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| REGISTRATION REPORT  Part B  Section 5  Analytical Methods  Detailed summary of the risk assessment |
| Product code: -  Product name(s): ULTRACENT 460 EC  Chemical active substance(s):  Prothioconazole, 160 g/L Spiroxamine, 300 g/L |
| Central Zone  Zonal Rapporteur Member State: Poland |
| CORE ASSESSMENT  (authorization) |
| Applicant: XXXX  Submission date: August 2023  update December 2023, update June 2024, update January 2025  MS Finalisation date: February 2025 |

Version history

|  |  |
| --- | --- |
| When | What |
| August 2023 | First submission – application according to Article 33 in connection with Article 34 of Regulation (EC) No. 1107/2009 with reference to unprotected data of the product INPUT 460 EC authorized in Poland |
| December 2023 | The dossier was updated to include available information on the unprotected data of the reference product INPUT 460 EC (R-61/2011). |
| June 2024 | The dossier was updated based on comments from the evaluating entity |
| January 2025 | The dossier was updated based on comments from the evaluating entity |
| February 2025 | Revised B5 in the context of the dossier update. |

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# Analytical methods

## Conclusion and summary of assessment

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

**In the context of the residue analytical methods, the submitted data are insufficient for evaluation.**

The unprotected data of the product Input needed to the authorisation requested was not provided.

It should be also considered that the requirements in the residue analytical methods area were changed from 2011.

The condition of the authorisation is the presentation of the data showing the access of the applicant to the currently required methods. The list of studies used for evaluation is also required.

Sufficiently sensitive and selective analytical methods are available for all analytes included in the residue definitions. The data requested were completed by the applicant.

| Commodity/crop | Supported/ Not supported |
| --- | --- |
| Winter wheat |  |
| Spring wheat |  |
| Spring barley |  |
| Winter barley |  |
| Winter triticale |  |
| Cereals | Supported |

No data is submitted in support of the application for authorization of ULTRACENT 460 EC. Reference is made to the unprotected data and dossier of INPUT 460 EC (R-61/2011, authorization holder Bayer AG), in accordance with Article 34 of Regulation 1107/2009/EC. It was not considered necessary to submit additional data and the evaluator is referred to the registration report of INPUT 460 EC.

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

### Analysis of the plant protection product (KCP 5.1.1)

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

~~No data is submitted in support of the application for authorization of ULTRACENT 460 EC. Reference is made to the unprotected data and dossier of INPUT 460 EC (R-61/2011, authorization holder Bayer AG), in accordance with Article 34 of Regulation 1107/2009/EC. It was not considered necessary to submit additional data and the evaluator is referred to the registration report of INPUT 460 EC.~~

An overview on the acceptable methods and possible data gaps for analysis of prothioconazole and spiroxamine in Prothioconazole 160 g/L + Spiroxamine 300 g/L EC is provided as follows:

**RMS comment on use of the art. 34 of the 1107/2009 to support ULTRACENT 460 EC registration in Poland**

From physicochemical perspective ULTRACENT 460 EC is considered equivalent/ comparable to already registered INPUT 460 EC in Poland under Composition’s comparison in accordance with Article 34 of Regulation 1107/2009. So, unprotected physicochemical data taken from INPUT 460 EC can be used to support ULTRACENT 460 EC registration in Poland.

|  |  |
| --- | --- |
| Comments of zRMS: | Accepted |

|  |  |
| --- | --- |
| Reference: | KCP 5.1.1/01 |
| Report | Accelerated Storage Stability Test by Heating at 54 ± 2°C of Prothioconazole 160 g/L + Spiroxamine 300 g/L EC, Kishora, K.S., 2023a, Report no. AG-G1571 |
| Guideline(s): | Yes, SANCO 3030/99 rev. 5 |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes |

Materials and methods

Test item:

Name: Prothioconazole 160 g/L + Spiroxamine 300 g/L EC

Active ingredients: Prothioconazole, Spiroxamine

Batch no.: JMG22X03A

Manufacturing date: 08/03/2023

Expiry date: 07/03/2025

Reference item:

Active substance: Prothioconazole

CAS no.: 178928-70-6

Purity: 99.91 %

Batch no.: CIPL/PTC/WRS/23/0120

Expiry date: December 2024

Active substance: Spiroxamine

CAS no.: 118134-30-8

Purity: 97.93 %

Batch no.: G1232128

Expiry date: 21/02/2026

Method:

Prothioconazole

Preparation of test item sample solution

Accurately 0.1 g of test item was weighed into a 100 mL volumetric flask, the contents were dissolved in 60 mL of acetonitrile by sonicating for 5 minutes. After equilibrated to room temperature, the volume was made up to the mark with acetonitrile. Further, an aliquot of 5.0 mL was diluted to 50 mL volumetric flask with acetonitrile and shaken thoroughly. These solutions were analysed for the active ingredient content by injecting to HPLC.

Chromatographic Conditions

The estimation of active ingredient content in the samples was carried out by means of HPLC operated under the following conditions:

Instrument: HPLC equipped with auto sampler, DA detector and PC based data system

Column: Eclipse XDB-C18, 5.0 µm, 150 mm long, 4.6 mm

Colum temperature: 30 °C

Mobile phase: 0.1 % H3PO4 in MQW/Acetonitrile/35/65 % v/v

Solvent Flow Rate: 1.0 mL/min

Detector wavelength: 254 nm

Injection Volume: 10 µL

Running time: 8.00 min

Spiroxamine

Preparation of test item sample solution

Accurately 0.1 g of test item was weighed into a 100 mL volumetric flask, the contents were dissolved in 60 mL of acetonitrile by sonicating for 2 minutes. After equilibrated to room temperature, the volume was made up to the mark with acetonitrile. Further, an aliquot of 5.0 mL was diluted to 50 mL volumetric flask with acetonitrile and shaken thoroughly. These solutions were analysed for the active ingredient content by injecting to GC.

Chromatographic Conditions

The estimation of active ingredient content in the samples was carried out by means of GC operated under the following conditions:

Instrument: HPLC equipped with auto sampler, DA detector and PC based data system

Instrument: Gas Chromatograph equipped with PC based data system

Detector: Flame Ionisation detector (FID)

Column: DC-1, 30 meters (length), 0.25 mm (i.d.), 0.25 µm film thickness

Gas Flow Rates: Carrier (Nitrogen): 3.0 mL/min

Hydrogen: 30 mM/min

Air 300 mL/min

Make up flow (N2): 30.0 mL/min

Injector Temperature: 260 °C

Detector temperature: 310 °C

Column Oven: Initial temperature: 150 °C

Initial hold time: 5 min

Ramp rate 1: 5°C/min

Final temperature: 280 °C

Final hold time: 5 min

Injection: Split

Split ratio: 2:1

Injection volume: 1µL

Validation - Results and discussions

Table 5.2‑1: Methods suitable for the determination of active substances prothioconazole and spiroxamine in plant protection product Prothioconazole 160 g/L + Spiroxamine 300 g/L EC

|  | Prothioconazole | Spiroxamine |
| --- | --- | --- |
| Author(s), year | Kishora, K.S., 2023a | Kishora, K.S., 2023a |
| Principle of method | HPLC-DA | HPLC-GC |
| Linearity  (linear between  mg/L / % range of the declared content)  (correlation coefficient, expressed as r) | The instrument method was linear  r = 0.99992  Range = 5.125 % to 51.254 %  y = 23546x + 1890 | The insturment method was linear  r= 0.99943  Range = 10.42 % to 102.44 %  y = 14.444x – 0.179 |
| Precision – Repeatability Mean  n = 10  (%RSD) | |  |  |  |  |  | | --- | --- | --- | --- | --- | | **AI content per sample [% w/w]** | **Average found**  **[% w/w]** | **RSD [%]** | **RSDr [%]** | **Hr** | | 16.37 | 16.3\* | 0.61\* | 1.76 | 0.35\* | | 16.32 | | 16.42 | | 16.31 | | 16.21 | | 16.32 | 16.3\*\* | 0.61\*\* | 1.76 | 0.35\*\* | | 16.24 | | 16.18 | | 16.36 | | 16.46 |   \* results from the precision test  \*\*results from the intermediate precision test | |  |  |  |  |  | | --- | --- | --- | --- | --- | | **AI content per sample [% w/w]** | **Average found**  **[% w/w]** | **RSD [%]** | **RSDr [%]** | **Hr** | | 30.46 | 30.4\* | 0.33\* | 1.60 | 0.21\* | | 30.32 | | 30.33 | | 30.43 | | 30.33 | | 30.48 | 30.4\*\* | 0.33\*\* | 1.60 | 0.21\*\* | | 30.24 | | 30.34 | | 30.41 | | 30.36 |   \* results from the precision test  \*\*results from the intermediate precision test |
| Accuracy  n = 3 at 3 fortification levels  (% Recovery) | |  |  |  | | --- | --- | --- | | **Fortification level [% w/w]** | **Recovery [%]** | **Mean Recovery [%]** | | 7.9 | 99.1 | 99.4 | | 15.9 | 100.7 | | 23.2 | 98.5 |   Acceptance criteria of per cent recovery was in the range ot 97 to 103 % for a.i. ≥10 (nominal). | |  |  |  | | --- | --- | --- | | **Fortification level [% w/w]** | **Recovery [%]** | **Mean Recovery [%]** | | 14.5 | 98.5 | 100.8 | | 29.9 | 101.7 | | 46.0 | 102.2 |   Acceptance criteria of per cent recovery was in the range ot 97 to 103 % for a.i. ≥10 (nominal). |
| Interference/ Specificity | Method is specific for Prothioconazole analysis | Method is specific for Spiroxamine analysis |
| Comment | The results comply with the requirements laid down in SANCO 3030/99 rev. 5. | The results comply with the requirements laid down in SANCO 3030/99 rev. 5. |

Conclusion

The analytical method for the determination of Prothioconazole and Spiroxamine in the test item Prothioconazole 160 g/L + Spiroxamine 300 g/L EC have been validated according to SANCO 3030/99 rev. 5. Therefore, the analytical method is considered acceptable.

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

~~No data is submitted in support of the application for authorization of ULTRACENT 460 EC. Reference is made to the unprotected data and dossier of INPUT 460 EC (R-61/2011, authorization holder Bayer AG), in accordance with Article 34 of Regulation 1107/2009/EC. It was not considered necessary to submit additional data and the evaluator is referred to the registration report of INPUT 460 EC.~~

*~~The following information can be found in the evaluation reports that were compiled for the authorization of INPUT 460 EC (R-61/2011) in Poland:~~*

~~There is no CIPAC method available for the determination of the active substance content of prothioconazole (JAU 6476) and spiroxamine (KWG 4168) in the fungicide INPUT 460 EC.~~

~~Determination of the prothioconazole content of the fungicide, INPUT 460 EC, was performed by reversed-phase high-performance liquid chromatography (HPLC) with isocratic concentration gradient and UV detection at 254 nm. Quantitative evaluation was performed by comparing the peak area with that of a reference substance used as an external standard.~~

~~For details of the method for the determination of prothioconazole, see document 5.1.1/01 (Seidel E., 2000).~~

~~The determination of spiroxamine (KWG 4168) in the product was performed by gas chromatography. After the addition of dipentylphthalate as an internal standard and dilution with dichloromethane, the spiroxamine content was determined by capillary gas chromatography with an FID detector. The internal standard was calibrated with the known content of KWG 4168. The spiroxamine content was calculated as the sum of the two enantiomers. For details of the method for the determination of spiroxamine, see document 5.1.1/02 (Teller M. G., 2000).~~

~~Validation of the method for the determination of prothioconazole was performed by Odendahl, A., 2000 (5.1.3/01), Odendahl A., 2001a) 5.1.3/02) and validation of the method for the determination of spiroxamine by Odendahl, A., 2001b (5.1.3/03), Odendahl A., 2001c) 5.1.3/04).~~

~~Validation data for prothioconazole~~

~~Specificity: no interference of the prothioconazole assay by the components contained in the formulation. The retention times of prothioconazole and the reference substance were the same.~~

~~Linearity: tests for linearity of the method were carried out on 6 samples of the formulation. A linear relationship was found over the range of concentrations tested, and the value of the correlation coefficient was 0.99999. The results of the linearity tests of the analytical method are in accordance with the criteria established in the European Union.~~

~~Accuracy (recovery): Recovery studies were performed on 6 formulation samples with a known amount of prothioconazole. The average recovery value was 99.925%, the relative deviation value was 0.122%.~~

~~Precision of the method: Precision tests were performed on 6 formulation samples. The coefficient of variation value was 0.222%.~~

~~Validation data for spiroxamine~~

~~Specificity: there was no interference with the determination of spiroxamine by the components contained in the formulation. The retention times of spiroxamine and the reference substance were the same.~~

~~Linearity: linearity studies of the method were carried out on 3 samples of the formulation (duplicate trials). A linear relationship was found over the range of concentrations tested, with a correlation coefficient value of 0.992. The results of the linearity tests of the analytical method are in accordance with the criteria established in the European Union.~~

~~Accuracy (recovery): Recovery studies were performed on 4 samples of the formulation with a known amount of spiroxamine. The mean recovery value was 99.7%, the relative deviation value was 0.5317%.~~

~~Precision of the method: Precision tests were performed on 5 formulation samples. Relative deviation value – 0.4137%.~~

~~Summary:~~

~~The information presented in the documents submitted on the methods of analysis of the plant protection product shows that the methods used for the determination of active substances in the plant protection product are specific methods, are characterised by sufficient linearity, accuracy and repeatability.~~

Technical spiroxamine does not contain any relevant impurities. However, an overview on the acceptable methods for analysis of relevant prothioconazole impurities in the plant protection product is provided as follows:

|  |  |
| --- | --- |
| Comments of zRMS: | Accepted |

|  |  |
| --- | --- |
| Reference: | KCP 5.1.1/02 |
| Report | Accelerated Storage Stability Test by Heating at 54 ± 2°C of Prothioconazole 160 g/L + Spiroxamine 300 g/L EC, Kishora, K.S., 2023a, Report no. AG-G1571 |
| Guideline(s): | Yes, SANCO 3030/99 rev. 5 |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes |

Materials and methods

Test item:

Name: Prothioconazole 160 g/L + Spiroxamine 300 g/L EC

Active ingredients: Prothioconazole, Spiroxamine

Batch no.: JMG22X03A

Manufacturing date: 08/03/2023

Expiry date: 07/03/2025

Reference item:

Desthio Prothioconazole

Purity: 97.15 %

Batch no.: SPPL/PRO-DT/04/23

Expiry date: 10/02/2024

Deschloro Prothioconazole

Purity: 95.84 %

Batch no.: VL-2711-F

Expiry date: 29/05/2025

Toluene

Purity: 99.9 %

Batch no.: D402016

Expiry date: 01/10/2024

Method for desthio prothioconazole and deschloro prothioconazole

For precision tests accurately 0.05 g of test item was weighed into a 50 mL volumetric flask, the contents were dissolved in 30 mL of acetonitrile by sonicating for 5 minutes. After equilibrated to room temperature, the volume was made up to the mark with acetonitrile. These solutions were analysed for impurity content by injecting to LC/MS.

For accuracy tests, about 0.01 g of the test item was weighted in five replications at each of two fortification levels into 10 mL volumetric flasks, the contents were dissolved in diluent by sonicating for 1 minutes. After equilibrated to room temperature, the lower and higher fortification levels, test item was fortified with about 0.12 and 0.6 mL of impurity reference standard stock solution. Then the volume was made up to the mark with acetonitrile. These solutions were analysed for impurity content by injecting to LC/MS.

Chromatographic Conditions

The estimation of impurity ingredient content in the samples was carried out by means of LC/MS operated under the following conditions:

Instrument: Applied Biosystems/API 3200 mass spectrometer operating Analyst software with Shimadzu liquid chromatograph

Column: Shim-pack C18, 5.0 µm, 250 mm long, 4.6 mm i.d.

Colum temperature: 40 °C

Mobile phase A: 0.1 % Formic acid in MQW

Mobile phase B: 0.1 % Formic acid in Acetonitrile

Gradient: Time (min) A% B%

0.01 50 50

4.00 50 50

5.00 20 80

12.00 20 80

12.50 50 50

Solvent Flow Rate: 1.0 mL/min

Injection Volume: 20 µL

Running time: 16.00 min

Ionization Mode: ESI (+)

Ion source: Turbo spray

Mass transitions: quantification confirmation

Desthio Prothioconazole *m/z* 312->70 *m/z* 312->125

Deschloro Prothioconazole *m/z* 310->155 *m/z* 310->292

Method for toluene

For precision tests accurately 0.1 g of test item was weighed into GC headspace vial and 1 mL of diluent (N-methyl-2-pyrrolidinone) was added into the vial. Headspace vials were sealed with aluminium cap and rubber septa using crimper. Vials were analysed by injecting into GC/FID.

For accuracy tests, about 0.1 g of test item was weighed in five replications at each fortification level into GC headspace vial and were fortified with 1 mL of impurity reference stock solution. Later, headspace vials were sealed with aluminium cap and rubber septa using crimper. Vials were analysed by injecting into GC/FID.

Chromatographic Conditions

The estimation of impurity ingredient content in the samples was carried out by means of GC/FID operated under the following conditions:

Instrument: Gas chromatograph equipped with FID and PC based data system

Column: Rxi-624 Sil MS, 30 m long, 0.32 i.d., 1.8 µm film thickness

Split ratio: 10:1

Detector: 290 °C

Colum oven: Initial 50 °C, hold for 3 min.

Ramp 1: 50 °C/min to 100 °C, hold for 0 min

Ramp 2: 30 °C/min to 280 °C, hold for 0 min

Flow rate: 2.0 ml/min (carrier), 40 mL/min (hydrogen), 300 mL/min (air), Nitrogen/Helium (make up) 25 mL/min

Oven temp.: 95 °C

Sample line temp.: 105 °C

Transfer line temp.: 115 °C

Validation - Results and discussions

Table 5.2‑2: Methods suitable for the determination of the relevant impurities in plant protection product (PPP) ULTRACENT 460 EC

|  | Desthio Prothioconazole  (max 0.5 g/kg) | Deschloro Prothioconazole  (max 0.08 g/kg) | Toluene  (max. 5 g/kg) |
| --- | --- | --- | --- |
| Author(s), year | Kishora, K.S., 2024a | Kishora, K.S., 2024a | Kishora, K.S., 2024a |
| Principle of method | LC-MS/MS | LC-MS/MS | GC/FID |
| Linearity  (linear between  mg/L)  (correlation coefficient, expressed as r) | 0.024 mg/L to 0.482 mg/L (corresponding to 0.0024 to 0.0482% w/w)  Regression Model: y = a + b\*x.  y = 2022174x - 5193, r = 0.99905. | 0.024 mg/L to 0.472 mg/L (corresponding to 0.0024 to 0.0472% w/w)  Regression Model: y = a + b\*x.  y = 182101x - 599, r = 0.99805. | 1.528 mg/L to 50.949 mg/L (corresponding to 0.0015 to 0.0509% w/w)  Regression Model: y = a + b\*x.  y = 6624x + 3526, r = 0.99939. |
| Precision – Repeatability Mean  n = 10  (%RSD) | results for the precision test prepared by analyst 1:  Mean contend: 0.00656% w/w (n=5)  %RSD: 2.287  %RSDr: 5.713  Hr: 0.40  results for precision test prepared by analyst 2:  Mean contend: 0.00695% w/w (n=5)  %RSD: 0.576  %RSDr: 5.662  Hr: 0.10 | results for the precision test prepared by analyst 1:  Mean contend: 0.00642% w/w (n=5)  %RSD: 1.713  %RSDr: 5.729  Hr: 0.30  results for precision test prepared by analyst 2:  Mean contend: 0.00665% w/w (n=5)  %RSD: 3.308  %RSDr: 5.701  Hr: 0.58 | results for the precision test prepared by analyst 1:  Mean contend: 0.0158% w/w (n=5)  %RSD: 1.899  %RSDr: 5.005  Hr: 0.38  results for precision test prepared by analyst 2:  Mean contend: 0.0160% w/w (n=5)  %RSD: 2.500  %RSDr: 4.994701  Hr: 0.50 |
| Accuracy  n = 2  (% Recovery) | |  |  |  | | --- | --- | --- | | **Fortification level [mg/kg]** | **Recovery [%]** | **Mean Recovery [%]** | | 0.004 | 103.11 | 100.36 | | 0.03 | 97.60 | | |  |  |  | | --- | --- | --- | | **Fortification level [mg/kg]** | **Recovery [%]** | **Mean Recovery [%]** | | 0.005 | 96.02 | 100.72 | | 0.03 | 105.42 | | |  |  |  | | --- | --- | --- | | **Fortification level [mg/kg]** | **Recovery [%]** | **Mean Recovery [%]** | | 0.002 | 90.02 | 94.31 | | 0.02 | 98.61 | |
| Interference/ Specificity | Interferences at the retention time of the analyte were not observed when injecting reagent blank samples. | Interferences at the retention time of the analyte were not observed when injecting reagent blank samples. | Interferences at the retention time of the analyte were not observed when injecting reagent blank samples. |
| LOQ | 0.005% w/w | 0.005% w/w | 0.00178% w/w |
| LOD | 0.00291 mg/L corresponding to 0.00011% w/w | 0.00288 mg/L corresponding to 0.00013% w/w | 0.255 mg/L corresponding to 0.000197% w/w |
| Comment | Primary method is highly selevtive, no confirmation required | Primary method is highly selevtive, no confirmation required | - |

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

~~No data is submitted in support of the application for authorization of ULTRACENT 460 EC. Reference is made to the unprotected data and dossier of INPUT 460 EC (R-61/2011, authorization holder Bayer AG), in accordance with Article 34 of Regulation 1107/2009/EC. It was not considered necessary to submit additional data and the evaluator is referred to the registration report of INPUT 460 EC~~.

No formulants with toxicological or ecotoxicological relevant compounds are present in the formulation. Therefore, no analytical methods for the determination of formulants are necessary

#### Applicability of existing CIPAC methods (KCP 5.1.1)

~~No data is submitted in support of the application for authorization of ULTRACENT 460 EC. Reference is made to the unprotected data and dossier of INPUT 460 EC (R-61/2011, authorization holder Bayer AG), in accordance with Article 34 of Regulation 1107/2009/EC. It was not considered necessary to submit additional data and the evaluator is referred to the registration report of INPUT 460 EC.~~

No existing CIPAC method was found to be applicable for analysis of spiroxamine in the technical material or plant protection product. However, applicable CIPAC methods are available for prothioconazole (CIPAC 745) using HPLC/MS-MS.

### Methods for the determination of residues (KCP 5.1.2)

No data is submitted in support of the application for authorization of ULTRACENT 460 EC. Reference is made to the unprotected data and dossier of INPUT 460 EC (R-61/2011, authorization holder Bayer AG), in accordance with Article 34 of Regulation 1107/2009/EC. It was not considered necessary to submit additional data and the evaluator is referred to the registration report of INPUT 460 EC.

*The following information can be found in the evaluation reports that were compiled for the authorization of INPUT 460 EC (R-61/2011) in Poland:*

Validated analytical methods are provided for the determination of residues of Prothioconazole (JAU 6476) and Spiroxamine (KWG 4168) in material of agricultural and animal origin and in soil, water and air.

The limits of quantification (LOQ) of the included analytical methods are sufficient to verify compliance with the respective reference values for food of agricultural origin, material of animal origin, water, soil and air. Determinations were performed at different levels of fortification including the proposed limits of quantification of the analytical methods.

