REGISTRATION REPORT

Part B

Section 5

Analytical Methods

Detailed summary of the risk assessment

Product code: ADM.00900.I.1.C

Product name: COSAYR

Chemical active substance:

Chlorantraniliprole, 200 g/L SC

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(New authorization)

Applicant: Adama country organisation / representative as specified in Part A

Submission date: October 2022, updated June 2023

MS Finalisation date: August 2023 (initial Core Assessment)
November 2023 (final Core Assessment)

Version history

When	What
October 2022	Part B - Section 5 - Core Assessment – Central Zone, Initial version
June 2023	Updated following requests by zRMS
August 2023	Initial zRMS assessment The report in the dRR format has been prepared by the Applicant, therefore all comments, additional evaluations and conclusions of the zRMS are presented in grey commenting boxes. Minor changes are introduced directly in the text and highlighted in grey. Not agreed or not relevant information are ~~struck through~~ and shaded for transparency.
November 2023	Final report (Core Assessment updated following the commenting period) Additional information/assessments included by the zRMS in the report in response to comments received from the cMS and the Applicant are highlighted in yellow. Not agreed or not relevant information are ~~struck through~~ and shaded for transparency.

DATA PROTECTION CLAIM

Under Article 59, Regulation 1107/2009/EC, on behalf of the Sponsor Company the applicant claims data protection for these studies conducted with ADM.00900.I.1.C (former code ADM.0900.I.1.C). The data protection status and corresponding justification as valid for the respective country will be confirmed in the respective PART A.

STATEMENT FOR OWNERSHIP

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Table of Contents

5 Analytical methods 5

5.1 Conclusion and summary of assessment 5
5.2 Methods used for the generation of pre-authorization data (KCP 5.1) 8
5.2.1 Analysis of the plant protection product (KCP 5.1.1) 8
5.2.1.1 Determination of active substance and/or variant in the plant protection product (KCP 5.1.1) 8
5.2.1.2 Description of analytical methods for the determination of relevant impurities (KCP 5.1.1) 13
5.2.1.3 Description of analytical methods for the determination of formulants (KCP 5.1.1) 18
5.2.1.4 Applicability of existing CIPAC methods (KCP 5.1.1) 18
5.2.2 Methods for the determination of residues (KCP 5.1.2) 18
5.3 Methods for post-authorization control and monitoring purposes (KCP 5.2) 19
5.3.1 Analysis of the plant protection product (KCP 5.2) 19
5.3.2 Description of analytical methods for the determination of residues of Chlorantraniliprole (KCP 5.2) 19
5.3.2.1 Overview of residue definitions and levels for which compliance is required 20
5.3.2.2 Description of analytical methods for the determination of residues in plant matrices (KCP 5.2) 20
5.3.2.3 Description of analytical methods for the determination of residues in animal matrices (KCP 5.2) 21
5.3.2.4 Description of methods for the analysis of soil (KCP 5.2) 22
5.3.2.5 Description of methods for the analysis of water (KCP 5.2) 23
5.3.2.6 Description of methods for the analysis of air (KCP 5.2) 23
5.3.2.7 Description of methods for the analysis of body fluids and tissues (KCP 5.2) 23
5.3.2.8 Other studies/ information 24

Appendix 1 Lists of data considered in support of the evaluation 25

Appendix 2 Detailed evaluation of submitted analytical methods 28

A 2.1 Analytical methods for Chlorantraniliprole 28
A 2.1.1 Methods used for the generation of pre-authorization data (KCP 5.1) 28
A 2.1.2 Methods for post-authorization control and monitoring purposes (KCP 5.2) 57
Appendix 3: Supplemental Information 67

5 Analytical methods

This document summarizes the analytical methods on the plant protection product ADM.00900.I.1.C (SC formulation containing 200 g/L of Chlorantraniliprole).

The dossier follows the data requirements of
- Regulation (EC) No. 1199/2013 for the active substance Chlorantraniliprole.

Deviations from this are justified where relevant.

5.1 Conclusion and summary of assessment

zRMS summary and conclusions:

The Letter of Access for chlorantraniliprole is provided separately to this submission (FMC Corporation, PPP of Coragen).

During the peer review, an analytical methods were evaluated and validated for the determination of chlorantraniliprole in plant matrices and in food of animal origin, in soil, air and water.

In the EFSA Journal 2013;11(6):3143 – "Peer review of the pesticide risk assessment of the active substance chlorantraniliprole" it is stated that *Appropriate LC-MS/MS methods are available for the post-registration monitoring of chlorantraniliprole in food of plant and animal origin with LOQs of 0.01 mg/kg.*
Validated analytical methods based on HPLC-MS/MS or GC-ECD exist for the determination of chlorantraniliprole in soil with LOQs of 0.5 µg/kg or 0.01 mg/kg respectively. Residues of chlorantraniliprole in ground water and surface water can be monitored by HPLC-MS/MS method with LOQ of 0.1 µg/L. Pending on the final residue definition for monitoring, additional information might be required. LC-MS/MS method is available for the determination of chlorantraniliprole in air with LOQ of 0.5 µg/m^3. A method for residues in body fluids and tissues is not required as the active substance is not classified as toxic or very toxic.

EFSA Scientific Report (2013):

Analytical methods for residues (Annex IIA, point 4.2)

Food/feed of plant origin (principle of method and LOQ for methods for monitoring purposes)	The DGF S 19 (L00.00-34) multi-residue procedure with LC/MS/MS detection is proposed for the analysis of chlorantraniliprole crop residues in regions which accept this multi residue method. The DFG S 19 procedure extracts chlorantraniliprole from crops using water and acetone. The extracts are purified using gel permeation chromatography and residues are quantified using LC/MS/MS detection. The limit of quantitation for this method is 0.01 mg/kg.
Food/feed of animal origin (principle of method and LOQ for methods for monitoring purposes)	The DGF S 19 (L00.00-34) multi-residue procedure with LC/MS/MS detection is proposed for the analysis of chlorantraniliprole animal tissue residues in regions which accept this multi residue method. The DFG S 19 procedure extracts chlorantraniliprole from animal tissue using water and acetone. The extracts are purified using gel permeation chromatography and residues are quantified using LC/MS/MS detection. The limit of quantitation for this method is 0.01 mg/kg.
Soil (principle of method and LOQ)	HPLC-MS/MS: LOQ = 0.5 µg/kg (chlorantraniliprole) GC-ECD: LOQ = 0.01 mg/kg (chlorantraniliprole)
Water (principle of method and LOQ)	Chlorantraniliprole and potential degradation products (IN-F9N04, IN-GAZ70, IN-EQW78, IN-ECD73, and IN-F6L99) were extracted from water samples using a liquid/liquid partition and analyzed using a LC/MS/MS system. The analysis of a polar potential breakdown product (IN-F6L99) was completed using solid phase extraction followed by LC/MS/MS detection. The limit of quantitation for this method is 0.1 µg/L for all analytes.
Air (principle of method and LOQ)	The analytical method for air consisted of sampling by adsorption in cartridges filled with XAD-2.Chlorantraniliprole was extracted from the XAD-2 cartridges with acetone and the extracts were analyzed

	using LC-MS/MS. The limit of quantitation for this method is 0.5 µg/m^3.
Body fluids and tissues (principle of method and LOQ)	No methods of analysis for chlorantraniliprole for body fluids and tissues were submitted by the notifier on the basis that chlorantraniliprole is not classified as toxic or highly toxic.

According to the EFSA Journal 2020;18(9):6235 – "Review of the existing MRLs for chlorantraniliprole":
The multiresidue analytical method DFG S19 based on HPLC coupled to MS/MS detection was validated for the determination of chlorantraniliprole in high water (tomato), high acid (orange), high oil content (almond) and dry commodities (wheat grain) with an LOQ of 0.01 mg/kg. An independent laboratory validation (ILV) was also available. The studies were assessed in the framework of the peerreview (Ireland, 2010; EFSA, 2013a).
A single residue method (LC-MS/MS) provided in the DAR (Ireland, 2010) can be used for the enforcement of chlorantraniliprole in maize/corn stover, sorghum stover, rice and common millet straw, with LOQ 0.01 mg/kg, in view of the future need to set MRLs in feed items. An ILV on these matrices difficult to analyse was not conducted, and it is considered desirable.
During the completeness check, the EURLs provided validation results on QuEChERS multi-residue method using LC-MS/MS with an LOQ of 0.01 mg/kg in high water content, high acid content, high oil content and dry commodities for the enforcement of chlorantraniliprole in routine analysis (EURL, 2018). During the Member States consultation, EURLs provided additional information on the enforcement LOQ achieved in routine analysis for dry matrices. The new reported value is 0.005 mg/kg (EFSA, 2020b).

Plant residue definition for monitoring (RD-Mo)	Chlorantraniliprole
Plant residue definition for risk assessment (RD-RA)	Chlorantraniliprole
Methods of analysis for monitoring of residues (analytical technique, matrix groups, LOQs)	High water, high acid, high oil content commodities, dry commodities, hops and coffee beans (EFSA, 2013a; EFSA, 2018a): - Multiresidue Method DFG S19 (LC–MS/MS) - LOQ 0.01 mg/kg - Confirmation by monitoring 1 additional MRM transition - ILV (LC–MS/MS) available - No specific validation details for coffee beans (desirable) - QuEChERS (LC–MS/MS) for enforcement in routine analysis, LOQ 0.01 mg/kg for high water, high acid, and high oil content commodities; LOQ 0.005 mg/kg for dry commodities (EURL, 2018; EFSA, 2020b). Maize/corn stover, sorghum stover, rice and common millet straw (Ireland, 2010): - Single residue Method (LC–MS/MS) - LOQ 0.01 mg/kg - Confirmation by monitoring 1 additional MRM transition - ILV not available (desirable)

According to the SANTE/2020/12830:
- The extraction procedures used in the methods for risk assessment and post-approval control and monitoring purposes for the determination of residues in food/feed of plant and animal origin should be verified.
- Analytical methods for monitoring residues in body fluids and tissues are required for detection of active substances and/or metabolites in humans and animals after possible intoxications or for biomonitoring purposes, regardless of their toxicological classification.

Therefore, an analytical methods for the residues of chlorantraniliprole in body fluids and tissues are required.

The Applicant submitted information that a 2021 extraction efficiency study was performed to which the Applicant has access. Study FMC-51880 (submitted in the renewal dossier Document M-CA, Section 4, Annex Point 4.2/01) compares a number of methods, including the previously assessed monitoring method and the QuEChERS method used in the magnitude of residues studies in this submission, and demonstrates acceptable extraction efficiency in all standard crop matrix types. The above-mentioned study has been provided by the Applicant and evaluated in this registration report by zRMS-PL (see Appendix 2).

A body fluids method for the determination of residues of chlorantraniliprole in plasma and urine has been submitted by Applicant. The limit of quantification was established at 1.0 µg/L. This study has been evaluated and

accepted by zRMS-FR in the Registration Report for Chlorantraniliprole 200 g/L SC (April 2022).
The details of the evaluation of new and additional studies are referred in Appendix 2.
No additional data are required to support the intended uses for ADM.00900.I.1.C.

Sufficiently sensitive and selective analytical methods are available for the active substance Chlorantraniliprole and the relevant impurities in the plant protection product.
Noticed data gaps are:

- None.

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

- None.

Commodity/crop	Supported/Not supported
Head cabbage	Supported
Cauliflower	Supported
Broccoli	Supported
Wine grape	Supported
Table grape	Supported
Corn (grain and silage)	Supported
Apple	Supported
Pear	Supported
Quince	Supported
Potato	Supported

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

5.2.1 Analysis of the plant protection product (KCP 5.1.1)

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

An overview on the acceptable methods and possible data gaps for analysis of active substance, Chlorantraniliprole in ADM.00900.I.1.C is provided as follows:

Comments of zRMS:	The method is sufficiently described and validated according to SANCO/3030/99 rev. 5 (22 March 2019) and is suitable for the determination of active substance in a plant protection product.

Reference:	KCP 5.1.1/01, Tsesin, N. (2019a)
Report	Determination of storage stability and phys-chem properties of chlorantraniliprole 200 sc (ADM.0900.I.1.C) stored at for 14 days and at 0 ºC for 7 days (Submitted in KCP 2.1_01)
Report No.	000102562.054FL
Sponsor study No.	000102562
Guideline(s):	Commission Regulation (EU) No 284/2013 SANCO/3030/99 rev.5, 22 March 2019
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Reference:	KCP 5.1.1/02, Tsesin, N. (2019b)
Report	Quantification of active ingredient in formulation product Chlorantraniliprole 200SC (ADM.0900.I.1.C)
Report No.	000103659.0SOFL
Sponsor study No.	000103659
Guideline(s):	SANCO/3030/99 rev.5, 22 March 2019
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Executive summary

The analysis was done by high performance liquid chromatograph (HPLC) with diode array detection (DAD) using external standard technique. The HPLC method, used to quantify the active ingredients in ADM.0900.I.1.C was fully validated. Method validation included linearity, specificity and confirmation of analyte identification, precision and accuracy.

Materials and methods

Material: One representative sample of the plant protection product ADM.0900.I.1.C manufactured (Batch no.: **3188-20519-01**) was used for the study.

External standards:

- **Chlorantraniliprole** (CAS: 500009-45-7; batch No.: 581-046-00, purity: 97.9 %, supplier: Adama Makhteshim Standards Laboratory).

Analytical methods:

HPLC system	Agilent 1260 infinity II series equipped with an autosampler, column oven and degasser (LC-5)
Column	YMC-Triart c18, 150 x 4.6 mm., D. S – 3 µm, 12 nm, P/N: TA12S031546PTH, S/N: 124XA80131, Lot: 16168
Column temperature	35 ºC
Injection volume	5 µL
Flow rate	1.0 ml/min
Wavelength	254 nm
Retention time	Approx. 20.2 min

About 100 mg of the formulated product were weighted into a 50 mL volumetric flask. About 40 ml of Acetonitrile were added as a solvent and solutions were sonicated well for about 40 minutes. After the solutions reached the room temperature about 0.5 ml DMF was added, sonication for 5 min was done and Acetonitrile was added up to the mark. The solutions were mixed well, filtrated with 0.45 µm Nylon filter and measured by injection of a 5 µL aliquots of these solutions into the HPLC/DAD. The calibrating solutions were injected in the same sequence.

Results: The parameters linearity, precision, accuracy and specificity were checked. Typical calibration curves and chromatograms are presented in the report. Information concerning the validation of the method please refer to **Table 5.2.1.1-04** and the following text.

Conclusions: The method was validated according to guideline SANCO/3030/99 rev.5 with regard to specificity, linearity of detector response, accuracy and precision for Chlorantraniliprole in ADM.0900.I.1.C and is considered acceptable.

Validation - Results and discussions

Specificity

The specificity of the method was checked by comparing the chromatograms obtained from the analysis of Chlorantraniliprole in Chlorantraniliprole 200 SC formulation batch with the one of the blank samples.
It was found that the blanks chromatograms do not contain any interfering peak at the retention time corresponding to the active ingredient. As a result, it can be concluded that the analytical method is specific for the determination of Chlorantraniliprole in Chlorantraniliprole 200 SC formulation product. The identification of the active ingredient was done by HPLC-MS method.

Linearity

The linearity for active ingredients was tested in linear range covering at least ± 20% of analyte nominal concentration (2 mg/ml for formulation) studied.

<u>High linearity range</u>

Six different solutions containing various concentrations of Chlorantraniliprole (Batch: 581-046-00) standard were prepared separately. About 40 ml of acetonitrile were added as a solvent and solutions were sonicated well. 0.5 ml of DMF was added and additional sonication for 5 min was done. Acetonitrile was added up to the mark and solutions were mixed well. These solutions were injected into the HPLC under method analysis conditions. The detector response was found linear over the concentration range studied, from ~0.3 mg/ml (about 75% concentration level) to ~0.7 mg/ml (about 175% concentration level) for Chlorantraniliprole. A good fit of the points to the regression line was obtained. The linear correlation coefficient R is about 0.9999.

<u>Low linearity range</u>

To prepare stock solutions, six solutions containing various concentrations of Chlorantraniliprole (Batch: 581-046-00) standard were prepared. About 40 ml Acetonitrile were added as a solvent and solutions were

sonicated well. 0.5 ml DMF was added and additional sonication for 5 min was done. Acetonitrile was added up to the mark and solutions were mixed well.

Six different solutions containing various concentrations of Chlorantraniliprole (Batch: 581-046-00) standard were prepared by dilution of stock solutions that were prepared separately. Different volumes were transferred to l0 ml volumetric flasks and Acetonitrile was added up to the mark. These solutions were mixed well and injected into the HPLC. The detector response was found linear over the concentration range studied, from - 0.06 mg/ml (15% concentration level) to - 0.14 mg/ml (70% concentration level) for Chlorantraniliprole. A good fit of the points to the regression line was obtained and the linear correlation coefficient R was found to be about 0.9999. The resulting linearity curves have correlation coefficients R^2 > 0.99 (as required by SANCO/3030/99 rev. 5, 22 March 2019) indicating that the active ingredient is linear in the range of interest.

Repeatability and intermediate repeatability
The precision of the method was evaluated by a repeatability assessment. Five samples solutions of the batch were prepared and analysed for the active ingredients content. Two repeatability assays were performed on different days. The relative standard deviation of the RF, obtained for active ingredient from 10 injections from two assays was taken as the indication of analytical method intermediate precision. The % RSD of the results was calculated to ensure it meets Horwitz criterion. The repetitive analysis of the formulation resulted in the following average contents. The obtained repeatability RSD values of 1.2% and 0.13% are less than the threshold value 1.72% (calculated by Horwitz equation) acceptable for ~19% analyte concentration according to SANCO 3030/99 rev.5 guidelines. Therefore, it can he concluded that the analytical method has a good analytical method. The obtained repeatability RSD value of 1.00 is less than the threshold value ~ 2.57 (calculated by Horwitz equation). Therefore, it can be concluded that the analytical method has a good analytical method intermediate precision.

System repeatability (precision)
In order to determine the active ingredient system repeatability, one sample of Chlorantraniliprole 200 SC formulation product (Batch No: 3188-220519-01) was prepared by weighting 103.6 mg of formulated product into 50 ml volumetric flask. The solutions were mixed well, filtrated with 0.45 µm Nylon filter and measured by injection of a 5µl aliquots of these solutions into the HPLC/DAD. The relative standard deviation of the response factor obtained for these injections was considered as an indication for system repeatability. The RSD value of 0.22 is less than the threshold values ~ 1.72% for Chlorantraniliprole acceptable for ~ 19% analyte concentration according to SANCO 3030/99 rev.5 guideline. Therefore, it can be concluded that the analytical method has good system repeatability (precision).

For the intermediate precision, the obtained repeatability RSD value of 1.00 is less than the threshold value ≤ ~~2.57~~ 1.72 (calculated by Horwitz equation) acceptable for ~ 19% analyte concentration.

Recovery (accuracy)
To three sets of two Matrix blank samples containing appropriate amount of material each, Chlorantraniliprole standard (ID: 581-046-00) was added at maximal, medium and minimal concentration levels in final solutions in 50 ml final volume. A blank, containing about 80 mg matrix blank without standard addition was prepared and used to obtain an indication of the contribution of the AI content in the sample to overall peak area. Prepared samples were assayed for Chlorantraniliprole content, under conditions of analysis using external standard solutions. Mean recoveries for a.i. were calculated and used as an indication of the method accuracy. According to SANCO 3030/99 rev 5. guideline the acceptance criterion for mean recoveries in accuracy study at >10% concentration levels of a.i. in sample is 97 - 103%. Statistical evaluation tests were performed and mean standard deviation and relative standard deviation were calculated. The accuracy results for a.i. in Chlorantraniliprole 200 SC formulation product at all concentration levels met the SANCO 3030/99 rev 5. acceptance criteria.

