|  |
| --- |
| REGISTRATION REPORT  Part B  Section 5  Analytical Methods  Detailed summary of the risk assessment |
| Product code: ADM.00150.I.2.A  Product name(s): LEAXO  Chemical active substance:  Acetamiprid, 200 g/L |
| Central Zone  Zonal Rapporteur Member State: Poland |
| CORE ASSESSMENT  (authorization according to Art. 33) |
| Sponsor: ADAMA Makhtesheim Ltd. Applicant: Country organisation / representative of ADAMA,  as given in Part A  Submission date: August 2023  MS Finalisation date: July 2024 (initial Core Assessment)  December 2024 (final Core Assessment), updated June 2025,  updated August 2025 |

Version history

|  |  |
| --- | --- |
| When | What |
| August 2023 | 1st application Art 33 Central Zone |
| July 2024 | Initial zRMS assessment  The report in the dRR format has been prepared by the Applicant, therefore all comments, additional evaluations and conclusions of the zRMS are presented in grey commenting boxes. Minor changes are introduced directly in the text and highlighted in grey. Not agreed or not relevant information are ~~struck through~~ and shaded for transparency. |
| October 2024 | Dossier updated after following the commenting period:   * References to the ongoing monitoring methods for the determination of metabolites IM-2-1 and IC-0 in body fluids and tissues have been added to Section 5.3.2.7 as well as Appendix 1 (List of data submitted by the applicant and relied on). * Appendix 1 reference list (List of data submitted or referred to by the applicant and relied on, but already evaluated at EU peer review) has been updated with Adama´s methods for determination of residues that are equivalent to the ones evaluated in the RAR. |
| December 2024 | Final report (Core Assessment updated following the commenting period)  Additional information/assessments included by the zRMS in the report in response to comments received from the cMS and the Applicant are highlighted in yellow. Not agreed or not relevant information are ~~struck through~~ and shaded for transparency. |
| June 2025 | Applicant’s update requested by zRMS  Due to changes in the residue definition and reduced MRL values, analytical methods for both risk assessment and monitoring must be included. These methods demonstrate appropriate LOQs for the relevant matrices and are aligned with the current residue definition.  Changes are highlighted in turquoise |
| June 2025 | Final report (Core Assessment updated following changes to the residue definition and MRL values)  The report in the dRR format has been prepared by the Applicant, therefore all comments, additional evaluations and conclusions of the zRMS are presented in grey commenting boxes. Minor changes are introduced directly in the text and highlighted in green. Not agreed or not relevant information are ~~struck through~~ and shaded for transparency. |
| August 2025 | Final report (Core Assessment updated following the commenting period)  Additional information/assessments included by the zRMS in the report in response to comments received from the cMS and the Applicant are highlighted in purple. Not agreed or not relevant information are ~~struck through~~ and shaded for transparency. |

Table of Contents

[5 Analytical methods 4](#_Toc201673432)

[5.1 Conclusion and summary of assessment 4](#_Toc201673433)

[5.2 Methods used for the generation of pre-authorization data (KCP 5.1) 5](#_Toc201673434)

[5.2.1 Analysis of the plant protection product (KCP 5.1.1) 5](#_Toc201673435)

[5.2.1.1 Determination of active substance and/or variant in the plant protection product (KCP 5.1.1) 5](#_Toc201673436)

[5.2.1.2 Description of analytical methods for the determination of relevant impurities (KCP 5.1.1) 7](#_Toc201673437)

[5.2.1.3 Description of analytical methods for the determination of formulants (KCP 5.1.1) 7](#_Toc201673438)

[5.2.1.4 With respect to toxicological, eco-toxicological or environmental aspects the formulation MCW-2222 does not contain any relevant formulants. Therefore, an ad-hoc analytical method and validation is not needed. Applicability of existing CIPAC methods (KCP 5.1.1) 7](#_Toc201673439)

[5.2.2 Methods for the determination of residues (KCP 5.1.2) 7](#_Toc201673440)

[5.3 Methods for post-authorization control and monitoring purposes (KCP 5.2) 11](#_Toc201673441)

[5.3.1 Analysis of the plant protection product (KCP 5.2) 11](#_Toc201673442)

[5.3.2 Description of analytical methods for the determination of residues of acetamiprid (KCP 5.2) 11](#_Toc201673443)

[5.3.2.1 Overview of residue definitions and levels for which compliance is required 11](#_Toc201673444)

[5.3.2.2 Description of analytical methods for the determination of residues in plant matrices (KCP 5.2) 13](#_Toc201673445)

[5.3.2.3 Description of analytical methods for the determination of residues in animal matrices (KCP 5.2) 15](#_Toc201673446)

[5.3.2.4 Description of methods for the analysis of soil (KCP 5.2) 18](#_Toc201673447)

[5.3.2.5 Description of methods for the analysis of water (KCP 5.2) 19](#_Toc201673448)

[5.3.2.6 Description of methods for the analysis of air (KCP 5.2) 20](#_Toc201673449)

[5.3.2.7 Description of methods for the analysis of body fluids and tissues (KCP 5.2) 21](#_Toc201673450)

[Appendix 1 Lists of data considered in support of the evaluation 24](#_Toc201673451)

[Appendix 2 Detailed evaluation of submitted analytical methods 40](#_Toc201673452)

[A 2.1 Analytical methods for acetamiprid 40](#_Toc201673453)

[A 2.1.1 Methods used for the generation of pre-authorization data (KCP 5.1) 40](#_Toc201673454)

[A 2.1.2 Methods for post-authorization control and monitoring purposes (KCP 5.2) 130](#_Toc201673455)

# 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.

Noticed data gaps are:

* none

|  |
| --- |
| **Evaluator comments (June 2025):**  Assessment of updated version considering EFSA Statement on the toxicological properties and maximum residue levels of acetamiprid and its metabolites (EFSA Journal. 2024;22:e8759):  For acetamiprid the toxicological reference values have been modified. EFSA proposed to lower the acceptable daily intake (ADI) and acute reference dose (ARfD) from 0.025 to 0.005 mg/kg body weight (per day). A revised residue definition for risk assessment was proposed for leafy and fruit crops as sum of acetamiprid and N-desmethyl-acetamiprid (IM-2-1), expressed as acetamiprid. Regarding pulses/oilseeds, root crops and cereals, the new data received did not indicate a need to modify the existing residue definition for risk assessment, which therefore remains as parent acetamiprid. Regarding the residue definition for enforcement, the available data did not indicate a need to modify the existing definition because acetamiprid is still a sufficient marker of the residues in all crop groups.  In the context of changed MRLs values for acetamiprid, no additional methods are required.  Additionally, Applicant provided two studies on the validation of an analytical methods for residues of acetamiprid metabolites IM-2-1 and IC-0 in body tissues and body fluids. The analytical methods were fully validated according to SANTE/2020/12830 rev.2. guidelines.  More details are presented in Appendix 2. |

| Commodity/crop | Supported/Not supported |
| --- | --- |
| Apple | Suported |
| Potato | Suported |
| Oilseed rape | Suported |
| Barley, Oat | Suported |
| Corn/maize | Suported |
| Wheat, Rye | Suported |
| Sugar beet | Suported |

## 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)

This application is for ADM.00150.I.2.A. ADM.00150.I.2.A and former MCW-2222 are the same product.

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

|  |  |
| --- | --- |
| Comments of zRMS: | The analytical method for analysis of acetamiprid in plant protection product is sufficiently described and validated in accordance with the requirements of the guidelines: SANCO/3030/99 rev. 4 ~~and SANTE/2020/12830 Rev. 1~~. The method also fulfils the requirements of SANCO/3030/99 rev. 5 guidelines. |

|  |  |
| --- | --- |
| Reference: | KCP 5.1.1/01 |
| Report | Development and validation of an analytical method for the determination of acetamiprid in MCW-2222 - Walter, D., 2014, Study No. S13-03099, Sponsor Reference No. R-33405 |
| Guideline(s): | SANCO/3030/99 rev. 4 |
| Deviations: | None |
| GLP: | Yes |
| Acceptability: | Yes |

1. **Materials**

|  |  |
| --- | --- |
| 1. Standards |  |
| Test item 1: | MCW-2222 formulation (acetamiprid 200 g/L SL) |
| CAS No.: | 135410-20-7 |
| Content of a.i.: | 201 g/L |
| Lot/batch No.: | 611-280413-01 |
| Expiry date: | 28/04/2015 |
|  |  |
| Blank formulation: | MCW-2222 blank formulation |
| Batch No.: | 120513 |
| Content of a.i.: | None |
| Expiry date: | 12/05/2015 |
|  |  |
| Reference item: | Acetamiprid |
| CAS No.: | 135410-20-7 |
| Batch No.: | SZBC110XV |
| Purity: | 99.9% |
| Expiry date: | 19/04/2017 |
|  |  |

**B. Sample preparation and processing**

An HPLC-UV method for the determination of acetamiprid in plant protection product MCW-2222 was validated according to the requirements of SANCO/3030/99 rev. 4 guidelines. The target analyte was detected using a UV detector at 250 nm.

The method also fulfils the requirements of SANCO/3030/99 rev. 5 guidelines.

1. **Analytical instrumentation and analysis**

|  |  |
| --- | --- |
| HPLC- parameters: | Agilent HP 1100 |
| Column: | Synergi Hydro-RP µm 80A, 150 x 4.6 mm |
| Mobile phase: | A: Acetonitrile containing acetic acid (1.1 L acetonitrile + 0.5 mL acetic acid)  B: Water containing acetic acid (1 L water + 100mL acetonitrile + 0.5 mL acetic acid) |
| Flow rate: | 0.4 mL/min |
| Injection volume: | 10 µL |
| Detector: | UV absorbance at 254 nm |

Results and discussions

An HPLC-UV method for the determination of acetamiprid in MCW-2222 was validated according to the requirements of SANCO/3030/99 rev. 4 guidelines. The quantification of acetamiprid was achieved by analysing ten concentration levels ranging between 101 and 1000 ng and the detector response was shown to be linear with a coefficient of determination r = 0.9999. The detector response was linear over the range of concentrations tested. For the instrument precision, five independent samples were injected into the HPLC-UV. For repeatability, fortified samples were prepared by adding known amounts of technical grade analyte to blank formulation to provide five replicates at each of two concentration levels (low and high). Precision and recovery data are given in the tables below. Representative chromatograms are provided in the study report.

**Table 5.2-1: Precision (repeatability) of measured concentrations of acetamiprid in** **plant protection product MCW-2222 reported in study S13-03099**

| **Analyte** | **% w/w Content1** | **Overall Mean Found**  **Content (%w/w)** | **Overall RSD (%)**  **(n = 5)** | **Horwitz value (%RSDr)** | **Horrat value2** |
| --- | --- | --- | --- | --- | --- |
|  | 18.3 |  |  |  |  |
|  | 18.1 |  |  |  |  |
| Acetamiprid | 17.9 | 18.0 | 1.2 | 1.73 | 0.69 |
|  | 17.8 |  |  |  |  |
|  | 17.8 |  |  |  |  |

1Mean of two injections.

2 Calculated for the purpose of this summary.

**Table 5.2-2: Method validation recovery data for the determination of acetamiprid in plant protection product MCW-2222 reported in study S13-03099.**

| **Analyte** | **Fortification level (mg/L)** | **Mean  recovery (%)**  **n=5** | **RSD (%)** | **Comments** |
| --- | --- | --- | --- | --- |
| Acetamiprid | 20.0 | 100.9 | 1.0 | - |
| 69.9 | 99.7 | 1.7 | - |

Table 5.2-3: Characteristics of the analytical method used for the determination of acetamiprid in plant protection product MCW-2222

|  |  |
| --- | --- |
| Specificity | Comparison of the chromatograms produced by the reference item and the test item revealed peaks with similar retention times that did not deviate by more than 0.2%. Comparison of the UV spectra of acetamiprid in the reference item with those of the test item revealed a 100% match between 200 and 400 nm. Analysis of blank formulation samples showed no interference at the retention time for acetamiprid. |
| Calibration (type, number of data points) | Ten-point linear calibration  Equation: y = 0.2151 x – 2.5059, r = 0.9999 |
| Calibration range | 101 – 1000 ng/injection (5 to 15μL injection volume) |
| Limit of determination/quantification | LOQ: 20.0 mg/L |

Conclusion

An analytical method based on HPLC-UV was validated for the determination of acetamiprid in plant protection product MCW-2222. All validation data meet the requirements of SANCO/3030/99 rev. 4 and rev. 5 guidelines and the validation requirements of Section 4.1.5 of SANTE/2020/12830 Rev. 1 guidelines. The method should therefore be considered suitable for its intended purpose and acceptable for the determination of acetamiprid in MCW-2222.

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

The formulation under consideration contains no relevant impurities.

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

#### With respect to toxicological, eco-toxicological or environmental aspects the formulation MCW-2222 does not contain any relevant formulants. Therefore, an ad-hoc analytical method and validation is not needed. Applicability of existing CIPAC methods (KCP 5.1.1)

A CIPAC analytical method currently exists for the determination of acetamiprid. CIPAC method no. 649 has been developed for the determination of acetamiprid technical in soluble concentrates (649/SL/(M)/) as well as wettable powders (649/WP/(M)/), water soluble powders (649/SP/(M)/), water soluble granules (649/SG/(M)/) and emulsifiable concentrates (649/EC/(M)/).

The guideline ‘Guidance for generating and reporting methods of analysis in support of pre- and post-registration data requirements for Regulation (EU) 283/2013 and Regulation (EU) 545/2011 of Regulation 1107/2009/EC’ states: ‘The applicability of existing CIPAC methods shall be assessed and reported. In case of use of a CIPAC method, further validation data shall not be required, but example chromatograms shall be submitted, where available.’

The CIPAC Method 649 for the active substance acetamiprid was collaboratively tested on equivalent SL-formulation(s).

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

Table 5.2‑4: Validated methods for the generation of pre-authorization data

| Component of residue definition: acetamiprid | | | | |
| --- | --- | --- | --- | --- |
| Matrix type | Method type | Method LOQ | Principle of method  (i.e. GC-MS or HPLC-UV) | Author(s), year / missing / EU agreed |
| Plant matrices  (Residues) | Primary | 0.01 mg/kg  *(wheat)* | HPLC-MS/MS | Barbier, G 2018, Report No. B17G-A4-A-02, New data  KCP 5.1.2/01 |
| Primary | 0.01 mg/kg *(barley whole plant, grain and straw)* | HPLC-MS/MS | Chevallier, E., 2014, Report No. 14SGS034, New data  KCP 5.1.2/02 |
| Primary | 0.01 mg/kg *(wheat whole plant, grain and straw)* | HPLC-MS/MS | Chevallier, E., 2014, Report No. 14SGS033, New data  KCP 5.1.2/03 |
| Primary | * 1. mg/kg   *(arthropods and ground vegetation)* | HPLC-MS/MS | Henkes, K. 2017, Report No. R1640039 , New data  KCP 5.1.2/04 |
| Primary | * 1. mg/kg   *(flowers, sucrose solution, pollen, bee larvae and bee wax)* | HPLC-MS/MS | Mayer, O. 2018, Report No. R1640035, New data  KCP 5.1.2/05 |
| Primary | * 1. mg/kg   *(all matrices)* | HPLC-MS/MS | Lefresne, S. 2014, Report No. B13-M1-A-01, New data\*  KCP 5.1.2/06 |
| Primary | * 1. mg/kg   *(all matrices)* | HPLC-MS/MS | Lang, A., 2014, Report No. 13M06017-01-VMPL, New data\*  KCP 5.1.2/07 |
| Primary | * 1. mg/kg   *(Apple)* | HPLC-MS/MS | Méric, D., 2013, Report No. DMC-13-16134, New data  KCP 5.1.2/08 |
| Primary | * 1. mg/kg   *(Apple and processed fractions)* | HPLC-MS/MS | Roussel, Ch. H., 2014, Report No. ChR-14-17311, New data  KCP 5.1.2/09 |
| Primary | * 1. mg/kg   *(maize whole plant, cobs and grain)* | HPLC-MS/MS | Lebrun F., 2014, Report No. 14SGS039, New data  KCP 5.1.2/10 |
| Primary | * 1. mg/kg   *(sugar beet roots)* | HPLC-MS/MS | Roussel Ch. H., 2022, Report No. SPK-20-46380, New data  KCP 5.1.2/11 |
| Primary | 0.01 mg/kg *(tomato)* | HPLC-MS/MS | Grall E., 2021, Study No. EGL-20-46375, New data  KCP 5.1.2/12 |
| Primary | * 1. mg/kg   *(sugar beet whole plant, roots, leaves + tops)* | HPLC-MS/MS | Roussel Ch.H., 2022, Report No. ChR-21-48246, New data  KCP 5.1.2/13 |
| Primary | 0.01 mg/kg *(cucumber)* | HPLC-MS/MS | Domingo, S., 2022, Report No. SDO-21-48624, New data  KCP 5.1.2/14 |
| Primary | 0.01 mg/kg *(plum whole fruit and flesh)* | HPLC-MS/MS | Grall, E. 2022, Study No. EGL-20-46374, New data  KCP 5.1.2/15 |
| Primary | 0.01  *(peach fruits)* | HPLC-MS/MS | Méric, D., 2014, Study No. DMC-13-16126, New data  KCP 5.1.2/16 |
| Animal products (residues) | Primary | 0.01 mg/kg *(honey)* | HPLC-MS/MS | Schrag K., 2022, Report No. 21A14030-01-VMHN, New data  KCP 5.1.2/17 |
| Primary | 0.01 mg/kg *(honey)* | HPLC-MS/MS | Boileau, G., 2022, Report no GBU-21-48185, New data  KCP 5.1.2/18 |
| Soil, water, sediment  (Environmental fate) | Primary | Not provided | HPLC | Mamouni, A. , 1997, Report No. 383826, EU agreed |
| Primary | Not provided | HPLC, NMR, IC-MS | Emeric, G.T., 1998, Report No. 98-47, EU agreed |
| Primary | Not provided | HPLC | Shiotani, H., 2003, Report No. C030709, EU agreed |
| Air  (Exposure) | Primary | - | Gravimetric analysis | xxxxx, 2013, Report No. 12/445-004P, New data  KCP 5.1.2/19 |
| Residue dissipation, Dislodgeable Foliar Residue  (Toxicology) | Primary | 0.2 µg/L  *(pome fruit)* | HPLC-MS/MS | Wilson, A., 2016, Report No. ACI16-010 (VV57LS), New data  KCP 5.1.2/20 |
| Arthropods, vegetation  (Ecotoxicology) | Primary | * 1. mg/kg   *(arthropods and ground vegetation)* | HPLC-MS/MS | Henkes, K. 2017, Report No. R1640039, New data filed and summarized under  KCP 5.1.2/04 |
| Primary | * 1. mg/kg   *(wheat and pea plants)* | HPLC-MS/MS | Staffel, J. 2021, Report No. R2040056, New data  KCP 5.1.2/21 |
| Primary | * 1. mg/kg   *(wheat and pea plants)* | HPLC-MS/MS | Staffel, J. 2021, Report No. R2040057, New data  KCP 5.1.2/22 |
| Primary | * 1. mg/kg   *(wheat and pea plants)* | HPLC-MS/MS | Staffel, J. 2022. Report No. R2040059, New data  KCP 5.1.2/23 |
| Primary | * 1. mg/kg   *(wheat and pea plants)* | HPLC-MS/MS | Gräf, K. 2022. Report No. R2040060, New data  KCP 5.1.2/24 |
| Primary | 0.005 mg/kg *(soil)* | HPLC-MS/MS | Schulz, L., 2022, Report No. 21 48 FCM 0002, New data  KCP 5.1.2/25 |
| Water, sediment  (Ecotoxicology) | Primary | 0.185 mg/L  *(water)* | HPLC-UV | xxxxx 2014, Report No. 141048005 W, New data  KCP 5.1.2/26 |
| Primary | 0.367 mg/L  *(M4 aqueous test medium)* | HPLC-UV | Juckeland, D. 2014, Report No. 141048006 W, New data  KCP 5.1.2/27 |
| Primary | 0.47 µg/L  *(M4 aqueous test medium)* | HPLC-MS/MS | Juckeland, D. 2015, Report No. 141048057 W, New data\*  KCP 5.1.2/28 |
| Primary | 0.5 µg/L  *(pond water)* | HPLC-MS/MS | Taylor, S. and Joyce, F. 2015, Report No. CEA.1510 (XCE2008), New data  KCP 5.1.2/29 |
| Primary | 0.344 mg/L  *(OECD aqueous test medium)* | HPLC-UV | Juckeland, D. 2014, Report No. 141048007 W, New data  KCP 5.1.2/30 |
| Mesocosm  (Exotoxicology) | Primary | 10 ng a.s/L *(water)*  50 ng a.s/kg *(sediment)* | UHPLC-MS/MS | Hennecke, N. 2020, Report No. ADM-026/6-22, New data  KCP 5.1.2/31 |
| Bee feeding  (Ecotoxicology) | Primary | 272.1 mg/L  *(sugar solution)* | HPLC-UV DAD | Kleebaum K. 2015, Report No. 141048078 B, New data  KCP 5.1.2/32 |
| Primary | * 1. mg a.s/kg   *(flowers, sucrose solution, pollen, larvae, honey, wax)* | HPLC-MS/MS | Hecht-Rost, S. and Mayer, O. 2018, Report No. R1640035, New data  KCP 5.1.2/04 |
| Primary | * 1. mg a.s/kg   *(bee bread, flowers, nectar/honey, pollen)* | HPLC-MS/MS | Molitor, C. 2015, Report No. 215-2014 + Amendment 1, New data  KCP 5.1.2/33 |
| Primary | 0.01 mg a.s/kg  *(bee bread, flowers, nectar/honey, pollen, wax)* | HPLC-MS/MS | Molitor, C. 2015, Report No. 230-2015, New data  KCP 5.1.2/34 |
| Honey bee, Arthropods  (Ecotoxicology) | Primary | 0.01 mg/L  *(nectar, larvae)* | HPLC-MS/MS | Aucejo, S. 2015, Report No. 307SRES15C02, New data  KCP 5.1.2/35 |
| Vegetative vigor test  (Ecotoxicology) | Primary | 130.4 mg/L  *(water)* | HPLC-UV | Friedrich, S. 2014, Report No. 14 10 48 002 P, New data  KCP 5.1.2/36 |

\*Matching data have been obtained and provided by ADAMA. These data were submitted to the RMS Netherlands in order to demonstrate access to a complete data package according to Reg. (EU) 283/2013 and for the data matching process. ~~The RMS Opinion on GLP compliance, guidance compliance and equivalence of the endpoint will be provided as soon as Data Matching process is finalized.~~ Matching active substance data necessary for the renewal of the approval of acetamiprid is available on CIRCA BC.

An overview on the acceptable methods and possible data gaps for analysis of residues of acetamiprid metabolite 6-chloronicotinic acid for the generation of pre-authorization data is given in the following table. For the detailed evaluation of new/additional studies it is referred to Appendix 2

Table 5.2‑5: Validated methods for the generation of pre-authorization data

| Component of residue definition: 6-chloronicotinic acid | | | | |
| --- | --- | --- | --- | --- |
| Matrix type | Method type | Method LOQ | Principle of method  (i.e. GC-MS or HPLC-UV) | Author(s), year / missing / EU agreed |
| Water, sediment  (Ecotoxicology) | Primary | 10 mg/L  *(M4 aqueous test medium)* | HPLC-UV | Hengsberger, A. and Wydra, V. 2015, Report No. 102461251, New data  KCP 5.1.2/37 |

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

### 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.

### Description of analytical methods for the determination of residues of acetamiprid (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 (Netherlands, 2016, incl. its addenda) the current legal residue definition is identical.

A residue definition for acetamiprid in honey and apiculture products was introduced with Reg. (EU) No 750/2010 and was added to Table 5.3-1, according to the upcoming Regulation.

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 | Acetamiprid | * 1. mg/kg   *(lowest MRL for potato and sugar beet)* | ~~SANTE/11278/2021~~  ~~(yet to be approved)~~  Reg. (EU) 2019/88  Reg. (EU) 2025/158  apply from 19 August 2025  Reg. (EU) 2025/1212  apply from 20 August 2025 |
| Plant, high acid content | 0.01 mg/kg  *(LOQ for whole orange)* | ~~SANTE/11278/2021~~  ~~(yet to be approved)~~  Reg. (EU) 2019/88  Reg. (EU) 2025/158  apply from 19 August 2025  Reg. (EU) 2025/1212  apply from 20 August 2025 |
| Plant, high protein/high starch content (dry commodities) | 0.01 mg/kg  *(lowest MRL for rye)* | ~~SANTE/11278/2021~~  ~~(yet to be approved)~~  Reg. (EU) 2019/88  Reg. (EU) 2025/158  apply from 19 August 2025  Reg. (EU) 2025/1212  apply from 20 August 2025 |
| Plant, high oil content | ~~0.4~~ 0.01 mg/kg  *(lowest MRL for ~~oilseed rape~~ lineseed)* | ~~SANTE/11278/2021~~  ~~(yet to be approved)~~  Reg. (EU) 2019/88  Reg. (EU) 2025/158  apply from 19 August 2025  Reg. (EU) 2025/1212  apply from 20 August 2025 |
| Muscle | *~~N~~*~~-desmethyl-acetamiprid (IM-2-1), expressed as acetamiprid~~  Sum of acetamiprid and N-desmethyl-acetamiprid (IM-2-1), expressed as acetamiprid | ~~0.5~~ 0.02 mg/kg (MRL) | ~~SANTE/11278/2021~~  ~~(yet to be approved)~~  Reg. (EU) 2019/88  Reg. (EU) 2025/158  apply from 19 August 2025  Reg. (EU) 2025/1212  apply from 20 August 2025 |
| Milk | 0.2 mg/kg (MRL) | ~~SANTE/11278/2021~~  ~~(yet to be approved)~~  Reg. (EU) 2019/88  Reg. (EU) 2025/158  apply from 19 August 2025  Reg. (EU) 2025/1212  apply from 20 August 2025 |
| Eggs | 0.02 mg/kg (MRL) | ~~SANTE/11278/2021~~  ~~(yet to be approved)~~  Reg. (EU) 2019/88  Reg. (EU) 2025/158  apply from 19 August 2025  Reg. (EU) 2025/1212  apply from 20 August 2025 |
| Fat | ~~0.3~~ 0.02 mg/kg (MRL) | ~~SANTE/11278/2021~~  ~~(yet to be approved)~~  Reg. (EU) 2019/88  Reg. (EU) 2025/158  apply from 19 August 2025  Reg. (EU) 2025/1212  apply from 20 August 2025 |
| Liver, kidney | ~~1.0~~ 0.1 mg/kg (MRL) | ~~SANTE/11278/2021~~  ~~(yet to be approved)~~  Reg. (EU) 2019/88  Reg. (EU) 2025/158  apply from 19 August 2025  Reg. (EU) 2025/1212  apply from 20 August 2025 |
| Honey | Acetamiprid | ~~0.3~~ 0.05 mg/kg (MRL)  0.3 mg/kg | ~~SANTE/11278/2021~~  ~~(yet to be approved)~~  Reg. (EU) 2019/88  Reg. (EU) 2025/158  apply from 19 August 2025  PLAN/2024/2431  Reg. (EU) 2025/1212  apply from 20 August 2025 |
| Soil  (Ecotoxicology) | Acetamiprid | 0.18 mg a.s./kg soil dry weight | NOAEC for *Folsomia candida* |
| Drinking water  (Human toxicology) | Acetamiprid and IM-1-5 | 0.1 µg/L | General limit for drinking water |
| Surface water  (Ecotoxicology) | Acetamiprid | 0.87 µg a.i./L | NOEC from Mesocosm higher tier study (Hommen, U., 2021; Report No. 000106190) |
| Air | Acetamiprid | ~~\*~~~~\*2.1 µg/m~~~~3~~ ~~based on AOELsystemic of 0.07 mg/kg bw/d~~  7.5 µg/m3 based on AOEL = 0.025 mg/kg bw/d | EFSA Conclusion 2016  . |
| Tissue (meat or liver) | Acetamiprid | 0.01 mg/kg | SANTE/2020/12830, Rev.~~1~~2 |
| Body fluids | 0.01 mg/L | SANTE/2020/12830, Rev.~~1~~2 |

~~\*MRL/Limit for air matrix was calculated using the EU agreed AOEL systemic value of 0.07 mg/kg bw/day from EFSA Conclusion 2016. The calculation was done according to the equation in SANTE/2020/12830, Rev.12 guidelines: c = AOEL-systemic \* 300 [µg/m~~~~3~~~~].~~

#### 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 acetamiprid 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: acetamiprid | | | | |
| --- | --- | --- | --- | --- |
| Matrix type | Method type | Method LOQ | Principle of method (i.e. GC-MS or HPLC-UV) | Author(s), year / missing / EU agreed |
| High water content | Primary | * 1. mg/kg   *in apple fruit* | HPLC-MS/MS | Schwarz, T., 2008, Report No. RD-01937, EU agreed  RAR, 2015, Netherlands\*  to which is equivalent Lang, A., 2015, R-33644, 13M06017-01- VMPL |
| Primary | * 1. mg/kg   *in potatoes* | Weber, H., 2013, Report No. RD-02603, EU agreed  RAR, 2015, Netherlands\*  to which is equivalent Lang, A., 2015, R-33644, 13M06017-01- VMPL |
| ILV | * 1. mg/kg   *in lettuce* | Brown, S. 2022, Report No. RES-00418, New data  KCP 5.2/01 |
| ILV | 0.01 mg/kg  *in cabbage heads* | Brown, S. 2022, Report No. RES-00419, New data  KCP 5.2/02 |
| Confirmatory  (if required) | - |  | - |
| High acid content | Primary | * 1. mg/kg   *in whole orange* | HPLC-MS/MS | Schwarz, T., 2008, Report No. RD-01937, EU agreed RAR, 2015, Netherlands\*  to which is equivalent Lefresne, S., 2015, R-33645, B13-M1-A-01 |
| ILV | * 1. mg/kg   *in orange pulp and peel* | Brown, S. 2022, Report No. RES-00418, New data  KCP 5.2/01 |
| Confirmatory  (if required) | - |  | - |
| High oil content | Primary | * 1. mg/kg   *in sunflower seeds* | HPLC-MS/MS | Schwarz, T., 2008, Report No. RD-01937, EU agreed  RAR, 2015, Netherlands\*  to which is equivalent Lefresne, S., 2015, R-33645, B13-M1-A-01 |
| ILV | 0.01 mg/kg *in oilseed rape seeds* | Brown, S. 2022, Report No. RES-00418, New data  KCP 5.2/01 |
| Confirmatory  (if required) | - |  | - |
| High protein/high starch content (dry) | Primary | 0.01 mg/kg *in maize grain* | HPLC-MS/MS | Schwarz, T., 2008, Report No. RD-01937, EU agreed  RAR, 2015, Netherlands\*  to which is equivalent Lefresne, S., 2015, R-33645, B13-M1-A-01 |
| ILV | 0.01 mg/kg *in dry bean seeds* | Brown, S. 2022, Report No. RES-00418, New data  KCP 5.2/01 |
| Confirmatory  (if required) | - |  | - |

\*Matching data have been obtained and provided by ADAMA. These data were submitted to the RMS Netherlands in order to demonstrate access to a complete data package according to Reg. (EU) 283/2013 and for the data matching process. ~~The RMS Opinion on GLP compliance, guidance compliance and equivalence of the endpoint will be provided as soon as Data Matching process is finalized.~~ Matching active substance data necessary for the renewal of the approval of acetamiprid is available on CIRCA BC.

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: | The efficiency of extraction procedures used in several residue trials and monitoring method was verified during a cross-validation study. The study aimed at assesing the efficiency of acetonitrile to extract acetamiprid from plant material with high water content, plant material with high acid content, plant material with high oil content and dry plant material with high protein content.  For more details please refer to the full summary in Appendix 2, KCP 5.2/03 and KCP 5.2/04 |
| Not required, because: |  |

|  |
| --- |
| **Evaluator comments:**  According to the evaluation presented in “Matching active substance data necessary for the renewal of the approval of acetamiprid” (RMS: The Netherlands, June 2023) RMS - The Netherlands concluded that  *“In the study by Lefresne, S. (2015), the method determined the residues of Acetamiprid in Dry bean (seed and straw), mandarin (peel, pulp and whole fiuit), oilseed rape (pod, seed, whole plant and whole plant without pod), olive (oil and whole fiuit) and orange (peel, pulp and whole fiuit) with an LOQ of 0.01 mg/kg.*  *In the study by Lang, A. (2014), the method determined Acetamiprid in head cabbage, apple fruits, potato tubers and peach fruits with an LOQ of 0.01 mg/kg.*  *In the study by Schwarz, T., (2008), acetamiprid was determined with an LOQ of 0.01 mg/kg in apple, whole orange, maize grains, sunflower seeds and honey.*  *Therefore, the methods presented in Lefresne, S. (2015) and Lang, A. (2014), cover all crop matrixes (high water, high acid, high protein/starch and high oil content matrixes) except difficult matrices.*  *Furthermore, the methods do not determine residues in honey as the method validated in the study by Schwarz, T., (2008), however this is considered acceptable as it is not specifically required according to the data requirements Reg. (EU) 283/2013.*  *The end-point is covered by an acceptable study.”*  Therefore the analytical method of Lang, A., 2015 has been matched for matrices with high water content and the analytical method of Lefresne, S., 2015 has been matched for matrices with high acid, high oil and high starch/dry content.  Two new analytical methods have been provided by the applicant. The studies by Brown, S. (RES-00418 & RES-00419) are ILVs to the primary methods by Lefresne, S. (2015) and Lang, A. (2014). In the opinion of RMS - The Netherlands, the ILV analytical method has been matched for all matrices.  All studies are considered acceptable, therefore the end-point is fully covered and the study by Schwarz, T. (2008) is matched.  No additional methods are required.  June 2025, August 2025:  In the context of changed MRLs values for acetamiprid, no additional methods are required. |

#### 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 acetamiprid in animal matrices is given in the following tables. Additionally, ADAMA has provided primary and ILV for the determination of acetamiprid residues in honey. For the detailed evaluation of the new study, it is referred to Appendix 2.

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

| Component of residue definition: N-desmethyl-acetamiprid (IM-2-1), expressed as acetamiprid | | | | |
| --- | --- | --- | --- | --- |
| 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 | Miya, K., 2010, Report No. RD-02080, EU agreed  RAR, 2015, Netherlands\*  to which is equivalent Lang, A., 2016, R-37837, 16A08133-01-VMAT |
| ILV | 0.01 mg/kg | Knoch, E., 2010, Report No. RD-02156, EU agreed  RAR, 2015, Netherlands\*  to which is equivalent Barbier, G., 2016, R-37912, B16G-A4-A-01 |
| Confirmatory  (if required) | - |  | - |
| Eggs | Primary | 0.01 mg/kg | HPLC-MS/MS | Miya, K., 2010, Report No. RD-02080, EU agreed  RAR, 2015, Netherlands\*  to which is equivalent Lang, A., 2016, R-37837, 16A08133-01-VMAT |
| ILV | 0.01 mg/kg | Knoch, E., 2010, Report No. RD-02156, EU agreed  RAR, 2015, Netherlands\*  to which is equivalent Barbier, G., 2016, R-37912, B16G-A4-A-01 |
| Confirmatory  (if required) | - |  | - |
| Muscle | Primary | 0.01 mg/kg | HPLC-MS/MS | Miya, K., 2010, Report No. RD-02080, EU agreed  RAR, 2015, Netherlands\*  to which is equivalent Lang, A., 2016, R-37837, 16A08133-01-VMAT |
| ILV | 0.01 mg/kg | Knoch, E., 2010, Report No. RD-02156, EU agreed  RAR, 2015, Netherlands\*  to which is equivalent Barbier, G., 2016, R-37912, B16G-A4-A-01 |
| Confirmatory  (if required) | - |  | - |
| Fat | Primary | 0.01 mg/kg | HPLC-MS/MS | Miya, K., 2010, Report No. RD-02080, EU agreed  RAR, 2015, Netherlands\*  to which is equivalent Lang, A., 2016, R-37837, 16A08133-01-VMAT |
| ILV | 0.01 mg/kg | Knoch, E., 2010, Report No. RD-02156, EU agreed  RAR, 2015, Netherlands\*  to which is equivalent Barbier, G., 2016, R-37912, B16G-A4-A-01 |
| Confirmatory  (if required) | - |  | - |
| Kidney, liver | Primary | 0.01 mg/kg | HPLC-MS/MS | Miya, K., 2010, Report No. RD-02080, EU agreed  RAR, 2015, Netherlands\*  to which is equivalent Lang, A., 2016, R-37837, 16A08133-01-VMAT |
| ILV | 0.01 mg/kg | Knoch, E., 2010, Report No. RD-02156, EU agreed  RAR, 2015, Netherlands\*  to which is equivalent Barbier, G., 2016, R-37912, B16G-A4-A-01 |
| Confirmatory  (if required) | - |  | - |
| Honey | Primary | 0.01 mg/kg | HPLC-MS/MS | Schrag K., 2022, Report No. 21A14030-01-VMHN, New data  KCP 5.1.2/05 |
| ILV | 0.01 mg/kg | LC-MS/MS | Brown, S., 2022, Report No. RES-00415, New data  KCP 5.2/06 |
| Confirmatory  (if required) | - |  | - |

\*Matching data have been obtained and provided by ADAMA. These data were submitted to the RMS Netherlands in order to demonstrate access to a complete data package according to Reg. (EU) 283/2013 and for the data matching process. ~~The RMS Opinion on GLP compliance, guidance compliance and equivalence of the endpoint will be provided as soon as Data Matching process is finalized.~~ Matching active substance data necessary for the renewal of the approval of acetamiprid is available on CIRCA BC.

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

Table 5.3‑5: Statement on extraction efficiency

|  | Method for products of animal origin |
| --- | --- |
| Required, available from: | The efficiency of the following extraction procedures has been demonstrated using incurred residues in previously submitted and reviewed metabolism studies (please refer to DAR section B.7.1).  Goat Liver, Kidney, Muscle  Liver, kidney and muscle were extracted twice with acetone by shaking for 30 minutes.  Recoveries: 59.2-66.7% for liver, 72.9-74.5% for kidney, 67.1% for muscle.  Hen Liver, Muscle, Eggs  Liver, muscle and eggs (white and yolk) were extracted twice with acetone by shaking for 30 minutes.  Recoveries: 70.9-76.8% for liver, 66.2% for muscle, 74.4-82.1% for egg white, 70.5-77.9% for egg yolk.  The use of acetone in a number of the recovery determinations presented above, indicates that the QuEChERS extraction using acetonitrile would be equally efficient, as acetone and acetonitrile are both polar aprotic solvents, with similar polarity and solubility parameters (extent of dispersion, polar and hydrogen bonding).  Honey A solvent combination comprising (acetonitrile/water, 1:1, v/v) has been demonstrated to be efficient in extracting Acetamiprid and its two metabolites IM-1-4 and IM-1-5 from honey, see Ringli D., 2020 (Study No. RD-11285). The results from the Ringli study were used for the purpose of setting MRLs (see, EFSA Reasoned Opinion on the Modification of the Existing Maximum Residue Levels for Acetamiprid in Various Crops, 2021). The same solvent combination (i.e. acetonitrile/water, 1:1, v/v) was used in the current studies. The extraction process used in the current studies should therefore be considered suitably efficient.  Moreover, acetone and acetonitrile are both aprotic solvents and have similar solubility characteristics (extent of dispersion, polar and hydrogen bonding). Accordingly, when used for extraction purposes, acetonitrile is comparable to acetone in its ability to extract a range of organic target analytes from various animal and plant matrices. The use of acetonitrile was therefore considered comparable to acetone for the extraction of acetamiprid and its metabolite IM-2-1 from honey (see section B5 the RAR, 2016). |
| Not required, because: | - |

|  |
| --- |
| **Evaluator comments:**  According to the evaluation presented in “Matching active substance data necessary for the renewal of the approval of acetamiprid” (RMS: The Netherlands, June 2023) RMS - The Netherlands concluded that *The method in the study by Lang, A. (2016) determines Acetamiprid and IM-2-1 in/on milk, eggs, liver/ kidney, muscle and fat with an LOQ of 0.01 mg/kg. The method in the study by Miya, K., (2010), determines Acetamiprid and IM-2-1 in/on milk, eggs, liver, muscle, kidney and fat with an LOQ of 0.01 mg/kg*  *The end-point is covered by an acceptable study, therefore data matching is shown.*  *In the study by Barbier, G. (2017), the primary method is independently validated for the determination of Acetamiprid and IM-2-1 in/on meat, egg, milk, fat and liver with an LOQ of 0.01 mg/kg.*  *In the study by Knoch, E., (2010), the primary method is independently validated for the determination of Acetamiprid and IM-2-1 in/on milk, eggs, liver, muscle, kidney and fat with an LOQ of 0.01 mg/kg.*  *The end-point is covered by an acceptable study, therefore data matching is shown.*  No additional methods are required.  June 2025, August 2025:  In the context of changed MRLs values for acetamiprid, no additional methods are required. |

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

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

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

| Component of residue definition: acetamiprid and its metabolite and IM-1-5 | | | |
| --- | --- | --- | --- |
| Method type | Method LOQ | Principle of method  (*i.e.* GC-MS or HPLC-UV) | Author(s), year / missing |
| Primary | 0.01 mg/kg  (*acetamiprid and its metabolite IM 1-5*) | LC-MS/MS | Täufer, A. and Weber, H., 2010, Report No. RD-02062N , EU agreed  RAR, 2015, Netherlands\*  to which is equivalent the amended study report R-35750 of Semrau, J., 2017 |
| Confirmatory  (if required) | - |  | - |

\*Matching data have been obtained and provided by ADAMA. The LOQ of ADAMA´s matching method is 0.01 mg/kg, in accordance with the requirements for the quantification of residues in soil (LOQ ≤ 0.05 mg/kg). Furthermore, the relevant ecotoxicological concentrations (NOEC) for the most sensitive terrestrial non-target organisms in soil range from 0.18 to 200 mg a.s./kg soil and are therefore above the selected LOQ. The current study´s LOQ complies with Guideline requirements as well as NOEC values. These data were submitted to the RMS Netherlands in order to demonstrate access to a complete data package according to Reg. (EU) 283/2013 and for the data matching process. ~~The RMS Opinion on GLP compliance, guidance compliance and equivalence of the endpoint will be provided as soon as Data Matching process is finalized.~~ Matching active substance data necessary for the renewal of the approval of acetamiprid is available on CIRCA BC.

|  |
| --- |
| **Evaluator comments:**  According to the evaluation presented in “Matching active substance data necessary for the renewal of the approval of acetamiprid” (RMS: The Netherlands, June 2023) *In the study by Semrau, J. (2017) a HPLC-MS/MS method was validated for the determination of acetamiprid and IM-1-5 in soil with an LOQ 0.01 mg/kg for each analyte.*  *In the study by Täufer, A. and Weber, H., (2010), a method HPLC-MS/MS was validated for the determination of acetamiprid and IM-1-5 in soil with an LOQ 0.002 mg/kg for each analyte.*  *Therefore, the LOQ of the method by Täufer, A. and Weber, H., (2010) is not matched to that evaluated in the RAR. However, according to the SANTE/2020/12830, Rev.1 the LOQ for the determination of residues in soil should not be more than 0.05 mg/kg. This is taking into account the ecotoxicological concentration (ER50, LC50, NOEC) for the most sensitive terrestrial non-target organism (<0.05 mg/kg). The LOQ should also comply with the lowest application rate showing 50% effect (vegetative vigour 329 or seedling emergence/growth ER50-value) in the plant tested.*  *Since the LOQ of the method is still less than 0.05 mg/kg, it is accepted. Therefore, the end-point is covered by an acceptable study, therefore data matching is shown.*  No additional methods are required. |

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

An overview on the acceptable methods and possible data gaps for analysis of acetamiprid 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: acetamiprid and its metabolite IM-1-5 | | | | |
| --- | --- | --- | --- | --- |
| Matrix type | Method type | Method LOQ | Principle of method (i.e. GC-MS or HPLC-UV) | Author(s), year / missing |
| Drinking water | Primary | * 1. µg/L   *(acetamiprid)* | HPLC-MS/MS | Miya, K., 2007, Report No. RD-01204, EU agreed  RAR, 2015, Netherlands\*  to which is equivalent Merdian, H., 2015, R-35910, S15-04647 |
| ILV | * 1. µg/L   *(acetamiprid)* | Senciuc, M., 2014a, Report No. RD-02951, EU agreed  RAR, 2015, Netherlands\*  to which is equivalent Wiesner, F., Breyer, N., 2016, R-35910A, S15-00166 |
| Confirmatory  (if required) | - |  | - |
| Primary | 0.05 µg/L  *(acetamiprid metabolite IM-1-5)* | HPLC-MS/MS | Giesau, A., and Weber, H., 2012, Report No. RD-02604, EU agreed  RAR, 2015, Netherlands\*  to which is equivalent Merdian, H., 2015, R-35911, S15-04648 |
| ILV | 0.05 µg/L  *(acetamiprid metabolite IM-1-5)* | Senciuc, M., 2014b, Report No. RD-02952, EU agreed, RAR, 2015, Netherlands\*  to which is equivalent Wiesner, F., Feddersen, T.2017, R-35911A, S15-00167 |
| Confirmatory  (if required) | - |  | - |
| Surface water | Primary | 0.1 µg/L *(acetamiprid)* | HPLC-MS/MS | Miya, K., 2007, Report No. RD-01204, EU agreed  RAR, 2015, Netherlands\*  to which is equivalent Merdian, H., 2015, R-35910, S15-04647 |
| Confirmatory | - | Not required | - |
| Primary | * 1. µg/L   *(acetamiprid metabolite IM-1-5)* | HPLC-MS/MS | Giesau, A., and Weber, H., 2012, Report No. RD-02604, EU agreed  RAR, 2015, Netherlands \*  to which is equivalent Merdian, H., 2015, R-35911, S15-04648 |
| Confirmatory  (if required) | - |  | - |

\*Matching data have been obtained and provided by ADAMA. These data were submitted to the RMS Netherlands in order to demonstrate access to a complete data package according to Reg. (EU) 283/2013 and for the data matching process. ~~The RMS Opinion on GLP compliance, guidance compliance and equivalence of the endpoint will be provided as soon as Data Matching process is finalized.~~ Matching active substance data necessary for the renewal of the approval of acetamiprid is available on CIRCA BC.

|  |
| --- |
| **Evaluator comments:**  According to the evaluation presented in “Matching active substance data necessary for the renewal of the approval of acetamiprid” (RMS: The Netherlands, June 2023) *In the study by Merdian, H. (2015), HPLC-MS/MS was validated for the determination of Acetamiprid in drinking and surface water with an LOQ of respectively 0.1 µg/L.*  *In the study by Miya, K., (2007), HPLC-MS/MS was validated for the determination of Acetamiprid in drinking, ground and surface water with an LOQ of 0.1 µg/L.*  *Ground water was not tested in the study by Merdian, H. (2015). This is however acceptable since surface and drinking water were tested. Surface water is regarded worst-case.*  *Therefore, the end-point is covered by an acceptable study, therefore data matching is shown.*  *In the study by Wiesner, F. Breyer, N. (2016), the HPLC-MS/MS method was independently validated for the determination of Acetamiprid in drinking with an LOQ of 0.1 µg/L.*  *In the study by Senciuc, M., (2014a), the HPLC-MS/MS was independently validated for the determination of Acetamiprid in drinking water with an LOQ of 0.1 µg/L.*  *Therefore, the end-point is covered by an acceptable study, therefore data matching is shown.*  *In the study by Merdian, H. (2015), HPLC-MS/MS method was validated for the determination of metabolite IM-1-5 in drinking and surface water with an LOQ of 0.10 μg/L.*  *In the study by Giesau, A. and Weber, H., (2012), HPLC-MS/MS was validated for the determination of IM-1-5 in drinking, ground and surface water with an LOQ of 0.05 µg/L.*  *Note that the LOQ is not matched. However, it is acceptable since it still comply with the requirement as stated in SANTE/2020/12830, Rev. 1*  *i.e. LOQ: 0.1 μg/L for drinking/ground and surface water. For surface water unless it is triggered to be lower due to relevant ecotoxicological concentration (EC50, LC50, 372 NOEC).*  *Furthermore, ground water was not tested in the study by Merdian, H. (2015). This is however acceptable since surface and drinking water were tested. Surface water is regarded worst-case.*  *Therefore, the end-point is covered by an acceptable study, therefore data matching is shown.*  *In the study by Wiesner, F. Feddersen, T. (2017), the HPLC-MS/MS method was independently validated for the determination of IM-1-5 in drinking with an LOQ of 0.1 µg/L.*  *In the study by Senciuc, M., (2014b), the HPLC-MS/MS was independently validated for the determination of IM-1-5 in drinking water with an LOQ of 0.05 µg/L.*  *Note that the LOQ is not matched. However, it is acceptable since it still complies with the requirement as stated in SANTE/2020/12830, Rev. 1. i.e. LOQ: 0.1 μg/L for drinking/ground and surface water. For surface water unless it is triggered to be lower due to relevant ecotoxicological concentration (EC50, LC50, 372 NOEC).*  *The end-point is covered by an acceptable study, therefore data matching is shown.*  No additional methods are required. |

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

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

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

| Component of residue definition: acetamiprid | | | |
| --- | --- | --- | --- |
| Method type | Method LOQ | Principle of method  (i.e. GC-MS or HPLC-UV) | Author(s), year / missing |
| Primary | 0.002 μg/m3 | HPLC-MS/MS | Beck, T., Class, T., 2009, Report No. RD-01863  EU agreed  RAR, 2015, Netherlands\*  to which is equivalent Lang, A., 2016, R-37839, 16A08133-01-VMAI |
| Confirmatory  (if required) | - |  | - |

\*Matching data have been obtained and provided by ADAMA. These data were submitted to the RMS Netherlands in order to demonstrate access to a complete data package according to Reg. (EU) 283/2013 and for the data matching process. ~~The RMS Opinion on GLP compliance, guidance compliance and equivalence of the endpoint will be provided as soon as Data Matching process is finalized.~~ Matching active substance data necessary for the renewal of the approval of acetamiprid is available on CIRCA BC.

|  |
| --- |
| **Evaluator comments:**  According to the evaluation presented in “Matching active substance data necessary for the renewal of the approval of acetamiprid” (RMS: The Netherlands, June 2023) *In the study by Lang, A. (2016), an HPLC-MS/MS method was validated for the determination of Acetamiprid in air with an LOQ of 2.1 µg/m3 .*  *The new AOEL value of 0.025 body weight/day set during renewal was taken into account. According to SANTE/2020/12830 rev.1 guidelines: LOQ should comply with the concentration c calculated from the AOELinhalative or the AOELsystemic (in [mg/kg bw d] , according to the following equation:*  *c = AOEL-systemic \* 300 [µg/m3]*  *As such the end-point is covered by an acceptable study by Lang. A. (2016) and the study by Beck, I. & Class, T. (2009) is matched.*  No additional methods are required. |

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

An overview on the acceptable methods and possible data gaps for analysis of acetamiprid in body fluids and tissues is given in ~~the following~~ table 5.3-9. A new study has been conducted by ADAMA for the determination of acetamiprid residues in body fluids, to comply with the most recent LOQ requirements of Guidelines SANTE/2020/12830 rev. 1. For the detailed evaluation of this study, it is referred to Appendix 2.

