REGISTRATION REPORT

Part B

Section 5

Analytical Methods

Detailed summary of the risk assessment

Product code: CA3573

Product name(s): Carnadine/Kestrel

Chemical active substance:

Acetamiprid, 200 g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(Re-authorisation acc. to Art. 43)

Applicant: Nufarm Europe GmbH

Submission date: July 2020, updated: December 2020

MS Finalisation date: May 2021 (initial Core Assessment)

November 2021, January 2022 (final Core Assessment)

Version history

When	What
July 2020	Initial dRR – Nufarm Europe GmbH – Version 1
October 2020	Update Analytical Methods – zRMS request
December 2020	Update on the analytical section of the storage stability study in honey in Section A 2.1.1.1.12.1 Method validation 20N08133-01-SSHN – Version 2
May 2021	Initial zRMS assessment (re-authorization). The report in the dRR format has been prepared by the Applicant, therefore all comments, additional evaluations and conclusions of the zRMS are presented in grey commenting boxes. Minor changes are introduced directly in the text and highlighted in grey. Not agreed or not relevant information are ~~struck through and shaded for transparency~~.
November 2021	Final report (Core Assessment updated following the commenting period) No additional information or assessments after the commenting period.
January 2022	Final report (Core Assessment after additional round of the commenting period) No additional information or assessments after the commenting period.

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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
Apple	Suported
Potato	Suported
Oilseed rape	Suported
Corn/maize	Suported

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)

This application is for CA3573 with the Tradename Carnadine (Acetamiprid 200 SL) by Nufarm GmbH & Co.KG. The product was formerly owned by Adama ADAMA Makhteshim Ltd. under the product code MCW-2222. The two products are identical. Therefore, all studies conducted with MCW-2222 can be used for CA3573, without any restrictions. Further details are given in Part C.

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 method was suitably validated in accordance with SANCO/3030/99 rev.4. The proposed HPLC with DAD and UV detection at 250 nm method is appropriate for the determination of acetamiprid in CA3573.

Reference:	KCP 5.1.1/01
Report	Development and validation of an analytical method for the determination of acetamiprid in MCW-2222, Walter, D., 2014, Study No. S13-03099
Guideline(s):	SANCO/3030/99 rev. 4
Deviations:	None
GLP:	Yes
Acceptability:	Yes

Materials and methods

A. Materials

1. Standards

Test item 1:	MCW-2222 formulation (acetamiprid 200 g/L SL)
CAS No.:	135410-20-7

Content of a.i.:	201 g/L
Lot/batch No.:	611-280413-01
Expiry date:	28/04/2015
Blank formulation:	MCW-2222 blank formulation
Batch No.:	120513
Content of a.i.:	None
Expiry date:	12/05/2015
Reference item:	Acetamiprid
CAS No.:	135410-20-7
Batch No.:	SZBC110XV
Purity:	99.9%
Expiry date:	19/04/2017

B. Sample preparation and processing

The content of acetamiprid in MCW-2222 was determined by dissolving the test item in acetonitrile and analysing the resulting solutions using HPLC with UV detection at 250 nm.

Specificity
Acetamiprid was identified in samples of the test item by comparing retention times and UV spectra to those produced by samples of the reference item.

Linearity
The response of the detector to acetamiprid was assessed by analysing ten concentration levels ranging between 101 and 1000 ng per injection with UV detection at 250 nm. A calibration curve was subsequently plotted and a correlation coefficient calculated.

Accuracy
The accuracy of the methodology was determined by measuring recovery rates for the target analyte. Fortified samples were prepared by adding known amounts of technical grade analyte to blank formulation to provide five replicates at each of two concentration levels (low and high). The nominal concentration of acetamiprid in the low level samples was 20.0 mg/L and in the high level samples was 69.9 mg/L, equivalent to 7.2% w/w and 22.2% w/w, respectively.

Precision
The precision of the methodology was determined by analysing five independent samples of the test item (MCW-2222). Each sample was analysed twice and the mean concentration of acetamiprid was then calculated along with its associated standard deviation and relative standard deviation.

C. Analytical instrumentation and analysis

1. UV parameters	Agilent HP 1100 with Diode Array detector
Column:	Synergi Hydro-RP µm 80A, 150 x 4.6 mm
Absorption:	250 nm

Validation - Results and discussions
Specificity
Comparison of the chromatograms produced by the reference item and the test item revealed peaks with similar retention times that did not deviate by more than 0.2%. Comparison of the UV spectra of acetamiprid in the reference item with those of the test item revealed a 100% match between 200 and 400 nm. Analysis of blank formulation samples showed no interference at the retention time for acetamiprid. The method is therefore considered specific for the determination of acetamiprid in MCW-2222.

Linearity
A correlation coefficient (R^2) of 0.9999 was calculated for the calibration curve produced by acetamiprid over the concentration range 101 – 1000 ng/injection. The detector response was linear over the range of concentrations tested.

Concentration range: 101 – 1000 ng
Equation of the curve: y = 0.2151 x – 2.5059

Accuracy (recovery)
The mean recovery rate at the 7.2% w/w (low) concentration level was 100.9% (n = 5). The mean recovery rate at the 22.2% w/w (high) concentration level was 99.7% (n = 5). Acetamiprid recovery rates at the low and high concentration levels were therefore well within their acceptable ranges of 97 – 103% and 98 – 102%, respectively.

Precision
The mean content of acetamiprid in MCW-2222 was determined as 18% w/w. At 1.2%, the RSD for the recovery of 18% w/w acetamiprid was below the 1.73% acceptability value calculated using the modified Horwitz equation. The precision of the method is therefore good and acceptable for acetamiprid. See Table 5.2.-1.

Table 5.2-1: Methods suitable for the determination of active substance acetamiprid in plant protection product MCW-2222

Author(s), year	Walter, D. 2014
Principle of method	HPLC-DAD
Linearity (linear between mg/L / % range of the declared content) (correlation coefficient, expressed as r)	101 – 1000ng/injection r = 0.9999
Precision – Repeatability Mean n = 5 (%RSD)	Mean content = 18.0 % w/w; n= 5 %RSD = 1.2 Within Horwitz value of = 1.73
Accuracy n = 5 (% Recovery)	Mean % of recovery = 100.9 % (n=5; at low fortification level) Mean % of recovery = 99.7 % (n=5; at high fortification level
Interference/ Specificity	No relevant interference occurred at the retention time of acetamiprid
Comment	-

Conclusion
In this study, an analytical method for the determination of acetamiprid in MCW-2222 was validated. The method complies with the relevant guidance and is therefore considered valid for the determination of acetamiprid in MCW-2222 in terms of specificity, linearity, precision, accuracy and recovery.

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

The formulation under consideration contains no relevant impurities.

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

CONFIDENTIAL information - data provided separately (Part C)

5.2.1.4 Applicability of existing CIPAC methods (KCP 5.1.1)

A CIPAC analytical method currently exists for the determination of acetamiprid. CIPAC method no. 649 has been developed for the determination of acetamiprid technical in soluble concentrates (649/SL/(M)/) as

well as wettable powders (649/WP/(M)/), water soluble powders (649/SP/(M)/), water soluble granules (649/SG/(M)/) and emulsifiable concentrates (649/EC/(M)/)
The guideline 'Guidance for generating and reporting methods of analysis in support of pre- and post-registration data requirements for Regulation (EU) 283/2013 and Regulation (EU) 545/2011 of Regulation 1107/2009/EC' states: 'The applicability of existing CIPAC methods shall be assessed and reported. In case of use of a CIPAC method, further validation data shall not be required, but example chromatograms shall be submitted, where available.'
The CIPAC Method 649 for the active substance acetamiprid was collaboratively tested on equivalent SL-formulation(s).

5.2.2 Methods for the determination of residues (KCP 5.1.2)

An overview on the acceptable methods and possible data gaps for analysis of residues of acetamiprid for the generation of pre-authorization data is given in the following table. For the detailed evaluation of new studies it is referred to Appendix 2.

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

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**
Plant matrices (residues)	Primary	Not provided *(in potatoes)*	LC-MS/MS	Netzband, D., 2003, Study No. RD-00243, EU agreed, Netherlands, RAR, 2015
	Confirmatory (if required)	-	Not required	-
	Primary	0.01 mg/kg	GC-ECD	Goller, G., 1999, Study No. RPA/NI-25/97051, EU agreed, Netherlands, RAR, 2015
	Confirmatory (if required)	-	Not required	-
	Primary	n.a.	LC-MS/MS	Jean-Baptiste C., 2009, Study No. A7125, EU agreed, Netherlands, RAR, 2015
	Confirmatory (if required)	-	Not required	-
	Primary	n..a.	GC-ECD (non cirtrus) HPLC-UV (citrus)	Gieseke L.D., 1999, Study No. 10201, EU agreed, Netherland, RAR, 2015
	Confirmatory (if required)	-	Not required	-
	Primary	0.01 mg/kg *(dry bean seed and straw, apple, olive, orange peel and pulp)*	HPLC-MS/MS	Lefresne, S., 2014, Study No. B13-M1-A-02, New data, KCP 5.1.2/01
	Confirmatory (if required)	-	Not required	-
	Primary	0.01 mg/kg *(in wheat)*	HPLC-MS/MS	Chevallier, E., 2014, Study No. B14C-S1-A-01, New data, KCP 5.1.2/02
	Confirmatory (if required)	-	Not required	-
	Primary	0.01 mg/kg *(in barley)*	HPLC-MS/MS	Chevallier, E., 2014, Study No. B14C-S1-A-03, New data, KCP

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**
				5.1.2/03
	Confirmatory (if required)	-	Not required	-
	Primary	0.01 mg/kg *(in wheat)*	LC-MS/MS	Barbier, G., 2018, Study No. B17G-A4-A-02, New data, KCP 5.1.2/04
	Confirmatory (if required)	-	Not required	-
	Primary	0.01 mg/kg *(peaches)*	HPLC-MS/MS	Méric, D., 2013, Study No. DMC-13-16126, New data, KCP 5.1.2/05
	Confirmatory (if required)	-	Not required	-
	Primary	0.01 mg/kg *(apple and peaches)*	HPLC-MS/MS	Méric, D., 2014, Study No. DMC-13-16134, New data, KCP 5.1.2/06
	Confirmatory (if required)	-	Not required	-
	Primary	0.01 mg/kg *(apple and processed fractions)*	HPLC-MS/MS	Roussel, Ch., 2014, Study No. ChR-14-17311, New data, KCP 5.1.2/07
	Confirmatory (if required)	-	Not required	-
	Primary	0.01 mg/kg *(oilseed rape)*	HPLC-MS/MS	Méric, D., 2014, Study No. DMC-13-16129, New data, KCP 5.1.2/08
	Confirmatory (if required)	-	Not required	-
	Primary	0.01 mg/kg *(oilseed rape)*	HPLC-MS/MS	Chevallier, E., 2014, Study No. 14SGS035, New data, KCP 5.1.2/09
	Confirmatory (if required)	-	Not required	-
	Primary	0.01 mg/kg *(potato)*	HPLC-MS/MS	Bousquet, C., 2014, Study No. 13SGS102, New data, KCP 5.1.2/10
	Confirmatory (if required)	-	Not required	-
	Primary	0.01 mg/kg *(in wheat, turnip, spinach)* [(a)]	LC-MS/MS	Raufer, B., 2013, Study No. S10-02822, EU agreed, Netherlands, RAR, 2015 *
	Confirmatory (if required)	-	Not required	-
	Primary	0.01 mg/kg [(a)]	HPLC-MS/MS	Semrau, J., 2017, Study No. S15-02364-L2, New data, KCP 5.1.2/11 , filed under KCP 5.2
	Confirmatory (if required)	-	Not required	-
	Primary	0.01 mg/kg *in honey*	HPLC-MS/MS	Hecht-Ross, S., 2020, Study No. R1940050, New data, KCP 5.1.2/12
	Confirmatory (if required)	-	Not required	-
	Primary	Ongoing *(storage*		Müller, S., 2020 Study No. CIP

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**
		stability in honey)		20N08133-01-SSHN KCP 5.1.2/13
	Confirmatory (if required)	-	Not required	-
Soil (Environmental fate)	Primary	Not provided	HPLC	Mamouni, A., 1997, EU agreed, Netherlands, RAR, 2015 and DAR 2001
	Confirmatory (if required)	-	Not required	-
	Primary	Not provided	HPLC	Liu, A.C., 1997, EU agreed, Netherlands, RAR, 2015 and DAR 2001
	Confirmatory (if required)	-	Not required	-
	Primary	Not provided	HPLC	Sugiyama, H., 2010, Study No. RD-02101, EU agreed, Netherlands, RAR, 2015 *
	Confirmatory (if required)	-	Not required	-
Water (Environmental fate)	Primary	Not provided	HPLC, NMR, IC-MS	Emeric, G.T., 1998, Study No. 96 – 82, EU agreed, Netherlands, RAR, 2015 and DAR 2001
	Confirmatory (if required)	-	Not required	-
	Primary	Not provided	HPLC	Shiotani, H., 2003, EU agreed, Netherlands, RAR, 2015
	Confirmatory (if required)	-	Not required	-
Residue dissipation (Toxicology)	Primary	0.2 µg/L *(in pome fruit)*	HPLC-MS/MS	Wilson, A., 2016, Study No. ACI16-010 (VV57LS), New data, KCP 5.1.2/14
	Confirmatory (if required)	-	Not required	-
Water (Ecotoxicology)	Primary	0.185 mg/L	HPLC-UV	Juckeland, D., 2014, Study No. 141048005 W, New data, KCP 5.1.2/15
	Confirmatory (if required)	-	Not required	-
	Primary	0.367 mg/L	HPLC-UV	Juckeland, D., 2014, Study No. 141048006 W, New data, KCP 5.1.2/16
	Confirmatory (if required)	-	Not required	-
	Primary	0.47 µg/L	HPLC-MS/MS	Juckeland, D., 2015, Study No. 141048057 W, New data, KCP 5.1.2/17
	Confirmatory (if required)	-	Not required	-
	Primary	Not provided	HPLC-UV	Putt,A.E., 2003b, Study No. 12681.6105, EU agreed, Netherlands, RAR 2015 *

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**
	Confirmatory (if required)	-	Not required	-
	Primary	Not provided	HPLC-UV	Kley, A., and Wydra, V., 2012b, Study No. RD-02374, EU agreed, Netherlands, RAR 2015 *
	Confirmatory (if required)	-	Not required	-
	Primary	0.344 mg/L	HPLC-UV	Juckeland, D., 2014, Study No. 141048007 W, New data, KCP 5.1.2/18
	Confirmatory (if required)	-	Not required	-
Bee feeding (Ecotoxicology)	Primary	0.010 mg/kg *(in wax, pollen, flowers, honey and nectar)*	HPLC-MS/MS	Molitor, C., 2014, Study No. 215-2014 + Ammendmend 1, New data, KCP 5.1.2/19
	Confirmatory (if required)	-	Not required	-
	Primary	0.01 mg/kg *(in wax, pollen, flowers, honey, and nectar)*	HPLC-MS/MS	Molitor, C., 2014, Study No. 230-2015, New data, KCP 5.1.2/20
	Confirmatory (if required)	-	Not required	-
	Primary	0.01 mg/kg *(larvae,pollen and nectar)*	HPLC-MS/MS	Aucejo, S., 2015, Stud No. 307SRES15C01, New data, KCP 5.1.2/21
	Confirmatory (if required)	-	Not required	-
	Primary	272.1 mg/L	HPLC-DAD	Kleebaum,K., 2015, Study No. 141048078 B, New data, KCP 5.1.2/ 22
	Confirmatory (if required)	-	Not required	-
Vegetative vigor test (Ecotoxicology)	Primary	130 mg/L	HPLC-UV	Friedrich, S., 2014, Study No. 141048002 WP, New data, KCP 5.1.2/23
	Confirmatory (if required)	-	Not required	-
Honey bee, Arthropods (Ecotoxicology)	Primary	0.01 mg/kg *(larvae, nectar, pollen)*	LC-MS/MS	Aucejo, S., 2015, Study No. 307SRES15C02 + Amendment No. 1, New data, KCP 5.1.2/24
		0.02		
	Confirmatory (if required)	-	Not required	-
	Primary	0.01 mg/kg *(honey, pollen, larvae)*	LC-MS/MS	Mayer, O., 2017, Study No. R1640035, New data, KCP 5.1.2/25
	Confirmatory (if required)	-	Not required	-

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	2.712 mg/L	HPLC-DAD	Dressler, K., 2019, Study No. 1948BAC 0028, New data, KCP 5.1.2/26
	Confirmatory (if required)	-	Not required	-
	Primary	0.096 mg/kg	HPLC-MS/MS	Scheller, K., 2020, Study No 1948BLC 0033, New data KCP 5.1.2/27
	Confirmatory (if required)	-	Not required	-
Mesocosm (Exotoxicology)	Primary	10 ng a.s/L *(water)* 50 ng a.s/kg *(sediment)*	UHPLC-MS/MS	Hennecke, S., 2020, Study No. NFM-001/7-52, New data KCP 5.1.2/28
	Confirmatory (if required)	-	Not required	-
	Primary	10 ng a.s/L *(water)* 50 ng a.s/kg *(sediment)*	UHPLC-MS/MS	Hennecke, S., 2020, Study No. NFM-002/6-22, New data KCP 5.1.2/29
	Confirmatory (if required)	Confirmatory (if required)	-	Not required

(a) LOQ of acetamiprid and its metabolite IM-1-4 and IM-1-5

* Matching data have been conducted and submitted to Ctgb to demonstrate access to a complete package according to Reg (EU) 283/2013 and for data matching step. RMS Opinion on GLP compliance, guidance compliance and equivalent endpoint can be provided as soon as evaluation is finalised.

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

5.3.1 Analysis of the plant protection product (KCP 5.2)

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

5.3.2 Description of analytical methods for the determination of residues of 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 Re-assessment Report, Netherlands, 2016 (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	Reg. (EU) 2019/88
Plant, high acid content		0.01 mg/kg	Reg. (EU) 2019/88
Plant, high protein/high starch content (dry commodities)		0.01 mg/kg	Reg. (EU) 2019/88
Plant, high oil content		0.01 mg/kg	Reg. (EU) 2019/88

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Plant, difficult matrices (hops, spices, tea)		0.05 mg/kg	Reg. (EU) 2019/88
Muscle	*N*-desmethyl-acetamiprid (IM-2-1), expressed as acetamiprid	0.5 mg/kg	Reg. (EU) 2019/88
Milk		0.2 mg/kg	Reg. (EU) 2019/88
Eggs		0.02 mg/kg	Reg. (EU) 2019/88
Fat		0.3 mg/kg	Reg. (EU) 2019/88
Liver, kidney		1.0 mg/kg	Reg. (EU) 2019/88
Soil (Ecotoxicology)	Acetamiprid	0.18 mg a.s./kg soil dry weight	NOAEC for *Folsomia candida*
Drinking water (Human toxicology)	Acetamiprid and IM-1-5	0.1 µg/L	general limit for drinking water
Surface water (Ecotoxicology)	Acetamiprid	0.00096 mg/ a.i/L	NOEC for *Chironomus riparius*
Air	Acetamiprid	*2.1 µg/m^3 based on $AOEL_{systemic}$ of 0.07 mg/kg bw/day	EFSA conclusion 2016
Tissue (meat or liver)	No residue definition provided	0.5 mg/kg	Reg. (EU) 2019/88
Body fluids (blood)		0.05 mg/L	SANCO/825/00 rev. 8.1.

*MRL/Limit for air matrix was calculated using the AOEL systemic value of 0.02 mg/kg bw/day from EFSA conclusion 2017. The calculation was done according to the equation in SANCO/825/00 rev 8.1 guidelines: c = $AOEL_{systemic}$ * 300 [µg/m^3].

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 *in apple fruit*	HPLC-MS/MS	Schwarz, T., 2008, Study No. RD-01937, EU agreed, Netherlands, RAR, 2015 * to which is equivalent Lang, A., 2015, R-33644, 13M06017-01-VMPL
	Primary	0.01 mg/kg *in potatoes*		Weber, H., 2013, Study No. RD-02603, EU agreed, Netherlands, RAR, 2015 * to which is equivalent Lefresne, S., 2015, R-33645, B13-M1-A-01
	ILV	0.01 mg/kg *in apple fruit*		Giseau, A., and Weber, H., 2012, Study No. RD-02454, EU agreed, Netherlands, RAR, 2015 * to which is equivalent method S20-00531
	Confirmatory	-	Not required	-

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**
	(if required)			
High acid content	Primary	0.01 mg/kg *whole orange*	HPLC-MS/MS	Schwarz, T., 2008, Study No. RD-01937, EU agreed, Netherlands, RAR, 2015* to which is equivalent Lang, A., 2015, R-33644, 13M06017-01-VMPL
	ILV	0.01 mg/kg		Giseau, A., and Weber, H., 2012, Study No. RD-02454, EU agreed, Netherlands, RAR, 2015* to which is equivalent method S20-00536
	Confirmatory (if required)	-	Not required	-
High oil content	Primary	0.01 mg/kg *in sunflower seed*	HPLC-MS/MS	Schwarz, T., 2008, Study No. RD-01937, EU agreed, Netherlands, RAR, 2015* to which is equivalent Lang, A., 2015, R-33644, 13M06017-01-VMPL
	ILV	0.01 mg/kg *in sunflower seed*		Giseau, A. and Weber, H., 2012, Study No. RD-02454, EU agreed, Netherlands, RAR, 2015* to which is equivalent method S20-00536
	Confirmatory (if required)	-	Not required	-
High protein/high starch content (dry)	Primary	0.01 mg/kg *in maize grain*	HPLC-MS/MS	Schwarz, T., 2008, Study No. RD-01937, EU agreed, Netherlands, RAR, 2015 * to which is equivalent Lang, A., 2015, R-33644, 13M06017-01-VMPL
	ILV	0.01 mg/kg		Giseau, A., and Weber, H., 2012, Study No. RD-02454, EU agreed, Netherlands, RAR, 2015 * to which is equivalent method S20-00536
	Confirmatory (if required)	-	Not required	-
No group	Primary	0.01 mg/kg *in honey*	HPLC-MS/MS	Schwarz, T., 2008, Study No. RD-01937, EU agreed, Netherlands, RAR, 2015 * to which is equivalent Lang, A., 2015, R-33644, 13M06017-01-VMPL
	ILV	-	Not required	-
	Confirmatory (if required)	-	Not required	-

* Matching data have been conducted and submitted to Ctgb to demonstrate access to a complete package according to Reg (EU) 283/2013 and for data matching step. RMS Opinion on GLP compliance, guidance compliance and equivalent endpoint can be provided as soon as evaluation is finalised.

Evaluator comments:

According to the evaluation presented in "Matching active substance data necessary for the renewal of the approval of acetamiprid" (RMS: The Netherlands, December 2020) RMS - The Netherlands concluded that the analytical method of Lang, A., 2015 (R-33644, 13M06017-01- VMPL) is validated for 4 matrices (head cabbage, apple fruits, potato tubers and peach fruits), which are matrices of high water content with an LOQ of 0.01 mg/kg. However, the analytical method in the RAR is validated for all matrix types (high water, high acid, high oil, high starch/dry and honey) with an LOQ of 0.01 mg/kg. Nevertheless, the method provided under CA 4.2/02 (Lefresne, S., 2015, R-33645, B13-M1-A-01) covers the matrices with high acid, high oil and high starch/dry content. Therefore the analytical method of Lang, A., 2015 has been matched for matrices with high water content and the analytical method of Lefresne, S., 2015 has been matched for matrices with high acid, high oil and high starch/dry content.

An ILV to the primary methods should be provided, as required according to Reg. (EU) 283/2013 and SANCO/825/00 rev. 8.1.
RMS - The Netherlands in "Matching active substance data necessary for the renewal of the approval of acetamiprid" (12-06-2020) concluded that two new ILVs have been provided by Applicant: S20-00531 & S20-00536.
The analytical method S20-00531 was validated for 1 matrix (apple fruits, high water) with an LOQ of 0.01 mg/kg according to SANCO/825/00 rev. 8.1.
The analytical method (S20-00536) was validated for 3 matrices (dry bean, dry/high starch/protein, oilseed rape, high oil and orange, high acid) with an LOQ of 0.01 mg/kg according to SANCO/825/00 rev. 8.1
Therefore in the opinion of RMS - The Netherlands, the ILV analytical method has been matched for all matrices.

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

Table 5.3-3: Statement on extraction efficiency

	Method for products of plant origin
Required, available from:	The efficiency of the following extraction procedures has been demonstrated using incurred residues in previously submitted and reviewed metabolism studies (please refer to DAR section B.7.1). Apple Leaves and fruits were washed with methanol and extracted twice with methanol/water, 3/1, v/v. Recoveries: 93-99% for leaf and 68-96% for fruits. Cabbage Leaves were washed with methanol and leaves and head were extracted three times with methanol/water, 3/1, v/v. Recoveries: 79-100% for leaf. Carrot Roots (flesh and peel seperately) were extracted once with acetone and twice with acetone/water (80/20 and 50/50, v/v). Recoveries: 52-65% for peel, 66-88% for flesh. Aubergine Leaves and fruits were washed with methanol and extracted twice with methanol/water, 3/1, v/v. Recoveries: 99% for leaf and 97-99% for fruits. The use of acetone in a number of the recovery determinations presented above, indicates that the QuEChERS extraction using acetonitrile would be equally efficient, as acetone and acetonitrile are both polar aprotic solvents, with similar polarity and solubility parameters (extent of dispersion, polar and hydrogen bonding).
Not required, because:	-

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	HPLC-MS/MS	Miya, K., 2010, Study No. RD-02080, EU agreed, Netherlands, RAR, 2015 * to which is equivalent Lang, A., 2016, R-37837, 16A08133-01-VMAT
	ILV	0.01 mg/kg		Knoch, E., 2010, Study No. RD-02156, EU agreed, Netherlands, RAR, 2015 * to which is equivalent Barbier, G., 2016, R-37912, B16G-A4-A-01
	Confirmatory (if required)	-	Not required	-
Eggs	Primary	0.01 mg/kg	HPLC-MS/MS	Miya, K., 2010, Study No. RD-02080, EU agreed, Netherlands, RAR, 2015 * to which is equivalent Lang, A., 2016, R-37837, 16A08133-01-VMAT
	ILV	0.01 mg/kg		Knoch, E., 2010, Study No. RD-02156, EU agreed, Netherlands, RAR, 2015 * to which is equivalent Barbier, G., 2016, R-37912, B16G-A4-A-01
	Confirmatory (if required)	-	Not required	-
Muscle (Meat)	Primary	0.01 mg/kg	HPLC-MS/MS	Miya, K., 2010, Study No. RD-02080, EU agreed, Netherlands, RAR, 2015 * to which is equivalent Lang, A., 2016, R-37837, 16A08133-01-VMAT
	ILV	0.01 mg/kg		Knoch, E., 2010, Study No. RD-02156, EU agreed, Netherlands, RAR, 2015 * to which is equivalent Barbier, G., 2016, R-37912, B16G-A4-A-01
	Confirmatory (if required)	-	Not required	-
Fat	Primary	0.01 mg/kg	HPLC-MS/MS	Miya, K., 2010, Study No. RD-02080, EU agreed, Netherlands, RAR, 2015 * to which is equivalent Lang, A.,

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**
				2016, R-37837, 16A08133-01-VMAT
	ILV	0.01 mg/kg		Knoch, E., 2010, Study No. RD-02156, EU agreed, Netherlands, RAR, 2015 * to which is equivalent Barbier, G., 2016, R-37912, B16G-A4-A-01
	Confirmatory (if required)	-	Not required	-
Liver	Primary	0.01 mg/kg	HPLC-MS/MS	Miya, K., 2010, Study No. RD-02080, EU agreed, Netherlands, RAR, 2015 * to which is equivalent Lang, A., 2016, R-37837, 16A08133-01-VMAT
	ILV	0.01 mg/kg		Knoch, E., 2010, Study No. RD-02156, EU agreed, Netherlands, RAR, 2015 * to which is equivalent Barbier, G., 2016, R-37912, B16G-A4-A-01
	Confirmatory (if required)	-	Not required	-
Kidney	Primary	0.01 mg/kg	HPLC-MS/MS	Miya, K., 2010, Study No. RD-02080, EU agreed, Netherlands, RAR, 2015 * to which is equivalent Lang, A., 2016, R-37837, 16A08133-01-VMAT
	ILV	0.01 mg/kg		Knoch, E., 2010, Study No. RD-02156, EU agreed, Netherlands, RAR, 2015 * to which is equivalent Barbier, G., 2016, R-37912, B16G-A4-A-01
	Confirmatory (if required)	-	Not required	-

* Matching data have been conducted and submitted to Ctgb to demonstrate access to a complete package according to Reg(EU)283/2013 and for data matching step. RMS Opinion on GLP compliance, guidance compliance and equivalent endpoint can be provided as soon as evaluation is finalised.