Table 5.2.1.1-01: Accuracy (recovery) of Chlorantraniliprole in formulated product ADM.0900.I.1.C at maximum concentration level

Concentration	C_A (g/kg)	C_F (g/kg)	Recovery [%]
Maximum concentration level (125 %)	330.3	336.1	101.8
	330.3	336.9	102.0
	315.8	319.6	101.2
	315.8	319.9	101.3
Mean recovery (%)			102
SD			0.37
% RSD [(SD / mean) * 100]			0.36

Table 5.2.1.1-02: Accuracy (recovery) of Chlorantraniliprole in formulated product ADM.0900.I.1.C at medium concentration level

Concentration	C_A (g/kg)	C_F (g/kg)	Recovery [%]
Medium concentration level (~~100~~ 80 %)	251.2	336.1	101.1
	251.2	336.9	101.0
	246.3	249.8	101.4
	246.3	250.1	101.5
Mean recovery (%)			101
SD			0.28
% RSD [(SD / mean) * 100]			0.28

Table 5.2.1.1-03: Accuracy (recovery) of Chlorantraniliprole in formulated product ADM.0900.I.1.C at minimum concentration level

Concentration	C_A (g/kg)	C_F (g/kg)	Recovery [%]
Minimum concentration level (~~100~~ 80 %)	170.4	172.1	101.0
	170.4	172.3	101.2
	173.2	176.2	101.7
	173.2	176.1	101.6
Mean recovery (%)			101
SD			0.33
% RSD [(SD / mean) * 100]			0.32

Table 5.2.1.1-04: Methods suitable for the determination of Chlorantraniliprole in plant protection product ADM.0900.I.1.C

	Chlorantraniliprole
Author(s), year	Tsesin, N. (2019)
Principle of method	HPLC-UV DAD
Linearity **(linear between mg/L / %** **range of the declared content)** **(correlation coefficient)**	High range y = 7461.9409 x + 85.54 R = 0.9999 (range: 0.3 mg/mL to 0.7 mg/mL, Based on a product sample concentration of 2 mg/mL, this corresponds to 150 g/kg to 350 g/kg) Low range y = 7759.3064 x – 1.8134 R = 0.9999 (range: 0.06 mg/mL to 0.14 mg/mL, Based on a product sample concentration of 2 mg/mL, this corresponds to 30 g/kg to 70 g/kg)
System Repeatability **n = 5** **(%RSD)**	RSD = 0.22 %
Intermediate precision **n = 10** **(% RSD)**	RSD = 1.00 % < RSD_r (calculated by Horwitz equation) = ~~2.57~~ 1.72 % Horwitz ratio (Horrat value) = ~~0.39~~ 0.58
Accuracy	125 % / 100% / 80% Mean recovery / RSD / Mean recovery / RSD / Mean recovery / RSD 102 / 0.36 / 101 / 0.28 / 101 / 0.32
Interference/ Specificity	Specific method, no interference

Conclusion

The analytical method was validated according to guideline SANCO/3030/99 rev.5 with regard to specificity, linearity, precision and accuracy for Chlorantraniliprole active substance in product ADM.0900.I.1.C and it is considered acceptable.

Tsesin, N. (2019b)

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

The relevant impurities in Chlorantraniprole reference source are: acetonitrile up to 3 g/kg, 3-picoline up to 3 g/kg and Methanesulfonic acid up to 2 g/kg (Commission Implementing Regulation (EU) 1199/2013 of 25 November 2013).

Comments of zRMS:	The method is sufficiently described and validated according to SANCO/3030/99 rev. 5 (22 March 2019) and is suitable for the determination of relevant impurities in a plant protection product.

Reference:	KCP 5.1.1/03, Rutyna, A. (2021)
Report	Methods validation and 1 batch analysis of Chlorantraniliprole 200 SC formulation
Report No.	K479/JS
Guideline(s):	SANCO/3030/99 rev.5. Brazilian Standard ABNT NBR 14029 3rd Ed. 09/12/2016 Australian Pesticides & Veterinary Medicines Authority 27/06/2018– Agrochemicals
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Impurity 1: 3-Picoline

Principle of the method

The goal of this study was to validate an analytical method for the determination of impurity 1 (3-Picoline) in the formulated product by using GC-FID (and MS for identity confirmation) according to SANCO/3030/99 rev. 5

Materials and methods

Material: One representative sample of the plant protection product Chlorantraniliprole 200 SC (Batch no.: **647421244**) was used for the study.

External standards:
- **Impurity 1 (3-Picoline)** (batch No.: MKCJ9205, purity: 99,7 %, supplier: Sigma).

Analytical methods: **Impurity 1**

The content of the Impurity 1 was determined by GC-FID.

Results: The parameters linearity, precision, accuracy and specificity were checked. Typical calibration curves and chromatograms are presented in the report. Information concerning the validation of the method please refer to **Table 5.2.1.2-1** and the following text.

Conclusions: The method was validated according to guideline SANCO/3030/99 rev.5 with regard to specificity, linearity of detector response, accuracy and precision for Impurity 1 in Chlorantraniliprole 200 SC and is considered acceptable.

Validation - Results and discussions

Specificity

This procedure checks for interferences that may have occurred from other species that might mask the result of the expected analyte.

In the Specificity chromatograms, the Impurity 1 reference standard has a retention time of 5.67 minutes. Other significant peaks were accounted for by assaying the diluent, the Formulation Blank and reference standards for impurities and the test item.

There were no significant peaks present in these chromatograms at the same retention time as Impurity 1. This demonstrates that there were no analyte interferences and the method is specific to Impurity 1.

Linearity
Six standard concentrations were prepared and injected once. The detector response was shown to be linear ranging from 0.0015 mg/mL to 0.012 mg/L (0.01-0.08%w/w).

$y = 2351.1 x + 0.4236$; $R^2 = 0.9967$

Precision (repeatability)
Precision was established by analyzing six (6) replicates of Chlorantraniliprole 200 SC test item batch number 647421244 (Selvita no. 1673/20/P) spiked with 3-Picoline standard solution at 0.06% (w/w). Analysis were performed by different analyst in a different day. Relative standard deviation (% RSD) of 3-Picoline content in Chlorantraniliprole 200SC test item spiked with solution at 0.06% w/w level calculated from six (n=6) test item solutions was 3.4 %. The result was lower than RSD_r calculated by modified Horowitz equation (4.1 %). Calculated Horrat value was below 1 (Hr = 0.4).

Recovery precision (accuracy)
Accuracy was established at three (3) levels (LOQ, 0.06% and about 0.08% of nominal concentration) by preparation 2 independent Matrix Blank item spiked with Chlorantraniliprole and 3-Picoline standard on each level. Results for mean recovery are between 75-125% for 3-Picoline. Acceptance criteria were met. The method is accurate.

Validation - Results and discussions

Table 5.2.1.2-1: Methods suitable for the determination of Impurity 1 in plant protection product Chlorantraniliprole 200 SC

	Impurity 1
Author(s), year	Rutyna, A. (2021)
Principle of method	GC-FID
Linearity (linear between mg/L / % range of the declared content) (correlation coefficient, expressed as r)	Linear between 0.0015 mg/mL and 0.012 mg/L (0.01-0.08%w/w) $y = 2351.1 x + 0.4236$; $R^2 = 0.9967$
Precision (repeatability) n = 6 (%RSD)	RSD (0.06% w/w level) = 3.4 % RSDr = 4.1 % Horrat value < 1
Accuracy (% Recovery) (%RSD)	Recovery precision at 0.01% (LOQ level) 114 % (n = 6) Recovery precision at 0.06% 108 % (n = 6) Recovery precision at 0.08% 109 % (n = 6)
Interference/ Specificity	Specific method. There are no interfering peaks
Selectivity	The MS Spectra obtained for Impurity 1 confirm the species identification
Limit of Quantification (LOQ)	LOQ = 0.0015 mg/mL = 0.001 g/kg (0.01% w/w).
Limit of Detection (LOD)	LOD calculated = 0.00005 g/kg (0.0005 % w/w)

Conclusion
The GC-FID method, used to quantify impurity 1 in Chlorantraniliprole 200 SC was fully validated in accordance to SANCO/3030/99 rev. 5.

Impurity 2: Acetonitrile

Principle of the method

The goal of this study was to validate an analytical method for the determination of impurity 2 (Acetonitrile) in the formulated product by using HS-GC-FID according to SANCO/3030/99 rev. 5

Materials and methods

Material: One representative sample of the plant protection product Chlorantraniliprole 200 SC (Batch no.: **647421244**) was used for the study.

External standards:
- **Impurity 2 (Acetonitrile)** (batch No.: I1122630, purity: 99,9 %, supplier: Merck).

Analytical methods:

Impurity 2
The content of the Impurity 2 was determined by HS-GC-FID.

Results: The parameters linearity, precision, accuracy and specificity were checked. Typical calibration curves and chromatograms are presented in the report. Information concerning the validation of the method please refer to **Table 5.2.1.2-2** and the following text.

Conclusions: The method was validated according to guideline SANCO/3030/99 rev.5 with regard to specificity, linearity of detector response, accuracy and precision for Impurity 2 in Chlorantraniliprole 200 SC and is considered acceptable.

Validation - Results and discussions

Specificity

This procedure checks for interferences that may have occurred from other species that might mask the result of the expected analyte.

In the Specificity chromatograms, the Impurity 2 reference standard has a retention time of 3.95 minutes. Other significant peaks were accounted for by assaying the diluent, the Formulation Blank and reference standards for impurities and the test item.

There were no significant peaks present in these chromatograms at the same retention time as Impurity 2. This demonstrates that there were no analyte interferences and the method is specific to Impurity 2.

Linearity

Six standard concentrations were prepared and injected once. The detector response was shown to be linear ranging from 0.01 mg/mL to 0.08 mg/mL (0.01 – 0.08%w/w).

$y = 16{,}713\ x + 0{,}0289$; $R^2 = 0.991$

Precision (repeatability)

Precision was established by analyzing six (6) replicates of Chlorantraniliprole 200 SC test item batch number 647421244 (Selvita no. 1673/20/P) spiked with Acetonitrile standard solution at 0.06% (w/w) Analysis were performed by different analyst in a different day. Relative standard deviation (% RSD) of Acetonitrile content in Chlorantraniliprole 200SC test item spiked with solution at 0.06% w/w level calculated from six (n=6) test item solutions was 3.9 %. The result was lower than RSD_r calculated by modified Horowitz equation (4.2 %). Calculated Horrat value was below 1 (Hr = 0.9).

Recovery precision (accuracy)

Accuracy was established at three (3) levels (LOQ, 0.06% and about 0.08% of nominal concentration) by preparation 2 independent Matrix Blank item spiked with Chlorantraniliprole and Acetonitrile standard on

each level. Results for mean recovery are between 75-125% for Acetonitrile. Acceptance criteria were met. The method is accurate.

Validation - Results and discussions

Table 5.2.1.2-2: Methods suitable for the determination of Impurity 2 in plant protection product Chlorantraniliprole 200 SC

	Impurity 2
Author(s), year	Rutyna, A. (2021)
Principle of method	HS-GC-FID
Linearity (linear between mg/L / % range of the declared content) (correlation coefficient, expressed as r)	Linear between 0.01 and 0.08 mg/mL (0.01 % w/w to 0.08 %w/w). y = 16,713x + 0,0289; R^2 = 0.991
Precision (repeatability) n = 6 (%RSD)	RSD (0.05% w/w level) = 3.9 % RSD_r = 4.2 % Horrat value < 1
Accuracy (% Recovery) (%RSD)	Recovery precision at 0.01% (LOQ level) 111 % (n = 6) Recovery precision at 0.06% 113 % (n = 6) Recovery precision at 0.08% 113 % (n = 6)
Interference/ Specificity	Specific method. There are no interferfing peaks
Selectivity	The MS Spectra obtained for Impurity 2 confirm the species indentification
Limit of Quantification (LOQ)	LOQ = 0.01 mg/mL = 0.001 g/kg (0.01%w/w).
Limit of Detection (LOD)	$LOD_{calculated}$ = 0.0002 % (w/w)

Conclusion
The HS-GC-FID method, used to quantify impurity 2 in Chlorantraniliprole 200 SC was fully validated in accordance to SANCO/3030/99 rev.5.

Impurity 3: Methanesulfonic acid

Principle of the method
The goal of this study was to validate an analytical method for the determination of impurity 3 (Methanesulfonic acid) in the formulated product by using GC-FID (and MS for identity confirmation) according to SANCO/3030/99 rev. 5

Materials and methods

Material: One representative sample of the plant protection product Chlorantraniliprole 200 SC (Batch no.: **647421244**) was used for the study.

External standards:
- **Impurity 3 (Methanesulfonic acid)** (batch No.: STBJ6677, purity: 99,75 %, supplier: Sigma).

Analytical methods: **Impurity 3**
The content of the Impurity 3 was determined by LC-HRMS.

Results: The parameters linearity, precision, accuracy and specificity were checked. Typical calibration curves and chromatograms are presented in the report. Information

concerning the validation of the method please refer to **Table 5.2.1.2-3** and the following text.

Conclusions: The method was validated according to guideline SANCO/3030/99 rev.5 with regard to specificity, linearity of detector response, accuracy and precision for Impurity 3 in Chlorantraniliprole 200 SC and is considered acceptable.

Validation - Results and discussions

Specificity

This procedure checks for interferences that may have occurred from other species that might mask the result of the expected analyte.

In the Specificity chromatograms, the Impurity 3 reference standard has a retention time of 6,1 minutes. Other significant peaks were accounted for by assaying the diluent, the Formulation Blank and reference standards for impurities and the test item.

There were no significant peaks present in these chromatograms at the same retention time as Impurity 3. This demonstrates that there were no analyte interferences and the method is specific to Impurity 3.

Linearity

Six standard concentrations were prepared and injected once. The detector response was shown to be linear ranging from 0.000124 to 0.001198 mg/mL (0.006 – 0.048 % w/w).

$y = 2707x + 0.0462$; $R^2 = 0.9982$

Precision (repeatability)

Precision was established by analyzing six (6) replicates of Chlorantraniliprole 200 SC test item batch number 647421244 (Selvita no. 1673/20/P) spiked with Methanesulfonic acid standard solution at 0.04 w/w)

Analysis were performed by different analyst in a different day. Relative standard deviation (% RSD) of Methanesulfonic acid content in Chlorantraniliprole 200SC test item spiked with solution at 0.04% w/w level calculated from six (n=6) test item solutions was 1.6 %. The result was lower than RSD_r calculated by modified Horowitz equation (4,3 %). Calculated Horrat value was below 1 (Hr = 0.4).

Recovery precision (accuracy)

Accuracy was established at three (3) levels (LOQ, 100% and about 120% of specification limit) by preparation 2 independent Matrix Blank item spiked with Chlorantraniliprole and Methanesulfonic acid standard on each level. Results for mean recovery are between 70-130% for Methanesulfonic acid. Acceptance criteria were met. The method is accurate.

Validation - Results and discussions

Table 5.2.1.2-3: Methods suitable for the determination of Impurity 3 in plant protection product Chlorantraniliprole 200 SC

	Impurity 3
Author(s), year	Rutyna, A. (2021)
Principle of method	LC-HRMS
Linearity (linear between mg/L / % range of the declared content) (correlation coefficient, expressed as r)	Linear between 0.000124 to 0.001198 mg/mL (0.006 – 0.048 % w/w) $y = 2707x + 0.0462$; $R^2 = 0.9982$

	Impurity 3
Precision (repeatability) **n = 6** **(%RSD)**	RSD (0.04% w/w level) = 1.6 % RSDr = 4.3 % Horrat value < 1
Accuracy **(% Recovery)** **(%RSD)**	Recovery precision at 0.05 % (w/w) 118 % (n = 6) Recovery precision at 0.04% (w/w) level 104 % (n = 6) Recovery precision at 0.048% (w/w) level 98 % (n = 6)
Interference/ Specificity	Specific method. There are no interfering peaks
Selectivity	The MS Spectra obtained for Impurity 3 confirm the species identification
Limit of Quantification (LOQ)	LOQ = 0.000125 mg/mL = 0.0005 g/kg (0.005% w/w)
Limit of Detection (LOD)	LOD calculated = 0.00003g/kg (0.0003 % w/w)

Conclusion
The GC-FID method, used to quantify impurity 3 in Chlorantraniliprole 200 SC was fully validated in accordance to SANCO/3030/99 rev.5.

Rutyna, A. (2021)

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

None of the formulants is of toxicological, environmental or ecotoxicological relevance within the formulation ADM.00900.I.1.C. Therefore, no analytical method is required.

5.2.1.4 Applicability of existing CIPAC methods (KCP 5.1.1)

For technical Chlorantraniliprole a CIPAC method is available:
CIPAC method 794

5.2.2 Methods for the determination of residues (KCP 5.1.2)

An overview on the acceptable methods and possible data gaps for analysis of residues of Chlorantraniliprole and for the generation of pre-authorization data is given in **Table 5.2.2-01**. For the detailed evaluation of new/additional studies it is referred to Appendix 2.

Table 5.2.2-01: Validated methods for the generation of pre-authorization data of Chlorantraniliprole

Component of residue definition: **Please refer to the respective matrix below for the residue definition**				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Bee products	Residues in Oilseed rape flowers, pollen, nectar, honey and sugar beet leaves	0.010 mg/kg	LC-MS/MS	Barbier, G. (2022) Report No: B20G-A4-C-02 KCP 5.1.2/01
	Confirmatory (no required)			
Daphnia and fish (Ecotoxicology)	Daphnia Iso medium: and fish ISO medium + 0.01% DMF:	<u>Daphnia:</u> Iso medium: LOQ: 8.9088 mg/L	LC-MS/MS	Fifi, A.P. (2020) Report No: BT281/20 KCP 5.1.2/02

Component of residue definition: Please refer to the respective matrix below for the residue definition				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
	Confirmatory (no required)	Fish: ISO medium + 0.01% DMF: LOQ: 0.3959 µg/L		
Algae and lemna (Ecotoxicology)	Algae and lemna	EPA medium LOQ = 0.0102 µg/L SIS medium LOQ = 0.0101 µg/L	LC-MS/MS	Fifi, A.P. (2020) Report No: BT207/19 KCP 5.1.2/03
	Confirmatory (no required)			
Bee diet (chronic and larval) (Ecotoxicology)	Water and sugar feeding solutions coming from honeybee's laboratory tests	Ultrapure water: LOQ = 3.7804 mg/L Sugar solution: LOQ = 7.5950 mg/kg	LC-MS/MS	Fifi, A.P. (2022) Report No: BT208/19 KCP 5.1.2/04
	Confirmatory (no required)			
Plant matrices	Peach, grape (bunches), wheat grain, oilseed rape seed and dry broad bean	LOQ = 0.010 mg /kg -	LC-MS/MS	Barbier, G. (2021) Report No: B20G-A4-C-01 KCP 5.1.2/05
	Confirmatory (no required)			
Water solutions	Coming from terrestrial plants laboratory tests	LOQ = 9.5 g/L	LC-MS/MS	Fifi, A.P. (2020c) Report No: BT209/19 KCP 5.1.2/06
	Confirmatory (no required)			
Feed, body fluids,... (Toxicology)	Primary	-	-	Not required
	Confirmatory (if required)	-	-	Not required
Body fluids, air,.... (Exposure)	Primary	-	-	Not required
	Confirmatory (if required)	-	-	Not required

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

5.3.1 Analysis of the plant protection product (KCP 5.2)

Analytical methods for the determination of the active substance and relevant impurities in the plant protection product shall be submitted, unless the applicant shows that these methods already submitted in accordance with the requirements set out in point 5.2.1 can be applied (KCP 5.1.1).

