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| REGISTRATION REPORT  Part B  Section 5  Analytical Methods  Detailed summary of the risk assessment |
| Product code: BAS 743 03 F  Product name(s): DIVEXO  Chemical active substance(s):  Ametoctradin 120 g/L Propamocarb hydrochloride 451 g/L |
| Central Zone  Zonal Rapporteur Member State: Poland |
| CORE ASSESSMENT  (authorization of product) |
| Applicant: XXXX  Submission date: October 2023 (update September 2024)  Evaluation date: May 2024  MS Finalisation date: November 2024 |

Version history

|  |  |
| --- | --- |
| When | What |
| October 2023 | Initial dRR – XXXX DocID 2023/2030651 |
| May 2024 | Initial RR of zRMS |
| September 2024 | Updating following the Commenting phase (XXXX Doc ID 2024/2031092).  1. Inclusion of report header information for KCP 5.1.1/4, Pecorelli, P., 2023, Doc ID 2023/2004017)  2. Removal of Control procedure 21/01288\_01, Wagner, I. (2022), Doc ID 2022/2034983 in both Ametoctradin and Propamocarb sections of the dossier  (A 2.1.1.10.1 and A 2.2.1.8.1 respectively) – method is not relevant to the BAS 743 03 F application  3. Annex point renumbering in light of insertions/deletions  4. Appendix 1 update (numbering and typographical corrections)  Minor typographical corrections |
| November 2024 | Updated dRR – after MSs consultation |

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# Analytical methods

## Conclusion and summary of assessment

Sufficiently sensitive and selective analytical methods are available for the active substances 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.

The applicant's dRR was not rewritten by the zRMS. In the resulted RR comments/corrections/addons were placed on the grey background.

Noticed data gaps are: none

| Commodity/crop | Supported/ Not supported |
| --- | --- |
| Plant matrices | supported |
| Animal matrices | supported |
| Soil | supported |
| Water | supported |
| Air | supported |
| Body fluids | supported |

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

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

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

An overview on the acceptable methods for analysis of Ametoctradin and Propamocarb in plant protection product is provided below. In some instances, reference may be made to a closely related SC formulation, BAS 743 02 F, which contains 515.4 g/L Propamocarb and 137.1 g/L Ametoctradin and the same co-formulants. Similarly, some methods were developed using BAS 743 00 F which is the formulation which preceeded BAS 743 03 F. The compositions of BAS 743 02 F and BAS 743 00 F are given in the confidential section (Part C) for information.

|  |  |
| --- | --- |
| Comments of zRMS: | The presented below, GC with FID detection analytical method has been validated according to EU Guidance SANCO/3030/99 rev.5. The method is acceptable for the simultaneous determination of ametoctradin and propamocarb in the formulation BAS 743 03 F |

|  |  |
| --- | --- |
| Reference: | CP 5.1.1/1 |
| Report | Analytical Method AFL1028/03  Determination of the active Ingredients Propamocarb and Ametoctradin in SC formulations BAS 743 00F, BAS 743 02F and BAS 743 03F by GC, including Suspensibility  Pecorelli, P.M., 2023  2023/2007064 |
| Guideline(s): | Not applicable |
| Deviations: | No |
| GLP: | No, no subject to GLP regulations |
| Acceptability: | Yes | |

|  |  |
| --- | --- |
| Reference: | CP 5.1.1/2 |
| Report | Validation of analytical method AFL1028/01 : Determination of the active ingredients Propamocarb and Ametoctradin in formulations by GC (including Suspensibility)  Stickland, L., 2021  report No 886959\_1, MX/21/012/1  2020/2106327 |
| Guideline(s): | OECD Principles of Good Laboratory Practice and Regulation (EU) 1107/2009 as set out in Regulation (EU) 284/2013, EC Guideline SANCO/3030/99 rev.5, EC Guideline SANCO/3029/99 rev.4 |
| Deviations: | No |
| GLP: | Yes (certified by MHRA, United Kingdom) |
| Acceptability: | Yes | |

|  |  |
| --- | --- |
| Reference: | CP 5.1.1/3 |
| Report | Additional Validation of Analytical Method AFL1028/01 : “Determination of the active ingredients Propamocarb and Ametoctradin in SC formulation BAS 743 00 F by GC, including Suspensibility”  Pecorelli, P.M., 2022  report No 919949\_1  2022/2027441 |
| Guideline(s): | SANCO/3030/99 rev 5 (active in product)  SANCO/3029/99 rev.4, SANTE/2020/12830 rev.1 (suspensibility) |
| Deviations: | Yes (none affect the integrity of the validation) |
| GLP: | Yes (certified by Landesamt für Umwelt, Mainz, Germany) |
| Acceptability: | Yes | |
|  |  | |
| Reference: | CP 5.1.1/4 | |
| Report | Amendment No 1 - Additional Validation of Analytical Method AFL1028/01 : “Determination of the active ingredients Propamocarb and Ametoctradin in SC formulation BAS 743 00 F by GC, including Suspensibility”  Pecorelli, P.M., 2023  report No 919949\_1  2023/2004017 | |
| Guideline(s): | SANCO/3030/99 rev 5 (active in product)  SANCO/3029/99 rev.4, SANTE/2020/12830 rev.1 (suspensibility) | |
| Deviations: | No (minor update to remove heating of syringe) | |
| GLP: | Yes (certified by Landesamt für Umwelt, Mainz, Germany) | |
| Acceptability: | Yes | |

Materials and methods

A quantity of sample (200 mg) is weighed into a 100 mL volumetric flask and water (10 mL) is added to make a slurry. Internal standard solution (triphenylphosphate, 2 mg/mL in acetone) is added together with acetone (60 mL). The solution is ultrasonicated for 5 minutes, allowed to equilibrate to room temperature and made to volume with acetone. Following mixing, an aliquot is passed through a 0.22 µm single-use syringe filter prior to quantification by gas chromatography utilising an RTX-5 Amine column with internal standardisation and external standard calibration.

A sample of formulation (25 mg for 0.1 % or 2500 mg for 10 %) is weighed into a 150 mL beaker. CIPAC water D (25 mL) is added added to make a slurry and the solutions placed for about 5 minutes in the ultrasonic bath. The solutions are evaporated (overnight) to dryness at approximately 50 °C. For 0.1 % samples residues are slurried with water (10 mL,) and transferred to a volumetric flask with acetone then internal standard solution (triphenylphosphate, 2 mg/mL in acetone, 10 mL) is added and the samples made up to volume with acetone. For 10 % samples residues are filled up with acetone (100 mL) placed for about 5 minutes in the ultrasonic bath. An aliquot (5 mL)is transferred to a 100 mL volumetric flask and (triphenylphosphate, 2 mg/mL in acetone, 10 mL) and the samples made up to volume with acetone. Samples are passed through a 0.22 µm single-use syringe filter prior to quantification by gas chromatography utilising an RTX-5 Amine column with internal standardisation and external standard calibration.

The analytical method is used for the determination of the active ingredients Ametoctradin and Propamocarb in the formulation BAS 743 03 F and for the determination of suspensibility. Validation was carried out using a comparable formulation, BAS 743 00 F (SC formulation containing 378 g/L Propamocarb and 120 g/L Ametoctradin).

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Column | RTX-5 Amine, 30 m x 0.32 mm, 1 µm or equivalent | | | | |
| Mobile phase | Helium | | | | |
| Flow rate | 1.5 mL/min (contant flow) | | | | |
| Column temperature | Rate (ºC/min) | Temp (ºC) | Hold Time (min) | | Run Time (min) |
| - | 150 | - | | Initial |
| 15 | 300 | 10 | | 20 |
| Injection volume | 1 μL | | | | |
| Syringe temp | 90 ºC (v1 and v2 of the method only) | | | | |
| Injection split | 1:10 | | | | |
| Injection temp. | 250 ºC | | | | |
| Detection | FID | | | | |
| Detection temp. | 320 ºC | | | | |
| Retention time | Component | | | Approx. Retention Time (min) | |
| Propamocarb | | | 5 | |
| Triphenylphosphate (ISTD) | | | 12.2 | |
| Ametoctradin | | | 15 | |
| Total run time | 20 min | | | | |
| Version control | v1 New method | | | | |
| v2 Preparation 0.1% level suspensibility The active ingredient propamocarb removed from the suspensibility  Stability added | | | | |
| v3 Syringe temperature (90ºC) removed | | | | |

Validation - Results and discussions

Table 5.2‑1: Methods suitable for the determination of active substances Ametoctradin and Prompamocarb in plant protection product BAS 743 03 F

|  | Ametoctradin | Prompamocarb |
| --- | --- | --- |
| Author(s), year | Stickland, L., 2021 | Stickland, L., 2021 |
| Principle of method | GC-FID | GC-FID |
| Linearity  (linear between  mg/L / % range of the declared content)  (correlation coefficient, expressed as r) | **Active in formulation**  Range: ~0.089 – ~0.445 mg/mL (approx 40-180% of nominal value, ~48-~216 g/L Ametoctradin in formulation BAS 743 00 F\*)  R: 1.0000  Slope: 4.2228  Intercept: -0.0126  n=5 x 2 Range >±20% of nominal concentration  r>0.99  **Suspensibility**  Range: ~0.023 – ~0.197 mg/mL (approx -20 to +20% of nominal value in formulation BAS 743 00 F\*)  R: 1.0000  Slope: 4.1537  Intercept: -0.0012  n=5 x 2  Range >±20% of nominal concentration  r>0.99 | **Active in formulation**  Range: ~0.271 – ~1.353 mg/mL (approx 40-180% of nominal value, ~151-~680 g/L propamocarb in formulation BAS 743 00 F\*)  R: 1.0000  Slope: 3.3874  Intercept: 0.0051  n=5 x 2  **Suspensibility**  Range: ~0.067 – ~0.583 mg/mL (approx -20 to +20% of nominal value in formulation BAS 743 00 F\*)  R: 1.0000  Slope: 3.2289  Intercept: -0.0089  n=5 x 2  Range >±20% of nominal concentration  r>0.99 |
| Precision – Repeatability Mean  n = 5 in each case  (%RSD) | **Active in formulation**  Mean Ametoctradin content = 11.3 %w/w  RSD = 0.372%  Horrat value = 0.200  Mean Ametoctradin content = 11.4 %w/w  RSD = 0.497%  Horrat value = 0.268  Overall precision (n=10)  11.4 %w/w  RSD = 0.590%  Horrat value = 0.32  Hr<1  **Suspensibility**  Mean Ametoctradin content at 0.1% rate: 9.02 %w/w  RSD = 4.07%  RSD<10% | **Active in formulation**  Mean propamocarb content =  36.1 %w/w  RSD = 0.117%  Horrat value = 0.075  Mean propamocarb content =  36.0 %w/w  RSD = 0.234%  Horrat value = 0.150  Overall precision (n=10)  36.1 %w/w  RSD = 0.189%  Horrat value = 0.12  Hr<1  **Suspensibility**  Mean propamocarb content at 0.1% rate: 30.0 %w/w  RSD = 7.68%  RSD<10% |
| Accuracy  n = 5 in each case  (% Recovery) | **Active in formulation**  Spiked blank formulation:  Nominal concn = 120 g/L  At 50% nominal:  Mean recovery = 99.7% At 100% nominal:  Mean recovery = 100.9% At 150% nominal: Mean recovery = 102.1%  **Suspensibility**  Recovery solutions prepared at suspensibility in-use rates of 0.1% and 10%  Mean recovery at 0.1% rate:  79.1%  Mean recovery at 10% rate:  93.7% | **Active in formulation**  Spiked blank formulation:  Nominal concn = 378 g/L  At 50% nominal:  Mean recovery = 101.2% At 100% nominal:  Mean recovery = 101.1% At 150% nominal:  Mean recovery = 101.1%  **Suspensibility**  Recovery solutions prepared at suspensibility in-use rates of 0.1% and 10%  Mean recovery at 0.1% rate:  83.0%  Mean recovery at 10% rate:  91.3% |
| Interference/ Specificity | No interference on test item response from co-formulants/reagents in the retention time region of the active from control samples.  Analyte i.d. confirmed by retention time and mass spectrometry. | No interference on test item response from co-formulants/reagents in the retention time region of the active from control samples.  Analyte i.d. confirmed by retention time and mass spectrometry. |
| Solution stability | Standard and sample solutions were shown to be stable for at least 63 hours | Standard and sample solutions were shown to be stable for at least 63 hours |
| Detection limits | **Suspensibility**  The LOQ was determined to be 0.023 mg/mL | **Suspensibility**  The LOQ was determined to be 0.075 mg/mL |
| Comment | \* BAS 743 00 F is an SC formulation containing Ametoctradin 120 g/L & Propamocarb 378 g/L and was replaced by BAS 743 03 F following reformulation | \* BAS 743 00 F is an SC formulation containing Ametoctradin 120 g/L & Propamocarb 378 g/L and was replaced by BAS 743 03 F following reformulation |
| **Active in formulation**  The method was shown to be valid for the determination of Ametoctradin and propamocab in BAS 743 00 F SC formulations in accordance with SANCO/3030/99 rev.5  **Suspensibility**  The method was shown to be valid for the determination of suspensibility of Ametoctradin and propamocab in BAS 743 00 F SC formulations in accordance with SANCO/3029/99 rev.4 (the guidance in place at the time of study initiation) | |
|  | | |
|  | Ametoctradin | Prompamocarb |
| Author(s), year | Pecorelli, P.M., 2022 | Pecorelli, P.M., 2022 |
| Principle of method | GC-FID | GC-FID |
| Linearity  (linear between  mg/L / % range of the declared content)  (correlation coefficient, expressed as r) | **Suspensibility**  0.1 % and the 10 % suspensibility level  Range: 24.17 – 185.96 mg/L n= 6  0.1 % suspensibility level  Range: 28.14 – 36.55 mg/L n= 6 | **-** |
| Precision – Repeatability Mean  n = 5 in each case  (%RSD) | **Active in formulation**  Mean Ametoctradin content = 11.54 %w/w  RSD = 1.35%  Horrat value = 0.73  Hr<1  **Suspensibility**  Using data from accuracy section below  RSD at 0.1% level: 1.22% RSD at 10% level: 7.76%  RSD<10% | **Active in formulation**  Mean Propamocarb content =  35.83 %w/w  RSD = 1.20%  Horrat value = 0.77  Hr<1  **Suspensibility**  See comments section below |
| Accuracy  (% Recovery) | **Active in formulation**  Spiked blank formulation:  At 100% nominal:  Mean recovery = 99.8%  n=3 (range 98.5 – 102%) recovery in the correct range (97 to 103%)  **Suspensibility**  Recovery solutions prepared at suspensibility in-use rates of 0.1% and 10%  Mean recovery at 0.1% rate:  108.4%  Mean recovery at 10% rate:  90.6%  n=5 at each rate  recovery in the correct range (70 to 110%) | **Active in formulation**  Spiked blank formulation:  At 100% nominal:  Mean recovery = 98.0%  n=3 (range 97.3 – 98.3%)  recovery in the correct range (97 to 103%)  **Suspensibility**  See comments section below |
| Interference/ Specificity | No interference on test item response from reagents or co-formulantss of BAS 743 03 F in the retention time region of the active from control samples.  Analyte i.d. confirmed by retention time and mass spectrometry. | No interference on test item response from reagents or co-formulants of BAS 743 03F in the retention time region of the active from control samples.  Analyte i.d. confirmed by retention time and mass spectrometry. |
| Solution stability | Standard and sample solutions were shown to be stable for at least 54 hours | Standard and sample solutions were shown to be stable for at least 54 hours |
| Comment |  | **Suspensibility**  The suspensibility of the active ingredient Propamocarb is no longer determined. Propamocarb is fully soluble in water, and it is also fully solved in formulation. Therefore, it is not possible to keep it in dispersion for suspensibility testing. |
| **Active in formulation**  The method was shown to be valid for the determination of Ametoctradin and Propamocab in BAS 743 03 F in accordance with SANCO/3030/99 rev.5  **Suspensibility**  The method was shown to be valid for the determination of suspensibility of Ametoctradin and Propamocab in BAS 743 03 F, in accordance with SANTE/2020/12830 rev.1 | |

Conclusion

The method has been demonstrated to be suitable for the determination of Ametrocradin and Propamocarb in solutions of BAS 743 02 F for the determination of the active ingredients and for the determination of suspensibility. All validated paramters (identity, specificity, linearity, accuracy & precision etc) are acceptable.

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

An overview on the acceptable methods for analysis of relevant impurities in plant protection product is provided as follows:

Ametoctradin contains max. 50 mg/kg amitrole (Reg.No. 900093, equivalent to 6.12 mg/L (5.71 mg/kg mg/kg) Amitrole in BAS 743 03 F) and max. 2 g/kg o-Xylene (Reg.No. 4108046, equivalent to 0.245 g/L (0.23 g/kg) o-Xylene in BAS 743 03F), which are considered relevant impurities.

The analytical method AFL1070/01 has been developed for the determination of the impurity Amitrole (Reg.No.: 900093) in the BAS 743 03 F SC-formulation containing Ametoctradin (Reg.No. 4993353) and Propamocarb (Reg. No. 4628172). This method has not previously been reviewed and is provided in support of this assessment.

|  |  |
| --- | --- |
| Comments of zRMS: | This analytical method is adequately validated according to EU Guidance SANCO/3030/99 rev.5. The method is acceptable for the determination of the relevant impurity amitrole in the formulation BAS 743 03 F. |

|  |  |
| --- | --- |
| Reference: | CP 5.1.1/~~4~~5 |
| Report | Validation of the Analytical Methode AFL1070/01: “Determination of Amitrole in Formulations containing Ametoctradin (BAS 650 F) and Propamocarb by HPLC-MS  Schubring, M., 2022  report No 919953\_1  2022/2028342 |
| Guideline(s): | OECD Principles of Good Laboratory Practice, Directive 2004/10/EC, EU Regulation 1107/2009, SANCO/3030/99 rev. 5 (22 March 2019) |
| Deviations: | No |
| GLP: | Yes  (certified by Landesamt fuer Umwelt, Mainz, Germany ) |
| Acceptability: | Yes | |

|  |  |
| --- | --- |
| Comments of zRMS: | The analytical method is acceptable for determination of the impurity amitrole in the formulation BAS 743 03 F. |

|  |  |
| --- | --- |
| Reference: | CP 5.1.1/~~5~~6 |
| Report | Analytical Method AFL1070/01  Determination of Amitrole in Formulations containing Amectoctradin (BAS 650 F) and Propamocarb by HPLC-MS  Schubring, M., 2022  2022/2028343 |
| Guideline(s): | No guidelines available |
| Deviations: | No |
| GLP: | No, not subject to GLP regulations |
| Acceptability: | Yes | |

**Material and methods**

The method AFL1070/01 is applicable to the determination of the impurity Amitrole (Reg.No.: 900093) in the SC-formulation containing Ametoctradin (Reg.No. 4993353) and Propamocarb (Reg.No.: 4628172). The samples are analyzed using high-performance liquid chromatograph with MS-detector. The analyte is detected using a MS detector.

|  |  |
| --- | --- |
| Column | Primesep 100, 5µm (Sielc), 250 x 3.2mm (or equivalent type) |
| Column temperature | 25 °C |
| Mobile phase | A: Water/Formic acid 1000/6.25 v/v  B: Acetonitrile |
| Gradient | |  |  |  | | --- | --- | --- | | Time [min] | A [%] | B [%] | | 0 | 90 | 10 | | 15 | 90 | 10 | | 16 | 10 | 90 | | 30 | 10 | 90 | | 31 | 90 | 10 | | 36 | 90 | 10 | |
| Flow rate | 1.0 mL/min |
| GVV | 0.165 mL |
| Injection volume | 10 µL |
| Run time | 36 min |
| Approximate retention time | approx.7.5 mins |

Specific Agilent MSD parameters:

|  |  |
| --- | --- |
| Capillary Voltage (V) | 4000 |
| Gas temperature (°C) | 300 |
| Gas Flow (L/min) | 11.0 |
| Nebuliser (psi) | 15 |
| Fragmentor (V) | 100 |

**Validation - Results and discussions**

Table 5.2‑2: Methods suitable for the determination of Amitrole in the plant protection product BAS 743 03 F

|  | Amitrole  Maximum content in BAS 743 03 F  6.12 mg/L / 5.71 mg/kg \* |
| --- | --- |
| Author(s), year | Schubring, M., 2022 |
| Principle of method | HPLC-MS |
| Linearity  (linear between  mg/L / % range of the declared content)  (correlation coefficient, expressed as r) | In solvent:  Range: 0.016 – 1.2 mg/L (equivalent to 0.16 mg/kg – 12.0 mg/kg of amitrole in BAS 743 03 F)  R2: 0.9990  Slope: 1907500.69  Intercept: -4012.91  Matrix-matched:  Range: 0.016 – 1.2 mg/L (equivalent to 0.16 mg/kg – 12.0 mg/kg of amitrole in BAS 743 03 F)  R2: 0.9999  Slope: 133990.49  Intercept: 602.07  Range >±20% of nominal concentration  r>0.99 |
| Precision – Repeatability Mean  n =5  (%RSD) | Mean: 0.000066% (0.66 mg/kg amitrole in BAS 743 03 F).  RSD: 1.45%  Horrat value: 0.13  Hr<1 |
| Accuracy  n = 3 in each case  (% Recovery) | 0.000020% level (0.2 mg/kg, marginal recovery):  Recovery range: 73.6 – 78.6%  Mean: 76.9% (marginal recovery)  %RSD: 3.75%  Recovery limit: 70 – 130 %  0.00057% level (5.7 mg/kg, marginal recovery):  Recovery range: 88.0 –91.8%  Mean: 89.3% (marginal recovery)  %RSD: 2.43%  Recovery limit: 70 – 130 %  0.00080% level (8.0 mg/kg, total recovery):  Recovery range: 83.6 –85.2%  Mean: 84.4% (total recovery)  %RSD: 1.00%  Recovery limit: 70 - 130 % |
| Interference / Specificity | No interferences in blank and control samples |
| LOQ | 0.2 mg/kg (0.21mg/L) relative to the formulation |
| Solution stability | Standard addition samples were found to be stable for 119 hours at room temperature |
| Comment | The method meets the requirements of SANCO/3030/99 Rev. 5. |

\*Based on the specified limit of 50 mg/kg amitrole in TG ametoctradin (see 1.2.3.1) and relative density of the formulation = 1.071

**Conclusion**

With respect to the conditions described for the analytical method AFL1070/01 all validation parameters (linearity, precision, accuracy, identity, specificity, stability and LOQ) are acceptable. Therefore, the method is valid without restriction in the tested concentration range and is suitable for the determination of Amitrole in BAS 743 03 F.

The analytical method AFL1073/01 has been developed for the determination of the impurity o-Xylene (Reg.No.: 4108046) in formulations containing Ametoctradin (Reg.No. 4993353) and Propamocarb (Reg.No.: 4628172). This method has not previously been reviewed and is provided in support of this assessment.

|  |  |
| --- | --- |
| Comments of zRMS: | This analytical method is adequately validated according to EU Guidance SANCO/3030/99 rev.5. The method is acceptable for the determination of the relevant impurity o-xylene in the formulation BAS 743 03 F. |

|  |  |
| --- | --- |
| Reference: | CP 5.1.1/~~6~~7 |
| Report | Validation for the Analytical Method AFL1073/01: “Determination of o-Xylene in Formulations containing Ametoctradin (BAS 650 F) and Propamocarb by GC-MS Headspace”  Schubring, M. & Kimani, M., 2022  report No 9919952\_1  2022/2038541 |
| Guideline(s): | Directive 2004/10/EC, EU Regulation 1107/2009, SANCO/3030/99 rev. 5 (22 March 2019) |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes | |

|  |  |
| --- | --- |
| Comments of zRMS: | The analytical method is acceptable for determination of the impurity o-xylene in the formulation BAS 743 03 F . |

|  |  |
| --- | --- |
| Reference: | CP 5.1.1/~~7~~8 |
| Report | Analytical Method AFL1073/01  Determination of o-Xylene in Formulations containing Ametoctradin (BAS 650 F) and Propamocarb by GC-MS Headspace  Schubring, 2022  2022/2038543 |
| Guideline(s): | No guidelines available |
| Deviations: | No |
| GLP: | No, not subject to GLP regulations |
| Acceptability: | Yes | |

**Material and methods**

The method AFL1073/01 is applicable for the determination of the content of the impurity o-Xylene (Reg.No. 4108046) in formulations containing Ametoctradin (Reg.No. 4993353) and Propamocarb (Reg.No.: 4628172). The samples are prepared by weighing 100 mg of the test item into 10 mL headspace vials. 1 mL of internal standard (12.5 mg/L toluene in DMSO) and 1 mL DMSO is added to each headspace vial. Samples are analyzed using a gas chromatograph with MS-detector and Headspace sampler.

|  |  |  |  |
| --- | --- | --- | --- |
| Column | DB-624, 60 m x 0.25 mm, 1.4 µm | | |
| Injector temperature | 260 °C | | |
| Oven temperature | Rate [°C/min] | Value [°C] | Hold Time [min] |
| - | 85 | 4 |
| 12 | 210 | 1 |
| 25 | 260 | 2.5 |
| Carrier gas | Helium |  |  |
| Detector | MSD | | |
| Split ratio | 10:1 | | |
| Column flow | 1.1 mL/min (constant flow) | | |
| Injection volume | 1000 µL | | |
| Analysis time | 20 min | | |
| Approximate retention time | Toluene (internal std) approx. 11.8 min | | |
| o-Xylene approx. 14.3 min | | |

**Validation - Results and discussions**

Table 5.2‑3: Methods suitable for the determination of o-Xylene in the plant protection product BAS 743 03 F

|  | o-xylene  Maximum content in BAS 743 03 F  0.245 g/L / 0.23 g/kg\* |
| --- | --- |
| Author(s), year | Schubring, M. & Kimani, M., 2022 |
| Principle of method | GC-MS |
| Linearity  (linear between  mg/L / % range of the declared content)  (correlation coefficient, expressed as r) | Range: 4.558 – 19.553 mg/L (equivalent to 91.2 mg/kg – 391.1 mg/kg of o-Xylene in BAS 743 03 F)  R2: 0.9998  Slope: 0.0593  Intercept: 0.0207  n = 6  Range >±20% of nominal concentration  r>0.99 |
| Precision – Repeatability Mean  n =10 (one outlier rejected as Dixon outlier)  (%RSD) | Mean: 0.0229% (229 mg/kg o-xylene in BAS 743 03 F)  RSD: 0.59%,  Horrat value: 0.12  Hr<1 |
| Accuracy  n = 6/10/6  (% Recovery) | **Accuracy of Assay:**  0.0182% level (182 mg/kg):  Recovery range: 100.9-104.1%  Mean: 101.7%  %RSD: 1.18%  n=6  Recovery limit: 75-125%  0.0228% level (228 mg/kg):  Recovery range: 99.6-101.3%  Mean: 100.6%  %RSD: 0.59%  n=10 (one outlier rejected as Dixon outlier)  Recovery limit: 75-125%  0.0311% level (311 mg/kg):  Recovery range: 96.75-97.7%  Mean: 97.0%  %RSD: 0.34%  n=6  Recovery limit: 75-125% |
| Interference / Specificity | No interferences in blank and control samples |
| LOQ | 182 mg/kg (195mg/L) relative to the formulation |
| Solution stability | Calibration solutions were found to be stable for 77 hours and sample solutions for 90 hours, at room temperature |
| Comment | The method meets the requirements of SANCO/3030/99 Rev. 5. |

\*Based on the specified limit of 2 g/kg o-xylene in TG ametoctradin (see 1.2.3.1, dRR B1) and relative density of the formulation = 1.071

**Conclusion**

With respect to the conditions described in the analytical method AFL1073/01 all validation parameters (identity, specificity, linearity, accuracy and precision, stability, LOQ) are acceptable. Therefore, the method is valid without restriction in the tested concentration range and is suitable for the determination of o-Xylene in BAS 743 03 F.

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

Analytical methods for the determination of formulants are not required.

**zRMS comment**: with respect to toxicological, eco-toxicological or environmental aspects the product BAS 743 03 F does not contain any relevant formulants. Therefore, a special analytical method and validation is not needed.

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

No CIPAC method is available for the determination of ametoctradin and propamocarb in the plant protection product BAS 743 03 F.

### 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 Ametoctradin 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: Ametoctradin | | | | |
| --- | --- | --- | --- | --- |
| Matrix type | Method type | Method LOQ | Principle of method  (i.e. GC-MS or HPLC-UV) | Author(s), year / missing / EU agreed |
| **Plants, plant products** | | | | |
| Wheat (grain) | Primary  (No confirmation method required, two mass transitions employed) | 0.01 mg/kg | LC-MS/MS | Mackenroth C., Schweda Z., 2008  EU agreed, DAR 2009  Method no. L0078/01  XXXX DocID 2008/1022139  Provided in Appendix 2 for completeness |
| Potato (tuber) |
| Lettuce (head) |
| Tomato (fruit) |
| Grape (fruit) |
| Orange (sweet, whole fruit) |
| Onion (bulb) |
| Sunflower (seed) |
| Melon (fruit) | Primary  (No confirmation method required, two mass transitions employed) | 0.01 mg/kg | LC-MS/MS | Schneider, E., 2020  Not peer-reviewed  Method no. L0078/01  XXXX DocID 2008/1022139  For detailed evaluation refer to Appendix 2. |
| Melon (peel) |
| Melon (pulp) |
| Lettuce  (open head, whole plant) | Primary  (No confirmation method required, two mass transitions employed) | 0.01 mg/kg | LC-MS/MS | Vagt, I. & Meyer, M., 2022  Not peer-reviewed  Method no. L0078/01  XXXX DocID 2022/2041753  For detailed evaluation refer to Appendix 2. |
| Lettuce  (open head, head – open leaves) |
| Lettuce (Lamb’s,  whole plant) |
| Lettuce (Lamb’s, leaves) |
| Tomato (fruit) | Primary  (No confirmation method required, two mass transitions employed) | 0.01 mg/kg | LC-MS/MS | Gálvez, O., 2021  Not peer-reviewed  Method no. L0078/01  XXXX DocID 2020/2103085  For detailed evaluation refer to Appendix 2. |
| Potato (shoot) | Primary  (No confirmation method required, two mass transitions employed) | 0.01 mg/kg | LC-MS/MS | Gálvez, O., 2021  Not peer-reviewed  Method no. L0078/01  XXXX DocID 2020/2103083  For detailed evaluation refer to Appendix 2. |
| Potato (tuber) |
| Onion (bulbs) | Primary  (No confirmation method required, two mass transitions employed) | 0.01 mg/kg | LC-MS/MS | Schneider, E., 2021  Not peer-reviewed  Method no. L0078/01  XXXX DocID 2021/2019659  For detailed evaluation refer to Appendix 2. |
| Onion (whole plant, no roots) |
| Lettuce (head) | Primary  (No confirmation method required, two mass transitions employed) | 0.01 mg/kg | LC-MS/MS | Xiaodan, Du., 2022  Not peer-reviewed  Method no. L0078/02  XXXX DocID 2022/2027748  For detailed evaluation refer to Appendix 2. |
| Grapes (fruit) |
| Wheat (grain) |
| Soybean (seed) |
| Cucumber (fruit) | Primary  (No confirmation method required, two mass transitions employed) | 0.01 mg/kg | LC-MS/MS | Schneider, E., 2023  Not peer-reviewed  Method no. L0078/02  XXXX DocID 2022/2041754  For detailed evaluation refer to Appendix 2. |
| Zucchini (fruit) |
| Tomato (fruit) | Primary  (No confirmation method required, two mass transitions employed) | 0.01 mg/kg | LC-MS/MS | Martin, T., 2022  Not peer-reviewed  Method no. L0078/02  XXXX DocID 2021/2054075  For detailed evaluation refer to Appendix 2. |
| **Animal products, food of animal origin** | | | | |
| - | - | - | - | No method necessary (no residue definition defined) |
| **Soil, water, air (Environmental fate)** | | | | |
| - | - | - | - | No new methods. |
| **Ecotoxicology methods** | | | | |
| Reconstituted water | Primary  (No confirmation method required, two mass transi-tions employed) | 0.0954 mg/L | LC-MS/MS | Wendling, K., 2023  Not peer-reviewed  Method APL0500/02  XXXX DocID 2022/2033712  Toxicity to Daphnia magna, Straus (Acute Immobilisation Test – Static)  For detailed evaluation refer to Appendix 2. |
|  |  |  |  | Obert-Rauser, P., 2023  Not peer-reviewed  Method APL0500/02  XXXX DocID 2022/2033713  Toxicity to the Single Cell Green Alga Pseudokirchneriella subcapitata Hindák under Laboratory Conditions  For detailed evaluation refer to Appendix 2. |
| Wendling, K., 2023  Not peer-reviewed  Method APL0500/02  XXXX DocID 2022/2033714  Toxicity to the Rainbow Trout Oncorhynchus mykiss under Laboratory Conditions (Acute Toxicity Test – Semi-Static)  For detailed evaluation refer to Appendix 2. |
| Reconstituted water | Primary  (No confirmation method required, two mass transi-tions employed) | 0.1550 mg/L | LC-MS/MS | Renner, P., 2023  Not peer-reviewed  XXXX DocID 2022/2033730  Acute toxicity of BAS 743 03 F on Daphnia magna in a 48-hour static test  For detailed evaluation refer to Appendix 2. |
| Aqueous application solutions | Primary  (No confirmation method required, two mass transi-tions employed) | 0.170 g/L | LC-MS/MS | Maleck, A., 2023  Not peer-reviewed  Method L0208/02  XXXX DocID 2022/2033722  Seedling emergence/growth study  For detailed evaluation refer to Appendix 2. |
| Maleck, A., 2023  Not peer-reviewed  Method L0208/02  XXXX DocID 2022/2033723  Vegetative vigour study  For detailed evaluation refer to Appendix 2. |
| Bee feeding solutions | Primary  (No confirmation method required, two mass transi-tions employed) | 0.459 mg Ametocradin/kg | LC-MS/MS | Ruhland, S., 2023  Not peer-reviewed  XXXX DocID 2022/2033709  Chronic toxicity of BAS 743 02 F to the honey bee Apis mellifera L. under laboratory conditions  For detailed evaluation refer to Appendix 2. |
| Bee larvae feeding solutions | Primary  (No confirmation method required, two mass transi-tions employed) | 0.469 mg Ametocradin/kg | LC-MS/MS | Schmidt, K., 202~~2~~3  Not peer-reviewed  XXXX DocID 2022/2033710  Repeated exposure of honey bee (Apis mellifera L.) larvae to BAS 743 02 F under laboratory conditions  For detailed evaluation refer to Appendix 2. |
| Bumble bee: Contact and oral toxicity solutions | Primary  (No confirmation method required, two mass transi-tions employed) | 0.459 mg/kg (contact toxicity solutions)  0.482 mg/kg (oral toxicity solutions) | LC-MS/MS | Amsel, K., 2023  Not peer-reviewed  XXXX Doc ID 2022/2033711  Acute toxicity of BAS 743 02 F to the bumblebee Bombus terrestris L. under laboratory conditions  For detailed evaluation refer to Appendix 2. |
| **Toxicology methods** | | | | |
| ~~Inhalation tox (analysis of Ametoctradin in acetonitrile / 10mM disodium hydrogen~~  ~~phosphate dihydrate with phosphoric acid (85%) pH 2.9 (50+50 v/v)~~ | ~~Primary~~ | ~~0.2 mg/100 mL~~ | ~~HPLC-UV~~ | ~~Wagner, I., 2022~~  ~~Not peer reviewed~~  ~~Control procedure 21/0288\_01~~  ~~XXXX DocID 2022/2034983~~  ~~For detailed evaluation refer to Appendix 2.~~ |

| Component of residue definition: Metabolite M650F003 | | | | |
| --- | --- | --- | --- | --- |
| Matrix type | Method type | Method LOQ | Principle of method  (i.e. GC-MS or HPLC-UV) | Author(s), year / missing / EU agreed |
| **Plants, plant products** | | | | |
| Wheat (grain) | Primary  (No confirmation method required, two mass transitions employed) | 0.01 mg/kg | LC-MS/MS | Mackenroth C., Schweda Z., 2008  EU agreed  Method no. L0078/01  XXXX DocID 2008/1022139  Provided in Appendix 2 for completeness |
| Potato (tuber) |
| Lettuce (head) |
| Tomato (fruit) |
| Grape (fruit) |
| Orange (sweet, whole fruit) |
| Onion (bulb) |
| Sunflower (seed) |
| Melon (fruit) | Primary  (No confirmation method required, two mass transitions employed) | 0.01 mg/kg | LC-MS/MS | Schneider, E., 2020  Not peer-reviewed  Method no. L0078/01  XXXX DocID 2008/1022139  For detailed evaluation refer to Appendix 2. |
| Melon (peel) |
| Melon (pulp) |
| Lettuce  (open head, whole plant) | Primary  (No confirmation method required, two mass transitions employed) | 0.01 mg/kg | LC-MS/MS | Vagt, I. & Meyer, M., 2022  Not peer-reviewed  Method no. L0078/01  XXXX DocID 2022/2041753  For detailed evaluation refer to Appendix 2. |
| Lettuce  (open head, head – open leaves) |
| Lettuce (Lamb’s,  whole plant) |
| Lettuce (Lamb’s, leaves) |
| Tomato (fruit) | Primary  (No confirmation method required, two mass transitions employed) | 0.01 mg/kg | LC-MS/MS | Gálvez, O., 2021  Not peer-reviewed  Method no. L0078/01  XXXX DocID 2020/2103085  For detailed evaluation refer to Appendix 2. |
| Potato (shoot) | Primary  (No confirmation method required, two mass transitions employed) | 0.01 mg/kg | LC-MS/MS | Gálvez, O., 2021  Not peer-reviewed  Method no. L0078/01  XXXX DocID 2020/2103083  For detailed evaluation refer to Appendix 2. |
| Potato (tuber) |
| Onion (bulbs) | Primary  (No confirmation method required, two mass transitions employed) | 0.01 mg/kg | LC-MS/MS | Schneider, E., 2021  Not peer-reviewed  Method no. L0078/01  XXXX DocID 2021/2019659  For detailed evaluation refer to Appendix 2. |
| Onion (whole plant, no roots) |
| Lettuce (head) | Primary  (No confirmation method required, two mass transitions employed) | 0.01 mg/kg | LC-MS/MS | Xiaodan, Du., 2022  Not peer-reviewed  Method no. L0078/02  XXXX DocID 2022/2027748  For detailed evaluation refer to Appendix 2. |
| Grapes (fruit) |
| Wheat (grain) |
| Soybean (seed) |
| Cucumber (fruit) | Primary  (No confirmation method required, two mass transitions employed) | 0.01 mg/kg | LC-MS/MS | Schneider, E., 2023  Not peer-reviewed  Method no. L0078/02  XXXX DocID 2022/2041754  For detailed evaluation refer to Appendix 2. |
| Zucchini (fruit) |
| Tomato (fruit) | Primary  (No confirmation method required, two mass transitions employed) | 0.01 mg/kg | LC-MS/MS | Martin, T., 2022  Not peer-reviewed  Method no. L0078/02  XXXX DocID 2021/2054075  For detailed evaluation refer to Appendix 2. |
| **Animal products, food of animal origin** | | | | |
| - | - | - | - | No method necessary (no residue definition defined) |
| **Soil, water, air (Environmental fate)** | | | | |
| - | - | - | - | No new methods. |
| **Ecotoxicology methods** | | | | |
| - | - | - | - | No new methods. |
| **Toxicology methods** | | | | |
| - | - | - | - | No new methods. |

| Component of residue definition: Metabolite M650F004 | | | | |
| --- | --- | --- | --- | --- |
| Matrix type | Method type | Method LOQ | Principle of method  (i.e. GC-MS or HPLC-UV) | Author(s), year / missing / EU agreed |
| **Plants, plant products** | | | | |
| Wheat (grain) | Primary  (No confirmation method required, two mass transitions employed) | 0.01 mg/kg | LC-MS/MS | Mackenroth C., Schweda Z., 2008  EU agreed, DAR 2009  Method no. L0078/01  XXXX DocID 2008/1022139  Provided in Appendix 2 for completeness |
| Potato (tuber) |
| Lettuce (head) |
| Tomato (fruit) |
| Grape (fruit) |
| Orange (sweet, whole fruit) |
| Onion (bulb) |
| Sunflower (seed) |
| Melon (fruit) | Primary  (No confirmation method required, two mass transitions employed) | 0.01 mg/kg | LC-MS/MS | Schneider, E., 2020  Not peer-reviewed  Method no. L0078/01  XXXX DocID 2008/1022139  For detailed evaluation refer to Appendix 2. |
| Melon (peel) |
| Melon (pulp) |
| Lettuce  (open head, whole plant) | Primary  (No confirmation method required, two mass transitions employed) | 0.01 mg/kg | LC-MS/MS | Vagt, I. & Meyer, M., 2022  Not peer-reviewed  Method no. L0078/01  XXXX DocID 2022/2041753  For detailed evaluation refer to Appendix 2. |
| Lettuce  (open head, head – open leaves) |
| Lettuce (Lamb’s,  whole plant) |
| Lettuce (Lamb’s, leaves) |
| Tomato (fruit) | Primary  (No confirmation method required, two mass transitions employed) | 0.01 mg/kg | LC-MS/MS | Gálvez, O., 2021  Not peer-reviewed  Method no. L0078/01  XXXX DocID 2020/2103085  For detailed evaluation refer to Appendix 2. |
| Potato (shoot) | Primary  (No confirmation method required, two mass transitions employed) | 0.01 mg/kg | LC-MS/MS | Gálvez, O., 2021  Not peer-reviewed  Method no. L0078/01  XXXX DocID 2020/2103083  For detailed evaluation refer to Appendix 2. |
| Potato (tuber) |
| Onion (bulbs) | Primary  (No confirmation method required, two mass transitions employed) | 0.01 mg/kg | LC-MS/MS | Schneider, E., 2021  Not peer-reviewed  Method no. L0078/01  XXXX DocID 2021/2019659  For detailed evaluation refer to Appendix 2. |
| Onion (whole plant, no roots) |
| Lettuce (head) | Primary  (No confirmation method required, two mass transitions employed) | 0.01 mg/kg | LC-MS/MS | Xiaodan, Du., 2022  Not peer-reviewed  Method no. L0078/02  XXXX DocID 2022/2027748  For detailed evaluation refer to Appendix 2. |
| Grapes (fruit) |
| Wheat (grain) |
| Soybean (seed) |
| Cucumber (fruit) | Primary  (No confirmation method required, two mass transitions employed) | 0.01 mg/kg | LC-MS/MS | Schneider, E., 2023  Not peer-reviewed  Method no. L0078/02  XXXX DocID 2022/2041754  For detailed evaluation refer to Appendix 2. |
| Zucchini (fruit) |
| Tomato (fruit) | Primary  (No confirmation method required, two mass transitions employed) | 0.01 mg/kg | LC-MS/MS | Martin, T., 2022  Not peer-reviewed  Method no. L0078/02  XXXX DocID 2021/2054075  For detailed evaluation refer to Appendix 2. |
| **Animal products, food of animal origin** | | | | |
| - | - | - | - | No method necessary (no residue definition defined) |
| **Soil, water, air (Environmental fate)** | | | | |
| - | - | - | - | No new methods. |
| **Ecotoxicology methods** | | | | |
| - | - | - | - | No new methods. |
| **Toxicology methods** | | | | |
| - | - | - | - | No new methods. |

| **Component of residue definition: Propamocarb (Sum of Propamocarb and its salts expressed as Propamocarb)** | | | | |
| --- | --- | --- | --- | --- |
| **Matrix type** | **Method type** | **Method LOQ** | **Principle of method**  **(i.e. GC-MS or HPLC-UV)** | **Author(s), year / missing / EU agreed** |
| **Plants, plant products** | | | | |
| Tomato (fruit) | Primary  (No confirmation method required, two mass transitions employed) | 0.01 mg/kg | LC-MS/MS | Denim, R., 2022  Not peer-reviewed  Method no. L0450/01  XXXX DocID 2022/2032351  For detailed evaluation refer to Appendix 2. |
| Pea (dry) |
| Soybean (seed) |
| Grapes (fruit) |
| Melon (fruit) | Primary  (No confirmation method required, two mass transitions employed) | 0.01 mg/kg | LC-MS/MS | Schneider, E., 202~~0~~1  Not peer-reviewed  Method no. L0450/01  XXXX DocID 2021/2019512  For detailed evaluation refer to Appendix 2. |
| Melon (peel) |
| Melon (pulp) |
| Lettuce  (open head, whole plant) | Primary  (No confirmation method required, two mass transitions employed) | 0.01 mg/kg | LC-MS/MS | Vagt, I. & Meyer, M., 2022  Not peer-reviewed  Method no. L00450/01  XXXX DocID 2022/2041753  For detailed evaluation refer to Appendix 2. |
| Lettuce  (open head, head – open leaves) |
| Lettuce (Lamb’s,  whole plant) |
| Lettuce (Lamb’s, leaves) |
| Tomato (fruit) | Primary  (No confirmation method required, two mass transitions employed) | 0.01 mg/kg | LC-MS/MS | Gálvez, O., 2021  Not peer-reviewed  Method no. L0450/01  XXXX DocID 2020/2103085  For detailed evaluation refer to Appendix 2. |
| Potato (shoot) | Primary  (No confirmation method required, two mass transitions employed) | 0.01 mg/kg | LC-MS/MS | Gálvez, O., 2021  Not peer-reviewed  Method no. L0450/01  XXXX DocID 2020/2103083  For detailed evaluation refer to Appendix 2. |
| Potato (tuber) |
| Onion (bulbs) | Primary  (No confirmation method required, two mass transitions employed) | 0.01 mg/kg | LC-MS/MS | Schneider, E., 2021  Not peer-reviewed  Method no. L00450/01  XXXX DocID 2021/2019659  For detailed evaluation refer to Appendix 2. |
| Onion (whole plant, no roots) |
| Cucumber (fruit) | Primary  (No confirmation method required, two mass transitions employed) | 0.01 mg/kg | LC-MS/MS | Schneider, E., 2023  Not peer-reviewed  Method no. L0450/01  XXXX DocID 2022/2041754  For detailed evaluation refer to Appendix 2. |
| Zucchini (fruit) |
| **Animal products, food of animal origin** | | | | |
| - | - | - | - | No new methods. |
| **Soil, water, air (Environmental fate)** | | | | |
| - | - | - | - | No new methods. |
| **Ecotoxicology methods** | | | | |
| Reconstituted water | Primary  (No confirmation method required, two mass transi-tions employed) | 0.0954 mg/L | LC-MS/MS | Wendling, K., 2023  Not peer-reviewed  Method APL0500/02  XXXX DocID 2022/2033712  Toxicity to Daphnia magna, Straus (Acute Immobilisation Test – Static)  For detailed evaluation refer to Appendix 2. |
| Obert-Rauser, P., 2023  Not peer-reviewed  Method APL0500/02  XXXX DocID 2022/2033713  Toxicity to the Single Cell Green Alga Pseudokirchneriella subcapitata Hindák under Laboratory Conditions  For detailed evaluation refer to Appendix 2. |
| Wendling, K., 2023  Not peer-reviewed  Method APL0500/02  XXXX DocID 2022/2033714  Toxicity to the Rainbow Trout Oncorhynchus mykiss under Laboratory Conditions (Acute Toxicity Test – Semi-Static)  For detailed evaluation refer to Appendix 2. |
| Reconstituted water | Primary  (No confirmation method required, two mass transi-tions employed) | 0.1550 mg/L | LC-MS/MS | Renner, P., 2023  Not peer-reviewed  XXXX DocID 2022/2033730  Acute toxicity of BAS 743 03 F on Daphnia magna in a 48-hour static test  For detailed evaluation refer to Appendix 2. |
| Bee feeding solutions | Primary  (No confirmation method required, two mass transi-tions employed) | 0.459 mg Ametocradin/kg | LC-MS/MS | Ruhland, S., 2023  Not peer-reviewed  XXXX DocID 2022/2033709  Chronic toxicity of BAS 743 02 F to the honey bee Apis mellifera L. under laboratory conditions  For detailed evaluation refer to Appendix 2. |
| Bee larvae feeding solutions | Primary  (No confirmation method required, two mass transi-tions employed) | 1.48 mg Propamocarb/kg | LC-MS/MS | Schmidt, K., 202~~2~~3  Not peer-reviewed  XXXX DocID 2022/2033710  Repeated exposure of honey bee (Apis mellifera L.) larvae to BAS 743 02 F under laboratory conditions  For detailed evaluation refer to Appendix 2. |
| Bumble bee: Contact and oral toxicity solutions | Primary  (No confirmation method required, two mass transi-tions employed) | 1.45 mg/kg (contact toxicity solutions)  1.52 mg/kg (oral toxicity solutions) | LC-MS/MS | Amsel, K., 2023  Not peer-reviewed  XXXX Doc ID 2022/2033711  Acute toxicity of BAS 743 02 F to the bumblebee Bombus terrestris L. under laboratory conditions  For detailed evaluation refer to Appendix 2. |
| **Toxicology methods** | | | | |
| ~~Inhalation tox (analysis of Propamocarb in acetonitrile / 10mM disodium hydrogen~~  ~~phosphate dihydrate with phosphoric acid (85%) pH 2.9 (50+50 v/v)~~ | ~~Primary~~ | ~~1.0 mg/100 mL~~ | ~~HPLC-UV~~ | ~~Wagner, I., 2022~~  ~~Not peer reviewed~~  ~~Control procedure 21/0288\_01~~  XXXX ~~DocID 2022/2034983~~  ~~For detailed evaluation refer to Appendix 2.~~ |

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

Analytical methods for the determination of Ametoctradin have previously been submitted in the Draft Assessment Report (DAR, The Netherlands, 2009 [and addendum of 2012]) and subsequent AIR submissions. Analytical methods for the determination of Propamocarb have previously been submitted in the Draft Assessment Report (DAR, Ireland, 2005) and Renewal Assessment Report (RAR, Portugal (co-RMS Belgium), 2017). The applicant is the data owner for Ametoctradin. Data pertaining to Propamocarb is owned by Bayer, the notifier of the active substance, and the competent regulatory authority is authorized to access the data package of Propamocarb in support of the application of DIVEXO (BAS 743 03 F). Please, refer to *Letter of Access* in part A.

### 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 Ametoctradin (KCP 5.2)

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

Compared to the residue definition proposed in the Draft Assessment Report (incl. its addenda) the current legal residue definition is not identical.

Table 5.3‑1: Relevant residue definitions for monitoring/enforcement and levels for which com pliance is required

| Matrix | Residue definition | MRL / limit | Reference for MRL/level Remarks |
| --- | --- | --- | --- |
| Plant, high water content | Ametoctradin | 0.01 mg/kg | Reg. (EU) 2022/1290 |
| Plant, high acid content | 0.01 mg/kg | Reg. (EU) 2022/1290 |
| Plant, high protein/high starch content (dry commodities) | 0.01 mg/kg | Reg. (EU) 2022/1290 |
| Plant, high oil content | 0.01 mg/kg | Reg. (EU) 2022/1290 |
| Plant, difficult matrices (hops, spices, tea) | 0.01 mg/kg | Reg. (EU) 2022/1290 |
| Muscle | Ametoctradin  + M650F01  + M650F06  Expressed as Ametoctradin | 0.03 mg/kg | Reg. (EU) 2022/1290 |
| Milk | 0.03 mg/kg | Reg. (EU) 2022/1290 |
| Eggs | 0.03 mg/kg | Reg. (EU) 2022/1290 |
| Fat | 0.03 mg/kg | Reg. (EU) 2022/1290 |
| Liver, kidney | 0.03 mg/kg | Reg. (EU) 2022/1290 |
| Honey | Ametoctradin | 5 mg/kg | Reg. (EU) 2022/1290 |
| Soil  (Ecotoxicology) | Ametoctradin  (calculated from study with test item BAS 651 02 F) | NOECCORR= 100 mg/kg dry soil  NOECCORR = 22.1 mg a.s./kg dry soil 1), 2) (equivalent to 12.05 mg ametoctradin and 10.05 mg dimethomorph/kg dry soil) 1) | NOEC, *E. fetida* |
| Soil  (Ecotoxicology) | Metabolite: M650F03 | 50.0 mg/kg dry soil | NOEC, *F. candida* |
| Soil  (Ecotoxicology) | Metabolite: M650F04 | ≥ 100.0 mg/kg dry soil | NOECcorr, *E. fetida* |
| Drinking water  (Human toxicology) | Ametoctradin | 0.1 µg/L | general limit for drinking water |
| Surface water  (Ecotoxicology) | Ametoctradin | 0.0646 mg/L | LC50 fish acute  mean measured |
| Surface water  (Ecotoxicology) | Metabolite: M650F01 | > 100 mg/L | EC50  *D. magna* acute  nominal |
| Surface water  (Ecotoxicology) | Metabolite: M650F03 | 41.75 mg/L | NOEC, *D. magna*  nominal |
| Surface water  (Ecotoxicology) | Metabolite: M650F04 | > 100 mg/L | EC50  *D. magna* acute  nominal |
| Air | Ametoctradin | 0.09 µg/m3 | AOEL: 2 mg/kg bw/d |
| Tissue | Ametoctradin | 0.01 mg/kg | SANTE/2020/12830, Rev.2 |
| Metabolite: M650F06 | 0.01 mg/kg | SANTE/2020/12830, Rev.2 |
| Body fluids | Ametoctradin | 0.01 mg/L | SANTE/2020/12830, Rev.2 |
| Metabolite: M650F06 | 0.01 mg/L | SANTE/2020/12830, Rev.2 |
| 1) Corrected value derived by dividing the endpoint by a factor of 2 in accordance with the EPPO earthworm scheme 2002.  2) Endpoint based on sum of active substances (nominal) and taking into account a density of BAS 651 02 F of 0.995 g/cm3. | | | |

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

An overview on the acceptable methods for analysis of ametoctradin in plant matrices is given in the following tables. For the detailed evaluation of new/additional studies it is referred to Appendix 2.

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

| Component of residue definition: ametoctradin | | | | |
| --- | --- | --- | --- | --- |
| Matrix type | Method type | Method LOQ | Principle of method (i.e. GC-MS or HPLC-UV) | Author(s), year / missing / EU agreed |
| High water content | Primary | 0.01 mg/kg | LC-MS/MS | Andrews, R., 2020  Appendix 2  Method No. R0077/01  report No 809021  XXXX DocID 2020/2036124  Mackenroth, C., Schweda, Z., 2008 EU agreed (DAR 2009 & addendum to DAR 2012)  Method no. L0117/01  XXXX DocID 2008/1028661  Mackenroth, C., Schweda, Z., 2016  EU agreed Amendment 1 to Method L0117/01  XXXX DocID 2015/1257669 |
| ILV | 0.01 mg/kg | LC-MS/MS | Schwarz, T., 2008 EU agreed (DAR 2009 & addendum to DAR 2012)  Method no. L0117/01  XXXX DocID 2008/1037015 |
| High acid content | Primary | 0.01 mg/kg | LC-MS/MS | Andrews, R., 2020  Appendix 2  Method No. R0077/01  report No 809021  XXXX DocID 2020/2036124  Mackenroth, C., Schweda, Z., 2008 EU agreed (DAR 2009 & addendum to DAR 2012)  Method no. L0117/01  XXXX DocID 2008/1028661  Mackenroth, C., Schweda, Z., 2016  EU agreed Amendment 1 to Method L0117/01  XXXX DocID 2015/1257669 |
| ILV | 0.01 mg/kg | LC-MS/MS | Schwarz, T., 2008 EU agreed (DAR 2009 & addendum to DAR 2012)  Method no. L0117/01  XXXX DocID 2008/1037015 |
| High oil content | Primary | 0.01 mg/kg | LC-MS/MS | Andrews, R., 2020  Appendix 2  Method No. R0077/01  report No 809021  XXXX DocID 2020/2036124  Mackenroth, C., Schweda, Z., 2008 EU agreed (DAR 2009 & addendum to DAR 2012)  Method no. L0117/01  XXXX DocID 2008/1028661  Mackenroth, C., Schweda, Z., 2016  EU agreed Amendment 1 to Method L0117/01  XXXX DocID 2015/1257669 |
| ILV | 0.01 mg/kg | LC-MS/MS | Schwarz, T., 2008 EU agreed (DAR 2009 & addendum to DAR 2012)  Method no. L0117/01  XXXX DocID 2008/1037015 |
| High protein/high starch content (dry) | Primary | 0.01 mg/kg | LC-MS/MS | Andrews, R., 2020  Appendix 2  Method L0117/01  XXXX DocID 2020/2031000  Andrews, R., 2020  Appendix 2  Method No. R0077/01  report No 809021  XXXX DocID 2020/2036124 |
| ILV | 0.01 mg/kg | LC-MS/MS | Homazava, N., 2019  Appendix 2  Method no. L0117/01  XXXX DocID 2019/2051445 |
| Difficult (if required, depends on intended use) | Primary | 0.01 mg/kg | LC-MS/MS | Richter, S., 2016  Appendix 2  Method no. L0117/02  XXXX DocID 2016/1271225 |
| ILV | 0.01 mg/kg | LC-MS/MS | Homazava, N., 2019  Appendix 2  Method no. L0117/02  XXXX DocID 2019/2051445 |

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: | Extraction efficiency was investigated in a separate study (XXXX DocID 2008/1037092) which is already peer-reviewed in the DAR for the evaluation of ametoctradin (The Netherlands, 2009). |
| Not required, because: | Extraction efficiency was determined using the solvent mixture methanol/water (50/50, v/v) of the data generation method L0078/01 (see section 5.2.2, for detail see Appendix 2) and enforcement method L0117/01 (see section 5.2.2 and section 5.3.2.2, for detail see Appendix 2) for the extraction of ametoctradin and its metabolites M650F03 and M650F04. Thereby, residues from selected samples of the tomato and potato metabolism studies and the confined rotational crop study were extracted and results compared with those of the extracts investigated by the metabolism and confined rotational crop studies. The extractabilities with the solvent mixture used for the analytical methods are comparable to those obtained during the course of the metabolism and confined rotational crop studies. Obtained extracts were analyzed by radio-HPLC to get metabolite pattern. The chromatograms show comparable metabolite distribution in relation to the chromatograms recorded during metabolism studies.  The extraction efficiency guideline (SANTE/2017/10632) states: “Solvent mixtures are considered as being identical if their composition varies by not more than 20 vol.-%”, thus covering the solvent mixture methanol/water (70/30, v/v) used in L0117/02.  Therefore, it can be concluded that the extraction solvent used in the analytical methods is sufficiently able to extract ametoctradin and its metabolites M650F03 and M650F04. |

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

An overview on the acceptable methods for analysis of ametoctradin in animal matrices is given in the following tables. For the detailed evaluation of new/additional studies it is referred to Appendix 2.

Table 5.3‑4: Validated methods for food and feed of animal origin

| Component of residue definition: Ametoctradin + metabolites M650F01 + M650F06  expressed as Ametoctradin | | | | |
| --- | --- | --- | --- | --- |
| Matrix type | Method type | Method LOQ | Principle of method (*i.e.* GC-MS or HPLC-UV) | Author(s), year / missing |
| Milk | Primary | 0.03 mg/kg | LC-MS/MS | Schweda Z., Mackenroth C., 2008  EU agreed (DAR 2009)  Method no. L0104/01  XXXX DocID 2008/1022140  Amendment 1  EU agreed  XXXX DocID 2015/1258815 |
| ILV | 0.03 mg/kg | LC-MS/MS | Macdougall J., 2008  EU agreed (DAR 2009)  Method no. L0104/01  XXXX DocID 2008/1022841 |
| Eggs | Primary | 0.03 mg/kg | LC-MS/MS | Schweda Z., Mackenroth C., 2008  EU agreed (DAR 2009)  Method no. L0104/01  XXXX DocID 2008/1022140  Amendment 1  EU agreed  XXXX DocID 2015/1258815 |
| ILV | 0.03 mg/kg | LC-MS/MS | Macdougall J., 2008  EU agreed (DAR 2009)  Method no. L0104/01  XXXX DocID 2008/1022841 |
| Muscle | Primary | 0.03 mg/kg | LC-MS/MS | Schweda Z., Mackenroth C., 2008  EU agreed (DAR 2009)  Method no. L0104/01  XXXX DocID 2008/1022140  Amendment 1  EU agreed  XXXX DocID 2015/1258815 |
| ILV | - | - | - |
| Fat | Primary | 0.03 mg/kg | LC-MS/MS | Schweda Z., Mackenroth C., 2008  EU agreed (DAR 2009)  Method no. L0104/01  XXXX DocID 2008/1022140  Amendment 1  EU agreed  XXXX DocID 2015/1258815 |
| ILV | 0.03 mg/kg | LC-MS/MS | Macdougall J., 2008  EU agreed (DAR 2009)  Method no. L0104/01  XXXX DocID 2008/1022841 |
| Kidney, liver | Primary | 0.03 mg/kg | LC-MS/MS | Schweda Z., Mackenroth C., 2008  EU agreed (DAR 2009)  Method no. L0104/01  XXXX DocID 2008/1022140  Amendment 1  EU agreed  XXXX DocID 2015/1258815 |
| ILV | 0.03 mg/kg | LC-MS/MS | Macdougall J., 2008  EU agreed (DAR 2009)  Method no. L0104/01  XXXX DocID 2008/1022841 |
| Honey/Pollen | Primary | 0.01 mg/kg | LC-MS/MS | Gordon, B., 2020  Appendix 2  Method no. R0072/01  XXXX DocID 2020/2032164 |
| ILV | 0.01 mg/kg | LC-MS/MS | Warnick, J., 2020  Appendix 2  Method no. R0072/01  XXXX DocID 2020/2032165 |

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: | DAR (The Netherlands, 2009) |
| Not required, because: | Residues ≥ LOQ are not expected (EFSA 2012) |
| Comment on extraction efficiency: | See DAR (The Netherlands, 2009) |

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

An overview on the acceptable methods for analysis of ametoctradin and metabolites M650F03 and M650F04 in soil is given in the following tables. For the detailed evaluation of new/ additional studies it is referred to Appendix 2.

Table 5.3‑6: Validated methods for soil

| Component of residue definition: Ametoctradin | | | |
| --- | --- | --- | --- |
| Method type | Method LOQ | Principle of method  (*i.e.* GC-MS or HPLC-UV) | Author(s), year / missing |
| Primary (No confirmation method required, two mass transitions employed) | 0.01 mg/kg | HPLC-MS/MS | Schelling, D. & Schatte, S., 2020  Appendix 2  Method no. L0091/03  XXXX DocID 2020/2034611 |

Table 5.3‑7: Validated methods for soil

| Component of residue definition: M650F03 & M650F04 | | | |
| --- | --- | --- | --- |
| Method type | Method LOQ | Principle of method  (*i.e.* GC-MS or HPLC-UV) | Author(s), year / missing |
| Primary (No confirmation method required, two mass transitions employed) | 0.01 mg/kg | HPLC-MS/MS | Schelling, D. & Schatte, S., 2020  Appendix 2  Method no. L0091/03  XXXX DocID 2020/2034611 |
| Primary (No confirmation method required, two mass transitions employed) | 1 µg/kg | HPLC-MS/MS | Karrer, C. & Albani, K., 2020  Appendix 2  Method no. L0110/02  XXXX DocID 2020/2034612 |

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

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

An overview on the acceptable methods for analysis of Ametoctradin in surface and drinking water is given in the following tables. For the detailed valuation of new/ additional studies it is referred to Appendix 2.

Table 5.3‑8: Validated methods for water

| Component of residue definition: Ametoctradin | | | | |
| --- | --- | --- | --- | --- |
| Matrix type | Method type | Method LOQ | Principle of method (i.e. GC-MS or HPLC-UV) | Author(s), year / missing |
| Drinking water | Primary | 0.03 µg/L | LC-MS/MS | Schelling, D. & Schatte, S., 2020  Appendix 2  Method no. L0208/02  XXXX DocID 2020/2034573 |
| ILV | 0.03 µg/L | LC-MS/MS | Tzelepi, E , 2020  Appendix 2  Method no. L0208/02  XXXX DocID 2020/2034827 |
| Surface water | Primary | 0.03 µg/L | LC-MS/MS | Schelling, D. & Schatte, S., 2020  Appendix 2  Method no. L0208/02  XXXX DocID 2020/2034573 |

Table 5.3‑9: Validated methods for water

| Component of residue definition: M650F01, M650F03 & M650F04 | | | | |
| --- | --- | --- | --- | --- |
| Matrix type | Method type | Method LOQ | Principle of method (i.e. GC-MS or HPLC-UV) | Author(s), year / missing |
| Drinking water | Primary | 0.03 µg/L | LC-MS/MS | Schelling, D. & Schatte, S., 2020  Appendix 2  Method no. L0113/03  XXXX DocID 2020/2034594 |
| ILV | 0.03 µg/L | LC-MS/MS | Tzelepi, E , 2020  Appendix 2  Method no. L0113/03  XXXX DocID 2020/2034828 |
| Surface water | Primary | 0.03 µg/L | LC-MS/MS | Schelling, D. & Schatte, S., 2020  Appendix 2  Method no. L0113/03  XXXX DocID 2020/2034594 |

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

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

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

Table 5.3‑10: Validated methods for air

| Component of residue definition: Ametoctradin | | | |
| --- | --- | --- | --- |
| Method type | Method LOQ | Principle of method  (i.e. GC-MS or HPLC-UV) | Author(s), year / missing |
| Primary | 0.0833 ng/L air | HPLC-MS/MS | Karrer, C. & Schatte, S., 2020  Appendix 2  Method no. L0108/02  XXXX DocID 2020/2034610 |

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

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

No analytical method for residues in body fluids and tissues was required at the time of the EU approval process as Ametoctradin is not classified as toxic or very toxic (EFSA Conclusion, 2012 (EFSA Journal 2012;10(11):2921)).

In order to meet the data requirements of Reg. EU No 284/2013 Annex Section 5, a validation study for a method was performed to determine ametoctradin in blood and urine. A method for the determination of Ametoctradin in body tissue is the same as the presented for food of animal origin (see Section 5.3.2.3 above).

