FINAL REGISTRATION REPORT

Part B

Section 5

Analytical Methods

Detailed summary of the risk assessment

Product code: SHA 3600 B

Product name(s): LABAMBA

Chemical active substance:

Lambda cyhalothrin, 100 g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

Applicant: Sharda Cropchem España S.L.

Submission date: March 2022

MS Finalisation date: July 2022; November 2022

Version history

When	What
July 2022	ZRMs Assessment
November 2022	Final Registration Report

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5 Analytical methods

5.1 Conclusion and summary of assessment

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

Sufficiently sensitive and selective analytical methods are available for all analytes included in the residue definitions.
Noticed data gaps are:
none

Commodity/crop	Supported/ Not supported
High water content commodities / Brassicas (Cabbage, Brussels sprouts, cauliflower), Tomato	**Supported**
Dry commodities / Winter cereals (wheat, barley, rye, oats, triticale)	**Supported**
High oil content commodities / Winter Oilseed rape	**Supported**

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

5.2.1 Analysis of the plant protection product (KCP 5.1.1)

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

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

Reference:	KCP 5.1.1
Report	Accelerated storage stability test by heating at elevated temperature lambda cyhalothrin 10% CS.
Guideline(s):	SANCO/3030/99 rev. 4
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The analytical method for the determination of active ingredient content in test item was validated by establishing specifity, linearity, range precision, LOD, LOQ and accuracy.

Preparation of blank formulation solution

Accurately 0.0250 g of blank formulation was weighed into a 50 mL volumetric flask, dissolved in about 20 mL of acetonitrile by sonicating for 2 minutes. After equilibrating to room temperature, volume was made up to the mark with acetonitrile and shaken thoroughly.
The specifity of the method for active ingredient was studied by injecting individual solutions of working standard solutions, test item solution, blank formulation solution and analytical blank (acetonitrile) to the HPLC operated under the conditions of the method being validated.

Preparation of calibration (standard) solution.

From the stock solution of Lambda Cyhalothrin prepared for accuracy test (1000µg/mL) an aliquot of 0.53 mL was pipetted out into 10 mL volumetric flask, volume was made up to the mark with acetonitrile.

Preparation of sample solution
About 0.05 g of the test item, in three replications (each ambient and aged sample) was taken into separate 100 mL volumetric flasks, dissolved in about 20 mL of acetonitrile and sonicated for 2 minutes. After attaining room temperature, the contents were made up to the mark using acetonitrile and shaken thoroughly. The solutions were analysed for the active ingredient content by injecting to HPLC.

Validation - Results and discussions

Table 5.2-1: Methods suitable for the determination of active substances lambda-cyhalothrin in plant protection product Lambda cyhalothrin 10% CS/SHA 3600 B

	Lambda-cyhalothrin
Author(s), year	D. Prakash, 2019
Principle of method	HPLC
Linearity (linear between mg/L / % range of the declared content) (correlation coefficient, expressed as r)	5 points (2.06, 20.55, 51.38, 102.75 and 205.50 µg/mL) Y = 24550.889x +3136.770 R = 1.00
Precision – Repeatability Mean n = 5 (%RSD)	%RSD = 1.25%
Accuracy n = 6 (% Recovery)	Mean recovery = 100.13%
Interference/ Specificity	No interference
Comment	LOD = 1.017 µg/mL LOQ = 3.090 µg/mL

Conclusion

According to the SANCO/3030/99 rev.4 (the test was started in April 2019) guidance document, the analytical method for the determination of lambda-cyhalothrin in the test item Lambda cyhalothrin 10% CS is validated and the method is acceptable.

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

No method was submitted during the EU review of lambda cyhalothrin since the active substance has no relevant impurities.

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

5.2.1.4 Applicability of existing CIPAC methods (KCP 5.1.1)

A CIPAC method No. 463 is available for lambda-cyhalothrin.

5.2.2 Methods for the determination of residues (KCP 5.1.2)

Please refer to the post-registration method.

