# FINAL REGISTRATION REPORT

## Part B

## Section 5

## Analytical Methods

Detailed summary of the risk assessment

Product code: CHR/H/IMA 40 SL

Product name(s):

Mazzam 40 SL

Zemax 40 SL

Chemical active substance:

Imazamox, 40 g/L

Central Zone

Zonal Rapporteur Member State: Poland

Co-Rapporteur Member State: Hungary, Romania

CORE ASSESSMENT

(authorization)

Applicant: Innvigo Sp. z o.o.

Submission date: 09.2022

Update: 02.2024

MS Finalisation date: 12/07/2024

# Version history

| When | What |
|---|---|
| January 2023 | Dossier sent for evaluation |
| February 2024 | Applicant update |
| April 2024 | zRMS evaluation of dRR |
| July 2024 | Final version prepared by zRMS after Commenting period |

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zRMS comments:
This report has been completed by the Applicant.
The text highlighted in grey was provided by the zRMS.

New, not previously evaluated information and studies are highlighted in yellow.

# 5 Analytical methods

Considering winter oilseed rape magnitude of residue studies we are obliged to relied upon following studies taking account that according to Regulation (EC) No 1107/2009 Article 59 Data protection: The period of data protection is 30 months starting at the date of renewal in accordance to art. 43 in that Member State. Renewal of the product in Poland was in 20.11.2017 (R-45/2017), therefore data protection is over, and other applicants can refer to studies performed during inclusion and extensions of uses of the product Clentiga 262.5 SC.

## 5.1 Conclusion and summary of assessment

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

| Commodity/crop | Supported/ Not supported |
|---|---|
| Peas, beans, broad bean, lentils, lupine | Supported |
| Soy, linseed, oilseed, poppy, sesame, mustard, sunflower, safflower, borage, hemp, castor beans, cotton, pumpkin for seeds | Supported |
| Tobacco, ornamental plants, wicker | Supported |

Noticed data gaps are:

- methods for the analysis of body fluids and tissues (EU data gap).

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

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

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

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

| | |
|---|---|
| Reference: | KCP 5.1.1 |
| Report | Gutowska I., *Development and validation of the method for determination of the active substance content in the preparation*, Institute of Industrial Organic Chemistry Warsaw 2018, study code: BA-85/18 |

| | |
|---|---|
| Guideline(s): | SANCO/3030/99 rev. 5 |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes |

**Materials and methods**

Test item:
Test item: **CHR/H/IMA 40 SL**
Manufacturer: Chemirol Sp. z o. o.
Batch number: 2018.08.21
Production date: 08/2018
Expiry date: 08/2020
Test item code: 122/BA-85/18

Reference item:
The following standard was used as reference items:
- Imazamox, 99.7%, IPO 942, Batch No 1A/18 (Annex 3)

Reagents:

– Acetonitrile for HPLC, POCh

- Deionized water, ultra-pure, Millipore

- *orto*-phosphoric acid, analytical grade, AppliChem

- Analytical standard (**3.2.**)

Equipment:

– Shimadzu liquid chromatograph equipped with UV-DAD

- Column: Luna C18(2) 100Å (5 μm), 250 - 4.6mm (Phenomenex)

- Analytical balance Mettler Toledo XS 205 DU/M, accuracy 0.01 mg

- Glass pipettes

- Glass graduated flasks

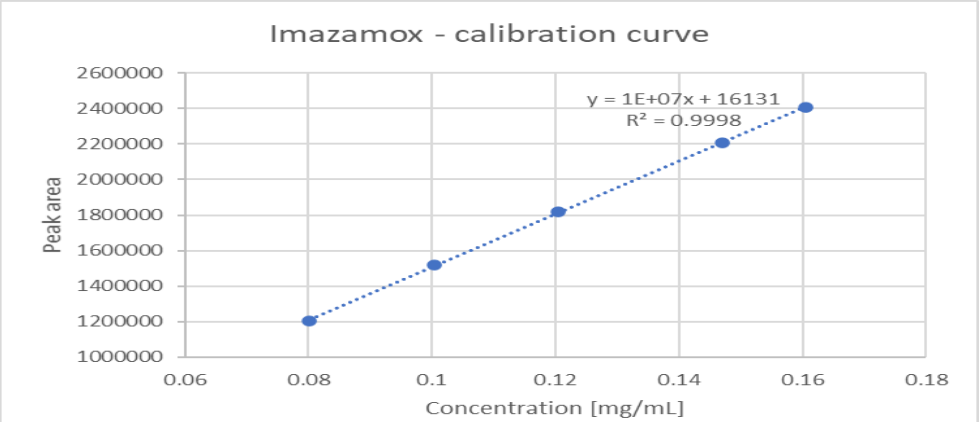
- Ultrasonic bath

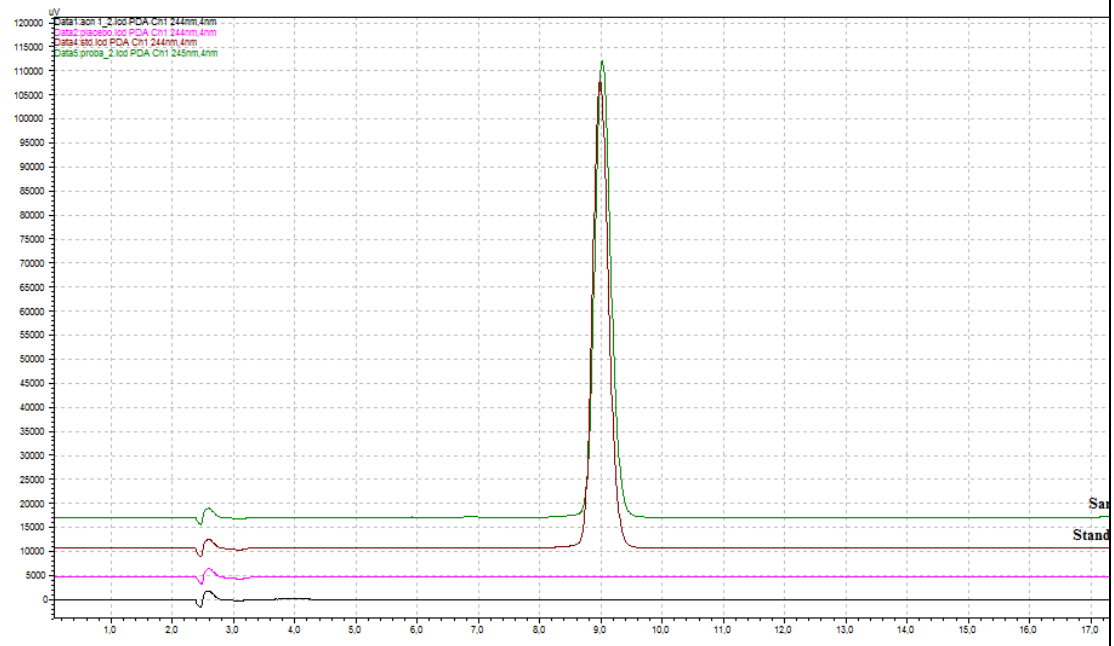
- Automatic pipettes
- Typical laboratory equipment

Analytical method:
The method is based on determination of imazamox using reversed phase high performance liquid chromatography (RP-HPLC) with UV-DAD detection at wavelength 244 nm and external standard.

**Validation - Results and discussions**

**Table 5.2-1: Methods suitable for the determination of active substance imazamox in plant protection product CHR/H/IMA 40 SL**

| Active substance | Imazamox |
|---|---|
| **Author(s), year** | Gutowska I., 2018 |
| **Principle of method** | RP-HPLC with UV-DAD |
| **Linearity (linear between mg/L / % range of the declared content) (correlation coefficient, expressed as r)** |  <br>Range of linearity: 0.08022 – 0.16044 mg/mL |
| **Precision – Repeatability Mean n = 6 (%RSD)** | 0.19 % RSD |
| **Accuracy n = 6 (% Recovery)** | Level I: 100.2%<br>Level II: 100.3%<br>Average: 100.3%<br>The result of 100.3% fulfils the acceptance criterion (97 – 103%). |
| **Interference/ Specificity** | To prove specificity of the developed method, chromatograms of: solvent (acetonitrile), placebo (Annex 4), standard of imazamox (Annex 5) and sample of CHR/H/IMA 40 SL preparation (Annex 6) were performed and superimposed.<br>There are no other peaks that could interfere with peak of imazamox under the specified chromatographic conditions. Overlaid chromatograms are presented below: |

| Active substance | Imazamox |
|---|---|
| |  |
| **Comment** | - |

**Conclusion**

In accordance to SANCO/3030/99 rev. 5 the method for determination of active substance imazamox in CHR/H/IMA 40 SL preparation is specific. The validation parameters for linearity, instrument precision, repeatability and accuracy are within the acceptance range. The determined average content of imazamox in CHR/H/IMA 40 SL is: 3.820 ± 0.007%.