Evaluator comments:

According to the evaluation presented in "Matching active substance data necessary for the renewal of the approval of acetamiprid" (RMS: The Netherlands, December 2020) RMS - The Netherlands concluded that the analytical method (Lang, 2016, R-37837, 16A08133-01-VMAT) for acetamiprid and its metabolite IM 2-1 has been validated in 5 matrices (eggs, milk, meat, fat and liver) with an LOQ of 0.01 mg/kg according to SANCO/825/00 rev. 8.1. Therefore the studies have been matched.

Remark:
The matrix kidney was not used in the validation of the analytical method, in contrast to the study by Miya, K. (2010). However this is not required according to SANCO/825/00 rev. 8.1, as kidney or liver can be chosen instead of both.

The ILV analytical method (Barbier, G., 2016, R-37912, B16G-A4-A-01) is validated for acetamiprid and its metabolite IM 2-1 in 5 matrices (eggs, milk, meat, fat and liver), with an LOQ of 0.01 mg/kg. Therefore the study has been matched.

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

Table 5.3-5: Statement on extraction efficiency

	Method for products of animal origin
Required, available from:	The efficiency of the following extraction procedures has been demonstrated using incurred residues in previously submitted and reviewed metabolism studies (please refer to DAR section B.7.1). Goat Liver, Kidney, Muscle Liver, kidney and muscle were extracted twice with acetone by shaking for 30 minutes. Recoveries: 59.2-66.7% for liver, 72.9-74.5% for kidney, 67.1% for muscle. Hen Liver, Muscle, Eggs Liver, muscle and eggs (white and yolk) were extracted twice with acetone by shaking for 30 minutes. Recoveries: 70.9-76.8% for liver, 66.2% for muscle, 74.4-82.1% for egg white, 70.5-77.9% for egg yolk. The use of acetone in a number of the recovery determinations presented above, indicates that the QuEChERS extraction using acetonitrile would be equally efficient, as acetone and acetonitrile are both polar aprotic solvents, with similar polarity and solubility parameters (extent of dispersion, polar and hydrogen bonding).
Not required, because:	-

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 and its metabolite and IM-1-5			
Method type	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	0.002 mg/kg [(a)]	LC-MS/MS	Täufer, A. and Weber, H., 2010, Study No. RD-02062N , EU agreed, Netherlands, RAR, 2015 * to which is equivalent the amended study report R-35750 of Semrau, J., 2017
Confirmatory	-	Not required	-
Primary	0.01 mg/kg [(b)]	HPLC-MS/MS	Semrau, J., 2017, Study No. S15-02364, New data KCP 5.2.1/01
Confirmatory	-	Not required	-

(a) Limit of quantification of acetamiprid and its metabolite IM 1-5
(b) Limit of quantification of acetamiprid and its metabolites IM 1-4 and IM 1-5

* Matching data have been conducted and submitted to Ctgb to demonstrate access to a complete package according to Reg(EU)283/2013 and for data matching step. RMS Opinion on GLP compliance, guidance compliance and equivalent endpoint can be provided as soon as evaluation is finalised.

Evaluator comments:

According to the evaluation presented in "Matching active substance data necessary for the renewal of the approval of acetamiprid" (RMS: The Netherlands, December 2020) validation of an analytical method for the determination of acetamiprid and its soil metabolites IM-1-4 and IM-1-5 in soil (ADAMA Report n° R-35750 Eurofins Agrosciences Services Chem GmbH, S15-02364) is available. The Netherlands (CTGB 30-09-2019) concluded that the analytical method is validated for soil for acetamiprid and its metabolites IM 1-4 and IM 1-5 with an LOQ of 0.01 mg/kg. However, the required LOQ is 0.002 mg/kg for acetamiprid and its metabolite IM 1-5 as validated in the study by Täufer, A. & Weber, H. (2010) and therefore considered not equivalent. The Applicant submitted the explanation. The rationale provided by the applicant, stating that the method's LOQ of 0.01 mg/kg is sufficiently low with regard to ecotox-endpoints, is valid. The Netherlands (CTGB 10-01-2020) concluded that the applicant has provided the amended study report R-35750, in which it has been shown that the analytical method was validated according to SANCO/3029/99 rev. 4 and also SANCO/825/00 rev. 8.1, therefore concluding that the study is acceptable to be used as monitoring method for the determination of acetamiprid and its metabolites IM 1-4 and IM 1-5 in soil and therefore matches the study from the DAR.

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 and its metabolite 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	0.1 µg/L[(a)]	HPLC-MS/MS	Miya, K., 2007, Study No. RD-01204, EU agreed, Netherlands, RAR, 2015 * to which is equivalent Merdian, H., 2015, R-35910, S15-04647
	ILV	0.1 µg/L[(a)]		Senciuc, M., 2014a, Study No. RD-01951, EU agreed, Netherlands, RAR, 2015 * to which is equivalent Wiesner, F., Breyer, N., 2016, R-35910A, S15-00166
	Primary	0.05 µg/L[(b)]	HPLC-MS/MS	Giesau, A., and Weber, H., 2012, Study No. RD-02604, EU agreed, Netherlands, RAR, 2015 * to which is equivalent Merdian, H., 2015, R-35911, S15-04648
	ILV	0.05 µg/L[(b)]		Senciuc, M., 2014b, Study No. RD-02952, EU agreed, Netherlands, RAR, 2015 * to which is equivalent Wiesner, F., Feddersen, T.2017, R-35911A, S15-00167
	Confirmatory	-	Not required	-
Surface water	Primary	0.1 µg/L[(a)]	HPLC-MS/MS	Miya, K., 2007, Study No. RD-01204, EU agreed, Netherlands, RAR, 2015 * to which is equivalent Merdian, H., 2015, R-35910, S15-04647
	Confirmatory	-	Not required	-
	Primary	0.1 µg/L[(b)]	HPLC-MS/MS	Giesau, A., and Weber, H., 2012, Study No. RD-02604, EU agreed,

Component of residue definition: acetamiprid and its metabolite IM-1-5				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
				Netherlands, RAR, 2015 * to which is equivalent Merdian, H., 2015, R-35911, S15-04648
	Confirmatory	-	Not required	-

(a) LOQ of acetamiprid
(b) LOQ of the acetamiprid metabolite IM-1-5

* Matching data have been conducted and submitted to Ctgb to demonstrate access to a complete package according to Reg (EU) 283/2013 and for data matching step. RMS Opinion on GLP compliance, guidance compliance and equivalent endpoint can be provided as soon as evaluation is finalised.

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

Evaluator comments:

According to the evaluation presented in "Matching active substance data necessary for the renewal of the approval of acetamiprid" (RMS: The Netherlands, December 2020) RMS - The Netherlands concluded that the analytical methods for
- the determination of acetamiprid in surface and drinking water (Merdian, H., 2015, R-35910, S15-04647),
- the metabolite IM 1-5 in water (drinking water and surface water) (Merdian, H., 2015, R-35911, S15-04648)
have been validated according to SANCO/825/00 rev. 8.1 with an LOQ of 0.1 µg/L (for acetamiprid) and 0.05 µg/L (for IM-1-5). Therefore the studies have been matched.
Remark:
Although no validation for groundwater is included, as is included in the study by Miya, K. (2007), it is found acceptable as drinking water or groundwater is required for validation according to SANCO/825/00 rev. 8.1.

Independent Laboratory Validation (ILV) of the analytical methods for
- the determination of acetamiprid in water (Wiesner, F., Breyer, N., 2016, R-35910A, S15-00166),
- the determination of metabolite IM 1-5 in water (Wiesner, F., Feddersen, T.2017, R-35911A, S15-00167)
are available.
RMS - The Netherlands concluded that the ILV analytical methods have been validated according to SANCO/825/00 rev. 8.1 for acetamiprid in water (drinking water) with an LOQ of 0.1 µg/L of 0.1 µg/L (for acetamiprid) and 0.05 µg/L (for IM-1-5). Therefore the study has been matched.
Remark:
ILV for drinking water is only 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	HPLC-MS/MS	Beck, T., and Class, T., 2009, Study No. ~~RD-02943~~ RD-01863, EU agreed, Netherlands, RAR, 2015 * to which is equivalent Lang, A., 2016, R-37839, 16A08133-01-VMAI
Confirmatory	-	Not required	-

* Matching data have been conducted and submitted to Ctgb to demonstrate access to a complete package according to Reg(EU)283/2013 and for data matching step. RMS Opinion on GLP compliance, guidance compliance and

equivalent endpoint can be provided as soon as evaluation is finalised.

Evaluator comments:

According to the evaluation presented in "Matching active substance data necessary for the renewal of the approval of acetamiprid" (RMS: The Netherlands, December 2020) validation of an analytical method for the determination of acetamiprid in air (Lang, A., 2016, R-37839, 1608133-01-VMAI) is available. The Netherlands (CTGB 23-07-2018) concluded that the analytical method has been validated according to SANCO/825/00 rev. 8.1 for acetamiprid in air with an LOQ of 2.1 $\mu g/m^3$. Therefore the study has been matched.

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 *in blood*	HPLC-MS/MS RD-02943	Senciuc, M., 2014c, Study No. RD-02943, EU agreed, Netherlands, RAR, 2015 * to which is equivalent Lang, A., 2016, R-37838, 16A08133-01-VMBF
Confirmatory	-	Not required	-
Primary	0.01 mg/kg *in muscle and liver and kidney*	HPLC-MS/MS	Miya, K., 2010, Study No. RD-02080, EU agreed, Netherlands, RAR, 2015 * to which is equivalent Lang, A., 2016, R-37837, 16A08133-01-VMAT
Confirmatory	-	Not required	-

* Matching data have been conducted and submitted to Ctgb to demonstrate access to a complete package according to Reg (EU) 283/2013 and for data matching step. RMS Opinion on GLP compliance, guidance compliance and equivalent endpoint can be provided as soon as evaluation is finalised.

Evaluator comments:

According to the evaluation presented in "Matching active substance data necessary for the renewal of the approval of acetamiprid" (RMS: The Netherlands, December 2020) RMS - The Netherlands concluded that
- the analytical methods for acetamiprid in blood has been validated according to SANCO/825/00 rev. 8.1 with an LOQ of 0.05 mg/L.
- the analytical method for acetamiprid and its metabolite IM 2-1 has been validated in 5 matrices (eggs, milk, meat, fat and liver) with an LOQ of 0.01 mg/kg according to SANCO/825/00 rev. 8.1.
Therefore the studies have been matched.

Remark: Body tissues has been covered by the analytical method in animal matrices.

Reference list

EFSA, 2016: EFSA Scientific Report (2016), 1-26, Conclusion on the peer review of active substance acetamiprid.

EFSA, 2016: EFSA Scientific Report (2016), 1-91, Appendix A – List of end points for the active substance and the representative formulation.

Netherlands, 2016: Draft Re-Assessment Report (RAR) of active substance acetamiprid, Volume 3 – Annex B (AS), June 2016, under Regulation (EC) 1107/2009

Data matching list, Netherlands, 2020

Appendix 1 Lists of data considered in support of the evaluation

List of data submitted by the applicant and relied on

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	Vertebrate study Y/N	Owner*
KCP 5.1.1/01	Walter, D.	2014	Development and validation of an analytical method for the determination of acetamiprid in MCW-2222 Eurofins Agroscience Services Sponsor no.: R-33405 Study No. S13-03099 GLP / GEP Unpublished	N	Adama
KCP 5.1.2/01	Lefresne,S.	2014	Freezing storage stability of acetamiprid in 4 plant matrices: Dry (dry bean seed and straw, water (apple), fat (olive whole fruit) and acid (orange peel and pulp) at/below -18°C during 1 year (0,3,6 and 12 months) Report No. B13-M1-A-02 GIRPA GLP Unpublished	N	Adama**
KCP 5.1.2/02	Chevallier, E.	2014	Magnitude of the residue of acetamiprid in wheat (Raw Agricultural Commodity) after two applications of MCW-2222 - four decline curve trials and four harvest trials in Northern Europe (Northern France, Poland, Germany, Hungary and Austria) – 2014 Report No. B14C-S1-A-01 GIRPA GLP Unpublished	N	Adama
KCP 5.1.2/03	Chevallier, E.	2014	Magnitude of the residue of acetamiprid in barley (Raw Agricultural Commodity) after two applications of MCW-2222 – four decline curve trials and four harvest trials in Northern Europe (Northern France, Poland, Germany, Hungary and Austria) – 2014 GIRPA GLP Unpublished	N	Adama
KCP 5.1.2/04	Barbier, G.	2018	Freezing storage stability of acetamiprid in wheat (grain) at/ below -18°C during 15 months (0 and 15 months) Report No. B17G-A4-A-02 GIRPA GLP Unpublished	N	Adama
KCP	Méric, D.	2013	Magnitude of the residues of acetamiprid in peaches (RAC fruits) following two applications of MCW-2222 in three	N	Adama

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*
5.1.2/05			trials (1DC + 2 HS), Southern Europe (Southern France and Italy) – 2013 Study Report. DMC-13-16126 Staphyt (France) GLP Unpubished		
KCP 5.1.2/06	Méric, D.	2014	Magnitude of the residues of acetamiprid in apples (RAC fruits) following two applications of MCW-2222 in two trials (1DC + 1 HS), Northern Europe (Northern France) – 2013 Study Report DMC-13-16134 Staphyt (France) GLP Unpublished	N	Adama**
KCP 5.1.2/07	Roussel, Ch. H.	2014	Magnitude of the residues of acetmipprid in apple (RAC fruit and processed fractions), following one or two applications of MCW-2222 in six trials (3 DCS + 3 HS), Northern Europe (Northern France, Germany, Poland and Belgium) – 2014 Study No. ChR-14-17311 Staphyt (France) GLP Unpublished	N	Adama**
KCP 5.1.2/08	Méric, D.	2014	Magnitude of the residues of acetamiprid in oilseed rape (RAC whole plants, pods, and seeds) Following one or two applications of MCW-2222 in two trials (1 DC + 1 HS), Northern europe (Germany and Northern France) – 2013 Study no. DMC-16129 GLP Unpublished	N	Adama**
KCP 5.1.2/09	Chevallier, E.	2014	Magnitude of the residue of acetamiprid in winter oil seed rape (Raw Agricultural Commodity) after one or two applications of MCW-2222 – three decline curve trials and three harvest trials in Northern Europe (Northern France, Poland, Germany, Czech Republic and Hungary) – 2014 Study No. 14SGS035 SGS France GLP Unpublished	N	Adama**
KCP 5.1.2/10	Bousquet, C.	2014	Magnitude of the Residue of acetamiprid in potato Raw Agricultural Commodity after two applications of MCW-2222 in three decline curve trials (Poland, United Kingdom and Northern France) and in one harvest trial (Poland) in Northern Europe – 2013 Study No. 13SGS102 SGS France	N	Adama**

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner*
			GLP Unpublished		
KCP 5.1.2/11 filed under KCP 5.2/01	Semrau, J.	2017	Determination of residues of acetamiprid and its metabolites IM 1-4 and IM 1-5 after one application of MCW-2222 to bare soil in rotational crops (radish, spinach and wheat) at 1 site in Northern Europe and 1 site in southern Europe 2016/2017 Study no. S15-02364 Eurofins GLP Unpublished	N	Adama
KCP 5.1.2/ 12	Hecht-Ross, S.	2020	Semi-field study for determining the magnitude of residues of Carnadine (CA3573) (a.s. acetamiprid) in honey GLP Study No. 467, Report No. R1940050 RIFCON GmbH GLP Unpublished	N	Nufarm
KCP 5.1.2/13	Müller, S.	2020	~~**Error! Bookmark not defined.**~~ Determination of the Storage Stability of Acetamiprid in Honey for a period of 8 months at ≤ -18 °C Study No. 20N08133-01-SSHN (interim report) CIP GLP Unpublished	N	Nufarm
KCP 5.1.2/14	Wilson, A.	2016	Acetamiprid – foliar dislodgeable residues dissipation on pome fruit in southern and northern Europe (Spain, Italy and chzech republic), 2016 Study No. ACI16- 010 Agrochemex International Ltd. UK GLP Unpublished	N	Adama
KCP 5.1.2/15	Juckeland, D.	2014	Acute toxicity of MCW-2222 to the rainbow trout *Oncorhynchus mykiss* in a 96-hour static test Study No. 141048005 W BioChem Agrar GLP Unpublished	Y	Adama**
KCP 5.1.2/16	Juckeland, D.	2015	Acute toxicity of MCW-2222 to *Daphnia magna* in a 48-hour static test Study No. 141048006 W BioChemAgrar	N	Adama**

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Vertebrate study Y/N**	**Owner***
			GLP Unpublished		
KCP 5.1.2/17	Juckeland, D.	2015	Acute toxicity of MCW-2222 to *Chironomus riparius* in a 48-hour static test Study No. 141048057W BioChemAgrar GLP Unpublished	N	Adama**
KCP 5.1.2/18	Juckeland, D.	2014	Effects of MCW-2222 on *Desmodesmus subspicatus* in an algal growth inhibition test Study No. 141048007 W BioChemAgrar GLP Unpublished	N	Adama**
KCP 5.1.2/19	Molitor, C.	2014	Field Study to Evaluate Potential Side Effects of the product MCW-2222 (acetamiprid 200 g/L) on Brood Development, Foraging Activity, Mortality and Behaviour of Adult Honeybees Apis mellifera L. (Hymenoptera: Apidae) Following Application after Bee-Flight on Phacelia tanacetifolia Study No. 215-2014 TESTAPI France GLP Unpublished	N	Adama**
KCP 5.1.2/20	Molitor, C.	2014	Field Study to Evaluate Potential Side Effects of MCW-2222 on Brood Development, Foraging Activity, Mortality and Behaviour of Adult Honeybees (*Apis mellifera*) on Oilseed Rape Study No. 230 – 2015 TESTAPI France GLP Unpublished	N	Adama**
KCP 5.1.2./21	Aucejo, S.	2015	Effects and Determination of Residues of Acetamiprid 200 SL on the Honeybee (*Apis mellifera* L.) Brood in Apple, under Field Conditions, in Italy 2015 Study No. 307SRE15C01 GIRPA GLP Unpublished	N	Adama**
KCP 5.1.2/22	Kleebaum, K.	2015	Chronic toxcitiy of MCW-2222 to honey bee larvae (Apis mellifera L.) under laboratory conditions (in vitro) Study No. 141048078B BioChem agrar	N	Adama**

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner*
			GLP Unpublished		
KCP 5.12/23	Friedirsch, S.	2014	Terrestrial plant test with MCW-2222: Vegetative vigour test Study No. 141048002 W BioChemagrar GLP Unpublished	N	Adama**
KCP 5.1.2./24	Aucejo, S.	2015	Effects and Determination of Residues of Acetamiprid 200 SL on the Honeybee (*Apis mellifera L.*) Brood in Citrus, under Field Conditions, in Spain 2015. Study No. 307SRE15C02 SynTECH research center Spain GLP Unpublished	N	Adama
KCP 5.1.2/25	Mayer, O.	2017	Semi-field brood study to evaluate potential effects of MCW-2222 on brood development of honeybees (*Apis mellifera L.*) Study No. R1640035 Eurofins GLP Unpublished	N	Adama
KCP 5.1.2/26	Dressler, K.	2019	Chronic oral toxcitiy of CA3573 Acetamiprid 200 SL (Carnadine) to the honey bee Apis mellifera L. under laboratory condtions. Study No. 1948 BAC0028 BioChem agrar GLP Unpublsihed	N	Nufarm
KCP 5.1.2/27	Scheller, K.	2020	CA3573 Acetamiprid 200 SL (Carnadine)- repeated exposure of honey bee larvae (Apris mellifera L.) under laboratory conditions. Study No. 19 48 BLC 0033 BioChem agrar GLP Unpublished	N	Nufarm
KCP 5.1.2/28	Hennecke, S. .	2020	Carnadine – Outdoor mesocosm study Study number: NFM-001/7-52 Fraunhofer IME	N	Nufarm

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Vertebrate study Y/N**	**Owner***
			GLP Unpublished		
KCP 5.1.2/29	Hennecke, S. .	2020	Metod validation of the anlytical method for water and sediment Study number: NFM-002/6-22 Fraunhofer IME Non GLP Unpublished	N	Nufarm
KCP 5.2/ 01	Semrau,J.	2017	Determination of residues of acetamiprid and its metabolites IM 1-4 and IM 1-5 after one application of MCW-2222 to bare soil in rotational crops (radish, spinach and wheat) at 1 site in Northern Europe and 1 site in Southern Europe 2016/2017 Study No. S15-02364 Eurofins GLP Unpublished	N	Adama

* For all Adama studies a Letter of access exists for Nufarm
** These Adama studies were already submitted but newly summarized respecting the new dRR format

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**
CP 5.1.2	Netzband, D.	2003	Stability study of Acetamiprid in potatoes during frozen storage, USA, 2002 in freezer at or below -18°C, Report No. RD-00243 Bayer Crop Science GLP Unpublished	N	Nippon Soda
CP 5.1.2	Goller, G.	1999	Stability Study of NI-25 (Acetamiprid) in apple and tomato samples after storage in freezer at or below -18 °C - Fortification experiments with active ingredient Report No RPA/NI-25/97051 A.D.M.E. - Bioanalyses, France GLP Unpublished	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
CP 5.1.2	Jean-Baptiste, C.	2009	Frozen Storage Stability of Residues of Acetamiprid in Fodder Pea Report No A7125 Anadiag Laboratories GLP Unpublished	N	Nippon Soda
CP 5.1.2	Gieseke L.D.	1999	NI-25 (acetamiprid): Freezer storage stability of acetamiprid residues in various raw agricultural commodities and processing fractions (plant matrices) Report No 10201 Horizon Laboratories, Inc. GLP Unpublished	N	Nippon Soda
CP 5.1.2	Raufer, B.	2013	Residue Study on Rotational Crops after one Application of Acetamiprid on Bare Soil at 2 Sites in Europe in 2010 to 2012 Study No. S10-02822 Eurofins GLP Unpublished	N	Nippon Soda
CP 5.1.2	Mamouni, A.	1997	Adsorption and Desorption of IM-1-4 on five soils GLP Unpublsiehd	N	Nippon Soda
CP 5.1.2	Liu, A.C.	1997	6-Chloronicotinic Acid (Acetamiprid metabolite) soil adsorption/desorption study GLP Unpublished	N	Nippon Soda
CP 5.1.2	Sugiyama H.	2010	Adsorption/Desorption Study of IM 1-5 on Soils Study no. RD-02101 GLP Unpublished	N	Nippon Soda
CP 5.1.2	Emeric, G.T.	1998	Acetamiprid- Verification of the identity of the photolyte obtained at pH 7 Study No. 96-82 GLP Unpublished	N	Nippon Soda
CP 5.1.2	Shiotani H.	2003	Photodegradation of IM-1-5 in water Study No. 2-9-16	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 Unpublished		
CP 5.1.2.	Putt, A. E.	2003b	Acute toxicity to gammarids (*Gammarus fasciatus*) under static conditions Study No. 12681.6105 Springborn Smithers Laboratories, NC, USA GLP Unpublished	N	Nippon Soda
CP 5.1.2.	Kley, A. & Wydra, V.	2012	Acute toxicity of IM-1-2 to larvae of *Chrironimus riparius* in a static 48 hours immobilisation-test Study No. RD-02374 Ibacon GLP Unpublished	N	Nippon Soda
CA 5.2.	Schwarz, T.	2008	Acetamiprid: Validation of an Enforcement Method for Plant Materials Study P/B1447G PTRL Europe Nippon-Soda Report No. RD-01937 GLP Upublished	N	Nippon soda
CA 5.2.	Weber, H.	2013	Validation of a Multiresidue Method (Fillion) with Modified Cleanup and Detection for the Determination of Acetamiprid in Potato Study No. S13-02134, Document ID RD-02603 Eurofins Agroscience Services GLP Unpublished	N	Nippon soda
CA 5.2.	Giesau, A.	2013	Independent laboratory Validation of an Enforcement Method (“QuEChERS”) for the Determination of Residues of Acetamiprid in Crops using LC-MS/MS Study No. S12-02718, Document ID RD-02454 Eurofins Agroscience Services GLP Unpublished	N	Nippon soda
CA 5.2.	Miya, K.	2010	Validation Study of the Analytical Method for the Determination of the Residues of Acetamiprid and Its Metabolite (IM-2-1) in Animal Commodities Report No. NCAS 10-144, Document ID RD-02080 Nisso Chemical Analysis Service Co., Japan	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 Unpublished		
CA 5.2.	Knoch, E.	2010	Independent Laboratory Validation: Analytical Method for the Determination of the Residues of Acetamiprid and its Metabolite (IM-2-1) in Animal Commodities Report No. IF-10/01687868, Document ID RD-02156 SGS Institut Fresenius GmbH GLP Unpublished	N	Nippon soda
CA 5.2.	Täufer, A. & Weber H.	2010	Validation of an Analytical Method for the Determination of Residues of Acetamiprid and Acetamiprid Soil Metabolite IM-1-5 in Calcareous Soil using LC-MS/MS Report No. S09-03287, Document ID RD-02062N Eurofins Dr. Specht, Germany GLP Unpublished	N	Nippon soda
CA 5.2.	Miya, K.	2007	Validation Study of the Confirmatory Method for the Determination of Acetamiprid in Water, Report No. NCAS 06-209, Document ID RD-01204 Nisso Chemical Analysis Service Co., Japan GLP Unpublished	N	Nippon soda
CA 5.2.	Senciuc, M.	2014a	Independent Laboratory Validation (ILV) of a Residues Analytical Method for the Determination of Acetamiprid in Drinking Water Report No. P 3244 G, Document ID RD-02951 PTRL Europe GmbH, Germany GLP, Unpublished	N	Nippon soda
CA 5.2.	Gieseau, A. & Weber, H.	2012	Validation of an Analytical Method for the Determination of Residues of Acetamiprid Metabolite IM-1-5 in Water using LC-MS/MS, Report No. S12-02719, Document ID RD-02604 Eurofins Agroscience Services, Germany, GLP, not published	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
CA 5.2.	Senciuc, M.	2014b	Independent Laboratory Validation (ILV) of a Residues Analytical Method for the Determination of Acetamiprid Metabolite IM-1-5 in Drinking Water Report No. P 3245 G, Document ID RD-02952 PTRL Europe GmbH, Germany GLP Unpublished	N	Nippon soda
CA 5.2.	Senciuc, M.	2014c	Development and Validation of an Analytical Method for the Determination of Acetamiprid in Blood Report No. P3208 G, Document ID RD-02943 PTRL Europe, Germany GLP Unpublished	N	Nippon soda

List of data submitted by the applicant and not relied on

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

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

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

Appendix 2 Detailed evaluation of submitted analytical methods

A 2.1 Analytical methods for acetamiprid

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

A 2.1.1.1 Description of analytical methods for the determination of residues (KCP 5.1.2)

A 2.1.1.1.1 Analytical method B13-M1-A-02

A 2.1.1.1.1.1 Method validation B13-M1-A-01 & 13M06017-01-VMPL

Comments of zRMS:	The study demonstrated that acetamiprid is stable in 4 plant matrices (dry (dry bean seed and straw), water (apple), fat (olive whole fruit) and acid (orange peel and pulp) at/below -18°C for a storage period up to 12 months. The limit of quantification of acetamiprid is 0.01 mg/kg for each specimen with mean recoveries ranging from 70% to 110% at a relative standard deviation (RSD) of ≤ 20%. The storage stability results of acetamiprid using the analytical method are presented below:

6. STORAGE STABILITY RESULTS

Storage		Residues and recoveries in specimens stored frozen (not corrected for procedural recoveries)					Residues and recoveries in specimens stored frozen (recovery corrected)		
Commodity	Period	Uncorrected residue results				Corrected results with day 0 as 100 %	Procedural recoveries for freshly fortified samples	Corrected results	
	Months	Sample 1 mg/kg	Sample 2 mg/kg	Sample 3 mg/kg	Mean mg/kg			Corr.	Day-0 as 100 %
Dry bean (seed)	0	0.081	0.082	0.078	0.080	100	81	0.099	100
	3	0.089	0.090	na	0.089	111	84	0.106	107
	6	0.098	0.098		0.098	122	98	0.100	102
	12	0.104	0.105		0.104	130	99	0.105	107
Dry bean (straw)	0	0.084	0.091	0.075	0.084	100	87	0.096	100
	3	0.091	0.085	na	0.088	105	81	0.109	113
	6	0.108	0.106		0.107	128	100	0.107	111
	12	0.106	0.089		0.098	117	97	0.101	105
Apple (fruit)	0	0.098	0.102	0.099	0.100	100	102	0.098	100
	3	0.096	0.094	na	0.095	96	87	0.109	111
	6	0.089	0.085		0.087	87	86	0.101	103
	12	0.097	0.098		0.097	98	93	0.104	106
Olive (Whole fruit)	0	0.088	0.082	0.081	0.084	100	85	0.098	100
	3	0.109	0.108	na	0.109	130	94	0.116	118
	6	0.093	0.095		0.094	112	100	0.094	96
	12	0.089	0.090		0.089	106	90	0.100	101
Orange (peel)	0	0.096	0.098	0.093	0.096	100	90	0.106	100
	3	0.088	0.086	na	0.087	91	82	0.107	101
	6	0.082	0.086		0.084	88	85	0.099	93
	12	0.085	0.087		0.086	90	85	0.101	95
Orange (pulp)	0	0.094	0.091	0.088	0.091	100	94	0.097	100
	3	0.110	0.095	na	0.102	112	98	0.104	107
	6	0.091	0.092		0.092	100	95	0.096	99
	12	0.087	0.089		0.088	97	87	0.101	104
Nominal fortification level: 0.100 mg/kg									

na: not applicable.

