REGISTRATION REPORT

# Part B

# Section 5

# Analytical Methods

Detailed summary of the risk assessment

Product code: A23282A

Product name: **KAYAK ERA**

Chemical active substances:

Cyprodinil, 225 g/L
Prothioconazole, 75 g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(New product authorization)

Applicant: XXXX

Submission date: July 2022

Evaluation date: March 2023

MS Finalisation date: December 2023

# Version history

| When | What |
|---|---|
| July 2022 | dRR submission |
| March 2023 | Initial RR |
| December 2023 | RR by zRMS after comments |
| | |

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# 5 Analytical methods

## 5.1 Conclusion and summary of assessment

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

Noticed data gaps are: none

Sufficiently sensitive and selective analytical methods are available for all analytes included in the residue definitions.
The applicant's dRR was not rewritten by the ZRMS and the RR resulting from the ZRMS' evaluation was prepared by an insertion on the grey background into the original dRR ZRMS' coments/correction.
Cyprodinil: The applicant (see Appendix 2) provided a wide range of acceptable validated LC-MS/MS methods (AG-631B, AG-597B modifications, REM 141.10, ECO_019_01B, GRM010.06A), HPLC/UV method (ECO_019_03B) - for data generation in oily, watery, acidic, and dry plant matrices or various eco media and animal matrices (*orange, apple, lettuce, barley and wheat grain, barley straw, wheat forage and hay, tomato, almond nut meat and hull, carrot, potato, cherry, peach, kiwi, strawberry, grape, blackcurrant, onion, fresh peas (with pods), dried beans, melon, asparagus, celery, witloof chicory, canola oil, oilseed rape, sucrose solution, Elendt M4 test medium, salt water, reconstituted water, OECD test medium, milk, fat, liver, kidney, muscle and eggs*).
Moreover the applicant provided a set of acceptable fully validated methods for post-authorization control and monitoring purposes: LC-MS/MS method DFG S 19 applicable in high acid matrices (ILV for *strawberry*), and dry matrices (ILV for *barley grain*); QuEChERS/LC-MS/MS method applicable in high water matrices (ILV for *lettuce*), high oil matrices (ILV for *oilseed rape*), and high starch (ILV for *barley grain*). For animal matrices (ILV for *milk, whole egg and liver*) the fully validated LC-MS/MS method GRM010.06A was provided. For *blood* matrix the QuEChERS/LC-MS/MS method was validated with no ILV. For honey the provided QuEChERS/LC-MS/MS was fully validated.
Furthermore fully validated LC-MS/MS GRM010.07A method for determination of cyprodinil, CGA249287, and CGA275535 in water was provided as well as the validated LC-MS/MS methods for cyprodinil, CGA249287, CGA275535 and CGA321915 in soil (method GRM010.08B) and cyprodinil in air (method GRM010.09A).
Prothioconazole:
Methods for the determination of prothioconazole in plant and animal matrices and body fluids (method QuEChERS, 00655/M002, 01009, 01471) were evaluated during the EU review and were considered acceptable. For prothioconazole residues in soil, water, and air the applicant did not provide the original studies on method modifications which were presented in Appendix 2 for completion as new data (method 00610/ M001, 00684/M001, 00731/M001, 01387/M002). Therefore, the assessment of these data has been omitted. The data, as mostly confirmatory for already agreed validated prothioconazole methods were not necessary in the context of the approval. In this context noticed no data gaps for both actives.

| Commodity/crop | Supported/ Not supported |
|---|---|
| Cereals/wheat | Yes |
| Cereals/barley | Yes |
| Cereals/rye | Yes |
| Cereals/oat | Yes |
| Cereals/triticale | Yes |

## 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)

The plant protection product A23282A has not been reviewed at EU level as a consequence of the review of Prothioconazole or Cyprodinil.

An overview on the acceptable methods and possible data gaps for analysis of Prothioconazole and Cyprodinil in plant protection product A23282A is provided as follows:

| | |
|---|---|
| Comments of zRMS: | This method is accepted for analysing Prothioconazole and Cyprodinil in the PPP. |

| | |
|---|---|
| Reference: | KCP 5.1.1 |
| Report | SF-1115/1 – Determination of Prothioconazole and Cyprodinil in Formulation EC by HPLC, Kirchkesler, A., Mink, C.2021, Method No. SF-1115/1 XXXX File No. VV-928725 |
| Guideline(s): | None (no guideline required) |
| Deviations: | None |
| GLP: | No |
| Acceptability: | Yes |

| | |
|---|---|
| Reference: | KCP 5.1.1 |
| Report | A23282A – Validation of Analytical Method SF-1115/1, Mink C., 2021, Report No. CHMU201133, XXXX File No. VV-928727 |
| Guideline(s): | SANCO/3030/99/rev.5<br>"Validation of analytical methods for active constituents and agricultural products" document dated 1 July 2014 by the Australian Pesticides and Veterinary Medicines Authority (APVMA) |
| Deviations: | None |
| GLP: | Yes |
| Acceptability: | Yes |

**Materials and methods**

Prothioconazole and Cyprodinil are determined simultaneously with analytical method SF-1115/1, a liquid chromatography method, using a Thermo Scientific HPLC system with a Kinetex C18 column (Length: 100 mm, internal diameter: 4.6 mm, particle size 2.6 µm, at 40°C), UV detection and an external standard. For separation, an Acetonitrile/0.1 % v/v aqueous Phosphoric acid gradient as mobile phase was used. Quantification was obtained by comparing peak areas of test samples with the areas from calibrated analytical reference solutions.

**Table 5.2-1: Material and method of SF-1115/1 for the determination of active substances Prothioconazole and Cyprodinil in plant protection product A23282A**

| | | | |
|---|---|---|---|
| **Instrument** | Thermo Scientific Ultimate 3000 | | |
| Dwell Volume | 800 µL | | |
| **Detector** | | | |
| Wavelength | 290 nm (0 min.)<br>254 nm (3.5 min.) | | |
| Bandwidth | 4 nm (0 min.)<br>4 nm (3.5 min.) | | |
| **Column Description** | | | |
| Stationary Phase | Kinetex C18 | | |
| Length | 100 mm | | |
| Internal Diameter | 4.6 mm | | |
| Particle Size | 2.6 µm | | |
| **Column Temperature** | 40°C | | |
| **Injection Volume** | 5 µL | | |
| **Total Run Time** | 10 min. | | |
| **Flow Rate** | 1.0 mL/min. | | |
| **Typical Backpressure** | 300 bar (at start, just for information) | | |
| **Mobile Phase** | A Acetonitrile<br>B 0.1% v/v aqueous phosphoric acid | | |
| | **Time (min.)** | **%A** | **%B** |
| | 0 | 40 | 60 |
| | 5.0 | 90 | 10 |
| | 7.0 | 90 | 10 |
| | 7.1 | 40 | 60 |
| | 10 | 40 | 60 |
| **Retention time** | Cyprodinil: 2.33 min.<br>Prothioconazole: 4.33 min. | | |

**Validation - Results and discussions**

The following validation of the analytical method for the determination of Prothioconazole and Cyprodinil in formulation A23282A has not previously been reviewed and is provided in support of this assessment.

Full validation of the method SF-1115/1 has been conducted for A23282A. The details are summarized in the table 5.2.-2 below:

**Table 5.2-2: Methods suitable for the determination of active substances Prothioconazole and Cyprodinil in plant protection product A23282A**

| | **Prothioconazole** | **Cyprodinil** |
|---|---|---|
| **Author(s), year** | Mink C., 2021 | |
| **Principle of method** | HPLC, UV | |
| **Linearity**<br>n = 6 (2 determinations each)<br><br>(correlation coefficient, expressed as r) | Linear between 32.8 µg/mL – 98.4 µg/mL, corresponding to 50 % - 150 % of prescribed weight of active ingre-dient(s)<br><br>r = 0.99995<br>Y = 0.171*X + 0.062 | Linear between 95 µg/mL – 285 µg/mL, corresponding to 50 % - 150 % of prescribed weight of active ingre-dient(s)<br><br>r = 0.99987<br>Y = 0.102*X + 0.436 |
| **Precision – Repeatability Mean**<br>n = 6 (double injection) | Mean concentration = 7.30 % w/w<br>RSD = 1.21 %<br>$RSD_r$ (mod. Horwitz) = 1.99 %<br>Horrat = 0.607 | Mean concentration = 21.8 % w/w<br>RSD = 1.15 %<br>$RSD_r$ (mod. Horwitz) = 1.69 %<br>Horrat = 0.685 |
| **Accuracy - Recovery**<br>n = 4 (2 determinations each)<br><br>in a range of 70 % to 130 % of pre-scribed weight of active ingredi-ent(s) | Recovery obtained L70 102.7 %<br>Recovery obtained L90 98.7 %<br>Recovery obtained L110 98.8 %<br>Recovery obtained L130 98.5 %<br><br>Mean recovery: 99.7 % | Recovery obtained L70 100.5 %<br>Recovery obtained L90 99.8 %<br>Recovery obtained L110 99.1 %<br>Recovery obtained L130 99.1 %<br><br>Mean recovery: 99.6 % |
| **Interference/ Specificity** | No significant co-elution | No significant co-elution |
| **Comment** | The method is acceptably validated | The method is acceptably validated |

**Conclusion**

The method has been shown to be specific for the determination of Prothioconaole and Cyprodinil in the product A23282A and no significant interference was observed. Based on the results for linearity, precision, accuracy and specificity the method is suitable for the specific, accurate and precise determination of Prothi-oconazole and Cyprodinil in product A23282A.

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

Toluene and Prothioconazole-desthio (2-(1-chlorocyclopropyl)1-(2-chlorophenyl)-3-(1,2,4-triazol-1-yl)-propan-2-ol (EXC5578) are relevant impurities prothioconazole technical material, that could be present in A23282A. Analytical methods have been used for the determination of toluene and Prothioconazole-desthio (2-(1-chlorocyclopropyl)1-(2-chlorophenyl)-3-(1,2,4-triazol-1-yl)-propan-2-ol (EXC5578) in A23282A.

**Toluene (CIPAC MT 198)**

The determination of toluene in product A23282A was conducted by analytical method SD-1540/1, that has previously developed for the determination of toluene in formulated products and validated for formulation A16283D. Method SD-1540/1 (headspace GC) is equivalent to CIPAC method MT 198.

| | |
|---|---|
| Comments of zRMS: | The method is accepted for analysing Toluene in the PPP. Applicant provided the validation study for the different formulation containing difenoconazole as the a.s. (A16283D). Validation data presented in the VV-942444 document confirmed the method's usefulness for the A23282A (chromatograms, revovery, specificity, linearity range and the LOQ) as well. There are no data on precision tested for the A23282A formulation. Nevertheless. from pragmatic point of view, knoving that this SD-1540/1 method is equivalent to CIPAC method MT 198 it allows to use and accept the method for quantification purpose in the A23282A regardless of some potential deficiencies under the Sanco/3030/99 rev.5. After all, CIPAC MT 198 doen't require any validation to be accepted. |

| | |
|---|---|
| Reference: | KCP 5.1.1 |
| Report | Analytical Method SD-1540/1– Determination of Toluene in Formulation by Headspace Gas Chromatography, Adolph S., 2011, XXXX File No. VV-127729 |
| Guideline(s): | None (no guideline required) |
| Deviations: | None |
| GLP: | No |
| Acceptability: | Yes |

| | |
|---|---|
| Reference: | KCP 5.1.1 |
| Report | A16283D - Validation of analytical method SD-1540/1 - toluene in A16283D, de Benedictis S., 2011, Report No. 123787, XXXX File No. VV-400661 |
| Guideline(s): | SANCO/3030/99/rev.4 |
| Deviations: | None |
| GLP: | Yes |
| Acceptability: | Yes |

| | |
|---|---|
| Reference: | KCP 5.1.1 |
| Report | Statement on Validation of the analytical method SD-1540/1 for the determination of toluene in A23282A prothioconazole/cyprodinil EC (075/225), Krauss S., 2011, XXXX File No. VV-942444 |
| Guideline(s): | SANCO/3030/99/rev.4 |
| Deviations: | None |
| GLP: | Yes |
| Acceptability: | Yes |

**Materials and methods**

The relevant impurity toluene is determined with headspace gas chromatography (analytical method SD-1540/1) on a 30m fused silica DB-624 column using helium as carrier gas. Column temperature: 50-280°C. Detection was by FID and quantification by standard addition method (internal standard).

**Validation - Results and discussions**

Full validation of the method SD-1540/1 has been conducted for A6283D.

The following validation of the analytical method for the determination of toluene in formulation performed on A23282A has not previously been reviewed and is provided in support of this assessment.

The details are summarized in the Table 5.2-3 below:

**Table 5.2-3: Method suitable for the determination of the relevant impurity EXC5578 in plant protection product (PPP) A23282A**

| **Relevant impurities in prothioconazole** | **toluene**<br>**Max. content in A23282A < 0.4 g/kg**<br>**( < 5 g/kg compared to prothioconazole tech.)** |
|---|---|
| **Author(s), year** | Krauss S. |
| **Principle of method** | Headspace GC |

| **Relevant impurities in prothioconazole** | **toluene**<br>**Max. content in A23282A < 0.4 g/kg**<br>**( < 5 g/kg compared to prothioconazole tech.)** |
|---|---|
| **Linearity**<br>n = 3 (2 determinations each)<br><br>(correlation coefficient, expressed as r) | Linear between 0.05% to 1.00% relative to the content of Prothioconazole.<br><br>r = 1.0000<br>Y = 15.661*X – 0.011 |
| **Precision – Repeatability Mean**<br>n = 5 (double injection) | Mean concentration = 0.2485 % (relative to content of active substance)<br>RSD = 0.93 % |
| **Accuracy - Recovery**<br>n = 3 (2 determinations each) | Recovery obtained (Level: 0.05%) 102.4 %<br>Recovery obtained (Level: 0.26%) 100.2 %<br>Recovery obtained (Level: 1.02%) 100.3 %<br><br>Mean recovery: 101.5 % |
| **Limit of Quantification (LoQ)** | 500 mg/kg (0.05%) |
| **Interference/ Specificity** | No significant interference |
| **Comment** | The method is acceptably validated |

**Conclusion**

The method has been shown to be specific for the determination of toluene in the product performed on A23282A and no significant interference was observed. Based on the results for linearity, precision, accuracy, specificity and Limit of Quantification (LoQ) the method is suitable for the specific, accurate and precise determination of toluene in product A23282A.

**Prothioconazole-desthio (2-(1-chlorocyclopropyl)1-(2-chlorophenyl)-3-(1,2,4-triazol-1-yl)-propan-2-ol (EXC5578)**

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

| | |
|---|---|
| Reference: | KCP 5.1.1 |
| Report | Analytical Method SD-2433/1 – Determination of Prothioconazole Relevant Impurity EXC5578 in Formulations by LC/MS$^2$, Burkhard R. & Heintz K., 2021, Method No. SD-2433/1 available, XXXX File No. VV-928726 |
| Guideline(s): | None (no guideline required) |
| Deviations: | None |
| GLP: | No |
| Acceptability: | Yes |

| | |
|---|---|
| Reference: | KCP 5.1.1 |
| Report | A23282A – Validation of Analytical Method SD-2433/1, Heintz K., 2021, Report No. CHMU210197, XXXX File No. VV-928724 |
| Guideline(s): | SANCO/3030/99/rev.5<br>"Validation of analytical methods for active constituents and agricultural products" document dated 1 July 2014 by the Australian Pesticides and Veterinary Medicines Authority (APVMA) |
| Deviations: | None |
| GLP: | Yes |
| Acceptability: | Yes |

**Materials and methods**

The relevant impurity Prothioconazole-desthio (2-(1-chlorocyclopropyl)1-(2-chlorophenyl)-3-(1,2,4-triazol-1-yl)-propan-2-ol (EXC5578) is determined with analytical method SD-2433/1, a liquid chromatography method using a Thermo Vanquish UHPLC system with a Kinetex C18 column (Length: 150 mm, internal diameter: 4.6 mm, particle size 2.6 µm, at 30°C) and MS detection. For separation, an Acetonitrile / 0.1 % v/v aqueous Trifluoroacetic acid gradient as mobile phase was used. This method uses the standard addition procedure, which implies that test substance samples are spiked with several levels of EXC5578 to obtain a multi-level calibration curve. One of the samples is prepared without the addition of EXC5578, as it is from this sample that the actual content of EXC5578 can be calculated using the generated calibration curve Due to the fact that the analyte of interest, in this case EXC5578, is directly added to the sample, all sample matrix effects with a potential influence on specificity, linearity, recovery, repeatability or the limit of quantification, can be accounted for.

**Table 5.2-4: Material and method of SD-2433/1 for the determination of relevant impurity EXC5578 in plant protection product A23282A**

| | | | | |
|---|---|---|---|---|
| **Instrument** | Thermo Vanquish UHPLC | | | |
| **Detector** | Thermo Orbitrap ID-X | | | |
| **Scan Description** | Orbitrap resolution 15000 | | | |
| | Quadrupole isolaton m/z 309 to m/z 318 | | | |
| **Column Description** | | | | |
| Type | Kinetex polar C18 | | | |
| Length | 150 mm | | | |
| Inside Diameter | 4.6 mm | | | |
| Particle Size | 2.6 µm | | | |
| **Column Temperature** | 30°C | | | |
| **Injection Volume** | 5 µL | | | |
| **Total Run Time** | 10 min. | | | |
| **Mobile Phase** | A Acetonitrile<br>B 0.1 % v/v aqueous trifluoroacetic acid | | | |
| | **Time (min.)** | **%A** | **%B** | **Flow rate (mL/min.)** |
| | 0 | 30 | 70 | 0.8 |
| | 10 | 95 | 5 | 0.8 |
| **Retention time** | EXC5578: 7.2 min. | | | |

**Validation - Results and discussions**

The following validation of the analytical method for the determination of EXC5578 in formulation performed on A23282A has not previously been reviewed and is provided in support of this assessment.

Full validation of the method SD-2433/1 has been conducted for A23282A. The details are summarized in the Table 5.2-5 below:

**Table 5.2-5: Method suitable for the determination of the relevant impurity EXC5578 in plant protection product (PPP) A23282A**

| **Relevant impurities in prothioconazole** | **EXC5578**<br>**Max. content in A23282A < 0.4 g/kg**<br>**( < 5 g/kg compared to prothioconazole tech.)** |
|---|---|
| **Author(s), year** | Heintz K. |
| **Principle of method** | UHPLC, MS |
| **Linearity**<br>n = 6 (2 determinations each)<br><br>(correlation coefficient, expressed as r) | Linear between 194 mg/kg to 618 mg/kg relative to the content of Prothioconazole.<br><br>r = 0.9996<br>Y = 28340.36*X + 81062.64 |
| **Precision – Repeatability Mean**<br>n = 6 (double injection) | Mean concentration = 389.01 mg/kg<br>RSD = 5.08 %<br>$RSD_r$ (mod. Horwitz) = 6.18 %<br>Horrat = 0.82 |
| **Accuracy - Recovery**<br>n = 5 (2 determinations each) | Recovery obtained (Level: 194.79 mg/kg) 101.4 %<br>Recovery obtained (Level: 293.30 mg/kg) 99.8 %<br>Recovery obtained (Level: 379.49 mg/kg) 102.4 %<br>Recovery obtained (Level: 492.71 mg/kg) 97.9 %<br>Recovery obtained (Level: 617.53 mg/kg) 100.4 %<br><br>Mean recovery: 100.4 % |
| **Limit of Quantification (LoQ)** | 200 mg/kg |
| **Interference/ Specificity** | The spcificity and interference are established using a specific detection technique (MS) and standard addition mode |
| **Comment** | The method is acceptably validated |

**Conclusion**

The method has been shown to be specific for the determination of EXC5578 in the product performed on A23282A and no significant interference was observed. Based on the results for linearity, precision, accuracy, specificity and Limit of Quantification (LoQ) the method is suitable for the specific, accurate and precise determination of EXC5578 in product A23282A.

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

There are no relevant formulants in formulation A23282A, therefore no method is required.

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

No CIPAC method is available for the determination of Cyprodinil or Prothioconazole in A23282A.

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

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

**Table 5.2-6: Validated methods for the generation of pre-authorization data for Cyprodinil in soil, water, air (KCP 5.1.2.1 in support of environmental fate studies)**

| **.Component of residue definition: Cyprodinil** | | | | |
|---|---|---|---|---|
| **Method type** | **Matrix type** | **Method LOQ** | **Principle of method (i.e. GC-MS or HPLC-UV)** | **Author(s), year / missing / EU agreed** |
| Primary | *Soil* | 0.01 mg/kg | HPLC-UV<br>Cyprodinil, CGA249287 | Method:<br>Dieterle, 1992<br>Report No. REM 141.03 (VV-375196)<br><br>EU agreed (2005) |
| Primary | | 0.01 mg/kg | HPLC-UV<br>Cyprodinil, CGA249287, CGA275535 | Method:<br>Tribolet, 2000<br>Report No. REM 141.08 (VV-311756)<br><br>EU agreed (2005) |
| Primary / Confirmatory* | | 0.01mg/kg | LC-MS/MS<br>Cyprodinil, CGA249287, CGA275535, CGA321915 | Method:<br>Allen, 2018<br>Report No. GRM010.08B (VV-128139)<br><br>Validation:<br>Allen, 2015<br>Report No. CEMR-6716-REG (VV-411986)<br><br>**New data** |
| Primary | Water | Potable:<br>0.05 µg/L | HPLC-UV | Method:<br>Lanter, 1990/Kissling, 1995<br>Report No. REM 141.02 (VV-125159)<br><br>EU agreed (2005) |
| Primary | | Potable:<br>0.05 µg/L<br>Surface water:<br>0.10 µg/L | HPLC-UV | Method:<br>Tribolet 2000<br>Report No. REM 141.07 (VV-123949)<br><br>EU agreed (2005) |
| Primary | | Potable:<br>0.05 µg/L<br>Surface water:<br>0.10 µg/L | HPLC-UV | Method:<br>Tribolet, 2000<br>Report No. REM 141.08 (VV-123948)<br><br>EU agreed (2005) |

| .Component of residue definition: Cyprodinil | | | | |
|---|---|---|---|---|
| **Method type** | **Matrix type** | **Method LOQ** | **Principle of method (i.e. GC-MS or HPLC-UV)** | **Author(s), year / missing / EU agreed** |
| Primary / Confirmatory* | | 0.05 µg/L | LC-MS/MS Cyprodinil, CGA249287, CGA275535 | Method:<br>Allen, Brooks, Crook, 2015<br>Report No. GRM010.07A (VV-128422)<br><br>Validation:<br>Allen, 2015<br>Report No. CEMR-6728-REG (VV-411056)<br><br>**New data** |
| Primary | Air | 0.5 µg/m$^3$ | HPLC-UV | Method:<br>Tribolet, 2001<br>Report No. REM 141.05 (VV-125054)<br><br>EU agreed (2005) |
| Primary / Confirmatory* | | 0.5 µg/m$^3$ | LC-MS/MS | Method:<br>Edwards & Wiltshire, 2015<br>Report No. GRM010.09A (VV-128327)<br><br>Validation:<br>Wiltshire, 2015<br>Report No. CEMR-6992-REG (VV-411794)<br><br>**New data** |

* Confirmatory method/transition are not required for pre-authorisation methods according to new SANTE/2020/12830, rev 1. Guidance but as confirmatory methods from the EU methods are available and required for monitoring purpose, these are provided for completeness and consistency with section 5.3.2.

**Table 5.2-7: Validated methods for the generation of pre-authorization data for Cyprodinil in soil, water (KCP 5.1.2.2 in support of efficacy studies)**

**Table not included;**

No specific analytical methods were used to support the efficacy data generated on this product.

**Table 5.2-8: Validated methods for the generation of pre-authorization data for Cyprodinil in feed, body fluids and tissues and air (KCP 5.1.2.3 in support of toxicological studies)**

**Table not included;**

No analytical methods were used to support the toxicology data generated on this product.

**Table 5.2-9: Validated methods for the generation of pre-authorization data for Cyprodinil in body fluids, air and any additional matrices used (KCP 5.1.2.4 in support of operator, worker, resident and bystander exposure studies)**

**Table not included;**

No specific operator, worker, resident or bystander exposure studies were conducted to support this product. Consequently no analytical methods were required.

**Table 5.2-10: Validated methods for the generation of pre-authorization data for Cyprodinil in plant and animal products (KCP 5.1.2.5 in support of residues studies)**

| **Component of residue definition for plant and animal products: cyprodinil** | | | | |
|---|---|---|---|---|
| **Method type** | **Matrix type** | **Method LOQ** | **Principle of method (i.e. GC-MS or HPLC-UV)** | **Author(s), year / missing / EU agreed** |
| Primary (REM 141.01) | Dry commodities content *barley grain, wheat grain, barley straw, wheat straw* | 0.01/0.02/0.05 mg/kg | HPLC-UV | ***Method:*** Dieterle, R., 1989 Reports: REM 141.01 (VV-125142)<br><br>***Validation:*** Tribolet, R., 2001 Report: 215/00 (VV-311755) Wurz R. E. M., 1995 Report: ABR-94088 (VV-375091) Beidler, W. T., 1996 Report: AG-631A[(a)] ( VV-125534) Doran, A. M., 2001 Report: 18961 (VV-312893)<br><br>EU agreed (France, 2005) |
| | High water content *apples, cherries, pear, stone fruit (peach, plum, prune and cherry), prune (processed), apple leaves* | 0.01/0.02 mg/kg | | |
| | High acid content *grapes, wine* | 0.01/0.02 mg/kg | | |
| | High oil content *almond hulls, almond nutmeat, pecan nutmeat, canola seed and canola meal* | 0.01/0.1 mg/kg | LC-MS/MS | ***Validation:*** Mazlo, J., 2010 Report: T003062-07 (VV-467356) Sagen, K., 2009 Report: CER 04169/07 (VV-117239)<br><br>**New data** |
| Primary (REM 141.10) | Dry commodities *barley grain, dried beans* | 0.01 mg/kg | LC-MS/MS | ***Method:*** Chaggar, S., 2005 Report: REM 141.10 (VV-125643)<br><br>***Validation:*** Chaggar, S., 2005 Report: RJ3583B (lettuce, orange, sunflower seeds, barley grain) (VV-333019) |
| | High water content *lettuce, apple, lettuce, cherries, peach, onion, fresh peas (with pods), carrot, tomato, melon, celery, asparagus, witloof* | 0.01 mg/kg | | |

| | | | | |
|---|---|---|---|---|
| | *chicory* | | | Richter, S., 2017,<br>Report: P 4186 G (apple) (VV-466898)<br><br>Stouvenot, C, 2018<br>Report: R B8040 (apple, barley grain, barley whole plant) (VV-469881)<br><br>Stouvenot, C, 2018<br>Report: R B7375 (lettuce, cherries, peach, onion, fresh peas (with pods), carrot, tomato, melon, celery, asparagus, witloof chicory, dried beans, strawberry, grapes, blackcurrant) (VV-469301)<br><br>Stouvenot, C., 2020<br>Report: R B9170 (kiwi) (VV-875665)<br><br>**New data** |
| | High acid content *whole orange, strawberry, grapes, blackcurrant, kiwi* | 0.01 mg/kg | | |
| | High oil content *sunflower seed* | 0.01 mg/kg | | |
| Primary (GRM010.02A) | Dry commodities *wheat hay, wheat grain, almond hulls* | 0.01 mg/kg | LC-MS/MS | **See section 5.3.2.2** |
| | High water content *carrot, potato, melon, tomato, wheat forage, apple* | 0.01 mg/kg | | |
| | High oil content *almond nut meat, rape seed* | 0.01 mg/kg | | |
| **Component of residue definition for animal products: cyprodinil** | | | | |
| **Method type** | **Matrix type** | **Method LOQ** | **Principle of method (i.e. GC-MS or HPLC-UV)** | **Author(s), year / missing / EU agreed** |
| Primary (REM 141.06) | Muscle/meat<br>Liver<br>Kidney<br>Blood | 0.01 mg/kg | HPLC-UV (single analyte) | ***Method:***<br>Kissling, M., 1995<br>Report: REM 141.06 (VV-375091)<br><br>***Validation:***<br>Kissling, M., 1995<br>Report: ABR-95075 (blood, liver, kidney, meat, muscle) (VV-375095)<br><br>***ILV:***<br>Van Geluwe, C., 1995<br>Report: AG-635 (liver, kidney, muscle) (VV-125515)<br><br>EU agreed (France, 2005) |

(a) Data derived using HPLC-UV and GC-NPD detectors.

**Table 5.2-11: Methods and relationship to studies presented in document Part B, Section 7**

| Method | Suppored study (Part B Section 7) | |
|---|---|---|
| **Identifier** | **Data Point** | **Report Reference** |
| AG-631B | KCA1 6.1 | TK0003759 |
| | | CER04169/07 |
| REM 141.10 | KCA1 6.3.1 | TK0357541 |
| | KCA1 6.3.2 | TK0223253-REG |
| | | TK0178711 |
| | | TK0178712-REG |
| | | R B5092 |
| | | TK0223256-REG |
| | KCA1 6.6.2 | 37SRX09R03 |
| | | 37SRX09R04 |
| GRM010.02A | | IF-14/03024493 |

**Table 5.2-12: Statement on extraction efficiency**

| | **Method for products of plant and animal origin** |
|---|---|
| Not required, because: | **Extraction Efficiency (SANTE 2017/10632 Rev. 4)**<br><br>Based on SANTE 2017/10632, for renewal of product authorisations for which no change of the MRL is needed, the data requirements used for the latest renewal or approval should be considered. In the case of cyprodinil as an AIR3 compound this application follows the data requirements for the active substance laid down in Regulation (EU) No. 544/2011 and the data requirements for the plant protection product laid down in Regulation (EU) No. 545/2011. Therefore, when considering these data requirements, no additional proof of extraction efficiency is required in the context of this product submission as in SANTE 2017/10632 Rev. 4 guidance (page 19).<br><br>However, should extraction efficiency be required, the extractability of cyprodinil and metabolite residues from crop matrices using 80:20 methanol:water extract solutions has been investigated in peach samples (crop metabolism study reference ABR-97002) and potato and soil samples (crop metabolism study reference PMR 03/96). Both crop metabolism studies were evaluated under Council Directive 91/414/EEC and are presented in the cyprodinil draft Assessment Report (Vol.3, Annex B, Section B.7.1, November 2003); see MCA Section 6.2.1. The majority of radioactive residue was extractable from mature peach fruit (80.1% to 101.4% TRR), peach leaves (103.3% to 119.1% TRR), potato foliage (93.7%), and whole potato and soil (87.3%). These values demonstrate that the solvent system used in AG-631B, REM141.10 and GRM010.02A is adequate to extract residues of cyprodinil and metabolites from crop commodities. |

**Table 5.2-13: Validated methods for the generation of pre-authorization data for Cyprodinil and CGA321915 metabolite in soil, water and other matrices (KCP 5.1.2.6 in support of ecotoxicological studies)**

| **Method type** | **Matrix type** | **Method LOQ** | **Principle of method (i.e. GC-MS or HPLC-UV)** | **Author(s), year / missing / EU agreed** |
|---|---|---|---|---|
| **Cyprodinil** | | | | |
| Primary | Test medium: Elendt M4 media | 0.01 mg a.s./L | HPLC-UV | Method: Maynard S, 2011 Report No. CEMR-5069 (VV-397982) **New data** |
| Primary | Test medium: Saltwater | Not reported | HPLC-UV | Method: Ward T. *et al*, 1995 Report No. 827-CG (VV-372679) **New data** |
| Primary | Test medium: Saltwater | 0.400 µg/L | HPLC-UV | Method: Drottar KR & Krueger HO, 1999 Report No. 108A-205 (VV-311558) **New data** |
| Primary | Test medium: Pond water/sediment | 0.75 µg/L | LC/MS/MS | Method: Ashwell et al., 2007 Report No. T008777-05 (VV-339018) **New data** |
| **CGA321915** | | | | |
| **Method type** | **Matrix type** | **Method LOQ** | **Principle of method (i.e. GC-MS or HPLC-UV)** | **Author(s), year / missing / EU agreed** |
| Primary | Test medium: Reconstituted test water | 1.03 mg/L | HPLC-UV | Method: Eckenstein H, 2015 Report No. 96733 (VV-411573) **New data** |
| Primary | Test medium: OECD test medium | 1.003 mg/L | HPLC-UV | Method: Eckenstein H, 2015 Report No. 96711 (VV-411271) **New data** |

**Table 5.2-14: Validated methods for the generation of pre-authorization data for A23282A in soil, water and other matrices (KCP 5.1.2.6 in support of ecotoxicological studies)**

| **Method type** | **Matrix type** | **Method LOQ** | **Principle of method (i.e. GC-MS or HPLC-UV)** | **Author(s), year / missing / EU agreed** |
|---|---|---|---|---|
| Primary | Water | 2.11 µg a.s./L | LC-MS/MS | Method:<br>Schuler, 2021<br>Report S21-05725 (VV-931771)<br><br>Validation:<br>Heinicke, 2021<br>Report S21-05703 (VV-928453)<br><br>**New data** |
| Primary | Water | 56.6 µg a.s./L | LC-MS/MS | Method:<br>Schuler, 2021<br>Report S21-05724 (VV-931772)<br><br>Validation:<br>Heinicke, 2021<br>Report S21-05703 (VV-928453)<br><br>**New data** |
| Primary | Bee adult oral feeding solution | 1.0 mg/kg | LC-MS/MS | Method:<br>Ripperger, 2021<br>Report No S21-02794 (VV-946992)<br><br>Validation:<br>Ringli, 2021<br>Report S21-03983 (VV-944813)<br><br>**New data** |
| Primary | Bee larval diet | 1.0 mg/kg | LC-MS/MS | Method:<br>Ripperger, 2021<br>Report No S21-02796 (VV-947029)<br><br>Validation:<br>Ringli, 2021<br>Report S21-03983 (VV-944813)<br><br>**New data** |

**Table 5.2-15: Methods and relationship to studies presented in document Part B, Section 9**

| Method | Supported Study (Part B Section 9) | |
|---|---|---|
| **Identifier** | **Data Point** | **Report Reference** |
| Elendt M4 media | KCP 10.2 | Report CEMR-5069 (study target organism: *"Asellus aquaticus* nymphs*"*) |
| Saltwater HPLC Method 1 | KCP 10.2 | Report 827-CG (study target organism: *"Mysidopsis bahia"*) |
| Saltwater HPLC Method 2 | KCP 10.2 | Report 108A-205 (study target organism: *"Mysidopsis bahia"*) |
| Pond water/sediment | KCP 10.2 | Report T008777-05 *(*study target organism: *"*Aquatic microcosm*")* |
| Reconstituted test water | KCP 10.2 | Report 96733 (study target organism: *"Daphnia magna")* |
| OECD test medium | KCP 10.2 | Report 96711 (study target organism: *"Pseudokirchneriella subcapitata")* |
| ECO_019_01B | KCP 10.2 | Report S21-05725 (study target organism: "*Daphnia magna*") |
| | | Report S21-05724 (study target organism: "*Raphidocelis subcapitata*") |
| ECO_019_03B | KCP 10.3.1 | Report No. S21-02794 (study target organism "*Apis mellifera* adults") |
| | | Report No. S21-02796 (study target organism "*Apis mellifera* larvae") |

**Table 5.2-16: Validated methods for the generation of pre-authorization data for Cyprodinil in water, buffer solutions (KCP 5.1.2.7 in support of physical and chemical properties tests)**

**Table not included;**

No specific analytical methods were used to support the physical and chemical properties generated on this product.

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

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

**Table 5.2-17: Validated methods for the generation of pre-authorization data for Prothioconazole in soil, water, air (KCP 5.1.2.1 in support of environmental fate studies)**

| Component of residue definition: prothioconazole and prothioconazole-desthio | | | | |
|---|---|---|---|---|
| **Method type** | **Matrix type** | **Method LOQ** | **Principle of method (i.e. GC-MS or HPLC-UV)** | **Author(s), year / missing / EU agreed** |
| Primary (00610) | Soil | 0.006 mg/kg (prothioconazole) | HPLC-MS/MS (1 MRM transition) | Schramel, 2000<br>EU agreed (UK, 2007) |
| Confirmatory* | | 0.006 mg/kg | HPLC-MS/MS | **Brumhard, 2005** |

| Component of residue definition: prothioconazole and prothioconazole-desthio | | | | |
|---|---|---|---|---|
| **Method type** | **Matrix type** | **Method LOQ** | **Principle of method (i.e. GC-MS or HPLC-UV)** | **Author(s), year / missing / EU agreed** |
| (00610/M001) | | (prothioconazole) | ($2^{nd}$ MRM transition) | **New data (see 5.3.3.5)** |
| Primary (00086/M038) | | 0.010 mg/kg (prothioconazole-desthio) | GC-MS | Steinhauer, 2001<br>EU agreed (UK, 2007) |
| Primary (00684) | Water | 0.001 mg/kg (prothioconazole)<br>0.05 mg/kg (prothioconazole-desthio) | HPLC-MS/MS (1 MRM transition) | Sommer, 2001<br>EU agreed (UK, 2007) |
| Confirmatory* (00684/M001) | | 0.05 mg/kg (prothioconazole)<br>0.05 mg/kg (prothioconazole-desthio) | HPLC-MS/MS ($2^{nd}$ MRM transition) | **Brumhard, 2005**<br>**New data (see 5.3.3.6)** |
| Primary (00724) | Air | 0.015 µg/$m^3$ (prothioconazole) | HPLC-MS/MS | Maasfeld, 2002<br>EU agreed (UK, 2007) |
| Primary (00731) | | 0.0006 µg/$m^3$ (prothioconazole-desthio) | HPLC-MS/MS (1 MRM transition) | Maasfeld, 2002<br>EU agreed (UK, 2007) |
| Confirmatory* (00731/M001) | | 0.0003 µg/$m^3$ (prothioconazole-desthio) | HPLC-MS/MS ($2^{nd}$ MRM transition) | **Anft & Bardel, 2005**<br>**New data (see 5.3.3.7)** |

* Confirmatory method/transition are not required for pre-authorisation methods according to new SANTE/2020/12830, rev 1. Guidance but as confirmatory methods from the EU methods are available and required for monitoring purpose, these are provided for completeness and consistency with section 5.3.3

**Table 5.2-18: Methods and relationship to studies presented in document Part B, Section 8**

| **Method** | **Supported study (Part B Section 8)** | |
|---|---|---|
| **Identifier** | **Data Point** | **Report Reference** |
| No new studies submitted for prothioconazole | | |

**Table 5.2-19: Validated methods for the generation of pre-authorization data for Prothioconazole in soil, water (KCP 5.1.2.2 in support of efficacy studies)**

**Table not included;**

No specific analytical methods were used to support the efficacy data generated on this product.

**Table 5.2-20: Validated methods for the generation of pre-authorization data for Prothioconazole in feed, body fluids and tissues and air (KCP 5.1.2.3 in support of toxicological studies)**

**Table not included;**

No analytical methods were used to support the toxicology data generated on this product.

**Table 5.2-21: Validated methods for the generation of pre-authorization data for Prothioconazole in body fluids, air and any additional matrices used (KCP 5.1.2.4 in support of operator, worker, resident and bystander exposure studies)**

**Table not included;**

No specific operator, worker, resident or bystander exposure studies were conducted to support this product. Consequently no analytical methods were required.

**Table 5.2-22: Validated methods for the generation of pre-authorization data for Prothioconazole in plant and animal products (KCP 5.1.2.5 in support of residues studies)**

| **Component of residue definition for plant and animal products: Prothioconazole-desthio and all metabolites containing the 2-(1-chlorocyclopropyl)-3-(2- chlorophenyl)-2-hydroxypropyl-2H-1,2,4-triazole moiety, expressed as prothioconazole-desthio (sum of isomers)** | | | | |
|---|---|---|---|---|
| **Method type** | **Matrix type** | **Method LOQ** | **Principle of method (i.e. GC-MS or HPLC-UV)** | **Author(s), year / missing / EU agreed** |
| Primary (00598[a]) | Dry commodities *wheat & barley grain and straw* | 0.01 mg/kg<br>0.05 mg/kg | LC-MS/MS | ***Validation:***<br>Heinemann, 2000<br>Report: 00598<br><br>EU agreed (United Kingdom, 2004, 2018) |
| | High water content *wheat & barley green material* | 0.05 mg/kg | | |
| Primary (00598/M001[a]) | Dry commodities *wheat & barley grain and straw* | 0.01 mg/kg<br>0.05 mg/kg | LC-MS/MS | ***Validation:***<br>Heinemann, 2000<br>Report: 00598/M001<br><br>EU agreed (United Kingdom, 2004, 2018) |
| | High oil content *oilseed rape seed* | 0.01 mg/kg | | |
| | High water content *wheat & barley green material* | 0.05 mg/kg | | |
| | No group *oilseed rape straw, pods & green material* | 0.05 mg/kg | | |
| Primary (00647[b]) | Dry commodities *wheat & barley grain and straw, barley brewing malt* | 0.01 mg/kg<br>0.05 mg/kg<br>0.02 mg/kg | LC-MS/MS | ***Validation:***<br>Heinemann, 2001<br>Report: 00647<br><br>EU agreed (United Kingdom, |
| | High oil content | 0.01 mg/kg | | |

| | | | | |
|---|---|---|---|---|
| | *oilseed rape seed* | | | 2004, 2018) |
| | High water content *wheat & barley green material* | 0.05 mg/kg | | |
| | No group *oilseed rape straw, pods & green material* | 0.05 mg/kg | | |
| Primary (00647/E001[(b)]) | High water content *broccoli & cauliflower curd, Brussels sprout, head & savoy cabbage head, leek shoot, tomato fruit, sugar beet body, sugar beet leaf with root collar, pea pod, pea with pod, pea without pod, spinach leaves* | 0.01 mg/kg | LC-MS/MS | ***Validation:*** Freitag, 2004 Report: 00647/E001 (MR-066/03)<br><br>EU agreed (United Kingdom, 2018) |
| | Dry commodities *Dried pea* | | | |
| Primary (00979[(c)]) | Dry commodities *wheat grain* | 0.01 mg/kg | LC-MS/MS | ***Validation:*** Freitag, 2006 Report: MR-06/023<br><br>EU agreed (United Kingdom, 2004, 2018) |
| Primary (00979/M001[(d)]) | Dry commodities *wheat grain* | 0.01 mg/kg | LC-MS/MS | ***Validation:*** Freitag & Daniels, 2009 Report: MR-08/023<br><br>EU agreed (United Kingdom, 2018) |
| | High water content *potato tuber, tomato fruit* | 0.01 mg/kg | | |
| | High oil content *oilseed rape seed* | 0.01 mg/kg | | |
| | High acid content *orange fruit* | 0.01 mg/kg | | |
| Primary (01013[(a)]) | Dry commodities *wheat grain* | 0.01 mg/kg | LC-MS/MS | ***Validation:*** Brumhard & Stuke, 2008 Report: MR-06/138<br><br>EU agreed (United Kingdom, 2018) |
| | High water content *peas fruit, corn green material* | 0.01 mg/kg | | |
| | High acid content *citrus fruit* | 0.01 mg/kg | | |
| | High oil content *oilseed rape seed* | 0.01 mg/kg | | |
| Primary (00655[(e)]) | Meat<br>Liver<br>Kidney<br>Fat | 0.01 mg/kg | LC-MS/MS | ***Validation:*** Heinemann, 2001 Report: 00655 (MR-537/00) |

| | | | | |
|---|---|---|---|---|
| | | | | EU agreed (United Kingdom, 2004, 2018) |
| Primary (00655/M001[e]) | Milk | 0.004 mg/kg | LC-MS/MS | ***Validation:***<br>Heinemann, 2001<br>Report: 00655/M001 (MR-170/01)<br><br>EU agreed (United Kingdom, 2004, 2018) |
| Primary (JA-009-A08-01[f]) | Eggs<br>Fat<br>Liver<br>Muscle | 0.005 mg/kg | LC-MS/MS | ***Validation:***<br>Sanitised author, 2008<br>Report: RAJAL001<br><br>EU agreed (United Kingdom, 2018) |
| **Component of residue definition for plant and animal commodities:** ***Triazole Alanine, Triazole Acetic Acid, Triazole Lactic Acid, 1,2,4-Triazole*** | | | | |
| **Method type** | **Matrix type** | **Method LOQ** | **Principle of method (i.e. GC-MS or HPLC-UV)** | **Author(s), year / missing / EU agreed** |
| Primary (GRM053.01A[g]) | High water content *potato tuber, barley whole plant, sugar beet top, apple, peach, tomato, lettuce, kale, onion bulb, leek peas with pods, maize whole plant* | 0.01 mg/kg | LC-MS/MS | ***Method:***<br>Gemrot F., 2011<br>Report: GRM053.01A<br><br>***Validation:***<br>Gemrot F., 2011<br>Report: S10-02599-REG<br><br>EU agreed (UK 2018, EFSA 2018) |
| | Dry commodities *wheat grain, maize kernels, wheat straw* | 0.01 mg/kg | | |
| | High acid content *grape, raspberry* | 0.01 mg/kg | | |
| | High oil content *rape seed* | 0.01 mg/kg | | |
| | No group *maize cob without kernels* | 0.01 mg/kg | | |
| | Difficult matrices *tobacco leaves* | 0.01 mg/kg | | |
| Primary (01062/M002) | High water content *apples* | 0.01 mg/kg | LC-MS/MS | ***Method and Validation:***<br>Schmeer, K., Krusell L., 2009<br>Report: MR-09/092 (M-360738-01-1)<br><br>EU agreed (UK 2018, EFSA 2018) |
| | Dry commodities *wheat grain, bean seed* | 0.01 mg/kg | | |
| | High acid content *orange* | 0.01 mg/kg | | |

| | | | | |
|---|---|---|---|---|
| | High oil content *linseed* | 0.01 mg/kg | | |
| Primary (01062/M003) | High water content *tomato, carrot root* | 0.01 mg/kg | LC-MS/MS | ***Method and Validation:*** Class, T., Goecer, M., 2009 Report: P/B 1690 G<br><br>***Validation:*** Class, T., 2010 Report: P 1981 G<br><br>EU agreed (UK 2018, EFSA 2018) |
| | Dry commodities *maize grain, rice grain, barley grain, rice straw* | 0.01 mg/kg | | |
| | High oil content *oilseed rape seed oilseed rape oil* | 0.01 mg/kg | | |
| Primary (01062/M004) | High water content *tomatoes, cucumber, lettuce, cereal green plant, tomato, cucumber, lettuce, apple, melon, pepper, carrot (root), carrot (leaf)* | 0.01 mg/kg | LC-MS/MS | ***Method and validation:*** Class, T., 2011 Reports: P 2383G, M-420638-01-1<br><br>EU agreed (UK 2018, EFSA 2018) |
| | Dry commodities *cereal grain, dry bean seed, cereal straw* | | | |
| | High acid content *grape, orange* | | | |
| | High oil content *oilseed rape (seed)* | | | |
| Primary (001132) | Milk | 0.01 mg/kg | LC-MS/MS | ***Method and Validation***[(g)]: Billian, P, Druskus, M., 2010 Reports: MR-08/201, M-357719-01-1<br><br>EU agreed (UK 2018, EFSA 2018) |
| | Meat | 0.01 mg/kg | | |
| | Liver | 0.01 mg/kg | | |
| | Fat | 0.01 mg/kg | | |
| | Kidney | 0.01 mg/kg | | |
| | Eggs | 0.01 mg/kg | | |