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 Lambda-cyhalothrin (KCP 5.2)

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

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

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

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Plant, high water content	Lambda-cyhalothrin	0.01 mg/kg	Reg. (EU) 2021/590
Plant, high acid content		0.01 mg/kg	Reg. (EU) 2021/590
Plant, high protein/high starch content (dry commodities)		0.01 mg/kg	Reg. (EU) 2021/590
Plant, high oil content		0.01 mg/kg	Reg. (EU) 2021/590
Plant, difficult matrices		0.01 mg/kg	Reg. (EU) 2021/590

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
(hops, spices, tea)			
Muscle	Lambda-cyhalothrin	0.01 mg/kg	Reg. (EU) 2021/590
Milk		0.02 mg/kg	Reg. (EU) 2021/590
Eggs		0.01 mg/kg	Reg. (EU) 2021/590
Fat		0.01 mg/kg	Reg. (EU) 2021/590
Liver, kidney		0.01 mg/kg	Reg. (EU) 2021/590
Soil (Ecotoxicology)	Lambda-cyhalothrin (as the sum of cyhalothrin isomers)	0.05 mg/kg	common limit
Drinking water (Human toxicology)	Lambda-cyhalothrin (as the sum of cyhalothrin isomers)	0.1 µg/L	general limit for drinking water
Surface water (Ecotoxicology)	Lambda-cyhalothrin (as the sum of cyhalothrin isomers)	0.00022 µg/L	Lowest NOEC from aquatic toxicity study on *Mysidopsis bahia*
Air	Lambda-cyhalothrin	0.189 µg/m^3	AOEL sys: 0.00063 mg/kg bw/d
Tissue (meat or liver)	Lambda cyhalothrin	0.1 mg/kg	classified as T / T+
Body fluids		0.05 mg/kg	classified as T / T+

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

An overview on the acceptable methods and possible data gaps for analysis of Lambda-cyhalothrin in plant matrices is given in the following tables. For the detailed evaluation of new studies it is referred to Appendix 2.

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: Lambda-cyhalothrin				
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	STF Multi-residue method QuEChERS GC-MSD	H. Weber, 2011 Report No. S-11-00715 RAR, Sweden, 2013 EU Agreed
	ILV	0.01 mg/kg	STF Multi-residue method QuEChERS	T. Class, T. Kuhn, 2001 Report No. B 2179 G RAR, Sweden, 2013

Component of residue definition: Lambda-cyhalothrin				
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)	-	-	Selectivity of method is confirmed by additional fragment ion, therefore other confirmatory method is not required.
	Primary	0.01 mg/kg	TFL Multi-residue method LC-MS/MS	N. Robinson, 2011 Report No. 20110043 RAR, Sweden, 2013 EU Agreed
	ILV	0.01 mg/kg	TFL Multi-residue method LC-MS/MS	O. Buttler, 2012 Report No. 111013IR RAR, Sweden, 2013
	Confirmatory	-	-	LC/MS/MS is highly specific method and no confirmation is required.
	Primary	0.01 mg/kg	LC-MS/MS	KCP 5.2.1 M. Pivato, 2016 Report No. 16.554813.0006
	ILV	0.01 mg/kg	LC-MS/MS	KCP 5.2.1.1 P. Sikorski, 2017 Report No. ZBBZ-2016/03/DPL/1
	Confirmatory	-	-	LC/MS/MS is highly specific method therefore no other confirmatory method is required.
High acid content	Primary	0.01 mg/kg	STF Multi-residue method QuEChERS GC-MSD	H. Weber, 2011 Report No. S-11-00715 RAR, Sweden, 2013 EU Agreed
	ILV	-	-	Not provided

Component of residue definition: Lambda-cyhalothrin				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
				during EU review.
	Confirmatory (if required)	-	-	Selectivity of method is confirmed by additional fragment ion, therefore other confirmatory method is not required.
	Primary	0.01 mg/kg	TFL Multi-residue method LC-MS/MS	N. Robinson, 2011 Report No. 20110043 RAR, Sweden, 2013 EU Agreed
	ILV	-	-	Not provided during EU review.
	Confirmatory	-	-	LC-MS/MS is highly specific method and no confirmation is required.
	Primary	0.01 mg/kg	LC-MS/MS	KCP 5.2.2 M. Pivato, 2016 Report No. 16.554813.0008
	ILV	0.01 mg/kg	LC-MS/MS	KCP 5.2.2.1 P. Sikorski, 2017 Report No. ZBBZ-2016/03/DPL/3
	Confirmatory	-	-	LC/MS/MS is highly specific method therefore no other confirmatory method is required.
High oil content	Primary	0.01 mg/kg	STF Multi-residue method QuEChERS GC-MSD	H. Weber, 2011 Report No. S-11-00715 RAR, Sweden, 2013 EU Agreed
	ILV	-	-	Not provided

