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| REGISTRATION REPORT  Part B  Section 5  Analytical Methods  Detailed summary of the risk assessment |
| Product code: FRE 001/08/2020  Product name: FUNABEN® 018 PA  Chemical active substance:  Thiabendazole, 18 g/kg (1,8 %) |
| Central Zone  Zonal Rapporteur Member State: Poland |
| CORE ASSESSMENT/Poland  (authorization) |
| Applicant: XXXX  Submission date: 07/07/2023  Evaluation date: 12/2023  Finalisation date: 03/2024 |

Version history

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| --- | --- |
| When | What |
| December 2023 | Initial RR |
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# Analytical methods

## Conclusion and summary of assessment

Sufficiently sensitive and selective analytical methods are ~~not~~ available for the active substance and relevant impurities in the plant protection product.

~~Noticed data gaps are:~~

* ~~data gap 1~~
* ~~data gap 2~~
* ~~data gap 3~~

Sufficiently sensitive and selective analytical methods are available for all analytes included in the residue definitions. The applicant's dRR text was not rewritten by the zRMS. In the resulting RR all comments /corrections/add-ons were placed on the grey background.

The applicant submitted a relevant validation of LC-MS/MS method which was accepted.

EFSA Journal 2014;12(7):3750: The multi-residue QuEChERS method in combination with LC-MS/MS is reported for the analysis of parent thiabendazole with an LOQ of 0.01 mg/kg in high water content, high oil content and acidic commodities. However, Thiabendazole can be enforced in food of plant origin with an LOQ of 0.01 mg/kg in high water content and acidic commodities.

Noticed data gaps are: none

| Commodity/crop | Supported/ Not supported |
| --- | --- |
| Pome, stone fruits type matrices | 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 Thiabendazole in plant protection product is provided as follows:

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

|  |  |
| --- | --- |
| Reference: | KCP 5.1.1 |
| Report | REPORT  FRE 01/08/2020 – 1,8 %  Method validation for determination of the active substance content in the preparation  K.Bajdor, S.Kowalska, 2022, Study code no. BA-11/22 |
| Guideline: | Yes: SANCO/3030/99 rev. 5 (22/03/2019) |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes |

Materials and methods

The standard solution was prepared by weighing 80.78 mg of thiabendazole (accuracy to 0.01 mg) into a 10 ml volumetric flask and filled with methanol to the nominal volume. The flask was placed in an ultrasonic bath (25°C) for 5 minutes, and after cooling the solution was diluted and analyzed.

The test sample solution was prepared by weighing approximately 60 mg of the test sample (with an accuracy of 0.01 mg) into a 10 ml flask and adding 2 ml of water and methanol. The flask was placed in an ultrasonic bath for 5 minutes (25°C). After cooling, methanol was added to nominal volume, filtered through a syringe filter and analysed.

Analyses were performed using high-performance liquid chromatography (HPLC) with UV-VIS detection. Chromatography conditions: oven temperature 30°C, eluent flow (acetonitrile + 0.02 M NaH2PO4 30 + 70 v/v), wavelength λ = 290 nm, sample volume: 20 μl. Under these conditions, the thiabendazole retention time was 6.1 min ± 0.1 min. The total analysis time is 15 minutes.

Validation - Results and discussions

Table 5.2‑1: Methods suitable for the determination of active substance Thiabendazole in plant protection product FUNABEN® 018 PA/FRE 01/08/2020 – 1,8 %