**Component of residue definition for plant and animal commodities: *Triazole Alanine, Triazole Acetic Acid, 1,2,4-Triazole***

| **Method type** | **Matrix type** | **Method LOQ** | **Principle of method (i.e. GC-MS or HPLC-UV)** | **Author(s), year / missing / EU agreed** |
|---|---|---|---|---|
| Primary (The Morse method 160: Method 01062) | High water content *tomatoes* | 0.01 mg/kg | LC/MS-MS | ***Method and Validation:*** Maliani N., 2004 Report: ML03-1081-TTF<br><br>EU agreed (UK 2018, EFSA |
| | High acid content *grapes* | 0.01 mg/kg | | |

| | | | | |
|---|---|---|---|---|
| | High oil content *soybeans* | 0.01 mg/kg | | 2018) |
| | Milk | 0.01 mg/kg[(h)] | | |
| Primary (01062/M001) | High water content *Apple, leek* | 0.01 mg/kg | LC-MS/MS | ***Method and Validation:*** Philipowski, C., Schmeer, K., Billian, P., 2009 Report: MR 08/082<br><br>***Additional Validation:*** Murphy I., 2008 Report: RAJAY006<br><br>EU agreed (UK 2018, EFSA 2018) |
| | Dry commodities *wheat grain, bean seed* | 0.01 mg/kg | | |
| | High acid content *lemon* | 0.01 mg/kg | | |
| | High oil content *linseed* | 0.01 mg/kg | | |
| **Component of residue definition for plant and animal commodities: *Triazole Alanine*** | | | | |
| **Method type** | **Matrix type** | **Method LOQ** | **Principle of method (i.e. GC-MS or HPLC-UV)** | **Author(s), year / missing / EU agreed** |
| Primary (2280) | Dry commodities *cereal grain, cereal straw* | 0.05 mg/kg | GC-NPD | ***Method and Validation:*** Zini G., 1999 Report: 2280<br><br>EU agreed (UK 2018, EFSA 2018) |
| **Component of residue definition for plant and animal commodities: *Triazole Acetic Acid*** | | | | |
| **Method type** | **Matrix type** | **Method LOQ** | **Principle of method (i.e. GC-MS or HPLC-UV)** | **Author(s), year / missing / EU agreed** |
| Primary (2281) | Dry commodities *cereal grain, cereal straw* | 0.04 mg/kg | GC-NPD | ***Method and Validation:*** Zini G., 1999 Report: 2280<br><br>EU agreed (UK 2018, EFSA 2018) |
| Primary (ARAM 217) | High water content *cabbage, sugarbeet root* | 0.5 mg/kg | GC-NPD | ***Method and Validation:*** Davy, G.S., Harradine, K.J., Newcombe, A. and Wheals, I.B., 1992 Report: RJ 1201B<br><br>***Additional recovery data:*** Kwiatkowski, A.S., Robinson N.J., 1995 Report: RJ1932B |
| | Dry commodities *wheat grain, pea* | 0.5 mg/kg | | |

| | | | | |
|---|---|---|---|---|
| | *seed, wheat straw* | | | EU agreed (UK 2018, EFSA 2018) |
| | High oil content *oilseed rape seed* | 0.5 mg/kg | | |
| **Component of residue definition for plant and animal commodities: *Triazole Lactic Acid*** | | | | |
| **Method type** | **Matrix type** | **Method LOQ** | **Principle of method (i.e. GC-MS or HPLC-UV)** | **Author(s), year / missing / EU agreed** |
| Primary (D0905) | High water content *lettuce* | 0.01 mg/kg | LC-MS/MS | ***Method and validation:*** Saha, M., Perez, R., Perez, S., Smith, M. & Patel, D., 2010 Report: 366866 (2010/7013002)<br><br>EU agreed (UK 2018, EFSA 2018) |
| | Dry commodities *wheat grain, navy bean, cereal straw* | 0.01 mg/kg<br>0.05 mg/kg | | |
| | High acid content *orange* | 0.01 mg/kg | | |
| | High oil content *oilseed rape seed* | 0.01 mg/kg | | |
| **Component of residue definition for plant and animal commodities: *1,2,4-Triazole*** | | | | |
| **Method type** | **Matrix type** | **Method LOQ** | **Principle of method (i.e. GC-MS or HPLC-UV)** | **Author(s), year / missing / EU agreed** |
| Primary (2175) | Milk | 0.015 mg/L | GC-NPD | ***Method and Validation:*** Zini G., 1996 Report: 2175<br><br>EU agreed (UK 2018, EFSA 2018) |
| Primary (2199) | Fat | 0.02 mg/kg | GC-NPD | ***Method and Validation:*** Zini G., 1997 Report: 2199<br><br>EU agreed (UK 2018, EFSA 2018) |
| | Muscle | 0.02 mg/kg | | |
| | Liver | 0.02 mg/kg | | |

(a) Analytes measured: Prothioconazole-desthio (and prothioconazole)
(b) Analytes measured: Prothioconazole-desthio
(c) Analytes measured: Prothioconazole-3-hydroxy-desthio, prothioconazole-4-hydroxy-desthio, prothioconazole-5-hydroxy-desthio and prothioconazole-6-hydroxy-desthio (expressed as prothioconazole-desthio)
(d) Analytes measured: Prothioconazole-3-hydroxy-desthio, prothioconazole-4-hydroxy-desthio, prothioconazole-5-hydroxy-desthio, prothioconazole-6-hydroxy-desthio and prothioconazole-α-hydroxy-desthio (expressed as prothioconazole-desthio)
(e) Analytes measured: Prothioconazole-desthio, prothioconazole-3-hydroxy-desthio and prothioconazole-4-hydroxy-desthio
(f) Analytes measured: Prothioconazole-desthio and prothioconazole-4-hydroxy-desthio (and prothioconazole)
(g) The method has been radiovalidated (Koester, J., Weber, E., 2010 report: MEF-09/839 and Koester, J., Weber, E., 2010 report: MEF-09/699)

(h) LOQ for 1,2,4-T in milk is 0.005 mg/kg

**Table 5.2-23: Methods and relationship to studies presented in document Part B, Section 7**

| Method | Suppored study (Part B Section 7) | |
|---|---|---|
| **Identifier** | **Data Point** | **Report Reference** |
| No new studies submitted for prothioconazole | | |

**Table 5.2-24: Statement on extraction efficiency**

| | **Method for products of plant and animal origin** |
|---|---|
| Required, available from: | Desmaris, 2015; Report MR-15/117<br><br>The extraction efficiency was demonstrated by method 01300/M018. The extraction efficiency of the method was evaluated using barley grain, wheat green material, wheat straw and rape seed matrices from nature of residue metabolism studies. Results obtained using the analytical method were equivalent to those obtained in the metabolism study, demonstrating the suitability of this analytical method for the determination of prothioconazole in plant matrices. The extraction efficiency was calculated as the ratio (expressed as percentage) between the average residues measured after extracting the samples according to the procedure and the average residues measured using the procedure of the corresponding metabolism study. Method 01300/M018 meet all necessary criteria (at least 70% of residues extracted compared to metabolism method corresponding to 100%) to sufficiently extract and determine the residues of prothioconazole in plant matrices.<br><br>Heinemann, 2001; Report 00655<br><br>The comparison of the residue analytical method of extraction for animal matrices with the extraction method used in the metabolism study demonstrated the suitability of the analytical method (extracting with an acetonitrile/water solvent system) for the determination of the relevant residue in animal matrices. The extraction efficiency is demonstrated.<br><br>Crook, 2020; Report TK0332801-01<br><br>Extractability data to support solvent extraction systems used in pre-registration residue methodology (e.g. Modification M004 of BCS residue analytical method 01062, and GRM053.01A) has been demonstrated with 14C metabolism studies using an identical solvent system methanol/water (80/20 v/v). The system utilised in the residue methodology extracts > 90% of the total extractable residue which includes triazole metabolites.<br><br>In addition, a number of 14C metabolism studies use extraction using methanol/water mixtures in different |

| | |
|---|---|
| | compositions or the Bligh-Dyer extraction system. Although not identical, the solvent systems comprise methanol/water mixtures with the addition of chloroform (in the case of the Bligh/Dyer & Ting/Dugger) to provide differentiation between lipophilic and hydrophobic residues into separate liquid phases. Triazole metabolite residues are contained in the aqueous/organic methanol/water phase. These studies provide additional supporting data to confirm that methanol/water mixtures are efficient extraction solvents for triazole metabolites. High levels of extractability are achieved ( > 90% of the extractable residue which includes triazole metabolites).<br><br>Analytical method 001132:<br><br>Radio validation of the extraction system was conducted as part of the triazole alanine livestock metabolism studies (Koester and Weber, 2010, VV- 393636; Koester and Weber, 2010, VV- 393635) and therefore extraction efficiency has been demonstrated for this method. |

**Table 5.2-25: Validated methods for the generation of pre-authorization data for Prothioconazole in soil, water and other matrices (KCP 5.1.2.6 in support of ecotoxicological studies)**

**Table not included;** refer to EFSA Conclusion 2007.

**Table 5.2-26: Validated methods for the generation of pre-authorization data for A23282A in soil, water and other matrices (KCP 5.1.2.6 in support of ecotoxicological studies)**

**Table not included;**

No specific analytical methods were used to support the ecotoxicological data on this product.

**Table 5.2-27: Validated methods for the generation of pre-authorization data for Prothioconazole in water, buffer solutions (KCP 5.1.2.7 in support of physical and chemical properties tests)**

**Table not included;**

No specific analytical methods were used to support the physical and chemical properties generated on this product.

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

### 5.3.1 Analysis of the plant protection product (KCP 5.2)

Analytical methods for the determination of the active 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 of cyprodinil (KCP 5.2)

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

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

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

| Matrix | Residue definition | MRL / limit | Reference for MRL/level Remarks |
|---|---|---|---|
| Plant, high water content | Cyprodinil | 0.02 mg/kg | Reg. (EU) 2021/1810 (default LOQ) |
| Plant, high acid content | | 0.02 mg/kg | Reg. (EU) 2021/1810 (default LOQ) |
| Dry commodities | | 0.02 mg/kg | Reg. (EU) 2021/1810 (default LOQ) |
| Plant, high oil content | | 0.02 mg/kg | Reg. (EU) 2021/1810 (default LOQ) |
| Plant, difficult matrices (hops, spices, tea) | | 0.1 mg/kg | Reg. (EU) 2021/1810 (default LOQ) |
| Muscle | The sum of cyprodinil and CGA 304075 (free) expressed as cyprodinil except milk, sum of cyprodinil and CGA 304075 (free and conjugated) expressed as cyprodinil | 0.02 mg/kg | Reg. (EU) 2021/1810 (default LOQ) |
| Milk | | 0.02 mg/kg | Reg. (EU) 2021/1810 (default LOQ) |
| Eggs | | 0.02 mg/kg | Reg. (EU) 2021/1810 (default LOQ) |
| Fat | | 0.02 mg/kg | Reg. (EU) 2021/1810 (default LOQ) |
| Liver, kidney | | 0.02 mg/kg | Reg. (EU) 2021/1810 (default LOQ) |
| Honey | Cyprodinil | 0.05 mg/kg | Reg. (EU) 2021/1810 (default LOQ) |
| Soil (Ecotoxicology) | Cyprodinil | 0.05 mg/kg | Common limit |
| Drinking water (Human toxicology) | Cyprodinil | 0.1 µg/L | General limit for drinking water |
| Surface water (Ecotoxicology) | Cyprodinil | 0.9 µg/L | NOEC mesocosm, SF = 2 Ashwell, J., Benyon, K., Powley, W. and Richardson, M., 2007, XXXX file number: CGA219417/1683 |
| Air | Cyprodinil | 0.09 mg/kg | AOEL systemic: 0.03 mg/kg bw/d (EFSA Scientific report (2005) 51, 1-78) |

| Matrix | Residue definition | MRL / limit | Reference for MRL/level Remarks |
|---|---|---|---|
| Tissue (meat or liver) | Cyprodinil | 0.01 mg/kg | Default LOQ |
| Body fluids | | 0.01 mg/L | Default LOQ |

### 5.3.2.2 Description of analytical methods for the determination of residues cyprodinil in plant matrices (KCP 5.2.1)

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

**Table 5.3-2: Validated methods for food and feed of plant origin**

| Component of residue definition: cyprodinil | | | | |
|---|---|---|---|---|
| **Matrix type** | **Method type** | **Method LOQ** | **Principle of method (i.e. GC-MS or HPLC-UV)** | **Author(s), year / missing / EU agreed** |
| High water content | Primary/confirmatory (DFG S19) | 0.02 mg/kg | GC--MS | **DFG S19 (extended version)**<br>***Validation:***<br>Pelz, S., 2001<br>Report: SYN-0108V (tomatoes, oranges, rape seed, wheat grain) (VV-324358)<br><br>***ILV:***<br>Steinhauer, S., 2001<br>Report: SYN-0109V (tomatoes, wheat grain) (VV-319385)<br><br>EU agreed (France, 2005)<br>---<br>**DFG S19***<br>***Validation:***<br>Lakaschus, S., 2005<br>Report: SYN-0502V (apple, strawberry, rape seed, barley grain) (VV-379854)<br><br>***ILV:***<br>Reichert, N., 2006<br>Report: IF-05/00362978 (strawberry, barley grain) (VV-379810)<br><br>**New data**<br>---<br>**GRM010.02A** |
| | ILV (DFG S19) | 0.02 mg/kg | | |
| | Primary/confirmatory (DFG S19) | 0.01 mg/kg | LC-MS/MS (multi-residue) | |
| | ILV (DFG S19) | 0.01 mg/kg | | |
| | Primary/confirmatory (QuEChERs) | 0.01 mg/kg | LC-MS/MS (multi-residue) | |
| | ILV (QuEChERs) | 0.01 mg/kg | | |
| | Primary/confirmatory (GRM010.02A) | 0.01 mg/kg | | |
| High acid content | Primary/confirmatory (DFG S19) | 0.02 mg/kg | GC--MS | |
| | ILV (DFG S19) | 0.02 mg/kg | | |
| | Primary/confirmatory (DFG S19) | 0.01 mg/kg | LC-MS/MS (multi-residue) | |
| | ILV (DFG S19) | 0.01 mg/kg | | |
| | Primary/confirmatory (QuEChERs) | 0.01 mg/kg | LC-MS/MS (multi-residue) | |
| | ILV (QuEChERs) | 0.01 mg/kg | | |
| High oil content | Primary/confirmatory (DFG S19) | 0.02 mg/kg | GC--MS | |
| | ILV (DFG S19) | 0.02 mg/kg | | |
| | Primary/confirmatory (DFG S19) | 0.01 mg/kg | LC-MS/MS (multi-residue) | |

| Component of residue definition: cyprodinil | | | | |
|---|---|---|---|---|
| **Matrix type** | **Method type** | **Method LOQ** | **Principle of method (i.e. GC-MS or HPLC-UV)** | **Author(s), year / missing / EU agreed** |
| | ILV (DFG S19) | 0.01 mg/kg | | ***Method:***<br>Lin, K. and Manuli, M., 2011<br>Report: GRM010.02A (VV-185044)<br><br>***Validation:***<br>Lin, K., 2011<br>Report: TK0021500 (wheat forage, hay and grain, apples, tomato, almond nut meat and almond hull) (VV-413174)<br><br>***Validation:***<br>Rabello, P., 2019<br>Report: 037SRBR18V16 (carrot roots, potato tubers, melon fruits and tomato fruits) (VV-635386)<br><br>***ILV:***<br>Asekunowo, J., 2015<br>Report: P3866 G (rape seed) (VV-414907)<br><br>**New data**<br>----------<br><br>**<u>QuEChERs</u>**<br>***Validation:***<br>Richter, S., 2017<br>Report: TK0319684 (lettuce, orange, oilseed rape seed, barley grain) (VV-467144)<br><br>***ILV:***<br>Airs, D., 2017<br>Report: TK0319685 QuEChERs (lettuce, barley grain, oilseed rape seed) (VV-467339)<br><br>**New data** |
| | Primary/confirmatory (GRM010.02A) | 0.01 mg/kg | LC-MS/MS (single analyte) | |
| | ILV (GRM010.02A) | 0.01 mg/kg | | |
| | Primary/confirmatory (QuEChERs) | 0.01 mg/kg | LC-MS/MS (multi-residue) | |
| | ILV (QuEChERs) | 0.01 mg/kg | | |
| Dry commodities | Primary/confirmatory (DFG S19) | 0.02 mg/kg | GC--MS | |
| | ILV (DFG S19) | 0.02 mg/kg | | |
| | Primary/confirmatory (DFG S19) | 0.01 mg/kg | LC-MS/MS (multi-residue) | |
| | ILV (DFG S19) | 0.01 mg/kg | | |
| | Primary/confirmatory (QuEChERs) | 0.01 mg/kg | LC-MS/MS (multi-residue) | |
| | ILV (QuEChERs) | 0.01 mg/kg | | |
| | Primary/confirmatory (GRM010.02A) | 0.01 mg/kg | LC-MS/MS (single analyte) | |

* The ILV for DFG S19 method by Steinhauer 2001 was not accepted by EFSA (2005) due to both validations being performed in the same laboratory. Moreover, the LOQ was 0.02 mg/kg, and many of the MRLs for cyprodinil have been set at 0.01 mg/kg. The method has subsequently been validated (Lakaschus, S. 2005) using LC-MS/MS and 2 ion transitions with the LOQ of 0.01 mg/kg. The method has been also independently validated (Reichert, N., 2006).

**Table 5.3-3: Statement on extraction efficiency**

See Table 5.2-12.

### 5.3.2.3 Description of analytical methods for the determination of residues of cyprodinil in animal matrices (KCP 5.2.2)

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

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

| **Component of residue definition: cyprodinil and CGA304075 (free and conjugated), expressed as cyprodinil** | | | | |
|---|---|---|---|---|
| **Matrix type** | **Method type** | **Method LOQ** | **Principle of method (i.e. GC-MS or HPLC-UV)** | **Author(s), year / missing / EU agreed** |
| Milk | Primary/confirmatory (GRM010.06B) | 0.01 mg/kg | LC-MS/MS (single analyte) | **REM141.06**<br>***Validation:***<br>Kissling, M., 1995<br>Report: ABR-95075 (blood, liver, kidney, meat, muscle) (VV-375095)<br><br>***ILV:***<br>Van Geluwe, C., 1995<br>Report: AG-635 (liver, kidney, muscle**)** (VV-125515)<br><br>EU agreed (France, 2005)<br>----------<br>**GRM010.06B[(a)]**<br>Bradford, W. and Langridge, G., 2015<br>Report: GRM010.06A (VV-128138)<br>Bradford, W. and Langridge, G., 2015<br>Report: GRM010.06B (VV-128329)<br><br>***Validation:***<br>Langridge G., 2015<br>Report: CEMR-6729 (animal matrices) (VV-412216)<br><br>***ILV***:<br>Knoch E., 2015<br>Report: IF-15/03135929 (bovine liver, milk, eggs) (VV-412515)<br><br>**New data** |
| | ILV (GRM1010.06B) | 0.01 mg/kg | | |
| Eggs | Primary/confirmatory (GRM010.06B) | 0.01 mg/kg | LC-MS/MS (single analyte) | |
| | ILV (GRM1010.06B) | 0.01 mg/kg | | |
| Muscle/meat | Primary (REM141.06) | 0.01 mg/kg | HPLC-UV (single analyte) | |
| | ILV (REM141.06) | 0.01 mg/kg | | |
| | Primary/confirmatory (GRM010.06B) | 0.01 mg/kg | LC-MS/MS (single analyte) | |
| Fat | Primary/confirmatory (GRM010.06B) | 0.01 mg/kg | LC-MS/MS (single analyte) | |
| Liver | Primary/confirmatory (REM141.06) | 0.01 mg/kg | HPLC-UV (single analyte) | |
| | ILV (REM141.06) | 0.01 mg/kg | | |
| | Primary/confirmatory (GRM010.06B) | 0.01 mg/kg | LC-MS/MS (single residue) | |
| | ILV (GRM1010.06B) | 0.01 mg/kg | | |
| Kidney | Primary/confirmatory (REM141.06) | 0.01 mg/kg | HPLC-UV (single analyte) | |
| | ILV (REM141.06) | 0.01 mg/kg | | |
| | Primary/confirmatory (GRM010.06B) | 0.01 mg/kg | LC-MS/MS (single analyte) | |

(a) The analytical method (GRM010.06A) was updated in order to include additional footnotes in Tables 3 and 4 (recovery tables for CGA304075) to indicate recoveries excluded as outliers via the Grubb's test. This updated method is entitled GRM010.06B. Barring the clarification of outliers in the table, both analytical methods are identical. The update of the method was performed after validation of the analytical method (GRM010.06A).

| Component of residue definition: cyprodinil | | | | |
|---|---|---|---|---|
| **Matrix type** | **Method type** | **Method LOQ** | **Principle of method (i.e. GC-MS or HPLC-UV)** | **Author(s), year / missing / EU agreed** |
| Honey | Primary/confirmatory (QuEChERS) | 0.01 mg/kg | LC-MS/MS (multi-residue) | ***Validation***<br>Harper H., 2022<br>Report: 8485604 (VV-939118)<br>**New data** |
| | ILV (QuEChERS) | 0.01 mg/kg | LC-MS/MS (multi-residue) | ***ILV***:<br>Mechelke J., 2022<br>Report: 20210437 (VV-945895)<br>**New data** |

**Table 5.3-5: Statement on extraction efficiency**

| | **Method for products of animal origin** |
|---|---|
| Not required, because: | **Extraction Efficiency (SANTE 2017/10632 Rev. 4)**<br><br>Based on SANTE 2017/10632, for renewal of product authorisations for which no change of the MRL is needed, the data requirements used for the latest renewal or approval should be considered. In the case of cyprodinil as an AIR3 compound this application follows the data requirements for the active substance laid down in Regulation (EU) No. 544/2011 and the data requirements for the plant protection product laid down in Regulation (EU) No. 545/2011. Therefore, when considering these data requirements, no additional proof of extraction efficiency is required in the context of this product submission as in SANTE 2017/10632 Rev. 4 guidance (page 19).<br><br>However, a study (T019338-04) was conducted to extract and quantify residues of CGA304075, and to optimise the hydrolysis conditions to cleave conjugates of CGA304075 in edible tissues and milk. Radio-labelled cyprodinil (labelled in the 2-position of the pyrimidinyl ring) was administered to a goat to generate milk and tissue samples containing incurred residues of cyprodinil and metabolites for use in method development work. The extraction system used was the same as for method GRM010.06A. Using the acid reflux step, extractable residues were 93.2% TRR in liver and 97.7% in milk. These values demonstrate that a 1 hour reflux in 0.5N HCl followed by extraction in an acetonitrile/water as described for GRM010.06B is adequate to extract residues of cyprodinil and CGA304075 (free and conjugated) from animal commodities. |

### 5.3.2.4 Description of methods for the analysis of cyprodinil in body fluids and tissues (KCP 5.2.3)

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

**Table 5.3-6: Methods for body fluids and tissues**

| Component of residue definition: cyprodinil | | | | |
|---|---|---|---|---|
| **Matrix type** | **Method type** | **Method LOQ** | **Principle of method (i.e. GC-MS or HPLC-UV)** | **Author(s), year / missing / EU agreed** |
| Blood | Primary (REM141.06) | 0.01 mg/kg | HPLC-UV (single analyte) | see Table 5.3-4 |
| | Primary/confirmatory (QuEChERS) | 0.01 mg/kg | LC-MS/MS (multi-residue) | ***Validation***: Richter, S., 2017 Report: TK0319684 (blood) (VV-467144)<br><br>**New data** |

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

### 5.3.2.5 Description of methods for the analysis of cyprodinil in soil (KCP 5.2.4)

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

**Table 5.3-7: Validated methods for soil**

| Component of residue definition: Cyprodinil and metabolites | | | |
|---|---|---|---|
| **Method type** | **Method LOQ** | **Principle of method (*i.e.* GC-MS or HPLC-UV)** | **Author(s), year / missing** |
| Primary | 0.01 mg/kg | HPLC-UV Cyprodinil, CGA249287 | Method: Dieterle, 1992 Report No. REM 141.03 (VV-375196)<br><br>EU agreed (2005) |
| Primary | 0.01 mg/kg | HPLC-UV Cyprodinil, CGA249287, CGA275535 | Method: Tribolet, 2000 Report No. REM 141.08 (VV-311756)<br><br>EU agreed (2005) |
| Primary / Confirmatory * | 0.01mg/kg | LC-MS/MS Cyprodinil, CGA249287, CGA275535, CGA321915 | Method: Allen, 2018 Report No. GRM010.08B (VV-128139)<br><br>Validation: Allen, 2015 Report No. CEMR-6716-REG (VV-411986) |

| Component of residue definition: Cyprodinil and metabolites | | | |
|---|---|---|---|
| **Method type** | **Method LOQ** | **Principle of method (*i.e.* GC-MS or HPLC-UV)** | **Author(s), year / missing** |
| | | | **New data** |

*New data was prepared to provide additional validation data for cyprodinil and its metabolites CGA249287, CGA275535, CGA321915 using a second transition.

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

### 5.3.2.6 Description of methods for the analysis of cyprodinil of water (KCP 5.2.5)

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

**Table 5.3-8: Validated methods for water**

| Component of residue definition: Cyprodinil and metabolites | | | | |
|---|---|---|---|---|
| **Matrix type** | **Method type** | **Method LOQ** | **Principle of method (i.e. GC-MS or HPLC-UV)** | **Author(s), year / missing** |
| Drinking water / Surface water | Primary | Potable: 0.05 µg/L | HPLC-UV | Method: Lanter, 1990/Kissling, 1995 Report No. REM 141.02 (VV-125159)<br><br>EU agreed (2005) |
| | Primary | Potable: 0.05 µg/L Surface water: 0.10 µg/L | HPLC-UV | Method: Tribolet 2000 Report No. REM 141.07 (VV-123949)<br><br>EU agreed (2005) |
| | Primary | Potable: 0.05 µg/L Surface water: 0.10 µg/L | HPLC-UV | Method: Tribolet, 2000 Report No. REM 141.08 (VV-123948)<br><br>EU agreed (2005) |
| | Primary / Confirmatory* | 0.05 µg/L | LC-MS/MS Cyprodinil, CGA249287, CGA275535 | Method: Allen, Brooks, Crook, 2015 Report No. GRM010.07A (VV-128422)<br><br>Validation: Allen, 2015 Report No. CEMR-6728-REG (VV-411056) |

| Component of residue definition: Cyprodinil and metabolites | | | | |
|---|---|---|---|---|
| **Matrix type** | **Method type** | **Method LOQ** | **Principle of method (i.e. GC-MS or HPLC-UV)** | **Author(s), year / missing** |
| | | | | **New data** |
| | ILV | 0.05 µg/L | LC-MS/MS | Kotthof (2015; SYN-036/6-22) |

*New data was prepared to provide additional validation data for cyprodinil and its metabolites CGA249287, CGA275535 sing a second transition.

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

### 5.3.2.7 Description of methods for the analysis of cyprodinil in air (KCP 5.2.6)

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

**Table 5.3-9: Validated methods for air**

| Component of residue definition: Cyprodinil | | | |
|---|---|---|---|
| **Method type** | **Method LOQ** | **Principle of method (i.e. GC-MS or HPLC-UV)** | **Author(s), year / miss-ing** |
| Primary | 0.5 µg/m$^3$ | HPLC-UV | Method:<br>Tribolet, 2001<br>Report No. REM 141.05 (VV-125054)<br><br>EU agreed (2005) |
| Primary / Confirmatory* | 0.5 µg/m$^3$ | LC-MS/MS | Method:<br>Edwards & Wiltshire, 2015<br>Report No. GRM010.09A (VV-128327)<br><br>Validation:<br>Wiltshire, 2015<br>Report No. CEMR-6992-REG (VV-411794)<br><br>**New data** |

*New data was prepared to provide additional validation data for cyprodinil using a second transition.

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

#### 5.3.2.8 Other studies/ information

No other studies and information are submitted in the framework of this application.

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

#### 5.3.3.1 Overview of residue definitions and levels of prothioconazole 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 5.3-10: Relevant residue definitions for monitoring/enforcement and levels of prothioconazole for which compliance is required**

| Matrix | Residue definition | MRL / limit | Reference for MRL/level Remarks |
|---|---|---|---|
| Plant, high water content | Prothioconazole-desthio (sum of isomers) | 0.02 mg/kg | MRL Regulation (EU) 2019/552 (lowest MRL) |
| Plant, high acid content | | 0.15 mg/kg | MRL Regulation (EU) 2019/552 (lowest MRL) |
| Dry commodities | | 0.05 mg/kg | MRL Regulation (EU) 2019/552 (lowest MRL) |
| Plant, high oil content | | 0.04 mg/kg | MRL Regulation (EU) 2019/552 (lowest MRL) |
| Plant, difficult matrices (hops, spices, tea) | | 0.05 mg/kg | MRL Regulation (EU) 2019/552 (default LOQ) |
| Muscle | Prothioconazole-desthio (sum of isomers) | 0.01 mg/kg | MRL Regulation (EU) 2019/552 (lowest MRL) |
| Milk | | 0.01 mg/kg | MRL Regulation (EU) 2019/552 (default LOQ) |
| Eggs | | 0.01 mg/kg | MRL Regulation (EU) 2019/552 (default LOQ) |
| Fat | | 0.02 mg/kg | MRL Regulation (EU) 2019/552 (lowest MRL) |
| Liver, kidney | | 0.1 mg/kg | MRL Regulation (EU) 2019/552 (lowest MRL) |
| Soil (Ecotoxicology) | Prothioconazole | 1.98 mg/kg | NOEC for earthworms |

| Matrix | Residue definition | MRL / limit | Reference for MRL/level Remarks |
|---|---|---|---|
| Drinking water (Human toxicology) | Prothioconazole JAU 6476-desthio | 0.1 µg/L | general limit for drinking water |
| Surface water (Ecotoxicology) | Prothioconazole JAU 6476-desthio | 4.6 µg/L 0.334 µg/L | Overall RACs for aquatic organisms |
| Air | Prothioconazole JAU 6476-desthio | 0.06 mg/kg (Prothioconazole) 0.003 mg/kg (JAU 6476-desthio) | AOEL sys: Prothioconazole 0.2 mg/kg bw/d JAU 6476-desthio 0.01 mg/kg bw/d |
| Tissue (meat or liver) | Prothioconazole-desthio (sum of isomers) | 0.01 mg/kg | Default LOQ |
| Body fluids | Prothioconazole-desthio (sum of isomers) | 0.05 mg/L | Default LOQ |

### 5.3.3.2 Description of analytical methods for the determination of residues of prothioconazole in plant matrices (KCP 5.2.1)

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

**Table 5.3-11: Validated methods for food and feed of plant origin**

| Component of residue definition: prothioconazole-desthio (sum of isomers) | | | | |
|---|---|---|---|---|
| **Matrix type** | **Method type** | **Method LOQ** | **Principle of method (i.e. GC-MS or HPLC-UV)** | **Author(s), year / missing / EU agreed** |
| High water content | QuEChERS | 0.01 mg/kg | LC-MS/MS (multi-residue) | **QuEChERS (01300/M018)** ***Validation:*** Chambers & Jarrett, 2014 Report: VC/13/017 ***ILV:*** Thies, 2014 Report: 2014/0110/01 EU agreed (United Kingdom, 2018) |
| | ILV (QuEChERS) | 0.01 mg/kg | | |
| High acid content | QuEChERS | 0.01 mg/kg | LC-MS/MS (multi-residue) | |
| | ILV (QuEChERS) | 0.01 mg/kg | | |
| High oil content | QuEChERS | 0.01 mg/kg | LC-MS/MS (multi-residue) | |
| | ILV (QuEChERS) | 0.01 mg/kg | | |
| Dry commodities | QuEChERS | 0.01 mg/kg | LC-MS/MS (multi-residue) | |
| | ILV (QuEChERS) | 0.01 mg/kg | | |

**Table 5.3-12: Statement on extraction efficiency**

| | Method for products of plant origin |
|---|---|
| Required, available from: | Desmaris, 2015; Report MR-15/117<br><br>The extraction efficiency was demonstrated by method 01300/M018. The extraction efficiency of the method was evaluated using barley grain, wheat green material, wheat straw and rape seed matrices from nature of residue metabolism studies. Results obtained using the analytical method were equivalent to those |

| | Method for products of plant origin |
|---|---|
| | obtained in the metabolism study, demonstrating the suitability of this analytical method for the determination of prothioconazole in plant matrices. The extraction efficiency was calculated as the ratio (expressed as percentage) between the average residues measured after extracting the samples according to the procedure and the average residues measured using the procedure of the corresponding metabolism study. Method 01300/M018 meet all necessary criteria (at least 70% of residues extracted compared to metabolism method corresponding to 100%) to sufficiently extract and determine the residues of prothioconazole in plant matrices. |

### 5.3.3.3 Description of analytical methods for the determination of residues of prothioconazole in animal matrices (KCP 5.2.2)

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

**Table 5.3-13: Validated methods for food and feed of animal origin**

| **Component of residue definition: prothioconazole-desthio (JAU6467-desthio) (sum of isomers)** | | | | |
|---|---|---|---|---|
| **Matrix type** | **Method type** | **Method LOQ** | **Principle of method (*i.e.* GC-MS or HPLC-UV)** | **Author(s), year / missing** |
| Milk | 00655/M002 | 0.004 mg/kg | LC-MS/MS (single residue) | **<u>00655/M002</u>**<br>***Validation:***<br>Freitag, 2013<br>Report: MR-06/199<br><br>***ILV:***<br>Schwarz & Class, 2007<br>Report: P/B 1226 G<br><br>EU agreed (United Kingdom, 2018)<br>----------<br>**<u>01009</u>**<br>***Validation:***<br>Schulte & Oel, 2014<br>Report: M-279725-03-1<br><br>***ILV:***<br>Bacher, 2006<br>Report: P/B 1111 G<br><br>EU agreed (United Kingdom, 2018) |
| | ILV (00655/M002) | 0.004 mg/kg | | |
| | 01009 | 0.01 mg/kg | LC-MS/MS (single residue) | |
| | ILV (01009) | 0.01 mg/kg | | |
| Eggs | 01009 | 0.01 mg/kg | LC-MS/MS (single residue) | |
| | ILV (01009) | 0.01 mg/kg | | |
| Muscle/meat | 00655/M002 | 0.01 mg/kg | LC-MS/MS (single residue) | |
| | ILV (00655/M002) | 0.01 mg/kg | | |
| | 01009 | 0.01 mg/kg | LC-MS/MS (single residue) | |
| | ILV (01009) | 0.01 mg/kg | | |
| Fat | 00655/M002 | 0.01 mg/kg | LC-MS/MS (single residue) | |
| | ILV (00655/M002) | 0.01 mg/kg | | |
| | 01009 | 0.01 mg/kg | LC-MS/MS (single residue) | |
| Liver | 00655/M002 | 0.01 mg/kg | LC-MS/MS (single residue) | |
| | ILV (00655/M002) | 0.01 mg/kg | | |
| | 01009 | 0.01 mg/kg | LC-MS/MS (single residue) | |
| Kidney | 00655/M002 | 0.01 mg/kg | LC-MS/MS (single residue) | |
| | | | | |

| | | | | |
|---|---|---|---|---|
| | 01009 | 0.01 mg/kg | LC-MS/MS (single residue) | |

**Table 5.3-14: Statement on extraction efficiency**

| | **Method for products of animal origin** |
|---|---|
| Required, available from: | Heinemann, 2001; Report 00655<br><br>The comparison of the residue analytical method of extraction for animal matrices with the extraction method used in the metabolism study demonstrated the suitability of the analytical method (extracting with an acetonitrile/water solvent system) for the determination of the relevant residue in animal matrices. The extraction efficiency is demonstrated. |

### 5.3.3.4 Description of methods for the analysis of prothioconazole in body fluids and tissues (KCP 5.2.3)

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

**Table 5.3-15: Methods for body fluids and tissues**

| **Component of residue definition: prothioconazole-desthio (sum of isomers)** | | | | |
|---|---|---|---|---|
| **Matrix type** | **Method type** | **Method LOQ** | **Principle of method (*i.e.* GC-MS or HPLC-UV)** | **Author(s), year / missing** |
| Blood | 01471 | 0.05 mg/L | LC-MS/MS (single residue) | ***Validation:***<br>Hoeppner, 2015<br>Report: M-535874-02-1 |

### 5.3.3.5 Description of methods for the analysis of prothioconazole in soil (KCP 5.2.4)

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

**Table 5.3-16: Validated methods for soil**

| Component of residue definition: Prothioconazole and prothioconazole-desthio | | | |
|---|---|---|---|
| **Method type** | **Method LOQ** | **Principle of method (*i.e.* GC-MS or HPLC-UV)** | **Author(s), year / missing** |
| Primary (00610) | 0.006 mg/kg | HPLC-MS/MS (1 MRM transition) | Schramel, 2000<br><br>EU agreed |
| Confirmatory* (00610/M001) | 0.006 mg/kg | HPLC-MS/MS ($2^{nd}$ MRM transition) | **Brumhard, 2005**<br>Report No 00610/M001<br><br>**New data** |
| Primary (00086/M038) | 0.010 mg/kg | GC-MS (JAU 7476-desthio) | Steinhauer, 2001<br><br>EU agreed |

*New data was prepared to provide additional validation data for prothioconazole and its metabolite prothioconazole-desthio using a second transition.

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

### 5.3.3.6 Description of methods for the analysis of prothioconazole in water (KCP 5.2.5)

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

**Table 5.3-17: Validated methods for water**

| Component of residue definition: Prothioconazole and prothioconazole-desthio | | | | |
|---|---|---|---|---|
| **Matrix type** | **Method type** | **Method LOQ** | **Principle of method (i.e. GC-MS or HPLC-UV)** | **Author(s), year / missing** |
| Drinking water | Primary (00684) | Prothioconazole 0.1 µg/L<br>Prothioconazole-desthio 0.05 µg/L | HPLC-MS/MS (1 MRMs) | Sommer, 2001<br><br>EU agreed |
| | Confirmatory* (00684/M001) | Prothioconazole 0.05 µg/L<br>Prothioconazole-desthio 0.05 µg/L | HPLC-MS/MS (2 MRMs) | Brumhard, 2005<br>Report No 00684/M001<br><br>**New data** |
| Drinking water | Primary/confirmatory* (01387/M002) | Prothioconazole 0.05 µg/L<br>Prothioconazole-desthio 0.05 µg/L | HPLC-MS/MS | Krebber & Sandau, 2015<br>Report No MR-15/025<br><br>**New data** |
| | ILV (01387/M002) | Prothioconazole 0.05 µg/L<br>Prothioconazole-desthio 0.05 µg/L | HPLC-MS/MS | Thies, 2015<br>Report No 2015/0034/01<br><br>**New data** |

*New data was prepared to provide additional validation data for prothioconazole and its metabolite prothioconazole-desthio using a second transition.

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

### 5.3.3.7 Description of methods for the analysis of prothioconazole in air (KCP 5.2.6)

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

**Table 5.3-18: Validated methods for air**

| **Component of residue definition: Prothioconazole and prothioconazole-desthio** | | | |
|---|---|---|---|
| **Method type** | **Method LOQ** | **Principle of method (i.e. GC-MS or HPLC-UV)** | **Author(s), year / missing** |
| Primary (00724) | Prothiconazole 0.015 µg/m$^3$ | HPLC-MS/MS | Maasfeld, 2002<br><br>EU agreed |
| Primary (00731) | Prothiconazole-desthio 0.0006 µg/m$^3$ | HPLC-MS/MS (1 MRMs) | Maasfeld, 2002<br><br>EU agreed |
| Confirmatory* (00731/M001) | Prothiconazole-desthio 0.0003 µg/m$^3$ | HPLC-MS/MS (2 MRMs) | Anft & Bardel, 2005 Report No 007321/M001<br><br>**New data** |

*New data was prepared to provide additional validation data for prothioconazole and its metabolite prothioconazole-desthio using a second transition.

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

### 5.3.3.8 Other studies/ information

None.

## 5.4 References

Cyprodinil

EFSA (European Food Safety Authority), 2006 . Conclusion regarding the peer review of the pesticide risk assessment of the active substance cyprodinil. EFSA Journal 2006;4(1):RN-51, 78 pp. https://doi.org/10.2903/ j.efsa.2006.51r

France, 2005a. Draft assessment report on the active substance cyprodinil prepared by the rapporteur Member State France in the framework of Council Directive 91/414/EEC, June 2005.

France, 2005b. Final addendum to the draft assessment report on the active substance cyprodinil prepared by the rapporteur Member State France in the framework of Council Directive 91/414/EEC, September 2005.

Prothioconazole

EFSA (European Food Safety Authority), 2007. Conclusion on the peer review of the pesticide risk assessment of the active substance prothioconazole. The EFSA Journal 2007, 106r, 1-98.

United Kingdom, 2004. Draft assessment report on the active substance Prothioconazole prepared by the rapporteur Member State United Kingdom in the framework of Council Directive 91/414/EEC, October 2004.

United Kingdom, 2007. Final addendum to the additional report and the draft assessment report on the active substance prothioconazole prepared by the rapporteur Member State United Kingdom in the framework of Council Regulation (EC) No 33/2008, compiled by EFSA, May 2007.

United Kingdom, 2018. Draft (renewal) assessment report on the active substance Prothioconazole prepared by the rapporteur Member State United Kingdom according to the Commission Regulation (EU) No 1107/2009, February 2018.

# 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 Y/N | Owner |
|---|---|---|---|---|---|
| XXX | XXXX | XXX | XXXX | XX | XX |
| **Prothioconazole** | | | | | |
| ~~No new data submitted~~ | | | | | |

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

| Data point | Author(s) | Year | Title<br>Company Report No.<br>Source (where different from company)<br>GLP or GEP status<br>Published or not | Verte-brate study Y/N | Owner |
|---|---|---|---|---|---|
| XXX | XXXX | XXX | XXXX | XX | XX |

# Appendix 2 Detailed evaluation of submitted analytical methods

## A 2.1 Analytical methods for cyprodinil

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

#### A 2.1.1.1 Description of analytical methods for the determination of residues in support of environmental fate studies (KCP 5.1.2.1)

No new or additional studies have been submitted

#### A 2.1.1.2 Description of analytical methods for the determination of residues in support of efficacy studies (KCP 5.1.2.2)

No new or additional studies have been submitted

#### A 2.1.1.3 Description of analytical methods for the determination of residues in support of toxicological studies (KCP 5.1.2.3)

No new or additional studies have been submitted

#### A 2.1.1.4 Description of analytical methods for the determination of residues in support of operator, worker, resident and bystander exposure studies (KCP 5.1.2.4)

No new or additional studies have been submitted

#### A 2.1.1.5 Description of analytical methods for the determination of residues in support of residues studies (KCP 5.1.2.5)

##### A 2.1.1.5.1 AG-631B (REM 141.01)

###### A 2.1.1.5.1.1 Method validation (report CER04169/07)

| | |
|---|---|
| Comments of zRMS: | The validation is acceptable.<br>The study and method has been accepted in the context of its stability purpose in Section B7 of the present report. Sixteen canola residue trials were conducted in Canada to determine the magnitude of the residues of fludioxonil and cyprodinil after a single foliar application corresponding to 365.6 g cyprodinil/ha and 243.8 g fludioxonil/ha.<br>The analytical methods (Novartis method AG-631B and XXXX method AG-597B) were modified to make them suitable for LC/MS/MS and to improve the method's ruggedness. Also the complications of extraction from an oily matrix were addressed. The LOQ for fludioxonil was 0.0100 ppm and for cyprodinil was 0.0200 ppm in seed and meal and 0.0100 ppm in oil. |

| | |
|---|---|
| Reference: | KCP 5.1.2.5 |
| Report | Fludioxonil/Cyprodinil WG (A9219B) - Residue Levels on Canola Seed and Processed Fractions, Meal and Refined Oil, from Trials Conducted with SWITCH® 62.5 WG in Canada During 2007 (MRID 47644301) Final Report Amendment 1, Sagan, K., 2009, XXXX Report No. CER 04169/07, XXXX File No. VV-263966 |
| Guideline(s): | Codex "Guidelines on Minimum Sample Size for Agricultural Commodities from Supervised Field Trials for Residue analysis"<br>ALINORM 87/24A (1987)<br>PMRA Regulatory Directive Dir98-02 "Residue Chemistry Guidelines"<br>PMRA Regulatory Directive 98-01 and 98-02 |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes |

Analytical method REM 141.01 was re-issued as AG-631, this was superseded AG631B.

Residues of cyprodinil were analysed according the method AG-631B with modifications. These modifications were replacement of the column switching HPLC UV system with a single column system with MS/MS determination. AG-631B with these modifications was issued as REM 141.10. REM 141.10 was validated on high oil crops (sunflower) in a study conducted according to SANCO/3029/99 rev.4 and SANCO/825/00 rev.6 guidelines, which were in force at the time the study was carried out (Chaggar 2005).

Analytical method AG597B was written for fludioxonil, however it was successfully applied to cyprodinil and verified within this study.

**Materials and methods**

Prior to the analysis of the canola seed and meal samples, the modified version of AG-631B was verified. Method AG-579B was verified for canola refined oil. Triplicate control samples of canola seed, meal and refined oil were fortified at 0.02 mg/kg and duplicate at two higher levels. Homogenised sub-samples of each test commodity (10 g) were fortified with standard solutions of cyprodinil in methanol.

Principle of the method AG-631B

A 10-g sample of canola seed and meal (high oil matrix) was weighed into 4 oz. amber glass bottles. Samples were extracted in 80 ml of 80:20 (v:v) methanol: water by shaking 1 hour shake at room temperature. After centrifugation an aliquot was taken and 1M hydrochloric acid (2 mL) was added to the extracts. The extract was cleaned up using solid phase extraction (SCX). The eluate was evaporated to near dryness and reconstituted methanol/water. A portion of the final fraction was transferred to an auto sampler vial for analysis by LC-MS/MS.

Principle of the method AG-597B

Canola refined oil samples were extracted by shaking with acetonitrile saturated with hexane. The extraction was repeated four more times. The acetonitrile was combined and evaporated to a small volume. The extract was diluted prior to analysis by LC-MS/MS.

Quantification was done with external standards in solvent using mass transitions m/z 226.0 to 108.2.

**Results and discussions**

Recoveries of cyprodinil obtained from each matrix at each fortification level using the modified method

AG-631B and AG-597B are presented in the table below.

The mean recoveries at each fortification level from 0.02 to 0.2 mg/kg for each commodity tested during method validation were in the range of 70-110% and the relative standard deviations (RSDs) were <15%, which is in accordance with the EU guidance SANTE/2020/12830, Rev.1.

**Table A 1: Recovery results from method verification and concurrent recoveries of cyprodinil using method AG-631B in canola seed, meal and AG-597B in refined oil**

| Matrix | Analyte | Fortification level (mg/kg) (*n* = x) | Individual recoveries (%) | Range of recoveries (%) (*n* = x) | Mean recovery (%) | RSD (%) | Comments |
|---|---|---|---|---|---|---|---|
| Canola seed | Cyprodinil | Mass transition m/z = 226.0 → 108.2 | | | | | |
| | | 0.02* | 73, 85, 82, 88, 73, 73, 85, 71 | 71 - 88 (n = 8) | 79 | 8,8 | Acceptable |
| | | 0.1 | 79, 92 | 79 - 92 (n = 2) | 86 | N/A | Acceptable |
| | | 0.2 | 86, 76, 73, 73, 84, 72 | 72 - 86 (n = 6) | 77 | 7.9 | Acceptable |
| | | Overall | | 71 -92 (n = 16) | 79 | 8.7 | Acceptable |
| Canola meal | Cyprodinil | Mass transition m/z = 226.0 → 108.2 | | | | | |
| | | 0.02* | 97, 88, 80, 86, 102, 77 | 77 - 102 (n = 6) | 88 | 10.9 | Acceptable |
| | | 0.1 | 102, 113, 89, 87, 86 | 86 - 113 (n = 5) | 95 | 12.3 | Acceptable |
| | | 0.2 | 104, 109 | 104 – 109 (n = 2) | 107 | N/A | Acceptable |
| | | Overall | | 77 – 113 (n = 13) | 94 | 12.1 | Acceptable |
| Canola refined oil | Cyprodinil | Mass transition m/z = 226.0 → 108.2 | | | | | |
| | | 0.01* | 107, 104, 92, 84, 85, 99 | 84 - 107 (n = 3) | 95 | 10.2 | Acceptable |
| | | 0.05 | 107, 89, 99, 108, 120 | 89 - 120 (n = 5) | 105 | 11.0 | Acceptable |
| | | 0.1 | 96, 99 | 96 - 99 (n = 2) | 98 | N/A | Acceptable |
| | | Overall | | 84 – 120 (n = 10) | 99 | 10.3 | Acceptable |

* Limit of quantitation, defined by the lowest validated fortification level

**Table A 2: Characteristics of the data-generation analytical method used for the quantification of cyprodinil residues in crop matrices**

| | **Cyprodinil** |
|---|---|
| Specificity | LC-MS/MS is considered to be a highly specific detection technique.<br>A second transition was not validated, but this is not required for data generation methods (SANTE/2020/12830, Rev.1). There were no significant (i.e. 30% of LOQ) interfering peaks in control matrices. |
| Calibration (type, number of data points) | Calibration was performed using one or more standard injections at each of 6 concentrations. Solvent standards were used. The detector response was linear (correlation coefficients (r) were ≥ 0.9950) |
| Calibration range | 0.0001 ppm to 0.1 ppm |
| Assessment of matrix effects is presented | Not assessed, the recoveries were in acceptable limits. Therefore, matrix effects are not considered significant. |
| Limit of determination/quantification | Limit of quantification (LOQ): 0.02 mg/kg canola seed and meal and 0.01 mg/kg for refined oil<br>Limit of detection (LOD): 0.006 mg/kg for canola seed and meal and 0.003 mg/kg for refined oil |

**Conclusion**

The modified method AG-631B has been validated for the determination of residues of cyprodinil in canola seed and meal with a limit of quantification (LOQ) of 0.02 mg/kg and in refined oil with a limit of quantification (LOQ) of 0.01 mg/kg.