Conclusions:

1) Average recovery rates obtained: 70 to 110%.

2) Relative standard deviation obtained: <20%.

3) The proposed analytical methods are specific for the analytes determined.

4) No interfering compounds were found to be present in the matrices tested (< 30% quantification limit). limit of quantification).

5) Methods meet Sanco requirements: "Guidance document on residue analytical methods SANCO/825/00 rev.7 (17.03.04)' and 'Guidance document Quality control procedures for pesticide residues analysis SANCO/10232/2006 (24.03.06)'.

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

Table 5.2‑3: Validated methods for the generation of pre-authorization data (spiroxamine)

| Component of residue definition: Sum of Spiroxamine and metabolites containing the 4-*tert*-butylcyclo-hexanone moiety, expressed as Spiroxamine | | | | |
| --- | --- | --- | --- | --- |
| Matrix type | Method type | Method LOQ | Principle of method  (i.e. GC-MS or HPLC-UV) | Author(s), year / missing / EU agreed |
| Cereals (Residues) | Primary | 0.05 mg/kg | LC-MS/MS | Heinemann (2002), Report No. 00769 !M-077994-01-1!MR-253/02 / EU agreed |
| Confirmatory  (if required) | Not required | | |
| Animal products, food of animal origin  (Residues from livestock feeding studies) | No risk assessment methods are submitted (EFSA Journal 2010;8(10):1719). | | | |
| Soil, water, sediment  (Environmental fate) | Not relevant, no studies submitted. | | | |
| Soil, water, etc.  (Efficacy) | Not relevant, no studies submitted. | | | |
| Feed, body fluids, etc.  (Toxicology) | Not relevant, no studies submitted. | | | |
| Body fluids, air, etc.  (Exposure) | Not relevant, no studies submitted. | | | |
| Water, buffer solutions, etc. (Properties) | Not relevant, no studies submitted. | | | |

Table 5.2‑4: Validated methods for the generation of pre-authorization data (prothioconazole)

| Component of residue definition: Prothioconazole | | | | |
| --- | --- | --- | --- | --- |
| Matrix type | Method type | Method LOQ | Principle of method  (i.e. GC-MS or HPLC-UV) | Author(s), year / missing / EU agreed |
| Cereals (Residues) | Primary | 0.01 mg/kg for PTZ-hydroxy desthio and TDMs | LC-MS/MS | KCP 5.1.2/01 Jooß, S. (2023), Report No. S22-05883  KCP 5.1.2/02 Jooß, S. (2023), Report No. S22-05884 |
| Confirmatory  (if required) | Not required | | |
| Honey  (Residues) | Primary | 0.01 mg/kg for PTZ-hydroxy desthio and TDMs | LC-MS/MS | KCP 5.1.2/01 Jooß, S. (2023), Report No. S22-05883  KCP 5.1.2/02 Jooß, S. (2023), Report No. S22-05884  KCP 5.2/01 Peris Mestre D. (2024), Report No. E23-0116  *submitted under KCA 6.10/01* |
| Confirmatory  (if required) | Not required | | |
| Storage Stability (Honey) | Primary | 0.01 mg/kg for PTZ-hydroxy desthio and TDMs | LC-MS/MS | KCP 5.1.2/03 Jooß, S. (2024), Report No. S23-102955  *submitted under KCA 6.1/02* |
| Confirmatory  (if required) | Not required | | |
| Soil, water, sediment  (Environmental fate) | Not relevant, no studies submitted. | | | |
| Soil, water, etc.  (Efficacy) | Not relevant, no studies submitted. | | | |
| Feed, body fluids, etc.  (Toxicology) | Not relevant, no studies submitted. | | | |
| Body fluids, air, etc.  (Exposure) | Not relevant, no studies submitted. | | | |
| Water, buffer solutions, etc. (Properties) | Not relevant, no studies submitted. | | | |

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

### 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.

No data is submitted in support of the application for authorization of ULTRACENT 460 EC. Reference is made to the unprotected data and dossier of INPUT 460 EC (R-61/2011, authorization holder Bayer AG), in accordance with Article 34 of Regulation 1107/2009/EC. It was not considered necessary to submit additional data and the evaluator is referred to the registration report of INPUT 460 EC.

### Description of analytical methods for the determination of residues of prothioconazole (KCP 5.2)

*The following information can be found in the evaluation reports that were compiled for the authorization of INPUT 460 EC (R-61/2011) in Poland:*

Conclusions:

1) The limits of quantification of the provided analytical methods are adequate for checking compliance with the relevant reference values for food, water, soil and air.

2) Average recovery factor levels: 70 to 110%.

3) Relative standard deviation: <20%.

4) No interfering compounds (below 30% analytical method limit of quantification).

5) The analytical equipment used and the techniques employed are appropriate for the determination of residues.

6) The analytical methods included are specific (HPLC-MS/MS, GC-MS) so no additional confirmatory methods are required.

The analytical methods listed are therefore suitable for the analysis of residues of prothioconazole, spiroxamine and their metabolites in foodstuffs of plant and animal origin, air, water and soil.

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

~~No data is submitted in support of the application for authorization of ULTRACENT 460 EC. Reference is made to the unprotected data and dossier of INPUT 460 EC (R-61/2011, authorization holder Bayer AG), in accordance with Article 34 of Regulation 1107/2009/EC. It was not considered necessary to submit additional data and the evaluator is referred to the registration report of INPUT 460 EC.~~

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

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

| Matrix | Residue definition | MRL / limit | Reference for MRL/level Remarks |
| --- | --- | --- | --- |
| Plant, high water content | Prothioconazole-desthio  (sum of isomers) | 0.01 mg/kg | Regulation (EU) 2019/552,  annex II |
| Plant, high acid content | 0.01 mg/kg |
| Plant, high protein/high starch content (dry commodities) | 0.01 mg/kg |
| Plant, high oil content | 0.01 mg/kg |
| Plant, difficult matrices (hops, spices, tea) | 0.01 mg/kg |
| Muscle | Prothioconazole-desthio  (sum of isomers) | 0.01 mg/kg |
| Milk | 0.01 mg/kg |
| Eggs | 0.01 mg/kg |
| Fat | 0.01 mg/kg |
| Liver, kidney | 0.1 mg/kg |
| Soil  (Ecotoxicology) | Prothioconazole, prothioconazole-desthio | 0.05 mg/kg | common limit |
| Drinking water  (Human toxicology) | Prothioconazole, prothioconazole-desthio | 0.1 µg/L | general limit for drinking water |
| Surface water  (Ecotoxicology) | Prothioconazole, prothioconazole-desthio | 0.308 mg/L (prothioconazole)    3.34 µg/L (JAU-6476-  desthio) | NOEC *Oncorhynchus mykiss*  NOEC *Oncorhynchus mykiss*  EFSA scientific report (2007) 106, 1-98 |
| Air | Prothioconazole, prothioconazole-desthio | 60 µg/m³ (prothioconazole)  3 µg/m³ (JAU 6476-  desthio) | AOEL sys: 0.2 mg/kg  bw/d;  AOEL sys: 0.01 mg/kg  bw/d  EFSA scientific report (2007) 106, 1-98 |
| Tissue (meat or liver) | Prothioconazole-desthio | 0.1 mg/kg | Not classified as T / T+ but  required according to  Regulation (EU) 283/2013 |
| Body fluids | 0.05 mg/L | Not classified as T / T+ but  required according to  Regulation (EU) 283/2013 |

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

An overview on the acceptable methods and possible data gaps for analysis of prothioconazole residues in plant matrices is given in the following tables.

Table 5.3‑2: Validated methods for food and feed of plant origin (required for all matrix types, “difficult” matrix only when indicated by intended GAP)

| Component of residue definition: Prothioconazole-desthio (sum of isomers) | | | | |
| --- | --- | --- | --- | --- |
| Matrix type | Method type | Method LOQ | Principle of method (i.e. GC-MS or HPLC-UV) | Author(s), year / missing / EU agreed |
| High water content | Primary | 0.01 mg/kg | LC-MS/MS | KCA 4.2 Chambers & Jarret (2014), Report No. VC/13/017 / Yes (RAR 2023) |
| ILV | 0.01 mg/kg | LC-MS/MS | KCA 4.2 Thies (2014), Report No. 2014/0110/01 / Yes (RAR 2023) |
| Confirmatory  (if required) | Not required | | - |
| High acid content | Primary | 0.01 mg/kg | LC-MS/MS | KCA 4.2 Chambers & Jarret (2014), Report No. VC/13/017 / Yes (RAR 2023) |
| ILV | 0.01 mg/kg | LC-MS/MS | KCA 4.2 Thies (2014), Report No. 2014/0110/01 / Yes (RAR 2023) |
| Confirmatory  (if required) | Not required | | - |
| High oil content | Primary | 0.01 mg/kg | LC-MS/MS | KCA 4.2 Chambers & Jarret (2014), Report No. VC/13/017 / Yes (RAR 2023) |
| ILV | 0.01 mg/kg | LC-MS/MS | KCA 4.2 Thies (2014), Report No. 2014/0110/01 / Yes (RAR 2023) |
| Confirmatory  (if required) | Not required | | - |
| High protein/high starch content (dry) | Primary | 0.01 mg/kg | LC-MS/MS | KCA 4.2 Chambers & Jarret (2014), Report No. VC/13/017 / Yes (RAR 2023) |
| ILV | 0.01 mg/kg | LC-MS/MS | KCA 4.2 Thies (2014), Report No. 2014/0110/01 / Yes (RAR 2023) |
| Confirmatory  (if required) | Not required | | - |
| Difficult (if required, depends on intended use) | Primary | Not required | | - |
| ILV |
| Confirmatory  (if required) |

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

Table 5.3‑3: Statement on extraction efficiency

|  | Method for products of plant origin |
| --- | --- |
| Required, available from: | KCA 4.2 Desmaris, F. (2015), Report No. MR-15/117, RAR 2023, vol. 3 B5 |
| Not required, because: | - |

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. Method meets 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.

~~No data is submitted in support of the application for authorization of ULTRACENT 460 EC. Reference is made to the unprotected data and dossier of INPUT 460 EC (R-61/2011, authorization holder Bayer AG), in accordance with Article 34 of Regulation 1107/2009/EC. It was not considered necessary to submit additional data and the evaluator is referred to the registration report of INPUT 460 EC.~~

*~~The following information can be found in the evaluation reports that were compiled for the authorization of INPUT 460 EC (R-61/2011) in Poland:~~*

~~PROTHIOCONAZOLE~~

~~Prothioconazole, metabolite JAU6476-destio:~~

~~- HPLC-MS/MS method (Heineman, O, 2001);~~

~~LOQ: 0,01mg/kg for wheat, barley, rape seed~~

~~LOQ: 0.05 mg/kg for other plant material.~~

~~- GC-MS method (Weeren, Pelz, 2000) equivalent to method 00086/ M033, DFG S19~~

~~LOQ: 0.05mg/kg for cereal grains~~

~~LOQ: 0.05mg/kg fruit, vegetables~~

~~Analytical methods included in the dossier (prothioconazole)~~

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **~~Reference~~** | **~~Heinemann~~**  **~~2000a~~**  **~~(00598)~~** | | | | **~~Heinemann~~**  **~~2000a~~**  **~~(00598)~~** | | | | **~~Heinemann 2000b~~** |
| ~~Linearity~~ | **~~Yes~~** | **~~Yes~~** | **~~Yes~~** | **~~Yes~~** | **~~Yes~~** | **~~Yes~~** | **~~Yes~~** | **~~Yes~~** | **~~Yes~~** |
| **~~RSD~~**  **~~(%) (n)~~** | **~~5.4-5.7 (5)~~** | **~~1.7-5.7 (5)~~** | **~~2.0-2.1 (5)~~** | **~~3.1-8.8 (5)~~** | **~~7.2-8.2 (5)~~** | **~~1.3-5.0 (5)~~** | **~~3.3-4.5 (5)~~** | **~~2.4-4.6 (5)~~** | **~~0.5-11.0 (5)~~** |
| **~~Recovery~~**  **~~%~~** | **~~92-112~~**  **~~(98)~~** | **~~80-99~~** | **~~91-104~~** | **~~67-100~~**  **~~(82)~~** | **~~82-104~~** | **~~84-98~~** | **~~77-86~~** | **~~72-100~~** | **~~81-106~~** |
| **~~Fortification level~~**  **~~(mg/kg)~~** | **~~0.01-0.1~~** | **~~0.05-5.0~~** | **~~0.01-0.1~~** | **~~0.05-5.0~~** | **~~0.01-0.1~~** | **~~0.05-5.0~~** | **~~0.01-0.1~~** | **~~0.05-5.0~~** | **~~0.01-0.1~~** |
| **~~LOQ (mg/kg)~~** | **~~0.01~~** | **~~0.05~~** | **~~0.01~~** | **~~0.05~~** | **~~0.01~~** | **~~0.05~~** | **~~0.01~~** | **~~0.05~~** | **~~0.1~~** |
| **~~Id Detection~~** | **~~HPLC-MS/MS~~** | **~~“~~** | **~~“~~** | **~~“~~** | **~~HPLC-MS/MS~~** | **~~“~~** | **~~“~~** | **~~“~~** | **~~HPLC-MS/MS~~** |
| **~~Division, purification~~** | **~~liquid-liquid~~**  **~~hexane and dichloromethane~~** | **~~“~~** | **~~“~~** | **~~“~~** | **~~liquid-liquid~~**  **~~hexane and dichloromethane~~** | **~~“~~** | **~~“~~** | **~~“~~** | **~~liquid-liquid~~**  **~~hexane and dichloromethane~~** |
| **~~Extraction~~** | **~~acetonitrile/water~~** | **~~“~~** | **~~“~~** | **~~“~~** | **~~acetonitrile/water~~** | **~~“~~** | **~~“~~** | **~~“~~** | **~~acetonitrile/water~~** |
| **~~Analyte~~** | **~~JAU6476~~** | **~~“~~** | **~~“~~** | **~~“~~** | **~~JAU6476-detio~~** | **~~“~~** | **~~“~~** | **~~“~~** | **~~JAU6476~~** |
| **~~Tested material~~** | **~~Wheat grain~~** | **~~Wheat straw and green material~~** | **~~Barley grain~~** | **~~Barley green material~~** | **~~Wheat grain~~** | **~~Wheat straw and green material~~** | **~~Barley grain~~** | **~~Barley green material~~** | **~~Wheat grain~~** |

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **~~Reference~~** | **~~(00598/M001)~~** | | | | | | **~~Heinemann~~**  **~~2000b~~**  **~~(00598/M001)~~** | | **~~Heinemann~~**  **~~2000b~~**  **~~(00598/M001)~~** | | | | | | | | |
| ~~Linearity~~ | **~~Yes~~** | | **~~Yes~~** | **~~Yes~~** | **~~Yes~~** | | **~~Yes~~** | | **~~Yes~~** | | | **~~Yes~~** | | **~~Yes~~** | **~~Yes~~** | **~~Yes~~** | |
| **~~RSD~~**  **~~(%) (n)~~** | **~~2.5-11.9~~** | | **~~6.7-7.9~~** | **~~2.6-8.2 (5)~~** | **~~10.7-17.9 (5)~~** | | **~~1.6-8.3 (5)~~** | | **~~1.8-11.0 (5)~~** | | | **~~1.4-4.8 (5)~~** | | **~~4.6-7.7 (5)~~** | **~~1.1-6.8 (5)~~** | **~~10.8-11.7 (5)~~** | |
| **~~Recovery~~**  **~~%~~** | **~~65-100~~**  **~~(89)~~** | | **~~72-103~~** | **~~79-104~~** | **~~69-118~~**  **~~(89)~~** | | **~~74-117~~**  **~~(94)~~** | | **~~71-96~~** | | | **~~86-97~~** | | **~~76-94~~** | **~~76-95~~** | **~~70-95~~** | |
| **~~Fortification level~~**  **~~(mg/kg)~~** | **~~0.05-5.0~~** | | **~~0.01-0.1~~** | **~~0.05-5.0~~** | **~~0.01-0.1~~** | | **~~0.05-5.0~~** | | **~~0.01-0.1~~** | | | **~~0.05-5.0~~** | | **~~0.01-0.1~~** | **~~0.05-5.0~~** | **~~0.01-0.1~~** | |
| **~~LOQ (mg/kg)~~** | **~~0.05~~** | | **~~0.01~~** | **~~0.05~~** | **~~0.01~~** | | **~~0.05~~** | | **~~0.01~~** | | | **~~0.05~~** | | **~~0.01~~** | **~~0.05~~** | **~~0.01~~** | |
| **~~Detection~~** | **~~“~~** | | **~~“~~** | **~~“~~** | **~~“~~** | | **~~“~~** | | **~~HPLC-MS/MS~~** | | | **~~“~~** | | **~~“~~** | **~~“~~** | **~~“~~** | |
| **~~Division, purification~~** | **~~“~~** | | **~~“~~** | **~~“~~** | **~~“~~** | | **~~“~~** | | **~~liquid-liquid~~**  **~~hexane and dichloromethane~~** | | | **~~“~~** | | **~~“~~** | **~~“~~** | **~~“~~** | |
| **~~Extraction~~** | **~~“~~** | | **~~“~~** | **~~“~~** | **~~“~~** | | **~~“~~** | | **~~acetonitrile/water~~** | | | **~~“~~** | | **~~“~~** | **~~“~~** | **~~“~~** | |
| **~~Analyte~~** | **~~“~~** | | **~~“~~** | **~~“~~** | **~~“~~** | | **~~“~~** | | **~~JAU6476-detio~~** | | | **~~“~~** | | **~~“~~** | **~~“~~** | **~~“~~** | |
| **~~Tested material~~** | **~~Wheat straw and green material~~** | | **~~Barley grain~~** | **~~Barley green material~~** | **~~Rape seed~~** | | **~~Rapeseed green material~~** | | **~~Wheat seed~~** | | | **~~Wheat straw and green material~~** | | **~~Barley grain~~** | **~~Barley green material~~** | **~~Seed rapeseed~~** | |
| **~~Reference~~** | | |  | **~~Heinemann~~**  **~~2001a~~**  **~~(00647)~~** | | | | | | | | | | **~~Heinemann~~**  **~~2001a~~**  **~~(00647)~~** | | **~~Weeren, Pelz 2000~~**  **~~(00086/ M033, DFG Method S19)~~** | | | |
| ~~Linearity~~ | | | **~~Yes~~** | **~~Yes~~** | **~~Yes~~** | | **~~Yes~~** | | **~~Yes~~** | | **~~Yes~~** | **~~Yes~~** | | **~~Yes~~** | | **~~Yes~~** | | **~~Yes~~** | |
| **~~RSD~~**  **~~(%) (n)~~** | | | **~~1.3-5.4 (5)~~** | **~~1.5-3.7 (5)~~** | **~~1.2-5.4 (5)~~** | | **~~1.6-2.1 (5)~~** | | **~~1.5-4.7 (5)~~** | | **~~0.6-0.9 (5)~~** | **~~1.8-3.1 (5)~~** | | **~~0.8-6.0 (5)~~** | | **~~3.9-5.3 (5)~~** | | **~~5.3-9.9 (5)~~** | |
| **~~Recovery~~**  **~~%~~** | | | **~~80-98~~** | **~~96-105~~** | **~~95-113~~** | | **~~98-103~~** | | **~~93-113~~** | | **~~96-100~~** | **~~87-97~~** | | **~~95-116~~** | | **~~91-119~~**  **~~(104)~~** | | **~~75-98~~** | |
| **~~Fortification level~~**  **~~(mg/kg)~~** | | | **~~0.05-5~~** | **~~0.01-0.1~~** | **~~0.05-5.0~~** | | **~~0.01-0.1~~** | | **~~0.05-5.0~~** | | **~~0.02-0.2~~** | **~~0.01-0.1~~** | | **~~0.05-5.0~~** | | **~~0.02-0.2~~** | | **~~0.02-0.2~~** | |
| **~~LOQ (mg/kg)~~** | | | **~~0.05~~** | **~~0.01~~** | **~~0.05~~** | | **~~0.01~~** | | **~~0.05~~** | | **~~0.02~~** | **~~0.01~~** | | **~~0.05~~** | | **~~0.02~~** | | **~~0.02~~** | |
| **~~Detection~~** | | | **~~“~~** | **~~HPLC-MS/MS~~** | **~~“~~** | | **~~“~~** | | **~~“~~** | | **~~“~~** | **~~“~~** | | **~~“~~** | | **~~GC/MS~~** | | **~~“~~** | |
| **~~Division, purification~~** | | | **~~“~~** | **~~liquid-liquid~~**  **~~hexane and dichloromethane~~** | **~~“~~** | | **~~“~~** | | **~~“~~** | | **~~“~~** | **~~“~~** | | **~~“~~** | | **~~liquid-liquid~~**  **~~ethyl acetate and cyclohexane.~~**  **~~Gel chromatography~~** | | **~~“~~** | |
| **~~Extraction~~** | | | **~~“~~** | **~~acetonitrile/water~~** | **~~“~~** | | **~~“~~** | | **~~“~~** | | **~~“~~** | **~~“~~** | | **~~“~~** | | **~~aceton/e/water~~** | | **~~“~~** | |
| **~~Analyte~~** | | | **~~“~~** | **~~JAU6476-detio~~** | **~~“~~** | | **~~“~~** | | **~~“~~** | | **~~“~~** | **~~“~~** | | **~~“~~** | | **~~JAU6476-detio~~** | | **~~“~~** | |
| **~~Tested material~~** | | | **~~Rapeseed green material~~** | **~~Wheat Grain~~** | **~~Wheat straw and green material~~** | | **~~Barley grain~~** | | **~~Barley green material~~** | | **~~Barley malt~~** | **~~Rape seed~~** | | **~~Rape seed green material~~** | | **~~Tomatoes and oranges~~** | | **~~Wheat seed~~** | |

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **~~Reference~~** |  | | **~~Class 2001 (00086/ M033, DFG Method S19, ILV)~~** | |
| ~~Linearity~~ | **~~Yes~~** | **~~Yes~~** | **~~Yes~~** | **~~Yes~~** |
| **~~RSD~~**  **~~(%) (n)~~** | **~~4.8-13.0 (5)~~** | **~~3.9-10.0 (5)~~** | **~~4.0-5.0 (5)~~** | **~~3.0-4.0 (5)~~** |
| **~~Recovery~~**  **~~%~~** | **~~77-121~~** | **~~64-84~~**  **~~(72)~~** | **~~91-108~~** | **~~95-105~~** |
| **~~Fortification level~~**  **~~(mg/kg)~~** | **~~0.05-5.0~~** | **~~0.02-0.2~~** | **~~0.02-0.2~~** | **~~0.02-0.2~~** |
| **~~LOQ (mg/kg)~~** | **~~0.05~~** | **~~0.02~~** | **~~0.02~~** | **~~0.02~~** |
| **~~Detection~~** | **~~“~~** | **~~“~~** | **~~GC/MS~~** | **~~“~~** |
| **~~Division, purification~~** | **~~“~~** | **~~Gel chromatography~~** | **~~liquid-liquid~~**  **~~ethyl acetate and cyclohexane.~~**  **~~Gel chromatography~~** | **~~“~~** |
| **~~Extraction~~** | **~~“~~** | **~~acetone/water and acetonitrile~~** | **~~acetone/water~~** | **~~“~~** |
| **~~Analyte~~** | **~~“~~** | **~~“~~** | **~~JAU6476-detio~~** | **~~“~~** |
| **~~Tested material~~** | **~~Wheat straw and green material~~** | **~~Rape seed~~** | **~~Tomato~~** | **~~Wheat seed~~** |

#### 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-desthio in animal matrices is given in the following tables.

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

| Component of residue definition: Prothioconazole-desthio (sum of isomers) | | | | |
| --- | --- | --- | --- | --- |
| Matrix type | Method type | Method LOQ | Principle of method (*i.e.* GC-MS or HPLC-UV) | Author(s), year / missing / EU agreed |
| Milk | Primary | 0.01 mg/kg | LC-MS/MS | KCA 4.2 Freitag (2007), Report No. MR-06/199 / Yes (RAR 2023) |
| ILV | 0.01 mg/kg | LC-MS/MS | KCA 4.2 Schwarz & Class (2007), Report No. P/B 1226 G / Yes (RAR 2023) |
| Confirmatory  (if required) | Not required | | - |
| Eggs | Primary | 0.01 mg/kg | LC-MS/MS | KCA 4.2 Schulte & Oel (2006), Report No. M-279725-03-01 / Yes (RAR 2023) |
| ILV | 0.01 mg/kg | LC-MS/MS | KCA 4.2 Bacher (2006), Report No. P/B 1111 G / Yes (RAR 2023) |
| Confirmatory  (if required) | Not required | | - |
| Muscle | Primary | 0.01 mg/kg | LC-MS/MS | KCA 4.2 Freitag (2007), Report No. MR-06/199 / Yes (RAR 2023) |
| ILV | 0.01 mg/kg | LC-MS/MS | KCA 4.2 Schwarz & Class (2007), Report No. P/B 1226 G / Yes (RAR 2023) |
| Confirmatory  (if required) | Not required | | - |
| Fat | Primary | 0.01 mg/kg | LC-MS/MS | KCA 4.2 Freitag (2007), Report No. MR-06/199 / Yes (RAR 2023) |
| ILV | 0.01 mg/kg | LC-MS/MS | KCA 4.2 Schwarz & Class (2007), Report No. P/B 1226 G / Yes (RAR 2023) |
| Confirmatory  (if required) | Not required | | - |
| Kidney, liver | Primary | 0.01 mg/kg | LC-MS/MS | KCA 4.2 Freitag (2007), Report No. MR-06/199 / Yes (RAR 2023) |
| ILV | 0.01 mg/kg | LC-MS/MS | KCA 4.2 Schwarz & Class (2007), Report No. P/B 1226 G / Yes (RAR 2023) |
| Confirmatory  (if required) | Not required | | - |
| Honey | Primary | 0.01 mg/kg | LC-MS/MS | KCP 5.2/01 Peris, D., Morsiani, S. (2024), Report No. E23-0116 (*Analytical phase report: 23306-01R*) |
| ILV | 0.01 mg/kg | LC-MS/MS | KCP 5.2/02 Sala, A. (2024), Report No. LBN-0085-2024 |
| Confirmatory  (if required) | Not required | | - |

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

Table 5.3‑5: Statement on extraction efficiency

|  | Method for products of animal origin |
| --- | --- |
| Required, available from: | Heinemann (2001), Report No. 00655, DAR, vol. 3 B1-5 (KIIA 4.2.1.1) |
| Not required, because: | - |

The suitability of the analytical method (extracting with an acetonitrile/water solvent system) for the determination of the relevant residue in animal matrices has been demonstrated.

