FINAL REGISTRATION REPORT

Part B

Section 5

Analytical Methods

Detailed summary of the risk assessment

Product code: **CHR/ZF/PROTI 100 FS**

Product name(s):

Gamelan 100 FS

Doraltes 100 FS

Chemical active substance(s):

Prothioconazole, 100 g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(authorization)

Applicant: Innvigo Sp. z o.o.

Submission date: 05.2022

MS Finalisation date: 05/09/2022

Version history

When	What
October 2021	Dossier sent for evaluation
May 2022	Updates based on feedback from zRMS Poland
June 2022	zRMS evaluation of dRR
September 2022	Final version prepared by zRMS after Commenting period

Table of Contents

Evaluator comments:
The text highlighted in grey was provided by the evaluator.

New and additional information were highlighted in yellow.

5.1 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.
Noticed data gaps are: none

Sufficiently sensitive and selective analytical methods are available for all analytes included in the residue definitions.
The applicant's report was not rewritten. The zRMS text is on grey background.

Data gaps: none

Commodity/crop	Supported/ Not supported
Cereals	Supported

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

5.2.1 Analysis of the plant protection product (KCP 5.1.1)

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

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

Comments of zRMS:	This method is validated and can be used for analysing prothioconazole in the PPP. No information on reagent stability and storage of validation samples prior to analysis.

Reference:	KCP 5.1/01
Report	CHR/ZF/PROTI 100 FS Stage I: Determination of physicochemical properties of the initial preparation, after accelerated storage and after low temperature storage, E. Arevalo, 2021, Study code: BF – 10/21
Guideline(s):	SANCO/3030/99 rev. 5
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Shimadzu liquid chromatograph equipped with DAD detector
· Column: Luna C18, 250 x 4.6 mm, 5µm
· Analytical balance Mettler Toledo XS 205 DU/M, accuracy 0.01 mg
· Ultrasonic bath
· Volumetric flasks
· Pipettes

· Syringe filters Pureland HPPTFE 0.22 µm
· Deionized water, ultra-pure, Millipore
· Acetonitrile for HPLC-Super Gradient, POCh
· Orthophosphoric acid, Chempur
· analytical standard – Prothioconazole (Dr.Ehrenstorfer)

The determination of the active ingredient – Prothioconazole content in CHR/ZF/PROTI 100 FS preparation were carried out in accordance with the method – MT/BA-08/21 – developed and validated (description of the method validation see Appendix no.14) according to EU requirements described in SANCO/3030/99 rev. 5 guideline and according to the Standard Operating Procedure SPO/BA/090/b. The method (MT/BA-08/21) is based on determination of Prothioconazole using reversed phase high performance liquid chromatography (RP-HPLC) with DAD detection at wavelength 206 nm and external standard at the initial stage and after accelerated storage

Validation - Results and discussions

Table 5.2-1: Methods suitable for the determination of active substances prothioconazole in plant protection product CHR/ZF/PROTI

<table>
<tr><th></th><th>Prothioconazole</th></tr>
<tr><td>Author(s), year</td><td>E. Arevalo, 2021</td></tr>
<tr><td>Principle of method</td><td>HPLC-DAD</td></tr>
<tr><td>Linearity (linear between mg/L / % range of the declared content) (correlation coefficient, expressed as r) n = 5</td><td>In order to check the linearity, the calibration curves were prepared using five standard solutions of a Prothoconazole ~~with the following standard solution prothioconazole~~ in the concentration~~s~~ in range from 0.1340 to 0.3350 [mg/mL]. A graph of the peak area to the concentration of prothioconazole was plotted. The resulting curves are linear in the tested concentrations. ~~Linearity range of prothioconazole is from 0.1340 to 0.3350 [mg/mL].~~ Correlation coefficient R^2 is 0.9998.</td></tr>
<tr><td>Precision – Repeatability Mean n = 6 (%RSD)</td><td>Acceptable relative standard deviation for analyte in preparation (~ 9.628%) is RSDr ≤ 1.91 %.
The obtained result 1.32% is acceptable.
<table>
<tr><th></th><th>Mass [mg]</th><th>Concentration [mg/ml]</th><th>Peak area</th><th>Content [%]</th></tr>
<tr><td>Sample 1</td><td>21.21</td><td>2.121</td><td>3831096</td><td>9.864</td></tr>
<tr><td>Sample 2</td><td>24.01</td><td>2.401</td><td>4177758</td><td>9.517</td></tr>
<tr><td>Sample 3</td><td>23.51</td><td>2.351</td><td>4143609</td><td>9.638</td></tr>
<tr><td>Sample 4</td><td>23.06</td><td>2.306</td><td>4022471</td><td>9.534</td></tr>
<tr><td>Sample 5</td><td>22.52</td><td>2.252</td><td>3944630</td><td>9.571</td></tr>
<tr><td>Sample 6</td><td>21.47</td><td>2.147</td><td>3791091</td><td>9.641</td></tr>
<tr><td></td><td></td><td></td><td>Average</td><td>9.628</td></tr>
<tr><td></td><td></td><td></td><td>SD</td><td>0.127</td></tr>
<tr><td></td><td></td><td></td><td>RSD [%]</td><td>1.32</td></tr>
</table>
Horwitz ratio is 0.69 and fulfils acceptance criterion Hr ≤ 1.</td></tr>
</table>

<table>
<tr><th></th><th>Prothioconazole</th></tr>
<tr><td>Accuracy
n = 2 levels (6 samples per each fortification level)
(% Recovery)</td><td>
<table>
<tr><th></th><th>Prothioconazole added [mg/mL]</th><th>Peak area</th><th>Determined of Prothioconazole [mg/mL]</th><th>Total Recovery [%]</th></tr>
<tr><td rowspan="6">Level I</td><td rowspan="6">0.2234</td><td>4078822</td><td>0.2230</td><td>99.8</td></tr>
<tr><td>4055496</td><td>0.2217</td><td>99.3</td></tr>
<tr><td>4184145</td><td>0.2288</td><td>102.5</td></tr>
<tr><td>4172152</td><td>0.2282</td><td>102.2</td></tr>
<tr><td>4170823</td><td>0.2281</td><td>102.1</td></tr>
<tr><td>4168978</td><td>0.2280</td><td>102.1</td></tr>
<tr><td rowspan="2">Lev</td><td rowspan="2">0.2792</td><td>5120556</td><td>0.2809</td><td>100.6</td></tr>
<tr><td>5112482</td><td>0.2804</td><td>100.4</td></tr>
<tr><td rowspan="4"></td><td rowspan="4"></td><td>5119789</td><td>0.2809</td><td>100.6</td></tr>
<tr><td>5126576</td><td>0.2812</td><td>100.7</td></tr>
<tr><td>5147224</td><td>0.2824</td><td>101.1</td></tr>
<tr><td>5136874</td><td>0.2818</td><td>100.9</td></tr>
<tr><td></td><td></td><td></td><td>Average</td><td>101.0</td></tr>
<tr><td></td><td></td><td></td><td>SD</td><td>1.00</td></tr>
<tr><td></td><td></td><td></td><td>RSD %</td><td>0.99%</td></tr>
</table>
The result of 101.0% fulfils the acceptance criterion (90 – 110%).
</td></tr>
<tr><td>Interference/ Specificity</td><td>Specificity of the method was evaluated based on the analysis of chromatograms for blank samples (placebo) against samples of placebo spiked with prothioconazole standards. Analysis showed no overlapping of determined substances signal with the signals of matrix components under method conditions hence method specificity criterion is fulfilled.</td></tr>
<tr><td>LOQ</td><td>The limit of quantification (LOQ) was defined as the lowest concentration of standard – 0.00046 mg/ml (0.011 g/kg of the preparation i.e. 0.105 g/kg of Prothioconazole), which was determined with an acceptable recovery.</td></tr>
<tr><td>Comment</td><td></td></tr>
</table>