	The analytical method was fully validated according to the requirements of the SANCO/825/00 rev. 8.1 guidelines. The study is acceptable.

Reference:	KCP 5.1.2/01
Report	Freezing storage stability of acetamiprid in 4 plant matrices: dry (dry bean seed and straw, water (apple), fat (olive whole fruit) and acid (orange peel

	and pulp) at/below -18°C during 1 year (0, 3, 6 and 12 months), Lefresne, S., 2014, Study No. B13-M1-A-02
Guideline(s):	Yes, SANCO 3029/99 rev. 4
Deviations:	None
GLP:	Yes
Acceptability:	Yes

A. Materials

1. Standards

Reference item:	Acetamiprid
Lot/Batch number:	20202
Purity:	98.1 ± 0.5 %
CAS No.:	135410-20-7
Expiry date:	February 2016
Standards for calibration	As above
Matrix:	Dry bean (seed), mandarin (peel & pulp), olive (whole fruit) and orange (peel & pulp)

B. Sample preparation and processing

Frozen samples were homogenized and frozen again until preparation for the storage stability test. 2 g (for either olive or dry bean) and 10 g (for either orange or mandarin) were weighed into a 50 mL PP tube. The recovery samples were fortified to 0.1 mg/kg (10 x LOQ) respectively. The samples were stored in a freezer at –18°C. For each sample after storage periods of 0, 3, 6 and 12 months the frozen samples were taken out and analysed by HPLC-MS/MS.

C. Chromatographic parameters

HPLC parameters	
Instrumentation	API6500
Column:	C18 Hydro RP (100 x 3 mm ID x 2.5 µm PD
Column temperature:	60°C
Injection volume:	10µl – 220µL
MS/MS - parameters	
Instrumentation	4000QTrap
Mode:	ESI (electrospray ionisation) positive
Ion source:	Turbospray
Scan type:	MRM
Transitions:	223 -> 126 m/z (quantifier) 223 -> 90 m/z (qualifier)

Results and discussions

The specific identification of acetamiprid was conducted by HPLC-MS/MS with two transitions monitored. Therefore no confirmatory data needs to be provided. The analytical method was validated according to SANCO/825/00 rev. 8.1 in B13-M1-A-01 and 13M06017-01-VMPL. The full method validation was previously evaluated by CTGB during the data-matching list process. The limit of quantification in the main validation is 0.01 mg/kg and is defined as the lowest fortification level with mean recoveries ranging from 70% to 110% at a relative standard deviation (RSD) of ≤ 20% and blanks not exceeding 30% of the LOQ level. These criteria were fulfilled for the procedural recoveries of the 10 × LOQ for acetamiprid. The detector response for acetamiprid was linear within the range from 1.5 µg/L to 100 µg/L (orange, dry bean) and 0.3 µg/L to 50 µg/L

for olive and 0.5 µg/L to 30 µg/L for apple with $r^2 \geq 0.99$. The procedural recovery data are presented in the table below.

Table A 1: Procedural recoveries from method validation of acetamiprid using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (*n* = 4)	Mean recovery (%) for 0, 3, 6, 12 months	Comments
Dry bean (seed)	Acetamiprid	0.1 mg/kg	81, 84, 98, 99	-
Dry bean (straw)	Acetamiprid	0.1 mg/kg	87, 81, 100, 97	-
Apple	Acetamiprid	0.1 mg/kg	102, 87, 86, 93	-
Olive	Acetamiprid	0.1 mg/kg	85, 94, 100, 90	-
Orange (peel)	Acetamiprid	0.1 mg/kg	90, 82, 85, 85	-
Orange (pulp)	Acetamiprid	0.1 mg/kg	94, 98, 95, 87	-

Table A 2: Characteristics for the analytical method used for validation of acetamiprid residues in plant matrices

	Acetamiprid
Specificity	The HPLC-MS/MS with two ions monitored is highly specific for the determination of acetamiprid. No interference above ≥ 30% LOQ
Calibration (type, number of data points)	Dry bean (seed): y= 93507.40x + 45609.79 r^2= 0.9955 Dry bean (straw): y= 57920.47x + 92300.72 r^2= 0.9976 Apple: y= 109185.15x + 4765.26 r^2= 0.9999 Olive: y= 112735.08 + 10529.82 r^2= 0.9981 Orange (peel): y= 45884.24 + 4719.09 r^2= 0.9998 Orange (pulp): y= 64297.76x + 6597.04 r^2= 0.9999
Calibration range	1.5 µg/L to 100µg/L (orange, dry bean) 0.3 µg/L to 50 µg/L (olive) 0.5 µg/L to 30 µg/L (apple)
Assessment of matrix effects is presented	No
Limit of determination/quantification	LOQ for all matrices: 0.01 mg/kg

Conclusion

The method validation in B13-M1-A-01 and 13M06017-01-VMPL for the determination of acetamiprid in plant material was fully validated according to the requirements of the SANCO/825/00 rev. 8.1 guidelines. The procedural recoveries of the storage stability study prove to be within the requirements of the guidelines and therefore the method can be fully accepted.

A 2.1.1.1.2 Analytical method B14C-S1-A-01

A 2.1.1.1.2.1 Method validation B14C-S1-A-03

Comments of zRMS:	The method was successfully validated according to the guidance document SANCO/3029/99 rev.4 on barley (whole plant, grains and straw) and reported in GIRPA report B14C-S1-A-03, SGS Agri min study 14SGS034. The limit of quantification (LOQ) for acetamiprid was 0.01 mg/kg. The limit of detection (LOD) was defined as 0.0033 mg/kg within the validation study. The summary of validation is presented below:

Summary of validation:

Specimen	Reference item	Level spiked (mg.kg^{-1})	Recovery rate (%)	Relative standard deviation (%)	Number of recovery rates (n)
Barley whole plant	acetamiprid	0.010	87	4	5
		0.100	90	4	5
		all	89	4	10
Barley grains		0.010	78	9	5
		0.100	76	5	5
		all	77	7	10
Barley straw		0.010	74	5	5
		0.100	83	4	5
		all	78	7	10

The method was checked within this study on wheat matrix by a reduced validation, performing on wheat (whole plant, grain and straw) 6 spiked samples, 3 recovery experiments fortified at the limit of quantification, 3 recovery experiment fortified at ten times the LOQ level and one control sample.

The reduced validation performed on wheat is summarised below:

Summary of validation:

Specimen	Reference item	Level spiked (mg.kg^{-1})	Recovery rate (%)	Relative standard deviation (%)	Number of recovery rates (n)
Wheat (whole plant)	acetamiprid	0.010	71	2	3
		0.100	75	0.4	3
		all	73	3	6
Wheat (grain)		0.010	72	0.3	3
		0.100	78	2	3
		all	75	5	6
Wheat (straw)		0.010	77	7	3
		0.100	79	3	3
		all	78	5	6

For each matrix (wheat whole plant, wheat grains and wheat straw), the limit of quantification (LOQ) of the method of acetamiprid is 0.01 mg/kg. The limit of detection (LOD) was defined as 0.0033 mg/kg. The mean recovery was between 70% and 110% with a RSD less than 20% at each level fortification.
The method was successfully validated according to the guidance document SANCO/3029/99 rev.4, so the method is acceptable.

Reference:	KCP 5.1.2/02
Report	Magnitude of the residue of acetamiprid in wheat (RAC) after two application of MCW-2222 – four decline curve trials and four harvest trials in Northern Europe (Northern France, Poland, Germany, Hungary and Austria) – 2014, Chevallier, E., 2014, B14C-S1-A-01.
Guideline(s):	Yes, SANCO 3029/99 rev. 4
Deviations:	None
GLP:	Yes
Acceptability:	Yes

A. Materials

1. Standards

Test item:	MCW-2222
Batch No.:	659-030314-01
Expiry date:	March 2016
Active ingredient:	Nominal: 200 g/L Analysed: 199.2 g/L
Reference item:	Acetamiprid
Lot/Batch number:	20202
Purity:	98.1 ± 0.5 %
CAS No.:	135410-20-7
Expiry date:	February 2016
Standards for calibration	As above
Matrix:	Wheat (grain, straw and whole plant)

B. Sample preparation and processing

Approximately 2 g are weighed into a 50 mL centrifuge tube. The samples are fortified respectively. For wheat grains the samples are shaken for one minute horizontally. For all matrices 10 mL of ultra-pure water were added. For wheat (whole plant and straw) 10 mL of acetonitrile was added. Afterwards all samples for all wheat compounds were shaken for 20 minutes. The aliquots are transferred into a QuEChERS tube and shaken by hand. The samples were centrifuged for 5 minutes at 4000 rpm and afterwards filtered (0.45 µm). The final extracts will be analysed by HPLC-MS/MS.

C. Chromatographic parameters

HPLC- parameters	
Instrumentation	API6500
Column:	C18 Hydro RP (100 x 3 mm ID x 2.5 µm PD
Column temperature:	60 °C
Injection volume:	10µl – 220µL
MS/MS - parameters	
Instrumentation	4000QTrap
Mode:	ESI (electrospray ionisation) positive
Ion source	Turbospray
Scan type:	MRM
Transitions:	223 -> 126 m/z (quantifier) 223 -> 90 m/z (qualifier)

Results and discussions

The specific identification of acetamiprid was conducted by HPLC-MS/MS with two transitions monitored. Therefore no confirmatory data needs to be provided. The analytical method was fully validated in Chevallier, E., Study No. B14C-S1-A-03, therefore a reduced validation was conducted. The limit of quantification in the main validation is 0.01 mg/kg and is defined as the lowest fortification level with mean recoveries ranging from 70% to 110% at a relative standard deviation (RSD) of ≤ 20% and blanks not exceeding 30% of the LOQ level. These criteria were fulfilled for all recoveries at each fortification level at LOQ and 10x LOQ for acetamiprid. The detector response for acetamiprid was linear with a five point calibration curve and a correlation coefficient of $r^2 \geq 0.99$. The recovery data are presented in the table below.

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

Matrix	Analyte	Fortification level (mg/kg)	Mean recovery (%)	RSD (%)	Mean (%) (RSD (%))	Comments
Wheat Whole plant	Acetamiprid	0.01 (n=2)	83	-	93 (15)	-
		4.0 (n=1)	102	-		-
		2.0 (n=1)	102	-		
Wheat grain		0.01 (n=1)	70	-	74 (-)	-
		0.1 (n=1)	77	-		-
Wheat straw		0.01 (n=2)	90	-	94 (15)	-
		0.1 (n=1)	104	-		-
		1.0 (n=2)	85	-		

Table A 4: Characteristics for the analytical method used for validation of acetamiprid residues in wheat

	Acetamiprid
Specificity	The HPLC-MS/MS with two ions monitored is highly specific for the determination of acetamiprid. No interferences above ≥ 30% LOQ
Calibration (type, number of data points)	5-point linear calibration range Wheat (grain): y= 35030.78x – 215.74 r^2= 0.9995 Wheat (whole plant): y= 120717.57x – 1211.73 r^2= 0.999 Wheat (straw) : y= 24344.59 – 2252.30 r^2= 0.9979
Calibration range	N/A
Limit of determination/quantification	LOQ for all matrices: 0.01 mg/kg

Conclusion

The reduced validation in study B14C-S1-A-01 for wheat fulfils the requirements of the SANCO/3029/99 rev. 4 guidelines and can be therefore considered as fully acceptable.

A 2.1.1.1.3 Analytical method B14C-S1-A-03

A 2.1.1.1.3.1 Method validation B14C-S1-A-03

Comments of zRMS:	The method was successfully validated according to the guidance document SANCO/3029/99 rev.4 on barley (whole plant, grains and straw) and reported in GIRPA report B14C-S1-A-03, SGS Study number: 14SGS034, Sponsor Reference: R-34898A. The limit of quantification (LOQ) for acetamiprid was 0.01 mg/kg. The limit of detection (LOD) was defined as 0.0033 mg/kg within the validation study. The mean recovery was between 70% and 110% with a RSD less than 20% at each level fortification. The method was successfully validated according to the guidance document SANCO/3029/99 rev.4, so the method is acceptable.

Reference:	KCP 5.1.2/03
Report	Magnitude of residue of acetamiprid in barley (RAC) after two applications of MCW-2222- four decline curve trials and four harvest trials in northern Europe (Northern France, Poland, Germany, Hungary and Austria) - 2014, Chevallier, E., Study No. B14C-S1-A-03.
Guideline(s):	Yes, SANCO 3029/99 rev. 4
Deviations:	None
GLP:	Yes
Acceptability:	Yes

A. Materials

1. Standards

Test item:	MCW-2222
Batch No.:	659-030314-01
Expiry date:	March 2016
Active ingredient:	Nominal: 200 g/L Analysed: 199.2 g/L
Reference item:	Acetamiprid
Lot/Batch number:	20202
Purity:	98.1 ± 0.5 %
CAS No.:	135410-20-7
Expiry date:	February 2016
Standards for calibration	As above
Matrix:	Barley (grain and straw)

B. Sample preparation and processing

Approximately 2 g are weighed into a 50 mL centrifuge tube. The samples are fortified respectively. For barley grains the samples are shaken for one minute horizontally. For all matrices 10 mL of ultra-pure water was added. For barley (whole plant and straw) 10 mL of acetonitrile was added. Afterwards all samples for all barley compounds were shaken for 20 minutes. The aliquots are transferred into a QuEChERS tube and shaken by hand. The samples will be centrifuge for 5 minutes at 4000 rpm and afterwards filtered (0.45 µm). The final extracts were analysed by HPLC-MS/MS.

C. Chromatographic parameters

HPLC- parameters	
Instrumentation:	API6500
Column:	C18 Hydro RP (100 x 3 mm ID x 2.5 µm PD
Column temperature:	60°C
Injection volume:	10µl – 220µL
MS/MS - parameters	
Instrumentation:	4000QTrap
Mode:	ESI (electrospray ionisation) positive
Ion source	Turbospray
Scan type:	MRM
Transitions:	223 -> 126 m/z (quantifier) 223 -> 90 m/z (qualifier)

Results and discussions

The specific identification of acetamiprid was conducted by HPLC-MS/MS with two transitions monitored. Therefore no confirmatory data needs to be provided. The limit of quantification in the main validation is 0.01 mg/kg and is defined as the lowest fortification level with mean recoveries ranging from 70% to 110% at a relative standard deviation (RSD) of ≤ 20% and blanks not exceeding 30% of the LOQ level. These criteria were fulfilled for all recoveries at each fortification level at LOQ and 10x LOQ for acetamiprid. The detector response for acetamiprid was linear with 0.3 to µg/L for grains and 0.3 to 10 µg/L for straw $r^2 \geq 0.99$. The recovery data are presented in the table below.

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

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
Barley (grains)	Acetamiprid	0.01	78	9	-
		0.1	76	5	-
Barley (whole plant)		0.01	87	4	-
		0.1	90	4	-
Barley (straw)		0.01	74	5	-
		0.1	83	4	-

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

	Acetamiprid
Specificity	The HPLC-MS/MS with two ions monitored is highly specific for the determination of acetamiprid. No interference above ≥ 30% LOQ
Calibration (type, number of data points)	5-point linear calibration range Barley (grain): y= 46479.58x + 2652.26 r^2= 0.995 Barley (whole plant): y= 1008331.54x + 7110.05 r^2= 0.999 Barley (straw) : y= 16507.86x + 223.12 r^2= 0.999
Calibration range	N/A
Limit of determination/quantification	LOQ for all matrices: 0.01 mg/kg

Conclusion

The method validation in study B14C-S1-A-03 for barley fulfils the requirements of the SANCO/3029/99 rev. 4 guidelines and can be therefore considered as fully acceptable.

A 2.1.1.1.4 Analytical method B17- A4-A-02

A 2.1.1.1.4.1 Method validation B14C-S1-A-03 & B14C-S1-A-01

Comments of zRMS:	The analytical method was successfully validated according to the guidance document SANCO/3029/99 rev.4 on barley (whole plant, grains and straw) and reported in GIRPA report B14C-S1-A-03, SGS Agri min study 14SGS034 (Chevallier, E., full validation in barley). The analytical method was validated (reduce validation) according to the guidance document SANCO/3029/99 rev.4 on wheat grains during another analytical phase performed at GIRPA in 2014 (report B14C-S1-A-01, SGS Study number: 14SGS033). The limit of quantification (LOQ) for acetamiprid was 0.01 mg/kg. The limit of detection (LOD) was defined as 0.0033 mg/kg within the validation study. As analyses at T=0 and T+15 months were performed the same day, they have the same procedural recoveries. Thus correct results with day 0 as 100% in the table below are the same.

Table 11 : storage stability results for wheat grains

Period	Residues and recoveries in specimens stored frozen (not corrected for procedural recoveries)					Residues and recoveries in specimens stored frozen (recovery corrected)		
	Uncorrected residue results[1]				Corrected results with day 0 as 100 %[2]	Procedural recoveries for freshly fortified samples	Corrected results	
Months (extraction date-injection date)	Sample 1 (mg/kg)	Sample 2 (mg/kg)	Sample 3 (mg/kg)	Mean (mg/kg)			Corrected[3]	Day-0 as 100 %[4]
0 (20/04/18 – 20/04/18)	0.074	0.073	0.081	0.076	**100**	76	0.100	**100**
15 (20/04/18 – 20/04/18)	0.074	0.076	-	0.075	**98**	76	0.098	**98**
Nominal fortification at 0.100 mg/kg								

[1] *calculated as detailed in paragraph 8.8.1*
[2] *(mean mg/kg at x months) / (mean mg/kg at 0 month) * 100*
[3] *(mean mg/kg at x months) / (procedural recoveries at x months) * 100*
[4] *(corr at x months) / (corr at 0 month) * 100*

The acetamiprid residue results of the three freshly fortified samples were 0.074, 0.073 and 0.081 mg/kg corresponding to recoveries respectively at 74, 73 and 81% (mean 76% - RSD 6%). In conclusion, the procedural recoveries of the storage stability study prove to be within the requirements of guidance document SANCO/3029/99 rev.4, therefore the method is acceptable.

Reference:	KCP 5.1.2/04
Report	Freezing storage stability of acetamiprid in wheat (grain) at/below -18°C during 15 months (0 and 15 months), Barbier, G., 2018, Study No. B17G-A4-A-02.
Guideline(s):	Yes, SANCO 3029/99 rev. 4
Deviations:	None
GLP:	Yes
Acceptability:	Yes

A. Materials

Reference item:	Acetamiprid
Lot/Batch number:	41007
Purity:	98.1 ± 0.5 %
CAS No.:	135410-20-7
Expiry date:	April 2019
Standards for calibration	As above
Matrix:	Wheat

B. Sample preparation and processing

Approximately 2 g are weighed into a 50 mL centrifuge tube. The samples are fortified respectively. 10 mL of acetonitrile was added. Afterwards all samples were shaken for 20 minutes and frozen for two months at -18°C. The aliquots of the acetonitrile phase are transferred into a centrifuge tube and 10 mL of ultra-pure water was added. The samples were shaken again for 20 minutes and then transferred into a 50 mL QuEChERS tube containing a salt mix. Afterwards the samples were shaken again for 5 minutes and centrifuged for 5 minutes at 4000 rpm. The aliquots of the organic phase were transferred into a second QuEChERS tube containg salts and PSA. The samples were shaken again and centrifuge again 5 minutes at 4000 rpm and afterwards filtered (0.45 µm). The final extracts were analysed by HPLC-MS/MS.

C. Chromatographic parameters

HPLC- parameters:	
Instrumentation:	API6500
Column:	C18 Hydro RP (100 x 3 mm ID x 2.5 µm PD
Column temperature:	60°C

Injection volume:	10µl – 220 µL
MS/MS - parameters	
Instrumentation:	4000QTrap
Mode:	ESI (electrospray ionisation) positive
Ion source:	Turbospray
Scan type:	MRM
Transitions:	223 -> 126 m/z (quantifier) 223 -> 90 m/z (qualifier)

Results and discussions

The specific identification of acetamiprid was conducted by HPLC-MS/MS with two transitions monitored. Therefore no confirmatory data needs to be provided. The analytical method were validated according to SANCO/3029/00 rev. 4 in B14C-S1-A-03 (Chevallier, E., full validation in barley) and in B14C-S1-A-031 (Chevallier, E., reduced validation in wheat). The limit of quantification in the main validation is 0.01 mg/kg and is defined as the lowest fortification level with mean recoveries ranging from 70% to 110% at a relative standard deviation (RSD) of ≤ 20% and blanks not exceeding 30% of the LOQ level. These criteria were fulfilled for the procedural recoveries of the 10 x LOQ for acetamiprid. The detector response for acetamiprid was linear within the range from 0.3 µg/L to 20 µg/L with $r^2 \geq 0.999$. The procedural recovery data are presented in the table below.

Table A 7: Procedural recovery results from method validation of acetamiprid using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Mean recovery (%) 0 & 15 months	RSD (%)	Comments
Wheat	Acetamiprid	0.1	76, 76	-	-

Table A 8: Characteristics for the analytical method used for validation of acetamiprid residues in wheat

	Acetamiprid
Specificity	The HPLC-MS/MS with two ions monitored is highly specific for the determination of acetamiprid. No interference above ≥ 30% LOQ
Calibration (type, number of data points)	5-point linear calibration rrange: Wheat: y= 8645.43X + 338.07 r^2= 0.9998
Calibration range	0.3 µg/L to 20 µg/L
Limit of determination/quantification	LOQ for all matrices: 0.01 mg/kg

Conclusion

The method validation in B14C-S1-A-03 and reduced validation in B14C-S1-A-01 for the determination of acetamiprid in plant material was fully validated according to the requirements of the SANCO/3029/99 rev. 4 guidelines. The procedural recoveries of the storage stability study prove to be within the requirements of the guidelines and therefore the method can be fully accepted.

A 2.1.1.1.5 Analytical method DMC-13-16126

A 2.1.1.1.5.1 Method validation 13M06017-01-VMPL

Comments of zRMS:	The analytical method was derived from the QuEChERS multi-residue method and based on an extraction procedure with final analysis by HPLC with MS/MS detection. The analytical method was fully validated according to guideline SANCO/3029/99.during a previous study performed in 2013 by CIP on head cabbage, apple fruits, potato tubers and peach fruits (Lang, A. 2014; CIP Study code : 13M06017-01-VMPL). Therefore, in the current study only a procedural recovery was performed for the LOQ (level of quantification; 0.01 mg/kg) by fortifying control (untreated) specimen. A higher fortification level for residues of acetamiprid (5 mg/kg) was introduced and fully validated in the current study.

	The samples of the Staphyt Studies DMC-13-16126 and DMC-13-16134 were analysed together and therefore, the results of the recoveries and the validation data will be reported in both studies. The method achieves a limit of quantification (LOQ) of 0.01 mg/kg. Limit of detection (LOD) is 0.003 mg/kg. Acceptance criteria for method validation were met, with average recoveries ranging from 70% to 110% and relative standard deviations ≤20%: The study is acceptable.

Reference:	KCP 5.1.2/05
Report	Magnitude of residues of acetamiprid in peaches (RAC), following two applications of MCW-2222, in three trials (1 DCS + 2 HS) Southern Europe (Southern France and Italy) – 2013, Méric, D., 2013, Study No. DMC-13-16126
Guideline(s):	Yes, SANCO 3029/99 rev. 4
Deviations:	None
GLP:	Yes
Acceptability:	Yes

A. Materials

1. Standards

Test item:	MCW-2222
Batch no.	611-280413-01
Active substance:	Nominal: 200 g/L Analysed: 202.7 g/L
Expiry date:	April 2015
Reference item:	Acetamiprid
Lot/Batch number:	20203
Purity:	99 %
CAS No.:	135410-20-7
Expiry date:	February 2016
Standards for calibration	As above
Matrix:	Peaches

B. Sample preparation and processing

Approximately 10 g of the homogenised peach samples were weighed into 50 mL centrifugation tubes and fortified respectively. 10 mL acetonitrile were added and the samples were extracted using a sample homogeniser. Afterwards, a QuEChERS salt mix was added, shaken well and vortexed for at least one minute. The samples were then centrifuged at 3500 rpm for at least 10 minutes. An aliquot was transferred into a tube prepared with 25 mg PSA, 150 mg anhydrous magnesium sulfate and 2.5 mg GCB and shaken. The extract was filtered through a 0.45 µm filter into an autosampler vial (1.8 mL). The final extracts were diluted 1:10 and used directly for analysis by HPLC-MS/MS.