~~No data is submitted in support of the application for authorization of ULTRACENT 460 EC. Reference is made to the unprotected data and dossier of INPUT 460 EC (R-61/2011, authorization holder Bayer AG), in accordance with Article 34 of Regulation 1107/2009/EC. It was not considered necessary to submit additional data and the evaluator is referred to the registration report of INPUT 460 EC.~~

*~~The following information can be found in the evaluation reports that were compiled for the authorization of INPUT 460 EC (R-61/2011) in Poland:~~*

~~PROTHIOCONAZOLE~~

~~Prothioconazole, metabolite JAU6476-destio, metabolite JAU64763-3-hydroxydestio, metabolite JAU64763-4-hydroxydestio,~~

~~-method HPLC-MS/MS (Heinemann, O, 2001b)~~

~~LOQ: 0.004mg/kg milk~~

~~LOQ: 0.01 mg/kg meat liver, kidney, animal fat~~

~~Analytical methods included in the dossier (prothioconazole)~~

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **~~Reference~~** | **~~Heinemann 2001b~~** | | **~~Heinemann 2001b~~** | **~~Heinemann 2001b~~** | **~~Heinemann 2001c~~**  **~~Heinemann 2001c~~**  **~~Heinemann 2001c~~** | **~~(ILV)~~**  **~~Dubey 2001~~** | **~~“~~** | **~~(ILV)~~**  **~~Dubey 2001~~** |
| ~~Linearity~~ | **~~Yes~~** | | **~~Yes~~** | **~~Yes~~** | **~~Yes~~** | **~~Yes~~** | **~~Yes~~** | **~~Yes~~** |
| **~~RSD~~**  **~~(%) (n)~~** | **~~0.6-5.8 (5)~~** | | **~~1.1-6.5 (5)~~** | **~~1.2-6.4 (5)~~** | **~~3.1-3.9 (5)~~**  **~~3.4-3.6 (5)~~**  **~~3.4-4.1 (5)~~** | **~~5.4-11.8~~** | **~~4.0-5.7~~** | **~~3.4-11.5 (4)~~** |
| **~~Recovery~~**  **~~%~~** | **~~80-97~~** | | **~~80-103~~** | **~~86-114~~**  **~~(102)~~** | **~~92-102~~**  **~~94-104~~**  **~~90-103~~** | **~~77-106~~** | **~~100-112~~**  **~~(105)~~** | **~~77-107~~** |
| **~~Fortification level~~**  **~~(mg/kg)~~** | **~~0.01-0.1~~** | | **~~0.01-0.1~~** | **~~0.01-0.1~~** | **~~0.004-0.04~~**  **~~0.01-0.1~~**  **~~0.01-0.1~~** | **~~0.01-0.1~~** | **~~0.004-0.04~~** | **~~0.01-0.1~~** |
| **~~LOQ (mg/kg)~~** | **~~0.01~~** | | **~~0.01~~** | **~~0.01~~** | **~~0.004~~**  **~~0.004~~**  **~~0.004~~** | **~~0.01~~** | **~~0.004~~** | **~~0.01~~** |
| **~~Detection~~** | **~~HPLC-MS/MS~~** | | **~~HPLC-MS/MS~~** | **~~HPLC-MS/MS~~** | **~~HPLC-MS/MS~~**  **~~HPLC-MS/MS~~**  **~~HPLC-MS/MS~~** | **~~HPLC-MS/MS~~** | **~~“~~** | **~~HPLC-MS/MS~~** |
| **~~Division, purification~~** | **~~ChemElut cyclohexane/ethyl acetate columns~~** | | **~~ChemElut cyclohexane/ethyl acetate columns~~** | **~~ChemElut cyclohexane/ethyl acetate columns~~** | **~~ChemElut cyclohexane/ethyl acetate columns~~**  **~~ChemElut cyclohexane/ethyl acetate columns~~**  **~~ChemElut cyclohexane/ethyl acetate columns~~** | **~~ChemElut cyclohexane/ethyl acetate columns~~** | **~~“~~** | **~~ChemElut cyclohexane/ethyl acetate columns~~** |
| **~~Extraction~~** | **~~acetonitrile/water~~**  **~~HCl~~** | | **~~acetonitrile/water~~**  **~~HCl~~** | **~~acetonitrile/water~~**  **~~HCl~~** | **~~acetonitrile/water~~**  **~~HCl~~**  **~~acetonitrile/water~~**  **~~HCl~~**  **~~acetonitrile/water~~**  **~~HCl~~** | **~~acetonitrile/water~~**  **~~HCl~~** | **~~“~~** | **~~acetonitrile/water~~**  **~~HCl~~** |
| **~~Analyte~~** | **~~JAU6476-detio~~** | | **~~JAU6476-3-hydroxy‑~~**  **~~detio~~** | **~~JAU6476-4-hydroxy‑~~**  **~~detio~~** | **~~JAU6476-detio~~**  **~~JAU6476-3-hydroxy-detio~~**  **~~JAU6476-4-hydroxy-detio~~** | **~~JAU6476-detio~~** | **~~“~~** | **~~JAU6476-3-hydroxy‑~~**  **~~detio~~** |
| **~~Tested material~~** | **~~Milk~~**  **~~Meat~~**  **~~Intestines~~** | | **~~Milk~~**  **~~Meat~~**  **~~Intestines~~** | **~~Milk~~**  **~~Meat~~**  **~~Intestines~~** | **~~Milk~~**  **~~Milk~~**  **~~Milk~~** | **~~Meat~~**  **~~Intestines~~** | **~~Milk~~** | **~~Meat~~**  **~~Intestines~~** |
| **~~Reference~~** | **~~“~~** | **~~(ILV)~~**  **~~Dubey, 2001~~** | **~~“~~** |
| ~~Linearity~~ | **~~Yes~~** | **~~Yes~~** | **~~Yes~~** |
| **~~RSD~~**  **~~(%) (n)~~** | **~~3.5-4.6 (5)~~** | **~~3.3-12.7~~** | **~~3.6-4.3~~** |
| **~~Recovery~~**  **~~%~~** | **~~99-112~~** | **~~76-106~~** | **~~101-112~~** |
| **~~Fortification level~~**  **~~(mg/kg)~~** | **~~0.004-0.04~~** | **~~0.01-0.1~~** | **~~0.004-0.04~~** |
| **~~LOQ (mg/kg)~~** | **~~0.004~~** | **~~0.01~~** | **~~0.004~~** |
| **~~Detection~~** | **~~“~~** | **~~HPLC-MS/MS~~** | **~~“~~** |
| **~~Division, purification~~** | **~~“~~** | **~~ChemElut cyclohexane/ethyl acetate columns~~** | **~~“~~** |
| **~~Extraction~~** | **~~“~~** | **~~acetonitrile/water~~**  **~~HCl~~** | **~~“~~** |
| **~~Analyte~~** | **~~“~~** | **~~JAU6476-4-hydroxy‑~~**  **~~detio~~** | **~~“~~** |
| **~~Tested material~~** | **~~Milk~~** | **~~Meat~~**  **~~Intestines~~** | **~~Milk~~** |

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

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

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

| Component of residue definition: Prothioconazole and prothioconazole-desthio | | | |
| --- | --- | --- | --- |
| Method type | Method LOQ | Principle of method  (*i.e.* GC-MS or HPLC-UV) | Author(s), year / missing / EU agreed |
| Primary | 0.006 mg/kg | HPLC-MS/MS | Schrammel (2000), Report No. 00610 / Yes (DAR 2007, KIIA 4.2.2.1) |
| Confirmatory | 0.006 mg/kg | HPLC-MS/MS | KCA 4.2 Brumhard (2005), Report No. 00610/M001 / Yes (RAR 2023) |
| Primary | 0.002 mg/kg | HPLC-MS/MS | KCA 4.2 Freitag & Koch (2014), Report No. MR-13/042 / Yes (RAR 2023) |
| Confirmatory | Not required | |

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

~~No data is submitted in support of the application for authorization of ULTRACENT 460 EC. Reference is made to the unprotected data and dossier of INPUT 460 EC (R-61/2011, authorization holder Bayer AG), in accordance with Article 34 of Regulation 1107/2009/EC. It was not considered necessary to submit additional data and the evaluator is referred to the registration report of INPUT 460 EC.~~

*~~The following information can be found in the evaluation reports that were compiled for the authorization of INPUT 460 EC (R-61/2011) in Poland:~~*

~~PROTHIOCONAZOLE~~

~~Prothioconazole, JAU6476-destio metabolite, JAU6476-S-methyl metabolite (not required for monitoring):~~

~~-HPLC-MS/MS method (Schramel, 2000)~~

~~LOQ: 0.006mg/kg~~

~~-GC-MS method for the metabolite JAU6476-detio (Steinhauer, 2001)~~

~~LOQ: 0.01mg/kg~~

~~Analytical methods included in the dossier (prothioconazole)~~

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **~~Reference~~** | **~~Schramel 2000~~** | **~~Schramel 2000~~** | **~~Schramel 2000~~** | **~~Steinhauer 2001~~** |
| ~~Linearity~~ | **~~Yes~~** | **~~Yes~~** | **~~Yes~~** | **~~Yes~~** |
| **~~RSD~~**  **~~(%) (n)~~** | **~~2.8-9.3 (5)~~** | **~~3.2-8.5 (5)~~** | **~~1.3-8.7 (5)~~** | **~~10.0-13.0 (5)~~** |
| **~~Recovery~~**  **~~%~~** | **~~79-110~~**  **~~(95)~~** | **~~87-118~~**  **~~(102)~~** | **~~83-122~~** | **~~84-114~~**  **~~(97)~~** |
| **~~Fortification level~~**  **~~(mg/kg)~~** | **~~0.006-0.2~~** | **~~0.006-0.2~~** | **~~0.006-0.2~~** | **~~0.01-0.1~~** |
| **~~LOQ (mg/kg)~~** | **~~0.006~~** | **~~0.006~~** | **~~0.006~~** | **~~0.01~~** |
| **~~Detection~~** | **~~HPLC-MS/MS~~** | **~~HPLC-MS/MS~~** | **~~HPLC-MS/MS~~** | **~~GC/MS~~** |
| **~~Division, purification~~** | **~~Filtration~~** | **~~-~~** | **~~‑~~** | **~~liquid-liquid~~**  **~~ethyl acetate and cyclohexane.~~**  **~~Gel chromatography~~** |
| **~~Extraction~~** | **~~acetonitriles/water / cysteine hydrochloride monohydrate~~** | **~~acetonitriles/water / cysteine hydrochloride monohydrate~~** | **~~acetonitriles/water / cysteine hydrochloride monohydrate~~** | **~~Method DFG S19 acetone/water~~** |
| **~~Analyte~~** | **~~JAU6476~~** | **~~JAU6476-detio~~** | **~~JAU6476-3-hydroxy-detio~~** | **~~JAU6476-detio~~** |
| **~~Tested material~~** | **~~Soil~~** | **~~Soil~~** | **~~Soil~~** | **~~Soil~~** |

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

An overview on the acceptable methods and possible data gaps for analysis of prothioconazole residues in surface and drinking water is given in the following tables.

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

| Component of residue definition: Prothioconazole and prothioconazole-desthio | | | | |
| --- | --- | --- | --- | --- |
| Matrix type | Method type | Method LOQ | Principle of method (i.e. GC-MS or HPLC-UV) | Author(s), year / missing |
| Drinking water | Primary | 0.05 μg/L | HPLC-MS/MS | KCA 4.2 Krebber & Sandau (2015), Report No. MR-15/025 / Yes (RAR 2023) |
| ILV | 0.05 μg/L | HPLC-MS/MS | KCA 4.2 Thies (2015), Report No. 2015/0034/01 / Yes (RAR 2023) |
| Confirmatory | Not required | | - |
| Surface water | Primary | 0.05 μg/L | HPLC-MS/MS | KCA 4.2 Krebber & Sandau (2015), Report No. MR-15/025 / Yes (RAR 2023) |
| Confirmatory | Not required | | - |

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

~~No data is submitted in support of the application for authorization of ULTRACENT 460 EC. Reference is made to the unprotected data and dossier of INPUT 460 EC (R-61/2011, authorization holder Bayer AG), in accordance with Article 34 of Regulation 1107/2009/EC. It was not considered necessary to submit additional data and the evaluator is referred to the registration report of INPUT 460 EC.~~

*~~The following information can be found in the evaluation reports that were compiled for the authorization of INPUT 460 EC (R-61/2011) in Poland:~~*

~~PROTHIOCONAZOLE~~

~~Prothioconazole, metabolite JAU6476-destio:~~

~~-HPLC-MS/MS method (Sommer, 2001b)~~

~~LOQ: 0,1 µg/L for JAU6476 and 0,05 µg/L for JAU6476-destio~~

~~Analytical methods included in the dossier (prothioconazole)~~

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **~~Reference~~** | **~~Sommer 1999~~** | **~~Sommer 1999~~** | **~~Sommer 2001a~~** | **~~Sommer 2001b~~** | **~~Sommer 2001b~~** |
| ~~Linearity~~ | **~~Yes~~** | **~~Yes~~** | **~~Yes~~** | **~~Yes~~** | **~~Yes~~** |
| **~~RSD~~**  **~~(%) (n)~~** | **~~0.3-6.4~~**  **~~(10)~~** | **~~0.3-6.4~~**  **~~(10)~~** | **~~1.6-2.3~~**  **~~(10)~~** | **~~3.2-4.3~~**  **~~(10)~~** | **~~6.8~~**  **~~(10)~~** |
| **~~Recovery~~**  **~~%~~** | **~~70-110~~** | **~~70-110~~** | **~~70-110~~** | **~~70-110~~** | **~~70-110~~** |
| **~~Fortification level~~**  **~~(mg/kg)~~** | **~~6.4-1276 µg/l~~** | **~~5.9-1185 µg/l~~** | **~~9.3-1861 µg/l~~** | **~~0.1-1 µg/l~~** | **~~0.05-0.5 µg/l~~** |
| **~~LOQ (mg/kg)~~** | **~~6 µg/l~~** | **~~6 µg/l~~** | **~~10 µg/l~~** | **~~0.1 µg/l~~** | **~~0.05 µg/l~~** |
| **~~Detection~~** | **~~HPLC-UV~~** | **~~HPLC-UV~~** | **~~HPLC-UV~~** | **~~HPLC-MS/MS~~** | **~~HPLC-MS/MS~~** |
| **~~Division, purification~~** | **~~-~~** | **~~-~~** | **~~-~~** | **~~-~~** | **~~-~~** |
| **~~Extraction~~** | **~~Direct injection~~** | **~~Direct injection~~** | **~~Direct injection~~** | **~~Direct injection~~** | **~~Direct injection~~** |
| **~~Analyte~~** | **~~JAU6476~~** | **~~JAU6476-detio~~** | **~~JAU6476-S-metyl~~** | **~~JAU6476~~** | **~~JAU6476-detio~~** |
| **~~Tested material~~** | **~~Water~~** | **~~Water~~** | **~~Water~~** | **~~Water~~** | **~~Water~~** |

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

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

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

| Component of residue definition: Prothioconazole and prothioconazole-desthio | | | |
| --- | --- | --- | --- |
| Method type | Method LOQ | Principle of method  (i.e. GC-MS or HPLC-UV) | Author(s), year / missing |
| Primary | 3 μg/m3 | HPLC-MS/MS | KCA 4.2 Anft & Bardel (2005); Report No. 00731/M001 / Yes (RAR 2023) |
| Confirmatory | Not required | | - |

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

~~No data is submitted in support of the application for authorization of ULTRACENT 460 EC. Reference is made to the unprotected data and dossier of INPUT 460 EC (R-61/2011, authorization holder Bayer AG), in accordance with Article 34 of Regulation 1107/2009/EC. It was not considered necessary to submit additional data and the evaluator is referred to the registration report of INPUT 460 EC.~~

*~~The following information can be found in the evaluation reports that were compiled for the authorization of INPUT 460 EC (R-61/2011) in Poland:~~*

~~PROTHIOCONAZOLE~~

~~Prothioconazole:~~

~~-HPLC-MS/MS method (Maasfeld, 2002a)~~

~~LOQ: 0.015mg/m3~~

~~-HPLC-MS/MS method (Maasfeld, 2002b - metabolite JAU6476-destio) -not required~~

~~LOQ: 0.0006mg/m3~~

~~Analytical methods included in the dossier (prothioconazole)~~

|  |  |  |
| --- | --- | --- |
| **~~Reference~~** | **~~Maasfeld 2002a~~** | **~~Maasfeld 2002b~~** |
| ~~Linearity~~ | **~~Yes~~** | **~~Yes~~** |
| **~~RSD~~**  **~~(%) (n)~~** | **~~3.8-13.7 (5)~~** | **~~1.8-7.9 (5)~~** |
| **~~Recovery~~**  **~~%~~** | **~~56-109~~**  **~~(94)~~** | **~~91-107~~** |
| **~~Fortification level~~**  **~~(mg/kg)~~** | **~~0.015-0.15 µg/l~~** | **~~0.0006‑~~**  **~~0.006 µg/l~~** |
| **~~LOQ (mg/kg)~~** | **~~0.015 µg/l~~** | **~~0.0006 µg/l~~** |
| **~~Detection~~** | **~~HPLC-MS/MS~~** | **~~HPLC-MS/MS~~** |
| **~~Division, purification~~** | **~~-~~** | **~~‑~~** |
| **~~Extraction~~** | **~~TENAX (6 hours; 2 L/min). extraction acetonitrile.~~** | **~~TENAX (6 hours; 2 L/min). extraction acetonitrile.~~** |
| **~~Analyte~~** | **~~JAU6476~~** | **~~JAU6476-detio~~** |
| **~~Tested material~~** | **~~Air~~** | **~~Air~~** |

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

~~No data is submitted in support of the application for authorization of ULTRACENT 460 EC. Reference is made to the unprotected data and dossier of INPUT 460 EC (R-61/2011, authorization holder Bayer AG), in accordance with Article 34 of Regulation 1107/2009/EC. It was not considered necessary to submit additional data and the evaluator is referred to the registration report of INPUT 460 EC.~~

*~~The following information can be found in the evaluation reports that were compiled for the authorization of INPUT 460 EC (R-61/2011) in Poland:~~*

~~Not required.~~

An overview on the acceptable methods and possible data gaps for analysis of prothioconazole residues in body fluids and tissues is given in the following table.

Table 5.3‑9: Methods for body fluids and tissues (if appropriate)

| Component of residue definition: Prothioconazole-desthio | | | |
| --- | --- | --- | --- |
| Method type | Method LOQ | Principle of method (i.e. GC-MS or HPLC-UV) | Author(s), year / missing |
| Primary | 0.05 mg/L | LC-MS/MS | KCA 4.2 Hoeppner (2015), Report No. M-535874-02-1 / Yes (RAR 2023) |
| Confirmatory | Not required | | - |

For any special comments or remarkable points concerning the analytical methods for body fluids and tissues please refer to Appendix 2.

#### Other studies/ information

~~No data is submitted in support of the application for authorization of ULTRACENT 460 EC. Reference is made to the unprotected data and dossier of INPUT 460 EC (R-61/2011, authorization holder Bayer AG), in accordance with Article 34 of Regulation 1107/2009/EC. It was not considered necessary to submit additional data and the evaluator is referred to the registration report of INPUT 460 EC.~~

Not relevant. No additional studies submitted.

### Description of analytical methods for the determination of residues of spiroxamine (KCP 5.2)

*The following information can be found in the evaluation reports that were compiled for the authorization of INPUT 460 EC (R-61/2011) in Poland:*

Validated analytical methods are provided for the determination of residues of Prothioconazole (JAU 6476) and Spiroxamine (KWG 4168) in material of agricultural and animal origin and in soil, water and air.

The limits of quantification (LOQ) of the included analytical methods are sufficient to verify compliance with the respective reference values for food of agricultural origin, material of animal origin, water, soil and air. Determinations were performed at different levels of fortification including the proposed limits of quantification of the analytical methods.

Conclusions:

1) Average recovery rates obtained: 70 to 110%.

2) Relative standard deviation obtained: <20%.

3) The proposed analytical methods are specific for the analytes determined.

