REGISTRATION REPORT

# Part B

# Section 5

# Analytical Methods

Detailed summary of the risk assessment

Product code: IN002B1760

Product name(s): Cymofil

Chemical active substance:

Cymoxanil, 450 g/kg

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(New authorisation)

Applicant: Indofil Industries (Netherlands) B.V.

Submission date: August 2022, updated May 2023, January 2024

MS Finalisation date: May 2023 (initial Core Assessment)

September 2023, updated February 2024 (Core Assessment following the commenting period - 1st tour)

April 2024, updated May 2024

(final Core Assessment following the commenting period - 2 nd tour)

# Version history

| When | What |
|---|---|
| August 2022 | Original version from applicant Indofil Industries (Netherlands) B.V. for submission to z-RMS, Poland, in the frame of the PPP Authorization according to Article 33 of Regulation (EC) No. 1107/2009 |
| May 2023 | Update according to Data Gap Clarification requests |
| May 2023 | Initial zRMS assessment<br>The report in the dRR format has been prepared by the Applicant, therefore all comments, additional evaluations and conclusions of the zRMS are presented in grey commenting boxes. Minor changes are introduced directly in the text and highlighted in grey. Not agreed or not relevant information are ~~struck through and shaded for transparency~~. |
| September 2023 | Core Assessment updated following the commenting period<br>Additional information/assessments included by the zRMS in the report in response to comments received from the cMS and the Applicant are highlighted in yellow. Information no longer relevant ~~is struck through and shaded~~. |
| January 2024 | Applicant update following the conclusion of a new toxicity test with IN002B1760 on green alga (highlighted in turquoise). |
| February 2024 | zRMS assessment after submission of the additional data by the applicant<br>The updated report has been prepared by the Applicant, therefore all comments, additional evaluations and conclusions of the zRMS are presented in grey commenting boxes. Minor changes are introduced directly in the text and highlighted in green. Not agreed or not relevant information are ~~struck through~~ and shaded for transparency. |
| April 2024 | Core Assessment updated following the commenting period 2 nd tour).<br>Additional information/assessments and new study for algae (submitted on February 2024) included by the zRMS in the report in response to comments received from the cMS and the Applicant are highlighted in purple. Information no longer relevant ~~is struck through~~ and shaded. |
| May 2024 | Final report (National Assessment updated after the correction of Appendix 4 prepared by the Applicant)<br>In order to facilitate tracking of changes in the Lists of data considered for national authori-zation (Appendix 4), amendments are highlighted in blue, while not agreed use pattern ~~is struck through~~ and shaded. |

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# 5 Analytical methods

This document aims to apply for the registration of a new product IN002B1760 on potato in field according to the conditions of use described in the GAP. Some analytical methods have already been evaluated and accepted and in this case, only a reference to the provided study is submitted. Moreover, considering that current authorized conditions are not changed, it is considered not necessary to submit any additional documentation or information in some parts of this section.

## 5.1 Conclusion and summary of assessment

Sufficiently sensitive and selective analytical methods are available for the active substance and relevant impurities in the plant protection product.
Noticed data gaps are: none

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

- ILV for drinking or ground water,
- fully validated method for the determination of cymoxanil in body fluids and tissues,
- new analytical method for the determination of residues of cymoxanil in soil,
- analytical methods for monitoring/enforcement purposes for food and feed of plant origin required for all matrix types with LOQ of 0.01* mg/kg.
-

zRMS-PL considers that these data gaps are anticipated to be addressed at active substance level in context with the renewal of cymoxanil and will be subject of the art.43 re-authorisation process for the product.

| Commodity/crop | Supported/ Not supported |
|---|---|
| ~~Grape~~ | |
| Potato | supported |
| ~~Tomato (field and glass house)~~ | |
| ~~Aubergine (field and glass house)~~ | |

## 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)

Analytical methods for determination of Cymoxanil in IN002B1760 were not already been evaluated at EU level for the registration of the IN002B1760 (cymoxanil 450 g/kg). An overview on the acceptable method is provided as follows:

| | |
|---|---|
| Comments of zRMS: | The method is sufficiently described and validated according to SANCO/3030/99 rev. 5 (22 March 2019) and is suitable for the determination of active substance in a plant protection product. |

| | |
|---|---|
| Reference: | KCP 5.1.1/01 |
| Report | Cymoxanil 45 WG (IN 002B1760): Validation of the Analytical Method for the Determination of Active Ingredient Content<br>Rigamonti E., 2021<br>Report: **CH-0526/2021**<br>Testing facility: ChemService S.r.l. Controlli e Ricerche |
| Guideline(s): | Yes.<br>SANCO/3030/99 rev. 5 dated 22nd March 2019 |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes |

**Materials and methods**
The content of Cymoxanil in the formulated is analysed by HPLC-UV/DAD analytical method validated according to SANCO 3030/99 rev. 5 guidance documents.

In order to demonstrate the validity of the analytical method, the following validity criteria have to be respected:

- Specificity / Interference: for a.s. interference not >3% of total peak area for target analyte.

- Linearity: Calibration appropriate to the nominal concentration range at least ± 20% of relevant analytical solutions
• duplicate determinations at 3 concentrations, or
• single determinations at 5 concentrations.

- Repeatability (Precision):
Minimum of 5 replicate sample determinations.
Acceptability criteria:
Hr ≤ 1, acceptable
1 < Hr ≤ 2, acceptable in case of a suggested explanation
Hr > 2, not acceptable
Hr = Horrat value

-Recovery (Trueness):
At least 2 independent recovery determination (two weights).

Each recovery value considering the active ingredient content:

70 - 130 % for content < 0.01 % w/w;
75 - 125 % for content ≥ 0.01 % w/w and < 0.1 % w/w;
80 - 120 % for content ≥ 0.1 % w/w and < 1 % w/w;
90 - 110 % for content ≥ 1 % w/w and < 10 % w/w;
97 - 103 % for content ≥ 10 % w/w.

Chromatographic conditions

| | | |
|---|---|---|
| HPLC column | | |
| Phenomenex or equivalent | : | Luna C18, 25 cm x 4.6 mm, 5 µm |
| Detector | : | 230 nm |
| Column temperature | : | 25°C |
| Eluent A | : | Water |
| Eluent B | : | Acetonitrile |
| Eluent D | : | Phosphoric acid at 10 % v/v |
| Gradient | : | From A:B:D 70:20:10 to A:B:D 10:80:10 in 25 minutes<br>A:B:D 10:80:10 for 5 minutes |
| Eluent flow | : | 1.0 mL/min |
| Volume of injection | : | 10 µL |
| R. T. Cymoxanil | : | about 11.3 minutes |
| R. T. Dimethyl phthalate | : | about 15.1 minutes |
| Total Analysis Time | : | 30 minutes |
| Post time | : | 5 minutes |

Active ingredient content
The content of Cymoxanil was determined according to the HPLC-UV/DAD analytical method validated. The Cymoxanil content in the test item stored at 20 ± 2 °C was obtained from the precision data of the method validation. Five determinations were performed using the same procedure, for the test item stored two weeks at 54 ± 2 °C.

**Description of the method validation**
Specificity
The specificity test was conducted injecting, in the adjusted chromatographic conditions, the following samples, comparing the chromatograms in order to check possible cross contaminations.

| **Injected solution samples** | **Nominal injected Concentration (µg/mL)** |
|---|---|
| Solvent wash (acetonitrile) | - |
| Cymoxanil reference material | 50 |
| Cymoxanil test substance | 50 |
| Dimethyl phthalate internal standard | 50 |
| Test item | 50 |
| Placebo | - |
| Placebo with Cymoxanil test substance | 50 |
| Fortified sample solution | 50 |

Linearity
Linear regression analysis was performed using the least squares method.
The correlation coefficient was calculated using regression analysis.

Preparation of the stock reference material solution and stock internal standard solution

Using the analytical balance and volumetric flasks, stock reference material solution and stock internal standard solution were prepared as follows:

| Stock solutions | Weight (mg) | Purity (%) | Solvent | Total volume (mL) | Concentration (µg/mL) |
|---|---|---|---|---|---|
| Cymoxanil | 20.4 | 100.0 | Acetonitrile | 20.00 | 1020.0 |
| Dimethyl phthalate | 20.9 | - | Acetonitrile | 10.00 | 2090.0 |

Preparation of the working standard solutions

Using volumetric flasks and volumetric pipettes, five working standard solutions for linear calibration were prepared as follows:

| Working Standard Solution | Stock Cymoxanil reference material solution (mL) | Stock Dimethyl phthalate internal standard solution (mL) | Solvent | Final Volume (mL) |
|---|---|---|---|---|
| WSS 1 | 0.60 | 0.50 | Acetonitrile | 20.00 |
| WSS 2 | 0.80 | 0.50 | Acetonitrile | 20.00 |
| WSS 3 | 1.00 | 0.50 | Acetonitrile | 20.00 |
| WSS 4 | 1.20 | 0.50 | Acetonitrile | 20.00 |
| WSS 5 | 1.40 | 0.50 | Acetonitrile | 20.00 |

| Working Standard Solution | Cymoxanil concentration (µg/mL) | Dimethyl phthalate concentration (µg/mL) |
|---|---|---|
| WSS 1 | 30.60 | 52.25 |
| WSS 2 | 40.80 | 52.25 |
| WSS 3 | 51.00 | 52.25 |
| WSS 4 | 61.20 | 52.25 |
| WSS 5 | 71.40 | 52.25 |

After the injection of the working standard solutions, from the lowest to the highest concentration, a solvent wash was also injected to verify if memory peaks were detected.

Repeatability (Precision)
The precision was assessed as follows: five solutions of the test item (labelled from A to E) were prepared and injected as described in Internal Analytical Method No. 0526/2021.

Precision (repeatability) of the analytical method was assessed with the data obtained.

Preparation of the standard solutions (in duplicate)

Using the analytical balance, the standard solutions in duplicate were prepared as follows:

| Reference Material or Internal Standard | Weight (mg) | Volume (mL) | Solvent | Dilution | Solvent |
|---|---|---|---|---|---|
| Cymoxanil | 25 | 25.0 | Acetonitrile | 1:20 | Acetonitrile |
| Dimethyl phthalate | 25 | | | | |

Precision of the analytical method was assessed with the data obtained.

Recovery (Trueness**)**

The test was performed by spiking two aliquots of the Placebo with the Cymoxanil test substance at one level, corresponding to additions of 100 % of the nominal concentration of active ingredient.

The standard solutions are the same already prepared for Repeatability.

Preparation of the fortified placebo solutions

| | Spike A (~ 100 %) | Spike B (~ 100 %) |
|---|---|---|
| Placebo (mg) | 252.8 | 252.6 |
| Cymoxanil Test Substance (mg) | 200.7 | 201.0 |
| Total weight (mg) (*) | 453.5 | 453.6 |
| Dimethyl phthalate Internal standard (mg) | 203.9 | 203.4 |

(*) Sum of Placebo and Test substance

The fortified samples were treated and analyzed as described for the test item in Internal Analytical Method No. 0526/2021.

Recovery of the analytical method was assessed with the data obtained.

**Validation - Results and discussions**

**Table 5.2-1: Methods suitable for the determination of active substance Acetamiprid in plant protection product IN002B1760**

| | **Cymoxanil** | **Validity Criteria** |
|---|---|---|
| **Author(s), year** | Rigamonti E., 2021 | |
| **Principle of method** | HPLC-UV/DAD analytical method validated according to SANCO/3030/99 rev.5 | |
| **Linearity (linear between mg/L / % range of the declared content) (correlation coefficient, expressed as r)** | Linearity 26.0 – 60.7% w/w Cymoxanil (from 30.60 µg/mL to 71.40 µg/mL)<br><br>Y = 1.00110 x + 0.03298<br>r = 0.99965 | Correlation coefficient r > 0.99 |
| **Precision – Repeatability Mean n = 5 (%RSD)** | RSD = 0.40 %<br>RSDr = 1.51%<br>Hr = 0.27 | Hr ≤ 1 |
| **Accuracy n = 2 for each fortification level (total n = 4) (% Recovery)** | Accuracy (Recovery) A: 101.31 %<br>Accuracy (Recovery) B: 100.94 % | 97 – 103 % |
| **Interference/ Specificity** | For a.s. interference not >3% of total peak area for target analyte. | Interference < 3 % |
| **Comment** | | |

**Conclusion**

According to the results, the HPLC/UV-DAD analytical method for the determination of cymoxanil in the test item IN002B1760 was successfully validated, according to SANCO/3030/99 rev. 5 (22 March 2019) guidance document.

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

The technical material contains no relevant impurities. No method was required for the registration of the product IN002B1760.

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

There are no formulants of toxicological, ecotoxicological or environmental concern used in the preparation.

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

A CIPAC analytical method high performance liquid chromatography on a reversed phase column (C8) using acetonitrile + water (25+75 v/v) at pH 2.8 as eluent, UV detection at 254 nm and internal standardisation. The method is available for the determination of cymoxanil in the TC, WP and WG-formulations (CIPAC handbook J, method 419).

