FINAL REGISTRATION REPORT

Part B

Section 5

Analytical Methods

Detailed summary of the risk assessment

Product code: SHA 5500 A

Product name(s): ASSET ~~(ZUXION)~~

Chemical active substance:

Acetamiprid, 200 g/kg

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

Applicant: Sharda Cropchem España S.L.

Submission date: April 2020

Finalisation date: 10/2020; 07/2021

Version history

When	What
October 2020	Applicant update
July 2021	Final Version

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5 Analytical methods

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.
Noticed data gaps are:

- None

Commodity/crop	Supported/ Not supported
High oil content (Oilseed rape)	Supported
High water content (Pome fruits)	Supported

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

5.2.1 Analysis of the plant protection product (KCP 5.1.1)

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

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

Comments of zRMS:	The analytical method meets the criteria of specificity, linearity, precision and accuracy. The method is acceptable and is suitable for determination of Acetamiprid in plant protection product SHA 5500 A (Asset/~~Zuxion~~).

Reference:	KCP 5.1.1
Report	Physico-Chemical Characterization of ACETAMIPRID 20% SG. J. A. Escudero, 2016, Report No. 15-4150-03
Guideline(s):	Yes (SANCO/3030/99 rev. 4)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Samples preparation

Preparation of reference item solutions
22.96 mg of acetamiprid reference item were weighed on a 25 ml volumetric flask and bought to volume with acetonitrile:water HPLC (50:50). Sonicate. From this solution four solutions were prepared to develop the calibration curve to quantify the formulation.

Sample	Colume (ml) of Cal M	Final volume with acetonitrile:water HPLC (50:50) (ml)	Level of Acetamiprid (mg/L)
Cal-1	1	10	91.74816
Cal-2	2	10	183.49632
Cal-3	3	10	275.2448
Cal-4	5	10	458.7408

Preparation of test item solutions
Weigh (to the nearest 0.1 mg) 45-55 mg of test item into a 50 ml volumetric flask and up to volume with acetonitrile:water HPLC (50:50). Weigh five independent samples of test item.

Preparation of spiked solutions

Test item sample
305.37 mg of test item were weighed into a 50 ml volumetric flask and dilute to volume with acetonitrile:water HPLC (50:50)

80% recovery
Pipette 1 ml of the test item solution into three 10 ml volumetric flask and add 0.5 ml of a stock solution of acetamiprid to each flask as to obtain a concentration of 170.220744 mg/l. Up to volume with acetonitrile:water HPLC (50:50)

120% Recovery
Pipette 1 ml of the test item solution into three 10 ml volumetric flask and add 1.5 ml of a stock solution of acetamiprid to each flasks as to obtain a concentration of 261.968904 mg/l. Up to volume with acetonitrile:water HPLC (50:50)

Specificity
Specificity was demonstrated using HPLC-UV. There are no interferences from other substances present in the formulation with peak corresponding to the acetamiprid.

Linearity
The linearity of response of the analyte has been demonstrated over the nominal analyte concentration ± 20% at least. ~~Five~~ Four concentrations have been measured with duplicate measurements. The analytical methos shows an excellent correlation between response and analytical concentration over the range 91.748 – 458.741 mg/l of acetamiprid. The correlation coefficient (R^2) was 0.99981.

Precision (Repeatability)
To show the system precision, five independent samples were injected by duplicate. The average is 20.36 ± 0.07 % w/w, acetamiprid. Relative standard deviation %RSD = 0.36 and is lower than %RSD based on the Horwitz equation (1.70%), so it shows an excellent repeatability of the analytical method.

Accuracy
Determination of accuracy is based on the recovery of known amounts of analyte from a representative sample matrix, at level of 80% and 120% of the certified value. The average recovery is 100.5 ± 0.6 % (RSD% = 0.6) for acetamiprid. This result shows that the recovery value is adequate because it is in the confidence interval 98-102%.

Validation - Results and discussions

Table 5.2-1: Methods suitable for the determination of active substances Acetamiprid in plant protection product ~~ZUXION~~ ASSET/SHA 5500 A

	Acetamiprid
Author(s), year	Jose Angel Escudero, 2016
Principle of method	Liquid chromatography (HPLC) and UV detection
Linearity (linear between mg/L / % range of the declared content) (correlation coefficient, expressed as r^2)	Linear between 91.75 mg/L and 458.74 mg/L R^2: 0.99981
Precision – Repeatability Mean n = 5 (%RSD)	RSD% = 0.36
Accuracy n = 6 (% Recovery)	100.5 ± 0.6 %
Interference/ Specificity	No interferences
Comment	-

Conclusion

The method of analysis of Acetamiprid in the test item has been conveniently validated. The method is suitable to determine the Acetamiprid content in Acetamiprid 20% SG (SHA 5500 A).

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

There are no relevant impurities.

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

Not relevant.

5.2.1.4 Applicability of existing CIPAC methods (KCP 5.1.1)

A CIPAC method is available: 649.

5.2.2 Methods for the determination of residues (KCP 5.1.2)

Please refer to the post-registration methods.

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

5.3.1 Analysis of the plant protection product (KCP 5.2)

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

5.3.2 Description of analytical methods for the determination of residues Acetamiprid (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	MRL / limit	Reference for MRL/level Remarks
Plant, high water content	Acetamiprid	0.01 mg/kg	Regulation (EU) No. 2019/88
Plant, high acid content		0.5 mg/kg	Regulation (EU) No. 2019/88
Plant, high protein/high starch content (dry commodities)		0.01 mg/kg	Regulation (EU) No. 2019/88
Plant, high oil content		0.01 mg/kg	Regulation (EU) No. 2019/88
Plant, difficult matrices (hops, spices, tea)		0.05 mg/kg	Regulation (EU) No. 2019/88
Muscle	N-desmethyl-acetamiprid (IM-2-1), expressed as acetamiprid	0.02 mg/kg	Regulation (EU) No. 2019/88
Milk		0.2 mg/kg	Regulation (EU) No. 2019/88
Eggs		0.02 mg/kg	Regulation (EU) No. 2019/88
Fat		0.02 mg/kg	Regulation (EU) No. 2019/88
Liver, kidney		0.1 mg/kg	Regulation (EU) No. 2019/88
Soil (Ecotoxicology)	Acetamiprid	0.025 mg/kg	AOEL
Drinking water (Human toxicology)	Acetamiprid, IM-1-5	0.1 µg/L	general limit for drinking water
Surface water (Ecotoxicology)	Acetamiprid	3.7 µg/L	Lowest EC_{50} for *Simulium latigonium*
Air	Acetamiprid	7.5 µg/m^3	AOEL sys: 0.025 mg/kg bw/d
Tissue (meat or liver)	No residue definition provided	Not required	Not classified as T / T+
Body fluids		Not required	Not classified as T / T+

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 Acetamiprid in plant matrices is given in the following tables.

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

Component of residue definition: Acetamiprid				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
High water content	Primary	0.01 mg/kg	QuEChERS LC-MS/MS	T. Schwarz, 2008 Report No. RD-01937 RAR, The Netherlands, 2015 EU Agreed
	Primary	0.01 mg/kg	QuEChERS method using HPLC-MS/MS	KCP 5.2.1 Sikorski P., 2017 Study code: ZBBZ-2016/62/DPL/1ES European Standard EN 15662:2008
	ILV	0.01 mg/kg	QuEChERS LC-MS/MS	A. Giesau, H. Weber, 2012 Report No. RD-02454 RAR, The Netherlands, 2015 EU Agreed
	ILV	0.01 mg/kg	LC-MS	KCP 5.2.2 xxx, 2019 Study code: FR 18.650724.0002
	Confirmatory (if required)	-	-	LC-MS/MS is highly selective mthod, therefore no confirmatory method is required.
High acid content	Primary	0.01 mg/kg	QuEChERS LC-MS/MS	T. Schwarz, 2008 Report No. RD-01937 RAR, The Netherlands, 2015 EU Agreed
	ILV	0.01 mg/kg	QuEChERS LC-MS/MS	A. Giesau, H. Weber, 2012 Report No. RD-02454 RAR, The Netherlands, 2015 EU Agreed
	Primary	0.02 mg/kg	LC-MS	KCP 5.2.5 xxx, 2019 Study code: Report FR 19.515448.0001
	Confirmatory (if required)	-	-	LC-MS/MS is highly selective mthod, therefore no confirmatory method is required.
High oil content	Primary	0.01 mg/kg	QuEChERS LC-MS/MS	T. Schwarz, 2008 Report No. RD-01937 RAR, The Netherlands, 2015 EU Agreed

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	0.01 mg/kg	QuEChERS method using HPLC-MS/MS	KCP 5.2.3 Sikorski P., 2018 Study code: ZBBZ-2016/14/DPL/1DE European Standard EN 15662:2008
	ILV	0.01 mg/kg	QuEChERS LC-MS/MS	A. Giesau, H. Weber, 2012 Report No. RD-02454 RAR, The Netherlands, 2015 EU Agreed
	ILV	0.01 mg/kg	LC-MS	KCP 5.2.4 xxx, 2019 Study code: FR 18.650724.0001
	Confirmatory (if required)	-	-	LC-MS/MS is highly selective mthod, therefore no confirmatory method is required.
High protein/high starch content (dry)	Primary	0.01 mg/kg	QuEChERS LC-MS/MS	T. Schwarz, 2008 Report No. RD-01937 RAR, The Netherlands, 2015 EU Agreed
	ILV	0.01 mg/kg	QuEChERS LC-MS/MS	A. Giesau, H. Weber, 2012 Report No. RD-02454 RAR, The Netherlands, 2015 EU Agreed
	Primary	0.03 mg/kg	LC-MS	KCP 5.2.6 xxx, 2019 Study code: Report FR 19.515448.0002
	Confirmatory (if required)	-	-	LC-MS/MS is highly selective method, therefore no confirmatory method is required.

Table 5.3-3: Statement on extraction efficiency

	Method for products of plant origin
Required, available from:	The efficiency of the solvent extraction procedures has been demonstrated using incurred residues in previously submitted and reviewed metabolism studies (please refer to DAR section B.7.1).

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 acetamiprid 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: 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	QuEChERS LC-MS/MS	K. Miya, 2010 Report No. NCAS 10-144 RAR, The Netherlands, 2015 EU Agreed
	Primary	0.01 mg/kg	HPLC-MS/MS	KCP 5.3.5 xxx, 2019 Report E19085
	Primary	0.01 mg/kg	HPLC-MS/MS	KCP 5.3.6 xxx, 2019 Report E19086
	ILV	0.01 mg/kg	QuEChERS LC-MS/MS	E. Knoch, 2010 Report No. IF-10/01687868 RAR, The Netherlands, 2015 EU Agreed
	ILV	0.01 mg/kg	LC-MS/MS	KCP 5.3.10 xxx, 2019 Report 19.542434.0002
	Confirmatory (if required)	-	-	LC-MS/MS is highly selective method, therefore no confirmatory method is required.
Eggs	Primary	0.01 mg/kg	QuEChERS LC-MS/MS	K. Miya, 2010 Report No. NCAS 10-144 RAR, The Netherlands, 2015 EU Agreed
	Primary	0.01 mg/kg	HPLC-MS/MS	KCP 5.3.7 xxx, 2019 Report E19087
	Primary	0.01 mg/kg	HPLC-MS/MS	KCP 5.3.8 xxx, 2019 Report E19088
	ILV	0.01 mg/kg	QuEChERS LC-MS/MS	E. Knoch, 2010 Report No. IF-10/01687868 RAR, The Netherlands, 2015 EU Agreed
	ILV	0.01 mg/kg	LC-MS/MS	KCP 5.3.11 xxx. 2020 Report 132/2020
	Confirmatory (if required)	-	-	LC-MS/MS is highly selective method, therefore no confirmatory method is required.
Muscle	Primary	0.01 mg/kg	QuEChERS LC-MS/MS	K. Miya, 2010 Report No. NCAS 10-144 RAR, The Netherlands, 2015 EU Agreed
	Primary	0.01 mg/kg	HPLC-MS/MS	KCP 5.3.1 xxx 2019

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**
				Report E19081
	Primary	0.01 mg/kg	HPLC-MS/MS	KCP 5.3.2 xxx 2019 Report E19082
	ILV	0.01 mg/kg	QuEChERS LC-MS/MS	E. Knoch, 2010 Report No. IF-10/01687868 RAR, The Netherlands, 2015 EU Agreed
	ILV	0.01 mg/kg	LC-MS/MS	KCP 5.3.9 xxx, 2019 Report 19.542434.0001
	Confirmatory (if required)	-	-	LC-MS/MS is highly selective method, therefore no confirmatory method is required.
Fat	Primary	0.01 mg/kg	QuEChERS LC-MS/MS	K. Miya, 2010 Report No. NCAS 10-144 RAR, The Netherlands, 2015 EU Agreed
	Primary	0.01 mg/kg	HPLC-MS/MS	KCP 5.3.5 xxx., 2019 Report E19085
	Primary	0.01 mg/kg	HPLC-MS/MS	KCP 5.3.6 xxx., 2019 Report E19086
	ILV	0.01 mg/kg	QuEChERS LC-MS/MS	E. Knoch, 2010 Report No. IF-10/01687868 RAR, The Netherlands, 2015 EU Agreed
	ILV	0.01 mg/kg	LC-MS/MS	KCP 5.3.11 xxx. 2020 Report 132/2020
	Confirmatory (if required)	-	-	LC-MS/MS is highly selective method, therefore no confirmatory method is required.
Kidney, liver	Primary	0.01 mg/kg	QuEChERS LC-MS/MS	K. Miya, 2010 Report No. NCAS 10-144 RAR, The Netherlands, 2015 EU Agreed
	Primary	0.01 mg/kg	HPLC-MS/MS	KCP 5.3.3 xxx, 2019 Report E19083
	Primary	0.01 mg/kg	HPLC-MS/MS	KCP 5.3.4 xxx, 2019 Report E19084
	ILV	0.01 mg/kg	QuEChERS LC-MS/MS	E. Knoch, 2010 Report No. IF-10/01687868 RAR, The Netherlands, 2015

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**
				EU Agreed
	ILV	0.01 mg/kg	LC-MS/MS	KCP 5.3.11 xxx. 2020 Report 132/2020
	Confirmatory (if required)	-	-	LC-MS/MS is highly selective method, therefore no confirmatory method is required.

Table 5.3-5: Statement on extraction efficiency

	Method for products of animal origin
Required, available from:	The efficiency of the solvent extraction procedures has been demonstrated using incurred residues in previously submitted and reviewed metabolism studies (please refer to DAR section B.7.1).

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 Acetamiprid in soil is given in the following tables.

Table 5.3-6: 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	A. Taufer, H. Weber, 2010 Report No. S09-03287 RAR, The Netherlands, 2015 EU Agreed
Primary	0.002 mg/kg	LC-MS/MS	KCP 5.4.1 – xxx, 2019. Report YV/18/010
Confirmatory	-	-	LC-MS/MS is highly selective method, therefore no confirmatory method is required.