**zRMS conclusion**

The method for determination of content of the active substance – imazamox – in CHR/H/IMA 40 SL preparation was developed and validated in GLP Laboratory - Analytical Department of the Institute of Industrial Organic Chemistry (IPO), Warsaw, according to EU requirements described in SANCO/3030/99 rev. 5. The determination was performed using high performance liquid chromatography (HPLC) with UV-DAD and external standard. The following validation parameters were determined: specificity, linearity, precision (repeatability) and accuracy.
The method described in study report BA-85/18: *Development and validation of the method for determination of the active substance content in the preparation* is applicable for the determination of the content of active substance – imazamox in CHR/H/IMA 40 SL.

### 5.2.1.2 Description of analytical methods for the determination of relevant impurities: cyanides ($CN^-$) (KCP 5.1.1)

| | |
|---|---|
| Reference: | KCP 5.1.1 |
| Report | Foster B., *Validation of Method for Determination of Cyanide Ion in CHR/H/IMA 40 SL*, Concept Life Sciences Analytical & Development Services Limited, Unit 69, Listerhills Science Park, Campus Road, Bradford, BD7 1HR, UK, 2020, study code: CLS3_0339_0002 |
| Guideline(s): | SANCO/3030/99 rev. 5 |

| | |
|---|---|
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes |

**SUMMARY OF THE STUDY**

An ion chromatography method with amperometric detection (Method IC_CLS3_011 version 2 and version 3) was validated for the analysis of cyanide in CHR/H/IMA 40 SL (soluble concentrate containing 40 g/L of Imazamox).

**Purpose of the study**

The objective of this study was to develop and validate an ion chromatography method for the analysis of cyanide ion in CHR/H/IMA 40 SL.

The test item was a soluble concentrate (SL) formulation containing Imazamox as the active ingredient. The formulation is an herbicidal product. The objective of this study was to develop and validate an analytical method to measure the content of cyanide, a possible impurity, in the test item.

**Regulatory**

This study was performed in compliance with the Study Plan, Company standard operating procedures and MHRA GLP regulations and OECD Principles of Good Laboratory Practice. Data are accepted by regulatory authorities within countries that are signatories to the OECD Mutual Acceptance of Data Agreement. Routine activities performed within this study are detailed within Concept Life Sciences, Bradford Standard Operating Procedures.

**MATERIALS AND METHODS**

**TEST ITEM**

The test item analysed in this study is an SL formulation containing Imazamox, which acts as a herbicide, as the active ingredient.

The test item used for this study was:

Test Item Name: Imazamox 40 SL (CHR/H/IMA)
Batch: 2018.08.21
Active Content: 3.820 ± 0.007%
Appearance: Brown liquid
Expiry: August 2020
Supplier: Sponsor
Storage Details: Room temperature (not lower than 0 °C and not exceeding 30 °C)

The Concept Life Sciences, Bradford identity number QC-T007-01 was given to the test item for reference at Concept Life Sciences, Bradford.

Unless otherwise instructed by the sponsor, unused test item samples will be disposed of on completion of the study.

**GENERAL REFERENCE ITEM**

Identification: Cyanide Standard for Ion Chromatography
Concept ID Number: QC-C231-02
Batch No: BCBX5093
Expiration Date: 30 June 2021
Physical Description: Colourless liquid
Concentration: 1001mg/L
Storage Conditions: Room temperature until opened and then stored refrigerated (2-8°C)
Supplier: Sigma-Aldrich
Supplier Part Number: 90157

**CHECK STANDARD REFERENCE ITEM**

Identification: Cyanide Anion Standard (SPEX Certi Prep Simple Cyanide)
Concept ID Number: QC-C231-03

Lot No: 13-79YPX
Expiration Date: 30 May 2019
Physical Description: Colourless liquid
Concentration: 1000µg/mL ± 50µg/mL
Storage Conditions: Room temperature
Supplier: Fisher Scientific
Supplier Part Number: 10497292
Identification: Cyanide Anion Standard (SPEX Certi Prep Simple Cyanide)
Concept ID Number: QC-C231-04
Lot No: 13-82YPX
Expiration Date: 15 August 2019
Physical Description: Colourless liquid
Concentration: 1000µg/mL ± 50µg/mL
Storage Conditions: Room temperature until opened and then stored refrigerated (2-8°C)
Supplier: Fisher Scientific
Supplier Part Number: 10497292

**METHODS**
The analytical method IC_CLS3_011 was followed with the exception of the deviations listed in Section 6. The instrument used was a Metrohm 940 high pressure gradient ion chromatograph (IC) with Metrohm 889 cooled autosampler and amperometric detection, MagIC Net 3.2 software and Metrosep A Supp 10, 100mm X 4.0mm analytical column.

Version 1 of the method was used for the validation runs made on 02 Apr 19 and 03 Apr 19.

Version 2 of the method, issued after further method development to separate interfering peak, was used for validation runs on 19 Jun 19, 20 Jun 19 and 12 Jul 19.

Version 3 of the method was issued on 11 Jul 19, a day before validation run on 12 Jul 19. The only change was to sample preparation procedure and as this was not actually used for method validation this version of the method was also covered by runs made on 19 Jun 19, 20 Jun 19 and 12 Jul 19.

**Validation - Results and discussions**

**Table 5.2-2: Methods suitable for the determination of relevant impurities: cyanides ($CN^-$) in plant protection product CHR/H/IMA 40 SL**

| Active substance | Imazamox |
|---|---|
| **Author(s), year** | Foster B., 2020 |
| **Principle of method** | High pressure gradient ion chromatography (IC) |
| **Specificity** | The method was shown to be specific for cyanide ion with no interfering peaks in blank samples. |
| **Linearity** | The method was shown to be linear with 1/x weighting over the range of 0.04 to 1.00µg/mL, equivalent to 1.9 to 46.5 mg/kg of cyanide. The correlation coefficient was within the acceptable range. |
| **Accuracy** | The mean recoveries were within the acceptance criteria for low, medium and high levels of 2.5mg/kg, 5.0mg/kg and 7.5mg/kg, respectively. |
| **Precision (Repeatability)** | 1 The method showed good precision with the percent relative standard deviation (%RSD) being within the accepted range. |
| **Precision (System)** | The method showed good precision with the percent relative standard deviation |

| Active substance | Imazamox |
|---|---|
| | (%RSD) being within the accepted range. |
| **Range** | The range of the method based on acceptable linearity, recoveries and precision was 0.04 to 1.00 µg/mL, equivalent to 1.9 to 46.5 mg/kg of cyanide. |
| **Robustness** | The method was robust. |
| **Limit of Detection (LOD)** | The calculated limit of detection was 0.00054µg/mL, equivalent to 0.027mg/kg. |
| **Limit of Quantification (LOQ)** | The limit of quantification was 2.5mg/kg. |

**Conclusions**

The analytical method has been successfully validated across the analytical range equivalent to 1.9 to 46.5mg/kg.
The limit of quantification is confirmed as 2.5mg/kg for cyanide.
All SANCO 3030/99/rev5 guidelines have been adhered to and acceptance criteria as stated in the study plan were met.