An overview on the acceptable methods and possible data gaps for analysis of ametoctradin in body fluids and tissues is given in the following table. For the detailed evaluation of new/additional studies it is referred to Appendix 2.

Table 5.3‑11: Methods for body fluids and tissues

| Component of residue definition: Ametoctradin | | | |
| --- | --- | --- | --- |
| Method type | Method LOQ | Principle of method (i.e. GC-MS or HPLC-UV) | Author(s), year / missing |
| Primary | 0.01 mg/kg (muscle meat) | LC-MS/MS | Schweda Z., Mackenroth C., 2008  EU agreed  Method no. L0104/01  XXXX DocID 2008/1022140  Amendment 1  EU agreed  XXXX DocID 2015/1258815 |
| 0.01 mg/kg (blood & urine) | LC-MS/MS | Richter S., Djedovic S., 2016  Appendix 2  Method no. L0347/01  XXXX DocID 2016/1235194 |

Table 5.3‑12: Methods for body fluids and tissues

| Component of residue definition: M650F006 | | | |
| --- | --- | --- | --- |
| Method type | Method LOQ | Principle of method (i.e. GC-MS or HPLC-UV) | Author(s), year / missing |
| Primary | 0.01 mg/kg (blood & urine) | LC-MS/MS | Horowitz, M., 2020  Appendix 2  Method no. R0066/01  XXXX DocID 2020/2032355 |

For any special comments or remarkable points concerning the analytical methods for body fluids and tissues please refer to Appendix 2.

#### Other studies/ information

None

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

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

Compared to the residue definition proposed in the Draft Assessment Report (incl. its addenda) the current legal residue definition is not identical.

Table 5.3‑13: 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 | Propamocarb and its salts, expressed as Propamocarb | 0.01 mg/kg | Reg. (EU) 2024/1439 |
| Plant, high acid content | 0.01 mg/kg | Reg. (EU) 2024/1439 |
| Plant, high protein/high starch content (dry commodities) | 0.01 mg/kg | Reg. (EU) 2024/1439 |
| Plant, high oil content | 0.01 mg/kg | Reg. (EU) 2024/1439 |
| Plant, difficult matrices (hops, spices, tea) | 0.01 mg/kg | Reg. (EU) 2024/1439 |
| Food of animal origin: Milk, pig and ruminant tissues: Muscle | N-oxide Propamocarb | 0.01 mg/kg | Reg. (EU) 2024/1439 |
| Fat | 0.01 mg/kg | Reg. (EU) 2024/1439 |
| Liver | 0.2 mg/kg | Reg. (EU) 2024/1439 |
| Kidney | 0.05 mg/kg | Reg. (EU) 2024/1439 |
| Edible offals (other than liver and kidney) | 0.2 mg/kg | Reg. (EU) 2024/1439 |
| Food of animal origin: Poultry tissues: Muscle | N-desmethyl Propamocarb | 0.01 mg/kg | Reg. (EU) 2024/1439 |
| Fat | 0.01 mg/kg | Reg. (EU) 2024/1439 |
| Liver | 0.2 mg/kg | Reg. (EU) 2024/1439 |
| Kidney | 0.05 mg/kg | Reg. (EU) 2024/1439 |
| Edible offals (other than liver and kidney) | 0.2 mg/kg | Reg. (EU) 2024/1439 |
| Soil  (Ecotoxicology) | Propamocarb and its salts, expressed as propamocarb | 0.29 mg/kg bw/day | AOEL for propamocarb hydrochloride  EFSA Scientific Report (2006) 78, 1-80 |
| Drinking water  (Human toxicology) | Propamocarb and its salts, expressed as propamocarb | 0.1 µg/L | general limit for drinking water |
| Surface water  (Ecotoxicology) | Propamocarb and its salts, expressed as propamocarb | 6.3 mg/L (NOEC Bluegill sunfish) | lowest NOEC/EC 50 from aquatic toxicity study  EFSA Scientific Report (2006) 78, 1-80 |
| Air | Propamocarb and its salts, expressed as propamocarb | 87 µg/m3 | AOEL sys/AOEL inhal: 0.29 mg/kg bw/d  EFSA Scientific Report (2006) 78, 1-80 |
| Tissue (meat or liver) | Propamocarb | 0.01 mg/kg | SANTE/2020/12830, Rev.2 |
| Body fluids | 0.01 mg/L | SANTE/2020/12830, Rev.2 |

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

An overview on the acceptable methods and possible data gaps for analysis of Propamocarb in plant matrices is given in the following tables. For the detailed evaluation of new/additional studies it is referred to Appendix 2. Please note that the reports supporting methods for post-authorization control/monitoring are owned by Bayer, the notifier of the active substance Propamocarb. Competent regulatory authority is authorized to access the data package of Propamocarb in support of the application of BAS 743 03 F. Please, refer to Letter of Access in part A. Excerpts of these methods are provided in Appendix 2 for information, taken from the Renewal Assessment Report (RAR, Portugal, 2017) for Propamocarb.

Table 5.3‑14: 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: Propamocarb  (Sum of Propamocarb and its salts expressed as Propamocarb) | | | | |
| --- | --- | --- | --- | --- |
| Matrix type | Method type | Method LOQ | Principle of method (i.e. GC-MS or HPLC-UV) | Author(s), year / missing / EU agreed |
| High water content | Primary | 0.01 mg/kg | HPLC-MS/MS | Rosati, D.; Valcarce, M. H., 2006  EU agreed (RAR 2017)  Provided in Appendix 2 for information |
| ILV | 0.01 mg/kg | HPLC-MS/MS | Freitag, T.; Wolters, A., 2006  EU agreed (RAR 2017)  Provided in Appendix 2 for information |
| High acid content | Primary | 0.01 mg/kg | HPLC-MS/MS | Rosati, D.; Valcarce, M. H., 2006  EU agreed (RAR 2017)  Provided in Appendix 2 for information |
| ILV | 0.01 mg/kg | HPLC-MS/MS | Freitag, T.; Wolters, A., 2006  EU agreed (RAR 2017)  Provided in Appendix 2 for information |
| High oil content | Primary | 0.01 mg/kg | HPLC-MS/MS | Rosati, D.; Valcarce, M. H., 2006  EU agreed (RAR 2017)  Provided in Appendix 2 for information |
| ILV | 0.01 mg/kg | HPLC-MS/MS | Freitag, T.; Wolters, A., 2006  EU agreed (RAR 2017)  Provided in Appendix 2 for information |
| High protein/high starch content (dry) | Primary | 0.01 mg/kg | HPLC-MS/MS | Rosati, D.; Valcarce, M. H., 2006  EU agreed (RAR 2017)  Provided in Appendix 2 for information |
| ILV | 0.01 mg/kg | HPLC-MS/MS | Freitag, T.; Wolters, A., 2006  EU agreed (RAR 2017)  Provided in Appendix 2 for information |

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 5.3‑15: Statement on extraction efficiency

|  | Method for products of plant origin |
| --- | --- |
| Required, available from: | RAR (Portugal, 2017) |
| Not required, because: | - |

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

An overview on the acceptable methods for analysis of Propamocarb in animal matrices is given in the following tables. For the detailed evaluation of new/additional studies it is referred to Appendix 2. A method (and a corresponding ILV) for the determination of Propamocarb in animal tissues is provided in Appendix 2 for information.

Table 5.3‑16: Validated methods for food and feed of animal origin

| Component of residue definition: N-oxide Propamocarb | | | | |
| --- | --- | --- | --- | --- |
| Matrix type | Method type | Method LOQ | Principle of method (*i.e.* GC-MS or HPLC-UV) | Author(s), year / missing |
| Milk, pig and ruminant tissues: Bovine Meat, fat, Liver, Kidney | Primary | 0.01 mg/kg | HPLC-MS/MS | Winter, O. & Amann, S., 2014  EU agreed (RAR 2017)  Provided in Appendix 2 for information |
| ILV | 0.01 mg/kg | HPLC-MS/MS | Mewis, A., 2015 EU agreed (RAR 2017)  Provided in Appendix 2 for information |

Table 5.3‑17: Validated methods for food and feed of animal origin

| Component of residue definition: N-desmethyl Propamocarb | | | | |
| --- | --- | --- | --- | --- |
| Matrix type | Method type | Method LOQ | Principle of method (*i.e.* GC-MS or HPLC-UV) | Author(s), year / missing |
| Poultry tissues (meat, fat, Liver, Kidney, Eggs) | Primary | 0.01 mg/kg | HPLC-MS/MS | Winter, O. & Amann, S., 2014  EU agreed (RAR 2017)  Provided in Appendix 2 for information |
| ILV | 0.01 mg/kg | HPLC-MS/MS | Mewis, A., 2015 EU agreed (RAR 2017)  Provided in Appendix 2 for information |

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 5.3‑18: Statement on extraction efficiency

|  | Method for products of animal origin |
| --- | --- |
| Required, available from: | RAR (Portugal, 2017) |
| Not required, because: | - |

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

An overview on the acceptable methods and possible data gaps for analysis of Propamocarb in soil is given in the following tables. For the detailed evaluation of new/additional studies it is referred to Appendix 2.

Table 5.3‑19: Validated methods for soil

| Component of residue definition: Propamocarb  (Sum of Propamocarb and its salts expressed as Propamocarb) | | | |
| --- | --- | --- | --- |
| Method type | Method LOQ | Principle of method  (*i.e.* GC-MS or HPLC-UV) | Author(s), year / missing |
| Primary | 2 µg/kg | HPLC-MS/MS | Freitag, T. & Koch, V., 2015  EU agreed (RAR 2017)  Provided in Appendix 2 for information |

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

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

An overview on the acceptable methods and possible data gaps for analysis of Propamocarb in surface/drinking water is given in the following tables. For the detailed valuation of new/additional studies it is referred to Appendix 2.

Table 5.3‑20: Validated methods for water

| Component of residue definition: Propamocarb  (Sum of Propamocarb and its salts expressed as Propamocarb) | | | | |
| --- | --- | --- | --- | --- |
| Matrix type | Method type | Method LOQ | Principle of method (i.e. GC-MS or HPLC-UV) | Author(s), year / missing |
| Drinking water | Primary | 0.04 μg/L | HPLC-MS/MS | Krebber, R. & Sandau, C., 2015  EU agreed (RAR 2017)  Provided in Appendix 2 for information |
| ILV | 0.04 μg/L | HPLC-MS/MS | Thies, S., 2015  EU agreed (RAR 2017)  Provided in Appendix 2 for information |
| Surface water | Primary | 0.04 μg/L | HPLC-MS/MS | Krebber, R. & Sandau, C., 2015  EU agreed (RAR 2017)  Provided in Appendix 2 for information |

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

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

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

Table 5.3‑21: Validated methods for air

| Component of residue definition: Propamocarb  (Sum of Propamocarb and its salts expressed as Propamocarb) | | | |
| --- | --- | --- | --- |
| Method type | Method LOQ | Principle of method  (i.e. GC-MS or HPLC-UV) | Author(s), year / missing |
| Primary | 9 μg/m3 |  | Class, T., 2004  EU agreed (RAR 2017)  Provided in Appendix 2 for information |

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

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

No analytical method for residues in body fluids and tissues was required at the time of the EU approval process as Propamocarb is not classified as toxic or very toxic (EFSA Scientific Report (2006) 78, 1-80), thus no methods have been submitted.

Nevertheless, a method for the determination of Propamocarb in body tissue is presented in Appendix 2 for food of animal origin for information.

An overview on the acceptable methods for analysis of Propamocarb in body fluids and tissues is given in the following table. For the detailed evaluation of studies it is referred to Appendix 2.

**Table 5.3‑22: Methods for body fluids and tissues**

| Component of residue definition: Propamocarb | | | |
| --- | --- | --- | --- |
| Method type | Method LOQ | Principle of method (i.e. GC-MS or HPLC-UV) | Author(s), year / missing |
| Primary (tissues) | 0.01 mg/kg (cattle meat) | HPLC-MS/MS | Weber, H. & Schernikau, N., 2010  EU agreed (RAR 2017)  Provided in Appendix 2 for information |

For any special comments or remarkable points concerning the analytical methods for body fluids and tissues please refer to Appendix 2.

#### Other studies/ information

No additional studies.

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 5.1.1/1 | Pecorelli, P. | 2023 | Analytische Methode AFL1028/03:Determination of the active Ingredients Propamocarb and Ametoctradin in SC formulations BAS 743 00 F, BAS 743 02 F and BAS 743 03 F by GC, including Suspensibility  2023/2007064  BASF SE, Limburgerhof, Germany Fed.Rep.  no  Unpublished | No | XXXX |
| KCP 5.1.1/2 | Stickland, L. | 2021 | Validation of the analytical method AFL1028/01: Determination of the active ingredients Propamocarb and Ametoctradin in formulations by GC (including Suspensibility)  2020/2106327  Battelle UK Ltd., Havant Hampshire PO9 1SA, United Kingdom  yes  Unpublished | No | XXXX |
| KCP 5.1.1/3 | Pecorelli, P. | 2022 | Additional Validation of Analytical Method AFL1028/01: "Determination of the active ingredients Propamocarb and Ametoctradin in SC formulation BAS 743 00 F by GC, including Suspensibility"  2022/2027441  BASF SE, Limburgerhof, Germany Fed.Rep.  yes  Unpublished | No | XXXX |
| KCP 5.1.1/4 | Pecorelli, P. | 2023 | Amendment No 1- Additional Validation of Analytical Method AFL1028/01: "Determination of the active ingredients Propamocarb and Ametoctradin in SC formulation BAS 743 00 F by GC, including Suspensibility"  2023/2004017  BASF SE, Limburgerhof, Germany Fed.Rep.  yes  Unpublished | No | XXXX |
| KCP 5.1.1/5 | Schubring, M. | 2022 | Validation of the Analytical Methode AFL1070/01: "Determination of Amitrole in Formulations containing Ametoctradin (BAS 650 F) and Propamocarb by HPLC-MS"  2022/2028342  BASF SE, Limburgerhof, Germany Fed.Rep.  yes  Unpublished | No | XXXX |
| KCP 5.1.1/6 | Schubring, M. | 2022 | Determination of Amitrole in Formulations containing Ametoctradin (BAS 650 F) and Propamocarb by HPLC-MS"  2022/2028343  BASF SE, Limburgerhof, Germany Fed.Rep.  yes  Unpublished | No | XXXX |
| KCP 5.1.1/7 | Schubring, M. | 2022 | Validation of the Analytical Methode AFL1073/01: "Determination of o-Xylene in Formulations containing Ametoctradin (BAS 650 F) and Propamocarb by GC-MS Headspace"  2022/2038541  BASF SE, Limburgerhof, Germany Fed.Rep.  yes  Unpublished | No | XXXX |
| KCP 5.1.1/8 | Schubring, M. | 2022 | Analytical Method AFL1073/01: Determination of o-Xylene in Formulations containing Ametoctradin (BAS 650 F) and Propamocarb by GC-MS Headspace  2022/2038543  BASF SE, Limburgerhof, Germany Fed.Rep.  no  Unpublished | No | XXXX |
| KCP 5.1.2/~~1~~2 | Homazava, N. | 2021 | Method Validation of Analytical Method L0078/01 for the Determination of BAS 650 F in plant matrices by LC-MS/MS  2021/2041685  IES - Innovative Environmental Services Ltd., Witterswil, Switzerland  yes  Unpublished | No | XXXX |
| KCP 5.1.2/~~2~~3 | Schneider, E. | 2021 | Study on the residue behaviour of Propamocarb (Reg.No. 4628172) and Ametoctradin (BAS 650 F) in Melon after treatment with BAS 743 01 F under field conditions in Southern Europe in 2020  2021/2019512  ANADIAG, Haguenau, France  yes  Unpublished | No | XXXX |
| KCP 5.1.2/~~3~~4 | Vagt, I & Meyer, M. | 2022 | Study on the residue behaviour of Propamocarb (Reg.No. 4628172) and Ametoctradin (BAS 650 F) in lettuce after two applications of BAS 743 01 F under field conditions in Southern Europe, 2021  2022/2041753  SGS Institut Fresenius GmbH, Taunusstein, Germany Fed.Rep.  yes  Unpublished | No | XXXX |
| KCP 5.1.2/~~4~~5 | G~~a~~álvez, O. | 2021 | Study on the residue behaviour of Ametoctradin (BAS 650 F) and Propamocarb (Reg. No. 4628172) on tomato after treatment with BAS 743 01 F under field conditions in Southern Europe, season 2020  2020/2103085  Agricultura y Ensayo S.L., Alcala de Guadaira, Spain  yes  Unpublished | No | XXXX |
| KCP 5.1.2/~~5~~6 | G~~a~~álvez, O. | 2021 | Study on the residue behaviour of Ametoctradin (BAS 650 F) and Propamocarb (Reg. No. 4628172) on potato after treatment with BAS 743 01 F under field conditions in Southern Europe, season 2020  2020/2103083  Agricultura y Ensayo S.L., Alcala de Guadaira, Spain  yes  Unpublished | No | XXXX |
| KCP 5.1.2/~~6~~7 | Schneider, E. | 2021 | Study on the residue behaviour of Propamocarb (Reg.No. 4628172) and Ametoctradin (BAS 650 F) in Onions after treatment with BAS 743 01 F under field conditions in Southern Europe in 2020  2021/2019659  ANADIAG, Haguenau, France  yes  Unpublished | No | XXXX |
| KCP 5.1.2/~~7~~8 | Xiaodan, D. | 2022 | Validation of analytical method L0078/02 for the analysis of BAS 650 F and its metabolites M650F003 and M650F004 in plant matrices  2022/2027748  BASF Metabolome Solutions GmbH, Berlin, Germany Fed.Rep.  yes  Unpublished | No | XXXX |
| KCP 5.1.2/~~8~~9 | Schneider, E. | 2023 | Study on the residue behaviour of Propamocarb (Reg.No. 4628172) and Ametoctradin (BAS 650 F) in cucumber and zucchini after two applications of BAS 743 01 F under greenhouse conditions in Northern and Southern Europe in 2021  2022/2041754  ANADIAG, Haguenau, France  yes  Unpublished | No | XXXX |
| KCP 5.1.2/~~9~~10 | Martin, T. | 2022 | Study on the residue behaviour of Propamocarb (Reg.No. 4628172) and Ametoctradin (BAS 650 F) in tomato after two applications of BAS 743 01 F under field conditions in Southern Europe, 2021  2021/2054075  BioChem Agrologia S.L.U., Utrera, Spain  yes  Unpublished | No | XXXX |
| ~~KCP 5.1.2/10~~ | ~~Wagner I.~~ | ~~2022~~ | ~~BAS 743 02 F - Validation of an analytical method for the analysis in acetonitrile / 10mM disodium hydrogen phosphate dihydrate with phosphoric acid (85%) pH 2.9 (50+50, V/V) using HPLC-UV (Control procedure: 21/0288\_01)~~  ~~2022/2034983~~  ~~BASF SE, Ludwigshafen, Germany Fed.Rep.~~  ~~yes~~  ~~Unpublished~~ | ~~No~~ | XXXX |
| KCP 5.1.2/~~11~~12 | Wendling, K. | 2023 | BAS 743 02 F: Toxicity to the Water Flea Daphnia magna Straus under Laboratory Conditions (Acute Immobilisation Test - Semi-static)  2022/2033712  Eurofins Agroscience Services EcoChem GmbH, Niefern-Oeschelbronn, Germany Fed.Rep.  yes  Unpublished | No | XXXX |
| KCP 5.1.2/~~12~~13 | Obert-Rauser, P. | 2023 | Toxicity to the Single Cell Green Alga Pseudokirchneriella subcapitata Hindak under Laboratory Conditions  2022/2033713  Eurofins Agroscience Services EcoChem GmbH, Niefern-Oeschelbronn, Germany Fed.Rep.  yes  Unpublished | No | XXXX |
| KCP 5.1.2/~~13~~14 | Wendling, K. | 2023 | BAS 743 02 F - Fish acute - Trout  2022/2033714  Eurofins Agroscience Services Ecotox GmbH, Niefern-Oeschelbronn, Germany Fed.Rep.  yes  Unpublished | Yes | XXXX |
| KCP 5.1.2/~~14~~15 | Maleck, A. | 2023 | Effect of BAS 743 02 F on seedling emergence and seedling growth of several species of terrestrial plants under greenhouse conditions  2022/2033722  Agro-Check Dr. Teresiak & Erdmann GbR, Lentzke, Germany Fed.Rep.  yes  Unpublished | No | XXXX |
| KCP 5.1.2/~~15~~16 | Maleck, A. | 2023 | Effect of BAS 743 02 F on vegetative vigour of several species of terrestrial plants under greenhouse conditions  2022/2033723  Agro-Check Dr. Teresiak & Erdmann GbR, Lentzke, Germany Fed.Rep.  yes  Unpublished | No | XXXX |
| KCP 5.1.2/~~16~~17 | Ruhland, S. | 2023 | Chronic toxicity of BAS 743 02 F to the honey bee Apis mellifera L. under laboratory conditions  2022/2033709  BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Machern OT Gerichshain, Germany Fed.Rep.  yes  Unpublished | No | XXXX |
| KCP 5.1.2/~~17~~18 | Schmidt, K. | 2022 | Repeated exposure of honey bee (Apis mellifera L.) larvae to BAS 743 02 F under laboratory conditions  2022/2033710  BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Machern OT Gerichshain, Germany Fed.Rep.  yes  Unpublished | No | XXXX |
| KCP 5.1.2/~~18~~19 | Amsel, K. | 2023 | Acute toxicity of BAS 743 02 F to the bumblebee Bombus terrestris L. under laboratory conditions  2022/2033711  BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Machern OT Gerichshain, Germany Fed.Rep.  yes  Unpublished | No | XXXX |
| KCP 5.1.2/~~19~~20 | Renner, P. | 2023 | Acute toxicity of BAS 743 03 F on Daphnia magna in a 48-hour static test  2022/2033730  BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Machern OT Gerichshain, Germany Fed.Rep.  yes  Unpublished | No | XXXX |
| KCP 5.1.2/~~20~~21 | Demin, R. | 2022 | Validation of analytical method L0450/01 for the analysis of Propamocarb in plant matrices  2022/2032351  BASF Metabolome Solutions GmbH, Berlin, Germany Fed.Rep.  yes  Unpublished | No | XXXX |
| KCP 5.2/~~1~~2 | Mackenroth, C., Schweda, Z. | 2016 | Report Amendment No. 1 to final report: Validation of BASF method L0117/01: Method for the determination of BAS 650 F in plant matrices  2015/1257669  BASF SE, Limburgerhof, Germany Fed.Rep.  yes  Unpublished | No | XXXX |
| KCP 5.2/~~2~~3 | Andrews, R. | 2020 | Validation of Method L0117/01: Method for the Determination of BAS 650 F (Ametoctradin, Reg.No. 4993353), in Dried Peas by LC-MS/MS  2020/2031000  BASF Corporation BASF Agricultural Solutions  yes  Unpublished | No | XXXX |
| KCP 5.2/~~3~~5 | Richter, S. | 2016 | Validation of BASF analytical method L0117/02 for the determination of BAS 650 F (Ametoctradin) in dried hops  2016/1271225  PTRL Europe, Ulm, Germany Fed.Rep.  yes  Unpublished | No | XXXX |
| KCP 5.2/~~4~~6 | Homazava, N. | 2019 | Independent Method Validation of BASF Analytical Method for the Determination of BAS 650 F in Dried Peas (L0117/01) and Hops (L0117/02) by LC-MS/MS  2019/2051445  IES - Innovative Environmental Services Ltd., Witterswil, Switzerland  yes  Unpublished | No | XXXX |
| KCP 5.2/~~5~~7 | Andrews, R. | 2020 | Validation of an analytical method (R0077/01, Quechers) for the determination of BAS 650 F in plant matrices  2020/2036124  BASF Corporation BASF Agricultural Solutions  yes  Unpublished | No | XXXX |
| KCP 5.2/~~6~~9 | Mackenroth, C., Schweda, Z. | 2016 | Report Amendment No.1: Validation of BASF method L0104/01: Method for the determination of BAS 650 F and its metabolites M650F01 and M650F06 in animal matrices  2015/1258815  BASF SE, Limburgerhof, Germany Fed.Rep.  yes  Unpublished | No | XXXX |
| KCP 5.2/~~7~~11 | Gordon, B. | 2020 | Validation of Method R0072/01: Method for the determination of BAS 181 S (Reg.No. 4117133), BAS 650 F (Reg.No. 4993353), M650F003 (Reg.No. 5178870, and M650F004 (Reg.No. 5211623) in pollen and honey by LC-MS/MS  2020/2032164  BASF Corporation BASF Agricultural Solutions  yes  Unpublished | No | XXXX |
| KCP 5.2/~~8~~12 | Warnick, J. | 2020 | Independent lab validation of BASF analytical method R0072/01: Method for the determination of BAS 181 S (Reg,No. 4117133), BAS 650 F (Reg.No. 4993353), M650F003 (Reg.No. 5178870), and M650F004 (Reg.No. 5211623) in pollen and honey by LC/MS/MS  2020/2032165  EPL Bio-Analytical Services Inc., Niantic IL, United States of America  yes  Unpublished | No | XXXX |
| KCP 5.2/~~9~~13 | Schelling, D. & Schatte, S. | 2020 | Validation of Analytical Method L0091/03 for the Determination of BAS 650 F and its Metabolites M650F001, M650F002, M650F003 and M650F004 in Soil by LC-MS/MS  2020/2034611  BASF SE, Limburgerhof, Germany Fed.Rep.  yes  Unpublished | No | XXXX |
| KCP 5.2/~~10~~14 | Karrer, C. & Albani, K. | 2020 | Validation of Analytical Method L0110/02 for the Determination of the BAS 650 F Metabolites M650F003 and M650F004 in Soil by LC-MS/MS  2020/2034612  BASF SE, Limburgerhof, Germany Fed.Rep.  yes  Unpublished | No | XXXX |
| KCP 5.2/~~11~~15 | Schelling, D. & Schatte, S. | 2020 | Validation of Analytical Method L0208/02 for the Determination of BAS 650 F (Reg.No. 4993353), in Surface Water and Groundwater by LC-MS/MS  2020/2034573  BASF SE, Limburgerhof, Germany Fed.Rep.  yes  Unpublished | No | XXXX |
| KCP 5.2/~~12~~16 | Tzelepi, E. | 2020 | Independent Laboratory Validation (ILV) of BASF Analytical Method L0208/02 for the Determination of BAS 650 F (Reg.No. 4993353), in Surface Water and Groundwater by LC-MS/MS  2020/2034827  CEMAS - CEM Analytical Services Ltd., Wokingham Berkshire RG41 2FD, United Kingdom  yes  Unpublished | No | XXXX |
| KCP 5.2/~~13~~17 | Schelling, D. & Schatte, S. | 2020 | Validation of Analytical Method L0113/03 for the Determination of BAS 650 F Metabolites M650F001, M650F002, M650F003 and M650F004 in Surface Water and Groundwater by LC-MS/MS  2020/2034594  BASF SE, Limburgerhof, Germany Fed.Rep.  yes  Unpublished | No | XXXX |
| KCP 5.2/~~14~~18 | Tzelepi, E. | 2020 | Independent Lab Validation (ILV) of BASF's Analytical Method L0113/03 for the Determination of BAS 650 F Metabolites M650F001, M650F002, M650F003 and M650F004 in Surface Water and Groundwater by LC-MS/MS  2020/2034828  CEMAS - CEM Analytical Services Ltd., Wokingham Berkshire RG41 2FD, United Kingdom  yes  Unpublished | No | XXXX |
| KCP 5.2/~~15~~19 | Karrer, C. | 2020 | Validation of Analytical Method L0108/02 for the Determination of BAS 650 F (Reg.No. 4993353) in Air by LC-MS/MS  2020/2034610  BASF SE, Limburgerhof, Germany Fed.Rep.  yes  Unpublished | No | XXXX |
| KCP 5.2/~~16~~20 | Richter, S. | 2016 | Validation of BASF analytical method L0347/01 for the determination of BAS 650 F (Ametoctradin) in body fluids  2016/1235194  PTRL Europe, Ulm, Germany Fed.Rep.  yes  Unpublished | No | XXXX |
| KCP 5.2/~~17~~21 | Horowitz, M. | 2020 | Validation of method R0066/01: Method for the determination of M650F006 (Reg. No. 5507462) in swine blood plasma and urine matrices by LC-MS/MS  2020/2032355  BASF Corporation BASF Agricultural Solutions  yes  Unpublished | No | XXXX |

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

| **Data point** | **Author(s)** | **Year** | **Title Company Report No.  Source (where different from company) GLP or GEP status Published or not** | **Vertebrate study**  **Y/N** | **Owner** |
| --- | --- | --- | --- | --- | --- |
| KCP 5.1.2/1 | Mackenroth, C., Schweda, Z. | 2008 | Validation of BASF method L0078/01: Method for the determination of BAS 650 F and its metabolites M650F03 and M650F04 in plant matrices  2008/1022139  BASF SE, Limburgerhof, Germany Fed.Rep.  yes  Unpublished | No | XXXX |
| KCP 5.2/1 | Mackenroth, C., Schweda, Z. | 2008 | Validation of BASF method L0117/01: Method for the determination of BAS 650 F in plant matrices  2008/1028661  BASF SE, Limburgerhof, Germany Fed.Rep.  yes  Unpublished | No | XXXX |
| KCP 5.2/~~2~~4 | Schwarz, T. | 2008 | Independent laboratory validation (ILV) of BASF method number L0117 for the determination of BAS 650 F in plant materials by LC-MS/MS  2008/1037015  PTRL Europe GmbH, Ulm, Germany Fed.Rep.  yes  Unpublished | No | XXXX |
| - | Rosati, D. & Valcarce, M. H., | 2006 | Modification M002 to the analytical method 00880 for the determination of residues of propamocarb free base (AE B0397744) in/on lettuce (head), chicory witloof (leaf), leek (shoot), cauliflower (curd), orange (whole fruit), avocado (pulp) and wheat (grain) by LC- MS/MS,  Report No MR-0036/06 (Method No.: 00880/M002)  Document No. M-268065-01-1 | No | XXXX |
| - | Thies, S., | 2016 | Storage stability of residues of propamocarb free base (AE B039744) in/on sunflower seed, orange fruit and dry bean seed by LC- MS/MS,  Report No 2013/0086/01  Document No. M-516661-02-1 | No | XXXX |
| - | Freitag, T. & Wolters, A., | 2006 | Independent laboratory validation of the modification M002 to the analytical method 00880 for the determination of residues of propamocarb free base (AE B039744) in/on plant material  Report No MR-158/05 (Method No.: 00880/M002); Study No.: P612051810  Document No. M-277122-01-1 | No | XXXX |
| - | Weber, H. & Schernikau, N., | 2010 | Validation of the QuEChERS Method (BCS method ID 01205) for the determination of propamocarb in animal tissues  Report No BAY-1032V  Document No. M-387185-01-1 | No | XXXX |
| - | Konrad, St. & Neuland, M., | 2010 | Determination of residues of propamocarb in animal tissues; Independent Lab Validation of QuEChERS method (BCS method 01205)  Report No 01205  Document No. M-398135-01-1 | No | XXXX |
| - | Winter, O. & Amann, S., | 2014 | Validation of the BCS-method 01300/M012 (based on QuEChERS) for the determination of propamocarb metabolites in/on animal tissues,  Report No S13-03821  Document No. M-490237-01-1 | No | XXXX |
| - | Mewis, A., | 2015 | Independent laboratory validation (ILV) of the BCS-method 01300/M012 (based on QuEChERS) for the determination of propamocarb metabolites in different matrices of animal origin  Report No S13-03822  Document No. M-517360-01-1 | No | XXXX |
| - | Freitag, T & Koch, V., | 2015 | Analytical method 01448 for the determination of propamocarb in soil by HPLC-MS/MS  Report No MR-15/013  Document No. M-525885-01-1 | No | XXXX |
| - | Krebber, R. & Sandau, C., | 2015 | Modification M002 of analytical method 01387 for the determination of various pesticides in drinking and surface water by HPLC-MS/MS,  Report No MR-15/025  Document No. M-526061-01-1 | No | XXXX |
| - | Thies, S., | 2015 | Independent laboratory validation of the BCS analytical method 01387/M002 for the determination of various pesticides in surface water by HPLC-MS/MS  Report No 2015/0034/01  Document No. M-536990-01-1 | No | XXXX |
| - | Class, T., | 2004 | Propamocarb: Analytical method for the determination of propamocarb in air  Report No P 755 G; C042611  Document No. M-232969-01-1 | No | XXXX |
| KCP 5.2/~~3~~8 | Mackenroth, C., Schweda, Z. | 2008 | Validation of BASF method L0104/01: Method for the determination of BAS 650 F and its metabolites M650F01 and M650F06 in animal matrices  2008/1022140  BASF SE, Limburgerhof, Germany Fed.Rep.  yes  Unpublished | No | XXXX |
| KCP 5.2/~~4~~10 | Macdougall J. | 2008 | Independent laboratory validation of BASF analytical method L0104 for the determination of BAS 650 F, M650F01 and M650F06 in bovine milk, liver, kidney, fat, and eggs by HPLC-MS/MS  2008/1022841  Charles River Laboratories, Tranent East Lothian EH33 2NE, United Kingdom  yes  Unpublished | No | XXXX |

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

The method for the determination of ametoctradin in plant matrices (L0078) has been previously assessed in the DAR (2009 or DAR addendum 2012) and is provided here for completeness. Data for the use of this method in additional crops (melon, lettuce, cucumber & zucchini) is provided below together with validation data to demonstrate method performance from laboratories not involved in the original validation of the method. The latter data have not been previously evaluated.

* + - 1. Method L0078/01: Method for the determination of ametoctradin in plant matrices
         1. Method validation

|  |  |
| --- | --- |
| Comments of zRMS: | Evaluated and accepted in DAR (2009)/in the addendum to the DAR (2012).  No comments.  Kindly please do not use Appendix 2 for evaluated already studies (this is remark also for all next cases in the present section). Kindly please also, remember in the future to list evaluated already studies within the list “already evaluated”. |

|  |  |
| --- | --- |
| Reference: | CP 5.1.2/1 |
| Report | Validation of XXXX method L0078/01: Method for the determination of BAS 650 F and its metabolites M650F03 and M650F004 in plant matrices  Mackenroth, C., Schweda, Z., 2008  report No 212554  XXXX DocID 2008/1022139  Authority registration No |
| Guideline(s): | EPA 860.1340; SANCO/825/00 rev.7 (17 March 2004); SANCO/3029/99 rev. 4 (11 July 2000) |
| Deviations: | No, except matrix effect was not directly evaluated |
| Previous evaluation: | Yes, evaluated and accepted in DAR (2009)/in the addendum to the DAR (2012) |
| GLP: | Yes |
| Acceptability: | Yes | |

**Study Summary**

The method L0078/01 was developed and validated for the determination of residues of Ametoctradin (BAS 650 F) and its metabolites M650F003 and M650F004 in wheat grain, potato, lettuce, orange, onion and sunflower matrices with a limit of quantification at 0.01 mg/kg. The brief description of method and the results are presented in the summary below.

Materials and methods

In method L0078/01, residues of Ametoctradin (BAS 650 F) and its metabolites M650F003 and M650F004 are extracted from plant matrices using a mixture of methanol-water (50:50, v/v). An aliquot of the extract is centrifuged and the supernatant is cleaned by solid phase extraction with two connected columns. After washing with MeOH/water, Ametoctradin (BAS 650 F) is bound on the SDB-L column, the metabolites M650F003 and M650F004 are washed off onto the second column (Strata X-AW). The final determination of Ametoctradin (BAS 650 F), M650F003 and M650F004 is performed by HPLC-MS/MS. Separation is achieved by using an Phenomenex, Synergi Fusion-RP column (150 mm x 4.6 mm) and a gradient of water (0.1% formic acid)/ methanol (0.1% formic acid) at a flow rate of 1.0 mL/min. Detection is accomplished in ESI positive mode using two different transitions. Detection is accomplished in ESI positive mode using two different transitions. For parent, BAS 650 F, mass transitions at 276 m/z > 176 m/z and 276 m/z > 149 m/z is used for quantification and confirmation, respectively. For metabolite, M650F003, mass transitions at 222 m/z > 176 m/z and 222 m/z > 121 m/z is used for quantification and confirmation, respectively. For metabolite, M650F004, mass transitions at 208 m/z > 190 m/z and 208 m/z > 123 m/z is used for quantification and confirmation respectively. Calibration standards are solvent based and were prepared in methanol/water/formic acid (50:50:0.1, v/v/v).

Results and discussions

The analytical method validation is summarised below. The HPLC-MS/MS determination was conducted by monitoring two MS/MS mass transitions and therefore no confirmatory method is required.

Recoveries were obtained by fortification of the matrices and all mean recoveries are within the permitted range required by the guideline SANTE/2020/12830 rev. 1.

Table A 1: Recovery results from method validation of ametoctradin and metabolites M650F003 & M650F004 using the analytical method

| Matrix | Analyte | Fortification level (mg/kg) (n = x) | Mean  recovery (%) | RSD (%) | Comments |
| --- | --- | --- | --- | --- | --- |
| ***Mass transition (Quantification)  Ametoctradin 276→176 m/z, M650F003 222→176 m/z, M650F004 208→190 m/z*** | | | | | |
| Wheat grain | Ametoctradin | 0.01 (n = 5) | 91.6 | 2.8 | Acceptable |
| 0.1 (n = 5) | 101.3 | 2.5 |
| M650F003 | 0.01 (n = 5) | 89.6 | 6.6 | Acceptable |
| 0.1 (n = 5) | 97.0 | 3.6 |
| M659F004 | 0.01 (n = 5) | 87.0 | 5.3 | Acceptable |
| 0.1 (n = 5) | 99.7 | 1.3 |
| Potato, tuber | Ametoctradin | 0.01 (n = 5) | 106.8 | 2.2 | Acceptable |
| 0.1 (n = 5) | 99.9 | 2.6 |
| M650F003 | 0.01 (n = 5) | 105.6 | 1.4 | Acceptable |
| 0.1 (n = 5) | 99.3 | 2.6 |
| M659F004 | 0.01 (n = 5) | 96.1 | 1.5 | Acceptable |
| 0.1 (n = 5) | 99.0 | 1.8 |
| Lettuce, head | Ametoctradin | 0.01 (n = 5) | 103.8 | 2.0 | Acceptable |
| 0.1 (n = 5) | 103.4 | 5.3 |
| M650F003 | 0.01 (n = 5) | 100.7 | 1.9 | Acceptable |
| 0.1 (n = 5) | 102.5 | 3.2 |
| M659F004 | 0.01 (n = 5) | 96.9 | 2.6 | Acceptable |
| 0.1 (n = 5) | 100.3 | 3.9 |
| Tomato, fruit | Ametoctradin | 0.01 (n = 5) | 106.6 | 1.4 | Acceptable |
| 0.1 (n = 5) | 92.0 | 3.4 |
| M650F003 | 0.01 (n = 5) | 99.0 | 1.6 | Acceptable |
| 0.1 (n = 5) | 93.5 | 2.6 |
| M659F004 | 0.01 (n = 5) | 96.6 | 2.8 | Acceptable |
| 0.1 (n = 5) | 91.0 | 1.0 |
| Grape, fruit | Ametoctradin | 0.01 (n = 5) | 94.9 | 5.8 | Acceptable |
| 0.1 (n = 5) | 99.2 | 2.8 |
| M650F003 | 0.01 (n = 5) | 95.4 | 3.5 | Acceptable |
| 0.1 (n = 5) | 93.6 | 3.8 |
| M659F004 | 0.01 (n = 5) | 87.8 | 2.8 | Acceptable |
| 0.1 (n = 5) | 94.9 | 2.2 |
| Orange, sweet, whole fruit | Ametoctradin | 0.01 (n = 5) | 96.0 | 3.9 | Acceptable |
| 0.1 (n = 5) | 104.6 | 1.0 |
| M650F003 | 0.01 (n = 5) | 103.4 | 1.1 | Acceptable |
| 0.1 (n = 5) | 96.9 | 5.4 |
| M659F004 | 0.01 (n = 5) | 92.4 | 1.5 | Acceptable |
| 0.1 (n = 5) | 94.9 | 1.9 |
| Onion, bulb | Ametoctradin | 0.01 (n = 5) | 95.2 | 1.1 | Acceptable |
| 0.1 (n = 5) | 91.8 | 2.1 |
| M650F003 | 0.01 (n = 5) | 86.3 | 1.6 | Acceptable |
| 0.1 (n = 5) | 95.9 | 1.6 |
| M659F004 | 0.01 (n = 5) | 91.7 | 2.5 | Acceptable |
| 0.1 (n = 5) | 98.0 | 2.8 |
| Sunflower, seed | Ametoctradin | 0.01 (n = 5) | 93.6 | 1.2 | Acceptable |
| 0.1 (n = 5) | 88.0 | 2.2 |
| M650F003 | 0.01 (n = 5) | 71.8 | 2.3 | Acceptable |
| 0.1 (n = 5) | 78.8 | 2.2 |
| M659F004 | 0.01 (n = 5) | 90.6 | 2.8 | Acceptable |
| 0.1 (n = 5) | 97.0 | 2.5 |
| ***Mass transition (Confirmation) Ametoctradin 276→149 m/z, M650F003 222→121 m/z, M650F004 208→123 m/z*** | | | | | |
| Wheat grain | Ametoctradin | 0.01 (n = 5) | 92.8 | 2.1 | Acceptable |
| 0.1 (n = 5) | 100.6 | 3.4 |
| M650F003 | 0.01 (n = 5) | 94.9 | 6.5 | Acceptable |
| 0.1 (n = 5) | 95.3 | 4.6 |
| M659F004 | 0.01 (n = 5) | 90.3 | 3.9 | Acceptable |
| 0.1 (n = 5) | 97.0 | 3.1 |
| Potato, tuber | Ametoctradin | 0.01 (n = 5) | 108.4 | 1.7 | Acceptable |
| 0.1 (n = 5) | 101.9 | 2.7 |
| M650F003 | 0.01 (n = 5) | 106.9 | 1.6 | Acceptable |
| 0.1 (n = 5) | 99.4 | 2.3 |
| M659F004 | 0.01 (n = 5) | 96.2 | 2.0 | Acceptable |
| 0.1 (n = 5) | 97.8 | 2.6 |
| Lettuce, head | Ametoctradin | 0.01 (n = 5) | 105.4 | 3.2 | Acceptable |
| 0.1 (n = 5) | 102.1 | 4.4 |
| M650F003 | 0.01 (n = 5) | 102.9 | 1.6 | Acceptable |
| 0.1 (n = 5) | 102.8 | 3.0 |
| M659F004 | 0.01 (n = 5) | 95.4 | 2.5 | Acceptable |
| 0.1 (n = 5) | 97.7 | 3.9 |
| Tomato, fruit | Ametoctradin | 0.01 (n = 5) | 104.5 | 1.4 | Acceptable |
| 0.1 (n = 5) | 93.0 | 2.4 |
| M650F003 | 0.01 (n = 5) | 98.2 | 7.1 | Acceptable |
| 0.1 (n = 5) | 90.9 | 5.3 |
| M659F004 | 0.01 (n = 5) | 98.4 | 3.6 | Acceptable |
| 0.1 (n = 5) | 89.8 | 3.5 |
| Grape, fruit | Ametoctradin | 0.01 (n = 5) | 91.4 | 4.5 | Acceptable |
| 0.1 (n = 5) | 101.9 | 2.9 |
| M650F003 | 0.01 (n = 5) | 99.3 | 3.8 | Acceptable |
| 0.1 (n = 5) | 92.8 | 2.5 |
| M659F004 | 0.01 (n = 5) | 88.7 | 5.0 | Acceptable |
| 0.1 (n = 5) | 91.2 | 2.6 |
| Orange, sweet, whole fruit | Ametoctradin | 0.01 (n = 5) | 95.0 | 2.5 | Acceptable |
| 0.1 (n = 5) | 104.6 | 2.1 |
| M650F003 | 0.01 (n = 5) | 99.7 | 3.3 | Acceptable |
| 0.1 (n = 5) | 98.6 | 1.8 |
| M659F004 | 0.01 (n = 5) | 92.3 | 3.7 | Acceptable |
| 0.1 (n = 5) | 96.7 | 4.0 |
| Onion, bulb | Ametoctradin | 0.01 (n = 5) | 98.4 | 1.9 | Acceptable |
| 0.1 (n = 5) | 92.6 | 2.1 |
| M650F003 | 0.01 (n = 5) | 88.8 | 7.1 | Acceptable |
| 0.1 (n = 5) | 97.8 | 4.0 |
| M659F004 | 0.01 (n = 5) | 92.5 | 2.6 | Acceptable |
| 0.1 (n = 5) | 98.5 | 2.7 |
| Sunflower, seed | Ametoctradin | 0.01 (n = 5) | 93.4 | 1.2 | Acceptable |
| 0.1 (n = 5) | 88.6 | 3.6 |
| M650F003 | 0.01 (n = 5) | 68.7 | 4.3 | Acceptable |
| 0.1 (n = 5) | 78.7 | 2.8 |
| M659F004 | 0.01 (n = 5) | 92.6 | 1.7 | Acceptable |
| 0.1 (n = 5) | 94.1 | 3.5 |

Table A 2: Characteristics for the method used for validation of ametoctradin and metabolites M650F003 & M650F004 residues in plant matrices

|  | Ametoctradin and metabolites M650F003 & M650F004 |
| --- | --- |
| Specificity | LC-MS/MS is a highly specific method. No interference (> 30 % LOQ) of total peak area for the target analyte at the retention time, was found in unfortified control samples. |
| Calibration (type, number of data points) | Calibration was performed with solvent based standards at a minimum of six concentrations ranging 0.025 to 2.5 ng/mL and individual calibration data was presented in the study report. Good linearity was observed (r ≥0.99). The resulting test substance peak areas versus theoretical test substance concentration data were fit to the linear function. |
| Calibration range | Accepted calibration range 0.025 ng/mL to 2.5 ng/mL |
| Assessment of matrix effects is presented | Not assessed. No significant matrix effects were observed considering the recoveries at LOQ and ten times LOQ are similar (<20%). |
| Solution Stability | Standard solutions and extract stability were evaluated in the study. Standards in fortification solvent (methanol) and calibration standards in methanol/water/formic acid (50:50:0.1, v/v/v) is shown to be stable (>80%) at least for 60 days for ametoctradin (BAS 650 F) and its metabolites, M650F003 and M650F004 under refrigerated conditions.  Extract stability was also evaluated in extraction solution [methanol/water (50:50, v/v)] and in final volume solution [methanol/water/formic acid (50:50:0.1, v/v/v)] for 14 days. The results shown that BAS 650 F and its metabolites, M650F003 and M650F004 were stable (>80%) for at least 14 days under refrigerated conditions. |
| Limit of determination/quantification | The limit of quantification (LOQ) of 0.01 mg/kg was confirmed. The limit of detection (LOD), defined as the lowest standard employed was 0.025 ng/mL. |

Conclusion

The analytical procedure, method L0078/01, for the determination of residues of ametoctradin (BAS 650 F) and its metabolites M650F003 and M650F004 in plant matrices has been fully validated in terms of specificity, linearity, precision, accuracy, solution stability and LOQ, in accordance with the requirements of SANCO/3029/99 rev.4, SANCO/825/00 rev.7 and meets the minimum requirements of SANTE/2020/12830 rev.1 for existing risk assessment methods to be considered fit for purpose also.

* + - 1. Method L0078/01: Method for the determination of ametoctradin in plant matrices
         1. Method validation

|  |  |
| --- | --- |
| Comments of zRMS: | The method validation has been accepted.  The method widely used within the section B7 of this submission for residue data generation in plant matrices.  Ametoctradin and its metabolites M650F003 and M650F004 were extracted, an aliquot was cleaned -up by solid phase extraction. The final determination was performed by LC-MS/MS which is highly sensitive and selective. The LOQ was set at 0.010 mg/kg for all analytes in all matrices.  Two mass transitions were monitored for each analyte. Untreated control samples were free from interference and no residues were above 30% of the LOQ for each mass transition for any analyte. Matrix matched calibration standards were used for the quantification of each analyte in all plant matrices.  The method L0078/01 was successfully validated in lettuce (head), soybean (seed), grapes and peas (dry) at a limit of quantification of 0.010 mg/kg. The mean recovery values were between 71.6% and 97.8% of the nominal value for both mass transitions in all matrices tested for all analytes. The relative standard deviations (RSD, %) for all fortification levels were ≤ 7.5%.  Thus, it could be confirmed that the analytical method L0078/01 fulfils requirements of SANTE/2020/12830, Rev.1 regarding specificity, repeatability, limit of quantification, recoveries and linearity and is therefore applicable to correctly determine residues of Ametoctradin and its metabolites M650F003 and M650F004 in validated plant matrices. |

|  |  |
| --- | --- |
| Reference: | CP 5.1.2/2 |
| Report | Method Validation of Analytical Method L0078/01 for the Determination of BAS 650 F in plant matrices by LC-MS/MS  Homazava, N., 2021  Report No: 919340  XXXX DocID: 2021/2041685  Authority registration No |
| Guideline(s): | EPA 860.1340 (1996); OECD ENV/JM/MONO(2007)17, SANTE/2020/12830 Rev. 1 |
| Deviations: | No |
| Previous evaluation: | No |
| GLP: | Yes |
| Acceptability: | Yes | |

**Study Summary**

The method L0078/01 was revalidated according to the recent guideline document SANTE/2020/12830 (Rev.1) for the determination of residues of Ametoctradin (BAS 650 F) and its metabolites M650F003 and M650F004 in lettuce (head), soybean (seed), grapes, and peas (dry) with a limit of quantification of 0.01 mg/kg.

Materials and methods

The method L0078/01 is as described in 2008/1022139 above.

Results and discussions

The analytical method validation is summarised below. The HPLC-MS/MS determination was conducted by monitoring two MS/MS mass transitions and therefore no confirmatory method is required.

Recoveries were obtained by fortification of the matrices and all mean recoveries are within the permitted range required by the guideline SANTE/2020/12830 rev. 1.

Table A 3: Recovery results from method validation of ametoctradin and metabolites M650F003 & M650F004 using the analytical method

| Matrix | Analyte | Fortification level (mg/kg) (n = x) | Mean  recovery (%) | RSD (%) | Comments |
| --- | --- | --- | --- | --- | --- |
| ***Mass transition (Quantification)  Ametoctradin 276→176 m/z, M650F003 222→176 m/z, M650F004 208→190 m/z*** | | | | | |
| Lettuce (head) | Ametoctradin | 0.01 (n = 5) | 83.7 | 2.8 | Acceptable |
| 0.1 (n = 5) | 79.3 | 1.6 |
| M650F003 | 0.01 (n = 5) | 86.0 | 2.4 | Acceptable |
| 0.1 (n = 5) | 90.8 | 0.9 |
| M659F004 | 0.01 (n = 5) | 85.5 | 2.7 | Acceptable |
| 0.1 (n = 5) | 90.1 | 0.7 |
| Soybean (seed) | Ametoctradin | 0.01 (n = 5) | 73.8 | 2.1 | Acceptable |
| 0.1 (n = 5) | 71.9 | 3.4 |
| M650F003 | 0.01 (n = 5) | 89.0 | 3.1 | Acceptable |
| 0.1 (n = 5) | 91.6 | 3.5 |
| M659F004 | 0.01 (n = 5) | 89.8 | 4.5 | Acceptable |
| 0.1 (n = 5) | 93.5 | 3.2 |
| Grapes | Ametoctradin | 0.01 (n = 5) | 83.9 | 1.8 | Acceptable |
| 0.1 (n = 5) | 83.4 | 2.1 |
| M650F003 | 0.01 (n = 5) | 95.4 | 3.9 | Acceptable |
| 0.1 (n = 5) | 93.5 | 6.4 |
| M659F004 | 0.01 (n = 5) | 93.5 | 1.8 | Acceptable |
| 0.1 (n = 5) | 97.8 | 2.3 |
| Peas (dry) | Ametoctradin | 0.01 (n = 5) | 78.8 | 0.9 | Acceptable |
| 0.1 (n = 5) | 76.8 | 1.9 |
| M650F003 | 0.01 (n = 5) | 90.4 | 1.4 | Acceptable |
| 0.1 (n = 5) | 94.0 | 2.5 |
| M659F004 | 0.01 (n = 5) | 92.1 | 2.2 | Acceptable |
| 0.1 (n = 5) | 94.2 | 2.3 |
| ***Mass transition (Confirmation) Ametoctradin 276→149 m/z, M650F003 222→121 m/z, M650F004 208→123 m/z*** | | | | | |
| Lettuce (head) | Ametoctradin | 0.01 (n = 5) | 83.0 | 2.7 | Acceptable |
| 0.1 (n = 5) | 78.4 | 1.7 |
| M650F003 | 0.01 (n = 5) | 85.7 | 4.3 | Acceptable |
| 0.1 (n = 5) | 91.2 | 0.8 |
| M659F004 | 0.01 (n = 5) | 84.8 | 1.8 | Acceptable |
| 0.1 (n = 5) | 90.9 | 0.8 |
| Soybean (seed) | Ametoctradin | 0.01 (n = 5) | 73.4 | 1.1 | Acceptable |
| 0.1 (n = 5) | 71.6 | 3.2 |
| M650F003 | 0.01 (n = 5) | 88.8 | 4.3 | Acceptable |
| 0.1 (n = 5) | 92.2 | 3.7 |
| M659F004 | 0.01 (n = 5) | 90.5 | 1.1 | Acceptable |
| 0.1 (n = 5) | 94.0 | 3.2 |
| Grapes | Ametoctradin | 0.01 (n = 5) | 83.3 | 1.4 | Acceptable |
| 0.1 (n = 5) | 83.3 | 2.0 |
| M650F003 | 0.01 (n = 5) | 91.6 | 3.8 | Acceptable |
| 0.1 (n = 5) | 92.8 | 7.5 |
| M659F004 | 0.01 (n = 5) | 92.1 | 1.4 | Acceptable |
| 0.1 (n = 5) | 97.5 | 2.3 |
| Peas (dry) | Ametoctradin | 0.01 (n = 5) | 78.4 | 1.9 | Acceptable |
| 0.1 (n = 5) | 76.6 | 1.3 |
| M650F003 | 0.01 (n = 5) | 90.3 | 1.8 | Acceptable |
| 0.1 (n = 5) | 93.3 | 2.7 |
| M659F004 | 0.01 (n = 5) | 91.7 | 1.6 | Acceptable |
| 0.1 (n = 5) | 93.9 | 1.9 |

Table A 4: Characteristics for the method used for validation of ametoctradin and metabolites M650F003 & M650F004 residues in plant matrices

|  | Ametoctradin and metabolites M650F003 & M650F004 |
| --- | --- |
| Specificity | LC-MS/MS is a highly specific method. No interference (> 30 % LOQ) of total peak area for the target analyte at the retention time, was found in unfortified control samples. |
| Calibration (type, number of data points) | Calibration was performed with matrix-matched standards using seven concentrations ranging 0.025 to 2.5 ng/mL (equivalent to 0.0025 mg/kg to 0.25 mg/kg at sample level) for Ametoctradin and 0.05 ng/ml to 5.0 ng.mL (equivalent to 0.002 mg/kg to 0.2 mg/kg at sample level) for both M650F003 and M650F004. Good linearity was observed (r ≥0.99). The resulting test substance peak areas versus test substance concentration data were fit to the linear function and residuals were randomly scattered.  **Lettuce (head)**   |  |  |  |  |  | | --- | --- | --- | --- | --- | | Analyte | m/z | Slope | Intercept | r | | Ametoctradin | 276→ 176 | 6142815 | 107460 | 0.99817719 | | 276→ 149 | 5909652 | 102587 | 0.99748831 | | M650F003 | 222→ 176 | 2689326 | 2099.465 | 0.99802638 | | 222→ 121 | 237079.2 | 2405.617 | 0.99819895 | | M650F004 | 208→ 190 | 3032410 | -9099.089 | 0.99819654 | | 208→ 123 | 1227780 | 4300.619 | 0.99815890 |   **Soybean (seed)**   |  |  |  |  |  | | --- | --- | --- | --- | --- | | Analyte | m/z | Slope | Intercept | r | | Ametoctradin | 276→ 176 | 5222514 | 7697.61 | 0.99979104 | | 276→ 149 | 4966798 | 7996.199 | 0.99974803 | | M650F003 | 222→ 176 | 6688619 | 138420.6 | 0.99934900 | | 222→ 121 | 596653.4 | 10063.73 | 0.99935612 | | M650F004 | 208→ 190 | 3807968 | 38118.21 | 0.99949952 | | 208→ 123 | 1549752 | 34601.49 | 0.99934310 |   **Grapes**   |  |  |  |  |  | | --- | --- | --- | --- | --- | | Analyte | m/z | Slope | Intercept | r | | Ametoctradin | 276→ 176 | 6019598 | 16070.22 | 0.99905398 | | 276→ 149 | 5742118 | 13853.14 | 0.99875182 | | M650F003 | 222→ 176 | 3822069 | 7139.9 | 0.99764341 | | 222→ 121 | 340395.4 | 3480 | 0.99794111 | | M650F004 | 208→ 190 | 3904050 | 18134.93 | 0.99804756 | | 208→ 123 | 1602189 | 6179.991 | 0.99742350 |   **Peas (dry)**   |  |  |  |  |  | | --- | --- | --- | --- | --- | | Analyte | m/z | Slope | Intercept | r | | Ametoctradin | 276→ 176 | 6601638 | -9232.97 | 0.99878066 | | 276→ 149 | 6274404 | -9814.571 | 0.99878010 | | M650F003 | 222→ 176 | 3482242 | 5983.675 | 0.99795640 | | 222→ 121 | 313319.3 | 1809.289 | 0.99868540 | | M650F004 | 208→ 190 | 3824352 | -9347.677 | 0.99813479 | | 208→ 123 | 1564628 | 5002.452 | 0.99770713 | |
| Calibration range | 0.025 to 2.5 ng/mL (equivalent to 0.0025 mg/kg to 0.25 mg/kg at sample level) for Ametoctradin.  0.05 ng/ml to 5.0 ng.mL (equivalent to 0.002 mg/kg to 0.2 mg/kg at sample level) for both M650F003 and M650F004 |
| Assessment of matrix effects is presented | **Matrix effects**   |  |  |  |  | | --- | --- | --- | --- | | Matrix | Analyte | m/z | Overall Mean Matrix Effect (%) | | Lettuce (head) | Ametoctradin | 276→ 176 | ± 8.8 | | 276→ 149 | ± 10.1 | | M650F003 | 222→ 176 | ± 6.4 | | 222→ 121 | ± 14.1 | | M650F004 | 208→ 190 | ± 9.0 | | 208→ 123 | ± 9.4 | | Soybean (seed) | Ametoctradin | 276→ 176 | ± 3.5 | | 276→ 149 | ± 3.4 | | M650F003 | 222→ 176 | ± 111.3 | | 222→ 121 | ± 94.6 | | M650F004 | 208→ 190 | ± 6.2 | | 208→ 123 | ± 11.2 | | Grapes | Ametoctradin | 276→ 176 | ± 11.1 | | 276→ 149 | ± 10.0 | | M650F003 | 222→ 176 | ± 7.8 | | 222→ 121 | ± 8.7 | | M650F004 | 208→ 190 | ± 5.1 | | 208→ 123 | ± 5.4 | | Peas (dry) | Ametoctradin | 276→ 176 | ± 7.1 | | 276→ 149 | ± 8.3 | | M650F003 | 222→ 176 | ± 7.0 | | 222→ 121 | ± 7.5 | | M650F004 | 208→ 190 | ± 7.6 | | 208→ 123 | ± 8.4 |   Significant matrix effects were observed (i.e. >±20%) in some matrices, therefore matrix-matched standards were used throughout. |
| Solution Stability | See XXXX Doc ID 2008/1022139 above. |
| Limit of determination/quantification | The limit of quantification (LOQ) of 0.01 mg/kg was confirmed. The limit of detection (LOD), defined as the lowest standard employed was 0.025 ng/mL. |

Conclusion

The analytical procedure, method L0078/01, for the determination of residues of ametoctradin (BAS 650 F) and its metabolites M650F003 and M650F004 in plant matrices has been fully validated in terms of specificity, linearity, precision, accuracy, matrix effects, solution stability and LOQ, in accordance with the requirements of SANTE/2020/12830 Rev.1.

* + - 1. Method L0078/01: Method for the determination of ametoctradin in melon (fruit, peel & pulp)
         1. Method validation

|  |  |
| --- | --- |
| Comments of zRMS: | The method has been accepted.  The method widely used within the section B7 of this submission for residue data generation in plant matrices. The final determination of Ametoctradin and its metabolites (M650F003, M650F004) in melon matrices (fruit, peel, pulp) was performed by LC-MS/MS which is highly sensitive and selective. The LOQ was set at 0.010 mg/kg for all analytes.  The mean recovery values were between the required range for all analytes. The relative standard deviations (RSD, %) for all fortification levels were ≤ 20 %.  Thus, it could be confirmed that the analytical method L0078/01 fulfils requirements of SANCO/3029/99 rev. 4. |

|  |  |
| --- | --- |
| Reference: | CP 5.1.2/3 |
| Report | Study on the residue behaviour of Propamocarb (Reg.No. 4628172) and Ametoctradin (BAS 650 F) in Melon after treatment with BAS 743 01 F under field conditions in Southern Europe in 2020  Schneider, E., 2021  report No 890088  XXXX DocID 2021/2019512  Authority registration No |
| Guideline(s): | EPA 860.1340; SANCO/3029/99 rev. 4 (11 July 2000); OECD ENV/JM/MONO(2007)17 - Guidance Document on Pesticide Residue Analytical Methods |
| Deviations: | None which affect integrity of the method validation |
| Previous evaluation: | No |
| GLP: | Yes |
| Acceptability: | Yes | |

**Study Summary**

The method L0078/01 was validated for the determination of residues of Ametoctradin (BAS 650 F) and its metabolites M650F003 and M650F004 in melon matrices (fruit, peel, pulp), with a limit of quantification at 0.01 mg/kg. The brief description of method and the results are presented in the summary below.

Materials and methods

The method is that described in XXXX Doc ID 2008/1022139 above, with the exception that the injection volume was decreased from 50 µL to 30 µL to reduce matrix effects.

Results and discussions

The analytical method validation is summarised below. The HPLC-MS/MS determination was conducted by monitoring two MS/MS mass transitions and therefore no confirmatory method is required.

Procedural recoveries were obtained by fortification of the matrices and all mean recoveries are within the permitted range required by the guideline SANTE/2020/12830 rev. 1. While two mass transitions were analysed, only the ones for quantification are reported.

Table A 5: Recovery results from method validation of ametoctradin and metabolites M650F003 & M650F004 in melon matrices using the analytical method

| Matrix | Analyte | Fortification level (mg/kg) (n = x) | Recovery (%) | Mean  recovery (%) | RSD (%) | Comments |
| --- | --- | --- | --- | --- | --- | --- |
| ***Mass transition (Quantification)  Ametoctradin 276→176 m/z, M650F003 222→176 m/z, M650F004 208→190 m/z*** | | | | | | |
| Melon  (fruit) | Ametoctradin | 0.01 (n = 4) | 109, 106, 94.7, 97.5 | 102 | 6.8 | Acceptable |
| 0.1 (n = 6) | 91.6, 96.8, 96.9, 103.1, 97.0, 95.3 | 96.8 | 3.8 |
| 30 (n=1) | 109 | 109 | - |
| M650F003 | 0.01 (n = 4) | 105, 103, 109, 110 | 107 | 2.8 | Acceptable |
| 0.1 (n = 6) | 103, 106, 104, 104, 109, 110 | 106 | 2.6 |
| M659F004 | 0.01 (n = 4) | 103, 110, 106, 109 | 107 | 3.0 | Acceptable |
| 0.1 (n = 6) | 105, 104, 111, 111, 108, 108 | 108 | 2.6 |
| Melon  (peel) | Ametoctradin | 0.01 (n = 3) | 88.8, 90.4, 90.4 | 89.9 | 1.0 | Acceptable |
| 0.1 (n = 3) | 84.4, 91.8, 87.0 | 87.7 | 4.2 |
| 10 (n = 1) | 93.2 | 93.2 | - |
| 30 (n = 1) | 97.4 | 97.4 | - |
| M650F003 | 0.01 (n = 3) | 108, 106, 108 | 107 | 1.0 | Acceptable |
| * 1. (n = 3) | 104, 106, 105 | 105 | 0.85 |
| M659F004 | 0.01 (n = 3) | 103, 105, 103 | 103 | 0.94 | Acceptable |
| 0.1 (n = 3) | 99.2, 101, 102 | 101 | 1.4 |
| Melon (pulp) | Ametoctradin | 0.01 (n = 3) | 94.7, 95.3, 93.1 | 94.0 | 1.2 | Acceptable |
| 0.1 (n = 3) | 92.0, 98.1, 97 | 95.7 | 3.4 |
| M650F003 | 0.01 (n = 3) | 73.1, 75.2, 77.7 | 75.4 | 3.1 | Acceptable |
| 0.1 (n = 3) | 70.6, 70.1, 69.9 | 70.2 | 0.46 |
| M659F004 | 0.01 (n = 3) | 74.3, 72.2, 75.2 | 73.9 | 2.1 | Acceptable |
| 0.1 (n = 3) | 71.4, 69.0, 69.9 | 70.1 | 1.7 |

Table A 6: Characteristics for the method used for validation of ametoctradin and metabolites M650F003 & M650F004 residues in plant matrices

|  | Ametoctradin and metabolites M650F003 & M650F004 |
| --- | --- |
| Specificity | LC-MS/MS is a highly specific method. No interference (> 30 % LOQ) of total peak area for the target analyte at the retention time, was found in unfortified control samples. |
| Calibration (type, number of data points) | Calibration was performed with solvent based standards at least seven concentrations ranging 0.025 to 5 ng/mL and good linearity was observed (r ≥0.99). The resulting test substance peak areas versus theoretical test substance concentration data were fit to the linear function.  Regression data:  Amectoctradin:  Mass transition 276→ 176 m/z (quantification)  Slope = 4.27 x 106, Intercept = 1.01 x 104, r = 0.9987  M650F003:  Mass transition 222→ 176 m/z (quantification)  Slope = 2.36 x 106, Intercept = -4.49 x 103, r = 0.9990  M650F004:  Mass transition 208→ 190 m/z (quantification)  Slope = 3.66 x 106, Intercept = -5.73 x 103, r = 0.9997 |
| Calibration range | 0.025 ng/mL to 2.5 ng/mL |
| Assessment of matrix effects is presented | No significant matrix effects observed (i.e. <20%). Calibration standards were therefore prepared in solvent throughout. |
| Solution Stability | See XXXX Doc ID 2008/1022139 above for standard stability.  Storage stability for melon was determined in XXXX Doc ID 2020/2036187 and samples were found to be stable for 2 years in freezer (≤ -18°C)/dark conditions. |
| Limit of determination/quantification | The limit of quantification (LOQ) of 0.01 mg/kg was confirmed. The limit of detection (LOD), defined as the lowest standard employed was 0.025 ng/mL. |

Conclusion

The analytical procedure, method L0078/01, for the determination of residues of ametoctradin (BAS 650 F) and its metabolites M650F003 and M650F004 in melon matrices has been fully validated in terms of specificity, linearity, precision, accuracy, matrix effects, solution stability and LOQ, in accordance with the requirements of SANCO/3029/99 rev.4, and meets the minimum requirements of requirements of SANTE/2020/12830 rev.1 for existing risk assessment methods to be considered fit for purpose also.