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 acetamiprid in surface and drinking water is given in the following tables.

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

Component of residue definition: Acetamiprid				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Drinking water	Primary	0.1 µg/L	LC-MS/MS	K. Miya, 2007 Report No. NCAS 06-209 RAR The Netherlands, 2015 EU Agreed
	Primary	0.1 µg/l	HPLC-MS/MS	KCP 5.5.1 – xxx., 2019. Report E18140
	ILV	0.1 µg/L	LC-MS/MS	M. Senciuc, 2014a Report No. P 3244 G RAR, The Netherlands, 2015 EU Agreed
	ILV	0.1 µg/mL	LC-MS/MS	KCP 5.5.2– xxx, 2019. Report No. 146/2019
	Confirmatory	-	-	LC-MS/MS is highly selective method, therefore no confirmatory method is required.
Surface water	Primary	0.1 µg/L		K. Miya, 2007 Report No. NCAS 06-209 RAR The Netherlands, 2015 EU Agreed
	Primary	0.1 µg/l	HPLC-MS/MS	KCP 5.5.1– xxx, 2019. Report E18140
	Confirmatory	-	-	LC-MS/MS is highly selective method, therefore no confirmatory method is required.

Component of residue definition: 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	0.05 µg/L	LC-MS/MS	A. Giesau, H. Weber, 2012 Report No. S12-02719 RAR, The Netherlands, 2015 EU Agreed
	Primary	0.05 µg/L	HPLC-MS/MS	KCP 5.5.3 – xxx 2019. Report E20064
	ILV	0.05 µg/L	LC-MS/MS	M. Senciuc, 2014b Report No. P 3245 G RAR, The Nethelrands, 2015 EU Agreed
	ILV	0.05 µg/L	LC-MS/MS	KCP 5.5.4– xxx, 2020. Report 28/2020
	Confirmatory	-	-	LC-MS/MS is highly selective method, therefore no

Component of residue definition: IM-1-5				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
				confirmatory method is required.
Surface water	Primary	0.05 µg/L	LC-MS/MS	A. Giesau, H. Weber, 2012 Report No. S12-02719 RAR, The Netherlands, 2015 EU Agreed
	Primary	0.05 µg/L	HPLC-MS/MS	KCP 5.5.3 – xxx 2019. Report E20064
	Confirmatory	-	-	LC-MS/MS is highly selective method, therefore no confirmatory method is required.

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

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

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

Component of residue definition: Acetamiprid			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	0.002 µg/m^3	LC-MS/MS	I. Beck, T. Class, 2009 Report No. P/B 1603 G RAR, The Netherlands, 2015 EU Agreed
Primary	0.002 µg/m^3	LC-MS/MS	KCP 5.6.1 – xxx, 2019. Report E19080
Confirmatory	-	-	LC-MS/MS is highly selective method, therefore no confirmatory method is required.

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 Acetamiprid 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: Acetamiprid			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	0.05 mg/L	LC-MS/MS	M. Senciuc, 2014c Report No. P3208 G RAR, The Netherlands, 2015 EU Agreed
Primary	1.0 µg/L in blood	HPLC-MS/MS	KCP 5.7.1 – xxx., 2019. Report E19080
Confirmatory	-	-	LC-MS/MS is highly selective method, therefore no confirmatory method is required.

5.3.2.8 Other studies/ information

Not relevant.

Appendix 1 Lists of data considered in support of the evaluation

Tables considered not relevant can be deleted as appropriate.

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

List of data submitted by the applicant and relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 5.1.1	J. A. Escudero	2016	Physico-Chemical Characterization of ACETAMIPRID 20% SG. Laboratorios Munuera, Report No. 15-4150-03 GLP Unpublished	N	Sharda Cropchem Limited
KCP 5.2.1	P. Sikorski	2017	Determination of residues of Acetamiprid applied at “Acetamiprid 20% SG” in apricot at one site in Spain (2016). Final Report ZBBZ-2016/62/DPL/1ES GLP Unpublished	N	Sharda Cropchem Limited
KCP 5.2.2	xxx	2019	“Indipendent Laboratory Validation (ILV) of the analytical procedure for the determination of residues of Acetamiprid (CAS: 135410-20-7), in apples samples by LC-MS”. Study Code FR 18.650724.0002 GLP Unpublished	N	Sharda Cropchem Limited
KCP 5.2.3	P. Sikorski	2017	Determination of Acetamiprid residues in oilseed rape after application of “acetamiprid 20% SG” in one trial (1HS), Germany-2017. Final Report ZBBZ-2016/14/DPL/1DE GLP	N	Sharda Cropchem Limited

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 5.2.4	xxx	2019	"Independent Laboratory Validation (ILV) of the analytical procedure for the determination of residues of Acetamiprid (CAS: 135410-20-7), in oilseed rape (seeds) samples by LC-MS". Study Code FR 18.650724.0001 GLP Unpublished	N	Sharda Cropchem Limited
KCP 5.2.5	xxx	2019	Validation of the analytical procedure for the determination of residues of acetamiprid (CAS: 135410-20-7) in grapes by LC-MS Study Code Report FR 19.515448.0001 GLP Unpublished	N	Sharda Cropchem Limited
KCP 5.2.6	xxx	2019	Validation of the analytical procedure for the determination of residues of acetamiprid (CAS: 135410-20-7) in wheat grain by LC-MS Study Code Report FR 19.515448.0002 GLP Unpublished		
KCP 5.3.1	xxx	2019	"Analytical method validation of acetamiprid parent compound in muscle (meat)". Report E19081 GLP Unpublished	Y	Sharda Cropchem Limited
KCP 5.3.2	xxx	2019	"Analytical method validation of acetamiprid metabolite IM 2-1compound in muscle (meat)". Report E19082 GLP Unpublished	Y	Sharda Cropchem Limited
KCP 5.3.3	xxx	2019	"Analytical method validation of acetamiprid parent compound in kidney and liver". Report E19083 GLP	Y	Sharda Cropchem Limited

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 5.3.4	xxx	2019	"Analytical method validation of acetamiprid metabolite IM 2-1compound in kidney and liver". Report E19084 GLP Unpublished	Y	Sharda Cropchem Limited
KCP 5.3.5	xxx	2019	"Analytical method validation of acetamiprid parent compound in milk and fat". Report E19085 GLP Unpublished	Y	Sharda Cropchem Limited
KCP 5.3.6	xxx	2019	"Analytical method validation of acetamiprid metabolite IM 2-1compound in milk and fat". Report E19086 GLP Unpublished	Y	Sharda Cropchem Limited
KCP 5.3.7	xxx	2019	"Analytical method validation of acetamiprid parent compound in eggs". Report E19087 GLP Unpublished	Y	Sharda Cropchem Limited
KCP 5.3.8	xxx	2019	"Analytical method validation of acetamiprid metabolite IM 2-1compound in eggs". Report E19088 GLP Unpublished	Y	Sharda Cropchem Limited
KCP 5.3.9	xxx	2019	Independent Laboratory Validation (ILV) of the analytical procedure for the determination of residues of acetamiprid (CAS: 13540-20-7) and its metabolite IM-2-1 (CAS: 190604-92-3) in muscle by LC-MS/MS. Report No. 19.542434.0001 GLP Unpublished	Y	Sharda Cropchem Limited

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 5.3.10	xxx	2019	Independent Laboratory Validation (ILV) of the analytical procedure for the determination of residues of acetamiprid (CAS: 13540-20-7) and its metabolite IM-2-1 (CAS: 190604-92-3) in milk by LC-MS/MS. Report No. 19.542434.0002 GLP Unpublished	Y	Sharda Cropchem Limited
KCP 5.3.11	xxx	2020	"Independent laboratory validation of the analytical procedure for the determination of residues of acetamiprid (CAS 135410-20-07), metabolites IM 2-1, (CAS 190604-92-3) in fat, liver, kidney and eggs by liquid chromatography". xxx, Report 132/2020 GLP Unpublished	Y	Sharda Cropchem Limited
KCP 5.4.1	xxx	2019	"Terrestrial soil dissipation of acetamiprid after one application to bare soil in Europe" xxx.., Report YV/18/010 GLP Unpublished	Y	Sharda Cropchem Limited
KCP 5.5.1	xxx	2019	"Analytical method validation of acetamiprid in waters". xxx., Report E18140 GLP Unpublished	Y	Sharda Cropchem Limited
KCP 5.5.2	xxx	2019	Independent Laboratory Validation of analytical procedure for the determination of residues of acetamiprid in water Report No. 146/2019 GLP Unpublished	Y	Sharda Cropchem Limited
KCP 5.5.3	xxx	2020	"IM 1-5 (acetamiprid metabolite): analytical method validation in surface water, ground water and drinking water at LOQ of 0.05 µg/l" xxx., Report E200644 GLP Unpublished	Y	Sharda Cropchem Limited

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 5.5.4	xxx	2020	Independent Laboratory Validation of IM 1-5 (acetamiprid metabolite) in drinking water by LC-MS. Report No. 28/2020 GLP Unpublished	Y	Sharda Cropchem Limited
KCP 5.6.1	xxx	2018	"Validation of the analytical procedure for the determination of acetamiprid (CAS: 135410-20-7) in air by LC-MS xxx. Report 18.644636.0002 GLP Unpublished	Y	Sharda Cropchem Limited
KCP 5.7.1	xxx	2019	"Analytical method validation of acetamiprid parent compound in blood". xxx., Report E19080 GLP Unpublished	Y	Sharda Cropchem Limited

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
	T. Schwarz	2008	Acetamiprid: Validation of an enforcement method for plant materials, PTRL Europe, Report No. RD-01937 GLP Published	N	Nippon Soda
	A. Giesau, H. Weber	2012	Independent laboratory validation of an enforcement method (QuEChERS) for the Determination of Residues of Acetamiprid in Crops using LC-MS/MS, Eurofins Agroscience Services, Study No. RD-02454	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**
			GLP Published		
	K. Miya	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 Published	N	Nippon Soda
	E. Knoch	2010	Independent Laboratory Validation: Analytical Method for the Determination of the Residues of Acetamiprid and its Metabolite (IM-2-1) in Animal Commodities, SGS Institut Fresenius GmbH, Report No. IF -10/01687868, Document ID RD-02156 GLP Published	N	Nippon Soda
	A. Täufer, H. Weber	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 Published	N	Nippon Soda
	K. Miya	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 Published	N	Nippon Soda
	M. Scenciuc	2014a	Independent Laboratory Validation (ILV) of a Residues Analytical Method for the Determination of Acetamiprid in Drinking Water, PTRL Europe GmbH, Germany, Report No. P 3244 G, Document ID RD-02951 GLP Published	N	Nippon Soda
	A. Giesau, H. Weber	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	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
			ID RD-02604 GLP Published		
	M. Senciuc	2014b	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 Published	N	Nippon Soda
	I. Beck, T. Class	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, Document ID RD-01863 GLP Published	N	Nippon Soda
	M. Senciuc	2014c	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 Published	N	Nippon Soda

The following tables are to be completed by MS

List of data submitted by the applicant and not relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	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

~~No new data were submitted in the framework of this application.~~

A 2.1.1 Methods for post-authorization control and monitoring purposes (KCP 5.2)

A 2.1.1.1 Description of analytical methods for the determination of residues in plant matrices (KCP 5.2)

A 2.1.1.1.1 Analytical method 1

A 2.1.1.1.1.1 Method validation

Comments of zRMS:	Method is accepted

Report:	KCP 5.2.1
Authors (year)	Piotr Sikorski., 2017
Title:	Determination of residues of Acetamiprid applied at "Acetamiprid 20% SG" in apricot at one site in Spain (2016). Final Report
Study Code:	ZBBZ-2016/62/DPL/1ES
Performing Laboratory:	Food Safety Laboratory, Research Institute of Horticulture
Guideline:	SANCO/825/00 rev. 8.1 ; SANCO/3029/99 rev.4
GLP:	Yes

Materials and Methods

Test Item(s)

Common name:	Acetamiprid
Chemical name (IUPAC):	(E)-N-(6-chloro-3-pyridylmethyl)-N'-cyano-N-methylacetamidine
Supplier (batch/Lot number)	Dr. Ehrenstorfer (92774)
Purity:	98.1%
Expiry Date:	23.04.2019

Method Scope

This multiresidue method is applicable for the quantitative determination of residues of Acetamiprid in apricot (high water content crops), following the QuEChERS sample preparation technique (EN 15662:2008).

Method Principle

A LC-MS/MS QUEChERS-based method for the determination of Acetamiprid in apricot (high water content crops) with a limit of quantification (LOQ) of 0.01 mg/kg was developed and validated. In brief, samples were extracted with acetonitrile. After addition of a buffer-salt mixture containing magnesium

sulphate, sodium chloride and sodium citrate the extract was shaken. After centrifugation, an aliquot of the upper acetonitrile was cleaned by primary secondary amine (PSA) and dehydrated by magnesium suphate addition. Quantification was performed by use of highly selective liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) with electrospray ionization source (ESI; Positive Ion Mode). LC-MS/MS monitors for acetamiprid two parent-daughter ion transitions (MRMs) for quantitation (223.1>126.1 m/z) and confirmation (223.1> 90.1 m/z).

Results and discussions

Method validation was accomplished by analyzing blank control specimens for specificity.
Accuracy was determination 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 (LOQ), 0.1 mg/kg (10xLOQ) and 0.4 mg/kg (40xLOQ) for both ion mass transitions were all in the range 70-110% and thus comply with the guidance document SANCO/825/00 rev.8.1. All precision values at the fortification levels of 0.01 mg/kg, 0.1 mg/kg and 0.4 mg/kg for both ion mass transitions were < 20%.

Mean recovery and precision results for both ion mass transitions of Acetamiprid are shown in Table 10: Recovery results from method validation of Acetamiprid in apricot samples using the analytical method.

Table 10: Recovery results from method validation of Acetamiprid in apricot samples using the analytical method. Accuracy and precision results.

