

# **FINAL REGISTRATION REPORT**

## **Part B**

### **Section 5**

#### **Analytical Methods**

Detailed summary of the risk assessment

Product code: CHR/F/PROTAZO 375 SC

Product name(s): CLARO 375 SC, KAJMAN 375 SC

Chemical active substance(s):

Prothioconazole, 175 g/L

Azoxystrobin, 200 g/L

Central Zone

Zonal Rapporteur Member State: Poland

#### **CORE ASSESSMENT**

(authorization)

Applicant: Innvigo Sp. z o.o.

Submission date: May 2020

**MS Finalisation date: 28/04/2022**

## Version history

| When          | What   |
|---------------|--|
| May 2021      | Dossier sent for evaluation                            |
| December 2021 | Applicant updated dRR on the zRMS request              |
| January 2022  | zRMS finalised evaluation                              |
| April 2022    | Final version prepared by zRMS after Commenting period |

## Table of Contents

|                   |   |           |
|-------------------|---|-----------|
| 5.1               | Conclusion and summary of assessment.....   | 5         |
| 5.2               | Methods used for the generation of pre-authorization data (KCP 5.1).....                              | 5         |
| 5.2.1             | Analysis of the plant protection product (KCP 5.1.1) .....  | 5         |
| 5.2.1.1           | Determination of active substance and/or variant in the plant protection product (KCP 5.1.1).....     | 5         |
| 5.2.1.2           | Description of analytical methods for the determination of relevant impurities (KCP 5.1.1).....       | 7         |
| 5.2.1.3           | Description of analytical methods for the determination of formulants (KCP 5.1.1) .....               | 13        |
| 5.2.1.4           | Applicability of existing CIPAC methods (KCP 5.1.1).....  | 13        |
| 5.2.2             | Methods for the determination of residues (KCP 5.1.2).....  | 13        |
| 5.3               | Methods for post-authorization control and monitoring purposes (KCP 5.2) .....                        | 15        |
| 5.3.1             | Analysis of the plant protection product (KCP 5.2) .....  | 15        |
| 5.3.2             | Description of analytical methods for the determination of residues Prothioconazole (KCP 5.2) .....   | 15        |
| 5.3.2.1           | Overview of residue definitions and levels for which compliance is required .....                     | 20        |
| 5.3.2.2           | Description of analytical methods for the determination of residues in plant matrices (KCP 5.2).....  | 21        |
| 5.3.2.3           | Description of analytical methods for the determination of residues in animal matrices (KCP 5.2)..... | 22        |
| 5.3.2.4           | Description of methods for the analysis of soil (KCP 5.2).....  | 24        |
| 5.3.2.5           | Description of methods for the analysis of water (KCP 5.2).....                                       | 25        |
| 5.3.2.6           | Description of methods for the analysis of air (KCP 5.2).....   | 25        |
| 5.3.2.7           | Description of methods for the analysis of body fluids and tissues (KCP 5.2) .....                    | 26        |
| 5.3.2.8           | Other studies/ information .....  | 26        |
| 5.3.3             | Description of analytical methods for the determination of residues azoxystrobin (KCP 5.2) .....      | 29        |
| Reference:        | 29  |           |
| 5.3.3.1           | Overview of residue definitions and levels for which compliance is required .....                     | 31        |
| 5.3.3.2           | Description of analytical methods for the determination of residues in plant matrices (KCP 5.2).....  | 32        |
| 5.3.3.3           | Description of analytical methods for the determination of residues in animal matrices (KCP 5.2)..... | 32        |
| 5.3.3.4           | Description of methods for the analysis of soil (KCP 5.2).....  | 33        |
| 5.3.3.5           | Description of methods for the analysis of water (KCP 5.2).....                                       | 33        |
| 5.3.3.6           | Description of methods for the analysis of air (KCP 5.2).....   | 34        |
| 5.3.3.7           | Description of methods for the analysis of body fluids and tissues (KCP 5.2) .....                    | 34        |
| 5.3.3.8           | Other studies/ information .....  | 34        |
| <b>Appendix 1</b> | <b>Lists of data considered in support of the evaluation .....</b>                                    | <b>39</b> |
| <b>Appendix 2</b> | <b>Detailed evaluation of submitted analytical methods .....</b>                                      | <b>45</b> |
| A 2.1             | Analytical methods for Prothioconazole.....   | 45        |

|         |  |    |
|---------|--|----|
| A 2.1.1 | Methods used for the generation of pre-authorization data (KCP 5.1).....       | 45 |
| A 2.1.2 | Methods for post-authorization control and monitoring purposes (KCP 5.2) ..... | 45 |
| A 2.2   | Analytical methods for Azoxystrobin .....                                      | 50 |
| A 2.2.1 | Methods used for the generation of pre-authorization data (KCP 5.1).....       | 50 |
| A 2.2.2 | Methods for post-authorization control and monitoring purposes (KCP 5.2) ..... | 50 |

## 5.1 Conclusion and summary of assessment

zRMS comments:

This report has been completed by the applicant.

The text highlighted in grey was provided by the evaluator.

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

Noticed data gaps are:

-none

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

Noticed data gaps are:

-none

| Commodity/crop  | Supported/<br>Not supported |
|-----------------|-----------------------------|
| Cereals         | Supported                   |
| Oilseed rape    | Supported                   |
| Mustard         | Supported                   |
| Sunflower       | Supported                   |
| Soya            | Supported                   |
| Breadseed poppy | Supported                   |
| Tobacco         | Supported                   |
| Salix, Wicker   | Supported                   |
| Ornamentals     | Supported                   |
| Forestry tree   | 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: | The method is validated and may be applied for analysing prothioconazole and azoxystrobin in the PPP. |
|-------------------|---|

Reference: KCP 5.1

Report: Determination of physicochemical properties, I. Knapik, Study code: ICB/93/2019

Guideline(s): Determination of content of azoxystrobin and prothioconazole – validated own method SPB/139; Determination of content of Z-azoxystrobin - validated own method SPB/144; Determination of toluene validated own method SPB/134; Appearance – based on OPPTS 830.6302, 830.6303 and 830.6304; pH – CIPAC MT 75.3; Flash point – EEC A.9; Surface tension - EEC A.5; Relative density – EEC A.3; Persistent foaming – CIPAC MT 47.3; Suspensibility – CIPAC MT 184; Low temperature stability of liquid

formulations – CIPAC MT 39.3; Particle size – CIPAC MT 187; Stability of package - own method SPB/38; Spontaneity of dispersion – CIPAC MT 160; Pourability – CIPAC MT 148.1; Wet sieve – CIPAC MT 185; Effectiveness of cleaning - Efficacy Guideline 305; Accelerated storage stability – CIPAC 46.3..

Deviations: No  
GLP: Yes  
Acceptability: Yes

## Materials and methods

- acetonitrile HPLC (Honeywell)
- 85% phosphoric acid (Sigma Aldrich)
- water ultra-purification unit HLP 5UV
- azoxystrobin standard; Sigma Aldrich; batch BCBT1118V
- prothioconazole standard; Sigma Aldrich; batch SZBE225XV
- placebo
- standard solution azoxystrobin and prothioconazole in acetonitrile (Table 3)
- standard solution azoxystrobin concentration 2.3832 mg/mL in acetonitrile (for ULOQ)
- standard solution prothioconazole concentration 2.43756 mg/mL in acetonitrile (for ULOQ)
- working standard solution azoxystrobin and prothioconazole in acetonitrile (Table 4)
- analytical balance – accuracy 0.0001 g (Ohaus, Switzerland)
- liquid chromatography with diode array detection (Shimadzu, Japan;
- chromatography column type C18, 250 mm x 4.6 mm; 5µm (Agilent, Zorbax Eclipse Plus)
- chromatography column type C18, 150 mm x 4.6 mm; 5µm (Kinetex®, Phenomenex)
- chromatographic vials 1.5 ml with septa buthyl/teflon
- volumetric flask A class 10 mL
- measuring syringes 10 µL, 50 µL ,100 µL, 250 µL, 500 µL, 1000 µL.

Determination of the content of azoxystrobin and prothioconazole was conducted according to validated analytical method SPB/139. Content of azoxystrobin at a level of 200 g/L and prothioconazole at a level of 175 g/L in the test item accordingly were determined by liquid chromatography with diode array detection (HPLC-DAD).

## Validation - Results and discussions

**Table 5.2-1: Methods suitable for the determination of active substances prothioconazole and azoxystrobin in plant protection product CHR/F/PROTAZOIO**

|   | Prothioconazole  | Azoxystrobin   |
|---|--|--|
| <b>Author(s), year</b>  | I. Knapik, 2019  |  |
| <b>Principle of method</b>  | HPLC-DAD   |  |
| <b>Linearity (linear between mg/L / % range of the declared content) (correlation coefficient, expressed as</b> | In order to check the linearity of prothioconazole, calibration curve was prepared using standard solutions. A graph of the peak area to the concentration of prothioconazole was plotted. The resulting curve is linear in the tested concentrations. Linearity range of prothioconazole is - 0.579 to 57.94 µg/mL. Correlation coefficient R2 is 0.9998868 for primary chromatographic system. Correlation coefficient R2 is 0.9997652 for secondary chromatographic system. | In order to check the linearity of azoxystrobin, calibration curve was prepared using standard solutions. A graph of the peak area to the concentration of azoxystrobin was plotted. The resulting curve is linear in the tested concentrations. Linearity range of azoxystrobin is from 0.511 to 51.14 µg/mL. Correlation coefficient R2 is 0.9997865 for primary chromatographic system. Correlation coefficient R2 is 0.9997529 for secondary |

|  | Prothioconazole  | Azoxystrobin                        |                   |                       |     |                     |               |               |              |        |      |      |        |      |      |        |   |                                  |                   |                       |       |      |        |      |       |                                  |      |      |        |                     |               |               |              |     |      |      |       |     |      |      |       |      |      |      |       |      |      |      |       |  |                                |  |  |  |                     |               |               |              |     |      |      |      |     |      |      |       |      |      |      |       |      |      |      |       |                                  |  |  |  |                     |               |               |              |     |      |      |       |     |      |      |       |      |      |      |       |      |      |      |       |
|--|--|-------------------------------------|-------------------|-----------------------|-----|---------------------|---------------|---------------|--------------|--------|------|------|--------|------|------|--------|---|----------------------------------|-------------------|-----------------------|-------|------|--------|------|-------|----------------------------------|------|------|--------|---------------------|---------------|---------------|--------------|-----|------|------|-------|-----|------|------|-------|------|------|------|-------|------|------|------|-------|--|--------------------------------|--|--|--|---------------------|---------------|---------------|--------------|-----|------|------|------|-----|------|------|-------|------|------|------|-------|------|------|------|-------|----------------------------------|--|--|--|---------------------|---------------|---------------|--------------|-----|------|------|-------|-----|------|------|-------|------|------|------|-------|------|------|------|-------|
| r)<br>n=5  |  | chromatographic system.             |                   |                       |     |                     |               |               |              |        |      |      |        |      |      |        |   |                                  |                   |                       |       |      |        |      |       |                                  |      |      |        |                     |               |               |              |     |      |      |       |     |      |      |       |      |      |      |       |      |      |      |       |  |                                |  |  |  |                     |               |               |              |     |      |      |      |     |      |      |       |      |      |      |       |      |      |      |       |                                  |  |  |  |                     |               |               |              |     |      |      |       |     |      |      |       |      |      |      |       |      |      |      |       |
| Precision – Repeatability Mean<br>n=4<br>(%RSD)<br>Five samples per each fortification level | According to SPT/31, the parameters obtained as a result of validation should meet the following criteria:<br>- linearity $R^2 \geq 0.99$<br>- Horwitz ratio $\leq 1.0$ <table><tr><th>Level of validation prothioconazole</th><th>Max precision [%]</th><th>Range of accuracy [%]</th></tr><tr><td>LOQ</td><td>3.14</td><td>80-120</td></tr><tr><td>50%</td><td>1.93</td><td>90-110</td></tr><tr><td>100%</td><td>1.74</td><td>97-103</td></tr><tr><td>ULOQ</td><td>1.57</td><td>97-103</td></tr></table>   | Level of validation prothioconazole | Max precision [%] | Range of accuracy [%] | LOQ | 3.14                | 80-120        | 50%           | 1.93         | 90-110 | 100% | 1.74 | 97-103 | ULOQ | 1.57 | 97-103 | According to SPT/31, the parameters obtained as a result of validation should meet the following criteria:<br>- linearity $R^2 \geq 0.99$<br>- Horwitz ratio $\leq 1.0$ <table><tr><th>Level of validation azoxystrobin</th><th>Max precision [%]</th><th>Range of accuracy [%]</th></tr><tr><td>LOQ</td><td>3.08</td><td>80-120</td></tr><tr><td>50%</td><td>1.90</td><td>97-103</td></tr><tr><td>100%</td><td>1.71</td><td>97-103</td></tr><tr><td>ULOQ</td><td>1.54</td><td>97-103</td></tr></table> | Level of validation azoxystrobin | Max precision [%] | Range of accuracy [%] | LOQ   | 3.08 | 80-120 | 50%  | 1.90  | 97-103                           | 100% | 1.71 | 97-103 | ULOQ                | 1.54          | 97-103        |              |     |      |      |       |     |      |      |       |      |      |      |       |      |      |      |       |  |                                |  |  |  |                     |               |               |              |     |      |      |      |     |      |      |       |      |      |      |       |      |      |      |       |                                  |  |  |  |                     |               |               |              |     |      |      |       |     |      |      |       |      |      |      |       |      |      |      |       |
| Level of validation prothioconazole  | Max precision [%]  | Range of accuracy [%]               |                   |                       |     |                     |               |               |              |        |      |      |        |      |      |        |   |                                  |                   |                       |       |      |        |      |       |                                  |      |      |        |                     |               |               |              |     |      |      |       |     |      |      |       |      |      |      |       |      |      |      |       |  |                                |  |  |  |                     |               |               |              |     |      |      |      |     |      |      |       |      |      |      |       |      |      |      |       |                                  |  |  |  |                     |               |               |              |     |      |      |       |     |      |      |       |      |      |      |       |      |      |      |       |
| LOQ  | 3.14   | 80-120                              |                   |                       |     |                     |               |               |              |        |      |      |        |      |      |        |   |                                  |                   |                       |       |      |        |      |       |                                  |      |      |        |                     |               |               |              |     |      |      |       |     |      |      |       |      |      |      |       |      |      |      |       |  |                                |  |  |  |                     |               |               |              |     |      |      |      |     |      |      |       |      |      |      |       |      |      |      |       |                                  |  |  |  |                     |               |               |              |     |      |      |       |     |      |      |       |      |      |      |       |      |      |      |       |
| 50%  | 1.93   | 90-110                              |                   |                       |     |                     |               |               |              |        |      |      |        |      |      |        |   |                                  |                   |                       |       |      |        |      |       |                                  |      |      |        |                     |               |               |              |     |      |      |       |     |      |      |       |      |      |      |       |      |      |      |       |  |                                |  |  |  |                     |               |               |              |     |      |      |      |     |      |      |       |      |      |      |       |      |      |      |       |                                  |  |  |  |                     |               |               |              |     |      |      |       |     |      |      |       |      |      |      |       |      |      |      |       |
| 100%   | 1.74   | 97-103                              |                   |                       |     |                     |               |               |              |        |      |      |        |      |      |        |   |                                  |                   |                       |       |      |        |      |       |                                  |      |      |        |                     |               |               |              |     |      |      |       |     |      |      |       |      |      |      |       |      |      |      |       |  |                                |  |  |  |                     |               |               |              |     |      |      |      |     |      |      |       |      |      |      |       |      |      |      |       |                                  |  |  |  |                     |               |               |              |     |      |      |       |     |      |      |       |      |      |      |       |      |      |      |       |
| ULOQ   | 1.57   | 97-103                              |                   |                       |     |                     |               |               |              |        |      |      |        |      |      |        |   |                                  |                   |                       |       |      |        |      |       |                                  |      |      |        |                     |               |               |              |     |      |      |       |     |      |      |       |      |      |      |       |      |      |      |       |  |                                |  |  |  |                     |               |               |              |     |      |      |      |     |      |      |       |      |      |      |       |      |      |      |       |                                  |  |  |  |                     |               |               |              |     |      |      |       |     |      |      |       |      |      |      |       |      |      |      |       |
| Level of validation azoxystrobin   | Max precision [%]  | Range of accuracy [%]               |                   |                       |     |                     |               |               |              |        |      |      |        |      |      |        |   |                                  |                   |                       |       |      |        |      |       |                                  |      |      |        |                     |               |               |              |     |      |      |       |     |      |      |       |      |      |      |       |      |      |      |       |  |                                |  |  |  |                     |               |               |              |     |      |      |      |     |      |      |       |      |      |      |       |      |      |      |       |                                  |  |  |  |                     |               |               |              |     |      |      |       |     |      |      |       |      |      |      |       |      |      |      |       |
| LOQ  | 3.08   | 80-120                              |                   |                       |     |                     |               |               |              |        |      |      |        |      |      |        |   |                                  |                   |                       |       |      |        |      |       |                                  |      |      |        |                     |               |               |              |     |      |      |       |     |      |      |       |      |      |      |       |      |      |      |       |  |                                |  |  |  |                     |               |               |              |     |      |      |      |     |      |      |       |      |      |      |       |      |      |      |       |                                  |  |  |  |                     |               |               |              |     |      |      |       |     |      |      |       |      |      |      |       |      |      |      |       |
| 50%  | 1.90   | 97-103                              |                   |                       |     |                     |               |               |              |        |      |      |        |      |      |        |   |                                  |                   |                       |       |      |        |      |       |                                  |      |      |        |                     |               |               |              |     |      |      |       |     |      |      |       |      |      |      |       |      |      |      |       |  |                                |  |  |  |                     |               |               |              |     |      |      |      |     |      |      |       |      |      |      |       |      |      |      |       |                                  |  |  |  |                     |               |               |              |     |      |      |       |     |      |      |       |      |      |      |       |      |      |      |       |
| 100%   | 1.71   | 97-103                              |                   |                       |     |                     |               |               |              |        |      |      |        |      |      |        |   |                                  |                   |                       |       |      |        |      |       |                                  |      |      |        |                     |               |               |              |     |      |      |       |     |      |      |       |      |      |      |       |      |      |      |       |  |                                |  |  |  |                     |               |               |              |     |      |      |      |     |      |      |       |      |      |      |       |      |      |      |       |                                  |  |  |  |                     |               |               |              |     |      |      |       |     |      |      |       |      |      |      |       |      |      |      |       |
| ULOQ   | 1.54   | 97-103                              |                   |                       |     |                     |               |               |              |        |      |      |        |      |      |        |   |                                  |                   |                       |       |      |        |      |       |                                  |      |      |        |                     |               |               |              |     |      |      |       |     |      |      |       |      |      |      |       |      |      |      |       |  |                                |  |  |  |                     |               |               |              |     |      |      |      |     |      |      |       |      |      |      |       |      |      |      |       |                                  |  |  |  |                     |               |               |              |     |      |      |       |     |      |      |       |      |      |      |       |      |      |      |       |
| Accuracy<br>n=4<br>(% Recovery)<br>Five samples per each fortification level                 | <table><tr><th colspan="4">Primary chromatographic system</th></tr><tr><th>Level of validation</th><th>Precision [%]</th><th>Horwitz ratio</th><th>Accuracy [%]</th></tr><tr><td>LOQ</td><td>2.95</td><td>0.94</td><td>100.9</td></tr><tr><td>50%</td><td>0.99</td><td>0.51</td><td>101.5</td></tr><tr><td>100%</td><td>0.61</td><td>0.35</td><td>101.6</td></tr><tr><td>ULOQ</td><td>0.33</td><td>0.21</td><td>103.0</td></tr></table> <p>Obtained results meet the criteria for precision and accuracy included in the sub-point 4.15.3.1.</p> <table><tr><th colspan="4">Secondary chromatographic system</th></tr><tr><th>Level of validation</th><th>Precision [%]</th><th>Horwitz ratio</th><th>Accuracy [%]</th></tr><tr><td>LOQ</td><td>2.98</td><td>0.95</td><td>103.9</td></tr><tr><td>50%</td><td>1.20</td><td>0.62</td><td>101.9</td></tr><tr><td>100%</td><td>0.61</td><td>0.35</td><td>101.7</td></tr><tr><td>ULOQ</td><td>0.14</td><td>0.09</td><td>103.0</td></tr></table> | Primary chromatographic system      |                   |                       |     | Level of validation | Precision [%] | Horwitz ratio | Accuracy [%] | LOQ    | 2.95 | 0.94 | 100.9  | 50%  | 0.99 | 0.51   | 101.5   | 100%                             | 0.61              | 0.35                  | 101.6 | ULOQ | 0.33   | 0.21 | 103.0 | Secondary chromatographic system |      |      |        | Level of validation | Precision [%] | Horwitz ratio | Accuracy [%] | LOQ | 2.98 | 0.95 | 103.9 | 50% | 1.20 | 0.62 | 101.9 | 100% | 0.61 | 0.35 | 101.7 | ULOQ | 0.14 | 0.09 | 103.0 | <table><tr><th colspan="4">Primary chromatographic system</th></tr><tr><th>Level of validation</th><th>Precision [%]</th><th>Horwitz ratio</th><th>Accuracy [%]</th></tr><tr><td>LOQ</td><td>2.52</td><td>0.82</td><td>99.1</td></tr><tr><td>50%</td><td>0.51</td><td>0.27</td><td>102.0</td></tr><tr><td>100%</td><td>0.66</td><td>0.39</td><td>102.0</td></tr><tr><td>ULOQ</td><td>0.68</td><td>0.44</td><td>101.3</td></tr></table> <table><tr><th colspan="4">Secondary chromatographic system</th></tr><tr><th>Level of validation</th><th>Precision [%]</th><th>Horwitz ratio</th><th>Accuracy [%]</th></tr><tr><td>LOQ</td><td>0.67</td><td>0.22</td><td>103.7</td></tr><tr><td>50%</td><td>1.02</td><td>0.54</td><td>102.6</td></tr><tr><td>100%</td><td>0.44</td><td>0.26</td><td>102.1</td></tr><tr><td>ULOQ</td><td>0.65</td><td>0.42</td><td>101.2</td></tr></table> | Primary chromatographic system |  |  |  | Level of validation | Precision [%] | Horwitz ratio | Accuracy [%] | LOQ | 2.52 | 0.82 | 99.1 | 50% | 0.51 | 0.27 | 102.0 | 100% | 0.66 | 0.39 | 102.0 | ULOQ | 0.68 | 0.44 | 101.3 | Secondary chromatographic system |  |  |  | Level of validation | Precision [%] | Horwitz ratio | Accuracy [%] | LOQ | 0.67 | 0.22 | 103.7 | 50% | 1.02 | 0.54 | 102.6 | 100% | 0.44 | 0.26 | 102.1 | ULOQ | 0.65 | 0.42 | 101.2 |
| Primary chromatographic system   |  |                                     |                   |                       |     |                     |               |               |              |        |      |      |        |      |      |        |   |                                  |                   |                       |       |      |        |      |       |                                  |      |      |        |                     |               |               |              |     |      |      |       |     |      |      |       |      |      |      |       |      |      |      |       |  |                                |  |  |  |                     |               |               |              |     |      |      |      |     |      |      |       |      |      |      |       |      |      |      |       |                                  |  |  |  |                     |               |               |              |     |      |      |       |     |      |      |       |      |      |      |       |      |      |      |       |
| Level of validation  | Precision [%]  | Horwitz ratio                       | Accuracy [%]      |                       |     |                     |               |               |              |        |      |      |        |      |      |        |   |                                  |                   |                       |       |      |        |      |       |                                  |      |      |        |                     |               |               |              |     |      |      |       |     |      |      |       |      |      |      |       |      |      |      |       |  |                                |  |  |  |                     |               |               |              |     |      |      |      |     |      |      |       |      |      |      |       |      |      |      |       |                                  |  |  |  |                     |               |               |              |     |      |      |       |     |      |      |       |      |      |      |       |      |      |      |       |
| LOQ  | 2.95   | 0.94                                | 100.9             |                       |     |                     |               |               |              |        |      |      |        |      |      |        |   |                                  |                   |                       |       |      |        |      |       |                                  |      |      |        |                     |               |               |              |     |      |      |       |     |      |      |       |      |      |      |       |      |      |      |       |  |                                |  |  |  |                     |               |               |              |     |      |      |      |     |      |      |       |      |      |      |       |      |      |      |       |                                  |  |  |  |                     |               |               |              |     |      |      |       |     |      |      |       |      |      |      |       |      |      |      |       |
| 50%  | 0.99   | 0.51                                | 101.5             |                       |     |                     |               |               |              |        |      |      |        |      |      |        |   |                                  |                   |                       |       |      |        |      |       |                                  |      |      |        |                     |               |               |              |     |      |      |       |     |      |      |       |      |      |      |       |      |      |      |       |  |                                |  |  |  |                     |               |               |              |     |      |      |      |     |      |      |       |      |      |      |       |      |      |      |       |                                  |  |  |  |                     |               |               |              |     |      |      |       |     |      |      |       |      |      |      |       |      |      |      |       |
| 100%   | 0.61   | 0.35                                | 101.6             |                       |     |                     |               |               |              |        |      |      |        |      |      |        |   |                                  |                   |                       |       |      |        |      |       |                                  |      |      |        |                     |               |               |              |     |      |      |       |     |      |      |       |      |      |      |       |      |      |      |       |  |                                |  |  |  |                     |               |               |              |     |      |      |      |     |      |      |       |      |      |      |       |      |      |      |       |                                  |  |  |  |                     |               |               |              |     |      |      |       |     |      |      |       |      |      |      |       |      |      |      |       |
| ULOQ   | 0.33   | 0.21                                | 103.0             |                       |     |                     |               |               |              |        |      |      |        |      |      |        |   |                                  |                   |                       |       |      |        |      |       |                                  |      |      |        |                     |               |               |              |     |      |      |       |     |      |      |       |      |      |      |       |      |      |      |       |  |                                |  |  |  |                     |               |               |              |     |      |      |      |     |      |      |       |      |      |      |       |      |      |      |       |                                  |  |  |  |                     |               |               |              |     |      |      |       |     |      |      |       |      |      |      |       |      |      |      |       |
| Secondary chromatographic system   |  |                                     |                   |                       |     |                     |               |               |              |        |      |      |        |      |      |        |   |                                  |                   |                       |       |      |        |      |       |                                  |      |      |        |                     |               |               |              |     |      |      |       |     |      |      |       |      |      |      |       |      |      |      |       |  |                                |  |  |  |                     |               |               |              |     |      |      |      |     |      |      |       |      |      |      |       |      |      |      |       |                                  |  |  |  |                     |               |               |              |     |      |      |       |     |      |      |       |      |      |      |       |      |      |      |       |
| Level of validation  | Precision [%]  | Horwitz ratio                       | Accuracy [%]      |                       |     |                     |               |               |              |        |      |      |        |      |      |        |   |                                  |                   |                       |       |      |        |      |       |                                  |      |      |        |                     |               |               |              |     |      |      |       |     |      |      |       |      |      |      |       |      |      |      |       |  |                                |  |  |  |                     |               |               |              |     |      |      |      |     |      |      |       |      |      |      |       |      |      |      |       |                                  |  |  |  |                     |               |               |              |     |      |      |       |     |      |      |       |      |      |      |       |      |      |      |       |
| LOQ  | 2.98   | 0.95                                | 103.9             |                       |     |                     |               |               |              |        |      |      |        |      |      |        |   |                                  |                   |                       |       |      |        |      |       |                                  |      |      |        |                     |               |               |              |     |      |      |       |     |      |      |       |      |      |      |       |      |      |      |       |  |                                |  |  |  |                     |               |               |              |     |      |      |      |     |      |      |       |      |      |      |       |      |      |      |       |                                  |  |  |  |                     |               |               |              |     |      |      |       |     |      |      |       |      |      |      |       |      |      |      |       |
| 50%  | 1.20   | 0.62                                | 101.9             |                       |     |                     |               |               |              |        |      |      |        |      |      |        |   |                                  |                   |                       |       |      |        |      |       |                                  |      |      |        |                     |               |               |              |     |      |      |       |     |      |      |       |      |      |      |       |      |      |      |       |  |                                |  |  |  |                     |               |               |              |     |      |      |      |     |      |      |       |      |      |      |       |      |      |      |       |                                  |  |  |  |                     |               |               |              |     |      |      |       |     |      |      |       |      |      |      |       |      |      |      |       |
| 100%   | 0.61   | 0.35                                | 101.7             |                       |     |                     |               |               |              |        |      |      |        |      |      |        |   |                                  |                   |                       |       |      |        |      |       |                                  |      |      |        |                     |               |               |              |     |      |      |       |     |      |      |       |      |      |      |       |      |      |      |       |  |                                |  |  |  |                     |               |               |              |     |      |      |      |     |      |      |       |      |      |      |       |      |      |      |       |                                  |  |  |  |                     |               |               |              |     |      |      |       |     |      |      |       |      |      |      |       |      |      |      |       |
| ULOQ   | 0.14   | 0.09                                | 103.0             |                       |     |                     |               |               |              |        |      |      |        |      |      |        |   |                                  |                   |                       |       |      |        |      |       |                                  |      |      |        |                     |               |               |              |     |      |      |       |     |      |      |       |      |      |      |       |      |      |      |       |  |                                |  |  |  |                     |               |               |              |     |      |      |      |     |      |      |       |      |      |      |       |      |      |      |       |                                  |  |  |  |                     |               |               |              |     |      |      |       |     |      |      |       |      |      |      |       |      |      |      |       |
| Primary chromatographic system   |  |                                     |                   |                       |     |                     |               |               |              |        |      |      |        |      |      |        |   |                                  |                   |                       |       |      |        |      |       |                                  |      |      |        |                     |               |               |              |     |      |      |       |     |      |      |       |      |      |      |       |      |      |      |       |  |                                |  |  |  |                     |               |               |              |     |      |      |      |     |      |      |       |      |      |      |       |      |      |      |       |                                  |  |  |  |                     |               |               |              |     |      |      |       |     |      |      |       |      |      |      |       |      |      |      |       |
| Level of validation  | Precision [%]  | Horwitz ratio                       | Accuracy [%]      |                       |     |                     |               |               |              |        |      |      |        |      |      |        |   |                                  |                   |                       |       |      |        |      |       |                                  |      |      |        |                     |               |               |              |     |      |      |       |     |      |      |       |      |      |      |       |      |      |      |       |  |                                |  |  |  |                     |               |               |              |     |      |      |      |     |      |      |       |      |      |      |       |      |      |      |       |                                  |  |  |  |                     |               |               |              |     |      |      |       |     |      |      |       |      |      |      |       |      |      |      |       |
| LOQ  | 2.52   | 0.82                                | 99.1              |                       |     |                     |               |               |              |        |      |      |        |      |      |        |   |                                  |                   |                       |       |      |        |      |       |                                  |      |      |        |                     |               |               |              |     |      |      |       |     |      |      |       |      |      |      |       |      |      |      |       |  |                                |  |  |  |                     |               |               |              |     |      |      |      |     |      |      |       |      |      |      |       |      |      |      |       |                                  |  |  |  |                     |               |               |              |     |      |      |       |     |      |      |       |      |      |      |       |      |      |      |       |
| 50%  | 0.51   | 0.27                                | 102.0             |                       |     |                     |               |               |              |        |      |      |        |      |      |        |   |                                  |                   |                       |       |      |        |      |       |                                  |      |      |        |                     |               |               |              |     |      |      |       |     |      |      |       |      |      |      |       |      |      |      |       |  |                                |  |  |  |                     |               |               |              |     |      |      |      |     |      |      |       |      |      |      |       |      |      |      |       |                                  |  |  |  |                     |               |               |              |     |      |      |       |     |      |      |       |      |      |      |       |      |      |      |       |
| 100%   | 0.66   | 0.39                                | 102.0             |                       |     |                     |               |               |              |        |      |      |        |      |      |        |   |                                  |                   |                       |       |      |        |      |       |                                  |      |      |        |                     |               |               |              |     |      |      |       |     |      |      |       |      |      |      |       |      |      |      |       |  |                                |  |  |  |                     |               |               |              |     |      |      |      |     |      |      |       |      |      |      |       |      |      |      |       |                                  |  |  |  |                     |               |               |              |     |      |      |       |     |      |      |       |      |      |      |       |      |      |      |       |
| ULOQ   | 0.68   | 0.44                                | 101.3             |                       |     |                     |               |               |              |        |      |      |        |      |      |        |   |                                  |                   |                       |       |      |        |      |       |                                  |      |      |        |                     |               |               |              |     |      |      |       |     |      |      |       |      |      |      |       |      |      |      |       |  |                                |  |  |  |                     |               |               |              |     |      |      |      |     |      |      |       |      |      |      |       |      |      |      |       |                                  |  |  |  |                     |               |               |              |     |      |      |       |     |      |      |       |      |      |      |       |      |      |      |       |
| Secondary chromatographic system   |  |                                     |                   |                       |     |                     |               |               |              |        |      |      |        |      |      |        |   |                                  |                   |                       |       |      |        |      |       |                                  |      |      |        |                     |               |               |              |     |      |      |       |     |      |      |       |      |      |      |       |      |      |      |       |  |                                |  |  |  |                     |               |               |              |     |      |      |      |     |      |      |       |      |      |      |       |      |      |      |       |                                  |  |  |  |                     |               |               |              |     |      |      |       |     |      |      |       |      |      |      |       |      |      |      |       |
| Level of validation  | Precision [%]  | Horwitz ratio                       | Accuracy [%]      |                       |     |                     |               |               |              |        |      |      |        |      |      |        |   |                                  |                   |                       |       |      |        |      |       |                                  |      |      |        |                     |               |               |              |     |      |      |       |     |      |      |       |      |      |      |       |      |      |      |       |  |                                |  |  |  |                     |               |               |              |     |      |      |      |     |      |      |       |      |      |      |       |      |      |      |       |                                  |  |  |  |                     |               |               |              |     |      |      |       |     |      |      |       |      |      |      |       |      |      |      |       |
| LOQ  | 0.67   | 0.22                                | 103.7             |                       |     |                     |               |               |              |        |      |      |        |      |      |        |   |                                  |                   |                       |       |      |        |      |       |                                  |      |      |        |                     |               |               |              |     |      |      |       |     |      |      |       |      |      |      |       |      |      |      |       |  |                                |  |  |  |                     |               |               |              |     |      |      |      |     |      |      |       |      |      |      |       |      |      |      |       |                                  |  |  |  |                     |               |               |              |     |      |      |       |     |      |      |       |      |      |      |       |      |      |      |       |
| 50%  | 1.02   | 0.54                                | 102.6             |                       |     |                     |               |               |              |        |      |      |        |      |      |        |   |                                  |                   |                       |       |      |        |      |       |                                  |      |      |        |                     |               |               |              |     |      |      |       |     |      |      |       |      |      |      |       |      |      |      |       |  |                                |  |  |  |                     |               |               |              |     |      |      |      |     |      |      |       |      |      |      |       |      |      |      |       |                                  |  |  |  |                     |               |               |              |     |      |      |       |     |      |      |       |      |      |      |       |      |      |      |       |
| 100%   | 0.44   | 0.26                                | 102.1             |                       |     |                     |               |               |              |        |      |      |        |      |      |        |   |                                  |                   |                       |       |      |        |      |       |                                  |      |      |        |                     |               |               |              |     |      |      |       |     |      |      |       |      |      |      |       |      |      |      |       |  |                                |  |  |  |                     |               |               |              |     |      |      |      |     |      |      |       |      |      |      |       |      |      |      |       |                                  |  |  |  |                     |               |               |              |     |      |      |       |     |      |      |       |      |      |      |       |      |      |      |       |
| ULOQ   | 0.65   | 0.42                                | 101.2             |                       |     |                     |               |               |              |        |      |      |        |      |      |        |   |                                  |                   |                       |       |      |        |      |       |                                  |      |      |        |                     |               |               |              |     |      |      |       |     |      |      |       |      |      |      |       |      |      |      |       |  |                                |  |  |  |                     |               |               |              |     |      |      |      |     |      |      |       |      |      |      |       |      |      |      |       |                                  |  |  |  |                     |               |               |              |     |      |      |       |     |      |      |       |      |      |      |       |      |      |      |       |
| Interference/ Specificity  | Specificity of the method was evaluated based on the analysis of chromatograms for blank samples (placebo) against samples of placebo spiked with azoxystrobin and 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.   |                                     |                   |                       |     |                     |               |               |              |        |      |      |        |      |      |        |   |                                  |                   |                       |       |      |        |      |       |                                  |      |      |        |                     |               |               |              |     |      |      |       |     |      |      |       |      |      |      |       |      |      |      |       |  |                                |  |  |  |                     |               |               |              |     |      |      |      |     |      |      |       |      |      |      |       |      |      |      |       |                                  |  |  |  |                     |               |               |              |     |      |      |       |     |      |      |       |      |      |      |       |      |      |      |       |
| Comment  |  |                                     |                   |                       |     |                     |               |               |              |        |      |      |        |      |      |        |   |                                  |                   |                       |       |      |        |      |       |                                  |      |      |        |                     |               |               |              |     |      |      |       |     |      |      |       |      |      |      |       |      |      |      |       |  |                                |  |  |  |                     |               |               |              |     |      |      |      |     |      |      |       |      |      |      |       |      |      |      |       |                                  |  |  |  |                     |               |               |              |     |      |      |       |     |      |      |       |      |      |      |       |      |      |      |       |