|  | Thiabendazole |
| --- | --- |
| Author, year | K. Bajdor, S. Kowalska, 2022 |
| Principle of method | The validated method of determination of active substance content was performed using high performance liquid chromatography (HPLC) with UV-VIS detector. |
| Linearity  (linear between  mg/L / % range of the declared content)  (correlation coefficient, expressed as r) | The linearity of the detector response was assessed using five standards solutions of Thiabendazol in the concentration range from 0.0564 mg/ml to 0.1209 mg/ml.  Correlation coefficient R2 = 0,996 |
| Precision – Repeatability Mean  (%RSD) | The content of Thiabendazol in the **FRE 01/08/2020- 1,8%** preparation was determined by analysis of six - about 6 mg/ml of the specimen solutions. Average content was 1,94 %. Acceptable relative standard deviation for analyte in preparation at concentration 1.94% is RSDr ≤ 2.43%. The obtained result 1.16% is acceptable. |
| Accuracy  (% Recovery) | Recovery of the method for determination of Thiabendazole content in FRE 01/08/2020- 1,8% preparation was assessed by total recovery. To twelve 5 ml flasks 1 ml placebo solution in methanol (concentration 30.357 mg/ml) was added. To six of them 0.50 ml (level I; concentration of Thiabendazol standard solution – 0.806 mg/ml) and to other six 0.70 ml (level II; concentration of Thiabendazol standard solution – 0.6045 mg/ml of Thiabendazol standard solution were added. Methanol was added up to the nominal volume and the mixture was put into an ultrasonic bath (5 min, t = 25 °C). Solutions were passed through syringe filters and analyzed. The result of 102.28% fulfils the acceptance criterion (90 – 110%). |
| Interference/ Specificity | To prove specificity of the developed method (chromatographic conditions are in the point 3.2), chromatograms of: solvent (methanol), placebo solution, standard solution and specimen FRE 01/08/2020 – 1,8% solution were performed and superimposed. There are no other peaks that could interfere with peak of Thiabendazol under the specified chromatographic conditions. |
| Comment | - |

Conclusion

The method for determination of Thiabendazole content in **FRE 01/08/2020 – 1,8%** preparation was developed and validated in Analytical Research Laboratory of the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry (Ł-IPO) in Warsaw according to EU requirements described in SANCO/3030/99 rev.5, 22 March 2019 guideline. The study was performed in accordance with the study plan and procedures. The aim of the study indicated in the study plan has been reached.

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

Not relevant - the plant protection product FUNABEN® 018 PA and the active substance Thiabendazole do not contain significant impurities from the toxicological and ecotoxicological point of view.

|  |  |
| --- | --- |
| Comments of zRMS: | Not applicable |

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

Not relevant.

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

Not available for this kind of preparation.

### 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 Thiabendazole 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: Thiabendazole | | | | |
| --- | --- | --- | --- | --- |
| Matrix type | Method type | Method LOQ | Principle of method  (i.e. GC-MS or HPLC-UV) | Author(s), year / missing / EU agreed |
| Apple  (Residues) | Primary | LOQ = 0,01 mg/kg | LC-MS/MS | D. Gąszczyk, K.Felczak-Konarska, E. Markiewicz, 2022 / Validation of method for determination of Thiabendazole by Liquid Chromatography (LC-MS/MS); VALIDATION STUDY NUMBER **PW-2021-10** |
| Apple  (Residues) | Primary | LOQ = 0,01 mg/kg | LC-MS/MS | D. Gąszczyk, K.Felczak-Konarska, E.Markiewicz, 2022 / Quantitative analysis of Thiabendazole residues in apple in field conditions (Raw Agricultural Commodity) after one application of a formulated product FRE 001/08/2020 – two harvest trials in Northern Europe – Poland 2021; STUDY NUMBER **PB-2022-01** |
| Confirmatory  (if required) | LOQ = 0,01 mg/kg | LC-MS/MS | D. Gąszczyk, K.Felczak-Konarska, E.Markiewicz, 2023 / Quantitative analysis of Thiabendazole residues in apple in field conditions (Raw Agricultural Commodity) after one application of a formulated product FRE 001/08/2020 – two harvest trials in Northern Europe – Poland 2021; STUDY NUMBER **PB-2023-25** |
| Peach  (Residues) | Primary | LOQ = 0,01 mg/kg | LC-MS/MS | D. Gąszczyk, K.Felczak-Konarska, E.Markiewicz, 2022 / Quantitative analysis of Thiabendazole residues in peaches in field conditions (Raw Agricultural Commodity) after one aplication of a formulated product FRE 001/08/2020 – two harvest trials in Northern Europe – Poland 2021; STUDY NUMBER **PB-2022-02** |
| Confirmatory  (if required) | LOQ = 0,01 mg/kg | LC-MS/MS | D. Gąszczyk, K.Felczak-Konarska, E.Markiewicz, 2023 / Quantitative analysis of Thiabendazole residues in peach in field conditions (Raw Agricultural Commodity) after one aplication of a formulated product FRE 001/08/2020 – two harvest trials in Northern Europe – Poland 2022; STUDY NUMBER **PB-2023-26** |

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

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

Please refer to point 5.2.1.