Matrix	Analyte	Fortification level (mg/kg) (*n* = x)	Mean recovery (%)	RSD (%)	Overall Mean Recovery (%)	Overall RSD (%)	Ion mass transition
Apricot (fruit flesh)	Acetamiprid	0.01 mg/kg (n=5)	106	0.6	104	1.9	m/z 223.1>126.1 quantifier
Apricot (fruit flesh)	Acetamiprid	0.1 mg/kg (n=5)	105	0.5			
Apricot (fruit flesh)	Acetamiprid	0.4 mg/kg (n=5)	102	1.1			
Apricot (fruit flesh)	Acetamiprid	0.01 mg/kg (n=5)	105	1.3	104	1.7	m/z 223.1->90.1 qualifier
Apricot (fruit flesh)	Acetamiprid	0.1 mg/kg (n=5)	105	0.8			
Apricot (fruit flesh)	Acetamiprid	0.4 mg/kg (n=5)	102	1.0			

Table 11: Characteristics for the analytical method used for validation of Acetamiprid in apricot samples

	Acetamiprid
Selectivity/specificity	Two ion mass transitions were evaluated in order to demonstrate that the method achives high level of selectivity. Confirmation ratios for Acetamiprid in all samples were within ± 30% of the average found for the standards. No significant interferences above 30% of LOQ was detected in any of the reagent blanks or control specimen extracts for apricot fruit flesh matrix, so that a highly level of selectivity was demonstrated and an additional confirmatory method is not necessary.
Calibration (type, number of data points, range)	The correlation between the injected concentration of analyte standard and detected response was demonstrated to be linear by single determination of matrix-matched calibration standards at 8 concentration levels ranging from 0.0005 mg/L to 0.1 mg/L (it corresponds from 0.0025 mg/kg to 0.5 mg/kg for apricot fruit and thus cover 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 with both ion mass transitions of Acetamiprid were linear with the coefficients of correlation (R) grater than 0.99.
Assessment of matrix effects is presented	Matrix effects on detection of Acetamiprid in extracts of apricot fruit flesh were less than 20% and thus considered insignificant, according to SANCO guidelines. However, determination was performed using matrix-matched calibrations standards.
Limit of determination/quantification	The method has a limit of quantification (LOQ) of 0.01 mg/kg for Acetamiprid in apricot samples. The method has a limit of determination (LOD) of 0.0025 mg/kg for tested matrix, which is < 30% of the LOQ.

Conclusion

It is concluded that method fulfils the requirements as defined in EC Guideline document on residue analytical methods (SANCO/3029/99, rev.4 and SANCO/825/00 rev. 8.1) and is applicable as enforcement and data generation method for determination of Acetamiprid in apricot after application of Acetamiprid 20% SG.

A 2.1.1.1.1.2 Independent laboratory validation

Comments of zRMS:	Method is accepted

Reference:	KCP 5.2.2
Report	"Indipendent Laboratory Validation (ILV) of the analytical procedure for the determination of residues of Acetamiprid (CAS: 135410-20-7), in apples samples by LC-MS". xxx 2019, Study Code FR 18.650724.0002
Guideline(s):	Yes (EU Guidance Document SANCO/3029/99 rev. 4, EU Guidance

	Document SANCO/825/00 rev. 8.1 and OECD-204/2014 guidance)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and Methods

Test Item(s)

Common name:	Acetamiprid
Chemical name (IUPAC):	(E)-N-[(6-chloro-3-pyridyl)methyl]-N'-cyano-N-methyl-acetamidine
Supplier (batch/Lot number)	HPC Standards GmbH, 672808/Lot
Purity:	99.9%
Expiry Date:	01.06.2021.

Method Scope

Purpose of this short term analytical study is to execute an Independent Laboratory Validation (ILV) of the analytical method for the determination of residues of acetamiprid (CAS: 135410-20-7) in apples samples.
LOQ required and verified was 0.01 mg/kg.
For this study, apples samples were used as representative matrix, according to SANCO/825/00 rev.8.1.

Method Principle

The analytical procedure for the determination of Acetamiprid in apples samples is described in the Primary Method ″Study code ″ZBBZ-2016/62/DPL/1ES″ and in the analytical method codified as SOPa-453-LABCHI-Rev.0, internally developed and validated. Briefly, samples were extracted with acetonitrile. After addition of a buffer-salt mixture containing magnesium sulphate, sodium chloride and sodium citrate the extract was vortexed. The Extract was centrifugeted and after, an aliquot of the upper acetonitrile was cleaned by primary secondary amine (PSA) and dehydrated by magnesium suphate addition. Prepered samples were vortexed and filtered through the Teflon filter directly into amber of HPLC vial and injected.
Two SRM transitions were monitored:
Acetamiprid:

- Transition 1: 223 m/z (parent ion) > 125.89 m/z (daughter ion)
- Transition 2: 223 m/z (parent ion) > 89.89 m/z (daughter ion)

Results and discussions

The validity of the method was performed by analyzing blind control samples for specificity. The method is capable to determine the analyte in the presence of the sample matrix. No significant peaks (≤ 30% LOQ) are detected at RT of the target analyte in the Blank and Test Solution with respect to the Spiked Test Solution for both transition 1 and 2.
The Linearity was evaluatedat 5 different levels of concentration, ranging from at least 24% LOQ to about 34×LOQ of analyte on the sample. Coefficient of determination (R^2) for transition 1 and 2 were ≥ 0.99.
The mean recovery values at the fortification levels of 0.01 mg/kg (LOQ), 0.1 mg/kg (10xLOQ) for both ion mass transitions were all in the range 70-110% in accordance with acceptable criteria.
Accuracy of the analytical method expresses the closeness of the consistency between the acceptable true value and the value found.
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 of Acetamiprid are shown in Table 10: Recovery results from method validation of Acetamiprid in apricot samples using the analytical method.

Table 13: Recovery results from method validation of Acetamiprid in apples samples using the analytical method. Accuracy and precision results.

Matrix	Analyte	Fortification level (mg/kg) (*n* = x)	Mean recovery (%)	RSD (%)	Overall Mean Recovery (%)	Overall RSD (%)	Ion mass transition
Apples (fruit flesh)	Acetamiprid	0.01 mg/kg (n=5)	109	0.2	109	0.6	m/z 223 > 125.89 quantifier
Apples (fruit flesh)	Acetamiprid	0.1 mg/kg (n=5)	108	0.5			
Apples (fruit flesh)	Acetamiprid	0.01 mg/kg (n=5)	107	1.6	107	1.2	m/z 223 > 89.89 qualifier
Apples (fruit flesh)	Acetamiprid	0.1 mg/kg (n=5)	107	0.7			

Table 1412: Characteristics for the analytical method used for validation of Acetamiprid in apples samples

	Acetamiprid
Selectivity/specificity	Two ion mass transitions were evaluated in order to demonstrate that the method achives high level of selectivity. Confirmation ratios for Acetamiprid in all samples were within ± 30% of the average found for the standards. No significant interferences above 30% of LOQ was detected in any of the reagent blanks or control specimen extracts for apples fruit flesh matrix, so that a highly level of selectivity was demonstrated and an additional confirmatory method is not necessary.
Calibration (type, number of data points, range)	The correlation between the injected concentration of analyte standard and detected response was demonstrated to be linear by single determination of matrix-matched calibration standards at 5 concentration levels ranging from 0.01 mg/kg to 0.1 mg/L (it corresponds from 0.0024 mg/kg to 0.34 mg/kg for apples fruit and thus cover 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 with both ion mass transitions of Acetamiprid were linear with the coefficients of correlation (R) grater than 0.99.
Assessment of matrix effects is presented	Matrix effects on detection of Acetamiprid in extracts of apples fruit flesh were less than 20% and thus considered insignificant, according to SANCO guidelines.
Limit of determination/quantification	The method has a limit of quantification (LOQ) of 0.01 mg/kg for Acetamiprid in apples samples. The method has a limit of determination (LOD) of 0.0025 mg/kg for tested matrix, which is < 30% of the LOQ.

Conclusion

It is concluded that method fulfils the requirements as defined in EC Guideline document on residue analytical methods (SANCO/3029/99, rev.4 and SANCO/825/00 rev. 8.1) and is applicable as enforcement and data generation method for determination of Acetamiprid in apple after application of Acetamiprid 20% SG.

A 2.1.1.1.2 Analytical method 2

A 2.1.1.1.2.1 Method validation

Comments of zRMS:	Method is accepted

Report:	**KCP 5.2.3**
Authors (year)	**Piotr S., 2018**
Title:	Determination of Acetamiprid residues in oilseed rape after application of "acetamiprid 20% SG" in one trial (1HS), Germany-2017. Final Report
Study Code:	ZBBZ-2016/14/DPL/1DE
Performing Laboratory:	Food Safety Laboratory, Research Institute of Horticulture
Guideline:	SANCO/825/00 rev. 8.1 ; SANCO/3029/99 rev.4
GLP:	Yes

Materials and Methods

Test Item(s)

Common name:	Acetamiprid
Chemical name (IUPAC):	(E)-N-(6-chloro-3-pyridylmethyl)-N'-cyano-N-methylacetamidine
Supplier (batch/Lot number)	Dr. Ehrenstorfer (92774)
Purity:	98.1%
Expiry Date:	23.04.2019

Method Scope

This multiresidue method is applicable for the quantitative determination of residues of Acetamiprid in Oilseed rape (seeds) (high oil content crops), following the QuEChERS sample preparation technique (EN 15662:2008).

Method Principle

A LC-MS/MS QUEChERS-based method for the determination of Acetamiprid in Oilseed rape plants and seeds with a limit of quantification (LOQ) of 0.01 mg/kg was developed and validated. In brief, samples were extracted with acetonitrile. After addition of a buffer-salt mixture containing magnesium sulphate, sodium chloride and sodium citrate the extract was shaken. After centrifugation, an aliquot of the upper acetonitrile was cleaned by primary secondary amine (PSA), silica sorbent (C18EC) and dehydrated by magnesium suphate addition. Quantification was performed by use of highly selective liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) with electrospray ionization source (ESI; Positive Ion Mode). LC-MS/MS monitors for acetamiprid two parent-daughter ion transitions (MRMs) for quantitation (223.1>126.1 m/z) and confirmation (223.1> 73.1 m/z).

Results and discussions

Method validation was accomplished by analyzing blank control specimens for specificity. Accuracy was determination 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).

Five recovery determinations were performed at the fortification levels of 0.01 mg/kg (LOQ) and 0.1 mg/kg (10xLOQ) for oilseed rape plants, and 0.01 mg/kg (LOQ) and 1 mg/kg (100xLOQ) for oilseed rape seeds, respectively. Mean recovery values for both ion mass transitions were all in the range 70-110% and thus comply with the guidance document SANCO/825/00 rev.8.1. All precision values at the fortification levels of 0.01 mg/kg, 0.1 mg/kg and 1 mg/kg for both ion mass transitions were < 20%.

Mean recovery and precision results for both ion mass transitions of Acetamiprid are shown in Table 13

Table 13: Recovery results from method validation of Acetamiprid in oilseed rape pants and seeds using the analytical method. Accuracy and precision results.

Matrix	Analyte	Fortification level (mg/kg) (*n* = x)	Mean recovery (%)	RSD (%)	Overall Mean Recovery (%)	Overall RSD (%)	Ion mass transition
Oilseed rape (plants)	Acetamiprid	0.01 mg/kg (n=5)	102	4.1	101	3.2	m/z 223.1>126.1 quantifier
	Acetamiprid	0.1 mg/kg (n=5)	100	1.8			
	Acetamiprid	0.01 mg/kg (n=5)	105	5.1	103	4.4	m/z 223.1->73.1 qualifier
	Acetamiprid	0.1 mg/kg (n=5)	100	1.7			
Oilseed rape (seeds)	Acetamiprid	0.01 mg/kg (n=5)	98	1.4	97	1.6	/z 223.1>126.1 quantifier
	Acetamiprid	1 mg/kg (n=5)	96	1.5			
	Acetamiprid	0.01 mg/kg (n=5)	99	2.4	97	2.3	m/z 223.1->73.1 qualifier
	Acetamiprid	1 mg/kg (n=5)	96	1.3			

Table 14: Characteristics for the analytical method used for validation of Acetamiprid in apricot samples

	Acetamiprid
Selectivity/specificity	Two ion mass transitions were evaluated in order to demonstrate that the method achives high level of selectivity. Confirmation ratios for Acetamiprid in all samples were within ± 30% of the average found for the standards. No significant interferences above 30% of LOQ was detected in any of the reagent blanks or control specimen extracts for oilseed rape matrix, so that a highly level of selectivity was demonstrated and an additional confirmatory method is not necessary.
Calibration (type, number of data points, range)	The correlation between the injected concentration of analyte standard and detected response was demonstrated to be linear

	Acetamiprid
Selectivity/specificity	Two ion mass transitions were evaluated in order to demonstrate that the method achives high level of selectivity. Confirmation ratios for Acetamiprid in all samples were within ± 30% of the average found for the standards. No significant interferences above 30% of LOQ was detected in any of the reagent blanks or control specimen extracts for oilseed rape matrix, so that a highly level of selectivity was demonstrated and an additional confirmatory method is not necessary.
	by single determination of matrix-matched calibration standards at 7 concentration levels (for each matrix: oilseed rape plant and pilseed rape seeds) ranging from 0.0002 mg/L to 0.5 mg/L (it corresponds from 0.002 mg/kg to 5 mg/kg) for oilseed rape plants and 0.0002 mg/L to 0.1 mg/L (it corresponds from 0.002 mg/kg to 1 mg/kg) for oilseed rape seeds. It 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 with both ion mass transitions of Acetamiprid were linear with the coefficients of correlation (R) grater than 0.99.
Assessment of matrix effects is presented	Matrix effects on detection of Acetamiprid in extracts of Oilseed rape pants and seeds were less than 20% and thus considered insignificant, according to SANCO guidelines. However, determination was performed using matrix-matched calibrations standards.
Limit of determination/quantification	The method has a limit of quantification (LOQ) of 0.01 mg/kg for Acetamiprid in oilseed rape pants and seeds. The method has a limit of determination (LOD) of 0.002 mg/kg for tested matrix, which is < 30% of the LOQ.

Conclusion

It is concluded that method fulfils the requirements as defined in EC Guideline document on residue analytical methods (SANCO/3029/99, rev.4 and SANCO/825/00 rev. 8.1) and is applicable as enforcement and data generation method for determination of Acetamiprid in oilseed rape (plants and seeds) after application of Acetamiprid 20% SG.

A 2.1.1.1.2.2 Independent laboratory validation

Comments of zRMS:	Method is accepted

Reference:	KCP 5.2.4
Report	"Independent Laboratory Validation (ILV) of the analytical procedure for the determination of residues of Acetamiprid (CAS: 135410-20-7), in oilseed rape (seeds) samples by LC-MS". xxx, 2019, Study Code FR 18.650724.0001

Guideline(s):	Yes (EU Guidance Document SANCO/3029/99 rev. 4, EU Guidance Document SANCO/825/00 rev. 8.1 and OECD-204/2014 guidance)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and Methods

Test Item(s)

Common name:	Acetamiprid
Chemical name (IUPAC):	(E)-N-[(6-chloro-3-pyridyl)methyl]-N'-cyano-N-methyl-acetamidine
Supplier (batch/Lot number)	HPC Standards GmbH, 672808/Lot
Purity:	99.9%
Expiry Date:	01.06.2021.

Method Scope

Purpose of this short term analytical study is to execute an Independent Laboratory Validation (ILV) of the analytical method for the determination of residues of acetamiprid (CAS: 135410-20-7) in oilseed rape (seeds) samples.
LOQ required and verified was 0.01 mg/kg.
For this study, oilseed rape (seeds) samples were used as representative matrix, according to SANCO/825/00 rev.8.1.

