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| FINAL REGISTRATION REPORT  Part B  Section 5  Analytical Methods  Detailed summary of the risk assessment |
| Product code: CHR/RW/PROH 100 WG  Product name(s): Heksal 100 WG, Jamur 100 WG  Chemical active substance(s):  Prohexadione calcium, 100 g/kg |
| Central Zone  Zonal Rapporteur Member State: Poland |
| CORE ASSESSMENT |
| Applicant: Innvigo Sp. z o.o.  Submission date: July 2021  MS Finalisation date: 22/04/2022 |

Version history

|  |  |
| --- | --- |
| When | What |
| September 2021 | Dossier sent for evaluation to Merit Mark (PL) |
| December 2021 | zRMS finalised evaluation |
| April 2022 | Final version prepared by zRMS after Commenting period |
|  |  |

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Evaluator comments:

The text highlighted in grey was provided by the evaluator.

# Analytical methods

## Conclusion and summary of assessment

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

Noticed data gaps are: none

Sufficiently sensitive and selective analytical methods are available for all analytes included in the residue definitions (see DAR). The applicant’s dRR was not rewritten. zRMS text is on grey background.

Noticed data gaps are: none

| Commodity/crop | Supported/ Not supported |
| --- | --- |
| ~~Apples~~ pome fruits | Supported |
| ~~Winter whea~~t cereals | Supported |

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

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

#### 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 prohexadione calcium in plant protection product is provided as follows:

|  |  |
| --- | --- |
| Comments of zRMS: | This method is accepted and can be used for analysing Prohexadione calcium in the PPP. |

|  |  |
| --- | --- |
| Reference: | KCP 5.1.1 |
| Report | *CHR/RW/PROH 100 WG Determination of physicochemical properties,* Patrzałek, M., 2019, ICB/91/2019 |
| Guideline(s): | SANCO/3029/99 - rev.4. |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes |

Materials and methods

Determination of the content of prohexadion-calcium was conducted according to validated analytical method SPB/138. Content of prohexadion-calcium in the test item at a level of 10 % accordingly was determined by liquid chromatography with diode array detection (HPLC-DAD).

The content of active substance in **CHR/RW/PROH 100 WG** formulation is:

Prohexadione calcium – **100.3 g/L ± 5%**

Validation - Results and discussions

Table 5.2‑1: Methods suitable for the determination of active substances Prohexadione calcium in plant protection product CHR/RW/PROH 100 WG

|  | Prohexadione calcium |
| --- | --- |
| Author(s), year | Patrzałek, M., 2019 |
| Principle of method | HPLC-DAD |
| Linearity  (linear between  mg/L / % range of the declared content)  (correlation coefficient, expressed as r) | According to SPT/31, the parameters obtained as a result of validation should meet the following criteria:  - linearity R2 ≥ 0.99,  - Horwitz ratio ≤ 1.0.  The resulting curves are linear in the tested concentrations. Linearity range is from 0.515 to 309.246 [μg/mL]. Correlation coefficients R2 are 0.9996180 and R2 = 0.9996514. |
| Precision – Repeatability Mean and Accuracy  (%RSD) | According to SPT/31, the parameters obtained as a result of validation should meet the following criteria:  - Horwitz ratio ≤ 1.0 |
| Interference/ Specificity | Specificity of the method was evaluated based on the analysis of chromatograms for blank samples (placebo) against samples of placebo spiked with prohexadione-calcium standards. Analysis showed no overlapping of determined substances signal with the signals of matrix components under method conditions hence method specificity criterion is fulfilled. |
| Comment | The determined validation parameters such as specificity, linearity, limit of quantification (LOQ), repeatability (precision) and accuracy are compliant with EU requirements given in SANCO/3030/99 rev.4. |

Conclusion

It was confirmed that the method is specific. There were no peaks from placebo interfering with deter-mined compounds. The validation parameters (specificity, linearity, instrument precision, repeatability and accuracy) are within the acceptance range and fulfil EU requirements given in SANCO/3030/99 -rev.4.