Conclusion

It was confirmed that the method is specific. There were no peaks from placebo interfering with determined compounds. The validation parameters (specificity, linearity, instrument precision, repeatability and accuracy) are within the acceptance range and fulfil EU requirements given in SANCO /3030/99 rev.5.

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

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

Comments of zRMS:	This method is validated and can be used for analysing relevant impurities in the PPP. no information on reagent stability and storage of validation samples prior to analysis.

Reference:	KCP 5.1/02
Report	CHR/ZF/PROTI 100 FS Stage I: Determination of physicochemical properties of the initial preparation, after accelerated storage and after low temperature storage, E. Arevalo, 2021, Study code: BF – 10/21
Guideline(s):	SANCO/3030/99 rev. 5
Deviations:	No

GLP:	Yes
Acceptability:	Yes

Materials and methods

Shimadzu liquid chromatograph equipped with DAD detector
· Column: Gemini NX-C18, 250 x 4.6 mm, 5µm
· Analytical balance Mettler Toledo XS 205 DU/M, accuracy 0.01 mg
· Ultrasonic bath
· Volumetric flasks
· Pipettes
· Syringe filters Pureland HPPTFE 0.22 µm
· Deionized water, ultra-pure, Millipore
· Acetonitrile for HPLC-Super Gradient, POCh
· Orthophosphoric acid, Chempur
· analytical standard – Prothioconazole-desthio (Dr.Ehrenstorfer)

The determination of impurity – Prothioconazole-desthio content in CHR/ZF/PROTI 100 FS preparation were carried out in accordance with the method – MT/BA-09/21 – developed and validated (description of the method validation see Appendix no.14) according to EU requirements described in SANCO/3030/99 rev. 5 (22/03/19) guideline and according to the Standard Operating Procedure SPO/BA/090/b.
The method (MT/BA-09/21) is based on determination of Prothioconazole-desthio using reversed phase high performance liquid chromatography (RP-HPLC) with DAD detection at wavelength 206 nm and external standard at the initial stage and after accelerated storage.

Reference:	KCP 5.1/03
Report	CHR/ZF/PROTI 100 FS Stage I: Determination of physicochemical properties of the initial preparation, after accelerated storage and after low temperature storage, E. Arevalo, 2021, Study code: BF – 10/21
Guideline(s):	SANCO/3030/99 rev. 5
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

VARIAN CP-3800 Gas Chromatograph with FID
· Teledyne Tekmar HT-3 Headspace Autosampler
· Rxi®-1301Sil MS capillary column, 30 m × 0.25 mm × 1.0 µm (RESTEK)
· Analytical balance Mettler Toledo XS205 Dual Range, accuracy of 0.01 mg
· Glass pipettes
· Automatic pipettes
· Glass graduated flasks
· 20 mL headspace vials with alumina caps and Teflon-silicone septa
· Laboratory crimper
· Autosampler vials

· Typical laboratory equipment
· Dimethyl sulfoxide (DMSO), for headspace analysis (99,99 %), VWR Chemicals,
· analytical standard - Toluene

The content of Toluene in the examined preparation was determined using headspace analysis in combination with gas chromatography and flame ionization detection (HS-GC-FID) using external standard method (MT/BA-11/21).

Validation - Results and discussions

Table 5.2-2: Methods suitable for the determination of the relevant impurities in plant protection product (PPP) CHR/ZF/PROTI

	Toluene **max. g mg/kg**	**Prothioconazole-desthio** **max. 0.5 g/kg**
Author(s), year	E. Arevalo, 2021	E. Arevalo, 2021
Principle of method	HS-GC-FID	HPLC-DAD
Linearity (linear between mg/L) (correlation coefficient, expressed as r)	n=5 In order to check the linearity, calibration curve was prepared using standard solutions with the following concentrations.. A graph of the peak area to the concentration of toluene was plotted against the ratio of toluene concentration to the internal standard concentration. The resulting curve is linear in the tested concentrations. Linearity range of toluene is from 0.001 to 0.020 [mg/mL]. Correlation coefficient R^2 is 0.9997	n=5 In order to check the linearity, calibration curve was prepared using five standard solutions ~~with the following~~ in the concentrations~~.range from~~ 0.00046 to 0.00217 [mg/mL]. A graph of the peak area to the concentration of toluene was plotted against the ratio of ~~toluene~~ Prothoconazole-desthio concentration to the internal standard concentration. The resulting curve is linear in the tested concentrations. ~~Linearity range of toluene is from 0.00046 to 0.00217 [mg/mL].~~ Correlation coefficient R^2 is 0.9989
Precision – Repeatability Mean n = 6 (%RSD)	Relative standard deviation of determination of toluene fulfils acceptance criterion. RSD for substance at the concentration of ~ 0.006 % should be less than or equal to 5.77.	Acceptable relative standard deviation for analyte in preparation (~ 0.0028%) is RSDr ≤ 6.49 %. The obtained result 5.09% is acceptable.