Method Principle

The analytical procedure for the determination of Acetamiprid in oilseed rape (seeds) samples is described in the Primary Method ″Study code ″ZBBZ-2016/14/DPL/1DE″ and in the analytical method codified as SOPa-454-LABCHI-Rev.0, internally developed and validated. Briefly, samples were extracted with acetonitrile. After addition of a buffer-salt mixture containing magnesium sulphate, sodium chloride and sodium citrate the extract was vortexed. The Extract was centrifugeted and after, an aliquot of the upper acetonitrile was cleaned by primary secondary amine (PSA) and dehydrated by magnesium suphate addition. Prepered samples were vortexed and filtered through the Teflon filter directly into amber of HPLC vial and injected.
Two SRM transitions were monitored:
Acetamiprid:

- Transition 1: 223 m/z (parent ion) > 126.1m/z (daughter ion)
- Transition 2: 223 m/z (parent ion) > 89.89 m/z (daughter ion)

Results and discussions

The validity of the method was performed by analyzing blind control samples for specificity. The method is capable to determine the analyte in the presence of the sample matrix. No significant peaks (≤ 30% LOQ) are detected at RT of the target analyte in the Blank and Test Solution with respect to the Spiked Test Solution for both transition 1 and 2.
The Linearity was evaluatedat 5 different levels of concentration, ranging from at least 30% LOQ to about 30×LOQ of analyte on the sample. Coefficient of determination (R^2) for transition 1 and 2 were ≥ 0.99.
The mean recovery values at the fortification levels of 0.01 mg/kg (LOQ), 0.1 mg/kg (10xLOQ) for both ion mass transitions were all in the range 70-110% in accordance with acceptable criteria.

Accuracy of the analytical method expresses the closeness of the consistency between the acceptable true value and the value found.
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 of Acetamiprid are shown in Table 10: Recovery results from method validation of Acetamiprid in apricot samples using the analytical method.

Table 17: Recovery results from method validation of Acetamiprid in oilseed rape (seeds) samples using the analytical method. Accuracy and precision results.

Matrix	Analyte	Fortification level (mg/kg) (*n* = x)	Mean recovery (%)	RSD (%)	Overall Mean Recovery (%)	Overall RSD (%)	Ion mass transition
Oilseed rape (seeds)	Acetamiprid	0.01 mg/kg (n=5)	100	2	90	11	m/z 223 > 126.1 quantifier
Oilseed rape (seeds)	Acetamiprid	0.1 mg/kg (n=5)	80	2			
Oilseed rape (seeds)	Acetamiprid	0.01 mg/kg (n=5)	98	2	89	12	m/z 223 > 89.89 qualifier
Oilseed rape (seeds)	Acetamiprid	0.1 mg/kg (n=5)	80	1			

Table 1815: Characteristics for the analytical method used for validation of Acetamiprid in oilseed rape (seeds) samples

	Acetamiprid
Selectivity/specificity	Two ion mass transitions were evaluated in order to demonstrate that the method achives high level of selectivity. Confirmation ratios for Acetamiprid in all samples were within ± 30% of the average found for the standards. No significant interferences above 30% of LOQ was detected in any of the reagent blanks or control specimen extracts for oilseed rape (seeds) matrix, so that a highly level of selectivity was demonstrated and an additional confirmatory method is not necessary.
Calibration (type, number of data points, range)	The correlation between the injected concentration of analyte standard and detected response was demonstrated to be linear by single determination of matrix-matched calibration standards at 5 concentration levels ranging from 0.01 mg/kg to 0.1 mg/L (it corresponds from 0.003 mg/kg to 0.3 mg/kg for oilseed rape (seeds) and thus cover 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 with both ion mass transitions of Acetamiprid were linear with the coefficients of correlation (R) grater than 0.99.
Assessment of matrix effects is presented	Matrix effects on detection of Acetamiprid in extracts of

	oilseed rape (seeds) were less than 20% and thus considered insignificant, according to SANCO guidelines.
Limit of determination/quantification	The method has a limit of quantification (LOQ) of 0.01 mg/kg for Acetamiprid in oilseed rape (seeds) samples. The method has a limit of determination (LOD) of 0.003 mg/kg for tested matrix, which is < 30% of the LOQ.

Conclusion

It is concluded that method fulfils the requirements as defined in EC Guideline document on residue analytical methods (SANCO/3029/99, rev.4 and SANCO/825/00 rev. 8.1) and is applicable as enforcement and data generation method for determination of Acetamiprid in oilseed rape (seeds) after application of Acetamiprid 20% SG.

A 2.1.1.1.3 Analytical method 3

Comments of zRMS:	Method is accepted

Reference:	KCP 5.2.5
Report	"Validation of the analytical procedure for the determination of residues of acetamiprid (CAS: 135410-20-7) in grapes by LC-MS". xxx, 2019, Chelab s.r.l., Report FR 19.515448.0001
Guideline(s):	Yes. SANCO/825/00 rev. 8.1 SANCO/3029/99 rev.4 OECD 204/2014
Deviations:	No
GLP:	Yes
Acceptability:	Yes/No/Supplementary

Materials and methods

Graps are mixed with the content of one Quechers extract pouch and extracted with acetonitrile. The sample was shaken and centrifuged, then, supernatant was transferred into a plastic tuve containing $MgSO_4$ and PSA resin. The sample was shaken and centrifuged. An aliquiot of supernatant was diluted with 0.1 % formic acid in acetonitrile, ultracentrifuged and inyected into LC-MS.

MRM:

	Precursor ion m/c		*m/z*	*Collision energy*
Acetamiprid	223	Quantifier ion (trans 1)	125.89	15
	223	Quantifier ion (trans 2)	89.89	25

Results and discussions

Specificity

No significant peaks (≤ 30% LOQ) are detected at RT of the target analyte in the Blank and Test Solution with respect to the Spiked Test Solution.

Linearity

Eight different levels of concentration were used for calibration, ranging from 0.0006 mg/L to 0.10 mg/L, that includes values from below 30 % of LOQ (0.003 mg/kg) to above 50xLOQ (0.51 mg/kg) ($R^2 \geq 0.99$ were achieved for both transitions, demonstrating acceptable linearity for each water.

Precision & Accuracy

Average recoveries at each fortification level were all within the acceptance range of 70-120 % (for both confirmatory transitions). The relative standard deviation (RSD) did not exceed 20 % at any fortification level.

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

Matrix	Analyte	Fortification level (mg/kg) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
Grapes	Acetamiprid	LOQ = 0.01 mg/kg (n = 5)	88	1	Trans 1
		10×LOQ = 0.10 mg/kg (n = 5)	96	7	
Grapes	Acetamiprid	LOQ = 0.01 mg/kg (n = 5)	87	2	Trans 2
		10×LOQ = 0.10 mg/kg (n = 5)	94	6	

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

	Acetamiprid
Specificity	No significant peaks (≤ 30% LOQ) are detected.
Calibration (type, number of data points)	Transition 1: y = 23522372x ; R = 0.9976 (n = 5) Transition 2: y = 3665705x ; R = 0.9976 (n = 5)
Calibration range	Accepted calibration range in concentration units: 0.0006 – 0.10 mg/L (corresponding to 0.003 – 0.51 mg/kg)
Assessment of matrix effects is presented	yes
Limit of determination/quantification	LOQ = 0.01 mg/kg LOD = 0.003 mg/kg

Conclusion

The method was successfully validated and is suitable for the determination of residues of acetamiprid un grapes.

A 2.1.1.1.4 Analytical method 4

Comments of zRMS:	Method is accepted

Reference:	KCP 5.2.6
Report	"Validation of the analytical procedure for the determination of residues of acetamiprid (CAS: 135410-20-7) in wheat grain by LC-MS". xxx, 2019, Chelab s.r.l., Report FR 19.515448.0002
Guideline(s):	Yes.

	SANCO/825/00 rev. 8.1 SANCO/3029/99 rev.4 OECD 204/2014
Deviations:	No
GLP:	Yes
Acceptability:	Yes/No/Supplementary

Materials and methods

Graps are mixed with the content of one Quechers extract pouch and extracted with acetonitrile. The sample was shaken and centrifuged, then, supernatant was transferred into a plastic tuve containing $MgSO_4$ and PSA resin. The sample was shaken and centrifuged. An aliquiot of supernatant was diluted with 0.1 % formic acid in acetonitrile, ultracentrifuged and inyected into LC-MS.

MRM:

	Precursor ion m/c		*m/z*	*Collision energy*
Acetamiprid	223	Quantifier ion (trans 1)	125.89	15
	223	Quantifier ion (trans 2)	89.89	25

Results and discussions

Specificity
No significant peaks (≤ 30% LOQ) are detected at RT of the target analyte in the Blank and Test Solution with respect to the Spiked Test Solution.

Linearity
Eight different levels of concentration were used for calibration, ranging from 0.0005 mg/L to 0.20 mg/L, that includes values from below 20 % of LOQ (0.003 mg/kg) to above 100xLOQ (1.02 mg/kg) ($R^2 \geq 0.99$ were achieved for both transitions, demonstrating acceptable linearity for each water.

Precision & Accuracy
Average recoveries at each fortification level were all within the acceptance range of 70-120 % (for both confirmatory transitions). The relative standard deviation (RSD) did not exceed 20 % at any fortification level.

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

Matrix	Analyte	Fortification level (mg/kg) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
Wheat grain	Acetamiprid	LOQ = 0.01 mg/kg (n = 5)	97	1	Trans 1
		10×LOQ = 0.10 mg/kg (n = 5)	91	7	
Wheat grain	Acetamiprid	LOQ = 0.01 mg/kg (n = 5)	98	1	Trans 2
		10×LOQ = 0.10 mg/kg (n = 5)	92	5	

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

	Acetamiprid
Specificity	No significant peaks (≤ 30% LOQ) are detected.
Calibration (type, number of data points)	Transition 1: y = 26513180x ; R = 0.9999 (n = 5) Transition 2: y = 4056309x ; R = 0.9999 (n = 5)

	Acetamiprid
Specificity	No significant peaks (≤ 30% LOQ) are detected.
Calibration range	Accepted calibration range in concentration units: 0.0005 – 0.20 mg/L (corresponding to 0.003 – 1.02 mg/kg)
Assessment of matrix effects is presented	yes
Limit of determination/quantification	LOQ = 0.01 mg/kg LOD = 0.003 mg/kg

Conclusion

The method was successfully validated and is suitable for the determination of residues of acetamiprid in wheat grain..

A 2.1.1.2 Description of analytical methods for the determination of residues in animal matrices (KCP 5.2)

A 2.1.1.2.1 Analytical method 1

Comments of zRMS:	Method is accepted

Reference:	KCP 5.3.1
Report	"Analytical method validation of acetamiprid parent compound in muscle (meat)". xxx, 2019, xxx, Report E19081
Guideline(s):	Yes. SANCO/825/00 rev. 8.1, SANCO/3029/99 rev. 4
Deviations:	No
GLP:	Yes
Acceptability:	Yes/No/Supplementary

Materials and methods

Criogenic meat was weighted and mixed with water and acetonitrile. It was stirred and then QuEChERS were added. Then was homogenized and centrifuged. The upper liquid was passed to a QuEChERs fatty samples cleanup tube and stirred and centrifuged.

MRM:

	Precursor ion m/c		*m/z*	*Collision energy*
Acetamiprid	222.94	Quantifier ion (trans 1)	125.93	20
	222.94	Quantifier ion (trans 2)	56.10	20

Results and discussions

Specificity

No significant peaks (≤ 30% LOQ) are detected at RT of the target analyte in the Blank and Test Solution with respect to the Spiked Test Solution.

Linearity

Five different levels of concentration were used for calibration, ranging from 0.1 µg/L to 24.93 µg/L, that includes values from below 20 % of LOQ (0.1 µg/L) to above 30xLOQ 25 µg/L)
($R^2 \geq 0.99$ were achieved for both transitions, demonstrating acceptable linearity.

Precision & Accuracy
Average recoveries at each fortification level were all within the acceptance range of 70-110 % (for both confirmatory transitions). The relative standard deviation (RSD) did not exceed 20 % at any fortification level.

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

Matrix	Analyte	Fortification level (mg/kg) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
Muscle (meat)	Acetamiprid	LOQ = 0.01 mg/kg (n = 5)	98.94	5.3	Trans 1
		10×LOQ = 0.1 mg/kg (n = 5)	96.16	19.8	
Muscle (meat)	Acetamiprid	LOQ = 0.01 mg/kg (n = 5)	98.91	6.6	Trans 2
		10×LOQ = 0.1 mg/kg (n = 5)	92.63	19.4	

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

	Acetamiprid
Specificity	No significant peaks (≤ 30% LOQ) are detected.
Calibration (type, number of data points)	Transition 1: y = 83.1760 + 3598.5154x ; R = 0.9996 (n = 8) Transition 2: y = 43.1395 + 1737.5689x ; R = 0.9996 (n = 8)
Calibration range	Accepted calibration range in concentration units: 0.1 – 24.93 µg/L equivalent to 0.002 – 0.5 mg/kg
Assessment of matrix effects is presented	yes
Limit of determination/quantification	LOQ = 0.01 mg/kg LOD = 0.002 mg/kg

Conclusion

The method was successfully validated and is suitable for the determination of residues of Acetamiprid in muscle (meat).

A 2.1.1.2.2 Analytical method 2

Comments of zRMS:	Method is accepted

Reference:	KCP 5.3.2
Report	"Analytical method validation of acetamiprid metabolite IM 2-1compound in muscle (meat)". xxx, 2019, xxx, Report E19082
Guideline(s):	Yes. SANCO/825/00 rev. 8.1, SANCO/3029/99 rev. 4
Deviations:	No
GLP:	Yes

Acceptability: Yes/No/Supplementary

Materials and methods

Criogenic meat was weighted and mixed with water and acetonitrile. It was stirred and then QuEChERS were added. Then was homogenized and centrifuged. The upper liquid was passed to a QuEChERs fatty samples cleanup tube and stirred and centrifuged.

MRM:

	Precursor ion m/c		*m/z*	*Collision energy*
IM 2-1	208.97	Quantifier ion (trans 1)	125.98	34
	208.97	Quantifier ion (trans 2)	90.04	20

Results and discussions

Specificity
No significant peaks (≤ 30% LOQ) are detected at RT of the target analyte in the Blank and Test Solution with respect to the Spiked Test Solution.

Linearity
Five different levels of concentration were used for calibration, ranging from 0.1 µg/L to 23.94 µg/L, that includes values from below 20 % of LOQ (0.03 µg/L) to above 30xLOQ (25 µg/L)
($R^2 \geq 0.99$ were achieved for both transitions, demonstrating acceptable linearity.

Precision & Accuracy
Average recoveries at each fortification level were all within the acceptance range of 70-110 % (for both confirmatory transitions). The relative standard deviation (RSD) did not exceed 20 % at any fortification level.