5.3.2 Description of analytical methods for the determination of residues of Chlorantraniliprole (KCP 5.2)

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

The residue definition given in **Table 5.3.2.1-01** below is identical to the one proposed in the EFSA Peer review of the pesticide risk assessment - EFSA Journal 2013;11(6):3143- in the approval of Chlorantraniliprole. For any special comments or remarkable points concerning the analytical methods for the determination of residues, please refer to Appendix 2.

Table 5.3.2.1-01: 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	Chlorantraniliprole	LOQ = 0.01 mg/kg 0.01 mg/kg (MRL)	EFSA Journal 2013;11(6):3143 Reg. (EU) 2022/1343
Plant, high acid content		LOQ = 0.01 mg/kg 0.01 mg/kg (MRL)	EFSA Journal 2013;11(6):3143 Reg. (EU) 2022/1343
Plant, high protein/high starch content (dry commodities)		LOQ = 0.01 mg/kg 0.02 mg/kg (MRL)	EFSA Journal 2013;11(6):3143 Reg. (EU) 2022/1343
Plant, high oil content		LOQ = 0.01 mg/kg 0.01 mg/kg (MRL)	EFSA Journal 2013;11(6):3143 Reg. (EU) 2022/1343
Muscle	Chlorantraniliprole	LOQ = 0.01 mg/kg 0.02 mg/kg (MRL, poultry)	EFSA Journal 2013;11(6):3143 Reg. (EU) 2022/1343
Milk		LOQ = 0.01 mg/kg 0.05 mg/kg (MRL)	EFSA Journal 2013;11(6):3143 Reg. (EU) 2022/1343
Eggs		LOQ = 0.01 mg/kg 0.2 mg/kg (MRL)	EFSA Journal 2013;11(6):3143 Reg. (EU) 2022/1343
Fat		LOQ = 0.01 mg/kg 0.08 mg/kg (MRL, poultry)	EFSA Journal 2013;11(6):3143 Reg. (EU) 2022/1343
Liver, kidney		LOQ = 0.01 mg/kg 0.07 mg/kg (MRL, liver, poultry) 0.01 mg/kg (MRL, kidney, poultry)	EFSA Journal 2013;11(6):3143 Reg. (EU) 2022/1343
Honey		LOQ = 0.01 mg/kg 0.05 mg/kg (MRL)	EFSA Journal 2013;11(6):3143 Reg. (EU) 2022/1343
Soil	Chlorantraniliprole	LOQ = 0.5 µg/kg	EFSA Journal 2013;11(6):3143
Drinking water	Chlorantraniliprole.	LOQ = 0.1 µg/L	EFSA Journal 2013;11(6):3143 general limit for drinking water
Surface water	Chlorantraniliprole	LOQ = 0.1 µg/L	EFSA Journal 2013;11(6):3143 general limit for drinking water
Air	Chlorantraniliprole	LOQ = 0.5 µg/m^3	EFSA Journal 2013;11(6):3143
Tissue (meat or liver)	Chlorantraniliprole	Not required 0.01 mg/kg	EFSA Journal 2013;11(6):3143 General limit according to SANTE/2020/12830, Rev.1
Body fluids		Not required 0.01 mg/L	EFSA Journal 2013;11(6):3143 General limit according to SANTE/2020/12830, Rev.1

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

Analytical methods for the determination of residues in plant matrices were already evaluated during the

EU Review of Chlorantraniliprole (EFSA Journal 2013;11(6):3143). An overview on the acceptable methods and possible data gaps for analysis of Chlorantraniliprole in plant matrices is given in the following table.

Table 5.3.2.2-01: Validated methods for food and feed of plant origin

Component of residue definition: Chlorantraniliprole				
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	EFSA Journal 2013;11(6):3143
	ILV	0.01 mg/kg	LC-MS/MS	EFSA Journal 2013;11(6):3143
	Confirmatory (if required)	-	-	-
High acid content	Primary	0.01 mg/kg	LC-MS/MS	EFSA Journal 2013;11(6):3143
	ILV	0.01 mg/kg	LC-MS/MS	EFSA Journal 2013;11(6):3143
	Confirmatory (if required)	Not required	-	-
High oil content	Primary	0.01 mg/kg	LC-MS/MS	EFSA Journal 2013;11(6):3143
	ILV	0.01 mg/kg	LC-MS/MS	EFSA Journal 2013;11(6):3143
	Confirmatory (if required)	-	-	-
High protein/high starch content (dry)	Primary	0.01 mg/kg	LC-MS/MS	EFSA Journal 2013;11(6):3143
	ILV	0.01 mg/kg	LC-MS/MS	EFSA Journal 2013;11(6):3143
	Confirmatory (if required)	-	-	-

Table 5.3.2.2-02: Statement on extraction efficiency

	Method for products of plant origin
Required, available from:	A 2021 extraction efficiency study was performed to which the applicant has access. Study FMC-51880 (submitted in the renewal dossier Document M-CA, Section 4, Annex Point 4.2/01) compares a number of methods, including the previously assessed monitoring method and the QuEChERS method used in the magnitude of residues studies in this submission, and demonstrates acceptable extraction efficiency in all standard crop matrix types
Not required, because:	-

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

Analytical methods for the determination of residues in animal matrices were already evaluated during the EU Review of Chlorantraniliprole (EFSA Journal 2013;11(6):3143). An overview on the acceptable methods and possible data gaps for analysis of Chlorantraniliprole in animal matrices is given in the following table.

Table 5.3.2.3-01: Validated methods for food and feed of animal origin

Component of residue definition: Chlorantraniliprole				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Milk	Primary	0.01 mg/kg	LC-MS/MS	EFSA Journal 2013;11(6):3143

Component of residue definition: Chlorantraniliprole				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
	ILV	0.01 mg/kg	LC-MS/MS	EFSA Journal 2013;11(6):3143
	Confirmatory (if required)	-	-	Not required
Eggs	Primary	0.01 mg/kg	LC-MS/MS	EFSA Journal 2013;11(6):3143
	ILV	0.01 mg/kg	LC-MS/MS	EFSA Journal 2013;11(6):3143
	Confirmatory (if required)	-	-	Not required
Muscle	Primary	0.01 mg/kg	LC-MS/MS	EFSA Journal 2013;11(6):3143
	ILV			EFSA Journal 2013;11(6):3143
	Confirmatory (if required)	-	-	Not required
Fat	Primary	0.01 mg/kg	LC-MS/MS	EFSA Journal 2013;11(6):3143
	Confirmatory (if required)	-	-	Not required
Kidney, liver	Primary	0.01 mg/kg	LC-MS/MS	EFSA Journal 2013;11(6):3143
	ILV	0.01 mg/kg	LC-MS/MS	EFSA Journal 2013;11(6):3143
	Confirmatory (if required)	-	-	Not required
Honey	Primary	0.01 mg/kg	LC-MS/MS	KCP 5.1.2/01 Barbier, G. (2022)
	ILV	0.01 mg/kg	LC-MS/MS	KCP 5.2/01 Brown, D. (2022)
	Confirmatory	-	-	Not required

Table 5.3.2.3-02: Statement on extraction efficiency

	Method for products of animal origin
Required, available from:	-
Not required, because:	only existing studies are relied on and extraction efficiency for this method is justifed in the renewal dossier.

zRMS comments:
In the Registration Report for Chlorantraniliprole 200 g/L SC, the following information is presented by Applicant and accepted by zRMS-FR (April 2022):
These methods have been previously reviewed at EU level and considered acceptable. At the time of the original submission extraction efficiency data was not required for multi residue methods. Since then extraction efficiency data is required for multi residue method. However, the new requirement states that additional animal testing should not be conducted to satisfy this requirement. Since the DFG S 19 method uses a robust extraction that has been demonstrated to be effective for a wide range of compounds. Additional animal tests to satisfy this data point have not been conducted.
zRMS-FR: Extraction efficiency should be demonstrated after the renewal of the active substance.

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

Analytical method for the determination of residues in soil was already evaluated during the EU Review of EU Review of Chlorantraniliprole (EFSA Journal 2013;11(6):3143). An overview on the acceptable methods and possible data gaps for analysis of Chlorantraniliprole in soil is given in the following table.

Table 5.3.2.4-01: Validated methods for soil

Component of residue definition: Chlorantraniliprole, IN EQW78, IN ECD73, IN F6L99, IN GAZ70 and IN F9NO4			
Method type	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	0.5 µg/kg	HPLC-MS/MS	EFSA Journal 2013;11(6):3143
Primary	0.01 mg/kg	CD-ECD	EFSA Journal 2013;11(6):3143
Confirmatory	Not required	-	-

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

Analytical methods for the determination of residues in water matrices were already evaluated during the EU Review of Chlorantraniliprole (EFSA Journal 2013;11(6):3143). An overview on the acceptable methods and possible data gaps for analysis of Chlorantraniliprole in surface and drinking water is given in the following table.

Table 5.3.2.5-01: Validated methods for water

Component of residue definition: Chlorantraniliprole and the metabolites IN-GAZ70, IN-EQW78, IN-F9N04, IN-ECD73 and IN-F6L99				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Drinking water, groundwater, Surface water	Primary	0.1 µg/L	LC-MS/MS	EFSA Journal 2013;11(6):3143
	ILV	0.1 µg/L	LC-MS/MS	EFSA Journal 2013;11(6):3143
	Confirmatory	Not required	-	-

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

Analytical method for the determination of residues in air was already evaluated during the EU Review of Chlorantraniliprole (EFSA Journal 2013;11(6):3143). An overview on the acceptable methods and possible data gaps for analysis of Chlorantraniliprole in air is given in the following table.

Table 5.3.2.6-01: Validated methods for air

Component of residue definition: Chlorantraniliprole			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	0.5 µg/m^3	LC-MS/MS	EFSA Journal 2013;11(6):3143
Confirmatory	Not required	-	-

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

Analytical methods for the determination of residues in body fluids and tissues are not required (EFSA Journal 2013; 11 (6): 3143).

Table 5.3.2.7-01: Methods for body fluids and tissues (if appropriate)

Component of residue definition: Chlorantraniliprole			
Method type	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year/missing**
Primary	0.0010 mg/L	HPLC-MS/MS	Pentz, A.M., Cabusas, M.E.Y., 2017 (DuPont-49234)/New*
Confirmatory	0.0010 mg/L	HPLC-MS/MS	Pentz, A.M., Cabusas, M.E.Y., 2017 (DuPont-49234)/New*

*the study conducted to satisfy the current data requirements.

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

zRMS comments:
According to the SANTE/2020/12830 an analytical methods for the residues of chlorantraniliprole in body fluids and tissues are required.
A body fluids method for the determination of residues of chlorantraniliprole in plasma and urine with LOQ of 1.0 µg/L has been submitted by Applicant. This study has been evaluated and accepted by zRMS-FR in Registration Report for Chlorantraniliprole 200 g/L SC (April 2022).
No additional study is required.

5.3.2.8 Other studies/ information

~~Table 5.3.~~

No further studies are required.

Appendix 1 Lists of data considered in support of the evaluation

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Vertebrate study Y/N**	**Owner***
KCP 5.1.1/01	Tsesin, N.	2019a	DETERMINATION OF STORAGE STABILITY AND PHYS-CHEM PROPERTIES OF CHLORANTRANILIPROLE 200 SC (ADM.0900.I.1.C) STORED AT 54 ºC FOR 14 DAYS AND AT 0 ºC FOR 7 DAYS Adama Makhteshim Ltd., Israel Report No.:000102562.054FL Sponsor No.: 000102562 GLP: yes Published: no **Submitted in KCP 2.1/01**	N	ADM

KCP 5.1.1/02	Tsesin, N.	2019b	Quantification of active ingredient in formulation product Chlorantraniliprole 200SC (ADM.0900.I.1.C) Report No.: 000103659.0SOFL Sponsor No.: 000103659 GLP: yes Published: no	N	ADM
KCP 5.1.1/03	Rutyna, A.	2021	Methods validation and 1 batch analysis of Chlorantraniliprole 200 SC formulation Selvita services Sp. Z o.o. Poland Report No.: K479/JS Sponsor No.: 000107858 GLP: yes Published: no	N	ADM
KCP 5.1.2/01	Barbier, G.	2022	Validation of an analytical method for the determination of chlorantraniliprole in oilseed rape flowers, pollen, nectar, honey and sugar beet leaves. Girpa, France Report No.: B20G-A4-C-02 Sponsor No.: 000105720 GLP: yes Published: no	N	ADM
KCP 5.1.2/02	Fifi, A.P.	2020a	Validation of the analytical method for the determination of Chlorantraniliprole in ISO test medium solutions with Chlorantraniliprole 200 SC (product code ADM.00900.I.1.C) BioTecnologie BT Srl, Italy Report No.: BT281/20 Sponsor No.: 000105396 GLP: yes Published: no	N	ADM
KCP 5.1.2/03	Fifi, A.P.	2020b	Validation of the analytical method for the determination of Chlorantraniliprole in test media of aquatic studies (algae and lemna) with Chlorantraniliprole 200 SC (product code ADM.0900.I.1.C) Adama Makhteshim Ltd., Israel Report No.: BT207/19 Sponsor No.: 000103373 GLP: yes Published: no	N	ADM
KCP 5.1.2/04	Fifi, A.P.	2022	Validation of the analytical method for the determination of Chlorantraniliprole in the water and sugar feeding solutions with test item Chlorantraniliprole 200 SC (product code ADM.0900.I.1.C) coming from honeybee's laboratory tests BioTecnologie BT Srl, Italy Report No.: BT208/19 Sponsor No.: 000103886 GLP: yes Published: no	N	ADM

KCP 5.1.2/05	Barbier, G.	2021	Validation of an analytical method for the determination of chlorantraniliprole in plant matrices: peach, grape (bunches), wheat grain, oilseed rape seed and dry broad bean. POLLENIZ/GIRPA, France Report No.: B20G-A4-C-01 Sponsor No.: 000105719 GLP: yes Published: no	N	ADM
KCP 5.1.2/06	Fifi, A.P.	2020c	Validation of the analytical method for the determination of Chlorantraniliprole in the water solutions with test item Chlorantraniliprole 200 SC (product code ADM.0900.I.1.C) coming from terrestrial plants laboratory tests BioTecnologie BT Srl, Italy Report No.: BT209/19 Sponsor No.: 000105397 GLP: yes Published: no	N	ADM
KCP 5.2/01	Brown, D.	2022	Independent laboratory validation of analytical method B20G-A4-C-02 (Adama study No. 000105720) for determination of chlorantraniliprole in honey. ResChem Analytical Limited Report No.: RES-00420 Sponsor No.: 000111801 GLP: yes Published: no	N	ADM
KCP 5.2/02	Pentz, A.M., Cabusas, E.M.	2018	Analytical method for the determination of cyantraniliprole (DPX-HGW86) and chlorantraniliprole (DPX-E2Y45) in plasma and urine by HPLC/ESI-MS/MS Report No. 49234 GLP: yes Published: no	N	FMC
KCA 4.2/01	Brown, D.	2021	Determination of the extraction efficiency of chlorantraniliprole (E2Y45) residues using multiple extraction procedures and analytical methods FMC-51880 GLP: yes Published: no	N	FMC

* ADM = proprietary of ADAMA Agricultural Solutions and all affiliates.

Appendix 2 Detailed evaluation of submitted analytical methods

A 2.1 Analytical methods for Chlorantraniliprole

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

The studies have been evaluated in the renewal of the active substance (AIR), for more detail please refer to EFSA Journal 2013;11(6):3143.

A 2.1.1.1 Determination of active substance and/or variant in the plant protection product (KCP 5.1.1)

Reference:	KCP 5.1.1/01, Tsesin, N. (2019a)
Title:	Determination of storage stability and phys-chem properties of chlorantraniliprole 200 sc (ADM.0900.I.1.C) stored at for 14 days and at 0 ºc for 7 days
Report No.:	000102562.054FL
Authority registration No:	000102562
Guideline(s):	Commission Regulation (EU) No 284/2013 SANCO/3030/99 rev.5, 22 March 2019
Deviations:	No
GLP/GEP:	Yes
Acceptability:	Yes
Reference:	KCP 5.1.1/02, Tsesin, N. (2019b)
Report	Quantification of active ingredient in formulation product Chlorantraniliprole 200SC (ADM.0900.I.1.C)
Report No.	000103659.0SOFL
Sponsor study No.	000103659
Guideline(s):	SANCO/3030/99 rev.5, 22 March 2019
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Summary

The analysis was done by high performance liquid chromatograph (HPLC) with diode array detection (DAD) using external standard technique. The HPLC method, used to quantify the active ingredients in ADM.0900.I.1.C was fully validated. Method validation included linearity, specificity and confirmation of analyte identification, precision and accuracy.

Materials and methods

Material: One representative sample of the plant protection product ADM.0900.I.1.C manufactured (Batch no.: **3188-20519-01**) was used for the study.

External standards:
- **Chlorantraniliprole** (CAS: 500009-45-7; batch No.: 581-046-00, purity: 97.9 %, supplier: Adama Makhteshim Standards Laboratory).

HPLC system	**Agilent 1260 infinity II series equipped with an autosampler, column oven and degasser (LC-5)**
Column	YMC-Triart c18, 150 x 4.6 mm., D. S – 3 µm, 12 nm, P/N: TA12S031546PTH, S/N: 124XA80131, Lot: 1618
Column temperature	35 ºC
Injection volume	5 µL
Flow rate	1.0 ml/min
Wavelenght	254 nm
Retantio time	Approx. 20.2 min

Results and discussions

Table A 2.1.1.1-01: Recovery of Chlorantraniliprole in formulated product at maximum concentration level

Concentration	**C_A (g/kg)**	**C_F (g/kg)**	**Recovery [%]**
Maximum concentration level (125 %)	330.3	336.1	101.8
	330.3	336.9	102.0
	315.8	319.6	101.2
	315.8	319.9	101.3
Mean recovery (%)			102
SD			0.37
% RSD [(SD / mean) * 100]			0.236

Table A 2.1.1.1-02: Recovery of Chlorantraniliprole in formulated product at medium concentration level

Concentration	**C_A (g/kg)**	**C_F (g/kg)**	**Recovery [%]**
Medium concentration level (100 %)	251.2	336.1	101.1
	251.2	336.9	101.0
	246.3	249.8	101.4
	246.3	250.1	101.5
Mean recovery (%)			101
SD			0.28
% RSD [(SD / mean) * 100]			0.28

Table A 2.1.1.1-03: Recovery of Chlorantraniliprole in formulated product at minimum concentration level

Concentration	**C_A (g/kg)**	**C_F (g/kg)**	**Recovery [%]**
Minimum concentration level (100 %)	170.4	172.1	101.0
	170.4	172.3	101.2
	173.2	176.2	101.7
	173.2	176.1	101.6
Mean recovery (%)			101
SD			0.33
% RSD [(SD / mean) * 100]			0.32

Table A 2.1.1.1-04: Characteristics for the analytical method used for validation of Chlorantraniliprole in formulated product

Specificity	The specificity of the method was checked by comparing the chromatograms obtained from the analysis of Chlorantraniliprole in Chlorantraniliprole 200 SC formulation batch with the one of the blank samples. It was found that the blanks chromatograms do not contain any interfering peak at the retention time corresponding to the active ingredient. As a result, it can be concluded that the analytical method is specific for the determination of Chlorantraniliprole in Chlorantraniliprole 200 SC for-mulation product. The identification of the active ingredient was done by HPLC-MS method
Linearity	The linearity for active ingredients was tested in linear range covering at least ± 20% of analyte nominal concentration (2 mg/ml for formulation) studied.