<table>
<tr>
<td></td>
<td>
<table>
<tr><th rowspan="2">No</th><th rowspan="2">Sample mass [mg]</th><th rowspan="2">Toluene peak area</th><th colspan="2">Result</th></tr>
<tr><th>[%]</th><th>[g/kg]</th></tr>
<tr><td>1</td><td>50.24</td><td>942.7</td><td>0.0063</td><td>0.0631</td></tr>
<tr><td>2</td><td>49.74</td><td>901.1</td><td>0.0061</td><td>0.0610</td></tr>
<tr><td>3</td><td>50.18</td><td>902.9</td><td>0.0061</td><td>0.0606</td></tr>
<tr><td>4</td><td>50.68</td><td>919.3</td><td>0.0061</td><td>0.0610</td></tr>
<tr><td>5</td><td>51.67</td><td>939.7</td><td>0.0061</td><td>0.0612</td></tr>
<tr><td>6</td><td>52.29</td><td>961.3</td><td>0.0062</td><td>0.0618</td></tr>
<tr><td></td><td></td><td>Mean</td><td>0.0061</td><td>0.0614</td></tr>
<tr><td></td><td></td><td>SD</td><td>0.00009</td><td>0.0009</td></tr>
<tr><td></td><td></td><td>RSD %</td><td>1.45</td><td>1.45</td></tr>
</table>
Horwitz ratio is 0.25 and fulfils acceptance criterion Hr ≤ 1.
</td>
<td>
<table>
<tr><th></th><th>Mass [mg]</th><th>Concentration [mg/ml]</th><th>Peak area</th><th>Content [%]</th></tr>
<tr><td>Sample 1</td><td>209.20</td><td>41.840</td><td>21107</td><td>0.0030</td></tr>
<tr><td>Sample 2</td><td>204.41</td><td>40.882</td><td>18836</td><td>0.0028</td></tr>
<tr><td>Sample 3</td><td>204.05</td><td>40.810</td><td>18582</td><td>0.0027</td></tr>
<tr><td>Sample 4</td><td>201.96</td><td>40.392</td><td>20507</td><td>0.0030</td></tr>
<tr><td>Sample 5</td><td>208.66</td><td>41.732</td><td>20099</td><td>0.0029</td></tr>
<tr><td>Sample 6</td><td>204.59</td><td>40.918</td><td>18260</td><td>0.0027</td></tr>
<tr><td></td><td></td><td></td><td>Average</td><td>0.0028</td></tr>
<tr><td></td><td></td><td></td><td>SD</td><td>0.0001</td></tr>
<tr><td></td><td></td><td></td><td>RSD [%]</td><td>5.09</td></tr>
</table>
Horwitz ratio is 0.78 and fulfils acceptance criterion Hr ≤ 1.
</td>
</tr>
<tr>
<td>Accuracy n = 6 (% Recovery)</td>
<td>
<table>
<tr><th></th><th>No</th><th>Sample mass [mg]</th><th>C_U [mg]</th><th>C_A [mg]</th><th>(C_U+C_A) [mg]</th><th>Toluene peak area</th><th>C_F [mg]</th><th>Recovery [%]</th></tr>
<tr><td rowspan="6">Level I</td><td>1</td><td>n/a*</td><td>n/a*</td><td>0.0010</td><td>0.0010</td><td>268.8</td><td>0.0010</td><td>98.7</td></tr>
<tr><td>2</td><td>n/a*</td><td>n/a*</td><td>0.0010</td><td>0.0010</td><td>261.1</td><td>0.0010</td><td>96.2</td></tr>
<tr><td>3</td><td>n/a*</td><td>n/a*</td><td>0.0010</td><td>0.0010</td><td>268.1</td><td>0.0010</td><td>98.4</td></tr>
<tr><td>4</td><td>n/a*</td><td>n/a*</td><td>0.0010</td><td>0.0010</td><td>265.9</td><td>0.0010</td><td>97.7</td></tr>
<tr><td>5</td><td>n/a*</td><td>n/a*</td><td>0.0010</td><td>0.0010</td><td>277.7</td><td>0.0010</td><td>101.5</td></tr>
<tr><td>6</td><td>n/a*</td><td>n/a*</td><td>0.0010</td><td>0.0010</td><td>278.4</td><td>0.0010</td><td>101.8</td></tr>
<tr><td rowspan="6">Level II</td><td>13</td><td>27.04</td><td>0.0017</td><td>0.0020</td><td>0.0037</td><td>1061.6</td><td>0.0036</td><td>97.0</td></tr>
<tr><td>14</td><td>26.28</td><td>0.0016</td><td>0.0020</td><td>0.0036</td><td>1063.2</td><td>0.0036</td><td>98.3</td></tr>
<tr><td>15</td><td>26.48</td><td>0.0016</td><td>0.0020</td><td>0.0036</td><td>1028.7</td><td>0.0034</td><td>94.9</td></tr>
<tr><td>16</td><td>25.88</td><td>0.0016</td><td>0.0020</td><td>0.0036</td><td>1052.7</td><td>0.0035</td><td>98.1</td></tr>
<tr><td>17</td><td>24.42</td><td>0.0015</td><td>0.0020</td><td>0.0035</td><td>983.6</td><td>0.0033</td><td>94.2</td></tr>
<tr><td>18</td><td>25.00</td><td>0.0015</td><td>0.0020</td><td>0.0035</td><td>1023.3</td><td>0.0034</td><td>96.9</td></tr>
<tr><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td>Mean</td><td>97.8</td></tr>
<tr><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td>SD</td><td>2.27</td></tr>
<tr><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td>RSD %</td><td>2.32</td></tr>
</table>
The result of 97.8 % fulfils acceptance criterion (70 – 130%).
</td>
<td>
<table>
<tr><th></th><th>Prothioconazole-desthio added [mg/mL]</th><th>Peak area</th><th>Determined of Prothioconazole-desthio [mg/mL]</th><th>Total Recovery [%]</th></tr>
<tr><td rowspan="7">Level I</td><td rowspan="7">0.00046</td><td>7100</td><td>0.000473</td><td>103.9</td></tr>
<tr><td>7064</td><td>0.000471</td><td>103.4</td></tr>
<tr><td>7145</td><td>0.000476</td><td>104.4</td></tr>
<tr><td>7347</td><td>0.000487</td><td>106.9</td></tr>
<tr><td>7383</td><td>0.000489</td><td>107.3</td></tr>
<tr><td>7289</td><td>0.000484</td><td>106.2</td></tr>
<tr><td rowspan="7">Level II</td><td rowspan="7">0.00109</td><td>18567</td><td>0.001112</td><td>102.5</td></tr>
<tr><td>18885</td><td>0.001130</td><td>104.2</td></tr>
<tr><td>18958</td><td>0.001134</td><td>104.5</td></tr>
<tr><td>18986</td><td>0.001136</td><td>104.7</td></tr>
<tr><td>19040</td><td>0.001139</td><td>104.9</td></tr>
<tr><td>18713</td><td>0.001121</td><td>103.3</td></tr>
<tr><td></td><td></td><td>Average</td><td>104.69</td></tr>
<tr><td></td><td></td><td>SD</td><td>1.00</td></tr>
<tr><td></td><td></td><td>RSD %</td><td>0.99%</td></tr>
</table>
The result of 104.69% fulfils the acceptance criterion (70 – 130%).
</td>
</tr>
<tr>
<td>Interference/ Specificity</td>
<td>Specificity of the method was evaluated based on the analysis of chromatograms for blank samples (placebo) against samples of placebo spiked with toluene standards. On the basis of the blank analysis of the test substance was not detected. Analysis showed no overlapping of determined substances signal with the signals of matrix components under method conditions hence method specificity criterion is fulfilled.</td>
<td>Specificity of the method was evaluated based on the analysis of chromatograms for blank samples (placebo) against samples of placebo spiked with prothioconazole-desthio standards. Analysis showed no overlapping of determined substances signal with the signals of matrix components under method conditions hence method specificity criterion is fulfilled</td>
</tr>
</table>

LOQ	Limit of quantification is 0.0010 mg what corresponds to 0.002 % (0.020 g/kg) of Toluene content in CHR/ZF/PROTI 100 FS preparation.	The limit of quantification (LOQ) was defined as the lowest concentration of standard – 0.00046 mg/ml (0.011 g/kg of the preparation i.e. 0.105 g/kg of Prothioconazole), which was determined with an acceptable recovery.
Comment		

Conclusion

It was confirmed that the methods are specific. There were no peaks from placebo interfering with determined compounds. The validation parameters (specificity, linearity, instrument precision, repeatability and accuracy) are within the acceptance range and fulfil EU requirements given in SANCO /3030 /99 rev.5.