New monitoring methods for the determination of metabolites IM-2-1 and IC-0 in body fluids and tissues are currently ongoing and will be provided when available. References to the ongoing studies are given in tables 5.3-10 and 5.3-11.

Table 5.3‑9: Methods for body fluids and tissues (if appropriate)

| Component of residue definition: acetamiprid | | | |
| --- | --- | --- | --- |
| Method type | Method LOQ | Principle of method (i.e. GC-MS or HPLC-UV) | Author(s), year / missing |
| Primary (Tissue) | * 1. mg/kg   *(muscle and liver and kidney)* | HPLC-MS/MS | Miya, K., 2010, Report No. RD-02080  EU agreed  RAR, 2015, Netherlands\*  to which is equivalent Lang, A., 2016, R-37837, 16A08133-01-VMAT |
| Primary(body fluid | 0.01mg/L  *(blood)* | HPLC-MS/MS | Brown, S., 2022, Report No. RES-00416, New data  KCP 5.2/07 |

\*Matching data have been obtained and provided by ADAMA. These data were submitted to the RMS Netherlands in order to demonstrate access to a complete data package according to Reg. (EU) 283/2013 and for the data matching process. ~~The RMS Opinion on GLP compliance, guidance compliance and equivalence of the endpoint will be provided as soon as Data Matching process is finalized.~~ Matching active substance data necessary for the renewal of the approval of acetamiprid is available on CIRCA BC.

Table 5.3‑10: Methods for body fluids and tissues

| Component of residue definition: IM-2-1 | | | |
| --- | --- | --- | --- |
| Method type | Method LOQ | Principle of method (i.e. GC-MS or HPLC-UV) | Author(s), year / missing |
| Primary (Tissue) | 0.01 mg/kg  (*muscle and liver*) | HPLC-MS/MS | ~~Brown, D., 2024-~~  Watson, G.,2025  ~~Reference~~ Report No. ~~000119484~~RES-00539, New Data  KCP 5.2/08  ~~(study ongoing)~~ |
| Primary (body fluids) | 0.01 mg/L  (*blood and urine*) | HPLC-MS/MS | ~~Brown, D., 2024-~~  Watson, G.,2025  ~~Reference~~ Report No. ~~000119483~~RES-00538, New Data  KCP 5.2/09  ~~(study ongoing)~~ |

Table 5.3‑11: Methods for body fluids and tissues

| Component of residue definition: IC-0 | | | |
| --- | --- | --- | --- |
| Method type | Method LOQ | Principle of method (i.e. GC-MS or HPLC-UV) | Author(s), year / missing |
| Primary (Tissue) | 0.01 mg/kg  (*muscle and liver*) | HPLC-MS/MS | ~~Brown, D., 2024-~~  Watson, G.,2025  ~~Reference~~ Report No. ~~000119484~~RES-00539, New Data  KCP 5.2/08  ~~(study ongoing)~~ |
| Primary (body fluids) | 0.01 mg/L  (*blood and urine*) | HPLC-MS/MS | ~~Brown, D., 2024-~~  Watson, G.,2025  ~~Reference~~ Report No. ~~000119483~~RES-00538, New Data  KCP 5.2/09  ~~(study ongoing)~~ |

|  |
| --- |
| **Evaluator comments:**  The LOQ required for body fluids according to SANTE/2020/12830 rev. 2 is 0.01 mg/L. Applicant provided new analytical method for determination of acetamiprid in blood with LOQ of 0.01 mg/L.  The method is acceptable. More details please see in Appendix 1.  Additionally, Adama is currently conducting two studies validation of an analytical method for residues of acetamiprid metabolites IM-2-1 and IC-0 in body fluids and in body liquids.  Placeholders have been added for the ongoing studies.  ~~No additional methods are required.~~  June 2025:  Applicant provided final versions of two studies on the validation of an analytical methods for residues of acetamiprid metabolites IM-2-1 and IC-0 in body tissues (meat (muscle) and liver) with LOQ of 0.01 mg/kg and body fluids (urine and blood) with and 0.01 mg/L. The analytical methods were fully validated according to SANTE/2020/12830 rev.2. guidelines.  More details are presented in Appendix 2. |

Reference list

Data Matching List, The Netherlands, ~~2022~~ June 2023

EFSA, 2016: EFSA Scientific Report (2016), 1-26, Conclusion on the peer review of active substance acetamiprid.

EFSA, 2016: EFSA Scientific Report (2016), 1-91, Appendix A – List of end points for the active substance and the representative formulation.

Ellas (Greece), Draft Assessment Report (DAR) of acetamiprid, October 2002

Netherlands, 2016. Revised Renewal Assessment Report (RAR) on acetamiprid prepared by the rapporteur

Member State the Netherlands, in the framework of Commission Implementing Regulation (EU) No 844/2012, August 2016.

EFSA, 2024: EFSA Journal. 2024;22:e8759, Statement on the toxicological properties and maximum residue levels of acetamiprid and its metabolites. ﻿

# 

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 5.1.1/01 | Walter, D. | 2014 | Development and validation of an analytical method for the determination of acetamiprid in MCW-2222 Report No . S13-03099  Eurofins Agroscience Services, Germany  GLP  Unpublished | N | ADAMA |
| KCP 5.1.2/01 | Barbier, G. | 2018 | Freezing storage stability of acetamiprid in wheat (grain) at/below -18°C during 15 months (0 and 15 months)  Report No. B17G-A4-A-02  Fredon Pays de la Loire / GIRPA  GLP  Unpublished | N | ADAMA |
| KCP 5.1.2/02 | Chevallier, E. | 2014 | Magnitude of residue of acetamiprid in barley (RAC) after two applica-tions of MCW-2222- four decline curve trials and four harvest trials in northern Europe (Northern France, Poland, Germany, Hungary and Austria) – 2014  Report No. 14SGS034  SGS AGRI MIN, France  GLP  Unpublished | N | ADAMA |
| KCP 5.1.2/03 | Chevallier, E. | 2014 | Magnitude of the residue of acetamiprid in wheat (Raw Agricultural Commodity) after two applications of MCW-2222 – four decline curve trials and four harvest trials in Northern Europe (Northern France, Poland, Germany, Hungary and Austria) – 2014  Report No. 14SGS033  SGS AGRI MIN, France  GLP  Unpublished | N | ADAMA |
| KCP 5.1.2/04 | Henkes, K. | 2017 | Residues of acetamiprid in foliage-dwelling arthropods and ground vegetation after spray application of Acetamiprid 200 SL in a pome fruit orchard in Italy – magnitude of residues and time course of resi-due decline  Report No. R1640039  RIFCON GmbH, Germany  GLP  Unpublished | N | ADAMA |
| KCP 5.1.2/05 | Mayer, O. | 2018 | Semi-field brood study to evaluate potential effects of MCW-2222 on brood development of honeybees (Apis mellifera L.)  Report No. R1640035  RIFCON GmbH, Germany  GLP  Unpublished | N | ADAMA |
| KCP 5.1.2/06 | Lefresne, S. | 2014 | Validation of the analytical method for the determination of residues of acetamiprid in plant matrices: Dry bean (seed and straw), mandarin (peel, pulp and whole fruit), oilseed rape (pod, seed, whole plant and whole fruit without pod), olive (oil and whole fruit) and orange (peel, pulp and whole fruit).  Report No. B13-M1-A-01  GIRPA, France  GLP  Unpublished | N | ADAMA |
| KCP 5.1.2/07 | Lang, A. | 2014 | Validation of an analytical method for the determination of residues of Acetamiprid in 4 different plant commodities (head cabbage, apple fruits, potato tubers and peach fruits)  Report No. 13M06017-01-VMPL  CIP, Germany  GLP  Unpublished | N | ADAMA |
| KCP 5.1.2/08 | Méric, D. | 2013 | Magnitude of residues of acetamiprid in apples (RAC), following one or two applications of MCW-2222, in two trials (1 DCS + 1 HS) North-ern Europe (Northern France) – 2013  Report No. DMC-13-16134  STAPHYT, France  GLP  Unpublished | N | ADAMA |
| KCP 5.1.2/09 | Roussel, Ch. H. | 2014 | Magnitude of the residues of acetamiprid in apple (RAC fruits and pro-cessed fractions), following one or two applications of MCW-2222 in six trials (3 DCS + 3 HS), Northern Europe (Northern France, Germany, Poland and Belgium) – 2014  Report No. ChR-14-17311  STAPHYT, France  GLP  Unpublished | N | ADAMA |
| KCP 5.1.2/10 | Lebrun, F. | 2014 | Magnitude of the residue of acetamiprid in maize (Raw Agricultural Commodity) after one application of MCW-2222 – four semi decline curve trials and four decline curve trials in Northern Europe (Northern France, Poland, Germany, Hungary and Austria) – 2014  Report No. 14SGS039  SGS AGRI MIN, France  GLP  Unpublished | N | ADAMA |
| KCP 5.1.2/11 | Roussel, Ch. H. | 2022 | Magnitude of the residues of acetamiprid in sugar beet (RAC whole plants, roots and leaves+tops), following two applications of Acetam-iprid 200 SL in three trials (two HS + one DCS) – Northern Europe (Po-land and Hungary) – 2020  Report No. SPK-20-46380  STAPHYT, France  GLP  Unpublished | N | ADAMA |
| KCP 5.1.2/13 | Roussel, Ch. H. | 2022 | Magnitude of the residues of acetamiprid, after application of Acetam-iprid 200 SL in sugar beet in Northern Europe – 2021  Report No. ChR-21-48246  STAPHYT, France  GLP  Unpublished | N | ADAMA |
| KCP 5.1.2/14 | Domingo S. | 2022 | Magnitude of the residues of acetamiprid, after application of Acetam-iprid 200 SL in indoor cucumber in Southern Europe – 2021  Report No. SDO-21-48624  STAPHYT, Spain  GLP  Unpublished | N | ADAMA |
| KCP 5.1.2/15 | Grall, E. | 2022 | Magnitude of the residues of acetamiprid in plum (RAC fruits), follow-ing one application of ACETAMIPRID 200 SL in four trials (two HS + two DCS) - Southern Europe (Spain, Greece and Italy) – 2020  Report No. EGL-20-46374  STAPHYT, Spain  GLP  Unpublished | N | ADAMA |
| KCP 5.1.2/16 | Méric, D. | 2014 | Magnitude of the residues of acetamiprid in peaches (rac fruits), following two applications of mcw-2222 in three trials (1 dcs + 2 hs), southern europe (southern france and italy) – 2013  Report No. DMC-13-16126  STAPHYT, France  GLP  Unpublished | N | ADAMA |
| KCP 5.1.2/17 | Schrag K. | 2022 | Validation of an Analytical Method for the Determination of Residues of Acetamiprid in Honey  Report No. 21A14030-01-VMHN  CIP, Germany  GLP  Unpublished | N | ADAMA |
| KCP 5.1.2/18 | Boileau, G. | 2022 | Magnitude of the residues of acetamiprid after application of ACETAMIPRID 200 SL in honey of phacelia in Northern and Southern Europe – 2021-2022  Report No. GBU-21-48185  STAPHYT, France  GLP  Unpublished | N | ADAMA |
| KCP 5.1.2/19 | xxxxx | 2013 | ACETAMIPRID 200 SL – Acute Inhalation Toxicity Study (Nose-only) in the Rat  Report No. 12/445-004P  xxxxx  GLP  Unpublished | Y | ADAMA |
| KCP 5.1.2/20 | Wilson, A. | 2016 | Foliar dislodgeable residues dissipation on pome fruit in Southern and Northern Europe (Spain, Italy and Czech Republic), 2016  Report No. ACI16-010  AgroChemex International Ltd., UK  GLP  Unpublished | N | ADAMA |
| KCP 5.1.2/21 | Staffel, J. | 2021 | Residues of acetamiprid in monocotyledonous and dicotyledonous plants after spray application of Acetamiprid 200 SL in early vegetation stages in Spain – magnitude of residues and time course of residue de-cline.  Report No. R2040056  RIFCON GmbH  GLP  Unpublished | N | ADAMA |
| KCP 5.1.2/22 | Staffel, J. | 2021 | Residues of acetamiprid in monocotyledonous and dicotyledonous plants after spray application of Acetamiprid 200 SL in early vegetation stages in Germany – magnitude of residues and time course of residue decline  Report No. R2040057  RIFCON GmbH  GLP  Unpublished | N | ADAMA |
| KCP 5.1.2/23 | Staffel, J. | 2022 | Residues of acetamiprid in monocotyledonous and dicotyledonous plants after spray application of Acetamiprid 200 SL in early vegetation stages in Spring in Germany – magnitude of residues and time course of residue decline  Report No. R2040059  RIFCON GmbH  GLP  Unpublished | N | ADAMA |
| KCP 5.1.2/24 | Gräf, K. | 2022 | Residues of acetamiprid in monocotyledonous and dicotyledonous plants after spray application of Acetamiprid 200 SL in early vegetation stages in Northern Europe – magnitude of residues and time course of residue decline  Report No. R2040060  RIFCON GmbH  GLP  Unpublished | N | ADAMA |
| KCP 5.1.2/25 | Schulz, L. | 2022 | Effects of Acetamiprid 200 SL on Collembola under field conditions  Report No. 21 48 FCM 0002  BioChem agrar, Germany  GLP  Unpublished | N | ADAMA |
| KCP 5.1.2/26 | xxxxx | 2014 | Acute toxicity of MCW-2222 to the rainbow trout Oncorhynchus mykiss in a 96-hour static test  Report No. 141048005 W  xxxxx  GLP  Unpublished | Y | ADAMA |
| KCP 5.1.2/27 | Juckeland, D. | 2014 | Acute toxicity of MCW-2222 to Daphnia magna in a 48-hour static test  Report No. 141048006 W  BioChemAgrar, Germany  GLP  Unpublished | N | ADAMA |
| KCP 5.1.2/28 | Juckeland, D. | 2015 | Acute toxicity of MCW-2222 to *Chironomus riparius* in a 48-hour static test  Study No. 141048057W  BioChemAgrar, Germany  GLP  Unpublished | N | ADAMA |
| KCP 5.1.2/29 | Taylor, S. and Joyce | 2015 | Acetamiprid 200 SL – Acute toxicity to aquatic organisms  Report No. CEA.1510 (XCE2008)  Smithers Viscient (ESG) Ltd, UK  GLP  Unpublished | N | ADAMA |
| KCP 5.1.2/30 | Juckeland, D. | 2014 | Effects of MCW-2222 on *Desmodesmus subspicatus* in an algal growth inhibition test  Study No. 141048007 W  BioChemAgrar, Germany  GLP  Unpublished | N | ADAMA |
| KCP 5.1.2/31 | Hennecke, N. | 2020 | Validation of the analytical methods for water and sediment  Report No. ADM-026/6-22  Fraunhofer IME, Germany  GLP  Unpublished | N | ADAMA |
| KCP 5.1.2/32 | Kleebaum, K. | 2015 | Chronic toxicity of MCW-2222 to the honeybee larvae Apis mellifera L. under laboratory conditions (in vitro)  Report No. 141048078 B  BioChem agrar, Germany  GLP  Unpublished | N | ADAMA |
| KCP 5.1.2/33 | Molitor, C. | 2015 | Field Study to Evaluate Potential Side Effects of the product MCW-2222 (acetamiprid 200 g/L) on Brood Development, Foraging Activity, Mortality and Behaviour of Adult Honeybees Apis mellifera L. (Hymenoptera: Apidae) Following Application after Bee-Flight on Phacelia tanacetifolia  Report No. 215-2014  TESTAPI, France  GLP  Unpublished | N | ADAMA |
| KCP 5.1.2/34 | Molitor, C. | 2015 | Field Study to Evaluate Potential Side Effects of MCW-2222 on Brood Development, Foraging Activity, Mortality and Behaviour of Adult Honeybees (Apis mellifera) on Oilseed Rape  Report No. 230-2015  TESTAPI, France  GLP  Unpublished  Unpublished | N | ADAMA |
| KCP 5.1.2/35 | Aucejo, S. | 2015 | Effects and Determination of Residues of Acetamiprid 200 SL on the Honeybee (*Apis mellifera L*.) Brood in Citrus, under Field Conditions, in Spain 2015.  Study No. 307SRE15C02  SynTECH research center, Spain  GLP  Unpublished | N | ADAMA |
| KCP 5.1.2/36 | Friedrich, S. | 2014 | Terrestrial plant test with MCW-2222: Vegetative vigour test  Report No. 14 10 48 002 P  BioChem agrar, Germany  GLP  Unpublished | N | ADAMA |
| KCP 5.1.2/37 | Hengsberger, A. and Wydra, V. | 2015 | IC-0: Acute Toxicity to Larvae of Chironomus riparius in a Static 48-hour Immobilisation Limit-Test  Report No. 102461251  Ibacon GmbH, Germany  GLP  Unpublished | N | ADAMA |
| KCP 5.2/01 | Brown, S. | 2022 | Independent laboratory validation of analytical method B13-M1-A-01 (Sponsor code R-33645) for determination of Acetamiprid in food of plant origin  Report No. RES-00418  ResChem Analytical Limited, UK  GLP  Unpublished | N | ADAMA |
| KCP 5.2/02 | Brown, S. | 2022 | Independent laboratory validation of analytical method 13M06017-01-VMPL (Sponsor code R-33644) for determination of Acetamiprid in food of plant origin.  Report No. RES-00419  ResChem Analytical Limited, UK  GLP  Unpublished | N | ADAMA |
| KCP 5.2/03 | Lefresne, S. | 2014 | Comparison of the extraction efficiency of two solvents used in the analytical methods for the determination of acetamiprid residues in various plant matrices (dry, acid, water and oily)  Report No. B14S-M1-A-01  FREDON Pays de la Loire / GIRPA  GLP  Unpublished | N | ADAMA |
| KCP 5.2/04 | Lefresne, S. | 2014 | Amendment No. 1 to study: Comparison of the extraction efficiency of two sol-vents used in the analytical methods for the determination of acetamiprid residues in various plant matrices (dry, acid, water and oily)  Report No. B14S-M1-A-01  FREDON Pays de la Loire / GIRPA  GLP  Unpublished | N | ADAMA |
| KCP 5.2/05 | Schrag K. | 2022 | Validation of an Analytical Method for the Determination of Residues of Acetamiprid in Honey  Report No. 21A14030-01-VMHN  CIP, Germany  GLP  Unpublished | N | ADAMA |
| KCP 5.2/ 06 | Brown, S. | 2022 | Independent laboratory validation of analytical method 21A14030-01-VMHN (Adama study No. 000107274) for residues of acetamiprid in honey.  Report No. RES-00415  ResChem Analytical Limited, UK  GLP  Unpublished | N | ADAMA |
| KCP 5.2/ 07 | Brown, S. | 2022 | Validation of an analytical method for the determination of residues of acetamiprid in body fluids (blood) by LC-MS/MS  Report No. RES-00416  ResChem Analytical Limited, UK  GLP  Unpublished | N | ADAMA |
| KCP 5.2/ 08 | ~~Brown, D.~~  Watson, G. | ~~2024-~~2025  ~~(study is ongoing)~~ | Validation of an analytical method for residues of acetamiprid metabolites IM-2-1 and IC-0 in body tissues  ~~Reference No. 000119484~~ Report No. RES-00539  ResChem Analytical Limited, UK  GLP  Unpublished | N | ADAMA |
| KCP 5.2/ 09 | ~~Brown, D.~~  Watson, G. | ~~2024-~~2025  ~~(study is ongoing)~~ | Validation of an analytical method for residues of acetamiprid metabolites IM-2-1 and IC-0 in body fluids  ~~Reference No. 000119483~~ Report No. RES-00538  ResChem Analytical Limited, UK  GLP  Unpublished | N | ADAMA |

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 |
| --- | --- | --- | --- | --- | --- |
| CP 5.1.2 | Mamouni, A. | 1997 | Adsorption/Desorption of IM-1-4 on Five Soils  Report No. 383826  GLP  Published | N | Nippon Soda |
| CP 5.1.2 | Emeric, G.T. | 1998 | Acetamiprid - Verification of the Identity of the Photolyte obtained at pH 7  Report No. 98-47  GLP  Published | N | Nippon Soda |
| CP 5.1.2 | Shiotani, H. | 2003 | Photodegradation of IM-1-5 in Water  Report No. C030709  GLP  Published | N | Nippon Soda |
| CA 5.2 | Schwarz, T. | 2008 | Acetamiprid: Validation of an Enforcement Method for Plant Materials  Study P/B1447G  PTRL Europe  Nippon-Soda Report No. RD-01937  GLP  Upublished | N | Nippon soda |
| CA 5.2 | Weber, H. | 2013 | Validation of a Multiresidue Method (Fillion) with Modified Cleanup and Detection for the Determination of Acetamiprid in Potato  Study No. S13-02134, Document ID RD-02603  Eurofins Agroscience Services  GLP  Unpublished | N | Nippon soda |
| CP 5.1.2 | Goller G. | 1999 | Stability Study of NI-25 (Acetamiprid) in apple and tomato samples after storage in freezer at or below -18 °C - Fortification experiments with active ingredient  Report No RPA/NI-25/97051  A.D.M.E. - Bioanalyses, France  GLP  Unpublished | N | Nippon Soda |
| CP 5.1.2 | Netzband D.J. | 2003 | Stability study of Acetamiprid in potatoes during frozen storage, USA, 2002 in freezer at or below -18°C  Report No RD-00243  Bayer CropScience  GLP  Unpublished | N | Nippon Soda |
| CP 5.1.2 | Jean-Baptiste C. | 2009 | Frozen Storage Stability of Residues of Acetamiprid in Fodder Pea  Report No A7125  Anadiag Laboratories  GLP  Unpublished | N | Nippon Soda |
| CP 5.1.2 | Gieseke L.D. | 1999 | NI-25 (acetamiprid): Freezer storage stability of acetamiprid residues in various raw agricultural commodities and processing fractions (plant matrices)  Report No 10201  Horizon Laboratories, Inc.  GLP  Unpublished | N | Nippon Soda |
| CP 5.1.2 | Raufer B. | 2013 | Residue study on rotational crops after one application of Acetamiprid on bare soil at 2 sites in Europe in 2010 to 2012.  Report No RD-02495N2  GLP  Unpublished | N | Nippon Soda |
| CP 5.1.2 | Raufer B. | 2014 | Residue study on rotational crop (turnip) after one application of Acetamiprid on bare soil at 1 site in Europe in 2012 to 2013.  Report No RD-02930  GLP  Unpublished | N | Nippon Soda |
| CP 5.1.2 | Kowite W.J. | 1999 | NI-25: Magnitude of Residues in Apple Processed Commodities Resulting from Foliar Applications of EXP 80667A Insecticide  Report No 97512650  Rhône- Poulenc Agriculture Ltd  GLP, GEP  Unpublished | N | Nippon Soda |
| CP 5.1.2 | Venet, C.  Barriere, I. | 2000a | Acetamiprid (NI-25) – Formulation EXP60707A (SP) - Trials France 1999 - Residues in Apple + Processed products  Study No. R&D/CRLD/AN/mba 0015360  Aventis crop science  GLP  Unpublished | N | Nippon Soda |
| CP 5.1.2 | Richard, M.  Maestracci,  M | 1997a | Acetamiprid (NI-25) – Formulation EXP60707A (SP) - Trial Spain 1996 - Residues in Tomato (Greenhouse) - Decline study  Study No. 9716021  Rhône Poulenc Agro  GLP  Unpublished | N | Nippon Soda |
| CP 5.1.2 | Richard, M.  Maestracci,  M | 1997b | Acetamiprid (NI-25) – Formulation EXP60707A (SP) - Trial Italy 1996 - Residues in Tomato (Greenhouse) - Decline study  Study No. 9715986  Rhône Poulenc Agro  GLP  Unpublished | N | Nippon Soda |
| CP 5.1.2 | Richard, M.  Maestracci,  M | 1997c | Acetamiprid (NI-25) – Formulation EXP60707A (SP) - Trial France 1997 -  Residues in Tomato (in Greenhouse)  Study No. 9716514  Rhône Poulenc Agro  GLP  Unpublished | N | Nippon Soda |
| CP 5.1.2 | Richard, M.  Maestracci,  M | 1997d | Acetamiprid (NI-25) – Formulation EXP60707A (SP) - Trial France 1997 - Residues in Tomato (in Greenhouse)  Study No. 9716513  Rhône Poulenc Agro  GLP  Unpublished | N | Nippon Soda |
| CP 5.1.2 | Venet, C.  Barriere, I. | 2000b | Acetamiprid (NI-25) – Formulation EXP60707A (SP) - South/Italy/1999 – 1 harvest study trial - Residues in Tomato (fruit).(in Greenhouse)  Study No. 0015356  Aventis crop science  GLP  Unpublished | N | Nippon Soda |
| CP 5.1.2 | xxxxx | 1999b | Acetamiprid (Code No.: NI-25) – Magnitude of Residues in Poultry Tissue and Eggs.  Report No RD-09988  xxxxx  Unpublished | Y | Nippon Soda |
| CP 5.1.2 | xxxxx | 1999a | Acetamiprid: Magnitude of Residues in Cairy Cow Milk and Tissues  Report No RD-9989  xxxxx  Unpublished | Y | Nippom Soda |
| CP 5.1.2 | Liu, A.C. | 1997 | 6-Chloronicotinic Acid (Acetamiprid metabolite) soil adsorption/desorption study  GLP  Unpublished | N | Nippon Soda |
| CP 5.1.2 | Sugiyama H. | 2010 | Adsorption/Desorption Study of IM 1-5 on Soils  Study no. RD-02101  GLP  Unpublished | N | Nippon Soda |
| CP 5.1.2 | Kellner, T. | 2018 | Field Soil Dissipation Study with IM-1-5 (a metabolite of Acetamiprid) on three Sites in Europe (2016 – 2017)  Eurofins, Germany  Report No. R1640068  Study reference: R-37999 (000110074)  GLP  Unpublished | N | ADAMA |
| CP 5.1.2 | Weber, H.  Zetzsch, A. | 2016 | Storage stability of acetamiprid and its metabolite IM-1-15 in soil under deep frozen conditions  Eurofins, Germany  Report No. S15-04842  Study reference: R-36488 (000083670)  GLP  Unpublished | N | ADAMA |
| CP 5.1.2 | Semrau, J. | 2017 | Determination of residues of acetamiprid and its soil metabolites IM-1-4 and IM-1-5 after one application of MCW-2222 to bare soil in rotational crops (radish, spinach and wheat) at 1 site in Northern Europe and 1 site in Southern Europe 2015 / 2016  Eurofins, Germany  Report No. S15-02364  Study reference: R-35750  GLP  Unpublished | N | ADAMA |
| CA 5.2 | Miya, K. | 2010 | Validation Study of the Analytical Method for the Determination of the Residues of Acetamiprid and Its Metabolite (IM-2-1) in Animal Commodities  Report No. NCAS 10-144, Document ID RD-02080  Nisso Chemical Analysis Service Co., Japan  GLP  Unpublished | N | Nippon soda |
| CA 5.2 | Knoch, E. | 2010 | Independent Laboratory Validation: Analytical Method for the Determination of the Residues of Acetamiprid and its Metabolite (IM-2-1) in Animal Commodities  Report No. IF-10/01687868, Document ID RD-02156  SGS Institut Fresenius GmbH  GLP  Unpublished | N | Nippon soda |
| CA 5.2 | Täufer, A. & Weber H. | 2010 | Validation of an Analytical Method for the Determination of Residues of Acetamiprid and Acetamiprid Soil Metabolite IM-1-5 in Calcareous Soil using LC-MS/MS  Report No. S09-03287, Document ID RD-02062N  Eurofins Dr. Specht, Germany  GLP  Unpublished | N | Nippon soda |
| CA 5.2 | Miya, K. | 2007 | Validation Study of the Confirmatory Method for the Determination of Acetamiprid in Water,  Report No. NCAS 06-209, Document ID RD-01204  Nisso Chemical Analysis Service Co., Japan  GLP  Unpublished | N | Nippon soda |
| CA 5.2 | Senciuc, M. | 2014a | Independent Laboratory Validation (ILV) of a Residues Analytical Method for the Determination of Acetamiprid in Drinking Water  Report No. P 3244 G, Document ID RD-02951  PTRL Europe GmbH, Germany  GLP,  Unpublished | N | Nippon soda |
| CA 5.2 | Gieseau, A. & Weber, H. | 2012 | Validation of an Analytical Method for the Determination of Residues of Acetamiprid Metabolite IM-1-5 in Water using LC-MS/MS,  Report No. S12-02719, Document ID RD-02604  Eurofins Agroscience Services, Germany,  GLP,  not published | N | Nippon soda |
| CA 5.2 | Senciuc, M. | 2014b | |  | | --- | | Independent Laboratory Validation (ILV) of a Residues Analytical Method for the Determination of Acetamiprid Metabolite IM-1-5 in Drinking Water  Report No. P 3245 G, Document ID RD-02952  PTRL Europe GmbH, Germany  GLP  Unpublished | | N | Nippon soda |
| CA 5.2 | Lang, A. | 2016 | Validation of an Analytical Method for the Determination of Acetamiprid in Air  CIP, Germany  Report No.: 16A08133-01-VMAI  Study reference: R-37839 (000086048)  GLP  Unpublished | N | ADAMA |
| CA 5.2 | Lang, A. | 2016 | Validation of an Analytical Method for the Determination of Residues of Acetamiprid and its Metabolite IM-2-1 in five Matrices of Animal Origin (Milk, Eggs, Meat, Fat and Kidney/Liver)  CIP, Germany  Report No. 16A08133-01-VMAT  Study reference: R-37837 (000086046)  GLP  Unpublished | N | ADAMA |
| CA 5.2 | Barbier, G. | 2017 | Independent Laboratory Validation of an analytical method for the determination of residues of acetamiprid and its metabolite N desmethylacetamiprid (IM-2-1) in animal tissues: meat, egg, milk, fat and liver  FREDON Pays de la Loire / GIRPA, France  Report No. B16G-A4-A-01  Study reference: R-37912 (000086201)  GLP  Unpublished | N | ADAMA |
| CA 5.2 | Merdian, H. | 2015 | Validation of the analytical method for the determination of acetamiprid  in surface and drinking water  Eurofins, UK  Report No. S15-04647  Study reference: R-35910 (000024803)  GLP  Unpublished | N | ADAMA |
| CA 5.2 | Wiesner, F.  Breyer, N. | 2016 | Indipendent Laboratory Validation (ILV) of an Analytical Method for the Determination of Acetamiprid in Water  Eurofins, Germany  Report No. S16-00166  Study reference: R-35910A (000085643)  GLP  Unpublished | N | ADAMA |
| CA 5.2 | Merdian, H. | 2015 | Validation of the analytical method for the determination of acetamiprid metabolite I M-1-5 in surface and drinking water  Eurofins, UK  Report No. S15-04648  Study reference: R-35911 (000024804)  GLP  Unpublished | N | ADAMA |
| CA 5.2 | Wiesner, F.  Feddersen, T. | 2017 | Indipendent Laboratory Validation (ILV) of an Analytical Method for the Determination of Acetamiprid Metabolite IM-1-5 in Water  Eurofins, Germany  Report No. S16-00167  Study reference: R-35911A (000085644)  GLP  Unpublished | N | ADAMA |
| CA 5.2 | Lang, A. | 2016 | Validation of an Analytical Method for the Determination of Residues of Acetamiprid in Blood  CIP, Germany  Report No. 16A08133-01-VMBF  Study reference: R-37838 (000086047)  GLP  Unpublished | N | ADAMA |

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 |
| --- | --- | --- | --- | --- | --- |
| - | - | - | - | - | - |

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 |
| --- | --- | --- | --- | --- | --- |
| - | - | - | - | - | - |

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

No new or additional studies have been submitted.

**A 2.1.1.1 Description of analytical method for the determination of residues (KCP 5.1.2)**

* + - * 1. Analytical method B17G-A4-A-02

Method validation 14SGS034 & 14SGS033

|  |  |
| --- | --- |
| Comments of zRMS: | The study has been evaluated and accepted in Registration Report, Section 5 for CA3573 / Carnadine / Kestrel, Nufarm (August 2021).  Conclusions:  *The analytical method was successfully validated according to the guidance document SANCO/3029/99 rev.4 on barley (whole plant, grains and straw) and reported in GIRPA report B14C-S1-A-03, SGS Agri min study 14SGS034 (Chevallier, E., full validation in barley).*  *The analytical method was validated (reduce validation) according to the guidance document SANCO/3029/99 rev.4 on wheat grains during another analytical phase performed at GIRPA in 2014 (report B14C-S1-A-01, SGS Study number: 14SGS033).*  *The limit of quantification (LOQ) for acetamiprid was 0.01 mg/kg. The limit of detection (LOD) was defined as 0.0033 mg/kg within the validation study.*  *As analyses at T=0 and T+15 months were performed the same day, they have the same procedural recoveries. Thus correct results with day 0 as 100% in the table below are the same.*    *The acetamiprid residue results of the three freshly fortified samples were 0.074, 0.073 and 0.081 mg/kg corresponding to recoveries respectively at 74, 73 and 81% (mean 76% - RSD 6%). In conclusion, the procedural recoveries of the storage stability study prove to be within the requirements of guidance document SANCO/3029/99 rev.4, therefore the method is acceptable.* |

|  |  |
| --- | --- |
| Reference: | KCP 5.1.2/01 |
| Report | Freezing storage stability of acetamiprid in wheat (grain) at/below -18°C during 15 months (0 and 15 months), Barbier, G., 2018, Study No. B17G-A4-A-02, Sponsor No. R-38589. |
| Guideline(s): | Yes, SANCO 3029/99 rev. 4 |
| Deviations: | None |
| GLP: | Yes |
| Acceptability: | Yes |

The multi-residue QuEChERS-based analytical method used in the current study was fully validated for the determination of acetamiprid in barley (grain) according to the requirements of SANCO 3029/99 rev. 4 guidelines in a previous study prior to 1st March 2021 (Chevallier E., 2014, Study No. 14SGS034, see KCP 5.1.2/02). Reduced validation data according to the requirements of SANCO 3029/99 rev. 4 for the determination of acetamiprid in wheat (grain) was generated during another study (Chevallier E., 2014, Study No. 14SGS033, see KCP 5.1.2/03).

In the current study procedural recovery data was generated.

1. Materials

|  |  |
| --- | --- |
| Reference item: | Acetamiprid |
| Lot/Batch number: | 41007 |
| Purity: | 98.1 ± 0.5 % |
| CAS No.: | 135410-20-7 |
| Expiry date: | April 2019 |
| Standards for calibration | As above |
| Matrix: | Wheat |

1. **Sample preparation and processing**

Approximately 2 g are weighed into a 50 mL centrifuge tube. The samples are fortified respectively. 10 mL of acetonitrile was added. Afterwards all samples were shaken for 20 minutes and frozen for two months at -18°C. The aliquots of the acetonitrile phase are transferred into a centrifuge tube and 10 mL of ultra-pure water was added. The samples were shaken again for 20 minutes and then transferred into a 50 mL QuEChERS tube containing a salt mix. Afterwards the samples were shaken again for 5 minutes and centrifuged for 5 minutes at 4000 rpm. The aliquots of the organic phase were transferred into a second QuEChERS tube containg salts and PSA. The samples were shaken again and centrifuge again 5 minutes at 4000 rpm and afterwards filtered (0.45 µm). The final extracts were analysed by HPLC-MS/MS.

1. **Chromatographic parameters**

|  |  |
| --- | --- |
| HPLC- parameters:  Instrumentation: | API6500 |
| Column: | C18 Hydro RP (100 x 3 mm ID x 2.5 µm PD |
| Column temperature: | 60°C |
| Injection volume: | 10µl – 220 µL |
| MS/MS - parameters  Instrumentation: | 4000QTrap |
| Mode: | ESI (electrospray ionisation) positive |
| Ion source: | Turbospray |
| Scan type: | MRM |
| Transitions: | 223 -> 126 m/z (quantifier)  223 -> 90 m/z (qualifier) |

Results and discussions

The specific identification of acetamiprid was conducted by HPLC-MS/MS with two transitions monitored. Therefore, no confirmatory data needs to be provided. The analytical method were validated according to SANCO/3029/00 rev. 4 in 14SGS034 (Chevallier, E., full validation in barley) and in 14SGS033 (Chevallier, E., reduced validation in wheat). The limit of quantification in the main validation is 0.01 mg/kg and is defined as the lowest fortification level with mean recoveries ranging from 70% to 110% at a relative standard deviation (RSD) of ≤ 20% and blanks not exceeding 30% of the LOQ level. These criteria were fulfilled for the procedural recoveries of the 10 x LOQ for acetamiprid. The detector response for acetamiprid was linear within the range from 0.3 µg/L to 20 µg/L with r2 ≥ 0.999. The procedural recovery data are presented in the table below.

Table A 1: Procedural recovery results from method validation of acetamiprid using the analytical method

| Matrix | Analyte | Fortification level (mg/kg) (*n* = 5) | Mean  recovery (%)  0 & 15 months | RSD (%) | Comments |
| --- | --- | --- | --- | --- | --- |
| Wheat | Acetamiprid | 0.1 | 76, 76 | - | - |

Table A 2: Characteristics for the analytical method used for validation of acetamiprid residues in wheat

|  | Acetamiprid |
| --- | --- |
| Specificity | A highly specific HPLC-MS/MS method was used for the determination of the target analyte. Two mass transitions (m/z 223→126, quantification and m/z 223→90, qualification) were monitored during each analysis.  Target analyte concentrations in controls did not exceed 30% of the method LOQ. No interference from co-eluting components at the retention time of the target analyte. |
| Calibration (type, number of data points) | Five-point linear matrix matched calibration:  Wheat: y= 8645.43x + 338.07 r2= 0.9998 |
| Assessment of matrix effects is presented | No, however matrix matched calibration was used throughout the study |
| Calibration range | 0.3 µg/L to 20 µg/L |
| Limit of determination/quantification | LOQ: 0.01 mg/kg  LOD: 0.003 mg/kg |

Conclusion

The method validation in B14C-S1-A-03 and reduced validation in B14C-S1-A-01 for the determination of acetamiprid in plant material were fully conducted according to the requirements of the SANCO/3029/99 rev. 4 guidelines in studies prior 1st of March 2021. The procedural recoveries of the storage stability study prove to be within the requirements of guidelines SANCO/3029/99 rev. 4 guidelines as well as the minimum validation requirements given in Section 4.2 of SANTE/2020/12830 rev. 1 and therefore the method can be fully accepted.

* + - * 1. Analytical method 14SGS034

Method validation 14SGS034

|  |  |
| --- | --- |
| Comments of zRMS: | The study has been evaluated and accepted in Registration Report, Section 5 for CA3573 / Carnadine / Kestrel, Nufarm (August 2021).  Conclusions:  *The method was successfully validated according to the guidance document SANCO/3029/99 rev.4 on barley (whole plant, grains and straw) and reported in GIRPA report B14C-S1-A-03, SGS Study number: 14SGS034, Sponsor Reference: R-34898A. The limit of quantification (LOQ) for acetamiprid was 0.01 mg/kg. The limit of detection (LOD) was defined as 0.0033 mg/kg within the validation study.*  *The mean recovery was between 70% and 110% with a RSD less than 20% at each level fortification.*  *The method is acceptable.* |

|  |  |
| --- | --- |
| Reference: | KCP 5.1.2/02 |
| Report | Magnitude of residue of acetamiprid in barley (RAC) after two applications of MCW-2222- four decline curve trials and four harvest trials in northern Europe (Northern France, Poland, Germany, Hungary and Austria) - 2014, Chevallier, E., Study No. 14SGS034, Adama Reference No. R-34898A. |
| Guideline(s): | Yes, SANCO 3029/99 rev. 4 |
| Deviations: | None |
| GLP: | Yes |
| Acceptability: | Yes |

A multi-residue QuEChERS-based analytical method for the determination of acetamiprid in barley (whole plant, grain and straw) was fully validated according to the requirements of SANCO/3029/99 rev. 4 prior to 1st March 2021 and used to support residue studies.

1. Materials

|  |  |
| --- | --- |
| 1. Standards |  |
| Test item: | MCW-2222 |
| Batch No.: | 659-030314-01 |
| Expiry date: | March 2016 |
| Active ingredient: | Nominal: 200 g/L  Analysed: 199.2 g/L |
| Reference item: | Acetamiprid |
| Lot/Batch number: | 20202 |
| Purity: | 98.1 ± 0.5 % |
| CAS No.: | 135410-20-7 |
| Expiry date: | February 2016 |
| Standards for calibration | As above |
| Matrix: | Barley (whole plant, grain and straw) |

1. **Sample preparation and processing**

Approximately 2 g are weighed into a 50 mL centrifuge tube. The samples are fortified respectively. For barley grains the samples are shaken for one minute horizontally. For all matrices 10 mL of ultra-pure water was added. For barley (whole plant and straw) 10 mL of acetonitrile was added. Afterwards all samples for all barley compounds were shaken for 20 minutes. The aliquots are transferred into a QuEChERS tube and shaken by hand. The samples will be centrifuge for 5 minutes at 4000 rpm and afterwards filtered (0.45 µm). The final extracts were analysed by HPLC-MS/MS.

1. **Chromatographic parameters**

|  |  |
| --- | --- |
| HPLC- parameters | API6500 |
| Column: | C18 Hydro RP (100 x 3 mm ID x 2.5 µm PD |
| Mobile phase: | A: Ultra-pure water/glacial acetic acid (100/0.1) (v/v) + 5mM ammonium acetate  B: Methanol/glacial acetic acid (100/0.1) (v/) + 5mM ammonium acetate |
| Flow rate: | 0.7 mL.min-1 |
| Injection volume: | 10µl – 220µL |
| MS/MS – parameters: | 4000QTrap |
| Ionisation type: | ESI (electrospray ionisation) positive |
| Transitions: | 223 → 126 m/z (quantification)  223→90 m/z (confirmation) |

Results and discussion

The analytical method used in the current study was fully validated for the determination of acetamiprid in barley (whole plant, grain and straw) according to the requirements of SANCO 3029/99 rev. 4 guidelines prior to 1st March 2021. The target analyte was determined using HPLC-MS/MS by monitoring two highly specific mass transitions. The detector response was linear over the range 0.3 -5 µg/L for barley (grain) and 0.3 -10 µg/L for barley (whole plant and straw) with associated correlation coefficients (r) > 0.99. The LOQ of the method is 0.01 mg/kg. All mean recovery values and associated RSDs meet the requirements of SANCO/3029/99 rev. 4 and the minimum validation requirements given in Section 4.2 of SANTE/2020/12830 Rev. 1. guidelines and are summarised in the table below.

Table A 3: Method validation recovery data for the determination of acetamiprid in barley reported in study 14SGS034

| Matrix | Analyte | Fortification level (mg/kg) (*n* = 5) | Mean  recovery (%) | RSD (%) | Comments |
| --- | --- | --- | --- | --- | --- |
| Quantification transition m/z 223→126 | | | | | |
| Barley (grains) | Acetamiprid | 0.01 | 78 | 9 | - |
| 0.1 | 76 | 5 | - |
| Barley  (whole plant) | 0.01 | 87 | 4 | - |
| 0.1 | 90 | 4 | - |
| Barley  (straw) | 0.01 | 74 | 5 | - |
| 0.1 | 83 | 4 | - |

Table A 4: Characteristics of the analytical method validated for the determination of acetamiprid in barley

|  | Acetamiprid |
| --- | --- |
| Specificity | A highly specific HPLC-MS/MS method was used for the determination of the target analyte. Two mass transitions (m/z 223→126, quantification and m/z 223→90, qualification) were monitored during each analysis.  Target analyte concentrations in controls did not exceed 30% of the method LOQ. No interference from co-eluting components at the retention time of the target analyte. |
| Calibration (type, number of data points) | 5-point linear calibration  Barley (grain): y= 46479.58x + 2652.26 r= 0.995  Barley (whole plant): y= 1008331.54x + 7110.05 r= 0.999  Barley (straw) : y= 16507.86x + 223.12 r= 0.999 |
| Calibration range | Barley (whole plant and straw): 0.3 -10 µg/L  Barley (grain): 0.3 -5 µg/L |
| Assessment of matrix effects is presented | Matrix effects were not assessed |
| Limit of determination/quantification | LOQ for all matrices: 0.01 mg/kg  LOD for all matrices: 0.003 mg/kg |

Conclusion

An analytical method for the determination of acetamiprid in barley (whole plant, grain and straw) was fully validated according to SANCO/3029/99 guidelines prior to March 21st 2021. The data provided exceed the minimum validation requirements given in Section 4.2 of SANTE/2020/12830 Rev. 1. The method should therefore be considered suitable for its intended purpose and acceptable for the determination of acetamiprid in barley (whole plant, grain and straw).

* + - * 1. Analytical method 14SGS033

Method validation 14SGS034

|  |  |
| --- | --- |
| Comments of zRMS: | The study has been evaluated and accepted in Registration Report, Section 5 for CA3573 / Carnadine / Kestrel, Nufarm (August 2021).  Conclusions:  *The method was successfully validated according to the guidance document SANCO/3029/99 rev.4 on barley (whole plant, grains and straw) and reported in GIRPA report B14C-S1-A-03, SGS Agri min study 14SGS034. The limit of quantification (LOQ) for acetamiprid was 0.01 mg/kg. The limit of detection (LOD) was defined as 0.0033 mg/kg within the validation study.*  *The summary of validation is presented below:*    *The method was checked within this study on wheat matrix by a reduced validation, performing on wheat (whole plant, grain and straw) 6 spiked samples, 3 recovery experiments fortified at the limit of quantification, 3 recovery experiment fortified at ten times the LOQ level and one control sample.*  *The reduced validation performed on wheat is summarised below:*    *For each matrix (wheat whole plant, wheat grains and wheat straw), the limit of quantification (LOQ) of the method of acetamiprid is 0.01 mg/kg. The limit of detection (LOD) was defined as 0.0033 mg/kg. The mean recovery was between 70% and 110% with a RSD less than 20% at each level fortification.*  *The method was successfully validated according to the guidance document SANCO/3029/99 rev.4, so the method is acceptable.* |

|  |  |
| --- | --- |
| Reference: | KCP 5.1.2/03 |
| Report | Magnitude of the residue of acetamiprid in wheat (RAC) after two applications of MCW-2222 – four decline curve trials and four harvest trials in Northern Europe (Northern France, Poland, Germany, Hungary and Austria) – 2014, Chevallier, E., 2014, 14SGS033, Adama Reference No. R-34897A. |
| Guideline(s): | Yes, SANCO 3029/99 rev. 4 |
| Deviations: | None |
| GLP: | Yes |
| Acceptability: | Yes |

The multi-residue QuEChERS-based analytical method used in the current study was fully validated for the determination of acetamiprid in barley (whole plant, grain and straw) according to the requirements of SANCO 3029/99 rev. 4 guidelines in a previous study prior to 1st March 2021 (Chevallier, E., Study No. 14SGS034, KCP 5.1.2/01). Reduced validation data according to the requirements of SANCO 3029/99 rev. 4 for the determination of acetamiprid in wheat (grain, straw and whole plant) was provided in the current study.

A. Materials

|  |  |
| --- | --- |
| 1. Standards |  |
| Test item: | MCW-2222 |
| Batch No.: | 659-030314-01 |
| Expiry date: | March 2016 |
| Active ingredient: | Nominal: 200 g/L  Analysed: 199.2 g/L |
| Reference item: | Acetamiprid |
| Lot/Batch number: | 20202 |
| Purity: | 98.1 ± 0.5 % |
| CAS No.: | 135410-20-7 |
| Expiry date: | February 2016 |
| Standards for calibration | As above |
| Matrix: | Wheat (grain, straw and whole plant) |

1. **Sample preparation and processing**

Approximately 2 g are weighed into a 50 mL centrifuge tube. The samples are fortified respectively. For wheat grains the samples are shaken for one minute horizontally. For all matrices 10 mL of ultra-pure water were added. For wheat (whole plant and straw) 10 mL of acetonitrile was added. Afterwards all samples for all wheat compounds were shaken for 20 minutes. The aliquots are transferred into a QuEChERS tube and shaken by hand. The samples were centrifuged for 5 minutes at 4000 rpm and afterwards filtered (0.45 µm). The final extracts will be analysed by HPLC-MS/MS.

1. **Chromatographic parameters**

|  |  |
| --- | --- |
| HPLC- parameters | API6500 |
| Column: | C18 Hydro RP, 100 x 3 mm ID x 2.5 µm PD |
| Mobile phase: | A: Ultra-pure water/glacial acetic acid (100/0.1) (v/v) + 5mM ammonium acetate  B: Methanol/glacial acetic acid (100/0.1) (v/) + 5mM ammonium acetate |
| Flow rate: | 0.7 mL/min |
| Injection volume: | 10µl – 220µL |
| MS/MS Parameters | 4000QTrap |
| Ionisation mode: | ESI (electrospray ionisation) positive |
| Transitions monitored: | 223 → 126 m/z (quantification)  223 → 90 m/z (confirmation) |

Results and discussion

The analytical method used in the current study was fully validated for the determination of acetamiprid in barley (whole plant, grain and straw) according to the requirements of SANCO 3029/99 rev. 4 guidelines in a previous study (See KCP 5.1.2/01). A reduced validation for the determination of acetamiprid in wheat (grain, straw and whole plant) was conducted in the current study. The target analyte was determined using HPLC-MS/MS by monitoring two highly specific mass transitions. The detector response was linear over the range 0.3 -10 µg/L for all matrices with associated correlation coefficients (r) > 0.99. The LOQ of the method is 0.01 mg/kg for all matrices. All mean recovery values and associated RSDs for both matrices meet the minimum validation requirements given in Section 4.2 of SANTE/2020/12830 Rev. 1. guidelines and are summarised in the table below.

Table A 5: Method validation recovery data for the determination of acetamiprid in wheat reported in study 14SGS033

| Matrix | Analyte | Fortification level (mg/kg) n=3 | Mean  recovery (%) | Overall RSD (%) | Comments |
| --- | --- | --- | --- | --- | --- |
| Quantification transition m/z 223→126 | | | | | |
| Wheat  Whole plant | Acetamiprid | 0.01 | 71 | 2 | - |
| 0.1 | 75 | 0.4 |
| Wheat (grain) | 0.01 | 72 | 0.3 | - |
| 0.1 | 78 | 3 |
| Wheat (straw) | 0.01 | 77 | 7 |
| 0.1 | 79 | 3 |

Table A 6: Characteristics of the analytical method validated for the determination of acetamiprid in wheat

|  | Acetamiprid |
| --- | --- |
| Specificity | A highly specific HPLC-MS/MS method was used for the determination of the target analyte. Two mass transitions (m/z 223→126, quantification and m/z 223→90, qualification) were monitored during each analysis.  Target analyte concentrations in controls did not exceed 30% of the method LOQ. No interference from co-eluting components at the retention time of the target analyte. |
| Calibration (type, number of data points) | 5-point linear calibration  Wheat (grain): y= 35030.78x – 215.74 r= 0.9995  Wheat (whole plant): y= 120717.57x – 1211.73 r= 0.999  Wheat (straw) : y= 24344.59 – 2252.30 r= 0.9979 |
| Calibration range | 0.3 -10 µg/L for all matrices |
| Assessment of matrix effects is presented | Matrix effects were not assessed |
| Limit of determination/quantification | LOQ for all matrices: 0.01 mg/kg  LOD for all matrices: 0.003 mg/kg |

Conclusion

An analytical method for the determination of acetamiprid in barley (whole plant, grain and straw) was fully validated according to the requirements of the SANCO/3029/99 rev 4 guidelines prior to 1st March 2021 (see KCP 5.1.2/01). The validation data provided in the current report represent a reduced validation of the method for wheat (whole plant and straw). The additional validation data provided in the current report exceed the minimum validation requirements given in Section 4.2 of SANTE/2020/12830 Rev. 1. guidelines and demonstrate that the method was functioning correctly when used. The method should therefore be considered suitable for its intended purpose and acceptable for the determination of acetamiprid in wheat (whole plant and straw).