	<u>High linearity range</u> y = 7461.9409 x + 85.54 R = 0.9999 (range: 0.3 mg/mL to 0.7 mg/mL) <u>Low linearity range</u> y = 7759.3064 x – 1.8134 R = 0.9999 (range: 0.06 mg/mL to 0.14 mg/mL) The resulting linearity curves have correlation coefficient R2 > 0.99 (as required by SANCO/3030/99 rev. 5) indicating that each one of the active ingredients are linear in the range of interest.
Repeatability (precision)	The precision of the method was evaluated by a repeatability assessment. Five samples solutions of the batch were prepared and analysed for the active ingredients content. Two repeatability assays were performed on different days. The relative standard deviation of the RF, obtained for active ingredient from 10 injections from two assays was taken as the indication of analytical method intermediate precision. The % RSD of the results was calculated to ensure it meets Horwitz criterion. RSD (1st analyst) = 1.2 % RSD (2nd analyst) = 0.3 % Intermediate precision RSD = 1.00 % RSDr = 2.57 % Hr = 0.39
Accuracy	According to SANCO 3030/99 rev 5. guideline the acceptance criterion for mean recoveries in accuracy study at > 10% concentration levels of a.i. in sample is 97 -103 %. Statistical evaluation tests were performed and mean standard deviation and relative standard deviation were calculated. The accuracy results for a.i. in Chlorantraniliprole 200 SC formulation product at all concentration levels met the SANCO 3030/99 rev 5. acceptance criteria.

Conclusion

The method was validated according to guideline SANCO/3030/99 rev. 5 with regard to specificity, linearity of detector response, accuracy and precision for Chlorantraniliprole plant protection product ADM.0900.I.1.C and is considered acceptable.

Tsesin, N (2019b)

A 2.1.1.2 Description of analytical methods for the determination of relevant impurities (KCP 5.1.1)

The relevant impurities in Chlorantraniprole reference source are: acetonitrile up to 3 g/kg, 3-picoline up to 3 g/kg and Methanesulfonic acid up to 2 g/kg (Commission Implementing Regulation (EU) 1199/2013 of 25 November 2013).

Reference:	KCP 5.1.1/03, Rutyna, A. (2021)
Report	Methods validation and 1 batch analysis of Chlorantraniliprole 200 SC formulation
Report No.	K479/JS
Guideline(s):	SANCO/3030/99 rev.5. Brazilian Standard ABNT NBR 14029 3rd Ed. 09/12/2016 Australian Pesticides & Veterinary Medicines Authority 27/06/2018– Agrochemicals
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Impurity 1

Summary

The goal of this study was to validate an analytical method for the determination of impurity 1 (3-Picoline) in the formulated product by using GC-FID according to SANCO/3030/99 rev. 5

Materials and methods

Material: One representative sample of the plant protection product Chlorantraniliprole 200 SC (Batch no.: **647421244**) was used for the study.

External standards:

- **Impurity 1 (3-Picoline)** (batch No.: MKCJ9205, purity: 99,7 %, supplier: Sigma)

GC-FID conditions

Column and packing:	DB-5MS 30m x 250µm x 1.0µm or equivalent
Column Oven Temp	70°C (2min), Ramp 25°C/min to 320°C (8min)
Detector:	FID
Detector Temperature:	300°C
Air Flow:	400 mL/min
Hydrogen flow:	35 mL/min
Make up flow	27 mL/min
Injector Temp:	270°C
Carrier Gas (He) Flow:	1.1 mL/min
Split Ratio:	1:25
Injection volume:	4µL
MS detector parameters	Ion Source: EI Source Temperature: 230°C Quad Temperature: 150°C Fixed Electron Energy: 70 eV Acquisition Type: Scan Stop time: 20 min Solvent Delay: 3 min
Retention time	Around 5 min

Results and discussions

Sample preparation
For example, weight accurate about 330 mg of sample into 20 mL volumetric flask. Dilute with Acetone and sonicate to full dissolution. Cool to room temperature and add Acetone to the mark. Mix well. Concentration of Sample Solution: 16.5 mg/mL; Concentration of AI in Sample Solution: 3 mg/mL

Standard preparation
For example, weight accurately about 30 mg of 3-Picoline into 50 mL volumetric flask. Dilute with Acetone to the mark and mix well.
Transfer 150 µL of the above solution into 10 mL volumetric flask and dilute to volume with Acetone. Mix well. Concentration of 3-Picoline: 0.009 mg/mL; 0.3 % w/w.

Table CA 2.1.1.2-1: Recovery results for Impurity 1 in Chlorantraniliprole 200 SC

Analyte	Concentration [%w/w]	Recoveries			n
		Single values [%]	Mean [%]	RSD [%]	
Impurity 1	0.01	113, 116	114	1.85	2
	0.06	109,107	108	1.31	2
	0.08	109, 109	109	0	2

Table CA 2.1.1.2-2: Characteristics for the analytical method used for validation of Impurity 1 in Chlorantraniliprole 200 SC

	Impurity 1
Specificity	There were no significant peaks present in these chromatograms at the same retention time as Impurity 1. This demonstrates that there were no analyte interferences and the method is specific to Impurity 1.
Linearity	To evaluate linearity, six standard concentrations were prepared and injected once. The detector response was shown to be linear between 0.0015 mg/mL and 0.012 mg/L (0,01-0,08%w/w). y = 2351.1 x + 0.4236; R2 = 0.9967 The resulting linearity curves have correlation coefficient R^2 > 0.99 (as required by SANCO/3030/99 rev. 5).
Precision (repeatability) **n = 6** (%RSD)	RSD (0.06% w/w level) = 3.4 % RSD_r =4.1 % Horrat value < 1
Accuracy **(% Recovery)** (%RSD)	Recovery precision at 0.01% (LOQ level) 114 % (n = 6) 1.85% Recovery precision at 0.06% 108 % (n = 6) 1.31% Recovery precision at 0.08% 109 % (n = 6) 0%
LOQ	LOQ = 0.0015 mg/mL = 0.001 g/kg (0.01%w/w).

Conclusions
The analytical method was validated according to guideline SANCO/3030/99 rev. 5 with regard to specificity, linearity of detector response, accuracy and precision for impurity 1 formulated product and is considered acceptable.

Rutyna, A. (2021)

Impurity 2 Acetonitrile

Summary
The goal of this study was to validate an analytical method for the determination of impurity 2 (Acetonitrile) in the formulated product by using HS-GC-FID according to SANCO/3030/99 rev. 5

Materials and methods

Material: One representative sample of the plant protection product Chlorantraniliprole 200 SC (Batch no.: **647421244**) was used for the study.

External standards:
- **Impurity 2 (Acetonitrile)** (batch No.: I1122630, purity: 99,9 %, supplier: Merck).

HS-GC-MS conditions

Chromatographic conditions:	
Column and packing:	ZB-624; 30m x 0.32mm x 1.8µm or equivalent
Carrier Gas	Helium
Carrier Gas Flow:	2 mL/min (constant flow)
Column Oven Temp	45°C (5min), Ramp 25°C/min to 250°C (6min)
Split ratio:	50:1
Injection volume:	1000 µL
Headspace parameters:	
Oven temperature:	90 °C
Loop temperature:	100 °C
Transfer line temperature:	120 °C
Vial equilibration time:	30 min
Injection time:	1 min
GC cycle time:	29 min
Vial shaking:	No shake
Fill pressure:	103 kPa
Transfer line (Thermal Aux)- for Agilent HS-GC systems:	150 °C
MS detector parameters	Ion Source: EI Source Temperature: 230°C Quad Temperature: 150°C Fixed Electron Energy: 70 eV Acquisition Type: Scan Stop time: 19.2 min
Retention time	Around 2.5 min

Results and discussions

Sample preparation

For example, weigh in triplicate accurately about 545 mg of sample into a 20 mL HS vial, add 4.5 mL of DMF and 100 µL of Internal Standard stock solution and immediately cap and crimp tightly. Concentration of Sample Solution: about 120 mg/mL; Concentration of AI in Sample Solution: 20 mg/mL

Standard preparation

Acetonitrile Standard stock solution:

For example, weigh in triplicate accurately about 25 mg of Acetonitrile into 25 mL volumetric flasks, partially filled with DMF. Make up to volume with the same solvent and mix well.

Acetonitrile Standard solution:
For example, into a 50 mL volumetric flask partially filled with DMF, transfer 3 mL of standard stock solution. Make up to volume with the same solvent.

Internal Standard (Ethanol) stock solution
For example, weigh accurately about 30 ml of Ethanol into 10 mL volumetric flasks, partially filled with DMF. Make up to volume with the same solvent and mix well.

Into a 20 mL HS vial transfer 5 mL of Acetonitrile standard solution and 100 µL of Internal Standard stock solution and immediately cap and crimp tightly.

Concentration of Acetonitrile: 0.06 mg/mL; 0.06 % w/w
Concentration of IS:0.06 mg/mL; 0.06 % w/w

Table CA 2.1.1.2-3: Recovery results for Impurity 2 in Chlorantraniliprole 200 SC

Analyte	Concentration %w/w	Recoveries			n
		Single values [%]	Mean [%]	RSD [%]	
Impurity 2	LOQ	107 , 114	111	4.5%	2
	0.06%	107, 119	113	7.5%	2
	0.08%	114, 113	114	0.6%	2

Table CA 2.1.1.2-4: Characteristics for the analytical method used for validation of Impurity 2 in Chlorantraniliprole 200 SC

	Impurity 2
Specificity	There were no significant peaks present in these chromatograms at the same retention time as Impurity 2. This demonstrates that there were no analyte interferences and the method is specific to Impurity 2.
Linearity	To evaluate linearity, six standard concentrations were prepared and injected once. The detector response was shown to be linear ranging 0.01 and 0.08 mg/mL (0.01 % w/w to 0.08 %w/w).. Each solution was injected once and peak areas were obtained. y = 16,713x + 0,0289; R2 = 0.991 The resulting linearity curves have correlation coefficient R^2 > 0.99 (as required by SANCO/3030/99 rev. 5).
Precision (repeatability) n = 6 (%RSD)	RSD (0.05% w/w level) = 3.9 % RSD_r =4.2 % Horrat value < 1
Accuracy (% Recovery) (%RSD)	Recovery precision was evaluated by injection of the six separate solutions at 3 levels:

	Impurity 2
	Recovery precision at 0.01% (LOQ level) 111 % (n = 6) 4.5% Recovery precision at 0.06% 113 % (n = 6) 7.5% Recovery precision at 0.08% 113 % (n = 6) 0.6%
LOQ	LOQ = 0.01 mg/mL = 0.001 g/kg (0.01%w/w).

Conclusions
The analytical method was validated according to guideline SANCO/3030/99 rev. 5 with regard to specificity, linearity of detector response, accuracy and precision for impurity 2 formulated product and is considered acceptable.

Rutyna, A. (2021)

Impurity 3

Summary
The goal of this study was to validate an analytical method for the determination of impurity 3 (1,2-dichloroethane) in the formulated product by using LC/HRMS according to SANCO/3030/99 rev. 5

Materials and methods

Material: One representative sample of the plant protection product Chlorantraniliprole 200 SC (Batch no.: **647421244**) was used for the study.

External standards:
- **Impurity 1 (Methanesulfonic acid)** (batch No.: STBJ6677, purity: 99,75 %, supplier: Sigma).

LC/HRMS conditions

Column and packing: Comosil Hilic 4.6 ID x 150 mm, 5 µm , code 07056-51
Eluent
Eluent A: Acetonitrile HPLC grade
Eluent B: 10 mmol/L Ammonium acetate in LC/MS grade water with 0.1 – 0.5 ppm of H3PO4 for precise MS locking

Time [min]	Eluent A [%]	Eluent B [%]
0	50	50
9	50	50

Column Temperature: 30°C
Flow rate: 1.2 mL/min, split 0.25mL/min onto mass detector
Detector: High resolution MS
Data collection GCMS Solutions
Retention time (approx.) Impurity 3 = 6,1

Results and discussions

Sample preparation

For example, weigh in triplicate accurately about 125 mg of Chlorantraniliprole 200 SC sample into a 50 mL volumetric flask, add 10 mL of DMF. Agitate in ultrasonic bath for about 10 min, add 0.05 mL of Ethanesulfonic acid IS Stock Solution and make up with acetonitrile to the mark, mix well.
0.5 mg/mL of Chlorantraniliprole. 2.5mg/ml formulation

Standard preparation

Ethanesulfonic acid IS stock solution:

For example: Weigh about 20 mg of Ethanesulfonic acid into a 100 mL volumetric flask and dissolve in diluent. Make up with diluent to the mark, mix well (c = 0.2 mg/mL)

Methanesulfonic acid STD stock solution:

For example: Weigh about 20 mg of Methanesulfonic acid into a 100 mL volumetric flask and dissolve in diluent. Make up with diluent to the mark, mix well (c = 0.2 mg/mL)

Standard solution:

Into a 100 mL volumetric flask transfer 0.1 mL of Ethanesulfonic acid IS Stock Solution, 0.5 mL of Methanesulfonic acid STD Stock Solution and make up with diluent to the mark, mix well.

0.01 mg/mL (1 ppm) of Methanesulfonic acid and 0.0002 mg/mL (0.2 ppm) of Ethanesulfonic acid.

Table CA 2.1.1.2-5: Recovery results for Impurity 3 in Chlorantraniliprole 200 SC

Analyte	Concentration [%w/w]	Recoveries			n
		Single values [%]	Mean [%]	RSD [%]	
Impurity 3	0.005	109.1, 127.2	118	10.8	2
	0.04	101.7, 105.6	104	2.7	2
	0.048	100.1, 96.7	98	2.4	2

Table CA 2.1.1.2-6: Characteristics for the analytical method used for validation of Impurity 3 in Chlorantraniliprole 200 SC

	Impurity 3
Specificity	There were no significant peaks present in these chromatograms at the same elution time as Impurity 3. This demonstrates that there were no analyte interferences and the method is specific to Impurity 3.
Linearity	To evaluate linearity, six standard concentrations were prepared and injected once. The detector response was shown to be linear ranging from 0.000124 to 0.001198 mg/mL (0.006 – 0.048 % w/w). Each solution was injected once and peak areas were obtained. y = 2707x + 0.0462; R2 = 0.9982 The resulting linearity curves have correlation coefficient R^2 > 0.99 (as required by SANCO/3030/99 rev. 5).

	Impurity 3
Precision (repeatability) **n = 6** (%RSD)	RSD (0.04% w/w level) = 1,6 % RSD_r =4.3 % Horrat value < 1
Accuracy **(% Recovery)** (%RSD)	Recovery precision was evaluated by injection of the six separate solutions at three levels: Recovery precision at 0,05 %(w/w) level 118 % (n = 6) 10.8% Recovery precision at 0.04% (w/w) level 104 % (n = 6) 2.7% Recovery precision at 0.048% (w/w) level 98 % (n = 6) 2.4%
LOQ	LOQ = 0.000125 mg/mL = 0.0005 g/kg (0.005%w/w).

Conclusions
The analytical method was validated according to guideline SANCO/3030/99 rev. 5 with regard to specificity, linearity of detector response, accuracy and precision for impurity 3 formulated product and is considered acceptable.

Rutyna, A. (2021)

A 2.1.1.3 Methods for the determination of residues in soil, water and non-target organisms (KCP 5.1.2)

New studies to support residues section are described below, not previously evaluated in a peer reviewed process at EU

Comments of zRMS:	The analytical method was successfully validated according to SANTE/2020/12830 Rev. 1 for the determination of chlorantraniliprole in oilseed rape flowers, pollen, nectar, honey and sugar beet leaves at a limit of quantitation (LOQ) of 0.01 mg/kg. The mean recoveries for chlorantraniliprole at each fortification level, and overall, for each of the matrices tested were within the acceptable range of 70-110% with the relative standard deviation (RSD) within the acceptable range of ≤ 20%. The method is acceptable.

Reference:	KCP 5.1.2/01 Barbier, G. (2022)
Report	Validation of an analytical method for the determination of chlorantraniliprole in oilseed rape flowers, pollen, nectar, honey and sugar beet leaves.
Report No.	B20G-A4-C-02
Guideline(s):	SANTE/2020/12830, Rev.1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Summary
The goal of this study was to validate an analytical method for the determination of chlorantraniliprole in oilseed rape flowers, pollen, nectar, honey and sugar beet leaves. Calibration linearity, specificity, blank samples analysis, recoveries, accuracy, repeatability, matrix effect, limit of quantification (LOQ) and limit of detection (LOD) were checked according to SANTE/2020/12830 Rev. 1. The analysis was performed

by LC-MS/MS.

Materials and methods

Material: Reference item:

- **Chlorantraniliprole** (CAS: 500008-45-7; batch No.: G1033981, purity: 97.28%, supplier: Dr. LGC Labor GmbH).

The study was performed on the following matrices:

- untreated oilseed rape flower and sugar beet leaf specimens provided by the Sponsor,
- organic multi-flower honey and organic pollen purchased by GIRPA,
- artificial nectar (30% w/v sucrose/ultra-pure water) made at the laboratory each day of analysis by weighing 30 g of sucrose into a 100 mL volumetric flask and making-up to 100 mL with ultra-pure water then shaking until full dissolution.

Stock solutions preparation

Between 10 and 50 mg of chlorantraniliprole was accurately weighed. A stock standard solution of exactly 1 g/L of chlorantraniliprole in acetone was prepared by taking the purity into account.

Standard solution preparation:

Standard solutions at 10, 2, 1, 0.2 and 0.1 mg/L were prepared by dilution of the stock standard solution in acetonitrile acidified with 1% formic acid (final concentration 0,2 to 0,003 mg/kg).

Sample preparation:

*Oilseed rape flowers: a*ccurately, weigh 2 g of ground laboratory sample into a 50 mL centrifuge tube. For recoveries, fortify the aliquots of laboratory sample with the appropriate spiking standard solutions using a pipette. Using a measuring cylinder, add 10 mL of ultra-pure water. Using a measuring cylinder, add 10 mL of acetonitrile acidified with 1% formic acid. Homogenize horizontally on a mechanical shaker at 200 cps/min during 20 minutes. Add one QuEChERS extraction salt packet containing 4 g $MgSO_4$, 1 g NaCl. Shake manually and vigorously during 1 minute. Centrifuge for 5 minutes at 4000 rpm. Dilute an aliquot of the supernatant two-fold into acetonitrile acidified with 1% formic acid in a 2 mL vial. This is the final extract (extraction ratio: 2 g in 20 mL of solvent).

*Pollen: a*ccurately, weigh 0.5 g of laboratory sample into a 50 mL centrifuge tube. For recoveries, fortify the aliquots of laboratory sample with the appropriate spiking standard solutions using a pipette. Using a measuring cylinder, add 10 mL of ultra-pure water. Using a measuring cylinder, add 5 mL of acetonitrile acidified with 1% formic acid. Homogenize horizontally on a mechanical shaker at 200 cps/min during 20 minutes. Add one QuEChERS extraction salt packet containing 4 g $MgSO_4$, 1 g NaCl. Shake manually and vigorously during 1 minute. Centrifuge for 5 minutes at 4000 rpm. Transfer an aliquot of the supernatant into a 2 mL vial. This is the final extract (extraction ratio: 0.5 g in 5 mL of solvent).