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

Prohexadione calcium possess no impurities.

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

Please refer to PART C – Confidential data.

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

Analytical methods for determination of Prohexadione calcium impurities and relevance of CIPAC methods in CHR/RW/PROH 100 WG were not evaluated as part of the EU review. Therefore, all relevant data are provided and are considered adequate.

### 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 Prohexadione calcium for the generation of pre-authorization data is given in the following table.

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

| Component of residue definition: Prohexadione calcium | | | | |
| --- | --- | --- | --- | --- |
| Matrix type | Method type | Method LOQ | Principle of method  (i.e. GC-MS or HPLC-UV) | Author(s), year / missing / EU agreed |
| Cereal  (forage) | Primary | 0.01 mg/kg | HPLC-MS/MS | Lehmann, A., Mackenroth, C., 2005, *Validation of the analytical method 564/0: Method for the determination of prohexadione-Ca in plant and animal matrices*  2005/1007581  Oppinger, M., Kerl, W., 2005, *Technical procedure: Method for the determination of prohexadione-Ca in plant and animal matrices*  2005/1007580 |
| Confirmatory  (if required) | N/A | N/A |
| Cereal  (straw) | Primary | 0.01 mg/kg | HPLC-MS/MS |
| Confirmatory  (if required) | N/A | N/A |
| Cereal  (grain) | Primary | 0.01 mg/kg | HPLC-MS/MS |
| Confirmatory  (if required) | N/A | N/A |
| Apple fruit | Primary | 0.01 mg/kg | HPLC-MS/MS |
| Confirmatory  (if required) | N/A | N/A |
| Lemon fruit | Primary | 0.01 mg/kg | HPLC-MS/MS |
| Confirmatory  (if required) | N/A | N/A |
| Oilseed rape seed | Primary | 0.01 mg/kg | HPLC-MS/MS |
| Confirmatory  (if required) | N/A | N/A |
| Bovine muscle | Primary | 0.01 mg/kg | HPLC-MS/MS |
| Confirmatory  (if required) | N/A | N/A |
| Bovine liver | Primary | 0.01 mg/kg | HPLC-MS/MS |
| Confirmatory  (if required) | N/A | N/A |
| Bovine kidney | Primary | 0.01 mg/kg | HPLC-MS/MS |
| Confirmatory  (if required) | N/A | N/A |
| Milk | Primary | 0.01 mg/kg | HPLC-MS/MS |
| Confirmatory  (if required) | N/A | N/A |
| Egg | Primary | 0.01 mg/kg | HPLC-MS/MS |
| Confirmatory  (if required) | N/A | N/A |
| Soil | Primary | 0.01 mg/kg | HPLC-MS/MS | Richter, T., Laik, W., 2004, *Validation of method No. 569/0 for the determination of BAS 125 W (prohexadione calcium) residues in soil by HPLC-MS/MS*  2004/1022530 |
| Confirmatory  (if required) | N/A | N/A | N/A |
| Surface water | Primary | 0.05 μg/L | HPLC-MS/MS | Richter, T., Laik, W., 2004, *Validation of analytical method 548/0 for the determination of prohexadione- (Calcium) in tap and surface water by HPLC-MS including storage stability investigations in solvents*  2004/1009167 |
| Confirmatory  (if required) | N/A | N/A | N/A |
| Tap water | Primary | 0.05 μg/L | HPLC-MS/MS | Richter, T., Laik, W., 2004, *Validation of analytical method 548/0 for the determination of prohexadione- (Calcium) in tap and surface water by HPLC-MS including storage stability investigations in solvents*  2004/1009167 |
| Confirmatory  (if required) | N/A | N/A | N/A |
| Air | Primary | 6.1 μg/m3 | HPLC/UV | Zangmeister, W., 2000, *Validation of analytical method 459. Determination of BAS 125 W (Reg. No. 285342) in air by HPLC-UV* |
| Confirmatory  (if required) | 6.1 μg/m3 | HPLC/UV | Zangmeister, W., 2005, *Validation of the confirmatory method to analytical method 459: Determination of BAS 125 W (Reg. No.285342) in air by HPLC-UV*  2005/1004788 |
| Body fluids and tissues | Primary | Not required, not a toxic compound | | |