* + - * 1. Analytical method R1640039

Method validation R1640039

|  |  |
| --- | --- |
| Comments of zRMS: | The analytical method was fully validated for the determination of acetamiprid in arthropods and plant material with high water content (ground vegetation) with a limit of quantification of 0.01 mg/kg.  The accuracy and precision of the method during specimen analysis were considered to be acceptable since single recoveries were in the range of 70 - 104% and the mean recoveries at each fortification level were in the range of 70 - 110% with relative standard deviations below 20%.  The method complies with the standard acceptance criteria of the SANTE/2020/12830 guidance document.  The method is acceptable. |

|  |  |
| --- | --- |
| Reference: | KCP 5.1.2/04 |
| Report | Residues of acetamiprid in foliage-dwelling arthropods and ground vegetation after spray application of Acetamiprid 200 SL in a pome fruit orchard in Italy – magnitude of residues and time course of residue decline. Henkes, K. 2017, Study No. R1640039. Adama Report No. R-37376 |
| Guideline(s): | SANCO 3029/99 rev. 4 |
| Deviations: | None |
| GLP: | Yes |
| Acceptability: | Yes |

A multi-residue QuEChERS-based analytical method for the determination of acetamiprid in foliage-dwelling arthropods and ground vegetation was fully validated according to the requirements of SANCO/3029/99 rev. 4 prior to 1st March 2021 and used to support a residue study conducted on honey bee related matrices, arthropods and ground vegetation.

**A. Materials**

|  |  |
| --- | --- |
| 1. Standards |  |
| Reference item: | Acetamiprid |
| Lot/Batch number: | 469-129-01 |
| Purity: | 99.8 % |
| CAS No.: | 135410-20-7 |
| Expiry date: | 21 January 2019 |
| Standards for calibration | As above |
| Matrices: | 1: Ground vegetation (i.e., plant material with high water content)  2: Arthropods |

1. **Sample preparation and processing**

*Arthropods*

Following homogenisation, 0.20 g were weighed into a 50 mL centrifuge tube. 10.0 mL water and 10 mL acetonitrile were added and the tubes were shaken be hand for 1 min and then on a mechanical shaker for 15 min. 4.0 g of magnesium sulfate, 1.0 g of sodium chloride, 1.0 g of trisodium citrate dihydrate and 0.5 g of disodium hydrogen citrate sesquihydrate were added. The tube was capped, shaken by hand for one (1) minute and then centrifuged at 3200×*g* for 5 min. 40 mg of PSA and 225 mg of magnesium sulphate was weighed into a 2-mL safe-lock tube. An aliquot of 1.5 mL of the supernatant was transferred into the tube containing the mixture of sorbents. The tube was intensively shaken by hand, vortexed for 30 and then centrifuged for 2 min at 3200 × g. A 1.0 mL of the purified extract is taken and added to 1.5 mL of 0.1% formic acid (v/v). The final extract was well mixed and an aliquot transferred to an HPLC vial ready for analysis by LC-MS/MS.

*Ground vegetation*

Following homogenisation, 5.0 ± 0.05g were weighed into a 50 mL centrifuge tube. 6.0 mL water and 10 mL acetonitrile were added and the tubes were shaken be hand for 1 min and then on a mechanical shaker for 15 min. 4.0 g of magnesium sulfate, 1.0 g of sodium chloride, 1.0 g of trisodium citrate dihydrate and 0.5 g of disodium hydrogen citrate sesquihydrate were added. The tube was capped, shaken by hand for one (1) minute and then centrifuged at 3200 × g for 5 min. 40 mg of PSA and 225 mg of magnesium sulphate was weighed into a 2-mL safe-lock tube. An aliquot of 1.5 mL of the supernatant was transferred into the tube containing the mixture of sorbents. The tube was intensively shaken by hand, vortexed for 30 and then centrifuged for 2 min at 3200 × g. 0.16 mL of the purified extract, 3.84 mL acetonitrile were made up to 10 mL with water containing 0.1% (v/v) formic acid. The final extract was well mixed and an aliquot transferred to an HPLC vial ready for analysis by LC-MS/MS.

1. **Chromatographic parameters**

|  |  |
| --- | --- |
| HPLC- parameters: | 1200 Infinity Binary LC System, Agilent Technologies |
| Column: | ZORBAX Eclipse XDB-C8, 150 mm × 2.1 mm, 3.5 µm |
| Mobile phase: | A: Acetonitrile containing 0.1 % formic acid (v/v)  B: Water containing 0.1 % formic acid (v/v) |
| Flow rate: | 800 µL/min |
| Injection volume: | 25 µL µL |
| MS/MS Parameters:  Ionisation mode:  Transitions monitored: | API 5000 System, SCIEX (Triple quadrupole mass spectrometer)  EI positive  m/z 223→126 (quantification)  m/z 223→90 (confirmation) |

**Results and discussion**

The analytical method used in the current study was fully validated for the determination of acetamiprid in arthropods and plant material with high water content (ground vegetation) according to the requirements of SANCO 3029/99 rev. 4 guidelines prior to 1st March 2021. The target analyte was determined using HPLC-MS/MS by monitoring two highly specific mass transitions. The detector response was linear over the range 0.020 – 2.0 ng/mL (corresponding to 0.003 – 0.25 mg/kg) with associated correlations coefficients (r2) > 0.99. The LOQ of the method is 0.01 mg acetamiprid/kg for both matrices. All mean recovery values and associated RSDs for both matrices meet the requirements of SANCO/3029/99 rev. 4 and the minimum validation requirements given in Section 4.2 of SANTE/2020/12830 rev.1 guidelines and are summarised in the table below.

**Table A 7: Method validation recovery data for the determination of acetamiprid in ground vegetation and arthropods reported in study R1640039.**

| **Matrix** | **Analyte** | **Fortification level (mg/kg) (n=5)** | **Mean  recovery (%)** | **RSD (%)** | **Comments** |
| --- | --- | --- | --- | --- | --- |
| Quantification transition m/z 223→126 | | | | | |
| Arthropods | Acetamiprid | 0.01 | 98 | 4.9 | - |
| 0.1 | 98 | 3.4 |
| Confirmation transition m/z 223→90 | | | | | |
| Arthropods | Acetamiprid | 0.01 | 98 | 0.9 | - |
| 0.1 | 99 | 3.7 |
| Quantification transition m/z 223→126 | | | | | |
| Ground vegetation | Acetamiprid | 0.01 | 106 | 2.1 | - |
| 0.1 | 101 | 0.5 |
| Confirmation transition m/z 223→90 | | | | | |
| Ground vegetation | Acetamiprid | 0.01 | 102 | 2.2 | - |
| 0.1 | 100 | 0.7 |

**Table A 8: Characteristics of the analytical method validated for the determination of acetamiprid in ground vegetation and arthropods**

|  | **Acetamiprid** |
| --- | --- |
| Specificity | A highly specific HPLC-MS/MS method was used for the determination of the target analyte. Two mass transitions (m/z 223→126, quantification and m/z 223→90, qualification) were monitored during each analysis.  Target analyte concentrations in controls did not exceed 30% of the method LOQ. No interference from co-eluting components at the retention time of the target analyte. |
| Calibration (type, number of data points) | Eight-point linear calibration  Arthropods: y = 486116.3611x + 982.7380, r2 = 0.9999  Ground vegetation: y = 483232.5905x + 873.0606, r2 = 0.9994 |
| Calibration range | 0.020 – 2.0 ng/mL corresponding to 0.003 – 0.25 mg/kg |
| Assessment of matrix effects is presented | Matrix effects were assessed and found to be insignificant (< ± 20%) for ground vegetation and arthropods. |
| Limit of determination/quantification | LOQ both matrices: 0.01 mg/kg  LOD both matrices: 0.003 mg/kg |

**Conclusion**

An analytical method for the determination of acetamiprid in arthropods and plant material with high water content (ground vegetation) was fully validated according to SANCO/3029/99 guidelines prior to March 21st 2021. The data provided exceed the minimum validation requirements given in Section 4.2 of SANTE/2020/12830 Rev. 1. The method should therefore be considered suitable for its intended purpose and acceptable for the determination of acetamiprid in arthropods and plant material with high water content (ground vegetation).

* + - * 1. Analytical method R1640035

Method validation R1640035

|  |  |
| --- | --- |
| Comments of zRMS: | The study has been evaluated and accepted in Registration Report, Section 5 for CA3573 / Carnadine / Kestrel, Nufarm (August 2021).  Conclusions:  *The method was successfully validated for determination of acetamiprid in Phacelia (pollen and flowers), nectar surrogate, honey bee larvae, honey and beeswax with an LOQ of 0.01 mg a.s./kg according to the guidance document SANCO/3029/99 rev. 4.*  *With regard to selectivity, accuracy and precision, the analytical method was applied successfully for each analytical set when analysing the specimens of the study.*  *The method is acceptable.* |

|  |  |
| --- | --- |
| Reference: | KCP 5.1.2/05 |
| Report | Semi-field brood study to evaluate potential effects of MCW-2222 on brood development of honeybees (*Apis mellifera* L.). Hecht-Rost, S. and Mayer, O. 2018, R1640035. Adama Reference No. R-37336. |
| Guideline(s): | SANCO 3029/99 rev. 4 |
| Deviations: | None |
| GLP: | Yes |
| Acceptability: | Yes |

An analytical method for the determination of acetamiprid in flowers, nectar, pollen, larvae, honey and wax was fully validated according to the requirements of SANCO/3029/99 rev. 4 prior to 1st March 2021 and used to support an ecotoxicology on bees and bee products.

**A. Materials**

|  |  |
| --- | --- |
| Reference item: | Acetamiprid |
| Lot/Batch number: | 469-129-01 |
| Purity: | 99.8% |
| CAS No.: | 135410-20-7 |
| Expiry date: | 21 January 2019 |
| Standards for calibration | As above |
| Matrices: | Flowers, nectar, pollen, larvae, honey and wax |

1. **Sample preparation and processing**

Flowers, nectar, larvae and honey

For each matrix type, 200 mg homogenised sample were weighed into a 50 mL centrifuge tube, 10 mL water and 10 mL acetonitrile were then added. The tube was shaken by hand for 1 min and then mechanically for 15 min. 4.0 g of magnesium sulfate, 1.0 g of sodium chloride, 1.0 g of trisodium citrate dihydrate and 0.50 g of disodium hydrogen citrate sesquihydrate were then added and the centrifuge tube was capped, shaken by hand for 1 min and then centrifuged at approximately 3200 × g for 5 min. 40 mg PSA plus 225 mg magnesium sulfate were weighed into a 2 mL Safe-lock tube and 1.5 mL of the extract supernatant were added. The tube was then shaken for 30 seconds by hand and centrifuged at approximately 3200 × g for 5 min. A 1.0 aliquot of the supernatant was transferred to clean vial and made up to 2.5 mL with 0.1% formic acid in water. The final extract was then analysed using HPLC-MS/MS.

Pollen and wax

For each matrix type, 200 mg homogenised sample were weighed into a 15 mL Lysing Matrix D tube containing 1.4 mm ceramic spheres. 2.5 mL water and 2.5 mL acetonitrile were then added. For wax, two 1/4" ceramic spheres were added and the sample was heated to 40 °C in a water bath. The samples were then shredded using a FastPrep for 1 min at 4.0 m/sec. 4.0 g of magnesium sulfate, 1.0 g of sodium chloride, 1.0 g of trisodium citrate dihydrate and 0.50 g of disodium hydrogen citrate sesquihydrate were then added and the centrifuge tube was then treated with a FastPrep for 1 min at 4.0 m/sec. For wax the sample tube was centrifuged at approximately 3200 × g for 5 min and the acetonitrile phase was frozen out at ≤ -18 °C for one hour. The sample tube was then centrifuged at approximately 3200 × g for 5 min. For both matrices, 40 mg PSA plus 225 mg magnesium sulfate were weighed into a 2 mL Safe-lock tube and 1.5 mL of the extract supernatant were added. The tube was then shaken for 30 seconds by hand and centrifuged at approximately 3200 × g for 5 min. A 1.0 aliquot of the supernatant was transferred to clean vial and made up to 2.5 mL with 0.1% formic acid in water. The final extract was then analysed using HPLC-MS/MS.

1. **Chromatographic parameters**

|  |  |
| --- | --- |
| HPLC- parameters  Instrumentation: | 1200 Binary Rapid Resolution LC System, Agilent Technologies |
| Column: | ZORBAX Eclipse XDB-C8, 150 mm × 2.1 mm, 3.5 μm, fitted with a SecurityGuard™ ULTRA Pre-column |
| Mobile phase: | A: Acetonitrile containing 0.1 % (v/v) formic acid  B: Water containing 0.1 % (v/v) formic acid |
| Flow rate: | 800 µL/min |
| Injection volume: | 25 μL or 35 μL (adjusted to the instrument’s performance when starting the sequence of injections) |
|  |  |

|  |  |
| --- | --- |
| MS/MS parameters | API 5000 System, SCIEX (Triple quadrupole mass spectrometer) |
| Ionisation mode: | ES positive |
| Scan type. | MRM |
| Transitions: | m/z 225→ 128 (quantification)  m/z 223 → 73 (confirmation)  m/z 223→ 126 (quantification wax)  m/z 223 → 90 (confirmation wax) |

**Results and discussion**

The method used for the determination of acetamiprid in flowers, nectar, pollen, larvae, honey and wax was fully validated according to the requirements of the SANCO/3029/99 rev. 4 guidelines prior to 1st March 2021. The target analyte was determined using HPLC-MS/MS with two transitions monitored during each analysis. For all matrices, the detector response was linear over the range 0.020 ng/mL to 2.0 ng/mL (corresponding to 0.0025 mg a.s./kg to 0.25 mg a.s./kg) with associated correlation coefficients (r2) ≥ 0.99. The LOQ of the method is 0.01 mg a.s/kg. Target analyte concentrations in controls were < 30% of the method LOQ. All mean recovery values and associated RSDs for both matrices meet the requirements of SANCO/3029/99 rev. 4 and SANTE/2020/12830 rev.1 guidelines and are summarised in the tables below.

**Table A 9: Method validation recovery data for the determination of acetamiprid in flowers, nectar, pollen, larvae, honey and wax reported in study R1640035**

| **Matrix** | **Analyte** | **Fortification level**  **(mg a.s./kg)** | **Mean  recovery (%)**  **n=5** | **RSD (%)** | **Comments** |
| --- | --- | --- | --- | --- | --- |
| Quantification transition m/z 225 → 128 | | | | | |
| Flowers | Acetamiprid | 0.01 | 98 | 8.3 | - |
| 0.1 | 95 | 1.6 | - |
| Sucrose solution\* | Acetamiprid | 0.01 | 96 | 4.3 | - |
| 0.1 | 91 | 2.1 | - |
| Pollen | Acetamiprid | 0.01 | 109 | 6.4 | - |
| 0.1 | 102 | 7.3 | - |
| Larvae | Acetamiprid | 0.01 | 109 | 3.3 | - |
| 0.1 | 101 | 2.9 | - |
| Honey | Acetamiprid | 0.01 | 84 | 4.4 | - |
| 0.1 | 98 | 2.2 | - |
| Quantification transition m/z 223 → 90 | | | | | |
| Wax | Acetamiprid | 0.01 | 80 | 5.0 | - |
| 0.1 | 97 | 2.1 | - |
| Confirmation transition m/z 223 → 73 | | | | | |
| Flowers | Acetamiprid | 0.01 | 81 | 14 | - |
| 0.1 | 94 | 2.9 | - |
| Sucrose solution\* | Acetamiprid | 0.01 | 92 | 5.6 | - |
| 0.1 | 91 | 2.3 | - |
| Pollen | Acetamiprid | 0.01 | 105 | 2.9 | - |
| 0.1 | 99 | 8.0 | - |
| Larvae | Acetamiprid | 0.01 | 105 | 5.4 | - |
| 0.1 | 102 | 3.4 | - |
| Honey | Acetamiprid | 0.01 | 83 | 7,8 | - |
| 0.1 | 97 | 1.7 | - |
| Confirmation transition m/z 223 → 90 | | | | | |
| Wax | Acetamiprid | 0.01 | 81 | 1.4 | - |
| 0.1 | 96 | 0.9 | - |

\* Validated as a surrogate for nectar

**Table A 10: Characteristics of the analytical method validated for the determination of acetamiprid in flowers, nectar, pollen, larvae, honey and wax**

|  | **Acetamiprid** |
| --- | --- |
| Specificity | A highly specific HPLC-MS/MS method was used for the determination of the target analyte. Two mass transitions (m/z 225 → 128, quantification and m/z 223 → 73, confirmation) were monitored during each analysis. Target analyte concentrations in controls did not exceed 30% of the method LOQ. No interference from co-eluting components at the retention time of the target analyte. |
| Calibration (type, number of data points) | Eight-point-linear calibration curves  Flowers: y = 113407.1637x + 6063973 r2 = 0.9992  Nectar: y = 124002.4541x – 147.8268 r2 = 0.9995  Pollen: y = 45165.7532x + 191.0036 r2 = 0.9980  Larvae y = 76548.5931x + 482.5289 r2 = 0.9996  Honey: y = 161932.3093x + 2640.9913 r2 = 0.9974  Wax: y = 482337.8464x + 5760.4337 r2 = 0.9974 |
| Assessment of matrix effects is presented | Matrix effects were assessed for all matrices and found to be significant (> ± 20%) for pollen, honey and wax and insignificant (< ± 20%) for nectar, larvae and flower. Matrix-matched calibration was for the quantitative determination of the target analyte all matrices. |
| Calibration range | All matrices: 0.020 ng/mL to 2.0 ng/mL  corresponding to 0.0025 mg a.s./kg to 0.25 mg a.s./kg |
| Limit of determination/quantification | LOQ: 0.01 mg a.s/kg |

**Conclusion**

An analytical method for the determination of acetamiprid in flowers, nectar, pollen, larvae, honey and wax was fully validated according to SANCO/3029/99 guidelines prior to March 21st 2021. The data provided exceed the minimum validation requirements given in Section 4.2 of SANTE/2020/12830 Rev. 1. The method should therefore be considered suitable for its intended purpose and acceptable for the determination of acetamiprid in flowers, nectar, pollen, larvae, honey and wax.

* + - * 1. Analytical method B13-M1-A-01

Method validation B13-M1-A-01

|  |  |
| --- | --- |
| Comments of zRMS: | The study has been evaluated and accepted in Registration Report, Section 2 for MCW-2222, Adama Makhteshim Ltd. (18.04.2018).  Conclusion:  *The analytical method of Lefresne, S. (2014; KIIIA 5.3.1/02) for the determination of residues of acetamiprid in plant matrices: dry bean (seed and straw), mandarin (peel, pulp and whole fruit), oilseed rape (pod, seed whole plant and whole plant without pod), olive (oil and fruit) and orange (peel, pulp and whole fruit) was found to be satisfactory in terms of linearity, specificity, accuracy and precision.*  *The limit of quantification (LOQ) was set to 0.01 mg/kg for acetamiprid for each matrix.*  *The recovery values for acetamiprid at each fortification level were within the EU guideline SANCO/825/00 rev. 8.1 and SANCO/3029/99 rev. 4 acceptance range of 70 - 110% with a relative standard deviation (RSD) that does not exceed 20%.*  *The analytical method (Lefresne, S., 2014) for the determination of residues of acetamiprid in various plant commodities was acceptable validated according to the SANCO/3029/99 rev. 4. and SANCO/825/00 rev 8.1.*  *This study is accepted.* |

|  |  |
| --- | --- |
| Reference: | KCP 5.1.2/06 |
| Report | Validation of the analytical method for the determination of residues of acetamiprid in plant matrics: Dry bean (seed and straw), mandarin (peel, pulp and whole fruit), oilseed rape (pod, seed, whole plant and whole fruit without pod), olive (oil and whole fruit) and orange (peel, pulp and whole fruit). Lefresne, S. 2014, Study No. B13-M1-A-01. Adama Report No. R-33645 |
| Guideline(s): | SANCO 3029/99 rev. 4 |
| Deviations: | None |
| GLP: | Yes |
| Acceptability: | Yes |

A multi-residue QuEChERS-based analytical method for the determination of acetamiprid in oilseed rape (whole plant, high water content), olive (whole fruit, high oil content), dry bean seed (high dry content), orange (peel and pulp, high acid content), dry bean (straw) was fully validated according to the requirements of SANCO/3029/99 rev. 4 prior to 1st March 2021. A reduced validation was performed on mandarin (peel, pulp and whole fruit, high acid content), oilseed rape (pod and whole plant without pod, high water content), oilseed rape (seed, high oil content) and olive (oil, high oil content) as part of the study. The method was used to support a residue study conducted on plant matrices with high dry, high water, high oil and high acid content.

**A. Materials**

|  |  |
| --- | --- |
| 1. Standards |  |
| Reference item: | Acetamiprid |
| Lot/Batch number: | 20202 |
| Purity: | 98.1 ± 0.5 % |
| CAS No.: | 135410-20-7 |
| Expiry date: | 02 February 2016 |
|  |  |
| Standards for calibration | As above |
|  |  |
| Matrices: | 1: oilseed rape, whole plant (high water content)  2: olive, whole fruit (high oil content)  3: dry bean, seed (high dry content)  4: dry bean, straw (no commodity group)  5: mandarin, peel (high acid content)  6: mandarin, pulp (high acid content)  7: mandarin, whole fruit (high acid content)  8: oilseed rape, pod (high water content)  9: oilseed rape, whole plant without pod (high water content)  7: oilseed rape, seeds (high oil content)  8: olive, oil (high oil content) |

1. **Sample preparation and processing**

*Dry bean (seed and straw)*

2 g of sample were weighed into a 50 mL centrifuge tube and 10 mL of cold water were added. Recovery samples were fortified at this stage with the appropriate standard solutions. 10 mL of acetonitrile were added to the tube, which was shaken for 1 minute by hand and for 20 min on mechanical shaker at maximum speed.

The extract was transferred into a 50 mL tube containing 4.0 g of magnesium sulphate, 1.0 g of sodium chloride, 1.0 g of sodium citrate and 0.50 g of disodium hydrogen citrate sesquihydrate. The tube was shaken for 1 minute by hand and for 5 min on mechanical shaker at maximum speed and subsequently centrifuged for 5 min at over 3000 g. An aliquot of 7 mL of acetonitrile phase were transferred into a 15 mL tube containing 900 mg of magnesium sulphate and 150 g of PSA for dry been seed. For dry bean straw, the tube would contain 885 mg of magnesium sulphate,150 g of PSA and 15 mg of GCB. The tube was shaken both manually (2 min) and mechanically (10 min) and subsequently centrifuged for 5 min at over 3000 g. an aliquot of 5 mL of the acetonitrile phase was transferred into a 100 mL flask. 40 μL of acetonitrile containing 5% of formic acid and about 50 μL of ethylene glycol were added and evaporated to dryness. The dry residue was dissolved into a 2 mL mixture methanol/water (50/50, v/v) and sonicated. If necessary, the extract was filtered before analysis by HPLC-MS/MS.

*Oilseed rape (seed), olive (oil and whole fruit)*

2 g of sample were weighed into a 50 mL centrifuge tube and 10 mL of cold water were added. Recovery samples were fortified at this stage with the appropriate standard solutions. 10 mL of acetonitrile were added to the tube, which was shaken for 1 minute by hand and for 20 min on mechanical shaker at maximum speed. After setting at -18°C for about 2 hours, the acetonitrile phase was transferred into a new centrifuge tube, without taking the oily precipitate. 10 mL of water were then added and the sample was shaken for about 1 min manually (or mechanically for 5 min), then shaken mechanically and horizontally for about 20 min at maximum speed and subsequently centrifuged for 5 min at over 3000 g. An aliquot of 7 mL of acetonitrile phase were transferred into a 15 mL tube containing 900 mg of magnesium sulphate and 150 g of PSA and 300 mg of C18. The tube was shaken manually (2 min) and subsequently centrifuged for 5 min at over 3000 g. Half of the extract was diluted into water and filtered on nylon 0.45 μm before analysis by HPLC-MS/MS.

*Mandarin and orange (peel, pulp and whole fruit), oilseed rape (whole plant, pod and whole plant)*

2 g (for oilseed rape) or 10 g (for mandaring and orange) were weighed ground frozen sample into a 50 mL centrifuge tube. 2.5 mL (for mandarin and orange peel) or 5 mL (for oilseed rape) of cold water were added. Recovery samples were fortified at this stage with the appropriate standard solutions.

10 mL of acetonitrile were added and the samples were shake manually for about 1 min (or mechanically for about 5 min) and then shaken mechanically for about 20 min at maximum speed. The extracts were transferred into a 50 mL tube containing 4 g of magnesium sulphate, 1 g of sodium chloride, 1 g sodium citrate dihydrate and 0.5 g of sodium citrate sesquihydrate. The samples were then shaken manually for about 1 min (or mechanically for about 5 min at maximum speed) and subsequently centrifuged for about 5 min at over 3000 g. half of the extracts were diluted into water and filtered on nylon 0.45 μm before analysis by HPLC-MS/MS

1. **Chromatographic parameters**

|  |  |
| --- | --- |
| HPLC- parameters: | 4000 QTRap or API 4000 |
| Column: | C18 Hydro RP, 100 mm × 3 mm, 2.5 µm |
| Mobile phase: | A: Ultra-pure water containing glacial acetic acid (100/0.1, v/v) and 5 mM ammonium acetate  B: Methanol containing glacial acetic acid (100/0.1, v/v) and 5 mM ammonium acetate |
| Flow rate: | 0.7 mL/min |
| Injection volume: | 20 µL |
| MS/MS Parameters:  Ionisation type:  Transitions monitored: | API 4000 System or 4000 QTRap (depending on date of analysis) with Triple quadruple detector  ESI positive  m/z 223→126 (quantification)  m/z 223→90 (confirmation) |

**Results and discussion**

The analytical method used in the current study was fully validated for the determination of acetamiprid in oilseed rape (whole plant, high water content), olive (whole fruit, high oil content), dry bean seed (high dry content), orange (peel and pulp, high acid content), dry bean (straw) according to the requirements of SANCO 3029/99 rev. 4 guidelines prior to 1st March 2021. A reduced validation was performed on mandarin (peel, pulp and whole fruit, high acid content), oilseed rape (pod and whole plant without pod, high water content), oilseed rape (seed, high oil content) and olive (oil, high oil content). The target analyte was determined using HPLC-MS/MS by monitoring two highly specific mass transitions. The detector response was linear over the range 0.0015 mg/L – 0.050 mg/L for dry bean (seeds and straw), mandarin (peel and pulp), mandarin whole fruit, orange (peel, pulp and whole fruit); over the range 0.0003 mg/L – 0.010 mg/L for oilseed rape (pods, whole plant and whole plant without pods); over the range 0.0003 mg/L – 0.008 mg/L for oilseed rape seeds and olive whole fruit and over the range 0.0003 mg/L – 0.005 mg/L for olive oil with associated determination coefficients (R2) > 0.99. The LOQ of the method is 0.010 mg/kg for all matrices. Mean recovery values and associated RSDs for all matrices meet the requirements of SANCO/3029/99 rev. 4 and the minimum validation requirements given in Section 4.2 of SANTE/2020/12830 rev.1 guidelines and are summarised in the table below.

**Table A 11: Method validation recovery data for the determination of acetamiprid in plant matrices (high water, high oil, high dry and high acid content) reported in study B13-M1-A-01**

| **Matrix** | **Analyte** | **Fortification level**  **(mg a.s./kg)** | **Mean  recovery (%)** | **RSD (%)** | **Comments** |
| --- | --- | --- | --- | --- | --- |
| Dry bean (seed) | Acetamiprid | 0.010 (n=5) | 80 | 3 | - |
| 0.100 (n=5) | 90 | 7 |
| *Dry bean (straw)* | Acetamiprid | 0.010 (n=5) | 81 | 1 | - |
| 0.100 (n=5) | 81 | 6 |
| Mandarin (peel) | Acetamiprid | 0.010 (n=3) | 98 | 2 | - |
| 0.100 (n=3) | 103 | 15 |
| Mandarin (pulp) | Acetamiprid | 0.010 (n=3) | 95 | 4 | - |
| 0.100 (n=3) | 98 | 7 |
| Mandarin (whole fruit) | Acetamiprid | 0.010 (n=3) | 92 | 2 | - |
| 0.100 (n=3) | 106 | 2 |
| Oilseed rape (pod) | Acetamiprid | 0.010 (n=3) | 83 | 4 | - |
| 0.100 (n=3) | 90 | 3 |
| Oilseed rape (seed) | Acetamiprid | 0.010 (n=5) | 76 | 9 | - |
| 0.100 (n=5) | 97 | 1 |
| Oilseed rape (whole plant) | Acetamiprid | 0.010 (n=5) | 70 | 4 | - |
| 0.100 (n=5) | 71 | 4 |
| Oilseed rape (whole plant without pod) | Acetamiprid | 0.010 (n=3) | 83 | 7 | - |
| 0.100 (n=3) | 73 | 20 |
| Olive (oil) | Acetamiprid | 0.010 (n=3) | 88 | 2 | - |
| 0.100 (n=3) | 93 | 5 |
| Olive (whole fruit) | Acetamiprid | 0.010 (n=5) | 95 | 8 | - |
| 0.100 (n=5) | 97 | 6 |
| Orange (peel) | Acetamiprid | 0.010 (n=5) | 100 | 1 | - |
| 0.100 (n=5) | 89 | 3 |
| Orange (pulp) | Acetamiprid | 0.010 (n=5) | 91 | 5 | - |
| 0.100 (n=5) | 91 | 6 |
| Orange (whole fruit) | Acetamiprid | 0.01 | 84 | 14 | - |
| 0.1 | 85 | 4 |

**Table A 12: Characteristics of the analytical method validated for the determination of acetamiprid in plant matrices (high water, high oil, high dry and high acid content)**

|  | **Acetamiprid** |
| --- | --- |
| Specificity | A highly specific HPLC-MS/MS method was used for the determination of the target analyte. Two mass transitions (m/z 223→126, quantification and m/z 223→90, qualification) were monitored during each analysis.  Target analyte concentrations in controls did not exceed 30% of the method LOQ. No interference from co-eluting components at the retention time of the target analyte. |
| Calibration (type, number of data points) | At least five-point linear calibration    *Dry bean (seed):*  y = 14185.11x + 3658.76, R2 = 0.9996  *Dry bean (straw):*  y = 8102.80x + 37.99, R2 = 1.0000  *Mandarin (peel):*  y = 11026.62x + 107.15, R2 = 0.9981  *Mandarin (pulp):*  y = 10008.14x – 182.09, R2 = 0.9991  *Mandarin (whole fruit):*  y = 9275.04x – 578.25, R2 = 0.9998  *Oilseed rape (pods):*  y =5878.98x + 194.50, R2 = 0.9991  *Oilseed rape (seeds):*  y = 5291.07x – 58.81, R2 = 0.9994  *Oilseed rape (whole plant):*  y = 10231.88x + 736.51, R2 = 0.9994  *Oilseed rape (whole plant without pods):*  y = 8061.99x + 499.22, R2 = 0.9986  *Olive (oil):*  y = 17613.43x + 1204.48, R2 = 0.9994  *Olive (whole fruit):*  Y = 17553.16x -397.49, R2 = 0.9999  *Orange (peel):*  Y = 12638.18x + 1276.22, R2 = 0.9990  *Orange (pulp):*  Y = 17874.11x + 2229.61, R2 = 0.9998  *Orange (whole fruit):*  Y = 18940.74x + 3315.82, R2 = 1.0000 |
| Calibration range | The calibration extended over a concentration range covered from 30 % of the LOQ to 20 % above the highest level ~~covering the lowest and highest nominal concentrations tested ± at least 20~~% |
| Assessment of matrix effects is presented | Matrix effects were not assessed. |
| Limit of determination/quantification | LOQ all matrices: 0.010 mg/kg  LOD: not indicated |

**Conclusion**

An analytical method for the determination of acetamiprid in oilseed rape (whole plant, high water content), olive (whole fruit, high oil content), dry bean seed (high dry content), orange (peel and pulp, high acid content), dry bean (straw) according to the requirements of SANCO 3029/99 rev. 4 guidelines prior to 1st March 2021. A reduced validation was performed on mandarin (peel, pulp and whole fruit, high acid content), oilseed rape (pod and whole plant without pod, high water content), oilseed rape (seed, high oil content) and olive (oil, high oil content). The data provided exceed the minimum validation requirements given in Section 4.2 of SANTE/2020/12830 Rev. 1. The method should therefore be considered suitable for its intended purpose and acceptable for the determination of acetamiprid in plant matrices with high water, high oil, high dry and high acid content.

* + - * 1. Analytical method 13M06017-01-VMPL

Method validation 13M06017-01-VMPL

|  |  |
| --- | --- |
| Comments of zRMS: | The study has been evaluated and accepted in Registration Report, Section 2 for MCW-2222, Adama Makhteshim Ltd. (18.04.2018).  Conclusion:  *The analytical method of Lang, A. (2014; KIIIA 5.3.1/01) for the determination of residues of acetamiprid in 4 different plant commodities (head cabbage, apple fruits, potato tubers and peach fruits) was found to be satisfactory in terms of linearity, specificity, accuracy and precision.*  *The limit of quantification (LOQ) was set to 0.01 mg/kg for acetamiprid for each matrix.*  *The recovery values for acetamiprid at each fortification level were within the EU guideline SANCO/825/00 rev. 8.1 and SANCO/3029/99 rev. 4 acceptance range of 70 - 110% with a relative standard deviation (RSD) that does not exceed 20%.*  *The analytical method (Lang, A., 2014) for the determination of residues of acetamiprid in plant commodities was acceptable validated according to the SANCO/3029/99 rev. 4. and SANCO/825/00 rev 8.1.*  *This study is accepted.* |

|  |  |
| --- | --- |
| Reference: | KCP 5.1.2/07 |
| Report | Validation of an analytical method for the determination of residues of Acetamiprid in 4 different plant commodities (head cabbage, apple fruits, potato tubers and peach fruits). Lang, A. 2014, Study No. 13M06017-01-VMPL. Adama Report No. R-33644 |
| Guideline(s): | SANCO 3029/99 rev. 4 |
| Deviations: | None |
| GLP: | Yes |
| Acceptability: | Yes |

A multi-residue QuEChERS-based analytical method for the determination of acetamiprid in head cabbage (high water content) was fully validated according to the requirements of SANCO/3029/99 rev. 4 prior to 1st March 2021. A reduced validation was performed on apple fruits, potato tubers and peach fruits (high water content). The analytical method was used to support several residue studies

**A. Materials**

|  |  |
| --- | --- |
| 1. Standards |  |
| Reference item: | Acetamiprid |
| Lot/Batch number: | 20203 |
| Purity: | 99.0 % |
| CAS No.: | 135410-20-7 |
| Expiry date: | 02 February 2016 |
|  |  |
|  |  |
| Standards for calibration | As above |
|  |  |
| Matrices: | 1: Head cabbage (high water content)  2: Apple, fruits ((high water content)  3: Potato, tubers ((high water content)  4: Peach, fruits (high water content) |

1. **Sample preparation and processing**

10 g of each sample were weighed into 50 mL centrifuge tubes. Recovery samples were fortified at this stage. 10 mL of acetonitrile were added and the samples were extracted applying homogenisation for at least 2 min at high speed. 4.0 g of magnesium sulphate, 1.0 g of sodium chloride and 0.50 g of sodium hydrogencitrate sesquihydrate were added. The tube was shaken by hand and mixed on a mixer for at least 1 min. The samples were then centrifuged at 3500 min-1 for at least 10 min. An aliquot of 1 mL of the supernatant was transferred into a 2 mL tube containing 25 mg of PSA and 150 mg anhydrous magnesia sulphate and 2.5 mg of GCB. The tube was shaken on a mixer for 30 seconds and the extract was filtered through a single-use syringe filter (0.45 μm) into a vial. The final extracts were diluted 1:10 (ACN) and analysed by HPLC-MS/MS

1. **Chromatographic parameters**

|  |  |
| --- | --- |
| HPLC- parameters: | Dionex Ultimate 3000 |
| Column: | Phenomenex Luna C18 (2), 100A 150 mm × 2.0 mm, 5.0 μm |
| Mobile phase: | A: Water/MeOH (90/10; v/v) +0.1% Formic acid + 5 mmol Ammonium formate  D: MeOH/Water (90/10; v/v) +0.1% Formic acid + 5 mmol Ammonium formate |
| Flow rate: | 300 μL/min |
| Injection volume: | 10 µL |
| MS/MS Parameters:  Ionisation type:  Transitions monitored: | AB Sciex API 5500 QTRAP  ESI positive  m/z 223→126 (quantification)  m/z 223→90 (confirmation) |

**Results and discussion**

The analytical method used in the current study was fully validated for the determination of acetamiprid in head cabbage (high water content) was fully validated according to the requirements of SANCO/3029/99 rev. 4 prior to 1st March 2021. A reduced validation was performed on apple fruits, potato tubers and peach fruits (high water content). The target analyte was determined using HPLC-MS/MS by monitoring two highly specific mass transitions. The detector response was linear over the range 0.25 μg/L to 20 μg/L (corresponding to 0.025 - 0.2 mg/kg) with associated correlation coefficients r ≥ 0.9994. The LOQ of the method is 0.01 mg/kg for all matrices. Mean recovery values and associated RSDs for all matrices meet the requirements of SANCO/3029/99 rev. 4 and the minimum validation requirements given in Section 4.2 of SANTE/2020/12830 rev.1 guidelines and are summarised in the table below.

**Table A 13: Method validation recovery data for the determination of acetamiprid in plant matrices (high water content) reported in study 13M06017-01-VMPL**

| **Matrix** | **Analyte** | **Fortification level**  **(mg/kg)** | **Mean  recovery (%)** | **RSD (%)** | **Comments** |
| --- | --- | --- | --- | --- | --- |
| **m/z 223→126 (quantification)** | | | | | |
| Head cabbage | Acetamiprid | 0.01 (n=5) | 96 | 4.1 | - |
| 0.10 (n=5) | 96 | 3.3 |
| **m/z 223→90 (confirmation)** | | | | | |
| Head cabbage | Acetamiprid | 0.01 (n=5) | 96 | 4.2 | - |
| 0.10 (n=5) | 96 | 3.0 |
| **m/z 223→126 (quantification)** | | | | | |
| Apple (fruits) | Acetamiprid | 0.01 (n=3) | 92 | 3.5 | - |
| 0.10 (n=3) | 93 | 1.1 |
| **m/z 223→90 (confirmation)** | | | | | |
| Apple (fruits) | Acetamiprid | 0.01 (n=3) | 91 | 2.9 | - |
| 0.10 (n=3) | 93 | 1.1 |
| **m/z 223→126 (quantification)** | | | | | |
| Potato (tubers) | Acetamiprid | 0.01 (n=3) | 87 | 3.3 | - |
| 0.10 (n=3) | 90 | 1.7 |
| **m/z 223→90 (confirmation)** | | | | | |
| Potato (tubers) | Acetamiprid | 0.01 (n=3) | 88 | 2.9 | - |
| 0.10 (n=3) | 90 | 1.3 |
| **m/z 223→126 (quantification)** | | | | | |
| Peach (fruits) | Acetamiprid | 0.01 (n=3) | 95 | 2.4 | - |
| 0.10 (n=3) | 93 | 2.2 |
| **m/z 223→90 (confirmation)** | | | | | |
| Peach (fruits) | Acetamiprid | 0.01 (n=3) | 96 | 4.2 | - |
| 0.10 (n=3) | 94 | 2.1 |

**Table A 14: Characteristics of the analytical method validated for the determination of acetamiprid in plant matrices (high water content)**

|  | **Acetamiprid** |
| --- | --- |
| Specificity | A highly specific HPLC-MS/MS method was used for the determination of the target analyte. Two mass transitions (m/z 223→126, quantification and m/z 223→90, qualification) were monitored during each analysis.  Target analyte concentrations in controls did not exceed 30% of the method LOQ. No interference from co-eluting components at the retention time of the target analyte. |
| Calibration (type, number of data points) | Seven-point linear calibration    *Head cabbage:*  Quantification transistion: y = 141756.0850x, R2 = 0.9996  Confirmation transition: y = 42754.7227x, R2 = 0.9995  *Apple (fruits):*  Quantification transistion: y = 150218.4795x, R2 = 0.9999  Confirmation transition: y = 45456.2826x, R2 = 0.9999  *Potato (tubers):*  Quantification transistion: y = 147130.7048x, R2 = 0.9999  Confirmation transition: y = 44632.9783x, R2 = 0.9989  *Peach (fruits):*  Quantification transistion: y = 133214.0810x, R2 = 0.9997  Confirmation transition: y = 39805.4045x, R2 = 0.9991 |
| Calibration range | 0.25 μg/L - 20 μg/L (corresponding to 0.025 - 0.2 mg/kg) |
| Assessment of matrix effects is presented | Matrix effects were assessed and found to be insignificant (< ± 20%) for all matrices. Nevertheless, matrix-matched calibration was used throughout the study. |
| Limit of determination/quantification | LOQ all matrices: 0.01 mg/kg  LOD: ≤ 0.003 mg/kg (< 30 % of the LOQ) |

**Conclusion**

An analytical method for the determination of acetamiprid in in head cabbage (high water content) was fully validated according to the requirements of SANCO/3029/99 rev. 4 prior to 1st March 2021. A reduced validation was performed on apple fruits, potato tubers and peach fruits (high water content). The data provided exceed the minimum validation requirements given in Section 4.2 of SANTE/2020/12830 Rev. 1. The method should therefore be considered suitable for its intended purpose and acceptable for the determination of acetamiprid in plant matrices with high water content.

* + - * 1. Analytical method DMC-13-16134

Method validation 13M06017-01-VMPL & DMC-13-16126

|  |  |
| --- | --- |
| Comments of zRMS: | The study has been evaluated and accepted in Registration Report, Section 5 for CA3573 / Carnadine / Kestrel, Nufarm (August 2021).  Conclusions:  *The analytical method was fully validated according to guideline SANCO/3029/99 rev.4 during a previous study performed in 2013 by CIP on head cabbage, apple fruits, potato tubers and peach fruits (Lang, A. 2014; CIP Study code : 13M06017-01-VMPL). Therefore, only procedural recoveries were performed for the LOQ (level of quantification; 0.01 mg/kg) by fortifying control (untreated) specimens. A higher fortification level for residues of acetamiprid (5 mg/kg) was validated in Staphyt Study DMC-13-16126 for matrix peaches (also aqueous matrix). Therefore, only a reduced validation had to be performed for the new fortification level at 0.5 mg/kg acetamiprid.*  *The samples of the Staphyt Studies DMC-13-16122 and DMC-13-16134 were analysed together and therefore, the results of the recoveries and the validation data will be reported in both studies.*  *The method achieves a limit of quantification (LOQ) of 0.01 mg/kg. Limit of detection (LOD) is 0.003 mg/kg.*  *Acceptance criteria for method validation were met, with average recoveries ranging from 70% to 110% and relative standard deviations ≤20%.*  *The study is acceptable.* |

|  |  |
| --- | --- |
| Reference: | KCP 5.1.2/08 |
| Report | Magnitude of residues of acetamiprid in apples (RAC), following one or two applications of MCW-2222, in two trials (1 DCS + 1 HS) Northern Europe (Northern France) – 2013. Méric, D., 2013, Study No. DMC-13-16134. Adama Reference No. R-33599 |
| Guideline(s): | Yes, SANCO 3029/99 rev. 4 |
| Deviations: | None |
| GLP: | Yes |
| Acceptability: | Yes |

The multi-residue QuEChERS-based method used in the current study for the determination of residues of acetamiprid in apple fruits was fully validated according to the requirements of SANCO 3029/99 rev. 4 guidelines in a previous study prior to 1st March 2021 (Lang, A. 2014: 13M06017-01-VMPL. KCP 5.1.2/06). Therefore, only procedural recoveries were performed for the recovery at LOQ (level of quantification; 0.01 mg/kg) by fortifying control (untreated) samples. A higher fortification level (5.0 mg/kg) was validated in Méric, D., 2013 Study No. DMC-13-16126 (KCP 5.1.2/18) according to the requirements of SANCO 3029/99 rev. 4 guidelines in a previous study prior to 1st March 2021 for the matrix peaches (also high water content matrix). Therefore, only a reduced validation had to be performed for the new fortification level at 0.5 mg/kg acetamiprid.

A. Materials

|  |  |
| --- | --- |
| 1. Standards |  |
| Test item: | MCW-2222 |
| Batch no. | 611-280413-01 |
| Active substance: | Nominal: 200 g/L  Analysed: 202.7 g/L |
| Expiry date: | April 2015 |
|  |  |
| Reference item: | Acetamiprid |
| Lot/Batch number: | 20203 |
| Purity: | 99 % |
| CAS No.: | 135410-20-7 |
| Expiry date: | February 2016 |
| Standards for calibration | As above |
| Matrix: | Apples |

1. **Sample preparation and processing**

Approximately 10 g of the homogenised apple samples were weighed into 50 mL centrifugation tubes and fortified respectively. 10 mL acetonitrile were added and the samples were extracted using a sample homogeniser. Afterwards, a QuEChERS salt mix was added, shaken well and vortexed for at least one minute. The samples were then centrifuged at 3500 rpm for at least 10 minutes. An aliquot was transferred into a tube prepared with 25 mg PSA, 150 mg anhydrous magnesium sulfate and 2.5 mg GCB and shaken. The extract was filtered through a 0.45 µm filter into an autosampler vial (1.8 mL). The final extracts were diluted 1:10 and used directly for analysis by HPLC-MS/MS.

1. **Chromatographic parameters**

|  |  |
| --- | --- |
| HPLC- parameters | Dionex Ultimate 3000 |
| Column: | Phenomenex Luna C18 (150 x 2 mm ID x 5 µm PD) |
| Mobile phase: | A: Water/MeOH (90/10; v/v) +0.1% Formic acid + 5 mmol  Ammonium formate  B: not used  C: not used  D: MeOH/Water (90/10; v/v) +0.1% Formic acid + 5 mmol  Ammonium formate |
| Flow rate: | 300 µL/min |
| Injection volume: | 10 µL |
| MS/MS - parameters | AB Sciex API 5500 QTrap |
| Ionisation type: | ESI (electrospray ionisation) positive |
| Transitions monitored: | 223 →126 m/z (quantification)  223 → 90 m/z (confirmation) |

Results and discussions

The multi-residue QuEChERS-based method used in the current study for the determination of residues of acetamiprid in apple fruits was fully validated in a previous study according to the requirements of SANCO 3029/99 rev. 4 guidelines in a previous study prior to 1st March 2021 (Lang, A. 2014: 13M06017-01-VMPL. KCP 5.1.2/06). Therefore, only procedural recoveries were performed for the recovery at LOQ (level of quantification; 0.01 mg/kg) by fortifying control (untreated) samples. A higher fortification level (5.0 mg/kg) was validated in Méric, D., 2013 Study No. DMC-13-16126 (KCP 5.1.2/18) according to the requirements of SANCO 3029/99 rev. 4 guidelines in a previous study prior to 1st March 2021 for the matrix peaches (also high water content matrix). Therefore, only a reduced validation had to be performed for the new fortification level at 0.5 mg/kg acetamiprid.

The target analyte was determined using HPLC-MS/MS by monitoring two highly specific mass transitions. The detector response was linear over the range 0.25 μg/L to 20 μg/L (corresponding to 0.025 - 0.2 mg/kg) with associated determination coefficient (R2) ≥ 0.9999. The LOQ of the method is 0.01 mg/kg. Mean recovery values and associated RSDs meet the requirements of SANCO/3029/99 rev. 4 and the minimum validation requirements given in Section 4.2 of SANTE/2020/12830 rev.1 guidelines and are summarised in the table below.

Table A 15: Method validation recovery results for acetamiprid in apples reported in study DMC-13-16134

| Matrix | Analyte | Fortification level (mg/kg) | Mean  recovery (%) | RSD (%) | Comments |
| --- | --- | --- | --- | --- | --- |
| m/z 223 → 126 | | | | | |
| Apple fruits | Acetamiprid | 0.01 mg/kg (n=3) | 98 | 5.1 | - |
|  |  | 0.5 mg/kg (n=4) | 94 | 0.9 | - |
| **m/z 223 → 90** | | | | | |
| Apple fruits | Acetamiprid | 0.01 mg/kg (n=3) | 98 | 6.6 | - |
|  |  | 0.5 mg/kg (n=4) | 94 | 1.5 | - |

Table A 16: Characteristics for the analytical method used for the determination of acetamiprid in apples

|  | Acetamiprid |
| --- | --- |
| Specificity | A highly specific HPLC-MS/MS method was used for the determination of the target analyte. Two mass transitions (m/z 223→126, quantification and m/z 223→90, qualification) were monitored during each analysis.  Target analyte concentrations in controls did not exceed 30% of the method LOQ. No interference from co-eluting components at the retention time of the target analyte. |
| Calibration (type, number of data points) | Seven-point linear calibration  y= 142528x R2= 0.9999 |
| Calibration range | 0.25 µg/L to 20µg/L (corresponding to 0.025 - 0.2 mg/kg) |
| Assessment of matrix effects is presented | Matrix effects were assessed and found to be insignificant (< ± 20%). Nevertheless, matrix-matched calibration was used throughout the study. |
| Limit of determination/quantification | LOQ: 0.01 mg/kg  LOD: 0.003 mg/kg (defined as 30% of the LOQ) |

Conclusion

An analytical method for the determination of acetamiprid in peaches was fully validated in study 13M06017-01-VMPL (KCP 5.1.2/06), as well as the validation at higher LOQ in DMC-13-16126 (KCP 5.1.2/18), according to the requirements of SANCO 3029/99 rev. 4 guidelines prior to 1st March 2021. The reduced validation data provided within the current study for the new fortification level (0.5 mg/kg acetamiprid) exceed the minimum validation requirements given in Section 4.2 of SANTE/2020/12830 Rev. 1. The method should therefore be considered suitable for its intended purpose and acceptable for the determination of acetamiprid in apple fruits.

