FINAL REGISTRATION REPORT

Part B

Section 5

Analytical Methods

Detailed summary of the risk assessment

Product code: CHR/I/ADEL 280 SC

Product name(s): ADEL 280 SC/ PYRIFOS ADE 280 SC

Chemical active substances:

Deltamethrin, 30 g/L
Acetamiprid, 250 g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(authorization)

Applicant: Innvigo Sp. z o.o.

Submission date: July 2021

Update: October 2024

MS Finalisation date: 24/10/2024, evaluation of additional data 02/06/2025

Version history

When	What
September 2021	Dossier sent for evaluation
December 2021	Updated by Applicant
February 2024	Updated by Applicant
April 2024	Updated by Applicant
June 2024	zRMS finalised evaluation
October 2024	Applicant provided corrections during commenting period.
October 2024	zRMS finalised evaluation after commenting period
March 2025	New and additional data added by the Applicant
July 2025	Evaluation of additional data by zRMS

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New and additional data added in February 2024 was highlighted in green.
New and additional data added in October 2024 was highlighted in yellow.
New and additional data added in March 2025 was highlighted in aquamarine.

zRMS comments:
The text highlighted in grey was provided by the evaluator. Changes made by zRMS after the comment period are marked in pink. The evaluation of new data provided by the applicant in March 2025 is marked in blue.

5 Analytical methods

Data matching studies for acetamiprid have been evaluated by RMS – Netherland and later by Poland. ~~As a result of the assessment all reports were accepted and considered as equivalent to pro-tected studies. Therefore, to support the authorization of CHR/I/ADEL 280 SC (ADEL 280 SC/ PYRIFOS ADE 280 SC) INNVIGO is allowed to refer to EU approved reports~~

In the following document, data for active substance deltamethrin was described during its inclusion on Annex 1 process in 2009. Were reference to active substance data in the current risk assessment has been made, it was based on the data presented by Bayer (AgroEvo).
In November 30th, 2009r Decis 2.5 EC product has been authorized in Poland thus according to the art. 59 reg. 1107/2009, data protection for mentioned data expired 10 years from date of first authorization of product containing that active substance (in this case December, 1st 2019).

5.1 Conclusion and summary of assessment

Sufficiently sensitive and selective analytical methods are available for the active substance(s) and relevant impurities in the plant protection product.
Noticed data gaps are: none

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

zRMS comments:
Deltamethrin
Plant residue definition for monitoring: deltamethrin (cis-deltamethrin), Reg. (EU) 2018/832 and Reg. (EU) 2024/1342 (not yet applicable)
Plant residue definition for risk assessment: sum of deltamethrin and its alpha-R isomer and trans-isomer, Journal 2015;13(11):4309
Animal residue definition for monitoring: deltamethrin (cis-deltamethrin), Reg. (EU) 2018/832 and Reg. (EU) 2024/1342 (not yet applicable)
Animal residue definition for risk assessment: sum of deltamethrin and its alpha-R isomer and trans-isomer, Journal 2015;13(11):4309

Acetamiprid
Nisso Chemical Europe GmbH is the notifier for the Annex I inclusion of the acetamiprid and its renewal. INNVIGO Sp. z o.o. has access to the Annex II data of. acetamiprid (renewal data), through a letter of access by members of Acetamiprid Task Force comprised by Proplan Plant Protection Company, S.L, PUH Chemirol and Exclusivas Sarabia S.A companies, which are owners of an Annex II data matching Table, available on CIRCABC, prepared by RMS NL (2022-08-29).
Plan residue definition for monitoring: acetamiprid, Reg. (EU) 2019/88
Plant residue definition for risk assessment: acetamiprid, EFSA Journal 2016;14(11):4610
Please note that in May 2024, EFSA published Statement on the toxicological properties and maximum residue levels of acetamiprid and its metabolites. EFSA Journal, 22(5), e875. It is recommended to

modify the residue definition for risk assessment in leafy and fruit crops as follows: 'sum of acetamiprid and N- desmethyl-acetamiprid (IM-2-1), expressed as acetamiprid.
It is not proposed to modify the residue definition for risk assessment in pulses/oilseeds, root crops and cereals, which therefore remains acetamiprid.
Regarding the residue definition for enforcement, the available data do not indicate a need to modify the existing definition because acetamiprid is still a sufficient marker of the residues in all crop groups.
Animal residue definition for monitoring (except honey): sum of acetamiprid and N-desmethyl-acetamiprid (IM-2-1), expressed as acetamiprid, Reg. (EU) 2019/88
Honey: acetamiprid
Animal residue definition for risk assessment: sum of acetamiprid and metabolite IM-2-1 (N-desmethyl-acetamiprid), expressed as acetamiprid, EFSA Journal 2016;14(11):4610
Residue definitions for monitoring (EFSA Journal 2016;14(11):4610):
Soil: acetamiprid
Surface water: acetamiprid
Drinking water: acetamiprid, IM-1-5
Body fluids and tissues: No residue definition provided, IM-2-1 and 6-chloronicotinic acid (IC-0) were the main residues identified in rat urine.

Noticed data gaps are:

- confirmatory methods for deltamethrin in soil, body fluids and tissues (data gap for active substance, have to be fulfilled after renewal of active substance);
- primary analytical method for deltamethrin in body tissues (data gap for active substance, have to be fulfilled after renewal of active substance);
- a fully validated method for acetamiprid in body fluids with LOQ 0.01 mg/L is required (data gap for active substance, have to be fulfilled after renewal of active substance);
- extraction efficiency for deltamethrin in plant and animal matrices (data gap for active substance, have to be fulfilled after renewal of active substance)

~~– the Applicant should provide primary and ILV methods for monitoring studies suitable for the all matrices (with LOQ 0.01 mg/kg) or indicate access to existing and already assessed studies in this area. **According to zRMS, the study may be provided post registration but no later than 2 years after receiving authorization.** –~~ additional information provided by the Applicant

- ~~the Applicant should provide study D. Longhi, 2019, GLP-STUDY-18-000079 (Equivalent to Senciuc, M., (2014c) Acetamiprid RAR, CA 4.2/12) or indicate access to existing and already assessed studies for body fluids and tissues. The study was not provided by the Applicant and not evaluated in this dRR. **According to zRMS, the study may be provided post registration but no later than 2 years after receiving authorization.**~~ – study provided by the Applicant, see Appendix 2

In the context of the authorisation no data gaps were found.

Commodity/crop	Supported/ Not supported
Winter oil seed rape	Supported
Winter cereals	Supported
Sugar beets	Supported

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

5.2.1 Analysis of the plant protection product (KCP 5.1.1)

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

Comments of zRMS:	The method is accepted and may be used for analysing active substances in the PPP

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

Reference:	KCP 5.1.1
Report	CHR/I/ADEL 280 SC Part I: Determination of physicochemical properties of the initial preparation, after accelerated storage and after low temperature. E. Arévalo, 2021, BF- 55/20, Authority registration No: 4/2020/DPL
Guideline(s):	SANCO/3030/99 rev. 5
Deviations:	NO
GLP:	YES
Acceptability:	YES

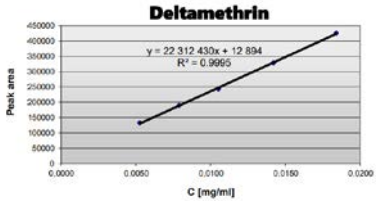
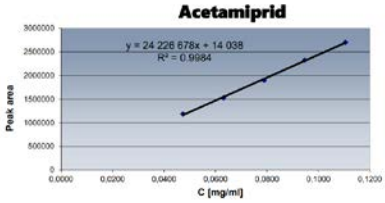
Materials and methods

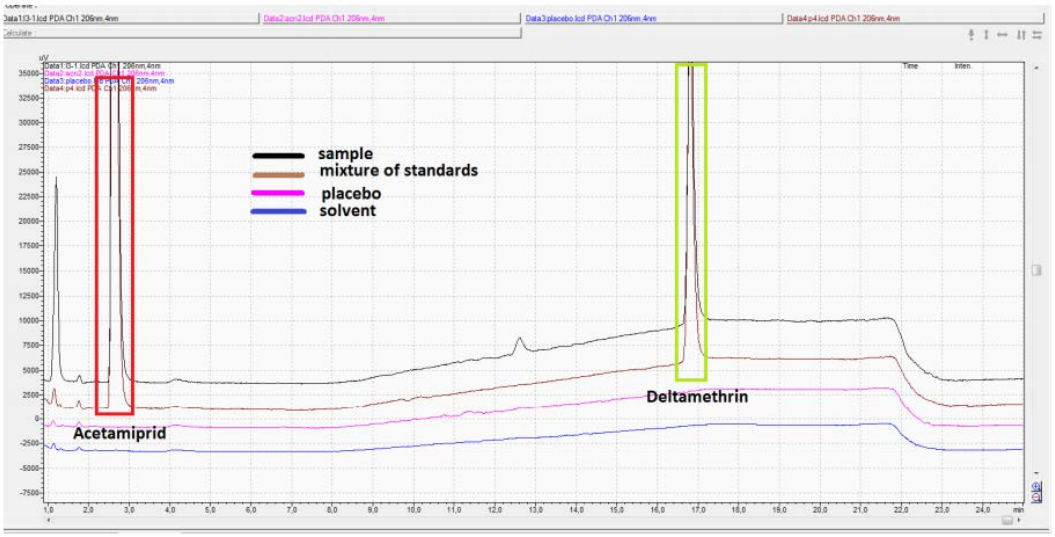
The content of active substances was determined by high performance liquid chromatography (HPLC) using reversed phase column with DAD detector. The method is specific, there are no interferences between the analytes and other components of the specimen, the method has good precision, accuracy, recovery and linearity and fulfils requirements of SANCO/3030/99 rev.5
The content of active substances in CHR/I/ADEL 280 SC determined by developed and validated method is respectively:
Deltamethrin: 3.04% (32.94 g/l)
Acetamiprid: 24.08% (261.29 g/l)

Validation - Results and discussions

Table 5.2-1: Methods suitable for the determination of active substances deltamethrin and acetamiprid in plant protection product CHR/I/ADEL 280 SC

	deltamethrin	**acetamiprid**
Author(s), year	E. Arévalo, 2021	E. Arévalo, 2021
Principle of method	HPLC/DAD	HPLC/DAD
Linearity (linear between mg/L / % range of the	The linearity of the detector response was assessed using five standards solutions at the concentration range of Deltamethrin 0.0053 mg/ml (5.26 mg/l) to 0.0184 mg/ml (18.41 mg/l), which corresponds to the concentration	The linearity of the detector response was assessed using five standards solutions at the concentration range of Acetamiprid 0.0474 mg/ml (47.35 mg/l) to 0.1105 mg/ml (110.49 mg/l), which corresponds to the concentration

	deltamethrin	acetamiprid
declared content) (correlation coefficient, expressed as r)	range of 48% to 169% of Deltamethrin content in the preparation. 	range of 57% to 132% of Acetamiprid content in the prepartion.  Correlation coefficient should be R2 ≥ 0.99. The obtained result is acceptable.
Precision – Repeatability Mean n = 6 (%RSD)	The method repeatability was assessed on the basis of six independent determinations of active substances content in CHR/I/ADEL 280 SC preparation. Chromatogram name / Specimen weight [mg] / Concentration C [mg/ml] / Peak area / Result [%] p1 / 43.55 / 0.348 / 251117 / 3.04 p2 / 43.39 / 0.347 / 253293 / 3.08 p3 / 50.13 / 0.401 / 296230 / 3.11 p4 / 42.60 / 0.341 / 246001 / 3.04 p5 / 48.13 / 0.385 / 275887 / 3.02 p6 / 45.16 / 0.361 / 261247 / 3.05 Average / 3.056 SD / 0.033 RSD [%] / 1.09 Data confidence interval x = 0.035% RSD for substance at the concentration of ~ 3.1 % should be less than or equal to 2.26 %. Horrat value is 0.48 and fulfils acceptance criterion Hr ≤ 1	The method repeatability was assessed on the basis of six independent determinations of active substances content in CHR/I/ADEL 280 SC preparation. Chromatogram name / Specimen weight [mg] / Concentration C [mg/ml] / Peak area / Result [%] p1 / 43.55 / 0.348 / 2088208 / 24.52 p2 / 43.39 / 0.347 / 2084835 / 24.57 p3 / 50.13 / 0.401 / 2458556 / 25.08 p4 / 42.60 / 0.341 / 2043494 / 24.53 p5 / 48.13 / 0.385 / 2307998 / 24.52 p6 / 45.16 / 0.361 / 2172847 / 24.61 Average / 24.64 SD / 0.2189 RSD [%] / 0.89 Data confidence interval x = 0.23% RSD for substance at the concentration of ~ 24.6 % should be less than or equal to 1.65 % Horrat value is 0.54 and fulfils acceptance criterion Hr ≤ 1
Accuracy n = 6 (% Recovery)	Recovery of active substances determination in CHR/I/ADEL 280 SC was assessed by total recovery value at two levels of concentration. For this purpose twelve 5 ml volumetric flasks were prepared and 0.75 ml of placebo solution at concentration 1.8045 mg/ml were added to each flask. 0.40 ml of the Acetamiprid standard solution at concentration of 0.7892 mg/ml and 0.05 ml of Deltamethrin standard solution at concentration of 0.5259 mg/ml were added to the each of the first six 5 ml flasks and acetonitrile were added up to the volume. To remaining six flasks 0.50 ml of Acetamiprid standard solution at concentration of 0.8014 mg/ml and 0.08 ml of Deltamethrin standard solution at concentration of 0.6763 mg/ml were added and acetonitrile was added up to the volume. The flasks were put into the ultrasonic bath for 5 min. Obtained final concentrations were examined and the nominal and calculated contents were compared. For the main ingredient at concentration of 1 - 10 % the average recovery value should be 100 ± 10%. The obtained result of 101.89% is acceptable.	Recovery of active substances determination in CHR/I/ADEL 280 SC was assessed by total recovery value at two levels of concentration. For this purpose twelve 5 ml volumetric flasks were prepared and 0.75 ml of placebo solution at concentration 1.8045 mg/ml were added to each flask. 0.40 ml of the Acetamiprid standard solution at concentration of 0.7892 mg/ml and 0.05 ml of Deltamethrin standard solution at concentration of 0.5259 mg/ml were added to the each of the first six 5 ml flasks and acetonitrile were added up to the volume. To remaining six flasks 0.50 ml of Acetamiprid standard solution at concentration of 0.8014 mg/ml and 0.08 ml of Deltamethrin standard solution at concentration of 0.6763 mg/ml were added and acetonitrile was added up to the volume. The flasks were put into the ultrasonic bath for 5 min. Obtained final concentrations were examined and the nominal and calculated contents were compared. For the main ingredient at concentration of >10 % the average recovery value should be 100 ± 3%. The obtained result of 100.02% is acceptable.
Interference/ Specificity	The chromatograms of solvent, placebo solution, mixture of standards solutions and the examined specimen solution were performed and superimposed. There are no interferences between the analytes and other components of the specimen.	

	deltamethrin	acetamiprid
		
Comment		

Conclusion

The method for determination of active substances in CHR/I/ADEL 280 SC preparation is specific. The validation parameters for linearity, instrument precision, repeatability and accuracy are within the acceptance range. The determined average content of active substance in CHR/I/ADEL 280 SC is respectively:
Deltamethrin: 3.04% (32.94 g/l)
Acetamiprid: 24.08% (261.29 g/l)

Comments of zRMS:	The method is accepted and may be used for analysing active substances in aqueous solutions and suspensions used for analyzing effectiveness of procedures for cleaning spray equipment in the physicochemical section

Reference:	KCP 5.1.1/2
Report	CHR/I/ADEL 280 SC Method validation for determination of the acetamiprid and deltamethrin residues in aqueous solutions and suspensions
Guideline(s):	SANTE/2020/12830 rev. 1
Deviations:	NO
GLP:	YES
Acceptability:	YES

The method for determination acetamiprid and deltamethrin residues were developed and validated in Analytical Department of the Lukasiewicz Research Network - Institute of Industrial Organic Chemistry (L-IPO) in Warsaw according to EU requirements described in SANTE/2020/12830 rev. 1 guideline relating to the assessing effectiveness of procedures for cleaning spray equipment (for full details please refer to the point KCP 2.11 from Part B section 1,2 and 4 of the registration report).
Method validation for determination of the acetamiprid and deltamethrin content was performed using high performance liquid chromatography (HPLC) with DAD detector and external standard method.

The following validation parameters were determined: specificity, linearity, limit of quantification and detection, precision (repeatability) and recovery ***Table 5.2-1a***.

Table 5.2-1a. Summary of validation parameters for active substances

Parameter	Acetamiprid		Deltamethrin	
	Acceptance criteria	Obtained result	Acceptance criteria	Obtained result
Specificity	Fulfilled		Fulfilled	
Range	0.00012 mg/ml - 0.0141 mg/ml (120.48 pg/l - 14055.83 pg/l)		0.00006 mg/ml - 0.0090 mg/ml (60.24 pg/l - 9035.81 pg/l)	
Linearity N=5	$R^2 > 0.99$	R^2=0.9999	$R^2 > 0.99$	R^2=0.9997
	Regression residual	Fulfilled	Regression residual	Fulfilled
Limit of quantification	0.0002 mg/ml (200.80 pg/l)		0.0001 mg/ml (100.40 pg/l)	
Precision (repeatability) [%] n=5	RSDr < 20	RSD=2.80	RSDr < 20	RSD=3.70
Recovery [%] N=5	70-120	96.97	70-120	98.78

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

No relevant impurities (SANCO Deltamethrin 6504/VI/99-final 17 October 2002)

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

Confidential data, please refer to Part C.

5.2.1.4 Applicability of existing CIPAC methods (KCP 5.1.1)

Analytical methods for determination of deltamethrin and acetamiprid impurities and relevance of CIPAC methods in CHR/I/ADEL 280 SC were not evaluated as part of the EU review of any of two active substances. Therefore, all relevant data are provided and are considered adequate.

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 deltamethrin for the generation of pre-authorization data is given in the following table. For the detailed evaluation of additional studies it is referred to KCP 5.2

Table 5.2-2a: Validated methods for the generation of pre-authorization data

Component of residue definition: Deltamethrin				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
High water	Primary	0.02 mg/kg	GC-ECD	Martens, 1998a, b and c, B.4.2.1,

Component of residue definition: Deltamethrin				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
content: (Potato, Peach, Onion, tomato, cucmber, melon, cauliflower, leek) High acid content (orange) High oil content(rape seed, cotton seed, corn oil) High starch content (Corn flour, wheat grain, sorghum starch, rice)		0.01 mg/kg		DAR, Deltamethrin, Addendum to the Monograph Annex B4: Methods of analysis Czarnecki, J.J., McKinney, F.R., Clayton, F.B. 1990 B.4.2.1, DAR, Deltamethrin – Volume 3, Annex B4: Methods of analysis Baldi, B.G., McKinney, F.R. 1994 B.4.2.1, DAR, Deltamethrin – Volume 3, Annex B4: Methods of analysis Addendum to the Monograph Annex B, 2002 Weber, H., 2009 / Report No: S09-00553 EU agreed
	Confirmatory	~~Not require~~ Required, SANTE/2020/12830, Rev.2 EFSA Journal 2022;20(7):7446: multi residue method DFG S19 GC-MSD, LOQ: 0.01 mg/kg The method allows separating the isomers of deltamethrin		
High starch/dry content (grain, straw)	Primary	0.01 mg/kg	LC-MS/MS	Niewelt-Stasiak, S., 2024, VAL/20/2023
	Confirmatory	~~Not require~~ ~~Required,~~ SANTE/2020/12830, Rev.2 multi-residue DFG S19 method, EFSA Journal 2022;20(7):7446 This method was provided for pre-authorisation purpose, with highly specific analythical method, therefore no confirmatory method is required.		
High oil content(rape seed)	Primary	0.01 mg/kg	LC-MS/MS	Agnes Perny, 2018, Study No. B7023
	Confirmatory	~~Not require~~ ~~Required~~, SANTE/2020/12830, Rev.2 multi-residue DFG S19 method, EFSA Journal 2022;20(7):7446 This method was provided for pre-authorisation purpose, with highly specific analythical method, therefore no confirmatory method is required.		
High water content (sugar beet roots)	Primary	0.01 mg/kg	LC-MS/MS	Faessel, V., 2022, C1145
	Confirmatory	This method was provided for pre-authorisation purpose, with highly specific analythical method, therefore no confirmatory method is required.		
Animal products, food of animal origin,... (Residues)	Primary	0.02 mg/kg	GC-ECD	Martens, 2000, B.4.2.2, DAR, Deltamethrin – Addendum to the Monograph Annex B4: Methods of analysis

Component of residue definition: Deltamethrin				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
muscle, fat, kidney, liver, milk and egg				
	Confirmatory	~~Not require~~ Required, SANTE/2020/12830, Rev.2 EFSA Journal 2022;20(7):7446: DFG S19 GC-MSD LOQ of 0.01 mg/kg. The method allows separation of alpha-R deltamethrin, cis-deltamethrin and trans-deltamethrin available.		
Honey, pollen	Primary	0.01 mg/kg	LC-MS/MS	Faessel, V., 2022, R C1199
	Confirmatory	Not required		
Soil Environmental fate)	Primary	0.001 mg/kg	GC-ECD	Benwell, L. (or Burden, A.N.) 1992 B.4.3.1, DAR, Deltamethrin – Volume 3, Annex B4: Methods of analysis
	Confirmatory	~~Not required~~ Required, SANTE/2020/12830, Rev.2		
Water (drinking water, surface water) (Efficacy)	Drinking water: extraction with hexane, analysis by GC-ECD. LOQ 0.05 µg/l (DAR)			
	Primary	Surface water: 0.003 µg/l	GC-ECD	Class T., 2001a, B.4.3.2, DAR, Deltamethrin –Addendum to the Monograph Annex B4: Methods of analysis
	Confirmatory	Surface water: 0.003 µg/l	GC/MS/MS	
Body fluids and tissues (Toxicology)	Primary	Human plasma: 20 ng/ml	GC-ECD	Tillier, C. 1988 B.4.4.1, DAR, Deltamethrin – Volume 3, Annex B4: Methods of analysis
	Confirmatory	~~Not require~~ Required, SANTE/2020/12830, Rev.2		
	Primary	Urine: 10 ng/ml	GC-ECD	Tillier, C., Devaux, P. 1981 B.4.4.2, DAR, Deltamethrin – Volume 3, Annex B4: Methods of analysis
		feaces: 10 ng/ml	GC-ECD	Akhtar, M. H., 1982, B.4.4.2, DAR, Deltamethrin – Volume 3, Annex B4: Methods of analysis
	Confirmatory	~~Not require~~ Required, SANTE/2020/12830, Rev.2		
Air (Exposure)	Primary	0.27 µg /m^3	GC-ECD	Class T., 2001b , B.4.3.3, DAR, Deltamethrin – Addendum to the Monograph Annex B4: Methods

Component of residue definition: Deltamethrin				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
	Confirmatory	0.27 µg /m^3	GC/MS	of analysis

An overview on the acceptable methods and possible data gaps for analysis of residues of acetamiprid for the generation of pre-authorization data is given in the following table. For the detailed evaluation of additional studies it is referred to KCP 5.2

RMS comments:
The Applicant provided data available for the approval of deltamethrin nevertheless in accordance with the SANTE/2020/12830, Rev.2: Confirmatory methods are required to demonstrate the selectivity of the primary method for all representative sample matrices. It has to be confirmed that the primary method detects the correct analyte (analyte identity) and that the analyte signal of the primary method is quantitatively correct and not affected by any other compound.
The Applicant provided new studies including the validation of methods for the determination of deltamethrin in high oil, high water and high starch content using the LC-MS/MS technique so no confirmatory method is required for these matrix.

There is no data on confirmatory methods for soil, body fluids and tissues.

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

Component of residue definition: Acetamiprid for food of plant origin. N-desmethyl-acetamiprid (IM-2-1), expressed as acetamiprid for food of animal origin. Acetamiprid for Air, soil and surface water. No residue definition for body fluids and tissues. Acetamiprid and IM-1-5 for drinking water				
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 ~~(Potato)~~ (Sugar beet)	~~Primary~~	~~0.01 mg/kg~~	~~LC-MS/MS~~	~~Weber H., 2013 Acetamiprid RAR, CA 4.2/02 (CA 6.3.3/3)~~ ~~Which is equivalent to: Łącka E., 2016, BA 17/15~~
	~~Confirmatory~~	~~Not required~~		
	Primary	0.005 mg/kg	LC-MS/MS	Niewelt-Stasiak, S., 2023, VAL/17/2023
	Confirmatory	Not required		
High acid content (Whole orange)	Primary	0.01 mg/kg	LC-MS/MS	Schwarz, T., (2008) Acetamiprid RAR, CA 4.2/01 (CA 6.3.3/1)

Component of residue definition: Acetamiprid for food of plant origin. N-desmethyl-acetamiprid (IM-2-1), expressed as acetamiprid for food of animal origin. Acetamiprid for Air, soil and surface water. No residue definition for body fluids and tissues. Acetamiprid and IM-1-5 for drinking water				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
			UPLC-MS/MS	Equivalent study: D. Longhi, 2019, GLP-STUDY-18-000081
	Confirmatory	Not required		
High oil content (sunflower seeds)	Primary	0.01 mg/kg	LC-MS/MS	Schwarz, T., (2008) Acetamiprid RAR, CA 4.2/01 (CA 6.3.3/1)
			UPLC-MS/MS	Which is equivalent to: D. Longhi, 2019, GLP-STUDY-18-000081
	Confirmatory	Not required		
High protein/high starch content (dry) (maze grain)	Primary	0.01 mg/kg	LC-MS/MS	Schwarz, T., (2008) Acetamiprid RAR, CA 4.2/01 (CA 6.3.3/1)
			UPLC-MS/MS	Which is equivalent to: D. Longhi, 2019, GLP-STUDY-18-000081
	Confirmatory	Not required		
	Primary	0.005 mg/kg	LC-MS/MS	Niewelt-Stasiak, S., 2023, VAL/16/2023
	Confirmatory	Not required		
Animal products, food of animal origin,... (Residues)	Primary	0.01 mg/kg	LC-MS/MS	Miya, K., Acetamiprid RAR, (2010) CA 4.2/04
			LC-QQQ	Equivalent study: D. Norris, 2019, DNA4036, addendum 2
	Confirmatory	Not required		
Honey, Pollen	Primary	0.01 mg/kg	LC-MS/MS	Faessel, V., 2020, R C0238
	Confirmatory	Not required		
Drinking and surface water	Primary	Acetamiprid: 0.1 µg/L	LC-MS/MS	Miya, K., (2007) Acetamiprid RAR, CA 4.2/07
		Acetamiprid: 0.05 µg/L	LC-QQQ	Equivalent study: D. Norris, 2017, DNA4037 addendum 3

Component of residue definition: Acetamiprid for food of plant origin. N-desmethyl-acetamiprid (IM-2-1), expressed as acetamiprid for food of animal origin. Acetamiprid for Air, soil and surface water. No residue definition for body fluids and tissues. Acetamiprid and IM-1-5 for drinking water				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
	Primary	IM-1-5: 0.05 µg/L	HPLC-MS/MS	Giesau, A. and Weber, H., (2012) Acetamiprid RAR, CA 4.2/09
			LC-QQQ	Equivalent study: D. Norris, 2017, DNA4518
	Confirmatory	Not required		
Soil Environmental fate)	Primary	0.002 mg/kg	LC-MS/MS	Täufer, A. and Weber, H., (2010) Acetamiprid RAR, CA 4.2/06 (CA 6.6.2/1)
			LC-QQQ	Equivalent study: D. Norris, 2018, Study Number: DNA4517
	Confirmatory (if required)	Not required		
Body fluids and tissues (Toxicology)	Primary	Blood: 0.05 mg/L	HPLC-MS/MS	Senciuc, M., (2014c) Acetamiprid RAR, CA 4.2/12 Equivalent study: D. Longhi, 2019, GLP-STUDY-18-000079 not provided by the Applicant and not evaluated in this dRR Method presented in Appendix 2.
	Confirmatory (if required)	Not required		
Air (Exposure)	Primary	0.002 µg /m^3	HPLC-MS/MS	Beck, I. and Class, T., (2009) Acetamiprid RAR, CA 4.2/11
			UPLC-MS/MS	Equivalent study: D. Longhi, 2019, GLP-STUDY-18-000080
	Confirmatory (if required)	Not required		

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

Data provided on Annex I inclusion is sufficient for post-authorizations methods. No new methods are

necessary since all data is described and presented in Table 5.2-3 in point KCP 5.1.2.

However since there are presented new residues studies for CHR/I/ADEL 280 SC in Oilseed Rape seeds and winter wheat, new methods for residues are presented in Appendix 2. In accordance to comment received during commenting period evaluation was moved to Appendix 2 from this chapter. Changes were not highlighted in yellow in Appendix 2 as no particular corrections were implemented.

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 deltamethrin (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 identical.