Component of residue definition: Lambda-cyhalothrin				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
				during EU review.
	Confirmatory (if required)	-	-	Selectivity of method is confirmed by additional fragment ion, therefore other confirmatory method is not required.
	Primary	0.01 mg/kg	TFL Multi-residue method LC-MS/MS	N. Robinson, 2011 Report No. 20110043 RAR, Sweden, 2013 EU Agreed
	ILV	-	-	Not provided during EU review.
	Confirmatory	-	-	LC-MS/MS is highly specific method and no confirmation is required.
	Primary	0.01 mg/kg	LC-MS/MS	KCP 5.2.3 M. Pivato, 2016 Report No. 16.554813.0005
	ILV	0.01 mg/kg	LC-MS/MS	KCP 5.2.3.1 P. Sikorski, 2017 Report No. ZBBZ-2016/03/DPL/2
	Confirmatory	-	-	LC/MS/MS is highly specific method therefore no other confirmatory method is required.
High protein/high starch content (dry)	Primary	0.01 mg/kg	STF Multi-residue method QuEChERS GC-MSD	H. Weber, 2011 Report No. S-11-00715 RAR, Sweden, 2013 EU Agreed
	ILV	0.01 mg/kg	STF Multi-residue method QuEChERS	T. Class, T.

Component of residue definition: Lambda-cyhalothrin				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
				Kuhn, 2001 Report No. B 2179 G RAR, Sweden, 2013
	Confirmatory (if required)	-	-	Selectivity of method is confirmed by additional fragment ion, therefore other confirmatory method is not required.
	Primary	0.01 mg/kg	TFL Multi-residue method LC-MS/MS	N. Robinson, 2011 Report No. 20110043 RAR, Sweden, 2013 EU Agreed
	ILV	0.01 mg/kg	TFL Multi-residue method LC-MS/MS	O. Buttler, 2012 Report no. 111013IR RAR, Sweden, 2013
	Confirmatory	-	-	LC-MS/MS is highly specific method and no confirmation is required.
	Primary	0.01 mg/kg	LC-MS/MS	KCP 5.2.4 M. Pivato, 2016 Report No. 16.554813.0007
	ILV	0.01 mg/kg	LC-MS/MS	KCP 5.2.4.1 P. Sikorski, 2017 Report No. ZBBZ-2016/03/DPL/4
	Confirmatory	-	-	LC/MS/MS is highly specific method therefore no other confirmatory method is required.

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

Table 5.3-3: Statement on extraction efficiency

	Method for products of plant origin
Required, available from:	Not required
Not required, because:	Residues ≥LOQ are not expected.

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 Lambda-cyhalothrin in animal matrices is given in the following tables. For the detailed evaluation of new studies it is referred to Appendix 2.

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

Component of residue definition: lambda cyhalothrin				
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	STF Multi-residue method DFG-S19 GC-MS	T. Kuhn, T. Class, 2010 Report No. B1866G RAR, Sweden, 2013 EU Agreed
	ILV	0.01 mg/kg	STF Multi-residue method DFG-S19 GC-MS	H. Weber, 2011a Report No. S10-03749 RAR, Sweden, 2013
	Confirmatory (if required)	-	-	Selectivity of method is confirmed by additional fragment ion, therefore other confirmatory method is not required.
	Primary	0.01 mg/kg	TFL Multi-residue method LC-MS/MS	N. Robinson, 2011a Report No. 20110044 RAR, Sweden, 2013 EU Agreed
	ILV	0.01 mg/kg	TFL Multi-residue method LC-MS/MS	O. Buttler, 2012 Report No.

Component of residue definition: lambda cyhalothrin				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
				111013IR RAR, Sweden, 2013
	Confirmatory (if required)	-	-	LC-MS/MS is highly specific method and no confirmation is required.
	Primary	0.01 mg/kg	LC-MS/MS	KCP 5.2.5 M. Pivato, 2016 Report No. 16.554813.0009
	ILV	0.01 mg/kg	LC-MS/MS	KCP 5.2.5.1 P. Sikorski, 2017 Report No. ZBBZ-2016/02/DPL/1
	Confirmatory	-	-	LC/MS/MS is highly specific method therefore no other confirmatory method is required.
Eggs	Primary	0.01 mg/kg	STF Multi-residue method DFG-S19 GC-MS	T. Kuhn, T. Class, 2010 Report No. B1866G RAR, Sweden, 2013 EU Agreed
	ILV	-	-	Not privided during EU review.
	Confirmatory (if required)	-	-	Selectivity of method is confirmed by additional fragment ion, therefore other confirmatory method is not required.
	Primary	0.01 mg/kg	TFL Multi-residue method LC-MS/MS	N. Robinson, 2011a Report No. 20110044 RAR, Sweden, 2013 EU Agreed