## A 2.1.1.5.1.2 Method validation (report T003062-07)

| | |
|---|---|
| Comments of zRMS: | The method validation is acceptable.<br>The study has been accepted in Section B7 in the context of stability tests. The study objective was to conduct ten trials in almond and pecan. Cyprodinil was applied and raw agricultural commodities were harvested at typical commercial maturity at PHI of 14 days.<br>The method imployed LC-MS/MS determination. The average procedural recoveries at fortification levels of 0.01 ppm, 0.10 ppm, and 10 ppm ranged from 71.0-98.2% for cyprodinil in almond hulls. For almond nutmeat and pecan nutmeat, the average procedural recoveries at fortifications levels of 0.01 ppm and 0.10 ppm ranged from 88.4-97.7% and 86.4-106% respectively. |

| | |
|---|---|
| Reference: | KCP 5.1.2.5 |
| Report | Cyprodinil – Magnitude of the Residues in or on Almond and Pecan as Representative Commodities of Tree Nuts, Group 14 and Storage Stability of Almonds (Hulls and Nutmeat), Mazlo, J., 2010, XXXX Report No. T003061-07, XXXX File No. VV-467356 |

| | |
|---|---|
| Guideline(s): | EPA OPPTS 860.1000 (background)<br>EPA OPPTS 860.1380 (storage stability)<br>EPA OPPTS 860.1500 (crop field trials) |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes |

Analytical method REM 141.01 was re-issued as AG-631, this was superseded AG631B.

Residues of cyprodinil were analysed according the method AG-631B with modifications. These modifications were replacement of the column switching HPLC UV system with a single column system with MS/MS determination. AG-631B with these modifications was issued as REM 141.10. REM141.10 was validated on high oil crops (sunflower) in a study conducted according to SANCO/3029/99 rev.4 and SANCO/825/00 rev.6 guidelines, which were in force at the time the study was carried out (Chaggar 2005).

**Materials and methods**

Prior to the analysis of the field samples, the modified version of AG-631B was verified. Duplicate control samples of almond nutmeat and hulls were fortified at 0.01 and 0.1 mg/kg. Homogenised sub-samples of each test commodity (10 g) were fortified with standard solutions of cyprodinil in methanol.

A 10-g sample of almond hulls or nutmeat (high oil matrix) was weighed into 4 oz. amber glass bottles. Samples were extracted in 80 ml of 80:20 (v:v) methanol: water by shaking 1 hour shake at room temperature. Depending on the matrix, a small volume of concentrated hydrochloric acid was added to the extracts (0.9 mL – 1.0 mL for hulls and 300 µL for nutmeat). The samples were then shaken an additional 5 minutes. Samples were centrifuged for 10 minutes at 4000 rpm and filtered through Reeve Angel 802 and Whatman 2V filter paper. An aliquot of the supernatant was diluted in an appropriate final volume with 0.1% ammonium acetate in water. A portion of the final fraction was transferred to an auto sampler vial for analysis by LC-MS/MS (226.1->93m/z).

Quantification was done with external standards in solvent.

**Results and discussions**

Recoveries of cyprodinil obtained from each matrix at each fortification level using the modified method AG-631B are presented in the table below.

The mean recoveries at each fortification level from 0.01 to 10 mg/kg for each commodity tested during method validation were in the range of 70-110% and the relative standard deviations (RSDs) at each fortification level from 0.01 to 0.1 mg/kg were <20%, which is in accordance with the EU guidance SANTE/2020/12830, Rev.1. The RSD at 10 mg/kg was 12.2% which is only slightly above 10% as required per SANTE/2020/12830, Rev.1 and therefore can be considered acceptable.

**Table A 3: Recovery results from method verification and concurrent recoveries of cyprodinil using method AG-631B in almond nutmeat, almond hulls and pecan**

| Matrix | Analyte | Fortification level (mg/kg) | Individual recoveries (%) | Range of recoveries (%) (*n* = x) | Mean recovery (%) | RSD (%) | Comments |
|---|---|---|---|---|---|---|---|
| Almond nutmeat | Cyprodinil | Mass transition m/z = 226.2 → 93.1 | | | | | |
| | | 0.01* | 102, 109, 105, 79, 103, 104 | 79 - 109 (n = 6) | 100 | 12.5 | Acceptable |
| | | 0.1 | 98, 98, 95, 71, 92, 96 | 71 - 98 (n = 6) | 92 | 12.9 | Acceptable |
| | | Overall | | 71 -109 (n = 12) | 96 | 11.5 | Acceptable |
| Almond hulls | Cyprodinil | Mass transition m/z = 226.2 → 93.1 | | | | | |
| | | 0.01* | 89, 86, 86, 73, 108, 126 | 73 - 126 (n = 6) | 95 | 24.8 | Acceptable |
| | | 0.1 | 80, 77, 87,87, 81 | 77 - 87 (n = 5) | 82 | 4.6 | Acceptable |
| | | 10 | 70, 73, 81, 60 | 60 – 81 (n = 4) | 71 | 12.2 | Acceptable |
| | | Overall | | 60 – 126 (n = 15) | 84 | 18.7 | Acceptable |
| Pecan | Cyprodinil | Mass transition m/z = 226.2 → 93.1 | | | | | |
| | | 0.01* | 100, 103, 115 | 100 - 115 (n = 3) | 106 | 7.5 | Acceptable |
| | | 0.1 | 79, 76, 104 | 76 - 104 (n = 3) | 86 | 17.8 | Acceptable |
| | | Overall | | 76 – 115 (n = 5) | 82 | 16.0 | Acceptable |

* Limit of quantitation, defined by the lowest validated fortification level

**Table A 4: Characteristics of the data-generation analytical method used for the quantification of cyprodinil residues in crop matrices**

| | **Cyprodinil** |
|---|---|
| Specificity | LC-MS/MS is considered to be a highly specific detection technique.<br>A second transition was not validated, but this is not required for data generation methods (SANTE/2020/12830, Rev.1). There were no significant (i.e. 30% of LOQ) interfering peaks in control matrices. |
| Calibration (type, number of data points) | Calibration was performed using one or more standard injections at each of 6 concentrations. The detector response was linear (correlation coefficients (r) were ≥ 0.9988) |
| Calibration range | ~~0.25 ng/mL to 10 ng/mL~~ 0,005 mg/kg 0,2 mg/kg |
| Assessment of matrix effects is presented | Not assessed, the recoveries were in acceptable limits. Therefore, matrix effects are not considered significant |

| | **Cyprodinil** |
|---|---|
| Limit of determination/quantification | Limit of quantification (LOQ): 0.01 mg/kg<br>Limit of detection (LOD): Not assessed, however from the example chromatography 30% of the LOQ is achievable |

**Conclusion**

The modified method AG-631B has been validated for the determination of residues of cyprodinil in almond hulls, almond nutmeat and pecan with a limit of quantification (LOQ) of 0.01 mg/kg.

### A 2.1.1.5.2 REM 141.10

### A 2.1.1.5.2.1 Method validation (reports REM 141.10 and RJ3583B)

| | |
|---|---|
| Comments of zRMS: | Both reports of REM 141.10 method have been accepted. The method can be considered valid for the determination of cyprodinil residues in validated matrices at the set LOQ.<br>The analytical procedure described in the study is for the determination of residues of cyprodinil (CGA219417) in crops with the LOQ 0.01 mg/kg. Residue method REM 141.10 has been determined in orange, lettuce, barley grain, and barley straw. The method procedure includes extraction by homogenisation with aqueous methanol, cleaning up of aliquots by solid phase and final determination by LC-MS/MS in multiple reaction monitoring mode. In Appendix 3 of the first study only a summary of the method validation is reported. All method validation data is described in Report no. RJ3583B (a second one).<br>Control samples were analysed in duplicate. The fortified samples were analysed in quintuplet at LOQ, 0.01 mg and in quintuplet at higher fortification levels.<br>Acceptable mean recoveries of between 70% and 110% with a relative standard deviation of < 20% were found for both cyprodinil transitions (primary m/z 226.1 → 92.9 and confirmatory m/z 226.1 → 77.0) on all matrices tested.<br>Residues of cyprodinil in the control samples were all below < 30% of the LOQ.<br>The MS/MS detector response to cyprodinil standard solutions was shown to be sufficiently linear.<br>The stability of cyprodinil in extracts was sufficient.<br>Only the commercially available laboratory equipment and reagents are required. |

| | |
|---|---|
| Reference: | KCP 5.1.2.5 |
| Report | Residue method for the determination of residues of cyprodinil (CGA219417) in crops. Final determination by LC-MS/MS, Chaggar, S., 2005, XXXX Method Reference: REM 141.10, Report No. REM 141.10, XXXX File No. CGA219417/1278, VV-125643 |
| Guideline(s): | Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/3029/99 rev.4)<br>Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/825/00 rev.6)<br>OPPTS 860.1340 |

| | |
|---|---|
| Deviations: | No |
| GLP: | No |
| Acceptability: | Yes |
| Reference: | KCP 5.1.2.5 |
| Report | Cyprodinil (CGA219417): Validation of analytical method REM 141.10 for the determination of residues in crops. Final determination by LC-MS/MS, Chaggar, S., 2005, Report No. RJ3583B, XXXX File No. CGA219417/1277, VV-333019 |
| Guideline(s): | Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/3029/99 rev.4)<br>Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/825/00 rev.6)<br>OPPTS 860.1340 |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes |

**Materials and methods**

Plant samples are homogenized and extracted with methanol/water (70:30). Aliquots of the extracts are acidified and cleaned-up using solid phase extraction cartridges (SCX phase). Cyprodinil is eluted in methanol/35% ammonia (95:5); the eluate is evaporated and dissolved in mobile phase. Final determination is by high performance liquid chromatography coupled to a triple quadrupole mass spectrometer (LC-MS/MS) in multiple reaction monitoring mode. Protonated molecular ions (m/z 226.1) generated in the ion source are selected and subjected to further fragmentation. The two most abundant ions in the resulting daughter spectra are then monitored. LC-MS/MS is considered to be highly specific therefore generally only the m/z 266.1 to 92.9 transition is used for quantitative analysis. A second transition (m/z 226.1 → 77.0) may also be monitored if further confirmation is required. The LOQ of the method is 0.01 mg/kg.

**Results and discussions**

Residue method REM 141.10 has been validated for the determination of residues of cyprodinil in crops, using orange, lettuce, and barley grain, barley straw and sunflower seed as representative matrices.

Fortified samples were analysed in quintuplet at the limit of quantification (LOQ, 0.01 mg/kg) and at relevant higher levels (2 mg/kg in orange, 10 mg/kg in lettuce, 2 mg/kg in grain, 3 mg/kg in straw and 0.1 mg/kg in sunflower seed). Acceptable mean recoveries of between 60% and 120% at 0.01 mg/kg, 70% and 120% at 0.1 mg/kg and 70% and 110% at > 0.1 mg/kg were found for both transitions on all matrices tested except for sunflower seed. For sunflower seed, the mean recovery was 41% with a relative standard deviation of 6% for the primary MRM transition. The relative standard deviations (RSDs) of the recoveries at each fortification level and overall for each commodity tested during method validation were ≤10%, which is in accordance with the EU guidance SANTE/2020/12830, Rev.1.

Re-analysis of stored cyprodinil final extracts at a temperature of <7 °C in glass HPLC vials demonstrated that cyprodinil is stable when for a period of at least 7 days. Re-analysis of cyprodinil lettuce, orange, barley grain and barley straw primary extracts stored at a temperature of <7 °C demonstrated that cyprodinil

is stable for a period of at least 28 days under these conditions.

**Table A 5: Recovery results from method validation of cyprodinil in crop matrices using the analytical method REM 141.10 (primary transition m/z 225.1→ 92.9)**

| Matrix | Fortification Level (mg/kg) | Recovery (%) | Number of Analyses (n) | Mean Recovery (%) | RSD (%) | Recovery Range (%) |
|---|---|---|---|---|---|---|
| Orange | 0.01* | 78, 75, 78, 71, 73 | 5 | 75 | 4 | 71-78 |
| | 2 | 72, 73, 81, 85, 89 | 5 | 80 | 9 | 72-89 |
| | Overall | | 10 | 78 | 8 | 71-89 |
| Lettuce | 0.01* | 75, 77, 85, 75, 81 | 5 | 79 | 6 | 75-85 |
| | 10 | 80, 76, 88, 97, 79 | 5 | 84 | 10 | 76-97 |
| | Overall | | 10 | 81 | 9 | 75-97 |
| Barley grain | 0.01* | 91, 94, 88, 87, 88 | 5 | 90 | 3 | 87-94 |
| | 2 | 80, 80, 81, 81, 84 | 5 | 81 | 2 | 80-84 |
| | Overall | | 10 | 86 | 6 | 80-94 |
| Barley straw | 0.01* | 88, 73, 72, 80, 78 | 5 | 78 | 8 | 72-88 |
| | 3 | 79, 83, 82, 77, 73 | 5 | 79 | 5 | 73-83 |
| | Overall | | 10 | 79 | 6 | 72-88 |
| Sunflower seed | 0.01* | 41, 44, 41, 36, 39 | 5 | 40 | 7 | 36-44 |
| | 0.1 | 43, 39, 43, 44, 42 | 5 | 42 | 5 | 39-44 |
| | Overall | | 10 | 41 | 6 | 36-44 |

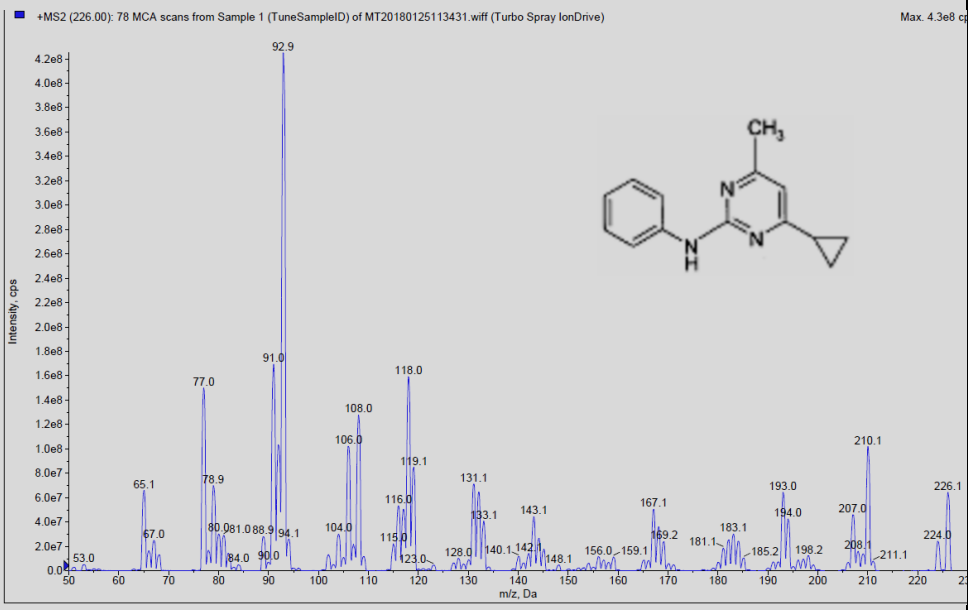
* Limit of quantitation, defined by the lowest validated fortification level

**Table A 6: Characteristics for the analytical method REM 141.10 used for validation of cyprodinil residues in crop matrices**

| | Cyprodinil |
|---|---|
| Specificity | LC-MS/MS with two transitions is considered to be a highly specific detection technique and therefore no further confirmatory technique is required (SANTE/2020/12830, Rev.1). No significant interferences arising from plant matrices, the lab ware, reagents or solvents have been observed at the retention times of interest. |
| Calibration (type, number of data points) | The linearity of the LC-MS/MS detector was tested using standard solutions in solvent. Linearity was tested for both MS/MS transitions. Standards at seven different concentrations were injected and the signal area plotted against concentration for all calibration points. Straight lines with correlation coefficients > 0.998 were obtained. |
| Calibration range | 0.0005 - 2 µg/mL |
| Assessment of matrix effects is presented | Yes<br>No significant enhancement or suppression of detector response was observed. |
| Limit of determination/quantification | The limit of quantification for cyprodinil residues in plant commodities was established at 0.01 mg/kg. No interfering |

| | Cyprodinil |
|---|---|
| | peaks around the retention time of cyprodinil were found in any of the control samples at levels above 30% of the limit of quantification. |

**Conclusion**

| | |
|---|---|
| Comments of zRMS: | REM141.10 validation in apple has been accepted.<br><br>The objective of this study was to adapt and to perform a method validation of the residue analytical method REM141.10 for the determination of cyprodinil (CGA219417) in apple (fruit) at LOQ of 0.01 mg/kg, using LC/MS/MS. Control samples were analysed in duplicate. The fortified samples were analysed in quintuplet at the LOQ (0.01 mg/kg). Additional higher level fortifications were performed in quintuplet. Additionally, one reagent blank per matrix set was analysed to show that no significant LC-MS/MS signal interference caused by the analytical method was observed.<br><br>The [M+H]+ ion of the analyte at 226 *m/z* was used as parent ion for MS/MS detection. For both characteristic LC-MS/MS mass transitions (primary transition to the daughter ion = *m/z* 226 → 108, confirmatory transition = *m/z* 226 → 93), acceptable mean recoveries between 70% and 110%, with relative standard deviations (RSDs) ≤ 20% (for levels ≤ 0.1 mg/kg) or ≤ 10% (for levels > 1 mg/kg), were obtained for apple. The method achieves a high level of specificity and no further confirmation on a different detector was necessary.<br>However, although the transitions set applied in the study and in much earlier validation study REM 141.10 are different (see previously: 226.1 → 92.9 and 226.1 → 77.0, and also other next studies), it does not affect the validations. Below for clarity cyprodinil ion (molecular Weight: 225.3 g/mol) spectrum acquired from the applicant study Report No. R B8040 (the next; the structure added by zRMS):<br><br>  <br><br>Reason for the study report amendment 1: Request by the Sponsor Representative / Study Manager front page and formatting of headers have been changed; Translations on page 3 and 24 have been added. Impact on the study: None |

Method REM 141.10 is considered valid for the determination of cyprodinil residues in crops (excluding oily crops) at the LOQ (0.01 mg/kg) and over concentration ranges typical of those for which the method will be used.

### A 2.1.1.5.2.2 Method validation (report P 4186G)

| | |
|---|---|
| Reference: | KCP 5.1.2.5 |
| Report | Cyprodinil (CGA219417): Validation of Analytical Method REM141.10 for the Determination of Residues of Cyprodinil in Crops by LC MS/MS, Richter, S., 2017, Report No. P 4186G, XXXX File No. CGA219417_11778, VV-466898<br>**Report Amendment 1** |
| Guideline(s): | European Commission Guidance Document on Residue Analytical Methods, SANCO/3029/99 rev. 4 (11 Jul 2000).<br>OECD Guidance Document on Pesticide Residue Analytical Methods ENV/JM/MONO(2007)17.<br>Residue Chemistry Test Guidelines OPPTS 860.1340 Residue Analytical Method, EPA 712-C-96-174, August 1996.<br>Regulation (EC) No 1107/2009 of the European Parliament and of the council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes |

**Materials and methods**

The analytical method is based on extraction/clean-up procedures and subsequent LC MS/MS determination.

Residues of cyprodinil were extracted from sample material with methanol/water (70/30, v/v; water content of the sample considered), following a homogenisation for 4 minutes. The extracts were then centrifuged. 1 M hydrochloric acid was added to aliquots of the extracts, and shaken afterwards. Conditioned Strata SCX SPE cartridges (3 mL methanol, then 3 mL of 0.1 M hydrochloric acid) were used for the clean-up of the samples. The cartridges were loaded with sample extracts and washed with 6 mL of methanol/water (50/50, v/v). Samples were eluted with 2 mL of methanol/ammonia (32%) (94.5/5.5, v/v). The eluates were evaporated to dryness under a stream of nitrogen (40 °C). Residues were then dissolved first in 0.35 mL of methanol and diluted with 0.15 mL of water. Afterwards samples are diluted with methanol/water (70/30, v/v; DF4) for final determination by high-performance liquid chromatography with mass-spectrometric detection (LC-MS/MS), monitoring for the primary (m/z 226→108) and the confirmatory transition (m/z 226→93) for cyprodinil.

The analytical method was validated for apple (fruit).

**Results and discussions**

Fortified samples were analysed in quintuplet at the limit of quantification (LOQ, 0.01 mg/kg) and at 200 x LOQ (2.0 mg/kg). Acceptable mean recoveries of between 60% and 120% at 0.01 mg/kg and 70% and 110% at > 0.1 mg/kg were found for both mass transitions and therefore, according to EU guidance (SANTE/2020/12830, Rev.1), demonstrate the method has satisfactory accuracy. The relative standard deviations (RSDs) of cyprodinil recoveries at each fortification level and overall were < 10% and therefore, according to the EU guidance (SANTE/2020/12830, Rev.1), demonstrate the method has satisfactory repeatability.

The stability of sample extracts originally fortified with cyprodinil at the LOQ level was assessed by reinjection, after a storage period of at least 7 days in a refrigerator at 6-8 °C, against freshly prepared calibration standards. The results proved that the cyprodinil residues in the stored fortified sample extracts were stable. The mean recovery values at the LOQ level were between 70% and 110%, with a RSD of ≤ 20% when re-analysed.

The stability of the stored stock and working solutions of cyprodinil was assessed after a storage period of at least 36 days (in methanol) or 29 days (in methanol/water, 70/30, v/v) in a refrigerator at 6-8 °C, against freshly prepared calibration standards at the same concentration. The mean peak areas of the stored solutions were found to be within ± 10% of the mean peak areas of the freshly prepared standard solutions for cyprodinil, demonstrating that residues of cyprodinil in the stored stock and working solutions were stable for the storage period assessed when stored under the described conditions.

**Table A 7: Recovery results from method validation of cyprodinil in apple using the analytical method REM 141.10 (primary transition m/z 226→108)**

| Matrix | Fortification Level (mg/kg) | Recovery (%) | Number of Analyses (n) | Mean Recovery (%) | RSD (%) | Recovery Range (%) |
|---|---|---|---|---|---|---|
| Apple | 0.01* | 90, 85, 84, 96, 95 | 5 | 90 | 6 | 84-96 |
| | 2.0 | 85, 87, 82, 84, 84 | 5 | 84 | 2 | 82-87 |
| | Overall | | 10 | 87 | 6 | 82-96 |

* Limit of quantitation, defined by the lowest validated fortification level

**Table A 8: Recovery results from method validation of cyprodinil in apple using the analytical method REM 141.10 (confirmatory transition m/z 226→93)**

| Matrix | Fortification Level (mg/kg) | Recovery (%) | Number of Analyses (n) | Mean Recovery(%) | RSD (%) | Recovery Range (%) |
|---|---|---|---|---|---|---|
| Apple | 0.01* | 90, 86, 85, 100, 94 | 5 | 91 | 7 | 85-100 |
| | 2.0 | 86, 87, 83, 84, 85 | 5 | 85 | 2 | 83-87 |
| | Overall | | 10 | 88 | 6 | 83-100 |

* Limit of quantitation, defined by the lowest validated fortification level

**Table A 9: Characteristics for the analytical method REM 141.10 used for validation of cyprodinil residues in apple**

| | Cyprodinil |
|---|---|
| Specificity | LC-MS/MS with two transitions is considered to be a highly specific detection technique and therefore, according to EU guidance (SANTE/2020/12830, Rev.1), no further confirmatory technique is required. The method includes two MS/MS transitions, both of which have been validated. No significant interferences arising from the matrices, the lab ware, reagents or solvents have been observed |

| | **Cyprodinil** |
|---|---|
| | at the retention times of interest. |
| Calibration (type, number of data points) | The linearity of the LC-MS/MS detector response was assessed using standard solutions in methanol/water (70/30, v/v). Linearity was assessed for both MS/MS transitions. Standards at ≥ 5 different concentrations were injected and the signal area plotted against concentration for all calibration points. Straight lines with correlation coefficients ranging from 0.9996 to 0.9999 were obtained for cyprodinil. |
| Calibration range | ~~0.050 ng/mL to 5.0 ng/mL~~ 0.002 mg/kg to 0,2 mg/kg |
| Assessment of matrix effects is presented | Yes<br>Matrix effects (signal suppression or enhancement; ≤ ± 20%) were considered not to be significant. Thus, calibration standards in methanol/water (70/30, v/v) were used for quantification of cyprodinil. |
| Limit of determination/quantification | The limit of quantification for cyprodinil residues in crop matrices using the analytical method was established at 0.01 mg/kg. No interfering peaks around the retention time of cyprodinil were found in any of the control samples at levels above 20% of the limit of quantification.<br>The limits of detection (LODs) were calculated to be 0.000155 mg/kg for the primary transition, and 0.000163 mg/kg for the confirmatory transition. |

**Conclusion**

The analytical method has been demonstrated to be a reliable and accurate procedure for the determination of cyprodinil in crops (exemplified by apple) to a limit of quantification of 0.01 mg/kg, using commercially available laboratory equipment and reagents.

## A 2.1.1.5.2.3 Method validation (report R B8040)

| | |
|---|---|
| Comments of zRMS: | The validation has been accepted.<br>The objective of the study was to validate the analytical method REM 141.10 for the analysis of cyprodinil in apple and barley (whole plant, grain and straw) at a LOQ) of 0.01. For the matrix tested and for both characteristic LC-MS/MS mass transitions (primary: *m/z* 226.2 → 93.0; confirmatory: *m/z* 226.2→ 77.0) acceptable mean recoveries between 70 and 110% with relative standard deviations < 20% were obtained.<br>The repeatability and specificity of the method have been demonstrated, and the analytical method REM 141.10 is therefore considered valid for the determination of residues of cyprodinil in apple and barley. |

| | |
|---|---|
| Reference: | KCP 5.1.2.5 |
| Report | Cyprodinil (CGA219417) - Validation of Analytical Method REM141.10 for the Determination of Residues of Cyprodinil in Apple and Barley (Whole Plant, Grain and Straw) Final Report, Stouvenot, C., 2018, Report No. R B8040, XXXX File No. CGA219417_11918, VV-~~229267~~ 469881 |

| | |
|---|---|
| Guideline(s): | Guidelines and Criteria for the Preparation and Presentation of Complete Dossiers and of Summary Dossiers for the Inclusion of Active Substances in Regulations (EU) 283/2013 and 284/2013 implementing Regulation (EC) 1107/2009.<br>European Commission Guidance for Generating and Reporting Methods of Analysis in Support of Pre-registration Requirements for Annex II (Part A, Section 4) of Directive 91/414, SANCO/3029/99 revision 4 (11 Jul 2000).<br>OECD Guidance Document on Pesticide Residue Analytical Methods, ENV/JM/MONO(2007)17 (Unclassified, 13 Aug 2007).<br>EPA Residue Chemistry Test Guidelines OPPTS 860.1340 Residue Analytical Method, EPA 712-C-96-174 (Aug 1996).<br>OECD Series on Principles of GLP and Compliance Monitoring No. 1 (as revised in 1997) "OECD Principles on Good Laboratory Practice", Paris 1998. ENV/MC/CHEM(98)17 and respective national regulations.<br>Article Annex II to Article D523-8 of the Environmental Code - 16 October 2007<br>Directive 2004/10/EC, 11 February 2004 |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes |

**Materials and methods**

Crop samples were extracted by homogenisation with methanol/$H_2O$ (70/30, v/v). Extracts were centrifuged and aliquots (0.5 mL or 1 mL for straw) were cleaned up by solid phase extraction using a SCX cartridge. Final determination was by high performance liquid chromatography with triple quadrupole mass spectrometric detection (LC-MS/MS), monitoring for the primary transition (m/z 226.2→93.0) and the confirmatory transition (m/z 226.2→77.0).

Analytical method REM 141.10 was validated in apple and barley (whole plant, grain and straw).

**Results and discussions**

Fortified samples were analysed in quintuplet at the limit of quantification (LOQ, 0.01 mg/kg) and at: 200 x LOQ (2 mg/kg) for apple, 600 x LOQ (6 mg/kg) for barley whole plant, 400 x LOQ (4 mg/kg) for barley grain and 200 x LOQ (2 mg/kg) for barley straw. Acceptable mean recoveries of between 60% and 120% at 0.01 mg/kg and 70% and 110% at > 0.1 mg/kg were found for both transitions on all matrices tested and therefore according to EU guidance (SANTE/2020/12830, Rev.1) demonstrate the method has satisfactory accuracy. The relative standard deviations (RSDs) of cyprodinil recoveries at each fortification level and overall for each crop tested during method validation were < 10% and therefore according to the EU guidance (SANTE/2020/12830, Rev.1) demonstrate the method has satisfactory repeatability.

Extracts solutions have been shown to be stable when stored under refrigerated conditions for up to 7 days for apple and 28 days for barley grain and straw.

Standard solutions have been shown to be stable when stored under refrigerated conditions (1 – 7 °C) for up to 31 days.

Spiking solutions have been shown to be stable when stored under refrigerated conditions (1 – 7 °C) for up to 31 days.

**Table A 10: Recovery results from method validation of cyprodinil in crops using the analytical method REM 141.10 (primary transition m/z 226.2→93)**

| Matrix | Fortification Level (mg/kg) | Recovery (%) | Number of Analysis (n) | Mean Recovery (%) | RSD (%) | Recovery Range (%) |
|---|---|---|---|---|---|---|
| Apple | 0.01* | 79, 77, 80, 82, 74 | 5 | 78 | 3.8 | 74 – 82 |
| | 2 | 65, 81, 80 ,78 ,73 | 5 | 75 | 8.4 | 65 – 81 |
| | Overall | - | 10 | 77 | 6.4 | 65 – 82 |
| Barley whole plant | 0.01* | 71, 74, 69, 69, 77 | 5 | 72 | 4.7 | 69 – 77 |
| | 6 | 68, 70, 83, 81, 82 | 5 | 77 | 9.2 | 68 – 83 |
| | Overall | - | 10 | 74 | 7.8 | 68 – 83 |
| Barley grain | 0.01* | 75, 75, 77, 78, 70 | 5 | 75 | 4.2 | 70 – 78 |
| | 4 | 79, 77, 74, 79, 74 | 5 | 77 | 3.5 | 74 – 79 |
| | Overall | - | 10 | 76 | 3.8 | 70 – 79 |
| Barley straw | 0.01* | 90, 82, 77, 82, 84 | 5 | 83 | 5.7 | 77 – 90 |
| | 2 | 86, 85, 80, 93, 86 | 5 | 86 | 5.7 | 80 – 93 |
| | Overall | - | 10 | 84 | 5.7 | 77 – 93 |

* Limit of quantitation, defined by the lowest validated fortification level

**Table A 11: Recovery results from method validation of cyprodinil in crops using the analytical method REM 141.10 (confirmatory transition m/z 226.2→77.0)**

| Matrix | Fortification Level (mg/kg) | Recovery (%) | Number of Analysis (n) | Mean Recovery (%) | RSD (%) | Recovery Range (%) |
|---|---|---|---|---|---|---|
| Apple | 0.01* | 78, 79, 81, 80, 72 | 5 | 78 | 4.4 | 72 – 81 |
| | 2 | 68, 81, 79, 79, 73 | 5 | 76 | 7.1 | 68 – 81 |
| | Overall | - | 10 | 77 | 5.8 | 68 – 81 |
| Barley whole plant | 0.01* | 70, 74, 70, 71, 78 | 5 | 73 | 4.9 | 70 – 78 |
| | 6 | 68, 72, 84, 82, 84 | 5 | 78 | 9.4 | 68 – 84 |
| | Overall | - | 10 | 75 | 8.1 | 68 – 84 |
| Barley grain | 0.01* | 76, 74, 82, 77, 73 | 5 | 76 | 4.8 | 73 – 82 |
| | 4 | 81, 78, 75, 81, 75 | 5 | 78 | 3.7 | 75 – 81 |
| | Overall | - | 10 | 77 | 4.2 | 73 – 82 |
| Barley straw | 0.01* | 91, 82, 77, 80, 81 | 5 | 82 | 6.5 | 77 - 91 |
| | 2 | 86, 85, 80, 94, 85 | 5 | 86 | 6.1 | 80 - 94 |
| | Overall | - | 10 | 84 | 6.4 | 77 - 94 |

* Limit of quantitation, defined by the lowest validated fortification level

**Table A 12: Characteristics for the analytical method REM 141.10 used for validation of cyprodinil residues in crops**

| | Cyprodinil |
|---|---|
| Specificity | LC-MS/MS with two transitions is considered to be a highly specific detection technique and therefore according to EU guidance (SANTE/2020/12830, Rev.1) no further confirmatory technique is required. The method includes two MS/MS transitions, both of which have been validated. No significant interferences arising from the crop matrices, the labware, reagents or solvents have been |

| | **Cyprodinil** |
|---|---|
| | observed at the retention times of interest. |
| Calibration (type, number of data points) | The linearity of the LC-MS/MS detector was tested using standard solutions. Linearity was tested for both MS/MS transitions. Standards at seven different concentrations were injected and the signal area plotted against concentration for all calibration points. Correlation coefficients (r) were ≥ 0.99 for both fragment ions monitored. |
| Calibration range | ~~0.3 ng/mL to 12 ng/mL.~~ 0,003 mg/kg to 0,012 mg/kg |
| Assessment of matrix effects is presented | Yes<br>No significant matrix effects were observed in the crop matrices tested during method validation, therefore non-matrix matched linearity standards were used for quantification. |
| Limit of determination/quantification | The limit of quantification for cyprodinil residues in crop matrices using method REM 141.10 was established at 0.01 mg/kg. No interfering peaks around the retention time of cyprodinil were found in any of the control samples and reagent blank at levels above 30% of the limit of quantification.<br>The limit of detections (LODs) were calculated to be 0.003 mg/kg for the primary and confirmatory transitions, for the lowest injected calibration standard (0.3 ng/mL) in methanol / water (70/30, v/v). |

**Conclusion**

Analytical method REM 141.10 has been demonstrated to be a reliable and accurate procedure for the determination of cyprodinil in apple and barley (whole plant, grain and straw) to a limit of quantification of 0.01 mg/kg, using commercially available laboratory equipment and reagents.

## A 2.1.1.5.2.4 Method validation (report R B7375)

| | |
|---|---|
| Comments of zRMS: | The validation of analytical method REM141.10 in cherry, peach, strawberry, grape, blackcurrant, lettuce, bulb onion, fresh peas (with pods), dried beans, carrot, tomato, melon, asparagus, celery and witloof chicory at a LOQ of 0.01 has been accepted.<br>For the matrices tested and for both characteristic LC-MS/MS mass transitions (primary: *m/z* 226.2 → 93.0; confirmatory: *m/z* 226.2→ 77.0) acceptable mean recoveries between 70 and 110% with relative standard deviations < 20% were obtained. |

| | |
|---|---|
| Reference: | KCP 5.1.2.5 |
| Report | Cyprodinil (CGA219417): Validation of Analytical Method REM141.10 for the Determination of Residues of Cyprodinil in multiple crops Final Report, Stouvenot, C., 2018, Report No. R B7375, XXXX File No. CGA219417_11883, VV-469301 |
| Guideline(s): | Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/825/00 rev. 8.1, 16/11/2010).<br>Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/3029/99 rev. 4, 11/07/2000). |

| | |
|---|---|
| | OECD Guidance Document on Pesticide Residue Analytical Methods ENV/JM/MONO(2007)17.<br><br>Regulation (EC) No 1107/2009 of the European Parliament and of the council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC.<br><br>Residue Chemistry Test Guidelines OPPTS 860.1340 Residue Analytical Method, EPA 712-C-96-174, August 1996.Directive 2004/10/EC, 11 February 2004 |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes |

**Materials and methods**

Crop samples were extracted by homogenisation with methanol/water (70/30, v/v). Extracts were centrifuged and aliquots (0.5 mL) were cleaned up by solid phase extraction using a SCX cartridge. Final determination was by high performance liquid chromatography with triple quadrupole mass spectrometric detection (LC-MS/MS), monitoring for the primary transition (m/z 226.2→93.0) and the confirmatory transition (m/z 226.2→77.0).

Analytical method REM 141.10 was validated in a wide range of crops: cherry, peach, strawberry, grape, blackcurrant, lettuce, bulb onion, fresh peas (with pods), dried beans, carrot, tomato, melon, asparagus, celery and witloof chicory (chicon).

**Results and discussions**

Fortified samples were analysed in quintuplet at the limit of quantification (LOQ, 0.01 mg/kg) and at:

- 200 x LOQ (2 mg/kg) for cherry,
- 200 x LOQ (2 mg/kg) for peach,
- 500 x LOQ (5 mg/kg) for strawberry,
- 300 x LOQ (3 mg/kg) for grape,
- 300 x LOQ (3 mg/kg) for blackcurrant,
- 1500 x LOQ (15 mg/kg) for lettuce,
- 30 x LOQ (0.3 mg/kg) for bulb onion,
- 200 x LOQ (2 mg/kg) for fresh peas (with pods),
- 20 x LOQ (0.2 mg/kg) for dried beans,
- 150 x LOQ (1.5 mg/kg) for carrot,
- 150 x LOQ (1.5 mg/kg) for tomato,
- 60 x LOQ (0.6 mg/kg) for melon,
- 10 x LOQ (0.1 mg/kg) for asparagus,
- 500 x LOQ (5 mg/kg) for celery,
- 10 x LOQ (0.1 mg/kg) for witloof chicory.

Acceptable mean recoveries of between 60% and 120% at 0.01 mg/kg, 70% and 120% at 0.1 mg/kg and

70% and 110% at > 0.1 mg/kg were found for both transitions on all matrices tested and therefore according to EU guidance (SANTE/2020/12830, Rev.1) demonstrate the method has satisfactory accuracy. The relative standard deviations (RSDs) of cyprodinil recoveries for each crop tested at ≤ 0.1 mg/kg fortification level were < 20%, at ≤ 1.0 mg/kg fortification level < 15% and at > 1 mg/kg fortification level ≤ 10% (except 12.3-12.4% in blackcurrant at 3 mg/kg and 13.6-14.9% in celery at 5 mg/kg), therefore according to the EU guidance (SANTE/2020/12830, Rev.1) demonstrate the method has satisfactory repeatability.

The stability of sample extracts fortified with cyprodinil at the LOQ level (0.01 mg/kg) was assessed up to 15 days for lettuce, tomato and bulb onion, 16 days for cherry, strawberry, blackcurrant, fresh peas (with pods), dried beans and asparagus, 18 days for peach, grape and melon and 23 days for carrot, celery and witloof chicory (chicon). The results demonstrated that the cyprodinil residues in the stored fortified samples were stable over these time periods. The mean recovery values at the LOQ level were between 70 and 110%, with a RSD of ≤ 20% and the difference from the original analysis was ≤ 20% when re-analysed.

The stability of the stored working standard solutions of cyprodinil at 10.2 ng/mL were assessed after a storage period of 31 days in a refrigerator between 1 – 7 °C against freshly prepared calibration standards. The results demonstrated that cyprodinil residues in the stored working standard solutions were stable. The mean response factors from three replicate measurements for each of two solutions (old and new) did not differ by more than 10%.

The stability of the stored working spiking solutions of cyprodinil at 1016 ng/mL was assessed after a storage period of 31 days in a refrigerator between 1 – 7 °C against freshly prepared spiking solution. The results demonstrated that cyprodinil residues in the stored working spiking solutions were stable. The mean response factors from three replicate measurements for each of two solutions (old and new) did not differ by more than 10%.

**Table A 13: Recovery results from method validation of cyprodinil in crops using the analytical method REM 141.10 (primary transition m/z 226.2→93)**

| Matrix | Fortification Level (mg/kg) | Recovery (%) | Number of Analysis (n) | Mean Recovery (%) | RSD (%) | Recovery Range (%) |
|---|---|---|---|---|---|---|
| Cherry | 0.01* | 65, 78, 69, 97, 73 | 5 | 76 | 16.2 | 65 - 97 |
| | 2 | 79, 82, 78, 73, 68 | 5 | 76 | 7.2 | 68 - 82 |
| | Overall | - | 10 | 76 | 11.9 | 65 - 97 |
| Peach | 0.01* | 82, 82, 80, 87, 78 | 5 | 82 | 4.1 | 78 - 87 |
| | 2 | 87, 82, 85, 85, 80 | 5 | 84 | 3.5 | 80 - 87 |
| | Overall | - | 10 | 83 | 3.8 | 78 - 87 |
| Strawberry | 0.01* | 94, 84, 83, 72, 81 | 5 | 83 | 9.6 | 72 - 94 |
| | 5 | 81, 79, 82, 82, 84 | 5 | 82 | 2.0 | 79 - 84 |
| | Overall | - | 10 | 82 | 6.7 | 72 - 94 |
| Grape | 0.01* | 79, 71, 73, 64, 73 | 5 | 72 | 7.2 | 64 - 79 |
| | 3 | 75, 75, 71, 73, 78 | 5 | 74 | 3.7 | 71 - 78 |
| | Overall | - | 10 | 73 | 5.6 | 64 - 79 |
| Blackcurrant | 0.01* | 67, 79, 74, 76, 57 | 5 | 70 | 12.6 | 57 - 79 |
| | 3 | 77, 83, 66, 71, 91 | 5 | 77 | 12.4 | 66 - 91 |
| | Overall | - | 10 | 74 | 12.9 | 57 - 91 |
| Lettuce | 0.01* | 95, 92, 93, 84, 70 | 5 | 87 | 12.0 | 70 - 95 |
| | 15 | 93, 92, 87, 97, 101 | 5 | 94 | 5.4 | 87 - 101 |
| | Overall | - | 10 | 90 | 9.6 | 70 - 101 |

| Matrix | Fortification Level (mg/kg) | Recovery (%) | Number of Analysis (n) | Mean Recovery (%) | RSD (%) | Recovery Range (%) |
|---|---|---|---|---|---|---|
| Bulb onion | 0.01* | 84, 75, 80, 95, 74 | 5 | 81 | 10.5 | 74 - 95 |
| | 0.3 | 93, 90, 91, 93, 97 | 5 | 93 | 2.9 | 90 - 97 |
| | Overall | - | 10 | 87 | 9.6 | 74 - 97 |
| Fresh peas (with pods) | 0.01* | 98, 85, 86, 73, 82 | 5 | 85 | 10.4 | 73 - 98 |
| | 2 | 79, 76, 80, 83, 79 | 5 | 79 | 3.1 | 76 - 83 |
| | Overall | - | 10 | 82 | 8.1 | 73 - 98 |
| Dried beans | 0.01* | 75, 66, 71, 64, 76 | 5 | 70 | 7.3 | 64 - 76 |
| | 0.2 | 81, 68, 68, 64, 78 | 5 | 72 | 10.3 | 64 - 81 |
| | Overall | - | 10 | 71 | 8.6 | 64 - 81 |
| Carrot | 0.01* | 81, 80, 73, 74, 73 | 5 | 76 | 5.2 | 73 - 81 |
| | 1.5 | 80, 78, 73, 72, 74 | 5 | 75 | 4.7 | 72 - 80 |
| | Overall | - | 10 | 76 | 4.7 | 72 - 81 |
| Tomato | 0.01* | 84, 88, 91, 75, 79 | 5 | 84 | 8.0 | 75 - 91 |
| | 1.5 | 82, 81, 79, 80, 97 | 5 | 84 | 8.9 | 79 - 97 |
| | Overall | - | 10 | 84 | 8.0 | 75 - 97 |
| Melon | 0.01* | 75, 74, 70, 95, 72 | 5 | 77 | 13.1 | 70 - 95 |
| | 0.6 | 91, 92, 92, 81, 89 | 5 | 89 | 5.3 | 81 - 92 |
| | Overall | - | 10 | 83 | 11.6 | 70 - 95 |
| Asparagus | 0.01* | 95, 99, 102, 102, 100 | 5 | 100 | 3.2 | 95 - 102 |
| | 0.1 | 99, 93, 99, 92, 95 | 5 | 96 | 3.3 | 92 - 99 |
| | Overall | - | 10 | 98 | 3.7 | 92 - 102 |
| Celery | 0.01* | 77, 65, 85, 80, 77 | 5 | 77 | 10.0 | 65 - 85 |
| | 5 | 73, 86, 68, 62, 63 | 5 | 70 | 13.6 | 62 - 86 |
| | Overall | - | 10 | 74 | 12.0 | 62 - 86 |
| Witloof chicory | 0.01* | 82, 88, 87, 68, 74 | 5 | 80 | 11.0 | 68 - 88 |
| | 0.1 | 74, 71, 70, 79, 75 | 5 | 74 | 4.5 | 70 - 79 |
| | Overall | - | 10 | 77 | 9.2 | 68 - 88 |

* Limit of quantitation, defined by the lowest validated fortification level

**Table A 14: Recovery results from method validation of cyprodinil in crops using the analytical method REM 141.10 (confirmatory transition m/z 226.2→77.0)**

| Matrix | Fortification Level (mg/kg) | Recovery (%) | Number of Analysis (n) | Mean Recovery (%) | RSD (%) | Recovery Range (%) |
|---|---|---|---|---|---|---|
| Cherry | 0.01* | 65, 78, 68, 96, 72 | 5 | 76 | 16.4 | 65 - 96 |
| | 2 | 79, 82, 78, 74, 69 | 5 | 76 | 6.4 | 69 - 82 |
| | Overall | - | 10 | 76 | 11.7 | 65 - 96 |
| Peach | 0.01* | 85, 85, 83, 90, 82 | 5 | 85 | 3.4 | 82 - 90 |
| | 2 | 87, 82, 85, 85, 79 | 5 | 84 | 3.6 | 79 - 87 |

| Matrix | Fortification Level (mg/kg) | Recovery (%) | Number of Analysis (n) | Mean Recovery (%) | RSD (%) | Recovery Range (%) |
|---|---|---|---|---|---|---|
| | Overall | - | 10 | 84 | 3.4 | 79 - 90 |
| Strawberry | 0.01* | 99, 85, 82, 80, 81 | 5 | 86 | 9.2 | 80 - 99 |
| | 5 | 80, 79, 80, 82, 84 | 5 | 81 | 2.2 | 79 - 84 |
| | Overall | - | 10 | 83 | 7.1 | 79 - 99 |
| Grape | 0.01* | 80, 72, 73, 69, 77 | 5 | 74 | 5.4 | 69 - 80 |
| | 3 | 74, 75, 71, 72, 78 | 5 | 74 | 3.7 | 71 - 78 |
| | Overall | - | 10 | 74 | 4.3 | 69 - 80 |
| Blackcurrant | 0.01* | 68, 77, 71, 74, 60 | 5 | 70 | 9.4 | 60 - 77 |
| | 3 | 77, 82, 66, 71, 91 | 5 | 78 | 12.3 | 66 - 91 |
| | Overall | | 10 | 74 | 11.8 | 60 - 91 |
| Lettuce | 0.01* | 93, 91, 92, 83, 67 | 5 | 85 | 12.8 | 67 - 93 |
| | 15 | 94, 93, 87, 98, 101 | 5 | 94 | 5.6 | 87 - 101 |
| | Overall | - | 10 | 90 | 10.5 | 67 - 101 |
| Bulb onion | 0.01* | 86, 77, 79, 94, 75 | 5 | 82 | 9.5 | 75 - 94 |
| | 0.3 | 92, 91, 91, 92, 96 | 5 | 92 | 2.2 | 91 - 96 |
| | Overall | - | 10 | 87 | 8.8 | 75 - 96 |
| Fresh peas (with pods) | 0.01* | 98, 84, 84, 73, 84 | 5 | 84 | 10.3 | 73 - 98 |
| | 2 | 80, 76, 81, 84, 79 | 5 | 80 | 3.6 | 76 - 84 |
| | Overall | - | 10 | 82 | 8.0 | 73 - 98 |
| Dried beans | 0.01* | 75, 68, 77, 65, 74 | 5 | 72 | 7.0 | 65 - 77 |
| | 0.2 | 82, 69, 68, 65, 79 | 5 | 73 | 10.1 | 65 - 82 |
| | Overall | - | 10 | 72 | 8.3 | 65 - 82 |
| Carrot | 0.01* | 79, 81, 72, 73, 72 | 5 | 75 | 5.9 | 72 - 81 |
| | 1.5 | 80, 77, 73, 72, 73 | 5 | 75 | 4.4 | 72 - 80 |
| | Overall | - | 10 | 75 | 4.9 | 72 - 81 |
| Tomato | 0.01* | 84, 88, 92, 79, 78 | 5 | 84 | 6.8 | 78 - 92 |
| | 1.5 | 82, 80, 78, 80, 95 | 5 | 83 | 8.1 | 78 - 95 |
| | Overall | - | 10 | 84 | 7.1 | 78 - 95 |
| Melon | 0.01* | 75, 79, 70, 98, 73 | 5 | 79 | 14.2 | 70 - 98 |
| | 0.6 | 90, 92, 91, 80, 89 | 5 | 88 | 5.4 | 80 - 92 |
| | Overall | - | 10 | 84 | 11.4 | 70 - 98 |
| Asparagus | 0.01* | 95, 96, 100, 102, 98 | 5 | 98 | 3.1 | 95 - 102 |
| | 0.1 | 100, 94, 101, 94, 96 | 5 | 97 | 3.4 | 94 - 101 |
| | Overall | - | 10 | 97 | 3.2 | 94 - 102 |
| Celery | 0.01* | 84, 74, 97, 91, 90 | 5 | 87 | 10.3 | 74 - 97 |
| | 5 | 73, 87, 68, 62, 62 | 5 | 70 | 14.9 | 62 - 87 |
| | Overall | - | 10 | 79 | 16.3 | 62 - 97 |
| Witloof chicory | 0.01* | 87, 89, 89, 67, 76 | 5 | 82 | 12.1 | 67 - 89 |

| Matrix | Fortification Level (mg/kg) | Recovery (%) | Number of Analysis (n) | Mean Recovery (%) | RSD (%) | Recovery Range (%) |
|---|---|---|---|---|---|---|
| | 0.1 | 75, 70, 70, 78, 75 | 5 | 73 | 5.0 | 70 - 78 |
| | Overall | - | 10 | 77 | 10.6 | 67 - 89 |

* Limit of quantitation, defined by the lowest validated fortification level

**Table A 15: Characteristics for the analytical method REM 141.10 used for validation of cyprodinil residues in crops**

| | Cyprodinil |
|---|---|
| Specificity | LC-MS/MS with two transitions is considered to be a highly specific detection technique and therefore according to EU guidance (SANTE/2020/12830, Rev.1) no further confirmatory technique is required. The method includes two MS/MS transitions, both of which have been validated. No significant interferences arising from the crop matrices, the labware, reagents or solvents have been observed at the retention times of interest. |
| Calibration (type, number of data points) | The linearity of the LC-MS/MS detector was tested using standard solutions. Linearity was tested for both MS/MS transitions. Standards at seven different concentrations were injected and the signal area plotted against concentration for all calibration points. Correlation coefficients (r) were ≥ 0.99 for both transitions monitored. |
| Calibration range | ~~0.3 ng/mL to 12 ng/mL.~~ 0,003 mg/kg to 0,012 mg/kg |
| Assessment of matrix effects is presented | Yes<br>No significant matrix effects were observed in the crop matrices tested during method validation, therefore non-matrix matched linearity standards were used for quantification. |
| Limit of determination/quantification | The limit of quantification for cyprodinil residues in crop matrices using method REM 141.10 was established at 0.01 mg/kg. No interfering peaks around the retention time of cyprodinil were found in any of the control samples and reagent blank at levels above 30% of the limit of quantification.<br>The limit of detections (LODs) were calculated to be 0.003 mg/kg for the primary and confirmatory transitions, for the lowest injected calibration standard (0.3 ng/mL) in methanol:water (70/30, v/v). |

**Conclusion**

Analytical method REM 141.10 has been demonstrated to be a reliable and accurate procedure for the determination of cyprodinil in crops to a limit of quantification of 0.01 mg/kg, using commercially available laboratory equipment and reagents.