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

Matrix	Residue definition	Method LOQ MRL/Limit	Reference for MRL/level Remarks
Plant, High water content: (Potato, Peach, Onion, tomato, cucmber, melon, cauliflower, leek)	Deltamethrin	0.02 mg/kg 0.01 mg/kg 0.01 mg/kg	DAR, Deltamethrin – Addendum to the Monograph Annex B4: Methods of analysis Reg. (EU) 2018/832 Reg. (EU) 2024/1342
Plant, high acid content (orange)		0.02 mg/kg 0.01 mg/kg 0.01 mg/kg	DAR, Deltamethrin – Addendum to the Monograph Annex B4: Methods of analysis Reg. (EU) 2018/832 Reg. (EU) 2024/1342
Plant, High starch content (Corn flour, wheat grain, sorghum starch, rice)		0.02 mg/kg 0.02 mg/kg 0.01 mg/kg	DAR, Deltamethrin – Volume 3, Annex B4: Methods of analysis Reg. (EU) 2018/832 Reg. (EU) 2024/1342
Plant, High oil content(rape seed, cotton seed, corn oil)		0.02 mg/kg	DAR, Deltamethrin – Volume 3, Annex B4: Methods of analysis

Matrix	Residue definition	Method LOQ MRL/Limit	Reference for MRL/level Remarks
		0.01 mg/kg 0.01 mg/kg	Reg. (EU) 2018/832 Reg. (EU) 2024/1342
Muscle	deltamethrin	0.02 mg/kg 0.02 mg/kg	DAR, Deltamethrin – Addendum to the Monograph Annex B4: Methods of analysis Reg. (EU) 2018/832 Reg. (EU) 2024/1342
Milk		0.02 mg/kg 0.05 mg/kg	DAR, Deltamethrin – Addendum to the Monograph Annex B4: Methods of analysis Reg. (EU) 2018/832 Reg. (EU) 2024/1342
Eggs		0.02 mg/kg 0.02 mg/kg	DAR, Deltamethrin – Addendum to the Monograph Annex B4: Methods of analysis Reg. (EU) 2018/832 Reg. (EU) 2024/1342
Fat		0.02 mg/kg 0.1 mg/kg	DAR, Deltamethrin – Addendum to the Monograph Annex B4: Methods of analysis Reg. (EU) 2018/832 Reg. (EU) 2024/1342
Liver, kidney		0.02 mg/kg 0.02 mg/kg	DAR, Deltamethrin – Addendum to the Monograph Annex B4: Methods of analysis Reg. (EU) 2018/832 Reg. (EU) 2024/1342
Honey, pollen	deltamethrin	0.01 mg/kg 0.05 mg/kg	Faessel, V., 2022, R C1199 Reg. (EU) 2018/832 Reg. (EU) 2024/1342
Soil (Ecotoxicology)	deltamethrin	0.001 mg/kg or 0.002 mg/kg 0.05 mg/kg	DAR, Deltamethrin – Volume 3, Annex B4: Methods of analysis common limit
Drinking water (Human toxicology)	deltamethrin	0.05 µg/l	general limit for drinking water
Surface water (Ecotoxicology)	deltamethrin	0.003 µg/l	DAR, Deltamethrin – Addendum to the Monograph Annex B4: Methods of analysis
Air	deltamethrin	0.27 µg/m^3	AOEL sys 7.5 mg/kg bw/d
Tissue (meat or liver)	deltamethrin	Human plasma: 20 ng/ml	DAR, Deltamethrin – Volume

Matrix	Residue definition	Method LOQ MRL/Limit	Reference for MRL/level Remarks
Body fluids		Urine and feaces: 10 g/ml 0.01 mg/kg for tissue 0.01 mg/L for body fluids	3, Annex B4: Methods of analysis SANTE/2020/12830, Rev.2

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 deltamethrin 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: deltamethrin Deltamethrin (cis-deltamethrin)				
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	0.02 mg/kg	GC-ECD	Martens, 1998c, B.4.2.1, DAR, Deltamethrin – Addendum to the Monograph Annex B4: Methods of analysis
		0.01 mg/kg	GC-MSD	Weber, H., 2009 / Report No: S09-00553 EU agreed
	ILV	0.02 mg/kg	GC-ECD	Haines BK, 2001, B.4.2.1, DAR, Deltamethrin – Addendum to the Monograph Annex B4: Methods of analysis
		0.01 mg/kg	GC-MSD	Merdian, H., 2009 / Report No: P 1681 G EU agreed
	Confirmatory	~~Not required~~ See Table 5.2-2a 0.01 mg/kg	GC-MSD	EFSA Journal 2022;20(7):7446: multi residue method DFG S19. The method allows separating the isomers of deltamethrin
High acid content	Primary	0.02 mg/kg	GC-ECD	Martens, 1998c, B.4.2.1, DAR, Deltamethrin – Addendum to the Monograph Annex B4: Methods of analysis
		0.01 mg/kg	GC-MSD	Weber, H., 2009 / Report No: S09-00553 EU agreed
	ILV	0.02 mg/kg	GC-ECD	Haines BK, 2001, B.4.2.1, DAR, Deltamethrin – Addendum to the Monograph Annex B4: Methods

Component of residue definition: deltamethrin Deltamethrin (cis-deltamethrin)				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
				of analysis
		0.01 mg/kg	GC-MSD	Merdian, H., 2009 / Report No: P 1681 G EU agreed
	Confirmatory	~~Not required~~ See Table 5.2-2a 0.01 mg/kg	GC-MSD	EFSA Journal 2022;20(7):7446: multi residue method DFG S19. The method allows separating the isomers of deltamethrin
High oil content	Primary	0.02mg/kg	GC-ECD	Martens, 1998a and b, B.4.2.1, DAR, Deltamethrin – Addendum to the Monograph Annex B4: Methods of analysis
	oilseed rape seeds	0.01 mg/kg	LC-MS/MS	Agnes Perny, 2018, Study No. B7023
		0.01 mg/kg	GC-MSD	Weber, H., 2009 / Report No: S09-00553 EU agreed
	ILV	~~Not required~~ 0.02mg/kg		Addendum to the Monograph Annex B4: Methods of analysis
		0.01 mg/kg	GC-MSD	Merdian, H., 2009 / Report No: P 1681 G EU agreed
	Confirmatory	~~Not required~~ See Table 5.2-2a 0.01 mg/kg	GC-MSD	EFSA Journal 2022;20(7):7446: multi residue method DFG S19. The method allows separating the isomers of deltamethrin
High protein/high starch content (dry)	Primary	0.02 mg/kg	GC-ECD	Martens, 1998a and b, B.4.2.1, DAR, Deltamethrin – Addendum to the Monograph Annex B4: Methods of analysis
	wheat	0.01 mg/kg	LC-MS/MS	Joanna Kicińska, 2018, Study Code: ZBBZ-2017/05/DPL/1 Niewelt-Stasiak, S. 2023, VAL/20/2023
		0.01 mg/kg	GC-MSD	Weber, H., 2009 / Report No: S09-00553 EU agreed
	ILV	~~Not required~~ 0.02 mg/kg		Addendum to the Monograph Annex B4: Methods of analysis
		0.01 mg/kg	GC-MSD	Merdian, H., 2009 / Report No: P 1681 G EU agreed
	Confirmatory	~~Not required~~ See Table 5.2-2a 0.01 mg/kg	GC-MSD	EFSA Journal 2022;20(7):7446: multi residue method DFG S19. The method allows separating the

Component of residue definition: deltamethrin Deltamethrin (cis-deltamethrin)				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
				isomers of deltamethrin

zRMS comments:
According to the EFSA Journal, 2025, 23(3), e931: "Analytical methods for the determination of deltamethrin residues in plants were assessed during the MRL review and in previous MRL applications (EFSA, 2015, 2018b). During the MRL review, an analytical method quantifying deltamethrin in plant matrices with high-water content, high-fat content, acidic and dry commodities using gas chromatography with electron capture detector (GC–ECD) was evaluated and validated at the LOQ of 0.02 mg/kg. However, as this method was not considered highly specific, a confirmatory method was required as Article 12 confirmatory data (EFSA, 2015). This data gap was addressed in the framework of the assessment of the confirmatory data of the MRL review (EFSA, 2022a). EFSA concluded that a full validation of a multi-residue DFG S19 method for the analysis of cis-deltamethrin residues by gas chromatography with mass selective detection (GC-MSD) is provided for high-water content, high-acid content, high-fat content and dry matrices at the LOQ of 0.01 mg/kg. The method allows separating the isomers of deltamethrin (EFSA, 2022a)."

Table 5.3-3: Statement on extraction efficiency

Method for products of plant origin
~~Not required, residue levels are below LOQ~~ Not evaluated in the Addendum to the Monograph, 2002. According to SANTE/2017/10632 Rev. 5.: the data requirements used for the latest renewal or approval should be considered. This means that no additional proof of extraction efficiency is required if it had not been required in the renewal of approval/approval procedure itself. Data on extraction efficiency should be provided for the reneval of approval of the active substance. ~~Considering that the methods are accepted on EU level no further statement regarding extraction efficiency is necessary.~~

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 deltamethrin 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: deltamethrin				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Milk	Primary	0.02 mg/kg	GC-ECD	Martens, 2000, B.4.2.2, DAR, Deltamethrin – Addendum to the Monograph Annex B4: Methods of analysis
	ILV	0.02 mg/kg	GC-ECD	Haines BK, 2001, B.4.2.1, DAR, Deltamethrin – Addendum to the

Component of residue definition: deltamethrin				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
				Monograph Annex B4: Methods of analysis
	Confirmatory	~~Not required~~ See Table 5.2-2a 0.01 mg/kg	GC-MSD	EFSA Journal 2022;20(7):7446: DFG S19 The method allows separation of alpha-R deltamethrin, cis-deltamethrin and trans-deltamethrin available.
Eggs	Primary	0.02 mg/kg	GC-ECD	Martens, 2000, B.4.2.2, DAR, Deltamethrin – Addendum to the Monograph Annex B4: Methods of analysis
	ILV	0.02 mg/kg	GC-ECD	Haines BK, 2001, B.4.2.1, DAR, Deltamethrin – Addendum to the Monograph Annex B4: Methods of analysis
	Confirmatory	~~Not required~~ See Table 5.2-2a 0.01 mg/kg	GC-MSD	EFSA Journal 2022;20(7):7446: DFG S19 The method allows separation of alpha-R deltamethrin, cis-deltamethrin and trans-deltamethrin available.
Muscle	Primary	0.02 mg/kg	GC-ECD	Martens, 2000, B.4.2.2, DAR, Deltamethrin – Addendum to the Monograph Annex B4: Methods of analysis
	ILV	0.02 mg/kg	GC-ECD	Haines BK, 2001, B.4.2.1, DAR, Deltamethrin – Addendum to the Monograph Annex B4: Methods of analysis
	Confirmatory	~~Not required~~ See Table 5.2-2a 0.01 mg/kg	GC-MSD	EFSA Journal 2022;20(7):7446: DFG S19 The method allows separation of alpha-R deltamethrin, cis-deltamethrin and trans-deltamethrin available.
Fat	Primary	0.02 mg/kg	GC-ECD	Martens, 2000, B.4.2.2, DAR, Deltamethrin – Addendum to the Monograph Annex B4: Methods of analysis
	ILV	0.02 mg/kg	GC-ECD	Haines BK, 2001, B.4.2.1, DAR, Deltamethrin – Addendum to the Monograph Annex B4: Methods

Component of residue definition: deltamethrin				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
				of analysis
	Confirmatory	~~Not required~~ See Table 5.2-2a 0.01 mg/kg	GC-MSD	EFSA Journal 2022;20(7):7446: DFG S19 The method allows separation of alpha-R deltamethrin, cis-deltamethrin and trans-deltamethrin available.
Kidney, liver	Primary	0.02 mg/kg	GC-ECD	Martens, 2000, B.4.2.2, DAR, Deltamethrin – Addendum to the Monograph Annex B4: Methods of analysis
	ILV	0.02 mg/kg	GC-ECD	Haines BK, 2001, B.4.2.1, DAR, Deltamethrin – Addendum to the Monograph Annex B4: Methods of analysis
	Confirmatory	~~Not required~~ See Table 5.2-2a 0.01 mg/kg	GC-MSD	EFSA Journal 2022;20(7):7446: DFG S19 The method allows separation of alpha-R deltamethrin, cis-deltamethrin and trans-deltamethrin available.
Honey, pollen	Primary	0.01 mg/kg	LC-MS/MS	Faessel, V., 2022, R C1199 Not presented on EU level. No method was presented in Peer review and RAR of Acetamiprid in bee products.
	ILV	Not required.		
	Confirmatory (if required)	Not required.		

zRMS comments: “According to the EFSA Journal 2022;20(7):7446: “a sufficiently validated multi residue DFG S19 method for enforcement using GC-MSD for milk, egg, muscle, liver and kidney and fat with an LOQ of 0.01 mg/kg for cis-deltamethrin was provided. The method allowed separation of the three isomers of deltamethrin (alpha-R-deltamethrin, cis-deltamethrin and trans-deltamethrin). The EMS noted that the extraction efficacy of the confirmatory method is not addressed (Austria, 2020; EFSA, 2022a). Therefore, it is recommended to consider this point during the ongoing renewal assessment.”

Table 5.3-5: Statement on extraction efficiency

Method for products of animal origin
Not required, residue levels are below LOQ
Not evaluated in the Addendum to the Monograph, 2002. According to SANTE/2017/10632 Rev. 5.: the data requirements used for the latest renewal or approval should be considered. This means that no additional proof of extraction efficiency is required if it had not been required in the

Method for products of animal origin
renewal of approval/approval procedure itself. Data on extraction efficiency should be provided for the reneval of approval of the active substance.

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 deltamethrin in soil is given in the following tables.

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

Component of residue definition: deltamethrin			
Method type	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	0.001 mg/kg	GC-ECD	Benwell, L. (or Burden, A.N.) 1992 B.4.3.1, DAR, Deltamethrin – Volume 3, Annex B4: Methods of analysis
Confirmatory	~~Not required~~ Required, data gap		

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 deltamethrin 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: deltamethrin				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Drinking water	Primary	0.05 µg/L	GC-ECD	Martens, 1999, B.4.3.2, DAR, Deltamethrin – Addendum to the Monograph Annex B4: Methods of analysis
	ILV	~~LOQ µg/L~~ 0.003 µg/L	GC-ECD (GC-MS/MS for confirmation)	~~author(s), year / missing / EU agreed~~ Class, 2001a) Addendum to the Monograph Annex B, 2002
	Confirmatory	~~Not required~~ See Table 5.2-2a 0.003 µg/l	GC-MS/MS	
Surface water	Primary	0.003 µg/L	GC-ECD	Class T., 2001a, B.4.3.2, DAR, Deltamethrin – Addendum to the Monograph Annex B4: Methods of analysis
	Confirmatory	0.003 µg/L	GC/MS/MS	

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 deltamethrin in air is given in the following tables.

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

Component of residue definition: deltamethrin			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	0.27 µg /m^3	GC-ECD	Class T., 2001b , B.4.3.3, DAR, Deltamethrin – Volume 3, Addendum to the Monograph Annex B4: Methods of analysis
Confirmatory	0.27 µg /m^3	GC/MS	

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

An overview on the acceptable methods and possible data gaps for analysis of deltamethrin in body fluids and tissues is given in the following table.

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

Component of residue definition: deltamethrin			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	Human plasma: 20 ng/ml	GC-ECD	Tillier, C. 1988 B.4.4.1, DAR, Deltamethrin – Volume 3, Annex B4: Methods of analysis
Confirmatory	~~Not required~~ See Table 5.2-2a Required, SANTE/2020/12830, Rev.2		
Primary	Urine and feaces: 10 ng/ml	GC-ECD	Akhtar, M. H. 1982 B.4.4.2, DAR, Deltamethrin – Volume 3, Annex B4: Methods of analysis
Confirmatory	~~Not required~~ See Table 5.2-2a Required, SANTE/2020/12830, Rev.2		

5.3.2.8 Other studies/ information

No other studies are provided.

5.3.3 Description of analytical methods for the determination of residues of acetamiprid (KCP 5.2)

zRMS comments:
According to the EFSA Journal 2016;14(11):4610: “Acetamiprid residues can be monitored in food and feed of plant origin with the quick, easy, cheap, effective and safe (QuEChERS) multiresidue method by high-performance liquid chromatography with tandem mass spectrometry (HPLC–MS/MS) with a limit of quantification (LOQ) of 0.01 mg/kg in all plant commodity groups. The QuEChERS multiresidue method with HPLC–MS/MS can also be used for the determination of the compound of the residue definition for monitoring in products of animal origin (N-desmethyl-acetamiprid (IM-2-1)) with an LOQ of 0.01 mg/kg in all animal matrices. Adequate HPLC–MS/MS methods are available for the determination of the residues of acetamiprid in soil and air with LOQs of 0.002 mg/kg and of 0.002 lg/m 3, respectively. Residues of acetamiprid in drinking water and surface water can be determined by HPLC–MS/MS with a LOQ of 0.1 lg/L, while monitoring metabolite IM-1-5 in drinking water and surface water can be done by HPLC–MS/MS with a LOQ of 0.05 lg/L. Acetamiprid residues in blood can be determined by HPLC–MS/MS with a LOQ of 0.05 mg/L”.

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

Compared to the residue definition proposed in the RAR (incl. its addenda) the current legal residue definition is identical.

Table 5.3-10: Relevant residue definitions for monitoring/enforcement and levels for which compliance is required

Matrix	Residue definition	LOQ mg/kg MRL / limit (mg/kg)	Reference for MRL/level Remarks
Potato, high water content	Acetamiprid	0.01 0.01	EFSA Journal 2016;14(11):4610 Reg. (EU)2019/88
Whole orange, high acid content		0.01 0.01	EFSA Journal 2016;14(11):4610 Reg. (EU)2019/88
Maize grain, high protein/high starch content (dry commodities)		0.01 0.01	EFSA Journal 2016;14(11):4610 Reg. (EU)2019/88
Sunflower seed, high oil content		0.01 0.01	EFSA Journal 2016;14(11):4610 Reg. (EU)2019/88
Muscle	N-desmethyl-acetamiprid (IM-2-1), expressed as acetamiprid	0.01 0.5	EFSA Journal 2016;14(11):4610 Reg. (EU)2019/88
Milk		0.01 0.2	EFSA Journal 2016;14(11):4610

Matrix	Residue definition	LOQ mg/kg MRL / limit (mg/kg)	Reference for MRL/level Remarks
			Reg. (EU)2019/88
Eggs		0.01 0.02	EFSA Journal 2016;14(11):4610 Reg. (EU)2019/88
Fat		0.01 0.3	EFSA Journal 2016;14(11):4610 Reg. (EU)2019/88
Liver, kidney		0.01 1.0	EFSA Journal 2016;14(11):4610 Reg. (EU)2019/88
Honey, Pollen	Acetamiprid	0.01 0.05	Faessel, V., 2020, R C 1199 Reg. (EU)2019/88
Soil (Ecotoxicology)	Acetamiprid	0.002 mg/kg	EFSA Journal 2016;14(11):4610
Drinking water (Human toxicology)	Acetamiprid, IM-1-5	Acetamiprid: 0.1 µg/L IM-1-5: 0.05 µg/L	general limit for drinking water
Surface water (Ecotoxicology)	Acetamiprid	Acetamiprid: 0.1 µg/L	EFSA Journal 2016;14(11):4610
Air	Acetamiprid	0.002 µg/m^3	EFSA Journal 2016;14(11):4610
Tissue (meat or liver)	No residue definition provided	Blood: 0.05 mg/L	EFSA Journal 2016;14(11):4610
Body fluids			

5.3.3.2 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 acetamiprid in plant matrices is given in the following tables.

Table 5.3-11: Validated methods for food and feed of plant origin (required for all matrix types, "difficult" matrix only when indicated by intended GAP

Component of residue definition: Acetamiprid				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
High water content ~~(Potato)~~ (sugar beet)	~~Primary~~	~~0.01 mg/kg~~	~~LC-MS/MS~~	~~Weber H., 2013 Acetamiprid RAR, CA 4.2/02 (CA 6.3.3/3)~~ ~~Which is equivalent to: Łącka E., 2016, BA 17/15~~

Component of residue definition: Acetamiprid				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
	Primary	0.005 mg/kg	LC-MS/MS	Niewelt-Stasiak, S., 2023, VAL/17/2023
High water content (apple)	ILV	0.01 mg/kg	LC-MS/MS	Giesau, A. and Weber, H., (2012), Acetamiprid RAR, CA 4.2/03
			HPLC-MS/MS	Equivalent study: S. Paronuzzi Ticco, 2019, GLP Study No. CH - 031/2019
	Confirmatory	Not required		
High acid content (whole orange)	Primary	0.01 mg/kg	LC-MS/MS	Schwarz, T., (2008) Acetamiprid RAR, CA 4.2/01 (CA 6.3.3/1)
			UPLC-MS/MS	Equivalent study: D. Longhi, 2019, GLP-STUDY-18-000081
High acid content (whole orange)	ILV	0.01 mg/kg	LC-MS/MS	Giesau, A. and Weber, H., (2012), Acetamiprid RAR, CA 4.2/03
			HPLC-MS/MS	Equivalent study: S. Paronuzzi Ticco, 2019, GLP Study No. CH - 031/2019
	Confirmatory	Not required		
High oil content (Sunflower seed)	Primary	0.01 mg/kg	LC-MS/MS	Schwarz, T., (2008) Acetamiprid RAR, CA 4.2/01 (CA 6.3.3/1)
			UPLC-MS/MS	Which is equivalent to: D. Longhi, 2019, GLP-STUDY-18-000081
High oil content (Sunflower seed)	ILV	0.01 mg/kg	LC-MS/MS	Giesau, A. and Weber, H., (2012), Acetamiprid RAR, CA 4.2/03
			HPLC-MS/MS	Equivalent study: S. Paronuzzi Ticco, 2019, GLP Study No. CH - 031/2019
	Confirmatory	Not required		
High protein/high starch content (dry) (Maze grain)	Primary	0.01 mg/kg	LC-MS/MS	Schwarz, T., (2008) Acetamiprid RAR, CA 4.2/01 (CA 6.3.3/1)
			UPLC-MS/MS	Which is equivalent to: D. Longhi, 2019, GLP-STUDY-18-000081

Component of residue definition: Acetamiprid				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
High protein/high starch content (dry) (Maze grain)	ILV	0.01 mg/kg	LC-MS/MS	Giesau, A. and Weber, H., (2012), Acetamiprid RAR, CA 4.2/03
			HPLC-MS/MS	Equivalent study : S. Paronuzzi Ticco, 2019, GLP Study No. CH - 031/2019
	Confirmatory	Not required		

Table 5.3-12: Statement on extraction efficiency

Method for products of plant origin
The efficiency of the following extraction procedures has been demonstrated using incurred residues in metabolism studies (DAR).
Apple Leaves and fruits were washed with methanol and extracted twice with methanol/water, 3/1, v/v. Acetamiprid – Volume 3 B.5 (AS) 54 Recoveries: 93-99% for leaf and 68-96% for fruits. Cabbage Leaves were washed with methanol and leaves and head were extracted three times with methanol/water, 3/1, v/v. Recoveries: 79-100% for leaf. Carrot Roots (flesh and peel seperately) were extracted once with acetone and twice with acetone/water (80/20 and 50/50, v/v). Recoveries: 52-65% for peel, 66-88% for flesh. Aubergine Leaves and fruits were washed with methanol and extracted twice with methanol/water, 3/1, v/v. Recoveries: 99% for leaf and 97-99% for fruits.

5.3.3.3 Description of analytical methods for the acetamiprid of residues in animal matrices (KCP 5.2)

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

Table 5.3-13: Validated methods for food and feed of animal origin (if appropriate)

Component of residue definition: N-desmethyl-acetamiprid (IM-2-1), expressed as acetamiprid				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Milk	Primary	0.01 mg/kg	LC-MS/MS	Miya, K., Acetamiprid RAR, (2010) CA 4.2/04
			LC-QQQ	Equivalent study: D. Norris, 2019, DNA4036, addendum 2
	ILV	0.01 mg/kg	LC-MS/MS	Knoch, E., Acetamiprid RAR,

Component of residue definition: N-desmethyl-acetamiprid (IM-2-1), expressed as acetamiprid				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
				(2010) CA 4.2/05 Equivalent study: Eichler, M., 2018, Study No. 133111101
	Confirmatory	Not required		
Eggs	Primary	0.01 mg/kg	LC-MS/MS	Miya, K., Acetamiprid RAR, (2010) CA 4.2/04
			LC-QQQ	Equivalent study: D. Norris, 2019, DNA4036, addendum 2
	ILV	0.01 mg/kg	LC-MS/MS	Knoch, E., Acetamiprid RAR, (2010) CA 4.2/05 Equivalent study: Eichler, M., 2018, Study No. 133111101
	Confirmatory	Not required		
Muscle	Primary	0.01 mg/kg	LC-MS/MS	Miya, K., Acetamiprid RAR, (2010) CA 4.2/04
			LC-QQQ	Equivalent study: D. Norris, 2019, DNA4036, addendum 2
	ILV	0.01 mg/kg	LC-MS/MS	Knoch, E., Acetamiprid RAR, (2010) CA 4.2/05 Equivalent study: Eichler, M., 2018, Study No. 133111101
	Confirmatory	Not required		
Fat	Primary	0.01 mg/kg	LC-MS/MS	Miya, K., Acetamiprid RAR, (2010) CA 4.2/04
			LC-QQQ	Equivalent study: D. Norris, 2019, DNA4036, addendum 2
	ILV	0.01 mg/kg	LC-MS/MS	Knoch, E., Acetamiprid RAR, (2010) CA 4.2/05 Equivalent study: Eichler, M., 2018, Study No. 133111101
	Confirmatory	Not required		

Component of residue definition: N-desmethyl-acetamiprid (IM-2-1), expressed as acetamiprid				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Kidney, liver	Primary	0.01 mg/kg	LC-MS/MS	Miya, K., Acetamiprid RAR, (2010) CA 4.2/04
			LC-QQQ	Equivalent study: D. Norris, 2019, DNA4036, addendum 2
	ILV	0.01 mg/kg	LC-MS/MS	Knoch, E., Acetamiprid RAR, (2010) CA 4.2/05 Equivalent study: Eichler, M., 2018, Study No. 133111101
	Confirmatory	Not required		
Honey, Pollen	Primary	0.01 mg/kg	LC-MS/MS	Faessel, V., 2020, R C 1199
	Confirmatory	Not required		

Table 5.3-14: Statement on extraction efficiency

Method for products of animal origin
The efficiency of the following extraction procedures has been demonstrated using incurred residues in metabolism studies (DAR).
Goat Liver, Kidney, Muscle Liver, kidney and muscle were extracted twice with acetone by shaking for 30 minutes. Recoveries: 59.2-66.7% for liver, 72.9-74.5% for kidney, 67.1% for muscle. Hen Liver, Muscle, Eggs Liver, muscle and eggs (white and yolk) were extracted twice with acetone by shaking for 30 minutes. Recoveries: 70.9-76.8% for liver, 66.2% for muscle, 74.4-82.1% for egg white, 70.5-77.9% for egg yolk

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

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

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

Table 5.3-15: Validated methods for soil (if appropriate)

Component of residue definition: Acetamiprid			
Method type	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	0.002 mg/kg	LC-MS/MS	Täufer, A. and Weber, H., (2010) Acetamiprid RAR, CA 4.2/06 (CA 6.6.2/1)
		LC-QQQ	Equivalent study: D. Norris, 2018, Study Number: DNA4517
ILV	0.002 mg/kg	LC-QQQ	Eichler M., Hermann S., 2018, Study number: 133113101 The study not provided, not assessed and not required.

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

zRMS comments:
The ILV study was not submitted and therefore was not evaluated.
Not required.
According to the SANTE/2020/12830, Rev.2: A validation of the primary monitoring method in an independent laboratory (ILV) is required for the determination of residues in food of plant and animal origin and in drinking water.

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

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

Table 5.3-16: Validated methods for water (if appropriate)

Component of residue definition: Acetamiprid, IM-1-5				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Drinking water	Primary	Acetamiprid: 0.1 µg/L	LC-MS/MS	Miya, K., (2007) Acetamiprid RAR, CA 4.2/07
		Acetamiprid: 0.05 µg/L	LC-QQQ	Equivalent study: D. Norris, 2017, DNA4037 addendum 3
	Primary	IM-1-5: 0.05 µg/L	HPLC-MS/MS	Giesau, A. and Weber, H., (2012) Acetamiprid RAR, CA 4.2/09
			LC-QQQ	Equivalent study: D. Norris, 2017, DNA4518

Component of residue definition: Acetamiprid, IM-1-5				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
	ILV	Acetamiprid: 0.1 µg/L	HPLC-MS/MS	Senciuc, M., (2014a) CA 4.2/08
		Acetamiprid: 0.05 µg/L	LC-MS/MS	Equivalent study: Eichler, M., Hermann S., 2018, Study no.: 133112101
	ILV	IM-1-5: 0.05 µg/L	HPLC-MS/MS	Senciuc, M., (2014b) Acetamiprid RAR, CA 4.2/10
			LC-MS/MS	Equivalent study: Eichler, M., Hermann S., 2018, Study no.: 133141101
	Confirmatory	Not required		
Surface water	Primary	Acetamiprid: 0.1 µg/L	LC-MS/MS	Miya, K., (2007) Acetamiprid RAR, CA 4.2/07
		Acetamiprid: 0.05 µg/L	LC-QQQ	Equivalent study: D. Norris, 2017, DNA4037 addendum 3
	Primary	IM-1-5: 0.05 µg/L	HPLC-MS/MS	Giesau, A. and Weber, H., (2012) Acetamiprid RAR, CA 4.2/09
			LC-QQQ	Equivalent study: D. Norris, 2017, DNA4518
	Confirmatory	Not required		

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

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

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

Table 5.3-17: Validated methods for air (if appropriate)

Component of residue definition: Acetamiprid			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	0.002 µg /m^3	HPLC-MS/MS	Beck, I. and Class, T., (2009) Acetamiprid RAR, CA 4.2/11
		UPLC-MS/MS	Equivalent study: D. Longhi, 2019, GLP-STUDY-18-000080
Confirmatory	Not required		

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

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

An overview on the acceptable methods and possible data gaps for analysis of acetamiprid in body fluids and tissues is given in the following table.

Table 5.3-18: Methods for body fluids and tissues (if appropriate)

Component of residue definition: No residue definition, IM-2-1 and 6-chloronicotinic acid (IC-0) were the main residues identified in rat urine (EFSA Journal 2016;14(11):4610)			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	Blood: 0.05 mg/L	HPLC-MS/MS	Senciuc, M., (2014c) Acetamiprid RAR, CA 4.2/12 Equivalent study: D. Longhi, 2019, GLP-STUDY-18-000079 – not provided by the Applicant and not evaluated in this dRR Method presented in Appendix 2.
Confirmatory	Not required		

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

zRMS comments:
According to EFSA Journal 2016;14(11):4610, on acetamiprid, no residue definition is proposed for monitoring purposes, however, an analytical method for the determination acetamiprid in body fluids (blood) is available with LOQ 0.05 mg/L. The Applicant provided a study deemed to be equivalent.

Nevertheless, according to SANTE/2020/12830 rev.2, a fully validated method for body fluids with LOQ 0.01 mg/L is required. This data gap should be addressed at active substance level.

5.3.3.8 Other studies/ information

No other studies are provided.