Component of residue definition: lambda cyhalothrin				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
	ILV	-	-	Not privided during EU review.
	Confirmatory (if required)	-	-	LC-MS/MS is highly specific method and no confirmation is required.
	Primary	0.01 mg/kg	LC-MS/MS	KCP 5.2.6 M. Pivato, 2016 Report No. 16.554813.0011
	ILV	0.01 mg/kg	LC-MS/MS	KCP 5.2.6.1 P. Sikorski, 2017 Report No. ZBBZ-2016/02/DPL/4
	Confirmatory	-	-	LC/MS/MS is highly specific method therefore no other confirmatory method is required.
Muscle	Primary	0.01 mg/kg	STF Multi-residue method DFG-S19 GC-MS	T. Kuhn, T. Class, 2010 Report No. B1866G RAR, Sweden, 2013 EU Agreed
	ILV	-	-	Not privided during EU review.
	Confirmatory (if required)	-	-	Selectivity of method is confirmed by additional fragment ion, therefore other confirmatory method is not required
	Primary	0.01 mg/kg	TFL Multi-residue method LC-MS/MS	N. Robinson, 2011a Report No. 20110044 RAR, Sweden, 2013 EU Agreed

Component of residue definition: lambda cyhalothrin				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
	ILV	0.01 mg/kg	TFL Multi-residue method LC-MS/MS	O. Buttler, 2012 Report No. 111013IR RAR, Sweden, 2013
	Confirmatory (if required)	-	-	LC-MS/MS is highly specific method and no confirmation is required.
	Primary	0.01 mg/kg	LC-MS/MS	KCP 5.2.7 xxxxxxx, 2016 Report No. 16.554813.0012
	ILV	0.01 mg/kg	LC-MS/MS	KCP 5.2.7.1 xxxxxx, 2017 Report No. ZBBZ-2016/02/DPL/3
	Confirmatory	-	-	LC/MS/MS is highly specific method there-fore no other confirmatory method is re-quired.
Fat	Primary	0.01 mg/kg	STF Multi-residue method DFG-S19 GC-MS	T. Kuhn, T. Class, 2010 Report No. B1866G RAR, Sweden, 2013 EU Agreed
	ILV	0.01 mg/kg	STF Multi-residue method DFG-S19 GC-MS	H. Weber, 2011a Report No. S10-03749 RAR, Sweden, 2013
	Confirmatory (if required)	Confirmatory (if required)	-	Selectivity of method is con-firmed by addi-tional fragment ion, therefore other confirma-tory method is not required.
	Primary	0.01 mg/kg	TFL Multi-residue method LC-MS/MS	N. Robinson, 2011a Report No.

Component of residue definition: lambda cyhalothrin				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
				20120107 RAR, Sweden, 2013 EU Agreed
	ILV	0.01 mg/kg	TFL Multi-residue method LC-MS/MS	O. Buttler, 2012 Report No. 111013IR RAR, Sweden, 2013
	Confirmatory (if required)	-	-	LC-MS/MS is highly specific method and no confirmation is required.
	Primary	0.01 mg/kg	LC-MS/MS	KCP 5.2.8 xxxxxxx, 2016 Report No. 16.554813.0010
	ILV	0.01 mg/kg	LC-MS/MS	KCP 5.2.8.1 xxxxxxx, 2017 Report No. ZBBZ-2016/02/DPL/5
	Confirmatory	-	-	LC/MS/MS is highly specific method therefore no other confirmatory method is required.
Kidney, liver	Primary	0.01 mg/kg	STF Multi-residue method DFG-S19 GC-MS	T. Kuhn, T. Class, 2010 Report No. B1866G RAR, Sweden, 2013 EU Agreed
	ILV	0.01 mg/kg	STF Multi-residue method DFG-S19 GC-MS	H. Weber, 2011a Report No. S10-03749 RAR, Sweden, 2013
	Confirmatory (if required)	-	-	Selectivity of method is confirmed by additional fragment ion, therefore other confirmatory method is