* + - * 1. Analytical method ChR-14-17311

Method validation 13M06017-01-VMPL & DMC-13-16126

|  |  |
| --- | --- |
| Comments of zRMS: | The study has been evaluated and accepted in Registration Report, Section 5 for CA3573 / Carnadine / Kestrel, Nufarm (August 2021).  Conclusions:  *The analytical method was fully validated on aqueous matrix (peach flesh) during previous study performed in 2013 by CIP (Lang, A. 2014; CIP Study Code: 13M06017-01-VMPL) for aqueous matrix validation.*  *Dry matrix (dried apples) was fully validated during this study with 5 validations at three different levels (LOQ, 100 LOQ and 500 LOQ).*  *Therefore, reduced validations were performed in the current study to confirm validation at LOQ and 100 LOQ levels on apple, washing water, juice, puree and on wet and dry pomace.*  *The method achieves a limit of quantification (LOQ) of 0.01 mg/kg. Limit of detection (LOD) is 0.003 mg/kg.*  *Acceptance criteria for method validation were met, with average recoveries ranging from 70% to 110% and relative standard deviations ≤20%.*  *The study is acceptable.* |

|  |  |
| --- | --- |
| Reference: | KCP 5.1.2/09 |
| Report | Magnitude of the residues of acetamiprid in apple (RAC fruits and processed fractions), following one or two applications of MCW-2222 in six trials (3 DCS + 3 HS), Northern Europe (Northern France, Germany, Poland and Belgium) – 2014. Roussel, Ch. H., 2014, Study No. ChR-14-17311. Adama Reference No. R-34915 |
| Guideline(s): | Yes, SANCO 3029/99 rev. 4 |
| Deviations: | None |
| GLP: | Yes |
| Acceptability: | Yes |

The multi-residue QuEChERS-based method used in the current study for the determination of residues of acetamiprid in apple, washed apples, washing water, apple juice, apple puree and wet apple pomace was fully validated in a previous study (Lang, A. 2014: 13M06017-01-VMPL, KCP 5.1.2/06) according to the requirements of SANCO 3029/99 rev. 4 prior to 1st March 2021. Therefore, only a reduced validation was performed for the recovery at LOQ (level of quantification; 0.01 mg/kg) by fortifying control (untreated) samples. A higher fortification level (5.0 mg/kg) was validated in Méric, D., 2013 Study No. DMC-13-16126 (KCP 5.1.2/18) for the matrix peaches (also high water content matrix) according the requirements of SANCO 3029/99 rev. 4 prior to 1st March 2021. Therefore, only a reduced validation had to be performed in the current study for the new high fortification level at 1.0 mg acetamiprid /kg. The dry matrix dry apples was fully validated in the current study at the following levels: LOQ (0.01 mg/kg), 500 x LOQ (5.0 mg/kg) and at 100 x LOQ (1.0 mg/kg). For the other dry matrix dry apple pomace, a reduced validation was performed at the same levels.

A. Materials

|  |  |
| --- | --- |
| 1. Standards |  |
| Test item: | MCW-2222 |
| Batch no. | 611-280413-01 |
| Active substance: | Nominal: 200 g/L  Analysed: 202.7 g/L |
| Expiry date: | April 2015 |
| Reference item: | Acetamiprid |
| Lot/Batch number: | 20203 |
| Purity: | 99 % |
| CAS No.: | 135410-20-7 |
| Expiry date: | February 2016 |
| Standards for calibration | As above |
| Matrix: | Apples and processed fractions |

1. **Sample preparation and processing**

Approximately 10 g of the homogenised apple samples and 5 g (dry apple, wet apple pomace and dry apple pomaces) were weighed into 50 mL centrifugation tubes and fortified respectively. 10 mL acetonitrile were added and the samples were extracted using a sample homogeniser. Afterwards a QuEChERS salt mix was added, shaken well and vortexed for at least one minute. The samples were then centrifuged at 3500 rpm for at least 10 minutes. An aliquot was transferred into a tube prepared with 25 mg PSA, 150 mg anhydrous magnesium sulfate and 2.5 mg GCB and shaken. The extract was filtered through a 0.45µm filter into an autosampler vial (1.8 mL). The final extracts were diluted 1:10 and 1:5 for matrix dry apples, wet pomace and dry apple pomaces, respectively. The diluted final extracts were used directly for analysis by HPLC-MS/MS.

1. **Chromatographic parameters**

|  |  |
| --- | --- |
| HPLC- parameters | Dionex Ultimate 3000 |
| Column: | Phenomenex Luna C18, 150 x 2 mm ID x 5 µm PD |
| Mobile phase: | A: not used  B: Water/MeOH (90/10; v/v) +0.1% Formic acid + 5 mmol  Ammonium formate  C: MeOH/Water (90/10; v/v) +0.1% Formic acid + 5 mmol  Ammonium formate  D: not used |
| Flow rate: | 300 µL/min |
| Injection volume: | 10 µL |
| MS/MS - parameters | AB Sciex API 5500 QTRAP |
| Ionisation type: | ESI (electrospray ionisation) positive |
| Transitions monitored: | 223 → 126 m/z (quantification)  223 → 90 m/z (confirmation) |

Results and discussions

The multi-residue QuEChERS-based method used in the current study for the determination of residues of acetamiprid in apple, washed apples, washing water, apple juice, apple puree and wet apple pomace was fully validated in a previous study (Lang, A. 2014: 13M06017-01-VMPL, KCP 5.1.2/06) according to the requirements of SANCO 3029/99 rev. 4 prior to 1st March 2021. Therefore, only a reduced validation was performed for the recovery at LOQ (level of quantification; 0.01 mg/kg) by fortifying control (untreated) samples. A higher fortification level (5.0 mg/kg) was validated in Méric, D., 2013 Study No. DMC-13-16126 (KCP 5.1.2/18) for the matrix peaches (also high water content matrix) according the requirements of SANCO 3029/99 rev. 4 prior to 1st March 2021. Therefore, only a reduced validation had to be performed in the current study for the new high fortification level at 1.0 mg acetamiprid /kg. The dry matrix dry apples was fully validated in the current study at the following levels: LOQ (0.01 mg/kg), 500 x LOQ (5.0 mg/kg) and at 100 x LOQ (1.0 mg/kg). For the other dry matrix dry apple pomace, a reduced validation was performed at the same levels.

The target analyte was determined using HPLC-MS/MS by monitoring two highly specific mass transitions. The detector response was linear over the range 0.25 μg/L to 100 μg/L with associated determination coefficient (R2) ≥ 0.999. The LOQ of the method is 0.01 mg/kg. Mean recovery values and associated RSDs meet the requirements of SANCO/3029/99 rev. 4 and the minimum validation requirements given in Section 4.2 of SANTE/2020/12830 rev.1 guidelines and are summarised in the table below.

Table A 17: Method validation recovery results for acetamiprid in apples and processed fractions reported in study ChR-14-17311

| Matrix | Analyte | Fortification level (mg/kg) (n=3) | Mean  recovery (%) | RSD (%) | Comments |
| --- | --- | --- | --- | --- | --- |
| Apple fruit | Acetamiprid | 0.01 | 95 | 4.7 | - |
| 1.0 | 103 | 3.5 | - |
| Dry apple | 0.01 (n=5) | 10 | 3.8 | - |
| 1.0 (n=5) | 106 | 4.3 | - |
| 5.0 (n=5) | 107 | 1.2 | - |
| Washing water | 0.01 | 97 | 3.1 | - |
| 1.0 | 97 | 1.2 | - |
| Apple juice | 0.01 | 92 | 3.5 | - |
| 1.0 | 96 | 2.1 | - |
| Apple puree | 0.01 | 101 | 1.0 | - |
| 1.0 | 103 | 2.4 | - |
| Wet apple pomace | 0.01 | 104 | 3.3 | - |
| 1.0 | 110 | 4.1 | - |
| Dry apple pomace | 0.01 (n=4) | 103 | 4.6 | - |
| 1.0 | 108 | 2.3 | - |
| 5.0 | 105 | 9.2 | - |

Table A 18: Characteristics of the analytical method used for the determination of acetamiprid residues in apples and processed fractions

|  | Acetamiprid |
| --- | --- |
| Specificity | A highly specific HPLC-MS/MS method was used for the determination of the target analyte. Two mass transitions (m/z 223→126, quantification and m/z 223→90, qualification) were monitored during each analysis.  Target analyte concentrations in controls did not exceed 30% of the method LOQ. No interference from co-eluting components at the retention time of the target analyte. |
| Calibration (type, number of data points) | Nine-point linear calibration  *Apple fruits:*  Quantification transition: y= 7.99437 e4x, r= 0.99994  Confirmation transition: y = 28851.39587x, r= 0.99996  *Dry apples*:  Quantification transition: y= 7.7413e4x, r= 0.99998  Confirmation transition: y= 27894.80316x, r= 0.99998  *Washing water*:  Quantification transition: y= 6.13117e4x, r= 0.99953  Confirmation transition: y= 22401.86922x, r= 0.99949  *Apple juice*:  Quantification transition: y= 6.94619 e4x, r= 0.99992  Confirmation transition: y= 25132.16767x, r= 0.99998  *Apple puree:*  Quantification transition: y= 6.685204 e4x, r= 0.99995  Confirmation transition: y= 24359.61806x, r= 0.99997  *Wet apple pomace*:  Quantification transition: y= 6.77067e4x r= 0.99992  Confirmation transition: y= 24481.53628x, r= 0.99990  *Dry apple pomace*:  Quantification transition: y= 7.16721e4x r= 0.99997  Confirmation transition: y= 25808.92525x, r= 0.99999 |
| Calibration range | 0.25 µg/L to 100µg/L |
| Assessment of matrix effects is presented | Matrix effects were assessed and found to be insignificant (< ± 20%). Nevertheless, matrix-matched calibration was used throughout the study. |
| Limit of determination/quantification | LOQ: 0.01 mg/kg  LOD: 0.003 mg/kg (30% of the LOQ) |

Conclusion

The analytical method for the determination of acetamiprid in plant matrices with high water content was fully validated according to the requirements of the SANCO/3029/99 rev 4 guidelines in Study 13M06017-01-VMPL, as well as the validation at higher LOQ in Study DMC-13-16126 (KCP 5.1.2/18), prior to 1st March 2021. The reduced validation data provided within the current study exceed the minimum validation requirements given in Section 4.2 of SANTE/2020/12830 Rev. 1. The method should therefore be considered suitable for its intended purpose and acceptable for the determination of acetamiprid in apple fruits and processed fractions.

* + - * 1. Analytical method 14SGS039

Method validation 13M06017-01-VMPL & 14SGS039

|  |  |
| --- | --- |
| Comments of zRMS: | The analytical method was fully validated on aqueous matrix during previous study performed in 2013 by CIP (Lang, A. 2014; CIP Study Code: 13M06017-01-VMPL).  Therefore, reduced validations were performed in the current study to confirm validation at LOQ and 100 LOQ levels on maize plant, maize cobs and grain.  The method achieves a limit of quantification (LOQ) of 0.01 mg/kg. Limit of detection (LOD) is 0.003 mg/kg.  Acceptance criteria for method validation were met, with average recoveries ranging from 70% to 110% and relative standard deviations ≤20%.  The study is acceptable. |

|  |  |
| --- | --- |
| Reference: | KCP 5.1.2/10 |
| Report | Magnitude of the residue of acetamiprid in maize (Raw Agricultural Commodity) after one application of MCW-2222 - four semi decline curve trials and four decline curve trials in Northern Europe (Northern France, Poland, Germany, Hungary and Austria) – 2014., Lebrun F., 2014, Study No. 14SGS039, Sponsor No. R-34912. |
| Guideline(s): | Yes, SANCO 3029/99 rev. 4 |
| Deviations: | None |
| GLP: | Yes |
| Acceptability: | Yes |

The multi-residue QuEChERS-based analytical method used in the current study was fully validated for the determination of acetamiprid in high water matrices according to the requirements of SANCO 3029/99 rev. 4 guidelines in a previous study prior to 1st March 2021 (see KCP 5.1.2/06, study No. 13M06017-01-VMPL). Reduced validation data for the determination of acetamiprid in maize (whole plants with and without cobs and kernel and maize cobs) was provided according to the requirements of SANCO 3029/99 rev. 4 in the current study and used to support a residue study conducted on maize. Additionally, an analytical method on the determination of acetamiprid in maize grain was fully validated according to the requirements of SANCO 3029/99 rev. 4 guidelines in this study.

A. Materials

|  |  |
| --- | --- |
| 1. Standards |  |
| Test item: | MCW-2222 |
| Batch No.: | 659-030314-01 |
| Expiry date: | 03 March 2016 |
| Active ingredient: | Nominal: 200 g/L  Analysed: 199.2 ± 3 g/L |
| Reference item: | Acetamiprid |
| Lot/Batch number: | 20202 |
| Purity: | 98.1 % |
| CAS No.: | 135410-20-7 |
| Expiry date: | 02 February 2016 |
| Standards for calibration | As above |
| Matrix: | Maize (whole plant, cobs and grain) |

1. **Sample preparation and processing**

Following homogenisation, 10 g ± 0.1 g maize plants or maize cobs (respectively 5 g ± 0.05 g maize grain) were weighed into a 50 mL centrifuge tube. 2.5 mL water for maize plants, 4 mL water for maize cobs and 8.5 mL were added for maize grain. 10 mL acetonitrile were added for each matrix and the samples homogenised for 2 min at high speed. Thereafter, 1 g sodium citrate, 0.5 g sodium hydrogencitrate sesquihydrate, 4 g magnesium sulphate and 1 g sodium chloride were added, the mixture thoroughly shaken and afterwards mixed on a vortex mixer for at least 1 min. The samples were centrifuged at 3500 rpm for at least 10 minutes. 25 mg PSA, 150 mg anhydrous magnesia sulphate and 25 mg GCB was weighed into a 2 mL tube. 1 mL of supernatant was transferred into the tube containing the mixture of sorbents and shaken on a vortex mixer for 30 s. Samples were filtered into an autosampler vial and diluted 1:10 with acetonitrile (1:5 for maize grain). Diluted extracts were analysed using HPLC-MS/MS.

1. **Chromatographic parameters**

|  |  |
| --- | --- |
| HPLC: | Dionex Ultimate 3000 |
| Column: | Phenomenex Luna C18 (2) 100 A, 150 mm x 2.0 mm, 5.0 μm particle  size |
| Mobile phase: | water/MeOH (90/10; v/v) + 0.1 % formic acid + 5 mmol ammonium formate |
| Flow rate: | 0.3 mL/min |
| Injection volume: | 10 µl |
| MS/MS Parameters: | AB Sciex API 5500 QTRAP |
| Mode: | ESI (electrospray ionisation) positive |
| Transitions monitored: | 223 → 126 m/z (quantification)  223 → 90 m/z (confirmation) |

Results and discussion

The analytical method used in the current study was fully validated for the determination of acetamiprid in high water matrices according to the requirements of SANCO 3029/99 rev. 4 guidelines in a previous study (See KCP 5.1.2/06). A reduced validation for the determination of acetamiprid in maize whole plant and maize cobs was conducted in the current study. Additionally, an analytical method on the determination of acetamiprid in maize grain was fully validated according to the requirements of SANCO 3029/99 rev. 4 guidelines in this study. The target analyte was determined using HPLC-MS/MS by monitoring two highly specific mass transitions. The detector response was linear over the range of 0.25 - 100 µg/L for all matrices with associated correlation coefficients (r) ≥ 0.9998. The LOQ of the method was 0.01 mg/kg for all matrices. The stability of the target analyte in final extracts was assessed. The target analyte was found to be stable in final extracts for at least 4 days (refrigerated at -18 °C). All mean recovery values and associated RSDs meet the validation requirements of SANCO 3029/99 rev. 4 guidelines and are summarised in the table below.

Table A 19: Method validation recovery data for the determination of acetamiprid in maize (whole plant, cobs and grain) reported in study 14SGS039

| Matrix | Analyte | Fortification level (mg/kg) | Mean  recovery (%) | Overall RSD (%) | Comments |
| --- | --- | --- | --- | --- | --- |
| Whole plant | Acetamiprid | Quantification transition m/z 223→126 | | | |
| 0.01 (n=3) | 90 | 1.4 | - |
| 1.0 (n=3) | 97 | 3.1 |
| Qualification transition m/z 223→90 | | | |
| 0.01 (n=3) | 89 | 1.3 | - |
| 1.0 (n=3) | 96 | 3.3 |
| Cobs | Quantification transition m/z 223→126 | | | |
| 0.01 (n=3) | 97 | 2.6 | - |
| 1.0 (n=3) | 99 | 2.1 |
| Qualification transition m/z 223→90 | | | |
| 0.01 (n=3) | 97 | 2.4 | - |
| 1.0 (n=3) | 102 | 2.3 |
| Grain | Quantification transition m/z 223→126 | | | |
| 0.01 (n=5) | 96 | 3.1 | - |
| 1.0 (n=5) | 94 | 3.9 |
| Qualification transition m/z 223→90 | | | |
| 0.01 (n=5) | 97 | 2.4 | - |
| 1.0 (n=5) | 96 | 5.3 |

Table A 20: Characteristics of the analytical method validated for the determination of acetamiprid in maize (whole plant, cobs and grain)

|  | Acetamiprid |
| --- | --- |
| Specificity | A highly specific HPLC-MS/MS method was used for the determination of the target analyte. Two mass transitions (m/z 223→126, quantification and m/z 223→90, qualification) were monitored during each analysis.  Target analyte concentrations in controls did not exceed 30% of the method LOQ. No interference from co-eluting components at the retention time of the target analyte were observed. |
| Calibration (type, number of data points) | Maize whole plants:  9-point linear calibration  y = 99189.5 x, r = 0.99982  Maize cobs:  9-point linear calibration  y = 102990 x, r = 0.99985  Maize grains:  9-point linear calibration  y = 97796.4 x, r = 0.99989 |
| Calibration range | 0.25 - 100 µg/L |
| Assessment of matrix effects is presented | Matrix effects were assessed and no matrix effects > 20 % were observed for all matrices. |
| Limit of determination/quantification | LOQ for all matrices: 0.01 mg/kg  LOD for all matrices: 0.003 mg/kg |

Conclusion

An analytical method for the determination of acetamiprid in high water matrices was fully validated according to the requirements of the SANCO/3029/99 rev 4 guidelines prior to 1st March 2021 (see KCP 5.1.2/06). The validation data provided in the current report represent a reduced validation of the method for maize whole plants and maize cobs and full validation for maize grains. The data provided exceed the minimum validation requirements given in Section 4.2 of SANTE/2020/12830 rev. 1. The method should therefore be considered suitable for its intended purpose and acceptable for the determination of acetamiprid in maize.

* + - * 1. Analytical method SPK-20-46380

Method validation SPK-20-46380

|  |  |
| --- | --- |
| Comments of zRMS: | The analytical method for the determination of acetamiprid was fully validated in matrices sugar beet roots and a reduced validation was performed on sugar beet whole plant and leaves + tops. Limit of quantification (LOQ) achieved was 0.01 mg/kg for all matrices.  Acceptance criteria for method validations were met, with average recoveries ranging from 70% to 110% and relative standard deviations ≤20%.  It can therefore be concluded, that the method was applicable on matrices sugar beet roots, sugar beet whole plants and sugar beet leaves + tops under investigation using HPLC with  MS/MS detection.  The method is acceptable. |

|  |  |
| --- | --- |
| Reference: | KCP 5.1.2/11 |
| Report | Magnitude of the residues of acetamiprid in sugar beet (RAC whole plants, roots and leaves+tops), following two applications of Acetamiprid 200 SL in three trials (two HS + one DCS) - Northern Europe (Poland and Hungary) – 2020. Roussel Ch. H., 2022, Study No. SPK-20-46380, Sponsor No. 000105979. |
| Guideline(s): | SANCO/3029/99 rev. 4 |
| Deviations: | None |
| GLP: | Yes |
| Acceptability: | Yes |

A multi-residue QuEChERS-based analytical method for the determination of acetamiprid residues in sugar beet roots was fully validated according to the requirements of SANCO/3029/99 rev. 4 prior to 1st March 2021 and used to support a field residue study.

**A. Materials**

|  |  |
| --- | --- |
| 1. Standards |  |
| Reference item: | Acetamiprid |
| Lot/Batch number: | BCBT9185 |
| Purity: | 100 % |
| CAS No.: | 160430-64-8 |
| Expiry date: | 28 February 2022 |
| Standards for calibration | As above |
| Matrices: | 1: Sugar beet root (i.e., plant commodities high water and high sugar content) |

**B. Sample preparation and processing**

*Sugar beet roots*

Following homogenisation, 10 g ± 0.1 g were weighed into a 50 mL centrifuge tube. 10 mL acetonitrile were added, and the samples were extracted by shaking for 15 min on a platform shaker at approximately 250 rpm. Thereafter 1 g sodium citrate, 0.5 g sodium hydrogencitrate sesquihydrate, 4 g magnesium sulphate and 1 g sodium chloride were added, the mixture thoroughly shaken by hand and afterwards mixed on a vortex mixer for at least 1 min. The samples were centrifuged at 4000 rpm for at least 5 minutes and the supernatant transferred to a 15 mL centrifuge tube. 25 mg PSA, 150 mg anhydrous magnesia sulphate and 25 mg C18e was weighed into a 2 mL tube. 1 mL of supernatant was transferred into the tube containing the mixture of sorbents, shaken on a vortex mixer for 30 s and centrifuged for 5 min (12 000 rpm) afterwards. The purified extracts were diluted 1:10 (with ACN) into an autosampler vial (1.8 mL) and used directly for analysis by HPLC-MS/MS. If necessary, the final extracts were diluted further.

**C. Chromatographic parameters**

|  |  |
| --- | --- |
| HPLC- parameters: | Dionex Ultimate 3000 |
| Column: | Phenomenex Luna C18 (2) 100 A, 150 mm x 2.0 mm, 5.0 μm particle size |
| Mobile phase: | A: Water containing 1 % formic acid (v/v)  B: Acetonitrile |
| Flow rate: | 500 µL/min |
| Injection volume: | 10 µL |
| MS/MS Parameters:  Ionisation type:  Transitions monitored: | AB Sciex API 5500 QTRAP (Triple quadrupole mass spectrometer)  EI positive  m/z 223 → 126 (quantification)  m/z 223 → 90 (confirmation) |

**Results and discussion**

The analytical method used in the current study was fully validated for the determination of acetamiprid in plant commodity dry/high protein content according to the requirements of SANCO/3029/99 rev. 4 guideline. The target analyte was determined using HPLC-MS/MS by monitoring two highly specific mass transitions. The detector response was linear over the range of 0.1 – 100 µg/L (corresponding to 0.001 - 1 mg/kg) with the correlation coefficient r > 0.999. The LOQ of the method is 0.01 mg acetamiprid/kg. All mean recovery values and associated RSDs for both matrices meet the requirements of SANCO/3029/99 rev. 4 guidelines and are summarised in the table below.

**Table A 21: Method validation recovery data for the determination of acetamiprid in sugar beet roots reported in study 000105979.**

| **Matrix** | **Analyte** | **Fortification level (mg/kg)**  **(n = 5)** | **Mean  recovery (%)** | **RSD (%)** | **Comments** |
| --- | --- | --- | --- | --- | --- |
| Quantification transition m/z 223→126 | | | | | |
| Dry beans seeds | Acetamiprid | 0.01 | 97 | 2.5 | - |
| 1 | 83 | 5.3 |
| Confirmation transition m/z 223→90 | | | | | |
| Dry beans seeds | Acetamiprid | 0.01 | 97 | 4.0 | - |
| 1 | 84 | 5.6 |

**Table A 22: Characteristics of the analytical method validated for the determination of acetamiprid in sugar beet root.**

|  | **Acetamiprid** |
| --- | --- |
| Specificity | A highly specific HPLC-MS/MS method was used for the determination of the target analyte. Two mass transitions (m/z 223→126, quantifiaction and m/z 223→90, qualification) were monitored during each analysis.  Target analyte concentrations in controls did not exceed 30% of the method LOQ. No interference from co-eluting components at the retention time of the target analyte was observed. |
| Calibration (type, number of data points) | Six-point linear calibration  y = 126591 x, r = 0.99976 |
| Calibration range | 0.1 – 100 µg/L (corresponding to 0.001 - 1 mg/kg) |
| Assessment of matrix effects is presented | Matrix effects were assessed and found to be insignificant (< ± 20%). Nevertheless, matrix-matched calibration was used. |
| Limit of determination/quantification | LOQ: 0.01 mg/kg  LOD: 0.003 mg/kg |
| Standard stability | The stability of the target anaylte in stock and fortification solutions has been tested in study No. EGL-20-46375 (KCP 5.1.2/12) where the solutions were found to be stable for 208 days when prepared in methanol and stored refrigerated in the dark. |
| Stability in final extracts | Freshly prepared matrix-matched standard solutions were compared with identical samples that had been stored refrigerated (-18 °C) and stability of acetamiprid in final extracts was proven for storage of at least 18 days for the matrix dry beans seeds. |

**Conclusion**

An analytical method for the determination of acetamiprid residues in dry beans seeds was fully validated according to SANCO/3029/99 rev. 4 guidelines. The data provided exceed the minimum validation requirements given in Section 4.2 of SANTE/2020/12830 Rev. 1. The method should therefore be considered suitable for its intended purpose and acceptable for the determination of acetamiprid in plant commodities dry/high protein content.

* + - * 1. Analytical method EGL-20-46375

Method validation EGL-20-46375

|  |  |
| --- | --- |
| Comments of zRMS: | The analytical method for the determination of acetamiprid was fully validated for the aqueous matrix plum whole fruit in the study EGL-20-46374 (CIP Phase ID: 20S13018-01-RAPM). A reduced validation for tomato fruit was done during this study.  Limit of quantification (LOQ) achieved was 0.01 mg/kg.  Acceptance criteria for method validations were met, with average recoveries ranging from 70% to 110% and relative standard deviations ≤20%.  The method is acceptable. |

|  |  |
| --- | --- |
| Reference: | KCP 5.1.2/12 |
| Report | Magnitude of the residues of acetamiprid in open field tomato (RAC fruits), following one or two applications of ACETAMIPRID 200 SL in four trials (two HS + two DCS) - Southern Europe (Spain and Italy) – 2020., Grall E., 2021, Study No. EGL-20-46375, Sponsor No. 000105974. |
| Guideline(s): | Yes, SANCO 3029/99 rev. 4 |
| Deviations: | None |
| GLP: | Yes |
| Acceptability: | Yes |

The multi-residue QuEChERS-based analytical method used in the current study was fully validated for the determination of acetamiprid in plum (whole fruit, flesh) according to the requirements of SANCO 3029/99 rev. 4 guidelines in a previous study prior to 1st March 2021 (study EGL-20-46374). Reduced validation data for the determination of acetamiprid in tomato was provided according to the requirements of SANCO 3029/99 rev. 4 in the current study and used to support a residue study conducted on tomato (Domingo S., 2021, Study No. SDO-20-46377).

This study is submitted in support of standard stability data of study SPK-20-46380 (see KCP 5.1.2/10).

A. Materials

|  |  |
| --- | --- |
| Standards |  |
| Test item: | ACETAMIPRID 200 SL |
| Batch No.: | 99191024 |
| Expiry date: | 08 June 2022 |
| Active ingredient: | Nominal: 200 g/L  Analysed: 200.1 ± 3 g/L |
|  |  |
| Reference item: | Acetamiprid |
| Lot/Batch number: | BCBT9185 |
| Purity: | 100 % |
| CAS No.: | 160430-64-8 |
| Expiry date: | 28 February 2022 |
|  |  |
|  |  |
| Standards for calibration | As above |
|  |  |
| Matrix: | Tomato |

1. **Sample preparation and processing**

Following homogenisation, 10 g ± 0.1 g tomato were weighed into a 50 mL centrifuge tube. 10 mL acetonitrile were added, and the samples were extracted by shaking for 15 min on a platform shaker at approximately 250 rpm. Thereafter, 1 g sodium citrate, 0.5 g sodium hydrogencitrate sesquihydrate, 4 g magnesium sulphate and 1 g sodium chloride were added, the mixture thoroughly shaken and afterwards mixed on a vortex mixer for at least 1 min. The samples were centrifuged at 4000 rpm for at least 5 minutes and the supernatant transferred to a 15 mL centrifuge tube. 25 mg PSA, 150 mg anhydrous magnesia sulphate and 2.5 mg GCB was weighed into a 2 mL tube. 1 mL of supernatant was transferred into the tube containing the mixture of sorbents, shaken on a vortex mixer for 30 s and centrifuged for 5 min (12 000 rpm) afterwards. The purified extracts were diluted 1:10 (with ACN) into an autosampler vial (1.8 mL) and used directly for analysis by HPLC-MS/MS. If necessary, the final extracts were diluted further.

1. **Chromatographic parameters**

|  |  |
| --- | --- |
| HPLC: | Dionex Ultimate |
| Column: | Phenomenex Luna C18 (2) 100 A, 150 mm x 2.0 mm, 5.0 μm particle  size |
| Mobile phase: | A: water + 1% formic acid  B: acetonitrile |
| Flow rate: | 0.5 mL/min |
| Injection volume: | 10 µl |
| MS/MS Parameters: | AB Sciex 5500 QTRAP |
| Mode: | ESI (electrospray ionisation) positive |
| Transitions monitored: | 223 → 126 m/z (quantification)  223 → 90 m/z (confirmation) |

Results and discussion

The analytical method used in the current study was fully validated for the determination of acetamiprid in plum (whole fruit and flesh) according to the requirements of SANCO 3029/99 rev. 4 guidelines in a previous study. A reduced validation for the determination of acetamiprid in tomato was conducted in the current study. The target analyte was determined using HPLC-MS/MS by monitoring two highly specific mass transitions. The detector response was linear over the range 0.1 - 100 µg/L (corresponding to 0.001 mg/kg to 1 mg/kg). for all matrices with associated correlation coefficients (r) ≥ 0.999. The LOQ of the method was 0.01 mg/kg. The stability of the target analyte in final extracts and working solutions was assessed. The target analyte was found to be stable in final extracts for at least 28 days (refrigerated at -18 °C). All mean recovery values and associated RSDs meet the validation requirements of SANCO/3029/99 rev. 4 and the minimum validation requirements given in Section 4.2 of SANTE/2020/12830 rev. 1 guidelines and are summarised in the table below.

Table 24: Method validation recovery data for the determination of acetamiprid in tomato reported in study EGL-20-46375

| Matrix | Analyte | Fortification level (mg/kg) | Mean  recovery (%) | Overall RSD (%) | Comments |
| --- | --- | --- | --- | --- | --- |
| Tomato | Acetamiprid | Quantification transition m/z 223→126 | | | |
| 0.01 (n=3) | 84 | 13.4 | - |
| 5.0 (n=3) | 92 | 4.3 |
| Qualification transition m/z 223→90 | | | |
| 0.01 (n=3) | 84 | 14.2 | - |
| 5.0 (n=3) | 94 | 4.3 |

Table 25: Characteristics of the analytical method validated for the determination of acetamiprid in tomato

|  | Acetamiprid |
| --- | --- |
| Specificity | A highly specific HPLC-MS/MS method was used for the determination of the target analyte. Two mass transitions (m/z 223→126, quantification and m/z 223→90, qualification) were monitored during each analysis.  Target analyte concentrations in controls did not exceed 30% of the method LOQ. No interference from co-eluting components at the retention time of the target analyte were observed. |
| Calibration (type, number of data points) | 6-point linear calibration  y = 93295.9 x, r ≥ 0. 999 |
| Calibration range | 0.1 - 100 µg/L (corresponding to 0.001 mg/kg to 1 mg/kg) |
| Assessment of matrix effects is presented | Matrix effects were assessed and no matrix effects > 20 % were observed. Nevertheless, matrix-matched calibration standards were used. |
| Limit of determination/quantification | LOQ: 0.01 mg/kg  LOD: 0.003 mg/kg |
| Standard stability | The stock solution of Acetamiprid and the fortification solutions were found to be stable for 208 days when prepared in methanol and stored refrigerated in the dark. |
| Stability in final extracts | Matrix-matched calibration was prepared at the day of extraction and stored refrigerated along with the samples. On the day of analysis, one  matrix-matched standard solution was freshly prepared and analysed against the stored one. If freshly prepared and stored standard solution differed by no more than 20% the stability of the analyte in the final extract is proven.  The stability of Acetamiprid in final extracts of tomato under refrigerated conditions was proven for at least 28 days. |

Conclusion

An analytical method for the determination of acetamiprid in plum was fully validated according to the requirements of the SANCO/3029/99 rev. 4 guidelines prior to 1st March 202. The validation data provided in the current report represent a reduced validation of the method at LOQ and 5.0 mg/kg acetamiprid in tomato. The data provided exceed the minimum validation requirements given in Section 4.2 of SANTE/2020/12830 rev. 1. The method should therefore be considered suitable for its intended purpose and acceptable for the determination of acetamiprid in tomato.

* + - * 1. Analytical method ChR-21-48246

Method validation SPK-20-46380 & ChR-21-48246

|  |  |
| --- | --- |
| Comments of zRMS: | The method for the determination of acetamiprid was fully validated in study SPK-20-46380 for the matrix sugar beet roots belonging to the group of plant commodities with high water content.and a reduced validation was performed on sugar beet whole plant and leaves + tops. Limit of quantification (LOQ) achieved was 0.01 mg/kg for all matrices.  Acceptance criteria for method validations were met, with average recoveries ranging from 70% to 110% and relative standard deviations ≤20%.  It can therefore be concluded, that the method was applicable on matrices sugar beet roots, sugar beet whole plants and sugar beet leaves + tops under investigation using HPLC with  MS/MS detection.  The method is acceptable. |

|  |  |
| --- | --- |
| Reference: | KCP 5.1.2/13 |
| Report | Magnitude of the residues of acetamiprid, after application of Acetamiprid 200 SL in sugar beet in Northern Europe – 2021. Roussel Ch.H., 2022, Study No. ChR-21-48246, Sponsor No. 000107604. |
| Guideline(s): | Yes, SANCO 3029/99 rev. 4 and SANTE 2020/12830 rev. 1 |
| Deviations: | None |
| GLP: | Yes |
| Acceptability: | Yes |

The multi-residue QuEChERS-based analytical method used in the current study was fully validated for the determination of acetamiprid in high water matrices according to the requirements of SANCO 3029/99 rev. 4 guidelines in a previous study prior to 1st March 2021 (see KCP 5.1.2/10, Study No. SPK-20-46380). Reduced validation data for the determination of acetamiprid in sugar beet (whole plants, roots and beets + tops) was provided according to the requirements of SANTE 12830/2020 rev. 1 in the current study and used to support a residue study conducted on sugar beet.

A. Materials

|  |  |
| --- | --- |
| 1. Standards |  |
| Test item: | Acetamiprid 200 SL |
| Batch No.: | 1242-050520-01 |
| Expiry date: | 26 February 2023 |
| Active ingredient: | Nominal: 200 g/L  Analysed: 203.4 |
| Reference item: | Acetamiprid |
| Lot/Batch number: | BCBT9185 |
| Purity: | 100 % |
| CAS No.: | 160430-64-8 |
| Expiry date: | 28 February 2022 |
| Standards for calibration | As above |
| Matrix: | Sugar beet (whole plant, roots and leaves + tops) |

1. **Sample preparation and processing**

Following homogenisation, 10 g ± 0.1 g sugar beet whole plant, roots or leaves + tops were weighed into a 50 mL centrifuge tube. 2.5 mL water were added for leaves + tops. 10 mL acetonitrile were added for each matrix and the samples extracted shaking for 15 min at 250 rpm. Thereafter, 1 g sodium citrate, 0.5 g sodium hydrogencitrate sesquihydrate, 4 g magnesium sulphate and 1 g sodium chloride were added, the mixture thoroughly shaken and afterwards mixed on a vortex mixer for at least 1 min. The samples were centrifuged at 4000 rpm for at least 5 minutes. 25 mg PSA and 150 mg anhydrous magnesia sulphate was weighed into a 2 mL tube. 1 mL of supernatant was transferred into the tube containing the mixture of sorbents, shaken on a vortex mixer for 30 s, centrifuged for 5 min and transferred to an autosampler vial. Samples were diluted 1:10 with acetonitrile, diluted extracts were analysed using HPLC-MS/MS.

1. **Chromatographic parameters**

|  |  |
| --- | --- |
| HPLC: | Shimadzu LC-40 |
| Column: | Phenomenex Luna C18 (2) 100 A, 150 mm x 2.0 mm, 5.0 μm particle  size |
| Mobile phase: | A: water + 0.1 % formic acid  B: acetonitrile |
| Flow rate: | 0.5 mL/min |
| Injection volume: | 10 µl |
| MS/MS Parameters: | AB Sciex QTRAP 5500 |
| Mode: | ESI (electrospray ionisation) positive |
| Transitions monitored: | 223 → 126 m/z (quantification)  223 → 90 m/z (confirmation) |

Results and discussion

The analytical method used in the current study was fully validated for the determination of acetamiprid in high water matrices according to the requirements of SANCO 3029/99 rev. 4 guidelines in a previous study (See KCP 5.1.2/10). A reduced validation for the determination of acetamiprid in sugar beet whole plant, roots and leaves + tops according to SANTE 12830/2020 rev. 1 was conducted in the current study. The target analyte was determined using HPLC-MS/MS by monitoring two highly specific mass transitions. The detector response was linear over the range of 0.3 - 30 µg/L (corresponding to 0.003 - 0.3 mg/kg) for all matrices with associated correlation coefficients (r) ≥ 0.9992. Regression residuals were plotted and showed no trend and a random distribution, demonstrating a linear calibration function was suitable for the quantitative determination of the target analyte. The LOQ of the method was 0.01 mg/kg for all matrices. The stability of the target analyte in standard solutions was assessed. The target analyte was found to be stable in standard solutions for 145 days (refrigerated at -18 °C). All mean recovery values and associated RSDs meet the validation requirements of SANTE 12830/2020 rev. 1 guidelines and are summarised in the table below.

Table A 26: Method validation recovery data for the determination of acetamiprid in sugar beet (whole plant, roots, leaves + tops) reported in study ChR-21-48246

| Matrix | Analyte | Fortification level (mg/kg) | Mean  recovery (%) | Overall RSD (%) | Comments |
| --- | --- | --- | --- | --- | --- |
| Whole plant | Acetamiprid | Quantification transition m/z 223→126 | | | |
| 0.01 (n=3) | 102 | 2.0 | - |
| 1.0 (n=3) | 96 | 2.2 |
| Qualification transition m/z 223→90 | | | |
| 0.01 (n=3) | 101 | 1.7 | - |
| 1.0 (n=3) | 96 | 1.0 |
| Roots | Quantification transition m/z 223→126 | | | |
| 0.01 (n=3) | 102 | 0.6 | - |
| 1.0 (n=3) | 90 | 1.7 |
| Qualification transition m/z 223→90 | | | |
| 0.01 (n=3) | 102 | 0.6 | - |
| 1.0 (n=3) | 91 | 2.3 |
| Leaves + Tops | Quantification transition m/z 223→126 | | | |
| 0.01 (n=5) | 101 | 4.0 | - |
| 1.0 (n=5) | 88 | 1.7 |
| Qualification transition m/z 223→90 | | | |
| 0.01 (n=5) | 102 | 4.5 | - |
| 1.0 (n=5) | 89 | 0.6 |

Table A 27: Characteristics of the analytical method validated for the determination of acetamiprid in sugar beet (whole plant, roots and leaves + tops)

|  | Acetamiprid |
| --- | --- |
| Specificity | A highly specific HPLC-MS/MS method was used for the determination of the target analyte. Two mass transitions (m/z 223→126, quantification and m/z 223→90, qualification) were monitored during each analysis.  Target analyte concentrations in controls did not exceed 30% of the method LOQ. No interference from co-eluting components at the retention time of the target analyte were observed. |
| Calibration (type, number of data points) | Sugar beet whole plants:  6-point linear calibration, 1/x weighing  y = 304418.90 x, r ≥ 0.9992  Sugar beet roots:  6-point linear calibration, 1/x weighing  y = 319887.37 x + 10783.39, r ≥ 0.9992  Sugar beet leaves + tops:  6-point linear calibration, 1/x weighing  y = 300091.73 x + 8939.63, r ≥ 0.9992 |
| Calibration range | 0.3 - 30 µg/L (corresponding to 0.003 - 0.3 mg/kg) |
| Assessment of matrix effects is presented | Matrix effects were assessed and no matrix effects > 20 % were observed for all whole plant and roots. Matrix effects > 20 % were observed for leaves + tops. Matrix-matched standards were used for all matrices. |
| Limit of determination/quantification | LOQ for all matrices: 0.01 mg/kg  LOD for all matrices: 0.003 mg/kg |
| Stability of standards | The stability of the stock and working solutions has been tested in separate study SDO-21-48624 (KCP 5.1.2/13), where analysis of stored stock and working solutions against freshly prepared standard solutions was performed. Stock and working solutions were found to be stable (difference to reference solution < 10%) for up to 145 when stored refrigerated. |
| Final extract stability | All sample extracts were analysed within 24 hours after extraction. An assessment of the stability of final extracts was not therefore required. |
| Storage stability | The maximum storage interval from sampling to analysis was 97 days for all specimens. The extracts were analysed within 24 hours of extraction. Storage stability data is not therefore required. |

Conclusion

An analytical method for the determination of acetamiprid in high water matrices was fully validated according to the requirements of the SANCO/3029/99 rev 4 guidelines prior to 1st March 2021 (see KCP 5.1.2/10). The additional reduced validation data provided in the current report meet the requirements of SANTE/2020/12830 rev 1 guidelines and also demonstrate that the method was functioning correctly when used in the magnitude of residues study. The method should therefore be considered suitable for its intended purpose and acceptable for the determination of acetamiprid in sugar beet.

* + - * 1. Analytical method SDO-21-48624

Method validation SDO-21-48624

|  |  |
| --- | --- |
| Comments of zRMS: | The method for the determination of acetamiprid was fully validated in STAPHYT study EGL-20-46374 (CIP phase ID 20S13018-01-RAPM) for matrix plum whole fruits belonging to the group of plant commodities with high water content. A reduced validation was carried out on cucumber fruit in this study according to guideline SANTE/2020/12830 rev.1.  Limit of quantification (LOQ) achieved was 0.01 mg/kg.  Acceptance criteria for method validations according to guideline SANTE/2020/12830 rev.1 were met. The method is acceptable. |

|  |  |
| --- | --- |
| Reference: | KCP 5.1.2/14 |
| Report | Magnitude of the residues of acetamiprid, after application of Acetamiprid 200 SL in indoor cucumber in Southern Europe – 2021, Domingo, S., 2022, Study No. SDO-21-48624, Sponsor No. 000108119. |
| Guideline(s): | Yes, SANCO 3029/99 rev. 4 and SANTE 2020/12830 rev. 1 |
| Deviations: | None |
| GLP: | Yes |
| Acceptability: | Yes |

The multi-residue QuEChERS-based analytical method used in the current study was fully validated for the determination of acetamiprid in plum (whole fruit, flesh) according to the requirements of SANCO 3029/99 rev. 4 guidelines in a previous study prior to 1st March 2021 (see KCP 5.1.2/17, study EGL-20-46374). Reduced validation data for the determination of acetamiprid in cucumber was provided according to the requirements of SANTE 2020/12830 rev. 1 in the current study and used to support a residue study conducted on cucumber.

This study was summarized solely in support of standard stability data of studies ChR-21-48246 and GBU-21-48185 (KCP 5.1.2/12 and KCP 5.1.2/15).

A. Materials

|  |  |
| --- | --- |
| 1. Standards |  |
| Test item: | ACETAMIPRID 200 SL |
| Batch No.: | 1242-050520-01 |
| Expiry date: | 26 February 2023 |
| Active ingredient: | Nominal: 200 g/L  Analysed: 203.4 ± 1.4 g/L |
| Reference item: | Acetamiprid |
| Lot/Batch number: | BCBT9185 |
| Purity: | 100 % |
| CAS No.: | 160430-64-8 |
| Expiry date: | 28 February 2022 |
| Standards for calibration | As above |
| Matrix: | Cucumber |

1. **Sample preparation and processing**

Following homogenisation, 10 g ± 0.1 g were weighed into a 50 mL centrifuge tube. 10 mL acetonitrile were added, and the samples were extracted by shaking for 15 min on a platform shaker at approximately 250 rpm. Thereafter, 1 g sodium citrate, 0.5 g sodium hydrogencitrate sesquihydrate, 4 g magnesium sulphate and 1 g sodium chloride were added, the mixture thoroughly shaken by hand and afterwards mixed on a vortex mixer for at least 1 min. The samples were centrifuged at 4000 rpm for at least 5 minutes and the supernatant transferred to a 15 mL centrifuge tube. 25 mg PSA, 150 mg anhydrous magnesia sulphate and 25 mg C18e was weighed into a 2 mL tube. 1 mL of supernatant was transferred into the tube containing the mixture of sorbents, shaken on a vortex mixer for 30 s and centrifuged for 5 min (12 000 rpm) afterwards. The purified extracts were diluted 1:10 (with ACN) into an autosampler vial (1.8 mL) and used directly for analysis by HPLC-MS/MS. If necessary, the final extracts were diluted further.

1. **Chromatographic parameters**

|  |  |
| --- | --- |
| HPLC: | Shimadzu LC-40 |
| Column: | Phenomenex Luna C18 (2) 100 A, 150 mm x 2.0 mm, 5.0 μm particle  size |
| Mobile phase: | A: water + 1% formic acid  B: acetonitrile |
| Flow rate: | 0.5 mL/min |
| Injection volume: | 10µl |
| MS/MS Parameters: | AB Sciex QTRAP 5500 |
| Mode: | ESI (electrospray ionisation) positive |
| Transitions monitored: | 223 → 126 m/z (quantification)  223 → 90 m/z (confirmation) |

Results and discussion

The analytical method used in the current study was fully validated for the determination of acetamiprid in plum (whole fruit and flesh) according to the requirements of SANCO 3029/99 rev. 4 guidelines in a previous study (See KCP 5.1.2/09). A reduced validation for the determination of acetamiprid in cucumber was conducted in the current study. The target analyte was determined using HPLC-MS/MS by monitoring two highly specific mass transitions. The detector response was linear over the range 0.3 - 30 µg/L (corresponding to 0.003 – 0.3 mg/kg for all matrices with associated correlation coefficients (r) ≥ 0.9986. Regression residuals were plotted and found to be randomly distributed demonstrating a linear calibration function was suitable for the quantitative determination of the target analyte. The LOQ of the method was 0.01 mg/kg. The stability of the target analyte in final extracts and working solutions was assessed. When stored at 8 °C in the dark, the target analyte was found to be stable in final extracts for at least 3 days and in working solutions for at least 145 days (refrigerated at -18 °C). All mean recovery values and associated RSDs meet the validation requirements of SANTE/2020/12830 rev. 1 guidelines and are summarised in the table below.

Table A 28: Method validation recovery data for the determination of acetamiprid in cucumber reported in study SDO-21-48624

| Matrix | Analyte | Fortification level (mg/kg) | Mean  recovery (%) | Overall RSD (%) | Comments |
| --- | --- | --- | --- | --- | --- |
| Cucumber | Acetamiprid | Quantification transition m/z 223→126 | | | |
| 0.01 (n=3) | 96 | 4.3 | - |
| 5.0 (n=3) | 100 | 2.5 |
| Qualification transition m/z 223→90 | | | |
| 0.01 (n=3) | 94 | 1.6 | - |
| 5.0 (n=3) | 101 | 4.1 |

Table A 29: Characteristics of the analytical method validated for the determination of acetamiprid in cucumber

|  | Acetamiprid |
| --- | --- |
| Specificity | A highly specific HPLC-MS/MS method was used for the determination of the target analyte. Two mass transitions (m/z 223→126, quantification and m/z 223→90, qualification) were monitored during each analysis.  Target analyte concentrations in controls did not exceed 30% of the method LOQ. No interference from co-eluting components at the retention time of the target analyte were observed. |
| Calibration (type, number of data points) | 6-point linear calibration  y = 266856.73 x – 27024.934 r ≥ 0. 9986 |
| Calibration range | 0.3 - 30 µg/L for all matrices (corresponds to 0.003 – 0.3 mg/kg) |
| Assessment of matrix effects is presented | Matrix effects were assessed and no matrix effects > 20 % were observed. Nevertheless, matrix-matched calibration standards were used. |
| Stability of target analyte in standard solutions | The stability of the analyte in stock and working solutions was examined by analysis of stored stock and working solutions against freshly prepared standard solutions. Stock and working solutions were found to be stable (difference to reference solution < 10%) for up to 145 days of refrigerated storage. |
| Stability of target analyte in final extracts | The sample extracts were analysed within 2 days after extraction. Stability of the analyte in the extracts was therefore assessed in a separate sequence afterwards by analysis of one matrix-matched standard solution stored for 3 days analysed against freshly prepared matrix-matched standards. As the stored standard solution differed less than 20% from the freshly prepared standards, the stability of the analyte in final extracts during refrigerated storage of up to 3 days was proven. |
| Limit of determination/quantification | LOQ for all matrices: 0.01 mg/kg  LOD for all matrices: 0.003 mg/kg |

Conclusion

An analytical method for the determination of acetamiprid in plum was fully validated according to the requirements of the SANCO/3029/99 rev 4 guidelines prior to 1st March 2021 (see KCP 5.1.2/17). The additional reduced validation data provided in the current report meet the requirements of SANTE/2020/12830 rev 1 guidelines and also demonstrate that the method was functioning correctly when used in the magnitude of residues study. The method should therefore be considered suitable for its intended purpose and acceptable for the determination of acetamiprid in cucumber.

* + - * 1. Analytical method EGL-20-46374

Method validation EGL-20-46374

|  |  |
| --- | --- |
| Comments of zRMS: | The analytical method for the determination of acetamiprid was fully validated for the aqueous matrix plum whole fruit in this study (CIP Phase ID: 20S13018-01-RAPM) and an additional reduced validation for plum flesh was also done during this study.  Limit of quantification (LOQ) achieved was 0.01 mg/kg for all matrices.  Acceptance criteria for method validations according to guideline SANTE/2020/12830 rev.1 were met. The method is acceptable. |

|  |  |
| --- | --- |
| Reference: | KCP 5.1.2/15 |
| Report | Magnitude of the residues of acetamiprid in plum (RAC fruits), following one application of ACETAMIPRID 200 SL in four trials (two HS + two DCS) - Southern Europe (Spain, Greece and Italy) – 2020. Grall, E. 2022, Study No. EGL-20-46374, Sponsor No. 000105973 |
| Guideline(s): | SANCO 3029/99 rev. 4 |
| Deviations: | None |
| GLP: | Yes |
| Acceptability: | Yes |

A multi-residue QuEChERS-based analytical method for the determination of acetamiprid residues in plum (whole fruit) was fully validated according to the requirements of SANCO/3029/99 rev. 4 prior to 1st March 2021 and used to support residue studies conducted in orchards, tomatoes, sweet pepper, melon and cucumber.

This study was summarized solely to provide method validation data in support of study SDO-21-48624 (KCP 5.1.2/16).