## 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.4.

### 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: | These methods are validated and may be used for analysing Z-azoxystrobin, toluene and prothioconazole-desthio impurities in the PPP. |
|-------------------|--|

Reference: KCP 5.1/01

Report: Determination of physicochemical properties, I. Knapik, Study code: ICB/93/2019

Guideline(s): Determination of content of azoxystrobin and prothioconazole – validated own method SPB/139; Determination of content of Z-azoxystrobin - validated own method SPB/144; Determination of toluene validated own method SPB/134; Appearance – based on OPPTS 830.6302, 830.6303 and 830.6304; pH – CIPAC MT 75.3; Flash point – EEC A.9; Surface tension - EEC A.5; Relative density – EEC A.3; Persistent foaming – CIPAC MT

47.3; Suspensibility – CIPAC MT 184; Low temperature stability of liquid formulations – CIPAC MT 39.3; Particle size – CIPAC MT 187; Stability of package - own method SPB/38; Spontaneity of dispersion – CIPAC MT 160; Pourability – CIPAC MT 148.1; Wet sieve – CIPAC MT 185; Effectiveness of cleaning - Efficacy Guideline 305; Accelerated storage stability – CIPAC 46.3

Deviations: No  
GLP: Yes  
Acceptability: Yes

### Materials and methods

- acetonitrile HPLC (Honeywell)
- 85% phosphoric acid (Sigma Aldrich)
- Z-azoxystrobin standard; HPC Standards; batch 789826
- standard solution Z-azoxystrobin in acetonitrile (Table 5)
- working standard solution Z-azoxystrobin in acetonitrile (Table 6)
- analytical balance – accuracy 0.0001 g (Ohaus, Switzerland)
- liquid chromatography with diode array detection (Shimadzu, Japan)
- chromatography column type C18, 250 mm x 4.6 mm; 5µm (Zorbax Eclipse Plus, Agilent)
- chromatography column type C18, 250 mm x 4.6 mm; 5µm (Luna®, Phenomenex)
- chromatographic vials 1,5 mL with septa buthyl/teflon
- syringe filter with a 0.45 µm pore diameter
- volumetric flasks class A, 10 mL
- measuring syringes 10 µL, 50 µL, 250 µL and 500 µL.

Determination of the content of Z-azoxystrobin, as impurity, was conducted according to validated analytical method SPB/144. Content of Z-azoxystrobin in the test item at a level of about 25 µg/mL was determined by liquid chromatography with diode array detection (HPLC-DAD).

- acetone GC (VWR)
- fluorobenzene standard (internal standard); Sigma Aldrich; batch BCBW1454
- standard solution fluorobenzene in acetone (Table 7)
- toluene standard; Sigma Aldrich; batch STBH6243
- standard solution toluene in acetone (Table 8)
- working standard solution toluene in acetone (Table 9)
- analytical balance – accuracy 0.0001 g (Ohaus, Switzerland)
- gas chromatograph Shimadzu GC2010 with mass detector (GC-MS) with column Phenomenex ZB-624; l. 30 m; diameter 0.25 mm, film thickness 1.4 µm; (Shimadzu, Japan)
- chromatographic vials 1,5 mL with septa buthyl/teflon;
- volumetric flasks class A, 10 mL;
- measuring syringes 10 µL, 50 µL, 100 µL, 250 µL, 500 µL, 1000 µL.

Determination of the content of toluene was conducted according to validated analytical method SPB/134. Content of toluene in the test item at a level of about 10 µg/mL was determined by gas chromatography with mass detector (GC- MS).

Reference: KCP 5.1/02

Report Prothioconazol + Azoxystrobin (175 + 200) SC Determination of the rele-



vant impurity of prothioconazole (prothioconazole-desthio) in the formulation at initial time and after accelerated storage, M. Wołoszynowska, Study code: BA-19/19

Guideline(s): Determination of content of prothioconazole – validated own method SPB/140;  
Determination of content of toluene - validated own method SPB/142; Appearance – based on OPPTS 830.6302, 830.6303 and 830.6304; Flash point – EEC A.9; pH – CIPAC MT 75.3; Surface tension - EEC A.5; Relative density – EEC A.3; Persistent foam – CIPAC MT 47.3; Pourability – CIPAC MT 148.1; Emulsion stability – CIPAC MT 36.3; Low temperature stability of liquid formulations – CIPAC MT 39.3; Stability of package - own method SPB/38; Efficiency of cleaning - Efficacy Guideline 305; Accelerated storage stability – CIPAC 46.3.

Deviations: No

GLP: Yes

Acceptability: Yes

## Materials and methods

- Shimadzu liquid chromatograph equipped with DAD detector, a thermostated column oven and autosampler
- Nucleodur 100-5, C18ec column (5µm), 250 x 4.6 mm
- Analytical balance Mettler Toledo XS 205 DU/M accuracy 0.01 mg
- Volumetric flasks
- Ultrasonic bath
- Disposable syringes
- Pureland PTFE syringe filters, 0.22µm
- Glass pipettes
- Other typical laboratory equipment
- Water for HPLC, Millipore
- Acetonitrile for HPLC – Super gradient, POCh
- Formic acid, reagent grade ≥ 95%, Sigma-Aldrich

The content of relevant impurity in the xamined specimen was determined by high performance liquid chromatography HPLC with DAD detector using reversed phase column.

## Validation - Results and discussions

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

|                                      | <b>Toluene<br/>max. g mg/kg</b>  | <b>Prothioconazole-desthio<br/>max. 0.5 g/kg</b>                                      | <b>Z-azoxystrobin</b>  |
|--------------------------------------|--|---|--|
| <b>Author(s),<br/>year</b>           | I. Kanpik, 2019  | M. Wołoszynowska, 2019  | I. Kanpik 2019   |
| <b>Principle of<br/>method</b>       | GC-MS  | HPLC-DAD  | HPLC-DAD   |
| <b>Linearity<br/>(linear between</b> | In order to check the linearity, calibration curve was prepared using standard | The linearity of the detector response was assessed using seven standard solutions at | In order to check the linearity of Z-azoxystrobin, calibration curve was prepared using standard solutions.. A |

|  | <b>Toluene<br/>max. g mg/kg</b>  | <b>Prothioconazole-desthio<br/>max. 0.5 g/kg</b>   | <b>Z-azoxystrobin</b>   |
|--|--|--|---|
| <b>mg/L)<br/>(correlation<br/>coefficient,<br/>expressed as<br/>r)<br/>n=5</b> | 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 1.16 to 17.37 [µg/mL]. Correlation coefficient R <sub>2</sub> is 0.998904 | the concentration range of prothioconazole-desthio from 0.000106 mg/ml to 0.00529 mg/ml which corresponds to concentration range of 8.96% to 447% of prothioconazole-desthio content in the preparations. All solutions were analysed twice.<br><br>y= 35628255x +2381<br>Correlation coefficient should be R <sup>2</sup> ≥0.99. The obtained result is acceptable. | graph of the peak area to the concentration of Z-azoxystrobin was plotted. The resulting curve is linear in the tested concentrations. Linearity range of Z-azoxystrobin is from 1.759 to 35.18 µg/mL. Correlation coefficient R <sub>2</sub> is 0.9999727 for primary chromatographic first. Correlation coefficient R <sub>2</sub> is 0.9999758 for secondary chromatographic system. |
| <b>Precision –<br/>Repeatability<br/>Mean<br/>n = 6<br/>(%RSD)</b>             | According to SPT/31, the parameters obtained as a result of validation should meet the following criteria:<br>- linearity R <sub>2</sub> ≥ 0.99;<br>- Horwitz ratio ≤ 1.0.   | The method repeatability was assessed on the basis of six independent determination of prothioconazole-desthio content in Prothioconazol + Azoxystrobin (175 + 200) SC preparation. Acceptable relative standard deviation for main ingredient (0.000190%) is RSDr≤9.78%. The confidence interval x=0.000008%. The obtained result 4.05 % is acceptable.             | According to SPT/31, the parameters obtained as a result of validation should meet the following criteria:<br>- linearity R <sub>2</sub> ≥ 0.99;<br>- Horwitz ratio ≤ 1.0.  |

|                                   | Toluene<br>max. g mg/kg   | Prothioconazole-desthio<br>max. 0.5 g/kg  | Z-azoxystrobin   |
|-----------------------------------|---|---|--|
| Accuracy<br>n = 6<br>(% Recovery) | <p>Level of validation    Precision [%]    Horwitz ratio    Accuracy [%]</p> <p>LOQ    2.39    0.46    89.2</p> <p>50%    2.53    0.62    98.2</p> <p>100%    2.27    0.62    100.9</p> |   | <p><b>Primary chromatographic system</b></p> <p>Level of validation    Precision [%]    Horwitz ratio    Accuracy [%]</p> <p>LOQ    1.76    0.42    107.1</p> <p>50%    0.60    0.18    102.9</p> <p>100%    0.44    0.15    100.1</p> |
|                                   |   |   | <p>Obtained results meet the criteria for precision and accuracy included in the sub-point 4.16.3.1.</p>   |
|                                   |   |   | <p><b>Secondary chromatographic</b></p> <p>Level of validation    Precision [%]    Horwitz ratio    Accuracy [%]</p> <p>LOQ    1.06    0.25    112.0</p> <p>50%    1.56    0.47    99.9</p> <p>100%    0.34    0.11    99.9</p>        |
|                                   |   |   |  |
|                                   |   |   |  |
|                                   |   | <p>Accuracy of prothioconazole-desthio determination in Prothioconazole 250 EC was assessed by recovery value at two levels concentration. Twelve 10 mL volumetric flasks were charged with approximately 100 mg placebo. 0.1ml of the prothioconazole -desthio standard solution at concentration of 0.00968 mg/ml was added to the each of the first six flasks and acetonitille was added up to the volume. To each of the remaining six flasks 1.0 ml of ptothioconazole-desthio standard solution at concentration of 0.01059 mg/ml was added up to the volume. The flasks were put into the ultrasonic bath for 5 min. The concentration of analyte in each solution was calculated from the equation of the valibration curve. Obtained final concentrations were examined and the nominal and calculated content were compared.</p> <p>For the impurities at concentration of &lt;0.01% the average recover value should be 100 ±30%. The obtained results of 95.17% is acceptable.</p> |  |

|                                      | <b>Toluene<br/>max. g mg/kg</b>  | <b>Prothioconazole-desthio<br/>max. 0.5 g/kg</b>  | <b>Z-azoxystrobin</b>  |
|--------------------------------------|--|---|--|
| <b>Interference/<br/>Specificity</b> | 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. Specificity and selectivity of validated method for toluene determination was assessed at the point of optimizing conditions of analysis, by obtaining parameters for the best ingredients separation while maintaining interference impact at its lowest. The compound is identified by the presence of specific fragmentation ions and determine by the target ions for each analyte. The method was set up in the way the percentage ratio between value of the main ion to identified reference ions doesn't exceed 30% value of the error from the mass spectrum. Ions have been selected by NIST 11 library. | The chromatograms of placebo, solvent, standard solution and the examined specimen solution were performed and superimposed. There are no interferences between the analyte and other components of the specimen.   | Specificity of the method was evaluated based on the analysis of chromatograms for blank samples (placebo) against samples of placebo spiked with Z-azoxystrobin standards. Analysis showed no overlapping of determined substances signal with the signals of matrix components under method conditions, hence method specificity criterion is fulfilled. |
| <b>LOQ</b>                           | LOQ is 1.16 µg/mL  | The limit of quantification (LOQ) was defined as the lowest concentration i.e. 0.000042 mg/ml (0.00137 g/kg of prothioconazole-desthio content in the preparation and 0.00785 g/kg for prothioconazole). Limit of detection (LOD) is LOQ/2 i.e. about 0.0006534 g/kg. | LOQ is 1.759 µg/mL   |
| <b>Comment</b>                       |  |   |  |

## Conclusion

It was confirmed that the method is specific. There were no peaks from placebo interfering with deter-

mined 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.4.

### 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 and azoxystrobin impurities and relevance of CIPAC methods in CHR/F/PROTAZO 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 and azoxystrobin 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                                       | xxxxxxxxxxxxxxxxxx<br>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 |   |                                       |

| Component of residue definition: Prothioconazole-desthio |                               |              |  |                                       |
|--|-------------------------------|--------------|--|---------------------------------------|
| Matrix type  | Method type                   | Method LOQ   | Principle of method<br>(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<br>(if required) | Not required |  |                                       |
| Soil   | Primary                       | 0.006 mg/kg  | HPLC-MS/MS                                     | Schramel, 2000                        |
|  | Confirmatory<br>(if required) | 0.01 mg/kg   | GC/MS  | Steinhauer, 2001                      |
| Water  | Primary                       | 6 µg/L       | HPLC-UV  | Sommer, 1999                          |
|  | Confirmatory<br>(if required) | 0.05 µg/L    | HPLC-MS/MS                                     | Sommer, 2001                          |
| Air  | Primary                       | 0.0006 µg/L  | HPLC-MS/MS                                     | Maasfeld, 2002                        |
|  | Confirmatory<br>(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<br>(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<br>(if required) | Not required |  |                                       |
| Soil   | Primary                       | 0.006 mg/kg  | HPLC-MS/MS                                     | Schramel, 2000                        |
|  | Confirmatory<br>(if required) | Not required |  |                                       |

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

| Component of residue definition: Azoxystrobin |                               |              |  |   |
|---|-------------------------------|--------------|--|---|
| Matrix type                                   | Method type                   | Method LOQ   | Principle of method<br>(i.e. GC-MS or HPLC-UV) | Author(s), year / missing / EU agreed           |
| Plants, plant products,...                    | Primary                       | 0.01 mg/kg   | LC-MS/MS                                       | Robinson, N.J. et al, 1999<br>Chaggar, S., 2004 |
|   | Confirmatory<br>(if required) | Not required |  |   |
| Soil  | Primary                       | 0.02 mg/kg   | HPLC-MS/MS                                     | Johnson, R.I., et al., 2000                     |
|   | Confirmatory<br>(if required) | Not required |  |   |
| Water   | Primary                       | 0.1 µg/L     | GC-MSD   | Robinson, N.J., 2000                            |

| Component of residue definition: Azoxystrobin |                               |              |  |                                       |
|---|-------------------------------|--------------|--|---------------------------------------|
| Matrix type                                   | Method type                   | Method LOQ   | Principle of method<br>(i.e. GC-MS or HPLC-UV) | Author(s), year / missing / EU agreed |
|   | Confirmatory<br>(if required) | Not required |  |                                       |
| Air   | Primary                       | 3 µg/L       | GC-MSD   | Crawford, N., 2001                    |
|   | Confirmatory<br>(if required) | Not required |  |                                       |
| Food of animal                                | Primary                       | 0.01 mg/kg   | GC-NPD   | Sapiets, A., et al., 1997             |
|   | Confirmatory<br>(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)

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

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

|                   |                        |
|-------------------|------------------------|
| Comments of zRMS: | The study is accepted. |
|-------------------|------------------------|

|                |   |
|----------------|---|
| Reference:     | KCP 5.2/01  |
| Report         | Validation of the Analytical Method for the Analysis of Prothioconazole-desthio (sum of isomers) in Oilseed rape (Whole Plant and Seeds), C. Stouvenot, Study Code B9154, |
| Guideline(s):  | Regulation (EC) No. 1107/2009<br>SANCO/3029/99 rev.4<br>SANCO/825/00 rev.8.1<br>ENV/JM/MONO(2007)17   |
| Deviations:    | NO  |
| GLP:           | YES   |
| Acceptability: | YES   |

The method under discussion describes the determination of residues of prothioconazole-desthio in oilseed rape whole plant and seeds. The method was validated at 0.01 mg/kg in oilseed rape whole plant and seeds.