Appendix 1 Lists of data considered in support of the evaluation

List of data submitted by the applicant and relied on

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	Vertebrate study Y/N	Owner
KCP 5.1.1	E. Arévalo	2021	CHR/I/ADEL 280 SC Part I: Determination of physicochemical properties of the initial preparation, after accelerated storage and after low temperature. BF-55/20 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry GLP Unpublished	N	Chemirol
KCP 5.1.1/2	M. Woloszynowska	2021	CHR/I/ADEL 280 SC Method validation for determination of the acetamiprid and deltamethrin residues in aqueous solutions and suspensions Ref No: BA.4023.20.2020.3 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry GLP Unpublished	N	Chemirol
~~KCP 5.1.2 KCP 5.2~~	~~E. Łącka~~	~~2016~~	~~CHEMIROL ACETAMIPRID determination of residues in potatoes BA 17/15 IPO Warszawa GLP, Not published~~	~~N~~	~~Chemirol~~
KCP 5.1.2 KCP 5.2	D. Longi	2019	Validation of an analytical method for the determination of Acetamiprid residues in high acid content, high oil content and dry/high starch content matrices GLP-STUDY-18-000081 LabAnalysis s.r.l. GLP	N	Chemirol

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 5.1.2 KCP 5.2	S. Paronuzzi Ticco	2019	Independent Laboratory Validation (ILV) of the analytical method for the determination of Acetamiprid residues in high acid content, high oil content and dry/high starch content matrices CH – 031/2019 ChemService S.r.l. Controlli e Ricerche GLP	N	Chemirol
KCP 5.1.2 KCP 5.2	D. Norris	2019	Validation of the Methods of Analysis used for the Determination of Acetamiprid and a specified metabolite in animal commodities, in Compilance with Good Laboratory Practice, and referencing SANCO/3029/99 DNA4036 DAVID NORRIS ANALYTICAL LABORATORIES LTD GLP Unpublished	N	Chemirol
KCP 5.1.2 KCP 5.2	Faessel, V.	2020	Validation of the Analytical Method for the analysis of Acetamiprid in Honey and Pollen. R C0238 Anadiag, Haguenau, France GLP Unpublished	N	Chemirol
KCP 5.1.2 KCP 5.2	Faessel, V.	2022	Validation of the Analytical Method for the analysis of Deltamethrin and its alpha-R-isomer and trans-isomer in Honey and Pollen. R C1199 Anadiag, Haguenau, France GLP Unpublished	N	Chemirol
KCP 5.1.2 KCP 5.2	D. Norris	2017	Validation of the Methods of Analysis used for the Determination of Acetamiprid in Water, in Compliance with Good Laboratory Practice, and referencing SANCO/3029/99 DNA4037 DAVID NORRIS ANALYTICAL LABORATORIES LTD GLP Unpublished	N	Chemirol
KCP 5.1.2	D. Norris	2017	Validation of the Methods of Analysis used for the Determination of a Metabolite of Acetamiprid in	N	Chemirol

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 5.2			Drinking Water, in Compilance with Good Laboratory Practice, and referencing SANCO/825/00 rev. 8.1 DNA4518 DAVID NORRIS ANALYTICAL LABORATORIES LTD GLP Unpublished		
KCP 5.1.2 KCP 5.2	D. Norris	2018	Validation of the Methods of Analysis used for the Determination of Acetamiprid and two Acetamiprid Metabolites in Caclareous Soil, in Compliance with Good Laboratory Practice, and referencing SANCO/825/00 rev. 8.1. DNA4517 DAVID NORRIS ANALYTICAL LABORATORIES LTD GLP Unpublished	N	Chemirol
KCP 5.1.2 KCP 5.2	D. Longhi	2019	Validation of an analytical method for the determination of Acetamiprid residues in air GLP-STUDY-18-000080 LabAnalysis s.r.l. GLP Unpublished	N	Chemirol
KCP 5.1.2 KCP 5.2	D. Longhi	2019	Validation of an analytical method for the determination of Acetamiprid residues in blood LabAnalysis s.r.l. GLP Unpublished	N	Chemirol
KCP 5.1.2	S. Niewelt-Stasiak	2023	Validation of an analytical method for the determination of residues of acetamiprid and acetamiprid-N-desmethyl in wheat (grain, plant, straw) Study No. VAL/16/2023 SGS Polska Sp. z o.o. GLP Unpublished	N	Chemirol
KCP 5.1.2	S. Niewelt-Stasiak	2023	Validation of an analytical method for the determination of residues of acetamiprid and acetamiprid-N-desmethyl in sugar beet (leaves, roots) Study No. VAL/17/2023	N	Chemirol

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Vertebrate study Y/N**	**Owner**
			SGS Polska Sp. z o.o. GLP Unpublished		
KCP 5.1.2	S. Niewelt-Stasiak	2023	Validation of an analytical method for the determination of residues of Deltamethrin (+ alpha R isomer + trans isomer) in wheat (grain, plant, straw) Study No. VAL/20/2023 SGS Polska Sp. z o.o. GLP Unpublished	N	Chemirol
KCP 5.1.2	Faessel, V.	2022	Validation of the Analytical Method for the Analysis of Deltamethrin and its alpha-R-isomer and trans-isomer metabolites in Sugar beet. Study No. R C 1145 SGS Polska Sp. z o.o. GLP Unpublished	N	Chemirol
KCP 5.2.1/01	A.Perny	2018	Validation of the Analytical Method for the Analysis of Deltamethrin in Oilseed Rape Seeds B7023 Anadiag GLP Unpublished	N	Chemirol
KCP 5.2.1/02	J. Kicińska	2018	DETERMINATION OF RESIDUES OF DELTAMETHRIN IN WINTER WHEAT APPLIED AS “DELCAPS 050 CS” AND “DELTAMETHRIN 100 SC” IN NORTHERN EUROPE IN 2017 ZBBZ-2017/05/DPL/1 Food Safety Laboratory GLP Unpublished	N	Chemirol
KCP 5.1.2 KCP 5.2	Eichler, M.	2018	Acetamiprid and its metabolite IM-2-1: Independent Laboratory Validation of an Analytical Method for the Determination in Animal Commodities Study No. 133111101 Ibacon GmbH	N	Chemirol

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Vertebrate study Y/N**	**Owner**
			GLP Unpublished		
~~KCP 5.2~~	~~Eichler, M.~~	~~2018~~	~~Acetamiprid and its Metabolites IM-1-2 and IM-1-4: Independent Laboratory Validation of an Analytical Method for the Determination in Calcareous Soil~~ ~~Study No. 133113101~~ ~~Ibacon GmbH~~ ~~GLP~~ ~~Unpublished~~	~~N~~	~~Chemirol~~
KCP 5.1.2 KCP 5.2	Eichler, M.	2018	Acetamiprid: Independent Laboratory Validation of an Analytical Method for the Determination in Drinking Water Study No. 133112101 Ibacon GmbH GLP Unpublished	N	Chemirol
KCP 5.1.2 KCP 5.2	Eichler, M.	2018	IM-1-5 (Metabolite of Acetamiprid): Independent Laboratory Validation of an Analytical Method for the Determination in Drinking Water Study No. 133141101 Ibacon GmbH GLP Unpublished	N	Chemirol

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

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 5.2	Weber, H.	2009	Validation of enforcement method DFG S19 (L 00.00-34) (BCS method ID 00086/M089) for the determination of cis-deltamethrin (AE F032640) in/on foodstuff of plant origin Eurofins Analytik GmbH, Dr. Specht Laboratorien, Hamburg, Germany Report No.: S09-00553, GLP: yes unpublished	N	Bayer AG
KCP 5.2	Merdian, H.	2009	Independent laboratory validation of the DFG method S19 (BCS method 00086/M089) for the determination of residues of cis-deltamethrin (AE F032640) in plant materials, using GC/MS PTRL Europe GmbH, Ulm, Germany Report No.: P/B 1681 G, GLP: yes unpublished	N	Bayer AG
KCP 5.1.2	Martens R.	1998	Deltamethrin Endosulfan AE F032640 AE F002671. Analytical method and validation for the determination of residues of Endosulfan and Deltamethrin by GC. – 1st Addendum. Report No. C001652 Hoechst Schering AgrEvo GmbH, Frankfurt am Main, Germany GLP, unpublished	N	AgrEvo
KCP 5.1.2	Martens R.	1998	Deltamethrin Endosulfan AE F032640 AE F002671. Analytical method and validation for the determination of residues of Endosulfan and Deltamethrin by GC. Report No. C000413 Hoechst Schering AgrEvo GmbH, Frankfurt am Main, Germany GLP, unpublished	N	AgrEvo
KCP 5.1.2	Akhtar, M. H.	1982	Gas Chromatographic Determination of Deltamethrin in Biological Samples. 15016P Non-GLP Published	N	AgrEvo
KCP 5.1.2	Czarnecki, J. J.,	1990	Validation of the Analytical Methodology for Determination of Combined Residues of Deltamethrin &	N	AgrEvo

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 5.2	McKinney, F. R., Clayton, F. B.		trans-Deltamethrin in Cottonseed & Cottonseed Processed Fractions. 890016 Hoechst-.Roussel, USA and EN-CAS Analytical Laboratories, USA GLP Unpublished		
KCP 5.1.2	Benwell, L.	1992	Deltamethrin: the validation of the analytical method for the determination of residues in field beans and soil GB49410 Hazleton UK, England GLP Unpublished	N	AgrEvo
KCP 5.1.2 KCP 5.2	Baldi, B. G., McKinney, F. R.	1994	Analytical Method for the Gas Chromatographic Determination of cis-Deltamethrin, trans-Deltamethrin and alpha-R-Deltamethrin in Selected Processed Grain Fractions, Grain Dusts and Whole Grain From Corn, Wheat, Sorghum and Rice. ENC692 EN-CAS Analytical Laboratories, USA GLP Unpublished	N	AgrEvo
KCP 5.1.2 KCP 5.2	Martens R.	2000	Validation of analytical method DGM F01/97-1 for foodstuff of animal origin (milk, eggs, meat, fat, liver, kidney Report No. C009558 Aventis CropScience GmbH, Frankfurt am Main, Germany GLP Unpublished	N	Aventis
KCP 5.1.2 KCP 5.2	Martens R.	1999	Enforcement method and validation for water by GC. Deltamethrin Endosulfan. Codes: AE F032640 AE F002671 Report No. C005528 Hoechst Schering AgrEvo GmbH, Frankfurt am Main Germany GLP	N	AgrEvo

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			Unpublished		
KCP 5.2	Martens R.	1998	Validation of analytical method DGM F01/97-0 for residues of Endosulfan and Deltamethrin in cucmber, orange, melon and tomato. Report No. C001152 Hoechst Schering AgrEvo GmbH, Frankfurt am Main, Germany GLP Unpublished	N	AgrEvo
KCP 5.1.2 KCP 5.2	Class T.	2001	Analytical Method for the Determination of Deltamethrin in Surface Water. Report No. B003535 PTRL Europe, ULM, Germany GLP Unpublished	N	Aventis
KCP 5.1.2 KCP 5.2	Tillier, C., Devaux, P.	1981	Quantitative determination of deltamethrin in urine. 812409 Roussel Uclaf, France Not GLP Unpublished	N	AgrEvo
KCP 5.1.2 KCP 5.2	Tillier, C.	1988	Assay procedure for the analysis of deltamethrin residues in human plasma. FR0588 Roussel Uclaf, France Not GLP Unpublished	N	AgrEvo
KCP 5.1.2 KCP 5.2	Class T.	2001	Validation of an Analytical Method for the Determination of Deltamethrin in Air Report No. B003367 PTRL, Europe, Ulm, Germany GLP Unpublished	N	Aventis
KCP 5.2	Haines BK	2001	Independent Laboratory Validation for the Determination of Residiues of Deltamethrin in Lettuce, Oranges, Milk and Fat and Endosulfan in Lettuce and Oranges Using Method DGM F01/97-1	N	Aventis

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Vertebrate study Y/N**	**Owner**
			Report No. B003259 Xenos Laboratories, Inc., Ottawa, Ontario GLP Unpublished		
KCP 5.1.2 KCP 5.2	Weber, H.	2013	Validation of a Multiresidue Method (Fillion) with Modified Cleanup and Detection for the Determination of Acetamiprid in Potato, Eurofins Agroscience Services, Study No. S13-02134, Document ID RD-02603 GLP, not published	N	Nippon Soda
KCP 5.2	Giesau, A. & Weber, H.	2012	Validation of an Analytical Method for the Determination of Residues of Acetamiprid Metabolite IM-1-5 in Water using LC-MS/MS, Eurofins Agroscience Services, Germany, Report No. S12-02719, Document ID RD-02604 GLP, not published	N	Nippon Soda
KCP 5.1.2 KCP 5.2	Schwarz, T.	2008	Acetamiprid: Validation of an Enforcement Method for Plant Materials, PTRL Europe Study P/B1447G Nippon-Soda Report No. RD-01937 GLP, not published	N	Nippon Soda
KCP 5.1.2 KCP 5.2	Miya, K.	2010	Validation Study of the Analytical Method for the Determination of the Residues of Acetamiprid and Its Metabolite (IM-2-1) in Animal Commodities, Nisso Chemical Analysis Service Co., Japan, Report No. NCAS 10-144, Document ID RD-02080 GLP, not published	N	Nippon Soda
KCP 5.2	Knoch, E.	2010	Independent Laboratory Validation: Analytical Method for the Determination of the Residues of	N	Nippon Soda

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			Acetamiprid and its Metabolite (IM-2-1) in Animal Commodities, SGS Institut Fresenius GmbH, Report No. IF-10/01687868, Document ID RD-02156 GLP, not published		
KCP 5.1.2 KCP 5.2	Täufer, A. & Weber, H.	2010	Validation of an Analytical Method for the Determination of Residues of Acetamiprid and Acetamiprid Soil Metabolite IM-1-5 in Calcareous Soil using LC-MS/MS, Eurofins Dr. Specht, Germany, Report No. S09-03287, Document ID RD-02062N GLP, not published	N	Nippon Soda
KCP 5.1.2 KCP 5.2	Miya, K.	2007	Validation Study of the Confirmatory Method for the Determination of Acetamiprid in Water, Nisso Chemical Analysis Service Co., Japan, Report No. NCAS 06-209, Document ID RD-01204 GLP, not published	N	Nippon Soda
KCP 5.2	Senciuc, M.	2014	Independent Laboratory Validation (ILV) of a Residues Analytical Method for the Determination of Acetamiprid Metabolite IM-1-5 in Drinking Water, PTRL Europe GmbH, Germany, Report No. P 3245 G, Document ID RD-02952 GLP, not published	N	Nippon Soda
KCP 5.1.2 KCP 5.2	Beck, I. & Class, T.	2009	Acetamiprid: Development and Validation of an Analytical Method(s) for the Determination of Residues on Operator Exposure Dosimeters from Field Studies, PTRL Europe, Germany, Report No. P/B 1603 G	N	Nippon Soda

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			Document ID RD-01863 GLP, not published		
KCP 5.1.2 KCP 5.2	Senciuc, M.	2014	Development and Validation of an Analytical Method for the Determination of Acetamiprid in Blood, PTRL Europe, Germany, Report No. P3208 G, Document ID RD-02943 GLP, not published	N	Nippon Soda

List of data submitted by the applicant and not relied on

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

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

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

Appendix 2 Detailed evaluation of submitted analytical methods

A 2.1 Analytical methods for Acetamiprid

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)

Comments of zRMS: BA-17/15	The Applicant supplemented the dRR with a study summary but did not provide a study report (document K). The study was not assessed and is not required for this documentation.
Reference:	KCP 5.1.2 KCP 5.2
Report	ACETAMIPRID determination of residues in potatoes, E. Łącka, 2016, BA-17/15
Guideline(s):	SANTE/2020/12830 Rev.2
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The analytical method consisted of extraction of acetamiprid from homogenized potatoes' bulbs with acetone, separation of matrix constituents by extraction with solvatation using QuEChERS mixture (4g MgSO4, 1g NaCl, 1g SCTD, 0.5g SCDS) and dispersive solid phase extraction using QuEChERS reagent (900 mg MgSO4, 150 mg PSA).
Acetamiprid was analysed using GC-ECD technique. The result of the analysis was confirmed by GC-MS technique.

Specimen preparation

a) 15.0 g of homogenized potatoes' bulbs were weighed into a 50 mL test tube
b) 10 mL of acetone was added
c) the tube was shaken for 2 minutes (2000 mot/min)
d) mixture of extracting salts (4g MgSO4, 1g NaCl, 1g SCTD, 0.5g SCDS) was added to the tube
e) the tube was shaken for 5 minutes (2000 mot/min)
f) acetone phase, separated by centrifuging (5000 rpm, -20 °C, 10 min), was transferred to test tube (vol. 15 mL) with second extracting mixture (900 mg MgSO4, 150 mg PSA)
g) the tube was shaken for 5 minutes (2000 mot/min)
h) phases were separated by centrifuging (5000 rpm, -20 °C, 10 min)
i) the acetone layer was transferred to glass tube and then evaporated to dryness in nitrogen stream at 50

°C
j) dry matter was dissolved in 0.5 mL of acetone
k) the extract was placed in an autosampler vial
l) acetamiprid was determined by GC-ECD

Chromatographic parameters
For the determination of acetamiprid Varian CP3800 with electrone capture detector (ECD) was used. The GC-ECD apparatus was equipped with autosampler, thermostated capillary column and split/splitless injector.
Column VF-5MS (30 m × 0.25 mm; 0.25 □m).
Oven temperature:
150 °C (1 min) → 10 °C/min → 220 °C (1 min) → 3 °C/min → 250 °C (1 min) → 20 °C/min → 270 °C (2 min)
Detector temperature: 300 °C
Injector: splitless 0.5 min → split 10:1; temp. 240 °C
Carrier gas helium, flow 1.0 mL/min
Make-up gas nitrogen 30 mL/min

Volume of injection 1 µL

Total time of analysis 23 minutes.
Retention time of acetamiprid is 17.7 ± 0.2 min.

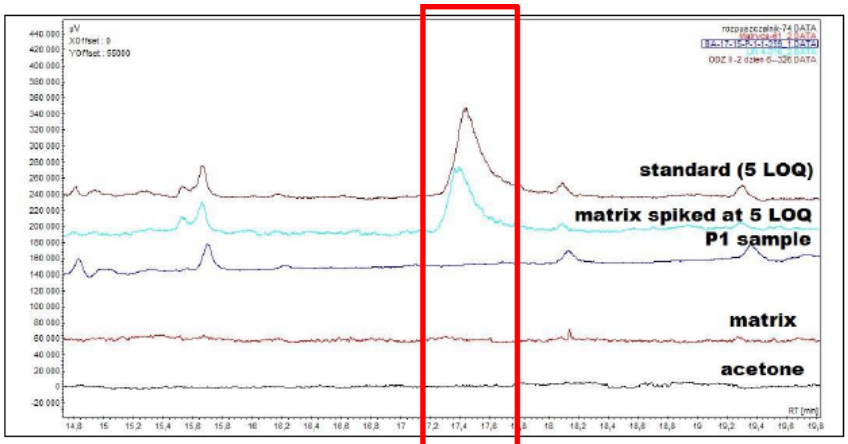
Results and discussions

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

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
Potato (bulbs)	Acetamiprid	0.01	89.8	3.79	-
		0.05	83.4	7.05	-

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

	Residues
Author(s), year	Łącka, E., 2016
Principle of method	GC MS
Linearity (linear between mg/L) (correlation coefficient, expressed as r)	The linearity of detector response was assessed using samples of potatoes fortified with adequate standard solutions. The coefficient of determination (R^2) were determined. R^2 were greater than 0.990. Calibration covers the range from 30% of the LOQ to 20% above the highest level. Calibration curve for Acetamiprid in potato: Y= 27199010X - 216038 R^2= 0.9962
Specificity	In this method specific detection system GC-ECD was employed. Additionally, to prove method specificity, analyses were confirmed by GC-MS. Retention time of examined subtance was confirmed by standard in solvent and in matrix. No interferences were detected in control potatoes at the retention time of acetamiprid for the range of residues > 30% of the LOQ.

	 Fig. 3 Method specificity
Calibration (type, number of data points)	Control samples of potatoes were spiked with acetamiprid working solutions for calibration standard curve in matrix. The fit of the calibration curve, constructed by plotting the acetamiprid content against the detector response, was investigated for standard in matrix. Peak area value of first point in calibration curve is mean from five measurements at LOQ level. The detector response was linear within the range 0.01 – 0.10 mg/kg of acetamiprid in potatoes with R^2 = 0.9962.
Calibration range	0.01 – 0.10 mg/kg
Matrix effect	Matrix effects, expressed in % enhancement or suppression of signal, are considered insignificant as they do not exceed ±20%.
LOQ **LOD**	The limit of quantification was defined by the lowest fortification level successfully tested and was 0.01 mg/kg. The limit of detection (LOD) for acetamiprid was determined as 0.005 mg/kg.
Extraction 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 took place and samples were analyzed within 24 hours of extraction. Then extract stability is not considered to be an issue.
Extraction efficiency	Working standard that were used for quantification were always prepared on the same day as the work up of the specimen for residue analysis took place and samples were analyzed within 24 hours of extraction. Then extract stability is not considered to be an issue.

Conclusion

The results acquired during validation of the analytical method (accuracy and repeatability) are in the range of 70 – 120% and RSD < 20 % for average recovery.
The limit of quantitation of the method is established at 0.01 mg/kg.
There were no interfering signals at retention time of analyzed compound in examined control matrix.
The validated analytical method used for determination of acetamiprid residues in potatoes fulfils criteria of acceptance restricted by SANCO/12571/2013 document.
There were no potatoes' samples where acetamiprid level was above MRL.

The analytical method is considered fully suitable for the analysis of acetamiprid in potato.

Comments of zRMS: GLP-STUDY-18-000081	The method was successfully validated for the determination of residues of acetamiprid in high acid content (oranges), high oil content (sunflower seeds) and dry/high starch content (maize grain). This method meets criteria according SANCO/825/00 rev.8.1. The study is accepted.

Reference:	KCP 5.2.1/01
Report	Validation of an analytical method for the determination of Acetamiprid residues in high acid content, high oil content and dry/high starch content matrices, D. Longhi, 2019, GLP-STUDY-18-000081
Guideline(s):	SANCO/825/00 rev. 8.1
Deviations:	NO
GLP:	YES
Acceptability:	YES

VALIDATION RESULTS SUMMARY

The validated method consisted in an extraction of the analyte from the matrices with acetonitrile under ultrasonic conditions. After extraction, water was added to the extracts, followed by a QuEChERS salts mixture. After centrifugation, an aliquot of the organic supernatant was filtered and injected in a UPLC-MS/MS system for the final determination, setting the instrument in the multi reaction monitoring mode (MRM) on 2 transitions: m/z 223 to m/z 126 (primary quantifier detection 223/126) and m/z 223 to m/z 56 (secondary confirmation detection 223/56). The applied analytical method was validated under GLP compliance according to the SANCO/825/00 rev.8.1 guideline.

LINEARITY

The linearity was evaluated on the same calibration range from 1.041 to 52.05 µg/L (equal to from 4.16 to 208 µg/kg in the original samples) in 5 levels for each matrix, monitoring both the MRM transitions: 223/126 and the confirmatory 223/56. The linearity was checked analysing matrix matched standard solutions. The determination coefficients obtained are hereunder reported:

Matrix	R^2 primary detection	R^2 confirmatory detection
High acid content (oranges)	1.0000	1.0000
High oil content (sunflower seeds)	0.9998	0.9999
Dry/high starch content (maize grain)	0.9998	1.0000

ACCURACY AND PRECISION

The results of accuracy and precision were found in accordance with the SANCO/825/00 rev.8.1 requirements, obtaining recoveries values in the range of 70-110% with a RSD% < 20% for each spiking level.

Table A1: Recovery results from method validation of acetamiprid using the analytical method

Matrix	Fortification Level (mg/kg)	Accuracy and precision per level		Overall accuracy and precision	
		Mean Recovery (%) n=5	RSD (%) n=5	Mean Recovery (%) n=10	RSD (%) n=10
High acid content (Oranges)	0.01 (LOQ)	92.5	2.0	94.8	4.1
	0.1 (10 x LOQ)	97.0	4.4		
High oil content (Sunflower seeds)	0.01 (LOQ)	95.8	2.1	93.8	2.7
	0.1 (10 x LOQ)	91.7	0.5		
Dry/high starch content (maize grain)	0.01 (LOQ)	102.9	0.8	102.1	1.3
	0.1 (10 x LOQ)	101.3	1.2		

SELECTIVITY

The method was found to be selective for the determination of the analyte Acetamiprid in the selected matrices for both the monitored transitions, that gave very similar results. No interfering signals were detected in the untreated matrix in amounts higher than 30% of the LOQ level, that is in compliance with the guideline requirements.

LIMIT OF DETECTION AND QUANTIFICATION

- Limit of detection: the less concentrated matrix matched standard injected, 0.521 µg/L can be considered the instrumental limit of detection for each matrix. The signal/noise ratio measured at this level for all matrices analysed was higher than 3. This amount corresponds to 0.0021 mg/kg on the samples.
- Limit of quantification: the limit of quantification of this method is 0.0104 mg/kg of Acetamiprid in all matrices analysed, accuracy and precision data at this level resulted in compliance with the mentioned guidelines.

Conclusion

It could be demonstrated that the method fulfils the requirements with regard to linearity, specificity, repeatability, limit of quantification and recoveries and therefore applicable to determine residues in foodstuff of plant origin.

Comments of zRMS: Study No. CH - 031/2019	The method was successfully validated for the determination of residues of acetamiprid in high acid content (oranges), high oil content (sunflower seeds) and dry/high starch content (maize grain). This method meets criteria according SANCO/825/00 rev.8.1 and SANCO/3029/99 rev. 4. The method described below is acceptable as ILV for the primary method "Validation of an analytical method for the determination of Acetamiprid residues in high acid content, high oil content and dry/high starch content matrices", GLP-STUDY-18-000081; Longhi, D. (2019)".

Reference:	KCP 5.2.1/02
Report	Independent Laboratory Validation (ILV) of the analytical method for the determination of Acetamiprid residues in high acid content, high oil content and dry/high starch content matrices. S. Paronuzzi Ticco, 2019, GLP Study No. CH - 031/2019
Guideline(s):	SANCO/825/00 rev. 8.1 and SANCO/3029/99 rev. 4

Deviations: NO

GLP: YES

Acceptability: YES

SUMMARY OF RESULTS

The method described below is acceptable as ILV for the primary method "Validation of an analytical method for the determination of Acetamiprid residues in high acid content, high oil content and dry/high starch content matrices", GLP-STUDY-18-000081; Longhi, D. (2019)". The analytical method adjusted and validated by LabAnalysis s.r.l. was shown to be specific for Acetamiprid residues in high acid content, high oil content and dry/high starch content matrices sample. The analysis was conducted using an HPLC hyphenated with a triple quadrupole mass detector run in multiple reaction monitoring mode. The confirmatory test has been run by following two different transitions and processing the data of both transitions, obtaining acceptable data for linearity, repeatability and recovery for each one for each matrix. The linearity test was performed with working standard solutions from 1.03 ng/mL to 51.50 ng/mL concentrations of Acetamiprid for each matrix, corresponding to an Acetamiprid residue content in each matrix samples from 0.004 mg/kg to 0.200 mg/kg. For all matrices, no significant memory effect was detected in the wash sample injected after the highest working standard solution and the range tested was found to be linear for the two product ions (quantifier at m/z 126.0 and qualifier at m/z 56.0, each correlation coefficient > 0.99). The limit of quantification (L.O.Q.) was the low fortification level at 0.01 mg/kg, as nominal value, for Acetamiprid in each matrix samples, corresponding to a final injected solution of 2.575 ng/mL. The limit of detection (L.O.D.), defined as half of the lowest calibration level, was 0.515 ng/mL, corresponding to 0.002 mg/kg for Acetamiprid in each matrix samples. The interference values obtained in each matrix for both product ions were lower than 30% of the L.O.Q. and comply with the SANCO/825/00 rev. 8.1 guideline. For precision, the SANCO/3029/99 rev. 4 and SANCO/825/00 rev. 8.1 guidelines require a RSD% lower than 20 % for each level; since this criteria was fulfilled, the precision of the analytical method is considered to be acceptable in each matrix for both product ions. For accuracy, the SANCO/3029/99 rev. 4 guideline requires mean recoveries for each level in the range from 70% to 110%, ideally with the mean in the range from 80% to 100%, considering the obtained results, the accuracy of the analytical method is considered to be acceptable in each matrix for both product ions.

Specificity

For each matrix, a comparison of the chromatograms of the wash (acetonitrile), the middle working standard solution (WSS 3 at 10.30 ng/mL), matrix sample and fortified matrix sample at low level (at 0.01 mg/kg) was done in order to verify possible interferences with the Acetamiprid peak. The method was demonstrated to be highly specific for the determination of Acetamiprid residues in each matrix samples by virtue of the HPLC/MS/MS technique. The analysis was conducted using the HPLC triple quadrupole in the MRM mode (two product ions at m/z 126.0 (quantifier) and 56.0 (qualifier) from the same precursor ion at m/z 223.0.

Linearity and System Precision

The linearity test was performed with working standard solutions from 1.03 ng/mL to 51.50 ng/mL concentrations of Acetamiprid for each matrix. Considering the matrices sample preparation with a nominal weight of 5.00 g, described in Appendix A, this range corresponds to an Acetamiprid residue content in each matrix samples from 0.004 mg/kg to 0.200 mg/kg. After the injection of the working standard solutions, from the lowest to the highest concentration, a solvent wash was also injected to verify that no memory peak was detected.

Repeatability (Precision) and Recovery (Accuracy)

Both repeatability and recovery tests were performed using fortified matrices samples. The tests were performed by spiking the matrices sample with the fortification standard solutions (FSS) five times at two fortification levels: - Low level at the L.O.Q. (nominal value is 0.01 mg/kg considering the nominal 5.00 g matrix weight and the fortification solution concentration at 515.00 ng/mL); - High level at 10 x L.O.Q. (nominal value is 0.10 mg/kg considering the nominal 5.00 g matrix weight and the fortification solution concentration at 5150.00 ng/mL) Preparation of the fortification standard solution for spike low (FSSL) Using a volumetric pipette, a 515.00 ng/mL fortification standard solution for spike low (FSSL) was prepared by transferring 0.50 mL from the first diluted standard solution, prepared for the linearity test, into a 10.00 mL volumetric flask and making up to volume with acetonitrile. Preparation of the fortification standard solution for spike high (FSSH) Using a volumetric pipette, a 5150.00 ng/mL fortification standard solution for spike high (FSSH) was prepared by transferring 5.00 mL from the first diluted standard solution, prepared for the linearity test, into a 10.00 mL volumetric flask and making up to volume with acetonitrile. Preparation of the Control solutions Using a technical balance, two nominal aliquots 5.00 g of each matrix (one for Control Low and one for Control High) were weighed into two 50 mL centrifuge test tubes and processed as described in the Analytical Method reported in Appendix A. Preparation of the Low fortification level (at 0.01 mg/kg). Using a technical balance, five nominal aliquots of 5.00 g of each matrix (labelled Spike Low from A to E) were weighed into five 50 mL centrifuge test tubes. Using volumetric pipettes, an aliquot of 0.10 mL of the FSSL was added to each matrix sample. The fortified samples were processed as described in the Analytical Method reported in Appendix A after 2 hours from the spiking, in order to allow the conditioning of the analyte in the matrix. Preparation of the High fortification level (at 0.10 mg/kg). Using a technical balance, five nominal aliquots of 5.00 g of each matrix (labelled Spike High from A to E) were weighed into five 50 mL centrifuge test tubes. Using volumetric pipettes, an aliquot of 0.10 mL of the FSSH was added to each matrix sample. The fortified samples were processed as described in the Analytical Method reported in Appendix A after 2 hours from the spiking, in order to allow the conditioning of the analyte in the matrix.

