellifera</i> L., in a 10 Day Chronic Feeding Test under Laboratory Conditions DAS# 170077 Eurofins Agrosience Services EcoChem / Eurofins Agrosience Services Ecotox GmbH GLP/GEP (Y/N): Yes Published (Y/N): No	N	Corteva Agriscience

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.3.1.5/1	Kleinhenz, M.	2018	GF-3307 (Fenpicoxamid + Prothioconazole): Brood Development of the Honeybee ( <i>Apis mellifera</i> L.) in a Semi-Field Tunnel Study in <i>Phacelia tanacetifolia</i> in Germany 2017 DAS Report No. 170673 Eurofins Agrosience Services EcoChem GmbH / Eurofins Agrosience Services Ecotox GmbH, Niefern-Öschelbronn, Germany GLP: Yes Published: No	N	Corteva Agriscience
KCP 10.3.1.6/2	Gonsoir, G.	2021	Assessment of Side-Effects on the GF-3307 (Fenpicoxamid and Prothioconazole): Brood Development of the Honey Bee ( <i>Apis mellifera</i> L.) in a Colony Feeding Test in Germany 2020 DAS Report No. 200660 Eurofins Agrosience Services EcoChem GmbH / Eurofins Agrosience Services Ecotox GmbH, Niefern-Öschelbronn, Germany GLP: Yes Published: No	N	Corteva Agriscience
KCP 10.3.1.1.1/3	Cornement, M., Morgenthal, K.	2022	XDE-777 TGAI - Acute Oral and Contact Toxicity to Bumble Bees ( <i>Bombus terrestris</i> ) under Laboratory Conditions Corteva Report No. 201076 IES GLP/GEP (Y/N): Yes Published (Y/N): No	N	Corteva Agriscience
KCP 10.3.1.1.1/4	Cornement, M., Morgenthal, K.	2022	GF-3307 - Acute Oral and Contact Toxicity to Bumble Bees ( <i>Bombus terrestris</i> ) under Laboratory Conditions Corteva Report No. 201075 IES GLP/GEP (Y/N): Yes Published (Y/N): No	N	Corteva Agriscience
KCP 5.2.3/06	Kalathoor, R.	2020a	Amendment no. 01: Residue analytical method 01600 and short term storage stability of prothioconazole (JAU 6476) and its Metabolite JAU 6476-desthio in/on honey by HPLC-MS/MS Report No. M-68023-02-1, Reference No. S19-01124 Eurofins Agrosience Services EcoChem GmbH / Eurofins Agrosience Services Ecotox GmbH, Niefern-Oeschelbronn, Germany GLP/GEP (Y/N): Yes Published (Y/N): No	N	BCS*
KCP 5.2.3/07	Kalathoor, R.	2020b	Residue analytical method 01601 and short term storage stability of the metabolites JAU 6476-alpha-hydroxy-desthio, JAU 6476-3-hydroxy-desthio, JAU 6476-4-hydroxy-desthio, JAU 6476-5-hydroxy-desthio and JAU 6476-6-hydroxy-desthio in/on honey by HPLC-MS/MS	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
			Report No. M-681477-01-1, Reference No. S19-01125 Eurofins Agroscience Services EcoChem GmbH / Eurofins Agroscience Services Ecotox GmbH, Niefern-Oeschelbronn, Germany GLP/GEP (Y/N): Yes Published (Y/N): No		
KCP 5.2.3/08	Kalathoor, R.	2020c	Amendment no. 02: Residue analytical method 01602 and short term storage stability of 1,2,4-triazole, triazole alanine, triazole acetic acid and triazole lactic acid in/on honey by HPLC-DMS-MS/MS Report No. M-680825-03-1, Reference No. S19-01126 Eurofins Agroscience Services EcoChem GmbH / Eurofins Agroscience Services Ecotox GmbH, Niefern-Oeschelbronn, Germany GLP/GEP (Y/N): Yes Published (Y/N): No	N	BCS*
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	Corteva Agriscience
KCP 5.3.2.3/03	Skaggs, C.	2021	Independent Laboratory Validation of Fenpicoxamid (XDE-777) in Honey Lab Study No. SGS-21-S-04, DAS No. 210700 SGS North America, Inc. GLP/GEP (Y/N): Yes Published (Y/N): No	N	Corteva Agriscience
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 Bayer CropScience, Report No.: VC/13/017, Edition Number: M-498384-01-1, Method Report No.: VC/13/017, Date: 2014-09-30 Battelle UK Ltd., Chelmsford, Essex, United Kingdom GLP/GEP (Y/N): Yes Published (Y/N): No	N	BCS*
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 Bayer CropScience, Report No.: 2014/0110/01, Edition Number: M-508116-03-1, Date: 2014-12-17 Currenta GmbH & Co. OHG, Leverkusen, Germany	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
			GLP/GEP (Y/N): Yes Published (Y/N): No		
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 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 Bayer S.A.S., Bayer CropScience, Lyon, France GLP/GEP (Y/N): Yes Published (Y/N): No	N	BCS*
KCP 5.3.3.3/02	Freitag, Th..	2007	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*
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 Method no. 00655/M002, Report no. P/B 1226 G Bayer CropScience GLP/GEP (Y/N): Yes Published (Y/N): No	N	BCS*
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 ... 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 Bayer CropScience, GLP/GEP (Y/N): Yes Published (Y/N): No	N	BCS*
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	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
			PTRL Europe GmbH, Ulm, Germany; Bayer CropScience GLP/GEP (Y/N): Yes Published (Y/N): No		
KCP 5.3.3.3/07	Fritzsich, S.	2020	Independent laboratory validation of the analytical method 01600 for the determination of prothioconazole (JAU 6476) and its metabolite JAU 6476-desthio in/on honey Report No. M-684857-01-1, Reference No. S19-22668 Eurofins Agrosience Services Chem GmbH (EAS Chem), Hamburg, Germany GLP/GEP (Y/N): Yes Published (Y/N): No		BCS*
KCP 5.3.3.3/08	Senciuc, M., Przybylek, A.	2022	Method Validation for the Determination of X12326349 in Animal Matrices; Lab Study No. S22-03479; Sponsor Study No. 220575; Eurofins Agrosience Services EAG Laboratories GmbH, GLP/GEP (Y/N): Yes Published (Y/N): No	N	Corteva Agriscience
KCP 5.3.3.3/09	Moore, S., Shepherd, J.	2023	Independent Laboratory Validation of an Analytical Method for the Determination of Residues of X12326349 (XDE-777 Metabolite) in Animal Matrices Lab Study No. 598SRUS23R0052; Sponsor Study No. 230145 SynTech Research, GLP/GEP (Y/N): Yes Published (Y/N): No	N	Corteva Agriscience
KCP 5.3.3.3/10	Senciuc, M., Przybylek, A	2022	Validation of the Analytical Method for the Determination of Fenpicoxamid (XDE-777) in Honey; Lab Study No. S22-03480; Sponsor Study No. 220576 ; Eurofins Agrosience Services EAG Laboratories GmbH GLP/GEP (Y/N): Yes Published (Y/N): No	N	Corteva Agriscience
KCP 5.3.3.3/11	Moore, S., Shepherd, J.	2023	Independent Laboratory Validation of an Analytical Method for the Determination of Residues of XDE-777 in Honey Lab Study No. 598SRUS23R0053; Sponsor Study No. 230146 SynTech Research GLP/GEP (Y/N): Yes Published (Y/N): No	N	Corteva Agriscience
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 Report No.: MR-15/025, Edition Number: M-526061-01-1, Date: 2015-06-01 TF- BCS-Adama Agan,	N	TF- BCS*- Adama Agan

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): 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 Report No.: 2015/0034/01, Edition Number: M-536990-01-1, Date: 2015-10-27 TF- BCS-Adama Agan, GLP/GEP (Y/N): Yes Published (Y/N): No	N	TF- BCS*- Adama Agan
KCP 5.3.3.7/01	Hoepfner, S.	2015	Validation of the BCS analytical method 01471 for the determination of prothiconazole-desthio in body fluid by HPLC-MS/MS 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 Currenta GmbH & Co. OHG, Leverkusen, Germany GLP/GEP (Y/N): Yes Published (Y/N): No	N	BCS*
KCP 5.3.3.7/02	Senciuc, M.	2023	Method Validation for the Determination of Fenpicoxamid (XDE-777) in Body Fluids Lab Study No. S22-08468; Sponsor Study No. 221208 Eurofins Agrosience Services EAG Laboratories GmbH, GLP/GEP (Y/N): Yes Published (Y/N): No	No	Corteva Agriscience
KCP 10.2.3/2	Hicks, S.	2017	XDE-777: Population Effects Study in an Indoor Aquatic Microcosm with <i>Daphnia magna</i> DAS# 160125 ABC Laboratories, Inc., 7200 E. ABC Lane Columbia, Missouri 65202, USA GLP/GEP (Y/N): Yes Published (Y/N): No	No	Corteva Agriscience
KCP 10.2.3/3	Hicks, S.	2016	GF-3308: Population Effects Study in an Indoor Aquatic Microcosm with <i>Daphnia magna</i> DAS# 160126 ABC Laboratories, Inc., 7200 E. ABC Lane Columbia, Missouri 65202, USA GLP/GEP (Y/N): Yes Published (Y/N): No	No	Corteva Agriscience
KCP 10.2.3/4	Lamichhane, K.	2015	X642188 (a metabolite of XDE-777): Population Effects Study in an Indoor Aquatic Microcosm with <i>Daphnia magna</i> DAS# 131295	N	Corteva Agriscience

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
			ABC Laboratories, Inc., Columbia, Missouri, USA GLP/GEP (Y/N): Yes Published (Y/N): No		
KCP 10.2.3/7	Brüggemann, M., Böhmer, W., Kosak, L	2020	GF-3307: Population Effects Study in an Indoor Aquatic Microcosm with <i>Daphnia magna</i> DAS Study No. 181382 Fraunhofer Institute for Molecular Biology and Applied Ecology (IME), Schmallenberg, Germany GLP/GEP (Y/N): Yes Published (Y/N): No	No	Corteva Agriscience

\*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	Corteva Agriscience
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	Corteva Agriscience
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	Corteva Agriscience

<b>Data point</b>	<b>Author(s)</b>	<b>Year</b>	<b>Title Company Report No. Source (where different from company) GLP or GEP status Published or not</b>	<b>Vertebrate study Y/N</b>	<b>Owner</b>
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	Corteva Agriscience
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 DAS Report No.: 120615 Eurofins Agrosience Services Ltd GLP/GEP (Y/N): Yes Published (Y/N): No	N	Corteva Agriscience
KCP 5.2.2/03  (KCA 6.4.2/01)	██████	2013	Data generation method for XDE-777 Livestock Feeding Study: Magnitude of Residue in Milk, Muscle, Liver, Kidney and Fat of Lactating Dairy Cattle DAS Report No.: 130949 ██████ GLP/GEP (Y/N): Y Published (Y/N): N	Y	Corteva Agriscience
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	Corteva Agriscience
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*

<b>Data point</b>	<b>Author(s)</b>	<b>Year</b>	<b>Title Company Report No. Source (where different from company) GLP or GEP status Published or not</b>	<b>Vertebrate study Y/N</b>	<b>Owner</b>
KCP 5.2.3/03	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 5.2.3/04	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 Bayer AG, Leverkusen, Germany, Bayer CropScience GLP/GEP (Y/N): Yes Published (Y/N): No	N	BCS*
KCP 5.2.3/09	Freitag, Th., Daniels, M.	2009	Analytical Method 00979/M001 for the determination of residues of JAU 6476- $\alpha$ -hydroxy-desthio, JAU 6476-3-hydroxy-desthio, JAU 6476-4-hydroxy-desthio, JAU 6476-5-hydroxy-desthio, and JAU 6476-6-hydroxy-desthio in/on matrices of plant origin by HPLC-MS/MS Method-No. 00979/M001, Report No.: MR-08/023 Bayer AG, Leverkusen, Germany, Bayer CropScience GLP/GEP (Y/N): Yes Published (Y/N): No	N	BCS*
KCP 5.3.2.2/01	Chambers, J., Jarrett H.	2013	Independent Laboratory Validation: XDE-777 and X641288 Residue Determination in Crops (Revision) DAS Report No.: 120951 Battelle UK Ltd GLP/GEP (Y/N): Yes Published (Y/N): No	N	Corteva Agriscience
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 DAS Report No.: 120998 Eurofins Agrosience Services Ltd GLP/GEP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience
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 DAS Report No.: 130114 Eurofins Agrosience Services Chem SAS	N	Corteva Agriscience

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 5.3.2.2/04	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 DAS Report No.: 121023 Dow AgroSciences LLC GLP/GEP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience
KCA 6.2.1/1	Ma, M Jackson, U	2013	A NATURE OF THE RESIDUE STUDY WITH [ <sup>14</sup> 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	Corteva Agriscience
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 DAS Report No.: 131027 CEM Analytical Services GLP/GEP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience
KCP 5.3.2.3/02	Lindner M Grewé D	2014	Independent Laboratory Validation of an Analytical Method for the Determination of XDE-777 and its Metabolite X12326349 in Matrices of Animal Origin DAS Report No.: 130712 Eurofins Agrosiences Services GLP/GEP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience
KCA 6.2.3	██████	2013	A NATURE OF THE RESIDUE STUDY IN THE RUMINANT WITH [ <sup>14</sup> C]-XR-777 DAS Report No.: 110766 ██████ GLP/GEP (Y/N): Y Published (Y/N): N	Y	Corteva Agriscience
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 DAS Report No.: 131045 Eurofins Agrosiences Services	N	Corteva Agriscience

<b>Data point</b>	<b>Author(s)</b>	<b>Year</b>	<b>Title Company Report No. Source (where different from company) GLP or GEP status Published or not</b>	<b>Vertebrate study Y/N</b>	<b>Owner</b>
			GLP/GEP (Y/N): Y Published (Y/N): N		
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 DAS Report No.: 131046 Battelle UK Ltd. GLP/GEP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience
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 DAS Report No.: 130711 Eurofins Agrosiences Services GLP/GEP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience
KCP 5.3.2.6/01	Bacher R	2012	The Development and Validation of a Method for the Analysis of XDE-777 in Air DAS Report No.: 120681 PTRL Europe GmbH GLP/GEP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience
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) DAS Report No.: 120682 PTRL Europe GmbH GLP/GEP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience
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. Bayer AG, Report No.: 0086/M033, Date 200-11-20 Dr. Specht Partner, Chemische Laboratorien GmbH, Hamburg, Germany GLP/GEP (Y/N): Yes Published (Y/N): No	N	BCS*
KCP 5.3.3.2/02	Class, Th	2001	Independent laboratory validation of DFG method S19 (extended revision) for the determination of residues of JAU 6476-desthio (Bayer method 00086/M033) in plant materials PTRL Europe, Ulm, Germany. Report No.: P/B 484 G, Date: 2001-05-15 PTRL Europe, Ulm, Germany	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
			GLP/GEP (Y/N): Yes Published (Y/N): No		
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 Report No.: MR-084/01, Date:2001-05-15 Bayer AG, Leverkusen, Germany, Bayer CropScience GLP/GEP (Y/N): Yes Published (Y/N): No	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 matrices of animal origin by HPLC-MS/MS Bayer AG, Report No.: A-14-01-01, Date:2001-10-16 Battelle, Geneva Research Centres, Carouge/Geneva, Switzerland GLP/GEP (Y/N): Yes Published (Y/N): No	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 BVL-2283223, BVL-2295522, ASB2010-11620 GLP: Yes Published: No	N	BCS*
KCA 6.2.2/01	██████	2001	(Phenyl-UL-14C)JAU6476 Absorption, distribution, excretion and metabolism in the lactating goat Report No.: MR-092/01 ██████ GLP/GEP (Y/N): Yes Published (Y/N): No	⚠ Y	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 Dr. Specht & Partner, Chemische Laboratorien GmbH, Hamburg, Germany, Bayer CropScience GLP: Yes Published: No	N	BCS*

<b>Data point</b>	<b>Author(s)</b>	<b>Year</b>	<b>Title Company Report No. Source (where different from company) GLP or GEP status Published or not</b>	<b>Vertebrate study Y/N</b>	<b>Owner</b>
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 Bayer AG, Leverkusen, Germany, Bayer CropScience GLP: Yes Published: No	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, BVL-2283232, BVL-2291546, MET2005-358 Bayer AG, Leverkusen, Germany, Bayer CropScience GLP: Yes Published: No	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, BVL-2291528, MET2002-411 Bayer AG, Leverkusen, Germany, Bayer CropScience GLP: Yes Published: No	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 BVL-2283234, BVL-2291531, MET2005-359 GLP: Yes Published: No	N	BCS*
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*

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 5.3.3.6/03	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 BVL-2283237, BVL-2291532, MET2005-360 GLP: Yes Published: No	N	BCS*
CA 8.1.1.3 /1	██████	2013	XDE-777 TGAI: A Reproduction Study with the Northern Bobwhite DAS Report No.: 120384 ██████ GLP/GEP (Y/N): Yes Published (Y/N): No	Yes	Corteva Agriscience
CA 8.1.1.3/2	██████	2015	XDE-777: Reproductive Toxicity Test with the Northern Bobwhite ( <i>Colinus virginianus</i> ) (Amended report) DAS Report No.: 140424 ██████ GLP/GEP (Y/N): Yes Published (Y/N): No	Yes	Corteva Agriscience
CA 8.2.1/4	██████	2014	X11963422 (a metabolite of XDE-777): Acute Toxicity to the Rainbow Trout, <i>Oncorhynchus mykiss</i> , Determined Under Static-Renewal Test Conditions (Revision) DAS Report No.: 130361 ██████ GLP/GEP (Y/N): Yes Published (Y/N): No	Yes	Corteva Agriscience
CA 8.2.1/5	██████	2014	X12264475 (a metabolite of XDE-777): Acute Toxicity to the Rainbow Trout, <i>Oncorhynchus mykiss</i> , Determined Under Static-Renewal Test Conditions DAS Report No.: 130360 ██████ GLP/GEP (Y/N): Yes Published (Y/N): No	Yes	Corteva Agriscience
CA 8.2.1/6	██████	2014	X12313581 (a metabolite of XDE-777): Acute Toxicity to the Rainbow Trout, <i>Oncorhynchus mykiss</i> , Determined Under Static-Renewal Test Conditions DAS Report No.: 130362 ██████ GLP/GEP (Y/N): Yes Published (Y/N): No	Yes	Corteva Agriscience

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
CA 8.2.1/7	████	2014	X696872 (a metabolite of XDE-777): Acute Toxicity to the Rainbow Trout, <i>Oncorhynchus mykiss</i> , Determined Under Static-Renewal Test Conditions DAS Report No.: 130363 ████ GLP/GEP (Y/N): Yes Published (Y/N): No	Yes	Corteva Agriscience
CA 8.2.1/8	████	2014	X696476 (a metabolite of XDE-777): Acute Toxicity to the Rainbow Trout, <i>Oncorhynchus mykiss</i> , Determined Under Static-Renewal Test Conditions DAS Report No.: 130364 ████ GLP/GEP (Y/N): Yes Published (Y/N): No	Yes	Corteva Agriscience
CA 8.2.1/9	████	2014	X12314005 (a metabolite of XDE-777): Acute Toxicity to the Rainbow Trout, <i>Oncorhynchus mykiss</i> , Determined Under Static-Renewal Test Conditions DAS Report No.: 130365 ████ GLP/GEP (Y/N): Yes Published (Y/N): No	Yes	Corteva Agriscience
CA 8.2.1/10	████	2015	X12255349 (a metabolite of XDE-777): Acute toxicity to the Rainbow Trout, <i>Oncorhynchus mykiss</i> , Determined Under Static-Renewal Test Conditions DAS Report No.: 141000 ████ GLP/GEP (Y/N): Yes Published (Y/N): No	Yes	Corteva Agriscience
CA 8.2.1	████	2016	XDE-777: Acute Toxicity to the Zebra Fish, <i>Danio rerio</i> , Determined Under Flow-Through Test Conditions DAS Report No. 160129 ████ GLP/GEP (Y/N): Yes Published (Y/N): No	Yes	Corteva Agriscience
CA 8.2.1	████	2016	XDE-777: Acute Toxicity to the Fathead minnow, <i>Pimephales promelas</i> , Determined Under Flow-Through Test Conditions DAS Report No. 160130 ████ GLP/GEP (Y/N): Yes Published (Y/N): No	Yes	Corteva Agriscience

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
CA 8.2.1	██████	2016	XDE-777: Acute Toxicity to the Bluegill, <i>Lepomis macrochirus</i> , Determined Under Flow-Through Test Conditions DAS Report No. 161022 ██████ GLP/GEP (Y/N): Yes Published (Y/N): No	Yes	Corteva Agriscience
CA 8.2.2.1 /1	██████	2012	XR-777 TGAI – Early Life-Stage Toxicity Test with Fathead Minnow, <i>Pimephales promelas</i> , Following OECD Guideline #210 DAS Report No.: 110214 ██████ GLP/GEP (Y/N): Yes Published (Y/N): No	Yes	Corteva Agriscience
CA 8.2.2.1/2	██████	2016	XDE-777: Investigation of Larval Toxicity to the Fathead Minnow ( <i>Pimephales promelas</i> ) Under Static Conditions in a Water-Sediment System DAS Report No. 160128 ██████ GLP/GEP (Y/N): Yes Published (Y/N): No	Yes	Corteva Agriscience
CA 8.2.2.3/1	██████	2014	XDE-777: Investigation of bioconcentration in zebrafish ( <i>Danio rerio</i> ) under flow-through conditions DAS Report No.: 130983 ██████ GLP/GEP (Y/N): Yes Published (Y/N): No	Yes	Corteva Agriscience
CA 8.2.4.1 /1	Fournier A	2012	XR-777 TGAI - Acute Toxicity to Water Fleas ( <i>Daphnia magna</i> ) Under Static-Renewal Conditions, Following OECD Guideline #202 and JMAFF 12 NohSan, No. 8147 <i>Daphnia</i> Acute Immobilization Test (2-7-2-1) Data Requirement OECD Guideline 202 JMAFF 12 NohSan, No. 8147 (Revision) DAS Report No.: 110215 Smithers Viscient GLP/GEP (Y/N): Yes Published (Y/N): No	No	Corteva Agriscience

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
CA 8.2.4.1 /2	Holou M	2013	X642188 Metabolite: Acute Toxicity to the Cladoceran, <i>Daphnia magna</i> , Determined Under Static-Renewal Test Conditions DAS Report No.: 120381 ABC Laboratories GLP/GEP (Y/N): Yes Published (Y/N): No	No	Corteva Agriscience
CA 8.2.4.1/03	Romine J	2014	X11963422 (a metabolite of XDE-777): Acute Toxicity to the Cladoceran, <i>Daphnia magna</i> , Determined Under Static-Renewal Test Conditions DAS Report No.: 130372 ABC Laboratories GLP/GEP (Y/N): Yes Published (Y/N): No	No	Corteva Agriscience
CA 8.2.4.1/04	Huffman	2014	X12264475 (a metabolite of XDE-777): Acute Toxicity to the Cladoceran, <i>Daphnia magna</i> , Determined Under Static-Renewal Test Conditions DAS Report No.: 130371 ABC Laboratories GLP/GEP (Y/N): Yes Published (Y/N): No	No	Corteva Agriscience
CA 8.2.4.1/05	Romine J	2014	X12313581 (a metabolite of XDE-777): Acute Toxicity to the Cladoceran, <i>Daphnia magna</i> , Determined Under Static-Renewal Test Conditions DAS Report No.: 130373 ABC Laboratories GLP/GEP (Y/N): Yes Published (Y/N): No	No	Corteva Agriscience
CA 8.2.4.1/06	Stadler T	2014	X696872 (a metabolite of XDE-777): Acute Toxicity to the Cladoceran, <i>Daphnia magna</i> , Determined Under Static-Renewal Test Conditions DAS Report No.: 130374 ABC Laboratories GLP/GEP (Y/N): Yes Published (Y/N): No	No	Corteva Agriscience