**Table 5.3-1: Methods suitable for the determination of the residues in plant protection product (PPP) CHR/F/PROTAZO**

|   | Residues   |                             |                              |                             |                                       |                                 |  |         |                         |                             |                              |                             |                                       |                                 |                         |                          |      |                             |      |      |      |      |                                  |               |               |               |               |   |       |       |       |                         |                    |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |
|---|--|-----------------------------|------------------------------|-----------------------------|---------------------------------------|---------------------------------|--|---------|-------------------------|-----------------------------|------------------------------|-----------------------------|---------------------------------------|---------------------------------|-------------------------|--------------------------|------|-----------------------------|------|------|------|------|----------------------------------|---------------|---------------|---------------|---------------|---|-------|-------|-------|-------------------------|--------------------|------|-------|------|------|---|------|-------|------|------|---|------------|-------|------|------|----|
| Author(s), year   | C. Stouvenot   |                             |                              |                             |                                       |                                 |  |         |                         |                             |                              |                             |                                       |                                 |                         |                          |      |                             |      |      |      |      |                                  |               |               |               |               |   |       |       |       |                         |                    |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |
| Principle of method   | LC MS/MS   |                             |                              |                             |                                       |                                 |  |         |                         |                             |                              |                             |                                       |                                 |                         |                          |      |                             |      |      |      |      |                                  |               |               |               |               |   |       |       |       |                         |                    |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |
| Linearity (linear between mg/L) (correlation coefficient, expressed as r) | The linearity of the method was studied between 1.5 ng/mL and 60.3 ng/mL of prothioconazole-desthio in matrix-matched calibration solutions (corresponding to 0.003 to 0.12 mg/kg). The linear correlation coefficients were typically > 0.990, showing a good linearity.  |                             |                              |                             |                                       |                                 |  |         |                         |                             |                              |                             |                                       |                                 |                         |                          |      |                             |      |      |      |      |                                  |               |               |               |               |   |       |       |       |                         |                    |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |
| Recovery  | <table><tr><th>Analyte</th><th>Matrix</th><th>Fortification level (mg/kg)</th><th>Mean recovery Percentage (%)</th><th>Standard deviation (SD) (%)</th><th>Relative standard deviation (RSD) (%)</th><th>Number of fortified samples (n)</th></tr><tr><td rowspan="3">Prothioconazole-desthio</td><td rowspan="3">Oilseed rape whole plant</td><td>0.01</td><td>81.0%</td><td>2.6%</td><td>3.3%</td><td>5</td></tr><tr><td>0.10</td><td>88.3%</td><td>1.4%</td><td>1.6%</td><td>5</td></tr><tr><td>All levels</td><td>84.7%</td><td>4.3%</td><td>5.1%</td><td>10</td></tr><tr><td rowspan="3">Prothioconazole-desthio</td><td rowspan="3">Oilseed rape seeds</td><td>0.01</td><td>70.2%</td><td>4.3%</td><td>6.2%</td><td>5</td></tr><tr><td>0.10</td><td>74.7%</td><td>3.1%</td><td>4.2%</td><td>5</td></tr><tr><td>All levels</td><td>72.4%</td><td>4.3%</td><td>5.9%</td><td>10</td></tr></table> |                             |                              |                             |                                       |                                 |  | Analyte | Matrix                  | Fortification level (mg/kg) | Mean recovery Percentage (%) | Standard deviation (SD) (%) | Relative standard deviation (RSD) (%) | Number of fortified samples (n) | Prothioconazole-desthio | Oilseed rape whole plant | 0.01 | 81.0%                       | 2.6% | 3.3% | 5    | 0.10 | 88.3%                            | 1.4%          | 1.6%          | 5             | All levels    | 84.7%                                   | 4.3%  | 5.1%  | 10    | Prothioconazole-desthio | Oilseed rape seeds | 0.01 | 70.2% | 4.3% | 6.2% | 5 | 0.10 | 74.7% | 3.1% | 4.2% | 5 | All levels | 72.4% | 4.3% | 5.9% | 10 |
| Analyte   | Matrix   | Fortification level (mg/kg) | Mean recovery Percentage (%) | Standard deviation (SD) (%) | Relative standard deviation (RSD) (%) | Number of fortified samples (n) |  |         |                         |                             |                              |                             |                                       |                                 |                         |                          |      |                             |      |      |      |      |                                  |               |               |               |               |   |       |       |       |                         |                    |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |
| Prothioconazole-desthio   | Oilseed rape whole plant   | 0.01                        | 81.0%                        | 2.6%                        | 3.3%                                  | 5                               |  |         |                         |                             |                              |                             |                                       |                                 |                         |                          |      |                             |      |      |      |      |                                  |               |               |               |               |   |       |       |       |                         |                    |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |
|   |  | 0.10                        | 88.3%                        | 1.4%                        | 1.6%                                  | 5                               |  |         |                         |                             |                              |                             |                                       |                                 |                         |                          |      |                             |      |      |      |      |                                  |               |               |               |               |   |       |       |       |                         |                    |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |
|   |  | All levels                  | 84.7%                        | 4.3%                        | 5.1%                                  | 10                              |  |         |                         |                             |                              |                             |                                       |                                 |                         |                          |      |                             |      |      |      |      |                                  |               |               |               |               |   |       |       |       |                         |                    |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |
| Prothioconazole-desthio   | Oilseed rape seeds   | 0.01                        | 70.2%                        | 4.3%                        | 6.2%                                  | 5                               |  |         |                         |                             |                              |                             |                                       |                                 |                         |                          |      |                             |      |      |      |      |                                  |               |               |               |               |   |       |       |       |                         |                    |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |
|   |  | 0.10                        | 74.7%                        | 3.1%                        | 4.2%                                  | 5                               |  |         |                         |                             |                              |                             |                                       |                                 |                         |                          |      |                             |      |      |      |      |                                  |               |               |               |               |   |       |       |       |                         |                    |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |
|   |  | All levels                  | 72.4%                        | 4.3%                        | 5.9%                                  | 10                              |  |         |                         |                             |                              |                             |                                       |                                 |                         |                          |      |                             |      |      |      |      |                                  |               |               |               |               |   |       |       |       |                         |                    |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |
| Accuracy  | <p>The accuracy of the method was assessed on the basis of the determined recovery rates.</p> <table><tr><td></td><td colspan="2">Prothioconazole-desthio</td><td colspan="2">Prothioconazole-desthio</td></tr><tr><td>Matrix</td><td colspan="2">Oilseed rape whole plant</td><td colspan="2">Oilseed rape seeds</td></tr><tr><td>Fortification level (mg/kg)</td><td>0.01</td><td>0.10</td><td>0.01</td><td>0.10</td></tr><tr><td>Single recovery rates</td><td>77.7% - 84.6%</td><td>86.6% - 90.2%</td><td>63.7% - 73.7%</td><td>71.9% - 79.2%</td></tr><tr><td>Mean recoveries per fortification level</td><td>81.0%</td><td>88.3%</td><td>70.2%</td><td>74.7%</td></tr></table> <p>Average recoveries at each spiking level are in the range 70-110%, showing a good accuracy of the method.</p>  |                             |                              |                             |                                       |                                 |  |         | Prothioconazole-desthio |                             | Prothioconazole-desthio      |                             | Matrix                                | Oilseed rape whole plant        |                         | Oilseed rape seeds       |      | Fortification level (mg/kg) | 0.01 | 0.10 | 0.01 | 0.10 | Single recovery rates            | 77.7% - 84.6% | 86.6% - 90.2% | 63.7% - 73.7% | 71.9% - 79.2% | Mean recoveries per fortification level | 81.0% | 88.3% | 70.2% | 74.7%                   |                    |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |
|   | Prothioconazole-desthio  |                             | Prothioconazole-desthio      |                             |                                       |                                 |  |         |                         |                             |                              |                             |                                       |                                 |                         |                          |      |                             |      |      |      |      |                                  |               |               |               |               |   |       |       |       |                         |                    |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |
| Matrix  | Oilseed rape whole plant   |                             | Oilseed rape seeds           |                             |                                       |                                 |  |         |                         |                             |                              |                             |                                       |                                 |                         |                          |      |                             |      |      |      |      |                                  |               |               |               |               |   |       |       |       |                         |                    |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |
| Fortification level (mg/kg)   | 0.01   | 0.10                        | 0.01                         | 0.10                        |                                       |                                 |  |         |                         |                             |                              |                             |                                       |                                 |                         |                          |      |                             |      |      |      |      |                                  |               |               |               |               |   |       |       |       |                         |                    |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |
| Single recovery rates   | 77.7% - 84.6%  | 86.6% - 90.2%               | 63.7% - 73.7%                | 71.9% - 79.2%               |                                       |                                 |  |         |                         |                             |                              |                             |                                       |                                 |                         |                          |      |                             |      |      |      |      |                                  |               |               |               |               |   |       |       |       |                         |                    |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |
| Mean recoveries per fortification level                                   | 81.0%  | 88.3%                       | 70.2%                        | 74.7%                       |                                       |                                 |  |         |                         |                             |                              |                             |                                       |                                 |                         |                          |      |                             |      |      |      |      |                                  |               |               |               |               |   |       |       |       |                         |                    |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |
| Precision and repeatability   | <p>Repeatability tests (5 recoveries at each fortification level) were performed at the LOQ level and at 10 x LOQ for oilseed rape whole plant and seeds.</p> <table><tr><td></td><td colspan="2">Prothioconazole-desthio</td><td colspan="2">Prothioconazole-desthio</td></tr><tr><td>Matrix</td><td colspan="2">Oilseed rape whole plant</td><td colspan="2">Oilseed rape seeds</td></tr><tr><td>Fortification level (mg/kg)</td><td>0.01</td><td>0.10</td><td>0.01</td><td>0.10</td></tr><tr><td>RSD for each fortification level</td><td>3.3%</td><td>1.6%</td><td>6.2%</td><td>4.2%</td></tr></table> <p>All RSD determined were less than 20%, the method therefore fulfils the requirements of residue analytical methods.</p>  |                             |                              |                             |                                       |                                 |  |         | Prothioconazole-desthio |                             | Prothioconazole-desthio      |                             | Matrix                                | Oilseed rape whole plant        |                         | Oilseed rape seeds       |      | Fortification level (mg/kg) | 0.01 | 0.10 | 0.01 | 0.10 | RSD for each fortification level | 3.3%          | 1.6%          | 6.2%          | 4.2%          |   |       |       |       |                         |                    |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |
|   | Prothioconazole-desthio  |                             | Prothioconazole-desthio      |                             |                                       |                                 |  |         |                         |                             |                              |                             |                                       |                                 |                         |                          |      |                             |      |      |      |      |                                  |               |               |               |               |   |       |       |       |                         |                    |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |
| Matrix  | Oilseed rape whole plant   |                             | Oilseed rape seeds           |                             |                                       |                                 |  |         |                         |                             |                              |                             |                                       |                                 |                         |                          |      |                             |      |      |      |      |                                  |               |               |               |               |   |       |       |       |                         |                    |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |
| Fortification level (mg/kg)   | 0.01   | 0.10                        | 0.01                         | 0.10                        |                                       |                                 |  |         |                         |                             |                              |                             |                                       |                                 |                         |                          |      |                             |      |      |      |      |                                  |               |               |               |               |   |       |       |       |                         |                    |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |
| RSD for each fortification level  | 3.3%   | 1.6%                        | 6.2%                         | 4.2%                        |                                       |                                 |  |         |                         |                             |                              |                             |                                       |                                 |                         |                          |      |                             |      |      |      |      |                                  |               |               |               |               |   |       |       |       |                         |                    |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |
| LOQ   | The limit of quantification (LOQ) is the lowest validated level where a mean recovery within the   |                             |                              |                             |                                       |                                 |  |         |                         |                             |                              |                             |                                       |                                 |                         |                          |      |                             |      |      |      |      |                                  |               |               |               |               |   |       |       |       |                         |                    |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |



|                | Residues   |
|----------------|--|
|                | range 70-110% with a RSD less than 20% could be obtained. The LOQ was set at 0.01 mg/kg in oilseed rape whole plant and seeds. |
| <b>Comment</b> | The validation parameters are within the acceptance range and fulfil EU requirements given in SANCO/3029/99 rev.4.             |

## 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) SANCO /3029 /99 rev.4. 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 study is accepted.

Reference: KCP 5.2/03

Report Validation of the Analytical Method for the Analysis of Azoxystrobin, Prothioconazole and Prothioconazole-desthio in Honey and Pollen, P. Schlewitz, Study Code: C0240

Guideline(s): Regulation (EC) No. 1107/2009  
 SANCO/3029/99 rev.4  
 SANCO/825/00 rev.8.1  
 ENV/JM/MONO(2007)17

Deviations: NO

GLP: YES

Acceptability: YES

## Materials and methods

The method under discussion describes the determination of residues of azoxystrobin, prothioconazole and prothioconazole-desthio in honey and pollen. The method was validated at 0.01 mg/kg in honey and pollen for azoxystrobin (quantifier transition 404.2 > 372.1 and qualifier transition 404.2 > 329.0), prothioconazole (quantifier transition 342.1 > 306.0 and qualifier transition 342.1 > 179.9) and prothioconazole-desthio (quantifier transition 312.0 > 89.0 and qualifier transition 312.0 > 124.9)..

## Validation - Results and discussions

**Table 5.3-2: Methods suitable for the determination of the residues in plant protection product (PPP) CHR/F/PROTAZO**

|                              | Residues  |
|------------------------------|---|
| <b>Author(s), year</b>       | P. Schlewitz  |
| <b>Principle of method</b>   | LC MS/MS  |
| <b>Linearity (linear be-</b> | The linearity of the method was studied between $\approx 0.7$ ng/mL and $\approx 30$ ng/mL of azoxystrobin, prothioconazole and prothioconazole-desthio in matrix-matched calibration solutions |

|  | <b>Residues</b>  |                             |                              |                             |                                       |                                 |                                       |                                 |                              |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |                                 |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |   |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |                              |        |      |        |      |      |   |      |       |      |      |   |            |       |      |      |    |                                 |        |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |   |        |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |
|--|--|-----------------------------|------------------------------|-----------------------------|---------------------------------------|---------------------------------|---------------------------------------|---------------------------------|------------------------------|-------|------|--------|------|------|---|------|--------|------|------|---|------------|--------|------|------|----|---------------------------------|-------|------|--------|------|------|---|------|--------|------|------|---|------------|--------|------|------|----|---|-------|------|--------|------|------|---|------|--------|------|------|---|------------|--------|------|------|----|------------------------------|--------|------|--------|------|------|---|------|-------|------|------|---|------------|-------|------|------|----|---------------------------------|--------|------|-------|------|------|---|------|-------|------|------|---|------------|-------|------|------|----|---|--------|------|-------|------|------|---|------|-------|------|------|---|------------|-------|------|------|----|
| <b>tween mg/L)</b><br><b>(correlation coefficient, expressed as r)</b> | (corresponding to $\approx 0.003$ to $\approx 0.12$ mg/kg). The linear correlation coefficients were $> 0.990$ , showing a good linearity.   |                             |                              |                             |                                       |                                 |                                       |                                 |                              |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |                                 |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |   |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |                              |        |      |        |      |      |   |      |       |      |      |   |            |       |      |      |    |                                 |        |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |   |        |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |
| <b>Recovery</b>  | <table><tr><th>Analyte (Transition)</th><th>Matrix</th><th>Fortification level (mg/kg)</th><th>Mean recovery Percentage (%)</th><th>Standard deviation (SD) (%)</th><th>Relative standard deviation (RSD) (%)</th><th>Number of fortified samples (n)</th></tr><tr><td rowspan="3">Azoxystrobin (404.2 &gt; 372.1)</td><td rowspan="3">Honey</td><td>0.01</td><td>105.1%</td><td>2.5%</td><td>2.3%</td><td>5</td></tr><tr><td>0.10</td><td>101.6%</td><td>1.4%</td><td>1.4%</td><td>5</td></tr><tr><td>All levels</td><td>103.4%</td><td>2.7%</td><td>2.6%</td><td>10</td></tr><tr><td rowspan="3">Prothioconazole (342.1 &gt; 306.0)</td><td rowspan="3">Honey</td><td>0.01</td><td>107.0%</td><td>3.8%</td><td>3.5%</td><td>5</td></tr><tr><td>0.10</td><td>101.7%</td><td>2.3%</td><td>2.3%</td><td>5</td></tr><tr><td>All levels</td><td>104.4%</td><td>4.1%</td><td>3.9%</td><td>10</td></tr><tr><td rowspan="3">Prothioconazole-desethio (312.0 &gt; 89.0)</td><td rowspan="3">Honey</td><td>0.01</td><td>107.8%</td><td>2.1%</td><td>2.0%</td><td>5</td></tr><tr><td>0.10</td><td>108.9%</td><td>1.9%</td><td>1.8%</td><td>5</td></tr><tr><td>All levels</td><td>108.4%</td><td>2.0%</td><td>1.9%</td><td>10</td></tr><tr><td rowspan="3">Azoxystrobin (404.2 &gt; 372.1)</td><td rowspan="3">Pollen</td><td>0.01</td><td>100.5%</td><td>1.7%</td><td>1.7%</td><td>5</td></tr><tr><td>0.10</td><td>98.0%</td><td>2.0%</td><td>2.1%</td><td>5</td></tr><tr><td>All levels</td><td>99.3%</td><td>2.2%</td><td>2.2%</td><td>10</td></tr><tr><td rowspan="3">Prothioconazole (342.1 &gt; 306.0)</td><td rowspan="3">Pollen</td><td>0.01</td><td>97.7%</td><td>7.9%</td><td>8.1%</td><td>5</td></tr><tr><td>0.10</td><td>93.4%</td><td>2.3%</td><td>2.5%</td><td>5</td></tr><tr><td>All levels</td><td>95.6%</td><td>5.9%</td><td>6.2%</td><td>10</td></tr><tr><td rowspan="3">Prothioconazole-desethio (312.0 &gt; 89.0)</td><td rowspan="3">Pollen</td><td>0.01</td><td>99.3%</td><td>1.9%</td><td>2.0%</td><td>5</td></tr><tr><td>0.10</td><td>97.2%</td><td>0.8%</td><td>0.8%</td><td>5</td></tr><tr><td>All levels</td><td>98.3%</td><td>1.8%</td><td>1.8%</td><td>10</td></tr></table> | Analyte (Transition)        | Matrix                       | Fortification level (mg/kg) | Mean recovery Percentage (%)          | Standard deviation (SD) (%)     | Relative standard deviation (RSD) (%) | Number of fortified samples (n) | Azoxystrobin (404.2 > 372.1) | Honey | 0.01 | 105.1% | 2.5% | 2.3% | 5 | 0.10 | 101.6% | 1.4% | 1.4% | 5 | All levels | 103.4% | 2.7% | 2.6% | 10 | Prothioconazole (342.1 > 306.0) | Honey | 0.01 | 107.0% | 3.8% | 3.5% | 5 | 0.10 | 101.7% | 2.3% | 2.3% | 5 | All levels | 104.4% | 4.1% | 3.9% | 10 | Prothioconazole-desethio (312.0 > 89.0) | Honey | 0.01 | 107.8% | 2.1% | 2.0% | 5 | 0.10 | 108.9% | 1.9% | 1.8% | 5 | All levels | 108.4% | 2.0% | 1.9% | 10 | Azoxystrobin (404.2 > 372.1) | Pollen | 0.01 | 100.5% | 1.7% | 1.7% | 5 | 0.10 | 98.0% | 2.0% | 2.1% | 5 | All levels | 99.3% | 2.2% | 2.2% | 10 | Prothioconazole (342.1 > 306.0) | Pollen | 0.01 | 97.7% | 7.9% | 8.1% | 5 | 0.10 | 93.4% | 2.3% | 2.5% | 5 | All levels | 95.6% | 5.9% | 6.2% | 10 | Prothioconazole-desethio (312.0 > 89.0) | Pollen | 0.01 | 99.3% | 1.9% | 2.0% | 5 | 0.10 | 97.2% | 0.8% | 0.8% | 5 | All levels | 98.3% | 1.8% | 1.8% | 10 |
| Analyte (Transition)   | Matrix   | Fortification level (mg/kg) | Mean recovery Percentage (%) | Standard deviation (SD) (%) | Relative standard deviation (RSD) (%) | Number of fortified samples (n) |                                       |                                 |                              |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |                                 |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |   |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |                              |        |      |        |      |      |   |      |       |      |      |   |            |       |      |      |    |                                 |        |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |   |        |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |
| Azoxystrobin (404.2 > 372.1)   | Honey  | 0.01                        | 105.1%                       | 2.5%                        | 2.3%                                  | 5                               |                                       |                                 |                              |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |                                 |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |   |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |                              |        |      |        |      |      |   |      |       |      |      |   |            |       |      |      |    |                                 |        |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |   |        |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |
|  |  | 0.10                        | 101.6%                       | 1.4%                        | 1.4%                                  | 5                               |                                       |                                 |                              |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |                                 |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |   |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |                              |        |      |        |      |      |   |      |       |      |      |   |            |       |      |      |    |                                 |        |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |   |        |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |
|  |  | All levels                  | 103.4%                       | 2.7%                        | 2.6%                                  | 10                              |                                       |                                 |                              |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |                                 |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |   |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |                              |        |      |        |      |      |   |      |       |      |      |   |            |       |      |      |    |                                 |        |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |   |        |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |
| Prothioconazole (342.1 > 306.0)  | Honey  | 0.01                        | 107.0%                       | 3.8%                        | 3.5%                                  | 5                               |                                       |                                 |                              |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |                                 |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |   |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |                              |        |      |        |      |      |   |      |       |      |      |   |            |       |      |      |    |                                 |        |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |   |        |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |
|  |  | 0.10                        | 101.7%                       | 2.3%                        | 2.3%                                  | 5                               |                                       |                                 |                              |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |                                 |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |   |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |                              |        |      |        |      |      |   |      |       |      |      |   |            |       |      |      |    |                                 |        |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |   |        |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |
|  |  | All levels                  | 104.4%                       | 4.1%                        | 3.9%                                  | 10                              |                                       |                                 |                              |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |                                 |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |   |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |                              |        |      |        |      |      |   |      |       |      |      |   |            |       |      |      |    |                                 |        |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |   |        |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |
| Prothioconazole-desethio (312.0 > 89.0)                                | Honey  | 0.01                        | 107.8%                       | 2.1%                        | 2.0%                                  | 5                               |                                       |                                 |                              |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |                                 |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |   |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |                              |        |      |        |      |      |   |      |       |      |      |   |            |       |      |      |    |                                 |        |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |   |        |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |
|  |  | 0.10                        | 108.9%                       | 1.9%                        | 1.8%                                  | 5                               |                                       |                                 |                              |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |                                 |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |   |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |                              |        |      |        |      |      |   |      |       |      |      |   |            |       |      |      |    |                                 |        |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |   |        |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |
|  |  | All levels                  | 108.4%                       | 2.0%                        | 1.9%                                  | 10                              |                                       |                                 |                              |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |                                 |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |   |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |                              |        |      |        |      |      |   |      |       |      |      |   |            |       |      |      |    |                                 |        |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |   |        |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |
| Azoxystrobin (404.2 > 372.1)   | Pollen   | 0.01                        | 100.5%                       | 1.7%                        | 1.7%                                  | 5                               |                                       |                                 |                              |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |                                 |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |   |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |                              |        |      |        |      |      |   |      |       |      |      |   |            |       |      |      |    |                                 |        |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |   |        |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |
|  |  | 0.10                        | 98.0%                        | 2.0%                        | 2.1%                                  | 5                               |                                       |                                 |                              |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |                                 |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |   |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |                              |        |      |        |      |      |   |      |       |      |      |   |            |       |      |      |    |                                 |        |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |   |        |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |
|  |  | All levels                  | 99.3%                        | 2.2%                        | 2.2%                                  | 10                              |                                       |                                 |                              |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |                                 |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |   |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |                              |        |      |        |      |      |   |      |       |      |      |   |            |       |      |      |    |                                 |        |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |   |        |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |
| Prothioconazole (342.1 > 306.0)  | Pollen   | 0.01                        | 97.7%                        | 7.9%                        | 8.1%                                  | 5                               |                                       |                                 |                              |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |                                 |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |   |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |                              |        |      |        |      |      |   |      |       |      |      |   |            |       |      |      |    |                                 |        |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |   |        |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |
|  |  | 0.10                        | 93.4%                        | 2.3%                        | 2.5%                                  | 5                               |                                       |                                 |                              |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |                                 |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |   |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |                              |        |      |        |      |      |   |      |       |      |      |   |            |       |      |      |    |                                 |        |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |   |        |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |
|  |  | All levels                  | 95.6%                        | 5.9%                        | 6.2%                                  | 10                              |                                       |                                 |                              |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |                                 |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |   |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |                              |        |      |        |      |      |   |      |       |      |      |   |            |       |      |      |    |                                 |        |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |   |        |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |
| Prothioconazole-desethio (312.0 > 89.0)                                | Pollen   | 0.01                        | 99.3%                        | 1.9%                        | 2.0%                                  | 5                               |                                       |                                 |                              |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |                                 |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |   |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |                              |        |      |        |      |      |   |      |       |      |      |   |            |       |      |      |    |                                 |        |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |   |        |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |
|  |  | 0.10                        | 97.2%                        | 0.8%                        | 0.8%                                  | 5                               |                                       |                                 |                              |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |                                 |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |   |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |                              |        |      |        |      |      |   |      |       |      |      |   |            |       |      |      |    |                                 |        |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |   |        |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |
|  |  | All levels                  | 98.3%                        | 1.8%                        | 1.8%                                  | 10                              |                                       |                                 |                              |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |                                 |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |   |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |                              |        |      |        |      |      |   |      |       |      |      |   |            |       |      |      |    |                                 |        |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |   |        |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |

|                                     | <b>Residues</b>  |                  |                  |                                |
|-------------------------------------|--|------------------|------------------|--------------------------------|
| <b>Accuracy</b>                     | <b>Azoxystrobin</b><br>(Transition 404.2 > 372.1)  |                  |                  |                                |
|                                     | Matrix   | Honey            |                  | Pollen                         |
|                                     | Fortification level (mg/kg)  | 0.01             | 0.10             | 0.01 0.10                      |
|                                     | Single recovery rates  | 102.3% to 108.7% | 100.3% to 103.5% | 98.2% to 102.3% 94.5% to 99.5% |
|                                     | Mean recoveries per fortification level  | 105.1%           | 101.6%           | 100.5% 98.0%                   |
|                                     | <b>Prothioconazole</b><br>(Transition 342.1 > 306.0)   |                  |                  |                                |
|                                     | Matrix   | Honey            |                  | Pollen                         |
|                                     | Fortification level (mg/kg)  | 0.01             | 0.10             | 0.01 0.10                      |
|                                     | Single recovery rates  | 103.8% to 113.3% | 98.3% to 104.5%  | 87.5% to 108.4% 89.7% to 95.4% |
|                                     | Mean recoveries per fortification level  | 107.0%           | 101.7%           | 97.7% 93.4%                    |
|                                     | <b>Prothioconazole-desthio</b><br>(Transition 312.0 > 89.0)  |                  |                  |                                |
|                                     | Matrix   | Honey            |                  | Pollen                         |
|                                     | Fortification level (mg/kg)  | 0.01             | 0.10             | 0.01 0.10                      |
|                                     | Single recovery rates  | 104.8% to 110.8% | 106.3% to 110.9% | 96.5% to 101.8% 96.5% to 98.5% |
|                                     | Mean recoveries per fortification level  | 107.8%           | 108.9%           | 99.3% 97.2%                    |
|                                     | The accuracy of the method fulfils the requirements for residue analytical methods which demand that the mean recoveries per fortification level should be in the range 70-110% according to SANCO/3029/99 rev.4.  |                  |                  |                                |
| <b>Precision and repeat-ability</b> | <b>Azoxystrobin</b><br>(Transition 404.2 > 372.1)  |                  |                  |                                |
|                                     | Matrix   | Honey            |                  | Pollen                         |
|                                     | Fortification level (mg/kg)  | 0.01             | 0.10             | 0.01 0.10                      |
|                                     | RSD for each fortification level   | 2.3%             | 1.4%             | 1.7% 2.1%                      |
|                                     | <b>Prothioconazole</b><br>(Transition 342.1 > 306.0)   |                  |                  |                                |
|                                     | Matrix   | Honey            |                  | Pollen                         |
|                                     | Fortification level (mg/kg)  | 0.01             | 0.10             | 0.01 0.10                      |
|                                     | RSD for each fortification level   | 3.5%             | 2.3%             | 8.1% 2.5%                      |
|                                     | <b>Prothioconazole-desthio</b><br>(Transition 312.0 > 89.0)  |                  |                  |                                |
|                                     | Matrix   | Honey            |                  | Pollen                         |
|                                     | Fortification level (mg/kg)  | 0.01             | 0.10             | 0.01 0.10                      |
|                                     | RSD for each fortification level   | 2.0%             | 1.8%             | 2.0% 0.8%                      |
|                                     | All RSD determined were less than 20%, the method therefore fulfils the requirements of residue analytical methods according to SANCO/3029/99 rev.4.   |                  |                  |                                |
|                                     |  |                  |                  |                                |
|                                     |  |                  |                  |                                |
| <b>Specificity</b>                  | The method is able to determine azoxystrobin, prothioconazole and prothioconazole-desthio in presence of honey and pollen. This was checked by analysing control and spiked specimens to verify the absence of interfering peaks. No interfering peaks were present at > 30% of the LOQ. The analyses were carried out by LC-MS/MS, monitoring two transitions for each analyte. The method was considered highly specific, thus the use of an alternative method was not necessary. |                  |                  |                                |