*Nectar: a*ccurately, weigh 0.2 g (corresponding to about 175 µL) of laboratory sample into a 15 mL centrifuge tube. For recoveries, fortify the aliquots of laboratory sample with the appropriate spiking standard solutions using a pipette. Using an automatic pipette, add 1.83 mL of acetonitrile acidified with 1% formic acid /ultrapure water (50/50 v/v) mixture. Homogenize for 5 seconds using a Vortex. Check there is not two phases otherwise homogenize again. Aliquot into a 2 mL vial. This is the final extract (extraction ratio: 0.2 g in 2 mL of solvent).

Honey: accurately, weigh 10 g of laboratory sample into a 50 mL centrifuge tube. For recoveries, fortify the aliquots of laboratory sample with the appropriate spiking standard solutions using a pipette. Using a measuring cylinder, add 10 mL of ultra-pure water. Using a measuring cylinder, add 10 mL of acetonitrile acidified with 1% formic acid. Homogenize horizontally on a mechanical shaker at 200 cps/min during 20 minutes. Add one QuEChERS extraction salt packet containing 4 g $MgSO_4$, 1 g NaCl. Shake manually and vigorously during 1 minute. Centrifuge for 5 minutes at 4000 rpm. Dilute an aliquot of the supernatant ten-fold into acetonitrile acidified with 1% formic acid in a 2 mL vial. This is the final extract (extraction ratio: 10 g in 100 mL of solvent).

*Sugar beet leaves: a*ccurately, weigh 10 g of ground laboratory sample into a 50 mL centrifuge tube. For recoveries, fortify the aliquots of laboratory sample with the appropriate spiking standard solutions using a pipette. Using a measuring cylinder, add 10 mL of acetonitrile acidified with 1% formic acid. Homogenize horizontally on a mechanical shaker at 200 cps/min during 20 minutes. Add one QuEChERS extraction salt packet containing 4 g $MgSO_4$, 1 g NaCl. Shake manually and vigorously during 1 minute. Centrifuge for 5 minutes at 4000 rpm. Dilute an aliquot of the supernatant ten-fold into acetonitrile acidified with 1% formic acid in a 2 mL vial. This is the final extract (extraction ratio: 10 g in 100 mL of solvent).

Fortification procedure
All fortifications were performed directly to aliquots of the laboratory sample in a 50 mL centrifuge tube for oilseed rape flowers, pollen, honey and sugar beet leaves and in a 15 mL centrifuge tube for nectar after weighing the sample. They were performed with the appropriate spiking standard solutions using a volumetric pipette. Fortification 0,01 and 0,1 mg/kg for each matrix.

HPLC conditions

HPLC system: - Liquid Chromatograph (LC-MS/MS QTrap 7500)
Analytical column: - Column C18 Kinetex (100 mm X 4.6 mm ID X 2.6 µm)
Mobile phases: Solvent A: ultra-pure water + 0.1% formic acid
Solvent B: acetonitrile + 0.1% formic acid

Time table (min)	Phase A (%)	Phase B (%)
0	95	5
3.0	0	100
5.0	0	100

Flow rate: 0.7 mL/min
Column Temperature: 40 ºC
Injection volume: 2 µL
Retention time about 3.6 min.

MS System PE-Sciex API 7500 QTRAP tandem mass Spectrometer
484 → 453 m/z (quantification)
484 → 286 m/z (confirmation)
Ion Mode : positive Multiple reaction Monitoring (MRM)

Results and discussions

Table CA 2.1.1.3-1: Accuracy results for validation of chlorantraniliprole in following matrix

Analyte	Matrix	Fortification level [mg/kg]	Recoveries			n	Overall recovery	
			Single values [%]	Mean [%]	RSD [%]		Mean [%]	RSD [%]
Chlorantraniliprole	Oilseed rape flowers	primary mass transition 484 → 453 (m/z)						
		0,01	97, 98, 97, 102, 104	100	3	5	104	3
		0,1	106, 107, 111, 104, 113	108	3	5		
	Oilseed rape flowers	confirmatory mass transition 484 → 286 (m/z)						
		0,01	96, 102, 96, 99, 102	99	3	5	101	2
		0,1	101, 103, 102, 105, 103	103	1	5		
Chlorantraniliprole	Organic pollen	primary mass transition 484 → 453 (m/z)						
		0,01	93, 93, 95, 92, 101	95	3	5	96	1
		0,1	91, 94, 97, 100, 101	97	4	5		
		confirmatory mass transition 484 → 286 (m/z)						

Analyte	Matrix	Fortification level [mg/kg]	Recoveries			n	Overall recovery	
			Single values [%]	Mean [%]	RSD [%]		Mean [%]	RSD [%]
	Organic pollen	0,01	94, 95, 100, 97, 94	96	3	5	98	2
		0,1	96, 96, 103, 103, 105	100	4	5		
Chlorantraniliprole	Artificial nectar	primary mass transition 484 → 453 (m/z)						
		0,01	99, 84, 103, 107, 102	99	8	5	101	2
		0,1	101, 102, 108, 100, 105	103	3	5		
	Artificial nectar	confirmatory mass transition 484 → 286 (m/z)						
		0,01	84, 79, 84, 89, 88	85	4	5	92	7
		0,1	100, 102, 98, 96, 94	98	3	5		
Chlorantraniliprole	Organic honey	primary mass transition 484 → 453 (m/z)						
		0,01	102, 100, 109, 104, 103	104	3	5	105	1
		0,1	109, 105, 107, 103, 103	105	2	5		
	Organic honey	confirmatory mass transition 484 → 286 (m/z)						
		0,01	101, 102, 107, 104, 100	103	2	5	104	1
		0,1	104, 102, 104, 107, 105	104	2	5		
Chlorantraniliprole	Sugar beet leaves	primary mass transition 484 → 453 (m/z)						
		0,01	96, 87 , 99, 93, 95	94	4	5	94	0
		0,1	94, 93, 91, 96, 95	94	2	5		
	Sugar beet leaves	confirmatory mass transition 484 → 286 (m/z)						
		0,01	88, 93, 90, 91, 94	91	2	5	92	1
		0,1	94, 92, 95, 93, 92	93	1	5		

Table CA 2.1.1.3-2: Characteristics for the analytical method used for validation of chlorantraniliprole in following matrix

	Chlorantraniliprole
Specificity	For each matrix, the specificity of the method was checked by the analysis of two samples of blank matrix (non-fortified sample or control sample), that had undergone the same sample preparation process as the fortified samples. Analysis of blank matrix (non-fortified sample or control sample) with MS/MS did not yield residues of chlorantraniliprole above 30 % of the limit of quantification, indicating that no interference was present at the retention time of chlorantraniliprole in the laboratory samples. This was in accordance with the level specified in the guideline, which demands blank values (non-fortified samples) less than 30 % of the LOQ. The selectivity and specificity of the method were demonstrated.
Linearity	For each matrix, the linearity of the method was determined by measuring the detector response (peak area) versus the concentration of a series of at least 5 calibration standard solutions and covered a maximum of two orders of magnitude. The analytical calibration covered the range from 30% of the LOQ to 20% above the highest level. Liniarity primary mass transition: <u>Oilseed rape flowers</u> $Y= 3361,4614x^2+ 1553142,2688 x + 18868,7671$ R2=0.9994 Calibration range: 0.3-20 µg/L and 0.010 – 0.100 mg/kg <u>Pollen</u>

	Chlorantraniliprole
	$Y= -3015,6351x^2 + 553845,8941x - 12730,8726$ R2=0.9994 Calibration range: 0.3-20 µg/L and 0.010 – 0.100 mg/kg <u>Nectar</u> $Y= -353,969\ x^2 + 1470991,6374\ x + 80724,3865$ R2=0.9952 Calibration range: 0.3-20 µg/L and 0.010 – 0.100 mg/kg <u>Honey</u> $Y= -9717,0719x^2 + 1812134,7307\ x + 9282,1395$ R=0.9990 Calibration range: 0.3-20 µg/L and 0.010 – 0.100 mg/kg <u>Sugar beet leaves</u> $Y= 4646,5771\ x^2 + 723199,6267\ x + 18085,2559$ R2=0.9994 Calibration range: 0.3-20 µg/L and 0.010 – 0.100 mg/kg
Matrix effects	Oilseed rape flowers – Chlorantraniliprole (m/z 484 → 453) – -11% Chlorantraniliprole (m/z 484 → 286) – -3% Pollen – Chlorantraniliprole (m/z 484 → 453) – -30% Chlorantraniliprole (m/z 484 → 286) – -26% Nectar – Chlorantraniliprole (m/z 484 → 453) – -1% Chlorantraniliprole (m/z 484 → 286) – +6% Honey – Chlorantraniliprole (m/z 484 → 453) – +2% Chlorantraniliprole (m/z 484 → 286) – +3% Sugar beet leaves – Chlorantraniliprole (m/z 484 → 453) – -22% Chlorantraniliprole (m/z 484 → 286) – -11% Matrix effects were not considered significant as they didn't exceed ± 20% except in pollen and for primary mass transition in sugar beet leaves. However, all analyses were carried out using matrix-matched standard calibration solutions for all matrices.
Stability of extracts	For oilseed rape flowers, pollen and sugar beet leaves, the final sample extracts were analysed within 24 hours after initial extraction thus no stability study was performed. For nectar and honey, as final sample extracts were not injected within 24 hours following the extraction, a study of stability of chlorantraniliprole in final sample extracts was performed during this study. The final sample extracts were stored respectively at about +4°C for nectar and -18°C for honey before injection in LC-MS/MS. A freshly prepared standard calibration solution was injected with the calibration standard solutions prepared on the day of extraction. The deviation of the freshly prepared standard calibration solution was lower than or equal to 20 % and thus validated the stability in the final sample extracts.
Precision and recovery	Mean recoveries and relative standard deviations per fortification level fulfil the criteria of guideline SANTE/2020/12830 Rev.1.
Limit of determination / quantification	The limit of quantification (LOQ)= 0.010 mg/kg The limit of detection (LOD)= 0.003 mg/kg

The method was validated according to guideline SANTE/2020/12830 Rev.1. with regard to specificity, linearity of detector response, accuracy and precision for Chlorantraniliprole in oilseed rape flowers, pollen, nectar, honey and sugar beet leaves and is considered acceptable.

Barbier, G. (2022)

Comments of zRMS:	The method was sufficiently validated for the quantification of chlorantraniliprole in test media of aquatic studies (ISO medium (with and without 0.01% DMF) solutions). For chlorantraniliprole in ISO medium the LOQ was established at 8.9088 mg/L. For chlorantraniliprole in ISO medium with 0.01% DMF the LOQ was established at 0.3959 µg/L. The mean recoveries for chlorantraniliprole at each fortification level, and overall, were within the acceptable range of 70-110% with the relative standard deviation (RSD) within the acceptable range of ≤ 20%. The method is acceptable for risk assessment.

Reference:	KCP 5.1.2/02, Fifi, A.P. (2020a)
Title:	Validation of the analytical method for the determination of Chlorantraniliprole in ISO test medium solutions with Chlorantraniliprole 200 SC (product code ADM.00900.I.1.C)
Report No.:	BT281/20
Sponsor No.:	000105396
Guideline(s):	SANCO/3029/99 rev.4
Deviations:	No
GLP/GEP:	Yes
Acceptability:	Yes

The goal of this study was to validate an analytical method for the determination of Chlorantraniliprole in test media of aquatic studies **(Fish study : KCP 10.2.1/01; Daphnia study : KCP 10.2.1/02).** The range of linearity, specificity, precision, recovery and LOQ and LOD of analytes were determined. Determination was performed by LC with MS/MS detection.

Material: Test item:
One batch (**1221-010320-0111**) of Chlorantraniliprole 200 SC (product code ADM.0900.I.1.C) as manufactured.
Reference item:
- **Chlorantraniliprole** (CAS No.: 500008-45-7, batch No.: **G1033981**, purity: 97;28%, supplier: Dr. Ehrenstorfer (LGC)).

The analytical method adopted for the determination of Chlorantraniliprole content in ~~in~~ ISO medium (with and without 0.01% DMF) solutions was validated in accordance with the validity criteria required in the SANCO/3029/99 rev.4 (11/07/2000) guidance document.

Equipment and conditions for Chlorantraniliprole determination

LC system	Agilent UHPLC 1290 series with 6495b Triple Quad. Spectrometer
Column:	Agilent Zorbax Eclipse Plus C18 RRHD 2.1 x 50 mm 1.8 µm
Column Temperature:	30ºC
Injection Volume:	3 µL
Mobile phases:	A: Ultrapure water acidified with 0.1 % formic acid B: Acetonitrile
Flow rate:	0.4 mL/min
Detector:	MassHunter Quantitative Analysis for QQQ, Version B.08.00 - Agilent Technologies - 2016
Ionisation:	Electro Spray (ESI) + AJS (Agilent Jet Stream)
Polarity:	Positive
Ion mass transition monitored (m/z)	*Chlorantraniliprole* Transition 1 (Qualifierr): 483.9 → 452.9 Transition 2 (Quantifier): 483.9 → 285.9
Retention time (approx.)	Approx. 1.5 min

Table A 2.1.1.3-3: Recovery results of Chlorantraniliprole in test medium

Matrix	**Analyte**	**Fortification level [mg/L]**	**Recoveries**			**n**	**Overall recovery**	
			Single values [%]	**Mean [%]**	**RSD [%]**		**Mean [%]**	**RSD [%]**
ISO medium	Chlorantraniliprole	Mass transition 483.9 → 285.9 m/z						
		8.9088	98.38, 87.70, 99.93, 86.23, 86.33	91.71	7.46	5	90.98	5.30
		22.2721	93.30, 89.86, 90.42, 87.49, 90.17	90.25	2.29	5		
		Mass transition 483.9 → 452.9 m/z						
		8.9088	100.62, 86.03, 95.76, 82.32, 86.18	90.18	8.51	5	90.18	8.51
ISO medium with 0.01 % DMF		Mass transition 483.9 → 285.9 m/z						
		0.3959E-3	102.50, 93.28, 94.14, 96.26, 93.61	95.96	4.00	5	96.19	2.72
		22.6205E-3	96.43, 96.34, 97.30, 95.25, 96.80	96.42	0.79	5		
		Mass transition 483.9 → 452.9 m/z						
		0.3959E-3	100.73, 95.35, 90.53, 99.92, 92.93	95.89	4.59	5	95.89	4.59

All mean recovery values at ~~at~~ LOQ and the high fortification level of Chlorantraniliprole in ISO medium and ISO medium with 0.01% DMF medium comply with the standard acceptance criteria of SANCO/3029/99 rev 4, since mean recoveries were in the range of 70% - 110% with a relative standard deviation ≤ 20%.

Table A 2.1.1.3-4: Characteristics for the analytical method used for validation of Chlorantraniliprole in test medium

	Chlorantraniliprole
Specificity	A confirmatory analysis was performed to demonstrate the selectivity of the primary detection transitions of quantifier for Chlorantraniliprole (Q1: m/z = 483.9 → m/z = 285.9; Q2: 483.9 → m/z = 452.9). The specificity of the method has been demonstrated. No signal higher than 30% of the lowest fortified solution was detected at the retention time of Chlorantraniliprole in the blank samples.
Calibration (type, number of data points) **Calibration range**	The linearity of the detector response was demonstrated by double determination of calibration solutions at five concentration levels ranging from 0.1020 µg/L to 10.2027 µg/L for both

	Chlorantraniliprole
	medium. This range covers the range from no more than 30 % of the LOQ and at least + 20 % of the highest analyte concentration detected in any diluted sample. The response of Chlorantraniliprole in ISO medium was found to be linear in the range of concentrations 00.1020 µg/L – 10.2027 µg/L. Mass transition (Quantifier) y= 95324.99x-1741.77 R2 = 0.9975 Mass transition (Confirmatory) Y=106874.63x-1711.07 R2 = 0.9980 The response of Chlorantraniliprole in ISO medium with 0.01% DMF was found to be linear in the range of concentrations 0.1020 µg/L to 10.2027 µg/L. Mass transition (Quantifier) Y=183939.57x-2381.34 R2 = 0.9994 Mass transition (Confirmatory) Y=208473.60x-1791.56 R2 = 0.9991
Precision and recovery	Mean recoveries and relative standard deviations per fortification level fulfil the criteria of guideline SANCO/3029/99 rev. 4, 11/07/2000 (70 - 110 % mean recovery, ≤ 20 % RSD).
Limit of determination / quantification	The limit of quantification is defined as the lowest fortification level at which an acceptable mean recovery was obtained (normally 70% - 110% with a relative standard deviation preferably ≤ 20%). For Chlorantraniliprole in ISO medium the LOQ was thus successfully established at the nominal value of 8.9088 mg/L. For Chlorantraniliprole in ISO medium with 0.01% DMF the LOQ was thus successfully established at the nominal value of 0.3959 µg/L. The minimum level established for the LOD was the one at which the analyte has a signal at least 3 times higher than the background noise of the response in the control solutions. LOD for both test medium was found to be 0.1020 µg/L.

Conclusion

The analytical method for the determination of Chlorantraniliprole in ISO medium and ISO medium + 0.01% DMF was demonstrated to be satisfactory in terms of accuracy, precision, linearity, and specificity. **(Used in Fish study : KCP 10.2.1/01; Daphnia study : KCP 10.2.1/02).**

Fifi, A.P. (2020a)

Comments of zRMS:	The method was sufficiently validated for the quantification of chlorantraniliprole in test media of aquatic studies (EPA medium and SIS medium). For chlorantraniliprole in EPA medium the LOQ was established at 0.0102 µg/L. For chlorantraniliprole in SIS medium the LOQ was established at 0.0101 µg/L. The mean recoveries for chlorantraniliprole at each fortification level, and overall, were within the acceptable range of 70-110% with the relative standard deviation (RSD) within the acceptable range of ≤ 20%. The method is acceptable for risk assessment.

Reference:	KCP 5.1.2/03, Fifi, A.P. (2020b)
Title:	Validation of the analytical method for the determination of Chlorantraniliprole in test media of aquatic studies (algae and lemna) with Chlorantraniliprole 200 SC (product code ADM.0900.I.1.C)
Report No.:	BT207/19
Sponsor No.:	000103373
Guideline(s):	SANCO/3029/99 rev.4
Deviations:	No
GLP/GEP:	Yes
Acceptability:	Yes

The goal of this study was to validate an analytical method for the determination of Chlorantraniliprole in test media **(Used in Algae : KCP 10.2.1/03 and Lemna : KCP 10.2.1/04).** The range of linearity, specificity, precision, recovery and LOQ and LOD of analytes were determined. Determination was performed by HPLC with MS/MS detection.

Material: Test item:
One batch (**3188-220519-01**) of Chlorantraniliprole 200 SC (product code ADM.0900.I.1.C) as manufactured.
Reference item:
- **Chlorantraniliprole** (CAS No.: 500008-45-7, batch No.: **BCBZ7553**, purity: 98.1 %, supplier: Sigma-Aldrich).