Component of residue definition: lambda cyhalothrin				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
				not required.
	Primary	0.01 mg/kg	TFL Multi-residue method LC-MS/MS	N. Robinson, 2011a Report No. 20110044 RAR, Sweden, 2013 EU Agreed
	ILV	-	-	Not privided during EU review.
	Confirmatory (if required)	-	-	LC-MS/MS is highly specific method and no confirmation is required.
	Primary	0.01 mg/kg	LC-MS/MS	KCP 5.2.9 xxxxx, 2016 Report No. 16.554813.0013
	ILV	0.01 mg/kg	LC-MS/MS	KCP 5.2.9.1 xxxxxx, 2017 Report No. ZBBZ-2016/02/DPL/2
	Confirmatory	-	-	LC/MS/MS is highly specific method there-fore no other confirmatory method is re-quired.

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

Table 5.3-5: Statement on extraction efficiency

	Method for products of animal origin
Required, available from:	Lambda-cyhalothrin – The metabolism of [14C]- Lambda-cyhalothrin in Lactating Goat, Report No. 32458, RAR Sweden 2013
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 Lambda-cyhalothrin in soil

is given in the following tables. For the detailed evaluation of new studies it is referred to Appendix 2.

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

Component of residue definition: lambda-cyhalothrin (as the sum of cyhalothrin isomers)			
Method type	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	0.01 mg/kg	STF Multi-residue method GC-MS	F. Gemrot, 2012 Report No. GRM043.05A RAR, Sweden, 2013 EU Agreed
Confirmatory	-	-	Selectivity of method is confirmed by additional fragment ion, therefore other confirmatory method is not required.
Primary	0.05 mg/kg	TFL Multi-residue method LC-MS/MS	N. Robinson, 2011c Report No. 20110095 RAR, Sweden, 2013 EU Agreed
Confirmatory	-	-	LC-MS/MS is highly specific method, therefore confirmatory is not required.
Primary	0.01 mg/kg	LC-MS/MS	KCP 5.2.10 M. Pivato, 2016 Report No. 16.554813.0003
Confirmatory	-	-	LC/MS/MS is highly specific method therefore no other confirmatory method is required.

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

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 Lambda-cyhalothrin in

surface and drinking water is given in the following tables. For the detailed evaluation of new studies it is referred to Appendix 2.

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

Component of residue definition: lambda-cyhalothrin (as the sum of cyhalothrin isomers)				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Drinking water	Primary	0.002 µg/L	STF Multi-residue method GC-MS	N. Robinson, F. Gemrot, S. Braid, 2010 Report No. GRM043.02A RAR, Sweden, 2013 EU Agreed
	ILV	-	-	-
	Confirmatory	-	-	Selectivity of method is confirmed by additional fragment ion, therefore other confirmatory method is not required.
	Primary	0.1 µg/L	TFL Multi-residue method LC-MS/MS	N. Robinson, 2011d Report No. 20110045 RAR, Sweden, 2013 EU Agreed
	ILV	-	-	-
	Confirmatory	-	-	LC-MS/MS is highly specific method, therefore confirmatory is not required.
	Primary	0.0001 mg/L	LC-MS/MS	KCP 5.2.11 M. Pivato, 2016 Report No. 16.554813.0001
	Confirmatory	-	-	LC/MS/MS is highly specific method therefore no other confirmatory method is required.
Surface water	Primary	0.0002 µg/L	LC-MS	KCP 5.2.12 M. Pivato, 2016 Report No. 16.554813.002

Component of residue definition: lambda-cyhalothrin (as the sum of cyhalothrin isomers)				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
	Confirmatory	-	-	Selectivity of method is confirmed by additional fragment ion, therefore other confirmatory method is not required.

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

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 Lambda-cyhalothrin in air is given in the following tables. For the detailed evaluation of new studies it is referred to Appendix 2.

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

Component of residue definition: lambda-cyhalothrin			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	0.075 µg/m^3	STF Multi-residue method GC-MS	F. Gemrot, 2012a Report No. GRM043.06A RAR, Sweden, 2013 EU Agreed
Confirmatory	-	-	Selectivity of method is confirmed by additional fragment ion, therefore other confirmatory method is not required.
Primary	0.075 µg/m^3	LC-MS/MS	KCP 5.2.13 M. Pivato, 2016 Report No. 16.554813.0004
Confirmatory	-	-	LC/MS/MS is highly specific method therefore no other confirmatory method is required.

