FINAL REGISTRATION REPORT

Part B

Section 5

Analytical Methods

Detailed summary of the risk assessment

Product code: CLOROL 200 SC

Product name(s): Catanga 200 SC, Boland 200 SC

Chemical active substance:

Chlorantraniliprole, 200 g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(authorization)

Applicant: Innvigo Sp. z o.o

Submission date: September 2024

zRMS Assessment: 12 September 2025

Following commenting period/Verification List of References: 18 November 2025

Version history

When	What
September 2025	zRMS assessment
November 2025	Following commenting period/Verification List of References

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No comments during commenting period.

5 Analytical methods

5.1 Conclusion and summary of assessment

State whether submitted data are sufficient for evaluation. Data gaps and conditions for authorization should be listed, if appropriate.

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

- none

Sufficiently sensitive and selective analytical methods are not available for all analytes included in the residue definitions.
Noticed data gaps are:

- data gap 1 According to Commission Regulation (EU) No Reg. No 283/2013 and Reg. No 284/2013 and SAN-TE/2020/12830 rev.1, a fully validated analytical method for monitoring chlorantraniliprole residues in body fluids and tissue is required. Methods for the analysis of body fluids and tissues must be provided at the renewal of the active substance.

Commodity/crop	Supported/ Not supported
oilseed rape	supported
sugar beet	supported

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

Studies for chlorantraniliprole and Coragen 200 SC have been evaluated during inclusion of it on Annex 1 and approved in 02.08.2007. Also First registration on Coragen 200 SC in Poland was in 2011 (Authorization number: R-65/2011). According to reg. 1107/2009 data protection is expiring after 10 years since first registration, so data for chlorantraniliprole and Coragen 200 SC is already not longer protect. Additionaly Clorol 200 SC and Coragen 200 SC have the same compostion of formulation.
Therefore, to sup-port the authorization of Clorol 200 SC (Catanga 200 SC, Boland 200 SC) INNVIGO is allowed to refer to EU and Coragen 200 SC approved reports.

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 chlorantraniliprole in plant protection product is provided as follows:

Comments of zRMS:	The analytical method code: ICB/131/2023 was fully validated in term of specificity, linearity, repeatability, accuracy according to SANCO/3030/99 rev.5. The results of analytical method validation confirm that this method is suitable for determination of chlorantraniliprole. The method is successfully validated and accepted.

Reference:	KCP 5.1.1/01
Report	Validation of analytical method for CLOROL 200 SC for determination of Chlorantraniliprole, Study: ICB/131/2023, E. Łata, 2023
Guideline(s):	Conducting of analytical method validation – own Standard Operational Procedure SPB/179
Deviations:	No
GLP:	GLP
Acceptability:	Yes

Materials and methods

Validation was carried out according to Standard Operational Procedure SPB/179. Content of chlorantraniliprole at a level of 200 g/L in the test item was determined accordingly by liquid chromatography with diode array detection (HPLC-DAD).

Equipment and materials.
- methanol (VWR);
- 85% phosphoric acid (VWR);
- chlorantraniliprole standard; LGC; batch G1365311, prod. date: April 25, 2023; exp. date: April 25, 2027; storage conditions: 20±4°C;;
- working standard solutions of chlorantraniliprole in methanol (for calibration);
- standard stock solution of chlorantraniliprole in methanol (for recovery) ;
- standard stock solution of chlorantraniliprole in mathanol (for LOQ and ULOQ);
- analytical balance – accuracy 0.0001 g (Ohaus, Switzerland), WP/16;
- ultrasonic bath, W/29 (Branson, Taiwan);
- liquid chromatograph with diode array detection (Shimadzu, Japan), WP/19, WP/42; - chromatography column type C18, 100Å, 250 mm x 4,6 mm, 5 µm; Phenomenex, Luna 5 µm C18(2) 100Å, K/4/HPLC, K/19/HPLC;
- chromatographic vials 1.5 mL with septa buthyl/Teflon;
- volumetric flasks A class 10 mL, 25 mL;
- measuring syringes 10 µl, 50 µL, 100 µL, 250 µL, 500 µL, 1000 µL;
- measuring pipette 5 mL.

Comments of zRMS:
Sample preparation for determination average content of chlorantraniliprole and precision. Sample of test item was standardized by gentle mixing for 1 minute. Approximately 55 mg of test item was weighed out into 10 mL volumetric flask and was filled up to the mark with methanol.

The contents of the flask was sonicated until the test material was completely dissolved. Afterwards 250 µL of solution was transferred into 10 mL volumetric flask and was filled up to the mark with methanol. Then final solution was placed in chromatographic vial and analysed. The test item was prepared in five repetitions.

Chromatography parameters.
Primary chromatographic system.

Column:	K/19/HPLC
Analysis time:	25 min
Flow:	1.0 mL/min
Column temperature:	25°C
Injection:	10 µL
Detector DAD:	270 nm
Mobile phase:	A – Methanol, D – 0.1% H_3PO_4
Isocratic program:	phase A - 70%, D – 30%

Secondary chromatographic system.

Column:	K/4/HPLC
Analysis time:	25 min
Flow:	1.2 mL/min
Column temperature:	25°C
Injection:	10 µL
Detector DAD:	270 nm
Mobile phase:	A – Methanol, D – 0.1% H_3PO_4
Isocratic program:	phase A - 60%, D – 40%

Validation - Results and discussions

Table 5.2-1: Methods suitable for the determination of chlorantraniliprole in plant protection product CLOROL 200 SC

	Chlorantraniliprole
Author(s), year	E. Łata
Principle of method	HPLC-DAD
Linearity (linear between mg/L / % range of the declared content) (correlation coefficient, expressed	In order to check the linearity of chlorantraniliprole, calibration curve was prepared using standard solutions with concentrations contained in table below:

	Chlorantraniliprole

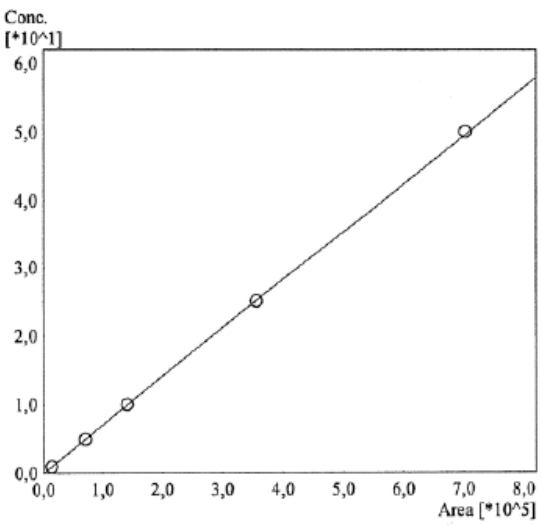
as r)

Concentration of standard solution [mg/mL]	Volume of standard solution [µL]	Flask volume [mL]	Concentration of working standard solutions [µg/mL]	Calibration level
1.0017	10	10	1.0017	Cal 1
1.0017	50	10	5.0085	Cal 2
1.0017	100	10	10.0170	Cal 3
1.0017	250	10	25.0425	Cal 4
1.0017	500	10	50.0850	Cal 5

A graph of the peak area to the concentration of chlorantraniliprole was plotted. The resulting curve is linear in the tested concentrations. Linearity range of chlorantraniliprole is from 1.0575 to 352.5 µg/mL. Correlation coefficient R2 is 0.9999533 and the linear regression is described by equation: $f(x$ 7 06226 10 5 x 0 0250060 for primary chromatographic system:

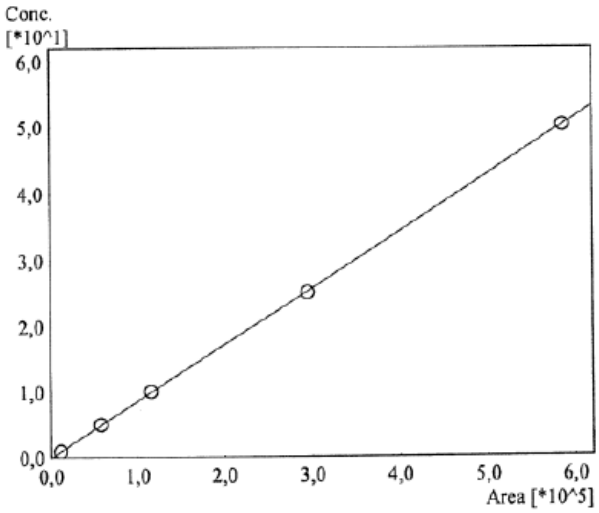
Calibration Curve

ID# : 1
Name : Chlorantraniliprole
Quantitative Method : External Standard
Function : f(x)=7,06226e-005*x+0,0250060
Rr1=0,9999766 Rr2=0,9999533 RSS=1,514734e-004
MeanRF: 7,111592e-005 RFSD: 8,663218e-007 RFRSD: 1,218183
FitType : Linear
ZeroThrough : Not Through
Weighted Regression : 1/C^2
Detector Name : PDA



#	Conc.	Area
1	1,0017	13813
2	5,0085	71059
3	10,0170	141172
4	25,0425	355956
5	50,0850	702768

Correlation coefficient R2 is 0.9999554 and the linear regression is described by equation: $f(x$ 8 54657 10 5 x 0 0274598 for secondary chromatographic system:

	Chlorantraniliprole
	Calibration Curve ID# : 1 Name : Chlorantraniliprole Quantitative Method : External Standard Function : f(x)=8,54657e-005*x+0,0274598 Rr1=0,9999777 Rr2=0,9999554 RSS=1,446917e-004 MeanRF: 8,612179e-005 RFSD: 1,114991e-006 RFRSD: 1,294668 FitType : Linear ZeroThrough : Not Through Weighted Regression : I/C^2 Detector Name : PDA  | # | Conc. | Area | |---|---|---| | 1 | 1,0017 | 11414 | | 2 | 5,0085 | 57980 | | 3 | 10,0170 | 116151 | | 4 | 25,0425 | 295247 | | 5 | 50,0850 | 586429 |
Precision – Repeatability Mean n = 5 (%RSD)	Weights, concentrations of chlorantraniliprole from chromatograms for all five independent test item samples used for determination of average active ingredient content and precision. | Chromatographic system | Sample | Weight [mg] | Concentrations [µg/mL] *chlorantraniliprole* | |---|---|---|---| | Primary | 1 | 54.9 | 24.525 | | | 2 | 55.3 | 24.648 | | | 3 | 55.8 | 24.717 | | | 4 | 54.8 | 24.451 | | | 5 | 55.5 | 24.727 | | Secondary | 1 | 54.9 | 24.626 | | | 2 | 55.3 | 24.780 | | | 3 | 55.8 | 24.899 | | | 4 | 54.8 | 24.607 | | | 5 | 55.5 | 24.873 | Precision was calculated according to following equations: $RSD\ [\%] = \frac{SD}{\overline{C_U}} * 100\%$ $SD = \sqrt{\frac{\sum_{i=1}^{n}(C_{U_i} - \overline{C_U})}{n-1}}$ $\overline{C_U} = \frac{\sum_{i=1}^{n} C_{U_i}}{n}$

	Chlorantraniliprole

Where:
CUi– concentration of active ingredient [µg/mL],
n – amount of measurements.

Results of precision and Horwitz ratio at 100% validation level for active ingredient.

Chromatographic system	Precision [%]	Horwitz ratio
Primary	0.33	0.19
Secondary	0.24	0.14

Concentrations of chlorantraniliprole from chromatograms for all five samples of solvent (methanol) with standard addition and average recovery for validation level LOQ:

Chromatographic system	Sample	Standard addition [µg/mL]	Concentrations [µg/mL]	Average recovery [%]
Primary	1	1.0575	1.111	103.1
	2		1.082	
	3		1.100	
	4		1.110	
	5		1.051	
		Average	1.0908	
Secondary	1	1.0575	1.062	102.1
	2		1.074	
	3		1.080	
	4		1.092	
	5		1.089	
		Average	1.0794	

Results of average recovery, precision and Horwitz ratio for chlorantraniliprole for validation level LOQ:

Chromatographic system	Precision [%]	Horwitz ratio	Average recovery [%]
Primary	2.30	0.82	103.1
Secondary	1.12	0.40	102.1

Concentrations of chlorantraniliprole from chromatograms for all five samples of solvent (methanol) with standard addition and average recovery for validation level ULOQ:

	Chlorantraniliprole

Chromatographic system	Sample	Standard addition [µg/mL]	Concentrations [µg/mL]	Average recovery [%]
Primary	1	352.5	350.085	98.8
	2		346.884	
	3		345.721	
	4		351.200	
	5		347.657	
		Average	348.3094	
Secondary	1	352.5	350.745	99.1
	2		348.890	
	3		346.908	
	4		351.656	
	5		348.545	
		Average	349.3488	

Results of average recovery, precision and Horwitz ratio for chlorantraniliprole for validation level ULOQ:

Chromatographic system	Precision [%]	Horwitz ratio	Average recovery [%]
Primary	0.65	0.40	98.8
Secondary	0.54	0.33	99.1

Accuracy n = 5 (% Recovery)

Recovery was calculated according to following equation:

$$Rec[\%] = \frac{C_F - \overline{C_U}}{C_A} \times 100\%$$

where:
CF – concentration of active ingredient in the test item in the sample with standard addition [µg/mL],
$\overline{CU}$ – average concentration of active ingredient [µg/mL],
CA – standard addition [µg/mL].

weights, concentrations of active ingredient from chromatograms for all five independent test item samples used for determination of recovery for both chromatographic systems:

Chromatographic system	Sample	Weight [mg]	Concentrations [µg/mL] *chlorantraniliprole*
Primary	1	56.1	32.059
	2	56.0	32.022
	3	56.2	32.041
	4	56.0	31.960
	5	55.8	31.974
Secondary	1	56.1	32.379
	2	56.0	32.199
	3	56.2	32.243
	4	56.0	32.133
	5	55.8	32.233

Concentrations of chlorantraniliprole and recovery for all five independent test item samples used for determination of recovery:

Chlorantraniliprole

Chromatographic system	Sample	Average concentration without standard addition [µg/mL]	Standard addition [µg/mL]	Concentrations [µg/mL]	Recovery [%]
Primary	1	24.49821	6.9275	31.43039	100.07
	2			31.45018	100.35
	3			31.35685	99.01
	4			31.38929	99.47
	5			31.51559	101.30
Secondary	1	24.64084	6.9275	31.74412	102.54
	2			31.62402	100.80
	3			31.55454	99.80
	4			31.55920	99.87
	5			31.77088	102.92

Results of recovery for chlorantraniliprole:

Chromatographic system	Recovery [%]	Standard addition [%]
Primary	99.01 – 101.30	28.28
Secondary	99.80 – 102.92	28.11

Concentrations of chlorantraniliprole from chromatograms for all five samples of solvent (methanol) with standard addition and average recovery for validation level LOQ:

Chromatographic system	Sample	Standard addition [µg/mL]	Concentrations [µg/mL]	Average recovery [%]
Primary	1	1.0575	1.111	103.1
	2		1.082	
	3		1.100	
	4		1.110	
	5		1.051	
		Average	1.0908	
Secondary	1	1.0575	1.062	102.1
	2		1.074	
	3		1.080	
	4		1.092	
	5		1.089	
		Average	1.0794	

Results of average recovery, precision and Horwitz ratio for chlorantraniliprole for validation level LOQ:

Chromatographic system	Precision [%]	Horwitz ratio	Average recovery [%]
Primary	2.30	0.82	103.1
Secondary	1.12	0.40	102.1

<table>
<tr><th></th><th>Chlorantraniliprole</th></tr>
<tr><td></td><td>Concentrations of chlorantraniliprole from chromatograms for all five samples of solvent (methanol) with standard addition and average recovery for validation level ULOQ:
<table>
<tr><th>Chromatographic system</th><th>Sample</th><th>Standard addition [µg/mL]</th><th>Concentrations [µg/mL]</th><th>Average recovery [%]</th></tr>
<tr><td rowspan="5">Primary</td><td>1</td><td rowspan="5">352.5</td><td>350.085</td><td rowspan="5">98.8</td></tr>
<tr><td>2</td><td>346.884</td></tr>
<tr><td>3</td><td>345.721</td></tr>
<tr><td>4</td><td>351.200</td></tr>
<tr><td>5</td><td>347.657</td></tr>
<tr><td></td><td></td><td>Average</td><td>348.3094</td><td></td></tr>
<tr><td rowspan="5">Secondary</td><td>1</td><td rowspan="5">352.5</td><td>350.745</td><td rowspan="5">99.1</td></tr>
<tr><td>2</td><td>348.890</td></tr>
<tr><td>3</td><td>346.908</td></tr>
<tr><td>4</td><td>351.656</td></tr>
<tr><td>5</td><td>348.545</td></tr>
<tr><td></td><td></td><td>Average</td><td>349.3488</td><td></td></tr>
</table>
Results of average recovery, precision and Horwitz ratio for chlorantraniliprole for validation level ULOQ:
<table>
<tr><th>Chromatographic system</th><th>Precision [%]</th><th>Horwitz ratio</th><th>Average recovery [%]</th></tr>
<tr><td>Primary</td><td>0.65</td><td>0.40</td><td>98.8</td></tr>
<tr><td>Secondary</td><td>0.54</td><td>0.33</td><td>99.1</td></tr>
</table></td></tr>
<tr><td>Interference/ Specificity</td><td>Specificity of the method was evaluated based on the analysis of chromatogram for sample against chromatogram of chlorantraniliprole standard and peak purity. Analysis showed no overlapping of determined ingredient signal with the signals of matrix components under method conditions, hence method specificity criterion is fulfilled.</td></tr>
<tr><td>Comment</td><td></td></tr>
</table>

Conclusion

In accordance to SANCO/3030/99 rev. 5 the method for determination of chlorantraniliprole in CLOROL 200 SC preparation is specific. The validation parameters for linearity, instrument precision, repeatability and accuracy are within the acceptance range.

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

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

Comments of zRMS:	The analytical method code: C3188 was fully validated in term of specificity, linearity, repeatability, accuracy according to SANCO/3030/99 rev.5. The results of analytical method validation confirm that this method is suitable for determination of relevant impurities (acetornitrile, 3-picoline and methanesulfonic

	acid) content. The method is successfully validated and accepted.

Reference:	KCP 5.1.1/02
Report	Method valiation and determination of relevant impurities (acetornitrile, 3-picoline and methanesulfonic acid) content in one batch of CLOROL 200 SC. S. Lobstein, 2024, Study code: C3188
Guideline(s):	SANCO/3030/99 rev. 5 Regulation (EC) No. 1107/2099 Commision Regulation (EU) No. 284/2013
Deviations:	NO
GLP:	Yes
Acceptability:	Yes

Materials and methods

The method used in the present study is an ANADIAG In-House GC/FID method for acetonitrile and 3-picoline.