Equipment and conditions for Chlorantraniliprole determination

UHPLC system	Agilent UHPLC 1290 series with 6495b Triple Quad. Spectrometer
Column:	Agilent Zorbax Eclipse Plus C 18 RRHD 1.8 µm 2.1 x 50 mm
Column Temperature:	30ºC
Injection Volume:	3 µL
Mobile phases:	A: Ultrapure water acidified with 0.1 % formic acid B: Acetonitrile
Flow rate:	0.4 mL/min
Detector:	MassHunter Quantitative Analysis for QQQ, Version B.08.00 - Agilent Technologies - 2016
Ionisation:	Electro Spray (ESI) + AJS (Agilent Jet Stream)
Polarity:	Positive
Ion mass transition monitored (m/z)	*Chlorantraniliprole* Transition 1 (Quantifier): 483.9 → 452.9 Transition 2 (Qualifier): 483.9 → 285.9
Retention time (approx.)	Approx. 1.6 min

Table A 2.1.1.3-5: Recovery results of Chlorantraniliprole in test medium

Matrix	Analyte	Fortification level [mg/L]	Recoveries			n	Overall recovery	
			Single values [%]	Mean [%]	RSD [%]		Mean [%]	RSD [%]
EPA medium	Chlorantraniliprole	Mass transition 483.9 → 452.9 m/z						
		0.0102	84.55, 81.60, 85.53, 93.39, 80.61	85.14	5.92	5	87.94	9.22
		0.1017	94.67, 103.13, 95.36, 81.69, 78.94	90.76	11.17	5		
		Mass transition 483.9 → 285.9 m/z						
		0.0102	87.5, 92.41, 100.28, 108.14, 97.33	97.13	8.08	5	97.13	8.08
SIS medium		Mass transition 483.9 → 452.9 m/z						
		0.0101	87.77, 82.84, 86.79, 90.73, 84.81	86.59	3.45	5	86.74	3.95
		0.1014	84.02, 89.45, 80.97, 89.94, 90.14	86.90	4.80	5		
		Mass transition 483.9 → 285.9 m/z						
		0.0101	88.76, 107.50, 89.74, 89.74, 106.51	96.45	10.01	5	96.45	10.01

All mean recovery values at LOQ and 10LOQ fortification levels of Chlorantraniliprole in EPA medium and SIS medium comply with the standard acceptance criteria of SANCO/3029/99 rev 4, since mean recoveries were in the range of 70 % - 110 % with a relative standard deviation ≤ 20 %.

Table A 2.1.1.3-6: Characteristics for the analytical method used for validation of Chlorantraniliprole in test medium

	Chlorantraniliprole
Specificity	A confirmatory analysis was performed to demonstrate the selectivity of the primary detection transitions of quantifier for Chlorantraniliprole (Q1: m/z = 483.9 → m/z = 285.9). The specificity of the method has been demonstrated. No signal higher than 30% of the lowest fortified solution was detected at the retention time of Chlorantraniliprole in the blank samples.
Calibration (type, number of data points) Calibration range	The linearity of the detector response was demonstrated by double determination of calibration solutions at five concentration levels ranging from 0.0049 µg/L to 0.49 µg/mL for both medium. This range covers the range from no more than 30 % of the LOQ and at least + 20 % of the highest analyte concentration detected in any diluted sample. The response of Chlorantraniliprole in EPA medium was found to be linear in the range of concentrations 0.0049 µg/L – 0.4905 µg/L. Mass transition 483.9 → 452.9 m/z (Quantifier) y= 1198.147237 + 70582.270435 x r^2= 0.99764676 Mass transition 483.9 → 285.9 (Confirmatory) y= 1181.30 + 66523.43 x r^2= 0.9987 The response of Chlorantraniliprole in SIS medium was found to be linear in the range of concentrations 0.0049 µg/L – 0.4905 µg/L. Mass transition 483.9 → 452.9 m/z (Quantifier) y= 1462.936507 + 200667.375244 x r^2= 0.99911129 Mass transition 483.9 → 285.9 (Confirmatory) y= 1346.81 + 197428.12 x r^2= 0.9989
Precision and recovery	Mean recoveries and relative standard deviations per fortification level fulfil the criteria of guideline SANCO/3029/99 rev. 4, 11/07/2000 (70 - 110 % mean recovery, ≤ 20 % RSD).
Limit of determination / quantification	The limit of quantification is defined as the lowest fortification level at which an acceptable mean recovery was obtained (normally 70% - 110% with a relative standard deviation preferably ≤ 20%).

	Chlorantraniliprole
	For Chlorantraniliprole in EPA medium the LOQ was thus successfully established at the nominal value of 0.0102 µg/L. For Chlorantraniliprole in SIS medium the LOQ was thus successfully established at the nominal value of 0.0101 µg/L The minimum level established for the LOD was the one at which the analyte has a signal at least 3 times higher than the background noise of the response in the control solutions. LOD for both test medium was found to be 0.0049 µg/L.

Conclusion
The analytical method for the determination of Chlorantraniliprole in EPA test medium and SIS test medim was demonstrated to be satisfactory in terms of accuracy, precision, linearity, and specificity. Although only three replicates (instead of five) at three fortification levels have been performed for accuracy and repeatability the method is considered as fit for purpose for supporting ecotoxicological toxicity section. **(Used in Algae: KCP 10.2.1/03 and Lemna: KCP 10.2.1/04).**

Fifi, A.P. (2020b)

Comments of zRMS:	The method was sufficiently validated for the quantification of chlorantraniliprole in water and sugar feeding solutions coming from honeybee's laboratory. For chlorantraniliprole in water the LOQ was established at 3.7804 mg/L. For chlorantraniliprole in sugar feeding solutions the LOQ was established at 7.5970 mg/kg. The mean recoveries for chlorantraniliprole at each fortification level, and overall, were within the acceptable range of 70-110% with the relative standard deviation (RSD) within the acceptable range of ≤ 20%. The method is acceptable for risk assessment.

Reference:	KCP 5.1.2/04 Fifi, A.P. (2022)
Report	Validation of the analytical method for the determination of Chlorantraniliprole in the water and sugar feeding solutions with test item Chlorantraniliprole 200 SC (product code ADM.0900.I.1.C) coming from honeybee's laboratory tests.
Report No.	BT208/19
Guideline(s):	SANCO/3029/99 rev.4 (11/07/2000)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Summary
The goal of this study was to validate an analytical method for the determination of chlorantraniliprole in water and sugar feeding solutions coming from honeybee's laboratory. Calibration linearity, specificity, blank samples analysis, recoveries, accuracy, repeatability, matrix effect, limit of quantification (LOQ) and limit of detection (LOD) were checked according to SANCO/3029/99 rev.4. The analysis was performed by LC-MS/MS. The method was used in these studies: **Chronic oral toxicity to adult bees : KCP 10.3.1.2/01 and Toxicity to larvae: KCP 10.3.1.3/01.**

Materials and methods

Material: Test item:
Chlorantraniliprole 200 SC (ADM.0900.I.1.C) (Chlorantraniliprole CAS: 500008-45-7; batch No.: 3188-220519-01, Chlorantraniliprole (w/v): 206 g/L
Chlorantraniliprole (w/w): 18.9%).

Reference item: Chlorantraniliprole CAS: 500008-45-7; batch No.: BCBZ7553, Purity: 98.1 %, Supplier: Sigma-Aldrich.
The study was performed on the following matrices:
- ultrapure water,
- Sugar solution: Aqueous sucrose solution (50 % w/v).

Stock solutions preparation
10.4 mg of chlorantraniliprole was accurately weighed. A stock standard solution of 1 g/L of chlorantraniliprole in acetonitrile was prepared. Used for LOD, matrix effect and linearity.

Standard solution preparation:
Standard solutions at 1020,24 mg/L, 10,2024 mg/L, 102,024 µg/L, 20,4048 µg/L and 81,6192 µg/Lwere prepared by dilution of the stock standard solution in acetonitrile.

Water solutions: the stock solution for accuracy and precision test with ultrapure water was prepared using the Test Item (BT code: 187/19/C, Purity: 18.9 %). 200 mg test item/mL was prepared. Then diluted 0,01mg /mL. The final solutions are 37803.78 mg c chlorantraniliprole /L and 378.0378 mg chlorantraniliprole /L.

Sugar feeding solutions: the stock solution for accuracy and precision test with sugar feeding solution (= aqueous sucrose solution (50 % w/v)) was prepared using the Test Item (BT code: 187/19/C, Purity: 18,9 %) 100 mg test item/mL was prepared. Then diluted 0,01mg /mL. The final solutions are 19000,17 mg c chlorantraniliprole /L and 190,0017 mg chlorantraniliprole /L.

Fortification procedure
Water solutions: Five solutions were prepared by weighing about 2.0 g of the Test Item (BT code: 187/19/C, batch: 3188-220519-01) in a 10 mL volumetric flask (37837,422 mg/L of Chlorantraniliprole). Five solutions were prepared by diluting 0.10 mL in a 10 mL volumetric flask (3;7804 mg/L of Chlorantraniliprole).

Sugar feeding solutions: 1 g of sugar feeding solution was weighed in a 15 mL centrifuged tube and spiked to have 1823.0523 mg/kg of Chlorantraniliprole. The sample was extracted and the extract was diluted. 1g of sugar feeding solution was weighed in a 15 mL centrifuged tube and spiked to obtain the lowest fortification LOQ solutions at the mean nominal concentration 7.5950 mg/kg of Chlorantraniliprole. The sample was extracted and diluted.

HPLC conditions

HPLC system:	- Agilent UHPLC 1290 series with 6495 Triple Quad. Spectrometer
Analytical column:	- Agilent Zorbax Eclipse Plus C 18 RRHD 1.8 µm 3 x 50 mm
Mobile phases:	Solvent A: ultra-pure water + 0.1% formic acid Solvent B: acetonitrile Isocratic, 55/45
Flow rate:	0.4 mL/min
Column Temperature:	30 ºC
Injection volume:	2 µL
Stop time	about 4 min.
MS System	MassHunter Quantitative Analysis for QQQ, Version B.08.00 - Agilent Technologies - 2016 483,9→ 452,9 m/z (quantification) 483,9 → 285,9 m/z (confirmation) Ion Mode : positive
Retention time for Chlorantraniliprole:	approx. 1.5 minutes.

Results and discussions

Table CA 2.1.1.3-7: Accuracy results for validation of chlorantraniliprole in water and sugar feeding solutions

Analyte	Matrix	Fortification level	Recoveries			n	Overall recovery	
			Single values [%]	Mean [%]	RSD [%]		Mean [%]	RSD [%]
Chlorantraniliprole	water	primary mass transition 484 → 453 (m/z)						
		3.7804 mg/L	99.38, 98.57, 97.73, 97.57, 96.79	98.01	1.01	5	97.65	0.95
		37837.442 mg/L	96.63, 96.56, 98.24, 97.01, 98.16	97.32	0.84	5		
	water	confirmatory mass transition 484 → 286 (m/z)						
		3.7804 mg/L	98.03, 97.86, 100.95, 97.44, 95.61	97.98	1.96	5	97.98	1.96
Chlorantraniliprole	sugar feeding solutions	primary mass transition 484 → 453 (m/z)						
		7.5970 mg/kg	91.85, 91.88, 90.21, 89.46, 89.89	90.66	1.25	5	93.67	3.51
		1824.0000 mg/kg	97.65, 95.77, 96.43, 96.67, 96.85	96.67	0.70	5		
	sugar feeding solutions	confirmatory mass transition 484 → 286 (m/z)						
		7.5970 mg/kg	90.71, 90.31, 88.51, 90.31, 90.27	90.02	0.96	5	90.02	0.96

Table CA 2.1.1.3-8: Characteristics for the analytical method used for validation of chlorantraniliprole in water and sugar feeding solutions

	Chlorantraniliprole
Specificity	For each matrix, the specificity of the method was checked by the analysis of blank matrix (water and sugar feeding matrices). Analysis of blank matrix did not yield residues of chlorantraniliprole above 30 % of the limit of quantification, indicating that no interference was present at the retention time of chlorantraniliprole in the laboratory samples. This was in accordance with the level specified in the guideline, which demands blank values (non-fortified samples) less than 30 % of the LOQ. The selectivity and specificity of the method were demonstrated.
Linearity	For each matrix, the linearity of the method was determined by measuring the detector response (peak area) versus the concentration of a series of at least 5 calibration standard solutions and covered a maximum of two orders of magnitude. Linearity primary mass transition: Water (1.0202 µg/L – 10.2024 µg/L) y= 11390.1172x-2739.1895 R2 = 0.9979 Sugar feeding solutions (1.0202 µg/L – 10.2024 µg/L) y=10445.2022x-2570.8707 R2 = 0.9966 Linearity confirmatory mass transition: Water (1.0202 µg/L – 10.2024 µg/L) y=10804.7927-2296.1331 R2 = 0.9983 Sugar feeding solutions (1.0202 µg/L – 10.2024 µg/L) y=9747.4277-1959.3367 R2 = 0.9977

<table>
<tr><td></td><td colspan="3">Chlorantraniliprole</td></tr>
<tr><td>Matrix effects</td><td colspan="3">
<table>
<tr><td rowspan="2">Water</td><td>LOQ</td><td>-6,03%; -8,19%</td></tr>
<tr><td>High fortification level</td><td>-6,57%; -7,51%</td></tr>
<tr><td rowspan="2">Sugar feeding solutions</td><td>LOQ</td><td>0,42%; -4,65%</td></tr>
<tr><td>High fortification level</td><td>0,10%; -3,56%</td></tr>
</table>
Matrix effects were not considered significant as they didn’t exceed ± 20% All analyses were carried out using matrix-matched standard calibration solutions for all matrices.
</td></tr>
<tr><td>Stability of extracts</td><td colspan="3">Final sample extracts were analysed within 24 hours after initial extraction thus no stability study was performed.</td></tr>
<tr><td>Precision and recovery</td><td colspan="3">Mean recoveries and relative standard deviations per fortification level fulfil the criteria of guideline SANCO/3029/99 rev 4</td></tr>
<tr><td>Limit of determination / quantification</td><td colspan="3">Sugar feeding solutions:
The limit of quantification (LOQ)= 7.5970 mg/kg
The limit of detection (LOD)= 1.0202 µg/L
Water:
The limit of quantification (LOQ)= 3.7804 mg/L
The limit of detection (LOD)= 1.0202 µg/L</td></tr>
</table>

Conclusion
The method was validated according to guideline SANCO/3029/99 rev.4 (11/07/2000). with regard to specificity, linearity of detector response, accuracy and precision for Chlorantraniliprole in water and sugar feeding solutions is considered acceptable. (**Used in Chronic oral toxicity to adult bees : KCP 10.3.1.2/01 and Toxicity to larvae: KCP 10.3.1.3/01).**

Fifi, A.P. (2019)

Comments of zRMS:	The analytical method has been demostrated to be reliable and accurate procedure for the determination of chlorantraniliprole in plant matrices: peach, grape (bunches), wheat grain, oilseed rape seed and dry broad bean. The method complies withe the guideline SANTE/2020/12830, Rev.1. Chlorantraniliprole was determined by HPLC-MS/MS. The LOQ was established at 0.01 mg/kg for each matrix. The method is acceptable.

Reference:	KCP 5.1.2/05, Barbier, G. (2021)
Report	Validation of an analytical method for the determination of chlorantraniliprole in plant matrices: peach, grape (bunches), wheat grain, oilseed rape seed and dry broad bean.
Report No.	B20G-A4-C-01
Guideline(s):	SANTE/2020/12830 Rev.1.
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Summary
The goal of this study was to validate an analytical method, based on the QuEChERS extraction, for the determination of chlorantraniliprole in plant matrices: peach (commodity with high water content), grape

(bunches) (commodity with high acid content), wheat grain (dry commodity with high starch content), oilseed rape seed (commodity with high oil content) and dry broad bean (dry commodity with high protein content). Calibration linearity, specificity, blank samples analysis, recoveries, accuracy, repeatability, matrix effect, limit of quantification (LOQ) and limit of detection (LOD) were checked according to SANTE/2020/12830 Rev. 1. The analysis was performed by LC-MS/MS detection. **(Used in KCP 8.3/01 to 13; KCP 8.5.3/01 to 04).**

Materials and methods

Material: Reference item:

- **Chlorantraniliprole** (CAS: 500008-45-7; batch No.: G1033981, purity: 97,28 %, supplier: LGC Labor GmbH).

Stock solutions preparation
Between 10 and 50 mg of chlorantraniliprole was accurately weighed. A stock standard solution of exactly 1 g/L of chlorantraniliprole in acetone was prepared by taking the purity into account. This solution was shaken thoroughly until complete dissolution using an ultrasonic bath. The stock standard solution was stored in a brown flask at a temperature of about -18°C.

Standard solutions, Fortification solutions, Matrix-matched calibration solutions:
Standard solutions at 10 and 1 mg/L were prepared by dilution of the stock standard solution in the acetonitrile/1% formic acid mixture. The standard solutions were stored in a brown flask at a temperature of about - 18°C.

Fortification procedure:
All fortifications were performed directly to aliquots of the laboratory sample in a 50 mL centrifuge tube after weighing the sample. They were performed with the appropriate spiking standard solutions using a volumetric pipette.
2 fortification levels were prepared and analysed:
- 1 * LOQ
- 10 * LOQ

Sample preparation **Peach and Grape (bunches)**
Accurately, weigh 10 g of ground laboratory sample into a 50 mL centrifuge tube. For recoveries, fortify the aliquots with the appropriate spiking standard solutions using a pipette.
Using a measuring cylinder, add 10 mL of acetonitrile/1% formic acid mixture. Homogenise horizontally on a mechanical shaker at 200 cps/min during 20 minutes. Add one QuEChERS extraction salt packet containing $MgSO_4$/NaCl. Shake manually and vigorously during 1 minute. Centrifuge for 5 minutes at 4000 rpm. Dilute an aliquot of the supernatant 10-fold into the acetonitrile/1% formic acid mixture in a 2 mL vial. This is the final extract.
A typical sample set consists of 36 analyses per day and per person, from extraction to preparation of final extract.

Sample preparation **Wheat grain** and **Dry broad bean**
Accurately, weigh 2 g of ground laboratory sample into a 50 mL centrifuge tube. For recoveries, fortify the aliquots of laboratory sample with the appropriate spiking standard solutions using a pipette.
Using measuring cylinders, add 10 mL of ultra-pure water then 10 mL of acetonitrile/1% formic acid mixture. Homogenize horizontally on a mechanical shaker at 200 cps/min during 20 minutes.
Add one sachet of $MgSO_4$/NaCl. Shake manually and vigorously during 1 minute. Centrifuge for 5 minutes at 4000 rpm. Dilute an aliquot of the supernatant two-fold into the acetonitrile/1% formic acid mixture in a 2 mL vial. This is the final extract.
A typical sample set consists of 36 analyses per day and per person, from extraction to preparation of final extract.

Sample preparation **Oilseed rape seed**

Accurately, weigh 2 g of ground laboratory sample into a 50 mL centrifuge tube. For recoveries, fortify the aliquots of laboratory sample with the appropriate spiking standard solutions using a pipette. Using measuring cylinders, add 40 mL of acetonitrile/1% formic acid mixture. Homogenize horizontally on a mechanical shaker at 200 cps/min during 20 minutes. Centrifuge for 5 minutes at 4000 rpm. Put the 50 mL centrifuge tube during two hours into a freezer at about -18°C. Aliquot the supernatant into a 2 mL vial. This is the final extract.

A typical sample set consists of 36 analyses per day and per person, from extraction to preparation of final extract.