* + - 1. Method L0078/01: Method for the determination of ametoctradin in lettuce (open head & lambs)
         1. Method validation

|  |  |
| --- | --- |
| Comments of zRMS: | The method has been accepted.  The method widely used within the section B7 of this submission for residue data generation in plant matrices. The final determination of Ametoctradin and its metabolites (M650F003, M650F004) in lettuce matrices was performed by LC-MS/MS which is highly sensitive and selective. The LOQ was set at 0.010 mg/kg for all analytes.  The mean recovery values were between the required range for all analytes. The relative standard deviations (RSD, %) for all fortification levels were ≤ 20 %.  The method was already validated in lettuce. Thus, it could be confirmed that the analytical method L0078/01 fulfils requirements of SANTE/2020/12830 rev.1. |

|  |  |
| --- | --- |
| Reference: | CP 5.1.2/4 |
| Report | Study on the residue behaviour of Propamocarb (Reg.No. 4628172) and Ametoctradin (BAS 650 F) in lettuce after two applications of BAS 743 01 F under field conditions in Southern Europe, 2021  Vagt, I. & Meyer, M., 2022  report No 890083  XXXX DocID 2022/2041753  Authority registration No |
| Guideline(s): | EPA 860.1340; SANTE/2020/12830, Rev.1; OECD ENV/JM/MONO(2007)17 - Guidance Document on Pesticide Residue Analytical Methods |
| Deviations: | None which affect integrity of the method validation |
| Previous evaluation: | No |
| GLP: | Yes |
| Acceptability: | Yes | |

**Study Summary**

The method L0078/01 was validated for the determination of residues of Ametoctradin (BAS 650 F) and its metabolites M650F003 and M650F004 in lettuce matrices (open head [whole plant and open leaves] and Lamb’s [whole plant and leaves]), with a limit of quantification at 0.01 mg/kg. The brief description of method and the results are presented in the summary below.

Materials and methods

The method is that described in XXXX Doc ID 2008/1022139 above.

Results and discussions

The analytical method validation is summarised below. The HPLC-MS/MS determination was conducted by monitoring two MS/MS mass transitions and therefore no confirmatory method is required.

Procedural recoveries were obtained by fortification of the matrices and all mean recoveries are within the permitted range required by the guideline SANTE/2020/12830 rev. 1. While two mass transitions were analysed, only the ones for quantification are reported.

Table A 7: Recovery results from method validation of ametoctradin and metabolites M650F003 & M650F004 in lettuce matrices using the analytical method

| Matrix | Analyte | Fortification level (mg/kg) (n = x) | Recovery (%) | Mean  recovery (%) | RSD (%) | Comments |
| --- | --- | --- | --- | --- | --- | --- |
| ***Mass transition (Quantification)  Ametoctradin 276→176 m/z, M650F003 222→176 m/z, M650F004 208→190 m/z*** | | | | | | |
| Lettuce  (Open head, whole plant) | Ametoctradin | 0.01 (n = 4) | 107, 111, 107, 106 | 108 | 2.2 | Acceptable |
| 0.1 (n = 4) | 107, 107, 107, 111 | 108 | 2.0 |
| 100 (n=1) | 104 | 104 | - |
| M650F003 | 0.01 (n = 3) | 109, 107, 110 | 109 | 1.6 | Acceptable |
| 0.1 (n = 3) | 108, 107, 110 | 108 | 1.7 |
| M659F004 | 0.01 (n = 3) | 107, 105, 110 | 107 | 2.6 | Acceptable |
| 0.1 (n = 3) | 109, 111, 109 | 110 | 1.1 |
| Lettuce  (Open head, head – open leaves) | Ametoctradin | 0.01 (n = 5) | 109, 113, 105, 110, 107 | 109 | 2.5 | Acceptable |
| 0.1 (n = 5) | 107, 103, 108, 103, 109 | 106 | 2.7 |
| 50 (n = 1) | 109 | 109 | - |
| M650F003 | 0.01 (n = 4) | 107, 108, 111, 106 | 108 | 2.2 | Acceptable |
| * 1. (n = 4) | 109, 109, 111, 108 | 109 | 1.1 |
| M659F004 | 0.01 (n = 4) | 110, 107, 107, 107 | 108 | 1.2 | Acceptable |
| 0.1 (n = 4) | 109, 109, 110, 110 | 109 | 0.4 |
| Lettuce (Lamb’s, whole plant) | Ametoctradin | 0.01 (n = 3) | 103, 104, 104 | 104 | 0.8 | Acceptable |
| 0.1 (n = 3) | 107, 106, 101 | 105 | 3.3 |
| 100 (n = 1) | 102 | 102 | - |
| M650F003 | 0.01 (n = 3) | 96.9, 97.9, 98.8 | 97.9 | 1.0 | Acceptable |
| 0.1 (n = 3) | 111, 110, 106 | 109 | 2.6 |
| M659F004 | 0.01 (n = 3) | 105, 103, 105 | 105 | 1.2 | Acceptable |
| 0.1 (n = 3) | 112, 107, 107 | 108 | 2.5 |
| Lettuce (Lamb’s, leaves) | Ametoctradin | 0.01 (n = 3) | 108, 106, 107 | 107 | 0.9 | Acceptable |
| 0.1 (n = 3) | 107, 107, 107 | 107 | 0.3 |
| 50 (n = 1) | 110 | 110 | - |
| M650F003 | 0.01 (n = 3) | 98.4, 105, 102 | 102 | 3.1 | Acceptable |
| 0.1 (n = 3) | 107, 105, 106 | 106 | 1.0 |
| M659F004 | 0.01 (n = 3) | 102, 104, 102 | 103 | 1.3 | Acceptable |
| 0.1 (n = 3) | 106, 108, 109 | 108 | 1.5 |

Table A 8: Characteristics for the method used for validation of ametoctradin and metabolites M650F003 & M650F004 residues in lettuce

|  | Ametoctradin and metabolites M650F003 & M650F004 |
| --- | --- |
| Specificity | LC-MS/MS is a highly specific method. No interference (> 30 % LOQ) of total peak area for the target analyte at the retention time, was found in unfortified control samples. |
| Calibration (type, number of data points) | Calibration was performed with solvent based standards at least seven concentrations ranging 0.025 to 2.5 ng/mL (5 ng/mL for M650F003 & M650F004) and good linearity was observed (r ≥0.99). The resulting test substance peak areas versus theoretical test substance concentration data were fit to the linear function.  Regression data:  Amectoctradin:  Mass transition 276→ 176 m/z (quantification)  Slope = 2948036.2, Intercept = 20832.163, r = 0.99964  M650F003:  Mass transition 222→ 176 m/z (quantification)  Slope = 1786048.5, Intercept = 13779.387, r = 0.99909  M650F004:  Mass transition 208→ 190 m/z (quantification)  Slope = 2552737.8, Intercept = 4518.843, r = 0.99990 |
| Calibration range | 0.025 ng/mL to 2.5 ng/mL (5 ng/mL for M650F003 & M650F004) |
| Assessment of matrix effects is presented | No significant matrix effects observed (i.e. <20%). Calibration standards were therefore prepared in solvent throughout. |
| Solution Stability | See XXXX Doc ID 2008/1022139 above for standard and sample stability. |
| Limit of determination/quantification | The limit of quantification (LOQ) of 0.01 mg/kg was confirmed. The limit of detection (LOD), defined as the lowest standard employed was 0.025 ng/mL. |

Conclusion

The analytical procedure, method L0078/01, for the determination of residues of ametoctradin (BAS 650 F) and its metabolites M650F003 and M650F004 in lettuce matrices has been fully validated in terms of specificity, linearity, precision, accuracy, matrix effects, solution stability and LOQ, in accordance with the requirements of SANTE/2020/12830 rev.1.

* + - 1. Method L0078/01: Method for the determination of ametoctradin in tomato
         1. Method validation

|  |  |
| --- | --- |
| Comments of zRMS: | The method has been accepted.  The method widely used within the section B7 of this submission for residue data generation in plant matrices. The final determination of Ametoctradin and its metabolites (M650F003, M650F004) in tomato matrices was performed by LC-MS/MS which is highly sensitive and selective. The LOQ was set at 0.010 mg/kg for all analytes.  The mean recovery values were between the required range for all analytes. The relative standard deviations (RSD, %) for all fortification levels were ≤ 20 %.  Thus, it could be confirmed that the analytical method L0078/01 fulfils requirements of SANCO/3029/99 rev. 4. |

|  |  |
| --- | --- |
| Reference: | CP 5.1.2/5 |
| Report | Study on the residue behaviour of Ametoctradin (BAS 650 F) and Propamocarb (Reg. No. 4628172) on tomato after treatment with BAS 743 01 F under field conditions in Southern Europe, season 2020  Gálvez, O., 2021  report No 890073  XXXX DocID 2020/2103085  Authority registration No |
| Guideline(s): | SANCO/3029/99 rev.4; OECD ENV/JM/MONO(2007)17 - Guidance Document on Pesticide Residue Analytical Methods |
| Deviations: | No |
| Previous evaluation: | No |
| GLP: | Yes |
| Acceptability: | Yes | |

**Study Summary**

The method L0078/01 was validated for the determination of residues of Ametoctradin (BAS 650 F) and its metabolites M650F003 and M650F004 in tomato (fruit), with a limit of quantification at 0.01 mg/kg. The brief description of method and the results are presented in the summary below.

Materials and methods

The method is that described in XXXX Doc ID 2008/1022139 above.

Results and discussions

The analytical method validation is summarised below. The HPLC-MS/MS determination was conducted by monitoring two MS/MS mass transitions and therefore no confirmatory method is required.

Procedural recoveries were obtained by fortification of the matrices and all mean recoveries are within the permitted range required by the guideline SANTE/2020/12830 rev. 1, with the exception of ametoctradin at the 0.1 mg/kg fortification level, where the mean recovery was marginally outside the guideline requirements (69.0% receovery). While two mass transitions were analysed, only the ones for quantification are reported.

Table A 9: Recovery results from method validation of ametoctradin and metabolites M650F003 & M650F004 in tomato using the analytical method

| Matrix | Analyte | Fortification level (mg/kg) (n = x) | Recovery (%) | Mean  recovery (%) | RSD (%) | Comments |
| --- | --- | --- | --- | --- | --- | --- |
| ***Mass transition (Quantification)  Ametoctradin 276→176 m/z, M650F003 222→176 m/z, M650F004 208→190 m/z*** | | | | | | |
| Tomato (fruit) | Ametoctradin | 0.01 (n = 3) | 72.2, 71.8, 78.9 | 74.3 | 5.4 | Acceptable |
| 0.1 (n = 3) | 70.3, 65.2, 71.5 | 69.0 | 4.8 |
| 2.0 (n = 3) | 102, 99.6, 100 | 100.5 | 1.3 |
| M650F003 | 0.01 (n = 3) | 90.0, 99.9, 91.4 | 93.8 | 5.7 | Acceptable |
| 0.1 (n = 3) | 92.6, 99.9, 87.0 | 93.2 | 6.9 |
| 2.0 (n = 3) | 90.4, 89.8, 91.3 | 90.5 | 0.8 |
| M659F004 | 0.01 (n = 3) | 84.8, 88.3, 82.1 | 85.1 | 3.7 | Acceptable |
| 0.1 (n = 3) | 84.5, 78.3, 90.6 | 84.5 | 7.3 |
| 2.0 (n = 3) | 93.8, 88.4, 89.3 | 90.5 | 3.2 |

Table A 10: Characteristics for the method used for validation of Ametoctradin and metabolites M650F003 & M650F004 residues in tomato

|  | Ametoctradin and metabolites M650F003 & M650F004 |
| --- | --- |
| Specificity | LC-MS/MS is a highly specific method. No signal interference at the target analyte retention time, was found in unfortified control samples. |
| Calibration (type, number of data points) | Calibration was performed with matrix-matched standards using at least seven concentrations ranging 0.025 to 3.0 ng/mL for ametoctradin (equiv. 0.0025 – 0.30 mg/kg at sample level) (0.050 – 3.0 ng/mL for M650F003 & M650F004 [equiv. 0.0020 – 0.12 mg/kg at sample level]) and good linearity was observed (r ≥0.99). The resulting test substance peak areas versus theoretical test substance concentration data were fit to the linear function.  Regression data:  Amectoctradin:  Mass transition 276→ 176 m/z (quantification)  Slope = 601457.1232405, Intercept = 506.419992400, r = 0.9993  M650F003:  Mass transition 222→ 176 m/z (quantification)  Slope = 1999664.5333584, Intercept = 10079.707592518, r = 0.9993  M650F004:  Mass transition 208→ 190 m/z (quantification)  Slope = 1895431.9595182, Intercept = 4998.714223664, r = 0.9993 |
| Calibration range | 0.025 to 3.0 ng/mL for ametoctradin (equiv. 0.0025 – 0.30 mg/kg at sample level)  0.050 to 3.0 ng/mL for M650F003 & M650F004 (equiv. 0.0020 – 0.12 mg/kg at sample level) |
| Assessment of matrix effects is presented | Not evaluated. However, matrix-matched calibration standards were used throughout. |
| Solution Stability | See XXXX Doc ID 2008/1022139 above for standard and XXXX Doc ID 2008/1062330 for sample storage stability (240 days in frozen (≤-18°C) conditions). |
| Limit of determination/quantification | The limit of quantification (LOQ) of 0.01 mg/kg was confirmed for Ametoctradin, M650F003 & M650F004 . The limit of detection (LOD) was 0.0025 mg/kg for Ametoctradin and 0.002 mg/kg for both M650F003 & M650F004. |

Conclusion

The analytical procedure, method L0078/01, for the determination of residues of ametoctradin (BAS 650 F) and its metabolites M650F003 and M650F004 in tomato has been fully validated in terms of specificity, linearity, precision, accuracy, matrix effects, solution stability and LOQ, in accordance with the requirements of SANTE/2020/12830 rev.1.

* + - 1. Method L0078/01: Method for the determination of ametoctradin in potato
         1. Method validation

|  |  |
| --- | --- |
| Comments of zRMS: | The method has been accepted.  The method widely used within the section B7 of this submission for residue data generation in plant matrices. The final determination of Ametoctradin and its metabolites (M650F003, M650F004) in potato matrices was performed by LC-MS/MS which is highly sensitive and selective. The LOQ was set at 0.010 mg/kg for all analytes.  The mean recovery values were between the required range for all analytes. The relative standard deviations (RSD, %) for all fortification levels were ≤ 20 %.  Thus, it could be confirmed that the analytical method L0078/01 fulfils requirements of SANCO/3029/99 rev. 4. |

|  |  |
| --- | --- |
| Reference: | CP 5.1.2/6 |
| Report | Study on the residue behaviour of Ametoctradin (BAS 650 F) and Propamocarb (Reg. No. 4628172) on potato after treatment with BAS 743 01 F under field conditions in Southern Europe, season 2020  Gálvez, O., 2021  report No 890078  XXXX DocID 2020/2103083  Authority registration No |
| Guideline(s): | EPA 860.1340; SANCO/3029/99 rev.4; OECD ENV/JM/MONO(2007)17 - Guidance Document on Pesticide Residue Analytical Methods |
| Deviations: | No |
| Previous evaluation: | No |
| GLP: | Yes |
| Acceptability: | Yes | |

**Study Summary**

The method L0078/01 was validated for the determination of residues of Ametoctradin (BAS 650 F) and its metabolites M650F003 and M650F004 in potato (shoot and tuber), with a limit of quantification at 0.01 mg/kg. The brief description of method and the results are presented in the summary below.

Materials and methods

The method is that described in XXXX Doc ID 2008/1022139 above, with the exception that the injection volume was decreased from 50 µL to 30 µL to reduce matrix effects.

Results and discussions

The analytical method validation is summarised below. The HPLC-MS/MS determination was conducted by monitoring two MS/MS mass transitions and therefore no confirmatory method is required.

Procedural recoveries were obtained by fortification of the matrices and all mean recoveries are within the permitted range required by the guideline SANTE/2020/12830 rev. 1. While two mass transitions were analysed, only the ones for quantification are reported.

Table A 11: Recovery results from method validation of ametoctradin and metabolites M650F003 & M650F004 in potato matrices using the analytical method

| Matrix | Analyte | Fortification level (mg/kg) (n = x) | Recovery (%) | Mean  recovery (%) | RSD (%) | Comments |
| --- | --- | --- | --- | --- | --- | --- |
| ***Mass transition (Quantification)  Ametoctradin 276→176 m/z, M650F003 222→176 m/z, M650F004 208→190 m/z*** | | | | | | |
| Potato (shoot) | Ametoctradin | 0.01 (n = 3) | 79.3, 81.5, 83.1 | 81.3 | 2.4 | Acceptable |
| 0.1 (n = 3) | 84.4, 89.3, 86.5 | 86.7 | 2.8 |
| 10 (n = 1) | 98.6 | 98.6 | - |
| 50 (n = 1) | 84.3 | 84.3 | - |
| M650F003 | 0.01 (n = 3) | 103, 109, 109 | 107 | 3.2 | Acceptable |
| 0.1 (n = 3) | 106, 105, 106 | 106 | 0.6 |
| M659F004 | 0.01 (n = 3) | 95.7, 99.3, 97.0 | 97.3 | 1.9 | Acceptable |
| 0.1 (n = 3) | 95.1, 101, 99.9 | 98.6 | 3.0 |
| Potato (tuber) | Ametoctradin | 0.01 (n = 4) | 75.0, 73.6, 77.8, 87.2 | 78.4 | 7.8 | Acceptable |
| 0.1 (n = 4) | 84.6, 88.6, 80.7, 73.4 | 81.8 | 7.9 |
| M650F003 | 0.01 (n = 4) | 99.0, 106, 105, 105 | 104 | 3.2 | Acceptable |
| 0.1 (n = 4) | 106, 103, 105, 107 | 105 | 1.8 |
| M659F004 | 0.01 (n = 4) | 105, 103, 101, 103 | 103 | 1.3 | Acceptable |
| 0.1 (n = 4) | 100, 105, 105, 105 | 104 | 2.2 |

Table A 12: Characteristics for the method used for validation of ametoctradin and metabolites M650F003 & M650F004 residues in potato matrices

|  | Ametoctradin and metabolites M650F003 & M650F004 |
| --- | --- |
| Specificity | LC-MS/MS is a highly specific method. No signal interference was observed for the target analytes at their retention time in unfortified control samples. |
| Calibration (type, number of data points) | Calibration was performed with solvent based standards throughout with the exception of potato shoot samples for ametoctradin where matrix-match standards were employed. In each case, at least five concentrations ranging 0.025 to 5 ng/mL and good linearity was observed (r ≥0.99). The resulting test substance peak areas versus theoretical test substance concentration data were fit to the linear function.  Regression data:  Solvent standards  Amectoctradin:  Mass transition 276→ 176 m/z (quantification)  Slope = 3.78 x 106, Intercept = 1.62 x 104, r = 0.9998  M650F003:  Mass transition 222→ 176 m/z (quantification)  Slope = 2.33 x 106, Intercept = -188, r = 0.9998  Mass transition 222→ 121 m/z (confirmation) – used for quantification of untreated tuber samples  Slope = 1.46 x 105, Intercept = -408, r = 0.9990  M650F004:  Mass transition 208→ 190 m/z (quantification)  Slope = 3.29 x 106, Intercept = -9.18 x 103, r = 0.9997  Matrix-matched standards (ametoctradin potato shoot samples)  Amectoctradin:  Mass transition 276→ 176 m/z (quantification)  Slope = 3.05 x 106, Intercept = 4.1 x 104, r = 0.9985 |
| Calibration range | 0.025 ng/mL to 5 ng/mL for all analytes |
| Assessment of matrix effects is presented | No significant matrix effects observed (i.e. <20%) in all cases except potato shoot samples for ametoctradin. Calibration standards were therefore prepared in solvent but in matrix-matched solutions for ametoctradin potato shoot analyses. |
| Solution Stability | See XXXX Doc ID 2008/1022139 above for standard and and XXXX Doc ID 2010/1062330 for sample stability (at least 226 days in frozen (≤-18°C) conditions). |
| Limit of determination/quantification | The limit of quantification (LOQ) of 0.01 mg/kg was confirmed. The limit of detection (LOD) was 0.0025 mg/kg for ametoctradin and 0.001 mg/kg for both M650F003 and M650F004. |

Conclusion

The analytical procedure, method L0078/01, for the determination of residues of ametoctradin (BAS 650 F) and its metabolites M650F003 and M650F004 in potato matrices has been fully validated in terms of specificity, linearity, precision, accuracy, matrix effects, solution stability and LOQ, in accordance with the requirements of SANTE/2020/12830 rev.1.

* + - 1. Method L0078/01: Method for the determination of ametoctradin in onion
         1. Method validation

|  |  |
| --- | --- |
| Comments of zRMS: | The method has been accepted.  The method widely used within the section B7 of this submission for residue data generation in plant matrices. The final determination of Ametoctradin and its metabolites (M650F003, M650F004) in onion matrices was performed by LC-MS/MS which is highly sensitive and selective. The LOQ was set at 0.010 mg/kg for all analytes.  The mean recovery values were between the required range for all analytes. The relative standard deviations (RSD, %) for all fortification levels were ≤ 20 %.  Thus, it could be confirmed that the analytical method L0078/01 fulfils requirements of SANCO/3029/99 rev. 4. |

|  |  |
| --- | --- |
| Reference: | CP 5.1.2/7 |
| Report | Study on the residue behaviour of Propamocarb (Reg.No. 4628172) and Ametoctradin (BAS 650 F) in Onions after treatment with BAS 743 01 F under field conditions in Southern Europe in 2020  Schneider, E., 2021  report No 890084  XXXX DocID 2021/2019659  Authority registration No |
| Guideline(s): | EPA 860.1340; SANCO/3029/99 rev.4; OECD ENV/JM/MONO(2007)17 - Guidance Document on Pesticide Residue Analytical Methods |
| Deviations: | No |
| Previous evaluation: | No |
| GLP: | Yes |
| Acceptability: | Yes | |

**Study Summary**

The method L0078/01 was validated for the determination of residues of Ametoctradin (BAS 650 F) and its metabolites M650F003 and M650F004 in onion (bulbs and whole plant (no roots)), with a limit of quantification at 0.01 mg/kg. The brief description of method and the results are presented in the summary below.

Materials and methods

The method is that described in XXXX Doc ID 2008/1022139 above, with the exception that the injection volume was decreased from 50 µL to 30 µL to reduce matrix effects.

Results and discussions

The analytical method validation is summarised below. The HPLC-MS/MS determination was conducted by monitoring two MS/MS mass transitions and therefore no confirmatory method is required.

Procedural recoveries were obtained by fortification of the matrices and all mean recoveries are within the permitted range required by the guideline SANTE/2020/12830 rev. 1. While two mass transitions were analysed, only the ones for quantification are reported.

Table A 13: Recovery results from method validation of ametoctradin and metabolites M650F003 & M650F004 in onion matrices using the analytical method

| Matrix | Analyte | Fortification level (mg/kg) (n = x) | Recovery (%) | Mean  recovery (%) | RSD (%) | Comments |
| --- | --- | --- | --- | --- | --- | --- |
| ***Mass transition (Quantification)  Ametoctradin 276→176 m/z, M650F003 222→176 m/z, M650F004 208→190 m/z*** | | | | | | |
| Onion (bulbs) | Ametoctradin | 0.01 (n = 4) | 103, 99.6, 100, 98.8 | 101 | 2.0 | Acceptable |
| 0.1 (n = 5) | 89.6, 90.3, 90.9, 103, 101 | 95.0 | 6.8 |
| M650F003 | 0.01 (n = 4) | 107, 109, 107, 106 | 107 | 0.9 | Acceptable |
| 0.1 (n = 5) | 109, 108, 108, 108, 108 | 108 | 0.4 |
| M659F004 | 0.01 (n = 4) | 101, 103, 106, 96.0 | 101 | 4.2 | Acceptable |
| 0.1 (n = 5) | 103, 102, 101, 99.0, 99.2 | 101 | 1.7 |
| Onion (whole plant, no roots) | Ametoctradin | 0.01 (n = 3) | 82.4, 92.3, 87 | 87.2 | 5.7 | Acceptable |
| 0.1 (n = 3) | 88.3, 91.8, 84.5 | 88.2 | 4.2 |
| 10 (n = 1) | 109 | 109 | - |
| M650F003 | 0.01 (n = 3) | 105, 108, 104 | 106 | 1.9 | Acceptable |
| 0.1 (n = 3) | 107, 107, 110 | 108 | 1.3 |
| M659F004 | 0.01 (n = 3) | 95.5, 97.8, 94.5 | 96.0 | 1.8 | Acceptable |
| 0.1 (n = 3) | 101, 96.1, 101 | 99.4 | 3.0 |

Table A 14: Characteristics for the method used for validation of ametoctradin and metabolites M650F003 & M650F004 residues in onion matrices

|  | Ametoctradin and metabolites M650F003 & M650F004 |
| --- | --- |
| Specificity | LC-MS/MS is a highly specific method. No signal interference was observed for the target analytes at their retention time in unfortified control samples. |
| Calibration (type, number of data points) | Calibration was performed with solvent based standards using at least five concentrations ranging 0.025 to 5 ng/mL and good linearity was observed (r ≥0.99). The resulting test substance peak areas versus theoretical test substance concentration data were fit to the linear function.  Regression data:  Amectoctradin:  Mass transition 276→ 176 m/z (quantification)  Slope = 4.82 x 106, Intercept = 1.89 x 104, r = 0.9998  M650F003:  Mass transition 222→ 176 m/z (quantification)  Slope = 2.47 x 106, Intercept = -9.4 x 103, r = 0.9993  M650F004:  Mass transition 208→ 190 m/z (quantification)  Slope = 3.81 x 106, Intercept = -5.73 x 103, r = 0.9999 |
| Calibration range | 0.025 ng/mL to 5 ng/mL |
| Assessment of matrix effects is presented | No significant matrix effects observed (i.e. <20%) in all cases thus calibration standards were prepared in solvent throughout. |
| Solution Stability | See XXXX Doc ID 2008/1022139 above for standard and and XXXX Doc ID 2010/1062330 for sample stability (at least 196 days in frozen (≤-18°C) conditions). |
| Limit of determination/quantification | The limit of quantification (LOQ) of 0.01 mg/kg was confirmed. The limit of detection (LOD) was 0.0025 mg/kg for ametoctradin and 0.001 mg/kg for both M650F003 and M650F004. |

Conclusion

The analytical procedure, method L0078/01, for the determination of residues of ametoctradin (BAS 650 F) and its metabolites M650F003 and M650F004 in onion matrices has been fully validated in terms of specificity, linearity, precision, accuracy, matrix effects, solution stability and LOQ, in accordance with the requirements of SANTE/2020/12830 rev.1.

* + - * 1. Method validation

|  |  |
| --- | --- |
| Comments of zRMS: | The method validation has been accepted.  The objective of this study was to validate the determination of BAS 650 F and its metabolite M650F003 and M650F004 in plant matrices with XXXX method no. L0078/02 according to the current guidelines.  Quantification of the analytes was achieved by LC-MS/MS, monitoring 2 mass transitions per analyte, one proposed for quantification and one for qualification.  The method was validated at 2 fortification levels (0.010 mg/kg and 0.10 mg/kg) for lettuce head, grapes fruit, wheat grain and soybean seed. For each matrix at each fortification level at least 5 replicates and 2 untreated control samples, as well as 1 reagent blank were analyzed. The following mass transitions were used for the analysis: for BAS 650 F m/z 276 → 176 proposed for quantification and m/z 276 → 149 proposed for qualification; for M650F003 m/z 222 → 176 proposed for quantification and m/z 222 → 121 proposed for qualification; for M650F004 m/z 208 → 190 proposed for quantification and m/z 208 → 123 proposed for qualification. The LC-MS/MS is highly selective and sensitive method. 2 characteristic mass transitions were monitored and therefore, no further confirmatory method is required.  Solvent and matrix-matched standards were analysed to assess potential matrix effects. As significant matrix effects on LC-MS/MS response were identified for all analytes, matrix-matched calibration standards were used for the evaluation of the results.  The LOQ of the analytical method was 0.010 mg/kg. The LOD of the method was defined as the lowest standard concentration injected. The LOD was 0.0020 mg/kg for all analytes and matrices. The interferences of the analyte measured in the control samples were below 30 % of the LOQ for all matrices at each mass transition. Correlation coefficients (r 2) were always ≥ 0.9919.  Stability of stock solutions, fortification solutions and calibration solutions was proven in another study. The stability of final sample volumes is considered sufficiently proven for these analytes for at least 7 days under refrigerated storage conditions. No residues were detected in the reagent blank showing that no contamination was present.  The mean recovery values were between 76.7 % and 107 % for all fortification level, analytes, and mass transitions. The relative standard deviations (RSDs, %) for all fortification levels were ≤ 8.7 %.  XXXX method no. L0078/02 was proven to be suitable to determine residues of Ametoctradin, M650F003, and M650F004 in lettuce head, grapes fruit, wheat grain and soybean seed at a LOQ of 0.010 mg/kg consistently with the requirements of SANTE/2020/12830 Rev.1. |

|  |  |
| --- | --- |
| Reference: | CP 5.1.2/8 |
| Report | Validation of analytical method L0078/02 for the analysis of BAS 650 F and its metabolites M650F003 and M650F004 in plant matrices  Xiaodan, Du., 2022  report No 919338  XXXX DocID 2022/2027748  Authority registration No |
| Guideline(s): | SANTE/2020/12830 Rev.1; OECD ENV/JM/MONO(2007)17; OPPTS 860.1340 |
| Deviations: | None |
| Previous evaluation: | No |
| GLP: | Yes |
| Acceptability: | Yes | |

**Study Summary**

The method L0078/02 was developed and validated for the determination of residues of Ametoctradin (BAS 650 F) and its metabolites M650F003 and M650F004 in lettuce (head), grapes (fruit), wheat (grain) and soybean (seed) with a limit of quantification at 0.01 mg/kg. The brief description of method and the results are presented in the summary below.

**Materials and methods**

In method L0078/02, residues of Ametoctradin (BAS 650 F) and its metabolites M650F003 and M650F004 are extracted from samples (5g) of plant matrices using a mixture of methanol-water (50:50, v/v, 100 mL). Samples are homogenised using a Polytron for about 3 minutes at approxuimately 10000 rpm. An aliquot of the extract (10 mL) is transferred to a culture tube and centrifuged for about 3 minutes at approximately 4000 rpm.

For lettuce (head), grapes (fruit) and wheat (grain), an aliquot (0.5 mL) of the extract is transferred to an autosample vial together with 0.2% formic acid (0.5 mL) and mixed well (final volume = 1 mL).

For soybean (seed), an aliquot (0.5 mL) of the extract is transferred to an Eppendorf tube and 0.2% formic acid (0.5 mL) is added. The solution is mixed well (final volume 1 mL). The sample is centrifuged for about 5 minutes at approximately 14000 rpm before 800 µL is transferred to an autosample vial.

Ametoctradin (BAS 650 F), M650F003 and M650F004 are determined by HPLC-MS/MS. Separation is achieved by using an Phenomenex, Synergi Fusion-RP column (50 mm x 2 mm, 4µm) and a gradient of water (0.1% formic acid)/ methanol (0.1% formic acid) at a flow rate of 0.7 mL/min. Detection is accomplished in ESI positive mode using two different transitions. For parent, Ametoctradin (BAS 650 F), mass transitions at 276 m/z > 176 m/z and 276 m/z > 149 m/z is used for quantification and confirmation, respectively. For metabolite, M650F003, mass transitions at 222 m/z > 176 m/z and 222 m/z > 121 m/z is used for quantification and confirmation, respectively. For metabolite, M650F004, mass transitions at 208 m/z > 190 m/z and 208 m/z > 123 m/z is used for quantification and confirmation, respectively External matrix-matched calibration standards were used throughout.

Results and discussions

The analytical method validation is summarised below. The HPLC-MS/MS determination was conducted by monitoring two MS/MS mass transitions and therefore no confirmatory method is required.

Recoveries were obtained by fortification of the matrices and all mean recoveries are within the permitted range required by the guideline SANTE/2020/12830 rev. 1.

Table A 15: Recovery results from method validation of ametoctradin using the analytical method

| Matrix | Analyte | Fortification level (mg/kg) (n = x) | Mean  recovery (%) | RSD (%) | Comments |
| --- | --- | --- | --- | --- | --- |
| ***Mass transition (Quantification)  Ametoctradin 276→176 m/z, M650F003 222→176 m/z, M650F004 208→170 m/z*** | | | | | |
| Lettuce (head) | Ametoctradin | 0.01 (n = 5) | 89.3 | 2.9 | Acceptable |
| 0.1 (n = 5) | 91.0 | 1.3 |
| M650F003 | 0.01 (n = 5) | 95.6 | 0.45 | Acceptable |
| 0.1 (n = 5) | 94.2 | 1.3 |
| M659F004 | 0.01 (n = 5) | 93.2 | 1.7 | Acceptable |
| 0.1 (n = 5) | 94.2 | 0.85 |
| Grapes (fruit) | Ametoctradin | 0.01 (n = 5) | 93.4 | 2.8 | Acceptable |
| 0.1 (n = 5) | 97.5 | 0.75 |
| M650F003 | 0.01 (n = 5) | 99.2 | 1.4 | Acceptable |
| 0.1 (n = 5) | 101 | 0.55 |
| M659F004 | 0.01 (n = 5) | 99.7 | 1.5 | Acceptable |
| 0.1 (n = 5) | 98.0 | 0.48 |
| Wheat (grain) | Ametoctradin | 0.01 (n = 5) | 92.5 | 1.7 | Acceptable |
| 0.1 (n = 5) | 92.2 | 2.5 |
| M650F003 | 0.01 (n = 5) | 99.3 | 3.0 | Acceptable |
| 0.1 (n = 5) | 94.8 | 1.3 |
| M659F004 | 0.01 (n = 5) | 101 | 2.8 | Acceptable |
| 0.1 (n = 5) | 96.1 | 1.8 |
| Soybean (seed) | Ametoctradin | 0.01 (n = 5) | 82.7 | 8.9 | Acceptable |
| 0.1 (n = 5) | 74.1 | 2.1 |
| M650F003 | 0.01 (n = 5) | 101 | 1.3 | Acceptable |
| 0.1 (n = 5) | 96.3 | 0.76 |
| M659F004 | 0.01 (n = 5) | 111 | 7.1 | Acceptable |
| 0.1 (n = 5) | 102 | 0.46 |
| ***Mass transition (Confirmation) Ametoctradin 276→149 m/z, M650F003 222→121 m/z, M650F004 208→123 m/z*** | | | | | |
| Lettuce (head) | Ametoctradin | 0.01 (n = 5) | 86.0 | 1.9 | Acceptable |
| 0.1 (n = 5) | 83.4 | 1.7 |
| M650F003 | 0.01 (n = 5) | 96.2 | 0.87 | Acceptable |
| 0.1 (n = 5) | 92.0 | 1.3 |
| M659F004 | 0.01 (n = 5) | 93.7 | 1.8 | Acceptable |
| 0.1 (n = 5) | 92.3 | 1.0 |
| Grapes (fruit) | Ametoctradin | 0.01 (n = 5) | 81.0 | 3.7 | Acceptable |
| 0.1 (n = 5) | 87.5 | 1.5 |
| M650F003 | 0.01 (n = 5) | 98.1 | 0.75 | Acceptable |
| 0.1 (n = 5) | 97.3 | 0.27 |
| M659F004 | 0.01 (n = 5) | 101 | 1.0 | Acceptable |
| 0.1 (n = 5) | 97.1 | 0.82 |
| Wheat (grain) | Ametoctradin | 0.01 (n = 5) | 89.5 | 2.3 | Acceptable |
| 0.1 (n = 5) | 91.6 | 1.9 |
| M650F003 | 0.01 (n = 5) | 101 | 2.1 | Acceptable |
| 0.1 (n = 5) | 96.1 | 1.2 |
| M659F004 | 0.01 (n = 5) | 101 | 1.7 | Acceptable |
| 0.1 (n = 5) | 96.3 | 0.90 |
| Soybean (seed) | Ametoctradin | 0.01 (n = 5) | 80.8 | 7.4 | Acceptable |
| 0.1 (n = 5) | 72.7 | 1.9 |
| M650F003 | 0.01 (n = 5) | 98.8 | 4.3 | Acceptable |
| 0.1 (n = 5) | 95.8 | 1.6 |
| M659F004 | 0.01 (n = 5) | 101 | 1.1 | Acceptable |
| 0.1 (n = 5) | 102 | 0.75 |

Table A 16: Characteristics for the method used for validation of ametoctradin residues in plant matrices

|  | Ametoctradin |
| --- | --- |
| Specificity | LC-MS/MS is a highly specific method. No interference (> 30 % LOQ) of total peak area for the target analyte at the retention time, was found in unfortified control samples. |
| Calibration (type, number of data points) | Calibration was performed with matrix-matched standards at a minimum of five concentrations ranging 0.050 to 5.0 ng/mL (0.0020 to 0.20 mg/kg at sample level) and good linearity was observed (r ≥0.99). The resulting test substance peak areas versus theoretical test substance concentration data were fit to the linear function.  Regression data:  **Lettuce (head)**   |  |  |  |  |  | | --- | --- | --- | --- | --- | | Analyte | m/z | Slope | Intercept | r | | Ametoctradin | 276→ 176 | 4.93463 x 105 | -7260.50275 | 0.99834 | | 276→ 149 | 5.20252 x 105 | -15620.27353 | 0.99595 | | M650F003 | 222→ 176 | 5.08106 x 106 | -12907.53223 | 0.99979 | | 222→ 121 | 7.59739 x 105 | -2894.89077 | 0.99973 | | M650F004 | 208→ 190 | 3.38984 x 106 | -27005.80018 | 0.99964 | | 208→ 123 | 1.89808 x 106 | -17181.97006 | 0.99956 |   **Grapes (fruit)**   |  |  |  |  |  | | --- | --- | --- | --- | --- | | Analyte | m/z | Slope | Intercept | r | | Ametoctradin | 276→ 176 | 4.63266 x 105 | 5098.39138 | 0.99839 | | 276→ 149 | 5.18137 x 105 | -6888.49111 | 0.99710 | | M650F003 | 222→ 176 | 6.13314 x 106 | -3.33988 x 104 | 0.99987 | | 222→ 121 | 9.64205 x 105 | -9.122.39779 | 0.99915 | | M650F004 | 208→ 190 | 2.86528 x 106 | -12986.98009 | 0.99960 | | 208→ 123 | 1.59848 x 106 | -3364.73272 | 0.99941 |   **Wheat (grain)**   |  |  |  |  |  | | --- | --- | --- | --- | --- | | Analyte | m/z | Slope | Intercept | r | | Ametoctradin | 276→ 176 | 5.96561 x 105 | 7092.25339 | 0.99957 | | 276→ 149 | 5.88417 x 105 | 4428.03963 | 0.99949 | | M650F003 | 222→ 176 | 1.18025 x 106 | -10392.78668 | 0.99983 | | 222→ 121 | 2.07911 x 105 | -2489.63105 | 0.99958 | | M650F004 | 208→ 190 | 1.30964 x 106 | -18418.29149 | 0.99971 | | 208→ 123 | 6.70174 x 105 | -3486.69324 | 0.99960 |   **Soybean (seed)**   |  |  |  |  |  | | --- | --- | --- | --- | --- | | Analyte | m/z | Slope | Intercept | r | | Ametoctradin | 276→ 176 | 7.31324 x 105 | 25.36257 | 0.99963 | | 276→ 149 | 7.30724 x 105 | -4123.83016 | 0.99920 | | M650F003 | 222→ 176 | 1.15707 x 106 | 2790.08024 | 0.99992 | | 222→ 121 | 2.02386 x 105 | -2163.09419 | 0.99952 | | M650F004 | 208→ 190 | 1.14186 x 106 | -22784.71690 | 0.99973 | | 208→ 123 | 5.71898 x 105 | -4904.60888 | 0.99996 | |
| Calibration range | Accepted calibration range 0.050 to 5.0 ng/mL (0.0020 to 0.20 mg/kg at sample level) |
| Assessment of matrix effects is presented | Matrix effects were significant (i.e. >20%) for all analytes in most matrices and thus matrix matched standards were used throughout. |
| Solution Stability | Standard solution stability was not performed in this study. Please refer to XXXX Doc ID: 2008/1022139.  Raw extracts and final volumes were demonstrated to be stable for at least 7 days under refrigerated conditions. |
| Limit of determination/quantification | The limit of quantification (LOQ) of 0.01 mg/kg was confirmed. The limit of detection (LOD), defined as the lowest standard employed was 0.05 ng/mL (=0.002 mg/kg). |

Conclusion

The analytical procedure, method L0078/02, for the determination of residues of ametoctradin (BAS 650 F) and its metabolites M650F003 and M650F004 in plant matrices (lettuce (head), grapes (fruit), wheat (grain) and soybean (seed)) has been fully validated in terms of specificity, linearity, precision, accuracy, matrix effects, solution stability and LOQ, in accordance with the requirements of SANTE/2020/12830 rev.1.

* + - 1. Method L0078/02: Method for the determination of ametoctradin in cucumber & zucchini
         1. Method validation

|  |  |
| --- | --- |
| Comments of zRMS: | The method has been accepted.  For the clarity it is stated here that in this study XXXX method no. L0078/02 was used in the analytical phase of the study to determine the residues of ametoctradin, M650F003 and M650F004, but still XXXX method no. L0450/01 was used to determine the residues of Propamocarb (see the relevant part of the Appendix 2).  The full validation of the analytical method L0078/02 was reported in XXXX DocID 2022/2027748 (see previous study).  The final determination of ametoctradin, M650F003 and M650F004 in cucumber and zucchini matrices was performed by LC with tandem MS (LC-MS/MS) which is highly sensitive and selective. The ionization source employed was Electro Turbo Spray IonDrive (ESI). 2 transitions were monitored.  The LOQ of the method is 0.010 mg/kg for BAS 650 F, 0.012 mg/kg for M650F003 (expressed as parent equivalent) and 0.013 mg/kg for M650F004 (expressed as parent equivalent). Matrix-matched standards were used for the determination of BAS 650 F, M650F003 and M650F004 in cucumber and zucchini.  The mean recovery values were within the acceptable range of 70 % and 120 %, with a relative standard deviation (RSD) ≤ 20 %. No residues ≥ 0.010 mg/kg (LOQ) were found in any of the untreated control specimens. The results prove that no interferences of the samples material with the analytical procedure occurred. Thus, it could be confirmed that the analytical method L0078/02 fulfils requirements of SANTE/2020/12830 rev.1. |

|  |  |
| --- | --- |
| Reference: | CP 5.1.2/9 |
| Report | Study on the residue behaviour of Propamocarb (Reg.No. 4628172) and Ametoctradin (BAS 650 F) in cucumber and zucchini after two applications of BAS 743 01 F under greenhouse conditions in Northern and Southern Europe in 2021  Schneider, E., 2023  report No 890095  XXXX DocID 2022/2041754  Authority registration No |
| Guideline(s): | OECD ENV/JM/MONO(2007)17 - Guidance Document on Pesticide Residue Analytical Methods; SANTE/2020/12830 Rev.1 |
| Deviations: | None |
| Previous evaluation: | No |
| GLP: | Yes |
| Acceptability: | Yes | |

**Study Summary**

The method L0078/02 was validated for the determination of residues of Ametoctradin (BAS 650 F) and its metabolites M650F003 and M650F004 in cucumber and zucchini with a limit of quantification at 0.01 mg/kg. The brief description of method and the results are presented in the summary below.

Materials and methods

The method is that described in XXXX Doc ID 2022/2027748 for the analysis of ametoctradin.

Results and discussions

The analytical method validation is summarised below. The HPLC-MS/MS determination was conducted by monitoring two MS/MS mass transitions and therefore no confirmatory method is required.

Procedural recoveries were obtained by fortification of the matrices and all mean recoveries are within the permitted range required by the guideline SANTE/2020/12830 rev. 1., with the exception of two single determinations of M659F004 at the 10 mg/kg fortification which exceeded the upper guideline limits (>110 % recovery). While two mass transitions were analysed, only the ones for quantification are reported.

Table A 17: Recovery results from method validation of ametoctradin in cucumber and zucchini using the analytical method

| Matrix | Analyte | Fortification level (mg/kg) (n = x) | Recovery (%) | Mean  recovery (%) | RSD (%) | Comments |
| --- | --- | --- | --- | --- | --- | --- |
| ***Mass transition (Quantification)  Ametoctradin 276→176 m/z, M650F003 222→176 m/z, M650F004 208→190 m/z*** | | | | | | |
| Cucumber (fruit) | Ametoctradin | 0.01 (n = 4) | 90.9, 100, 93.7, 101 | 96.4 | 4.4 | Acceptable |
| 0.1 (n = 4) | 86.9, 91.9, 85.0, 88.4 | 88.1 | 2.9 |
| 10 (n = 1) | 97.2 | 97.2 | - |
| M650F003 | 0.01 (n = 4) | 94.9, 98.9, 90.2, 89.4 | 93.4 | 4.1 | Acceptable |
| 0.1 (n = 4) | 92.4, 94.8, 88.7, 89.6 | 91.4 | 2.6 |
| 10 (n = 1) | 100 | 100 | - |
| M659F004 | 0.01 (n = 4) | 94.2. 99.2. 92.0. 91.4 | 94.2 | 3.3 | Acceptable |
| 0.1 (n = 4) | 94.4. 92.8. 88.3. 87.7 | 90.8 | 3.2 |
| 10 (n = 1) | *137\** | 137 | - |
| Zucchini (fruit) | Ametoctradin | 0.01 (n = 4) | 94.9, 97.9, 87.5, 92.4 | 93.2 | 4.1 | Acceptable |
| 0.1 (n = 4) | 89.5, 92.2, 93.0, 88.1 | 90.7 | 2.2 |
| 10 (n = 1) | 97.2 | 97.2 | - |
| M650F003 | 0.01 (n = 4) | 93.2, 92.6, 96.6, 86.2 | 92.2 | 4.1 | Acceptable |
| 0.1 (n = 4) | 93.9, 94.8, 91.1, 90.3 | 92.5 | 2.0 |
| 10 (n = 1) | 98.6 | 98.6 | - |
| M659F004 | 0.01 (n = 4) | 95.7, 95.1, 91.1, 88.8 | 92.7 | 3.1 | Acceptable |
| 0.1 (n = 4) | 96.0, 92.2, 92.0, 91.8 | 93.0 | 1.9 |
| 10 (n = 1) | *138\** | 138 | - |

\* Outside guideline requirements (70-110%).

Table A 18: Characteristics for the method used for validation of ametoctradin residues in plant matrices

|  | Ametoctradin |
| --- | --- |
| Specificity | LC-MS/MS is a highly specific method. No interference (> 30 % LOQ) of total peak area for the target analyte at the retention time, was found in unfortified control samples. |
| Calibration (type, number of data points) | Calibration was performed with matrix-matched standards with at least seven concentrations ranging 0.05 to 5 ng/mLand good linearity was observed (r ≥0.99). The resulting test substance peak areas versus theoretical test substance concentration data were fit to the linear function.  Regression data:  **Cucumber**   |  |  |  |  |  | | --- | --- | --- | --- | --- | | Analyte | m/z | Slope | Intercept | r | | Ametoctradin | 276→ 176 | 2.62930 x 105 | 101.67037 | 0.99794 | | M650F003 | 222→ 176 | 3.27042 x 106 | -26660.07395 | 0.99924 | | M650F004 | 208→ 190 | 3.13078 x 106 | -19501.57651 | 0.99894 |   **Zucchini**   |  |  |  |  |  | | --- | --- | --- | --- | --- | | Analyte | m/z | Slope | Intercept | r | | Ametoctradin | 276→ 176 | 2.14086 x 105 | -1188.36091 | 0.99721 | | M650F003 | 222→ 176 | 3.08833 x 106 | -18601.03137 | 0.99946 | | M650F004 | 208→ 190 | 3.70007 x 106 | -3.66324 x 104 | 0.99864 | |
| Calibration range | 0.050 ng/mL to 5 ng/mL |
| Assessment of matrix effects is presented | No assessed, however matrix-matched calibration standards were used throughout. |
| Solution Stability | See XXXX Doc ID 2008/1022139 above for standard stability.  Please refer to XXXX Doc ID 2022/2006182 for extract stability data. |
| Limit of determination/quantification | The limit of quantification (LOQ) of 0.01 mg/kg was confirmed. The limit of detection (LOD) was 0.0020 mg/kg for ametoctradin, 0.0025 mg/kg for M650F003 & 0.0027 mg/kg for M650F004). |

Conclusion

The analytical procedure, method L0078/02, for the determination of residues of ametoctradin (BAS 650 F) and its metabolites M650F003 and M650F004 in cucumber and zucchini has been fully validated in terms of specificity, linearity, precision, accuracy, solution stability and LOQ, in accordance with the requirements of SANTE/2020/12830 rev.1.

* + - 1. Method L0078/02: Method for the determination of ametoctradin in tomato
         1. Method validation

|  |  |
| --- | --- |
| Comments of zRMS: | The method has been accepted.  The full validation of the analytical method L0078/02 was reported in XXXX DocID 2022/2027748 (see on previous pages).  The final determination of residues in tomato matrices was performed by LC with tandem MS (LC-MS/MS) which is highly sensitive and selective. The ionization source employed was Electro Turbo Spray IonDrive (ESI). 2 transitions were monitored.  The LOQ of the method is 0.010 mg/kg for ametoctradin, 0.012 mg/kg for M650F003 (expressed as parent equivalent) and 0.013 mg/kg for M650F004 (expressed as parent equivalent). Matrix-matched standards were used for the determination of ametoctradin, M650F003 and M650F004 in tomato.  The mean recovery values were within the acceptable range of 70 % and 120 %, with a relative standard deviation (RSD) ≤ 20 %. No residues ≥ LOQ were found in any of the untreated control samples. The results prove that no interferences of the samples material with the analytical procedure occurred. Thus, it could be confirmed that the analytical method L0078/02 fulfils requirements of SANTE/2020/12830 rev.1.  *For the technical clarity it is stated here that in this study XXXX method no. L0078/02 was used in the analytical phase of the study to determine the residues of ametoctradin, M650F003 and M650F004, but in the same study still XXXX method no. L0450/01 was used to determine the residues of propamocarb. However, propamocarb determination in tomato matrices with the method L0450/01 from this study is not discussed within the present Appendix 2. The reason is that the method L0450/01 was fully validated for the determination of residues of propamocarb (BAS 9068F) inter alia in tomato (fruit) in study under XXXX DocID 2022/2032351 (see in the present Appendix 2), and then applied in the field residue study XXXX DocID 2020/2103085 also commented in propamocarb part of the present Appendix 2.* |

|  |  |
| --- | --- |
| Reference: | CP 5.1.2/10 |
| Report | Study on the residue behaviour of Propamocarb (Reg.No. 4628172) and Ametoctradin (BAS 650 F) in tomato after two applications of BAS 743 01 F under greenhouse conditions in Northern and Southern Europe in 2021  Martin, T., 2022  report No 890074  XXXX DocID 2021/2054075  Authority registration No |
| Guideline(s): | OECD ENV/JM/MONO(2007)17; SANTE/2020/12830 Rev.1 |
| Deviations: | None |
| Previous evaluation: | No |
| GLP: | Yes |
| Acceptability: | Yes | |

**Study Summary**

The method L0078/02 was validated for the determination of residues of Ametoctradin (BAS 650 F) and its metabolites M650F003 and M650F004 in tomato with a limit of quantification at 0.01 mg/kg. The brief description of method and the results are presented in the summary below.

Materials and methods

The method is that described in XXXX Doc ID 2022/2041754 above for the analysis of ametoctradin.

Results and discussions

The analytical method validation is summarised below. The HPLC-MS/MS determination was conducted by monitoring two MS/MS mass transitions and therefore no confirmatory method is required.

Procedural recoveries were obtained by fortification of the matrices and all mean recoveries are within the permitted range required by the guideline SANTE/2020/12830 rev. 1. While two mass transitions were analysed, only the ones for quantification are reported.

Table A 19: Recovery results from method validation of ametoctradin in tomato using the analytical method

| Matrix | Analyte | Fortification level (mg/kg) (n = x) | Recovery (%) | Mean  recovery (%) | RSD (%) | Comments |
| --- | --- | --- | --- | --- | --- | --- |
| ***Mass transition (Quantification)  Ametoctradin 276→176 m/z, M650F003 222→176 m/z, M650F004 208→190 m/z*** | | | | | | |
| Tomato (fruit) | Ametoctradin | 0.01 (n = 6) | 71.0, 72.7, 90.5, 89.6, 89.2, 89.0 | 83.7 | 10 | Acceptable |
| 0.1 (n = 6) | 85.9, 90.8, 88.5, 92.7, 88.3, 84.1 | 88.4 | 3.2 |
| 10 (n = 2) | 103, 90.0 | 96.5 | - |
| M650F003 | 0.01 (n = 6) | 73.2, 73.8, 92.2, 91.1, 91.2, 92.7 | 85.7 | 10 | Acceptable |
| 0.1 (n = 6) | 92.2, 92.4, 92.0, 92.3, 91.0, 86.4 | 91.0 | 2.3 |
| 10 (n = 2) | 102, 89.1 | 95.7 | - |
| M659F004 | 0.01 (n = 6) | 74.2, 74.9, 94.4, 95.6, 91.2, 95.5 | 87.6 | 11 | Acceptable |
| 0.1 (n = 6) | 93.6, 94.1, 92.8, 95.4, 93.0, 86.9 | 92.6 | 2.9 |

Table A 20: Characteristics for the method used for validation of ametoctradin residues in tomato

|  | Ametoctradin |
| --- | --- |
| Specificity | LC-MS/MS is a highly specific method. No interference (> 30 % LOQ) of total peak area for the target analyte at the retention time, was found in unfortified control samples. |
| Calibration (type, number of data points) | Calibration was performed with matrix-matched standards with at least seven concentrations ranging 0.05 to 5 ng/mLand good linearity was observed (r ≥0.99). The resulting test substance peak areas versus test substance concentration data were fit to the linear function.  Regression data:  Tomato   |  |  |  |  |  | | --- | --- | --- | --- | --- | | Analyte | m/z | Slope | Intercept | r | | Ametoctradin | 276→ 176 | 2.21733 x 105 | 4226.29180 | 0.99943 | | M650F003 | 222→ 176 | 3.05425 x 106 | 5093.37583 | 0.99980 | | M650F004 | 208→ 190 | 3.44636 x 106 | 3.71488 x 104 | 0.99984 | |
| Calibration range | 0.050 ng/mL to 5 ng/mL |
| Assessment of matrix effects is presented | No assessed, however matrix-matched calibration standards were used throughout. |
| Solution Stability | See XXXX Doc ID 2008/1022139 above for standard stability.  Please refer to XXXX Doc ID 2022/2006182 for extract stability data. |
| Limit of determination/quantification | The limit of quantification (LOQ) of 0.01 mg/kg was confirmed. The limit of detection (LOD) was 0.0020 mg/kg for ametoctradin, 0.0025 mg/kg for M650F003 & 0.0027 mg/kg for M650F004). |

Conclusion

The analytical procedure, method L0078/02, for the determination of residues of ametoctradin (BAS 650 F) and its metabolites M650F003 and M650F004 in tomato has been fully validated in terms of specificity, linearity, precision, accuracy, solution stability and LOQ, in accordance with the requirements of SANTE/2020/12830 rev.1.

* + - 1. Methods for the determination of ametoctradin in support of toxicological studies

None

* + - * 1. ~~Method validation~~

|  |  |
| --- | --- |
| ~~Comments of zRMS:~~ | ~~The procedure could not be accepted with no explanation.~~  ~~The title of the study is unclear. In conclusion below ametoctradin is stated.~~  ~~The aim of this study was the validation of a quantitative HPLC-UV method for BAS 743 02 F formulation (the test item) containing propamocarb (431.0 g/L) and ametoctradin (137.7 g/L) in the medium (vehicle, matrix) of acetonitrile / 10mM disodium hydrogen phosphate dihydrate with phosphoric acid (85%) pH 2.9 (50+50, V/V).~~  ~~However, the method and its usefulness need explanation of the applicant. It is unclear what is the basis of the below study description by the applicant.~~  ~~Based on the study it seems that the method should not be reported as a method for determining propamocarb and/or ametoctradin. The test item is a mixture of chemical compounds. The LOQ of the method was defined as follows: the LOQ was assessed and confirmed at a concentration of 1 mg/100 mL for active ingredient 1 and 0.2 mg/100 mL for active ingredient 2 in the vehicle with sufficient results for accuracy and precision (correlating to a concentration of 0.1 mg/100 mL for active ingredient 1 and 0.2 mg/100 mL for active ingredient 2 in the final sample extract).~~  ~~So, the evaluated study operates with the active ingredient 1 and active ingredient 2 as analytes being determined. In the study there is no clear explanation of what they are. While the applicant in the study description uses the names ametoctradin and propamocarb that are not included in the text of the study (except a place with information about the formulation content) and they are not the same as “active ingredients”. Therefore, it is unclear what the applicant means.~~  ~~Moreover, according to the applicant data provided in the present section the formulation BAS 743 02 F contains 515.4 g/L propamocarb and 137.1 g/L ametoctradin.~~  ~~Apart from above the procedure within the study is described correctly e.g.: The method was validated at 3 fortification levels for each active ingredient with 5 fortification samples at each level. 2 independently prepared blank medium extract with no samples were analyzed as controls. Detection of the test item was performed at 195 nm for active ingredient 1 and 294 nm for active ingredient 2.~~  ~~(UV 195 nm and 294 nm cannot be the only source for identifying analytes).~~ |

|  |  |
| --- | --- |
| ~~Reference:~~ | ~~CP 5.1.2/11~~ |
| ~~Report~~ | ~~Validation of an analytical method for the analysis in acetonitrile/10 mM disodium hydrogen phosphate dihydrate with phosphoric acid (85%) pH 2.9 (50+50, V/V) using HPLC-UV~~  ~~Control procedure: 21/0288\_01~~  ~~Wagner, I.., 2022~~  ~~XXXX DocID 2022/2034983~~  ~~Authority registration No~~ |
| ~~Guideline(s):~~ | ~~SANTE/2020/12830 Rev.1~~ |
| ~~Deviations:~~ | ~~None which affect quality~~ |
| ~~Previous evaluation:~~ | ~~No~~ |
| ~~GLP:~~ | ~~Yes~~ |
| ~~Acceptability:~~ | ~~Yes~~ | |

**~~Study Summary~~**

~~Control procedure 21/0288\_01 was validated for the determination of Ametoctradin and Propamocab formulated in BAS 743 02 F in the vehicle acetonitrile / 10mM disodium hydrogen phosphate dihydrate with phosphoric acid (85%) pH 2.9 (50+50, v/v) to demonstrate the suitability and correctness of the method in the vehicle.~~

**~~Materials and methods~~**

~~In control procedure 21/0288\_01, the sample solutions for Ametoctradin analysis are used directly without any further sample preparation. Samples are diluted into the calibration range with blank vehicle, if necessary, and centrifuged at 13000 rpm for 5 minutes. Samples are analysed by high performance liquid chromatography with ultra-violet detection (HPLC-UV) at 294 nm (reference wavelength of 420 nm for diode-array) using an Xbridge Shield RP 18 column (100 mm x 4.6 mm, 3.5 µm) and isocratic elution with mobile phases of acetonitrile:water (95:5) and 10 mM disodium hydrogen phosphate dihydrate adjusted to pH 2.8-2.9 with phosphoric acid (85%) in a ratio of 65:35 v/v respectively, at a flow rate of 1.5 mL/min. Calibration is by external matrix-matched standards.~~

~~Results and discussions~~

~~The analytical method validation is summarised below. No confirmatory method is required for methods for risk-assessment according to SANTE/2020/12830 Rev.1.~~

~~Recoveries were obtained by fortification of blank vehicle at three fortification levels. All mean recoveries are within the permitted range required by the guideline SANTE/2020/12830 rev. 1.~~

~~Table A 21: Recovery results from method validation of ametoctradin using the analytical method~~

| ~~Matrix~~ | ~~Analyte~~ | ~~Nominal fortification level (mg/100 mL) (n = 5)~~ | ~~Mean  recovery (%)~~ | ~~RSD (%)~~ | ~~Comments~~ |
| --- | --- | --- | --- | --- | --- |
| ~~acetonitrile:10 mM disodium hydrogen phosphate dihydrate adjusted to pH 2.8-2.9 with phosphoric acid (85%) (50:50 v/v)~~ | ~~Ametoctradin~~ | ~~0.2~~ | ~~98.9~~ | ~~1.2~~ | ~~Acceptable~~ |
| ~~5~~ | ~~99.3~~ | ~~0.3~~ | ~~Acceptable~~ |
| ~~30~~ | ~~101.4~~ | ~~1.5~~ | ~~Acceptable~~ |

~~Table A 22: Characteristics for the method used for validation of ametoctradin in sample matrix~~

|  | ~~Ametoctradin~~ |
| --- | --- |
| ~~Specificity~~ | ~~No interference (> 30 % LOQ) was found in unfortified control samples. Peak identification was confirmed by retention time match with reference material.~~ |
| ~~Calibration (type, number of data points)~~ | ~~Calibration was performed with matrix-matched standards at a minimum of seven concentrations ranging 0.06 to 2.0 mg/100 mL (slope = 2.061938, intercept = 0.003335). Good linearity was observed (r~~~~2~~ ~~= 0.999968). The resulting test substance peak areas versus test substance concentration data were fit to the linear function. Residuals were randomly scattered.~~ |
| ~~Calibration range~~ | ~~Nominal calibration range 0.06 mg/100 mL to 2.0 mg/100 mL~~ |
| ~~Assessment of matrix effects is presented~~ | ~~No significant matrix effects were observed (i.e. the matrix effect was found to be ≤ ±20%). Matrix-matched standards were used throughout nevertheless.~~ |
| ~~Solution Stability~~ | ~~Not necessary as standards were prepared daily and samples analysed within 24 hours.~~ |
| ~~Limit of determination/quantification~~ | ~~The limit of quantification (LOQ) of 0.2 mg/100 mL was confirmed. The limit of detection (LOD) was 0.06 mg/100 mL.~~ |

~~Conclusion~~

~~The control procedure 21/0288\_01, for the determination of Ametoctradin (BAS 650 F) in a matrix of acetonitrile:10 mM disodium hydrogen phosphate dihydrate adjusted to pH 2.8-2.9 with phosphoric acid (85%) (50:50 v/v) has been fully validated in terms of specificity, linearity, precision, accuracy, matrix effects, solution stability and LOQ, in accordance with the requirements of SANTE/2020/12830 Rev.1.~~

* + - 1. Methods for the determination of ametoctradin in support of ecotoxicological studies
         1. Method validation

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| --- | --- |
| Comments of zRMS: | The method has been accepted.  Below studies are related with XXXX analytical method APL0500/02.  The objective of the analytical part was to perform concentration control of BAS 742 02 F in test medium in the context of an ecotoxicological study on the Water Flea Daphnia magna Straus.  For this purpose, XXXX analytical method APL0500/02 was modified for the analysis of ametoctradin and propamocarb. Final determination was accomplished by LC-MS/MS, using 2 transitions. The set LOQs vary among the submitted reports. The validity of the analytical method APL0500/02 was proven by recovery experiments. Results showed that the mean recovery and repeatability was within the acceptable range, with RSD < 20%. No significant peak interferences occurred at the retention time and mass transitions of ametoctradin or propamocarb. Ametoctradin and propamocarb were sufficiently stable. XXXX analytical method APL0500/02 was validated consistently with SANTE/2020/12830 rev.1. |

|  |  |
| --- | --- |
| Reference: | CP 5.1.2/12 |
| Report | BAS 743 02 F: Toxicity to the Water Flea *Daphnia* *magna* Straus under Laboratory Conditions (Acute Immobilisation Test – Static)  Wendling, K., 2023  Study No.: 933752-4  XXXX DocID: 2022/2033712  Authority registration No |
| Guideline(s): | SANTE/2020/12830 Rev.1; OECD ENV/JM/MONO(2007)17; OPPTS 860.1340 |
| Deviations: | None |
| Previous evaluation: | No |
| GLP: | Yes |
| Acceptability: | Yes | |

|  |  |
| --- | --- |
| Reference: | CP 5.1.2/13 |
| Report | BAS 743 02 F: Toxicity to the Single Cell Green Alga *Pseudokirchneriella subcapitata* Hindák under Laboratory Conditions  Obert-Rauser, P., 2023  Study No.: 933752\_5  XXXX DocID: 2022/2033713  Authority registration No |
| Guideline(s): | SANTE/2020/12830 Rev.1; OECD ENV/JM/MONO(2007)17; OPPTS 860.1340 |
| Deviations: | None |
| Previous evaluation: | No |
| GLP: | Yes |
| Acceptability: | Yes | |

|  |  |
| --- | --- |
| Reference: | CP 5.1.2/14 |
| Report | BAS 743 02 F: Toxicity to the Rainbow Trout *Oncorhynchus mykiss* under Laboratory Conditions (Acute Toxicity Test – Semi-Static)  Wendling, K., 2023  Study No.: 933752\_6  XXXX DocID: 2022/2033714  Authority registration No |
| Guideline(s): | SANTE/2020/12830 Rev.1; OECD ENV/JM/MONO(2007)17; OPPTS 860.1340 |
| Deviations: | None |
| Previous evaluation: | No |
| GLP: | Yes |
| Acceptability: | Yes | |

Study Summary

The method APL0500/02 was validated in the above studies (CP 5.1.2/12 – CP 5.1.2/14) for the determination of Ametoctradin (BAS 650 F) in test medium with a limit of quantification at 0.0954 mg/L. The brief description of method and the results are presented in the summary below.