The method used in the present study is an ANADIAG In-House HPLC/Qtof method for methanesulfonic acid

Reagents for acetonitrile and 3-picoline.
Acetone Ultrapure grade
0.2 µm PET filter

Reagents for methanesulfonic acid
Acetonitrile HPLC grade
Formic acid HPLC grade
Water Type 1 (Ultra-pure)
0.2 µm PET filter

Validation - Results and discussions

Table 5.2-2: Methods suitable for the determination of the relevant impurities in plant protection product (PPP) CLOROL 200 SC

	Acetonitrile **max. 0.6 g/kg in CLOROL 200 SC**	**3-picoline** **max. 0.6 g/kg in CLOROL 200 SC**
Author(s), year	S. Lobstein, 2024	
Principle of method	GC/FID	

Linearity (linear be-tween mg/L) (correlation coefficient, expressed as r)

Sequence Name	Analyte	Min – Max (µg/mL)	Min – Max (g/kg)	Number of Levels	R^2	r
seq231219	Acetonitrile	1.04 – 26.5	0.05 – 1.33	11	0.99954	0.99977
	3-picoline	1.01 – 26.2	0.05 – 1.31	11	0.99937	0.99969
seq240108	Acetonitrile	1.00 – 26.1	0.05 – 1.31	11	0.99952	0.99976
	3-picoline	1.00 – 26.2	0.05 – 1.31	11	0.99980	0.99990

The linear correlation coefficients were > 0.99, showing a good linearity.

Precision – Repeatability Mean n = 5/6 (%RSD)

Analyte	Average of peak area (pA)	Number of determinations	Conc. (µg/mL)	Conc. (w/w in sample) (g/kg)	RSD (%)	RSD_r (%)
Acetonitrile	2.007	6	3.126	0.16	1.86	4.99
3-picoline	3.328	6	3.024	0.15	1.25	5.04

The results obtained show a relative standard deviation < RSDr given by the modified Horwitz equation based on the concentration, in g/kg, of the calibration solution which indicates a good consistency of the data according to SANCO/3030/99 rev. 5.

Analyte	Average Percentage of recovery (%)	Number of determinations	Average theoretical amount (g/kg)	RSD (%)	RSD_r (%)	Horrat value H_r
Acetonitrile	98.3	5	0.20	1.50	4.83	0.31

Analyte	Average content g/kg	Number of determinations	RSD (%)	RSD_r (%)	Horrat value H_r
3-picoline	0.280	5	1.15	4.59	0.25

The results obtained indicate a good precision of the data (RSD < RSDr given by modified Horwitz equation based on the average theoretical amount in spiked samples at the LOQ or on the average content in the test item and the Horrat Value Hr ≤ 1).

Accuracy n = 5 (% Recovery)

Analyte	Spiking level (g/kg)	Average Percentage of recovery (%)	Number of determinations	Average theoretical amount (g/kg)	RSD (%)	RSD_r (%)
Acetonitrile	~ 0.15	98.3	5	0.20	1.50	4.83
	~ 0.60	89.9	5	0.65	1.05	4.04
3-picoline	~ 0.15	102.0	5	0.43	1.54	4.30
	~ 0.60	104.1	5	0.85	2.16	3.88

The results obtained show a good accuracy which indicates acceptable data (acceptability according to the values given in SANCO/3030/99 rev.5 (22/03/19), mean recovery must be between 75 % and 125 % for an average theoretical amount in spiked samples between 0.1 g/kg and 1.0 g/kg and RSD < RSDr given by modified Horwitz equation based on the average theoretical amount in spiked samples).

Intermediate precision

Analyte	Average Percentage of recovery (%)	Number of determinations	Average theoretical amount (g/kg)	RSD (%)	RSD_R (%)
Acetonitrile	100.1	10	0.19	2.17	7.26

Analyte	Average content g/kg	Number of determinations	RSD (%)	RSD_R (%)
3-picoline	0.277	10	1.25	6.86

The results obtained show a good intermediate precision (as the relative standard deviation obtained is below the RSDR given by Horwitz equation).

Interference/ Specificity

No interference can be seen in the area of the impurities retention times during the analysis of the solvent.

LOQ

The limit of quantification was validated at 0.15 g/kg for Acetonitrile and 3-picoline (fivefold determination at this level gave a good accuracy and a good precision).

LOD

Analyte	LOD* (µg/mL)	LOD* (g/kg)
Acetonitrile	0.58	0.03
3-picoline	0.39	0.02

* upper rounded value.

Confirmation of analyte identification (MS spectrum)

The MS spectrum of acetonitrile extracted from the standard solution is in accordance with the structure of acetonitrile
The MS spectrum of acetonitrile extracted from the test item solution is not available as no acetonitrile was detected in the test item solution.
The MS spectrum of 3-picoline extracted from the test item solution is consistent with the MS spectrum of 3-picoline extracted from the standard solution.
Low mass (m/z = 43, 45…) come from solvent. The background noise is more important in the test item solution than in the standard solution, because the analyte concentration is low in the test item solution.
MS spectra recorded are in accordance with the structure of 3-picoline.

Methanesulfonic acid
Max. 0.4 g/kg in CLOROL 200 SC

Author(s), year

S. Lobstein, 2024

Principle of method

HPLC/Qtof

Linearity (linear between mg/L) (correlation coefficient, expressed as r)

Sequence Name	Min – Max (µg/mL)	Min – Max (g/kg)	Number of Levels	R^2	r
seq240201	0.50 – 9.03	0.05 – 0.90	11	0.99245	0.99622
seq240202	0.50 – 9.03	0.05 – 0.90	11	0.99545	0.99772
seq240206	0.51 – 8.07	0.05 – 0.81	10*	0.99687	0.99843

Eleven levels were prepared but some of them were discarded (see *Appendix B.1*: Linearity/Tabulated data). The linear correlation coefficients were > 0.99, showing a good linearity.

Precision – Repeatability Mean n = 5/6 (%RSD)

Average of peak area (counts)	Number of determinations	Conc. (µg/mL)	Conc. (w/w in sample) (g/kg)	RSD (%)	RSD_r (%)
91476	6	1.505	0.15	3.96	5.04

The results obtained show a relative standard deviation < RSDr given by the modified Horwitz equation based on the concentration, in g/kg, of the calibration solution which indicates a good consistency of the data according to SANCO/3030/99 rev. 5.

Average content g/kg	Number of determinations	RSD (%)	RSD_r (%)	Horrat value H_r
0.202	5	3.16	4.82	0.66

The results obtained indicate a good precision of the data (RSD < RSDr given by modified Horwitz equation based on the average theoretical amount in spiked samples at the LOQ or on the average content in the test item and the Horrat Value Hr ≤ 1).

<table>
<tr><td>Accuracy
n = 5
(% Recovery)</td><td>
<table>
<tr><th>Spiking level (g/kg)</th><th>Average Percentage of recovery (%)</th><th>Number of determinations</th><th>Average theoretical amount (g/kg)</th><th>RSD (%)</th><th>RSD_r (%)</th></tr>
<tr><td>~ 0.15</td><td>100.6</td><td>5</td><td>0.35</td><td>4.18</td><td>4.44</td></tr>
<tr><td>~ 0.40</td><td>94.2</td><td>4*</td><td>0.60</td><td>1.61</td><td>4.09</td></tr>
</table>
* Five spiked samples were prepared but one of them was discarded by the Grubbs Test.

The results obtained show a good accuracy which indicates acceptable data (acceptability according to the values given in SANCO/3030/99 rev.5 (22/03/19), mean recovery must be between 75 % and 125 % for an average theoretical amount in spiked samples between 0.10 g/kg and 1.0 g/kg and RSD < RSDr given by modified Horwitz equation based on the average theoretical amount in spiked samples).
</td></tr>
<tr><td>Intermediate precision</td><td>
<table>
<tr><th>Average content g/kg</th><th>Number of determinations</th><th>RSD (%)</th><th>RSD_R (%)</th></tr>
<tr><td>0.207</td><td>10</td><td>3.68</td><td>7.17</td></tr>
</table>
The results obtained show a good intermediate precision (as the relative standard deviation obtained is below the RSDR given by Horwitz equation).
</td></tr>
<tr><td>Interference/ Specificity</td><td>No interference can be seen in the area of the impurities retention times during the analysis of the solvent.</td></tr>
<tr><td>LOQ</td><td>The limit of quantification was validated at 0.15 g/kg (fivefold determination at this level gave a good accuracy and a good precision).</td></tr>
<tr><td>LOD</td><td>
<table>
<tr><th>LOD* (µg/mL)</th><th>LOD* (g/kg)</th></tr>
<tr><td>0.07</td><td>0.01</td></tr>
</table>
* upper rounded value.
</td></tr>
<tr><td>Confirmation of analyte identification (MS spectrum)</td><td>The MS spectrum of methanesulfonic acid extracted from the test item solution is consistent with the MS spectrum of methanesulfonic acid extracted from the standard solution.
MS spectra recorded are in accordance with the structure of methanesulfonic acid.
Higher mass observed come from polymerized forms of methanesulfonic acid.</td></tr>
<tr><td>Comment</td><td></td></tr>
</table>

Conclusion

The analytical method has been successfully validated. The limit of quantification is confirmed as 0.15 mg/kg for methanesulfonic acid, acetonitrile and 3-picoline.
All SANCO 3030/99/rev5 guidelines have been adhered to and acceptance criteria as stated in the study plan
were met.

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

Not required..

5.2.1.4 Applicability of existing CIPAC methods (KCP 5.1.1)

Not relevant.

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

Table 5.2-3: Validated methods for the generation of pre-authorization data

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**
Plants, plant products,... (Residues)	Primary	0.01 mg/kg	LC-MS/MS	(2006a) DuPont-15025
	Confirmatory (if required)	LOQ mg/kg		Stry, J.J, 2013b, DuPont-13261, Supplement No. 1
Animal products, food of animal origin,... (Residues)	Primary	0.01 mg/kg	LC-MS/MS	Rzepka, S., 2005 DuPont-13261
Soil	Primary	0.01 mg/kg	GC-ECD	Bilas, J.M. Gagnon, M.R. Stry, J.J 2005 DuPont-14942
	Confirmatory (if required)	0.01 mg/kg	LC-MS	Bilas, J.M., Gagnon, M.R., Stry, J.J, 2005 DuPont-14942
Water	Primary	0.01 µg/L	LC-Ms/MS	Bilas, J.M., Stry, J.J. (2005); DuPont-16058
Air	Primary	0.5 µg/m3	LC-MS/MS	van Schaik, F. (2006), DuPont-18612
Honey	Primary	0.01 mg/kg	LC-MS/MS	K. Głowiak, 2024, VAL/10/2024
Oilseed Rape for risk assessment	Pimary	0.01 mg/kg	LC-MS/MS	K.Głowiak, 2024, VAL/11/2024
Sugar beet for risk assesment	Primary	0.01 mg/kg	LC-MS/MS	M. Jedrusik,2024,VAL/17/2024
Sucrose solution for chronic oral Toxicity Test	Primary	20 mg/kg	HPLC-DAD	A. Fulczyk, 2024, B-93-23
Water for Larval Toxicity Test	Primary	0.07 mg/L	HPLC-DAD	A. Fulczyk, 2024, B-92-23

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**
Soil for Folsomia Candida	Primary	91 µg/kg	UHPLC-MS/MS	E. Kulec-Płoszczyca, ETOX-2023-13
Soil for Hypoaspis aculeifer	Primary	91 µg/kg	UHPLC-MS/MS	S. Groch, ETOX -2024-65
Soil for Earthworm	Primary	91 µg/kg	UHPLC-MS/MS	K. Wesołowska, ETOX-2024-64

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.

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

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

Table 5.3-1: Relevant residue definitions for monitoring/enforcement and levels for which compliance is required

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Plant, high water content	Chlorantraniliprole	2 mg/kg	Reg. (EU) 2022/1343
Plant, high acid content		0.7 mg/kg	Reg. (EU) 2022/1343
Plant, high protein/high starch content (dry commodities)		0.02 mg/kg	Reg. (EU) 2022/1343
Plant, high oil content		2 mg/kg	Reg. (EU) 2022/1343
Plant, difficult matrices (hops, spices, tea)		0.05 mg/kg	Reg. (EU) 2022/1343
Muscle	Chlorantraniliprole	0.03 mg/kg	Reg. (EU) 2022/1343
Milk		0.05 mg/kg	Reg. (EU) 2022/1343

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Eggs		0.2 mg/kg	Reg. (EU) 2022/1343
Fat		0.2 mg/kg	Reg. (EU) 2022/1343
Liver, kidney		0.2 mg/kg	Reg. (EU) 2022/1343
Soil (Ecotoxicology)	Chlorantraniliprole	0.05 mg/kg	common limit or NOEAC/AOEL for XXX (which ever is the lowest)
Drinking water (Human toxicology)	Chlorantraniliprole	0.1 µg/L	general limit for drinking water
Surface water (Ecotoxicology)	Chlorantraniliprole	5.8 µg/L	
Air	Chlorantraniliprole	Xxx µg/m^3	AOEL sys/AOEL inhal: xxx mg/kg bw/d
Body fluids	Chlorantraniliprole	0.01 mg/L	Reg. (EU) 2022/1343
Tissue	Chlorantraniliprole	0.2 mg/L	Reg. (EU) 2022/1343

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

An overview on the acceptable methods and possible data gaps for analysis of Chlorantraniliprole in plant matrices is given in the following tables.

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

Component of residue definition: 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	Rzepka, S., 2005 DuPont-13261
	ILV	0.01 mg/kg	LC-MS/MS	van Schaik, 2006a DuPont-18611, Revision No. 1
	Confirmatory (if required)	0.01 mg/kg	LC-MS/MS	Stry, J.J, 2013b, DuPont-13261, Supplement No. 1
High acid content	Primary	0.01 mg/kg	LC-MS/MS	Rzepka, S., 2005 DuPont-13261
	ILV	0.01 mg/kg	LC-MS/MS	van Schaik,

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**
				2006a DuPont-18611, Revision No. 1
	Confirmatory (if required)	0.01 mg/kg	LC-MS/MS	Stry, J.J, 2013b, DuPont-13261, Supplement No. 1
High oil content	Primary	0.01 mg/kg	LC-MS/MS	Rzepka, S., 2005 DuPont-13261
	ILV	0.01 mg/kg	LC-MS/MS	van Schaik, 2006a DuPont-18611, Revision No. 1
	Confirmatory (if required)	0.01 mg/kg	LC-MS/MS	Stry, J.J, 2013b, DuPont-13261, Supplement No. 1
High protein/high starch content (dry)	Primary	0.01 mg/kg	LC-MS/MS	Rzepka, S., 2005 DuPont-13261
	ILV	0.01 mg/kg	LC-MS/MS	van Schaik, 2006a DuPont-18611, Revision No. 1
	Confirmatory (if required)	0.01 mg/kg	LC-MS/MS	Stry, J.J, 2013b, DuPont-13261, Supplement No. 1

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

Table 5.3-3: Statement on extraction efficiency

	Method for products of plant origin
Required, available from:	Please refer to DAR Chlorantraniliprole, Section B5: Extraction efficiency of this method was not demonstrated, due to the fact that multi-residue methods are intended to analyze many classes of compounds. The extraction solvents used for this procedure are consistent with solvent used to extract DPX-E2Y45 incurred 14C residues from crops. The extraction procedure used for the German DFG S 19 is a pre-soak in water followed by an acetone extraction. The DPX-E2Y45 extraction efficiency study demonstrated that incurred residues could be efficiently extracted using a water pre-soak and an acetonitrile extraction. The extraction solvent is very similar and based on the physicochemical properties of the technical grade DPX-E2Y45 test compound, acetone is a suitable solvent for the extraction of incurred DPX-E2Y45 residues.

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

An overview on the acceptable methods and possible data gaps for analysis of Chlorantraniliprole in animal matrices is given in the following tables.

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

Component of residue definition: 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	(2006a) DuPont-15025
	ILV	0.01 mg/kg	LC-MS/MS	(2006b) DuPont-18610
	Confirmatory (if required)	0.01 mg/kg	LC-MS/MS	DuPont-20979, 2006
Eggs	Primary	0.01 mg/kg	LC-MS/MS	(2006a) DuPont-15025
	ILV	0.01 mg/kg	LC-MS/MS	(2006b) DuPont-18610
	Confirmatory (if required)	0.01 mg/kg	LC-MS/MS	DuPont-20979, 2006
Muscle	Primary	0.01 mg/kg	LC-MS/MS	(2006a) DuPont-15025
	ILV	0.01 mg/kg	LC-MS/MS	(2006b) DuPont-18610
	Confirmatory (if required)	0.01 mg/kg	LC-MS/MS	DuPont-20979, 2006
Fat	Primary	0.01 mg/kg	LC-MS/MS	(2006a) DuPont-15025
	ILV	0.01 mg/kg	LC-MS/MS	(2006b) DuPont-18610
	Confirmatory (if required)	0.01 mg/kg	LC-MS/MS	DuPont-20979, 2006
Kidney, liver	Primary	0.01 mg/kg	LC-MS/MS	(2006a) DuPont-15025
	ILV	0.01 mg/kg	LC-MS/MS	(2006b) DuPont-18610
	Confirmatory (if required)	0.01 mg/kg	LC-MS/MS	DuPont-20979, 2006

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

Table 5.3-5: Statement on extraction efficiency

	Method for products of animal origin
Not required, because:	Please refer to DAR Chlorantraniliprole, Section B5 Extraction efficiency of this method was not demonstrated, due to the fact that multi-residue methods are intended to analyze many classes of compounds. The extraction solvents used for this procedure are consistent with solvents used to extract DPX-E2Y45 incurred 14C residues from tissues. The extraction procedure used for the German DFG S 19 procedure is a pre-soak in water followed by an acetone extraction. The DPX-E2Y45 metabolism study DuPont-14377 provided in DPX-E2Y45 OECD Dossier, Annex IIA, Document M-II, Section 4, DuPont-18272, Point IIA 6.2.3 demonstrated that incurred residues could be completely extracted using a water pre-soak and an acetonitrile extraction. The extraction solvent is very similar and based on the physicochemical properties of the technical grade DPX-E2Y45 test compound acetone is a suitable solvent for the extraction of incurred DPX-E2Y45 residus

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

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

Table 5.3-6: Validated methods for soil (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	0.01 mg/kg	GC-ECD	Bilas, J.M. Gagnon, M.R. Stry, J.J 2005 DuPont-14942
Confirmatory	0.01 mg/kg	LC/MS	Bilas, J.M., Gagnon, M.R., Stry, J.J, 2005 DuPont-14942

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

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

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

Component of residue definition: Chlorantraniliprole				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Drinking water	Primary	0.01 µg/L	LC-MS/MS	Bilas, J.M., Stry, J.J. (2005); DuPont-16058
	ILV	0.01 µg/L	LC-MS/MS	Buscher, B.A.P. (2006); DuPont-16708, Revision No. 1
	Confirmatory	Not required		
Surface water	Primary	0.01 µg/L	LC-MS/MS	Bilas, J.M., Stry, J.J. (2005); DuPont-16058
	Confirmatory	Not required		

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

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

Table 5.3-8: Validated methods for air (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	0.50 µg/m3	LC-MS/MS	van Schaik, F. (2006), DuPont-18612
Confirmatory	Not required		

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

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

This data requirement is not relevant for chlorantraniliprole. Chlorantraniliprole is not classified as highly toxic or toxic to humans and would not bioaccumulate in human tissues in case of accidental contact or ingestion. Therefore, methods for the determination of chlorantraniliprole in body fluids and tissues would be of no practical use. No further studies were required or conducted.