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

Matrix	**Analyte**	**Fortification level (mg/kg) (*n* = x)**	**Mean recovery (%)**	**RSD (%)**	**Comments**
Muscle (meat)	IM 2-1	LOQ = 0.01 mg/kg (n = 5)	116.70	2.3	Trans 1
		10×LOQ = 0.1 mg/kg (n = 5)	114.44	5.2	
Muscle (meat)	IM 2-1	LOQ = 0.01 mg/kg (n = 5)	112.81	5.8	Trans 2
		10×LOQ = 0.1 mg/kg (n = 5)	114.12	4.5	

Table A 2: Characteristics for the analytical method used for validation of IM 2-1 in muscle (meat)

	Acetamiprid
Specificity	No significant peaks (≤ 30% LOQ) are detected.
Calibration (type, number of data points)	Transition 1: y = -86.9187 + 3880.4147x ; R = 0.9972 (n = 8) Transition 2: y = -14.7571 + 798.4372x ; R = 0.9979 (n = 8)
Calibration range	Accepted calibration range in concentration units: 0.1 – 25 µg/L Equivalent to 0.002 – 0.5 mg/kg
Assessment of matrix effects is presented	yes
Limit of determination/quantification	LOQ = 0.01 mg/kg LOD = 0.002 mg/kg

Conclusion

The method was successfully validated and is suitable for the determination of residues of Acetamiprid in muscle (meat).

A 1.1.1.1 Analytical method 3

Comments of zRMS:	Method is accepted

Reference:	KCP 5.3.3
Report	"Analytical method validation of acetamiprid parent compound in kidney and liver". xxx, 2019, xxx., Report E19083
Guideline(s):	Yes. SANCO/825/00 rev. 8.1, SANCO/3029/99 rev. 4
Deviations:	No
GLP:	Yes
Acceptability:	Yes/No/Supplementary

Materials and methods

Criogenic matrix was weighted and mixed with water and acetonitrile. It was stirred and then QuEChERS were added. Then was homogenized and centrifuged. The upper liquid was passed to a QuEChERs fatty samples cleanup tube and stirred and centrifuged.

MRM:

	Precursor ion m/c		*m/z*	*Collision energy*
Acetamiprid	222.94	Quantifier ion (trans 1)	125.93	20
	222.94	Quantifier ion (trans 2)	56.10	20

Results and discussions

Specificity
No significant peaks (≤ 30% LOQ) are detected at RT of the target analyte in the Blank and Test Solution with respect to the Spiked Test Solution each matrix.

Linearity
Five different levels of concentration were used for calibration, ranging from 0.1 µg/L to 23.94 µg/L, that includes values from below 20 % of LOQ (0.03 µg/L) to above 30xLOQ (25 µg/L)
($R^2 \geq 0.99$ were achieved for both transitions, demonstrating acceptable linearity.

Precision & Accuracy
Average recoveries at each fortification level were all within the acceptance range of 70-110 % (for both confirmatory transitions). The relative standard deviation (RSD) did not exceed 20 % at any fortification level.

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

Matrix	Analyte	Fortification level (mg/kg) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
Kidney	Acetamiprid	LOQ = 0.01 mg/kg (n = 5)	89.50	2.7	Trans 1

Matrix	Analyte	Fortification level (mg/kg) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
		10×LOQ = 0.1 mg/kg (n = 5)	91.29	4.7	
Kidney	Acetamiprid	LOQ = 0.01 mg/kg (n = 5)	91.59	5.7	Trans 2
		10×LOQ = 0.1 mg/kg (n = 5)	92.05	5.6	
Liver	Acetamiprid	LOQ = 0.01 mg/kg (n = 5)	90.95	2.2	Trans 1
	Acetamiprid	10×LOQ = 0.1 mg/kg (n = 5)	99.47	1.9	
Liver	Acetamiprid	LOQ = 0.01 mg/kg (n = 5)	95.26	3.2	Trans 2
	Acetamiprid	10×LOQ = 0.1 mg/kg (n = 5)	102.79	1.6	

Table A 2: Characteristics for the analytical method used for validation of acetamiprid in kidney and liver

	Acetamiprid
Specificity	No significant peaks (≤ 30% LOQ) are detected.
Calibration (type, number of data points)	Kidney Transition 1: y = 58.5015 + 3855.1345x; R = 0.9998 (n = 8) Transition 2: y = 19.6995 + 1840.7092x; R = 0.9995 (n = 8) Liver Transition 1: y = 97.1267 + 3966.6256x; R = 0.9987 (n = 8) Transition 2: y = 32.2936 + 1847.3500x; R = 0.9978 (n = 8)
Calibration range	Accepted calibration range in concentration units: 0.1 – 25 µg/L Equivalent to 0.002 – 0.5 mg/kg
Assessment of matrix effects is presented	yes
Limit of determination/quantification	LOQ = 0.01 mg/kg LOD = 0.002 mg/kg

Conclusion

The method was successfully validated and is suitable for the determination of residues of Acetamiprid in kidney and liver.

A 1.1.1.2 Analytical method 4

Comments of zRMS:	Method is accepted

Reference:	KCP 5.3.4
Report	"Analytical method validation of acetamiprid metabolite IM 2-1 compound in kidney and liver". xxx, 2019, xxx, Report E19084
Guideline(s):	Yes. SANCO/825/00 rev. 8.1, SANCO/3029/99 rev. 4
Deviations:	No
GLP:	Yes
Acceptability:	Yes/No/Supplementary

Materials and methods

Criogenic matrix was weighted and mixed with water and acetonitrile. It was stirred and then QuEChERS were added. Then was homogenized and centrifuged. The upper liquid was passed to a QuEChERs fatty samples cleanup tube and stirred and centrifuged.

MRM:

	Precursor ion m/c		*m/z*	*Collision energy*
Acetamiprid	208.97	Quantifier ion (trans 1)	125.98	34
	208.97	Quantifier ion (trans 2)	90.04	20

Results and discussions

Specificity
No significant peaks (≤ 30% LOQ) are detected at RT of the target analyte in the Blank and Test Solution with respect to the Spiked Test Solution each matrix.

Linearity
Five different levels of concentration were used for calibration, ranging from 0.1 µg/L to 23.94 µg/L, that includes values from below 20 % of LOQ (0.03 µg/L) to above 30xLOQ (25 µg/L)
($R^2 \geq 0.99$ were achieved for both transitions, demonstrating acceptable linearity.

Precision & Accuracy
Average recoveries at each fortification level were all within the acceptance range of 70-110 % (for both confirmatory transitions). The relative standard deviation (RSD) did not exceed 20 % at any fortification level.

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

Matrix	Analyte	Fortification level (mg/kg) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
Kidney	IM 2-1	LOQ = 0.01 mg/kg (n = 5)	101.69	1.7	Trans 1
		10×LOQ = 0.1 mg/kg (n = 5)	101.15	1.1	
Kidney	IM 2-1	LOQ = 0.01 mg/kg (n = 5)	102.56	2.2	Trans 2
		10×LOQ = 0.1 mg/kg (n = 5)	99.72	2.3	
Liver	IM 2-1	LOQ = 0.01 mg/kg (n = 5)	96.91	2.3	Trans 1
		10×LOQ = 0.1 mg/kg (n = 5)	101.89	2.9	
Liver	IM 2-1	LOQ = 0.01 mg/kg (n = 5)	97.17	10.0	Trans 2
		10×LOQ = 0.1 mg/kg (n = 5)	102.74	2.8	

Table A 2: Characteristics for the analytical method used for validation of IM 2-1 in kidney and liver

	Acetamiprid
Specificity	No significant peaks (≤ 30% LOQ) are detected.
Calibration (type, number of data points)	Kidney Transition 1: y = 52.9518 + 5101.5096x; R = 0.9997 (n = 8) Transition 2: y = 4.3233 + 1047.1686x; R = 0.9998 (n = 8) Liver Transition 1: y = 72.7901 + 2994.0925x; R = 0.9995 (n = 8) Transition 2: y = 15.4545 + 606.2520x; R = 0.9998 (n = 8)
Calibration range	Accepted calibration range in concentration units: 0.1 – 25 µg/L

	Acetamiprid
Specificity	No significant peaks (≤ 30% LOQ) are detected.
	Equivalent to 0.002 – 0.5 mg/kg
Assessment of matrix effects is presented	yes
Limit of determination/quantification	LOQ = 0.01 mg/kg LOD = 0.002 mg/kg

Conclusion

The method was successfully validated and is suitable for the determination of residues of IM 2-1 in kidney and liver.

A 1.1.1.3 Analytical method 5

Comments of zRMS:	Method is accepted

Reference:	KCP 5.3.5
Report	"Analytical method validation of acetamiprid parent compound in milk and fat". xxx, 2019, xxx, Report E19085
Guideline(s):	Yes. SANCO/825/00 rev. 8.1, SANCO/3029/99 rev. 4
Deviations:	No
GLP:	Yes
Acceptability:	Yes/No/Supplementary

Materials and methods

Matrix was mixed with acetonitrile. Then extraction salts QuEChERS were added and homogenized and centrifuged. The upper liquid was passed to a QuEChERS fatty samples cleanup and stirred. Supernatant was adquired.

MRM:

	Precursor ion m/c		*m/z*	*Collision energy*
Acetamiprid	222.94	Quantifier ion (trans 1)	125.93	20
	222.94	Quantifier ion (trans 2)	56.10	20

Results and discussions

Specificity

No significant peaks (≤ 30% LOQ) are detected at RT of the target analyte in the Blank and Test Solution with respect to the Spiked Test Solution each matrix.

Linearity

Five different levels of concentration were used for calibration, ranging from 0.1 µg/L to 23.94 µg/L, that includes values from below 20 % of LOQ (0.03 µg/L) to above 30xLOQ (25 µg/L)
($R^2 \geq 0.99$ were achieved for both transitions, demonstrating acceptable linearity.

Precision & Accuracy

Average recoveries at each fortification level were all within the acceptance range of 70-110 % (for both confirmatory transitions). The relative standard deviation (RSD) did not exceed 20 % at any fortification level.

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

Matrix	Analyte	Fortification level (mg/kg) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
Milk	Acetamiprid	LOQ = 0.01 mg/kg (n = 5)	95.68	3.2	Trans 1
		10×LOQ = 0.1 mg/kg (n = 5)	109.73	3.0	
Milk	Acetamiprid	LOQ = 0.01 mg/kg (n = 5)	89.43	6.9	Trans 2
		10×LOQ = 0.1 mg/kg (n = 5)	105.01	5.3	
Fat	Acetamiprid	LOQ = 0.01 mg/kg (n = 5)	95.02	1.7	Trans 1
		10×LOQ = 0.1 mg/kg (n = 5)	99.93	2.2	
Fat	Acetamiprid	LOQ = 0.01 mg/kg (n = 5)	95.69	4.4	Trans 2
		10×LOQ = 0.1 mg/kg (n = 5)	99.25	1.5	

Table A 2: Characteristics for the analytical method used for validation of acetamiprid in milk and fat

	Acetamiprid
Specificity	No significant peaks (≤ 30% LOQ) are detected.
Calibration (type, number of data points)	Milk Transition 1: y = 1.1997 + 937.9811x; R = 0.9990 (n = 8) Transition 2: y = 5.2052 + 516.5804x; R = 0.9998 (n = 8) Fat Transition 1: y = 79.7563 + 2799.7908x; R = 0.9989 (n = 8) Transition 2: y = 13.0867 + 1463.1610x; R = 0.9989 (n = 8)
Calibration range	Accepted calibration range in concentration units: 0.1 – 25 µg/L Equivalent to 0.002 – 0.5 mg/kg
Assessment of matrix effects is presented	yes
Limit of determination/quantification	LOQ = 0.01 mg/kg LOD = 0.002 mg/kg

Conclusion

The method was successfully validated and is suitable for the determination of residues of acetamiprid in milk and fat.

A 1.1.1.4 Analytical method 6

Comments of zRMS:	Method is accepted

Reference:	KCP 5.3.6
Report	"Analytical method validation of acetamiprid metabolite IM 2-1 compound in milk and fat". xxx, 2019, xxx, Report E19086

Guideline(s):	Yes. SANCO/825/00 rev. 8.1, SANCO/3029/99 rev. 4
Deviations:	No
GLP:	Yes
Acceptability:	Yes/No/Supplementary

Materials and methods

Matrix was mixed with acetonitrile. Then extraction salts QuEChERS were added and homogenized and centrifuged. The upper liquid was passed to a QuEChERS fatty samples cleanup and stirred. Supernatant was adquired.

MRM:

	Precursor ion m/c		*m/z*	*Collision energy*
Acetamiprid	208.97	Quantifier ion (trans 1)	125.98	20
	208.97	Quantifier ion (trans 2)	90.04	20

Results and discussions

Specificity
No significant peaks (≤ 30% LOQ) are detected at RT of the target analyte in the Blank and Test Solution with respect to the Spiked Test Solution each matrix.

Linearity
Five different levels of concentration were used for calibration, ranging from 0.1 µg/L to 23.94 µg/L, that includes values from below 20 % of LOQ (0.03 µg/L) to above 30xLOQ (25 µg/L)
($R^2 \geq 0.99$ were achieved for both transitions, demonstrating acceptable linearity.

Precision & Accuracy
Average recoveries at each fortification level were all within the acceptance range of 70-110 % (for both confirmatory transitions). The relative standard deviation (RSD) did not exceed 20 % at any fortification level.

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

Matrix	Analyte	Fortification level (mg/kg) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
Milk	Acetamiprid	LOQ = 0.01 mg/kg (n = 5)	99.71	6.2	Trans 1
		10×LOQ = 0.1 mg/kg (n = 5)	104.50	2.7	
Milk	Acetamiprid	LOQ = 0.01 mg/kg (n = 5)	107.99	14.1	Trans 2
		10×LOQ = 0.1 mg/kg (n = 5)	102.07	5.7	
Fat	Acetamiprid	LOQ = 0.01 mg/kg (n = 5)	110.78	3.2	Trans 1
		10×LOQ = 0.1 mg/kg (n = 5)	112.17	3.0	
Fat	Acetamiprid	LOQ = 0.01 mg/kg (n = 5)	108.87	6.2	Trans 2
		10×LOQ = 0.1 mg/kg (n = 5)	114.63	3.1	

Table A 2: Characteristics for the analytical method used for validation of acetamiprid in milk and fat

	IM 2-1
Specificity	No significant peaks (≤ 30% LOQ) are detected.

	IM 2-1
Specificity	No significant peaks (≤ 30% LOQ) are detected.
Calibration (type, number of data points)	Milk Transition 1: y = -5.8677 + 830.4829x; R = 0.9994 (n = 8) Transition 2: y = -4.5340 + 178.1872x; R = 0.9995 (n = 8) Fat Transition 1: y = -26.3951 + 4238.4341x; R = 0.9998 (n = 8) Transition 2: y = -9.4868 + 858.1227x; R = 0.9998 (n = 8)
Calibration range	Accepted calibration range in concentration units: 0.1 – 25 µg/L Equivalent to 0.002 – 0.5 mg/kg
Assessment of matrix effects is presented	yes
Limit of determination/quantification	LOQ = 0.01 mg/kg LOD = 0.002 mg/kg

Conclusion

The method was successfully validated and is suitable for the determination of residues of IM 2-1 in milk and fat.