**zRMS conclusion**

The method for determination of content of Cyanide Ion (the impurity cyanide ion ($CN^-$) shall not exceed 5 mg/kg in the technical material - imazamox) – in CHR/H/IMA 40 SL preparation was developed and validated in GLP Laboratory according to EU requirements described in SANCO/3030/99 rev. 5. An ion chromatography method with amperometric detection was validated for the analysis of cyanide in CHR/H/IMA 40 SL (soluble concentrate containing 40 g/L of Imazamox). The following validation parameters were determined: specificity, linearity, precision, accuracy, precision (repeatability), precision (system), robustness – intra-assay solution stability and Expiration Date Testing, LOQ and LOD.
The method described in study report CLS3_0339_0002: *Validation of method for determination of Cyanide Ion in CHR/H/IMA 40 SL* is applicable for the determination of the cyanide ion in CHR/H/IMA 40 SL.

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

Not required – CHR/H/IMA 40 SL does not contain other formulants.

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

Not relevant

### 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 imazamox for the generation of pre-authorization data is given in the following table. For the detailed evaluation of new studies it is referred to Appendix 2.

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

| Component of residue definition: imazamox and its salts, expressed as imazamox | | | | |
|---|---|---|---|---|
| **Matrix type** | **Method type** | **Method LOQ** | **Principle of method (i.e. GC-MS or HPLC-UV)** | **Author(s), year / missing / EU agreed** |
| Food/feed of plant origin (Residues) | Primary – own method | Imazamox: 0.0056 mg/kg in dry pea<br>0.0052 mg/kg in whole plant w/o roots | LC-MS/MS | Wołoszynowska M., 2022, Reprort no. 1/21/019/K |
| | Primary – method evaluated at EU level | Imazamox: 0.01 mg/kg | LC-MS/MS | Lehmann A., Lewis A., 2013, Data point: 4.,EU agreed |
| | Confirmatory (if required) | Not required | | |
| Food/feed of animal origin (Residues) | Primary | 0.01 mg/kg for imazamox and its metabolite CL 263284 in liver, kidney, muscle, milk, fat and egg | LC-MS/MS | Stewart J., 2003a, Data piont: 4.2/5, EU agreed |
| | Primary | 0.01 mg/kg for honey | LC-MS/MS | Sahvorost, N., 2022, Data point: KCP 5.2/02 |
| | Confirmatory (if required) | Not required | | |
| Soil (Environmental fate) | Primary | 0.001 ppm | LC-MS/MS | Gooding R., 2013b, Data point: 4.1.2/1, EU agreed |
| | Confirmatory (if required) | Not required | | |
| Water (groundwater, surface water) (Environmental fate) | Primary | 0.025 µg/L | LC-MS/MS | Toledo F., 2013a, Data point 4.1.2/3, EU agreed<br>Holzer S.., 2013a, Data point 4.1.2/4, EU agreed |
| | Confirmatory (if required) | Not required | | |
| Air (Environmental fate) | Primary | 444 µg/$m^3$ | LC-MS/MS | Bacher R., 2013b, Data point: 4.1.2/2, EU agreed |
| | Confirmatory (if required) | Not required | | |

**Table 5.2-3a: Validated methods for the generation of pre-authorization data – metabolite CL 189215**

| Component of residue definition: metabolite CL 189215 | | | | |
|---|---|---|---|---|
| **Matrix type** | **Method type** | **Method LOQ** | **Principle of method (i.e. GC-MS or HPLC-UV)** | **Author(s), year / missing / EU agreed** |
| Food/feed of plant origin (Residues) | Primary – own method | 0.0041 mg/kg – dry pea<br>0.0030 mg/kg – whole plant w/o roots | LC-MS/MS | Wołoszynowska M., 2022, Reprort no. 1/21/019/K |
| | Primary – method evaluated at EU level | 0.01 mg/kg | LC-MS/MS | Lehmann A., Lewis A., 2013, Data point: 4.,EU agreed |
| | Confirmatory (if required) | Not required | | |
| Food/feed of animal origin (Residues) | Primary | 0.01 mg/kg | LC-MS/MS | Stewart J., 2003a, Data piont: 4.2/5, EU agreed |
| | Primary | 0.00674 mg/kg for honey | LC-MS/MS | Sahvorost, N., 2022, Data point: KCP 5.2/02 |
| | Confirmatory (if required) | Not required | | |
| Soil (Environmental fate) | Primary | Not required – this is not a soil metabolite | | |
| | Confirmatory (if required) | | | |
| Water (groundwater, surface water) (Environmental fate) | Primary | Not required – this is not a water metabolite | | |
| | Confirmatory (if required) | | | |
| Air (Environmental fate) | Primary | Not required – this is not an air metabolite | | |
| | Confirmatory (if required) | | | |

**Table 5.2-4b: Validated methods for the generation of pre-authorization data – metabolite CL 263284**

| Component of residue definition: metabolite CL 263284 | | | | |
|---|---|---|---|---|
| **Matrix type** | **Method type** | **Method LOQ** | **Principle of method (i.e. GC-MS or HPLC-UV)** | **Author(s), year / missing / EU agreed** |
| Food/feed of plant origin (Residues) | Primary – own method | 0.0079 mg/kg – dry pea<br>0.0079 mg/kg – whole plant w/o roots | LC-MS/MS | Wołoszynowska M., 2022, Reprort no. 1/21/019/K |
| | Primary – method | 0.01 mg/kg | LC-MS/MS | Lehmann A., Lewis A., 2013, |

| Component of residue definition: metabolite CL 263284 | | | | |
|---|---|---|---|---|
| **Matrix type** | **Method type** | **Method LOQ** | **Principle of method (i.e. GC-MS or HPLC-UV)** | **Author(s), year / missing / EU agreed** |
| | evaluated at EU level | | | Data point: 4.,EU agreed |
| | Confirmatory (if required) | Not required | | |
| Food/feed of animal origin (Residues) | Primary | 0.01 mg/kg | LC-MS/MS | Stewart J., 2003a, Data piont: 4.2/5, EU agreed |
| | Primary | 0.01 mg/kg for honey | LC-MS/MS | Sahvorost, N., 2022, Data point: KCP 5.2/02 |
| | Confirmatory (if required) | Not required | | |
| Soil (Environmental fate) | Primary | Not required – this is not a soil metabolite | | |
| | Confirmatory (if required) | | | |
| Water (groundwater, surface water) (Environmental fate) | Primary | Not required – this is not a water metabolite | | |
| | Confirmatory (if required) | | | |
| Air (Environmental fate) | Primary | Not required – this is not an air metabolite | | |
| | Confirmatory (if required) | | | |
| | Confirmatory (if required) | | | |

**Table 5.2-5c: Validated methods for the generation of pre-authorization data – metabolite CL 354825**

| Component of residue definition: metabolite CL 354825 | | | | |
|---|---|---|---|---|
| **Matrix type** | **Method type** | **Method LOQ** | **Principle of method (i.e. GC-MS or HPLC-UV)** | **Author(s), year / missing / EU agreed** |
| Food/feed of plant origin (Residues) | Primary – own method | Not required – this is not a plant metabolite | | |
| | Confirmatory (if required) | | | |
| Food/feed of animal origin (Residues) | Primary | Not required – this is not an animal metabolite | | |
| | Confirmatory (if required) | | | |
| Soil (Environmental fate) | Primary | 0.001 ppm | LC-MS/MS | Gooding R., 2013b, Data point: 4.1.2/1, EU agreed |
| | Confirmatory (if required) | Not required | | |

| Component of residue definition: metabolite CL 354825 | | | | |
|---|---|---|---|---|
| **Matrix type** | **Method type** | **Method LOQ** | **Principle of method (i.e. GC-MS or HPLC-UV)** | **Author(s), year / missing / EU agreed** |
| Water (groundwater, surface water) (Environmental fate) | Primary | 0.025 µg/L | LC-MS/MS | Toledo F., 2013a, Data point 4.1.2/3, EU agreed<br>Holzer S.., 2013a, Data point 4.1.2/4, EU agreed |
| | Confirmatory (if required) | Not required | | |
| Air (Environmental fate) | Primary | Not required – there is not an air metabolite | | |

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

Data provided on Annex I inclusion is sufficient for post-authorizations methods. No new methods are necessary since all data is described and presented in Table 5.2-3 in point KCP 5.1.2.

Therefore, the new studies for CHR/H/IMA 40 SL have not been performed.