### Description of analytical methods for the determination of residues Thiabendazole (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 |
| --- | --- | --- | --- |
| Apple | Thiabendazole | MRL = 4 mg/kg | Commission Regulation (EU) 2023/377 of 15 February 2023 amending Annexes II, III, IV and V to Regulation (EC) No 396/2005 of the European Parliament and of the Council as regards maximum residue levels for benzalkonium chloride (BAC), chlorpropham, didecyldimethylammonium chloride (DDAC), flutriafol, metazachlor, nicotine, profenofos, quizalofop-P, sodium aluminium silicate, thiabendazole and triadimenol in or on certain products. |
| Peach | MRL = 0,01 mg/kg | Commission Regulation (EU) 2023/377 of 15 February 2023 amending Annexes II, III, IV and V to Regulation (EC) No 396/2005 of the European Parliament and of the Council as regards maximum residue levels for benzalkonium chloride (BAC), chlorpropham, didecyldimethylammonium chloride (DDAC), flutriafol, metazachlor, nicotine, profenofos, quizalofop-P, sodium aluminium silicate, thiabendazole and triadimenol in or on certain products. |

#### 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 Thiabendazole 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: Thiabendazole | | | | |
| --- | --- | --- | --- | --- |
| Matrix type | Method type | Method LOQ | Principle of method (i.e. GC-MS or HPLC-UV) | Author(s), year / missing / EU agreed |
| Apple | Primary | LOQ = 0,01 mg/kg | LC-MS/MS | D. Gąszczyk, K.Felczak-Konarska, E.Markiewicz, 2022 / Quantitative analysis of Thiabendazole residues in apple in field conditions (Raw Agricultural Commodity) after one application of a formulated product FRE 001/08/2020 – two harvest trials in Northern Europe – Poland 2021; STUDY NUMBER **PB-2022-01** |
| Confirmatory  (if required) | LOQ = 0,01 mg/kg | LC-MS/MS | D. Gąszczyk, K.Felczak-Konarska, E.Markiewicz, 2023 / Quantitative analysis of Thiabendazole residues in apple in field conditions (Raw Agricultural Commodity) after one application of a formulated product FRE 001/08/2020 – two harvest trials in Northern Europe – Poland 2021; STUDY NUMBER **PB-2023-25** |
| Peach | Primary | LOQ = 0,01 mg/kg | LC-MS/MS | D. Gąszczyk, K.Felczak-Konarska, E.Markiewicz, 2022 / Quantitative analysis of Thiabendazole residues in peaches in field conditions (Raw Agricultural Commodity) after one aplication of a formulated product FRE 001/08/2020 – two harvest trials in Northern Europe – Poland 2021; STUDY NUMBER **PB-2022-02** |
| Confirmatory  (if required) | LOQ = 0,01 mg/kg | LC-MS/MS | D. Gąszczyk, K.Felczak-Konarska, E.Markiewicz, 2023 / Quantitative analysis of Thiabendazole residues in peach in field conditions (Raw Agricultural Commodity) after one aplication of a formulated product FRE 001/08/2020 – two harvest trials in Northern Europe – Poland 2022; STUDY NUMBER **PB-2023-26** |

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: | N/A |
| Not required, because: | No residues exceeding LOQ are expected. |

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

Not relevant due to intended uses and specific mode of application: painting of wounds on trees with brush (locally) and form of product (thick paste; additionally, the present polyvinyl acetate creates an impermeable and indelible coating on the wound surface, which prevents from getting through of active substance and other co-formulants into environment compartments – they are “trapped”). Therefore, there is no possibility to expect for Thiabendazole and/or its metabolites for getting through to animal matrices, soil, groundwater, surface-water and air, when plant protection product is used in accordance with the label and good agricultural practice.