<b>Data point</b>	<b>Author(s)</b>	<b>Year</b>	<b>Title Company Report No. Source (where different from company) GLP or GEP status Published or not</b>	<b>Vertebrate study Y/N</b>	<b>Owner</b>
CA 8.2.4.1/07	Stadler T	2014	X696476 (a metabolite of XDE-777): Acute Toxicity to the Cladoceran, <i>Daphnia magna</i> , Determined Under Static-Renewal Test Conditions DAS Report No.: 130375 ABC Laboratories GLP/GEP (Y/N): Yes Published (Y/N): No	No	Corteva Agriscience
CA 8.2.4.1/08	Dinehart S	2014	X12314005 (a metabolite of XDE-777): Acute Toxicity to the Cladoceran, <i>Daphnia magna</i> , Determine Under Static-Renewal Test Conditions DAS Report No.: 130376 ABC Laboratories GLP/GEP (Y/N): Yes Published (Y/N): No	No	Corteva Agriscience
CA 8.2.4.1/09	Stadler T	2014	X12386481 (a metabolite of XDE-777): Acute Toxicity to the Cladoceran, <i>Daphnia magna</i> , Determined Under Static-Renewal Test Conditions DAS Report No.: 130379 ABC Laboratories GLP/GEP (Y/N): Yes Published (Y/N): No	No	Corteva Agriscience
CA 8.2.4.1/10	Romine J	2014	X763024 (a metabolite of XDE-777): Acute Toxicity to the Cladoceran, <i>Daphnia magna</i> , Determined Under Static-Renewal Test Conditions DAS Report No.: 130378 ABC Laboratories GLP/GEP (Y/N): Yes Published (Y/N): No	No	Corteva Agriscience
CA 8.2.4.1/11	Romine J	2014	X12019520 (a metabolite of XDE-777): Acute Toxicity to the Cladoceran, <i>Daphnia magna</i> , Determined Under Static-Renewal Test Conditions DAS Report No.: 130380 ABC Laboratories GLP/GEP (Y/N): Yes Published (Y/N): No	No	Corteva Agriscience

<b>Data point</b>	<b>Author(s)</b>	<b>Year</b>	<b>Title Company Report No. Source (where different from company) GLP or GEP status Published or not</b>	<b>Vertebrate study Y/N</b>	<b>Owner</b>
CA 8.2.4.1/12	Dinehart S	2014	X12335723 (a metabolite of XDE-777): Acute Toxicity to the Cladoceran, <i>Daphnia magna</i> , Determined Under Static-Renewal Test Conditions DAS Report No.: 130377 ABC Laboratories GLP/GEP (Y/N): Yes Published (Y/N): No	No	Corteva Agriscience
CA 8.2.4.1/13	Romine J	2014	X12393285 (a metabolite of XDE-777): Acute Toxicity to the Cladoceran, <i>Daphnia magna</i> , Determined Under Static-Renewal Test Conditions DAS Report No.: 130383 ABC Laboratories GLP/GEP (Y/N): Yes Published (Y/N): No	No	Corteva Agriscience
CA 8.2.4.1/14	Lamichhane K	2014	X12255349 (a metabolite of XDE-777): Acute Toxicity to the Cladoceran, <i>Daphnia magna</i> , Exposed Under Static-Renewal Test Conditions DAS Report No.: 140484 ABC Laboratories GLP/GEP (Y/N): Yes Published (Y/N): No	No	Corteva Agriscience
CA 8.2.4.1/15	Lamichhane K	2014	X12446477 (a metabolite of XDE-777): Acute Toxicity to the Cladoceran, <i>Daphnia magna</i> , Exposed Under Static-Renewal Test Conditions DAS Report No.: 140485 ABC Laboratories GLP/GEP (Y/N): Yes Published (Y/N): No	No	Corteva Agriscience
CA 8.2.4.1/16	Romine J	2014	X12442397 (sodium salt of X12399889, a metabolite of XDE-777): Acute Toxicity to the Cladoceran, <i>Daphnia magna</i> , Determined Under Static-Renewal Test Conditions DAS Report No.: 130382 ABC Laboratories GLP/GEP (Y/N): Yes Published (Y/N): No	No	Corteva Agriscience



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
CA 8.2.4.1/17	Dinehart S	2015	X12442403 (a metabolite of XDE-777): Acute Toxicity to the Cladoceran, <i>Daphnia magna</i> , Determined Under Static-Renewal Test Conditions DAS Report No.: 140486 ABC Laboratories GLP/GEP (Y/N): Yes Published (Y/N): No	No	Corteva Agriscience
CA 8.2.4.2/1	Lamichhane K	2014	XDE-777 TGAI: Acute Toxicity to the Cladoceran, <i>Daphnia pulex</i> , Exposed Under Static-Renewal Test Conditions DAS Report No.: 140483 ABC Laboratories GLP/GEP (Y/N): Yes Published (Y/N): No	No	Corteva Agriscience
CA 8.2.4.2/2	VanHooser, A.	2015a	XDE-777: Acute toxicity to the Freshwater Midge, <i>Chironomus riparius</i> , Determined Under Static-Renewal Test Conditions DAS Report No.: 141002 ABC Laboratories, Inc. GLP/GEP (Y/N): Yes Published (Y/N): No	No	Corteva Agriscience
CA 8.2.4.2/3	VanHooser, A.	2015b	X642188 (a metabolite of XDE-777): Acute toxicity to the Freshwater Midge, <i>Chironomus riparius</i> , Determined Under Static-Renewal Test Conditions DAS Report No.: 141003 ABC Laboratories, Inc. GLP/GEP (Y/N): Yes Published (Y/N): No	No	Corteva Agriscience
CA 8.2.4.2/4	Hadsell, R.	2015	X12255349 (a metabolite of XDE-777): Acute toxicity to the Freshwater Midge, <i>Chironomus riparius</i> , Determined Under Static-Renewal Test Conditions DAS Report No.: 141004 ABC Laboratories, Inc. GLP/GEP (Y/N): Yes Published (Y/N): No	No	Corteva Agriscience
CA 8.2.5/2	Lamichhane, K.	2015	X12255349 (a metabolite of XDE-777): Population Effects Study in an Indoor Aquatic Microcosm with <i>Daphnia magna</i> DAS Report No. 140999 ABC Laboratories GLP/GEP (Y/N): Yes	No	Corteva Agriscience

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 (Y/N): No		
CA 8.2.5.1 /1	Fournier A	2012	XR-777 TGAI: Full Life-Cycle Toxicity Test with Water Fleas, <i>Daphnia magna</i> , Under Static Renewal Conditions Following OECD Guideline #211 DAS Report No.: 110216 Smithers Viscient GLP/GEP (Y/N): Yes Published (Y/N): No	No	Corteva Agriscience
CA 8.2.6.1 /1	Rebstock M	2013	XDE-777: Growth Inhibition Test with the Unicellular Green Alga, <i>Pseudokirchneriella subcapitata</i> DAS Report No.: 120383 ABC Laboratories, Inc GLP/GEP (Y/N): Yes Published (Y/N): No	No	Corteva Agriscience
CA 8.2.6.1 /2	Rebstock M	2013	X642188 metabolite: Growth Inhibition Test with the Unicellular Green Alga, <i>Pseudokirchneriella subcapitata</i> DAS Report No.: 120380 ABC Laboratories, Inc GLP/GEP (Y/N): Yes Published (Y/N): No	No	Corteva Agriscience
CA 8.2.6.1 /3	Bergfield A	2014	X11963422 (a metabolite of XDE-777): Growth Inhibition Test with the Unicellular Green Alga, <i>Pseudokirchneriella subcapitata</i> DAS Report No.: 130385 ABC Laboratories, Inc GLP/GEP (Y/N): Yes Published (Y/N): No	No	Corteva Agriscience
CA 8.2.6.1 /4	Aufderheide, J.	2014	X12264475 (a metabolite of XDE-777): Growth Inhibition Test with the Unicellular Green Alga, <i>Pseudokirchneriella subcapitata</i> DAS Report No.: 130384 ABC Laboratories, Inc GLP/GEP (Y/N): Yes Published (Y/N): No	No	Corteva Agriscience

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
CA 8.2.6.1 /5	Aufderheide, J.	2015	X12255349 (a metabolite of XDE-777): Growth Inhibition Test with the Unicellular Green Alga, <i>Pseudokirchneriella subcapitata</i> DAS Report No.: 141001 ABC Laboratories, Inc GLP/GEP (Y/N): Yes Published (Y/N): No	No	Corteva Agriscience
CP 10.2.1/1	██████	2013	GF-2925: Acute Toxicity to the Rainbow Trout, <i>Oncorhynchus mykiss</i> , Determined Under Static-Renewal Test Conditions (Revision) DAS Report No.: 120374 ██████ GLP/GEP (Y/N): Y Published (Y/N): N	Y	Corteva Agriscience
CP 10.2.1/2	Stadler T Lamichhane K Goudie, O	2013, amended 2014, revised 2017	GF-2925: Acute Toxicity to the Cladoceran, <i>Daphnia magna</i> , Determined Under Static-Renewal Test Conditions (Revision) DAS Report No.: 120375 ABC Laboratories GLP/GEP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience
CP 10.2.1/3	Holou M	2013	GF-2925: Growth inhibition test with the unicellular green alga, <i>Pseudokirchneriella subcapitata</i> DAS Report No.: 120376 ABC Laboratories, Inc. GLP/GEP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience
CP 10.2.3/04	██████	2014	XDE-777: Community level study in outdoor aquatic mesocosms DAS Report No.: 130984 ██████ GLP/GEP (Y/N): Y Published (Y/N): N	Y	Corteva Agriscience
CP 10.2.3/06	Mueller, J.	2015	XDE-777 metabolites: Analysis in aqueous and sediment samples of the outdoor mesocosm study DAS Report No.: 140860 Fraunhofer Institute GLP/GEP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience

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
CA 8.2.1 /1	██████	2012	XR-777 - Acute Toxicity to Rainbow Trout ( <i>Oncorhynchus mykiss</i> ) Under Flow-Through Conditions, Following OECD Guideline #203 DAS Report No.: 110213 ██████ GLP/GEP (Y/N): Yes Published (Y/N): No	<del>N</del> Y	Corteva Agriscience
CA 8.2.1 /2	██████	2012	XDE-777 Technical: Acute Toxicity to the Common Carp, <i>Cyprinus carpio</i> , Determined Under Flow-Through Test Conditions DAS Report No.: 120392 ██████ GLP/GEP (Y/N): Yes Published (Y/N): No	<del>N</del> Y	Corteva Agriscience
CA 8.2.1 /3	██████	2012	X642188 Metabolite: Acute Toxicity Test with the Rainbow Trout, <i>Oncorhynchus mykiss</i> , Determined Under Flow-Through Test Conditions DAS Report No.: 120382 ██████ GLP/GEP (Y/N): Yes Published (Y/N): No	<del>N</del> Y	Corteva Agriscience

\*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 10.3.1.6/1	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. DAS Report No.: 200670 Eurofins Agriscience Services Ltd GLP/GEP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience
KCA 6.10.1/2	Appeltauer, A	2020	Determination of residues of prothioconazole and its metabolites in honey after two applications of PTZ EC 250 in winter oilseed rape at 5 sites in Northern and Southern Europe in 2019. Bayer Report No.: M-682401-01-1/ Study Number: S19-00902	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
			Eurofins Agroscience Services Ltd GLP/GEP (Y/N): Y Published (Y/N): N		

**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:	<p>The methods (A, B, C, D, E) were successfully validated for the determination of fenpicoxamid and its metabolites (X642188 (X'188), X12314005 (X'D05), X12D1952D (X'520), X12335723 (X'723), X12264475 (X'475) and prothioconazole-desthio (PTZ-desthio) and its prothioconazole <math>\alpha</math>-, 3-, 4-, 5- and 6-hydroxy desthio metabolites in sugar beet including processed fractions in accordance to guidance document SANTE/2020/12830, rev. 2 for risk assessment.</p> <p>The limit of detection (LOD) and limit of quantitation (LOQ) for each analyte and each matrix were 0.003 mg/kg and 0.01 mg/kg respectively.</p> <p>The methods are acceptable.</p>
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<b>Data Point:</b>	KCA 6.3.1
<b>Report author:</b>	Munro, M..
<b>Report year:</b>	2024
<b>Report title:</b>	Fenpicoxamid Residues in Sugar Beet Including Processed Fractions Following Two Applications of GF-3307, Europe, 2022. DAS Report No.: 220541/ Study Number: 685066
<b>Report No.:</b>	220541
<b>Testing Facility Report No.:</b>	685066
<b>Method(s) used:</b>	120615, 140696, MR-06/138, 00979/M002,
<b>Guidelines followed in study:</b>	SANTE/2020/12830, rev. 2
<b>Deviation from current test guidelines:</b>	No
<b>Analytical Performing Laboratory:</b>	Eurofins Agrosience Services Chem GmbH Hamburg, Germany
<b>GLP/Officially recognised testing facilities:</b>	Yes
<b>Acceptability/Reliability:</b>	Yes

## MATERIALS AND METHODS

### Method Principle

Test Method “A” for Sugar Beet Tops and Roots: Residues of fenpicoxamid and X642188 were extracted with an acetonitrile/ultra-pure water solution. The samples were homogenized and shaken on a flatbed shaker. After centrifugation, an aliquot was diluted using acetonitrile/ultra-pure water/formic acid. The final sample was analysed for fenpicoxamid and X642188 by LC-MS/MS.

Test Method “B” for Sugar Beet Tops and Roots: Residues of X12314005 were extracted with an acetonitrile/ultra-pure water/orthophosphoric acid solution. The samples were homogenized and shaken on a flatbed shaker. After centrifugation, an aliquot was diluted using acetonitrile/ultra-pure water/orthophosphoric acid. The final sample was analysed for X12314005 by LC-MS/MS.

Test Method “C” for Processing Fractions: Residues of fenpicoxamid, X12019520, and X12314005 were extracted with an acetonitrile/ultra-pure water/orthophosphoric acid solution. The samples were homogenized and shaken on a flatbed shaker. After centrifugation, an aliquot was diluted using acetonitrile/ultra-pure water/orthophosphoric acid. The final sample was analysed for fenpicoxamid, X12019520, X12314005, X12335723, and X12264475 by LC-MS/MS.

Test Method “D” in Sugar Beet Tops and Roots: Residues of prothioconazole-desthio were extracted with an acetonitrile/ultra-pure water solution containing cysteine hydrochloride. . The samples were cleaned-up using liquid-liquid partition with hexane and then dichloromethane. After evaporation to dryness of the organic phase, the samples were dissolved in acetonitrile/ultra-pure water. The final sample was analysed for prothioconazole-desthio by LC-MS/MS

Test Method “E” in Sugar Beet Tops and Roots: Residues of  $\alpha$ -hydroxy-desthio, 3-hydroxy-desthio, 4-hydroxy-desthio, 5-hydroxy-desthio and 6-hydroxy-desthio were extracted with an acetonitrile/water solution. After filtration and evaporation to the aqueous remainder, the extract was diluted and acidified with 5 N hydrochloric acid prior to reflux for 2 hours. An aliquot was then neutralized with sodium hydrogencarbonate and purified on a Chromabond® XTR cartridge. The eluate was evaporated to dryness and resolved in acetonitrile. An aliquot was diluted with acetonitrile and water. The final sample was analysed for  $\alpha$ -hydroxy-desthio, 3-hydroxy-desthio, 4-hydroxy-desthio, 5-hydroxy-desthio and 6-hydroxy-desthio.

## RESULTS AND DISCUSSION

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

**Table A 1: Recovery results from method validation of fenpicoxamid ( $m/z$  615.0/239.0) using the analytical method**

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Sugar Beet (Roots)	Fenpicoxamid	0.01	110	6.5	5	
Sugar Beet (Roots)	Fenpicoxamid	0.1	103	5.5	5	
Sugar Beet (Tops)	Fenpicoxamid	0.01	104	8.2	5	
Sugar Beet (Tops)	Fenpicoxamid	0.1	107	4.8	5	
Sugar Beet (Roots Prior to Processing)	Fenpicoxamid	0.01	91	9.5	5	
Sugar Beet (Roots Prior to Processing)	Fenpicoxamid	0.1	94	2.1	5	
Press Water	Fenpicoxamid	0.01	102	4.7	7	
Press Water	Fenpicoxamid	0.1	90	6.2	7	
Wet Pulp	Fenpicoxamid	0.01	87	15	7	
Wet Pulp	Fenpicoxamid	0.1	84	4.6	7	
Pressed Pulp	Fenpicoxamid	0.01	92	12	7	
Pressed Pulp	Fenpicoxamid	0.1	85	11	7	
Dried Pulp	Fenpicoxamid	0.01	89	14	10	
Dried Pulp	Fenpicoxamid	0.1	89	6.5	10	
Ensiled Pulp	Fenpicoxamid	0.01	79	20	7	
Ensiled Pulp	Fenpicoxamid	0.1	84	10	7	
Raw Juice	Fenpicoxamid	0.01	99	8.6	7	
Raw Juice	Fenpicoxamid	0.1	102	11	7	
Thick Juice	Fenpicoxamid	0.01	94	8.1	7	
Thick Juice	Fenpicoxamid	0.1	97	5.2	7	
Raw Sugar	Fenpicoxamid	0.01	101	10	4	
Raw Sugar	Fenpicoxamid	0.1	99	4.9	4	
Molasses	Fenpicoxamid	0.01	85	17	4	
Molasses	Fenpicoxamid	0.1	97	8.9	4	
Refined Sugar	Fenpicoxamid	0.01	91	7.0	5	

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Refined Sugar	Fenpicoxamid	0.1	99	7.8	5	

**Table A 2:** Recovery results from method validation of X642188 (m/z 515.0/239.0) using the analytical method

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Sugar Beet(Roots)	X642188	0.01	97	7.1	5	
Sugar Beet(Roots)	X642188	0.1	95	2.6	5	
Sugar Beet(Tops)	X642188	0.01	93	6.6	5	
Sugar Beet(Tops)	X642188	0.1	97	4.0	5	

**Table A 3:** Recovery results from method validation of X12314005 (m/z 277.1/143.1) using the analytical method

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Sugar Beet(Roots)	X12314005	0.01	90	5.4	5	
Sugar Beet(Roots)	X12314005	0.1	86	3.9	5	
Sugar Beet(Tops)	X12314005	0.01	89	4.5	5	
Sugar Beet(Tops)	X12314005	0.1	89	1.9	5	
Sugar Beet (Roots Prior to Processing)	X12314005	0.01	93	3.3	5	
Sugar Beet (Roots Prior to Processing)	X12314005	0.1	93	3.4	5	

**Table A 4:** Recovery results from method validation of Prothioconazole-desthio (m/z 312.0/70.0) using the analytical method

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Sugar Beet(Roots)	Prothioconazole-desthio	0.01	85	3.8	5	
Sugar Beet(Roots)	Prothioconazole-desthio	0.1	57	2.8	5	Mean recovery outside the acceptable range
Sugar Beet(Tops)	Prothioconazole-desthio	0.01	92	3.5	5	
Sugar Beet(Tops)	Prothioconazole-desthio	0.1	87	4.1	5	

**Table A 5:** Recovery results from method validation of  $\alpha$ -hydroxy-desthio (m/z 328.1/69.9) using the analytical method

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Sugar Beet(Roots)	$\alpha$ -hydroxy-desthio	0.01	80	1.9	5	
Sugar Beet(Roots)	$\alpha$ -hydroxy-desthio	0.1	79	1.5	5	
Sugar Beet(Tops)	$\alpha$ -hydroxy-desthio	0.01	83	3.2	5	
Sugar Beet(Tops)	$\alpha$ -hydroxy-desthio	0.1	82	1.8	5	



**Table A 6: Recovery results from method validation of 3-hydroxy-desthio (*m/z* 328.1/69.9) using the analytical method**

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Sugar Beet(Roots)	3-hydroxy-desthio	0.01	99	5.2	5	
Sugar Beet(Roots)	3-hydroxy-desthio	0.1	95	2.5	5	
Sugar Beet(Tops)	3-hydroxy-desthio	0.01	100	2.4	5	
Sugar Beet(Tops)	3-hydroxy-desthio	0.1	96	2.7	5	

**Table A 7: Recovery results from method validation of 4-hydroxy-desthio (*m/z* 328.1/69.9) using the analytical method**

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Sugar Beet(Roots)	4-hydroxy-desthio	0.01	93	3.1	5	
Sugar Beet(Roots)	4-hydroxy-desthio	0.1	94	2.0	5	
Sugar Beet(Tops)	4-hydroxy-desthio	0.01	98	3.6	5	
Sugar Beet(Tops)	4-hydroxy-desthio	0.1	95	1.8	5	

**Table A 8: Recovery results from method validation of 5-hydroxy-desthio (*m/z* 328.1/69.9) using the analytical method**

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Sugar Beet(Roots)	5-hydroxy-desthio	0.01	91	34.2	5	
Sugar Beet(Roots)	5-hydroxy-desthio	0.1	95	2.1	5	
Sugar Beet(Tops)	5-hydroxy-desthio	0.01	90	4.6	5	
Sugar Beet(Tops)	5-hydroxy-desthio	0.1	91	2.8	5	

**Table A 9: Recovery results from method validation of 6-hydroxy-desthio (*m/z* 328.1/69.9) using the analytical method**

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Sugar Beet(Roots)	6-hydroxy-desthio	0.01	99	2.9	5	
Sugar Beet(Roots)	6-hydroxy-desthio	0.1	97	2.9	5	
Sugar Beet(Tops)	6-hydroxy-desthio	0.01	100	7.0	5	
Sugar Beet(Tops)	6-hydroxy-desthio	0.1	97	2.3	5	

**Table A10 Recovery results from method validation of X12335723 (*m/z* 357/257) using the analytical method**

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Sugar Beet(Roots Prior to Processing)	X12335723	0.01	75	5.7	5	
Sugar Beet(Roots Prior to Processing)	X12335723	0.1	80	1.5	5	

**Table A11** 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
Sugar Beet(Roots Prior to Processing)	X12019520	0.01	90	3.7	5	
Sugar Beet(Roots Prior to Processing)	X12019520	0.1	96	2.0	5	

**Table A12:** Recovery results from method validation of X12264475 (*m/z* 257/152.0) using the analytical method

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Sugar Beet (Roots Prior to Processing)	X12264475	0.01	103	4.5	5	
Sugar Beet (Roots Prior to Processing)	X12264475	0.1	99	4.6	5	
Press Water	X12264475	0.01	87	19	5	
Press Water	X12264475	0.1	77	17	5	
Wet Pulp	X12264475	0.01	91	20	8	
Wet Pulp	X12264475	0.1	96	20	8	
Pressed Pulp	X12264475	0.01	86	13	5	
Pressed Pulp	X12264475	0.1	93	16	5	
Dried Pulp	X12264475	0.01	86	19	6	
Dried Pulp	X12264475	0.1	90	20	5	
Ensiled Pulp	X12264475	0.01	90	10	5	
Ensiled Pulp	X12264475	0.1	99	6.5	5	
Raw Juice	X12264475	0.01	82	11	5	
Raw Juice	X12264475	0.1	87	13	5	
Thick Juice	X12264475	0.01	101	16	5	
Thick Juice	X12264475	0.1	110	3.1	5	
Raw Sugar	X12264475	0.01	105	7.2	5	
Raw Sugar	X12264475	0.1	98	3.7	5	
Molasses	X12264475	0.01	81	17	5	
Molasses	X12264475	0.1	91	3.8	5	
Refined Sugar	X12264475	0.01	106	16	5	
Refined Sugar	X12264475	0.1	96	12	5	

**Table A 3:** Procedural recovery results of fenpicoxamid (*m/z* 615.0/239.0) using the analytical method

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Sugar Beet (Roots)	Fenpicoxamid	0.01	96	10	4	
Sugar Beet (Roots)	Fenpicoxamid	0.1	81	.3	4	
Sugar Beet (Tops)	Fenpicoxamid	0.01	107	3.6	6	
Sugar Beet (Tops)	Fenpicoxamid	0.1	96	7.6	4	