Comments of zRMS:

According to Commission Regulation (EU) No Reg. No 283/2013 and Reg. No 284/2013 and SANTE/2020/12830 rev.1, a fully validated analytical method for monitoring chlorantraniliprole residues in body fluids and tissue is required.

Appendix 1 Lists of data considered in support of the evaluation

Tables considered not relevant can be deleted as appropriate.

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

List of data submitted by the applicant and relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Verte-brate study Y/N	Owner
KCP 5.1.1/01	E. Łata	2023	Validation of analytical method for CLOROL 200 SC for determination of Chlorantraniliprole, Study: ICB/131/2023 ICB Pharma GLP Unpublished	N	PUH Chemirol Sp. Z o.o.
KCP 5.1.1/02	S. Lobstein	2024	Method valiation and determination of relevant impurities (acetornitrile, 3-picoline and methanesulfonic acid) content in one batch of CLOROL 200 SC. Study code: C3188 GLP Unpublished	N	PUH Chemirol Sp. Z o.o.
KCP 5.2/01	K. Głowiak	2024	Validation of an analytical method for the determination of residues of chlorantraniliprole in oilseed rape (seed, plant) Study code: VAL/11/2024 SGS POLSKA Sp. z o.o. Jerozolimskie Business Park, Al. Jerozolimskie 146A, 02-305 Warszawa LABORATORIUM SGS POLSKA ul. Cieszyńska 52 A 43-200 Pszczyna, Poland GLP	N	PUH Chemirol Sp. Z o.o.

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Verte-brate study Y/N	Owner
			Unpublished		
KCP 5.2/02	M. Jedrusik	2024	Validation of an analytical method for the determination of residues of Chlorantraniliprole in sugar beet (roots and leaves) Study code: VAL/17/2024 SGS POLSKA Sp. z o.o. Jerozolimskie Business Park, Al. Jerozolimskie 146A, 02-305 Warszawa LABORATORIUM SGS POLSKA ul. Cieszyńska 52 A 43-200 Pszczyna, Poland GLP Unpublished	N	PUH Chemirol Sp. Z o.o.
KCP 5.2/03	K. Głowiak	2024	Validation of an analytical method for the determination of residues of chlorantraniliprole in honey Study code: VAL/10/2024 SGS POLSKA Sp. z o.o. Jerozolimskie Business Park, Al. Jerozolimskie 146A, 02-305 Warszawa LABORATORIUM SGS POLSKA ul. Cieszyńska 52 A 43-200 Pszczyna, Poland GLP Unpublished	N	PUH Chemirol Sp. Z o.o.
KCP 5.2/04	A. Fulczyk	2024	PART II (Analytical Phase) CLOROL 200 SC Honeybees (Apis mellifera L.), Chronic Oral Toxicity Test Study code: B-93-23 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna Ecotoxicology Research Group, Doświadczalna 27, 43-200 Pszczyna, Poland GLP Unpublished	N	PUH Chemirol Sp. Z o.o.
KCP 5.2/05	A. Fulczyk	2024	PART II (Analytical Phase) CLOROL 200 SC Honeybees (Apis mellifera L.), Larval Toxicity Test, Repeated Exposure Study code: B-92-23	N	PUH Chemirol Sp. Z o.o.

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Verte-brate study Y/N	Owner
			Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna Ecotoxicology Research Group, Doświadczalna 27, 43-200 Pszczyna, Poland GLP Unpublished		
KCP 5.2/06	E. Kulec-Płoszczyca	2024	Collembolan (Folsomia candida) Reproduction Test in soil, APPENDIX No. 5 – Copy of the results of the analytical measurements – Chlorantraniliprole ETOX-2023-13 EcoTox Alliance Sp. z o. o. Kalinowa 2, 43-520 Zaborze, Poland GLP Unpublished	N	PUH Chemirol Sp. Z o.o.
KCP 5.2/07	K. Wesołowska	2025	Earthworm Reproduction Test (Eisenia andrei), APPENDIX No. 3 Chemical analysis ETOX-2024-64 EcoTox Alliance Sp. z o. o., ul. Michałowicza 12, 43-300 Bielsko-Biała GLP Unpublished	N	PUH Chemirol Sp. Z o.o.
KCP 5.2/08	S. Groch	2025	Predatory mite (Hypoaspis (Geolaelaps) aculieifer) reproduction test in soil, APPENDIX No. 3 Chemical analysis ETOX-2024-65 EcoTox Alliance Sp. z o. o., ul. Michałowicza 12, 43-300 Bielsko-Biała GLP Unpublished	N	PUH Chemirol Sp. Z o.o.

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

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Verte-brate study Y/N	Owner
KCP 5.1.2/01		2006	Validation of multi-residue method DFG S 19 (L 00.00-34) for the determination of residues of DPX-E2Y45 and its metabolites IN-EQW78; IN-GAZ70; IN-HXH44; and IN-K9T00 in different animal matrices with LC-MS/MS detection DuPont-15025 GLP Unpublished	N	DuPont
KCP 5.1.2/02	Rzepka, S.	2005	Validation of multi-residue method DFG S 19 (L00.00-34) for the determination of residues of DPX-E2Y45 in different plant matrices with LC-MS/MS detection Dr. Specht & Partner Chemische Laboratorien GmbH DuPont-13261 GLP Unpublished	N	DuPont
KCP 5.1.2/03	Bilas, J.M., Gagnon, M.R., Stry, J.J.	2005	Analytical method for the determination of DPX-E2Y45 in soil using GC-ECD DuPont Stine-Haskell Research Center DuPont-14942 GLP Unpublished	N	DuPont
KCP 5.1.2/04	Bilas, J.M., Stry, J.J.	2005	Analytical method for the determination of DPX-E2Y45 and degradation products in water using LC/MS/MS DuPont Stine-Haskell Research Center DuPont-16058 GLP Unpublished	N	DuPont
KCP 5.1.2/05	van Schaik, F.	2006	Development of an analytical enforcement method in air (by adsorption on XAD-2 and LC-MS/MS) for DPX-E2Y45 TNO Quality of Life DuPont-18612	N	DuPont

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Verte-brate study Y/N	Owner
			GLP Unpublished		
KCP 5.2/01	Rzepka, S.	2005	Validation of multi-residue method DFG S 19 (L00.00-34) for the determination of residues of DPX-E2Y45 in different plant matrices with LC-MS/MS detection Dr. Specht & Partner Chemische Laboratorien GmbH DuPont-13261 GLP Unpublished	N	DuPont
KCP 5.2/02	van Schaik, F.	2006	Validation of multi-residue method DFG S 19 (L 00.00-34) for the determination of residues of DPX-E2Y45 in different animal matrices with LC-MS/MS detection DuPont-20979 GLP Unpublished	N	DuPont
KCP 5.2/03		2006	Validation of multi-residue method DFG S 19 (L 00.00-34) for the determination of residues of DPX-E2Y45 and its metabolites IN-EQW78; IN-GAZ70; IN-HXH44; and IN-K9T00 in different animal matrices with LC-MS/MS detection DuPont-15025 GLP Unpublished	N	DuPont
KCP 5.2/04	Bilas, J.M., Stry, J.J.	2005	Analytical method for the determination of DPX-E2Y45 and degradation products in water using LC/MS/MS DuPont Stine-Haskell Research Center DuPont-16058 GLP Unpublished	N	DuPont
KCP 5.2/05		2006	ILV of DPX-E2Y45 and it metabolites IN-EQW78, IN-GAZ70, IN-HXH44 and IN-K9T00 in different animal matrices with LC-MS/MS detection as described in DuPont-15025 DuPont-18610	N	DuPont

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Verte-brate study Y/N	Owner
			GLP Unpublished		
KCP 5.2/06	Buscher, B.A.P.	2006	Inter laboratory validation of DuPont-16058 "Analytical method for the determination of DPX-E2Y45 and degradation products in water using LC/MS/MS" TNO Nutrition and Food Research DuPont-16708, Revision No. 1 GLP Unpublished	N	DuPont
KCP 5.2/07		2006	Analytical method for the determination of DPX-E2Y45 in bovine tissues, milk, and eggs using LC/MS/MS DuPont-20978 GLP Unpublished	N	DuPont
KCP 5.2/08		2006	Validation of multi-residue method DFG S 19 (L 00.00-34) for the determination of residues of DPX-E2Y45 and its metabolites IN-EQW78; IN-GAZ70; IN-HXH44; and IN-K9T00 in different animal matrices with LC-MS/MS detection DuPont-15025 GLP Unpublished	N	DuPont
KCP 5.2/09	van Schaik, F.	2006	Development of an analytical enforcement method in air (by adsorption on XAD-2 and LC-MS/MS) for DPX-E2Y45 TNO Quality of Life DuPont-18612 GLP Unpublished	N	DuPont
KCP 5.2/10	Bilas, J.M., Gagnon, M.R., Stry, J.J.	2005	Analytical method for the determination of DPX-E2Y45 in soil using GC-ECD DuPont Stine-Haskell Research Center DuPont-14942	N	DuPont

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Verte-brate study Y/N	Owner
			GLP Unpublished		

The following tables are to be completed by MS

List of data submitted by the applicant and not relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Verte-brate study Y/N	Owner
KCP XX	Author	YYYY	Title Company Report N Source GLP/non GLP/GEP/non GEP Published/Unpublished	Y/N	Owner

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

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Verte-brate study Y/N	Owner
KCP XX	Author	YYYY	Title Company Report N Source GLP/non GLP/GEP/non GEP Published/Unpublished	Y/N	Owner

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)

No new or additional studies have been submitted

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

A 2.1.2.1 Description of analytical methods for the determination of residues in plant matrices (KCP 5.2)

No new or additional studies have been submitted

A 2.1.2.1.1 Analytical method 1

A 2.1.2.1.1.1 Method validation

Comments of zRMS:	The analytical method code: VAL/11/2024 was fully validated in term of specificity, linearity, repeatability, accuracy according to SANTE/2020/12830, rev. 2 The results of analytical method validation confirm that this method is suitable for determination of residues of chlorantraniliprole in oilseed rape (seed, plant),. The method is successfully validated and accepted.
Reference:	KCP 5.2/01
Report	Validation of an analytical method for the determination of residues of chlorantraniliprole in oilseed rape (seed, plant), K. Głowiak, 2024, Study code: VAL/11/2024,
Guideline(s):	Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC Guidance Document on Pesticide Analytical Methods for Risk Assessment and Post-approval Control and Monitoring Purposes, SANTE/2020/12830 Rev.2. 14. February 2023.
Deviations:	NO
GLP:	YES

Acceptability: YES

Materials and methods

The method for the determination of residues of chlorantraniliprole was validated in oilseed rape (seed, plant). The method was validated over the concentration range of 0.010-0.10 mg/kg (µg/g) with an estimated limit of detection at 0.002 mg/kg. This study was conducted to fulfill data requirements outlined in the SANTE/2020/12830 Rev.2.
Validation was carried out using oilseed rape that was spiked with active substance at three different concentration levels (LOD, LOQ and 10 x LOQ). Linearity, specificity, precision, recovery, expanded uncertainty and the limit of detection and quantification were determined

Following sequence of samples was analyzed during validation process, separately for each representative matrix:

- blank calibration solution with no addition of a matrix (checking the purity of the reagents used in the method
- 6 levels of calibration (matrix-matched calibration)
- 2 samples as "blank matrix"
- 2 samples fortified at the limit of detection (LOD = 0.002 mg/kg)
- 5 samples fortified at the limit of quantification (LOQ = 0.010 mg/kg)
- 5 samples fortified with standard at the 10-fold higher concentration than the LOQ (10 x LOQ = 0.10 mg/kg)

EQUIPMENT AND REAGENTS

EQUIPMENT
• Electronic balance Class I; WP-011/W/S/LF
• Electronic balance Class II; WP-012/W/S/LF, WP-013/W/S/LF
• Freezers - storage of:
- analytical standards; W-082/S/LF
- analytical samples before the analytical part; W-093/LF
- analytical samples extracts–freezing during extraction/preparation of samples; W-084/S/LF
- analytical samples extracts until the end of the instrumental analysis; W-084/S/LF
• Centrifuge (> 3000 rpm); W-076/LF
• Cutter (knife grinder); W-133/LF, W-096/LF
• Polypropylene centrifuge tubes, with screw caps, 25 and 50 mL
• Plastic cups (stackable), 100 mL, for the storage of archived samples
• Volumetric flasks, 5 and 10 mL (class A)
• Automatic pipettes: 10–200 µL (WP-111/S/LF); 0.1–1 mL (WP-101/S/LF); 0.5-5.00 mL (WP-102/S/LF); 1.00-10.0 mL (WP-103/S/LF);
• Vials with screw caps, amber glass, 10 mL
• Injection vials, 2.5 mL, amber glass, screw caps with septa butyl/PTFE
• Syringe filters RC 0.22 µm, 25 mm
• Liquid Chromatography Instrument LC-MS/MS Sciex Qtrap 6500+ with SelexION+; WP-144/K/S/ko/LF
• Chromatographic column: Gemini 3 µm, C18, 110 Å, 150 x 4.6 mm (Phenomenex); Lot: 5526-0079
REAGENTS
• Water for LC-MS, Lot: 24G084018

• Acetonitrile, CH3CN, hypergrade for LC-MS, Lot: I1342629420
• Formic acid, HCOOH, 98 – 100%, for analysis, Lot: 23I054006
• Acetone, C3H6O, Lot: 240417A004
• Buffer salt mixture, Lot: 3624, containing:
- 4g of anhydrous magnesium sulfate, MgSO4
- 1g of sodium chloride, NaCl
- 1g of trisodium citrate dihydrate, C5H5Na3O7·2H2O
- 0.50g of disodium hydrogencitrate sesquihydrate, C6H6Na2O7·1,5H2O

DETAILED DESCRIPTION OF THE ANALYTICAL PROCEDURE:

INITIAL SAMPLE PREPARATION AND HOMOGENISATION
Untreated samples for validation purposes were obtained from commercial source.
The field specimens arrived at the Test Facility in good conditions and were further stored at ≤ -18°C until beginning of analysis.

EXTRACTION
5g of the homogenized sample was weighed into a 50 mL centrifuge tube. 10 mL of acetonitrile and 10 mL of water was added together with 50 µL of internal standard solution (1.4) and the mixture was shaken vigorously by hand for one minute. After addition of buffering salts (4 g anhydrous magnesium sulfate, 1 g sodium chloride, 1 g trisodium citrate dehydrate, 0.5 g disodium hydrogencitrate sesquihydrate), the mixture was shaken again intensively for 1 min, then centrifuged at 4700 rpm for 5 min for phase separation and finally subjected to a freezing process at ≤ -18 oC for 1.5 h. After that, the extract (organic phase) was filtered through a membrane filter and the final extract was directly employed for LC-MS/MS analysis. Quantification was performed using an internal standard, which was added to the extract after the initial addition of acetonitrile.

Fortification samples
For each analytical sequence two samples blank matrix, two samples at limit of detection (LOD), five procedural recoveries at the level of LOQ and five at the level 10 x LOQ were prepared.
5 g of the homogenized untreated sample was weighed into a 50 mL centrifuge tube. Appropriate active substance standard solution was added. Extraction of all samples was performed on 09.10.2024, after that the samples were directly employed for LC-MS/MS analysis, that was started on the same day.
Chlorantraniliprole in oilseed rape (seed, plant) - duration of the extraction process was about 3 h and duration of the chromatographic analysis was 950 min (15.8 h), the total analytical procedure was performed and completed within 1 day.
As required in SANTE/2020/12830 Rev.2, 14 February 2023, If the extracts contain an IL-IS (isotopically labelled internal standard) for quantification, testing of final extract stability is not required since the IL-IS will compensate for losses during extract storage. As there was used IL-IS in this validation study, in addition total analytical procedure was performed and completed within 24h (in case of each matrix), and recoveries in the fortified samples are within the acceptable range of 70-120 %, stability is sufficiently proven.
Working standards that were used for quantification were always prepared on the same day as the work up of the specimen for residue analysis take place (then extract stability should not be considered to be an issue)

ANALYSIS

The extracts were analyzed using liquid chromatography coupled with mass spectrometry, by single extraction and single injection to the detection system. Final extracts were employed for LC-MS/MS analysis directly after completion of the extraction procedure (on the same day). Data acquisition was carried out in the MRM mode. The analysis was performed using internal standard addition. For each analyte, two mass transitions were evaluated and used for quantification. Representative chromatograms are shown in this report. A third mass transition was monitored for confirmation of peak identity, but was not used for quantification.