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

Please refer to PART C – Confidential data.

5.2.1.4 Applicability of existing CIPAC methods (KCP 5.1.1)

Analytical methods for determination of prothioconazole impurities and relevance of CIPAC methods in CHR/ZF/PROTI were not evaluated as part of the EU review. Therefore, all relevant data are provided and are considered adequate.

5.2.2 Methods for the determination of residues (KCP 5.1.2)

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

Table 5.2-3: Validated methods for the generation of pre-authorization data

Component of residue definition: Prothioconazole				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Plants, plant products,...	Primary	0.01 mg/kg	HPLC-MS/MS	Heinemann 2000
	Primary	0.02 mg/kg	GC/MS	Weeren, Pelz 200 Class, 2001
	Confirmatory (if required)	Not required		
Soil	Primary	0.006 mg/kg	HPLC-MS/MS	Schramel, 2000
	Confirmatory (if required)	Not required		
Water	Primary	6 µg/L	HPLC-UV	Sommer, 1999
	Confirmatory (if required)	0.1 µg/L	HPLC-MS/MS	Sommer, 2001
Air	Primary	0.015 µg/L	HPLC-MS/MS	Maasfeld, 2002
	Confirmatory (if required)	Not required		

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

Component of residue definition: Prothioconazole-desthio				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Plants, plant products,...	Primary	0.01 mg/kg	HPLC-MS/MS	Heinemann 2000
	Confirmatory (if required)	Not required		
Food of animal	Primary	0.01 mg/kg	HPLC-MS/MS	Heinemann 2001

Component of residue definition: Prothioconazole-desthio				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
	Confirmatory (if required)	Not required		
Soil	Primary	0.006 mg/kg	HPLC-MS/MS	Schramel, 2000
	Confirmatory (if required)	0.01 mg/kg	GC/MS	Steinhauer, 2001
Water	Primary	6 µg/L	HPLC-UV	Sommer, 1999
	Confirmatory (if required)	0.05 µg/L	HPLC-MS/MS	Sommer, 2001
Air	Primary	0.0006 µg/L	HPLC-MS/MS	Maasfeld, 2002
	Confirmatory (if required)	Not required		

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

Component of residue definition: Prothioconazole-3-hydroxy-desthio				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Food of animal	Primary	0.01 mg/kg	HPLC-MS/MS	Heinemann 2001
	Confirmatory (if required)	Not required		
Soil	Primary	0.006 mg/kg	HPLC-MS/MS	Schramel, 2000
	Confirmatory (if required)	Not required		

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

Data provided on Annex I inclusion is sufficient for post-authorizations methods. All data is described in EU approved documents for :

- Methods are described and presented in Table 5.2-3 in point KCP 5.1.2.

5.3.1 Analysis of the plant protection product (KCP 5.2)

No method on product available, but methods for active substance presented on EU level can be considered sufficient, therefore no additional study has to be provided.

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

Reference:	KCP 5.2/01
Report	*Validation of an analytical method for the determination of residues of Triazole Derivative Metabolites (TAA, TA, 1,2,4-T, TLA) in wheat - grain, straw, plant*, Jędrusik, M., VAL/09/2021
Guideline(s):	Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC Guidance Document on Pesticide Analytical Methods for Risk Assessment and Post-approval Control and Monitoring Purposes, SANTE/2020/12830 Rev.1, 24 February 2021
Deviations:	NO
GLP:	YES
Acceptability:	YES

Materials and methods

The purpose of this study was to validate an analytical method for the determination of residues of Triazole Derivative Metabolites (TDMs = 1,2,4-Triazole, Triazole-alanine, Triazole-acetic acid, Triazole-lactic acid) in wheat - grain, straw, plant. Specimen extraction and determination of residues of TDMs was performed using the QuPPe method. The specimens were prepared, extracted and analyzed following an ANALYTICAL PROCEDURE DPL-23 Determination of residues of Triazole Derivative Metabolites (TAA, TA, 1,2,4-T, TLA) in food of plant origin using the QuPPe method and liquid chromatography technique with LC-MS / MS tandem mass spectrometry detection – Version 02, that is available at the Test Facility.

Quantification was performed by use of LC-MS/MS detection system. The limit of detection (LOD) and quantification (LOQ) of the analytical method were subsequently: LOD: 0.004* (grain, plant), 0.005* (straw) and LOQ: 0.010 mg/kg for TAA, TA, 1,2,4-T, TLA.

The extracts were analyzed using liquid chromatography coupled with mass spectrometry, by single extraction and single injection to the detection system. Final extracts were employed for LC-MS/MS analysis directly after completion of the extraction procedure (on the same day). Data acquisition was carried out in the MRM mode. The analysis was performed using internal standard addition.

For TA, TAA and TLA, a third MRM transition was used to confirm the identity of the analyte. For internal standards, only one MRM transition was used.

The results acquired during validation of the analytical method (accuracy and repeatability) were in the range of 70 – 120%.

The limit of detection (LOD) that was expressed as the lowest calibration standard and limit of quantification (LOQ) of the analytical method was established at 0.010 mg/kg for each of compounds (TAA, TA, 1,2,4-T, TLA) in wheat.

There were no interfering signals at retention time of analyzed compound in examined control matrix.

The analytical method for determining the residues of triazole derivative metabolites (TAA, TA, 1,2,4-T, TLA) meets the criteria of SANTE/2020/12830, Rev.1 guidelines in terms of precision, accuracy and uncertainty.

Reference:	KCP 5.2/02
Report	Determination of Residues of Prothioconazole and PTZ-Desthio in Winter Wheat Seeds and Seedlings after Drilling of Coated Seeds at 4 Sites in Central Europe in 2021, Sahvorost, N., S21-06525
Guideline(s):	SANTE/2020/12830, rev.1: Guidance document on pesticide analytical methods for risk assessment and post-approval control and monitoring purposes Regulations (EU) 283/2013 and 284/2013 implementing Regulation (EC) 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC
Deviations:	NO
GLP:	YES
Acceptability:	YES