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

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 lambda cyhalothrin in body fluids and tissues is given in the following table. For the detailed evaluation of new studies it is referred to Appendix 2.

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

Component of residue definition: Lambda-cyhalothrin			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	0.01 mg/kg	STF Multi-residue method DFG-S19 GC-MS	T. Kuhn, T. Class, 2010 Report No. B1866G RAR, Sweden, 2013 EU Agreed
Confirmatory	-	-	Selectivity of method is confirmed by additional fragment ion, therefore other confirmatory method is not required.
Primary	0.05 mg/L	TFL Multi-residue method LC-MS/MS	N. Robinson, 2011e Report No. 20110046 RAR, Sweden, 2013 EU Agreed
Confirmatory	-	-	LC-MS/MS is highly specific method, therefore confirmatory is not required.
Primary	0.05 mg/kg	LC-MS/MS	KCP 5.2.14 M. Pivato, 2016 Report No. 16.554813.0014
Confirmatory	-	-	LC/MS/MS is highly specific method therefore no other confirmatory method is required.

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

5.3.2.8 Other studies/ information

Not relevant, not required.

Appendix 1 Lists of data considered in support of the evaluation

Tables considered not relevant can be deleted as appropriate.

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

List of data submitted by the applicant and relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 5.1.1	D. Prakash	2019	Accelerated storage stability test by heating at elevated temperature Lambda cyhalothrin 10% CS Eurofins Advinus Limited, Report G13955, 2019 GLP Unpublished	N	Sharda Cropchem Limited
KCP 5.2.1	M. Pivato	2016	Validation of the analytical procedure for the determination of lambda cyhaolothrin in potatoes by liquid chromatography CHELAB S.r.l., Report No. 16.554813.0006 GLP Unpublished	N	Sharda Cropchem Limited
KCP 5.2.1.1	P. Sikorski	2017	Independent Laboratory Validation of the Method for Determination of Lambda-Cyhalothrin in Potatoes by Liquid Chromatography Food Safety Labolatory Research Institute of Horticulture, Report No. ZBBZ-2016/03/DPL/1 GLP Unpublished	N	Sharda Cropchem Limited
KCP 5.2.2	M. Pivato	2016	Validation of the analytical procedure for the determination of lambda cyhalothrin in oranges by liquid chromatography CHELAB S.r.l., Report No. 16.554813.0008 GLP Unpublished	N	Sharda Cropchem Limited
KCP	P. Sikorski	2017	Independent Laboratory Validation of the Method for Determination of Lambda-Cyhalothrin in Oranges	N	Sharda

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
5.2.2.1			by Liquid Chromatography Food Safety Labolatory Research Institute of Horticulture, Report No. ZBBZ-2016/03/DPL/3 GLP Unpublished		Cropchem Limited
KCP 5.2.3	M. Pivato	2016	Validation of the analytical procedure for the determination of lambda cyhalothrin in rape seeds by liquid chromatography CHELAB S.r.l., Report No. 16.554813.0005 GLP Unpublished	N	Sharda Cropchem Limited
KCP 5.2.3.1	P. Sikorski	2017	Independent Laboratory Validation of the Method for Determination of Lambda-Cyhalothrin in Rape Seeds by Liquid Chromatography Food Safety Labolatory Research Institute of Horticulture, Report No. ZBBZ-2016/03/DPL/2 GLP Unpublished	N	Sharda Cropchem Limited
KCP 5.2.4	M. Pivato	2016	Validation of the analytical procedure for the determination of lambda cyhalothrin in wheat by liquid chromatography CHELAB S.r.l., Report No. 16.554813.0007 GLP Unpublished	N	Sharda Cropchem Limited
KCP 5.2.4.1	P. Sikorski	2017	Independent Laboratory Validation of the Method for Determination of Lambda-Cyhalothrin in Wheat by Liquid Chromatography Food Safety Labolatory Research Institute of Horticulture, Report No. ZBBZ-2016/03/DPL/4 GLP Unpublished	N	Sharda Cropchem Limited
KCP 5.2.5	M. Pivato	2016	Validation of the analytical procedure for the determination of lambda cyhalothrin in milk by liquid chromatography CHELAB S.r.l., Report No. 16.554813.0009 GLP Unpublished	N	Sharda Cropchem Limited