Table A2: Recovery results from method validation of acetamiprid using the analytical method

Maize Grains matrix	Mean Found	Mean Recovery
Quantifier (m/z 126.0)		
0.01 mg/kg	0.010 mg/kg	104.5%
0.10 mg/kg	0.102 mg/kg	100.3%
Quantifier (m/z 126.0)		
0.01 mg/kg	0.010 mg/kg	93.1%
0.10 mg/kg	0.102 mg/kg	101.2%

Table A3: Recovery results from method validation of acetamiprid using the analytical method

Sunflower seeds matrix	Mean Found	Mean Recovery
Quantifier (m/z 126.0)		
0.01 mg/kg	0.010 mg/kg	94.5%
0.10 mg/kg	0.102 mg/kg	93.7%
Quantifier (m/z 126.0)		
0.01 mg/kg	0.010 mg/kg	89.1%
0.10 mg/kg	0.102 mg/kg	94.5%

Table A4: Recovery results from method validation of acetamiprid using the analytical method

Orange matrix	Mean Found	Mean Recovery
Quantifier (m/z 126.0)		
0.01 mg/kg	0.010 mg/kg	105.8%

0.10 mg/kg	0.102 mg/kg	96.2%
Quantifier (m/z 126.0)		
0.01 mg/kg	0.010 mg/kg	103.9%
0.10 mg/kg	0.102 mg/kg	97.1%

Conclusion

It could be demonstrated that the method fulfils the requirements with regard to linearity, specificity, repeatability, limit of quantification and recoveries and therefore applicable to determine residues in foodstuff of plant origin.

Comments of zRMS: VAL/16/2023	The method was successfully validated for the determination of residues of acetamiprid and acetamiprid-N-desmethyl (IM-2-1) in wheat (grain, straw, plant). This method meets criteria according SANTE/2020/12830 Rev.2 The study is accepted.

Reference:	KCP 5.1.2
Report	Validation of an analytical method for the determination of residues of acetamiprid and acetamiprid-N-desmethyl in wheat (grain, plant, straw), Niewelt-Stasiak, S., VAL/16/2023
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 acetamiprid and acetamiprid-N-desmethyl was validated on wheat (grain, plant, straw).
The method was validated over the concentration range of 0.005-0.05 mg/kg (µg/g) for acetamiprid and 0.005-0.05 mg/kg for N-desmethyl-acetamiprid (IM-2-1). The limit of detection (LOD) that was expressed as the lowest calibration standard was 0.001 mg/kg (grain, plant) / 0.0015 (straw) for acetamiprid, and 0.001 mg/kg (grain, plant) / 0.0015 (straw) for acetamiprid-N-desmethyl (IM-2-1).

Specimen preparation

5 g (grain, plant) / 2g (straw) of the homogenized sample was weighed into a 50 mL centrifuge tube. 10 mL of acetonitrile and 10 mL of deionized water was added together with 50 µL (grain, plant) / 20 µL (straw) 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. Afterwards, 6 mL of the supernatant was transferred to a polypropylene centrifuge tube containing of cleanup mixture (900 mg of anhydrous magnesium sulphate, 150 mg of C18, 150 mg of PSA), next the mixture was shaken again intensively for 0.5 min, then centrifuged at 4700 rpm for 5 min for phase separation.

After that, the extract 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.

Validation - Results and discussions

Table A 3: Recovery results from method validation of Acetamiprid using the analytical method

Matrix	Analyte	Fortification level (mg/kg) ~~(n = x)~~ n=5	Mean recovery (%)	RSD (%)	Comments
Wheat grain	Acetamiprid 223.10→126.00	LOQ (0.005)	91.7	1.57	-
	Acetamiprid 223.10→126.00	10 x LOQ (0.05)	97.0	6.89	-
	Acetamiprid 223.10→56.10	LOQ (0.005)	94.4	3.69	-
	Acetamiprid 223.10→56.10	10 x LOQ (0.05)	98.0	5.14	-
	Acetamiprid-N-Desmethyl 210.90→128.10	LOQ (0.005)	87.2	2.92	-
	Acetamiprid-N-Desmethyl 210.90→128.10	10 x LOQ (0.05)	89.5	3.58	-
	Acetamiprid-N-Desmethyl 208.80→73.10	LOQ (0.005)	83.3	1.66	-
	Acetamiprid-N-Desmethyl 208.80→73.10	10 x LOQ (0.05)	88.9	2.72	-
Wheat straw	Acetamiprid 223.10→126.00	LOQ (0.005)	87.9	1.94	-
	Acetamiprid 223.10→126.00	10 x LOQ (0.05)	93.1	1.38	-
	Acetamiprid 223.10→56.10	LOQ (0.005)	84.6	3.22	-
	Acetamiprid 223.10→56.10	10 x LOQ (0.05)	91.2	1.08	-
	Acetamiprid-N-Desmethyl 210.90→128.10	LOQ (0.005)	88.4	6.45	-
	Acetamiprid-N-Desmethyl 210.90→128.10	10 x LOQ (0.05)	85.8	1.31	-
	Acetamiprid-N-Desmethyl	LOQ (0.005)	97.5	8.03	-

Matrix	Analyte	Fortification level (mg/kg) ~~(n = x)~~ n=5	Mean recovery (%)	RSD (%)	Comments
	208.80→73.10				
	Acetamiprid-N-Desmethyl 208.80→73.10	10 x LOQ (0.05)	85.2	0.67	-
Wheat plant	Acetamiprid 223.10→126.00	LOQ (0.005)	104.4	3.01	-
	Acetamiprid 223.10→126.00	10 x LOQ (0.05)	99.7	2.78	-
	Acetamiprid 223.10→56.10	LOQ (0.005)	102.8	6.14	-
	Acetamiprid 223.10→56.10	10 x LOQ (0.05)	100.8	1.76	-
	Acetamiprid-N-Desmethyl 210.90→128.10	LOQ (0.005)	85.9	2.12	-
	Acetamiprid-N-Desmethyl 210.90→128.10	10 x LOQ (0.05)	92.9	1.61	-
	Acetamiprid-N-Desmethyl 208.80→73.10	LOQ (0.005)	85.9	4.07	-
	Acetamiprid-N-Desmethyl 208.80→73.10	10 x LOQ (0.05)	92.9	1.76	-

Table A 2: Methods suitable for the determination of the residues in plant protection product (PPP) CHR/I/ACE 200 SE

	Residues
Author(s), year	Niewelt-Stasiak, S., 2023
Principle of method	LC MS/MS
Linearity (linear between mg/L) (correlation coefficient, expressed as r)	The linearity of the detector response was demonstrated by single determination of calibration standards at six concentration levels ranging from 0.5 to 500 ppb for acetamiprid and acetamiprid-N-desmethyl in wheat (grain, plant), and from 0.3 to 500 ppb for acetamiprid and acetamiprid-N-desmethyl in wheat (straw).The coefficient of determination (R^2) were determined. R^2 were greater than 0.990. Calibration covers the range from 30% of the LOQ to 20% above the highest level. Calibration Acetamiprid in wheat grain (223.10→126.00): Y= 0.461403X – 0.0001761132 R^2= 0.9990890 Calibration Acetamiprid in wheat grain (223.10→56.10): Y= 0.279891X + 9.31891e-005 R^2= 0.9994837 Calibration Acetamiprid-N-desmethyl in wheat grain (210.90→128.10): Y= 0.2722721X – 0.000187968

	Residues
	R^2= 0.99955647 Calibration Acetamiprid-N-desmethyl in wheat grain (210.90→73.10): Y= 0.180107X + 0.000174131 R^2= 0.9997703 Calibration Acetamiprid in wheat straw (223.10→126.00): Y= 0.470777X – 0.000241215 R^2= 0.9991639 Calibration Acetamiprid in wheat straw (223.10→56.10): Y= 0.287882X + 2.15234e-0.05 R^2= 0.9992715 Calibration Acetamiprid-N-desmethyl in straw (210.90→128.10): Y= -0.274313X + 0.000583573 R^2= 0.9972415 Calibration Acetamiprid-N-desmethyl in straw (210.90→73.10): Y= 0.178843X + 0.000441317 R^2= 0.9975958 Calibration Acetamiprid in whole plant (223.10→126.00): Y= 0.477418X + 0.000530854 R^2= 0.9998917 Calibration Acetamiprid in wheat straw (223.10→56.10): Y= 0.9998583X + 0.000278547 R^2= 0.9998583 Calibration Acetamiprid-N-desmethyl in straw (210.90→128.10): Y= 0.242316X – 0.000193835 R^2= 0.9997150 Calibration Acetamiprid-N-desmethyl in straw (210.90→73.10): Y= 0.155349X – 0.000355415 R^2= 0.9994348
Precision, accuracy and uncertainty	Recovery data was generated from five samples fortified at the limit of quantification (LOQ) and five samples fortified at the 10-fold higher concentration than the LOQ (10 x LOQ). Precision of the method was determined as the relative standard deviation (RSD) of recovery at each fortification level. The mean recovery at fortification level of 0.01 mg/kg (LOQ) should be in the range of 60 – 120% with RSD ≤30 %, and recovery at fortification level of 0.10 mg/kg (10xLOQ) should be in the range of 70 – 120% with RSD ≤ 20 %. RSD were determined only during validation process. Grains:

Residues

Acetamiprid

Transition: 223.10→126.00

Fortification level [mg/kg]	Obtained result [mg/kg]	Recovery [%]	Fortification level [mg/kg]	Obtained result [mg/kg]	Recovery [%]
0.005	0.0046	91.8	0.05	0.047	94.6
	0.0045	90.2		0.045	89.2
	0.0047	93.4		0.047	94.1
	0.0046	92.8		0.050	100.8
	0.0045	90.2		0.053	106.4
Average	0.0046	91.7	Average	0.049	97.0
SD	0.000072	1.44	SD	0.0033	6.68
RSD [%]	1.57		**RSD [%]**	6.89	
Uncertainty [%]	16.9		**Uncertainty [%]**	15.0	

Transition: 223.10→ 56.10

Fortification level [mg/kg]	Obtained result [mg/kg]	Recovery [%]	Fortification level [mg/kg]	Obtained result [mg/kg]	Recovery [%]
0.005	0.0046	91.0	0.05	0.048	96.3
	0.0050	99.5		0.046	92.1
	0.0047	94.2		0.048	95.1
	0.0046	91.4		0.051	102.5
	0.0048	95.9		0.052	103.9
Average	0.0047	94.4	Average	0.049	98.0
SD	0.00017	3.49	SD	0.0025	5.04
RSD [%]	3.69		**RSD [%]**	5.14	
Uncertainty [%]	13.4		**Uncertainty [%]**	11.0	

Residues

Acetamiprid-N-desmethyl

Transition: 210.90→128.10

Fortification level [mg/kg]	Obtained result [mg/kg]	Recovery [%]	Fortification level [mg/kg]	Obtained result [mg/kg]	Recovery [%]
0.005	0.0042	83.4	0.05	0.045	90.2
	0.0044	87.5		0.043	85.7
	0.0045	89.4		0.043	86.8
	0.0043	86.0		0.046	91.6
	0.0045	89.5		0.047	93.3
Average	0.0044	87.2	Average	0.045	89.5
SD	0.00013	2.54	SD	0.0016	3.20
RSD [%]	2.92		**RSD [%]**	3.58	
Uncertainty [%]	26.3		**Uncertainty [%]**	22.1	

Transition: 208.80→73.10

Fortification level [mg/kg]	Obtained result [mg/kg]	Recovery [%]	Fortification level [mg/kg]	Obtained result [mg/kg]	Recovery [%]
0.005	0.0041	82.9	0.05	0.045	89.5
	0.0041	81.8		0.043	85.9
	0.0041	82.1		0.043	86.8
	0.0042	84.6		0.045	90.4
	0.0042	84.8		0.046	91.7
Average	0.0042	83.3	Average	0.044	88.9
SD	0.000069	1.38	SD	0.0012	2.41
RSD [%]	1.66		**RSD [%]**	2.72	
Uncertainty [%]	33.7		**Uncertainty [%]**	22.9	

	Residues

Straw:

Acetamiprid

Transition: 223.10→126.00

Fortification level [mg/kg]	Obtained result [mg/kg]	Recovery [%]	Fortification level [mg/kg]	Obtained result [mg/kg]	Recovery [%]
0.005	0.0043	85.9	0.05	0.046	92.3
	0.0044	88.4		0.046	91.9
	0.0045	89.2		0.046	92.5
	0.0045	89.8		0.047	94.2
	0.0043	86.5		0.047	94.8
Average	0.0044	87.9	Average	0.047	93.1
SD	0.000085	1.71	SD	0.00064	1.28
RSD [%]	1.94		**RSD [%]**	1.38	
Uncertainty [%]	24.4		**Uncertainty [%]**	14.0	

Transition: 223.10→ 56.10

Fortification level [mg/kg]	Obtained result [mg/kg]	Recovery [%]	Fortification level [mg/kg]	Obtained result [mg/kg]	Recovery [%]
0.005	0.0042	84.1	0.05	0.045	90.7
	0.0043	86.5		0.045	90.3
	0.0043	85.8		0.045	90.7
	0.0043	86.4		0.046	91.4
	0.0040	80.0		0.046	92.8
Average	0.0042	84.6	Average	0.046	91.2
SD	0.00014	2.73	SD	0.00049	0.99
RSD [%]	3.22		**RSD [%]**	1.08	
Uncertainty [%]	31.5		**Uncertainty [%]**	17.8	

Residues

Acetamiprid-N-desmethyl

Transition: 210.90→128.10

Fortification level [mg/kg]	Obtained result [mg/kg]	Recovery [%]	Fortification level [mg/kg]	Obtained result [mg/kg]	Recovery [%]
0.005	0.0039	78.3	0.05	0.043	85.2
	0.0045	90.9		0.043	85.1
	0.0045	89.3		0.043	86.4
	0.0046	91.4		0.044	87.5
	0.0046	92.0		0.042	84.9
Average	0.0044	88.4	Average	0.043	85.8
SD	0.00029	5.70	SD	0.00056	1.13
RSD [%]	6.45		**RSD [%]**	1.31	
Uncertainty [%]	26.6		**Uncertainty [%]**	28.5	

Transition: 208.80→73.10

Fortification level [mg/kg]	Obtained result [mg/kg]	Recovery [%]	Fortification level [mg/kg]	Obtained result [mg/kg]	Recovery [%]
0.005	0.0045	89.1	0.05	0.042	84.9
	0.0054	107.9		0.043	85.4
	0.0052	103.4		0.043	85.1
	0.0047	94.1		0.042	84.7
	0.0046	92.9		0.043	86.1
Average	0.0049	97.5	Average	0.043	85.2
SD	0.00039	7.83	SD	0.00029	0.57
RSD [%]	8.03		**RSD [%]**	0.67	
Uncertainty [%]	16.8		**Uncertainty [%]**	29.5	

Residues

Whole plant:

Acetamiprid

Transition: 223.10→126.00

Fortification level [mg/kg]	Obtained result [mg/kg]	Recovery [%]	Fortification level [mg/kg]	Obtained result [mg/kg]	Recovery [%]
0.005	0.0053	106.9	0.05	0.051	101.9
	0.0050	100.5		0.051	102.6
	0.0051	102.0		0.048	95.5
	0.0054	107.9		0.050	99.5
	0.0052	104.5		0.050	99.1
Average	0.0052	104.4	Average	0.050	99.7
SD	0.00016	3.14	SD	0.0014	2.78
RSD [%]	3.01		**RSD [%]**	2.78	
Uncertainty [%]	10.6		**Uncertainty [%]**	5.6	

Transition: 223.10→ 56.10

Fortification level [mg/kg]	Obtained result [mg/kg]	Recovery [%]	Fortification level [mg/kg]	Obtained result [mg/kg]	Recovery [%]
0.005	0.0055	110.0	0.05	0.051	102.1
	0.0047	94.6		0.052	103.2
	0.0049	99.0		0.049	99.0
	0.0051	102.7		0.050	100.3
	0.0054	107.8		0.050	99.5
Average	0.0051	102.8	Average	0.050	100.8
SD	0.00032	6.31	SD	0.00089	1.78
RSD [%]	6.14		**RSD [%]**	1.76	
Uncertainty [%]	13.5		**Uncertainty [%]**	3.9	

	Residues

Acetamiprid-N-desmethyl

Transition: 210.90→128.10

Fortification level [mg/kg]	Obtained result [mg/kg]	Recovery [%]	Fortification level [mg/kg]	Obtained result [mg/kg]	Recovery [%]
0.005	0.0053	106.9	0.05	0.049	97.1
	0.0052	105.0		0.049	97.5
	0.0052	103.5		0.047	94.0
	0.0054	109.0		0.048	96.3
	0.0052	104.0		0.047	94.7
Average	0.0053	105.7	Average	0.048	95.9
SD	0.00011	2.24	SD	0.00077	1.55
RSD [%]	2.12		**RSD [%]**	1.61	
Uncertainty [%]	12.1		**Uncertainty [%]**	8.8	

Transition: 208.80→73.10

Fortification level [mg/kg]	Obtained result [mg/kg]	Recovery [%]	Fortification level [mg/kg]	Obtained result [mg/kg]	Recovery [%]
0.005	0.0043	86.6	0.05	0.047	93.0
	0.0040	81.0		0.048	95.1
	0.0042	84.8		0.045	90.5
	0.0045	90.7		0.046	92.7
	0.0043	86.3		0.047	93.1
Average	0.0043	85.9	Average	0.046	92.9
SD	0.00017	3.50	SD	0.00082	1.64
RSD [%]	4.07		**RSD [%]**	1.76	
Uncertainty [%]	29.4		**Uncertainty [%]**	14.6	

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. No interferences at above 30% of the LOQ were detected at the retention time of active substance in matrix blank samples
Matrix Effects	For acetamiprid and acetamiprid-N-desmethyl matrix effects in wheat grain calculated using equation are <±20%, in wheat straw and plant matrix effect calculated using equation exceed ±20%. To compensate matrix effect, there was used matrix-matched calibrations.
LOQ **LOD**	Limit of quantification (LOQ) - 0.005 mg/kg Limit of detection (LOD) - 0.001 mg/kg
Extraction 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 took place and samples were analyzed within 24 hours of extraction. Then extract stability is not considered to be an issue.
Comment	The analytical method for determining the residues of acetamiprid and

	Residues
	acetamiprid-N-desmethyl in wheat (grain, straw, plant) meets the criteria of SANTE/2020/12830 Rev.2, 14. February 2023 documents in terms of precision, accuracy and uncertainty. The method was validated over the concentration range of 0.005-0.05 mg/kg (µg/g) for acetamiprid and 0.005-0.05 mg/kg for N-desmethyl-acetamiprid (IM-2-1). Limit of detection was established at 0.001 mg/kg for wheat (grain, plant) and at 0.0015 for wheat (straw).

Conclusion

The analytical method for determining the residues of acetamiprid and acetamiprid-N-desmethyl in wheat (grain, straw, plant) meets the criteria of SANTE/2020/12830 Rev.2, 14. February 2023 documents in terms of precision, accuracy and uncertainty.
The method was validated over the concentration range of 0.005-0.05 mg/kg (µg/g) for acetamiprid and 0.005-0.05 mg/kg for N-desmethyl-acetamiprid (IM-2-1). Limit of detection was established at 0.001 mg/kg for wheat (grain, plant) and at 0.0015 for wheat (straw).

Comments of zRMS: VAL/17/2023	The method was successfully validated for the determination of residues of acetamiprid and acetamiprid-N-desmethyl (IM-2-1) in sugar beet (leaves, roots). This method meets criteria according SANTE/2020/12830 Rev.2 The study is accepted.

Reference:	KCP 5.1.2
Report	Validation of an analytical method for the determination of residues of acetamiprid and acetamiprid-N-desmethyl in sugar beet (leaves, roots), Niewelt-Niewelt-Stasiak, S., VAL/17/2023
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 acetamiprid and acetamiprid-N-desmethyl was validated on wheat (grain, plant, straw).
The method was validated over the concentration range of 0.005-0.05 mg/kg (µg/g) for acetamiprid and 0.005-0.05 mg/kg for N-desmethyl-acetamiprid (IM-2-1). The limit of detection (LOD) that was expressed as the lowest calibration standard was 0.001 mg/kg (grain, plant) / 0.0015 (straw) for acetamiprid, and 0.001 mg/kg (grain, plant) / 0.0015 (straw) for acetamiprid-N-desmethyl (IM-2-1).

Specimen preparation

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. Afterwards, 6 mL of the supernatant was transferred to a polypropylene centrifuge tube containing of cleanup mixture (900 mg of anhydrous magnesium sulphate, 150 mg of C18, 150 mg of PSA), next the mixture was shaken again intensively for 0.5 min, then centrifuged at 4700 rpm for 5 min for phase separation. After that, the extract 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.

Validation - Results and discussions

Table A 4: Recovery results from method validation of Acetamiprid using the analytical method

Matrix	Analyte	Fortification level (mg/kg) ~~(n = x)~~ n=5	Mean recovery (%)	RSD (%)	Comments
Beet root	Acetamiprid 223.10→126.00	LOQ (0.005)	98.5	6.68	-
	Acetamiprid 223.10→126.00	10 x LOQ (0.05)	113.5	5.50	-
	Acetamiprid 223.10→56.10	LOQ (0.005)	93.6	7.36	-
	Acetamiprid 223.10→56.10	10 x LOQ (0.05)	114.4	5.79	-
	Acetamiprid-N-Desmethyl 210.90→128.10	LOQ (0.005)	91.2	6.89	-
	Acetamiprid-N-Desmethyl 210.90→128.10	10 x LOQ (0.05)	102.1	5.52	-
	Acetamiprid-N-Desmethyl 208.80→73.10	LOQ (0.005)	81.3	6.96	-
	Acetamiprid-N-Desmethyl 208.80→73.10	10 x LOQ (0.05)	105.2	6.52	-
Beet leaves	Acetamiprid 223.10→126.00	LOQ (0.005)	106.3	4.72	-
	Acetamiprid 223.10→126.00	10 x LOQ (0.05)	98.9	1.12	-
	Acetamiprid 223.10→56.10	LOQ (0.005)	100.2	4.78	-
	Acetamiprid 223.10→56.10	10 x LOQ (0.05)	99.0	0.35	-
	Acetamiprid-N-Desmethyl 210.90→128.10	LOQ (0.005)	110.5	4.84	-

Matrix	Analyte	Fortification level (mg/kg) ~~(n = x)~~ n=5	Mean recovery (%)	RSD (%)	Comments
	Acetamiprid-N-Desmethyl 210.90→128.10	10 x LOQ (0.05)	94.7	2.95	-
	Acetamiprid-N-Desmethyl 208.80→73.10	LOQ (0.005)	99.8	2.91	-
	Acetamiprid-N-Desmethyl 208.80→73.10	10 x LOQ (0.05)	95.3	1.54	-

Table A 2: Methods suitable for the determination of the residues in plant protection product (PPP) CHR/I/ACE 200 SE

	Residues
Author(s), year	Niewelt-Stasiak, S., 2023
Principle of method	LC MS/MS
Linearity (linear between mg/L) (correlation coefficient, expressed as r)	The linearity of the detector response was demonstrated by single determination of calibration standards at six concentration levels ranging from 0.5 to 500 ppb for acetamiprid and acetamiprid-N-desmethyl in wheat (grain, plant), and from 0.3 to 500 ppb for acetamiprid and acetamiprid-N-desmethyl in wheat (straw).The coefficient of determination (R^2) were determined. R^2 were greater than 0.990. Calibration covers the range from 30% of the LOQ to 20% above the highest level. Calibration Acetamiprid in sugar beet roots (223.10→126.00): Y= 0.510586X + 0.000260807 R^2= 0.9997827 Calibration Acetamiprid in sugar beet roots (223.10→56.10): Y= 0.300388X + 0.00134989 R^2= 0.9999442 Calibration Acetamiprid-N-desmethyl in sugar beet roots (210.90→128.10): Y= 0.320534X + 0.000344395 R^2= 0.9994719 Calibration Acetamiprid-N-desmethyl in sugar beet roots (210.90→73.10): Y= 0.205916X + 0.00123548 R^2= 0.9997703 Calibration Acetamiprid in sugar beet leaves (223.10→126.00): Y= 0.443940X + 0.000133867 R^2= 0.9996646 Calibration Acetamiprid in sugar beet leaves (223.10→56.10): Y= 0.280775X + 0.00109521 R^2= 0.9993465 Calibration Acetamiprid-N-desmethyl in sugar beet leaves (210.90→128.10): Y= 0.257287X – 0.000931123 R^2= 0.9978962 Calibration Acetamiprid-N-desmethyl in sugar beet leaves (210.90→73.10): Y= 0.165735X + 0.000414614 R^2= 0.9966578
Precision, accuracy and	Recovery data was generated from five samples fortified at the limit of

	Residues
uncertainty	quantification (LOQ) and five samples fortified at the 10-fold higher concentration than the LOQ (10 x LOQ). Precision of the method was determined as the relative standard deviation (RSD) of recovery at each fortification level. The mean recovery at fortification level of 0.01 mg/kg (LOQ) should be in the range of 60 – 120% with RSD ≤30 %, and recovery at fortification level of 0.10 mg/kg (10xLOQ) should be in the range of 70 – 120% with RSD ≤ 20 %. RSD were determined only during validation process. Roots:

Acetamiprid

Transition: 223.10→126.00

Fortification level [mg/kg]	Obtained result [mg/kg]	Recovery [%]	Fortification level [mg/kg]	Obtained result [mg/kg]	Recovery [%]
0.005	0.0048	96.3	0.05	0.051	102.8
	0.0054	107.2		0.059	118.4
	0.0052	103.6		0.059	117.6
	0.0046	92.0		0.057	113.8
	0.0047	93.6		0.057	114.9
Average	0.0049	98.5	Average	0.057	113.5
SD	0.00033	6.58	SD	0.0031	6.24
RSD [%]	6.68		**RSD [%]**	5.50	
Uncertainty [%]	13.7		**Uncertainty [%]**	29.1	

Transition: 223.10→ 56.10

Fortification level [mg/kg]	Obtained result [mg/kg]	Recovery [%]	Fortification level [mg/kg]	Obtained result [mg/kg]	Recovery [%]
0.005	0.0047	94.2	0.05	0.051	103.0
	0.0051	101.7		0.060	119.1
	0.0049	98.7		0.059	118.4
	0.0043	85.8		0.057	114.5
	0.0044	87.4		0.059	117.1
Average	0.0047	93.6	Average	0.057	114.4
SD	0.00034	6.89	SD	0.0033	6.63
RSD [%]	7.36		**RSD [%]**	5.79	
Uncertainty [%]	19.6		**Uncertainty [%]**	31.1	

Residues

Acetamiprid-N-desmethyl

Transition: 210.90→128.10

Fortification level [mg/kg]	Obtained result [mg/kg]	Recovery [%]	Fortification level [mg/kg]	Obtained result [mg/kg]	Recovery [%]
0.005	0.0045	89.3	0.05	0.046	92.7
	0.0050	99.3		0.053	106.9
	0.0048	96.3		0.053	106.0
	0.0043	85.8		0.051	102.3
	0.0043	85.4		0.051	102.8
Average	0.0046	91.2	Average	0.051	102.1
SD	0.00031	6.29	SD	0.0028	5.64
RSD [%]	6.89		**RSD [%]**	5.52	
Uncertainty [%]	22.3		**Uncertainty [%]**	11.9	

Transition: 208.80→73.10

Fortification level [mg/kg]	Obtained result [mg/kg]	Recovery [%]	Fortification level [mg/kg]	Obtained result [mg/kg]	Recovery [%]
0.005	0.0041	81.1	0.05	0.047	93.2
	0.0045	89.5		0.055	110.0
	0.0042	83.5		0.055	109.2
	0.0039	77.1		0.053	106.3
	0.0038	75.1		0.054	107.5
Average	0.0041	81.3	Average	0.053	105.2
SD	0.00028	5.66	SD	0.0034	6.86
RSD [%]	6.96		**RSD [%]**	6.52	
Uncertainty [%]	40.0		**Uncertainty [%]**	16.7	

Leaves:

Residues

Acetamiprid

Transition: 223.10→126.00

Fortification level [mg/kg]	Obtained result [mg/kg]	Recovery [%]	Fortification level [mg/kg]	Obtained result [mg/kg]	Recovery [%]
0.005	0.0049	98.1	0.05	0.050	99.6
	0.0053	106.1		0.049	98.7
	0.0053	106.6		0.049	97.4
	0.0055	110.0		0.049	98.6
	0.0055	110.7		0.050	100.4
Average	0.0053	106.3	Average	0.049	98.9
SD	0.00025	5.02	SD	0.00056	1.11
RSD [%]	4.72		**RSD [%]**	1.12	
Uncertainty [%]	15.8		**Uncertainty [%]**	3.1	

Transition: 223.10→ 56.10

Fortification level [mg/kg]	Obtained result [mg/kg]	Recovery [%]	Fortification level [mg/kg]	Obtained result [mg/kg]	Recovery [%]
0.005	0.0047	93.5	0.05	0.050	99.4
	0.0049	98.6		0.049	98.8
	0.0050	99.4		0.049	99.0
	0.0053	106.0		0.049	98.5
	0.0052	103.3		0.050	99.2
Average	0.0050	100.2	Average	0.049	99.0
SD	0.00024	4.78	SD	0.00017	0.35
RSD [%]	4.78		**RSD [%]**	0.35	
Uncertainty [%]	9.6		**Uncertainty [%]**	2.1	

	Residues
	Acetamiprid-N-desmethyl *Transition: 210.90→128.10* **Transition: 208.80→73.10**

Transition: 210.90→128.10

Fortification level [mg/kg]	Obtained result [mg/kg]	Recovery [%]	Fortification level [mg/kg]	Obtained result [mg/kg]	Recovery [%]
0.005	0.0051	102.0	0.05	0.046	92.2
	0.0057	113.5		0.046	92.5
	0.0055	109.7		0.047	93.4
	0.0058	116.1		0.049	97.7
	0.0056	111.0		0.049	97.9
Average	0.0055	110.5	Average	0.047	94.7
SD	0.00027	5.34	SD	0.0014	2.80
RSD [%]	4.84		**RSD [%]**	2.95	
Uncertainty [%]	23.0		**Uncertainty [%]**	12.1	

Transition: 208.80→73.10

Fortification level [mg/kg]	Obtained result [mg/kg]	Recovery [%]	Fortification level [mg/kg]	Obtained result [mg/kg]	Recovery [%]
0.005	0.0048	95.1	0.05	0.046	92.9
	0.0049	98.9		0.048	95.0
	0.0051	102.2		0.048	95.7
	0.0051	101.6		0.048	96.4
	0.0051	101.1		0.048	96.5
Average	0.0050	99.8	Average	0.048	95.3
SD	0.00015	2.90	SD	0.00074	1.47
RSD [%]	2.91		**RSD [%]**	1.54	
Uncertainty [%]	5.8		**Uncertainty [%]**	9.9	

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. No interferences at above 30% of the LOQ were detected at the retention time of active substance in matrix blank samples
Matrix Effects	For acetamiprid and acetamiprid-N-desmethyl in sugar beet roots matrix effects calculated using equation are <±20%, but for acetamiprid and acetamiprid-N-desmethyl in leaves matrix effects exceeds value ±20%.
LOQ **LOD**	Limit of quantification (LOQ) - 0.005 mg/kg Limit of detection (LOD) - 0.001 mg/kg
Extraction 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 took place and samples were analyzed within 24 hours of extraction. Then extract stability is not considered to be an issue.
Comment	The analytical method for determining the residues of acetamiprid and acetamiprid-N-desmethyl in sugar beet (roots and leaves) meets the criteria of SANTE/2020/12830 Rev. 2, 14 February 2023, documents in terms of precision, accuracy and uncertainty. The method was validated over the concentration range of 0.005 - 0.05 mg/kg (µg/g) for acetamiprid and 0.005 - 0.05 mg/kg for N-desmethyl-acetamiprid (IM-2-1). Limit of detection was established at 0.001 mg/kg

Conclusion

The analytical method for determining the residues of acetamiprid and acetamiprid-N-desmethyl in sugar beet (roots and leaves) meets the criteria of SANTE/2020/12830 Rev. 2, 14 February 2023, documents in terms of precision, accuracy and uncertainty.
The method was validated over the concentration range of 0.005 - 0.05 mg/kg (µg/g) for acetamiprid and 0.005 - 0.05 mg/kg for N-desmethyl-acetamiprid (IM-2-1). Limit of detection was established at 0.001 mg/kg

A 2.1.2.1.1 Analytical method 1

A 2.1.2.1.1.1 Method validation

No new or additional studies have been submitted.