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

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

### 5.3.2 Description of analytical methods for the determination of residues imazamox (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: grapes, rice (whole plant) | Imazamox and metabolites CL 263284, CL 189215, expressed as imazamox | LOQ: 0.01 mg/kg<br>MRL: 0.05 mg/kg | EFSA Journal 2016;14(4):4432<br>Reg. (EU) 2021/2202 |
| Plant, high protein/high starch content (dry commodities): dry pea, | | LOQ: 0.01 mg/kg<br>MRL: 0.05 mg/kg | EFSA Journal 2016;14(4):4432<br>Reg. (EU) 2021/2202 |

| Matrix | Residue definition | MRL / limit | Reference for MRL/level Remarks |
|---|---|---|---|
| rice (grain and straw), gren beans | | | |
| Plant, high oil content: sunflower (seeds) | | LOQ: 0.01 mg/kg<br>MRL: 0.05 mg/kg | EFSA Journal 2016;14(4):4432<br>Reg. (EU) 2021/2202 |
| Muscle | Imazamox and its metabolite CL 263284, expressed as imazamox | LOQ: 0.01 mg/kg<br>MRL: 0.01 mg/kg mg/kg | EFSA Journal 2016;14(4):4432<br>Reg. (EU) 2021/2202 |
| Milk | | LOQ: 0.01 mg/kg<br>MRL: 0.01 mg/kg mg/kg | EFSA Journal 2016;14(4):4432<br>Reg. (EU) 2021/2202 |
| Eggs | | LOQ: 0.01 mg/kg<br>MRL: 0.01 mg/kg mg/kg | EFSA Journal 2016;14(4):4432<br>Reg. (EU) 2021/2202 |
| Fat | | LOQ: 0.01 mg/kg<br>MRL: 0.01 mg/kg mg/kg | EFSA Journal 2016;14(4):4432<br>Reg. (EU) 2021/2202 |
| Liver, kidney | | LOQ: 0.01 mg/kg<br>MRL: 0.01 mg/kg mg/kg | EFSA Journal 2016;14(4):4432<br>Reg. (EU) 2021/2202 |
| Soil (Ecotoxicology) | Imazamox and metabolites CL 312622, Cl 354825, expressed as imazamox | 0.001 mg/kg | EFSA Journal 2016;14(4):4432 |
| Drinking water (Human toxicology) | Imazamox and metabolites CL 312622, Cl 354825, expressed as imazamox | 0.025 µg/L | EFSA Journal 2016;14(4):4432 |
| Surface water (Ecotoxicology) | Imazamox and metabolites CL 312622, Cl 354825, expressed as imazamox | 0.025 µg a.s /L from most sensitive species for imazamox *Lemna gibba* | EFSA Journal 2016;14(4):4432 |
| Air | imazamox | 444 µg/$m^3$ | EFSA Journal 2016;14(4):4432 |
| Tissue (meat or liver) | Sum of imazamox and metabolites CL 312622 and CL 354825 | 0.01 mg/kg | Limit based on SANCO 825 |
| Body fluids | | 0.01 mg/kg | Limit based on SANCO 825 |

### 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 imazamox in plant matrices is given in the following tables. For the detailed evaluation of new studies it is referred to Appendix 2.

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

| Component of residue definition: imazamox and metabolites CL 263284, CL 189215 | | | | |
|---|---|---|---|---|
| **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 (grapes, rice (whole plant)) | Primary | 0.01 mg/kg | HPLC-MS/MS ESI+ | Lehmann A., 2013a, Data point: 4.2/1, 4.2/2 |
| | ILV | 0.01 mg/kg | HPLC-MS/MS ESI+ | Mewis A., 2013a, Data point: 4.2/1, 4.2/2 |
| | Confirmatory (if required) | Not required | | |
| High oil content (sunflower) | Primary | 0.01 mg/kg | HPLC-MS/MS ESI+ | Lehmann A., 2013a,b, Data point: 4.2/1, 4.2/2 |
| | ILV | 0.01 mg/kg | HPLC-MS/MS ESI+ | Mewis A., 2013a, Data point: 4.2/3 |
| | Confirmatory (if required) | Not required | | |
| High protein/high starch content (dry) (green beans, peas (dry), rice (grain, straw)) | Primary | 0.01 mg/kg | HPLC-MS/MS ESI+ | Lehmann A., 2013a,b, Data point: 4.2/1, 4.2/2 |
| | ILV | 0.01 mg/kg | HPLC-MS/MS ESI+ | Mewis A., 2013a, Data point: 4.2/3 |
| | Confirmatory (if required) | Not required | | |

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

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

| | **Method for products of plant origin** |
|---|---|
| Required, available from: | RAR 2016: Lehmann A., 2013a,b, Data point: 4.2/1, 4.2/2: Extraction efficiency for the solvent system used in method L0188/01 (acidic methanol/water) was proven in the rapeseed and wheat metabolism studies (2011/1281377 Radzom, Klöppner, 2013 see KCA 6.2.1/2 and 2012/1064722 Grosshans, Lutz, 2012 see KCA 6.2.1/4 ). In these studies, wheat forage, hay, straw and grain and rapeseed seed and straw which were treated with radio-labeled imazamox were extracted with the solvent system used in both method M3519 and L0188/01 - methanol/water/1N HCl 60/39/1. Extraction efficiency of imazamox and its relevant metabolites was acceptable for each of these matrices when compared to the metabolism extraction scheme except for oilseed rape seed (31% TRR). |
| Not required, because: | N/D |

For the detailed evaluation of (additional) studies on extraction efficiency, it is referred to Appendix 2.

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

An overview on the acceptable methods and possible data gaps for analysis of imazamox in animal matrices is given in the following tables. There are no new or additional studies.

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

| Component of residue definition: imazamox | | | | |
|---|---|---|---|---|
| **Matrix type** | **Method type** | **Method LOQ** | **Principle of method (*i.e.* GC-MS or HPLC-UV)** | **Author(s), year / missing** |
| Milk | Primary | 0.01 mg/kg | HPLC-MS/MS ESI+ | Stewart J., 2003, Data point: 4.2/5 |
| | ILV | 0.01 mg/kg | HPLC-MS/MS ESI+ | Sears K., 2013a, Data point: 4.2/7 |
| | Confirmatory (if required) | Not required | | |
| Eggs | Primary | 0.01 mg/kg | HPLC-MS/MS ESI+ | Sears K., 2013a, Data point: 4.2/5 |
| | ILV | 0.01 mg/kg | HPLC-MS/MS ESI+ | Stewart J., 2003, Data point: 4.2/7 |
| | Confirmatory (if required) | Not required | | |
| Muscle | Primary | 0.01 mg/kg | HPLC-MS/MS ESI+ | Stewart J., 2003, Data point: 4.2/5 |
| | ILV | 0.01 mg/kg | HPLC-MS/MS ESI+ | Sears K., 2013a, Data point: 4.2/7 |
| | Confirmatory (if required) | Not required | | |
| Fat | Primary | 0.01 mg/kg | HPLC-MS/MS ESI+ | Stewart J., 2003, Data point: 4.2/5 |
| | ILV | 0.01 mg/kg | HPLC-MS/MS ESI+ | Sears K., 2013a, Data point: 4.2/7 |
| | Confirmatory (if required) | Not required | | |
| Kidney, liver | Primary | 0.01 mg/kg | HPLC-MS/MS ESI+ | Stewart J., 2003, Data point: 4.2/5 |
| | ILV | 0.01 mg/kg | HPLC-MS/MS ESI+ | Sears K., 2013a, Data point: 4.2/7 |
| | Confirmatory (if required) | Not required | | |

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

| | **Method for products of animal origin** |
|---|---|
| Required, available from: | RAR 2016: Stewart J., 2003, Data point: 4.2/5: Analytical method D0303 uses acidified methanol/water to extract imazamox residues from livestock tissues and<br>acidified acetonitrile for milk and fat. Acidified methanol/water has been shown to adequately extract<br>imazamox residues from plant matrices, since the residues of concern in animal matrices (parent and the hydroxy<br>metabolite CL 263284) are the same as for the plant matrices, their extraction efficiency can be regarded as<br>being covered by the work provided in the plant metabolism studies.<br>In addition, in the two goat metabolism studies (IA-440-001 Kao 1994 and ID-440-002 Johnson 1994), |

| | **Method for products of animal origin** |
|---|---|
| | extraction solvents of methanol and 80/20 methanol/water extracted 91-99% of the total radioactivity, respectively, from goat kidney (the only organ with significant radioactive residue). |
| Not required, because: | N/D |

### 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 imazamox in soil is given in the following tables. For the detailed evaluation of new studies it is referred to Appendix 2.