C. Chromatographic parameters

HPLC- parameters

Instrumentation:	Dionex Ultimate 3000

Column:	C18 (150 x 2 mm ID x 5 µm PD
Column temperature:	40°C
Injection volume:	10 µL
MS/MS - parameters	
Instrumentation:	5500QTrap
Mode:	ESI (electrospray ionisation) positive
Ion source	Turbospray
Scan type:	SRM
Transitions:	223 -> 126 m/z (quantifier) 223 -> 90 m/z (qualifier)

Results and discussions

The method used for the determination of residues of acetamiprid in peach fruits was validated in a previous study (Lang, A. 2014: 13M06017-01-VMPL). The study of Lang is already EU evaluated during the data-matching-list process from CTGB Netherlands in 2020. Therefore, only procedural recoveries were performed for the recovery at LOQ (level of quantification; 0.01 mg/kg) by fortifying control (untreated) samples. A higher fortification level (5.0 mg/kg) had to be introduced for quantification of residues. The specific identification of acetamiprid was conducted by HPLC-MS/MS with two transitions monitored. Therefore, no confirmatory data need to be provided. The limit of quantification in the main validation is 0.01 mg/kg and is defined as the lowest fortification level with mean recoveries ranging from 70% to 110% at a relative standard deviation (RSD) of ≤ 20% and blanks not exceeding 30% of the LOQ level. These criteria were fulfilled for the procedural recoveries of the LOQ for acetamiprid, as well as for the full validation at 500 × LOQ (5.0 mg/kg). The detector response for acetamiprid was linear within the range from 0.25 µg/L to 20 µg/L with $r^2 \geq 0.9996$. The procedural recovery data are presented in the table below.

Table A 9: Procedural- and recovery results from method validation of acetamiprid using the analytical method

Matrix	Analyte	Fortification level (mg/kg)	Mean recovery (%)	RSD (%)	Comments
Acetamiprid SRM 223→126 (quantification)					
Peach fruit	Acetamiprid	0.01 mg/kg (n=2)	97	-	-
		5.0 mg/kg (n=6)	95	4.2	-
Acetamiprid SRM 223→90 (confirmation)					
Peach fruit	Acetamiprid	0.01 mg/kg (n=2)	97	-	-
		5.0 mg/kg (n=6)	94	4.2	-

Table A 10: Characteristics for the analytical method used for validation of acetamiprid residues in peaches

	Acetamiprid
Specificity	The HPLC-MS/MS with two ions monitored is highly specific for the determination of acetamiprid. No interference above ≥ 30% LOQ
Calibration (type, number of data points)	Seven-point linear calibration curve y= 141683x r^2= 0.9992
Calibration range	0.25 µg/L to 20 µg/L
Assessment of matrix effects is presented	Matrix-matched standard solutions were used. No matrix effects above 5 % were observed
Limit of determination/quantification	LOQ: 0.01 mg/kg

Conclusion

The method validation for 500 × LOQ for the determination of acetamiprid in peaches was fully validated

according to the requirements of the SANCO/3029/99 rev 4 guidelines. The procedural recoveries of the method DMC-13-16126 validated in 13M06017-01-VMPL prove to be within the requirements of the guidelines and therefore the method can be fully accepted.

A 2.1.1.1.6 Analytical method DMC-13-16134
A 2.1.1.1.6.1 Method validation 13M06017-01-VMPL & DMC-13-16126

Comments of zRMS:	The analytical method was fully validated according to guideline SANCO/3029/99 rev.4 during a previous study performed in 2013 by CIP on head cabbage, apple fruits, potato tubers and peach fruits (Lang, A. 2014; CIP Study code : 13M06017-01-VMPL). Therefore, only procedural recoveries were performed for the LOQ (level of quantification; 0.01 mg/kg) by fortifying control (untreated) specimens. A higher fortification level for residues of acetamiprid (5 mg/kg) was validated in Staphyt Study DMC-13-16126 for matrix peaches (also aqueous matrix). Therefore, only a reduced validation had to be performed for the new fortification level at 0.5 mg/kg acetamiprid. The samples of the Staphyt Studies DMC-13-16122 and DMC-13-16134 were analysed together and therefore, the results of the recoveries and the validation data will be reported in both studies. The method achieves a limit of quantification (LOQ) of 0.01 mg/kg. Limit of detection (LOD) is 0.003 mg/kg. Acceptance criteria for method validation were met, with average recoveries ranging from 70% to 110% and relative standard deviations ≤20%. The study is acceptable.

Reference:	KCP 5.1.2/06
Report	Magnitude of residues of acetamiprid in apples (RAC), following one or two applications of MCW-2222, in two trials (1 DCS + 1 HS) Northern Europe (Northern France) – 2013, Méric, D., 2013, Study No. DMC-13-16134
Guideline(s):	Yes, SANCO 3029/99 rev. 4
Deviations:	None
GLP:	Yes
Acceptability:	Yes

A. Materials

1. Standards

Test item:	MCW-2222
Batch no.	611-280413-01
Active substance:	Nominal: 200 g/L Analysed: 202.7 g/L
Expiry date:	April 2015
Reference item:	Acetamiprid
Lot/Batch number:	20203
Purity:	99 %
CAS No.:	135410-20-7
Expiry date:	February 2016
Standards for calibration	As above
Matrix:	Apples

B. Sample preparation and processing

Approximately 10 g of the homogenised apple samples were weighed into 50 mL centrifugation tubes and fortified respectively. 10 mL acetonitrile were added and the samples were extracted using a sample homogeniser. Afterwards, a QuEChERS salt mix was added, shaken well and vortexed for at least one minute. The samples were then centrifuged at 3500 rpm for at least 10 minutes. An aliquot was transferred into a tube prepared with 25 mg PSA, 150 mg anhydrous magnesium sulfate and 2.5 mg GCB and shaken. The extract was filtered through a 0.45 µm filter into an autosampler vial (1.8 mL). The final extracts were diluted 1:10 and used directly for analysis by HPLC-MS/MS.

C. Chromatographic parameters

HPLC- parameters	
Instrumentation:	Dionex Ultimate 3000
Column:	C18 (150 x 2 mm ID x 5 µm PD
Column temperature:	40°C
Injection volume:	10 µL
MS/MS - parameters	
Instrumentation:	5500QTrap
Mode:	ESI (electrospray ionisation) positive
Ion source	Turbospray
Scan type:	SRM
Transitions:	223 -> 126 m/z (quantifier)
	223 -> 90 m/z (qualifier)

Results and discussions

The method used for the determination of residues of acetamiprid in apple fruits was validated in a previous study (Lang, A. 2014: 13M06017-01-VMPL). The study of Lang is already EU evaluated during the data-matching-list process from CTGB Netherlands in 2020. Therefore, only procedural recoveries were performed for the recovery at LOQ (level of quantification; 0.01 mg/kg) by fortifying control (untreated) samples. A higher fortification level (5.0 mg/kg) was validated in Méric, D., 2013 Study No. DMC-13-16126 for the matrix peaches (also high water content matrix). Therefore only a reduced validation had to be performed for the new fortification level at 0.5 mg/kg acetamiprid. The specific identification of acetamiprid was conducted by HPLC-MS/MS with two transitions monitored. Therefore no confirmatory data need to be provided. The limit of quantification in the main validation is 0.01 mg/kg and is defined as the lowest fortification level with mean recoveries ranging from 70% to 110% at a relative standard deviation (RSD) of ≤ 20% and blanks not exceeding 30% of the LOQ level. These criteria were fulfilled for the procedural recoveries of the LOQ for acetamiprid, as well as for the reduced validation of the temper LOQ (0.5 mg/kg). The detector response for acetamiprid was linear within the range from 0.25 µg/L to 20 µg/L with $r^2 \geq 0.9999$. The procedural recovery data and reduced validation data are presented in the table below.

Table A 11: Procedural- and recovery results from method validation of acetamiprid using the analytical method

Matrix	Analyte	Fortification level (mg/kg)	Mean recovery (%)	RSD (%)	Comments
Acetamiprid SRM 223→126 (quantification)					
Apple fruit	Acetamiprid	0.01 mg/kg (n=3)	98	5.1	-
		0.5 mg/kg (n=4)	94	0.9	-
Acetamiprid SRM 223→90 (confirmation)					
Apple fruit	Acetamiprid	0.01 mg/kg (n=3)	98	6.6	-
		0.5 mg/kg (n=4)	94	1.5	-

Table A 12: Characteristics for the analytical method used for validation of acetamiprid residues in apples

	Acetamiprid
Specificity	The HPLC-MS/MS with two ions monitored is highly specific for the determination of acetamiprid. No interference above ≥ 30% LOQ
Calibration (type, number of data points)	Seven-point linear calibration curve y= 142528x r^2= 0.9999
Calibration range	0.25 µg/L to 20µg/L
Assessment of matrix effects is presented	Matrix-matched standard solutions were used. No matrix effects above 5 % were detected
Limit of determination/quantification	LOQ: 0.01 mg/kg

Conclusion
The method for the determination of acetamiprid in peaches was fully validated according to the requirements of the SANCO/3029/99 rev 4 guidelines in 13M06017-01-VMPL, as well as the validation at higher LOQ in DMC-13-16126. The procedural recoveries and the reduced validation of the method DMC-13-16134 prove to be within the requirements of the guidelines and therefore the method can be fully accepted.

A 2.1.1.1.7 Analytical method ChR-14-17311
A 2.1.1.1.7.1 Method validation 13M06017-01-VMPL & DMC-13-16126

Comments of zRMS:	The analytical method was fully validated on aqueous matrix (peach flesh) during previous study performed in 2013 by CIP (Lang, A. 2014; CIP Study Code: 13M06017-01-VMPL) for aqueous matrix validation. Dry matrix (dried apples) was fully validated during this study with 5 validations at three different levels (LOQ, 100 LOQ and 500 LOQ). Therefore, reduced validations were performed in the current study to confirm validation at LOQ and 100 LOQ levels on apple, washing water, juice, puree and on wet and dry pomace. The method achieves a limit of quantification (LOQ) of 0.01 mg/kg. Limit of detection (LOD) is 0.003 mg/kg. Acceptance criteria for method validation were met, with average recoveries ranging from 70% to 110% and relative standard deviations ≤20%. The study is acceptable.

Reference:	KCP 5.1.2/07
Report	Magnitude of the residues of acetamiprid in apple (RAC fruits and processed fractions), following one or two applications of MCW-2222 in six trials (3 DCS + 3 HS), Northern Europe (Northern France, Germany, Poland and Belgium) – 2014, Roussel, Ch. H., 2014, Study No. ChR-14-17311
Guideline(s):	Yes, SANCO 3029/99 rev. 4
Deviations:	None
GLP:	Yes
Acceptability:	Yes

A. Materials
1. Standards

Test item:	MCW-2222
Batch no.	611-280413-01
Active substance:	Nominal: 200 g/L Analysed: 202.7 g/L

Expiry date:	April 2015
Reference item:	Acetamiprid
Lot/Batch number:	20203
Purity:	99 %
CAS No.:	135410-20-7
Expiry date:	February 2016
Standards for calibration	As above
Matrix:	Apples and processed fractions

B. Sample preparation and processing

Approximately 10 g of the homogenised apple samples and 5 g (dry apple, wet apple pomace and dry apple pomaces) were weighed into 50 mL centrifugation tubes and fortified respectively. 10 mL acetonitrile were added and the samples were extracted using a sample homogeniser. Afterwards a QuEChERS salt mix was added, shaken well and vortexed for at least one minute. The samples were then centrifuged at 3500 rpm for at least 10 minutes. An aliquot was transferred into a tube prepared with 25 mg PSA, 150 mg anhydrous magnesium sulfate and 2.5 mg GCB and shaken. The extract was filtered through a 0.45µm filter into an autosampler vial (1.8 mL). The final extracts were diluted 1:10 and 1:5 for matrix dry apples, wet pomace and dry apple pomaces, respectively. The diluted final extracts were used directly for analysis by HPLC-MS/MS.

C. Chromatographic parameters

HPLC- parameters	
Instrumentation:	Dionex Ultimate 3000
Column:	C18 (150 x 2 mm ID x 5 µm PD
Column temperature:	40°C
Injection volume:	10 µL
MS/MS - parameters	
Instrumentation	5500QTrap
Mode:	ESI (electrospray ionisation) positive
Ion source	Turbospray
Scan type:	SRM
Transitions:	223 -> 126 m/z (quantifier)
	223 -> 90 m/z (qualifier)

Results and discussions

The method used for the determination of residues of acetamiprid in apple, washed apples, washing water, apple juice, apple puree and wet apple pomace was validated in a previous study (Lang, A. 2014: 13M06017-01-VMPL). The study of Lang is already EU evaluated during the data-matching-list process from CTGB Netherlands in 2020. Therefore, only a reduced validation was performed for the recovery at LOQ (level of quantification; 0.01 mg/kg) by fortifying control (untreated) samples. A higher fortification level (5.0 mg/kg) was validated in Méric, D., 2013 Study No. DMC-13-16126 for the matrix peaches (also high water content matrix). Therefore only a reduced validation had to be performed for the new high fortification level at 1.0 mg/kg acetamiprid. The dry matrix dry apples was fully validated at the following levels: LOQ (0.01 mg/kg), 500 x LOQ (5.0 mg/kg) and at 100 x LOQ (1.0 mg/kg). For the other dry matrix dry apple pomace, a reduced validation was performed at the same levels. The specific identification of acetamiprid was conducted by HPLC-MS/MS with two transitions monitored. Therefore no confirmatory data need to be provided. The limit of quantification in the main validation is 0.01 mg/kg and is defined as the lowest fortification level with mean recoveries ranging from 70% to 110% at a relative standard deviation (RSD)

of ≤ 20% and blanks not exceeding 30% of the LOQ level. These criteria were fulfilled for the reduced validation of the LOQ for acetamiprid, as well as for the reduced validation of the temper LOQ (1.0 mg/kg). The detector response for acetamiprid was linear within the range from 0.25 µg/L to 100 µg/L with $r^2 \geq 0.999$. The reduced validation data are presented in the table below.

Table A 13: Recovery results from method validation of acetamiprid using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (n=3)	Mean recovery (%)	RSD (%)	Comments
Apple fruit	Acetamiprid	0.01	95	4.7	-
		1.0	103	3.5	-
Dry apple		0.01 (n=5)	10	3.8	-
		1.0 (n=5)	106	4.3	-
		5.0 (n=5)	107	1.2	-
Washing water		0.01	97	3.1	-
		1.0	97	1.2	-
Apple juice		0.01	92	3.5	-
		1.0	96	2.1	-
Apple puree		0.01	101	1.0	-
		1.0	103	2.4	-
Wet apple pomace		0.01	104	3.3	-
		1.0	110	4.1	-
Dry apple pomace		0.01 (n=4)	103	4.6	-
		1.0	108	2.3	-
		5.0	105	9.2	-

Table A 14: Characteristics for the analytical method used for validation of acetamiprid residues in apples and processed fractions

	Acetamiprid
Specificity	The HPLC-MS/MS with two ions monitored is highly specific for the determination of acetamiprid. No interference above ≥ 30% LOQ
Calibration (type, number of data points)	Nine-point linear calibration curve Apple: y= 7.99437 e4x r^2= 0.999 Dry apples: : y= 7.7413e4x r^2= 0.999 Washing water: y= 6.13117e4x r^2= 0.999 Apple juice: y= 6.94619 e4x r^2= 0.999 Apple puree: y= 6.685204 e4x r^2= 0.999 Wet apple pomace: y= 6.77067e4x r^2= 0.999 Dry apple pomace: y= 7.16721e4x r^2= 0.999
Calibration range	0.25 µg/L to 100µg/L
Assessment of matrix effects is presented	Matrix-matched standard solutions were used. No matrix effects above 5 % were observed.
Limit of determination/quantification	LOQ: 0.01 mg/kg

Conclusion

The method validation for the determination of acetamiprid in plant matrices with high water content was fully validated according to the requirements of the SANCO/3029/99 rev 4 guidelines in 13M06017-01-VMPL, as well as the validation at higher LOQ in DMC-13-16126. The reduced validation of the method ChR-14-17311 proof to be within the requirements of the guidelines and therefore the method can be fully

accepted.

A 2.1.1.1.8 Analytical method DMC-13-16129
A 2.1.1.1.8.1 Method validation B13-M1-A-01

Comments of zRMS:	The analytical method for determination of acetamiprid on olive (whole fruit) (representative crop for high oil content group) and on oilseed rape (whole plant) (representative crop for high content group) was fully validated in study GIRPA study code B13-M1-A-01, sponsor code R_33645 (Lefresne, S, 2014) according to guideline SANCO/3029/99 rev.4 at the levels of 0.01 mg/kg (LOQ) and 0.1 mg/kg (10 fold LOQ). Within this study, reduced validations were carried out at the levels of 0.01 mg/kg (LOQ) and 0.1 mg/kg (10 fold LOQ) at each sampling point on oilseed rape (pod, seed and whole plant without pod) to verify the validity of the used method. The limit of quantification of the method is the lowest validated level where a mean recovery within the range 70-110% and with RSD less or equal to 20% could be obtained. LOQ was 0.01 mg/kg for acetamiprid. The data presented demonstrate that the method permits the determination of residues of acetamiprid in oilseed rape (pod, seed and whole plant without pod) with satisfactory accuracy, precision and repeatability according to guideline SANCO/3029/99 rev.4. **Summary of validations:** The study is acceptable.

Specimen	Reference item	Level spiked ($mg.kg^{-1}$)	Mean of Recovery rates (%)	Relative standard deviation (%)	Number of recovery rates (n)
Olive (whole fruit)	acetamiprid	0.010	95	8	5
		0.100	97	6	5
		All	96	7	10
Oilseed rape (whole plant)		0.010	70	4	5
		0.100	71	4	5
		All	70	3	10
Oilseed rape (pods)		0.010	83	4	3
		0.100	90	3	3
		All	87	5	6
Oilseed rape (seeds)		0.010	76	9	3
		0.100	97	1	3
		All	87	14	6
Oilseed rape (whole plant without pods)		0.010	83	7	3
		0.100	73	20	3
		All	78	14	6

Reference:	KCP 5.1.2/08
Report	Magnitude of the residues of acetamiprid in oilseed rape (RAC, whole plants, pods and seeds), following one or two applications of MCW-2222– in two trials (1 DCS + 1 HS), Northern Europe (Germany and Northern France) - 2013, Méric, D., 2014, Study No. DMC-13-16129
Guideline(s):	Yes, SANCO 3029/99 rev. 4
Deviations:	None
GLP:	Yes
Acceptability:	Yes

A. Materials

1. Standards

Test item:	MCW-2222
Batch no.	611-280413-01
Active substance:	Nominal: 200 g/L Analysed: 202.7 g/L
Expiry date:	April 2015

Reference item:	Acetamiprid
Lot/Batch number:	20202
Purity:	98.1 %
CAS No.:	135410-20-7
Expiry date:	February 2016
Standards for calibration	As above
Matrix:	Oilseed rape (whole plant, pods, whole plant without pods) and seeds

B. Sample preparation and processing

Approximately 2 g of the homogenised oil seed rape samples were weighed into 50 mL centrifugation tubes and fortified respectively, additionally for oilseed rape (whole plant) 5 mL ultra-pure water was added and 10 mL acetonitrile for all matrix compounds . The samples were shaken for 20 minutes. Afterwards a QuEChERS salt mix was added, shaken well and vortexed for at least one minute. The samples were then centrifuged at 3000 rpm for at least 10 minutes. The extract was filtered through a 0.45µm filter. The diluted final extracts were used directly for analysis by HPLC-MS/MS.

C. Chromatographic parameters

HPLC- parameters	
Instrumentation:	Dionex Ultimate 3000
Column:	C18 (150 x 2 mm ID x 5 µm PD
Column temperature:	40°C
Injection volume:	10 µL
MS/MS - parameters	
Instrumentation:	5500QTrap
Mode:	ESI (electrospray ionisation) positive
Ion source	Turbospray
Scan type:	MRM
Transitions:	223 -> 126 m/z (quantifier) 223 -> 90 m/z (qualifier)

Results and discussions

The analytical method was fully validated during a previous study from Lefresne; Study No. B13-M1-A-01 (EU agreed, during the data-matching-list process by CTGB, Netherlands 2020) on olives (whole plant) and oilseed rape (whole plant). Therefore, only a reduced validation was performed at LOQ (0.01 mg/kg) and 10 × LOQ (0.1 mg/kg) for oilseed rape (pod, seed, whole plant and whole plant without pod). The specific identification of acetamiprid was conducted by HPLC-MS/MS with two transitions monitored. Therefore no confirmatory data need to be provided. The limit of quantification in the main validation is 0.01 mg/kg and is defined as the lowest fortification level with mean recoveries ranging from 70% to 110% at a relative standard deviation (RSD) of ≤ 20% and blanks not exceeding 30% of the LOQ level. These criteria were fulfilled for the reduced validation of the LOQ for acetamiprid, as well as for 10 × LOQ (0.1 mg/kg). The detector response for acetamiprid was linear within the range from 0.3 µg/L to 10 µg/L for oilseed rape (whole plant, pods and whole plant without pods) and 0.3 µg/L to 8 µg/L for oilseed rape seeds with $r^2 \geq 0.999$. The reduced validation data are presented in the table below.

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

Matrix	Analyte	Fortification level (mg/kg) (n=3)	Mean recovery (%)	RSD (%)	Comments
Oilseed rape (seed)	Acetamiprid	0.01	70	-	-
Oilseed rape (whole plant)		0.01	89	-	-
		0.1	86	-	-
Oilseed rape (pods)		0.01	71	-	-
		0.1	86	-	-
Oilseed rape (whole plant without pods)		0.01	79	-	-
		0.1	72	-	-

Table A 16: Characteristics for the analytical method used for validation of acetamiprid residues in oilseed rape (pod, seed, whole plant and whole plant without pod)

	Acetamiprid
Specificity	The HPLC-MS/MS with two ions monitored is highly specific for the determination of acetamiprid. No interference above ≥ 30% LOQ
Calibration (type, number of data points)	Nine-point linear calibration curve Oilseed rape (pod) : y= 5878.98x + 194.50 r^2= 0.999 Oilseed rape (seed) y= 5291. 07x + 58.81 r^2= 0.999 Oilseed rape (whole plant): y= 10231.88x + 736.51 r^2= 0.999 Oilseed rape (whole plant without pod): y= 8061.99x + 499.22 r^2= 0.998
Calibration range	0.3 µg/L to 10 µg/L for oilseed rape (whole plant, pods and whole plant without pods) 0.3 µg/L to 8 µg/L for oilseed rape seeds
Assessment of matrix effects is presented	Matrix-matched standard solutions were used. No matrix effects above 10 % could be obtained
Limit of determination/quantification	LOQ: 0.01 mg/kg

Conclusion

The method for the determination of acetamiprid in plant matrices with high water content and high oil content was fully validated according to the requirements of the SANCO/3029/99 rev 4 guidelines in B13-M1-A-01. The reduced validation of the method DMC-13-16129 proved to be within the requirements of the guidelines and therefore the method can be fully accepted.

A 2.1.1.1.9 Analytical method 14SGS035

A 2.1.1.1.9.1 Method validation B13-M1-A-01

Comments of zRMS:	The analytical method for determination of acetamiprid on olive (whole fruit) (representative crop for high oil content group) and on oilseed rape (whole plant) (representative crop for high content group) was fully validated in study GIRPA study code B13-M1-A-01, sponsor code R_33645 (Lefresne, S, 2014) according to guideline SANCO/3029/99 rev.4 at the levels of 0.01 mg/kg (LOQ) and 0.1 mg/kg (10 fold LOQ). Within this study, reduced validations were carried out at the levels of 0.01 mg/kg (LOQ) and 0.1 mg/kg (10 fold LOQ) at each sampling point on oilseed rape (pod, seed and whole plant without pod) to verify the validity of the used method. The limit of quantification of the method is the lowest validated level where a mean recovery within the range 70-110% and with RSD less or equal to 20% could be obtained. LOQ was 0.01 mg/kg for acetamiprid. The data presented demonstrate that the method permits the determination of residues of acetamiprid in oilseed rape (pod, seed and whole plant without pod) with satisfactory accuracy, precision and repeatability according to guideline SANCO/3029/99 rev.4. The study is acceptable.

Reference:	KCP 5.1.2/09
Report	Magnitude of the residues of acetamiprid in winter oilseed rape (RAC), after one or two applications of MCW-2222–-three decline curve trials and three harvest trials in Northern Europe (Northern France, Poland, Germany, Czech Republic and Hungary) - 2014, Chevallier, E., 2014, Study No. 14SGS035
Guideline(s):	Yes, SANCO 3029/99 rev. 4
Deviations:	None
GLP:	Yes
Acceptability:	Yes

A. Materials

1. Standards

Test item:	MCW-2222
Batch no.	659- 030314-01
Active substance:	Nominal: 200 g/L Analysed: 199.2 g/L
Expiry date:	March 2016
Reference item:	Acetamiprid
Lot/Batch number:	20202
Purity:	98.1 %
CAS No.:	135410-20-7
Expiry date:	February 2016
Standards for calibration	As above
Matrix:	Oilseed rape

A. Sample preparation and processing

Approximately 2 g of the homogenised oil seed rape samples were weighed into 50 mL centrifugation tubes and fortified respectively, additionally for oilseed rape (whole plant, pods, whole plant without pods)) 5 mL ultra-pure water was added and 10 mL acetonitrile for all matrix compounds . The samples were shaken for 20 minutes. Afterwards a QuEChERS salt mix was added, shaken well and vortexed for at least one minute. The samples were then centrifuged at 3000 rpm for at least 10 minutes. The extract was filtered through a 0.45µm filter. The diluted final extracts were used directly for analysis by HPLC-MS/MS.

B. Chromatographic parameters

HPLC- parameters	
Instrumentation:	API6500
Column:	C18 (100 x 3 mm ID x 2.5 µm PD
Column temperature:	60°C
Injection volume:	10 µL
MS/MS - parameters	5500QTrap
Mode:	ESI (electrospray ionisation) positive
Ion source	Turbospray
Scan type:	MRM
Transitions:	223 -> 126 m/z (quantifier) 223 -> 90 m/z (qualifier)

HPLC- parameters

Instrumentation:	API 4000
Column:	C18 (100 mm x 3 mm ID x 2.5 µm PD)
Column temperature:	60°C
Injection volume:	10 µL
MS/MS - parameters	
Mode:	ESI
Ion source:	Turbospray
Scan type:	MRM
Transitions:	223 -> 126 m/z (quantifier) 223 -> 90 m/z (qualifier)

Results and discussions

The analytical method was fully validated during a previous study from Lefresne; Study No. B13-M1-A-01 (EU agreed, during the data-matching-list process by CTGB, Netherlands 2020) on olives (whole plant) and oilseed rape (whole plant). Reduced validation were also performed on oilseed rape (whole plant without pod, pod and seed). Therefore, only a reduced validation was performed at LOQ (0.01 mg/kg) and 10 × LOQ (0.1 mg/kg) for oilseed rape (pod, seed, whole plant and whole plant without pod). The specific identification of acetamiprid was conducted by HPLC-MS/MS with two transitions monitored. Therefore no confirmatory data need to be provided. The limit of quantification in the main validation is 0.01 mg/kg and is defined as the lowest fortification level with mean recoveries ranging from 70% to 110% at a relative standard deviation (RSD) of ≤ 20% and blanks not exceeding 30% of the LOQ level. These criteria were fulfilled for the reduced validation of the LOQ for acetamiprid, as well as for 10 x LOQ (0.1 mg/kg). The detector response for acetamiprid was linear within the range from 0.3 µg/L to 10 µg/L for oilseed rape (whole plant and seeds) and 0.3 µg/L to 5 µg/L for oilseed rape (whole plant without pods) with $r^2 \geq 0.999$. The reduced validation data are presented in the table below.