A 2.1.2.1.1.2 Independent laboratory validation

No new or additional studies have been submitted.

A 2.1.2.1.1.3 Confirmatory method (if required)

No confirmatory method is required

A 2.1.2.1.2 Analytical method 2

No new or additional studies have been submitted.

A 2.1.2.1.3 Extraction efficiency

No new or additional studies have been submitted.

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

A 2.1.2.2.1 Analytical method 1

A 2.1.2.2.1.1 Method validation

Comments of zRMS: DNA4036	The method was successfully validated for the determination of residues of acetamiprid and acetamiprid-N-desmethyl (IM-2-1) in animal commodities (liver, muscle, fat, milk and eggs). This method meets criteria according SANCO/825/00 rev. 8.1 and SANCO/3029/99 rev. 4. The study is accepted.

Reference:	KCP 5.2.1/03
Report	Validation of the Methods of Analysis used for the Determination of acetamiprid and a specified metabolite in animal commodities, in Compliance with good laboratory practice, and referencing SANCO/3029/99, D. Norris 2017 (addendum II 2019), DNA4036
Guideline(s):	SANCO/825/00 rev. 8.1 and SANCO/3029/99 rev. 4
Deviations:	NO
GLP:	YES
Acceptability:	YES

Materials and methods

Approximately 10g of homogenised samples were weight into a 50mL centrifuge tube. 10mL acetonitrile was added and the samples shaken for 1 minute. Aliquots of sodium chloride, sodium citrate, sodium hydrogen citrate sesquihydrate and magnesium sulfate were added and the samples were shaken for 1 minute and centrifuged at 3000rpm for 5 minutes. The supernatant was transferred to a dispersive SPE tube containing PSA, C18 and magnesium sulfate and shaken for 1 minute and centrifuged at 3000rpm for 5 minutes. The supernatant was diluted with 30% Methanol and syringe filtered. The samples were analysed by HPLC-MS/MS in positive polarity mode using a Phenomenex Luna C18 column, 150 mm x 2.0 mm, 3 µm and a gradient elution with mobile phase of 0.1% Formic Acid in Methanol and 0.1% Formic Acid in Deionised Water. The Acetamiprid molecular ion used was 223m/z and the fragment ions used were 90m/z, 99m/z and 126m/z.

Results and discussions

In all matrices tested, the mean recovery values were between 70% and 110%. The relative standard deviations (RSD) for all fortification levels were below 20%. The detailed results are given in the table below.

Table A 5: Recovery results from method validation of acetamiprid and IM-2-1 using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (n = 6)	Mean recovery (%)	RSD (%)
Liver	Acetamiprid	10 mg/kg	93.06	1.504
		1.0 mg/kg	92.04	2.146
		0.1 mg/kg	89.93	3.063
		0.01 mg/kg	94.47	2.320
	IM-2-1	10 mg/kg	88.55	1.401
		1.0 mg/kg	86.65	2.609
		0.1 mg/kg	86.67	2.453
		0.01 mg/kg	87.16	1.660
Muscle	Acetamiprid	10 mg/kg	91.18	1.812
		1.0 mg/kg	89.57	2.801
		0.1 mg/kg	90.09	1.321

Matrix	Analyte	Fortification level (mg/kg) (n = 6)	Mean recovery (%)	RSD (%)
		0.01 mg/kg	105.2	5.565
	IM-2-1	10 mg/kg	87.80	0.696
		1.0 mg/kg	85.01	2.618
		0.1 mg/kg	84.45	1.807
		0.01 mg/kg	89.59	7.848
Fat	Acetamiprid	10 mg/kg	92.24	2.182
		1.0 mg/kg	90.25	2.213
		0.1 mg/kg	91.07	2.034
		0.01 mg/kg	96.49	2.328
	IM-2-1	10 mg/kg	87.18	1.963
		1.0 mg/kg	86.24	2.769
		0.1 mg/kg	86.13	1.567
		0.01 mg/kg	86.24	0.781
Milk	Acetamiprid	10 mg/kg	93.54	1.328
		1.0 mg/kg	88.78	1.030
		0.1 mg/kg	89.81	1.454
		0.01 mg/kg	87.81	1.137
	IM-2-1	10 mg/kg	82.38	1.521
		1.0 mg/kg	80.20	1.259
		0.1 mg/kg	80.31	1.542
		0.01 mg/kg	80.68	1.321
Eggs	Acetamiprid	10 mg/kg	99.20	0.938
		1.0 mg/kg	97.26	2.420
		0.1 mg/kg	98.42	1.598
		0.01 mg/kg	100.4	12.28
	IM-2-1	10 mg/kg	87.01	1.750
		1.0 mg/kg	82.03	0.756
		0.1 mg/kg	80.73	2.668
		0.01 mg/kg	83.98	1.339

Table A 6: Characteristics for the analytical method used for validation of acetamiprid and IM-2-1 residues in foodstuff of animal origin

	acetamiprid	IM-2-1
Specificity	Acetamiprid eluated at 3.6 minutes. The compound was specifically extracted from the chromatogram using accurate high resolution mass spectrometry, and there were no	IM-2-1 eluted at 3.0 minutes. The compound was specifically extracted from the chromatogram using accurate high resolution mass spectrometry, and there were no

	acetamiprid	IM-2-1
	other peaks present at the same elution time as Acetamiprid.	other peaks present at the same elution time as IM-2-1.
Calibration (type, number of data points)	The linearity was determined form twenty injections of ten concentrations of standard ranging from a blank to 200µg/L Acetamiprid. The plot possesses a correlation coefficient higher than 0.99.	The linearity was determined form twenty injections of ten concentrations of standard ranging from a blank to 200µg/L IM-2-1. The plot possesses a correlation coefficient higher than 0.99.
Calibration range	blank to 200µg/L Acetamiprid	blank to 200µg/L IM-2-1
Assessment of matrix effects is presented	no	no
Limit of determination/quantification	limit of quantification 0.01 mg/kg	limit of quantification 0.01 mg/kg

Conclusion

It could be demonstrated that the method fulfils the requirements with regard to linearity, specificity, repeatability, limit of quantification and recoveries and therefore applicable to determine residues in foodstuff of animal origin.

Comments of zRMS: R C0238	The method was successfully validated for the determination of residues of acetamiprid in honey. This method meets criteria according SANTE/2020/12830 Rev.2. The study is accepted.

Reference:	KCP 5.2
Report	Validation of the Analytical Method for the analysis of Acetamiprid in Honey and Pollen, V. Faessel, R C0238
Guideline(s):	SANTE/2020/12830 Rev.2
Deviations:	NO
GLP:	YES
Acceptability:	YES

Materials and methods

The objective of the study was to validate the analytical method for the analysis of acetamiprid in Honey and pollen. The method was validated according to SANCO/3029/99, rev. 4 guidelines, but results are in accordance with SANTE/2020/12830 Rev.2.
The results acquired during validation of the analytical method (accuracy and repeatability) were in the range of 70 – 110% and RSD ≤ 20% for average recovery.
The limit of quantification of the method was established at 0.010 mg/kg for honey and pollen.
There were no interfering signals at retention time of analysed compound in examined control matrix.

Specimen preparation

-Honey
The Honey sample (thawed at room temperature) was homogenized.
The amount required by the analytical method (5 g) was weighed from this homogeneous matrix.
1. Weigh 5.0 (±0.03) g of the homogenized sample material into a 50 mL centrifuge tube.

2. Fortify if necessary.
3. Add 10 mL of Type I water and 10 mL of extraction solution and manually shake vigorously for 1 min.
4. Add 6.5 (±0.03) g of the bulk citrates salts mixture.
Attach the centrifuge tubes on the mechanical shaker for centrifuge tubes.
Shake vigorously for 10 min and centrifuge for 5 min at 3000 rpm.
5. Transfer about 6 mL of the acetonitrile layer into a 15 mL centrifuge tube.
Add 150 (±5) mg of PSA and 900 (±5) mg of magnesium sulfate.
6. Attach the centrifuge tubes on the mechanical shaker for centrifuge tubes.
Shake vigorously for 10 min and centrifuge for 5 min at 3000 rpm.
7. Transfer a 0.5 mL aliquot of the supernatant to an autosampler vial. Add 0.5 mL of extraction solution and mix.
8. Transfer into an Eppendorf tube and centrifuge at 13000 rpm for 1 min.
Store the samples at ≤-18°C before analysis.
9. Analyze by LC/MS/MS.

-Pollen
The whole sample was blended with dry ice.
The sample was then placed at ≤-18°C for at least 12 hours until the dry ice is completely sublimated.
The amount required by the analytical method (2.5 g) was weighed from this homogeneous matrix.
1. Weigh 2.5000 (register the actual weight) g of the homogenized sample material into a 50 mL centrifuge tube.
2. Fortify if necessary.
3. Add 10 mL of Type I water and 10 mL of extraction solution and manually shake vigorously for 1 min.
4. Add 6.5 (±0.03) g of the bulk citrates salts mixture.
Attach the centrifuge tubes on the mechanical shaker for centrifuge tubes.
Shake vigorously for 10 min and centrifuge for 5 min at 3000 rpm.
5. Transfer about 6 mL of the acetonitrile layer into a 15 mL centrifuge tube.
Add 150 (±5) mg of PSA and 900 (±5) mg of magnesium sulfate.
6. Attach the centrifuge tubes on the mechanical shaker for centrifuge tubes.
Shake vigorously for 10 min and centrifuge for 5 min at 3000 rpm.
7. Transfer a 0.5 mL aliquot of the supernatant to an autosampler vial. Add 0.5 mL of extraction solution and mix.
8. Transfer into an Eppendorf tube and centrifuge at 13000 rpm for 1 min.
Store the samples at ≤-18°C before analysis.
9. Analyze by LC/MS/MS.

Bulk citrates salts mixture: 4g MgSO4 + 1g NaCl + 0.5g HOC(COOH)(CH2COONa)2 ·1.5H2O + 1g HOC(COONa)(CH2COONa)2 · 2H2O
In a 2 L glass bottle, weigh:
-400g (±0.4, between 399.6 and 400.4) g of anhydrous magnesium sulfate (MgSO4),
Into 3 separate 500 mL beakers weigh,
-100g (±0.1, between 99.9 and 100.1) g of sodium chloride (NaCl),
-50g (±0.05, between 49.95 and 50.05) g of Sodium citrate dibasic sesquihydrate (HOC(COOH)(CH2COONa)2 ·1.5H2O)
-100g (±0.1, between 99.9 and 100.1) g of sodium citrate tribasic dihydrate (HOC(COONa)(CH2COONa)2 · 2H2O.
Transfer the content of the 3 beakers into the 2 L glass bottle.
Add 5 magnetic bars.
Hand shake upside down during 30 seconds.
Rotate the bottle from a quarter of tour and hand shake upside down during 30 seconds.
Repeat 6 times (equivalent to 2 full tours).
Weight uncertainty =0.1x4=0.4 %
Individual citrates salts mixture: 4g MgSO4 + 1g NaCl + 0.5g HOC(COOH)(CH2COONa)2 ·1.5H2O +

1g HOC(COONa)(CH2COONa)2 · 2H2O
Weigh 6.5g (±0.03, between 6.47 and 6.53) g of the bulk citrates salts mixture into a 15 mL centrifuge tube.
Use one tube per sample.
Weight uncertainty =0.46 %

Chromatographic parameters

Apparatus	XEVO LC /MS /MS

Column

Description	Aquasil C18	Supplier	Thermo	Particles	3 µm
Internal diam. x length	3*150 mm	Supplier reference	10010637	Temperature	30 °C
Development Column ANADIAG Number	197	Stationary Phase	C18	Comment	-

Mobile phase

A =	H_2O HPLC + 0.1 % formic acid
B =	MeOH HPLC + 0.1 % formic acid

Sample temperature	15 ° C

Elution

Elution	Time min	Flow mL/min	Composition (%) A	B	C	D	Curve (type)
Pg1	0.00	0.25	80	20	-	-	-
Pg2	3.00	0.25	80	20	-	-	6
Pg3	6.50	0.25	0	100	-	-	6
Pg4	9.00	0.25	0	100	-	-	6
Pg5	9.10	0.25	80	20	-	-	6
Pg6	13.00	0.25	80	20	-	-	-

Detector

IONISATION mode	ES
Polarity	Pos

Active ingredient(s)	Cone voltage	Collision Energy	Dwell time (ms)	TRANSITION 1	TRANSITION 2
Acetamiprid	25	20	250	223.1 > 126.0*	-
	25	35	250	-	223.1 > 90.1**

*used as quantification transition
**used as qualification transition

Date of application of analytical conditions: 09/06/2020

Study	C0238	Column ANADIAG number	293
Matrix	Honey and polen	Retention time	Acetamiprid:≈ 8.8 min.
Sample temperature	+15 °C	Injected volume	5 µL

Validation - Results and discussions

Table A 5: Recovery results from method validation of Acetamiprid using the analytical method

Matrix	Analyte	Fortification level (mg/kg) ~~(n = x)~~ n=5	Mean recovery (%)	RSD (%)	Comments
Honey	Acetamiprid	LOQ (0.01)	79.0	4.2	-
	Acetamiprid	10 x LOQ (0.1)	83.7	5.8	-

Matrix	Analyte	Fortification level (mg/kg) ~~(n = x)~~ n=5	Mean recovery (%)	RSD (%)	Comments
Pollen	Acetamiprid	LOQ (0.01)	93.1	8.9	-
	Acetamiprid	10 x LOQ (0.1)	83.6	8.1	-

Table A 2: Methods suitable for the determination of the residues in plant protection product (PPP) CHR/I/ACE 200 SE

	Residues
Author(s), year	V. Faessel, 2020
Principle of method	LC MS/MS
Linearity (linear between mg/L) (correlation coefficient, expressed as r)	The linearity of the method was checked by injecting into the analytical system matrix-matched calibration solutions of Acetamiprid, at 7 concentration levels, over the range: -for Honey 0.75 ng/mL to 30.1 ng/mL (corresponding to 0.003 to 0.12 in mg/kg). - for Pollen 0.38 ng/mL to 15.1 ng/mL (corresponding to 0.003 to 0.12 in mg/kg). Calibration curves were run for each analysis sequence. The linear correlation coefficients were > 0.990, showing a good linearity. Calibration covers the range from 30% of the LOQ to 20% above the highest level. Calibration curve for honey: C= 9.6360E-05 × S (Peak area) + 0.402 R^2= 0.99846
Precision, accuracy and uncertainty	Recovery data was generated from five samples fortified at the limit of quantification (LOQ) and five samples fortified at the 10-fold higher concentration than the LOQ. Precision of the method was determined as the relavite 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.
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. No interferences at above 30% of the LOQ were detected at the retention time of active substance in matrix blank samples.
Matrix Effects	Matrix effects enhancement on the instrument response were considered significant as experimental amount found is out of the range 80-120% of the theoretical concentration. Consequently matrix-matched calibration solutions were used for all calibrations.
LOQ **LOD**	The limit of quantification (LOQ) is the lowest validated level where a mean recovery within the range 70-110% with a RSD less than 20% could be obtained. The LOQ was set at 0.01 mg/kg in Honey and Pollen. The limit of detection (LOD= 0.003 mg/kg) is the lowest measurable standard concentration estimated at 3 times the background noise with the analytical conditions used.

	Residues
Extraction stability	The storage stability of the analyte in extracts was evaluated by analysing spiked samples extracts after frozen storage. The storage stability of standard solutions was evaluated by comparing response factors obtained for frozen stored solutions to freshly prepared solutions. Spiked samples at 10xLOQ level were stored frozen for: -19 days for Honey; -14 days for Pollen, after samples extraction, and analysed to check the stability of the final extracts.
Extraction efficiency	In accordance to SANTE/2017/10632 Rev. 4: Extraction efficiency should be addressed if for a product authorization a different analytical methodology (in methods for risk assessment and/or monitoring) is used, compared to that of the approval/renewal procedure of the active substance. Used method is similar to one presented in RAR for estimating residues in animal matrices, therefore in applicants opinion no further testing is required.
Comment	The validation parameters are within the acceptance range and fulfil EU requirements given in SANTE/2020/12830 Rev.2.

Conclusion

The method was successfully validated for determination of all analytes in all matrices with an LOQ of 0.01 mg/kg according to the guidance document(s) SANTE/2020/12830 Rev.2. With regard to selectivity, accuracy and precision, the analytical methods were applied successfully for each analytical set when analysing the specimens of the study.

A 2.1.2.2.1.2 Independent laboratory validation

Comments of zRMS: Study No. 133111101	The method was successfully validated for the determination of residues of acetamiprid and its metabolite IM-2-1 in milk, liver and muscle tissue. This method meets criteria according SANCO/825/00 rev. 8.1. The study is accepted.

Reference:	KCP 5.2.1/04
Report	Acetamiprid and its metabolite IM-2-1: Independent Laboratory Validation of an Analytical Method for the Determination in Animal Commodities Eichler M., Hermann S., 2018, Study No. 133111101
Guideline(s):	SANCO/825/00 rev. 8.1
Deviations:	YES
GLP:	YES
Acceptability:	YES

Analytical Method

An analytical method to determine Acetamiprid in surface water was provided by the Sponsor (“Validation of the Methods of Analysis used for the Determination of Acetamiprid and a specified metabolite in animal commodities, in Compliance with Good Laboratory Practice, and referencing SANCO/3029/99.”(Norris, D.; 2017; Study No. DNA4036)). The analytical method was independently validated at the performing laboratory. The method was validated for the matrices milk, liver and muscle

tissue with a Limit of Quantification (LOQ) of 0.01 mg/kg. Method for Determination: LC-MS/MS

Deviations to the Original Method

Concerning: Analytical Column

According to Original Method: Phenomenex Luna –C18, 150 * 2 mm

Deviation to Original Method: Synergi 4-Hydro-RP 80A, (150 * 3 mm)

Reason for Deviation: The column of the original method was not available at ibacon.

Impact on the Study: Both columns have the same stationary phase (C18) and comparable dimensions. The chromatograms and results confirmed the suitability of the present column. Therefore, this modification of the original method is considered to be insignificant.

Results and discussions

Table A 8: Recovery results from method validation of acetamiprid and IM-2-1 using the analytical method

sample description	replicates	% of nominal[1] concentration	RSD [%]	replicates	% of nominal[1] concentratior	RSD [%]
	Acetamiprid in milk			**IM-2-1 in milk**		
Fortified 0.01 mg/kg (LOQ)	6	112	4	6	114	3
Fortified 0.1 mg/kg	6	114	3	6	115	3
Fortified 1 mg/kg	6	120	2	6	119	2
Fortified 10 mg/kg	6	120	3	6	119	3
overall mean value	**24**	**117**	**5**	**24**	**117**	**4**
	Acetamiprid in liver			**IM-2-1 in liver**		
Fortified 0.01 mg/kg (LOQ)	6	101	6	6	104	8
Fortified 0.1 mg/kg	6	103	3	6	103	2
Fortified 1 mg/kg	6	111	1	6	112	1
Fortified 10 mg/kg	6	112	1	6	113	2
overall mean value	**24**	**107**	**6**	**24**	**108**	**6**
	Acetamiprid in muscle tissue			**IM-2-1 in muscle tissue**		
Fortified 0.01 mg/kg (LOQ)	6	113	4	6	113	2
Fortified 0.1 mg/kg	6	106	3	6	106	2
Fortified 1 mg/kg	6	110	3	6	110	3
Fortified 10 mg/kg	6	107	2	6	107	2
overall mean value	**24**	**109**	**4**	**24**	**109**	**4**

[1] mean value of all measured samples per fortification level

RSD: relative standard deviation per treatment group

Table A 9: Characteristics for the analytical method used for validation of acetamiprid and IM-2-1

residues in foodstuff of animal origin

	acetamiprid	**IM-2-1**
Specificity	Specificity: Specificity was established by monitoring two different mass transitions for acetamiprid: quantifier 223 → 126 m/z qualifier 223 → 90 m/z Interference: There was no interference from blank values and therefore the recommendation by SANCO guideline (< 30 % of the mean peak area at LOQ level) is fulfilled.	Specificity was established by monitoring two different mass transitions for IM-2-1: • quantifier 209 → 126 m/z • qualifier 209 → 90 m/z There was no interference from blank values and therefore the recommendation by SANCO guideline (< 30 % of the mean peak area at LOQ level) is fulfilled.
Calibration (type, number of data points)	The linearity was determined form twenty injections of ten concentrations of standard ranging from a blank to 200µg/L Acetamiprid. The plot possesses a correlation coefficient higher than 0.99.	The linearity was determined form twenty injections of ten concentrations of standard ranging from a blank to 200µg/L IM-2-1. The plot possesses a correlation coefficient higher than 0.99.
Calibration range	1.2 to 200 µg/L	1.2 to 200 µg/L
Assessment of matrix effects is presented	no	no
Limit of determination/quantification	limit of quantification 0.01 mg/kg	limit of quantification 0.01 mg/kg

Conclusion:
For the method validation purpose, the matrices milk, liver and muscle tissue were spiked at four concentration levels and an LOQ of 0.01 mg/kg was established for each matrix. The validity criteria specificity, linearity, accuracy and precision were fulfilled for analysis of the test item in milk, liver and muscle tissue.

A 2.1.2.2.1.3 Confirmatory method (if required)

No confirmatory method is required

A 2.1.2.2.1.4 Extraction efficiency

No new or additional studies have been submitted.

A 2.1.2.2.2 Analytical method 2

No new or additional studies have been submitted.

A 2.1.2.2.2.1 Extraction efficiency

No new or additional studies have been submitted.

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

Comments of zRMS: Study No. DNA4517	The method was successfully validated for the determination of residues of acetamiprid and its metabolites IM-1-4 and IM-1-2 in calcareous soil. This method meets criteria according SANCO/825/00 rev. 8.1. The study is accepted.

Reference:	KCP 5.2.1/05
Report	Validation of the Methods of Analysis used for the Determination of Acetamiprid and two Acetamiprid Metabolites in Calcareous Soil, in Compliance with Good Laboratory Practice, and referencing SANCO/825/00 rev. 8.1. D. Norris, 2018, DNA4517
Guideline(s):	SANCO/825/00 rev. 8.1
Deviations:	NO
GLP:	YES
Acceptability:	YES

Materials and method

Approximately 10 g of homogenized soils were weighed into a 150 ml glass jars. 90 ml 80:20 Acetonitrilie:Water containing 1% Acetic Acid was added and the samples which were sonicated in a sonic bath for 5 minutes. The samples were then shaken on an orbital shaker for 30 minutes and filtered into a 200 ml volumetric flask. These steps were repeated with 60 ml of 50:50 Acetonitrilie:water containing 1% Acetic Acid to extract the residual soil and 40 ml of water containing 1% acetic acid as a third extraction. The combined extracts were made to volume in the 200 ml volumetric flask with water containing 1% acetic acid. The samples were analysed by LC-QQQ in positive polarity mode using a Phenomenex Luna C18 column, 150 mm x 2.0 mm 5 um and a isocratic elution with mobile phases of acetonitrilie and 1.0% acetic acid in deionised water.

Results and discussion

The detailed results are given in the tables below.

Table A10: Recovery results from method validation of acetamiprid using the analytical method

Validation Parameter	Results Obtained
Linearity	$R^2 = 0.9967$ And $R^2 = 0.9999$
Recovery at 0.02mg/Kg Acetamiprid	Mean Recovery = 92.62%
Precision at 0.02mg/Kg Acetamiprid	%RSD = 4.14
LOQ Recovery at 0.002mg/Kg Acetamiprid	Mean Recovery = 86.52%
LOQ Precision at 0.002mg/Kg Acetamiprid	%RSD = 29.62

Table A11: Recovery results from method validation of IM-1-4 using the analytical method

Validation Parameter	Results Obtained
Linearity	$R^2 = 0.9996$
Recovery at 0.02mg/Kg IM-1-4	Mean Recovery = 81.69%
Precision at 0.02mg/Kg IM-1-4	%RSD = 1.98
Recovery at 0.002mg/Kg IM-1-4	Mean Recovery = 85.23%
Precision at 0.002mg/Kg IM-1-4	%RSD = 11.52

Table A12: Recovery results from method validation of IM-1-2 using the analytical method

Validation Parameter	Results Obtained
Linearity	$R^2 = 0.9996$
Recovery at 0.02mg/Kg IM-1-2	Mean Recovery = 89.57%
Precision at 0.02mg/Kg IM-1-2	%RSD = 2.18
LOQ Recovery at 0.002mg/Kg IM-1-2	Mean Recovery = 94.32%
LOQ Precision at 0.002mg/Kg IM-1-2	%RSD = 6.31

Table A 13: Characteristics for the analytical method used for validation of acetamiprid, IM-2-1 and IM-1-4 residues in Calcareous Soil

	acetamiprid	IM-1-2	IM-1-4
Specificity	Acetamiprid aluted at 4.3 minutes. The compund was specifically extracted from the chromatogram using MRM mass spectrometry, and there	IM-1-2 aluted at 2.5 minutes. The compund was specifically extracted from the chromatogram using MRM mass spectrometry, and there	IM-1-4 eluted at 3.3 minutes. The compund was specifically extracted from the chromatogram using accurate high resolution mass spectrometry, and

	acetamiprid	IM-1-2	IM-1-4
	were no other peaks present at the same elution time as acetamiprid	were no other peaks present at the same elution time as IM-1-2	there were no other peaks present at the same elution time as IM-1-4.
Calibration (type, number of data points)	The linearity was determined form sixteen injections of eight concentrations of standard ranging from a blank to 5.0 ug/l Acetamiprid. The plot possesses a correlation coefficient of 0.9967.	The linearity was determined form sixteen injections of eight concentrations of standard ranging from a blank to 10.0 ug/l IM-1-2. The plot possesses a correlation coefficient of 0.9996	The linearity was determined form sixteen injections of eight concentrations of standard ranging from a blank to 5.0 ug/l IM-1-4. The plot possesses a correlation coefficient of 0.9996
Calibration range	blank to 5µg/L Acetamiprid	blank to 10µg/L Acetamiprid	blank to 5µg/L Acetamiprid
Assessment of matrix effects is presented	no	no	no
Limit of determination/quantification	limit of quantification 0.002 mg/kg	limit of quantification 0.002 mg/kg	limit of quantification 0.002 mg/kg

Conclusion

It could be demonstrated that the method fulfils the requirements with regard to linearity, specificity, repeatability, limit of quantification and recoveries and therefore applicable to determine residues in Calcareous Soil

Comments of zRMS: Study No. 133113101	The study was not submitted and therefore was not evaluated. Not required. According to the SANTE/2020/12830, Rev.2: A validation of the primary monitoring method in an independent laboratory (ILV) is required for the determination of residues in food of plant and animal origin and in drinking water.

Reference:	KCP 5.2.1/6
Report	Acetamiprid and its Metabolites IM-1-2 and IM-1-4: Independent Laboratory Validation of an Analytical Method for the Determination in Calcareous Soil Eichler M., Herrmann S., 2018, Study No. 133113101
Guideline(s):	SANCO/825/00 rev. 8.1
Deviations:	YES
GLP:	YES
Acceptability:	YES

Analytical Method

An analytical method to determine Acetamiprid and two Acetamiprid Metabolites in Calcareous Soil was provided by the Sponsor (“Validation of the Methods of Analysis used for the Determination of Acetamiprid and two Acetamiprid Metabolites in Calcareous Soil, in Compliance with Good Laboratory Practice, and referencing SANCO/825/00 rev 8.1.”(Norris, D.; 2018; Study No. DNA4517)). The

analytical method was independently validated at the performing laboratory. The method was validated for the matrix calcareous soil with a Limit of Quantification (LOQ) of 0.002 mg/kg.
Method for Determination: LC-MS/MS

Deviations to the Original Method

Concerning: Analytical Column
According to Original Method: Phenomenex Luna –C18, 150 * 2 mm
Deviation to Original Method: Synergi 4-Hydro-RP 80A, (150 * 3 mm)
Reason for Deviation: The column of the original method was not available at ibacon.
Impact on the Study: Both columns have the same stationary phase (C18) and comparable dimensions. The chromatograms and results confirmed the suitability of the present column. Therefore, this modification of the original method is considered to be insignificant.

Method validation

Specificity
Specificity was established by monitoring two different mass transitions for IM-1-2:
• quantifier 241 → 126 m/z
• qualifier 241 → 98 m/z

Calibration Range: 0.03 to 10 µg/L

Calibration Curves: Quantifier: y = 128725 * x + 2884
Qualifier: y = 89167 * x + 3047

Linearity of Response: Quantifier: 0.9999
Qualifier: 0.9999

Limit of Detection (LOD): Quantifier: 0.006 µg/L corresponding to 0.116 µg/kg calcareous soil
Qualifier: 0.014 µg/L corresponding to 0.277 µg/kg calcareous soil

Limit of Quantification (LOQ): 0.002 mg/kg calcareous soil

Results and discussion:

Table A14: Recovery results from method validation of acetamiprid, IM-1-2 and IM-1-4 using the analytical method

sample description	replicates	% of nominal[1] concentration	RSD [%]
Acetamiprid in soil			
Fortified 0.002 mg/kg (LOQ)	6	86	4
Fortified 0.02 mg/kg	6	90	1
overall mean value	**12**	**88**	**4**
IM-1-2 in soil			
Fortified 0.002 mg/kg (LOQ)	6	85	3
Fortified 0.02 mg/kg	6	103	3
overall mean value	**12**	**94**	**10**
IM-1-4 in soil			
Fortified 0.002 mg/kg (LOQ)	6	88	6
Fortified 0.02 mg/kg	6	90	6
overall mean value	**12**	**89**	**6**

[1] mean value of all measured samples per fortification level
RSD: relative standard deviation per treatment group

Conclusion:
For method validation purpose, calcareous soil was spiked at two concentration levels and an LOQ of 0.002 mg/kg was established. The validity criteria specificity, linearity, accuracy and precision were fulfilled for analysis of the test items in calcareous soil.