|   | Residues   |                             |                              |                             |                                       |                                 |                                       |                                 |  |       |      |        |      |      |   |        |      |       |      |      |   |   |       |      |        |      |      |   |        |      |       |      |      |   |   |       |      |        |      |      |   |        |      |        |      |      |   |
|---|--|-----------------------------|------------------------------|-----------------------------|---------------------------------------|---------------------------------|---------------------------------------|---------------------------------|--|-------|------|--------|------|------|---|--------|------|-------|------|------|---|---|-------|------|--------|------|------|---|--------|------|-------|------|------|---|---|-------|------|--------|------|------|---|--------|------|--------|------|------|---|
| Recoveries  | <table><tr><th>Analyte</th><th>Matrix</th><th>Fortification level (mg/kg)</th><th>Mean recovery Percentage (%)</th><th>Standard deviation (SD) (%)</th><th>Relative standard deviation (RSD) (%)</th><th>Number of fortified samples (n)</th></tr><tr><td rowspan="2">Azoxystrobin<br/>(Transition 404.2 &gt; 329.0)</td><td>Honey</td><td>0.01</td><td>105.0%</td><td>2.3%</td><td>2.2%</td><td>5</td></tr><tr><td>Pollen</td><td>0.01</td><td>99.4%</td><td>2.3%</td><td>2.3%</td><td>5</td></tr><tr><td rowspan="2">Prothioconazole<br/>(Transition 342.1 &gt; 179.9)</td><td>Honey</td><td>0.01</td><td>109.9%</td><td>4.0%</td><td>3.6%</td><td>5</td></tr><tr><td>Pollen</td><td>0.01</td><td>97.0%</td><td>5.8%</td><td>6.0%</td><td>5</td></tr><tr><td rowspan="2">Prothioconazole-desthio<br/>(Transition 312.0 &gt; 124.9)</td><td>Honey</td><td>0.01</td><td>109.9%</td><td>2.1%</td><td>1.9%</td><td>5</td></tr><tr><td>Pollen</td><td>0.01</td><td>101.0%</td><td>1.8%</td><td>1.8%</td><td>5</td></tr></table> <p>Recoveries and precision data for the qualifier transition comply with the requirements of residue analytical methods as mean recoveries at the LOQ level are within the range 70-110% and RSD is less than 20%.</p> | Analyte                     | Matrix                       | Fortification level (mg/kg) | Mean recovery Percentage (%)          | Standard deviation (SD) (%)     | Relative standard deviation (RSD) (%) | Number of fortified samples (n) | Azoxystrobin<br>(Transition 404.2 > 329.0) | Honey | 0.01 | 105.0% | 2.3% | 2.2% | 5 | Pollen | 0.01 | 99.4% | 2.3% | 2.3% | 5 | Prothioconazole<br>(Transition 342.1 > 179.9) | Honey | 0.01 | 109.9% | 4.0% | 3.6% | 5 | Pollen | 0.01 | 97.0% | 5.8% | 6.0% | 5 | Prothioconazole-desthio<br>(Transition 312.0 > 124.9) | Honey | 0.01 | 109.9% | 2.1% | 1.9% | 5 | Pollen | 0.01 | 101.0% | 1.8% | 1.8% | 5 |
| Analyte   | Matrix   | Fortification level (mg/kg) | Mean recovery Percentage (%) | Standard deviation (SD) (%) | Relative standard deviation (RSD) (%) | Number of fortified samples (n) |                                       |                                 |  |       |      |        |      |      |   |        |      |       |      |      |   |   |       |      |        |      |      |   |        |      |       |      |      |   |   |       |      |        |      |      |   |        |      |        |      |      |   |
| Azoxystrobin<br>(Transition 404.2 > 329.0)            | Honey  | 0.01                        | 105.0%                       | 2.3%                        | 2.2%                                  | 5                               |                                       |                                 |  |       |      |        |      |      |   |        |      |       |      |      |   |   |       |      |        |      |      |   |        |      |       |      |      |   |   |       |      |        |      |      |   |        |      |        |      |      |   |
|   | Pollen   | 0.01                        | 99.4%                        | 2.3%                        | 2.3%                                  | 5                               |                                       |                                 |  |       |      |        |      |      |   |        |      |       |      |      |   |   |       |      |        |      |      |   |        |      |       |      |      |   |   |       |      |        |      |      |   |        |      |        |      |      |   |
| Prothioconazole<br>(Transition 342.1 > 179.9)         | Honey  | 0.01                        | 109.9%                       | 4.0%                        | 3.6%                                  | 5                               |                                       |                                 |  |       |      |        |      |      |   |        |      |       |      |      |   |   |       |      |        |      |      |   |        |      |       |      |      |   |   |       |      |        |      |      |   |        |      |        |      |      |   |
|   | Pollen   | 0.01                        | 97.0%                        | 5.8%                        | 6.0%                                  | 5                               |                                       |                                 |  |       |      |        |      |      |   |        |      |       |      |      |   |   |       |      |        |      |      |   |        |      |       |      |      |   |   |       |      |        |      |      |   |        |      |        |      |      |   |
| Prothioconazole-desthio<br>(Transition 312.0 > 124.9) | Honey  | 0.01                        | 109.9%                       | 2.1%                        | 1.9%                                  | 5                               |                                       |                                 |  |       |      |        |      |      |   |        |      |       |      |      |   |   |       |      |        |      |      |   |        |      |       |      |      |   |   |       |      |        |      |      |   |        |      |        |      |      |   |
|   | Pollen   | 0.01                        | 101.0%                       | 1.8%                        | 1.8%                                  | 5                               |                                       |                                 |  |       |      |        |      |      |   |        |      |       |      |      |   |   |       |      |        |      |      |   |        |      |       |      |      |   |   |       |      |        |      |      |   |        |      |        |      |      |   |
| LOQ   | <p>The limit of quantification (LOQ) is the lowest validated level where a mean recovery within the range 70-110% with a RSD less than 20% could be obtained.</p> <p>The LOQ was set at 0.01 mg/kg in honey and pollen for each analyte.</p>   |                             |                              |                             |                                       |                                 |                                       |                                 |  |       |      |        |      |      |   |        |      |       |      |      |   |   |       |      |        |      |      |   |        |      |       |      |      |   |   |       |      |        |      |      |   |        |      |        |      |      |   |
| Comment   | <p>Stability results for extracts</p> <p>The stability of extracts during frozen storage (<math>\leq -18^{\circ}\text{C}</math>) was investigated. The results indicate a good stability for at least 7 days for honey and 14 days for pollen for all analytes</p> <p>Stability results for matrix matched standard solutions</p> <p>The stability of matrix matched standard solutions during frozen storage (<math>\leq -18^{\circ}\text{C}</math>) was investigated.</p> <p>The results</p>   |                             |                              |                             |                                       |                                 |                                       |                                 |  |       |      |        |      |      |   |        |      |       |      |      |   |   |       |      |        |      |      |   |        |      |       |      |      |   |   |       |      |        |      |      |   |        |      |        |      |      |   |

## 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.

### 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 5.3-3: Relevant residue definitions for monitoring/enforcement and levels for which compliance is required**

| Matrix  | Residue definition                          | MRL / limit | Reference for MRL/level<br>Remarks |
|---|---|-------------|------------------------------------|
| Plant, high water content                                 | Prothioconazole,<br>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<br>Heinemann, 2001 |
| Plant, high oil content                                   |   | 0.01 mg/kg  | Heinemann, 2000                    |
| Muscle  | Prothioconazole-desthio                     | 0.01 mg/kg  | xxxxxxxxxxxxxxxxxxxx               |
| Milk  | Prothioconazole-3-hydroxy-desthio           | 0.004 mg/kg | xxxxxxxxxxxxxxxxxxxx               |
| Liver, kidney   | Prothioconazole-4-hydroxy-desthio           | 0.01 mg/kg  | xxxxxxxxxxxxxxxxxxxx               |
| Soil<br>(Ecotoxicology)                                   | Prothioconazole<br>Prothioconazole-desthio  | 0.006 mg/kg | Schramel 2000                      |

| Matrix                               | Residue definition   | MRL / limit              | Reference for MRL/level<br>Remarks     |
|--------------------------------------|--|--------------------------|--|
|                                      | Prothioconazole-3-hydroxy-<br>desthio<br>Prothioconazole-4-hydroxy-<br>desthio |                          |  |
| Drinking water<br>(Human toxicology) | Prothioconazole  | 0.1 µg/L                 | general limit for drinking<br>water    |
| Surface water<br>(Ecotoxicology)     | Prothioconazole-desthio  | 3.34 µg/L                |  |
| Air                                  | Prothioconazole  | 0.015 µg/m <sup>3</sup>  | AOEL sys/AOEL inhal:<br>xxx mg/kg bw/d |
|                                      | Prothioconazole-desthio  | 0.0006 µg/m <sup>3</sup> |  |
| Tissue (meat or liver)               | Prothioconazole-desthio  | 0.01 mg/kg               | notclassified as T / T+                |
| Body fluids                          |  | not required             | notclassified 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 5.3-4: 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<br>(i.e. GC-MS or<br>HPLC-UV) | Author(s), year / missing / EU<br>agreed |
| High oil content                                | Primary                       | 0.01 mg/kg   | HPLC-MS/MS  | Heinemann, 2000a                         |
|   | ILV                           | 0.01 mg/kg   | GC-MS   | Heinemann, 2001                          |
|   | Confirmatory<br>(if required) |              |   |  |
| High<br>protein/high<br>starch content<br>(dry) | Primary                       | 0.01 mg/kg   | HPLC-MS/MS  | Heinemann, 2000a                         |
|   | ILV                           | 0.01 mg/kg   | HPLC-MS/MS  | Heinemann, 2000b                         |
|   | Confirmatory<br>(if required) | Not required |   |  |

**Table 5.3-5: 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<br>(i.e. GC-MS or<br>HPLC-UV) | Author(s), year / missing / EU<br>agreed |
| High water<br>content                                   | Primary     | 0.02 mg/kg | GC/MS   | Weeren, Pelz 2000                        |
|   | ILV         | 0.02 mg/kg | GC/MS   | Class, 2001                              |

| Component of residue definition: Prothiconazole-desthio |                               |              |  |                                       |
|---|-------------------------------|--------------|--|---------------------------------------|
| Matrix type   | Method type                   | Method LOQ   | Principle of method<br>(i.e. GC-MS or HPLC-UV) | Author(s), year / missing / EU agreed |
|   | Confirmatory<br>(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<br>(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<br>(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<br>(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 5.3-6: 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 5.3-7: 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<br>(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<br>(if required) | Not required |  |                           |
| Muscle   | Primary                       | 0.01 mg/kg   | HPLC-MS/MS                                     | xxxxxxxxxxxxxxxxxxxx      |
|  | ILV                           | 0.01 mg/kg   | HPLC-MS/MS                                     | xxxxxxxxxxxxxxxxxxxx      |
|  | Confirmatory<br>(if required) | Not required |  |                           |

| Component of residue definition: Prothioconazole-desthio |                               |              |  |                           |
|--|-------------------------------|--------------|--|---------------------------|
| Matrix type  | Method type                   | Method LOQ   | Principle of method<br>(i.e. GC-MS or HPLC-UV) | Author(s), year / missing |
| Kidney, liver  | Primary                       | 0.01 mg/kg   | HPLC-MS/MS                                     | XXXXXXXXXXXXXXXXXXXX      |
|  | ILV                           | 0.01 mg/kg   | HPLC-MS/MS                                     | XXXXXXXXXXXXXXXXXXXX      |
|  | Confirmatory<br>(if required) | Not required |  |                           |

**Table 5.3-8: 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<br>(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<br>(if required) | Not required |  |                           |
| Muscle   | Primary                       | 0.01 mg/kg   | HPLC-MS/MS                                     | XXXXXXXXXXXXXXXXXXXX      |
|  | ILV                           | 0.01 mg/kg   | HPLC-MS/MS                                     | XXXXXXXXXXXXXXXXXXXX      |
|  | Confirmatory<br>(if required) | Not required |  |                           |
| Kidney, liver  | Primary                       | 0.01 mg/kg   | HPLC-MS/MS                                     | XXXXXXXXXXXXXXXXXXXX      |
|  | ILV                           | 0.01 mg/kg   | HPLC-MS/MS                                     | XXXXXXXXXXXXXXXXXXXX      |
|  | Confirmatory<br>(if required) | Not required |  |                           |

**Table 5.3-9: 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<br>(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<br>(if required) | Not required |  |                           |
| Muscle   | Primary                       | 0.01 mg/kg   | HPLC-MS/MS                                     | XXXXXXXXXXXXXXXXXXXX      |
|  | ILV                           | 0.01 mg/kg   | HPLC-MS/MS                                     | XXXXXXXXXXXXXXXXXXXX      |
|  | Confirmatory<br>(if required) | Not required |  |                           |
| Kidney, liver  | Primary                       | 0.01 mg/kg   | HPLC-MS/MS                                     | XXXXXXXXXXXXXXXXXXXX      |
|  | ILV                           | 0.01 mg/kg   | HPLC-MS/MS                                     | XXXXXXXXXXXXXXXXXXXX      |

| Component of residue definition: Prothioconazole-4-hydroxy-desthio |                               |              |  |                           |
|--|-------------------------------|--------------|--|---------------------------|
| Matrix type  | Method type                   | Method LOQ   | Principle of method<br>(i.e. GC-MS or HPLC-UV) | Author(s), year / missing |
|  | Confirmatory<br>(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 5.3-10: 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 5.3-11: Validated methods for soil (if appropriate)**

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

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

| Component of residue definition: Prothioconazole-desthio |              |  |                           |
|--|--------------|--|---------------------------|
| Method type  | Method LOQ   | Principle of method<br>(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 5.3-13: Validated methods for soil (if appropriate)**

| Component of residue definition: Prothioconazole-3-hydroxy-desthio |              |  |                           |
|--|--------------|--|---------------------------|
| Method type  | Method LOQ   | Principle of method<br>(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 additional studies it is referred to Appendix 2.

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

| Component of residue definition: Prothioconazole |              |            |  |                           |
|--|--------------|------------|--|---------------------------|
| Matrix type                                      | Method type  | Method LOQ | Principle of method<br>(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 5.3-15: Validated methods for water (if appropriate)**

| Component of residue definition: Prothioconazole-desthio |              |            |  |                           |
|--|--------------|------------|--|---------------------------|
| Matrix type  | Method type  | Method LOQ | Principle of method<br>(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 5.3-16: Validated methods for air (if appropriate)**

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

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

| Component of residue definition: Prothiconazole-desthio |                          |  |                           |
|---|--------------------------|--|---------------------------|
| Method type   | Method LOQ               | Principle of method<br>(i.e. GC-MS or HPLC-UV) | Author(s), year / missing |
| Primary   | 0.0006 µg/m <sup>3</sup> | 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 5.3-18: Methods for body fluids and tissues (if appropriate)**

| Component of residue definition: Prothioconazole-desthio |              |  |                           |
|--|--------------|--|---------------------------|
| Method type  | Method LOQ   | Principle of method<br>(i.e. GC-MS or HPLC-UV) | Author(s), year / missing |
| Primary  | 0.01 mg/kg   | HPLC-MS/MS                                     | XXXXXXXXXXXXXXXXXXXX      |
| 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

|                   |                        |
|-------------------|------------------------|
| Comments of zRMS: | The study is accepted. |
|-------------------|------------------------|

|                |  |
|----------------|--|
| Reference:     | KCP 5.2/03   |
| Report         | Validation of the Analytical Method for the Analysis of Azoxystrobin, Prothioconazole and Prothioconazole-desthio in Honey and Pollen, P. Schlewitz, Study Code: C0240 |
| Guideline(s):  | Regulation (EC) No. 1107/2009<br>SANCO/3029/99 rev.4<br>SANCO/825/00 rev.8.1<br>ENV/JM/MONO(2007)17  |
| Deviations:    | NO   |
| GLP:           | YES  |
| Acceptability: | YES  |

## Materials and methods

The method under discussion describes the determination of residues of azoxystrobin, prothioconazole and prothioconazole-desthio in honey and pollen. The method was validated at 0.01 mg/kg in honey and pollen for azoxystrobin (quantifier transition 404.2 > 372.1 and qualifier transition 404.2 > 329.0), prothioconazole (quantifier transition 342.1 > 306.0 and qualifier transition 342.1 > 179.9) and prothioconazole-desthio (quantifier transition 312.0 > 89.0 and qualifier transition 312.0 > 124.9)..

## Validation - Results and discussions

**Table 5.3-19: Methods suitable for the determination of the residues in plant protection product (PPP) CHR/F/PROTAZO**

|   | Residues   |        |                             |                              |                             |                                       |                                 |
|---|--|--------|-----------------------------|------------------------------|-----------------------------|---------------------------------------|---------------------------------|
| Author(s), year   | P. Schlewitz   |        |                             |                              |                             |                                       |                                 |
| Principle of method   | LC MS/MS   |        |                             |                              |                             |                                       |                                 |
| Linearity (linear between mg/L) (correlation coefficient, expressed as r) | The linearity of the method was studied between $\approx 0.7$ ng/mL and $\approx 30$ ng/mL of azoxystrobin, prothioconazole and prothioconazole-desthio in matrix-matched calibration solutions (corresponding to $\approx 0.003$ to $\approx 0.12$ mg/kg). The linear correlation coefficients were $> 0.990$ , showing a good linearity. |        |                             |                              |                             |                                       |                                 |
| Recovery  | Analyte (Transition)   | Matrix | Fortification level (mg/kg) | Mean recovery Percentage (%) | Standard deviation (SD) (%) | Relative standard deviation (RSD) (%) | Number of fortified samples (n) |
|   | Azoxystrobin (404.2 > 372.1)   | Honey  | 0.01                        | 105.1%                       | 2.5%                        | 2.3%                                  | 5                               |
|   |  |        | 0.10                        | 101.6%                       | 1.4%                        | 1.4%                                  | 5                               |
|   |  |        | All levels                  | 103.4%                       | 2.7%                        | 2.6%                                  | 10                              |
|   | Prothioconazole (342.1 > 306.0)  | Honey  | 0.01                        | 107.0%                       | 3.8%                        | 3.5%                                  | 5                               |
|   |  |        | 0.10                        | 101.7%                       | 2.3%                        | 2.3%                                  | 5                               |
|   |  |        | All levels                  | 104.4%                       | 4.1%                        | 3.9%                                  | 10                              |
|   | Prothioconazole-desthio (312.0 > 89.0)   | Honey  | 0.01                        | 107.8%                       | 2.1%                        | 2.0%                                  | 5                               |
|   |  |        | 0.10                        | 108.9%                       | 1.9%                        | 1.8%                                  | 5                               |
|   |  |        | All levels                  | 108.4%                       | 2.0%                        | 1.9%                                  | 10                              |
|   | Azoxystrobin (404.2 > 372.1)   | Pollen | 0.01                        | 100.5%                       | 1.7%                        | 1.7%                                  | 5                               |
|   |  |        | 0.10                        | 98.0%                        | 2.0%                        | 2.1%                                  | 5                               |
|   |  |        | All levels                  | 99.3%                        | 2.2%                        | 2.2%                                  | 10                              |
|   | Prothioconazole (342.1 > 306.0)  | Pollen | 0.01                        | 97.7%                        | 7.9%                        | 8.1%                                  | 5                               |
|   |  |        | 0.10                        | 93.4%                        | 2.3%                        | 2.5%                                  | 5                               |
|   |  |        | All levels                  | 95.6%                        | 5.9%                        | 6.2%                                  | 10                              |
|   | Prothioconazole-desthio (312.0 > 89.0)   | Pollen | 0.01                        | 99.3%                        | 1.9%                        | 2.0%                                  | 5                               |
|   |  |        | 0.10                        | 97.2%                        | 0.8%                        | 0.8%                                  | 5                               |
|   |  |        | All levels                  | 98.3%                        | 1.8%                        | 1.8%                                  | 10                              |

|   |  |  |                  |                 |                |
|---|--|--|------------------|-----------------|----------------|
|   | <b>Residues</b>  |  |                  |                 |                |
| <b>Accuracy</b>   | <b>Azoxystrobin</b><br>(Transition 404.2 > 372.1)  |  |                  |                 |                |
|   | Matrix   | Honey  |                  | Pollen          |                |
|   | Fortification level (mg/kg)  | 0.01   | 0.10             | 0.01            | 0.10           |
|   | Single recovery rates  | 102.3% to 108.7%   | 100.3% to 103.5% | 98.2% to 102.3% | 94.5% to 99.5% |
|   | Mean recoveries per fortification level  | 105.1%   | 101.6%           | 100.5%          | 98.0%          |
|   | <b>Prothioconazole</b><br>(Transition 342.1 > 306.0)   |  |                  |                 |                |
|   | Matrix   | Honey  |                  | Pollen          |                |
|   | Fortification level (mg/kg)  | 0.01   | 0.10             | 0.01            | 0.10           |
|   | Single recovery rates  | 103.8% to 113.3%   | 98.3% to 104.5%  | 87.5% to 108.4% | 89.7% to 95.4% |
|   | Mean recoveries per fortification level  | 107.0%   | 101.7%           | 97.7%           | 93.4%          |
|   | <b>Prothioconazole-desthio</b><br>(Transition 312.0 > 89.0)  |  |                  |                 |                |
|   | Matrix   | Honey  |                  | Pollen          |                |
|   | Fortification level (mg/kg)  | 0.01   | 0.10             | 0.01            | 0.10           |
|   | Single recovery rates  | 104.8% to 110.8%   | 106.3% to 110.9% | 96.5% to 101.8% | 96.5% to 98.5% |
|   | Mean recoveries per fortification level  | 107.8%   | 108.9%           | 99.3%           | 97.2%          |
| The accuracy of the method fulfils the requirements for residue analytical methods which demand that the mean recoveries per fortification level should be in the range 70-110% according to SANCO/3029/99 rev.4. |  |  |                  |                 |                |
| <b>Precision and repeat-ability</b>   | <b>Azoxystrobin</b><br>(Transition 404.2 > 372.1)  |  |                  |                 |                |
|   | Matrix   | Honey  |                  | Pollen          |                |
|   | Fortification level (mg/kg)  | 0.01   | 0.10             | 0.01            | 0.10           |
|   | RSD for each fortification level   | 2.3%   | 1.4%             | 1.7%            | 2.1%           |
|   | <b>Prothioconazole</b><br>(Transition 342.1 > 306.0)   |  |                  |                 |                |
|   | Matrix   | Honey  |                  | Pollen          |                |
|   | Fortification level (mg/kg)  | 0.01   | 0.10             | 0.01            | 0.10           |
|   | RSD for each fortification level   | 3.5%   | 2.3%             | 8.1%            | 2.5%           |
|   | <b>Prothioconazole-desthio</b><br>(Transition 312.0 > 89.0)  |  |                  |                 |                |
|   | Matrix   | Honey  |                  | Pollen          |                |
|   | Fortification level (mg/kg)  | 0.01   | 0.10             | 0.01            | 0.10           |
|   | RSD for each fortification level   | 2.0%   | 1.8%             | 2.0%            | 0.8%           |
|   | All RSD determined were less than 20%, the method therefore fulfils the requirements of residue analytical methods according to SANCO/3029/99 rev.4. |  |                  |                 |                |
|   | <b>Specificity</b>   | The method is able to determine azoxystrobin, prothioconazole and prothioconazole-desthio in presence of honey and pollen. This was checked by analysing control and spiked specimens to verify the absence of interfering peaks. No interfering peaks were present at > 30% of the LOQ. The analyses were carried out by LC-MS/MS, monitoring two transitions for each analyte. The method was considered highly specific, thus the use of an alternative method was not necessary. |                  |                 |                |

|   | Residues   |                             |                              |                             |                                       |                                 |                                       |                                 |  |       |      |        |      |      |   |        |      |       |      |      |   |   |       |      |        |      |      |   |        |      |       |      |      |   |   |       |      |        |      |      |   |        |      |        |      |      |   |
|---|--|-----------------------------|------------------------------|-----------------------------|---------------------------------------|---------------------------------|---------------------------------------|---------------------------------|--|-------|------|--------|------|------|---|--------|------|-------|------|------|---|---|-------|------|--------|------|------|---|--------|------|-------|------|------|---|---|-------|------|--------|------|------|---|--------|------|--------|------|------|---|
| Recoveries  | <table><tr><th>Analyte</th><th>Matrix</th><th>Fortification level (mg/kg)</th><th>Mean recovery Percentage (%)</th><th>Standard deviation (SD) (%)</th><th>Relative standard deviation (RSD) (%)</th><th>Number of fortified samples (n)</th></tr><tr><td rowspan="2">Azoxystrobin<br/>(Transition 404.2 &gt; 329.0)</td><td>Honey</td><td>0.01</td><td>105.0%</td><td>2.3%</td><td>2.2%</td><td>5</td></tr><tr><td>Pollen</td><td>0.01</td><td>99.4%</td><td>2.3%</td><td>2.3%</td><td>5</td></tr><tr><td rowspan="2">Prothioconazole<br/>(Transition 342.1 &gt; 179.9)</td><td>Honey</td><td>0.01</td><td>109.9%</td><td>4.0%</td><td>3.6%</td><td>5</td></tr><tr><td>Pollen</td><td>0.01</td><td>97.0%</td><td>5.8%</td><td>6.0%</td><td>5</td></tr><tr><td rowspan="2">Prothioconazole-desthio<br/>(Transition 312.0 &gt; 124.9)</td><td>Honey</td><td>0.01</td><td>109.9%</td><td>2.1%</td><td>1.9%</td><td>5</td></tr><tr><td>Pollen</td><td>0.01</td><td>101.0%</td><td>1.8%</td><td>1.8%</td><td>5</td></tr></table> <p>Recoveries and precision data for the qualifier transition comply with the requirements of residue analytical methods as mean recoveries at the LOQ level are within the range 70-110% and RSD is less than 20%.</p> | Analyte                     | Matrix                       | Fortification level (mg/kg) | Mean recovery Percentage (%)          | Standard deviation (SD) (%)     | Relative standard deviation (RSD) (%) | Number of fortified samples (n) | Azoxystrobin<br>(Transition 404.2 > 329.0) | Honey | 0.01 | 105.0% | 2.3% | 2.2% | 5 | Pollen | 0.01 | 99.4% | 2.3% | 2.3% | 5 | Prothioconazole<br>(Transition 342.1 > 179.9) | Honey | 0.01 | 109.9% | 4.0% | 3.6% | 5 | Pollen | 0.01 | 97.0% | 5.8% | 6.0% | 5 | Prothioconazole-desthio<br>(Transition 312.0 > 124.9) | Honey | 0.01 | 109.9% | 2.1% | 1.9% | 5 | Pollen | 0.01 | 101.0% | 1.8% | 1.8% | 5 |
| Analyte   | Matrix   | Fortification level (mg/kg) | Mean recovery Percentage (%) | Standard deviation (SD) (%) | Relative standard deviation (RSD) (%) | Number of fortified samples (n) |                                       |                                 |  |       |      |        |      |      |   |        |      |       |      |      |   |   |       |      |        |      |      |   |        |      |       |      |      |   |   |       |      |        |      |      |   |        |      |        |      |      |   |
| Azoxystrobin<br>(Transition 404.2 > 329.0)            | Honey  | 0.01                        | 105.0%                       | 2.3%                        | 2.2%                                  | 5                               |                                       |                                 |  |       |      |        |      |      |   |        |      |       |      |      |   |   |       |      |        |      |      |   |        |      |       |      |      |   |   |       |      |        |      |      |   |        |      |        |      |      |   |
|   | Pollen   | 0.01                        | 99.4%                        | 2.3%                        | 2.3%                                  | 5                               |                                       |                                 |  |       |      |        |      |      |   |        |      |       |      |      |   |   |       |      |        |      |      |   |        |      |       |      |      |   |   |       |      |        |      |      |   |        |      |        |      |      |   |
| Prothioconazole<br>(Transition 342.1 > 179.9)         | Honey  | 0.01                        | 109.9%                       | 4.0%                        | 3.6%                                  | 5                               |                                       |                                 |  |       |      |        |      |      |   |        |      |       |      |      |   |   |       |      |        |      |      |   |        |      |       |      |      |   |   |       |      |        |      |      |   |        |      |        |      |      |   |
|   | Pollen   | 0.01                        | 97.0%                        | 5.8%                        | 6.0%                                  | 5                               |                                       |                                 |  |       |      |        |      |      |   |        |      |       |      |      |   |   |       |      |        |      |      |   |        |      |       |      |      |   |   |       |      |        |      |      |   |        |      |        |      |      |   |
| Prothioconazole-desthio<br>(Transition 312.0 > 124.9) | Honey  | 0.01                        | 109.9%                       | 2.1%                        | 1.9%                                  | 5                               |                                       |                                 |  |       |      |        |      |      |   |        |      |       |      |      |   |   |       |      |        |      |      |   |        |      |       |      |      |   |   |       |      |        |      |      |   |        |      |        |      |      |   |
|   | Pollen   | 0.01                        | 101.0%                       | 1.8%                        | 1.8%                                  | 5                               |                                       |                                 |  |       |      |        |      |      |   |        |      |       |      |      |   |   |       |      |        |      |      |   |        |      |       |      |      |   |   |       |      |        |      |      |   |        |      |        |      |      |   |
| LOQ   | <p>The limit of quantification (LOQ) is the lowest validated level where a mean recovery within the range 70-110% with a RSD less than 20% could be obtained.</p> <p>The LOQ was set at 0.01 mg/kg in honey and pollen for each analyte.</p>   |                             |                              |                             |                                       |                                 |                                       |                                 |  |       |      |        |      |      |   |        |      |       |      |      |   |   |       |      |        |      |      |   |        |      |       |      |      |   |   |       |      |        |      |      |   |        |      |        |      |      |   |
| Comment   | <p>Stability results for extracts</p> <p>The stability of extracts during frozen storage (<math>\leq -18^{\circ}\text{C}</math>) was investigated. The results indicate a good stability for at least 7 days for honey and 14 days for pollen for all analytes</p> <p>Stability results for matrix matched standard solutions</p> <p>The stability of matrix matched standard solutions during frozen storage (<math>\leq -18^{\circ}\text{C}</math>) was investigated.</p> <p>The results</p>   |                             |                              |                             |                                       |                                 |                                       |                                 |  |       |      |        |      |      |   |        |      |       |      |      |   |   |       |      |        |      |      |   |        |      |       |      |      |   |   |       |      |        |      |      |   |        |      |        |      |      |   |

## 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.

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

Comments of zRMS: The study is accepted.