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

An overview on the acceptable methods and possible data gaps for analysis of residues of Cymoxanil for the generation of pre-authorization data is given in the following table. For the detailed evaluation of new studies please refer to Appendix 2.

**Table 5.2-2: Validated methods for the generation of pre-authorization data**

| **Component of residue definition: Cymoxanil** | | | | |
|---|---|---|---|---|
| **Matrix type** | **Method type** | **Method LOQ** | **Principle of method (i.e. GC-MS or HPLC-UV)** | **Author(s), year / missing / EU agreed** |
| **Plants,** plant products,... (Residues) | Primary and confirmatory | 0.04 mg/kg potato, grapes, tomato, hopes cones, post-cured tobacco | DFG S-19 modified GC NPD | Kretschmer S., Class T. 1999 EU Agreed |
| | ILV | 0.04 mg/kg potato, grapes, tomato 0.1 mg/kg tobacco 0.33 mg/kg hops | DFG S-19 modified GC NPD (confirmed GC-MSD) | Linkerhägner M. 1999 EU Agreed |
| | Primary | 0.05 mg/kg lettuce | DFG-513, GC NPD | Freschi G., 2001a EU Agreed |
| | Primary | 0.05 mg/kg potato | DFG-513, GC NPD | Freschi G., 2001b EU Agreed |
| | Primary | 0.05 mg/kg potato, grapes, tomato, lettuce | GC NPD | Wasser C., 2002 EU Agreed |
| | Confirmatory | | RP-HPLC-UV | |
| | Primary and confirmatory | 0.01 mg/kg potato | LC-MS/MS | **Semrau J. 2010 [KCP 5.1.2_06]** |
| | Primary and confirmatory | 0.01 mg/kg tomato | HPLC-MS/MS | **Sala A. 2021a [KCP 5.1.2_01]** |
| | Primary and | 0.01 mg/kg | HPLC-MS/MS | **Sala A. 2021b** |

| Component of residue definition: Cymoxanil | | | | |
|---|---|---|---|---|
| **Matrix type** | **Method type** | **Method LOQ** | **Principle of method (i.e. GC-MS or HPLC-UV)** | **Author(s), year / missing / EU agreed** |
| | confirmatory | tomato | | **[KCP 5.1.2_02]** |
| **Animal products**, food of animal origin,... (Residues) | Primary | -- | -- | No residue definition is proposed, therefore no analytical method is required |
| **Soil, water, sediment**,... (Environmental fate) | Primary | 0.01 mg/kg (soil) | HPLC-UV | Melkebeke T., 2000a EU Agreed |
| | Confirmatory | | HPLC-DAD | |
| | Primary and confirmatory | 0.10 µg/l (water) | HPLC-UV | Cabusas M.E.Y. 1999 EU Agreed |
| **Soil, water**,... (Efficacy) | Primary | -- | -- | -- |
| | Confirmatory | -- | -- | -- |
| **Feed, body fluids**,... (Toxicology) | Primary | -- | -- | Cymoxanil is not classified as toxic or highly toxic, therefore no analytical method is required |
| Fresh water Algae (Ecotoxicology) | Primary | 0.1 mg/ml | HPLC-DAD | **Venkanna, B. 2023a [KCP 5.1.2/06a]** |
| | Confirmatory | 0.1 mg/ml | HPLC-DAD | **Venkanna, B. 2023b [KCP 5.1.2/06b]** |
| **Air** | Primary | 0.46 µg/m$^3$ | HPLC-UV | Melkebeke T., 2000b EU Agreed |
| | Confirmatory | | HPLC-DAD | |

A description of the new methods set up to support ecotoxicological studies with the determination of cymoxanil in different matrices is here reported. For a detailed description of them please refer to Appendix 2.

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

| Component of residue definition: Cymoxanil | | | | |
|---|---|---|---|---|
| **Matrix type** | **Method type** | **Method LOQ** | **Principle of method** | **Author(s), year** |
| Stock solutions (Ecotoxicological tests) | Primary and confirmatory | 0.31 mg/L | HPLC/MS/MS | **D. Garagna, 2021a [KCP 5.1.2_03]** |
| Feeding solutions (Ecotoxicological tests) | Primary and confirmatory | 1.52 mg/L | HPLC/MS/MS | **D. Garagna, 2021b [KCP 5.1.2_04]** |
| Artificial soil (Ecotoxicological tests) | Primary and confirmatory | 0.052 mg/kg | HPLC/MS/MS | **D. Garagna, 2021c [KCP 5.1.2_05]** |

~~**Table 5.2-4: Validated methods for the generation of pre-authorization data – toxicology**~~

| ~~Component of residue definition: Cymoxanil~~ | | | | |
|---|---|---|---|---|
| ~~**Matrix type**~~ | ~~**Method type**~~ | ~~**Method LOQ**~~ | ~~**Principle of method**~~ | ~~**Author(s), year**~~ |
| ~~Grape plants~~ | ~~Primary and confirmatory~~ | ~~0.01 mg/L grapes~~ | ~~HPLC/HRMS/MS~~ | ~~**Sala A. 2021c [KCP 5.1.2_06]**~~ |
| ~~Tomato plants~~ | ~~Primary and confirmatory~~ | ~~0.01 mg/L tomato~~ | ~~HPLC/HRMS/MS~~ | ~~**Sala A. 2021d [KCP 5.1.2_07]**~~ |

## 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)

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

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

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

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

| Matrix | Residue definition | MRL / limit | Reference for MRL/level Remarks |
|---|---|---|---|
| Plant, high water content | Cymoxanil | 0.01 mg/kg | Reg. (EU) ~~832/2018~~ 2022/1363 |
| Plant, high acid content | | 0.01 mg/kg | Reg. (EU) ~~832/2018~~ 2022/1363 |
| Plant, high protein/high starch content (dry commodities) | | 0.01 mg/kg | Reg. (EU) ~~832/2018~~ 2022/1363 |
| Plant, high oil content | | 0.01 mg/kg | Reg. (EU) ~~832/2018~~ 2022/1363 |
| Muscle | Cymoxanil | 0.01 mg/kg | Reg. (EU) ~~832/2018~~ 2022/1363 |
| Milk | | 0.01 mg/kg | Reg. (EU) ~~832/2018~~ 2022/1363 |
| Eggs | | 0.01 mg/kg | Reg. (EU) ~~832/2018~~ 2022/1363 |
| Fat | | 0.01 mg/kg | Reg. (EU) ~~832/2018~~ 2022/1363 |
| Liver, kidney | | 0.01 mg/kg | Reg. (EU) ~~832/2018~~ 2022/1363 |
| Soil (Ecotoxicology) | Cymoxanil | 0.01 mg/kg bw/d | $EC_{50}$ for Cymoxanil *EFSA Scientific Report (2008) 167, 1-116* |
| Drinking water (Human toxicology) | Cymoxanil and IN-KQ960 | 0.1 µg/L | general limit for drinking water SANTE/2020/12830, Rev.1 |
| Surface water (Ecotoxicology) | Cymoxanil and IN-KQ960 | 0.067 mg/L | NOEC (21 d) *EFSA Scientific Report (2008) 167, 1-116* |
| Air | Cymoxanil | 0.01 mg/kg bw/d | AOEL for Cymoxanil *EFSA Scientific Report (2008) 167, 1-116* |
| Tissue (meat or liver) | Cymoxanil | ~~Not required~~ 0.01 mg/kg | Not classified as T / T+ Requested under (EU) No 283/2013 |
| Body fluids | | ~~Not Required~~ 0.01 mg/L | Not classified as T / T+ Requested under (EU) No 283/2013 and SANTE/2020/12830, Rev.1 |

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

An overview on the acceptable methods and possible data gaps for analysis of **Cymoxanil** in plant matrices is given in the following tables. New studies are available for tomatoes whereas studies on potatoes were already evaluated by zRMS Greece in 2014 in the context of an application for the authorisation of a new product. For the detailed evaluation of new studies please refer to Appendix 2.

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

| Component of residue definition: Cymoxanil | | | | |
|---|---|---|---|---|
| **Matrix type** | **Method type** | **Method LOQ** | **Principle of method (i.e. GC-MS or HPLC-UV)** | **Author(s), year / missing / EU agreed** |
| High water content | Primary | 0.04 mg/kg (grape, potato, tomato, hops cones, post-cured tobacco) | DFG S19 GC NPD | Kretschmer S., Class T., 1999 EU agreed Report: DuPont 2158 |
| | ILV | 0.04 mg/kg (potato, grapes, tomato) 0.1 mg/kg (tobacco) 0.33 mg/kg (hops) | GC-NPD (Confirmed GC MSD) | Linkerhaegner M., 1999 EU agreed Report: DuPont 2946 |
| | Primary | 0.05 mg/kg (potato, grapes, tomato) | GC-NPD RP-HPLC (confirmatory) | Wasser C., 2002 EU agreed Report: Oxon A0087 |
| | Primary | 0.05 mg/kg (lettuce) | DFG 513 GC NPD | Freschi G., 2001a EU agreed Report: Oxon SIP1279 |
| | Primary and confirmatory | 0.01 mg/kg potato | LC-MS/MS | **Semrau J. 2010 [KCP 5.1.2_06]** |
| | Primary and confirmatory | 0.01 mg/kg (tomato) | HPLC-MS/MS | **Sala A. 2021a [KCP 5.1.2_01]** |
| | Primary and confirmatory | 0.01 mg/kg (tomato) | HPLC-MS/MS | **Sala A. 2021b [KCP 5.1.2_02]** |
| | ILV | 0.01 mg/kg (tomato) | HPLC-MS/MS | **Pardo Matinez M. [KCP 5.2_01]** |
| High acid content | Primary | 0.04 mg/kg (grape, potato, tomato, hops cones, post-cured tobacco) | DFG S19 GC NPD | Kretschmer S., Class T., 1999 EU agreed Report: DuPont 2158 |
| | ILV | 0.04 mg/kg (potato, grapes, tomato) 0.1 mg/kg (tobacco) 0.33 mg/kg (hops) | GC-NPD (Confirmed GC MSD) | Linkerhaegner M., 1999 EU agreed Report: DuPont 2946 |
| | Primary | 0.05 mg/kg (potato, grapes, tomato) | GC-NPD RP-HPLC (confirmatory) | Wasser C., 2002 EU agreed Report: Oxon A0087 |
| High protein/high starch content (dry) | Primary | -- | -- | -- |
| Difficult | Primary | 0.04 mg/kg (grape, potato, tomato, hops cones, post-cured tobacco | DFG S19 GC NPD | Kretschmer S., Class T., 1999 EU agreed Report: DuPont 2158 |

For any special comments or remarkable points concerning the analytical methods for the determination

of residues in plant matrices, please refer to Appendix 2.

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

| | **Method for products of plant origin** |
|---|---|
| Not required, because: | Multiresidue methods are available for the extraction of this active substance. In compliance with SANTE/2020/12830 rev.1, it is not necessary to address extraction efficiency since there aren't matrix groups for which residues are ≥LOQ. |

**zRMS comments:**

According to the SANTE/2020/12830, Rev.1, 24. February 2021 potato belongs to high water content commodities analytical group.
According to the EFSA Journal 2015;13(12):4355 *In the framework of the peer review under Directive 91/414/EEC, a method using GC-NPD was sufficiently validated with a limit of quantification (LOQ) of 0.04 mg/kg for the determination of cymoxanil in commodities with high water and high acid content, and with an LOQ of 0.1 mg/kg for the determination of cymoxanil in hops. A confirmatory method using GC-NPD is available for the determination of cymoxanil residues in commodities with high water and high acid content but is still missing for hops (EFSA, 2008).*
*Furthermore, the multi-residue QuEChERS method is also applicable for the determination of cymoxanil. The LC-MS/MS analyses cymoxanil residues in high water, high acid content and dry commodities with an LOQ of 0.01 mg/kg (CEN, 2008).*
*In addition, the RMS has evaluated the DFG S19 method for enforcement of cymoxanil in high oil content commodities. The HPLC-MS/MS was validated for enforcement of cymoxanil in high oil content commodities with an LOQ of 0.01 mg/kg (Austria, 2013). An ILV is not required for this method.*
*Hence, it is concluded that cymoxanil can be enforced in the four main plant matrices with an LOQ of 0.01 mg/kg. The enforcement of cymoxanil is also achievable in difficult matrices such as hops and herbal infusions (dried) with an LOQ of 0.1 mg/kg. This conclusion was confirmed by the EURLs during the Member States consultation. Nevertheless, a confirmatory method is still required for these specific matrices.*

In accordance with Regulations 283/2013 and 284/2013 and guide SANTE/2020/12830, Rev.1, 24. February 2021, validated methods should be available for the determination of cymoxanil residues for monitoring purposes in all plant matrices. Generally, an LOQ of at least 0.01 mg/kg should be met, except for MRLs which have been established at an even lower level (e.g. for compounds with a very low toxicological reference value) which then has to be covered by the LOQ.
According to the current Reg. (EU) 2022 /1363 most of the MRLs are 0.01* mg/kg.