**A. Materials**

|  |  |
| --- | --- |
| 1. Standards |  |
| Reference item: | Acetamiprid |
| Lot/Batch number: | BCBT9185 |
| Purity: | 100 % |
| CAS No.: | 160430-64-8 |
| Expiry date: | 28 February 2022 |
| Standards for calibration | As above |
| Matrices: | 1: Plum whole fruit (i.e., plant commodities with high water content)  2: Plum flesh (i.e., plant commodities with high water content) |

1. **Sample preparation and processing**

*Plum whole fruit and flesh*

Following homogenisation, 10 g ± 0.1 g sample were weighed into a 50 mL centrifuge tube. 10 mL acetonitrile were added and the samples were extracted by shaking for 15 min on a platform shaker at approximately 250 rpm. Thereafter 1 g sodium citrate, 0.5 g sodium hydrogencitrate sesquihydrate, 4 g magnesium sulphate and 1 g sodium chloride were added, the mixture thoroughly shaken by hand and afterwards mixed on a vortex mixer for at least 1 min. The samples were centrifuged at 4000 rpm for at least 5 minutes and the supernatant transferred to a 15 mL centrifuge tube. 25 mg PSA, 150 mg anhydrous magnesia sulphate and 2.5 mg GCB was weighed into a 2 mL tube. 1 mL of supernatant was transferred into the tube containing the mixture of sorbents, shaken on a vortex mixer for 30 s and centrifuged for 5 min (12 000 rpm) afterwards. The purified extracts were diluted 1:10 (with ACN) into an autosampler vial (1.8 mL) and used directly for analysis by HPLC-MS/MS. If necessary, the final extracts were diluted further.

1. **Chromatographic parameters**

|  |  |
| --- | --- |
| HPLC- parameters: | Dionex Ultimate 3000 |
| Column: | Phenomenex Luna C18 (2) 100 A, 150 mm x 2.0 mm, 5.0 μm particle size |
| Mobile phase: | A: Water containing 1 % formic acid (v/v)  B: Acetonitrile |
| Flow rate: | 500 µL/min |
| Injection volume: | 10 µL |
| MS/MS Parameters:  Ionisation type:  Transitions monitored: | AB Sciex API 5500 QTRAP (Triple quadrupole mass spectrometer)  EI positive  m/z 223 → 126 (quantification)  m/z 223 → 90 (confirmation) |

**Results and discussion**

The analytical method used in the current study was fully validated for the determination of acetamiprid in plant commodities with high water content (RAC fruit) according to the requirements of SANCO 3029/99 rev. 4 guidelines. The target analyte was determined using HPLC-MS/MS by monitoring two highly specific mass transitions. The detector response was linear over the range 0.1 – 100.0 µg/L (corresponding to 0.001 – 1 mg/kg) with associated correlations coefficients r > 0.999). The LOQ of the method is 0.01 mg acetamiprid/kg for both matrices. All mean recovery values and associated RSDs for both matrices meet the minimum validation requirements of SANTE/2020/12830 rev.1 guidelines and are summarised in the table below.

**Table A 30: Method validation recovery data for the determination of acetamiprid in plum whole fruits and plum flesh reported in study EGL-20-46374**

| **Matrix** | **Analyte** | **Fortification level (mg/kg)** | **Mean  recovery (%)** | **RSD (%)** | **Comments** |
| --- | --- | --- | --- | --- | --- |
| Quantification transition m/z 223→126 | | | | | |
| Plum whole fruit  n = 5 | Acetamiprid | 0.01 | 93 | 14.9 | - |
| 5 | 93 | 4.7 |
| Confirmation transition m/z 223→90 | | | | | |
| Plum whole fruit  n = 5 | Acetamiprid | 0.01 | 93 | 13.3 | - |
| 5 | 93 | 5.7 |
| Quantification transition m/z 223→126 | | | | | |
| Plum flesh  n = 3 | Acetamiprid | 0.01 | 91 | 2.3 | - |
| 5 | 91 | 3.9 |
| Confirmation transition m/z 223→90 | | | | | |
| Plum flesh  n = 3 | Acetamiprid | 0.01 | 92 | 3.3 | - |
| 5 | 91 | 2.9 |

**Table A 31: Characteristics of the analytical method validated for the determination of acetamiprid in plums (whole fruit and flesh)**

|  | **Acetamiprid** |
| --- | --- |
| Specificity | A highly specific HPLC-MS/MS method was used for the determination of the target analyte. Two mass transitions (m/z 223→126, quantifiaction and m/z 223→90, qualification) were monitored during each analysis.  Target analyte concentrations in controls did not exceed 30% of the method LOQ. No interference from co-eluting components at the retention time of the target analyte was observed. |
| Calibration (type, number of data points) | Six-point linear calibration  Whole fruit: y = 45193.8 x, r = 0.99908  Flesh: y = 60410.6 x, r = 0.99990 |
| Calibration range | 0.1 – 100 µg/L (corresponding to 0.001 – 1 mg/kg) |
| Assessment of matrix effects is presented | Matrix effects were assessed and found to be insignificant (< ± 20%). Still, matrix-matched calibration was used. |
| Stability of target analyte in standard solutions | The stability of Acetamiprid in working solutions has been tested in study EGL-20-46375 (KCP 5.1.2/12). The stock solution of Acetamiprid (1000 mg/L) and the fortification solutions were found to be stable  for 208 days when prepared in methanol and stored refrigerated in the dark. |
| Stability of target analyte in final extracts | Not all sample extracts were analysed within 24 h after extraction of Acetamiprid. Therefore, stability testing in final extracts was performed. The matrix-matched calibration was prepared at the day of extraction and stored refrigerated along with the samples. On the day of analysis, one matrix-matched standard solution was freshly prepared and analysed against the stored one. The freshly prepared and stored standard solution differed by no more than 20%, therefore the stability of the analyte in the final extract of plum under refrigerated conditions for at least 28 days is proven. |
| Limit of determination/quantification | LOQ: 0.01 mg/kg (both matrices)  LOD: 0.003 mg/kg |

**Conclusion**

An analytical method for the determination of acetamiprid residues in plant commodities with high water content (RAC fruit; plum whole fruit and flesh) was fully validated according to SANCO/3029/99 guidelines prior to March 21st 2021. The data provided exceed the minimum validation requirements given in Section 4.2 of SANTE/2020/12830 Rev. 1. The method should therefore be considered suitable for its intended purpose and acceptable for the determination of acetamiprid in plant commodities with high water content.

* + - * 1. Analytical method DMC-13-16126

Method validation DMC-13-16126 & 13M06017-01-VMPL

|  |  |
| --- | --- |
| Comments of zRMS: | The study has been evaluated and accepted in Registration Report, Section 5 for CA3573 / Carnadine / Kestrel, Nufarm (August 2021).  Conclusions:  *The analytical method was derived from the QuEChERS multi-residue method and based on an extraction procedure with final analysis by HPLC with MS/MS detection.*  *The analytical method was fully validated according to guideline SANCO/3029/99.during a previous study performed in 2013 by CIP on head cabbage, apple fruits, potato tubers and peach fruits (Lang, A. 2014; CIP Study code : 13M06017-01-VMPL).*  *Therefore, in the current study only a procedural recovery was performed for the LOQ (level of quantification; 0.01 mg/kg) by fortifying control (untreated) specimen. A higher fortification level for residues of acetamiprid (5 mg/kg) was introduced and fully validated in the current study.*  *The samples of the Staphyt Studies DMC-13-16126 and DMC-13-16134 were analysed together and therefore, the results of the recoveries and the validation data will be reported in both studies.*  *The method achieves a limit of quantification (LOQ) of 0.01 mg/kg. Limit of detection (LOD) is 0.003 mg/kg.*  *Acceptance criteria for method validation were met, with average recoveries ranging from 70% to 110% and relative standard deviations ≤20%:*  *The study is acceptable.* |

|  |  |
| --- | --- |
| Reference: | KCP 5.1.2/16 |
| Report | Magnitude of the residues of acetamiprid in peaches (rac fruits), following two applications of mcw-2222 in three trials (1 dcs + 2 hs), southern europe (southern france and italy) – 2013. Méric, D., 2014, Study No. DMC-13-16126. Adama Report No. R-33596 |
| Guideline(s): | SANCO 3029/99 rev. 4 |
| Deviations: | None |
| GLP: | Yes |
| Acceptability: | Yes |

The multi-residue QuEChERS-based analytical method used in the current study was fully validated for the determination of acetamiprid in peach fruits according to the requirements of SANCO 3029/99 rev. 4 guidelines in a previous study prior to 1st March 2021 (Lang, A. 2014, Study No. 13M06017-01-VMPL, KCP 5.1.2/06). A higher fortification level (5.0 mg/kg) was validated in peach fruits according to the requirements of SANCO 3029/99 rev. 4 guidelines in the current study. The analytical method was used to support several residue studies.

**A. Materials**

|  |  |
| --- | --- |
| 1. Standards |  |
| Reference item: | Acetamiprid |
| Lot/Batch number: | 20203 |
| Purity: | 99.0 % |
| CAS No.: | 135410-20-7 |
| Expiry date: | 02 February 2016 |
| Standards for calibration | As above |
| Matrix: | Peach (fruits) |

1. **Sample preparation and processing**

10 g of each sample were weighed into 50 mL centrifuge tubes. Recovery samples were fortified at this stage. 10 mL of acetonitrile were added and the samples were extracted applying homogenisation for at least 2 min at high speed. 4.0 g of magnesium sulphate, 1.0 g of sodium chloride, 1.0 g of sodium citrate and 0.50 g of sodium hydrogencitrate sesquihydrate were added. The tube was shaken by hand and mixed on a mixer for at least 1 min. The samples were then centrifuged at 3500 min-1 for at least 10 min. An aliquot of 1 mL of the supernatant was transferred into a 2 mL tube containing 25 mg of PSA and 150 mg anhydrous magnesium sulphate and 2.5 mg of GCB. The tube was shaken on a mixer for 30 seconds and the extract was filtered through a single-use syringe filter (0.45 μm) into a vial. The final extracts were diluted 1:10 (100 μL sample + 900 μL ACN) and analysed by HPLC-MS/MS.

1. **Chromatographic parameters**

|  |  |
| --- | --- |
| HPLC- parameters: | Dionex Ultimate 3000 |
| Column: | Phenomenex Luna C18 (2), 100A 150 mm × 2.0 mm, 5.0 μm |
| Mobile phase: | A: Water/MeOH (90/10; v/v) +0.1% Formic acid + 5 mmol Ammonium formate  D: MeOH/Water (90/10; v/v) +0.1% Formic acid + 5 mmol Ammonium formate |
| Flow rate: | 300 μL/min |
| Injection volume: | 10 µL |
| MS/MS Parameters:  Ionisation type:  Transitions monitored: | AB Sciex API 5500 QTRAP  ESI positive  m/z 223→126 (quantification)  m/z 223→90 (confirmation) |

**Results and discussion**

The analytical method used in the current study was fully validated for the determination of acetamiprid in peach fruits according to the requirements of SANCO 3029/99 rev. 4 guidelines in a previous study prior to 1st March 2021 (see KCP 5.1.2/02). A higher fortification level (5.0 mg/kg) was validated in peach fruits according to the requirements of SANCO 3029/99 rev. 4 guidelines in the current study. The target analyte was determined using HPLC-MS/MS by monitoring two highly specific mass transitions. The detector response was linear over the range 0.25 μg/L to 20 μg/L with associated correlation coefficient (r) ≥ 0.9996. The LOQ of the method is 0.01 mg/kg. Mean recovery values and associated RSDs meet the requirements of SANCO/3029/99 rev. 4 and the minimum validation requirements given in Section 4.2 of SANTE/2020/12830 rev.1 guidelines and are summarised in the table below.

**Table A 32: Method validation recovery data for the determination of acetamiprid in peach fruits reported in study DMC-13-16126**

| **Matrix** | **Analyte** | | **Fortification level**  **(mg/kg)** | **Recovery values (%)** | **Mean  recovery (%)** | **RSD (%)** | **Comments** |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | | **m/z 223→126 (quantification)** | | | | | |
| Peach (fruits) | Acetamiprid | | 0.01 (n=2) | 99, 95 | - | - | - |
| 5.0 (n=6) | 99, 96, 91, 93, 100, 91 | 95 | 4.2 |
|  | | **m/z 223→90 (confirmation)** | | | | | |
| Peach (fruits) | Acetamiprid | | 0.01 (n=2) | 98, 95 | - | - | - |
| 5.0 (n=6) | 97, 95, 89, 94, 100, 91 | 94 | 4.2 |

**Table A 33: Characteristics of the analytical method validated for the determination of acetamiprid in peach fruits**

|  | **Acetamiprid** |
| --- | --- |
| Specificity | A highly specific HPLC-MS/MS method was used for the determination of the target analyte. Two mass transitions (m/z 223→126, quantification and m/z 223→90, qualification) were monitored during each analysis.  Target analyte concentrations in controls did not exceed 30% of the method LOQ. No interference from co-eluting components at the retention time of the target analyte. |
| Calibration (type, number of data points) | Seven-point linear calibration    Quantification transistion: y = 141683x, R2 = 0.9992  Confirmation transition: y = 46343x, R2 = 0.9994 |
| Calibration range | 0.25 μg/L - 20 μg/L |
| Assessment of matrix effects is presented | Matrix effects were assessed and found to be insignificant (< ± 20%) for all matrices. Nevertheless, matrix-matched calibration was used throughout the study. |
| Limit of determination/quantification | LOQ: 0.01 mg/kg  LOD: 0.003 mg/kg |

**Conclusion**

An analytical method for the determination of acetamiprid in peach fruits was fully validated according to the requirements of SANCO 3029/99 rev. 4 guidelines in a previous study prior to 1st March 2021 (see KCP 5.1.2/02). A higher fortification level (5.0 mg/kg) was validated in peach fruits according to the requirements of SANCO 3029/99 rev. 4 guidelines in the current study. The data provided exceed the minimum validation requirements given in Section 4.2 of SANTE/2020/12830 Rev. 1. The method should therefore be considered suitable for its intended purpose and acceptable for the determination of acetamiprid in peach fruits.

* + - * 1. Analytical method 21A14030-01-VMHN

Method validation 21A14030-01-VMHN

|  |  |
| --- | --- |
| Comments of zRMS: | The analytical method for the determination of acetamiprid was fully validated in samples of matrix honey according to SANTE/2020/12830 with a limit of quantification of 0.01 mg/kg using HPLC-MS/MS for quantification and confirmation.  Acceptance criteria for method validations according to guideline SANTE/2020/12830 rev.1 were met. The method is acceptable |

|  |  |
| --- | --- |
| Reference: | KCP 5.1.2/17 |
| Report | Validation of an Analytical Method for the Determination of Residues of  Acetamiprid in Honey - Schrag, K. 2022, Report No. 21A14030-01-VMHN, Sponsor Reference No. 000107274 |
| Guideline(s): | SANTE/2020/12830 rev.1 |
| Deviations: | None |
| GLP: | Yes |
| Acceptability: | Yes |

A multi-residue QuEChERS-based analytical method for the determination of acetamiprid residues in honey was fully validated according to the requirements of SANTE/2020/12830 rev.1 and used in support of a storage stability study.

**A. Materials**

|  |  |
| --- | --- |
| 1. Standards |  |
| Reference item: | Acetamiprid |
| Lot/Batch number: | BCBT9185 |
| Purity: | 100 % |
| CAS No.: | 160430-64-8 |
| Expiry date: | 28 February 2022 |
| Standards for calibration | As above |
| Matrix: | Honey |

1. **Sample preparation and processing**

Following homogenisation, 5 g were weighed into a 50 mL centrifuge tube. 10.0 mL water and 10 mL acetonitrile were added and the tubes were homogenised for at least 2 min using a vortex mixer. 1 g sodium citrate, 0.5 g sodium hydrogencitrate sequihydrate, 4 g magnesium sulphate, 1 g sodium chloride were added, thoroughly shaken and mixed again on a vortex mixer for at least 1 minute and then centrifuged at 4000 min-1 for at least 5 minutes. The supernatant was transferred into a 15 mL centrifuge tube.

An aliquot of 1 mL of the supernatant was transferred into a tube (2 mL) prepared with 25 mg PSA

and 150 mg anhydrous magnesia sulphate and 25 mg C18e, shaken on a vortex mixer for 30 s, centrifuged for 5 min (12 000 rpm) and transferred into an autosampler vial of 1.8 mL. The final extracts were diluted 1:10 with acetonitrile into an autosampler vial (1.8 mL) and directly analysed by LC-MS/MS. Eventually final extracts would be further diluted in final extract of the matrix, if necessary to obtain a concentration falling within the linear range of the calibration curve.

1. **Chromatographic parameters**

|  |  |
| --- | --- |
| HPLC- parameters: | Shimadzu LC-40 |
| Column: | Phenomenex Luna C18 (2) 100 A, 150 mm x 2.0 mm, 5.0 μm |
| Mobile phase: | A: Water containing 1 % formic acid (v/v)  B: Acetonitrile |
| Flow rate: | 0.5 mL/min |
| Injection volume: | 10 µL |
| MS/MS Parameters:  Ionisation type:  Transitions monitored: | AB Sciex QTRAP 5500  ESI positive  m/z 223→126 (quantification)  m/z 223→90 (confirmation) |

**Results and discussion**

The analytical method used in the current study was fully validated for the determination of acetamiprid in honey according to the requirements of SANTE/2020/12830 rev.1. guidelines. The target analyte was determined using HPLC-MS/MS by monitoring two highly specific mass transitions. The detector response was linear over the range 0.15 - 100 μg/L (corresponding to 0.003 mg/kg to 2 mg/kg) with associated correlation coefficient (r) ≥ 0.9959. The LOQ of the method is 0.01 mg/kg. All mean recovery values and associated RSDs meet the requirements of SANTE/2020/12830 rev.1 guidelines and are summarised in the table below.

**Table A 34: Method validation recovery data for the determination of acetamiprid in honey reported in study 21A14030-01-VMHN.**

| **Matrix** | **Analyte** | **Fortification level (mg/kg) (n=5)** | **Mean  recovery (%)** | **RSD (%)** | **Comments** |
| --- | --- | --- | --- | --- | --- |
| Quantification transition m/z 223→126 | | | | | |
| Honey | Acetamiprid | 0.01 | 98 | 1.5 | - |
| 0.1 | 97 | 0.9 |
| Confirmation transition m/z 223→90 | | | | | |
| Honey | Acetamiprid | 0.01 | 97 | 3.0 | - |
| 0.1 | 96 | 1.5 |

**Table A 35: Characteristics of the analytical method validated for the determination of acetamiprid in honey**

|  | **Acetamiprid** |
| --- | --- |
| Specificity | A highly specific HPLC-MS/MS method was used for the determination of the target analyte. Two mass transitions (m/z 223→126, quantifiaction and m/z 223→90, qualification) were monitored during each analysis.  Target analyte concentrations in controls did not exceed 30% of the method LOQ. No interference from co-eluting components at the retention time of the target analyte. |
| Calibration (type, number of data points) | Eight-point matrix-matched linear calibration    Calibration range 0.15 - 15 μg/L:  quantifier mass transition: y = 659027.76x + 0.00, r ≥ 0.9959  qualifier mass transition: y = 194316.75x - 0.00, r ≥ 0.9959    Calibration range 5 – 100 μg/L:  quantifier mass transition: y= 580319.83x -0.00, r ≥ 0.9959  qualifier mass transition: y = 179691.75x - 0.00, r ≥ 0.9959 |
| Calibration range | 0.15 - 100 μg/L (corresponding to 0.003 mg/kg to 2 mg/kg) |
| Assessment of matrix effects is presented | Matrix effects were assessed and found to be insignificant (< ± 20%) for honey. Nevertheless, matrix matched calibrations were used for target analyte quantification. |
| Limit of determination/quantification | LOQ: 0.01 mg/kg  LOD: 0.003 mg/kg |
| Stability of the target analyte in standards | The stability of the analyte in stock and working solutions was examined by comparison of stored stock and working solutions against freshly prepared standard solutions. Stock and working solutions were found to be stable, as the difference in the means from six (6) replicates (for each solution tested) to the reference solution was below 10% for up to 145 days of refrigerated storage. |
| Stability of the target analyte in final extracts | All sample extracts were analysed within 24 hours after extraction, therefore the stability of the analyte is sufficiently proven. |
| Storage stability | A storage stability experiment was conducted in study GBU-21-48185 (see KCP 5.1.2/15) and demonstrated that acetamiprid residues in freshly fortified samples at day 7 were within 70-120% and residues in the control sample were < 30% of the LOQ. Therefore, acetamiprid can be regarded as stable for up to 7 days after storage at 30°C |

**Conclusion**

An analytical method for the determination of acetamiprid residues in honey was fully validated according to SANTE/2020/12830 rev.1. guidelines. The method was sufficiently accurate and precise to be able to provide reliable data on target analyte concentrations in honey and should therefore be considered acceptable.

* + - * 1. Analytical method GBU-21-48185

Method validation GBU-21-48185

|  |  |
| --- | --- |
| Comments of zRMS: | The method for the determination of acetamiprid in honey was fully validated in this study, together with ADAMA study CIP Study Code 21A14030-01-VMHN according to SANTE/2020/12830 rev.1 in matrix honey.  LOQ was 0.01 mg/kg for acetamiprid  Acceptance criteria for method validations according to guideline SANTE/2020/12830 rev.1 were met. The method is acceptable |

|  |  |
| --- | --- |
| Reference: | KCP 5.1.2/18 |
| Report | Magnitude of the residues of acetamiprid after application of ACETAMIPRID 200 SL in honey of phacelia in Northern and Southern Europe – 2021-2022. Boileau, G., 2022, Study No. GBU-21-48185, Sponsor No. 000107273. |
| Guideline(s): | Yes, SANTE/2020/12830 rev. 1 |
| Deviations: | None |
| GLP: | Yes |
| Acceptability: | Yes |

A multi-residue QuEChERS-based analytical method for the determination of acetamiprid in honey was validated and used to support a magnitude of residues study. The method was fully validated according to the requirements of SANTE/2020/12830 rev. 1 Guidelines.

Method validation took place together with the validation in matrix honey of study 21A14030-01-VMHN (see KCP 5.1.2/12).

A. Materials

|  |  |  |
| --- | --- | --- |
| Reference item 1: | |  |
| Lot/Batch number: | | BCCG5809 |
| Purity: | | 99.9 % |
| CAS No.: | | 160430-64-8 |
| Expiry date: | | 31 August 2026 |
| Reference item 2: | |  |
| Lot/Batch number: | | BCBT9185 |
| Purity: | | 100 % |
| CAS No.: | | 160430-64-8 |
| Expiry date: | | 28 February 2022 |
| Standards for calibration | As above | | |

1. **Sample preparation and processing**

5 g (+/- 0.05 g) of honey samples were weighed into a 50 mL centrifugation tube and diluted with 10 mL of water. Recovery samples were fortified at this step. Then, 10 mL acetonitrile were added and the samples were homogenised for at least 2 minutes using a vortex mixer.

QuEChERS alt mixture was added, shaken and mixed again on a vortex mixer for at least 1 minute. The samples were centrifuged at 4000 min-1 for at least 5 minutes. The supernatant was transferred

into a 15 mL centrifuge tube. Final extracts were diluted 1:10 (with acetonitrile) and directly analysed by HPLC MS/MS.

1. **Chromatographic parameters**

|  |  |
| --- | --- |
| HPLC: Shimadzu LC-40 |  |
| Column: Phenomenex Luna C18 (2) 100 A, 150 mm x 2.0 mm, 5.0 μm |  |
| Mobile phase: A: water + 1% formic acid  B: acetonitrile |  |
| Flow rate: 0.5 mL/min |  |
| Injection volume: 10 μL |  |
| MS/MS Parameters: |  |
| Mode: ESI Positive Turbo Spray |  |
| Transitions monitored: 223 → 126 m/z (quantification)  223 → 90 m/z (confirmation) |  |

Results and discussion

A multi-residue QuEChERS-based analytical method was fully validated for the determination of acetamiprid in honey according to guidelines SANTE/2020/12830 rev.1 in support of a residue study. The target analyte was determined using HPLC-MS/MS by monitoring two highly specific mass transitions. The detector response was split into two concentration ranges of 0.15 - 15 μg/L and 5 – 100 μg/L (corresponding to 0.003 mg/kg to 2 mg/kg) which were found to be linear and with associated correlation coefficients (r) ≥ 0.9958. The LOQ of the method was 0.01 mg/kg. All mean recovery values and associated RSDs meet the validation requirements of SANTE/2020/12830 rev.1 and are summarised in the table below.

Table 36: Method validation recovery data for the determination of acetamiprid in honey reported in study GBU-21-48185

| Matrix | Analyte | Fortification level (mg/kg) | Mean  recovery (%) | RSD (%) | Comments |
| --- | --- | --- | --- | --- | --- |
| Honey | Acetamiprid | Quantification transition m/z 223→126 | | | |
| 0.01 (n=5) | 98 | 1.5 | - |
| 1 (n=5) | 97 | 0.9 |
| Qualification transition m/z 223→90 | | | |
| 0.01 (n=5) | 97 | 3.0 | - |
| 1 (n=5) | 96 | 1.5 |

Table 37: Characteristics of the analytical method validated for the determination of acetamiprid in honey

|  | Acetamiprid |
| --- | --- |
| Specificity | A highly specific HPLC-MS/MS method was used for the determination of the target analyte. Two mass transitions (m/z 223→126, quantification and m/z 223→90, qualification) were monitored during each analysis.  Target analyte concentrations in controls did not exceed 30% of the method LOQ. No interference from co-eluting components at the retention time of the target analyte were observed. |
| Calibration (type, number of data points) | Regression residuals were plotted and found to be randomly distributed. Accordingly, a linear calibration function was considered suitable for quantitative determination of the target analyte. 5 point linear matrix-matched calibration was used for target analyte quantification and is presented in the study.  Equation of the calibration curves are:  Quantification transition: y= 659027.76x , r ≥ 0.9958  Confirmation transition : y= 194316.75x , r ≥ 0.9958 |
| Calibration range | 0.15 - 15 μg/L and 5 – 100 μg/L (corresponding to 0.003 mg/kg to 2 mg/kg) |
| Determination of matrix effects | Matrix effects were tested and were determined as insignificant (< 20%). Nevertheless, matrix-matched calibration was used for the quantification of acetamiprid. |
| Limit of determination/quantification | LOQ for all matrices: 0.01 mg/kg  LOD for all matrices: 0.003 mg/kg |
| Stability of the target analyte in standards | The stability of the stock and working solutions has been tested in separate study SDO-21-48624 (KCP 5.1.2/13), where analysis of stored stock and working solutions against freshly prepared standard solutions was performed. Stock and working solutions were found to be stable (difference to reference solution < 10%) for up to 145 when stored refrigerated. |
| Stability of the target analyte in final extracts | Final extracts were analysed within 24 hours. An assessment of the stability of final extracts was not therefore required. |
| Storage stability | A storage stability experiment was conducted and demonstrated that acetamiprid residues in freshly fortified samples at day 7 were within 70-120% and residues in the control sample were < 30% of the LOQ. Therefore, acetamiprid can be regarded as stable for up to 7 days after storage at 30°C. |

Conclusion

The analytical method was fully validated according to SANTE/2020/12830 rev. 1 Guidelines. The method was sufficiently accurate and precise to be able to provide reliable data on target analyte concentrations in honey and should therefore be considered reliable.

* + - * 1. Analytical method 12/445-004P

Method validation 12/445-004P

|  |  |
| --- | --- |
| Comments of zRMS: | The method is acceptable and fit for purpose. |

|  |  |
| --- | --- |
| Reference: | KCP 5.1.2/19 |
| Report | ACETAMIPRID 200 SL - Acute Inhalation Toxicity Study (Nose-only) in the Rat. xxxxx 2013. Report No. 12/445-004P, Sponsor No. R-31125 |
| Guideline(s): | No analytical Guidelines |
| Deviations: | n.a |
| GLP: | Yes |
| Acceptability: | Yes |

The method used to provide dose verification in this study is based on gravimetric analysis. According to OECD Guidelines 403, Section 21, the gravimetric analytical method used for dose verification in this study is fully acceptable. Gravimetric methods are based solely on the accurate determination of the weight of filter papers. As such, method validation data are not obtained and no requirement for validation data from gravimetric methods are given in either the older SANCO guidelines or the current SANTE/2020/12830 rev. 1 guidelines. This has no influence on the outcome of the corresponding toxicology study and a full summary of the toxicological study along with gravimetrically-obtained dose verification data are provided in Part B6, under KCP 7.1.3/01.

* + - * 1. Analytical method ACI16-010

Method validation ACI16-010

|  |  |
| --- | --- |
| Comments of zRMS: | The study has been evaluated and accepted in Registration Report, Section 5 for CA3573 / Carnadine / Kestrel, Nufarm (August 2021).  Conclusions:  *A method of analysis for the determination of acetamiprid in dislodging solution was validated according to the guidance document SANCO/3029/99 rev. 4. All criteria are fulfilled:*  *- blank values do not exceed 30% of the lowest validated concentration,*  *- the mean recoveries for each level are in the range 70-110%,*  *- the RSD is < 20% per level.*  *The limit of quantitation (LOQ) was 0.2 μg/L and the limit of detection (LOD) was 0.02 μg/L.*  *The study is acceptable.* |

|  |  |
| --- | --- |
| Reference: | KCP 5.1.2/20 |
| Report | Foliar dislodgeable residues dissipation on pome fruit in Southern and Northern Europe (Spain, Italy and Czech Republic), 2016. Wilson, A., 2016 Report No. ACI16-010, Sponsor No. R-37353 |
| Guideline(s): | Yes, SANCO 3029/99 rev. 4 |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes |

An analytical method was fully validated in the current study for the determination of acetamiprid in DFR leaf wash solutions of pome fruits according to the requirements of SANCO 3029/99 rev. 4 guidelines prior to 1st March 2021 and used to support an operator exposure study.

A. Materials

|  |  |
| --- | --- |
| 1. Standards |  |
| Test item: | MCW-2222 |
| Batch no. | 611-280413-01 |
| Active substance: | Nominal: 200 g/L  Analysed: 205.1 g/L |
| Expiry date: | May 2016 |
| Standards for calibration | As above |
| Matrix: | DFR leaf wash solution (pome fruit) |

1. **Sample preparation and processing**

10 mL of the sample of the dislodge solution were measured into a scintillation vial (20 mL) and fortified respectively. Afterwards 10 mL of a methanol-formic acid (100:0.2, v: v) was added and shaken. An aliquot of the sample was transferred to a suitable vial prior quantitation by liquid chromatography using tandem mass spectrometric detection (LC-MS/MS).

1. **Chromatographic parameters**

|  |  |
| --- | --- |
| HPLC- parameters | Waters Acquity TQD |
| Column: | C18 (50 x 2.0 mm, 1.7 µm) |
| Mobile phase: | A: Water: methanol: formic acid (90:10:0.1 v:v:v) containing 0.01 M ammonium formate  B: Methanol: formic acid (100:0.1 v:v) |
| Flow rate: | 0.4 mL/min |
| Injection volume: | 20 µL |
| MS/MS Parameters |  |
| Ionisation type: | ESP (electrospray ionisation) positive |
| Transitions monitored: | m/z 223→126 (quantification)  m/z 223→56 (confirmation) |

Results and discussions

The analytical method used in the current study was fully validated for the determination of residues of acetamiprid in pome fruit according to the requirements of SANCO/3029/99 rev. 4 guidelines. The target analyte was determined using HPLC-MS/MS by monitoring two highly specific mass transitions. The detector response was linear over the range 0.01 - 1.0 ng/L with associated coefficients of correlation (r) ≥ 0.999. The LOQ of the method is 0.2 µg/L. All mean recovery values and associated RSDs meet the requirements of SANCO/3029/99 rev. 4 and SANTE/2020/12830 rev.1 guidelines and are summarised in the table below.

Table A 38: Method validation recovery data for the determination of acetamiprid in DFR pome fruit leaves solution report in study ACI16-010

| Matrix | Analyte | Fortification level (µg/L) | Mean  recovery (%)  n=5 | RSD (%) | Comments |
| --- | --- | --- | --- | --- | --- |
| Quantification transition m/z 223→126 | | | | | |
| DFR leaf wash | Acetamiprid | 0.2 | 94 | 3 | - |
| 1000 | 87 | 5.5 |
| **Confirmation transition m/z 223→56** | | | | | |
| DFR leaf wash | Acetamiprid | 0.2 | 91 | 5.1 | - |
| 1000 | 83 | 5.3 |

Table A 39: Characteristics of the analytical method used for the determination of acetamiprid in DFR pome fruit leaves solution

|  | Acetamiprid |
| --- | --- |
| Specificity | A highly specific HPLC-MS/MS method was used for the determination of the target analyte. Two mass transitions (m/z 223→126, quantification and m/z 223→90, qualification) were monitored during each analysis.  Target analyte concentrations in controls did not exceed 30% of the method LOQ. No interference from co-eluting components at the retention time of the target analyte. |
| Calibration (type, number of data points) | Nine-point-linear calibration  Equation 1 (quantification transition): y= 38511.8x + 73.2054, r= 0.9998  Equation 2 (confirmation transition): y= 21769.7x + 60.3831, r= 0.9995 |
| Calibration range | 0.01 - 1.0 ng/L |
| Assessment of matrix effects is presented | Since all mean recovery values and associated RSDs meet the requirements of SANCO/3029/99 rev. 4 and the minimum validation requirements of the current SANTE/2020/12830 rev.1 guidelines, matrix effect is being considered insignificant. |
| Limit of determination/quantification | LOQ: 0.2 µg/L  LOD: 0.01 ng/mL (equivalent to 0.02 µg/L in dislodge solution) |

Conclusion

An analytical method for the determination of acetamiprid in DFR pome fruit leaves solutions was fully validated according to the requirements of the SANCO/3029/99 rev 4 guidelines prior to 1st March 2021. The data provided exceed the minimum validation requirements given in Section 4.2 of SANTE/2020/12830 Rev. 1. The method should therefore be considered suitable for its intended purpose and acceptable for the determination of acetamiprid in DFR pome fruit leaves solutions.

* + - * 1. Analytical method R2040056

Method validation R2040056

|  |  |
| --- | --- |
| Comments of zRMS: | The analytical method for the determination of acetamiprid was fully validated for the high water content matrix grass (Henkes, K. 2017, Study No. R1640039) and an additional reduced validation for wheat plants and pea plants.  The LOQ for acetamiprid was 0.01 mg/kg.  Acceptance criteria for method validations according to guideline SANTE/2020/12830 rev.1 were met. The method is acceptable. |

|  |  |
| --- | --- |
| Reference: | KCP 5.1.2/21 |
| Report | Residues of acetamiprid in monocotyledonous and dicotyledonous plants after spray application of Acetamiprid 200 SL in early vegetation stages in Spain – magnitude of residues and time course of residue decline. Staffel, J. 2021, Study No. R2040056. Adama Reference No.000106551. |
| Guideline(s): | SANCO 3029/99 rev. 4 |
| Deviations: | None |
| GLP: | Yes |
| Acceptability: | Yes |

The multi-residue QuEChERS-based analytical method used in the current study was fully validated for the determination of acetamiprid in plant material with high water content (ground vegetation) according to the requirements of SANCO 3029/99 rev. 4 guidelines in a previous study prior to 1st March 2021 (Henkes, K. 2017, Study No. R1640039. KCP 5.1.2/03). Concurrent validation data for the determination of acetamiprid in early growth stage wheat and pea plants (high water plant matrices) was provided according to the requirements of SANTE/2020/12830 rev. 1 in the current study.

**A. Materials**

|  |  |
| --- | --- |
| 1. Standards |  |
| Reference item: | Acetamiprid |
| Lot/Batch number: | G1050293 |
| Purity: | 99.75 % |
| CAS No.: | 135410-20-7 |
| Expiry date: | 03 February 2024 |
| Standards for calibration | As above |
| Matrix: | Plant material with high water content (early growth stage wheat and pea plants) |

1. **Sample preparation and processing**

For each sample type, 5.0 ± 0.05g were weighed into a 50 mL centrifuge tube. 6.0 mL water and 10 mL acetonitrile were added and the tubes were shaken be hand for 1 min and then on a mechanical shaker for 15 min. 4.0 g of magnesium sulfate, 1.0 g of sodium chloride, 1.0 g of trisodium citrate dihydrate and 0.5 g of disodium hydrogen citrate sesquihydrate were added. The tube was capped, shaken by hand for one (1) minute and then centrifuged at 3200 × *g* for 5 min. 40 mg of PSA and 225 mg of magnesium sulphate was weighed into a 2-mL safe-lock tube. An aliquot of 1.5 mL of the supernatant was transferred into the tube containing the mixture of sorbents. The tube was intensively shaken by hand, vortexed for 30 and then centrifuged for 2 min at 3200 × g. 0.16 mL of the purified extract, 3.84 mL acetonitrile were made up to 10 mL with water containing 0.1% (v/v) formic acid. The final extract was well mixed and an aliquot transferred to an HPLC vial ready for analysis by LC-MS/MS.

1. **Chromatographic parameters**

|  |  |
| --- | --- |
| HPLC- parameters: | 1200 Infinity Binary LC System, Agilent Technologies |
| Column: | Phenomenex Luna C8, 150 mm × 2.0 mm, 5 µm, 100A |
| Mobile phase: | A: Acetonitrile containing 0.1 % formic acid (v/v)  B: Water containing 0.1 % formic acid (v/v) |
| Flow rate: | 800 µL/min |
| Injection volume: | 25 µL µL |
| MS/MS Parameters:  Ionisation type:  Transitions monitored: | API 5000 System, SCIEX (Triple quadrupole mass spectrometer)  EI positive  m/z 223→126 (quantification)  m/z 223→90 (confirmation) |

**Results and discussion**

The analytical method used in the current study was fully validated for the determination of acetamiprid in ground vegetation with high water content according to the requirements of SANCO 3029/99 rev. 4 guidelines in a previous study (See KCP 5.1.2/03). A concurrent validation for the determination of acetamiprid in early growth stage pea and wheat plants (high water plant matrices) was conducted in the current study. The target analyte was determined using HPLC-MS/MS by monitoring two highly specific mass transitions. The detector response was linear over the range 0.020 – 1.6 ng/mL (corresponding to 0.0025 – 0.20 mg/kg) with associated correlations coefficients (r2) > 0.99. The LOQ of the method is 0.01 mg acetamiprid/kg for both matrices. All mean recovery values and associated RSDs for both matrices meet the requirements of SANTE/2020/12830 rev.1 guidelines and are summarised in the table below.

**Table A 40: Method validation recovery data for the determination of acetamiprid in early growth stage wheat and pea plants reported in study R2040056.**

| **Matrix** | **Analyte** | **Fortification level (mg/kg)** | **Mean  recovery (%)**  **n=3** | **RSD (%)** | **Comments** |
| --- | --- | --- | --- | --- | --- |
| Quantification transition m/z 223→126 | | | | | |
| Wheat plant | Acetamiprid | 0.01 | 98 | 1.8 | - |
| 0.1 | 102 | 2.2 |
| Confirmation transition m/z 223→90 | | | | | |
| Wheat plant | Acetamiprid | 0.01 | 102 | 2.8 | - |
| 0.1 | 103 | 3.3 |
| Quantification transition m/z 223→126 | | | | | |
| Pea plant | Acetamiprid | 0.01 | 104 | 2.3 | - |
| 0.1 | 109 | 3.8 |
| Confirmation transition m/z 223→90 | | | | | |
| Pea plant | Acetamiprid | 0.01 | 104 | 1.4 | - |
| 0.1 | 108 | 3.3 |

**Table A 41: Characteristics of the analytical method validated for the determination of acetamiprid in early growth stage wheat and pea plants**

|  | **Acetamiprid** |
| --- | --- |
| Specificity | A highly specific HPLC-MS/MS method was used for the determination of the target analyte. Two mass transitions (m/z 223→126, quantification and m/z 223→90, qualification) were monitored during each analysis.  Target analyte concentrations in controls did not exceed 30% of the method LOQ. No interference from co-eluting components at the retention time of the target analyte. |
| Calibration (type, number of data points) | Eight-point linear calibration  Wheat: y = 1467808.056x + 2208.3240, r2= 0.9962  Pea: y = 698727.7137 + 3007.9439, r2= 0.9987 |
| Calibration range | 0.020 – 1.6 ng/mL corresponding to 0.0025 – 0.20 mg/kg |
| Assessment of matrix effects is presented | Matrix effects were assessed and found to be significant for wheat plants (≥ ± 20%) but insignificant (< ± 20%) for pea plants. Nevertheless, matrix-matched calibration was used for the quantitiative determination of the target analyte in both matrix types. |
| Limit of determination/quantification | LOQ both matrices: 0.01 mg/kg  LOD both matrices: 0.003 mg/kg |

**Conclusion**

An analytical method for the determination of acetamiprid in plant material with high water content (ground vegetation) was fully validated according to the requirements of the SANCO/3029/99 rev 4 guidelines prior to 1st March 2021 (see KCP 5.1.2/03). The validation data provided in the current report represent a concurrent validation (n =3 at each of two fortification levels) of the method for two additional plant matrices with high water content (early growth stage wheat and pea plants). The additional concurrent validation data provided in the current report meet the requirements of SANTE/2020/12830 rev 1 guidelines and demonstrate that the method was functioning correctly when used. The method should therefore be considered suitable for its intended purpose and acceptable for the determination of acetamiprid in early growth stage wheat and pea plants.

* + - * 1. Analytical method R2040057

Method validation R2040057

|  |  |
| --- | --- |
| Comments of zRMS: | The analytical method for the determination of acetamiprid was fully validated for the high water content matrix grass (Henkes, K. 2017, Study No. R1640039) and an additional reduced validation for wheat plants and pea plants.  The LOQ for acetamiprid was 0.01 mg/kg in both, wheat and pea plants..  Acceptance criteria for method validations according to guideline SANTE/2020/12830 rev.1 were met. The method is acceptable. |

|  |  |
| --- | --- |
| Reference: | KCP 5.1.2/22 |
| Report | Residues of acetamiprid in monocotyledonous and dicotyledonous plants after spray application of Acetamiprid 200 SL in early vegetation stages in Germany – magnitude of residues and time course of residue decline. Staffel, J. 2021, Study No. R2040057. Adama Reference No. 000106552. |
| Guideline(s): | SANCO 3029/99 rev. 4 and SANTE/2020/12830 rev.1 |
| Deviations: | None |
| GLP: | Yes |
| Acceptability: | Yes |

The multi-residue QuEChERS-based analytical method used in the current study was fully validated for the determination of acetamiprid in plant material with high water content (ground vegetation) according to the requirements of SANCO 3029/99 rev. 4 guidelines in a previous study prior to 1st March 2020 (Henkes, K. 2017, Study No. R1640039. KCP 5.1.2/03). Concurrent validation data for the determination of acetamiprid in early growth stage wheat and pea plants (high water plant matrices) was provided according to the requirements of SANTE/2020/12830 rev. 1 in the current study.

**A. Materials**

|  |  |
| --- | --- |
| 1. Standards |  |
| Reference item: | Acetamiprid |
| Lot/Batch number: | G1050293 |
| Purity: | 99.75 % |
| CAS No.: | 135410-20-7 |
| Expiry date: | 03 February 2024 |
| Standards for calibration | As above |
| Matrix: | Plant material with high water content (early growth stage wheat and pea plants) |

1. **Sample preparation and processing**

For each sample type, 5.0 ± 0.05g were weighed into a 50 mL centrifuge tube. 6.0 mL water and 10 mL acetonitrile were added and the tubes were shaken be hand for 1 min and then on a mechanical shaker for 15 min. 4.0 g of magnesium sulfate, 1.0 g of sodium chloride, 1.0 g of trisodium citrate dihydrate and 0.5 g of disodium hydrogen citrate sesquihydrate were added. The tube was capped, shaken by hand for one (1) minute and then centrifuged at 3200 × *g* for 5 min. 40 mg of PSA and 225 mg of magnesium sulphate was weighed into a 2-mL safe-lock tube. An aliquot of 1.5 mL of the supernatant was transferred into the tube containing the mixture of sorbents. The tube was intensively shaken by hand, vortexed for 30 and then centrifuged for 2 min at 3200 × g. 0.16 mL of the purified extract, 3.84 mL acetonitrile were made up to 10 mL with water containing 0.1% (v/v) formic acid. The final extract was well mixed and an aliquot transferred to an HPLC vial ready for analysis by LC-MS/MS.

1. **Chromatographic parameters**

|  |  |
| --- | --- |
| HPLC- parameters: | 1200 Infinity Binary LC System, Agilent Technologies |
| Column: | Phenomenex Luna C8, 150 mm × 2.0 mm, 5 µm, 100A |
| Mobile phase: | A: Acetonitrile containing 0.1 % formic acid (v/v)  B: Water containing 0.1 % formic acid (v/v) |
| Flow rate: | 800 µL/min |
| Injection volume: | 25 µL µL |
| MS/MS Parameters:  Ionisation type:  Transitions monitored: | API 5000 System, SCIEX (Triple quadrupole mass spectrometer)  EI positive  m/z 223→126 (quantification)  m/z 223→ 90 (confirmation) |

**Results and discussion**

The analytical method used in the current study was fully validated for the determination of acetamiprid in ground vegetation with high water content according to the requirements of SANCO 3029/99 rev. 4 guidelines in a previous study (See KCP 5.1.2/03). A concurrent validation for the determination of acetamiprid in early growth stage pea and wheat plants (high water plant matrices) was conducted in the current study. The target analyte was determined using HPLC-MS/MS by monitoring two highly specific mass transitions. The detector response was linear over the range 0.020 – 2.0 ng/mL (corresponding to 0.0025 – 0.25 mg/kg) with associated correlations coefficients (r2) > 0.99. The LOQ of the method is 0.01 mg acetamiprid/kg for both matrices. All mean recovery values and associated RSDs for both matrices meet the requirements of SANTE/2020/12830 rev.1 guidelines and are summarised in the table below.

**Table A 42: Method validation recovery data for the determination of acetamiprid in early growth stage wheat and pea plants reported in study R2040057.**

| **Matrix** | **Analyte** | **Fortification level (mg/kg)** | **Mean  recovery (%)**  **n=3** | **RSD (%)** | **Comments** |
| --- | --- | --- | --- | --- | --- |
| Quantification transition m/z 223→126 | | | | | |
| Wheat plant | Acetamiprid | 0.01 | 78 | 2.3 | - |
| 0.1 | 83 | 2.0 |
| Confirmation transition m/z 223→90 | | | | | |
| Wheat plant | Acetamiprid | 0.01 | 78 | 3.7 | - |
| 0.1 | 83 | 1.3 |
| Quantification transition m/z 223→126 | | | | | |
| Pea plant | Acetamiprid | 0.01 | 97 | 4.7 | - |
| 0.1 | 103 | 3.3 |
| Confirmation transition m/z 223→90 | | | | | |
| Pea plant | Acetamiprid | 0.01 | 104 | 4.0 | - |
| 0.1 | 104 | 3.8 |

**Table A 43: Characteristics of the analytical method validated for the determination of acetamiprid in early growth stage wheat and pea plants**

|  | **Acetamiprid** |
| --- | --- |
| Specificity | A highly specific HPLC-MS/MS method was used for the determination of the target analyte. Two mass transitions (m/z 223→126, quantification and m/z 223→90, qualification) were monitored during each analysis.  Target analyte concentrations in controls did not exceed 30% of the method LOQ. No interference from co-eluting components at the retention time of the target analyte. |
| Calibration (type, number of data points) | Eight-point linear calibration  Wheat: y = 591891.7201x + 3034.5699, r2= 0.9985  Pea: y = 727897.7738 - 7001.8197, r2= 0.9995 |
| Calibration range | 0.020 – 2.0 ng/mL corresponding to 0.0025 – 0.25 mg/kg |
| Assessment of matrix effects is presented | Matrix effects were assessed and found to be significant for pea plants (≥ ± 20%) but insignificant (< ± 20%) for wheat plants. Nevertheless, matrix-matched calibration was used for the quantitiative determination of the target analyte in both matrix types. |
| Limit of determination/quantification | LOQ both matrices: 0.01 mg/kg  LOD both matrices: 0.003 mg/kg |

**Conclusion**

An analytical method for the determination of acetamiprid in plant material with high water content (ground vegetation) was fully validated according to the requirements of the SANCO/3029/99 rev 4 guidelines prior to 1st March 2020 (see KCP 5.1.2/03). The validation data provided in the current report represent a concurrent validation (n =3 at each of two fortification levels) of the method for two additional plant matrices with high water content (early growth stage wheat and pea plants). The additional concurrent validation data provided in the current report meet the requirements of SANTE/2020/12830 rev 1 guidelines and also demonstrate that the method was functioning correctly when used to analyse the field samples. The method should therefore be considered suitable for its intended purpose and acceptable for the determination of acetamiprid in early growth stage wheat and pea plants.

* + - * 1. Analytical method R2040059

Method validation R2040059

|  |  |
| --- | --- |
| Comments of zRMS: | The analytical method for the determination of acetamiprid was fully validated for the high water content matrix grass (Henkes, K. 2017, Study No. R1640039) and an additional reduced validation for wheat plants and pea plants.  The LOQ for acetamiprid was 0.01 mg/kg.  Acceptance criteria for method validations according to guideline SANTE/2020/12830 rev.1 were met. The method is acceptable. |

|  |  |
| --- | --- |
| Reference: | KCP 5.1.2/23 |
| Report | Residues of acetamiprid in monocotyledonous and dicotyledonous plants after spray application of Acetamiprid 200 SL in early vegetation stages in Spring in Germany – magnitude of residues and time course of residue decline. Staffel, J. 2022. Study No. R2040059. Adama Reference No. 000106554. |
| Guideline(s): | SANCO 3029/99 rev. 4 and SANTE/2020/12830 rev.1 |
| Deviations: | None |
| GLP: | Yes |
| Acceptability: | Yes |

The multi-residue QuEChERS-based analytical method used in the current study was fully validated for the determination of acetamiprid in plant material with high water content (ground vegetation) according to the requirements of SANCO 3029/99 rev. 4 guidelines in a previous study prior to 1st March 2020 (Henkes, K. 2017, Study No. R1640039. KCP 5.1.2/03). Concurrent validation data for the determination of acetamiprid in early growth stage wheat and pea plants (high water plant matrices) was provided according to the requirements of SANTE/2020/12830 rev. 1 in the current study.

**A. Materials**

|  |  |
| --- | --- |
| 1. Standards |  |
| Reference item: | Acetamiprid |
| Lot/Batch number: | G1050293 |
| Purity: | 99.75 % |
| CAS No.: | 135410-20-7 |
| Expiry date: | 03 February 2024 |
| Standards for calibration | As above |
| Matrix: | Plant material with high water content (early growth stage wheat and pea plants) |

1. **Sample preparation and processing**

For each sample type, 5.0 ± 0.05g were weighed into a 50 mL centrifuge tube. 6.0 mL water and 10 mL acetonitrile were added and the tubes were shaken be hand for 1 min and then on a mechanical shaker for 15 min. 4.0 g of magnesium sulfate, 1.0 g of sodium chloride, 1.0 g of trisodium citrate dihydrate and 0.5 g of disodium hydrogen citrate sesquihydrate were added. The tube was capped, shaken by hand for one (1) minute and then centrifuged at 3200 × *g* for 5 min. 40 mg of PSA and 225 mg of magnesium sulphate was weighed into a 2-mL safe-lock tube. An aliquot of 1.5 mL of the supernatant was transferred into the tube containing the mixture of sorbents. The tube was intensively shaken by hand, vortexed for 30 and then centrifuged for 2 min at 3200 × g. 0.16 mL of the purified extract, 3.84 mL acetonitrile were made up to 10 mL with water containing 0.1% (v/v) formic acid. The final extract was well mixed and an aliquot transferred to an HPLC vial ready for analysis by LC-MS/MS.