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

| **Component of residue definition: imazamox + CL 312622 + CL 354825** | | | |
|---|---|---|---|
| **Method type** | **Method LOQ** | **Principle of method (*i.e.* GC-MS or HPLC-UV)** | **Author(s), year / missing** |
| Primary | 0.001 mg/kg for imazamox, CL 312622 and CL 354825 | LC-MS/MS | Gooding R., 2013b, Data point: 4.1.2/1 |
| Confirmatory | Not required | | |

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

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

An overview on the acceptable methods and possible data gaps for analysis of imazamox in surface and drinking water is given in the following tables. There are no new or additional studies.

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

| **Component of residue definition: imazamox+CL312622+354825** | | | | |
|---|---|---|---|---|
| **Matrix type** | **Method type** | **Method LOQ** | **Principle of method (i.e. GC-MS or HPLC-UV)** | **Author(s), year / missing** |
| Drinking water | Primary | 0.025 µg/L | LC-MS/MS | Toledo F., 2013a, Data point 4.1.2/3<br>Holzer S.., 2013a, Data point 4.1.2/4 |
| | ILV | 0.025 µg/L | LC-MS/MS | Schmitt J.L.,Patel D., 2013a, Data point 4.1.2/5 |
| | Confirmatory | Not required | | |
| Surface water | Primary | 0.025 µg/L | LC-MS/MS | Toledo F., 2013a, Data point 4.1.2/3<br>Holzer S.., 2013a, Data point 4.1.2/4 |
| | Confirmatory | Not required | | |

For any special comments or remarkable points concerning the analytical methods for water please refer

to Appendix 2.

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

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

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

| **Component of residue definition: imazamox** | | | |
|---|---|---|---|
| **Method type** | **Method LOQ** | **Principle of method (i.e. GC-MS or HPLC-UV)** | **Author(s), year / missing** |
| Primary | 444 µg/m$^3$ | HPLC-MS/MS | Bacher R., 2013b, Data point: 4.1.2/2 |
| Confirmatory | Not required | | |

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

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

Not performed, not required for this preparation.

zRMS comments:
According to the Commission Regulation (EU) No 283/2013 methods with a full description shall be submitted for the analysis in body fluids and tissues for active substances and relevant metabolites.

### 5.3.2.8 Other studies/ information

Not performed, not required for this preparation.

# 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 5.1.1/1 | Gutowska I. | 2018 | *Development and validation of the method for determination of the active substance content in the preparation*<br>Testing facility: Institute of Industrial Organic Chemistry Warsaw 2018<br>Report No.: BA-85/18<br>GLP: Yes<br>Unpublished | N | Chemirol |
| KCP 5.1.1/2 | Foster B. | 2020 | *Validation of Method for Determination of Cyanide Ion in CHR/H/IMA 40 SL*<br>Testing facility: Concept Life Sciences Analytical & Development Services Limited, Unit 69, Listerhills Science Park, Campus Road, Bradford, BD7 1HR, UK, 2020,<br>Report No.: CLS3_0339_0002<br>GLP: Yes<br>Unpublished | N | Chemirol |
| KCP 5.2/01 | Wołoszynowska M. | 2022 | *Method validation for determination of the residues of Imazamox and its salts in dry pea and whole plants w/o roots*<br>Testing facility: Łukasiewicz Research Network, Institute of Industrial Organic Chemistry, Warsaw, Poland, 2022,<br>Project no. 1/21/019 K<br>GLP: Yes<br>Unpublished | N | Chemirol |
| KCP 5.2/02 | Sahvorost, N. | 2022 | *Validation of the Analytical Method for Determination of Imazamox and Metabolites in Honey*<br>Testing facility: Eurofins Agroscience Services EcoChem GmbH, Niefern-Öschelbronn, Germany<br>Project no. S22-02937<br>GLP: Yes<br>Unpublished | N | Chemirol |

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

| Data point | Author(s) | Year | Title<br>Company Report No.<br>Source (where different from company)<br>GLP or GEP status<br>Published or not | Vertebrate study<br>Y/N | Owner |
|---|---|---|---|---|---|
| KCP 5.2/01 | Lehmann A. | 2013a | Validation of BASF method no. L0188/01:<br>Method for the determination of Imazamox (BAS 720 H, Reg.No. 4096483) and its metabolites Reg.No. 4110542 (CL312622), Reg.No. 4110773 (CL263284) and Reg.No. 4110445 (CL189215) in plant matrices<br>2012/1294678 BASF SE, Limburgerhof, Germany Fed.Rep.<br>yes<br>Unpublished | N | BASF |
| KCP 5.2/02 | Mewis A. | 2013a | Independent laboratory validation (ILV) of an analytical method L0188/01 for the determination of BAS 720 H and 3 metabolites in plant matrices 2013/1249356<br>Eurofins Agroscience Services GmbH, Niefern Oeschelbronn,<br>Germany Fed.Rep.<br>yes<br>Unpublished | N | BASF |
| KCP 5.2/03 | Stewart J. | 2003a | Method validation of BASF Analytical Method D0303 entitled Method for the Determination of BAS 720 H (CL 299263) and its metabolite CL 263284 in bovine matrices using LC/MS/MS 2003/5000116 BASF Agro Research RTP, Research Triangle Park NC, United States of America<br>yes<br>Unpublished | N | BASF |
| KCP 5.2/04 | Gooding R.F. | 2013a | Validation of BASF analytical method D0303:<br>Method for the determination of BAS 720 H (CL 299263) and its metabolite CL 263284 in animal matrices using LC-MS/MS 2013/7002842 BASF Crop Protection, Research Triangle Park<br>NC, United States of America<br>yes | N | BASF |

| 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 |
|---|---|---|---|---|---|
| | | | Unpublished | | |
| KCP 5.2/05 | 2013 a | Sears K. | Independent laboratory validation of analytical method number D0303: Method for determination of BAS 720 H (CL 299263) and its metabolite CL 263284 residues in animal matrices using LC-MS/MS 2013/7002962 Pyxant Labs Inc., Colorado Springs CO, United States of America<br>yes<br>Unpublished | N | BASF |
| KCP 5.2/06 | Bacher R. | 2013 b | Development and validation of an analytical method for the determination of Imazamox in air 2013/1134980 PTRL Europe, Ulm, Germany Fed.Rep.<br>yes<br>Unpublished | N | BASF |
| KCP 5.2/07 | Toledo F. | 2013 a | Method development and validation of an analytical method for the determination of BAS 720 H and its 2 metabolites Reg.No 411060 and Reg.No 4110542 in water (analytical method L0209)2013/1224024 SGS Institut Fresenius GmbH, Taunusstein, Germany Fed. Rep.<br>yes<br>Unpublished | N | BASF |
| KCP 5.2/08 | Holzer S. | 2013 a | Method development and validation of an analytical method for the determination of BAS 720 H and its 2 metabolites Reg.No 4110603 and Reg.No 4110542 in water (analytical method L0209) 2013/1327750 SGS Institut Fresenius GmbH, Taunusstein, Germany Fed. Rep.<br>yes<br>Unpublished | N | BASF |

**List of data submitted by the applicant and not relied on**

| | | | | **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 Y/N** | **Owner** |
|---|---|---|---|---|---|
| | | | | | |
| | | | | | |

# Appendix 2 Detailed evaluation of submitted analytical methods

## A 2.1 Analytical methods for imazamox

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

No new or additional studies have been submitted.