Data are available to the Applicant from a study on potatoes that was already evaluated by zRMS Greece in the context of an application for the authorisation of the product (Moximate 505 WG) in 2014. This study demonstrated a determined LOQ of 0.01 mg/kg.

Applicant submitted additional data supporting the MRL of 0.01 mg/kg for tomatoes from study GLP-STUDY-21-58, Sala A. 2021a. The results of an ILV study are available and the dRR was updated accordingly (CH-0061-2023, Pardo Martinez M.).
Analytical methods for determination of cymoxanil in high water content matrix have been assessed.

During the commenting period Applicant submitted additional information:
*According to the agreed decision of the Interzonal Steering Committee of March 2023 on Data Gaps in EFSA Conclusions, as no data gaps were reported in the review report or EFSA Conclusions or requested at Member State level, "data gap is not to be considered at PPP level because it is not relevant".*
*Considering that no residues of cymoxanil above the LOQ were found in any of the raw commodities analysed in the dossier, no additional data have to be provided by the Applicant.*
*However, the Applicant is a component of the Task Force for the renewal of the active ingredient and studies were provided and are currently under evaluation in the renewal procedure.*
*The applicant resubmitted the Pardo-Martinez (2023) study to the BVL in Germany through the Portal on the 10/07/2023.*

**zRMS conclusion:**
As the current Reg. (EU) 2022 /1363 most of the MRLs are 0.01* mg/kg, new analytical methods (with confirmatory data and ILV) for food and feed of plant origin required for all matrix types are required. It should be noted that sufficiently validated methods have been submitted in the framework of active substance renewal.

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

No residue definition is proposed; therefore, no analytical method is required.

**zRMS comments:**

No analytical method is available at EU level. A definition residue with MRL are fixed as cymoxanil at LOQ of 0.01*mg/kg in animal products according to Reg. (EU) 2022/1363.
Considering the intended use, potatoes are addressed as feed items.
According to the residue level found in potatoes (not quantifiable) and to the results of dietary burden calculations for livestock where the trigger was not exceed for any of the animal group considered, it is considered that no residues should be available in any animal commodity.
Additionally, Reg. (EU) 2022/1363 did not change the MRL levels for animal commodities, as they have been set by default at the LOQ level. Therefore no analytical methods are required for food/feed of animal origin in the current application.

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

An overview on the acceptable methods and possible data gaps for analysis of **Cymoxanil** in soil is given in the following tables.
Methods presented hereunder have already been evaluated and peer reviewed at EU level.

**Table 5.3-4: Validated methods for soil**

| Component of residue definition: Cymoxanil | | | |
|---|---|---|---|
| **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 | Melkebeke T., 2000a EU agreed Report: Oxon 281802 |
| Confirmatory | 0.01 mg/kg | HPLC-DAD | |

**zRMS comments:**

An analytical method using HPLC-UV (Melkebeke, 2000) has been provided and validated in the DAR of cymoxanil (2007) for the determination of the residues of active substance in soil with a LOQ = 0.01 mg/kg. The confirmatory method is HPLC-DAD.
~~No further data are required.~~
As dichloromethane is used in the method of Melkebeke (2000) (is not acceptable anymore according to SANTE/2020/12830) new analytical method for the determination of residues of cymoxanil in soil is required. It should be noted that sufficiently validated methods have been submitted in the framework of active substance renewal. zRMS-PL considers that this data gap will be the subject of the art.43 re-authorisation process for the product.

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

An overview on the acceptable methods and possible data gaps for analysis of **Cymoxanil** in surface and drinking water is given in the following tables.
Methods presented hereunder have already been evaluated and peer reviewed at EU level.

**Table 5.3-5: Validated methods for water**

| Component of residue definition: Cymoxanil and IN-KQ960 | | | | |
|---|---|---|---|---|
| **Matrix type** | **Method type** | **Method LOQ** | **Principle of method (i.e. GC-MS or HPLC-UV)** | **Author(s), year / missing** |
| Drinking, ground and surface water | Primary and Confirmatory | 0.10 µg/L | HPLC-UV | Cabusas M.E.Y., 1999 EU agreed |

| Component of residue definition: Cymoxanil and IN-KQ960 | | | | |
|---|---|---|---|---|
| **Matrix type** | **Method type** | **Method LOQ** | **Principle of method (i.e. GC-MS or HPLC-UV)** | **Author(s), year / missing** |
| | | | | Report: DuPont 2126 |

**zRMS comments:**

An analytical method using HPLC-UV (Cabusas MEY, 1999) has been provided and fully validated in the DAR of cymoxanil (2007) for the determination of the residues of active substance in water (surface, drinking and ground) with LOQ = 0.10 µg/L.

EFSA in EFSA Scientific Report (2008) 167, 1-116 – "Conclusion on the peer review of cymoxanil" concluded that *Single methods for the determination of residues in soil, water and air are available. However, a new data gap has been identified as the metabolite IN-KQ9602 has been included in the residue definition for surface water and ground water.*

To address the data gap stated in the EFSA Scientific Report no. 167 (2008): "*An analytical method for IN-KQ960 in surface water with an LOQ lower than 0.3 mg/L and in ground water with an LOQ of 0.1 µg/L is required*" the Applicant submitted the method of Caine, J., 2010 (Belchim/Indofil report no. OZ/10/001). This method was already evaluated by zRMS Greece in the context of an application for the authorisation of the product (Moximate 505 WG) in 2014:

LC-MS/MS (2 transitions)
LOQ = 0.1 µg/L for ground and drinking water,
LOQ = 0.1 mg/L for surface water,
Validation: complete
Monitoring method: yes.

It should be noted that the method of Caine (2010) is not acceptable for surface water as the LOQ doesn't meet the RAC for IN-KQ960 of 8 µg/L. Consequently, an analytical method for the determination of residues of IN-KQ960 in surface water is missing (data gap).

In accordance with guide SANTE/2020/12830, Rev.1, 24. February 2021, the ILV method for drinking water or ground water should be conducted and provided.
Applicant's response:
*It is Applicant opinion that no additional studies are necessary for the purpose of the present application. Any possible data gap will be addressed at EU level during the renewal of the active ingredient.*

**Conclusion:**
According to the SANTE/2020/12830, Rev.1, 24. February 2021 the ILV for drinking or ground water is required (data gap).
It should be added that the Applicant is a component of the Task Force for the renewal of the active ingredient and sufficiently validated methods were provided and are currently under evaluation in the renewal procedure (dRAR for Cymoxanil, February 2020, Cymoxanil Task Force).
zRMS-PL considers that these data gaps are anticipated to be addressed at active substance level in context with the renewal of cymoxanil and will be subject of the art.43 re-authorisation process for the product.

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

An overview on the acceptable methods and possible data gaps for analysis of **Cymoxanil** in air is given in the following tables. Methods presented hereunder have already been evaluated and peer reviewed at EU level.

**Table 5.3-6: Validated methods for air**

| Component of residue definition: Cymoxanil | | | |
|---|---|---|---|
| **Method type** | **Method LOQ** | **Principle of method (i.e. GC-MS or HPLC-UV)** | **Author(s), year / missing** |
| Primary and Confirmatory | 0.46 µg/m$^3$ | HPLC UV | Melkebeke T, 2000b<br>EU agreed<br>Report: Oxon 257805 |

**zRMS comments:**

An analytical method using HPLC-DAD (Melkebeke, 2000) has been provided and fully validated in the DAR of cymoxanil for the determination of the residues of active substance in air with LOQ = 0.46 µg/m$^3$ (according to the AOEL).
No further data are required.

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

Cymoxanil is not classified as toxic or highly toxic, therefore no analytical method is required.

**zRMS comments:**

According to the Regulation No. 283/2013 and to the SANTE/2020/12830, Rev.1, 24. February 2021 an analytical method for the determination of residues in body fluids and tissues for enforcement/monitoring purposes is required (data gap).
Applicant's response:
*It is Applicant opinion that no additional studies are necessary for the purpose of the present application, as no residues were identified in the raw commodities for the requested uses.*
*Any possible data gap will be addressed at EU level during the renewal of the active ingredient.*

**Conclusion:**
zRMS-PL considers that this data gap is anticipated to be addressed at active substance level in context with the renewal of cymoxanil and will be subject of the art.43 re-authorisation process for the product.

### 5.3.2.8 Other studies/ information

Not necessary.

# 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 |
|---|---|---|---|---|---|
| KCP 5.1.1/01 | Rigamonti E. | 2021 | Cymoxanil 45 WG (IN 002B1760): Validation of the Analytical Method for the Determination of Active Ingredient Content<br>Report N. **CH-0526/2021**<br>ChemService S.r.l. Controlli e Ricerche<br>GLP: Yes<br>Unpublished | N | Indofil Industries B.V. |
| KCP 5.1.2/01 | Sala A. | 2021a | Determination of cymoxanil in raw agricultural commodity **tomato** following five applications of the formulated product Cymoxanil 45 WG (Southern Europe – 2 open field trials year 2021)<br>Report N. **GLP-STUDY-21-58**<br>LabAnalysis S.r.l.<br>GLP: Yes<br>Unpublished | N | Indofil Industries B.V. |
| KCP 5.1.2/02 | Sala A. | 2021b | Determination of cymoxanil in raw agricultural commodity **tomato** following five applications of the formulated product Cymoxanil 45 WG in protected condition (Southern Europe – 2 greenhouse trial year 2021)<br>Report N. **GLP-STUDY-21-59**<br>LabAnalysis S.r.l.<br>GLP: Yes<br>Unpublished | N | Indofil Industries B.V. |
| KCP 5.1.2/03a | Garagna D. | 2021b | Validation of the Analytical Method for the Determination of Cymoxanil content in **stock solutions** of Cymoxanil 45 WG (IN 002B1760) coming from the Ecotoxicological tests<br>Report N. **CH-0351/2021**<br>ChemService S.r.l. Controlli e Ricerche<br>GLP: Yes<br>Unpublished | N | Indofil Industries B.V. |
| KCP 5.1.2/03b | Garagna D. | 2021b | Amendment N.1 to final report<br>Validation of the Analytical Method for the Determination of Cymoxanil content in **stock solutions** of Cymoxanil 45 WG (IN 002B1760) coming from the Ecotoxicological tests<br>Report N. **CH-0351/2021**<br>ChemService S.r.l. Controlli e Ricerche<br>GLP: Yes<br>Unpublished | N | Indofil Industries B.V. |

| | | | | | |
|---|---|---|---|---|---|
| KCP 5.1.2/04a | Garagna D. | 2021c | Validation of the Analytical Method for the Determination of Cymoxanil Content in **Feeding Solutions** of Cymoxanil 45 WG (IN 002B1760) coming from the Ecotoxicological tests<br>Report N. **CH-0352/2021**<br>ChemService S.r.l. Controlli e Ricerche<br>GLP: Yes<br>Unpublished | N | Indofil Industries B.V. |
| KCP 5.1.2/04b | Garagna D. | 2021c | Amendment N.1 to final report<br>Validation of the Analytical Method for the Determination of Cymoxanil Content in **Feeding Solutions** of Cymoxanil 45 WG (IN 002B1760) coming from the Ecotoxicological tests<br>Report N. **CH-0352/2021**<br>ChemService S.r.l. Controlli e Ricerche<br>GLP: Yes<br>Unpublished | N | Indofil Industries B.V. |
| KCP 5.1.2/05a | Garagna D. | 2021a | Validation of the Analytical Method for the Determination of Cymoxanil Content in **Soil** Sample of Cymoxanil 45 WG (IN 002B1760) coming from the Ecotoxicological tests<br>Report N. **CH-0353/2021**<br>ChemService S.r.l. Controlli e Ricerche<br>GLP: Yes<br>Unpublished | N | Indofil Industries B.V. |
| KCP 5.1.2/05b | Garagna D. | 2021a | Amendment N.1 to final report<br>Validation of the Analytical Method for the Determination of Cymoxanil Content in **Soil** Sample of Cymoxanil 45 WG (IN 002B1760) coming from the Ecotoxicological tests<br>Report N. **CH-0353/2021**<br>ChemService S.r.l. Controlli e Ricerche<br>GLP: Yes<br>Unpublished | N | Indofil Industries B.V. |
| KCP 5.1.2/06a | Venkanna, B. | 2023a | Analytical report for the fresh water algae growth inhibition test with Cymoxanil 45% WG<br>Vivo Bio Tech LTD<br>Report N: 23/0177<br>GLP<br>Unpublished | N | ~~Vivo Bio Tech LTD~~ Indofil Industries Limited ~~(Netherlands) B.V.~~ |
| KCP 5.1.2/06b | Venkanna, B. | 2023b | Validation of the analytical method for determination of cymoxanil active ingredient in Cymoxanil 45% WG for the fresh water algae growth inhibition test with Cymoxanil 45% WG<br>Vivo Bio Tech LTD<br>Report N: 23/0181<br>GLP<br>Unpublished | N | ~~Vivo Bio Tech LTD~~ Indofil Industries Limited ~~(Netherlands) B.V.~~ |

| | | | | | |
|---|---|---|---|---|---|
| KCP 5.2/01 | Pardo Martinez M. | 2023 | Independent Laboratory Validation (ILV) of the Analytical Method for the Determination of Cymoxanil in Tomato<br>Report N. CH-0061-2023<br>ChemService S.r.l. Controlli e Ricerche<br>GLP: Yes<br>Unpublished | N | Indofil Industries B.V. |