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

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

-*DAR, Prohexadione calcium - Volume 3, Annex B.5: Analytical methods (2009)*

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

For Prohexadione calcium all presented methods are sufficient and no new methods are necessary.

### Description of analytical methods for the determination of residues Prohexadione calcium (KCP 5.2)

#### 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 |
| --- | --- | --- | --- |
| Cereal  (forage) | Prohexadione calcium | LOQ = 0.01 mg/kg | Lehmann, A., Mackenroth, C., 2005, *Validation of the analytical method 564/0: Method for the determination of prohexadione-Ca in plant and animal matrices*  2005/1007581  Oppinger, M., Kerl, W., 2005, *Technical procedure: Method for the determination of prohexadione-Ca in plant and animal matrices*  2005/1007580 |
| Cereal  (grain) | Prohexadione calcium | LOQ = 0.01 mg/kg |
| Cereal  (straw) | Prohexadione calcium | LOQ = 0.01 mg/kg |
| Apple fruit | Prohexadione calcium | LOQ = 0.01 mg/kg |

#### 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 Prohexadione calcium in plant matrices is given in the following tables.

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: Prohexadione calcium | | | | |
| --- | --- | --- | --- | --- |
| 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  (Apples) | Primary | 0.01 mg/kg | HPLC-MS/MS | Lehmann, A., Mackenroth, C., 2005, *Validation of the analytical method 564/0: Method for the determination of prohexadione-Ca in plant and animal matrices*  2005/1007581  Oppinger, M., Kerl, W., 2005, *Technical procedure: Method for the determination of prohexadione-Ca in plant and animal matrices*  2005/1007580 |
| ILV | 0.05 mg/kg | GC-MS | Bacher, R., 2004, *Independent laboratory validation (ILV) on BASF analytical method D9608 used for the detemination of Prohexadione-calcium in plant material*  2004/1015913 |
| Confirmatory  (if required) | N/A | N/A | N/A |
| High acid content  (Lemon) | Primary | 0.01 mg/kg | HPLC-MS/MS | Lehmann, A., Mackenroth, C., 2005, *Validation of the analytical method 564/0: Method for the determination of prohexadione-Ca in plant and animal matrices*  2005/1007581  Oppinger, M., Kerl, W., 2005, *Technical procedure: Method for the determination of prohexadione-Ca in plant and animal matrices*  2005/1007580 |
| ILV | 0.05 mg/kg | GC-MS | Bacher, R., 2004, *Independent laboratory validation (ILV) on BASF analytical method D9608 used for the detemination of Prohexadione-calcium in plant material*  2004/1015913 |
| Confirmatory  (if required) | N/A | N/A | N/A |
| High oil content  (Oilseed rape seed) | Primary | 0.01 mg/kg | HPLC-MS/MS | Lehmann, A., Mackenroth, C., 2005, *Validation of the analytical method 564/0: Method for the determination of prohexadione-Ca in plant and animal matrices*  2005/1007581  Oppinger, M., Kerl, W., 2005, *Technical procedure: Method for the determination of prohexadione-Ca in plant and animal matrices*  2005/1007580 |
| ILV | 0.05 mg/kg | GC-MS | Bacher, R., 2004, *Independent laboratory validation (ILV) on BASF analytical method D9608 used for the detemination of Prohexadione-calcium in plant material*  2004/1015913 |
| Confirmatory  (if required) | N/A | N/A | N/A |
| High starch content  (cereals) | Primary | 0.01 mg/kg | HPLC-MS/MS | Lehmann, A., Mackenroth, C., 2005, *Validation of the analytical method 564/0: Method for the determination of prohexadione-Ca in plant and animal matrices*  2005/1007581  Oppinger, M., Kerl, W., 2005, *Technical procedure: Method for the determination of prohexadione-Ca in plant and animal matrices*  2005/1007580 |
| ILV | 0.05 mg/kg | GC-MS | Bacher, R., 2004, *Independent laboratory validation (ILV) on BASF analytical method D9608 used for the detemination of Prohexadione-calcium in plant material*  2004/1015913 |
| Confirmatory  (if required) | N/A | N/A | N/A |