1) ANALYSIS OF TRIBENURON METHYL
Instrument settings:
Liquid Chromatograph LC-MS/MS Sciex Qtrap 6500+ with SelexION+ (WP-144/K/S/ko/LF),consists of:
Degazer DGU-405
Two pumps LC-40D XR
Autosampler SIL-40C XR
Column oven CTO-40C
Compressor, generator PEAK Genius 1024
HPLC Column – Gemini 3 µm, C18, 110 Å, 150 x 4.6 mm (Phenomenex); Lot: 5526-0079
Pumps:
Mode – Binary gradient
Total Flow – 0.50 mL/min
- Mobile Phase A – 0.1% formic acid in deionized water
- Mobile Phase B – 0.1% formic acid in acetonitrile
A Conc – 90%
B Conc – 10%

Stop time: 19 min
Cooler Temp.: 4°C
Oven Temp.: 40°C
Injection volume: 3 µL
Curtain Gas: 30.0
Collision Gas: Medium
IonSpray Voltage: 5500.0
Temperature: 400.0°C
Ion Source Gas 1 (GS1): 50.0
Ion Source Gas 2 (GS2): 50.0

Chlorantraniliprole
Acquisition Mode: MRM
Polarity: Positive
Retention Time: 13.24 min

Chlorantraniliprole D3
Acquisition Mode: MRM
Polarity: Positive
Retention Time: 13.22 min

	Chlorantraniliprole
Author(s), year	K. Głowiak 2024
Principle of method	LC-MS/MS
Matrix Effects	For chlorantraniliprole in oilseed rape (seed, plant) matrix effects calculated using equation are <20%. Nevertheless, there was used matrix-matched calibration..
Linearity (linear between mg/L / % range of the declared content) (correlation coefficient, expressed as r)	The linearity of the detector response was demonstrated by single determination of calibration standards at concentration levels ranging from 1 ppb to 250 ppb. The coefficients of determination (R2) were determined. Linear regression analysis with 1/x weighting was used to describe the detector response as a function of the calibration standard concentrations. For the least squares regression equations describing the detector response as a function of the standard calibration curve concentrations, the coefficients of determination (r) were greater than 0.990 for all of the calibration curve determinations during the method validation. The results indicate linearity of the detector response as a function of the standard concentration.
	Grain: Transition : 483.90 → 286.00 – y=0.67237x + 8.12087E-04 Transition: 486.00 → 452.00 – y=1.13349x + 0.00190 Whole Plant: Transition : 483.90 → 286.00 – y=0.69879x + 0.00226 Transition: 483.90 → 452.90 – y=1.15395x + 0.00473
Specificity/selectivity	LC-MS/MS method was used during the study. Two mass transitions were evaluated and used for quantification. The specificity of the method was evaluated on the basis of the analysis of chromatograms recorded for the matrix blank samples.
Quantification (target) ion	Grain: 483.90 → 452.90 483.90 → 286.00 Plant: 483.90 → 452.90 483.90 → 286.00
Qualification (ref) Ion(s)	Grain: 483.90 → 286.00 486.00 → 455.00 Plant: 483.90 → 286.00 486.00 → 455.00
Precision, Accuracy and Uncertainity	Recover data was generated from five sample fortified at the limit of quantification and five samples fortified at the 10-fold higher concentration than the LOQ. Precision of the method was determined as the relative standard deviation (RSD) of recovery at each fortification level. The mean recovery at each fortification level should be in the range of 70 – 120%. Wherever

Chlorantraniliprole

applicable (n≥3), the relative standard deviation was determined and should be ≤ 20% for each level (RSD were determined only during validation process):

Grain:

Transition: 483.90→ 452.90

Fortification level [mg/kg]	Obtained result [mg/kg]	Recovery [%]	Fortification level [mg/kg]	Obtained result [mg/kg]	Recovery [%]
0.010	0.010	103.0	0.10	0.11	105.6
	0.010	101.6		0.10	102.2
	0.011	105.6		0.10	104.1
	0.011	107.6		0.10	102.5
	0.010	104.8		0.11	105.2
Average	0.010	104.5	Average	0.10	103.9
SD	0.00023	2.34	SD	0.0015	1.54
RSD [%]	2.24		**RSD [%]**	1.48	
Uncertainty [%]	10.1		**Uncertainty [%]**	8.4	

Transition: 483.90→ 286.00

Fortification level [mg/kg]	Obtained result [mg/kg]	Recovery [%]	Fortification level [mg/kg]	Obtained result [mg/kg]	Recovery [%]
0.010	0.010	102.8	0.10	0.11	106.8
	0.010	103.1		0.10	102.5
	0.010	102.1		0.10	102.4
	0.011	106.1		0.10	103.2
	0.010	103.6		0.10	102.5
Average	0.010	103.6	Average	0.10	103.5
SD	0.00015	1.54	SD	0.0019	1.86
RSD [%]	1.49		**RSD [%]**	1.80	
Uncertainty [%]	7.7		**Uncertainty [%]**	7.8	

Plant:

	Chlorantraniliprole

Transition: 483.90→ 452.90

Fortification level [mg/kg]	Obtained result [mg/kg]	Recovery [%]	Fortification level [mg/kg]	Obtained result [mg/kg]	Recovery [%]
0.010	0.010	102.4	0.10	0.11	106.3
	0.010	103.2		0.10	102.1
	0.011	105.9		0.11	106.0
	0.011	107.8		0.10	101.5
	0.010	102.2		0.11	105.1
Average	0.010	104.3	Average	0.10	104.2
SD	0.00024	2.45	SD	0.0023	2.25
RSD [%]	2.35		**RSD [%]**	2.16	
Uncertainty [%]	9.8		**Uncertainty [%]**	9.4	

Transition: 483.90→ 286.00

Fortification level [mg/kg]	Obtained result [mg/kg]	Recovery [%]	Fortification level [mg/kg]	Obtained result [mg/kg]	Recovery [%]
0.010	0.010	101.7	0.10	0.10	104.9
	0.010	100.2		0.10	101.5
	0.010	104.2		0.10	103.8
	0.011	105.1		0.097	97.1
	0.010	99.5		0.10	102.0
Average	0.010	102.1	Average	0.10	101.9
SD	0.00024	2.42	SD	0.0030	3.02
RSD [%]	2.37		**RSD [%]**	2.96	
Uncertainty [%]	6.4		**Uncertainty [%]**	7.0	

Limit of quantification (LOQ)	The LOQ is the lowest validated fortification level for which an average recovery in the range of 70 –120% (60 – 120 % in case of level ≤ 0.01 mg/kg) and RSD ≤ 20 % (≤ 30 % in case of level ≤ 0.01 mg/kg) is achieved. LOQ was successfully established at 0.010 mg/kg for chlorantraniliprole in oilseed rape (seed, plant).
Limit of detection (LOD)	Limit of detection (LOD) was established at 0.002 mg/kg. The limit of detection (LOD) was expressed as the lowest calibration standard.
Stability	Working standard that were used for quantification were always prepared on the same day as the work up of the specimen for residue analysis take place (then extract stability should not be considered to be an issue)

The analytical method for determining the residues of chlorantraniliprole in oilseed rape (seed, plant) meets the criteria of SANTE/2020/12830. Rev. 2. 14 February 2023 guidelines document in terms of precision. accuracy and uncertainty. The method was validated over the concentration range of 0.01 - 0.10 mg/kg (µg/g) for chlorantraniliprole in oilseed rape (seed, plant). The limit of detection (LOD) that was expressed as the lowest calibration standard was established at 0.002 mg/kg in oilseed rape (seed, plant).

Limit of quantification (LOQ) of the analytical method was established at 0.010 mg/kg for chlorantraniliprole in oilseed rape (seed, plant).

A 2.1.2.1.2 Analytical method 2

A 2.1.2.1.2.1 Method validation

Comments of zRMS:	The analytical method code: VAL/17/2024 was fully validated in term of specificity, linearity, repeatability, accuracy according to SANTE/2020/12830, rev. 2 The results of analytical method validation confirm that this method is suitable for determination of residues of chlorantraniliprole in sugar beet (roots and leaves). The method is successfully validated and accepted.

Reference:	KCP 5.2/02
Report	Validation of an analytical method for the determination of residues of Chlorantraniliprole in sugar beet (roots and leaves), M. Jędrusik, 2024, Study code: VAL/17/2024,
Guideline(s):	Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC Guidance Document on Pesticide Analytical Methods for Risk Assessment and Post-approval Control and Monitoring Purposes, SANTE/2020/12830 Rev.2. 14. February 2023.
Deviations:	NO
GLP:	YES
Acceptability:	YES

Materials and methods

The method for the determination of residues of chlorantraniliprole was validated in sugar beet (roots, leaves). The method was validated over the concentration range of 0.010-0.10 mg/kg (µg/g) with an estimated limit of detection at 0.001 mg/kg.. This study was conducted to fulfill data requirements outlined in the SANTE/2020/12830 Rev.2. Validation was carried out using sugar beet (roots, leaves) that was spiked with active substance at three different concentration levels (LOD, LOQ and 10 x LOQ). Linearity, specificity, precision, recovery, expanded uncertainty and the limit of detection and quantification were determined.

Following sequence of samples was analyzed during validation process, separately for each representative matrix:

- blank calibration solution with no addition of a matrix (checking the purity of the reagents used in the method
- 6 levels of calibration (matrix-matched calibration)
- 2 samples as "blank matrix"
- 2 samples fortified at the limit of detection (LOD = 0.002 mg/kg)
- 5 samples fortified at the limit of quantification (LOQ = 0.010 mg/kg)

- 5 samples fortified with standard at the 10-fold higher concentration than the LOQ (10 x LOQ = 0.10 mg/kg)

EQUIPMENT AND REAGENTS

EQUIPMENT
• Electronic balance Class I; WP-011/W/S/LF
• Electronic balance Class II; WP-012/W/S/LF, WP-013/W/S/LF
• Freezers - storage of:
- analytical standards; W-082/S/LF
- analytical samples before the analytical part; W-093/LF
- analytical samples extracts–freezing during extraction/preparation of samples; W-084/S/LF
- analytical samples extracts until the end of the instrumental analysis; W-084/S/LF
• Centrifuge (> 3000 rpm); W-076/LF
• Cutter (knife grinder); W-133/LF, W-096/LF
• Polypropylene centrifuge tubes, with screw caps, 25 and 50 mL
• Plastic cups (stackable), 100 mL, for the storage of archived samples
• Volumetric flasks, 5 and 10 mL (class A)
• Automatic pipettes: 10–200 µL (WP-111/S/LF); 0.1–1 mL (WP-101/S/LF); 0.5-5.00 mL (WP-102/S/LF); 1.00-10.0 mL (WP-103/S/LF);
• Vials with screw caps, amber glass, 10 mL
• Injection vials, 2.5 mL, amber glass, screw caps with septa butyl/PTFE
• Syringe filters RC 0.22 µm, 25 mm
• Liquid Chromatography Instrument LC-MS/MS Sciex Qtrap 6500+ with SelexION+; WP-144/K/S/ko/LF
• Chromatographic column: Gemini 3 µm, C18, 110 Å, 150 x 4.6 mm (Phenomenex); Lot: 5526-0079

REAGENTS
• Water for LC-MS, Lot: 24G084018
• Acetonitrile, CH3CN, hypergrade for LC-MS, Lot: I1342629420
• Formic acid, HCOOH, 98 – 100%, for analysis, Lot: 23I054006
• Acetone, C3H6O, Lot: 2404171004
• Buffer salt mixture, Lot: 3624, containing:
- 4g of anhydrous magnesium sulfate, MgSO4
- 1g of sodium chloride, NaCl
- 1g of trisodium citrate dihydrate, C5H5Na3O7·2H2O
- 0.50g of disodium hydrogencitrate sesquihydrate, C6H6Na2O7·1,5H2O

DETAILED DESCRIPTION OF THE ANALYTICAL PROCEDURE:

INITIAL SAMPLE PREPARATION AND HOMOGENISATION
Untreated samples for validation purposes were obtained from commercial source. The field specimens arrived at the Test Facility in good conditions and were further stored at ≤ -18°C until beginning of analysis.

EXTRACTION
10 g of the homogenized sample was weighed into a 50 mL centrifuge tube. 10 mL of acetonitrile was added together with 100 µL of internal standard solution (1.4) and the mixture was shaken

vigorously by hand for one minute. After addition of buffering salts (4 g anhydrous magnesium sulfate, 1 g sodium chloride, 1 g trisodium citrate dehydrate, 0.5 g disodium hydrogencitrate sesquihydrate), the mixture was shaken again intensively for 1 min, then centrifuged at 4700 rpm for 5 min for phase separation and finally subjected to a freezing process at ≤ -18°C for 1.5 h. After that, the extract (organic phase) was filtered through a membrane filter and the final extract was directly employed for LC-MS/MS analysis.
Quantification was performed using an internal standard, which was added to the extract after the initial addition of acetonitrile.

Fortification samples
For each analytical sequence two samples blank matrix, two samples at limit of detection (LOD), five procedural recoveries at the level of LOQ and five at the level 10 x LOQ were prepared.
10 g of the homogenized untreated sample was weighed into a 50 mL centrifuge tube. Appropriate active substance standard solution was added and the sample was extracted as described above. Extraction of all samples was performed on 21.10.2024, after that the samples were directly employed for LC-MS/MS analysis, that was started on the same day.
Chlorantraniliprole in sugar beet (roots, leaves) - duration of the extraction process was about 4 h and duration of the chromatographic analysis was 988 min (16.5 h), the total analytical procedure was performed and completed within 1 day.
As required in SANTE/2020/12830 Rev.2, 14 February 2023, If the extracts contain an IL-IS (isotopically labelled internal standard) for quantification, testing of final extract stability is not required since the IL-IS will compensate for losses during extract storage. As there was used IL-IS in this validation study, in addition total analytical procedure was performed and completed within 24h (in case of each matrix), and recoveries in the fortified samples are within the acceptable range of 70-120 %, stability is sufficiently proven. Working standards that were used for quantification were always prepared on the same day as the work up of the specimen for residue analysis take place (then extract stability should not be considered to be an issue).

ANALYSIS
The extracts were analyzed using liquid chromatography coupled with mass spectrometry, by single extraction and single injection to the detection system. Final extracts were employed for LC-MS/MS analysis directly after completion of the extraction procedure (on the same day). Data acquisition was carried out in the MRM mode. The analysis was performed using internal standard addition.
For each analyte, two mass transitions were evaluated and used for quantification. Representative chromatograms are shown in this report. A third mass transition was monitored for confirmation of peak identity, but was not used for quantification

1) ANALYSIS OF TRIBENURON METHYL
Instrument settings:
Liquid Chromatograph LC-MS/MS Sciex Qtrap 6500+ with SelexION+ (WP-144/K/S/ko/LF), consists of:
Degazer DGU-405
Two pumps LC-40D XR
Autosampler SIL-40C XR
Column oven CTO-40C
Compressor, generator PEAK Genius 1024
HPLC Column – Gemini 3 µm, C18, 110 Å, 150 x 4.6 mm (Phenomenex); Lot: 5526-0079

Pumps:
Mode – Binary gradient
Total Flow – 0.50 mL/min
- Mobile Phase A – 0.1% formic acid in deionized water
- Mobile Phase B – 0.1% formic acid in acetonitrile
A Conc – 90%
B Conc – 10%

Stop time: 19 min
Cooler Temp.: 4°C
Oven Temp.: 40°C
Injection volume: 2 µL

Curtain Gas: 30.0
Collision Gas: Medium
IonSpray Voltage: 5500.0
Temperature: 400.0°C
Ion Source Gas 1 (GS1): 50.0
Ion Source Gas 2 (GS2): 50.0

Chlorantraniliprole
Acquisition Mode: MRM
Polarity: Positive
Retention Time: 13.18 min

Chlorantraniliprole D3
Acquisition Mode: MRM
Polarity: Positive
Retention Time: 13.16 min

	Chlorantraniliprole
Author(s), year	M. Jędrusik, 2024
Principle of method	LC-MS/MS
Matrix Effects	For chlorantraniliprole in sugar beet roots matrix effects calculated using equation are <20%. For chlorantraniliprole in sugar beet leaves matrix effect calculated using equation exceed ±20%. However to compensate matrix effects, the matrix-matched calibrations was used in case of each matrix.
Linearity (linear between mg/L / % range of the declared content)	The linearity of the detector response was demonstrated by single determination of calibration standards at concentration levels ranging from 1 ppb to 250 ppb. The coefficients of determination (R2) were determined. Linear regression analysis with 1/x weighting was used to describe the detector response as a function of the calibration standard concentrations. For the least squares regression equations describing the detector response as a function of the standard calibration curve concentrations, the coefficients of determination (r) were greater than 0.990 for all of the calibration curve determinations during the method validation. The results indicate linearity of the detector

	Chlorantraniliprole
(correlation coefficient, expressed as r)	response as a function of the standard concentration.
	Roots: Transition : 483.90 → 286.00 – y=0.64111x + 6.08204E-04 Transition: 486.00 → 452.90 – y=1.05828x + 0.00173 Leaves: Transition : 483.90 → 286.00 – y=0.60258x + 3.38688E-04 Transition: 483.90 → 452.90 – y=1.03788x + 0.00117
Specific-ity/selectiv-ity	LC-MS/MS method was used during the study. Two mass transitions were evaluated and used forquantification. The specificity of the method was evaluated on the basis of the analysis of chromatograms recorded for the matrix blank samples..
Quantifica-tion (tar-get) ion	Roots: 483.90 → 452.90 483.90 → 286.00 Leaves: 483.90 → 452.90 483.90 → 286.00
Qualifica-tion (ref) Ion(s)	Roots:: 483.90 → 286.00 486.00 → 455.00 Leaves: 483.90 → 286.00 486.00 → 455.00
Precision, Accuracy and Uncer-tainity	Recover data was generated from five sample fortified at the limit of quantification and five samples fortified at the 10-fold higher concentration than the LOQ. Precision of the method was determined as the relative standard deviation (RSD) of recovery at each fortification level. The mean recovery at each fortification level should be in the range of 70 – 120%. Wherever applicable (n≥3), the relative standard deviation was determined and should be ≤ 20% for each level (RSD were determined only during validation process): Roots:

Chlorantraniliprole

Transition: 483.90→ 452.90

Fortification level [mg/kg]	Obtained result [mg/kg]	Recovery [%]	Fortification level [mg/kg]	Obtained result [mg/kg]	Recovery [%]
0.010	0.011	105.4	0.10	0.10	102.2
	0.010	104.6		0.10	104.9
	0.011	106.4		0.11	105.5
	0.011	105.0		0.10	102.3
	0.010	101.9		0.10	102.2
Average	0.010	104.7	Average	0.10	103.4
SD	0.00017	1.70	SD	0.0016	1.64
RSD [%]	1.63		**RSD [%]**	1.59	
Uncertainty [%]	9.87		**Uncertainty [%]**	7.51	

Transition: 483.90→ 286.00

Fortification level [mg/kg]	Obtained result [mg/kg]	Recovery [%]	Fortification level [mg/kg]	Obtained result [mg/kg]	Recovery [%]
0.010	0.010	102.2	0.10	0.10	101.9
	0.010	102.7		0.10	102.1
	0.010	101.8		0.10	101.7
	0.010	100.5		0.10	102.7
	0.010	102.3		0.10	101.5
Average	0.010	101.9	Average	0.10	102.0
SD	0.000086	0.86	SD	0.00043	0.43
RSD [%]	0.84		**RSD [%]**	0.43	
Uncertainty [%]	4.13		**Uncertainty [%]**	4.04	

Leaves:

Chlorantraniliprole

Transition: 483.90→ 452.90

Fortification level [mg/kg]	Obtained result [mg/kg]	Recovery [%]	Fortification level [mg/kg]	Obtained result [mg/kg]	Recovery [%]
0.010	0.010	103.3	0.10	0.11	105.1
	0.010	101.8		0.11	106.3
	0.010	103.2		0.10	103.5
	0.011	105.5		0.10	103.8
	0.011	105.0		0.10	101.7
Average	0.010	103.7	Average	0.10	104.1
SD	0.00015	1.51	SD	0.0017	1.73
RSD [%]	1.45		**RSD [%]**	1.66	
Uncertainty [%]	8.03		**Uncertainty [%]**	8.82	

Transition: 483.90→ 286.00

Fortification level [mg/kg]	Obtained result [mg/kg]	Recovery [%]	Fortification level [mg/kg]	Obtained result [mg/kg]	Recovery [%]
0.010	0.010	99.8	0.10	0.10	103.7
	0.010	100.6		0.10	104.8
	0.010	100.7		0.10	102.8
	0.010	104.6		0.11	105.5
	0.010	102.2		0.10	102.1
Average	0.010	101.6	Average	0.10	103.8
SD	0.00019	1.91	SD	0.0014	1.39
RSD [%]	1.88		**RSD [%]**	1.34	
Uncertainty [%]	4.92		**Uncertainty [%]**	8.01	

Limit of quantification (LOQ)	The LOQ is the lowest validated fortification level for which an average recovery in the range of 70 –120% (60 – 120 % in case of level ≤ 0.01 mg/kg) and RSD ≤ 20 % (≤ 30 % in case of level ≤ 0.01 mg/kg) is achieved. LOQ was successfully established at 0.010 mg/kg for chlorantraniliprole in sugar beet (roots, leaves).
Limit of detection (LOD)	Limit of detection (LOD) was established at 0.001 mg/kg. The limit of detection (LOD) was expressed as the lowest calibration standard..
Stability	Working standard that were used for quantification were always prepared on the same day as the work up of the specimen for residue analysis take place (then extract stability should not be considered to be an issue)

The analytical method for determining the residues of chlorantraniliprole in sugar beet (roots, leaves) meets the criteria of SANTE/2020/12830. Rev. 2. 14 February 2023 guidelines document in terms of precision. accuracy and uncertainty. The method was validated over the concentration range of 0.01 - 0.10 mg/kg (µg/g) for chlorantraniliprole in sugar beet (roots, leaves). The limit of detection (LOD) that was expressed as the lowest calibration standard was established at 0.001 mg/kg in sugar beet (roots, leaves). Limit of quantification (LOQ) of the analytical method was established at 0.010 mg/kg for

chlorantraniliprole in sugar beet (roots, leaves).