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

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

#### A 2.1.2.1.1 Analytical method 1 – residues determination in plant origin matrices

##### A 2.1.2.1.1.1 Methods validation

**Method 1**

| | |
|---|---|
| Comments of zRMS | The main study report indicated to the too high levels of fortification in the recovery studies (both levels above the applicable MRL, which is 0.05 mg/kg for peas). According to the SANTE/2020/12830 rev. 2 recovery and repeatability (as precision, % RSD) data must be reported for the following fortification levels: LOQ (n=5) and 10 times LOQ, or MRL (n=5) or other relevant level.<br>The Applicant provided Annex No 1 to validation report (January 2024) with the following explanation:<br>*Some parts of the report 'Method validation for determination of the residues of Imazamox and its salts in dry pea and whole plants w/o roots' were unclear, therefore the owner of the study requested verification of the calculations performed in the final report and included in annex I to the report. Some tables were presented briefly and were not clearly described. Below corrections, explanations and tables with full data were presented. Preparation of this annex need no additional new analysis not included in the original validation report CHR/H/IMA 40 SL Method validation for determination of the residues of Imazamox and its salts in dry pea and whole plants w/o roots. All calculations were based on the data from original report.*<br>Based on the data presented in Annex I to the main study report, it can be concluded that the method is validated in accordance with SANTE/2020/12830, Rev. 2 and can be accepted. |

| | |
|---|---|
| Reference: | KCP 5.2/01 |
| Report | Wołoszynowska M., Method validation for determination of the residues of Imazamox and its salts in dry pea and whole plants w/o roots, Łukasiewicz |

| | |
|---|---|
| | Research Network, Institute of Industrial Organic Chemistry, Warsaw, Poland, 2022, Project no. 1/21/019 K |
| Guideline(s): | SANTE/2020/12830 Rev., 24 February 2021 (2000) |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes |

**Materials and methods**
**Analyitcal standard**

- Imazamox, IPO 942, batch no. 2A/21, purity 99,7%, storage conditions - refrigerator (Appendix no.1)
- 5-(hydroxymethyl)-2-(4-isopropyl-4-methyl-5-oxo-4,5-dihydro-1H-imidazol-2-yl)nicotinic acid (CL 263284), batch no. 1_210324_2_18_RR_02_04, purity 99,04%, storage conditions – freezer (Appendix no.2)
- 2-(4-isopropyl-4-methyl-5-oxo-4,5-dihydro-1H-imidazol-2-yl)-5-(((2R,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)methyl)nicotinic acid (CL 189215), batch no. 1_210324_2_2_8_RR_16, purity 98,71%, storage conditions – freezer (Appendix no.3)
- Imazapic, Dr Ehrenstorfer, batch no. G1001373, purity 96,96%, storage conditions – refrigerator (Appendix no.4)

**Reagents**

- Deionized water, ultra-pure, Millipore
- Acetonitrile hypergrade for LC-MS, Supelco
- Formic acid > 95%, Sigma-Aldrich
- Ammonium acetate ≥ 99%, HyperSolv CHROMANORM

**Chromatographic conditions**

- Column temperature: 40 °C
- Mobile phase: 5 mmol aqueous solution of ammonium acetate + 0,1% aqueous solution of formic acid (A) + 5 mmol acetonitrile solution of ammonium acetate + 0,1% acetonitrile solution of formic acid (B) (A+B; v/v)
- Flow rate: 0.4 ml/min
- Volume of sample injected: 5 µl

**Results and discussions**

**Table A 1: Recovery results from method validation of imazamox and metabolites CL 189215, CL 263284 using the analytical method**

| Matrix | Analyte | Fortification level (mg/kg) ~~(n = x)~~ (n=5) | Mean recovery (%) | RSD (%) | Comments |
|---|---|---|---|---|---|
| Dry pea | Imazamox | 0.005563 | 106.72 | 2.42 | - |
| | | 0.04172 | 107.02 | 1.69 | - |
| | CL 189215 | 0.004110 | 101.12 | 3.54 | - |
| | | 0.04110 | 98.42 | 11.28 | - |
| | CL 263284 | 0.003970 | 80.75 | 1.45 | - |
| | | 0.04962 | 85.08 | 1.39 | - |
| Whole plant w/o roots | Imazamox | 0.005249 | 89.25 | 2.08 | - |
| | | 0.03150 | 105.04 | 0.81 | - |
| | CL 189215 | 0.00304 | 79.35 | 1.66 | - |
| | | 0.0203 | 98.56 | 3.56 | - |
| | CL 263284 | 0.007939 | 85.71 | 9.70 | - |
| | | 0.07939 | 79.55 | 1.88 | - |

**Table A 2: Characteristics for the analytical method used for validation of imazamox residues in dry pea**

| | | Imazamox | CL 189215 | CL 263284 |
|---|---|---|---|---|
| **Specificity** | | The chromatograms of standard(s) at the lowest calibration level, matrix blanks and samples fortified at the lowest fortification level for each analyte/matrix combination were performed and superimposed. The obtained results are presented in ***Fig. 1 - 3***. **There are no interferences between the analytes and other components of the specimen.** | | |
| Calibration (type, number of data points) | | Matrix matched calibration curve; 5 data points | Matrix matched calibration curve; 5 data points | Matrix matched calibration curve; 5 points |
| Calibration range | | 0.0022 – ~~0.0113~~ 0.1113 mg/kg | 0.0021 – 0.4110 mg/kg | 0.0040 – 0.1985 mg/kg |
| Assessment of matrix effects is presented | | 3.74% - negligible matrix effect (<20%) | 7.62% - negligible matrix effect (<20%) | 3.34% - negligible matrix effect (<20%) |
| Limit of determination/quantification | LOD | 0.0022 mg/kg | 0.0021 mg/kg | 0.0040 mg/kg |
| | LOQ | 0.0056 mg/kg | 0.0041 mg/kg | ~~0.0079 mg/kg~~ 0.004 mg/kg |

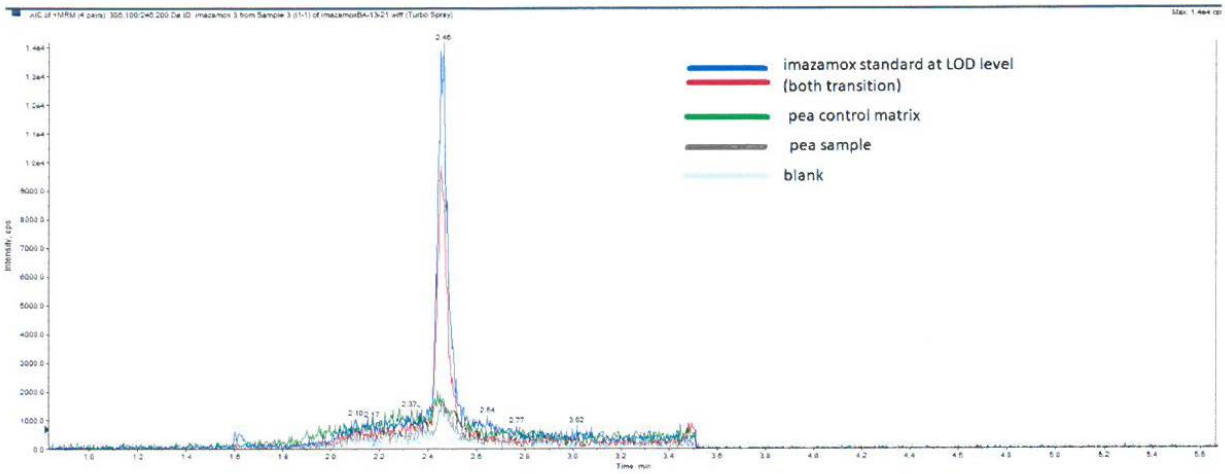



**Fig. 1. Specificity of imazamox** Overlaid chromatograms of: standard solution of imazamox at LOD level, pea control matrix, pea sample and blank solvent.