## A 2.1.1.5.2.5 Method validation (report R B9170)

| | |
|---|---|
| Comments of zRMS: | The validation of analytical method REM141.10 has been accepted.<br>For the matrices tested and for both characteristic LC-MS/MS mass transitions (primary: *m/z* 226.2 → 93.0; confirmatory: *m/z* 226.2→ 77.0) acceptable mean recoveries between 70 and 110% with relative standard deviations < 20% were obtained. The LOQ was set at 0.01 mg/kg in kiwi. |

| | |
|---|---|
| Reference: | KCP 5.1.2.5 |
| Report | CYPRODINIL (CGA219417): Validation of Analytical Method REM141.10 for the Determination of Residues of Cyprodinil in Kiwi, Stouvenot, C., 2020, Report No. R B9170, XXXX File No. VV- 875665 |
| Guideline(s): | OECD Series on Principles of GLP and Compliance Monitoring: Number 1, OECD Principles on Good Laboratory Practice (as revised in 1997) (ENV/MC/CHEM(98)17)<br>Article Annexe II à l'Article D523-8 du Code de l'Environnement, October 16 2007<br>Directive 2004/10/EC, 11 February 2004<br>Regulation (EC) No. 1107/2009; Concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC<br>Regulation (EU) No.284/2013<br>SANCO/3029/99 rev.4, 11 July 2000<br>OECD Guidance document on pesticide residue analytical methods ENV/JM/MONO(2007)17, 13 August 2007<br>Residue Chemistry Test Guideline EPA OPPTS 860.1340 (1996) |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes |

**Materials and methods**

Prepared samples are extracted by homogenisation with aqueous methanol. After centrifugation, an aliquot is removed and cleaned up with solid phase extraction using SCX cartridge. Final determination is by high performance liquid chromatography coupled to a triple quadrupole mass spectrometry (LC-MS/MS) in multiple reaction monitoring mode.

The effect of crop matrices on the LC-MS/MS response was assessed by analysing a matrix-matched standard solution against calibration solutions prepared in methanol/$H_2O$ (70/30, v/v).

A standard solution in methanol/$H_2O$ (70/30, v/v) at 10.2 ng/mL was analysed after 15 days of refrigerated storage, and the average response factor (3 injections) obtained was compared with the average response factor obtained for a freshly prepared solution.

**Results and discussions**

Fortified samples were analysed in quintuplet at the limit of quantification (LOQ, 0.01 mg/kg) and at 10 x LOQ for kiwi. Acceptable mean recoveries of between 60% and 120% at 0.01 mg/kg and 70% and 120% at 0.1 mg/kg were found and therefore according to EU guidance (SANTE/2020/12830, Rev.1) demonstrate the method has satisfactory accuracy. The relative standard deviations (RSDs) of cyprodinil recoveries at each fortification level and overall were < 10% and therefore according to the EU guidance (SANTE/2020/12830, Rev.1) demonstrate the method has satisfactory repeatability.

The difference between average response factors was below 10% showing good stability of cyprodinil in

standard solutions prepared in methanol/$H_2O$ (70/30, v/v) upon refrigerated storage up to 15 days.

**Table A 16: Recovery results from method validation of cyprodinil in kiwi using the analytical method REM 141.10**

| Matrix | Fortification Level (mg/kg) | Recovery (%) | Number of Analysis (n) | Mean Recovery (%) | RSD (%) | Recovery Range (%) |
|---|---|---|---|---|---|---|
| Kiwi | 0.01* | 68.6, 71.3, 74.8, 70.6, 70.0 | 5 | 71.1 | 2.3 | 68.6-74.8 |
| | 0.10 | 64.3, 88.6, 82.2, 77.8, 70.1 | 5 | 76.5 | 9.6 | 64.3-88.6 |
| | Overall | | 10 | 73.8 | 7.2 | 64.3-88.6 |

* Limit of quantitation, defined by the lowest validated fortification level

**Table A 17: Characteristics for the analytical method REM 141.10 used for validation of cyprodinil residues in kiwi**

| | Cyprodinil |
|---|---|
| Specificity | LC-MS/MS with two transitions m/z 226.2 to 77.0 and 226.2 to 93.0<br>blank value < 30 % LOQ |
| Calibration (type, number of data points) | Straight lines with correlation coefficients typically > 0.990 were obtained |
| Calibration range | Accepted calibration range in concentration units working range of ~~0.3 ng/mL to 12.2 ng/mL~~<br>0,003 mg/kg to 0,012 mg/kg |
| Assessment of matrix effects is presented | Yes<br>No significant interferences arising from the matrices, the lab ware, reagents or solvents observed |
| Limit of determination/quantification | 0.01 mg/kg limit of quantification representing the lowest validated level with sufficient recovery and precision |

**Conclusion**

Method REM 141.10 is considered to be sufficiently validated for the analysis of residues of cyprodinil in kiwi.

## A 2.1.1.6 Description of analytical methods for the determination of residues in support of ecotoxicological studies (KCP 5.1.2.6)

### A 2.1.1.6.1 Analytical method “ECO_019_01B”

#### A 2.1.1.6.1.1 Method validation

| | |
|---|---|
| Comments of zRMS: | The validation of the method ECO_019_01B has been accepted.<br>In study S21-05703 analytical method ECO_019_01B for the determination of cyprodinil in test medium (applied in studies S21-05724 and S21-05725) was verified with regard to recovery, linearity of detector response, repeatability, specificity, matrix effect, extract stability, limit of quantification and limit of detection. The method was validated in Elendt M4 test medium at LOQ of 0.001 mg/L. The analytical method fulfils the requirements of guideline |

| | |
|---|---|
| | SANTE/2020/12830 rev. 1.<br><br>Quantification was performed by use of LC-MS/MS detection. 2 mass transitions (226 93 *m/z* (Quantification) 226 77 *m/z* (Confirmation)) were evaluated in order to demonstrate that the method achieves a high level of selectivity. Recoveries were within the required range. No significant interference above LOD (30 % of LOQ) was detected in any of the untreated matrix so that a high level of selectivity was demonstrated. |

| | |
|---|---|
| Reference: | KCP 5.1.2.6 |
| Report | Method for the Determination of Cyprodinil in Aquatic Ecotoxicology Test Medium, Heineke, 2021, XXXX Method Reference: ECO_019_01B,_Report No S21-05725, XXXX File No. VV-931771 and Report No S21-05724, XXXX File No. VV-931772 |
| Guideline: | SANTE/2020/12830 Rev. 1 |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes |

| | |
|---|---|
| Reference: | KCP 5.1.2.6 |
| Report | Cyprodinil – Analytical Method ECO_019_01B and Validation for the Determination of Cyprodinil in Aquatic Ecotoxicology Test Medium, Heinicke, 2021, Report No S21-05703, XXXX File No. VV-928453 |
| Guideline: | SANTE/2020/12830 Rev. 1 |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes |

**Materials**

| | |
|---|---|
| **Test Material** | Cyprodinil |
| **Lot/Batch #:** | AMS 452/3 |
| **Purity (%):** | 99.9 % w/w |
| **IUPAC name:** | (4-cyclopropyl-6-methyl-pyrimidin-2-yl)-phenyl-amine |
| **CAS number:** | 121552-61-2 |

**Study Design and Methods**

Test facility: Eurofins Agroscience Services EcoChem GmbH (EAS EcoChem GmbH)

Study start date: 27 July 2021

Study end date: 14 Oct 2021

Analytical phase dates: 29th July to 10th Aug 2021

For recovery samples, 10 mL of homogenised Elendt M4 test medium were fortified with standard solutions of cyprodinil in acetonitrile. Five samples of test medium were fortified at the limit of quantification (LOQ; 0.001 mg/L) and five at a higher level (30.0 mg/L). The fortified samples were analysed alongside untreated control samples.

**Principle of the Method**

Samples of aquatic media (10 mL Elendt M4/algal medium) with residues in ranges from 0.00100 mg/L to 30.0 mg/L for were diluted with 10 mL acetonitrile and shaken on a vortex mixer. If necessary, samples were then diluted further with acetonitrile/test medium (1:1, v/v) to bring the sample within the calibration range. The diluted samples were then quantified by LC-MS/MS, monitoring two mass transitions of cyprodinil ($m/z = 226 \rightarrow 93$ and $m/z = 226 \rightarrow 77$). The limit of quantification (LOQ) for the method was 0.00100 mg cyprodinil/L. Cyprodinil was determined by high-performance liquid chromatography with mass-spectrometric detection (LC-MS/MS).

**HPLC-MS/MS Conditions**

| | |
|---|---|
| HPLC system: | Shimadzu |
| Pumps: | LC-30 AD HPLC pump |
| Degasser: | DGU-20A5R |
| Column Oven: | CTO-20AC |
| Detector: | SCIEX API 5500 |
| Autosampler: | SIL-30ACMP |
| Column: | Phenomenex Kinetex 2.6 µm Biphenyl 100A, 100 mm x 2.1 mm i.d., 2.6 µm mean particle size (No. 00D-4622-AN) with 2.1 mm C18 UHPLC guard column |
| Mobile phase: | A: Water + 0.5 % formic acid<br>B: Acetonitrile |

| Time | %A | %B | Gradient |
|---|---|---|---|
| 0.00 | 90 | 10 | - |
| 1.80 | 5 | 95 | Linear |
| 2.50 | 5 | 95 | - |
| 2.60 | 90 | 10 | Linear |
| 3.60 | 90 | 10 | - |

| | |
|---|---|
| Flow rate: | 0.5 ml/min |
| Column oven temperature | 40°C |
| Injection volume: | 2 µL/ 5°µL |
| Retention time: | 1.7 min |

| | | |
|---|---|---|
| Detector | SCIEX API 5500 | |
| | Ionisation mode | Electrospray ionisation ESI |
| | Source polarity: | Positive |
| | Curtain gas (CUR): | 40 (arbitrary units) |
| | Gas 1 (GSI): | 40 (arbitrary units) |
| | Gas 2 (GSI): | 60 (arbitrary units) |
| | Temperature (TEM): | 400 °C |
| | Ionspray voltage (IS): | 4500V |
| | Collision gas setting (CAD): | 10 |
| | Entrance potential (EP): | 10 V |
| | Dwell time | 50 msec |
| | Resolution Q1 and Q2 | 0.7 |

Source and detection parameters for MS/MS experiments:

| Compound | Parent m/z | CE (V) | DP (V) | CXP (V) | Fragment ions (m/z) | |
|---|---|---|---|---|---|---|
| Cyprodinil | 226 | 45<br>60 | 70<br>70 | 10<br>10 | 93<br>77 | Quantification<br>Confirmation |

CE: Collision energy; CXP: Collision cell exit potential; DP: Declustering Potential
Quantification: Peak areas of fragment ion at m/z = 93, external standards in matrix Confirmation: Peak areas of fragment ion at m/z = 77, external standards in matrix

**Recovery Data**

Recovery and precision data of cyprodinil obtained from test medium (Elendt M4) at each fortification level using method ECO_019_01B are presented in the table below.

**Table A 18: Accuracy and precision results from validation of ECO_019_01B for cyprodinil in test medium.**

| Matrix | Fortification Level (mg/L) | Accuracy (%) | Number of Analysis (n) | Mean Accuracy (%) | RSD (%) | Accuracy Range (%) |
|---|---|---|---|---|---|---|
| **Elendt M4 Test Medium** | ***Mass transition 266 → 93 m/z (Primary)*** | | | | | |
| | 0.00100 | 101 / 102 / 101 / 102 / 102 | 5 | 102 | 1 | 101 - 102 |
| | 30.0 | 98 / 99 / 98 / 98 / 100 | 5 | 99 | 1 | 98 - 100 |
| | **Overall** | **-** | **10** | **100** | **2** | **98 -102** |
| **Elendt M4 Test Medium** | ***Mass transition 266 → 77 m/z (Confirmatory)*** | | | | | |
| | 0.00100 | 97 / 103 / 100 / 98 / 98 | 5 | 99 | 2 | 97 - 103 |
| | 30.0 | 97 / 99 / 98 / 100 / 100 | 5 | 99 | 1 | 97 - 100 |
| | **Overall** | **-** | **10** | **99** | **2** | **97 - 103** |

*Limit of quantitation, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ

**Table A 19: Characteristics of the analytical method used for the quantification of cyprodinil residues in test medium**

| **Analyte** | **Cyprodinil** |
|---|---|
| Equipment/ Chromatographic method | HPLC-MS/MS |
| Accuracy/ Precision (repeatability) | Fortified samples were analysed in quintuplet at the limit of quantification (LOQ) of 0.00100 mg/L and at 30.0 mg/L for cyprodinil in test medium. Acceptable mean accuracy values of between 70 % and 120 % with < 20% RSD were found and therefore according to SANTE/2020/12830 rev. 1 demonstrate the method has satisfactory accuracy and repeatability. |
| Precision (reproducibility) | The relative standard deviations (RSDs) of cyprodinil recovery values at each fortification level and overall during method validation were <20 % and therefore according to SANTE/2020/12830 rev. 1 demonstrate the method has satisfactory repeatability. |
| Specificity | LC-MS/MS with two transitions is considered to be a highly specific detection technique and therefore, according to SANTE/2020/12830 rev. 1, no further confirmatory technique is required. No significant interferences from the sample matrix, the labware, reagents or solvents were detected in the LC-MS/MS |

| | |
|---|---|
| | chromatograms at the retention time corresponding to cyprodinil in any of the control samples tested. |
| Confirmatory method | Since two LC-MS/MS mass transitions were used to monitor cyprodinil, the method achieves a high level of specificity, hence no further confirmatory method is required. |
| Assessment of matrix effects is presented | yes,<br>matrix effects were < ± 20 % and deemed to be insignificant. However, matrix-matched standards were used for quantification throughout the study. |
| Calibration/Linearity | Standards at a minimum of five different concentrations were injected and the signals area were plotted against concentrations for all calibration points.<br>The linearity of the LC-MS/MS detector were tested using matrix-matched standard solutions from 0.1 – 10 ng/mL cyprodinil (corresponding to 0.0002 – 0.02 mg/L) in test medium. The linear range was from 30% of the LOQ to at least 20% above the highest residue measured.<br>Quantification – y = 4.67e+005 x + 213 (r = 0.9999)<br>Confirmation - y = 3.3e+005 x + -4.44e+003 (r = 0.9999)<br>Residual plots were generated to assess the suitability of the chosen function by visual inspection. The calibration models were considered suitable since the residuals were randomly distributed |
| Limit of quantification (LOQ) | Limit of quantification representing the lowest validated level with acceptable recovery and precision<br>The LOQ for cyprodinil in test medium using method ECO_019_01B was established at 0.00100 mg cyprodinil /L. No interfering peaks around the retention time of cyprodinil were found in any of the control samples at levels above 30% of the LOQ. |
| Limit of detection (LOD) | 0.000200 mg cyprodinil /L |
| Standard Solution Stability | Stock solution of cyprodinil in HPLC grade water are stable when stored at 1 °C to 10 °C in the dark for 48 days. |

**Conclusion:**

Analytical method ECO_019_01B has been demonstrated to be a reliable and accurate procedure for the determination of cyprodinil in Elendt M4 test medium with a limit of quantification (LOQ) of 0.00100 mg/L in accordance SANTE/2020/12830 Rev. 1, using commercially available laboratory equipment and reagents.

(Heinicke, 2021)

### A 2.1.1.6.1.2 Confirmatory method

No confirmatory method is required according to new SANTE/2020/12830, rev 1.

## A 2.1.1.6.2 Analytical method “ECO_019_03B”

### A 2.1.1.6.2.1 Method validation

| | |
|---|---|
| Comments of zRMS: | The method validation has been accepted.<br>The objective of the study S21-03983 was to fully validate an analytical method ECO_019_03B for the determination of cyprodinil in 50 % aqueous sucrose solution and in larval diet in accordance with SANTE/2020/12830, rev.1. This method was applied in studies S21-02794 and S21-02796.<br>The limit of quantification (1 mg/kg for both matrices) was sufficient for the determination of cyprodinil in 50 % aqueous sucrose solution and in larval diet in parallel honey bee chronic feeding test studies and honey bee larval toxicity test studies. |

| | |
|---|---|
| | Quantification was performed by HPLC-MS/MS detection. 2 mass transitions (226 93 *m/z* (Quantification) 226 77 *m/z* (Confirmation)) were evaluated in order to demonstrate that the method achieves a high level of selectivity. Mean recoveries for both mass transitions at each fortification level are within 70 – 110 % and the associated RSD are < 5%. No significant interference above LOD (30 % of LOQ) was detected in any of the untreated matrix so that a high level of selectivity was demonstrated. |

| | |
|---|---|
| Reference: | KCP 5.1.2.6 |
| Report | Method for the Determination of Cyprodinil in Feeding Solution (50% Aqueous Sucrose Solution) and Larval Diet, Ringli, 2021, XXXX Method Reference: ECO_019_03B,_Report No S21-02794, XXXX File No. VV-946992 and Report No S21-02796, XXXX File No. VV-947029 |
| Guideline: | SANTE/2020/12830 Rev. 1 |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes |

| | |
|---|---|
| Reference: | KCP 5.1.2.6 |
| Report | Cyprodinil – Analytical Method ECO_019_03B and Validation for the Determination of Cyprodinil in Feeding Solution (50 % Aqueous Sucrose Solution) and Larval Diet, Ringli D., 2021, S21-03983 (XXXX File No. VV-944813) |
| Guideline(s): | SANTE/2020/12830 rev. 1 |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes |

**Materials**

| **Test Material** | **Cyprodinil** |
|---|---|
| **Lot/Batch #:** | **AMS 452/3** |
| **Purity (%):** | **99.9% W/W** |
| **IUPAC name:** | **4-cyclopropyl-6-methyl-pyrimidin-2-yl)-phenyl-amine** |
| **CAS number:** | **121552-61-2** |

**Study Design and Methods**

Test facility: Eurofins Agroscience Services EcoChem GmbH, Eutinger Str. 24, 75223 Niefern-Öschelbronn, Germany

Study start date: 28 May 2021

Study end date: 19 Aug 2021

Analytical phase dates: 01 Jun – 15 Jun 2021

Feeding solutions (Adult honeybees 50% w/v sucrose solution and larvae diet) were fortified with standard

solutions of Cyprodinil in acetonitrile. Five samples were fortified at the limit of quantification (LOQ; 1.0 mg/kg), and five at a higher level (10 mg/kg). The fortified samples were analysed alongside untreated control samples.

**Principle of the Method**

Feeding solutions (Samples of 50 % aqueous sucrose solution (2 mL) and larval diet samples (500 mg) were extracted with 10 mL acetonitrile / water (1:1, v/v) and shaken for 10 minutes. One QuEChERS-Citrat Kit was added to the sample before centrifugation at 4000 rpm for 5 minutes. The acetonitrile phase was then diluted further using acetonitrile / water (1:1, v/v), by a factor of 200 for 50% aqueous sucrose solution samples, and a factor of 50 for larval diet samples. If necessary, further dilution with blank matrix was performed to bring the sample within the calibration range. The sample extraction and dilution process was then followed by quantification by LC-MS/MS using two mass transitions; primary transition (m/z = 226/93) and confirmatory transition (m/z = 226/77). The limit of quantification (LOQ) of the method was 1.0 mg/kg.

**HPLC Conditions**

| | |
|---|---|
| HPLC system: | Shimadzu HPLC |
| Column: | Phenomenex Kinetex 2.6 um Biphenyl 100A, 100 mm x 2.1 mm, 2.6 µm, (Part No. 00D-4622-AN) with 2.1 mm C18 UHPLC guard column (Phenomenex, AJ0-9000 and AJ0-8782) |
| Mobile phase: | A: Water + 0.5% Formic Acid<br>B: Acetonitrile |

| Time | % mobile phase A | % mobile phase B | Gradient |
|---|---|---|---|
| 0.01 | 90 | 10 | - |
| 1.80 | 5 | 95 | Linear |
| 2.50 | 5 | 95 | - |
| 2.60 | 90 | 10 | Linear |
| 3.60 | 90 | 10 | - |

| | |
|---|---|
| Flow rate: | 0.5 ml/min |
| Column oven temperature | 40°C |
| Injection volume: | 5 µL |
| Stop Time | 3.61 minutes |
| Injection protocol | Standard injections spread over sequence with a maximum of six sample injections between two standards |
| Retention time: | Cyprodinil: 1.8 mins |

**MS/MS Conditions**

| | | |
|---|---|---|
| Detector | Sciex API 5500<br>Interface<br>Source polarity:<br>Curtain gas (CUR):<br>Gas 1 (GS1):<br>Gas 2 (GS2):<br>Temperature (TEM):<br>Ionspray voltage (IS):<br>Collision gas setting (CAD):<br>Entrance potential (EP):<br>Scan type | <br>TurboIonSpray<br>Positive<br>Nitrogen set at 40 (arbitrary units)<br>Nitrogen set at 40 (arbitrary units)<br>Nitrogen set at 60 (arbitrary units)<br>400°C<br>4500V<br>Nitrogen set at 40 (arbitrary units)<br>10 V<br>MRM |

Source and detection parameters for MS/MS experiments:

| Compound | Parent m/z | CE (V) | DP (V) | CXP (V) | Fragment ions (m/z) | |
|---|---|---|---|---|---|---|
| Cyprodinil | 226 | 45 | 70 | 10 | 93 | Quantification |
| | | 60 | 70 | 10 | 77 | Confirmation |

CE: Collision energy; CXP: Collision cell exit potential; DP: Declustering Potential

Quantification: Peak areas of fragment ion at m/z = 93, external standards in matrix Confirmation: Peak areas of fragment ion at m/z = 77, external standards in matrix

**Recovery data**

Recovery and precision data of Cyprodinil obtained from 50% aqueous sucrose solution and larval diet at each fortification level using method ECO_019_03B are presented in the table below

**Table A 20: Accuracy and precision results from validation of ECO_019_03B for Cyprodinil in 50% aqueous sucrose solution and larval diet.**

| Matrix | Fortification Level (mg/kg) | Accuracy (%) | Number of Analysis (n) | Mean Accuracy (%) | RSD (%) | Accuracy Range (%) |
|---|---|---|---|---|---|---|
| ***Mass transition 226 → 93 m/z (Primary)*** | | | | | | |
| Feeding solution (50 % aqueous sucrose solution) | 1.00 | 92 / 92 / 90 /95 / 97 | 5 | 93 | 3 | 90-97 |
| | 10.0 | 101 / 92 / 92 / 91 / 94 | 5 | 94 | 4 | 91-101 |
| | **Overall** | | **10** | **94** | **4** | **90-101** |
| Larval Diet | 1.00 | 92 / 84 / 88 / 89 / 88 | 5 | 88 | 3 | 88-92 |
| | 10.0 | 93 / 91 / 92 / 90 / 91 | 5 | 91 | 1 | 90-93 |
| | **Overall** | | **10** | **90** | **3** | **88-93** |
| ***Mass transition 226 → 77 m/z (Confirmatory)*** | | | | | | |
| Feeding solution (50 % | 1.00 | 92 / 92 / 92 / 94 / 96 | 5 | 93 | 2 | 92-96 |
| | 10.0 | 101 / 92 / 91 / 92 / 93 | 5 | 94 | 4 | 91-101 |

| | | | | | | |
|---|---|---|---|---|---|---|
| aqueous sucrose solution) | **Overall** | | **10** | **94** | **3** | **91-101** |
| Larval Diet | 1.00 | 90 / 87 / 84 / 88 / 89 | 5 | 88 | 3 | 84-90 |
| | 10.0 | 90 / 90 / 91 / 90 / 87 | 5 | 90 | 2 | 87-91 |
| | **Overall** | | **10** | **89** | **2** | **84-91** |

*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

**Table A 21: Characteristics of the analytical method used for the quantification of Cyprodinil in 50% aqueous sucrose solution and larval diet**

| | |
|---|---|
| Analyte | Cyprodinil |
| Equipment/ Chromatographic method | HPLC-MS/MS |
| Accuracy/ Precision (repeatability) | Acceptable mean accuracy values of between 70 % and 110 % were found in water and therefore according to EU guidance demonstrate the method has satisfactory accuracy.<br>Fortified feeding solutions were analysed in quintuplet at the limit of quantification (LOQ) of 1.0 mg/kg, and at higher level 10 mg/kg . Acceptable mean accuracy values of between 70 % and 120 % were found in feeding solutions and therefore according to EU guidance demonstrate the method has satisfactory accuracy. |
| Precision (reproducibility) | The relative standard deviations (RSDs) of analyte(s) recovery values at each fortification level and overall during method validation were <20 % and therefore according to the EU guidance (see guidance section of this summary) demonstrate the method has satisfactory repeatability. |
| Specificity | LC-MS/MS provides high specificity for the analysis and detection of Cyprodinil for the purpose of ecotoxicity studies i.e. clean, well described test matrix analysing a pre-defined quantity of test item. No significant interferences from the sample matrix, the labware, reagents or solvents were detected in the LC-MS/MS chromatograms at the retention time corresponding to Cyprodinil in any of the control samples tested. No significant interference at or above 30 % of LOQ was detected in the reagent blank or the control samples. |
| Confirmatory method | Two LC-MS/MS mass transitions were used to monitor Cyprodinil , and therefore the method achieves a high level of specificity. |
| Assessment of matrix effects is presented | Untreated 50 % aqueous sucrose solution samples and untreated larval diet samples were analysed according to the method to investigate the presence of residue and/or background interference at the retention time of cyprodinil. Two (2) mass transitions were evaluated. The samples showed no significant interference (above 30 % of LOQ) at the retention time of the analyte in any investigated 50 % aqueous sucrose solution and in any investigated larval diet, therefore showing that the method is highly specific.<br>The matrix effects observed for 50 % aqueous sucrose solution and larval diet were deemed to be insignificant (< ± 20 %). Nevertheless, matrix-matched standards were used for quantification for 50 % aqueous sucrose solution and for larval diet.<br>No significant matrix effects (<20 %) were found for Cyprodinil in the feeding solution during the method validation. |
| Calibration/Linearity | A linear calibration curve was constructed by single determination of matrix-matched or solvent standards at seven (7) concentration levels ranging from 0.7 ng/mL (or 0.6 ng/mL, respectively for larval diet) to 15 ng/mL for the |

| | |
|---|---|
| | quantifier and qualifier transition. This range corresponds to a fortification level of 0.294 mg/kg to 6.30 mg/kg for 50 % aqueous sucrose solution and of 0.300 mg/kg to 7.50 mg/kg for larval diet and thus covers the range from no more than 30 % of the limit of quantification (LOQ) and at least + 30 % of the highest analyte concentration detected in a diluted sample extract.<br>The equation of the line and coefficient of determination were:<br>**50% w/v sucrose**<br>Primary transition - y = 4.36e+005 x + -4.05e+003 (r = 0.9999)<br>Confirmatory transition - y = 3.03e+005 x + -1.12e+004 (r = 0.9999)<br>**Larval diet**<br>Primary transition - y = 4.27e+005 x + 1.37e+004 (r = 0.9998)<br>Confirmatory transition - y = 3.02e+005 x + 2.34e+003 (r = 0.9999) |
| Limit of quantification (LOQ) | The LOQ for Cyprodinil using method ECO_019_03B was established at 1.00 mg/kg representing the lowest validated level with acceptable recovery and precision. |
| Limit of detection (LOD) | The LOD for Cyprodinil in feeding solutions using method ECO_019_03B was established as 0.294 mg/kg in 50 % aqueous sucrose solution (equivalent to 29.4 % of the LOQ) and 0.300 mg/kg in larval diet (equivalent to 30 % of the LOQ) . The LOD is defined as the lowest analyte concentration detectable above the mean amplitude of the background noise in an untreated sample and corresponds to the lowest calibration standard. |
| Extract Stability | No significant degradation of Cyprodinil was observed when final samples were stored 1°C to 10°C for 11 days (50 % aqueous sucrose solution) or eight (8) days (larval diet). The mean recovery value was within 70-120% with an RSD of ≤20% and was within ± 20% of the original value. |
| Standard stability | No significant degradation of Cyprodinil was observed when standard samples prepared in acetonitrile were stored 1°C to 10°C for 48 days. The mean recovery value was within 70-120% with an RSD of ≤20% and was within ± 20% of the original value. |
| Extractability | Adequate recovery data from the method validation verify the extraction of residues of Cyprodinil from feeding solutions of adult honeybees (50% aqueous sucrose solution) and final diets of honey bee larvae (larval diet). |

**Conclusion:**
Analytical method ECO_019_03B has been demonstrated to be a reliable and accurate procedure for the determination of Cyprodinil in 50% aqueous sucrose solution and in larval diet with a limit of quantification (LOQ) of 1.00 mg/kg in accordance with SANTE/2020/12830 rev. 1, using commercially available laboratory equipment and reagents.

(Ringli, 2021)

### A 2.1.1.6.2.2 Confirmatory method

No confirmatory method is required according to new SANTE/2020/12830, rev 1.

### A 2.1.1.6.3 Analytical method "Elendt M4 Media"

### A 2.1.1.6.3.1 Method validation

| | |
|---|---|
| Comments of zRMS: | The validation has been accepted.<br><br>Concentrations of CGA219417 were determined using a HPLC/UV method of analysis. The method was validated at 0.01 mg/L and 20 mg/L cyprodinil tech. Five determinations were carried out at each fortification level. The mean recovery at each level and the overall mean recovery were determined and were in the range 80 to 110%. The % RSD level and the overall % RSD were all less than 20%.<br><br>The LOQ is 0.01 mg/L cyprodinil, based on the lowest fortification level where a mean recovery falls within the range 70 to 110%. Samples from the tests were analysed in batches containing a control sample and two procedural recoveries. Procedural recoveries were in the range 70 to 120%. |

| | |
|---|---|
| Reference: | KCP 5.1.2.6 |
| Report | Maynard, S. K., (2011), CGA219417 – 96 Hour Acute Toxicity to Juvenile *Asellus aquaticus*, CEM Analytical Services Limited (CEMAS), UK, Report No. CEMS-5069. (XXXX File No: CGA219417_11453; VV-397982 ) |
| Guideline(s): | SANCO/3029/99 rev. 4 |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes |

**Principle of the Method**

Samples were diluted with methanol and analysed by high performance liquid chromatography with ultra violet detection (HPLC-UV) at 270 nm, using a Nucleosil 100-5C 18 column (120 mm x 4.0 mm) and gradient elution with mobile phases of 0.05 M aqueous ammonia acetate/methanol (90:10, v/v) and methanol. Quantification was performed using external standards.

**Specificity**

No interferences were observed at the retention time of interest in control matrix samples, demonstrating the specificity of the method. Analyte identity was confirmed by retention time match with an analytical standard.

**Linearity**

The linearity of detector response was demonstrated using nine external standard solutions across the concentration range of 0.0025 to 10.0 µg/mL. The coefficient of determination ($R^2$) was determined to be 0.9998 (slope = 350.1850).

**Precision (Repeatability)**

Repeatability data was generated from samples fortified at the LOQ and 2000 x LOQ. The relative standard deviations (RSD) obtained for each fortification level were within the guideline requirements of less than 20% and are presented in the table below.

**Accuracy (Recovery)**

Recovery data was generated from samples fortified at the LOQ and 2000 x LOQ. The mean percentage recoveries obtained for each fortification level were within the guideline requirements of 70 – 110% and are presented in the table below.

**Limit of quantification (LOQ)**

The limit of quantification (LOQ), defined as the lowest fortification level where acceptable precision and accuracy data were obtained has been demonstrated to be 0.01 mg/L.

**Conclusion**
The analytical procedures have been successfully validated in terms of specificity, linearity, precision, accuracy and LOQ in accordance with all the requirements of SANCO/3029/99 rev. 4.

**Table A 22: Precision and Accuracy Data**

| Matrix | Analyte | Fortification Level (mg/L) | Number of Samples (n) | Mean Recovery (%) | RSD (%) |
|---|---|---|---|---|---|
| Elendt M4 Media | Cyprodinil | 0.01 | 5 | 95 | 5.2 |
| | | 20.0 | 5 | 101 | 4.1 |
| | | Overall | 10 | 98 | 5.4 |

(Maynard, 2011)

### A 2.1.1.6.3.2 Confirmatory method

No confirmatory method is required according to new SANTE/2020/12830, rev 1.

## A 2.1.1.6.4 Analytical method "Saltwater HPLC Method 1"

### A 2.1.1.6.4.1 Method validation

| | |
|---|---|
| Comments of zRMS: | The validation has not been accepted.<br>The analytical method was validated by preparing triplicate samples of CGA-219417 in a representative dilution water at nominal concentrations of 0.001 and 2.5 mg/L. The validation data are too poor. Recoveries in pretest samples ranged from 71 to 140%. |

| | |
|---|---|
| Reference: | KCP 5.1.2.6 |
| Report | Ward, T. J., Boeri, R. L., Magazu, J. P., (1995), Acute Flow-Through Toxicity of CGA-219417 to the Mysid, *Mysidopsis bahia*, T.R. Wilbury Laboratories, Inc., USA, Report No. 827-CG. (XXXXX File No: CGA219417/0649 / VV-372679) |
| Guideline(s): | FIFRA - Guideline No.: 72-3(c) |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes |

**Principle of the Method**
Samples were diluted with phosphate buffer solution and methanol, and were cleaned up using pre-conditioned BOND-ELUT C18 solid phase extraction cartridges. The samples were eluted with methanol into

centrifuge tubes containing 2 g/L ammonium acetate in methanol/water (50:50 v/v). The samples were analysed by high performance liquid chromatography with ultra violet detection (HPLC-UV) at 270 nm, using a Hypersil-ODS column (100 mm x 4.6 mm, 5 µm particle size) fitted with a Hypersil ODS guard column (20 mm x 4 mm) and isocratic elution with a mobile phase of 2 g/L ammonium acetate in water/methanol (20:80 v/v). Quantification was performed using external standards.

**Specificity**
No data provided in the report.

**Linearity**
No data provided in the report.

**Precision (Repeatability)**
No data provided in the report.

**Accuracy (Recovery)**
Recovery data was generated from samples fortified at 0.001 mg/L and 2.5 mg/L. The mean percentage recoveries obtained for each fortification level were within the guideline requirements of 70 – 110% and are presented in the table below.

**Limit of quantification (LOQ)**
No data provided in the report.

**Conclusion**
The reported method validation is not fully compliant with current analytical reporting requirements under SANCO/3029/99 rev. 4. Nevertheless, the analytical method can be considered as fit for purpose for dose verification, confirming dosing of CGA219417 concentrations in test water in this aquatic flow-through system.

**Table A 23: Precision and Accuracy Data**

| Matrix | Analyte | Fortification Level (mg/L) | Number of Samples (n) | Recovery range (%) | Mean Recovery (%) |
|---|---|---|---|---|---|
| Test water | Cyprodinil | 0.001 | 3 | 53.2 – 90.5 | 76.1 |
| | | 2.5 | 3 | 65.2 – 89.6 | 76.0 |

(Boeri *et al.*, 1995)

### A 2.1.1.6.4.2 Confirmatory method

No confirmatory method is required according to new SANTE/2020/12830, rev 1.

## A 2.1.1.6.5 Analytical method “Saltwater HPLC Method 2”

### A 2.1.1.6.5.1 Method validation

| | |
|---|---|
| Comments of zRMS: | The method has been accepted.<br>The analysis of CGA-219417 technical in saltwater was done by HPLC method |

| | |
|---|---|
| | with UV detection. No interferences were observed at or above the LOQ during the sample analysis. Saltwater samples were fortified at 0.400, 2.00 and 10.0 µg/L. The mean recoveries and RSDs obtained were within required range.<br>The method fits the purpose. |

| | |
|---|---|
| Reference: | KCP 5.1.2.6 |
| Report | Drottar, K. R., Krueger, H. O., (1999), CGA-219417: A Flow-Through Life-Cycle Toxicity Test with the Saltwater Mysid (*Mysidopsis bahia*), Wildlife International Ltd., USA, Report No.: 108A-205, Study No.: 35-99. (XXXX File No. CGA219417/0926 / VV-311558) |
| Guideline(s): | EPA Guideline No. 72-4, OPPTS No. 850.1350 Draft |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes |

**Principle of the Method**

Saltwater samples were extracted with methanol and hexane, combining the hexane layers in round bottom flasks. Aqueous hydrochloric acid was added; the samples were concentrated and transferred to centrifuge tubes with methanol. The samples were concentrated and diluted with methanol/water (50:50, v/v) for analysis. The samples were analysed by high performance liquid chromatography with ultra violet detection (HPLC-UV) at 270 nm, using a Phenomenex Luna C18 column (250 mm x 4.6 mm, 5 µm particle size) and gradient elution with mobile phases of methanol/aqueous ammonium acetate (50:50, v/v) and methanol/aqueous ammonium acetate (90:10, v/v). Quantification was performed using external standards.

**Specificity**

No interferences were observed at the retention time of interest in control matrix samples, demonstrating the specificity of the method. Analyte identity was confirmed by retention time match with an analytical standard.

**Linearity**

The linearity of detector response was demonstrated using five external standard solutions across the concentration range of 5.00 to 50.0 µg/L. The coefficient of determination ($R^2$) was determined to be 0.9998 (slope = 259.76, intercept = -49.63579).

**Precision (Repeatability)**

Repeatability data was generated from samples fortified at 0.400 µg/L and higher fortification levels. The relative standard deviation (RSD) obtained were within the guideline requirements of less than 20% and are presented in the table below.

**Accuracy (Recovery)**

Recovery data was generated from samples fortified at 0.400 µg/L and higher fortification levels. The mean percentage recoveries obtained for each fortification level were within the guideline requirements of 70 – 110% and are presented in the table below.

**Limit of quantification (LOQ)**

The limit of quantification (LOQ), defined as the lowest fortification level where acceptable precision and accuracy data were obtained has been demonstrated to be 0.400 µg/L.

**Conclusion**
The analytical procedures have been successfully validated in terms of specificity, linearity, precision, accuracy and LOQ in accordance with all the requirements of SANCO/3029/99 rev. 4.

**Table A 24: Precision and Accuracy Data**

| Matrix | Analyte | Fortification Level (µg/L) | Number of Samples (n) | Mean Recovery (%) | RSD (%) |
|---|---|---|---|---|---|
| Saltwater | CGA219417 | 0.400 | 6 | 108 | 4.1 |
| | | 2.00 | 6 | 102 | 1.5 |
| | | 10.0 | 6 | 99.3 | 1.1 |
| | | Overall | 18 | 103 | 4.29 |

(Drottar & Krueger, 1999)

### A 2.1.1.6.5.2 Confirmatory method

No confirmatory method is required according to new SANTE/2020/12830, rev 1.

## A 2.1.1.6.6 Analytical method "Pond Water/Sediment"

### A 2.1.1.6.6.1 Method validation

| | |
|---|---|
| Comments of zRMS: | The acceptable method applied in the study is a modification of the validated LC-MS/MS cyprodinil method REM 141.10.<br>Residues of cyprodinil in the microcosm water were measured following application to the microcosms as A14325E (EC, 303 g/L) with the LOQ 0.75 µg ai/L.<br>The method fits the purpose. |

| | |
|---|---|
| Reference: | KCP 5.1.2.6 |
| Report | Ashwell, J., Benyon, K., Powley, W., Richardson, M., (2007), Cyprodinil (CGA219417) 300 g/L EC Formulation A14325E Effects on Aquatic Organisms in an Outdoor Microcosm T008777-05, XXXX, UK, Report No. T008777-05-REG, (XXXX File No. CGA219417/1683; VV-339018) |
| Guideline(s): | SANCO/3029/99 rev. 4 |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes |

**Principle of the Method**
Application solution samples were diluted with water prior to analysis. Microcosm samples were analysed directly. All the samples were analysed by high performance liquid chromatography with tandem mass specific detection (HPLC-MS/MS) in positive ionisation mode, using an Ace C18 column (50 mm x 3.2 mm, 5 µm particle size) and gradient elution with mobile phases of acetonitrile and 0.2% acetic acid in

water. Quantification was performed using external standards. The ion transition m/z 226.1 > 92.9 was used for quantification.

**Specificity**
No interferences were observed at the retention time of interest in control matrix samples, demonstrating the specificity of the method. Analyte identity was confirmed by retention time match with an analytical standard.

**Linearity**
No data provided in the report.

**Precision (Repeatability)**
Repeatability data was generated from samples fortified at 0.75 µg/L and at higher fortification levels. The relative standard deviation (RSD) obtained were within the guideline requirements of less than 20% and are presented in the table below.

**Accuracy (Recovery)**
Recovery data was generated from samples fortified at 0.75 µg/L and at higher fortification levels. The mean percentage recoveries obtained for each fortification level were within the guideline requirements of 70 – 110% and are presented in the table below.

**Limit of quantification (LOQ)**
The limit of quantification (LOQ), defined as the lowest fortification level where acceptable precision and accuracy data were obtained has been demonstrated to be 0.75 µg/L.

**Conclusion**
The reported method validation is not fully compliant with current analytical reporting requirements under SANCO/3029/99 rev. 4. Nevertheless, the analytical method can be considered as fit for purpose based on the concentrations of cyprodinil determined in the sample solutions.

**Table A 25: Precision and Accuracy Data**

| Analyte | Fortification Level (µg/L) | Number of Samples (n) | Mean Recovery (%) | RSD (%) |
|---|---|---|---|---|
| Cyprodinil | 0.75 | 2 | 104 | - |
| | 20.0 | 2 | 96 | - |
| | 40.0 | 2 | 108 | - |
| | 150 | 2 | 88 | - |
| | Overall | 8 | 99 | 10 |

(Ashwell *et al.*, 2007)

### A 2.1.1.6.6.2 Confirmatory method

No confirmatory method is required according to new SANTE/2020/12830, rev 1.

## A 2.1.1.6.7 Analytical method "Reconstituted Test Water"

### A 2.1.1.6.7.1 Method validation

| | |
|---|---|
| Comments of zRMS: | The method has been accepted.<br>CGA321915 concentration in the test media samples was determined by HPLC/UV. On the basis of a method provided by the sponsor, an analytical method was adapted and implemented by Harlan Lab (Harlan D96711, see next study).<br>The $R^2$ fits of the calibration curves used were 0.9995 and 0.9989. The average recoveries for the samples were found to be 100% of the spiked values with relative standard deviations of 6% and 2%, respectively. The method was considered to be sufficiently accurate and precise for the purposes of this test. The test sample results were not corrected for recovery. The LOQ is 1.03 mg/L. The method of analysis was validated and proven to be suitable for the intended use. |

| | |
|---|---|
| Reference: | KCP 5.1.2.6 |
| Report | Eckenstein, H., (2015), CGA321915 – Acute Toxicity to *Daphnia magna* in a 48-Hour Immobilization Test, Harlan Laboratories Ltd., Switzerland, Report No.: D96733. (XXXX File No. CGA321915_10005; VV-411573) |
| Guideline(s): | SANCO/3029/99 rev. 4 |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes |

**Principle of the Method**
Reconstituted test water samples were directly analysed by high performance liquid chromatography with ultra violet detection (HPLC-UV) at 300 nm, using an Inertsil ODS-3 C18 column (50 mm x 4.6 mm, 5 µm particle size) and gradient elution with mobile phases of 0.1% heptafluorobutyric acid in water and acetonitrile. Quantification was performed using external standards.

**Specificity**
No interferences were observed at the retention time of interest in control matrix samples, demonstrating the specificity of the method. Analyte identity was confirmed by retention time match with an analytical standard.

**Linearity**
The linearity of detector response was demonstrated using eight external standard solutions across the concentration range of 1.03 to 115 mg/L. The coefficient of determination ($R^2$) was determined to be 0.9995 (slope = 31805, intercept = -3542.3).

**Precision (Repeatability)**

Repeatability data was generated from samples fortified at 5.09 mg/L and 102 mg/L. The relative standard deviation (RSD) obtained were within the guideline requirements of less than 20% and are presented in the table below.