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

Not relevant due to intended uses and specific mode of application: painting of wounds on trees with brush (locally) and form of product (thick paste; additionally, the present polyvinyl acetate creates an impermeable and indelible coating on the wound surface, which prevents from getting through of active substance and other co-formulants into environment compartments – they are “trapped”). Therefore, there is no possibility to expect for Thiabendazole and/or its metabolites for getting through to animal matrices, soil, groundwater, surface-water and air, when plant protection product is used in accordance with the label and good agricultural practice. Description of methods for the analysis of water (KCP 5.2)

Not relevant due to intended uses.

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

Not relevant due to intended uses and specific mode of application: painting of wounds on trees with brush (locally) and form of product (thick paste; additionally, the present polyvinyl acetate creates an impermeable and indelible coating on the wound surface, which prevents from getting through of active substance and other co-formulants into environment compartments – they are “trapped”). Therefore, there is no possibility to expect for Thiabendazole and/or its metabolites for getting through to animal matrices, soil, groundwater, surface-water and air, when plant protection product is used in accordance with the label and good agricultural practice

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

Not relevant due to intended uses and specific mode of application: painting of wounds on trees with brush (locally) and form of product (thick paste; additionally, the present polyvinyl acetate creates an impermeable and indelible coating on the wound surface, which prevents from getting through of active substance and other co-formulants into environment compartments – they are “trapped”). Therefore, there is no possibility to expect for Thiabendazole and/or its metabolites for getting through to animal matrices, soil, groundwater, surface-water and air, when plant protection product is used in accordance with the label and good agricultural practice.

#### Other studies/ information

No additional studies/information are required.

# 

1. Lists of data considered in support of the evaluation

Tables considered not relevant can be deleted as appropriate.

MS to blacken authors of vertebrate studies in the version made available to third parties/public.

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 | K. Bajdor  J. Kupiec  A. Gralak  S. Kowalska | 2022 | REPORT  FRE 01/08/2020 – 1,8 %  Method validation for determination of the active substance content in the preparation  Study code no: **BA-11/22**  Source: Sieć Badawcza Łukasiewicz – Instytut Przemysłu Organicznego  GLP: Yes  Unpublished | N | XXXX |
| KCP 5.1.2  KCP 5.2 | D. Gąszczyk, K.Felczak-Konarska, E. Markiewicz | 2022 | Validation of method for determination of Thiabendazole by Liquid Chromatography (LC-MS/MS); VALIDATION STUDY NUMBER **PW-2021-10**  Source: Fertico Sp. z o.o. – Laboratorium  GLP: Yes  Unpublished | N | XXXX |
| KCP 5.1.2  KCP 5.2 | D. Gąszczyk, K.Felczak-Konarska, E.Markiewicz | 2022 | Quantitative analysis of Thiabendazole residues in peaches in field conditions (Raw Agricultural Commodity) after one aplication of a formulated product FRE 001/08/2020 – two harvest trials in Northern Europe – Poland 2021; STUDY NUMBER **PB-2022-02**  Source: Fertico Sp. z o.o. – Laboratorium  GLP: Yes  Unpublished | N | XXXX |
| KCP 5.1.2  KCP 5.2 | D. Gąszczyk, K.Felczak-Konarska, E.Markiewicz | 2022 | Quantitative analysis of Thiabendazole residues in apple in field conditions (Raw Agricultural Commodity) after one application of a formulated product FRE 001/08/2020 – two harvest trials in Northern Europe – Poland 2021; STUDY NUMBER **PB-2022-01**  Source: Fertico Sp. z o.o. – Laboratorium  GLP: Yes  Unpublished | N | XXXX |
| KCP 5.1.2  KCP 5.2 | D. Gąszczyk, K.Felczak-Konarska, E.Markiewicz | 2023 | Quantitative analysis of Thiabendazole residues in peach in field conditions (Raw Agricultural Commodity) after one aplication of a formulated product FRE 001/08/2020 – two harvest trials in Northern Europe – Poland 2022; STUDY NUMBER **PB-2023-26**  Source: Fertico Sp. z o.o. – Laboratorium  GLP: Yes  Unpublished | N | XXXX |
| KCP 5.1.2  KCP 5.2 | D. Gąszczyk, K.Felczak-Konarska, E.Markiewicz | 2023 | Quantitative analysis of Thiabendazole residues in apple in field conditions (Raw Agricultural Commodity) after one application of a formulated product FRE 001/08/2020 – two harvest trials in Northern Europe – Poland 2021; STUDY NUMBER **PB-2023-25**  Source: Fertico Sp. z o.o. – Laboratorium  GLP: Yes  Unpublished | N | XXXX |