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 5.2.5.1	P. Sikorski	2017	Independent Laboratory Validation of the Method for Determination of Lambda-Cyhalothrin in Milk by Liquid Chromatography Food Safety Labolatory Research Institute of Horticulture, Report No. ZBBZ-2016/02/DPL/1 GLP Unpublished	N	Sharda Cropchem Limited
KCP 5.2.6	M. Pivato	2016	Validation of the analytical procedure for the determination of lambda cyhalothrin in eggs by liquid chromatography CHELAB S.r.l., Report No. 16.554813.0011 GLP Unpublished	N	Sharda Cropchem Limited
KCP 5.2.6.1	P. Sikorski	2017	Independent Laboratory Validation of the Method for Determination of Lambda-Cyhalothrin in Eggs by Liquid Chromatography Food Safety Labolatory Research Institute of Horticulture, Report No. ZBBZ02916/02/DPL/4 GLP Unpublished	N	Sharda Cropchem Limited
KCP 5.2.7	xxxxxxxxxx	2016	Validation of the analytical procedure for the determination of lambda cyhalothrin in meat by liquid chromatography xxxxxxxxxxx GLP Unpublished	N	Sharda Cropchem Limited
KCP 5.2.7.1	xxxxxxxxxx	2017	Independent Laboratory Validation of the Method for Determination of Lambda-Cyhalothrin in Meat by Liquid Chromatography xxxxxxxxxxxxxxxxxxxx, Report No. ZBBZ-2016/02/DPL/3 GLP Unpublished	N	Sharda Cropchem Limited
KCP 5.2.8	xxxxxxxxx	2016	Validation of the analytical procedure for the determination of lambda cyhalothrin in fat by liquid chromatography xxxxxxxx Report No. 16.554813.0010 GLP Unpublished	N	Sharda Cropchem Limited

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 5.2.8.1	xxxxxxxxxxx	2017	Independent Laboratory Validation of the Method for Determination of Lambda-Cyhalothrin in Fat by Liquid Chromatography xxxxxxxxxxxxxxxxxxxxx, Report No. ZBBZ-2016/02/DPL/5 GLP Unpublished	N	Sharda Cropchem Limited
KCP 5.2.9	xxxxxxxxxxx	2016	Validation of the analytical procedure for the determination of lambda cyhalothrin in liver by liquid chromatography xxxxxxxxxxxxx Report No. 16.554813.0013 GLP Unpublished	N	Sharda Cropchem Limited
KCP 5.2.9.1	xxxxxxxxxxx	2017	Independent Laboratory Validation of the Method for Determination of Lambda-Cyhalothrin in Liver by Liquid Chromatography xxxxxxxxxxxxxxxxxxxxx, Report No. ZBBZ-2016/02/DPL/2 GLP Unpublished	N	Sharda Cropchem Limited
KCP 5.2.10	M. Pivato	2016	Validation of the analytical procedure for the determination of Lambda Cyhalothrin in sand by liquid chromatography CHELAB S.r.l., Report No. 16.554813.0003 GLP Unpublished	N	Sharda Cropchem Limited
KCP 5.2.11	M. Pivato	2016	Validation of the analytical procedure for the determination of Lambda Cyhalothrin in drinking water by liquid chromatography CHELAB S.r.l., Report No. 16.554813.0001 GLP Unpublished	N	Sharda Cropchem Limited
KCP 5.2.12	M. Pivato	2016	Validation of the analitycal procedure for the determination of lambda cyhalothrin in surface water by liquid chromatography Chelab S.r.l., Report No. 16.554813.0002 GLP Unpublished	N	Sharda Cropchem Limited

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 5.2.13	M. Pivato	2016	Validation of the analytical procedure for the determination of lambda cyhalothrin in ambiental air by liquid chromatography CHELAB S.r.l., Report No. 16.554813.0004 GLP Unpublished	N	Sharda Cropchem Limited
KCP 5.2.14	M. Pivato	2016	Validation of the analytical procedure for the determination of lambda cyhalothrin in blood by liquid chromatography CHELAB S.r.l., Report No. 16.554813.0014 GLP Unpublished	N	Sharda Cropchem Limited

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

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

The following tables are to be completed by MS

List of data submitted by the applicant and not relied on

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

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

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

Appendix 2 Detailed evaluation of submitted analytical methods

A 2.1 Analytical methods for Lambda-Cyhalothrin

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

Please refer to the post-authorisation method.