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Sugar Beet (Tops)	Fenpicoxamid	3.0	75	2.7	5	
Sugar Beet (Roots Prior to Processing)	Fenpicoxamid	0.01	95	7.3	4	
Sugar Beet (Roots Prior to Processing)	Fenpicoxamid	0.1	93	19	4	
Press Water	Fenpicoxamid	0.01	100	4.7	7	
Press Water	Fenpicoxamid	0.1	90	6.2	7	
Wet Pulp	Fenpicoxamid	0.01	87	15	7	
Wet Pulp	Fenpicoxamid	0.1	84	1.6	7	
Pressed Pulp	Fenpicoxamid	0.01	92	12	7	
Pressed Pulp	Fenpicoxamid	0.1	85	11	7	
Dried Pulp	Fenpicoxamid	0.01	94	8.7	8	
Dried Pulp	Fenpicoxamid	0.1	91	4.6	7	
Ensiled Pulp	Fenpicoxamid	0.01	79	20	7	
Ensiled Pulp	Fenpicoxamid	0.1	84	10	7	
Raw Juice	Fenpicoxamid	0.01	99	8.6	7	
Raw Juice	Fenpicoxamid	0.1	102	11	7	
Thick Juice	Fenpicoxamid	0.01	94	8.1	7	
Thick Juice	Fenpicoxamid	0.1	97	5.2	7	
Raw Sugar	Fenpicoxamid	0.01	103	8.0	7	
Raw Sugar	Fenpicoxamid	0.1	105	7.9	7	
Molasses	Fenpicoxamid	0.01	87	13	7	
Molasses	Fenpicoxamid	0.1	97	6.3	7	
Refined Sugar	Fenpicoxamid	0.01	98	10	4	
Refined Sugar	Fenpicoxamid	0.1	97	1.3	4	

**Table A 4: Procedural recovery results of X642188 (m/z 515.0/239.0) using the analytical method**

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Sugar Beet(Roots)	X642188	0.01	95	8.7	4	
Sugar Beet(Roots)	X642188	0.1	94	8.1	4	
Sugar Beet(Tops)	X642188	0.01	103	12.6	6	
Sugar Beet(Tops)	X642188	0.1	89	7.5		
Sugar Beet(Tops)	X642188	1.0	107	1.1	5	

**Table A 5: Procedural recovery results of X12314005 (m/z 277.1/189.1) using the analytical method**

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Sugar Beet(Roots)	X12314005	0.01	96	7.1	20	
Sugar Beet(Roots)	X12314005	0.1	95	6	20	
Sugar Beet(Tops)	X12314005	0.01	95	5.7	20	

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Sugar Beet(Tops)	X12314005	0.1	94	4.6	20	
Sugar Beet (Roots Prior to Processing)	X12314005	0.01	101	3.1	4	
Sugar Beet (Roots Prior to Processing)	X12314005	0.1	103	0.2	4	

**Table A 6: Procedural recovery results of Prothioconazole-desthio ( $m/z$  312.0/70.0) using the analytical method**

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Sugar Beet(Roots)	Prothioconazole-desthio	0.01	92	5.2	6	
Sugar Beet(Roots)	Prothioconazole-desthio	0.1	93	4.8	6	
Sugar Beet(Tops)	Prothioconazole-desthio	0.01	93	5.7	11	
Sugar Beet(Tops)	Prothioconazole-desthio	0.1	93	7.2	11	
Sugar Beet(Tops)	Prothioconazole-desthio	1.0	82	4.2	5	

**Table A 7: Procedural recovery results of  $\alpha$ -hydroxy-desthio ( $m/z$  328.1/69.9) using the analytical method**

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Sugar Beet(Roots)	$\alpha$ -hydroxy-desthio	0.01	83	8.4	6	
Sugar Beet(Roots)	$\alpha$ -hydroxy-desthio	0.1	87	12	6	
Sugar Beet(Tops)	$\alpha$ -hydroxy-desthio	0.01	85	8	6	
Sugar Beet(Tops)	$\alpha$ -hydroxy-desthio	0.1	81	3.3	4	
Sugar Beet(Tops)	$\alpha$ -hydroxy-desthio	1.0	11	1.3	5	

**Table A 8: Procedural recovery results of 3-hydroxy-desthio ( $m/z$  328.1/69.9) using the analytical method**

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Sugar Beet(Roots)	3-hydroxy-desthio	0.01	98	4.3	6	
Sugar Beet(Roots)	3-hydroxy-desthio	0.1	99	4.7	6	
Sugar Beet(Tops)	3-hydroxy-desthio	0.01	98	5.5	10	
Sugar Beet(Tops)	3-hydroxy-desthio	0.1	95	4.0	8	
Sugar Beet(Tops)	3-hydroxy-desthio	1.0	81	3.3	5	

**Table A 9: Procedural recovery results of 4-hydroxy-desthio ( $m/z$  328.1/69.9) using the analytical method**

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Sugar Beet(Roots)	4-hydroxy-desthio	0.01	97	7.9	6	
Sugar Beet(Roots)	4-hydroxy-desthio	0.1	96	7.6	6	
Sugar Beet(Tops)	4-hydroxy-desthio	0.01	98	9.3	10	

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Sugar Beet(Tops)	4-hydroxy-desthio	0.1	95	3.4	8	
Sugar Beet(Tops)	4-hydroxy-desthio	1.0	82	2.7	5	

**Table A 20:** Procedural recovery results of 5-hydroxy-desthio (*m/z* 328.1/69.9) using the analytical method

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Sugar Beet(Roots)	5-hydroxy-desthio	0.01	95	8.6	6	
Sugar Beet(Roots)	5-hydroxy-desthio	0.1	95	9.4	6	
Sugar Beet(Tops)	5-hydroxy-desthio	0.01	95	7.6	6	
Sugar Beet(Tops)	5-hydroxy-desthio	0.1	94	2.4	4	
Sugar Beet(Tops)	5-hydroxy-desthio	1.0	80	2.0	5	

**Table A 21:** Procedural recovery results of 6-hydroxy-desthio (*m/z* 328.1/69.9) using the analytical method

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Sugar Beet(Roots)	6-hydroxy-desthio	0.01	93	4.3	6	
Sugar Beet(Roots)	6-hydroxy-desthio	0.1	99	6.2	6	
Sugar Beet(Tops)	6-hydroxy-desthio	0.01	94	9.6	6	
Sugar Beet(Tops)	6-hydroxy-desthio	0.1	101	3.7	4	
Sugar Beet(Tops)	6-hydroxy-desthio	1.0	70	3.7	5	

**Table A22** Procedural results from method validation of X12335723 (*m/z* 357/257) using the analytical method

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Sugar Beet(Roots Prior to Processing)	X12335723	0.01	69	7.3	4	
Sugar Beet(Roots Prior to Processing)	X12335723	0.1	78	12	4	

**Table A23** Procedural 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
Sugar Beet(Roots Prior to Processing)	X12019520	0.01	107	12	4	
Sugar Beet(Roots Prior to Processing)	X12019520	0.1	100	5.5	4	

**Table A 24:** Procedural results from method validation of X12264475 (*m/z* 257/152.0) using the analytical method

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Sugar Beet (Roots Prior to Processing)	X12264475	0.01	77	8.2	4	
Sugar Beet (Roots Prior to Processing)	X12264475	0.1	83	1.3	4	

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
to Processing)						
Press Water	X12264475	0.01	87	19	5	
Press Water	X12264475	0.1	77	17	5	
Wet Pulp	X12264475	0.01	91	20	8	
Wet Pulp	X12264475	0.1	96	20	8	
Pressed Pulp	X12264475	0.01	86	13	5	
Pressed Pulp	X12264475	0.1	93	16	5	
Dried Pulp	X12264475	0.01	86	19	6	
Dried Pulp	X12264475	0.1	90	20	5	
Ensiled Pulp	X12264475	0.01	90	10	5	
Ensiled Pulp	X12264475	0.1	99	6.5	5	
Raw Juice	X12264475	0.01	82	11	5	
Raw Juice	X12264475	0.1	87	13	5	
Thick Juice	X12264475	0.01	101	16	5	
Thick Juice	X12264475	0.1	110	3.1	5	
Raw Sugar	X12264475	0.01	101	9.0	8	
Raw Sugar	X12264475	0.1	98	3.7	5	
Molasses	X12264475	0.01	91	3.8	5	
Molasses	X12264475	0.1	85	7.2	5	
Refined Sugar	X12264475	0.01	101	n.a.	2	
Refined Sugar	X12264475	0.1	99	n.a.	2	

**Table A 25: Characteristics for the analytical method used for validation of fenpicoxamid and X642188 residues in sugar beet tops and roots.**

	Fenpicoxamid	X642188
Specificity	<i>m/z</i> 615.0/239.0 Q blank value <30% LOQ	<i>m/z</i> 515.0/239.0 Q blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting $r \geq 0.995$ 5 data points	linear regression analysis with 1/x weighting $r \geq 0.995$ 5 data points
Calibration range	Concentration range of 0.0075- 0.5ng/mL	Concentration range of 0.0075- 0.50 ng/mL
Limit of quantitation	LOQ= 0.01 mg/kg	LOQ= 0.01 mg/kg

**Table A 6: Characteristics for the analytical method used for validation of X12314005 residues in sugar beet tops and roots.**

	X12314005
Specificity	<i>m/z</i> 277.1/189.1 Q blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting $r \geq 0.995$ 5 data points
Calibration range	Concentration range of 0.075-5.0 ng/mL
Limit of quantitation	LOQ= 0.01 mg/kg

**Table A 7: Characteristics for the analytical method used for validation of prothioconazole-desthio residues in sugar beet tops and roots.**

	Prothioconazole-desthio
Specificity	<i>m/z</i> 312.0/70.0 Q blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting $r \geq 0.995$ 5 data points
Calibration range	Concentration range of 0.030-3.0 ng/mL
Limit of quantitation	LOQ= 0.01 mg/kg

**Table A 8: Characteristics for the analytical method used for validation of  $\alpha$ -hydroxy-desthio, 3-hydroxy-desthio, 4-hydroxy-desthio, 5-hydroxy-desthio and 6-hydroxy-desthio residues in sugar beet tops and roots.**

	$\alpha$ -hydroxy-desthio, 3-hydroxy-desthio, 4-hydroxy-desthio, 5-hydroxy-desthio and 6-hydroxy-desthio
Specificity	<i>m/z</i> 328.1/69.9 Q blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting $r \geq 0.995$ 5 data points
Calibration range	Concentration range of 0.060-6.0 ng/mL
Limit of quantitation	LOQ= 0.01 mg/kg

**Table A 29: Characteristics for the analytical method used for validation of fenpicoxamid, X12019520, X12314005, X12335723, and X12264475 in processed fractions**

	fenpicoxamid, X12019520, X12314005, X12335723, and X12264475
Specificity	<i>m/z</i> 328.1/69.9 Q blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting $r \geq 0.995$ 5 data points
Calibration range	XDE-777, X'520 and X'005 0.075 – 5.0 ng/mL X'475 0.15 – 10 ng/mL X'723 0.15 – 10 ng/mL
Limit of quantitation	LOQ= 0.01 mg/kg

## ASSESSMENT AND CONCLUSION

This method was successfully validated per SANCO/3029/99 rev.4 for the determination of fenpicoxamid, X642188, X12314005, prothioconazole-desthio,  $\alpha$ -hydroxy-desthio, 3-hydroxy-desthio, 4-hydroxy-desthio, 5-hydroxy-desthio and 6-hydroxy-desthio in ~~oilseed rape whole plant, seeds and remaining plant~~ sugar beet roots, tops and in processing fractions.

## A 2.1.1.2 Analytical method 2

### A 2.1.1.2.1 Method validation

Comments of zRMS:	The study of Appeltauer, A (2021, Report No. 200670)) has been not evaluated and an analytical method is not necessary in the assessment to support this application.
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<b>Report author:</b>	Appeltauer, A.
<b>Report year:</b>	2021
<b>Report title:</b>	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
<b>Report No.:</b>	200670
<b>Testing Facility Report No.:</b>	S20-01926
<b>Method(s) used:</b>	200670
<b>Guidelines followed in study:</b>	SANCO/3029/99 rev. 4
<b>Deviation from current test guidelines:</b>	No
<b>Analytical Performing Laboratory:</b>	Eurofins Agrosience Services EcoTox GmbH Niefern- Öschelbronn , Germany
<b>GLP/Officially recognised testing facilities:</b>	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 prothioconazole-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 10: 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 11: 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 12: 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 13: 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 14: 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 15: 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 16: 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	<i>m/z</i> 615/239Q <i>m/z</i> 615/515C blank value <30% LOQ	<i>m/z</i> 344/189Q <i>m/z</i> 344/154C blank value <30% LOQ	<i>m/z</i> 312/70Q <i>m/z</i> 312/125C blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting $r \geq 0.9984$ 8 data points	linear regression analysis with 1/x weighting $r \geq 0.9994$ 8 data points	linear regression analysis with 1/x weighting $r \geq 0.9998$ 8 data points
Calibration range	Concentration range of 0.015 ng/mL(equivalent sample concentration 0.0003 – 0.15 mg/kg)	Concentration range of 0.015 ng/mL(equivalent sample concentration 0.0003 – 0.15 mg/kg)	Concentration range of 0.015 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 17: Characteristics for the analytical method used for determination of residues of Fenpicoxamid, Prothioconazole and Prothioconazole-desthio in Nectar**

Analyte	Fenpicoxamid	Prothioconazole	Prothioconazole-desthio
Matrix	Nectar	Nectar	Nectar
Technique	LC-MS/MS	LC-MS/MS	LC-MS/MS
Specificity	<i>m/z</i> 615/239Q <i>m/z</i> 615/515C blank value <30% LOQ	<i>m/z</i> 344/189Q <i>m/z</i> 344/154C blank value <30% LOQ	<i>m/z</i> 312/70Q <i>m/z</i> 312/125C blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting $r \geq 0.9998$ 8 data points	linear regression analysis with 1/x weighting $r \geq 0.9998$ 8 data points	linear regression analysis with 1/x weighting $r \geq 0.9997$ 8 data points
Calibration range	Concentration range of 0.015 ng/mL(equivalent sample concentration 0.0003 – 0.15 mg/kg)	Concentration range of 0.015 ng/mL(equivalent sample concentration 0.0003 – 0.15 mg/kg)	Concentration range of 0.015 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 18: 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	<i>m/z</i> 615/239Q <i>m/z</i> 615/515C blank value <30% LOQ	<i>m/z</i> 344/154Q <i>m/z</i> 344/189C blank value <30% LOQ	<i>m/z</i> 312/70Q <i>m/z</i> 312/125C blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting $r \geq 0.9991$ 8 data points	linear regression analysis with 1/x weighting $r \geq 0.9990$ 8 data points	linear regression analysis with 1/x weighting $r \geq 0.9999$ 8 data points
Calibration range	Concentration range of 0.035 ng/mL(equivalent sample concentration 0.0003 – 0.05 mg/kg)	Concentration range of 0.035 ng/mL(equivalent sample concentration 0.0003 – 0.05 mg/kg)	Concentration range of 0.035 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 2.1.1.3 Analytical method 3

#### A 2.1.1.3.1 Method validation

Comments of zRMS:	The study was evaluated and accepted in RR for Queen (January 2023) and is not being re-assessed in this application.
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Method Identifier No.: 140479 Amendment 1

Performing Laboratory: XXXXXXXXXX

Reference: KCP 10.2.1/1

Report: XXXXXXXXXX; 2014; GF-3307: Acute Toxicity to the Rainbow Trout, *Oncorhynchus mykiss*, Determined Under Static-Renewal Test Conditions; XXXXXXXXXX; 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 (LCMS/MS).

## RESULTS AND DISCUSSION

Mean recovery values at each fortification concentration were within the acceptance range (mean recovery 70110%; RSD ≤ 20%). The results obtained are summarised in the following tables.

**Table A 19: 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 20: 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	<i>m/z</i> 615.0/239.2 blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting $r \geq 0.995$ 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 2.1.1.4 Analytical method 4

#### A 2.1.1.4.1 Method validation

Comments of zRMS:	The study was evaluated and accepted in RR for Queen (January 2023) and is not being re-assessed in this application.
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Method Identifier No.:	180975 Protocol
Performing Laboratory:	██████
Reference:	KCP 10.2.1/8
Report:	██████; 2018; GF-3307: Acute Toxicity to the Rainbow Trout, <i>Oncorhynchus mykiss</i> , Determined Under Flow-Through Test Conditions; ██████; 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 21:** 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 22: 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 23: Characteristics for the analytical method used for validation of GF-3307, based on XDE-777 and prothioconazole active ingredients analysis, residues in freshwater**

	<b>GF-3307, based on XDE-777 active ingredient analysis</b>	<b>GF-3307, based on prothioconazole active ingredients analysis</b>
Specificity	<i>m/z</i> 615.0/239.0 <i>m/z</i> 615.0/515.0 <i>m/z</i> 615.0/124.0 blank value <30% LOQ	<i>m/z</i> 342.0/100.0 <i>m/z</i> 342.0/125.0 <i>m/z</i> 342.0/180.0 <i>m/z</i> 342.0/264.0 blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting $r \geq 0.997$ 6 data points	linear regression analysis with 1/x weighting $r \geq 0.996$ 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 GF3307/L, equivalent to 0.123 ng a.i./mL	LOQ=5.00 µg GF3307/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 2.1.1.5 Analytical method 5

#### A 2.1.1.5.1 Method validation

Comments of zRMS:	The study was evaluated in the Registration Report, Part B5 for GF-3308 on 24.08.2022 and is not being re-assessed in this application.
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Method Identifier No.:	160102
Performing Laboratory:	ABC Laboratories, Inc. (now EAG Laboratories) Columbia, Missouri, USA
Reference:	KCP 10.2.1/7
Report:	Goudie, O.; 2016; GF-3308: Acute Toxicity to the Cladoceran, <i>Daphnia magna</i> , 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 GF3308/L and this study had an MQL of 0.0042 mg GF3308/L. The original method used fortification levels of 0.985, 2.25, 45.9, and 65.6 mg GF3308/L while this study used fortification levels of 0.0279 and 0.572 mg GF3308/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 (LCMS/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 24: 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 25: 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	<i>m/z</i> 615.0/239.2 <i>m/z</i> 615.0/515.4 blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting $r^2 \geq 0.995$ 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 2.1.1.6 Analytical method 6

#### A 2.1.1.6.1 Method validation

Comments of zRMS:	The study was evaluated in the Registration Report, Part B5 for GF-3308 on 24.08.2022 and is not being re-assessed in this application.
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Reference:	KCP 10.2.1/4
Report:	Goudie, O; 2018; X642188 (a metabolite of XDE-777): Acute Toxicity to the Cladoceran, <i>Daphnia magna</i> , 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 (LCMS/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 26:** 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 27: Characteristics for the analytical method used for validation of X642188 residues in freshwater**

	<b>X642188</b>
Specificity	<i>m/z</i> 515.000/124.00 <i>m/z</i> 515.000/152.00 <i>m/z</i> 515.000/239.00 blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting $r \geq 0.994$ 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 2.1.1.7 Analytical method 7

#### A 2.1.1.7.1 Method validation

Comments of zRMS:	The study was evaluated in the Registration Report, Part B5 for GF-3308 on 24.08.2022 and is not being re-assessed in this application.
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Method Identifier No.:	191366
Performing Laboratory:	Eurofins EAG Agrosience, LLC, Easton, Maryland, USA
Reference:	KCP 10.2.1/9
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 ( <i>Daphnia magna</i> ); Eurofins EAG Agrosience, 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 70110%; RSD ≤ 20%). The results obtained are summarised in the following tables.

**Table A 28:** 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 29:** 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%
freshwater	prothioconazole	520	102	18.2	5	5 QC samples from definitive test analyses, ranging from 90.0 to 134%

**Table A 30:** Characteristics for the analytical method used for determination of GF-3307, analyzed for fenpicoxamid and prothioconazole, residues in freshwater

	fenpicoxamid	prothioconazole
Specificity	<i>m/z</i> 615.200/239.000 blank value <30% LOQ	<i>m/z</i> 334.100/326.000 blank value <30% LOQ
Calibration (type, number of data points)	Linear regression analysis with 1/x weighting $r \geq 0.998$ 5 data points	Linear regression analysis with 1/x weighting $r \geq 0.999$ 5 data points
Calibration range	Concentration range of 0.240 – 4.00 µg a.i./L Sample equivalent range of 0.511-85.1 µg GF-3307/L	Concentration range of 0.240 – 4.00 µg a.i./L Sample equivalent range of 2.47 – 41.2 µg GF-3307/L
Limit of determination/quantification	LOQ=15.0 µg GF-3307/L (7.05 µg fenpicoxamid/L) LOD = 4.50 µg GF-3307/L (2.12 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 2.1.1.8 Analytical method 8

### A 2.1.1.8.1 Method validation

Comments of zRMS:	The study was evaluated in the Registration Report, Part B5 for GF-3308 on 24.08.2022 and is not being re-assessed in this application.
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Method Identifier No.:	202284 Appendix 6
Performing Laboratory:	Eurofins EAG Agrosience, LLC Easton, Maryland, U.S.A.
Reference:	KCP 10.2.1/10
Report:	Goudie, O.J., Schneider, S.Z., Sneckenberger, G., and Zhang, L.; 2021; GF-2925: A Static-Renewal Acute Toxicity Test with the Cladoceran ( <i>Daphnia magna</i> ); Eurofins EAG Agrosience, 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 31:** 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 32: 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	<i>m/z</i> 615.2/239.0 (Q) <i>m/z</i> 615.2/515.1 (C) blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting $r \geq 0.999$ 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 GF2925/L (5.90 ng a.i./L) LOQ=0.160 µg GF2925/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 2.1.1.9 Analytical method 9

#### A 2.1.1.9.1 Method validation

Comments of zRMS:	The study was evaluated in the Registration Report, Part B5 for GF-3308 on 24.08.2022 and is not being re-assessed in this application.
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Method Identifier No.:	140489 Amendment 1
Performing Laboratory:	ABC Laboratories, Inc. (now EAG, Inc.) Columbia, Missouri, USA
Reference:	KCP 10.2.1/2
Report:	Hadsell, R.; Erin Hoover, 2014; GF-3307: Acute Toxicity to the Cladoceran, <i>Daphnia magna</i> , 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 33: 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 34: 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	<i>m/z</i> 615.0/239.2 blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting $r \geq 0.995$ 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 2.1.1.10 Analytical method 10

#### A 2.1.1.10.1 Method validation

Comments of zRMS:	The study was evaluated and accepted in RR for Queen (January 2023) and is not being re-assessed in this application.
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Method Identifier No.:	140491 Amendment 1
Performing Laboratory:	ABC Laboratories, Inc. (now EAG, Inc.) Columbia, Missouri, USA
Reference:	KCP 10.2.1/3
Report:	Hicks, S.; 2014; GF-3307: Growth Inhibition Test with the Unicellular Green Alga, <i>Pseudokirchneriella subcapitata</i> ; 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 35: 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 36: 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 37: 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	<i>m/z</i> 615.0/239.2 <i>m/z</i> 615.0/515.4 blank value <LOQ	<i>m/z</i> 342.0/100.0 <i>m/z</i> 342.0/306.0 and <i>m/z</i> 342.0/180.0 blank value < LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting $r \geq 0.995$ 7 data points	linear regression analysis with 1/x weighting $r \geq 0.995$ 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 2.1.1.11 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 and is not being re-assessed in this application.
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Reference:	KCP 10.2.1/5
Report:	██████; 2018; X12019520 (a metabolite of XDE-777): Acute Toxicity to the Rainbow Trout, <i>Oncorhynchus mykiss</i> , Determined Un-der Static-Renewal Test Conditions; ██████; Lab Study No. 87146; DAS Study No. 180560 ; 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 38: 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 39: Characteristics for the analytical method used for validation of X12019520 residues in freshwater**

	X12019520
Specificity	<i>m/z</i> 189.00/143.00 <i>m/z</i> 189.00/128.00 blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting $r \geq 0.999$ 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 2.1.1.12 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
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	and is not being re-assessed in this application.
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Reference: KCP 10.2.1/6

Report: [REDACTED]; 2018; X12446477 (a metabolite of XDE-777): Acute Tox-icity to the Rainbow Trout, *Oncorhynchus mykiss*, Determined Un-der Static-Renewal Test Conditions; [REDACTED]; Lab Study No. 87147; DAS Study No. 180561 ; 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 70110%; RSD ≤ 20%). The results obtained are summarised in the following tables.