**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** | Vertebrate study Y/N | Owner |
|---|---|---|---|---|---|
| KCA 4.2.1/01 | Kretschmer S., Class T. | 1999 | Method assessment and validation of an analytical multi-residue enforcement method (DFG S19 modified) for the determination of residues of cymoxanil in plant material (grape, potato, tomato, hops cones, post-cured tobacco).<br>PRTL Europe<br>Report N.: Dupont-2158<br>GLP<br>Unpublished | N | DuPont |
| KCA 4.2.1/02 | Linkerhägner M. | 1999 | Indipendent laboratory validation (ILV) of a multi-residue enforcement method (DFG S19 modified) for the determination of cymoxanil in dry, high water and oily crops.<br>Dr. Specht & Partner, Germany<br>Report N.: DuPont-2946<br>GLP<br>Unpublished | N | DuPont |
| KCA 4.2.1/05 | Freschi G. | 2001a | Validation of the analytical method for determination of residues of cymoxanil in lettuce (plant)<br>SIPCAM S.p.A. Salerano sul Lambro, Italy<br>Report N.: SIP1279<br>GLP<br>Unpublished | N | Oxon |
| KCA 4.2.1/06 | Freschi G. | 2001b | Validation of the analytical method for determination of residues of cymoxanil in potato (tuber)<br>SIPCAM S.p.A. Salerano sul Lambro, Italy<br>Report N.: SIP1277<br>GLP<br>Unpublished | N | Oxon |

| | | | | | |
|---|---|---|---|---|---|
| KCA 4.2.1/07 | Wasser C. | 2002 | Validation of the analytical method for determination of residues of cymoxanil in specimens of tomato, grapes, potatoes and lettuce.<br>Anadiag S.A. Haguenau, France<br>Report N.: A0087<br>GLP<br>Unpublished | N | Oxon |
| KCA 4.2.2/01 | Melkebeke T. | 2000a | Validation of the analytical method for determination of residues of cymoxanil in soil.<br>Notox B.V., 's-Hertogenbosch, The Nederlands<br>Report N.: 281802<br>GLP<br>Unpublished | N | Oxon |
| KCA 4.2.3/01 | Cabusas M.E.Y. | 1999 | Analytical method for the determination of cymoxanil in drinking, ground and surface water using liquid chromatography with ultraviolet detection.<br>DupPont Experimental Station<br>Report N.: DuPont-2126<br>GLP<br>Unpublished | N | DuPont |
| KCA 4.2.4/01 | Melkebeke T. | 2000b | Validation of the analytical method for determination of cymoxanil in air.<br>Notox B.V., 's-Hertogenbosch, The Nederlands<br>Report N:. 257805<br>GLP<br>Unpublished | N | Oxon |
| KCA 5.1.2-06 | Semrau J. | 2010 | Determination of Residues of Cymoxanil and Mancozeb After Six Applications Cymoxanil/Mancozeb 4.5/68 % w/w in Field Potatoes, Northern Europe, 2007/2008<br>Eurofins Report no.: 20074095/E1-FPPO<br>Doc. No. 634-1105<br>GLP, Unpublished | N | Indofil Industries Limited<br>~~IND~~ |

**List of data submitted by the applicant and not 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 |
|---|---|---|---|---|---|
| - | - | - | - | - | - |

**List of data relied on not submitted by the applicant but necessary for evaluation**

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

# Appendix 2 Detailed evaluation of submitted analytical methods

## A 2.1 Analytical methods for Cymoxanil

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

For the active ingredient please refer to the study described at the point 5.2.1.1 ((KCP 5.1.1_01 Rigamonti E., 2021).

New residue studies have been conducted and are submitted and summarised in this section.

Furthermore, three new analytical methods have been validated in the context of ecotoxicological studies and two analytical methods available for the determination of Dislodgeable Foliar Residues (DFR) and are hereunder presented in support of this new dossier. A summary of these studies is presented in this Appendix.

#### A 2.1.1.1 Description of analytical methods for the determination of residues in plant matrices (KCP 5.1.2)

#### A 2.1.1.1.1 Analytical method 1 (Tomato)

#### A 2.1.1.1.1.1 Method validation

| | |
|---|---|
| Comments of zRMS: | The analytical method was successfully validated for the determination of residues of cymoxanil in tomatoes in compliance with requirements reported in guideline SANTE/2020/12830 rev. 1 for the analyte.<br><br>For cymoxanil the limit of quantification (LOQ) was set at 0.01 mg/kg.<br><br>According to SANTE 2017/10632, chapter 5, the extraction efficiency was not tested: Cymoxanil residues resulted not detectable (lower than LOD). The SANTE guideline states that extraction efficiency is only necessary for pesticide showing significant residues, i.e residues at or above the LOQ of the analytical method.<br>The method is acceptable. |

Reference: KCP 5.1.2/01

Report: Determination of cymoxanil in raw agricultural commodity **tomato** following five applications of the formulated product Cymoxanil 45 WG (Southern Europe – 2 open field trials year 2021), Sala A. 2021a
Report **GLP-STUDY-21-58**
LabAnalysis Srl

Guideline(s): Yes.

- European Commission, Guidance Document on Pesticide Analytical Methods for Risk Assessment and Post-approval Control and Monitoring Purposes, SANTE/2020/12830, Rev.1 (dated 24th February 2021).
- European Commission (2017): SANTE 2017/10632 rev. 3, dated 22 November 2017: Technical Guideline on the Evaluation of Extraction Efficiency of Residue Analytical Methods.
- "European Committee for Standardisation (CEN) EN 15662:2018. Foods of plant origin - Multi-method for the determination of pesticide residues using GC- and LC-based analysis following acetonitrile extraction/partitioning and clean-up by dispersive SPE - Modular QuEChERS-method".

Deviations: No

GLP: Yes

Acceptability: Yes

**Materials and methods**

The validation of the analytical method was carried out under GLP compliance to SANTE/2020/12830 Rev.1 guideline. The analytical method for the determination of cymoxanil in the tested matrices (GLP-STUDY-21-58) was based on the QuEChERS method (EN 15662_2018). The instrumental determination was carried out using a HPLC-HRMS/MS system (high-performance liquid chromatography + triple quadrupole mass spectrometry).

**Description of the method**

*Stability of final extracts and standard*

Plant matrix (tomato): aliquot of 10 g of specimen were taken from the homogenised frozen samples and placed in a 50 mL screw capped centrifuge PE test tube. For recovery tests the sample was spiked at this stage. Then, 20 mL of acetonitrile were added to the sample and the mixture was vigorously shaken for one minute. After that, a packet of QuEChERS extraction salt (4.0 g $MgSO_4$, 1.0 g NaCl, 1.0 g trisodium citrate dehydrate, 0.5 g disodium hydrogen citrate sesquihydrate) was added and the mixture shaken for 1 minute again. The separation of the organic phase was achieved by centrifugation at 4500 rpm for 5 minutes. An aliquot of about 1 mL of the organic supernatant was taken and transferred in a 2 mL HPLC glass vial and analysed with a HPLC-HRMS/MS system.

Reference solution preparation

Stock solution: A stock solution of Cymoxanil was prepared in acetonitrile starting from certified reference material as summarised in the following table:

| Analyte | Weight (mg)/ Volume (µL) | Final volume (mL) | Standard purity (%) | Final concentration (mg/L) |
|---|---|---|---|---|
| Cymoxanil | 10.09 | 10 | 99.86 | 1008 |
| Solution A (~ 10 mg/L) | 100 µL | 10 | -- | 10.08 |
| Solution B (~ 1 mg/L) | 100 µL | 1 | -- | 1.008 |

Matrix-matched analytical standard solutions were prepared using the final extract of an untreated tomato control sample (GLP-SMPL-21-845) to dilute solution B on the basis of the following scheme:

| Solution | µL of Solution B | Final volume (mL) | Nominal concentration (µg/L) | Nominal concentration on the sample[1] (µg/kg) |
|---|---|---|---|---|
| L1 | 1 | 1 | 1.008 | 2.016 |
| L2 | 5 | 1 | 5.040 | 10.08 |
| L3 | 10 | 1 | 10.08 | 20.16 |
| L4 | 50 | 1 | 50.40 | 100.8 |
| L5 | 100 | 1 | 100.08 | 201.6 |

1: calculated considering the nominal sample preparation (10.0 g to a final volume of 20.0 mL)

The analyses were carried out using a HPLC-HRMSS system according to the following conditions:

Instrument: HPLC Thermo Vanquish coupled with HRMS Orbitrap Q-Exactive
Column: Waters Acquity UPLC HSS PFP, 1.8 µm, 2.1 x 150 mm
Column temperature: 40°C
Flow: 0.3 mL/min
Injection volume: 1.00 µL
Mobile phase A: 100% water + 0.2% formic acid
Mobile phase B: 100% methanol + 0.2% formic acid
Elution gradient:

| Time (min) | % A | % B |
|---|---|---|
| 0.00 | 80.0 | 20.0 |
| 1.00 | 80.0 | 20.0 |

| | | |
|---|---|---|
| 5 | 0 | 100 |
| 6 | 0 | 100 |
| 6 | 80 | 20 |
| 7 | 80 | 20 |

Run time: 7.0 min
Source type: ESI
Capillary temperature: 250°C
Sheath gas flow (L/min): 50
Auxiliary gas flow rate (L/min): 20
Auxiliary gas temperature: 380°C
Spray voltage: 4500 V
Acquiring mode: ESI positive, PRM (parallel reaction monitoring)

*Calibration*
The quantification of each analyte was made through the building of a calibration straight line with the external standard method. 5 matrix-matched analytical standard solutions were analysed in single injections in order to obtain a calibration curve (1/x weighed) interpolated with a linear regression.

*Recovery (Accuracy) and Repeatability (Precision*
Recovery and repeatability (as precision, % RSD) data will be reported for the following fortification levels:

- LOQ level (5 replicates): 0.01 mg/kg
- 10xLOQ level (5 replicates): 0.1 mg/kg - or alternatively at a spiking level higher than maximum residue level found on field specimens

*Specificity (Selectivity)*
This parameter will be evaluated in order to demonstrate that the applied method detects the right analyte and that the analytical signal is quantitatively correct and not affected by other analytes or by matrix interferences.
Representative, clearly labelled chromatograms of standard at the lowest calibrated level, matrix blanks and samples fortified at the lowest fortification level for each analyte/matrix combination will be provided to prove selectivity of the method. Labelling will include sample description, chromatographic scale and identification of all relevant components in the chromatogram.
A product ion spectrum will be provided to justify the selection of ions used for determination.
Blank values (non-fortified samples) will be determined from the matrices used in fortification experiments and should not be higher than 30% of the LOQ.

*Matrix effect*
Assessment of matrix effects will be performed by comparing the analyte response of at least one individual standard prepared in solvent to at least one prepared in blank matrix, for all sample matrix used in the study. In order to nullify matrix effect, calibration curves for all matrices analysed will be prepared using matrix matched analytical standards.

*Limit of detection (LOD)*
The limit of detection (LOD) is defined as the lowest detectable concentration or amount of an analyte in a sample. It is the lowest concentration at which an analyte produces an instrumental signal at least 3 times higher than background noise of the chromatogram. It should be not higher than 30% of LOQ value and it will be considered as the lowest point of the instrumental calibration.

*Limit of quantification (LOQ)*
Limit of quantitation (LOQ) is defined as the lowest validated level with sufficient recovery and precision. Target LOQ for Cymoxanil in the sample analysed will be set to 0.01 mg/kg.

*Confirmation:*
A confirmation simultaneous to the primary detection will be used monitoring one additional MS/MS transition. The following data will be provided for the additional ion: calibration data as recorded for primary detection, recovery and precision data as recorded for primary detection (at least for the 5

replicates at LOQ level) the recovery and precision mean values calculated on confirmatory ion must fulfill the same acceptable range reported above for primary detection.