Reference: KCP 5.2/02

Report VALIDATION OF THE METHOD FOR DETERMINATION OF AZOXYSTROBIN IN OILSEED RAPE BY GAS CHROMATOGRAPHY, J. Kicińska, Study Code ZBBZ-2018/11/DPL/1A,

Guideline(s): Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21-Oct-2009 concerning the placing of plant protection products on the market and repealing council Directives 79/117/EEC and 91/414/EC  
EU Guidance Document SANCO/3029/99 rev. 4  
EU Guidance Document SANCO/825/00 rev. 8.1

Deviations: NO

GLP: YES

Acceptability: YES

## Materials and methods

The objective of the study is to validate a method for the determination of Azoxystrobin in Oilseed rape (pods and seeds), in order to provide a method for residue analysis, and to meet residue regulatory requirements. To achieve the objective appropriate analytical method for determination of Azoxystrobin was validated in accordance to the guidance document SANCO/825/00, rev. 8.1. and SANCO/3029/99, rev. 4 of the European Commission and to meet residue regulatory requirements. The validated limit of quantification is 0.01 mg/kg.

## Validation - Results and discussions

**Table 5.3-20: Methods suitable for the determination of the residues in plant protection product (PPP) CHR/F/PROTAZO**

|  | <b>Residues</b>   |                             |                   |                             |                   |                           |                 |                           |                 |              |  |  |  |  |  |  |  |                     |       |     |     |   |     |     |     |     |     |   |                      |       |    |     |   |    |     |     |    |     |   |  |  |  |  |  |  |  |                     |       |     |     |   |     |     |     |     |     |   |                      |       |    |     |   |    |     |     |    |     |   |
|--|---|-----------------------------|-------------------|-----------------------------|-------------------|---------------------------|-----------------|---------------------------|-----------------|--------------|--|--|--|--|--|--|--|---------------------|-------|-----|-----|---|-----|-----|-----|-----|-----|---|----------------------|-------|----|-----|---|----|-----|-----|----|-----|---|--|--|--|--|--|--|--|---------------------|-------|-----|-----|---|-----|-----|-----|-----|-----|---|----------------------|-------|----|-----|---|----|-----|-----|----|-----|---|
| <b>Author(s), year</b>   | J. Kicińska   |                             |                   |                             |                   |                           |                 |                           |                 |              |  |  |  |  |  |  |  |                     |       |     |     |   |     |     |     |     |     |   |                      |       |    |     |   |    |     |     |    |     |   |  |  |  |  |  |  |  |                     |       |     |     |   |     |     |     |     |     |   |                      |       |    |     |   |    |     |     |    |     |   |
| <b>Principle of method</b>   | GC-MS/MS  |                             |                   |                             |                   |                           |                 |                           |                 |              |  |  |  |  |  |  |  |                     |       |     |     |   |     |     |     |     |     |   |                      |       |    |     |   |    |     |     |    |     |   |  |  |  |  |  |  |  |                     |       |     |     |   |     |     |     |     |     |   |                      |       |    |     |   |    |     |     |    |     |   |
| <b>Linearity<br/>(linear between<br/>mg/L)<br/>(correlation coefficient, expressed as r)</b> | <p>The correlation between the injected concentration of analyte standard and detector response was demonstrated to be linear by single determination of matrix-matched calibration standards at nine concentration levels ranging from 0.001 µg/mL to 0.5 µg/mL for Oilseed rape pods and seeds. Those ranges correspond from 0.002 mg/kg to 1 mg/kg for Oilseed rape pods and seeds. thus, covers the range from no more than 30 % of the LOQ and at least + 20 % of the highest analyte concentration level detected in samples.</p> <p>The calibration curves obtained for both ion mass transitions of Azoxystrobin were linear with the coefficients of correlation (R) greater than 0.99. Linear regression was performed with 1/x weighting.</p>  |                             |                   |                             |                   |                           |                 |                           |                 |              |  |  |  |  |  |  |  |                     |       |     |     |   |     |     |     |     |     |   |                      |       |    |     |   |    |     |     |    |     |   |  |  |  |  |  |  |  |                     |       |     |     |   |     |     |     |     |     |   |                      |       |    |     |   |    |     |     |    |     |   |
| <b>Recovery</b>  | <table><tr><th>Analyte</th><th>Matrix</th><th>Fortification Level (mg/kg)</th><th>Mean Recovery (%)</th><th>RSD (%)</th><th>n</th><th>Overall Mean Recovery (%)</th><th>Overall RSD (%)</th></tr><tr><td rowspan="10">Azoxystrobin</td><td colspan="7">Ion Mass Transition 344.0→172.0 (Quantification)</td></tr><tr><td rowspan="2">Oilseed rape (pods)</td><td>0.01*</td><td>107</td><td>1.8</td><td>5</td><td rowspan="2">107</td><td rowspan="2">2.3</td></tr><tr><td>0.1</td><td>107</td><td>2.9</td><td>5</td></tr><tr><td rowspan="2">Oilseed rape (seeds)</td><td>0.01*</td><td>97</td><td>1.6</td><td>5</td><td rowspan="2">98</td><td rowspan="2">2.4</td></tr><tr><td>0.1</td><td>99</td><td>2.9</td><td>5</td></tr><tr><td colspan="7">Ion Mass Transition 344.0→156.0 (Confirmation)</td></tr><tr><td rowspan="2">Oilseed rape (pods)</td><td>0.01*</td><td>107</td><td>3.3</td><td>5</td><td rowspan="2">107</td><td rowspan="2">2.7</td></tr><tr><td>0.1</td><td>107</td><td>2.4</td><td>5</td></tr><tr><td rowspan="2">Oilseed rape (seeds)</td><td>0.01*</td><td>99</td><td>3.4</td><td>5</td><td rowspan="2">98</td><td rowspan="2">3.7</td></tr><tr><td>0.1</td><td>98</td><td>4.3</td><td>5</td></tr></table> <p>*-Limit of quantification, defined by the lowest validated fortification level</p> | Analyte                     | Matrix            | Fortification Level (mg/kg) | Mean Recovery (%) | RSD (%)                   | n               | Overall Mean Recovery (%) | Overall RSD (%) | Azoxystrobin | Ion Mass Transition 344.0→172.0 (Quantification) |  |  |  |  |  |  | Oilseed rape (pods) | 0.01* | 107 | 1.8 | 5 | 107 | 2.3 | 0.1 | 107 | 2.9 | 5 | Oilseed rape (seeds) | 0.01* | 97 | 1.6 | 5 | 98 | 2.4 | 0.1 | 99 | 2.9 | 5 | Ion Mass Transition 344.0→156.0 (Confirmation) |  |  |  |  |  |  | Oilseed rape (pods) | 0.01* | 107 | 3.3 | 5 | 107 | 2.7 | 0.1 | 107 | 2.4 | 5 | Oilseed rape (seeds) | 0.01* | 99 | 3.4 | 5 | 98 | 3.7 | 0.1 | 98 | 4.3 | 5 |
| Analyte  | Matrix  | Fortification Level (mg/kg) | Mean Recovery (%) | RSD (%)                     | n                 | Overall Mean Recovery (%) | Overall RSD (%) |                           |                 |              |  |  |  |  |  |  |  |                     |       |     |     |   |     |     |     |     |     |   |                      |       |    |     |   |    |     |     |    |     |   |  |  |  |  |  |  |  |                     |       |     |     |   |     |     |     |     |     |   |                      |       |    |     |   |    |     |     |    |     |   |
| Azoxystrobin   | Ion Mass Transition 344.0→172.0 (Quantification)  |                             |                   |                             |                   |                           |                 |                           |                 |              |  |  |  |  |  |  |  |                     |       |     |     |   |     |     |     |     |     |   |                      |       |    |     |   |    |     |     |    |     |   |  |  |  |  |  |  |  |                     |       |     |     |   |     |     |     |     |     |   |                      |       |    |     |   |    |     |     |    |     |   |
|  | Oilseed rape (pods)   | 0.01*                       | 107               | 1.8                         | 5                 | 107                       | 2.3             |                           |                 |              |  |  |  |  |  |  |  |                     |       |     |     |   |     |     |     |     |     |   |                      |       |    |     |   |    |     |     |    |     |   |  |  |  |  |  |  |  |                     |       |     |     |   |     |     |     |     |     |   |                      |       |    |     |   |    |     |     |    |     |   |
|  |   | 0.1                         | 107               | 2.9                         | 5                 |                           |                 |                           |                 |              |  |  |  |  |  |  |  |                     |       |     |     |   |     |     |     |     |     |   |                      |       |    |     |   |    |     |     |    |     |   |  |  |  |  |  |  |  |                     |       |     |     |   |     |     |     |     |     |   |                      |       |    |     |   |    |     |     |    |     |   |
|  | Oilseed rape (seeds)  | 0.01*                       | 97                | 1.6                         | 5                 | 98                        | 2.4             |                           |                 |              |  |  |  |  |  |  |  |                     |       |     |     |   |     |     |     |     |     |   |                      |       |    |     |   |    |     |     |    |     |   |  |  |  |  |  |  |  |                     |       |     |     |   |     |     |     |     |     |   |                      |       |    |     |   |    |     |     |    |     |   |
|  |   | 0.1                         | 99                | 2.9                         | 5                 |                           |                 |                           |                 |              |  |  |  |  |  |  |  |                     |       |     |     |   |     |     |     |     |     |   |                      |       |    |     |   |    |     |     |    |     |   |  |  |  |  |  |  |  |                     |       |     |     |   |     |     |     |     |     |   |                      |       |    |     |   |    |     |     |    |     |   |
|  | Ion Mass Transition 344.0→156.0 (Confirmation)  |                             |                   |                             |                   |                           |                 |                           |                 |              |  |  |  |  |  |  |  |                     |       |     |     |   |     |     |     |     |     |   |                      |       |    |     |   |    |     |     |    |     |   |  |  |  |  |  |  |  |                     |       |     |     |   |     |     |     |     |     |   |                      |       |    |     |   |    |     |     |    |     |   |
|  | Oilseed rape (pods)   | 0.01*                       | 107               | 3.3                         | 5                 | 107                       | 2.7             |                           |                 |              |  |  |  |  |  |  |  |                     |       |     |     |   |     |     |     |     |     |   |                      |       |    |     |   |    |     |     |    |     |   |  |  |  |  |  |  |  |                     |       |     |     |   |     |     |     |     |     |   |                      |       |    |     |   |    |     |     |    |     |   |
|  |   | 0.1                         | 107               | 2.4                         | 5                 |                           |                 |                           |                 |              |  |  |  |  |  |  |  |                     |       |     |     |   |     |     |     |     |     |   |                      |       |    |     |   |    |     |     |    |     |   |  |  |  |  |  |  |  |                     |       |     |     |   |     |     |     |     |     |   |                      |       |    |     |   |    |     |     |    |     |   |
|  | Oilseed rape (seeds)  | 0.01*                       | 99                | 3.4                         | 5                 | 98                        | 3.7             |                           |                 |              |  |  |  |  |  |  |  |                     |       |     |     |   |     |     |     |     |     |   |                      |       |    |     |   |    |     |     |    |     |   |  |  |  |  |  |  |  |                     |       |     |     |   |     |     |     |     |     |   |                      |       |    |     |   |    |     |     |    |     |   |
|  |   | 0.1                         | 98                | 4.3                         | 5                 |                           |                 |                           |                 |              |  |  |  |  |  |  |  |                     |       |     |     |   |     |     |     |     |     |   |                      |       |    |     |   |    |     |     |    |     |   |  |  |  |  |  |  |  |                     |       |     |     |   |     |     |     |     |     |   |                      |       |    |     |   |    |     |     |    |     |   |
| <b>Accuracy</b>  | Accuracy was determined by fortification of control samples with known amounts of the reference items (Azoxystrobin) and subsequent determination of the recoveries when applying the extraction procedure. Precision was determined by repeatability (relative standard deviation – RSD).  |                             |                   |                             |                   |                           |                 |                           |                 |              |  |  |  |  |  |  |  |                     |       |     |     |   |     |     |     |     |     |   |                      |       |    |     |   |    |     |     |    |     |   |  |  |  |  |  |  |  |                     |       |     |     |   |     |     |     |     |     |   |                      |       |    |     |   |    |     |     |    |     |   |
| <b>Precision and re-peatability</b>  | The mean recoveries values for Azoxystrobin at the fortification levels: 0.01 mg/kg and 0.1 mg/kg for both tested matrices (Oilseed rape pods and seeds) were in the range 70 – 110 % and thus comply with the standard acceptance criteria of the guidance document SANCO/825/00 rev. 8.1. The precision values at the above fortification levels for both ion mass transitions were < 20% for all tested analytes.  |                             |                   |                             |                   |                           |                 |                           |                 |              |  |  |  |  |  |  |  |                     |       |     |     |   |     |     |     |     |     |   |                      |       |    |     |   |    |     |     |    |     |   |  |  |  |  |  |  |  |                     |       |     |     |   |     |     |     |     |     |   |                      |       |    |     |   |    |     |     |    |     |   |
| <b>LOQ</b>   | The limit of quantification (LOQ) is the lowest validated fortification level and was thus  |                             |                   |                             |                   |                           |                 |                           |                 |              |  |  |  |  |  |  |  |                     |       |     |     |   |     |     |     |     |     |   |                      |       |    |     |   |    |     |     |    |     |   |  |  |  |  |  |  |  |                     |       |     |     |   |     |     |     |     |     |   |                      |       |    |     |   |    |     |     |    |     |   |

|                | Residues   |
|----------------|--|
|                | successfully established at 0.01 mg/kg for both ion mass transitions of Azoxystrobin in Oilseed rape (pods and seeds).<br>The limit of detection (LOD) for Azoxystrobin was set at 0.002 mg/kg for Oilseed rape (pods and seeds), which is < 30% of the LOQ. |
| <b>Comment</b> | The validation parameters are within the acceptance range and fulfil EU requirements given in SANCO/3029/99 rev.4.   |

## Conclusion

The method was shown to be highly selective, as it includes two parent-daughter ion transitions for Azoxystrobin and it yields accurate and repeatable results. The limit of quantification (LOQ) was established at 0.01 mg/kg for all analytes, interfering signals in control specimen were negligible, and thus the limit of detection (LOD) is 0.002 mg/kg for Oilseed rape (pods and seeds).

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

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

**Table 5.3-21: Relevant residue definitions for monitoring/enforcement and levels for which compliance is required**

| Matrix  | Residue definition | MRL / limit         | Reference for MRL/level<br>Remarks |
|---|--------------------|---------------------|------------------------------------|
| Plant, high water content                                 | Azoxystrobin       | 0.01 mg/kg          | Robinson, N.J. et al, 1999         |
| Plant, high acid content                                  |                    | 0.02 mg/kg          | Chaggar, S., 2004                  |
| Plant, high protein/high starch content (dry commodities) |                    | 0.01 mg/kg          | Robinson, N.J. et al, 1999         |
| Plant, high oil content                                   |                    | 0.01 mg/kg          | Chaggar, S., 2004                  |
| Muscle  | Azoxystrobin       | 0.01 mg/kg          | Sapiets, A., et al., 1997          |
| Milk  |                    | 0.001 mg/kg         | Sapiets, A., et al., 1997          |
| Liver, kidney   |                    | 0.01 mg/kg          | Sapiets, A., et al., 1997          |
| Soil  | Azoxystrobin       | 0.02 mg/kg          | Johnson, R.I., et al., 2000        |
| Drinking water (Human toxicology)                         | Azoxystrobin       | 0.1 µg/L            | general limit for drinking water   |
| Surface water (Ecotoxicology)                             | Azoxystrobin       | 3.34 µg/L           |                                    |
| Air   | Azoxystrobin       | 3 µg/m <sup>3</sup> | Crawford, N., 2001                 |
| Tissue (meat or liver)                                    | Azoxystrobin       | 0.05 µg/L           | notclassified as T / T+            |
| Body fluids   |                    |                     | notclassified as T / T+            |

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

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

**Table 5.3-22: 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: Azoxystrobin |                            |              |   |                                       |
|---|----------------------------|--------------|---|---------------------------------------|
| 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   | LC-MS/MS                                    | Chaggar, S., 2004                     |
|   | ILV                        | -            | -   | -                                     |
|   | Confirmatory (if required) |              |   |                                       |
| High protein/high starch content (dry)        | Primary                    | 0.01 mg/kg   | LC-MS/MS                                    | Robinson, N.J. et al, 1999            |
|   | ILV                        | -            | -   | -                                     |
|   | Confirmatory (if required) | Not required |   |                                       |

**Table 5.3-23: Statement on extraction efficiency**

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

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

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

**Table 5.3-24: Validated methods for food and feed of animal origin (if appropriate)**

| Component of residue definition: Azoxystrobin |                            |              |   |                           |
|---|----------------------------|--------------|---|---------------------------|
| Matrix type                                   | Method type                | Method LOQ   | Principle of method (i.e. GC-MS or HPLC-UV) | Author(s), year / missing |
| Milk  | Primary                    | 0.001 mg/kg  | GC-NPD                                      | Sapiets, A., et al., 1996 |
|   | ILV                        | -            | -   | -                         |
|   | Confirmatory (if required) | Not required |   |                           |
| Muscle  | Primary                    | 0.01 mg/kg   | GC-NPD                                      | Sapiets, A., et al., 1996 |
|   | ILV                        | -            | -   | -                         |
|   | Confirmatory (if required) | Not required |   |                           |



| Component of residue definition: Azoxystrobin |                               |              |  |                           |
|---|-------------------------------|--------------|--|---------------------------|
| Matrix type                                   | Method type                   | Method LOQ   | Principle of method<br>(i.e. GC-MS or HPLC-UV) | Author(s), year / missing |
| Kidney, liver                                 | Primary                       | 0.01 mg/kg   | GC-NPD   | Sapiets, A., et al., 1996 |
|   | ILV                           | -            | -  | -                         |
|   | Confirmatory<br>(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 5.3-25: Statement on extraction efficiency**

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

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

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

**Table 5.3-26: Validated methods for soil (if appropriate)**

| Component of residue definition: Azoxystrobin |            |  |                             |
|---|------------|--|-----------------------------|
| Method type                                   | Method LOQ | Principle of method<br>(i.e. GC-MS or HPLC-UV) | Author(s), year / missing   |
| Primary                                       | 0.02 mg/kg | GC-MSD   | Johnson, R.I., et al., 2000 |
| Confirmatory                                  | -          |  |                             |

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

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

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

**Table 5.3-27: Validated methods for water (if appropriate)**

| Component of residue definition: Azoxystrobin |              |            |  |                           |
|---|--------------|------------|--|---------------------------|
| Matrix type                                   | Method type  | Method LOQ | Principle of method<br>(i.e. GC-MS or HPLC-UV) | Author(s), year / missing |
| Drinking water                                | Primary      | 0.1 µg/L   | GC-MSD   | Robinson, N.J., 2000      |
|   | ILV          | -          |  |                           |
|   | Confirmatory |            |  |                           |
| Surface water                                 | Primary      | 0.1 µg/L   | GC-MSD   | Robinson, N.J., 2000      |
|   | Confirmatory |            |  |                           |

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

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

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

**Table 5.3-28: Validated methods for air (if appropriate)**

| Component of residue definition: Azoxystrobin |                     |   |                           |
|---|---------------------|---|---------------------------|
| Method type                                   | Method LOQ          | Principle of method (i.e. GC-MS or HPLC-UV) | Author(s), year / missing |
| Primary                                       | 3 µg/m <sup>3</sup> | GC-MSD                                      | Crawford, N., 2001        |
| Confirmatory                                  | Not required        |   |                           |

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

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

An overview on the acceptable methods and possible data gaps for analysis of azoxystrobin 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 5.3-29: Methods for body fluids and tissues (if appropriate)**

| Component of residue definition: Azoxystrobin |            |   |                           |
|---|------------|---|---------------------------|
| Method type                                   | Method LOQ | Principle of method (i.e. GC-MS or HPLC-UV) | Author(s), year / missing |
| Primary                                       | 0.05 mg/kg | HPLC-UV                                     | XXXXXXXXXXXXX             |
| Confirmatory                                  | 0.05       | LC-MS                                       | XXXXXXXXXXXXX             |

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

### 5.3.3.8 Other studies/ information

Comments of zRMS: The study is accepted.

Reference: KCP 5.2/03

Report Validation of the Analytical Method for the Analysis of Azoxystrobin, Prothioconazole and Prothioconazole-desthio in Honey and Pollen, P. Schlewitz, Study Code: C0240

Guideline(s): Regulation (EC) No. 1107/2009  
 SANCO/3029/99 rev.4  
 SANCO/825/00 rev.8.1  
 ENV/JM/MONO(2007)17

Deviations: NO

GLP: YES

Acceptability: YES

## Materials and methods

The method under discussion describes the determination of residues of azoxystrobin, prothioconazole and prothioconazole-desthio in honey and pollen. The method was validated at 0.01 mg/kg in honey and pollen for azoxystrobin (quantifier transition 404.2 > 372.1 and qualifier transition 404.2 > 329.0), prothioconazole (quantifier transition 342.1 > 306.0 and qualifier transition 342.1 > 179.9) and prothioconazole-desthio (quantifier transition 312.0 > 89.0 and qualifier transition 312.0 > 124.9)..