A 2.1.2.1.2.2 Independent laboratory validation

A 2.1.2.2 Description of analytical methods for the determination of residues in animal matrices (KCP 5.2)

No new or additional studies have been submitted

A 2.1.2.3 Description of Methods for the Analysis of Soil (KCP 5.2)

No new or additional studies have been submitted

A 2.1.2.4 Description of Methods for the Analysis of Water (KCP 5.2)

No new or additional studies have been submitted

A 2.1.2.5 Description of Methods for the Analysis of Air (KCP 5.2)

No new or additional studies have been submitted

A 2.1.2.6 Description of Methods for the Analysis of Body Fluids and Tissues (KCP 5.2)

No new or additional studies have been submitted

A 2.1.2.7 A.2.A.9 Other Studies/ Information

No new or additional studies have been submitted

A 2.1.2.7.1 Analytical method 3

A 2.1.2.7.1.1 Method validation

Comments of zRMS:	The analytical method code: VAL/10/2024 was fully validated in term of specificity, linearity, repeatability, accuracy according to SANTE/2020/12830, rev. 2 The results of analytical method validation confirm that this method is suitable for determination of chlorantraniliprole in honey. The method is successfully validated and accepted.

Reference:	KCP 5.2/03
Report	Validation of an analytical method for the determination of residues of chlorantraniliprole in honey, K. Głowiak, 2024, Study code: VAL/10/2024.
Guideline(s):	Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC Guidance Document on Pesticide Analytical Methods for Risk Assessment and Post-approval Control and Monitoring Purposes, SANTE/2020/12830 Rev.2. 14. February 2023.
Deviations:	NO
GLP:	YES
Acceptability:	YES

Materials and methods

The method for the determination of residues of chlorantraniliprole was validated in honey. The method was validated over the concentration range of 0.010-0.10 mg/kg (µg/g) with an estimated limit of detection at 0.002 mg/kg.. This study was conducted to fulfill data requirements outlined in the SANTE/2020/12830 Rev.2.
Validation was carried out using oilseed rape that was spiked with active substance at three different concentration levels (LOD, LOQ and 10 x LOQ). Linearity, specificity, precision, recovery, expanded uncertainty and the limit of detection and quantification were determined

Following sequence of samples was analyzed during validation process, separately for each representative matrix:

- blank calibration solution with no addition of a matrix (checking the purity of the reagents used in the method
- 6 levels of calibration (matrix-matched calibration)
- 2 samples as "blank matrix"
- 2 samples fortified at the limit of detection (LOD = 0.002 mg/kg)
- 5 samples fortified at the limit of quantification (LOQ = 0.010 mg/kg)
- 5 samples fortified with standard at the 10-fold higher concentration than the LOQ (10 x LOQ = 0.10 mg/kg)

EQUIPMENT AND REAGENTS

EQUIPMENT
• Electronic balance Class I; WP-011/W/S/LF
• Electronic balance Class II; WP-012/W/S/LF, WP-013/W/S/LF
• Freezers - storage of:
- analytical standards; W-082/S/LF
- analytical samples before the analytical part; W-093/LF
- analytical samples extracts–freezing during extraction/preparation of samples; W-084/S/LF
- analytical samples extracts until the end of the instrumental analysis; W-084/S/LF
• Centrifuge (> 3000 rpm); W-076/LF
• Cutter (knife grinder); W-133/LF, W-096/LF
• Polypropylene centrifuge tubes, with screw caps, 25 and 50 mL

• Plastic cups (stackable), 100 mL, for the storage of archived samples
• Volumetric flasks, 5 and 10 mL (class A)
• Automatic pipettes: 10–200 µL (WP-111/S/LF); 0.5-5.00 mL (WP-102/S/LF); 1.00-10.0 mL (WP-
103/S/LF); 50-1000 µL (WP-138/S/LF);
• Vials with screw caps, amber glass, 10 mL
• Injection vials, 2.5 mL, amber glass, screw caps with septa butyl/PTFE
• Syringe filters RC 0.22 µm, 25 mm
• Liquid Chromatography Instrument LC-MS/MS Sciex Qtrap 6500+ with SelexION+; WP-144/K/S/ko/LF
• Chromatographic column: Gemini 3 µm, C18, 110 Å, 150 x 4.6 mm (Phenomenex); Lot: 5526-0079

REAGENTS
• Water for LC-MS, Lot: Z0911433406
• Acetonitrile, CH3CN, hypergrade for LC-MS, Lot: I1342529418
• Formic acid, HCOOH, 98 – 100%, for analysis, Lot: 23I054006
• Acetone, C3H6O, Lot: 211007A002
• Buffer salt mixture, Lot: 1124, containing:
- 4g of anhydrous magnesium sulfate, MgSO4
- 1g of sodium chloride, NaCl
- 1g of trisodium citrate dihydrate, C5H5Na3O7·2H2O
- 0.50g of disodium hydrogencitrate sesquihydrate, C6H6Na2O7·1,5H2O

DETAILED DESCRIPTION OF THE ANALYTICAL PROCEDURE:

INITIAL SAMPLE PREPARATION AND HOMOGENISATION
Untreated samples for validation purposes were obtained from commercial source.
The field specimens arrived at the Test Facility in good conditions and were further stored at ≤ -18°C until beginning of analysis.

EXTRACTION
5 g of the homogenized sample was weighed into a 50 mL centrifuge tube. 10 mL of acetonitrile and 10 mL of water was added together with 50 µL of internal standard solution (1.4) and the mixture was shaken vigorously by hand for one minute. After addition of buffering salts (4 g anhydrous magnesium sulfate, 1 g sodium chloride, 1 g trisodium citrate dehydrate, 0.5 g disodium hydrogencitrate sesquihydrate), the mixture was shaken again intensively for 1 min, then centrifuged at 4700 rpm for 5 min for phase separation and finally subjected to a freezing process at ≤ -18 °C for 1.5 h. After that, the extract (organic phase) was filtered through a membrane filter and the final extract was directly employed for LC-MS/MS analysis. Quantification was performed using an internal standard, which was added to the extract after the initial addition of acetonitrile.

Fortification samples
For each analytical sequence two samples blank matrix, two samples at limit of detection (LOD), five procedural recoveries at the level of LOQ and five at the level 10 x LOQ were prepared.
5 g of the homogenized untreated sample was weighed into a 50 mL centrifuge tube. Appropriate active substance standard solution was added and the sample was extracted as described above.
Chlorantraniliprole in honey - duration of the extraction process was about 3 h and duration of the chromatographic analysis was 532 min (8.9 h), the total analytical procedure was performed and

completed within 1 day. As required in SANTE/2020/12830 Rev.2, 14 February 2023, If the extracts contain an IL-IS (isotopically labelled internal standard) for quantification, testing of final extract stability is not required since the IL-IS will compensate for losses during extract storage. As there was used IL-IS in this validation study, in addition total analytical procedure was performed and completed within 24h (in case of each matrix), and recoveries in the fortified samples are within the acceptable range of 70-120 %, stability is sufficiently proven.
Working standards that were used for quantification were always prepared on the same day as the work up of the specimen for residue analysis take place (then extract stability should not be considered to be an issue)

ANALYSIS
The extracts were analyzed using liquid chromatography coupled with mass spectrometry, by single extraction and single injection to the detection system. Final extracts were employed for LC-MS/MS analysis directly after completion of the extraction procedure (on the same day). Data acquisition was carried out in the MRM mode. The analysis was performed using internal standard addition. For each analyte, two mass transitions were evaluated and used for quantification. Representative chromatograms are shown in this report. A third mass transition was monitored for confirmation of peak identity, but was not used for quantification

1) ANALYSIS OF TRIBENURON METHYL
Instrument settings:
Liquid Chromatograph LC-MS/MS Sciex Qtrap 6500+ with SelexION+ (WP-144/K/S/ko/LF),consists of:
Degazer DGU-405
Two pumps LC-40D XR
Autosampler SIL-40C XR
Column oven CTO-40C
Compressor, generator PEAK Genius 1024
HPLC Column – Gemini 3 µm, C18, 110 Å, 150 x 4.6 mm (Phenomenex); Lot: 5526-0079

Pumps:
Mode – Binary gradient
Total Flow – 0.50 mL/min
- Mobile Phase A – 0.1% formic acid in deionized water
- Mobile Phase B – 0.1% formic acid in acetonitrile
A Conc – 90%
B Conc – 10%

Stop time: 19 min
Cooler Temp.: 4°C
Oven Temp.: 40°C
Injection volume: 10 µL
Curtain Gas: 30.0
Collision Gas: Medium
IonSpray Voltage: 5500.0
Temperature: 400.0°C
Ion Source Gas 1 (GS1): 50.0
Ion Source Gas 2 (GS2): 50.0

Chlorantraniliprole
Acquisition Mode: MRM
Polarity: Positive
Retention Time: 13.28 min

Chlorantraniliprole D3
Acquisition Mode: MRM
Polarity: Positive
Retention Time: 13.26 min

	Chlorantraniliprole
Author(s), year	K. Głowiak 2024
Principle of method	LC-MS/MS
Matrix Effects	For chlorantraniliprole in honey matrix effects calculated using equation are <20%. Nevertheless, therewas used matrix-matched calibration.
Linearity (linear between mg/L / % range of the declared content) (correlation coefficient, expressed as r)	The linearity of the detector response was demonstrated by single determination of calibration standards at concentration levels ranging from 1 ppb to 500 ppb. The coefficients of determination (R2) were determined. Linear regression analysis with 1/x weighting was used to describe the detector response as a function of the calibration standard concentrations. For the least squares regression equations describing the detector response as a function of the standard calibration curve concentrations, the coefficients of determination (r) were greater than 0.990 for all of the calibration curve determinations during the method validation. The results indicate linearity of the detector response as a function of the standard concentration.
	Transition : 483.90 → 286.00 – y=0.78018x + -0.00175 Transition: 483.90 → 452.90 – y=1.26586x + -0.00111
Specificity/selectivity	LC-MS/MS method was used during the study. Two mass transitions were evaluated and used for quantification. The specificity of the method was evaluated on the basis of the analysis of chromatograms recorded for the matrix blank samples.
Quantification (target) ion	483.90 → 452.90 483.90 → 286.00
Qualification (ref) Ion(s)	483.90 → 286.00 486.00 → 455.00

<table>
<tr><th></th><th>Chlorantraniliprole</th></tr>
<tr><td>Precision, Accuracy and Uncer-tainity</td><td>Recover data was generated from five sample fortified at the limit of quantification and five samples fortified at the 10-fold higher concentration than the LOQ. Precision of the method was determined as the relative standard deviation (RSD) of recovery at each fortification level. The mean recovery at each fortification level should be in the range of 70 – 120%. Wherever applicable (n≥3), the relative standard deviation was determined and should be ≤ 20% for each level (RSD were determined only during validation process):

Transition: 483.90→ 452.90
<table>
<tr><th>Fortification level [mg/kg]</th><th>Obtained result [mg/kg]</th><th>Recovery [%]</th><th>Fortification level [mg/kg]</th><th>Obtained result [mg/kg]</th><th>Recovery [%]</th></tr>
<tr><td rowspan="5">0.010</td><td>0.0094</td><td>93.7</td><td rowspan="5">0.10</td><td>0.10</td><td>104.2</td></tr>
<tr><td>0.0098</td><td>98.2</td><td>0.097</td><td>97.4</td></tr>
<tr><td>0.0099</td><td>98.9</td><td>0.10</td><td>102.1</td></tr>
<tr><td>0.0096</td><td>96.4</td><td>0.098</td><td>98.2</td></tr>
<tr><td>0.0097</td><td>96.9</td><td>0.095</td><td>95.0</td></tr>
<tr><td>Average</td><td>0.0097</td><td>96.8</td><td>Average</td><td>0.099</td><td>99.4</td></tr>
<tr><td>SD</td><td>0.00020</td><td>2.01</td><td>SD</td><td>0.0037</td><td>3.71</td></tr>
<tr><td>RSD [%]</td><td colspan="2">2.08</td><td>RSD [%]</td><td colspan="2">3.73</td></tr>
<tr><td>Uncertanity [%]</td><td colspan="2">7.6</td><td>Uncertanity [%]</td><td colspan="2">7.6</td></tr>
</table>
Transition: 483.90→ 286.00
<table>
<tr><th>Fortification level [mg/kg]</th><th>Obtained result [mg/kg]</th><th>Recovery [%]</th><th>Fortification level [mg/kg]</th><th>Obtained result [mg/kg]</th><th>Recovery [%]</th></tr>
<tr><td rowspan="5">0.010</td><td>0.010</td><td>99.8</td><td rowspan="5">0.10</td><td>0.094</td><td>94.1</td></tr>
<tr><td>0.0092</td><td>91.8</td><td>0.099</td><td>99.3</td></tr>
<tr><td>0.0089</td><td>89.1</td><td>0.097</td><td>97.4</td></tr>
<tr><td>0.0093</td><td>92.6</td><td>0.094</td><td>93.8</td></tr>
<tr><td>0.0094</td><td>94.1</td><td>0.092</td><td>91.8</td></tr>
<tr><td>Average</td><td>0.0093</td><td>93.5</td><td>Average</td><td>0.095</td><td>95.3</td></tr>
<tr><td>SD</td><td>0.00040</td><td>4.00</td><td>SD</td><td>0.0030</td><td>3.00</td></tr>
<tr><td>RSD [%]</td><td colspan="2">4.28</td><td>RSD [%]</td><td colspan="2">3.15</td></tr>
<tr><td>Uncertanity [%]</td><td colspan="2">15.6</td><td>Uncertanity [%]</td><td colspan="2">11.3</td></tr>
</table></td></tr>
<tr><td>Limit of quantifica-tion (LOQ)</td><td>The LOQ is the lowest validated fortification level for which an average recovery in the range of 70 –120% (60 – 120 % in case of level ≤ 0.01 mg/kg) and RSD ≤ 20 % (≤ 30 % in case of level ≤ 0.01 mg/kg) is achieved.
LOQ was successfully established at 0.010 mg/kg for chlorantraniliprole in honey.</td></tr>
<tr><td>Limit of de-tection (LOD)</td><td>Limit of detection (LOD) was established at 0.002 mg/kg. The limit of detection (LOD) was expressed as the lowest calibration standard.</td></tr>
</table>

	Chlorantraniliprole
Stability	Working standard that were used for quantification were always prepared on the same day as the work up of the specimen for residue analysis take place (then extract stability should not be considered to be an issue)

The analytical method for determining the residues of chlorantraniliprole in honey meets the criteria of SANTE/2020/12830, Rev. 2, 14 February 2023 guidelines document in terms of precision, accuracy and uncertainty. The method was validated over the concentration range of 0.01 - 0.10 mg/kg (µg/g) for chlorantraniliprole in honey. The limit of detection (LOD) that was expressed as the lowest calibration standard was established at 0.002 mg/kg in honey. Limit of quantification (LOQ) of the analytical method was established at 0.010 mg/kg for chlorantraniliprole in honey.

A 2.1.2.7.2 Analytical method 4

A 2.1.2.7.2.1 Method validation

Comments of zRMS:	The analytical method code: B-93-23 was fully validated in term of specificity, linearity, repeatability, accuracy according to SANTE/2020/12830, rev. 2 The results of analytical method validation confirm that this method is suitable for determination of chlorantraniliprole in sucrose solution. The method is successfully validated and accepted.

Reference:	KCP 5.2/04
Report	PART II (Analytical Phase) CLOROL 200 SC Honeybees (Apis mellifera L.), Chronic Oral Toxicity Test, A. Fulczyk, 2024, Study code: B-93-23
Guideline(s):	SANTE/2020/12830, Rev. 2 [1] and Standard Operating Procedure SOP/C/9.
Deviations:	NO
GLP:	YES
Acceptability:	YES

Materials and methods

The validation of analytical method was performed according to SANTE/2020/12830, Rev. 2 [1] and Standard Operating Procedure SOP/C/9. The range of linearity of the analytical graph, the regression residual (di), selectivity and specificity, precision, accuracy, stock solution stability and limit of quantification and detection were determined

Active substance of test item:	Chlorantraniliprole Cas No.: 500008-45-7
Matrix:	Sucrose solution
Method:	Dilution method
Chemical analyses:	HPLC with DAD, Luna Omega 5µm PS C18 100 Å, 150 mm×4.6 mm
Calibration curve:	Linear; 1/C
Working solution concentrations:	0.5, 1.0, 2.0, 5.0, 10.0, 20.0, and 50.0 mg/L
Range of linearity of calibration curve:	0.5 – 50.0 mg/L
Equivalent calibration range of Linearity:	5 – 500 mg/kg matrix
Coefficient r^2:	0.9999458
Fortification levels:	Control – 0 mg/kg – two replicates LOQ – 20 mg/kg – five replicates 10xLOQ – 200 mg/kg – five replicates
Matrix effect:	not determined
Final extract stability:	not determined
Stock solution stability:	186 days
Mean recoveries:	97.3 – 99.8%
RSD:	0.0 – 0.1%
Limit of quantification (LOQ):	20 mg/kg
Limit of detection (LOD):	5 mg/kg

The concentrations of chlorantraniliprole were chemically determined using the validated high performance liquid chromatographic method with DAD detection [SOP/C/603, SOP/C/328]. Fresh samples of the test item concentration and the control at test initiation and at the end of the maximum storage period (after 4 days) were analysed in order to confirm correct preparation of the tested sucrose solution [SOP/C/603]. The summary results of determination of active substance of test item in the test samples are shown in table below.