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 XX | Author | YYYY | Title  Company Report N  Source  GLP/non GLP/GEP/non GEP  Published/Unpublished | Y/N | Owner |
|  |  |  |  |  |  |

The following tables are to be completed by MS

List of data submitted by the applicant and not 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 XX | Author | YYYY | Title  Company Report N  Source  GLP/non GLP/GEP/non GEP  Published/Unpublished | Y/N | Owner |
|  |  |  |  |  |  |

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

| 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 XX | Author | YYYY | Title  Company Report N  Source  GLP/non GLP/GEP/non GEP  Published/Unpublished | Y/N | Owner |
|  |  |  |  |  |  |

1. Detailed evaluation of submitted analytical methods
   1. Analytical methods for Thiabendazole
      1. Methods used for the generation of pre-authorization data (KCP 5.1)

No new or additional studies have been submitted.

* + 1. Methods for post-authorization control and monitoring purposes (KCP 5.2)
       1. Description of analytical methods for the determination of residues in plant matrices (KCP 5.2)

No new or additional studies have been submitted.

* + - * 1. Validation of method for determination of Thiabendazole by Liquid Chromatography (LC-MS/MS)

Method validation

|  |  |
| --- | --- |
| Comments of zRMS: | The method has been accepted. |

|  |  |
| --- | --- |
| Reference: | KCP 5.1.2 |
| Report | Validation of method for determination of Thiabendazole by Liquid Chromatography (LC-MS/MS); VALIDATION STUDY NUMBER **PW-2021-10** |
| Guideline: | Principles of Good Laboratory Practice contained in OECD ENV/MC/CHEM (98) 17. And Directive 2004/9/EC of the European Parliament of the Council of 11th February 2004 |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes |

Materials and methods

The study in the analytical phase consist of a quantitative analysis of thiabendazole. Reference material Thiabendazole (batch number: G147404) was bought by Fertico Laboratory. The producer of analyte is Dr. Ehrenstorfer GmbH. Analytical standard of Thiabendazole was used to determine the content of active substance in the samples. Triphenylphosphate standard was also used for analysis as the internal standard. The test was carried out using liquid chromatography (LC-MSMS). Analysis was performed on apple matrix. The lower limit of quantification (LOQ) of thiabendazole is 0,01 mg/kg.

Untreated reserve sample was delivered to the laboratory in controlled manner. Sample was not treated with the analysed substance. Matrix sample was weighed and registered. Whole sample was homogenized by laboratory mill and weight. From homogenized matrix 10 g of test sample was weight to 50 ml centrifuge tubes for validation.

The control sample material was used to determine recovery if the zero values at the expected retention time of the analyte do not exceed 30 % LOQ. Recovery was calculated based on the concentration value read for fortified samples. During the test, two blank samples were analysed, i.e. reagent samples, which are samples without the presence of matrix.

Untreated homogenous matrix samples were weigh at 10 g ± 0.05 g into a 50 ml centrifuge tube. Spiking solution was added and then proper amount of acetonitrile was added to reach the final volume of 10 ml. The tube was closed and shaken vigorously by hand in room temperature for 1 min to 3 min. Then the buffer-salt mixture (Quechers) was added and samples were shaken vigorously for 5 min using shaker and centrifuged for 5 min at 5500 rpm. After this time 0.5 ml of sample and 10 μl of Triphenylphosphate was transferred into Eppendorf tube. Samples were diluted to the final volume of 1 ml by water containing 0.1 % of formic acid and 5 mM ammonium formate.

Prepared samples were filtered with 0.22 μm PTFE into the injection vial for LC-MSMS. The prepared samples were analyzed by means of an Agilent Technologies LC MS Triple Quad liquid chromatograph, in one sequence with standard samples.