Materials and methods

The objective of the analytical phase S21-06525-L1 of this study was to analyse samples of seeds and seedlings for residues of Prothioconazole and PTZ-desthio in accordance to guidance document SANTE/2020/12830, rev. 1 for risk assessment with an intended limit of quantification of 0.01 mg/kg. Sample extraction and determination of residues were performed according to an analytical procedure that was validated within this analytical phase.
In brief, for Prothioconazole and Prothioconazole-desthio samples of Seeds and Seedlings were extracted with acetonitrile/water (1/1, v/v) after addition of cysteine hydrochloride solution (250 g/L) (3%). The samples have been shaken with a flatbed shaker for 30 minutes. Afterwards, a salt mixture containing magnesium sulphate, sodium chloride and sodium citrate was added and the extract was shaken. After centrifugation an aliquot of the acetonitrile phase was cleaned-up by adding primary secondary amine (PSA) and diluted with methanol/water (40/60 v/v) containing 50 g/L cysteine hydrochloride. Quantification was performed by use of
LC-MS/MS detection.
The limit of quantification (LOQ) of the analytical method was 0.01 mg/kg for each analyte and each matrix with a limit of detection (LOD) set at 0.003 mg/kg (defined as the lowest calibration standard, which is 30 % of the LOQ).
The analytes were determined in the final sample extracts by use of LC-MS/MS detection.
For each analyte, one mass transition was evaluated. A second mass transition was monitored for confirmation of peak identity but was not used for quantification of samples.
Untreated samples for accompanying control sample work up, for determination of (procedural) recoveries and, if needed, for preparation of matrix-matched calibration standards originated from the current study or were supplied by the Test Facility. At least one control sample per each matrix type and analytical set was analysed to investigate the residue level of the analytes and to check for any background interferences at the expected retention times of the analytes.
Since blank peaks were not observed, blank correction was not necessary.
Furthermore, at least one reagent blank sample, which is a sample work up without matrix present, was conducted with each analytical set. Blank peaks were not observed in the reagent blank samples.
The method was successfully validated for determination of all analytes in all matrices with an LOQ of 0.01 mg/kg and up to 0.1 mg/kg (seedlings) and 80 mg/kg (seeds) according to guidance document SANTE/2020/12830, rev.1, (for risk assessment). With regard to selectivity, accuracy and precision, the analytical method was applied successfully for each analytical set when analysing the samples of the study.

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

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

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

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Plant, high water content	Prothioconazole, Prothioconazole-desthio	0.02 mg/kg	Weeren, Pelz, 2000
Plant, high acid content		0.02 mg/kg	Weeren, Pelz, 2000
Plant, high protein/high starch content (dry commodities)		0.01 mg/kg	Heinemann, 2000 Heinemann, 2001
Plant, high oil content		0.01 mg/kg	Heinemann, 2000
Muscle	Prothiconazole-desthio Prothioconazole-3-hydroxy-desthio Prothioconazole-4-hydroxy-desthio	0.01 mg/kg	Heinemann, 2001
Milk		0.004 mg/kg	Heinemann, 2001
Liver, kidney		0.01 mg/kg	Heinemann, 2001
Soil (Ecotoxicology)	Prothioconazole Prothiconazole-desthio Prothioconazole-3-hydroxy-desthio Prothioconazole-4-hydroxy-desthio	0.006 mg/kg	Schramel 2000
Drinking water (Human toxicology)	Prothioconazole	0.1 µg/L	general limit for drinking water
Surface water (Ecotoxicology)	Prothioconazole-desthio	3.34 µg/L	
Air	Prothicoconazole Prothioconazole-desthio	0.015 µg/m^3 0.0006 µg/m^3	AOEL sys/AOEL inhal: xxx mg/kg bw/d
Tissue (meat or liver)	Prothioconazole-desthio	0.01 mg/kg	Not classified as T / T+
Body fluids		not required	Not classified as T / T+

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

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

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

Component of residue definition: Prothiconazole				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
High oil content	Primary	0.01 mg/kg	HPLC-MS/MS	Heinemann, 2000a
	ILV	0.01 mg/kg	GC-MS	Heinemann, 2001
	Confirmatory (if required)			
High protein/high starch content (dry)	Primary	0.01 mg/kg	HPLC-MS/MS	Heinemann, 2000a
	ILV	0.01 mg/kg	HPLC-MS/MS	Heinemann, 2000b
	Confirmatory (if required)	Not required		

Table 5.3-3: 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: Prothiconazole-desthio				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
High water content	Primary	0.02 mg/kg	GC/MS	Weeren, Pelz 2000
	ILV	0.02 mg/kg	GC/MS	Class, 2001
	Confirmatory (if required)	Not required		
High acid content	Primary	0.02 mg/kg	GC/MS	Weeren, Pelz 2000
	ILV	0.02 mg/kg	GC/MS	Class, 2001
	Confirmatory (if required)	Not required		
High oil content	Primary	0.01 mg/kg	GC-MS	Heinemann, 2000
	ILV	0.01 mg/kg	GC/MS	Heinemann, 2001
	Confirmatory (if required)	Not required		
High protein/high starch content (dry)	Primary	0.01 mg/kg	HPLC-MS/MS	Heinemann, 2000
	ILV	0.01 mg/kg	HPLC-MS/MS	Heinemann, 2001
	Confirmatory (if required)	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 5.3-4: Statement on extraction efficiency

	Method for products of plant origin
Not required, because:	Residues below LOQ

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

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

Table 5.3-5: Validated methods for food and feed of animal origin (if appropriate)

Component of residue definition: Prothioconazole-desthio				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Milk	Primary	0.004 mg/kg	HPLC-MS/MS	Heinemann, 2001
	ILV	0.004 mg/kg	HPLC-MS/MS	Dubey, 2001
	Confirmatory (if required)	Not required		
Muscle	Primary	0.01 mg/kg	HPLC-MS/MS	Heinemann, 2001
	ILV	0.01 mg/kg	HPLC-MS/MS	Dubley, 2001
	Confirmatory (if required)	Not required		
Kidney, liver	Primary	0.01 mg/kg	HPLC-MS/MS	Heinemann, 2001
	ILV	0.01 mg/kg	HPLC-MS/MS	Dubley, 2001
	Confirmatory (if required)	Not required		

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

Component of residue definition: Prothioconazole-3-hydroxy-desthio				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Milk	Primary	0.004 mg/kg	HPLC-MS/MS	Heinemann, 2001
	ILV	0.004 mg/kg	HPLC-MS/MS	Dubey, 2001
	Confirmatory (if required)	Not required		
Muscle	Primary	0.01 mg/kg	HPLC-MS/MS	Heinemann, 2001
	ILV	0.01 mg/kg	HPLC-MS/MS	Dubley, 2001
	Confirmatory (if required)	Not required		

Component of residue definition: Prothioconazole-3-hydroxy-desthio				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Kidney, liver	Primary	0.01 mg/kg	HPLC-MS/MS	Heinemann, 2001
	ILV	0.01 mg/kg	HPLC-MS/MS	Dubley, 2001
	Confirmatory (if required)	Not required		

Table 5.3-7: Validated methods for food and feed of animal origin (if appropriate)

Component of residue definition: Prothioconazole-4-hydroxy-desthio				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Milk	Primary	0.004 mg/kg	HPLC-MS/MS	Heinemann, 2001
	ILV	0.004 mg/kg	HPLC-MS/MS	Dubey, 2001
	Confirmatory (if required)	Not required		
Muscle	Primary	0.01 mg/kg	HPLC-MS/MS	Heinemann, 2001
	ILV	0.01 mg/kg	HPLC-MS/MS	Dubley, 2001
	Confirmatory (if required)	Not required		
Kidney, liver	Primary	0.01 mg/kg	HPLC-MS/MS	Heinemann, 2001
	ILV	0.01 mg/kg	HPLC-MS/MS	Dubley, 2001
	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 5.3-8: Statement on extraction efficiency