## Validation - Results and discussions

**Table 5.3-30: Methods suitable for the determination of the residues in plant protection product (PPP) CHR/F/PROTAZO**

|   | Residues   |
|---|--|
| Author(s), year   | P. Schlewitz   |
| Principle of method   | LC MS/MS   |
| Linearity (linear between mg/L) (correlation coefficient, expressed as r) | The linearity of the method was studied between $\approx 0.7$ ng/mL and $\approx 30$ ng/mL of azoxystrobin, prothioconazole and prothioconazole-desthio in matrix-matched calibration solutions (corresponding to $\approx 0.003$ to $\approx 0.12$ mg/kg). The linear correlation coefficients were $> 0.990$ , showing a good linearity. |

Residues

Recovery

| Analyte<br>(Transition)                       | Matrix | Fortification<br>level<br>(mg/kg) | Mean recovery<br>Percentage<br>(%) | Standard<br>deviation<br>(SD) (%) | Relative<br>standard<br>deviation<br>(RSD) (%) | Number of<br>fortified<br>samples<br>(n) |
|---|--------|-----------------------------------|------------------------------------|-----------------------------------|--|--|
| Azoxystrobin<br>(404.2 > 372.1)               | Honey  | 0.01                              | 105.1%                             | 2.5%                              | 2.3%   | 5  |
|   |        | 0.10                              | 101.6%                             | 1.4%                              | 1.4%   | 5  |
|   |        | All levels                        | 103.4%                             | 2.7%                              | 2.6%   | 10                                       |
| Prothioconazole<br>(342.1 > 306.0)            | Honey  | 0.01                              | 107.0%                             | 3.8%                              | 3.5%   | 5  |
|   |        | 0.10                              | 101.7%                             | 2.3%                              | 2.3%   | 5  |
|   |        | All levels                        | 104.4%                             | 4.1%                              | 3.9%   | 10                                       |
| Prothioconazole-<br>desthio<br>(312.0 > 89.0) | Honey  | 0.01                              | 107.8%                             | 2.1%                              | 2.0%   | 5  |
|   |        | 0.10                              | 108.9%                             | 1.9%                              | 1.8%   | 5  |
|   |        | All levels                        | 108.4%                             | 2.0%                              | 1.9%   | 10                                       |
| Azoxystrobin<br>(404.2 > 372.1)               | Pollen | 0.01                              | 100.5%                             | 1.7%                              | 1.7%   | 5  |
|   |        | 0.10                              | 98.0%                              | 2.0%                              | 2.1%   | 5  |
|   |        | All levels                        | 99.3%                              | 2.2%                              | 2.2%   | 10                                       |
| Prothioconazole<br>(342.1 > 306.0)            | Pollen | 0.01                              | 97.7%                              | 7.9%                              | 8.1%   | 5  |
|   |        | 0.10                              | 93.4%                              | 2.3%                              | 2.5%   | 5  |
|   |        | All levels                        | 95.6%                              | 5.9%                              | 6.2%   | 10                                       |
| Prothioconazole-<br>desthio<br>(312.0 > 89.0) | Pollen | 0.01                              | 99.3%                              | 1.9%                              | 2.0%   | 5  |
|   |        | 0.10                              | 97.2%                              | 0.8%                              | 0.8%   | 5  |
|   |        | All levels                        | 98.3%                              | 1.8%                              | 1.8%   | 10                                       |

Accuracy

|   |                  |  |                 |                |  |
|---|------------------|--|-----------------|----------------|--|
|   |                  | Azoxystrobin<br>(Transition 404.2 > 372.1)           |                 |                |  |
| Matrix                                  | Honey            |  | Pollen          |                |  |
| Fortification level (mg/kg)             | 0.01             | 0.10   | 0.01            | 0.10           |  |
| Single recovery rates                   | 102.3% to 108.7% | 100.3% to 103.5%                                     | 98.2% to 102.3% | 94.5% to 99.5% |  |
| Mean recoveries per fortification level | 105.1%           | 101.6%   | 100.5%          | 98.0%          |  |
|   |                  | Prothioconazole<br>(Transition 342.1 > 306.0)        |                 |                |  |
| Matrix                                  | Honey            |  | Pollen          |                |  |
| Fortification level (mg/kg)             | 0.01             | 0.10   | 0.01            | 0.10           |  |
| Single recovery rates                   | 103.8% to 113.3% | 98.3% to 104.5%                                      | 87.5% to 108.4% | 89.7% to 95.4% |  |
| Mean recoveries per fortification level | 107.0%           | 101.7%   | 97.7%           | 93.4%          |  |
|   |                  | Prothioconazole-desthio<br>(Transition 312.0 > 89.0) |                 |                |  |
| Matrix                                  | Honey            |  | Pollen          |                |  |
| Fortification level (mg/kg)             | 0.01             | 0.10   | 0.01            | 0.10           |  |
| Single recovery rates                   | 104.8% to 110.8% | 106.3% to 110.9%                                     | 96.5% to 101.8% | 96.5% to 98.5% |  |
| Mean recoveries per fortification level | 107.8%           | 108.9%   | 99.3%           | 97.2%          |  |

The accuracy of the method fulfils the requirements for residue analytical methods which demand that the mean recoveries per fortification level should be in the range 70-110% according to SANCO/3029/99 rev.4.

|  |  |        |  |                              |                             |                                       |                                 |
|--|--|--------|--|------------------------------|-----------------------------|---------------------------------------|---------------------------------|
|  | Residues   |        |  |                              |                             |                                       |                                 |
| Precision and repeat-ability   |  |        | Azoxystrobin<br>(Transition 404.2 > 372.1)           |                              |                             |                                       |                                 |
|  | Matrix   | Honey  |  | Pollen                       |                             |                                       |                                 |
|  | Fortification level (mg/kg)  | 0.01   | 0.10   | 0.01                         | 0.10                        |                                       |                                 |
|  | RSD for each fortification level   | 2.3%   | 1.4%   | 1.7%                         | 2.1%                        |                                       |                                 |
|  |  |        | Prothioconazole<br>(Transition 342.1 > 306.0)        |                              |                             |                                       |                                 |
|  | Matrix   | Honey  |  | Pollen                       |                             |                                       |                                 |
|  | Fortification level (mg/kg)  | 0.01   | 0.10   | 0.01                         | 0.10                        |                                       |                                 |
|  | RSD for each fortification level   | 3.5%   | 2.3%   | 8.1%                         | 2.5%                        |                                       |                                 |
|  |  |        | Prothioconazole-desthio<br>(Transition 312.0 > 89.0) |                              |                             |                                       |                                 |
|  | Matrix   | Honey  |  | Pollen                       |                             |                                       |                                 |
| Fortification level (mg/kg)  | 0.01   | 0.10   | 0.01   | 0.10                         |                             |                                       |                                 |
| RSD for each fortification level   | 2.0%   | 1.8%   | 2.0%   | 0.8%                         |                             |                                       |                                 |
| All RSD determined were less than 20%, the method therefore fulfils the requirements of residue analytical methods according to SANCO/3029/99 rev.4. |  |        |  |                              |                             |                                       |                                 |
| Specificity  | The method is able to determine azoxystrobin, prothioconazole and prothioconazole-desthio in presence of honey and pollen. This was checked by analysing control and spiked specimens to verify the absence of interfering peaks. No interfering peaks were present at > 30% of the LOQ. The analyses were carried out by LC-MS/MS, monitoring two transitions for each analyte. The method was considered highly specific, thus the use of an alternative method was not necessary. |        |  |                              |                             |                                       |                                 |
| Recoveries   | Analyte  | Matrix | Fortification level (mg/kg)                          | Mean recovery Percentage (%) | Standard deviation (SD) (%) | Relative standard deviation (RSD) (%) | Number of fortified samples (n) |
|  | Azoxystrobin<br>(Transition 404.2 > 329.0)   | Honey  | 0.01   | 105.0%                       | 2.3%                        | 2.2%                                  | 5                               |
|  |  | Pollen | 0.01   | 99.4%                        | 2.3%                        | 2.3%                                  | 5                               |
|  | Prothioconazole<br>(Transition 342.1 > 179.9)  | Honey  | 0.01   | 109.9%                       | 4.0%                        | 3.6%                                  | 5                               |
|  |  | Pollen | 0.01   | 97.0%                        | 5.8%                        | 6.0%                                  | 5                               |
|  | Prothioconazole-desthio<br>(Transition 312.0 > 124.9)  | Honey  | 0.01   | 109.9%                       | 2.1%                        | 1.9%                                  | 5                               |
|  |  | Pollen | 0.01   | 101.0%                       | 1.8%                        | 1.8%                                  | 5                               |
|  | Recoveries and precision data for the qualifier transition comply with the requirements of residue analytical methods as mean recoveries at the LOQ level are within the range 70-110% and RSD is less than 20%.   |        |  |                              |                             |                                       |                                 |
| LOQ  | The limit of quantification (LOQ) is the lowest validated level where a mean recovery within the range 70-110% with a RSD less than 20% could be obtained.<br>The LOQ was set at 0.01 mg/kg in honey and pollen for each analyte.  |        |  |                              |                             |                                       |                                 |
| Comment  | Stability results for extracts<br>The stability of extracts during frozen storage (≤ -18°C) was investigated. The results indicate a good stability for at least 7 days for honey and 14 days for pollen for all analytes<br>Stability results for matrix matched standard solutions<br>The stability of matrix matched standard solutions during frozen storage (≤ -18°C) was investigated. The results   |        |  |                              |                             |                                       |                                 |

## 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 selectivi-

ty, accuracy and precision, the analytical methods were applied successfully for each analytical set when analysing the specimens of the study,

## 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<br>Company Report No.<br>Source (where different from company)<br>GLP or GEP status<br>Published or not  | Vertebrate study<br>Y/N | Owner                                       |
|----------------|------------------|------|--|-------------------------|---|
| KCP<br>5.1./01 | I. Knapik        | 2019 | Final Report Determination of physicochemical properties<br>Study code: ICB/93/2019<br>ICB Pharma, ul. Lema 10, 43-600, Jaworzno, POLAND<br>GLP<br>Unpublished   | N                       | Chemiroł Sp.<br>z o.o.                      |
| KCP<br>5.1/02  | M. Wołoszynowska | 2019 | Protiokonazol + Azoxystrobin (175 + 200) SC Determination of the relevant impurity of prothioconazole (prothioconazole-desthio) in the formulation at initial time and after accelerated storage<br>Study code: BA-19/19<br>Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, 6 Annopol St., 09-236 Warsaw<br>GLP<br>Unpublished | N                       | Chemiroł Sp.<br>z o.o.                      |
| KCP<br>5.2/01  | C. Stouvenot     | 2019 | Validation of the Analytical Method for the Analysis of prothioconazole-desthio (sum of isomers) in oilseed rape (whole plant and seeds)<br>Study code: B9154<br>Anadiag, Ampere, 67500 HAGUENAU, France<br>GLP<br>Unpublished   | N                       | Chemiroł Sp.<br>z o.o.<br>Finchimica<br>SPA |
| KPC<br>5.2/02  | J. Kicińska      | 2018 | VALIDATION OF THE METHOD FOR DETERMINATION OF AZOXYSTROBIN IN OILSEED RAPE BY GAS CHROMATOGRAPHY<br>Study code: ZBBZ-2018/11/DPL/1A<br>Food Safety Laboratory, Research Institute of Horticulture, Skierniewice, Poland<br>GLP<br>Unpublished  | N                       | Chemiroł<br>Sp. z o.o.                      |
| KCP<br>5.2/03  | P. Schlewitz     | 2020 | Validation of the Analytical Method for the Analysis of Azoxystrobin, Prothioconazole and Prothioconazole-desthio in Honey and Pollen<br>Study code: C0240   | N                       | Chemiroł Sp.<br>z o.o.                      |

| <b>Data point</b> | <b>Author(s)</b> | <b>Year</b> | <b>Title</b><br><b>Company Report No.</b><br><b>Source (where different from company)</b><br><b>GLP or GEP status</b><br><b>Published or not</b> | <b>Vertebrate study</b><br><b>Y/N</b> | <b>Owner</b> |
|-------------------|------------------|-------------|--|---------------------------------------|--------------|
|                   |                  |             | Anadiag, Ampere, 67500 HAGUENAU, France<br>GLP<br>Unpublished  |                                       |              |

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

| <b>Data point</b>              | <b>Author(s)</b> | <b>Year</b> | <b>Title</b><br><b>Company Report No.</b><br><b>Source (where different from company)</b><br><b>GLP or GEP status</b><br><b>Published or not</b>   | <b>Vertebrate study</b><br><b>Y/N</b> | <b>Owner</b> |
|--------------------------------|------------------|-------------|--|---------------------------------------|--------------|
| Prothioconazole                |                  |             |  |                                       |              |
| KCP<br>5.1/01<br>KCP<br>5.2/01 | Heinemann, O.    | 2000        | Analytical determination of residues of JAU 6476 and desthio-JAU 6476 in/on cereals by HPLC/MS/MS<br>Report No.: 00598<br>Bayer AG<br>GLP<br>Unpublished   | N                                     | BAY          |
| KCP<br>5.1/02<br>KCP<br>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)<br>Report No.: 00598/M001<br>Bayer AG<br>GLP<br>Unpublished | N                                     | BAY          |
| KCP<br>5.1/03<br>KCP<br>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<br>Report No.: 00647<br>Bayer AG<br>GLP<br>Unpublished                        | N                                     | BAY          |



| Data point                     | Author(s)          | Year | Title<br>Company Report No.<br>Source (where different from company)<br>GLP or GEP status<br>Published or not  | Vertebrate<br>study<br>Y/N | Owner |
|--------------------------------|--------------------|------|--|----------------------------|-------|
| KCP<br>5.1/04<br>KCP<br>5.2/04 | xxxxxxxxxxxxxxxxxx | 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<br>Report No.: 00655<br>xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx<br>GLP<br>Unpublished   | N                          | BAY   |
| KCP<br>5.1/05<br>KCP<br>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)<br>Report No.: 00655/M001<br>Bayer AG<br>GLP<br>Unpublished   | N                          | BAY   |
| KCP<br>5.1/06<br>KCP<br>5.2/06 | xxxxxxxxxxxxxxxxxx | 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<br>Report No.: 00086/M033<br>xx<br>GLP<br>Unpublished           | N                          | BAY   |
| KCP<br>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<br>Report No.: P/B 484 G<br>PTRL Europe, Ulm, germany<br>GLP<br>Unpublished  | N                          | BAY   |
| KCP<br>5.2/08                  | xxxxxxxxxxxxxxxxxx | 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<br>Report No.: A-14-01-01<br>xx<br>GLP | N                          | BAY   |

| <b>Data point</b>              | <b>Author(s)</b> | <b>Year</b> | <b>Title<br/>Company Report No.<br/>Source (where different from company)<br/>GLP or GEP status<br/>Published or not</b>   | <b>Vertebrate<br/>study<br/>Y/N</b> | <b>Owner</b> |
|--------------------------------|------------------|-------------|--|-------------------------------------|--------------|
|                                |                  |             | Unpublished  |                                     |              |
| KCP<br>5.1/07<br>KCP<br>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<br>Report No.: 00610<br>Bayer AG<br>GLP<br>Unpublished  | N                                   | BAY          |
| KCP<br>5.1/08<br>KCP<br>5.2/10 | Sommer, H.       | 1998        | Method 00520 (MR-342/98) for liquid chromatographic determination of JAU 6476 and SXX 0665 on application verification pads<br>Report No.: 00520<br>Bayer AG<br>GLP<br>Unpublished   | N                                   | BAY          |
| KCP<br>5.1/09<br>KCP<br>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)<br>Report No.: 00086/M038<br>DR. Specht&Partner, Chemische Laboratorien GmbH, Hamburg, Germany<br>Bayer AG<br>GLP<br>Unpublished | N                                   | BAY          |
| KCP<br>5.1/10<br>KCP<br>5.2/12 | Maasfeld, W.     | 2002        | Method for the determination of JAU 6476 in air by HPLC-MS/MS<br>Report No.: 00724<br>Bayer AG<br>GLP<br>Unpublished   | N                                   | BAY          |
| KCP<br>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]<br>Report No.: 00586<br>Bayer AG<br>GLP   | N                                   | BAY          |

| Data point                     | Author(s)            | Year | Title<br>Company Report No.<br>Source (where different from company)<br>GLP or GEP status<br>Published or not   | Vertebrate<br>study<br>Y/N | Owner    |
|--------------------------------|----------------------|------|---|----------------------------|----------|
|                                |                      |      | Unpublished   |                            |          |
| KCP<br>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<br>Report No.: 00699<br>Bayer AG<br>GLP<br>Unpublished   | N                          | BAY      |
| KCP<br>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<br>Report No.: 00684<br>Bayer AG<br>GLP<br>Unpublished                                       | N                          | BAY      |
| Azoxystrobin                   |                      |      |   |                            |          |
| KCP<br>5.1/11<br>KCP<br>5.2/16 | Robinson, N.J. et al | 1999 | Residue Analytical Method for the Determination of Azoxystrobin and R230130 in Crops<br>Report No.: ICI5504/1022<br>Syngenta<br>GLP<br>Unpublished  | N                          | syngenta |
| KCP<br>5.1/12<br>KCP<br>5.2/17 | Chaggar, S.          | 2004 | Residue Analytical Method for the Determination of Residues of Azoxystrobin (ICI5504) and R230310 in Crop Samples.<br>Report No. RAM 305/03 Syngenta File No. ICI5504/2686<br>GLP<br>Unpublished                    | N                          | Syngenta |
| KCP<br>5.1/13<br>KCP<br>5.2/18 | xxxxxxxxxxxxxxxx     | 1996 | ICIA5504 and R230310: Validation of a method for the determination of residues in animal tissue, eggs and milk. RAM 255/03<br>Report number: RJ1809B xx<br>GLP<br>Unpublished | N                          | Syngenta |

| <b>Data point</b>              | <b>Author(s)</b>     | <b>Year</b> | <b>Title<br/>Company Report No.<br/>Source (where different from company)<br/>GLP or GEP status<br/>Published or not</b>  | <b>Vertebrate<br/>study<br/>Y/N</b> | <b>Owner</b> |
|--------------------------------|----------------------|-------------|---|-------------------------------------|--------------|
| KCP<br>5.1/14<br>KCP<br>5.2/19 | Johnson, R.I., et al | 2000        | Residue Analytical Method for the Analysis of Azoxystrobin, R230310, R2334886, R401553 and R402173 (in soil). RAM 269/03<br>Syngenta File No. ICI5504/0751<br>GLP<br>unpublished      | N                                   | Syngenta     |
| KCP<br>5.1/15<br>KCP<br>5.2/20 | Robinson, N.J.       | 2000        | Analytical Method for the Determination of Residues of Azoxystrobin in Water. Report Number: RAM 358/01.<br>Syngenta File No. ICI5504/0758<br>GLP<br>unpublished                      | N                                   | Syngenta     |
| KCP<br>5.1/16<br>KCP<br>5.2/21 | Crawford, N.         | 2001        | Azoxystrobin: Validation of an Analytical Method for the Determination of Residues in Air. RAM 376/01<br>Report number: TMJ4658B Syngenta File No. ICI5504/0011<br>GLP<br>Unpublished | N                                   | Syngenta     |
| KCP<br>5.1/17<br>KCP<br>5.2/22 | xxxxxxxxxx           | 1999        | Azoxystrobin and R234886: Determination in Human and Animal Plasma by LC-UV and LC-MS.<br>CTL/R/1401<br>Report number: CTL/R/1401 xxxxxxxxxxxxxxxxxxxxxx<br>GLP<br>unpublished        | N                                   | Syngenta     |

## 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 study is accepted. |
|-------------------|------------------------|

Reference: KCP 5.2/01

Report Validation of the Analytical Method for the Analysis of Prothioconazole-desthio (sum of isomers) in Oilseed rape (Whole Plant and Seeds), C. Stouvenot, Study Code B9154,

Guideline(s): Regulation (EC) No. 1107/2009  
 SANCO/3029/99 rev.4  
 SANCO/825/00 rev.8.1  
 ENV/JM/MONO(2007)17

Deviations: NO

GLP: YES

Acceptability: YES

### Linearity

The linearity of the method was checked by injecting into the analytical system matrix-matched calibration solutions of prothioconazole-desthio, at 7 concentration levels, over the range 1.5 ng/mL to 60.3 ng/mL (corresponding to 0.003 to 0.12 mg/kg). Calibration curves were run for each analysis sequence. The linear correlation coefficients were > 0.990, showing a good linearity.

### LOD

The limit of detection (LOD) is the lowest measurable standard concentration estimated at 3 times the background noise with the analytical conditions used.

| Analyte                 | Matrix                   | C <sub>LOD</sub> (ng/mL)* | LOD (mg/kg)** |
|-------------------------|--------------------------|---------------------------|---------------|
| Prothioconazole-desthio | Oilseed rape whole plant | 0.2                       | 0.003         |
| Prothioconazole-desthio | Oilseed rape seeds       | 0.7                       | 0.003         |

\* in standard solution

\*\* in sample (rounded value to 30% of the LOQ)

### LOQ

The limit of quantification (LOQ) is the lowest validated level where a mean recovery in the range 70-110% with a RSD less than 20% could be obtained. It was set at 0.01 mg/kg for oilseed rape whole plant and seeds.

### Accuracy, precision and repeatability

In order to determine blank values, two untreated specimens of oilseed rape whole plant and seeds were worked up for prothioconazole-desthio and analysed according to the method. No interferences above 30% of the limit of quantification were recorded.

### Accuracy

The accuracy of the method was assessed on the basis of the determined recovery rates.

|   | Prothioconazole-desthio  |               | Prothioconazole-desthio |               |
|---|--------------------------|---------------|-------------------------|---------------|
| Matrix                                  | Oilseed rape whole plant |               | Oilseed rape seeds      |               |
| Fortification level (mg/kg)             | 0.01                     | 0.10          | 0.01                    | 0.10          |
| Single recovery rates                   | 77.7% - 84.6%            | 86.6% - 90.2% | 63.7% - 73.7%           | 71.9% - 79.2% |
| Mean recoveries per fortification level | 81.0%                    | 88.3%         | 70.2%                   | 74.7%         |

Average recoveries at each spiking level are in the range 70-110%, showing a good accuracy of the method.

### Precision and repeatability

Repeatability tests (5 recoveries at each fortification level) were performed at the LOQ level and at 10 x LOQ for oilseed rape whole plant and seeds.

|                                  | Prothioconazole-desthio  |      | Prothioconazole-desthio |      |
|----------------------------------|--------------------------|------|-------------------------|------|
| Matrix                           | Oilseed rape whole plant |      | Oilseed rape seeds      |      |
| Fortification level (mg/kg)      | 0.01                     | 0.10 | 0.01                    | 0.10 |
| RSD for each fortification level | 3.3%                     | 1.6% | 6.2%                    | 4.2% |

All RSD determined were less than 20%, the method therefore fulfils the requirements of residue analytical methods.

### Matrix Effect

The effect of crop matrices on the LC-MS/MS response was assessed by analysing standard solutions prepared in solvent against matrix-matched calibration solutions for each matrix.

| Matrix                                 | Analyte                 | Theoretical concentration (ng/mL)<br>C<br>in acetonitrile | Experimental Concentration (ng/mL)<br>Ce<br>against matrix-matched calibration solutions | Recovery (%)<br>100 x Ce / C |
|--|-------------------------|---|--|------------------------------|
| QUANTIFICATION TRANSITION 312.0 > 69.9 |                         |   |  |                              |
| Oilseed rape whole plant               | Prothioconazole-desthio | 50.3  | 105.14   | 209.0%                       |
| Oilseed rape seeds                     |                         | 50.3  | 66.19  | 131.6%                       |
| QUALIFICATION TRANSITION 312.0 > 124.9 |                         |   |  |                              |
| Oilseed rape whole plant               | Prothioconazole-desthio | 50.3  | 111.00   | 220.7%                       |
| Oilseed rape seeds                     |                         | 50.3  | 66.16  | 131.5%                       |

Matrix effects on the instrument response were considered significant. Consequently, matrix-matched calibration solutions were used for calibration.

### 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

Comments of zRMS: The study is accepted.

Reference: KCP 5.2/03

Report Validation of the Analytical Method for the Analysis of Azoxystrobin, Prothioconazole and Prothioconazole-desthio in Honey and Pollen, P. Schlewitz, Study Code: C0240

Guideline(s): Regulation (EC) No. 1107/2009  
 SANCO/3029/99 rev.4  
 SANCO/825/00 rev.8.1  
 ENV/JM/MONO(2007)17

Deviations: NO

GLP: YES

Acceptability: YES

### Materials and methods

The method under discussion describes the determination of residues of azoxystrobin, prothioconazole and prothioconazole-desthio in honey and pollen. The method was validated at 0.01 mg/kg in honey and pollen for azoxystrobin (quantifier transition 404.2 > 372.1 and qualifier transition 404.2 > 329.0), prothioconazole (quantifier transition 342.1 > 306.0 and qualifier transition 342.1 > 179.9) and prothioconazole-desthio (quantifier transition 312.0 > 89.0 and qualifier transition 312.0 > 124.9)..

### Validation - Results and discussions

**Table 5.3-1: Methods suitable for the determination of the residues in plant protection product (PPP) CHR/F/PROTAZO**

|                       | Residues  |
|-----------------------|---|
| Author(s), year       | P. Schlewitz  |
| Principle of method   | LC MS/MS  |
| Linearity (linear be- | The linearity of the method was studied between $\approx 0.7$ ng/mL and $\approx 30$ ng/mL of azoxystrobin, prothioconazole and prothioconazole-desthio in matrix-matched calibration solutions |