**Table A 40: 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 41: 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 2.1.1.13 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 and is not being re-assessed in this application.
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Reference:	KCP 10.2.2/1
Report:	Beasley, J.; 2018; X642188 (a metabolite of XDE-777): Chronic Toxicity in Whole Sediment to Freshwater Midge, <i>Chironomus riparius</i> , 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 (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 was analysed for X642188 by liquid chromatography coupled with positive-ion electrospray tandem mass spectrometry (LCMS/MS).

## RESULTS AND DISCUSSION

Mean recovery values at each fortification concentration in sediment and freshwater (overlying water), and at the 14 mg X642188/L in overlying and pore water were within the acceptance range (mean recovery 70-110%;  $RSD \leq 20\%$ ). 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 42: 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 43: 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 44: Recovery results from method validation of X642188 (*m/z* 515.000/124.000) in freshwater (overlying water) using the analytical method**

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

**Table A 45: Characteristics for the analytical method used for validation of X642188 residues in sediment and freshwater (pore and overlying water)**

	X642188
Specificity	<i>m/z</i> 515.000/124.000 <i>m/z</i> 515.000/152.000 <i>m/z</i> 515.000/239.000 blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting $r \geq 0.997$ 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 2.1.1.14 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 and is not being re-assessed in this application.
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Reference:	KCP 10.2.2/2
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 (LCMS/MS).

## RESULTS AND DISCUSSION

Mean recovery values at each fortification concentration were within the acceptance range (mean recovery 70110%; RSD ≤ 20%). The results obtained are summarised in the following tables.

**Table A 46: 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 47: 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 48: 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 49: 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 2.1.1.15 Analytical method 15

#### A 2.1.1.15.1 Method validation

Comments of zRMS:	The study was evaluated and accepted in RR for Queen (January 2023) and is not being re-assessed in this application.
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Method Identifier No.:	171043
Performing Laboratory:	Eurofins Agrosience Services EcoChem GmbH (EAS EcoChem GmbH) / Eurofins Agrosience 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 ( <i>Apis mellifera</i> L.) 22 Day Larval Toxicity Test (Repeated Exposure); Eurofins Agrosience Services EcoChem GmbH (EAS EcoChem GmbH) / Eurofins Agrosience 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

### 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 70110%; RSD ≤ 20%). The results obtained are summarised in the following tables.

**Table A 50: 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
		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 51: 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 52: Characteristics for the analytical method used for validation of GF-3307 residues, based on fenpicoxamid analysis, in larval diet (Diet C)**

	Fenpicoxamid
Specificity	<i>m/z</i> 615/239 (Q) <i>m/z</i> 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 53: Characteristics for the analytical method used for validation of GF-3307 residues, based on fenpicoxamid residues, in water**

	Fenpicoxamid
Specificity	<i>m/z</i> 615/239 (Q) <i>m/z</i> 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 2.1.1.16 Analytical method 16

#### A 2.1.1.16.1 Method validation

Comments of zRMS:	The study was evaluated and accepted in RR for Queen (January 2023) and is not being re-assessed in this application.
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Method Identifier No.:	170077
Performing Laboratory:	Eurofins Agrosience Services EcoChem GmbH (EAS EcoChem GmbH) / Eurofins Agrosience 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, <i>Apis mellifera</i> L., in a 10 Day Chronic Feeding Test under Laboratory Conditions; Eurofins Agrosience Services EcoChem GmbH (EAS EcoChem GmbH) / Eurofins Agrosience 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 70110%; RSD ≤ 20%). The results obtained are summarised in the following tables.

**Table A 54: 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
		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 55: 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	<i>m/z</i> 615/239 (Q) <i>m/z</i> 615/515 (C) blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting $r \geq 0.999$ 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 2.1.1.17 Analytical method 17

#### A 2.1.1.17.1 Method validation

Comments of zRMS:	The study was evaluated and accepted in RR for Queen (January 2023) and is not being re-assessed in this application.
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Method Identifier No.:	170673
Performing Laboratory:	Eurofins Agrosience Services EcoChem GmbH (EAS EcoChem GmbH) / Eurofins Agrosience 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 ( <i>Apis mellifera</i> L.) in a Semi-Field Tunnel Study in <i>Phacelia tanacetifolia</i> in Germany 2017; Eurofins Agrosience Services EcoChem GmbH (EAS EcoChem GmbH) / Eurofins Agrosience 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 56:** 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	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	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	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 57:** 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	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	86	8	5	Individual recoveries: 78, 79, 79, 90, 93
		0.01	87	12	5	Individual recoveries: 93, 96, 85, 90, 70

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
		1	76	8	5	Individual recoveries: 83, 72, 75, 68, 82
Plant	prothioconazole	0.001	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 58: 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	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	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	108	5	5	Individual recoveries: 106, 107, 114, 113, 102
		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 59: 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	<i>m/z</i> 615/515 (Q) <i>m/z</i> 615/239 (C) blank value <30% LOQ	<i>m/z</i> 344/100 (Q) <i>m/z</i> 344/58 (C) blank value <30% LOQ	<i>m/z</i> 312/70 (Q) <i>m/z</i> 312/125 (C) blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting $r \geq 0.995$ 6 data points	linear regression analysis with 1/x weighting $r \geq 0.995$ 6 data points	linear regression analysis with 1/x weighting $r \geq 0.995$ 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 2.1.1.18 Analytical method 18

#### A 2.1.1.18.1 Method validation

Comments of zRMS:	The study was evaluated and accepted in RR for Queen (January 2023) and is not being re-assessed in this application.
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Reference: KCP 10.3.1.6/2

<b>Report author:</b>	Gonsoir, G.
<b>Report year:</b>	2021
<b>Report title:</b>	GF-3307 (Fenpicoxamid and Prothioconazole) Brood Development of the Honey Bee ( <i>Apis mellifera</i> L.) in a Colony Feeding Test in Germany 2020
<b>Report No.:</b>	200660
<b>Testing Facility Report No.:</b>	S20-02058
<b>Method(s) used:</b>	200660
<b>Guidelines followed in study:</b>	SANCO/3029/99 rev. 4 OR SANCO/825/00 rev. 8.1 (for matrix honey only)
<b>Deviation from current test guidelines:</b>	No
<b>Analytical Performing Laboratory:</b>	Eurofins Agrosience Services EcoChem GmbH 75223 Niefern-Öschelbronn, Eutinger Str. 24 Germany
<b>GLP/Officially recognised testing facilities:</b>	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 60: 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 61: 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 62: 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 63: 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 64: 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 65: 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 66: 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 67: 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 68: 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 69: 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 70: 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 71: 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 72: 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 73: 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 74: 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 75: 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 $\geq$ 0.995 $\geq$ 5 data points	linear regression analysis with 1/x weighting r $\geq$ 0.995 $\geq$ 5 data points	linear regression analysis with 1/x weighting r $\geq$ 0.995 $\geq$ 5 data points	linear regression analysis with 1/x weighting r $\geq$ 0.995 $\geq$ 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 76: 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	<i>m/z</i> 615/239Q <i>m/z</i> 615/515C blank value <30% LOQ	<i>m/z</i> 615/239Q <i>m/z</i> 615/515C blank value <30% LOQ	<i>m/z</i> 615/239Q <i>m/z</i> 615/515C blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting $r \geq 0.995$ $\geq 5$ data points	linear regression analysis with 1/x weighting $r \geq 0.995$ $\geq 5$ data points	linear regression analysis with 1/x weighting $r \geq 0.995$ $\geq 5$ 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 77: 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	<i>m/z</i> 344/154Q <i>m/z</i> 344/189C blank value <30% LOQ	<i>m/z</i> 344/189Q <i>m/z</i> 344/154C blank value <30% LOQ	<i>m/z</i> 342/58Q <i>m/z</i> 344/58C blank value <30% LOQ	<i>m/z</i> 344/189Q <i>m/z</i> 344/189C blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting $r \geq 0.995$ $\geq 5$ data points	linear regression analysis with 1/x weighting $r \geq 0.995$ $\geq 5$ data points	linear regression analysis with 1/x weighting $r \geq 0.995$ $\geq 5$ data points	linear regression analysis with 1/x weighting $r \geq 0.995$ $\geq 5$ 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 78: 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	<i>m/z</i> 344/189Q <i>m/z</i> 344/154C blank value <30% LOQ	<i>m/z</i> 344/189Q <i>m/z</i> 344/154C blank value <30% LOQ	<i>m/z</i> 344/189Q <i>m/z</i> 344/154C blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting $r \geq 0.995$ $\geq 5$ data points	linear regression analysis with 1/x weighting $r \geq 0.995$ $\geq 5$ data points	linear regression analysis with 1/x weighting $r \geq 0.995$ $\geq 5$ 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 79: 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	<i>m/z</i> 312/70Q <i>m/z</i> 312/125C blank value <30% LOQ	<i>m/z</i> 312/70Q <i>m/z</i> 312/125C blank value <30% LOQ	<i>m/z</i> 312/70Q <i>m/z</i> 312/125C blank value <30% LOQ	<i>m/z</i> 312/70Q <i>m/z</i> 312/125C blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting $r \geq 0.995$ $\geq 5$ data points	linear regression analysis with 1/x weighting $r \geq 0.995$ $\geq 5$ data points	linear regression analysis with 1/x weighting $r \geq 0.995$ $\geq 5$ data points	linear regression analysis with 1/x weighting $r \geq 0.995$ $\geq 5$ 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 80: 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	<i>m/z</i> 312/70Q <i>m/z</i> 312/125C blank value <30% LOQ	<i>m/z</i> 312/70Q <i>m/z</i> 312/125C blank value <30% LOQ	<i>m/z</i> 312/70Q <i>m/z</i> 312/125C blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting $r \geq 0.995$ $\geq 5$ data points	linear regression analysis with 1/x weighting $r \geq 0.995$ $\geq 5$ data points	linear regression analysis with 1/x weighting $r \geq 0.995$ $\geq 5$ 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 81: 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	<i>m/z</i> 230/199Q <i>m/z</i> 230/125C blank value <30% LOQ	<i>m/z</i> 302/88Q <i>m/z</i> 302/116C blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting $r \geq 0.995$ $\geq 5$ data points	linear regression analysis with 1/x weighting $r \geq 0.995$ $\geq 5$ 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 2.1.1.19 Analytical method 19

### A 2.1.1.19.1 Method validation

Comments of zRMS:	The analytical method 01601 has been validated for the determination of metabolites JAU 6476-alpha-hydroxy-desthio, JAU 6476-3-hydroxy-desthio, JAU 6476-4-hydroxydesthio, JAU 6476-5-hydroxy-desthio and JAU 6476-6-hydroxy-desthio residues in/on honey with LOQ of 0.01 mg/kg. The method is acceptable.
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Title:	Residue analytical method 01601 and short term storage stability of the metabolites JAU 6476-alpha-hydroxy-desthio, JAU 6476-3-hydroxy-desthio, JAU 6476-4-hydroxy-desthio, JAU 6476-5-hydroxy-desthio and JAU 6476-6-hydroxy-desthio in/on honey by HPLC-MS/MS
Author:	Kalathoor, R.
Edition Date:	26.03.2020
Report No:	M-681477-01-1
Reference No:	S19-01125
Guideline(s)	Regulation (EC) No 1107/2009 of the European Parliament and 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 Guidance document on residue analytical methods, SANCO/825/00/rev. 8.1, European Commission, Directorate General Health and Consumer Protection 16/11/2010 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, 11/07/00 OECD 506, 2007; OECD Guideline for the Testing of Chemicals - Stability of Pesticide Residues in Stored Commodities SANTE/11956/2016 rev.9
Guideline Deviation(s)	None
GLP/GEP	yes
Testing Facility:	Eurofins Agroscience Services EcoChem GmbH / Eurofins Agroscience Services Ecotox GmbH, Niefern-Oeschelbronn, Germany
Sponsor:	Bayer
Owner:	BAY

#### Materials and methods

The analytical method 01601 was developed for the determination of metabolites JAU 6476-alpha-hydroxy-desthio, JAU 6476-3-hydroxy-desthio, JAU 6476-4-hydroxydesthio, JAU 6476-5-hydroxy-desthio and JAU 6476-6-hydroxy-desthio residues in/on honey by HPLC-MS/MS detection, in accordance to the guidance document SANCO/825/00, rev. 8.1 of the European Commission.

In all the method, results will be expressed as JAU 6476-desthio.

In addition, a short-term storage stability provided data about the storage stability of all five hydroxy-metabolites in matrix honey at  $\leq 18$  °C (target) in the dark over a storage period up to 6 months in accordance to OECD Guideline 506.

Stabilities of stock standard solutions and diluted standard solutions were as well established.

1 g of homogenized sample of honey was introduced into a 50 mL plastic tube and diluted with 12 mL of acetonitrile/water (1/1, v/v). For recoveries, the fortification solution was added to the control sample material and recovery samples were extracted about 5 min after spiking.

The sample was shake by hand 1 minute and further 10 minutes on a flatbed shaker (300 rpm) until the honey is completely dissolved. The volume was adjusted to 40 mL, with acetonitrile/water (1/1, v/v).

The sample was transferred into a 100 mL round bottom flask. The tube was rinsed with 2 x 2 mL

acetonitrile/water (1/1, v/v), transferred into the flask. Then the extract was evaporated to an aqueous remainder (ca. 20 mL) using a rotary evaporator with water temperature at 40°C

2 mL of N hydrochloric solution was added to the sample (check PH with PH paper ca.1) and the sample was mixed well and hydrolysed for 2 hours under reflux conditions.

The reflux coil was rinsed with 2 x 5 mL methanol with the round bottom flask below to collect any condensed residues.

About 6 micro spatulas (HSN 426-18) of ammonia carbonate were added to the solution and the solution was gently mixed until no CO<sub>2</sub> bubbles appeared anymore. The PH was adjusted to PH 5-7 with HCL 1 N or ammonia carbonate.

The solution was transferred into a 50 mL volumetric flask, the round bottom flask was rinsed with 10 mM ammonium formate. The rinsing solution was combined with the sample extract the adjusted to 50 mL. The volumetric flask was shake manually and the PH re-checked using PH paper, PH~ 5-7.

An aliquot was transferred into an autosampler vial to be injected into the high-performance liquid chromatograph and subjected to reversed phase gradient chromatography coupled with tandem mass spectrometry (MS/MS) with electrospray ionisation.

For each analyte two sets of HPLC-MS/MS parameters (using either different MRM transitions or different HPLC columns) were validated.

The high selectivity of the method resulted from the separation on HPLC in combination with MS/MS detection. A MS/MS-based confirmatory method was established as well:

**Table A 82: Quantitation and Confirmation Mass Transitions**

Analytes	Mode ionisation	Mass transition First MRM Quantitation	Mass transition Second MRM Confirmation
JAU 6476-alpha-hydroxy-desthio	Positive	m/z = 328® 141*	m/z = 328® 70
JAU 6476-3-hydroxy-desthio	Positive	m/z = 328® 70*	m/z = 328® 141
JAU 6476-4-hydroxydesthio	Positive	m/z = 328® 70*	m/z = 328® 141
JAU 6476-5-hydroxy-desthio	Positive	m/z = 328® 70*	m/z = 328® 141
JAU 6476-6-hydroxy-desthio	Positive	m/z = 328® 70*	m/z = 328® 141

\*proposed (and used) for quantification but both of the mass transitions listed can be used for quantification

JAU 6476-alpha-hydroxy-desthio, JAU 6476-3-hydroxy-desthio, JAU 6476-4-hydroxydesthio, JAU 6476-5-hydroxy-desthio and JAU 6476-6-hydroxy-desthio residues were quantified using matrix-matched calibration standards. Mixed standards were adjusted to parent (JAU6476-desthio) equivalent, conversion factor 1.0512.



## Results and discussions

The effect of honey on the HPLC-MS/MS response was assessed by comparing the mean response factor of matrix-matched standards of at least 90% matrix amount with the mean response factor of solvent standards within the same calibration range: Matrix effects in honey were  $\geq \pm 20\%$  and deemed to be significant for JAU 6476- $\alpha$ -hydroxy-desthio, JAU 6476-3-hydroxy-desthio, JAU 6476-4-hydroxydesthio, JAU 6476-5-hydroxy-desthio and JAU 6476-6-hydroxy-desthio. Therefore, matrix-matched standards were used for quantification throughout the analytical phase.

In course of the study the matrix effect of each sample seems to vary depending on reflux conditions during hydrolysis resulting in high RSD for recoveries and storage samples. Therefore, at storage stability timing of 4 months onwards standard addition method was used to compensate possible matrix effects of each sample. For this, reagent blanks which were obtained during sample workup (containing same amount of all reagents as each sample) was used for preparation of calibration standards.

Quantification was performed using matrix matched standards for validation and storage stability testing of 36-41 days (approx. 1 month). For 119-120 days (4 months, back up of 3 m) and 182 days (6 m) standard addition method was used with solvent calibration. For both MRM transitions of each analyte the lower margin of the linearity test was at 30% of the LOQ and the higher margin was at minimum 20% above the highest concentration as demanded in SANCO/825/00 rev. 8.1. The correlation between the injected amount of analyte and the detector response was linear for standards ranging from 0.03 ng/mL to 10 ng/mL (corresponding to 0.003 mg/kg to approx. 0.50 mg/kg). The correlation coefficients of the 1/x weighted linear regressions were always  $\geq 0.995$ .

The two MRM transitions were successfully validated for JAU 6476- $\alpha$ -hydroxy-desthio, JAU 6476-3-hydroxy-desthio, JAU 6476-4-hydroxydesthio, JAU 6476-5-hydroxy-desthio and JAU 6476-6-hydroxy-desthio in the tested matrix honey. Therefore, an additional confirmatory method is not necessary.

The apparent residues in the control samples were below 30% of the LOQ. The recoveries were not corrected for interferences.

The lowest fortification level providing a mean recovery between 70 and 110% with a relative standard deviation of  $< 20\%$  was defined as the Limit of Quantification (LOQ), provided that the blank values were below 30% at this level. Based on the validation results, the LOQ was established at 0.01 mg/kg for all analytes in honey. The limit of detection (LOD) was estimated to be  $\leq 30\%$  of the LOQ. The LOD for all analytes was set at 0.003 mg/kg.

The peak areas of the stock standards stored diluted solutions were within  $\pm 20\%$  of the peak areas of the stock standards freshly prepared diluted solutions indicating that solutions of JAU 6476- $\alpha$ -hydroxydesthio, JAU 6476-3-hydroxy-desthio, JAU 6476-4-hydroxy-desthio, and JAU 6476-5-hydroxy-desthio are stable when stored at 1 °C to 10 °C in the dark for 91 days and JAU 6476-6-hydroxy-desthio was stable for 49 days.

Results obtained are summarised in the tables below.

**Table A 83: Stability of stock solutions**

Analyte	Solvent of secondary standard solution	Concentration of dilution [ng/mL]	Storage period [d]	Mean difference (in %) of stored solution compared to freshly prepared solution
JAU 6476-alpha-hydroxy-desthio	Acetonitrile	10	91	+2.4
JAU 6476-3-hydroxy-desthio	Acetonitrile	10	91	+1.1
JAU 6476-4-hydroxydesthio	Acetonitrile	10	91	-2.8
JAU 6476-5-hydroxy-desthio	Acetonitrile	10	91	-2.2
JAU 6476-6-hydroxy-desthio	Acetonitrile	10	49	+2.1

The secondary standard solutions prepared in acetonitrile were stored at 1 °C to 10 °C for a period in the dark, which was sufficient to cover the length of time they were used in this study (i.e. 62 days).

**Table A 84: Stability of secondary standard solutions**

Analyte	Solvent of secondary standard solution	Concentration of dilution [ng/mL]	Storage period [d]	Mean difference (in %) of stored solution compared to freshly prepared solution
JAU 6476-alpha-hydroxy-desthio	Acetonitrile	10	63	-2.5
JAU 6476-3-hydroxy-desthio	Acetonitrile	10	63	-3.3
JAU 6476-4-hydroxydesthio	Acetonitrile	10	63	-4.5
JAU 6476-5-hydroxy-desthio	Acetonitrile	10	63	+4.9
JAU 6476-6-hydroxy-desthio	Acetonitrile	10	63	+4.0

The stability of residues of all analytes in the final extract was determined after 8 days for honey at the 0.10 mg/kg level. The analytes were stable in the final extracts stored at 1-10°C in the dark for at least 8 days for JAU 6476-alpha-hydroxy-desthio, JAU 6476-3-hydroxy-desthio, JAU 6476-4-hydroxydesthio, JAU 6476-5-hydroxy-desthio and JAU 6476-6-hydroxy-desthio.

Results obtained are summarised in the table below.

**Table A 85: Stability of Prothioconazole (JAU 6476) and its metabolite in Final Extracts of Honey**

Sample material	Fortification Level [mg/kg]	Analyte <sup>1)</sup>	Analytical series <sup>#</sup>	Recovery rates					Mean
Honey	0.10	JAU 6476-alpha-hydroxy-desthio	Initial analysis	84	98	103	95	92	94
			7 days reanalysis	94	98	99	95	98	97
			deviation*						3
	0.10	JAU 6476-3-hydroxy-desthio	Initial analysis	93	97	99	95	94	96
			7 days reanalysis	97	96	97	98	100	98
			deviation*						2
	0.10	JAU 6476-4-hydroxydesthio	Initial analysis	95	103	104	99	99	100
			7 days reanalysis	96	105	96	101	98	99
			deviation*						-1
	0.10	JAU 6476-5-hydroxy-desthio	Initial analysis	93	99	101	97	94	97
			6 days reanalysis	100	101	98	100	96	99
			deviation*						2
	0.10	JAU 6476-6-hydroxy-desthio	Initial analysis	89	92	93	88	88	90
			6 days reanalysis	85	77	74	76	70	76
			deviation*						-15

LOQ = 0.01 mg/kg (fortified and determined as analyte for JAU 6476-alpha-hydroxy-desthio, JAU 6476-3-hydroxy-desthio, JAU 6476-4-hydroxydesthio, JAU 6476-5-hydroxy-desthio and JAU 6476-6-hydroxy-desthio and calculated as JAU 6476-desthio.

Calculation of Deviation:  $(100 \times (\text{mean reanalysis} - \text{mean initial analysis}) / \text{mean initial analysis})$

\* For the calculation of deviations between initial analysis and reanalysis, unrounded mean values were used.

Therefore, minor deviations may occur by calculating the deviations between the initial analysis and the reanalysis with the above-shown rounded mean values.

After a deep-freezer storage ( $\leq -18^{\circ}\text{C}$ ) period of about 6 months, the mean recovery rates were 85% for JAU 6476-alpha-hydroxy-desthio, 84% for JAU 6476-3-hydroxy-desthio, 83% for JAU 6476-4-hydroxy-desthio, 84% for JAU 6476-5-hydroxy-desthio, and 77% for JAU 6476-6-hydroxy-desthio in honey.

Recovery rates were determined at fortification levels of 0.01 mg/kg and 0.10 mg/kg. The recovery experiments were conducted by fortification of untreated control samples with defined amounts of the analytes prior to analysis. The mean recoveries at each fortification level and the overall mean recovery were within the 70 - 110% range and the relative standard deviations for each fortification level were below 20%.