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

Comments of zRMS: Study No. DNA4518	The method was successfully validated for the determination of residues of acetamiprid and its metabolite IM-1-5 in drinking water. This method meets criteria according SANCO/825/00 rev. 8.1 and SANCO/3029/99 rev. 4. The study is accepted.

Reference:	KCP 5.2.1/7
Report	Validation of the Methods of Analysis used for the determination of a Metabolite of Acetamiprid in Drinking Water, in Compliance with Good Laboratory Practice, and referencing SANCO/825/00 rev. 8.1, D. Norris, 2018, DNA4518
Guideline(s):	SANCO/825/00 rev. 8.1 and SANCO/3029/99 rev. 4
Deviations:	NO
GLP:	YES
Acceptability:	YES

Materials and method

The analysis is conducted by direct injection of drinking water samples. The mixture is spiked as required

and then analysed on the LC-MS/MS using a Phenomenex Aqua C18 125A column (150 x 4.6 mm, 5um) column with a isocratic elution using a mobile phase of 1.0% acetic acid in HPLC grade water/HPLC grade acetonitrile.

Table A15: Recovery results from method validation of IM-1-5 using the analytical method

Validation Parameter	Results Obtained
Linearity	$R^2 = 0.9973$
Recovery at 0.5µg/L IM-1-5	Mean Recovery = 107.3%
Precision at 0.5µg/L IM-1-5	%RSD = 1.07
Recovery at 0.05µg/L IM-1-5	Mean Recovery = 113.4%
Precision at 0.05µg/L IM-1-5	%RSD = 1.47

Table A 16: Characteristics for the analytical method used for validation of acetamiprid and metabolite residues in water

	IM-1-5
Specificity	IM-1-5eluated at 1.4 minutes. The compound was specifically extracted from the chromatogram using accurate MRM mass spectrometry, and there were no other peaks present at the same elution time as IM-1-5.
Calibration (type, number of data points)	The linearity was determined form twenty injections of ten concentrations of standard ranging from a blank to 5µg/L Acetamiprid. The plot possesses a correlation coefficient higher than 0.9973.
Calibration range	blank to 5µg/L Acetamiprid
Assessment of matrix effects is presented	no
Limit of determination/quantification	limit of quantification 0.5 µg/kg

Conclusion

It could be demonstrated that the method fulfils the requirements with regard to linearity, specificity, repeatability, limit of quantification and recoveries and therefore applicable to determine residues in water

Comments of zRMS: Study No. DNA4037	The method was successfully validated for the determination of residues of acetamiprid in water. This method meets criteria according SANCO/825/00 rev. 8.1 and SANCO/3029/99 rev. 4. The study is accepted.

Reference: KCP 5.2.1/8

Report Validation of the Methods of Analysis used for the Determination of Acetamiprid in Water, in Compliance with Good Laboratory Practice, and referencing SANCO/3029/99, D. Norris, 2017 (addendum 3 2019), DNA 4037

Guideline(s): SANCO/825/00 rev. 8.1 and SANCO/3029/99 rev. 4

Deviations: NO

GLP: YES

Acceptability: YES

Materials and method

Mega Bond Elut C18 cartridges were conditioned with 5 ml Acetonirile and 5 ml deionised water, 200ml water samples were passed through the cartridge followed by an aliquot of 10 ml deionised water. The eluates were discarded. The analyte was eluted using 30 ml of a mix of acetonitrile and deionised water (3/17). The eluate was collected and evaporated to dryness under reduced pressure and the residue reconstituted in 10 ml deionised water. Sep-Pak plus C18 cartridges were conditioned with 5 ml acetonitrile and 5ml deionised water. The reconstituted sample was passed through the cartridge and the eluate discarded. The analyte was eluted using 30 ml of a mix of acetonitrile and deionised water (3/17) evaporated to dryness under reduced pressure and reconstituted in a mix of acetonitrile and deionised water (1/1). The samples were analysed by HPLC-MS/MS in a positive polarity mode using a YMC-Pack of acetonitrile and 1.0% acetic acid in deionised water. The acetamiprid molecular ion used was 223m/z and the fragment ions used were 56m/z, 99m/z and 126m/z

Results and discussion

Table A17: Recovery results from method validation of acetamiprid using the analytical method

Validation Parameter	Results Obtained
Linearity	$R^2 = 0.9994$
Recovery at 10µg/L Acetamiprid	Mean Recovery = 92.20%
Precision at 10µg/L Acetamiprid	%RSD = 1.54
Recovery at 1.0µg/L Acetamiprid	Mean Recovery = 93.09%
Precision at 1.0µg/L Acetamiprid	%RSD = 3.60
Recovery at 0.1µg/L Acetamiprid	Mean Recovery = 91.79%
Precision at 0.1µg/L Acetamiprid	%RSD = 1.42
LOQ Recovery at 0.05µg/L Acetamiprid	Mean Recovery = 88.33%

Table A 18: Characteristics for the analytical method used for validation of acetamiprid and metabolite residues in water

	acetamiprid
Specificity	Acetamiprid eluated at 2.9 minutes. The compound was specifically extracted from the chromatogram using accurate high resolution mass spectrometry, and there were no other peaks present at the same elution time as Acetamiprid.
Calibration (type, number of data points)	The linearity was determined form eighteen injections of nine concentrations of standard ranging from a blank to 100µg/L Acetamiprid. The plot possesses a correlation coefficient higher than 0.9994.
Calibration range	blank to 100µg/L Acetamiprid
Assessment of matrix effects is presented	no
Limit of determination/quantification	limit of quantification 0.05 µg/kg

Conclusion

It could be demonstrated that the method fulfils the requirements with regard to linearity, specificity, repeatability, limit of quantification and recoveries and therefore applicable to determine residues in water.

Comments of zRMS: Study No. 133112101	The method was successfully validated for the determination of residues of acetamiprid in water. This method meets criteria according SANCO/825/00 rev. 8.1. The study is accepted.

Reference: KCP 5.2.1/9

Report: Acetamiprid: Independent Laboratory Validation of an Analytical Method for the Determination in Drinking Water, Eichler M., Herrmann S., 2018, Study No. 133112101

Guideline(s): SANCO/825/00 rev. 8.1

Deviations: YES

GLP: YES

Acceptability: YES

Analytical Method

An analytical method to determine Acetamiprid in surface water was provided by the Sponsor ("Validation of the Methods of Analysis used for the Determination of Acetamiprid in Water, in Compliance with Good Laboratory Practice, and referencing SANCO/3029/99."(Norris, D.; 2017; Study No. DNA4037)). The analytical method was independently validated at the performing laboratory. Any addition or modification of the original method were discussed with the Sponsor´s monitor before implementation and are reported and justified in this report. The method was validated for drinking water with a Limit of Quantification (LOQ) of 0.05 µg/L. Method for Determination: LC-MS/MS

Deviations to the Original Method

In the present method an analytical column was used that was different to the column used in the original method, which was not available at the performing laboratory. In non-GLP pre-experiments it was demonstrated that the Synergi 4-Hydro-RP 80A column was a suitable replacement. This modification of the original method is considered to be negligible. A further modification to the original method was to increase of the evaporation temperature from 45 to 55 °C due to inefficient evaporation of the aqueous solution at the lower temperature. This modification is also considered to be negligible.

Method Validation

Specificity / Interference: Specificity was established by monitoring two different mass fragments, one as a quantifier (223 → 126 m/z) and one as a qualifier (223 → 90 m/z).

Interference: There was no interference from blank values and therefore the recommendation by SANCO guideline (< 30 % of the mean peak area at LOQ level) is fulfilled.

Matrix Effects: The peak areas obtained from measurement of the solvent standards and matrix-matched standards were compared. The mean difference between peak areas was 7 % for quantifier and qualifier mass transition. Therefore, it was decided to use calibration standards prepared from solvent for calibration and evaluation of samples.

Calibration Ranges: 0.7 to 100 µg/L

Calibration Curves: Quantifier: y = 121513 * x + 35366
Qualifier: y = 32570 * x + 15768

Linearity of Response: Regression coefficient of calibration curves was determined for both mass transitions: Quantifier: 0.9999
Qualifier: 0.9999

Limit of Detection (LOD): Quantifier: 0.027 µg/L
Qualifier: 0.053 µg/L

Limit of Quantification (LOQ): 0.05 µg/L

Accuracy and Precision:

Table A19: Recovery results from method validation of acetamiprid using the analytical method

Results for the Determination of the Test Item in Fortified Samples (Quantifier)

sample description	replicate	measured [µg/L]	d.f.	concentration[1] calculated [µg/L]	nominal [µg/L]	% of nominal
Acetamiprid in Drinking Water (223 m/z > 126 m/z; for quantitation)						
Control	1	< LOD	1	n.a.	0.000	n.a.
Control	2	< LOD	1	n.a.	0.000	n.a.
Control	3	< LOD	1	n.a.	0.000	n.a.
Control	4	< LOD	1	n.a.	0.000	n.a.
Fortified 0.05 µg/L (LOQ)	A	1.544	0.025	0.039	0.050	77
Fortified 0.05 µg/L (LOQ)	B	1.709	0.025	0.043	0.050	86
Fortified 0.05 µg/L (LOQ)	C	1.659	0.025	0.041	0.050	83
Fortified 0.05 µg/L (LOQ)	D	1.635	0.025	0.041	0.050	82
Fortified 0.05 µg/L (LOQ)	E	1.626	0.025	0.041	0.050	81
Fortified 0.05 µg/L (LOQ)	F	1.602	0.025	0.040	0.050	80
					mean value (n=6):	**82**
					SD (n=6):	**3**
					RSD:	**3**
Fortified 0.1 µg/L	A	3.314	0.025	0.083	0.100	83
Fortified 0.1 µg/L	B	3.495	0.025	0.087	0.100	87
Fortified 0.1 µg/L	C	3.100	0.025	0.077	0.100	78
Fortified 0.1 µg/L	D	3.231	0.025	0.081	0.100	81
Fortified 0.1 µg/L	E	3.643	0.025	0.091	0.100	91
Fortified 0.1 µg/L	F	3.264	0.025	0.082	0.100	82
					mean value (n=6):	**84**
					SD (n=6):	**5**
					RSD:	**6**
Fortified 1 µg/L	A	36.413	0.025	0.910	0.999	91
Fortified 1 µg/L	B	42.256	0.025	1.056	0.999	106
Fortified 1 µg/L	C	35.919	0.025	0.898	0.999	90
Fortified 1 µg/L	D	35.096	0.025	0.877	0.999	88
Fortified 1 µg/L	E	34.602	0.025	0.865	0.999	87
Fortified 1 µg/L	F	34.520	0.025	0.863	0.999	86
					mean value (n=6):	**91**
					SD (n=6):	**7**
					RSD:	**8**
Fortified 10 µg/L	A	36.166	0.250	9.041	9.987	91
Fortified 10 µg/L	B	35.261	0.250	8.815	9.987	88
Fortified 10 µg/L	C	35.672	0.250	8.918	9.987	89
Fortified 10 µg/L	D	35.919	0.250	8.980	9.987	90
Fortified 10 µg/L	E	35.425	0.250	8.856	9.987	89
Fortified 10 µg/L	F	35.754	0.250	8.939	9.987	89
					mean value (n=6):	**89**
					SD (n=6):	**1**
					RSD:	**1**
					overall mean value (n=24):	**86**
					SD (n=24):	**6**
					RSD:	**7**

[1] all values calculated from exact raw data, taking into account the purity and weighing factors

LOD: Limit of Detection = 0.027 µg/L

LOQ: Limit of Quantification

d.f.: dilution factor

SD: standard deviation

RSD: relative standard deviation

Table A20: Recovery results from method validation of acetamiprid using the analytical method

Results for the Determination of the Test Item in Fortified Samples (Qualifier)

sample description	replicate	measured [µg/L]	d.f.	concentration[1] calculated [µg/L]	nominal [µg/L]	% of nominal
Acetamiprid in Drinking Water (223 m/z > 90 m/z; for confirmation)						
Control	1	< LOD	1	n.a.	0.000	n.a.
Control	2	< LOD	1	n.a.	0.000	n.a.
Control	3	< LOD	1	n.a.	0.000	n.a.
Control	4	< LOD	1	n.a.	0.000	n.a.
Fortified 0.05 µg/L (LOQ)	A	1.395	0.025	0.035	0.050	70
Fortified 0.05 µg/L (LOQ)	B	1.530	0.025	0.038	0.050	77
Fortified 0.05 µg/L (LOQ)	C	1.456	0.025	0.036	0.050	73
Fortified 0.05 µg/L (LOQ)	D	1.462	0.025	0.037	0.050	73
Fortified 0.05 µg/L (LOQ)	E	1.490	0.025	0.037	0.050	75
Fortified 0.05 µg/L (LOQ)	F	1.419	0.025	0.035	0.050	71
					mean value (n=6):	**73**
					SD (n=6):	**2**
					RSD:	**3**
Fortified 0.1 µg/L	A	3.262	0.025	0.082	0.100	82
Fortified 0.1 µg/L	B	3.354	0.025	0.084	0.100	84
Fortified 0.1 µg/L	C	2.985	0.025	0.075	0.100	75
Fortified 0.1 µg/L	D	3.139	0.025	0.078	0.100	79
Fortified 0.1 µg/L	E	3.415	0.025	0.085	0.100	85
Fortified 0.1 µg/L	F	3.108	0.025	0.078	0.100	78
					mean value (n=6):	**80**
					SD (n=6):	**4**
					RSD:	**5**
Fortified 1 µg/L	A	36.973	0.025	0.924	0.999	93
Fortified 1 µg/L	B	42.500	0.025	1.062	0.999	106
Fortified 1 µg/L	C	36.359	0.025	0.909	0.999	91
Fortified 1 µg/L	D	35.438	0.025	0.886	0.999	89
Fortified 1 µg/L	E	34.824	0.025	0.871	0.999	87
Fortified 1 µg/L	F	34.824	0.025	0.871	0.999	87
					mean value (n=6):	**92**
					SD (n=6):	**7**
					RSD:	**8**
Fortified 10 µg/L	A	36.666	0.250	9.167	9.987	92
Fortified 10 µg/L	B	35.745	0.250	8.936	9.987	89
Fortified 10 µg/L	C	36.359	0.250	9.090	9.987	91
Fortified 10 µg/L	D	36.666	0.250	9.167	9.987	92
Fortified 10 µg/L	E	35.745	0.250	8.936	9.987	89
Fortified 10 µg/L	F	36.052	0.250	9.013	9.987	90
					mean value (n=6):	**91**
					SD (n=6):	**1**
					RSD:	**1**
					overall mean value (n=24):	**84**
					SD (n=24):	**9**
					RSD:	**11**

[1] all values calculated from exact raw data, taking into account the purity and weighing factors

LOD: Limit of Detection = 0.027 µg/L

LOQ: Limit of Quantification

d.f.: dilution factor

SD: standard deviation

Conclusion

In conclusion, the analytical method for the determination of the test item in drinking water was independently validated in this project. Results for specificity, linearity, accuracy and precision are given and fulfill the demanded validity criteria

Comments of zRMS: Study No. 133141101	The method was successfully validated for the determination of residues of IM-1-5 in water. This method meets criteria according SANCO/825/00 rev. 8.1. The study is accepted.

Reference:	KCP 5.2.1/10
Report	IM-1-5 (Metabolite of Acetamiprid): Independent Laboratory Validation of an Analytical Method for the Determination in Drinking Water, Eichler M., Herrmann S., 2018, Study No. 133141101
Guideline(s):	SANCO/825/00 rev. 8.1
Deviations:	YES
GLP:	YES
Acceptability:	YES

Analytical Method

An analytical method to determine IM-1-5 (Metabolite of Acetamiprid) in drinking water was provided by the Sponsor ("Validation of the Methods of Analysis used for the Determination of a Metabolite of Acetamiprid in Drinking Water, in Compliance with Good Laboratory Practice, and referencing SANCO/825/00 rev. 8.1."(Norris, D.; 2018; Study No. DNA4518)). The analytical method was independently validated at the performing laboratory. Any addition or modification of the original method was discussed with the Sponsor´s monitor before implementation and is reported and justified in this report. The method was validated for drinking water with a Limit of Quantification (LOQ) of 0.05 µg/L. Method for Determination: LC-MS/MS

Deviations to the Study Plan

Concerning: Chromatographic Conditions
According to the Study Plan: Kinetex C18 100A (100 * 3 mm)
Deviation to the Study Plan: Acquity UPLC BEH C18 (100*2.1 mm; 1.7 µm)
Reason for Deviation: Error, the Acquity column was another column used in the pre-experiments.
Presumable Effect on the Study: None, both columns showed similar performances in pre-tests. The method was fully validated using the Acquity column.

Deviations to the Original Method

In the present method an analytical column was used that was different to the column used in the original method, which was not available at the performing laboratory. In non-GLP pre-experiments it was demonstrated that the Acquity column was a suitable replacement. This modification of the original method is considered to be negligible

Method Validation

Specificity was established by monitoring two different mass fragments, one as a quantifier (198 → 126 m/z) and one as a qualifier (198 → 90 m/z).

Matrix Effects: The peak areas obtained from measurement of the solvent standards and matrix-matched standards were compared. The mean difference between peak areas was 30%. Therefore, it was decided to use the matrix-matched calibration standards for calibration and evaluation.

Calibration Ranges: 0.015 to 5 µg/L (high range)
0.015 to 0.25 µg/L (low range)
The control samples and samples prepared at LOQ level were evaluated using calibration data of the lower concentration range; i.e. 0.015 to 0.25 µg/L. The fortified samples of the 10*LOQ level were evaluated using calibration data of the higher concentration range; i.e. 0.015 to 5 µg/L.

Calibration Curves:
Quantifier (high): y = 1801305 * x + 91989
Qualifier (high): y = 501952 * x + 17203

Quantifier (low): y = 2188640 * x – 2478
Qualifier (low): y = 559202 * x + 130

Linearity of Response: Regression coefficient of calibration curves was determined for both mass transitions:
Quantifier (high): 0.9991
Qualifier (high): 0.9995

Quantifier (low): 0.9997
Qualifier (low): 0.9999

Limit of Detection (LOD):
Quantifier: 0.0012 µg/L
Qualifier: 0.0014 µg/L

Table A21: Recovery results from method validation of acetamiprid using the analytical method

Results for the Determination of IM-1-5 in the Fortified Samples (Quantifier)

sample description	concentration					
	[µg/L]	found [µg/L][1]	d.f.	calculated [µg/L][1]	nominal [µg/L][1]	% of nominal [2]
IM-1-5 in Drinking Water (quantifier m/z 198 amu → 126 amu)						
control sample	0	0.0017	1	< LOQ	0	n.a.
control sample	0	0.0016	1	< LOQ	0	n.a.
solvent control	0	0.0018	1	< LOQ	0	n.a.
fortified sample	0.05	0.047	1	0.047	0.050	95
fortified sample	0.05	0.053	1	0.053	0.050	106
fortified sample	0.05	0.047	1	0.047	0.050	95
fortified sample	0.05	0.045	1	0.045	0.050	90
fortified sample	0.05	0.052	1	0.052	0.050	103
fortified sample	0.05	0.049	1	0.049	0.050	98
					mean value (n=6):	**98**
					RSD (n=6):	**6**
fortified sample	0.5	0.510	1	0.510	0.499	102
fortified sample	0.5	0.532	1	0.532	0.499	107
fortified sample	0.5	0.537	1	0.537	0.499	108
fortified sample	0.5	0.526	1	0.526	0.501	105
fortified sample	0.5	0.526	1	0.526	0.501	105
fortified sample	0.5	0.510	1	0.510	0.501	102
					mean value (n=6):	**105**
					RSD (n=6):	**2**
					mean value (n=12):	**101**
					RSD (n=12):	**5**

[1] The tabulated results represent rounded results calculated on the exact raw data

[2] The results represent rounded values

LOD Limit of Detection = 0.0012 µg/L

n.a. not applicable

RSD Relative Standard Deviation

d.f. dilution factor

Table A22: Recovery results from method validation of acetamiprid using the analytical method

Results for the Determination of IM-1-5 in the Fortified Samples (Qualifier)

sample description	concentration found [µg/L]	[µg/L][1]	d.f.	calculated [µg/L][1]	nominal [µg/L][1]	% of nominal [2]
IM-1-5 in Drinking Water (qualifier m/z 198 amu → 90 amu)						
control sample	0	< LOD	1	n.a.	0	n.a.
control sample	0	< LOD	1	n.a.	0	n.a.
solvent control	0	< LOD	1	n.a.	0	n.a.
fortified sample	0.05	0.048	1	0.048	0.050	97
fortified sample	0.05	0.054	1	0.054	0.050	108
fortified sample	0.05	0.048	1	0.048	0.050	96
fortified sample	0.05	0.046	1	0.046	0.050	91
fortified sample	0.05	0.053	1	0.053	0.050	106
fortified sample	0.05	0.049	1	0.049	0.050	98
					mean value (n=6):	**99**
					RSD (n=6):	**6**
fortified sample	0.5	0.506	1	0.506	0.499	101
fortified sample	0.5	0.516	1	0.516	0.499	103
fortified sample	0.5	0.518	1	0.518	0.499	104
fortified sample	0.5	0.524	1	0.524	0.501	105
fortified sample	0.5	0.516	1	0.516	0.501	103
fortified sample	0.5	0.514	1	0.514	0.501	103
					mean value (n=6):	**103**
					RSD (n=6):	**1**
					mean value (n=12):	**101**
					RSD (n=12):	**5**

[1] The tabulated results represent rounded results calculated on the exact raw data

[2] The results represent rounded values

LOD Limit of Detection = 0.0014 µg/L

n.a. not applicable

RSD Relative Standard Deviation

d.f. dilution factor

Conclusion

In conclusion, the analytical method for the determination of the test item in drinking water was independently validated in this project. Results for specificity, linearity, accuracy and precision are given and fulfill the demanded validity criteria

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

Comments of zRMS: Study No. GLP-STUDY-18-000080	The method was successfully validated for the determination of residues of acetamiprid in water. This method meets criteria according SANCO/825/00 rev. 8.1. The study is accepted.

Reference:	KCP 5.2.1/11
Report	Validation of an analytical method for the determination of Acetamiprid residues in air, D. Longhi, 2019, GLP-STUDY-18-000080
Guideline(s):	SANCO/825/00 rev. 8.1
Deviations:	NO
GLP:	YES
Acceptability:	YES

Validation of the Method

The validated method consisted in a simple extraction of the analyte from the filters with acetonitrile. After sonication, an aliquot of the organic solvent was filtered and injected in a UPLC-MS/MS system for the final determination, setting the instrument in the multi reaction monitoring mode (MRM) on 2 transitions: m/z 223 to m/z 126 (primary quantifier detection 223/126) and m/z 223 to m/z 56 (secondary confirmation detection 223/56). The applied analytical method was validated under GLP compliance according to the SANCO/825/00 rev.8.1 guideline. The results of the evaluated validation parameters are hereunder summarised

Linearity

The linearity was evaluated on the calibration range from 0.624 ng to 41.6 ng (that are equal to the range from 0.00065 to 0.043 µg/m3 of air considering a fluxing volume of 0.96 m3) in 6 levels, monitoring both the MRM transitions: 223/126 and the confirmatory 223/56. The linearity was checked analysing standard solutions prepared in solvent (the matrix effect was proved to be less than 20%). The determination coefficients obtained are hereunder reported:

Table A23: summary of R2 values

R^2 primary detection	R^2 confirmatory detection
0.9996	0.9997

ACCURACY AND PRECISION

The results of accuracy and precision were found in accordance with the SANCO/825/00 rev.8.1 requirements, obtaining recovery values in the range of 70-120% with a RSD% < 20%. The obtained results are summarised in the following tables:

Table A24: summary of accuracy and precision - Primary detection (MRM transition 223/126)

Test	Fortification Level (ng/filter)	Fortification Level (µg/m³) – considering a fluxed volume of 0.96 m³	Accuracy and precision per level	
			Mean Recovery (%) n=5	RSD (%) n=5
Extraction efficiency	1.926 (LOQ)	0.0020	103.7	1.4
	19.26 (10xLOQ)	0.020	87.6	1.6
Overall recovery (fluxing of 0.96 m³ of ambient air at 35°C and 80% of relative humidity)	1.926 (LOQ)	0.0020	93.2	2.2
	19.26 (10xLOQ)	0.020	83.4	3.3

Table A25: summary of accuracy and precision - Confirmatory detection (MRM transition 223/56)

Test	Fortification Level *(ng/filter)*	Fortification Level (µg/m³) – considering a fluxed volume of 0.96 m³	Accuracy and precision per level	
			Mean Recovery (%) n=5	RSD (%) n=5
Extraction efficiency	1.926 (LOQ)	0.0020	103.1	0.8
	19.26 (10xLOQ)	0.020	87.2	1.9
Overall recovery (fluxing of 0.96 m³ of ambient air at 35°C and 80% of relative humidity)	1.926 (LOQ)	0.0020	94.3	2.5
	19.26 (10xLOQ)	0.020	83.6	2.4

BREAKTHROUGH

The evaluation of the breakthrough was made comparing the mean value of % recovery obtained for each level in the extraction efficiency test with the mean value of % recovery obtained for each level in the overall recovery tests. The breakthrough was evaluated as the difference between these series of values, obtaining the results hereunder summarised:

Test	Fortification Level (ng/filter)	Fortification Level (µg/m³) – considering a fluxed volume of 0.96 m³	Calculated breakthrough (%)
Primary detection (223/126)	1.926 (LOQ)	0.0020	10.5
	19.26 (10xLOQ)	0.020	4.2
Confirmatory detection (223/56)	1.926 (LOQ)	0.0020	8.8
	19.26 (10xLOQ)	0.020	3.6

(a): calculated as difference between the mean extraction efficiency and the mean overall recovery.

The loss of the analyte amount after the fluxing of 0.96 m3 (that is a volume much greater than that the minimum of 100 L required by the SANCO/825/00 rev8.1 guideline) was calculated to be less than 20%. The overall recovery remained in the range 70-120%, so the breakthrough can be considered not significant.

SELECTIVITY

The method was found to be selective for the determination of the analyte Acetamiprid for both the monitored transitions, that gave very similar results. No interfering signals were detected in the untreated matrix in amount higher than 30% of the LOQ level, that complies with the guideline requirements.

LIMIT OF DETECTION AND QUANTIFICATION

- Limit of detection: the less concentrated matrix matched standard injected, 0.312 µg/L can be considered the instrumental limit of detection for each matrix. The signal/noise ratio measured at this level was higher than 3. Considering a fluxing volume of 0.96 m3 , this amount corresponds to 0.00065 µg/m3 on the samples.

- Limit of quantification: the limit of quantification of this method is 0.0020 µg/m3 of Acetamiprid; accuracy and precision data at this level resulted in compliance with the mentioned guideline.

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~~

Comments of zRMS: Study No. GLP-STUDY-18-000079	The method was successfully validated for the determination of residues of acetamiprid in blood. This method meets criteria according SANCO/825/00 rev. 8.1. The study is accepted.

Reference:	KCP 5.1.2 KCP 5.2
Report	Validation of an analytical method for the determination of Acetamiprid residues in blood. Diego Longhi, Report No: GLP-STUDY-18-000079, 2019
Guideline(s):	Yes (SANCO/825/00 rev.8.1)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods
The aim of this Study was the Validation of an analytical method for the determination of Acetamiprid in bovine blood.
The validated method consisted in a 2-times-extraction of the analyte from the matrix with acetone. After centrifugation, an aliquot of the organic supernatant was filtered, dried under a nitrogen stream, dissolved in acetonitrile/water, filtered and injected in a UPLC-MS/MS system for the final determination, setting the instrument in the multi reaction monitoring mode (MRM) on 2 transitions: m/z 223 to m/z 126 (primary quantifier detection 223/126) and m/z 223 to m/z 56 (secondary confirmation detection 223/56).
The applied analytical method was validated under GLP compliance according to the guideline.

Results and discussions
The results of accuracy and precision were found in accordance with the current requirements, obtaining recovery values in the range of 70-120% with a RSD% < 20%.

Table A 1: Recovery results from method validation of acetamiprid in blood using the analytical method

Quantifier transition 223/126					
Sample code	**Fortification level (mg/L)**	**Recovery (%)**	**Mean Recovery (%)**	**SD**	**RSD%**
BPL-SMPL-18-000800 LOQ1	0.0520 (LOQ, nominal 0.05 mg/L)	99.9	100.9	2.0	2.0
BPL-SMPL-18-000800 LOQ2		98.9			
BPL-SMPL-18-000800 LOQ3		99.6			
BPL-SMPL-18-000800 LOQ4		102.5			
BPL-SMPL-18-000800 LOQ5		103.6			

Confirmation transition 223/56					
Sample code	Fortification level (mg/L)	Recovery (%)	Mean Recovery (%)	SD	RSD%
BPL-SMPL-18-000800 LOQ1	0.0520 (LOQ, nominal 0.05 mg/L)	99.2	101.6	3.3	3.2
BPL-SMPL-18-000800 LOQ2		99.2			
BPL-SMPL-18-000800 LOQ3		99.1			
BPL-SMPL-18-000800 LOQ4		104.8			
BPL-SMPL-18-000800 LOQ5		105.5			

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

	Acetamiprid
Specificity	This parameter was evaluated in order to demonstrate that the applied method detects the right analyte and that the analytical signal is quantitatively correct not affected by other compounds. Using a MS/MS mass spectrometer detector, the selectivity was evaluated comparing the following chromatograms: a blank, a sample, a fortified sample and a reference solution at the LOQ level, in order to assess the presence or absence of interfering signals. No interfering signals were detected in the untreated matrix in amount higher than 30% of the LOQ level, that is in compliance with the guideline requirements.
Calibration (type, number of data points)	The linearity was evaluated on the same calibration range from 2.082 to 20.82 µg/L (equal to from 0.0260 to 260 mg/L in the blood samples) in 5 levels, monitoring both the MRM transitions: 223/126 and the confirmatory 223/56. The linearity was checked analysing matrix matched standard solutions. The determination coefficients obtained are hereunder reported:
Calibration range	0.0260 to 260 mg/L
Assessment of matrix effects is presented	Referring to the requirements of SANTE/2020/12830 Rev.2 if the matrix effect exceeds 20% LOQ, the "matrix-matched" calibration should be introduced. This effect was not observed during validation, but the method of preparing working calibration standards based on blank sample was used.
Limit of determination/quantification	**LIMIT OF DETECTION AND QUANTIFICATION** - **Limit of detection**: the less concentrated matrix matched standard injected, 1.041 µg/L can be considered the instrumental limit of detection for each matrix. The signal/noise ratio measured at this level was higher than 3. This amount corresponds to 0.013 mg/L on the samples. - **Limit of quantification**: the limit of quantification of this method is 0.0520 mg/L of Acetamiprid; accuracy and precision data at this level resulted in compliance with the mentioned guideline.