Nominal test item concentration [mg/kg]	**Nominal concentration of active substance [mg/kg]**	**Mean concentration of active substance determined (n=3) in samples collected**			
		[mg/kg]	%± RSD of nominal concentration		
Day 0 (01.08.2024)					
Control	0.000	ND	---		
3000	548.4	570.4	104	±	0.2
Day 4 (05.08.2024)					
Control	0.000	ND	---		
3000	548.4	574.9	105	±	1.0

LOQ = 20 mg chlorantraniliprole/kg; LOD = 5 mg chlorantraniliprole/kg;
--- not calculated; ND – not detected

EQUIPMENT AND REAGENTS

EQUIPMENT

Equipment	Size, description	Manufacturer/Supplier	Standard Operating Procedure
Analytical Balance	Adventurer Pro AV 114CM	Ohaus Corporation (USA)	SOP/C/122
Balance	PS 600.X2	Radwag (Poland)	SOP/C/587
Volumetric flasks	Various volumes	Glassco (Germany)	SOP/C/12
Variable volume single-channel pipettes	Various volumes	Eppendorf AG (Germany)	
Autosampler vials with PTFE/silicone septa and screw caps	Clear glass, 2 mL	Alwsci Technologies (China)	-
Chromatograph	Prominence-*i*	Shimadzu Corp. (Japan)	SOP/C/328

Reagents and solvents [SOP/C/7, SOP/C/8]:
- 0.05% ortho-phosphoric acid solution in deionized water (v/v),
- 50% sucrose solution in deionized water (v/v),
- mixture of acetonitrile for HPLC and deionized water (50:50, v/v) i.e. 50% acetonitrile for HPLC solution,
- mixture of 50% sucrose solution and 50% acetonitrile for HPLC solution (1:9, v/v),
- standard solution of 1 mg/mL of chlorantraniliprole in methanol for LC-MS,
- working solutions of chlorantraniliprole at concentration 50.0, 20.0, 10.0, 5.0, 2.0, 1.0, 0.5 µg/mL in mixture of 50% sucrose solution and 50% acetonitrile for HPLC solution (1:9, v/v).

DETAILED DESCRIPTION OF THE ANALYTICAL PROCEDURE:

Stock and standard solutions

The stock solution with a concentration of 1.0 mg/mL was prepared by weighting 10.0 mg of standard into a volumetric flask with a capacity of 10.0 mL, dissolving in methanol for LC-MS, and next the volume was made up to 10.0 mL with the same solvent. The working solution of chlorantraniliprole with a concentration of 100 µg/mL was prepared by dilution of the stock solution with acetonitrile for HPLC. The working (calibration) solution of chlorantraniliprole with a concentration of 50 µg/mL was prepared by dilution of the stock solution with mixture of 50% sucrose solution and 50% acetonitrile for HPLC solution (1:9, v/v). The working (calibration) solutions were prepared by diluting standard solutions of higher concentrations.

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Fortification samples
For the preparation of procedural recoveries and validation experiments, fortified samples were prepared from standard solution. The appropriate amount of spiking solutions was added to the matrix to prepare LOQ and 10xLOQ

Taken solution at concentration [µg/mL]	Aliquot volume [mL]	Dilute with D2 solution to a final volume of [mL]	Final concentration [µg/mL]
1000* (Stock)	1	10	100.0*

* Spiking solution D2 – acetonitrile for HPLC

The following fortification scheme was used:

Sample type	Matrix	Number of replicates	Weight of spiked sample [g]	Concentration of spiking solution [mg/L]	Volume of spiking solution [mL]	Level of fortification [mg/kg]
Control	Sucrose solution	2	1.0	---	---	0.0
Fortification (LOQ)		5	1.0	100	0.2	20.0
Fortification (10x LOQ)		5	1.0	1000	0.2	200.0

Fortified samples were prepared and analyzed to ensure the result fits within the range of the respective standard curve.

Sample preparation for the chromatographic analysis

First, 1 g of sucrose solution was weighted into a volumetric flask with a capacity of 10 mL and the volume was made up to 10 ml with mixture of acetonitrile for HPLC and deionized water (50:50; v/v). The sample was diluted mixture of acetonitrile for HPLC and deionized water (50:50; v/v), if necessary. An aliquot of the final volume was transferred into a HPLC vial for further quantification using HPLC-DAD [SOP/C/5, SOP/C/12, SOP/C/328, SOP/C/603, SOP/OG/5].

1) Instrumentation and conditions:

	Parameter
Chromatographic System	High Performance Liquid Chromatography (HPLC)
Chromatograph	Shimadzu, Prominence-*i* (Shimadzu Corporation Japan)
Analytical Column	Luna Omega 5µm PS C18 100 Å, 150 mm×4.6 mm
Oven temperature	35°C
Injection Volume	20 µL
Mobile Phase	Acetonitrile for HPLC : ortho-phosphoric acid solution 0.05 % (70 : 30, *v/v*)
Flow Rate	0.50 mL/min
Wave length	240 nm
Retention time	approx. 4.5 min.
Detection System	Diode Array Detector

	Chlorantraniliprole
Author(s), year	A. Fulczyk, 2024
Principle of method	HPLC-DAD
Matrix Effects	Since potential matrix effects were compensated by using matrix matched calibration standards (same matrix load), no instrument recovery samples were prepared and analysed in addition.
Linearity (linear between mg/L / % range of the declared content) (correlation coefficient, expressed as r)	Working solutions at all calibration levels were injected successively to the chromatographic column and the chromatograms were recorded. The range of linearity of the analytical graph and the ranges of calibration curve corresponding to the real samples level are presented in the table below.

Analyte	Working solution concentrations [mg/L]	Range of linearity of calibration curve [mg/L]	Equivalent calibration range of linearity [mg/kg sucrose solution]
Chlorantraniliprole	0.5, 1.0, 2.0, 5.0, 10.0, 20.0, 50.0	0.5 – 50.0	5 – 500

The equation of the calibration line is presented as the linear equation; y = ax + b (a – slope, b – intercept). The linear coefficient r2 must be higher than 0.99. Range of the linearity was given in µg/mL (equal to mg/L).

Range of linearity of calibration curve [mg/L]	Analyte	Slope	Intercept	Coefficient r²
0.5 – 50.0	Chlorantraniliprole	1.33372e-005	0.0109039	0.9999458

<table>
<tr><th></th><th>Chlorantraniliprole</th></tr>
<tr><td>Specificity/selectivity</td><td>The analytical method specificity was estimated on the basis of the analysis of the chromatograms obtained for the control, and fortified samples of matrix. Considering the results of the analysis, no signal of detected substance was overlapping with matrix signal of the control samples in the experiments conditions. Therefore, the specificity of the method was demonstrated. Furthermore, any interference are directly apparent and would be observed in the chromatograms of the matrix control sample..</td></tr>
<tr><td>Precision, Accuracy and Uncertainity</td><td>Precision of a particular methods is defined as its repeatability (RSD – relative standard deviation [%]). The repeatability for detected substance analyzed are presented in table below. The RSD is ≤ 20% per each level

The accuracy of the method is reported as mean recovery ± relative standard deviation. Recovery was reported as mean recovery ± relative standard deviation (RSD). Mean recoveries for each level is in the range 70-120%.
A summary of the recovery data of control and fortified samples are presented in the table below

<table>
<tr><th>Method</th><th>Analyte</th><th>Fortification Level [mg/kg]</th><th>Number of Replicates</th><th>Mean Recovery [%]</th><th>RSD [%]</th></tr>
<tr><td rowspan="2">Dilution method</td><td rowspan="2">Chlorantraniliprole</td><td>20</td><td>5</td><td>97.3</td><td>0.1</td></tr>
<tr><td>200</td><td>5</td><td>99.8</td><td>0.0</td></tr>
</table>
</td></tr>
<tr><td>Limit of quantification (LOQ)</td><td>Limit of Quantification was estimated as the lowest concentration of a detected substance at which an acceptable mean recovery and precision (normally 70 – 120% with a relative standard deviation of preferably ≤ 20%).
LOQ was successfully established at 20 mg/kg for chlorantraniliprole</td></tr>
<tr><td>Limit of detection (LOD)</td><td>Limit of detection (LOD) was established at 5 mg/kg. The limit of detection (LOD) is defined as the lowest detectable concentration or amount of an analyte in a sample. LOD is expressed as the lowest calibration standard. LOD is less than or equal to 30% of LOQ.</td></tr>
<tr><td>Stability</td><td>Final extract stability was not determined. The final extracts were analysed within 24 h.

The stability of stock solution in methanol was tested at concentrations 1 mg/mL i.e. 1000 mg chlorantraniliprole /L. The results for stability were obtained after 0, 13, 33, 61, 90, 114, 141, 155, 178 and 186 days of storage at cool temperature i.e. from 2ºC to 8ºC. Compared to the mean recoveries measured at 0 day, significant decline was not observed after 186 days. The mean recoveries for stock solution did not differ by more than 10%.</td></tr>
</table>

The results of the procedural recovery samples showed that the recovery efficiency was within an acceptable range. The mean recovery for chlorantraniliprole ranged from 97.3% to 99.8% for all fortification levels. The overall RSDs were < 20%. Hence, a good performance of the analytical method is proven. Therefore, the requirements of the cited guideline were fulfilled. The regression residuals are presented in a residual plot in range equal to range of linearity of calibration curve. The analyzed untreated control samples showed no residues above the LOD of 5 mg/L. The specificity of the method was also demonstrated.

A 2.1.2.7.3 Analytical method 5

A 2.1.2.7.3.1 Method validation

Comments of zRMS:	The analytical method code: B-92-23 was fully validated in term of specificity, linearity, repeatability, accuracy according to SANTE/2020/12830, rev. 2 The results of analytical method validation confirm that this method is suitable for determination of chlorantraniliprole in water. The method is successfully validated and accepted.

Reference:	KCP 5.2/05
Report	PART II (Analytical Phase) CLOROL 200 SC Honeybees (Apis mellifera L.), Larval Toxicity Test, Repeated Exposure, A. Fulczyk, 2024, Study code: B-92-23,
Guideline(s):	SANTE/2020/12830, Rev.2 [1] and Standard Operating Procedure SOP/C/9.
Deviations:	NO
GLP:	YES
Acceptability:	YES

Materials and methods

The validation of analytical method was performed according to SANTE/2020/12830, Rev. 2 [1] and Standard Operating Procedure SOP/C/9. The range of linearity of the analytical graph, the regression residual (di), selectivity and specificity, precision, accuracy, stock solution stability and limit of quantification and detection were determined

Active substance of test item:	Chlorantraniliprole Cas No.: 500008-45-7
Matrix:	Water
Method:	Dilution method
Chemical analyses:	HPLC with DAD, Luna Omega 5µm PS C18 100 Å, 150 mm×4.6 mm
Calibration curve:	Linear; 1/C
Working solution concentrations:	0.02, 0.05, 0.1, 0.2, 0.5, 1.0, 2.0 and 5.0 mg/L
Range of linearity of calibration curve:	0.02 – 5.0 mg/L
Equivalent calibration range of Linearity:	0.02 – 5.0 mg/L matrix
Coefficient r^2:	0.9994151
Fortification levels:	Control – 0.00 mg/L – two replicates LOQ – 0.07 mg/L – five replicates 10xLOQ – 0.70 mg/L – five replicates
Matrix effect:	not determined
Final extract stability:	not determined
Stock solution stability:	186 days
Mean recoveries:	99.1 - 107%
RSD:	0.9 – 3.5%
Limit of quantification (LOQ):	0.07 mg/L
Limit of detection (LOD):	0.02 mg/L

The concentrations of chlorantraniliprole were chemically determined using the validated high performance liquid chromatographic method with DAD detection [SOP/C/603, SOP/C/328]. Fresh samples of the test item concentration and the control at test initiation and at the end of the maximum storage period (after 3 days) were analysed in order to confirm correct preparation of the tested water [SOP/C/603].The summary results of determination of active substance of test item in the test samples are shown in table below.

Nominal test item concentration	Nominal concentration of active substance [mg/L]	Mean concentration of active substance determined (n=3) in samples collected			
		[mg/L]	% ± RSD of nominal concentration		
Day 0 (12.08.2024)					
Control	0.000	<LOD	---		
0.82 µg/140 µL	1.071	1.042	97.3	±	1.4
80.0 µg/140 µL	104.46	101.02	96.7	±	1.5
Day 3 (15.08.2024)					
Control	0.000	<LOD	---		
0.82 µg/140 µL	1.071	0.920	85.9	±	0.5
80.0 µg/140 µL	104.46	101.20	96.9	±	1.2

LOQ = 0.07 mg chlorantraniliprole/L; LOD = 0.02 mg chlorantraniliprole/L;
--- not calculated;

EQUIPMENT AND REAGENTS

EQUIPMENT

Equipment	Size, description	Manufacturer/Supplier	Standard Operating Procedure
Analytical Balance	Adventurer Pro AV 114CM	Ohaus Corporation (USA)	SOP/C/122
Volumetric flasks	Various volumes	Glassco (Germany)	SOP/C/12
Variable volume single-channel pipettes	Various volumes	Eppendorf AG (Germany)	
Autosampler vials with PTFE/silicone septa and screw caps	Clear glass, 2 mL	Alwsci Technologies (China)	-
Chromatograph	Prominence-*i*	Shimadzu Corp. (Japan)	SOP/C/328

Reagents and solvents [SOP/C/7, SOP/C/8]:
- 0.05% ortho-phosphoric acid solution in deionized water (v/v),
- standard solution of 1 mg/mL of chlorantraniliprole in methanol LC-MS,
- fortification solutions of chlorantraniliprole at concentration 70.0 and 7.0 µg/mL in methanol LC-MS,
- working solutions of chlorantraniliprole at concentration 100, 70.0, 50.0, 10.0, 7.0, 5.0, 2.0, 1.0, 0.5, 0.2, 0.1, 0.05 and 0.02 µg/mL in deionized water.

DETAILED DESCRIPTION OF THE ANALYTICAL PROCEDURE:

Stock and standard solutions

The stock solution with a concentration of 1.0 mg/mL was prepared by weighting 10.0 mg of standard into a volumetric flask with a capacity of 10.0 mL, dissolving in methanol LC-MS, and next the volume was made up to 10.0 mL with the same solvent. The working solutions of chlorantraniliprole were prepared by dilution of the stock solution with deionized water HPLC. The working solutions, fortification and calibration were prepared by diluting standard solutions of higher concentrations.

Fortification samples
For the preparation of procedural recoveries and validation experiments, fortified samples were prepared from standard solution. The appropriate amount of spiking solutions was added to the matrix to prepare LOQ and 10xLOQ

Taken solution at concentration [µg/mL]	Aliquot volume [mL]	Dilute with D2 solution to a final volume of [mL]	Final concentration [µg/mL]
1000* (Stock)	0.07	1	70.0*
70	0.1	1	7.0*

* Spiking solution, D2 – methanol LC-MS

The following fortification scheme was used:

Sample type	Matrix	Number of replicates	Volume of spiked sample [mL]	Concentration of spiking solution [mg/L]	Volume of spiking solution [mL]	Level of fortification [mg/L]
Control	Water	2	1.0	---	---	0.00
Fortification (LOQ)		5	0.99	7.0	0.01	0.07
Fortification (10x LOQ)		5	0.99	70.0	0.01	0.70

Sample preparation for the chromatographic analysis

First, 1 ml of sample was taken and shake. The sample was diluted deionized water, if necessary. An aliquot of the final volume was transferred into a HPLC vial for further quantification using HPLC-DAD [SOP/C/5, SOP/C/12, SOP/C/328, SOP/C/603, SOP/OG/5].

1) Instrumentation and conditions:

	Parameter
Chromatographic System	High Performance Liquid Chromatography (HPLC)
Chromatograph	Shimadzu, Prominence-*i* (Shimadzu Corporation Japan)
Analytical Column	Luna Omega 5µm PS C18 100 Å, 150 mm×4.6 mm
Oven temperature	35°C
Injection Volume	20 µL
Mobile Phase	Acetonitrile for HPLC : ortho-phosphoric acid solution 0.05 % (70 : 30, *v/v*)
Flow Rate	0.50 mL/min
Wave length	240 nm
Retention time	approx. 4.5 min.
Detection System	Diode Array Detector

<table>
<tr><th></th><th>Chlorantraniliprole</th></tr>
<tr><td>Author(s), year</td><td>A. Fulczyk, 2024</td></tr>
<tr><td>Principle of method</td><td>HPLC-DAD</td></tr>
<tr><td>Matrix Effects</td><td>Since potential matrix effects were compensated by using matrix matched calibration standards (same matrix load), no instrument recovery samples were prepared and analysed in addition.</td></tr>
<tr><td>Linearity (linear between mg/L / % range of the declared content) (correlation coefficient, expressed as r)</td><td>Working solutions at all calibration levels were injected successively to the chromatographic column and the chromatograms were recorded. The range of linearity of the analytical graph and the ranges of calibration curve corresponding to the real samples level are presented in the table below.
<table>
<tr><th>Analyte</th><th>Working solution concentrations [mg/L]</th><th>Range of linearity of calibration curve [mg/L]</th><th>Equivalent calibration range of linearity [mg/L water]</th></tr>
<tr><td>Chlorantraniliprole</td><td>0.02, 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, 5.0</td><td>0.02 – 5.0</td><td>0.02 – 5.0</td></tr>
</table>
The equation of the calibration line is presented as the linear equation; y = ax + b (a – slope, b – intercept). The linear coefficient r2 must be higher than 0.99. Range of the linearity was given in µg/mL (equal to mg/L).
<table>
<tr><th>Range of linearity of calibration curve [mg/L]</th><th>Analyte</th><th>Slope</th><th>Intercept</th><th>Coefficient r²</th></tr>
<tr><td>0.02 – 5.0</td><td>Chlorantraniliprole</td><td>1.16826e-005</td><td>-0.0192363</td><td>0.9994151</td></tr>
</table></td></tr>
<tr><td>Specificity/selectivity</td><td>The analytical method specificity was estimated on the basis of the analysis of the chromatograms obtained for the control, and fortified samples of matrix. Considering the results of the analysis, no signal of detected substance was overlapping with matrix signal of the control samples in the experiments conditions. Therefore, the specificity of the method was demonstrated. Furthermore, any interference are directly apparent and would</td></tr>
</table>

<table>
<tr><td></td><td>Chlorantraniliprole</td></tr>
<tr><td></td><td>be observed in the chromatograms of the matrix control sample.</td></tr>
<tr><td>Precision, Accuracy and Uncertainity</td><td>Precision of a particular methods is defined as its repeatability (RSD – relative standard deviation [%]). The repeatability for detected substance analyzed are presented in table below. The RSD is ≤ 20% per each level

The accuracy of the method is reported as mean recovery ± relative standard deviation. Recovery was reported as mean recovery ± relative standard deviation (RSD). Mean recoveries for each level is in the range 70-120%.
A summary of the recovery data of control and fortified samples are presented in the table below

<table>
<tr><th>Method</th><th>Analyte</th><th>Fortification Level [mg/L]</th><th>Number of Replicates</th><th>Mean Recovery [%]</th><th>RSD [%]</th></tr>
<tr><td rowspan="2">Direct injection method</td><td rowspan="2">Chlorantraniliprole</td><td>0.07</td><td>5</td><td>107</td><td>3.5</td></tr>
<tr><td>0.70</td><td>5</td><td>99.1</td><td>0.9</td></tr>
</table>
</td></tr>
<tr><td>Limit of quantification (LOQ)</td><td>Limit of Quantification was estimated as the lowest concentration of a detected substance at which an acceptable mean recovery and precision (normally 70 – 120% with a relative standard deviation of preferably ≤ 20%).
LOQ was successfully established at 0.07mg/kg for chlorantraniliprole</td></tr>
<tr><td>Limit of detection (LOD)</td><td>Limit of detection (LOD) was established at 0.02 mg/kg. The limit of detection (LOD) is defined as the lowest detectable concentration or amount of an analyte in a sample. LOD is expressed as the lowest calibration standard. LOD is less than or equal to 30% of LOQ.</td></tr>
<tr><td>Stability</td><td>Final extract stability was not determined. The final extracts were analysed within 24 h.