**Results and discussions**
Method validation data can be summarised in the tables below.

**Table A 1: Recovery results from method validation of Cymoxanil using the analytical method**

| Matrix | Analyte | Fortification level (mg/kg) (*n* = 5) | Mean recovery (%) | RSD (%) | Comments |
|---|---|---|---|---|---|
| Tomato | Cymoxanil (Quantifier ion) | 0.01 mg/kg (LOQ) | 95.6 | 5.1 | -- |
| | Cymoxanil (Qualifier ion) | 0.1 mg/kg (10xLOQ) | 97.4 | 3.8 | -- |

**Table A 2: Characteristics for the analytical method used for validation of Cymoxanil**

| | Cymoxanil |
|---|---|
| Specificity | MS spectrum provided: Yes<br>m/z 199.1 – 129.03 (quantification)<br>m/z 199.1 – 83.03 (confirmatory, 1 transition)<br><br>Cymoxanil signals resulted lower than the instrumental limit of detection (LOD) for both primary and confirmatory detection, therefore the selectivity of the analytical method resulted proven |
| Calibration (type, number of data points) | n=5<br>*Quantifier ion*<br>y= $1.142*10^4$x +$1.401*10^3$<br>$R^2$=0.999<br><br>*Qualifier ion*<br>y= $6.533*10^3$x -$1.296*10^3$<br>$R^2$=0.998 |
| Calibration range | Calibration range: from 1.008-100.8 µg/L (0.002016-0.2016 mg/kg) |
| Assessment of matrix effects is presented | Matrix effects, expressed in % enhancement or suppression of signal, resulted relevant with a value of -29% (higher than < ±20%),therefore the calibration curves were prepared using matrix matched standard to nullify the matrix effect. |
| Limit of determination/quantification | LOD: 1.008 µg/L (corresponding to 0.002016 mg/kg)<br>LOQ: 0.01 mg/kg |
| Stability | Final extract stability: -8.2% (< ±10%)<br>Stock solution stability: -4.54% (< ±10%) |

**Conclusions**
A mean recovery of 70% - 110% with a Relative Standard Deviation lower than 20% was adopted as acceptability criteria.

The results obtained concerning matrix effects, linearity, selectivity, accuracy (recovery), precision (repeatability), specificity, limit of quantification and limit of detection are in compliance with requirements reported in guideline SANTE/2020/12830 rev. 1 for the analyte.

## A 2.1.1.1.2 Analytical method 2 (Tomato)

## A 2.1.1.1.2.1 Method validation

| | |
|---|---|
| Comments of zRMS: | The determination of Cymoxanil on field specimens was carried out applying the |

| | |
|---|---|
| | QuEChERS method (EN 15662 2018). The analytical method applied (AM1-GLP-STUDY-21-58) was validated under GLP compliance according to SANTE/2020/12830 Rev.1, dated 24. February 2021 guideline in this GLP study.<br>The following parameters were evaluated in this study to check method performances: linearity, procedural recovery, LOD, LOQ and stability.<br>For cymoxanil the limit of quantification (LOQ) was set at 0.01 mg/kg.<br>The method is acceptable. |

Reference: KCP 5.1.2/02

Report: Determination of cymoxanil in raw agricultural commodity **tomato** following five applications of the formulated product IN002B1760 (Southern Europe – 2 greenhouse trial year 2021), Sala A. 2021b
Report **GLP-STUDY-21-59**
LabAnalysis S.r.l.

Guideline(s): Yes.

- European Commission, Guidance Document on Pesticide Analytical Methods for Risk Assessment and Post-approval Control and Monitoring Purposes, SANTE/2020/12830, Rev.1 (dated 24th February 2021).
- European Commission (2017): SANTE 2017/10632 rev. 3, dated 22 November 2017: Technical Guideline on the Evaluation of Extraction Efficiency of Residue Analytical Methods.
- "European Committee for Standardisation (CEN) EN 15662:2018. Foods of plant origin - Multi-method for the determination of pesticide residues using GC- and LC-based analysis following acetonitrile extraction/partitioning and clean-up by dispersive SPE - Modular QuEChERS-method".

Deviations: No

GLP: Yes

Acceptability: Yes

**Materials and methods**
The validation of the analytical method was carried out under GLP compliance to SANTE/2020/12830 Rev.1 guideline. The analytical determination was carried out using a HPLC-HRMS/MS method validated in the GLP study coded **GLP-STUDY-21-58** *("Determination of cymoxanil in raw agricultural commodity **tomato** following five applications of the formulated product Cymoxanil 45 WG (Southern Europe – 2 open field trials year 2021"*).

**Description of the method**
*Stability of final extracts and standard*
Plant matrix (tomato): aliquot of 10 g of specimen were taken from the homogenised frozen samples and placed in a 50 mL screw capped centrifuge PE test tube. For recovery tests the sample was spiked at this stage. Then, 20 mL of acetonitrile were added to the sample and the mixture was vigorously shaken for one minute. After that, a packet of QuEChERS extraction salt (4.0 g $MgSO_4$, 1.0 g NaCl, 1.0 g trisodium citrate dehydrate, 0.5 g disodium hydrogen citrate sesquihydrate) was added and the mixture shaken for 1 minute again. The separation of the organic phase was achieved by centrifugation at 4500 rpm for 5 minutes. An aliquot of about 1 mL of the organic supernatant was taken and transferred in a 2 mL HPLC glass vial and analysed with a HPLC-HRMS/MS system.

Reference solution preparation
Stock solution: A stock solution of Cymoxanil was prepared in acetonitrile starting from certified reference material as summarised in the following table:

| Analyte | Weight (mg)/ Volume (µL) | Final volume (mL) | Standard purity (%) | Final concentration (mg/L) |
|---|---|---|---|---|
| Cymoxanil | 10.09 | 10 | 99.86 | 1008 |
| Solution A (~ 10 mg/L) | 100 µL | 10 | -- | 10.08 |
| Solution B (~ 1 mg/L) | 100 µL | 1 | -- | 1.008 |

Matrix-matched analytical standard solutions were prepared using the final extract of an untreated tomato control sample (GLP-SMPL-21-845) to dilute solution B on the basis of the following scheme:

| Solution | µL of Solution B | Final volume (mL) | Nominal concentration (µg/L) | Nominal concentration on the sample[1] (µg/kg) |
|---|---|---|---|---|
| L1 | 1 | 1 | 1.008 | 2.016 |
| L2 | 5 | 1 | 5.040 | 10.08 |
| L3 | 10 | 1 | 10.08 | 20.16 |
| L4 | 50 | 1 | 50.40 | 100.8 |
| L5 | 100 | 1 | 100.08 | 201.6 |

1: calculated considering the nominal sample preparation (10.0 g to a final volume of 20.0 mL)

The analyses were carried out using a HPLC-HRMS/MS system according to the following conditions:

Instrument: HPLC Thermo Vanquish coupled with HRMS Orbitrap Q-Exactive
Column: Waters Acquity UPLC HSS PFP, 1.8 µm, 2.1 x 150 mm
Column temperature: 40°C
Flow: 0.3 mL/min
Injection volume: 1.00 µL
Mobile phase A: 100% water + 0.2% formic acid
Mobile phase B: 100% methanol + 0.2% formic acid
Elution: gradient of the following composition:

| Time (min) | % A | % B |
|---|---|---|
| 0.00 | 80.0 | 20.0 |
| 1.00 | 80.0 | 20.0 |
| 5 | 0 | 100 |
| 6 | 0 | 100 |
| 6 | 80 | 20 |
| 7 | 80 | 20 |

Run time: 7.0 min
Source type: ESI
Capillary temperature: 250°C
Sheath gas flow (L/min): 50
Auxiliary gas flow rate (L/min): 20
Auxiliary gas temperature: 380°C
Spray voltage: 4500 V
Acquiring mode: ESI positive, PRM (parallel reaction monitoring)

*Calibration*

The quantification of each analyte was made through the building of a calibration straight line with the external standard method. 5 matrix-matched analytical standard solutions were analysed in single injections in order to obtain a calibration curve (1/x weighed) interpolated with a linear regression.

*Recovery (Accuracy) and Repeatability (Precision*

Recovery and repeatability (as precision, % RSD) data will be reported for the following fortification levels:

- LOQ level (5 replicates): 0.01 mg/kg
- 10xLOQ level (5 replicates): 0.1 mg/kg - or alternatively at a spiking level higher than maximum residue level found on field specimens

*Specificity (Selectivity)*
This parameter will be evaluated in order to demonstrate that the applied method detects the right analyte and that the analytical signal is quantitatively correct and not affected by other analytes or by matrix interferences.
Representative, clearly labelled chromatograms of standard at the lowest calibrated level, matrix blanks and samples fortified at the lowest fortification level for each analyte/matrix combination will be provided to prove selectivity of the method. Labelling will include sample description, chromatographic scale and identification of all relevant components in the chromatogram.
A product ion spectrum will be provided to justify the selection of ions used for determination.
Blank values (non-fortified samples) will be determined from the matrices used in fortification experiments and should not be higher than 30% of the LOQ.

*Matrix effect*
Assessment of matrix effects will be performed by comparing the analyte response of at least one individual standard prepared in solvent to at least one prepared in blank matrix, for all sample matrix used in the study. In order to nullify matrix effect, calibration curves for all matrices analysed will be prepared using matrix matched analytical standards.

*Limit of detection (LOD)*
The limit of detection (LOD) is defined as the lowest detectable concentration or amount of an analyte in a sample. It is the lowest concentration at which an analyte produces an instrumental signal at least 3 times higher than background noise of the chromatogram. It should be not higher than 30% of LOQ value and it will be considered as the lowest point of the instrumental calibration.

*Limit of quantification (LOQ)*
Limit of quantitation (LOQ) is defined as the lowest validated level with sufficient recovery and precision. Target LOQ for Cymoxanil in the sample analysed will be set to 0.01 mg/kg.

*Confirmation:*
A confirmation simultaneous to the primary detection will be used monitoring one additional MS/MS transition. The following data will be provided for the additional ion: calibration data as recorded for primary detection, recovery and precision data as recorded for primary detection (at least for the 5 replicates at LOQ level) the recovery and precision mean values calculated on confirmatory ion must fulfill the same acceptable range reported above for primary detection.

**Results and discussions**
Method validation data can be summarised in the tables below.

**Table A 3: Recovery results from method validation of Cymoxanil using the analytical method GLP-STUDY-21-58**

| Matrix | Analyte | Fortification level (mg/kg) (*n* = 2) | Mean recovery (%) | RSD (%) | Comments |
|---|---|---|---|---|---|
| Tomato | Cymoxanil | 0.01 mg/kg (LOQ) | 88.3 | -- | -- |
| | Cymoxanil | 0.1 mg/kg (10xLOQ) | 94.0 | -- | -- |

The spiked samples were prepared and analysed according to the validated analytical method (GLP-STUDY-21-58).

**Table A 4: Characteristics for the analytical method used for validation of Cymoxanil**

| | Cymoxanil |
|---|---|
| Specificity | -- |
| Calibration (type, number of data points) | n=5<br>$y= 3.318*10^4x +2.374*10^4$ |

| | **Cymoxanil** |
|---|---|
| | $R^2$=1.0000 |
| Calibration range | Calibration range: from 1.008-100.8 µg/L (0.002016-0.2016 mg/kg) |
| Assessment of matrix effects is presented | The calibration curve was built in all analytical sequence by using matrix matched standards in order to nullify the matrix effect, therefore no assessment of matrix effect was required. |
| Limit of determination/quantification | LOD: 1.008 µg/L (corresponding to 0.002016 mg/kg)<br>LOQ: 0.01 mg/kg (corresponding to 5.040 µg/L in the final extract) |
| Stability | Samples were analysed within 24 hours from preparation. |

**Conclusions**
A mean recovery of 70% - 110% with a Relative Standard Deviation lower than 20% was adopted as acceptability criteria.

The results obtained concerning matrix effects, linearity, selectivity, accuracy (recovery), precision (repeatability), specificity, limit of quantification and limit of detection are in compliance with requirements reported in guideline SANTE/2020/12830 rev. 1 for the analyte.

## A 2.1.1.1.2.2 Independent Laboratory Validation

| | |
|---|---|
| Comments of zRMS: | An Independent Laboratory Validation (ILV) of the analytical method was validated for the determination of cymoxanil residues in tomato with LOQ of 0.01 mg/kg according to SANTE/2020/12830 rev. 1 guideline.<br>The method is acceptable. |

Reference: KCP 5.2-01

Report: Independent Laboratory Validation (ILV) of the Analytical Method for the Determination of Cymoxanil in Tomato
Pardo Martinez M., 2023, report no CH-0061-2023

Guideline(s): Yes

- Regulation (EC) No 1107/2009
- SANTE/2020/12830 rev. 1 dated 24/02/21: Guidance Document on Pesticide Analytical Methods for Risk Assessment and Post-approval Control and Monitoring Purposes
- Main method validated in GLP study Code GLP-STUDY-21-58 performed by LabAnalysis s.r.l.: “Validation of an analytical method for the quantification of Cymoxanil in tomato”
- Council Decision [C(97)186/Final] amending Annex II to the council decision concerning the mutual acceptance of data in the assessment of chemicals [C(81) 30 (final)].
- ENV/MC/CHEM(98)17 OECD Principles on Good Laboratory Practice, No. 1 and all subsequent OECD consensus documents.
- Directive 2004/9/CE and directive 2004/10/CE of the European Parliament and of the Council of February 11th, 2004 enforced by Italian Legislative Decree No. 50 of March 2nd, 2007 as published in G.U. No. 86 of April 13th, 2007

Deviations: No

GLP: Yes

Acceptability: Yes

## Materials and methods

The Test Facility had revalidated the analytical method already adjusted and validated by LabAnalysis S.r.l. in the GLP study Code GLP-STUDY-21-58, to verify its applicability in the Test Facility laboratory and check specificity, linearity, limit of quantification (LOQ) and limit of detection (LOD), accuracy, precision and matrix effect of the analytical method for the determination of Cymoxanil residues in tomato.