	Method for products of animal origin
Not required, because:	Residue below LOQ

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

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

Table 5.3-9: Validated methods for soil (if appropriate)

Component of residue definition: Prothioconazole			
Method type	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	0.0006 mg/kg	HPLC-MS/MS	Schramel, 2000
Confirmatory	-		

Table 5.3-10: Validated methods for soil (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	0.0006 mg/kg	HPLC-MS/MS	Schramel, 2000
Confirmatory	0.01 mg/kg	GC/MS	Steinhauer, 20001

Table 5.3-11: Validated methods for soil (if appropriate)

Component of residue definition: Prothioconazole-3-hydroxy-desthio			
Method type	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	0.0006 mg/kg	HPLC-MS/MS	Schramel, 2000
Confirmatory	-		

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

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

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

Table 5.3-12: Validated methods for water (if appropriate)

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**
Drinking water	Primary	6 µg/L	HPLC-UV	Sommer, 1999
	ILV	-		
	Confirmatory	0.1 µg/L	HPLC-MS/Ms	Sommer, 2001
Surface water	Primary	6 µg/L	HPLC-UV	Sommer, 1999
	Confirmatory	0.1 µg/L	HPLC-MS/Ms	Sommer, 2001

Table 5.3-13: Validated methods for water (if appropriate)

Component of residue definition: Prothioconazole-desthio				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Drinking water	Primary	6 µg/L	HPLC-UV	Sommer, 1999
	ILV	-		
	Confirmatory	0.05 µg/L	HPLC-MS/Ms	Sommer, 2001
Surface water	Primary	6 µg/L	HPLC-UV	Sommer, 1999
	Confirmatory	0.05 µg/L	HPLC-MS/Ms	Sommer, 2001

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

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

An overview on the acceptable methods and possible data gaps for analysis of Prothiconazole in air is given in the following tables. For the detailed evaluation of additional studies please refer to Appendix 2.

Table 5.3-14: Validated methods for air (if appropriate)

Component of residue definition: Prothiconazole			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	0.015 µg/m^3	HPLC-MS/MS	Maasfeld, 2002
Confirmatory	Not required		

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

Component of residue definition: Prothiconazole-desthio			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	0.0006 µg/m^3	HPLC-MS/MS	Maasfeld, 2002
Confirmatory	Not required		

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

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

An overview on the acceptable methods and possible data gaps for analysis of Prothioconazole in body fluids and tissues is given in the following table. For the detailed evaluation of additional studies it is referred to Appendix 2.

Table 5.3-16: 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	0.01 mg/kg	HPLC-MS/MS	Heinemann, 2001
Confirmatory	Not required		

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

5.3.2.8 Other studies/ information

Not required

Appendix 1 Lists of data considered in support of the evaluation

List of data submitted by the applicant and relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Verte-brate study Y/N	Owner
KCP 5.1./01, 5.1./02, 5.1./03	E. Arevalo	2021	*CHR/ZF/PROTI 100 FS Stage I: Determination of physicochemical properties of the initial preparation, after accelerated storage and after low temperature storage.* Study code: BF-10/21 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Warsaw, Poland GLP Unpublished	N	Chemirol
KCP 5.2/01	Jędrusik, M.	2022	*Validation of an analytical method for the determination of residues of Triazole Derivative Metabolites (TAA, TA, 1,2,4-T, TLA) in wheat - grain, straw, plant* SGS Polska Sp.z o.o., Pszczyna, Poland Study code: VAL/09/2021 GLP Unpublished	N	Chemirol
KCP 5.2/02	Appeltauer, A., Sahvorost, N.	2022	*Determination of Residues of Prothioconazole and PTZ-Desthio in Winter Wheat Seeds and Seedlings after Drilling of Coated Seeds at 4 Sites in Central Europe in 2021* Eurofins Agroscience Services Ecotox GmbH, Niefern-Öschelbronn, Germany Study code: S21-06525 GLP Unpublished	N	Chemirol

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	Verte-brate study Y/N	Owner
KCP 5.1/01 KCP 5.2/01	Heinemann, O.	2000	Analytical determination of residues of JAU 6476 and desthio-JAU 6476 in/on cereals by HPLC/MS/MS Report No.: 00598 Bayer AG GLP Unpublished	N	BAY
KCP 5.1/02 KCP 5.2/02	Heinemann, O.	2000	Analytical determination of residues of JAU6476 and JAU6476-desthio in/on cereals and caola by HPLC-MS/MS (method modification 00598/M001) Report No.: 00598/M001 Bayer AG GLP Unpublished	N	BAY
KCP 5.1/03 KCP 5.2/03	Heinemann, O.	2001	Analytical determination of residues of JAU6476-sulfonic acid and JAU6476-desthio in/on cereals and canola by HPLC-MS/MS Report No.: 00647 Bayer AG GLP Unpublished	N	BAY
KCP 5.1/04 KCP 5.2/04	Heinemman, 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 Report No.: 00655 Bayer AG GLP Unpublished	N	BAY
KCP 5.1/05 KCP 5.2/05	Heinemann, O.	2001	Analytical determination of residues of JAU6476-3-hydroxy-desthio, JAU6476-4-hydroxy-desthio, and JAU6476-desthio in milk by HPLC-MS/MS (00655/M001) Report No.: 00655/M001 Bayer AG	N	BAY

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Verte-brate study Y/N	Owner
			GLP Unpublished		
KCP 5.1/06 KCP 5.2/06	Weeren, R.D. Pelz, S.	2000	Modification M033 of method 00086: Validation of DFG method S 19 (extended revision) for the determination of residues of JAU 6476-desthio in materials of plant and animal origin Report No.: 00086/M033 Dr. Specht&Partner, Chemische Laboratorien GmbH, Hamburg, Germany GLP Unpublished	N	BAY
KCP 5.2/07	Class, Th.	2001	Independent laboratory validation of DFG method S19 (extended revision) for the determination of residues of JAU6476-desthio (BAYER method 00086/M033) in plant materials Report No.: P/B 484 G PTRL Europe, Ulm, gGermany GLP Unpublished	N	BAY
KCP 5.2/08	Dubey, L.	2001	Independent laboratory validation of bayer methods 00655 and 00655/M001 for the determination of residues of JAU6476-3-hydroxy-desthio, JAU6476-4-hydroxy-desthio and JAU6476-desthio in/on matreces of animal origin by HPLC-MS/MS Report No.: A-14-01-01 Battelle, Geneva Research Cetres, Carouge/Geneva, Switzerland GLP Unpublished	N	BAY
KCP 5.1/07 KCP 5.2/09	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 Report No.: 00610 Bayer AG GLP Unpublished	N	BAY
KCP	Sommer, H.	1998	Method 00520 (MR-342/98) for liquid chromatographic determination of JAU 6476 and SXX 0665 on	N	BAY