4) No interfering compounds were found to be present in the matrices tested (< 30% quantification limit). limit of quantification).

5) Methods meet Sanco requirements: "Guidance document on residue analytical methods SANCO/825/00 rev.7 (17.03.04)' and 'Guidance document Quality control procedures for pesticide residues analysis SANCO/10232/2006 (24.03.06)'.

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

~~No data is submitted in support of the application for authorization of ULTRACENT 460 EC. Reference is made to the unprotected data and dossier of INPUT 460 EC (R-61/2011, authorization holder Bayer AG), in accordance with Article 34 of Regulation 1107/2009/EC. It was not considered necessary to submit additional data and the evaluator is referred to the registration report of INPUT 460 EC.~~

Table 5.3‑10: Relevant residue definitions for monitoring/enforcement and levels for which compliance is required

| Matrix | Residue definition | MRL / limit | Reference for MRL/level Remarks |
| --- | --- | --- | --- |
| Plant, high water content | Spiroxamine | 0.01 mg/kg | Regulation (EU) 2016/452 (2016), annex II |
| Plant, high acid content | 0.01 mg/kg |
| Plant, high protein/high starch content (dry commodities) | 0.01 mg/kg |
| Plant, high oil content | 0.05 mg/kg |
| Plant, difficult matrices (hops, spices, tea) | 0.05 mg/kg |
| Muscle | Spiroxamine carboxylic acid, expressed as Spiroxamine | 0.02 mg/kg |
| Milk | 0.015 mg/kg |
| Eggs | 0.02 mg/kg |
| Fat |
| Liver, kidney |
| Soil (Ecotoxicology) | Spiroxamine | 0.05 mg/kg | Common limit |
| Drinking water  (Human toxicology) | 0.1 µg/L | general limit for drinking water |
| Surface water (Ecotoxicology) | 24 µg/L | NOEC *Pimephales promelas*  EFSA Journal (2010) 8(10):1719 |
| Air | 4.5 μg/m³ | AOEL sys: 0.015 mg/kg  bw/d;  EFSA Journal (2010) 8(10):1719 |
| Tissue (meat or liver) | Spiroxamine carboxylic acid, expressed as Spiroxamine | 0.02 mg/kg | Regulation (EU) 2016/452 (2016), annex II |
| Body fluids | Not available. Will be an issue on EU level | | |

#### 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 spiroxamine in plant matrices is given in the following tables.

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

| Component of residue definition: Spiroxamine | | | | |
| --- | --- | --- | --- | --- |
| Matrix type | Method type | Method  LOQ | Principle of method  (i.e. GC-MS or HPLC-UV) | Author(s), year / missing / EU agreed |
| High protein/high  starch content (dry)  (Wheat flour) | Primary | 0.01 mg/kg | HPLC-MS/MS, QuEChERS | Anonymous (2008), Report No. ASB2008-5464 / EU agreed (KIIA 4.3) |
| ILV | Not required for international official methods with included ILV data | | |
| Confirmatory  (if required) | Not required. | | |
| High protein/high  starch content (dry)  (Barley grain & hops) | Primary | 0.05 mg/kg | HPLC-MS/MS | Heinemann (2002), Report No. 00769 !M-077994-01-1!MR-253/02 / EU agreed (KIIA 4.3) |
| ILV | 0.05 mg/kg | HPLC-MS/MS | Sommer (2002), Report No. MR-357/02 ! M-053290-01-1 / EU agreed (KIIA 4.3) |
| Confirmatory  (if required) | Not required. | | |
| High water content cucumber) | Primary | 0.01 mg/kg | HPLC-MS/MS, QuEChERS | Anonymous (2008), Report No. ASB2008-5464 / EU agreed (KIIA 4.3) |
| ILV | Not required for international official methods with included ILV data | | |
| Confirmatory  (if required) | Not required. | | |
| High water content  (tomato) | Primary | 0.05 mg/kg | HPLC-MS/MS | Heinemann (2002), Report No. 00769 !M-077994-01-1!MR-253/02 / EU agreed (KIIA 4.3) |
| ILV | 0.05 mg/kg | HPLC-MS/MS | Sommer (2002), Report No. MR-357/02 ! M-053290-01-1 / EU agreed(KIIA 4.3) |
| Confirmatory  (if required) | Not required. | | |
| High acid content (lemon) | Primary | 0.01 mg/kg | HPLC-MS/MS, QuEChERS | Anonymous (2008), Report No. ASB2008-5464 / EU agreed (KIIA 4.3) |
| ILV | Not required for international official methods with included ILV data | | |
| Confirmatory  (if required) | Not required. | | |
| High acid content  (orange) | Primary | 0.05 mg/kg | HPLC-MS/MS | Heinemann (2002), Report No. 00769 !M-077994-01-1!MR-253/02 / EU agreed (KIIA 4.3) |
| ILV | 0.05 mg/kg | HPLC-MS/MS | Sommer (2002), Report No. MR-357/02 ! M-053290-01-1 / EU agreed (KIIA 4.3) |
| Confirmatory  (if required) | Not required. | | |
| High oil content (rape seed) | Primary | 0.05 mg/kg | HPLC-MS/MS | Heinemann (2002), Report No. 00769 !M-077994-01-1!MR-253/02 / EU agreed |
| ILV | 0.05 mg/kg | HPLC-MS/MS | Sommer (2002), Report No. MR-357/02 ! M-053290-01-1 / EU agreed (KIIA 4.3) |
| Confirmatory  (if required) | Not required. | | |
| Difficult (if required,  depends on intended use) | Not required | | | - |

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

Table 5.3‑12: Statement on extraction efficiency

|  | Method for products of plant origin |
| --- | --- |
| Required, available from: | - KCP 5.2 Nuesslein, F. (2001), Report No. 00709!M-082616-01-1 !MR-448/01/ EU agreed  - KCP 5.2 Nuesslein, F. (2004), Report No. MR-025/04 ! M-002917-01-1  / EU agreed |
| Not required, because: | - |

Sufficient extraction efficiency for acetone/water and for acetonitrile/water as extraction solvents was demonstrated for the extraction of residues of spiroxamine in plant matrices barley and wheat (green material, grain, straw).

~~No data is submitted in support of the application for authorization of ULTRACENT 460 EC. Reference is made to the unprotected data and dossier of INPUT 460 EC (R-61/2011, authorization holder Bayer AG), in accordance with Article 34 of Regulation 1107/2009/EC. It was not considered necessary to submit additional data and the evaluator is referred to the registration report of INPUT 460 EC.~~

*~~The following information can be found in the evaluation reports that were compiled for the authorization of INPUT 460 EC (R-61/2011) in Poland:~~*

~~SPIROXAMINE~~

~~Spiroxamine~~

~~- HPLC-MS/MS method (Nuesslein, F, 2001)~~

~~LOQ: 0,05mg/kg plant material~~

~~- GC-MS method (Allmendinger, H, 1991, 1993)~~

~~LOQ: 0,05mg/kg plant material~~

~~Analytical methods included in the dossier (spiroxamine)~~

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **~~Reference~~** | **~~Nüsslein, 2001~~** | **~~Nüsslein, 2001~~** | **~~Allmendinger, H, 1991~~** | **~~Allmendinger, H, 1993~~** |
| ~~Linearity~~ | **~~0.0001-0.1 mg/l~~** | **~~0.0001-0.1 mg/l~~** | **~~Yes~~** | **~~Yes~~** |
| **~~RSD~~**  **~~(%) (n)~~** | **~~4.4~~**  **~~(30)~~**  **~~3.7~~**  **~~4.2~~**  **~~3.5~~**  **~~1.4~~**  **~~1.4~~**  **~~2,6~~** | **~~5.3~~**  **~~(30)~~**  **~~9.3~~**  **~~3.0~~**  **~~2.0~~**  **~~1.4~~**  **~~0.5~~**  **~~1,0~~** | **~~8.0~~** | **~~10.0~~** |
| **~~Recovery~~**  **~~%~~** | **~~94-112~~**  **~~100~~**  **~~108~~**  **~~102~~**  **~~99~~**  **~~108~~**  **~~105~~** | **~~81-109~~**  **~~105~~**  **~~107~~**  **~~96~~**  **~~106~~**  **~~105~~**  **~~107~~** | **~~98~~** | **~~90~~** |
| **~~Fortification level~~**  **~~(mg/kg)~~** | **~~0.05-0.5~~**  **~~0.05~~**  **~~0.5~~**  **~~0.05~~**  **~~0.5~~**  **~~0.05~~**  **~~0,5~~** | **~~0.05-0.5~~**  **~~0.05~~**  **~~0.5~~**  **~~0.05~~**  **~~0.5~~**  **~~0.05~~**  **~~0,5~~** | **~~0.05-0.5~~** | **~~0.05-0.5~~** |
| **~~LOQ (mg/kg)~~** | **~~0.05~~** | **~~0.05~~** | **~~0.05~~** | **~~0.05~~** |
| **~~Detection~~** | **~~HPLC-MS/MS Turbo Ion-spray~~** | **~~HPLC-MS/MS Turbo Ion-spray~~** | **~~GCMS~~** | **~~GCMs~~** |
| **~~Division, purification~~** | **~~filtration~~** | **~~filtration~~** | **~~RP-18 columns~~**  **~~reversed phase chromatography~~** | **~~Gel chromatography~~** |
| **~~Extraction~~** | **~~acetonitryle/water~~** | **~~acetonitryle/water~~** | **~~Acetone/water~~** | **~~Methanole/HCL~~** |
| **~~Analyte~~** | **~~KWG~~**  **~~4168~~** | **~~KWG~~**  **~~4168~~** | **~~KWG~~**  **~~4168~~** | **~~KWG~~**  **~~4168 +~~**  **~~metabolites containing 4-t-butylcyclohexanone~~** |
| **~~Tested material~~** | **~~Wheat~~**  **~~grain~~**  **~~straw~~**  **~~green material~~** | **~~Barley~~**  **~~seed~~**  **~~straw~~**  **~~green material~~** | **~~Cereals~~** | **~~Cereals~~** |

#### 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 spiroxamine in animal matrices is given in the following tables.

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

| Component of residue definition: Spiroxamine carboxylic acid as Spiroxamine | | | | |
| --- | --- | --- | --- | --- |
| Matrix type | Method type | Method LOQ | Principle of method (i.e. GC-MS or HPLC-UV) | Author(s), year / missing |
| Bovine: muscle, liver, kidney | Primary | 0.02 mg/kg | HPLC-MS/MS | Allmendinger (1995), Report No. 0035!M-0190-51-02-1!MR-683/95 / EU agreed (KIIA 4.3) |
| ILV | 0.02 mg/kg | HPLC-MS/MS | KCP 5.2 Class (2010), Report No. P/B 1898 G ! M-362276-01-1 / EU agreed |
| Confirmatory | Not required. | | |
| Milk (bovine) | Primary | 0.01 mg/kg | HPLC-MS/MS | Allmendinger (1995), Report No. 0035!M-0190-51-02-1!MR-683/95 / EU agreed (KIIA 4.3) |
| ILV | 0.01 mg/kg | HPLC-MS/MS | KCP 5.2 Class (2010), Report No. P/B 1898 G ! M-362276-01-1 / EU agreed |
| Confirmatory | Not required. | | |
| Fat | Primary | 0.02 mg/kg | GC-MSD | KCP 5.2 Meyer (2010), Report No. IF-09/01560496 ! M-388215-01-1 / EU agreed |
| ILV | 0.02 mg/kg | GC-MSD | KCP 5.2 Class & Merdian (2009), Report No. P 1693 G / EU agreed |
| Confirmatory | 0.02 mg/kg | GC-MSD of a silylated derivative | KCP 5.2 Meyer (2010), Report No. IF-09/01560496 ! M-388215-01-1 / EU agreed |
| Eggs | Primary | 0.02 mg/kg | GC-MSD | KCP 5.2 Meyer (2010), Report No. IF-09/01560496 ! M-388215-01-1 / EU agreed |
| ILV | 0.02 mg/kg | GC-MSD | KCP 5.2 Class & Merdian (2009), Report No. P 1693 G / EU agreed |
| Confirmatory | 0.02 mg/kg | GC-MSD of a silylated derivative | KCP 5.2 Meyer (2010), Report No. IF-09/01560496 ! M-388215-01-1 / EU agreed |

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

Table 5.3‑12: Statement on extraction efficiency

|  | Method for products of animal origin |
| --- | --- |
| Required, available from: | - KCP 5.2 Allmendinger, H. (1995), Report No. MR-911/95!M-058266-01-1/ EU agreed |
| Not required, because: | - |

Sufficient extraction efficiency for acetonitrile/water and for acetonitrile/methanol/cyclohexane/acetic acid as extraction solvents was demonstrated for the extraction of residues of spiroxamine in animal matrices.

~~No data is submitted in support of the application for authorization of ULTRACENT 460 EC. Reference is made to the unprotected data and dossier of INPUT 460 EC (R-61/2011, authorization holder Bayer AG), in accordance with Article 34 of Regulation 1107/2009/EC. It was not considered necessary to submit additional data and the evaluator is referred to the registration report of INPUT 460 EC.~~

*~~The following information can be found in the evaluation reports that were compiled for the authorization of INPUT 460 EC (R-61/2011) in Poland:~~*

~~SPIROXAMINE~~

~~Spiroxamine~~

~~-CG-MS method (Allmendinger, H, 1997,1995a, 1991, 1993)~~

~~LOQ: 0.02 mg/kg eggs, meat~~

~~LOQ: 0.05 mg/kg fat, liver~~

~~-Method HPLC-MS/MS (Allmendinger, H, 1995,)~~

~~LOQ: 0.01 mg/kg milk~~

~~LOQ: 0.02 mg/kg fat, liver, kidney, meat~~

~~Analytical methods included in the dossier (spiroxamine)~~

|  |  |  |
| --- | --- | --- |
| **~~Reference~~** | **~~Allmendinger,~~**  **~~1997,1995a~~** | **~~Allmendinger,~~**  **~~1995~~** |
| ~~Linearity~~ | **~~Yes~~** | **~~Yes~~** |
| **~~RSD~~**  **~~(%) (n)~~** | **~~12~~** | **~~12.1~~** |
| **~~Recovery~~**  **~~%~~** | **~~91~~** | **~~90-98~~** |
| **~~Fortification level~~**  **~~(mg/kg)~~** | **~~0.02-0.5~~** | **~~0.01-2.0~~** |
| **~~LOQ (mg/kg)~~** | **~~LOQ: 0.02 mg/kg eggs, meat~~**  **~~0.05 mg/kg fat, lever~~** | **~~0.01 mg/kg~~**  **~~milk~~**  **~~0.02 mg/kg~~**  **~~fat, liver, kidney, meat~~** |
| **~~Detection~~** | **~~GCMS~~** | **~~LCMSMS~~** |
| **~~Division, purification~~** | **~~Stirring with n heptane, evaporation and dissolution in solution HCL/methanol~~** | **~~Gel chromatography~~** |
| **~~Extraction~~** | **~~Acetonitrile/water or ethanol/water~~** | **~~Acetonitrile/water - muscle, kidney~~**  **~~Methanol - milk~~**  **~~Mixture acetonitrile, methanol, cyclohexane and acetic acid~~**  **~~- fat~~** |
| **~~Analyte~~** | **~~KWG~~**  **~~4168 +~~**  **~~metabolites containing 4-t-butylcyclohexanone~~** | **~~KWG~~**  **~~4168~~** |
| **~~Tested material~~** | **~~Material of animal origin~~** | **~~Material of animal origin~~** |

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

An overview on the acceptable methods and possible data gaps for analysis of spiroxamine in soil is given in the following tables.

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

| Component of residue definition: Spiroxamine | | | |
| --- | --- | --- | --- |
| Method type | Method LOQ | Principle of method  (*i.e.* GC-MS or HPLC-UV) | Author(s), year / missing |
| Primary | 0.001 mg/kg | HPLC-MS/MS | KCP 5.2 Freitag & Daniels (2008), Report No. 10188!MR-08/028!M-298750-01-1 /EU agreed |
| Confirmatory | Not required | | |
| Primary | 0.01 mg/kg | GC-MSD | KCP 5.2 Sommer (1994), Report No. 00374!M-019207-02-1!MR-607/94 / EU agreed |
| Confirmatory | Not required | | |

~~No data is submitted in support of the application for authorization of ULTRACENT 460 EC. Reference is made to the unprotected data and dossier of INPUT 460 EC (R-61/2011, authorization holder Bayer AG), in accordance with Article 34 of Regulation 1107/2009/EC. It was not considered necessary to submit additional data and the evaluator is referred to the registration report of INPUT 460 EC.~~

*~~The following information can be found in the evaluation reports that were compiled for the authorization of INPUT 460 EC (R-61/2011) in Poland:~~*

~~SPIROXAMINE~~

~~Spiroxamine and metabolites: desethyl (KWG 4557) and despropyl (KWG 4669)~~

~~-GC-MS method (Sommer,1994 a, 1994 b)~~

~~LOQ: 0.01mg/kg (spiroxamine), 0.02 mg/kg KWG 4557, KWG 4669)~~

~~-GC-MS/MS method~~

~~LOQ: 0.005mg/kg~~

~~Analytical methods included in the dossier (spiroxamine)~~

|  |  |  |
| --- | --- | --- |
| **~~Reference~~** | **~~Sommer, 1994a~~** | **~~Sommer, 1994b~~** |
| ~~Linearity~~ | **~~Yes~~** | **~~Yes~~** |
| **~~RSD~~**  **~~(%) (n)~~** |  |  |
| **~~Recovery~~**  **~~%~~** | **~~KWG~~**  **~~4168: 97.3~~**  **~~KWG~~**  **~~4557: 81.6,~~**  **~~KWG~~**  **~~4669:72.5~~** | **~~KWG~~**  **~~4168: 87.1~~**  **~~KWG~~**  **~~4557: 89.8,~~**  **~~KWG~~**  **~~4669:91.1~~** |
| **~~Fortification level~~**  **~~(mg/kg)~~** | **~~0.005 – 0.5~~** | **~~0.005 – 0.5~~** |
| **~~LOQ (mg/kg)~~** | **~~0.01 - KWG 4168~~**  **~~0.02 - KWG~~**  **~~4557,~~**  **~~KWG 4669~~** | **~~0.005~~** |
| **~~Detection~~** | **~~GCMS~~** | **~~GCMS/MS~~** |
| **~~Division, purification~~** | **~~Evaporation and redissolution in 2-propanol~~** | **~~Centrifugation~~** |
| **~~Extraction~~** | **~~Methanole~~** | **~~Methanol + aqueous solution of ammonia~~** |
| **~~Analyte~~** | **~~KWG~~**  **~~4168,~~**  **~~Metabolity: KWG 4557, KWG 4669~~** | **~~KWG~~**  **~~4168,~~**  **~~Metabolity: KWG 4557, KWG 4669~~** |
| **~~Tested material~~** | **~~Soil~~** | **~~Soil~~** |

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

An overview on the acceptable methods and possible data gaps for analysis of spiroxmine residues in surface and drinking water is given in the following tables.

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

| Component of residue definition: Spiroxamine | | | | |
| --- | --- | --- | --- | --- |
| Matrix type | Method type | Method LOQ | Principle of method (i.e. GC-MS or HPLC-UV) | Author(s), year / missing |
| Drinking water | Primary | 0.01 µg/ | GC-MSD | KCP 5.2 Sommer (1994), Report No. 00769!M-077994-01-1!MR-253/02/ EU agreed |
| ILV | Not available. This issue will be addressed on EU level | | |
| Confirmatory | 0.01 µg/L | GC-MSD, second GC column of different polarity | KCP 5.2 Sommer (1994), Report No. 00769!M-077994-01-1!MR-253/02/ EU agreed |
| Surface water | Primary | 0.01 µg/L | GC-MSD | KCP 5.2 Sommer (1994), Report No. 00769!M-077994-01-1!MR-253/02/ EU agreed |
| ILV | Not required | | - |
| Confirmatory | 0.01 µg/L | GC-MSD, second GC column of different polarity | KCP 5.2 Sommer (1994), Report No. 00769!M-077994-01-1!MR-253/02/ EU agreed |

~~No data is submitted in support of the application for authorization of ULTRACENT 460 EC. Reference is made to the unprotected data and dossier of INPUT 460 EC (R-61/2011, authorization holder Bayer AG), in accordance with Article 34 of Regulation 1107/2009/EC. It was not considered necessary to submit additional data and the evaluator is referred to the registration report of INPUT 460 EC.~~

*~~The following information can be found in the evaluation reports that were compiled for the authorization of INPUT 460 EC (R-61/2011) in Poland:~~*

~~SPIROXAMINE~~

~~GC-MS method (Köning, 1993, 1994)~~

~~LOQ: 0,1 µg/L~~

~~Analytical methods included in the dossier (spiroxamine)~~

|  |  |
| --- | --- |
| **~~Reference~~** | **~~Köning, 1993, 1994~~** |
| ~~Linearity~~ | **~~Yes~~** |
| **~~RSD~~**  **~~(%) (n)~~** | **~~9.8~~** |
| **~~Recovery~~**  **~~%~~** | **~~83-115~~** |
| **~~Fortification level~~**  **~~(mg/kg)~~** |  |
| **~~LOQ (mg/kg)~~** | **~~0.1~~** ~~µ~~**~~g/l~~** |
| **~~Detection~~** | **~~GCMS~~** |
| **~~Division, purification~~** | **~~Evaporation and dissolution in ethyl acetate~~** |
| **~~Extraction~~** | **~~RP-18 columns Mixture of ammonia and methanol~~** |
| **~~Analyte~~** | **~~KWG~~**  **~~4168~~** |
| **~~Tested material~~** | **~~Water~~** |

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

An overview on the acceptable methods and possible data gaps for analysis of spiroxamine in air is given in the following tables.

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

| Component of residue definition: Spiroxamine | | | |
| --- | --- | --- | --- |
| Method type | Method LOQ | Principle of method  (i.e. GC-MS or HPLC-UV) | Author(s), year / missing |
| Primary | 7.7 μg/m3 | GC/PND | KCP 5.2 Riegner (1995), Report No. 00408!MR-746/95!M-019447-02-1 / EU agreed |
| Confirmatory | Not required | | |

~~No data is submitted in support of the application for authorization of ULTRACENT 460 EC. Reference is made to the unprotected data and dossier of INPUT 460 EC (R-61/2011, authorization holder Bayer AG), in accordance with Article 34 of Regulation 1107/2009/EC. It was not considered necessary to submit additional data and the evaluator is referred to the registration report of INPUT 460 EC.~~

*~~The following information can be found in the evaluation reports that were compiled for the authorization of INPUT 460 EC (R-61/2011) in Poland:~~*

~~SPIROXAMINE~~

~~-Method GC -NP (Riegner, 1995) GO: 0.0077 mg/ m3~~

~~Analytical methods included in the dossier (spiroxamine)~~

|  |  |
| --- | --- |
| **~~Reference~~** | **~~Riegner, 1995~~** |
| ~~Linearity~~ | **~~Yes~~** |
| **~~RSD~~**  **~~(%) (n)~~** | **~~6.8~~** |
| **~~Recovery~~**  **~~%~~** | **~~87-101~~** |
| **~~Fortification level~~**  **~~(mg/kg)~~** |  |
| **~~LOQ (mg/kg)~~** | **~~0.0077~~**  **~~mg/m3~~**  **~~35-C, 80%~~**  **~~humidity~~** |
| **~~Detection~~** | **~~GCNPD~~** |
| **~~Division, purification~~** |  |
| **~~Extraction~~** | **~~Adsorption Extraction with n butyl acetate~~** |
| **~~Analyte~~** | **~~KWG~~**  **~~4168~~** |
| **~~Tested material~~** | **~~Air~~** |

#### 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 spiroxamine in body fluids and tissue is given in the following table.