1. **Chromatographic parameters**

|  |  |
| --- | --- |
| HPLC- parameters: | 1200 Infinity Binary LC System, Agilent Technologies |
| Column: | Phenomenex Luna C8, 150 mm × 2.0 mm, 5 µm, 100A |
| Mobile phase: | A: Acetonitrile containing 0.1 % formic acid (v/v)  B: Water containing 0.1 % formic acid (v/v) |
| Flow rate: | 800 µL/min |
| Injection volume: | 25 µL µL |
| MS/MS Parameters:  Ionisation type:  Transition monitored: | API 5000 System, SCIEX (Triple quadrupole mass spectrometer)  EI positive  m/z 223→126 (quantification)  m/z 223→ 90 (confirmation) |

**Results and discussion**

The analytical method used in the current study was fully validated for the determination of acetamiprid in ground vegetation with high water content according to the requirements of SANCO 3029/99 rev. 4 guidelines in a previous study (See KCP 5.1.2/03). A concurrent validation for the determination of acetamiprid in early growth stage pea and wheat plants (high water plant matrices) was conducted in the current study. The target analyte was determined using HPLC-MS/MS by monitoring one specific mass transition. The detector response was linear over the range 0.020 – 2.0 ng/mL (corresponding to 0.0025 – 0.25 mg/kg) with associated correlations coefficients (r2) > 0.99. Regression residuals were plotted and found to be randomly distributed. Linear calibration was therefore considered suitable for quantitative determination of the target analyte. The LOQ of the method is 0.01 mg acetamiprid/kg for both matrices. The target analyte was shown to be stable in final extracts for up to 11 days and 16 days, for wheat and pea seedlings, respectively. The target analyte was shown to be stable in working solutions for up to 181 days in acetonitrile, 21 days in methanol and 11 days in 0.1 % formic acid/acetonitrile. All mean recovery values and associated RSDs for both matrices meet the requirements of SANTE/2020/12830 rev.1 guidelines and are summarised in the table below.

**Table A 44: Method validation recovery data for the determination of acetamiprid in early growth stage wheat and pea plants reported in study R2040059.**

| **Matrix** | **Analyte** | **Fortification level (mg/kg)** | **Mean  recovery (%)**  **n=3** | **RSD (%)** | **Comments** |
| --- | --- | --- | --- | --- | --- |
| Quantification transition m/z 223→126 | | | | | |
| Wheat plant | Acetamiprid | 0.01 | 110 | 3.0 | - |
| 0.10 | 95 | 2.4 |
| 10 | 104 | 0.3 |
| Pea plant | 0.01 | 102 | 0.5 | - |
| 0.10 | 110 | 1.0 |
| 10 | 108 | 1.3 |

**Table A 45: Characteristics of the analytical method validated for the determination of acetamiprid in early growth stage wheat and pea plants**

|  | **Acetamiprid** |
| --- | --- |
| Specificity | A highly specific HPLC-MS/MS method was used for the determination of the target analyte. Two mass transitions (m/z 223→126, quantification and m/z 223→90, qualification) were monitored during each analysis. Target analyte concentrations in controls did not exceed 30% of the method LOQ. No interference from co-eluting components at the retention time of the target analyte. |
| Calibration (type, number of data points) | Eight-point linear calibration  Wheat: y = 1011492.0133 – 17963.6186, r2= 0.9962  Pea: y = 700710.5262 + 1597.4869, r2= 0.9996 |
| Calibration range | 0.020 – 2.0 ng/mL corresponding to 0.0025 – 0.25 mg/kg |
| Assessment of matrix effects is presented | Matrix effects were assessed and found to be significant for pea plants (≥ ± 20%) but insignificant (< ± 20%) for wheat plants. Nevertheless, matrix-matched calibration was used for the quantitiative determination of the target analyte in both matrix types. |
| Limit of determination/quantification | LOQ both matrices: 0.01 mg/kg  LOD both matrices: 0.0025 mg/kg |

**Conclusion**

An analytical method for the determination of acetamiprid in plant material with high water content (ground vegetation) was fully validated according to the requirements of the SANCO/3029/99 rev 4 guidelines prior to 1st March 2020 (see KCP 5.1.2/03). The validation data provided in the current report represent a concurrent validation (n =3 at each of two fortification levels) of the method for two additional plant matrices with high water content (early growth stage wheat and pea plants). The additional concurrent validation data provided in the current report meet the requirements of SANTE/2020/12830 rev 1 guidelines and also demonstrate that the method was functioning correctly when used to analyse the field samples. The method should therefore be considered suitable for its intended purpose and acceptable for the determination of acetamiprid in early growth stage wheat and pea plants.

* + - * 1. Analytical method R2040060

Method validation R2040060

|  |  |
| --- | --- |
| Comments of zRMS: | The analytical method for the determination of acetamiprid was fully validated for the high water content matrix grass (Henkes, K. 2017, Study No. R1640039) and an additional reduced validation for wheat plants and pea plants.  The LOQ for acetamiprid was 0.01 mg/kg.  Acceptance criteria for method validations according to guideline SANTE/2020/12830 rev.1 were met. The method is acceptable. |

|  |  |
| --- | --- |
| Reference: | KCP 5.1.2/24 |
| Report | Residues of acetamiprid in monocotyledonous and dicotyledonous plants after spray application of Acetamiprid 200 SL in early vegetation stages in Northern Europe – magnitude of residues and time course of residue decline. Gräf, K. 2022. Study No. R2040060. Adama Reference No. 000106555. |
| Guideline(s): | SANCO 3029/99 rev. 4 and SANTE/2020/12830 rev.1 |
| Deviations: | None |
| GLP: | Yes |
| Acceptability: | Yes |

The multi-residue QuEChERS-based analytical method used in the current study was fully validated for the determination of acetamiprid in plant material with high water content (ground vegetation) according to the requirements of SANCO 3029/99 rev. 4 guidelines in a previous study prior to 1st March 2020 (Henkes, K. 2017, Study No. R1640039. KCP 5.1.2/03). Concurrent validation data for the determination of acetamiprid in early growth stage wheat and pea plants (high water plant matrices) was provided according to the requirements of SANTE/2020/12830 rev. 1 in the current study.

**A. Materials**

|  |  |
| --- | --- |
| 1. Standards |  |
| Reference item: | Acetamiprid |
| Lot/Batch number: | G1050293 |
| Purity: | 99.75 % |
| CAS No.: | 135410-20-7 |
| Expiry date: | 03 February 2024 |
| Standards for calibration | As above |
| Matrix: | Plant material with high water content (early growth stage wheat and pea plants) |

1. **Sample preparation and processing**

For each sample type, 5.0 ± 0.05g were weighed into a 50 mL centrifuge tube. 6.0 mL water and 10 mL acetonitrile were added and the tubes were shaken be hand for 1 min and then on a mechanical shaker for 15 min. 4.0 g of magnesium sulfate, 1.0 g of sodium chloride, 1.0 g of trisodium citrate dihydrate and 0.5 g of disodium hydrogen citrate sesquihydrate were added. The tube was capped, shaken by hand for one (1) minute and then centrifuged at 3200 × *g* for 5 min. 40 mg of PSA and 225 mg of magnesium sulphate was weighed into a 2-mL safe-lock tube. An aliquot of 1.5 mL of the supernatant was transferred into the tube containing the mixture of sorbents. The tube was intensively shaken by hand, vortexed for 30 and then centrifuged for 2 min at 3200 × g. 0.16 mL of the purified extract, 3.84 mL acetonitrile were made up to 10 mL with water containing 0.1% (v/v) formic acid. The final extract was well mixed and an aliquot transferred to an HPLC vial ready for analysis by LC-MS/MS.

1. **Chromatographic parameters**

|  |  |
| --- | --- |
| HPLC- parameters: | 1200 Infinity Binary LC System, Agilent Technologies |
| Column: | Phenomenex Luna C8, 150 mm × 2.0 mm, 5 µm, 100A |
| Mobile phase: | A: Acetonitrile containing 0.1 % formic acid (v/v)  B: Water containing 0.1 % formic acid (v/v) |
| Flow rate: | 800 µL/min |
| Injection volume: | 25 µL µL |
| MS/MS Parameters:  Ionisation type:  Transition monitored: | API 5000 System, SCIEX (Triple quadrupole mass spectrometer)  EI positive  m/z 223→126 (quantification)  m/z 223→ 90 (confirmation) |

**Results and discussion**

The analytical method used in the current study was fully validated for the determination of acetamiprid in ground vegetation with high water content according to the requirements of SANCO 3029/99 rev. 4 guidelines in a previous study (See KCP 5.1.2/03). A concurrent validation for the determination of acetamiprid in early growth stage pea and wheat plants (high water plant matrices) was conducted in the current study. The target analyte was determined using HPLC-MS/MS by monitoring one specific mass transition. The detector response was linear over the range 0.020 – 2.0 ng/mL (corresponding to 0.0025 – 0.25 mg/kg) with associated correlations coefficients (r2) > 0.99. Regression residuals were plotted and found to be randomly distributed. Linear calibration was therefore considered suitable for quantitative determination of the target analyte. The LOQ of the method is 0.01 mg acetamiprid/kg for both matrices. The target analyte was shown to be stable in final extracts for up to 9 days for both wheat and pea seedlings. The target analyte was shown to be stable in working solutions for up to 181 days in acetonitrile, 21 days in methanol and 20 days in 0.1 % formic acid/acetonitrile. All mean recovery values and associated RSDs for both matrices meet the requirements of SANTE/2020/12830 rev.1 guidelines and are summarised in the table below.

**Table A 46: Method validation recovery data for the determination of acetamiprid in early growth stage wheat and pea plants reported in study R2040060.**

| **Matrix** | **Analyte** | **Fortification level (mg/kg)** | **Mean  recovery (%)**  **n=3** | **RSD (%)** | **Comments** |
| --- | --- | --- | --- | --- | --- |
| Quantification transition m/z 223→126 | | | | | |
| Wheat plant | Acetamiprid | 0.01 | 109 | 5.8 | - |
| 0.10 | 91 | 5.1 |
| 20 | 95 | 7.5 |
| Pea plant | 0.01 | 73 | 2.1 | - |
| 0.10 | 92 | 1.7 |
| 20 | 98 | 8.5 |

**Table A 47: Characteristics of the analytical method validated for the determination of acetamiprid in early growth stage wheat and pea plants**

|  | **Acetamiprid** |
| --- | --- |
| Specificity | A highly specific HPLC-MS/MS method was used for the determination of the target analyte. Two mass transitions (m/z 223→126, quantification and m/z 223→90, qualification) were monitored during each analysis. Target analyte concentrations in controls did not exceed 30% of the method LOQ. No interference from co-eluting components at the retention time of the target analyte. |
| Calibration (type, number of data points) | Eight-point linear calibration  Wheat: y = 332901.0842 – 691.0534, r2= 0.9979  Pea: y = 313147.5662 + 99.5613, r2= 0.9996 |
| Calibration range | 0.020 – 2.0 ng/mL (corresponding to 0.0025 – 0.25 mg/kg) |
| Assessment of matrix effects is presented | Matrix effects were assessed and found to be insignificant (< ± 20%) for both matrix types. Nevertheless, matrix-matched calibration was used for the quantitiative determination of the target analyte in both matrix types. |
| Limit of determination/quantification | LOQ both matrices: 0.01 mg/kg  LOD both matrices: 0.0025 mg/kg |

**Conclusion**

An analytical method for the determination of acetamiprid in plant material with high water content (ground vegetation) was fully validated according to the requirements of the SANCO/3029/99 rev 4 guidelines prior to 1st March 2020 (see KCP 5.1.2/03). The validation data provided in the current report represent a concurrent validation (n =3 at each of two fortification levels) of the method for two additional plant matrices with high water content (early growth stage wheat and pea plants). The additional concurrent validation data provided in the current report meet the requirements of SANTE/2020/12830 rev 1 guidelines and also demonstrate that the method was functioning correctly when used to analyse the field samples. The method should therefore be considered suitable for its intended purpose and acceptable for the determination of acetamiprid in early growth stage wheat and pea plants.

* + - * 1. Analytical method 21 48 FCM 0002

Method validation 21 48 FCM 0002

|  |  |
| --- | --- |
| Comments of zRMS: | The method was validated for the determination of acetamiprid in soil samples with LOQ of 0.005 mg/kg.  Acceptance criteria for method validations according to guideline SANTE/2020/12830 rev.1 were met. The method is acceptable. |

|  |  |
| --- | --- |
| Reference: | KCP 5.1.2/25 |
| Report | Effects of Acetamiprid 200 SL on Collembola under field conditions - Schulz, L. 2022, Report No. 21 48 FCM 0002, Sponsor Reference No. 00108239 |
| Guideline(s): | SANTE/2020/12830 rev.1 |
| Deviations: | None |
| GLP: | Yes |
| Acceptability: | Yes |

An analytical method for the determination of acetamiprid residues in soil was fully validated according to the requirements of SANTE/2020/12830 rev.1 and used to support an ecotoxicology study conducted on collembola.

**A. Materials**

|  |  |
| --- | --- |
| 1. Standards |  |
| Reference item: | Acetamiprid |
| Lot/Batch number: | BCBT9185 |
| Purity: | 100% |
| CAS No.: | 160430-64-8 |
| Expiry date: | 28 February 2022 |
| Standards for calibration | As above |
| Matrix: | Soil |

**B. Sample preparation and processing**

50 g (± 0.1 g) of the sample were weighed into a 250 mL glass bottle. 10 mL demineralised water was added. For fortified samples, a solution of acetamiprid in methanol was added. 200 mL of acetonitrile was added, and the samples were extracted on a flatbed shaker for at least 30 min at around 250 rpm. Sodium chloride was added, and the phases were allowed to separate. An aliquot of the upper clear organic phase was transferred into a HPLC vial and were diluted if necessary, with control matrix extract (in acetonitrile). The vials were capped and mixed on a Vortex mixer for a few seconds. The samples were analysed by HPLC-MS/MS analysis. Time required for one sample set (consisting of 10 recoveries and 2 control samples) is about 1.5 hour (without time required for analysis). An interruption of the method is possible after weighing in the sample into the glass bottle(s).

**C. Sample preparation and processing**

|  |  |
| --- | --- |
| HPLC- parameters: | Thermo Scientific TSQ Quantum Access |
| Column: | Phenomenex Luna C18 (2) 100 A, 150 mm x 2.0 mm, 5.0 μm |
| Mobile phase: | A: Water containing 1 % formic acid (v/v)  B: Acetonitrile |
| Flow rate: | 0.5 mL/min |
| Injection volume: | 20 µL |
| MS/MS Parameters:  Ionisation type:  Transitions monitored: | Surveyor MS Pump Plus  m/z 223→126 (quantification)  m/z 223→90 (confirmation) |

**Results and discussion**

The analytical method used in the current study was fully validated for the determination of acetamiprid residues in soil according to the requirements of SANTE/2020/12830 rev.1. guidelines. The target analyte was determined using HPLC-MS/MS by monitoring two highly specific mass transitions. The detector response was linear over the range 0.25 - 25 μg/L (corresponding to 0.001 mg/kg to 0.10 mg/kg) with associated correlation coefficient (r) ≥ 0.9975. Regression residuals were plotted and found to be randomly distributed demonstrating a linear calibration function was suitable for the quantitative determination of the target analyte. The LOQ of the method is 0.005 mg/kg. All mean recovery values and associated RSDs meet the requirements of SANTE/2020/12830 rev.1 guidelines and are summarised in the table below.

**Table A 48: Method validation recovery data for the determination of acetamiprid in soil reported in study 21 48 FCM 0002.**

| **Matrix** | **Analyte** | **Fortification level (mg/kg) (n=5)** | **Mean  recovery (%)** | **RSD (%)** | **Comments** |
| --- | --- | --- | --- | --- | --- |
| Quantification transition m/z 223→126 | | | | | |
| Soil | Acetamiprid | 0.005 | 72 | 3.6 | - |
| 1.00 | 79 | 6.9 |
| Confirmation transition m/z 223→90 | | | | | |
| Soil | Acetamiprid | 0.005 | 70 | 4.0 | - |
| 1.00 | 80 | 7.1 |

**Table A 49: Characteristics of the analytical method validated for the determination of acetamiprid in soil**

|  | **Acetamiprid** |
| --- | --- |
| Specificity | A highly specific HPLC-MS/MS method was used for the determination of the target analyte. Two mass transitions (m/z 223→126, quantifiaction and m/z 223→90, qualification) were monitored during each analysis.  Target analyte concentrations in controls did not exceed 30% of the method LOQ. No interference from co-eluting components at the retention time of the target analyte. |
| Calibration (type, number of data points) | Eight-point matrix-matched linear calibration    Calibration range 0.25 - 25 μg/L:  quantifier mass transition: y = 2050899.44x + 126523.11, r ≥ 0.9975  qualifier mass transition: y = 687313.29x + 20465.25, r ≥ 0.9975 |
| Calibration range | 0.25 - 25 μg/L (corresponding to 0.001 mg/kg to 0.10 mg/kg) |
| Assessment of matrix effects is presented | Matrix effects were assessed and found to be insignificant (< ± 20%) for soil. Nevertheless, matrix matched calibrations were used for target analyte quantification. |
| Limit of determination/quantification | LOQ: 0.005 mg/kg  LOD: 0.0015 mg/kg |
| Stability of the target analyte in standards | The stability of the analyte in stock and working solutions was examined by comparison of stored stock and working solutions against freshly prepared standard solutions. Stock and working solutions were found to be stable, as the difference in the means from five (5) replicates (for each solution tested) to the reference solution was below 10% for up to 14 days when prepared in methanol and stored refrigerated (at ≤ 8 °C) in the dark. |
| Stability of the target analyte in final extracts | All sample extracts were analysed within 24 hours after extraction, therefore the stability of the analyte is sufficiently proven. |

**Conclusion**

An analytical method for the determination of acetamiprid residues in soil was fully validated according to SANTE/2020/12830 rev.1. guidelines. The method was sufficiently accurate and precise to be able to provide reliable data on target analyte concentrations in soil and should therefore be considered acceptable.

* + - * 1. Analytical method 141048005 W

Method validation 141048005 W

|  |  |
| --- | --- |
| Comments of zRMS: | The study has been evaluated and accepted in Registration Report, Section 5 for CA3573 / Carnadine / Kestrel, Nufarm (August 2021).  Conclusions:  *The purpose of the analytical phase of the study was the verification of the test solution concentrations of the active ingredient.*  *The analytical method was validated according to the guidance document SANCO/3029/99 rev. 4. All criteria are fulfilled:*  *- blank values do not exceed 30% of the lowest validated concentration,*  *- the mean recoveries for each level are in the range 70-110%,*  *- the RSD is < 20% per level.*  *The LOQ of acetamiprid was defined in the context of this study as the lowest successfully validated fortification level, i.e. 0.185 mg/L.*  *The study is acceptable.* |

|  |  |
| --- | --- |
| Reference: | KCP 5.1.2/26 |
| Report | Acute toxicity of MCW-2222 to the rainbow trout *Oncorhynchus mykiss* in a 96-hour static test. xxxxx 2014, Study No. 141048005 W. Adama Reference No. R-33831 |
| Guideline(s): | SANCO 3029/99 rev. 4 |
| Deviations: | None |
| GLP: | Yes |
| Acceptability: | Yes |

An analytical method for the determination of acetamiprid in water was fully validated according to the requirements of SANCO/3029/99 rev. 4 prior to 1st March 2021 and used to support an ecotoxicology study conducted on fish.

**A. Materials**

|  |  |
| --- | --- |
| 1. Standards |  |
| Test item: | MCW-2222 |
| Batch no. | 611-280413-01 |
| Active substance: | Nominal: 200 g/L  Analysed: 202.7 g/L |
| Expiry date: | April 2015 |
| Reference item: | Acetamiprid |
| Lot/Batch number: | 772827 |
| Purity: | 99.9 % |
| CAS No.: | 135410-20-7 |
| Expiry date: | August 2017 |
| Standards for calibration | As above |
| Matrix: | Water |

1. **Sample preparation and processing**

The samples were allowed to thaw at room temperature and were analysed without any further preparation.

1. **Chromatographic parameters**

|  |  |
| --- | --- |
| HPLC- parameters  Instrumentation: | Shimadzu LC-10 HPLC |
| Column: | 2.6 µm C18, 100 x 2.1 mm |
| Mobile phase: | 25: 75 methanol: water (v/v) |
| Flow rate: | 0.4 mL/min |
| Injection volume: | 10 µL |
| Detection: | UV at 245 nm |
|  |  |

**Results and discussion**

The method used for the determination of residues of acetamiprid in water was fully validated according to the requirements of the SANCO/3029/99 rev. 4 guidelines prior to 1st March 2021. The target analyte was determined using HPLC-UV and monitoring at 245 nm. The detector response was linear over the range from 0.149 – 114.5 mg/L with associated correlations coefficients (r2) ≥ 0.999. The LOQ of the method is 0.185 mg/L. Target analyte concentrations in controls were < 30% of the method LOQ. All mean recovery values and associated RSDs meet the requirements of SANCO/3029/99 rev. 4 and SANTE/2020/12830 rev.1 guidelines and are summarised in the table below.

**Table A 50: Method validation recovery data for the determination of acetamiprid in water reported in study 141048005.**

| **Matrix** | **Analyte** | **Fortification level (mg/L)** | **Mean  recovery (%)**  **n=5** | **RSD (%)** | **Comments** |
| --- | --- | --- | --- | --- | --- |
| Water | Acetamiprid | 0.185 | 92.3 | 1.7 | - |
| 92.65 | 93.2 | 0.6 | - |

**Table A 51: Characteristics of the analytical method validated for the determination of acetamiprid in water**

|  | **Acetamiprid** |
| --- | --- |
| Specificity | The HPLC-UV method is sufficiently specific for the determination of acetamiprid.  Target analyte concentrations in controls did not exceed 30% of the method LOQ. No interference from co-eluting components at the retention time of the target analyte. |
| Calibration (type, number of data points) | Ten-point-linear calibration  y = 148054x + 3544.07, r2= 0.999 |
| Assessment of matrix effects is presented | Matrix effects were not assessed but matrix-matched calibration standards were used |
| Calibration range | 0.149 – 114.5 mg/L |
| Limit of determination/quantification | LOQ: 0.185 mg/L |

**Conclusion**

An analytical method for the determination of acetamiprid in water was fully validated according to SANCO/3029/99 guidelines prior to March 21st 2021. The data provided exceed the minimum validation requirements given in Section 4.2 of SANTE/2020/12830 Rev. 1. The method should therefore be considered suitable for its intended purpose and acceptable for the determination of acetamiprid in water.

* + - * 1. Analytical method 141048006 W

Method validation 141048006 W

|  |  |
| --- | --- |
| Comments of zRMS: | The study has been evaluated and accepted in Registration Report, Section 5 for CA3573 / Carnadine / Kestrel, Nufarm (August 2021).  Conclusions:  *The purpose of the analytical phase of the study was the verification of the test solution concentrations of the active ingredient.*  *The analytical method was validated according to the guidance document SANCO/3029/99 rev. 4. All criteria are fulfilled:*  *- blank values do not exceed 30% of the lowest validated concentration,*  *- the mean recoveries for each level are in the range 70-110%,*  *- the RSD is < 20% per level.*  *The LOQ of acetamiprid was defined in the context of this study as the lowest successfully validated fortification level, i.e. 0.367 mg/L.*  *The study is acceptable.* |

|  |  |
| --- | --- |
| Reference: | KCP 5.1.2/27 |
| Report | Acute toxicity of MCW-2222 to *Daphnia magna* in a 48-hour static test. Juckeland, D. 2014, Study No. 141048006 W. Adama Reference No. R-33832 |
| Guideline(s): | SANCO 3029/99 rev. 4 |
| Deviations: | None |
| GLP: | Yes |
| Acceptability: | Yes |

An analytical method for the determination of acetamiprid in M4 aqueous test medium was fully validated according to the requirements of SANCO/3029/99 rev. 4 prior to 1st March 2021 and used to support an ecotoxicology study conducted on Daphnia.

**A. Materials**

|  |  |
| --- | --- |
| 1. Standards |  |
| Test item: | MCW-2222 |
| Batch no. | 611-280413-01 |
| Active substance: | Nominal: 200 g/L  Analysed: 202.7 g/L |
| Expiry date: | April 2015 |
| Reference item: | Acetamiprid |
| Lot/Batch number: | 772827 |
| Purity: | 99.9 % |
| CAS No.: | 135410-20-7 |
| Expiry date: | August 2017 |
| Standards for calibration | As above |
| Matrix: | M4 aqueous test medium |

1. **Sample preparation and processing**

The samples were allowed to thaw at room temperature and were analysed without any further preparation.

1. **Chromatographic parameters**

|  |  |
| --- | --- |
| HPLC- parameters  Instrumentation: | Shimadzu LC-10 HPLC |
| Column: | 2.6 µm C18, 100 x 2.1 mm |
| Mobile phase: | 25: 75 methanol: water (v/v) |
| Flow rate: | 0.4 mL/min |
| Injection volume: | 10 µL |
| Detection: | UV at 245 nm |
|  |  |

**Results and discussion**

The method used for the determination of residues of acetamiprid in M4 aqueous test medium was fully validated according to the requirements of the SANCO/3029/99 rev. 4 guidelines prior to 1st March 2021. The target analyte was determined using HPLC-UV and monitoring at 245 nm. The detector response was linear over the range from 0.149 – 114.5 mg/L with associated correlations coefficients (r2) ≥ 0.999. The LOQ of the method is 0.367 mg/L. Target analyte concentrations in controls were < 30% of the method LOQ. All mean recovery values and associated RSDs meet the requirements of SANCO/3029/99 rev. 4 and SANTE/2020/12830 rev.1 guidelines and are summarised in the table below.

**Table A 52: Method validation recovery data for the determination of acetamiprid in M4 aqueous test medium reported in study 141048006.**

| **Matrix** | **Analyte** | **Fortification level (mg/L)** | **Mean  recovery (%)**  **n=5** | **RSD (%)** | **Comments** |
| --- | --- | --- | --- | --- | --- |
| M4 aqueous test medium | Acetamiprid | 0.367 | 98.9 | 1.1 | - |
| 91.740 | 95.1 | 0.2 | - |

**Table A 53: Characteristics of the analytical method validated for the determination of acetamiprid in M4 aqueous test medium**

|  | **Acetamiprid** |
| --- | --- |
| Specificity | The HPLC-UV method is sufficiently specific for the determination of acetamiprid.  Target analyte concentrations in controls did not exceed 30% of the method LOQ. No interference from co-eluting components at the retention time of the target analyte. |
| Calibration (type, number of data points) | Ten-point-linear calibration  y = 148054x + 3544.07, r2= 0.999 |
| Assessment of matrix effects is presented | Matrix effects were not assessed but matrix-matched calibration standards were used |
| Calibration range | 0.149 – 114.5 mg/L |
| Limit of determination/quantification | LOQ: 0.367 mg/L |

**Conclusion**

An analytical method for the determination of acetamiprid in M4 aqueous test medium was fully validated according to SANCO/3029/99 guidelines prior to March 21st 2021. The data provided exceed the minimum validation requirements given in Section 4.2 of SANTE/2020/12830 Rev. 1. The method should therefore be considered suitable for its intended purpose and acceptable for the determination of acetamiprid in M4 aqueous test medium.

* + - * 1. Analytical method 141048057 W

Method validation 141048057 W

|  |  |
| --- | --- |
| Comments of zRMS: | The study has been evaluated and accepted in Registration Report, Section 5 for CA3573 / Carnadine / Kestrel, Nufarm (August 2021).  Conclusions:  *The purpose of the analytical phase of the study was the verification of the test solution concentrations of the active ingredient.*  *The analytical method was validated according to the guidance document SANCO/3029/99 rev. 4. All criteria are fulfilled:*  *- blank values do not exceed 30% of the lowest validated concentration,*  *- the mean recoveries for each level are in the range 70-110%,*  *- the RSD is < 20% per level.*  *The LOQ of acetamiprid was defined in the context of this study as the lowest successfully validated fortification level, i.e. 0.47 µg/L.*  *The study is acceptable.* |

|  |  |
| --- | --- |
| Reference: | KCP 5.1.2/28 |
| Report | Acute toxicity of MCW-2222 to *Chironomus riparius* in a 48-hour static test, Juckeland, D. 2015, Study No. 141048057 W. Adama Reference No. R-34873 |
| Guideline(s): | SANCO 3029/99 rev. 4 |
| Deviations: | None |
| GLP: | Yes |
| Acceptability: | Yes |

An analytical method for the determination of acetamiprid in M4 aqueous test medium was fully validated according to the requirements of SANCO/3029/99 rev. 4 prior to 1st March 2021 and used to support an ecotoxicology study conducted on Chironomids.

**A. Materials**

|  |  |
| --- | --- |
| 1. Standards |  |
| Test item: | MCW-2222 |
| Batch no. | 611-280413-01 |
| Active substance: | Nominal: 200 g/L  Analysed: 202.7 g/L |
| Expiry date: | April 2015 |
| Reference item: | Acetamiprid |
| Lot/Batch number: | 772827 |
| Purity: | 99.9 % |
| CAS No.: | 135410-20-7 |
| Expiry date: | August 2017 |
| Standards for calibration | As above |
| Matrix: | M4 aqueous test medium |

1. **Sample preparation and processing**

The samples were allowed to thaw at room temperature and were analysed without any further preparation.

1. **Chromatographic parameters**

|  |  |
| --- | --- |
| HPLC- parameters: | Agilent 1260 HPLC |
| Column: | 2.6 µm C18, 100 x 2.1 mm |
| Flow rate: | 0.420 mL/min |
| MS/MS | Agilent 6460 |
| Ionisation source: | ESI |
| Transitions monitored: | 223.1 => 126 m/z (quantifier)  223.1 => 90 m/z (qualifier) |
|  |  |

**Results and discussion**

The method used for the determination of residues of acetamiprid in M4 aqueous test medium was fully validated according to the requirements of the SANCO/3029/99 rev. 4 guidelines prior to 1st March 2021. The target analyte was determined using HPLC-MS/MS by monitoring two specific mass transitions. The detector response was linear over the range from 0.364 – 22.24 µg/L with associated correlation coefficients (r2) ≥ 0.999. The LOQ of the method is 0.47 µg/L. Target analyte concentrations in controls were < 30% of the method LOQ. All mean recovery values and associated RSDs for both matrices meet the requirements of SANCO/3029/99 rev. 4 and SANTE/2020/12830 rev.1 guidelines and are summarised in the table below.

**Table A 54: Method validation recovery data for the determination of acetamiprid in M4 aqueous test medium reported in study 141048057.**

| **Matrix** | **Analyte** | **Fortification level (µg/L)** | **Mean  recovery (%)**  **n=5** | **RSD (%)** | **Comments** |
| --- | --- | --- | --- | --- | --- |
| M4 Aqueous medium | Acetamiprid | 0.470 | 86.2 | 1.2 | - |
| 14.40 | 101.7 | 1.2 | - |

**Table A 55: Characteristics of the analytical method validated for the determination of acetamiprid in M4 aqueous test medium**

|  | **Acetamiprid** |
| --- | --- |
| Specificity | A highly specific HPLC-MS/MS method was used for the determination of the target analyte. Two mass transitions (m/z 223.1→126, quantification and m/z 223.1→90, qualification) were monitored during each analysis. Target analyte concentrations in controls did not exceed 30% of the method LOQ. No interference from co-eluting components at the retention time of the target analyte. |
| Calibration (type, number of data points) | Nine-point-linear calibration  y = 50.455967x + 17062.645969, r2= 0.999 |
| Assessment of matrix effects is presented | Matrix effects were not assessed but matrix-matched calibration standards were used |
| Calibration range | 0.364 – 22.24 µg/L |
| Limit of determination/quantification | LOQ: 0.47 µg/L |

**Conclusion**

An analytical method for the determination of acetamiprid in M4 aqueous test medium was fully validated according to SANCO/3029/99 guidelines prior to March 21st 2021. The data provided exceed the minimum validation requirements given in Section 4.2 of SANTE/2020/12830 Rev. 1. The method should therefore be considered suitable for its intended purpose and acceptable for the determination of acetamiprid in M4 aqueous test medium.

* + - * 1. Analytical method CEA.1510 (XCE2008)

Method validation CEA.1510 (XCE2008)

|  |  |
| --- | --- |
| Comments of zRMS: | An analytical method for the determination of acetamiprid in pond water was validated with LOQ of 0.5 µg/L.  The method is acceptable and fit for purpose. |

|  |  |
| --- | --- |
| Reference: | KCP 5.1.2/29 |
| Report | Acetamiprid 200 SL – Acute toxicity to aquatic organisms. Taylor, S. and Joyce, F. 2015, Study No. CEA.1510 (XCE2008). Adama Reference No. R-35057 |
| Guideline(s): | SANCO 3029/99 rev. 4 |
| Deviations: | None |
| GLP: | Yes |
| Acceptability: | Yes |

An analytical method for the determination of acetamiprid in pond water was fully validated according to the requirements of SANCO/3029/99 rev. 4 prior to 1st March 2021 and used to support an ecotoxicology study conducted on aquatic organisms.

**A. Materials**

|  |  |
| --- | --- |
| 1. Standards |  |
| Test item: | Acetamiprid |
| Batch no. | SZBC110XV |
| Purity: | 99.9%L |
| Expiry date: | 19 April 2015 |
| Standards for calibration | As above |
| Matrix: | Pond water |

1. **Sample preparation and processing**

Frozen samples of 30 µm filtered pond water were allowed to thaw at room temperature and if required were diluted with acetonitrile/water (2/8, v/v) containing 1% acetic acid. The samples were then analysed without any further preparation.

1. **Chromatographic parameters**

|  |  |
| --- | --- |
| HPLC- parameters: | Shimadzu SIL HTc HPLC |
| Column:  Mobile phase | Waters Xbridge BEH C18 C18, 50 × 3.0 mm, 2.5 µm  A: 0.1% Acetic acid in water  B: 0.1% Acetic acid in acetonitrile |
| Flow rate:  Injection volume: | 0.4 mL/min  10µL |
| MS/MS | API 5000 |
| Ionisation source: | ESI positive |
| Transitions monitored: | m/z 223.1 => 126.1 (quantifier)  m/z 223.1 => 90 (qualifier) |
|  |  |

**Results and discussion**

The method used for the determination of residues of acetamiprid in pond water was fully validated according to the requirements of the SANCO/3029/99 rev. 4 guidelines prior to 1st March 2021. The target analyte was determined using HPLC-MS/MS by monitoring two specific mass transitions. The detector response was linear over the range from 0.0001 – 0.01 µg/mL with an associated correlation coefficient (r) ≥ 0.999. The LOQ of the method is 0.5 µg/L. When store froze at < -10°C, the target analyte was found to be stable for up to 62 days. Target analyte concentrations in controls were < 30% of the method LOQ. All mean recovery values and associated RSDs for both matrices meet the requirements of SANCO/3029/99 rev. 4 and SANTE/2020/12830 rev.1 guidelines and are summarised in the table below.

**Table A 56: Method validation recovery data for the determination of acetamiprid in pond water reported in study CEA.1510.**

| **Matrix** | **Analyte** | **Fortification level (µg/mL)** | **Mean  recovery (%)**  **n=5** | **RSD (%)** | **Comments** |
| --- | --- | --- | --- | --- | --- |
| Quantification transition m/z 223.1 => 126.1 | | | | | |
| Pond water | Acetamiprid | 0.0005 | 94.8 | 1.54 | - |
| 0.10 | 96.7 | 1.09 | - |
| 2.50 | 99.2 | 1.87 |  |
| 2600 | 99.9 | 3.44 |  |
| Confirmatory transition m/z 223.1 => 90 | | | | | |
| Pond water | Acetamiprid | 0.0005 | 93.2 | 2.01 | - |
| 0.10 | 96.2 | 0.97 | - |
| 2.50 | 98.3 | 1.81 | - |
| 2600 | 99.1 | 4.60 | - |

**Table A 57: Characteristics of the analytical method validated for the determination of acetamiprid in pond water**

|  | **Acetamiprid** |
| --- | --- |
| Specificity | A highly specific HPLC-MS/MS method was used for the determination of the target analyte. Two mass transitions (m/z 223.1→126.1, quantification and m/z 223.1→90, qualification) were monitored during each analysis. Target analyte concentrations in controls did not exceed 30% of the method LOQ. No interference from co-eluting components at the retention time of the target analyte. |
| Calibration (type, number of data points) | Eight-point-linear calibration  y = 7.7e+007x + 652, r= 1.000 |
| Assessment of matrix effects is presented | No, however since all mean recovery values and associated RSDs meet the validation requirements of SANCO/3029/99 rev. 4 and the mini-mum validation requirements given in Section 4.2 of SAN-TE/2020/12830 rev.1 guidelines, matrix effects are being considered insignificant. |
| Calibration range | 0.0001 – 0.01 µg/mL |
| Limit of determination/quantification | LOQ: 0.5 µg/L |

**Conclusion**

An analytical method for the determination of acetamiprid in pond water was fully validated according to SANCO/3029/99 guidelines prior to March 21st 2021. The data provided exceed the minimum validation requirements given in Section 4.2 of SANTE/2020/12830 Rev. 1. The method should therefore be considered suitable for its intended purpose and acceptable for the determination of acetamiprid in pond water.

* + - * 1. Analytical method 141048007 W

Method validation 141048007 W

|  |  |
| --- | --- |
| Comments of zRMS: | The study has been evaluated and accepted in Registration Report, Section 5 for CA3573 / Carnadine / Kestrel, Nufarm (August 2021).  Conclusions:  *The purpose of the analytical phase of the study was the verification of the test solution concentrations of the active ingredient.*  *The analytical method was validated according to the guidance document SANCO/3029/99 rev. 4. All criteria are fulfilled:*  *- blank values do not exceed 30% of the lowest validated concentration,*  *- the mean recoveries for each level are in the range 70-110%,*  *- the RSD is < 20% per level.*  *The LOQ of acetamiprid was defined in the context of this study as the lowest successfully validated fortification level, i.e. 0.344 mg/L.*  *The study is acceptable.* |

|  |  |
| --- | --- |
| Reference: | KCP 5.1.2/30 |
| Report | Effects of MCW-2222 on *Desmodesmus subspicatus* in an algal growth inhibition test, Juckeland, D. 2014, Study No. 141048007 W. Adama Reference No. R-33833 |
| Guideline(s): | SANCO 3029/99 rev. 4 |
| Deviations: | None |
| GLP: | Yes |
| Acceptability: | Yes |

An analytical method for the determination of acetamiprid in OECD aqueous test medium was fully validated according to the requirements of SANCO/3029/99 rev. 4 prior to 1st March 2021 and used to support an ecotoxicology study conducted on algae.

**A. Materials**

|  |  |
| --- | --- |
| 1. Standards |  |
| Test item: | MCW-2222 |
| Batch no. | 611-280413-01 |
| Active substance: | Nominal: 200 g/L  Analysed: 202.7 g/L |
| Expiry date: | April 2015 |
| Reference item: | Acetamiprid |
| Lot/Batch number: | 772827 |
| Purity: | 99.9 % |
| CAS No.: | 135410-20-7 |
| Expiry date: | August 2017 |
| Standards for calibration | As above |
| Matrix: | OECD aqueous test medium |

1. **Sample preparation and processing**

The samples were allowed to thaw at room temperature and were analysed without any further preparation.

1. **Chromatographic parameters**

|  |  |
| --- | --- |
| HPLC- parameters  Instrumentation: | Shimadzu LC-10 HPLC |
| Column: | 2.6 µm C18, 100 x 2.1 mm |
| Mobile phase: | 25: 75 methanol: water (v/v) |
| Flow rate: | 0.4 mL/min |
| Injection volume: | 10 µL |
| Detection: | UV at 245 nm |
|  |  |

**Results and discussion**

The method used for the determination of residues of acetamiprid in OECD aqueous test medium was fully validated according to the requirements of the SANCO/3029/99 rev. 4 guidelines prior to 1st March 2021. The target analyte was determined using HPLC-UV and monitoring at 245 nm. The detector response was linear over the range from 0.172 – 80.14 mg/L with associated correlations coefficients (r2) ≥ 0.999. The LOQ of the method is 0.344 mg/L. Target analyte concentrations in controls were < 30% of the method LOQ. All mean recovery values and associated RSDs meet the requirements of SANCO/3029/99 rev. 4 and SANTE/2020/12830 rev.1 guidelines and are summarised in the table below.

**Table A 58: Method validation recovery data for the determination of acetamiprid in OECD aqueous test medium reported in study 141048007.**

| **Matrix** | **Analyte** | **Fortification level (mg/L)** | **Mean  recovery (%)**  **n=5** | **RSD (%)** | **Comments** |
| --- | --- | --- | --- | --- | --- |
| OECD aqueous test medium | Acetamiprid | 0.344 | 96.4 | 0.7 | - |
| 55.49 | 100.1 | 0.1 | - |

**Table A 59: Characteristics of the analytical method validated for the determination of acetamiprid in OECD aqueous test medium**

|  | **Acetamiprid** |
| --- | --- |
| Specificity | The HPLC-UV method is sufficiently specific for the determination of acetamiprid.  Target analyte concentrations in controls did not exceed 30% of the method LOQ. No interference from co-eluting components at the retention time of the target analyte. |
| Calibration (type, number of data points) | Ten-point-linear calibration  y = 146604x + 3503.21, r2= 0.999 |
| Assessment of matrix effects is presented | Matrix effects were not assessed but matrix-matched calibration standards were used |
| Calibration range | 0.172 – 80.14 mg/L |
| Limit of determination/quantification | LOQ: 0.344 mg/L |

**Conclusion**

An analytical method for the determination of acetamiprid in OECD aqueous test medium was fully validated according to SANCO/3029/99 guidelines prior to March 21st 2021. The data provided exceed the minimum validation requirements given in Section 4.2 of SANTE/2020/12830 Rev. 1. The method should therefore be considered suitable for its intended purpose and acceptable for the determination of acetamiprid in OECD aqueous test medium.

* + - * 1. Analytical method ADM-026/6-22

Method validation ADM-026/6-22

|  |  |
| --- | --- |
| Comments of zRMS: | The study has been evaluated and accepted in Registration Report, Section 5 for CA3573 / Carnadine / Kestrel, Nufarm (August 2021).  Conclusions:  *The method was validated for the determination of acetamiprid in mesocosm water and sediment. The methods were developed non-GLP experiments, these experiments were completed before start of the respective GLP activities. The methods were validated under GLP conditions and according to the requirements of SANCO/3029/00 rev.4.*  *The limit of quantification is 10 ng a.s/L (mesocosm water) and 50 ng a.s/kg (mesocosm-sediment). All mean recovery values at fortification levels are within the required range of 70 – 110%, the overall RSD are below 20% for both mass transitions.*  *The analytical method NFM-002/6-22 for acetamiprid has been fully validated according to the requirements of SANCO/3029/00 rev.4. The method is therefore suitable for the determination of acetamiprid in the aqueous and sediment test media used in the corresponding mesocosm study.*  *The study is acceptable.* |

|  |  |
| --- | --- |
| Reference: | KCP 5.1.2/31 |
| Report | Validation of the analytical methods for water and sediment. Hennecke, N. 2020, ADM-026/6-22. Adama Reference No. 000106075 |
| Guideline(s): | SANCO 3029/99 rev. 4 |
| Deviations: | None |
| GLP: | Yes |
| Acceptability: | Yes |

An analytical method for the determination of acetamiprid in water and sediment was fully validated according to the requirements of SANCO/3029/99 rev. 4 prior to 1st March 2021 and used to support an ecotoxicology mesocosm study.

**A. Materials**

|  |  |
| --- | --- |
| Reference item: | Acetamiprid |
| Lot/Batch number: | BCBT9185 |
| Purity: | > 98% |
| CAS No.: | 135410-20-7 |
| Expiry date: | February 2022 |
| Standards for calibration | As above |
| Matrices: | Water and sediment |

1. **Sample preparation and processing**

Mesocosm water

The samples were prepared by dilution of 80 µL of the working solution and 80 µL of the solvent solution into separate 1.5 mL sample vials prepared with 40 µL acetonitrile and 800 µL aqueous test medium. The samples were then analysed by UHPLC-MS/MS.

Mesocosm sediment

Untreated sediment was centrifuged for 5 minutes at 4000 rpm. The supernatant was discarded. The pellet was mixed with a spoon and approximately 7.5 g corresponding to 5 g (dw) were weighed into each 50 mLPP vials. The samples were prepared by adding 50 µl of the internal standard stock solution and 50 µL of the working solutions. The untreated control samples were prepared by adding 50 µL of the internal standard stock solution and 50 µL acetonitrile. All samples were homogenized and allowed to stand for one hour before being analysed by UHPLC-MS/MS.

1. **Chromatographic parameters**

|  |  |
| --- | --- |
| HPLC- parameters  Instrumentation: | Waters Acquity UHPLC System |
| Column: | BEH C18; 50 × 2.1 mm; 1.7 µm |
| Mobile phase: | A: Water/methanol/formic acid (89.9 : 10 : 0.1 v:v:v) including 2 mM AcNH4  B: Methanol / formic acid (99.9: 0.1 v:v) including 2 mM AcNH4 |
| Flow rate: | 10.350 mL/min |
| Injection volume: | 10 µL |
|  |  |

|  |  |
| --- | --- |
| MS/MS parameters | Waters LC-MS/MS Xevo TQ-D Detector |
| Ionisation mode: | ES positive |
| Scan type. | MRM |
| Transitions: | m/z 223.0 → 126 (quantifier)  m/z 223.0 → 56 (qualifier)  m/z 226.0 →126 (internal standard) |

**Results and discussion**

The method used for the determination of acetamiprid in water and sediment was fully validated according to the requirements of the SANCO/3029/99 rev. 4 guidelines prior to 1st March 2021. The target analyte was determined using UHPLC-MS/MS with two transitions monitored during each analysis. The detector response was linear over the range 3.0 – 300 ng a.s/L and 10 – 1000 ng a.s/L (corresponding to 10 – 1000 ng a.s/kg) with associated correlation coefficients (r2) ≥ 0.999. The LOQ of the method is 10 ng a.s/L for water and 50 ng a.s/kg for sediment. Target analyte concentrations in controls were < 30% of the method LOQ. All mean recovery values and associated RSDs for both matrices meet the requirements of SANCO/3029/99 rev. 4 and SANTE/2020/12830 rev.1 guidelines and are summarised in the tables below.

**Table A 60: Method validation recovery data for the determination of acetamiprid in mesocosm water reported in study ADM-026-22**

| **Matrix** | **Analyte** | **Fortification level (ng a.s./L)** | **Mean  recovery (%)**  **n=5** | **RSD (%)** | **Comments** |
| --- | --- | --- | --- | --- | --- |
| Mesocosm water | Acetamiprid | 10 | 92.7 | 2.9 | - |
| 100 | 92.0 | 2.7 | - |

**Table A 61: Method validation recovery data for the determination of acetamiprid in mesocosm sediment reported in study ADM-026-22**

| **Matrix** | **Analyte** | **Fortification level (ng a.s/kg)** | **Mean  recovery (%)**  **n=5** | **RSD (%)** | **Comments** |
| --- | --- | --- | --- | --- | --- |
| Mesocosm sediment | Acetamiprid | 50 | 107 | 1.9 | - |
| 500 | 100 | 2.7 | - |

**Table A 62: Characteristics of the analytical method validated for the determination of acetamiprid in water and sediment**

|  | **Acetamiprid** |
| --- | --- |
| Specificity | A highly specific HPLC-MS/MS method was used for the determination of the target analyte. Two mass transitions (m/z 223.0 → 126, quantification and m/z 223.0 → 56, qualification) were monitored during each analysis. Target analyte concentrations in controls did not exceed 30% of the method LOQ. No interference from co-eluting components at the retention time of the target analyte. |
| Calibration (type, number of data points) | Multiple-point-linear calibration curve  Water: y = 1.03786x + 0.409849 r2 = 0.999  Sediment: y = 0.978249x – 1.0934 r2 = 0.999 |
| Assessment of matrix effects is presented | Matrix effects were assessed and found to be insignificant (< ± 20%) |
| Calibration range | 3.0 – 300 ng a.s/L  10 – 1000 ng a.s/L (corresponding to 10 to 1000 ng a.s/kg) |
| Limit of determination/quantification | Water: LOQ: 10 ng a.s/ L  Sediment: LOQ: 50 ng a.s/kg |

**Conclusion**

An analytical method for the determination of acetamiprid in mesocosm water and sediment was fully validated according to SANCO/3029/99 guidelines prior to March 21st 2021. The data provided exceed the minimum validation requirements given in Section 4.2 of SANTE/2020/12830 Rev. 1. The method should therefore be considered suitable for its intended purpose and acceptable for the determination of acetamiprid in water and sediment.

* + - * 1. Analytical method 14 10 48 078 B

Method validation 14 10 48 078 B

|  |  |
| --- | --- |
| Comments of zRMS: | The study has been evaluated and accepted in Registration Report, Section 5 for CA3573 / Carnadine / Kestrel, Nufarm (August 2021).  Conclusions:  *The method was successfully validated according to the guidance document SANCO/3029/99 rev. 4 and used for the analytical determination of acetamiprid in sugar solution.*  *The LOQ of acetamiprid was 272.1 mg/L.*  *The method is acceptable.* |

|  |  |
| --- | --- |
| Reference: | KCP 5.1.2/32 |
| Report | Chronic toxicity of MCW-2222 to the honeybee larvae *Apis mellifera* L. under laboratory conditions (*in vitro*). Kleebaum K. 2015, Study No. 141048078 B Adama Reference No. R-33835 |
| Guideline(s): | SANCO 3029/99 rev. 4 |
| Deviations: | None |
| GLP: | Yes |
| Acceptability: | Yes |

An analytical method for the determination of acetamiprid in sugar solution was fully validated according to the requirements of SANCO/3029/99 rev. 4 prior to 1st March 2021 and used to support an ecotoxicology study conducted on Bees.

**A. Materials**

|  |  |
| --- | --- |
| 1. Standards |  |
| Test item: | MCW-2222 |
| Batch no. | 611-280413-01 |
| Active substance: | Nominal: 200 g/L  Analysed: 202.7 g/L |
| Expiry date: | April 2015 |
| Reference item: | Acetamiprid |
| Lot/Batch number: | 772827 |
| Purity: | 99.9 % |
| CAS No.: | 135410-20-7 |
| Expiry date: | August 2017 |
| Standards for calibration | As above |
| Matrix: | Sugar solution (bee feed; 18% (w/v) glucose, 18% (w/v) fructose, 4% (w/v) yeast) |

1. **Sample preparation and processing**

The samples were allowed to thaw at room temperature, diluted by a factor of 50 with the dilution medium containing a water: methanol mixture (50/50/v/v). The control samples were analysed without any further dilution. All samples were analysed using HPLC-UV DAD. Target analyte confirmation was achieved by comparing UV absorbance spectra produced by standards and samples.