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Verte-brate study Y/N	Owner
5.1/08 KCP 5.2/10			application verification pads Report No.: 00520 Bayer AG GLP Unpublished		
KCP 5.1/09 KCP 5.2/11	Steinhauer, S.	2001	Enforcement method 00086/M038 for the determination of the residues of JAU 6476-desthio in soil – validation of DFG method S 19 (extended revision) Report No.: 00086/M038 DR. Specht&Partner, Chemische Laboratorien GmbH, Hamburg, Germany Bayer AG GLP Unpublished	N	BAY
KCP 5.1/10 KCP 5.2/12	Maasfeld, W.	2002	Method for the determination of JAU 6476 in air by HPLC-MS/MS Report No.: 00724 Bayer AG GLP Unpublished	N	BAY
KCP 5.2/13	Sommer, H.	1999	Method for the determination of JAU 6476 and SXX 0665 in test water from aquatic toxicity test by HPLC [TOX/Ecotox method] Report No.: 00586 Bayer AG GLP Unpublished	N	BAY
KCP 5.2/14	Sommer, H.	2001	Tox/Ecotox method: Method for determination JAU6476-S-methyl in test water from aquatic toxicity test by HPLC-UV Report No.: 00699 Bayer AG GLP	N	BAY

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Verte-brate study Y/N	Owner
			Unpublished		
KCP 5.2/15	Sommer, H.	2001	Enforcement method 00684 for determination of JAU6476 and JAU 6476-desthio in drinking and surface water by HPLC-MS/MS Report No.: 00684 Bayer AG GLP Unpublished	N	BAY

Appendix 2 Detailed evaluation of submitted analytical methods

A 2.1 Analytical methods for Prothioconazole

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

No new or additional studies have been submitted

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

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

Comments of zRMS:	The method validation has been accepted. The purpose of this study was the validation of **LC-MS/MS** method for determination of TDMs in wheat. The analytical method for determining *1,2,4-Triazole, Triazole-alanine, Triazole-acetic acid and Triazole-lactic acid* in wheat - grain, straw, plant, meets the criteria of SANTE/2020/12830, Rev.1 in terms of precision, accuracy and uncertainty. The results obtained during validation of the analytical method (accuracy and repeatability) were in the range of 70 – 120%. The LOD that was expressed as the lowest calibration standard and the LOQ of the analytical method was established at 0.010 mg/kg for each of compounds (TAA, TA, 1,2,4-T, TLA) in wheat. There were no interfering signals at retention time of analyzed compound in examined control matrix.

Reference:	KCP 5.2/01
Report	*Validation of an analytical method for the determination of residues of Triazole Derivative Metabolites (TAA, TA, 1,2,4-T, TLA) in wheat - grain, straw, plant*, Jędrusik, M., VAL/09/2021
Guideline(s):	Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC Guidance Document on Pesticide Analytical Methods for Risk Assessment and Post-approval Control and Monitoring Purposes, SANTE/2020/12830 Rev.1, 24 February 2021
Deviations:	NO
GLP:	YES
Acceptability:	YES

Materials and methods

The purpose of this study was to validate an analytical method for the determination of residues of Triazole Derivative Metabolites (TDMs = 1,2,4-Triazole, Triazole-alanine, Triazole-acetic acid, Triazole-lactic acid) in wheat - grain, straw, plant. Specimen extraction and determination of residues of TDMs was performed using the QuPPe method. The specimens were prepared, extracted and analyzed following an ANALYTICAL PROCEDURE DPL-23 Determination of residues of Triazole Derivative Metabolites (TAA, TA, 1,2,4-T, TLA) in food of plant origin using the QuPPe method and liquid chromatography technique with LC-MS / MS tandem mass spectrometry detection – Version 02, that is available at the Test Facility.

Quantification was performed by use of LC-MS/MS detection system. The limit of detection (LOD) and quantification (LOQ) of the analytical method were subsequently: LOD: 0.004* (grain, plant), 0.005* (straw) and LOQ: 0.010 mg/kg for TAA, TA, 1,2,4-T, TLA.
The extracts were analyzed using liquid chromatography coupled with mass spectrometry, by single extraction and single injection to the detection system. Final extracts were employed for LC-MS/MS analysis directly after completion of the extraction procedure (on the same day). Data acquisition was carried out in the MRM mode. The analysis was performed using internal standard addition.
For TA, TAA and TLA, a third MRM transition was used to confirm the identity of the analyte. For internal standards, only one MRM transition was used.
The results acquired during validation of the analytical method (accuracy and repeatability) were in the range of 70 – 120%.
The limit of detection (LOD) that was expressed as the lowest calibration standard and limit of quantification (LOQ) of the analytical method was established at 0.010 mg/kg for each of compounds (TAA, TA, 1,2,4-T, TLA) in wheat.
There were no interfering signals at retention time of analyzed compound in examined control matrix.
The analytical method for determining the residues of triazole derivative metabolites (TAA, TA, 1,2,4-T, TLA) meets the criteria of SANTE/2020/12830, Rev.1 guidelines in terms of precision, accuracy and uncertainty.

Validation - Results and discussions

Table 5.3-1: Methods suitable for the determination of the residues in plant protection product (PPP) CHR/I/ACE 200 SE

	Residues
Author(s), year	M. Jędrusik, 2022
Principle of method	LC MS/MS
Linearity (linear between mg/L) (correlation coefficient, expressed as r)	Linear regression analysis with 1/x weighting was used to describe the detector response as a function of the calibration standard concentrations. For the least squares regression equations describing the detector response as a function of the standard calibration curve concentrations, the coefficients of determination (r) were greater than 0.990 for all of the calibration curve determinations during the method validation. The results indicate linearity of the detector response as a function of the standard concentration.
Precision, accuracy and uncertainty	Recovery data was generated from five samples fortified at the limit of quantification (LOQ) and five samples fortified at the 10-fold higher concentration than the LOQ. Precision of the method was determined as the relavite standard deviation (RSD) of recovery at each fortification level. The mean recovery at each fortification level was in the range of 70-120%. Wherever applicable (n≥3), the relative standard deviation was determined and was ≤20% for each level.
Selectivity	LC-MS/MS method was used during the study. Two mass transition were evaluated and used for quantification. The specificity of the method was evaluated on the basis of the analysis of chromatograms recorded for the matrix blank samples. The method was considered highly specific, thus the use of an alternative method was not necessary. The absence of interferences due to reagents was evaluated (procedural blank reagents).
Matrix Effects	Referring to the requirements of SANTE/2020/12830 Rev.1, if the matrix effect exceeds 20% LOQ, the "matrix-matched" calibration should be introduced. This effect was not observed during validation, but the method of preparing working calibration standards based on blank sample was used.
LOQ	The LOQ is the lowest validated fortification level for which an average recovery

	Residues
	in the range of 70 – 120% and RSD ≤ 20 % is achieved. For TDMs LOQ was successfully established at 0.010 mg/kg for wheat (grain, straw, plant). LOD (limit of detection) was established at 0.004 mg/kg (grain, plant) and 0.005 (straw). The limit of detection (LOD) that was expressed as the lowest calibration standard.
Comment	The validation parameters are within the acceptance range and fulfil EU requirements given in SANTE/2020/12830 Rev.1.