**Accuracy (Recovery)**
Recovery data was generated from samples fortified at 5.09 mg/L and 102 mg/L. The mean percentage recoveries obtained for each fortification level were within the guideline requirements of 70 – 110% and are presented in the table below.

**Limit of quantification (LOQ)**
The limit of quantification (LOQ), defined as the lowest calibration standard has been demonstrated to be 1.03 mg/L.

**Conclusion**
The analytical procedures have been successfully validated in terms of specificity, linearity, precision, accuracy and LOQ.

**Table A 26: Precision and Accuracy Data**

| Matrix | Analyte | Fortification Level (mg/L) | Number of Samples (n) | Mean Recovery (%) | RSD (%) |
|---|---|---|---|---|---|
| Reconstituted test water | CGA321915 | 5.09 | 5 | 100 | 6 |
| | | 102 | 5 | 100 | 2 |
| | | Overall | 10 | 100 | 4 |

(Eckenstein, 2014)

### A 2.1.1.6.7.2 Confirmatory method

No confirmatory method is required according to new SANTE/2020/12830, rev 1.

## A 2.1.1.6.8 Analytical method “OECD Test Medium”

### A 2.1.1.6.8.1 Method validation

| | |
|---|---|
| Comments of zRMS: | The method has been accepted.<br>CGA321915 concentration in the test media was determined by HPLC/UV. The method was adapted and implemented based on sponsor’s method.<br>The $R^2$ fits of the calibration curves used were 0.9995 and 0.9989. This reflects the linearity of the analytical system within the calibration range of 1.03 - 115 mg test item/L. The average recoveries for the non-centrifuged samples were found to be 102% and 105% of the spiked values with relative standard deviations of 3% and 1%, respectively. The average recoveries for the centrifuged samples were found to be 96% and 108% of the spiked values, with an overall mean of 102% (n = 4). The method was considered to be sufficiently accurate and precise for the purposes of this test. The test sample results were not corrected for recovery. |

| | |
|---|---|
| | The LOQ is 1.03 mg/L. The method of analysis was validated and proven to be suitable for the intended use. |

| | |
|---|---|
| Reference: | KCP 5.1.2.6 |
| Report | Eckenstein, H., (2015), CGA321915 – Toxicity to *Pseudokirchneriella subcapitata* in a 96-hour Algal Growth Inhibition Test. Report Number D96711. Harlan Laboratories Ltd, Zelgiweg 1, 4452 Itingen / Switzerland.<br>(XXXX File No. CGA321915_10004, VV-411271) |
| Guideline(s): | SANCO/3029/99 rev. 4 |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes |

**Principle of the Method**
In summary, test samples were analysed by HPLC with UV-VIS detection at 300 nm. The limit of quantitation (LOQ) was 1.03 mg CGA321915/L and the limit of detection (LOD) was not reported. The analytical method was validated for reconstituted water ("AAP").

**Specificity**
Representative chromatograms of standard solutions at 1.03 and 115 mg CGA321915/L and spiked media at 2.04 mg CGA321915/L are presented alongside a biological control, demonstrating no co-eluting peaks at the retention time of CGA321915 (2.1 to 2.2 minutes).

**Recovery Findings**
A summary of the results for CGA321915 is reported in the analytical phase report.

**Table A 27: Recovery and precision results from validation of the analytical method for CGA321915 in reconstituted water as used in the test**

| Matrix | Fortification Level (mg CGA321915/L) | Recovery | | | |
|---|---|---|---|---|---|
| | | Individual Measurements (%) | Mean (%) | RSD (%) | Range (%) |
| Reconstituted water | 2.04[1] | 98, 102, 98, 106, 104 | 102 | 3 | 98 – 106 |
| | 2.04[2] | 86, 107 | 96 | NR | 86 – 107 |
| | 102[1] | 104, 104, 104, 104, 107 | 105 | 1 | 104 – 107 |
| | 102[2] | 111, 105 | 108 | NR | 105 - 111 |
| | Overall[2] | - | 102 | - | - |

[1] Non-centrifuged spiked recovery samples
[2] Centrifuged recovery spiked samples
NR: Not reported

**Linearity**
Nine calibration standards in the range 1.03 to 115 mg CGA321915/L were used to generate a calibration curve. The regression analysis gave the following values: Slope = 31805; Intercept = 3542.3; $R^2$ = 0.9995.

**Recovery**
The mean recovery for the 2.04 mg CGA321915/L non-centrifuged fortified samples was 102 % with a relative standard deviation (RSD) of 3 %, and for the 2.04 mg CGA321915/L centrifuged fortified samples was 96 %. The mean recovery for the 102 mg CGA321915/L non-centrifuged fortified samples was 105 % with a RSD of 1 %, and for the 102 mg CGA321915/L centrifuged fortified samples was 108 %.

**Repeatability**
The overall RSD of CGA321915 values for the non-centrifuged fortification samples tested during method validation were ≤ 10 % (actual 1 and 3 %), demonstrating that the method has satisfactory repeatability.

**Limit of Quantification**
The Limit of Quantification was 1.03 mg CGA321915/L based on the lowest standard solution which fits into the calibration curve. The Limit of Detection (LOD) was not reported.

**Matrix Extract**
Matrix effects were not explicitly assessed, however the report states that test water was used in the analytical phase, so it can be assumed that matrix effects were appropriately accounted for.

**Conclusion**
This analytical method can be considered as fit for purpose for confirming CGA321915 concentrations in reconstituted water in a static aquatic system.

### A 2.1.1.6.8.2 Confirmatory method

No confirmatory method is required according to new SANTE/2020/12830, rev 1.

## A 2.1.1.7 Description of analytical methods for the determination of residues in support of physical and chemical properties tests (KCP 5.1.2.7)

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 5.2.1)

#### A 2.1.2.1.1 DFG S19

##### A 2.1.2.1.1.1 Method validation

| | |
|---|---|
| Comments of zRMS: | The validation of the method DFG S 19 has been accepted.<br>The method DFG S 19 was validated for the determination of the residues of cyprodinil by LC-MS/MS in apple, strawberry, barley grain and rape seed.<br>Control samples were analysed in duplicate. The fortified samples were analysed in quintuplet for each fortification level with one additional control for each matrix. Since two transitions were used to monitor cyprodinil, the method achieves a high level of specicity and no confirmation on a different detector was necessary. The transition 226 → 77 was selected for quantification and the transition 226 → 93 was used for confirmation (Appendix 5 of the original study shows the spectrum). Fortication experiments were performed at LOQ level and additionally at 3 mg/kg for strawberry and barley grain, 2 mg/kg for apple and 0.1 mg/kg for rape seed. For cyprodinil in apple, strawberry, barley grain and rape seed the LOQ was 0.01 mg/kg with a LOD of 0.003 mg/kg. Mean recovery values obtained for cyprodinil at both fortication levels (70 – 110%) are consistent with the requirements. The Multi Method DFG S 19 is applicable for the determination of residues of cyprodinil in apple, strawberry, barley grain and rape seed. |

| | |
|---|---|
| Reference: | KCP 5.2.1 |
| Report | Validation of multi-residue method DFG S19 (L00.00-34) for the determination of residues of cyprodinil in different plant matrices with LC-MS/MS detection, Lakaschus, S., 2005, Report No. SYN-0502V, XXXX File No. CGA219417/1388, VV-379854 |
| Guideline(s): | Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/825/00 rev. 7)<br>BA Guideline: Residue Analytical Methods for Post-Registration Control Purposes of July 21, 1998 |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes/No/Supplementary |

**Materials and methods**

The multi-residue method DFG S19 (extended revision L00.00-34) is a modular procedure. Apple and

strawberry samples were extracted using module E1. Barley grain was extracted using module E2 and rape seed by module E7. Extracts of each crop type were cleaned-up using the GPC module. All determinations were by LC-MS/MS using positive ion multiple reaction monitoring (MRM). The primary MRM transition 226→77 m/z was used for quantification and the MRM transition 226→93 m/z was used for confirmation.

**Results and discussions**

Residue method DFG S19 has been validated for the determination of residues of cyprodinil in crops, using strawberry, apple, barley grain and rape seed as representative matrices.

Fortified samples were analysed in quintuplet at the limit of quantification (LOQ, 0.01 mg/kg) and at relevant higher levels (3 mg/kg for strawberry and barley grain; 2 mg/kg for apple and 0.1 mg/kg for rape seed). Acceptable mean recoveries of between 60% and 120% at 0.01 mg/kg, 70% and 120% at 0.1 mg/kg and 70% and 110% at > 0.1 mg/kg were found for both transitions on all matrices tested, therefore according to the EU guidance (SANTE/2020/12830, Rev.1) demonstrate the method has satisfactory accuracy. The relative standard deviations (RSDs) of cyprodinil recoveries for each crop tested at ≤ 0.1 mg/kg fortification level were < 20% and at > 1 mg/kg fortification level ≤ 10% (except 11% in strawberry at 3.04 mg/kg), therefore according to the EU guidance (SANTE/2020/12830, Rev.1) demonstrate the method has satisfactory repeatability.

**Table A 28: Recovery results from method validation of cyprodinil using the analytical method DFG S19 (primary transition m/z 226→77)**

| Matrix | Fortification Level (mg/kg) | Recovery (%) | Number of Analyses (n) | Mean Recovery (%) | RSD (%) | Recovery Range (%) |
|---|---|---|---|---|---|---|
| Strawberry | 0.01* | 91, 112, 101, 106, 100 | 5 | 102 | 7.6 | 91-112 |
| | 3.04 | 87, 68, 90, 81, 88 | 5 | 83 | 11 | 68-90 |
| | Overall | | 10 | 92 | 14 | 68-112 |
| Apple | 0.01* | 94, 101, 107, 103, 111 | 5 | 103 | 6.2 | 94-111 |
| | 2.00 | 79, 87, 70, 75, 80 | 5 | 78 | 8.1 | 70-87 |
| | Overall | | 10 | 91 | 16 | 70-111 |
| Barley grain | 0.01* | 89, 101, 105, 104, 101 | 5 | 100 | 6.4 | 89-105 |
| | 3.04 | 109, 109, 120, 106, 104 | 5 | 110 | 5.6 | 104-120 |
| | Overall | | 10 | 105 | 7.4 | 89-120 |
| Rape seed | 0.01* | 95, 103, 107, 103, 77 | 5 | 97 | 12 | 77-107 |
| | 0.1 | 103, 101, 114, 106, 114 | 5 | 108 | 5.6 | 101-114 |
| | Overall | | 10 | 102 | 10 | 77-114 |

* Limit of quantitation, defined by the lowest validated fortification level

**Table A 29: Recovery results from method validation of cyprodinil using the analytical method DFG S19 (confirmatory transition m/z 226→93)**

| Matrix | Fortification Level (mg/kg) | Recovery (%) | Number of Analyses (n) | Mean Recovery (%) | RSD (%) | Recovery Range (%) |
|---|---|---|---|---|---|---|
| Strawberry | 0.01* | 98, 108, 105, 108, 105 | 5 | 105 | 3.9 | 98-108 |
| | 3.04 | 84, 70, 89, 81, 91 | 5 | 83 | 10 | 70-91 |
| | Overall | | 10 | 94 | 14 | 70-108 |
| Apple | 0.01* | 103, 104, 104, 103, 106 | 5 | 104 | 1.2 | 103-106 |
| | 2.00 | 79, 83, 69, 77, 77 | 5 | 77 | 6.6 | 69-83 |
| | Overall | | 10 | 91 | 16 | 69-106 |
| Barley grain | 0.01* | 91, 99, 99, 102, 99 | 5 | 98 | 4.2 | 91-102 |
| | 3.04 | 110, 110, 120, 106, 106 | 5 | 110 | 5.2 | 106-120 |
| | Overall | | 10 | 104 | 7.8 | 91-120 |
| Rape seed | 0.01* | 106, 98, 101, 97, 78 | 5 | 96 | 11 | 78-106 |
| | 0.1 | 103, 103, 115, 106, 114 | 5 | 108 | 5.5 | 103-115 |
| | Overall | | 10 | 102 | 10 | 78-115 |

* Limit of quantitation, defined by the lowest validated fortification level

**Table A 30: Characteristics for the analytical method DFG S19 used for validation of cyprodinil residues in crops**

| | Cyprodinil |
|---|---|
| Specificity | LC-MS/MS with two transitions is considered to be a highly specific detection technique and therefore according to EU guidance (SANTE/2020/12830, Rev.1) no further confirmatory technique is required. The method includes two MS/MS transitions, both of which have been validated. No significant interferences arising from plant matrices, the lab ware, reagents or solvents have been observed at the retention times of interest. |
| Calibration (type, number of data points) | The linearity of the LC-MS/MS detector was tested using standard solutions in solvent. Linearity was tested for both MS/MS transitions. Standards at 6 different concentrations were injected and the signal area plotted against concentration for all calibration points. Straight lines with correlation coefficients > 0.999 were obtained. |
| Calibration range | ~~0.25 - 20 ng/mL~~ 0,0025mg/kg to 0,02 mg/kg |
| Assessment of matrix effects is presented | Yes<br>No significant enhancement or suppression of detector response was observed. |
| Limit of determination/quantification | The limit of quantification for cyprodinil residues in plant commodities was established at 0.01 mg/kg. Analysis of control specimens of apple and rape seed yielded no residues of cyprodinil above the LOD indicating no interferences were present. During analysis of strawberry and barley grain specimens fortified at 3 mg/kg, the controls analysed in the |

| | |
|---|---|
| | same set showed residues above the LOQ for one transition (strawberry) and both transitions (barley). These blanks were caused by a carryover from the high fortification level samples but had no impact on the results, because the very high levels in the fortified samples were exceeding the blank level by one to two orders of magnitude. The control specimens analysed along with the LOQ fortifications of strawberry and barley did not show any residues above the LOD. This is in accordance with the level specified in EU Guidance Document SANTE/2020/12830, Rev.1, which demands a blank level of less than 30% of the LOQ. |

**Conclusion**

The multi-residue method DFG S19 has been sufficiently validated on a representative commodity included in the categories high water (apple), high acid (strawberry), dry commodities (barley grain) and high oil (rape seed) for the determination of cyprodinil residues at the LOQ (0.01 mg/kg).

## A 2.1.2.1.1.2 Independent laboratory validation

| | |
|---|---|
| Comments of zRMS: | The independent validation of the method DFG S 19 in strawberry and barley grain has been accepted.<br>The residue concentrations of Cyprodinil in the control specimens were < 30 % of LOQ. The specicity of the analytical method is acceptable, since no signicant interferences from the specimen matrices were detected at the retention time of interest. The evaluation of the second transition (226→93) showed the same recovery range (70 — llO %) as the evaluation using the first transition (226→77), demonstrating that both ions are valid for quantification. Taking into account the approved recovery range of 70 - 110 % (accuracy) and a relative standard deviation of 20 % (precision) for each fortication level and for each matrix, the DFG method for the determination of Cyprodinil in barley grain and strawberry was successfully validated. |

| | |
|---|---|
| Reference: | KCP 5.2.1 |
| Report | Independent Laboratory Validation of the DFG Method S19 for the determination of residues of cyprodinil in plant matrices (barley grain and strawberry), Reichert, N., 2006, Report No. IF-05/00362978, XXXX File No. CGA219417/1469, VV-379810 |
| Guideline(s): | Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/825/00 rev. 6) |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes/No/Supplementary |

**Materials and methods**

The principle of the method was the same than in the primary method.

**Results and discussions**

Fortified samples were analysed in quintuplet at the limit of quantification (LOQ, 0.01 mg/kg) and at 3 mg/kg. Acceptable mean recoveries of between 60% and 120% at 0.01 mg/kg and 70% and 110% at > 0.1 mg/kg were found for both transitions on all matrices tested, and therefore according to EU guidance (SANTE/2020/12830, Rev.1) demonstrate the method has satisfactory accuracy. The relative standard deviations (RSDs) of the recoveries at each fortification level and overall for each commodity tested during method validation were < 10%, therefore according to the EU guidance (SANTE/2020/12830, Rev.1) demonstrate the method has satisfactory repeatability.

**Table A 31: Recovery results from independent laboratory validation of cyprodinil using the analytical method DFG S19 (primary transition m/z 226→77)**

| Matrix | Fortification Level (mg/kg) | Recovery (%) | Number of Analyses (n) | Mean Recovery (%) | RSD (%) | Recovery Range (%) |
|---|---|---|---|---|---|---|
| Barley grain | 0.01* | 97, 97, 92, 92, 97 | 5 | 95 | 3 | 92-97 |
| | 3 | 99, 102, 96, 95, 98 | 5 | 98 | 3 | 95-102 |
| | Overall | | 10 | 97 | 3 | 92-102 |
| Strawberry | 0.01* | 87, 87, 87, 85, 92 | 5 | 88 | 3 | 85-92 |
| | 3 | 94, 90, 99, 92, 105 | 5 | 96 | 6 | 90-105 |
| | Overall | | 10 | 92 | 7 | 85-105 |

*Limit of quantitation, defined by the lowest validated fortification level

**Table A 32: Characteristics for the analytical method DFG S19 used for independent laboratory validation of cyprodinil residues in crops**

| | Cyprodinil |
|---|---|
| Specificity | LC-MS/MS with two transitions is considered to be a highly specific detection technique and therefore according to EU guidance (SANTE/2020/12830, Rev.1) no further confirmatory technique is required. The method includes two MS/MS transitions, both of which have been validated. No significant interferences arising from plant matrices, the lab ware, reagents or solvents have been observed at the retention times of interest. |
| Calibration (type, number of data points) | The linearity of the LC-MS/MS detector was tested using standard solutions in solvent. Linearity was tested for both MS/MS transitions. Standards at 7 different concentrations were injected and the signal area plotted against concentration for all calibration points. Straight lines with correlation coefficients > 0.999 were obtained. |
| Calibration range | ~~0.25 – 5 ng/mL~~ 0,0026 -0,053 mg/kg |
| Assessment of matrix effects is presented | Yes<br>No significant enhancement or suppression of detector response was observed. |
| Limit of determination/quantification | The limit of quantification for cyprodinil residues in plant commodities was established at 0.01 mg/kg. The residue concentrations of cyprodinil in the control specimens were <30% of the LOQ. |

**Conclusion**

The multi-residue method DFG S19 was successfully independently validated for the determination of cyprodinil residues in strawberry (high acid) and barley grain (dry commodities).

### A 2.1.2.1.1.3 Confirmatory method

No confirmatory method is required, because the method was validated at two mass transitions (primary and confirmatory).

### A 2.1.2.1.2 GRM010.02A

### A 2.1.2.1.2.1 Method validation (reports GRM010.02A and TK0021500)

| | |
|---|---|
| Comments of zRMS: | The validation of Analytical Method GRM010.02A has been accepted.<br><br>This method is a modified version of Method REM141.01. The second study (TK0021500) demonstrates the validation process. LC-MS/MS is a highly specific detection technique. Interference arising from the matrices tested has not been observed. 2 transitions were applied: primary *m/z* = 226.0 → *m/z* = 93.0; confirmatory *m/z* = 226.0 → *m/z* = 77.0. Residues of cyprodinil have been shown to be efficiently extracted from the matrices in a previous validation study (Tim, Oakes, XXXX Final Report 362-94).<br><br>The method GRM010.02A has been successfully validated for determination of residues of cyprodinil in wheat forage, wheat hay, wheat grain, apple, tomato, almond nut meat and almond hull (rep. TK0021500). For each of the crop commodities, two untreated controls and 5 recovery samples at 0.01 mg/kg (LOQ) and 5 recovery samples at 0.1 ppm (10 X LOQ) were analysed. Procedural recoveries ranged from 69 – 98% with an average of 82% (n = 70). The standard deviation was 6.02% with a relative standard deviation (RSD) of 7.31%. The procedural recoveries from confirmatory transition ranged from 71 – 99% with an average of 84% (n = 70). The standard deviation was 5.43% with a RSD of 6.50%. The LOQ for this analytical method was established as 0.01 ppm (mg/kg) for cyprodinil in all matrices. Residues of cyprodinil in untreated crop commodities were <30% of the LOQ. This procedure has been demonstrated to be a reliable and accurate procedure for the determination of residues of cyprodinil in crops and tree nuts. |

| | |
|---|---|
| Reference: | KCP 5.2.1 |
| Report | Cyprodinil: Analytical method for the determination of residues of cyprodinil in crops and tree nuts by LC-MS/MS, Lin, K., Manuli, M., 2011, Report No. GRM010.02A, XXXX File No. CGA219417_50141, VV-185044 |
| Guideline(s): | OECD Guidance Document on Pesticide Residue Analytical Methods ENV/JM/MONO(2007)17.<br><br>Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/825/00 rev. 7, 2004).<br><br>Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/3029/99 rev. 4, 11/07/2000). |

| | |
|---|---|
| | EPA Residue Chemistry Test Guideline OPPTS 860.1340 (712C-96-174) Residue Analytical Method, 1996 |
| Deviations: | No |
| GLP: | No |
| Acceptability: | Yes |
| Reference: | KCP 5.2.1 |
| Report | Cyprodinil: Validation of analytical method GRM010.02A for the determination of residues of cyprodinil in crops and tree nuts by LC-MS/MS, Lin, K., 2011, Report No. TK0021500, XXXX File No. CGA219417_50142, VV-413174 |
| Guideline(s): | OECD Guidance Document on Pesticide Residue Analytical Methods ENV/JM/MONO(2007)17.<br>Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/825/00 rev. 7, 2004).<br>Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/3029/99 rev. 4, 11/07/2000).<br>EPA Residue Chemistry Test Guideline OPPTS 860.1340 (712C-96-174) Residue Analytical Method, 1996 |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes |

**Materials and methods**

Analytical method GRM010.02A is a modified version of method REM141.01. Plant samples are homogenized and extracted with methanol/water (80:20). Nut samples are extracted for a further 5 minutes with acidified methanol/water (80:20). Aliquots of the extracts are cleaned-up using solid phase extraction cartridges (HLB phase). Cyprodinil is eluted in 0.1% ammonium acetate/acetonitrile (40:60). Final determination is by high performance liquid chromatography coupled to a triple quadrupole mass spectrometer (LC-MS/MS) in multiple reaction monitoring mode using the ion transition m/z 226→93 for quantitative analysis and the transition m/z 226→77 for confirmation. The LOQ of the method is 0.01 mg/kg.

**Results and discussions**

Residue method GRM010.02A has been validated for the determination of residues of cyprodinil in crops, using wheat forage, hay and grain, apples, tomato, almond nut meat and almond hull as representative matrices.

Fortified samples were analysed in quintuplet at the limit of quantification (LOQ, 0.01 mg/kg) and at ten times the LOQ (0.1 mg/kg). Acceptable mean recoveries of between 60% and 120% at 0.01 mg/kg and 70% and 120% at 0.1 mg/kg were found for both transitions on all matrices tested and therefore according to EU guidance (SANTE/2020/12830, Rev.1) demonstrate the method has satisfactory accuracy. The relative standard deviations (RSDs) of the recoveries at each fortification level and overall for each commodity

tested during method validation were < 20%, therefore according to the EU guidance (SANTE/2020/12830, Rev.1) demonstrate the method has satisfactory repeatability.

Re-analysis of stored cyprodinil final extracts at a temperature of 4 °C in glass HPLC vials demonstrated that cyprodinil is stable when for a period of 6-12 days.

**Table A 33: Recovery results from method validation of cyprodinil in crop matrices using the analytical method GRM010.02A (primary transition m/z 226→93)**

| Matrix | Fortification Level (mg/kg) | Recovery (%) | Number of Analysis (n) | Mean Recovery (%) | RSD (%) | Recovery Range (%) |
|---|---|---|---|---|---|---|
| Tomato | 0.01* | 83, 82, 83, 86, 83 | 5 | 83 | 2 | 82-86 |
| | 0.1 | 81, 83, 81, 94, 83 | 5 | 84 | 6 | 81-94 |
| | Overall | | 10 | 84 | 4 | 81-94 |
| Wheat hay | 0.01* | 79, 80, 83, 80, 79 | 5 | 78 | 6 | 79-83 |
| | 0.1 | 79, 82, 83, 82, 83 | 5 | 82 | 2 | 79-83 |
| | Overall | | 10 | 81 | 5 | 79-83 |
| Almond nut meat | 0.01* | 84, 82, 85, 83, 77 | 5 | 83 | 4 | 77-85 |
| | 0.1 | 93, 88, 72, 83, 89 | 5 | 85 | 10 | 72-93 |
| | Overall | | 10 | 84 | 7 | 72-93 |
| Almond hull | 0.01* | 76, 73, 69, 72, 77 | 5 | 73 | 4 | 69-77 |
| | 0.1 | 87, 94, 78, 79, 76 | 5 | 83 | 9 | 76-94 |
| | Overall | | 10 | 78 | 10 | 69-94 |
| Wheat grain | 0.01* | 76, 80, 83, 85, 71 | 5 | 80 | 7 | 71-85 |
| | 0.1 | 86, 86, 77, 81, 72 | 5 | 78 | 6 | 72-86 |
| | Overall | | 10 | 80 | 6 | 71-86 |
| Wheat forage | 0.01* | 81, 98, 83, 82, 82 | 5 | 85 | 8 | 81-98 |
| | 0.1 | 94, 85, 87, 84, 91 | 5 | 88 | 5 | 84-94 |
| | Overall | | 10 | 87 | 7 | 81-98 |
| Apple | 0.01* | 90, 86, 82, 86, 86 | 5 | 86 | 3 | 82-90 |
| | 0.1 | 88, 86, 70, 71, 87 | 5 | 80 | 12 | 70-88 |
| | Overall | | 10 | 83 | 8 | 70-90 |

* Limit of quantitation, defined by the lowest validated fortification level

**Table A 34: Recovery results from method validation of cyprodinil in crop matrices using the analytical method GRM010.02A (confirmatory transition m/z 226→77)**

| Matrix | Fortification Level (mg/kg) | Recovery (%) | Number of Analysis (n) | Mean Recovery (%) | RSD (%) | Recovery Range (%) |
|---|---|---|---|---|---|---|
| Tomato | 0.01* | 86, 87, 92, 86, 88 | 5 | 87 | 2 | 86-92 |
| | 0.1 | 91, 78, 81, 89, 80 | 5 | 84 | 7 | 78-91 |
| | Overall | | 10 | 86 | 5 | 78-92 |
| Wheat hay | 0.01* | 78, 84, 77, 80, 82 | 5 | 80 | 4 | 78-84 |
| | 0.1 | 87, 84, 88, 87, 86 | 5 | 86 | 2 | 84-88 |

| Matrix | Fortification Level (mg/kg) | Recovery (%) | Number of Analysis (n) | Mean Recovery (%) | RSD (%) | Recovery Range (%) |
|---|---|---|---|---|---|---|
| | Overall | | 10 | 83 | 5 | 78-88 |
| Almond nut meat | 0.01* | 88, 88, 81, 83, 86 | 5 | 85 | 4 | 81-88 |
| | 0.1 | 91, 87, 81, 84, 88 | 5 | 86 | 4 | 81-91 |
| | Overall | | 10 | 86 | 4 | 81-91 |
| Almond hull | 0.01* | 76, 82, 75, 80, 84 | 5 | 79 | 5 | 75-84 |
| | 0.1 | 83, 84, 81, 74, 79 | 5 | 81 | 5 | 74-84 |
| | Overall | | 10 | 80 | 5 | 74-84 |
| Wheat grain | 0.01* | 84, 82, 75, 84, 73 | 5 | 80 | 7 | 73-84 |
| | 0.1 | 79, 83, 76, 80, 71 | 5 | 78 | 6 | 71-83 |
| | Overall | | 10 | 79 | 6 | 71-84 |
| Wheat forage | 0.01* | 85, 96, 83, 82, 78 | 5 | 85 | 8 | 78-96 |
| | 0.1 | 87, 90, 88, 80, 87 | 5 | 86 | 4 | 80-90 |
| | Overall | | 10 | 86 | 6 | 78-96 |
| Apple | 0.01* | 88, 80, 89, 87, 84 | 5 | 86 | 4 | 80-89 |
| | 0.1 | 91, 87, 75, 76, 99 | 5 | 86 | 12 | 75-99 |
| | Overall | | 10 | 86 | 8 | 75-99 |

* Limit of quantitation, defined by the lowest validated fortification level

**Table A 35: Characteristics for the analytical method GRM010.02A used for validation of cyprodinil residues in crop matrices**

| | Cyprodinil |
|---|---|
| Specificity | LC-MS/MS with two transitions is considered to be a highly specific detection technique (SANTE/2020/12830, Rev.1) and therefore no further confirmatory technique is required. No significant interferences arising from plant matrices, the lab ware, reagents or solvents have been observed at the retention times of interest. |
| Calibration (type, number of data points) | The linearity of the LC-MS/MS detector was tested using standard solutions in solvent. Linearity was tested for both MS/MS transitions. Standards at ≥5 different concentrations were injected and the signal area plotted against concentration for all calibration points. Straight lines with correlation coefficients > 0.99 were obtained. |
| Calibration range | 0.01 - 0.2 ng/mL |
| Assessment of matrix effects is presented | Yes<br>No significant enhancement or suppression of detector response was observed. |
| Limit of determination/quantification | The limit of quantification for cyprodinil residues was established at 0.01 mg/kg. No interfering peaks around the retention time of cyprodinil were found in any of the control samples at levels above 30% of the limit of quantification. |

**Conclusion**

Method GRM010.02A is considered valid for the determination of cyprodinil residues in high water content (tomato, apple, wheat forage), dry (wheat hay, wheat grain and almond hull) and high oil content (almond nut meat) commodities with an LOQ of 0.01 mg/kg.

### A 2.1.2.1.2.2 Method validation (report 037SRBR18V16)

| | |
|---|---|
| Comments of zRMS: | Another validation of the Method GRM010.02A has been accepted.<br>This method is a modified version of Method REM141.01. Analytical Method GRM010.02A was successfully validated for the determination of cyprodinil residues in carrot roots, potato tubers, melon fruits and tomato fruits. 2 transitions were applied: primary *m/z* = 226 → *m/z* = 93; confirmatory *m/z* = 226 → *m/z* = 77. The LOQ of the analytical method was successfully validated at 0.01 mg/kg for cyprodinil in all matrices. 5 recoveries samples were analyzed per matrix at the LOQ concentration level and 5 at the 100 x LOQ. The mean values were between 70% and 120% with a relative standard deviation of ≤ 20% and ≤ 15%, respectively. The analytical method is suitable for the determination of cyprodinil in all matrices tested. |

| | |
|---|---|
| Reference: | KCP 5.2.1 |
| Report | Cyprodinil - Validation of Analytical Method GRM010.02A for the Determination of Residues of Cyprodinil in Carrot Roots, Potato Tubers, Melon Fruits and Tomato Fruits by LC-MS/MS, Rabello, P., 2019, Report No. 037SRBR18V16, XXXX File No. VV-635386 |
| Guideline(s): | OECD ENV/JM/MONO(2007)17<br>ANVISA RDC No 4 (2012) |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes |

**Materials and methods**

A mixture of methanol:water (80:20, 50 mL) was added to the sample material, before homogenising in a mechanical shaker for approximately 1 hour. An aliquot (10 mL) of the extract was decanted and the remaining material centrifuged (3500 rpm, 5 min). The supernatant (0.1 mL) was decanted and evaporated to dryness using a compressed air evaporator. The extract was re-dissolved in methanol:water (50:50, 1 mL) and vortex mixed. The solution was transferred to an Eppendorf tube and centrifuged (14000 rpm, 5 min). The supernatant was decanted for final determination by high-performance liquid chromatography with triple quadrupole mass-spectrometric detection (LC-MS/MS), monitoring for the primary transition (m/z 226.19→93.2) and the confirmatory transition (m/z 226.19→77.1).

The purpose of this study was to conduct the validation of XXXX analytical method GRM010.02A for determination of residues of cyprodinil in carrot roots, potato tubers, melon fruits and tomato fruits by LC-MS/MS.

**Results and discussions**

Fortified samples were analysed in quintuplet at the limit of quantification (LOQ, 0.01 mg/kg) and at 100 x LOQ (1.0 mg/kg). Acceptable mean recoveries of between 60% and 120% at 0.01 mg/kg and 70% and 110% at > 0.1 mg/kg were found for both mass transitions and therefore, according to EU guidance (SANTE/2020/12830, Rev.1), demonstrate the method has satisfactory accuracy. The relative standard deviations (RSDs) of cyprodinil recoveries at each fortification level and overall were ≤ 15% and therefore, according to the EU guidance (SANTE/2020/12830, Rev.1), demonstrate the method has satisfactory repeatability.

Cyprodinil was stable in final extracts of all matrices stored under refrigerated conditions (approx. 4 °C) for at least 5 days.

Cyprodinil was stable in final extracts of all matrices stored under ambient conditions (approx. 20 °C) for at least 5 days.

Cyprodinil was stable in standard solution in methanol:water (50:50) stored under refrigerated conditions (2 – 8 °C) for at least 114 days.

**Table A 36: Recovery results from method validation of cyprodinil in crops using the analytical method GRM010.02A (primary transition m/z 226.19→93.2)**

| Matrix | Fortification Level (mg/kg) | Recovery (%) | Number of Analyses (n) | Mean Recovery (%) | RSD (%) | Recovery Range (%) |
|---|---|---|---|---|---|---|
| Carrot root | 0.01* | 95, 88, 88, 94, 86 | 5 | 90 | 4 | 88-95 |
| | 1.0 | 88, 85, 87, 89, 87 | 5 | 87 | 2 | 85-87 |
| | Overall | | 10 | 89 | 4 | 85-95 |
| Potato tuber | 0.01* | 72, 73, 75, 75, 80 | 5 | 75 | 4 | 72-80 |
| | 1.0 | 80, 74, 82, 84, 83 | 5 | 81 | 5 | 74-84 |
| | Overall | | 10 | 78 | 6 | 72-84 |
| Melon fruit | 0.01* | 80, 78, 76, 74, 90 | 5 | 80 | 8 | 74-90 |
| | 1.0 | 81, 84, 83, 83, 75 | 5 | 81 | 5 | 75-84 |
| | Overall | | 10 | 80 | 6 | 74-90 |
| Tomato fruit | 0.01* | 72, 75, 65, 73, 83 | 5 | 74 | 9 | 65-83 |
| | 1.0 | 75, 76, 71, 83, 74 | 5 | 76 | 6 | 71-83 |
| | Overall | | 10 | 75 | 7 | 65-83 |

* Limit of quantitation, defined by the lowest validated fortification level

**Table A 37: Recovery results from method validation of cyprodinil in crops using the analytical method GRM010.02A (confirmatory transition m/z 226.19→77.1)**

| Matrix | Fortification Level (mg/kg) | Recovery (%) | Number of Analyses (n) | Mean Recovery (%) | RSD (%) | Recovery Range (%) |
|---|---|---|---|---|---|---|
| Carrot root | 0.01* | 95, 100, 82, 92, 84 | 5 | 90 | 8 | 82-100 |
| | 1.0 | 89, 88, 89, 89, 87 | 5 | 88 | 1 | 87-89 |
| | Overall | | 10 | 89 | 6 | 82-100 |
| Potato tuber | 0.01* | 77, 75, 71, 67, 74 | 5 | 73 | 5 | 67-77 |
| | 1.0 | 76, 74, 79, 83, 81 | 5 | 79 | 5 | 74-83 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | Overall | | 10 | 76 | 6 | 67-83 |
| Melon fruit | 0.01* | 72, 75, 69, 71, 79 | 5 | 73 | 5 | 69-79 |
| | 1.0 | 78, 81, 86, 83, 74 | 5 | 80 | 6 | 74-86 |
| | Overall | | 10 | 77 | 7 | 69-86 |
| Tomato fruit | 0.01* | 71, 73, 65, 68, 77 | 5 | 71 | 7 | 65-77 |
| | 1.0 | 79, 79, 72, 81, 73 | 5 | 77 | 5 | 72-81 |
| | Overall | | 10 | 74 | 7 | 65-81 |

* Limit of quantitation, defined by the lowest validated fortification level

**Table A 38: Characteristics for the analytical method GRM010.02A used for validation of cyprodinil residues in crops**

| | **Cyprodinil** |
|---|---|
| Specificity | LC-MS/MS with two transitions is considered to be a highly specific detection technique and therefore, according to EU guidance (SANTE/2020/12830 rev.1), no further confirmatory technique is required.<br>There were no significant (i.e. 30% of LOQ) interfering peaks in control matrices. |
| Calibration (type, number of data points) | Calibration was performed using one or more standard injections at each of 6 concentrations. The calibration range extended from 30% of the LOQ to 16x the LOQ. The detector response was linear (correlation coefficients (r) were ≥ 0.99 for both mass transitions monitored) |
| Calibration range | from 0.03 ng/mL to 1.6 ng/mL |
| Assessment of matrix effects is presented | Yes.<br>There were no significant matrix effects (i.e. ≥ 20% suppression or enhancement) in any of the matrices tested, therefore all matrices were quantified using non-matrix calibration standards. |
| Limit of determination/quantification | The limit of quantification for cyprodinil residues in crop matrices using the analytical method was established at 0.01 mg/kg.<br>The limit of detection (LOD) was 0.003 mg/kg. |

**Conclusion**

The method GRM010.02A has been successfully validated for the determination of residues of cyprodinil in carrot, potato, melon and tomato, with a limit of quantification (LOQ) of 0.01 mg/kg in each matrix.

## A 2.1.2.1.2.3 Independent laboratory validation

| | |
|---|---|
| Comments of zRMS: | The validation of the Method GRM010.02A in oilseed rape has been accepted, however this is not an ILV – because, to zRMS knowledge, there was no such matrix validated before in other laboratory with this method. So, it is another validation. And this validation was successfully performed.<br>For both mass transitions (quantification: 226 → 93 m/z; confirmation: 226 →77 m/z) the mean recoveries for all fortification levels (0.01 and 0.10 mg/kg) were in the range of 70 - 110 % with a relative standard deviation (RSD) of ≤ 20 %. The method is considered valid for the determination of residues of cyprodinil in oilseed rape at the LOQ of 0.01 mg/kg. |

| | |
|---|---|
| Reference: | KCP 5.2.1 |
| Report | Cyprodinil - Independent Laboratory Validation of GRM010.02A Method for the Determination of Residues of Cyprodinil in Crop Matrices by LC-MS/MS, Asekunowo, J., 2015, Report No. P 3866 G, XXXX File No. CGA219417_11672, VV-414907 |
| Guideline(s): | OECD Guidance Document on Pesticide Residue Analytical Methods, ENV/JM/MONO(2007)17 (Unclassified, 13 Aug 2007).<br>EPA Residue Chemistry Test Guideline OPPTS 860.1340 (712C-96-174) Residue Analytical Method, 1996.<br>European Commission Guidance for Generating and Reporting Methods of Analysis in Support of Pre-registration Data Requirements for Annex II (Part A, Section 4) of Directive 91/414, SANCO/3029/99 revision 4 (11 Jul 2000).<br>European Commission Guidance Documents on Pesticide Residue Analytical Methods, SANCO/825/00 rev. 8.1 (16 Nov 2010).<br>The OECD Principles of Good Laboratory Practice |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes |

**Materials and methods**

Analytical method GRM010.02A is a modified version of method REM141.01. The method GRM010.02A was originally validated for the determination of cyprodinil in various crops and in tree nuts and provides a limit of quantitation (LOQ) of 0.01 mg/kg. In the ILV study oilseed rape seed samples are homogenized and extracted with methanol/water (80:20) and extracted for a further 5 minutes with acidified methanol/water (80:20). Aliquots of the extracts are cleaned-up using solid phase extraction cartridges (HLB phase). Cyprodinil is eluted in 0.1% ammonium acetate/acetonitrile (40:60). Final determination is by high performance liquid chromatography coupled to a triple quadrupole mass spectrometer (LC-MS/MS) in multiple reaction monitoring mode using the ion transition m/z 226 →93 for quantitative analysis and the transition m/z 226 → 77 for confirmation. The LOQ of the method is 0.01 mg/kg.

**Results and discussions**

Recovery of cyprodinil was assessed by fortifying five aliquots of the untreated matrix with the appropriate fortification solution (in methanol) at fortification levels 0.01 and 0.1 mg/kg. Acceptable mean recoveries of between 60% and 120% at 0.01 mg/kg and 70% and 120% at 0.1 mg/kg were found for both transitions on all matrices tested and therefore according to EU guidance (SANTE/2020/12830, Rev.1) demonstrate the method has satisfactory accuracy. The relative standard deviations (RSDs) of the recoveries at each fortification level and overall for each commodity tested during method validation were < 20%, therefore according to the EU guidance (SANTE/2020/12830, Rev.1) demonstrate the method has satisfactory repeatability.

**Table A 39: Recovery results from independent laboratory validation of cyprodinil using the analytical method GRM010.02A (primary transition m/z 226→93)**

| Matrix | Fortification Level (mg/kg) | Recovery (%) | Number of Analyses (n) | Mean Recovery (%) | RSD (%) | Recovery Range (%) |
|---|---|---|---|---|---|---|
| Oilseed rape seed | 0.01* | 100, 98, 99, 100, 98 | 5 | 99 | 1 | 98-100 |
| | 0.1 | 74, 73, 78, 71, 69 | 5 | 73 | 5 | 69-78 |
| | Overall | | 10 | 86 | 16 | 69-100 |

*Limit of quantitation, defined by the lowest validated fortification level

**Table A 40: Recovery results from independent laboratory validation of cyprodinil using the analytical method GRM010.02A (confirmatory transition m/z 226→77)**

| Matrix | Fortification Level (mg/kg) | Recovery (%) | Number of Analyses (n) | Mean Recovery (%) | RSD (%) | Recovery Range (%) |
|---|---|---|---|---|---|---|
| Oilseed rape seed | 0.01* | 100, 101, 97, 101, 99 | 5 | 100 | 1 | 97-101 |
| | 0.1 | 73, 73, 76, 71, 70 | 5 | 73 | 3 | 70-76 |
| | Overall | | 10 | 86 | 17 | 70-101 |

*Limit of quantitation, defined by the lowest validated fortification level

**Table A 41: Characteristics for the analytical method GRM010.02A (used for independent laboratory validation of cyprodinil residues in oilseed rape seed**

| | Cyprodinil |
|---|---|
| Specificity | LC-MS/MS with two transitions is considered to be a highly specific detection technique and therefore no further confirmatory technique is required. No significant interferences arising from plant matrices, the lab ware, reagents or solvents have been observed at the retention times of interest. |
| Calibration (type, number of data points) | The linearity of the LC-MS/MS detector was demonstrated using matrix matched standard solutions in solvent (0.01-1.0 ng/mL). Linearity was tested for both MS/MS transitions. Standards at 5 different concentrations were injected and the signal area plotted against concentration for all calibration points. A straight line with correlation coefficients > 0.99 were obtained. |
| Calibration range | 0.01-1.0 ng/mL |
| Assessment of matrix effects is presented | Yes<br>Matrix effects on detector response were considered to be significant (> ± 20%). Thus matrix-matched standards were used for quantification. |
| Limit of determination/quantification | The limit of quantification for cyprodinil residues was established at 0.01 mg/kg. No interfering peaks around the retention time of cyprodinil were found in any of the control samples at levels above 30% of the limit of quantification. |

**Conclusion**

Method GRM010.02A is considered independently validated for the determination of cyprodinil residues

in oily crops with an LOQ of 0.01 mg/kg.

#### A 2.1.2.1.2.4 Confirmatory method

No confirmatory method is required, because the method was validated at two mass transitions (primary and confirmatory).

### A 2.1.2.1.3 QuEChERs

#### A 2.1.2.1.3.1 Method validation (report P 4185 G)

| | |
|---|---|
| Comments of zRMS: | The validation of the method in lettuce, orange, oilseed rape, barley grain, barley straw and blood has been accepted.<br><br>The samples were extracted and analysed based on the multi-residue analytical method QuEChERS (EN 15662:2009-2). For all matrices tested and for both characteristic LC-MS/MS mass transitions (primary transition = *m/z* 226 → 108, confirmatory transition = *m/z* 226 → 93), acceptable mean recoveries between 70% and 110%, with relative standard deviations (RSDs) ≤ 20% (for levels ≤ 0.1 mg/kg) or ≤ 10% (for levels > 1 mg/kg), were obtained. The LOQ of the method for cyprodinil was confirmed to be 0.01 mg/kg for all matrices tested, and for both mass transitions. The LOD was determined to be ≤ 0.0020 mg/kg (i.e. 20% of the LOQ) in all cases. The method is valid for the determination of residues of cyprodinil in all matrices tested at the LOQ of 0.01 mg/kg and achieves a high level of specificity thus no further confirmation on a different detector is necessary. |

| | |
|---|---|
| Reference: | KCP 5.2.1 |
| Report | Cyprodinil (CGA219417): Validation of the QuEChERS Method for the Determination of Residues of Cyprodinil in Crop Matrices and Body Fluid by LC-MS/MS, Richter, S., 2017, Report No. P 4185 G (XXXX Task No. TK0319684), XXXX File No. CGA219417_11774; VV-467144 |
| Guideline(s): | Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/825/00 rev. 8.1, 16/11/2010).<br><br>OECD Guidance Document on Pesticide Residue Analytical Methods ENV/JM/MONO(2007)17.<br><br>EPA Residue Chemistry Test Guideline OPPTS 860.1340 (712-C-96-174) Pesticide Residue Analytical Method, 1996.<br><br>Regulation (EC) No 1107/2009 of the European Parliament and of the council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC |
| Deviations: | No |
| GLP: | Work was performed in a GLP compliant facility. |
| Acceptability: | Yes/No/Supplementary |

**Materials and methods**

The analytical method was derived from the QuEChERS (EN 15662:2009-02) multi-residue method. It is based on extraction/clean-up procedures and subsequent LC-MS/MS determination. Residues of cyprodinil were extracted from sample material with acetonitrile, following the addition of a suitable volume of water. A salt mixture (magnesium sulphate, sodium chloride, sodium citrate tribasic dihydrate and sodium citrate dibasic sesquihydrate; available commercially pre-mixed - Supelco 55227-U) was added, and the extracts were shaken and then centrifuged. Then aliquots of the extracts were transferred into pre-mixed, commercially available dispersive SPE PSA clean-up tubes (Supelco 55228 U). After shaking, samples were centrifuged. Sample extracts were then diluted with acetonitrile/water (20/80, v/v) + 0.1 % formic acid or with final extract of control specimen (depending on matrix) for final determination by high-performance liquid chromatography with mass-spectrometric detection (LC-MS/MS), monitoring for the primary (m/z 226→108) and the confirmatory transition (m/z 226→93) for cyprodinil.

**Results and discussions**

Fortified samples were analysed in quintuplet at the limit of quantification (LOQ, 0.01 mg/kg) and at higher level fortification (lettuce 15 x LOQ (0.15 mg/kg), oilseed rape seed 100 x LOQ (1.0 mg/kg), barley grain 400 x LOQ (4.0 mg/kg), barley straw 500 x LOQ (5.0 mg/kg), orange 300 x LOQ (3.0 mg/kg)). Acceptable mean recoveries of between 60% and 120% at 0.01 mg/kg and 70% and 110% at > 0.1 mg/kg were found for both mass transitions on all matrices tested and therefore, according to EU guidance (SANTE/2020/12830, Rev.1), demonstrate the method has satisfactory accuracy. The relative standard deviations (RSDs) of cyprodinil recoveries for each crop tested at ≤ 0.1 mg/kg fortification level were < 20%, at ≤ 1.0 mg/kg fortification level < 15% and at > 1 mg/kg fortification level ≤ 10%, therefore according to the EU guidance (SANTE/2020/12830, Rev.1) demonstrate the method has satisfactory repeatability.

The stability of sample extracts originally fortified with cyprodinil at the LOQ level was assessed by reinjection, after a storage period of at least 8 days in a refrigerator at 6-8 °C, against freshly prepared calibration standards. The results proved that the cyprodinil residues in the stored fortified sample extracts were stable. The mean recovery values at the LOQ level were between 70% and 110%, with a RSD of ≤ 20% when re-analysed.

The stability of the stored stock and working solutions of cyprodinil was assessed after a storage period of at least 32 days in a refrigerator at 6-8 °C, against freshly prepared calibration standards at the same concentration. The mean peak areas of the stored solutions were found to be within ± 10% of the mean peak areas of the freshly prepared standard solutions for cyprodinil, demonstrating that residues of cyprodinil in the stored stock and working solutions were stable for the storage period assessed when stored under the described conditions.