1. **Chromatographic parameters**

|  |  |
| --- | --- |
| HPLC- parameters  Instrumentation: | Shimadzu LC-10 HPLC |
| Column: | Phenomenex Kinetex C18, 100 × 2.1mm, 2.6 µm |
| Mobile phase: | A: water with 0.1% (v/v) phosphoric acid (85%)  B: Acetonitrile with 0.1 % (v/v) phosphoric acid (85%) |
| Flow rate: | 0.4 mL/min |
| Detection: | UV DAD at 246 nm |
|  |  |

**Results and discussion**

The method used for the determination of residues of acetamiprid in sugar solution was fully validated according to the requirements of the SANCO/3029/99 rev. 4 guidelines prior to 1st March 2021. The target analyte was determined using HPLC-UV DAD and monitoring at 246 nm. The detector response was linear over the range from 4.24 – 12.84 mg/L with an associated correlation coefficient (r2) ≥ 0.999. The LOQ of the method is 272.1 mg/L. Target analyte concentrations in controls were < 30% of the method LOQ. All mean recovery values and associated RSDs meet the requirements of SANCO/3029/99 rev. 4 and SANTE/2020/12830 rev.1 guidelines and are summarised in the table below.

**Table A 63: Method validation recovery data for the determination of acetamiprid in sugar solution reported in study 141048078 B**

| **Matrix** | **Analyte** | **Fortification level (mg/L)** | **Mean  recovery (%)**  **n=5** | **RSD (%)** | **Comments** |
| --- | --- | --- | --- | --- | --- |
| Sugar solution | Acetamiprid | 272.1 | 91 | 0.2 | - |
| 537.8 | 95 | 0.2 | - |

**Table A 64: Characteristics of the analytical method validated for the determination of acetamiprid in sugar solution**

|  | **Acetamiprid** |
| --- | --- |
| Specificity | The HPLC-UV method is sufficiently specific for the determination of acetamiprid.  Target analyte concentrations in controls did not exceed 30% of the method LOQ. No interference from co-eluting components at the retention time of the target analyte. |
| Calibration (type, number of data points) | Five-point-linear calibration  y = 141457x - 4576.44, r2 = 0.999 |
| Assessment of matrix effects is presented | Matrix effects were not taken into account because validation samples and test samples from the biological phase were diluted by a factor of 50.The influence of the matrix solution is therefore regarded as negligible when using UV-detection. |
| Calibration range | 4.24 – 12.84 mg/L |
| Limit of determination/quantification | LOQ: 272.1 mg/L |

**Conclusion**

An analytical method for the determination of acetamiprid in sugar solution was fully validated according to SANCO/3029/99 guidelines prior to March 21st 2021. The data provided exceed the minimum validation requirements given in Section 4.2 of SANTE/2020/12830 Rev. 1. The method should therefore be considered suitable for its intended purpose and acceptable for the determination of acetamiprid in sugar solution.

* + - * 1. Analytical method 215-2014

Method validation 215-2014

|  |  |
| --- | --- |
| Comments of zRMS: | The study has been evaluated and accepted in Registration Report, Section 5 for CA3573 / Carnadine / Kestrel, Nufarm (August 2021).  Conclusions:  *The analytical method was successfully validated according to the guidance document SANCO/3029/99 rev. 4 for bee bread, flowers, nectar and pollen by10 spiked samples: 5 recovery experiments fortified at the LOQ level and 5 recovery experiments fortified at 10 times the LOQ level. 2 control samples and a reagent blank were prepared.*  *Honey and nectar are considered as similar matrices. So no validation on honey was realized.*  *The LOQ of acetamiprid and acetamiprid-N-desmethyl is 0.01 mg/kg.*  *The study is acceptable.* |

|  |  |
| --- | --- |
| Reference: | KCP 5.1.2/33 |
| Report | Field Study to Evaluate Potential Side Effects of the product MCW-2222 (acetamiprid 200 g/L) on Brood Development, Foraging Activity, Mortality and Behaviour of Adult Honeybees *Apis mellifera* L. (Hymenoptera: Apidae) Following Application after Bee-Flight on *Phacelia tanacetifolia*. Molitor, C. 2015, Study No. 215-2014 + Amendment 1. Adama Reference No. R-34877. |
| Guideline(s): | SANCO 3029/99 rev. 4 |
| Deviations: | None |
| GLP: | Yes |
| Acceptability: | Yes |

An analytical method for the determination of acetamiprid and acetamiprid-N-desmethyl in bee bread, flowers, nectar/honey and pollen was fully validated according to the requirements of SANCO/3029/99 rev. 4 prior to 1st March 2021 and used to support an ecotoxicology on bee products.

**A. Materials**

|  |  |
| --- | --- |
| Test item: | MCW-2222 |
| Batch no. | 93191024 |
| Active substance: | Nominal: 200 g/L  Analysed: 198 g/L |
| Expiry date: | October 2015 |
| Standards for calibration | As above |
| Matrices: | Bee bread, flowers, nectar/honey and pollen |

1. **Sample preparation and processing**

For bee bread, flowers and nectar/honey, residues of acetamiprid and acetamiprid-N-desmethyl were extracted from samples in frozen conditions by agitation in acetonitrile and ultra-pure water. The extracts were then purified by dispersive solid phase extraction (SPE). For pollen residues of acetamiprid and acetamiprid-N-desmethyl were extracted from pollen with ethyl acetate using Dionex ASE 300 automatic extractor. All sample extracts were then analysed using HPLC-MS/MS

1. **Chromatographic parameters**

|  |  |
| --- | --- |
| HPLC- parameters  Instrumentation: | Shimadzu API 4000 and API 6000 LC-MS/MS |
| Column: | C18, 100 × 3.0 mm, 2.5 µm |
| Mobile phase: | A: Ultra-pure water/glacial acetic acid (100/0.1, v/v) + 5 nM ammonium acetate  B: Methanol/glacial acetic acid (100/0.1, v/v) + 5 nM ammonium acetate |
| Flow rate: | 0.7 ml/min |
| Injection volume: | 20 µL |
|  |  |

|  |  |
| --- | --- |
| MS/MS parameters | API 5500 QTrap |
| Ionisation mode: | ES positive |
| Scan type. | MRM |
| Transitions: | m/z 223→ 126 (quantification)  m/z 223 → 73 (confirmation) |

**Results and discussion**

The method used for the determination of acetamiprid and acetamiprid-N-desmethyl in bee bread, flowers, nectar/honey and pollen was fully validated according to the requirements of the SANCO/3029/99 rev. 4 guidelines prior to 1st March 2021. The target analyte was determined using HPLC-MS/MS with two transitions monitored during each analysis. The detector response was linear over the range 0.3 – 1 0 µg/L for flowers, 1.5 – 50 µg/L for nectar and honey, 3.0 – 100 µg/L for bee bread and pollen with associated correlation coefficients (r) ≥ 0.99. The LOQ of the method is 0.01 mg a.s/kg for all matrices. Target analyte concentrations in controls were < 30% of the method LOQ. All mean recovery values and associated RSDs for matrix-analyte combinations meet the requirements of SANCO/3029/99 rev. 4 and SANTE/2020/12830 rev.1 guidelines and are summarised in the tables below.

**Table A 65: Method validation recovery data for the determination of acetamiprid in bee bread, flowers, nectar/honey and pollen reported in study 215-2014**

| **Matrix** | **Analyte** | **Fortification level**  **(mg/kg)** | **Mean  recovery (%)**  **n=5** | **RSD (%)** | **Comments** |
| --- | --- | --- | --- | --- | --- |
| Quantification transition m/z 223 → 126 | | | | | |
| Bee bread | Acetamiprid | 0.01 | 90 | 7 | - |
| 0.1 | 101 | 2 | - |
| Flowers | 0.01 | 86 | 7 | - |
| 0.1 | 89 | 3 | - |
| Nectar/honey | 0.01 | 76 | 5 | - |
| 0.1 | 86 | 3 | - |
| Pollen | 0.01 | 93 | 6 | - |
| 0.1 | 93 | 6 | - |

**Table A 66: Method validation recovery data for the determination of acetamiprid-N-desmethyl in bee bread, flowers, nectar/honey and pollen reported in study 215-2014**

| **Matrix** | **Analyte** | **Fortification level**  **(mg/kg)** | **Mean  recovery (%)**  **n=5** | **RSD (%)** | **Comments** |
| --- | --- | --- | --- | --- | --- |
| Quantification transition m/z 223 → 126 | | | | | |
| Bee bread | acetamiprid-N-desmethyl | 0.01 | 93 | 4 | - |
| 0.1 | 89 | 2 | - |
| Flowers | 0.01 | 88 | 3 | - |
| 0.1 | 95 | 4 | - |
| Nectar/honey | 0.01 | 86 | 3 | - |
| 0.1 | 87 | 4 | - |
| Pollen | 0.01 | 90 | 5 | - |
| 0.1 | 86 | 5 | - |

**Table A 67: Characteristics of the analytical method validated for the determination of acetamiprid an acetamiprid-N-desmethyl in bee bread, flowers, nectar/honey and pollen**

|  | **Acetamiprid** | **Acetamiprid-N-desmethyl** |
| --- | --- | --- |
| Specificity | A highly specific HPLC-MS/MS method was used for the determination of the target analytes. Two mass transitions (m/z 223 → 126, quantification and m/z 223 → 73, confirmation) were monitored during each analysis. Target analyte concentrations in controls did not exceed 30% of the method LOQ. | |
| Calibration (type, number of data points) | Five-point-linear calibration curves  Flowers: y = 6129.38x + 424.97  r= 0.9998  Pollen: y = 33027.95x + 2595.56  r = 1.00  Nectar: y = 7472.18x + 5680.45  r = 0.9996  Honey: y= 5152.76x + 1764.30  r= 0.9998    Bee bread: y = 2111.78x + 2403.75 r = 0.9999 | Five-point-linear calibration curves  Flowers: y = 4054.24x -22.48  r = 0.9993  Pollen: y = 17883.10x + 6454.25  r = 1.00  Nectar: y = 5227.80x + 289.79  r = 0.9999  Honey: y = 3303.73x – 642.70  r = 0.9987  Bee bread: y = 1667.49x + 1141.08 r = 0.9997 |
| Assessment of matrix effects is presented | Matrix effects were not assessed but matrix-matched calibration was for the quantitative determination of the target analyte all matrices. | |
| Calibration range | Flowers: 0.3 – 10 µg/L for  Nectar and honey: 1.5 – 50 µg/L for  Bee bread and pollen: 3.0 – 100 µg/L for | |
| Limit of determination/quantification | All matrices LOQ: 0.01 mg a.s/kg | |

**Conclusion**

An analytical method for the determination of acetamiprid and acetamiprid-N-desmethyl in bee bread, flowers, nectar/honey and pollen was fully validated according to SANCO/3029/99 guidelines prior to March 21st 2021. The data provided exceed the minimum validation requirements given in Section 4.2 of SANTE/2020/12830 Rev. 1. The method should therefore be considered suitable for its intended purpose and acceptable for the determination of acetamiprid and acetamiprid-N-desmethyl in bee bread, flowers, nectar/honey and pollen.

* + - * 1. Analytical method 230-2015

Method validation 230-2015

|  |  |
| --- | --- |
| Comments of zRMS: | The study has been evaluated and accepted in Registration Report, Section 5 for CA3573 / Carnadine / Kestrel, Nufarm (August 2021).  Conclusions:  *The objective of the analytical phase was to determine the residues of acetamiprid and its metabolite acetamiprid-N-desmethyl in pollen, honey, nectar, flower, bee bread and wax.*  *For wax, the analytical method was fully validated within the analytical phase by 10 spiked samples: 5 recovery experiments fortified at the LOQ level and 5 recovery experiments fortified at 10 times the LOQ level. 2 control samples and a reagent blank were prepared.*  *For pollen, bee bread, flowers and nectar, the analytical method used was previously validated during another analytical phase in a parallel study number 215-2014, S. Lefresne, 2014 performed by 10 spiked samples: 5 recovery experiments fortified at the LOQ level and 5 recovery experiments fortified at 10 times the LOQ level. 2 control samples and a reagent blank were prepared.*  *Honey and nectar were considered as similar matrices. So no validation on honey were realized.*  *The LOQ: 0.01 mg/kg for each reference item and for each specimen.*  *The method was successfully validated according to the guidance document SANCO/3029/99 rev. 4.*  *The study is acceptable.* |

|  |  |
| --- | --- |
| Reference: | KCP 5.1.2/34 |
| Report | Field study to evaluate potential side effects of MCW-2222 on brood development, foraging activity, mortality and behaviour of adult honeybees (*Apis mellifera*) on oilseed rape. Molitor, C. 2015, Study No. 230-2015. Adama Reference No. R-35844. |
| Guideline(s): | SANCO 3029/99 rev. 4 |
| Deviations: | None |
| GLP: | Yes |
| Acceptability: | Yes |

An analytical method for the determination of acetamiprid and acetamiprid-N-desmethyl in bee bread, flowers nectar/honey, pollen and wax was fully validated according to the requirements of SANCO/3029/99 rev. 4 prior to 1st March 2021 and used to support an ecotoxicology on bee products.

**A. Materials**

|  |  |
| --- | --- |
| Reference item 1: | Acetamiprid |
| Batch no. | 20202 |
| Purity:  Expiry date: | 98.1 ± 0.5%  February 2016 |
| Reference item 2: | Acetamiprid-*N*-desmethyl |
| Batch No.: | SZBE066XV |
| Purity: | 99.8 % |
| Expiry date: | March 2017 |
| Matrices: | Bee bread, flowers and nectar/honey, pollen and wax |

1. **Sample preparation and processing**

For bee bread, flowers nectar/honey, pollen and wax, residues of acetamiprid and acetamiprid-N-desmethyl were extracted from samples in frozen conditions by agitation in acetonitrile and ultra-pure water. The extracts were then purified by dispersive solid phase extraction (SPE). For pollen residues of acetamiprid and acetamiprid-N-desmethyl were extracted from pollen with ethyl acetate using Dionex ASE 300 automatic extractor. All sample extracts were then analysed using HPLC-MS/MS

1. **Chromatographic parameters**

|  |  |
| --- | --- |
| HPLC- parameters  Instrumentation: | Shimadzu API 4000 and API 6000 LC-MS/MS |
| Column: | C18 Hydro, 100 × 3.0 mm, 2.5 µm |
| Mobile phase: | A: Ultra-pure water/glacial acetic acid (100/0.1, v/v) + 5 nM ammonium acetate  B: Methanol/glacial acetic acid (100/0.1, v/v) + 5 nM ammonium acetate |
| Flow rate: | 0.7 ml/min |
| Injection volume: | 20 µL |
|  |  |

|  |  |
| --- | --- |
| MS/MS parameters | API 5500 QTrap |
| Ionisation mode: | ES positive |
| Scan type. | MRM |
| Transitions: | m/z 223→ 126 (quantification)  m/z 223 → 73 (confirmation) |

**Results and discussion**

The method used for the determination of acetamiprid and acetamiprid-N-desmethyl in bee bread, flowers nectar/honey, pollen and wax was fully validated according to the requirements of the SANCO/3029/99 rev. 4 guidelines prior to 1st March 2021. The target analyte was determined using HPLC-MS/MS with two transitions monitored during each analysis. The detector response was linear over the range 0.3 – 1 0 µg/L for flowers, 3.0 – 100 µg/L for pollen, 1.5 – 50 µg/L for nectar, 1.5 – 30 µg/L for honey and 3.0 – 100 µg/L for bee bread and pollen with associated correlation coefficients (r) ≥ 0.99. The LOQ of the method is 0.01 mg a.s/kg for all matrices. Target analyte concentrations in controls were < 30% of the method LOQ. All mean recovery values and associated RSDs for matrix-analyte combinations meet the requirements of SANCO/3029/99 rev. 4 and SANTE/2020/12830 rev.1 guidelines and are summarised in the tables below.

**Table A 68: Method validation recovery data for the determination of acetamiprid in bee bread, flowers nectar/honey, pollen and wax reported in study 230-2015.**

| **Matrix** | **Analyte** | **Fortification level**  **(mg/kg)** | **Mean  recovery (%)**  **n=5** | **RSD (%)** | **Comments** |
| --- | --- | --- | --- | --- | --- |
| Quantification transition m/z 223 → 126 | | | | | |
| Bee bread | Acetamiprid | 0.01 | 90 | 7 | - |
| 0.1 | 101 | 2 | - |
| Flowers | 0.01 | 86 | 7 | - |
| 0.1 | 89 | 3 | - |
| Nectar/honey | 0.01 | 76 | 5 | - |
| 0.1 | 86 | 3 | - |
| Pollen | 0.01 | 93 | 6 | - |
| 0.1 | 93 | 6 | - |
| Wax | 0.01 | 93 | 6 | - |
| 0.1 | 94 | 7 | - |

**Table A 69: Method validation recovery data for the determination of acetamiprid-N-desmethyl in bee bread, flowers nectar/honey, pollen and wax reported in study 230-2015**

| **Matrix** | **Analyte** | **Fortification level**  **(mg/kg)** | **Mean  recovery (%)**  **n=5** | **RSD (%)** | **Comments** |
| --- | --- | --- | --- | --- | --- |
| Quantification transition m/z 223 → 126 | | | | | |
| Bee bread | acetamiprid-N-desmethyl | 0.01 | 93 | 4 | - |
| 0.1 | 89 | 2 | - |
| Flowers | 0.01 | 88 | 3 | - |
| 0.1 | 95 | 4 | - |
| Nectar/honey | 0.01 | 86 | 3 | - |
| 0.1 | 87 | 4 | - |
| Pollen | 0.01 | 90 | 5 | - |
| 0.1 | 86 | 5 | - |
| Wax | 0.01 | 90 | 7 | - |
| 0.1 | 89 | 8 | - |

**Table A 70: Characteristics of the analytical method validated for the determination of acetamiprid an acetamiprid-N-desmethyl in bee bread, flowers nectar/honey, pollen and wax**

|  | **Acetamiprid** | **Acetamiprid-N-desmethyl** |
| --- | --- | --- |
| Specificity | A highly specific HPLC-MS/MS method was used for the determination of the target analytes. Two mass transitions (m/z 223 → 126, quantification and m/z 223 → 73, confirmation) were monitored during each analysis. Target analyte concentrations in controls did not exceed 30% of the method LOQ. | |
| Calibration (type, number of data points) | Five-point-linear calibration curves  Flowers: y = 127675.05x – 1254.20  r = 0.9999  Pollen: y = 208107.32x + 80131.31 r= 0.9999  Nectar: y = 17552.64x +31898.13  r = 0.9973  Honey: y = 155731.04x -21445.38  r= 0.9996    Bee bread: y = 58150.61x + 5672.63 r= 0.9994  Wax: y = 196261.67x + 14116.74  r = 0.9996 | Five-point-linear calibration curves  Flowers: y = 72462.78x + 2728.20  r = 0.9996  Pollen: y = 145592.01x +37369.42 r= 0.9995  Nectar: y = 101699.51x + 9307.13  r= 0.9989  Honey: y = 90882.09x – 12532.93  r = 0.9997  Bee bread: y = 31660.29x + 12219.80  r= 0.9994  Wax: y = 153856.92.67x + 5500.59  r = 0.9996 |
| Assessment of matrix effects is presented | Matrix effects were not assessed but matrix-matched calibration was for the quantitative determination of the target analyte all matrices. | |
| Calibration range | Flowers 0.3 – 10 µg/L  Pollen: 3.0 – 100 µg/L  Nectar: 1.5 – 50 µg/L  Honey: 1.5 – 30 µg/L  Bee bread and pollen: 3.0 – 100 µg/L  Wax: 0.3 – 10 µg/L | |
| Limit of determination/quantification | LOQ all matrices: 0.01 mg a.s/kg | |

**Conclusion**

An analytical method for the determination of acetamiprid and acetamiprid-N-desmethyl in bee bread, flowers nectar/honey, pollen and wax was fully validated according to SANCO/3029/99 guidelines prior to March 21st 2021. The data provided exceed the minimum validation requirements given in Section 4.2 of SANTE/2020/12830 Rev. 1. The method should therefore be considered suitable for its intended purpose and acceptable for the determination of acetamiprid and acetamiprid-N-desmethyl in bee bread, flowers nectar/honey, pollen and wax.

* + - * 1. Analytical method 307SRES15C02

Method validation 307SRES15C02

|  |  |
| --- | --- |
| Comments of zRMS: | The study has been evaluated and accepted in Registration Report, Section 5 for CA3573 / Carnadine / Kestrel, Nufarm (August 2021).  Conclusions:  *For nectar and larvae, the method was successfully validated according to the guidance document SANCO/3029/99 rev. 4. For each specimen, the LOQ of the method was the lowest validated level where a mean recovery within the range 70-110% and with a RSD less or equal to 20% could be obtained. The LOQ of acetamiprid was 0.01 mg/kg.*  *The method is acceptable.* |

|  |  |
| --- | --- |
| Reference: | KCP 5.1.2/35 |
| Report | Effects and determination of residues of acetamiprid 200 SL on the Honeybee (*Apis mellifera L*) Brood in citrus, under field conditions, in Spain 2015. Aucejo, S. 2015, Study No. 307SRES15C02. Adama Reference No. R-35955 |
| Guideline(s): | SANCO 3029/99 rev. 4 |
| Deviations: | None |
| GLP: | Yes |
| Acceptability: | Yes |

An analytical method for the determination of acetamiprid in nectar and larvae was fully validated according to the requirements of SANCO/3029/99 rev. 4 prior to 1st March 2021 and used to support an ecotoxicology conducted on bees. The analytical method was also used to confirm target analyte concentrations in the spray solutions used in the biological phase of the study.

**A. Materials**

|  |  |
| --- | --- |
| Reference item: | Acetamiprid |
| Lot/Batch number: | 20202 |
| Purity: | > 98.1 ± 0.5% |
| CAS No.: | 135410-20-7 |
| Expiry date: | 02 February 2016 |
| Standards for calibration | As above |
| Matrices: | Nectar and bee larvae |

1. **Sample preparation and processing**

Nectar and larvae

Either 1 g (larvae) or 2 g (nectar) of frozen sample were weighed into a 50 mL tube. For larvae, 5 mL water and 5 mL acetonitrile were added, for nectar 10 mL water and 10 mL acetonitrile were added. The samples were then shaken manually before being placed on a horizontally shaker for 20 minutes. The extracts were transferred into a QuEChERS tube containing MgSO4/NaCl buffer salts and shaken manually before being centrifuged for approx. 5 min at 4000 rpm. The organic phase was diluted with ultrapure water, filtered through a 0.45µm Nylon filter and an aliquot analysed using HPLC-MS/MS.

Spray solution

The sample was homogenised by shaking mechanically for 15 minutes. 1 mL of the sample was transferred into a 10 mL (untreated) or 100 mL (treated sample) measuring cylinder containing a methanol/ultra-pure water mix (50/50 v/v). For treated samples the extract was adjusted to 50 mL with acetone for analyses. All samples were diluted to be within the calibration range and analysed using HPLC-MS/MS.

1. **Chromatographic parameters**

|  |  |
| --- | --- |
| HPLC- parameters  Instrumentation: | API 4000 LC-MS/MS |
| Column: | Phenomenex Hydro RP C18; 100 × 3.0 mm; 2.5 µm |
| Mobile phase: | A: Ultra-pure water/glacial acetic acid (100/0.1, v/v) + 5 nM ammonium acetate B: Methanol/glacial acetic acid (100/0.1, v/v) + 5 nM ammonium acetate |
| Flow rate: | 0.7 mL/min |
| Injection volume: | 20 µL |
|  |  |

|  |  |
| --- | --- |
| MS/MS parameters | Appleied Biosystems 5500 QTrap |
| Ionisation mode: | ES positive |
| Scan type. | MRM |
| Transitions: | m/z 223 → 126 (quantifier)  m/z 223 → 90 (qualifier) |

**Results and discussion**

The method used for the determination of acetamiprid in nectar and larvae was fully validated according to the requirements of the SANCO/3029/99 rev. 4 guidelines prior to 1st March 2021. The target analyte was determined using HPLC-MS/MS with two transitions monitored during each analysis. The detector response was linear over the range 0.3 – 12 µg/L for nectar and 0.3 – 50 µg/L for larvae with associated correlation coefficients (r2) ≥ 0.999. The LOQ of the method is 0.01mg/L. Target analyte concentrations in controls were < 30% of the method LOQ. All mean recovery values and associated RSDs for both matrices meet the requirements of SANCO/3029/99 rev. 4 and SANTE/2020/12830 rev.1 guidelines and are summarised in the tables below.

**Table A 71: Method validation recovery data for the determination of acetamiprid in nectar and larvae reported in study 307SRES15C02**

| **Matrix** | **Analyte** | **Fortification level (mg/kg)** | **Mean  recovery (%)**  **n=5** | **RSD (%)** | **Comments** |
| --- | --- | --- | --- | --- | --- |
| Nectar | Acetamiprid | 0.01 | 75 | 3 | - |
| 0.1 | 80 | 6 | - |
| Larvae | 0.01 | 104 | 6 | - |
| 0.1 | 110 | 5 | - |

**Table A 72: Characteristics of the analytical method validated for the determination of acetamiprid in nectar and larvae**

|  | **Acetamiprid** |
| --- | --- |
| Specificity | A highly specific HPLC-MS/MS method was used for the determination of the target analyte. Two mass transitions (m/z 223→ 126, quantification and m/z 223→ 90, qualification) were monitored during each analysis. Target analyte concentrations in controls did not exceed 30% of the method LOQ. No interference from co-eluting components at the retention time of the target analyte. |
| Calibration (type, number of data points) | Seven-point-linear calibration curves  Nectar: y = 161766.14x + 9397.78, r2= 0.999 ()  Larvae: y = 160947.03 + 13365.45, r2= 1 () |
| Assessment of matrix effects is presented | Matrix effects were not assessed but matrix-matched calibration was for the quantitative determination of the target analyte all matrices. |
| Calibration range | Nectar: 0.3 – 12 µg/L  Larvae: 0.3 – 50 µg/L  Spray solution: 0.3 – 50 µg/L |
| Limit of determination/quantification | LOQ Nectar and larvae: 0.01mg/kg |

**Conclusion**

An analytical method for the determination of acetamiprid in nectar and larvae was fully validated according to SANCO/3029/99 guidelines prior to March 21st 2021. The data provided exceed the minimum validation requirements given in Section 4.2 of SANTE/2020/12830 Rev. 1. The method should therefore be considered suitable for its intended purpose and acceptable for the determination of acetamiprid in nectar and larvae.

* + - * 1. Analytical method 14 10 48 002 P

Method validation 14 10 48 002 P

|  |  |
| --- | --- |
| Comments of zRMS: | The study has been evaluated and accepted in Registration Report, Section 5 for CA3573 / Carnadine / Kestrel, Nufarm (August 2021).  Conclusions:  *The method was successfully validated according to the guidance document SANCO/3029/99 rev. 4 and used for the analytical determination of acetamiprid in water.*  *The LOQ of acetamiprid was 130 mg/L.*  *The method is acceptable.* |

|  |  |
| --- | --- |
| Reference: | KCP 5.1.2/36 |
| Report | Terrestrial plant test with MCW-2222: Vegetative vigour test. Friedrich, S. 2014, Study No. 14 10 48 002 P. Adama Reference No. 000024860 |
| Guideline(s): | SANCO 3029/99 rev. 4 |
| Deviations: | None |
| GLP: | Yes |
| Acceptability: | Yes |

An analytical method for the determination of acetamiprid in water was fully validated according to the requirements of SANCO/3029/99 rev. 4 prior to 1st March 2021 and used to support an ecotoxicology study conducted on non-target plants.

**A. Materials**

|  |  |
| --- | --- |
| 1. Standards |  |
| Test item: | MCW-2222 |
| Batch no. | 611-280413-01 |
| Active substance: | Nominal: 200 g/L  Analysed: 202.7 g/L |
| Expiry date: | April 2015 |
| Reference item: | Acetamiprid |
| Lot/Batch number: | 772827 |
| Purity: | 99.9 % |
| CAS No.: | 135410-20-7 |
| Expiry date: | August 2017 |
| Standards for calibration | As above |
| Matrix: | Water |

1. **Sample preparation and processing**

Method validation samples were prepared by fortifying water with appropriate amounts of the test item. The samples were then diluted 1/20 with water and analysed using HPLC-UV

1. **Chromatographic parameters**

|  |  |
| --- | --- |
| HPLC- parameters  Instrumentation: | Shimadzu LC-10 HPLC |
| Column: | Phenomenex Kinetex C18, 100 × 2.1 mm, 2.6 µm |
| Mobile phase: | A: 250 mL methanol, 750 mL water, 1 mL phosphoric acid  B: 900 mL methanol, 100 mL water, 1 mL phosphoric acid |
| Flow rate: | 0.4 mL/min |
| Injection volume: | 5 µL |
| Detection: | UV at 245 nm |
|  |  |

**Results and discussion**

The method used for the determination of residues of acetamiprid in water was fully validated according to the requirements of the SANCO/3029/99 rev. 4 guidelines prior to 1st March 2021. The target analyte was determined using HPLC-UV and monitoring at 245 nm. The detector response was linear over the range from 4.8 – 80.7 mg/L with an associated correlation coefficient (r2) ≥ 0.999. The LOQ of the method is 130.4 mg/L. Target analyte concentrations in controls were < 30% of the method LOQ. All mean recovery values and associated RSDs meet the requirements of SANCO/3029/99 rev. 4 and SANTE/2020/12830 rev.1 guidelines and are summarised in the table below.

**Table A 73: Method validation recovery data for the determination of acetamiprid in water reported in study 141048002 W.**

| **Matrix** | **Analyte** | **Fortification level (mg/L)** | **Mean  recovery (%)**  **n=5** | **RSD (%)** | **Comments** |
| --- | --- | --- | --- | --- | --- |
| Water | Acetamiprid | 130.4 | 99.3 | 0.4 | - |
| 1340 | 99.9 | 0.4 | - |

**Table A 74: Characteristics of the analytical method validated for the determination of acetamiprid in water**

|  | **Acetamiprid** |
| --- | --- |
| Specificity | The HPLC-UV method is sufficiently specific for the determination of acetamiprid in water.  Target analyte concentrations in controls did not exceed 30% of the method LOQ. No interference from co-eluting components at the retention time of the target analyte. |
| Calibration (type, number of data points) | Five-point-linear calibration  y = 1.37038e005x + 0.144862 r2= 0.999 |
| Assessment of matrix effects is presented | Matrix effects were not assessed but matrix-matched calibration standards were used |
| Calibration range | 4.8 – 80.7 mg/L |
| Limit of determination/quantification | LOQ: 130.4 mg/L |

**Conclusion**

An analytical method for the determination of acetamiprid in water was fully validated according to SANCO/3029/99 guidelines prior to March 21st 2021. The data provided exceed the minimum validation requirements given in Section 4.2 of SANTE/2020/12830 Rev. 1. The method should therefore be considered suitable for its intended purpose and acceptable for the determination of acetamiprid in water.

* + - * 1. Analytical method 102461251

Method validation 102461251

|  |  |
| --- | --- |
| Comments of zRMS: | An analytical method for the determination of 6-Chloronicotinic acid in M4 aqueous test medium was validated with LOQ was 10 mg test item/L.  The method is acceptable and fit for purpose. |

|  |  |
| --- | --- |
| Reference: | KCP 5.1.2/37 |
| Report | IC-0: Acute Toxicity to Larvae of *Chironomus riparius* in a Static 48-hour Immobilisation Limit-Test. Hengsberger, A. and Wydra, V. 2015, Study No. 102461251. Adama Reference No. R-36482 |
| Guideline(s): | SANCO 3029/99 rev. 4 |
| Deviations: | None |
| GLP: | Yes |
| Acceptability: | Yes |

An analytical method for the determination of acetamiprid in 6-Chloronicotinic acid in M4 aqueous test medium was fully validated according to the requirements of SANCO/3029/99 rev. 4 prior to 1st March 2021 and used to support an ecotoxicology study conducted on Chironomids.

**A. Materials**

|  |  |
| --- | --- |
| 1. Standards |  |
| Test item: | 6-Chloronicotinic acid (IC-0) |
| CAS No:  Batch No: | 5326-23-8  516-070-00 |
| Purity: | 99.4% |
| Expiry date: | May 03 2015 |
| Reference item: | As above |
| Standards for calibration | As above |
| Matrix: | M4 aqueous test medium |

1. **Sample preparation and processing**

The samples were allowed to thaw at room temperature, shaken well, treated with ultrasound and were analysed without any further preparation.

1. **Chromatographic parameters**

|  |  |
| --- | --- |
| HPLC- parameters  Instrumentation: | LaChrom, Merck Hitachi HPLC |
| Column: | US RP 18, 250 × 4 mm, 5 μm |
| Mobile phase: | A: Acetonitrile/water (95/5, v/v)  B: Water containing 0.1 % phosphoric acid |
| Flow rate: | 1 mL/min |
| Injection volume: | 10 µL |
| Detection: | UV at 268 nm |
|  |  |

**Results and discussion**

The method used for the determination of residues of 6-Chloronicotinic acid in M4 aqueous test medium was fully validated according to the requirements of the SANCO/3029/99 rev. 4 guidelines prior to 1st March 2021. The target analyte was determined using HPLC-UV and monitoring at 268 nm. The detector response was linear over the range from 5 – 150 mg/L with an associated correlation coefficient (r2) ≥ 0.999. The LOQ of the method is 10 mg/L. Target analyte concentrations in controls were < 30% of the method LOQ. All mean recovery values and associated RSDs meet the requirements of SANCO/3029/99 rev. 4 and SANTE/2020/12830 rev.1 guidelines and are summarised in the table below.

**Table A 75: Method validation recovery data for the determination of acetamiprid in M4 aqueous test medium reported in study 102461251**

| **Matrix** | **Analyte** | **Fortification level (mg/L)** | **Mean  recovery (%)**  **n=5** | **RSD (%)** | **Comments** |
| --- | --- | --- | --- | --- | --- |
| M4 Aqueous medium | Acetamiprid | 10 | 106 | 1 | - |
| 120 | 101 | 1 | - |

**Table A 76: Characteristics of the analytical method validated for the determination of acetamiprid in M4 aqueous test medium**

|  | **Acetamiprid** |
| --- | --- |
| Specificity | The HPLC-UV method is sufficiently specific for the determination of acetamiprid.  Target analyte concentrations in controls did not exceed 30% of the method LOQ. No interference from co-eluting components at the retention time of the target analyte. |
| Calibration (type, number of data points) | Seven-point-linear calibration  y = 58963x - 39834, r2= 0.9998 |
| Assessment of matrix effects is presented | Matrix effects were not assessed but matrix-matched calibration standards were used |
| Calibration range | 5 – 150 mg/L |
| Limit of determination/quantification | LOQ: 10 mg/L  LOD: 0.1 mg/L |

**Conclusion**

An analytical method for the determination of 6-Chloronicotinic acid in M4 aqueous test medium was fully validated according to SANCO/3029/99 guidelines prior to March 21st 2021. The data provided exceed the minimum validation requirements given in Section 4.2 of SANTE/2020/12830 Rev. 1. The method should therefore be considered suitable for its intended purpose and acceptable for the determination of 6-Chloronicotinic acid in M4 aqueous test medium.

* + 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)

Matching data have been obtained and provided by ADAMA. These data were submitted to the RMS Netherlands in order to demonstrate access to a complete data package, according to Reg. (EU) 283/2013 and for the data matching process. The RMS Opinion on GLP compliance, guidance compliance and equivalence of the endpoints will be provided as soon as Data Matching process is finalized. Additionally, ADAMA has provided ILVs for the determination of acetamiprid residues in plant material with high water content, plant material with high acid content, plant material with high oil content and dry plant material with high protein content. A detailed evaluation of the new studies can be found below.

* + - * 1. Analytical method B13-M1-A-01

Method validation RES-00418

|  |  |
| --- | --- |
| Comments of zRMS: | An independent laboratory validation (ILV) of analytical method B13-M1-A-01 (Adama study code R-33645) for the determination of residues of acetamiprid in dry bean (seed) (high protein content), lettuce (high water content), oilseed rape seed (high oil content), orange pulp and orange peel (high acid content) with an LOQ of 0.01 mg/kg by LC-MS/MS was conducted.  The independent laboratory validation met the criteria detailed in SANTE/2020/12830, Rev.1. |

|  |  |
| --- | --- |
| Reference: | KCP 5.2/01 |
| Report | Independent laboratory validation of analytical method B13-M1-A-01 (Sponsor code R-33645) for determination of Acetamiprid in food of plant origin. Brown, S. 2022, Report No. RES-00418, Sponsor Reference No. 000111705 |
| Guideline(s): | SANTE/2020/12830 rev.1 |
| Deviations: | None |
| GLP: | Yes |
| Acceptability: | Yes |
|  |  |

A multi-residue QuEChERS-based analytical method for the determination of acetamiprid residues in dry bean (seed) (high protein content), lettuce (high water content), oilseed rape seed (high oil content), orange pulp and orange peel (high acid content) was fully independently validated according to the requirements of SANTE/2020/12830 rev.1.

**A. Materials**

|  |  |
| --- | --- |
| 1. Standards |  |
| Reference item: | Acetamiprid |
| Lot/Batch number: | G1157118 |
| Purity: | 99.78 % |
| CAS No.: | 135410-20-7 |
| Expiry date: | 19 July 2025 |
| Standards for calibration | As above |
| Matrix: | Dry bean (seed) (high protein content)  Lettuce (high water content)  Oilseed rape seed (high oil content)  Orange pulp and orange peel (high acid content) |

1. **Sample preparation and processing**

*Dry Bean (seed)*

A 2.0 g (± 0.05 g) aliquot of homogenised dry bean (seed) sample was dispensed into a 50 mL centrifuge tube. 10 mL of water were added Procedural recoveries were prepared at this stage by fortifying sub-samples of untreated matrix.

10 mL of acetonitrile were added and the tubes were shaken by hand for 1 min and subsequently shaken in an orbital shaker for 20 minutes at 200 rpm.

4 g magnesium sulphate, 1 g sodium chloride, 1 g trisodium citrate dehydrate and 0.5 g disodium hydrogencitrate sesquihydrate were added and thoroughly shaken for 1 minute and then centrifuged at 3500 rpm for 5 minutes. A 7 mL aliquot of the supernatant was transferred to a clean-up tube and mixed by hand for 2 minutes. Samples were centrifuged at 3500 rpm for 5 minutes.

A 5 mL aliquot of the supernatant was transferred to a 100 mL flask where 40 μL of 5% formic acid in acetonitrile was added along with 50 μL of ethylene glycol. The sample was evaporated to just dryness by RFE (40°C). Extracts were re-dissolved in 2 mL of methanol / water (50/50, v/v) with the aid of an ultrasonic bath. An aliquot was transferred to an autosampler vial which was analysed by LC-MS/MS.

*Oilseed Rape (seed)*

A 2.0 g (± 0.05 g) aliquot of homogenised dry bean (seed) sample was dispensed into a 50 mL centrifuge tube. 10 mL of water were added Procedural recoveries were prepared at this stage by fortifying sub-samples of untreated matrix.

10 mL of water were added and the tubes were shaken by hand for 1 min and subsequently shaken in an orbital shaker for 20 minutes at 200 rpm.

4 g magnesium sulphate, 1 g sodium chloride, 1 g trisodium citrate dehydrate and 0.5 g disodium hydrogencitrate sesquihydrate were added and thoroughly shaken for 1 minute and then centrifuged at 3500 rpm for 5 minutes. A 7 mL aliquot of the supernatant was transferred to a clean-up tube and mixed by hand for 2 minutes. Samples were centrifuged at 3500 rpm for 5 minutes.

Extracts were diluted with water, clean-up extract and de-ionised water. An aliquot was transferred to an autosampler vial which was analysed by LC-MS/MS.

*Lettuce*

A 2.0 g (± 0.05 g) aliquot of homogenised dry bean (seed) sample was dispensed into a 50 mL centrifuge tube. 5 mL of water were added Procedural recoveries were prepared at this stage by fortifying sub-samples of untreated matrix.

10 mL of acetonitrile were added and the tubes were shaken by hand for 1 min and subsequently shaken in an orbital shaker for 20 minutes at 200 rpm.

4 g magnesium sulphate, 1 g sodium chloride, 1 g trisodium citrate dehydrate and 0.5 g disodium hydrogencitrate sesquihydrate were added and thoroughly shaken for 1 minute and then centrifuged at 3500 rpm for 5 minutes. A 7 mL aliquot of the supernatant was transferred to a clean-up tube and mixed by hand for 2 minutes. Samples were centrifuged at 3500 rpm for 5 minutes.

Extracts were diluted with water, clean-up extract and de-ionised water. An aliquot was transferred to an autosampler vial which was analysed by LC-MS/MS.

*Orange Pulp and Peel*

A 10.0 g (± 0.05 g) aliquot of homogenised dry bean (seed) sample was dispensed into a 50 mL centrifuge tube. 2.5 mL of water were added Procedural recoveries were prepared at this stage by fortifying sub-samples of untreated matrix.

10 mL of acetonitrile were added and the tubes were shaken by hand for 1 min and subsequently shaken in an orbital shaker for 20 minutes at 200 rpm.

4 g magnesium sulphate, 1 g sodium chloride, 1 g trisodium citrate dehydrate and 0.5 g disodium hydrogencitrate sesquihydrate were added and thoroughly shaken for 1 minute and then centrifuged at 3500 rpm for 5 minutes. A 7 mL aliquot of the supernatant was transferred to a clean-up tube and mixed by hand for 2 minutes. Samples were centrifuged at 3500 rpm for 5 minutes.

Extracts were diluted with water, clean-up extract and de-ionised water. An aliquot was transferred to an autosampler vial which was analysed by LC-MS/MS.

1. **Chromatographic parameters**

|  |  |
| --- | --- |
| HPLC- parameters: | Agilent 1100 Binary |
| Column: | Phenomenex Luna C18, 50 mm x 2.0 mm, 5 μm |
| Mobile phase: | A: 1% Formic acid in ultra-pure water  B: Acetonitrile |
| Flow rate: | 1.0 mL/min |
| Injection volume: | 2 µL |
| MS/MS Parameters:  Ionisation type:  Transitions monitored: | AB Sciex 5500  Turbo Ion Spray positive  m/z 223→126 (quantification)  m/z 223→90 (confirmation) |

**Results and discussion**

The analytical method used in the current study was fully independently validated for the determination of acetamiprid in cabbage heads according to the requirements of SANTE/2020/12830 rev.1. guidelines. The target analyte was determined using HPLC-MS/MS by monitoring two highly specific mass transitions. The detector response was linear over the range 0.0003 – 0.012 μg/mL (oilseed rape seed and lettuce) and 0.0015 – 0.06 μg/mL (orange pulp / peel and dry bean seed). This corresponds to a range of 0.003 to 0.12 mg/kg with associated correlation coefficients (r) ≥ 0.995 for each matrix. The LOQ of the method is 0.01 mg/kg. All mean recovery values and associated RSDs meet the requirements of SANTE/2020/12830 rev.1 guidelines and are summarised in the table below.

**Table A 77: Method validation recovery data for the determination of acetamiprid in plant matrices reported in study RES-00418**

| **Matrix** | **Analyte** | **Fortification level (mg/kg) (n=5)** | **Mean  recovery (%)** | **RSD (%)** | **Comments** |
| --- | --- | --- | --- | --- | --- |
| **Quantification transition m/z 223→126** | | | | | |
| Dry bean (seed) | Acetamiprid | 0.01 | 110 | 4.2 | - |
| 0.1 | 112 | 3.4 |
| **Confirmation transition m/z 223→90** | | | | | |
| Dry bean (seed) | Acetamiprid | 0.01 | 112 | 3.5 | - |
| 0.1 | 111 | 3.6 |
| **Quantification transition m/z 223→126** | | | | | |
| Lettuce | Acetamiprid | 0.01 | 92 | 6.3 | - |
| 0.1 | 95 | 2.5 |
| **Confirmation transition m/z 223→90** | | | | | |
| Lettuce | Acetamiprid | 0.01 | 88 | 2.8 | - |
| 0.1 | 94 | 3.9 |
| **Quantification transition m/z 223→126** | | | | | |
| Orange Pulp | Acetamiprid | 0.01 | 101 | 3.4 | - |
| 0.1 | 104 | 3.2 |
| **Confirmation transition m/z 223→90** | | | | | |
| Orange Pulp | Acetamiprid | 0.01 | 103 | 3.8 | - |
| 0.1 | 105 | 3.1 |
| **Quantification transition m/z 223→126** | | | | | |
| Orange Peel | Acetamiprid | 0.01 | 102 | 4.2 | - |
| 0.1 | 99 | 3.1 |
| **Confirmation transition m/z 223→90** | | | | | |
| Orange Peel | Acetamiprid | 0.01 | 104 | 2.6 | - |
| 0.1 | 99 | 3.2 |
| **Quantification transition m/z 223→126** | | | | | |
| Oilseed Rape (seed) | Acetamiprid | 0.01 | 96 | 3.0 | - |
| 0.1 | 100 | 2.3 |
| **Confirmation transition m/z 223→90** | | | | | |
| Oilseed Rape (seed) | Acetamiprid | 0.01 | 100 | 3.2 | - |
| 0.1 | 99 | 2.6 |

**Table A 78: Characteristics of the analytical method validated for the determination of acetamiprid in plant matrices**

|  | **Acetamiprid** |
| --- | --- |
| Specificity | A highly specific HPLC-MS/MS method was used for the determination of the target analyte. Two mass transitions (m/z 223→126, quantifiaction and m/z 223→90, qualification) were monitored during each analysis.  Target analyte concentrations in controls did not exceed 30% of the method LOQ. No interference from co-eluting components at the retention time of the target analyte. |
| Calibration (type, number of data points) | Regression residuals were plotted and found to be randomly distributed. Accordingly, a linear calibration function was considered suitable for quantitative determination of the target analyte. Seven-point linear matrix-matched calibration was used for target analyte quantification and is presented in the study.  Equation of the calibration curves are:  quantifier mass transition: y = 4713236.5 x + 597.20606, r = 0.9994  qualifier mass transition: y = 3044807.9 x + -132.8824, r = 0.9994 |
| Calibration range | 0.0003 – 0.012 μg/mL (oilseed rape seed and lettuce) and 0.0015 – 0.06 μg/mL (orange pulp / peel and dry bean seed), corresponding to a range of 0.003 to 0.12 mg/kg |
| Assessment of matrix effects is presented | Matrix effects were assessed and found to be significant (≥20%) in orange pulp, orange peel and dry been (seed).  Therefore, matrix matched calibrations were used for target analyte quantification in all matrices. |
| Limit of determination/quantification | LOQ: 0.01 mg/kg  LOD: 0.003 mg/kg |
| Stability of the target analyte in standards | Stability of a 18-day stored 0.05 μg/mL acetamiprid standard solution prepared in acetonitrile was assessed by diluting the stored and freshly prepared standard in acetonitrile/water (50:50) to 0.001 μg/mL. The peak area of the stored standard (mean of 5 injections) was compared to the peak area of the freshly prepared standard (mean of 5 injections) showing that the stored standard was stable when stored refrigerated (2°C to 8°C), as the difference when compared to the fresh standard was 4.0% ( ≤ 10%). |
| Stability of the target analyte in final extracts | Extract stability was assessed by re-injection of the LOQ recoveries using freshly prepared calibration standards after 7 days (dry been seed), 8 days (oilseed rape seed and lettuce) and 9 days (orange pulp and peel) of refrigerated storage (2°C to 8°C). Mean recovery was in the range 70 – 120% with a relative standard deviation of ≤ 20% for all matrices and was within ± 20% of the original value. Extracts were therefore deemed to be stable for at least 7 days when stored refrigerated. |
| Extraction efficiency | Refer to the cross-validation study summarized under 5.2/03 and 5.2/04 |

**Conclusion**

An analytical method for the determination of acetamiprid residues in dry bean (seed) (high protein content), lettuce (high water content), oilseed rape seed (high oil content), orange pulp and orange peel (high acid content) was fully independently validated according to SANTE/2020/12830 rev.1. guidelines. The method was sufficiently accurate and precise to be able to provide reliable data on target analyte concentrations in plant matrices and should therefore be considered acceptable.

* + - * 1. Analytical method 13M06017-01-VMPL

Method validation RES-00419

|  |  |
| --- | --- |
| Comments of zRMS: | The analytical method 13M06017-01-VMPL for acetamiprid (Adama study code R-33644) was independently validated in cabbage heads with an LOQ of 0.01 mg/kg. The independent laboratory validation met the criteria detailed in SANTE/2020/12830, Rev.1. |

|  |  |
| --- | --- |
| Reference: | KCP 5.2/02 |
| Report | Independent laboratory validation of analytical method 13M06017-01-VMPL (Sponsor code R-33644) for determination of Acetamiprid in food of plant origin. Brown, S. 2022, Report No. RES-00419, Sponsor Reference No. 000111704 |
| Guideline(s): | SANTE/2020/12830 rev.1 |
| Deviations: | None |
| GLP: | Yes |
| Acceptability: | Yes |

A multi-residue QuEChERS-based analytical method for the determination of acetamiprid residues in cabbage heads (high water content) was fully independently validated according to the requirements of SANTE/2020/12830 rev.1.

**A. Materials**

|  |  |
| --- | --- |
| 1. Standards |  |
| Reference item: | Acetamiprid |
| Lot/Batch number: | G1157118 |
| Purity: | 99.78 % |
| CAS No.: | 135410-20-7 |
| Expiry date: | 19 July 2025 |
| Standards for calibration | As above |
| Matrix: | Cabbage heads |

1. **Sample preparation and processing**

A 10.0 g (± 0.05 g) aliquot of homogenised cabbage heads sample was dispensed into a 50 mL centrifuge tube. Procedural recoveries were prepared at this stage by fortifying sub-samples of untreated matrix.

10 mL of acetonitrile were added and the tubes were homogenised for at least 2 minutes. 4 g magnesium sulphate, 1 g sodium chloride, 1 g trisodium citrate dehydrate and 0.5 g disodium hydrogencitrate sesquihydrate were added, thoroughly shaken and mixed again on a vortex mixer for at least 1 minute and then centrifuged at 3500 rpm for at least 10 minutes. A 1 mL aliquot of the supernatant was transferred to a

clean-up tube and mixed by vortex mixer for 30 seconds.

Samples were then filtered into an autosampler vial. A 0.1mL aliquot was diluted to 1 mL using acetonitrile in an autosampler vial which was analysed by LC-MS/MS.