|  | <b>Residues</b>  |                             |                              |                             |                                       |                                 |                                       |                                 |                              |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |                                 |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |   |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |                              |        |      |        |      |      |   |      |       |      |      |   |            |       |      |      |    |                                 |        |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |   |        |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |
|--|--|-----------------------------|------------------------------|-----------------------------|---------------------------------------|---------------------------------|---------------------------------------|---------------------------------|------------------------------|-------|------|--------|------|------|---|------|--------|------|------|---|------------|--------|------|------|----|---------------------------------|-------|------|--------|------|------|---|------|--------|------|------|---|------------|--------|------|------|----|---|-------|------|--------|------|------|---|------|--------|------|------|---|------------|--------|------|------|----|------------------------------|--------|------|--------|------|------|---|------|-------|------|------|---|------------|-------|------|------|----|---------------------------------|--------|------|-------|------|------|---|------|-------|------|------|---|------------|-------|------|------|----|---|--------|------|-------|------|------|---|------|-------|------|------|---|------------|-------|------|------|----|
| <b>tween mg/L)<br/>(correlation coefficient, expressed as r)</b> | (corresponding to $\approx 0.003$ to $\approx 0.12$ mg/kg). The linear correlation coefficients were $> 0.990$ , showing a good linearity.   |                             |                              |                             |                                       |                                 |                                       |                                 |                              |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |                                 |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |   |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |                              |        |      |        |      |      |   |      |       |      |      |   |            |       |      |      |    |                                 |        |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |   |        |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |
| <b>Recovery</b>  | <table><tr><th>Analyte (Transition)</th><th>Matrix</th><th>Fortification level (mg/kg)</th><th>Mean recovery Percentage (%)</th><th>Standard deviation (SD) (%)</th><th>Relative standard deviation (RSD) (%)</th><th>Number of fortified samples (n)</th></tr><tr><td rowspan="3">Azoxystrobin (404.2 &gt; 372.1)</td><td rowspan="3">Honey</td><td>0.01</td><td>105.1%</td><td>2.5%</td><td>2.3%</td><td>5</td></tr><tr><td>0.10</td><td>101.6%</td><td>1.4%</td><td>1.4%</td><td>5</td></tr><tr><td>All levels</td><td>103.4%</td><td>2.7%</td><td>2.6%</td><td>10</td></tr><tr><td rowspan="3">Prothioconazole (342.1 &gt; 306.0)</td><td rowspan="3">Honey</td><td>0.01</td><td>107.0%</td><td>3.8%</td><td>3.5%</td><td>5</td></tr><tr><td>0.10</td><td>101.7%</td><td>2.3%</td><td>2.3%</td><td>5</td></tr><tr><td>All levels</td><td>104.4%</td><td>4.1%</td><td>3.9%</td><td>10</td></tr><tr><td rowspan="3">Prothioconazole-desethio (312.0 &gt; 89.0)</td><td rowspan="3">Honey</td><td>0.01</td><td>107.8%</td><td>2.1%</td><td>2.0%</td><td>5</td></tr><tr><td>0.10</td><td>108.9%</td><td>1.9%</td><td>1.8%</td><td>5</td></tr><tr><td>All levels</td><td>108.4%</td><td>2.0%</td><td>1.9%</td><td>10</td></tr><tr><td rowspan="3">Azoxystrobin (404.2 &gt; 372.1)</td><td rowspan="3">Pollen</td><td>0.01</td><td>100.5%</td><td>1.7%</td><td>1.7%</td><td>5</td></tr><tr><td>0.10</td><td>98.0%</td><td>2.0%</td><td>2.1%</td><td>5</td></tr><tr><td>All levels</td><td>99.3%</td><td>2.2%</td><td>2.2%</td><td>10</td></tr><tr><td rowspan="3">Prothioconazole (342.1 &gt; 306.0)</td><td rowspan="3">Pollen</td><td>0.01</td><td>97.7%</td><td>7.9%</td><td>8.1%</td><td>5</td></tr><tr><td>0.10</td><td>93.4%</td><td>2.3%</td><td>2.5%</td><td>5</td></tr><tr><td>All levels</td><td>95.6%</td><td>5.9%</td><td>6.2%</td><td>10</td></tr><tr><td rowspan="3">Prothioconazole-desethio (312.0 &gt; 89.0)</td><td rowspan="3">Pollen</td><td>0.01</td><td>99.3%</td><td>1.9%</td><td>2.0%</td><td>5</td></tr><tr><td>0.10</td><td>97.2%</td><td>0.8%</td><td>0.8%</td><td>5</td></tr><tr><td>All levels</td><td>98.3%</td><td>1.8%</td><td>1.8%</td><td>10</td></tr></table> | Analyte (Transition)        | Matrix                       | Fortification level (mg/kg) | Mean recovery Percentage (%)          | Standard deviation (SD) (%)     | Relative standard deviation (RSD) (%) | Number of fortified samples (n) | Azoxystrobin (404.2 > 372.1) | Honey | 0.01 | 105.1% | 2.5% | 2.3% | 5 | 0.10 | 101.6% | 1.4% | 1.4% | 5 | All levels | 103.4% | 2.7% | 2.6% | 10 | Prothioconazole (342.1 > 306.0) | Honey | 0.01 | 107.0% | 3.8% | 3.5% | 5 | 0.10 | 101.7% | 2.3% | 2.3% | 5 | All levels | 104.4% | 4.1% | 3.9% | 10 | Prothioconazole-desethio (312.0 > 89.0) | Honey | 0.01 | 107.8% | 2.1% | 2.0% | 5 | 0.10 | 108.9% | 1.9% | 1.8% | 5 | All levels | 108.4% | 2.0% | 1.9% | 10 | Azoxystrobin (404.2 > 372.1) | Pollen | 0.01 | 100.5% | 1.7% | 1.7% | 5 | 0.10 | 98.0% | 2.0% | 2.1% | 5 | All levels | 99.3% | 2.2% | 2.2% | 10 | Prothioconazole (342.1 > 306.0) | Pollen | 0.01 | 97.7% | 7.9% | 8.1% | 5 | 0.10 | 93.4% | 2.3% | 2.5% | 5 | All levels | 95.6% | 5.9% | 6.2% | 10 | Prothioconazole-desethio (312.0 > 89.0) | Pollen | 0.01 | 99.3% | 1.9% | 2.0% | 5 | 0.10 | 97.2% | 0.8% | 0.8% | 5 | All levels | 98.3% | 1.8% | 1.8% | 10 |
| Analyte (Transition)   | Matrix   | Fortification level (mg/kg) | Mean recovery Percentage (%) | Standard deviation (SD) (%) | Relative standard deviation (RSD) (%) | Number of fortified samples (n) |                                       |                                 |                              |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |                                 |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |   |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |                              |        |      |        |      |      |   |      |       |      |      |   |            |       |      |      |    |                                 |        |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |   |        |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |
| Azoxystrobin (404.2 > 372.1)                                     | Honey  | 0.01                        | 105.1%                       | 2.5%                        | 2.3%                                  | 5                               |                                       |                                 |                              |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |                                 |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |   |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |                              |        |      |        |      |      |   |      |       |      |      |   |            |       |      |      |    |                                 |        |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |   |        |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |
|  |  | 0.10                        | 101.6%                       | 1.4%                        | 1.4%                                  | 5                               |                                       |                                 |                              |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |                                 |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |   |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |                              |        |      |        |      |      |   |      |       |      |      |   |            |       |      |      |    |                                 |        |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |   |        |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |
|  |  | All levels                  | 103.4%                       | 2.7%                        | 2.6%                                  | 10                              |                                       |                                 |                              |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |                                 |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |   |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |                              |        |      |        |      |      |   |      |       |      |      |   |            |       |      |      |    |                                 |        |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |   |        |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |
| Prothioconazole (342.1 > 306.0)                                  | Honey  | 0.01                        | 107.0%                       | 3.8%                        | 3.5%                                  | 5                               |                                       |                                 |                              |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |                                 |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |   |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |                              |        |      |        |      |      |   |      |       |      |      |   |            |       |      |      |    |                                 |        |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |   |        |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |
|  |  | 0.10                        | 101.7%                       | 2.3%                        | 2.3%                                  | 5                               |                                       |                                 |                              |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |                                 |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |   |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |                              |        |      |        |      |      |   |      |       |      |      |   |            |       |      |      |    |                                 |        |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |   |        |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |
|  |  | All levels                  | 104.4%                       | 4.1%                        | 3.9%                                  | 10                              |                                       |                                 |                              |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |                                 |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |   |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |                              |        |      |        |      |      |   |      |       |      |      |   |            |       |      |      |    |                                 |        |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |   |        |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |
| Prothioconazole-desethio (312.0 > 89.0)                          | Honey  | 0.01                        | 107.8%                       | 2.1%                        | 2.0%                                  | 5                               |                                       |                                 |                              |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |                                 |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |   |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |                              |        |      |        |      |      |   |      |       |      |      |   |            |       |      |      |    |                                 |        |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |   |        |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |
|  |  | 0.10                        | 108.9%                       | 1.9%                        | 1.8%                                  | 5                               |                                       |                                 |                              |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |                                 |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |   |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |                              |        |      |        |      |      |   |      |       |      |      |   |            |       |      |      |    |                                 |        |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |   |        |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |
|  |  | All levels                  | 108.4%                       | 2.0%                        | 1.9%                                  | 10                              |                                       |                                 |                              |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |                                 |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |   |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |                              |        |      |        |      |      |   |      |       |      |      |   |            |       |      |      |    |                                 |        |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |   |        |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |
| Azoxystrobin (404.2 > 372.1)                                     | Pollen   | 0.01                        | 100.5%                       | 1.7%                        | 1.7%                                  | 5                               |                                       |                                 |                              |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |                                 |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |   |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |                              |        |      |        |      |      |   |      |       |      |      |   |            |       |      |      |    |                                 |        |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |   |        |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |
|  |  | 0.10                        | 98.0%                        | 2.0%                        | 2.1%                                  | 5                               |                                       |                                 |                              |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |                                 |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |   |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |                              |        |      |        |      |      |   |      |       |      |      |   |            |       |      |      |    |                                 |        |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |   |        |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |
|  |  | All levels                  | 99.3%                        | 2.2%                        | 2.2%                                  | 10                              |                                       |                                 |                              |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |                                 |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |   |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |                              |        |      |        |      |      |   |      |       |      |      |   |            |       |      |      |    |                                 |        |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |   |        |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |
| Prothioconazole (342.1 > 306.0)                                  | Pollen   | 0.01                        | 97.7%                        | 7.9%                        | 8.1%                                  | 5                               |                                       |                                 |                              |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |                                 |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |   |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |                              |        |      |        |      |      |   |      |       |      |      |   |            |       |      |      |    |                                 |        |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |   |        |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |
|  |  | 0.10                        | 93.4%                        | 2.3%                        | 2.5%                                  | 5                               |                                       |                                 |                              |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |                                 |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |   |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |                              |        |      |        |      |      |   |      |       |      |      |   |            |       |      |      |    |                                 |        |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |   |        |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |
|  |  | All levels                  | 95.6%                        | 5.9%                        | 6.2%                                  | 10                              |                                       |                                 |                              |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |                                 |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |   |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |                              |        |      |        |      |      |   |      |       |      |      |   |            |       |      |      |    |                                 |        |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |   |        |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |
| Prothioconazole-desethio (312.0 > 89.0)                          | Pollen   | 0.01                        | 99.3%                        | 1.9%                        | 2.0%                                  | 5                               |                                       |                                 |                              |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |                                 |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |   |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |                              |        |      |        |      |      |   |      |       |      |      |   |            |       |      |      |    |                                 |        |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |   |        |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |
|  |  | 0.10                        | 97.2%                        | 0.8%                        | 0.8%                                  | 5                               |                                       |                                 |                              |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |                                 |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |   |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |                              |        |      |        |      |      |   |      |       |      |      |   |            |       |      |      |    |                                 |        |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |   |        |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |
|  |  | All levels                  | 98.3%                        | 1.8%                        | 1.8%                                  | 10                              |                                       |                                 |                              |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |                                 |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |   |       |      |        |      |      |   |      |        |      |      |   |            |        |      |      |    |                              |        |      |        |      |      |   |      |       |      |      |   |            |       |      |      |    |                                 |        |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |   |        |      |       |      |      |   |      |       |      |      |   |            |       |      |      |    |



|  |   |  |                  |   |   |   |
|--|---|--|------------------|---|---|---|
|  | <b>Residues</b>   |  |                  |   |   |   |
| <b>Accuracy</b>  |   |  |                  |   | <b>Azoxystrobin</b><br>(Transition 404.2 > 372.1)           |   |
|  | Matrix  | Honey  |                  | Pollen  |   |   |
|  | Fortification level (mg/kg)   | 0.01   | 0.10             | 0.01  | 0.10  |   |
|  | Single recovery rates   | 102.3% to 108.7%   | 100.3% to 103.5% | 98.2% to 102.3%   | 94.5% to 99.5%  |   |
|  | Mean recoveries per fortification level   | 105.1%   | 101.6%           | 100.5%  | 98.0%   |   |
|  |   |  |                  |   | <b>Prothioconazole</b><br>(Transition 342.1 > 306.0)        |   |
|  | Matrix  | Honey  |                  | Pollen  |   |   |
|  | Fortification level (mg/kg)   | 0.01   | 0.10             | 0.01  | 0.10  |   |
|  | Single recovery rates   | 103.8% to 113.3%   | 98.3% to 104.5%  | 87.5% to 108.4%   | 89.7% to 95.4%  |   |
|  | Mean recoveries per fortification level   | 107.0%   | 101.7%           | 97.7%   | 93.4%   |   |
|  |   |  |                  |   | <b>Prothioconazole-desthio</b><br>(Transition 312.0 > 89.0) |   |
|  | Matrix  | Honey  |                  | Pollen  |   |   |
|  | Fortification level (mg/kg)   | 0.01   | 0.10             | 0.01  | 0.10  |   |
|  | Single recovery rates   | 104.8% to 110.8%   | 106.3% to 110.9% | 96.5% to 101.8%   | 96.5% to 98.5%  |   |
|  | Mean recoveries per fortification level   | 107.8%   | 108.9%           | 99.3%   | 97.2%   |   |
|  | The accuracy of the method fulfils the requirements for residue analytical methods which demand that the mean recoveries per fortification level should be in the range 70-110% according to SANCO/3029/99 rev.4. |  |                  |   |   |   |
|  | <b>Precision and repeat-ability</b>   |  |                  |   |   | <b>Azoxystrobin</b><br>(Transition 404.2 > 372.1) |
| Matrix   |   | Honey  |                  | Pollen  |   |   |
| Fortification level (mg/kg)  |   | 0.01   | 0.10             | 0.01  | 0.10  |   |
| RSD for each fortification level   |   | 2.3%   | 1.4%             | 1.7%  | 2.1%  |   |
|  |   |  |                  | <b>Prothioconazole</b><br>(Transition 342.1 > 306.0)        |   |   |
| Matrix   |   | Honey  |                  | Pollen  |   |   |
| Fortification level (mg/kg)  |   | 0.01   | 0.10             | 0.01  | 0.10  |   |
| RSD for each fortification level   |   | 3.5%   | 2.3%             | 8.1%  | 2.5%  |   |
|  |   |  |                  | <b>Prothioconazole-desthio</b><br>(Transition 312.0 > 89.0) |   |   |
| Matrix   |   | Honey  |                  | Pollen  |   |   |
| Fortification level (mg/kg)  |   | 0.01   | 0.10             | 0.01  | 0.10  |   |
| RSD for each fortification level   |   | 2.0%   | 1.8%             | 2.0%  | 0.8%  |   |
| All RSD determined were less than 20%, the method therefore fulfils the requirements of residue analytical methods according to SANCO/3029/99 rev.4. |   |  |                  |   |   |   |
| <b>Specificity</b>   |   | The method is able to determine azoxystrobin, prothioconazole and prothioconazole-desthio in presence of honey and pollen. This was checked by analysing control and spiked specimens to verify the absence of interfering peaks. No interfering peaks were present at > 30% of the LOQ. The analyses were carried out by LC-MS/MS, monitoring two transitions for each analyte. The method was considered highly specific, thus the use of an alternative method was not necessary. |                  |   |   |   |

|  | <b>Residues</b>   |                             |                              |                             |                                       |                                 |  |         |        |                             |                              |                             |                                       |                                 |   |       |      |        |      |      |   |        |      |       |      |      |   |  |       |      |        |      |      |   |        |      |       |      |      |   |  |       |      |        |      |      |   |        |      |        |      |      |   |
|--|---|-----------------------------|------------------------------|-----------------------------|---------------------------------------|---------------------------------|--|---------|--------|-----------------------------|------------------------------|-----------------------------|---------------------------------------|---------------------------------|---|-------|------|--------|------|------|---|--------|------|-------|------|------|---|--|-------|------|--------|------|------|---|--------|------|-------|------|------|---|--|-------|------|--------|------|------|---|--------|------|--------|------|------|---|
| <b>Recoveries</b>  | <table><tr><th>Analyte</th><th>Matrix</th><th>Fortification level (mg/kg)</th><th>Mean recovery Percentage (%)</th><th>Standard deviation (SD) (%)</th><th>Relative standard deviation (RSD) (%)</th><th>Number of fortified samples (n)</th></tr><tr><td rowspan="2"><b>Azoxystrobin</b><br/>(Transition 404.2 &gt; 329.0)</td><td>Honey</td><td>0.01</td><td>105.0%</td><td>2.3%</td><td>2.2%</td><td>5</td></tr><tr><td>Pollen</td><td>0.01</td><td>99.4%</td><td>2.3%</td><td>2.3%</td><td>5</td></tr><tr><td rowspan="2"><b>Prothioconazole</b><br/>(Transition 342.1 &gt; 179.9)</td><td>Honey</td><td>0.01</td><td>109.9%</td><td>4.0%</td><td>3.6%</td><td>5</td></tr><tr><td>Pollen</td><td>0.01</td><td>97.0%</td><td>5.8%</td><td>6.0%</td><td>5</td></tr><tr><td rowspan="2"><b>Prothioconazole-desthio</b><br/>(Transition 312.0 &gt; 124.9)</td><td>Honey</td><td>0.01</td><td>109.9%</td><td>2.1%</td><td>1.9%</td><td>5</td></tr><tr><td>Pollen</td><td>0.01</td><td>101.0%</td><td>1.8%</td><td>1.8%</td><td>5</td></tr></table> <p>Recoveries and precision data for the qualifier transition comply with the requirements of residue analytical methods as mean recoveries at the LOQ level are within the range 70-110% and RSD is less than 20%.</p> |                             |                              |                             |                                       |                                 |  | Analyte | Matrix | Fortification level (mg/kg) | Mean recovery Percentage (%) | Standard deviation (SD) (%) | Relative standard deviation (RSD) (%) | Number of fortified samples (n) | <b>Azoxystrobin</b><br>(Transition 404.2 > 329.0) | Honey | 0.01 | 105.0% | 2.3% | 2.2% | 5 | Pollen | 0.01 | 99.4% | 2.3% | 2.3% | 5 | <b>Prothioconazole</b><br>(Transition 342.1 > 179.9) | Honey | 0.01 | 109.9% | 4.0% | 3.6% | 5 | Pollen | 0.01 | 97.0% | 5.8% | 6.0% | 5 | <b>Prothioconazole-desthio</b><br>(Transition 312.0 > 124.9) | Honey | 0.01 | 109.9% | 2.1% | 1.9% | 5 | Pollen | 0.01 | 101.0% | 1.8% | 1.8% | 5 |
| Analyte  | Matrix  | Fortification level (mg/kg) | Mean recovery Percentage (%) | Standard deviation (SD) (%) | Relative standard deviation (RSD) (%) | Number of fortified samples (n) |  |         |        |                             |                              |                             |                                       |                                 |   |       |      |        |      |      |   |        |      |       |      |      |   |  |       |      |        |      |      |   |        |      |       |      |      |   |  |       |      |        |      |      |   |        |      |        |      |      |   |
| <b>Azoxystrobin</b><br>(Transition 404.2 > 329.0)            | Honey   | 0.01                        | 105.0%                       | 2.3%                        | 2.2%                                  | 5                               |  |         |        |                             |                              |                             |                                       |                                 |   |       |      |        |      |      |   |        |      |       |      |      |   |  |       |      |        |      |      |   |        |      |       |      |      |   |  |       |      |        |      |      |   |        |      |        |      |      |   |
|  | Pollen  | 0.01                        | 99.4%                        | 2.3%                        | 2.3%                                  | 5                               |  |         |        |                             |                              |                             |                                       |                                 |   |       |      |        |      |      |   |        |      |       |      |      |   |  |       |      |        |      |      |   |        |      |       |      |      |   |  |       |      |        |      |      |   |        |      |        |      |      |   |
| <b>Prothioconazole</b><br>(Transition 342.1 > 179.9)         | Honey   | 0.01                        | 109.9%                       | 4.0%                        | 3.6%                                  | 5                               |  |         |        |                             |                              |                             |                                       |                                 |   |       |      |        |      |      |   |        |      |       |      |      |   |  |       |      |        |      |      |   |        |      |       |      |      |   |  |       |      |        |      |      |   |        |      |        |      |      |   |
|  | Pollen  | 0.01                        | 97.0%                        | 5.8%                        | 6.0%                                  | 5                               |  |         |        |                             |                              |                             |                                       |                                 |   |       |      |        |      |      |   |        |      |       |      |      |   |  |       |      |        |      |      |   |        |      |       |      |      |   |  |       |      |        |      |      |   |        |      |        |      |      |   |
| <b>Prothioconazole-desthio</b><br>(Transition 312.0 > 124.9) | Honey   | 0.01                        | 109.9%                       | 2.1%                        | 1.9%                                  | 5                               |  |         |        |                             |                              |                             |                                       |                                 |   |       |      |        |      |      |   |        |      |       |      |      |   |  |       |      |        |      |      |   |        |      |       |      |      |   |  |       |      |        |      |      |   |        |      |        |      |      |   |
|  | Pollen  | 0.01                        | 101.0%                       | 1.8%                        | 1.8%                                  | 5                               |  |         |        |                             |                              |                             |                                       |                                 |   |       |      |        |      |      |   |        |      |       |      |      |   |  |       |      |        |      |      |   |        |      |       |      |      |   |  |       |      |        |      |      |   |        |      |        |      |      |   |
| <b>LOQ</b>   | <p>The limit of quantification (LOQ) is the lowest validated level where a mean recovery within the range 70-110% with a RSD less than 20% could be obtained.</p> <p>The LOQ was set at 0.01 mg/kg in honey and pollen for each analyte.</p>  |                             |                              |                             |                                       |                                 |  |         |        |                             |                              |                             |                                       |                                 |   |       |      |        |      |      |   |        |      |       |      |      |   |  |       |      |        |      |      |   |        |      |       |      |      |   |  |       |      |        |      |      |   |        |      |        |      |      |   |
| <b>Comment</b>   | <p>Stability results for extracts</p> <p>The stability of extracts during frozen storage (<math>\leq -18^{\circ}\text{C}</math>) was investigated. The results indicate a good stability for at least 7 days for honey and 14 days for pollen for all analytes</p> <p>Stability results for matrix matched standard solutions</p> <p>The stability of matrix matched standard solutions during frozen storage (<math>\leq -18^{\circ}\text{C}</math>) was investigated.</p> <p>The results</p>  |                             |                              |                             |                                       |                                 |  |         |        |                             |                              |                             |                                       |                                 |   |       |      |        |      |      |   |        |      |       |      |      |   |  |       |      |        |      |      |   |        |      |       |      |      |   |  |       |      |        |      |      |   |        |      |        |      |      |   |

## 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 2.2 Analytical methods for Azoxystrobin

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

No new or additional studies have been submitted

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

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

|                   |                        |
|-------------------|------------------------|
| Comments of zRMS: | The study is accepted. |
|-------------------|------------------------|

Reference: KCP 5.2/02

Report VALIDATION OF THE METHOD FOR DETERMINATION OF AZOXYSTROBIN IN OILSEED RAPE BY GAS CHROMATOGRAPHY, J. Kicińska, Study Code: ZNNZ-2018/11/DPL/1A,

Guideline(s): Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21-Oct-2009 concerning the placing of plant protection products on the market and repealing council Directives 79/117/EEC and 91/414/EC  
 EU Guidance Document SANCO/3029/99 rev. 4  
 EU Guidance Document SANCO/825/00 rev. 8.1

Deviations: NO

GLP: YES

Acceptability: YES

### Selectivity and Confirmation on Residues Identity

Final determination by GC-MS/MS was conducted by monitoring two MS/MS ion mass transitions for each analyte.

| Analyte      | Ion mass transition        |
|--------------|----------------------------|
| Azoxystrobin | 344.0→172.0 (Quantitation) |
|              | 344.0→156.0 (Confirmation) |

Transitions proposed for quantification were chosen due to their enhanced sensitivity, but both ion mass transitions were applicable and interchangeably for quantification and confirmation. Reagents blanks and control specimens were extracted and analysed according to the method to investigate the presence of residue and/or background interference at the retention time of the analyte. For both ion mass transitions, the specimen showed no significant interference (above 30 % of the LOQ) at the retention time of each analyte. Confirmation of the presence of each analyte was conducted by observation of a precursor ion plus one structurally significant product ion observed at the same retention time. By monitoring MS/MS ion transitions for Azoxystrobin the confirmation ratios were calculated for sample set and compared to the average for the calibration standards. The retention times of analytes in extracts corresponds to that of the calibration standards with a tolerance of  $\leq \pm 0.1$  min. also confirmation ratios for all analytes in all samples were within  $\pm 30$  % of the average found for the standards. Exemplary chromatograms for Azoxystrobin in Oilseed rape pods and seeds matrices representing control specimens, the calibration levels correspond to LOD, LOQ and 10 x LOQ specimens fortified at the LOQ, and at 10 x LOQ also solvent blank and reagent blank (procedural) are included in the figures section together with a MS/MS product ion spectrum.

### Matrix Effects

The matrices effects that the samples extracts have upon the Azoxystrobin during analysis were determined. Matrices effects were evaluated by comparing peak areas of matrix-matched standards with standards at equivalent concentrations prepared in neat solvents. A negative value of the matrix effect indicates matrix suppression and a positive value for matrix effect indicates matrix enhancement. Matrix effects for the quantitative and confirmatory transitions were calculated as described below.

| Matrix effect (%)       | = $[(100 \cdot A_{\text{Matrix-Std}} \cdot C_{\text{Solv-Std}}) / (A_{\text{Solv-Std}} \cdot C_{\text{Matrix-Std}})] - 100$ |                                    |                            |
|-------------------------|---|------------------------------------|----------------------------|
| A <sub>Solv-Std</sub>   | Peak area of solvent standard   |                                    |                            |
| A <sub>Matrix-Std</sub> | Peak area of matrix-matched standard  |                                    |                            |
| C <sub>Solv-Std</sub>   | Nominal Concentration of solvent standard in µg/mL  |                                    |                            |
| C <sub>Matrix-Std</sub> | Nominal Concentration of matrix-matched standard in µg/mL   |                                    |                            |
| Matrix / Commodity      | Standard Concentration (µg/mL)  | Matrix Effect for Azoxystrobin (%) |                            |
|                         |   | Quantification (344.0→172.0)       | Confirmation (344.0→156.0) |
| Oilseed rape (pods)     | 0.001   | 8227                               | 10970                      |
|                         | 0.002   | 4586                               | 57149                      |
|                         | 0.005   | 3306                               | 60763                      |
|                         | 0.01  | 4746                               | 5030                       |
|                         | 0.02  | 7296                               | 6049                       |
|                         | 0.05  | 4641                               | 6362                       |
|                         | 0.1   | 4785                               | 4717                       |
|                         | 0.2   | 4742                               | 4662                       |
|                         | 0.5   | 7926                               | 5395                       |
|                         | Mean:   | 5583.7                             | 17899.6                    |
| Oilseed rape (seeds)    | 0.001   | 35                                 | 1020                       |
|                         | 0.002   | 31                                 | 276                        |
|                         | 0.005   | 17                                 | 371                        |
|                         | 0.01  | 21                                 | 87                         |
|                         | 0.02  | 24                                 | 203                        |
|                         | 0.05  | 29                                 | 691                        |
|                         | 0.1   | 25                                 | 490                        |
|                         | 0.2   | 25                                 | 113                        |
|                         | 0.5   | 25                                 | 48                         |
|                         | Mean:   | 25.7                               | 366.5                      |

(+) matrix enhancement; (-) matrix suppression

For Oilseed rape mean matrix enhancement was > 20 % and thus deemed to be significant. Matrix-matched standards were used for quantification for Oilseed rape matrix.

### Linearity

The correlation between the injected concentration of each analyte standard and detector response was demonstrated to be linear by single determination of matrix-matched calibration standards at nine concentration levels ranging from 0.001 µg/mL to 0.5 µg/mL for Oilseed rape pods and seeds. Those ranges correspond from 0.002 mg/kg to 1 mg/kg for Oilseed rape pods and seeds thus covers the range from no more than 30 % of the LOQ and at least + 20 % of the highest analyte concentration level detected in samples.

The calibration curve was not forced through zero (y-intercept was calculated) and was derived from a weighted (1/x) linear regression. For Azoxystrobin both ion mass transitions calibration curves (quantification, confirmation) were linear with the coefficients of correlation (R) greater than 0.99. Each linearity was spread evenly over the whole analytical sequence

### System Suitability Test

System suitability test (SST) was performed to verify the performance of GC-MS/MS system and to ensure its adequacy for Azoxystrobin determination in Oilseed rape matrix. For the purpose, at the beginning of analytical sequence the matrix-matched calibration at a concentration level corresponding to LOQ in the sample was injected in triplicate. %RSD of Azoxystrobin peak area was calculated for the first and

second transition. The results of system suitability test were found within the acceptable range (peak area %RSD  $\leq$  15%) indicating that the system was suitable for the intended analysis

| Parameters           |              | Azoxystrobin        |             |
|----------------------|--------------|---------------------|-------------|
|                      |              | Ion Mass Transition |             |
|                      |              | Quant.              | Conf.       |
| Oilseed rape (seeds) | Replicate #1 | 20459               | 16302       |
|                      | Replicate #2 | 19545               | 15468       |
|                      | Replicate #3 | 22090               | 15915       |
| Average              |              | 20698               | 15895       |
| Std. Dev.            |              | 1289                | 417         |
| %RSD                 |              | 6.2                 | 2.6         |
| Parameter Limit %RSD |              | $\leq$ 15 %         | $\leq$ 15 % |
| Conformity           |              | YES                 | YES         |

### Stability of Analyte in Final Dilution

Recoveries of the fortified samples within the acceptable range of 70-110% obtained with calibration solutions and the use of bracketing standards at LOQ level (SST samples) to insure integrity of the analytical sequence sufficiently demonstrate the stability of analyte in the final dilution.

### Quantification

Quantification was performed by using the linear regression with 1/x weighting over the required concentration range as described in the section “Linearity”.