HPLC –MS/MS conditions

Analytical column: Column C18 Kinetex (100 mm X 4.6 mm ID X 2.6 µm)
Mobile phases: Phase A: ultra-pure water + 0.1% formic acid
Phase B: acetonitrile + 0.1% formic acid

Gradient:

Time (min)	A %	B %
0	95	5
3	0	100
5	0	100
5.1	95	5
7.0	95	5

Flow rate: 0.7 mL/min
Injection volume: 2 µL
Scan type: MS/MS
Ionisation type: Positive Multiple reaction Monitoring (MRM)
Polarity: Negative
Ion mass transition monitored (m/z) Transition 1 (Quantifier):
484 → 453
Transition 2 (Qualifier):
484 → 286
Retention time (approx.) Chlorantraniliprole = 3.6 – 3.7 min

Results and discussions

Table CA 2.1.1.3-9: Accuracy results for validation of Chlorantraniliprole in plant matrix

Analyte	Matrix	Fortification level [mg/kg]	Recoveries			n	Overall recovery	
			Single values [%]	Mean [%]	RSD [%]		Mean [%]	RSD [%]
Chlorantraniliprole	Peach (whole fruit without stones)	primary mass transition 484 → 453 (m/z)						
		0.01	95, 100, 100, 97, 101	99	2	5	99	2
		0,1	99, 101, 97, 102, 102	100	2	5		
	Peach (whole fruit without stones)	confirmatory mass transition 484 → 286 (m/z)						
		0.01	94 , 94 , 95, 98, 101	97	3	5	98	3
		0,1	101, 95, 95, 99, 101	98	3	5		
Chlorantraniliprole	Organic grape (bunches)	primary mass transition 484 → 453 (m/z)						
		0.01	97, 96, 97, 95, 97	96	1	5	98	2
		0,1	100, 100, 98, 103, 99	100	2	5		
	Organic grape (bunches)	confirmatory mass transition 484 → 286 (m/z)						
		0.01	95, 100, 98, 94, 97	97	2	5	98	3
		0,1	101, 98, 100, 100, 94	99	3	5		

Analyte	Matrix	Fortification level [mg/kg]	Recoveries			n	Overall recovery	
			Single values [%]	Mean [%]	RSD [%]		Mean [%]	RSD [%]
Chlorantraniliprole	Wheat grain	primary mass transition 484 → 453 (m/z)						
		0.01	87, 93, 88, 92, 90	90	2	5	91	2
		0,1	93, 95, 93, 91, 91	92	2	5		
	Wheat grain	confirmatory mass transition 484 → 286 (m/z)						
		0.01	90, 93, 92, 93, 93	92	1	5	94	2
		0,1	97, 93, 97, 95, 97	96	2	5		
Chlorantraniliprole	Oilseed rape seed	primary mass transition 484 → 453 (m/z)						
		0.01	94, 87, 84, 92, 85	88	4	5	89	5
		0,1	85, 96, 84, 92, 88	89	5	5		
	Oilseed rape seed	confirmatory mass transition 484 → 286 (m/z)						
		0.01	100, 85, 90, 90, 85	90	6	5	89	5
		0,1	85, 90, 90, 90, 83	87	3	5		
Chlorantraniliprole	Dry broad bean	primary mass transition 484 → 453 (m/z)						
		0.01	74, 75, 79, 77, 74	76	2	5	79	5
		0,1	80, 80, 77, 86, 84	81	4	5		
	Dry broad bean	confirmatory mass transition 484 → 286 (m/z)						
		0.01	74, 75, 76, 81, 76	76	3	5	78	4
		0,1	80, 83, 81, 79, 81	81	2	5		

Table CA 2.1.1.3-10: Characteristics for the analytical method used for validation of Chlorantraniliprole in plant matrix

	Chlorantraniliprole
Specificity	Chlorantraniliprole was analysed by the LC-MS/MS highly specific detection system. No interference above 30% of LOQ was observed in control samples at retention time of target analyte.
Linearity	Peach To evaluate linearity, calibration solutions were prepared by dilution of a stock solution ranging from 0.3 µg/L to 20 µg/L; 0.010 to 0.100 mg/kg. $y = -2345{,}5182\ x^2 + 711798{,}9616\ x - 7273{,}9709$ $r = 0.9998$ Organic grape (bunches) To evaluate linearity, calibration solutions were prepared by dilution of a stock solution ranging from 0.3 µg/L to 20 µg/L; 0.010 to 0.100 mg/kg $y = -1120{,}8818\ x^2 + 730855{,}4296\ x - 4953{,}2869$ $r = 0.9992$ Wheat grain To evaluate linearity, calibration solutions were prepared by dilution of a stock solution ranging from 0.3 µg/L to 20 µg/L; 0.010 to 0.100 mg/kg $y = -3980{,}4593\ x^2 + 630711{,}1333\ x - 2808{,}4556$ $r = 0.9998$ Oilseed rape seed To evaluate linearity, calibration solutions were prepared by dilution of a stock solution ranging from 0.15 µg/L to 10 µg/L; 0.010 to 0.100 mg/kg $y = -2102{,}1103\ x^2 + 552150{,}3228\ x - 16878{,}8626$ $r = 0.9982$

	Chlorantraniliprole
	Dry broad bean To evaluate linearity, calibration solutions were prepared by dilution of a stock solution ranging from 0.3 µg/L to 20 µg/L; 0.010 to 0.100 mg/kg $y = -5343{,}8786\ x^2 + 680197{,}1507\ x - 10079{,}9051$ $r = 0.9992$
Matrix effects	No significant matrix effect (< 20% for all matrices) However, matrix-matched standard solutions were used for calibration
Stability of extracts	For peach, grape, wheat grain, oilseed rape seed and dry broad bean, the final sample extracts were analysed within 24 hours after initial extraction thus no stability study was performed The stability of Chlorantraniliprole in sample extracts was sufficiently proven, as the recoveries in the fortified samples are within the acceptable range.
Precision and recovery	Mean recoveries and relative standard deviations per fortification level fulfil the criteria of guideline SANTE/2020/12830 Rev.1.
Limit of determination / quantification	LOD = 0,003 mg Chlorantraniliprole /kg LOQ = 0.010 mg Chlorantraniliprole /kg

Conclusion
The method was validated according to guideline SANTE/2020/12830 Rev.1. with regard to specificity, linearity of detector response, accuracy and precision for Chlorantraniliprole in peach, grape, wheat grain, oilseed rape seed and dry broad bean. **(Used in KCP 8.3/01 to 13; KCP 8.5.3/01 to 04).**

Barbier, G. (2021)

Comments of zRMS:	The method was sufficiently validated for the quantification of chlorantraniliprole in water solutions coming from terrestrial plants laboratory tests. For chlorantraniliprole in water the LOQ was established at 9.5 g/L. The mean recoveries for chlorantraniliprole at each fortification level, and overall, were within the acceptable range of 70-110% with the relative standard deviation (RSD) within the acceptable range of ≤ 20%. The method is acceptable for risk assessment.

Reference:	KCP 5.1.2/06 Fifi, A.P. (2020c)
Report	Validation of the analytical method for the determination of Chlorantraniliprole in the water solutions with test item Chlorantraniliprole 200 SC (product code ADM.0900.I.1.C) coming from terrestrial plants laboratory tests.
Report No.	BT209/19
Guideline(s):	SANCO/3029/99 rev.4 (11/07/2000)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Summary
The goal of this study was to validate an analytical method for the determination of chlorantraniliprole content in the water solutions coming from terrestrial plants laboratory tests. Specificity (= Confirmatory analysis), Linearity, Accuracy (as mean recovery), Precision (as Relative Standard Deviation), Limit of Detection (LOD) and Limit of Quantification (LOQ) were determined.according to SANCO/3029/99 rev.4. The analysis was performed by LC-MS/MS. **(Used in KCP 10.6/01).**

Materials and methods

Material: Test item:
Chlorantraniliprole 200 SC (ADM.0900.I.1.C) (Chlorantraniliprole CAS: 500008-45-7; batch No.: 3188-220519-01, Chlorantraniliprole (w/v): 206 g/L Chlorantraniliprole (w/w): 18.9%).

Reference item: Chlorantraniliprole CAS: 500008-45-7; batch No.: BCCC3567, Purity: 96.6 %, Supplier: Sigma-Aldrich.
The study was performed on the following matrices:
- ultrapure water,

Stock solutions preparation
10.3 mg of chlorantraniliprole was accurately weighed. A stock standard solution of 1 g/L of chlorantraniliprole in acetonitrile was prepared. Used for LOD, matrix effect and linearity.

Standard solution preparation:
Standard solutions at 994.9800 mg/L, 9.9498 mg/L, 99.4980 µg/L, 79.5984 µg/L, 19.8996 µg/L were prepared by dilution of the stock standard solution in acetonitrile.

Water solutions: the stock solution for accuracy and precision test with diluent (ultrapure water / acetonitrile). The Test Item used was BT code: 187/19/C, Purity: 18.9 %.

Fortification procedure
Water solutions: Five solutions were prepared by weighing about 1.2 g of the Test Item (BT code: 187/19/C, batch: 3188-220519-01) in a 10 mL volumetric flask (22.5g/L of Chlorantraniliprole). Five solutions were prepared by diluting 4.17 mL in a 10 mL volumetric flask (9.5 mg/L of Chlorantraniliprole).

HPLC conditions

HPLC system:	- Agilent UHPLC 1290 series with 6495 Triple Quad. Spectrometer
Analytical column:	- Agilent Zorbax Eclipse Plus C 18 RRHD 1.8 µm 3 x 50 mm
Mobile phases:	Solvent A: ultra-pure water + 0.1% formic acid Solvent B: acetonitrile Isocratic, 55/45
Flow rate:	0.4 mL/min
Column Temperature:	30 ºC
Injection volume:	2 µL
Stop time	about 4 min.
MS System	MassHunter Quantitative Analysis for QQQ, Version B.08.00 - Agilent Technologies - 2016 483,9→ 452,9 m/z (qualifier) 483,9 → 285,9 m/z (quantifier) Ion Mode : positive
Retention time for Chlorantraniliprole:	approx. 1.5 - 1.6 minutes.

Results and discussions

Table CA 2.1.1.3-11: Accuracy and precision results for validation of chlorantraniliprole in water solutions

Analyte	Matrix	Fortification level	Recoveries			n	Overall recovery	
			Single values [%]	Mean [%]	RSD [%]		Mean [%]	RSD [%]
Chlorantraniliprole	water	primary mass transition 484 → 286 (m/z)						

Analyte	Matrix	Fortification level	Recoveries			n	Overall recovery	
			Single values [%]	Mean [%]	RSD [%]		Mean [%]	RSD [%]
		9.5 g/L	99.94, 99.23, 103.86, 99.45, 101.73	100.84	1.93	5	100.52	1.96
		22.8 g/L	101.75, 101.92, 97.01, 100.53 99.77	100.20	1.99	5		
	water	confirmatory mass transition 484 → 453 (m/z)						
		9.5 g/L	100.45 100.83 109.78 101.80 101.52	102.88	3.79	5	102.88	3.79

Table CA 2.1.1.3-12: Characteristics for the analytical method used for validation of chlorantraniliprole in water solutions

	Chlorantraniliprole
Specificity	The specificity of the method was checked by the analysis of blank matrix (water). Analysis of blank matrix did not yield residues of chlorantraniliprole above 30 % of the limit of quantification, indicating that no interference was present at the retention time of chlorantraniliprole in the laboratory samples. This was in accordance with the level specified in the guideline, which demands blank values (non-fortified samples) less than 30 % of the LOQ. The selectivity and specificity of the method were demonstrated.
Linearity	The linearity of the method was determined by measuring the detector response (peak area) versus the analyte concentration of a series of at least 5 calibration standard solutions and covered a maximum of two orders of magnitude. Linearity primary mass transition: Water (0.9950 µg/L – 9.9498/L) y= 16970.29x-1492.09 R2 = 0.9969 Linearity confirmatory mass transition: Water (0.9950 µg/L – 9.9498/L) y= 14592.72x-1125.40 R2 = 0.9977
Matrix effects	The percentage of Blank samples signal compared to LOQ Water | LOQ | 0.13%, 0.09% No signal higher than 30% of the lowest fortified solution was detected at the retention time of Chlorantraniliprole in the blank samples.
Precision and recovery	Mean recoveries and relative standard deviations per fortification level fulfil the criteria of guideline SANCO/3029/99 rev 4
Limit of determination / quantification	The limit of quantification (LOQ)= 9.5308 g/L The limit of detection (LOD)= 0.9950 µg/L

Conclusion

The method was validated according to guideline SANCO/3029/99 rev.4 (11/07/2000). with regard to specificity, linearity of detector response, accuracy and precision for Chlorantraniliprole in water solutions is considered acceptable. **(Used in KCP 10.6/01).**

Fifi, A.P. (2020)

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

The primary method (KCP 5.1.2/01) summarized in A.2.1.1.3. Here below the ILV of this primary study is presented.

Comments of zRMS:	The analytical method (Barbier, G. (2022)) for the determination of concentrations of chlorantraniliprole in honey by HPLC-MS/MS using two MRM trasitions has been independly validated. The limit of quantitation (LOQ) is 0.01 mg/kg in honey. the mean recovery value for chlorantraniliprole was between 70 – 120% with a relative standard deviation of ≤ 20% at each fortification level for both mass transitions monitored. The method is acceptable.

Reference:	KCP 5.2/01 Brown, D. (2022)
Report	Independent laboratory validation of analytical method B20G-A4-C-02 (Adama study No. 000105720) for determination of chlorantraniliprole in honey.
Report No.	RES-00420
Guideline(s):	SANTE/2020/12830, Rev.1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Summary

The goal of this study was to validate an analytical method for the determination of chlorantraniliprole residues in honey. Calibration linearity, specificity, blank samples analysis, recoveries, accuracy, repeatability, matrix effect, limit of quantification (LOQ) and limit of detection (LOD) were checked according to SANTE/2020/12830 Rev. 1. The analysis was performed by LC-MS/MS.

Materials and methods

Material: Test/Reference item: Chlorantraniliprole CAS: 500008-45-7; batch No.: 644-021-05, Purity: 99.1 %,

The study was performed on the following matrices:

- honey

Stock solutions preparation

A stock solution of chlorantraniliprole was prepared on 30/08/2022 at a concentration of 1000 µg/mL in acetone with the aid of an ultrasonic bath, by dissolving ca. 20.0 mg in 20 mL of solvent (1 g/L).

Standard solution preparation:

Fortification standard solutions were prepared by serial dilution of the stock solution using 1% formic acid in acetonitrile 0.1-10 µg/mL.

Intermediate calibration standard solutions were prepared by serial dilution of the fortification standard solutions using 1% formic acid in acetonitrile on the day of analysis 0.05-2.0 µg/mL.

Fortification procedure

Fortification 0,01 and 0,1 mg/kg. Procedural recoveries were prepared by fortifying sub-samples of untreated matrix with the appropriate fortification solution.

HPLC conditions

HPLC system:	-AB Sciex 5500 Mass Spectrometer with an Agilent 1260 Binary HPLC Pump, Degasser and Column Oven, a CTC Analytics HTC PAL Autosampler and a Peak Scientific ABN2ZA Gas Generator was used in the study.

Analytical column:	- Kinetex C18, 50 x 2.0 mm, 2.6 µm Particle Size (Phenomenex)		
Mobile phases:	Solvent A: 0.1% Formic acid in ultra-pure water B Solvent B: 0.1% Formic acid in Acetonitrile		
	Time (minutes)	A (%)	B (%)
	0.00	95	5
	3.00	0	100
	5.00	0	100
	5.10	95	5
	7.00	95	5
Flow rate:	0.7 mL/min		
Column Temperature:	40 °C		
Injection volume:	2 µL		
MS System	Turbo Ion Spray (Electrospray)		
	484.0 → 453.0 m/z (qualifier) 484.0 → 286.0 m/z (quantifier) Ion Mode : positive		

Results and discussions

Table CA 2.1.2-1: Accuracy and precision results for validation of chlorantraniliprole in honey

Analyte	**Matrix**	**Fortification level**	**Recoveries**			**n**	**Overall recovery**	
			Single values [%]	**Mean [%]**	**RSD [%]**		**Mean [%]**	**RSD [%]**
Chlorantraniliprole	honey	primary mass transition 484.0 → 453.0 (m/z)						
		0.01mg/kg	113 105 97 105 104	105	5.2	5	106	4.0
		0.1mg/kg	108 110 106 101 107	106	2.9	5		
	honey	confirmatory mass transition 484.0 → 286.0 (m/z)						
		0.01mg/kg	107 105 94 101 102	102	4.7	5	104	4.4
		0.1mg/kg	108 109 106 99 107	106	3.7	5		

Table CA 2.1.2-2: Characteristics for the analytical method used for validation of chlorantraniliprole in honey

	Chlorantraniliprole
Specificity	The specificity of the method was demonstrated by 2 mass transitions. The selectivity of the method for chlorantraniliprole was demonstrated by LC-MS/MS where no significant interferences ≥ 30% of the LOQ were detected in any of the reagent blank or control specimens.

<table>
<tr><td></td><td>Chlorantraniliprole</td></tr>
<tr><td>Linearity</td><td>The linearity of the detector was confirmed on a run-by-run basis by single injection of matrix matched calibration standards at 8 concentration levels.
The linearity of the detector response was confirmed over the range 0.3 – 20 ng/mL. This corresponds to a range of 0.003 mg/kg (30% LOQ) to 0.2 mg/kg (200% of the higher fortification level).

Linearity primary mass transition: 484.0 → 453.0 (m/z)
y = 5314.9 x + 248.0
r = 0.9974

Linearity confirmatory mass transition: 484.0 → 286.0 (m/z)
y = 5176.2 x + 333.8
r = 0.9976</td></tr>
<tr><td>Matrix effects</td><td>Matrix effects on detection were evaluated by triplicate injection of a matrix-matched standard solution equivalent to the LOQ and triplicate injection of a solvent standard of the same concentration.
<table>
<tr><td>Honey</td><td>Mass Transition</td><td>Matrix effect (%)</td></tr>
<tr><td></td><td>484 → 453 m/z</td><td>-17.9</td></tr>
<tr><td></td><td>484 → 286 m/z</td><td>-18.9</td></tr>
</table>
No signal higher than 30% of the lowest fortified solution was detected at the retention time of Chlorantraniliprole in the blank samples.</td></tr>
<tr><td>Stability of extracts</td><td>For chlorantraniliprole the peak area of the stored standard (mean of 5 injections) was compared to the peak area of the freshly prepared standard (mean of 5 injections).
The difference when compared to the fresh standard was ≤ 10%. Difference (%)=-8.5</td></tr>
<tr><td>Precision and recovery</td><td>The accuracy and precision of the method was successfully demonstrated as the mean recovery value for chlorantraniliprole was between 70 – 120% with a relative standard deviation of ≤ 20% at each fortification level for both mass transitions monitored.</td></tr>
<tr><td>Limit of determination / quantification</td><td>The limit of quantification (LOQ)= 0.01 mg/kg
The limit of detection (LOD)= 0.003 mg/kg</td></tr>
</table>

Conclusion

The method was validated according to guideline SANTE/2020/12830 Rev.1. with regard to specificity, linearity of detector response, accuracy and precision for Chlorantraniliprole in honey is considered acceptable. **The method is suitable for post-monitoring control analysis (LOQ=0.01 mg/kg; Matrix=honey).**

Brown, D. (2022)

A 2.1.2.1 Description of methods for the analysis of body fluids and tissues (KCP 5.2)

A 2.1.2.1.1 DuPont-49234

Comments of zRMS:	This study has been evaluated and accepted by zRMS-FR in RR of Chlorantraniliprole 200 g/L SC (April 2022). zRMS-FR conclusion: Acceptable. This method is validated for the determination of Chlorantraniliprole in Plasma and urine using HPLC/ESI-MS/MS at the LOQ of 1µg/L.