Materials and methods

In method APL0500/02, deep frozen samples of test medium (10 mL + 50 µL formic acid) are thawed to ambient temperature. Acetonitrile (10 mL) is added and shaken well using a vortex mixer. The samples are further diluted into the linear range of the calibration curve with acetonitrile/test medium + 0.5 % formic acid (1:1, v/v) prior to analysis.

Ametoctradin (BAS 650 F) is determined by HPLC-MS/MS. Separation is achieved by using an YMC YMC-Triart C18 column (150 x 3 mm, 3 µm) and a gradient of water (0.1% formic acid)/acetonitrile (0.1% formic acid) at a flow rate of 0.5 mL/min.

Detection is accomplished in ESI positive mode using two different transitions; m/z 276 > 176 and m/z 276 > 149 used for quantification and confirmation, respectively. External matrix-matched calibration standards are used throughout.

Results and discussions

The analytical method validation is summarised below. The HPLC-MS/MS determination was conducted by monitoring two MS/MS mass transitions and therefore no confirmatory method is required.

Recoveries were obtained by fortification of the matrices and all mean recoveries are within the permitted range required by the guideline SANTE/2020/12830 rev. 1.

Table A 23: Recovery results from validation of method APL0500/02 for determination of Ametoctradin in test medium (example data from Study 933752-5)

| **Matrix** | **Analyte** | **Fortification level (mg/L) (n=x)** | **Mean  recovery (%)** | **RSD (%)** | **Comments** |
| --- | --- | --- | --- | --- | --- |
| ***Mass transition (Quantification) Ametoctradin m/z 276→149*** | | | | | |
| Reconstituted water | Ametoctradin | 0.1550 (n=5) | 100 | 1.83 | Acceptable |
| 7.749 ( n=5) | 98.8 | 1.57 |

Table A 24: Characteristics for method APL0500/02 used for validation of Ametoctradin in test medium (example data from Study 933752-5)

|  | **Ametoctradin** |
| --- | --- |
| Specificity | LC-MS/MS is a highly specific method. No interference (> 30 % LOQ) of total peak area for the target analyte at the retention time, was found in unfortified control samples. |
| Calibration (type, number of data points) | Calibration was performed with matrix-matched standards at a minimum of five concentrations ranging 0.360 – 6.00 ng/mL and good linearity was observed (r ≥0.99). The resulting test substance peak areas versus theoretical test substance concentration data were fit to the linear function.  Regression data:   |  |  |  |  | | --- | --- | --- | --- | | **Set No.** | **Intercept** | **Slope** | **r** | | 1 | -94300 | 405000 | 0.9967 | | 2 | 4960 | 331000 | 0.9967 | | 3 | -181 | 38500 | 0.9998 | | 4 | 1480 | 43700 | 0.9993 | | 5 | -2380 | 41500 | 0.9999 | | 6 | -1040 | 39900 | 0.9999 | | 7 | -841 | 38600 | 0.9999 | | 8 | -5650 | 30700 | 0.9972 | |
| Calibration range | Accepted calibration range 0.360 – 6.00 ng/mL |
| Assessment of matrix effects is presented | Matrix matched standards were used throughout. |
| Solution Stability | Working calibration solutions are stable when stored at 1 °C to 10 °C in the dark for at least 91 days. Extracts are considered to be stable when stored at 1 °C to 10 °C for 12 days in the dark. |
| Limit of determination/quantification | The limit of quantification (LOQ) of 0.0954 mg/L was confirmed. The limit of detection (LOD), defined as the lowest standard employed was 0.360 ng/mL. |

Conclusion

The method APL0500/02 for the determination of ametoctradin (BAS 650 F) in test medium has been fully validated in terms of specificity, linearity, precision, accuracy, matrix effects, solution stability and LOQ, in accordance with the requirements of SANTE/2020/12830 rev.1.

* + - * 1. Method validation

|  |  |
| --- | --- |
| Comments of zRMS: | The method has been accepted.  The validation of the method L0208/02 is presented in both studies below.  The analytical method approach was based on method L0208/02 for the determination of Ametoctradin in water by LC-MS/MS. The lowest concentration level verified within this study was 0.180 g/L, which represents the LOQ of the method as adapted for this study.  Results of the procedural recovery experiments obtained during the experimental phase showed that the recovery efficiency and repeatability were within the acceptable range of 80% to 110% of the intended concentrations for analyte BAS 650 F. No significant peak interferences occurred at the retention time and mass transition of BAS 650 F in the control samples.  In the context of the sample storage stability the analysis of the application solutions was performed within 86 days after preparation of the samples. Thus, no additional storage stability testing was necessary.  No relevant matrix effects were observed for the LC-MS/MS determination of BAS 650 F. Therefore, evaluation was performed using calibration solutions in solvent.  The method L0208/02 for the determination of ametoctradin (BAS 650 F) in test medium has been validated consistently with SANTE/2020/12830 Rev.1. |

|  |  |
| --- | --- |
| Reference: | CP 5.1.2/15 |
| Report | Effect of BAS 743 02 F on seedling emergence and seedling growth of several species of terrestrial plants under greenhouse conditions  Maleck, A., 2023  Study No.: 933752-13  XXXX DocID: 2022/2033722  Authority registration No |
| Guideline(s): | SANTE/2020/12830 Rev.1; OECD ENV/JM/MONO(2007)17; OPPTS 860.1340 |
| Deviations: | None |
| Previous evaluation: | No |
| GLP: | Yes |
| Acceptability: | Yes | |

|  |  |
| --- | --- |
| Reference: | CP 5.1.2/16 |
| Report | Effect of BAS 743 02 F on vegetative vigour of several species of terrestrial plants under greenhouse conditions  Maleck, A., 2023  Study No.: 933752-14  XXXX DocID: 2022/2033723  Authority registration No |
| Guideline(s): | SANTE/2020/12830 Rev.1; OECD ENV/JM/MONO(2007)17; OPPTS 860.1340 |
| Deviations: | None |
| Previous evaluation: | No |
| GLP: | Yes |
| Acceptability: | Yes | |

Study Summary

Method L0208/02 was validated in the above studies (CP 5.1.2/15 and CP 5.1.2/16) for the determination of Ametoctradin (BAS 650 F) in aqueous application solutions with a limit of quantification at 0.179 g/L. A brief description of method and the results are presented in the summary below.

Materials and methods

In method L0208/02, samples are defrosted to room temperature for 2 hours or more (max. 6 hours). The defrosted application solution samples are then homogenised by treatment in an ultrasonic bath for 20 minutes and then further homogenised using a Vortex mixer. An aliquot of 100 μL is transferred into a 10 mL volumetric flask which contains 9 mL of acetonitrile/water (50/50, v/v). This is made to volume with acetonitrile/water (50/50, v/v) and manually mixed thoroughly. A 100 μL aliquot of that solution is added to a 10 mL volumetric flask containing 9 mL acetonitrile/water (50/50, v/v) and made to volume with acetonitrile/water (50/50, v/v) with manual mixing. A 100 μL aliquot of this solution is transferred into an autosampler vial containing 900 μL acetonitrile/water (50/50, v/v) and manually mixed thoroughly. The final solution has a total dilution factor of 100000.

Only data for Ametoctradin (BAS 650 F) is performed and this is determined by HPLC-MS/MS. Separation is achieved by using an Thermo Betasil C18 column (100 x 2.1 mm, 5 µm) and a gradient of water (0.1% formic acid)/ acetonitrile (0.1% formic acid) at a flow rate of 0.6 mL/min.

Detection is accomplished in ESI positive mode using two different transitions; m/z 276 > 149 and m/z 276 > 176 used for quantification and confirmation, respectively. External calibration standards were used throughout.

Results and discussions

The analytical method validation is summarised below. The HPLC-MS/MS determination was conducted by monitoring two MS/MS mass transitions and therefore no confirmatory method is required.

Recoveries were obtained by fortification of the matrices and all mean recoveries are within the permitted range required by the guideline SANTE/2020/12830 rev. 1.

Table A 25: Recovery results from validation of method for determination of Ametoctradin in aqueous application solutions (example data from Study 933752-14)

| **Matrix** | **Analyte** | **Fortification level (mg/L) (n=x)** | **Mean  recovery (%)** | **RSD (%)** | **Comments** |
| --- | --- | --- | --- | --- | --- |
| ***Mass transition (Quantification) Ametoctradin m/z 276→149*** | | | | | |
| Aqueous application  solutions | Ametoctradin | 0.179 (n=5) | 103 | 4.79 | Acceptable |
| 3.79 ( n=5) | 91.5 | 3.16 |

Table A 26: Characteristics for method L0208/02 used for validation of Ametoctradin in aqueous application solutions (example data from Study 933752-14)

|  | **Ametoctradin** |
| --- | --- |
| Specificity | LC-MS/MS is a highly specific method. Neither the analyte nor relevant interferences were detected in the untreated application solution |
| Calibration (type, number of data points) | Calibration was performed with matrix-matched standards at a minimum of five concentrations ranging 0.5 – 50.0 ng/mL and good linearity was observed (r ≥0.99). The resulting test substance peak areas versus theoretical test substance concentration data were fit to the linear function.  Regression data:   |  |  |  | | --- | --- | --- | | **Intercept** | **Slope** | **r** | | 8710 | 1360 | 0.9998 | |
| Calibration range | Accepted calibration range 0.5 – 50.0 ng/mL |
| Assessment of matrix effects is presented | Due to the high total dilution factor (100,000), no relevant matrix effects were observed for the LC-MS/MS determination of BAS 650 F. |
| Solution Stability | The actual storage period for the tested application solutions was 84 days. Thus no separate analysis of lab made storage stability samples was considered to be necessary within this study. |
| Limit of determination/quantification | The limit of quantification (LOQ) of 0.170 g/L was confirmed. The limit of detection (LOD), defined as the lowest standard employed was 0.5 ng/mL. |

Conclusion

The method L0208/02 for the determination of ametoctradin (BAS 650 F) in test medium has been fully validated in terms of specificity, linearity, precision, accuracy, matrix effects, solution stability and LOQ, in accordance with the requirements of SANTE/2020/12830 rev.1.

* + - * 1. Method validation

|  |  |
| --- | --- |
| Comments of zRMS: | The method has been accepted.  The determination was conducted by an in-house developed method using reversed phase high performance liquid chromatography (RP-HPLC) with tandem mass spectrometric (MS/MS) detection. The concentration of both active ingredients in the highest and lowest test item feeding solution applied on the first and last day of application (D0 and D9) was determined. The recovery rates of Propamocarb were 104% on D0 and 99.1% on D9 in samples of test item feeding solution AT (highest applied concentration) and 104% on D0 and 97.3% on D9 in samples of test item feeding solution ET (lowest applied concentration).  The recovery rates of Ametoctradin were 104% on D0 and 102% on D9 in samples of test item feeding solution AT and 100% on D0 and 92.6% on D9 in samples of test item feeding solution ET. Furthermore, no residues of the active ingredients were found in the control samples, i.e., the concentrations of active ingredients were below 30% of the LOQ.  The method was validated at LOQ of 1.44 mg/kg Propamocarb and 0.459 mg/kg BAS 650 F. The mean recovery was within the acceptable range of 70% to 120% for all active ingredients, with relative standard deviations RSD < 20%. No significant peak interferences (> LOD) occurred at the retention time and mass transition of any active ingredient in the control samples. The observed matrix effects were -7.25% for Propamocarb and +1.79% for BAS 650 F and considered insignificant.  The method was fully validated according to the requirements of SANTE/2020/12830, Rev.1 guideline. |

|  |  |
| --- | --- |
| Reference: | CP 5.1.2/17 |
| Report | Chronic toxicity of BAS 743 02 F to the honey bee *Apis mellifera* L. under laboratory conditions  Ruhland, S., 2023  Study No.: 933752-2  XXXX DocID: 2022/2033709  Authority registration No |
| Guideline(s): | SANTE/2020/12830 Rev.1; OECD ENV/JM/MONO(2007)17; OPPTS 860.1340 |
| Deviations: | None |
| Previous evaluation: | No |
| GLP: | Yes |
| Acceptability: | Yes | |

**Study Summary**

An analytical method was validated for the determination of Ametoctradin in bee feeding solutions of BAS 743 02 F (containing the active ingredients Ametocradin (BAS 650 F) and Propamocarb (BAS 9068 F), with an LOQ of 0.459 mg/kg. A brief description of method and the results are presented in the summary below.

**Materials and methods**

An aliquot of bee feeding solution (0.2 g) is shaken with acetonitrile:0.5% formic acid solution (50:50 v/v, 10 mL) using a Fast Prep system at 5 m/s for 5 minutes and centrifuged at 4000 rpm for 5 minutes. The resulting extract is further filuted with dilution medium (blank extract) into the range of the calibration curve. Samples are analysed by high performance liquid chromatography with tandem mass detection (HPLC-MS/MS) in positive ion mode using an ACE Excel 3 C18 column (100 mm x 2.1 mm, 3 µm) and gradient elution with mobile phases of water with 0.1% (v/v) formic acid + 5mM ammonium formate and methanol + 0.1% (v/v) formic acid. Calibration is by external matrix-matched standards monitoring the ion transitions *m/z* 276 → 149 and *m/z* 276 → 176 for the quantification and confirmation of Ametoctradin, respectively.

**Results and discussions**

The analytical method validation is summarised below and only the quantifying transition is reported.

Recoveries were evaluated with the fortification of sample matrix (sucrose solution containing 50% (w/v) sucrose +0.1% (w/v) xanthan) with BAS 743 02 F at two fortification levels (LOQ and approx 7500 x LOQ). The mean recoveries were within the permitted range required by the guideline SANTE/2020/12830 rev. 1.

**Table A 27: Recovery results from method validation of Ametoctradin using the analytical method**

| **Matrix** | **Analyte** | **n** | **Nominal fortification level (mg/kg)** | **Corresponding fortification level as  BAS 743 02 F (mg/kg)** | **Mean  recovery (%)** | **RSD (%)** | **Comments** |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Bee feeding solution  (sucrose solution containing 50% (w/v) sucrose + 0.1% (w/v) xanthan) | Ametoctradin | 5 | 0.459 | 3.61 | 101 | 0.490 | Acceptable |
| 5 | 3469 | 27315 | 100 | 2.04 | Acceptable |

**Table A 28: Characteristics for the method used for validation of Ametoctradin in bee feeding solutions**

|  | **Ametoctradin** |
| --- | --- |
| Specificity | No significant peak interferences (> 30% LOQ) occurred at the retention time and mass transition of any active ingredient in the control samples. Peak identification was confirmed by retention time match with reference material. |
| Calibration (type, number of data points) | Calibration was performed with matrix-matched standards at six concentrations ranging from 1.94 to 43.2 ng Ametoctradin/mL (slope = 3783, intercept = -658). Good linearity was observed (r2 = 0.99998). The resulting test substance peak areas versus test substance concentration data were fit to the linear function. Residuals were randomly scattered. |
| Calibration range | Calibration range 1.94 to 43.2 ng Ametoctradin/mL |
| Assessment of matrix effects is presented | No significant matrix effects were observed (i.e. the matrix effect was found to be ≤ ±20%). Matrix-matched standards were used throughout nevertheless. |
| Solution Stability | Stability of Ametoctradin in bee feeding solution were proven over a time period of 191 days under deep frozen conditions in the dark. |
| Limit of determination/quantification | The limit of quantification (LOQ) of 0.459 mg Ametoctradin/kg was confirmed. The limit of detection (LOD) was 0.0988 mg Ametoctradin/kg. |

**Conclusion**

The analytical method for the determination of Ametoctradin (BAS 650 F) in bee feeding solution (sucrose solution containing 50% (w/v) sucrose + 0.1% (w/v) xanthan) has been fully validated in terms of specificity, linearity, precision, accuracy, matrix effects, solution stability and LOQ/LOD, in accordance with the requirements of SANTE/2020/12830 Rev.1.

* + - * 1. Method validation

|  |  |
| --- | --- |
| Comments of zRMS: | The method has been accepted.  The purpose of the analytical phase of the study was the determination of the concentrations of Propamocarb and Ametoctradin in final diets of honeybee larvae Apis mellifera L. The determination was conducted by an in-house developed method using reversed phase high performance liquid chromatography (RP-HPLC)  with tandem mass spectrometric (MS/MS) detection.  Results of the procedural recovery experiments obtained during analysis of BAS 743 02 F showed that the mean recovery efficiency and repeatability was within the acceptable range of 70% to 120% of the intended concentrations for all active ingredients, with relative standard deviations RSD < 20%. No significant peak interferences (> LOD) occurred at the retention time and mass transition of any active ingredient in the control samples.  No significant interferences occurred at the retention time and mass transition of any active ingredient in the control samples. The observed matrix effects were considered insignificant.  The method was fully validated according to the requirements of SANTE/2020/12830, Rev.1. |

|  |  |
| --- | --- |
| Reference: | CP 5.1.2/18 |
| Report | Repeated exposure of honey bee (*Apis mellifera* L.) larvae to BAS 743 02 F under laboratory conditions  Schmidt, K., 202~~2~~3  Study No.: 933752-3  XXXX DocID: 2022/2033710  Authority registration No |
| Guideline(s): | SANTE/2020/12830 Rev.1; OECD ENV/JM/MONO(2007)17; OPPTS 860.1340 |
| Deviations: | None |
| Previous evaluation: | No |
| GLP: | Yes |
| Acceptability: | Yes | |

**Study Summary**

An analytical method was validated for the determination of Ametoctradin from final diets of honey bee larvae containing BAS 743 02 F, with an LOQ of 0.469 mg/kg. A brief description of method and the results are presented in the summary below.

**Materials and methods**

A 0.2g aliquot of bee feeding solution (50:50 (w/w) royal jelly/aqueous sugar solution (containing 4% (w/v) yeast extract, 18% (w/v) glucose and 18% (w/v) fructose)) is shaken with acetonitrile:0.5% formic acid solution (50:50 v/v, 10 mL) using a Fast Prep system at 5 m/s for 5 minutes and centrifuged at 4000 rpm for 5 minutes. The resulting extract is further diluted with dilution medium (blank extract) into the range of the calibration curve. Samples are analysed by high performance liquid chromatography with tandem mass detection (HPLC-MS/MS) in positive ion mode using an ACE Excel 3 C18 column (100 mm x 2.1 mm, 3 µm) and gradient elution with mobile phases of water with 0.1% (v/v) formic acid + 5mM ammonium formate and methanol + 0.1% (v/v) formic acid. Calibration is by external matrix-matched standards monitoring the ion transitions *m/z* 276 → 149 and *m/z* 276 → 176 for the quantification and confirmation of Ametoctradin, respectively.

**Results and discussions**

The analytical method validation is summarised below and only the quantifying transition is reported.

Recoveries were evaluated with the fortification of sample matrix with BAS 743 02 F at two fortification levels (LOQ and approx 800 x LOQ). The mean recoveries were within the permitted range required by the guideline SANTE/2020/12830 rev. 2.

**Table A 29: Recovery results from method validation of Ametoctradin using the analytical method**

| **Matrix** | **Analyte** | **n** | **Nominal fortification level (mg/kg)** | **Corresponding fortification level as  BAS 743 02 F (mg/kg)** | **Mean  recovery (%)** | **RSD (%)** | **Comments** |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Bee feeding solution extract | Ametoctradin | 5 | 0.469 | 3.70 | 99.3 | 5.02 | Acceptable |
| 5 | 375 | 2957 | 109 | 0.443 | Acceptable |

**Table A 30: Characteristics for the method used for validation of Ametoctradin in bee feeding solutions**

|  | **Ametoctradin** |
| --- | --- |
| Specificity | No significant peak interferences (> 30% LOQ) occurred at the retention time and mass transition of any active ingredient in the control samples. Peak identification was confirmed by retention time match with reference material. |
| Calibration (type, number of data points) | Calibration was performed with matrix-matched standards at six concentrations ranging from 1.94 to 43.2 ng Ametoctradin/mL (slope = 1901, intercept = 716). Good linearity was observed (r2 = 0.99985). The resulting test substance peak areas versus test substance concentration data were fit to the linear function. Residuals were randomly scattered. |
| Calibration range | Calibration range 1.94 to 43.2 ng Ametoctradin/mL (corresponding to 0.0988 to 2.20 mg/kg) |
| Assessment of matrix effects is presented | The mean matrix effect was determined to be -22.0% and thus considered to be significant (i.e. the matrix effect was ≥±20%). Matrix-matched standards were used throughout. |
| Solution Stability | Stability of Ametoctradin in bee feeding solution were proven over a time period of 203 days under deep frozen conditions in the dark. |
| Limit of determination/quantification | The limit of quantification (LOQ) of 0.469 mg Ametoctradin/kg was confirmed. The limit of detection (LOD) was 0.0988 mg Ametoctradin/kg. |

**Conclusion**

The analytical method for the determination of Ametoctradin (BAS 650 F) from final diets of honey bee larvae containing BAS 743 02 F has been fully validated in terms of specificity, linearity, precision, accuracy, matrix effects, solution stability and LOQ/LOD, in accordance with the requirements of SANTE/2020/12830 Rev.2

* + - * 1. Method validation

|  |  |
| --- | --- |
| Comments of zRMS: | The method has been accepted.  The purpose of the analytical phase of the study was to determine the concentrations of the test item BAS 743 02 F via its active ingredients Propamocarb and BAS 650 F (Ametoctradin) in test item solutions resulting from acute toxicity tests with BAS 743 02 F on bumblebees (Bombus terrestris L.). The determination was conducted by HPLC with mass-spectrometric (MS-MS) detection.  The method has LOQ of 1.45 mg/kg for Propamocarb and 0.459 mg/kg for BAS 650 F (contact toxicity test) and 1.52 mg/kg for Propamocarb and 0.482 mg/kg for  BAS 650 F (oral toxicity test). Results of the validation experiments showed that the mean recovery efficiency and repeatability was within the acceptable range of 70% to 120% of the intended concentrations for Propamocarb and BAS 650 F, with relative standard deviations RSD < 20%. No significant peak interferences (>LOD) occurred at the retention time and mass transition in the control samples.  Matrix effects were considered by the addition of the same amount of blank extract to calibration samples as included in the analysis samples. Thus, all measured samples contained the same amount of original sample matrix.  The method was fully validated according to the requirements of SANTE/2020/12830, Rev.1. |

|  |  |
| --- | --- |
| Reference: | CP 5.1.2/19 |
| Report | Acute toxicity of BAS 743 02 F to the bumblebee *Bombus terrestris* L. under laboratory conditions  Amsel, K., 2023  Study No.: 933752-18  XXXX DocID: 2022/2033711  Authority registration No |
| Guideline(s): | SANTE/2020/12830 Rev.1; OECD ENV/JM/MONO(2007)17; OPPTS 860.1340 |
| Deviations: | None |
| Previous evaluation: | No |
| GLP: | Yes |
| Acceptability: | Yes | |

**Study Summary**

An analytical method was validated for the determination of Ametoctradin from test item solutions containing BAS 743 02 F produced during acute toxicity tests. A brief description of method and the results are presented in the summary below.

**Materials and methods**

For all samples of the contact toxicity test, which are present in water containing 0.5% (v/v) TritonX, no extraction is necessary. They are diluted with 50/50 (v/v) (acetonitrile + 0.5% (v/v) formic acid) / water and, if necessary to obtain equal amount of sample matrix in all samples for analysis, with sample matrix into the range of the calibration curve before injecting into the HPLC-system.

All samples of the oral toxicity test, which are present in 50% (w/v) sucrose solution, are extracted prior to sample measurement. A 0.2 g aliquot of sample test solution is shaken with acetonitrile:0.5% formic acid solution (50:50 v/v, 10 mL) using a Fast Prep system at 5 m/s for 5 minutes and centrifuged at 4000 rpm for 5 minutes. The resulting extract is further diluted with dilution medium (blank extract) into the range of the calibration curve

Samples are analysed by high performance liquid chromatography with tandem mass detection (HPLC-MS/MS) in positive ion mode using an ACE Excel 3 C18 column (100 mm x 2.1 mm, 3 µm) and gradient elution with mobile phases of water with 0.1% (v/v) formic acid + 5mM ammonium formate and methanol + 0.1% (v/v) formic acid. Calibration is by external matrix-matched standards monitoring the ion transitions *m/z* 276 → 149 and *m/z* 276 → 176 for the quantification and confirmation of Ametoctradin, respectively.

**Results and discussions**

The analytical method validation is summarised below and only the quantifying transition is reported.

Recoveries were evaluated with the fortification of sample matrix with BAS 743 02 F at two fortification levels (LOQ & approx 92000 x LOQ for contact toxicity samples and LOQ & approx 3675 x LOQ for oral toxicity samples). The mean recoveries were within the permitted range required by the guideline SANTE/2020/12830 rev. 2.

**Table A 31: Recovery results from method validation of Ametoctradin using the analytical method**

| **Matrix** | **Analyte** | **n** | **Nominal fortification level (mg/kg)** | **Corresponding fortification level as  BAS 743 02 F (mg/kg)** | **Mean  recovery (%)** | **RSD (%)** | **Comments** |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Water containing 0.5% (v/v) TritonX (Contact toxicity test) | Ametoctradin | 5 | 0.459 | 3.62 | 101 | 3.50 | Acceptable |
| 5 | 42222 | 332505 | 105 | 2.04 | Acceptable |
| 50% (w/v) sucrose solution  (Oral toxicity test) | Ametoctradin | 5 | 0.482 | 3.80 | 105 | 8.40 | Acceptable |
| 5 | 1772 | 13951 | 107 | 1.29 | Acceptable |

**Table A 32: Characteristics for the method used for validation of Ametoctradin in contact and oral toxicity samples**

|  | **Ametoctradin** |
| --- | --- |
| Specificity | No significant peak interferences (> 30% LOQ) occurred at the retention time and mass transition of any active ingredient in the control samples. Peak identification was confirmed by retention time match with reference material. |
| Calibration (type, number of data points) | Calibration was performed with matrix-matched standards at six concentrations.  Contact toxicity analysis:  Fit: Linear, 6 points Range: 1.94 to 48.6 ng/mL  Slope = 4267, Intercept = 45.1, r2 = 0.999996).  Residuals were randomly scattered.  Oral toxicity sample analysis:  Fit: Quandratic (a𝑥2 +b𝑥 +c), 6 points Range: 2.05 to 51.3 ng/mL  a= 16.7, b=2185, c= 613, r2 = 0.999955.  Residuals were randomly scattered confirming correct fit type. |
| Calibration range | Calibration range  Contact toxicity analysis:  1.94 to 48.6 ng/mL (corresponding to 0.0971 to 2.43 mg/kg)  2.05 to 51.3 ng/mL (corresponding to 0.104 to 2.61 mg/kg) |
| Assessment of matrix effects is presented | The mean matrix effect was determined to be -3.60% and -14.2% for contact and oral toxicity samples respectively thus not considered to be significant (i.e. the matrix effect was ≤±20%). |
| Solution Stability | Stability of Ametoctradin in contact and oral toxicity samples was proven over a time period of at least 185 days and 188 days respectively under deep frozen conditions in the dark. |
| Limit of determination/quantification | The limit of quantification (LOQ) and limit of detection (LOD) were:  Contact toxicity solutions: LOQ = 0.459 mg/kg (9.19 ng/mL)  LOD = 0.0971 mg/kg (1.94 ng/mL)  Oral toxicity solutions: LOQ = 0.482 mg/kg (9.49 ng/mL)  LOD = 0.104 mg/kg (2.05 ng/mL) |

**Conclusion**

The analytical method for the determination of Ametoctradin (BAS 650 F) from contact and oral toxicity samples containing BAS 743 02 F has been fully validated in terms of specificity, linearity, precision, accuracy, matrix effects, solution stability and LOQ/LOD, in accordance with the requirements of SANTE/2020/12830 Rev.2

* + - * 1. Method validation

|  |  |
| --- | --- |
| Comments of zRMS: | The method has been accepted.  The purpose of the analytical phase of the study was to determine Propamocarb and Ametoctradin in test item solutions resulting from an acute toxicity test with BAS 743 03 F. The determination was conducted by using HPLC with mass-spectrometric (MS-MS) detection. The LOQ was set at 0.4882 mg/L (Propamocarb) and at 0.1550 mg/L (BAS 650 F).  The mean recovery was within the acceptable range of 70% to 120% for Propamocarb and BAS 650 F, with RSD < 20%. No significant peak interferences (>LOD) occurred at the retention time and mass transition in the control samples. The method was fully validated according to the requirements of SANTE/2020/12830, Rev.1. |

|  |  |
| --- | --- |
| Reference: | CP 5.1.2/20 |
| Report | Acute toxicity of BAS 743 03 F on *Daphnia magna* in a 48-hour static test  Renner, P., 2023  Study No.: 933750-2  XXXX DocID: 2022/2033730  Authority registration No |
| Guideline(s): | SANTE/2020/12830 Rev.1; OECD ENV/JM/MONO(2007)17; OPPTS 860.1340 |
| Deviations: | None |
| Previous evaluation: | No |
| GLP: | Yes |
| Acceptability: | Yes | |

**Study Summary**

The method was validated in the above study for the determination of Ametoctradin (BAS 650 F) in reconstituted water with a limit of quantification at 0.1550 mg/L. The brief description of method and the results are presented in the summary below.

**Materials and methods**

Samples were diluted with 50/50 (v/v) test matrix/ 0.2% formic acid (v/v) in acetonitrile (v/v) into the range of the calibration curve before analysis.

Ametoctradin (BAS 650 F) is determined by HPLC-MS/MS. Separation is achieved by using an ACE Excel 3 C18-PFP column (100 x 2.1 mm, 3 µm) and a gradient of water/formic acid/5 mM ammonium formate (1000/1, v/v) and acetonitrile/formic acid/5 mM ammonium formate (1000/1, v/v) at a flow rate of 0.2 mL/min.

Detection is accomplished in ESI positive mode monitoring three transitions; *m/z* 276 > 149 used for quantification and *m/z* 276 > 70 and *m/z* 276 > 123 used for confirmation. External matrix-matched calibration standards were used throughout.

**Results and discussions**

The analytical method validation is summarised below. The HPLC-MS/MS determination was conducted by monitoring three MS/MS mass transitions and therefore no confirmatory method is required.

Recoveries were obtained by fortification of the matrices and all mean recoveries are within the permitted range required by the guideline SANTE/2020/12830 rev. 1.

**Table A 33: Recovery results from method validation of Ametoctradin using the analytical method**

| **Matrix** | **Analyte** | **Fortification level (mg/L) (n=x)** | **Mean  recovery (%)** | **RSD (%)** | **Comments** |
| --- | --- | --- | --- | --- | --- |
| ***Mass transition (Quantification) Ametoctradin m/z 276→149*** | | | | | |
| Reconstituted water | Ametoctradin | 0.1550 (n=5) | 100 | 1.83 | Acceptable |
| 7.749 ( n=5) | 98.8 | 1.57 |

**Table A 34: Characteristics for the method used for validation of Ametoctradin in Reconstituted Water**

|  | **Ametoctradin** |
| --- | --- |
| Specificity | LC-MS/MS is a highly specific method. No significant peak interferences (>LOD) occurred at the retention time and mass transition in the control samples. |
| Calibration (type, number of data points) | Calibration was performed with matrix-matched standards at a minimum of five concentrations ranging 2.087 ng/mL to 37.95 ng/mL and good linearity was observed (r ≥0.99). The resulting test substance peak areas versus theoretical test substance concentration data were fit to the quadratic function.  Regression data: y = ax2 + bx +c   |  |  |  |  | | --- | --- | --- | --- | | **a** | **b** | **c** | **r** | | -106.068 | 49733.1 | 7384.00 | 0.99974 | |
| Calibration range | Accepted calibration range 2.087 ng/mL to 37.95 ng/mL |
| Assessment of matrix effects is presented | Matrix effects were evaluated by comparing of the analyte responses of each calibration level. The matrix effect for the sample matrix of BAS 650 F was -1.94%. Thus, the matrix effect is not significant. Nevertheless, matrix-matched standards were used throughout the analytical phase for quantification of the test samples. |
| Solution Stability | All samples were measured within less than 30 days. Therefore, no storage stability was analysed. |
| Limit of determination/quantification | The limit of quantification (LOQ) of 0.1550 mg/L was confirmed. The limit of detection (LOD), defined as the lowest standard employed was 2.087 ng/mL ng/mL. |

**Conclusion**

The validation data for the method for the determination of ametoctradin (BAS 650 F) in reconstituted water has been fully validated in terms of specificity, linearity, precision, accuracy, matrix effects, solution stability and LOQ, in accordance with the requirements of SANTE/2020/12830 rev.1.

* + 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)

The method for the determination of ametoctradin in plant matrices (L0117) has been previously assessed in the DAR (The Netherlands, 2009 or addendum 2012) and is provided here for completeness. Additional data for the use of this method in dried pea seeds and hops is provided below also.

* + - * 1. Analytical method 1

Method validation

The method L0117/01 (XXXX DocID 2008/1028661) for the determination of Ametoctradin residues in food of plant origin is already peer-reviewed. The summary is presented below for reasons of completeness.

|  |  |
| --- | --- |
| Comments of zRMS: | The method L0117/01 already peer reviewed. No comments.  CP 5.2/2: The amendment is accepted.  Information was provided to demonstrate the influence of matrix load on the analysis using quality control samples. Detailed appendix tables show the raw data of quality control samples. Additional information regarding linearity, ionization mode, calibration Curve and chromatograms of the qualifier mass transition are shown. Additional information that was required for re-registration purposes according to SANCO/825/00 rev 8.1 (16/11/2010) and SANCO/3029/99 rev. 4. |

|  |  |
| --- | --- |
| Reference: | CP 5.2/1 |
| Report | Validation of XXXX method L0117/01: Method for the determination of BAS 650 F in plant matrices  Mackenroth, C., Schweda, Z., 2008  report No 250381  XXXX DocID 2008/1028661  Authority registration No |
| Guideline(s): | OPPTS 860.1340: US EPA Residue Chemistry Test Guidelines - Residue Analytical Method; European Commission; Guidance document on residue analytical methods; SANCO/825/00 rev.7; 17/03/04; European Commission; Residues: Guidance for generating and reporting methods of analysis in support of pre-registration data requirements for Annex II; SANCO/3029/99 rev.4; 11/07/00 |
| Deviations: | No |
| Previous evaluation: | Yes, evaluated and accepted in DAR (2009)/in the addendum to the DAR (2012) |
| GLP: | Yes  (laboratory certified by Landesamt fuer Umweltschutz und Gewerbeaufsicht, Mainz, Germany Fed. Rep.) |
| Acceptability: | Yes | |

|  |  |
| --- | --- |
| Reference: | CP 5.2/2 |
| Report | Report Amendment No. 1 to final report: Validation of XXXX method L0117/01: Method for the determination of BAS 650 F in plant matrices  Mackenroth, C., Schweda, Z., 2016  report No 250381  XXXX DocID 2015/1257669  Authority registration No |
| Guideline(s): | EPA 860.1340, SANCO/3029/99 rev. 4 (11 July 2000), SANCO/825/00 rev. 8.1 (16 November 2010) OPPTS 860.1340: US EPA Residue Chemistry Test Guidelines - Residue Analytical Method; European Commission; Guidance document on residue analytical methods; SANCO/825/00 rev.7; 17/03/04; European Commission; Residues: Guidance for generating and reporting methods of analysis in support of pre-registration data requirements for Annex II; SANCO/3029/99 rev.4; 11/07/00 |
| Deviations: | No |
| Previous evaluation: | Amendment was not evaluated previously; but the analytical method and validation data have previously been evaluated and accepted in DAR (2009)/in the addendum to the DAR (2012) |
| GLP: | Yes |
| Acceptability: | Yes | |

Study Summary

The method L0117/01 was developed and validated for the determination of residues of Ametoctradin (BAS 650 F) in representative plant matrices with a limit of quantification at 0.01 mg/kg. The brief description of the method and the results are presented in the summary below.

Materials and methods

In method L0117/01, the residues of Ametoctradin (BAS 650 F) extracted with a mixture of methanol/water. An aliquot of the extract is centrifuged and partitioned against dichloromethane. The final determination of Ametoctradin (BAS 650 F) is performed by HPLC-MS/MS. Separation is achieved by using an Phenomenex, Synergi Fusion-RP column (150 mm x 4.6 mm) and a gradient of water (0.1% formic acid)/ methanol (0.1% formic acid) at a flow rate of 1.0 mL/min. Detection is accomplished in ESI positive mode for BAS 650 F at mass transition 276 m/z > 176 m/z and 276 m/z > 149 m/z for quantification and confirmation, respectively. Calibration standards are solvent based and were prepared in methanol/water/formic acid (50:50:0.1, v/v/v).

Results and discussions

The analytical method validation is summarised below. The HPLC-MS/MS determination was conducted by monitoring two MS/MS mass transitions and therefore no confirmatory method is required.

Recoveries were obtained by fortification of the matrices and all mean recoveries are within the permitted range required by the guideline SANTE/2020/12830 rev. 1.

Table A 35: Recovery results from method validation of ametoctradin using the analytical method

| Matrix | Analyte | Fortification level (mg/kg) (n = x) | Mean  recovery (%) | RSD (%) | Comments |
| --- | --- | --- | --- | --- | --- |
| ***Mass transition 276→176 m/z (Quantification)*** | | | | | |
| Wheat grain | Ametoctradin | 0.01 (n = 5) | 80.9 | 13.4 | Acceptable |
| 0.1 (n = 5) | 88.1 | 2.9 |
| Potato tuber | Ametoctradin | 0.01 (n = 5) | 107.4 | 4.6 | Acceptable |
| 0.1 (n = 5) | 96.3 | 11.7 |
| Lettuce | Ametoctradin | 0.01 (n = 5) | 97.3 | 4.3 | Acceptable |
| 0.1 (n = 5) | 91.1 | 13.1 |
| Tomato | Ametoctradin | 0.01 (n = 5) | 103.3 | 3.2 | Acceptable |
| 0.1 (n = 5) | 90.7 | 5.9 |
| Grape | Ametoctradin | 0.01 (n = 5) | 91.9 | 10.8 | Acceptable |
| 0.1 (n = 5) | 92.0 | 8.3 |
| Orange | Ametoctradin | 0.01 (n = 5) | 91.7 | 1.5 | Acceptable |
| 0.1 (n = 5) | 101.9 | 3.8 |
| Onion | Ametoctradin | 0.01 (n = 5) | 100.8 | 6.2 | Acceptable |
| 0.1 (n = 5) | 95.6 | 6.6 |
| Sunflower | Ametoctradin | 0.01 (n = 5) | 88.2 | 5.9 | Acceptable |
| 0.1 (n = 5) | 86.8 | 2.6 |
| ***Mass transition 276→149 m/z (Confirmation)*** | | | | | |
| Wheat grain | Ametoctradin | 0.01 (n = 5) | 85.3 | 12.1 | Acceptable |
| 0.1 (n = 5) | 87.4 | 3.6 |
| Potato tuber | Ametoctradin | 0.01 (n = 5) | 104.6 | 2.5 | Acceptable |
| 0.1 (n = 5) | 92.2 | 9.2 |
| Lettuce | Ametoctradin | 0.01 (n = 5) | 97.0 | 5.5 | Acceptable |
| 0.1 (n = 5) | 89.7 | 12.4 |
| Tomato | Ametoctradin | 0.01 (n = 5) | 96.2 | 4.4 | Acceptable |
| 0.1 (n = 5) | 91.9 | 5.5 |
| Grape | Ametoctradin | 0.01 (n = 5) | 89.5 | 11.8 | Acceptable |
| 0.1 (n = 5) | 94.1 | 7.5 |
| Orange | Ametoctradin | 0.01 (n = 5) | 95.1 | 5.6 | Acceptable |
| 0.1 (n = 5) | 101.7 | 4.8 |
| Onion | Ametoctradin | 0.01 (n = 5) | 100.3 | 6.5 | Acceptable |
| 0.1 (n = 5) | 95.5 | 3.4 |
| Sunflower | Ametoctradin | 0.01 (n = 5) | 88.4 | 4.6 | Acceptable |
| 0.1 (n = 5) | 83.9 | 5.4 |

Table A 36: Characteristics for the method used for validation of ametoctradin residues in plant matrices

|  | Ametoctradin |
| --- | --- |
| Specificity | LC-MS/MS is a highly specific method. No interference (> 30 % LOQ) of total peak area for the target analyte at the retention time, was found in unfortified control samples. |
| Calibration (type, number of data points) | Calibration was performed with solvent based standards at six concentrations ranging 0.025 to 1.0 ng/mL and good linearity was observed (r ≥0.998). The resulting test substance peak areas versus theoretical test substance concentration data were fit to the linear function. |
| Calibration range | 0.025 ng/mL to 1.0 ng/mL |
| Assessment of matrix effects is presented | Matrix effects were not found to be significant (i.e. they were not greater than 20%). |
| Solution Stability | Standard solutions stability was not evaluated in the study, but was demonstrated in a separate study (refer to XXXX DocID: 2008/1022139 below). Standards in fortification solvent (methanol) and calibration standards in methanol/water/formic acid (50:50:0.1, v/v/v) is shown to be stable (>80%) at least for 60 days for ametoctradin (BAS 650 F) under refrigerated conditions.  Extract stability was also evaluated in extraction solution [methanol/water (50:50, v/v)] and in final volume solution [methanol/water/formic acid (50:50:0.1, v/v/v)] for 14 days. Ametoctradin (BAS 650 F) was found to be stable (>80%) for at least 14 days under refrigerated conditions. |
| Limit of determination/quantification | The limit of quantification (LOQ) of 0.01 mg/kg was confirmed for ametoctradin. The limit of detection (LOD), defined as the lowest standard employed was 0.025 ng/mL for ametoctradin. |

Conclusion

The analytical procedure of method L0117/01 has been fully validated in terms of specificity, linearity, precision, accuracy, matrix effects, solution stability and LOQ, in accordance with the requirements of SANCO/825/00 rev.8.1 and meets the requirements of SANTE/2020/12830 rev.1 also. It is suitable as a monitoring method for determination of residues of ametoctradin (BAS 650 F) in the plant matrices types analysed.

* + - * 1. Analytical method 2

Method validation

|  |  |
| --- | --- |
| Comments of zRMS: | The validation has been accepted.  The objective was to validate XXXX analytical method No. L0117/01 applied for the determination of ametoctradin residues in dried pea seed by LC-MS/MS. This method was originally validated in various plant matrices covering all crop groups (high starch, high water, high acid, high oil), except the high protein. The subject study was conducted in dry peas to address the data gap.  It is confirmed that the method L0117/01 has been fully validated in terms of specificity, linearity, precision, accuracy, matrix effects, solution stability and LOQ, consistently with SANTE/2020/12830 rev.1 |

|  |  |
| --- | --- |
| Reference: | CP 5.2/3 |
| Report | Validation of Method L0117/01: Method for the Determination of BAS 650 F (Ametoctradin, Reg.No. 4993353), in Dried Peas by LC-MS/MS  Andrews, R., 2020  report No 872721  XXXX DocID 2020/2031000  Authority registration No |
| Guideline(s): | OECD ENV/JM/MONO(2967)17, OPPTS 860.1340, SANCO 3029/99 Rev.4 |
| Deviations: | No |
| Previous evaluation: | No |
| GLP: | Yes |
| Acceptability: | Yes | |

Study Summary

In this study, the method L0117/01 was developed and validated for the determination of Ametoctradin (BAS 650 F) in dried peas, with a limit of quantification of 0.01 mg/kg. The brief description of the method and the results are presented in the summary below.

Materials and methods

Method L0117/01 is validated to determine residues of Ametoctradin (Reg.No. 4993353, BAS 650 F) in dried pea seeds by LC-MS/MS. Ametoctradin residues in crop commodity samples (5 g each) are extracted with methanol/water (50/50, v/v), centrifuged, and an aliquot of the extract is cleaned up by liquid-liquid partitioning against dichloromethane. Residues in the organic (DCM) layer are evaporated to dryness, re-dissolved in methanol/water/formic acid (50/50/0.1, v/v/v) and then determined by LC MS/MS in ESI positive mode at mass transition 276 → 176 for quantification and 276 → 149 for confirmation. Separation is accomplished with a Phenomenex Synergi Fusion RP column (150 mm x 4.6 mm, 4 µm) by using a gradient mixture of water/methanol, each acidified with 0.1% formic acid at a flow rate of 1.0 mL/min.

Results and discussions

The analytical method validation is summarised below. The HPLC-MS/MS determination was conducted by monitoring two MS/MS mass transitions and therefore no confirmatory method is required.

Recoveries were obtained by fortification of the matrix and all mean recoveries are within the permitted range required by the guideline SANTE/2020/12830 rev. 1.

Table A 37: Recovery results from method validation of ametoctradin using the analytical method

| Matrix | Analyte | Fortification level (mg/kg) (n = x) | Mean  recovery (%) | RSD (%) | Comments |
| --- | --- | --- | --- | --- | --- |
| ***Mass transition 276→176 m/z (Quantification)*** | | | | | |
| Pea  (dried seed) | Ametoctradin | 0.01 (n = 5) | 85 | <1 | Acceptable |
| 0.1 (n = 5) | 91 | 2 |
| ***Mass transition 276→149 m/z (Confirmation)*** | | | | | |
| Pea  (dried seed) | Ametoctradin | 0.01 (n = 5) | 88 | 4 | Acceptable |
| 0.1 (n = 5) | 91 | 4 |

Table A 38: Characteristics for the method used for validation of ametoctradin residues in pea (dried seeds)

|  | Ametoctradin |
| --- | --- |
| Specificity | LC-MS/MS is a highly specific method. No interference (> 30 % LOQ) of total peak area for the target analyte at the retention time, was found in unfortified control samples. |
| Calibration (type, number of data points) | Calibration was performed with solvent-based standards, prepared in methanol/water/formic acid (50/50/0.1, v/v/v), at six concentrations ranging from 0.025 to 1.0 ng/mL and good linearity was observed (r ≥ 0.9998) for both mass transitions. The resulting test substance peak areas versus theoretical test substance concentration data were fit to the linear function.  Calibration data  Mass transition 276→ 176 m/z  Slope = 2.83 x 106 , Intercept = -5.77 x 103 (r = 0.9998)  Mass transition 276→ 149 m/z  Slope = 2.79 x 106 , Intercept = -5.53 x 103 (r = 0.9998) |
| Calibration range | Accepted calibration range 0.025 ng/mL to 1.0 ng/mL |
| Assessment of matrix effects is presented | Matrix effects were not found to be significant (i.e. they were not greater than 20%). |
| Solution Stability | Standard solutions stability was not evaluated in the study, but was demonstrated in a separate study (refer to XXXX DocID: 2008/1022139 below). Standards in fortification solvent (methanol) and calibration standards in methanol/water/formic acid (50:50:0.1, v/v/v) is shown to be stable (>80%) at least for 60 days for ametoctradin (BAS 650 F) under refrigerated conditions.  Extract stability was also evaluated in extraction solution [methanol/water (50:50, v/v)] and in final volume solution [methanol/water/formic acid (50:50:0.1, v/v/v)] for 14 days. Ametoctradin (BAS 650 F) was found to be stable (>80%) for at least 14 days under refrigerated conditions. |
| Limit of determination/quantification | The limit of quantification (LOQ) of 0.01 mg/kg was confirmed for ametoctradin. The limit of detection (LOD), defined as the lowest standard employed was 0.025 ng/mL for ametoctradin. |

Conclusion

The analytical procedure of method L0117/01 has been fully validated in terms of specificity, linearity, precision, accuracy, matrix effects, solution stability and LOQ, in accordance with the requirements of SANTE/2020/12830 rev.1. It is suitable as a monitoring method for determination of residues of ametoctradin (BAS 650 F) in pea (dried seed).

Please refer to XXXX Doc Id 2019/2051445 for the ILV corresponding to the analysis of ametoctradin for pea (dried seeds) using the method above.

* + - * 1. Independent laboratory validation

Analytical method 3

Method validation

|  |  |
| --- | --- |
| Comments of zRMS: | Evaluated and accepted in the addendum to the DAR (2012).  No comments. |

|  |  |
| --- | --- |
| Reference: | CP 5.2/4 |
| Report | Independent laboratory validation (ILV) of XXXX method number L0117 for the determination of BAS 650 F in plant materials by LC-MS/MS  Schwarz, T., 2008  report No EU-P/B1507G,EU-250522  XXXX DocID 2008/1037015  Authority registration No |
| Guideline(s): | EEC 91/414 Annex II (Part A Section 4.2), OECD Guidance Document on Pesticide Residue Analytical Methods (ENV/JM/MONO(2007)17 - 13-Aug-07, SANCO/3029/99 rev. 4 (11 July 2000), SANCO/825/00 rev. 7 (17 March 2004) |
| Deviations: | No |
| Previous evaluation: | Yes, evaluated and accepted in DAR (2009)/in the addendum to the DAR (2012) |
| GLP: | Yes |
| Acceptability: | Yes | |

Study Summary

In this study, the method L0117 for the determination of residues of Ametoctradin (BAS 650 F) in several plant matrices (wheat grain, potato tuber, lettuce, orange, and sunflower), was independently validated in a different laboratory, with a limit of quantification of 0.01 mg/kg. A brief description of the method and the results are presented in the summary below.

Materials and methods

Ametoctradin (BAS 650 F) is extracted with a mixture of methanol/water. An aliquot of the extract is centrifuged and partitioned against dichloromethane. The final determination of BAS 650 F is performed by HPLC-MS/MS. Separation is achieved by using an Phenomenex, Synergi Fusion-RP column (150 mm x 4.6 mm) and a gradient of water (0.1% formic acid)/ methanol (0.1% formic acid) at a flow rate of 1.0 mL/min. Detection is accomplished in ESI positive mode for BAS 650 F at mass transition 276 m/z > 176 m/z and 276 m/z > 149 m/z for quantification and confirmation, respectively. Calibration standards are solvent based and were prepared in methanol/water/formic acid (50:50:0.1, v/v/v)

Results and discussions

The analytical method validation is summarised below. The HPLC-MS/MS determination was conducted by monitoring two MS/MS mass transitions and therefore no confirmatory method is required.

Recoveries were obtained by fortification of the matrices and all mean recoveries are within the permitted range required by the guideline SANTE/2020/12830 rev. 1 with the exception of sunflower at the 0.1 mg/kg fortification level which was marginally outside the guideline level.

Table A 39: Recovery results from method validation of ametoctradin using the analytical method

| Matrix | Analyte | Fortification level (mg/kg) (n = x) | Mean  recovery (%) | RSD (%) | Comments |
| --- | --- | --- | --- | --- | --- |
| ***Mass transition 276→176 m/z (Quantification)*** | | | | | |
| Wheat grain | Ametoctradin | 0.01 (n = 5) | 77.4 | 4.4 | Acceptable |
| 0.1 (n = 5) | 73.6 | 0.7 |
| Potato tuber | Ametoctradin | 0.01 (n = 5) | 85.4 | 2.4 | Acceptable |
| 0.1 (n = 5) | 77.8 | 2.5 |
| Lettuce | Ametoctradin | 0.01 (n = 5) | 78.4 | 3.8 | Acceptable |
| 0.1 (n = 5) | 73.8 | 3.6 |
| Orange | Ametoctradin | 0.01 (n = 5) | 98.2 | 5.7 | Acceptable |
| 0.1 (n = 5) | 84.0 | 4.9 |
| Sunflower | Ametoctradin | 0.01 (n = 5) | 76.2 | 3.5 | Acceptable |
| 0.1 (n = 5) | 69.0 | 5.7 |
| ***Mass transition 276→149 m/z (Confirmation)*** | | | | | |
| Wheat grain | Ametoctradin | 0.01 (n = 5) | 75.4 | 4.0 | Acceptable |
| 0.1 (n = 5) | 71.0 | 1.0 |
| Potato tuber | Ametoctradin | 0.01 (n = 5) | 83.8 | 2.6 | Acceptable |
| 0.1 (n = 5) | 76.6 | 2.0 |
| Lettuce | Ametoctradin | 0.01 (n = 5) | 72.8 | 0.6 | Acceptable |
| 0.1 (n = 5) | 73.4 | 3.0 |
| Orange | Ametoctradin | 0.01 (n = 5) | 82.6 | 4.7 | Acceptable |
| 0.1 (n = 5) | 83.0 | 5.1 |
| Sunflower | Ametoctradin | 0.01 (n = 5) | 72.0 | 2.8 | Acceptable |
| 0.1 (n = 5) | 67.6 | 7.2 |

Table A 40: Characteristics for the method used for independant laboratory validation of ametoctradin residues in plant matrices

|  | Ametoctradin |
| --- | --- |
| Specificity | LC-MS/MS is a highly specific method. No interference (> 30 % LOQ) of total peak area for the target analyte at the retention time, was found in unfortified control samples. |
| Calibration (type, number of data points) | Calibration was performed with solvent based standards at six concentrations ranging 0.025 to 5.0 ng/mL and good linearity was observed (r ≥0.998). The resulting test substance peak areas versus theoretical test substance concentration data were fit to the linear function. |
| Calibration range | Accepted calibration range 0.025 ng/mL to 5.0 ng/mL |
| Assessment of matrix effects is presented | Matrix effects were not found to be significant (i.e. they were not greater than 20%, determined in XXXX Doc ID 2015/1257669 above). |
| Solution Stability | Standard solutions stability was not evaluated in the study, but was demonstrated in a separate study (refer to XXXX DocID: 2008/1022139 below). Standards in fortification solvent (methanol) and calibration standards in methanol/water/formic acid (50:50:0.1, v/v/v) is shown to be stable (>80%) at least for 60 days for ametoctradin (BAS 650 F) under refrigerated conditions.  Extract stability was also evaluated in extraction solution [methanol/water (50:50, v/v)] and in final volume solution [methanol/water/formic acid (50:50:0.1, v/v/v)] for 14 days. Ametoctradin (BAS 650 F) was found to be stable (>80%) for at least 14 days under refrigerated conditions. |
| Limit of determination/quantification | The limit of quantification (LOQ) of 0.01 mg/kg was confirmed for ametoctradin. The limit of detection (LOD), defined as the lowest standard employed was 0.025 ng/mL for ametoctradin. |

Conclusion

The analytical procedure of method L0117/01 has been fully validated in terms of specificity, linearity, precision, accuracy, matrix effects, solution stability and LOQ, in accordance with the requirements of SANCO/825/00 rev.7 and meets the requirements of SANTE/2020/12830 rev.1 also. It is suitable as a monitoring method for determination of residues of ametoctradin (BAS 650 F) in the plant matrices types analysed.

* + - * 1. Analytical method 4

Method validation

|  |  |
| --- | --- |
| Comments of zRMS: | The method has been accepted.  The objective of this study was to validate the analytical method L0117/02 for the determination of Ametoctradin in dried hops. The LOQ was 0.010 mg/kg, using LC-MS/MS with two mass transitions. Consequently, no further confirmatory method is required. The interferences of the analyte measured in the control samples were below 20 % of the LOQ for both mass transitions.  The mean recovery values were between 89.7 % and 94.6 % of the nominal values.  The relative standard deviations (RSD, %) for all fortification levels were below 7.2%. It could be demonstrated that the method L0117/02 fulfils the requirements regarding specificity, repeatability, limit of quantification, recoveries and linearity and is therefore applicable to correctly determine residues of BAS 650 F in dried hops consistently with SANTE/2020/12830 Rev. 1. |

|  |  |
| --- | --- |
| Reference: | CP 5.2/5 |
| Report | Validation of XXXX analytical method L0117/02 for the determination of BAS 650 F (Ametoctradin) in dried hops  Richter, S., 2016  report No EU-P4029G,EU-772934,P 4029 G  2016/1271225  Authority registration No |
| Guideline(s): | EPA 860.1340 (1996), OECD-ENV/JM/MONO/(2007)17, SANCO/825/00 rev. 8.1 (16 November 2010) |
| Deviations: | No |
| Previous evaluation: | No |
| GLP: | Yes  (certified by Landesanstalt fuer Umwelt, Messungen und Naturschutz Baden-Wuerttemberg, Karlsruhe, Germany ), |
| Acceptability: | Yes | |

Study Summary

In this study, the method L0117/02 for the determination of residues of Ametoctradin (BAS 650 F) in hops was validated with a limit of quantification of 0.01 mg/kg. A brief description of the method and the results are presented in the summary below.

Materials and methods

In the analytical method L0117/02, residues of ametoctradin (BAS 650 F) were extracted from crop commodities (hops cones (dried) (representing a difficult matrix)) using methanol/water (70/30, v/v) followed by cleaning under alkaline conditions using liquid/liquid partition against dichloromethane. An aliquot of the organic phase was concentrated, residues redissolved in acetonitrile and analysis performed by LC-MS/MS. Separation is achieved by using a Phenomenex Synergi Hydro column (150 mm x 3.0 mm, 4 µm) and a gradient of water and acetonitrile, each acidified with 0.1% formic acid at a flow rate of 0.8 mL/min. Detection was accomplished in ESI positive mode at mass transitions 276 m/z → 176 m/z for quantification and 276 m/z → 149 m/z for qualification. Calibration standards were prepared as matrix-matched standards.

Results and discussions

The mean recovery values were between 70 and 120% with relative standard deviations (RSDs) of ≤ 20%. Due to the high specificity of LC-MS/MS using two mass transitions, a confirmatory method is not necessary. The detailed results are given in the table below.

**Table A 41: Recovery results from method validation of ametoctradin in crop commodities using the analytical method L0117/02**

| **Matrix** | **Analyte** | **Fortification level (mg/kg) (*n* = x)** | **Mean  recovery (%)** | **RSD (%)** | **Comments** |
| --- | --- | --- | --- | --- | --- |
| Hops  (dried cones) | Ametoctradin | 0.01 (n=5) | 94.6 | 4.5 | 276 m/z → 176 m/z Quantifier |
| 0.1 (n=5) | 89.7 | 1.4 |
| 0.01 (n=5) | 93.5 | 7.2 | 276 m/z → 149 m/z Qualifier |
| 0.1 (n=5) | 90.0 | 1.5 |

**Table A 42: Characteristics for the analytical method used for validation of ametoctradin in crop commodities**

|  | **Ametoctradin** |
| --- | --- |
| Specificity | LC-MS/MS, using two mass transitions, is a highly specific detection technique and therefore a confirmatory technique is not required. No interfering peaks were found at the retention time for phosphorous acid. Mass spectra are provided. |
| Calibration (type, number of data points) | Calibration standards were prepared as matrix-matched calibration standards. Eight calibration points were used. The calibration function of the analyte was linear and a correlation coefficient of ≥ 0.99 was obtained.  Typical line of best fit:  mass transition 276→ 176 m/z  Slope = 2.78 x 105  Intercept = 1.64 x 103  r = 0.9997  mass transition 276→ 149 m/z  Slope = 2.78 x 105  Intercept = 4.73 x 103  r = 0.9993 |
| Calibration range | Standards in the range of 0.02 ng/mL to 2.0 ng/mL were used. |
| Assessment of matrix effects is presented | The experiment to evaluate any potential matrix effects showed that the matrix load in the samples had a significant influence on analysis (matrix effects >20%); therefore, the validation samples were analyzed using matrix-matched calibration standard solutions. |
| Standard Stability | The analyte indicates sufficient stability in stock (methanol) / fortification (methanol) as well as in calibration solutions (acetonitrile/water 1/9) after storage at ~ 8°C for 30 days. |
| Extract Stability | The analyte indicates sufficient stability in final extracts for 7 days and in raw extracts for 4 days. |
| Limit of determination/quantification | The limit of quantification (LOQ) defined by the lowest successfully tested fortification level is 0.01 mg/kg. The limit of detection (LOD) is 0.02 ng/mL (0.002 mg/kg) at test sample level, corresponding to the lowest calibration level used. |

**Conclusion**

The analytical method L0117/02 met the requirements of SANCO/825/00 rev. 8.1 is valid for the analysis of ametoctradin in crop commodities (hops cones (dried) (representing a difficult matrix)). The requirements for method validation under SANTE/2020/12830 Rev. 1 were also met. Independent laboratory validation is provided in XXXX Doc ID 2019/2051445 below.

Analytical method 5

Method validation

The study below outlines the independent method validation for analytical method L0117/01 for the determination of ametoctradin in pea (dried seed) and for analytical method L0117/02 for the determination of ametoctradin in hops (dried cones).

|  |  |
| --- | --- |
| Comments of zRMS: | The methods have been accepted.  The purpose of the study was to validate the XXXX analytical methods L0117/01 in dried peas and L0117/02 in dried hops cones for the determination of BAS 650 F by LC-MS/MS.  The methods L0117/01 and L0117/02 are suitable to determine BAS 650 F in dried peas and dried hop cones at a LOQ of 0.010 mg/kg. The mean recovery values were between 70% and 110% for both mass transitions for both test systems. The relative standard deviations (RSD, %) for all fortification levels were below 20%.  All requirements were met consistently with SANTE/2020/12830 Rev. 1. |

|  |  |
| --- | --- |
| Reference: | CP 5.2/6 |
| Report | Independent Method Validation of XXXX Analytical Method for the Determination of BAS 650 F in Dried Peas (L0117/01) and Hops (L0117/02) by LC-MS/MS  Homazava, N., 2019  report No 872723, 20190101  XXXX Doc ID: 2019/2051445  Authority registration No |
| Guideline(s): | EPA 860.1340 (1996), OECD-ENV/JM/MONO/(2007)17, SANCO/3029/99 rev. 4 (11 July 2000), SANCO/825/00 rev. 8.1 (16 November 2010) |
| Deviations: | No |
| GLP: | yes |
| Acceptability: | Yes | |

**Materials and methods**

In the analytical method L0117/01, residues of ametoctradin (BAS 650 F, Reg.No. 4993353) were extracted from pea(dried seeds) using methanol/water (50/50, v/v).

In the analytical method L0117/02, residues of ametoctradin (BAS 650 F, Reg.No. 4993353) were extracted from hops cones (dried) using methanol/water (70/30, v/v).

For both matrices, samples were cleaned up using liquid/liquid partition against dichloromethane. An aliquot of the organic phase was then concentrated. For dried peas the residue was taken up in methanol:water:formic acid (500:500:1 v/v/v) while those for hops were redissolved in acetonitrile prior to analysis performed by LC-MS/MS. Separation was achieved by using a Synergi Hydro RP column (150 mm x 3.0 mm, 4 µm) and a gradient of water and acetonitrile, each acidified with 0.1% formic acid at a flow rate of 0.8 mL/min. Detection was accomplished in ESI positive mode at mass transitions 276 m/z → 176 m/z for quantification and 276 m/z → 149 m/z for qualification. Calibration standards were prepared as matrix-matched standards.

**Results and discussions**

The mean recovery values were between 70 and 120% with relative standard deviations (RSDs) of ≤ 20%. Due to the high specificity of LC-MS/MS using two mass transitions, a confirmatory method is not necessary. The detailed results are given in the table below.

**Table A 43: Recovery results from method validation of ametoctradin in crop commodities using the analytical methods L0117/01 and L0117/02**

| **Matrix** | **Analyte** | **Fortification level (mg/kg) (*n* = x)** | **Mean  recovery (%)** | **RSD (%)** | **Comments** |
| --- | --- | --- | --- | --- | --- |
| Pea (dried seed) | Ametoctradin | 0.01 (n=5) | 97.7 | 2.9 | 276 m/z → 176 m/z Quantifier |
| 0.1 (n=5) | 103 | 1.8 |
| 0.01 (n=5) | 96.7 | 3.4 | 276 m/z → 149 m/z Qualifier |
| 0.1 (n=5) | 104 | 1.9 |
| Hops  (dried cones) | Ametoctradin | 0.01 (n=5) | 95.2 | 1.6 | 276 m/z → 176 m/z Quantifier |
| 0.1 (n=5) | 90.6 | 2.7 |
| 0.01 (n=5) | 96.4 | 3.6 | 276 m/z → 149 m/z Qualifier |
| 0.1 (n=5) | 91.5 | 3.2 |

**Table A 44: Characteristics for the analytical method used for validation of ametoctradin in peas (dried seed) and hops**

|  | **Ametoctradin** |
| --- | --- |
| Specificity | LC-MS/MS, using two mass transitions, is a highly specific detection technique and therefore a confirmatory technique is not required. No interfering peaks were found at the retention time for ametoctradin. Mass spectra are provided. |
| Calibration (type, number of data points) | Calibration standards were prepared as matrix-matched calibration standards. Seven calibration points were used. The calibration function of the analyte was linear and a correlation coefficient of ≥ 0.99 was obtained.  Typical line of best fit:  Peas (dried seed)  mass transition 276→ 176 m/z  Slope = 2.8 x 106  Intercept = 2.4 x 104  r = 0.9989  mass transition 276→ 149 m/z  Slope = 2.68 x 106  Intercept = 2.36 x 104  r = 0.9990  Hops (dried cones)  mass transition 276→ 176 m/z  Slope = 1.19 x 106  Intercept = -1.73 x 104  r = 0.9989  mass transition 276→ 149 m/z  Slope = 1.1 x 106  Intercept = -1.43 x 104  r = 0.9988 |
| Calibration range | Standards in the range of 0.025 ng/mL to 2.5 ng/mL were used. |
| Assessment of matrix effects is presented | The experiment to evaluate any potential matrix effects showed that the matrix load in the samples had a significant influence on analysis (matrix effects >20%) for hops (dried cones), but not for pea (dried seed); nevertheless, the validation samples were analyzed using matrix-matched calibration standard solutions. |
| Standard Stability | Stock, fortification and calibration solutions were found to be stable for 30 days when stored refrigerated (2 – 8 °C) in the dark |
| Extract Stability | The analyte indicates sufficient stability in final extracts for 7 & 9 days for dried peas and hops respectively and in final volume samples for 8 & 13 days respectively. |
| Limit of determination/quantification | The limit of quantification (LOQ) defined by the lowest successfully tested fortification level is 0.01 mg/kg. The limit of detection (LOD) is 0.025 ng/mL (0.0025 mg/kg) at test sample level, corresponding to the lowest calibration level used. |

**Conclusion**

The analytical methods L0017/01 & L0117/02 met the requirements of SANCO/825/00 rev. 8.1 and are valid for the analysis of ametoctradin in crop commodities (peas (dried seeds) and hops cones (dried)). The requirements for method validation under SANTE/2020/12830 Rev. 1 were also met.