**Table A 42: Recovery results from method validation of cyprodinil using the analytical method QuEChERS (primary transition m/z 226→108)**

| Matrix | Fortification Level (mg/kg) | Recovery (%) | Number of Analyses (n) | Mean Recovery (%) | RSD (%) | Recovery Range (%) |
|---|---|---|---|---|---|---|
| Lettuce | 0.01* | 109, 110, 110, 110, 100 | 5 | 108 | 4 | 100-110 |
| | 15 | 98, 98, 94, 96, 95 | 5 | 96 | 2 | 94-98 |
| | Overall | | 10 | 102 | 7 | 94-110 |
| Oilseed rape seed | 0.01* | 92, 93, 94, 89, 91 | 5 | 92 | 2 | 89-94 |
| | 1.0 | 92, 86, 90, 91, 88 | 5 | 89 | 2 | 86-92 |
| | Overall | | 10 | 91 | 2 | 86-94 |
| Barley grain | 0.01* | 104, 102, 108, 104, 109 | 5 | 106 | 3 | 102-109 |
| | 4.0 | 99, 100, 101, 104, 103 | 5 | 101 | 2 | 99-104 |

| Matrix | Fortification Level (mg/kg) | Recovery (%) | Number of Analyses (n) | Mean Recovery (%) | RSD (%) | Recovery Range (%) |
|---|---|---|---|---|---|---|
| | Overall | | 10 | 103 | 3 | 99-109 |
| Barley straw | 0.01* | 66, 75, 81, 92, 101 | 5 | 83 | 17 | 66-101 |
| | 5.0 | 82, 88, 94, 91, 94 | 5 | 90 | 5 | 82-94 |
| | Overall | | 10 | 86 | 12 | 66-101 |
| Orange | 0.01* | 103, 96, 99, 100, 100 | 5 | 99 | 2 | 96-103 |
| | 3.0 | 109, 111, 113, 109, 109 | 5 | 110 | 1 | 109-113 |
| | Overall | | 10 | 105 | 6 | 96-113 |

*Limit of quantitation, defined by the lowest validated fortification level.

**Table A 43: Recovery results from method validation of cyprodinil using the analytical method QuEChERS (confirmatory transition m/z 226→93)**

| Matrix | Fortification Level (mg/kg) | Recovery (%) | Number of Analyses (n) | Mean Recovery(%) | RSD (%) | Recovery Range (%) |
|---|---|---|---|---|---|---|
| Lettuce | 0.01* | 105, 110, 112, 111, 102 | 5 | 108 | 4 | 102-112 |
| | 15 | 97, 100, 93, 96, 95 | 5 | 96 | 3 | 93-100 |
| | Overall | | 10 | 102 | 7 | 93-112 |
| Oilseed rape seed | 0.01* | 93, 96, 94, 93, 92 | 5 | 94 | 2 | 92-96 |
| | 1.0 | 90, 84, 87, 90, 87 | 5 | 87 | 3 | 84-90 |
| | Overall | | 10 | 91 | 4 | 84-96 |
| Barley grain | 0.01* | 105, 102, 111, 109, 111 | 5 | 108 | 4 | 102-111 |
| | 4.0 | 100, 100, 102, 103, 104 | 5 | 102 | 2 | 100-104 |
| | Overall | | 10 | 105 | 4 | 100-111 |
| Barley straw | 0.01* | 64, 76, 80, 90, 96 | 5 | 81 | 15 | 64-96 |
| | 5.0 | 83, 88, 94, 92, 93 | 5 | 90 | 5 | 83-94 |
| | Overall | | 10 | 86 | 12 | 64-96 |
| Orange | 0.01* | 101, 96, 98, 99, 102 | 5 | 99 | 3 | 96-102 |
| | 3.0 | 111, 111, 111, 108, 108 | 5 | 110 | 1 | 108-111 |
| | Overall | | 10 | 105 | 6 | 96-111 |

*Limit of quantitation, defined by the lowest validated fortification level.

**Table A 44: Characteristics for the analytical method QuEChERS used for validation of cyprodinil residues in crops**

| | Cyprodinil |
|---|---|
| Specificity | LC-MS/MS with two transitions is considered to be a highly specific detection technique and therefore, according to EU guidance (SANTE/2020/12830, Rev.1), no further confirmatory technique is required. The method includes two MS/MS transitions, both of which have been validated. No significant interferences arising from the matrices, the lab ware, reagents or solvents have been observed at the retention times of interest. |

| | **Cyprodinil** |
|---|---|
| Calibration (type, number of data points) | The linearity of the LC-MS/MS detector response was assessed using standard solutions in acetonitrile/water (20/80, v/v) + 0.1% formic acid (for lettuce, orange) or matrix-matched standards (for oilseed rape seed, barley grain and straw). Linearity was assessed for both MS/MS transitions. Standards at ≥ 5 different concentrations were injected and the signal area plotted against concentration for all calibration points. Straight lines with correlation coefficients ranging from 0.9993 to 0.9999 were obtained for cyprodinil. |
| Calibration range | 0.050 ng/mL to 5.0 ng/mL |
| Assessment of matrix effects is presented | Yes<br>Matrix effects (signal suppression or enhancement; ≤ ± 20%) were considered not to be significant for lettuce, orange. Thus, calibration standards in acetonitrile/water (20/80, v/v) + 0.1% formic acid were used for quantification of cyprodinil in these matrices. For oilseed rape seed, barley straw and grain the matrix effect were considered to be significant, thus matrix-matched standard calibrations were used to evaluate results. |
| Limit of determination/quantification | The limit of quantification for cyprodinil residues in crop matrices using the QuEChERS method was established at 0.01 mg/kg. No interfering peaks around the retention time of cyprodinil were found in any of the control samples at levels above 20% of the limit of quantification.<br>The limits of detection (LODs) were calculated to be 0.000124 mg/kg for the primary transition, and 0.000153 mg/kg for the confirmatory transition, for lettuce and orange, respectively 0.000099 mg/kg for the primary transition, and 0.000094 mg/kg for the confirmatory transition for oilseed rape seed. The LOD were calculated to be 0.000124 mg/kg for the primary transition, and 0.000149 mg/kg for the confirmatory transition for barley grain, respectively 0.000228 mg/kg for the primary transition, and 0.000258 mg/kg for the confirmatory transition for barley straw. |

**Conclusion**

The analytical multi-residue QuEChERS method has been demonstrated to be a reliable and accurate procedure for the determination of cyprodinil in crop matrices to a limit of quantification of 0.01 mg/kg, using commercially available laboratory equipment and reagents.

## A 2.1.2.1.3.2 Independent laboratory validation

| | |
|---|---|
| Comments of zRMS: | The ILV in lettuce, oilseed rape and barley grain has been accepted.<br>This independent laboratory validationwas performed in all matrices at the limit of quantification (LOQ: 0.01 mg/kg) and at 100 x LOQ (1 mg/kg) for OSR seeds, 400 x LOQ (4 mg/kg) for barley grain and 1500 x LOQ (15 mg/kg) for lettuce. The mean recoveries for each fortification level, and overall, for all matrices and LC-MS/MS transitions (primary: *m/z* 226 → 108; confirmatory: *m/z* 226 → 93) tested, were within the acceptable range of 70 to 110% (with 1 insignificant exception) demonstrating the accuracy of the method. The RSD obtained at each fortification level, and overall, for all matrices tested, was within the acceptable |

| | |
|---|---|
| | range of ≤ 20%, demonstrating the precision of the method. |

| | |
|---|---|
| Reference: | KCP 5.2.1 |
| Report | Cyprodinil (CGA219417) - Independent Laboratory Validation of the QuEChERS Method for the Determination of Cyprodinil Residues in Crop Matrices by LC-MS/MS, Airs, D., 2017, Report No. CS93XJ (XXXX Task No. TK0319685), XXXX File Number CGA219417_11782, VV-467339 |
| Guideline(s): | Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/825/00 rev. 8.1, 16/11/2010).<br>OECD Guidance Document on Pesticide Residue Analytical Methods ENV/JM/MONO(2007)17.<br>EPA Residue Chemistry Test Guideline OPPTS 860.1340 (712-C-96-174) Pesticide Residue Analytical Method, 1996.<br>Regulation (EC) No 1107/2009 of the European Parliament and of the council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes/No/Supplementary |

**Materials and methods**

Prepared matrix (10 g for lettuce, 5 g for barley grain and 2.5 g for oilseed rape seed) was extracted by shaking with acetonitrile (10 mL) for lettuce and barley grain or acetonitrile containing 1% acetic acid (15 mL) for oilseed rape seeds. For barley grain, water was added prior to extraction to give a total water volume of 10 mL in the extraction tube (taking in to account the water content of the crop). After shaking, the contents of a dispersive SPE Citrate Extraction tube were added (containing sodium citrate tribasic dehydrate, sodium citrate dibasic sesquihydrate, magnesium sulphate and sodium chloride) and the mixture re-shaken. Mixtures were placed in a centrifuge to separate the acetonitrile and aqueous phase. An aliquot of the upper acetonitrile extract was transferred to a clean-up tube containing magnesium sulphate and primary secondary amine, the tube was capped and shaken to mix. Lettuce extracts were then diluted (x40) with acetonitrile:water (20:80 v:v) containing 0.1% formic acid, barley grain extracts were diluted (x20) with acetonitrile:water (20:80 v:v) containing 0.1% formic acid and oilseed rape seed extracts were diluted (x6.66) with acetonitrile:water (20:80 v:v) containing 0.1% formic acid. Extracts were analysed for cyprodinil by high-performance liquid chromatography with mass-spectrometric detection (LC-MS/MS), monitoring for the primary transition (m/z 226 to 108) and the confirmatory transition (m/z 226 to 93).

**Results and discussions**

Fortified samples were analysed in replicates of five at the limit of quantification (LOQ, 0.01 mg/kg) and at one hundred times the LOQ (1 mg/kg) for OSR seeds, four hundred times (4 mg/kg) for barley grain and fifteen hundred times (15 mg/kg) for lettuce. Acceptable mean recoveries of between 60% and 120% at 0.01 mg/kg and 70% and 110% at > 0.1 mg/kg were found for both transitions on both matrices tested and therefore according to EU guidance (SANTE/2020/12830, Rev.1) demonstrate the method has satisfactory accuracy. The relative standard deviations (RSDs) of cyprodinil recoveries for each crop tested at ≤ 0.1 mg/kg fortification level were < 20%, at ≤ 1.0 mg/kg fortification level < 15% and at > 1 mg/kg fortification level ≤ 10% (except 11.1% in barley grain at 4 mg/kg), therefore according to the EU guidance

(SANTE/2020/12830, Rev.1) demonstrate the method has satisfactory repeatability.

**Table A 45: Recovery results from independent laboratory validation of cyprodinil using the analytical method QuEChERS (primary transition m/z 226→108)**

| Matrix | Fortification Level (mg/kg) | Recovery (%) | Number of Analysis (n) | Mean Recovery(%) | RSD (%) | Recovery Range (%) |
|---|---|---|---|---|---|---|
| Lettuce | 0.01* | 84, 120, 102, 91, 89 | 5 | 97 | 14.8 | 84 – 120 |
| | 15 | 113, 107, 115, 108, 109 | 5 | 110 | 3.1 | 107 - 115 |
| | Overall | - | 10 | 104 | 11.6 | 84 - 120 |
| Oilseed rape seeds | 0.01* | 62, 72, 84, 69, 67 | 5 | 71 | 11.6 | 62 – 84 |
| | 1 | 66, 71, 70, 74, 75 | 5 | 71 | 5.0 | 66 – 75 |
| | Overall | - | 10 | 71 | 8.4 | 62 – 84 |
| Barley grain | 0.01* | 89, 92, 97, 106, 100 | 5 | 97 | 6.9 | 89 – 106 |
| | 4 | 77, 82, 83, 85, 102 | 5 | 86 | 11.1 | 77 – 102 |
| | Overall | - | 10 | 91 | 10.6 | 77 - 106 |

*Limit of quantitation, defined by the lowest validated fortification level

**Table A 46: Recovery results from independent laboratory validation of cyprodinil using the analytical method QuEChERS (confirmatory transition m/z 226→93)**

| Matrix | Fortification Level (mg/kg) | Recovery (%) | Number of Analysis (n) | Mean Recovery(%) | RSD (%) | Recovery Range (%) |
|---|---|---|---|---|---|---|
| Lettuce | 0.01* | 85, 120, 102, 92, 90 | 5 | 98 | 14.2 | 85 – 120 |
| | 15 | 114, 108, 116, 108, 110 | 5 | 111 | 3.3 | 108 – 116 |
| | Overall | - | 10 | 105 | 11.4 | 85 - 120 |
| Oilseed rape seeds | 0.01* | 64, 73, 85, 70, 68 | 5 | 72 | 11.1 | 64 – 85 |
| | 1 | 67, 71, 69, 73, 75 | 5 | 71 | 4.5 | 67 – 75 |
| | Overall | - | 10 | 72 | 8.0 | 64 - 85 |
| Barley grain | 0.01* | 88, 93, 91, 100, 97 | 5 | 94 | 5.1 | 88 – 100 |
| | 4 | 77, 82, 83, 86, 102 | 5 | 86 | 11.1 | 77 – 102 |
| | Overall | - | 10 | 90 | 9.1 | 77 - 102 |

*Limit of quantitation, defined by the lowest validated fortification level

**Table A 47: Characteristics for the analytical method QuEChERS used for independent laboratory validation of cyprodinil residues in crops**

| | Cyprodinil |
|---|---|
| Specificity | LC-MS/MS with two transitions is considered to be a highly specific detection technique and therefore according to EU guidance (SANTE/2020/12830, Rev.1) no further confirmatory technique is required. The method includes two MS/MS transitions, both of which have been validated. No significant interferences arising from the crop matrices, the labware, reagents or solvents have been observed at the retention times of interest. |
| Calibration (type, number of data points) | The linearity of the LC-MS/MS detector was tested using solvent and matrix matched standard solutions. Linearity was tested for |

| | **Cyprodinil** |
|---|---|
| | both MS/MS transitions. Standards at eight different concentrations were injected and the signal area plotted against concentration for all calibration points. Straight lines with correlation coefficients ranging from 0.9971 to 0.9994 were obtained for cyprodinil. |
| Calibration range | 0.05 ng/mL to 10 ng/mL for solvent standards and 0.05 ng/mL to 5 ng/mL for matrix matched standards |
| Assessment of matrix effects is presented | Yes<br>Matrix matched standards were used for quantification of oilseed rape seed and barley grain only. |
| Limit of determination/quantification | The limit of quantification for cyprodinil residues in crop matrices using the QuEChERS method was established at 0.01 mg/kg. No significant interfering peaks around the retention time of cyprodinil were found in any of the control samples at levels above 30% of the limit of quantification.<br>The limit of detection (LOD) was defined in this study as the lowest prepared instrument calibration solution that gave rise to a measureable chromatographic response. For this study, it was shown to be 0.05 ng/mL (equivalent to 0.002 mg/kg in sample matrix). |

**Conclusion**

The QuEChERS analytical method has been demonstrated to be a reliable and accurate procedure for the determination of cyprodinil in crops to a limit of quantification of 0.01 mg/kg, using commercially available laboratory equipment and reagents.

### A 2.1.2.1.3.3 Confirmatory method

No confirmatory method is required, because the method was validated at two mass transitions (primary and confirmatory).

## A 2.1.2.2 Description of analytical methods for the determination of residues in animal matrices (KCP 5.2.2)

### A 2.1.2.2.1 GRM010.06B

#### A 2.1.2.2.1.1 Method validation (reports GRM010.06A, GRM010.06B AND CEMR-6729)

| | |
|---|---|
| Comments of zRMS: | The validation of the method GRM010.06A (updated as GRM010.06B) in animal tissue matrices has been accepted.<br><br>Analytical method GRM010.06A has been updated in the study on Analytical Method GRM010.06B (XXXX File No. CGA219417_11632) and independently validated in milk, whole egg and liver (XXXX File No. CGA218417_11614).<br><br>This method is suitable for the determination of cyprodinil ((4-cyclopropyl-6-methyl-pyrimidin-2-yl)-phenyl-amine) and its metabolite CGA304075 (4-(4-cyclopropyl-6-methyl-pyrimidin-2-ylamino)-phenol) in ruminant milk, fat, liver, kidney, muscle and poultry eggs. The method validation data for these matrices are reported in the mentioned above report CEMR-6729.<br><br>The LOQ of the method has been established at 0.01 mg/kg. Cyprodinil and CGA304075 were efficiently extracted using the conditions described in analytical procedure of a $^{14}C$ radiolabelled study (W. Anderson, 2006, XXXX report T019338-04). Final determination was done by LC-MS/MS with two transitions for both compounds (Cyprodinil primary transition 226.2 → 93.1; confirmatory transition 226.2 → 76.9; CGA304075 primary transition 242.1 → 93.1; confirmatory transition 242.1 → 108.0). The mean recovery from 5 replicates fortified at the LOQ (0.01 mg/kg) and 5 replicates fortified at 10 × LOQ (0.1 mg/kg) was between 60% and 120% (0.01 mg/kg) or 70% and 120% (0.1 mg/kg) of the nominal fortified concentration, with a relative standard deviation lower than 20%, for cyprodinil and CGA304075 for both the primary and confirmatory transitions in all matrices. The method can be considered valid for the determination of residues of cyprodinil and CGA304075 in animal matrices tested. |

| | |
|---|---|
| Reference: | KCP 5.2.2 |
| Report | Analytical Method (GRM010.06A) for the Determination of Residues of Cyprodinil and its Metabolite CGA304075 in Ruminant Livestock Commodities and Poultry Eggs, Bradford W., Langridge G., 2015, XXXX Analytical Method GRM010.06A, Report No. GRM010.06A, XXXX File No. CGA219417_11608, VV-128138 |
| Guideline(s): | Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/825/00 rev. 8.1, 16/11/2010).<br><br>Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/3029/99 rev. 4, 11/07/2000).<br><br>OECD Guidance Document on Pesticide Residue Analytical Methods ENV/JM/MONO (2007)17. |

| | |
|---|---|
| | Residue Chemistry Test Guidelines OPPTS 860.1340 Environmental Chemistry Methods and Associated Independent Laboratory Validation, EPA 712-C-96-174, August 1996 |
| Deviations: | No |
| GLP: | No |
| Acceptability: | Yes/No/Supplementary |
| Reference: | KCP 5.2.2 |
| Report | Analytical Method (GRM010.06B) for the Determination of Residues of Cyprodinil and its Metabolite CGA304075 in Ruminant Livestock Commodities and Poultry Eggs, Bradford W., Langridge G., 2015, Report No. GRM010.06B, XXXX File No. CGA219417_11632, VV-128329 |
| Guideline(s): | Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/825/00 rev. 8.1, 16/11/2010).<br>Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/3029/99 rev. 4, 11/07/2000).<br>OECD Guidance Document on Pesticide Residue Analytical Methods ENV/JM/MONO (2007)17.<br>Residue Chemistry Test Guidelines OPPTS 860.1340 Environmental Chemistry Methods and Associated Independent Laboratory Validation, EPA 712-C-96-174, August 1996 |
| Deviations: | No |
| GLP: | No |
| Acceptability: | Yes/No/Supplementary |
| Reference: | KCP 5.2.2 |
| Report | Cyprodinil - Validation of an Analytical Method (GRM010.06A) for the Determination of Cyprodinil and its Metabolite CGA304075 in Animal Matrices, Langridge G., 2015, CEMAS Report No. CEMR-6729, XXXX File No. CGA219417_11607, VV-412216 |
| Guideline(s): | Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/825/00 rev. 8.1, 16/11/2010).<br>Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/3029/99 rev. 4, 11/07/2000).<br>OECD Guidance Document on Pesticide Residue Analytical Methods ENV/JM/MONO (2007)17.<br>Residue Chemistry Test Guidelines OPPTS 860.1340 Environmental Chemistry Methods and Associated Independent Laboratory Validation, EPA 712-C-96-174, August 1996 |
| Deviations: | No |

GLP: No

Acceptability: Yes/No/Supplementary

**Materials and methods**

Sample material was refluxed in 0.5M hydrochloric acid to extract residues and hydrolyse conjugated residues of CGA304075. Once cooled, the samples were filtered and an aliquot was taken though an SPE clean up using Bond-Elut SCX cartridges. Samples were eluted with methanol/ammonium hydroxide (95/5, v/v) and evaporated to dryness. Samples were reconstituted in acetonitrile/water (30/70, v/v). Final determination was by high-performance liquid chromatography with triple quadrupole mass-spectrometric detection (LC-MS/MS), monitoring for the primary transition (m/z 226.2→93.1) and the confirmatory transition (m/z 226.2→76.9) for cyprodinil and the primary transition (m/z 242.1→ 93.1) and the confirmatory transition (m/z 242.1→108.0) for its metabolite CGA304075.

**Results and discussions**

Fortified samples were analysed in quintuplet at the limit of quantification (LOQ, 0.01 mg/kg) and at ten times the LOQ (0.1 mg/kg). Acceptable mean recoveries of between 60% and 120% at 0.01 mg/kg and 70 and 120% at 0.1 mg/kg were found for both transitions in all matrices tested, which is compliant with the EU guidance (SANTE/2020/12830, Rev.1). Three recovery values are below (54 x 2, 58 in muscle) and two are above (131, 134 in eggs), however as the mean values are within the acceptable ranges, it is concluded that the method has satisfactory accuracy. The relative standard deviations (RSDs) of cyprodinil and CGA304075 recoveries at each fortification level and overall for each animal commodity tested during method validation were < 20% and therefore, according to the EU guidance (SANTE/2020/12830, Rev.1), demonstrate the method has satisfactory repeatability.

Final sample extracts (in acetonitrile/water (30/70, v/v)) of fortified samples (fortified at 0.01 mg/kg) were re analysed after 5-8 days of refrigerated storage (2-8 °C) in clear glass vials. In the case of liver, kidney, muscle, fat, and milk extracts, the overall mean recoveries of cyprodinil and CGA304075 in the stored final extracts were within the acceptable range of 60-120%, with an RSD of ≤ 20%, and within ± 20% of their initial values. The recovery results for final stored extracts of egg matrix demonstrated that cyprodinil and CGA304075 were not stable in fortified sample extracts when stored in vials at between 2-8 °C for at least 7 days. Whilst there is no indication of instability of cyprodinil and CGA304075 in final extracts of liver, kidney, muscle, fat and milk matrices during storage, it is recommended that all final sample extracts are analysed immediately after preparation.

The stability of the stored working standard solutions of cyprodinil and CGA304075 in acetonitrile/water (30/70, v/v) were checked after a storage period of 108 days at 2-8 °C against freshly prepared calibration standards. The mean response values for stored and fresh solutions (at a concentration of 7.5 ng/mL; equivalent to 0.03 mg/kg) were within 10% of each other, demonstrating that cyprodinil and CGA304075 residues in the stored working standard solutions were stable when stored refrigerated

**Table A 48: Recovery results from method validation of cyprodinil using the analytical method GRM010.06A (primary transition m/z 226.2→93.1)**

| Matrix | Fortification Level (mg/kg) | Recovery (%) | Number of Analyses (n) | Mean Recovery (%) | RSD (%) | Recovery Range (%) |
|---|---|---|---|---|---|---|
| Liver | 0.01* | 79, 67, 76, 95, 90 | 5 | 82 | 13.8 | 67-95 |
| | 0.1 | 71, 76, 77, 81, 78 | 5 | 77 | 4.9 | 71-81 |
| | Overall | | 10 | 79 | 10.5 | 67-95 |
| Kidney | 0.01* | 77, 88, 82, 72, 90 | 5 | 82 | 9.4 | 72-90 |

| Matrix | Fortification Level (mg/kg) | Recovery (%) | Number of Analyses (n) | Mean Recovery (%) | RSD (%) | Recovery Range (%) |
|---|---|---|---|---|---|---|
| | 0.1 | 89, 85, 81, 80, 74 | 5 | 82 | 6.7 | 74-89 |
| | Overall | | 10 | 82 | 7.7 | 72-90 |
| Muscle | 0.01* | 65, 60, 54, 67, 64 | 5 | 62 | 8.5 | 54-67 |
| | 0.1 | 68, 78, 72, 72, 85 | 5 | 75 | 9.2 | 68-85 |
| | Overall | | 10 | 68 | 13.1 | 54-85 |
| Fat | 0.01* | 86, 90, 93, 99, 94 | 5 | 92 | 5.2 | 86-99 |
| | 0.1 | 96, 90, 93, 107, 99 | 5 | 97 | 6.7 | 90-107 |
| | Overall | | 10 | 95 | 6.3 | 86-107 |
| Eggs | 0.01* | 91, 97, 87, 108, 100 | 5 | 97 | 8.5 | 87-108 |
| | 0.1 | 93, 95, 131, 79, 96 | 5 | 99 | 19.2 | 79-131 |
| | Overall | | 10 | 98 | 14.2 | 79-131 |
| Milk | 0.01* | 100, 81, 77, 78, 81 | 5 | 84 | 10.9 | 77-100 |
| | 0.1 | 79, 78, 72, 71, 76 | 5 | 75 | 4.5 | 71-79 |
| | Overall | | 10 | 79 | 9.9 | 71-100 |

*Limit of quantitation, defined by the lowest validated fortification level.
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ
% Mean and % RSD calculated using unrounded values

**Table A 49: Recovery results from method validation of cyprodinil using the analytical method GRM010.06A (confirmatory transition m/z 226.2→76.9)**

| Matrix | Fortification Level (mg/kg) | Recovery (%) | Number of Analyses (n) | Mean Recovery(%) | RSD (%) | Recovery Range (%) |
|---|---|---|---|---|---|---|
| Liver | 0.01* | 87, 72, 82, 93, 95 | 5 | 86 | 10.8 | 72-95 |
| | 0.1 | 74, 79, 78, 77, 79 | 5 | 77 | 3.0 | 74-79 |
| | Overall | | 10 | 79 | 10.5 | 72-95 |
| Kidney | 0.01* | 81, 92, 94, 75, 95 | 5 | 88 | 9.9 | 75-95 |
| | 0.1 | 87, 86, 81, 80, 77 | 5 | 82 | 5.5 | 77-87 |
| | Overall | | 10 | 85 | 8.4 | 75-95 |
| Muscle | 0.01* | 65, 58, 54, 65, 61 | 5 | 61 | 7.8 | 54-65 |
| | 0.1 | 69, 78, 71, 73, 84 | 5 | 75 | 8.3 | 69-84 |
| | Overall | | 10 | 68 | 13.5 | 54-84 |
| Fat | 0.01* | 90, 101, 88, 106, 99 | 5 | 97 | 7.9 | 88-106 |
| | 0.1 | 92, 93, 92, 105, 98 | 5 | 96 | 5.8 | 92-105 |
| | Overall | | 10 | 96 | 6.5 | 88-106 |
| Eggs | 0.01* | 84, 95, 94, 89, 97 | 5 | 92 | 5.4 | 84-97 |
| | 0.1 | 95, 96, 134, 83, 103 | 5 | 102 | 18.6 | 83-134 |
| | Overall | | 10 | 97 | 14.7 | 83-134 |
| Milk | 0.01* | 93, 87, 76, 81, 84 | 5 | 84 | 7.6 | 76-93 |
| | 0.1 | 78, 78, 70, 71, 75 | 5 | 74 | 4.8 | 70-78 |
| | Overall | | 10 | 79 | 9.0 | 70-93 |

*Limit of quantitation, defined by the lowest validated fortification level.
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ
% Mean and % RSD calculated using unrounded values

**Table A 50: Recovery results from method validation of CGA304075 using the analytical method GRM010.06A (primary transition m/z 242.1→93.1)**

| Matrix | Fortification Level (mg/kg) | Recovery (%) | Number of Analyses (n) | Mean Recovery(%) | RSD (%) | Recovery Range (%) |
|---|---|---|---|---|---|---|
| Liver | 0.01* | 66, 77, 92, 102, 87 | 5 | 85 | 16.2 | 66-102 |
| | 0.1 | 80, 89, 89, 85, 88 | 5 | 86 | 4.3 | 80-89 |
| | Overall | | 10 | 85 | 11.1 | 66-102 |
| Kidney | 0.01* | 84, 77, 83, 83, 85 | 5 | 82 | 3.7 | 77-85 |
| | 0.1 | 87, 94, 88, 84, 88 | 5 | 88 | 4.1 | 84-94 |
| | Overall | | 10 | 85 | 5.1 | 77-94 |
| Muscle | 0.01* | 79, 67, 79, 80, 74 | 5 | 76 | 7.2 | 67-80 |
| | 0.1 | 75, 80, 76, 72, 76 | 5 | 76 | 3.9 | 72-80 |
| | Overall | | 10 | 76 | 5.5 | 67-80 |
| Fat | 0.01* | 90, 86, 92, 84, 87 | 5 | 88 | 3.4 | 84-92 |
| | 0.1 | 87, 95, 84, 82, 85 | 5 | 86 | 5.6 | 82-95 |
| | Overall | | 10 | 87 | 4.4 | 82-95 |
| Eggs | 0.01* | 80, 75, 82, 80, 90 | 5 | 82 | 6.3 | 75-90 |
| | 0.1 | 81, 90, 86, 86, 85 | 5 | 85 | 3.6 | 81-90 |
| | Overall | | 10 | 84 | 5.3 | 75-90 |
| Milk | 0.01* | 87, 159**, 82, 79, 84 | 5 | 83 | 4.4 | 79-87 |
| | 0.1 | 82, 82, 80, 80, 78 | 5 | 81 | 2.2 | 78-82 |
| | Overall | | 10 | 82 | 3.6 | 78-87 |

*Limit of quantitation, defined by the lowest validated fortification level.
**This is shown to be an outlier using the Grubb's test and therefore is not included in the mean and %RSD calculations.
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ
% Mean and % RSD calculated using unrounded values

**Table A 51: Recovery results from method validation of CGA304075 using the analytical method GRM010.06A (confirmatory transition m/z 242.1→108.0)**

| Matrix | Fortification Level (mg/kg) | Recovery (%) | Number of Analyses (n) | Mean Recovery(%) | RSD (%) | Recovery Range (%) |
|---|---|---|---|---|---|---|
| Liver | 0.01* | 65, 79, 95, 102, 88 | 5 | 86 | 16.8 | 65- 102 |
| | 0.1 | 79, 88, 87, 87, 90 | 5 | 86 | 5.0 | 79-90 |
| | Overall | | 10 | 86 | 11.7 | 65-102 |
| Kidney | 0.01* | 82, 82, 86, 87, 88 | 5 | 85 | 3.3 | 82-88 |
| | 0.1 | 88, 91, 90, 83, 86 | 5 | 88 | 3.4 | 83-91 |
| | Overall | | 10 | 86 | 3.6 | 82-91 |
| Muscle | 0.01* | 75, 68, 82, 77, 74 | 5 | 75 | 6.5 | 68-82 |
| | 0.1 | 74, 81, 76, 71, 75 | 5 | 75 | 5.1 | 71-81 |

| Matrix | Fortification Level (mg/kg) | Recovery (%) | Number of Analyses (n) | Mean Recovery(%) | RSD (%) | Recovery Range (%) |
|---|---|---|---|---|---|---|
| | Overall | | 10 | 75 | 5.5 | 68-82 |
| Fat | 0.01* | 91, 87, 89, 81, 86 | 5 | 87 | 4.3 | 81-91 |
| | 0.1 | 86, 97, 85, 86, 86 | 5 | 88 | 5.9 | 85-97 |
| | Overall | | 10 | 87 | 4.9 | 81-97 |
| Eggs | 0.01* | 81, 74, 81, 82, 92 | 5 | 82 | 7.8 | 74-92 |
| | 0.1 | 83, 88, 83, 85, 83 | 5 | 85 | 2.7 | 83-88 |
| | Overall | | 10 | 83 | 5.7 | 74-92 |
| Milk | 0.01* | 90, 163**, 85, 78, 88 | 5 | 85 | 5.8 | 78-90 |
| | 0.1 | 80, 83, 83, 81, 78 | 5 | 81 | 2.6 | 78-83 |
| | Overall | | 10 | 83 | 5.0 | 78-90 |

*Limit of quantitation, defined by the lowest validated fortification level.
**This is shown to be an outlier using the Grubb's test and therefore is not included in the mean and %RSD calculations.
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ
% Mean and % RSD calculated using unrounded values

**Table A 52: Characteristics for the analytical method GRM010.06A used for validation of cyprodinil and CGA304075 residues in crops**

| | Cyprodinil and CGA304075 |
|---|---|
| Specificity | LC-MS/MS with two transitions is considered to be a highly specific detection technique and therefore according to EU guidance (SANTE/2020/12830, Rev.1) no further confirmatory technique is required. The method includes two MS/MS transitions, both of which have been validated. No significant interferences arising from the soil matrices, the labware, reagents or solvents have been observed at the retention times of interest. |
| Calibration (type, number of data points) | The linearity of the LC-MS/MS detector was tested using both non-matrix calibration standard solutions and matrix-matched standard solutions. Standards at seven different concentrations were injected and the signal area plotted against concentration for all calibration points. Straight lines with correlation coefficients ranging from 0.9961 to 0.9999 were obtained for cyprodinil and CGA304075. |
| Calibration range | 0.75 to 50 ng/mL |
| Assessment of matrix effects is presented | Yes<br>Significant matrix effects (suppression, ≥ −20 %) were observed in muscle, fat, liver, kidney and egg commodities during the method validation. No significant matrix effects (≥ ±20 %) were observed in milk. Matrix matched linearity standards were used for quantification of cyprodinil and CGA304075 in all commodities tested. |
| Limit of determination/quantification | The limit of quantification for cyprodinil and CGA304075 residues in animal commodities using method GRM010.06A was established at 0.01 mg/kg. No interfering peaks around the retention time of cyprodinil and CGA304075 were found |

| | **Cyprodinil and CGA304075** |
|---|---|
| | in any of the control samples at levels above 30% of the limit of quantification. |

**Conclusion**

Analytical method GRM010.06A has been demonstrated to be a reliable and accurate procedure for the determination of cyprodinil and CGA304075 in animal commodities to a limit of quantification of 0.01 mg/kg, using commercially available laboratory equipment and reagents.

## A 2.1.2.2.1.2 Independent laboratory validation

| | |
|---|---|
| Comments of zRMS: | The independent validation of the method GRM010.06A in milk, whole egg and liver has been accepted.<br><br>Acceptable mean recoveries between 70 % and 110 % with a relative standard deviation less than 20 %, for each fortification level and LC-MS/MS mass transition of cyprodinil (i.e. primary transition: m/z 226 → 93; confirmatory transition: m/z 226 → 77) and its metabolite CGA304075 (i.e. primary transition: m/z 242 → 93 and confirmatory transition: m/z 242 → 108) were obtained in the animal matrices tested. The method achieved a high level of specificity, because two characteristic LC-MS/MS mass transitions were used to monitor cyprodinil and CGA304075. No further confirmation on a different detector was necessary. |

| | |
|---|---|
| Reference: | KCP 5.2.2 |
| Report | Cyprodinil – Independent Laboratory Validation of analytical method GRM010.06A for the determination of residues of cyprodinil and its metabolite CGA304075 in animal matrices, Knoch, E., 2015, Report No. IF-15/03135929, XXXX File No. CGA218417_11614, VV-412515 |
| Guideline(s): | Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/825/00 rev. 8.1, 16/11/2010).<br><br>Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/3029/99 rev. 4, 11/07/2000).<br><br>OECD Guidance Document on Pesticide Residue Analytical Methods ENV/JM/MONO (2007)17.<br><br>Residue Chemistry Test Guidelines OPPTS 860.1340 Environmental Chemistry Methods and Associated Independent Laboratory Validation, EPA 712-C-96-174, August 1996 |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes/No/Supplementary |

**Materials and methods**

Homogenised samples were refluxed in 0.5 M hydrochloric acid to extract residues and hydrolyse conjugated residues of CGA304075. Once cooled, the samples were filtered and an aliquot was taken through an SPE clean-up using Bond-Elut SCX cartridges. Samples were eluted with methanol/ammonium hydroxide (95/5, v/v), reduced in volume under a stream of nitrogen, and subsequently made up to a defined volume with acetonitrile/water (30/70, v/v). Final determination was by high-performance liquid chromatography with triple quadrupole mass-spectrometric detection (LC-MS/MS), monitoring for the primary transition (m/z 226.2→93) and the confirmatory transition (m/z 226.2→77) for cyprodinil, and the primary transition (m/z 242.1→93) and the confirmatory transition (m/z 242.1→108) for its metabolite CGA304075.

Analytical method GRM010.06A, as described in CEMAS report number CEMR-6729, was independently validated for the analysis of cyprodinil and CGA304075 in animal matrices (liver, milk and eggs).

**Results and discussions**

Fortified samples were analysed in quintuplet at the limit of quantification (LOQ, 0.01 mg/kg) and at ten times the LOQ (0.1 mg/kg). Acceptable mean recoveries of between 60% and 120% at 0.01 mg/kg and 70% and 120% at 0.1 mg/kg were found for both transitions in all matrices tested and therefore, according to EU guidance (SANTE/2020/12830, Rev.1), demonstrate the method has satisfactory accuracy. The relative standard deviations (RSDs) of cyprodinil and CGA304075 recoveries at each fortification level and overall for each animal commodity tested during method validation were < 20% and therefore according to the EU guidance (SANTE/2020/12830, Rev.1) demonstrate the method has satisfactory repeatability.

**Table A 53: Recovery results from independent laboratory validation of cyprodinil using the analytical method GRM010.06A (primary transition m/z 226→93)**

| Matrix | Fortification Level (mg/kg) | Recovery (%) | Number of Analysis (n) | Mean Recovery(%) | RSD (%) | Recovery Range (%) |
|---|---|---|---|---|---|---|
| Milk | 0.01* | 89, 81, 83, 83, 91 | 5 | 85.4 | 5.1 | 81 - 91 |
| | 0.1 | 87, 87, 87, 86, 87 | 5 | 86.8 | 0.5 | 86 - 87 |
| | overall | | 10 | 86.1 | 3.5 | 81 - 91 |
| Chicken Whole Egg | 0.01* | 84, 84, 86, 77, 81 | 5 | 82.4 | 4.3 | 77 - 86 |
| | 0.1 | 78, 78, 80, 78, 77 | 5 | 78.2 | 1.4 | 77 - 80 |
| | overall | | 10 | 80.3 | 4.1 | 77 - 86 |
| Bovine Liver | 0.01* | 82, 90, 85, 82, 80 | 5 | 83.8 | 4.7 | 80 - 90 |
| | 0.1 | 84, 83, 85, 89, 89 | 5 | 86.0 | 3.3 | 83 - 89 |
| | overall | | 10 | 84.9 | 4.0 | 80 - 90 |

*Limit of quantitation, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ
% Mean and % RSD calculated using un-rounded values

**Table A 54: Recovery results from independent laboratory validation of cyprodinil using the analytical method GRM010.06A (confirmatory transition m/z 226→77)**

| Matrix | Fortification Level (mg/kg) | Recovery (%) | Number of Analysis (n) | Mean Recovery(%) | RSD (%) | Recovery Range (%) |
|---|---|---|---|---|---|---|
| Milk | 0.01* | 90, 86, 81, 91, 81 | 5 | 85.8 | 5.6 | 81 - 91 |
| | 0.1 | 86, 86, 87, 84, 84 | 5 | 85.4 | 1.6 | 84 - 87 |
| | overall | | 10 | 86.5 | 3.8 | 81 - 91 |
| | 0.01* | 83, 82, 81, 73, 78 | 5 | 79.4 | 5.1 | 73 - 83 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Chicken Whole Egg | 0.1 | 78, 78, 79, 77, 75 | 5 | 77.4 | 2.0 | 75 - 79 |
| | overall | | 10 | 78.4 | 3.9 | 73 - 83 |
| Bovine Liver | 0.01* | 77, 86, 85, 78, 78 | 5 | 80.8 | 5.4 | 77 - 86 |
| | 0.1 | 85, 83, 85, 89, 89 | 5 | 86.2 | 3.1 | 83 - 89 |
| | overall | | 10 | 83.5 | 5.3 | 77 - 89 |

*Limit of quantitation, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ
% Mean and % RSD calculated using unrounded values

**Table A 55: Recovery results from independent laboratory validation of CGA304075 using the analytical method GRM010.06A (primary transition m/z 242→93)**

| Matrix | Fortification Level (mg/kg) | Recovery (%) | Number of Analysis (n) | Mean Recovery(%) | RSD (%) | Recovery Range (%) |
|---|---|---|---|---|---|---|
| Milk | 0.01* | 88, 85, 89, 85, 79 | 5 | 85.2 | 4.6 | 79 - 89 |
| | 0.1 | 85, 83, 83, 82, 83 | 5 | 83.2 | 1.3 | 82 - 85 |
| | overall | | 10 | 84.2 | 3.4 | 79 - 89 |
| Chicken Whole Egg | 0.01* | 90, 90, 93, 86, 86 | 5 | 89.0 | 3.4 | 86 - 93 |
| | 0.1 | 91, 90, 91, 94, 89 | 5 | 91.0 | 2.1 | 89 - 94 |
| | overall | | 10 | 90.0 | 2.9 | 86 - 94 |
| Bovine Liver | 0.01* | 87, 93, 88, 87, 83 | 5 | 87.6 | 4.1 | 83 - 93 |
| | 0.1 | 89, 89, 94, 99, 100 | 5 | 94.2 | 5.6 | 89 - 100 |
| | overall | | 10 | 90.9 | 6.0 | 83 - 100 |

*Limit of quantitation, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ
% Mean and % RSD calculated using un-rounded values

**Table A 56: Recovery results from independent laboratory validation of CGA304075 using the analytical method GRM010.06A (confirmatory transition m/z 242→108)**

| Matrix | Fortification Level (mg/kg) | Recovery (%) | Number of Analysis (n) | Mean Recovery(%) | RSD (%) | Recovery Range (%) |
|---|---|---|---|---|---|---|
| Milk | 0.01* | 85, 84, 85, 85, 77 | 5 | 83.2 | 4.2 | 77 - 85 |
| | 0.1 | 85, 83, 82, 82, 84 | 5 | 83.2 | 1.6 | 82 - 85 |
| | overall | | 10 | 84.6 | 2.9 | 77 - 85 |
| Chicken Whole Egg | 0.01* | 90, 87, 90, 87, 88 | 5 | 88.4 | 1.7 | 87 - 90 |
| | 0.1 | 90, 89, 92, 95, 90 | 5 | 91.2 | 2.6 | 89 - 95 |
| | overall | | 10 | 89.8 | 2.7 | 87 - 95 |
| Bovine Liver | 0.01* | 90, 95, 89, 88, 85 | 5 | 89.4 | 4.1 | 85 - 95 |
| | 0.1 | 88, 88, 93, 97, 99 | 5 | 93.0 | 5.4 | 88 - 99 |
| | overall | | 10 | 91.2 | 5.0 | 85 - 99 |

*Limit of quantitation, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ
% Mean and % RSD calculated using unrounded values

**Table A 57: Characteristics for the analytical method GRM010.06A used for independent laboratory validation of cyprodinil and CGA304075 residues in crops**

| | **Cyprodinil** |
|---|---|
| Specificity | LC-MS/MS with two transitions is considered to be a highly specific detection technique and therefore according to EU guidance (SANTE/2020/12830, Rev.1) no further confirmatory technique is required. The method includes two MS/MS transitions, both of which have been validated. No significant interferences arising from the animal matrices, the labware, reagents or solvents have been observed at the retention times of interest. |
| Calibration (type, number of data points) | The linearity of the LC-MS/MS detector was tested using both solvent standard solutions in acetonitrile/ultra-pure water (30/70, v/v). Standards at seven different concentrations were injected and the signal area plotted against concentration for all calibration points. Straight lines with correlation coefficients ranging from 0.99975 to 0.99999 were obtained for cyprodinil and CGA304075. |
| Calibration range | Solvent standard solutions 0.2 - 12.5 ng/mL, and liver matrix-matched standard solutions, covering a concentration range of 0.06 - 7.5 ng/mL |
| Assessment of matrix effects is presented | Yes<br>No significant matrix effects (suppression or enhancement ≥ ± 20%) were observed for cyprodinil and CGA304075 in the liver, milk and egg matrices tested during independent laboratory validation. Non-matrix matched linearity standards in acetonitrile/ultra-pure water (30/70, v/v) were used for quantification of cyprodinil and CGA304075 in milk and egg matrices. Matrix matched linearity standards were used for quantification of cyprodinil and CGA304075 in liver matrix. |
| Limit of determination/quantification | The limit of quantification for cyprodinil and CGA304075 residues in animal commodities using analytical method GRM010.06A was confirmed at 0.01 mg/kg. No interfering peaks around the retention time of cyprodinil and CGA304075 were found in any of the control samples at levels above 30% of the limit of quantification.<br>The limit of detection (LOD) for cyprodinil and CGA304075 was calculated to be ≤ 0.003 mg/kg for both primary and confirmatory transitions for liver, milk and egg matrices. |

**Conclusion**

The repeatability and specificity of the method have been independently demonstrated, and GRM010.06A is therefore considered valid for the determination of residues of cyprodinil and CGA304075 in animal commodities to a limit of quantification of 0.01 mg/kg, using commercially available laboratory equipment and reagents.

### A 2.1.2.2.1.3 Confirmatory method

No confirmatory method is required, because the method was validated at two mass transitions (primary and confirmatory).

### A 2.1.2.2.1 QuEChERs

### A 2.1.2.2.1.1 Method validation

| | |
|---|---|
| Comments of zRMS: | The method validation in honey has been accepted.<br>The objective of this study was to validate an analytical residue QuEChERS method to determine residues of cyprodinil in honey, to meet the requirements of the guideline SANTE/2020/12830, Rev. 1 (2021). The methodology validated was based on the following documents P 4185 G and EN 15662:2009-02 (QuEChERS).<br>Acceptable mean recovery values between 70% and 120 % and precision of the relative standard deviation (RSD) ≤ 20% were achieved at LOQ fortification level (0.01 mg/kg) and at 10 x LOQ fortification level (0.10 mg/kg) for cyprodinil in honey for both the primary (*m/z* 226 → 108) and confirmatory (*m/z* 226 → 93) mass transitions for cyprodinil. Since two characteristic mass transitions are used to monitor cyprodinil, the method achieves a high level of specificity and no further confirmation on a different detector was necessary. |

| | |
|---|---|
| Reference: | KCP 5.2.2 |
| Report | Cyprodinil (CGA219417) - Validation of the Analytical QuEChERS Method for the Determination of Residues of Cyprodinil in Honey Matrices by LC-MS/MS, Harper H (2022), Report number 8485604<br>XXXX File No. VV-939118 |
| Guideline(s): | Yes:<br>OECD ENV/JM/MONO (2007)17<br>EPA OPPTS 860.1340<br>SANTE/2020/12830 rev. 1<br>EC 1107/2009 |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes |

**Materials**

| | |
|---|---|
| **Test Material** | Cyprodinil |
| **Lot/Batch #:** | G130349 |
| **Purity (%):** | 99.96 |
| **IUPAC name:** | (4-Cyclopropyl-6-methyl-pyrimidin-2-yl)-phenyl-amine |
| **CAS number:** | 121552-61-2 |

| **Test commodities** | | | |
|---|---|---|---|
| **Crop** | **Commodity** | **Commodity type** | **Source** |
| Honey Matrices | Honey | Multifloral | Colonies set up at Labcorp, Huntingdon, UK |

**Study Design and Methods**

Test facility: Labcorp Early Development Laboratories Ltd.

Study start date: 10 November 2021

Study end date: 10 January 2022

Analytical phase dates: 16 November 2021 to 26 November 2021

Sub-samples of the test commodity (2 g) were fortified with standard solutions of cyprodinil in methanol. Five samples of the matrix were fortified at the limit of quantification (LOQ; 0.01 mg/kg) and five at 10x LOQ (0.1 mg/kg). Matrix used was honey. The fortified samples were analysed alongside untreated control samples.