**Table A 86: Recoveries using the HPLC-MS/MS settings for quantification, 1.0 g honey weight**

Analyte	Matrix	Fortification Level [mg/kg]	n	Mean [%]	RSD [%]	Comments
JAU 6476-alpha-hydroxy-desthio (BCS-AA10057)	Honey	0.01	5	101	12.1	(1 <sup>st</sup> MRM) m/z = 328® 141
		0.10	5	94	7.5	
JAU 6476-3-hydroxy-desthio (BCSAA10056)	Honey	0.01	5	105	10.3	(1 <sup>st</sup> MRM) m/z = 328® 70
		0.10	5	96	2.5	
JAU 6476-4-hydroxydesthio (BCSAA10048)	Honey	0.01	5	110	11.8	(1 <sup>st</sup> MRM) m/z = 328® 70
		0.10	5	100	3.6	
JAU 6476-5-hydroxy-desthio (BCSAA10058)	Honey	0.01	5	110	8.9	(1 <sup>st</sup> MRM) m/z = 328® 70
		0.10	5	97	3.5	
JAU 6476-6-hydroxy-desthio (BCSAA10049)	Honey	0.01	5	102	10.9	(1 <sup>st</sup> MRM) m/z = 328® 70
		0.10	5	90	2.6	

LOQ = 0.01 mg/kg, LOD = 0.003 mg/kg, fortified and determined as analyte for JAU 6476-alpha-hydroxy-desthio, JAU 6476-3-hydroxy-desthio, JAU 6476-4-hydroxydesthio, JAU 6476-5-hydroxy-desthio and JAU 6476-6-hydroxy-desthio and calculated as JAU 6476-desthio

n: number of single results per fortification

RSD: Relative Standard Deviation

**Table A 87: Recoveries using the HPLC-MS/MS settings for confirmation, 1.0 g honey weight**

Analyte	Matrix	Fortification Level [mg/kg]	n	Mean [%]	RSD [%]	Comments
JAU 6476-alpha-hydroxy-desthio (BCS-AA10057)	Honey	0.01	5	106	8.3	(2 <sup>nd</sup> MRM) m/z = 328® 70
		0.10	5	95	5.6	
JAU 6476-3-hydroxy-desthio (BCSAA10056)	Honey	0.01	5	105	5.2	(2 <sup>nd</sup> MRM) m/z = 328® 141
		0.10	5	96	3.7	
JAU 6476-4-hydroxydesthio (BCSAA10048)	Honey	0.01	5	108	13.4	(2 <sup>nd</sup> MRM) m/z = 328® 141
		0.10	5	98	3.7	
JAU 6476-5-hydroxy-desthio (BCSAA10058)	Honey	0.01	5	105	9.4	(2 <sup>nd</sup> MRM) m/z = 328® 141
		0.10	5	96	2.9	
JAU 6476-6-hydroxy-desthio (BCSAA10049)	Honey	0.01	5	100	10.4	(2 <sup>nd</sup> MRM) m/z = 328® 141
		0.10	5	89	3.8	

LOQ = 0.01 mg/kg, LOD = 0.003 mg/kg, fortified and determined as analyte for JAU 6476-alpha-hydroxy-desthio, JAU 6476-3-hydroxy-desthio, JAU 6476-4-hydroxydesthio, JAU 6476-5-hydroxy-desthio and JAU 6476-6-hydroxy-desthio and calculated as JAU 6476-desthio

n: number of single results per fortification

RSD: Relative Standard Deviation

**Table A 88:** **Characteristics for the analytical method used for validation of JAU 6476-alpha-hydroxy-desthio, JAU 6476-3-hydroxy-desthio, JAU 6476-4-hydroxydesthio, JAU 6476-5-hydroxy-desthio and JAU 6476-6-hydroxy-desthio in honey**

Method 01601	JAU 6476-alpha-hydroxy-desthio	JAU 6476-3-hydroxy-desthio	JAU 6476-4- hydroxy-desthio	JAU 6476-5-hydroxy-desthio	JAU 6476-6-hydroxy-desthio
Specificity	Mass spectra provided in Appendix 5 of the method report blank value < 30% LOQ)	Mass spectra provided in Appendix 5 of the method report blank value < 30% LOQ)	Mass spectra provided in Appendix 5 of the method report blank value < 30% LOQ)	Mass spectra provided in Appendix 5 of the method report blank value < 30% LOQ)	Mass spectra provided in Appendix 5 of the method report blank value < 30% LOQ)
Calibration (type, number of data points)	Calibration data presented in Appendix 6 calibration line equations presented number of data points $\geq 6$ R >0.995	Calibration data presented in Appendix 6 calibration line equations presented number of data points $\geq 6$ R >0.995	Calibration data presented in Appendix 6 calibration line equations presented number of data points $\geq 6$ R >0.995	Calibration data presented in Appendix 6 calibration line equations presented number of data points $\geq 6$ R >0.995	Calibration data presented in Appendix 6 calibration line equations presented number of data points $\geq 6$ R >0.995
Calibration range	Excellent linear correlation between the injected amount and detector response of the HPLC-MS/MS system was observed within the range from 0.03 ng/mL to 10 ng/mL (corresponding to 0.003 mg/kg to approx. 0.50 mg/kg expressed as JAU 6476-desthio equivalent)	Excellent linear correlation between the injected amount and detector response of the HPLC-MS/MS system was observed within the range from 0.03 ng/mL to 10 ng/mL (corresponding to 0.003 mg/kg to approx. 0.50 mg/kg expressed as JAU 6476-desthio equivalent )	Excellent linear correlation between the injected amount and detector response of the HPLC-MS/MS system was observed within the range from 0.03 ng/mL to 10 ng/mL (corresponding to 0.003 mg/kg to approx. 0.50 mg/kg expressed as JAU 6476-desthio equivalent )	Excellent linear correlation between the injected amount and detector response of the HPLC-MS/MS system was observed within the range from 0.03 ng/mL to 10 ng/mL (corresponding to 0.003 mg/kg to approx. 0.50 mg/kg expressed as JAU 6476-desthio equivalent )	Excellent linear correlation between the injected amount and detector response of the HPLC-MS/MS system was observed within the range from 0.03 ng/mL to 10 ng/mL (corresponding to 0.003 mg/kg to approx. 0.50 mg/kg expressed as JAU 6476-desthio equivalent )
Assessment of matrix effects is presented	Yes. Matrix effects in honey were $\geq \pm 20\%$ and deemed to be significant Therefore, matrix-matched standards were used for quantification throughout the analytical phase	Yes. Matrix effects in honey were $\geq \pm 20\%$ and deemed to be significant Therefore, matrix-matched standards were used for quantification throughout the analytical phase	Yes. Matrix effects in honey were $\geq \pm 20\%$ and deemed to be significant Therefore, matrix-matched standards were used for quantification throughout the analytical phase	Yes. Matrix effects in honey were $\geq \pm 20\%$ and deemed to be significant Therefore, matrix-matched standards were used for quantification throughout the analytical phase	Yes. Matrix effects in honey were $\geq \pm 20\%$ and deemed to be significant Therefore, matrix-matched standards were used for quantification throughout the analytical phase
Limit of determination/quantification	LOQ: 0.01 mg/kg LOD: 0.003 mg/kg	LOQ: 0.01 mg/kg LOD: 0.003 mg/kg	LOQ: 0.01 mg/kg LOD: 0.003 mg/kg	LOQ: 0.01 mg/kg LOD: 0.003 mg/kg.	LOQ: 0.01 mg/kg LOD: 0.003 mg/kg.

## Conclusion

The analytical method 01601 was developed for the determination of JAU 6476- $\alpha$ -hydroxy-desthio, JAU 6476-3-hydroxy-desthio, JAU 6476-4-hydroxydesthio, JAU 6476-5-hydroxy-desthio and JAU 6476-6-hydroxy-desthio which is the official residue definition for plants and is also proposed to be the residue definition for the honey. The limit of quantification is 0.01 mg/kg.

Two MRM transitions were monitored for each analyte in honey.

The HPLC-MS/MS method is highly specific, and an additional confirmatory method is not necessary.

All analytes can be considered stable in honey under deep-freezer storage conditions ( $\leq -18^{\circ}\text{C}$ ) for at least 6 months.

The analytical method complies with all guidance criteria according to SANTE/2020/12830, Rev.1 and is therefore suitable as an enforcement method for the determination of JAU 6476- $\alpha$ -hydroxy-desthio, JAU 6476-3-hydroxy-desthio, JAU 6476-4-hydroxydesthio, JAU 6476-5-hydroxy-desthio and JAU 6476-6-hydroxy-desthio in honey by HPLC-MS/MS.

## A 2.1.1.20 Analytical method 20

### A 2.1.1.20.1 Method validation

Comments of zRMS:	The analytical method 01602 has been validated for determination of 1,2,4-triazole, triazole alanine, triazole acetic acid and triazole lactic acid residues in/on honey with LOQ of 0.01 mg/kg. The method is acceptable.
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Title:	Amendment no. 02: Residue analytical method 01602 and short term storage stability of 1,2,4-triazole, triazole alanine, triazole acetic acid and triazole lactic acid in/on honey by HPLC-DMS-MS/MS
Author:	Kalathoor, R.
Edition Date:	25.01.2021
Report No:	<a href="#">M-680825-03-1</a>
Reference No:	S19-01126
Guideline(s)	Regulation (EC) No 1107/2009 of the European Parliament and 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  Guidance document on residue analytical methods, SANCO/825/00/rev. 8.1, European Commission, Directorate General Health and Consumer Protection 16/11/2010  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, 11/07/00  OECD 506, 2007; OECD Guideline for the Testing of Chemicals – Stability of Pesticide Residues in Stored Commodities  Technical guidelines for determining the magnitude of pesticide residues in honey and setting Maximum Residue Levels in honey, SANTE/11956/2016 rev.9
Guideline Deviation(s)	None
GLP/GEP	yes
Testing Facility:	Eurofins Agroscience Services EcoChem GmbH / Eurofins Agroscience Services Ecotox GmbH, Niefern-Oeschelbronn, Germany
Sponsor:	Bayer
Owner:	BAY

## Materials and methods

The analytical method 01602 was developed for the determination of 1,2,4-triazole, triazole alanine, triazole acetic acid and triazole lactic acid residues in/on honey by HPLC-DMS-MS/MS detection, in accordance

with relevant guidelines.

In all the method, results will be expressed as themselves for 1,2,4-triazole, triazole alanine, triazole acetic acid and triazole lactic acid.

In addition, a short-term storage stability will provide data about the storage stability of 1,2,4-triazole, triazole alanine, triazole acetic acid and triazole lactic acid in the matrix honey at  $\leq 18$  °C (target) in the dark over a storage period up to 5 months in accordance to OECD Guideline 506.

Stabilities of stock standard solutions and diluted standard solutions were as well established.

1 g of homogenized sample of honey was diluted in a 15 mL plastic tube with 3.5 mL of HPLC water. The sample was mixed 15 min and on a flatbed shaker at highest rpm until the honey is completely dissolved. The volume was adjusted to 4 mL, if necessary. Then the sample was put 1 hour in a refrigerator. After that, the sample was filtered using syringe filter (0.45  $\mu$ m, PTFE) in a 4 mL glass vial. 0.5 mL aliquot of the sample was diluted to 1mL with 0.5 HPLC water.

For each analyte two sets of HPLC-DMS-MS/MS parameters (using either different MRM transitions or different HPLC columns) were validated.

The high selectivity of the method resulted from the separation on HPLC different columns (a Thermo Hypercarb or a Phenomenex Kinetex Biphenyl chromatographic column) in combination with DMS-MS/MS detection. A DMS-MS/MS-based confirmatory method was established as well:

**Table A 89: Quantitation and Confirmation Mass Transitions**

Analytes	Mode ionisation	Mass transition First MRM Quantitation	Mass transition Second MRM Confirmation
1,2,4-triazole	Positive	m/z = 70® 43* Column Thermo Hypercarb	m/z = 70® 43 Column Phenomenex Kinetex Biphenyl
triazole alanine	Positive	m/z = 157® 70*	m/z = 157® 88
triazole acetic acid	Positive	m/z = 128® 70*	m/z = 128® 43
triazole lactic acid	Positive	m/z = 158® 70*	m/z = 158® 43

\*proposed (and used) for quantification but both of the mass transitions listed can be used for quantification

1,2,4-triazole, triazole alanine, triazole acetic acid and triazole lactic acid residues were quantified using matrix-matched calibration standards.



## Results and discussions

The effect of honey on the HPLC-DMS-MS/MS response was assessed by comparing the mean response factor of matrix-matched standards of at least 90% matrix amount with the mean response factor of solvent standards within the same calibration range: Matrix effects in honey were  $\geq \pm 20\%$  and deemed to be significant for 1,2,4-triazole, triazole alanine, triazole acetic acid and triazole lactic acid. Therefore, matrix-matched standards were used for quantification throughout the analytical phase.

For all analytes and using the HPLC-DMS-MS/MS settings for both quantification and confirmation the lower margin of the linearity test was below 30% of the LOQ and the higher margin was at minimum 20% above the highest concentration. The correlation between the injected amount of analyte and the detector response was linear for standards ranging from 0.38 ng/mL to 20 ng/mL (corresponding to 0.003 mg/kg to approx. 0.16 mg/kg). The correlation coefficients of the 1/x weighted linear regressions were always  $\geq 0.995$ .

The two MRM transitions were successfully validated for 1,2,4-triazole, triazole alanine, triazole acetic acid and triazole lactic acid in the tested matrix honey. Therefore, an additional confirmatory method is not necessary.

The apparent residues in the control samples were below 30% of the LOQ. The recoveries were not corrected for interferences.

The lowest fortification level providing a mean recovery between 70 and 110% with a relative standard deviation of  $< 20\%$  was defined as the Limit of Quantification (LOQ), provided that the blank values were below 30% at this level. Based on the validation results, the LOQ was established at 0.01 mg/kg for all analytes in honey. The limit of detection (LOD) was estimated to be  $\leq 30\%$  of the LOQ. The LOD for all analytes was set at 0.003 mg/kg.

The stability of 1,2,4-triazole, triazole alanine, triazole acetic acid and triazole lactic acid stock solutions in water was tested over a period of 63 days at 1–10 °C under dark conditions. The mean peak areas of the stored diluted solutions were within  $\pm 20\%$  of the mean peak areas of the freshly prepared diluted solutions indicating that solutions of all analytes are stable when stored at 1 °C to 10 °C in the dark for 63 days.

Results obtained are summarised in the tables below.

**Table A 90: Stability of stock solutions**

Analyte	Solvent of secondary standard solution	Concentration of dilution [ng/mL]	Storage period [d]	Mean difference (in %) of stored solution compared to freshly prepared solution
1,2,4-triazole m/z = 70® 43*	Water	10	63	-6
triazole alanine m/z = 157® 70	Water	10	63	-7
triazole acetic acid m/z = 128® 70	Water	10	63	+3
triazole lactic acid m/z = 158® 70	Water	10	63	+1

\*: column: Thermo Hypercarb

The stability of the analytes in the final extracts was checked for the sample material honey. The analytes were stable in the final extracts stored at 1-10°C in the dark over a time period of at least 46 days for triazole acetic acid, triazole lactic acid and 1,2,4-triazole (confirmation transition tested), at least 7 days for 1,2,4-triazole (quantification transition tested) and triazole alanine.

Investigations showed that the final extracts of all sample materials were stable for at least 7 days under refrigerated and dark conditions.

Results obtained are summarised in the table below.

**Table A 91: Stability of Prothioconazole (JAU 6476) and its metabolite in Final Extracts of Honey**

Sample material	Fortification Level [mg/kg]	Analyte <sup>1)</sup>	Analytical series <sup>#</sup>	Recovery rates					Mean
Honey	0.10	1,2,4-Triazole Quantification MRM	Initial analysis	84	88	88	89	94	89
			7 days reanalysis	74	79	76	81	78	78
			deviation*						-12.4
	0.10	1,2,4-Triazole Confirmation MRM	Initial analysis	94		93		90	92
			46 days reanalysis	91		100		98	96
			deviation*						4.3
	0.10	Triazole Alanine Quantification MRM	Initial analysis	90	92	93	94	92	92
			7 days reanalysis	92	88	102	90	98	94
			deviation*						+2.0
	0.10	Triazole Acetic Acid Quantification MRM MRM	Initial analysis	96	96	95	97	94	96
			6 days reanalysis	94	94	94	96	95	95
			deviation*						-1.0
	0.10	Triazole Acetic Acid Quantification MRM	Initial analysis	96		97		94	96
			46 days reanalysis	95		95		93	94
			deviation*						-1.4
	0.10	Triazole Lactic Acid Quantification MRM	Initial analysis	93	94	92	96	91	93
			6 days reanalysis	95	97	98	98	95	97
			deviation*						+3.6
	0.10	Triazole Lactic Acid Quantification MRM	Initial analysis	94		96		91	94
			46 days reanalysis	91		94		95	93
			deviation*						-0.4

LOQ = 0.01 mg/kg

Fortified, determined and calculated as analyte 1,2,4-triazole, triazole alanine, triazole acetic acid and triazole lactic acid.

Calculation of Deviation<sup>2)</sup>:  $(100 \times (\text{mean reanalysis} - \text{mean initial analysis}) / \text{mean initial analysis})$

\*For the calculation of deviations between initial analysis and reanalysis, unrounded mean values were used.

Therefore, minor deviations may occur by calculating the deviations between the initial analysis and the reanalysis with the above-shown rounded mean values.

<sup>#</sup> For 1,2,4-Triazole 2nd MRM is also given since extract stability had longer coverage.

<sup>1)</sup> Longer coverage of extract stability was additionally proven in the study and needed to be adjusted accordingly. For TLA the first re-analyses was done 6 days after initial analyses and some initial mean recovery values of 1,2,4-Triazole, TA, TAA and TLA contained typographical errors in Table 19 which were also corrected.

<sup>2)</sup> Formula for “Calculation of Deviation” was corrected

Recovery rates were determined at fortification levels of 0.01 mg/kg and 0.10 mg/kg. The recovery experiments were conducted by fortification of untreated control samples with defined amounts of the analytes prior to analysis. The mean recoveries at each fortification level and the overall mean recovery were within the 70 - 110% range and the relative standard deviations for each fortification level were below 20%.

**Table A 92: Recoveries using the HPLC-DMS-MS/MS settings for quantification, 1.0 g honey weight**

Analyte	Matrix	Fortification Level [mg/kg]	n	Mean [%]	RSD [%]	Comments
1,2,4-Triazole	Honey	0.01	5	95	6.8	(1 <sup>st</sup> MRM) m/z 70 → 43*
		0.10	5	89	4.0	
Triazole Alanine	Honey	0.01	5	101	7.0	(1 <sup>st</sup> MRM) m/z 157 → 70
		0.10	5	92	1.6	
Triazole Acetic Acid	Honey	0.01	5	97	0.6	(1 <sup>st</sup> MRM) m/z 128 → 70
		0.10	5	96	1.2	
Triazole Lactic Acid	Honey	0.01	5	97	1.3	(1 <sup>st</sup> MRM) m/z 158 → 70
		0.10	5	93	2.1	

LOQ = 0.01 mg/kg, LOD = 0.003 mg/kg, fortified, determined and calculated as analyte for 1,2,4-triazole, triazole alanine, triazole acetic acid and triazole lactic acid.

\*: column= Thermo Hypercarb

n: number of single results per fortification

RSD: Relative Standard Deviation

**Table A 93: Recoveries using the HPLC-DMS-MS/MS settings for confirmation, 1.0 g honey weight**

Analyte	Matrix	Fortification Level [mg/kg]	n	Mean [%]	RSD [%]	Comments
1,2,4-Triazole	Honey	0.01	5	98	14.3	(2 <sup>nd</sup> MRM) m/z 70 → 43*
		0.10	5	95	3.6	
Triazole Alanine	Honey	0.01	5	92	15.2	(2 <sup>nd</sup> MRM) m/z 157 → 88
		0.10	5	88	1.7	
Triazole Acetic Acid	Honey	0.01	5	105	7.8	(2 <sup>nd</sup> MRM) m/z 128 → 43
		0.10	5	96	1.9	
Triazole Lactic Acid	Honey	0.01	5	94	4.0	(2 <sup>nd</sup> MRM) m/z 158 → 43
		0.10	5	95	1.9	

LOQ = 0.01 mg/kg, LOD = 0.003 mg/kg, fortified, determined and calculated as analyte for 1,2,4-triazole, triazole alanine, triazole acetic acid and triazole lactic acid.

\*: column= Phenomenex Kinetex Biphenyl

n: number of single results per fortification

RSD: Relative Standard Deviation

**Table A 94: Characteristics for the analytical method used for validation of 1,2,4-triazole, triazole alanine, triazole acetic acid and triazole lactic acid in honey**

Method 01602	1,2,4-Triazole	Triazole Alanine	Triazole Acetic Acid	Triazole Lactic Acid
Specificity	Mass spectra provided in Appendix 5 of the method report blank value < 30% LOQ)	Mass spectra provided in Appendix 5 of the method report blank value < 30% LOQ)	Mass spectra provided in Appendix 5 of the method report blank value < 30% LOQ)	Mass spectra provided in Appendix 5 of the method report blank value < 30% LOQ)
Calibration (type, number of data points)	Calibration data presented in Appendix 6 calibration line equations presented number of data points ≥ 6 R >0.995	Calibration data presented in Appendix 6 calibration line equations presented number of data points ≥ 6 R >0.995	Calibration data presented in Appendix 6 calibration line equations presented number of data points ≥ 6 R >0.995	Calibration data presented in Appendix 6 calibration line equations presented number of data points ≥ 6 R >0.995
Calibration range	Excellent linear correlation between the injected amount and detector response of the HPLC-MS/MS system was observed within the range from 0.38 ng/mL to 20 ng/mL corresponding to 0.003 to 0.16 mg/kg	Excellent linear correlation between the injected amount and detector response of the HPLC-MS/MS system was observed within the range from 0.38 ng/mL to 20 ng/mL corresponding to 0.003 to 0.16 mg/kg	Excellent linear correlation between the injected amount and detector response of the HPLC-MS/MS system was observed within the range from 0.38 ng/mL to 20 ng/mL corresponding to 0.003 to 0.16 mg/kg	Excellent linear correlation between the injected amount and detector response of the HPLC-MS/MS system was observed within the range from 0.38 ng/mL to 20 ng/mL corresponding to 0.003 to 0.16 mg/kg
Assessment of matrix effects is presented	Yes. Matrix effects in honey were <± 20 % and deemed to be significant Therefore, matrix-matched standards were used for quantification throughout the analytical phase	Yes. Matrix effects in honey were <± 20 % and deemed to be significant Therefore, matrix-matched standards were used for quantification throughout the analytical phase	Yes. Matrix effects in honey were <± 20 % and deemed to be significant Therefore, matrix-matched standards were used for quantification throughout the analytical phase	Yes. Matrix effects in honey were <± 20 % and deemed to be significant Therefore, matrix-matched standards were used for quantification throughout the analytical phase
Limit of determination/quantification	LOQ: 0.01 mg/kg LOD: 0.003 mg/kg	LOQ: 0.01 mg/kg LOD: 0.003 mg/kg	LOQ: 0.01 mg/kg LOD: 0.003 mg/kg	LOQ: 0.01 mg/kg LOD: 0.003 mg/kg.

## Conclusion

The analytical method 01602 was developed for the determination of 1,2,4-triazole, triazole alanine, triazole acetic acid and triazole lactic acid which is the official residue definition for plants and is also proposed to be the residue definition for the honey. The limit of quantification is 0.01 mg/kg.

Two MRM transitions were monitored for each analyte in honey.

The HPLC-DMS-MS/MS method is highly specific, and an additional confirmatory method is not necessary.

The analytical method complies with all guidance criteria according to SANTE/2020/12830, Rev.1 and is therefore suitable as an enforcement method for the determination of 1,2,4-triazole, triazole alanine, triazole acetic acid and triazole lactic acid in honey by HPLC-MS/MS.