Extraction efficiency

Extraction efficiency was investigated in a separate study (XXXX DocID 2008/1037092) which is already peer-reviewed in the DAR for the evaluation of ametoctradin (The Netherlands, 2009). Please refer to Table 5.3-3 above.

* + - * 1. Analytical method 6

Method validation

A QuEChERS method R0077/01 (XXXX DocID 2020/2036124) for the determination of Ametoctradin residues in food of plant origin is presented below.

|  |  |
| --- | --- |
| Comments of zRMS: | The method has been accepted.  The objective of the study was to validate XXXX analytical method No. R0077/01, a multi-residue method based on the European Standards QuEChERS method (EN 15562) and used for the determination of ametoctradin (BAS 650 F) residues in crop commodities. The residues were determined by LC positive ion electrospray ionization tandem mass spectrometry (MS/MS-ESI), monitoring ion transitions m/z 276→149 and 276→176 for primary and confirmatory quantitation, respectively.  For validation, untreated crop commodities samples - apple fruit, grape fruit, wheat grain, kidney bean (dried seed), canola dried seed, representing high water, high acid, high starch, high protein, and high oil plant matrices, respectively - were fortified with ametoctradin and analyzed according to the established method validation guidelines. The typical analytical set consisted of a reagent blank, two controls, five replicates fortified with ametoctradin at the method limit of quantitation, and five replicates fortified at a higher level, corresponding to 10X the limit of quantitation. The two mass transitions used for monitoring for ametoctradin were evaluated. The LOQ for ametoctradin residues in crop commodities was 0.01 mg/kg. In the subject study, matrix-matched standards were also analyzed to evaluate any potential matrix effects.  Mean overall recoveries from crop commodity samples fortified with ametoctradin at 0.01 and 0.1 mg/kg ranged from 101 to 109% (RSD, ≤4%), considering results obtained using both the primary and secondary transitions (n=10/ transition/matrix).  XXXX Analytical Method No. R0077/01 fulfils the requirements regarding specificity, repeatability, limit of quantification, and recoveries and is, therefore, applicable to correctly determine ametoctradin residues in crop commodity samples |

|  |  |
| --- | --- |
| Reference: | CP 5.2/7 |
| Report | Validation of an analytical method (R0077/01, QuEChERS) for the determination of BAS 650 F in plant matrices  Andrews, R., 2020  report No 809021  XXXX DocID 2020/2036124  Authority registration No |
| Guideline(s): | OECD-ENV/JM/MONO/(2007)17, US EPA OPPTS 860.1340, SANCO/3029/99 (11 July 2000), SANCO/825/00 rev. 8.1 (16/11/2010) |
| Deviations: | No |
| Previous evaluation: | No |
| GLP: | Yes  (Laboratory certified by US EPA GLP Officials) |
| Acceptability: | Yes | |

**Study Summary**

The objective of this validation study was to demonstrate the applicability and repeatability of XXXX analytical method No. R0077/01, a multi-residue method based on the European Standards QuEChERS method (DIN EN 15662:2018 Foods of plant origin, Multimethod), to determine residues of Ametoctradin in plant matrices by LC-MS/MS. with a limit of quantification at 0.01 mg/kg. This method is also intended to be used as multiresidue methods using only Ametoctradin as an analyte. The brief description of the method and the results are presented in the summary below.

Materials and methods

QuEChERS analytical method R0077/01 was validated to determine residues of Ametoctradin in crop commodities by LC-MS/MS. Samples (after hydration for dry matrices) were extracted by mechanical shaking with acetonitrile. The extract was partitioned using a mixture of QuEChERS salts (MgSO4, NaCl, trisodium citrate, and disodium hydrogencitrate) and an aliquot of the resulting organic layer was treated with MgSO4 and dispersive SPE material (primary-secondary amine, or PSA). An aliquot of the extract was then diluted in methanol/water/formic acid (50/50/0.1, v/v/v) and the residues were determined by liquid chromatography (LC) positive ion electrospray ionization tandem mass spectrometry (MS/MS-ESI), monitoring ion transitions m/z 276→149 and m/z 276→176 for primary and confirmatory quantitation, respectively. Separation is accomplished with a Acquity HSS T3 column (100 mm x 2.1 mm, 1.8 µm) by using a gradient mixture of water/methanol, each acidified with 0.1% formic acid at a flow rate of 1.0 mL/min.

**Results and discussions**

The analytical method validation is summarised below. The HPLC-MS/MS determination was conducted by monitoring two MS/MS mass transitions and therefore no confirmatory method is required.

Recoveries were obtained by fortification of the matrices and all mean recoveries are within the permitted range required by the guideline SANTE/2020/12830 rev. 1.

Table A 45: Recovery results from method validation of Ametoctradin using the analytical method

| Matrix | Analyte | Fortification level (mg/kg) (n = x) | Mean  recovery (%) | RSD (%) | Comments |
| --- | --- | --- | --- | --- | --- |
| ***Mass transition 276→149 m/z (Quantification)*** | | | | | |
| Grape (fruit) | Ametoctradin | 0.01 (n = 5) | 106 | 1.5 | Acceptable |
| 0.1 (n = 5) | 107 | 1.0 |
| Apple (fruit) | Ametoctradin | 0.01 (n = 5) | 100 | 1.0 | Acceptable |
| 0.1 (n = 5) | 104 | 1.9 |
| Wheat (grain) | Ametoctradin | 0.01 (n = 5) | 105 | 1.5 | Acceptable |
| 0.1 (n = 5) | 102 | 2.4 |
| Canola (dried seed) | Ametoctradin | 0.01 (n = 5) | 103 | 1.4 | Acceptable |
| 0.1 (n = 5) | 98 | 4.3 |
| Kidney bean (dried seed) | Ametoctradin | 0.01 (n = 5) | 113 | 2.3 | Acceptable |
| 0.1 (n = 5) | 105 | 1.8 |
| ***Mass transition 276→176 m/z (Confirmation)*** | | | | | |
| Grape (fruit) | Ametoctradin | 0.01 (n = 5) | 107 | 1.1 | Acceptable |
| 0.1 (n = 5) | 107 | 0.4 |
| Apple (fruit) | Ametoctradin | 0.01 (n = 5) | 100 | 1.0 | Acceptable |
| 0.1 (n = 5) | 104 | 1.9 |
| Wheat (grain) | Ametoctradin | 0.01 (n = 5) | 105 | 1.9 | Acceptable |
| 0.1 (n = 5) | 103 | 2.7 |
| Canola (dried seed) | Ametoctradin | 0.01 (n = 5) | 104 | 2.0 | Acceptable |
| 0.1 (n = 5) | 98 | 4.4 |
| Kidney bean (dried seed) | Ametoctradin | 0.01 (n = 5) | 112 | 1.9 | Acceptable |
| 0.1 (n = 5) | 106 | 0.5 |

Table A 46: Characteristics for the method used for validation of Ametoctradin residues in plant matrices

|  | Ametoctradin |
| --- | --- |
| Specificity | LC-MS/MS is a highly specific method. No interference (> 30 % LOQ) of total peak area for the target analyte at the retention time, was found in unfortified control samples. |
| Calibration (type, number of data points) | Calibration was performed with solvent based standards at a minimum of five concentrations ranging 0.02 to 1.0 ng/mL and good linearity was observed (r ≥0.99). The resulting test substance peak areas versus theoretical test substance concentration data were fit to the linear function.  Calibration data  Mass transition 276→ 149 m/z  Slope = 2.52 x 106 , Intercept = 1.47 x 103 (r = 1.0000)  Mass transition 276→ 176 m/z  Slope = 2.5 x 106 , Intercept = -617 (r = 1.0000) |
| Calibration range | 0.02 ng/mL to 1.0 ng/mL |
| Assessment of matrix effects is presented | Matrix effects were not found to be significant (i.e. they were not greater than 20%). |
| Solution Stability | Ametoctradin was previously shown to be stable in standards prepared in methanol and in calibration standards solution prepared by serial dilution of the intermediate standards using methanol/water/formic acid (50/50/0.1, v/v/v), for at least 2 months (60 days), each when held under refrigeration (refer to XXXX DocID 2008/1022139). During the course of this study, the test/reference substance solutions were stored in a refrigerator and all solutions were used within the demonstrated time period of stability.  The method validation fortification sample extracts were analyzed within 1 day of extraction. Acceptable method recoveries obtained during analysis demonstrate the storage stability of ametoctradin residues in the extracts in the brief period prior to analysis. In addition, the recoveries from stored solutions generated during extract stability experiments performed in conjunction with this study, which included tests on the initial extracts and HPLC final volume stored under refrigeration, indicate that Ametoctradin is stable in crop matrix extracts (raw extracts and final volumes) for at least the time period tested, approximately 2 weeks (12-14 days). |
| Limit of determination/quantification | The limit of quantification (LOQ) of 0.01 mg/kg was confirmed for ametoctradin. The limit of detection (LOD), defined as the lowest standard employed was 0.002 mg/kg for ametoctradin. |

Conclusion

The analytical procedure of method R0077/01 has been fully validated in terms of specificity, linearity, precision, accuracy, matrix effects, solution stability and LOQ, in accordance with the requirements of SANCO/825/00 rev.8.1 for determination of residues of ametoctradin (BAS 650 F) in the plant matrices types analysed The method meets the requirements of SANTE/2020/12830 rev.1 also.

Please note that an ILV for method R0077/01 is ongoing and is expected in Q3 2023.

Extraction efficiency

Extraction efficiency of the method was submitted as part of the renewal of approval process for Ametoctradin (RAR). Please refer to this evaluation.

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

The validation study for the method L0104/01 (XXXX DocID 2008/1022140) is already peer-reviewed. Due to additional generated validation data (amendment no. 1, XXXX DocID 2015/1258815), and for reasons of completeness the whole data are reported below.

* + - * 1. Analytical method 7

Method validation

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Comments of zRMS: | The study already peer-reviewed.  No comments. | | | |
| Reference: | | CP 5.2/8 |
| Report | | Validation of XXXX method L0104/01: Method for the determination of BAS 650 F and its metabolites M650F01 and M650F06 in animal matrices  Schweda Z., Mackenroth C., 2008  report No 250519  XXXX DocID 2008/1022140  Authority registration No |
| Guideline(s): | | SANCO/825/00 rev. 7 (17 March 2004), SANCO/3029/99 rev. 4 (11 July 2000) |
| Deviations: | | No |
| Previous evaluation: | | Yes, DAR (The Netherlands, 2010) |
| GLP: | | Yes  (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany) |
| Acceptability: | | Yes | |

|  |  |
| --- | --- |
| Comments of zRMS: | The amendment has been accepted.  Additional information was provided to demonstrate the influence of matrix load on the analysis using quality control samples. Detailed appendix tables show the raw data of quality control samples, extract and standard stability. Additional information regarding linearity, ionization mode, calibration curve and chromatograms of the qualifier mass transition are shown. Additional information was required for re-registration purposes according to SANCO/825/00 rev 8.1 and SANCO/3029/99 rev. 4. |

|  |  |
| --- | --- |
| Reference: | CP 5.2/9 |
| Report | Report Amendment 1: Validation of XXXX method L0104/01: Method for the determination of BAS 650 F and its metabolites M650F01 and M650F06 in animal matrices  Schweda Z., Mackenroth C., Studenroth S., 2016  report No 250519  XXXX DocID 2015/1258815  Authority registration No |
| Guideline(s): | SANCO/825/00 rev. 7 (17 March 2004), SANCO/3029/99 rev. 4 (11 July 2000) |
| Deviations: | No |
| Previous evaluation: | No |
| GLP: | Yes  (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany) |
| Acceptability: | Yes | |

Materials and methods

In method L0104/01, residues of Ametoctradin (BAS 650 F) and its metabolites, M650F01 and M650F06 are extracted from animal matrices using a mixture of methanol-water (50:50, v/v). An aliquot of the extract is centrifuged, and the supernatant is cleaned by solid phase extraction with a cation exchange column (Strata X-C). The final determination of Ametoctradin, M650F01 and M650F06 is performed by HPLC-MS/MS.

Separation is achieved by using a Phenomenex, Synergi Fusion-RP column (150 mm x 4.6 mm) and a gradient of water (0.1% formic acid)/ methanol (0.1% formic acid) at a flow rate of 1.0 mL/min.

Detection is accomplished in ESI positive mode using two different transitions. For parent, Ametoctradin, mass transitions at 276 m/z → 176 m/z and 276 m/z → 149 m/z is used for quantification and confirmation, respectively. For metabolite, M650F001, mass transitions at 250 m/z → 176 m/z and 250 m/z → 149 m/z is used for quantification and confirmation, respectively. For metabolite, M650F006, mass transitions at 278 m/z → 217 m/z and 278 m/z → 176 m/z is used for quantification and confirmation, respectively Calibration standards are solvent based and were prepared in methanol/water/formic acid (50:50:0.1, v/v/v).

**Results and discussions**

The mean recovery values were between 70 and 110% with relative standard deviations (RSDs) of < 20%. Due to the high specificity of LC-MS/MS using two mass transitions a confirmatory method is not necessary. The detailed results are given in the table below.

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Table A 47 Validation results of method L0104/01: Ametoctradin and metabolites in animal matrices | | | | | | | | | | |
| **Matrix** | **Analyte** | **No. of tests** | **Fortification level [mg/kg]** | **1. Transition \*** | | | **2. Transition \*\*** | | |
| **mean [%]** | **SD [+/-]** | **CV [%]** | **mean [%]** | **SD [+/-]** | **CV [%]** |
| Cow, liver | Ametoctradin | 5 | 0.01 | 75.9 | 1.0 | 1.3 | 75.6 | 1.0 | 1.3 |
| 5 | 0.1 | 73.7 | 1.4 | 1.9 | 73.8 | 1.0 | 1.4 |
| M650F01 | 5 | 0.01 | 95.1 | 2.5 | 2.6 | 93.9 | 1.1 | 1.2 |
| 5 | 0.1 | 91.8 | 1.5 | 1.6 | 90.9 | 1.2 | 1.3 |
| M650F06 | 5 | 0.01 | 80.9 | 2.4 | 2.9 | 79.2 | 2.1 | 2.7 |
| 5 | 0.1 | 81.6 | 0.9 | 1.1 | 78.7 | 2.1 | 2.7 |
| Cow, kidney | Ametoctradin | 5 | 0.01 | 74.4 | 1.0 | 1.3 | 75.0 | 1.6 | 2.1 |
| 5 | 0.1 | 74.6 | 2.1 | 2.9 | 75.5 | 1.0 | 1.4 |
| M650F01 | 5 | 0.01 | 84.3 | 3.9 | 4.6 | 85.1 | 1.9 | 2.2 |
| 5 | 0.1 | 84.4 | 2.6 | 3.1 | 85.8 | 4.5 | 5.3 |
| M650F06 | 5 | 0.01 | 74.7 | 0.7 | 1.0 | 75.9 | 4.0 | 5.3 |
| 5 | 0.1 | 78.6 | 9.3 | 11.8 | 76.6 | 6.1 | 7.9 |
| Cow, muscle | Ametoctradin | 5 | 0.01 | 75.2 | 2.0 | 2.6 | 75.7 | 1.1 | 1.5 |
| 5 | 0.1 | 74.9 | 2.4 | 3.2 | 74.8 | 1.3 | 1.8 |
| M650F01 | 5 | 0.01 | 83.4 | 2.4 | 2.9 | 86.5 | 2.8 | 3.3 |
| 5 | 0.1 | 79.5 | 3.5 | 4.4 | 80.6 | 5.2 | 6.4 |
| M650F06 | 5 | 0.01 | 78.6 | 2.8 | 3.5 | 80.8 | 2.2 | 2.8 |
| 5 | 0.1 | 77.9 | 1.9 | 2.4 | 77.9 | 3.9 | 5.0 |
| Cow, fat | Ametoctradin | 5 | 0.01 | 81.8 | 5.9 | 7.3 | 80.3 | 2.4 | 3.0 |
| 5 | 0.1 | 78.3 | 3.3 | 4.2 | 76.5 | 2.3 | 3.0 |
| M650F01 | 5 | 0.01 | 90.0 | 3.1 | 3.4 | 93.0 | 4.6 | 4.9 |
| 5 | 0.1 | 87.5 | 4.2 | 4.8 | 88.4 | 2.5 | 2.8 |
| M650F06 | 5 | 0.01 | 85.7 | 1.3 | 1.6 | 89.6 | 4.3 | 4.8 |
| 5 | 0.1 | 86.8 | 4.7 | 5.4 | 91.2 | 3.1 | 3.4 |
| Cow, milk | Ametoctradin | 5 | 0.01 | 84.7 | 3.4 | 4.0 | 85.5 | 4.1 | 4.8 |
| 5 | 0.1 | 79.8 | 2.6 | 3.2 | 83.2 | 1.2 | 1.4 |
| M650F01 | 5 | 0.01 | 88.0 | 4.2 | 4.8 | 89.9 | 3.7 | 4.1 |
| 5 | 0.1 | 83.6 | 2.8 | 3.3 | 85.2 | 3.7 | 4.3 |
| M650F06 | 5 | 0.01 | 90.2 | 1.9 | 2.2 | 91.4 | 4.6 | 5.0 |
| 5 | 0.1 | 87.5 | 2.4 | 2.8 | 88.1 | 3.6 | 4.1 |
| Cow, cream | Ametoctradin | 5 | 0.01 | 78.6 | 1.0 | 1.3 | 80.8 | 4.4 | 5.5 |
| 5 | 0.1 | 76.1 | 4.5 | 5.9 | 78.0 | 3.2 | 4.1 |
| M650F01 | 5 | 0.01 | 82.6 | 4.3 | 5.2 | 84.8 | 6.5 | 7.6 |
| 5 | 0.1 | 82.1 | 7.5 | 9.1 | 85.5 | 4.1 | 4.8 |
| M650F06 | 5 | 0.01 | 81.8 | 3.3 | 4.0 | 87.2 | 4.6 | 5.3 |
| 5 | 0.1 | 88.2 | 3.0 | 3.4 | 85.4 | 2.7 | 3.1 |
| Hen,  egg | Ametoctradin | 5 | 0.01 | 88.5 | 2.5 | 2.8 | 87.4 | 2.3 | 2.6 |
| 5 | 0.1 | 88.2 | 3.1 | 3.5 | 88.8 | 3.6 | 4.1 |
| M650F01 | 5 | 0.01 | 89.5 | 2.6 | 3.0 | 88.2 | 2.1 | 2.4 |
| 5 | 0.1 | 91.5 | 2.7 | 3.0 | 90.4 | 1.7 | 1.9 |
| M650F06 | 5 | 0.01 | 86.7 | 2.0 | 2.3 | 87.4 | 2.1 | 2.4 |
| 5 | 0.1 | 90.1 | 1.2 | 1.3 | 90.2 | 2.3 | 2.6 |

**\*1. Transition (for quant.)** Ametoctradin: 276 m/z → 176 m/z \*\***2. Transition** 276 m/z → 149 m/z

M650F03: 222 m/z → 176 m/z 222 m/z → 121 m/z

M650F04: 208 m/z → 170 m/z 208 m/z → 123 m/z

Table A 48: Characteristics for the analytical method used for validation of Ametoctradin residues in animal matrices

|  | Ametoctradin |
| --- | --- |
| Specificity | LC-MS/MS, using two mass transitions is a highly specific detection technique and therefore a confirmatory technique is not required. No interfering peaks were found at the retention time of concern for each analyte. Mass spectra are provided. |
| Calibration (type, number of data points) | Calibration standards (solvent based) were used for the analysis. At least six calibration points were used, and individual calibration data was presented in the study report. Linear correlations with coefficients >0.99 were obtained for Ametoctradin and its metabolites, M650F01 and M650F06. |
| Calibration range | Standards in the range of 0.025 to 2.5 ng/mL were injected and the response was plotted against the concentration. |
| Standard solution and extract stability | Standard solutions and extract stability were evaluated in the study (Amendment1; XXXX DocID. 2015/1258815). Standards in fortification solvent (methanol) and calibration standards in methanol/water/formic acid (50:50:0.1, v/v/v) is shown to be stable (>80%) at least for 28 days for Ametoctradin and its metabolites, M650F01 and M650F06 under refrigerated conditions. Extract stability was also evaluated in extraction solution [methanol/water (50:50, v/v)] and in final volume solution [methanol/water/formic acid (50:50:0.1, v/v/v)] for 9 days. The results shown that Ametoctradin and its metabolites, M650F01 and M650F06 were stable (>80%) for at least 9 days under refrigerated conditions. |
| Assessment of matrix effects | Matrix effects were evaluated in the study (Amendment1; XXXX DocID. 2015/1258815). No significant matrix effects (<20%) were observed |
| Limit of determination/quantification | The limit of quantification (LOQ) defined by the lowest successfully tested fortification level was 0.01 mg/kg for all analytes. The LOD was not defined in the study, but the lowest standard (0.025 ng/mL) was used for calibration. |

Conclusion

The method L0104/01 was validated according to the requirements of SANCO/825/00 rev. 7 and SANCO/3029/99 rev. 4. It also meets the requirements of SANTE/2020/12830 Rev.1 for the analysis of Ametoctradin and its metabolites M650F01 and M650F06 in animal matrices.

Independent laboratory validation

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Comments of zRMS: | Evaluated already in DAR (The Netherlands, 2010).  No comments. | | | |
| Reference: | | CP 5.2/10 |
| Report | | Independent laboratory validation of XXXX analytical method L0104 for the determination of BAS 650 F, M650F01, and M650F06 in bovine milk, liver, kidney, fat, and eggs by HPLC-MS/MS  Macdougall J., 2008  report No 250516  XXXX DocID. 2008/1022841  Authority registration No |
| Guideline(s): | | SANCO/825/00 rev. 7 (17 March 2004); SANCO/3029/99 rev. 4 (11 July 2000); EEC 91/414 Annex II (Part A Section 4); EEC 91/414 Annex III (Part A Section 5); EPA 860.1340 |
| Deviations: | | No |
| Previous evaluation: | | Yes, DAR (The Netherlands, 2010) |
| GLP: | | Yes  (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany) |
| Acceptability: | | Yes | |

**Materials and methods**

In method L0104/01, residues of BAS 650 F and its metabolites, M650F01 and M650F06 are extracted from animal matrices using a mixture of methanol:water (50:50, v/v). An aliquot of the extract is centrifuged, and the supernatant is cleaned by solid phase extraction with a cation exchange column (Strata X-C). The final determination of BAS 650 F, M650F01 and M650F06 is performed by HPLC-MS/MS.

Separation is achieved by using a Phenomenex, Synergi Fusion-RP column (150 mm x 4.6 mm) and a gradient of water (0.1% formic acid)/ methanol (0.1% formic acid) at a flow rate of 1.0 mL/min.

Detection is accomplished in ESI positive mode using two different transitions. For parent, BAS 650 F, mass transitions at 276 m/z → 176 m/z and 276 m/z → 149 m/z is used for quantification and confirmation, respectively. For metabolite, M650F001, mass transitions at 250 m/z → 176 m/z and 250 m/z → 149 m/z is used for quantification and confirmation, respectively. For metabolite, M650F006, mass transitions at 278 m/z → 217 m/z and 278 m/z → 176 m/z is used for quantification and confirmation, respectively Calibration standards are solvent based and were prepared in methanol/water/formic acid (50:50:0.1, v/v/v).

**Results and discussions**

The mean recovery values were between 70 and 110% with relative standard deviations (RSDs) of < 20%, for primary quantitation ion. The analysis was repeated for confirmation, only for milk, liver and egg. The mean recovery values from egg analysis were only acceptable for all analytes, between 70 and 110% with relative standard deviations (RSDs) of < 20%. For milk confirmatory analysis, only M650F01 and M650F06 provided acceptable data (mean recoveries were between 70 and 110% with relative standard deviations (RSDs) of < 20%). For liver confirmatory analysis, only BAS 650 F and M650F01 provided acceptable data (mean recoveries were between 70 and 110% with relative standard deviations (RSDs) of < 20%). Due to the high specificity of LC-MS/MS using two mass transitions a confirmatory method is not necessary. The detailed results from both analyses are provided in tables below.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Table A 49 Validation results of method L0104/01: Ametoctradin and metabolites in animal matrices** | | | | | | | |
| **Matrix** | **Analyte** | **No. of tests** | **Fortification level [mg/kg]** | **1. Transition \*** | | |
| **mean [%]** | **SD [+/-]** | **RSD [%]** |
| Cow, liver | Ametoctradin | 5 | 0.01 | 72.0 | 5.22 | 7.25 |
| 5 | 0.1 | 86.8 | 7.25 | 8.35 |
| M650F01 | 5 | 0.01 | 99.5 | 5.99 | 6.03 |
| 5 | 0.1 | 106 | 11.2 | 10.6 |
| M650F06 | 5 | 0.01 | 72.8 | 2.99 | 4.10 |
| 5 | 0.1 | 84.4 | 6.62 | 7.84 |
| Kidney | Ametoctradin | 5 | 0.01 | 82.8 | 2.14 | 2.58 |
| 5 | 0.1 | 81.0 | 1.50 | 1.85 |
| M650F01 | 5 | 0.01 | 99.8 | 2.54 | 2.51 |
| 5 | 0.1 | 90.7 | 2.29 | 2.53 |
| M650F06 | 5 | 0.01 | 87.3 | 3.56 | 4.08 |
| 5 | 0.1 | 78.2 | 2.07 | 2.65 |
| Fat | Ametoctradin | 5 | 0.01 | 73.2 | 8.15 | 11.1 |
| 5 | 0.1 | 85.2 | 9.90 | 11.6 |
| M650F01 | 5 | 0.01 | 98.0 | 5.07 | 5.18 |
| 5 | 0.1 | 98.0 | 6.26 | 6.38 |
| M650F06 | 5 | 0.01 | 84.6 | 4.93 | 5.83 |
| 5 | 0.1 | 89.3 | 5.67 | 6.35 |
| Milk | Ametoctradin | 5 | 0.01 | 78.8 | 3.26 | 4.14 |
| 5 | 0.1 | 86.7 | 1.91 | 2.20 |
| M650F01 | 5 | 0.01 | 90.2 | 1.95 | 2.16 |
| 5 | 0.1 | 92.7 | 2.56 | 2.77 |
| M650F06 | 5 | 0.01 | 71.6 | 1.88 | 2.63 |
| 5 | 0.1 | 74.9 | 2.04 | 2.73 |
| Egg | Ametoctradin | 5 | 0.01 | 70.9 | 7.87 | 11.1 |
| 5 | 0.1 | 86.4 | 2.41 | 2.79 |
| M650F01 | 5 | 0.01 | 92.7 | 2.62 | 2.83 |
| 5 | 0.1 | 97.3 | 1.77 | 1.82 |
| M650F06 | 5 | 0.01 | 81.0 | 4.36 | 5.38 |
| 5 | 0.1 | 89.9 | 0.865 | 0.962 |

**\*1. Transition (for quant.)** Ametoctradin: 276 m/z → 176 m/z

M650F03: 222 m/z → 176 m/z

M650F04: 208 m/z → 170 m/z

**Table A 50 Validation results of method L0104/01: Ametoctradin and metabolites in animal matrices (Egg)**

| **Matrix** | **Analyte** | **No. of tests** | **Fortification level [mg/kg]** | **1. Transition \*** | | | **1. Transition \*\*** | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **mean [%]** | **SD [+/-]** | **RSD [%]** | **mean**  **[%]** | **SD [+/-]** | **RSD [%]** |
| Egg | Ametoctradin | 5 | 0.01 | 77.1 | 9.96 | 12.9 | 84.8 | 7.79 | 9.19 |
| 5 | 0.1 | 70.5 | 1.33 | 1.88 | 72.6 | 1.52 | 2.09 |
| M650F01 | 5 | 0.01 | 92.7 | 3.17 | 3.42 | 87.6 | 1.93 | 2.21 |
| 5 | 0.1 | 86.5 | 2.62 | 3.03 | 87.5 | 3.79 | 4.33 |
| M650F06 | 5 | 0.01 | 93.3 | 5.02 | 5.38 | 96.8 | 3.22 | 3.33 |
| 5 | 0.1 | 89.9 | 2.94 | 3.26 | 91.3 | 3.03 | 3.32 |

**\*1. Transition (for quant.)** BAS 650 F: 276.3 → 176.1 \*\***2. Transition (for confirmation)** 276.3 → 149.1

M650F001: 250.2 → 176.1 250.2 → 149.1

M650F006: 278.2 → 217.2 278.2 → 176.1

**Table A 51: Characteristics for the analytical method used for validation of Ametoctradin residues in animal matrices**

|  | **Ametoctradin** |
| --- | --- |
| Specificity | LC-MS/MS, using two mass transitions is a highly specific detection technique and therefore a confirmatory technique is not required. No interference (< 30 % LOQ) of total peak area for the target analyte at the retention time, was found in unfortified control samples. |
| Calibration (type, number of data points) | Calibration standards (solvent based) were used for the analysis. At least six calibration points were used, and individual calibration data was presented in the study report. Linear correlations with coefficients >0.99 were obtained for Ametoctradin and its metabolites, M650F01 and M650F06. |
| Calibration range | Standards in the range of 0.05 to 2.5 ng/mL were injected and the response was plotted against the concentration. |
| Standard solution and extract stability | Standard solutions and extract stability were evaluated in the validation study (refer to XXXX DocID. 2015/1258815). Standards in fortification solvent (methanol) and calibration standards in methanol/water/formic acid (50:50:0.1, v/v/v) is shown to be stable (>80%) at least for 28 days for Ametoctradin (BAS 650 F) and its metabolites, M650F001 and M650F006 under refrigerated conditions.  Extract stability was also evaluated in extraction solution [methanol/water (50:50, v/v)] and in final volume solution [methanol/water/formic acid (50:50:0.1, v/v/v)] for 9 days. The results shown that Ametoctradin (BAS 650 F) and its metabolites, M650F001 and M650F006 were stable (>80%) for at least 9 days under refrigerated conditions. |
| Assessment of matrix effects | Matrix effects were evaluated in the study. No significant matrix effects (<20%) were observed |
| Limit of determination/quantification | The limit of quantification (LOQ) defined by the lowest successfully tested fortification level was 0.01 mg/kg for all analytes. The LOD, defined as the lowest calibration standard was 0.05 ng/mL. |

**Conclusion**

The analytical procedure, method L0104/01, has been fully validated in an independent laboratory in representative animal matrices in terms of specificity, linearity, matrix effects, precision, accuracy and LOQ, in accordance with the requirements of SANCO/825/00 rev. 7 and SANCO/3029/99 rev. 4. It also meets the requirements of SANTE/2020/12830 Rev.1 for the analysis of Ametoctradin and its metabolites M650F01 and M650F06 in animal matrices.

Confirmatory method

No confirmatory technique is required as two different, highly specific mass transitions are used for quantitation and qualification in the primary method as well as in the independent laboratory validation study.

Extraction efficiency

Investigations on the extractability of the residues of ametoctradin in animal matrices with different solvents were performed during the goat metabolism study. Please refer to this evaluation in the DAR (The Netherlands, 2010).

* + - * 1. Analytical method 8

Method R0072/01 is used for the determination of Ametoctradin (BAS 650 F) in honey and pollen. The data can also be used for the determination of another active ingredient, BAS 181 S. It is not relevant here, and the data for the latter active ingredient has not been reported below.

Method validation

|  |  |
| --- | --- |
| Comments of zRMS: | The method has been accepted.  The objective of this validation study was to demonstrate the applicability and repeatability of XXXX Analytical Method No. R0072/01 used for the determination of phosphorous acid residues (BAS 181 S) and residues of ametoctradin (BAS 650 F), including its metabolites M650F003 and M650F004, in pollen and honey by LC-MS/MS.  Following the extraction and clean-up residues of ametoctradin are determined by LC-MS/MS monitoring in the positive mode ion transitions m/z 276→176 (primary quantitation ion) and 276→149 (confirmatory ion) for parent ametoctradin; m/z 222→176 (primary quantitation ion) and 222→ 121 (confirmatory ion) for M650F003; and m/z 208→123 (primary quantitation ion) and 208→95 (confirmatory ion) for M650F004. In lieu of a secondary ion transition for M650F003, for pollen only, confirmatory analysis is performed using an alternate chromatographic technique comprised of a different mobile phase.  For validation, untreated honey and pollen were fortified with ametoctradin, M650F003 and M650F004. The analytical sets typically consisted of a reagent blank, two controls, five replicates fortified at the method limit of quantitation, and five replicates fortified at a higher level, corresponding to 10X the limit of quantitation. The two mass transitions used for monitoring of each analyte. In the subject study, matrix- and solvent-matched standards were analyzed in a separate experiment to evaluate any potential matrix effects. The LOQ and LOD for residues of ametoctradin are 0.01 and 0.002 mg/kg, respectively, in honey and pollen, for each analyte.  Mean overall recoveries from honey and pollen fortified with ametoctradin, M650F003 and M650F004 at 0.01 and 0.1 mg/kg (each) ranged from 73.9 to 90.2% (RSD, 2.1-8.5%), again considering results obtained using the primary and secondary transitions (n=10/analyte/matrix/transition or confirmatory technique).  XXXX Analytical Method No R0072/01 fulfils the requirements with regard to specificity, repeatability, limit of quantification, and recoveries and is, therefore, applicable to correctly determine residues of ametoctradin (including its metabolites M650F003 and M650F004) in the bee matrices, honey and pollen. |

|  |  |
| --- | --- |
| Reference: | CP 5.2/11 |
| Report | Validation of Method R0072/01: Method for the determination of BAS 181 S (Reg.No. 4117133), BAS 650 F (Reg.No. 4993353), M650F003 (Reg.No. 5178870, and M650F004 (Reg.No. 5211623) in pollen and honey by LC-MS/MS  Gordon, B., 2020  report No 886594  XXXX DocID 2020/2032164  Authority registration No |
| Guideline(s): | EPA 860.1340: Residue Chemistry Test Guidelines - Residue Analytical Method, SANCO 3029/99 rev.4, SANCO/825/00 rev. 8.1 (16/11/2010) |
| Deviations: | No |
| Previous evaluation: | No |
| GLP: | Yes  (certified by United States Environmental Protection Agency) |
| Acceptability: | Yes | |

Study Summary

The method R0072/01 was developed and validated for the determination of residues of Ametoctradin (BAS 650 F) and its metabolites M650F003 and M650F004 in honey and pollen by HPLC-MS/MS with a limit of quantification (LOQ) of 0.01 mg/kg. The brief description of the method and the results are presented in the summary below.

Materials and methods

Residues of Ametoctradin in honey samples are extracted using methanol:water (50:50, v/v) and, for pollen samples, sequential extraction using methanol:water (50:50, v/v) followed by methanol. Residues in a separate aliquot of the extract (or combined extract) are cleaned up on a solid phase extraction (SPE) column eluted with buffered methanol, evaporated to dryness, and then re-dissolved and diluted in methanol/water/formic acid (50/50/0.1, v/v/v).

Following the extraction and clean-up described above residues of ametoctradin are determined by LC-MS/MS monitoring in the positive mode ion transitions m/z 276→149 (primary quantitation ion) and 276→176 (confirmatory ion) for parent ametoctradin; m/z 222→176 (primary quantitation ion) and 222→ 121 (confirmatory ion) for M650F03; and m/z 208→123 (primary quantitation ion) and 208→95 (confirmatory ion) for M650F04. In lieu of a secondary ion transition for M650F03, for pollen only, confirmatory analysis is performed using an alternate chromatographic technique comprised of a different mobile phase. The results are calculated by direct comparison of the sample peak responses to those of external standards.

Ametoctradin (BAS 650 F) and its metabolites M650F003 and M650F004 are analysed using an XSelect HSS T3 column (150 mm x 3 mm, 2.5 µm) by using a gradient mixture of acidified water/buffered methanol at a flow rate of 0.6 mL/min.

**Results and discussions**

The mean recovery values were between 70 and 110% with relative standard deviations (RSDs) of < 20%. The detailed results are given in the table below.

Table A 52 Validation results of method R0072/01: Ametoctradin and metabolites M650F003 and M650F004 in honey and pollen

| **Analyte** | **Matrix** | **Mass transition** | **Fortification Level [mg/kg]** | **Number of replicates** | **Mean recovery [%]** | **RSD [%]** | **Overall recovery [%]** | **Overall RSD [%]** |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Ametoctradin | Honey | 276→176 | 0.01 | 5 | 83.6 | 1.8 | 82.2 | 2.3 |
| 0.1 | 5 | 80.9 | 1.1 |
| 276→149 | 0.01 | 5 | 83.0 | 1.7 | 81.7 | 2.1 |
| 0.1 | 5 | 80.5 | 1.1 |
| Pollen | 276→176 | 0.01 | 5 | 89.7 | 4.2 | 89.7 | 3.1 |
| 0.1 | 5 | 89.7 | 2.2 |
| 276→149 | 0.01 | 5 | 89.4 | 2.8 | 89.3 | 2.6 |
| 0.1 | 5 | 89.3 | 2.8 |
| M650F003 | Honey | 222→176 | 0.01 | 5 | 75.8 | 1.9 | 78.7 | 4.3 |
| 0.1 | 5 | 81.6 | 1.9 |
| 222→121 | 0.01 | 5 | 79.4 | 8.2 | 80.3 | 5.8 |
| 0.1 | 5 | 81.1 | 2.8 |
| Pollen | 222→176 | 0.01 | 5 | 89.4 | 4.8 | 90.2 | 4.5 |
| 0.1 | 5 | 91.0 | 4.5 |
| 222→176 1) | 0.01 | 5 | 78.6 | 7.2 | 83.3 | 7.8 |
| 0.1 | 5 | 88.0 | 3.1 |
| M650F004 | Honey | 208→123 | 0.01 | 5 | 73.4 | 3.1 | 73.9 | 2.5 |
| 0.1 | 5 | 74.4 | 1.7 |
| 208→95 | 0.01 | 5 | 79.3 | 4.4 | 78.3 | 5.1 |
| 0.1 | 5 | 77.4 | 5.9 |
| Pollen | 208→123 | 0.01 | 5 | 81.1 | 2.7 | 83.4 | 3.8 |
| 0.1 | 5 | 85.7 | 2.6 |
| 208→95 | 0.01 | 5 | 75.9 | 8.1 | 81.1 | 8.5 |
| 0.1 | 5 | 86.2 | 2.0 |
| RSD = Relative standard deviation  1) Confirmatory chromatographic technique | | | | | | | | |

Table A 53: Characteristics for the analytical method used for validation of Ametoctradin and metabolites M650F003 and M650F004 in honey and pollen

|  | Ametoctradin |
| --- | --- |
| Specificity | LC-MS/MS is a highly specific self-confirmatory technique and was used for determination of the analytes monitoring two characteristic mass transitions for quantification and qualification. Under the described conditions the method is specific for the determination of Ametoctradin and its metabolites M650F003 and M650F004 in honey and pollen. In lieu of a secondary ion transition for M650F003, for pollen only, confirmatory analysis is performed using an alternate chromatographic technique comprised of a different mobile phase. No interfering peaks were found at the retention time for each analyte. |
| Calibration (type, number of data points) | Calibration standards (matrix-matched) were used for the analysis. At least seven calibration points were used. Linear correlations with coefficients >0.99 were obtained for Ametoctradin and its metabolites, M650F01 and M650F06.  **Calibration data:**   |  |  |  |  |  | | --- | --- | --- | --- | --- | | Analyte | m/z | Slope | Intercept | r | | Ametoctradin | 276→ 176 | 1.51 x 106 | -3.53 x 103 | 0.9999 | | 276→149 | 1.51 x 106 | -2.87 x 103 | 0.9999 | | M650F003 | 222→ 176 | 1.2 x 106 | 3.28 x 103 | 0.9995 | | 222→121\* | 1.29 x 105 | 2.07 x 103 | 0.9962 | | M650F004 | 208→ 123 | 9.81 x 105 | 9.91 x 103 | 0.9993 | | 208→95 | 1.78 x 105 | 771 | 0.9990 |   \*In lieu of a secondary ion transition for M650F003 in pollen, confirmatory analysis is performed using an alternate chromatographic technique comprised of a different LC mobile phase gradient. |
| Calibration range | Standards in the range of 0.025 to 2 ng/mL were injected and the response was plotted against the concentration. |
| Standard solution and extract stability | Ametoctradin, M650F03 and M650F04 have been previously shown to be stable in standards prepared in methanol and in mixed calibration standards solution prepared by serial dilution of the intermediate standards using methanol:water:formic acid (50:50:0.1, v/v/v), for at least 2 months (60 days), each when held under refrigeration (refer to XXXX DocID 2008/1022139)  The method validation fortification sample extracts were analyzed within 1 day of extraction. The generally acceptable method recoveries obtained during analysis demonstrate the storage stability of each analyte in the extracts in the brief period prior to analysis. In addition, the recoveries from stored solutions generated during extract stability experiments performed in conjunction with this study, which included tests on the initial extracts and HPLC final volume (both matrices) stored under refrigeration, indicated that residues of Ametoctradin (including M650F03 and M650F04) are stable for at least 10 days, the maximum time periods tested. |
| Assessment of matrix effects | Matrix effects were evaluated in the study and were found to be significant (i.e. >20%), thus matix-matched calibration standards were used throughout. |
| Limit of determination/quantification | The limit of quantification (LOQ) defined by the lowest successfully tested fortification level was 0.01 mg/kg for all analytes. The LOD, defined as the lowest standard, was 0.025 ng/mL (= 0.002 mg/kg at sample level). |

Conclusion

The method R0072/01 was validated according to the requirements of SANCO 3029/99 rev.4, SANCO/825/00 rev. 8.1. It also meets the requirements of SANTE/2020/12830 Rev.1 for the analysis of Ametoctradin and its metabolites M650F01 and M650F06 in honey and pollen matrices.

Independent laboratory validation

|  |  |
| --- | --- |
| Comments of zRMS: | The ILV of XXXX Analytical Method R0072/01 has been accepted.  As previously the final determination of Ametoctradin was performed using the LC-MS/MS. The transition at m/z 276→176 was monitored in positive mode for primary quantification; the transition at m/z 276→149 was monitored in positive mode for confirmation. The overall mean recovery value for Ametoctradin (quantitation ion) in honey was found to be 98.2% (n=10) with an overall RSD of 3.48%. The overall mean recovery value for Ametoctradin (confirmatory ion) in honey was found to be 99.5% (n=10) with an overall RSD of 3.52%.  The method R0072/01 was independently validated for Ametoctradin in honey consistently with SANTE/2020/12830 Rev.1. |

|  |  |
| --- | --- |
| Reference: | CP 5.2/12 |
| Report | Independent lab validation of XXXX analytical method R0072/01: Method for the determination of BAS 181 S (Reg,No. 4117133), BAS 650 F (Reg.No. 4993353), M650F003 (Reg.No. 5178870), and M650F004 (Reg.No. 5211623) in pollen and honey by LC/MS/MS  Warnick, J. (2020)  report No 886595, 137F2694  XXXX DocID. 2020/2032165  Authority registration No |
| Guideline(s): | EPA 860.1340, OECD-ENV/JM/MONO/(2007)17, SANCO/825/00 Rev.8.1 (2010) |
| Deviations: | No |
| Previous evaluation: | No |
| GLP: | Yes  (certified by United States Environmental Protection Agency) |
| Acceptability: | Yes | |

Study Summary

The method R0072/01 was developed and validated for the determination of residues of Ametoctradin (BAS 650 F) and its metabolites M650F003 and M650F004 in honey and pollen by HPLC-MS/MS with a limit of quantification (LOQ) of 0.05 and 0.01 mg/kg, respectively (see XXXX Doc ID 2020/2032164). In this independent laboratory method validation study, only honey was used as matrix and Ametoctradin (BAS 650 F) was used as the relevant analyte for enforcement purposes. A brief description of the method and the results are presented in the summary below. Data for an additional active substance, BAS 181 S, is not relevant and has not been provided in the summary below.

**Materials and methods**

Residues of Ametoctradin (BAS 650 F) were extracted from honey by vortex in 20 mL of methanol and water (50:50 v/v), followed by centrifugation for 5 minutes at about 3750 rpm. An aliquot was removed and cleaned up on a solid phase extraction (SPE) column, eluted with buffered methanol, evaporated to dryness, and then re-dissolved and diluted in methanol/water/formic acid (50/50/0.1, v/v/v). The final determination of Ametoctradin (BAS 650 F) was performed by LC-MS/MS monitoring in the positive mode ion transitions m/z 276→176 (primary quantification ion) and m/z 276→149 (confirmatory ion). The results are calculated by direct comparison of the sample peak responses to those of external standards. Separation is accomplished with a XSelect HSS T3 column (150 mm x 3 mm, 2.5 µm) by using a gradient mixture of water with 0.1% formic acid /buffered methanol with 5 mM ammonium acetate and 0.5 % formic acid at a flow rate of 0.6 mL/min.

**Results and discussions**

The mean recovery values were between 70 and 110% with relative standard deviations (RSDs) of < 20%. The detailed results are given in the table below.

Table A 54 Validation results of method R0072/01: Ametoctradin in honey

| **Analyte** | **Matrix** | **Mass transition** | **Fortification Level [mg/kg]** | **Number of replicates** | **Mean recovery [%]** | **RSD [%]** |
| --- | --- | --- | --- | --- | --- | --- |
| Ametoctradin | Honey | 276→176 | 0.01 | 5 | 98.0 | 1.52 |
| 1.0 | 5 | 98.3 | 4.99 |
| 276→149 | 0.01 | 5 | 99.6 | 1.62 |
| 1.0 | 5 | 99.4 | 5.03 |

**Table A 55: Characteristics for the analytical method used for validation of Ametoctradin residues in honey**

|  | **Ametoctradin** |
| --- | --- |
| Specificity | LC-MS/MS, using two mass transitions is a highly specific detection technique and therefore a confirmatory technique is not required. No interfering peaks were found at the retention time of concern. |
| Calibration (type, number of data points) | Calibration standards (matrix-matched) were used for the analysis. A minimum of five calibration points were used, and individual calibration data was presented in the study report. A linear correlation with good fit (r >0.98) was obtained.  **Calibration data:**   |  |  |  |  |  | | --- | --- | --- | --- | --- | | Analyte | m/z | Slope | Intercept | r | | Ametoctradin | 276→ 176 | 2.96718 x 107 | 3.19678 x 105 | 0.99942 | |
| Calibration range | Standards in the range of 0.020 to 0.5 ng/mL were injected and the response was plotted against the concentration. |
| Standard solution and extract stability | Standard solution stability was not determined in this ILV study and used according to information established the original method validation study (XXXX DocID 2020/2032164]. Ametoctradin has been previously shown to be stable in standards prepared in methanol and in mixed calibration standards solution prepared by serial dilution of the intermediate standards using methanol/water/formic acid (50:50:0.1, v/v/v), for at least 2 months (60 days), each when held under refrigeration [refer to XXXX DocID 2008/1022139].  Extract stability was not determined in this ILV study and used according to information established in the original method validation study [XXXX DocID 2020/2032164]. The method validation fortification sample extracts were analyzed within 1 day of extraction. The generally acceptable method recoveries obtained during analysis demonstrate the storage stability of each analyte in the extracts in the brief period prior to analysis. |
| Assessment of matrix effects | Not evaluated in this ILV. Please refer to XXXX Doc ID 2020/2032164. Matrix-matched standards were employed throughout. |
| Limit of determination/quantification | The limit of quantification (LOQ) defined by the lowest successfully tested fortification level was 0.01 mg/kg. The LOD was 0.002 mg/kg. |

**Conclusion**

The method R0072/01 was validated according to the requirements of SANCO/825/00 rev. 8.1. It also meets the requirements of SANTE/2020/12830 Rev.1 for the analysis of Ametoctradin in honey.

Confirmatory method

No confirmatory technique is required as two different, highly specific mass transitions are used for quantitation and qualification in the study.

* + - 1. Description of Methods for the Analysis of Soil (KCP 5.2)
         1. Analytical method 9

Method L0091/03 is used for the determination of Ametoctradin (BAS 650 F) and its metabolites M650F001, M650F002, M650F003 & M650F004 in soil. The method was submitted in the AIR5 dossier for Ametoctradin and has not yet been peer reviewed. Thus, it is provided for information below.

Method validation.

|  |  |
| --- | --- |
| Comments of zRMS: | The method L0091/03 has been accepted.  The study objective was to validate analytical method L0091/03 for the determination of Ametoctradin, and its metabolites M650F001, M650F002, M650F003, and M650F004 in soil by LC-MS/MS. The method was validated at two fortification levels (0.01 mg/kg (LOQ) and 0.1 mg/kg (10xLOQ)) for two soil types. For each fortification level and matrix, at least five replicates were analyzed. Per matrix at least two control samples were measured. Two mass transitions were evaluated and reported for each analyte.  It was proven that the analytical method L0091/03 is suitable to determine residues of BAS 650 F and its metabolites M650F001, M650F002, M650F003 and M650F004 in soil. The mean recovery values ranged between 95.0 % and 104.9 % for all analytes, matrices and fortification levels. The relative standard deviations (RSD, %) for all mass transitions and fortification levels were below 12 %.  The method meets criteria of SANTE/2020/12830 Rev.1. |

|  |  |
| --- | --- |
| Reference: | CP 5.2/13 |
| Report | Validation of Analytical Method L0091/03 for the Determination of BAS 650 F and its Metabolites M650F001, M650F002, M650F003 and M650F004 in Soil by LC-MS/MS  Schelling, D. & Schatte, S., 2020  report No 808961  XXXX DocID 2020/2034611  Authority registration No |
| Guideline(s): | EPA 850.6100, SANCO/825/00 rev. 8.1 (16 November 2010), SANCO/3029/99 (11 July 2000) |
| Deviations: | No |
| Previous evaluation: | No |
| GLP: | Yes |
| Acceptability: | Yes | |

Study Summary

The method L0091/03, was developed and validated for the determination of residues of Ametoctradin (BAS 650 F, Reg.No. 4993353) and its metabolites M650F001 (Reg.No. 5178872), M650F002 (Reg.No. 5178871), M650F003 (Reg.No. 5178870) and M650F004 (Reg.No. 5211623) in soil by LC-MS/MS with an LOQ of 0.01 mg/kg.

The brief description of the methods and the results are presented in the summary below.

Materials and methods

Residues of BAS 650 F (Ametoctradin, Reg.No. 4993353) and its metabolites M650F001 (Reg.No.: 5178872), M650F002 (Reg.No.: 5178871), M650F003 (Reg.No.: 5178870) and M650F004 (Reg.No.: 5211623) are extracted with acetonitrile and twice with acetonitrile/water (50/50, v/v) each with subsequent centrifugation. The combined extracts are diluted to the final volume with water to achieve a solvent ratio of acetonitrile/water (24/76, v/v). The final determination of BAS 650 F and its metabolites M650F001, M650F002, M650F003 and M650F004 is performed by LC-MS/MS, monitoring at least two mass transitions for each analyte in positive ion ESI mode. For quantification, the mass transitions m/z 276→149 (BAS 650 F), m/z 250→176 (M650F001), m/z 236→176 (M650F002), m/z 222→176 (M650F003), m/z 208→123 (M650F004) are proposed and for confirmation, the mass transitions m/z 276→176 (BAS 650 F), m/z 250→149 (M650F001), m/z 236→121 (M650F002), m/z 222→121 (M650F003), m/z 208→95 (M650F004) are proposed.

Separation is accomplished with a Waters X Terra C18 column (50 mm x 4.6 mm; 3.5 µm) applying a gradient mixture of water and methanol each with 0.1 % formic acid as modifier at a flow rate of 0.6 mL/min.

**Results and discussions**

The mean recovery values were between 70 and 110% with relative standard deviations (RSDs) of < 20%. The detailed results are given in the table below.

Table A 56 Validation results of the method L0091/03: Ametoctradin and its metabolites M650F001, M650F002, M650F003 and M650F004 in soil matrices

| **Analyte** | **Matrix** | **m/z** | **Fortification Level [mg/kg]** | **Number of replicates** | **Mean recovery [%]** | **RSD [%]** | **Overall recovery [%]** | **Overall RSD [%]** |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| BAS 650 F (Reg. No. 4993353) | Soil LUFA 2.2 | 276 → 176 | 0.010 | 5 | 98.4 | 5.8 | 99.8 | 4.1 |
| 0.10 | 5 | 101.1 | 0.8 |
| 276 → 149 | 0.010 | 5 | 99.0 | 4.5 | 100.7 | 3.5 |
| 0.10 | 5 | 102.4 | 0.7 |
| Soil Li 10 | 276 → 176 | 0.010 | 5 | 95.8 | 4.9 | 99.9 | 5.5 |
| 0.10 | 5 | 104.0 | 2.1 |
| 276 → 149 | 0.010 | 5 | 96.4 | 5.8 | 100.7 | 6.0 |
| 0.10 | 5 | 104.9 | 2.0 |
| M650F001 (Reg. No. 5178872) | Soil LUFA 2.2 | 250 → 149 | 0.010 | 5 | 95.2 | 8.9 | 96.2 | 6.0 |
| 0.10 | 5 | 97.1 | 0.4 |
| 250 → 176 | 0.010 | 5 | 95.1 | 10.3 | 95.9 | 6.9 |
| 0.10 | 5 | 96.7 | 1.0 |
| Soil Li 10 | 250 → 149 | 0.010 | 5 | 99.0 | 1.7 | 100.1 | 1.8 |
| 0.10 | 5 | 101.2 | 1.4 |
| 250 → 176 | 0.010 | 5 | 99.6 | 4.7 | 100.5 | 3.5 |
| 0.10 | 5 | 101.3 | 1.8 |
| M650F002 (Reg. No. 5178871) | Soil LUFA 2.2 | 236 → 176 | 0.010 | 5 | 95.6 | 10.3 | 95.5 | 6.9 |
| 0.10 | 5 | 95.5 | 0.9 |
| 236 → 121 | 0.010 | 5 | 95.8 | 9.9 | 95.4 | 6.7 |
| 0.10 | 5 | 95.0 | 0.6 |
| Soil Li 10 | 236 → 176 | 0.010 | 5 | 98.6 | 3.1 | 99.3 | 2.6 |
| 0.10 | 5 | 100.0 | 2.1 |
| 236 → 121 | 0.010 | 5 | 99.1 | 2.3 | 99.6 | 2.1 |
| 0.10 | 5 | 100.1 | 2.0 |
| M650F003 (Reg. No. 5178870) | Soil LUFA 2.2 | 222 → 176 | 0.010 | 5 | 95.7 | 11.0 | 96.5 | 7.3 |
| 0.10 | 5 | 97.2 | 0.7 |
| 222 → 121 | 0.010 | 5 | 96.6 | 11.3 | 97.0 | 7.5 |
| 0.10 | 5 | 97.3 | 0.7 |
| Soil Li 10 | 222 → 176 | 0.010 | 5 | 99.8 | 1.7 | 101.4 | 2.3 |
| 0.10 | 5 | 102.9 | 1.7 |
| 222 → 121 | 0.010 | 5 | 101.2 | 6.2 | 102.5 | 4.6 |
| 0.10 | 5 | 103.7 | 2.5 |
| M650F004 (Reg. No. 5211623) | Soil LUFA 2.2 | 208 → 123 | 0.010 | 5 | 97.3 | 9.6 | 97.0 | 6.4 |
| 0.10 | 5 | 96.7 | 0.6 |
| 208 → 95 | 0.010 | 5 | 97.5 | 11.9 | 97.6 | 7.9 |
| 0.10 | 5 | 97.8 | 0.5 |
| Soil Li 10 | 208 → 123 | 0.010 | 5 | 98.6 | 1.9 | 100.3 | 2.4 |
| 0.10 | 5 | 102.1 | 1.2 |
| 208 → 95 | 0.010 | 5 | 98.2 | 3.3 | 100.3 | 3.3 |
| 0.10 | 5 | 102.5 | 1.7 |
|  | | | | | | | | |

Table A 57: Characteristics for the analytical method L0091/03: Ametoctradin and metabolites M650F001, M650F002, M650F003 and M650F004 in soil

|  | Ametoctradin and metabolites M650F001, M650F002, M650F003 and M650F004 |
| --- | --- |
| Specificity | The quantification of BAS 650 F and its metabolites M650F001, M650F002, M650F003 and M650F004 is based on the monitoring of two mass transitions. Due to the high selectivity and specificity of LC MS/MS, an additional confirmatory technique is not required. In the untreated control samples, no interferences (> 30 % LOQ) at the relevant retention time and mass transitions were observed. |
| Calibration (type, number of data points) | Calibration standards (solvent-based) were used for the analysis. At least six calibration points were used. Linear correlations with coefficients >0.9950 were obtained for Ametoctradin and its metabolites M650F001, M650F002, M650F003 and M650F004.  **Calibration data:**   |  |  |  |  |  | | --- | --- | --- | --- | --- | | Analyte | m/z | Slope | Intercept | r | | Ametoctradin | 276→ 176 | 1.09 x 106 | 3.45 x 104 | 0.9971 | | 276→ 149 | 9.1 x 105 | 2.54 x 104 | 0.9970 | | M650F001 | 250→ 149 | 4.52 x 105 | 9.02 x 103 | 0.9989 | | 250→ 176 | 5.32 x 105 | 1.01 x 104 | 0.9991 | | M650F002 | 236→ 176 | 8.84 x 105 | 2.05 x 104 | 0.9985 | | 236→ 121 | 1.29 x 105 | 2.57 x 103 | 0.9988 | | M650F003 | 222→ 176 | 1.26 x 106 | 1.25 x 104 | 0.9991 | | 222→ 121 | 1.24 x 105 | 1.57 x 103 | 0.9991 | | M650F004 | 208→ 123 | 6.09 x 105 | 1.26 x 104 | 0.9993 | | 208→ 95 | 1.3 x 105 | 2.83 x 103 | 0.9994 | |
| Calibration range | Standards in the range of 0.12 to 6.0 ng/mL were injected and the response was plotted against the concentration. |
| Standard solution and extract stability | Stability tests showed that BAS 650 F and its metabolites M650F001, M650F002 and M650F004 indicate sufficient stability (less than 20 % difference) in working solutions; i.e., calibration solutions (2.0 and 4.0 ng/mL) and fortification solutions (1000 and 10000 ng/mL) for 14 days when stored refrigerated at approximately +4°C in the dark.  Stability in calibration and fortification solutions was confirmed for the metabolite M650F003 for 16 days, when stored refrigerated at approximately +4°C in the dark. Fortification solutions were prepared in acetonitrile/water (50/50, v/v), calibration solutions in acetonitrile/water (24/76, v/v).  Stability tests showed that BAS 650 F indicate sufficient stability (less than 20 % difference) in stock solutions prepared in methanol (0.5 mg/mL) for at least 33 days when stored under refrigerated conditions in the dark (refer to XXXX DocID 2020/2034594).  Stability tests showed that metabolites M650F001, M650F003 and M650F004 indicate sufficient stability (less than 20 % difference) in stock solutions (1.0 mg/mL) for 37 days and metabolite M650F002 for 15 days, when stored refrigerated at approximately +4 °C in the dark. Stock solutions of M650F001, M650F002 and M650F003 were prepared in acetonitrile/water (10/90, v/v), while the stock solution of M650F004 was prepared in methanol/water (80/20, v/v) [refer to CA 4.1.2/8; DocID 2020/2034594].  Extract stability experiments demonstrated that BAS 650 F and its metabolites M650F001, M650F002, M650F003 and M650F004 were stable in final volume extracts, prepared in acetonitrile/water (24/76, v/v, FV) at a concentration of 0.1 mg/kg (10x LOQ, corresponding to a measurement concentration of 4 ng/mL), over a time period of 10 days, when stored refrigerated in the dark at approximately +4°C. |
| Assessment of matrix effects | The influence of the matrix load on the analysis of BAS 650 F and its metabolites M650F001, M650F002, M650F003 and M650F004 was determined by comparing matrix-matched standards prepared in acetonitrile/water (24/76, v/v, FV, matrix load 100 %) with solvent standards, which were prepared in acetonitrile/water (24/76, v/v, FV).The results demonstrated that the matrix load in the tested matrix-matched standards had no significant influence (differences < 20 %) on the detection of BAS 650 F and its metabolites M650F001, M650F002, M650F003 and M650F004 in soil Li 10 and soil LUFA 2.2. Therefore, solvent-based standards were used for quantification and no matrix-matched standards were needed for further experiments |
| Limit of determination/quantification | The limit of quantification (LOQ) defined by the lowest successfully tested fortification level was 0.01 mg/kg for all analytes. The LOD, defined as the lowest standard, was 0.12 ng/mL (= 0.003 mg/kg at sample level). |

Conclusion

The method L0091/03 was validated according to the requirements of SANCO/825/00 rev. 8.1. It also meets the requirements of SANTE/2020/12830 Rev.1 for the analysis of Ametoctradin and its metabolites M650F001, M650F002, M650F003 and M650F004 in soil matrices.

* + - * 1. Analytical method 10

Method L0110/02 is used for the determination of Ametoctradin metabolites M650F003 & M650F004 in soil. The method was submitted in the AIR5 dossier for Ametoctradin and has not yet been peer reviewed. Thus, it is provided for information below.

Method validation

|  |  |
| --- | --- |
| Comments of zRMS: | The method L0110/02 has been accepted.  The study objective was to validate analytical method L0110/02 for the determination of Ametoctradin, and its metabolites M650F003, and M650F004 in soil by LC-MS/MS. The method was validated at two fortification levels 1.0 µg/kg (LOQ) and 10.0 µg/kg (10x LOQ) for two soil types. For each fortification level and matrix, five replicates were analyzed. Per matrix at least two control samples were measured. Two mass transitions were evaluated and reported for the analytes.  It was proven that the analytical method L0110/02 is suitable to determine residues of BAS 650 F and its metabolites M650F003 and M650F004 in soil. The mean recovery values ranged consistently with the requirements for all analytes, matrices and fortification levels. The relative standard deviations (RSD, %) for all mass transitions and fortification levels were below 20 %.  The method meets criteria of SANTE/2020/12830 Rev.1. |

|  |  |
| --- | --- |
| Reference: | CP 5.2/14 |
| Report | Validation of Analytical Method L0110/02 for the Determination of Residues of BAS 650 F Metabolites M650F003 (Reg.No. 5178870) and M650F004 (Reg.No. 5211623) in Soil Samples with a Limit of Quantification of 1.0 µg/kg by LC-MS/MS  Karrer, C. & Albani, K., 2020  report No 808962  XXXX DocID 2020/2034612  Authority registration No |
| Guideline(s): | SANCO/3029/99 rev. 4 (11 July 2000), US EPA 850.6100, SANCO/825/00 rev. 8.1 (16/11/2010) |
| Deviations: | No |
| Previous evaluation: | No |
| GLP: | Yes |
| Acceptability: | Yes | |

Study Summary

The method L0110/02, was developed and validated for the determination of residues of Ametoctradin (BAS 650 F) Metabolites M650F003 (Reg.No. 5178870) and M650F004 (Reg.No. 5211623) in Soil Samples by LC-MS/MS with a Limit of Quantification (LOQ) of 1.0 µg/kg. The brief description of the methods and the results are presented in the summary below.

Materials and methods

Residues of M650F003 and M650F004 are extracted with a mixture of methanol/water (50/50, v/v). An aliquot of the extract is then centrifuged. For high residues, samples are further diluted before measurement. The final determination of metabolites M650F003 and M650F004 is performed by LC-MS/MS, monitoring at least two mass transitions for each analyte in positive ion ESI mode. For quantification, the mass transitions m/z 222→176 (M650F003), m/z 208→190 (M650F004) are proposed and for confirmation, the mass transitions m/z 222→121 (M650F003), m/z 208→123 (M650F004) are proposed.

Separation was accomplished with a Phenomenex Synergi Fusion RP column (150 mm x 4.6 mm, 4 µm) applying a gradient mixture of water and methanol each with 0.1 % formic acid as modifier at a flow rate of 1.0 mL/min.

**Results and discussions**

The mean recovery values were between 70 and 110% with relative standard deviations (RSDs) of < 20%. The detailed results are given in the table below.