The stability of stock solution in methanol was tested at concentrations 1 mg/mL i.e. 1000 mg chlorantraniliprole /L. The results for stability were obtained after 0, 13, 33, 61, 90, 114, 141, 155, 178 and 186 days of storage at cool temperature i.e. from 2ºC to 8ºC. Compared to the mean recoveries measured at 0 day, significant decline was not observed after 186 days. The mean recoveries for stock solution did not differ by more than 10%.</td></tr>
</table>

The results of the procedural recovery samples showed that the recovery efficiency was within an acceptable range. The mean recovery for chlorantraniliprole ranged from 99.1% to 107% for all fortification levels. The overall RSDs were < 20%. Hence, a good performance of the analytical method is proven. Therefore, the requirements of the cited guideline were fulfilled. The regression residuals are presented in a residual plot in range equal to range of linearity of calibration curve. The analyzed untreated control samples showed no residues above the LOD of 0.02 mg/L. The specificity of the method was also demonstrated.

A 2.1.2.7.4 Analytical method 6

A 2.1.2.7.4.1 Method validation

Comments of zRMS:	The analytical method code: ETOX-2023-13 was fully validated in term of specificity, linearity, repeatability, accuracy according to SANTE/2020/12830, rev. 2 The results of analytical method validation confirm that this method is suitable for determination of chlorantraniliprole.

	The method is successfully validated and accepted.

Reference:	KCP 5.2/06
Report	Collembolan (Folsomia candida) Reproduction Test in soil, APPENDIX No. 5 – Copy of the results of the analytical measurements – Chlorantraniliprole, E.Kulec-Płoszczyca, 2024, Study code: ETOX-2023-13,
Guideline(s):	SANTE/2020/12830, Rev.2
Deviations:	NO
GLP:	YES
Acceptability:	YES

Materials and methods

The analytical method was validated according to SANTE/2020/12830, Rev.2. The concentration of Chlorantraniliprole in artificial soil was determined using a ultrahigh performance liquid chromatographic method with tandem mass spectrometry detection (UHPLC-MS/MS). The analytical method involves extracting the sample with water, acetonitrile and a mixture of salts and analysing using UHPLC-MS/MS. The analytical method was validated using standard solutions of Chlorantraniliprole and the Test Item CLOROL 200 SC. The calibration curve used for calculations should be prepared on the same day as the samples to be analysed. The range of the calibration curve should not exceed the range of linearity determined during validation

EQUIPMENT

Equipment	**Inventory number**
Laboratory glass	Test: G1, G16, G20, G21, G23, G24, G67, G68 Validation: G1, G23, G24, G33, G67, G68, G69, G70, G71
Automatic pipettes	EQ90, EQ96, EQ98
UHPLC	EQ13
Analytical balance	EQ5
MilliQ water treatment system	EQ10
Centrifuge	EQ40

Reagents and solvents:

Reagent	**Supplier**	**LOT**	**Purity**	**Expiry date**
Chlorantraniliprole (CoA - Annex 1)	LGC Standards	G1365311	98.21%	04.2027
CLOROL 200 SC (CoA - Annex 1)	PUH Chemirol Sp. Z o.o.	CHR/03/2023	199.35 g/L	-
Disodium hydrogen citrate sesquihydrate	Pol-Aura	500LNF	pure p.a.	09.2024

Magnesium sulphate anhydrous	Chempur	200131272	pure p.a.	01.2024
Sodium chloride	Chempur	200911019	pure p.a.	09.2024
Trisodium citrate di-hydrate	Chempur	200824193	pure p.a.	08.2024
Formic acid	Chempur	200205013	pure p.a.	01.2024
Acetonitrile	Chempur	230505304	LC-MS	05.2024
Ultrapure water	-	-	-	-

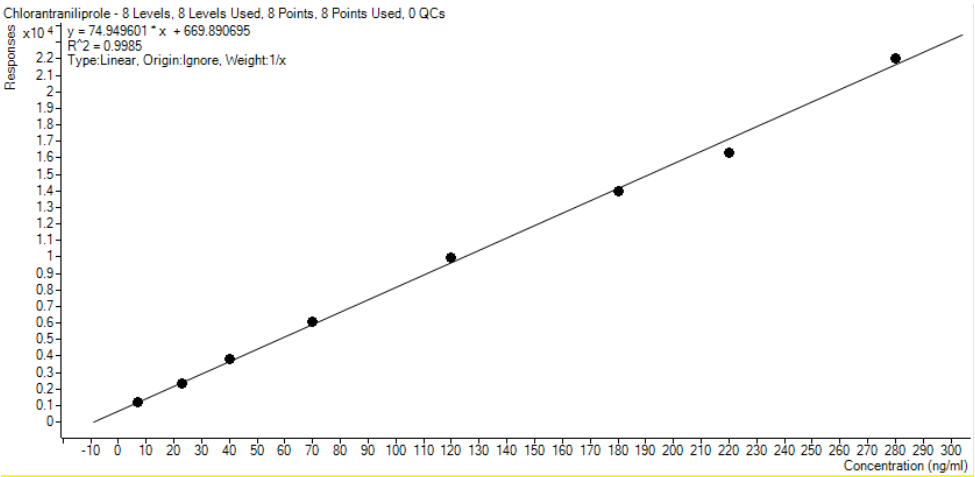
SAMPLE PREPARATION:

At least 2 g of sample should be taken for chemical analysis. 4 mL of water and 4 mL of acetonitrile were added to 1.3 g ± 0.3 g of sample and vigorously shaken by hand (approximately 1 min). Then 2.6 g ± 0.2 g of the salt mixture (8:2:2:1, w:w:w:w, magnesium sulfate anhydrous : sodium chloride : sodium citrate dihydrate : sodium hydrogen citrate sesquihydrate) was added, vigorously shaken by hand (approximately 1 min) and centrifuged (5 min, 5000 rcf). The upper phase was collected. If the concentration of Chlorantraniliprole in the upper phase is greater than the concentration of the highest standard solution used to prepare the calibration curve, this sample should be diluted with acetonitrile so that the estimated concentration is within the range of the calibration curve after this dilution. The collected samples are then analysed using UHPLC-MS/MS..

1) Instrumentation and conditions:

UHPLC-MS/MS	Agilent Infinity 1290: HiP Sampler G4226A, Binary Pump G4220A, Column Comp. G1316C, Guard Column Zorbax SB-C18 2.1×5 mm, 1.8 µm, Column Zorbax SB-C18 RRHT 2.1×50 mm, 1.8 µm, 600 bar, 6460 Triple Quad Mass Spectrometer (Ion Source AJS ESI)	
Injection	1 µL	
Elution	Isocratic	
Mobile phases	58%: Water + formic acid (0.05%), 42%: Acetonitrile	
Flow [mL/min]	0.5	
Stop time	2.5 min	
Column temperature	40 °C	
MS/MS	**Gas Temperature [°C]**	320
	Gas Flow [L/min]	10
	Nebulizer [psi]	40
	Sheath Gas Heater [°C]	320
	Sheath Gas Flow [L/min]	12

Capillary [V]					3000			
Nozzle Voltage [V]					0			
Transition	**Precursor m/z**		**Product m/z**	**Dwell**	**Frag [V]**	**CE [V]**	**Cell Acc [V]**	**Polarity**
Target	482.7	→	204.1	100	128	10	2	Negative
Qualifier	482.7	→	147.0	100	128	30	2	Negative
Ion ratio [%]	23.6% ± 30% (relative)							
Integrator	Agile 2 or manual integration if needed.							

	Chlorantraniliprole
Author(s), year	
Principle of method	UHPLC-MS/MS
Matrix Effects	Assessment of matrix effects were performed by comparing the analyte response of the 120 µg/L standard solution of Chlorantraniliprole to spiked matrix blank sample at the same concentration. The matrix effect did not exceed ±20%, so it is not considered significant.
Linearity (linear between mg/L / % range of the declared content) (correlation coefficient, expressed as r)	Linearity was determined by preparing a series of standard solutions of Chlorantraniliprole at the concentrations of 7, 23, 40, 70, 120, 180, 220 and 280 µg/L.  Calibration curve equation: A = 74.95 C + 670 Coefficient of determination R^2: 0.9985 Linearity range: 7 – 280 µg/L Range of analytical method: 91 µg/kg – 219 mg/kg
Specificity/selectivity	Representative chromatograms of standard at the lowest calibrated level (LOD), matrix blanks and samples fortified at the LOQ level are provided to prove selectivity of the method. Comparison of chromatograms of blank matrix samples, spiked matrix samples to LOD and LOQ level the Y-axis scale on all chromatograms was matched to the chromatogram of the matrix spiked sample at LOQ level. No signal of the detected substance was overlapping with the matrix signal of the control samples under the experimental conditions. The specificity of the method was confirmed.

Chlorantraniliprole

To further confirm the specificity of the analytical method, two ion transitions were recorded:

Target: 482.7 → 204.1
Qualifier: 482.7 → 147.0

Specificity was verified using the ion transition ratio of 23.6% ± 30% (relative). Specificity of the method was confirmed.

Precision, Accuracy and Uncertainty

Five LOQ samples and five MAX samples were preparedand analysed 3 times on the same day. MAX samples were diluted to 230 µg/L of Chlorantraniliprole.
The mean (recovery) and the RSD (repeatability) were calculated from the average recoveries for each concentration level. The outlier was checked using the test Q (α = 0.95). No outliers were found.
Determined precision 4.3% (n = 10) meets the acceptance criteria (≤ 20%).
Determined accuracy 102.2% (n = 10) meets the acceptance criteria (70-120%).

No	Nominal concentration [ ]	Area A	Sample volume [ml]	Volume after dilution [ml]	Additional dilution	Sample weight [g]	Dry weight DW	Measured concentration	Final concentration	Recovery [%]	Recovery [%]	SD	RSD [%]	Outlier	Recovery [%]	SD	RSD	n	Accuracy [%]	Precision [%]	n
1	0.091	2524	1.0	1.0	1	1.2923	81.4%	24.7	0.094	102.9	101.4	2.8	2.7	No	102.7	6.4	6.2	5	102.2	4.3	10
		2440						23.6	0.090	98.2											
		2529						24.8	0.094	103.2											
2		2973	1.0	1.0	1	1.4995	81.4%	30.7	0.101	110.2	110.7	0.6	0.5	No							
		2981						30.8	0.101	110.6											
		2997						31.1	0.102	111.3											
3		2043	1.0	1.0	1	1.074	81.4%	18.3	0.089	97.8	97.5	1.0	1.1	No							
		2023						18.1	0.088	96.4											
		2052						18.4	0.090	98.4											
4		2460	1.0	1.0	1	1.3209	81.4%	23.9	0.089	97.2	96.1	1.4	1.4	No							
		2412						23.2	0.086	94.6											
		2447						23.7	0.088	96.5											
5		2274	1.0	1.0	1	1.0129	81.4%	21.4	0.104	113.6	107.8	5.0	4.7	No							
		2150						19.8	0.096	104.8											
		2152						19.8	0.096	104.9											
6	219.3	18028	0.0412	10	243	1.2484	81.5%	232	221	100.8	99.5	2.9	2.9	No	101.7	1.3	1.3	5			
		18161						233	223	101.6											
		17228						221	211	96.2											
7		17789	0.0374	10	267	1.3775	81.5%	228	218	99.3	101.9	3.3	3.2	No							
		18062						232	221	100.9											

	Chlorantraniliprole																				
			18868						243	231	105.5										
8			18431	0.0328	10	305	1.5690	81.5%	237	226	103.1	102.8	1.4	1.4	No						
			18594						239	228	104.1										
			18114						233	222	101.3										
9			18037	0.0370	10	270	1.3905	81.5%	232	221	100.8	101.6	2.8	2.7	No						
			18701						241	230	104.7										
			17767						228	218	99.3										
10			18332	0.0440	10	227	1.1680	81.5%	236	225	102.7	102.8	1.1	1.0	No						
			18556						239	228	104.0										
			18196						234	223	101.9										
Limit of quantification (LOQ)	The Limit of Quantification (LOQ) was determined as the lowest concentration of a detected substance at which the acceptable mean recovery is obtained (70 – 120% with a relative standard deviation (RSD) ≤ 20%). LOQ: 91 µg/kg																				
Limit of detection (LOD)	The calculated Limit of Detection (LOD) was 30% of the LOQ. LOD: 27 µg/kg																				
Stability	Sample will be analysed within 24 h after collection so sample stability were not assessed in the process of the analytical method validation.																				

A 2.1.2.7.5 Analytical method 7

A 2.1.2.7.5.1 Method validation

Comments of zRMS:	The analytical method code: ETOX-2024-64 was fully validated in term of specificity, linearity, repeatability, accuracy according to SANTE/2020/12830, rev. 2 The results of analytical method validation confirm that this method is suitable for determination of chlorantraniliprole. The method is successfully validated and accepted.

Reference:	KCP 5.2/07
Report	Earthworm Reproduction Test (Eisenia andrei), APPENDIX No. 3 Chemical analysis, K. Wesołowska, 2025, Study code: ETOX-2024-64,
Guideline(s):	SANTE/2020/12830, Rev.2
Deviations:	NO
GLP:	YES
Acceptability:	YES

Materials and methods

The analytical method was validated according to SANTE/2020/12830, Rev.2. The concentration of Chlorantraniliprole in artificial soil was determined using a ultrahigh performance liquid chromatographic method with tandem mass spectrometry detection (UHPLC-MS/MS). The analytical method involves extracting the sample with water, acetonitrile and a mixture of salts and analysing using UHPLC-MS/MS. The analytical method was validated using standard solutions of Chlorantraniliprole and the Test Item CLOROL 200 SC. The calibration curve used for calculations should be prepared on the same day as the samples to be analysed. The range of the calibration curve should not exceed the range of linearity determined during validation

EQUIPMENT

Equipment	**Inventory number**
Laboratory glass	G16, G23, G24, G33, G67, G68, G69, G70, G71, G95
Automatic pipettes	EQ110, EQ111, EQ112, EQ113
UHPLC	EQ13
Analytical balance	EQ118
MilliQ water treatment system	EQ10
Centrifuge	EQ40

Reagents and solvents:

Reagent	**Supplier**	**LOT**	**Purity**	**Expiry date**
Chlorantraniliprole (CoA - Annex 1)	LGC Standards	G1365311	98.21%	04.2027
CLOROL 200 SC (CoA - Annex 1)	PUH Chemirol Sp. Z o.o.	CHR/03/2023	199.35 g/L	-
Disodium hydrogen citrate sesquihydrate	Pol-Aura	924FSV	pure p.a.	09.2029
Magnesium sulphate anhydrous	Chempur	240109133	pure p.a.	01.2028
Sodium chloride	Pol-Aura	APA409	pure p.a.	06.2026
Trisodium citrate dihydrate	Stanlab	19.05.2024	pure p.a.	05.2026

Formic acid	Chempur	240109178	pure p.a.	01.2028
Acetonitrile	Chem solve	P4B526064B	LC-MS	02.2026
Ultrapure water	-	-	-	-

SAMPLE PREPARATION:

At least 2 g of sample should be taken for chemical analysis. 4 mL of water and 4 mL of acetonitrile were added to 1.3 g ± 0.3 g of sample and vigorously shaken by hand (approximately 1 min). Then 2.6 g ± 0.2 g of the salt mixture (8:2:2:1, w:w:w:w, magnesium sulfate anhydrous : sodium chloride : sodium citrate dihydrate : sodium hydrogen citrate sesquihydrate) was added, vigorously shaken by hand (approximately 1 min) and centrifuged (5 min, 5000 rcf). The upper phase was collected. If the concentration of Chlorantraniliprole in the upper phase is greater than the concentration of the highest standard solution used to prepare the calibration curve, this sample should be diluted with acetonitrile so that the estimated concentration is within the range of the calibration curve after this dilution. The collected samples are then analysed using UHPLC-MS/MS.