Table 5.3‑18: Validated methods for animal matrices (if appropriate) – Spiroxamine

| Component of residue definition: Spiroxamine | | | | |
| --- | --- | --- | --- | --- |
| Matrix type | Method type | Method LOQ | Principle of method (i.e. GC-MS or HPLC-UV) | Author(s), year / missing |
| Liver (bovine) | Primary | 0.02 mg/kg | HPLC-MS/MS | Allmendinger (1995), Report No. 0035!M-0190-51-02-1!MR-683/95 / EU agreed (KIIA 4.3) |
| Confirmatory | Not required | | |
| Body fluid | Primary | Not available. This issue will be addressed on the EU level. | | |
| Confirmatory |

~~No data is submitted in support of the application for authorization of ULTRACENT 460 EC. Reference is made to the unprotected data and dossier of INPUT 460 EC (R-61/2011, authorization holder Bayer AG), in accordance with Article 34 of Regulation 1107/2009/EC. It was not considered necessary to submit additional data and the evaluator is referred to the registration report of INPUT 460 EC.~~

*~~The following information can be found in the evaluation reports that were compiled for the authorization of INPUT 460 EC (R-61/2011) in Poland:~~*

~~Not required.~~

#### Other studies/ information

No data is submitted in support of the application for authorization of ULTRACENT 460 EC. Reference is made to the unprotected data and dossier of INPUT 460 EC (R-61/2011, authorization holder Bayer AG), in accordance with Article 34 of Regulation 1107/2009/EC. It was not considered necessary to submit additional data and the evaluator is referred to the registration report of INPUT 460 EC.

1. Lists of data considered in support of the evaluation

No data is submitted in support of the application for authorization of ULTRACENT 460 EC. Reference is made to the unprotected data and dossier of INPUT 460 EC (R-61/2011, authorization holder Bayer AG), in accordance with Article 34 of Regulation 1107/2009/EC. It was not considered necessary to submit additional data and the evaluator is referred to the registration report of INPUT 460 EC.

List of data submitted by the applicant and relied on

| Data point | Author(s) | Year | Title Company Report No.  Source (where different from company) GLP or GEP status Published or not | Vertebrate study  Y/N | Owner |
| --- | --- | --- | --- | --- | --- |
| **KCP 5.1.1/01** | Kishora, K. S. | 2023a | Accelerated Storage Stability Test by Heating at 54 ± 2°C of Prothioconazole 160 g/L + Spiroxamine 300 g/L EC  Report No. AG-G1571  Eurofins Advinius Agrosciences Services India Private Limited, Karnataka, India  GLP/GEP: yes, unpublished | N | XXXX |
| KCP 5.1.1/02  *Submitted under KCP 5.1.1/01* | Kishora, K. S. | 2023a | Accelerated Storage Stability Test by Heating at 54 ± 2°C of Prothioconazole 160 g/L + Spiroxamine 300 g/L EC  Report No. AG-G1571  Eurofins Advinius Agrosciences Services India Private Limited, Karnataka, India  GLP/GEP: yes, unpublished | N | XXXX |
| KCP 5.1.2/01 | Jooß, S. | 2023 | Validation of a residue analytical method for determination of TDMs in cereal matrices and honey  Report No.: S22-05883  Eurofins agroscience services, EAG Laboratories GmbH, Germany  GLP, unpublished | N | XXXX |
| KCP 5.1.2/02 | Jooß, S. | 2023 | Development and validation of a residue analytical method for the determination of 5 metabolites of Prothioconazole in cereal matrices, oilseed rape (seed) and honey  Report No.: S22-05884  Eurofins agroscience services, EAG Laboratories GmbH, Germany  GLP, unpublished | N | XXXX |
| KCP 5.1.2/03  *Submitted under KCA 6.1/02* | Jooß, S. | 2024 | Storage Stability of Prothioconazole OH-desthio Metabolites (alpha-, 3-, 4-, 5- and 6-hydroxy desthio) and Triazole Derivative Metabolites (TDMs) in Honey under Deep Frozen Conditions  Report No.: S23-102955  Eurofins agroscience services, EAG Laboratories GmbH, Germany  GLP, unpublished | N | XXXX |
| KCP 5.2/01  *Also submitted under KCA 6.10/01* | Peris, D.,  Morsiani, S. | 2024 | Determination of Residues of Prothioconazole and its metabolites in Honey after Two Applications of Prothioconazole 250g/L EC to *Phacelia tancetifolia* under Semi-Field Conditions in Northern and Southern Europe in 2023  Report No.: E23-0116 (*Analytical phase report: 23306-01R*)  Renolab S.r.l., Italy  GLP, unpublished | N | XXXX |
| KCP 5.2/02 | Sala, A. | 2024 | Independent Laboratory Validation of an Analytical Method for the determination of Prothioconazole-desthio in honey samples  Report No.: LBN-0085-2024  LabAnalysis Life Science s.r.l. sede di Pavia, Italy  GLP, unpublished | N | XXXX |

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 |
| --- | --- | --- | --- | --- | --- |
| KIIA 4.2.1.1 | Heinemann, O. | 2001 | Analytical determination of residues of JAU6476-3-hydroxy-desthio, JAU6476-4-hydroxy-desthio, and JAU6476-desthio in/on matrices of animal origin by HPLC-MS/MS  Bayer CropScience,  Report No.: 00655  GLP/GEP: yes, unpublished | N | XXXX |
| KCA 4.2 | Chambers, J., Jarret, H. | 2014 | Modification M018 of the analytical method 01300 (based on QuEChERS method) for the determination of residues of prothioconazole-desthio and iprovalicarb in wheat grain, grapes, rapeseed, dry bean and  cucumber  Battelle UK Ltd., Chelmsford, Essex, United Kingdom  Report No.: VC/13/017,  GLP/GEP: yes, unpublished | N | XXXX |
| KCA 4.2 | Thies, S. | 2014 | Amendment no.2 to study 2014/0110/01 - Independent laboratory validation of BCS method 01300/M018 (based on "QuEChERS" method) for the determination of residues of prothioconazole-desthio and iprovalicarb in/on plant matrices by LC/MS/MS  Currenta GmbH & Co. OHG, Leverkusen, Germany  Report No.: 2014/0110/01,  GLP/GEP: yes, unpublished | N | XXXX |
| KCA 4.2 | Freitag, T. | 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 - Bayer CropScience  Report No.: MR-06/199  GLP/GEP: yes, unpublished | N | XXXX |
| KCA 4.2 | 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  GLP/GEP: yes, unpublished | N | XXXX |
| KCA 4.2 | Schulte, G., Oel, G. | 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 HPLC-MS/MS – Amendment No. 2 - incl. amendment No. 1 dated 25.01.2013 - incl. report dated 26.10.2006  Bayer CropScience  Report No.: M-279725-03-01  GLP/GEP: yes, unpublished | N | XXXX |
| KCA 4.2 | 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,  Report No.: P/B 1111 G  GLP/GEP: yes, unpublished | N | XXXX |
| KCA 4.2 | Desmaris, F. | 2015 | Amendment no. 1 to the final report - Cross validation of extraction methods for the determination of residues of prothioconazole-desthio in plant material by HPLC-MS/MS  Bayer S.A.S., Bayer CropScience, Lyon, France  Report No.: MR-15/117  GLP/GEP: yes, unpublished | N | XXXX |
| KIIA 4.2.2.1 | Schramel, O. | 2000 | Residue analytical method 00610 (MR-643/99) for the determination of JAU 6476 and the metabolites JAU6476-desthio and JAU6476-S-methyl in soil by HPLC-MS/MS  Bayer CropScience  Report No.: 00610  GLP/GEP: yes, unpublished | N | XXXX |
| KCA 4.2 | 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 HPLC-MS/MS  Bayer CropScience,  Report No.: 00610/M001,  GLP/GEP: yes, unpublished | N | XXXX |
| KCA 4.2 | Freitag, T., Koch, V. | 2014 | Analytical method 01372 for the determination of various pesticides in soil by HPLC-MS/MS  TF- BCS-Adama Agan  Report No.: MR-13/042  GLP/GEP: yes, unpublished | N | XXXX |
| KCA 4.2 | Krebber, R., Sandau, C. | 2015 | Modification M002 of analytical method 01387 for the determination of various pesticides in drinking and surface water by HPLC-MS/MS  TF-BCS-Adama Agan,  Report No.: MR-15/025,  GLP/GEP: yes, unpublished | N | XXXX |
| KCA 4.2 | 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,  GLP/GEP: yes, unpublished | N | XXXX |
| KCA 4.2 | Anft, T., Bardel, P. | 2005 | Modification M001 of method 00731 for the determination of residues of JAU 6476-desthio (SXX 0665) in air by HPLCMS/MS  Bayer CropScience,  Report No.: 00731/M001  GLP/GEP: yes, unpublished | N | XXXX |
| KCA 4.2 | Hoeppner, S. | 2015 | Validation of the BCS analytical method 01471 for the determination of prothiconazole-desthio in body fluid by HPLC-MS/MS  Currenta GmbH & Co. OHG, Leverkusen, Germany  Bayer CropScience,  Report No.: M-535874-02-1,  GLP/GEP: yes, unpublished | N | XXXX |
| KIIA 4.3 | Anonymous | 2008 | Foods of plant origin- Determination of pesticide residues using GC-MS and/or LC-MS/MS following acetonitrile extraction/partitioning and clean-up by dispersive SPE – QuEChERS-method EN 15662:2008  published | N | XXXX |
| KIIA 4.3 | Heinemann, O. | 2002 | Enforcement method 00769 for the determination of residues of Spiroxamine in/on matrices of plant origin by HPLC-MS/MS  Report No. 00769!M-077994-01-1!MR-253/02  GLP/GEP: yes, unpublished | N | XXXX |
| KIIA 4.3 | Sommer H. | 2002 | Independent laboratory validation of the enforcement method 00769 for the determination of residues of Spiroxamine in/on matrices of plant origin by HPLC-MS/MS  Report No. M-053290-01-1!MR-357/02  GLP/GEP: yes, unpublished | N | XXXX |
| KCP 5.2. | Nuesslein, F. | 2001 | Method 00709 for the determination of residues of KWG 4168 in/on sample materials of wheat and barley  Report No.: 00709!M-082616-01-1 !MR-448/01  GLP/GEP: yes, unpublished | N | XXXX |
| KCP 5.2. | Nuesslein, F. | 2004 | Residue data of KWG 4168 in wheat and barley after comparative extraction with acetonitrile/water and acetone/water according to method 00709  Report No.: MR-025/04 ! M-002917-01-1  GLP/GEP: yes, unpublished | N | XXXX |
| KCP 5.2 | Allmendinger H. | 1995 | Method for the determination of residues of KWG 4168 carboxylic acid in bovine tissues and milk with LC/MS/MS  Report No. 0035!M-0190-51-02-1!MR-683/95  GLP/GEP: yes, unpublished | N | XXXX |
| KCP 5.2 | Class, T. | 2010 | Independent laboratory validation of method 00355 for the determination of residues of spiroxamine carboxylic acid metabolite in milk, muscle and in liver using LC/MS/MS  P/B 1898 G ! M-362276-01-1  GLP/GEP: yes, unpublished | N | XXXX |
| KCP 5.2 | Meyer, M. | 2010 | Validation of PTRL Europe study P 1693 G (Bayer CropScience methods 00395 and 00395/M001) for the determination of residues of the spiroxamine carboxylic acid metabolite in egg and fat using GC/MS  IF-09/01560496 ! M-388215-01-1  GLP/GEP: yes, unpublished | N | XXXX |
| KCP 5.2 | Class T., Merdian H. | 2009 | Independent laboratory validation of method 00395 and 00395/M001 for the determination of residues of Spiroxamine carboxylic acid metabolite in egg and fat, using GC/MS  Report No. P 1693 G, P613090697, M-344343-01-1  GLP/GEP: yes, unpublished | N | XXXX |
| KCP 5.2 | Allmendinger H. | 1995 | Extraction efficiency of the total residue of KWG 4168 from goat tissues and milk  Report No. MR-911/95!M-058266-01-1  GLP/GEP: yes, unpublished | N | XXXX |
| KCP 5.2 | Freitag T.; Daniels M. | 2008 | Analytical method 01088 for the determination of residues of KWG 4168 (Spiroxamine) in soil and sediment by HPLC-MS/MS  Report No.: 10188!MR-08/028!M-298750-01-1  GLP/GEP: yes, unpublished | N | XXXX |
| KCP 5.2 | Sommer H. | 1995 | Validation of method 00374 (MR-607/94) for liquid chromatographic determination of KWG 4168 and the metabolites KWG 4557 and KWG 4669 in soil  Report No.: 00374!M-019207-02-1!MR-607/94  GLP/GEP: yes, unpublished | N | XXXX |
| KCP 5.2 | Sommer H. | 1999 | Enforcement and confirmatory method for determination of KWG 4168 in drinking water and surface water by GC/MS  Report No. 00769!M-077994-01-1!MR-253/02  GLP/GEP: yes, unpublished | N | XXXX |
| KCP 5.2. | Riegner R. | 1995 | Method for the determination of KWG 4168 in air  Report No. 00408!MR-746/95!M-019447-02-1  GLP/GEP: yes, unpublished | N | XXXX |

1. Detailed evaluation of submitted analytical methods
   1. Analytical methods for prothioconazole
      1. Methods used for the generation of pre-authorization data (KCP 5.1)

~~No new or additional studies have been submitted.~~

* + - 1. Method validation of active substance in plant protection product (KCP 5.1.1)

A Summary of the method has already been presented under 5.2.1.1.

* + - 1. Description of analytical methods for the determination of residues in support of residues studies (KCP 5.1.2)
         1. Method validation TDMs in cereal and honey

The objective of this study was to validate a residue analytical method for the determination of triazole derivative metabolites (TDMs) in/on barley and wheat specimens and honey in accordance to guidance document SANTE/2020/12830, rev.1.

|  |  |
| --- | --- |
| Reference: | KCP 5.1.2/01 |
| Report | Validation of a residue analytical method for determination of TDMs in cereal matrices and honey  Jooß, S., (2023)  Report No. S22-05883 |
| Guideline(s): | Yes, SANTE/2020/12830 rev. 1 |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes |

Materials and methods

Cereals:

In brief, for 1,2,4-triazole (T), triazole alanine (TA), triazole acetic acid (TAA) and triazole lactic acid (TLA) homogenized samples of barley (whole plant, grain and straw) and wheat (whole plant, grain and straw), were extracted with methanol/water (4/1, v/v) using a high-speed homogenizer. After rinsing the blender, celite filter was added to the extract and mixed well. The homogenate was filtered through a filter paper placed on a Buchner funnel, collected in a volumetric flask and make up to a defined volume with extraction solution.

Subsequently, to an aliquot of the supernatant an internal standard solution was added and the extract was evaporated to the aqueous remainder and filled up with water. Finally, to the end volume a small spatula tip of C18 material was added. After centrifugation the sample was filtered through a spin centrifuge tube filter. Quantification was performed by use of LC-DMS-MS/MS detection with isotopically labelled internal standard(s).

Honey:

In brief, for 1,2,4-triazole (T), triazole alanine (TA), triazole acetic acid (TAA) and triazole lactic acid (TLA) homogenized samples of honey were extracted with methanol/water (4/1, v/v) by shaking on a horizontal shaker, sonication and shaking by hand. The extracts were collected in a volumetric flask and, after rinsing the bottle, made up to a defined volume with extraction solution. Subsequently, to an aliquot of the supernatant an internal standard solution was added and the extract was evaporated to the aqueous remainder and filled up with water. Finally, to the end volume a small spatula tip of C18 material was added. After centrifugation the sample was filtered through a spin centrifuge tube filter. Quantification was performed by use of LC-DMS-MS/MS detection with isotopically labelled internal standard(s).

LC-DMS-MS/MS determination was conducted by monitoring two mass transitions per analyte. A summary is given below:

|  |  |  |
| --- | --- | --- |
| Analyte | Mass transition proposed for Quantification | Mass transition proposed for Confirmation |
| 1,2,4-Triazole | *m*/*z* 70 -> 43 | *m*/*z* 70 -> 70 |
| Triazole Acetic Acid | *m*/*z* 128 -> 70 | *m*/*z* 128 -> 43 |
| Triazole Lactic Acid | *m*/*z* 158 -> 70 | *m*/*z* 158 -> 43 |
| Triazole Alanine | *m*/*z* 157 -> 70 | *m*/*z* 157 -> 88 |

Results and discussions

The analytical method was fully validated in terms of specificity, linearity of detector’s response, LOQ, recovery and repeatability (by means of precision) according to Guidance Document SANTE/2020/12830 rev. 1 The results are summarized below.

Table A 1: Recovery results from method validation of 1,2,4-Triazole using the analytical method

| Matrix | Analyte | Fortification level [mg/kg] (*n* = 5) | Recovery range [%] | Mean  recovery [%] | RSD [%] | Comments |
| --- | --- | --- | --- | --- | --- | --- |
| Barley (Whole plant) | 1,2,4-Triazole | 0.01 | 75.7 – 83.6 | 80.6 | 3.7 | Quantification |
| 0.10 | 69.2 – 80.2 | 76.2 | 5.8 |
| **Overall** | 69.2 – 83.6 | 78.4 | 5.4 |
| 0.01 | 71.9 – 78.9 | 75.9 | 3.8 | Confirmation |
| 0.10 | 70.7 – 84.2 | 76.7 | 7.5 |
| **Overall** | 70.7 – 84.2 | 76.3 | 5.6 |
| Barley (Grain) | 1,2,4-Triazole | 0.01 | 70.0 – 83.4 | 79.6 | 7.0 | Quantification |
| 0.10 | 80.4 – 84.0 | 82.6 | 1.7 |
| **Overall** | 70.0 – 84.0 | 81.1 | 5.1 |
| 0.01 | 74.5 – 83.3 | 80.9 | 4.5 | Confirmation |
| 0.10 | 79.1 – 83.6 | 81.3 | 2.2 |
| **Overall** | 74.5 – 83.6 | 81.1 | 3.3 |
| Barley (Straw) | 1,2,4-Triazole | 0.01 | 80.7 – 96.5 | 88.5 | 7.6 | Quantification |
| 0.10 | 93.4 – 99.3 | 96.1 | 3.1 |
| **Overall** | 80.7 – 99.3 | 92.3 | 6.8 |
| 0.01 | 85.1 – 96.5 | 91.1 | 5.0 | Confirmation |
| 0.10 | 93.1 – 99.1 | 96.6 | 2.3 |
| **Overall** | 85.1 – 99.1 | 93.9 | 4.7 |
| Wheat (Whole plant) | 1,2,4-Triazole | 0.01\* | 86.4 – 109 | 94.8 | 11 | Quantification |
| 0.10 | 85.9 – 91.8 | 89.6 | 3.0 |
| **Overall** | 85.9 – 109 | 91.9 | 8.0 |
| 0.01 | 71.2 – 87.8 | 80.9 | 8.7 | Confirmation |
| 0.10 | 82.4 – 94.4 | 91.7 | 5.7 |
| **Overall** | 71.2 – 94.4 | 86.9 | 9.2 |
| Wheat (Grain) | 1,2,4-Triazole | 0.01 | 103 – 109 | 107 | 2.2 | Quantification |
| 0.10 | 98.1 – 109 | 102 | 4.1 |
| **Overall** | 98.1 – 109 | 104 | 3.9 |
| 0.01 | 108 – 112 | 110 | 1.7 | Confirmation |
| 0.10 | 100 – 108 | 104 | 2.8 |
| **Overall** | 100 – 112 | 107 | 3.8 |
| Wheat (Straw) | 1,2,4-Triazole | 0.01 | 74.1 – 82 | 79.5 | 4.2 | Quantification |
| 0.10 | 76.4 – 84.4 | 80.9 | 3.7 |
| **Overall** | 74.1 – 84.4 | 80.2 | 3.8 |
| 0.01 | 78.3 – 83.9 | 81.8 | 2.6 | Confirmation |
| 0.10 | 78.1 – 86.3 | 82.9 | 3.7 |
| **Overall** | 78.1 – 86.3 | 82.3 | 3.1 |
| Honey | 1,2,4-Triazole | 0.01 | 97.4 – 112 | 103 | 5.5 | Quantification |
| 0.10 | 86.1 – 89.6 | 88 | 1.6 |
| **Overall** | 86.1 – 112 | 95.8 | 9.4 |
| 0.01 | 91.3 – 110 | 105 | 7.4 | Confirmation |
| 0.10 | 91.3 – 97.2 | 93.9 | 2.7 |
| **Overall** | 91.3 – 110 | 99.5 | 8.0 |

\* Outlier value excluded through Dixon test (90% confidence) and not taken into account for mean and relative standard deviation calculations

Table A 2: Recovery results from method validation of Triazole Acetic Acid using the analytical method

| Matrix | Analyte | Fortification level [mg/kg] (*n* = 5) | Recovery range [%] | Mean  recovery [%] | RSD [%] | Comments |
| --- | --- | --- | --- | --- | --- | --- |
| Barley (Whole plant)a | Triazole Acetic Acid | 0.01 | 101 – 107 | 105 | 2.4 | Quantification |
| 0.10 | 86.6 – 93.7 | 89.0 | 3.3 |
| **Overall** | 86.6 – 107 | 97.1 | 9.2 |
| 0.01 | 94.3 – 112 | 105 | 6.2 | Confirmation |
| 0.10 | 86.5 – 92.3 | 88.5 | 2.6 |
| **Overall** | 86.5 – 112 | 96.6 | 10 |
| Barley (Grain) | Triazole Acetic Acid | 0.01 b | 73.5 – 85.4 | 82.0 | 6.1 | Quantification |
| 0.10 b | 81.2 – 85.5 | 82.8 | 2.2 |
| **Overall** b | 73.5 – 85.5 | 82.4 | 4.4 |
| 0.01 d | 67.8 – 83.3 | 78.0 | 7.7 | Confirmation |
| 0.10 d | 78.9 – 83.8 | 81.3 | 2.8 |
| **Overall** d | 67.8 – 83.8 | 79.6 | 5.8 |
| Barley (Straw) | Triazole Acetic Acid | 0.01 c | 100 – 109 | 104 | 3.8 | Quantification |
| 0.10 c | 99.4 – 104 | 102 | 1.8 |
| **Overall** c | 99.4 – 109 | 103 | 3.1 |
| 0.01 e | 96.6 – 108 | 99.9 | 4.4 | Confirmation |
| 0.10 e | 95.3 – 104 | 102 | 3.5 |
| **Overall** e | 95.3 – 108 | 101 | 3.9 |
| Wheat (Whole plant) | Triazole Acetic Acid | 0.01\* f | 95.8 – 101 | 98.2 | 2.4 | Quantification |
| 0.10 f | 96.1 – 100 | 98.1 | 1.6 |
| **Overall** f | 95.8 – 101 | 98.2 | 1.8 |
| 0.01\* i | 93.8 – 103 | 98.8 | 4.7 | Confirmation |
| 0.10 i | 93.6 – 101 | 98.1 | 3.3 |
| **Overall** i | 93.6 – 103 | 98.4 | 3.8 |
| Wheat (Grain) | Triazole Acetic Acid | 0.01 g | 70.9 – 81.9 | 75.6 | 5.6 | Quantification |
| 0.10 g | 86.5 – 95.2 | 91.1 | 3.6 |
| **Overall** g | 70.9 – 95.2 | 83.3 | 11 |
| 0.01 j | 73.5 – 91.5 | 81.4 | 9.5 | Confirmation |
| 0.10 j | 90.4 – 99.2 | 94.3 | 3.4 |
| **Overall** j | 73.5 – 99.2 | 87.9 | 10 |
| Wheat (Straw) | Triazole Acetic Acid | 0.01 h | 83 – 96 | 86.8 | 6.1 | Quantification |
| 0.10 h | 80.6 – 84.2 | 82.2 | 1.8 |
| **Overall** h | 80.6 – 96 | 84.5 | 5.2 |
| 0.01k | 75 – 92 | 86.8 | 7.9 | Confirmation |
| 0.10 k | 80.8 – 83.7 | 82.5 | 1.5 |
| **Overall** k | 75 – 92 | 84.7 | 6.1 |
| Honey | Triazole Acetic Acid | 0.01\* l | 88 – 119 | 104 | 15 | Quantification |
| 0.10 l | 92.2 – 112 | 98.6 | 8.2 |
| **Overall** l | 88 – 119 | 101 | 12 |
| 0.01 m | 103 – 119 | 111 | 5.7 | Confirmation |
| 0.10 m | 81.8 – 105 | 95.6 | 9.6 |
| **Overall** m | 81.8 – 119 | 103 | 11 |