Chromatographic parameters:

Autosampler with cooling (constant temperature 10°C), injection volume 2 μl, injection mode: 200 μl/min

Chromatographic column: InfinityLab Poroshell 120 EC-C18 column with dimensions of 3.0 x 150 mm and grain diameter 2.7μm, series number USCFW17005 and guard column: InfinityLab Poroshell EC-C18 guard column with dimensions of 3.0 x 5 mm and grain 2.7 μm, series number USCEC11811 maintaining a constant temperature of 35°C at the entrance and 35°C at the exit of the chromatographic column. Binary Pump: solvent A: 0.1 % formic acid, 5 mM ammonium formate in water, solvent B: 0.1 % formic acid in methanol with LC-MS purity, flow rate 0.5 ml/min.

MS Triple Quad parameters:

Time of analysis: 20 min

Ionisation type: positive

Collision gas: nitrogen, temperature 300°C, flow 8 l/min, sheath gas: temperature 350°C, flow 11 l/min

Results and discussions

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

| **Matrix** | **Analyte** | **Fortification level (μg/kg) (n = x)** | **Mean  recovery (%)** | **RSD (%)** | **Comments** |
| --- | --- | --- | --- | --- | --- |
| apple | thiabendazole | 10 | 90,88 | 2,35 | For quanfifier ion (202.0 → 175.0) |
| apple | thiabendazole | 10 | 90.15 | 2,31 | For quanfifier ion (202.0 → 131.0) |
| apple | thiabendazole | 100 | 113,40 | 1,15 | For quanfifier ion (202.0 → 175.0) |
| apple | thiabendazole | 100 | 113,84 | 1,18 | For quanfifier ion (202.0 → 131.0) |

Table A 2: Characteristics for the analytical method used for validation of Thiabendazole residues in apple matrix

|  | Thiabendazole |
| --- | --- |
| Specificity | The applied Lc MS Triple Quad method is specific due to chromatographic separation and selective detection systems. Confirmation of the presence of the analytes was obtained by comparing the signal ratios of the two MRM pairs. For the standards and samples tested, the relative signal responseratio for specific MRM transitions was determined using the MassHunter application, expressed as a percentage by the ratio of the qualifying ion response to the quantifier ion response. The obtained ratios for the test samples were related to the results obtained for the standard analyzed three times (in the same time) (twice before the test samples and once after the test samples) for LC-MS/MS and to the coefficients for the calibration standards. The presence of the analyte was confirmed when the retention time (RT) of the samples corresponded to the retention time of the analyte determined with the tolerance of RT ± 0.1 min and the confirmation factor within ± 30 %. Thiabendazole was analysed by the LC-MS/MS specific detection system. Response of matrix samples were < 30 % of LOQ samples. |
| Calibration (type, number of data points) | Six recovery values for two concentration levels 0.01 and 0.1 mg/kg in commodity: apple on LC-MS/MS. In all of cases the quantitative results (70-120 %) with RSD < 20 % for most analytes/matrix combinations were obtained. |
| Calibration range | For monitoring purposes the quantification is done in standard diluted in apple matrix. The method showed to be linear up to 0.4 mg/kg for all analytes in matrix with LC-MS/MS. A criterion of linearity (R2≥0,99( is fullfilled for all analytes in the method.  Equation (for the first transition 202.0→175.0): y=0.7967x+0.0118; R2=0.9924  Equation (for the second transition 202.0→131.0): y=0.7265x+0.0111 |
| Assessment of matrix effects is presented | yes |
| Limit of determination/quantification | 0.01 mg/kg / 0.003 mg/kg |

Conclusion

Method has been validated. All acceptance criteria are met.

* + - 1. Description of analytical methods for the determination of residues in animal matrices (KCP 5.2)

Not relevant.

* + - 1. Description of Methods for the Analysis of Soil (KCP 5.2)

Not relevant.

* + - 1. Description of Methods for the Analysis of Water (KCP 5.2)

Not relevant.

* + - 1. Description of Methods for the Analysis of Air (KCP 5.2)

Not relevant.

* + - 1. Description of Methods for the Analysis of Body Fluids and Tissues (KCP 5.2)

Not relevant.

* + - 1. A.2.A.9 Other Studies/ Information

No new or additional studies have been submitted