**Principle of the method**

Honey (2 g) was weighed into a polypropylene centrifuge tube (50 mL) and the samples fortified if required. Water (10 mL) was added along with acetonitrile (10 mL) and the sample was shaken vigorously for approximately 1 minute. The contents of a Supel ™ QuE citrate tube (Suplco Part No. 55227-U) was added to the sample and the sample was shaken vigorously for approximately 1 minute. The sample was then centrifuged at 3500 rpm for 5 minutes. An aliquot of the upper acetonitrile phase (6 mL) was transferred into a Supel ™ QuE PSA tube (Suplco Part No. 55228-U) and shaken vigorously by hand for approximately 1 minute. The sample was then centrifuged at 3500 rpm for 5 minutes. An aliquot of the upper acetonitrile layer (1 mL) was transferred into 15 mL polypropylene tube and made to volume (8 mL) with the addition of acetonitrile/water (20/80 v/v) containing 0.1% formic acid. The matrix:solvent ratio in the final extract is 0.025 g/mL. Cyprodinil is determined by high-performance liquid chromatography with mass-spectrometric detection (LC-MS/MS).

**HPLC-MS/MS Conditions**

| | | | | |
|---|---|---|---|---|
| HPLC system: | Waters Acquity UPLC System | | | |
| Detector: | Applied Biosystems API 4000 triple quadrupole mass | | | |
| Column: | Acquity UPLC BEH $C_{18}$ 50 mm x 2.1 mm, 1.7 µm | | | |
| Mobile phase: | A: 0.1 % formic acid in water<br>B: 0.1 % formic acid in acetonitrile | | | |
| | Time | %A | %B | Gradient |
| | 0.0 | 90 | 10 | - |
| | 2.0 | 90 | 10 | - |
| | 3.0 | 5 | 95 | Linear |
| | 5.0 | 5 | 95 | - |
| | 5.1 | 90 | 10 | Linear |
| | 8.0 | 90 | 10 | - |
| Flow rate: | 0.4 mL/min | | | |
| Column oven temperature | 40°C | | | |
| Injection volume: | 10 µL | | | |
| Retention time: | Cyprodinil: 3.2 min | | | |

| | | |
|---|---|---|
| Detector | API 4000 | |
| | Ionisation mode | Ionspray |
| | Source polarity: | Positive |
| | Curtain gas (CUR): | 30 (arbitrary units) |
| | Gas 1 (GSI): | 40 (arbitrary units) |
| | Gas 2 (GSI): | 40 (arbitrary units) |
| | Temperature (TEM): | 450°C |
| | Ionspray voltage (IS): | 4200V |
| | Collision gas setting (CAD): | 6 |
| | Entrance potential (EP): | 10 V |

Dwell time 100 msec

Source and detection parameters for MS/MS experiments:

| Compound | Parent m/z | CE (V) | DP (V) | CXP (V) | Fragment ions (m/z) | |
|---|---|---|---|---|---|---|
| Cyprodinil | 226 | 33 | 50 | 12 | 108 | Quantification |
| | | 46 | 50 | 14 | 93 | Confirmation |

CE: Collision energy; CXP: Collision cell exit potential ; DP : Declustering Potential

Quantification: Peak areas of fragment ion at m/z = 108, external standards in solvent

Confirmation: Peak areas of fragment ion at m/z = 93, external standards in solvent

**Results**

Recoveries of cyprodinil obtained from honey at each fortification level using the QuEChERS method validated in this study, and other validation parameters of the method are presented in tables below.

**Table A 58: Recovery results from method validation of cyprodinil using the QuEChERS analytical method validated in this study in honey.**

| Matrix | Analyte | Fortification level (mg/kg) | Individual recoveries (%) | Range of recoveries (%) (*n* = x) | Mean recovery (%) | RSD (%) | Comments |
|---|---|---|---|---|---|---|---|
| Honey | Cyprodinil | Mass transition m/z = 226 → 108 (quantification) | | | | | |
| | | 0.01 | 75, 84, 73, 76, 75 | 73 – 84 (n = 5) | 77 | 5.6 | |
| | | 0.1 | 88, 91, 90, 86, 97 | 86 – 97 (n = 5) | 90 | 4.6 | |
| | | Overall | | 73 – 97 (n = 10) | 84 | 9.9 | |
| | | Mass transition m/z = 226 → 93 (confirmation) | | | | | |
| | | 0.01 | 81, 90, 89, 96, 85 | 81 – 96 (n = 5) | 88 | 6.4 | |
| | | 0.1 | 90, 94, 91, 85, 96 | 85 – 96 (n = 5) | 91 | 4.6 | |
| | | Overall | | 81 – 96 (n = 10) | 90 | 5.5 | |

**Table A 59: Characteristics of the analytical method used for the quantification of cyprodinil residues in honey.**

| | |
|---|---|
| Analytes | Cyprodinil |
| Equipment/ Chromatographic method | HPLC-MS/MS |
| Accuracy/ Precision (repeatability) | **Cyprodinil:**<br>Honey: mean recoveries were in the range of 77 – 91% and RSD in the range 4.6 – 6.4% (for both fortification levels). |
| Specificity | HPLC-MS/MS with two ion transitions is considered to be a highly specific detection technique and therefore, according to EU guidance (SANTE/2020/12830, Rev.1), no further confirmatory technique is required.<br>No peaks in controls above 30% of LOQ were detected. |
| Assessment of matrix effects is presented | Matrix effects on detector response caused by honey were found to be insignificant for cyprodinil for both transitions monitored (< ±20%) therefore solvent standards were used for quantification. |
| Calibration/Linearity | Calibration was performed using at least 5 single levels. |
| | Calibration performed over the range 0.05 ng/mL to 5 ng/mL, equivalent to 0.002 mg/kg to 0.2 mg/kg in matrix. The calibration range was from 20% of the LOQ to at least 20% above the highest residue measured. |
| | The detector response was linear with 1/x weighing used.<br>**Honey - Cyprodinil**<br>Quantification - y= 20641.6 x + 3300.91 (r = 0.9979)<br>Confirmation - y = 34562.6 x + 5311.61 (r = 0.9985) |
| Limit of quantification (LOQ) | 0.01 mg/kg |
| Limit of detection (LOD) | 0.05 ng/mL equivalent to 0.002 mg/kg (20% of the LOQ) |
| Extract Stability | Residues of cyprodinil in final extract demonstrated to be stable when stored at 2 – 8°C for a period of upto 8 days. |

**Conclusion**

The method has been successfully validated for the determination of residues of cyprodinil in honey with a limit of quantification (LOQ) of 0.01 mg/kg.

## A 2.1.2.2.1.2 Independent laboratory validation

| | |
|---|---|
| Comments of zRMS: | The ILV in honey of the QuEChERS analytical method (as described in Report 8485604) has been accepted. For both fortification levels (0.010 mg/kg and 0.10 mg/kg) in honey matrix, acceptable mean recoveries in the range of 70 - 120% with an RSD of < 20% were found for cyprodinil for both the quantification and confirmation mass transition. The method is valid for the determination of cyprodinil in honey at the LOQ of 0.010 mg/kg. |

| | |
|---|---|
| Reference: | KCP 5.2.2 |
| Report | Cyprodinil - ILV of Analytical QuEChERS Method for the Determination of Residues of Cyprodinil in Honey by LC-MS/MS, Mechelke J (2022), Report number 20210437<br>XXXX File No. VV-945895 |
| Guideline(s): | Yes:<br>OECD ENV/JM/MONO(2007)17 |

| | |
|---|---|
| | EPA 860.1340<br>EC No 1107/2009<br>SANTE/2020/12830, Rev.1 |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes |

**Study Design and Methods**

Test facility: Innovative Environmental Services (IES) Ltd, Switzerland

Study start date: 18 Jan 2022

Study end date: 31 Mar 2022

Homogenised sub-samples of the test commodity (2.0 g honey) were fortified with standard solutions of Cyprodinil in methanol. Five samples were fortified at the limit of quantification (LOQ; 0.010 mg/kg) and five at a higher level (10x LOQ). The matrix used was honey. The fortified samples were analysed alongside untreated control samples.

**Principle of the method**

Samples of honey are extracted with water and acetonitrile. Samples are shaken vigorously by hand before adding the QuEChERS extraction salts (4 g $MgSO_4$, 1 g NaCl, 1 g trisodium citrate dehydrate, 0.5 g disodium hydrogencitrate sesquihydrate) and then shaken vigorously. After centrifugation, an aliquot of the upper acetonitrile layer is cleaned-up by dispersive solid phase extraction (d-SPE) employing bulk sorbent (PSA) and $MgSO_4$ for the removal of the residual water. After the clean-up the samples are centrifuged and further diluted. Cyprodinil is determined by high-performance liquid chromatography with mass-spectrometric detection (LC-MS/MS).

**HPLC-MS/MS Conditions**

| | | | |
|---|---|---|---|
| HPLC system: | Agilent 1290 Infinity II HPLC pump | | |
| Detector: | AB Sciex 6500+ QTrap mass spectrometer | | |
| Autosampler: | Agilent 1290 Infinity II autosampler | | |
| Column: | Waters Acquity UPLC BEH C18, 50 mm x 2.1 mm, 1.7 µm particle size | | |
| Mobile phase: | A: 0.1% formic acid in water<br>B: 0.1% formic acid in acetonitrile | | |
| | Time | %A | %B |
| | 0.00 | 90 | 10 |
| | 2.00 | 90 | 10 |
| | 3.00 | 5 | 95 |
| | 5.00 | 5 | 95 |
| | 5.10 | 90 | 10 |
| | 8.00 | 90 | 10 |
| Flow rate: | 0.4 mL/min | | |
| Column oven temperature | 40°C | | |
| Injection volume: | 5 µL | | |
| Retention time: | Cyprodinil: 3.9 min | | |

| | | |
|---|---|---|
| Detector | AB Sciex 6500+ | |
| | Interface: | ESI (ElectroSpray Ionisation) |
| | Polarity: | Positive |

| | |
|---|---|
| Curtain Gas: | 30 psi |
| Ion Source Gas 1: | 50 psi |
| Ion Source Gas 2: | 50 psi |
| Collision Gas: | Medium |
| Source Temperature: | 500 °C |
| Ion Spray Voltage: | 4500 V |
| Entrance Potential: | 10 V |
| Scan Type: | Multiple Reaction Monitoring (MRM) |
| Dwell time: | 100 msec |
| Resolution Q1 and Q3: | unit |

Source and detection parameters for MS/MS experiments:

| Compound | Parent m/z | CE (V) | DP (V) | CXP (V) | Fragment ions (m/z) | |
|---|---|---|---|---|---|---|
| *Cyprodinil* | 226 | 33 | 76 | 12 | 108 | Quantification |
| | | 41 | 76 | 12 | 93 | Confirmation |

CE: Collision energy; CXP: Collision cell exit potential; DP: Declustering Potential

Quantification: Peak areas of transition m/z 226 > 108, external standards in matrix Confirmation: Peak areas of transition m/z 226 > 93, external standards in matrix

**Results**

Recoveries of Cyprodinil obtained from honey at each fortification level using QuEChERS analytical method are presented in the table below. Other validation parameters of the method are presented in the following table.

**Table A 60: Recovery results from method validation of Cyprodinil using QuEChERS analytical method in honey.**

| Matrix | Analyte | Fortification level (mg/kg) | Individual recoveries (%) | Range of recoveries (%) (n = x) | Mean recovery (%) | RSD (%) | Comments |
|---|---|---|---|---|---|---|---|
| Honey | Cyprodinil | Mass transition m/z = 226 → 108 (quantification) | | | | | |
| | | 0.010 | 107, 106, 108, 104, 98 | 98 – 108 (n = 5) | 105 | 3.9 | |
| | | 0.10 | 101, 101, 103, 105, 106 | 101 – 106 (n = 5) | 103 | 2.1 | |
| | | Overall | - | 98 – 108 (n = 10) | 104 | 3.1 | |
| | | Mass transition m/z = 226 → 93 (confirmation) | | | | | |
| | | 0.010 | 104, 105, 106, 103, 97 | 97 – 106 (n = 5) | 103 | 3.4 | |
| | | 0.10 | 101, 101, 103, 105, 106 | 101 - 106 (n = 5) | 103 | 2.0 | |
| | | Overall | - | 97 – 106 (n = 10) | 103 | 2.7 | |

**Table A 61: Characteristics of the QuEChERS analytical method used for the quantification of Cyprodinil residues in honey.**

| | |
|---|---|
| Analyte | Name |
| Equipment/ Chromato-graphic method | HPLC-MS/MS |
| Accuracy/ Precision (repeatability) | For the LOQ fortification level (0.010 mg/kg) and 0.10 mg/kg fortification level, acceptable mean recoveries in the range of 70 - 120% with a relative standard deviation (RSD) of ≤ 20% were found for Cyprodinil for both the primary and confirmatory mass transitions |
| Precision (reproducibility) | This independent laboratory validation (ILV) study was conducted to verify the reliability of QuEChERS analytical method as described in Labcorp Report 8485604 for the determination of Cyprodinil in honey. The results indicate that the method is reproducible. |
| Specificity | LC-MS/MS with two transitions is considered to be a highly specific detection technique and therefore, according to EU guidance (SANTE/2020/12830, Rev.1), no further confirmatory technique is required.<br>There were no peaks in controls above 30% of LOQ. |
| Confirmatory method | Not applicable. |
| Assessment of matrix effects is presented | Yes<br>Matrix effects (enhancement or suppression) were assessed for Cyprodinil in honey matrix; these are deemed to be significant if greater than 20%. No significant matrix effects (i.e. >20% suppression or enhancement) on the LC-MS/MS detector response were observed for Cyprodinil in the honey matrix tested. Nevertheless, matrix-matched calibration standards were routinely used, and all sample extracts were evaluated with multi-point calibrations based on matrix-matched calibration standards in honey matrix. |
| Calibration/Linearity | The linearity of the detector response was confirmed by double injection of seven six matrix-matched calibration standards. |
| | A calibration range of 0.05 ng/mL to 3.0 ng/mL was used (equivalent to 0.002 mg/kg to 0.12 mg/kg). The lower margin of the linearity test was 20% of the LOQ and the upper margin was at least 20% above the highest concentration in the final measured extracts |
| | The detector response was linear. The correlation coefficients (r) were ≥ 0.99 for Cyprodinil in honey matrix: Residual assessment confirmed no bias. |
| | The detector response was linear.<br>**Cyprodinil in Honey**<br>Quantification – y = 530323 x + 8369.43 (r = 0.9995)<br>Confirmation – y = 663598 x + 7188.89 (r = 0.9995) |
| Limit of quantification (LOQ) | 0.010 mg/kg |
| Limit of detection (LOD) | 0.002 mg/kg |

**Conclusion**

QuEChERS analytical method as described in Labcorp Report 8485604 has been successfully independently validated for the determination of residues of Cyprodinil in honey with a limit of quantification (LOQ) of 0.010 mg/kg.

## A 2.1.2.2.1.3 Confirmatory method

No confirmatory method is required, because the method was validated at two mass transitions (primary and confirmatory).

## A 2.1.2.3 Description of Methods for the Analysis of Body Fluids and Tissues (KCP 5.2.3)

### A 2.1.2.3.1 QuEChERS

#### A 2.1.2.3.1.1 Method validation (report P 4185 G)

| | |
|---|---|
| Comments of zRMS: | The validation in blood matrix has been accepted.<br>(see A 2.1.2.1.3.1) |

| | |
|---|---|
| Reference: | KCP 5.2.3 (also filed under 5.2.1) |
| Report | Cyprodinil (CGA219417): Validation of the QuEChERS Method for the Determination of Residues of Cyprodinil in Crop Matrices and Body Fluid by LC-MS/MS, Richter, S., 2017, Report No. P 4185 G (XXXX Task No. TK0319684), XXXX File No. CGA219417_11774 VV-467144 |
| Guideline(s): | Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/825/00 rev. 8.1, 16/11/2010).<br>OECD Guidance Document on Pesticide Residue Analytical Methods ENV/JM/MONO(2007)17.<br>EPA Residue Chemistry Test Guideline OPPTS 860.1340 (712-C-96-174) Pesticide Residue Analytical Method, 1996.<br>Regulation (EC) No 1107/2009 of the European Parliament and of the council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC |
| Deviations: | No |
| GLP: | Work was performed in a GLP compliant facility. |
| Acceptability: | Yes |

**Materials and methods**

The analytical method was derived from the QuEChERS (EN 15662:2009-02) multi-residue method. It is based on extraction/clean-up procedures and subsequent LC-MS/MS determination. Residues of cyprodinil were extracted from sample material with acetonitrile, following the addition of a suitable volume of water. A salt mixture (magnesium sulphate, sodium chloride, sodium citrate tribasic dihydrate and sodium citrate dibasic sesquihydrate; available commercially pre-mixed - Supelco 55227-U) was added, and the extracts were shaken and then centrifuged. Then aliquots of the extracts were transferred into pre-mixed, commercially available dispersive SPE PSA clean-up tubes (Supelco 55228 U). After shaking, samples were centrifuged. Sample extracts were then diluted with acetonitrile/water (20/80, v/v) + 0.1 % formic acid or with final extract of control specimen (depending on matrix) for final determination by high-performance liquid chromatography with mass-spectrometric detection (LC-MS/MS), monitoring for the primary (m/z 226→108) and the confirmatory transition (m/z 226→93) for cyprodinil.

**Results and discussions**

Fortified samples were analysed in quintuplet at the limit of quantification (LOQ, 0.01 mg/kg) and at higher

level fortification (10 x LOQ (0.10 mg/kg) for blood). Acceptable mean recoveries of between 60% and 120% at 0.01 mg/kg and 70% and 120% at 0.1 mg/kg were found for both mass transitions and therefore, according to EU guidance (SANTE/2020/12830, Rev.1), demonstrate the method has satisfactory accuracy. The relative standard deviations (RSDs) of cyprodinil recoveries at each fortification level and overall were < 20% and therefore, according to the EU guidance (SANTE/2020/12830, Rev.1), demonstrate the method has satisfactory repeatability.

The stability of sample extracts originally fortified with cyprodinil at the LOQ level was assessed by reinjection, after a storage period of at least 8 days in a refrigerator at 6-8 °C, against freshly prepared calibration standards. The results proved that the cyprodinil residues in the stored fortified sample extracts were stable. The mean recovery values at the LOQ level were between 70% and 110%, with a RSD of ≤ 20% when re-analysed.

The stability of the stored stock and working solutions of cyprodinil was assessed after a storage period of at least 32 days in a refrigerator at 6-8 °C, against freshly prepared calibration standards at the same concentration. The mean peak areas of the stored solutions were found to be within ± 10% of the mean peak areas of the freshly prepared standard solutions for cyprodinil, demonstrating that residues of cyprodinil in the stored stock and working solutions were stable for the storage period assessed when stored under the described conditions.

**Table A 62: Recovery results from method validation of cyprodinil using the analytical method QuEChERS (primary transition m/z 226→108)**

| Matrix | Fortification Level (mg/kg) | Recovery (%) | Number of Analyses (n) | Mean Recovery (%) | RSD (%) | Recovery Range (%) |
|---|---|---|---|---|---|---|
| Blood | 0.01* | 101, 103, 99, 103, 102 | 5 | 101 | 2 | 99-103 |
| | 0.10 | 104, 104, 104, 102, 100 | 5 | 103 | 2 | 100-104 |
| | Overall | | 10 | 102 | 2 | 99-104 |

*Limit of quantitation, defined by the lowest validated fortification level.

**Table A 63: Recovery results from method validation of cyprodinil using the analytical method QuEChERS (confirmatory transition m/z 226→93)**

| Matrix | Fortification Level (mg/kg) | Recovery (%) | Number of Analyses (n) | Mean Recovery(%) | RSD (%) | Recovery Range (%) |
|---|---|---|---|---|---|---|
| Blood | 0.01* | 99, 99, 101, 101, 100 | 5 | 100 | 1 | 99-101 |
| | 0.10 | 104, 103, 103, 100 ,101 | 5 | 102 | 2 | 100-104 |
| | Overall | | 10 | 101 | 2 | 99-104 |

*Limit of quantitation, defined by the lowest validated fortification level.

**Table A 64: Characteristics for the analytical method QuEChERS used for validation of cyprodinil residues in blood**

| | Cyprodinil |
|---|---|
| Specificity | LC-MS/MS with two transitions is considered to be a highly specific detection technique and therefore, according to EU guidance (SANTE/2020/12830, Rev.1), no further confirmatory technique is required. The method includes two MS/MS transitions, both of which have been validated. No significant interferences arising from the matrices, the lab ware, reagents or solvents have been observed at the retention times of interest. |

| | **Cyprodinil** |
|---|---|
| Calibration (type, number of data points) | The linearity of the LC-MS/MS detector response was assessed using standard solutions in acetonitrile/water (20/80, v/v) + 0.1% formic acid. Linearity was assessed for both MS/MS transitions. Standards at ≥ 5 different concentrations were injected and the signal area plotted against concentration for all calibration points. Straight lines with correlation coefficients ranging from 0.9993 to 0.9999 were obtained for cyprodinil. |
| Calibration range | 0.050 ng/mL to 5.0 ng/mL |
| Assessment of matrix effects is presented | Yes<br>Matrix effects (signal suppression or enhancement; ≤ ± 20%) were considered not to be significant for blood. Thus, calibration standards in acetonitrile/water (20/80, v/v) + 0.1% formic acid were used for quantification of cyprodinil in this matrix. |
| Limit of determination/quantification | The limit of quantification for cyprodinil residues in blood using the QuEChERS method was established at 0.01 mg/kg. No interfering peaks around the retention time of cyprodinil were found in any of the control samples at levels above 20% of the limit of quantification.<br>The limits of detection (LODs) were calculated to be 0.000124 mg/kg for the primary transition, and 0.000153 mg/kg for the confirmatory transition, for blood. |

**Conclusion**

The analytical multi-residue QuEChERS method has been demonstrated to be a reliable and accurate procedure for the determination of cyprodinil in blood to a limit of quantification of 0.01 mg/kg, using commercially available laboratory equipment and reagents.

### A 2.1.2.3.1.2 Confirmatory method

No confirmatory method is required, because the method was validated at two mass transitions (primary and confirmatory).

## A 2.1.2.4 Description of Methods for the Analysis of Soil (KCP 5.2.4)

### A 2.1.2.4.1 Analytical method GRM010.08B

#### A 2.1.2.4.1.1 Method validation

| | |
|---|---|
| Comments of zRMS: | The validation of the method GRM010.08B in soil has been accepted.<br><br>In the LC-MS/MS method two transitions were also applied (for CGA219417 Primary transition *m/z* 226.0→93.0 confirmatory *m/z* 226.0→108.0; CGA249287 [4-cyclopropyl-6-methyl-pyrimidin-2-yl amine] primary transition *m/z* 150.0→118.0 confirmatory *m/z* 150.0→133.0; CGA275535 [3-(4-cyclopropyl-6-methyl-pyrimidin-2-ylamino)- phenol] primary transition *m/z* 242.2→93.0 confirmatory *m/z* 242.2→108.0; CGA321915 [4-cyclopropyl-6-methyl-pyrimidin-2-ol] primary transition *m/z* 151.0→93.0 confirmatory *m/z* 151.0→108.0). Final determination by LC-MS/MS with two transitions is considered to be highly specific. The method validation data are reported in the CEMAS report CEMS-6716.<br><br>The magnitude of the matrix effects were considered not to be significant (>20%) for the soil types tested (clay and sandy loam soil). No significant degradation of the analytes was observed when stored under the specified conditions in soil types tested. Recovery efficiency is generally considered acceptable when the mean values are between 70% and 120% and with a relative standard deviation of <20%. The procedure has been demonstrated to be a reliable and accurate procedure for the determination of CGA219417, CGA249287, CGA275535 and CGA321915 residues in soil. |

| | |
|---|---|
| Reference: | KCP 5.2.4 |
| Report | Cyprodinil - Residue Method GRM010.08B for the Determination of Cyprodinil, CGA249287, CGA 275535 and CGA321915 in Soil. XXXX Analytical Method GRM010.08B. XXXX Ltd, Jealott's Hill International Research Centre, Bracknell, Berkshire, RG42 6EY, UK. Report No. GRM010.08B (method), CEMR-6716-REG (validation). XXXX File No. VV-128139 (method), VV-411986 (validation)<br><br>Allen, L., 2018, Allen, L. 2015 |
| Guideline(s): | Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/825/00 rev. 8.1, 16/11/2010).<br><br>Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/3029/99 rev. 4, 11/07/2000).<br><br>Residue Chemistry Test Guidelines OCSPP 850.6100 Environmental Chemistry Methods and Associated Independent Laboratory Validation, EPA 712-C-001, January 2012. |
| Deviations: | No |
| GLP: | Validation: Yes |

Method: No

Acceptability: Yes

**Principle of the Method**

In summary, soil samples are extracted by reflux with methanol/water (80/20, v/v) before dilution with 10 mM ammonium acetate and analysis by high performance liquid chromatography with triple quadrupole mass spectrometry detection (LC-MS/MS) for cyprodinil, CGA249287, CGA275535 and CGA321915. The limit of quantification (LOQ) of the method is 0.01 mg/kg for each analyte.

**Recovery Findings**

Summaries of the results for cyprodinil, CGA249287, CGA275535 and CGA321915 are presented in **Błąd! Nie można odnaleźć źródła odwołania.** to **Błąd! Nie można odnaleźć źródła odwołania.**.

**Table A 65: Recovery and precision results from validation of GRM010.08A for cyprodinil in soil: primary transition *m*/z 226.0 → 93.0**

| Matrix | Fortification Level (mg/kg) | Recovery (%) | Mean (%) | RSD (%) | Range (%) |
|---|---|---|---|---|---|
| **(Primary Transition *m/z* 226.0 → 93.0)** | | | | | |
| Clay | 0.01 | 95, 91, 97, 90, 93 | 93 | 3.1 | 90-97 |
| | 0.1 | 95, 93, 93, 91, 91 | 93 | 1.8 | 91-95 |
| | Overall | | 93 | 2.4 | 90-97 |
| Sandy Loam | 0.01 | 96, 97, 97, 99, 93 | 96 | 2.3 | 93-99 |
| | 0.1 | 99, 96, 99, 97, 98 | 98 | 1.3 | 96-99 |
| | Overall | | 97 | 1.9 | 93-99 |

Limit of quantitation, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ
% Mean and % RSD calculated using rounded values

**Table A 66: Recovery and precision results from validation of GRM010.08A for cyprodinil in soil: confirmatory transition *m*/z 226.0 → 108.0**

| Matrix | Fortification Level (mg/kg) | Recovery (%) | Mean (%) | RSD (%) | Range (%) |
|---|---|---|---|---|---|
| **(Confirmatory Transition *m/z* 226.0 → 108.0)** | | | | | |
| Clay | 0.01 | 98, 96, 92, 87, 93 | 93 | 4.5 | 87-98 |
| | 0.1 | 95, 92, 93, 92, 90 | 92 | 2.0 | 90-95 |
| | Overall | | 93 | 3.3 | 87-98 |
| Sandy Loam | 0.01 | 99, 102, 98, 102, 97 | 100 | 2.3 | 97-102 |
| | 0.1 | 98, 95, 96, 95, 98 | 96 | 1.6 | 95-98 |
| | Overall | | 98 | 2.5 | 95-102 |

Limit of quantitation, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ
% Mean and % RSD calculated using rounded values

**Table A 67: Recovery and precision results from validation of GRM010.08A for CGA249287 in soil: primary transition *m*/z 150.0 → 118.0**

| Matrix | Fortification Level (mg/kg) | Recovery (%) | Mean (%) | RSD (%) | Range (%) |
|---|---|---|---|---|---|
| **(Primary Transition *m/z* 150.0 → 118.0)** | | | | | |
| Clay | 0.01 | 83, 104, 94, 103, 91 | 95 | 9.2 | 83-104 |
| | 0.1 | 95, 93, 95, 94, 98 | 95 | 2.0 | 93-98 |
| | Overall | | 95 | 6.3 | 83-104 |
| Sandy Loam | 0.01 | 88, 97, 86, 117, 99 | 97 | 12.6 | 86-117 |
| | 0.1 | 97, 95, 98, 99, 100 | 98 | 2.0 | 95-100 |
| | Overall | | 98 | 8.5 | 86-117 |

Limit of quantitation, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ
% Mean and % RSD calculated using rounded values

**Table A 68: Recovery and precision results from validation of GRM010.08A for CGA249287 in soil: confirmatory transition *m*/z 150.0 → 133.0**

| Matrix | Fortification Level (mg/kg) | Recovery (%) | Mean (%) | RSD (%) | Range (%) |
|---|---|---|---|---|---|
| **(Confirmatory Transition *m/z* 150.0 → 133.0)** | | | | | |
| Clay | 0.01 | 106, 99, 110, 99, 109 | 105 | 5.1 | 99-110 |
| | 0.1 | 98, 97, 97, 98, 99 | 98 | 0.9 | 97-99 |
| | Overall | | 101 | 5.0 | 97-110 |
| Sandy Loam | 0.01 | 100, 91, 105, 97, 101 | 99 | 5.3 | 91-105 |
| | 0.1 | 98, 98, 100, 92, 101 | 98 | 3.6 | 92-101 |
| | Overall | | 98 | 4.3 | 91-105 |

Limit of quantitation, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ
% Mean and % RSD calculated using rounded values.

**Table A 69: Recovery and precision results from validation of GRM010.08A for CGA275535 in soil: primary transition *m*/z 242.2 → 93.0**

| Matrix | Fortification Level (mg/kg) | Recovery (%) | Mean (%) | RSD (%) | Range (%) |
|---|---|---|---|---|---|
| **(Primary Transition *m/z* 242.2→ 93.0)** | | | | | |
| Clay | 0.01 | 100, 99, 100, 95, 93 | 97 | 3.3 | 93-100 |
| | 0.1 | 101, 95, 97, 94, 94 | 96 | 3.1 | 94-101 |
| | Overall | | 97 | 3.1 | 93-101 |
| Sandy Loam | 0.01 | 104, 99, 102, 98, 100 | 101 | 2.4 | 98-104 |
| | 0.1 | 98, 99, 101, 97, 101 | 99 | 1.8 | 97-101 |
| | Overall | | 100 | 2.1 | 97-104 |

Limit of quantitation, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ
% Mean and % RSD calculated using rounded values

**Table A 70: Recovery and precision results from validation of GRM010.08A for CGA275535 in soil: confirmatory transition *m*/z 242.2 → 108.0**

| Matrix | Fortification Level (mg/kg) | Recovery (%) | Mean (%) | RSD (%) | Range (%) |
|---|---|---|---|---|---|
| **(Confirmatory Transition *m/z* 242.2→ 108.0)** | | | | | |
| Clay | 0.01 | 101, 104, 97, 99, 103 | 101 | 2.8 | 97-104 |
| | 0.1 | 100, 97, 95, 95, 93 | 96 | 2.8 | 93-100 |
| | Overall | | 98 | 3.7 | 93-104 |
| Sandy Loam | 0.01 | 102, 106, 102, 94, 97 | 100 | 4.7 | 94-106 |
| | 0.1 | 99, 100, 101, 99, 100 | 100 | 0.8 | 99-101 |
| | Overall | | 100 | 3.2 | 94-106 |

Limit of quantitation, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ
% Mean and % RSD calculated using rounded values

**Table A 71: Recovery and precision results from validation of GRM010.08A for CGA321915 in soil: primary transition *m*/z 151.0 → 93.0**

| Matrix | Fortification Level (mg/kg) | Recovery (%) | Mean (%) | RSD (%) | Range (%) |
|---|---|---|---|---|---|
| **(Primary Transition *m/z* 151.0→ 93.0)** | | | | | |
| Clay | 0.01 | 92, 87, 88, 97, 86 | 90 | 5.0 | 86-97 |
| | 0.1 | 87, 84, 85, 85, 88 | 86 | 1.9 | 84-88 |
| | Overall | | 88 | 4.4 | 84-97 |
| Sandy Loam | 0.01 | 99, 98, 107, 103, 97 | 101 | 4.1 | 97-107 |
| | 0.1 | 94, 91, 92, 89, 94 | 92 | 2.3 | 89-94 |
| | Overall | | 96 | 5.8 | 89-107 |

Limit of quantitation, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ
% Mean and % RSD calculated using rounded values

**Table A 72: Recovery and precision results from validation of GRM010.08A for CGA321915 in soil: confirmatory transition *m*/z 151.0 → 108.0**

| Matrix | Fortification Level (mg/kg) | Recovery (%) | Mean (%) | RSD (%) | Range (%) |
|---|---|---|---|---|---|
| **(Confirmatory Transition *m/z* 151.0→ 108.0)** | | | | | |
| Clay | 0.01 | 73, 74, 76, 78, 74 | 75 | 2.7 | 73-78 |
| | 0.1 | 90, 83, 85, 88, 89 | 87 | 3.4 | 83-90 |
| | Overall | | 81 | 8.3 | 73-90 |
| Sandy Loam | 0.01 | 97, 100, 107, 108, 93 | 101 | 6.4 | 93-108 |
| | 0.1 | 95, 95, 94, 93, 100 | 95 | 2.8 | 93-100 |
| | Overall | | 98 | 5.6 | 93-108 |

Limit of quantitation, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ
% Mean and % RSD calculated using rounded values

**Specificity**
LC-MS/MS with two transitions is considered to be a highly specific detection technique and therefore according to EU guidance (see guidance section of this summary) no further confirmatory technique is required. The method includes two MS/MS transitions, both of which have been validated. No significant interferences arising from the soil matrices, the labware, reagents or solvents have been observed at the retention times of interest.

**Linearity**
The linearity of the LC-MS/MS detector was tested using both non-matrix calibration standard solutions and matrix-matched standard solutions (from 0.06 to 4.0 ng/mL). Standards at eight different concentrations were injected and the signal area plotted against concentration for all calibration points. Straight lines with correlation coefficients ranging from 0.9972 to 1.0000 were obtained for cyprodinil, CGA249287, CGA275535 and CGA321915.

**Recovery**
Fortified samples were analysed in quintuplicate at the limit of quantification (LOQ) of 0.01 mg/kg and at ten times the LOQ (0.1 mg/kg). Acceptable mean accuracy values of between 70% and 120% were found for both transitions on the matrices tested and therefore according to EU guidance (see guidance section of this summary), demonstrate the method has satisfactory accuracy.

**Repeatability**
The relative standard deviations (RSDs) of cyprodinil, CGA249287, CGA275535 and CGA321915 values at each fortification level and overall for the soil samples tested during method validation were <20% and therefore according to the EU guidance (see guidance section of this summary) demonstrate the method has satisfactory repeatability.

**Limit of Quantification**
The LOQ for cyprodinil, CGA249287, CGA275535 and CGA321915 residues in soil using method GRM010.08A was established at 0.01 mg/kg. No interfering peaks around the retention time of cyprodinil, CGA249287, CGA275535 and CGA321915 were found in any of the control samples at levels above 30% of the LOQ.

**Matrix Extract**
No significant matrix effects (suppression or enhancement) were observed for cyprodinil, CGA249287, CGA275535 and CGA321915 in either of the soil types used during the method validation and therefore, non matrix-matched linearity standards were used for quantification.

**Stability of Final Extracts**
The stability of the sample extracts fortified with cyprodinil, CGA249287, CGA275535 and CGA321915 was checked after a storage period of 7 days at 2-8ºC against freshly prepared calibration standards. The results proved that cyprodinil, CGA249287, CGA275535 and CGA321915 residues in the stored fortified soil extracts were stable.

**Stability of Standard Solutions**
The stability of the stored working standard solutions of cyprodinil, CGA249287, CGA275535 and CGA321915 were checked after a storage period of 167 days at 2-8ºC against freshly prepared calibration standards. The mean response values for stored and fresh solutions were within 10% of each other and the results demonstrated that cyprodinil, CGA249287, CGA275535 and CGA321915 residues were stable in the standard solutions.

**Conclusion**
Analytical method GRM010.08A has been demonstrated to be a reliable and accurate procedure for the determination of cyprodinil, CGA249287, CGA275535 and CGA321915 in soil to a limit of quantification of 0.01 mg/kg, using commercially available laboratory equipment and reagents.

### A 2.1.2.4.1.2 Confirmatory method

No confirmatory method is required, because the method was validated at two mass transitions (primary and confirmatory).

(Allen L, 2018 and 2015)

## A 2.1.2.5 Description of Methods for the Analysis of Water (KCP 5.2.5)

### A 2.1.2.5.1 Analytical method GRM010.07A

#### A 2.1.2.5.1.1 Method validation

| | |
|---|---|
| Comments of zRMS: | The validation of the method GRM010.07A has been accepted in surface and ground water.<br><br>Final determination by LC-MS/MS with two transitions was performed: for **CGA219417** primary transition *m/z* 226→93 confirmatory *m/z* 226→108; for **CGA249287** [4-cyclopropyl-6-methyl-pyrimidin-2-yl amine] primary transition *m/z* 150→118 confirmatory *m/z* 150→133; for **CGA275535** [3-(4-cyclopropyl-6-methyl-pyrimidin-2-ylamino)- phenol] primary transition *m/z* 242→93 confirmatory *m/z* 242→108. Recovery values were between 70% and 110% with a relative standard deviation of ≤ 20%. The method LOQ of 0.05 µg/L was set. The magnitude of the matrix effects were considered not to be significant (>15%) for the water types tested (ground and surface).<br><br>This procedure has been demonstrated to be a reliable and accurate procedure for the determination of cyprodinil, CGA249287 and CGA275535 residues in water. |

| | |
|---|---|
| Reference: | KCP 5.2.5 |
| Report | Cyprodinil - Residue Method GRM010.07A for the Determination of Cyprodinil, CGA249287 and CGA275535 in Water by Solid Phase Extraction and LC-MS/MS Analysis. XXXX Ltd, Jealott's Hill International Research Centre, Bracknell, Berkshire, RG42 6EY, UK. Allen, Brooks, Crook (2015), Report No. GRM010.07A. XXXX File No. VV-128422<br><br>Cyprodinil - Validation of Draft Residue Method GRM010.07A for the Determination of Cyprodinil, CGA249287 and CGA275535 in Water. CEMAS. CEM Analytical Services Ltd (CEMAS), Imperial House, Oaklands Business Centre, Oaklands Park, Wokingham, Berkshire, RG41 2FD UK. Allen (2015), Report No. CEMR-6728-REG. XXXX File No. VV-411056 |
| Guideline(s): | Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/825/00 rev. 8.1, 16/11/2010).<br><br>Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/3029/99 rev. 4, 11/07/2000).<br><br>Residue Chemistry Test Guidelines OCSPP 850.6100 Environmental Chemistry Methods and Associated Independent Laboratory Validation, EPA 712-C-001, January 2012. |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes |

**Principle of the Method**

In summary, water is extracted by solid phase extraction before analysis by high performance liquid chromatography with triple quadrupole mass spectrometry detection (LC-MS/MS) for cyprodinil, CGA249287 and CGA275535. The limit of quantification (LOQ) of the method is 0.05 µg/L for each analyte.

**Recovery Findings**

Summaries of the results for cyprodinil, CGA249287 and CGA275535 are presented in **Błąd! Nie można odnaleźć źródła odwołania.** to **Błąd! Nie można odnaleźć źródła odwołania.**.

**Table A 73: Recovery and precision results from validation of GRM010.07A for cyprodinil in water: primary transition *m*/z 226.0 → 93.0**

| Matrix | Fortification Level (µg/L) | Recovery (%) | Mean (%) | RSD (%) | Range (%) |
|---|---|---|---|---|---|
| (Primary Transition *m/z* 226.0 → 93.0) | | | | | |
| Surface Water | 0.05 | 89, 89, 86, 87, 90 | 88 | 1.9 | 86-90 |
| | 0.5 | 85, 86, 86, 86, 87 | 86 | 0.8 | 85-87 |
| | Overall | | 87 | 1.9 | 86-90 |
| Groundwater | 0.05 | 83, 82, 83, 82, 92 | 84 | 5.1 | 82-92 |
| | 0.5 | 79, 79, 79, 88, 87 | 82 | 5.7 | 79-88 |
| | Overall | | 83 | 5.2 | 79-92 |

Limit of quantitation, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ
% Mean and % RSD calculated using un-rounded values

**Table A 74: Recovery and precision results from validation of GRM010.07A for cyprodinil in water: confirmatory transition m/z 226.0 → 108.0**

| Matrix | Fortification Level (µg/L) | Recovery (%) | Mean (%) | RSD (%) | Range (%) |
|---|---|---|---|---|---|
| (Confirmatory Transition *m/z* 226.0 → 108.0) | | | | | |
| Surface Water | 0.05 | 87, 88, 92, 94, 96 | 91 | 4.2 | 87-96 |
| | 0.5 | 86, 87, 86, 85, 86 | 86 | 0.8 | 85-87 |
| | Overall | | 89 | 4.4 | 85-96 |
| Groundwater | 0.05 | 76, 79, 83, 80, 90 | 82 | 6.5 | 76-90 |
| | 0.5 | 80, 79, 78, 87, 88 | 82 | 5.7 | 78-88 |
| | Overall | | 82 | 5.8 | 76-90 |

*Limit of quantitation, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ
% Mean and % RSD calculated using un-rounded values

**Table A 75: Recovery and precision results from validation of GRM010.07A for CGA275535 in water: primary transition m/z 242.1 → 93.0**

| Matrix | Fortification Level (µg/L) | Recovery (%) | Mean (%) | RSD (%) | Range (%) |
|---|---|---|---|---|---|
| (Primary Transition *m/z* 242.1 → 93.0) | | | | | |
| Surface Water | 0.05 | 90, 92, 92, 87, 90 | 90 | 2.3 | 87-92 |
| | 0.5 | 86, 92, 89, 89, 89 | 89 | 2.4 | 86-92 |
| | Overall | | 90 | 2.3 | 86-92 |
| Groundwater | 0.05 | 92, 87, 89, 87, 91 | 89 | 2.6 | 87-92 |
| | 0.5 | 89, 88, 88, 93, 94 | 90 | 3.2 | 88-94 |
| | Overall | | 90 | 2.8 | 87-94 |

Limit of quantitation, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ
% Mean and % RSD calculated using un-rounded values

**Table A 76: Recovery and precision results from validation of GRM010.07A for CGA275535 in water: confirmatory transition m/z 242.1 → 108.0**

| Matrix | Fortification Level (µg/L) | Recovery (%) | Mean (%) | RSD (%) | Range (%) |
|---|---|---|---|---|---|
| (Confirmatory Transition *m/z* 242.1 → 108.0) | | | | | |
| Surface Water | 0.05 | 87, 93, 91, 90, 90 | 90 | 2.4 | 87-93 |
| | 0.5 | 85, 90, 89, 87, 89 | 88 | 2.3 | 85-90 |
| | Overall | | 89 | 2.6 | 85-93 |
| Groundwater | 0.05 | 93, 87, 90, 88, 90 | 90 | 2.6 | 87-93 |
| | 0.5 | 88, 88, 88, 94, 93 | 90 | 3.4 | 88-94 |
| | Overall | | 90 | 2.8 | 87-94 |

*Limit of quantitation, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ
% Mean and % RSD calculated using un-rounded values.

**Table A 77: Recovery and precision results from validation of GRM010.07A for CGA249287 in water: primary transition m/z 150.0 → 118.0**

| Matrix | Fortification Level (µg/L) | Recovery (%) | Mean (%) | RSD (%) | Range (%) |
|---|---|---|---|---|---|
| (Primary Transition *m/z* 150.0→ 118.0) | | | | | |
| Surface Water | 0.05 | 79, 85, 81, 75, 81 | 80 | 4.5 | 75-85 |
| | 0.5 | 73, 79, 79, 80, 79 | 78 | 3.6 | 73-80 |
| | Overall | | 79 | 4.1 | 73-85 |
| Groundwater | 0.05 | 76, 73, 70, 71, 74 | 73 | 3.3 | 70-76 |
| | 0.5 | 77, 77, 76, 82, 81 | 79 | 3.4 | 76-82 |
| | Overall | | 76 | 5.1 | 70-82 |

*Limit of quantitation, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ
% Mean and % RSD calculated using un-rounded values

**Table A 78: Recovery and precision results from validation of GRM010.07A for CGA249287 in water: confirmatory transition m/z 150.0 → 133.0**

| Matrix | Fortification Level (µg/L) | Recovery (%) | Mean (%) | RSD (%) | Range (%) |
|---|---|---|---|---|---|
| (Confirmatory Transition *m/z* 150.0→ 133.0) | | | | | |
| Surface Water | 0.05 | 78, 82, 75, 79, 75 | 78 | 3.8 | 75-82 |
| | 0.5 | 74, 79, 79, 78, 78 | 78 | 2.7 | 74-79 |
| | Overall | | 78 | 3.1 | 74-82 |
| Groundwater | 0.05 | 75, 74, 72, 68, 77 | 73 | 4.7 | 68-77 |
| | 0.5 | 75, 79, 74, 81, 83 | 78 | 4.9 | 74-83 |
| | Overall | | 76 | 5.8 | 68-83 |

*Limit of quantitation, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ
% Mean and % RSD calculated using un-rounded values

**Specificity**

LC-MS/MS with two transitions is considered to be a highly specific detection technique and therefore according to EU guidance (see guidance section of this summary) no further confirmatory technique is required. The method includes two MS/MS transitions, both of which have been validated. No significant interferences arising from the water matrices, the labware, reagents or solvents have been observed at the retention times of interest.

**Linearity**

The linearity of the LC-MS/MS detector was tested using both non-matrix calibration standard solutions and matrix-matched standard solutions (from 0.3 to 20.0 ng/mL). Standards at eight different concentrations were injected and the signal area plotted against concentration for all calibration points. Straight lines with correlation coefficients ranging from 0.9976 to 0.9999 were obtained for a cyprodinil, CGA249287 and CGA275535.

**Recovery**
Fortified samples were analysed in quintuplicate at the limit of quantification (LOQ) of 0.05 µg/L and at ten times the LOQ (0.5 µg/L). Acceptable mean accuracy values of between 70% and 120% were found for both transitions on matrices tested and therefore according to EU guidance (see guidance section of this summary), demonstrate the method has satisfactory accuracy.

**Repeatability**
The relative standard deviations (RSDs) of cyprodinil, CGA249287 and CGA275535 values at each fortification level and overall for the water samples tested during method validation were <20% and therefore according to the EU guidance (see guidance section of this summary) demonstrate the method has satisfactory repeatability.

**Limit of Quantification**
The LOQ for cyprodinil, CGA249287 and CGA275535 residues in water using method GRM010.07A was established at 0.05 µg/L. No interfering peaks around the retention time of cyprodinil, CGA249287 and CGA275535 were found in any of the control samples at levels above 30% of the LOQ.

**Matrix Extract**
No significant matrix effects (suppression or enhancement) were observed for cyprodinil, CGA249287 and CGA275535 in either of the water types used during the method validation and therefore, non matrix-matched linearity standards were used for quantification.

**Stability of Final Extracts**
The stability of the sample extracts fortified with a cyprodinil, CGA249287 and CGA275535 was checked after a storage period of 8 days at 2-8ºC against freshly prepared calibration standards. The results proved that cyprodinil, CGA249287 and CGA275535 residues in the stored fortified water samples were stable.

**Stability of Standard Solutions**
The stability of the stored working standard solutions were checked against freshly prepared calibration standards after a storage period of 23 days at 2-8ºC for cyprodinil and CGA249287 and after a storage period of 37 days at 2-8ºC for CGA275535. The mean response values for stored and fresh solutions were within 10% of each other and the results demonstrated that cyprodinil, CGA249287 and CGA275535 residues were stable in the standard solutions.

**Conclusion**
Analytical method GRM010.07A has been demonstrated to be a reliable and accurate procedure for the determination of cyprodinil, CGA249287 and CGA275535 in water to a limit of quantification of 0.05 µg/L, using commercially available laboratory equipment and reagents.

### A 2.1.2.5.1.2 Confirmatory method

No confirmatory method is required, because the method was validated at two mass transitions (primary and confirmatory).