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: | *DAR, Prohexadione calcium - Volume 3, Annex B.5: Analytical methods (2009)* |
| Not required, because: | No new methods submitted |

#### 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 Prohexadione calcium in animal matrices is given in the following tables.

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

| Component of residue definition: Prohexadione calcium | | | | |
| --- | --- | --- | --- | --- |
| 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 | Lehmann, A., Mackenroth, C., 2005, *Validation of the analytical method 564/0: Method for the determination of prohexadione-Ca in plant and animal matrices*  2005/1007581  Oppinger, M., Kerl, W., 2005, *Technical procedure: Method for the determination of prohexadione-Ca in plant and animal matrices*  2005/1007580 |
| ILV | 0.01 mg/kg | HPLC-MS/MS | Anspach, T., 2005, *Independent laboratory validation of BASF method 564/0: Method for the detemination of prohexadione-Ca in plant and animal matrices.*  2005/1006484 |
| Confirmatory  (if required) | N/A | N/A | N/A |
| Eggs | Primary | 0.01 mg/kg | HPLC-MS/MS | Lehmann, A., Mackenroth, C., 2005, *Validation of the analytical method 564/0: Method for the determination of prohexadione-Ca in plant and animal matrices*  2005/1007581  Oppinger, M., Kerl, W., 2005, *Technical procedure: Method for the determination of prohexadione-Ca in plant and animal matrices*  2005/1007580 |
| ILV | 0.01 mg/kg | HPLC-MS/MS | Anspach, T., 2005, *Independent laboratory validation of BASF method 564/0: Method for the detemination of prohexadione-Ca in plant and animal matrices.*  2005/1006484 |
| Confirmatory  (if required) | N/A | N/A | N/A |
| Bovine muscle | Primary | 0.01 mg/kg | HPLC-MS/MS | Lehmann, A., Mackenroth, C., 2005, *Validation of the analytical method 564/0: Method for the determination of prohexadione-Ca in plant and animal matrices*  2005/1007581  Oppinger, M., Kerl, W., 2005, *Technical procedure: Method for the determination of prohexadione-Ca in plant and animal matrices*  2005/1007580 |
| ILV | 0.01 mg/kg | HPLC-MS/MS | Anspach, T., 2005, *Independent laboratory validation of BASF method 564/0: Method for the detemination of prohexadione-Ca in plant and animal matrices.*  2005/1006484 |
| Confirmatory  (if required) | N/A | N/A | N/A |
| Bovine kidney, liver | Primary | 0.01 mg/kg | HPLC-MS/MS | Lehmann, A., Mackenroth, C., 2005, *Validation of the analytical method 564/0: Method for the determination of prohexadione-Ca in plant and animal matrices*  2005/1007581  Oppinger, M., Kerl, W., 2005, *Technical procedure: Method for the determination of prohexadione-Ca in plant and animal matrices*  2005/1007580 |
| ILV | 0.01 mg/kg | HPLC-MS/MS | Anspach, T., 2005, *Independent laboratory validation of BASF method 564/0: Method for the detemination of prohexadione-Ca in plant and animal matrices.*  2005/1006484 |
| Confirmatory  (if required) | N/A | N/A | N/A |

Table 5.3‑5: Statement on extraction efficiency

|  | Method for products of animal origin |
| --- | --- |
| Required, available from: | *DAR, Prohexadione calcium - Volume 3, Annex B.5: Analytical methods (2009)* |
| Not required, because: | No new methods submitted |

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

An overview on the acceptable methods and possible data gaps for analysis of Prohexadione calcium in soil is given in the following tables.