Table A 58 Validation results of the method L0110/02: Ametoctradin metabolites M650F003 and M650F004 in soil matrices

| **Analyte** | **Matrix** | **m/z** | **Fortification Level [µg/kg]** | **Number of replicates** | **Mean recovery [%]** | **RSD [%]** | **Overall recovery [%]** | **Overall RSD [%]** |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| M650F003 (Reg. No. 5178870) | Soil Li 10 | 222 → 176 | 1.0 | 5 | 106 | 1.9 | 105 | 1.6 |
| 10 | 5 | 104 | 1.0 |
| 222 → 121 | 1.0 | 5 | 101 | 6.2 | 102 | 4.4 |
| 10 | 5 | 103 | 1.4 |
| Soil LUFA 2.2 | 222 → 176 | 1.0 | 5 | 97.2 | 0.6 | 99.7 | 3.0 |
| 10 | 5 | 102 | 1.8 |
| 222 → 121 | 1.0 | 5 | 105 | 2.9 | 103 | 2.7 |
| 10 | 5 | 102 | 1.4 |
| M650F004 (Reg. No. 5211623) | Soil Li 10 | 208 → 190 | 1.0 | 5 | 95.9 | 2.8 | 95.9 | 2.0 |
| 10 | 5 | 95.8 | 1.0 |
| 208 → 123 | 1.0 | 5 | 96.4 | 2.6 | 95.7 | 2.0 |
| 10 | 5 | 95.0 | 1.0 |
| Soil LUFA 2.2 | 208 → 190 | 1.0 | 5 | 98.4 | 1.5 | 97.5 | 1.9 |
| 10 | 5 | 96.6 | 1.9 |
| 208 → 123 | 1.0 | 5 | 99.0 | 1.6 | 97.6 | 1.9 |
| 10 | 5 | 96.3 | 1.2 |
|  | | | | | | | | |

Table A 59: Characteristics for the analytical method L0110/02: Ametoctradin metabolites M650F003 and M650F004 in soil matrices

|  | Ametoctradin metabolites M650F003 and M650F004 |
| --- | --- |
| Specificity | The quantification of M650F003 and M650F004 is based on the monitoring of two mass transitions. Due to the high selectivity and specificity of LC MS/MS, an additional confirmatory technique is not required. In the untreated control samples, no interferences (> 30 % LOQ) at the relevant retention time and mass transitions were observed. |
| Calibration (type, number of data points) | Calibration standards (solvent-based) were used for the analysis. At least nine calibration points were used. Linear correlations with coefficients >0.9977 were obtained for Ametoctradin metabolites M650F003 and M650F004.  **Calibration data:**   |  |  |  |  |  | | --- | --- | --- | --- | --- | | Analyte | m/z | Slope | Intercept | r | | M650F003 | 222→ 176 | 5.51 x 105 | 3.55 x 103 | 0.9981 | | 222→ 121 | 8.61 x 104 | 639 | 0.9977 | | M650F004 | 208→ 190 | 6.04 x 105 | 3.94 x 103 | 0.9981 | | 208→ 123 | 3.63 x 105 | 2.35 x 103 | 0.9980 | |
| Calibration range | Standards in the range of 0.02 to 10.0 ng/mL were injected and the response was plotted against the concentration. |
| Standard solution and extract stability | Stability tests showed that M650F003 and M650F004 indicate sufficient stability (less than 20 % difference) in working solution prepared in methanol/water (50/50, v/v) for at least 25 days when stored refrigerated in the dark (XXXX Doc ID 2008/1017004 submitted in the DAR).  Extract stability experiments demonstrated that the metabolites M650F003 and M650F004 were stable in final volumes, prepared in methanol/water (50/50, v/v) over a time period of 7 days, when stored refrigerated in the dark at approximately +4°C. |
| Assessment of matrix effects | The influence of the matrix load on the analysis of metabolites M650F003 and M650F004 was determined by comparing matrix-matched standards prepared in methanol/water (50/50, v/v, matrix load 90 %) with solvent standards, which were prepared in methanol/water (50/50, v/v).The results demonstrated that the matrix load in the tested matrix-matched standards had no significant influence (differences < 20 %) on the detection of metabolites M650F003 and M650F004 in soil Li 10 and soil LUFA 2.2. Therefore, solvent-based standards were used for quantification and no matrix-matched standards were needed for further experiments. |
| Limit of determination/quantification | The limit of quantification (LOQ) is defined by the lowest fortification level successfully tested, hence 1.0 µg/kg (0.1 ng/mL measurement sample level, considering a sample aliquot of 5 g and a final volume of 50 mL). The limit of detection (LOD) is 0.2 µg/kg (0.02 ng/mL measurement sample level), corresponding to the lowest calibration level used. |

Conclusion

The method L0110/02 was validated according to the requirements of SANCO/825/00 rev. 8.1. It also meets the requirements of SANTE/2020/12830 Rev.1 for the analysis of Ametoctradin metabolites M650F003 and M650F004 in soil matrices.

* + - 1. Description of Methods for the Analysis of Water (KCP 5.2)
         1. Analytical method 11

Method L0208/02 is used for the determination of Ametoctradin in surface and ground water. The method and its corresponding ILV was submitted in the AIR5 dossier for Ametoctradin and have not yet been peer reviewed. Thus, they are provided for information below.

Method validation

|  |  |
| --- | --- |
| Comments of zRMS: | The method L0208/02 has been accepted.  The study objective was to validate analytical method L0208/02 for the determination of Ametoctradin in water by LC-MS/MS. The method was validated at two fortification levels (0.03 µg /L (LOQ) and 0.3 µg /L (10x LOQ)) for two water types. For each fortification level and matrix, at least five replicates were analyzed. Per matrix at least two control samples were measured. Two mass transitions were evaluated and reported for the analyte. Matrix and solvent standards were analyzed within the study to test for possible matrix effects.  The mean recovery values and RSD ranged consistently with the requirements for all analytes. The method meets criteria of SANTE/2020/12830 Rev.1. |

|  |  |
| --- | --- |
| Reference: | CP 5.2/15 |
| Report | Validation of Analytical Method L0208/02 for the Determination of BAS 650 F (Reg.No. 4993353), in Surface Water and Groundwater by LC-MS/MS  Schelling, D. & Schatte, S., 2020  report No 808963  XXXX DocID 2020/2034573  Authority registration No |
| Guideline(s): | SANCO/3029/99 rev. 4 (11 July 2000), US EPA 850.6100, SANCO/825/00 rev. 8.1 (16/11/2010) |
| Deviations: | No |
| Previous evaluation: | No |
| GLP: | Yes |
| Acceptability: | Yes | |

Study Summary

Method L0208/02 was developed and validated for the determination of residues of Ametoctradin (BAS 650 F) in tap and surface water, with a limit of quantification at 0.03 µg/L. The brief description of method and the results are presented in the summary below.

Materials and methods

Residues of Ametoctradin (BAS 650 F) is extracted from a 5 mL water aliquot sample by liquid-liquid extraction using 6 mL ethyl acetate. An aliquot of 4 mL of the organic phase is evaporated to dryness in an N-Vap at 40°C and the obtained residues are re-dissolved in 1 mL acetonitrile/water (1/1 v/v). The final determination of BAS 650 F is conducted with LC-MS/MS. For quantification, the mass transition m/z 276→m/z 149 is proposed for quantification and the mass transition m/z 276→m/z 176 is proposed for confirmation.

Separation is achieved by using a Waters Acquity UPLC Betasil C-18 column (100 mm x 2.1 mm, 5 µm) at a flow rate of 0.6 mL/min. A gradient flow of water/formic acid (1000/1, v/v) and acetonitrile/formic acid (1000/1, v/v) is applied.

**Results and discussions**

The mean recovery values were between 70 and 110% with relative standard deviations (RSDs) of < 20%. The detailed results are given in the table below.

Table A 60 Validation results of the method L0208/02: Determination of Ametoctradin in water

| **Analyte** | **Matrix** | **m/z** | **Fortification Level [µg/L]** | **Number of Replicates** | **Mean Recovery [%]** | **Mean RSD [%]** | **Overall Recovery [%]** | **Overall RSD [%]** |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| BAS 650 F | Groundwater | 276 → 149 | 0.03 | 5 | 98.2 | 1.9 | 97.6 | 2.8 |
| 0.3 | 5 | 97.0 | 3.7 |
| 276 → 176 | 0.03 | 5 | 96.7 | 1.9 | 97.0 | 2.6 |
| 0.3 | 5 | 97.3 | 3.4 |
| Surface Water | 276 → 149 | 0.03 | 5 | 99.4 | 2.1 | 98.5 | 3.2 |
| 0.3 | 5 | 97.7 | 4.1 |
| 276 → 176 | 0.03 | 5 | 101 | 2.6 | 99.1 | 3.6 |
| 0.3 | 5 | 97.6 | 4.2 |

Table A 61: Characteristics for the analytical method L0208/02: Determination of Ametoctradin in water matrices

|  | Ametoctradin |
| --- | --- |
| Specificity | The quantification of Ametoctradin (BAS 650 F) is based on the monitoring of two mass transitions. Due to the high selectivity and specificity of LC MS/MS, an additional confirmatory technique is not required. In the untreated control samples, no interferences (> 30 % LOQ) at the relevant retention time and mass transitions were observed. |
| Calibration (type, number of data points) | Calibration standards (solvent-based) were used for the analysis. At least six calibration points were used. Linear correlations with coefficients >0.99 were obtained for Ametoctradin.  **Calibration data:**   |  |  |  |  |  | | --- | --- | --- | --- | --- | | Analyte | m/z | Slope | Intercept | r | | Ametoctradin | 276→ 149 | 2.53 x 106 | 1.5 x 104 | 0.9998 | | 276→ 176 | 3.13 x 106 | 1.9 x 104 | 0.9998 | |
| Calibration range | Standards in the range of 0.03 to 1.5 ng/mL were injected and the response was plotted against the concentration. |
| Standard solution and extract stability | The standard solution stability of stock solutions was established in methanol at a concentration of 500000 ng/mL and fortification and calibration standard solutions at concentration levels of 0.50 ng/mL and 1.00 ng/mL, prepared in acetonitrile/water (1/1, v/v), were found to be stable for a duration of at least 33 days, when stored under refrigerated conditions in the dark.  The stability of extracts was established in final volume solution (acetonitrile/water, 1/1, v/v) at 10x LOQ were stable for a duration of at least 5 days, when stored under refrigerated conditions in the dark. |
| Assessment of matrix effects | Solvent- and matrix-matched standards were analyzed to assess potential matrix effects. No significant matrix effects were identified during analysis of quality control samples. Therefore, the use of matrix-matched standards was not required. |
| Limit of determination/quantification | The method has a limit of quantification (LOQ) of 0.03 µg/L (test sample level), corresponding to the lowest fortification level successfully tested with a concentration of 0.1 ng/mL at measurement sample level. The method has a limit of detection (LOD) of 0.009 µg/L (test sample level), corresponding to the lowest calibration standard (0.03 ng/mL at measurement sample level) used. |

Conclusion

The method L0208/02 was validated according to the requirements of SANCO/825/00 rev. 8.1. It also meets the requirements of SANTE/2020/12830 Rev.1 for the analysis of Ametoctradin in surface water and groundwater matrices.

Independent laboratory validation

|  |  |
| --- | --- |
| Comments of zRMS: | The independent validation of the method L0208/02 has been accepted.  The study objective was to independently validate analytical method L0208/02 for the determination of Ametoctradin in water by LC-MS/MS. The method was validated at two fortification levels (0.03 µg /L (LOQ) and 0.3 µg /L (10x LOQ)) for two water types. For each fortification level and matrix, at least five replicates were analyzed. Per matrix at least two control samples were measured. Two mass transitions were evaluated and reported for the analyte. Matrix and solvent standards were analyzed within the study to test for possible matrix effects.  The mean recovery values and RSD ranged consistently with the requirements for all analytes. The method meets criteria of SANTE/2020/12830 Rev.1. |

|  |  |
| --- | --- |
| Reference: | CP 5.2/16 |
| Report | Independent Laboratory Validation (ILV) of XXXX Analytical Method L0208/02 for the Determination of BAS 650 F (Reg.No. 4993353), in Surface Water and Groundwater by LC-MS/MS  Tzelepi, E., 2020  report No 808959  XXXX DocID. 2020/2034827  Authority registration No |
| Guideline(s): | SANCO/825/00 Rev.8.1 (2010) |
| Deviations: | No |
| Previous evaluation: | No |
| GLP: | Yes |
| Acceptability: | Yes | |

Study Summary

In this study, Method L0208/02, was independently validated for the determination of residues of Ametoctradin (BAS 650 F) in surface and ground water, with a limit of quantification at 0.03 µg/L. The brief description of the methods and the results are presented in the summary below.

**Materials and methods**

Residues of Ametoctradin (BAS 650 F) are extracted from a 5 mL water aliquot sample by liquid-liquid partition using 6 mL ethyl acetate. An aliquot of 4 mL of the organic phase is evaporated to dryness under nitrogen at ≤40 °C and the dry residues are re-dissolved in 1 mL acetonitrile/water (1/1 v/v). The final determination of Ametoctradin (BAS 650 F) is conducted with LC-MS/MS monitoring the mass transitions m/z 276 → 149 and m/z 276 → 176 for the quantification and confirmation of Ametoctradin (BAS 650 F) respectively. Separation is accomplished with a Betasil C18 column (100 mm x 2.1 mm, 5 µm) by using a gradient mixture of water/acetonitrile, each acidified with 0.1% formic acid, at a flow rate of 0.6 mL/min.

**Results and discussions**

The mean recovery values were between 70 and 110% with relative standard deviations (RSDs) of < 20%. The detailed results are given in the table below.

Table A 62 Validation results of method L0208/02: ILV for the determination of Ametoctradin in water

| **Matrix** | **Analyte** | **No. of tests** | **Fortification level [µg/L]** | **mean [%]** | **RSD [%]** | **mean [%]** | **RSD [%]** |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Surface water |  | | | Transition 276 → 149 1) | | Transition 276 → 176 | |
| Ametoctradin | 5 | 0.03 | 98.9 | 2.9 | 98.0 | 1.9 |
| 5 | 0.3 | 99.3 | 1.0 | 98.8 | 0.4 |
|  | Overall | 10 | Range:  0.03 – 0.3 | 99.1 | 2.1 | 98.4 | 1.4 |
| Ground water |  | | | Transition 276 → 149 1) | | Transition 276 → 176 | |
| Ametoctradin | 5 | 0.03 | 97.9 | 1.8 | 97.4 | 1.9 |
| 5 | 0.3 | 100 | 1.0 | 102 | 0.5 |
|  | Overall | 10 | Range:  0.03 – 0.3 | 99..2 | 1.9 | 99.9 | 2.9 |
| 1) Primary transition | | | | | | | |

**Table A 63: Characteristics for the analytical method L0208/02 ILV used for validation of Ametoctradin residues in water**

|  | **Ametoctradin** |
| --- | --- |
| Specificity | LC-MS/MS using two mass transitions is a highly specific, self-confirmatory method. The interferences/residues of the analyte measured in the control samples were demonstrated to be below 30% of the limit of quantification (LOQ) for each matrix and each mass transition. |
| Calibration (type, number of data points) | Calibration standards (solvent-based) were used for the analysis. A minimum of six calibration points were used. A linear correlation with good fit (r >0.9998) was obtained.  **Calibration data:**   |  |  |  |  |  | | --- | --- | --- | --- | --- | | Analyte | m/z | Slope | Intercept | r2 | | Ametoctradin | 276→ 149 | 885190.4 | 375.3 | 0.9999 | | 276→ 176 | 926475.9 | 1050.1 | 0.9998 | |
| Calibration range | Standards in the range of 0.03 to 1.5 ng/mL were injected and the response was plotted against the concentration. |
| Standard solution and extract stability | Standard solution and extract stability were not directly evaluated in the ILV study of Method L0208/02, but determined in the original method validation study (XXXX Doc ID 2020/2034573). Stability of the analytes in solutions and in extracts in this study was also demonstrated by consistent LC-MS/MS results throughout the duration of the experimental phase and acceptable mean recoveries in the fortified samples within the range of 70-110%. |
| Assessment of matrix effects | The matrix effect was tested for surface water and ground water. No significant (>20%) matrix effects were observed for Ametoctradin. For the evaluation of all results, solvent calibration standards were used.. |
| Limit of determination/quantification | The LOQ of the method is defined as the lowest analyte concentration in a sample at which the methodology has been successfully validated and is at 0.03 μg/L for all analytes.  The limit of detection (LOD) of the method is defined as the lowest analyte concentration injected as a calibration solution resulting in an LOD of 0.009 μg/L for all analytes and both quantification and confirmation transitions. |

**Conclusion**

The method L0208/02 was validated according to the requirements of SANCO/825/00 rev. 8.1. It also meets the requirements of SANTE/2020/12830 Rev.1 for the analysis of Ametoctradin in surface water and groundwater.

Confirmatory method

No confirmatory technique is required as two different, highly specific mass transitions are used for quantitation and qualification in the study.

* + - * 1. Analytical method 12

Method L0113/03 is used for the determination of Ametoctradin metabolites M650F001, M650F002, M650F003 & M650F004 in surface and ground water. The method and its corresponding ILV was submitted in the AIR5 dossier for Ametoctradin and have not yet been peer reviewed. Thus, they are provided for information below.

Method validation

|  |  |
| --- | --- |
| Comments of zRMS: | The validation of the method L0113/03 has been accepted.  The study objective was to validate analytical method L0113/03 for the determination of Ametoctradin metabolites M650F001, M650F002, M650F003 & M650F004 in water by LC-MS/MS. The method was validated at two fortification levels (0.03 μg/L (LOQ) and 0.3 μg/L (10x LOQ)) for two water types. For each fortification level and matrix, at least five replicates were analyzed. Per matrix at least two control samples were measured. Two mass transitions were evaluated and reported for each analyte. Matrix and solvent standards were analyzed within the study to test for possible matrix effects.  The mean recovery values and RSD ranged consistently with the requirements for all analytes. The method meets criteria of SANTE/2020/12830 Rev.1. |

|  |  |
| --- | --- |
| Reference: | CP 5.2/17 |
| Report | Validation of Analytical Method L0113/03 for the Determination of BAS 650 F Metabolites M650F001, M650F002, M650F003 and M650F004 in Surface Water and Groundwater by LC-MS/MS  Schelling, D. & Schatte, S., 2020  report No 808964  XXXX DocID 2020/2034594  Authority registration No |
| Guideline(s): | SANCO/825/00 rev. 8.1 (16 November 2010), SANCO/3029/99 (11 July 2000), US EPA 850.6100 |
| Deviations: | No |
| Previous evaluation: | No |
| GLP: | Yes |
| Acceptability: | Yes | |

Study Summary

The analytical method L0113/03 was developed and validated for the determination of Ametoctradin (BAS 650 F) Metabolites M650F001 (Reg.No.: 5178872), M650F002 (Reg.No.: 5178871), M650F003 (Reg.No.: 5178870) and M650F004 (Reg.No.: 5211623) in surface water and groundwater by LC-MS/MS with a limit of quantification (LOQ) of 0.03 µg/L. The brief description of method and the results are presented in the summary below.

Materials and methods

Using analytical method L0113/03, residues of Ametoctradin metabolites M650F001 (Reg.No.: 5178872), M650F002 (Reg.No.: 5178871), M650F003 (Reg.No.: 5178870) and M650F004 (Reg.No.: 5211623) were directly measured from water samples (1 mL aliquot) after being mixed with 0.1 mL acetonitrile/water (10/90, v/v) to achieve a matrix load of 91 % and a final volume of 1.1 mL.

Residues of Ametoctradin metabolites were determined from both surface water and ground water by LC MS/MS in ESI positive mode at the following mass transitions:

|  |  |  |
| --- | --- | --- |
| **Analyte** | **Primary Mass Transition (m/z)** | **Secondary Mass Transition (m/z)** |
| M650F001 | 250→149 | 250→176 |
| M650F002 | 236 → 176 | 236 → 121 |
| M650F003 | 222 → 176 | 222 → 121 |
| M650F004 | 208 → 123 | 208 → 95 |

Separation was accomplished with a Xterra® C18 column (50 mm x 4.6 mm, 3.5 µm) by using a gradient mixture of water/methanol, each acidified with 0.1% formic acid, at a flow rate of 0.6 mL/min.

**Results and discussions**

The mean recovery values were between 70 and 110% with relative standard deviations (RSDs) of < 20%. The detailed results are given in the table below.

Table A 64 Validation results of the method L0113/03: Determination of Ametoctradin metabolites M650F001, M650F002, M650F003 and M650F004 in surface water and groundwater

| **Analyte** | **Matrix** | **m/z** | **Fortification level**  **[µg/L]** | **Number of replicates** | **Mean recovery [%]** | **RSD [%]** | **Overall recovery [%]** | **Overall RSD [%]** |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| M650F001 | Groundwater | 250→149 | 0.03 | 5 | 105.4 | 1.9 | 104.6 | 1.7 |
| 0.3 | 5 | 103.8 | 1.0 |
| 250→176 | 0.03 | 5 | 106.3 | 1.7 | 105.0 | 1.9 |
| 0.3 | 5 | 103.6 | 0.9 |
| Surface Water | 250→149 | 0.03 | 5 | 103.5 | 1.1 | 102.7 | 1.2 |
| 0.3 | 5 | 101.9 | 0.7 |
| 250→176 | 0.03 | 5 | 105.9 | 2.4 | 104.1 | 2.5 |
| 0.3 | 5 | 102.3 | 0.6 |
| M650F002 | Groundwater | 236→176 | 0.03 | 5 | 105.4 | 1.3 | 103.5 | 2.1 |
| 0.3 | 5 | 101.7 | 0.7 |
| 236→121 | 0.03 | 5 | 104.3 | 4.2 | 103.7 | 2.9 |
| 0.3 | 5 | 103.1 | 0.5 |
| Surface Water | 236→176 | 0.03 | 5 | 102.4 | 1.5 | 101.8 | 1.3 |
| 0.3 | 5 | 101.2 | 0.8 |
| 236→121 | 0.03 | 5 | 103.0 | 2.6 | 102.5 | 1.9 |
| 0.3 | 5 | 101.9 | 0.7 |
| M650F003 | Groundwater | 222→176 | 0.03 | 5 | 101.9 | 0.8 | 101.5 | 0.9 |
| 0.3 | 5 | 101.2 | 1.0 |
| 222→121 | 0.03 | 5 | 97.5 | 5.1 | 98.5 | 3.7 |
| 0.3 | 5 | 99.6 | 1.4 |
| Surface Water | 222→176 | 0.03 | 5 | 102.5 | 1.8 | 100.9 | 2.1 |
| 0.3 | 5 | 99.2 | 0.6 |
| 222→121 | 0.03 | 5 | 102.2 | 4.0 | 100.9 | 3.2 |
| 0.3 | 5 | 99.5 | 1.3 |
| M650F004 | Groundwater | 208→123 | 0.03 | 5 | 102.0 | 1.4 | 101.6 | 1.5 |
|  | 0.3 | 5 | 101.3 | 1.7 |
| 208→95 | 0.03 | 5 | 97.7 | 5.6 | 98.6 | 4.0 |
|  | 0.3 | 5 | 99.5 | 1.5 |
| Surface Water | 208→123 | 0.03 | 5 | 102.9 | 2.3 | 100.6 | 2.9 |
|  | 0.3 | 5 | 98.3 | 1.1 |
| 208→95 | 0.03 | 5 | 98.3 | 3.1 | 98.4 | 2.2 |
|  | 0.3 | 5 | 98.5 | 1.0 |

Table A 65: Characteristics for the analytical method L0113/03: Determination of Ametoctradin metabolites M650F001, M650F002, M650F003 and M650F004 in surface water and groundwater

|  | M650F001, M650F002, M650F003 and M650F004 |
| --- | --- |
| Specificity | LC MS/MS is a highly specific self-confirmatory technique. Under the described conditions the method is specific for the determination of metabolites M650F001, M650F002, M650F003 and M650F004 in surface water and ground water. No residues of Ametoctradin metabolites, greater than the LOD, were found in any of the untreated water samples. |
| Calibration (type, number of data points) | Calibration standards (matrix-matched) were used for the analysis. At least six calibration points were used. Linear correlations with coefficients >0.9992 were obtained for Ametoctradin metabolites M650F001, M650F002, M650F003 and M650F004.  **Calibration data:**  **Groundwater**   |  |  |  |  |  | | --- | --- | --- | --- | --- | | Analyte | m/z | Slope | Intercept | r | | M650F001 | 250→ 149 | 1.26 x 106 | 1.63 x 103 | 0.9998 | | 250→ 176 | 1.49 x 106 | 2.31 x 103 | 0.9998 | | M650F002 | 236→ 176 | 4.59 x 106 | 4.41 x 103 | 1.0000 | | 236→ 121 | 6.84 x 105 | 1.32 x 103 | 0.9998 | | M650F003 | 222→ 176 | 3.44 x 106 | 2.54 x 103 | 1.0000 | | 222→ 121 | 3.42 x 105 | 608 | 0.9995 | | M650F004 | 208→ 123 | 1.58 x 106 | 1.76 x 103 | 1.0000 | | 208→ 95 | 3.43 x 105 | 914 | 0.9998 |   **Surface water**   |  |  |  |  |  | | --- | --- | --- | --- | --- | | Analyte | m/z | Slope | Intercept | r | | M650F001 | 250→ 149 | 3.56 x 106 | 4.17 x 103 | 1.0000 | | 250→ 176 | 4.19 x 106 | 6.09 x 103 | 1.0000 | | M650F002 | 236→ 176 | 1.06 x 107 | 2.97 x 104 | 0.9998 | | 236→ 121 | 1.58 x 106 | 3.68 x 103 | 0.9999 | | M650F003 | 222→ 176 | 1.31 x 107 | 2.32 x 104 | 0.9998 | | 222→ 121 | 1.3 x 106 | 2.12 x 103 | 0.9996 | | M650F004 | 208→ 123 | 2.62 x 106 | 1.68 x 103 | 0.9998 | | 208→ 95 | 5.51 x 105 | 516 | 0.9998 | |
| Calibration range | Standards in the range of 0.008 to 0.4 ng/mL were injected and the response was plotted against the concentration. |
| Standard solution and extract stability | Stability tests showed that metabolites M650F001, M650F002, M650F003 and M650F004 in calibration solutions is given for at least 37 days when stored refrigerated (+4 °C) in the dark. Stability in stock solutions was confirmed for metabolites M650F001, M650F003 and M650F004 for 37 days and M650F002 for 15 days, when stored refrigerated at approximately +4 °C in the dark. Working solutions were prepared in acetonitrile/water (10/90, v/v). The stock solution for M650F004 was prepared in methanol/water 80/20.  The extract stability of metabolites M650F001, M650F002, M650F003 and M650F004 in final volume was not tested, since no extraction procedure was used. Therefore, there are no extracts to be stored for a certain time period prior to LC-MS/MS analysis. Stability of the analytes in extracts was demonstrated by consistent LC-MS/MS results throughout the duration of the experimental phase and acceptable mean recoveries in the fortified samples within the range of 70-110%. |
| Assessment of matrix effects | Solvent- and matrix-matched standards were analysed to assess potential matrix effects. As significant matrix effects were identified, matrix-matched standards, prepared with acetonitrile/water (10/90, v/v) and a matrix load of 91 %, were used for calibration and quantification of metabolites M650F001, M650F002, M650F003 and M650F004. |
| Limit of determination/quantification | The limit of quantification (LOQ) is defined by the lowest successfully tested fortification level, corresponding to 0.03 µg/L for both water types tested and a concentration of 0.027 ng/mL in the sample extract after work-up. The limit of detection (LOD) is 0.009 µg/L, corresponding to the lowest calibration standard of 0.0082 ng/mL in the extract. |

Conclusion

The method L0113/03 was validated according to the requirements of SANCO/825/00 rev. 8.1. It also meets the requirements of SANTE/2020/12830 Rev.1 for the analysis of Ametoctradin metabolites M650F001, M650F002, M650F003 and M650F004 in surface water and groundwater matrices.

Independent laboratory validation

|  |  |
| --- | --- |
| Comments of zRMS: | The independent validation of the method L0113/03 has been accepted.  The study objective was to independently validate analytical method L0113/03 for the determination of Ametoctradin metabolites M650F001, M650F002, M650F003 & M650F004 in water matrix by LC-MS/MS. The method was validated at two fortification levels (0.03 μg/L (LOQ) and 0.3 μg/L (10x LOQ)) for two water types. For each fortification level and matrix, at least five replicates were analyzed. Per matrix at least two control samples were measured. Two mass transitions were evaluated and reported for each analyte. Matrix and solvent standards were analyzed within the study to test for possible matrix effects.  The mean recovery values and RSD ranged consistently with the requirements for all analytes. The method meets criteria of SANTE/2020/12830 Rev.1. |

|  |  |
| --- | --- |
| Reference: | CP 5.2/18 |
| Report | Independent Lab Validation (ILV) of XXXX's Analytical Method L0113/03 for the Determination of BAS 650 F Metabolites M650F001, M650F002, M650F003 and M650F004 in Surface Water and Groundwater by LC-MS/MS  Tzelepi, E., 2020  report No 89843  XXXX DocID. 2020/2034828  Authority registration No |
| Guideline(s): | SANCO/825/00 rev. 8.1 (16 November 2010), US EPA 860.1340, SANCO/3029/99 (11 July 2000) |
| Deviations: | No |
| Previous evaluation: | No |
| GLP: | Yes |
| Acceptability: | Yes | |

Study Summary

Method L0113/03 was independently validated for the determination of residues of Ametoctradin (BAS 650 F) Metabolites M650F001, M650F002, M650F003 and M650F004 in surface water and groundwater, with a limit of quantification at 0.03 µg/L. The brief description of the methods and the results are presented in the summary below.

Materials and methods

In Method L0113/03, the residues of Ametoctradin metabolites, M650F001, M650F002, M650F003 and M650F004 were determined by direct injection of the surface or ground water using LC-MS/MS.

Residues of Ametoctradin metabolites (M650F001, M650F002, M650F003 and M650F004) were determined from both surface water and groundwater, by LC MS/MS in ESI positive mode at the following mass transitions:

|  |  |  |
| --- | --- | --- |
| **Analyte** | **Primary Mass Transition (m/z)** | **Secondary Mass Transition (m/z)** |
| M650F001 | 250 → 149 | 250 → 176 |
| M650F002 | 236 → 176 | 236 → 121 |
| M650F003 | 222 → 176 | 222 → 121 |
| M650F004 | 208 → 123 | 208 → 95 |

Separation is accomplished with a Xterra® C18 column (50 mm x 4.6 mm, 3.5 µm) by using a gradient mixture of water/acetonitrile, each acidified with 0.1% formic acid, at a flow rate of 0.6 mL/min.

**Results and discussions**

The mean recovery values were between 70 and 110% with relative standard deviations (RSDs) of < 20%. The detailed results are given in the table below.

Table A 66 Validation results of method L0113/03: ILV for the determination of Ametoctradin metabolites (M650F001, M650F002, M650F003 and M650F004) in surface water and groundwater

| **Analyte** | **Matrix** | **m/z** | **Fortification level**  **[µg/L]** | **Number of replicates** | **Mean recovery [%]** | **RSD [%]** | **Overall recovery [%]** | **Overall RSD [%]** |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| M650F001 | Surface Water | 250→149 | 0.03 | 5 | 77.1 | 5.3 | 80.9 | 6.1 |
| 0.3 | 5 | 84.7 | 1.0 |
| 250→176 | 0.03 | 5 | 77.4 | 2.5 | 80.0 | 4.0 |
| 0.3 | 5 | 82.6 | 1.6 |
| Groundwater | 250→149 | 0.03 | 5 | 82.6 | 6.0 | 85.4 | 5.4 |
| 0.3 | 5 | 88.1 | 2.4 |
| 250→176 | 0.03 | 5 | 80.1 | 2.5 | 83.7 | 5.0 |
| 0.3 | 5 | 87.4 | 1.2 |
| M650F002 | Surface Water | 236→176 | 0.03 | 5 | 75.3 | 3.2 | 82.8 | 9.8 |
| 0.3 | 5 | 90.3 | 2.0 |
| 236→121 | 0.03 | 5 | 76.1 | 5.8 | 83.9 | 11.0 |
| 0.3 | 5 | 91.7 | 2.7 |
| Groundwater | 236→176 | 0.03 | 5 | 79.4 | 2.3 | 84.7 | 6.8 |
| 0.3 | 5 | 90.0 | 1.5 |
| 236→121 | 0.03 | 5 | 77.9 | 14 | 83.5 | 11.0 |
| 0.3 | 5 | 89.0 | 1.3 |
| M650F003 | Surface Water | 222→176 | 0.03 | 5 | 83.0 | 2.2 | 84.9 | 2.9 |
| 0.3 | 5 | 86.8 | 1.3 |
| 222→121 | 0.03 | 5 | 83.3 | 3.4 | 85.6 | 3.6 |
| 0.3 | 5 | 87.9 | 0.9 |
| Groundwater | 222→176 | 0.03 | 5 | 87.1 | 1.7 | 88.8 | 2.4 |
| 0.3 | 5 | 90.4 | 1.1 |
| 222→121 | 0.03 | 5 | 82.2 | 3.8 | 86.7 | 6.2 |
| 0.3 | 5 | 91.1 | 2.5 |
| M650F004 | Surface Water | 208→123 | 0.03 | 5 | 76.0 | 6.6 | 83.5 | 10.0 |
| 0.3 | 5 | 90.9 | 1.7 |
| 208→95 | 0.03 | 5 | 77.3 | 6.5 | 83.9 | 9.2 |
| 0.3 | 5 | 90.5 | 0.9 |
| Groundwater | 208→123 | 0.03 | 5 | 77.2 | 10 | 82.6 | 9.4 |
| 0.3 | 5 | 88.0 | 1.8 |
| 208→95 | 0.03 | 5 | 69.8 | 7.0 | 78.7 | 13 |
| 0.3 | 5 | 87.5 | 1.9 |

**Table A 67: Characteristics for the analytical method L0113/03 ILV used for validation of Ametoctradin metabolites (M650F001, M650F002, M650F003 and M650F004) in water**

|  | **M650F001, M650F002, M650F003 and M650F004** |
| --- | --- |
| Specificity | LC MS/MS is a highly specific self-confirmatory technique. LC-MS/MS method was used for determination of the analyte monitoring two characteristic mass transitions for quantification and qualification with regards to M650F001, M650F002, M650F003 and M650F004. LC MS/MS is a highly specific self-confirmatory technique. Under the described conditions the method is specific for the determination of metabolites M650F001, M650F002, M650F003 and M650F004 in surface water and groundwater. No residues of Ametoctradin metabolites greater than the LOD were found in any of the untreated water samples. |
| Calibration (type, number of data points) | A minimum of six calibration points were used. A linear correlation with good fit (r2 >0.9974) was obtained.  **Calibration data:**   |  |  |  |  |  | | --- | --- | --- | --- | --- | | Analyte | m/z | Slope | Intercept | r2 | | M650F001 | 250→ 149 | 1651420.9 | 4808.5 | 0.9999 | | 250→ 176 | 2008975.9 | 5909.9 | 0.9998 | | M650F002 | 236→ 176 | 5900281.8 | 25771.3 | 0.9997 | | 236→ 121 | 666622.6 | 3482.4 | 0.9998 | | M650F003 | 222→ 176 | 8085625.4 | 7391.7 | 0.9994 | | 222→ 121 | 814659.6 | 472.0 | 0.9992 | | M650F004 | 208→ 123 | 3230571.2 | 19872.0 | 0.9995 | | 208→ 95 | 704561.7 | 4117.3 | 0.9996 | |
| Calibration range | Standards in the range of 0.0082 to 0.41 ng/mL were injected and the response was plotted against the concentration. |
| Standard solution and extract stability | Standard solution and extract stability were not directly evaluated in the ILV study of Method L0113/03, but determined in a separate study (XXXX Doc ID 2008/1017003 submitted in the DAR). In this study, Ametoctradin metabolites, M650F001, M650F02, M650F003 and M650F004 was shown to be stable (>80%) at least for 23 days, in calibration standard solutions in acetonitrile-water (10:90, v/v) under refrigerated conditions. Although stability in methanol for all analytes was not directly evaluated, it could be assumed to be stable as these analytes are stable in calibration solution which were prepared from fortification solution, for at least 23 days.  Stability of the analytes in solutions and in extracts in this study was also demonstrated by consistent LC-MS/MS results throughout the duration of the experimental phase and acceptable mean recoveries in the fortified samples within the range of 70-110%. |
| Assessment of matrix effects | The matrix effect was tested for surface water and ground water. No significant (>20%) matrix effects were observed for Ametoctradin metabolites. For the evaluation of all results, solvent calibration standards were used. |
| Limit of determination/quantification | The LOQ of the method is defined as the lowest analyte concentration in a sample at which the methodology has been successfully validated and is at 0.03 μg/L for all analytes.  The limit of detection (LOD) of the method is defined as the lowest analyte concentration injected as a calibration solution resulting in an LOD of 0.009 μg/L for all analytes and both quantification and confirmation transitions, corresponding to the lowest calibration standard of 0.0082 ng/mL in the extract. |

**Conclusion**

The method L0113/03 was validated according to the requirements of SANCO/825/00 rev. 8.1. It also meets the requirements of SANTE/2020/12830 Rev.1 for the analysis of Ametoctradin metabolites (M650F001, M650F02, M650F003 and M650F004) in surface water and groundwater.

Confirmatory method

No confirmatory technique is required as two different, highly specific mass transitions are used for quantitation and qualification in the study.

* + - 1. Description of Methods for the Analysis of Air (KCP 5.2)
         1. Analytical method 13

Method L0108/02 is used for the determination of Ametoctradin in air. The method was submitted in the AIR5 dossier for Ametoctradin and has not yet been peer reviewed. Thus, it is provided for information below.

Method validation

|  |  |
| --- | --- |
| Comments of zRMS: | The method has been accepted.  The study objective was to validate analytical method L0108/02 for the determination of Ametoctradin in air by LC-MS/MS.  For the recovery experiments, two fortification levels, 45 ng (LOQ) and 450 ng (10x LOQ), were spiked onto the adsorber material (corresponding to 540 L air), which results in a fortified concentration at LOQ level of 0.0833 ng/L air. For each fortification level, five replicates were prepared and analyzed. To check for break-through in the backup part of the adsorber tubes, the front bed of the adsorber tubes was spiked with 4500 ng BAS 650 F (100x LOQ) and front bed and backup bed of the adsorber were individually analyzed for five replicates each. Matrix and solvent standards were analyzed within the study to test for possible matrix effects and to decide if matrix-matched standards are necessary. Additionally, unfortified samples were analyzed (untreated control samples). Two mass transitions were evaluated for quantification and confirmation of BAS 650 F.  The mean recovery values and RSD ranged consistently with the requirements for all analytes. The method meets criteria of SANTE/2020/12830 Rev.1. |

|  |  |
| --- | --- |
| Reference: | CP 5.2/19 |
| Report | Validation of Method L0108/02 for the Determination of BAS 650 F (Reg.No. 4993353) in Air using LC/MS-MS  Karrer, C. & Schatte, S., 2020  report No 808965  XXXX DocID 2020/2034610  Authority registration No |
| Guideline(s): | SANCO/825/00 rev. 8.1 (16/11/2010), SANCO/3029/99 rev. 4 (11/07/2000), U.S. EPA Ecological Effects Test Guideline, OCSPP 850.6100 Environmental Chemistry Methods and Associated ILV |
| Deviations: | No |
| Previous evaluation: | No |
| GLP: | Yes |
| Acceptability: | Yes | |

Study Summary

The method L0108/02, was developed and validated for the determination of residues of Ametoctradin (BAS 650 F) in air samples, with a limit of quantification at 0.0833 ng/L air. The brief description of the methods and the results are presented in the summary below.

Materials and methods

Residues of Ametoctradin (BAS 650 F) are extracted from ORBO™ - 402 Tenax adsorber tubes (either containing trapped residues or fortified for validation purposes) with methanol, assisted by ultrasonication. An aliquot of the extract is diluted with methanol/water/formic acid (500/500/1, v/v/v) prior to the final determination of Ametoctradin (BAS 650 F), which is conducted by LC-MS/MS. For quantification, the mass transition m/z 276→m/z 149 is proposed for quantification and the mass transition m/z 276→m/z 176 is proposed for confirmation.

Separation is achieved by using a Phenomenex Synergi Fusion -RP column (150x4.6, 4 µm particle size) at a flow rate of 1 mL/min. A gradient flow of water/formic acid (1000/1, v/v) and acetonitrile/formic acid (1000/1, v/v) is applied.

**Results and discussions**

The mean recovery values were between 70 and 110% with relative standard deviations (RSDs) of < 20%. The detailed results are given in the table below.

Table A 68 Validation results of the method L0108/02: Determination of Ametoctradin in air

| **Analyte** | **Matrix** | **m/z** | **Fortification Level [ng/L air]** | **Number of Replicates** | **Mean Recovery [%]** | **Mean RSD [%]** | **Overall Recovery [%]** | **Overall RSD [%]** |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| BAS 650 F | Air | 276 → 149 | 0.833 | 5 | 101 | 1.9 | 100 | 1.9 |
| 0.0833 | 5 | 99.0 | 1.3 |
| 276 → 176 | 0.833 | 5 | 102 | 1.9 | 101 | 2.0 |
| 0.0833 | 5 | 99.5 | 1.3 |
|  | | | | | | | | |

Table A 69: Characteristics for the analytical method L0108/02: Determination of Ametoctradin in air

|  | Ametoctradin |
| --- | --- |
| Specificity | The quantification of Ametoctradin (BAS 650 F) is based on the monitoring of two mass transitions. Due to the high selectivity and specificity of LC MS/MS, an additional confirmatory technique is not required. In the untreated control samples, no interferences (> 30 % LOQ) at the relevant retention time and mass transitions were observed. |
| Calibration (type, number of data points) | Calibration standards (solvent-based) were used for the analysis. At least eight calibration points were used. Linear correlations with coefficients r >0.99 were obtained for Ametoctradin.  **Calibration data:**   |  |  |  |  |  | | --- | --- | --- | --- | --- | | Analyte | m/z | Slope | Intercept | r | | Ametoctradin | 276→ 149 | 3.29 x 105 | 3.27 x 103 | 0.9997 | | 276→ 176 | 3.01 x 105 | 3.19 x 103 | 0.9998 | |
| Calibration range | Standards in the range of 0.1 to 8.0 ng/mL were injected and the response was plotted against the concentration. |
| Standard solution and extract stability | Stability tests showed that Ametoctradin (BAS 650 F) in stock, fortification and calibration solutions in methanol and in methanol/water (50:50, v/v/v), respectively is given for at least 33 days as determined within a separate study [XXXX DocID 2020/2034573], when stored refrigerated (+4°C) in the dark.  Extract stability was evaluated in initial extraction solvent (methanol) and in final volume solution [methanol/water (50:50, v/v)], fortified at 10x LOQ and were shown to be stable for 5 days of storage refrigerated (+4 °C) in the dark. BAS 650 F on ORBO™ - 402 Tenax adsorber tubes fortified at 10x LOQ was also shown to be stable 6 days after refrigerated (+4 °C) storage in the dark |
| Assessment of matrix effects | No significant matrix effects (i.e > ±20 % signal suppression or signal enhancement) were observed for Ametoctradin (BAS 650 F) in air adsorber material tested. Therefore, solvent calibration standards were used for the quantification. |
| Limit of determination/quantification | The method has a limit of quantification (LOQ) of 0.0833 ng/L air (45 ng in 540 L air), corresponding to the lowest fortification level successfully tested with a concentration of 0.45 ng/mL at measurement sample level. The method has a limit of detection (LOD) of 0.0185 ng/L air (10 ng in 540 L air), corresponding to the lowest calibration standard (0.01 ng/mL at measurement sample level) used. |

Conclusion

The method L0108/02 was validated according to the requirements of SANCO/825/00 rev. 8.1. It also meets the requirements of SANTE/2020/12830 Rev.1 for the analysis of Ametoctradin in air.

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

Please refer to Section A 2.1.2.2 above (Methods for the Analysis of Food of Animal Origin) for methodology for the determination of Ametoctradin and metabolite M650F006 in body tissues.

Method L0347/01 and R0066/01 are used for the determination of Ametoctradin and metabolite M650F006 respectively in body fluids (blood and urine). The methods were submitted in the AIR5 dossier for Ametoctradin and have not yet been peer reviewed. Thus, they are provided for information below.

* + - * 1. Analytical method 14

Method validation

|  |  |
| --- | --- |
| Comments of zRMS: | The method has been accepted.  The objective of this study was to validate an analytical method for the determination of ametoctradin in blood and urine. The LOQ of the analytical method was 0.01 mg/kg, using LC-MS/MS with two mass transitions. The analytical method was derived from the QuEChERS multi-residue method. The validation was performed at 2 fortification levels (0.01 and 0.10 mg/kg), each with 5 replicates and 2 untreated control samples and 1 reagent blank per matrix (blood and urine). The matrix effect was tested for each matrix. No significant matrix effects (i.e. > 20 % suppression or enhancement) on LC-MS/MS response were observed for the matrices.  The mean recovery values were between 94.5 % and 104 %. The relative standard deviations (RSD, %) for all fortification levels were below 7.4 %.  The method meets criteria of SANTE/2020/12830 Rev.1. |

|  |  |
| --- | --- |
| Reference: | CP 5.2/20 |
| Report | Validation of XXXX analytical method L0347/01 for the determination of BAS 650 F (Ametoctradin) in body fluids  Richter, S. & Diedovic, S., 2016  report No EU-P4000G,EU-809017,P 4000 G  XXXX DocID 2016/1235194  Authority registration No |
| Guideline(s): | OECD-ENV/JM/MONO/(2007)17, SANCO/3029/99 rev. 4 (11 July 2000), SANCO/825/00 rev. 8.1 (16 November 2010) |
| Deviations: | No |
| Previous evaluation: | No |
| GLP: | Yes |
| Acceptability: | Yes | |

Study Summary

The method L0347/01 was developed and validated for the determination of residues of Ametoctradin (BAS 650 F) in body fluid (blood and urine) matrices with a limit of quantification at 0.01 mg/kg using LC-MS/MS. The brief description of the method and the results are presented in the summary below.

Materials and methods

In the analytical method L0347/01, residues of Ametoctradin (BAS 650 F, Reg.No. 4993353) are extracted from blood and urine using acetonitrile. After addition of MgSO4, NaCl and buffering citrate salts, the mixture is shaken intensively and centrifuged. For urine samples, an extract aliquot is diluted prior to LC MS/MS analysis. For blood samples, an aliquot of the organic extract is cleaned-up by addition of PSA and MgSO4. After shaking and centrifugation, an extract aliquot is diluted followed by LC-MS/MS analysis. Separation is achieved by using a Phenomenex Synergy Fusion column (100 mm x 2 mm, 2.5 µm) and a gradient of water/formic acid (1000/1, v/v) and methanol/formic acid (1000/1, v/v) at a flow rate of 0.3 mL/min. Detection is accomplished in ESI positive mode at mass transitions 276 m/z → 176 m/z for quantification and 276 m/z → 149 m/z for qualification. Calibration standards are prepared as solvent based standards in acetonitrile/water (2/8, v/v) + 0.1% formic acid.

**Results and discussions**

The mean recovery values were between 70 and 110% with relative standard deviations (RSDs) of < 20%. The detailed results are given in the table below.

Table A 70 Validation results of the method L0347/01: Determination of Ametoctradin in body fluids (blood & urine)

| Matrix | Analyte | Fortification level (mg/kg) (*n* = x) | Mean  recovery (%) | RSD (%) | Comments |
| --- | --- | --- | --- | --- | --- |
| Urine | Ametoctradin | 0.01 (n=5) | 94.5 | 1.1 | 276 m/z → 176 m/z Quantifier |
| 0.10 (n=5) | 99.2 | 10 |
| 0.01 (n=5) | 96.9 | 1.3 | 276 m/z → 149 m/z Qualifier |
| 0.10 (n=5) | 99.6 | 9.8 |
| Blood | 0.01 (n=5) | 103 | 2.1 | 276 m/z → 176 m/z Quantifier |
| 0.10 (n=5) | 104 | 1.9 |
| 0.01 (n=5) | 103 | 1.6 | 276 m/z → 149 m/z Qualifier |
| 0.10 (n=5) | 104 | 2.3 |

|  | Ametoctradin |
| --- | --- |
| Specificity | LC-MS/MS is a highly specific method. No interference (< 30 % LOQ) of total peak area for the target analyte at the retention time, was found in unfortified control samples. |
| Calibration (type, number of data points) | Calibration standards (solvent-based) were used for the analysis. At least six calibration points were used. Linear correlations with coefficients r >0.99 were obtained for Ametoctradin.  **Calibration data:**   |  |  |  |  |  | | --- | --- | --- | --- | --- | | Analyte | m/z | Slope | Intercept | r | | Ametoctradin | 276→ 176 | 1.38 x 106 | 1.95 x 103 | 0.9992 | | 276→ 149 | 1.41 x 106 | -561 | 0.9992 | |
| Calibration range | Standards in the range of 0.02 to 2.0 ng/mL were injected and the response was plotted against the concentration with a linear fit. |
| Standard solution and extract stability | Standard solutions and extract stability were evaluated in the study. Standards in stock and fortification solvent (methanol) is shown to be stable (>80%) at least for 30 days for Ametoctradin (BAS 650 F), under refrigerated conditions. Calibration standards in acetonitrile/water (2:8, v/v) with 0.1% formic acid, is shown to be stable (>80%) at least for 61 days, for BAS 650 F under refrigerated conditions (dark).  Extract stability was also evaluated in initial raw extract and final sample extracts in acetonitrile/water (2/8, v/v) + 0.1 % FA for 8 days. The results shown that Ametoctradin (BAS 650 F), was stable (>80%) for at least 8 days under refrigerated conditions. |
| Assessment of matrix effects | The matrix effect was tested for each matrix. No significant matrix effects (i.e. > 20 % suppression or enhancement) on LC-MS/MS response were observed for the matrices. Therefore, solvent calibration standards were used for the quantification. |
| Limit of determination/quantification | The limit of quantification (LOQ) defined by the lowest successfully tested fortification level is 0.01 mg/kg. The limit of detection (LOD) of the method is defined 0.02 ng/mL corresponding to the lowest calibration level used. |

Conclusion

The method L0347/01 was validated according to the requirements of SANCO/825/00 rev. 8.1. It also meets the requirements of SANTE/2020/12830 Rev.1 for the analysis of Ametoctradin in body fluids (blood & urine).

* + - * 1. Analytical method 15

Method validation

|  |  |
| --- | --- |
| Comments of zRMS: | The method R0068/01 has been accepted.  The objective of this study was to validate an analytical method for the determination of M650F006 residues in swine (porcine) blood plasma and urine by liquid chromatography (LC) positive ion electrospray ionization tandem mass spectrometry (MS/MS-ESI), monitoring ion transitions m/z 278→149 and 278→217. The LOQ for M650F006 residues in swine blood plasma and urine is 10 µg/L (0.01 ppm). The validation was performed at 2 fortification levels (LOQ and 100xLOQ), each with 5 replicates and 2 untreated control samples and 1 reagent blank per matrix.  The mean recovery values were in the required range. The relative standard deviations (RSD, %) for all fortification levels were below 20 %.  The method meets criteria of SANTE/2020/12830 Rev.1. |

|  |  |
| --- | --- |
| Reference: | CP 5.2/21 |
| Report | Validation of method R0066/01: Method for the determination of M650F006 (Reg. No. 5507462) in swine blood plasma and urine matrices by LC-MS/MS  Horowitz, M., 2020  report No 897507  XXXX DocID 2020/2032355  Authority registration No |
| Guideline(s): | EPA 850.6100, SANCO 3029/99 Rev.4, SANCO/825/00 rev. 8.1 (16/11/2010) |
| Deviations: | No |
| Previous evaluation: | No |
| GLP: | Yes |
| Acceptability: | Yes | |

Study Summary

The method R0066/01 was developed and validated for the determination of residues of M650F006 (Reg.No. 5507462, a metabolite of Ametoctradin) in swine blood plasma and urine by LC-MS/MS with a limit of quantification at 0.01 mg/kg. The brief description of the method and the results are presented in the summary below

Materials and methods

In method R0068/01, residues in body fluid (blood plasma and urine) samples (1 mL each), were extracted with the addition of methanol, which precipitates/aggregates the body fluid proteins. After centrifugation, residues in the extract solution were diluted with methanol/water (50/50, v/v) and then determined by LC-MS/MS in ESI positive mode at mass transition m/z 278→149 for quantification and m/z 278→217 for confirmation. Separation was accomplished with an Acquity HSS T3 column (100 mm x 2.1 mm, 1.8 µm) by using a gradient mixture of water/methanol, each acidified with 0.1% formic acid at a flow rate of 450 µL/min

**Results and discussions**

The mean recovery values were between 70 and 110% with relative standard deviations (RSDs) of < 20%. The detailed results are given in the table below.

Table A 71 Validation results of the method R0068/01: Determination of Ametoctradin metabolite M650F006 in body fluids (blood & urine)

| **Matrix** | **Analyte** | **No. of tests** | **Fortification level [mg/L]** | **Transition 278 → 149 1)** | | **Transition 278 → 217** | |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **mean [%]** | **RSD [%]** | **mean [%]** | **RSD [%]** |
| Blood Plasma (Swine) | M650F006 | 5 | 0.01 | 98 | 9 | 96 | 11 |
| 5 | 1.00 | 94 | 9 | 88 | 3 |
| Urine (Swine) | 5 | 0.01 | 101 | 4 | 103 | 7 |
| 5 | 1.00 | 101 | 10 | 102 | 12 |
| 1) Primary transition | | | | | | | |

Table A 72: Characteristics for the analytical method R0068/01: Determination of Ametoctradin metabolite M650F006 in body fluids (blood & urine)

|  | Ametoctradin |
| --- | --- |
| Specificity | LC-MS/MS using two mass transitions is a highly specific, self-confirmatory method. No interfering peaks were found at the retention time for M650F006. |
| Calibration (type, number of data points) | Calibration standards (solvent-based) were used for the analysis. At least five calibration points were used. Linear correlations with coefficients r >0.9977 were obtained for M650F006.  **Calibration data:**   |  |  |  |  |  | | --- | --- | --- | --- | --- | | Analyte | m/z | Slope | Intercept | r | | M650F006 | 278→ 149 | 1.09 x 106 | -5.57 x 102 | 0.9980 | | 278→ 217 | 1.44 x 106 | 1.04 x 102 | 0.9977 | |
| Calibration range | Standards in the range of 0.005 to 0.1 ng/mL were injected and the response was plotted against the concentration with a linear fit. |
| Standard solution and extract stability | The standard solution stability of M650F006 was evaluated in a previous validation study [XXXX DocID 2008/1022140] and was shown to be stable in stock standards prepared in methanol, intermediate (fortification) standards prepared in methanol, and in calibration standards prepared in methanol/water (50/50, v/v), for at least 1 month (28 days), each when held under refrigeration. During the course of this study, the test/reference substance solutions were stored in a refrigerator and all solutions were used within the demonstrated time period of stability.  The method validation fortification sample extracts were analysed on the same day of extraction. The acceptable method recoveries obtained during analysis demonstrate the storage stability of M650F006 residues in the extracts in the brief period prior to analysis. In addition, the recoveries from stored solutions generated during extract stability experiments, which included tests on the initial extracts and HPLC final volume stored under refrigeration, indicated that M650F006 is stable in extracts of swine blood plasma and urine for at least the time period tested, 8 days. |
| Assessment of matrix effects | The experiment to evaluate any potential matrix effects showed that the matrix load in the samples did not have a significant influence on the analysis (matrix effects <20%). Therefore, solvent-based calibration standards, prepared in methanol/water (50/50, v/v), were used in the study. |
| Limit of determination/quantification | The limit of quantification (LOQ) defined by the lowest successfully tested fortification level was 0.01 mg/L. The limit of detection (LOD) of the method is defined 0.002 mg/L corresponding to the lowest calibration level used |

Conclusion

The method R0068/01 was validated according to the requirements of SANCO/825/00 rev. 8.1. It also meets the requirements of SANTE/2020/12830 Rev.1 for the analysis of the Ametoctradin metabolite M650F006 in body fluids (blood & urine).

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

No new or additional studies have been submitted

* 1. Analytical methods for Propamocarb
     1. Methods used for the generation of pre-authorization data (KCP 5.1)
        1. Method L0450/01: Method for the determination of propamocarb plant matrices
           1. Method validation

|  |  |
| --- | --- |
| Comments of zRMS: | The method validation has been accepted.  The purpose of this study was to validate the analytical method L0450/01 for the determination of Propamocarb in tomato fruit, grapefruit, dry pea, and soybean seeds using LC-MS/MS. The validation was performed at 2 fortification levels (LOQ and 10 x LOQ), each with 5 replicates and 2 untreated control samples. After the entire workup the selective and sensitive LC-MS/MS technique was used for determination of propamocarb monitoring two characteristic mass transitions. Therefore, no confirmatory method was needed.  The calibration curve was obtained by injection of 7 concentration levels of propamocarb in matrix-matched calibration solutions. Matrix-matched and solvent-based standards were analyzed within this study to assess potential matrix effects. In addition, a reagent blank was run in parallel to investigate potential contaminations.  Mean recoveries for all analytes, all matrices, MS/MS ion transitions were between 101 % and 106 %. The relative standard deviations (RSD, %) for all fortification levels were ≤ 10 %. The interferences of propamocarb were below 30 % LOQ.  The method L0450/01 was successfully validated in tomato fruit, grapefruit, dry pea, and soybean seeds at a LOQ of 0.010 mg/kg.  It could be demonstrated that XXXX method no. L0450/01 fulfills the requirements of SANTE/2020/12830, Rev.1 regarding recoveries, linearity, specificity (selectivity) and limit of quantification and is therefore applicable to correctly determine residues of propamocarb in plant matrices. |

|  |  |
| --- | --- |
| Reference: | CP 5.1.2/21 |
| Report | Validation of analytical method L0450/01 for the analysis of Propamocarb in plant matrices  Denim, R., 2022  report No 919333  XXXX DocID 2022/2032351  Authority registration No |
| Guideline(s): | EPA 860.1340; SANTE/2020/12830 Rev.1; OECD ENV/JM/MONO(2007)17 |
| Deviations: | None |
| Previous evaluation: | No |
| GLP: | Yes |
| Acceptability: | Yes | |

**Study Summary**

The method L0450/01 was validated for the determination of residues of Propamocarb (BAS 9068F) in plant matrices (tomato (fruit), pea (dry), soybean (seed) and grapes (fruit)), with a limit of quantification at 0.01 mg/kg. The brief description of method and the results are presented in the summary below.

Materials and methods

Sample material (2.5 g) is weighed into a 250 mL plastic containiner with screw cap. The sample is homogenised on a Polytron for 3 minutes at approximately 5000 rpm with extraction solvent (methanol:water, 2M HCl 80/15/5 v/v/v, 50 mL), transferred to a 50 mL culture tube and centrifuged for about 5 minutes at approximately 4000 U/min. The supernatant is transferred to a 100 mL volumetric flask. The extraction procedure is repeated with a fresh extraction solvent as before and the two supernatant extracts combined. The final volume is adjusted to 100 mL with extraction solvent. An aliquot (400 µL) is diluted with water (600 µL) and vortex mixed. The final determination of Propamocarb is performed by HPLC-MS/MS using a Synergi Fusion RP column (100 mm x 2 mm, 2.5 µm) and gradient elution with mobile phases of 5mM ammonium formate in water and 5mM ammonium formate in methanol at a flow rate of 0.5 mL/min. Detection is accomplished in ESI positive mode using two different transitions. Detection is accomplished in ESI positive mode using two different transitions. Mass transitions at m/z 189 > 102 and m/z 189 > 144 are used for quantification and confirmation of Propamocarb respectively. External matrix-matched calibration standards were employed.

Results and discussions

The analytical method validation is summarised below. The HPLC-MS/MS determination was conducted by monitoring two MS/MS mass transitions and therefore no confirmatory method is required.

Mean recoveries & precision are within the permitted range required by the guideline SANTE/2020/12830 rev. 1.

Table A 73: Recovery results from method validation of Propamocarb in plant matrices using the analytical method

| Matrix | Analyte | Fortification level (mg/kg) (n = x) | Recovery (%) | Mean  recovery (%) | RSD (%) | Comments |
| --- | --- | --- | --- | --- | --- | --- |
| ***Mass transition (Quantification) 189→102 m/z*** | | | | | | |
| Tomato (fruit) | Propamocarb | 0.01 (n = 5) | 99.9, 94.8, 91.6, 101, 100 | 97.6 | 3.8 | Acceptable |
| 0.1 (n = 5) | 104, 106, 105, 109, 105 | 106 | 1.5 |
| Pea (dry) | Propamocarb | 0.01 (n = 5) | 114, 105, 107, 102, 98.6 | 105 | 4.9 | Acceptable |
| 0.1 (n = 5) | 106, 105, 105, 106, 97.2 | 104 | 3.2 |
| Soybean (seeds) | Propamocarb | 0.01 (n = 5) | 123, 103, 106, 101, 98.8 | 107 | 8.1 | Acceptable |
| 0.1 (n = 5) | 98.6, 103, 106, 104, 100 | 102 | 2.7 |
| Grapes (fruit) | Propamocarb | 0.01 (n = 5) | 117, 104, 105, 107, 106 | 108 | 4.4 | Acceptable |
| 0.1 (n = 5) | 105, 104, 100, 104, 102 | 103 | 1.5 |
| ***Mass transition (Confirmation) 189→144 m/z*** | | | | | | |
| Tomato (fruit) | Propamocarb | 0.01 (n = 5) | 87.1, 101, 92.6, 105, 95.9 | 96.2 | 6.4 | Acceptable |
| 0.1 (n = 5) | 105, 108, 105, 107, 105 | 106 | 1.1 |
| Pea (dry) | Propamocarb | 0.01 (n = 5) | 103, 99.4, 102, 77.7, 90.7 | 94.5 | 10 | Acceptable |
| 0.1 (n = 5) | 110, 107, 108, 110, 99.0 | 107 | 3.8 |
| Soybean (seeds) | Propamocarb | 0.01 (n = 5) | 113, 102, 107, 105, 90.0 | 103 | 7.2 | Acceptable |
| 0.1 (n = 5) | 100, 107, 107, 105, 102 | 104 | 2.5 |
| Grapes (fruit) | Propamocarb | 0.01 (n = 5) | 115, 94.6, 106, 105, 115 | 107 | 7.1 | Acceptable |
| 0.1 (n = 5) | 105, 105, 102, 105, 103 | 104 | 1.0 |

Table A 74: Characteristics for the method used for validation of Propamocarb residues in plant matrices

|  | Propamocarb |
| --- | --- |
| Specificity | HPLC-MS/MS monitoring two ion transitions is a highly specific method. No interference (> 30 % LOQ) was observed at the retention time of interest in blank and control samples. |
| Calibration (type, number of data points) | Calibration was performed with matrix-matched standards using at least five concentrations ranging 0.030 to 2.5 ng/mL (equivalent to 0.0030 mg/kg to 0.25 mg/kg at sample level) and good linearity was observed (r ≥0.99). The resulting test substance peak areas versus test substance concentration plot were regressed using a linear fit.  Regression data for Propamocarb:   |  |  |  |  |  | | --- | --- | --- | --- | --- | | Matrix | m/z | Slope | Intercept | r | | Tomato (fruit) | 189→ 102 | 1.53857 x 106 | 24691.61279 | 0.99863 | | 189→ 144 | 4.18194 x 105 | 23479.65284 | 0.99811 | | Pea  (dry) | 189→ 102 | 1.86190 x 106 | 16668.15553 | 0.99993 | | 189→ 144 | 5.28207 x 105 | 8438.12142 | 0.99885 | | Soybean (seeds) | 189→ 102 | 1.80116 x 106 | 16358.28994 | 0.99989 | | 189→ 144 | 4.88335 x 105 | 5507.26878 | 0.99966 | | Grapes  (fruit) | 189→ 102 | 1.68588 x 106 | 18062.68192 | 0.99992 | | 189→ 144 | 4.56365 x 105 | 7513.62455 | 0.99989 | |
| Calibration range | 0.030 ng/mL to 2.5 ng/mL (equivalent to 0.0030 mg/kg to 0.25 mg/kg at sample level) |
| Assessment of matrix effects is presented | No significant matrix effects observed (i.e. <20%), nevertheless calibration standards were matrix-matched throughout. |
| Solution Stability | Solution stability was determined in XXXX Doc ID 2020/2106237:  Stock and calibration solutions were found to be stable following refrigerated storage at 2-8 ºC for 15 days. Raw and final volume extracts were found to be stable for at least 7 days following refrigerated storage at 2-8 ºC. |
| Limit of determination/quantification | The limit of quantification (LOQ) of 0.01 mg/kg was confirmed. The limit of detection (LOD), defined as the lowest standard employed was 0.030 ng/mL (=0.0030 mg/kg at sample level). |

Conclusion

The analytical procedure, method L0450/01 was validated for the determination of residues of Propamocarb (BAS 9068F) in plant matrices (tomato (fruit), pea (dry), soybean (seed) and grapes (fruit)) in terms of specificity, linearity, precision, accuracy, matrix effects, solution stability and LOQ, in accordance with the requirements of SANTE/2020/12830 Rev.1.

* + - 1. Method L0450/01: Method for the determination of propamocarb in melon (fruit, peel & pulp)
         1. Method validation

|  |  |
| --- | --- |
| Comments of zRMS: | The method has been accepted.  The method widely used within the section B7 of this submission for residue data generation in plant matrices. The final determination of propamocarb in melon matrices (fruit, peel, pulp) was performed by LC-MS/MS which is highly sensitive and selective. The LOQ was set at 0.010 mg/kg for all analytes.  The mean recovery values were between the required range for all analytes. The relative standard deviations (RSD, %) for all fortification levels were ≤ 20 %.  Thus, it could be confirmed that the analytical method L0450/01 fulfils requirements of SANCO/3029/99 rev. 4. and meets the requirements of SANTE/2020/12830 rev.1 also. |

|  |  |
| --- | --- |
| Reference: | CP 5.1.2/3 |
| Report | Study on the residue behaviour of Propamocarb (Reg.No. 4628172) and Ametoctradin (BAS 650 F) in Melon after treatment with BAS 743 01 F under field conditions in Southern Europe in 2020  Schneider, E., 202~~0~~1  report No 890088  XXXX DocID 2021/2019512  Authority registration No |
| Guideline(s): | EPA 860.1340; SANCO/3029/99 rev. 4 (11 July 2000); OECD ENV/JM/MONO(2007)17 - Guidance Document on Pesticide Residue Analytical Methods |
| Deviations: | None which affect integrity of the method validation |
| Previous evaluation: | No |
| GLP: | Yes |
| Acceptability: | Yes | |

**Study Summary**

The method L0450/01 was validated for the determination of residues of Propamocarb (BAS 9068 F) in melon matrices (fruit, peel, pulp), with a limit of quantification at 0.01 mg/kg. The brief description of the method and the results are presented in the summary below.