(Allen L, Brooks S, Crook S, 2015 and Allen L, 2015)

### A 2.1.2.5.1.3 Independent laboratory validation

| | |
|---|---|
| Comments of zRMS: | The independent validation of the XXXX method GRM010.07A for determination cyprodinil and its metabolites in drinking water has been accepted.<br><br>Final determination by LC-MS/MS with two transitions was performed: for cyprodinil **CGA219417** primary transition *m/z* 226→93 confirmatory *m/z* 226→108; for **CGA249287** [4-cyclopropyl-6-methyl-pyrimidin-2-yl amine] primary transition *m/z* 150→118 confirmatory *m/z* 150→133; for **CGA275535** [3-(4-cyclopropyl-6-methyl-pyrimidin-2-ylamino)- phenol] primary transition *m/z* 242→93 confirmatory *m/z* 242→108.<br><br>The LOQ of the method was confirmed in waters tested at 0.05 µg/L for cyprodinil and its metabolites CGA249287 and CGA275535. The mean recoveries at LOQ level for cyprodinil (CGA219417) were 76 % ± 5 % (primary ion), 76 % ± 5 % (confirmatory ion), for CGA249287 were 76 % ± 12 % (primary ion), 78 % ± 20 % (confirmatory ion) and for CGA275535 were 109 % ± 7 % (primary ion), 109 % ± 6 % (confirmatory ion). At 0.50 µg/L (10 times the LOQ) recovery values were also between 70% and 110% with a relative standard deviation of ≤ 20% for all waters and analytes tested.<br><br>The method GRM010.07A is valid to quantitatively determine residues of cyprodinil and its metabolites CGA249287 and CGA275535 in drinking water. |

| | |
|---|---|
| Reference: | KCP 5.2.5 |
| Report | Cyprodinil - Independent Laboratory Validation of Analytical Method GRM010.07A for the Determination of Cyprodinil (CGA219417) and its Metabolites CGA249287 and CGA275535 in Water. Kotthoff (2015), Report No. SYN-036/6-22. XXXX File No. VV-412795 |
| Guideline(s): | Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/825/00 rev. 8.1, 16/11/2010).<br>Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/3029/99 rev. 4, 11/07/2000) |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | |

**Principle of the Method**

Cyprodinil, CGA249287 and CGA275535 are extracted from water by solid phase extraction and analysed by high performance liquid chromatography with triple quadrupole mass spectrometric detection (LC-MS/MS).

**Recovery Findings**
Analytical method GRM010.07A was independent laboratory validated on drinking water samples by fortifying at the limit of quantification (LOQ) of the method (0.05 µg/L) and at 10 x LOQ (0.5 µg/L).
The recoveries obtained for cyprodinil, CGA249287 and CGA275535 are presented in **Błąd! Nie można odnaleźć źródła odwołania.** to **Błąd! Nie można odnaleźć źródła odwołania.**, respectively.

**Table A 79: Recovery Results Obtained During Independent Laboratory Validation of Method GRM010.07A for Cyprodinil in Drinking Water**

| Matrix | Analyte (Ion Transition) | Fortification Level (µg/L)* | Number of Analysis (n) | Mean Recovery (%) | RSD (%) | Recovery Range (%) |
|---|---|---|---|---|---|---|
| Drinking water | Cyprodinil (Primary transition; *m/z* 226 → 93) | 0.05 | 4* | 76 | 5 | 71 - 80 |
| | | 0.5 | 5 | 73 | 8 | 68 - 81 |
| | | Overall | 10 | 74 | 7 | 66 - 81 |
| Drinking water | Cyprodinil (Confirmatory transition; *m/z* 226 → 108) | 0.05 | 4* | 76 | 6 | 71 - 80 |
| | | 0.5 | 5 | 70 | 7 | 65 - 77 |
| | | Overall | 10 | 73 | 7 | 65 - 80 |

*One of the replicates was an outlier according to the Grubbs test.

**Table A 80: Recovery Results Obtained During Independent Laboratory Validation of Method GRM010.07A for CGA249287 in Drinking Water**

| Matrix | Analyte (Ion Transition) | Fortification Level (µg/L)* | Number of Analysis (n) | Mean Recovery (%) | RSD (%) | Recovery Range (%) |
|---|---|---|---|---|---|---|
| Drinking water | CGA249287 (Primary transition; m/z 150 → 118) | 0.05 | 5 | 76 | 12 | 69 – 91 |
| | | 0.5 | 5 | 101 | 15 | 78 – 114 |
| | | Overall | 10 | 88 | 20 | 69 - 114 |
| Drinking water | CGA249287 (Confirmatory transition; m/z 150 → 133) | 0.05 | 5 | 78 | 20 | 63 – 101 |
| | | 0.5 | 5 | 96 | 19 | 77 – 113 |
| | | Overall | 10 | 87 | 21 | 63 - 113 |

**Table A 81: Recovery Results Obtained During Independent Laboratory Validation of Method GRM010.07A for CGA275535 in Drinking Water**

| Matrix | Analyte (Ion Transition) | Fortification Level (µg/L)* | Number of Analysis (n) | Mean Recovery (%) | RSD (%) | Recovery Range (%) |
|---|---|---|---|---|---|---|
| Drinking water | CGA275535 (Primary transition; m/z 242 → 93) | 0.05 | 5 | 109 | 7 | 98 – 116 |
| | | 0.5 | 5 | 83 | 14 | 66 – 95 |
| | | Overall | 10 | 96 | 17 | 66 - 116 |
| Drinking water | CGA275535 (Confirmatory transition; m/z 242 → 108) | 0.05 | 5 | 109 | 6 | 99 – 116 |
| | | 0.5 | 5 | 81 | 14 | 65 - 92 |
| | | Overall | 10 | 95 | 18 | 65 - 116 |

**Specificity**

LC-MS/MS with two transitions is considered to be a highly specific detection technique and therefore according to the guidance (see guidance section of this summary) no further confirmatory technique is required. No significant interferences, above 30% of the LOQ, arising from the drinking water matrix, the labware, reagents or solvents have been observed at the retention times of interest.

**Linearity**

The linearity of the LC-MS/MS detector response was tested using both non-matrix calibration standard solutions and matrix-matched standard solutions over the range 0.1 µg/L to 20.0 µg/L (equivalent to 1.0 pg to 200 pg of analyte injected on to the column, based on a 10 µL injection). Standards at twenty one different concentrations were injected and the signal area plotted against concentration for all calibration

points. Straight lines with correlation coefficients ranging from 0.9974 to 0.9987 were obtained for cyprodinil (CGA219417) and its metabolites CGA249287 and CGA275535 in drinking water.

**Accuracy**
The mean cyprodinil, CGA249287 and CGA275535 recoveries, for both the primary and confirmatory ion transitions, at each fortification level and overall for the drinking water matrix tested during independent laboratory method validation were between 70% and 109%. These values are all at or between 70% and 110% and therefore according to the guidance (see guidance section of this summary) demonstrate the method has satisfactory accuracy.

**Repeatability**
The relative standard deviations (RSDs) of the cyprodinil, CGA249287 and CGA275535 recoveries, for both the primary and confirmatory ion transitions, at each fortification level and overall for the drinking water matrix tested during independent laboratory validation were between 5% and 21%. These values are all below or equal to 20% except for the overall RSD for the CGA249287 confirmatory transistion. For the CGA249287 confirmatory transistion, the individual fortification levels produced RSDs of 19 % and 20 %. These results, according to the guidance (see guidance section of this summary), demonstrate the method has satisfactory repeatability.

**Limit of Quantification**
The LOQ for cyprodinil, CGA249287 and CGA275535 in drinking water using method GRM010.07A was confirmed at 0.05 µg/L in the independent laboratory validation. No interfering peaks around the retention times of cyprodinil, CGA249287 and CGA275535 in drinking water were found in any of the control samples at levels above 30% of the LOQ.

**Matrix Extract**
The effect of the drinking water matrix on the LC-MS/MS response was assessed by preparing standards in the presence of the drinking water matrix and comparing the peak areas of cyprodinil, CGA249287 and CGA275535 against non-matrix standards at an equivalent concentration. No significant enhancement or suppression of the detector response was observed in the presence of the drinking water matrix tested. Therefore non matrix-matched calibration standards should generally be used for quantification. Non-matrix matched standards were used for the independent laboratory validation study.

**Conclusion**
Analytical method GRM010.07A has been demonstrated to be a reliable and accurate procedure for the determination of cyprodinil (CGA219417) and its metabolites CGA249287 and CGA275535 in drinking water to a limit of quantification of 0.05 µg/L, using commercially available laboratory equipment and reagents, in an independent laboratory validation study.

(Kotthoff M, 2015)

## A 2.1.2.6 Description of Methods for the Analysis of Air (KCP 5.2.6)

### A 2.1.2.6.1 Analytical method GRM010.09A

#### A 2.1.2.6.1.1 Method validation

| | |
|---|---|
| Comments of zRMS: | The validation is acceptable.<br>A defined volume of air was drawn through a sorbent tube. The different layers of the tube were separated (front and back separately) and cyprodinil was extracted on ultrasonic bath. Then the obtained sample was determined by LC-MS/MS. Two transitions were used: primary 226.2 → 93.1 and confirmatory 226.2 → 76.9. The limit of quantification of the method was set at 0.5 µg/m$^3$ (i.e. 0.09 µg/tube). Recovery values were between 70% and 110% with a relative standard deviation of ≤20%. This procedure has been demonstrated to be a reliable and accurate procedure for the determination of cyprodinil residues in air. |

| | |
|---|---|
| Reference: | KCP 5.2.6 |
| Report | Cyprodinil – Residue Method GRM010.09A for the Determination of Cyprodinil (CGA219417) in Air by LC-MS/MS. XXXX Analytical Method GRM010.09A. CEM Analytical Services Ltd (CEMAS), Imperial House, Oaklands Business Centre, Oaklands Park, Wokingham, Berkshire, RG41 2FD UK. Edwards & Wiltshire (2015), XXXX File No. VV-128327<br>Validation of Draft Residue Method GRM010.09A for the Determination of Cyprodinil (CGA219417) in Air by LC-MS/MS. CEMAS Report Number CEMR-6992-REG. CEM Analytical Services Ltd (CEMAS), Imperial House, Oaklands Business Centre, Oaklands Park, Wokingham, Berkshire, RG41 2FD UK. Wiltshire (2015), XXXX File No. VV-411794 |
| Guideline(s): | Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/825/00 rev. 8.1 (2010)).<br>Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/3029/99 rev. 4 (2000)). |
| Deviations: | No |
| GLP: | Method : No<br>Validation: Yes |
| Acceptability: | |

**Principle of the Method**
In summary, the contents of adsorbent air tubes were transferred to 12 mL glass specimen tubes and extracted with acetonitrile (2 × 5 mL portions). The total volume was adjusted to 20 mL with HPLC water,

and an aliquot was taken, ready for analysis by high performance liquid chromatography with triple quadrupole mass spectrometric detection (LC-MS/MS) for cyprodinil. The limit of quantification (LOQ) of the method is 0.5 µg/m$^3$ (≡ 0.09 µg/tube) for cyprodinil.

**Recovery Findings**
Summaries of the results for cyprodinil are presented in **Błąd! Nie można odnaleźć źródła odwołania.** to **Błąd! Nie można odnaleźć źródła odwołania.**.

**Table A 82: Accuracy and precision results from validation of GRM010.09A for cyprodinil in air: primary transition *m*/z 226.2 → 93.1**

| Matrix | Fortification Level (µg/m$^3$) | Accuracy (%) | Number of Analysis (n) | Mean Accuracy (%) | RSD (%) | Accuracy Range (%) |
|---|---|---|---|---|---|---|
| Air (Front section of tube) | 0.5* | 90, 93, 95, 95 | 4 | 93 | 2.5 | 90-95 |
| | 5 | 82, 88, 96, 93, 92 | 5 | 90 | 6.0 | 82-96 |
| | Overall | - | 9 | 92 | 4.8 | 82-96 |

*Limit of quantitation, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ
% Mean and % RSD calculated using rounded values

**Table A 83: Accuracy and precision results from validation of GRM010.09A for cyprodinil in air: confirmatory transition m/z 226.2 → 76.9**

| Matrix | Fortification Level (µg/m$^3$) | Accuracy (%) | Number of Analysis (n) | Mean Accuracy (%) | RSD (%) | Accuracy Range (%) |
|---|---|---|---|---|---|---|
| Air (Front section of tube) | 0.5* | 90, 93, 93, 93 | 4 | 92 | 1.6 | 90-93 |
| | 5 | 82, 87, 96, 92, 90 | 5 | 89 | 5.9 | 82-96 |
| | Overall | - | 9 | 91 | 4.5 | 82-96 |

*Limit of quantitation, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ
% Mean and % RSD calculated using un-rounded values

**Table A 84: Breakthrough results from validation of GRM010.09A for cyprodinil in air: primary transition m/z 226.2 → 93.1**

| Matrix | Fortification Level (µg/m$^3$) | Breakthrough (%) | Number of Analysis (n) | Mean Accuracy (%) | Breakthrough Range (%) |
|---|---|---|---|---|---|
| Air (Rear section of tube) | 0.5* | 2, 1, 0, 0 | 4 | 1 | 0-2 |
| | 5 | 0, 0, 0, 0, 0 | 5 | 0 | 0-0 |
| | Overall | - | 9 | 0 | 0-2 |

*Limit of quantitation, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ
% Mean and % RSD calculated using rounded values

**Table A 85: Breakthrough results from validation of GRM010.09A for cyprodinil in air: confirmatory transition m/z 226.2 → 76.9**

| Matrix | Fortification Level (µg/m$^3$) | Breakthrough (%) | Number of Analysis (n) | Mean Accuracy (%) | Breakthrough Range (%) |
|---|---|---|---|---|---|
| Air (Rear section of tube) | 0.5* | 3, 1, 0, 0 | 4 | 1 | 0-3 |
| | 5 | 0, 0, 0, 0, 0 | 5 | 0 | 0-0 |
| | Overall | - | 9 | 0 | 0-3 |

*Limit of quantitation, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ
% Mean and % RSD calculated using un-rounded values

**Specificity**
LC-MS/MS with two transitions is considered to be a highly specific detection technique and therefore according to EU guidance (see guidance section of this summary) no further confirmatory technique is required. The method includes two MS/MS transitions, both of which have been validated. No significant interferences arising from the air matrix, the labware, reagents or solvents have been observed at the retention time of interest.

**Linearity**
The linearity of the LC-MS/MS detector was tested using both non-matrix calibration standard solutions (from 0.001 to 0.1 µg/mL). Standards at eight different concentrations were injected and the signal area plotted against concentration for all calibration points. Straight lines with correlation coefficients ranging from 0.9986 to 0.9990 were obtained for cyprodinil.

**Accuracy**
Fortified samples were analysed in quadruplet at the limit of quantification (LOQ) of 0.5 µg/m$^3$ (≡ 0.09 µg/tube) and in quintuplet at ten times the LOQ (5 µg/m$^3$ ≡ 0.9 µg/tube). Acceptable mean accuracy values of between 70% and 110% were found for both transitions on matrices tested and therefore according to EU guidance (see guidance section of this summary) demonstrate the method has satisfactory accuracy.

**Repeatability**
The relative standard deviations (RSDs) of cyprodinil accuracy values at each fortification level and overall for the air tube samples tested during method validation were <20% and therefore according to the EU guidance (see guidance section of this summary) demonstrate the method has satisfactory repeatability.

**Limit of Quantification**
The LOQ for cyprodinil residues in air using method GRM010.09A was established at 0.5 µg/m$^3$ (≡ 0.09 µg/tube). No interfering peaks around the retention time of cyprodinil were found in any of the control samples at levels above 30% of the LOQ.

**Matrix Effects**
No significant matrix effects (suppression or enhancement) were observed for cyprodinil in air during the method validation and therefore, non-matrix matched linearity standards were used for quantification.

**Stability of Final Extracts**
The stability of the sample extracts fortified with cyprodinil was checked after a storage period of 7 days at 2-8 °C against freshly prepared calibration standards. The results proved that cyprodinil residues in the stored fortified air samples were stable (the mean accuracy values were between 70% and 120%, with a RSD of ≤ 20% when re-analysed).

**Stability of Standard Solutions**
The stability of the stored standard solutions of cyprodinil was checked after a storage period of 140 days at 2-8 °C against freshly prepared standard solutions. The mean response values for stored and fresh solutions were within 20% of each other and the results demonstrated that cyprodinil was stable in the standard solutions.

**Breakthrough**
The mean of cyprodinil breakthrough residues at each fortification level and overall for the rear section of the air tube samples tested during method validation was <1 % of the fortification. The breakthrough was tested at a temperature of approximately 35 °C and a relative humidity of approximately 80 %.

**Conclusion**
Analytical method GRM010.09A has been demonstrated to be a reliable and accurate procedure for the determination of cyprodinil in air to a limit of quantification of 0.5 µg/$m^3$ (≡ 0.09 µg/tube), using commercially available laboratory equipment and reagents.

### A 2.1.2.6.1.2 Confirmatory method

No confirmatory method is required, because the method was validated at two mass transitions (primary and confirmatory).

(Wiltshire K, Edwards J, 2015 and Wiltshire K, 2015)

### A 2.1.2.7 Other Studies/ Information

No new or additional studies have been submitted.

## A 2.2 Analytical methods for the prothioconazole

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

#### A 2.2.1.1 Description of analytical methods for the determination of residues in support of environmental fate studies (KCP 5.1.2.1)

No new or additional studies have been submitted.

#### A 2.2.1.2 Description of analytical methods for the determination of residues in support of efficacy studies (KCP 5.1.2.2)

No new or additional studies have been submitted

#### A 2.2.1.3 Description of analytical methods for the determination of residues in support of toxicological studies (KCP 5.1.2.3)

No new or additional studies have been submitted

#### A 2.2.1.4 Description of analytical methods for the determination of residues in support of operator, worker, resident and bystander exposure studies (KCP 5.1.2.4)

No new or additional studies have been submitted

#### A 2.2.1.5 Description of analytical methods for the determination of residues in support of residues studies (KCP 5.1.2.5)

No new or additional studies have been submitted

#### A 2.2.1.6 Description of analytical methods for the determination of residues in support of ecotoxicological studies (KCP 5.1.2.6)

No new or additional studies have been submitted.

#### A 2.2.1.7 Description of analytical methods for the determination of residues in support of physical and chemical properties tests (KCP 5.1.2.7)

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 5.2.1)

No new or additional studies have been submitted

### A 2.2.2.2 Description of analytical methods for the determination of residues in animal matrices (KCP 5.2.2)

No new or additional studies have been submitted

### A 2.2.2.3 Description of Methods for the Analysis of Body Fluids and Tissues (KCP 5.2.3)

No new or additional studies have been submitted

## A 2.2.2.4 Description of Methods for the Analysis of Soil (KCP 5.2.4)

### A 2.2.2.4.1 Method 00610/M001

A new study describing a modification of the soil method 00610 for prothioconazole and prothiocoazole-desthio and prothioconazol-desmethyl has been submitted.

#### A 2.2.2.4.1.1 Method validation

Schramel, 2000, EU agreed

#### A 2.2.2.4.1.2 Confirmatory method

| | |
|---|---|
| Comments of zRMS: | The applicant did not provide the original study. However, since these data, as additional confirmation for already agreed validated methods, are not necessary for the requested approval of the product, the assessment has been omitted here as not necessary. |

| | |
|---|---|
| Reference: | KCP 5.2.4 |
| Report | Modification M001 of Method 00610 for the determination of JAU6476 and the metabolites JAU6476-desthio and JAU6476-S-methyl in soil by HPLC-MS/MS, Brumhard, 2005, report No 00610/M001, Document No. M-243729-01-1 |
| Guideline(s): | Yes<br>EC Guidance Document on Residue Analytical Methods, SANCO/825/00 rev.7 of March 17, 2004<br>BBA Guideline: Residue Analytical Methods for Post-Registration Control Purposes of July 21, 1998<br>Commission Directive 96/46/EC amending Council Directive 91/414/EEC of 16 July 1996 |
| Deviations: | Not specified |
| GLP: | Yes |
| Acceptability: | Yes |

**Materials and methods**

Objective of the study was to validate the method 00610 for prothioconazole (JAU 6476) and its metabolite prothioconazole-desthio (JAU 6476-desthio, M04) using a second MRM transition (second product ion / qualifier ion). The original method 00610 describes the determination of the active ingredient prothioconazole and its metabolites prothioconazole-S-methyl (JAU 6476-S-methyl (M01)) and prothioconazole-desthio (M04) in soil by HPLC-MS/MS and provides validation data for one MRM transition. This modification M001 was prepared to provide additional validation data for prothioconazole and its metabolite prothioconazole-desthio (M04) using a second MRM transition.

The first MRM transition of prothioconazole is the daughter ion with the mass 326.1 [JAU 6476 (m/z 326.1)] and the second MRM transition is the daughter ion with the mass 189.1 [JAU 6476 (m/z 189.1)]. For prothioconazole-desthio (M04) the first MRM transition is the daughter ion with m/z 70.2 [JAU 6476-desthio (m/z 70.2)] and the second MRM transition is the daughter ion with the m/z 125.0 [JAU 6476-desthio (m/z 125.0)].

Soil samples of 25 g are extracted with approximately 100 mL of a mixture of acetonitrile/ water/cysteine hydrochloride monohydrate on a mechanical shaker for 60 minutes and filtered. 35 mL of the filtered solution are transferred into a 50 mL volumetric flask. The ingredient and the metabolite are done with HPLC using MS/MS detection in the Multiple Reaction Monitoring mode. Possible matrix effects in the MS/MS-detector were eliminated by using matrix matches standard solutions.

**Results and discussions**

For all mass transitions the mass spectrometric detector showed linear response in the range of about 0.5 µg/L to 50 µg/L for prothioconazole and its metabolite prothioconazole-desthio (M04) with correlation coefficients ranging from 0.9997 to 0.9999.

The mean recoveries determined at a fortification level of 6 µg/kg were 105% for prothioconazole (m/z 326.1) (relative standard deviation (RSD) = 1.6%), 103% for prothioconazole (m/z 189.1) (RSD = 2.6%), 102% for prothioconazole-desthio (m/z 125.0) (RSD = 1.2%) and 104% for prothioconazole-desthio (m/z 70.2) (RSD =2.3%). The mean recoveries, determined at a fortification level of 60 µg/L were 104% for prothioconazole (m/z 326.1) (RSD = 2.7%), 103% for prothioconazole (m/z 189.1) (RSD = 2.6%), 101% for prothioconazole-desthio (m/z 125.0) (RSD = 3.0%) and 101% for prothioconazole-desthio (m/z 70.2) (RSD =2.9%).

The mean recoveries over all single values (6 and 60 µg/L) were 104% for prothioconazole (m/z 326.1) (RSD = 2.1%), 103% for prothioconazole (m/z 189.1) (RSD = 2.5%), 102% for prothioconazole-desthio (M04) (m/z 125.0) (RSD = 2.3%) and 102% for prothioconazole-desthio (m/z 70.2) (RSD =2.9%).

The blank values of all control samples were below 2.0 µg/kg for prothioconazole and prothioconazole-desthio (M04).

The limit of quantitation of the method is 6 µg/kg for prothioconazole and its metabolite prothioconazole-desthio (M04).

The limit of detection of the method is 2.0 µg/kg for both analytes.

**Table A 86: Recovery results from method validation of prothioconazole using the analytical method**

| Soil | Fortification level [µg/kg] | Single values [%] | | | | | Mean [%] | RSD [%] |
|---|---|---|---|---|---|---|---|---|
| | | **Daughter ion: m/z 326.1 Quantitation** | | | | | | |
| Höfchen | 6.01 | 103 | 105 | 105 | 103 | 107 | 105 | 1.6 |
| | 60.1 | 99.1 | 104 | 104 | 107 | 105 | 104 | 2.7 |
| *overall* | | | | | | | *104* | *2.1* |
| | | **Daughter ion: m/z 189.1 Comfirmation** | | | | | | |
| Höfchen | 6.01 | 106 | 102 | 98.6 | 104 | 103 | 103 | 2.6 |
| | 60.1 | 98.6 | 103 | 103 | 105 | 106 | 103 | 2.6 |
| *overall* | | | | | | | *103* | *2.5* |

**Table A 87: Recovery results from method validation of prothioconazole-desthio (M04) using the analytical method**

| Soil | Fortification level [µg/kg] | Single values [%] | | | | | Mean [%] | RSD [%] |
|---|---|---|---|---|---|---|---|---|
| | | **Daughter ion: m/z 125.01 Quantitation** | | | | | | |
| Höfchen | 6.01 | 103 | 102 | 104 | 101 | 102 | 102 | 1.2 |
| | 60.1 | 97.8 | 103 | 97.4 | 104 | 102 | 101 | 3.0 |
| *overall* | | | | | | | *102* | *2.3* |
| | | **Daughter ion: m/z 70.2 Comfirmation** | | | | | | |
| Höfchen | 6.01 | 102 | 106 | 106 | 101 | 105 | 104 | 2.3 |
| | 60.1 | 97.2 | 104 | 98.9 | 103 | 101 | 101 | 2.9 |
| *overall* | | | | | | | **102** | **2.9** |

**Table A 88: Characteristics for the analytical method used for validation of prothioconazole and prothioconazole-desthio residues in soil**

| | |
|---|---|
| Specificity | The blank values of all control samples were below 2.0 µg/kg for prothioconazole and JAU 6476-desthio (M04) (<30% of LOQ). |
| Linearity | The linearity of the detector response was confirmed by solvent (3 concentrations) and matrix matched standard solutions (6 concentrations). For all mass transitions, the mass spectrometric detector showed linear response in the range of about 0.5 µg/L to 50 µg/L (corresponding to 3 µg/kg to 300 µg/kg) for prothioconazole and its metabolite JAU 6476-desthio *(M04)* with correlation coefficients ranging from 0.9997 to 0.9999. |
| Accuracy (recovery) | Mean recoveries for all analytes (prothioconazole and JAU 6476-desthio) at all fortification levels (LOQ and 10-fold LOQ) were well within the 70–120% range. The mean recoveries at each fortification for the matrices were between 101-105%. |
| Repeatability (precision) | The repeatability of the method was determined for all matrices by running five recoveries at concentrations at LOQ and 10xLOQ. The RSDs of the repeatability for each recovery set ranged from 1.2-3.0%. The results show good repeatability as all relative standard deviations were below 20%. |
| Limit of determination/quantification | The limit of quantitation of the method is 6 µg/kg for prothioconazole and JAU 6476-desthio *(M04).* |

**Conclusion**

Original method 00610 describes the determination of prothioconazole and its metabolites JAU 6476-desthio and JAU 6476-S-methyl in soil by HPLC-MS/MS and provides validation data for one MRM transition. This modification M001 provides additional validation data for prothioconazole and JAU 6476-desthio using a second MRM transition.

## A 2.2.2.5 Description of Methods for the Analysis of Water (KCP 5.2.5)

### A 2.2.2.5.1 Method 00684 (MR-184/04)

For confirmatory purposes, a method for the determination of residues of for prothioconazole (JAU 6476) and its metabolite prothioconazole-desthio (JAU 6476-desthio, M04) in water was validated to demonstrate

the use of a 2nd mass transition (modification M001 of method 00684 (MR-184/04)).

In addition, an analytical method for the determination of various pesticides (including prothioconazole and prothioconazole-desthio) in drinking water and surface water by HPLC-MS/MS and the corresponding independent laboratory validation (ILV) were delevoped (01387/M002 (MR-15/025 and 2015/0034/01, respectively).

### A 2.2.2.5.1.1 Method validation

Sommer, 2001, EU agreed

### A 2.2.2.5.1.2 Confirmatory method

| | |
|---|---|
| Comments of zRMS: | The applicant did not provide the original study. However, since these data, as additional confirmation for already agreed validated methods, are not necessary for the requested approval of the product, the assessment has been omitted here as not necessary. |

| | |
|---|---|
| Reference: | KCP 5.2.5 |
| Report | Modification M001 of Method 00684 for the determination of JAU6476 and the metabolites JAU6476-desthio and JAU6476-S-methyl in drinking and surface water by HPLC-MS/MS, Brumhard, 2005, report No 00684/M001, Document No. M-243734-01-1 |
| Guideline(s): | Yes<br>EC Guidance Document on Residue Analytical Methods, SANCO/825/00 rev.7 of March 17, 2004<br>BBA Guideline: Residue Analytical Methods for Post-Registration Control Purposes of July 21, 1998<br>Commission Directive 96/46/EC amending Council Directive 91/414/EEC of 16 July 1996 |
| Deviations: | Not specified |
| GLP: | Yes |
| Acceptability: | Yes/No/Supplementary |

**Materials and methods**

Objective of the study was to validate the method 00684 for prothioconazole (JAU 6476) and its metabolite prothioconazole-desthio (JAU 6476-desthio, M04) using a second MRM transition (second product ion / qualifier ion). The original method 00684 describes the determination of the active ingredient prothioconazole and its metabolites prothioconazole-S-methyl (JAU 6476-S-methyl (M01)) and prothioconazole-desthio (M04) in drinking and surface water by HPLC-MS/MS and provides validation data for one MRM transition. This modification M001 was prepared to provide additional validation data for prothioconazole and its metabolite prothioconazole-desthio (M04) using a second MRM transition.

Water samples are analysed by direct injectipn into a HPLC-MS/MS instrument after addition of acetic acid and cysteine hydrochloride monohydrate to achieve a final concentration of 50 mg/L cysteine hydrochloride

monohydrate and 0.1 mg/L of acetic acid. Because of the direct measurement of the samples, recovery rates cannot be calculated and these are peesnted belwo for completeness only.

The first MRM transition of prothioconazole is the daughter ion with the mass 326.1 [JAU 6476 (m/z 326.1)] and the second MRM transition is the daughter ion with the mass 189.1 [JAU 6476 (m/z 189.1)]. For prothioconazole-desthio (M04) the first MRM transition is the daughter ion with m/z 70.2 [JAU 6476-desthio (m/z 70.2)] and the second MRM transition is the daughter ion with the m/z 125.0 [JAU 6476-desthio (m/z 125.0)].

**Results**

**Table A 89: Recovery results from method validation of prothioconazole using the analytical method**

| Matrix | Fortification level [µg/kg] | Single values [%] | | | | | Mean [%] | RSD [%] |
|---|---|---|---|---|---|---|---|---|
| | | Daughter ion: m/z 326.1 Quantitation | | | | | | |
| Surface water | 0.05 | 96.8 | 101 | 96.8 | 96.7 | 91.9 | 96.7 | 3.4 |
| | 0.5 | 98.0 | 94.2 | 99.3 | 98.9 | 94.3 | 96.9 | 2.6 |
| | | | | | | | | |
| | | Daughter ion: m/z 189.1 Comfirmation | | | | | | |
| Surface water | 0.05 | 91.3 | 102 | 101 | 98.9 | 98.4 | 98.3 | 4.2 |
| | 0.5 | 97.5 | 97.0 | 101 | 100 | 100 | 99.0 | 1.6 |

**Table A 90: Recovery results from method validation of prothioconazole-desthio (M04) using the analytical method**

| Matrix | Fortification level [µg/kg] | Single values [%] | | | | | Mean [%] | RSD [%] |
|---|---|---|---|---|---|---|---|---|
| | | Daughter ion: m/z 125.01 Quantitation | | | | | | |
| Surface water | 0.05 | 93.5 | 91.1 | 100 | 97.1 | 96.3 | 95.6 | 3.6 |
| | 0.50 | 97.9 | 97.0 | 101 | 100 | 100 | 99.0 | 1.6 |
| | | | | | | | | |
| | | Daughter ion: m/z 70.2 Comfirmation | | | | | | |
| Surface water | 0.05 | 100 | 100 | 100 | 100 | 101 | 100 | 0.6 |
| | 0.50 | 97.0 | 97.4 | 100 | 101 | 101 | 99.4 | 2.0 |

**Table A 91: Characteristics for the analytical method used for validation of prothioconazole and prothioconazole-desthio residues in water**

| | |
|---|---|
| Specificity | Confirmation of identity was demonstrated by determining the recovery and precision for both MS/MS transitions. HPLC-MS/MS method is highly specific and an additional confirmatory method is not necessary. The blank values of all control samples were below 0.05 µg/L (<30% of LOQ). |
| Linearity | The linearity of the detector response was confirmed by standard solvent solutions at 6 concentrations, which is acceptable for aqueous samples analysed by direct injection. The MS/MS detection of prothioconazole is affected by the matrix - for both mass transitions, between 22-23% matrix effect was observed between the peak area in a surface water sample fortified at 0.5 µg/L and the corresponding peak area in milli-Q-water. No difference in the peak area was detected for JAU 6476-desthio. For all mass |

| | |
|---|---|
| Specificity | Confirmation of identity was demonstrated by determining the recovery and precision for both MS/MS transitions. HPLC-MS/MS method is highly specific and an additional confirmatory method is not necessary. The blank values of all control samples were below 0.05 µg/L (<30% of LOQ). |
| | transitions, the mass spectrometric detector showed linear response in the range of about 0.04 µg/L to 10.0 µg/L for prothioconazole and its metabolite JAU 6476-desthio *(M04)* with correlation coefficients ranging from 0.9992 to 0.9998. This is fit for purpose. |
| Accuracy (recovery) | Because of the direct measurement of the samples, recovery rates cannot be calculated and is presented for completeness only. |
| Repeatability (precision) | The repeatability of the method was determined for all matrices by running five recoveries at concentrations at LOQ and 10-fold LOQ. The RSDs of the repeatability for each recovery set ranged from 0.6-4.2%. The results show good repeatability as all relative standard deviations were below 20%. |
| Limit of determination/quantification | The limit of quantitation of the method is 0.05 µg/L for prothioconazole and the metabolite JAU 6476-desthio in surface water. |

**Conclusion**

A validation for drinking water was not necessary because the limit of quantitation for surface water is equal or below the drinking water limit of 0.1 µg/L. Method 00684/M001 has been sufficiently validated for the determination of prothioconazole and JAU 6476-desthio (M04) in drinking and surface water with a LOQ of 0.05 µg/L.

### A 2.2.2.5.1 Method 01387/M002

#### A 2.2.2.5.1.1 Method validation

| | |
|---|---|
| Comments of zRMS: | The applicant did not provide the original study. However, since these data, as additional confirmation for already agreed validated methods, are not necessary for the requested approval of the product, the assessment has been omitted here as not necessary. |

| | |
|---|---|
| Reference: | KCP 5.2.5 |
| Report | Modification M002 of analytical method 01387 for the determination of various pesticides in drinking and surface water by HPLC-MS/MS ,<br>Krebber and Sandau, 2015, report No MR-15/025, Document No. M-526061-01-1 |
| Guideline(s): | Yes<br>Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC<br>EC Guidance Document on Residue Analytical Methods, SANCO/825/00 rev. 8.1 of November 16, 2010<br>European Commission Guidance Document for Generating and Reporting Methods of Analysis in Support of Pre-Registration data Requirements for |

| | |
|---|---|
| | Annex II (part A, Section 4) and Annex III (part A, section 5) of directive 91/414, SANCO/3029/99 rev. 4, July 11, 2000 |
| Deviations: | Not specified |
| GLP: | Yes |
| Acceptability: | Yes/No/Supplementary |

**Materials and methods**

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

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

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

**Results**

**Table A 92: Method validation for prothioconazole**

| **Matrix** | **Fortification level [µg/kg]** | **Peak area (single values) [area counts]** | | | | | **Mean [%]** | **RSD [%]** |
|---|---|---|---|---|---|---|---|---|
| | | **Quantitation ion (m/z 344 → m/z 189)** | | | | | | |
| Surface water | 0.05 | 8645 | 8204 | 8566 | 8859 | 8723 | 8680 | 2.3 |
| | | 8741 | 8859 | 8691 | 8636 | 8859 | | |
| | 0.5 | 89774 | 85561 | 85395 | 85405 | 89321 | 87797 | 2.3 |
| | | 85820 | 89712 | 88393 | 89082 | 89505 | | |
| | | | | | | | | |
| | | **Comfirmation ion (m/z 344 → m/z 154)** | | | | | | |
| Surface water | 0.05 | 6790 | 6771 | 6958 | 6364 | 6920 | 6299 | 9.5 |
| | | 6207 | 6413 | 5472 | 5755 | 5336 | | |
| | 0.5 | 68113 | 67347 | 70861 | 76320 | 68686 | 69808 | 3.8 |
| | | 67232 | 69030 | 69063 | 70477 | 70946 | | |

**Table A 93: Method validation of prothioconazole-desthio (M04)**

| Matrix | Fortification level [µg/kg] | Peak area (single values) [area counts] | | | | | Mean [%] | RSD [%] |
|---|---|---|---|---|---|---|---|---|
| | | Quantitation ion (m/z 312 → m/z 70) | | | | | | |
| Surface water | 0.05 | 155867 | 151051 | 152289 | 148150 | 145810 | 151037 | 1.9 |
| | | 153369 | 151896 | 148989 | 151847 | 151105 | | |
| | 0.5 | 1511351 | 1514428 | 1556334 | 1524425 | 1533506 | 1522200 | 1.2 |
| | | 1500634 | 1523083 | 1542504 | 1506524 | 1509210 | | |
| | | | | | | | | |
| | | Comfirmation ion (m/z 312 → m/z 125) | | | | | | |
| Surface water | 0.05 | 94174 | 93527 | 92626 | 92165 | 91693 | 93164 | 1.6 |
| | | 92026 | 96571 | 93143 | 93830 | 91886 | | |
| | 0.5 | 950877 | 938876 | 949687 | 943186 | 921905 | 932259 | 1.6 |
| | | 916213 | 935352 | 938690 | 912477 | 915328 | | |

**Table A 94: Characteristics for the analytical method used for validation of prothioconazole and prothioconazole-desthio residues in water**

| | |
|---|---|
| Specificity | No signals/peaks interfering with the detection of the analytes were observed in solutions of untreated control specimens. The blank values of all control samples were below 0.05 µg/L (<30% of LOQ). Two MRM transitions were monitored for all analytes. HPLC-MS/MS method is highly specific and an additional confirmatory method is not necessary. |
| Limit of determination/quantification | The limit of quantitation (LOQ) is 0.05 µg/L for all analytes in surface water. |
| Linearity | Concentrations were quantified using external matrix-matched standard solutions. The correlation between the injected amount of substance and the detector response was linear (1/x weighted) for standard solutions in surface water (+ cysteine hydrochloride 50 mg/L) / formic acid / (1000 / 0.1, v/v) over at least 6 concentrations ranging from 0.015 µg/L to at least 1 µg/L for prothioconazole and ranging from 0.015 µg/L to 5 µg/L for JAU 6476-desthio. The correlation coefficients were ≥ 0.9990 and ≥ 0.9991 for these MRM transitions, respectively. |
| Accuracy (recovery) | Because of the direct measurement of the samples, recovery rates cannot be calculated and the corresponding peak areas are presented for completeness only. |
| Repeatability (precision) | The repeatability of the method was determined by running five surface water recoveries at concentrations at LOQ and 10-fold LOQ. The RSDs of the repeatability for each recovery set ranged from 1.2-9.5%. The results show good repeatability as all relative standard deviations were below 20%. |
| Storage stability of the analytes | JAU 6476-desthio was stable in surface water when stored in a freezer at ≤ -18°C for a period of 7 days. Prothioconazole can be stabilised by addition of cysteine hydrochloride. |
| Reproducibility (ILV) | An acceptable ILV was conducted; see Thies (2015); M-536990-01-1 below. |

**Conclusion**

A validation for drinking water was not necessary because the limit of quantitation for surface water is

equal or below the drinking water limit of 0.1 µg/L. Method 01387/M002 has been sufficiently validated for the determination of prothioconazole and JAU 6476-desthio (M04) in drinking and surface water with a LOQ of 0.05 µg/L.

### A 2.2.2.5.1.2 Confirmatory method

No confirmatory method is required, because the method was validated at two mass transitions (primary and confirmatory).

### A 2.2.2.5.1.1 Independent laboratory validation

| | |
|---|---|
| Comments of zRMS: | The applicant did not provide the original study. However, since these data, as additional confirmation for already agreed validated methods, are not necessary for the requested approval of the product, the assessment has been omitted here as not necessary. |

| | |
|---|---|
| Reference: | KCP 5.2.5 |
| Report | Independent laboratory validation of the BCS analytical method 01387/M002 for the determination of various pesticides in surface water by HPLC-MS/MS,<br>Thies, 2015, report No 2015/0034/01, Document No. M-536990-01-1 |
| Guideline(s): | Yes<br>Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC<br>European Commission Guidance Document for Generating and Reporting Methods of Analysis in Support of Pre-Registration data Requirements for Annex II (part A, Section 4) and Annex III (part A, section 5) of directive 91/414, SANCO/3029/99.<br>Guidance document on residue analytical methods; SANCO/825/00 rev. 8.1, European Commission, Directorate General Health and Consumer Protection; 2010-11-16.<br>OECD Guidance Document on Pesticide Residue analytical Methods; ENV/JM/Mono (2007); 2007-08-13 |
| Deviations: | Not specified |
| GLP: | Yes |
| Acceptability: | Yes |

**Materials and methods**

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

Water samples were determined by direct injection into the HPLC-MS/MS instrument using the positive ion mode for all analytes without further clean-up. Concentrations were quantified using external matrix-matched standard solutions. Because of the direct measurement of the samples, recovery rates cannot be calculated and the peak areas are presented below for completeness only.

**Results**

**Table A 95: Method validation for prothioconazole**

| Matrix | Fortification level [µg/kg] | Peak area (single values) [area counts] | | | | | Mean [%] | RSD [%] |
|---|---|---|---|---|---|---|---|---|
| | | Quantitation ion (m/z 344 Da→ m/z 189 Da) | | | | | | |
| Surface water | 0.05 | 7510 | 6130 | 7360 | 7310 | 7340 | 7130 | 7.9 |
| | 0.5 | 74700 | 62000 | 77300 | 75600 | 71800 | 72280 | 8.4 |
| | | | | | | | | |
| | | Comfirmation ion (m/z 344 Da → m/z 154 Da) | | | | | | |
| Surface water | 0.05 | 4010 | 5080 | 4750 | 5020 | 4430 | 4658 | 9.52.8 |
| | 0.5 | 56600 | 53400 | 56200 | 53800 | 53800 | 54760 | |

**Table A 96: Method validation of prothioconazole-desthio (M04)**

| Matrix | Fortification level [µg/kg] | Peak area (single values) [area counts] | | | | | Mean [%] | RSD [%] |
|---|---|---|---|---|---|---|---|---|
| | | Quantitation ion (m/z 312 Da → m/z 70) | | | | | | |
| Surface water | 0.05 | 71900 | 70300 | 59600 | 71700 | 73100 | 69320 | 8.0 |
| | 0.5 | 682000 | 691000 | 694000 | 690000 | 694000 | 690200 | 0.7 |
| | | | | | | | | |
| | | Comfirmation ion (m/z 312 Da → m/z 125 Da) | | | | | | |
| Surface water | 0.05 | 49600 | 53400 | 48500 | 53100 | 52300 | 51380 | 4.3 |
| | 0.5 | 606000 | 462000 | 523000 | 514000 | 481000 | 517200 | 11 |

**Table A 97: Characteristics for the analytical method used for validation of prothioconazole and prothioconazole-desthio residues in water**

| | |
|---|---|
| Specificity | Conformation of identity was demonstrated by determining the recovery and precision for both MS/MS transitions. HPLC-MS/MS methos is highly specific and an additonal confirmatory method is not necessary. Blank values of all control samples wer below 0.05 µg/L (<30% of LOQ). |
| Limit of determination/quantification | The limit of quantitation (LOQ) is 0.05 µg/L for all analytes in surface water. |
| Linearity | Concentrations were quantified using external matrix-matched standard solutions. The correlation between the injected amount of substance and the detector response was linear (1/x weighted) for standard solutions in surface water (+ cysteine hydrochloride for stabilisation of prothioconazole) over at least 5 concentration levels ranging from 0.015 µg/L to at least 1.0 µg/L for all analytes. Determined correlation coefficients for all analytes were ≥ 0.99 for both MRM transitions. |
| Accuracy (recovery) | Because of the direct measurement of the samples, recovery rates cannot be calculated and the corresponding peak areas are presented for completeness only. |
| Repeatability (precision) | The repeatability of the method was determined for all matrices by running five surface water recoveries at concentrations at LOQ and 10-fold LOQ. The repeatability for each recovery set ranged from 0.7-9.5%. The results show good repeatability as all relative standard deviations were below 20%. |

**Conclusion**

A validation for drinking water was not necessary because the limit of quantitation for surface water is equal or below the drinking water limit of 0.1 µg/L. The ILV confirms the LOQ for prothioconazole and JAU 6476-desthio (M04) is 0.05 µg/L in drinking and surface water.

## A 2.2.2.6 Description of Methods for the Analysis of Air (KCP 5.2.6)

### A 2.2.2.6.1 Method 00731/M001

For confirmatory purposes, the analytical method 00731/M001 for the determination of residues of prothioconazole-desthio (M04) in air was validated to demonstrate the use of a 2nd mass transition. This additional method was not reviewed for the EU review of the active substance, is summarised below and is considered to be adequate.

#### A 2.2.2.6.1.1 Method validation

Maasfeld, 2002, EU agreed

#### A 2.2.2.6.1.2 Confirmatory method

| | |
|---|---|
| Comments of zRMS: | The applicant did not provide the original study. However, since these data, as additional confirmation for already agreed validated methods, are not necessary for the requested approval of the product, the assessment has been omitted here as not necessary. |

| | |
|---|---|
| Reference: | KCP 5.2.6 |
| Report | Modification M001 of method 00731 for the determination of residues of JAU 6476-desthio (SXX 0665) in air by HPLC-MS/MS, Anft, T. and Bardel, P., 2005, report No 007321/M001, Document No. M-242870-01-1 |
| Guideline(s): | Not specified |
| Deviations: | Not specified |
| GLP: | Yes |
| Acceptability: | Yes |

**Materials and methods**

The analytical method 00731 demonstrated the determination of the prothioconazole metabolite JAU 6476-desthio (M04)) in air by HPLC-MS/MS. Its suitability was demonstrated by elution and desorption recoveries. The analytical method modification 00731/M001 presented here was validated for the deter-mination of the residues of JAU 6476-desthio in air by HPLC MS/MS using a second Multi Reaction Monitoring (MRM 312-125) mode.

Method 00731/M001 follows the same analytical methodology as method 00731. As the procedure itself is not in question, the suitability of a 2nd MRM is therefore only demonstrated by extraction recoveries.

JAU 6476-desthio was added to the Tenax ® tubes. Then air was drawn through the Tenax® tubes for 10 min with a rate of 2 L/min to remove the solvent. The adsorbed compound was then extracted by acetonitrile and its concentration was determined by LC MS/MS.

**Results**

**Table A 98: Recovery rates for prothioconazole-desthio**

| Test System | Fortification level [µg/kg] | Peak area (single values) [area counts] | | | | | Mean [%] | RSD [%] |
|---|---|---|---|---|---|---|---|---|
| | | Quantitation ion (m/z 312 → m/z 70) | | | | | | |
| Tenax® | 0.0003 | 104 | 101 | 99 | 100 | 97 | 100 | 2.6 |
| | 0.06 | 103 | 103 | 106 | 108 | 103 | 103 | 2.1 |
| | | | | | | | | |
| | | Comfirmation ion (m/z 312 → m/z 125) | | | | | | |
| Tenax® | 0.0003 | 106 | 104 | 102 | 103 | 99 | 103 | 2.5 |
| | 0.06 | 105 | 105 | 108 | 111 | 104 | 107 | 2.7 |

**Table A 99: Characteristics for the analytical method used for validation of prothioconazole-desthio residues in air**

| | |
|---|---|
| Specificity | Confirmation of identity was demonstrated by determining the recovery and precision for both MS/MS transitions. HPLC-MS/MS method is highly specific and an additional confirmatory method is not necessary. Apparent residues in control samples were all below 30% x LOQ. |
| Limit of determination/quantification | The limit of quantitation of the method is 0.0003 mg JAU 6476-desthio/m³ air. |
| Linearity | The linearity of the method was validated from 0.59 µg/L to 176 µg/L over 5 concentrations with correlation coefficients of 1.0000 for the MRM 312-70 and 0.99997 for MRM 312-125. |
| Accuracy (recovery) | Recovery rates were determined for five replicate samples of the matrices spiked with JAU 6476-desthio at 0.0003 and 0.06 mg/m³ air. The mean recoveries at each fortification level were between 100-107%. Results were within guideline requirements (mean recovery 70-120%). |
| Repeatability (precision) | The repeatability of the method was determined for all matrices by running five recoveries at concentrations at 0.0003 and 0.06 mg/m$^3$ in air. The RSDs of the repeatability for each recovery ranged from 2.1-2.7%. The results show good repeatability as all relative standard deviations were below 20%. |

**Conclusion**

Method modfification M001 (MR-003/02) validates the use of a 2$^{nd}$ MRM (312 – 125 amu) to measure JAU 6476-desthio in air by HPLC-MS/MS with a LOQ of 0.003 mg/m$^3$ in air.

## A 2.2.2.7 Other Studies/ Information

No new or additional studies have been submitted.