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

| Component of residue definition: Prohexadione calcium | | | |
| --- | --- | --- | --- |
| Method type | Method LOQ | Principle of method  (*i.e.* GC-MS or HPLC-UV) | Author(s), year / missing |
| Primary | 0.01 mg/kg | HPLC-MS/MS | Richter, T., Laik, W., 2004, *Validation of method No. 569/0 for the determination of BAS 125 W (prohexadione calcium) residues in soil by HPLC-MS/MS*  2004/1022530 |
| Confirmatory | N/A | N/A | N/A |

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

An overview on the acceptable methods and possible data gaps for analysis of Prohexadione calcium in surface and drinking water is given in the following tables.

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

| Component of residue definition: Prohexadione calcium | | | | |
| --- | --- | --- | --- | --- |
| Matrix type | Method type | Method LOQ | Principle of method (i.e. GC-MS or HPLC-UV) | Author(s), year / missing |
| Drinking water | Primary | 0.05 μg/L | HPLC-MS/MS | Richter, T., Laik, W., 2004, *Validation of analytical method 548/0 for the determination of prohexadione- (Calcium) in tap and surface water by HPLC-MS including storage stability investigations in solvents*  2004/1009167 |
| ILV | N/A | N/A | N/A |
| Confirmatory | N/A | N/A | N/A |
| Surface water | Primary | 0.05 μg/L | HPLC-MS/MS | Richter, T., Laik, W., 2004, *Validation of analytical method 548/0 for the determination of prohexadione- (Calcium) in tap and surface water by HPLC-MS including storage stability investigations in solvents*  2004/1009167 |
| Confirmatory | N/A | N/A | N/A |

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

An overview on the acceptable methods and possible data gaps for analysis of Prohexadione calcium in air is given in the following tables.

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

| Component of residue definition: Prohexadione calcium | | | |
| --- | --- | --- | --- |
| Method type | Method LOQ | Principle of method  (i.e. GC-MS or HPLC-UV) | Author(s), year / missing |
| Primary | 6.1 μg/m3 | HPLC/UV | Zangmeister, W., 2000, *Validation of analytical method 459. Determination of BAS 125 W (Reg. No. 285342) in air by HPLC-UV*  2000/1000126 |
| Confirmatory | 6.1 μg/m3 | HPLC/UV | Zangmeister, W., 2005, *Validation of the confirmatory method to analytical method 459: Determination of BAS 125 W (Reg. No.285342) in air by HPLC-UV*  2005/1004788 |

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

No methods required as Prohexadione calcium is not classified as toxic or highly toxic.

#### Other studies/ information

Not required

# 

1. Lists of data considered in support of the evaluation

List of data submitted by the applicant and relied on

| Data point | Author(s) | Year | Title Company Report No.  Source (where different from company) GLP or GEP status Published or not | Vertebrate study  Y/N | Owner |
| --- | --- | --- | --- | --- | --- |
| KCP 5.1.1 | Patrzałek, M. | 2019 | *CHR/RW/PROH 100 WG Determination of physicochemical properties*  ICB/91/2019  ICB Pharma, Poland  GLP  Unpublished | N | Chemirol |