Matrix	R^2 primary detection	R^2 confirmatory detection
Whole bovine blood	1.0000	1.0000

Conclusion

It could be demonstrated that the method fulfils the requirements with regard to linearity, specificity, repeatability, limit of quantification and recoveries and therefore applicable to determine residues in blood.

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

No new or additional studies have been submitted

A 2.2 Analytical methods for the deltamethrin

A 2.2.1 Methods used for the generation of pre-authorization data (KCP 5.1)

No new or additional studies have been submitted

A 2.2.2 Methods for post-authorization control and monitoring purposes (KCP 5.2)

A 2.2.2.1 Description of analytical methods for the determination of residues in plant matrices (KCP 5.2)

Comments of zRMS:	The method No. B7023 was successfully validated for the determination of residues of deltamethrin in oilseed rape seeds. This method meets criteria according to SANCO /3030 /99 rev.4. and SANCO/825/00 rev.8.1. The study is accepted.

Reference:	KCP 5.2.1/01
Report	Validation of the Analytical Method for the Analysis of Deltamethrin in Oilseed Rape Seeds, 2018, Agnes Perny, Study No. B7023
Guideline(s):	SANCO /3030 /99 rev.4. and SANCO/825/00 rev.8.1
Deviations:	NO
GLP:	YES
Acceptability:	YES

ANALYTICAL METHOD

Samples were analysed using a method developed by ANADIAG:
Outline of ANADIAG method:
Residues are extracted with acetonitrile. The extract obtained after centrifugation is then analysed by LC-MS/MS. Principle of the method ANADIAG references for the application of the method were:
- MP 561 (for extraction and purification)
- MA 1258 (for analytical determination)

SUMMARY:

The method under discussion describes the determination of residues of deltamethrin in oilseed rape seeds. The method was validated at 0.01 mg/kg in oilseed rape seeds. The following points were examined during the study:

Linearity of the analytical method:

The linearity of the method was studied between 0.8 ng/mL and 30 ng/mL of deltamethrin in matrix-matched calibration solutions (corresponding to 0.003 to 0.12 in mg/kg). The linear correlation coefficients were typically > 0.990, showing a good linearity.

Sensitivity:

The limit of quantification (LOQ) is the lowest validated level where a mean recovery within the range 70-110% with a RSD less than 20% could be obtained. The LOQ was set at 0.01 mg/kg in oilseed rape seeds.

Recovery results:

Analyte	Matrix	Fortification level (mg/kg)	Mean Percentage recovery (%)	Standard deviation (SD) (%)	Relative standard deviation (RSD) (%)	Number of fortified samples (n)
Deltamethrin	oilseed rape seeds	LOQ	104.7%	2.3%	2.2%	5
		10 x LOQ	92.6%	1.8%	1.9%	5
		All levels	98.7%	6.7%	6.8%	10

Accuracy
The accuracy of the method was assessed on the basis of the determined recovery rates.

	Deltamethrin	
Matrix	oilseed rape seeds	
Fortification level (mg/kg)	0.01	0.10
Single recovery rates	101.3 to 107.7%	91.3 to 95.2%
Mean recoveries per fortification level	104.7%	92.6%

The accuracy of the method fulfils the requirements for residue analytical methods which demand that the mean recovery per fortification level should be in the range 70-110%.

Precision and repeatability
Repeatability tests (5 recoveries at each fortification level) were performed at the LOQ level and at 10 x LOQ for oilseed rape seeds.

	Deltamethrin	
Matrix	oilseed rape seeds	
Fortification level (mg/kg)	0.01	0.10
RSD for each fortification level	2.2%	1.9%

RSD determined were less than 20%, the method therefore fulfils the requirements for residue analytical methods.

Specificity
The method is able to determine deltamethrin in oilseed rape seeds. This was checked by analysing control and spiked specimens to verify the absence of interfering peaks. No interfering peaks were present at > 30% of the LOQ. The analyses were carried out by LC-MS/MS, monitoring two transitions. The method was considered highly specific, thus the use of an alternative method was not necessary.

Confirmatory method
Repeatability tests (5 recoveries) were performed at the LOQ level for oilseed rape seeds for the qualification transition.

Summary of recoveries

Analyte	Matrix	Fortification level (mg/kg)	Mean Percentage recovery (%)	Standard deviation (SD) (%)	Relative standard deviation (RSD) (%)	Number of fortified samples (n)
Deltamethrin	oilseed rape seeds	0.01	103.5%	0.9%	0.9%	5

Recoveries and precision data for the qualifier transition comply with the requirements of SANCO/3029/99 rev.4 as the mean recovery at the LOQ level is within the range 70-110% and RSD is less than 20%.

Stability results for extracts
The stability of extracts during frozen storage was investigated. The results indicate a good stability up to 16 days.

Stability results for matrix matched standard solutions
The stability of matrix matched standard solutions during frozen storage was investigated. The results indicate a good stability up to 15 days.

Matrix Effect
The effect of crop matrices on the LC-MS/MS response was assessed by analysing standard solutions prepared in solvent against matrix-matched calibration solutions.

Matrix	Analyte	Theoretical concentration (ng/mL) Ct in acetonitrile	Experimental Concentration (ng/mL) Ce against matrix-matched calibration solutions	Recovery (%) 100 x Ce / Ct
Quantification transition				
oilseed rape seeds	Deltamethrin	25.2	24.26	96.3%
Qualification transition				
oilseed rape seeds	Deltamethrin	25.2	24.29	96.4%

Validation results:

Linearity

The linearity of the method was checked by injecting into the analytical system matrix-matched calibration solutions of deltamethrin, at 7 concentration levels, over the range 0.8 ng/mL to 30 ng/mL (corresponding to 0.003 to 0.12 in mg/kg). Calibration curves were run for each analysis sequence. The linear correlation coefficients were > 0.990, showing a good linearity.

Sensitivity

The limit of detection (LOD) is the lowest measurable standard concentration estimated at 3 times the background noise with the analytical conditions used. With analytical conditions, the limit of detection for deltamethrin is:

Analyte	Matrix	C_{LOD} (ng/mL)*	LOD (mg/kg)**
Deltamethrin	oilseed rape seeds	0.3	0.003

The limit of quantification (LOQ) is the lowest validated level where a mean recovery in the range 70- 120% with a RSD less than 20% could be obtained. It was set at 0.01 mg/kg for oilseed rape seeds.

Accuracy, precision and repeatability

In order to determine blank values, two untreated specimens of oilseed rape seeds were worked up for deltamethrin and analysed according to the method. No interferences above 30% of the limit of quantification were recorded.

Sample ANADIAG No.	Matrix	Deltamethrin amount found (µg/kg) 522.9 > 506.0
B7023 01 01 71	oilseed rape seeds	NDR
B7023 01 01 81		NDR

NDR: No detectable residues (residues below the limit of detection)

Recovery tests were performed by analysing untreated control samples spiked with deltamethrin before extraction. Results are given in the table below.

Recoveries in oilseed rape seeds:

Sample ANADIAG No.	Fortification level (mg/kg)	Deltamethrin % Recovery * 522.9 > 506.0
B7023 01 01 AEA	0.01	101.3%
B7023 01 01 AFA		105.3%
B7023 01 01 AGA		105.4%
B7023 01 01 AHA		103.9%
B7023 01 01 AIA		107.7%
B7023 01 01 AJA	0.10	91.3%
B7023 01 01 AKA		91.4%
B7023 01 01 ALA		95.2%
B7023 01 01 AMA		93.7%
B7023 01 01 ANA		91.4%

* Amount in control is deducted if above the LOD level

Summary of recoveries:

Analyte	Matrix	Fortification level (mg/kg)	Mean Percentage recovery (%)	Standard deviation (SD) (%)	Relative standard deviation (RSD) (%)	Number of fortified samples (n)
Deltamethrin	oilseed rape seeds	0.01	104.7%	2.3%	2.2%	5
		0.10	92.6%	1.8%	1.9%	5
		All levels	98.7%	6.7%	6.8%	10

Accuracy

The accuracy of the method was assessed on the basis of the determined recovery rates.

	Deltamethrin	
Matrix	oilseed rape seeds	
Fortification level (mg/kg)	0.01	0.10
Single recovery rates	101.3 to 107.7%	91.3 to 95.2%
Mean recoveries per fortification level	104.7%	92.6%

The accuracy of the method fulfils the requirements for residue analytical methods which demand that the mean recovery per fortification level should be in the range 70-110%.

Precision and repeatability Repeatability tests (5 recoveries at each fortification level) were performed at the LOQ level and at 10 x LOQ for oilseed rape seeds.

	Deltamethrin	
Matrix	oilseed rape seeds	
Fortification level (mg/kg)	0.01	0.10
RSD for each fortification level	2.2%	1.9%

Specificity
No interfering signal was present accounting for more than 30% of the LOQ level. The identity of the analyte detected was confirmed by monitoring a second mass transition.

Comments of zRMS:	The method ZBBZ-2017/05/DPL/1 was successfully validated for the determination of residues of deltamethrin in wheat (ears, rest of plants, straw and grain). This method meets criteria according to SANCO /3030/99 rev.4. and SANCO/825/00 rev.8.1. The study is accepted.

Reference: KCP 5.2.1/02

Report: DETERMINATION OF RESIDUES OF DELTAMETHRIN IN WINTER WHEAT APPLIED AS “DELCAPS 050 CS” AND “DELTAMETHRIN 100 SC” IN NORTHERN EUROPE IN 2017, 2018, Joanna Kicińska, Study Code: ZBBZ-2017/05/DPL/1

Guideline(s): SANCO /3030 /99 rev.4. and SANCO/825/00 rev.8.1

Deviations: NO

GLP: YES

Acceptability: YES

SUMMARY:

The objective of this study is to determine the decline and the magnitude of residues of Deltamethrin in Winter Wheat samples taken from the field trials following application of DELCAPS 050 CS and DELTAMETHRIN 100 SC. To achieve the objective appropriate analytical method for the determination of Deltamethrin in Winter Wheat will be validated in accordance to the guidance documents SANCO/825/00, rev. 8.1. and SANCO/3029/99 rev. 4. The intended limit of quantification is 0.01 mg/kg.

The general principles of the analytical procedure were based on the normalized method EN 15662:2008. In brief, samples of Winter Wheat were extracted with acetonitrile. After addition of a buffer-salt mixture

containing magnesium sulphate, sodium chloride and sodium citrate the extract was shaken. Following centrifugation, an aliquot of the upper acetonitrile phase was cleaned by primary secondary amine (PSA) and dehydrated by magnesium sulphate addition.

Selectivity and Confirmation of Residue Identity:

Quantification was performed by use of highly selective gas chromatography coupled with tandem mass spectrometry (GC-MS/MS). Two selected ion mass transitions were evaluated in order to demonstrate that the method achieves a high level of selectivity. The retention times of analyte in extracts corresponds to that of the calibration standards with a tolerance of < ± 0.1 min. Also, confirmation ratios for Deltamethrin in all samples were within ± 30 % of the average found for the standards.

No significant interference above 30 % of LOQ was detected in any of the reagent blanks or control specimen extracts for Winter Wheat matrix, so that a highly level of selectivity was demonstrated and an additional confirmatory method is not necessary.

Matrix Effects:

Matrix effects on the detection of Deltamethrin in extracts of Winter Wheat were found to be significant (> 20 %). Thus matrix-matched standards were used for quantification.

Linearity:

The correlation between the injected concentration of analyte standard and detector response was demonstrated to be linear by single determination of matrix-matched calibration standards at seven concentration levels ranging from 0.0015 µg/mL to 0.2 µg/mL for Winter Wheat (ears, grain) and nine concentration levels ranging from 0.0005 µg/mL to 0.2 µg/mL for Winter Wheat (rest of plants and straw). Those ranges correspond from 0.003 mg/kg to 0.4 mg/kg for Winter Wheat (ears, grain) and 0.002 mg/kg to 0.8 mg/kg for Winter Wheat (rest of plants and straw) thus covers the range from no more than 30 % of the LOQ and at least + 20 % of the highest analyte concentration level detected in samples.
The calibration curves obtained for both ion mass transitions of Deltamethrin were linear with the coefficients of correlation (R) greater than 0.99. Linear regression was performed with 1/x weighting.

Quantification:

Quantification was performed by using weighted (1/x) linear regression as described in the section “Linearity”.

Accuracy and Precision

Accuracy was determined by fortification of control samples with known amounts of the reference item and subsequent determination of the recoveries when applying the extraction procedure. Precision was determined by repeatability (relative standard deviation – RSD).
The mean recovery values at the fortification levels of 0.01 mg/kg and 0.1 mg/kg for both ion mass transitions were all in the range 70 – 110 % and thus comply with the standard acceptance criteria of the guidance document SANCO/825/00 rev. 8.1 and SANCO/3029/99, rev. 4. All precision values at the fortification levels of 0.01 mg/kg and 0.1 mg/kg for both ion mass transitions were < 20%.

Mean recovery and precision results for both ion mass transitions Deltamethrin are shown in Table 1.

Table 1: Summary of recovery results:

Analyte	Matrix	Fortification Level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Overall Mean Recovery (%)	Overall RSD (%)
Deltamethrin	Ion Mass Transition 252.9→172.0 (Quantification)						
	Winter Wheat (ears)	0.01*	103	6.0	5	106	5.4
		0.1	110	2.8	5		
	Ion Mass Transition 250.9→172.0 (Confirmation)						
	Winter Wheat (ears)	0.01*	104	2.8	5	105	3.0
		0.1	106	3.3	5		
	Ion Mass Transition 252.9→172.0 (Quantification)						
	Winter Wheat (rest of plants)	0.01*	90	7.5	5	86	9.1
		0.1	83	9.7	5		
	Ion Mass Transition 250.9→172.0 (Confirmation)						
	Winter Wheat (rest of plants)	0.01*	87	7.3	5	83	7.9
		0.1	79	5.6	5		
	Ion Mass Transition 252.9→172.0 (Quantification)						
	Winter Wheat (straw)	0.01*	76	6.6	5	78	6.1
		0.1	81	4.4	5		
	Ion Mass Transition 250.9→172.0 (Confirmation)						
	Winter Wheat (straw)	0.01*	77	5.8	5	80	6.6
		0.1	83	6.1	5		
	Ion Mass Transition 252.9→172.0 (Quantification)						
	Winter Wheat (grain)	0.01*	106	2.4	5	104	3.7
		0.1	102	3.8	5		
	Ion Mass Transition 250.9→172.0 (Confirmation)						
	Winter Wheat (grain)	0.01*	104	5.8	5	102	5.5
		0.1	101	5.2	5		

*-Limit of quantification, defined by the lowest validated fortification level

Limit of Quantification (LOQ) and Limit of Detection (LOD)

The limit of quantification (LOQ) is the lowest validated fortification level and was thus successfully established at 0.01 mg/kg for both ion mass transitions of Deltamethrin in Winter Wheat matrix.
The limit of detection (LOD) for Deltamethrin was set at 0.002 mg/kg for tested matrix, which is < 30% of the LOQ.
The limit of detection (LOD) for Deltamethrin was set at 0.003 mg/kg for tested matrix, which is < 30% of the LOQ.

Conclusions:

The method was shown to be highly selective, as it includes two parent-daughter ion transitions for Deltamethrin, and it yields accurate and repeatable results. The limit of quantification (LOQ) was established at 0.01 mg/kg for Deltamethrin, interfering signals in control specimen were negligible, and thus the limit of detection (LOD) is 0.002 mg/kg for Winter Wheat (rest of plants and straw) and 0.003 mg/kg for Winter Wheat (ears and grain).
It is concluded that method fulfils the requirements as defined in EC Guidance document on residue analytical methods (and SANCO/825/00, rev. 8.1. SANCO/3029/99, rev. 4 and is, applicable as enforcement and data generation method for determination of Deltamethrin in Winter Wheat after application of “DELCAPS 050 CS” AND “DELTAMETHRIN 100 SC”.

Comments of zRMS: Study No. VAL/20/2023	The method was successfully validated for the determination of residues of deltamethrin (sum of cis-deltamethrin and its alpha-R-isomer and trans-isomer) in wheat (grain, plant,

	straw). Analytical procedure DPL-64 *Determination of residues of Deltamethrin (sum of cis-Deltamethrin and its alpha-R-isomer and trans-isomer) in food of plant origin using QuEChERS method and LC-MS/MS tandem mass spectrometry*, based on: EN 15662:2018 Foods of plant origin. Multimethod for the determination of pesticide residues using GC- and LC-based analysis following acetonitrile extraction/partitioning and clean-up by dispersive SPE. Modular QuEChERS-method. Quantification was performed by use of LC-MS/MS detection system. This method meets criteria according SANTE/2020/12830 Rev.2. The study is accepted.

Reference:	KCP 5.1.2
Report	Validation of an analytical method for the determination of residues of Deltamethrin (sum of cis-Deltamethrin and its alpha-R-isomer and trans-isomer) in wheat (grain, plant, straw), ~~Niewelt-~~Niewelt-Stasiak, S., VAL/20/2023
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 Deltamethrin (sum of cis-Deltamethrin and its alpha-R-isomer and trans-isomer) was validated in wheat (grain, plant, straw).
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 (in case of wheat grain and plant) and 0.0025 mg/kg (in case of wheat straw).

Specimen preparation

5 g (grain, plant) / 2 g (straw) of the homogenized sample was weighed into a 50 mL centrifuge tube. 10 mL of deionized water and 10 mL of acetonitrile was added together with 50 µL (grain, plant) / 20 µL (straw) 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 ≤ -18oC for 2 h. After that, the extract 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.

Validation - Results and discussions

Table A 6: Recovery results from method validation of Acetamiprid using the analytical method

Matrix	Analyte	Fortification level (mg/kg) ~~(n = x)~~ n=5	Mean recovery (%)	RSD (%)	Comments
Wheat straw	Cis-Deltamethrin 523.30→506.00	LOQ (0.010)	103.9	9.32	-
	Cis-Deltamethrin 523.30→506.00	10 x LOQ (0.05)	97.0	1.36	-
	Cis-Deltamethrin 523.30→281.00	LOQ (0.005)	102.2	4.06	-
	Cis-Deltamethrin 523.30→281.00	10 x LOQ (0.05)	99.4	0.67	-
	Alpha-R-Deltamethrin 523.30→506.00	LOQ (0.010)	108.4	9.74	-
	Alpha-R-Deltamethrin 523.30→506.00	10 x LOQ (0.05)	96.1	1.26	-
	Alpha-R-Deltamethrin 523.30→281.00	LOQ (0.005)	108.0	2.82	-
	Alpha-R-Deltamethrin 523.30→281.00	10 x LOQ (0.05)	95.6	0.53	-
	Trans-Deltamethrin 523.30→506.00	LOQ (0.010)	99.0	8.65	-
	Trans-Deltamethrin 523.30→506.00	10 x LOQ (0.05)	96.3	1.64	-
	Trans-Deltamethrin 523.30→281.00	LOQ (0.005)	98.5	2.24	-
	Trans-Deltamethrin 523.30→281.00	10 x LOQ (0.05)	97.2	1.25	-
Wheat grain	Cis-Deltamethrin 523.30→506.00	LOQ (0.010)	98.2	2.71	-
	Cis-Deltamethrin 523.30→506.00	10 x LOQ (0.05)	98.6	1.28	-

Matrix	Analyte	Fortification level (mg/kg) ~~(n = x)~~ n=5	Mean recovery (%)	RSD (%)	Comments
	Cis-Deltamethrin 523.30→281.00	LOQ (0.005)	100.8	3.13	-
	Cis-Deltamethrin 523.30→281.00	10 x LOQ (0.05)	101.8	1.20	-
	Alpha-R-Deltamethrin 523.30→506.00	LOQ (0.010)	99.5	1.97	-
	Alpha-R-Deltamethrin 523.30→506.00	10 x LOQ (0.05)	100.8	2.69	-
	Alpha-R-Deltamethrin 523.30→281.00	LOQ (0.005)	100.6	2.95	-
	Alpha-R-Deltamethrin 523.30→281.00	10 x LOQ (0.05)	102.9	2.17	-
	Trans-Deltamethrin 523.30→506.00	LOQ (0.010)	96.2	1.66	-
	Trans-Deltamethrin 523.30→506.00	10 x LOQ (0.05)	96.4	1.08	-
	Trans-Deltamethrin 523.30→281.00	LOQ (0.005)	96.9	2.50	-
	Trans-Deltamethrin 523.30→281.00	10 x LOQ (0.05)	98.3	1.56	-
Wheat plant	Cis-Deltamethrin 523.30→506.00	LOQ (0.010)	100.1	4.45	-
	Cis-Deltamethrin 523.30→506.00	10 x LOQ (0.05)	102.6	1.17	-
	Cis-Deltamethrin 523.30→281.00	LOQ (0.005)	100.0	2.43	-
	Cis-Deltamethrin 523.30→281.00	10 x LOQ (0.05)	103.6	1.31	-
	Alpha-R-Deltamethrin 523.30→506.00	LOQ (0.010)	98.1	7.69	-
	Alpha-R-	10 x LOQ (0.05)	97.2	3.04	-

Matrix	Analyte	Fortification level (mg/kg) ~~(n = x)~~ n=5	Mean recovery (%)	RSD (%)	Comments
	Deltamethrin 523.30→506.00				
	Alpha-R-Deltamethrin 523.30→281.00	LOQ (0.005)	94.4	3.39	-
	Alpha-R-Deltamethrin 523.30→281.00	10 x LOQ (0.05)	96.8	1.84	-
	Trans-Deltamethrin 523.30→506.00	LOQ (0.010)	94.7	4.53	-
	Trans-Deltamethrin 523.30→506.00	10 x LOQ (0.05)	98.8	0.68	-
	Trans-Deltamethrin 523.30→281.00	LOQ (0.005)	96.6	1.70	-
	Trans-Deltamethrin 523.30→281.00	10 x LOQ (0.05)	99.5	0.81	-

~~Table A 2: Methods suitable for the determination of the residues in plant protection product (PPP) CHR/I/ACE 200 SE~~

	Residues
Author(s), year	Niewelt-Stasiak, S., 2023
Principle of method	LC MS/MS
Linearity (linear between mg/L) (correlation coefficient, expressed as r)	The linearity of the detector response was demonstrated by single determination of calibration standards at six concentration levels ranging from 0.5 to 500 ppb for acetamiprid and acetamiprid-N-desmethyl in wheat (grain, plant), and from 0.3 to 500 ppb for acetamiprid and acetamiprid-N-desmethyl in wheat (straw).The coefficient of determination (R^2) were determined. R^2 were greater than 0.990. Calibration covers the range from 30% of the LOQ to 20% above the highest level. Calibration cis-deltamethrin in wheat straw (523.30→506.00): Y= 0.71994X + 2.68998e-04 R^2= 0.99994 Calibration cis-deltamethrin in wheat straw (523.30→281.00): Y= 0.56400X + 4.69872e-04 R^2= 0.99988 Calibration alpha-R-deltamethrin in wheat straw (523.30→506.00): Y= 0.18006X + 1.17914E-04 R^2= 0.99984 Calibration alpha-R-deltamethrin in wheat straw (523.30→281.00): Y= 0.14508X + 2.16253e-04 R^2= 0.99996 Calibration trans-deltamethrin in whole straw (523.30→506.00): Y= 0.40869X -1.37511e-04 R^2= 0.99996

	Residues
	Calibration trans-deltamethrin in wheat straw (523.30→281.00): Y= 0.31211X + 7.16139e-05 R^2= 0.99997 Calibration cis-deltamethrin in wheat grain (523.30→506.00): Y= 0.61911X + 7.78292e-04 R^2= 0.99969 Calibration cis-deltamethrin in wheat grain (523.30→281.00): Y= 0.46507 + 6.33472e-04 R^2= 0.99988 Calibration alpha-R-deltamethrin in wheat grain (523.30→506.00): Y= 0.14139X – 2.06763e-04 R^2= 0.99979 Calibration alpha-R-deltamethrin in wheat grain (523.30→281.00): Y= 0.10841X + 6.43436e-06 R^2= 0.99992 Calibration trans-deltamethrin in whole grain (523.30→506.00): Y= 0.34896X + 2.53571e-04 R^2= 0.99974 Calibration trans-deltamethrin in wheat grain (523.30→281.00): Y= 0.25392X + 2.36758e-04 R^2= 0.99951
Precision, accuracy and uncertainty	Recovery data was generated from five samples fortified at the limit of quantification (LOQ) and five samples fortified at the 10-fold higher concentration than the LOQ (10 x LOQ). Precision of the method was determined as the relative standard deviation (RSD) of recovery at each fortification level. The mean recovery at fortification level of 0.01 mg/kg (LOQ) should be in the range of 60 – 120% with RSD ≤30 %, and recovery at fortification level of 0.10 mg/kg (10xLOQ) should be in the range of 70 – 120% with RSD ≤ 20 %. RSD were determined only during validation process. Grains:

Residues

Cis-deltamethrin

523.30→ 506.00

Fortification level [mg/kg]	Obtained result [mg/kg]	Recovery [%]	Fortification level [mg/kg]	Obtained result [mg/kg]	Recovery [%]
0.010	0.0095	95.0	0.10	0.098	98.0
	0.0097	97.1		0.098	98.1
	0.0097	97.4		0.097	97.2
	0.010	101.9		0.10	100.3
	0.010	99.8		0.10	99.5
Average	0.0098	98.2	Average	0.099	98.6
SD	0.00027	2.66	SD	0.0013	1.27
RSD [%]	2.71		**RSD [%]**	1.28	
Uncertanity [%]	6.5		**Uncertanity [%]**	3.7	

Cis-deltamethrin

523.30→ 281.00

Fortification level [mg/kg]	Obtained result [mg/kg]	Recovery [%]	Fortification level [mg/kg]	Obtained result [mg/kg]	Recovery [%]
0.010	0.0098	97.5	0.10	0.10	100.9
	0.010	100.2		0.10	102.3
	0.0099	98.8		0.10	100.3
	0.011	105.7		0.10	102.5
	0.010	101.5		0.10	103.3
Average	0.010	100.8	Average	0.10	101.8
SD	0.00032	3.15	SD	0.0012	1.22
RSD [%]	3.13		**RSD [%]**	1.20	
Uncertanity [%]	6.4		**Uncertanity [%]**	4.4	

	Residues

Deltamethrin-alpha-R-isomer

523.30→ 506.00

Fortification level [mg/kg]	Obtained result [mg/kg]	Recovery [%]	Fortification level [mg/kg]	Obtained result [mg/kg]	Recovery [%]
0.010	0.010	99.7	0.10	0.10	101.3
	0.0098	98.4		0.098	98.1
	0.010	102.7		0.098	98.3
	0.0099	98.8		0.10	104.7
	0.0098	97.7		0.10	101.5
Average	0.0099	99.5	Average	0.10	100.8
SD	0.00020	1.96	SD	0.0027	2.71
RSD [%]	1.97		**RSD [%]**	2.69	
Uncertanity [%]	4.1		**Uncertanity [%]**	5.6	

Deltamethrin-alpha-R-isomer

523.30→ 281.00

Fortification level [mg/kg]	Obtained result [mg/kg]	Recovery [%]	Fortification level [mg/kg]	Obtained result [mg/kg]	Recovery [%]
0.010	0.0098	97.5	0.10	0.10	103.3
	0.010	100.0		0.10	101.2
	0.011	105.4		0.10	100.4
	0.010	99.0		0.10	103.4
	0.010	100.9		0.11	106.1
Average	0.010	100.6	Average	0.10	102.9
SD	0.00030	2.96	SD	0.0022	2.23
RSD [%]	2.95		**RSD [%]**	2.17	
Uncertanity [%]	6.0		**Uncertanity [%]**	7.2	

Residues

Deltamethrin-trans-isomer

523.30→ 506.00

Fortification level [mg/kg]	Obtained result [mg/kg]	Recovery [%]	Fortification level [mg/kg]	Obtained result [mg/kg]	Recovery [%]
0.010	0.0096	96.4	0.10	0.098	97.6
	0.0095	94.5		0.096	95.6
	0.0096	96.1		0.095	95.2
	0.0099	98.7		0.097	97.3
	0.0095	95.2		0.096	96.4
Average	0.0096	96.2	Average	0.096	96.4
SD	0.00016	1.60	SD	0.0010	1.04
RSD [%]	1.66		**RSD [%]**	1.08	
Uncertanity [%]	8.3		**Uncertanity [%]**	7.5	

Deltamethrin-trans-isomer

523.30→ 281.00

Fortification level [mg/kg]	Obtained result [mg/kg]	Recovery [%]	Fortification level [mg/kg]	Obtained result [mg/kg]	Recovery [%]
0.010	0.0098	97.9	0.10	0.10	100.1
	0.0094	93.5		0.097	97.0
	0.0096	95.9		0.096	96.4
	0.010	100.1		0.098	98.4
	0.0097	97.2		0.099	99.2
Average	0.0097	96.9	Average	0.098	98.3
SD	0.00024	2.43	SD	0.0015	1.54
RSD [%]	2.50		**RSD [%]**	1.56	
Uncertanity [%]	7.9		**Uncertanity [%]**	4.7	

Straw:

Residues

Cis-deltamethrin

523.30→ 506.00

Fortification level [mg/kg]	Obtained result [mg/kg]	Recovery [%]	Fortification level [mg/kg]	Obtained result [mg/kg]	Recovery [%]
0.010	0.011	111.5	0.10	0.099	98.7
	0.011	107.9		0.096	96.5
	0.011	113.2		0.096	95.8
	0.0094	93.7		0.096	95.8
	0.0093	93.4		0.098	97.9
Average	0.010	103.9	Average	0.097	97.0
SD	0.00097	9.69	SD	0.0013	1.32
RSD [%]	9.32		**RSD [%]**	1.36	
Uncertainty [%]	20.2		**Uncertainty [%]**	6.7	

Cis-deltamethrin

523.30→ 281.00

Fortification level [mg/kg]	Obtained result [mg/kg]	Recovery [%]	Fortification level [mg/kg]	Obtained result [mg/kg]	Recovery [%]
0.010	0.011	106.0	0.10	0.10	100.2
	0.011	106.0		0.10	99.8
	0.010	103.0		0.099	98.5
	0.0099	98.6		0.099	99.0
	0.0097	97.1		0.099	99.4
Average	0.010	102.2	Average	0.099	99.4
SD	0.00041	4.14	SD	0.00066	0.66
RSD [%]	4.06		**RSD [%]**	0.67	
Uncertainty [%]	9.2		**Uncertainty [%]**	1.8	

Residues

Deltamethrin alpha R isomer

523.30→ 506.00

Fortification level [mg/kg]	Obtained result [mg/kg]	Recovery [%]	Fortification level [mg/kg]	Obtained result [mg/kg]	Recovery [%]
0.010	0.010	101.5	0.10	0.097	96.7
	0.012	116.9		0.096	95.6
	0.0094	94.2		0.096	95.6
	0.011	109.9		0.095	94.7
	0.012	119.5		0.098	97.8
Average	0.011	108.4	Average	0.096	96.1
SD	0.0011	10.56	SD	0.0012	1.21
RSD [%]	9.74		**RSD [%]**	1.26	
Uncertanity [%]	25.7		**Uncertanity [%]**	8.2	