Reference:	KCP 5.2/02
Report:	Pentz, A.M., Cabusas, E.M., (2018); Analytical method for the determination of cyantraniliprole (DPX-HGW86) and chlorantraniliprole (DPX-E2Y45) in plasma and urine by HPLC/ESI-MS/MS
Report No.:	Report No. 49234
Testing Facility Report No.:	Report No. 49234
Guidelines	OPPTS 860.1340, SANCO/825/00 rev. 8.1 (2010)
Deviations:	None
GLP:	Yes

Method validation
The method was successfully validated for the analysis of chlorantraniliprole in plasma and urine.

Materials and methods
Residues of chlorantraniliprole were extracted from plasma or urine sample by two consecutive vigorous shaking in 9:1 acetonitrile: water and centrifugations. The supernatants/extracts were decanted and combined and then partitioned with hexane; the hexane fraction was discarded. A 500-µL extract aliquot was diluted with 500 µL of 0.02 M formic acid (aq) and filtered through a 0.45-µm PTFE disc. The sample extract was analyzed by reversed-phase HPLC with electrospray ionization mass spectrometry/mass spectrometry (ESI MS/MS) for detection. At least two ion transitions (primary/quantitative and confirmatory) were monitored during sample analyses.

Results and discussions

Table A 1: Quantitative data for analytical methods for the determination of chlorantraniliprole in plasma and urine using LC/MS/MS

Matrix	Fortification level (µg/L) [a, b]	Number of tests	Average recovery (%)	Standard deviation	% Relative standard deviation	Reference
Chlorantraniliprole (484→ 453)						
Plasma	1.0 10	5 5 Total = 10	98 109	10.1 7.1	10.3 9.8	DuPont-49234
Urine	1.0 10	5 5 Total = 10	98 98	6.2 3.8	6.3 3.8	

a Fortifications were performed with analyte reference standard solutions
b Limit of quantification, defined by a signal-to-noise ratio of approximately 10 and the lowest validated fortification level

Table A 2: Characteristics for the analytical method used for validation of chlorantraniliprole residues in plasma and urine

	Chlorantraniliprole
Specificity	A mass spectrum is provided, and the blank value is <30% LOQ
Calibration (type, number of data points)	Individual calibration data is presented, and a calibration line equation presented.

	Chlorantraniliprole
Specificity	A mass spectrum is provided, and the blank value is <30% LOQ
	Set #1: y=197279x-291.9; r^2=1.0000 A total of 7 points were used to generate the calibration curve.
Calibration range	Accepted calibration range from 0.010 to 5.0 ng/mL is provided. This calibration range corresponds to 0.25 to 25 µg/L of chlorantraniliprole in a sample.
Assessment of matrix effects is presented	Yes, matrix effects were evaluated and not observed. All calibration curves were generated in solvent.
Limit of determination/quantification	1.0 µg/L

Conclusion
A method was successfully validated for the analysis of chlorantraniliprole in plasma and urine.

Independent laboratory validation
An independent laboratory validation is not required for body fluid methods.

Confirmatory method
Materials and methods
Two ion transitions were used for the analysis of chlorantraniliprole in plasma and urine. The results using the confirmatory ion transition are reported below.

Results and discussions

Table A 3: Confirmation data for analytical methods for the determination of chlorantraniliprole in plasma and urine using LC/MS/MS

Matrix	Fortification level (µg/L) [a, b]	Number of tests	Average recovery (%)	Standard deviation	% Relative standard deviation	Reference
Chlorantraniliprole (484→ 286)						
Plasma	1.0 10	5 5 Total = 10	107 112	10.7 4.9	10.1 4.4	DuPont-49234
Urine	1.0 10	5 5 Total = 10	101 100	7.2 7.8	7.2 7.8	

a Fortifications were performed with analyte reference standard solutions
b Limit of quantification, defined by a signal-to-noise ratio of approximately 10 and the lowest validated fortification level

Table A 4: Characteristics for the confirmation method used for validation of chlorantraniliprole residues in plasma and urine

	Chlorantraniliprole
Specificity	A mass spectrum is provided, and the blank value is <30% LOQ
Calibration (type, number of data points)	Individual calibration data is presented, and a calibration line equation presented. Set #1: y=171936x-147.1; r^2=0.9992 A total of 7 points were used to generate the calibration curve.
Calibration range	Accepted calibration range from 0.010 to 5.0 ng/mL is provided. This calibration range corresponds to 0.25 to 25 µg/L of chlorantraniliprole in a sample.
Assessment of matrix effects is presented	Yes, matrix effects were evaluated and not observed. All calibration curves were generated in solvent.
Limit of determination/quantification	1.0 µg/L

Conclusion
A method and a confirmation method has been validated for the analysis of chlorantraniliprole in plasma and urine.

Extraction efficiency
Extraction efficiency is not required for body fluid methods. Since these samples are liquids the fortification data is reflective of the extraction efficiency.

A 2.1.2.2 Extraction efficiency (KCP 5.2)

Comments of zRMS:	Study FMC-51880 has been submitted in the renewal dossier of Chlorantraniliprole (Document M-CA, Section 4, Annex Point 4.2/01). A number of methods, including the previously assessed monitoring method and the QuEChERS method used in the magnitude of residues studies in this submission have been compared. It was shown that the extraction efficiency in all standard crop matrix types is acceptable. Acceptable.

Reference:	KCA 4.2/01
Report author:	Brown, D.
Report year	2021
Report title	Determination of the extraction efficiency of chlorantraniliprole (E2Y45) residues using multiple extraction procedures and analytical methods
Report No.:	FMC-51880
Test Facility Document No.:	230134
Guidelines followed:	SANTE 2017/10632 Rev 3, SANCO/825/00 rev.8.1 (2010)
Deviations from current guidelines:	None
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

An extraction efficiency bridging study was conducted to compare the ability to extract incurred residues of chlorantraniliprole using the metabolism method (DuPont-12265 Revision no. 1), the single analyte residue method (DuPont-13295), the DFG S 19 multi-residue method (DuPont-15025) and the QuEChERS multi-residue method (DuPont-27124). Incurred field treated residue samples of lettuce, hops, grapes, olives, and dried peas were generated as part of this study or used from a previous field study. The incurred residue samples were analysed using each method and the residue values determined. The average residue values determined using the single analyte residue method, the DFG S 19 multi-residue method and the QuEChERS multi-residue method were compared against the average residue value determined using the metabolism method to assess the extraction efficiency of each method in all EU crop groups.

Method validation
Prior to the analysis of the incurred residue samples, the metabolism method (DuPont-12265 Revision No. 1), the single analyte residue method (DuPont-13295), the DFG S 19 multi-residue method (DuPont-15025) and the QuEChERS multi-residue method (DuPont-27124) were validated at the facility conducting the bridging study. Since the incurred residue samples were known to contain chlorantraniliprole residues at a level greater than 0.05 mg/kg the method limit of quantitation of each method was increased to 0.05 mg/kg. Validating the method at an elevated limit of quantitation (above 0.01 mg/kg) allowed for modifications to

the methods to be made. All modifications were made to the extract purification steps; the extraction methods remained unchanged.

Materials and methods

The metabolism method (DuPont-12265 Revision No. 1) for lettuce, grapes, dried peas and hops involves blending the sample twice with acetonitrile and twice with acetonitrile: water (1:1, v/v) combined the extracts and analysing an aliquot by liquid chromatography with tandem mass spectrometry employing atmospheric pressure chemical ionisation in positive mode. The metabolism method for olives involves blending of the sample with acetonitrile: water (90:10, v/v) and then with acetonitrile: water (70:30, v/v) combined the extracts and analysing an aliquot by liquid chromatography with tandem mass spectrometry employing atmospheric pressure chemical ionisation in positive mode.

The single analyte residue method (DuPont-13295) for lettuce, grapes, dried peas, hops and olives involves blending the sample twice with water/acetonitrile and analysing an aliquot of the extract by liquid chromatography with tandem mass spectrometry employing atmospheric pressure chemical ionisation in positive mode

The DFG S 19 multi-analyte method (DuPont-15025) for lettuce, grapes, dried peas, and olives involves extraction with acetone. Water is added beforehand so that the acetone/water ratio remains constant at 2/1 (v/v). To form a liquid-liquid partition ethyl acetate/cyclohexane (1/1, v/v) and sodium chloride are added. An aliquot of the organic phase is filtered and then is evaporated. The extracts were reconstituted by adding acetonitrile at the ratio remain constant at (1/1, v/v). The residue extracts are then analysed by LC-MS/MS analysis.

The QuEChERS multi-analyte method (DuPont-27124) for lettuce, grapes, dried peas, and olives involves homogenization with acetonitrile/water. After addition of MgSO4, NaCl and buffering citrate salts (pH 5-5.5); the mixture is shaken intensively and centrifuged for phase separation. An aliquot of the organic phase is purified by dispersive SPE with PSA, MgSO4. Final extracts are acidified with formic acid, diluted with water and make up with 50:50 (v/v) acetonitrile: water for LC-MS/MS.

A Phenomenex Luna C18 (2), 4.6mm x 150mm, 3µm analytical column was used for the analysis of all of the sample extracts. The same chromatographic separation was run for all methods. All extracts were analysed on a Sciex API 5000 LC/MS/MS instrument.

Results and discussions

Table A 5 Quantitative validation data for analytical methods for the determination of chlorantraniliprole in crops using LC/MS/MS

Matrix	Fortification level (mg/kg) [a, b]	Number of tests	Average recovery (%)	Standard deviation	% Relative standard deviation	Reference
Metabolism Method - Chlorantraniliprole (284à 177)						
Lettuce	0.050 0.50	5 5 Total = 10	89 110	3 3	4 3	FMC-51880
Hops	0.050 0.50	5 5 Total = 10	74 87	5 8	7 10	
Grapes	0.050 0.50	5 5 Total = 10	88 97	3 6	4 7	
Olives	0.050 0.50	5 5 Total = 10	98 98	13 9	14 9	
Dried Peas	0.050 0.50	5 5 Total = 10	96 107	5 2	6 2	
Single Analyte Residue Method - Chlorantraniliprole (284à 177)						
Lettuce	0.050 0.50	5 5 Total = 10	112 103	20 7	18 7	FMC-51880

Matrix	Fortification level (mg/kg) [a, b]	Number of tests	Average recovery (%)	Standard deviation	% Relative standard deviation	Reference
Hops	0.050 0.50	5 5 Total = 10	89 91	9 3	10 3	
Grapes	0.050 0.50	5 5 Total = 10	111 105	5 2	4 2	
Olives	0.050 0.50	5 5 Total = 10	99 109	2 5	3 5	
Dried Peas	0.050 0.50	5 5 Total = 10	82 93	4 4	5 5	
DFG S 19 Multi-Residue Method - Chlorantraniliprole (284à 177)						
Lettuce	0.050 0.50	5 5 Total = 10	89 107	6 5	7 5	FMC-51880
Hops	0.050 0.50	5 5 Total = 10	93 100	12 17	13 17	
Grapes	0.050 0.50	5 5 Total = 10	85 91	5 2	6 2	
Olives	0.050 0.50	5 5 Total = 10	88 114	5 5	5 4	
Dried Peas	0.050 0.50	5 5 Total = 10	96 116	10 2	11 2	
QuEChERS Multi-Residue Method - Chlorantraniliprole (284à 177)						
Lettuce	0.050 0.50	5 5 Total = 10	87 100	3 3	4 3	FMC-51880
Hops	0.050 0.50	5 5 Total = 10	102 99	8 7	8 7	
Grapes	0.050 0.50	5 5 Total = 10	93 96	4 3	4 4	
Olives	0.050 0.50	5 5 Total = 10	98 96	11 2	11 2	
Dried Peas	0.050 0.50	5 5 Total = 10	113 100	7 1	6 1	

[a] Fortifications were performed with analyte reference standard solutions

[b] Limit of quantification, defined by a signal-to-noise ratio of approximately 10 and the lowest validated fortification level

Table A 6 Characteristics for the analytical methods used for validation of chlorantraniliprole residues in crops

	Chlorantraniliprole (Metabolism method)
Specificity	A mass spectrum is not provided however the ions monitored are consistent with the analysis of chlorantraniliprole.
Calibration (type, number of data points)	Individual calibration data is presented, and a calibration line equation presented. Solvent: y=7.05e3x + 1.22e3; r=0.9987 A total of 8 points were used to generate the calibration curve.
Calibration range	Accepted calibration range from 0.70 to 50 ng/mL is provided.
Assessment of matrix effects is presented	Matrix effects were not assessed as part of this study.

Extract Stability	Sample extracts should be analysed within 24 hours of preparation.
Limit of determination/quantification	0.050 mg/kg
Estimated Limit of Detection	0.015 mg/kg
	Chlorantraniliprole (Single analyte method)
Specificity	A mass spectrum is not provided however the ions monitored are consistent with the analysis of chlorantraniliprole.
Calibration (type, number of data points)	Individual calibration data is presented, and a calibration line equation presented. Solvent: y=1.45e4x+ -1.62e3; r=0.9992 A total of 8 points were used to generate the calibration curve.
Calibration range	Accepted calibration range from 0.70 to 50 ng/mL is provided.
Assessment of matrix effects is presented	Matrix effects were not assessed as part of this study.
Extract Stability	Sample extracts should be analysed within 24 hours of preparation.
Limit of determination/quantification	0.050 mg/kg
Estimated Limit of Detection	0.015 mg/kg
	Chlorantraniliprole (DFG S19 method)
Specificity	A mass spectrum is provided, and the blank value is <30 % LOQ
Calibration (type, number of data points)	Individual calibration data is presented, and a calibration line equation presented. Solvent: y=6.41e3x + 594; r=0.9998 A total of 8 points were used to generate the calibration curve.
Calibration range	Accepted calibration range from 0.70 to 50 ng/mL is provided.
Assessment of matrix effects is presented	Matrix effects were not assessed as part of this study.
Extract Stability	Sample extracts should be analysed within 24 hours of preparation.
Limit of determination/quantification	0.050 mg/kg
Estimated Limit of Detection	0.015 mg/kg
	Chlorantraniliprole (QuEChERS method)
Specificity	A mass spectrum is not provided however the ions monitored are consistent with the analysis of chlorantraniliprole.
Calibration (type, number of data points)	Individual calibration data is presented, and a calibration line equation presented. Solvent: y=9.89e3x + 43.1; r=0.9998 A total of 8 points were used to generate the calibration curve.
Calibration range	Accepted calibration range from 0.70 to 50 ng/mL is provided.
Assessment of matrix effects is presented	Matrix effects were not assessed as part of this study.
Extract Stability	Sample extracts should be analysed within 24 hours of preparation.
Limit of determination/quantification	0.050 mg/kg
Estimated Limit of Detection	0.015 mg/kg

Conclusion

The four methods tested were successfully validated for the analysis of chlorantraniliprole in lettuce, hops, grapes, olives and dried peas.

Independent laboratory validation

An independent laboratory validation was not conducted. However, the single analyte residue method, the DFG S 19 multi-analyte residue method and the QuEChERS multi-analyte residue method have been independently validated at the LOQ of 0.010 mg/kg as part of separate studies.

Confirmatory method

During method validation a two additional ion transitions were monitored. The results for one of the confirmatory ion transitions is presented below.

Table A 7 Confirmatory data for analytical methods for the determination of chlorantraniliprole in crops using LC/MS/MS

Matrix	Fortification level (mg/kg) [a, b]	Number of tests	Average recovery (%)	Standard deviation	% Relative standard deviation	Reference
Metabolism Method - Chlorantraniliprole (484à 452.9)						
Lettuce	0.050 0.50	5 5 Total = 10	92 115	3 2	3 2	FMC-51880
Hops	0.050 0.50	5 5 Total = 10	79 89	4 9	5 10	
Grapes	0.050 0.50	5 5 Total = 10	93 100	3 6	3 6	
Olives	0.050 0.50	5 5 Total = 10	102 101	13 8	13 8	
Dried Peas	0.050 0.50	5 5 Total = 10	96 109	6 1	7 1	
Single Analyte Residue Method - Chlorantraniliprole (484à 452.9)						
Lettuce	0.050 0.50	5 5 Total = 10	113 104	19 7	17 7	FMC-51880
Hops	0.050 0.50	5 5 Total = 10	88 90	7 1	8 2	
Grapes	0.050 0.50	5 5 Total = 10	112 109	3 2	3 2	
Olives	0.050 0.50	5 5 Total = 10	93 104	3 4	3 4	
Dried Peas	0.050 0.50	5 5 Total = 10	82 94	3 3	4 4	
DFG S 19 Multi-Residue Method - Chlorantraniliprole (484à 452.9)						
Lettuce	0.050 0.50	5 5 Total = 10	91 110	6 4	6 4	FMC-51880
Hops	0.050 0.50	5 5 Total = 10	94 101	11 17	11 17	
Grapes	0.050 0.50	5 5 Total = 10	81 93	5 3	6 3	
Olives	0.050 0.50	5 5 Total = 10	90 118	4 4	4 3	
Dried Peas	0.050 0.50	5 5 Total = 10	96 116	11 3	11 2	
QuEChERS Multi-Residue Method - Chlorantraniliprole (484à 452.9)						
Lettuce	0.050	5	87	3	4	FMC-51880

Matrix	Fortification level (mg/kg) [a, b]	Number of tests	Average recovery (%)	Standard deviation	% Relative standard deviation	Reference
	0.50	5 Total = 10	100	3	3	
Hops	0.050 0.50	5 5 Total = 10	106 98	7 7	7 8	
Grapes	0.050 0.50	5 5 Total = 10	93 96	4 3	5 3	
Olives	0.050 0.50	5 5 Total = 10	103 100	11 2	10 2	
Dried Peas	0.050 0.50	5 5 Total = 10	109 101	8 3	7 3	

[a] Fortifications were performed with analyte reference standard solutions
[b] Limit of quantification, defined by a signal-to-noise ratio of approximately 10 and the lowest validated fortification level

Extraction efficiency

Five field residue samples were analysed using each method and the residues were compared. The mean residue values determined are provided in the table below.

Table A 8 Bridging samples analysis of the incurred residues of chlorantraniliprole in treated specimens of lettuce, hops, grapes, olives and dried peas

Commodity	Mean Chlorantraniliprole Residue mg/kg (n=5)				Residue Method as % of Metabolism Method	DFG S19 Method as % of Metabolism Method	QuEChERS Method as % of Metabolism Method
	Metabolism Method	Residue method	DFG S19 Method	QuEChERS Method			
Lettuce	1.0	0.77	0.77	0.88	77%	77%	88%
Hops	19	14	14	11	74%	74%	58%
Grapes	0.074	0.088	0.10	0.11	119%	135%	149%
Olives	0.59	0.57	0.56	0.54	97%	95%	92%
Dried Peas	0.24	0.22	0.24	0.23	92%	100%	96%
	0.20	0.18	0.19	0.18	90%	95%	90%

The single analyte residue method (DuPont-13295), the DFG S 19 multi-residue method (DuPont-15025) and the QuEChERS multi-residue method (DuPont-27124) adequately extract chlorantraniliprole incurred residues from crop samples. The only exception is the QuEChERS method for the analysis of hop samples.

Conclusions

The bridging study, FMC-51880, adequately assess the ability of the singe analyte residue method, the DFG S 19 method and the QuEChERS method to analyse chlorantraniliprole residues in incurred crop samples. All four EU crop groups and the fifth difficult to analyse crop group was tested.

Appendix 3: Supplemental Information

None.