Table A 17: Recovery results from the reduced method validation of acetamiprid using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (n=3)	Mean recovery (%)	RSD (%)	Comments
Oilseed rape (whole plant)	Acetamiprid	0.01	97	-	-
		0.1	99	-	-
Oilseed rape (whole plant without pods)		0.01	90	-	-
		0.1	95	-	-
Oilseed rape (seeds)		0.01	90	-	-
		0.1	93	-	-
Oilseed rape (whole plant without pods) [a)]		0.01	70	-	-
		0.1	92	-	-
Oilseed rape (seeds) [a)]		0.01	107	-	-
		0.1	112[b)]	-	-

a) Samples are presented separated from the same matrix compounds in the table due to a different time of analysis

b) Recovery rate concerned by the deviation Nr. 57 of the report: "The recovery rate performed on seeds at 10×LOQ during the 29/09/14 sample set was more than 110 %. As the mean of recoveries performed on seeds at 10×LOQ was between 70 % and 110 % as required by SANCO/3029/99 rev 4 11/07/00 guideline, this deviation does not interfere with the quality of the results. No impact."

Table A 18: Characteristics for the analytical method used for validation of acetamiprid residues in oilseed rape (pod, seed, whole plant and whole plant without pod)

	Acetamiprid
Specificity	The HPLC-MS/MS with two ions monitored is highly specific for the determination of acetamiprid. No interference above ≥ 30% LOQ

	Acetamiprid
Calibration (type, number of data points)	Nine-point linear calibration curve Oilseed rape (pod) : y= 2906.56x + 191.64 r^2= 1 Oilseed rape (seed) y= 111.770x + 12802.64 r^2= 0.999 Oilseed rape (whole plant): y= 3713.54x + 16.47 r^2= 0.999 Oilseed rape (whole plant without pod): y= 443.02 – 320.50 r^2= 0.999
Calibration range	0.3 µg/L to 5 µg/L for oilseed rape (whole plant without pods) 0.3 µg/L to 10 µg/L for oilseed rape (whole plant and seeds)
Assessment of matrix effects is presented	Matrix-matched standard solutions were used. No matrix effects could be obtained
Limit of determination/quantification	LOQ: 0.01 mg/kg

Conclusion
The method validation for the determination of acetamiprid in plant matrices with high water content and high oil content was fully validated according to the requirements of the SANCO/3029/99 rev 4 guidelines in B13-M1-A-01. The reduced validation of the method 14SGS035 proved to be within the requirements of the guidelines and therefore the method can be fully accepted.

A 2.1.1.1.10 Analytical method 13SGS102
A 2.1.1.1.10.1 Method validation 13M06017-01-VMPL

Comments of zRMS:	The analytical method for specimens of matrix potato was validated in an extra study (Lang, A. 2014: 13M06017-01-VMPL) according to the SANCO/3029/00 rev.4, therefore only procedural recoveries was performed within this study. The recovery values obtained by HPLC-MS/MS for acetamiprid for all fortification levels comply with the standard acceptance criteria of guideline SANCO/3029/99 rev.4, which demands that the recovery at each fortification level should be in the range of 70-110%. The study is acceptable.

Reference:	KCP 5.1.2/10
Report	Magnitude of the residues of acetamiprid in potato (RAC), after two applications of MCW-2222 in three decline curve trials (Poland, United Kingdom, and Northern France) and in one harvest trials (Poland) in Northern Europe – 2013, Bousquet, C., 2014, Study No. 13SGS102
Guideline(s):	Yes, SANCO 3029/99 rev. 4
Deviations:	None
GLP:	Yes
Acceptability:	Yes

A. Materials

1. Standards

Test item:	MCW-2222
Batch no.	611-280413-01
Active substance:	Nominal: 200 g/L Analysed: 202.7 g/L
Expiry date:	April 2015
Reference item:	Acetamiprid
Lot/Batch number:	20203
Purity:	99 %
CAS No.:	135410-20-7
Expiry date:	February 2016

Standards for calibration	As above
Matrix:	Potato tubers

B. Sample preparation and processing

Approximately 10 g of the homogenised potato samples were weighed into 50 mL centrifugation tubes and fortified respectively. 10 mL acetonitrile were added and the samples were extracted using a sample homogeniser. Afterwards a QuEChERS salt mix was added, shaken well and vortexed for at least one minute. The samples were then centrifuged at 3500 rpm for at least 10 minutes. An aliquot was transferred into a tube prepared with 25 mg PSA, 150 mg anhydrous magnesium sulfate and 2.5 mg GCB and shaken. The extract was filtered through a 0.45µm filter into an autosampler vial (1.8 mL). The final extracts were diluted 1:10 and were used directly for analysis by HPLC-MS/MS.

C. Chromatographic parameters

HPLC- parameters	
Instrumentation:	Dionex Ultimate 3000
Column:	C18 (150 x 2 mm ID x 5 µm PD
Column temperature:	40°C
Injection volume:	10 µL
MS/MS - parameters	
Instrumentation:	5500QTrap
Mode:	ESI (electrospray ionisation) positive
Ion source	Turbospray
Scan type:	SRM
Transitions:	223 -> 126 m/z (quantifier) 223 -> 90 m/z (qualifier)

Results and discussions

The method used for the determination of residues of acetamiprid in potato was validated in a previous study (Lang, A. 2014: 13M06017-01-VMPL) the study of Lang is already EU evaluated during the data-matching-list process from CTGB Netherlands in 2020. Therefore, only procedural recoveries were performed for the recovery at LOQ (level of quantification; 0.01 mg/kg) and 10 × LOQ (0.1 mg/kg) by fortifying control (untreated) samples. The specific identification of acetamiprid was conducted by HPLC-MS/MS with two transitions monitored. Therefore no confirmatory data need to be provided. The limit of quantification in the main validation is 0.01 mg/kg and is defined as the lowest fortification level with mean recoveries ranging from 70% to 110% at a relative standard deviation (RSD) of ≤ 20% and blanks not exceeding 30% of the LOQ level. These criteria were fulfilled for the procedural recoveries of the LOQ for acetamiprid, as well as for the reduced validation of the temper LOQ (1.0 mg/kg). The detector response for acetamiprid was linear within the range from 0.25 µg/L to 20 µg/L with $r^2 \geq 0.999$. The procedural recoveries are presented in the table below.

Table A 19: Procedural recovery results from method validation of acetamiprid using the analytical method

Matrix	Analyte	Fortification level (mg/kg)	Mean recovery (%)	RSD (%)	Comments
SRM 223→126 (quantifier)					
Potato tubers	Acetamiprid	0.01	87	-	-
		1.0	85	-	-
SRM 223→90 (qualifier)					
Potato tubers	Acetamiprid	0.01	90	-	-
		1.0	85	-	-

Table A 20: Characteristics for the analytical method used for validation of acetamiprid residues in potato tubers

	Acetamiprid
Specificity	The HPLC-MS/MS with two ions monitored is highly specific for the determination of acetamiprid. No interference above ≥ 30% LOQ
Calibration (type, number of data points)	Seven-point-linear calibration curve Apple: y= 183320.2258x r^2= 0.999
Calibration range	0.25 µg/L to 20µg/L
Assessment of matrix effects is presented	Matrix-matched standard solutions were used. No matrix effects above 5 % were observed
Limit of determination/quantification	LOQ: 0.01 mg/kg

Conclusion
The method validation for the determination of acetamiprid in potato tubers was fully validated according to the requirements of the SANCO/3029/99 rev 4 guidelines in 13M06017-01-VMPL. The procedural recoveries of the method 13SGS102 proved to be within the requirements of the guidelines and therefore the method can be fully accepted.

A 2.1.1.1.11 Analytical method 1940050
A 2.1.1.1.11.1 Method validation 1940050

Comments of zRMS:	The data presented in the report demonstrate that the method used permits the determination of acetamiprid in honey and meets the requirements of SANCO/3029/99 rev. 4 guidelines. Mean recovery values obtained by HPLC-MS/MS for matrix honey comply with the standard acceptance criteria of guideline SANCO/3029/99 rev. 4, which require that the mean recoveries should be within the range of 70-110% for each fortification level and precision data must be ≤20%. It can therefore be concluded, that the method was applicable on matrix honey under investigation using HPLC with MS/MS detection. LOQ was 0.01 mg/kg for acetamiprid in matrix honey. The study is acceptable.

Reference:	KCP 5.1.2/ 12
Report	Semi-field study for determining the magnitude of residues of Carnadine (CA3573) (a.s. acetamiprid) in honey, Hecht-Ross, S., 2020, Study No. 1940050.
Guideline(s):	Yes, SANCO 3029/99 rev. 4
Deviations:	None
GLP:	Yes
Acceptability:	Yes

A. Materials
1. Standards

Test item:	CA3573 Acetamiprid 200 SL
Batch no.	981107246
Active substance:	Nominal: 200 g/L Analysed: 203.0 g/L
Expiry date:	November 2020
Reference item:	Acetamiprid
CAS No.:	160430-64-8

Batch No.:	BCBT9185
Purity:	100 %
Expiry date:	February 2022
Standards for calibration	As above
Matrix:	Honey

B. Sample preparation and processing

Honey samples of 5 g were added to 10 mL of demineralised water. The samples were weighed into 50 mL centrifugation tubes, 10 mL acetonitrile were added and the samples were homogenised for at least 2 min using a vortex mixer.
Thereafter, QuEChERS EN15662 salt-mixture (1 g sodium citrate, 0.5 g sodium hydrogencitrate sequihydrate, 4 g magnesium sulphate, 1 g sodium chloride (Bekolut Citrate-Kit-01) was added, thoroughly shaken and mixed again on a vortex mixer for at least 1 min.
The samples were centrifuged at 4000 min-1 for at least 5 minutes. The supernatant was transferred into a 15 mL centrifuge tube.
An aliquot of 1 mL of the supernatant was transferred into a tube (2 mL) prepared with 25 mg PSA and 150 mg anhydrous magnesia sulphate and 25 mg C18e (Bekolut PSA-Kit-03), shaken on a vortex mixer for 30 s, centrifuged for 5 min (12 000 rpm) and transferred into an autosampler vial (1.8 mL). The final extracts were diluted 1:10 (with ACN) into an autosampler vial (1.8 mL) and used directly for analysis by HPLC-MS/MS and analysed via HPLC-MS/MS. If necessary, the final extracts were diluted.

C. Chromatographic parameters

HPLC- parameters:	Dionex Ultimate 3000
Column:	Phenomenex Luna C18 (150 mm x 2.0 mm 5 µm)
Column temperature:	40°C
Injection volume:	10 µL
Mobile Phase:	A: Water + 1% formic acid B: Acetonitrile
MS/MS parameters:	AB Sciex API 5500 QTRAP
Ion source:	ESI positive
Ion mode:	MRM
Detection:	223.0 -> 126 (quantifier) 223.0 -> 90 (qualifier)

Results and discussions

The method used for the determination of residues of acetamiprid in honey was fully validated according to the requirements of the SANCO/3029/99 rev. 4 guidelines. The specific identification of acetamiprid was conducted by HPLC-MS/MS with two transitions monitored. Therefore no confirmatory data need to be provided. The limit of quantification is 0.01 mg/kg and is defined as the lowest fortification level with mean recoveries ranging from 70% to 110% at a relative standard deviation (RSD) of ≤ 20% and blanks not exceeding 30% of the LOQ level. These criteria were fulfilled for the recoveries of the method LOQ for acetamiprid, as well as for the temper LOQ. The detector response for acetamiprid was linear within the range within 0.1 µg/L to 100 µg/L (corresponds to 0.002 mg/kg to 2 mg/kg) with $r^2 \geq 0.99$. Matrix-matched standards were used. The recoveries are presented in the table below. Representative Chromatograms and calibration data are presented in the report.

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

Matrix	Analyte	Fortification level (mg/kg) (n=5)	Single values (%)	Mean recovery (%)	RSD (%)	~~Comments~~ Overall recovery	
						Mean (%)	RSD (%)
acetamiprid SRM 223 → 126 (quantification)							
Honey	Acetamiprid	0.01	95, 110, 105, 105, 105, 101	104	4.9	105	4.8
		0.1	113, 105, 114, 107, 108	109	3.6		
		1	98, 102, 103, 105, 101	102	2.5		
acetamiprid SRM 223 → 90 (confirmation)							
Honey	Acetamiprid	0.01	92, 111, 112, 106, 108, 101	105	7.1	103	6.2
		0.1	112, 100, 108, 104, 105	106	4.2		
		1	94, 99, 96, 98, 98	97	2.1		

Table A 22: Characteristics for the analytical method used for validation of acetamiprid residues in honey

	Acetamiprid
Specificity	The HPLC-MS/MS is highly specific for the determination of acetamiprid in bee feeding solution No interference above ≥ 30% LOQ
Calibration (type, number of data points)	Seven-point-linear calibration curve Y= 4.69449e4x r^2= 0.9986
Calibration range	0.1 to 100 µg/L (corresponds to 0.002 mg/kg to 2 mg/kg)
Assessment of matrix effects is presented	No significant matrix effect above 20% could be observed.
Limit of determination/quantification	LOQ: 0.01 mg/kg LOD: 0.003 mg/kg

Conclusion

The method validation for the determination of acetamiprid in honey was fully validated according to the requirements of the SANCO/3029/99 rev 4 guidelines and therefore the method can be fully accepted.

A 2.1.1.1.12 Analytical method 20N08133-01-SSHN

A 2.1.1.1.12.1 Method validation ~~20N08133-01-SSHN~~ 1940050

Comments of zRMS:	The analytical method for determination of acetamiprid in matrix honey was fully validated in study 20R08133-01-RAHN according to guideline SANCO/3029/99 rev.4 at the levels of 0.01 mg/kg (LOQ), 0.1 mg/kg (10 fold LOQ) and 1 mg/kg (100 fold LOQ). Within this study, reduced validations were carried out at the levels of 0.01 mg/kg (LOQ) and 0.1 mg/kg (10 fold LOQ) at each sampling point to verify the validity of the used method. Recovery values obtained by HPLC-MS/MS for acetamiprid at a level of 0.01 mg/kg and 0.1 mg/kg comply with the standard acceptance criteria of SANCO/3029/99 rev.4, which demands that the mean recovery at each fortification level should be in the range of 70 – 110%. In addition, these values also fulfil the criterion of the guideline requirements of the SANCO/3029/99 rev.4 for relative standard deviations (≤ 20%). No significant degradation of acetamiprid during storage at ≤ -18 °C was observed within 9 months for matrix honey. The data presented demonstrate that the method permits the determination of residues of acetamiprid in honey with satisfactory accuracy, precision and repeatability according to guideline SANCO/3029/99 rev.4. The study is acceptable.

Reference: KCP 5.1.2/13

Report	Determination of the Storage Stability of Acetamiprid in Honey for a period of 12 months at ≤ -18 °C, Müller, S., 2020, Study No. 20N08133-01-SSHN (interim report)
Guideline(s):	Yes, SANCO 3029/99 rev. 4
Deviations:	No
GLP:	Yes
Acceptability:	Yes

A. Materials

1. Standards

Test and reference item:	Acetamiprid
CAS No.:	160430-64-8
Batch no.	BCBT9185
Expiry date:	28 February 2022
Standards for calibration	As above
Matrix:	Honey, purchased from a local food store and checked prior usage for its content of acetamiprid

The analytical method for determination of acetamiprid in matrix honey was fully validated in RIFCON GmbH Project No. 1940050 (CIP Phase ID 20R08133-01-RAHN) according to guideline SANCO/3029/99 rev.4 at the levels of 0.01 mg/kg (LOQ), 0.1 mg/kg (10 fold LOQ) and 1 mg/kg (100 fold LOQ). Within this study, reduced validations were carried out at the levels of 0.01 mg/kg (LOQ) and 0.1 mg/kg (10 fold LOQ) at each sampling point to verify the validity of the used method.

B. Sample preparation and processing

Honey samples of 5 g were added to 10 mL of demineralised water. The samples were weighed into 50 mL centrifugation tubes, 10 mL acetonitrile were added and the samples were homogenised for at least 2 min using a vortex mixer.
Thereafter, QuEChERS EN15662 salt-mixture (1 g sodium citrate, 0.5 g sodium hydrogencitrate sequihydrate, 4 g magnesium sulphate, 1 g sodium chloride (Bekolut Citrate-Kit-01) was added, thoroughly shaken and mixed again on a vortex mixer for at least 1 min.
The samples were centrifuged at 4000 min-1 for at least 5 minutes. The supernatant was transferred into a 15 mL centrifuge tube.
An aliquot of 1 mL of the supernatant was transferred into a tube (2 mL) prepared with 25 mg PSA and 150 mg anhydrous magnesia sulphate and 25 mg C18e (Bekolut PSA-Kit-03), shaken on a vortex mixer for 30 s, centrifuged for 5 min (12 000 rpm) and transferred into an autosampler vial (1.8 mL). The final extracts were diluted 1:10 (with ACN) into an autosampler vial (1.8 mL) and used directly for analysis by HPLC-MS/MS and analysed via HPLC-MS/MS. If necessary, the final extracts were diluted.

C. Chromatographic parameters

HPLC- parameters:	Dionex Ultimate 3000
Column:	Phenomenex Luna C18 (150 mm x 2.0 mm 5 µm)
Column temperature:	40°C
Injection volume:	10 µL
Mobile Phase:	A: Water + 1% formic acid B: Acetonitrile
MS/MS parameters:	AB Sciex API 5500 QTRAP
Interface:	ESI
Source polarity:	Positive

Ion source:	Turbo spray
Ion mode:	ESI (positive)
Detection:	223.0 -> 126 (quantifier)
	223.0 -> 90 (qualifier)

Results and discussions

The method used for the determination of residues of acetamiprid in honey was fully validated according to the requirements of the SANCO/3029/99 rev. 4 guidelines. The specific identification of acetamiprid was conducted by HPLC-MS/MS with two transitions monitored. Therefore no confirmatory data need to be provided. The limit of quantification is 0.01 mg/kg and is defined as the lowest fortification level with mean recoveries ranging from 70% to 110% at a relative standard deviation (RSD) of ≤ 20% and blanks not exceeding 30% of the LOQ level. These criteria were fulfilled for the recoveries of the method LOQ for acetamiprid, as well as for the temper LOQ. The detector response for acetamiprid was linear within the range within 0.1 µg/L to 100 µg/L (corresponds to 0.002 mg/kg to 2 mg/kg) with $r^2 \geq 0.995$. Matrix-matched standards were used. The recoveries are presented in the table below. Representative Chromatograms and calibration data are presented in the report.

Table A 23: Recovery results from method validation of acetamiprid using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (n=8)	Mass fragments (m/z)	Single values recovery (%)	Mean recovery (%)	RSD (%)
Honey	Acetamiprid	0.01	223 → 126 Quantifier	110, 105, 105, 105, 101, 91, 90, 91	100	7.9
			223 → 90 Qualifier	111, 112, 106, 108, 101, 94, 93, 88	102	8.9
		0.1	223 → 126 Quantifier	113, 105, 114, 107, 108, 89, 87, 95	102	10.3
			223 → 90 Qualifier	112, 100, 108, 104, 105, 88, 87 ,94	100	9.2

Table A 24: Characteristics for the analytical method used for validation of acetamiprid residues in honey

	Acetamiprid
Specificity	The HPLC-MS/MS is highly specific for the determination of acetamiprid in bee feeding solution No interference above ≥ 30% LOQ
Calibration (type, number of data points)	Seven-point-linear calibration curve SRM 223 → 126, quantification: Y= 4.69449e4 x r^2= 0.9986 SRM 223 → 90, confirmation : Y= 12911.85614 x r^2=0.99913
Calibration range	0.1 to 100 µg/L (corresponds to 0.002 mg/kg to 2 mg/kg)
Assessment of matrix effects is presented	No significant matrix effect above 20% could be observed.
Limit of determination/quantification	LOQ: 0.01 mg/kg LOD: 0.003 mg/kg

Conclusion

The method validation for the determination of acetamiprid in honey was fully validated according to the requirements of the SANCO/3029/99 rev 4 guidelines and therefore the method can be fully accepted.

A 2.1.1.1.13 Analytical method ACI16-010
A 2.1.1.1.13.1 Method validation ACI16-010

Comments of zRMS:	A method of analysis for the determination of acetamiprid in dislodging solution was validated according to the guidance document SANCO/3029/99 rev. 4. All criteria are fulfilled: - blank values do not exceed 30% of the lowest validated concentration,

	- the mean recoveries for each level are in the range 70-110%, - the RSD is < 20% per level. The limit of quantitation (LOQ) was 0.2 µg/L and the limit of detection (LOD) was 0.02 µg/L. The study is acceptable.

Reference:	KCP 5.1.2/14
Report	Foliar dislodgeable residues dissipation on pome fruit in Southern and Northern Europe (Spain, Italy and Czech Republic), 2016, Wilson, A., Study No. ACI16-010
Guideline(s):	Yes, SANCO 3029/99 rev. 4
Deviations:	None
GLP:	Yes
Acceptability:	Yes

A. Materials

1. Standards

Test item:	MCW-2222
Batch no.	611-280413-01
Active substance:	Nominal: 200 g/L Analysed: 205.1 g/L
Expiry date:	May 2016
Reference item:	Acetamiprid
Lot/Batch number:	SZBF098XV
Purity:	99.9 %
CAS No.:	135410-20-7
Expiry date:	April 2020
Standards for calibration	As above
Matrix:	Pome fruit

B. Sample preparation and processing

10 mL of the sample of the dislodge solution were measured into a scintillation vial (20 mL) and fortified respectively. Afterwards 10 mL of a methanol-formic acid (100:0.2, v: v) was added and shaken. An aliquot of the sample was transferred to a suitable vial prior quantitation by liquid chromatography using tandem mass spectrometric detection (LC-MS/MS).

C. Chromatographic parameters

HPLC- parameters

Instrumentation:	Waters Acquity TQD
Column:	C18 (50 x 2.0 mm, 1.7 µm)
Column temperature:	45°C
Injection volume:	20 µL

MS/MS - parameters

Mode:	ESP (electrospray ionisation) positive
Transitions:	223 -> 126 m/z (quantifier) 223 -> 90 m/z (qualifier)

Results and discussions

The method used for the determination of residues of acetamiprid in pome fruit was fully validated according to the requirements of the SANCO/3029/99 rev. 4 guidelines. The specific identification of acetamiprid was conducted by HPLC-MS/MS with two transitions monitored. Therefore no confirmatory data need to be provided. The limit of quantification is 0.2 µg/L and is defined as the lowest fortification level with mean recoveries ranging from 70% to 110% at a relative standard deviation (RSD) of ≤ 20% and blanks not exceeding 30% of the LOQ level. These criteria were fulfilled for the recoveries of the method LOQ for acetamiprid, as well as for the temper LOQ (1000 µg/L). The detector response for acetamiprid was linear within the range from 0.01 ng/L to 1.0 ng/L with $r^2 \geq 0.999$. The recoveries are presented in the table below.

Table A 25: Recovery results from method validation of acetamiprid using the analytical method

Matrix	Analyte	Fortification level (µg/L) (n=5)	Mean recovery (%)	RSD (%)	Comments
Pome fruit	Acetamiprid	0.2	94	3	-
		1000	87	5.5	-

Table A 26: Characteristics for the analytical method used for validation of acetamiprid residues in pome fruit

	Acetamiprid
Specificity	The HPLC-MS/MS with two ions monitored is highly specific for the determination of acetamiprid. No interference above ≥ 30% LOQ
Calibration (type, number of data points)	Nine-point-linear calibration curve y= 38511.8x + 73.2054 r^2= 0.999
Calibration range	0.01 ng/L to 1.0 ng/L
Limit of determination/quantification	LOQ: 0.2 µg/L

Conclusion

The method for the determination of acetamiprid in pome fruit was fully validated according to the requirements of the SANCO/3029/99 rev 4 guidelines and therefore the method can be fully accepted.

A 2.1.1.1.14 Analytical method 141048005 W
A 2.1.1.1.14.1 Method validation 141048005 W

Comments of zRMS:	The purpose of the analytical phase of the study was the verification of the test solution concentrations of the active ingredient. The analytical method was validated according to the guidance document SANCO/3029/99 rev. 4. All criteria are fulfilled: - blank values do not exceed 30% of the lowest validated concentration, - the mean recoveries for each level are in the range 70-110%, - the RSD is < 20% per level. The LOQ of acetamiprid was defined in the context of this study as the lowest successfully validated fortification level, i.e. 0.185 mg/L. The study is acceptable.

Reference:	KCP 5.1.2/15
Report	Acute toxicity of MCW-2222 to the rainbow trout *Oncorhynchus mykiss* in a 96-hour static test, Juckeland, D., 2014, Study No. 141048005 W.
Guideline(s):	Yes, SANCO 3029/99 rev. 4
Deviations:	None
GLP:	Yes

Acceptability: Yes

A. Materials

1. Standards

Test item:	MCW-2222
Batch no.	611-280413-01
Active substance:	Nominal: 200 g/L Analysed: 202.7 g/L
Expiry date:	April 2015
Reference item:	Acetamiprid
Lot/Batch number:	772827
Purity:	99.9 %
CAS No.:	135410-20-7
Expiry date:	August 2017
Standards for calibration	As above
Matrix:	Water

B. Sample preparation and processing

The samples were allowed to thaw at room temperature and were analysed without any sample preparation.

C. Chromatographic parameters

HPLC- parameters

Instrumentation:	Shimadzu LC-10 HPLC
Column:	2.6 µm C18, 100 x 2.1 mm
Mobile phase:	25: 75 methanol: water (v/v)
Flow rate:	0.4 mL/min
Injection volume:	10 µL
Detection:	UV at 245 nm

Results and discussions

The method used for the determination of residues of acetamiprid in water was fully validated according to the requirements of the SANCO/3029/99 rev. 4 guidelines. The specific identification of acetamiprid was conducted by HPLC-UV. The limit of quantification is 0.185 mg/L and is defined as the lowest fortification level with mean recoveries ranging from 70% to 110% at a relative standard deviation (RSD) of ≤ 20% and blanks not exceeding 30% of the LOQ level. These criteria were fulfilled for the recoveries of the method LOQ for acetamiprid, as well as for the temper LOQ (92.65 mg/L). The detector response for acetamiprid was linear within the range from 0.149 to 114.5 mg/L with $r^2 \geq 0.999$. The recoveries are presented in the table below.