Conclusion

The method was successfully validated for determination of all analytes in all matrices with an LOQ of 0.01 mg/kg according to the guidance document(s) SANTE/2020/12830 Rev.1. With regard to selectivity, accuracy and precision, the analytical methods were applied successfully for each analytical set when analysing the specimens of the study.

Comments of zRMS:	The LC-MS/MS method validation has been accepted. The objective of this study was to determine the residues of prothioconazole and PTZ-desthio in winter wheat seedlings and seeds after drilling of untreated and coated seeds treated with CHR/ZF/PROTI 100 FS under representative growing conditions in Central Europe in the field. The residues and degradation kinetics of Prothioconazole and PTZ-desthio were investigated. 4 trials were performed. The calculation of the DT50 values and DT90 values as well as the fitting of the kinetic degradation models was done using the computer software KinGU II (version 2.2012). Recovery data were generated from 5 samples fortified at the LOQ and 5 samples fortified at the 10-fold higher concentration than the LOQ. Precision of the method was determined as the RSD of recovery at each fortification level. The mean recovery at each fortification level was in the range of 70-120%. RSD was ≤20% for each level. The analytes were fortified jointly and quantified separately. At least one reagent blank and two control samples were analysed. One mass transition was evaluated. A second ion transition was included to the detection method but used for monitoring only. Recovery data were not reported for this mass transition. ZRMS agrees that with regard to selectivity, accuracy and precision, the analytical method was applied successfully for each analytical set when analysing the samples of the study. The method was validated consistently with SANTE/2020/12830, rev. 1.

Reference:	KCP 5.2/02
Report	Determination of Residues of Prothioconazole and PTZ-Desthio in Winter Wheat Seeds and Seedlings after Drilling of Coated Seeds at 4 Sites in Central Europe in 2021, Sahvorost, N., S21-06525
Guideline(s):	SANTE/2020/12830, rev.1: Guidance document on pesticide analytical methods for risk assessment and post-approval control and monitoring purposes Regulations (EU) 283/2013 and 284/2013 implementing Regulation (EC) 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC
Deviations:	NO
GLP:	YES
Acceptability:	YES

Materials and methods

The objective of the analytical phase S21-06525-L1 of this study was to analyse samples of seeds and seedlings for residues of Prothioconazole and PTZ-desthio in accordance to guidance document SANTE/2020/12830, rev. 1 for risk assessment with an intended limit of quantification of 0.01 mg/kg. Sample extraction and determination of residues were performed according to an analytical procedure that was validated within this analytical phase.

In brief, for Prothioconazole and Prothioconazole-desthio samples of Seeds and Seedlings were extracted with acetonitrile/water (1/1, v/v) after addition of cysteine hydrochloride solution (250 g/L) (3%). The samples have been shaken with a flatbed shaker for 30 minutes. Afterwards, a salt mixture containing magnesium sulphate, sodium chloride and sodium citrate was added and the extract was shaken. After centrifugation an aliquot of the acetonitrile phase was cleaned-up by adding primary secondary amine

(PSA) and diluted with methanol/water (40/60 v/v) containing 50 g/L cysteine hydrochloride. Quantification was performed by use of
LC-MS/MS detection.
The limit of quantification (LOQ) of the analytical method was 0.01 mg/kg for each analyte and each matrix with a limit of detection (LOD) set at 0.003 mg/kg (defined as the lowest calibration standard, which is 30 % of the LOQ).
The analytes were determined in the final sample extracts by use of LC-MS/MS detection.
For each analyte, one mass transition was evaluated. A second mass transition was monitored for confirmation of peak identity but was not used for quantification of samples.
Untreated samples for accompanying control sample work up, for determination of (procedural) recoveries and, if needed, for preparation of matrix-matched calibration standards originated from the current study or were supplied by the Test Facility. At least one control sample per each matrix type and analytical set was analysed to investigate the residue level of the analytes and to check for any background interferences at the expected retention times of the analytes.
Since blank peaks were not observed, blank correction was not necessary.
Furthermore, at least one reagent blank sample, which is a sample work up without matrix present, was conducted with each analytical set. Blank peaks were not observed in the reagent blank samples.
The method was successfully validated for determination of all analytes in all matrices with an LOQ of 0.01 mg/kg and up to 0.1 mg/kg (seedlings) and 80 mg/kg (seeds) according to guidance document SANTE/2020/12830, rev.1, (for risk assessment). With regard to selectivity, accuracy and precision, the analytical method was applied successfully for each analytical set when analysing the samples of the study.

Validation - Results and discussions

Table 5.3-2: Methods suitable for the determination of the residues in plant protection product (PPP) CHR/I/ACE 200 SE

	Residues
Author(s), year	N. Sahvorost
Principle of method	LC MS/MS
Linearity (linear between mg/L) (correlation coefficient, expressed as r)	The linearity of the detector response was demonstrated by single determination of matrix-matched calibration standards at a minimum of ten concentration levels ranging from 0.075 ng/mL to 7.5 ng/mL. This range corresponds to a fortification level of 0.003 mg/kg to 0.3 mg/kg and thus covers the range from no more than 30 % of the LOQ and at least + 20 % of the highest analyte concentration detected in any (diluted) sample extract. The calibration curve did not exceed two orders of magnitude. Sample extracts were diluted as appropriate to be within the calibration range. The calibration curves obtained for both analytes and all matrices were linear since the regression residuals were randomly distributed. Furthermore, correlation coefficients (R) were greater than 0.990.
Precision, accuracy and uncertainty	Recovery data was generated from five samples fortified at the limit of quantification (LOQ) and five samples fortified at the 10-fold higher concentration than the LOQ. Precision of the method was determined as the relavite standard deviation (RSD) of recovery at each fortification level. The mean recovery at each fortification level was in the range of 70-120%. Wherever applicable (n≥3), the relative standard deviation was determined and was ≤20% for each level.
Selectivity	LC-MS/MS method was used during the study. Two mass transition were evaluated and used for quantification. The specificity of the method was evaluated on the basis of the analysis of chromatograms recorded for the matrix blank samples. The method was considered highly specific, thus the use of an alternative method was not necessary.

	Residues
Matrix Effects	Referring to the requirements of SANTE/2020/12830 Rev.1, if the matrix effect exceeds 20% LOQ, the "matrix-matched" calibration should be introduced. This effect was not observed during validation, but the method of preparing working calibration standards based on blank sample was used.
LOQ	The limit of quantification (LOQ) of the analytical method was 0.01 mg/kg for each analyte and each matrix with a limit of detection (LOD) set at 0.003 mg/kg (defined as the lowest calibration standard, which is 30 % of the LOQ).
Comment	The validation parameters are within the acceptance range and fulfil EU requirements given in SANTE/2020/12830 Rev.1.

Conclusion
The method was successfully validated for determination of all analytes in all matrices with an LOQ of 0.01 mg/kg according to the guidance document(s) SANTE/2020/12830 Rev.1. With regard to selectivity, accuracy and precision, the analytical methods were applied successfully for each analytical set when analysing the specimens of the study.

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted.

A 2.1.2.7 A.2.A.9 Other Studies/ Information

No new or additional studies have been submitted