Materials and methods

The method is that described in XXXX Doc ID 2022/2032351 above.

Results and discussions

The analytical method validation is summarised below. The HPLC-MS/MS determination was conducted by monitoring two MS/MS mass transitions and therefore no confirmatory method is required.

Procedural recoveries were obtained by fortification of the matrices and all mean recoveries are within the permitted range required by the guideline SANTE/2020/12830 rev. 1. While two mass transitions were analysed, only the one for quantification is reported.

Table A 75: Recovery results from method validation of propamocarb in melon matrices using the analytical method

| Matrix | Analyte | Fortification level (mg/kg) (n = x) | Recovery (%) | Mean  recovery (%) | RSD (%) | Comments |
| --- | --- | --- | --- | --- | --- | --- |
| ***Mass transition (Quantification): Propamocarb 189→102 m/z*** | | | | | | |
| Melon  (fruit) | Propamocarb | 0.01 (n = 6) | 97.1, 99.2, 93.6, 98.3, 96.5, 96.5 | 96.8 | 2.0 | Acceptable |
| 0.1 (n = 6) | 106, 107, 102, 106, 101, 104 | 104 | 2.2 |
| 10 (n = 1) | 102 | 102 | - |
| Melon  (peel) | Propamocarb | 0.01 (n = 3) | 100, 98.6, 95.7 | 98.2 | 2.4 | Acceptable |
| 0.1 (n = 3) | 92.7, 93.4, 93.9 | 93.3 | 0.64 |
| 10 (n = 1) | 92.7 | 92.7 | - |
| Melon (pulp) | Propamocarb | 0.01 (n = 3) | 103, 110, 105 | 106 | 3.4 | Acceptable |
| 0.1 (n = 3) | 98.3, 87.6, 101 | 95.7 | 7.4 |

Table A 76: Characteristics for the method used for validation of propamocarb residues in melon (fruit, peel, pulp) matrices

|  | Propamocarb |
| --- | --- |
| Specificity | LC-MS/MS is a highly specific method. No interference (> 30 % LOQ) of total peak area for the target analyte at the retention time, was found in unfortified control samples. |
| Calibration (type, number of data points) | Calibration was performed with solvent based standards at least five concentrations ranging 0.030 to 10 ng/mL and good linearity was observed (r ≥0.99). The resulting test substance peak areas versus test substance concentration data were fit to the linear function.  Regression data:  Mass transition 189→ 102 m/z (quantification)  Slope = 1.29 x 106, Intercept = 1.96 x 103, r = 0.9997 |
| Calibration range | 0.030 ng/mL to 10 ng/mL |
| Assessment of matrix effects is presented | No significant matrix effects observed (i.e. <20%). Calibration standards were therefore prepared in solvent throughout. |
| Solution Stability | Solution stability was determined in XXXX Doc ID 2020/2106237:  Stock and calibration solutions were found to be stable following refrigerated storage at 2-8 ºC for 15 days. Raw and final volume extracts were found to be stable for at least 7 days following refrigerated storage at 2-8 ºC. |
| Limit of determination/quantification | The limit of quantification (LOQ) of 0.01 mg/kg was confirmed. The limit of detection (LOD) was 0.003 mg/kg. |

Conclusion

The analytical procedure, method L0450/01, for the determination of residues of Propamocarb (BAS 9068 F) in melon matrices has been fully validated in terms of specificity, linearity, precision, accuracy, matrix effects, solution stability and LOQ, in accordance with the requirements of SANCO/3029/99 rev.4 and meets the requirements of SANTE/2020/12830 rev.1 also.

* + - 1. Method L0450/01: Method for the determination of propamocarb in lettuce (open head and Lamb’s)
         1. Method validation

|  |  |
| --- | --- |
| Comments of zRMS: | The method has been accepted.  The method widely used within the section B7 of this submission for residue data generation in plant matrices. The final determination of propamocarb in lettuce matrices (open head and Lamb’s) was performed by LC-MS/MS which is highly sensitive and selective. The LOQ was set at 0.010 mg/kg for all analytes.  The mean recovery values were between the required range for all analytes. The relative standard deviations (RSD, %) for all fortification levels were ≤ 20 %.  Thus, it could be confirmed that the analytical method L0450/01 fulfils requirements of SANCO/3029/99 rev. 4. |

|  |  |
| --- | --- |
| Reference: | CP 5.1.2/4 |
| Report | Study on the residue behaviour of Propamocarb (Reg.No. 4628172) and Ametoctradin (BAS 650 F) in lettuce after two applications of BAS 743 01 F under field conditions in Southern Europe, 2021  Vagt, I. & Meyer, M., 2022  report No 890083  XXXX DocID 2022/2041753  Authority registration No |
| Guideline(s): | EPA 860.1340; SANTE/2020/12830, Rev.1; OECD ENV/JM/MONO(2007)17 - Guidance Document on Pesticide Residue Analytical Methods |
| Deviations: | None which affect integrity of the method validation |
| Previous evaluation: | No |
| GLP: | Yes |
| Acceptability: | Yes | |

**Study Summary**

The method L0450/01 was validated for the determination of residues of Propamocarb (BAS 9068 F) in lettuce matrices (open head [whole plant and open leaves] and Lamb’s [whole plant and leaves]), with a limit of quantification at 0.01 mg/kg. The brief description of method and the results are presented in the summary below.

Materials and methods

The method is that described in XXXX Doc ID 2022/2032351 above.

Results and discussions

The analytical method validation is summarised below. The HPLC-MS/MS determination was conducted by monitoring two MS/MS mass transitions and therefore no confirmatory method is required.

Procedural recoveries were obtained by fortification of the matrices and all mean recoveries are within the permitted range required by the guideline SANTE/2020/12830 rev. 1. While two mass transitions were analysed, only the ones for quantification are reported.

Table A 77: Recovery results from method validation of propamocarb in lettuce matrices using the analytical method

| Matrix | Analyte | Fortification level (mg/kg) (n = x) | Recovery (%) | Mean  recovery (%) | RSD (%) | Comments |
| --- | --- | --- | --- | --- | --- | --- |
| ***Mass transition (Quantification): Propamocarb 189→102 m/z*** | | | | | | |
| Lettuce  (Open head, whole plant) | Propamocarb | 0.01 (n = 3) | 99.3, 102, 107 | 103 | 3.6 | Acceptable |
| 0.1 (n = 3) | 108, 93.7, 108 | 103 | 8.1 |
| 100 (n=1) | 108 | 108 | - |
| Lettuce  (Open head, head – open leaves) | Propamocarb | 0.01 (n = 4) | 98.5, 98.4, 100, 102 | 99.7 | 1.7 | Acceptable |
| 0.1 (n = 4) | 103, 105, 102, 107 | 104 | 2.2 |
| 100 (n = 1) | 105 | 105 | - |
| Lettuce (Lamb’s, whole plant) | Propamocarb | 0.01 (n = 3) | 82.0, 81.2, 88.4 | 83.9 | 4.7 | Acceptable |
| 0.1 (n = 3) | 107, 105, 106 | 106 | 0.7 |
| 100 (n = 1) | 104 | 104 | - |
| Lettuce (Lamb’s, leaves) | Propamocarb | 0.01 (n = 3) | 106, 106, 107 | 106 | 0.5 | Acceptable |
| 0.1 (n = 3) | 104, 106, 101 | 104 | 2.5 |
| 100 (n = 1) | 100 | 100 | - |

Table A 78: Characteristics for the method used for validation of Propamocarb residues in lettuce

|  | Propamocarb |
| --- | --- |
| Specificity | LC-MS/MS is a highly specific method. No interference (> LOQ) for the target analyte was found in unfortified control samples. |
| Calibration (type, number of data points) | Calibration was performed with solvent based standards using at least five concentrations ranging 0.030 to 3 ng/mL and good linearity was observed (r ≥0.99). The resulting test substance peak areas versus test substance concentration data were fit to the linear function.  Regression data:  Mass transition 189→ 102 m/z (quantification)  Slope = 1516987.7, Intercept = 8055.8557, r = 0.999896406 |
| Calibration range | 0.030 ng/mL to 3 ng/mL |
| Assessment of matrix effects is presented | No significant matrix effects observed (i.e. <20%). Calibration standards were therefore prepared in solvent throughout. |
| Solution Stability | Solution stability was determined in XXXX Doc ID 2020/2106237:  Stock and calibration solutions were found to be stable following refrigerated storage at 2-8 ºC for 15 days. Raw and final volume extracts were found to be stable for at least 7 days following refrigerated storage at 2-8 ºC. |
| Limit of determination/quantification | The limit of quantification (LOQ) of 0.01 mg/kg was confirmed. The limit of detection (LOD) was 0.003 mg/kg. |

Conclusion

The analytical procedure, method L0450/01, for the determination of residues of Propamocarb in lettuce matrices has been fully validated in terms of specificity, linearity, precision, accuracy, matrix effects, solution stability and LOQ, in accordance with the requirements of SANTE/2020/12830 rev.1.

* + - 1. Method L0450/01: Method for the determination of Propamocarb in tomato
         1. Method validation

|  |  |
| --- | --- |
| Comments of zRMS: | The method has been accepted.  The method widely used within the section B7 of this submission for residue data generation in plant matrices. The final determination of propamocarb in tomato matrices was performed by LC-MS/MS which is highly sensitive and selective. The LOQ was set at 0.010 mg/kg for all analytes.  The mean recovery values were between the required range for all analytes. The relative standard deviations (RSD, %) for all fortification levels were ≤ 20 %.  Thus, it could be confirmed that the analytical method L0450/01 fulfils requirements of SANCO/3029/99 rev. 4. |

|  |  |
| --- | --- |
| Reference: | CP 5.1.2/5 |
| Report | Study on the residue behaviour of Ametoctradin (BAS 650 F) and Propamocarb (Reg. No. 4628172) on tomato after treatment with BAS 743 01 F under field conditions in Southern Europe, season 2020  Gálvez, O., 2021  report No 890073  XXXX DocID 2020/2103085  Authority registration No |
| Guideline(s): | SANCO/3029/99 rev.4; OECD ENV/JM/MONO(2007)17 - Guidance Document on Pesticide Residue Analytical Methods |
| Deviations: | No |
| Previous evaluation: | No |
| GLP: | Yes |
| Acceptability: | Yes | |

**Study Summary**

The method L0450/01 was validated for the determination of residues of Propamocarb (BAS 9068 F) in tomato (fruit), with a limit of quantification at 0.01 mg/kg. The brief description of method and the results are presented in the summary below.

Materials and methods

The method is that described in XXXX Doc ID 2022/2032351 above.

Results and discussions

The analytical method validation is summarised below. The HPLC-MS/MS determination was conducted by monitoring two MS/MS mass transitions and therefore no confirmatory method is required.

Procedural recoveries were obtained by fortification of the matrices and all mean recoveries are within the permitted range required by the guideline SANTE/2020/12830 rev. 1. While two mass transitions were analysed, only the ones for quantification are reported.

Table A 79: Recovery results from method validation of Propamocarb in tomato using the analytical method

| Matrix | Analyte | Fortification level (mg/kg) (n = x) | Recovery (%) | Mean  recovery (%) | RSD (%) | Comments |
| --- | --- | --- | --- | --- | --- | --- |
| ***Mass transition (Quantification): Propamocarb 189→102 m/z*** | | | | | | |
| Tomato (fruit) | Propamocarb | 0.01 (n = 4) | 95.4, 93.4, 89.3, 87.3 | 91.4 | 4.1 | Acceptable |
| 0.1 (n = 4) | 101, 91.6, 91.8, 89.9 | 93.6 | 5.4 |
| 1.0 (n = 4) | 102, 92.6, 96.2, 95.1 | 96.5 | 4.1 |
| 2.0 (n = 3) | 93.8, 97.1, 97.2 | 96.0 | 2.0 |

Table A 80: Characteristics for the method used for validation of Propamocarb residues in tomato

|  | Propamocarb |
| --- | --- |
| Specificity | LC-MS/MS is a highly specific method. No signal interference at the target analyte retention time, was found in unfortified control samples. |
| Calibration (type, number of data points) | Calibration was performed with matrix-matched standards using at least seven concentrations ranging 0.030 to 10 ng/mL (equiv. 0.0030 – 1.0 mg/kg at sample level) and good linearity was observed (r ≥0.99). The resulting test substance peak areas versus test substance concentration data were fit to the linear function.  Regression data:  Mass transition 189→ 102 m/z (quantification)  Slope = 6017370.0370419, Intercept = 26278.813055418, r = 0.9997 |
| Calibration range | 0.030 to 10 ng/mL (equiv. 0.0030 – 1.0 mg/kg at sample level) |
| Assessment of matrix effects is presented | Not evaluated. However, matrix-matched calibration standards were used throughout. |
| Solution Stability | Solution stability was determined in XXXX Doc ID 2020/2106237:  Stock and calibration solutions were found to be stable following refrigerated storage at 2-8 ºC for 15 days. Raw and final volume extracts were found to be stable for at least 7 days following refrigerated storage at 2-8 ºC. |
| Limit of determination/quantification | The limit of quantification (LOQ) of 0.01 mg/kg was confirmed . The limit of detection (LOD) was 0.003 mg/kg. |

Conclusion

The analytical procedure, method L0450/01, for the determination of residues of Propamocarb (BAS 9068 F) in tomato has been fully validated in terms of specificity, linearity, precision, accuracy, matrix effects, solution stability and LOQ, in accordance with the requirements of SANCO/3029/99 rev.4 but also meets the requirements of SANTE/2020/12830 rev.1.

* + - 1. Method L0450/01: Method for the determination of Propamocarb in potato
         1. Method validation

|  |  |
| --- | --- |
| Comments of zRMS: | The method has been accepted.  The method widely used within the section B7 of this submission for residue data generation in plant matrices. The final determination of propamocarb in potato matrices was performed by LC-MS/MS which is highly sensitive and selective. The LOQ was set at 0.010 mg/kg for all analytes.  The mean recovery values were between the required range for all analytes. The relative standard deviations (RSD, %) for all fortification levels were ≤ 20 %.  Thus, it could be confirmed that the analytical method L0450/01 fulfils requirements of SANCO/3029/99 rev. 4. |

|  |  |
| --- | --- |
| Reference: | CP 5.1.2/6 |
| Report | Study on the residue behaviour of Ametoctradin (BAS 650 F) and Propamocarb (Reg. No. 4628172) on potato after treatment with BAS 743 01 F under field conditions in Southern Europe, season 2020  Gálvez, O., 2021  report No 890078  XXXX DocID 2020/2103083  Authority registration No |
| Guideline(s): | EPA 860.1340; SANCO/3029/99 rev.4; OECD ENV/JM/MONO(2007)17 - Guidance Document on Pesticide Residue Analytical Methods |
| Deviations: | No |
| Previous evaluation: | No |
| GLP: | Yes |
| Acceptability: | Yes | |

**Study Summary**

The method L0450/01 was validated for the determination of residues of Propamocarb (BAS 9068 F) in potato (shoot and tuber), with a limit of quantification at 0.01 mg/kg. The brief description of method and the results are presented in the summary below.

Materials and methods

The method is that described in XXXX Doc ID 2022/2032351 above.

Results and discussions

The analytical method validation is summarised below. The HPLC-MS/MS determination was conducted by monitoring two MS/MS mass transitions and therefore no confirmatory method is required.

Procedural recoveries were obtained by fortification of the matrices and all mean recoveries are within the permitted range required by the guideline SANTE/2020/12830 rev. 1. While two mass transitions were analysed, only the ones for quantification are reported.

Table A 81: Recovery results from method validation of Propamocarb in potato matrices using the analytical method

| Matrix | Analyte | Fortification level (mg/kg) (n = x) | Recovery (%) | Mean  recovery (%) | RSD (%) | Comments |
| --- | --- | --- | --- | --- | --- | --- |
| ***Mass transition (Quantification): Propamocarb 189→102 m/z*** | | | | | | |
| Potato (shoot) | Propamocarb | 0.01 (n = 4) | 103, 104, 101, 109 | 104 | 3.1 | Acceptable |
| 0.1 (n = 4) | 103, 105, 104, 92.5 | 101 | 5.7 |
| 50 (n = 1) | 97.1 | 97.1 | - |
| 70 (n = 1) | 96.6 | 96.6 | - |
| Potato (tuber) | Propamocarb | 0.01 (n = 4) | 110, 108, 95.7, 106 | 105 | 6.2 | Acceptable |
| 0.1 (n = 4) | 105, 106, 96.2, 97.8 | 101 | 5.0 |

Table A 82: Characteristics for the method used for validation of Propamocarb residues in potato matrices

|  | Propamocarb |
| --- | --- |
| Specificity | LC-MS/MS is a highly specific method. No signal interference was observed for the target analyte in unfortified control samples (with the exception of one untreated shoot sample L2002740001 where Propamocarb was present at 0.026 mg/kg and beleived to be contamnation). |
| Calibration (type, number of data points) | Calibration was performed with solvent based standardsusing at least five concentrations ranging 0.03 to 10 ng/mL and good linearity was observed (r ≥0.99). The resulting test substance peak areas versus test substance concentration data were fit to the linear function.  Regression data:  Mass transition 189→ 102 m/z (quantification)  Slope = 1.3 x 106, Intercept = -1.3 x 106, r = 0.9996 |
| Calibration range | 0.03 ng/mL to 10 ng/mL |
| Assessment of matrix effects is presented | No significant matrix effects observed (i.e. <20%). Calibration standards were therefore prepared in solvent. |
| Solution Stability | Solution stability was determined in XXXX Doc ID 2020/2106237:  Stock and calibration solutions were found to be stable following refrigerated storage at 2-8 ºC for 15 days. Raw and final volume extracts were found to be stable for at least 7 days following refrigerated storage at 2-8 ºC. |
| Limit of determination/quantification | The limit of quantification (LOQ) of 0.01 mg/kg was confirmed. The limit of detection (LOD) was 0.003 mg/kg. |

Conclusion

The analytical procedure, method L0450/01, for the determination of residues of Propamocarb (BAS 9068 F) in potato matrices has been fully validated in terms of specificity, linearity, precision, accuracy, matrix effects, solution stability and LOQ, in accordance with SANCO/3029/99 rev.4 and meets the requirements of SANTE/2020/12830 rev.1 also.

* + - 1. Method L0450/01: Method for the determination of Propamocarb in onion
         1. Method validation

|  |  |
| --- | --- |
| Comments of zRMS: | The method has been accepted.  The method widely used within the section B7 of this submission for residue data generation in plant matrices. The final determination of propamocarb in onion matrices was performed by LC-MS/MS which is highly sensitive and selective. The LOQ was set at 0.010 mg/kg for all analytes.  The mean recovery values were between the required range for all analytes. The relative standard deviations (RSD, %) for all fortification levels were ≤ 20 %.  Thus, it could be confirmed that the analytical method L0450/01 fulfils requirements of SANCO/3029/99 rev. 4. |

|  |  |
| --- | --- |
| Reference: | CP 5.1.2/7 |
| Report | Study on the residue behaviour of Propamocarb (Reg.No. 4628172) and Ametoctradin (BAS 650 F) in Onions after treatment with BAS 743 01 F under field conditions in Southern Europe in 2020  Schneider, E., 2021  report No 890084  XXXX DocID 2021/2019659  Authority registration No |
| Guideline(s): | EPA 860.1340; SANCO/3029/99 rev.4; OECD ENV/JM/MONO(2007)17 - Guidance Document on Pesticide Residue Analytical Methods |
| Deviations: | No |
| Previous evaluation: | No |
| GLP: | Yes |
| Acceptability: | Yes | |

**Study Summary**

The method L0450/01 was validated for the determination of residues of Propamocarb (BAS 9068 F) in onion (bulbs and whole plant (no roots)), with a limit of quantification at 0.01 mg/kg. The brief description of method and the results are presented in the summary below.

Materials and methods

The method is that described in XXXX Doc ID 2022/2032351 above.

Results and discussions

The analytical method validation is summarised below. The HPLC-MS/MS determination was conducted by monitoring two MS/MS mass transitions and therefore no confirmatory method is required.

Procedural recoveries were obtained by fortification of the matrices and all mean recoveries are within the permitted range required by the guideline SANTE/2020/12830 rev. 1. While two mass transitions were analysed, only the ones for quantification are reported.

Table A 83: Recovery results from method validation of Propamocarb in onion matrices using the analytical method

| Matrix | Analyte | Fortification level (mg/kg) (n = x) | Recovery (%) | Mean  recovery (%) | RSD (%) | Comments |
| --- | --- | --- | --- | --- | --- | --- |
| ***Mass transition (Quantification): Propamocarb 189→102 m/z*** | | | | | | |
| Onion (bulbs) | Propamocarb | 0.01 (n = 5) | 103, 96.2, 102, 106, 102 | 102 | 3.4 | Acceptable |
| 0.1 (n = 5) | 96.4, 106, 104, 104, 100 | 102 | 3.6 |
| Onion (whole plant, no roots) | Propamocarb | 0.01 (n = 3) | 94.2, 97.0, 100 | 97.1 | 3.0 | Acceptable |
| 0.1 (n = 3) | 104, 103, 104 | 104 | 0.3 |
| 10 (n = 1) | 107 | 107 | - |

Table A 84: Characteristics for the method used for validation of Propamocarb residues in onion matrices

|  | Propamocarb |
| --- | --- |
| Specificity | LC-MS/MS is a highly specific method. No signal interference was observed for the target analyte in unfortified control samples. |
| Calibration (type, number of data points) | Calibration was performed with solvent based standards using at least five concentrations ranging 0.030 to 10 ng/mL and good linearity was observed (r ≥0.99). The resulting test substance peak areas versus theoretical test substance concentration data were fit to the linear function.  Regression data:  Mass transition 189→ 102 m/z (quantification)  Slope = 1.21 x 106, Intercept = 6.49 x 103, r = 0.9975 |
| Calibration range | 0.030 ng/mL to 10 ng/mL |
| Assessment of matrix effects is presented | No significant matrix effects observed (i.e. <20%) in all cases thus calibration standards were prepared in solvent throughout. |
| Solution Stability | Sample stability was established as at least 197 days in frozen (≤-18°C) conditions.  Other solution stability was determined in XXXX Doc ID 2020/2106237:  Stock and calibration solutions were found to be stable following refrigerated storage at 2-8 ºC for 15 days. Raw and final volume extracts were found to be stable for at least 7 days following refrigerated storage at 2-8 ºC. |
| Limit of determination/quantification | The limit of quantification (LOQ) of 0.01 mg/kg was confirmed. The limit of detection (LOD) was 0.003 mg/kg. |

Conclusion

The analytical procedure, method L0450/01, for the determination of residues of Propamocarb (BAS 9068 F) in onion matrices has been fully validated in terms of specificity, linearity, precision, accuracy, matrix effects, solution stability and LOQ, in accordance with the requirements of SANCO/3029/99 rev.4. The requirements of SANTE/2020/12830 rev.1 were also met.

* + - 1. Method L0450/01: Method for the determination of Propamocarb in cucumber & zucchini
         1. Method validation

|  |  |
| --- | --- |
| Comments of zRMS: | The method has been accepted.  The L0450/01 method is widely used within the section B7 of this submission for residue data generation in plant matrices. The final determination of propamocarb in cucumber and zucchini matrices was performed by LC-MS/MS which is highly sensitive and selective. The LOQ was set at 0.010 mg/kg for all analytes.  The mean recovery values were between the required range for all analytes. The relative standard deviations (RSD, %) for all fortification levels were ≤ 20 %.  Thus, it could be confirmed that the analytical method L0450/01 fulfils requirements of SANCO/3029/99 rev. 4.  *Minor comment: For the clarity it is stated here that in this study XXXX method no. L0450/01 was used in the analytical phase of the study to determine the residues of propamocarb, but XXXX method no. L0078/02 was used in the analytical phase of the study to determine the residues of BAS 650 F, M650F003 and M650F004 (see the relevant part of the Appendix 2).* |

|  |  |
| --- | --- |
| Reference: | CP 5.1.2/9 |
| Report | Study on the residue behaviour of Propamocarb (Reg.No. 4628172) and Ametoctradin (BAS 650 F) in cucumber and zucchini after two applications of BAS 743 01 F under greenhouse conditions in Northern and Southern Europe in 2021  Schneider, E., 2023  report No 890095  XXXX DocID 2022/2041754  Authority registration No |
| Guideline(s): | OECD ENV/JM/MONO(2007)17 - Guidance Document on Pesticide Residue Analytical Methods; SANTE/2020/12830 Rev.1 |
| Deviations: | None |
| Previous evaluation: | No |
| GLP: | Yes |
| Acceptability: | Yes | |

**Study Summary**

The method L0450/01 was validated for the determination of residues of Propamocarb (BAS 9068 F) in cucumber and zucchini with a limit of quantification at 0.01 mg/kg. The brief description of method and the results are presented in the summary below.

Materials and methods

The method is that described in XXXX Doc ID 2022/2032351 above.

Results and discussions

The analytical method validation is summarised below. The HPLC-MS/MS determination was conducted by monitoring two MS/MS mass transitions and therefore no confirmatory method is required.

Procedural recoveries were obtained by fortification of the matrices and all mean recoveries are within the permitted range required by the guideline SANTE/2020/12830 rev. 1. While two mass transitions were analysed, only the ones for quantification are reported. The precision for cucumber samples at 0.01 mg/kg fortification was marginally outside (RSD = 22% ) the guideline value (RSD 20%) and not considered to be significant taking the data in the round (the overall RSD for cucumber data was <20%).

Table A 85: Recovery results from method validation of Propamocarb in cucumber and zucchini using the analytical method

| Matrix | Analyte | Fortification level (mg/kg) (n = x) | Recovery (%) | Mean  recovery (%) | RSD (%) | Comments |
| --- | --- | --- | --- | --- | --- | --- |
| ***Mass transition (Quantification): Propamocarb 189→102 m/z*** | | | | | | |
| Cucumber (fruit) | Propamocarb | 0.01 (n = 4) | 79.2, 138, 92.0, 96.3 | 101 | 22 | Acceptable |
| 0.1 (n = 4) | 101, 100, 109, 107 | 104 | 3.7 |
| 10 (n = 1) | 87.4 | 87.4 | - |
| Zucchini (fruit) | Propamocarb | 0.01 (n = 4) | 96.5, 97.1, 93.8, 96.8 | 96.1 | 1.4 | Acceptable |
| 0.1 (n = 4) | 105, 104, 104, 102 | 104 | 1.1 |
| 10 (n = 1) | 91.0 | - | - |

Table A 86: Characteristics for the method used for validation of Propamocarb residues in plant matrices

|  | Propamocarb |
| --- | --- |
| Specificity | LC-MS/MS is a highly specific method. No signal interference was observed for the target analyte in unfortified control samples. |
| Calibration (type, number of data points) | Calibration was performed with matrix-matched standards with at least seven concentrations ranging 0.030 to 10 ng/mL and good linearity was observed (r ≥0.99). The resulting test substance peak areas versus test substance concentration data were fit to the linear function.  Regression data:   |  |  |  |  |  | | --- | --- | --- | --- | --- | | Matrix | m/z | Slope | Intercept | r | | **Cucumber** | 189→102 | 1.98407 x 106 | 1.33115 x 105 | 0.99905 | | **Zucchini** | 189→102 | 1.71450 x 106 | 6530.31674 | 0.99981 | |
| Calibration range | 0.030 ng/mL to 10 ng/mL |
| Assessment of matrix effects is presented | Not assessed, however matrix-matched calibration standards were used throughout. |
| Solution Stability | See XXXX Doc ID 2020/2106237 above for standard stability.  Sample stability was established as at least 317 days in frozen (≤-18°C) conditions. |
| Limit of determination/quantification | The limit of quantification (LOQ) of 0.01 mg/kg was confirmed. The limit of detection (LOD) was 0.0030 mg/kg. |

Conclusion

The analytical procedure, method L0450/01, for the determination of residues of Propamocarb (BAS 9068 F) in cucumber and zucchini has been fully validated in terms of specificity, linearity, precision, accuracy, solution stability and LOQ, in accordance with the requirements of SANTE/2020/12830 rev.1.

* + - 1. Methods for the determination of Propamocarb in support of toxicological studies

None

* + - * 1. ~~Method validation~~

|  |  |
| --- | --- |
| ~~Comments of zRMS:~~ | ~~The procedure has been accepted itself. However, is unclearly described. Also, the title of the study is unclear. The applicant is kindly asked to clarify the study or remove it.~~  ~~The aim of this study was the validation of a quantitative HPLC-UV method for BAS 743 02 F formulation (the test item) containing propamocarb (431.0 g/L) and ametoctradin (137.7 g/L) in the medium (vehicle, matrix) of acetonitrile / 10mM disodium hydrogen phosphate dihydrate with phosphoric acid (85%) pH 2.9. It can be noted that in the present section the formulation BAS 743 02 F according to the applicant data contains 515.4 g/L propamocarb and 137.1 g/L ametoctradin.~~  ~~This is probably “the case” of the determining “BAS 743 02 F~~ *~~via~~* ~~propamocarb and ametoctradin” The evaluated study operates however, with only the active ingredient 1 and active ingredient 2 as analytes being determined. While the applicant in the study description uses the names ametoctradin and propamocarb that are not included in the text of the study (except a place with information about the formulation content).~~  ~~The LOQ of the method was defined as follows: the LOQ was assessed and confirmed at a concentration of 1 mg/100 mL for active ingredient 1 and 0.2 mg/100 mL for active ingredient 2 in the vehicle with sufficient results for accuracy and precision (correlating to a concentration of 0.1 mg/100 mL for active ingredient 1 and 0.2 mg/100 mL for active ingredient 2 in the final sample extract).~~  ~~Apart from above the procedure within the study is described correctly e.g.: The method was validated at 3 fortification levels for each active ingredient with 5 fortification samples at each level. 2 independently prepared blank medium extract with no samples were analyzed as controls. Detection of the test item was performed at 195 nm for active ingredient 1 and 294 nm for active ingredient 2.~~ |

|  |  |
| --- | --- |
| ~~Reference:~~ | ~~CP 5.1.2/11~~ |
| ~~Report~~ | ~~Validation of an analytical method for the analysis in acetonitrile/10 mM disodium hydrogen phosphate dihydrate with phosphoric acid (85%) pH 2.9 (50+50, V/V) using HPLC-UV~~  ~~Control procedure: 21/0288\_01~~  ~~Wagner, I.., 2022~~  ~~XXXX DocID 2022/2034983~~  ~~Authority registration No~~ |
| ~~Guideline(s):~~ | ~~SANTE/2020/12830 Rev.1~~ |
| ~~Deviations:~~ | ~~No~~ |
| ~~Previous evaluation:~~ | ~~No~~ |
| ~~GLP:~~ | ~~Yes~~ |
| ~~Acceptability:~~ | ~~Yes~~ | |

**~~Study Summary~~**

~~Control procedure 21/0288\_01 was validated for the determination of Ametoctradin and Propamocab formulated in BAS 743 02 F in the vehicle acetonitrile / 10mM disodium hydrogen phosphate dihydrate with phosphoric acid(85%) pH 2.9 (50+50, v/v) to demonstrate the suitability and correctness of the method in the vehicle.~~

**~~Materials and methods~~**

~~In control procedure 21/0288\_01, an aliquot of the sample solution for Propamocarb analysis is transferred to a voluemtric flask, diluted at least 1+9 with buffer (10mM disodium hydrogen phosphate dihydrate with phosphoric acid (85%) pH 2.9) and centrifuged at 13000 rpm for 5 minutes. Samples are diluted further with buffer as necessary into the calibration range. Samples are then analysed by high performance liquid chromatography with ultra-violet detection (HPLC-UV) at 195 nm (reference wavelength of 360 nm for diode-array) using an Xbridge Shield RP 18 column (100 mm x 4.6 mm, 3.5 µm) and gradient elution with mobile phases of acetonitrile:water (95:5) and 10 mM disodium hydrogen phosphate dihydrate adjusted to pH 2.8-2.9 with phosphoric acid (85%), at a flow rate of 1.5 mL/min. Calibration is by external matrix-matched standards.~~

~~Results and discussions~~

~~The analytical method validation is summarised below. No confirmatory method is required for methods for risk-assessment according to SANTE/2020/12830 Rev.1.~~

~~Recoveries were obtained by fortification of blank vehicle at three fortification levels. All mean recoveries are within the permitted range required by the guideline SANTE/2020/12830 rev. 1.~~

~~Table A 87: Recovery results from method validation of Propamocarb using the analytical method~~

| ~~Matrix~~ | ~~Analyte~~ | ~~Nominal fortification level (mg/100 mL) (n = 5)~~ | ~~Mean  recovery (%)~~ | ~~RSD (%)~~ | ~~Comments~~ |
| --- | --- | --- | --- | --- | --- |
| ~~acetonitrile:10 mM disodium hydrogen phosphate dihydrate adjusted to pH 2.8-2.9 with phosphoric acid (85%) (50:50 v/v)~~ | ~~Propamocarb~~ | ~~1~~ | ~~105.0~~ | ~~2.3~~ | ~~Acceptable~~ |
| ~~5~~ | ~~103.6~~ | ~~2.7~~ | ~~Acceptable~~ |
| ~~30~~ | ~~104.5~~ | ~~2.3~~ | ~~Acceptable~~ |

~~Table A 88: Characteristics for the method used for validation of Propamocarb in sample matrix~~

|  | ~~Ametoctradin~~ |
| --- | --- |
| ~~Specificity~~ | ~~No interference (> 30 % LOQ) was found in unfortified control samples. Peak identification was confirmed by retention time match with reference material.~~ |
| ~~Calibration (type, number of data points)~~ | ~~Calibration was performed with matrix-matched standards at a minimum of seven concentrations ranging 0.03 to 1.0 mg/100 mL (slope = 2.700949, intercept = -0.034788). Good linearity was observed (r~~~~2~~ ~~= 0.999294). The resulting test substance peak areas versus test substance concentration data were fit to the linear function. Residuals were randomly scattered.~~ |
| ~~Calibration range~~ | ~~Nominal calibration range 0.03 mg/100 mL to 1.0 mg/100 mL (0.003 mg/100 mL to 0.1 mg/100 mL in sample)~~ |
| ~~Assessment of matrix effects is presented~~ | ~~No significant matrix effects were observed (i.e. the matrix effect was found to be ≤ ±20%). Matrix-matched standards were used throughout nevertheless.~~ |
| ~~Solution Stability~~ | ~~Not necessary as standards were prepared daily and samples analysed within 24 hours.~~ |
| ~~Limit of determination/quantification~~ | ~~The limit of quantification (LOQ) of 1.0 mg/100 mL was confirmed. The limit of detection (LOD) was 0.03 mg/100 mL.~~ |

~~Conclusion~~

~~The control procedure 21/0288\_01, for the determination of Ametoctradin (BAS 650 F) in a matrix of acetonitrile:10 mM disodium hydrogen phosphate dihydrate adjusted to pH 2.8-2.9 with phosphoric acid (85%) (50:50 v/v) has been fully validated in terms of specificity, linearity, precision, accuracy, matrix effects, solution stability and LOQ, in accordance with the requirements of SANTE/2020/12830 Rev.1.~~

* + 1. Methods for the determination of Propamocarb in support of ecotoxicological studies
       - 1. Method validation

|  |  |
| --- | --- |
| Comments of zRMS: | The method has been accepted.  Below studies are related with XXXX analytical method APL0500/02.  The objective of the analytical part was to perform concentration control of BAS 742 02 F in test medium in the context of an ecotoxicological study on the Water Flea Daphnia magna Straus.  For this purpose, XXXX analytical method APL0500/02 was modified for the analysis of ametoctradin and propamocarb. Final determination was accomplished by LC-MS/MS, using 2 transitions. The set LOQs vary among the submitted reports. The validity of the analytical method APL0500/02 was proven by recovery experiments. Results showed that the mean recovery and repeatability was within the acceptable range, with RSD < 20%. No significant peak interferences occurred at the retention time and mass transitions of ametoctradin or propamocarb. Ametoctradin and propamocarb were sufficiently stable. XXXX analytical method APL0500/02 was validated consistently with SANTE/2020/12830 rev.1. |

|  |  |
| --- | --- |
| Reference: | CP 5.1.2/12 |
| Report | BAS 743 02 F: Toxicity to the Water Flea *Daphnia* *magna* Straus under Laboratory Conditions (Acute Immobilisation Test – Static)  Wendling, K., 2023  Study No.: 933752-4  XXXX DocID: 2022/2033712  Authority registration No |
| Guideline(s): | SANTE/2020/12830 Rev.1; OECD ENV/JM/MONO(2007)17; OPPTS 860.1340 |
| Deviations: | None |
| Previous evaluation: | No |
| GLP: | Yes |
| Acceptability: | Yes | |

|  |  |
| --- | --- |
| Reference: | CP 5.1.2/13 |
| Report | BAS 743 02 F: Toxicity to the Single Cell Green Alga *Pseudokirchneriella subcapitata* Hindák under Laboratory Conditions  Obert-Rauser, P., 2023  Study No.: 933752\_5  XXXX DocID: 2022/2033713  Authority registration No |
| Guideline(s): | SANTE/2020/12830 Rev.1; OECD ENV/JM/MONO(2007)17; OPPTS 860.1340 |
| Deviations: | None |
| Previous evaluation: | No |
| GLP: | Yes |
| Acceptability: | Yes | |

|  |  |
| --- | --- |
| Reference: | CP 5.1.2/14 |
| Report | BAS 743 02 F: Toxicity to the Rainbow Trout *Oncorhynchus mykiss* under Laboratory Conditions (Acute Toxicity Test – Semi-Static)  Wendling, K., 2023  Study No.: 933752\_6  XXXX DocID: 2022/2033714  Authority registration No |
| Guideline(s): | SANTE/2020/12830 Rev.1; OECD ENV/JM/MONO(2007)17; OPPTS 860.1340 |
| Deviations: | None |
| Previous evaluation: | No |
| GLP: | Yes |
| Acceptability: | Yes | |

Study Summary

The method APL0500/02 was validated in the above studies (CP 5.1.2/28 – CP 5.1.2/30) for the determination of Propamocarb (BAS 9068 F) in test medium with a limit of quantification at 0.0954 mg/L. The brief description of method and the results are presented in the summary below.

Materials and methods

In method APL0500/02, deep frozen samples of test medium (10 mL + 50 µL formic acid) are thawed to ambient temperature. 10 mL of acetonitrile is added and shaken well using a vortex mixer. The samples were further diluted with acetonitrile/test medium + 0.5 % formic acid (1:1, v/v) prior to analysis to be in the linear range of the calibration curve.

Propamocarb (BAS 9068 F) is determined by HPLC-MS/MS. Separation is achieved by using an YMC YMC-Triart C18 column (150 x 3 mm, 3 µm) and a gradient of water (0.1% formic acid)/ acetonitrile (0.1% formic acid) at a flow rate of 0.5 mL/min.

Detection is accomplished in ESI positive mode using two different transitions; *m/z* 189 > 102 and *m/z* 189 > 144 used for quantification and confirmation, respectively. External matrix-matched calibration standards were used throughout.

Results and discussions

The analytical method validation is summarised below. The HPLC-MS/MS determination was conducted by monitoring two MS/MS mass transitions and therefore no confirmatory method is required.

Recoveries were obtained by fortification of the matrices and all mean recoveries are within the permitted range required by the guideline SANTE/2020/12830 rev. 1.

Table A 89: Recovery results from validation of method APL0500/02 for determination of Propamocarb in test medium (example data from Study 933752-5)

| **Matrix** | **Analyte** | **Fortification level (mg/L) (n = x)** | **Mean  recovery (%)** | **RSD (%)** | **Comments** |
| --- | --- | --- | --- | --- | --- |
| ***Mass transition (Quantification) Ametoctradin m/z 189→102*** | | | | | |
| Test medium | Propamocarb | 0.0954 (n = 11) | 110 | 5.0 | Acceptable |
| 130 (n = 5) | 101 | 2.0 |

Table A 90: Characteristics for method APL0500/02 used for validation of Ametoctradin in test medium (example data from Study 933752-5)

|  | **Propamocarb** |
| --- | --- |
| Specificity | LC-MS/MS is a highly specific method. No interference (> 30 % LOQ) of total peak area for the target analyte at the retention time, was found in unfortified control samples. |
| Calibration (type, number of data points) | Calibration was performed with matrix-matched standards at a minimum of five concentrations ranging 1.08 – 18.0 ng/mL and good linearity was observed (r ≥0.99). The resulting test substance peak areas versus theoretical test substance concentration data were fit to the linear function.  Regression data:   |  |  |  |  | | --- | --- | --- | --- | | **Set No.** | **Intercept** | **Slope** | **r** | | 1 | 42400 | 183000 | 0.9984 | | 2 | 80700 | 103000 | 0.9932 | | 3 | -2920 | 32200 | 0.9999 | | 4 | -212 | 34600 | 0.9999 | | 5 | -600 | 32400 | 1.0000 | | 6 | -2740 | 31900 | 1.0000 | | 7 | -4350 | 31300 | 0.9999 | | 8 | -9150 | 32500 | 0.9997 | |
| Calibration range | Accepted calibration range 1.08 – 18.0 ng/mL |
| Assessment of matrix effects is presented | Matrix matched standards were used throughout. |
| Solution Stability | Working calibration solutions are stable when stored at 1 °C to 10 °C in the dark for at least 315 days for Propamocarb. Extracts are considered to be stable for Propamocarb when stored at 1 °C to 10 °C for 12 days in the dark. |
| Limit of determination/quantification | The limit of quantification (LOQ) of 0.0954 mg/L was confirmed. The limit of detection (LOD), defined as the lowest standard employed was 1.08 ng/mL. |

Conclusion

The additional validation data for method APL0500/02, for the determination of Propamocarb (BAS 9068 F) in test medium has been fully validated in terms of specificity, linearity, precision, accuracy, matrix effects, solution stability and LOQ, in accordance with the requirements of SANTE/2020/12830 rev.1.

* + - * 1. Method validation

|  |  |
| --- | --- |
| Comments of zRMS: | The method has been accepted.  The determination was conducted by an in-house developed method using reversed phase high performance liquid chromatography (RP-HPLC) with tandem mass spectrometric (MS/MS) detection. The concentration of both active ingredients in the highest and lowest test item feeding solution applied on the first and last day of application (D0 and D9) was determined. The recovery rates of Propamocarb were 104% on D0 and 99.1% on D9 in samples of test item feeding solution AT (highest applied concentration) and 104% on D0 and 97.3% on D9 in samples of test item feeding solution ET (lowest applied concentration).  The recovery rates of Ametoctradin were 104% on D0 and 102% on D9 in samples of test item feeding solution AT and 100% on D0 and 92.6% on D9 in samples of test item feeding solution ET. Furthermore, no residues of the active ingredients were found in the control samples, i.e., the concentrations of active ingredients were below 30% of the LOQ.  The method was validated at LOQ of 1.44 mg/kg Propamocarb and 0.459 mg/kg BAS 650 F. The mean recovery was within the acceptable range of 70% to 120% for all active ingredients, with relative standard deviations RSD < 20%. No significant peak interferences (> LOD) occurred at the retention time and mass transition of any active ingredient in the control samples. The observed matrix effects were -7.25% for Propamocarb and +1.79% for BAS 650 F and considered insignificant.  The method was fully validated according to the requirements of SANTE/2020/12830, Rev.1 guideline. |

|  |  |
| --- | --- |
| Reference: | CP 5.1.2/17 |
| Report | Chronic toxicity of BAS 743 02 F to the honey bee *Apis mellifera* L. under laboratory conditions  Ruhland, S., 2023  Study No.: 933752-2  XXXX DocID: 2022/2033709  Authority registration No |
| Guideline(s): | SANTE/2020/12830 Rev.1; OECD ENV/JM/MONO(2007)17; OPPTS 860.1340 |
| Deviations: | None |
| Previous evaluation: | No |
| GLP: | Yes |
| Acceptability: | Yes | |

**Study Summary**

An analytical method was validated for the determination of Propamocarb in bee feeding solutions of BAS 743 02 F (containing the active ingredients Ametocradin (BAS 650 F) and Propamocarb (BAS 9068 F), with an LOQ of 1.44 mg/kg. A brief description of method and the results are presented in the summary below.

**Materials and methods**

An aliquot of bee feeding solution (0.2 g) is shaken with acetonitrile:0.5% formic acid solution (50:50 v/v, 10 mL) using a Fast Prep system at 5 m/s for 5 minutes and centrifuged at 4000 rpm for 5 minutes. The resulting extract is further filuted with dilution medium (blank extract) into the range of the calibration curve. Samples are analysed by high performance liquid chromatography with tandem mass detection (HPLC-MS/MS) in positive ion mode using an ACE Excel 3 C18 column (100 mm x 2.1 mm, 3 µm) and gradient elution with mobile phases of water with 0.1% (v/v) formic acid + 5mM ammonium formate and methanol + 0.1% (v/v) formic acid. Calibration is by external matrix-matched standards monitoring the ion transitions *m/z* 189 → 74 and *m/z* 189 → 144 for the quantification and confirmation of Propamocarb, respectively.

**Results and discussions**

The analytical method validation is summarised below and only the quantifying transition is reported.

Recoveries were evaluated with the fortification of sample matrix (sucrose solution containing 50% (w/v) sucrose +0.1% (w/v) xanthan) with BAS 743 02 F at two fortification levels (LOQ and approx 7500 x LOQ). The mean recoveries were within the permitted range required by the guideline SANTE/2020/12830 rev. 1.

**Table A 91: Recovery results from method validation of Propamocarb using the analytical method**

| **Matrix** | **Analyte** | **n** | **Nominal fortification level (mg/kg)** | **Corresponding fortification level as  BAS 743 02 F (mg/kg)** | **Mean  recovery (%)** | **RSD (%)** | **Comments** |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Bee feeding solution  (sucrose solution containing 50% (w/v) sucrose + 0.1% (w/v) xanthan) | Popamocarb | 5 | 1.44 | 3.61 | 99.4 | 0.540 | Acceptable |
| 5 | 10926 | 27315 | 98.1 | 1.72 | Acceptable |

**Table A 92: Characteristics for the method used for validation of Propamocarb in bee feeding solutions**

|  | **Ametoctradin** |
| --- | --- |
| Specificity | No significant peak interferences (> 30% LOQ) occurred at the retention time and mass transition of any active ingredient in the control samples. Peak identification was confirmed by retention time match with reference material. |
| Calibration (type, number of data points) | Calibration was performed with matrix-matched standards at six concentrations ranging from 6.12 to 136 ng Propamocarb/mL (slope = 2697, intercept = -691). Good linearity was observed (r2 = 0.99995). The resulting test substance peak areas versus test substance concentration data were fit to the linear function. Residuals were randomly scattered. |
| Calibration range | Calibration range 6.12 to 136 ng Propamocarb/mL |
| Assessment of matrix effects is presented | No significant matrix effects were observed (i.e. the matrix effect was found to be ≤ ±20%). Matrix-matched standards were used throughout nevertheless. |
| Solution Stability | Stability of Propamocarb in bee feeding solution were proven over a time period of 191 days under deep frozen conditions in the dark. |
| Limit of determination/quantification | The limit of quantification (LOQ) of 1.44 mg Propamocarb/kg was confirmed. The limit of detection (LOD) was 0.311 mg Propamocarb/kg. |

**Conclusion**

The analytical method for the determination of Propamocarb (BAS 9068 F) in bee feeding solution (sucrose solution containing 50% (w/v) sucrose + 0.1% (w/v) xanthan) has been fully validated in terms of specificity, linearity, precision, accuracy, matrix effects, solution stability and LOQ/LOD, in accordance with the requirements of SANTE/2020/12830 Rev.1.

* + - * 1. Method validation

|  |  |
| --- | --- |
| Comments of zRMS: | The method has been accepted.  The purpose of the analytical phase of the study was the determination of the concentrations of Propamocarb and Ametoctradin in final diets of honeybee larvae Apis mellifera L. The determination was conducted by an in-house developed method using reversed phase high performance liquid chromatography (RP-HPLC)  with tandem mass spectrometric (MS/MS) detection.  Results of the procedural recovery experiments obtained during analysis of BAS 743 02 F showed that the mean recovery efficiency and repeatability was within the acceptable range of 70% to 120% of the intended concentrations for all active ingredients, with relative standard deviations RSD < 20%. No significant peak interferences (> LOD) occurred at the retention time and mass transition of any active ingredient in the control samples.  No significant interferences occurred at the retention time and mass transition of any active ingredient in the control samples. The observed matrix effects were considered insignificant.  The method was fully validated according to the requirements of SANTE/2020/12830, Rev.1. |

|  |  |
| --- | --- |
| Reference: | CP 5.1.2/18 |
| Report | Repeated exposure of honey bee (*Apis mellifera* L.) larvae to BAS 743 02 F under laboratory conditions  Schmidt, K., 202~~2~~3  Study No.: 933752-3  XXXX DocID: 2022/2033710  Authority registration No |
| Guideline(s): | SANTE/2020/12830 Rev.1; OECD ENV/JM/MONO(2007)17; OPPTS 860.1340 |
| Deviations: | None |
| Previous evaluation: | No |
| GLP: | Yes |
| Acceptability: | Yes | |

**Study Summary**

An analytical method was validated for the determination of Propamocarb from final diets of honey bee larvae containing BAS 743 02 F, with an LOQ of 1.48 mg/kg. A brief description of method and the results are presented in the summary below.

**Materials and methods**

A 0.2g aliquot of bee feeding solution (50:50 (w/w) royal jelly/aqueous sugar solution (containing 4% (w/v) yeast extract, 18% (w/v) glucose and 18% (w/v) fructose)) is shaken with acetonitrile:0.5% formic acid solution (50:50 v/v, 10 mL) using a Fast Prep system at 5 m/s for 5 minutes and centrifuged at 4000 rpm for 5 minutes. The resulting extract is further diluted with dilution medium (blank extract) into the range of the calibration curve. Samples are analysed by high performance liquid chromatography with tandem mass detection (HPLC-MS/MS) in positive ion mode using an ACE Excel 3 C18 column (100 mm x 2.1 mm, 3 µm) and gradient elution with mobile phases of water with 0.1% (v/v) formic acid + 5mM ammonium formate and methanol + 0.1% (v/v) formic acid. Calibration is by external matrix-matched standards monitoring the ion transitions *m/z* 189 → 74 and *m/z* 189 → 144 for the quantification and confirmation of Propamocarb, respectively.

**Results and discussions**

The analytical method validation is summarised below and only the quantifying transition is reported.

Recoveries were evaluated with the fortification of sample matrix with BAS 743 02 F at two fortification levels (LOQ and approx 800 x LOQ). The mean recoveries were within the permitted range required by the guideline SANTE/2020/12830 rev. 2.

**Table A 93: Recovery results from method validation of Propamocarb using the analytical method**

| **Matrix** | **Analyte** | **n** | **Nominal fortification level (mg/kg)** | **Corresponding fortification level as  BAS 743 02 F (mg/kg)** | **Mean  recovery (%)** | **RSD (%)** | **Comments** |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Bee feeding solution extract | Propamocarb | 5 | 1.48 | 3.70 | 103 | 0.572 | Acceptable |
| 5 | 1183 | 2957 | 104 | 0.684 | Acceptable |

**Table A 94: Characteristics for the method used for validation of Propamocarb in bee feeding solutions**

|  | **Propamocarb** |
| --- | --- |
| Specificity | No significant peak interferences (> 30% LOQ) occurred at the retention time and mass transition of any active ingredient in the control samples. Peak identification was confirmed by retention time match with reference material. |
| Calibration (type, number of data points) | Calibration was performed with matrix-matched standards at six concentrations ranging from 6.12 to 136 ng Propamocarb/mL (slope = 2190, intercept = 72.0). Good linearity was observed (r2 = 0.99997). The resulting test substance peak areas versus test substance concentration data were fit to the linear function. Residuals were randomly scattered. |
| Calibration range | Calibration range 6.12 to 136 ng Propamocarb/mL (corresponding to 0.311 to 6.92 mg/kg BAS 743 02 F) |
| Assessment of matrix effects is presented | The mean matrix effect was determined to be -16.4% and thus considered not to be significant (i.e. the matrix effect was ≤±20%). Matrix-matched standards were used throughout nevertheless. |
| Solution Stability | Stability of Propamocarb in bee feeding solution were proven over a time period of 203 days under deep frozen conditions in the dark. |
| Limit of determination/quantification | The limit of quantification (LOQ) of 1.48 mg Propamocarb/kg was confirmed. The limit of detection (LOD) was 0.311 mg Propamocarb/kg. |

**Conclusion**

The analytical method for the determination of Propamocarb from final diets of honey bee larvae containing BAS 743 02 F has been fully validated in terms of specificity, linearity, precision, accuracy, matrix effects, solution stability and LOQ/LOD, in accordance with the requirements of SANTE/2020/12830 Rev.2

* + - * 1. Method validation

|  |  |
| --- | --- |
| Comments of zRMS: | The method has been accepted.  The purpose of the analytical phase of the study was to determine the concentrations of the test item BAS 743 02 F via its active ingredients Propamocarb and BAS 650 F (Ametoctradin) in test item solutions resulting from acute toxicity tests with BAS 743 02 F on bumblebees (Bombus terrestris L.). The determination was conducted by HPLC with mass-spectrometric (MS-MS) detection.  The method has LOQ of 1.45 mg/kg for Propamocarb and 0.459 mg/kg for BAS 650 F (contact toxicity test) and 1.52 mg/kg for Propamocarb and 0.482 mg/kg for  BAS 650 F (oral toxicity test). Results of the validation experiments showed that the mean recovery efficiency and repeatability was within the acceptable range of 70% to 120% of the intended concentrations for Propamocarb and BAS 650 F, with relative standard deviations RSD < 20%. No significant peak interferences (>LOD) occurred at the retention time and mass transition in the control samples.  Matrix effects were considered by the addition of the same amount of blank extract to calibration samples as included in the analysis samples. Thus, all measured samples contained the same amount of original sample matrix.  The method was fully validated according to the requirements of SANTE/2020/12830, Rev.1. |

|  |  |
| --- | --- |
| Reference: | CP 5.1.2/19 |
| Report | Acute toxicity of BAS 743 02 F to the bumblebee *Bombus terrestris* L. under laboratory conditions  Amsel, K., 2023  Study No.: 933752-18  XXXX DocID: 2022/2033711  Authority registration No |
| Guideline(s): | SANTE/2020/12830 Rev.1; OECD ENV/JM/MONO(2007)17; OPPTS 860.1340 |
| Deviations: | None |
| Previous evaluation: | No |
| GLP: | Yes |
| Acceptability: | Yes | |

**Study Summary**

An analytical method was validated for the determination of Propamocarb from test item solutions containing BAS 743 02 F produced during acute toxicity tests. A brief description of method and the results are presented in the summary below.

**Materials and methods**

For all samples of the contact toxicity test, which are present in water containing 0.5% (v/v) TritonX, no extraction is necessary. They are diluted with 50/50 (v/v) (acetonitrile + 0.5% (v/v) formic acid) / water and, if necessary to obtain equal amount of sample matrix in all samples for analysis, with sample matrix into the range of the calibration curve before injecting into the HPLC-system.

All samples of the oral toxicity test, which are present in 50% (w/v) sucrose solution, are extracted prior to sample measurement. A 0.2 g aliquot of sample test solution is shaken with acetonitrile:0.5% formic acid solution (50:50 v/v, 10 mL) using a Fast Prep system at 5 m/s for 5 minutes and centrifuged at 4000 rpm for 5 minutes. The resulting extract is further filuted with dilution medium (blank extract) into the range of the calibration curve

Samples are analysed by high performance liquid chromatography with tandem mass detection (HPLC-MS/MS) in positive ion mode using an ACE Excel 3 C18 column (100 mm x 2.1 mm, 3 µm) and gradient elution with mobile phases of water with 0.1% (v/v) formic acid + 5mM ammonium formate and methanol + 0.1% (v/v) formic acid. Calibration is by external matrix-matched standards monitoring the ion transitions *m/z* 276 → 149 and *m/z* 276 → 176 for the quantification and confirmation of Ametoctradin, respectively.

**Results and discussions**

The analytical method validation is summarised below and only the quantifying transition is reported.

Recoveries were evaluated with the fortification of sample matrix with BAS 743 02 F at two fortification levels (LOQ & approx 92000 x LOQ for contact toxicity samples and LOQ & approx 3675 x LOQ for oral toxicity samples). The mean recoveries were within the permitted range required by the guideline SANTE/2020/12830 rev. 2.

**Table A 95: Recovery results from method validation of Propamocarb using the analytical method**

| **Matrix** | **Analyte** | **n** | **Nominal fortification level (mg/kg)** | **Corresponding fortification level as  BAS 743 02 F (mg/kg)** | **Mean  recovery (%)** | **RSD (%)** | **Comments** |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Water containing 0.5% (v/v) TritonX (Contact toxicity test) | Propamocarb | 5 | 1.45 | 3.62 | 99.2 | 0.236 | Acceptable |
| 5 | 133002 | 332505 | 102 | 1.72 | Acceptable |
| 50% (w/v) sucrose solution  (Oral toxicity test) | Propamocarb | 5 | 1.52 | 3.80 | 97.3 | 0.945 | Acceptable |
| 5 | 5581 | 13951 | 97.8 | 1.41 | Acceptable |

**Table A 96: Characteristics for the method used for validation of Propamocarb in contact and oral toxicity samples**

|  | **Propamocarb** |
| --- | --- |
| Specificity | No significant peak interferences (> 30% LOQ) occurred at the retention time and mass transition of any active ingredient in the control samples. Peak identification was confirmed by retention time match with reference material. |
| Calibration (type, number of data points) | Calibration was performed with matrix-matched standards at six concentrations.  Contact toxicity analysis:  Fit: Linear, 6 points Range: 6.12 to 153 ng/mL  Slope = 3033, Intercept = -287, r2 = 0.999897).  Residuals were randomly scattered.  Oral toxicity sample analysis:  Fit: Linear, 6 points Range: 6.46 to 162 ng/mL  Slope = 2423, Intercept = -100, r2 = 0.999962).  Residuals were randomly scattered confirming correct fit type. |
| Calibration range | Calibration range  Contact toxicity analysis:  6.12 to 153 ng/mL (corresponding to 0.306 to 7.65 mg/kg)  6.46 to 162 ng/mL (corresponding to 0.328 to 8.21 mg/kg) |
| Assessment of matrix effects is presented | The mean matrix effect was determined to be -0.191% and -2.44% for contact and oral toxicity samples respectively thus not considered to be significant (i.e. the matrix effect was ≤±20%). |
| Solution Stability | Stability of Propamocarb in contact and oral toxicity samples was proven over a time period of at least 185 days and 188 days respectively under deep frozen conditions in the dark. |
| Limit of determination/quantification | The limit of quantification (LOQ) and limit of detection (LOD) were:  Contact toxicity solutions: LOQ = 1.45 mg/kg (28.9 ng/mL)  LOD = 0.306 mg/kg (6.12 ng/mL)  Oral toxicity solutions: LOQ = 1.52 mg/kg (29.9 ng/mL)  LOD = 0.328 mg/kg (6.46 ng/mL) |

**Conclusion**

The analytical method for the determination of Propamocarb from contact and oral toxicity samples containing BAS 743 02 F has been fully validated in terms of specificity, linearity, precision, accuracy, matrix effects, solution stability and LOQ/LOD, in accordance with the requirements of SANTE/2020/12830 Rev.2

* + - * 1. Method validation

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Comments of zRMS: | The method has been accepted.  The purpose of the analytical phase of the study was to determine Propamocarb and Ametoctradin in test item solutions resulting from an acute toxicity test with BAS 743 03 F. The determination was conducted by using HPLC with mass-spectrometric (MS-MS) detection. The LOQ was set at 0.4882 mg/L (Propamocarb) and at 0.1550 mg/L (BAS 650 F).  The mean recovery was within the acceptable range of 70% to 120% for Propamocarb and BAS 650 F, with RSD < 20%. No significant peak interferences (>LOD) occurred at the retention time and mass transition in the control samples. The method was fully validated according to the requirements of SANTE/2020/12830, Rev.1. | | | |
| Reference: | | CP 5.1.2/20 |
| Report | | Acute toxicity of BAS 743 03 F on *Daphnia magna* in a 48-hour static test  Renner, P., 2023  Study No.: 933750-2  XXXX DocID: 2022/2033730  Authority registration No |
| Guideline(s): | | SANTE/2020/12830 Rev.1; OECD ENV/JM/MONO(2007)17; OPPTS 860.1340 |
| Deviations: | | None |
| Previous evaluation: | | No |
| GLP: | | Yes |
| Acceptability: | | Yes | |

**Study Summary**

The method was validated in the above study for the determination of Propamocarb in reconstituted water with a limit of quantification at 0.4882 mg/L. The brief description of method and the results are presented in the summary below.

**Materials and methods**

Samples of were diluted with 50/50 (v/v) test matrix/ 0.2% formic acid (v/v) in acetonitrile (v/v) into the range of the calibration curve before analysis.

Propamocarb is determined by HPLC-MS/MS. Separation is achieved by using an ACE Excel 3 C18-PFP column (100 x 2.1 mm, 3 µm) and a gradient of water/formic acid/5 mM Ammonium Formate (1000/1, v/v) and acetonitrile/formic acid/5 mM Ammonium Formate (1000/1, v/v) at a flow rate of 0.2 mL/min.

Detection is accomplished in ESI positive mode using three different transitions; *m/z* 189 > 102 used for quantification and *m/z* 189 > 74and *m/z* 189 > 144 used for confirmation. External matrix-matched calibration standards were used throughout.

**Results and discussions**

The analytical method validation is summarised below. The HPLC-MS/MS determination was conducted by monitoring three MS/MS mass transitions and therefore no confirmatory method is required.

Recoveries were obtained by fortification of the matrices and all mean recoveries are within the permitted range required by the guideline SANTE/2020/12830 rev. 1.

**Table A 97: Recovery results from method validation of Propamocarb using the analytical method**

| **Matrix** | **Analyte** | **Fortification level (mg/L) (n=x)** | **Mean  recovery (%)** | **RSD (%)** | **Comments** |
| --- | --- | --- | --- | --- | --- |
| ***Mass transition (Quantification) Propamocarb m/z 189→102*** | | | | | |
| Reconstituted water | Propamocarb | 0.4882 (n=5) | 101 | 1.39 | Acceptable |
| 24.41 ( n=5) | 97.7 | 2.17 |

**Table A 98: Characteristics for the method used for validation of Propamocarb in Reconstituted Water**

|  | **Ametoctradin** |
| --- | --- |
| Specificity | LC-MS/MS is a highly specific method. No significant peak interferences (>LOD) occurred at the retention time and mass transition in the control samples |
| Calibration (type, number of data points) | Calibration was performed with matrix-matched standards at a minimum of five concentrations ranging 6.575 – 119.5 ng/mL and good linearity was observed (r ≥0.99). The resulting test substance peak areas versus theoretical test substance concentration data were fit to the linear function.  Regression data: y = ax2 + bx +c   |  |  |  |  | | --- | --- | --- | --- | | **a** | **b** | **c** | **r** | | -17.9473 | 24090.3 | -3288.45 | 0.99993 | |
| Calibration range | Accepted calibration range 2.087 ng/mL to 37.95 ng/mL |
| Assessment of matrix effects is presented | Matrix effects were evaluated by comparing of the analyte responses of each calibration level. The matrix effect for the sample matrix of Propamocarb was -1.37% . Thus, the matrix effect for is not significant. Matrix matched standards were used throughout the analytical phase for quantification of the test samples. |
| Solution Stability | All samples were measured within less than 30 days. Therefore, no storage stability was analysed. |
| Limit of determination/quantification | The limit of quantification (LOQ) of 0.4882 mg/L was confirmed. The limit of detection (LOD), defined as the lowest standard employed was 6.575 ng/mL ng/mL. |

**Conclusion**

The validation data for the method for the determination of Propamocarb in reconstituted water has been fully validated in terms of specificity, linearity, precision, accuracy, matrix effects, solution stability and LOQ, in accordance with the requirements of SANTE/2020/12830 rev.1.

* + 1. Methods for post-authorization control and monitoring purposes (KCP 5.2)

The reports supporting methods for post-authorization control/monitoring are owned by Bayer, the notifier of the active substance Propamocarb. Competent regulatory authority is authorized to access the data package of Propamocarb in support of the application of BAS 743 02 F. Please, refer to Letter of Access in part A. Excerpts of these methods are provided below for information, taken from the Renewal Assessment Report (RAR, Portugal, 2017) for Propamocarb.