\* Outlier value excluded through Dixon test (90% confidence) and not taken into account for mean and relative standard deviation calculations

a No residue above 20 % of the LOQ level was detected in control sample extracts except when indicated differently. Recoveries are without any blank correction.

b Recoveries are corrected for corresponding mean control residue (0.00217 mg/kg)

c Recoveries are corrected for corresponding mean control residue (0.00357 mg/kg)

d Recoveries are corrected for corresponding mean control residue (0.00308 mg/kg)

e Recoveries are corrected for corresponding mean control residue (0.00314 mg/kg)

f Recoveries are corrected for corresponding mean control residue (0.00387 mg/kg)

g Recoveries are corrected for corresponding mean control residue (0.00422 mg/kg)

h Recoveries are corrected for corresponding mean control residue (0.0168 mg/kg)

i Recoveries are corrected for corresponding mean control residue (0.00332 mg/kg)

j Recoveries are corrected for corresponding mean control residue (0.00385 mg/kg)

k Recoveries are corrected for corresponding mean control residue (0.0163 mg/kg)

l Recoveries are corrected for corresponding mean control residue (0.0128 mg/kg)

m Recoveries are corrected for corresponding mean control residue (0.0132 mg/kg)

Table A 3: Recovery results from method validation of Triazole Lactic Acid using the analytical method

| Matrix | Analyte | Fortification level [mg/kg] (*n* = 5) | Recovery range [%] | Mean  recovery [%] | RSD [%] | Comments |
| --- | --- | --- | --- | --- | --- | --- |
| Barley (Whole plant) | Triazole Lactic Acid | 0.01 a | 84.8 – 96.8 | 88.6 | 5.4 | Quantification |
| 0.10 a | 80.2 – 91.1 | 85.5 | 4.8 |
| **Overall** a | 80.2 – 96.8 | 87.0 | 5.2 |
| 0.01 d | 77.7 – 93.7 | 84.9 | 7.8 | Confirmation |
| 0.10 d | 83.2 – 90.3 | 86.8 | 3.5 |
| **Overall** d | 77.7 – 93.7 | 85.8 | 5.8 |
| Barley (Grain) b | Triazole Lactic Acid | 0.01 | 69.7 – 82.1 | 76.9 | 6.0 | Quantification |
| 0.10 | 78.5 – 82.4 | 80.1 | 2.1 |
| **Overall** | 69.7 – 82.4 | 78.5 | 4.7 |
| 0.01 | 70.2 – 88.2 | 79.5 | 8.2 | Confirmation |
| 0.10 | 78.5 – 81.7 | 80.0 | 1.4 |
| **Overall** | 70.2 – 88.2 | 79.7 | 5.5 |
| Barley (Straw) | Triazole Lactic Acid | 0.01 c | 89.6 – 102 | 94.7 | 5.3 | Quantification |
| 0.10 c | 91.2 – 94.9 | 92.8 | 1.7 |
| **Overall** c | 89.6 – 102 | 93.7 | 3.9 |
| 0.01 e | 80.5 – 92.2 | 88.8 | 5.3 | Confirmation |
| 0.10 e | 91.3 – 95.4 | 93.1 | 1.7 |
| **Overall** e | 80.5 – 95.4 | 90.9 | 4.4 |
| Wheat (Whole plant) | Triazole Lactic Acid | 0.01\* f | 92.7 – 101 | 96.3 | 3.9 | Quantification |
| 0.10 f | 90.9 – 95.9 | 92.9 | 2.0 |
| **Overall** f | 90.9 – 101 | 94.4 | 3.4 |
| 0.01\* h | 87.2 – 99.2 | 91.4 | 6.0 | Confirmation |
| 0.10 h | 93.3 – 98.3 | 94.9 | 2.2 |
| **Overall** h | 87.2 – 99.2 | 93.4 | 4.4 |
| Wheat (Grain)b | Triazole Lactic Acid | 0.01 | 83.4 – 89.4 | 85.3 | 2.8 | Quantification |
| 0.10 | 85.5 – 90.3 | 87.2 | 2.1 |
| **Overall** | 83.4 – 90.3 | 86.3 | 2.6 |
| 0.01 | 81.5 – 92.6 | 86.0 | 5.0 | Confirmation |
| 0.10 | 83.9 – 89.5 | 85.9 | 2.5 |
| **Overall** | 81.5 – 92.6 | 86.0 | 3.7 |
| Wheat (Straw) | Triazole Lactic Acid | 0.01 g | 79.2 – 89.2 | 84.0 | 4.9 | Quantification |
| 0.10 g | 77.3 – 82.9 | 79.4 | 2.9 |
| **Overall** g | 77.3 – 89.2 | 81.7 | 4.8 |
| 0.01 i | 83.2 – 95.2 | 88.6 | 5.4 | Confirmation |
| 0.10 i | 77.5 – 82.1 | 80.2 | 2.1 |
| **Overall** i | 77.5 – 95.2 | 84.4 | 6.6 |
| Honeyb | Triazole Lactic Acid | 0.01 | 88.5 – 118 | 97.8 | 12 | Quantification |
| 0.10 | 88.4 – 131 | 107 | 17 |
| **Overall** | 88.4 – 131 | 102 | 15 |
| 0.01 | 87.8 – 119 | 108 | 12 | Confirmation |
| 0.10 | 88.3 – 121 | 104 | 12 |
| **Overall** | 87.8 – 121 | 106 | 11 |

\* Outlier value excluded through Dixon test (90% confidence) and not taken into account for mean and relative standard deviation calculations

a Recoveries are corrected for corresponding mean control residue (0.00443 mg/kg)

b No residue above 20 % of the LOQ level was detected in control sample extracts except when indicated differently. Recoveries are without any blank correction.

c Recoveries are corrected for corresponding mean control residue (0.00294mg/kg)

d Recoveries are corrected for corresponding mean control residue (0.00444 mg/kg)

e Recoveries are corrected for corresponding mean control residue (0.00269 mg/kg)

f Recoveries are corrected for corresponding mean control residue (0.00909 mg/kg)

g Recoveries are corrected for corresponding mean control residue (0.00769mg/kg)

h Recoveries are corrected for corresponding mean control residue (0.00969 mg/kg)

i Recoveries are corrected for corresponding mean control residue (0.00698 mg/kg)

Table A 4: Recovery results from method validation of Triazole Alanine using the analytical method

| Matrix | Analyte | Fortification level [mg/kg] (*n* = 5) | Recovery range [%] | Mean  recovery [%] | RSD [%] | Comments |
| --- | --- | --- | --- | --- | --- | --- |
| Barley (Whole plant) | Triazole Alanine | 0.01 a | 91.9 – 104 | 97.3 | 4.6 | Quantification |
| 0.10 a | 85.7 – 94 | 89.9 | 3.7 |
| **Overall** a | 85.7 – 104 | 93.6 | 5.8 |
| 0.01 d | 92.3 – 105 | 100 | 5.6 | Confirmation |
| 0.10 d | 83.2 – 91.7 | 87.9 | 4.1 |
| **Overall** d | 83.2 – 105 | 94 | 8.3 |
| Barley (Grain) | Triazole Alanine | 0.01 b | 73.5 – 94.5 | 86.5 | 9.8 | Quantification |
| 0.10 b | 85.5 – 91.9 | 88.7 | 2.6 |
| **Overall** b | 73.5 – 94.5 | 87.6 | 6.9 |
| 0.01 e | 80.3 – 96.3 | 90.5 | 7.3 | Confirmation |
| 0.10 e | 86.5 – 93 | 89.6 | 3.2 |
| **Overall** e | 80.3 – 96.3 | 90 | 5.3 |
| Barley (Straw) | Triazole Alanine | 0.01 c | 114 – 121 | 118 | 2.4 | Quantification |
| 0.10 c | 112 – 121 | 118 | 3.1 |
| **Overall** c | 112 – 121 | 118 | 2.6 |
| 0.01 f | 106 – 119 | 115 | 4.7 | Confirmation |
| 0.10 f | 112 – 118 | 115 | 1.9 |
| **Overall** f | 106 – 119 | 115 | 3.4 |
| Wheat (Whole plant) | Triazole Alanine | 0.01\* g | 98.5 – 116 | 104 | 7.6 | Quantification |
| 0.10 g | 110 – 120 | 116 | 3.1 |
| **Overall** g | 98.5 – 120 | 110 | 7.6 |
| 0.01 j | 97 – 164 | 119 | 23 | Confirmation |
| 0.10 j | 110 – 116 | 113 | 2.4 |
| **Overall** j | 97 – 164 | 116 | 16 |
| Wheat (Grain) | Triazole Alanine | 0.01\* h | 71.5 – 75 | 72.9 | 2.0 | Quantification |
| 0.10 h | 78.5 – 82 | 80 | 1.8 |
| **Overall** h | 71.5 – 82 | 76.8 | 5.2 |
| 0.01\* k | 66 – 73 | 70.3 | 4.3 | Confirmation |
| 0.10 k | 75.9 – 80 | 78.1 | 2.2 |
| **Overall** k | 66 – 80 | 74.6 | 6.3 |
| Wheat (Straw) i | Triazole Alanine | 0.01 | 84.9 – 97 | 89.3 | 6.2 | Quantification |
| 0.10 | 76.5 – 78.2 | 77.5 | 0.9 |
| **Overall** | 76.5 – 97 | 83.4 | 8.7 |
| 0.01 | 80.5 – 99.2 | 88.7 | 8.5 | Confirmation |
| 0.10 | 71.7 – 79.1 | 76.7 | 3.8 |
| **Overall** | 71.7 – 99.2 | 82.7 | 10 |
| Honey | Triazole Alanine | 0.01 l | 77.7 – 102 | 90 | 9.8 | Quantification |
| 0.10 l | 94.3 – 101 | 97.9 | 2.9 |
| **Overall** l | 77.7 – 102 | 93.9 | 7.9 |
| 0.01 m | 86.7 – 92.7 | 88.6 | 2.7 | Confirmation |
| 0.10 m | 80.7 – 88.7 | 83.6 | 3.8 |
| **Overall** m | 80.7 – 92.7 | 86.1 | 4.4 |

\* Outlier value excluded through Dixon test (90% confidence) and not taken into account for mean and relative standard deviation calculations

a Recoveries are corrected for corresponding mean control residue (0.00421 mg/kg)

b Recoveries are corrected for corresponding mean control residue (0.0101mg/kg)

c Recoveries are corrected for corresponding mean control residue (0.00273mg/kg)

d Recoveries are corrected for corresponding mean control residue (0.00448 mg/kg)

e Recoveries are corrected for corresponding mean control residue (0.00998 mg/kg)

f Recoveries are corrected for corresponding mean control residue (0.00224 mg/kg)

g Recoveries are corrected for corresponding mean control residue (0.0176 mg/kg)

h Recoveries are corrected for corresponding mean control residue (0.0145mg/kg)

i No residue above 20 % of the LOQ level was detected in control sample extracts except when indicated differently. Recoveries are without any blank correction.

j Recoveries are corrected for corresponding mean control residue (0.0162 mg/kg)

k Recoveries are corrected for corresponding mean control residue (0.0146 mg/kg)

l Recoveries are corrected for corresponding mean control residue (0.00773 mg/kg)

m Recoveries are corrected for corresponding mean control residue (0.00594 mg/kg)

Table A 5: Characteristics for the analytical method used for validation of prothioconazole metabolites in cereal and honey

|  | 1,2,4-Triazole, Triazole Acetic Acid, Triazole Lactic Acid, Triazole Alanine |
| --- | --- |
| Specificity | A highly specific detection system has been used; two transitions were simultaneously acquired: one transition for quantification and one transition for conﬁrmation. No interference was detected at the retention time of the analytes signal. Mass spectra a given in the original study report. |
| Calibration (type, number of data points) | five calibration levels, solvent calibration standards. Calibration standards contained isotopically-labelled standards at a constant concentration of 50 ng/mL (equivalent to 0.10 mg/kg). The peak area ratio of analyte and the internal standard was calculated and used for the generation of the calibration curves.  Regression Model: y = a + b\*x.  1,2,4-Triazole:  Quantification:  y = 0.759 x + 7.23e-005, r = 1.0000  Confirmation:  y = 4.78 x + 0.00623, r = 0.9999  Triazole Acetic Acid:  Quantification:  y = 1.23 x + 5.91e-005, r = 0.9999  Confirmation:  y = 0.0681 x + 4.22e-005, r = 0.9999  Triazole Lactic Acid:  Quantification:  y = 0.726 x – 0.000355, r = 0.9999  Confirmation:  y = 0.107 x + 0.000187, r = 0.9999  Triazole Alanine:  Quantification:  y = 1.22 x + 0.00126, r = 0.9998  Confirmation:  y = 0.541 x + 0.000273, r = 0.9999 |
| Calibration range | 1 ng/mL to 100 ng/mL (corresponding to 0.002 mg/kg to 0.2 mg/kg). |
| Assessment of matrix effects is presented | Isotopically labelled internal standards were used for quantification so that matrix effects on the detector response were automatically accounted for when using the response ratio of analyte and internal standard for quantification. Therefore, solvent standard solutions were used throughout the study and matrix effects have not been determined. |
| Limit of determination/quantification | |  |  |  | | --- | --- | --- | | LOQ | 0.01 mg/kg |  | | LOD | 0.002 mg/kg |  | |
| Stability of Analyte(s) in Working Solutions | All analytes were found to be stable for 179 days when prepared in water and stored at refrigerated conditions (typically 1 – 10 °C) in the dark. |
| Stability of Analyte(s) in Sample Extracts | As the extracts contain an isotopically labelled internal standard (IL-IS) for quantification, testing of final extract stability is not required since the IL-IS compensates for losses during extract storage. Final extracts of all matrices were stored for max. 22 days at typically 1 °C to 10 °C in the dark. |

Conclusion

The underlying analytical method was successfully validated in terms of linearity of detector response, recovery and repeatability (by means of precision), LOQ and specificity following requirements set out in Guidance Document SANTE/2020/12830 rev. 1 at LOQ und higher fortification level in cereal and honey samples. Requirements set out in the superseding guidance document SANTE/2020/12830, rev. 2 (2023) were taken into account and fulfilled. The analytical method is reliable without restrictions.

* + - * 1. Method validation prothioconazole metabolites in crop matrices and honey

The objective of this study was to validate a residue analytical method for the determination of five prothioconazole hydroxy-desthio (PTZ-hydroxy desthio) metabolites in cereal matrices, oilseed rape (seed) and honey in accordance to guidance document SANTE/2020/12830, rev.2.

|  |  |
| --- | --- |
| Reference: | KCP 5.1.2/02 |
| Report | Development and validation of a residue analytical method for the determination of 5 metabolites of Prothioconazole in cereal matrices, oilseed rape (seed) and honey  Jooß, S., (2023)  Report No. S22-05884 |
| Guideline(s): | Yes, SANTE/2020/12830 rev. 2 |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes |

Materials and methods

In brief, samples of oilseed rape (seed), barley (grain), wheat (grain), barley (straw), wheat (straw) and honey were extracted with acetonitrile/water (4/1, v/v). An aliquot of the raw extract was concentrated to an aqueous remainder which was hydrolysed by heating with 5N hydrochloric acid. After neutralization the extracts were diluted with aqueous ammonium formate solution. Quantification was performed by use of LC-MS/MS detection. LC-MS/MS determination was conducted with evaluation of two mass transitions (m/z 328->70 and m/z 328->141) per analyte.

|  |  |
| --- | --- |
| **Analyte** | **Abbreviation** |
| Prothioconazole-3-hydroxy desthio | PTZ 3-OH desthio |
| Prothioconazole-4-hydroxy desthio | PTZ 4-OH desthio |
| Prothioconazole-5-hydroxy desthio | PTZ 5-OH desthio |
| Prothioconazole-6-hydroxy desthio | PTZ 6-OH desthio |
| Prothioconazole-α-hydroxy desthio | PTZ α-OH desthio |

Results and discussions

The analytical method was fully validated in terms of specificity, linearity of detector’s response, LOQ, recovery and repeatability (by means of precision) according to Guidance Document SANTE/2020/12830 rev. 2 The results are summarized below.

Table A 6: Recovery results from method validation of PTZ 3-OH desthio using the analytical method

| **Matrix** | **Analyte** | **Fortification level [mg/kg] (*n* = 5)** | **Recovery range [%]** | **Mean** | **RSD [%]** | **Comments** |
| --- | --- | --- | --- | --- | --- | --- |
| **recovery [%]** |
| Oilseed Rape (Seed) | PTZ 3-OH desthio | 0.01 (LOQ) | 89.5 – 104 | 96.3 | 5.4 | Quantification |
| 0.1 | 97.5 – 107 | 101 | 3.8 |
| **Overall** | 89.5 – 107 | 98.6 | 5.0 |
| 0.01 (LOQ) | 86 – 110 | 102 | 9.3 | Confirmation |
| 0.1 | 103 – 111 | 108 | 3.3 |
| **Overall** | 86 – 111 | 105 | 7.0 |
| Barley (Grain) | PTZ 3-OH desthio | 0.01 (LOQ) | 103 – 116 | 109 | 5.1 | Quantification |
| 0.1 | 102 – 112 | 106 | 4.1 |
| **Overall** | 102 – 116 | 107 | 4.6 |
| 0.01 (LOQ) | 96 – 116 | 107 | 7.1 | Confirmation |
| 0.1 | 106 – 115 | 110 | 3.7 |
| **Overall** | 96 – 116 | 109 | 5.5 |
| Wheat (Grain)\* | PTZ 3-OH desthio | 0.01 (LOQ) | 102 – 116 | 111 | 7.0 | Quantification |
| 0.1 | 110 – 119 | 115 | 3.9 |
| **Overall** | 102 – 119 | 113 | 5.4 |
| 0.01 (LOQ) | 98 – 115 | 109 | 8.5 | Confirmation |
| 0.1 | 112 – 120 | 117 | 3.4 |
| **Overall** | 98 – 120 | 113 | 6.8 |
| Barley (Straw) | PTZ 3-OH desthio | 0.01 (LOQ) | 86 – 98 | 92.2 | 5.6 | Quantification |
| 0.1 | 97.5 – 107 | 101 | 4.5 |
| **Overall** | 86 – 107 | 96.8 | 6.8 |
| 0.01 (LOQ) | 85 – 102 | 92.3 | 7.6 | Confirmation |
| 0.1 | 89.5 – 108 | 98.9 | 7.1 |
| **Overall** | 85 – 108 | 95.6 | 7.8 |
| Wheat (Straw)\* | PTZ 3-OH desthio | 0.01 (LOQ) | 79.5 – 104 | 94.1 | 14 | Quantification |
| 0.1 | 91.5 – 104 | 95.8 | 6.9 |
| **Overall** | 79.5 – 104 | 95 | 9.7 |
| 0.01 (LOQ) | 94.5 – 109 | 100 | 7.4 | Confirmation |
| 0.1 | 96.5 – 96.5 | 96.5 | 0 |
| **Overall** | 94.5 – 109 | 98.3 | 5.2 |
| Honey | PTZ 3-OH desthio | 0.01 (LOQ) | 84.5 – 102 | 94.7 | 7.5 | Quantification |
| 0.1 | 115 – 121 | 118 | 1.9 |
| **Overall** | 84.5 – 121 | 106 | 12 |
| 0.01 (LOQ) | 95 – 122 | 105 | 10 | Confirmation |
| 0.1 | 114 – 119 | 117 | 1.5 |
| **Overall** | 95 – 122 | 111 | 8.4 |

\* a reduced validation set of n = 3 has been used.

Table A 7: Recovery results from method validation of PTZ 4-OH desthio using the analytical method

| **Matrix** | **Analyte** | **Fortification level [mg/kg] (*n* = 5)** | **Recovery range [%]** | **Mean** | **RSD [%]** | **Comments** |
| --- | --- | --- | --- | --- | --- | --- |
| **recovery [%]** |
| Oilseed Rape (Seed) | PTZ 4-OH desthio | 0.01 (LOQ) | 101 – 109 | 105 | 3.4 | Quantification |
| 0.1 | 99.5 – 111 | 103 | 4.4 |
| **Overall** | 99.5 – 111 | 104 | 3.8 |
| 0.01 (LOQ) | 86 – 108 | 93.7 | 9.2 | Confirmation |
| 0.1 | 94 – 112 | 102 | 7.1 |
| **Overall** | 86 – 112 | 98 | 8.9 |
| Barley (Grain) | PTZ 4-OH desthio | 0.01 (LOQ) | 94.5 – 114 | 103 | 7.1 | Quantification |
| 0.1 | 104 – 117 | 111 | 4.3 |
| **Overall** | 94.5 – 117 | 107 | 6.7 |
| 0.01 (LOQ) | 106 – 116 | 111 | 3.6 | Confirmation |
| 0.1 | 101 – 116 | 110 | 6.0 |
| **Overall** | 101 – 116 | 111 | 4.7 |
| Wheat (Grain)\* | PTZ 4-OH desthio | 0.01 (LOQ) | 103 – 111 | 106 | 3.7 | Quantification |
| 0.1 | 107 – 119 | 113 | 5.1 |
| **Overall** | 103 – 119 | 109 | 5.1 |
| 0.01 (LOQ) | 102 – 109 | 106 | 3.4 | Confirmation |
| 0.1 | 114 – 120 | 117 | 2.8 |
| **Overall** | 102 – 120 | 112 | 6.1 |
| Barley (Straw) | PTZ 4-OH desthio | 0.01 (LOQ) | 82.5 – 102 | 91.0 | 7.6 | Quantification |
| 0.1 | 94 – 102 | 98.3 | 3.4 |
| **Overall** | 82.5 – 102 | 94.7 | 6.8 |
| 0.01 (LOQ) | 80.5 – 94.5 | 87.0 | 6.2 | Confirmation |
| 0.1 | 105 – 114 | 109 | 3.2 |
| **Overall** | 80.5 – 114 | 97.8 | 12 |
| Wheat (Straw)\* | PTZ 4-OH desthio | 0.01 (LOQ) | 96.5 – 106 | 101 | 4.5 | Quantification |
| 0.1 | 93 – 98.5 | 95.8 | 2.9 |
| **Overall** | 93 – 106 | 98.5 | 4.5 |
| 0.01 (LOQ) | 86 – 98 | 92.5 | 6.6 | Confirmation |
| 0.1 | 92.5 – 104 | 97.0 | 6.3 |
| **Overall** | 86 – 104 | 94.8 | 6.3 |
| Honey | PTZ 4-OH desthio | 0.01 (LOQ) | 98.5 – 110 | 106 | 4.7 | Quantification |
| 0.1 | 118 – 120 | 119 | 0.5 |
| **Overall** | 98.5 – 120 | 112 | 6.7 |
| 0.01 (LOQ) | 89.8 – 114 | 102 | 8.7 | Confirmation |
| 0.1 | 113 – 119 | 117 | 2.3 |
| **Overall** | 89.8 – 119 | 109 | 9.3 |

\* a reduced validation set of n = 3 has been used.