The Cymoxanil determination was conducted by LC-MS/MS monitoring two MS/MS ion mass transitions.

| **Analyte** | **Transition type** | **Precursor ion** (m/z) | **Product ion** (m/z) |
|---|---|---|---|
| Cymoxanil | quantifier | 199.0 | 128.1 |
| | 1st qualifier | | 83.0 |
| | 2nd qualifier | | 129.0(*) |

(*) The product ion (m/z) at 129.0, that was the quantifier product ion reported in the primary method validation, was also considered in this study but its intensity was much lower respect to the product ion (m/z) 128.1 that is considered the quantifier product ion in this study.

The reason of the difference at the m/z value for the quantifier ion could be due to the diverse instrument (mass detector) used.

## Results and discussions

**Table A 1: Summary of the results, Quality criteria**

| Parameter | Results |
|---|---|
| Specificity / Interference | The analytical method results to be specific for Cymoxanil in Tomato samples, as no interferences above the LOD at the analytes retention time were detected. |
| Matrix Effect | Not significant (Lower than 20% at low (LOQ) and high (10 x LOQ) levels).<br>Linearity should not be prepared in extracted matrix but working standard solutions (WSS) were prepared in tomato matrix following the primary method validation. |
| Linearity | Five Working Standard Solutions (matrix-matched).<br><br>Nominal injected range from 1 ng/mL to 100 ng/mL,<br>corresponding to a range from 0.002 mg/kg to 0.2 mg/kg in Tomato samples.<br>Correlation coefficient r > 0.99 with residuals randomly distributed around the zero line with no visible trend. |
| Confirmatory | Since the analysis by HPLC/MS-QQQ gave quantification and identification data, the confirmatory test using another instrumental technique was not necessary. |
| LOD | 0.002 mg/kg |
| LOQ | 0.01 mg/kg |
| Recovery (Accuracy) and Repeatability (Precision) | Control Tomato spiked 5 times at low level (LOQ) and 5 times at high level (10 x LOQ).<br>Recovery in the correct range for each spike level with RSD% lower than 20% were obtained. |
| Stability of stock, fortification and calibration solutions | Not assessed since solutions were analysed within 24 hours after preparation. |
| Stability of final extracts/dilution | Not assessed since extracts/ dilutions were analysed within 24 hours after preparation. |

**Table A 2: Recovery results from independent laboratory validation of cymoxanil using the analytical method**

| Matrix | Analyte | Fortification level (mg/kg) (n = x) | Mean recovery (%) | RSD (%) | Comments |
|---|---|---|---|---|---|
| Tomatoes | Cymoxanil Product ion 128.1 | 0.01 (n = 5) | 109.1 | 1.80 | Overall mean recovery: 103.9 ± 5.63 |
| | | 0.10 (n = 5) | 98.7 | 2.36 | |
| | Cymoxanil Product ion 83.0 | 0.01 (n = 5) | 209.8 | 2.96 | Overall mean recovery: 103.6 ± 1.07 |
| | | 0.10 (n = 5) | 97.3 | 1.07 | |

**Table A 3: Characteristics for the analytical method used for independent laboratory validation of cymoxanil residues in tomatoes**

| | Cymoxanil – quantifier 199.0 m/z -> 128.1 m/z | Cymoxanil – qualifier 199.0 m/z -> 83.0 m/z |
|---|---|---|
| Specificity | blank value < 30 % LOQ | blank value < 30 % LOQ |
| Calibration (type, number of data points) | n = 5<br>y = 756x – 90<br>$R^2$= 0.99981 | n = 5<br>y = 258x – 221<br>$R^2$= 0.99982 |
| Calibration range | Calibration range in concentration units (ng/mL): 1.00-99.70<br>Corresponding calibration range in mg/kg: 0.002-0.20 | Calibration range in concentration units (ng/mL): 1.00-99.70<br>Corresponding calibration range in mg/kg: 0.002-0.20 |
| Assessment of matrix effects is presented | Yes<br>Matrix-matched solutions were used for calibration | Yes<br>Matrix-matched solutions were used for calibration |
| Limit of determination/quantification | LOQ = 0.01 mg/kg<br>LOD = 0.002 mg/kg | LOQ = 0.01 mg/kg<br>LOD = 0.002 mg/kg |

**Conclusion**

Since all the experimental results are in compliance with the guideline requirements, this analytical method can be considered validated according to SANTE/2020/12830 rev. 1 guideline.

Considering the available data, the method can be considered acceptable as ILV for the primary method.

## A 2.1.1.2 Description of Methods for the Analysis of Soil (KCP 5.2)

### A 2.1.1.2.1 Analytical method 1 (soil – *coming from Hypoaspis study*)

#### A 2.1.1.2.1.1 Method validation

| | |
|---|---|
| Comments of zRMS: | The analytical method was successfully validated for the determination of cymoxanil residues in soil samples with LOQ of 0.052 mg/kg according to the SANTE/2020/12830, rev. 1.<br>The mean recovery was 70% - 110% with a RSD lower than 20%.<br>The method is acceptable. |

The aim of this study is to develop and validate an analytical method for the determination of cymoxanil content in stock solutions prepared with Cymoxanil 45 WG (IN 002B1760) that will come from the ecotoxicological tests (*Hypoaspis* in soil).

The determination of cymoxanil content in soil test was performed by HPLC using an external standard and MS triple quadrupole detector validated according to SANTE/2020/12830 rev. 1 dated 24/02/21.

Reference: KCP 5.1.2/~~03~~ 05

Report: Validation of the Analytical Method for the Determination of Cymoxanil Content in Soil Sample of Cymoxanil 45 WG (IN 002B1760) coming from the Ecotoxicological tests, Garagna D. (2021a)
Final report **CH-0353/2021**
ChemService S.r.l. Controlli e Ricerche

Guideline(s): Yes

- SANTE/2020/12830, rev. 1 dated 24/02/21: Guidance Document on Pesticide Analytical Methods for Risk Assessment and Post-approval Control and Monitoring Purposes.
- OECD Guideline for Testing of Chemicals No. 208, "Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test", 2006.
- OECD Guideline for Testing of Chemicals No. 227, "Terrestrial Plant Test: Vegetative Vigour Test", 2006.
- OECD Guideline No. 239 (ENV/JM/MONO (2016)34), "Guidance Document on Honeybee (Apis mellifera) Larval Toxicity Test, Repeated Exposure", 2016.
- Method validation will be performed as described in the "Standard Operating Procedures" in force at the involved laboratories.

Deviations: No

GLP: Yes

Acceptability: Yes

**Materials and methods**

Principle of the method

The determination of cymoxanil residues is performed by HPLC using an external standard and MS triple quadrupole detector.

Transitions (MS/MS):
- 199.1 → 128.1 (quantifier);
- 199.1 → 111.1 and 199.1 → 83 (qualifiers)

Its quantification is achieved using the calibration curve obtained by plotting standard solutions concentrations and the corresponding peak area.

Reference material
Cymoxanil Pestanal®

Instrument settings:

| Chromatographic conditions | | | |
|---|---|---|---|
| Column | HPLC column, Zorbax Eclipse Plus C18, 3.5 µm, 100 x 4.6 mm i.d.; Internal code LCN 306 (or equivalent) | | |
| Detector | MS Triple quadrupole (Scan in MRM mode) | | |
| Column temperature | Not Controlled | | |
| Eluent A | Water / formic acid 0.1% / ammonium formate 10 mM | | |
| Eluent B | Acetonitrile | | |
| Eluent flow | 0.6 mL/min | | |
| Eluention mode | gradient condition | | |
| Mixture | % A | % B | Time (min) |
| | 90 | 10 | 0 |
| | 2 | 98 | 15 |
| | 90 | 10 | 20 |
| Volume of injection | 2 µL | | |
| Retention time | Approximately 9.0 minutes | | |
| Total analysis time | 20 minutes + 5 minutes of post time | | |
| Mass scan parameters | | | |
| Compound | Cymoxanil | | |
| Ion mode | ESI, positive polarity | | |
| Scan type | MRM | | |
| Electro multiplier voltage (V) | 400 | | |
| Dry gas temperature (°C) | 300 | | |
| Dry gas flow (L/min) | 12 | | |
| Nebuliser (psi) | 60 | | |
| Capillary current (V) | 4000 | | |
| Precursor ion (m/z) - (fragmentor, V) | 199.1 - (50) | | |
| Product ions (m/z) - (Collision Energy, V) | quantifier: 128.1 - (0)<br>qualifier 1: 111.1 - (15)<br>qualifier 2: 83 - (25) | | |
| Dwell time (msec) | 200 | | |

**Results and discussions**
Summary of the obtained results

| ~~Parameter~~ | ~~Acceptability criteria~~ |
|---|---|
| ~~Matrix Effect~~ | ~~< ±20%~~ |
| ~~result~~ | ~~0.4%~~ |
| ~~Selectivity / Specificity~~ | ~~untreated blank < 30% LOQ~~ |
| ~~result~~ | ~~0.0%~~ |
| ~~Linearity / Calibration~~ | ~~r ≥ 0.99~~ |
| ~~result~~ | ~~Quantifier transition m/z 199.1 → m/z 128.1~~ |
| | ~~Range 0.01-0.51 mg/L, r = **0.99953**~~ |
| | ~~Qualifier 1 transition m/z 199.1 → m/z 111.1~~ |
| | ~~Range 0.01-0.51 mg/L, r = **0.99973**~~ |

| | |
|---|---|
| | Qualifier 2 transition m/z 199.1 → m/z 83 |
| | Range 0.01-0.51 mg/L, **r = 0.99967** |
| LOD | Lowest calibration level |
| result | 0.01 mg/L |
| LOQ | Lowest fortified level |
| result | 0.31 mg/L |
| Stability of final extract | Recovery Mean between 70% - 120% |
| Result (3 days) | Low level: 110.9% |
| Stability of standard | < ±10% |
| Result (3 days) | -2.6% |

| Repeatability (Precision) Recovery (Accuracy) | | |
|---|---|---|
| Matrix | Demineralized water | |
| Fortification level | Low (mg/L) | High (mg/L) |
| Nominal | 0.31 | 1175 |
| Corrected* | 0.31 | 1183.46 |
| Mean found | 0.33 | 1185.19 |
| Recovery | Mean between 70% - 120% | |
| result | 107.5 | 100.1 |
| Repeatability | as precision RSD % ≤ 20% | |
| result | 1 | 1 |

(*) corrected for exact test item weighed amount during fortification solution preparation.

| Parameter | Acceptability criteria |
|---|---|
| Matrix Effect | < ±20% |
| result | +4.1% |
| Selectivity / Specificity | untreated blank < 30% LOQ |
| result | +18% |
| Linearity / Calibration | r ≥ 0.99 |
| result | Quantifier transition m/z 199.1 → m/z 128.1 |
| | Range 7.7 - 257.5 µg/L, r = **0.99829** |
| | Corresponding to range 15.5 - 515.0 µg/kg<br>r = 0.99829 |
| | Qualifier 1 transition m/z 199.1 → m/z 111.1 |
| | Range 7.7 - 257.5 µg /L, r = **0.99576** |
| | Corresponding to range 15.5 - 515.0 µg/kg<br>r = 0.99576 |
| | Qualifier 2 transition m/z 199.1 → m/z 83 |
| | Range 7.7 - 257.5 µg/L, **r = 0.99631** |
| | Corresponding to range 15.5 - 515.0 µg/kg<br>r = 0.99631 |
| LOD | Lowest calibration level |
| result | 7.7 µg/L (corresponding to 15.5 µg/kg) |
| LOQ | Lowest fortified level |
| result | 0.052 mg/L |
| Stability of final extract | Recovery Mean between 70% - 120% |
| Result (3 days) | Low level: 97.3% |
| Stability of standard | < ±10% |
| Result (3 days) | -6.8% |

| Repeatability (Precision) Recovery (Accuracy) | | |
|---|---|---|
| Matrix | Demineralized water | |
| Fortification level | Low (mg/L) | High (mg/L) |

| | | |
|---|---|---|
| Nominal | 0.05 | 470 |
| Corrected* | 0.052 | 472.350 |
| Mean found | 0.051 | 438.879 |
| Recovery | Mean between 70% – 120% | |
| Result | 98.3 | 92.9 |
| Repeatability | as precision RSD % ≤ 20% | |
| Result | 3 | 3 |

(*) corrected for exact test item weighed amount during fortification solution preparation.

**Conclusions**
A mean recovery of 70% - 110% with a Relative Standard Deviation lower than 20% was adopted as acceptability criteria.

The results obtained concerning matrix effects, linearity, selectivity, accuracy (recovery), precision (repeatability), specificity, limit of quantification and limit of detection are in compliance with requirements reported in guideline SANTE/2020/12830 rev. 1 for the analyte.