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

Method validation

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| --- | --- |
| Comments of zRMS: | Excerpts of these methods are provided below for information, taken from the Renewal Assessment Report (RAR, Portugal, 2017) for Propamocarb  No comments. |

|  |  |
| --- | --- |
| Report | Modification M002 to the analytical method 00880 for the determination of residues of propamocarb free base (AE B0397744) in/on lettuce (head), chicory witloof (leaf), leek (shoot), cauliflower (curd), orange (whole fruit), avocado (pulp) and wheat (grain) by LC- MS/MS, Rosati, D. & Valcarce, M. H., 2006  Report No MR-0036/06 (Method No.: 00880/M002)  Document No. M-268065-01-1  Authority registration No |
| Guideline(s): | EU Council Directive 91/414/EEC amended by Commission Directive 96/68/EC; European Commission Guidance Document for Generating and Reporting Methods of Analysis in Support of Pre-Registration Data Requirements for Annex II (Part A, Section 4) and Annex III (Part A, Section 5) of Directive 91/414, SANCO/3029/99; European Commission Guidance Document for on Residue Analytical Methods, SANCO/825/00 |
| Deviations: | No |
| GLP: | yes |
| Acceptability: | Yes | |
| Report | Storage stability of residues of propamocarb free base (AE B039744) in/on sunflower seed, orange fruit and dry bean seed by LC- MS/MS,  Thies, S., 2016  Report No 2013/0086/01  Document No. M-516661-02-1  Authority registration No |
| Guideline(s): | Commission Regulation (EU) No 544/2011 of 10 June 2011 implementing Regulation (EC) No 1107/2009 of the European Parliament and of the Council as regards the data requirements for active substances (reference to document no. 7032/VI/95 rev.5 Appendix H); US EPA Residue Chemistry Test Guideline OCSPP 860.1380: Storage Stability Data; OECD Test Guideline 506, adopted 16 October 2007; PMRA Ref.: DACO 7.3, Storage Stability |
| Deviations: | No |
| GLP: | yes |
| Acceptability: | Yes | |

|  |  |
| --- | --- |
| Report | Independent laboratory validation of the modification M002 to the analytical method 00880 for the determination of residues of propamocarb free base (AE B039744) in/on plant material  Freitag, T. & Wolters, A., 2006  Report No MR-158/05 (Method No.: 00880/M002); Study No.: P612051810  Document No. M-277122-01-1  Authority registration No |
| Guideline(s): | SANCO/825/00 rev. 7 of March 17, 2004 of the European Commission; 91/414/EEC (Annex VI, Part C, No. 2.6.2) |
| Deviations: | No |
| GLP: | yes |
| Acceptability: | Yes | |

Materials and methods

The presented residue analytical method modification 00880/M002 is validated to suppress the step of C18 SPE cartridge clean up and for the determination of residues of Propamocarb free base (AE B039744) in lettuce, chicory witloof, leek, cauliflower, orange, avocado and wheat by LC/MS/MS. In addition, during the course of the storage stability study (Report no. 2013/0086/01) additional validation sets were performed on dry bean seed, orange fruit and sunflower seed to complete those obtained previously on the same group of matrices.

For all sample materials, Propamocarb is extracted from the sample material with a mixture of water / acetic acid (99/1). For cauliflower and orange sample material two extractions are necessary. After centrifugation and dilution of the extract, the residues are quantified by HPLC using an Hypercarb column and detected by tandem mass spectrometry with electrospray ionisation (HPLC-MS/MS). For oily matrices like avocado, after the extraction step, a clean-up using n-hexane is necessary.

The quantification is done by an external standardisation using matrix matched standards except for chicory witloof; in the last case, standards are prepared in solvents. The respective followed mass transitions are presented in Table A 99.

**Table A 99: Quantification and confirmatory mass transitions**

|  |  |  |
| --- | --- | --- |
|  | **Mass transition used for**  **quantification (1st MRM)** | **Mass transition used for**  **confirmation (2nd MRM)** |
| Propamocarb | 189 >102 m/z | 189 >144 m/z |

Results and discussions

In all matrices tested, the mean recovery values were between 70 and 110%, with one exception for dry bean seed (Study no 2013/0086/01) with 64% at the 0.01 mg/kg level, and with RSD values <20%. The detailed results are given in the table below.

**Table A 100: Recovery results from method validation of Propamocarb in plant material with the quantification MRM (189 >102 m/z)**

| **Matrix** | **Analyte** | **Fortification level (mg/kg) (*n* = x)** | **Mean  recovery (%)** | **RSD (%)** | **Comments** |
| --- | --- | --- | --- | --- | --- |
| Lettuce Head | Propamocarb | 0.01 (n=5) | 79 | 1.9 | MR-0036/06 |
| 0.1 (n=5) | 87 | 11.8 |
| Overall Recovery (n=10) | 83 | 9.8 |
| Chicory witloof Leaf | Propamocarb | 0.01 (n=5) | 87 | 15.6 |
| 0.1 (n=5) | 82 | 4.3 |
| Overall Recovery (n=10) | 85 | 11 |
| Cauliflower Curd | Propamocarb | 0.01 (n=5) | 92 | 2.7 |
| 0.1 (n=5) | 96 | 1.3 |
| Overall Recovery (n=10) | 94 | 3.2 |
| Orange Whole fruit | Propamocarb | 0.01 (n=5) | 78 | 5.6 |
| 0.1 (n=5) | 95 | 14.7 |
| Overall, Recovery (n=10) | 86 | 15 |
| Leek Shoot | Propamocarb | 0.01 (n=5) | 91 | 3.5 |
| 0.1 (n=5) | 97 | 4.6 |
| Overall Recovery (n=10) | 94 | 5.4 |
| Wheat Grain | Propamocarb | 0.01 (n=5) | 78 | 13.2 |
| 0.1 (n=5) | 85 | 12.4 |
| Overall Recovery (n=10) | 82 | 13 |
| Avocado Pulp | Propamocarb | 0.01 (n=5) | 79 | 9.4 |
| 0.1 (n=5) | 81 | 6.2 |
| Overall Recovery (n=10) | 80 | 7.6 |
| Dry bean Seed | Propamocarb | 0.01 (n=3) | 64 | 4.3 | study 2013/0086/01 |
| 0.1 (n=5) | 71 | 1.2 |
| Overall Recovery (n=8) | 68 | 5.5 |
| Orange Fruit | Propamocarb | 0.01 (n=3) | 90 | 4 |
| 0.1 (n=5) | 98 | 1.3 |
| Overall Recovery (n=8) | 95 | 4.6 |
| Sunflower Seed | Propamocarb | 0.01 (n=3) | 91 | 3.9 |
| 0.1 (n=5) | 92 | 0.7 |
| Overall Recovery (n=8) | 92 | 2.2 |

**Table A 101: Recovery results from method validation of Propamocarb in plant material with the confirmation MRM (189 >144 m/z)**

| **Matrix** | **Analyte** | **Fortification level (mg/kg) (*n* = x)** | **Mean  recovery (%)** | **RSD (%)** | **Comments** |
| --- | --- | --- | --- | --- | --- |
| Lettuce Head | Propamocarb | 0.01 (n=5) | 81 | 5.1 | MR-0036/06 |
| 0.1 (n=5) | 84 | 10.4 |
| Overall Recovery (n=10) | 82 | 8 |
| Chicory witloof Leaf | Propamocarb | 0.01 (n=5) | 88 | 14.3 |
| 0.1 (n=5) | 82 | 4.5 |
| Overall Recovery (n=10) | 85 | 11 |
| Cauliflower Curd | Propamocarb | 0.01 (n=5) | 100 | 4.3 |
| 0.1 (n=5) | 97 | 2.2 |
| Overall Recovery (n=10) | 99 | 3.5 |
| Orange Whole fruit | Propamocarb | 0.01 (n=5) | 90 | 9.3 |
| 0.1 (n=5) | 95 | 12.8 |
| Overall, Recovery (n=10) | 93 | 11 |
| Leek Shoot | Propamocarb | 0.01 (n=5) | 97 | 9.1 |
| 0.1 (n=5) | 98 | 6.2 |
| Overall Recovery (n=10) | 97 | 7.4 |
| Wheat Grain | Propamocarb | 0.01 (n=5) | 83 | 5.2 |
| 0.1 (n=5) | 91 | 8.7 |
| Overall Recovery (n=10) | 87 | 8.2 |
| Avocado Pulp | Propamocarb | 0.01 (n=5) | 74 | 9.8 |
| 0.1 (n=5) | 82 | 7.9 |
| Overall Recovery (n=10) | 78 | 9.7 |

**Table A 102: Recovery results obtained by an independent laboratory for the determination of Propamocarb in plant material with the quantification MRM (m/z 189>102)**

| **Matrix** | **Analyte** | **Fortification level (mg/kg) (*n* = x)** | **Mean  recovery (%)** | **RSD (%)** | **Comments** |
| --- | --- | --- | --- | --- | --- |
| Lettuce Head | Propamocarb | 0.01 (n=5) | 85 | 4.6 | MR-158/05 |
| 0.1 (n=5) | 88 | 2.5 |
| Overall Recovery (n=10) | 87 | 3.7 |
| Cauliflower Curd | Propamocarb | 0.01 (n=5) | 93 | 2.9 |
| 0.1 (n=5) | 91 | 3.6 |
| Overall Recovery (n=10) | 92 | 3.3 |
| Avocado, Pulp | Propamocarb | 0.01 (n=4) | 73 | 5.2 |
| 0.1 (n=5) | 77 | 4.1 |
| Overall Recovery (n=9) | 75 | 5.1 |
| Wheat Grain | Propamocarb | 0.01 (n=5) | 80 | 1.8 |
| 0.1 (n=5) | 81 | 3.4 |
| Overall Recovery (n=10) | 80 | 2.6 |

**Table A 103: Characteristics for the analytical method used for validation of propamocarb**

|  | **Propamocarb** |
| --- | --- |
| Specificity | The apparent residues for all control samples were below 30% of the LOQ for Propamocarb, i.e. < 0.003 mg/kg. In addition the method is based on mass specific detection of the compound. Therefore the method is considered specific for the detection of Propamocarb. |
| Calibration (type, number of data points) | The linearity of the detector used was tested for Propamocarb using standards in solvent and matrix matched standards.  m/z 102, 6 levels r>0.999  m/z 144, 6 levels r [0.9983 – 0.9999] |
| Calibration range | The linearity was tested by injecting standards of Propamocarb at concentrations between 0.04 and 5.0 μg/L.  ILV  For lettuce (head), wheat (grain) and avocado (pulp) matrix-matched standards of propamocarb free base covering the range of 0.01-1.0 μg/L, for cauliflower (curd), matrix-matched standards of propamocarb hydrochloride covering the range of 0.02-2.0 μg/L |
| Assessment of matrix effects is presented | No investigation of matrix effects was performed in this study. Matrix-matched standards were used throughout. |
| Limit of determination/quantification | The limit of quantification (LOQ) was defined as the lowest fortification level where a mean recovery within the range of 70 to 110 % and an RSD of ≤ 20 % could be obtained. The LOQ was set at 0.01 mg/kg for Propamocarb. |
| Stability | The extract of recovery samples were left in the fridge at about 4°C after their preparation and the samples were re-analysed after a storage period of up to 5 days.  The final extract of recovery samples were left in the autosampler at about 10°C after initial analysis and re-analysed after a storage period of up to 5 days. |

Conclusion

The method is suitable and validated for the determination of residues of Propamocarb (determined and calculated as Propamocarb - AE B039744) in plant material (e.g. lettuce (head), chicory witloof (leaf), cauliflower (curd), orange (whole fruit), leek (shoot), wheat (grain) and avocado (pulp)) with a LOQ of 0.01 mg/kg

Extraction efficiency

Please refer to the RAR (RMS Portugal, 2017) where this was addressed.

* + - 1. Description of analytical methods for the determination of residues in animal matrices (KCP 5.2)
         1. Analytical method 2

|  |  |
| --- | --- |
| Comments of zRMS: | Both EU agreed (RAR 2017) Provided in Appendix 2 for information  No comments. |

|  |  |
| --- | --- |
| Report | Validation of the QuEChERS Method (BCS method ID 01205) for the determination of propamocarb in animal tissues  Weber, H. & Schernikau, N., 2010  Report No BAY-1032V  Document No. M-387185-01-1  Authority registration No |
| Guideline(s): | 91/414/EEC as amended by 96/46/EC 4.2.1; SANCO/825/00 rev. 7, 17/03/04; BBA Guideline of 21 July 1998; OECD ENV/JM/MONO(2007)17, 13-Aug-07 |
| Deviations: | No |
| GLP: | yes |
| Acceptability: | Yes | |

|  |  |
| --- | --- |
| Report | Determination of residues of propamocarb in animal tissues; Independent Lab Validation of QuEChERS method (BCS method 01205)  Konrad, St. & Neuland, M., 2010  Report No 01205  Document No. M-398135-01-1  Authority registration No |
| Guideline(s): | 91/414/EEC, SANCO/3029/99, SANCO/825/00 rev. 7, ENV/JM/Mono(2007) 17 |
| Deviations: | No |
| GLP: | yes |
| Acceptability: | Yes | |

Materials and methods

The study objective is to validate the QuEChERS Method (BCS Method ID 01205) for the determination of residues of Propamocarb in meat (cattle), liver (cattle), kidney (cattle), fat (cattle), milk (cattle) and egg (chicken).

The Multi-Method L00.00-115 “QuEChERS” of the Official Collection of Test Methods was performed according to § 64 LFGB with:

- extraction with water/acetonitrile (5 g homogenised specimens and 5 mL of water (7 mL for fat samples) and 10 mL of acetonitrile);

- addition of magnesium sulphate, sodium chloride and sodium citrate, liquid/liquid partition and subsequent centrifugation;

- clean up by Primary Secondary Amine (PSA) and/or by freezing-out;

- detection with liquid chromatography tandem mass spectrometry LC-MS/MS using two mass transitions.

The quantification was done by an external standardisation using matrix matched standards prepared in solvents. The respective followed mass transitions are presented in the table below.

**Table A 104: Quantification and confirmatory mass transitions**

|  |  |  |
| --- | --- | --- |
|  | **Mass transition used for quantification (1st MRM)** | **Mass transition used for confirmation  (2nd MRM)** |
| Propamocarb | 189 >102 m/z | 189 >74 m/z |

**Results and discussions**

In all matrices tested, the mean recovery values were between 70 and 110 %, single recoveries below 60% (e.g. for egg and fat) were accepted because mean values were between 70-110%, and with RSD values <20%. The detailed results are given in the table below. For the ILV the mean recovery values obtained for whole milk, fat and whole egg (fat containing matrices) for both levels (LOQ and ten times LOQ) did not comply with the standard acceptance criteria. It was assumed that the extraction step is not complete or that there was a loss of analyte in the extracts. With slightly changed conditions in the sample preparation procedure compared to original BCS method 01205 (extraction by shaking vigorously for five minutes instead of one, no freeze out) the recovery rates determined for whole milk, fat and whole egg for both levels (LOQ and ten times LOQ) did comply with the standard acceptance criteria, the detailed results are given in the tables below.

**Table A 105: Recovery results from method validation of Propamocarb in animal matrices with the quantification MRM (189 >102 m/z)**

| **Matrix** | **Analyte** | **Fortification level (mg/kg) (*n* = x)** | **Mean  recovery (%)** | **RSD (%)** | **Comments** |
| --- | --- | --- | --- | --- | --- |
| Cattle Liver | Propamocarb | 0.01 (n=5) | 85 | 4.9 | BAY-1032V (Method No.: 01205) |
| 0.1 (n=5) | 78 | 3.3 |
| Overall Recovery (n=10) | 82 | 6.0 |
| Cattle Meat | Propamocarb | 0.01 (n=5) | 83 | 1.9 |
| 0.1 (n=5) | 77 | 1.8 |
| Overall Recovery (n=10) | 80 | 4.3 |
| Cattle Kidney | Propamocarb | 0.01 (n=5) | 82 | 2.9 |
| 0.1 (n=5) | 72 | 3 |
| Overall Recovery (n=10) | 77 | 7.6 |
| Cattle Milk | Propamocarb | 0.01 (n=5) | 77 | 5.5 |
| 0.1 (n=5) | 76 | 3.8 |
| Overall Recovery (n=10) | 77 | 4.4 |
| Chicken Egg | Propamocarb | 0.01 (n=5) | 75 | 2.5 |
| 0.1 (n=5) | 70 | 16 |
| Overall Recovery (n=10) | 73 | 11 |
| Cattle Fat | Propamocarb | 0.01 (n=5) | 78 | 12 |
| 0.1 (n=5) | 78 | 3.7 |
| Overall Recovery (n=10) | 78 | 8.2 |

**Table A 106: Recovery results from method validation of Propamocarb in animal matrices with the quantification MRM (189 >74 m/z)**

| **Matrix** | **Analyte** | **Fortification level (mg/kg) (*n* = x)** | **Mean  recovery (%)** | **RSD (%)** | **Comments** |
| --- | --- | --- | --- | --- | --- |
| Cattle Liver | Propamocarb | 0.01 (n=5) | 79 | 7.1 | BAY-1032V (Method No.: 01205) |
| 0.1 (n=5) | 77 | 2.3 |
| Overall Recovery (n=10) | 78 | 5.2 |
| Cattle Meat | Propamocarb | 0.01 (n=5) | 81 | 3.8 |
| 0.1 (n=5) | 78 | 0.6 |
| Overall Recovery (n=10) | 79 | 3 |
| Cattle Kidney | Propamocarb | 0.01 (n=5) | 85 | 3.9 |
| 0.1 (n=5) | 74 | 4.3 |
| Overall Recovery (n=10) | 79 | 8.4 |
| Cattle Milk | Propamocarb | 0.01 (n=5) | 79 | 9.4 |
| 0.1 (n=5) | 75 | 4 |
| Overall Recovery (n=10) | 77 | 7.4 |
| Chicken Egg | Propamocarb | 0.01 (n=5) | 76 | 6.4 |
| 0.1 (n=5) | 70 | 19 |
| Overall Recovery (n=10) | 73 | 13 |
| Cattle Fat | Propamocarb | 0.01 (n=5) | 77 | 13 |
| 0.1 (n=5) | 80 | 2.4 |
| Overall Recovery (n=10) | 79 | 9.1 |

**Table A 107: Recovery results obtained by an independent laboratory for the determination of Propamocarb in animal matrices with the quantification MRM (m/z 189>102)**

| **Matrix** | **Analyte** | **Fortification level (mg/kg) (*n* = x)** | **Mean  recovery (%)** | **RSD (%)** | **Comments** |
| --- | --- | --- | --- | --- | --- |
| Cattle Whole Milk | Propamocarb | 0.01 (n=5) | 47 | 8 | 2010/0129/01 |
| 0.1 (n=5) | 50 | 9 |
| Overall Recovery (n=10) | 48 | 9 |
| Cattle Muscle | Propamocarb | 0.01 (n=5) | 93 | 4 |
| 0.1 (n=5) | 96 | 4 |
| Overall Recovery (n=10) | 95 | 4 |
| Cattle Kidney | Propamocarb | 0.01 (n=5) | 105 | 11 |
| 0.1 (n=5) | 106 | 3 |
| Overall Recovery (n=10) | 105 | 8 |
| Cattle Liver | Propamocarb | 0.01 (n=5) | 99 | 2 |
| 0.1 (n=5) | 101 | 2 |
| Overall Recovery (n=10) | 100 | 2 |
| Cattle Fat | Propamocarb | 0.01 (n=5) | 67 | 6 |
| 0.1 (n=5) | 65 | 6 |
| Overall Recovery (n=10) | 66 | 6 |
| Chicken Whole Egg | Propamocarb | 0.01 (n=5) | 37 | 5 |
| 0.1 (n=5) | 38 | 10 |
| Overall Recovery (n=10) | 37 | 8 |

**Table A 108: Recovery results obtained by an independent laboratory for the determination of Propamocarb in animal matrices with the quantification MRM (m/z 189>74)**

| **Matrix** | **Analyte** | **Fortification level (mg/kg) (*n* = x)** | **Mean  recovery (%)** | **RSD (%)** | **Comments** |
| --- | --- | --- | --- | --- | --- |
| Cattle Whole Milk | Propamocarb | 0.01 (n=5) | 45 | 12 | 2010/0129/01 |
| 0.1 (n=5) | 46 | 12 |
| Overall Recovery (n=10) | 46 | 11 |
| Cattle Muscle | Propamocarb | 0.01 (n=5) | 91 | 7 |
| 0.1 (n=5) | 95 | 4 |
| Overall Recovery (n=10) | 03 | 6 |
| Cattle Kidney | Propamocarb | 0.01 (n=5) | 102 | 11 |
| 0.1 (n=5) | 98 | 4 |
| Overall Recovery (n=10) | 100 | 8 |
| Cattle Liver | Propamocarb | 0.01 (n=5) | 92 | 4 |
| 0.1 (n=5) | 92 | 1 |
| Overall Recovery (n=10) | 92 | 3 |
| Cattle Fat | Propamocarb | 0.01 (n=5) | 60 | 4 |
| 0.1 (n=5) | 60 | 3 |
| Overall Recovery (n=10) | 60 | 3 |
| Chicken Whole Egg | Propamocarb | 0.01 (n=5) | 36 | 3 |
| 0.1 (n=5) | 35 | 7 |
| Overall Recovery (n=10) | 35 | 5 |

**Table A 109: Recovery results obtained by an independent laboratory for the determination of Propamocarb in animal matrices with the quantification MRM (m/z 189>102) adapted method**

| **Matrix** | **Analyte** | **Fortification level (mg/kg) (*n* = x)** | **Mean  recovery (%)** | **RSD (%)** | **Comments** |
| --- | --- | --- | --- | --- | --- |
| Cattle Whole Milk | Propamocarb | 0.01 (n=5) | 87 | 1 | 2010/0129/01 |
| 0.1 (n=5) | 85 | 8 |
| Overall Recovery (n=10) | 86 | 5 |
| Cattle Fat | Propamocarb | 0.01 (n=5) | 86 | 2 |
| 0.1 (n=5) | 85 | 7 |
| Overall Recovery (n=10) | 86 | 4 |
| Chicken Whole Egg | Propamocarb | 0.01 (n=5) | 81 | 3 |
| 0.1 (n=5) | 80 | 4 |
| Overall Recovery (n=10) | 80 | 3 |

**Table A 110: Recovery results obtained by an independent laboratory for the determination of Propamocarb in animal matrices with the quantification MRM (m/z 189>102) adapted method**

| **Matrix** | **Analyte** | **Fortification level (mg/kg) (*n* = x)** | **Mean  recovery (%)** | **RSD (%)** | **Comments** |
| --- | --- | --- | --- | --- | --- |
| Cattle Whole Milk | Propamocarb | 0.01 (n=5) | 85 | 3 | 2010/0129/01 |
| 0.1 (n=5) | 86 | 5 |
| Overall Recovery (n=10) | 85 | 4 |
| Cattle Fat | Propamocarb | 0.01 (n=5) | 88 | 2 |
| 0.1 (n=5) | 87 | 8 |
| Overall Recovery (n=10) | 87 | 5 |
| Chicken Whole Egg | Propamocarb | 0.01 (n=5) | 82 | 3 |
| 0.1 (n=5) | 80 | 3 |
| Overall Recovery (n=10) | 81 | 3 |

**Table A 111: Characteristics for the analytical method used for validation of Propamocarb**

|  | **Propamocarb** |
| --- | --- |
| Specificity | The concentration of Propamocarb in final extracts was determined by high performance liquid chromatography with MS/MS detection. In order to ensure unambiguous identification two mass transitions were monitored. No significant interferences from the specimen matrix were detected at the retention times corresponding to Propamocarb in any of the control specimens The apparent residues for all control samples were below 30% of the LOQ for Propamocarb, i.e. < 0.003 mg/kg. In addition the method is based on mass specific detection of the compound. |
| Calibration (type, number of data points) | The linearity of the detector used was tested for Propamocarb hydrochloride using standards in solvent. Linearity curves were performed by injecting eight standard solutions. Regression coefficients R were higher than 0.999 for the two mass transitions.  ILV  The linearity of the detector response was determined by injecting matrix-matched standards of Propamocarb hydrochloride for muscle (cattle), liver (cattle), kidney (cattle), fat (cattle), milk (cattle) and whole egg (chicken). Linearity curves were performed by injecting eight standard solutions. Regression coefficients R were higher than 0.999 for the two mass transitions. |
| Calibration range | The linearity was demonstrated by injecting standards of Propamocarb hydrochloride at concentrations between 0.100 and 20.0 ng/mL.  ILV  The linearity was demonstrated covering the range of 0.1-20.0 ng/mL Propamocarb hydrochloride. |
| Assessment of matrix effects is presented | The tests for meat showed a signal depression of up to 25%. Only slight effects were observed for liver, kidney, egg, fat and milk.  ILV  The tests showed effects for all matrices. Matrix matched standards were used in the ILV. |
| Limit of determination/quantification | The limit of quantification (LOQ) was defined as the lowest fortification level where a mean recovery within the range of 70 to 110 % and an RSD of ≤ 20 % could be obtained. The LOQ was set at 0.01 mg/kg for Propamocarb. |
| Stability | The extract of recovery samples were left in the dark between +3°C and +10°C after their preparation and the samples were re-analysed after a storage period of 14 days. This stability was checked by measuring three recovery samples at 10xLOQ (0.10 mg/kg) for each matrix. The second analysis was done using freshly prepared calibration standards. |

**Table A 112: Stability of extracts for a period of 14 days in the dark between +3°C and +10°C**

| **Matrix** | **Analyte** | **Ion transition / m/z** | **fortification level (mg/kg)** | **Recovery (%)** | | **Comments** |
| --- | --- | --- | --- | --- | --- | --- |
| **Initial** | **After 14 days storage** |
| Cattle Liver | Propamocarb | 189 > 102 | 0.1 | 78, 79, 81 | 77, 71, 76 | BAY-1032V (Method No.: 01205) |
| 189 > 74 | 0.1 | 76, 78, 78 | 72, 76, 74 |
| Cattle Meat | Propamocarb | 189 > 102 | 0.1 | 78, 78, 75 | 94, 90, 77 |
| 189 > 74 | 0.1 | 78, 79, 78 | 89, 73, 74 |
| Cattle Kidney | Propamocarb | 189 > 102 | 0.1 | 68, 73, 73 | 73, 78, 74 |
| 189 > 74 | 0.1 | 73, 79, 71 | 70, 76, 80 |
| Cattle Milk | Propamocarb | 189 > 102 | 0.1 | 77, 76, 74 | 73, 75, 73 |
| 189 > 74 | 0.1 | 76, 79, 71 | 72, 76, 77 |
| Chicken Egg | Propamocarb | 189 > 102 | 0.1 | 70, 82, 68 | 79, 74, 80 |
| 189 > 74 | 0.1 | 69, 86, 67 | 82, 72, 78 |
| Cattle Fat | Propamocarb | 189 > 102 | 0.1 | 82, 77, 74 | 83, 82, 83 |
| 189 > 74 | 0.1 | 83, 80, 78 | 83, 76, 78 |

Conclusion

The data presented demonstrate that the QuEChERS Method (BCS Method 01205) of the Official Collection of Test Methods according to § 64 LFGB is suitable and validated for the determination of residues of Propamocarb in animal tissues (meat, liver, kidney, fat, milk and egg) with a LOQ of 0.01 mg/kg. Satisfactory specificity, linearity, accuracy, precision, LOQ, matrix effect and stability data have been obtained and therefore have proven its applicability as enforcement method.

* + - * 1. Analytical method 3

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| --- | --- |
| Comments of zRMS: | Both studies are EU agreed (RAR 2017) Provided in Appendix 2 for information.  No comments. |

|  |  |
| --- | --- |
| Report | Validation of the BCS-method 01300/M012 (based on QuEChERS) for the determination of propamocarb metabolites in/on animal tissues, Winter, O. & Amann, S., 2014  Report No S13-03821  Document No. M-490237-01-1  Authority registration No |
| Guideline(s): | Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC  European Commission Guidance Document for Generating and Reporting  Methods of Analysis in Support of Pre-Registration data Requirements for Annex II  (part A, Section 4) and Annex III (part A, section 5) of directive 91/414,  SANCO/3029/99 rev. 4, 11/07/00  Guidance document on residue analytical methods, SANCO/825/00/rev. 8.1, European Commission, Directorate General Health and Consumer Protection 16/11/2010  US EPA Residue Chemistry Test Guideline OCSPP 860.1340: Residue Analytical Method |
| Deviations: | not specified |
| GLP: | Yes |
| Acceptability: | Yes | |

|  |  |
| --- | --- |
| Report | Independent laboratory validation (ILV) of the BCS-method 01300/M012 (based on QuEChERS) for the determination of propamocarb metabolites in different matrices of animal origin  Mewis, A., 2015  Report No S13-03822  Document No. M-517360-01-1  Authority registration No |
| Guideline(s): | Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC; Guidance document on residue analytical methods, SANCO/825/00/rev. 8.1, European Commission, Directorate General Health and Consumer Protection 16/11/2010; US EPA Residue Chemistry Test Guideline OCSPP 860.1340: Residue Analytical Method |
| Deviations: | not specified |
| GLP: | yes |
| Acceptability: | Yes | |

Materials and methods

Generally, only sufficiently characterized and certified substances were used as reference items. For the analysis the hydrochloride analogues of both substances were used as analytical standards. N-oxide Propamocarb hydrochloride (AE F155306) was used for N-oxide Propamocarb and N-desmethyl Propamocarb hydrochloride (BCS-AW15480) for N-desmethyl propamocarb. This has no impact on the analytical method as the free metabolites are existent in the solution.

N-desmethyl Propamocarb was extracted from poultry tissues with acetonitrile/water (1:1). After the samples were manually shaken for about 1 min, magnesium sulfate, sodium chloride and buffering citrate salts were added to the extract.

The samples were centrifuged and an aliquot was purified by addition of primary/secondary amine (PSA). The sample was centrifuged and the supernatant was deep-frozen for 1 hour.

Afterwards an aliquot of the supernatant was evaporated almost to dryness and diluted with methanol/0.05% acetic acid (15/85, v/v) for measurement by reversed phase HPLC-MS/MS in positive ion mode and multiple reaction monitoring (MRM).

N-oxide Propamocarb was extracted from ruminant tissues with acetonitrile/water (1:1). After the samples were manually shaken for about 1 min, magnesium sulfate, sodium chloride and buffering citrate salts were added to the extract.

The samples were centrifuged and the supernatant was deep-frozen for 1 hour.

Afterwards an aliquot of the supernatant was evaporated to dryness and diluted with methanol/0.05% acetic acid (15/85, v/v) for measurement by reversed phase HPLC-MS/MS in positive ion mode and multiple reaction monitoring (MRM).

Instrument/Detector: Agilent 1200 HPLC, AB Sciex API 5000 tandem mass spectrometer with Turbo Ion spray (ESI) in positive ion mode and multiple reaction monitoring (MRM). For the ILV, Agilent 1290 Infinity HPLC, API 6500 Tandem Mass Spectrometer with Turbo Ion spray (ESI) in positive ion mode and multiple reaction monitoring (MRM).

Column used: ZORBAX Eclipse XDB-C8, 150 mm length, 4.6 mm diameter, 5.0 μm particle size. For the ILV, a ZORBAX Eclipse XDB-C18, 150 mm x 4.6 mm x 5.0 μm, with 4 mm guard-column was used.

**Table A 113: MRM transitions (quantitation and confirmation) used for N-desmethyl Propamocarb and N-oxide Propamocarb**

|  |  |  |
| --- | --- | --- |
|  | **Mass transition used for  quantification (1st MRM)** | **Mass transition used for  confirmation (2nd MRM)** |
| N-desmethyl Propamocarb | 175 → 102 m/z | 175 → 74 m/z |
| N-oxide Propamocarb | 205 → 102 m/z | 205 → 74 m/z |

Results and discussions

For validation of the method, recovery experiments were performed at fortification levels of 0.01 mg/kg (LOQ) and 0.10 mg/kg for N-desmethyl Propamocarb and N-oxide Propamocarb by preparing and analyzing control and fortified samples of poultry tissues and eggs and ruminant tissues and milk. For each matrix there were five recovery experiments at LOQ and 10 fold LOQ levels.

**Table A 114: Recoveries from the method validation for N-desmethyl Propamocarb, Quantitation mass transition (m/z 175->102)**

| **Analyte** | **Matrix** | **Fortification level (mg/kg) (*n* = x)** | **Mean  recovery (%)** | **RSD (%)** | **Comments** |
| --- | --- | --- | --- | --- | --- |
| N-desmethyl Propamocarb.HCl\* | Poultry meat | 0.01 (n=5) | 88 | 2.4 | S13-03821 |
| 0.10 (n=5) | 81 | 4.6 |
| Overall Recovery (n=10) | 84 | 5.5 |
| Poultry fat | 0.01 (n=4†) | 83 | 12 |
| 0.10 (n=4†) | 78 | 3.5 |
| Overall Recovery (n=8) | 80 | 8.7 |
| Poultry Liver | 0.01 (n=5) | 87 | 4.6 |
| 0.10 (n=5) | 77 | 5.2 |
| Overall Recovery (n=10) | 82 | 7.7 |
| Poultry Kidney | 0.01 (n=5) | 84 | 3.1 |
| 0.10 (n=5) | 82 | 4.8 |
| Overall Recovery (n=10) | 83 | 4.1 |
| Poultry Eggs | 0.01 (n=5) | 89 | 7.7 |
| 0.10 (n=5) | 88 | 4.9 |
| Overall Recovery (n=10) | 89 | 6.2 |

\* molecular weight conversion factor from N-desmethyl Propamocarb.HCl to N-desmethyl Propamocarb = 0.83

† Outlier according to Dixon test, not taken into account for statistical evaluation

**Table A 115: Recoveries from the method validation for N-desmethyl Propamocarb, Confirmatory Mass Transition (m/z 175->74).**

| **Analyte** | **Matrix** | **Fortification level (mg/kg) (*n* = x)** | **Mean  recovery (%)** | **RSD (%)** | **Comments** |
| --- | --- | --- | --- | --- | --- |
| N-desmethyl Propamocarb.HCl\* | Poultry meat | 0.01 (n=5) | 86 | 6.0 | S13-03821 |
| 0.10 (n=5) | 82 | 4.4 |
| Overall Recovery (n=10) | 84 | 5.6 |
| Poultry fat | 0.01 (n=4†) | 81 | 13 |
| 0.10 (n=4†) | 73 | 5.2 |
| Overall Recovery (n=8) | 77 | 11 |
| Poultry Liver | 0.01 (n=5) | 88 | 4.7 |
| 0.10 (n=5) | 78 | 6.8 |
| Overall Recovery (n=10) | 83 | 8.2 |
| Poultry Kidney | 0.01 (n=5) | 82 | 1.6 |
| 0.10 (n=5) | 83 | 3.1 |
| Overall Recovery (n=10) | 83 | 2.4 |
| Poultry Eggs | 0.01 (n=5) | 86 | 4.8 |
| 0.10 (n=5) | 86 | 3.4 |
| Overall Recovery (n=10) | 86 | 4.0 |

\* molecular weight conversion factor from N-desmethyl Propamocarb.HCl to N-desmethyl Propamocarb = 0.83

† Outlier according to Dixon test, not taken into account for statistical evaluation

**Table A 116: Recoveries from the method validation for N-oxide Propamocarb, Quantitation mass transition (m/z 205->102)**

| **Analyte** | **Matrix** | **Fortification level (mg/kg) (*n* = x)** | **Mean  recovery (%)** | **RSD (%)** | **Comments** |
| --- | --- | --- | --- | --- | --- |
| N-oxide Propamocarb.HCl\* | Bovine Meat | 0.01 (n=5) | 89 | 11 | S13-03821 |
| 0.10 (n=5) | 104 | 1.7 |
| Overall Recovery (n=10) | 97 | 10 |
| Bovine Fat | 0.01 (n=5) | 78 | 4.6 |
| 0.10 (n=5) | 109 | 2.9 |
| Overall Recovery (n=10) | 94 | 18 |
| Bovine Liver | 0.01 (n=4†) | 74 | 4.8 |
| 0.10 (n=5) | 107 | 3.3 |
| Overall Recovery (n=9) | 92 | 19 |
| Bovine Kidney | 0.01 (n=5) | 71 | 3.2 |
| 0.10 (n=5) | 100 | 14 |
| Overall Recovery (n=10) | 86 | 21 |
| Milk | 0.01 (n=5) | 81 | 2.3 |
| 0.10 (n=5) | 110 | 0.6 |
| Overall Recovery (n=10) | 95 | 16 |

\* molecular weight conversion factor from N-oxide Propamocarb.HCl to N-oxide Propamocarb = 0.85

† Outlier according to Dixon test, not taken into account for statistical evaluation

**Table A 117: Recoveries from the method validation for N-oxide Propamocarb, Confirmatory Mass Transition (m/z 205-74).**

| **Analyte** | **Matrix** | **Fortification level (mg/kg) (*n* = x)** | **Mean  recovery (%)** | **RSD (%)** | **Comments** |
| --- | --- | --- | --- | --- | --- |
| N-oxide Propamocarb.HCl\* | Bovine Meat | 0.01 (n=5) | 85 | 12 | S13-03821 |
| 0.10 (n=5) | 102 | 0.9 |
| Overall Recovery (n=10) | 94 | 12 |
| Bovine Fat | 0.01 (n=5) | 74 | 4.1 |
| 0.10 (n=5) | 109 | 1.6 |
| Overall Recovery (n=10) | 92 | 20 |
| Bovine Liver | 0.01 (n=4†) | 71 | 6.1 |
| 0.10 (n=5) | 105 | 3.3 |
| Overall Recovery (n=9) | 90 | 20 |
| Bovine  Kidney | 0.01 (n=5) | 70 | 4.2 |
| 0.10 (n=5) | 101 | 15 |
| Overall Recovery (n=10) | 86 | 23 |
| Milk | 0.01 (n=5) | 75 | 1.7 |
| 0.10 (n=5) | 110 | 0.6 |
| Overall Recovery (n=10) | 93 | 20 |

\* molecular weight conversion factor from N-oxide Propamocarb.HCl to N-oxide Propamocarb = 0.85

† Outlier according to Dixon test, not taken into account for statistical evaluation

**Table A 118: Recoveries from the ILV for N-desmethyl Propamocarb,   
Quantitation mass transition (m/z 175-102)**

| **Analyte** | **Matrix** | **Fortification level (mg/kg) (*n* = x)** | **Mean  recovery (%)** | **RSD (%)** | **Comments** |
| --- | --- | --- | --- | --- | --- |
| N-desmethyl Propamocarb.HCl\* | Poultry meat | 0.01 (n=5) | 70 | 3.6 | S13-03822 |
| 0.10 (n=5) | 85 | 2.2 |
| Overall Recovery (n=10) | 78 | 10 |
| Poultry fat | 0.01 (n=5) | 80 | 5.7 |
| 0.10 (n=5) | 84 | 2.6 |
| Overall Recovery (n=10) | 82 | 4.8 |
| Poultry Liver | 0.01 (n=5) | 70 | 8.7 |
| 0.10 (n=5) | 80 | 2.6 |
| Overall Recovery (n=10) | 75 | 9.4 |
| Poultry  Kidney | 0.01 (n=5) | 79 | 9.1 |
| 0.10 (n=5) | 86 | 2.1 |
| Overall Recovery (n=10) | 83 | 7.2 |
| Poultry Eggs | 0.01 (n=5) | 70 | 0.8 |
| 0.10 (n=5) | 85 | 1.5 |
| Overall Recovery (n=10) | 78 | 9.9 |

\* molecular weight conversion factor from N-desmethyl Propamocarb.HCl to N-desmethyl Propamocarb = 0.83

**Table A 119: Recoveries from the ILV for N-desmethyl Propamocarb,   
 Confirmatory Mass Transition (m/z 175-74).**

| **Analyte** | **Matrix** | **Fortification level (mg/kg) (*n* = x)** | **Mean  recovery (%)** | **RSD (%)** | **Comments** |
| --- | --- | --- | --- | --- | --- |
| N-desmethyl Propamocarb.HCl\* | Poultry meat | 0.01 (n=5) | 71 | 3.4 | S13-03822 |
| 0.10 (n=5) | 85 | 1.7 |
| Overall Recovery (n=10) | 78 | 9.9 |
| Poultry fat | 0.01 (n=5) | 79 | 7.9 |
| 0.10 (n=5) | 83 | 2.8 |
| Overall Recovery (n=10) | 81 | 6.1 |
| Poultry Liver | 0.01 (n=5) | 70 | 9.6 |
| 0.10 (n=5) | 81 | 2.1 |
| Overall Recovery (n=10) | 76 | 10 |
| Poultry  Kidney | 0.01 (n=5) | 78 | 9.0 |
| 0.10 (n=5) | 84 | 1.3 |
| Overall Recovery (n=10) | 81 | 7.0 |
| Poultry Eggs | 0.01 (n=5) | 71 | 2.6 |
| 0.10 (n=5) | 84 | 1.5 |
| Overall Recovery (n=10) | 77 | 9.5 |

\* molecular weight conversion factor from N-desmethyl Propamocarb.HCl to N-desmethyl Propamocarb = 0.83

**Table A 120: Recoveries from the ILV for N-oxide Propamocarb,   
 Quantitation mass transition (m/z 205-102)**

| **Analyte** | **Matrix** | **Fortification level (mg/kg) (*n* = x)** | **Mean  recovery (%)** | **RSD (%)** | **Comments** |
| --- | --- | --- | --- | --- | --- |
| N-oxide Propamocarb.HCl\* | Bovine Meat | 0.01 (n=5) | 70 | 0.8 | S13-03822 |
| 0.10 (n=5) | 104 | 1.6 |
| Overall Recovery (n=10) | 87 | 20 |
| Bovine Fat | 0.01 (n=5) | 87 | 1.3 |
| 0.10 (n=5) | 108 | 7.6 |
| Overall Recovery (n=10) | 98 | 12 |
| Bovine Liver | 0.01 (n=5) | 70 | 12 |
| 0.10 (n=5) | 80 | 10 |
| Overall Recovery (n=10) | 75 | 12 |
| Bovine  Kidney | 0.01 (n=5) | 70 | 3.3 |
| 0.10 (n=5) | 91 | 3.0 |
| Overall Recovery (n=10) | 81 | 15 |
| Milk | 0.01 (n=5) | 74 | 3.5 |
| 0.10 (n=5) | 95 | 1.2 |
| Overall Recovery (n=10) | 84 | 13 |

\* molecular weight conversion factor from N-oxide Propamocarb.HCl to N-oxide Propamocarb = 0.85

**Table A 121: Recoveries from the ILV for N-oxide Propamocarb,   
 Confirmatory Mass Transition (m/z 205-74).**

| **Analyte** | **Matrix** | **Fortification level (mg/kg) (*n* = x)** | **Mean  recovery (%)** | **RSD (%)** | **Comments** |
| --- | --- | --- | --- | --- | --- |
| N-oxide Propamocarb.HCl\* | Bovine Meat | 0.01 (n=5) | 71 | 0.8 | S13-03822 |
| 0.10 (n=5) | 104 | 2.5 |
| Overall Recovery (n=10) | 87 | 20 |
| Bovine Fat | 0.01 (n=5) | 87 | 1.0 |
| 0.10 (n=5) | 108 | 8.3 |
| Overall Recovery (n=10) | 97 | 13 |
| Bovine Liver | 0.01 (n=5) | 70 | 9.4 |
| 0.10 (n=5) | 79 | 10 |
| Overall Recovery (n=10) | 74 | 11 |
| Bovine  Kidney | 0.01 (n=5) | 71 | 4.4 |
| 0.10 (n=5) | 91 | 3.4 |
| Overall Recovery (n=10) | 81 | 14 |
| Milk | 0.01 (n=5) | 74 | 2.6 |
| 0.10 (n=5) | 95 | 1.2 |
| Overall Recovery (n=10) | 84 | 13 |

\* molecular weight conversion factor from N-oxide Propamocarb.HCl to N-oxide Propamocarb = 0.85

**Table A 122: Characteristics for the analytical method used for validation of propamocarb**

|  | **N-desmethyl Propamocarb and N-oxide Propamocarb** |
| --- | --- |
| Specificity | Apparent residues in control samples were below 30 % of the LOQ. The recoveries were not corrected for interferences.  Two MRM transitions were monitored for N-desmethyl Propamocarb in poultry tissues (meat, fat, liver, and kidney) and eggs and for N-oxide Propamocarb in ruminant tissues (meat, fat, liver, and kidney) and milk. HPLC-MS/MS is a highly specific technique. |
| Calibration (type, number of data points) | The correlation between the injected amount and the detector response for N-desmethyl Propamocarb and N-oxide Propamocarb was confirmed by seven matrix matched standard solutions.  All matrix standards were prepared with constant matrix content. The coefficients of determination (R²) were always > 0.98.  ILV  All matrix standards were prepared with constant matrix content. The coefficients of determination (R²) were always > 0.99. |
| Calibration range | The linearity was shown:  from 1.24 ng/mL to 49.6 ng/mL (corresponding to 0.0025 mg/kg - 0.099 mg/kg) for N-desmethyl Propamocarb  from 0.042 ng/mL to 8.48 ng/mL (corresponding to 0.0008 mg/kg - 0.170 mg/kg) for N-oxide Propamocarb  The lower margin of the linearity test was at least 30 % of the LOQ. The higher margin was at least 20% above the highest fortification level.  ILV  The linearity was shown:  from 1.0 ng/mL to 100.0 ng/mL (corresponding to 0.002 mg/kg - 0.2 mg/kg) for N-desmethyl Propamocarb.HCl.  from 0.05 ng/mL to 100.0 ng/mL (corresponding to 0.001 mg/kg - 2.0 mg/kg) N-oxide Propamocarb.HCl.  The lower margin of the linearity test was at least 30 % of the LOQ. The higher margin was at least 20% above the highest fortification level. |
| Assessment of matrix effects is presented | Matrix effects of ≤ 20 % were measured for N-desmethyl Propamocarb in all matrices and considered to be not significant.  For N-oxide Propamocarb significant matrix effects of > 20% were detected for bovine meat, bovine kidney, bovine fat and milk. For bovine liver no significant matrix effects were observed. Nonetheless in all cases, quantification was performed with matrix-matched standards.  ILV  Matrix effects of < 20 % were measured for N-desmethyl Propamocarb in all matrices and considered to be not significant. For N-oxide Propamocarb a significant matrix effect was detected for bovine kidney. Hence, matrix-matched standards were used for determination. |
| Limit of determination/quantification | The limit of quantification (LOQ) was 0.01 mg/kg for N-desmethyl Propamocarb HCl and N-oxide Propamocarb HCl in all matrices. The corresponding LOQ is 0.0083 mg/kg for N-desmethyl Propamocarb and 0.0085 mg/kg for N-oxide Propamocarb in all matrices.  The limit of detection (LOD) was not higher than 30% of the LOQ for each analyte. |
| Stability | The stability of the compounds in solvent standard solutions was tested by comparing aged standard solutions against freshly prepared standard solutions. The stability in solvent standards in methanol/0.05% acetic acid (15/85, v/v) was checked over a period of six days for N-desmethyl Propamocarb and seven days for N-oxide Propamocarb. No significant alteration was observed. Therefore N-desmethyl Propamocarb is considered stable in solvent standards for at least six days and N-oxide Propamocarb for at least seven days when stored at 5 ± 4 °C in the dark in a refrigerator.  The stability in final extracts of samples fortified at the 10xLOQ was checked for the tested sample materials over a period of five days up to eight days. Therefore the stored extracts were quantified against fresh matrix-matched standard solutions by comparing the recoveries at the initial day of analysis and analysis after storage of the final sample extracts at 5 ± 4 °C under dark conditions over the given periods. N-oxide Propamocarb was found to be stable for at least five days in bovine kidney and for at least eight days in bovine meat when stored at 5 ± 4 °C under dark conditions.  In bovine liver, bovine fat and milk extracts a significant alteration was observed and the recoveries were outside the permitted range of 70 – 110% after storage at 5 ± 4 °C under dark conditions over the given periods.  It is therefore strongly recommended to analyse these samples within 24 hours after extraction.  N-desmethyl Propamocarb was found to be stable in all poultry matrices analysed within this study for at least seven days when stored at 5 ± 4 °C under dark conditions. |

Conclusion

The method meets all guideline criteria to determine residues of N-desmethyl Propamocarb hydrochloride and N-oxide Propamocarb hydrochloride at the LOQ of 0.01 mg/kg (0.0083 mg/kg as N-desmethyl Propamocarb and 0.0085 mg/kg as N-oxide Propamocarb) in animal tissues, milk and eggs.

* + - 1. Description of Methods for the Analysis of Soil (KCP 5.2)
         1. Analytical method 4

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| --- | --- |
| Comments of zRMS: | EU agreed (RAR 2017) Provided in Appendix 2 for information.  No comments. |

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| --- | --- |
| Report | Analytical method 01448 for the determination of propamocarb in soil by HPLC-MS/MS  Freitag, T & Koch, V., 2015  Report No MR-15/013  Document No. M-525885-01-1  Authority registration No |
| Guideline(s): | Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC  European Commission Guidance Document for Generating and Reporting Methods of Analysis in Support of Pre-Registration data Requirements for Annex II (part A, Section 4) and Annex III (part A, section 5) of directive 91/414, SANCO/3029/99 rev. 4, 11/07/00  Guidance document on residue analytical methods, SANCO/825/00/rev. 8.1, European Commission, Directorate General Health and Consumer Protection 16/11/2010  US EPA Residue Chemistry Test Guideline OCSPP 860.1340: Residue Analytical Method |
| Deviations: | not specified |
| GLP: | Yes |
| Acceptability: | Yes | |

Materials and methods

The analytical method 01448 was developed for the determination of Propamocarb in soil by LC/MS/MS with two MRM transitions. Soil samples of 25 g were extracted three times successively each by shaking with 50 mL of 1 N aqueous hydrochloric acid for 60 min followed by neutralization with aqueous (25%) ammonia solution and centrifugation. The combined soil extract was diluted and the analyte was quantified and its identity confirmed by high performance liquid chromatography coupled with MS/MS (HPLC-MS/MS) detection in the MRM mode. The method was validated using a silt loam (Höfchen, OM 1.58%) and a sandy loam soil (Laacher Hof, OM 2.06%). Quantitation was performed by using matrix-matched standards.

**Table A 123: Soil characteristics**

|  |  |  |
| --- | --- | --- |
| **Description** | **Soil Höfchen Plot 4011; 0-30 cm soil layer** | **Soil Laacher Hof Plot 712/18; 0-30 cm soil layer** |
| pH (in CaCl2 solution) | 6.7 | 6.8 |
| pH (in H2O) | 7.4 | 7.4 |
| Organic Carbon (%) | 0.92 | 1.20 |
| Organic matter (%)\* | 1.58 | 2.06 |
| Cation exchange capacity (meq/100 g dry soil) | 12.4 | 9.8 |
| Max. water holding capacity (g/100 g dry soil) | 39.5 | 37.9 |
| Textural description according to USDA (fraction %) | | |
| Clay (<0.002 mm) | 19.4 | 12.0 |
| Silt (0.002 – 0.050 mm) | 76.3 | 18.3 |
| Sand (0.050-2.000 mm) | 4.3 | 67.7 |
| Soil type | Silt loam | Sandy loam |
| \*Organic matter = organic carbon x1.72 | | |

**Table A 124: MRM transitions (quantitation and confirmation) used for Propamocarb**

|  |  |  |
| --- | --- | --- |
|  | **Mass transition used for**  **quantification (1st MRM)** | **Mass transition used for**  **confirmation (2nd MRM)** |
| Propamocarb | 189 → 102 m/z | 189 → 144 m/z |

Results and discussions

**Table A 125: Recovery results from method validation of Propamocarb in soil m/z = 189 → 102**

| **Analyte** | **Matrix** | **Fortification level (µg/kg) (*n* = x)** | **Mean  recovery (%)** | **RSD (%)** | **Comments** |
| --- | --- | --- | --- | --- | --- |
| Propamocarb | Soil Höfchen | 2 (n=5) | 104 | 2.6 | MR-15/013 |
| 20 (n=5) | 107 | 1.6 |
| Overall Recovery (n=10) | 106 | 2.3 |
| Soil Laacher Hof | 2 (n=5) | 111 | 4.9 |
| 20 (n=5) | 107 | 1.1 |
| Overall Recovery (n=10) | 109 | 3.7 |

**Table A 126: Recovery results from method validation of Propamocarb in soil m/z = 189 → 144**

| **Analyte** | **Matrix** | **Fortification level (µg/kg) (*n* = x)** | **Mean  recovery (%)** | **RSD (%)** | **Comments** |
| --- | --- | --- | --- | --- | --- |
| Propamocarb | Soil Höfchen | 2 (n=5) | 107 | 4.7 | MR-15/013 |
| 20 (n=5) | 107 | 0.8 |
| Overall Recovery (n=10) | 107 | 3.2 |
| Soil Laacher Hof | 2 (n=5) | 107 | 3.8 |
| 20 (n=5) | 105 | 0.8 |
| Overall Recovery (n=10) | 106 | 2.7 |

**Table A 127: Characteristics for the analytical method used for validation of propamocarb**

|  | **Propamocarb** |
| --- | --- |
| Specificity | The specificity of the method resulted from HPLC separation in combination with the very selective MS/MS detection. Apparent residues in control samples were below 0.3 × LOQ. The recoveries were not corrected for interferences. Two MRM transitions were monitored for each soil tested, i.e. m/z 189 → 102 for quantitation and m/z 189 → 144 for confirmation. Therefore, this HPLC-MS/MS method was regarded as highly specific. |
| Calibration (type, number of data points) | The mass spectrometric detector showed linear correlation between concentration and peak area for matrix matched standards (n=10). Correlation coefficients were > 0.998 |
| Calibration range | 0.05 to 60 μg/L, equivalent to concentrations in soil samples ranging from 0.555 to 666 μg/kg soil |
| Assessment of matrix effects is presented | Matrix matched standards were used. |
| Limit of determination/quantification | The limit of quantitation (LOQ) was 2 μg/kg in soil. The corresponding limit of determination (LOD) was 0.7 μg/kg soil. |
| Stability | The stock solutions were found to be stable for 84 days when stored in a refrigerator in the dark. |

**Conclusion**

The method fulfils the guideline criteria for determination of residues in soil as demonstrated by investigations for the two mass transitions and the two concentrations of 2 μg/kg soil (LOQ) and 20 μg/kg soil.

* + - 1. Description of Methods for the Analysis of Water (KCP 5.2)
         1. Analytical method 5

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| Comments of zRMS: | EU agreed (RAR 2017) Provided in Appendix 2 for information.  No comments. |

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| --- | --- |
| Report | Modification M002 of analytical method 01387 for the determination of various pesticides in drinking and surface water by HPLC-MS/MS, Krebber, R. & Sandau, C., 2015  Report No MR-15/025  Document No. M-526061-01-1  Authority registration No |
| Guideline(s): | Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC  EC Guidance Document on Residue Analytical Methods, SANCO/825/00 rev. 8.1 of November 16, 2010  European Commission Guidance Document for Generating and Reporting Methods of Analysis in Support of Pre-Registration data Requirements for Annex II (part A, Section 4) and Annex III (part A, section 5) of directive 91/414, SANCO/3029/99 rev. 4, July 11, 2000 |
| Deviations: | not specified |
| GLP: | Yes |
| Acceptability: | Yes | |

Materials and methods

Samples were injected directly into the HPLC-MS/MS instrument without further clean-up. Propamocarb was detected by using the positive ion mode and two Multiple Reaction Monitoring (MRM) transitions.

**Table A 128: Characteristics of the surface water from river Rhine, sampled on 2015-01-13 in Leverkusen-Hitdorf (Germany)**

|  |  |
| --- | --- |
| Parameter | Value |
| Total organic carbon (TOC) | 3 mg/L |
| Dissolved organic carbon (DOC) | 3 mg/L |
| Conductivity | 453 μS/cm |
| pH | 7.8 |
| Water hardness | 9.3 dH |
| Filtered solids | 33 mg/L |
| Dry sieve after filtration | 290 mg/L |

**Table A 129: MRM transitions (quantitation and confirmation) used for Propamocarb**

|  |  |  |
| --- | --- | --- |
|  | **Mass transition used for  quantification (1st MRM)** | **Mass transition used for  confirmation (2nd MRM)** |
| Propamocarb | 189 → 102 m/z | 189 → 144 m/z |

**Results and discussions**

**Table A 130: Method validation for Propamocarb hydrochloride for the confirmation ion (m/z = 189 → 144)**

| **Analyte** | **Matrix** | **Fortification level (µg/L) (*n* = x)** | **Mean Peak Area** | **RSD (%)** | **Comments** |
| --- | --- | --- | --- | --- | --- |
| Propamocarb | Surface Water | 0.05 (n=10) | 328837 | 2.5 | MR-15/025 |
| 0.5 (n=10) | 3495728 | 1.4 |

**Table A 131: Method validation for Propamocarb hydrochloride for the quantification ion (m/z = 189 → 102)**

| **Analyte** | **Matrix** | **Fortification level (µg/L) (*n* = x)** | **Mean Peak Area** | **RSD (%)** | **Comments** |
| --- | --- | --- | --- | --- | --- |
| Propamocarb | Surface Water | 0.05 (n=10) | 1021148 | 1.5 | MR-15/025 |
| 0.5 (n=10) | 104003842 | 1.3 |

**Table A 132: Characteristics for the analytical method used for validation of propamocarb**

|  | **Propamocarb** |
| --- | --- |
| Specificity | The specificity of the method resulted from HPLC separation in combination with the very selective MS/MS detection. Apparent concentrations in control samples were below 0.3 × LOQ. Two MRM transitions were monitored (m/z 189 → m/z 102 for quantification and m/z 109 → m/z 144 for confirmation). Therefore, the HPLC-MS/MS method is highly specific and an additional confirmatory method is not necessary. |
| Calibration (type, number of data points) | The correlation between the injected amount of substance and the detector response was linear (n=11). The correlation coefficients for standard solutions in surface water were ≥0.9990 for the two MRM transitions. |
| Calibration range | 0.015 μg/L to at least 1 μg/L |
| Assessment of matrix effects is presented | Matrix matched standards were used. |
| Limit of quantification | The limit of quantification (LOQ) in surface water was 0.05 μg/L. |
| Stability | The analyte was stable in surface water when stored in a freezer at ≤ -18 °C for a period of 7 days. |

Conclusion

The method is regarded to be straightforward since the water samples were directly measured by LC-MS/MS without further concentration or purification steps. The quantification by two distinct MS/MS transitions ensured the specificity of the method successfully validated at a limit of quantification (LOQ) of 0.05 μg/L. While being the less complex matrix than surface water, the method is applicable for the determination of Propamocarb residues in drinking water.

The method has been demonstrated to be suitable as enforcement method for monitoring of Propamocarb residues in drinking and surface water.

* + - * 1. Independent laboratory validation

Analytical method 6

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| --- | --- |
| Comments of zRMS: | EU agreed (RAR 2017) Provided in Appendix 2 for information.  No comments |

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| --- | --- |
| Report | Independent laboratory validation of the BCS analytical method 01387/M002 for the determination of various pesticides in surface water by HPLC-MS/MS  Thies, S., 2015  Report No 2015/0034/01  Document No. M-536990-01-1  Authority registration No |
| Guideline(s): | Regulation (EC) No 1107/2009; SANCO/3029/99; SANCO/825/00 rev. 8.1; OECD Guidance Document ENV/JM/Mono (2007) |
| Deviations: | not specified |
| GLP: | Yes |
| Acceptability: | Yes | |

Materials and methods

The independent validation of BCS Method 01387 in its modification M002 was performed for eight compounds in total including the active substance Propamocarb at an LOQ of 0.05 μg/L in surface water. The method was developed for the determination of residues by direct injection into the HPLC-MS/MS instrument. For Propamocarb the positive ion mode was used without further clean-up. An aliquot of the sample solution was injected into the high performance liquid chromatograph and subject to reversed phase chromatography (HPLC) coupled with tandem mass spectrometry (MS/MS) with electrospray ionisation. The MS/MS instrument was operated in the Multiple Reaction Monitoring mode (MRM).  
Concentrations were quantified using external matrix-matched standard solutions.

**Table A 133: Characteristics of the surface water from River Rhine, sampled on 2015-08-11 in Leverkusen (Germany)**

|  |  |
| --- | --- |
| Parameter | Value |
| Total organic carbon (TOC) | 2 mg/L |
| Dissolved organic carbon (DOC) | <2 mg/L |
| Conductivity | 550 μS/cm |
| pH | 7.2 |
| Water hardness | 11.1 dH |
| Filtered solids | <5 mg/L |
| Dry sieve after filtration | 690 mg/L |

**Table A 134: MRM transitions (quantitation and confirmation) used for Propamocarb**

|  |  |  |
| --- | --- | --- |
|  | **Mass transition used for  quantification (1st MRM)** | **Mass transition used for  confirmation (2nd MRM)** |
| Propamocarb | 189 → 102 m/z | 189 → 144 m/z |

Results and discussions

**Table A 135: Method validation for Propamocarb hydrochloride in water for the quantitation ion (m/z = 189 → 102)**

| **Analyte** | **Matrix** | **Fortification level (µg/L) (*n* = x)** | **Mean Peak Area** | **RSD (%)** | **Comments** |
| --- | --- | --- | --- | --- | --- |
| Propamocarb | Surface Water | 0.05 (n=5) | 385200 | 4.1 | 2015/0034/01 |
| 0.5 (n=5) | 3462000 | 2.1 |

**Table A 136: Method validation for Propamocarb hydrochloride in water for the confirmation ion (m/z = 189 → 144)**

| **Analyte** | **Matrix** | **Fortification level (µg/L) (*n* = x)** | **Mean Peak Area** | **RSD (%)** | **Comments** |
| --- | --- | --- | --- | --- | --- |
| Propamocarb | Surface Water | 0.05 (n=10) | 165600 | 2.4 | 2015/0034/01 |
| 0.5 (n=10) | 1514000 | 2.5 |

**Table A 137: Characteristics for the analytical method used for validation of propamocarb**

|  | **Propamocarb** |
| --- | --- |
| Specificity | Apparent concentrations in control samples were below 0.3 × LOQ. Two MRM transitions were monitored. HPLC-MS/MS using two characteristic MS/MS transitions for detection and quantitation ensures a high level of specificity. |
| Calibration (type, number of data points) | The correlation between the injected amount of substance and the detector response was linear (1/x weighted) for standard solutions in surface water (n=8) for Propamocarb hydrochloride. The correlation coefficients were ˃0.99 for all MRM transitions. |
| Calibration range | 0.015 μg/L to at least 1 μg/L |
| Assessment of matrix effects is presented | Matrix matched standards were used. |
| Limit of quantification | The limit of quantification (LOQ) in surface was 0.05 μg/L for propamocarb. The limit of detection (LOD) in surface water is 0.015 μg/L. |

**Conclusion**

The method meets all guideline criteria to determine concentrations in surface water of Propamocarb at 0.05 μg/L. The method is regarded to be straightforward since the water samples are directly measured by LC-MS/MS without further concentration or purification steps. The quantification by two distinct MS/MS transitions ensured the specificity of the method that was successfully validated at a limit of quantification (LOQ) of 0.05 μg/L. While being the less complex matrix than surface water, the objective of the study is to confirm that the method was also applicable for the determination of Propamocarb residues in drinking water.

The study demonstrated the validity of the enforcement method for monitoring of Propamocarb residues in drinking and surface water when being performed by an independent analytical laboratory

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

Analytical method 7

|  |  |
| --- | --- |
| Comments of zRMS: | EU agreed (RAR 2017) Provided in Appendix 2 for information.  No commens. |

|  |  |
| --- | --- |
| Report | Propamocarb: Analytical method for the determination of propamocarb in air  Class, T., 2004  Report No P 755 G; C042611  Document No. M-232969-01-1  Authority registration No |
| Guideline(s): | EU Directive 91/414/EEC Annex n (Part A, Section 4.2), as amended by Commission Directive 96/46/EC, EC Guidance document on residue analytical methods, SANCO/825/00 rev. 6 20/06/00 |
| Deviations: | not specified |
| GLP: | Yes |
| Acceptability: | Yes | |

Materials and methods

Samples of air were drawn through silica gel adsorption tubes at a flow rate of ~0.3 L/min. for a period of 6 hr. (total air sampling volume = 0.1 m3). The silica gel was extracted three times with a mixture of acetonitrile/water/acetic acid/ammonia (200:800:10:2, v/v/v/v). The total combined extract was analysed using LC/MS/MS with atmospheric pressure chemical ionisation (APCI) source. Quantification was based on MS of the daughter ion peak 144 m/z, resulting from the protonated molecular propamocarb ion observed at 189 m/z. For further confirmation a second transition resulting in a daughter ion at 102 m/z was included in the method.

Extraction efficiency was determined by fortifying the analyte (duplicates at 1 μg and 10 μg) onto adsorbent portions of sampling cartridges. Subsequently the analyte was extracted as described. Storage stability of adsorbed propamocarb was examined by fortifying the analyte with 10 μg, onto adsorbent portions of several sampling cartridges. The cartridges were sealed and stored for 1 day and 8 day periods at ambient temperature and analysed as above.

Results and discussions

**Table A 138: Recovery results from method validation of propamocarb in air**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Conditions** | | **Spike amount (μg)** | **Mean concentration in air (μg/m3)** | **Mean  recovery (%)** | **RSD (%)** | **n** |
| Extraction efficiency | | 1.0 | --- | 81 | 14 | 4 |
| 10.0 | --- |
| Storage stability | Ambient temp. 1 day | 10.0 | --- | 83 | 3 | 4 |
| Ambient temp. 8 day | 10.0 | --- |
| Sampling of Ambient Air (23ºC, 33% relative humidity approx.) | | 1.0 | 9.2 | 76 | 8 | 5 |
| 10.0 | 90 | 89 | 5 | 5 |
| Sampling of Warm Air (35ºC, 100% relative humidity) | | 1.0 | 9.1 | 74 | 9 | 4 |
| 10.0 | 92 | 73 | 5 | 5 |

**Table A 139: Characteristics for the analytical method used for validation of propamocarb**

|  | **Propamocarb** |
| --- | --- |
| Specificity | No signal (<0.3 μg/m3) was recorded at the retention time of propamocarb. The method used is considered to be specific for the analyte. |
| Calibration (type, number of data points) | Linear, (7 point), r2= 0.9997 |
| Calibration range | 20-2000 ng/mL |
| Limit of quantification | 9 μg/m3 |

**Conclusion**

The method is suitable for detection of residues in air to an LOQ of 9 μg/m3

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

No methods required as Propamocarb is neither toxic (T) or very toxic (T+) or classified according to GHS as acute toxic (Cat1-3), CMR (Cat.1) or STOT (Cat.1). However, analytical methods are provided for the determination of Propamocarb in animal tissue. Please refer to Section „Description of analytical methods for the determination of residues in animal matrices (KCP 5.2)“ above.

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

No new or additional studies have been submitted