Table A 8: Recovery results from method validation of PTZ 5-OH desthio using the analytical method

| **Matrix** | **Analyte** | **Fortification level [mg/kg] (*n* = 5)** | **Recovery range [%]** | **Mean** | **RSD [%]** | **Comments** |
| --- | --- | --- | --- | --- | --- | --- |
| **recovery [%]** |
| Oilseed Rape (Seed) | PTZ 5-OH desthio | 0.01 (LOQ) | 91.5 – 107 | 100 | 5.6 | Quantification |
| 0.1 | 97.5 – 107 | 102 | 4.1 |
| **Overall** | 91.5 – 107 | 101 | 4.7 |
| 0.01 (LOQ) | 102 – 122 | 113 | 7 | Confirmation |
| 0.1 | 102 – 103 | 103 | 0.44 |
| **Overall** | 102 – 122 | 108 | 6.9 |
| Barley (Grain) | PTZ 5-OH desthio | 0.01 (LOQ) | 98 – 119 | 109 | 7.3 | Quantification |
| 0.1 | 104 – 112 | 107 | 2.9 |
| **Overall** | 98 – 119 | 108 | 5.4 |
| 0.01 (LOQ) | 91 – 116 | 105 | 8.6 | Confirmation |
| 0.1 | 99.5 – 118 | 110 | 7.1 |
| **Overall** | 91 – 118 | 107 | 7.8 |
| Wheat (Grain)\* | PTZ 5-OH desthio | 0.01 (LOQ) | 101 – 120 | 110 | 8.6 | Quantification |
| 0.1 | 110 – 119 | 116 | 4.2 |
| **Overall** | 101 – 120 | 113 | 6.5 |
| 0.01 (LOQ) | 93 – 110 | 104 | 9.1 | Confirmation |
| 0.1 | 100 – 113 | 105 | 6.6 |
| **Overall** | 93 – 113 | 105 | 7.1 |
| Barley (Straw) | PTZ 5-OH desthio | 0.01 (LOQ) | 87 – 99.5 | 92.6 | 5.8 | Quantification |
| 0.1 | 99 – 109 | 105 | 3.6 |
| **Overall** | 87 – 109 | 98.8 | 8.0 |
| 0.01 (LOQ)\*\* | 85.5 – 98.5 | 92.6 | 6.3 | Confirmation |
| 0.1 | 100 – 110 | 106 | 3.5 |
| **Overall** | 85.5 – 110 | 100 | 8.3 |
| Wheat (Straw)\* | PTZ 5-OH desthio | 0.01 (LOQ) | 84.5 – 98 | 91.1 | 7.4 | Quantification |
| 0.1 | 90 – 98.5 | 93.8 | 4.6 |
| **Overall** | 84.5 – 98.5 | 92.5 | 5.7 |
| 0.01 (LOQ) | 95 – 113 | 105 | 8.6 | Confirmation |
| 0.1 | 88.5 – 93.5 | 91.7 | 3.0 |
| **Overall** | 88.5 – 113 | 98.4 | 9.7 |
| Honey | PTZ 5-OH desthio | 0.01 (LOQ) | 97 – 112 | 105 | 5.9 | Quantification |
| 0.1 | 110 – 121 | 115 | 3.9 |
| **Overall** | 97 – 121 | 110 | 6.5 |
| 0.01 (LOQ) | 100 – 111 | 107 | 4.3 | Confirmation |
| 0.1 | 111 – 119 | 115 | 2.7 |
| **Overall** | 100 – 119 | 111 | 5.3 |

\* a reduced validation set of n = 3 has been used.

\*\* Outlier value excluded through Dixon test, p<0.01; not taken into account for mean and relative standard deviation calculations

Table A 9: Recovery results from method validation of PTZ 6-OH desthio using the analytical method

| **Matrix** | **Analyte** | **Fortification level [mg/kg] (*n* = 5)** | **Recovery range [%]** | **Mean** | **RSD [%]** | **Comments** |
| --- | --- | --- | --- | --- | --- | --- |
| **recovery [%]** |
| Oilseed Rape (Seed) | PTZ 6-OH desthio | 0.01 (LOQ) | 84 – 113 | 99.8 | 13 | Quantification |
| 0.1 | 101 – 114 | 105 | 5.2 |
| **Overall** | 84 – 114 | 102 | 9.3 |
| 0.01 (LOQ) | 92.5 – 114 | 102 | 7.8 | Confirmation |
| 0.1 | 98 – 115 | 107 | 6.8 |
| **Overall** | 92.5 – 115 | 104 | 7.3 |
| Barley (Grain) | PTZ 6-OH desthio | 0.01 (LOQ) | 98 – 118 | 110 | 7.6 | Quantification |
| 0.1 | 111 – 119 | 115 | 2.8 |
| **Overall** | 98 – 119 | 112 | 5.7 |
| 0.01 (LOQ) | 105 – 113 | 110 | 3.1 | Confirmation |
| 0.1 | 110 – 118 | 115 | 2.9 |
| **Overall** | 105 – 118 | 113 | 3.5 |
| Wheat (Grain)\* | PTZ 6-OH desthio | 0.01 (LOQ) | 102 – 109 | 105 | 3.5 | Quantification |
| 0.1 | 113 – 119 | 115 | 2.6 |
| **Overall** | 102 – 119 | 110 | 5.9 |
| 0.01 (LOQ) | 104 – 111 | 107 | 3.3 | Confirmation |
| 0.1 | 114 – 119 | 117 | 2.1 |
| **Overall** | 104 – 119 | 112 | 5.1 |
| Barley (Straw) | PTZ 6-OH desthio | 0.01 (LOQ) | 82 – 104 | 92.4 | 10 | Quantification |
| 0.1 | 95 – 105 | 99.4 | 3.8 |
| **Overall** | 82 – 105 | 95.9 | 8.2 |
| 0.01 (LOQ)\*\* | 88 – 99 | 92.9 | 5.7 | Confirmation |
| 0.1 | 95.5 – 106 | 99.5 | 4.3 |
| **Overall** | 88 – 106 | 96.6 | 5.8 |
| Wheat (Straw)\* | PTZ 6-OH desthio | 0.01 (LOQ) | 70 – 91 | 78.8 | 14 | Quantification |
| 0.1 | 72 – 86.5 | 80.5 | 9.4 |
| **Overall** | 70 – 91 | 79.7 | 11 |
| 0.01 (LOQ) | 83 – 96.3 | 88.6 | 7.8 | Confirmation |
| 0.1 | 73 – 83 | 78.8 | 6.6 |
| **Overall** | 73 – 96.3 | 83.7 | 9.1 |
| Honey | PTZ 6-OH desthio | 0.01 (LOQ) | 87.5 – 107 | 98.6 | 8.4 | Quantification |
| 0.1 | 113 – 120 | 116 | 2.3 |
| **Overall** | 87.5 – 120 | 107 | 10 |
| 0.01 (LOQ) | 84.5 – 109 | 98.5 | 11 | Confirmation |
| 0.1 | 116 – 125 | 119 | 3.0 |
| **Overall** | 84.5 – 125 | 109 | 12 |

\* a reduced validation set of n = 3 has been used.

\*\* Outlier value excluded through Dixon test, p<0.01; not taken into account for mean and relative standard deviation calculations

Table A 10: Recovery results from method validation of PTZ α-OH desthio using the analytical method

| **Matrix** | **Analyte** | **Fortification level [mg/kg] (*n* = 5)** | **Recovery range [%]** | **Mean** | **RSD [%]** | **Comments** |
| --- | --- | --- | --- | --- | --- | --- |
| **recovery [%]** |
| Oilseed Rape (Seed) | PTZ α-OH desthio | 0.01 (LOQ) | 91 – 112 | 103 | 7.4 | Quantification |
| 0.1 | 103 – 112 | 109 | 3.4 |
| **Overall** | 91 – 112 | 106 | 6.2 |
| 0.01 (LOQ) | 103 – 109 | 105 | 2.4 | Confirmation |
| 0.1 | 107 – 115 | 110 | 3.1 |
| **Overall** | 103 – 115 | 107 | 3.4 |
| Barley (Grain) | PTZ α-OH desthio | 0.01 (LOQ) | 105 – 118 | 114 | 4.8 | Quantification |
| 0.1 | 111 – 119 | 115 | 3.2 |
| **Overall** | 105 – 119 | 114 | 3.9 |
| 0.01 (LOQ) | 114 – 120 | 117 | 2.5 | Confirmation |
| 0.1 | 111 – 120 | 117 | 3.6 |
| **Overall** | 111 – 120 | 117 | 2.9 |
| Wheat (Grain)\* | PTZ α-OH desthio | 0.01 (LOQ) | 106 – 116 | 111 | 4.8 | Quantification |
| 0.1 | 117 – 120 | 119 | 1.1 |
| **Overall** | 106 – 120 | 115 | 4.7 |
| 0.01 (LOQ) | 107 – 115 | 110 | 4 | Confirmation |
| 0.1 | 113 – 119 | 116 | 2.7 |
| **Overall** | 107 – 119 | 113 | 4 |
| Barley (Straw) | PTZ α-OH desthio | 0.01 (LOQ) | 96 – 123 | 105 | 10 | Quantification |
| 0.1 | 104 – 110 | 108 | 2.3 |
| **Overall** | 96 – 123 | 106 | 7.2 |
| 0.01 (LOQ) | 86.5 – 110 | 102 | 8.9 | Confirmation |
| 0.1 | 93.5 – 109 | 103 | 6.1 |
| **Overall** | 86.5 – 110 | 103 | 7.2 |
| Wheat (Straw)\* | PTZ α-OH desthio | 0.01 (LOQ) | 90 – 104 | 94.5 | 8.2 | Quantification |
| 0.1 | 95.5 – 102 | 99.2 | 3.4 |
| **Overall** | 90 – 104 | 96.8 | 6.1 |
| 0.01 (LOQ) | 92.5 – 107 | 100 | 7.2 | Confirmation |
| 0.1 | 99 – 106 | 103 | 3.4 |
| **Overall** | 92.5 – 107 | 102 | 5.2 |
| Honey | PTZ α-OH desthio | 0.01 (LOQ) | 93.5 – 119 | 105 | 9.1 | Quantification |
| 0.1 | 115 – 126 | 119 | 3.9 |
| **Overall** | 93.5 – 126 | 112 | 9.1 |
| 0.01 (LOQ) | 90.8 – 112 | 97.6 | 9 | Confirmation |
| 0.1 | 113 – 122 | 118 | 3.3 |
| **Overall** | 90.8 – 122 | 108 | 11 |

\* a reduced validation set of n = 3 has been used.

Table A 11: Characteristics for the analytical method used for validation of prothioconazole metabolites in cereal matrices, oilseed rape and honey

|  | PTZ 3-OH desthio, PTZ 4-OH desthio, PTZ 5-OH desthio, PTZ 6-OH desthio and PTZ α-OH desthio |
| --- | --- |
| Specificity | A reagent blank and two control samples per matrix/analyte were extracted and analysed according to themethod to investigate the presence of residue and/or background interference at the retention time of the analyte(s). For both mass transitions, the samples showed no significant interference that would correspond to 30% of LOQ at the retention times of the analytes in any investigated matrix, therefore showing that the method is highly specific. Blank correction was not performed. |
| Calibration (type, number of data points) | A minimum of five calibration levels using matrix-matched calibration standards.  Regression Model: y = a + b\*x.  PTZ 3-OH desthio:   |  |  | | --- | --- | | *Oilseed rape*  Quantification:  y = 3.14+003 x – 30.7, r = 0.9990  Confirmation:  y = 1.71e+003 x + 21.4, r = 0.9993 | *Barley (grain)*  Quantification  y = 5.17e+003 x + 27.1, r = 0.9997  Confirmation:  y = 3.06e+003 x + 40.6, r = 0.9997 | | *Wheat (grain)*  Quantification  y = 8.36e+003 x + 125, r = 0.9994  Confirmation:  y = 4.91e+003 x + 53.4, r = 0.9999 | *Barley (straw)*  Quantification  y = 1.7e+003 x + 88.4, r = 0.9992  Confirmation:  y = 975x + 65.8, r = 0.9972 | | *Wheat (straw)*  Quantification  y = 5.44e+003 x + 7.96, r = 0.9999  Confirmation:  y = 3.01e+003 x + 77.6, r = 0.9980 | *Honey*  Quantification  y = 7.16e+003 x + 418, r = 0.9990  Confirmation:  y = 4.22e+003 x + 125, r = 0.9993 |   PTZ 4-OH desthio:   |  |  | | --- | --- | | *Oilseed rape*  Quantification:  y = 2.85e+003 x – 20.2, r = 0.9997  Confirmation:  y = 3.28e+003 x + 4.72, r = 0.9987 | *Barley (grain)*  Quantification  y = 4.99e+003 x + 86.8, r = 0.9997  Confirmation:  y = 5.62e+003 x – 4.17, r = 0.9999 | | *Wheat (grain)*  Quantification  y = 7.93e+003 x + 92, r = 0.9994  Confirmation:  y = 8.87e+003 x + 85.9, r = 0.9997 | *Barley (straw)*  Quantification  y = 1.77e+003 x + 92.4, r = 0.9982  Confirmation:  y = 1.76e+003 x + 143, r = 0.9999 | | *Wheat (straw)*  Quantification  y = 4.86e+003 x – 72.3, r = 0.9991  Confirmation:  y = 5.83e+003 x + 44, r = 0.9994 | *Honey*  Quantification  y = 6.83e+003 x + 387, r = 0.9982  Confirmation:  y = 7.8e+003 x + 581, r = 0.9996 |   PTZ 5-OH desthio:   |  |  | | --- | --- | | *Oilseed rape*  Quantification:  y = 2.59e+003 x + 15.3, r = 0.9983  Confirmation:  y = 1.48e+003 x + 26.8, r = 0.9992 | *Barley (grain)*  Quantification  y = 4.4e+003 x + 59.5, r = 0.9995  Confirmation:  y = 2.57e+003 x + 54.7, r = 0.9993 | | *Wheat (grain)*  Quantification  y = 7.62e+003 x + 114, r = 0.9998  Confirmation:  y = 4.93e+003 x + 42.9, r = 0.9983 | *Barley (straw)*  Quantification  y = 1.54e+003 x + 71.9, r = 0.9995  Confirmation:  y = 868 x + 59.8, r = 0.9982 | | *Wheat (straw)*  Quantification  y = 4.55e+003 x – 4.04, r = 0.9998  Confirmation:  y = 3.19e+003 x – 52.2, r = 0.9982 | *Honey*  Quantification  y = 6.93e+003 x + 390, r = 0.9994  Confirmation:  y = 3.95e+003 x + 254, r = 0.9970 |   PTZ 6-OH desthio:   |  |  | | --- | --- | | *Oilseed rape*  Quantification:  y = 4.05e+003 x – 88.2, r = 0.9988  Confirmation:  y = 3.07e+003 x – 59.2, r = 0.9965 | *Barley (grain)*  Quantification  y = 7.48e+003 x – 0.137, r = 0.9993  Confirmation:  y = 5.83e+003 x + 81.5, r = 0.9991 | | *Wheat (grain)*  Quantification  y = 1.36e+004 x + 53.6, r = 0.9998  Confirmation:  y = 1.05e+004 x + 54, r = 0.9995 | *Barley (straw)*  Quantification  y = 2.23e+003 x + 74.8, r = 0.9997  Confirmation:  y = 1.79e+003 x + 43.3, r = 0.9992 | | *Wheat (straw)*  Quantification  y = 8.82e+003 x – 205, r = 0.9969  Confirmation:  y = 6.91e+003 x – 197, r = 0.9976 | *Honey*  Quantification  y = 1.2e+004 x + 535, r = 0.9990  Confirmation:  y = 9.33e+003 x +502, r = 0.9992 |   PTZ α-OH desthio:   |  |  | | --- | --- | | *Oilseed rape*  Quantification:  y = 4.86e+003 x – 25.7, r = 0.9977  Confirmation:  y = 2.29e+003 x + 5.62, r = 0.9998 | *Barley (grain)*  Quantification  y = 7.67e+003 x + 27.6, r = 0.9994  Confirmation:  y = 3.49e+003 x + 1.76, r = 0.9986 | | *Wheat (grain)*  Quantification  y = 1.19e+004 x + 52.6, r = 0.9999  Confirmation:  y = 5.63e+003 x – 9.27, r = 0.9999 | *Barley (straw)*  Quantification  y = 2.86e+003 x + 26.4, r = 0.9986  Confirmation:  y = 1.36e+003 x + 60.6, r = 0.9987 | | *Wheat (straw)*  Quantification  y = 9.15e+003 x – 135, r = 0.9980  Confirmation:  y = 4.28e+003 x – 27.1, r = 0.9976 | *Honey*  Quantification  y = 1.02e+004 x + 390, r = 0.9973  Confirmation:  y = 4.81e+003 x +283, r = 0.9982 | |
| Calibration range | 0.06 ng/mL to 6.0 ng/mL (0.06 ng/mL to 4.0 ng/mL only for barley (straw)), corresponding mass fraction range: 0.003 mg/kg to 0.3 mg/kg (0.003 mg/kg to 0.2 mg/kg only for barley (straw)). |
| Assessment of matrix effects is presented | Yes, matrix effects were assessed. Significant matrix effects were determined for all analytes in oilseed rape (seed), and for PTZ α-OH desthio and PTZ 5-OH desthio in wheat (straw) matrix. Hence, matrix matched calibration standards were used throughout the study. |
| Limit of determination/quantification | |  |  |  | | --- | --- | --- | | LOQ | 0.01 mg/kg |  | | LOD | 0.002 mg/kg |  | |
| Stability of Analytes in Standard Solutions | Analytes were found to be stable in acetonitrile for at least 43 days when stored at typically 1 °C to 10 °C in the dark.  Analytes were found to be stable in water containing 10 mmol/L ammonium formate for at least 50 days when stored at typically 1 °C to 10 °C in the dark. |
| Stability of Analytes in Sample Extracts | Analytes were found to be stable in all matrix extracts for at least 9 days when stored at typically 1 °C to 10 °C in the dark. |

Conclusion

The underlying analytical method was successfully validated in terms of linearity of detector response, recovery and repeatability (by means of precision), LOQ and specificity following requirements set out in Guidance Document SANTE/2020/12830 rev. 2 at LOQ und higher fortification level in cereal matrices, oilseed rape (seed) and honey samples. The analytical method is reliable without restrictions.

* + - * 1. Method validation Storage Stability of Prothioconazole OH-desthio Metabolites and Triazole Derivative Metabolites in honey

The objective was to obtain data about the storage stability of the PTZ hydroxy-desthio metabolites and TDMs in honey at ≤ 18 °C in the dark over a storage period of up to 6 months. Analytical methods were previously validated in studies submitted under KCP 5.1.2/01 and KCP 5.1.2/02 in accordance to guidance document SANTE/2020/12830, rev. 2.

|  |  |
| --- | --- |
| Reference: | KCP 5.1.2/03, submitted under *KCA 6.1/02* |
| Report | Storage Stability of Prothioconazole OH-desthio Metabolites (alpha-, 3-, 4-, 5- and 6-hydroxy desthio) and Triazole Derivative Metabolites (TDMs) in Honey under Deep Frozen Conditions  Jooß, S., (2024)  Report No. S23-102955 |
| Guideline(s): | Yes, SANTE/2020/12830 rev. 2 |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes |

Materials and methods

*PTZ hydroxy-desthio* (based on study S22-05884, KCP 5.1.2/10):

In brief, samples of honey were extracted with acetonitrile/water (4/1, v/v). An aliquot of the raw extract was concentrated to an aqueous remainder which was hydrolysed by heating with 5N hydrochloric acid. After neutralization the extracts were diluted with aqueous ammonium formate solution. Quantification was performed by use of LC-MS/MS detection. LC-MS/MS determination was conducted with evaluation of one mass transitions (m/z 328->70) for each analyte.

*TDMs* (based on study S22-05883, KCP 5.1.2/09):

In brief, for 1,2,4-triazole (T), triazole alanine (TA), triazole acetic acid (TAA) and triazole lactic acid (TLA) homogenized samples of honey were extracted with methanol/water (4/1, v/v). Clean-up of the extract was performed by dispersive SPE with C18 material. Quantification was performed by use of LC-DMS/MS/MS detection with isotopically labelled internal standard(s).

LC-DMS-MS/MS determination was conducted by monitoring one mass transitions per analyte. A summary is given below:

|  |  |
| --- | --- |
| Analyte | Mass transition proposed for Quantification |
| 1,2,4-Triazole | *m*/*z* 70 -> 43 |
| Triazole Acetic Acid | *m*/*z* 128 -> 70 |
| Triazole Lactic Acid | *m*/*z* 158 -> 70 |
| Triazole Alanine | *m*/*z* 157 -> 70 |

Results and discussions

The analytical methods were previously fully validated in terms of specificity, linearity of detector’s response, LOQ, recovery and repeatability (by means of precision) according to Guidance Document SANTE/2020/12830 rev. 2. Fortification of aliquots of homogenised sample material with defined amounts of the analyte(s) (10x LOQ) and analysis for remaining levels after defined intervals of storage has been performed. The results are summarized below.