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

| Data point | Author(s) | Year | Title Company Report No.  Source (where different from company) GLP or GEP status Published or not | Vertebrate study  Y/N | Owner |
| --- | --- | --- | --- | --- | --- |
| KCP 5.1.2/01  KCP 5.2/01 | Lehmann, A., Mackenroth, C. | 2005 | *Validation of the analytical method 564/0: Method for the determination of prohexadione-Ca in plant and animal matrices*  2005/1007581  GLP  Unpublished | N | BASF |
| KCP 5.1.2/02  KCP 5.2/02 | Oppinger, M., Kerl, W. | 2005 | *Technical procedure: Method for the determination of prohexadione-Ca in plant and animal matrices*  2005/1007580  GLP  Unpublished | N | BASF |
| KCP 5.1.2/03  KCP 5.2/03 | Richter, T., Laik, W. | 2004 | *Validation of method No. 569/0 for the determination of BAS 125 W (prohexadione calcium) residues in soil by HPLC-MS/MS*  2004/1022530  GLP  Unpublished | N | BASF |
| KCP 5.1.2/04  KCP 5.2/04 | Richter, T., Laik, W. | 2004 | *Validation of analytical method 548/0 for the determination of prohexadione- (Calcium) in tap and surface water by HPLC-MS including storage stability investigations in solvents*  2004/1009167  GLP  Unpublished | N | BASF |
| KCP 5.1.2/05  KCP 5.2/05 | Zangmeister, W. | 2000 | *Validation of analytical method 459. Determination of BAS 125 W (Reg. No. 285342) in air by HPLC-UV*  2000/1000126  GLP  Unpublished | N | BASF |
| KCP 5.1.2/06  KCP 5.2/06 | Zangmeister, W. | 2005 | *Validation of the confirmatory method to analytical method 459: Determination of BAS 125 W (Reg. No.285342) in air by HPLC-UV*  2005/1004788  GLP  Unpublished | N | BASF |
| KCP 5.2/07 | Bacher, R. | 2004 | *Independent laboratory validation (ILV) on BASF analytical method D9608 used for the detemination of Prohexadione-calcium in plant material*  2004/1015913  PTRL Europe GmbH; Ulm; Germany  GLP  Unpublished | N | BASF |
| KCP 5.2/08 | Anspach, T. | 2005 | *Independent laboraotry validation of BASF method 564/0: Method for the detemination of prohexadione-Ca in plant and animal matrices.*  2005/1006484  Dr. Specht & Partner Chemische Laboratorien GmbH; Hamburg; Germany  GLP  Unpublished | N | BASF |

1. Detailed evaluation of submitted analytical methods

|  |  |
| --- | --- |
| Report | *CHR/RW/PROH 100 WG Determination of physicochemical properties*, M. Patrzałek, 2019, ICB/91/2019 |
| Guideline(s): | *SANCO /3029 /99 rev.4.* |
| Deviations: | NO |
| GLP: | YES |
| Acceptability: | YES |

**Materials and methods**

In order to the validate determination of content of prohexadion-calcium in the WG formulation, series of measurements (n=5) were prepared by adding active ingredients. standards solutions to the placebo of test item. Validation was conducted in three levels (LOQ) 0.515 μg/mL, (50%) 25.771 μg/mL, (100%) 51.541 μg/mL.

The following points were examined during the study:

**Linearity**

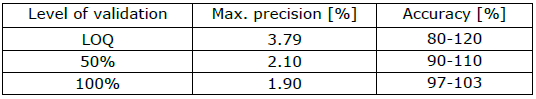
In order to check the linearity, the calibration curves were prepared using standard solutions with the following standard solution prohexadion-calcium concentrations: 0.515; 5.154; 25.771; 51.541; 103.082; 309.246 [μg/mL]. A graph of the peak area to the concentration of prohexadion-calcium was plotted. The resulting curves are linear in the tested concentrations. Linearity range is from 0.515 to 309.246 [μg/mL]. Correlation coefficients R2 are 0.9996180 and R2 = 0.9996514.

**Criteria for linearity, precision and accuracy.**

According to SPT/31, the parameters obtained as a result of validation should meet the following criteria:

- linearity R2 ≥ 0.99,

- Horwitz ratio ≤ 1.0.