1) Instrumentation and conditions:

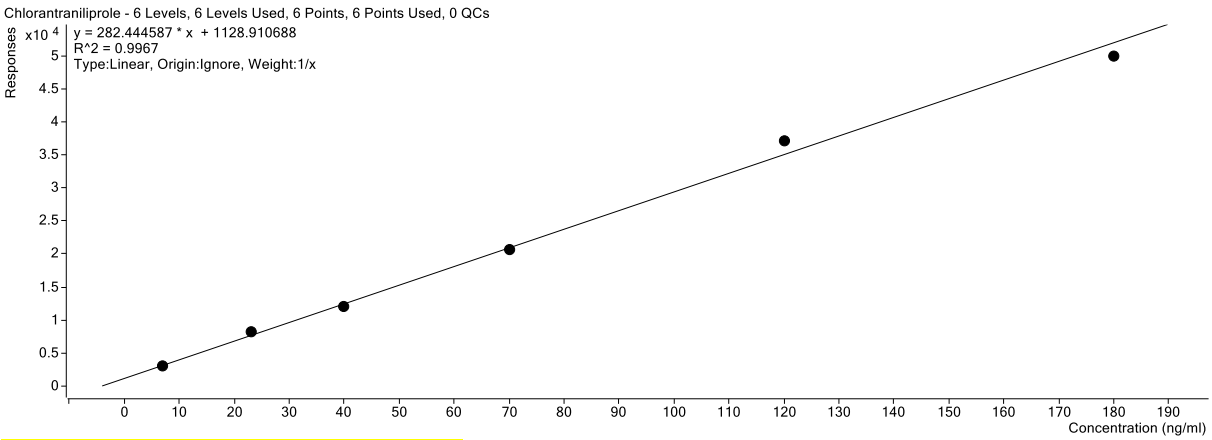
UHPLC-MS/MS	Agilent Infinity 1290: HiP Sampler G4226A, Binary Pump G4220A, Column Comp. G1316C, Guard Column Zorbax SB-C18 2.1×5 mm, 1.8 µm, Column Zorbax SB-C18 RRHT 2.1×50 mm, 1.8 µm, 600 bar, 6460 Triple Quad Mass Spectrometer (Ion Source AJS ESI)
Injection	2 µL
Elution	Isocratic
Mobile phases	58%: Water + formic acid (0.05%), 42%: Acetonitrile
Flow [mL/min]	0.5
Stop time	2.5 min
Column temperature	40 °C

MS/MS	**Gas Temperature [°C]**	320
	Gas Flow [L/min]	10
	Nebulizer [psi]	40
	Sheath Gas Heater [°C]	320
	Sheath Gas Flow [L/min]	12
	Capillary [V]	3000
	Nozzle Voltage [V]	0

Transition	**Precursor m/z**	**Product m/z**	**Dwell**	**Frag [V]**	**CE [V]**	**Cell Acc [V]**	**Polarity**

Target	482.7	→	204.1	200	128	10	2	Negative
Qualifier	482.7	→	147.0	200	128	30	2	Negative

Ion ratio [%] 20.5% ± 30% (relative)
Integrator Agile 2 or manual integration if needed.

	Chlorantraniliprole
Author(s), year	
Principle of method	UHPLC-MS/MS
Matrix Effects	Assessment of matrix effects were performed by comparing the analyte response of the 120 µg/L standard solution of Chlorantraniliprole to spiked matrix blank sample at the same concentration. The matrix effect did not exceed ±20%, so it is not considered significant.
Linearity (linear between mg/L / % range of the declared content) (correlation coefficient, expressed as r)	Linearity was determined by preparing a series of standard solutions of Chlorantraniliprole at the concentrations of 7, 23, 40, 70, 120 and 180 µg/L.  Calibration curve equation: A = 282.4 C + 1129 Coefficient of determination R2: 0.9967 Linearity range: 7 – 180 µg/L Range of analytical method: 91 µg/kg – 219 mg/kg
Specificity/selectivity	Representative chromatograms of standard at the lowest calibrated level (LOD), matrix blanks and samples fortified at the LOQ level are provided to prove selectivity of the method. Comparison of chromatograms of blank matrix samples, spiked matrix samples to LOD and LOQ level the Y-axis scale on all chromatograms was matched to the chromatogram of the matrix spiked sample at LOQ level. No signal of the detected substance was overlapping with the matrix signal of the control samples under the experimental conditions. The specificity of the method was confirmed. To further confirm the specificity of the analytical method, two ion transitions were recorded: Target: 482.7 → 204.1 Qualifier: 482.7 → 147.0 Specificity was verified using the ion transition ratio of 20.5% ± 30% (relative). Specificity of the method was confirmed.

Chlorantraniliprole

Precision, Accuracy and Uncertainty

Precision and accuracy were determined at 2 concentration levels of the Test Item CLOROL 200 SC in artificial soil: 0.5 mg/kg (LOQ) and 1200 mg/kg of (MAX). Five LOQ samples and five MAX samples were prepared and analysed 3 times on the same day. MAX samples were diluted to 230 µg/L of Chlorantraniliprole.
The mean (recovery) and the RSD (repeatability) were calculated from the average recoveries for each concentration level. The outlier was checked using the test Q (α = 0.95). No outliers were found.
Determined precision 7.0% (n = 9) meets the acceptance criteria (≤ 20%).
Determined accuracy 87.6% (n = 9) meets the acceptance criteria (70-120%).

No	Nominal concentration [ ]	Area A	Sample volume [ml]	Volume after dilution [ml]	Additional dilution	Sample weight [g]	Dry weight DW	Measured concentration	Final concentration	Recovery [%]	Recovery [%]	SD	RSD [%]	Outlier	Recovery [%]	SD	RSD [ ]	n	Accuracy [%]	Precision [%]	n
1	0.091	5769	1	1	1	1.1903	73.8%	16.4	0.075	82.2	84.5	2.0	2.3	No	83.5	5.0	6.0	5	87.6	7.0	9
		5948						17.1	0.078	85.4											
		5972						17.1	0.078	85.8											
2		5723						16.3	0.082	89.9	89.8	3.6	4.0	No							
		5524	1	1	1	1.0772	73.8%	15.6	0.078	86.0											
		5894						16.9	0.085	93.3											
3		5607						15.9	0.083	90.7	86.0	6.0	7.0	No							
		5476	1	1	1	1.0412	73.8%	15.4	0.080	88.0											
		5043						13.9	0.072	79.3											
4		4730						12.7	0.069	75.7	76.9	3.4	4.5	No							
		4975	1	1	1	1.0033	73.8%	13.6	0.074	80.8											
		4664						12.5	0.068	74.3											
5		5534						15.6	0.079	87.0	80.1	10.7	13.3	No							
		5458	1	1	1	1.0674	73.8%	15.3	0.078	85.5											
		4563						12.2	0.062	67.8											
6	219.3	325333	0.02	10	500	1.2535	84.4%	111	210	95.9	93.8	7.7	8.3	No	92.8	1.4	1.5	4			
		339224						116	219	100.2											
		290003						99	186	85.2											
7		223337	0.02	10	500	1.2351	84.4%	75	144	65.8	67.6	2.2	3.3	Yes							
		237239						80	154	70.1											
		227337						77	147	67.0											
8		247076	0.02	10	500	1.1040	84.4%	83	179	81.8	93.4	10.2	10.9	No							

	Chlorantraniliprole																					
			30159						103	221	100.7											
			29303						100	214	97.7											
9			24643	0.02	10	500	1.1222	84.4%	83	176	80.2	90.8	9.2	10.1	No							
			29351						100	211	96.3											
			29245						100	210	95.9											
10			36767	0.02	10	500	1.3254	84.4%	126	225	103.0	93.4	8.3	8.9	No							
			31858						109	194	88.8											
			31733						108	194	88.4											

Limit of quantification (LOQ)	The Limit of Quantification (LOQ) was determined as the lowest concentration of a detected substance at which the acceptable mean recovery is obtained (70 – 120% with a relative standard deviation (RSD) ≤ 20%). LOQ: 91 µg/kg
Limit of detection (LOD)	The calculated Limit of Detection (LOD) was 30% of the LOQ. LOD: 27 µg/kg
Stability	Sample will be analysed within 24 h after collection so sample stability were not assessed in the process of the analytical method validation.

A 2.1.2.7.6 Analytical method 8

A 2.1.2.7.6.1 Method validation

Comments of zRMS:	The analytical method code: ETOX-2024-65 was fully validated in term of specificity, linearity, repeatability, accuracy according to SANTE/2020/12830, rev. 2 The results of analytical method validation confirm that this method is suitable for determination of chlorantraniliprole. The method is successfully validated and accepted.

Reference: KCP 5.2/08

Report	Predatory mite (Hypoaspis (Geolaelaps) aculieifer) reproduction test in soil, APPENDIX No. 3 Chemical analysis, S. Groch, 2025, Study code: ETOX-2024-65,
Guideline(s):	SANTE/2020/12830, Rev.2
Deviations:	NO
GLP:	YES
Acceptability:	YES

Materials and methods

The analytical method was validated according to SANTE/2020/12830, Rev.2. The concentration of Chlorantraniliprole in artificial soil was determined using a ultrahigh performance liquid chromatographic method with tandem mass spectrometry detection (UHPLC-MS/MS). The analytical method involves extracting the sample with water, acetonitrile and a mixture of salts and analysing using UHPLC-MS/MS. The analytical method was validated using standard solutions of Chlorantraniliprole and the Test Item CLOROL 200 SC. The calibration curve used for calculations should be prepared on the same day as the samples to be analysed. The range of the calibration curve should not exceed the range of linearity determined during validation

EQUIPMENT

Equipment	**Inventory number**
Laboratory glass	G16, G23, G24, G33, G67, G68, G69, G70, G71
Automatic pipettes	EQ4, EQ110, EQ111, EQ112, EQ113
UHPLC	EQ13
Analytical balance	EQ118
MilliQ water treatment system	EQ10
Centrifuge	EQ40

Reagents and solvents:

Reagent	**Supplier**	**LOT**	**Purity**	**Expiry date**
Chlorantraniliprole (CoA - Annex 1)	LGC Standards	G1365311	98.21%	04.2027
CLOROL 200 SC (CoA - Annex 1)	PUH Chemirol Sp. Z o.o.	CHR/03/2023	199.35 g/L	-
Disodium hydrogen citrate sesquihydrate	Pol-Aura	924FSV	pure p.a.	09.2029
Magnesium sulphate anhydrous	Chempur	240109133	pure p.a.	01.2028
Sodium chloride	Pol-Aura	APA409	pure p.a.	06.2026
Trisodium citrate dihydrate	Stanlab	19.05.2024	pure p.a.	05.2026
Formic acid	Chempur	240109178	pure p.a.	01.2028

Acetonitrile	Chem solve	P4B526064B	LC-MS	02.2026
Ultrapure water	-	-	-	-

SAMPLE PREPARATION:

At least 2 g of sample should be taken for chemical analysis. 4 mL of water and 4 mL of acetonitrile were added to 1.3 g ± 0.3 g of sample and vigorously shaken by hand (approximately 1 min). Then 2.6 g ± 0.2 g of the salt mixture (8:2:2:1, w:w:w:w, magnesium sulfate anhydrous : sodium chloride : sodium citrate dihydrate : sodium hydrogen citrate sesquihydrate) was added, vigorously shaken by hand (approximately 1 min) and centrifuged (5 min, 5000 rcf). The upper phase was collected. If the concentration of Chlorantraniliprole in the upper phase is greater than the concentration of the highest standard solution used to prepare the calibration curve, this sample should be diluted with acetonitrile so that the estimated concentration is within the range of the calibration curve after this dilution. The collected samples are then analysed using UHPLC-MS/MS.

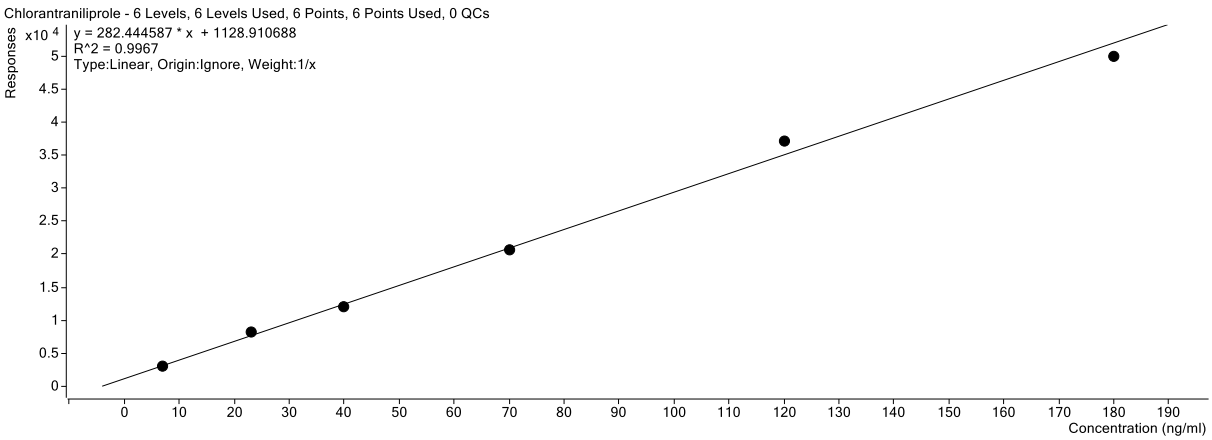
1) Instrumentation and conditions:

UHPLC-MS/MS	Agilent Infinity 1290: HiP Sampler G4226A, Binary Pump G4220A, Column Comp. G1316C, Guard Column Zorbax SB-C18 2.1×5 mm, 1.8 µm, Column Zorbax SB-C18 RRHT 2.1×50 mm, 1.8 µm, 600 bar, 6460 Triple Quad Mass Spectrometer (Ion Source AJS ESI)
Injection	2 µL
Elution	Isocratic
Mobile phases	58%: Water + formic acid (0.05%), 42%: Acetonitrile
Flow [mL/min]	0.5
Stop time	2.5 min
Column temperature	40 °C

MS/MS	**Gas Temperature [°C]**	320
	Gas Flow [L/min]	10
	Nebulizer [psi]	40
	Sheath Gas Heater [°C]	320
	Sheath Gas Flow [L/min]	12
	Capillary [V]	3000
	Nozzle Voltage [V]	0

Transition	**Precursor m/z**		**Product m/z**	**Dwell**	**Frag [V]**	**CE [V]**	**Cell Acc [V]**	**Polarity**
Target	482.7	→	204.1	200	128	10	2	Negative
Qualifier	482.7	→	147.0	200	128	30	2	Negative

Ion ratio [%]	20.5% ± 30% (relative)
Integrator	Agile 2 or manual integration if needed.

	Chlorantraniliprole
Author(s), year	
Principle of method	UHPLC-MS/MS
Matrix Effects	Assessment of matrix effects were performed by comparing the analyte response of the 120 µg/L standard solution of Chlorantraniliprole to spiked matrix blank sample at the same concentration. The matrix effect did not exceed ±20%, so it is not considered significant.
Linearity (linear between mg/L / % range of the declared content) (correlation coefficient, expressed as r)	Linearity was determined by preparing a series of standard solutions of Chlorantraniliprole at the concentrations of 7, 23, 40, 70, 120, 180~~, 220 and 280~~ µg/L.  Calibration curve equation: A = 282.4 C + 1129 Coefficient of determination R2: 0.9967 Linearity range: 7 – 180 µg/L Range of analytical method: 91 µg/kg – 219 mg/kg
Specificity/selectivity	Representative chromatograms of standard at the lowest calibrated level (LOD), matrix blanks and samples fortified at the LOQ level are provided to prove selectivity of the method. Comparison of chromatograms of blank matrix samples, spiked matrix samples to LOD and LOQ level the Y-axis scale on all chromatograms was matched to the chromatogram of the matrix spiked sample at LOQ level. No signal of the detected substance was overlapping with the matrix signal of the control samples under the experimental conditions. The specificity of the method was confirmed. To further confirm the specificity of the analytical method, two ion transitions were recorded: Target: 482.7 → 204.1 Qualifier: 482.7 → 147.0 Specificity was verified using the ion transition ratio of 23.6% ± 30% (relative). Specificity of the method was confirmed.

Chlorantraniliprole

Precision, Accuracy and Uncertainty

Precision and accuracy were determined at 2 concentration levels of the Test Item CLOROL 200 SC in artificial soil: 0.5 mg/kg (LOQ) and 1200 mg/kg of (MAX). Five LOQ samples and five MAX samples were preparedand analysed 3 times on the same day. MAX samples were diluted to 230 µg/L of Chlorantraniliprole.
The mean (recovery) and the RSD (repeatability) were calculated from the average recoveries for each concentration level. The outlier was checked using the test Q (α = 0.95). No outliers were found.
Determined precision 4.3% (n = 10) meets the acceptance criteria (≤ 20%).
Determined accuracy 102.2% (n = 10) meets the acceptance criteria (70-120%).

No	Nominal concentration [ ]	Area A	Sample volume [ml]	Volume after dilution [ml]	Additional dilution	Sample weight [g]	Dry weight DW	Measured concentration	Final concentration	Recovery [%]	Recovery [%]	SD	RSD [%]	Outlier	Recovery [%]	SD	RSD [%]	n	Accuracy [%]	Precision [%]	n
1	0.091	4561	1	1	1	1.0246	79.7%	12.2	0.060	65.4	80.4	13.1	16.3	No	83.3	2.1	2.5	5	83.3	2.8	10
		5654						16.0	0.078	86.3											
		5831						16.6	0.082	89.6											
2		6820	1	1	1	1.2546	79.7%	20.1	0.081	88.6	82.7	8.4	10.1	No							
		6681						19.7	0.079	86.4											
		5827						16.6	0.067	73.1											
3		5813	1	1	1	1.1042	79.7%	16.6	0.075	82.9	83.1	12.3	14.8	No							
		5137						14.2	0.065	70.9											
		6526						19.1	0.087	95.5											
4		5408	1	1	1	1.0056	79.7%	15.1	0.076	83.1	84.4	11.1	13.2	No							
		4938						13.5	0.067	74.0											
		6080						17.5	0.088	96.2											
5		6862	1	1	1	1.2223	79.7%	20.3	0.083	91.6	86.1	7.0	8.2	No							
		6021						17.3	0.071	78.2											
		6666						19.6	0.081	88.5											
6	219.3	31986	0.025	10	400	1.0823	90.5%	109	178	81.5	81.0	1.9	2.4	No	83.2	2.9	3.4	5			
		32410						111	181	82.6											
		30993						106	173	78.9											
7		34931	0.025	10	400	1.1771	90.5%	120	180	82.1	86.5	3.8	4.4	No							
		37510						129	193	88.4											
		37774						130	195	89.0											
8		31466	0.025	10	400	1.0561	90.5%	107	180	82.1	81.3	0.8	1.0	No							

	Chlorantraniliprole

		31164						106	178	81.3											
		30860						105	176	80.5											
9		33607	0.025	10	400	1.0792	90.5%	115	188	86.0	81.0	4.5	5.6	No							
		30309						103	169	77.3											
		31242						107	175	79.8											
10		33054	0.025	10	400	1.1066	90.5%	113	181	82.5	86.2	3.3	3.8	No							
		35511						122	195	88.8											
		34911						120	191	87.3											

Limit of quantification (LOQ)	The Limit of Quantification (LOQ) was determined as the lowest concentration of a detected substance at which the acceptable mean recovery is obtained (70 – 120% with a relative standard deviation (RSD) ≤ 20%). LOQ: 91 µg/kg
Limit of detection (LOD)	The calculated Limit of Detection (LOD) was 30% of the LOQ. LOD: 27 µg/kg
Stability	Sample will be analysed within 24 h after collection so sample stability were not assessed in the process of the analytical method validation.