Table A 12: Recovery results from storage stability and concurrent recovery samples of PTZ 3-OH desthio using the analytical method

| **Matrix** | **Analyte** | **Fortification level [mg/kg] (*n* = 3)** | **Recovery range [%]** | **Mean** | **RSD [%]** | **Mean concurrent recoveries [%]** | **Comments** |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **recovery [%]** |
| Honey | PTZ 3-OH desthio | 0.10 | 96.5 – 104 | 99.7 | 3.9 | 108\* | 0-month storage |
| 0.10 | 70.5 – 90.5 | 80.8 | 12 | 104 | 3-month storage |
| 0.10 | 89.0 – 92.0 | 90.5 | 1.7 | 103 | 6-month storage |

\* concurrent recoveries measured at 0.01 mg/kg (LOQ)

Table A 13: Recovery results from storage stability and concurrent recovery samples of PTZ 4-OH desthio using the analytical method

| **Matrix** | **Analyte** | **Fortification level [mg/kg] (*n* = 3)** | **Recovery range [%]** | **Mean** | **RSD [%]** | **Mean concurrent recoveries [%]** | **Comments** |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **recovery [%]** |
| Honey | PTZ 4-OH desthio | 0.10 | 95.0 – 106 | 99.0 | 6.1 | 111\* | 0-month storage |
| 0.10 | 72.0 – 89.5 | 80.3 | 11 | 94.3 | 3-month storage |
| 0.10 | 92.5 – 97.5 | 94.5 | 2.8 | 103 | 6-month storage |

\* concurrent recoveries measured at 0.01 mg/kg (LOQ)

Table A 14: Recovery results from storage stability and concurrent recovery samples of PTZ 5-OH desthio using the analytical method

| **Matrix** | **Analyte** | **Fortification level [mg/kg] (*n* = 3)** | **Recovery range [%]** | **Mean** | **RSD [%]** | **Mean concurrent recoveries [%]** | **Comments** |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **recovery [%]** |
| Honey | PTZ 5-OH desthio | 0.10 | 94.0 – 104 | 97.7 | 5.6 | 107\* | 0-month storage |
| 0.10 | 74.5 – 103 | 90.3 | 16 | 97 | 3-month storage |
| 0.10 | 98.5 – 103 | 100 | 2.5 | 99.5 | 6-month storage |

\* concurrent recoveries measured at 0.01 mg/kg (LOQ)

Table A 15: Recovery results from storage stability and concurrent recovery samples of PTZ 6-OH desthio using the analytical method

| **Matrix** | **Analyte** | **Fortification level [mg/kg] (*n* = 3)** | **Recovery range [%]** | **Mean** | **RSD [%]** | **Mean concurrent recoveries [%]** | **Comments** |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **recovery [%]** |
| Honey | PTZ 6-OH desthio | 0.10 | 80.5 – 90.5 | 84.3 | 6.4 | 91.3\* | 0-month storage |
| 0.10 | 70.0 – 83.0 | 76.0 | 8.6 | 91.8 | 3-month storage |
| 0.10 | 95.5 – 101 | 98.3 | 2.6 | 92.3 | 6-month storage |

\* concurrent recoveries measured at 0.01 mg/kg (LOQ)

Table A 16: Recovery results from storage stability and concurrent recovery samples of PTZ α-OH desthio using the analytical method

| **Matrix** | **Analyte** | **Fortification level [mg/kg] (*n* = 3)** | **Recovery range [%]** | **Mean** | **RSD [%]** | **Mean concurrent recoveries [%]** | **Comments** |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **recovery [%]** |
| Honey | PTZ α-OH desthio | 0.10 | 113 – 116 | 115 | 1.6 | 108\* | 0-month storage |
| 0.10 | 72.0 – 94.5 | 83.2 | 14 | 100 | 3-month storage |
| 0.10 | 99.0 – 101 | 100 | 1.0 | 117 | 6-month storage |

\* concurrent recoveries measured at 0.01 mg/kg (LOQ)

Table A 17: Recovery results from storage stability and concurrent recovery samples of 1,2,4-Triazole using the analytical method

| **Matrix** | **Analyte** | **Fortification level [mg/kg] (*n* = 3)** | **Recovery range [%]** | **Mean** | **RSD [%]** | **Mean concurrent recoveries [%]** | **Comments** |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **recovery [%]** |
| Honey | 1,2,4-Triazole | 0.10 | 84.4 – 87.9 | 86.2 | 2.0 | 76.2\* | 0-month storage |
| 0.10 | 79.5 – 88.6 | 83.4 | 5.6 | 79.7 | 3-month storage |
| 0.10 | 77.6 – 86.8 | 82.0 | 5.6 | 90.2 | 6-month storage |

\* concurrent recoveries measured at 0.01 mg/kg (LOQ)

Table A 18: Recovery results from storage stability and concurrent recovery samples of Triazole Acetic Acid using the analytical method

| **Matrix** | **Analyte** | **Fortification level [mg/kg] (*n* = 3)** | **Recovery range [%]** | **Mean** | **RSD [%]** | **Mean concurrent recoveries [%]** | **Comments** |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **recovery [%]** |
| Honey | Triazole Acetic Acid | 0.10 | 80.4 – 84.7 | 82.2 | 2.7 | 62.5\* | 0-month storage |
| 0.10 | 90.6 – 94.6 | 92.4 | 2.2 | 90.5 | 3-month storage |
| 0.10 | 99.0 – 103 | 101 | 2.1 | 93.6 | 6-month storage |

\* concurrent recoveries measured at 0.01 mg/kg (LOQ)

Table A 19: Recovery results from storage stability and concurrent recovery samples of Triazole Lactic Acid using the analytical method

| **Matrix** | **Analyte** | **Fortification level [mg/kg] (*n* = 3)** | **Recovery range [%]** | **Mean** | **RSD [%]** | **Mean concurrent recoveries [%]** | **Comments** |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **recovery [%]** |
| Honey | Triazole Lactic Acid | 0.10 | 76.6 – 85.2 | 80.7 | 5.3 | 62.4\* | 0-month storage |
| 0.10 | 88.7 – 96.9 | 92.9 | 4.4 | 89.6 | 3-month storage |
| 0.10 | 96.1 – 104 | 101 | 4.3 | 106 | 6-month storage |

\* concurrent recoveries measured at 0.01 mg/kg (LOQ)

Table A 20: Recovery results from storage stability and concurrent recovery samples of Triazole Alanine using the analytical method

| **Matrix** | **Analyte** | **Fortification level [mg/kg] (*n* = 3)** | **Recovery range [%]** | **Mean** | **RSD [%]** | **Mean concurrent recoveries [%]** | **Comments** |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **recovery [%]** |
| Honey | Triazole Alanine | 0.10 | 85.4 – 87.7 | 86.2 | 1.5 | 88.9\* | 0-month storage |
| 0.10 | 80.7 – 93.2  (85.6 – 98.1) | 85.7 | 7.7 | 85.2 | 3-month storage |
| 0.10 | 81.7 – 85.0  (86.5 – 89.8) | 83.0 | 2.1 | 88.1 | 6-month storage |

\* concurrent recoveries measured at 0.01 mg/kg (LOQ)

Recoveries were corrected for apperent blank residues if those were >20% of the LOQ; uncorrected recoveries are given in brackets.

Table A 21: Characteristics for the analytical method used for validation of PTZ-hydroxy desthio metabolites in crop matrices

|  | PTZ-hydroxy desthio metabolites |
| --- | --- |
| Specificity | The analytes were determined in the final sample extracts by use of LC-MS/MS detection with evaluation of one mass transition per analyte. The blank values at the expected retention times of the analytes resulting from reagents and/or the control sample materials used for recovery determinations and for preparation of matrix-matched calibration standards did not exceed a level that would correspond to 30% of the LOQ. |
| Calibration (type, number of data points) | A minimum of five calibration levels using matrix-matched calibration standards.  Regression Model: y = a + b\*x.  PTZ 3-OH desthio:  Quantification  y = 8.4e+003 x - 184, r = 0.9995  PTZ 4-OH desthio:  Quantification  y = 7.77e+003 x – 117, r = 0.9995  PTZ 5-OH desthio:  Quantification  y = 7.42e+003 x - 121, r = 0.9999  PTZ 6-OH desthio:  Quantification  y = 1.02e+004 x - 109, r = 0.9998  PTZ α-OH desthio:  Quantification  y = 1.2e+004 x – 11.8, r = 0.9980 |
| Calibration range | 0.06 ng/mL to 6.0 ng/mL, corresponding mass fraction range: 0.003 mg/kg to 0.3 mg/kg. |
| Assessment of matrix effects is presented | Matrix effects were assesed in primary validation study S22-05884, KCP 5.1.2/10. Matrix matched calibration standards were used throughout the study. |
| Limit of determination/quantification | |  |  | | --- | --- | | LOQ | 0.01 mg/kg | | LOD | 0.003 mg/kg (for PTZ-hydroxy desthio metabolites) | |

Table A 22: Characteristics for the analytical method used for validation of TDMs in crop matrices

|  | TDMs |
| --- | --- |
| Specificity | With the exception of triazole alanine, the control samples showed no significant interference (above 30 % of LOQ for the PTZ hydroxy-desthio metabolites and above 20 % of LOQ for the TDMs, respectively) at the retention time of the analyte(s), therefore showing that the methods are highly specific. |
| Calibration (type, number of data points) | The linearity of the detector response was demonstrated by single determination of solvent calibration standards at a minimum of five (5) concentrations. Calibration standards contained isotopically-labelled standards at a constant concentration of 50 ng/mL (equivalent to 0.10 mg/kg). The peak area ratio of analyte and the internal standard was calculated and used for the generation of the calibration curves.  Regression Model: y = a + b\*x.  1,2,4-Triazole:  Quantification:  y = 0.711 x + 0.00147, r = 0.9999  Triazole Acetic Acid:  Quantification:  y = 1.33 x + 0.000745, r = 0.9997  Triazole Lactic Acid:  Quantification:  y = 0.775 x + 0.000715, r = 0.9998  Triazole Alanine:  Quantification:  y = 0.858 x + 0.000957, r = 0.9999 |
| Calibration range | 1 ng/mL to 100 ng/mL (corresponding to 0.002 mg/kg to 0.2 mg/kg). |
| Assessment of matrix effects is presented | Isotopically labelled internal standards were used for quantification so that matrix effects on the detector response were automatically accounted for when using the response ratio of analyte and internal standard for quantification. Therefore, solvent standard solutions were used throughout the study and matrix effects have not been determined. |
| Limit of determination/quantification | |  |  | | --- | --- | | LOQ | 0.01 mg/kg | | LOD | 0.002 mg/kg (for TDMs) | |

Conclusion

The underlying analytical methods were previously validated in terms of linearity of detector response, recovery and repeatability (by means of precision), LOQ and specificity following requirements set out in Guidance Document SANTE/2020/12830 rev. 2, respectively at LOQ und higher fortification levels. Applicability of the method was confirmed by concurrent recovery determination during storage stability determination. The analytical methods are reliable without restrictions.

The study is deemed sufficient for assessing the stability of PTZ hydroxy-desthio metabolites (alpha-, 3-, 4-, 5- and 6-hydroxy desthio) and the Triazole Derivative Metabolites (TDMs) 1,2,4-triazole (T), triazole acetic acid (TAA), triazole lactic acid (TLA) and triazole alanine (TA) in honey upon storage at ≤ -18 °C for 6 months.

* + - 1. Description of analytical methods for the determination of residues in support of toxicological studies (KCP 5.1.2)

No new or additional studies have been submitted

* + - 1. Description of analytical methods for the determination of residues in support of ecotoxicological studies (KCP 5.1.2)

No new or additional studies have been submitted

* + 1. Methods for post-authorization control and monitoring purposes (KCP 5.2)
       1. Description of analytical methods for the determination of residues in plant matrices (KCP 5.2)

No new or additional studies have been submitted.

* + - 1. Description of analytical methods for the determination of residues in animal matrices (KCP 5.2)
         1. Analytical method in honey

Method validation

An analytical method for the determination of residues of prothioconazole in honey is given below:

|  |  |
| --- | --- |
| Reference: | KCP 5.2/01 |
| Report | Determination of Residues of Prothioconazole and its metabolites in Honey after Two Applications of Prothioconazole 250g/L EC to Phacelia tancetifolia under Semi-Field Conditions in Northern and Southern Europe in 2023, Peris Mestre, D., 2024, Report No.: 23306-01R |
| Guideline(s): | Yes, SANTE/2020/12830 rev. 2 |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes |

Materials and methods

Prothioconazole and prothioconazole-destio residues were extracted from homogenised honey specimens with acetonitrile after addition of an opportune amount of water. After QuEChERS salts addition, the acetonitrile phase was separated from the aqueous phase and an aliquot was diluted 1;2 with water. Final analysis was performed in positive ionisation mode by High Performance Liquid Chromatography, tandem Mass Spectrometry (LC-MS/MS). Two ion transitions were concurrently acquired for each analyte:

|  |  |  |
| --- | --- | --- |
| Analyte | Mass transition proposed for Quantification | Mass transition proposed for Confirmation |
| Prothioconazole | *m*/*z* 344 -> 326 | *m*/*z* 344 -> 125 |
| Prothioconazole-desthio | *m*/*z* 312 -> 70 | *m*/*z* 312 -> 125 |

Results and discussions

The analytical method was fully validated in terms of specificity, linearity of detector’s response, LOQ, recovery and repeatability (by means of precision) according to Guidance Document SANTE/2020/12830 rev. 2. The results are summarized below.

Table A 23: Recovery results from method validation of prothioconazole and its metabolite prothioconazole-desthio in honey using the analytical method

| Matrix | Analyte | Fortification level [mg/kg] (n = 5) | Recovery range [%] | Mean  recovery [%] | RSD [%] | Comments |
| --- | --- | --- | --- | --- | --- | --- |
| Honey | Prothioconazole | 0.01 | 78.0 – 93.3 | 85.0 | 6.6 | quantification |
| 0.1 | 104.7 – 110.9 | 107.2 | 2.3 |
| **Overall** | 78.0 – 110.9 | 96.1 | 12.9 |
| 0.01 | 79.5 – 87.6 | 84.3 | 3.9 | confirmation |
| 0.1 | 104.6 – 109.7 | 105.9 | 3.2 |
| **Overall** | 79.5 – 109.7 | 95.1 | 12.4 |
| Honey | Prothioconazole-desthio | 0.01 | 93.4 – 109.5 | 101.6 | 6.8 | quantification |
| 0.1 | 103.1 – 112.4 | 108.6 | 3.4 |
| **Overall** | 93.4 – 112.4 | 105.1 | 6.1 |
| 0.01 | 90.3 – 110.5 | 101.5 | 8.3 | confirmation |
| 0.1 | 104.0 – 113.5 | 109.4 | 3.4 |
| **Overall** | 90.3 – 113.5 | 105.5 | 7.0 |

Table A 24: Recovery results from storage stability of prothioconazole and its metabolite prothioconazole-desthio in honey using the analytical method

| Matrix | Analyte | Fortification level [mg/kg] (n = 2) | Recovery range [%] | Mean  recovery [%] | RSD [%] | Comments |
| --- | --- | --- | --- | --- | --- | --- |
| Honey | Prothioconazole | 0.1 | 100.8 – 105.0 | 102.9 | 2.9 | Quantification, Pre Storage T0 |
| 0.1 | 76.5 – 78.1 | 77.3 | 1.5 | Quantification, After Storage T181 |
| Honey | Prothioconazole-desthio | 0.1 | 105.0 – 108.0 | 106.5 | 2.0 | Quantification, Pre Storage T0 |
| 0.1 | 86.7 – 91.0 | 88.9 | 3.4 | Quantification, After Storage T181 |

Table A 25: Characteristics for the analytical method used for validation of prothioconazole and its metabolite prothioconazole-desthio residues in honey

|  | Prothioconazole | Prothioconazole-desthio |
| --- | --- | --- |
| Specificity | The analytes were determined in the final sample extracts by use of LC-MS/MS detection with evaluation of two mass transitions per analyte. No significant interferences exceeding 30% of the LOQ were found of the retention time of the analyte were found in blank samples. | The analytes were determined in the final sample extracts by use of LC-MS/MS detection with evaluation of two mass transitions per analyte. No significant interferences exceeding 30% of the LOQ were found of the retention time of the analyte were found in blank samples. |
| Calibration (type, number of data points) | A minimum of seven calibration levels using matrix-matched calibration standards.  Regression Model: y = a + b\*x.  Prothioconazole:  Quantification  y = 6.12e+004 x + 3.07e+003, r = 0.9996  Confirmation  y = 1.29e+004 x + 129, r = 0.9989 | A minimum of seven calibration levels using matrix-matched calibration standards.  Regression Model: y = a + b\*x.  Prothioconazole-desthio:  Quantification  y = 8.17e+004 x + 2.62e+003, r = 0.9993  Confirmation  y = 5.40e+004 x + 1.9e+003, r = 0.9995 |
| Calibration range | 0.25 ng/mL to 20.0 ng/mL, corresponding mass fraction range: 0.0025 mg/kg to 0.2 mg/kg in sample | 0.25 ng/mL to 20.0 ng/mL, corresponding mass fraction range: 0.0025 mg/kg to 0.2 mg/kg in sample |
| Assessment of matrix effects is presented | Yes, no significant matrix effects were detected. Nevertheless, matrix matched calibration standards were used throughout the study. | Yes, significant matrix effects were detected. Hence, matrix matched calibration standards were used throughout the study. |
| Limit of determination/quantification | |  |  | | --- | --- | | LOQ | 0.01 mg/kg | | LOD | 0.0025 mg/kg | | |  |  | | --- | --- | | LOQ | 0.01 mg/kg | | LOD | 0.0025 mg/kg | |
| Stability of Analyte(s) in Working Solutions | Stability of stock solutions were verified for a maximum storage period of 105 days at nominal temperature of ‑18 °C. | Stability of stock solutions were verified for a maximum storage period of 28 days at nominal temperature of ‑18 °C. |
| Stability of Analyte(s) in Sample Extracts | Final extracts were analysed within 24 h after extraction, hence no determination of extract stability is required. | Final extracts were analysed within 24 h after extraction, hence no determination of extract stability is required. |
| Frozen storage stability | Residues were found to be stable for 181 days under frozen conditions (< -18 °C) in honey matrix. | Residues were found to be stable for 181 days under frozen conditions (< -18 °C) in honey matrix. |

Conclusion

The underlying analytical method was validated in terms of linearity of detector response, recovery and repeatability (by means of precision), LOQ and specificity following requirements set out in Guidance Document SANTE/2020/12830 rev. 2 at LOQ und higher fortification level. Stability of residues in frozen storage samples has been determined for 181 days. The analytical method is reliable without restrictions.

Independent laboratory validation

|  |  |
| --- | --- |
| Reference: | KCP 5.2/02 |
| Report | Independent Laboratory Validation of an Analytical Method for the determination of Prothioconazole-desthio in honey samples, Sala, A., 2024, Report No.: LBN-0085-2024 |
| Guideline(s): | Yes, SANTE/2020/12830 rev. 2 |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes |

Materials and methods

Prothioconazole-destio residues were extracted from homogenised honey specimens with acetonitrile after addition of an opportune amount of water. After QuEChERS salts addition, the acetonitrile phase was separated from the aqueous phase and an aliquot was diluted with water. Final analysis was performed in positive ionisation mode by High Performance Liquid Chromatography, tandem Mass Spectrometry (LC-MS/MS). Two ion transitions were concurrently acquired:

|  |  |  |
| --- | --- | --- |
| Analyte | Mass transition proposed for Quantification | Mass transition proposed for Confirmation |
| Prothioconazole-desthio | *m*/*z* 312 -> 70 | *m*/*z* 312 -> 125 |

Results and discussions

The analytical method was fully validated in terms of specificity, linearity of detector’s response, LOQ, recovery and repeatability (by means of precision) according to Guidance Document SANTE/2020/12830 rev. 2. The results are summarized below.

**Table A 26: Recovery results from independent laboratory validation of prothioconazole-desthio in honey using the analytical method**

| **Matrix** | **Analyte** | **Fortification level [mg/kg] (n = 5)** | **Recovery range [%]** | **Mean  recovery [%]** | **RSD [%]** | **Comments** |
| --- | --- | --- | --- | --- | --- | --- |
| Honey | Prothioconazole-desthio | 0.01 | 96.3 – 99.6 | 98.0 | 1.4 | quantification |
| 0.1 | 101.7 – 104.7 | 103.1 | 1.0 |
| **Overall** | 96.3 – 104.7 | 100.6 | 2.9 |
| 0.01 | 97.0 – 100.5 | 98.9 | 1.4 | confirmation |
| 0.1 | 101.9 – 104.6 | 103.3 | 0.9 |
| **Overall** | 97.0 – 104.6 | 101.1 | 2.5 |

**Table A 27: Characteristics for the analytical method used for independent laboratory validation of prothioconazole-desthio residues in honey**

|  | **Prothioconazole-desthio** |
| --- | --- |
| Specificity | The analyte was determined in the final sample extracts by use of LC-MS/MS detection with evaluation of two mass transitions per analyte. No significant interferences exceeding 30% of the LOQ were found of the retention time of the analyte were found in blank samples. |
| Calibration (type, number of data points) | A minimum of seven calibration levels using matrix-matched calibration standards.  Regression Model: y = a + b\*x.  Prothioconazole-desthio:  Quantification  y = 8659.550752x + 315.147453, r = 0.9991  Confirmation  y = 2904.024849x + 179.023011, r = 0.9991 |
| Calibration range | 0.2 µg/L to 20.0 µg/L, corresponding mass fraction range: 0.002 mg/kg to 0.2 mg/kg in sample |
| Assessment of matrix effects is presented | Yes, significant matrix effects were detected in primary validation study. Hence, matrix matched calibration standards were used throughout the study. |
| Limit of determination/quantification | |  |  | | --- | --- | | LOQ | 0.01 mg/kg | | LOD | 0.002 mg/kg | |
| Stability of Analyte(s) in Working Solutions | Stability of stock solutions were verified for a maximum storage period of 28 days at nominal temperature of ‑18 °C in primary validation study. |
| Stability of Analyte(s) in Sample Extracts | Final extracts were analysed within 24 h after extraction, hence no determination of extract stability is required. |

Conclusion

The underlying analytical method 23306-01R was independently validated for prothioconazole-desthio in terms of linearity of detector response, recovery and repeatability (by means of precision), LOQ and specificity following requirements set out in Guidance Document SANTE/2020/12830 rev. 2 at LOQ und higher fortification level. The analytical method is reliable without restrictions.

* + - 1. Description of Methods for the Analysis of Soil (KCP 5.2)

No new or additional studies have been submitted.

* + - 1. Description of Methods for the Analysis of Water (KCP 5.2)

No new or additional studies have been submitted

* + - 1. Description of Methods for the Analysis of Air (KCP 5.2)

No new or additional studies have been submitted.

* + - 1. Description of Methods for the Analysis of Body Fluids and Tissues (KCP 5.2)

No new or additional studies have been submitted.

* + - 1. Other Studies/ Information

No new or additional studies have been submitted.

* 1. Analytical methods for spiroxamine
     1. Methods used for the generation of pre-authorization data (KCP 5.1)

~~No new or additional studies have been submitted.~~

* + - 1. Method validation of active substance in plant protection product (KCP 5.1.1)

A Summary of the method has already been presented under 5.2.1.1.

* + - 1. Description of analytical methods for the determination of residues in support of residues studies (KCP 5.1.2)

No new or additional studies have been submitted.

* + - 1. Description of analytical methods for the determination of residues in support of toxicological studies (KCP 5.1.2)

No new or additional studies have been submitted

* + - 1. Description of analytical methods for the determination of residues in support of ecotoxicological studies (KCP 5.1.2)

No new or additional studies have been submitted

* + 1. Methods for post-authorization control and monitoring purposes (KCP 5.2)
       1. Description of analytical methods for the determination of residues in plant matrices (KCP 5.2)

No new or additional studies have been submitted.

* + - 1. Description of analytical methods for the determination of residues in animal matrices (KCP 5.2)

No new or additional studies have been submitted.

* + - 1. Description of Methods for the Analysis of Soil (KCP 5.2)

No new or additional studies have been submitted.

* + - 1. Description of Methods for the Analysis of Water (KCP 5.2)

No new or additional studies have been submitted

* + - 1. Description of Methods for the Analysis of Air (KCP 5.2)

No new or additional studies have been submitted.

* + - 1. Description of Methods for the Analysis of Body Fluids and Tissues (KCP 5.2)

No new or additional studies have been submitted.

* + - 1. Other Studies/ Information

No new or additional studies have been submitted.