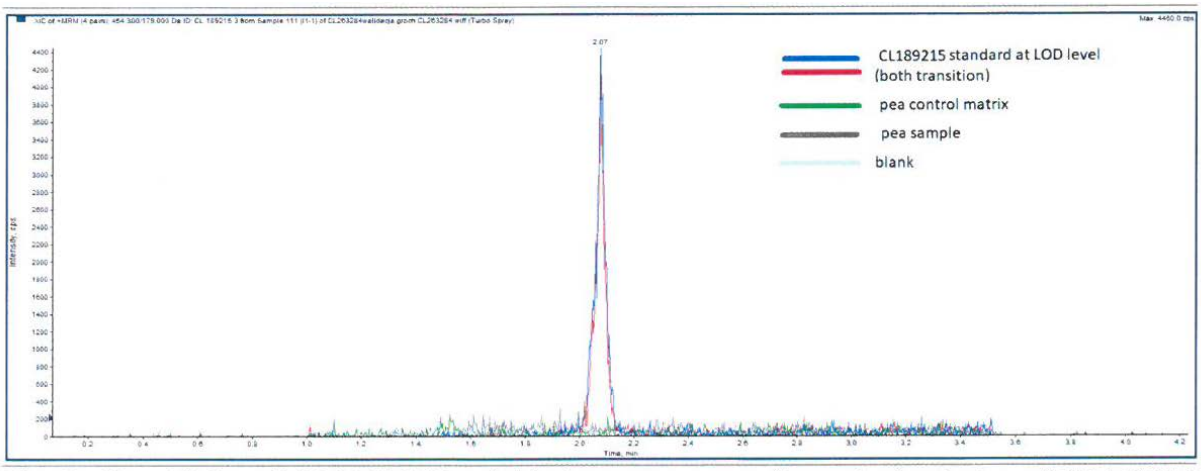



**Fig. 2. Specificity of CL189215**Overlaid chromatograms of: standard solution of CL189215 at LOD level, pea control matrix, pea sample and blank solvent.

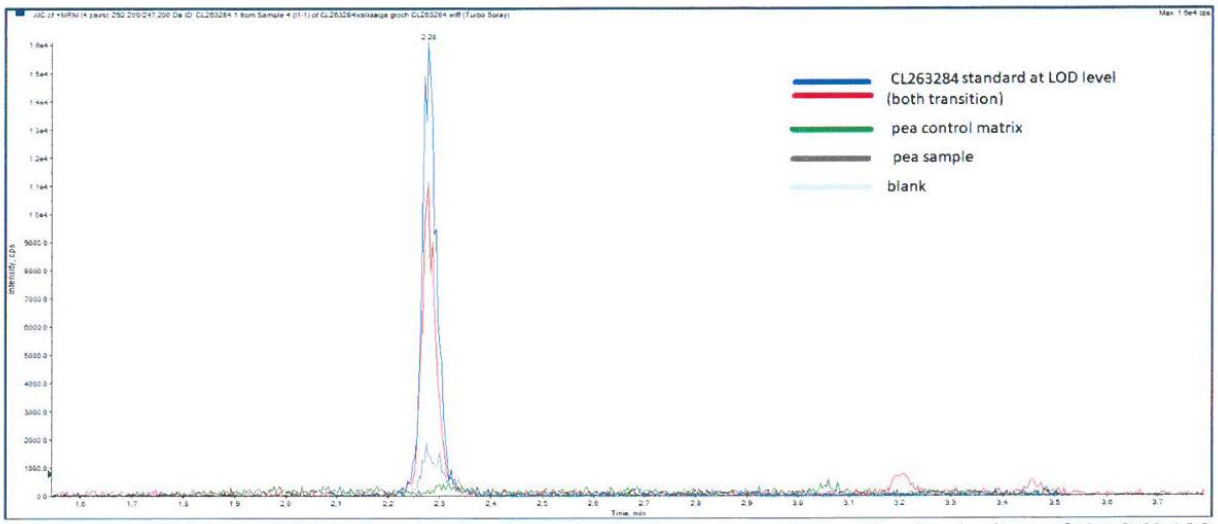



**Fig. 3. Specificity of CL263484.** Overlaid chromatograms of: standard solution of CL263482 at LOD level, pea control matrix, pea sample and blank solvent.

**Table A 3: Characteristics for the analytical method used for validation of imazamox residues in whole plant w/o roots**

| | | Imazamox | CL 189215 | CL 263284 |
|---|---|---|---|---|
| **Specificity** | | The chromatograms of standard(s) at the lowest calibrated level, matrix blanks and samples fortified at the lowest fortification level for each analyte/matrix combination were performed and superimposed. The obtained results are presented in ***Fig. 16-18***. **There are no interferences between the analytes and other components of the specimen.** | | |
| Calibration (type, number of data points) | | Matrix matched calibration curve; 5 points | Matrix matched calibration curve; 5 points | Matrix matched calibration curve; 5 points |
| Calibration range | | 0.0026-0.0840 mg/kg | 0.0020-0.0406 mg/kg | 0.0020-0.1985 mg/kg |
| Assessment of matrix effects is presented | | 27.93% - minor matrix effect (20-50%) | 16.95% - negligible matrix effect (<20%) | 29.52% - minor matrix effect (20-50%) |
| Limit of determination/quantification | LOD | 0.0026 mg/kg | 0.0020 mg/kg | 0.0020 mg/kg |
| | LOQ | 0.0052 mg/kg | 0.0030 mg/kg | 0.0079 mg/kg |

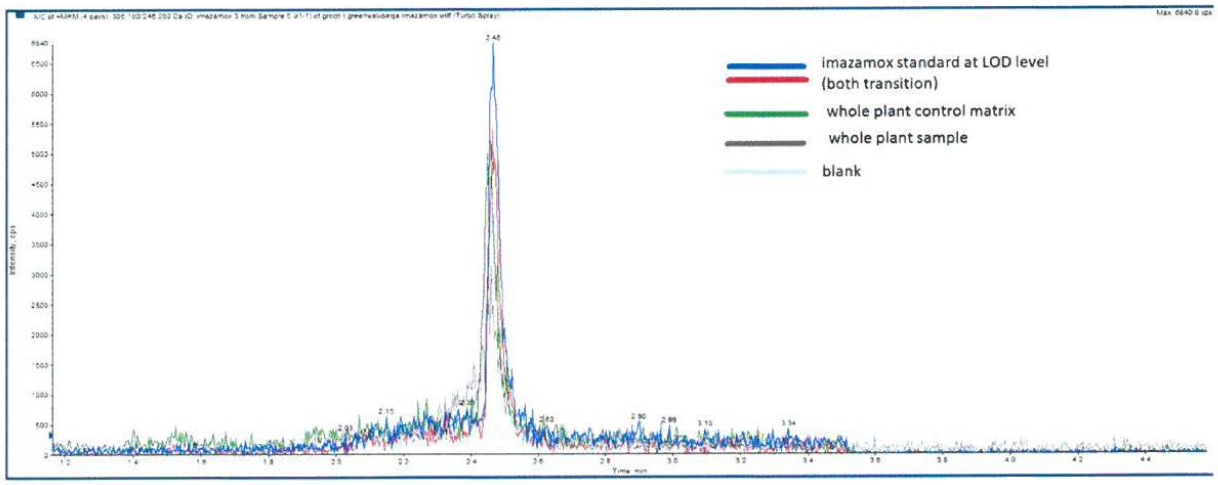



**Fig. 16. Specificity of imazamox** Overlaid chromatograms of: standard solution of imazamox at LOD level, whole plants w/o roots control matrix, whole plant w/o roots sample and blank solvent.

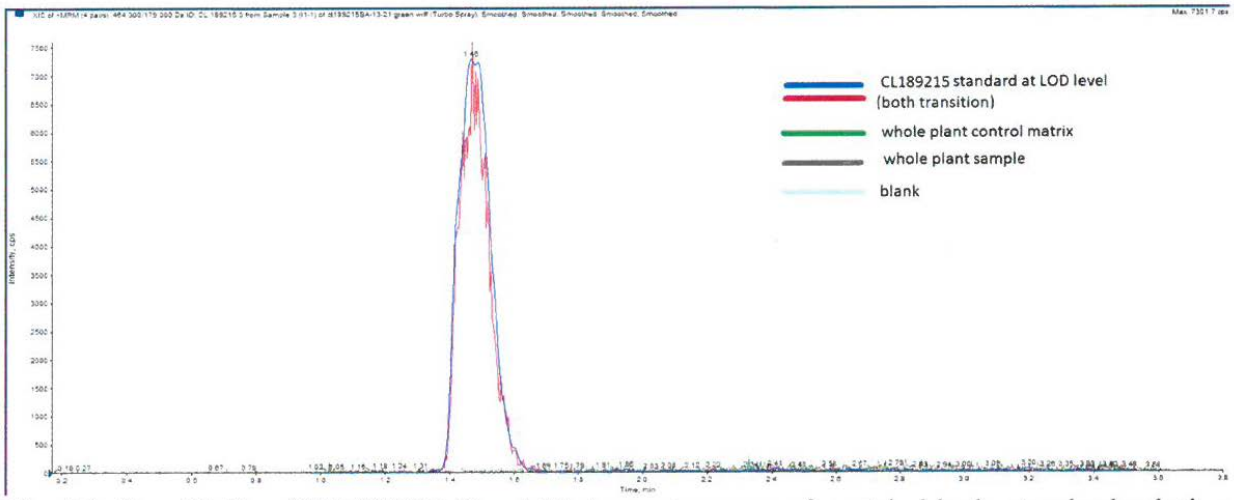



**Fig. 17. Specificity of CL189215** Overlaid chromatograms of: matrix blank, standard solution of CL189215 at LOD level, whole plants w/o roots control matrix, whole plant w/o roots sample and blank solvent.