## A 2.1.1.21 Analytical method 21

### A 2.1.1.21.1 Method validation

Comments of zRMS:	The analytical method of Semrau, J; Kühnel, S; 2016 for the determination of fenpicoxamid and metabolites X642188 and X12255349 in samples of freshwater by LC-MS/MS has been successfully validated in accordance with the EU guidance document SANCO/3029/99 rev. 4.
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	LOQ = 0.000050 mg/L for XDE-777 LOQ = 0.0000040 mg/L for X642188 LOQ = 0.0000090 mg/L for X12255349 The mean recovery of each fortification level and the overall mean recovery value was 70 – 120% with an RSD < 30%. For X642188 the mean recovery value was 122%. The validation parameters are acceptable. The method is considered fit for purpose.
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Method Identifier No.:	160126 Amendment 2
Performing Laboratory:	ABC Laboratories, Inc. (now EAG Laboratories) Columbia, Missouri, USA
Reference:	KCP 10.2.3/2
Report:	Semrau, J; Kühnel, S; 2016; Kiran Lamichhane, 2015, GF-3308: Population Effects Study in an Indoor Aquatic Microcosm with <i>Daphnia magna</i> ; Eurofins Agrosience Services Chem SAS, 75B Avenue de Pascalet30310 Vergeze France; Lab Study No. S18-01567; DAS Study No. DAS Study No. 131295 ; 07 December 2016; Unpublished
Guideline(s):	Yes, SANCO/3029/99 rev.4
Guideline Deviations:	Yes. The number of replicate recoveries (N = 1 or 2) assessed at some fortification levels was less than described in the guideline (N = 5)
GLP:	Yes
Acceptability:	Yes
Method Alterations:	160126 Amendment 1 was based on 160128 Amendment 2. The original method used fortification levels of 0.0500 and 120 µg XDE-777/L, 0.0040 and 30 µg X642188/L, and 0.0090 and 30 µg X12255349/L while this study used fortification levels of 0.000050, 0.00020, and 0.030 mg XDE777/L, 0.0000040, 0.000016, and 0.030 mg X642188/L, and 0.0000090, 0.000046, and 0.030 mg X12255349/L.

## MATERIALS AND METHODS

### Method Principle

Residues of GF-3308, based on analysis of XDE-777, and X642188 and X12255349 (XDE777 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 XDE-777, X642188, and X12255349 by liquid chromatography coupled with positive-ion electrospray tandem mass spectrometry (LC-MS/MS).

## RESULTS AND DISCUSSION

Mean recovery values of all XDE-777 and the 0.030 mg X642188/L fortification concentrations were within the acceptance range (mean recovery 70-110%; RSD ≤ 20%). Mean recovery values at 0.000016 mg X642188/L and all X12255349 fortification concentrations were higher than 110% but were still considered acceptable since the precision of the assay (%RSD) was less than 20%. Mean recovery values at 0.0000040 mg X642188/L fortification concentration were higher than 110% and the (%RSD) was greater than 20%. The results obtained are summarised in the following tables.

**Table A 95:** Recovery results from method validation of XDE-777 (*m/z* 615.0/239.2) using the analytical method

Matrix	Analyte	Fortification level (mg XDE-777/L)	Mean Recovery (%)	RSD (%)	n	Comments
Freshwater	XDE-777	0.000050	100	7	5	5 QC samples from definitive test analyses, ranging from 93 to 112%
Freshwater	XDE-777	0.00020	102	10	2	2 QC samples from

Matrix	Analyte	Fortification level (mg XDE-777/L)	Mean Recovery (%)	RSD (%)	n	Comments
						definitive test analyses, ranging from 95 to 109%
Freshwater	XDE-777	0.030	99	9	5	5 QC samples from definitive test analyses, ranging from 84 to 105%

**Table A 96:** Recovery results from method validation of X642188 (m/z 515.1/239.0) using the analytical method

Matrix	Analyte	Fortification level (mg X642188/L)	Mean Recovery (%)	RSD (%)	n	Comments
Freshwater	X642188	0.0000040	122	26	5	5 QC samples from definitive test analyses, ranging from 88 to 175%
Freshwater	X642188	0.000016	113	16	2	2 QC samples from definitive test analyses, ranging from 100 to 125%
Freshwater	X642188	0.030	107	7	5	5 QC samples from definitive test analyses, ranging from 100 to 117%

**Table A 97:** Recovery results from method validation of X12255349 (m/z 515.2/239.0) using the analytical method

Matrix	Analyte	Fortification level (mg X12255349/L)	Mean Recovery (%)	RSD (%)	n	Comments
Freshwater	X12255349	0.0000090	111	11	6	6 QC samples from definitive test analyses, ranging from 98 to 133%
Freshwater	X12255349	0.000046	111	NA	1	1 QC sample from definitive test analyses, at 111%
Freshwater	X12255349	0.030	111	12	6	6 QC samples from definitive test analyses, ranging from 90 to 127%

**Table A 98:** Characteristics for the analytical method used for validation of XDE-777 and X642188 residues in freshwater

	XDE-777	X642188	X12255349
Specificity	m/z 615.0/239.2 blank value <30% MQL	m/z 515.1/239.0 blank value <30% MQL	m/z 515.2/239.0 blank value <30% MQL
Calibration (type, number of data points)	linear regression analysis with 1/x weighting Representative y = 1,540,860x – 1,281.859 r≥0.995 6 data points	linear regression analysis with 1/x weighting Representative y = 1,161,291x + 353.8701 r≥0.995 6 data points	linear regression analysis with 1/x weighting Representative y = 636,955.4x + 235.2649 r≥0.99 6 data points
Calibration range	Concentration range of 0.0100-0.500 ng/mL Sample equivalent range of 0.0000200-0.00100 mg XDE-777/L	Concentration range of 0.00079-0.052 ng/mL Sample equivalent range of 0.0000016-0.000104 mg X642188/L	Concentration range of 0.0021-0.10 ng/mL Sample equivalent range of 0.0000042-0.00020 mg X12255349/L
Limit of determination/quantification	LOQ = 0.000050 mg/L	LOQ = 0.0000040 mg/L	LOQ = 0.0000090 mg/L

## CONCLUSION

The method was considered acceptable for the determination of GF-3308, based on analysis of XDE-777, and X642188 and X12255349 (XDE-777 metabolites) in natural surface water (freshwater) based on acceptable precision and accuracy demonstrated within this study.

### 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 and is not being re-assessed in this application.
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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 <i>Daphnia magna</i> ; 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  $\leq 20\%$ . 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 99: Recovery results from method validation of fenpicoxamid ( $m/z$  615.0/239.2) using the analytical method**

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

**Table A 100: Characteristics for the analytical method used for validation of fenpicoxamid residues in freshwater**

	Fenpicoxamid
Specificity	<i>m/z</i> 615.0/239.2 blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting $r \geq 0.995$ 6 data points
Calibration range	Concentration range of 0.0100-0.500 ng/mL
Limit of determination/quantification	LOQ = 0.0500 mg/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 2.1.1.23 Analytical method 23

#### A 2.1.1.23.1 Method validation

Comments of zRMS:	The study was evaluated and accepted in RR for Queen (January 2023) and is not being re-assessed in this application (please see Method 21).
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Method Identifier No.:	131295 Protocol
Performing Laboratory:	ABC Laboratories, Inc. (now EAG, Inc.) Columbia, Missouri, USA
Reference:	KCP 10.2.3/4
Report:	Semrau, J; Kühnel, S; 2015; Kiran Lamichhane, 2015; X642188 (a metabolite of XDE-777): Population Effects Study in an Indoor Aquatic Microcosm with <i>Daphnia magna</i> ; Eurofins Agrosience Services Chem SAS, 75B Avenue de Pascalet30310 Vergeze France; Lab Study No. S18-01567; DAS Study No. DAS Study No. 131295 ; 12 June 2015; 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 X642188 (a metabolite of XDE-777) were determined from samples of natural surface freshwater by diluting with 0.2% formic acid in acetonitrile (ACN), centrifuging at 3,600 rpm for 10 minutes, and further diluting, if needed, 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 101: Recovery results from method validation of X642188 (*m/z* 515.0/239.0) using the analytical method**

Matrix	Analyte	Fortification level (µg/L)	Mean Recovery (%)	RSD (%)	n	Comments
Freshwater	X642188	2.3	96	6	10	
Freshwater	X642188	6.0	101	9	8	
Freshwater	X642188	45	97	4	10	

**Table A 102: Characteristics for the analytical method used for validation of X642188 residues in freshwater**

	X642188
Specificity	<i>m/z</i> 515.0/239.0 <i>m/z</i> 515.0/124.0, <i>m/z</i> 515.0/211.0, and <i>m/z</i> 515.0/170.0 blank value <30% MQL
Calibration (type, number of data points)	linear regression analysis with 1/x weighting $r \geq 0.995$ 7 data points
Calibration range	Concentration range of 0.050-5.0 µg/L Sample equivalent range of 0.10-10 µg/L
Limit of determination/quantification	LOQ = 2.3 µg/L MQL (minimum quantifiable limit) = 0.10 µg/L sample equivalents

## CONCLUSION

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

### A 2.1.1.24 Analytical method 24

#### A 2.1.1.24.1 Method validation

Comments of zRMS:	The study was evaluated and accepted in RR for Queen (January 2023) and is not being re-assessed in this application.
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Method Identifier No.:	181382
Performing Laboratory:	Fraunhofer Institute for Molecular Biology and Applied Ecology (IME), Auf dem Aberg 1, 57392 Schmallenberg, Germany
Reference:	KCP 10.2.3/7
Report:	Brüggemann, M., Böhmer, W., Kosak, L.; 2020; GF-3307: Population Effects Study in an Indoor Aquatic Microcosm with <i>Daphnia magna</i> ; 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 70110%; RSD ≤ 20%). The results obtained are summarised in the following tables.

**Table A 103: 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 104: 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 dilution water	0.0015	94.5	5.90	5	
	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 105: 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 106: 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
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## 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 2.1.1.25 Analytical method 25

#### A 2.1.1.25.1 Method validation

Comments of zRMS:	The study was evaluated and accepted in RR for Queen (January 2023) and is not being re-assessed in this application.
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<b>Report author:</b>	Cornement, M.; Morgenthal, K.
<b>Report year:</b>	2022
<b>Report title:</b>	XDE-777 TGAI - Acute Oral and Contact Toxicity to Bumble Bees ( <i>Bombus terrestris</i> ) under Laboratory Conditions
<b>Report No.:</b>	201076
<b>Testing Facility Report No.:</b>	20200224
<b>Method(s) used:</b>	201076
<b>Guidelines followed in study:</b>	SANCO/3029/99 rev. 4
<b>Deviation from current test guidelines:</b>	No
<b>Analytical Performing Laboratory:</b>	Innovative Environmental Services (IES) Ltd Witterswil Switzerland
<b>GLP/Officially recognised testing facilities:</b>	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 $r \geq 0.99$ 8 data points	linear regression analysis without weighting $r \geq 0.99$ 8 data points
Calibration range	Concentration range of 0.0007100.0265 mg a.i./L	Concentration range of 0.0007100.0265 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 2.1.1.26 Analytical method 26

### A 2.1.1.26.1 Method validation

Comments of zRMS:	The study was evaluated and accepted in RR for Queen (January 2023) and is not being re-assessed in this application.
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<b>Report author:</b>	Cornement, M. and Dr. Morgenthal, K.
<b>Report year:</b>	2022
<b>Report title:</b>	GF-3307 - Acute Oral and Contact Toxicity to Bumble Bees ( <i>Bombus terrestris</i> ) under Laboratory Conditions
<b>Report No.:</b>	201075
<b>Testing Facility Report No.:</b>	20200222
<b>Method(s) used:</b>	HPLC/MS/MS
<b>Guidelines followed in study:</b>	SANTE/2020/12830/Rev.1
<b>Deviation from current test guidelines:</b>	No
<b>Analytical Performing Laboratory:</b>	Innovative Environmental Services (IES) Ltd Benkenstrasse, Witterswil, Switzerland
<b>GLP/Officially recognised testing facilities:</b>	Yes

## MATERIAL AND METHODS

### Method Principle

The concentrations of fenpicoxamid and prothioconazole as the active ingredients of the test item in application 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 fempicoxamid (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 fempicoxamid (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 fempicoxamid in application solutions**

Analyte	Fempicoxamid	Fempicoxamid
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 $r \geq 0.99$ 12 data points	linear regression analysis, no weighting $r \geq 0.99$ 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 $r \geq 0.99$ 14 data points	linear regression analysis, no weighting $r \geq 0.99$ 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 and is not being re-assessed in this application.
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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 XDE777 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 107: 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 108: 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 109: 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:	The study was evaluated and accepted in RR for Queen (January 2023) and is not being re-assessed in this application.
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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-desmethio and iprovalicarb 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-desmethio 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-desmethio 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  $\rightarrow$  70 was selected for all matrices tested for quantitation. For confirmation the mass transition  $m/z$  312  $\rightarrow$  125 was monitored for all matrices.

**Table A 110: Recovery results from method 01300/M018 for the determination of prothioconazole-desthio in various plant matrices**

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 (%)
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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 study was evaluated and accepted in RR for Queen (January 2023) and is not being re-assessed in this application.
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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 111: 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 (R<sup>2</sup>) 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.

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**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 %). 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 study was evaluated and accepted in RR for Queen (January 2023) and is not being re-assessed in this application.
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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 ( <i>Apis mellifera</i> 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

Reference: KCP 10.3.1.6/1

#### CITATION

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#### COMPLIANCE

Guideline(s):	SANCO/3029/99 rev. 4, SANCO/825/00 rev. 8.1
Guideline Deviations:	None

Test Item(s)

Method Scope

Method Principle

Linearity

Selectivity

Confirmation

Limits of Detection and Quantitation

#### RESULTS AND DISCUSSION

##### Summary of Recovery

Results obtained were within guideline requirements (mean recovery 70–110%; RSD ≤ 20%). For each analyte, the two ion mass transitions could be used interchangeably for quantification and confirmation. The results obtained are summarised in the following tables.

**Table A 112: Summary of quantitative recovery of Fenpicoxamid (m/z 615/239Q)**

Matrix group	Matrix	LOQ	Fortification level	Recovery (%)		SD	RSD	n
			mg/kg	mean	range	(%)	(%)	
Honey	Honey	0.001 mg/kg	0.001	101	93-106	N/A	5	5
Honey	Honey	0.001 mg/kg	0.01	109	105-113	N/A	5	5
Honey	Honey	0.001 mg/kg	7	105	120-108	N/A	3	5

**Table A 113: Summary of confirmatory recovery of Fenpicoxamid (m/z 615/515C)**

Matrix group	Matrix	LOQ	Fortification level	Recovery (%)		SD	RSD	n
			mg/kg	mean	range	(%)	(%)	
Honey	Honey	0.001 mg/kg	0.001	98	89-103	N/A	5	5
Honey	Honey	0.001 mg/kg	0.01	106	97-110	N/A	5	5
Honey	Honey	0.001 mg/kg	7	106	103-108	N/A	2	5

#### Repeatability

#### Working Solution Stability

#### Sample Extract Stability

#### Matrix Effects

#### Extraction Efficiency

### CONCLUSION

Method is acceptable based on current guidelines: SANCO/3029/99 rev.4 and SANCO/825/00 rev.8.1.

### 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 114: 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 115: 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 116: 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 117: 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 118: 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 119: 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 120: 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 121: 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 122: 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 123: 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 124: 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 125: 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 126: 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 127: 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 128: 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 129: 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 $\geq$ 0.995 $\geq$ 5 data points	linear regression analysis with 1/x weighting r $\geq$ 0.995 $\geq$ 5 data points	linear regression analysis with 1/x weighting r $\geq$ 0.995 $\geq$ 5 data points	linear regression analysis with 1/x weighting r $\geq$ 0.995 $\geq$ 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 130: 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 $\geq$ 0.995 $\geq$ 5 data points	linear regression analysis with 1/x weighting r $\geq$ 0.995 $\geq$ 5 data points	linear regression analysis with 1/x weighting r $\geq$ 0.995 $\geq$ 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 131: 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	<i>m/z</i> 344/154Q <i>m/z</i> 344/189C blank value <30% LOQ	<i>m/z</i> 344/189Q <i>m/z</i> 344/154C blank value <30% LOQ	<i>m/z</i> 342/58Q <i>m/z</i> 344/58C blank value <30% LOQ	<i>m/z</i> 344/189Q <i>m/z</i> 344/189C blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting $r \geq 0.995$ $\geq 5$ data points	linear regression analysis with 1/x weighting $r \geq 0.995$ $\geq 5$ data points	linear regression analysis with 1/x weighting $r \geq 0.995$ $\geq 5$ data points	linear regression analysis with 1/x weighting $r \geq 0.995$ $\geq 5$ 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 132: 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	<i>m/z</i> 344/189Q <i>m/z</i> 344/154C blank value <30% LOQ	<i>m/z</i> 344/189Q <i>m/z</i> 344/154C blank value <30% LOQ	<i>m/z</i> 344/189Q <i>m/z</i> 344/154C blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting $r \geq 0.995$ $\geq 5$ data points	linear regression analysis with 1/x weighting $r \geq 0.995$ $\geq 5$ data points	linear regression analysis with 1/x weighting $r \geq 0.995$ $\geq 5$ 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 133: 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	<i>m/z</i> 312/70Q <i>m/z</i> 312/125C blank value <30% LOQ	<i>m/z</i> 312/70Q <i>m/z</i> 312/125C blank value <30% LOQ	<i>m/z</i> 312/70Q <i>m/z</i> 312/125C blank value <30% LOQ	<i>m/z</i> 312/70Q <i>m/z</i> 312/125C blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting $r \geq 0.995$ $\geq 5$ data points	linear regression analysis with 1/x weighting $r \geq 0.995$ $\geq 5$ data points	linear regression analysis with 1/x weighting $r \geq 0.995$ $\geq 5$ data points	linear regression analysis with 1/x weighting $r \geq 0.995$ $\geq 5$ 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 134: 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	<i>m/z</i> 312/70Q <i>m/z</i> 312/125C blank value <30% LOQ	<i>m/z</i> 312/70Q <i>m/z</i> 312/125C blank value <30% LOQ	<i>m/z</i> 312/70Q <i>m/z</i> 312/125C blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting $r \geq 0.995$ $\geq 5$ data points	linear regression analysis with 1/x weighting $r \geq 0.995$ $\geq 5$ data points	linear regression analysis with 1/x weighting $r \geq 0.995$ $\geq 5$ 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

## 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 2.1.2.2.1.1 Method ILV

Comments of zRMS:	<p>The purpose of this study was to demonstrate that method “GF-3307 (Fenpicoxamid and Prothioconazole) Brood Development of the Honey Bee (<i>Apis mellifera</i> L.) in a Colony Feeding Test in Germany 2020” could be performed successfully at an outside facility with no prior experience with the method.</p> <p>During the independent laboratory validation of the method, the limit of quantitation (LOQ) of fenpicoxamid was confirmed to be 0.001 mg/kg for honey.</p> <p>The overall mean recovery values fell within the acceptable recovery range of 70-120% with RSD &lt; 20%.</p> <p>The results of this independent laboratory validation (ILV) study demonstrate that “GF-3307 (Fenpicoxamid and Prothioconazole) Brood Development of the Honey Bee (<i>Apis mellifera</i> L.) in a Colony Feeding Test in Germany 2020” fulfils the requirements with regard to specificity, repeatability, limit of quantification, and recoveries for honey.</p>
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Reference:	KCA 5.3.2.3/03
Report author:	Christopher Skaggs
Report year:	2021
Report title:	Independent Laboratory Validation of Fenpicoxamid (XDE-777) in Honey
Report No.:	SGS-21-S-04
Sponsor Study ID	210700

## CITATION

## COMPLIANCE

Guideline(s):	SANCO/825/00 Rev. 8.1 (2010)
US EPA Guideline(s):	U.S. EPA Guidance OPPTS 860.1000, Background, OCSPP 850.6100 and OPPTS 860.1340, Independent Laboratory Validation
Guideline Deviations:	N/A

## MATERIALS AND METHODS

### Test Item(s)

### Method Scope

### Method Principle

### Critical Steps

N/A

### Linearity

### Selectivity

### Confirmation

### Limits of Detection and Quantitation

## RESULTS AND DISCUSSION

### Summary of Recovery

Results obtained were within guideline requirements (mean recovery 70-110%; RSD ≤ 20%). For fenpicoxamid, the two ion mass transitions could be used interchangeably for quantification and confirmation. The results obtained are summarised in the following tables.

**Table A 135: Summary of quantitative recovery of Fenpicoxamide (m/z 615/239Q)**

Matrix group	Matrix	Fortification level	Recovery (%)		SD	RSD	n
		(mg/kg)	mean	range	(%)	(%)	
Crop	Honey	0.001	98	96-108	6.2	6.3	5
Crop	Honey	0.01	109	104-113	3.7	3.4	5
Crop	Honey	0.1	100	96-105	4.2	4.2	5

**Table A 136: Summary of quantitative recovery of Fenpicoxamide (m/z 615/515C)**

Matrix group	Matrix	Fortification level	Recovery (%)		SD	RSD	n
		(mg/kg)	mean	range	(%)	(%)	
Crop	Honey	0.001	98	92-108	6.4	6.5	5
Crop	Honey	0.01	108	103-112	3.4	3.1	5
Crop	Honey	0.1	99	95-104	4.2	4.2	5

### Repeatability

### Working Solution Stability

### Sample Extract Stability

### Matrix Effects

### Extraction Efficiency

### Changes to Method

No changes were made to the method during the conduct of the ILV.

## CONCLUSION

Method is acceptable based on current guidelines: EPA Residue Chemistry Test Guidelines OPPTS 860.1340 and OPPTS 860.1340, Independent Laboratory Validation and the requirements of SANCO/825/00 rev.8.1.

### A 2.1.2.2.2 Method validation

Comments of zRMS:	The study was evaluated and accepted in RR for Queen (January 2023) and is not being re-assessed in this application.
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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/M001 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 M001 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 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

The analytes were fortified, determined and expressed as themselves.

**Table A 137: 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 138: 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)
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### 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 2.1.2.2.2.1 Method ILV

Comments of zRMS:	The study was evaluated and accepted in RR for Queen (January 2023) and is not being re-assessed in this application.
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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 139: Independent laboratory validation results of analytical method 006556/M002 - Quantification**

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 140: 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)
	Meat		0.01	83-88 (85)	3 (5)
			0.10	83-94 (90)	5 (5)
JAU6476-3- hydroxy- desthio	Liver	0.01	0.01	83-90 (88)	3 (5)
			0.10	83-92 (90)	4 (5)
m/z 328 → 141 confirmation	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)
	Meat		0.01	83-87 (85)	2 (5)
			0.10	83-94 (90)	5 (5)
JAU6476-4- hydroxy- desthio	Liver	0.01	0.01	83-90 (86)	3 (5)
			0.10	82-91 (88)	4 (5)
m/z 328 → 141 confirmation	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 2.1.2.2.3 Method validation

Comments of zRMS:	The study was evaluated and accepted in RR for Queen (January 2023) and is not being re-assessed in this application.
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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 aglycones 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 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 141: Validation of method 01009 - Quantification**

Table A 141: Validation of method 61007 – 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 142: Validation of method 01009 - Confirmation**

Table 1-12: Validation of method 61007 - 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)
	Kidney		0.10	76-86 (81)	5.7 (5)
			0.01	77-104 (94)	10.9 (5)
	Liver		0.10	84-94 (90)	4.1 (5)
			0.01	89-101 (93)	4.8 (5)
	Fat		0.10	93-99 (96)	2.2 (5)
			0.01	89-105 (95)	6.7 (5)
	Egg		0.10	85-123 (101)	16.6 (5)
			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 residues 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 2.1.2.2.3.1 Method ILV

Comments of zRMS:	The study was evaluated and accepted in RR for Queen (January 2023) and is not being re-assessed in this application.
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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 143: 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 144: 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	Meat	0.01	0.01	84-92 (88)	4 (5)

344 → 157			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  $\geq 0.997$  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 2.1.2.2.4 Method validation

Comments of zRMS:	<p>The analytical method was successfully validated for the determination of X12326349 in animal matrices (bovine milk, bovine liver, bovine muscle and fat and poultry egg) in accordance to guidance document SANTE/2020/12830.</p> <p>The limit of quantification was 0.01 mg/kg.</p> <p>The individual recoveries for the analyte for both mass transitions in the tested matrices fell within the range of 80% to 100%. The average recoveries at each fortification level fell within the range of 70 to 110%. Relative standard deviations at each fortification level were all less than 15%.</p>
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	No significant matrix effects (<20%) were observed for X12326349 in animal matrices. The method is acceptable.
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Reference:	KCP 5.3.3.3/08
Report:	Senciuc, M., Przybylek, A.; 2022; Method Validation for the Determination of X12326349 in Animal Matrices; Eurofins Agrosience Services EAG Laboratories GmbH, Eiselaue Weg 4, Geb./Bldg. 5, 89081, Ulm; Lab Study No. S22-03479; Sponsor Study No. 220575 ; 21 December 2022; Published: No
Guideline(s):	SANTE/2020/12830 Rev.1 OPPTS 860.1340, DIR98-02
GLP:	Yes

### Method Scope

This method is applicable for the quantitative determination of residues of X12326349 in animal matrices (bovine whole milk, bovine muscle, bovine liver, bovine fat and poultry's eggs). The method was independently validated in bovine whole milk, bovine muscle, bovine liver, bovine fat and poultry's eggs over the concentration range of 0.010-1.0 mg/kg with a validated limit of quantitation of 0.010 mg/kg.