1. **Chromatographic parameters**

|  |  |
| --- | --- |
| HPLC- parameters: | Agilent 1100 Binary |
| Column: | Phenomenex Luna C18, 50 mm x 2.0 mm, 5 μm |
| Mobile phase: | A: 1% Formic acid in ultra-pure water  B: Acetonitrile |
| Flow rate: | 1.0 mL/min |
| Injection volume: | 10 µL |
| MS/MS Parameters:  Ionisation type:  Transitions monitored: | AB Sciex 5500  Turbo Ion Spray positive  m/z 223→126 (quantification)  m/z 223→90 (confirmation) |

**Results and discussion**

The analytical method used in the current study was fully independently validated for the determination of acetamiprid in cabbage heads according to the requirements of SANTE/2020/12830 rev.1. guidelines. The target analyte was determined using HPLC-MS/MS by monitoring two highly specific mass transitions. The detector response was linear over the range 0.3 – 20 μg/L (corresponding to 0.003 to 0.2 mg/kg) with associated correlation coefficients (r) ≥ 0.995. The LOQ of the method is 0.01 mg/kg. All mean recovery values and associated RSDs meet the requirements of SANTE/2020/12830 rev.1 guidelines and are summarised in the table below.

**Table A 79: Method validation recovery data for the determination of acetamiprid in cabbage heads reported in study RES-00419**

| **Matrix** | **Analyte** | **Fortification level (mg/kg) (n=5)** | **Mean  recovery (%)** | **RSD (%)** | **Comments** |
| --- | --- | --- | --- | --- | --- |
| Quantification transition m/z 223→126 | | | | | |
| Cabbage heads | Acetamiprid | 0.01 | 95 | 7.2 | - |
| 0.1 | 94 | 1.7 |
| Confirmation transition m/z 223→90 | | | | | |
| Cabbage heads | Acetamiprid | 0.01 | 98 | 6.8 | - |
| 0.1 | 93 | 2.1 |

**Table A 80: Characteristics of the analytical method validated for the determination of acetamiprid in cabbage heads**

|  | **Acetamiprid** |
| --- | --- |
| Specificity | A highly specific HPLC-MS/MS method was used for the determination of the target analyte. Two mass transitions (m/z 223→126, quantifiaction and m/z 223→90, qualification) were monitored during each analysis.  Target analyte concentrations in controls did not exceed 30% of the method LOQ. No interference from co-eluting components at the retention time of the target analyte. |
| Calibration (type, number of data points) | Regression residuals were plotted and found to be randomly distributed. Accordingly, a linear calibration function was considered suitable for quantitative determination of the target analyte. Seven-point linear matrix-matched calibration was used for target analyte quantification and is presented in the study.  Equation of the calibration curves are:  quantifier mass transition: y = 7192.3 x + 10.59, r = 0.9996  qualifier mass transition: y = 4688.5 x + -209.66, r = 0.9997 |
| Calibration range | 0.3 – 20 μg/L (corresponding to 0.003 to 0.2 mg/kg) |
| Assessment of matrix effects is presented | Matrix effects were assessed and found to be insignificant (< ± 20%) for cabbage heads. Nevertheless, matrix matched calibrations were used for target analyte quantification. |
| Limit of determination/quantification | LOQ: 0.01 mg/kg  LOD: 0.003 mg/kg |
| Stability of the target analyte in standards | Stability of a 25-day stored 1.0 μg/mL acetamiprid standard solution prepared in acetonitrile was assessed by diluting the stored and freshly prepared standard in acetonitrile to 5.0 ng/mL. The peak area of the stored standard (mean of 5 injections) was compared to the peak area of the freshly prepared standard (mean of 5 injections) showing that the stored standard was stable when stored refrigerated (2°C to 8°C), as the difference when compared to the fresh standard was 3.8% ( ≤ 10%). |
| Stability of the target analyte in final extracts | Extract stability was assessed by re-injection of the LOQ recoveries using freshly prepared calibration standards after 9 days refrigerated storage (2°C to 8°C). Mean recovery was in the range 70 – 120% with a relative standard deviation of 8.0% (≤ 20%) and was within ± 20% of the original value. Extracts were therefore deemed to be stable for at least 9 days when stored refrigerated. |
| Extraction efficiency | Refer to the study summarized under 5.2/03 and 5.2/04 |

**Conclusion**

An analytical method for the determination of acetamiprid residues in cabbage heads was fully independently validated according to SANTE/2020/12830 rev.1. guidelines. The method was sufficiently accurate and precise to be able to provide reliable data on target analyte concentrations in cabbage heads and should therefore be considered acceptable.

Extraction efficiency

|  |  |
| --- | --- |
| Comments of zRMS: | The efficiency of the extraction procedures used in metabolism studies as well as those used in residue trials meet the requirements of SANTE/2017/10632, rev. 4 Technical Guidelines on the Evaluation of Extraction Efficiency of Residue Analytical Methods. |

|  |  |
| --- | --- |
| Reference: | KCP 5.2/03 |
| Report | Comparison of the extraction efficiency of two solvents used in the analytical methods for the determination of acetamiprid residues in various plant matrices (dry, acid, water and oily). Lefresne, S., 2014, Report No. B14S-M1-A-01, Sponsor Reference No. R-34859 |
| Guideline(s): | Commission Regulation (EU) No. 283/2013  Regulation (EC) No. 1107/2009 |
| Deviations: | None |
| GLP: | Yes |
| Acceptability: | Yes |

|  |  |
| --- | --- |
| Reference: | KCP 5.2/04 |
| Report | Amendment No. 1 to study: Comparison of the extraction efficiency of two solvents used in the analytical methods for the determination of acetamiprid residues in various plant matrices (dry, acid, water and oily). Lefresne, S., 2014, Report No. B14S-M1-A-01, Sponsor Reference No. R-34859 |
| Guideline(s): | Commission Regulation (EU) No 283/2013  Regulation (EC) No. 1107/2009 |
| Deviations: | None |
| GLP: | Yes |
| Acceptability: | Yes |

The aim of this extraction efficiency cross-validation study was to assess the efficiency of acetonitrile to extract acetamiprid from plant material with high water content, plant material with high acid content, plant material with high oil content and dry plant material with high protein content. This was achieved by comparing the amount of acetamiprid extracted by acetonitrile from samples with incurred residues to the amount of acetamiprid extracted from similar samples using methanol as described in a metabolism study conducted using radiolabeled target analyte.

**Introduction**

An analytical method for the determination of acetamiprid residues in plant material with high water content, plant material with high acid content, plant material with high oil content and dry plant material with high protein content with high water, high oil, dry/high protein and high acid content was fully validated by Lefresne, S. in 2014 (Report No. B13-M1-A-01) according to the requirements of SANCO/3029/99 rev. 4 prior to 1st March 2021. The method was based on extraction with acetonitrile:water (50:50) followed by clean-up with QuEChERS salt mixture for the matrix dry bean seeds and straw (dry/high protein group), acetonitrile (100 %) for the matrices oilseed rape (seeds) and olive oil and whole fruit (high oil group), acetonitrile:water (80:20) for mandarin and orange peel, pulp and whole fruit (high acid group) and acetonitrile:water (66.6:33.4) for oilseed rape whole plant, pod and whole plant without pod (high water group). This method was used in residue studies for risk assessment purposes and also for monitoring/enforcement purposes. The same method was used to process and analyse acetamiprid residues in the current cross-validation study. A summary of the primary validation data for this method is given under KCP 5.1.2/05.

**A. Materials and methods**

|  |  |
| --- | --- |
|  |  |
| Reference item: | Acetamiprid |
| Lot/Batch number: | 20202 |
| Purity: | 98.1 % |
| CAS No.: | 135410-20-7 |
| Expiry date: | 02 February 2016 |
| Standards for calibration | As above |
| Matrices: | Oilseed rape (whole plant) (high water)  Olive (flesh) (high oil)  Orange (whole fruit) (high acid)  Wheat (straw) (dry/high protein) |

**Sample preparation and processing**

Once acetamiprid residues were extracted from all samples by agitation in either acetonitrile (residue studies extracting solvent) or methanol (metabolism studies extracting solvent), the primary extracts were processed using the following methodology:

2g of frozen sample for each matrix into a 50mL centrifuge tube. 10 mL of acetonitrile or methanol were added for olive (flesh) and orange (fruit) using a measuring cylinder. For wheat (straw), 10 mL of water followed by 10 mL of acetonitrile or methanol were added. For oilseed rape (whole plant), 5 mL of water followed by 10mL of acetonitrile or methanol were added.

All samples were shaken for about 1 minute manually and subsequently mechanically for about 20 minutes at maximum speed. Samples were then centrifuged for about 5 minutes at high speed. Extracts were diluted and homogenized at this point in order to fit into the calibration range. Final extracts were analyzed by HPLC-MS/MS.

**Analytical instrumentation and analysis**

|  |  |
| --- | --- |
| **LC-MS/MS parameters** |  |
| HPLC System  Instrument: | Shimadzu LC-20AD XR  API 6500 LC/MS/MS System |
| Column: | C18 Hydro, 100 mm x 3 mm, 2.5 μm |
| Mobile phase: | A: Water / acetic acid (100/0.1) (v/v) + 5mM ammonium acetate  B: Methanol / acetic acid (100/0.1) (v/v) + 5mM ammonium acetate |
| Injection volume:  Scan type:  Polarity: | 10 µL  ESI  Positive |

Transitions monitored: 223 → 126 (quantification)

223 → 90 (confirmation)

**Table A 81: Characteristics of the analytical methods used for the determination** **of acetamiprid in plant material in study B14S-M1-A-01**

|  | **Acetamiprid** |
| --- | --- |
| Specificity | The method is highly specific with two mass transitions monitored during each analysis.  The target analyte was not detecteted above 30% of the method LOQ in any controls.  Retention time of the target analyte on sample chromatograms matches that on a standard chromatogram.  The target analyte was sufficiently well resolved to allow quantitative determination. |
| Calibration (type, number of data points) | Seven-point linear calibration was used for target analyte quantification and is presented in the study.  Equation of the calibration curve for m/z 223 → 126 is:  Lettuce: y = 85057.95x + 9915.17  R = 0.9997 |
| Calibration range | 0.3 – 10 μg/mL |
| Limit of determination/quantification | LOQ 0.3 μg/mL |

**Extraction efficiency data**

Results for the efficiency of the extraction processes using method B13-M1-A-01 are given in table below. The mean recovery values for the solvent used in metabolism studies (methanol) are taken as 100%. In each case, mean recoveries are expressed as a percentage of the respective mean recovery obtained using methanol as extracting solvent.

**Table A 82: Extraction efficiency results reported in study B14S-M1-A-01**

|  | | **Acetamiprid (quantification transition 223 → 126)** | | | | | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Matrix** | **Analyte** | | **Solvent** | **Replicates** | **Mean**  **(mg/kg)** | **Relative Mean Recovery (%)** | **RSD**  **(%)** | **Comments** |
| Wheat (straw) | Acetamiprid | | Methanol 1 | 3 | 0.24 | 100 | 5.6 | - |
| Acetonitrile 2 | 3 | 0.26 | 107.7 | 10.2 | - |
| Olive (flesh) | Methanol 1 | 3 | 1.02 | 100 | 2.3 | - |
| Acetonitrile 2 | 3 | 1.18 | 113.6 | 3.0 | - |
| Oilseed rape (whole plant) | Methanol 1 | 3 | 0.78 | 100 | 8.4 | - |
| Acetonitrile 2 | 3 | 0.83 | 106.03 | 2.6 | - |
| Orange (whole fruit) | Methanol 1 | 3 | 0.31 | 100 | 3.8 | - |
| Acetonitrile 2 | 3 | 0.37 | 116.2 | 3.5 | - |

1 Solvent used in the metabolism studies

2 Solvent used in the residue trials

In all cases, the amount of acetamiprid extracted using acetonitrile (residue method) was greater than 100% of that extracted using methanol (metabolism method). Acetonitrile as used in the residue studies and monitoring methods was therfore more efficeint at exracting acetamiprid from plant material than the methanol used in the metabolism study. Accordingly, the efficiency of the extraction procedures used in metabolism studies as well as those used in residue trials meet the requirements of SANTE/2017/10632, rev. 4 Technical Guidelines on the Evaluation of Extraction Efficiency of Residue Analytical Methods.

**Conclusion**

Analytical method B13-M1-A-01 was fully validated according to SANCO/3029/99 rev. 4 prior to 1st March 2021 (see KCP 5.1.2/05) and is therefore suitable for use in the current extraction efficiency cross-validation study.

Acetonitrile has been demonstrated to be sufficiently efficient at extracting acetamiprid residues from plant material with high water content, plant material with high acid content, plant material with high oil content and dry plant material with high protein content in accordance with the requirements of SANTE/2017/10632, rev. 4 Technical Guidelines on the Evaluation of Extraction Efficiency of Residue Analytical Methods.

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

Matching data have been obtained and provided by ADAMA. These data were submitted to the RMS Netherlands in order to demonstrate access to a complete data package, according to Reg (EU)283/2013 and for the data matching process. The RMS Opinion on GLP compliance, guidance compliance and equivalence of the endpoints will be provided as soon as Data Matching process is finalized.

Additionally, ADAMA has provided a primary method and an ILV for the determination of acetamiprid residues in honey. A detailed evaluation of the new study can be found below.

* + - * 1. Analytical method 21A14030-01-VMHN

Method validation 21A14030-01-VMHN

|  |  |
| --- | --- |
| Comments of zRMS: | The analytical method for the determination of residues of acetamiprid in samples of matrix honey was fully validated according to SANTE/2020/12830 rev.1 with limit of quantification of 0.01 mg/kg LOQ  Mean recovery values obtained by HPLC-MS/MS for acetamiprid comply with the standard acceptance criteria of guideline SANTE/2020/12830 rev.1, which requires mean recoveries in the range of 60 - 120% at 0.01 mg/kg with RSD≤ 20%.  The method is acceptable. |

|  |  |
| --- | --- |
| Reference: | KCP 5.2/05 |
| Report | Validation of an Analytical Method for the Determination of Residues of  Acetamiprid in Honey - Schrag, K. 2022, Report No. 21A14030-01-VMHN, Sponsor Reference No. 000107274 |
| Guideline(s): | SANTE/2020/12830 rev.1 |
| Deviations: | None |
| GLP: | Yes |
| Acceptability: | Yes |

A multi-residue QuEChERS-based analytical method for the determination of acetamiprid residues in honey was fully validated according to the requirements of SANTE/2020/12830 rev.1.

**A. Materials**

|  |  |
| --- | --- |
| 1. Standards |  |
| Reference item: | Acetamiprid |
| Lot/Batch number: | BCBT9185 |
| Purity: | 100 % |
| CAS No.: | 160430-64-8 |
| Expiry date: | 28 February 2022 |
| Standards for calibration | As above |
| Matrix: | Honey |

1. **Sample preparation and processing**

Following homogenisation, 5 g were weighed into a 50 mL centrifuge tube. 10.0 mL water and 10 mL acetonitrile were added and the tubes were homogenised for at least 2 min using a vortex mixer. 1 g sodium citrate, 0.5 g sodium hydrogencitrate sequihydrate, 4 g magnesium sulphate, 1 g sodium chloride were added, thoroughly shaken and mixed again on a vortex mixer for at least 1 minute and then centrifuged at 4000 min-1 for at least 5 minutes. The supernatant was transferred into a 15 mL centrifuge tube.

An aliquot of 1 mL of the supernatant was transferred into a tube (2 mL) prepared with 25 mg PSA and 150 mg anhydrous magnesia sulphate and 25 mg C18e, shaken on a vortex mixer for 30 s, centrifuged for 5 min (12 000 rpm) and transferred into an autosampler vial of 1.8 mL. The final extracts were diluted 1:10 with acetonitrile into an autosampler vial (1.8 mL) and directly analysed by LC-MS/MS. Eventually final extracts would be further diluted in final extract of the matrix, if necessary to obtain a concentration falling within the linear range of the calibration curve.

1. **Chromatographic parameters**

|  |  |
| --- | --- |
| HPLC- parameters: | Shimadzu LC-40 |
| Column: | Phenomenex Luna C18 (2) 100 A, 150 mm x 2.0 mm, 5.0 μm |
| Mobile phase: | A: Water containing 1 % formic acid (v/v)  B: Acetonitrile |
| Flow rate: | 0.5 mL/min |
| Injection volume: | 10 µL |
| MS/MS Parameters:  Ionisation type:  Transitions monitored: | AB Sciex QTRAP 5500  ESI positive  m/z 223→126 (quantification)  m/z 223→90 (confirmation) |

**Results and discussion**

The analytical method used in the current study was fully validated for the determination of acetamiprid in honey according to the requirements of SANTE/2020/12830 rev.1. guidelines. The target analyte was determined using HPLC-MS/MS by monitoring two highly specific mass transitions. The detector response was linear over the range 0.15 - 100 μg/L (corresponding to 0.003 mg/kg - 2 mg/kg) with associated correlation coefficient (r) ≥ 0.9959. The LOQ of the method is 0.01 mg/kg. All mean recovery values and associated RSDs meet the requirements of SANTE/2020/12830 rev.1 guidelines and are summarised in the table below.

**Table A 83: Method validation recovery data for the determination of acetamiprid in honey reported in study 21A14030-01-VMHN.**

| **Matrix** | **Analyte** | **Fortification level (mg/kg) (n=5)** | **Mean  recovery (%)** | **RSD (%)** | **Comments** |
| --- | --- | --- | --- | --- | --- |
| Quantification transition m/z 223→126 | | | | | |
| Honey | Acetamiprid | 0.01 | 98 | 1.5 | - |
| 0.1 | 97 | 0.9 |
| Confirmation transition m/z 223→90 | | | | | |
| Honey | Acetamiprid | 0.01 | 97 | 3.0 | - |
| 0.1 | 96 | 1.5 |

**Table A 84: Characteristics of the analytical method validated for the determination of acetamiprid in honey**

|  | **Acetamiprid** |
| --- | --- |
| Specificity | A highly specific HPLC-MS/MS method was used for the determination of the target analyte. Two mass transitions (m/z 223→126, quantifiaction and m/z 223→90, qualification) were monitored during each analysis.  Target analyte concentrations in controls did not exceed 30% of the method LOQ. No interference from co-eluting components at the retention time of the target analyte. |
| Calibration (type, number of data points) | Eight-point matrix-matched linear calibration    Calibration range 0.15 - 15 μg/L:  quantifier mass transition: y = 659027.76x + 0.00, r ≥ 0.9959  qualifier mass transition: y = 194316.75x - 0.00, r ≥ 0.9959    Calibration range 5 – 100 μg/L:  quantifier mass transition: y= 580319.83x -0.00, r ≥ 0.9959  qualifier mass transition: y = 179691.75x - 0.00, r ≥ 0.9959 |
| Calibration range | 0.15 - 100 μg/L (corresponding to 0.003 mg/kg to 2 mg/kg) |
| Assessment of matrix effects is presented | Matrix effects were assessed and found to be insignificant (< ± 20%) for honey. Nevertheless, matrix matched calibrations were used for target analyte quantification. |
| Limit of determination/quantification | LOQ: 0.01 mg/kg  LOD: 0.003 mg/kg |
| Stability of the target analyte in standards | The stability of the analyte in stock and working solutions was examined by comparison of stored stock and working solutions against freshly prepared standard solutions. Stock and working solutions were found to be stable, as the difference in the means from six (6) replicates (for each solution tested) to the reference solution was below 10% for up to 145 days of refrigerated storage. |
| Stability of the target analyte in final extracts | All sample extracts were analysed within 24 hours after extraction, therefore the stability of the analyte is sufficiently proven. |
| Storage stability | A storage stability experiment was conducted in study GBU-21-48185 (see KCP 5.1.2/15) and demonstrated that acetamiprid residues in freshly fortified samples at day 7 were within 70-120% and residues in the control sample were < 30% of the LOQ. Therefore, acetamiprid can be regarded as stable for up to 7 days after storage at 30°C |

**Conclusion**

An analytical method for the determination of acetamiprid residues in honey was fully validated according to SANTE/2020/12830 rev.1. guidelines. The method was sufficiently accurate and precise to be able to provide reliable data on target analyte concentrations in honey and should therefore be considered acceptable.

Independent laboratory validation

|  |  |
| --- | --- |
| Comments of zRMS: | The analytical method 21A14030-01-VMHN for the determination of acetamiprid residues in honey was independently validated according to SANTE/2020/12830 rev.1. guideline. |

|  |  |
| --- | --- |
| Reference: | KCP 5.2/06 |
| Report | Independent laboratory validation of analytical method 21A14030-01-VMHN (Adama study No. 000107274) for residues of acetamiprid in honey. Brown, S. 2022, Report No. RES-00415, Sponsor Reference No. 000111247 |
| Guideline(s): | SANTE/2020/12830 rev.1 |
| Deviations: | None |
| GLP: | Yes |
| Acceptability: | Yes |

A multi-residue QuEChERS-based analytical method for the determination of acetamiprid residues in honey was fully independently validated according to the requirements of SANTE/2020/12830 rev.1.

**A. Materials**

|  |  |
| --- | --- |
| 1. Standards |  |
| Reference item: | Acetamiprid |
| Lot/Batch number: | G1157118 |
| Purity: | 99.78 % |
| CAS No.: | 135410-20-7 |
| Expiry date: | 19 July 2025 |
| Standards for calibration | As above |
| Matrix: | Honey |

1. **Sample preparation and processing**

A 5 g aliquot of honey was weighed into a 50 mL centrifuge tube. 10.0 mL of water and procedural recovery samples were fortified at this point. 10 mL of acetonitrile were added and the tubes were homogenised for at least 2 min using a vortex mixer. 1 g sodium citrate, 0.5 g sodium hydrogencitrate sequihydrate, 4 g magnesium sulphate, 1 g sodium chloride were added, thoroughly shaken and mixed again on a vortex mixer for at least 1 minute and then centrifuged at 4000 rpm for at least 5 minutes. The supernatant was transferred into a 15 mL centrifuge tube.

An aliquot of 1 mL of the supernatant was transferred into a tube (2 mL) prepared with 25 mg PSA and 150 mg anhydrous magnesia sulphate and 25 mg C18e, shaken on a vortex mixer for 30 s, centrifuged for 5 min (12 000 rpm) and transferred into an autosampler vial of 1.8 mL. Extracts were then diluted 10-fold in acetonitrile i.e., by adding 0.1 mL of acetonitrile extract to an autosampler vial containing 0.9 mL of acetonitrile and mixed thoroughly. A sample of final concentration 0.05 g/mL was analysed by LC-MS/MS.

1. **Chromatographic parameters**

|  |  |
| --- | --- |
| HPLC- parameters: | Agilent 1260 Binary |
| Column: | Phenomenex Luna C18, 50 mm x 2.0 mm, 5 μm |
| Mobile phase: | A: 1% Formic acid in ultra-pure water  B: Acetonitrile |
| Flow rate: | 1.0 mL/min |
| Injection volume: | 10 µL |
| MS/MS Parameters:  Ionisation type:  Transitions monitored: | AB Sciex 5500  Turbo Ion Spray positive  m/z 223→126 (quantification)  m/z 223→90 (confirmation) |

**Results and discussion**

The analytical method used in the current study was fully independently validated for the determination of acetamiprid in honey according to the requirements of SANTE/2020/12830 rev.1. guidelines. The target analyte was determined using HPLC-MS/MS by monitoring two highly specific mass transitions. The detector response was linear over the range 0.15 – 15 ng/mL (corresponding to 0.003 mg/kg to 0.3 mg/kg) with associated correlation coefficients (r) ≥ 0.995. The LOQ of the method is 0.01 mg/kg. All mean recovery values and associated RSDs meet the requirements of SANTE/2020/12830 rev.1 guidelines and are summarised in the table below.

**Table A 85: Method validation recovery data for the determination of acetamiprid in honey reported in study RES-00415**

| **Matrix** | **Analyte** | **Fortification level (mg/kg) (n=5)** | **Mean  recovery (%)** | **RSD (%)** | **Comments** |
| --- | --- | --- | --- | --- | --- |
| Quantification transition m/z 223→126 | | | | | |
| Honey | Acetamiprid | 0.01 | 108 | 3.3 | - |
| 0.1 | 107 | 2.7 |
| Confirmation transition m/z 223→90 | | | | | |
| Honey | Acetamiprid | 0.01 | 109 | 5.6 | - |
| 0.1 | 110 | 3.0 |

**Table A 86: Characteristics of the analytical method validated for the determination of acetamiprid in honey**

|  | **Acetamiprid** |
| --- | --- |
| Specificity | A highly specific HPLC-MS/MS method was used for the determination of the target analyte. Two mass transitions (m/z 223→126, quantifiaction and m/z 223→90, qualification) were monitored during each analysis.  Target analyte concentrations in controls did not exceed 30% of the method LOQ. No interference from co-eluting components at the retention time of the target analyte. |
| Calibration (type, number of data points) | Five-point matrix-matched linear calibration    quantifier mass transition: y = 78742.2 x + 1404.22, r = 1.0000  qualifier mass transition: y = 23420 x + 239.131, r = 0.9999 |
| Calibration range | 0.15 – 15 ng/mL (corresponding to 0.003 mg/kg to 0.3 mg/kg) |
| Assessment of matrix effects is presented | Matrix effects were assessed and found to be insignificant (< ± 20%) for honey. Nevertheless, matrix matched calibrations were used for target analyte quantification. |
| Limit of determination/quantification | LOQ: 0.01 mg/kg  LOD: 0.003 mg/kg |
| Stability of the target analyte in standards | Stability of a 17-day stored 1.0 μg/mL acetamiprid standard solution prepared in methanol was assessed by comparing the stored standard to a freshly prepared standard. The stored standard was stable when stored refrigerated (2°C to 8°C), as the difference when compared to the fresh standard was ≤ 10%. |
| Stability of the target analyte in final extracts | Extract stability was assessed by re-injection of the LOQ recoveries using freshly prepared calibration standards after 8 days refrigerated storage (2°C to 8°C). Mean recovery was in the range 70 – 120% with a relative standard deviation of ≤ 20% and was within ± 20% of the original value. Extracts were therefore deemed to be stable for at least 8 days when stored refrigerated. |

**Conclusion**

An analytical method for the determination of acetamiprid residues in honey was fully independently validated according to SANTE/2020/12830 rev.1. guidelines. The method was sufficiently accurate and precise to be able to provide reliable data on target analyte concentrations in honey and should therefore be considered acceptable.

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

Matching data have been obtained and provided by ADAMA. These data were submitted to the RMS Netherlands in order to demonstrate access to a complete data package, according to Reg (EU)283/2013 and for the data matching process. The RMS Opinion on GLP compliance, guidance compliance and equivalence of the endpoints will be provided as soon as Data Matching process is finalized.

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

Matching data have been obtained and provided by ADAMA. These data were submitted to the RMS Netherlands in order to demonstrate access to a complete data package, according to Reg (EU)283/2013 and for the data matching process. The RMS Opinion on GLP compliance, guidance compliance and equivalence of the endpoints will be provided as soon as Data Matching process is finalized.

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

Matching data have been obtained and provided by ADAMA. These data were submitted to the RMS Netherlands in order to demonstrate access to a complete data package, according to Reg (EU)283/2013 and for the data matching process. The RMS Opinion on GLP compliance, guidance compliance and equivalence of the endpoints will be provided as soon as Data Matching process is finalized.

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

Matching data have been obtained and provided by ADAMA. These data were submitted to the RMS Netherlands in order to demonstrate access to a complete data package, according to Reg (EU)283/2013 and for the data matching process. The RMS Opinion on GLP compliance, guidance compliance and equivalence of the endpoints will be provided as soon as Data Matching process is finalized.

A new study has been conducted by ADAMA for the determination of acetamiprid residues in body fluids, to comply with the most recent LOQ requirements of Guidelines SANTE/2020/12830 rev. 1. A detailed evaluation of this study can be found below.

* + - * 1. Analytical method 16A08133-01-VMBF

Method validation RES-00416

|  |  |
| --- | --- |
| Comments of zRMS: | The analytical method was found to be valid for the determination of acetamiprid in body fluids (blood) with an LOQ of 0.01 mg/L. The validation of the method met the criteria detailed in SANTE/2020/12830, Rev.1.  The method is acceptable. |

|  |  |
| --- | --- |
| Reference: | KCP 5.2/07 |
| Report | Validation of an analytical method for the determination of residues of acetamiprid in body fluids (blood) by LC-MS/MS.  Brown, S. 2022, Report No. RES-00416, Sponsor Reference No. 000111248 |
| Guideline(s): | SANTE/2020/12830 rev.1 |
| Deviations: | None |
| GLP: | Yes |
| Acceptability: | Yes |

A multi-residue QuEChERS-based analytical method for the determination of acetamiprid residues in body fluids (blood) was fully validated according to the requirements of SANTE/2020/12830 rev.1.

**A. Materials**

|  |  |
| --- | --- |
| 1. Standards |  |
| Reference item: | Acetamiprid |
| Lot/Batch number: | G1157118 |
| Purity: | 99.78 % |
| CAS No.: | 135410-20-7 |
| Expiry date: | 19 July 2025 |
| Standards for calibration | As above |
| Matrix: | Blood |

1. **Sample preparation and processing**

A 2 mL aliquot of sample was weighed into a 50 mL centrifuge tube. 10.0 mL of water and procedural recovery samples were fortified at this point. 10 mL of acetonitrile were added and the samples were homogenised for at least 2 min using a vortex mixer. QuEChERS citrate extraction salts were added, shaken for 10 seconds and mixed again on a vortex mixer for 1 minute. Samples were then centrifuged at 3500 rpm for 5 minutes.

Extracts were then diluted 10-fold in acetonitrile i.e., by adding 0.1 mL of acetonitrile extract to a vial containing 0.9 mL of acetonitrile and mixed thoroughly. The final sample was analysed by LC-MS/MS.

1. **Chromatographic parameters**

|  |  |
| --- | --- |
| HPLC- parameters: | Agilent 1260 Binary |
| Column: | Phenomenex Luna C18, 50 mm x 2.0 mm, 5 μm |
| Mobile phase: | A: 1% Formic acid in ultra-pure water  B: Acetonitrile |
| Flow rate: | 1.0 mL/min |
| Injection volume: | 10 µL |
| MS/MS Parameters:  Ionisation type:  Transitions monitored: | AB Sciex 5500  Turbo Ion Spray positive  m/z 223→126 (quantification)  m/z 223→90 (confirmation) |

**Results and discussion**

The analytical method used in the current study was fully validated for the determination of acetamiprid in body fluids (blood) according to the requirements of SANTE/2020/12830 rev.1 guidelines. The target analyte was determined using HPLC-MS/MS by monitoring two highly specific mass transitions. The detector response was linear over the range 0.05 ng/mL to 5 ng/mL (corresponding to 0.0025 mg/L to 0.25 mg/L) with associated correlation coefficients (r) ≥ 0.995. The LOQ of the method is 0.01 mg/L. All mean recovery values and associated RSDs meet the requirements of SANTE/2020/12830 rev.1 guidelines and are summarised in the table below.

**Table A 87: Method validation recovery data for the determination of acetamiprid in ~~honey~~ blood reported in study RES-00416**

| **Matrix** | **Analyte** | **Fortification level (mg/kg) (n=5)** | **Mean  recovery (%)** | **RSD (%)** | **Comments** |
| --- | --- | --- | --- | --- | --- |
| Quantification transition m/z 223→126 | | | | | |
| Blood | Acetamiprid | 0.01 | 113 | 5.6 | - |
| Confirmation transition m/z 223→90 | | | | | |
| Blood | Acetamiprid | 0.01 | 111 | 5.2 | - |

**Table A 88: Characteristics of the analytical method validated for the determination of acetamiprid in blood**

|  | **Acetamiprid** |
| --- | --- |
| Specificity | A highly specific HPLC-MS/MS method was used for the determination of the target analyte. Two mass transitions (m/z 223→126, quantifiaction and m/z 223→90, qualification) were monitored during each analysis.  Target analyte concentrations in controls did not exceed 30% of the method LOQ. No interference from co-eluting components at the retention time of the target analyte. |
| Calibration (type, number of data points) | Seven-point matrix-matched linear calibration    quantifier mass transition: y = 149109x -1471.18, r = 0.9996  qualifier mass transition: y = 44034.7x +4.67394, r = 0.9998 |
| Calibration range | 0.05 ng/mL to 5 ng/mL (corresponding to 0.0025 mg/L to 0.25 mg/L) |
| Assessment of matrix effects is presented | Matrix effects were assessed and found to be insignificant (< ± 20%) for blood. Nevertheless, matrix matched calibrations were used for target analyte quantification. |
| Limit of determination/quantification | LOQ: 0.01 mg/L  LOD: 0.0025 mg/L |
| Stability of the target analyte in standards | Stability of a 19-day stored 0.004 μg/mL acetamiprid standard solution prepared in acetonitrile was assessed by comparing the stored standard to a freshly prepared standard. The stored standard was stable when stored refrigerated (2°C to 8°C), as the difference when compared to the fresh standard was ≤ 10%. |
| Stability of the target analyte in final extracts | Extract stability was assessed by re-injection of the LOQ recoveries using freshly prepared calibration standards after 8 days refrigerated storage (2°C to 8°C). Mean recovery was in the range 70 – 120% with a relative standard deviation within ±20% of the original value. Extracts were therefore deemed to be stable for at least 8 days when stored refrigerated. |

**Conclusion**

An analytical method for the determination of acetamiprid residues in body fluids (blood) was fully validated according to SANTE/2020/12830 rev.1. guidelines. The method was sufficiently accurate and precise to be able to provide reliable data on target analyte concentrations in blood and should therefore be considered acceptable.

* + - * 1. Analytical method RES-00539

Method validation RES-00539

|  |  |
| --- | --- |
| Comments of zRMS: | ~~Study is ongoing.~~  The analytical method was found to be valid for the determination of residues of acetamiprid metabolites IM-2-1 and IC-O in meat (muscle) and liver, with an LOQ of 0.01 mg/kg. The validation of the method met the criteria detailed in SANTE/2020/12830, Rev.2.  The method is acceptable. |

|  |  |
| --- | --- |
| Reference: | KCP 5.2/08 |
| Report | Validation of an analytical method for residues of acetamiprid metabolites IM-2-1 and IC-0 in body tissues.  ~~Brown, D. 2024-~~Watson, G., 2025, Report No. RES-00539, Sponsor Reference No. 000119484 |
| Guideline(s): | SANTE/2020/12830 rev.2 |
| Deviations: | None |
| GLP: | Yes |
| Acceptability: | Yes |

A multi-residue QuEChERS-based analytical method for the determination of acetamiprid metabolites (IM-2-1 and C-0) residues in body tissues (muscle and liver) was fully validated according to the requirements of SANTE/2020/12830 rev.2.

**A. Materials**

|  |  |
| --- | --- |
| 1. Standards |  |
| Reference item 1: | IM-2-1, (*N*1-[(6-chloro-3-pyridyl)methyl]-*N*2-cyano-acetamidine) |
| Lot/Batch number: | 681-028-00 |
| Purity: | 99.8 % |
| CAS No.: | 190604-92-3 |
| Expiry date: | 23 October 2028 |
|  |  |
| Reference item 2: | IC-0 (6*-*chloronicotinic acid) |
| Lot/Batch number: | 644-095-01 |
| Purity: | 99.1 % |
| CAS No.: | 5326-23-8 |
| Expiry date: | 24 October 2026 |
|  |  |
| Standards for calibration and fortification | Reference items 1 and 2 |
| Matrix: | Body tissues (muscle and liver) |

1. **Sample preparation and processing**

An aliquot (2 mL) was weighed into a 50-mL centrifuge tube. Samples were fortified at this stage, if necessary. Deionised water (8 mL) and acetonitrile (10 mL) were added, and the samples were homogenised for 15 minutes on a mechanical shaker. QuEChERS citrate extraction salts were added, and the samples were shaken by hand for 1 minute. Samples were then centrifuged at 3500 rpm for 5 minutes. An aliquot of the acetonitrile extract (1 mL) was diluted with deionised water (1 ml) in a 7-mL glass vial and mixed by hand. The final extract was filtered over (nylon, 13 mm, 0.45 µm) into a autosampler vial prior analysis by LC-MS/MS. Final sample concentration was 0.1 g/mL.

1. **Chromatographic parameters**

|  |  |
| --- | --- |
| HPLC- parameters: | Agilent 1260 Binary |
| Column: | Waters Cortecs Phenyl, 100 x 3.0 mm, 2.7 µm |
| Mobile phase: | A: 0.01% formic acid in water  B: Acetonitrile |
| Flow rate: | 0.5 mL/min |
| Injection volume: | 10 µL |
| Retention times: | 4:2 minutes for IC-0  4.7 minutes for IM-2-1 |
|  |  |
| MS/MS Parameters:  Ionisation type:  Transitions monitored: | AB Sciex 5500  Turbo Ion Spray positive  m/z 209.0→126.0 (quantification for IM-2-1)  m/z 209.0→90.0 (confirmation for IM-2-1)  m/z 158.0→122.0 (quantification for IC-0)  m/z 160.0→122.0 (confirmation for IC-0) |

**Results and discussion**

The analytical method used in the current study was fully validated for the determination of acetamiprid metabolites (IM-2-1 and IC-0) in body tissues (muscle and liver) according to the requirements of SANTE/2020/12830 rev.2 guidelines. The target analytes were determined using HPLC-MS/MS by monitoring two highly specific mass transitions. The detector response was linear over the range 0.3 ng/mL to 20 ng/mL (corresponding to 0.003 mg/kg to 0.2 mg/kg) with associated correlation coefficients (r) ≥ 0.995. The LOQ of the method is 0.01 mg/kg for both analytes in both matrices. All mean recovery values and associated RSDs meet the requirements of SANTE/2020/12830 rev.2 guidelines and are summarised in the table below.

**Table A 89: Method validation recovery data for the determination of acetamiprid metabolites IM-2-1 in body tissues (muscle and liver) reported in study RES-00539**

| **Matrix** | **Analyte** | **Fortification level (mg/kg) (n=5)** | **Mean  recovery (%)** | **RSD (%)** | **Comments** |
| --- | --- | --- | --- | --- | --- |
| Quantification transition m/z 209→126 | | | | | |
| Meat (muscle) | IM-2-1 | 0.01 | 98 | 1.2 | - |
| 0.1 | 97 | 2.7 | - |
| Liver | 0.01 | 99 | 2.1 | - |
| 0.1 | 99 | 1.3 | - |
| Confirmation transition m/z 209→90 | | | | | |
| Meat (muscle) | IM-2-1 | 0.01 | 97 | 1.7 | - |
| 0.1 | 97 | 3.4 | - |
| Liver | 0.01 | 100 | 2.9 | - |
| 0.1 | 98 | 1.6 | - |

**Table A 90: Method validation recovery data for the determination of acetamiprid metabolites IC-0 in body tissues (muscle and liver) reported in study RES-00539**

| **Matrix** | **Analyte** | **Fortification level (mg/kg) (n=5)** | **Mean  recovery (%)** | **RSD (%)** | **Comments** |
| --- | --- | --- | --- | --- | --- |
| Quantification transition m/z 158→122 | | | | | |
| Meat (muscle) | IC-0 | 0.01 | 92 | 1.1 | - |
| 0.1 | 91 | 0.5 | - |
| Liver | 0.01 | 91 | 1.0 | - |
| 0.1 | 90 | 0.8 | - |
| Confirmation transition m/z 160→122 | | | | | |
| Meat (muscle) | IC-0 | 0.01 | 92 | 2.0 | - |
| 0.1 | 90 | 0.6 | - |
| Liver | 0.01 | 90 | 1.3 | - |
| 0.1 | 90 | 0.7 | - |

**Table A 91: Characteristics of the analytical method validated for the determination of acetamiprid metabolites IM-2-1 and IC-0 in body tissues (muscle and liver)**

|  | **IM-2-1 and IC-0** |
| --- | --- |
| Specificity | A highly specific HPLC-MS/MS method was used for the determination of the target analytes. Two mass transitions (m/z 209→126, quantifiaction and m/z 209→90, qualification for IM-2-1 and m/z 158→122, quantifiaction and m/z 160→122, qualification for IC-0) were monitored during each analysis.  Target analyte concentrations in controls did not exceed 30% of the method LOQ. No interference from co-eluting components at the retention time of the target analyte. |
| Calibration (type, number of data points) | Seven-point matrix-matched linear calibration, 1/x weighting     |  |  |  |  |  | | --- | --- | --- | --- | --- | | **Mass transition** | **Analyte** | **Matrix** | **Equation** | **r** | | quantification | IM-2-1 | Muscle | y = 307247x + 2487 | 0.9997 | | confirmation | y = 74519x + 1718 | 0.9997 | | quantification | Liver | y = 298994x + 1292 | 0.9999 | | confirmation | y = 72364x + 733 | 0.9998 | | quantification | IC-0 | Muscle | y = 106127x + 1027 | 0.9999 | | confirmation | y = 34360x + 260 | 0.9999 | | quantification | Liver | y = 104554x + 522 | 0.9998 | | confirmation | y = 33718x + 497 | 1.0000 | |
| Calibration range | 0.3 ng/mL to 20 ng/mL (corresponding to 0.003 mg/kg to 0.25mg/kg) |
| Assessment of matrix effects is presented | Matrix effects were assessed and found to be significant for IM-2-1 in muscle (-46.1%) and liver (-49.6%). Therefore, matrix matched calibrations were used for target analyte quantification.  Matrix effects were assessed and found to be insignificant for IC-0 in muscle (-3.9) and liver (-7.8%). Nevertheless, matrix matched calibrations were used for target analyte quantification. |
| Limit of determination/quantification | LOQ: 0.010 mg/kg  LOD: 0.3 ng/L corersponding to 0.003 mg/kg |
| Stability of the target analyte in standards | Stability of a 10-day stored 0.015 μg/mL of both analytes standard solution prepared in acetonitrile/water (50/50, v/v) were assessed by comparing the stored standard to a freshly prepared standard. The stored standard was stable when stored refrigerated (2°C to 8°C), as the difference when compared to the fresh standard was ≤ 10%. |
| Stability of the target analyte in final extracts | Extract stability was assessed by re-injection of the LOQ recoveries using freshly prepared calibration standards after 7 days refrigerated. Mean recovery was in the range 70 – 120% with a relative standard deviation within ±20% of the original value. Extracts of both analytes in both matrices were therefore deemed to be stable for at least 7 days when stored refrigerated. |

**Conclusion**

An analytical method for the determination of acetamiprid metabolites (IM-2-1 and IC-0) residues in body tissues (muscle and liver) was fully validated according to SANTE/2020/12830 rev.2. guidelines. The method was sufficiently accurate and precise to be able to provide reliable data on target analyte concentrations in body tissues and should therefore be considered acceptable.

* + - * 1. Analytical method RES-00538

Method validation RES-00538

|  |  |
| --- | --- |
| Comments of zRMS: | ~~Study is ongoing.~~  The analytical method was found to be valid for the determination of residues of acetamiprid  metabolites IM-2-1 and IC-O in blood and urine, with an LOQ of 0.01 mg/L. The validation of the method met the criteria detailed in SANTE/2020/12830, Rev.2.  The method is acceptable. |

|  |  |
| --- | --- |
| Reference: | KCP 5.2/09 |
| Report | Validation of an analytical method for residues of acetamiprid metabolites IM-2-1 and IC-0 in body fluids.  ~~Brown, D. 2024-~~Watson, G., 2025, Report No. RES-00538, Sponsor Reference No. 000119483 |
| Guideline(s): | SANTE/2020/12830 rev.2 |
| Deviations: | None |
| GLP: | Yes |
| Acceptability: | Yes |

A multi-residue QuEChERS-based analytical method for the determination of acetamiprid metabolites (IM-2-1 and C-0) residues in body fluids (blood and urine) was fully validated according to the requirements of SANTE/2020/12830 rev.2.

**A. Materials**

|  |  |
| --- | --- |
| 1. Standards |  |
| Reference item 1: | IM-2-1, (*N*1-[(6-chloro-3-pyridyl)methyl]-*N*2-cyano-acetamidine) |
| Lot/Batch number: | 681-028-00 |
| Purity: | 99.8 % |
| CAS No.: | 190604-92-3 |
| Expiry date: | 23 October 2028 |
|  |  |
| Reference item 2: | IC-0 (6*-*chloronicotinic acid) |
| Lot/Batch number: | 644-095-01 |
| Purity: | 99.1 % |
| CAS No.: | 5326-23-8 |
| Expiry date: | 24 October 2026 |
|  |  |
| Standards for calibration and fortification | Reference items 1 and 2 |
| Matrix: | Body fluids (blood and urine) |

1. **Sample preparation and processing**

An aliquot (2 mL) was weighed into a 50-mL centrifuge tube. Samples were fortified at this stage, if necessary. Deionised water (8 mL) and acetonitrile (10 mL) were added, and the samples were homogenised for 15 minutes on a mechanical shaker. QuEChERS citrate extraction salts were added, and the samples were shaken by hand for 1 minute. Samples were then centrifuged at 3500 rpm for 5 minutes. An aliquot of the acetonitrile extract (1 mL) was diluted with deionised water (1 ml) in a 7-mL glass vial and mixed by hand. The final extract was filtered over (nylon, 13 mm, 0.45 µm) into a autosampler vial prior analysis by LC-MS/MS. Final sample concentration was 0.1 g/mL.

1. **Chromatographic parameters**

|  |  |
| --- | --- |
| HPLC- parameters: | Agilent 1260 Binary |
| Column: | Waters Cortecs Phenyl, 100 x 3.0 mm, 2.7 µm |
| Mobile phase: | A: 0.01% formic acid in water  B: Acetonitrile |
| Flow rate: | 0.5 mL/min |
| Injection volume: | 10 µL |
| Retention times: | 4:2 minutes for IC-0  4.7 minutes for IM-2-1 |
|  |  |
| MS/MS Parameters:  Ionisation type:  Transitions monitored: | AB Sciex 5500  Turbo Ion Spray positive  m/z 209.0→126.0 (quantification for IM-2-1)  m/z 209.0→90.0 (confirmation for IM-2-1)  m/z 158.0→122.0 (quantification for IC-0)  m/z 160.0→122.0 (confirmation for IC-0) |

**Results and discussion**

The analytical method used in the current study was fully validated for the determination of acetamiprid metabolites (IM-2-1 and IC-0) in body fluids (blood and urine) according to the requirements of SANTE/2020/12830 rev.2 guidelines. The target analytes were determined using HPLC-MS/MS by monitoring two highly specific mass transitions. The detector response was linear over the range 0.3 ng/mL to 20 ng/mL (corresponding to 0.003 mg/L to 0.2 mg/L) with associated correlation coefficients (r) ≥ 0.995. The LOQ of the method is 0.01 mg/L for both analytes in both matrices. All mean recovery values and associated RSDs meet the requirements of SANTE/2020/12830 rev.2 guidelines and are summarised in the table below.

**Table A 92: Method validation recovery data for the determination of acetamiprid metabolites IM-2-1 in body fluids (blood and urine) reported in study RES-00538**

| **Matrix** | **Analyte** | **Fortification level (mg/L) (n=5)** | **Mean  recovery (%)** | **RSD (%)** | **Comments** |
| --- | --- | --- | --- | --- | --- |
| Quantification transition m/z 209→126 | | | | | |
| Blood | IM-2-1 | 0.01 | 103 | 2.9 | - |
| Urine | 0.01 | 98 | 2.0 | - |
| Confirmation transition m/z 209→90 | | | | | |
| Blood | IM-2-1 | 0.01 | 104 | 2.6 | - |
| Urine | 0.01 | 98 | 1.3 | - |

**Table A 93: Method validation recovery data for the determination of acetamiprid metabolites IC-0 in body fluids (blood and urine) reported in study RES-00538**

| **Matrix** | **Analyte** | **Fortification level (mg/L) (n=5)** | **Mean  recovery (%)** | **RSD (%)** | **Comments** |
| --- | --- | --- | --- | --- | --- |
| Quantification transition m/z 158→122 | | | | | |
| Blood | IC-0 | 0.01 | 84 | 1.9 | - |
| Urine | 0.01 | 94 | 1.1 | - |
| Confirmation transition m/z 160→122 | | | | | |
| Blood | IC-0 | 0.01 | 83 | 1.1 | - |
| Urine | 0.01 | 95 | 3.1 | - |

**Table A 94: Characteristics of the analytical method validated for the determination of acetamiprid metabolites IM-2-1 and IC-0 in body fluids (blood and urine)**

|  | **IM-2-1 and IC-0** |
| --- | --- |
| Specificity | A highly specific HPLC-MS/MS method was used for the determination of the target analytes. Two mass transitions (m/z 209→126, quantifiaction and m/z 209→90, qualification for IM-2-1 and m/z 158→122, quantifiaction and m/z 160→122, qualification for IC-0) were monitored during each analysis.  Target analyte concentrations in controls did not exceed 30% of the method LOQ. No interference from co-eluting components at the retention time of the target analyte. |
| Calibration (type, number of data points) | Seven-point matrix-matched linear calibration, 1/x weighting     |  |  |  |  |  | | --- | --- | --- | --- | --- | | **Mass transition** | **Analyte** | **Matrix** | **Equation** | **r** | | quantification | IM-2-1 | Blood | y = 257862x + 3665 | 0.9998 | | confirmation | y = 62462x + 4931 | 0.9998 | | quantification | Urine | y = 506336x + 3214 | 0.9997 | | confirmation | y = 123923x + 459 | 0.9996 | | quantification | IC-0 | Blood | y = 108590x + 1257 | 0.9999 | | confirmation | y = 35168x + 872 | 0.9999 | | quantification | Urine | y = 110333x + 3565 | 0.9991 | | confirmation | y = 35555x + 971 | 0.9992 | |
| Calibration range | 0.3 ng/mL to 20 ng/mL (corresponding to 0.003 mg/kg to 0.25 mg/kg) |
| Assessment of matrix effects is presented | Matrix effects were assessed and found to be significant for IM-2-1 in muscle (-60.1%) and insignificant in liver (-14.7%). Therefore, matrix matched calibrations were used for target analyte quantification.  Matrix effects were assessed and found to be insignificant for IC-0 in muscle (-10.8) and liver (+.03%). Nevertheless, matrix matched calibrations were used for target analyte quantification. |
| Limit of determination/quantification | LOQ: 0.010 mg/kg  LOD: 0.3 ng/L corersponding to 0.003 mg/kg |
| Stability of the target analyte in standards | Stability of a 10-day stored 0.015 μg/mL of both analytes standard solution prepared in acetonitrile/water (50/50, v/v) were assessed by comparing the stored standard to a freshly prepared standard. The stored standard was stable when stored refrigerated (2°C to 8°C), as the difference when compared to the fresh standard was ≤ 10%. |
| Stability of the target analyte in final extracts | Extract stability was assessed by re-injection of the LOQ recoveries using freshly prepared calibration standards after 8 days refrigerated. Mean recovery was in the range 70 – 120% with a relative standard deviation within ±20% of the original value. Extracts of both analytes in both matrices were therefore deemed to be stable for at least 8 days when stored refrigerated. |

**Conclusion**

An analytical method for the determination of acetamiprid metabolites (IM-2-1 and IC-0) residues in body fluids (blood and urine) was fully validated according to SANTE/2020/12830 rev.2. guidelines. The method was sufficiently accurate and precise to be able to provide reliable data on target analyte concentrations in body tissues and should therefore be considered acceptable.

* + - 1. Other Studies/ Information

No new or additional studies have been submitted.