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

Matrix	Analyte	Fortification level (mg/L) (n=5)	Mean recovery (%)	RSD (%)	Comments
Water	Acetamiprid	0.185	92.3	1.7	-
		92.65	93.2	0.6	-

Table A 28: Characteristics for the analytical method used for validation of acetamiprid residues in water

	Acetamiprid
Specificity	The HPLC-UV method is specific for the determination of acetamiprid. No interference above ≥ 30% LOQ

	Acetamiprid
Calibration (type, number of data points)	Nine-point-linear calibration curve y= 148054x + 3544.07 r^2= 0.999
Calibration range	0.149 to 114.5 mg/L
Limit of determination/quantification	LOQ: 0.185 mg/L

Conclusion
The method for the determination of acetamiprid in water was fully validated according to the requirements of the SANCO/3029/99 rev 4 guidelines and therefore the method can be fully accepted.

A 2.1.1.1.15 Analytical method 141048006 W
A 2.1.1.1.15.1 Method validation 141048006 W

Comments of zRMS:	The purpose of the analytical phase of the study was the verification of the test solution concentrations of the active ingredient. The analytical method was validated according to the guidance document SANCO/3029/99 rev. 4. All criteria are fulfilled: - blank values do not exceed 30% of the lowest validated concentration, - the mean recoveries for each level are in the range 70-110%, - the RSD is < 20% per level. The LOQ of acetamiprid was defined in the context of this study as the lowest successfully validated fortification level, i.e. 0.367 mg/L. The study is acceptable.

Reference:	KCP 5.1.2/16
Report	Acute toxicity of MCW-2222to *Daphnia magna* in a 48-hour static test, Juckeland, D., 2014, Study No. 141048006 W.
Guideline(s):	Yes, SANCO 3029/99 rev. 4
Deviations:	None
GLP:	Yes
Acceptability:	Yes

A. Materials

1. Standards

Test item:	MCW-2222
Batch no.	611-280413-01
Active substance:	Nominal: 200 g/L Analysed: 202.7 g/L
Expiry date:	April 2015
Reference item:	Acetamiprid
Lot/Batch number:	772827
Purity:	99.9 %
CAS No.:	135410-20-7
Expiry date:	August 2017
Standards for calibration	As above
Matrix:	Water

B. Sample preparation and processing

The samples were allowed to thaw at room temperature and were measured without any sample preparation.

C. Chromatographic parameters

HPLC- parameters:	Shimadzu LC-10 HPLC
Column:	2.6 µm C18, 100 x 2.1 mm
Mobile phase:	25: 75 methanol: water (v/v)
Flow rate:	0.4 mL/min
Injection volume:	10 µL
Detection:	UV at 245 nm

Results and discussions

The method used for the determination of residues of acetamiprid in water was fully validated according to the requirements of the SANCO/3029/99 rev. 4 guidelines. The specific identification of acetamiprid was conducted by HPLC-UV. The limit of quantification is 0.367 mg/L and is defined as the lowest fortification level with mean recoveries ranging from 70% to 110% at a relative standard deviation (RSD) of ≤ 20% and blanks not exceeding 30% of the LOQ level. These criteria were fulfilled for the recoveries of the method LOQ for acetamiprid, as well as for the temper LOQ (91.740 mg/L). The detector response for acetamiprid was linear within the range from 0.149 to 114.5 mg/L with $r^2 \geq 0.999$. The recoveries are presented in the table below.

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

Matrix	Analyte	Fortification level (mg/L) (n=5)	Mean recovery (%)	RSD (%)	Comments
Water	Acetamiprid	0.367	98.9	1.1	-
		91.740	95.1	0.2	-

Table A 30: Characteristics for the analytical method used for validation of acetamiprid residues in water

	Acetamiprid
Specificity	The HPLC-UV method is specific for the determination of acetamiprid. No interference above ≥ 30% LOQ
Calibration (type, number of data points)	Nine-point linear calibration curve y= 148054x + 3544.07 r^2= 0.999
Calibration range	0.149 to 114.5 mg/L
Assessment of matrix effects is presented	N/A
Limit of determination/quantification	LOQ: 0.367 mg/L

Conclusion

The method for the determination of acetamiprid in water was fully validated according to the requirements of the SANCO/3029/99 rev 4 guidelines and therefore the method can be fully accepted.

A 2.1.1.1.16 Analytical method 141048057 W

A 2.1.1.1.16.1 Method validation 141048057 W

Comments of zRMS:	The purpose of the analytical phase of the study was the verification of the test solution concentrations of the active ingredient. The analytical method was validated according to the guidance document SANCO/3029/99 rev. 4. All criteria are fulfilled: - blank values do not exceed 30% of the lowest validated concentration, - the mean recoveries for each level are in the range 70-110%, - the RSD is < 20% per level. The LOQ of acetamiprid was defined in the context of this study as the lowest successfully validated fortification level, i.e. 0.47 µg/L. The study is acceptable.

Reference:	KCP 5.1.2/17
Report	Acute toxicity of MCW-2222 to Chironomus riparius in a 48-hour static test, Juckeland, D., 2015, Study No. 141048057 W.
Guideline(s):	Yes, SANCO 3029/99 rev. 4
Deviations:	None
GLP:	Yes
Acceptability:	Yes

A. Materials

1. Standards

Test item:	MCW-2222
Batch no.	611-280413-01
Active substance:	Nominal: 200 g/L Analysed: 202.7 g/L
Expiry date:	April 2015
Reference item:	Acetamiprid
Lot/Batch number:	772827
Purity:	99.9 %
CAS No.:	135410-20-7
Expiry date:	August 2017
Standards for calibration	As above
Matrix:	Water

B. Sample preparation and processing

The samples were allowed to thaw at room temperature and were analyses without any sample preparation.

C. Chromatographic parameters

HPLC- parameters:	Agilent 1260 HPLC
Column:	2.6 µm C18, 100 x 2.1 mm
Flow rate:	0.420 mL/min
MS/MS	Agilent 6460
Ionisation source:	ESI
Ionisation mode:	MRM
Transitions:	223.1 => 126 m/z (quantifier) 223.1 => 90 m/z (qualifier)

Results and discussions

The method used for the determination of residues of acetamiprid in water was fully validated according to the requirements of the SANCO/3029/99 rev. 4 guidelines. The specific identification of acetamiprid was conducted by HPLC-MS/MS. The limit of quantification is 0.47 µg/L and is defined as the lowest fortification level with mean recoveries ranging from 70% to 110% at a relative standard deviation (RSD) of ≤ 20% and blanks not exceeding 30% of the LOQ level. These criteria were fulfilled for the recoveries of the method LOQ for acetamiprid, as well as for the temper LOQ . The detector response was quadratic due to a better fit than the linear curve. The correlation coefficient is ≥ 0.999. The recoveries are presented in the table below.

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

Matrix	Analyte	Fortification level (µg/L) (n=5)	Mean recovery (%)	RSD (%)	Comments
Water	Acetamiprid	0.470	86.2	1.2	-

Matrix	Analyte	Fortification level (µg/L) (n=5)	Mean recovery (%)	RSD (%)	Comments
		17.40	101.7	1.2	-

Table A 32: Characteristics for the analytical method used for validation of acetamiprid residues in water

	Acetamiprid
Specificity	The HPLC-MS//MS method is highly specific for the determination of acetamiprid. No interference above ≥ 30% LOQ
Calibration (type, number of data points)	Nine-point calibration curve y= 50.455967x + 17062.645969 r^2= 0.999
Calibration range	0.364 to 22.24 µg/L
Limit of determination/quantification	LOQ: 0.47 µg/L

Conclusion
The method for the determination of acetamiprid in water was fully validated according to the requirements of the SANCO/3029/99 rev 4 guidelines and therefore the method can be fully accepted.

A 2.1.1.1.17 Analytical method 141048007 W
A 2.1.1.1.17.1 Method validation 141048007 W

Comments of zRMS:	The purpose of the analytical phase of the study was the verification of the test solution concentrations of the active ingredient. The analytical method was validated according to the guidance document SANCO/3029/99 rev. 4. All criteria are fulfilled: - blank values do not exceed 30% of the lowest validated concentration, - the mean recoveries for each level are in the range 70-110%, - the RSD is < 20% per level. The LOQ of acetamiprid was defined in the context of this study as the lowest successfully validated fortification level, i.e. 0.344 mg/L. The study is acceptable.

Reference:	KCP 5.1.2/18
Report	Effects of MCW-2222 on *Desmodesmus subspicatus* in an algal growth inhibition test, Juckeland, D., 2014, Study No. 141048007 W.
Guideline(s):	Yes, SANCO 3029/99 rev. 4
Deviations:	None
GLP:	Yes
Acceptability:	Yes

A. Materials

1. Standards

Test item:	MCW-2222
Batch no.	611-280413-01
Active substance:	Nominal: 200 g/L Analysed: 202.7 g/L
Expiry date:	April 2015
Reference item:	Acetamiprid
Lot/Batch number:	772827
Purity:	99.9 %

CAS No.: 135410-20-7
Expiry date: August 2017

Standards for calibration: As above

Matrix: Water

B. Sample preparation and processing

The samples were allowed to thaw at room temperature and were measured without any sample preparation.

C. Chromatographic parameters

HPLC- parameters

Instrumentation:	Shimadzu LC-10 HPLC
Column:	2.6 µm C18, 100 x 2.1 mm
Mobile phase:	25: 75 methanol: water (v/v)
Flow rate:	0.4 mL/min
Injection volume:	10 µL
Detection:	UV at 245 nm

Results and discussions

The method used for the determination of residues of acetamiprid in water was fully validated according to the requirements of the SANCO/3029/99 rev. 4 guidelines. The specific identification of acetamiprid was conducted by HPLC-UV. The limit of quantification is 0.344 mg/L and is defined as the lowest fortification level with mean recoveries ranging from 70% to 110% at a relative standard deviation (RSD) of ≤ 20% and blanks not exceeding 30% of the LOQ level. These criteria were fulfilled for the recoveries of the method LOQ for acetamiprid, as well as for the temper LOQ (55.49 mg/L). The detector response for acetamiprid was linear within the range from 0.172 to 80.14 mg/L with $r^2 \geq 0.999$. The recoveries are presented in the table below.

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

Matrix	Analyte	Fortification level (mg/L) (n=5)	Mean recovery (%)	RSD (%)	Comments
Water	Acetamiprid	0.344	96.4	0.7	-
		55.49	100.1	0.1	-

Table A 34: Characteristics for the analytical method used for validation of acetamiprid residues in water

	Acetamiprid
Specificity	The HPLC-UV method is specific for the determination of acetamiprid. No interference above ≥ 30% LOQ
Calibration (type, number of data points)	Ten-point linear calibration curve y= 146604x + 3503.21 r^2= 0.999
Calibration range	0.172 to 80.14 mg/L
Assessment of matrix effects is presented	N/A
Limit of determination/quantification	LOQ: ~~0.367~~ 0.344 mg/L

Conclusion

The method for the determination of acetamiprid in water was fully validated according to the requirements of the SANCO/3029/99 rev 4 guidelines and therefore the method can be fully accepted.

A 2.1.1.1.18 Analytical method 215-2014

A 2.1.1.1.18.1 Method validation 215-2014

Comments of zRMS:	The analytical method was successfully validated according to the guidance document SANCO/3029/99 rev. 4 for bee bread, flowers, nectar and pollen by10 spiked samples: 5 recovery experiments fortified at the LOQ level and 5 recovery experiments fortified at 10 times the LOQ level. 2 control samples and a reagent blank were prepared. Honey and nectar are considered as similar matrices. So no validation on honey was realized. The LOQ of acetamiprid and acetamiprid-N-desmethyl is 0.01 mg/kg. The study is acceptable.

Reference:	KCP 5.1.2/19
Report	Field Study to Evaluate Potential Side Effects of the product MCW-2222 (acetamiprid 200 g/L) on Brood Development, Foraging Activity, Mortality and Behaviour of Adult Honeybees *Apis mellifera L*. (Hymenoptera: Apidae) Following Application after Bee-Flight on *Phacelia tanacetifolia*, Molitor, C., 2014, Study No. 215-2014 + Amendment 1
Guideline(s):	Yes, SANCO 3029/99 rev. 4
Deviations:	None
GLP:	Yes
Acceptability:	Yes

A. Materials

1. Standards

Test item:	MCW-2222
Batch no.	93191024
Active substance:	Nominal: 200 g/L Analysed: 198 g/L
Expiry date:	October 2015
Reference item:	N/A
Standards for calibration	As above
Matrix:	Bee bread, flowers, nectar, pollen

B. Sample preparation and processing

For bee bread, flowers and nectar, residues of acetamiprid and acetamiprid-*N*-desmethyl were extracted from samples in frozen conditions by agitation in acetonitrile and ultra-pure water. Then the extracts were purified by dispersive solid phase extraction (SPE). For pollen residues of acetamiprid and acetamiprid-*N*-desmethyl were extracted from pollen with ethyl acetate using Dionex ASE 300 automatic extractor. The quantification for all matrices was performed by liquid chromatography with tandem mass spectrometry detection (LC-MS/MS).

C. Chromatographic parameters

HPLC- parameters

Column:	C18 (1000 x 3.0 mm, 2.5 µm)
Column temperature:	60°C
Injection volume:	20 µL
MS/MS - parameters	API 5500 Qtrap
Ion source :	ESP (electrospray ionisation) positive
Ion mode:	MRM
Transitions:	223 -> 126 m/z (quantifier) 223 -> 73 m/z (qualifier)

Results and discussions

The method used for the determination of residues of acetamiprid in honey nectar, pollen and bee bread was fully validated according to the requirements of the SANCO/3029/99 rev. 4 guidelines. The specific identification of acetamiprid was conducted by HPLC-MS/MS with two transitions monitored. Therefore no confirmatory data need to be provided. The limit of quantification is 0.01 mg/kg and is defined as the lowest fortification level with mean recoveries ranging from 70% to 110% at a relative standard deviation (RSD) of ≤ 20% and blanks not exceeding 30% of the LOQ level. These criteria were fulfilled for the recoveries of the method LOQ for acetamiprid, as well as for the temper LOQ (0.1 mg/kg). The detector response for acetamiprid was linear within the range from 1.5 to 30 µg/L for honey, 1.5 to 100 µg/L for nectar and 3 to 100 µg/L for bee bread with $r^2 \geq 0.999$. The recoveries are presented in the table below.

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

Matrix	Analyte	Fortification level (mg/kg) (n=5)	Mean recovery (%)	RSD (%)	Comments
Bee bread	Acetamiprid	0.01	90	7	-
		0.1	101	2	-
	Acetamiprid-*N*-desmethyl	0.01	93	4	-
		0.1	89	2	-
Flowers	Acetamiprid	0.01	86	7	-
		0.1	89	3	-
	Acetamiprid-*N*-desmethyl	0.01	88	3	-
		0.1	95	4	-
Nectar	Acetamiprid	0.01	76	5	-
		0.1	86	3	-
	Acetamiprid-*N*-desmethyl	0.01	86	3	-
		0.1	87	4	-
Pollen	Acetamiprid	0.01	93	6	-
		0.1	93	6	-
	Acetamiprid-*N*-desmethyl	0.01	90	5	-
		0.1	86	5	-

Table A 36: Characteristics for the analytical method used for validation of acetamiprid residues in pollen, nectar and bee bread

	Acetamiprid
Specificity	The HPLC-MS/MS with two ions monitored is highly specific for the determination of acetamiprid. No interference above ≥ 30% LOQ
Calibration (type, number of data points)	five-point linear calibration curve y= 6129.38x + 424.97 (flowers) r^2= 0.99 y= 4054.24x -22.48 (flowers N-desmethyl) r2= 0.99 y= 33027.95x + 2595.56 (pollen) r^2= 1.00 y= 17883.10x + 6454.25 (pollen N-desmethyl) r^2= 1.00 y= 7472.18x + 5680.45 (nectar) r^2= 0.99 y= 5227.80x + 289.79 (nectar N-desmethyl) r^2= 0.99 y= 5152.76x + 1764.30 (honey) r^2= 0.99 y= 3303.73x – 642.70 (honey n-desmethyl) r^2= 0.99 y= 2111.78x + 2403.75 (bee bread) r^2= 0.99 y= 1667.49x + 1141,08 (bee bread N-desmethyl) r^2= 0.99
Calibration range	0.3 to 10 µg/L for flowers 1.5 to 50 µg/L for honey and nectar 3.0 to 100 µg/L for bee bread and pollen

	Acetamiprid
Limit of determination/quantification	LOQ: 0.01 mg/kg (for all matrices)

Conclusion
The method validation for the determination of acetamiprid in pollen, nectar and bee bread was fully validated according to the requirements of the SANCO/3029/99 rev 4 guidelines and therefore the method can be fully accepted.

A 2.1.1.1.19 Analytical method 230-2015
A 2.1.1.1.19.1 Method validation 230-2015

Comments of zRMS:	The objective of the analytical phase was to determine the residues of acetamiprid and its metabolite acetamiprid-N-desmethyl in pollen, honey, nectar, flower, bee bread and wax. For wax, the analytical method was fully validated within the analytical phase by 10 spiked samples: 5 recovery experiments fortified at the LOQ level and 5 recovery experiments fortified at 10 times the LOQ level. 2 control samples and a reagent blank were prepared. For pollen, bee bread, flowers and nectar, the analytical method used was previously validated during another analytical phase in a parallel study number 215-2014, S. Lefresne, 2014 performed by 10 spiked samples: 5 recovery experiments fortified at the LOQ level and 5 recovery experiments fortified at 10 times the LOQ level. 2 control samples and a reagent blank were prepared. Honey and nectar were considered as similar matrices. So no validation on honey were realized. The LOQ: 0.01 mg/kg for each reference item and for each specimen. The method was successfully validated according to the guidance document SANCO/3029/99 rev. 4. The study is acceptable.

Reference:	KCP 5.1.2/20
Report	Field study to evaluate potential side effects of MCW-2222 on brood development, foraging activity, mortality and behaviour of adult honeybees (*apis mellifera*) on oilseed rape, Molitor, C., 2014, Study No. 230-2015
Guideline(s):	Yes, SANCO 3029/99 rev. 4
Deviations:	None
GLP:	Yes
Acceptability:	Yes

A. Materials

1. Standards

Reference item 1 :	Acetamiprid
Batch no.	20202
Purity:	98.1 ± 0.5%
Expiry date:	February 2016
Reference item 2:	Acetamiprid-*N*-desmethyl
Batch No.:	SZBE066XV
Purity:	99.8 %
Expiry date:	March 2017
Standards for calibration	As above
Matrix:	Bee bread, flowers, nectar, pollen and wax

B. Sample preparation and processing

For bee bread, flowers and nectar and wax residues, acetamiprid and acetamiprid-*N*-desmethyl were extracted from samples in frozen conditions by agitation in acetonitrile and ultra-pure water. Then the extracts were purified by dispersive solid phase extraction (SPE). For pollen, residues of acetamiprid and acetamiprid-N-desmethyl were extracted with ethyl acetate using Dionex ASE 300 automatic extractor. The quantification for all matrices was performed by liquid chromatography with tandem mass spectrometry detection (LC-MS/MS).

C. Chromatographic parameters

HPLC- parameters:	
Column:	C18 (1000 x 3.0 mm, 2.5 µm)
Column temperature:	60°C
Injection volume:	20 µL
MS/MS - parameters	API 5500 Qtrap
Ion source :	ESP (electrospray ionisation) positive
Ion mode:	MRM
Transitions:	223 -> 126 m/z (quantifier)
	223 -> 73 m/z (qualifier)

Results and discussions

The method used for the determination of residues of acetamiprid in honey nectar, pollen, bee bread and wax was fully validated according to the requirements of the SANCO/3029/99 rev. 4 guidelines. The specific identification of acetamiprid was conducted by HPLC-MS/MS with two transitions monitored. Therefore no confirmatory data need to be provided. The limit of quantification is 0.01 mg/kg and is defined as the lowest fortification level with mean recoveries ranging from 70% to 110% at a relative standard deviation (RSD) of ≤ 20% and blanks not exceeding 30% of the LOQ level. These criteria were fulfilled for the recoveries of the method LOQ for acetamiprid, as well as for the temper LOQ (0.1 mg/kg). The detector response for acetamiprid was linear within the range from 1.5 to 30 µg/L for honey, 1.5 to 100 µg/L for nectar and 3 to 100 µg/L for bee bread and additionally 0.3 to 8 µg/L for wax with $r^2 \geq 0.99$. The recoveries are presented in the table below.

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

Matrix	Analyte	Fortification level (mg/kg) (n=5)	Mean recovery (%)	RSD (%)	Comments
Bee bread	Acetamiprid	0.01	90	7	-
		0.1	101	2	-
	Acetamiprid-*N*-desmethyl	0.01	93	4	-
		0.1	89	2	-
Flowers	Acetamiprid	0.01	86	7	-
		0.1	89	3	-
	Acetamiprid-*N*-desmethyl	0.01	88	3	--
		0.1	95	4	-
Nectar	Acetamiprid	0.01	76	5	-
		0.1	86	3	-
	Acetamiprid-*N*-desmethyl	0.01	86	3	-
		0.1	87	4	-
Pollen	Acetamiprid	0.01	93	6	-
		0.1	93	6	-
	Acetamiprid-	0.01	90	5	-

Matrix	Analyte	Fortification level (mg/kg) (n=5)	Mean recovery (%)	RSD (%)	Comments
	N-desmethyl	0.1	86	5	-
Wax	Acetamiprid	0.01	93	6	-
		0.1	94	7	-
	Acetamiprid-*N*-desmethyl	0.01	90	~~90~~ 7	-
		0.1	89	~~89~~ 8	-

Table A 38: Characteristics for the analytical method used for validation of acetamiprid residues in pollen, nectar, bee bread and wax

	Acetamiprid
Specificity	The HPLC-MS/MS with two ions monitored is highly specific for the determination of acetamiprid. No interference above ≥ 30% LOQ
Calibration (type, number of data points)	five-point linear calibration curve y= 127675.05x – 1254.20 (flowers) r^2= 0.99 y= 72462.78x + 2728.20 (flowers N-desmethyl) r2= 0.99 y= 208107.32x + 80131.31 (pollen) r^2= 0. 99 y= 145592.01x +37369.42 (pollen N-desmethyl) r^2= 0.99 y= 17552.64 +31898.13 (nectar) r^2= 0.99 y= 101699.51x + 9307.13 (nectar N-desmethyl) r^2= 0.99 y= 155731.04x -21445.38 (honey) r^2= 0.99 y= 90882.09 – 12532.93 (honey n-desmethyl) r^2= 0.99 y= 58150.61x + 5672.63 (bee bread) r^2= 0.99 y= 31660.29x + 12219.80 (bee bread N-desmethyl) r^2= 0.99
Calibration range	0.3 µg/L to 10 µg/L for flowers 3.0 to 100 µg/L for pollen 1.5 to 50 µg/L for nectar 1.5 to 30 µg/L for honey 3.0 to 100 µg/L for bee bread
Limit of determination/quantification	LOQ: 0.01 mg/kg

Conclusion

The method validation for the determination of acetamiprid in pollen, nectar, bee bread and wax was fully validated according to the requirements of the SANCO/3029/99 rev 4 guidelines and therefore the method can be fully accepted.

A 2.1.1.1.20 Analytical method 307SRES15C01

A 2.1.1.1.20.1 Method validation 307SRES15C02 & 215-2014

Comments of zRMS:	The objective of the analytical phase was to determine the residues of acetamiprid in the spray solutions, pollen, fresh nectar and larvae. For nectar and larvae, the analytical method used was previously validated during another analytical phase in a parallel study number 307SRES15C02, S. Lefresne, 2015 performed by 10 spiked samples: 5 recovery experiments fortified at the LOQ level and 5 recovery experiments fortified at 10 times the LOQ level. 2 control samples and a reagent blank were prepared. For pollen, the analytical method used was previously validated during another analytical phase in a parallel study number 215-2014, S. Lefresne, 2014 performed by 10 spiked samples: 5 recovery experiments fortified at the LOQ level and 5 recovery experiments fortified at 10 times the LOQ level. 2 control samples and a reagent blank were prepared. The LOQ: 0.01 mg/kg. The method was successfully validated according to the guidance document

	SANCO/3029/99 rev. 4 and used for the analytical determination of acetamiprid in pollen, fresh nectar and larvae. The study is acceptable.

Reference:	KCP 5.1.2/21
Report	Effects and determination of residues of acetamiprid 200 SL on the Honeybee (*Apis mellifera L*) Brood in apple, under field conditions, in Italy 2015, Aucejo, S., 2015, Study No. 307SRES15C01
Guideline(s):	Yes, SANCO 3029/99 rev. 4
Deviations:	None
GLP:	Yes
Acceptability:	Yes

A. Materials

1. Standards

Test item :	MCW – 2222
Batch no.	659-030314-01
Active content:	a.i. nominal: 200 g/L a.i. analysed 199.2 g/L
Expiry date:	March 2016
Reference item :	Acetamiprid
Batch No.:	20202
Purity:	98.1 ± 0.5%
Expiry date:	February 2016
Standards for calibration	As above
Matrix:	Nectar, larvae and pollen

B. Sample preparation and processing

For nectar and larvae residues, acetamiprid were extracted from samples in frozen conditions by agitation in acetonitrile and ultra-pure water. Then the extracts were purified by dispersive solid phase extraction (SPE). For pollen residues of acetamiprid were extracted from pollen with ethyl acetate using Dionex ASE 300 automatic extractor. For spray solution the samples were diluted before quantified. The quantification for all matrices was performed by liquid chromatography with tandem mass spectrometry detection (LC-MS/MS).

C. Chromatographic parameters

HPLC- parameters:

Column:	C18 (1000 x 3.0 mm, 2.5 µm PD)
Column temperature:	60°C
Injection volume:	20 µL
MS/MS - parameters	PE-Sciex API 4000 Qtrap
Ion source :	ESI (electrospray ionisation) positive
Ion mode:	MRM
Transitions:	223 -> 126 m/z (quantifier) 223 -> 90 m/z (qualifier)

Results and discussions

The method used for the determination of residues of acetamiprid on pollen was previous validated in Molitor, C., 2014, Study No. 215-2014, according to the requirements of the SANCO/3029/99 rev. 4 guidelines. Therefore, only procedural recoveries were performed.

The method used for the determination of residues of acetamiprid in nectar and larvae was also previously validated in Aucejo, S. 2015, Study No. 307SRES15C02 according to the requirements of the SANCO/3029/99 rev. 4 guidelines. Therefore, only procedural recoveries were performed.

The specific identification of acetamiprid was conducted by HPLC-MS/MS with two transitions monitored. Therefore no confirmatory data need to be provided. The limit of quantification is 0.01 mg/kg and is defined in the main validations as the lowest fortification level with mean recoveries ranging from 70% to 110% at a relative standard deviation (RSD) of ≤ 20% and blanks not exceeding 30% of the LOQ level. These criteria were fulfilled for the procedural recoveries of the method LOQ for acetamiprid, as well as for the temper LOQs. For spray solutions, no recovery rates were performed as the analyses consisted only of simple dilutions. The detector response for acetamiprid was linear within the range from 0.3 to 10 µg/L for nectar and larvae, 0.3 to 10 µg/L for spray solution and 3 to 100 µg/L for pollen with $r^2 \geq 0.99$. The procedural recoveries are presented in the table below.