### Method Principle

Residues of X12326349 are extracted from samples by acetonitrile and QuEChERS citrate salts. The final sample is analysed for X12326349 by liquid chromatography coupled with negative-ion electrospray tandem mass spectrometry (LC-MS/MS).

### Linearity

The linearity of detector response was evaluated using  $\geq 5$  matrix-matched standard solutions in the range of 0.15 to 15 ng/mL (sample equivalent range of 0.003 to 0.30 mg/kg). Calibration curves were calculated by linear regression analysis with 1/x weighting and correlation coefficient obtained was  $>0.999$ .

### Selectivity

The LC-MS/MS method is highly selective for both the quantitation and confirmation of X12326349. Significant peak response ( $>30\%$  of the LOQ peak area) is not observed in reagent blank and extracts of untreated blank control samples at the expected retention time of the analyte. Unambiguous identification is ensured by monitoring two MS/MS transitions characteristic for X12326349 as follows in the table below.

**Table 1: Transitions monitored**

X12326349	<i>m/z</i> 463/257Q (quantitative)
X12326349	<i>m/z</i> 463/152C (confirmatory)

### Confirmation

Confirmation of the presence of X12326349 was by comparison of retention times of recovery samples with the retention times of the calibration standards as well as by monitoring two structurally characteristic MS/MS transitions by tandem mass spectrometry. Validation data obtained using the confirmatory MS/MS transition met the same acceptance criteria as the validation data generated using the quantitative MS/MS transition, therefore demonstrating that the analyte signal of the quantitative MS/MS transition is correct and not affected by any other compound.

### Limits of Detection and Quantitation

The limit of quantitation, defined as the lowest concentration of an analyte tested at which an unambiguous identification of the analyte can be proven and at which an acceptable mean recovery with an acceptable relative standard deviation is obtained, is 0.01 mg/kg for all tested matrices. The limit of detection, defined as 30% of the LOQ is 0.003 mg/kg for all tested matrices.

## RESULTS AND DISCUSSION

### Summary of Recovery

Results obtained were within guideline requirements (mean recovery 70-110%; RSD  $\leq$  20%). The two ion mass transitions could be used interchangeably for quantification and confirmation. The results obtained are summarised in the following tables.

**Table 2: Summary of quantitative recovery of X12326349 (m/z 463/257Q)**

Matrix group	Matrix	LOQ	Fortification level	Recovery (%)		SD	RSD	n
			mg/kg	mean	range	(%)	(%)	
Animal	Bovine whole milk	0.01 mg/kg	0.01	90	85-93	4	4	5
Animal	Bovine whole milk	0.01 mg/kg	0.1	87	85-89	2	2	5
Animal	Bovine whole milk	0.01 mg/kg	1.0	87	84-89	2	2	5
Animal	Bovine muscle	0.01 mg/kg	0.01	84	82-87	2	3	5
Animal	Bovine muscle	0.01 mg/kg	0.1	83	80-89	3	4	5
Animal	Bovine muscle	0.01 mg/kg	1.0	87	81-90	4	4	5
Animal	Bovine liver	0.01 mg/kg	0.01	88	85-90	2	3	5
Animal	Bovine liver	0.01 mg/kg	0.1	88	86-90	2	2	5
Animal	Bovine liver	0.01 mg/kg	1.0	88	85-90	2	2	5
Animal	Bovine fat	0.01 mg/kg	0.01	91	88-95	3	3	5
Animal	Bovine fat	0.01 mg/kg	0.1	95	94-97	1	2	5
Animal	Bovine fat	0.01 mg/kg	1.0	96	93-100	2	2	5
Animal	Poultry egg	0.01 mg/kg	0.01	84	83-86	2	2	5
Animal	Poultry egg	0.01 mg/kg	0.1	87	86-89	1	1	5
Animal	Poultry egg	0.01 mg/kg	1.0	92	88-94	2	3	5

**Table 3: Summary of confirmatory recovery of X12326349 (m/z 363/152C)**

Matrix group	Matrix	LOQ	Fortification level	Recovery (%)		SD	RSD	n
			mg/kg	mean	range	(%)	(%)	
Animal	Bovine whole milk	0.01 mg/kg	0.01	92	87-95	5	5	5
Animal	Bovine whole milk	0.01 mg/kg	0.1	88	85-92	3	3	5
Animal	Bovine whole milk	0.01 mg/kg	1.0	87	84-89	2	2	5
Animal	Bovine muscle	0.01 mg/kg	0.01	85	82-87	2	3	5
Animal	Bovine muscle	0.01 mg/kg	0.1	83	81-87	3	3	5
Animal	Bovine muscle	0.01 mg/kg	1.0	87	82-91	4	4	5
Animal	Bovine liver	0.01 mg/kg	0.01	88	85-91	3	3	5

Matrix group	Matrix	LOQ	Fortification level	Recovery (%)		SD	RSD	n
			mg/kg	mean	range	(%)	(%)	
Animal	Bovine liver	0.01 mg/kg	0.1	87	85-90	2	3	5
Animal	Bovine liver	0.01 mg/kg	1.0	88	85-90	2	2	5
Animal	Bovine fat	0.01 mg/kg	0.01	92	89-94	2	2	5
Animal	Bovine fat	0.01 mg/kg	0.1	94	90-97	3	3	5
Animal	Bovine fat	0.01 mg/kg	1.0	96	94-98	2	2	5
Animal	Poultry egg	0.01 mg/kg	0.01	87	85-92	3	3	5
Animal	Poultry egg	0.01 mg/kg	0.1	88	86-90	2	2	5
Animal	Poultry egg	0.01 mg/kg	1.0	92	88-94	2	3	5

### Repeatability

Repeatability was not assessed as a part of this study.

### Working Solution Stability

Stock solutions X12326349 of prepared in acetonitrile containing 0.1% formic acid were tested after 35 days of storage at 1 – 10 °C and were found to be stable.

Calibration standard solutions of X1236349 prepared in acetonitrile:water (1:1, v/v) containing 0.1% formic acid were tested after 35 days of storage at 1 – 10 °C and were found to be stable.

### Sample Extract Stability

Sample extracts of X12326349 were tested after 23 days for bovine whole milk, for 15 days for bovine muscle and liver, for 14 days for bovine fat and for 8 days for poultry's eggs of storage at 1 – 10 °C and were found to be stable.

### Matrix Effects

Matrix effects were evaluated by comparing the response of the analyte fortified in a control extract after processing (for each matrix type) to the response of the analyte fortified in neat solvent. The results demonstrate that matrix effects are within  $\pm 20\%$ . Nevertheless, matrix matched standards were used for quantification for all matrices for this study.

### Extraction Efficiency

Extraction efficiency was not assessed as a part of this study.

## CONCLUSION

Method is acceptable based on current guidelines: EPA Residue Chemistry Test Guidelines OPPTS 860.1340, SANTE/2020/12830 Rev.1, as well as PMRA Regulatory Directive Dir98-02.

### A 2.1.2.2.4.1 Method ILV

Comments of zRMS:	<p>The analytical method S22-03479 of Senciuc, M., Przybylek, A.; 2022 for X12326349 in animal matrices (bovine liver and bovine muscle) was independently validated with LOQ of 0.01 mg/kg in accordance with SANTE/2020/12830 rev. 2.</p> <p>The average recoveries at each fortification level in both matrices fell within the range of 95-99% and 96-101% for the quantitative and confirmatory transitions, respectively. Relative standard deviations at each fortification level were all less than 20%.</p> <p>The method is acceptable.</p>
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Reference:	KCP 5.3.3.5/09
Report:	Moore, S., Shepherd, J.; 2023; Independent Laboratory Validation of an Analytical Method for the Determination of Residues of X12326349 (XDE-777 Metabolite) in Animal Matrices ; SynTech Research, 17745 Metcalf Ave., Stilwell, KS; Lab Study No. 598SRUS23R0052; Sponsor Study No. 230145 ; 13 June 2023; Published: No
Guideline(s):	OCSPP 850.1340, OCSPP 850.1600, SANTE/2020/12830 rev. 2, PMRA Dir98-02, DACO 7.2.3 Inter-laboratory Analytical Methodology Validation OCSPP 850.1340, OCSPP 850.1600, SANTE/2020/12830 rev. 2, PMRA Dir98-02, DACO 7.2.3 Inter-laboratory Analytical Methodology Validation. OCSPP 850.1340, OCSPP 850.1600
GLP:	Yes

## Method Scope

## Method Principle

## Critical Steps

None.

## Linearity

## Selectivity

## Confirmation

## Limits of Detection and Quantitation

## RESULTS AND DISCUSSION

### Summary of Recovery

Results obtained were within guideline requirements (mean recovery 70-110%;  $RSD \leq 20\%$ ). The two ion mass transitions could be used interchangeably for quantification and confirmation. The results obtained are summarised in the following tables.

**Table 2: Summary of quantitative recovery of X12326349 (m/z 463/257Q)**

Matrix group	Matrix	LOQ	Fortification level	Recovery (%)		SD	RSD	n
			mg/kg	mean	range	(%)	(%)	
Animal	Bovine liver	0.010 mg/kg	0.010	95	93-97	1.3	1.4	5
Animal	Bovine liver	0.010 mg/kg	0.10	97	95-99	2.2	2.3	5
Animal	Bovine liver	0.010 mg/kg	1.0	99	97-102	1.8	1.8	5
Animal	Bovine muscle	0.010 mg/kg	0.010	99	92-120	11.8	11.9	5
Animal	Bovine muscle	0.010 mg/kg	0.10	95	94-96	0.9	0.9	5
Animal	Bovine muscle	0.010 mg/kg	1.0	99	98-101	1.4	1.4	5

**Table 3: Summary of confirmatory recovery of X12326349 (m/z 463/152C)**

Matrix group	Matrix	LOQ	Fortification level	Recovery (%)		SD	RSD	n
			mg/kg	mean	range	(%)	(%)	
Animal	Bovine liver	0.010 mg/kg	0.010	97	91-104	5.3	5.5	5
Animal	Bovine liver	0.010 mg/kg	0.10	97	95-99	1.9	1.9	5
Animal	Bovine liver	0.010 mg/kg	1.0	101	99-104	2.0	2.0	5
Animal	Bovine muscle	0.010 mg/kg	0.010	99	91-118	11.2	11.3	5
Animal	Bovine muscle	0.010 mg/kg	0.10	96	95-96	0.6	0.6	5
Animal	Bovine muscle	0.010 mg/kg	1.0	100	98-102	1.3	1.3	5

#### Repeatability

#### Working Solution Stability

#### Sample Extract Stability

#### Matrix Effects

#### Extraction Efficiency

#### Changes to Method

No changes were made to the method during the conduct of the ILV.

### CONCLUSION

Method is acceptable based on current guidelines: EPA Residue Chemistry Test Guidelines OPPTS 860.1340, the requirements of SANTE/2020/12830 Rev.2, as well as PMRA Regulatory Directive Dir98-02.

#### A 2.1.2.2.5 Method validation

Comments of zRMS:	An analytical method 01600 was successfully validated for determination of prothioconazole (JAU 6476) and its metabolite JAU 6476-desthio in/on honey by HPLC-MS/MS in accordance with SANTE/2020/12830. The limit of quantification is 0.01 mg/kg for prothioconazole and prothioconazole-desthio in honey. The method is acceptable for monitoring purposes.
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Title:	Amendment no. 01: Residue analytical method 01600 and short term storage stability of prothioconazole (JAU 6476) and its Metabolite JAU 6476-desthio in/on honey by HPLC-MS/MS
Author:	Kalathoor, R.
Edition Date:	18.05.2020
Report No:	M-680823-02-1
Reference No:	S19-01124
Guideline(s)	Regulation (EC) No 1107/2009 of the European Parliament and 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  Guidance document on residue analytical methods, SANCO/825/00/rev. 8.1, European Commission, Directorate General Health and Consumer Protection 16/11/2010  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, 11/07/00  OECD 506, 2007; OECD Guideline for the Testing of Chemicals – Stability of Pesticide Residues in Stored Commodities  SANTE/11956/2016 rev.9
Guideline Deviation(s)	None
GLP/GEP	yes
Testing Facility:	Eurofins Agrosience Services EcoChem GmbH / Eurofins Agrosience Services Ecotox GmbH, Niefern-Oeschelbronn, Germany
Sponsor:	Bayer
Owner:	BAY

## Materials and methods

The analytical method 01600 was developed for the determination of prothioconazole (JAU 6476) and its Metabolite JAU 6476-desthio residues in/on honey by HPLC-MS/MS detection, in accordance to the guidance document SANCO/825/00, rev. 8.1 of the European Commission.

In all the method, results will be expressed as themselves for parent prothioconazole (JAU 6476) and its Metabolite JAU 6476-desthio.

In addition, a short-term storage stability will provide data about the storage stability of Prothioconazole (JAU 6476) and its metabolite JAU 6476-desthio in the matrix honey at  $\leq 18^\circ\text{C}$  (target) in the dark over a storage period up to 6 months in accordance to OECD Guideline 506.

Stabilities of 10-fold LOQ recoveries in honey were established. Stabilities of stock standard solutions and diluted standard solutions were as well established.

1 g of homogenized sample of honey was diluted in a 15 mL Falcon® tube with 8 mL of acetonitrile/water mixture (1/1, v/v) and 0.8 mL of a 250 g/L cysteine hydrochloride solution. After shaking manually for 1 min and further 15 min on a flatbed shaker at 300 rpm until the honey is completely dissolved, the sample was transferred into a 20 mL volumetric flask. The centrifuge tube was rinsed to 20 ml with acetonitrile/water mixture (1/1, v/v). The volume was adjusted to 20 mL. 200  $\mu\text{L}$  aliquot was diluted to 1 mL with 0.8 methanol/water (4/6 v/v).

Matrix-matched standards were used for quantification throughout the analytical phase.

The high selectivity of the method resulted from the separation on HPLC with a Reversed-Phase C18 chromatographic column in combination with MS/MS detection. A MS/MS-based confirmatory method was established as well:

**Table A 145: Quantitation and Confirmation Mass Transitions**

Analytes	Mode ionisation	Mass transition First MRM Quantitation	Mass transition Second MRM Confirmation
Prothioconazole (JAU 6476)	Positive	m/z = 344® 189*	m/z = 344® 154
JAU 6476-desthio	Positive	m/z = 312® 70*	m/z = 312® 125

\*proposed (and used) for quantification but both of the mass transitions listed can be used for quantification

Prothioconazole (JAU 6476) and its Metabolite JAU 6476-desthio residues were quantified using matrix-matched calibration standards.

### Results and discussions

The effect of honey on the HPLC-MS/MS response was assessed by comparing the mean response factor of matrix-matched standards of at least 90% matrix amount with the mean response factor of solvent standards within the same calibration range:

Matrix effects in honey were  $< \pm 20\%$  and deemed to be insignificant for Prothioconazole (JAU 6476) and for JAU 6476-desthio. Nevertheless, matrix-matched standards were used for quantification throughout the analytical phase.

For both MRM transitions of each analyte the lower margin of the linearity test was below 30% of the LOQ and the higher margin was at minimum 20% above the highest concentration as demanded in SANCO/825/00 rev. 8.1. The correlation between the injected amount of analyte and the detector response was linear for at least 6 standards ranging from 0.03 ng/mL to 5 ng/mL (corresponding to 0.003 mg/kg to 0.5 mg/kg for prothioconazole (JAU 6476) and JAU 6476-desthio). The correlation coefficients of the 1/x weighted linear regressions were always  $\geq 0.995$ .

The two MRM transitions were successfully validated for Prothioconazole (JAU 6476) and its metabolite in the tested matrix honey. Therefore, an additional confirmatory method is not necessary. The apparent residues in the control samples were below 30% of the LOQ. The recoveries were not corrected for interferences.

The limit of quantification (LOQ) for all the analytes was established at 0.01 mg/kg. The limit of detection (LOD) was estimated to be  $\leq 30\%$  of the LOQ. The LOD for all analytes was set at 0.003 mg/kg.

The stability of prothioconazole (JAU 6476) and JAU 6476-desthio stock and mixed secondary standard solutions in acetonitrile was tested over a period of 55 days and 114 days after storage, respectively. The analytes were stable in the stock solutions and mix solutions when stored at 1–10 °C under dark conditions.

Results obtained are summarised in the tables below.

**Table A 146: Stability of stock solutions of Prothioconazole (JAU 6476) and its metabolite**

Analyte	Solvent of secondary standard solution	Concentration of dilution [ng/mL]	Storage period [d]	Mean difference (in %) of stored solution compared to freshly prepared solution
Prothioconazole (JAU 6476)	Acetonitrile	10	55	-3.9
JAU 6476-desthio	Acetonitrile	10	55	-3.2

**Table A 147: Stability of secondary standard solutions of Prothioconazole (JAU 6476) and its metabolite**

Analyte	Solvent of secondary standard solution	Concentration of dilution [ng/mL]	Storage period [d]	Mean difference (in %) of stored solution compared to freshly prepared solution
Prothioconazole (JAU 6476)	Acetonitrile	10	114	+2.8
JAU 6476-desthio	Acetonitrile	10	114	+3.3

The stability of the analytes in the final extracts was checked for the sample material honey. Residues of both analytes were stable over a time period of 10 days (prothioconazole (JAU 6476) and JAU 6476-desthio) after storage of the final extracts at 1–10°C under dark conditions. One mass transition per analyte was evaluated.

Results obtained are summarised in the table below.

**Table A 148: Stability of Prothioconazole (JAU 6476) and its metabolite in Final Extracts of Honey**

Sample material	Fortification Level [mg/kg]	Analyte	1st MRM	Recovery rates					Mean
Honey	0.10	Prothioconazole (JAU 6476)	Initial analysis	101	102	102	88	100	99
			10 days reanalysis	104	105	99	108	109	105
			deviation*						6
	0.10	JAU 6476-desthio	Initial analysis	99	100	95	84	98	95
			10 days reanalysis	103	101	99	109	104	103
			deviation*						8

LOQ = 0.01 mg/kg

Fortified, determined and calculated as analyte for Prothioconazole (JAU 6476) and metabolite JAU 6476-desthio.

Calculation of Deviation: Absolute value (100 x (mean reanalysis - mean initial analysis) / mean initial analysis)

\* For the calculation of deviations between initial analysis and reanalysis, unrounded mean values were used.

Therefore, minor deviations may occur by calculating the deviations between the initial analysis and the reanalysis with the above-shown rounded mean values.

After a deep-freezer storage ( $\leq -18^{\circ}\text{C}$ ) period of about 6 months, the mean recovery rates were 87% for Prothioconazole (JAU 6476) and 97% for JAU 6476-desthio in honey. Altogether, the study results demonstrate that the residues of Prothioconazole (JAU 6476) and JAU 6476-desthio are stable in honey for at least 6 months under deep-freezer storage conditions ( $\leq -18^{\circ}\text{C}$ ).

Recovery rates were determined at fortification levels of 0.01 mg/kg and 0.1 mg/kg. The recovery experiments were conducted by fortification of untreated control samples with defined amounts of the analytes prior to analysis. The mean recoveries at each fortification level and the overall mean recovery were within the 70 - 110% range and the relative standard deviations for each fortification level were below 20%.

**Table A 149: Recoveries obtained using the quantitation MRM-transition, 1.0 g honey weight**

Analyte	Matrix	Fortification Level [mg/kg]	n	Mean [%]	RSD [%]	Comments
Prothioconazole (JAU 6476)	Honey	0.01	5	103	7	(1 <sup>st</sup> MRM) m/z = 344 ® m/z = 189
		0.10	5	99	6	
JAU 6476-desthio	Honey	0.01	5	100	6	(1 <sup>st</sup> MRM) m/z = 312 ® m/z = 70
		0.10	5	95	7	

LOQ = 0.01 mg/kg, LOD = 0.003 mg/kg, fortified, determined and calculated as analyte for Prothioconazole (JAU 6476) and metabolite JAU 6476-desthio.

n: number of single results per fortification

RSD: Relative Standard Deviation

**Table A 150: Recoveries obtained using the confirmation MRM-transition, 1.0 g honey weight**

Analyte	Matrix	Fortification Level [mg/kg]	n	Mean [%]	RSD [%]	Comments
Prothioconazole (JAU 6476)	Honey	0.01	5	109	4	(2 <sup>nd</sup> MRM) m/z = 344 ® m/z = 154
		0.10	5	100	6	
JAU 6476-desthio	Honey	0.01	5	102	5	(2 <sup>nd</sup> MRM) m/z = 312 ® m/z = 125
		0.10	5	96	7	

LOQ = 0.01 mg/kg, LOD = 0.003 mg/kg, fortified, determined and calculated as analyte for Prothioconazole (JAU 6476) and metabolite JAU 6476-desthio.

n: number of single results per fortification

RSD: Relative Standard Deviation

**Table A 151: Characteristics for the analytical method used for validation of Prothioconazole (JAU 6476) and its metabolite in honey**

Method 01600	Prothioconazole (JAU 6476)	JAU 6476-desthio
Specificity	Mass spectra provided in Appendix 5 of the method report blank value < 30% LOQ)	Mass spectra provided in Appendix 5 of the method report blank value < 30% LOQ)
Calibration (type, number of data points)	Calibration data presented in Appendix 6 calibration line equations presented number of data points ≥ 6 R >0.995	Calibration data presented in Appendix 6 calibration line equations presented number of data points ≥ 6 R >0.995
Calibration range	Excellent linear correlation between the injected amount and detector response of the HPLC-MS/MS system was observed within the range of 0.03 to 5 ng/mL corresponding to 0.003 to 0.5 mg/kg for Prothioconazole (JAU 6476)	Excellent linear correlation between the injected amount and detector response of the HPLC-MS/MS system was observed within the range of 0.03 to 5 ng/mL for the metabolite JAU 6476-desthio corresponding to 0.003 to 0.5 mg/kg in JAU 6476-desthio.
Assessment of matrix effects is presented	Yes. Matrix effects in honey were < ± 20 % and deemed to be insignificant for Prothioconazole (JAU 6476). Therefore, matrix-matched standards were used for quantification throughout the analytical phase	Yes. Matrix effects in honey were < ± 20 % and deemed to be insignificant for JAU 6476-desthio. Therefore, matrix-matched standards were used for quantification throughout the analytical phase
Limit of determination/quantification	LOQ: 0.01 mg/kg LOD: 0.003 mg/kg	LOQ: 0.01 mg/kg in JAU 6476-desthio. LOD: 0.003 mg/kg in JAU 6476-desthio.

## Conclusion

The analytical method 01600 was developed for the determination of Prothioconazole (JAU 6476) and JAU 6476-desthio which is the official residue definition for plants and is also proposed to be the residue definition for the honey. The limit of quantification is 0.01mg/kg.

For completing the picture, the analytical method 01600 was developed in order to determine also residues of

one metabolite of Prothioconazole (JAU 6476), JAU 6476-desthio, with a limit of quantification of 0.01 mg/kg in honey.

Two MRM transitions were monitored for each analyte in honey.

The HPLC-MS/MS method is highly specific, and an additional confirmatory method is not necessary.

All analytes can be considered stable in honey under deep-freezer storage conditions ( $\leq 18^{\circ}\text{C}$ ) for at least 6 months.

The analytical method complies with all guidance criteria according to SANTE/2020/12830, Rev.1 and is therefore suitable as an enforcement method for the determination of Prothioconazole (JAU 6476) and JAU 6476-desthio in honey by HPLC-MS/MS.