## A 2.1.1.3 Description of Methods for the Analysis of Water (KCP 5.1.2)

## A 2.1.1.3.1 Analytical method 1 (stock solutions – *coming from Terrestrial Plants*)

## A 2.1.1.3.1.1 Method validation

| | |
|---|---|
| Comments of zRMS: | The analytical method was successfully validated for the determination of cymoxanil content in stock solutions prepared with Cymoxanil 45 WG (IN 002B1760) with LOQ of 0.31 mg/L according to the SANTE/2020/12830, rev. 1.<br>The mean recovery was 70% - 110% with a RSD lower than 20%.<br>The method is acceptable. |

The aim of this study is to develop and validate an analytical method for the determination of cymoxanil content in stock solutions prepared with Cymoxanil 45 WG (IN 002B1760) that will come from the ecotoxicological tests (*Terrestrial Plants).*

The determination of cymoxanil content in stock solutions coming from terrestrial plant test was performed by HPLC using an external standard and MS triple quadrupole detector validated according to SANTE/2020/12830 rev. 1 dated 24/02/21.

Reference: KCP 5.1.2/04 03

Report: Validation of the Analytical Method for the Determination of Cymoxanil content in **stock solutions** of Cymoxanil 45 WG (IN 002B1760) coming from the Ecotoxicological tests, Garagna D. (2021b)
Final report **CH-0351/2021**
ChemService S.r.l. Controlli e Ricerche

Guideline(s): Yes

- SANTE/2020/12830, rev. 1 dated 24/02/21: Guidance Document on Pesticide Analytical Methods for Risk Assessment and Post-approval Control and Monitoring Purposes.
- Feeding solutions in sucrose will be prepared according to the following guidelines.
- OECD, Guideline for the Test of chemicals n°245: "Honeybee (Apis mellifera L.), chronic oral toxicity test (10-day feeding)", adopted on 2017.
- Method validation was performed as described in the "Standard Operating Procedures" in force at the involved laboratories.

Deviations: No

GLP: Yes

Acceptability: Yes

**Materials and methods**

Principle of the method

The determination of cymoxanil residues is performed by HPLC using an external standard and MS triple quadrupole detector.

Transitions (MS/MS):
- 199.1 → 128.1 (quantifier);
- 199.1 → 111.1 and 199.1 → 83 (qualifiers)

Its quantification is achieved using the calibration curve obtained by plotting standard solutions concentrations and the corresponding peak area.

Reference material

Cymoxanil Pestanal®

Instrument settings:

| Chromatographic conditions | | | |
|---|---|---|---|
| Column | HPLC column, Zorbax Eclipse Plus C18, 3.5 µm, 100 x 4.6 mm i.d.; Internal code LCN 306 (or equivalent) | | |
| Detector | MS Triple quadrupole (Scan in MRM mode) | | |
| Column temperature | 25°C | | |
| Eluent A | Water / formic acid 0.1% / ammonium formate 10 mM | | |
| Eluent B | Acetonitrile | | |
| Eluent flow | 0.6 mL/min | | |
| Eluention mode | gradient condition | | |
| Mixture | % A | % B | Time (min) |
| | 90 | 10 | 0 |
| | 2 | 98 | 15 |
| | 90 | 10 | 20 |
| Volume of injection | 2 µL | | |
| Retention time | Approximately 9.0 minutes | | |
| Total analysis time | 20 minutes + 5 minutes of post time | | |
| Mass scan parameters | | | |
| Compound | Cymoxanil | | |
| Ion mode | ESI, positive polarity | | |
| Scan type | MRM | | |
| Electro multiplier voltage (V) | 400 | | |
| Dry gas temperature (°C) | 300 | | |
| Dry gas flow (L/min) | 12 | | |
| Nebuliser (psi) | 60 | | |
| Capillary current (V) | 4000 | | |
| Precursor ion (m/z) - (fragmentor, V) | 199.1 - (50) | | |
| Product ions (m/z) - (Collision Energy, V) | quantifier: 128.1 - (0)<br>qualifier 1: 111.1 - (15)<br>qualifier 2: 83 - (25) | | |
| Dwell time (msec) | 200 | | |

**Results and discussions**

Summary of the obtained results

| Parameter | Acceptability criteria |
|---|---|
| Matrix Effect | < ±20% |
| result | +1.6% |
| Selectivity / Specificity | untreated blank < 30% LOQ |
| result | 0.0% |
| Linearity / Calibration | r ≥ 0.99 |
| result | Quantifier transition m/z 199.1 → m/z 128.1 |
| | Range 0.01-0.51 mg/L, r = **0.99897** |
| | Qualifier 1 transition m/z 199.1 → m/z 111.1 |
| | Range 0.01-0.51 mg/L, r = **0.99854** |
| | Qualifier 2 transition m/z 199.1 → m/z 83 |
| | Range 0.01-0.51 mg/L, **r = 0.99854** |
| LOD | Lowest calibration level |
| result | 0.01 mg/L |
| LOQ | Lowest fortified level |
| result | 1.52 mg/L |
| Stability of final extract | Recovery Mean between 70% – 120% |
| Result (3 days) | Low level: 117.1% |
| Stability of standard | < ±10% |
| Result (3 days) | ±7.3% |

| Repeatability (Precision) Recovery (Accuracy) | | |
|---|---|---|
| Matrix | Demineralized water | |
| Fortification level | Low (mg/L) | High (mg/L) |
| Nominal | 1.50 | 1269 |
| Corrected* | 1.52 | 1277.46 |
| Mean found | 1.62 | 1158.48 |
| Recovery | Mean between 70% – 120% | |
| result | 106.2 | 90.7 |
| Repeatability | as precision RSD % ≤ 20% | |
| result | 2 | 6 |

(*) corrected for exact test item weighed amount during fortification solution preparation.

| Parameter | Acceptability criteria |
|---|---|
| Matrix Effect | < ±20% |
| result | -0.4% |
| Selectivity / Specificity | untreated blank < 30% LOQ |
| result | 0.0% |
| Linearity / Calibration | r ≥ 0.99 |
| result | Quantifier transition m/z 199.1 → m/z 128.1 |
| | Range 0.01-0.51 mg/L, r = **0.99953** |
| | Qualifier 1 transition m/z 199.1 → m/z 111.1 |
| | Range 0.01-0.51 mg/L, r = **0.99973** |
| | Qualifier 2 transition m/z 199.1 → m/z 83 |
| | Range 0.01-0.51 mg/L, **r = 0.99967** |
| LOD | Lowest calibration level |
| result | 0.01 mg/L |
| LOQ | Lowest fortified level |
| result | 0.31 mg/L |

| | |
|---|---|
| Stability of final extract | Recovery Mean between 70% - 120% |
| Result (3 days) | Low level: 110.9% |
| Stability of standard | < ±10% |
| Result (3 days) | -2.6% |

| Repeatability (Precision) Recovery (Accuracy) | | |
|---|---|---|
| Matrix | Demineralized water | |
| Fortification level | Low (mg/L) | High (mg/L) |
| Nominal | 0.31 | 1175 |
| Corrected* | 0.31 | 1183.46 |
| Mean found | 0.33 | 1185.19 |
| Recovery | Mean between 70% – 120% | |
| result | 107.5 | 100.1 |
| Repeatability | as precision RSD % ≤ 20% | |
| result | 1 | 1 |

(*) corrected for exact test item weighed amount during fortification solution preparation.

**Conclusions**
A mean recovery of 70% - 110% with a Relative Standard Deviation lower than 20% was adopted as acceptability criteria.

The results obtained concerning matrix effects, linearity, selectivity, accuracy (recovery), precision (repeatability), specificity, limit of quantification and limit of detection are in compliance with requirements reported in guideline SANTE/2020/12830 rev. 1 for the analyte.

## A 2.1.1.4 Description of Methods for the Analysis of Water (KCP 5.1.2)

### A 2.1.1.4.1 Analytical method 21 (feeding solutions – *coming from Chronic oral toxicity on bees*)

#### A 2.1.1.4.1.1 Method validation

| | |
|---|---|
| Comments of zRMS: | The analytical method was successfully validated for the determination of cymoxanil content in feeding solutions prepared with Cymoxanil 45 WG (IN 002B1760) with LOQ of 1.52 mg/L according to the SANTE/2020/12830, rev. 1.<br>The mean recovery was 70% - 110% with a RSD lower than 20%.<br>The method is acceptable. |

The aim of this study is to develop and validate an analytical method for the determination of cymoxanil content in stock solutions prepared with Cymoxanil 45 WG (IN 002B1760) that will come from the ecotoxicological tests (*Chronic oral toxicity on bees).*

The determination of cymoxanil content in stock solutions coming from terrestrial plant test was performed by HPLC using an external standard and MS triple quadrupole detector validated according to SANTE/2020/12830 rev. 1 dated 24/02/21.

Reference: KCP 5.1.2/05 04

Report: Validation of the Analytical Method for the Determination of Cymoxanil Content in **Feeding Solutions** of Cymoxanil 45 WG (IN 002B1760) coming from the Ecotoxicological tests, Garagna D. (2021c)
Final report **CH-0352/2021**
ChemService S.r.l. Controlli e Ricerche

Guideline(s): Yes

- SANTE/2020/12830, rev. 1 dated 24/02/21: Guidance Document on Pesticide Analytical Methods for Risk Assessment and Post-

approval Control and Monitoring Purposes.

- OECD Guideline for Testing of Chemicals No. 222. “Earthworm Reproduction Test (Eisenia fetida/ Eisenia andrei)”, 2016.
- OECD Guideline for Testing of Chemicals No. 226. “Predatory mite (Hypoaspis (Geolaelaps) aculeifer) reproduction test in soil”, 2016.
- OECD Guideline for Testing of Chemicals, No. 232, “Collembolan reproduction test in soil”, 2016.
- Method validation will be performed as described in the "Standard Operating Procedures" in force at the involved laboratories.

Deviations: No

GLP: Yes

Acceptability: Yes

**Materials and methods**

Principle of the method

The determination of cymoxanil residues is performed by HPLC using an external standard and MS triple quadrupole detector.

Transitions (MS/MS):
- 199.1 → 128.1 (quantifier);
- 199.1 → 111.1 and 199.1 → 83 (qualifiers)

Its quantification is achieved using the calibration curve obtained by plotting standard solutions concentrations and the corresponding peak area.

Reference material

Cymoxanil Pestanal®

Instrument settings:

| Chromatographic conditions | | | |
|---|---|---|---|
| Column | HPLC column, Zorbax Eclipse Plus C18, 3.5 µm, 100 x 4.6 mm i.d.; Internal code LCN 306 (or equivalent) | | |
| Detector | MS Triple quadrupole (Scan in MRM mode) | | |
| Column temperature | Not Controlled | | |
| Eluent A | Water / formic acid 0.1% / ammonium formate 10 mM | | |
| Eluent B | Acetonitrile | | |
| Eluent flow | 0.6 mL/min | | |
| Eluention mode | gradient condition | | |
| Mixture | % A | % B | Time (min) |
| | 90 | 10 | 0 |
| | 2 | 98 | 15 |
| | 90 | 10 | 20 |
| Volume of injection | 2 µL | | |
| Retention time | Approximately 9.0 minutes | | |
| Total analysis time | 20 minutes + 5 minutes of post time | | |
| Mass scan parameters | | | |
| Compound | Cymoxanil | | |
| Ion mode | ESI, positive polarity | | |

| | |
|---|---|
| Scan type | MRM |
| Electro multiplier voltage (V) | 400 |
| Dry gas temperature (°C) | 300 |
| Dry gas flow (L/min) | 12 |
| Nebuliser (psi) | 60 |
| Capillary current (V) | 4000 |
| Precursor ion (m/z) - (fragmentor, V) | 199.1 - (50) |
| Product ions (m/z) - (Collision Energy, V) | quantifier: 128.1 - (0)<br>qualifier 1: 111.1 - (15)<br>qualifier 2: 83 - (25) |
| Dwell time (msec) | 200 |

## Results and discussions

Summary of the obtained results

| | |
|---|---|
| Parameter | Acceptability criteria |
| Matrix Effect | < ±20% |
| result | +4.1% |
| Selectivity / Specificity | untreated blank < 30% LOQ |
| result | ±18% |
| Linearity / Calibration | r ≥ 0.99 |
| result | Quantifier transition m/z 199.1 → m/z 128.1 |
| | Range 7.7 – 257.5 µg/L, r = **0.99829** |
| | Corresponding to range 15.5 – 515.0 µg/kg<br>r = 0.99829 |
| | Qualifier 1 transition m/z 199.1 → m/z 111.1 |
| | Range 7.7 – 257.5 µg /L, r = **0.99576** |
| | Corresponding to range 15.5 – 515.0 µg/kg<br>r = 0.99576 |
| | Qualifier 2 transition m/z 199.1 → m/z 83 |
| | Range 7.7 – 257.5 µg/L, **r = 0.99631** |
| | Corresponding to range 15.5 – 515.0 µg/kg<br>r = 0.99631 |
| LOD | Lowest calibration level |
| result | 7.7 µg/L (corresponding to 15.5 µg/kg) |
| LOQ | Lowest fortified level |
| result | 0.052 mg/L |
| Stability of final extract | Recovery Mean between 70% – 120% |
| Result (3 days) | Low level: 97.3% |
| Stability of standard | < ±10% |
| Result (3 days) | -6.8% |

| Repeatability (Precision) Recovery (Accuracy) | | |
|---|---|---|
| Matrix | Demineralized water | |
| Fortification level | Low (mg/L) | High (mg/L) |
| Nominal | 0.05 | 470 |
| Corrected* | 0.052 | 472.350 |
| Mean found | 0.051 | 438.879 |
| Recovery | Mean between 70% – 120% | |
| Result | 98.3 | 92.9 |
| Repeatability | as precision RSD % ≤ 20% | |
| Result | 3 | 3 |