Table A 39: Procedural recoveries results from method validation of acetamiprid using the analytical method

Matrix	Analyte	Fortification level (mg/kg)	Mean recovery (%)	RSD (%)	Comments
Nectar	Acetamiprid	0.01 (n=3)	90	12	-
		0.5 (n=1)	92	-	-
Larvae	Acetamiprid	0.01 (n=1)	76	-	-
		-	-	-	-
Pollen	Acetamiprid	0.01 (n=3)	70	6.3	-
		2.0 (n=1)	77	-	-

Table A 40: Characteristics for the analytical method used for validation of acetamiprid residues in pollen, nectar and larvae

	Acetamiprid
Specificity	The HPLC-MS/MS with two ions monitored is highly specific for the determination of acetamiprid. No interference above ≥ 30% LOQ
Calibration (type, number of data points)	seven-point linear calibration curve Nectar: y= 7724.59x + 1144.77 r^2= 0.999 Pollen: y= 14093.28x + 24266.53 r^2= 0.992 Larvae: y= 8860.75x + 4244.53 r^2= 0.999
Calibration range	0.3 to 10 µg/L for nectar and larvae, 0.3 to 10 µg/L for spray solution and 3 to 100 µg/L for pollen
Assessment of matrix effects is presented	N/A
Limit of determination/quantification	LOQ: 0.01 mg/kg

Conclusion

The method validation for the determination of acetamiprid in pollen, nectar, larvae and spray solution was fully validated according to the requirements of the SANCO/3029/99 rev 4 guidelines in studies No. 307SRES15C02 & 215-2014. The procedural recoveries of the method 307SRES15C01 prove to be within the requirements of the guidelines and therefore the method can be fully accepted.

A 2.1.1.1.21 Analytical method 141048078B

A 2.1.1.1.21.1 Method validation 141048078B

Comments of zRMS:	The method was successfully validated according to the guidance document SANCO/3029/99 rev. 4 and used for the analytical determination of acetamiprid in sugar solution. The LOQ of acetamiprid was 272.1 mg/L. The method is acceptable.

Reference:	KCP 5.1.2/22
Report	Chronic toxicity of MCW-2222 to the honeybee larvae Apis mellifera L. under laboratory conditions (in vitro) , Kleebaum K., 2015, Study No. 141048078 B
Guideline(s):	Yes, SANCO 3029/99 rev. 4
Deviations:	None
GLP:	Yes
Acceptability:	Yes

A. Materials

1. Standards

Test item :	MCW – 2222
Batch no.	611-280413-01
Active content:	a.i. nominal: 200 g/L a.i. analysed 202.7 g/L
Expiry date:	April 2015
Reference item :	Acetamiprid
Batch No.:	772827
Purity:	99.9
Expiry date:	August 2017
Standards for calibration	As above
Matrix:	Sugar solution (bee feed) (18% (w/v) glucose, 18% (w/v) fructose, 4% (w/v) yeast)

B. Sample preparation and processing

The samples of the biological phase of the study were allowed to reach room temperature and were analysed after dilution with factor 50 with the dilution medium containing a water: methanol mixture (50/50/v/v). The control samples were analysed without any further dilution. All samples were analysed with HPLC-DAD.

C. Chromatographic parameters

HPLC- parameters:

Column:	Phenomenex Kinetex C18 (100 x 2.1mm, 2.6 µm PD)
Flow rate :	0.4 mL/min
Gradient:	Isocratic
Mobile phase	A: water with 0.1% (v/v) phosphoric acid (85%) B: Acetonitrile with 0.1 % (v/v) phosphoric acid (85%)
Detection:	UV (DAD)

Results and discussions

The method used for the determination of residues of acetamiprid in sugar bee feeding solution was fully validated according to the requirements of the SANCO/3029/99 rev. 4 guidelines. The specific

identification of acetamiprid was conducted by HPLC-DAD. Therefore no confirmatory data need to be provided. The limit of quantification is 272.1 mg/L and is defined as the lowest successfully validated fortification level with mean recoveries ranging from 70% to 110% at a relative standard deviation (RSD) of ≤ 20% and blanks not exceeding 30% of the LOQ level. These criteria were fulfilled of the method LOQ for acetamiprid, as well as for the temper LOQs. The detector response for acetamiprid was linear within the range from 0.3 to 10 µg/L for nectar and larvae, 4.237to 12.84 mg/L with $r^2 \geq 0.99$. The recoveries are presented in the table below.

Table A 41: Recoveries results from method validation of acetamiprid using the analytical method

Matrix	Analyte	Fortification level (mg/L) with dilution factor 50	Mean recovery (%)	RSD (%)	Comments
Sugar solution	Acetamiprid	5.442	91	0.2	-
		10.76	95	0.2	-

Table A 42: Characteristics for the analytical method used for validation of acetamiprid residues in sugar solution

	Acetamiprid
Specificity	The HPLC-DAD is specific for the determination of acetamiprid. No interference above ≥ 30% LOQ
Calibration (type, number of data points)	Five-point linear calibration curve Sugar solution: y= 141457x-4576.44 r^2= 0.9996
Calibration range	4.237 to 12.84 g/L
Limit of determination/quantification	LOQ: 272.1 mg/L (5.442 mg/L with dilution factor)

Conclusion
The method validation for the determination of acetamiprid in sugar solution was fully validated according to the requirements of the SANCO/3029/99 rev 4 guidelines, therefore the method can be fully accepted.

A 2.1.1.1.22 Analytical method 141048002 W
A 2.1.1.1.22.1 Method validation 141048002 W

Comments of zRMS:	The method was successfully validated according to the guidance document SANCO/3029/99 rev. 4 and used for the analytical determination of acetamiprid in water. The LOQ of acetamiprid was 130 mg/L. The method is acceptable.

Reference:	KCP 5.1.2/23
Report	Terrestrial plant test with MCW-2222: Vegetative vigour test, Friedrich, S. 2014, Study No. 141048002 W
Guideline(s):	Yes, SANCO 3029/99 rev. 4
Deviations:	None
GLP:	Yes
Acceptability:	Yes

A. Materials
1. Standards

Test item:	MCW-2222
Batch no.	611-280413-01
Active substance:	Nominal: 200 g/L

	Analysed: 202.7 g/L
Expiry date:	April 2015
Reference item:	Acetamiprid
Lot/Batch number:	772827
Purity:	99.9 %
CAS No.:	135410-20-7
Expiry date:	August 2017
Standards for calibration	As above
Matrix:	Aqueous solution

B. Sample preparation and processing

Directly after preparation of the test solutions of the biological test, aliquots were transferred into glass containers. Before analysis the samples were homogenized by shaking. The treated sample was diluted 1/20 with water. From the samples, approximately 1 mL was pipetted into samples vials and measure immediately after fortification. The samples were analysed by HPLC with UV detection.

C. Chromatographic parameters

HPLC- parameters:	Shimadzu LC-10 HPLC
Column:	2.6 µm C18, 100 x 2.1 mm
Flow rate:	0.4 mL/min
Injection volume:	5 µL
Detection:	UV at 245 nm

Results and discussions

The method used for the determination of residues of acetamiprid in water was fully validated according to the requirements of the SANCO/3029/99 rev. 4 guidelines. The specific identification of acetamiprid was conducted by HPLC-UV. The limit of quantification is 130 mg/L (6.520 mg/L diluted by factor 20) and is defined as the lowest fortification level with mean recoveries ranging from 70% to 110% at a relative standard deviation (RSD) of ≤ 20% and blanks not exceeding 30% of the LOQ level. These criteria were fulfilled for the recoveries of the method LOQ for acetamiprid, as well as for the temper LOQ of 1304 mg/L (65.20 mg/L diluted by factor 20). The detector response for acetamiprid was linear within the range of 4.890 to 80.78 mg/L $r^2 \geq 0.999$. The recoveries are presented in the table below.

Table A 43: Recovery results from method validation of acetamiprid using the analytical method

Matrix	Analyte	Fortification level (mg/L) (n=5)	Mean recovery (%)	RSD (%)	Comments
Water	Acetamiprid	130.4	99.3	0.4	-
		1340	99.9	0.4	-

Table A 44: Characteristics for the analytical method used for validation of acetamiprid residues in water

	Acetamiprid
Specificity	The HPLC-UV (DAD) method is specific for the determination of acetamiprid. No interference above ≥ 30% LOQ
Calibration (type, number of data points)	five-point linear calibration curve y= 1.37038e005x + 0.144862 r^2= 0.999
Calibration range	4.8 to 80.7 mg/L
Assessment of matrix effects is presented	N/A
Limit of determination/quantification	LOQ: 130.4 mg/L

Conclusion
The method validation for the determination of acetamiprid in water was fully validated according to the requirements of the SANCO/3029/99 rev 4 guidelines and therefore the method can be fully accepted.

A 2.1.1.1.23 Analytical method 307SRES15C02
A 2.1.1.1.23.1 Method validation 307SRES15C02

Comments of zRMS:	For nectar and larvae, the method was successfully validated according to the guidance document SANCO/3029/99 rev. 4. For each specimen, the LOQ of the method was the lowest validated level where a mean recovery within the range 70-110% and with a RSD less or equal to 20% could be obtained. The LOQ of acetamiprid was 0.01 mg/kg. The method is acceptable.

Reference:	KCP 5.1.2/24
Report	Effects and determination of residues of acetamiprid 200 SL on the Honeybee (*Apis mellifera L*) Brood in citrus, under field conditions, in Spain 2015, Aucejo, S., 2015, Study No. 307SRES15C02
Guideline(s):	Yes, SANCO 3029/99 rev. 4
Deviations:	None
GLP:	Yes
Acceptability:	Yes

A. Materials

1. Standards

Test item :	MCW – 2222
Batch no.	659-030314-01
Active content:	nominal: 200 g/L analysed 199.2 g/L
Expiry date:	March 2016
Reference item :	Acetamiprid
Batch No.:	20202
Purity:	98.1 ± 0.5%
Expiry date:	February 2016
Standards for calibration	As above
Matrix:	Nectar, larvae and spray solution

B. Sample preparation and processing
For nectar and larvae residues, 1 g for larvae and 2 g for nectar of frozen sample were weighed into a 50 mL tube and fortified respectively. 5 mL (larvae) and 10 mL (nectar) of ultra-pure water was added following 5 mL for larvae and 10 mL for nectar of acetonitrile. Afterwards the samples were shaken manually and then place on a horizontally shaker for 20 minutes. The extracts were transferred into a QuEChERS tube containing a buffer salt mix. After shaken manually again the samples were centrifuged for about 5 minutes at about 4000 rpm. The extract was filtered with a 0.45 µm filter. Quantitation was performed by HPLC-MS/MS.
For spray solution the sample was homogenised by shaking the samples mechanically for 15 minutes. 1 mL of the sample was transferred into a 10 mL (untreated) or 100 mL (treated sample) measuring cylinder containing a methanol/ultra-pure water mix (50/50 v/v). For treated samples the extract was adjusted to 50

mL with acetone for analyses. All samples were diluted accordingly: untreated sample by a factor of 10 and untreated samples by a factor of 5000. The quantitation was performed by HPLC-MS/MS.

C. Chromatographic parameters

HPLC- parameters	
Column:	C18 (100 x 3.0 mm, 2.5 µm PD)
Column temperature:	60°C
Injection volume:	20 µL
MS/MS - parameters	API 4000 Qtrap or API 5500
Ion source :	ESI (electrospray ionisation) positive
Ion mode:	MRM
Transitions:	223 -> 126 m/z (quantifier)
	223 -> 90 m/z (qualifier)

Results and discussions

The method used for the determination of residues of acetamiprid in larvae and nectar and spray solution was fully validated according to the requirements of the SANCO/3029/99 rev. 4 guidelines. The specific identification of acetamiprid was conducted by HPLC-MS/MS with two transitions monitored. Therefore no confirmatory data need to be provided. The limit of quantification is 0.01 mg/kg and is defined as the lowest fortification level with mean recoveries ranging from 70% to 110% at a relative standard deviation (RSD) of ≤ 20% and blanks not exceeding 30% of the LOQ level. These criteria were fulfilled for the method LOQ for acetamiprid, as well as for the temper LOQ. For spray solutions, no recovery rates were performed as the analyses consisted only of simple dilutions. The detector response for acetamiprid was linear within the range from 0.3 to 12 µg/L for nectar, 0.3 to 5 µg/L for larvae and 0.3 to 50 µg/L for solution spray with $r^2 \geq 0.99$. The recoveries are presented in the table below.

Table A 45: Procedural recoveries results from method validation of acetamiprid using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (n=5)	Mean recovery (%)	RSD (%)	Comments
Nectar	Acetamiprid	0.01	75	3	-
		0.1	80	6	-
Larvae	Acetamiprid	0.01	104	6	-
		0.1	110	5	-

Table A 46: Characteristics for the analytical method used for validation of acetamiprid residues in nectar, larvae and spray solution

	Acetamiprid
Specificity	The HPLC-MS/MS with two ions monitored is highly specific for the determination of acetamiprid. No interference above ≥ 30% LOQ
Calibration (type, number of data points)	seven-point linear calibration curve Nectar: y= 161766.14x + 9397.78 r^2= 0.999 Larvae: y= 160947.03 + 13365.45 r^2= 1
Calibration range	0.3 to 12 µg/L for nectar 0.3 to 5 µg/L for larvae 0.3 to 50 µg/L for solution spray
Assessment of matrix effects is presented	N/A
Limit of determination/quantification	LOQ: 0.01 mg/kg

Conclusion

The method validation for the determination of acetamiprid in nectar, larvae and spray solution was fully validated according to the requirements of the SANCO/3029/99 rev 4 guidelines and therefore the method

can be fully accepted.

A 2.1.1.1.24 Analytical method S16-02168
A 2.1.1.1.24.1 Method validation S16-02168

Comments of zRMS:	The method was successfully validated for determination of acetamiprid in Phacelia (pollen and flowers), nectar surrogate, honey bee larvae, honey and beeswax with an LOQ of 0.01 mg a.s./kg according to the guidance document SANCO/3029/99 rev. 4. With regard to selectivity, accuracy and precision, the analytical method was applied successfully for each analytical set when analysing the specimens of the study. The method is acceptable.

Reference:	KCP 5.1.2/25
Report	Semi-field brood study to evaluate potential effects of MCW-2222 on brood development of honeybees (*Apis mellifera*), Mayer, O., 2017, Study No. R1640035
Guideline(s):	Yes, SANCO 3029/99 rev. 4
Deviations:	None
GLP:	Yes
Acceptability:	Yes

A. Materials

1. Standards

Reference item:	Acetamiprid
Lot/Batch number:	469-129-01
Purity:	99.8 %
CAS No.:	135410-20-7
Expiry date:	January 2019

Standards for calibration	As above
Matrix:	Honey bee related products

B. Sample preparation and processing

Sample preparation for flowers, nectar, larvae, honey:

200 mg homogenised samples of flowers, nectar, larvae, honey or arthropods were weighed into a 50-mL centrifuge tube and 10 mL of water are added. For extraction, exactly 10 mL of acetonitrile are added. The centrifuge tube is capped and shaken vigorously by hand for at least one minute followed by shaking on platform shaker for 15 minutes. Afterwards, a buffer salt mix is added and immediately shaken by hand for about one minute. The sample tube is centrifuged for 5 minutes at about 3200 rpm. An amount of 40 mg of PSA and 225 mg of magnesium sulfate is weighed into a 2-mL safe-lock tube. An aliquot of 1.5 mL of the acetonitrile phase is transferred into the tube containing the mixture of PSA and magnesium sulfate. The tube is shaken using a vortex mixer and by hand for 30 seconds followed by centrifugation for 5 minutes at about 3500 rpm. An aliquot of exactly 1.0 mL is taken and filled up with 0.1 % formic acid in water to a final volume of 2.5 mL. The final solution is mixed and stored at 1 °C – 10 °C (target) in the dark until injection into LC-MS/MS.

Sample preparation of pollen and wax samples:

200 mg homogenised samples of pollen or wax is weighed into a 15-mL Lysing Matrix D Tube containing the 1.4 mm ceramic spheres and 2.5 mL water are added. For extraction, exactly 2.5 mL of acetonitrile are added. For wax: The samples is heated to approx. 50 °C in a water bath. The centrifuge tube is capped and the sample material is shred twice for pollen or three times for wax with FastPrep for one minute

(4.0 m/sec). Afterwards, a buffer salt-mix is added. The centrifuge tube is capped again and shaken by FastPrep for about one minute (4.0m/sec). The sample tube is centrifuged for 5 minutes at about 3200 x g. For wax: The acetonitrile phase is frozen out at ≤ -18 °C for one hour and the sample tube is centrifuged for 5 minutes at about 3200 x g (in cooled state). An amount of 40 mg of PSA and 225 mg of magnesium sulfate is weighed into a 2-mL safe-lock tube. An aliquot of 1.5 mL of the acetonitrile phase is transferred into the tube containing the mixture of PSA and magnesium sulfate. The tube is intensively shaken using a vortex mixer and by hand for 30 seconds followed by centrifugation for 5 minutes at about 3500 x g. Finally, 0.25 mL of the extract of pollen or wax along with 0.75 mL of acetonitrile are filled up with 0.1 % formic acid in water to a final volume of 2.5 mL. The final solution is mixed and stored at 1 °C – 10 °C (target) in the dark until injection into LC-MS/MS.

C. Chromatographic parameters

HPLC- parameters	
Instrumentation:	200 Infinity Binary LC System, Agilent Technologies
Column:	Zorbax, 150 mm x 2.1 mm, 3.5 µm,
Column temperature:	30°C
Injection volume:	25µL or 35 µL
MS/MS - parameters	
Instrumentation:	SCIEX TripleQuad 5000 System
Mode:	ESI (electrospray ionisation) positive
Ion source:	Turbospray
Scan type:	MRM
Transitions:	223 -> 126 m/z (quantifier)
	225 -> 128 m/z (qualifier)
	223 -> 73 m/z (quantifier)
	223 -> 90 m/z (qualifier)

Results and discussions

The analytical method for the determination of acetamiprid in flowers, nectar, honey, bee larvae, pollen and wax was fully validated according to the SANCO/3029/99 rev. 4 guideline. The specific identification of acetamiprid was conducted by HPLC-MS/MS with two transitions monitored. Therefore no confirmatory data needs to be provided. The analytical method were validated according to SANCO/3029/99 rev. 4. The limit of quantification in the main validation is 0.01 mg/kg and is defined as the lowest fortification level with mean recoveries ranging from 70% to 110% at a relative standard deviation (RSD) of ≤ 20% and blanks not exceeding 30% of the LOQ level. These criteria were fulfilled for the recoveries of the method LOQ and the temper LOQ for acetamiprid. The detector response for acetamiprid was linear within the range from 0.02 ng/mL to 2.0 ng/mL (corresponds to 0.0025 mg/Kg to 0.25 mg/kg) with $r^2 \geq 0.99$. The recovery data are presented in the table below.

Table A 47: Recovery results from method validation of acetamiprid using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
Flowers	Acetamiprid	0.01	98	8.3	-
		0.1	95	1.6	-
Pollen		0.01	109	6.4	-
		0.1	102	7.3	-
Nectar		0.01	96	4.3	-
		0.1	91	2.1	-
Honey		0.01	84	4.4	-
		0.1	98	2.2	-
Wax		0.01	80	5.0	-
		0.1	97	2.1	-

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
Larvae		0.01	109	3.3	-
		0.1	101	2.9	-

Table A 48: Characteristics for the analytical method used for validation of acetamiprid residues in honey related matrices

	Acetamiprid
Specificity	The HPLC-MS/MS with two ions monitored is highly specific for the determination of acetamiprid. No interference above ≥ 30% LOQ
Calibration (type, number of data points)	Eight-point linear calibration curve: Flowers: y= 608.3973 + 113407.1637x, r^2= 999 Pollen: y= 191.0036 + 45165.7532x, r^2= 998 Nectar: y= -147.8268 + 124002.4541x, r^2= 999 Honey: y= 2640.9913 + 161932.3093x, , r^2= 997 Bee larvae: y= 482.5289 + 76548.5931x, , r^2= 999 Wax: y= 5760.4337 + 482337.8464x , r^2= 997
Calibration range	0.02 to 2.0 ng/mL for all matrices
Assessment of matrix effects is presented	Matrix effects on LC-MS/MS detection were investigated and found to be significant for pollen, honey and wax and insignificant for flowers, nectar and larvae. Matrix-matched standards were used for quantification for all matrices throughout the analytical phase.
Limit of determination/quantification	LOQ for all matrices: 0.01 mg/kg

Conclusion
The method validation described in S16-02168 for the determination of acetamiprid in honey related material was fully validated according to the requirements of the SANCO/3029/99 rev. 4 guidelines. The validation proves to be within the requirements of the guidelines and therefore the method can be fully accepted.

A 2.1.1.1.25 Analytical method 1948BAC 0028
A 2.1.1.1.25.1 Method validation 1948BAC 0028

Comments of zRMS:	The purpose of the analytical phase of the study was the verification of the concentration of the active ingredient acetamiprid in feeding solutions given to honeybees. The determination was conducted by an in-house developed method using reversed phase high performance liquid chromatography (RP-HPLC) with diode-array-detection (DAD). The limit of quantification (LOQ) was defined in the context of this analytical phase as the lowest successfully validated fortification level, i.e. 8.475 mg/L of acetamiprid (corresponding to 2.712 mg/L regarding DF). All validity criteria of the guidance document SANCO/3029/99 are fulfilled: – LOQ - blank values did not exceed 30% of the lowest validated concentration, – Accuracy was tested by fortifying sample matrix with the test item at 2 concentrations levels. Mean recoveries for each level were in the range 70-110%, – Repeatability with 5 replicates for each level with the RSD (relative standard deviation) was < 20% per level. – Linearity was tested for 5 points at a concentration range of at least ± 20% of a.i. in the analytical solution, with correlation coefficient of > 0.99. The analytical method was validated according to SANCO/3029/99 rev. 4 and is acceptable.

Reference:	KCP 5.1.2/ 26
Report	Chronic oral toxcitiy of CA3573 Acetamiprid 200 SL (Carnadine) to the honey bee Apis mellifera L. under laboratory conditions, Dressler, K., 2019, Study No. 1948BAC 008

Guideline(s):	Yes, SANCO 3029/99 rev. 4
Deviations:	None
GLP:	Yes
Acceptability:	Yes

A. Materials

1. Standards

Test item:	CA3573 Acetamiprid 200 SL
Batch no.	981101035
Active substance:	Nominal: 200 g/L Analysed: 195.5 g/L
Expiry date:	March 2020
Reference item:	Acetamiprid
CAS No.:	135410-20-7
Batch No.:	780664
Purity:	99.9 %
Expiry date:	April 2020
Standards for calibration	As above
Matrix:	Honey bee feeding solution

B. Sample preparation and processing

For sample measurement the samples were allowed to reach room temperature and homogenised by shaking. They were analysed after dilution by factor 3.125 and 50 with dilution medium and test matrix. The quantitation was performed by HPLC-DAD.

C. Chromatographic parameters

HPLC- parameters:	Shimadzu LC-20 HPLC
Column:	C18 (2.1 mm x 150 mm 5 µm)
Column temperature:	40°C
Injection volume:	10 µL
Flow rate:	0.50 mL/min
Detection:	246 nm

Results and discussions

The method used for the determination of residues of acetamiprid in honey bee feeding solution was fully validated according to the requirements of the SANCO/3029/99 rev. 4 guidelines. The specific identification of acetamiprid was conducted by HPLC-MS/MS with two transitions monitored. Therefore no confirmatory data need to be provided. The limit of quantification is 8.475 mg/L (corresponds to 2.712 mg/L regarding dilution factor) and is defined as the lowest fortification level with mean recoveries ranging from 70% to 110% at a relative standard deviation (RSD) of ≤ 20% and blanks not exceeding 30% of the LOQ level. These criteria were fulfilled for the recoveries of the method LOQ for acetamiprid, as well as for the temper LOQ (350 mg/L corresponds to 7.0 mg/L regarding the dilution factor). The detector response for acetamiprid was linear within the range within 0.540 to 10.79 mg/L with $r^2 \geq 0.99$. The recoveries are presented in the table below.

Table A 49: Recovery results from method validation of acetamiprid using the analytical method

Matrix	Analyte	Fortification level (mg/L) (n=5)	Mean recovery (%)	RSD (%)	Comments
Bee feeding		8.475	93	0.1	-

Matrix	Analyte	Fortification level (mg/L) (n=5)	Mean recovery (%)	RSD (%)	Comments
solution	Acetamiprid	350	94	1.2	-

Table A 50: Characteristics for the analytical method used for validation of acetamiprid residues in honey bee feeding solution

	Acetamiprid
Specificity	The HPLC-DAD is specific for the determination of acetamiprid in bee feeding solution No interference above ≥ 30% LOQ
Calibration (type, number of data points)	five-point-linear calibration curve 110614x – 1383.36 r^2= 0.999
Calibration range	0.540 to 10.79 mg/L
Assessment of matrix effects is presented	N/A
Limit of determination/quantification	LOQ: 8.475 mg/L

Conclusion
The method validation for the determination of acetamiprid in bee feeding solution was fully validated according to the requirements of the SANCO/3029/99 rev 4 guidelines and therefore the method can be fully accepted.

A 2.1.1.1.26 Analytical method 1948BLC 0033
A 2.1.1.1.26.1 Method validation 1948BLC 0033

Comments of zRMS:	The determination of the active ingredient acetamiprid in larval diet was conducted by an in-house developed method using high performance liquid chromatography (HPLC) with mass-spectrometric (MSMS) detection. The analytical method was validated according to SANCO/3029/99 rev. 4. The method is acceptable.

Reference:	KCP 5.1.2/27
Report	CA3573 Acetamiprid 200 SL (Carnadine) – repeated exposure of honey bee larvae (Apis mellifera) under laboratory conditions, Scheller K., 2020, Study No. 1948BLC 0033
Guideline(s):	Yes, SANCO 3029/99 rev. 4
Deviations:	None
GLP:	Yes
Acceptability:	Yes

A. Materials

1. Standards

Test item:	CA3573 Acetamiprid 200 SL
Batch no.	981101035
Active substance:	Nominal: 200 g/L Analysed: 195.5 g/L
Expiry date:	March 2020
Reference item:	Acetamiprid
CAS No.:	135410-20-7
Batch No.:	780664

Purity:	99.9 %
Expiry date:	April 2020
Standards for calibration	As above
Matrix:	Honey bee larvae

B. Sample preparation and processing

Samples were allowed to reach room temperature and then homogenised by shaking. 0.25 mg of each sample were vortexed with 5 mL dilution medium for 5 min and then centrifuged for 3 min at 3000 rpm. The supernatants were diluted with dilution medium and blank extract. The same extraction and dilution procedure was carried out for analysis of the validation samples. Quantitation was achieved by HPLC-MS/MS.

C. Chromatographic parameters

HPLC- parameters:	Agilent 1200 HPLC
Column:	C18 (100 mm x 2.1 mm 3 µm)
Column temperature:	40°C
Injection volume:	10 µL
MS/MS parameters:	6410 triple quadrupole
Ion source:	ESI
Ion mode:	MRM
Detection:	223.1 -> 126 (quantifier)
	223.1 -> 90 (qualifier)

Results and discussions

The method used for the determination of residues of acetamiprid in honey bee larvae was fully validated according to the requirements of the SANCO/3029/99 rev. 4 guidelines. The specific identification of acetamiprid was conducted by HPLC-MS/MS with two transitions monitored. Therefore no confirmatory data need to be provided. The limit of quantification is 0.096 mg/kg (corresponds to 1.146 µg/L regarding the dilution factor) and is defined as the lowest fortification level with mean recoveries ranging from 70% to 110% at a relative standard deviation (RSD) of ≤ 20% and blanks not exceeding 30% of the LOQ level. These criteria were fulfilled for the recoveries of the method LOQ for acetamiprid, as well as for the temper LOQ. The detector response for acetamiprid was linear within the range within 0.240 to 4.807 µg/L with $r^2 \geq 0.99$. The recoveries are presented in the table below.