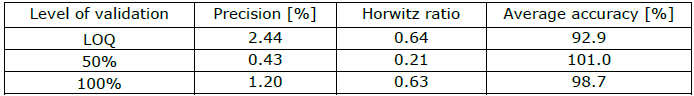


**Specificity**

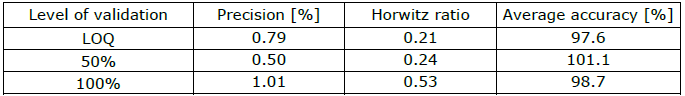
Specificity of the method was evaluated based on the analysis of chromatograms for blank samples (placebo) against samples of placebo spiked with prohexadion-calcium standards. Analysis showed no overlapping of determined substances signal with the signals of matrix components under method conditions hence method specificity criterion is fulfilled.

**Accuracy and precision**

* Primary chromatographic system

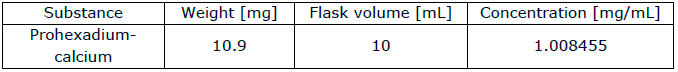


* Secondary chromatographic system

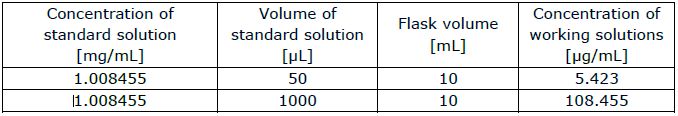


**Preparation of standard solutions**

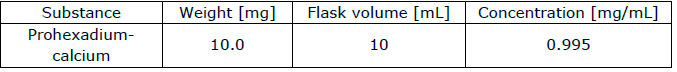
Preparation prohexadion-calcium standard solution in the mixture 0.1% H3PO4/ACN (95/5) (before accelerated storage test).



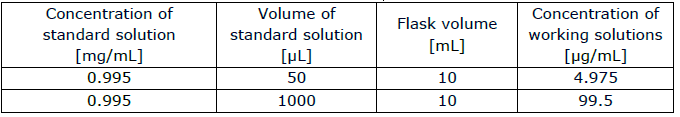
Preparation of prohexadion-calcium working solutions in the mixture 0.1% H3PO4/ACN (95/5) (before accelerated storage test).



Preparation prohexadion-calcium standard solution in the mixture 0.1% H3PO4/ACN (95/5) (after accelerated storage test).



Preparation of prohexadion-calcium working solutions in the mixture 0.1% H3PO4/ACN (95/5) (after accelerated storage test).



**Sample preparation for determination of prohexadion-calcium content.**

Sample of test item was standardized by gentle mixing for 1 minute. Approximately 250 mg of test item was weighed out into 100 mL volumetric flask was filled up to the mark with mixture 0.1% H3PO4/ACN (95/5). After thorough mixing, 2000 μL of solution was transferred into 10 mL volumetric flask and was filled up to the mark with mixture 0.1% H3PO4/ACN (95/5). Then final solution was placed in chromatographic vial and analysed according to conditions given in chapter 4.15.6. Sample was prepared in triplicate.

**Accelerate storage test.**

Accelerated storage test was conducted according to CIPAC MT 46.3 method. Test item was stored at 54±2°C for 2 weeks in a tightly closed commercial packaging, which simulates 2 years of storage of the test item at temperature of 20°C. After the end of the accelerated storage, test item was analysed for the content of prohexadium-calcium, appearance, pH, suspensibility, dispersibility of water dispersible granules, wettability, particle size distribution, wet sieve, attrition characteristics, dry sieve test and stability of packaging.

**Preparation of test item**

Test item was stored at 54±2°C for 2 weeks. The originally closed, 1L volume commercial bottle, prefilled with test item was provided by the study Sponsor.

**Course of experiment**

Test item was stored at 54 ± 2 °C for 14 days in a tightly closed commercial packaging. Time measurement was started one hour after the insertion of the packaging to the incubator on 29.08.2019. At the accelerated storage test end ± 1 h on 12.09.2019 packaging was taken out from the incubator and allowed to cool down to room temperature for 24 h.