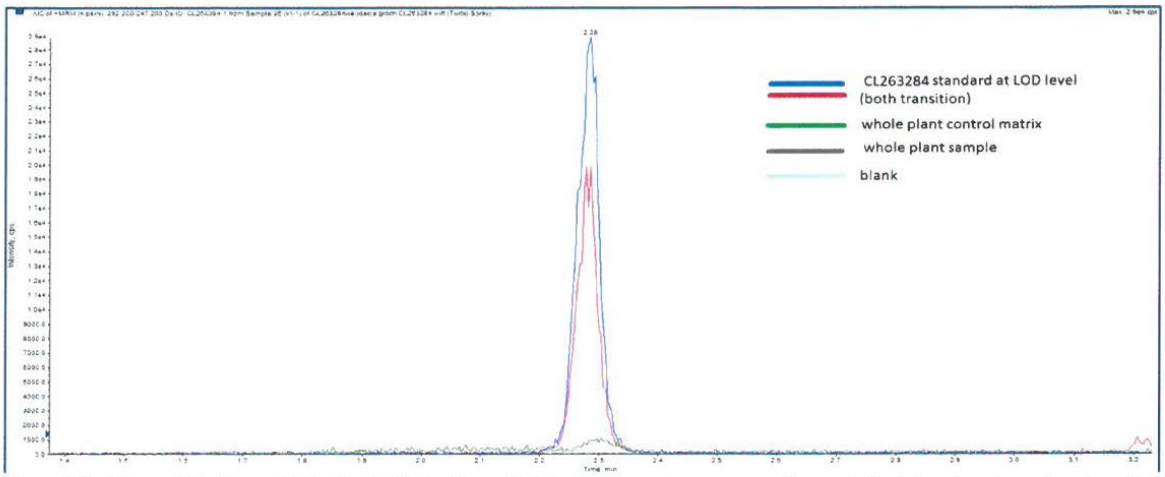



**Fig. 18. Specificity of CL263484.** Overlaid chromatograms of: matrix blank, standard solution of CL263482 at LOD level, whole plants w/o roots control matrix, whole plant w/o roots sample and blank solvent.

**Conclusion**

SANTE/2020/12830 Rev., 24 the method for determination of active substance imazamox in CHR/H/IMA 40 SL preparation is specific. The validation parameters for linearity, instrument precision, repeatability and accuracy are within the acceptance range. The advantage of this method is also the small or negligible influence of the matrix.

## Method 2

| | |
|---|---|
| Comments of zRMS | The method was successfully validated for the determination of imazamox, CL312622, CL189215 and CL263284 according to the SANTE/2020/12830, rev. 2. |

| | |
|---|---|
| Reference: | KCP 5.2/02 |
| Report | Sahvorost, N., Validation of the Analytical Method for Determination of Imazamox and Metabolites in Honey, Eurofins Agroscience Services EcoChem GmbH, Niefern-Öschelbronn, Germany, 2022, Project no. S22-02937 |
| Guideline(s): | SANTE/2020/12830 Rev., 24 February 2021 (2000) |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes |

## Materials and methods

The objective of the study was to validate a multi-residue method for the determination of imazamox and its metabolites (CL263284, CL189215 and CL312622) in honey in accordance to guidance document SANTE/2020/12830, rev.1 for monitoring. The limit of quantification was 0.01 mg/kg for imazamox and CL312622, 0.00674 mg/kg for CL189215 and 0.0105 mg/kg for CL263284 expressed as parent equivalent (imazamox).
In brief, samples of honey were dissolved in demineralized water and extracted by addition of methanol containing 1 % formic acid and addition of water by shaking on a flatbed shaker. After centrifuging the sample was used for LC-MS/MS detection.
Sample concentration in final extract: 0.05 g sample per mL of extract
Quantification: LC-MS/MS

**Table 5.3-5: Methods suitable for the determination of the residues in plant protection product (PPP) CHR/H/IMA 40 SL**

| | **Residues in honey** |
|---|---|
| **Author(s), year** | N. Sahvorost, 2022 |
| **Principle of method** | LC MS/MS |
| **Linearity (linear between mg/L) (correlation coefficient, expressed as r)** | The linearity of the detector response was demonstrated by single determination of matrix-matched calibration standards at a minimum of eight concentration levels ranging from 0.15 ng/mL to 10 ng/mL for Imazamox and CL312622 expressed as parent equivalent. For CL263284 concentration levels were ranging from 0.157 ng/mL to 10.5 ng/mL expressed as parent equivalent and for CL189215 concentration levels were ranging from 0.101 ng/mL to 6.74 ng/mL expressed as parent equivalent.<br>This ranges correspond to a mass fraction level of 0.003 mg/kg to 0.2 mg/kg or rather 0.00202 mg/kg to 0.135 mg/kg (for CL189215) and 0.00314 mg/kg to 0.210 mg/kg (CL263284) expressed as parent equivalent and thus cover the range from no more than 30 % of the limit of quantification (LOQ) and at least + 20 % of |

| | **Residues in honey** |
|---|---|
| | the highest analyte concentration detected in any sample extract. The calibration curve does not exceed two orders of magnitude.<br>Linear regression was performed with 1/x-weighting. The calibration curves obtained for both ion mass transitions were linear and the regression residuals were randomly distributed on visual inspection.<br>Furthermore, correlation coefficients (R) were > 0.995. |
| **Precision, accuracy and uncertainty** | Recovery data was generated from five samples fortified at the limit of quantification (LOQ) and five samples fortified at the 10-fold higher concentration than the LOQ. Precision of the method was determined as the relavite standard deviation (RSD) of recovery at each fortification level.<br>The mean recovery at each fortification level should be in the range of 70-120%. Wherever applicable (n≥3), the relative standard deviation was determined and should be ≤20% for each level. |
| **Selectivity** | LC-MS/MS method was used during the study. Two mass transitions were evaluated and used for quantification. The specificity of the method was evaluated on the basis of the analysis of chromatograms recorded for the matrix blank samples. No interferences at above 30% of the LOQ were detected at the retention time of active substance in matrix blank samples |
| **Matrix Effects** | Matrix effects was significant (≥ 20 %) for Imazamox and insignificant (< 20 %) for CL312622, CL189215 and CL263284. |
| **LOQ** | For Imazamox and CL312622:<br>0.01 mg/kg (lowest validated fortification level)<br>For CL189215:<br>0.00674 mg/kg (lowest validated fortification level)<br>For CL263284:<br>0.0105 mg/kg<br>(for metabolites defined as parent equivalent) |
| **Comment** | The validation parameters are within the acceptance range and fulfil EU requirements given in SANTE/2020/12830 Rev.1. |

**Conclusion**

The method was successfully validated for the determination of imazamox and CL312622 in honey from the tested LOQ of 0.01 mg/kg up to 0.1 mg/kg, for CL189215 from the tested LOQ of 0.0067 mg/kg up to 0.067 mg/kg and for CL263284 from the tested LOQ of 0.0105 mg/kg up to 0.105 mg/kg according to the guidance document SANTE/2020/12830, rev. 1 for monitoring.

### A 2.1.2.1.1.2 Independent laboratory validation

No new or additional studies have been submitted

### A 2.1.2.1.1.3 Confirmatory method (if required)

No confirmatory method is required

### A 2.1.2.1.1.4 Extraction efficiency

No new or additional studies have been submitted

### A 2.1.2.1.2 Analytical method 2

No new or additional studies have been submitted

### A 2.1.2.1.3 Extraction efficiency

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