#### A 2.1.2.2.5.1 Method ILV

Comments of zRMS:	The method 01600 is independently validated for the determination of prothioconazole (JAU 6476) and its metabolite JAU 6476-desthio in/on honey in accordance with the guidance document SANTE/2020/12830.
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Title:	Independent laboratory validation of the analytical method 01600 for the determination of prothioconazole (JAU 6476) and its metabolite JAU 6476-desthio in/on honey
Author:	Fritzsche, S.
Edition Date:	18.05.2020
Report No:	M-684857-01-1
Reference No:	S19-22668
Guideline(s)	Regulation (EC) No. 1107/2009 of the European Parliament and 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 on pesticide residue analytical methods SANCO/825/00 rev. 8.1  US EPA Residue Chemistry Test Guideline OCSPP 860.1340: Residue Analytical Method  OECD Guideline, OECD 506, 2007
Guideline Deviation(s)	None
GLP/GEP	yes
Testing Facility:	Eurofins Agrosience Services Chem GmbH (EAS Chem), Hamburg, Germany
Sponsor:	Bayer
Owner:	BAY

#### Materials and methods

The purpose of this study was to independently validate the HPLC-MS/MS method 01600 for the determination of residues of Prothioconazole (JAU 6476) and its metabolite JAU 6476-desthio in/on honey by HPLC-MS/MS detection, with a limit of quantification (LOQ) of 0.01 mg/kg, in accordance to regulation (EC) No. 1107/2009 of the European Parliament and the Council of 21 October 2009 and the guidance documents SANCO/825/00, rev. 8.1 of the European Commission and OECD 506, 2007.

1 g of sample material was weighed into a 50 mL Sarstedt tube. 100  $\mu\text{L}$  of the fortification solution were added for fortification experiments. 8 mL acetonitrile/water/ (1/1 v/v) and 0.8 mL cysteine hydrochloride solution (250 g/L) were added and specimens were homogenised by shaking on a platform shaker for 15 min at 150 rpm until the whole sample was diluted. The sample was filled up to 20 mL with acetonitrile/water/ (1/1 v/v). 200  $\mu\text{L}$  aliquot was diluted to 1 mL with methanol/water (4/6, v/v). Residues of Prothioconazole (JAU 6476) and its metabolite JAU 6476-desthio are quantified using matrix-matched calibration standards.

The high selectivity of the method resulted from the separation on HPLC with a Reversed-Phase C8 chromatographic column in combination with MS/MS detection. A MS/MS-based confirmatory method was established as well:

**Table A 152: Quantitation and Confirmatory Mass Transitions**

Analytes	Mass transition 1st MRM Quantitation	Mass transition 2st MRM Confirmatory
Prothioconazole (JAU 6476)	m/z = 344® 189*	m/z = 344® 154
JAU 6476-desthio	m/z = 312® 70*	m/z = 312® 125

\*proposed (and used) for quantification but both of the mass transitions listed can be used for quantification

## Results and discussions

The correlation between the injected amount of substance and the detector response was determined by double injections of matrix-matched calibration standards prepared from the extracts of the corresponding control material with a minimum of six concentration levels from 0.03 - 10 ng/mL for Prothioconazole (JAU 6476) and its metabolite JAU 6476-desthio, corresponding to 0.003 - 1.0 mg/kg (expressed as parent equivalent). The correlation coefficients of the linear regression were in all cases > 0.99.

In the course of this study, two untreated control samples were analysed to investigate the residue level of the analytes. Additionally, one reagent blank, which is a sample without matrix, was analysed to check for any background interferences at the expected retention time of the analyte.

All residues of prothioconazole (JAU 6476) and its metabolite JAU 6476-desthio were below the limit of detection (30% of the LOQ of 0.01 mg/kg), so a high level of selectivity was demonstrated. The limit of quantification (LOQ) in/on honey was set at 0.01 mg/kg for Prothioconazole (JAU 6476) and its metabolite JAU 6476-desthio.

Recovery experiments were conducted by fortification of untreated control material with defined amounts of each analyte prior to analysis.

Recovery rates were determined at fortification levels of 0.01 mg/kg (= LOQ level) and 0.10 mg/kg (= 10xLOQ level) for Prothioconazole (JAU 6476) and its metabolite JAU 6476-desthio, in/on honey. Recovery experiments were conducted by fortification of untreated control material with defined amounts of each analyte prior to analysis.

The lowest fortification level providing a mean recovery between 70 and 110% with a relative standard deviation of < 20% per definition corresponding to the Limit of Quantitation (LOQ), provided that the blank values were below 30% of LOQ.

Results are presented in the following tables.

As a measure for the precision of the method, the intra-laboratory repeatability (n = 5) is given as relative standard deviation (% RSD) at all fortification levels. The RSD of the repeatability was < 20%.

**Table A 153: Independent Laboratory Validation results of analytical method 01600 Recoveries and relative standard deviations (RSDs) for Prothioconazole (JAU 6476) and its metabolite JAU 6476-desthio - Quantitation transitions**

*Recoveries calculated via matrix-matched calibration standards*

Analyte	Crop, matrix	Fortification Level [mg/kg]	Recoveries [%] Data per fortification level			
			n	Mean [%]	RSD [%]	Comments
Prothioconazole (JAU 6476)	honey	0.01	5	110	3.0	m/z = 344® 189
		0.10	5	107	2.7	
JAU 6476-desthio	honey	0.01	5	100	1.7	m/z = 312® 70
		0.10	5	104	1.5	

n: number of replicates

RSD: relative standard deviation



**Table A 154: Independent Laboratory Validation results of analytical method 01600 Recoveries and relative standard deviations (RSDs) for Prothioconazole (JAU 6476) and its metabolite JAU 6476-desthio - Confirmatory transitions**

*Recoveries calculated via matrix-matched calibration standards*

Analyte	Crop, matrix	Fortification Level [mg/kg]	Recoveries [%] Data per fortification level			
			n	Mean [%]	RSD [%]	Comments
Prothioconazole (JAU 6476)	honey	0.01	5	110	1.8	m/z = 344® 154
		0.10	5	107	2.1	
JAU 6476-desthio	honey	0.01	5	100	2.7	m/z = 312® 125
		0.10	5	104	1.5	

n: number of replicates

RSD: relative standard deviation

**Table A 155: Characteristics for the analytical method used for Independent Laboratory Validation of Prothioconazole (JAU 6476) and its metabolite JAU 6476-desthio residues in honey**

ILV of Method 01600	Prothioconazole (JAU 6476)	JAU 6476-desthio
Specificity	Mass spectra provided in Appendix 5 of the method report blank value < 30% LOQ)	Mass spectra provided in Appendix 5 of the method report blank value < 30% LOQ)
Calibration (type, number of data points)	Calibration data presented in Appendix 6 calibration line equation presented number of data points ≥ 6 R > 0.99	Calibration data presented in Appendix 6 calibration line equation presented number of data points ≥ 6 R > 0.99
Calibration range	Excellent linear correlation between the injected amount and detector response Minimum of six concentration levels with concentrations from 0.03 – 10 ng/mL for Prothioconazole (JAU 6476) corresponding to 0.003 – 1.0 mg/kg	Excellent linear correlation between the injected amount and detector response Minimum of six concentration levels with concentrations from 0.03 – 10 ng/mL for JAU 6476-desthio corresponding to 0.003 – 1.0 mg/kg
Assessment of matrix effects is presented	Matrix-matched standards was used for quantification throughout the study.	Matrix-matched standards was used for quantification throughout the study.
Limit of determination and quantification	-LOQ: 0.01 mg/kg -LOD: 0.003 mg/kg	-LOQ: 0.01 mg/kg -LOD: 0.003 mg/kg

## Conclusion

The independent laboratory validation of the analytical method 01600 was successfully performed for the determination of Prothioconazole (JAU 6476) and its metabolite JAU 6476-desthio in/on honey. It is concluded that the independent laboratory validation fulfils the reproducibility requirements defined in the guidance document SANTE/2020/12830, Rev.1. Therefore, the method is considered applicable as an enforcement method.

### A 2.1.2.2.6 Method validation

Comments of zRMS:	An analytical method was successfully validated for determination of fenpicoxamid in/on honey by LC-MS/MS in accordance with SANTE/2020/12830. The limit of quantification is 0.05 mg/kg in honey. The average recoveries at each fortification level fell within the range of 70 to 110%. Relative standard deviations at each fortification level were all less than 10%. The concentration ranged from 0.050 to 5.0 mg/kg for XDE-777. The method is acceptable for monitoring purposes.
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Reference: KCP 5.3.3.5/10

Report: Senciuc, M., Przybylek, A.; 2022; Validation of the Analytical Method for the Determination of Fenpicoxamid (XDE-777) in Honey; Eurofins Agrosience Services EAG Laboratories GmbH, Eiselaer Weg 4, Geb./Bldg. 5, 89081 Ulm,

Germany; Lab Study No. S22-03480; Sponsor Study No. 220576 ; 15 December 2022; Published: No

Guideline(s): SANTE/2020/12830 rev.1  
OPPTS 860.1340, Dir98-02

GLP: Yes

### Method Scope

This method is applicable for the quantitative determination of residues of Fenpicoxamid (XDE-777) in Honey. The method was independently validated in Honey over the concentration range of 0.050-5.0 mg/kg with a validated limit of quantitation of 0.050 mg/kg.

### Method Principle

Residues of are extracted from samples by use of acetonitrile (QuEChERS). The final sample is analysed for Fenpicoxamid (XDE-777) by liquid chromatography coupled with positive-ion electrospray tandem mass spectrometry (LC-MS/MS).

### Linearity

The linearity of detector response was evaluated using matrix-matched standard solutions at > 5 concentration levels. Calibration curves were calculated by linear regression analysis with 1/x weighting resulting in a correlation coefficient R of > 0.99.

### Selectivity

The LC-MS/MS method is highly selective for both the quantitation and confirmation of Fenpicoxamid (XDE-777). Significant peak response (>30% of the LOQ peak area) is not observed in reagent blank and extracts of untreated blank control samples at the expected retention times of the analyte. Unambiguous identification is ensured by monitoring two MS/MS transitions characteristic as follows in the table below.

**Table 1:Transitions monitored**

Fenpicoxamid (XDE-777)	<i>m/z</i> 615/239Q (quantitative)
Fenpicoxamid (XDE-777)	<i>m/z</i> 615/515C (confirmatory)

### Confirmation

Confirmation of the presence of Fenpicoxamid (XDE-777) was by comparison of retention times of recovery samples with the retention times of the calibration standards as well as by monitoring two structurally characteristic MS/MS transitions by tandem mass spectrometry. Validation data obtained using the confirmatory MS/MS transition met the same acceptance criteria as the validation data generated using the quantitative MS/MS transition, therefore demonstrating that the analyte signal of the quantitative MS/MS transition is correct and not affected by any other compound.

### Limits of Detection and Quantitation

The limit of quantitation, defined as the lowest concentration of an analyte tested at which an unambiguous identification of the analyte can be proven and at which an acceptable mean recovery with an acceptable relative standard deviation is obtained, is 0.050 mg/kg for the analyte in the tested matrix.

The limit of detection, defined as 20% of the LOQ (i.e. the lowest concentration level of calibration curve injected, 0.01 mg/kg).

## RESULTS AND DISCUSSION

### Summary of Recovery

Results obtained were within guideline requirements (mean recovery 70-110%; RSD ≤ 20%). Two ion mass transitions could be used interchangeably for quantification and confirmation. The results obtained are summarised in the following tables.

**Table 2: Summary of quantitative recovery of fenpicoxamid (XDE-777) (m/z 615/239Q)**

Matrix group	Matrix	LOQ	Fortification level	Recovery (%)		SD	RSD	n
			mg/kg	mean	range	(%)	(%)	
Animal	Honey	0.05 mg/kg	0.05	101	94-111	7	7	5
Animal	Honey	0.05 mg/kg	0.5	91	83-98	7	8	5
Animal	Honey	0.05 mg/kg	5.0	93	87-96	3	4	5

**Table 3: Summary of confirmatory recovery of fenpicoxamid (XDE-777) (m/z 615/515C)**

Matrix group	Matrix	LOQ	Fortification level	Recovery (%)		SD	RSD	n
			mg/kg	mean	range	(%)	(%)	
Animal	Honey	0.05 mg/kg	0.05	95	91-98	4	4	5
Animal	Honey	0.05 mg/kg	0.5	89	84-94	4	5	5
Animal	Honey	0.05 mg/kg	5.0	89	77-97	8	9	5

### Repeatability

Repeatability was not assessed as a part of this study.

### Working Solution Stability

Stock solution of Fenpicoxamid (XDE-777) prepared in acetonitrile was tested after 41 days of storage at 1 to 10 °C and was found to be stable.

A calibration standard solution of Fenpicoxamid (XDE-777) prepared in acetonitrile/ water (1/1, v/v) containing 0.1% formic acid was tested after 35 days of storage at 1 to 10 °C and was found to be stable.

### Sample Extract Stability

Sample extracts of fenpicoxamid (XDE-777) were tested after 8 days of storage at 1 to 10 °C and were found to be stable.

### Matrix Effects

Matrix effects were evaluated by comparing the response of the analyte fortified in a control extract after processing to the response of the analyte fortified in neat solvent. The results demonstrate that matrix effects are within ±20%. Nevertheless, matrix-matched standards were used for quantification in this study.

### Extraction Efficiency

Extraction efficiency was not assessed as a part of this study.

## CONCLUSION

Method is acceptable based on current guidelines: EPA Residue Chemistry Test Guidelines OPPTS 860.1340, SANTE/2020/12830 Rev.1, as well as PMRA Regulatory Directive Dir98-02.

### A 2.1.2.2.6.1 Method ILV

Comments of zRMS:	The method was independently validated for the determination of fenpicoxamid in/on honey in accordance with the guidance document SANTE/2020/12830.
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Reference: KCP 5.3.3.5/11

Report: Moore, S., Shepherd, J.; 2023; Independent Laboratory Validation of an Analytical Method for the Determination of Residues of XDE-777 in Honey ; SynTech Research, 17745 Metcalf Avenue, Stilwell, KS 66085; Lab Study No. 598SRUS23R0053; Sponsor Study No. 230146 ; 13 June 2023; Published: No

Guideline(s): SANTE/2020/12830 rev. 1, Dir98-02, DACO 7.2.3  
OPPTS 860.1340, OCSPP 850.6100

GLP: Yes

## Method Scope

## Method Principle

## Critical Steps

None.

## Linearity

## Selectivity

## Confirmation

## Limits of Detection and Quantitation

## RESULTS AND DISCUSSION

### Summary of Recovery

Results obtained were within guideline requirements (mean recovery 70-110%; RSD ≤ 20%). The two ion mass transitions could be used interchangeably for quantification and confirmation. The results obtained are summarised in the following tables.

**Table 2: Summary of quantitative recovery of XDE-777 (m/z 615.0/239.0Q)**

Matrix group	Matrix	LOQ	Fortification level	Recovery (%)		SD	RSD	n
			mg/kg	mean	range	(%)	(%)	
Pollinator	Honey	0.05 mg/kg	0.05	93	82-108	12.3	13.3	5
Pollinator	Honey	0.05 mg/kg	0.50	103	85-117	13.4	13.0	5
Pollinator	Honey	0.05 mg/kg	5.0	106	100-110	3.9	3.7	5

**Table 3: Summary of confirmatory recovery of XDE-777 (m/z 615.0/515.0C)**

Matrix group	Matrix	LOQ	Fortification level	Recovery (%)		SD	RSD	n
			mg/kg	mean	range	(%)	(%)	
Pollinator	Honey	0.05 mg/kg	0.05	92	80-110	13.3	14.4	5
Pollinator	Honey	0.05 mg/kg	0.50	104	86-118	13.8	13.3	5
Pollinator	Honey	0.05 mg/kg	5.0	108	100-113	4.7	4.4	5

## Repeatability

## Working Solution Stability

## Sample Extract Stability

## Matrix Effects

Matrix effects were evaluated five times at 10X LOQ (0.50 mg/kg) by comparing the response of the analyte fortified in a control extract after processing to the response of the analyte fortified in neat solvent. The results demonstrate that matrix effects are within ±20%. Matrix matched standards were used for quantification for XDE-777 in honey for this study.

## Extraction Efficiency

## Changes to Method

No changes were made to the method during the conduct of the ILV.

## CONCLUSION

Method is acceptable based on current guidelines: EPA Residue Chemistry Test Guidelines OPPTS 860.1340, the requirements of SANTE/2020/12830 Rev.2, as well as PMRA Regulatory Directive Dir98-02.

### 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:	The analytical method was evaluated and accepted in RR for Queen (January 2023) and is not
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being re-assessed in this application.

Reference: KCP 5.3.3.5/01

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 ( <i>M04</i> )	quantitation	312	70
	confirmation	312	125

**Table A 156: 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 157: 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 158: 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 159: 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  $\geq 0.9990$  and  $\geq 0.9991$  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  $\leq -18^{\circ}\text{C}$  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 study was evaluated and accepted in RR for Queen (January 2023) and is not being re-assessed in this application.
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Reference:	KCP 5.3.3.5/02
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 160: 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 161: 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 162: 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 163: 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 0.05 µg/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 correlation 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 0.015 µg/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 recovery set 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 necessary 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:	<p>The analytical method was evaluated and accepted in RR for Queen (January 2023) and is not being re-assessed in this application.</p> <p>The method meets all criteria of guidelines SANCO/825/00 rev. 8.1 to determine concentrations of prothioconazole-desthio in body fluid at the LOQ level of 0.05 mg/L, but according to the SANTE/2020/12830, Rev.2, the LOQ should be lower - 0.01 mg/L for body fluids and 0.01 mg/kg for body tissues.</p>
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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):	<p>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.</p> <p>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.</p> <p>Guidance document on residue analytical methods; SANCO/825/00 rev. 8.1, European Commission, Directorate General Health and Consumer Protection; 2010-11-16.</p> <p>OECD Guidance Document on Pesticide Residue analytical Methods; ENV/JM/Mono (2007); 2007-08-13.</p>
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  $\rightarrow$  70 (quantitation) and  $m/z$  312  $\rightarrow$  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 164: Validation of the method 01471 for the determination of prothioconazole-desthio in blood**

Substrate	Fortification level ( $\mu\text{g/L}$ )	Number of replicates	$m/z$ 312 $\rightarrow$ 70		$m/z$ 312 $\rightarrow$ 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  $\mu\text{g/L}$ , 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  $\mu\text{g/L}$  to 10.0  $\mu\text{g/L}$  ( $0.01 \times 10^{-6}$  to  $1 \times 10^{-6}$  % w/w) for both MRM transitions. Correlation coefficients were  $\geq 0.9997$  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  $\mu\text{g/L}$ , 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 2.1.2.6.2 Method validation

Comments of zRMS:	<p>The analytical method was successfully validated for the determination of fenpicoxamid (XDE-777) in body fluids (blood or urine) in accordance to guidance document SANTE/2020/12830, rev. 2.</p> <p>The limit of quantification was 0.01 mg/L.</p> <p>The average recoveries at each fortification level fell within the range of 95% to 106%.</p> <p>Relative standard deviations at each fortification level were all less than 20%.</p> <p>Matrix effects &gt; 20% were not observed for fenpicoxamid (XDE-777).</p>
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	The method is acceptable.
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Reference:	KCP 5.3.3.7/02
Report:	Senciuc, M.; 2023; Method Validation for the Determination of fenpicoxamid (XDE-777) in Body Fluids; Eurofins Agrosience Services EAG Laboratories GmbH, Eiselaue Weg 4, Geb.&Bldg. 5, 89081 Ulm; Lab Study No. S22-08468; Sponsor Study No. 221208 ; 30 March 2023; Published: No
Guideline(s):	SANTE/2020/12830 rev.1 Dir98-02
GLP:	Yes

### Method Scope

This method is applicable for the quantitative determination of residues of fenpicoxamid (XDE-777) in body fluids. The method was independently validated in human urine over the concentration range of 0.01-0.10 mg/L with a validated limit of quantitation of 0.01 mg/L.

### Method Principle

Residues of fenpicoxamid (XDE-777) are extracted from samples by using water containing 0.2 % phosphoric acid and acetonitrile containing 0.1 % phosphoric acid. The final sample is analysed for fenpicoxamid (XDE-777) by liquid chromatography coupled with positive-ion electrospray tandem mass spectrometry (LC-MS/MS).

### Linearity

The linearity of detector response was evaluated using solvent standard solutions. Calibration curves resulting from the injection of  $\geq 5$  standards over a nominal concentration range of 0.050 – 5.0 ng/mL (sample equivalent range of 0.002 – 0.20 mg/L) demonstrated linearity with coefficients of determinations (r) of at least 0.99 and calculated by linear regression analysis with 1/x weighting.

### Selectivity

The LC-MS/MS method is highly selective for both the quantitation and confirmation of fenpicoxamid (XDE-777). Significant peak response (>30% of the LOQ peak area) is not observed in reagent blank and extracts of untreated blank control samples at the expected retention times of the analyte. Unambiguous identification is ensured by monitoring two MS/MS transitions characteristic of the analyte as follows in the table below.

**Table 1: Transitions monitored**

Fenpicoxamid (XDE-777)	<i>m/z</i> 615/239 Q (quantitative)
Fenpicoxamid (XDE-777)	<i>m/z</i> 615/515 C (confirmatory)

### Confirmation

Confirmation of the presence of fenpicoxamid (XDE-777) was by comparison of retention time (liquid chromatography) of recovery samples with the retention time of the calibration standards as well as by monitoring two structurally characteristic MS/MS transitions by tandem mass spectrometry. Validation data obtained using the confirmatory MS/MS transition met the same acceptance criteria as the validation data generated using the quantitative MS/MS transition, therefore demonstrating that the analyte signal of the quantitative MS/MS transition is correct and not affected by any other compound.

### Limits of Detection and Quantitation

The limit of quantitation, defined as the lowest concentration of an analyte tested at which an unambiguous identification of the analyte can be proven and at which an acceptable mean recovery with an acceptable relative standard deviation is obtained, is 0.01 mg/L for the analyte in human urine.

The limit of detection is defined as 30% of the LOQ (0.003 mg/L). Samples fortified in the range of the LOD level were analyzed only to demonstrate that observable peaks at the LOD level could be distinguished from untreated control samples; the results were not included for average percent recovery calculations.

## RESULTS AND DISCUSSION

### Summary of Recovery

Results obtained were within guideline requirements (mean recovery 70-110%; RSD  $\leq$  20%). The two ion mass transitions could be used interchangeably for quantification and confirmation. The results obtained are summarised in the following tables.

**Table 2: Summary of quantitative recovery of fencicoxamid (XDE-777) (m/z 615/239)**

Matrix group	Matrix	LOQ	Fortification level	Recovery (%)		SD	RSD	n
			mg/L	mean	range	(%)	(%)	
Body Fluids	Human Urine	0.01	0.01	102	93 - 108	6	6	5
Body Fluids	Human Urine	0.01	0.10	95	86 - 110	10	10	5

**Table 3: Summary of confirmatory recovery of fencicoxamid (XDE-777)(m/z 615/515)**

Matrix group	Matrix	LOQ	Fortification level	Recovery (%)		SD	RSD	n
			mg/L	mean	range	(%)	(%)	
Body Fluids	Human Urine	0.01	0.01	106	97 - 110	5	5	5
Body Fluids	Human Urine	0.01	0.10	97	87 - 110	9	10	5

### Repeatability

Repeatability was not assessed as a part of this study.

### Working Solution Stability

Stock solutions of fencicoxamid (XDE-777) prepared in acetonitrile were tested after 34 days of storage at typically 0-10°C and were found to be stable.

Calibration standard solutions of fencicoxamid (XDE-777) prepared in acetonitrile/water (1/1, v/v) containing 0.1% phosphoric acid were tested after 34 days of storage at typically 0-10°C and were found to be stable.

### Sample Extract Stability

Sample extracts of fencicoxamid (XDE-777) in acetonitrile/water (1/1, v/v) containing 0.1% phosphoric acid were tested after 7 days of storage at typically 0-10°C and were found to be stable.

### Matrix Effects

Matrix effects were evaluated by comparing the response of the analyte fortified in a control extract after processing to the response of the analyte fortified in neat solvent. The results demonstrate that matrix effects are within  $\pm 20\%$ . Therefore, calibrations in neat solvent were used for quantification for this study.

### Extraction Efficiency

Extraction efficiency was not assessed as a part of this study.

## CONCLUSION

Method is acceptable based on current guidelines: SANTE/2020/12830 Rev.1, as well as PMRA Regulatory Directive Dir98-02.