Table A 51: Recovery results from method validation of acetamiprid using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (n=5)	Mean recovery (%)	RSD (%)	Comments
Bee larvae	Acetamiprid	0.096	102	5	-
		3.682	90	1.6	-

Table A 52: Characteristics for the analytical method used for validation of acetamiprid residues in honey bee larvae

	Acetamiprid
Specificity	The HPLC-MS/MS is highly specific for the determination of acetamiprid in bee feeding solution No interference above ≥ 30% LOQ
Calibration (type, number of data points)	Six-point-linear calibration curve Y= 872.60953x + 49.544003 r^2= 0.999
Calibration range	0.240 to 4.807 µg/L
Assessment of matrix effects is presented	N/A

	Acetamiprid
Limit of determination/quantification	LOQ: 0.096 mg/kg

Conclusion
The method validation for the determination of acetamiprid in honey bee larvae was fully validated according to the requirements of the SANCO/3029/99 rev 4 guidelines and therefore the method can be fully accepted.

A 2.1.1.1.27 Analytical method NFM-001/7-52
A 2.1.1.1.27.1 Method validation NFM-002/6-22

Comments of zRMS:	The aim of this analytical study was to determine and evaluate the different test concentrations of the acetamiprid of the test item Carnadine in mesocosm water and sediment. The methods were developed in non-GLP experiments, these experiments were completed before start of the respective GLP activities in study NFM-002/6-22 (see point A2.1.1.1.28). The analytical method for the determination of acetamiprid used in the current study have been fully validated according to the requirements of SANCO/3029/00 rev.4. The validation data are reported in the study NFM-002/6-22. The method is therefore suitable for the determination of acetamiprid in the aqueous and sediment test media used in the corresponding mesocosm study. The procedural recoveries from method NFM-001/7-52 were all within the requirements and therefore the method acceptable.

Reference:	KCP 5.1.2/28
Report	Carnadine – Outdoor mesocosm study, Hennecke, S.,2020, Study No NFM-001/7-52
Guideline(s):	Yes, SANCO 3029/99 rev. 4
Deviations:	None
GLP:	Yes
Acceptability:	Yes

A. Materials

1. Standards

Reference item:	Acetamiprid
Batch no.	BCBT9185
Purity:	>98 %
CAS No.:	135410-20-7
Expiry date:	February 2022
Standards for calibration	As above
Matrix:	Water and sediment

B. Sample preparation and processing

Sample preparation – mesocosm water

The solutions of procedural recovery samples were prepared by dilution of 80 µL of the working solutions and 80 µL of stock solution into separate 1.5 mL sample vials prepared with 40 µL acetonitrile and 800 µl aqueous test medium. The samples were prepared at least three times and were analysed by UHPLC-MS/MS.

Sample preparation – sediment

Untreated sediment was centrifuged for 5 minutes. The supernatant was discarded. The pellet was homogenised with a spoon before six 7.44 g (ww), corresponding to 5 g (dw) subsamples were individually weighed into separate 50 mL PP vials.
The samples were prepared by adding 50 µL of the internal standard stock solution and were fortified with 50 µL of the working solutions and left for 1 hour. 7.5 mL water and 5 mL acetonitrile were then added to each sample. The samples were treated in an ultrasonic bath for 5 minutes. One package of QuEChERS salts was added to each sample. The samples were shaken and centrifuged for 5 minutes. The centrifuge was adjusted at 4000 rpm. Subsamples of 1.5 mL of the upper acetonitrile phases were individually transferred to separate 2 mL dSPE vials and manually shaken. The samples were then centrifuged for 5 minutes, adjusted at 4000 rpm. Subsamples of 1 mL were individually transferred to seperate 1.5 mL sample vials and evaporated to dryness under nitrogen at room temperature. The residues were reconstituted in 1 mL water / acetonitrile (80:20 v:v) and analysed by UHPLC-MS/MS.

C. Chromatographic parameters

HPLC- parameters:	Waters Acquity UHPLC System
Column:	BEH C18; 50 x 2.1 mm; 1.7 µm
Flow rate:	10.350 mL/min
Oven temperature:	55 °C
Injection volume:	50 µL
Mobile phase:	A: water / methanol / formic acid (89.9 : 10 : 0.1 v:v:v) including 2 mM $AcNH_4$
	B: methanol / formic acid (99.9 : 0.1 v:v) including 2 mM $AcNH_4$
MS/MS parameters	Waters LC-MS/MS Xevo TQ-D Detector
Ionisation mode:	ES positive
Scan type.	MRM
Transitions:	223.0 -> 126 m/z (quantifier)
	223.0 -> 56 m/z (qualifier)
	226. 0 -> 126 m/z (internal standard)

Results and discussions

The method used for the determination of residues of acetamiprid in water was fully validated in a previous study of Hennecke,S. (Study No. NFM-002/6-22) according to the requirements of the SANCO/3029/99 rev. 4 guidelines. Therefore only procedural recoveries were conducted. The specific identification of acetamiprid was conducted by UHPLC-MS/MS with two transitions monitored. The limit of quantification is 10 ng a.s/L (mesocosm water) and 50 ng a.s/kg (mesocosm-sediment) and is defined as the lowest fortification level with mean recoveries ranging from 70% to 110% at a relative standard deviation (RSD) of ≤ 20% and blanks not exceeding 30% of the LOQ level. These criteria were fulfilled for the procedural recoveries of the method LOQ for acetamiprid, as well as for the temper LOQ . Solvent-standards were used for the calibration. The detector response was linear within the range of 3.0 – 300 ng a.s/L and 10 – 1000 ng a.s/L (corresponds to 10 to 1000 ng a.s/kg). The correlation coefficients are ≥ 0.999. The recoveries are presented in the table below. Representative chromatograms are stated in the report.

Table A 53: Procedural recovery results from method validation NFM-002/6-22 of acetamiprid using the analytical method

Matrix	Analyte	Fortification level (ng a.s./L)	Mean recovery (%)	RSD (%)	Comments
Mesocosm – water Day 0 (Part 1)		10 (n=3)	96.1	3.4	-
		100 (n=3)	97.7	2.7	-
Mesocosm – water Day 0		10 (n=3)	93.8	3.9	-
		100 (n=3)	97.3	2.5	-

Matrix	Analyte	Fortification level (ng a.s./L)	Mean recovery (%)	RSD (%)	Comments
(Part 2)	Acetamiprid				
Mesocosm – water Day 1		10 (n=3)	92.9	3.1	-
		100 (n=3)	89.5	0.8	-
Mesocosm – water Day 3 and 6		10 (n=5)	95.1	2.4	-
		100 (n=5)	89.8	2.9	-
Mesocosm – water Day 8 and 10		10 (n=3)	95.3	3.3	-
		100 (n=3)	91.0	1.8	-
Mesocosm – water Day 7		10 (n=4)	92.2	3.2	-
		100 (n=4)	89.7	2.3	-
Mesocosm – water (depth samples) Day 15		10 (n=3)	108.2	2.6	day 15 and repeat measure-ments depth samples day 7*
		100 (n=3)	103.2	2.0	See above.
Mesocosm – water (depth samples) Day 22		10 (n=3)	106.9	3.7	day 22, day 30 and repeat measure-ment sample day 15*
		100 (n=3)	103.8	1.7	See above.
Mesocosm – water (depth samples) Day 42		10 (n=3)	103.2	9.3	day 42 and repeat measure-ment sample day 15*
		100 (n=3)	93.1	1.1	See above
Mesocosm – water (depth samples) Day 56		10 (n=3)	95.0	7.0	day 56 and repeat measurement sample day 42*
		100 (n=3)	95.1	1.4	See above.
Mesocosm – water (depth samples) Day 56		10 (n=3)	92.6	1.2	repeat measurement sample day 15, day 42 and day 56*
		100 (n=3)	90.8	1.0	See above.
Mesocosm – water (depth samples) Day 84		10 (n=3)	102.5	1.0	-
		100 (n=3)	97.1	2.5	-
Mesocosm – water (depth samples) Day 84		10 (n=3)	106.4	4.0	repeat measurement sample day 56 and day 84*
		100 (n=3)	100.5	5.7	See above.

- Samples of different sample days were prepared at the same time and analysed in one sample sequence.

Table A 54: Procedural recovery results from method validation NFM-002/6-22 of acetamiprid using the analytical method

Matrix*	Analyte	Fortification level (µg a.s/kg)	Mean recovery (%)	RSD (%)	Comments
Sediment Day 5 and 15		0.05 (n=4)	98.9	10.6	-
		0.5 (n=4)	86.9	3.6	-
Sediment Day 23 and 28		0.05 (n=3)	101.4	6.9	-
		0.5 (n=3)	95.5	4.0	-

Matrix*	Analyte	Fortification level (µg a.s/kg)	Mean recovery (%)	RSD (%)	Comments
Sediment Day 42	Acetamiprid	0.05 (n=3)	110.5	3.2	-
		0.5 (n=3)	103.7	3.8	-
Sediment Day 56		0.05 (n=4)	99.5	4.5	-
		0.5 (n=4)	105.1	2.1	-
Sediment Day 84		0.05 (n=3)	106.6	2.7	-
		0.5 (n=3)	98.6	1.9	-

- Samples of different sample days were prepared at the same time and analysed in one sample sequence.

Table A 55: Procedural storage stability recovery results from method validation NFM-002/6-22 of acetamiprid using the analytical method

Matrix	Analyte	Fortification level (ng a.s./L) (n=3)	Mean recovery (%)	RSD (%)	Comments
Day 0	Acetamiprid	10	105.2	2.2	-
		100	104	0.4	-
Day 7		10	102.2	1.5	-
		100	102.7	0.5	-
Day 14		10	105.2	5.8	-
		100	95.2	0.9	-
Day 29		10	89.9	5.8	-
		100	93.7	1.5	-
Day 56		10	105.5	7.8	-
		100	97.0	3.6	-

Table A 56: Characteristics for the analytical method used for validation of acetamiprid residues in water/sediment

	Acetamiprid
Specificity	The UHPLC-MS/MS method with two transitions is highly specific for the determination of acetamiprid. No interference above ≥ 30% LOQ was applicable.
Calibration (type, number of data points)	Multiple-point-linear calibration curve y= 1.04075x + 0.146002 r^2= 0.999 (mesocosm- water) y= 1.07158x + 2.99826 r^2= 0.999 (sediment)
Calibration range	3.0 – 300 ng a.s/L 10 – 1000 ng a.s/L (corresponds to 10 to 1000 ng a.s/kg)
Assessment of matrix effects is presented	N/A
Limit of determination/quantification	LOQ: 10 ng a.s/ L (mesocosm – water) LOQ: 50 ng a.s/kg (sediment)

Conclusion

The method validation NFM-002/6-22 for the determination of acetamiprid in mesocosm-water and mesocosm-sediment was fully validated according to the requirements of the SANCO/3029/99 rev 4 guidelines. The procedural recoveries from method NFM-001/7-52 were all within the requirements and

therefore the method can be fully accepted.

A 2.1.1.1.28 Analytical method NFM-002/6-22
A 2.1.1.1.28.1 Method validation NFM-002/6-22

Comments of zRMS:	The method was validated for the determination of acetamiprid in mesocosm water and sediment. The methods were developed non-GLP experiments, these experiments were completed before start of the respective GLP activities. The methods were validated under GLP conditions and according to the requirements of SANCO/3029/00 rev.4. The limit of quantification is 10 ng a.s/L (mesocosm water) and 50 ng a.s/kg (mesocosm-sediment). All mean recovery values at fortification levels are within the required range of 70 – 110%, the overall RSD are below 20% for both mass transitions. The analytical method NFM-002/6-22 for acetamiprid has been fully validated according to the requirements of SANCO/3029/00 rev.4. The method is therefore suitable for the determination of acetamiprid in the aqueous and sediment test media used in the corresponding mesocosm study. The study is acceptable.

Reference:	KCP 5.1.2/29
Report	Validation of the analytical methods for water and sediment, Hennecke, S., 2020, Study No. NFM-002/6-22
Guideline(s):	Yes, SANCO 3029/99 rev. 4
Deviations:	None
GLP:	Yes
Acceptability:	Yes

A. Materials

1. Standards

Reference item:	Acetamiprid
Batch no.	BCBT9185
Purity:	>98 %
CAS No.:	135410-20-7
Expiry date:	February 2022
Standards for calibration	As above
Matrix:	Water and sediment

B. Sample preparation and processing

Sample preparation – mesocosm water

The samples were prepared by dilution of 80 µL of the working solution and 80 µL of the solvent solution into separate 1.5 mL sample vials prepared with 40 µL acetonitrile and 800 µL aqueous test medium. The samples were analysed by UHPLC-MS/MS.

Sample preparation – sediment

Untreated sediment was centrifuged for 5 minutes at 4000 rpm. The supernatant was discarded. The pellet was mixed with a spoon and approximately 7.5 g corresponding to 5 g (dw) were weighed into each 50 mLPP vials. The samples were prepared by adding 50 µl of the internal standard stock solution and 50 µL of the working solutions. The untreated control samples were prepared by adding 50 µL of the internal standard stock solution and 50 µL acetonitrile. The samples were homogenized and sit for one hour. The samples were analysed by UHPLC-MS/MS.

C. Chromatographic parameters

HPLC- parameters:	Waters Acquity UHPLC System
Column:	BEH C18; 50 x 2.1 mm; 1.7 µm
Flow rate:	10.350 mL/min
Oven temperature:	55 °C
Injection volume:	50 µL
Mobile phase:	A: water / methanol / formic acid (89.9 : 10 : 0.1 v:v:v) including 2 mM $AcNH_4$ B: methanol / formic acid (99.9 : 0.1 v:v) including 2 mM $AcNH_4$

MS/MS parameters	Waters LC-MS/MS Xevo TQ-D Detector
Ionisation mode:	ES positive
Scan type.	MRM
Transitions:	223.0 -> 126 m/z (quantifier) 223.0 -> 56 m/z (qualifier) 226. 0 -> 126 m/z (internal standard)

Results and discussions

The method used for the determination of residues of acetamiprid in mesocosm- water/sediment was fully validated according to the requirements of the SANCO/3029/99 rev. 4 guidelines. The specific identification of acetamiprid was conducted by UHPLC-MS/MS with two transitions monitored. The limit of quantification is 10 ng a.s/L (mesocosm water) and 50 ng a.s/kg (mesocosm-sediment) and is defined as the lowest fortification level with mean recoveries ranging from 70% to 110% at a relative standard deviation (RSD) of < 20% and blanks not exceeding 30% of the LOQ level. These criteria were fulfilled for the procedural recoveries of the method LOQ for acetamiprid, as well as for the temper LOQ. The detector response was linear within the range of 3.0 – 300 ng a.s/L and 10 – 1000 ng a.s/L (corresponds to 10 to 1000 ng a.s/kg). The correlation coefficients are ≥ 0.999. The recoveries are presented in the table below. Representative chromatograms are stated in the report.

Table A 57: Recovery results from method validation NFM-002/6-22 of acetamiprid using the analytical method

Matrix	Analyte	Fortification level (ng a.s./L) (n=5)	Mean recovery (%)	RSD (%)	Comments
Mesocosm – water	Acetamiprid	10	92.7	2.9	-
		100	92.0	2.7	-

Table A 58: Recovery results from method validation NFM-002/6-22 of acetamiprid using the analytical method

Matrix	Analyte	Fortification level (ng a.s/kg) (n=5)	Mean recovery (%)	RSD (%)	Comments
Sediment	Acetamiprid	50	107	1.9	-
		500	100	2.7	-

Table A 59: Characteristics for the analytical method used for validation of acetamiprid residues in water/sediment

	Acetamiprid
Specificity	The UHPLC-MS/MS method with two transitions is highly specific for the determination of acetamiprid. No interference above ≥ 30% LOQ was applicable.
Calibration (type, number of data points)	Multiple-point-linear calibration curve y= 1.03786x + 0.409849 r^2= 0.999 (mesocosm- water) y= 0.978249x – 1.0934 r^2= 0.999 (sediment)

	Acetamiprid
Calibration range	3.0 – 300 ng a.s/L 10 – 1000 ng a.s/L (corresponds to 10 to 1000 ng a.s/kg)
Assessment of matrix effects is presented	No significant of ≥ 20% matrix effects were observed.
Limit of determination/quantification	LOQ: 10 ng a.s/ L (mesocosm – water) LOQ: 50 ng a.s/kg (sediment)

Conclusion
The method validation for the determination of acetamiprid in mesocosm-water/sediment was fully validated according to the requirements of the SANCO/3029/99 rev 4 guidelines and therefore the method can be fully accepted.

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

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

A 2.1.2.1.1 Analytical method

A 2.1.2.1.1.1 Method validation

Matching data have been conducted and submitted to Ctgb to demonstrate access to a complete package according to Reg (EU)283/2013 and for data matching step. RMS Opinion on GLP compliance, guidance compliance and equivalent endpoint can be provided as soon as evaluation is finalised.

A 2.1.2.1.1.2 Extraction efficiency

No new or additional studies have been submitted.

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

A 2.1.2.2.1 Analytical method

A 2.1.2.2.1.1 Method validation

Matching data have been conducted and submitted to Ctgb to demonstrate access to a complete package according to Reg (EU)283/2013 and for data matching step. RMS Opinion on GLP compliance, guidance compliance and equivalent endpoint can be provided as soon as evaluation is finalised.

A 2.1.2.2.1.2 Extraction efficiency

No new or additional studies have been submitted

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

A 2.1.2.3.1 Analytical method S15-02364-L2

A 2.1.2.3.1.1 Method v alidation S15-02364-L2

Comments of zRMS:	In the analytical phase S15-0236-L2 of this study, samples of radish, spinach, wheat and soil were analysed for residues of acetamiprid and its metabolites with a limit of quantification (LOQ) of 0.01 mg/kg for acetamiprid, IM-1-4 and IM-1-5 in soil and each crop type, with the exception of 0.05 mg/kg as LOQ for IM-1-5 in straw.

	The analytical method was successfully validated for determination of acetamiprid and its metabolites IM-1-4 and IM-1-5 for radish (leaves and roots), spinach (leaves), wheat (grain and straw) and soil according to the requirements of the SANCO/3029/99 rev.4 and SANCO/825/00 rev. 8.1 guidelines. . The analytical method was applied successfully for each analytical set when analysing the samples of the study. The residue levels reported for the residue samples can therefore be considered to be accurate. The study is acceptable.

Reference:	KCP 5.2/01
Report	Determination of residues of acetamiprid and its metabolites IM 1-4 and IM 1-5 after one application of MCW-2222 to bare soil in rotational crops (radish, spinach and wheat) at 1 site in Northern Europe and 1 site in Southern Europe 2016/2017, Semrau, J., 2017, Study No. S15-02364-L2
Guideline(s):	Yes, SANCO/825/00 rev. 8.1 and SANCO 3029/99 rev. 4
Deviations:	None
GLP:	Yes
Acceptability:	Yes

Please notice, that even if the study belongs to the data matching list (which was evaluated by the RMS (Ctgb)), the study needed to be presented. The B7 section needed to present the study to cover the GAP, therefore the study is summarized here.

A. Materials

1. Standards

Test item:	MCW-2222
Active substance content:	nominal: 200 g a.i./L analysed: 199.2 ± 1.3 g a.i./L
Batch No.:	659-030314-01
Expiry date:	March 2016
Reference item:	Acetamiprid
Lot/Batch number:	469-129-01
Purity:	99.8 %
CAS No.:	135410-20-7
Expiry date:	January 2019
Reference item:	IM 1-4
Lot/Batch number:	516-072-01
Purity:	98.9 %
CAS No.:	120739-62- 0
Expiry date:	May 2017
Reference item:	IM 1-5
Lot/Batch number:	516-15-00
Purity:	99.7 %
CAS No.:	365441-66-3
Expiry date:	
Standards for calibration	As above
Matrix:	Wheat, spinach, soil

B. Sample preparation and processing

Sample preparation for soil:

10 g of sample material were weighed into a 250 mL screw-capped glass container. 90 mL of a mixture of acetonitrile/ 1% acetic acid (80/20 v/v) were added and the sample material was extracted for 5 minutes using an ultrasonic-bath. Afterwards the samples were put on a mechanical shaker for 30 minutes. The remaining soil was extracted again with 60 mL of a mixture of acetonitrile/1% acetic acid (50/50 v/v) for 5 minutes using an ultra-sonic bath and subsequently shaken by a mechanical shaker for 30 minutes. The supernatant was decanted with a filter. The remaining extract was extracted again with 40 mL of 1% acetic acid following the same procedure as in the second extraction step. The samples were diluted 1:5 with a mix of acetonitrile/water before quantitation by LC-MS/MS.

Sample preparation for spinach, radish and wheat:

10 g of sample material (5 g for wheat) were weighed into a 250 mL screw-capped glass container. 70 mL of acetonitrile were added and the sample material was extracted for 5 minutes using an ultrasonic-bath. Afterwards the samples were decanted with a filter. The remaining sample material was extracted again with 60 mL of a mixture of acetonitrile/1% acetic acid (50/50 v/v) for 5 minutes using an ultra-sonic bath. The supernatant was decanted with a filter again. The remaining extract was extracted again with 50 mL of water following the same procedure as in the second extraction step. The samples were diluted 1:5 with a mix of acetonitrile/water for spinach and radish before quantitation by LC-MS/MS. For wheat the samples were diluted 1:2:5 with a mixture of acetonitrile/water (50 v/v) before quantitation by LC-MS/MS.

C. Chromatographic parameters

HPLC- parameters	
Instrumentation:	1200 Infinity Binary LC System, Agilent Technologies
Column:	Zorbax, 150 mm x 2.1 mm, 3.5 µm,
Column temperature:	30°C
Injection volume:	35µL
MS/MS - parameters	SCIEX TripleQuad 5000 System
Mode:	ESI (electrospray ionisation) positive
Ion source	Turbospray
Scan type:	MRM
Transitions:	223 -> 126 m/z (quantifier)
	223 -> 90 m/z (qualifier)
	157 -> 126 for IM 1-4
	159 -> 128 for IM 1-4
	198 -> 126 for IM 1-5
	198 -> 90 for IM 1-5

Results and discussions

The analytical method for the determination of acetamiprid IM 1-4 and IM 1-5 in radish, spinach, wheat and soil was validated according to the SANCO/3029/99 rev. 4 guidelines and SANCO/825/00 rev. 8.1 guidelines. The specific identification of acetamiprid was conducted by HPLC-MS/MS with two transitions monitored. Therefore no confirmatory data needs to be provided. The limit of quantification in the main validation is 0.01 mg/ kg exception wheat with 0.05 mg/kg and is defined as the lowest fortification level with mean recoveries ranging from 70% to 110% at a relative standard deviation (RSD) of ≤ 20% and blanks not exceeding 30% of the LOQ level. These criteria were fulfilled for the recoveries of the method LOQ and temper LOQ for acetamiprid. The detector response for acetamiprid was linear within the range from 0.025 ng/mL to 10.0 ng/mL with $r^2 \geq 0.98$. The recovery data are presented in the table below.

Table A 60: Recovery results from method validation of acetamiprid and IM 1-4 and Im 1-5 using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
Radish leaves	Acetamiprid	0.01	101	5.4	-
		0.1	96	4.1	-
Radish roots		0.01	106	2.6	-
		0.1	103	3	-
Wheat grain		0.01	100	3.7	-
		0.1	94	2.6	-
Wheat straw		0.01	81	3.8	-
		0.1	79	3	-
Soil		0.01	93	2	-
		0.1	91	3.5	-
IM 1-4					
Radish leaves		0.01	97	3.6	-
		0.1	102	3.2	-
Radish roots		0.01	98	4.7	-
		0.1	98	2.4	-
Wheat grain		0.01	89	5.2	-
		0.1	91	2.2	-
Wheat straw		0.01	95	2.0	-
		0.1	96	1.6	-
Soil		0.01	77	3.1	-
		0.1	78	3.8	-
IM 1-5					
Radish leaves		0.01	72	1.2	-
		0.1	102	2.2	-
Radish roots		0.01	72	2.4	-
		0.1	97	2.0	-
Wheat grain		0.01	79	4.3	-
		0.1	95	4.0	-
Wheat straw		0.05	77	3.2	-
		0.5	81	3	-
Soil		0.01	90	9.7	-
		0.1	89	6.4	-

Table A 61: Procedural recovery results from method validation of acetamiprid using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
Radish leaves	Acetamiprid	0.01	96	10	-
		0.1	94	9.3	-
Radish roots		0.01	96	9.8	-
		0.1	102	15	-

Spinach leaves		0.01	93	9.2	-
		0.1	89	11	-
Wheat grain		0.01 (n=2)	100	-	-
		0.1 (n=2)	97	-	-
Wheat straw		0.01 (n=2)	93	-	-
		0.1 (n=2)	90	-	-
Soil		0.01	97	5.1	-
		0.1	100	6.9	-

Table A 62: Characteristics for the analytical method used for validation of acetamiprid residues in soil and in rotational crops

	Acetamiprid
Specificity	The HPLC-MS/MS with two ions monitored is highly specific for the determination of acetamiprid. No interference above ≥ 30% LOQ
Calibration (type, number of data points)	Nine-point linear calibration curve: Equations for acetamiprid: Radish (leaves): y= 112650.6548x + 931883.8865; r^2= 996 Radish roots : y= 72713. 0952x + 750264.2030; r^2= 0.988 Wheat grain: y= 98000.5952x + 722872.311; r^2= 0.996 Wheat straw : y= 13905.0000x + 439189.4466; r^2= 0.999 Soil: y= 92166.1310x +757081.3293; r^2= 0.9970
Calibration range	0.025 to 10.0 ng/mL for all matrices
Assessment of matrix effects is presented	Mean matrix effects for acetamiprid were insignificant (<20%) neither for IM 1-4 and IM 1-5
Limit of determination/quantification	LOQ for all matrices: 0.01 mg/kg (except for wheat straw for IM 1-5: 0.05 mg/kg)

Conclusion
The method validation S15-02364-L2 for the determination of acetamiprid, IM 1-4 and IM 1-5 in rotational crops and soil was fully validated according to the requirements of the SANCO/3029/99 rev. 4 and in terms of soil of SANCO/825/00 rev. 8.1. guidelines. The validation proof to be within the requirements of the guidelines and therefore the method can be fully accepted.

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

Matching data have been conducted and submitted to Ctgb to demonstrate access to a complete package according to Reg (EU)283/2013 and for data matching step. RMS Opinion on GLP compliance, guidance compliance and equivalent endpoint can be provided as soon as evaluation is finalised

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

Matching data have been conducted and submitted to Ctgb to demonstrate access to a complete package according to Reg (EU)283/2013 and for data matching step. RMS Opinion on GLP compliance, guidance compliance and equivalent endpoint can be provided as soon as evaluation is finalised

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

Matching data have been conducted and submitted to Ctgb to demonstrate access to a complete package according to Reg (EU)283/2013 and for data matching step. RMS Opinion on GLP compliance, guidance compliance and equivalent endpoint can be provided as soon as evaluation is finalised

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

No new or additional studies have been submitted