A 1.1.1.5 Analytical method 7

Comments of zRMS:	Method is accepted

Reference:	KCP 5.3.7
Report	"Analytical method validation of acetamiprid parent compound in eggs". xxx, 2019, xxx, Report E19087
Guideline(s):	Yes. SANCO/825/00 rev. 8.1, SANCO/3029/99 rev. 4
Deviations:	No
GLP:	Yes
Acceptability:	Yes/No/Supplementary

Materials and methods

Homogeneized eggs (broken and beaten) were mixed with acetonitrile. Then extraction salts QuEChERS were added and homogenized and centrifuged. The upper liquid was passed to a QuEChERS fatty samples cleanup and stirred. Supernatant was adquired.

MRM:

	Precursor ion m/c		*m/z*	*Collision energy*
Acetamiprid	222.94	Quantifier ion (trans 1)	125.93	20
	222.94	Quantifier ion (trans 2)	56.10	20

Results and discussions

Specificity

No significant peaks (≤ 30% LOQ) are detected at RT of the target analyte in the Blank and Test Solution with respect to the Spiked Test Solution each matrix.

Linearity
Five different levels of concentration were used for calibration, ranging from 0.1 µg/L to 23.94 µg/L, that includes values from below 20 % of LOQ (0.03 µg/L) to above 30xLOQ (25 µg/L)
($R^2 \geq 0.99$ were achieved for both transitions, demonstrating acceptable linearity.

Precision & Accuracy
Average recoveries at each fortification level were all within the acceptance range of 70-110 % (for both confirmatory transitions). The relative standard deviation (RSD) did not exceed 20 % at any fortification level.

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

Matrix	Analyte	Fortification level (µg/L) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
Eggs	Acetamiprid	LOQ = 0.01 mg/kg (n = 5)	97.1	7.0	Trans 1
		10×LOQ = 0.1 mg/kg (n = 5)	109.4	3.3	
Eggs	Acetamiprid	LOQ = 0.01 mg/kg (n = 5)	94.6	6.2	Trans 2
		10×LOQ = 0.1 mg/kg (n = 5)	108.7	4.3	

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

	Acetamiprid
Specificity	No significant peaks (≤ 30% LOQ) are detected.
Calibration (type, number of data points)	Transition 1: y = -0.7874 + 858.6353x; R = 0.9997 (n = 8) Transition 2: y = -6.8266 + 475.0892; R = 0.9995 (n = 8)
Calibration range	Accepted calibration range in concentration units: 0.1 – 25 µg/L Equivalent to 0.002 – 0.5 mg/kg
Assessment of matrix effects is presented	yes
Limit of determination/quantification	LOQ = 0.01 mg/kg LOD = 0.002 mg/kg

Conclusion

The method was successfully validated and is suitable for the determination of residues of Acetamiprid in eggs.

A 1.1.1.6 Analytical method 8

Comments of zRMS:	Method is accepted

Reference:	KCP 5.3.8
Report	"Analytical method validation of acetamiprid metabolite IM 2-1 in eggs". xxx, 2019, xxx, Report E19088
Guideline(s):	Yes. SANCO/825/00 rev. 8.1, SANCO/3029/99 rev. 4
Deviations:	No

GLP: Yes

Acceptability: Yes/No/Supplementary

Materials and methods

Matrix was mixed with acetonitrile. Then extraction salts QuEChERS were added and homogenized and centrifuged. The upper liquid was passed to a QuEChERS fatty samples cleanup and stirred. Supernatant was adquired.

MRM:

	Precursor ion m/c		*m/z*	*Collision energy*
Acetamiprid	208.97	Quantifier ion (trans 1)	125.98	34
	208.97	Quantifier ion (trans 2)	90.04	34

Results and discussions

Specificity
No significant peaks (≤ 30% LOQ) are detected at RT of the target analyte in the Blank and Test Solution with respect to the Spiked Test Solution each matrix.

Linearity
Five different levels of concentration were used for calibration, ranging from 0.1 µg/L to 23.94 µg/L, that includes values from below 20 % of LOQ (0.03 µg/L) to above 30xLOQ (25 µg/L)
($R^2 \geq 0.99$ were achieved for both transitions, demonstrating acceptable linearity.

Precision & Accuracy
Average recoveries at each fortification level were all within the acceptance range of 70-110 % (for both confirmatory transitions). The relative standard deviation (RSD) did not exceed 20 % at any fortification level.

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

Matrix	Analyte	Fortification level (mg/kg) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
Eggs	IM 2-1	LOQ = 0.01 mg/kg (n = 5)	93.52	2.1	Trans 1
		10×LOQ = 0.1 mg/kg (n = 5)	101.69	3.2	
Eggs	IM 2-1	LOQ = 0.01 mg/kg (n = 5)	101.11	9.9	Trans 2
		10×LOQ = 0.1 mg/kg (n = 5)	97.93	2.3	

Table A 2: Characteristics for the analytical method used for validation of acetamiprid in milk and fat

	IM 2-1
Specificity	No significant peaks (≤ 30% LOQ) are detected.
Calibration (type, number of data points)	Transition 1: y = 13.7274 + 1305.1593x; R = 0.9992 (n = 8) Transition 2: y = -6.5489 + 271.9556x; R = 0.9990 (n = 8)
Calibration range	Accepted calibration range in concentration units: 0.1 – 25 µg/L Equivalent to 0.002 – 0.5 mg/kg
Assessment of matrix effects is presented	yes
Limit of determination/quantification	LOQ = 0.01 mg/kg LOD = 0.002 mg/kg

Conclusion

The method was successfully validated and is suitable for the determination of residues of IM 2-1 in eggs.

A 2.1.1.2.2.1 Independent laboratory validation

Comments of zRMS:	Method is accepted

Reference:	KCP 5.3.9
Report	Independent Laboratory Validation (ILV) of the analytical procedure for the determination of residues of acetamiprid (CAS: 13540-20-7) and its metabolite IM-2-1 (CAS: 190604-92-3) in muscle by LC-MS/MS. xxx, 2019, Report No. 19.542434.0001
Guideline(s):	Yes (EU Guidance Document SANCO/3029/99 rev. 4, EU Guidance Document SANCO/825/00 rev. 8.1 and OECD-204/2014 guidance)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Mobile Phase A: 0.1 % formic acid in milliQ water. In a 1000 ml volumetric flask containing about 500 ml of milliQ water, 1 ml of formic acid was added and then the solution was diluted to volume with milliQ water.

Mobile Phase B: 0.1 % formic acid in acetonitrile. In a 1000 ml volumetric flask containing about 500 ml of acetonitrile, 1 ml of formic acid was added and then the solution was diluted to volume with acetonitrile.

Recovery samples preparation
Ground muscle was weighted and mixed with water and acetonitrile. It was stirred and then QuEChERS were added. Then was homogenized and centrifuged. The upper liquid was passed to a QuEChERs fatty samples cleanup tube and stirred and centrifuged. Milli Q water was added to supernatant and transferred to a HPLC and inyected after shaking.

Results and discussions

Specificity
No significant peaks (≤ 30% LOQ) are detected at RT of the target analyte in the Blank and Test Solution with respect to the Spiked Test Solution..

Linearity
Eight different levels of concentration were used for calibration, ranging from 0.0001 mg/L to 0.0259 mg/L, equivalent to 0.002-0.5 mg/kg that includes values from below 30 % of LOQ (0.002 mg/kg) to above 30xLOQ (0.3 mg/kg)
($R^2 \geq 0.99$ were achieved for both transitions, demonstrating acceptable linearity.

Precision & Accuracy

Average recoveries at each fortification level were all within the acceptance range of 70-110 % (for both confirmatory transitions). The relative standard deviation (RSD) did not exceed 20 % at any fortification level.

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

Matrix	Analyte	Fortification level (mg/kg) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
Muscle	Acetamiprid	LOQ = 0.01 mg/kg (n = 5)	105	3	Trans 1
		10×LOQ = 0.10 mg/kg (n = 5)	105	3	
Muscle	Acetamiprid	LOQ = 0.01 mg/kg (n = 5)	108	2	Trans 2
		10×LOQ = 0.10 mg/kg (n = 5)	106	3	
Muscle	IM 2-1	LOQ = 0.01 mg/kg (n = 5)	94	2	Trans 1
		10×LOQ = 0.10 mg/kg (n = 5)	95	3	
Muscle	IM 2-1	LOQ = 0.01 mg/kg (n = 5)	94	5	Trans 2
		10×LOQ = 0.10 mg/kg (n = 5)	95	2	

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

	Acetamiprid
Specificity	No significant peaks (≤ 30% LOQ) are detected.
Calibration (type, number of data points)	Acetamiprid Transition 1: y = 36745361x ; R = 0.9999 (n = 8) Transition 2: y = 7228681x ; R = 0.9999 (n = 8) IM 2-1 Transition 1: y = 58397874x ; R = 0.9999 (n = 8) Transition 2: y 5028732x ; R = 0.9999 (n = 8)
Calibration range	Accepted calibration range in concentration units: 0.002 – 0.3 mg/kg
Assessment of matrix effects is presented	yes
Limit of determination/quantification	LOQ = 0.01 mg/kg LOD = 0.003 mg/kg

Conclusion

The method was successfully validated and is suitable for the determination of residues of Acetamiprid in muscle.

A 2.1.1.2.2.2 Independent laboratory validation

Comments of zRMS:	Method is accepted

Reference:	KCP 5.3.10
Report	Independent Laboratory Validation (ILV) of the analytical procedure for the determination of residues of acetamiprid (CAS: 135410-20-7) and its me-

	tabolite IM-2-1 (CAS: 190604-92-3) in milk by LC-MS/MS. xxx., 2019, Report No. 19.542434.0002
Guideline(s):	Yes (EU Guidance Document SANCO/3029/99 rev. 4, EU Guidance Document SANCO/825/00 rev. 8.1 and OECD-204/2014 guidance)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Mobile Phase A: 0.1 % formic acid in milliQ water. In a 1000 ml volumetric flask containing about 500 ml of milliQ water, 1 ml of formic acid was added and then the solution was diluted to volume with milliQ water.

Mobile Phase B: 0.1 % formic acid in acetonitrile. In a 1000 ml volumetric flask containing about 500 ml of acetonitrile, 1 ml of formic acid was added and then the solution was diluted to volume with acetonitrile.

Recovery samples preparation
Milk was weighted and mixed with water and acetonitrile. It was stirred and then QuEChERS were added. Then was homogenized and centrifuged. The upper liquid was passed to a QuEChERs fatty samples cleanup tube and stirred and centrifuged. Milli Q water was added to supernatant and transferred to a HPLC and inyected after shaking.

Results and discussions

Specificity
No significant peaks (≤ 30% LOQ) are detected at RT of the target analyte in the Blank and Test Solution with respect to the Spiked Test Solution.

Linearity
Eight different levels of concentration were used for calibration, ranging from 0.0001 mg/L to 0.0259 mg/L, equivalent to 0.002-0.5 mg/kg that includes values from below 30 % of LOQ (0.002 mg/kg) to above 30xLOQ (0.3 mg/kg)
($R^2 \geq 0.99$ were achieved for both transitions, demonstrating acceptable linearity.

Precision & Accuracy
Average recoveries at each fortification level were all within the acceptance range of 70-110 % (for both confirmatory transitions). The relative standard deviation (RSD) did not exceed 20 % at any fortification level.

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

Matrix	Analyte	Fortification level (mg/kg) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
Milk	Acetamiprid	LOQ = 0.01 mg/kg (n = 5)	92	0.5	Trans 1
		10×LOQ = 0.10 mg/kg (n = 5)	93	2.1	
Milk	Acetamiprid	LOQ = 0.01 mg/kg (n = 5)	92	3.3	Trans 2
		10×LOQ = 0.10 mg/kg (n = 5)	91	3.0	
Milk	IM 2-1	LOQ = 0.01 mg/kg (n = 5)	99	1	Trans 1

Matrix	Analyte	Fortification level (mg/kg) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
		10×LOQ = 0.10 mg/kg (n = 5)	98	2	
Milk	IM 2-1	LOQ = 0.01 mg/kg (n = 5)	95	2	Trans 2
		10×LOQ = 0.10 mg/kg (n = 5)	95	1	

Table A 2: Characteristics for the analytical method used for validation of acetamiprid and metabolite in milk

	Acetamiprid
Specificity	No significant peaks (≤ 30% LOQ) are detected.
Calibration (type, number of data points)	Acetamiprid Transition 1: y = 58954873x ; R = 0.9994 (n = 8) Transition 2: y = 11619337x ; R = 0.9998 (n = 8) IM 2-1 Transition 1: y = 74199208x ; R = 0.9992 (n = 8) Transition 2: y = 7276055x ; R = 0.9993 (n = 8)
Calibration range	Accepted calibration range in concentration units: 0.002 – 0.3 mg/kg
Assessment of matrix effects is presented	yes
Limit of determination/quantification	LOQ = 0.01 mg/kg LOD = 0.003 mg/kg

Conclusion

The method was successfully validated and is suitable for the determination of residues of Acetamiprid and its metabolite in milk.

A 1.1.1.1 Independent laboratory validation

Comments of zRMS:	Method is accepted

Reference:	KCP 5.3.11
Report	"Independent laboratory validation of the analytical procedure for the determination of residues of acetamiprid (CAS 135410-20-07), metabolites IM 2-1, (CAS 190604-92-3) in fat, liver, kidney and eggs by liquid chromatography". xxx 2020, xxx, Report 132/2020
Guideline(s):	Yes. SANCO/825/00 rev. 8.1, SANCO/3029/99 rev. 4
Deviations:	No
GLP:	Yes
Acceptability:	Yes/No/Supplementary

Materials and methods

Grinded matrix and water (except for eggs) was mixed, and then acetonitrile was added, and the mixture was stirred. Extraction salts QuEChERS was added, and the mixture was homogenized and centrifuged. The upper liquid was passed to a QuEChERS fatty samples cleanup tube and stirred and vortexed. The

supernatant liquid was mixed with Milli-Q wate and analyzed by HPLC-MS/MS

MRM:

	Precursor ion m/c		*m/z*	*Collision energy*
Acetamiprid	223	Quantifier ion (trans 1)	126	31
	223	Quantifier ion (trans 2)	56	53
IM 2-1	209	Quantifier ion (trans 1)	126	23
	209	Quantifier ion (trans 2)	90	43

Results and discussions

Specificity
No significant peaks (≤ 30% LOQ) are detected at RT of the target analyte in the Blank and Test Solution with respect to the Spiked Test Solution for each matrix.

Linearity
Five different levels of concentration were used for calibration, ranging from 0.002 mg/kg to 0.4 mg/kg, that includes values from below 20 % of LOQ (0.01 mg/kg) to at least 40xLOQ (0.4 mg/kg)
($R^2 \geq 0.99$ were achieved for both transitions, demonstrating acceptable linearity.

Precision & Accuracy
Average recoveries at each fortification level were all within the acceptance range of 70-110 % (for both confirmatory transitions). The relative standard deviation (RSD) did not exceed 20 % at any fortification level.