Deltamethrin alpha R isomer

523.30→ 281.00

Fortification level [mg/kg]	Obtained result [mg/kg]	Recovery [%]	Fortification level [mg/kg]	Obtained result [mg/kg]	Recovery [%]
0.010	0.011	112.0	0.10	0.095	95.3
	0.011	110.4		0.096	96.1
	0.011	106.8		0.095	95.1
	0.010	104.9		0.095	95.2
	0.011	106.0		0.096	96.2
Average	0.011	108.0	Average	0.096	95.6
SD	0.00030	3.04	SD	0.00051	0.51
RSD [%]	2.82		**RSD [%]**	0.53	
Uncertanity [%]	17.0		**Uncertanity [%]**	8.9	

Residues

Deltamethrin trans isomer

523.30→ 506.00

Fortification level [mg/kg]	Obtained result [mg/kg]	Recovery [%]	Fortification level [mg/kg]	Obtained result [mg/kg]	Recovery [%]
0.010	0.011	113.1	0.10	0.099	98.9
	0.0099	99.1		0.095	94.8
	0.0097	96.9		0.095	95.4
	0.0090	89.9		0.096	96.4
	0.0096	96.1		0.096	95.9
Average	0.0099	99.0	Average	0.096	96.3
SD	0.00086	8.57	SD	0.0016	1.58
RSD [%]	8.65		**RSD [%]**	1.64	
Uncertanity [%]	17.4		**Uncertanity [%]**	8.1	

Deltamethrin trans isomer

523.30→ 281.00

Fortification level [mg/kg]	Obtained result [mg/kg]	Recovery [%]	Fortification level [mg/kg]	Obtained result [mg/kg]	Recovery [%]
0.010	0.0099	99.5	0.10	0.099	99.4
	0.010	100.9		0.097	96.6
	0.010	99.7		0.097	96.6
	0.0096	95.6		0.097	97.0
	0.0097	96.8		0.097	96.6
Average	0.0098	98.5	Average	0.097	97.2
SD	0.00022	2.21	SD	0.0012	1.22
RSD [%]	2.24		**RSD [%]**	1.25	
Uncertanity [%]	5.4		**Uncertanity [%]**	6.1	

Whole plant:

Residues

Cis-deltamethrin

523.30→ 506.00

Fortification level [mg/kg]	Obtained result [mg/kg]	Recovery [%]	Fortification level [mg/kg]	Obtained result [mg/kg]	Recovery [%]
0.010	0.0095	95.1	0.10	0.10	102.1
	0.010	102.4		0.10	103.2
	0.0096	96.0		0.10	100.8
	0.011	105.8		0.10	103.7
	0.010	101.0		0.10	103.4
Average	0.010	100.1	Average	0.10	102.6
SD	0.00045	4.45	SD	0.0012	1.20
RSD [%]	4.45		**RSD [%]**	1.17	
Uncertainty [%]	8.9		**Uncertainty [%]**	5.7	

Cis-deltamethrin

523.30→ 281.00

Fortification level [mg/kg]	Obtained result [mg/kg]	Recovery [%]	Fortification level [mg/kg]	Obtained result [mg/kg]	Recovery [%]
0.010	0.0099	99.2	0.10	0.10	102.7
	0.010	101.7		0.10	103.7
	0.0096	96.2		0.10	102.0
	0.010	102.3		0.11	105.6
	0.010	100.7		0.10	104.1
Average	0.010	100.0	Average	0.10	103.6
SD	0.00024	2.43	SD	0.0014	1.36
RSD [%]	2.43		**RSD [%]**	1.31	
Uncertainty [%]	4.9		**Uncertainty [%]**	7.7	

Residues

Deltamethrin-alpha-R-isomer

523.30→ 506.00

Fortification level [mg/kg]	Obtained result [mg/kg]	Recovery [%]	Fortification level [mg/kg]	Obtained result [mg/kg]	Recovery [%]
0.010	0.0095	95.3	0.10	0.094	94.0
	0.0092	91.6		0.096	95.6
	0.0094	93.9		0.096	96.0
	0.011	110.8		0.10	101.1
	0.0099	98.8		0.099	99.5
Average	0.0098	98.1	Average	0.097	97.2
SD	0.00075	7.55	SD	0.0030	2.95
RSD [%]	7.69		**RSD [%]**	3.04	
Uncertainty [%]	15.9		**Uncertainty [%]**	8.2	

Deltamethrin-alpha-R-isomer

523.30→ 281.00

Fortification level [mg/kg]	Obtained result [mg/kg]	Recovery [%]	Fortification level [mg/kg]	Obtained result [mg/kg]	Recovery [%]
0.010	0.0094	93.7	0.10	0.095	94.8
	0.0091	90.5		0.095	95.3
	0.0093	93.3		0.097	97.0
	0.0095	95.2		0.099	99.3
	0.0099	99.3		0.097	97.2
Average	0.0094	94.4	Average	0.097	96.8
SD	0.00032	3.20	SD	0.0018	1.78
RSD [%]	3.39		**RSD [%]**	1.84	
Uncertainty [%]	13.1		**Uncertainty [%]**	7.5	

	Residues

Deltamethrin-trans-isomer

523.30→ 506.00

Fortification level [mg/kg]	Obtained result [mg/kg]	Recovery [%]	Fortification level [mg/kg]	Obtained result [mg/kg]	Recovery [%]
0.010	0.0092	92.5	0.10	0.099	98.6
	0.010	99.9		0.099	99.4
	0.0089	89.1		0.098	98.2
	0.0098	97.9		0.10	99.6
	0.0094	94.0		0.098	98.2
Average	0.0095	94.7	Average	0.099	98.8
SD	0.00043	4.29	SD	0.00067	0.67
RSD [%]	4.53		**RSD [%]**	0.68	
Uncertainty [%]	14.0		**Uncertainty [%]**	2.8	

Deltamethrin-trans-isomer

523.30→ 281.00

Fortification level [mg/kg]	Obtained result [mg/kg]	Recovery [%]	Fortification level [mg/kg]	Obtained result [mg/kg]	Recovery [%]
0.010	0.0094	94.3	0.10	0.099	99.1
	0.0098	97.7		0.100	100.4
	0.0095	95.4		0.098	98.4
	0.0098	97.9		0.10	100.2
	0.0098	97.8		0.10	99.6
Average	0.0097	96.6	Average	0.10	99.5
SD	0.00016	1.64	SD	0.00081	0.81
RSD [%]	1.70		**RSD [%]**	0.81	
Uncertainty [%]	7.6		**Uncertainty [%]**	1.9	

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. No interferences at above 30% of the LOQ were detected at the retention time of active substance in matrix blank samples.
Matrix Effects	For acetamiprid and acetamiprid-N-desmethyl matrix effects in wheat grain calculated using equation are <±20%, in wheat straw and plant matrix effect calculated using equation exceed ±20%. To compensate matrix effect, there was used matrix-matched calibrations.

LOQ
LOD

Limit of quantification (LOQ)	-	0.010 mg/kg
Limit of detection (LOD)	-	0.0025 mg/kg

Extraction 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 took place and samples were analyzed within 24 hours of extraction. Then extract stability is not considered to be an issue.
Comment	The analytical method for determining the residues of deltamethrin (sum of cis-Deltamethrin and its apha-R-isomer and trans-isomer) in wheat (grain, plant, straw) meets the criteria of SANTE/2020/12830 Rev.2, 14 February 2023 documents in terms of precision, accuracy and uncertainty. The method was validated over the concentration range of 0.010-0.10 mg/kg for each matrix with a

	Residues
	limit of quantification of 0.010 mg/kg for each of them. Limit of detection was established at 0.002 mg/kg for wheat (grain, plant) and 0.0025 mg/kg for wheat (straw).

Conclusion

The analytical method for determining the residues of deltamethrin (sum of cis-Deltamethrin and its apha-R-isomer and trans-isomer) in wheat (grain, plant, straw) meets the criteria of SANTE/2020/12830 Rev.2, 14 February 2023 documents in terms of precision, accuracy and uncertainty. The method was validated over the concentration range of 0.010-0.10 mg/kg for each matrix with a limit of quantification of 0.010 mg/kg for each of them. Limit of detection was established at 0.002 mg/kg for wheat (grain, plant) and 0.0025 mg/kg for wheat (straw).

Comments of zRMS: Study No. R C1145	The method was successfully validated for the determination of residues of deltamethrin and its alpha-R-isomer and trans-isomer metabolites in sugar beet leaves with top and sugar beet roots. The LOD was 0.75 ng/mL for deltamethrin, alpha-R-isomer of deltamethrin and trans-deltamethrin in sugar beet leaves with top, sugar beet roots and sugar beet roots (corresponding to 0.003 mg/kg). The LOQ was 0.01 mg/kg for deltamethrin, alpha-R-isomer of deltamethrin and trans-deltamethrin in sugar beet leaves with top and sugar beet roots. For samples fortified at 0.01 mg/kg and 0.1 mg/kg, mean recoveries were within the acceptable range 70-120% with RSD less than 20% for both primary and confirmatory methods. The stability of extracts during frozen storage was investigated. Deltamethrin, alpha-R-isomer of deltamethrin and trans-deltamethrin residues were stable in sugar beet leaves with top and sugar beet roots extracts for at least 15 days and 17 days respectively of frozen storage. This method meets criteria according SANTE/2020/12830 Rev.2. The study is accepted.

Reference:	KCP 5.1.2
Report	Validation of the Analytical Method for the Analysis of Deltamethrin and its alpha-R-isomer and trans-isomer metabolites in Sugar beet. Anadiag, Véronique Faessel, R C1145
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 objective of the study was to validate the analytical method for the analysis of Deltamethrin and its

alpha-R-isomer and trans-isomer metabolites in Sugar beet leaves with top and Sugar beet roots.

Specimen preparation
The specimens were prepared according to ANADIAG SOPs.
The specimen was blended with dry ice and placed at ≤ -18°C for at least 12 hours for sublimation of dry ice. The amount required by the analytical method (5 g) was weighed from this homogeneous matrix.
Mobile phase A: HPLC H_2O / HPLC Methanol 80/20 + 5mM ammonium acetate.
In a 1 L bottle, 385 mg of ammonium acetate were dissolved in 800 mL of HPLC H_2O, 200 mL of HPLC methanol were added and then homogenized.

Mobile phase B: HPLC H_2O / HPLC Methanol 10/90 + 5mM ammonium acetate.
In a 1 L bottle, 385 mg of ammonium acetate were dissolved in 100 mL of HPLC H_2O, 900 mL of HPLC methanol were added and then homogenized.

Validation - Results and discussions

Table A 7: Recovery results from method validation of Deltemethrin in sugar beets using the analytical method

Recoveries in sugar beet leaves with top

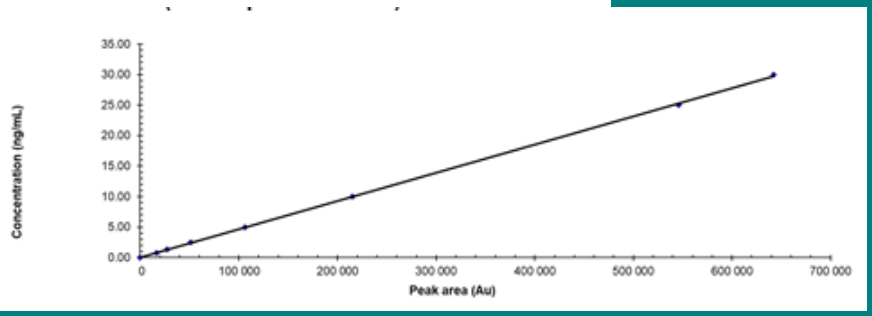
Sample ANADIAG No.	Fortification level (mg/kg)	Deltamethrin % Recovery Primary method 522.9 > 506.0	Alpha-R-isomer of deltamethrin % Recovery Primary method 522.9 > 506.0	Trans-deltamethrin % Recovery Primary method 522.9 > 506.0
C1145 01 01 KA	0.01	95.8%	96.8%	90.2%
C1145 01 01 LA		98.2%	100.6%	95.2%
C1145 01 01 MA		106.9%	108.3%	96.4%
C1145 01 01 NA		105.3%	107.5%	103.5%
C1145 01 01 OA		97.9%	104.7%	86.6%
C1145 01 01 PA	0.10	90.3%	95.0%	87.9%
C1145 01 01 QA		80.0%	82.1%	76.2%
C1145 01 01 RA		94.9%	97.2%	91.6%
C1145 01 01 SA		93.1%	94.7%	86.2%
C1145 01 01 TA		100.0%	104.4%	97.8%

Recoveries in sugar beet roots

Sample ANADIAG No.	Fortification level (mg/kg)	Deltamethrin % Recovery Primary method 522.9 > 506.0	Alpha-R-isomer of deltamethrin % Recovery Primary method 522.9 > 506.0	Trans-deltamethrin % Recovery Primary method 522.9 > 506.0
C1145 02 01 AA	0.01	79.6%	76.6%	77.6%
C1145 02 01 BA		80.4%	78.2%	80.3%
C1145 02 01 CA		83.8%	79.7%	80.2%
C1145 02 01 DA		75.6%	70.9%	70.1%
C1145 02 01 EA		74.2%	74.5%	76.8%
C1145 02 01 FA	0.10	72.5%	76.3%	72.2%

C1145 02 01 GA		**77.7%**	**80.5%**	**74.0%**
C1145 02 01 HA		**80.1%**	**85.0%**	**79.4%**
C1145 02 01 IA		**80.4%**	**83.1%**	**74.5%**
C1145 02 01 JA		**81.6%**	**82.2%**	**76.2%**

Table A 2: Methods suitable for the determination of the Deltamethrin residues in sugar beet.

	Residues
Author(s), year	Veronique Faessel, 2022
Principle of method	LC MS/MS
Linearity (linear between mg/L) (correlation coefficient, expressed as r)	The analytical calibration consisted of matrix-matched calibration solutions of deltamethrin, alpha-R-isomer of deltamethrin and trans-deltamethrin, at least at 5 concentration levels, ranged from 0.75 ng/mL to 30 ng/mL (corresponding to 0.003 to 0.12 mg/kg) for sugar beet leaves with top and of calibration solutions in acetonitrile of deltamethrin, alpha-R-isomer of deltamethrin and trans-deltamethrin, at least at 5 concentration levels, ranged from 0.75 ng/mL to 30 ng/mL (corresponding to 0.003 to 0.12 mg/kg) for sugar beet roots. The calibration covered two orders of magnitude and ranged from 30% of the LOQ to 20% above the highest level. Standard concentrations were distributed evenly over the full calibration range. Calibration curves were run for each analysis sequence for both primary and confirmatory methods. The linear correlation coefficients were > 0.990, showing good linearity with regression residuals randomly distributed for both primary and confirmatory methods. Please find below example for sugar beet leaves with top: Calibration function: C (Concentration) = 4.6294E-05 x S (Peak area) + 0.037 r = 0.99992 
Precision, accuracy and uncertainty	**Untreated samples** Two untreated samples were extracted concurrently with fortified samples for each matrix. Residue levels were reported below. No interferences above 30% of the limit of quantification were recorded.

Residues

Sample ANADIAG No.	Matrix	Deltamethrin amount found (mg/kg)	Alpha-R-isomer of deltamethrin amount found (mg/kg)	Trans-deltamethrin amount found (mg/kg)
		Primary method	Primary method	Primary method
		522.9 > 506.0	522.9 > 506.0	522.9 > 506.0
C1145 01 01 31	Sugar beet leaves with top	NDR	NDR	NDR
C1145 01 01 41		NDR	NDR	NDR
C1145 02 01 11	Sugar beet roots	NDR	NDR	NDR
C1145 02 01 21		NDR	NDR	NDR

NDR: No detectable residues (residues below the limit of detection)

Fortified samples

For the primary method, recovery and repeatability (as precision, % RSD) tests were performed by untreated control samples spiked with deltamethrin, alpha-R-isomer of deltamethrin and trans-deltamethrin before extraction at the following fortification levels for each matrix:
- LOQ (5 samples),
- 10 x LOQ (5 samples).

Sugar beet leaves

Fortification level (mg/kg)	Deltamethrin % Recovery	Alpha-R-isomer of deltamethrin % Recovery	Trans-deltamethrin % Recovery
	Primary method	Primary method	Primary method
	522.9 > 506.0	522.9 > 506.0	522.9 > 506.0
0.01	95.8%	96.8%	90.2%
	98.2%	100.6%	95.2%
	106.9%	108.3%	96.4%
	105.3%	107.5%	103.5%
	97.9%	104.7%	86.6%
0.10	90.3%	95.0%	87.9%
	80.0%	82.1%	76.2%
	94.9%	97.2%	91.6%
	93.1%	94.7%	86.2%
	100.0%	104.4%	97.8%

Sugar beet roots

	Residues

Fortification level (mg/kg)	Deltamethrin % Recovery	Alpha-R-isomer of deltamethrin % Recovery	Trans-deltamethrin % Recovery
	Primary method	Primary method	Primary method
	522.9 > 506.0	522.9 > 506.0	522.9 > 506.0
0.01	79.6%	76.6%	77.6%
	80.4%	78.2%	80.3%
	83.8%	79.7%	80.2%
	75.6%	70.9%	70.1%
	74.2%	74.5%	76.8%
0.10	72.5%	76.3%	72.2%
	77.7%	80.5%	74.0%
	80.1%	85.0%	79.4%
	80.4%	83.1%	74.5%
	81.6%	82.2%	76.2%

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. No interferences at above 30% of the LOQ were detected at the retention time of active substance in matrix blank samples.
Matrix Effects	No matrix effects (enhancement or suppression) on the instrument response were considered significant. Nevertheless, matrix-matched calibration solutions for sugar beet leaves with top and calibration solutions prepared in solvent for sugar beet roots were used for calibration.
LOQ **LOD**	Limit of detection The limit of detection (LOD) was expressed as lowest calibration standard. The LOD was 0.75 ng/mL for deltamethrin, alpha-R-isomer of deltamethrin and trans-deltamethrin in sugar beet leaves with top, sugar beet roots and sugar beet roots (corresponding to 0.003 mg/kg). Limit of quantification The limit of quantification (LOQ) was the lowest validated level with sufficient recovery and precision. The LOQ was 0.01 mg/kg for deltamethrin, alpha-R-isomer of deltamethrin and trans-deltamethrin in sugar beet leaves with top and sugar beet roots.
Extraction stability	The stability of extracts during frozen storage was investigated. Deltamethrin, alpha-R-isomer of deltamethrin and trans-deltamethrin residues were stable in sugar beet leaves with top and sugar beet roots extracts for at least 15 days and 17 days respectively of frozen storage.
Comment	For the method validation purpose, drinking water was spiked at two concentration levels. The validity criteria linearity, accuracy, precision and repeatability were fulfilled for analysis of the test item in sugar beet.

Conclusion

For the method validation purpose, drinking water was spiked at two concentration levels.
The validity criteria linearity, accuracy, precision and repeatability were fulfilled for analysis of the test item in sugar beet.

A 2.2.2.2 Description of analytical methods for the determination of residues in

animal matrices (KCP 5.2)

A 2.2.2.2.1 Analytical method 1

A 2.2.2.2.1.1 Method validation

Comments of zRMS: Study No. R C1199	The method was successfully validated for the determination of residues of deltamethrin and its alpha-R-isomer and trans-isomer in honey and pollen. This method meets criteria according SANTE/2020/12830 Rev.2. The study is accepted.

Reference:	KCP 5.2
Report	Validation of the Analytical Method for the analysis of Deltamethrin and its alpha-R-isomer and trans-isomer in Honey and Pollen, V. Faessel, R C1199, 2022
Guideline(s):	SANTE/2020/12830 Rev.2
Deviations:	NO
GLP:	YES
Acceptability:	YES

Materials and methods

The objective of the study was to validate the analytical method for the analysis of Deltamethrin and its alpha-R-isomer and trans-isomer in honey and pollen.
The method under discussion describes the determination of deltamethrin, alpha-R-isomer of deltamethrin and trans-deltamethrin in honey and pollen. The method was validated at 0.01 mg/kg for each matrix and each analyte.
The analytical method was validated according to SANTE/2020/12830, Rev.1.

Specimen preparation

The specimens were prepared according to ANADIAG SOP PG 0115.
The specimen of honey was allowed to thaw at ≈ +4°C. After homogenization, the amount required by the analytical method (1 g) was weighed from this homogeneous matrix.
After mixing of the specimen of pollen, the amount required by the analytical method (1 g) was weighed from this homogeneous matrix.
Mobile phase A: HPLC H2O / HPLC Methanol 80/20 + 5mM ammonium acetate.
In a 1 L bottle, 385 mg of ammonium acetate were dissolved in 800 mL of HPLC H2O, 200 mL of HPLC methanol were added and then homogenized.
Mobile phase B: HPLC H2O / HPLC Methanol 10/90 + 5mM ammonium acetate.
In a 1 L bottle, 385 mg of ammonium acetate were dissolved in 100 mL of HPLC H2O, 900 mL of HPLC methanol were added and then homogenized.
Acetonitrile / ultra-pure H2O 85/15 solution
Transfer 425 mL of acetonitrile into a 500 mL volumetric flask.
Add 75 mL of ultra-pure water. Mix well.

Chromatographic parameters

Analytical conditions LC-MS/MS – 6500 / n° MA_1738-01

Column

Description	**AQUASIL C18**	Supplier	**thermoscientific**	Particles	**3 µm**
Internal diam. x length	**3 X 150 mm**	Supplier reference	**77503-153030**	Comment	-
Development Column ANADIAG Number	**293**	Stationary Phase	**C18**		

Mobile phase

A =	**HPLC H_2O/HPLC methanol 80:20 + 5 mM ammonium acetate**
B =	**HPLC H_2O/HPLC methanol 10:90 + 5 mM ammonium acetate**

Sample temperature	**+15°C**
Column temperature	**+40°C**

Elution

Elution	Time min	Flow mL/min	Composition (%) A	Composition (%) B	Curve* (type)	Elution	Time min	Flow mL/min	Composition (%) A	Composition (%) B	Curve* (type)
Pg1	**0.00**	**0.4**	**50**	**50**	**0**	Pg5	**17.00**	**0.4**	**50**	**50**	**0**
Pg2	**3.00**	**0.4**	**15**	**85**	**0**	Pg6	-	-	-	-	-
Pg3	**14.00**	**0.4**	**15**	**85**	**0**	Pg7	-	-	-	-	-
Pg4	**14.50**	**0.4**	**50**	**50**	**0**	Pg8	-	-	-	-	-

*0=linear

Detector

IONISATION mode	ES		
Polarity	Pos		
Collision gas setting (CAD)	Nitrogen set at 7.00 (arbitrary units)	Gas flow 1 (GS1)	Air set at 50 (arbitrary units)
Curtain gas (CUR)	Nitrogen set at 30.00 (arbitrary units)	Gas flow 2 (GS2)	Air set at 60 (arbitrary units)
Ionspray turbo heater (TEM)	350°C		
Capillary voltage (IS)	5500V		

Active ingredient(s)	Dwell time (ms)	Declustering Potential DP (V)	Entrance Potential EP (V)	Collision Energy CE (V)	Collision Cell Exit Potential CXP (V)	TRANSITIONS
Trans-Deltamethrin + Deltamethrin + Alpha-R-isomer of deltamethrin	100	36.00	10.00	13.00	24.00	**522.900 > 506.000****
	100	36.00	10.00	21.00	16.00	**522.900 > 280.800**
	100	36.00	10.00	21.00	14.00	**520.900 > 278.800**
	100	36.00	10.00	15.00	26.00	**520.900 > 504.000**

** quantification transition

Valco valve

Prog.	Time (min.)	Position	Prog.	Time (min.)	Position
Pg1	**0.0**	**A (Waste)**	Pg3	**16.9**	**A (Waste)**
Pg2	**0.1**	**B (Detector)**	Pg4	-	-

Date of application of analytical conditions: 16/09/2021

Study	C1199	Column ANADIAG number	293
Matrix	All	Retention time	Trans-Deltamethrin: ≈ 11.4 min. Deltamethrin: ≈ 11.7 min. Alpha-R-isomer of deltamethrin: ≈ 12.2 min.
Sample temperature	+15 °C	Injected volume	5 µL

Validation - Results and discussions

Table A 8: Recovery results from method validation of Acetamiprid using the analytical method

Matrix	Analyte	Fortification level (mg/kg) ~~(n = x)~~ n=5	Mean recovery (%)	RSD (%)	Comments
Honey	Deltamethrin	LOQ (0.01)	110.8	3.0	-

Matrix	Analyte	Fortification level (mg/kg) ~~(n = x)~~ n=5	Mean recovery (%)	RSD (%)	Comments
		10 x LOQ (0.1)	108.2	1.8	-
	Alpha-R isomer of deltamethrin	LOQ (0.01)	110.4	7.7	-
		10 x LOQ (0.1)	109.2	1.6	-
	Transdeltamethrin	LOQ (0.01)	114.1	8.8	-
		10 x LOQ (0.1)	107.9	2.1	-
Pollen	Deltamethrin	LOQ (0.01)	92.0	3.9	-
		10 x LOQ (0.1)	95.2	3.7	-
	Alpha-R isomer of deltamethrin	LOQ (0.01)	96.7	11.8	-
		10 x LOQ (0.1)	107.0	6.4	-
	Transdeltamethrin	LOQ (0.01)	95.9	6.0	-
		10 x LOQ (0.1)	102.3	4.3	-

Table A 2: Methods suitable for the determination of the residues in plant protection product (PPP) CHR/I/ACE 200 SE

	Residues
Author(s), year	V. Faessel, 2022
Principle of method	LC MS/MS
Linearity (linear between mg/L) (correlation coefficient, expressed as r)	The linearity of the method was checked by injecting into the analytical system matrix-matched The analytical calibration consisted of matrix-matched calibration solutions of deltamethrin, alpha-Risomer of deltamethrin and trans-deltamethrin, at least at 5 concentration levels, ranged from 0.3 ng/mL to 12 ng/mL (corresponding to 0.003 to 0.12 mg/kg). The linear correlation coefficients were > 0.990, showing good linearity with regression residuals randomly distributed for both primary and confirmatory methods. The linear correlation coefficients were > 0.990, showing a good linearity. Calibration covers the range from 30% of the LOQ to 20% above the highest level. Calibration curve for Deltamethrin in honey (522.9 > 506.0): C= 4.0301E-05 × S (Peak area) + 0.067 R^2= 0.99934 Calibration curve for Deltamethrin in honey (522.9 > 280.8): C= 5.0364E-05 × S (Peak area) + 0.031 R^2= 0.99918 Calibration curve for Alpha-R-isomer of Deltamethrin in honey (522.9 > 506.0): C= 3.9240E-05 × S (Peak area) + 0.022 R^2= 0.99936 Calibration curve for Alpha-R-isomer of Deltamethrin in honey (522.9 > 280.8): C= 4.4535E-05 × S (Peak area) + 0.033

	Residues
	R^2= 0.99952 Calibration curve for Trans- Deltamethrin in honey (522.9 > 506.0): C= 9.4558E-05 × S (Peak area) + 0.001 R^2= 0.99754 Calibration curve for Trans- Deltamethrin in honey (522.9 > 280.8): C= 1.3149E-04 × S (Peak area) + 0.048 R^2= 0.99556 Calibration curve for Deltamethrin in pollen (522.9 > 506.0): C= 3.7729E-05 × S (Peak area) + 0.012 R^2= 0.99954 Calibration curve for Deltamethrin in pollen (522.9 > 280.8): C= 4.7798E-05 × S (Peak area) – 0.149 R^2= 0.99962 Calibration curve for Alpha-R-isomer of Deltamethrin in pollen (522.9 > 506.0): C= 3.9912E-05 × S (Peak area) – 0.169 R^2= 0.99948 Calibration curve for Alpha-R-isomer of Deltamethrin in pollen (522.9 > 280.8): C= 4.4327E-05 × S (Peak area) – 0.174 R^2= 0.99862 Calibration curve for Trans- Deltamethrin in pollen (522.9 > 506.0): C= 9.5851E-05 × S (Peak area) – 0.037 R^2= 0.99942 Calibration curve for Trans- Deltamethrin in pollen (522.9 > 280.8): C= 1.3944E-04 × S (Peak area) – 0.088 R^2= 0.99986
Precision, accuracy and uncertainty	Recovery data was generated from five samples fortified at the limit of quantification (LOQ) and five samples fortified at the 10-fold higher concentration than the LOQ. Precision of the method was determined as the relavite 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.
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. No interferences at above 30% of the LOQ were detected at the retention time of active substance in matrix blank samples.
Matrix Effects	Matrix effects enhancement on the instrument response are considered significant when experimental amount found is out of the range 80-120% of the theoretical concentration. Matrix effects (enhancement) on the instrument response were considered significant for transdeltamethrin in pollen. Consequently, matrix-matched calibration solutions were used for calibration.
LOQ **LOD**	The limit of quantification (LOQ) was the lowest validated level with sufficient recovery and precision. The LOQ was 0.01 mg/kg for deltamethrin, alpha-R-isomer of deltamethrin and

	Residues
	trans-deltamethrin in honey and pollen. The limit of detection (LOD) was expressed as lowest calibration standard. The LOD was 0.3 ng/mL for deltamethrin, alpha-R-isomer of deltamethrin and trans-deltamethrin in honey and pollen (corresponding to 0.003 mg/kg).
Extraction stability	Spiked samples at 10xLOQ level were stored frozen for 15 days for honey and for pollen after samples extraction, and analysed against freshly prepared standards to check the stability of the final extracts. The stability of the analyte(s) in the final extracts was sufficiently proven according to the SANTE/2020/12830, Rev.1 guideline, as mean recoveries in the fortified samples were within the range 70-120%, measured against freshly prepared standards. Deltamethrin, alpha-R-isomer of deltamethrin and trans-deltamethrin residues were stable in honey and pollen extracts for at least 15 days of frozen storage.
Extraction efficiency	In accordance to SANTE/2017/10632 Rev. 4: Extraction efficiency should be addressed if for a product authorization a different analytical methodology (in methods for risk assessment and/or monitoring) is used, compared to that of the approval/renewal procedure of the active substance. Used method is similar to one presented in RAR for estimating residues in animal matrices, therefore in applicants opinion no further testing is required.
Comment	The validation parameters are within the acceptance range and fulfil EU requirements given in SANTE/2020/12830 Rev.2.

Conclusion

The method was successfully validated for determination of all analytes in all matrices with an LOQ of 0.01 mg/kg according to the guidance document(s) SANTE/2020/12830 Rev.2. With regard to selectivity, accuracy and precision, the analytical methods were applied successfully for each analytical set when analysing the specimens of the study.