### Accuracy and Precision

Accuracy was determined by fortification of control (untreated) sample with known amounts of the reference items and subsequent determination of the recoveries when applying the extraction procedure. Precision was determined by repeatability (relative standard deviation – RSD). Five recovery determinations were performed at the LOQ (0.01 mg/kg) and at the 10 x LOQ (0.1 mg/kg) for both tested matrices (Oilseed rape pods and seeds). Analysis was performed by extraction and single injection. The mean recovery values at the fortification levels for both ion mass transitions of Azoxystrobin were all in the range of 70 – 110 % and thus comply with the standard acceptance criteria of the guidance document SANCO/825/00 rev. 8.1. and SANCO/3029/99 rev. 4. All precision values at the fortification levels for both ion mass transitions were < 20 %..

| Analyte      | Matrix               | Fort. Level (mg/kg)                              | Recovery** (%)      | Mean Rec. (%) | RSD (%) | n | Overall Mean Recovery (%) | Overall RSD (%) |
|--------------|----------------------|--|---------------------|---------------|---------|---|---------------------------|-----------------|
| Azoxystrobin | Oilseed rape (pods)  | Ion Mass Transition 344.0→172.0 (Quantification) |                     |               |         |   |                           |                 |
|              |                      | 0.01*  | 105/105/107/109/108 | 107           | 1.8     | 5 | 107                       | 2.3             |
|              |                      | 0.1  | 106/103/110/109/108 | 107           | 2.9     | 5 |                           |                 |
|              |                      | Ion Mass Transition 344.0→156.0 (Confirmation)   |                     |               |         |   |                           |                 |
|              |                      | 0.01*  | 110/109/109/104/102 | 107           | 3.3     | 5 | 107                       | 2.7             |
|              |                      | 0.1  | 107/103/110/107/106 | 107           | 2.4     | 5 |                           |                 |
|              | Oilseed rape (seeds) | Ion Mass Transition 344.0→172.0 (Quantification) |                     |               |         |   |                           |                 |
|              |                      | 0.01*  | 96/97/95/98/98      | 97            | 1.6     | 5 | 98                        | 2.4             |
|              |                      | 0.1  | 97/98/101/96/103    | 99            | 2.9     | 5 |                           |                 |
|              |                      | Ion Mass Transition 344.0→156.0 (Confirmation)   |                     |               |         |   |                           |                 |
|              |                      | 0.01*  | 101/99/103/94/98    | 99            | 3.4     | 5 | 98                        | 3.7             |
|              |                      | 0.1  | 97/96/99/93/104     | 98            | 4.3     | 5 |                           |                 |

\*- Limit of quantification, defined by the lowest validated fortification level

\*\* - Residues in duplicate control samples and reagent blanks were less than 30 % of the LOQ

### Limit of Quantification (LOQ) and Limit of Detection (LOD)

The LOQ of the method was defined as the lowest analytes concentration at which the methodology had been successfully validated. Thus, an LOQ of 0.01 mg/kg was confirmed for Azoxystrobin in Oilseed rape pods and seeds. The LOD was set at < 30 % of the LOQ.

### Stability of Analytes in Standard Solutions

Working solutions used for preparation calibration samples were stored in refrigerator at  $5 \pm 4^{\circ}\text{C}$  during study. At the end of experimental phase, the solution used for preparation calibration level at LOQ was reanalysed by quintuple injection and compared to freshly prepared. One (1) ion mass transition was used for evaluation and the other ion mass transition was monitored for confirmation purpose only. Difference between stored and newly prepared solution is < 10%. The results indicated that Azoxystrobin working solutions prepared in acetonitrile were stable during study under refrigerated conditions

| Calibration Level (µg/mL)                        | Date of preparation (dd.mm.yy) | Working solution ID | Relative peak area            | RSD (%) | Difference (%) |
|--|--------------------------------|---------------------|-------------------------------|---------|----------------|
| Azoxystrobin                                     |                                |                     |                               |         |                |
| Ion Mass Transition 344.0→172.0 (Quantification) |                                |                     |                               |         |                |
| 0.005 (LOQ)                                      | 06.11.2018                     | 1S1W7               | 18168/19095/17888/18439/17243 | 3.8     | 3.0            |
|  | 13.11.2018                     | 1S1W12              | 19014/17532/17635/17006/16999 | 4.7     |                |

### CONCLUSIONS

The method was shown to be highly selective, as it includes two parent-daughter ion transitions for Azoxystrobin and it yields accurate and repeatable results. The limit of quantification (LOQ) was established at 0.01 mg/kg for all analytes, interfering signals in control specimen were negligible, and thus the limit of detection (LOD) is 0.002 mg/kg for Oilseed rape (pods and seeds). It is concluded that method fulfils the requirements as defined in EC Guidance document on residue analytical methods (SANCO/3029/99, rev. 4 and SANCO/825/00, rev. 8.1.) and is, applicable as enforcement and data generation method for determination of Azoxystrobin, and Azoxystrobin in Oilseed rape.

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted.

#### A 2.2.2.7 A.2.A.9 Other Studies/ Information

|                   |                        |
|-------------------|------------------------|
| Comments of zRMS: | The study is accepted. |
|-------------------|------------------------|

Reference: KCP 5.2/03

Report Validation of the Analytical Method for the Analysis of Azoxystrobin,

Prothioconazole and Prothioconazole-desthio in Honey and Pollen, P. Schlewitz, Study Code: C0240

**Guideline(s):** Regulation (EC) No. 1107/2009  
 SANCO/3029/99 rev.4  
 SANCO/825/00 rev.8.1  
 ENV/JM/MONO(2007)17

**Deviations:** NO

**GLP:** YES

**Acceptability:** YES

## Materials and methods

The method under discussion describes the determination of residues of azoxystrobin, prothioconazole and prothioconazole-desthio in honey and pollen. The method was validated at 0.01 mg/kg in honey and pollen for azoxystrobin (quantifier transition 404.2 > 372.1 and qualifier transition 404.2 > 329.0), prothioconazole (quantifier transition 342.1 > 306.0 and qualifier transition 342.1 > 179.9) and prothioconazole-desthio (quantifier transition 312.0 > 89.0 and qualifier transition 312.0 > 124.9)..

## Validation - Results and discussions

**Table 5.3-2: Methods suitable for the determination of the residues in plant protection product (PPP) CHR/F/PROTAZO**

|  | Residues   |
|--|--|
| <b>Author(s), year</b>   | P. Schlewitz   |
| <b>Principle of method</b>   | LC MS/MS   |
| <b>Linearity (linear between mg/L) (correlation coefficient, expressed as r)</b> | The linearity of the method was studied between $\approx 0.7$ ng/mL and $\approx 30$ ng/mL of azoxystrobin, prothioconazole and prothioconazole-desthio in matrix-matched calibration solutions (corresponding to $\approx 0.003$ to $\approx 0.12$ mg/kg). The linear correlation coefficients were $> 0.990$ , showing a good linearity. |



|   | Residues                                      |  |                                   |                                    |                                   |  |  |  |
|---|---|--|-----------------------------------|------------------------------------|-----------------------------------|--|--|--|
| Recovery  | Analyte<br>(Transition)                       | Matrix                                     | Fortification<br>level<br>(mg/kg) | Mean recovery<br>Percentage<br>(%) | Standard<br>deviation<br>(SD) (%) | Relative<br>standard<br>deviation<br>(RSD) (%) | Number of<br>fortified<br>samples<br>(n) |  |
|   | Azoxystrobin<br>(404.2 > 372.1)               | Honey                                      | 0.01                              | 105.1%                             | 2.5%                              | 2.3%   | 5  |  |
|   |   |  | 0.10                              | 101.6%                             | 1.4%                              | 1.4%   | 5  |  |
|   |   |  | All levels                        | 103.4%                             | 2.7%                              | 2.6%   | 10                                       |  |
|   | Prothioconazole<br>(342.1 > 306.0)            | Honey                                      | 0.01                              | 107.0%                             | 3.8%                              | 3.5%   | 5  |  |
|   |   |  | 0.10                              | 101.7%                             | 2.3%                              | 2.3%   | 5  |  |
|   |   |  | All levels                        | 104.4%                             | 4.1%                              | 3.9%   | 10                                       |  |
|   | Prothioconazole-<br>desthio<br>(312.0 > 89.0) | Honey                                      | 0.01                              | 107.8%                             | 2.1%                              | 2.0%   | 5  |  |
|   |   |  | 0.10                              | 108.9%                             | 1.9%                              | 1.8%   | 5  |  |
|   |   |  | All levels                        | 108.4%                             | 2.0%                              | 1.9%   | 10                                       |  |
|   | Azoxystrobin<br>(404.2 > 372.1)               | Pollen                                     | 0.01                              | 100.5%                             | 1.7%                              | 1.7%   | 5  |  |
|   |   |  | 0.10                              | 98.0%                              | 2.0%                              | 2.1%   | 5  |  |
|   |   |  | All levels                        | 99.3%                              | 2.2%                              | 2.2%   | 10                                       |  |
|   | Prothioconazole<br>(342.1 > 306.0)            | Pollen                                     | 0.01                              | 97.7%                              | 7.9%                              | 8.1%   | 5  |  |
|   |   |  | 0.10                              | 93.4%                              | 2.3%                              | 2.5%   | 5  |  |
|   |   |  | All levels                        | 95.6%                              | 5.9%                              | 6.2%   | 10                                       |  |
|   | Prothioconazole-<br>desthio<br>(312.0 > 89.0) | Pollen                                     | 0.01                              | 99.3%                              | 1.9%                              | 2.0%   | 5  |  |
|   |   |  | 0.10                              | 97.2%                              | 0.8%                              | 0.8%   | 5  |  |
|   |   |  | All levels                        | 98.3%                              | 1.8%                              | 1.8%   | 10                                       |  |
|   | Accuracy                                      | Azoxystrobin<br>(Transition 404.2 > 372.1) |                                   |                                    |                                   |  |  |  |
|   |   | Matrix                                     | Honey                             |                                    | Pollen                            |  |  |  |
| Fortification level (mg/kg)   |   | 0.01                                       | 0.10                              | 0.01                               | 0.10                              |  |  |  |
| Single recovery rates   |   | 102.3% to 108.7%                           | 100.3% to 103.5%                  | 98.2% to 102.3%                    | 94.5% to 99.5%                    |  |  |  |
| Mean recoveries per fortification level   |   | 105.1%                                     | 101.6%                            | 100.5%                             | 98.0%                             |  |  |  |
| Prothioconazole<br>(Transition 342.1 > 306.0)   |   |  |                                   |                                    |                                   |  |  |  |
| Matrix  |   | Honey                                      |                                   | Pollen                             |                                   |  |  |  |
| Fortification level (mg/kg)   |   | 0.01                                       | 0.10                              | 0.01                               | 0.10                              |  |  |  |
| Single recovery rates   |   | 103.8% to 113.3%                           | 98.3% to 104.5%                   | 87.5% to 108.4%                    | 89.7% to 95.4%                    |  |  |  |
| Mean recoveries per fortification level   |   | 107.0%                                     | 101.7%                            | 97.7%                              | 93.4%                             |  |  |  |
| Prothioconazole-desthio<br>(Transition 312.0 > 89.0)  |   |  |                                   |                                    |                                   |  |  |  |
| Matrix  |   | Honey                                      |                                   | Pollen                             |                                   |  |  |  |
| Fortification level (mg/kg)   |   | 0.01                                       | 0.10                              | 0.01                               | 0.10                              |  |  |  |
| Single recovery rates   |   | 104.8% to 110.8%                           | 106.3% to 110.9%                  | 96.5% to 101.8%                    | 96.5% to 98.5%                    |  |  |  |
| Mean recoveries per fortification level   |   | 107.8%                                     | 108.9%                            | 99.3%                              | 97.2%                             |  |  |  |
| The accuracy of the method fulfils the requirements for residue analytical methods which demand that the mean recoveries per fortification level should be in the range 70-110% according to SANCO/3029/99 rev.4. |   |  |                                   |                                    |                                   |  |  |  |



|  | <b>Residues</b>   |                             |   |                             |                                       |                                 |  |         |        |   |                              |                             |                                       |                                 |   |       |      |        |        |      |   |                             |      |       |      |      |   |  |   |      |        |      |      |   |        |      |       |      |      |   |  |       |      |        |      |      |   |        |      |        |      |      |   |
|--|---|-----------------------------|---|-----------------------------|---------------------------------------|---------------------------------|--|---------|--------|---|------------------------------|-----------------------------|---------------------------------------|---------------------------------|---|-------|------|--------|--------|------|---|-----------------------------|------|-------|------|------|---|--|---|------|--------|------|------|---|--------|------|-------|------|------|---|--|-------|------|--------|------|------|---|--------|------|--------|------|------|---|
| <b>Precision and repeat-ability</b>  | <table><tr><td colspan="2"></td><td colspan="5"><b>Azoxystrobin</b><br/>(Transition 404.2 &gt; 372.1)</td></tr><tr><td>Matrix</td><td colspan="3">Honey</td><td colspan="3">Pollen</td></tr><tr><td>Fortification level (mg/kg)</td><td>0.01</td><td>0.10</td><td>0.01</td><td>0.10</td><td></td><td></td></tr><tr><td><b>RSD for each fortification level</b></td><td>2.3%</td><td>1.4%</td><td>1.7%</td><td>2.1%</td><td></td><td></td></tr></table>  |                             |   |                             |                                       |                                 |  |         |        | <b>Azoxystrobin</b><br>(Transition 404.2 > 372.1)           |                              |                             |                                       |                                 | Matrix  | Honey |      |        | Pollen |      |   | Fortification level (mg/kg) | 0.01 | 0.10  | 0.01 | 0.10 |   |  | <b>RSD for each fortification level</b> | 2.3% | 1.4%   | 1.7% | 2.1% |   |        |      |       |      |      |   |  |       |      |        |      |      |   |        |      |        |      |      |   |
|  |   |                             | <b>Azoxystrobin</b><br>(Transition 404.2 > 372.1)           |                             |                                       |                                 |  |         |        |   |                              |                             |                                       |                                 |   |       |      |        |        |      |   |                             |      |       |      |      |   |  |   |      |        |      |      |   |        |      |       |      |      |   |  |       |      |        |      |      |   |        |      |        |      |      |   |
|  | Matrix  | Honey                       |   |                             | Pollen                                |                                 |  |         |        |   |                              |                             |                                       |                                 |   |       |      |        |        |      |   |                             |      |       |      |      |   |  |   |      |        |      |      |   |        |      |       |      |      |   |  |       |      |        |      |      |   |        |      |        |      |      |   |
|  | Fortification level (mg/kg)   | 0.01                        | 0.10  | 0.01                        | 0.10                                  |                                 |  |         |        |   |                              |                             |                                       |                                 |   |       |      |        |        |      |   |                             |      |       |      |      |   |  |   |      |        |      |      |   |        |      |       |      |      |   |  |       |      |        |      |      |   |        |      |        |      |      |   |
|  | <b>RSD for each fortification level</b>   | 2.3%                        | 1.4%  | 1.7%                        | 2.1%                                  |                                 |  |         |        |   |                              |                             |                                       |                                 |   |       |      |        |        |      |   |                             |      |       |      |      |   |  |   |      |        |      |      |   |        |      |       |      |      |   |  |       |      |        |      |      |   |        |      |        |      |      |   |
|  | <table><tr><td colspan="2"></td><td colspan="5"><b>Prothioconazole</b><br/>(Transition 342.1 &gt; 306.0)</td></tr><tr><td>Matrix</td><td colspan="3">Honey</td><td colspan="3">Pollen</td></tr><tr><td>Fortification level (mg/kg)</td><td>0.01</td><td>0.10</td><td>0.01</td><td>0.10</td><td></td><td></td></tr><tr><td><b>RSD for each fortification level</b></td><td>3.5%</td><td>2.3%</td><td>8.1%</td><td>2.5%</td><td></td><td></td></tr></table>   |                             |   |                             |                                       |                                 |  |         |        | <b>Prothioconazole</b><br>(Transition 342.1 > 306.0)        |                              |                             |                                       |                                 | Matrix  | Honey |      |        | Pollen |      |   | Fortification level (mg/kg) | 0.01 | 0.10  | 0.01 | 0.10 |   |  | <b>RSD for each fortification level</b> | 3.5% | 2.3%   | 8.1% | 2.5% |   |        |      |       |      |      |   |  |       |      |        |      |      |   |        |      |        |      |      |   |
|  |   |                             | <b>Prothioconazole</b><br>(Transition 342.1 > 306.0)        |                             |                                       |                                 |  |         |        |   |                              |                             |                                       |                                 |   |       |      |        |        |      |   |                             |      |       |      |      |   |  |   |      |        |      |      |   |        |      |       |      |      |   |  |       |      |        |      |      |   |        |      |        |      |      |   |
|  | Matrix  | Honey                       |   |                             | Pollen                                |                                 |  |         |        |   |                              |                             |                                       |                                 |   |       |      |        |        |      |   |                             |      |       |      |      |   |  |   |      |        |      |      |   |        |      |       |      |      |   |  |       |      |        |      |      |   |        |      |        |      |      |   |
|  | Fortification level (mg/kg)   | 0.01                        | 0.10  | 0.01                        | 0.10                                  |                                 |  |         |        |   |                              |                             |                                       |                                 |   |       |      |        |        |      |   |                             |      |       |      |      |   |  |   |      |        |      |      |   |        |      |       |      |      |   |  |       |      |        |      |      |   |        |      |        |      |      |   |
|  | <b>RSD for each fortification level</b>   | 3.5%                        | 2.3%  | 8.1%                        | 2.5%                                  |                                 |  |         |        |   |                              |                             |                                       |                                 |   |       |      |        |        |      |   |                             |      |       |      |      |   |  |   |      |        |      |      |   |        |      |       |      |      |   |  |       |      |        |      |      |   |        |      |        |      |      |   |
|  | <table><tr><td colspan="2"></td><td colspan="5"><b>Prothioconazole-desthio</b><br/>(Transition 312.0 &gt; 89.0)</td></tr><tr><td>Matrix</td><td colspan="3">Honey</td><td colspan="3">Pollen</td></tr><tr><td>Fortification level (mg/kg)</td><td>0.01</td><td>0.10</td><td>0.01</td><td>0.10</td><td></td><td></td></tr><tr><td><b>RSD for each fortification level</b></td><td>2.0%</td><td>1.8%</td><td>2.0%</td><td>0.8%</td><td></td><td></td></tr></table>  |                             |   |                             |                                       |                                 |  |         |        | <b>Prothioconazole-desthio</b><br>(Transition 312.0 > 89.0) |                              |                             |                                       |                                 | Matrix  | Honey |      |        | Pollen |      |   | Fortification level (mg/kg) | 0.01 | 0.10  | 0.01 | 0.10 |   |  | <b>RSD for each fortification level</b> | 2.0% | 1.8%   | 2.0% | 0.8% |   |        |      |       |      |      |   |  |       |      |        |      |      |   |        |      |        |      |      |   |
|  |   |                             | <b>Prothioconazole-desthio</b><br>(Transition 312.0 > 89.0) |                             |                                       |                                 |  |         |        |   |                              |                             |                                       |                                 |   |       |      |        |        |      |   |                             |      |       |      |      |   |  |   |      |        |      |      |   |        |      |       |      |      |   |  |       |      |        |      |      |   |        |      |        |      |      |   |
| Matrix   | Honey   |                             |   | Pollen                      |                                       |                                 |  |         |        |   |                              |                             |                                       |                                 |   |       |      |        |        |      |   |                             |      |       |      |      |   |  |   |      |        |      |      |   |        |      |       |      |      |   |  |       |      |        |      |      |   |        |      |        |      |      |   |
| Fortification level (mg/kg)  | 0.01  | 0.10                        | 0.01  | 0.10                        |                                       |                                 |  |         |        |   |                              |                             |                                       |                                 |   |       |      |        |        |      |   |                             |      |       |      |      |   |  |   |      |        |      |      |   |        |      |       |      |      |   |  |       |      |        |      |      |   |        |      |        |      |      |   |
| <b>RSD for each fortification level</b>  | 2.0%  | 1.8%                        | 2.0%  | 0.8%                        |                                       |                                 |  |         |        |   |                              |                             |                                       |                                 |   |       |      |        |        |      |   |                             |      |       |      |      |   |  |   |      |        |      |      |   |        |      |       |      |      |   |  |       |      |        |      |      |   |        |      |        |      |      |   |
| All RSD determined were less than 20%, the method therefore fulfils the requirements of residue analytical methods according to SANCO/3029/99 rev.4. |   |                             |   |                             |                                       |                                 |  |         |        |   |                              |                             |                                       |                                 |   |       |      |        |        |      |   |                             |      |       |      |      |   |  |   |      |        |      |      |   |        |      |       |      |      |   |  |       |      |        |      |      |   |        |      |        |      |      |   |
| <b>Specificity</b>   | The method is able to determine azoxystrobin, prothioconazole and prothioconazole-desthio in presence of honey and pollen. This was checked by analysing control and spiked specimens to verify the absence of interfering peaks. No interfering peaks were present at > 30% of the LOQ. The analyses were carried out by LC-MS/MS, monitoring two transitions for each analyte. The method was considered highly specific, thus the use of an alternative method was not necessary.  |                             |   |                             |                                       |                                 |  |         |        |   |                              |                             |                                       |                                 |   |       |      |        |        |      |   |                             |      |       |      |      |   |  |   |      |        |      |      |   |        |      |       |      |      |   |  |       |      |        |      |      |   |        |      |        |      |      |   |
| <b>Recoveries</b>  | <table><tr><th>Analyte</th><th>Matrix</th><th>Fortification level (mg/kg)</th><th>Mean recovery Percentage (%)</th><th>Standard deviation (SD) (%)</th><th>Relative standard deviation (RSD) (%)</th><th>Number of fortified samples (n)</th></tr><tr><td rowspan="2"><b>Azoxystrobin</b><br/>(Transition 404.2 &gt; 329.0)</td><td>Honey</td><td>0.01</td><td>105.0%</td><td>2.3%</td><td>2.2%</td><td>5</td></tr><tr><td>Pollen</td><td>0.01</td><td>99.4%</td><td>2.3%</td><td>2.3%</td><td>5</td></tr><tr><td rowspan="2"><b>Prothioconazole</b><br/>(Transition 342.1 &gt; 179.9)</td><td>Honey</td><td>0.01</td><td>109.9%</td><td>4.0%</td><td>3.6%</td><td>5</td></tr><tr><td>Pollen</td><td>0.01</td><td>97.0%</td><td>5.8%</td><td>6.0%</td><td>5</td></tr><tr><td rowspan="2"><b>Prothioconazole-desthio</b><br/>(Transition 312.0 &gt; 124.9)</td><td>Honey</td><td>0.01</td><td>109.9%</td><td>2.1%</td><td>1.9%</td><td>5</td></tr><tr><td>Pollen</td><td>0.01</td><td>101.0%</td><td>1.8%</td><td>1.8%</td><td>5</td></tr></table> <p>Recoveries and precision data for the qualifier transition comply with the requirements of residue analytical methods as mean recoveries at the LOQ level are within the range 70-110% and RSD is less than 20%.</p> |                             |   |                             |                                       |                                 |  | Analyte | Matrix | Fortification level (mg/kg)                                 | Mean recovery Percentage (%) | Standard deviation (SD) (%) | Relative standard deviation (RSD) (%) | Number of fortified samples (n) | <b>Azoxystrobin</b><br>(Transition 404.2 > 329.0) | Honey | 0.01 | 105.0% | 2.3%   | 2.2% | 5 | Pollen                      | 0.01 | 99.4% | 2.3% | 2.3% | 5 | <b>Prothioconazole</b><br>(Transition 342.1 > 179.9) | Honey                                   | 0.01 | 109.9% | 4.0% | 3.6% | 5 | Pollen | 0.01 | 97.0% | 5.8% | 6.0% | 5 | <b>Prothioconazole-desthio</b><br>(Transition 312.0 > 124.9) | Honey | 0.01 | 109.9% | 2.1% | 1.9% | 5 | Pollen | 0.01 | 101.0% | 1.8% | 1.8% | 5 |
| Analyte  | Matrix  | Fortification level (mg/kg) | Mean recovery Percentage (%)                                | Standard deviation (SD) (%) | Relative standard deviation (RSD) (%) | Number of fortified samples (n) |  |         |        |   |                              |                             |                                       |                                 |   |       |      |        |        |      |   |                             |      |       |      |      |   |  |   |      |        |      |      |   |        |      |       |      |      |   |  |       |      |        |      |      |   |        |      |        |      |      |   |
| <b>Azoxystrobin</b><br>(Transition 404.2 > 329.0)  | Honey   | 0.01                        | 105.0%  | 2.3%                        | 2.2%                                  | 5                               |  |         |        |   |                              |                             |                                       |                                 |   |       |      |        |        |      |   |                             |      |       |      |      |   |  |   |      |        |      |      |   |        |      |       |      |      |   |  |       |      |        |      |      |   |        |      |        |      |      |   |
|  | Pollen  | 0.01                        | 99.4%   | 2.3%                        | 2.3%                                  | 5                               |  |         |        |   |                              |                             |                                       |                                 |   |       |      |        |        |      |   |                             |      |       |      |      |   |  |   |      |        |      |      |   |        |      |       |      |      |   |  |       |      |        |      |      |   |        |      |        |      |      |   |
| <b>Prothioconazole</b><br>(Transition 342.1 > 179.9)   | Honey   | 0.01                        | 109.9%  | 4.0%                        | 3.6%                                  | 5                               |  |         |        |   |                              |                             |                                       |                                 |   |       |      |        |        |      |   |                             |      |       |      |      |   |  |   |      |        |      |      |   |        |      |       |      |      |   |  |       |      |        |      |      |   |        |      |        |      |      |   |
|  | Pollen  | 0.01                        | 97.0%   | 5.8%                        | 6.0%                                  | 5                               |  |         |        |   |                              |                             |                                       |                                 |   |       |      |        |        |      |   |                             |      |       |      |      |   |  |   |      |        |      |      |   |        |      |       |      |      |   |  |       |      |        |      |      |   |        |      |        |      |      |   |
| <b>Prothioconazole-desthio</b><br>(Transition 312.0 > 124.9)   | Honey   | 0.01                        | 109.9%  | 2.1%                        | 1.9%                                  | 5                               |  |         |        |   |                              |                             |                                       |                                 |   |       |      |        |        |      |   |                             |      |       |      |      |   |  |   |      |        |      |      |   |        |      |       |      |      |   |  |       |      |        |      |      |   |        |      |        |      |      |   |
|  | Pollen  | 0.01                        | 101.0%  | 1.8%                        | 1.8%                                  | 5                               |  |         |        |   |                              |                             |                                       |                                 |   |       |      |        |        |      |   |                             |      |       |      |      |   |  |   |      |        |      |      |   |        |      |       |      |      |   |  |       |      |        |      |      |   |        |      |        |      |      |   |
| <b>LOQ</b>   | The limit of quantification (LOQ) is the lowest validated level where a mean recovery within the range 70-110% with a RSD less than 20% could be obtained.<br>The LOQ was set at 0.01 mg/kg in honey and pollen for each analyte.   |                             |   |                             |                                       |                                 |  |         |        |   |                              |                             |                                       |                                 |   |       |      |        |        |      |   |                             |      |       |      |      |   |  |   |      |        |      |      |   |        |      |       |      |      |   |  |       |      |        |      |      |   |        |      |        |      |      |   |
| <b>Comment</b>   | Stability results for extracts<br>The stability of extracts during frozen storage (≤ -18°C) was investigated. The results indicate a good stability for at least 7 days for honey and 14 days for pollen for all analytes<br>Stability results for matrix matched standard solutions<br>The stability of matrix matched standard solutions during frozen storage (≤ -18°C) was investigated. The results  |                             |   |                             |                                       |                                 |  |         |        |   |                              |                             |                                       |                                 |   |       |      |        |        |      |   |                             |      |       |      |      |   |  |   |      |        |      |      |   |        |      |       |      |      |   |  |       |      |        |      |      |   |        |      |        |      |      |   |

## 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 selectivi-

ty, accuracy and precision, the analytical methods were applied successfully for each analytical set when analysing the specimens of the study