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

Matrix	**Analyte**	**Fortification level (mg/kg) (*n* = x)**	**Mean recovery (%)**	**RSD (%)**	**Comments**
Chicken fat	Acetamiprid	LOQ = 0.01 mg/kg (n = 6)	91	3.5	Trans 1
		10×LOQ = 0.1 mg/kg (n = 6)	100	4.2	
Chicken fat	Acetamiprid	LOQ = 0.01 mg/kg (n = 6)	85	10.4	Trans 2
		10×LOQ = 0.1 mg/kg (n = 6)	97	6.6	
Chicken fat	IM 2-1	LOQ = 0.01 mg/kg (n = 6)	91	7.9	Trans 1
		10×LOQ = 0.1 mg/kg (n = 6)	103	4.0	
Chicken fat	IM 2-1	LOQ = 0.01 mg/kg (n = 6)	90	8.7	Trans 2
		10×LOQ = 0.1 mg/kg (n = 6)	98	6.3	
Bovine liver	Acetamiprid	LOQ = 0.01 mg/kg (n = 6)	105	3.0	Trans 1
		10×LOQ = 0.1 mg/kg (n = 6)	106	5.4	
Bovine liver	Acetamiprid	LOQ = 0.01 mg/kg (n = 6)	88	11.4	Trans 2
		10×LOQ = 0.1 mg/kg (n = 6)	74	7.1	
Bovine liver	IM 2-1	LOQ = 0.01 mg/kg (n = 6)	98	5.7	Trans 1
		10×LOQ = 0.1 mg/kg (n = 6)	102	6.5	
Bovine liver	IM 2-1	LOQ = 0.01 mg/kg (n = 6)	99	10.5	Trans 2
		10×LOQ = 0.1 mg/kg (n = 6)	105	5.6	
Bovine kidney	Acetamiprid	LOQ = 0.01 mg/kg (n = 6)	102	5.6	Trans 1
		10×LOQ = 0.1 mg/kg (n = 6)	98	3.6	
Bovine	Acetamiprid	LOQ = 0.01 mg/kg (n = 6)	106	3.7	Trans 2

Matrix	Analyte	Fortification level (mg/kg) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
kidney		10×LOQ = 0.1 mg/kg (n = 6)	95	4.2	
Bovine kidney	IM 2-1	LOQ = 0.01 mg/kg (n = 6)	82	8.6	Trans 1
		10×LOQ = 0.1 mg/kg (n = 6)	73	7.6	
Bovine kidney	IM 2-1	LOQ = 0.01 mg/kg (n = 6)	94	13.0	Trans 2
		10×LOQ = 0.1 mg/kg (n = 6)	96	2.9	
Chicken eggs	Acetamiprid	LOQ = 0.01 mg/kg (n = 6)	100	10.4	Trans 1
		10×LOQ = 0.1 mg/kg (n = 6)	102	6.3	
Chicken eggs	Acetamiprid	LOQ = 0.01 mg/kg (n = 6)	106	4.7	Trans 2
		10×LOQ = 0.1 mg/kg (n = 6)	107	3.8	
Chicken eggs	IM 2-1	LOQ = 0.01 mg/kg (n = 6)	105	5.8	Trans 1
		10×LOQ = 0.1 mg/kg (n = 6)	90	3.5	
Chicken eggs	IM 2-1	LOQ = 0.01 mg/kg (n = 6)	94	4.1	Trans 2
		10×LOQ = 0.1 mg/kg (n = 6)	85	4.2	

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

	Acetamiprid
Specificity	No significant peaks (≤ 30% LOQ) are detected.
Calibration (type, number of data points)	Chicken fat. Acetamiprid Transition 1: y = 1e+3 + 9.12e+3x; R = 0.9996 (n = 5) Transition 2: y = 233 + 1.61e+3x ; R = 0.9997 (n = 5) IM 2-1 Transition 1: y = 902 + 8.66e+3x ; R = 0.9990 (n = 5) Transition 2: y = 156 + 1.37e+3x ; R = 0.9997 (n = 5) Bovine liver. Acetamiprid Transition 1: y = 436 + 7.2e+3x ; R = 0.9995 (n = 5) Transition 2: y = 174 + 1.25e+3x ; R = 0.9995 (n = 5) IM 2-1 Transition 1: y = -216 + 1.15e+005x ; R = 0.9941 (n = 5) Transition 2: y = -58.3 + 2.68e+004x ; R = 0.9925 (n = 5) Bovine kidney. Acetamiprid Transition 1: y = 343 + 7.29e+3x ; R = 0.9998 (n = 5) Transition 2: y = 50 + 1.31e+3x; R = 0.9993 (n = 5) IM 2-1 Transition 1: y = 116 + 6.91e+3x ; R = 0.9993 (n = 5) Transition 2: y = 18.8 + 1.07e+3x ; R = 0.9992 (n = 5) Chicken eggs. Acetamiprid Transition 1: y = 674 + 5.56e+3x ; R = 0.9941 (n = 5) Transition 2: y = 43.3 + 962x; R = 0.9925 (n = 5) IM 2-1 Transition 1: y = 228 + 6.08e+3x ; R = 0.9969 (n = 5) Transition 2: y = 50.8 + 966x ; R = 0.9985 (n = 5)
Calibration range	Accepted calibration range in concentration units: 0.1 – 25 µg/mL. Corresponding to 0.002 – 0.5 mg/kg

	Acetamiprid
Specificity	No significant peaks (≤ 30% LOQ) are detected.
Assessment of matrix effects is presented	yes
Limit of determination/quantification	LOQ = 0.01 mg/kg LOD = 0.002 mg/kg

Conclusion

The method was successfully validated and is suitable for the determination of residues of residues of Acetamiprid and IM 2-1 in matrix (fat, liver, kidney and eggs).

A 2.1.1.3 Description of Methods for the Analysis of Soil (KCP 5.2)

A 2.1.1.3.1 Analytical method 1

Comments of zRMS:	Method is accepted

Reference:	KCP 5.4.1
Report	"Terrestrial soil dissipation of acetamiprid after one application to bare soil in Europe". xxx. 2019, xxx, Report YV/18/010
Guideline(s):	Yes. SANCO/825/00 rev. 8.1, SANCO/3029/99 rev. 4
Deviations:	No
GLP:	Yes
Acceptability:	Yes/No/Supplementary

Materials and methods

Resides of acetamiprid and IM-1-5 were extracted from soil with acetonitrile:acidified water (1 % acetic acid) solution 8:2 v/v by shaking. After centrifugation, the solution was decanted and the retained solid re-extracted with 1:1 v/v solution. Following centrifugation, extracts were combined and the solid retained re-extracted with acidified water. The combined extracts were made to volume (50 ml) with water. A portion of final extract was then taken for final determination by LC-MS/MS.

MRM:

	Precursor ion m/c		*m/z*	*Collision energy*
Acetamiprid	223	Quantifier ion (trans 1)	126	25
	223	Quantifier ion (trans 2)	56	17
IM 1-5	198	Quantifier ion (trans 1)	99	45
	198	Quantifier ion (trans 2)	90	37

Results and discussions

Specificity

No significant peaks (≤ 30% LOQ) are detected at RT of the target analyte in the Blank and Test Solution with respect to the Spiked Test Solution each water.

Linearity

Five different levels of concentration were used for calibration, ranging from 0.1 ng/mL to 20 ng/mL, that includes values from below 30 % of LOQ (0.1 ng/mL) to at leat 20 % above the highest concentration level (20 ng/mL)
($R^2 \geq 0.99$ were achieved for both transitions, demonstrating acceptable linearity.

Precision & Accuracy
Average recoveries at each fortification level were all within the acceptance range of 70-110 % (for both confirmatory transitions). The relative standard deviation (RSD) did not exceed 20 % at any fortification level.

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

Matrix	Analyte	Fortification level (µg/L) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
Soil	Acetamiprid	LOQ = 0.002 mg/kg (n = 6)	95	3.5	Trans 1
		10×LOQ = 0.02 mg/kg (n = 6)	99	2.7	
Soil	Acetamiprid	LOQ = 0.002 mg/kg (n = 6)	97	4.6	Trans 2
		10×LOQ = 0.02 mg/kg (n = 6)	101	3.3	
Soil	IM 1-5	LOQ = 0.002 mg/kg (n = 6)	72	3.2	Trans 1
		10×LOQ = 0.02 mg/kg (n = 6)	74	3.3	
Soil	IM 1-5	LOQ = 0.002 mg/kg (n = 6)	74	3.4	Trans 2
		10×LOQ = 0.02 mg/kg (n = 6)	75	3.1	

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

	Acetamiprid
Specificity	No significant peaks (≤ 30% LOQ) are detected.
Calibration (type, number of data points)	Acetamiprid Transition 1: y = 3.22e+003 + 3.82e+004x ; R = 0.9977 (n = 8) Transition 2: y = -708 + 5.05e+004x ; R = 0.9969 (n = 8) IM-1-5 Transition 1: y = 3.19e+003 + 1.33e+005x ; R = 0.9992 (n = 8) Transition 2: y = 4.4e+003 + 1.16e+005x ; R = 0.9993 (n = 8)
Calibration range	Accepted calibration range in concentration units: 0.1 – 20 ng/mL. Corresponding to 0.0005 – 0.1 mg/kg
Assessment of matrix effects is presented	yes
Limit of determination/quantification	LOQ = 0.002 mg/kg LOD = 0.0006 mg/kg

Conclusion

The method was successfully validated and is suitable for the determination of residues of residues of Acetamiprid and IM-1-5 in soil.

A 2.1.1.4 Description of Methods for the Analysis of Water (KCP 5.2)

A 2.1.1.4.1 Analytical Method 1

Comments of zRMS:	Method is accepted

Reference:	KCP 5.5.1
Report	"Analytical method validation of acetamiprid in waters". xxx, 2019, xxx, Report E18140
Guideline(s):	Yes. SANCO/825/00 rev. 8.1, SANCO/3029/99 rev. 4
Deviations:	No
GLP:	Yes
Acceptability:	Yes/No/Supplementary

Materials and methods

Mesotrione was extracted by shaking with water, acetonitrile, magnesium sulphate anhydrous, sodium chloride, sodium citrate dehydrate and sodium hydrogencitrate sesquidrate. After centrifugation, the supernatant was purified with magnesium sulphate anhydrous and C18 resin. After a second centrifugation, mobile phase A was added to the sample and analyzed by LC-MS/MS.

MRM:

	Precursor ion m/c		*m/z*	*Collision energy*
Acetamiprid	222.94	Quantifier ion (trans 1)	125.93	20
	222.94	Quantifier ion (trans 2)	56.10	20

Results and discussions

Specificity
No significant peaks (≤ 30% LOQ) are detected at RT of the target analyte in the Blank and Test Solution with respect to the Spiked Test Solution each water.

Linearity
Five different levels of concentration were used for calibration, ranging from 0.02 µg/L to 5.07 µg/L, that includes values from below 30 % of LOQ (0.03 µg/L) to above 30xLOQ (3 µg/L)
($R^2 \geq 0.99$ were achieved for both transitions, demonstrating acceptable linearity.

Precision & Accuracy
Average recoveries at each fortification level were all within the acceptance range of 70-110 % (for both confirmatory transitions). The relative standard deviation (RSD) did not exceed 20 % at any fortification level.

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

Matrix	Analyte	Fortification level (µg/L) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
Drinking water	Acetamiprid	LOQ = 0.1 µg/L (n = 5)	100.77	1.28	Trans 1
		10×LOQ = 1 µg/L (n = 5)	97.49	0.84	
Drinking water	Acetamiprid	LOQ = 0.1 µg/L (n = 5)	96.41	2.37	Trans 2
		10×LOQ = 1 µg/L (n = 5)	97.38	1.73	
Ground water	Acetamiprid	LOQ = 0.1 µg/L (n = 5)	107.52	2.78	Trans 1

Matrix	Analyte	Fortification level (µg/L) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
		10×LOQ = 1 µg/L (n = 5)	103.60	0.76	
Ground water	Acetamiprid	LOQ = 0.1 µg/L (n = 5)	104.47	5.18	Trans 2
		10×LOQ = 1 µg/L (n = 5)	100.63	0.94	
Surface water	Acetamiprid	LOQ = 0.1 µg/L (n = 5)	102.70	5.08	Trans 1
		10×LOQ = 1 µg/L (n = 5)	104.75	1.82	
Surface water	Acetamiprid	LOQ = 0.1 µg/L (n = 5)	98.19	5.67	Trans 2
		10×LOQ = 1 µg/L (n = 5)	100.64	2.45	

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

	Acetamiprid
Specificity	No significant peaks (≤ 30% LOQ) are detected.
Calibration (type, number of data points)	Transition 1: y = 68.3197 + 13738.1929x ; R = 0.9999 (n = 8) Transition 2: y = 50.6314 + 7407.6474x ; R = 0.9999 (n = 8)
Calibration range	Accepted calibration range in concentration units: 0.02 – 5.07 µg/L
Assessment of matrix effects is presented	yes
Limit of determination/quantification	LOQ = 0.1 µg/L LOD = 0.03 µg/L

Conclusion

The method was successfully validated and is suitable for the determination of residues of Acetamiprid in waters (drinking water, ground water and surface water).

A 2.1.1.4.1.1 Independent laboratory validation

Comments of zRMS:	Method is accepted

Reference:	KCP 5.5.2
Report	Independent Laboratory Validation of analytical procedure for the determination of residues of acetamiprid in water. xxx, 2019, Report No. 146/2019
Guideline(s):	Yes (EU Guidance Document SANCO/3029/99 rev. 4, EU Guidance Document SANCO/825/00 rev. 8.1 and OECD-204/2014 guidance)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Mobile Phase A: 10 mM ammonium formate buffer, 0.1% (v/v) Formic Acid in Water. About 0.62 g of ammonium formate was accurately weighed (+/-) 0.01 g) into 1000 ml volumetric flask and dissolved

with about 500 ml of milliQ water. 50 ml of methanol and 1 ml of formic acid were added and then the solution was diluted to volume with milliQ water closed tightly and mixed by inverting several times. Solvent was transferred to amber HPLC solvent reservoir. The adjusted pH was 4.

Mobile Phase B: Methanol. Solvent was transferred to amber HPLC solvent reservoir.

Recovery samples preparation
5 samples at two different concentrations were prepared for drinking water. For the preparation of the samples, in the indicated ml vvolumetric dlasks the amounts of reference item solution were added with micropipette. The final volume was made with drinking water.

Results and discussions

Specificity
No significant peaks (≤ 30% LOQ) are detected at RT of the target analyte in the Blank and Test Solution with respect to the Spiked Test Solution each water.

Linearity
Eight different levels of concentration were used for calibration, ranging from 0.02 µg/L to 5.1 µg/L, that includes values from below 30 % of LOQ (0.03 µg/L) to above 30xLOQ (3 µg/L)
($R^2 \geq 0.99$ were achieved for both transitions, demonstrating acceptable linearity.