*(*) corrected for exact test item weighed amount during fortification solution preparation.*

| Parameter | Acceptability criteria |
|---|---|
| Matrix Effect | < ±20% |
| result | +1.6% |
| Selectivity / Specificity | untreated blank < 30% LOQ |
| result | 0.0% |
| Linearity / Calibration | r ≥ 0.99 |
| result | Quantifier transition m/z 199.1 → m/z 128.1 |
| | Range 0.01-0.51 mg/L, r = **0.99897** |
| | Qualifier 1 transition m/z 199.1 → m/z 111.1 |
| | Range 0.01-0.51 mg/L, r = **0.99854** |
| | Qualifier 2 transition m/z 199.1 → m/z 83 |
| | Range 0.01-0.51 mg/L, **r = 0.99854** |
| LOD | Lowest calibration level |
| result | 0.01 mg/L |
| LOQ | Lowest fortified level |
| result | 1.52 mg/L |
| Stability of final extract | Recovery Mean between 70% - 120% |
| Result (3 days) | Low level: 117.1% |
| Stability of standard | < ±10% |
| Result (3 days) | +7.3% |

| Repeatability (Precision) Recovery (Accuracy) | | |
|---|---|---|
| Matrix | Demineralized water | |
| Fortification level | Low (mg/L) | High (mg/L) |
| Nominal | 1.50 | 1269 |
| Corrected* | 1.52 | 1277.46 |
| Mean found | 1.62 | 1158.48 |
| Recovery | Mean between 70% – 120% | |
| result | 106.2 | 90.7 |
| Repeatability | as precision RSD % ≤ 20% | |
| result | 2 | 6 |

(*) corrected for exact test item weighed amount during fortification solution preparation.

**Conclusions**
A mean recovery of 70% - 110% with a Relative Standard Deviation lower than 20% was adopted as acceptability criteria.

The results obtained concerning matrix effects, linearity, selectivity, accuracy (recovery), precision (repeatability), specificity, limit of quantification and limit of detection are in compliance with requirements reported in guideline SANTE/2020/12830 rev. 1 for the analyte.

## A 2.1.1.4.2 Analytical method 2 (Exposure solutions – *coming from Fresh water Algae Growth inhibition test)*

## A 2.1.1.4.2.1 Method validation

| | |
|---|---|
| Comments of zRMS: | Stability of the test item was analyzed with the samples prepared at concentrations of 0.3 and 100 mg/L at 0 and 72 hours after preparation. The mean measured % recovery at 0 h for concentrations 0.3 and 100 mg/L was 93.50% and 111.84%, respectively. Similarly, the mean measured % recovery at 72 h for concentrations 0.3 and 100 mg/L was 98.67% and 110.38%, respectively.<br>It can be concluded that the test item was stable at 0 hr and 72 hr in OECD medium under the experimental conditions. |

The aim of this study is to evaluate the stability of Cymoxanil by means of an analytical method that will come from the ecotoxicological tests (*Fresh water Algae Growth inhibition).*

The determination of cymoxanil stability is performed by HPLC using an external standard and Diode Array Detector (DAD)

| | |
|---|---|
| Reference: | KCP 5.1.2/06a |
| Report | Fresh water Algae Growth inhibition Test with Cymoxanil 45% WG, b. Venkanna (2024), Study number: 23/0177, ~~Vivo Bio Tech Ltd.~~ Indofil Industries (Netherlands) B.V. |
| Guideline(s): | Yes<br>– OECD Guideline for Testing of Chemicals, Section 2. No. 201. "Freshwater Alga and Cyanobacteria, Growth Inhibition Test" Adopted on 23rd March 2006. Annex 5 corrected: 28th July 2011. |
| Deviations: | Two amendments to the Study Plan were raised. No deviations occurred during the study |
| GLP: | Yes |
| Acceptability: | Yes |

**Materials and method**

Principle of the method

The determination of cymoxanil stability is performed by HPLC using an external standard and Diode Array Detector (DAD)

Its quantification is achieved using the calibration curve obtained by plotting standard solutions concentrations and the corresponding peak area.

Reference material

Cymoxanil Pestanal®

Instrument settings:

| Chromatographic conditions | | |
|---|---|---|
| Column | ACE 5 C18 (Avantor) | |
| Detector | Diode Array Detector (DAD) | |
| Column temperature | 30 °C | |
| Sample temperature | 20 °C | |
| Wavelength | 250 nm | |
| Eluent A | 0.1 % OPA in HPLC grade water | |
| Eluent B | Acetonitrile | |
| Eluent flow | 1 mL/min | |
| Eluention mode | Isocratic | |
| Mixture | % A | % B |
| | 70 | 30 |
| Volume of injection | 10 µL | |
| Retention time | Approximately 5.1 minutes | |
| Total analysis time | 15 minutes | |

**Results and discussions**

**See zRMS comments above.**

Summary of the obtained results

| | | |
|---|---|---|
| Parameter | Acceptability criteria | |
| Selectivity / Specificity | No interference | |
| Linearity / Calibration | r ≥ 1 | |
| result | Corresponding to range 0.03 – 149.97 µg/mL, r = 1 | |
| LOD | Lowest calibration standard | |
| result | 0.03 µg/mL | |
| LOQ | Recovery and repeatability test from OECD medium | |
| result | 0.1 mg/mL | |
| Stability of final extract | Recovery Mean between 70% – 120% | |
| Result (3 days) | Low level: 98.67% | |
| Stability of standard | < ±10% | |
| Result (3 days) | 0 % change | |
| % Recovery (Accuracy) | Mean between 70% – 120% | |
| Result | LOQ = 93.8 | 10 x LOQ = 77.58 |
| Precision (% RSD) | as precision RSD % ≤ 20% | |
| Result | LOQ = 5.41 | 10 x LOQ = 2.45 |

**Conclusions**
The Test item was stable at 0 hr and 72 hr in OECD medium under the experimental conditions.

## A 2.1.1.4.2.2 Method validation

| | |
|---|---|
| Comments of zRMS: | The analytical method has been validated for determination of cymoxanil concentration in test media (OECD medium) with LOQ of 0.1 µg/mL.<br>**Table 3 Summary of Recovery and Repeatability test**<br>The mean recovery was 70% - 110% with a RSD lower than 20%.<br>The method is fit for purpose. |

| Vehicle | LOQ | | 10 x LOQ | |
|---|---|---|---|---|
| | % Recovery ± S.D | % RSD | % Recovery ± S.D | % RSD |
| OECD Medium | 93.53 ± 5.07 | 5.41 | 77.58 ± 1.90 | 2.45 |

The objective of this study is to validate HPLC-DAD analytical method of determination of active ingredient, Cymoxanil, concentration in test media (OECD medium) for specificity, linearity, LOD, LOQ, recovery and repeatability.

| | |
|---|---|
| Reference: | KCP 5.1.2/06b |
| Report | Validation of Analytical Method for Determination of Cymoxanil Active Ingredient, Study number: 23/0181, Vivo Bio Tech Ltd. Indofil Industries (Netherlands) B.V. |
| Guideline(s): | n/a |

Deviations: No

GLP: Yes

Acceptability: Yes

**Materials and method**

Principle of the method

Validation of a HPLC-DAD analytical method for the determination of the active ingredient, Cymoxanil 45% WG, in test media (OECD medium) for specificity, linearity, LOD, LOQ, recovery and repeatability. Its quantification is achieved using the calibration curve obtained by plotting standard solutions concentrations and the corresponding peak area. Linearity was established in the range 0.03 µg/mL — 149.97 µg/mL by injecting six different concentrations of Cymoxanil reference item.

Specificity:

The quantity of 10.00 mg of the test item was weighed into 10 mL volumetric flask. The contents were dissolved and made up to mark with diluent. From the stock solution, aliquot of 1.0 mL was transferred into 5 mL volumetric flask the contents were made up to mark with diluent. Prepared solution used for specificity test of test item.

Specificity test was conducted by injecting Mobile phase A, B, diluent blank, vehicle blank OECD medium, reference item working solution, reference item in OECD medium and test item solution used in the analysis.

LOQ and LOD:

LOQ is defined as the lowest validated level with sufficient recovery and repeatability. Based on the recovery, the LOQ was determined.

LOD is defined as the lowest detectable concentration of an analyte in a sample, It was expressed as lowest calibration standard in linearity test.

Recovery and repeatability experiment:

At LOQ in OECD Medium: From the working standard solution stock-II (concentration of 100.11 µg/mL), aliquot of 0.010 mL was fortified into 5 mL of OECD Medium separately in 5 replications and volume made up to 10.0 mL mark with OECD Medium (Concentration 0:10 µg/mL).

At 10 x LOQ in OECD Medium: From the working standard solution stock-Il (concentration of 100.11 µg/mL), aliquot of 0.1 mL was fortified into 5 mL of OECD Medium separately in 5 replications and volume was made up to 10.0 mL mark with OECD Medium (Concentration 1.00 µg/mL).

Recovery was calculated as % of recovery, the precision at LOQ md LOQ x 10 was calculated as % of RSD

Reference material

Cymoxanil Pestanal®

Instrument settings:

| Chromatographic conditions | |
|---|---|
| Column | ACE 5 C18 (Avantor) |
| Detector | Diode Array Detector (DAD) |
| Column temperature | 30 °C |
| Sample temperature | 20 °C |
| Wavelength | 250 nm |
| Eluent A | 0.1 % OPA in HPLC grade water |
| Eluent B | Acetonitrile |
| Eluent flow | 1 mL/min |
| Eluention mode | Isocratic |

| | | |
|---|---|---|
| Mixture | % A | % B |
| | 70 | 30 |
| Volume of injection | 10 µL | |
| Retention time | Approximately 5.1 minutes | |
| Total analysis time | 15 minutes | |

**Results and discussions**

Specificity test:

No interference was observed at the retention time of Cymoxanil 45% WG in the diluent, mobile phase A and B and test medium (OECD medium) blank. The % difference of retention time of target analyte was within the range.

As there was no interference observed at the retention time of peak of interest and the % difference in retention time of analytical standard and test item, which is well within the acceptance limits, the HPLC-DAD method was found to be specific for analysis of test item.

Linearity:

The remaining volume was made up to the mark with diluent and shaken well. The equipment response (area) was found to be liner in the concentration range of 0.030 µg/mL to 149.97 µ/mL. The correlation coefficient (r) of the graph was found to be ≥ 0.99 (actual result 1.00). The results of linearity test were within the acceptance limits.

LOD and LOQ:

The LOD was established to be 0.030 µg/mL from Linearity test as a lowest calibration standard.
The LOQ was stablished to be 0.1 µg/mL in OECD medium from recovery and repeatability test.

Recovery and reproducibility:

Recovery test at LOQ and another at 10 x LOQ in OECD Medium: This was carried out in 5 replications. Recovery was calculated as % of RSD and the precision was calculated as % of RSD at LOQ and 10 x LOQ levels.

Summary of the obtained results

| Parameters | | Results |
|---|---|---|
| Test Medium | | OECD Medium |
| Specificity | | No interference |
| Linearity | Standard solutions | 6 |
| | Concentration range | 0.03-149.97 |
| | Intercept (a) | -2.38 |
| | Slope of the line | 23.33 |
| | Correlation Coefficient (r) | 1.000 |
| | Equation: Y = bX+a | Y=23.33X.2.38 |
| Limit of Detection (LOD) [WmL1 | | 0.03 |
| Limit of Quantification [µg/mL] | | 0.1 |
| Precision RSD) | LOQ | 5.41 |
| | 10 x LOQ | 2.45 |
| Accuracy (% Recovery) | LOQ | 93.80 |
| | 10 x LOQ | 77.58 |

**Conclusions**

Based on the study results, it was concluded that the method could be used for its intended purpose.

## A 2.1.1.5 Other studies

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)

No new or additional studies have been submitted.

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

No new or additional studies have been submitted.

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

No new or additional studies have been submitted.

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

No new or additional studies have been submitted.

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

No new or additional studies have been submitted.

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

No new or additional studies have been submitted.

### A 2.1.2.7 Other Studies/ Information

No new or additional studies have been submitted.