Precision & Accuracy
Average recoveries at each fortification level were all within the acceptance range of 70-110 % (for both confirmatory transitions). The relative standard deviation (RSD) did not exceed 20 % at any fortification level.

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

Matrix	Analyte	Fortification level (µg/L) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
Drinking water	Acetamiprid	LOQ = 0.1 µg/L (n = 5)	85	2.1	Trans 1
		10×LOQ = 1 µg/L (n = 5)	96	1.5	
Drinking water	Acetamiprid	LOQ = 0.1 µg/L (n = 5)	81	8.0	Trans 2
		10×LOQ = 1 µg/L (n = 5)	94	1.5	

Table A 4: Characteristics for the analytical method used for validation of acetamiprid in drinking water

	Acetamiprid
Specificity	No significant peaks (≤ 30% LOQ) are detected.
Calibration (type, number of data points)	Transition 1: y = 3320 + 206000x ; R = 0.9996 (n = 8) Transition 2: y = 54.5 + 32900x ; R = 0.9995 (n = 8)
Calibration range	Accepted calibration range in concentration units: 0.02 – 5.1 µg/L
Assessment of matrix effects is presented	yes
Limit of determination/quantification	LOQ = 0.1 µg/L LOD = 0.03 µg/L

Conclusion

The method was successfully validated and is suitable for the determination of residues of Acetamiprid in drinking water.

A 2.1.1.4.2 Analytical method 2

Comments of zRMS:	Method is accepted

Reference:	KCP 5.5.3
Report	"IM 1-5 (acetamiprid metabolite): analytical method validation in surface water, ground water and drinking water at LOQ of 0.05 µg/l". xxx, 2020, xxx, Report E20064
Guideline(s):	Yes. SANCO/825/00 rev. 8.1
Deviations:	No
GLP:	Yes
Acceptability:	Yes/No/Supplementary

Materials and methods

For the preparation of the samples, in glass vials the amounts indicated in the previous table of the reference item solution and each matrix (ground water, drinking water and surface water) were added using suitable micropipettes. The surface water was filtered before adding the amount in the glass vial. The final volume was made up with milli-Q water. The final extracts will be analyzed by liquid chromatography with tandem mass spectrometric detection (UPLC-MS/MS):

MRM:

	Precursor ion m/c		*m/z*	*Collision energy*
IM 1-5	197.99	Quantifier ion (trans 1)	125.96	20
	197.99	Quantifier ion (trans 2)	90.04	34

Results and discussions

Specificity

No significant peaks (≤ 30% LOQ) are detected at RT of the target analyte in the Blank and Test Solution with respect to the Spiked Test Solution each water.

Linearity

Eight different levels of concentration were used for calibration, ranging from 0.01 µg/L to 1.95 µg/L, that includes values from below 30 % of LOQ (0.015 µg/L) to above 30xLOQ (1.5 µg/L) ($R^2 \geq 0.99$ were achieved for both transitions, demonstrating acceptable linearity for each water.

Precision & Accuracy

Average recoveries at each fortification level were all within the acceptance range of 70-120 % (for both confirmatory transitions). The relative standard deviation (RSD) did not exceed 20 % at any fortification level.

Table A 1: Recovery results from method validation of IM 1-5 (acetamiprid metabolite) using the analytical method

Matrix	Analyte	Fortification level (µg/L) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
Drinking water	IM 1-5	LOQ = 0.05 µg/L (n = 5)	102.57	5.84	Trans 1
		10×LOQ = 0.5 µg/L (n = 5)	110.54	2.83	

Matrix	Analyte	Fortification level (µg/L) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
Drinking water	IM 1-5	LOQ = 0.05 µg/L (n = 5)	106.97	18.41	Trans 2
		10×LOQ = 0.5 µg/L (n = 5)	108.54	2.95	
Ground water	IM 1-5	LOQ = 0.05 µg/L (n = 5)	107.09	9.04	Trans 1
		10×LOQ = 0.5 µg/L (n = 5)	108.52	1.09	
Ground water	IM 1-5	LOQ = 0.05 µg/L (n = 5)	96.39	10.15	Trans 2
		10×LOQ = 0.5 µg/L (n = 5)	109.75	1.88	
Surface water	IM 1-5	LOQ = 0.05 µg/L (n = 5)	109.41	4.85	Trans 1
		10×LOQ = 0.5 µg/L (n = 5)	115.51	4.38	
Surface water	IM 1-5	LOQ = 0.05 µg/L (n = 5)	111.20	9.09	Trans 2
		10×LOQ = 0.5 µg/L (n = 5)	112.20	3.55	

Table A 2: Characteristics for the analytical method used for validation of IM 1-5 (acetamiprid metabolite) in waters

	IM 1-5 Acetamiprid metabolite
Specificity	No significant peaks (≤ 30% LOQ) are detected.
Calibration (type, number of data points)	Ground water: Transition 1: y = 44.15 + 6253.20x ; R = 0.9991 (n = 8) Transition 2: y = 19.64 + 1334.38x ; R = 0.9987 (n = 8) Drinking water: Transition 1: y = 58.02 + 6011.41x ; R = 0.9999 (n = 8) Transition 2: y = -0.72 + 1299.47x ; R = 0.9988 (n = 8) Surface water: Transition 1: y = 48.70 + 6061.56x ; R = 0.9997 (n = 8) Transition 2: y = 15.31 + 1272.35x ; R = 0.9982 (n = 8)
Calibration range	Accepted calibration range in concentration units: 0.01 – 1.95 µg/L
Assessment of matrix effects is presented	yes
Limit of determination/quantification	LOQ = 0.05 µg/L LOD = 0.01 µg/L

Conclusion

The method was successfully validated and is suitable for the determination of residues of IM 1-5 (Acetamiprid metabolite) in waters (drinking water, ground water and surface water).

A 2.1.1.4.2.1 Independent laboratory validation

Comments of zRMS:	Method is accepted

Reference:	KCP 5.5.4
Report	Independent Laboratory Validation of IM 1-5 (acetamiprid metabolite) in drinking water by LC-MS. xxx, 2020, Report No. 28/2020
(acetamiprid metabolite)	Yes (EU Guidance Document SANCO/3029/99 rev. 4, EU Guidance Document SANCO/825/00 rev. 8.1 and OECD-204/2014 guidance)

Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Mobile Phase A: 0.1 % formic acid in water. 1 ml of formic acid was added into 1000 ml volumetric flask half full with milli-Q water and then dissolved with milli-Q water to 1000 ml.

Mobile Phase B: 0.1 % acid formic in acetonitrile.1 ml of formic acid was added into 1000 ml volumetric flask half full with acetonitrile and then dissolved with acetonitrile to 1000 ml.

Recovery samples preparation
5 samples at two different concentrations were prepared for drinking water. For the preparation of the samples, in the indicated ml volumetric flasks the amounts of reference item solution were added with micropipette. The final volume was made with milli-Q water.

Results and discussions

Specificity
No significant peaks (≤ 30% LOQ) are detected at RT of the target analyte in the Blank and Test Solution with respect to the Spiked Test Solution.

Linearity
Five different levels of concentration were used for calibration, ranging from 0.01 µg/L to 2.0 µg/L, that includes values from below 20 % of LOQ (0.01 µg/L) to above 40xLOQ (2.0 µg/L)
($R^2 \geq 0.99$ were achieved for both transitions, demonstrating acceptable linearity.

Precision & Accuracy
Average recoveries at each fortification level were all within the acceptance range of 70-110 % (for both confirmatory transitions). The relative standard deviation (RSD) did not exceed 20 % at any fortification level.

Table A 1: Recovery results from method validation of IM 1-5 (acetamiprid metabolite) using the analytical method

Matrix	Analyte	Fortification level (µg/L) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
Drinking water	IM 1-5	LOQ = 0.05 µg/L (n = 5)	82	8.6	Trans 1
		10×LOQ = 0.5 µg/L (n = 5)	73	7.6	
Drinking water	IM 1-5	LOQ = 0.05 µg/L (n = 5)	88	11.4	Trans 2
		10×LOQ = 0.5 µg/L (n = 5)	74	7.1	

Table A 2: Characteristics for the analytical method used for validation of IM 1-5 (Acetamiprid metabolite) in drinking water

	Acetamiprid
Specificity	No significant peaks (≤ 30% LOQ) are detected.
Calibration (type, number of data points)	Transition 1: y = -216 + 1.15e+0.05x ; R = 0.9941 (n = 8) Transition 2: y = -58.3 + 2.68e+0.04x ; R = 0.9925 (n = 8)
Calibration range	Accepted calibration range in concentration units: 0.01 – 2.0 µg/L

	Acetamiprid
Specificity	No significant peaks (≤ 30% LOQ) are detected.
Assessment of matrix effects is presented	yes
Limit of determination/quantification	LOQ = 0.05 µg/L LOD = 0.01 µg/L

Conclusion

The method was successfully validated and is suitable for the determination of residues of IM 1-5 (Acetamiprid metabolite) in drinking water.

A 2.1.1.5 Description of Methods for the Analysis of Air (KCP 5.2)

Comments of zRMS:	Method is accepted

Reference:	KCP 5.6.1
Report	"Validation of the analytical procedure for the determination of acetamiprid (CAS: 135410-20-7) in air by LC-MS, xxx, 2018, xxx. Report 18.644636.0002
Guideline(s):	Yes. SANCO/825/00 rev. 8.1, SANCO/3029/99 rev.4 OECD-204/2014 ENV/MC/CH EM(98)17 ENV/JV/MON0 (2002)9 Directive 91/414/EEC
Deviations:	No.
GLP:	Yes
Acceptability:	Yes/No/Supplementary

Materials and methods

A polyurethane plug is soaked with acetone and dried. Then plug is put into a glass syringe connect to a pump and air is collected. After sampling the plug is transferred into a round bottomed flask and soak into extraction mixture. After sonication, an aliquot is transferred into a glass tube and dried by N2 flux. The dried sample is resuspended into blank solution.

MRM:

	Precursor ion m/c		*m/z*	*Collision energy*
Acetamiprid	223	Quantifier ion (trans 1)	125.89	15
	223	Confirmation ion (trans 2)	55.90	15

Results and discussions

Specificity

No significant peaks (≤ 30% LOQ) are detected at RT of the target analyte in the Blank and Test Solution with respect to the Spiked Test Solution for both transition 1 and 2.

Linearity
Method I:
Five different levels of concentration were used for calibration, ranging from 30 % LOQ (0.0006 µg/m^3) to 35xLOQ (0.069 µg/m^3) of analyte on the sample.
($R^2 \geq 0.99$ were achieved for both transitions, demonstrating acceptable linearity.

Precision & Accuracy
Average recoveries were within the acceptance range of 80-110 % (for both confirmatory transitions). The relative standard deviation (RSD) did not exceed 20 %.

Table A 1: Recovery results from method validation of acetamiprid.

Matrix	Analyte	Fortification level (µg/m^3)) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
Air	Acetamiprid	LOQ = 0.002 µg/m^3 (n = 5)	100	0.5	Trans 1
		LOQ = 0.002 µg/m^3 (n = 5)	101	1.2	Trans 2
		10xLOQ = 0.02 µg/m^3 (n = 5)	103	0.7	Trans 1
		10xLOQ = 0.02 µg/m^3 (n = 5)	101	0.6	Trans 2

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

	Acetamiprid
Specificity	No significant peaks (≤ 30% LOQ) are detected.
Calibration (type, number of data points)	Transition 1: y = 0 + 172018 ; R^2 = 0.9995 (n = 5) Transition 2: y = -685.800286 + 62367x ; R^2 = 0.9993 (n = 5)
Calibration range	Linear between 0.0006 µg/m^3 to 0.069 µg/m^3
Assessment of matrix effects is presented	yes
Limit of determination/quantification	LOQ = 0.002 µg/m^3 LOD = 0.0006 µg/m^3

Conclusion

It is concluded that extraction efficiency has been sufficiently proven, since method of extraction gives acceptable results, and is applicable as enforcement and data generation method for determination of Acetamiprid in air.

A 2.1.1.6 Description of Methods for the Analysis of Body Fluids and Tissues (KCP 5.2)

Comments of zRMS:	Method is accepted

Reference:	KCP 5.7.1
Report	"Analytical method validation of acetamiprid parent compound in blood". xxx, 2019, xxx, Report E19080

Guideline(s):	Yes. SANCO/825/00 rev. 8.1, SANCO/3029/99 rev. 4
Deviations:	Yes - Study plan deviation relating to some method conditions. Sample temperature and some conditions of Daughter scan mode were changed to improve method. - Study plan deviation relating spectra. PM7 was injected instead PM3 to obtain better spectra. - Study plan deviation relating concentration in linearity. Calculations regarding equivalent concentration in blood were included in the final report.
GLP:	Yes
Acceptability:	Yes/No/Supplementary

Materials and methods

Blood matrix extract was prepared by shaking blood and acetone. After centrifugation, the acetone phase was diluted in acetone.

MRM:

	Precursor ion m/c		*m/z*	*Collision energy*
Acetamiprid	222.94	Quantifier ion (trans 1)	125.93	20
	222.94	Quantifier ion (trans 2)	56.10	20

Results and discussions

Specificity
No significant peaks (≤ 30% LOQ) are detected at RT of the target analyte in the Blank and Test Solution with respect to the Spiked Test Solution each water.

Linearity
Five different levels of concentration were used for calibration, ranging from 0.25 µg/L to 25.33 µg/L, that includes values from below 30 % of LOQ (0.3 µg/L) to about 30xLOQ (30 µg/L).
($R^2 \geq 0.99$ were achieved for both transitions, demonstrating acceptable linearity.

Precision & Accuracy
Average recoveries were within the acceptance range of 70-110 % (for both confirmatory transitions). The relative standard deviation (RSD) did not exceed 20 %.

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

Matrix	Analyte	Fortification level (µg/L) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
Blood	Acetamiprid	LOQ = 1 µg/L (n = 5)	99.72	3.94	Trans 1
		LOQ = 1 µg/L (n = 5)	104.14	3.55	Trans 2

Table A 6: Characteristics for the analytical method used for validation of acetamiprid in blood

	Acetamiprid
Specificity	No significant peaks (≤ 30% LOQ) are detected.
Calibration (type, number of data points)	Transition 1: y = -4.9169 + 1527.5671x ; R = 0.9997 (n = 7)

	Acetamiprid
Specificity	No significant peaks (≤ 30% LOQ) are detected.
	Transition 2: y = -16.2129 + 705.1539x ; R = 0.9994 (n = 7)
Calibration range	Accepted calibration range in concentration units: 0.25 – 25.33 µg/L Corresponding to a equilavent concentration in blood of (1.25 – 125.0 µg/L).
Assessment of matrix effects is presented	yes
Limit of determination/quantification	LOQ = 1 µg/L LOD = 0.3 µg/L

Conclusion

The method was successfully validated and is suitable for the determination of residues of Acetamiprid in blood.

A 2.1.1.7 A.2.A.9 Other Studies/ Information

No new or additional studies have been submitted.