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 1

A 2.1.2.1.1.1 Method validation

Comments of zRMS:	Method is accepted

Reference:	KCP 5.2.1
Report	Validation of the analytical procedure for the determination of Lambda Cyhalothrin in potatoes by liquid chromatography. M. Pivato, 2016, Report No. 16.554813.0006
Guideline(s):	SANCO/3030/99 Rev. 4 SANCO/825/00 Rev. 8.1 SANCO/3029/99 Rev. 4 OECD-204/2014
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Short term study for the validation of analytical method, based on QuECheRs procedure for the determination of Lambda Cyhalothrin in potato.

Mobile phase A (10 mM ammonium formate buffer 4.0)
About 0.63 g of ammonium formate were accurately weighed into a 100 mL volumetric flask and dissolved with milliQ water. 0.45 mL of formic acid 50% (v/v) were added and then the solution was diluted to volume with milliQ water.

Mobile phase B

Methanol.

Blank solution
10 mM ammonium formate buffer: acetonitrile 50:50 (v/v).

Extraction mixture
About 20 mL of glacial acetic acid and 1 mL of internal standard (Chlorpyrifos ethyl-D10) solution were introduced into a 2000 mL volumetric flask (glacial acetic acid final concentration: 1% v/v; internal standard final concentration about 100 ng/mL). The solution was filled up to the volume with acetonitrile.

Sample extraction
About 10 g of grounded potatoes were introduced in a 50 mL plastic tube and 10 mL of extraction mixture were added. After vortexing for about 1 min, a pouch of sodium acetate and magnesium sulfate anhydrous was added to the sample and vortexed again for about 1 min. The tube was centrifuged at 4750 rpm for 5 min and purified. Test sample was prepared in triplicate.

Instrumental conditions:
- Column: Acquity UPLC BEH C18, 50 mm x 2.1 mm x 1.7 µm
- Mobile phase A: 10 mM ammonium formate buffer pH 4.0
- Mobile phase B: Methanol
- Flow: 0.2 ml/min
- Injection volume: 5 µl
- Detector: MS XEVO TQS (Waters-Micromass)
- Source: APCI
- Source temp.: 150 °C
- Probe temp.: 500 °C
- Cone gas: 150 l/h
- Desolvation gas: 800 l/h
- Run time: 13 minutes
- Run mode: MRM (First transition: 450 > 225 m/z; Second transition (confirmatory ion): 450 > 141 m/z)
- Elution: Gradient

Results and discussions

Table A 1: Recovery results from method validation of Lambda-Cyhalothrin using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
Potato	Lambda-cyhalothrin	0.01	100	2.3	First mass transition
		0.1	94	7.1	
		0.01	101	3.0	Second mass ransition
		0.1	97	5.7	

Table A 2: Characteristics for the analytical method used for validation of Lambda-cyhalothrin residues in potato

	Lambda-cyhalothrin
Specificity	For both ion mass transitions, the specimen showed no significant interference (above 30 % of the LOQ) at the retention time of the analyte. The method is specific.
Calibration (type, number of data points)	7 points 0.003 mg/kg to 0.321 mg/kg First mass transition y=270059x R^2=1.00 Second mass transition y=92804x R^2=1.00
Assessment of matrix effects is presented	Yes
Limit of determination/quantification	LOQ = 0.01 mg/kg LOD = 0.003 mg/kg

Conclusion

According to SANTE/2020/12830 Rev.1 the method was successfully validated and suitable for determination of residues of lambda-cyhalothrin in potato.

A 2.1.2.1.1.2 Independent laboratory validation

Comments of zRMS:	Validation is accepted

Reference:	KCP 5.2.1.1
Report	Independent Laboratory Validation of the Method for Determination of Lambda-Cyhalothrin in Potatoes by Liquid Chromatography. P. Sikorski, 2017, Report No. ZBBZ-2016/03/DPL/1
Guideline(s):	SANCO/3030/99 Rev. 4 SANCO/825/00 Rev. 8.1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The objective of the study was to independently validate a method for the determination of Lambda-Cyhalothrin in potatoes in accordance to the guidance document SANCO/825/00, rev. 8.1 of the European Commission. The validated limit of quantification is 0.01 mg/kg.
In brief sample of potatoes was extracted with acidified acetonitrile. After addition of a buffer-salt mixture containing magnesium sulphate and sodium acetate the extract was shaken. After centrifugation, an aliquot of the acetonitrile phase was cleaned by primary secondary amine (PSA) and dehydrated by magnesium sulphate addition.

Selectivity and Confirmation of Residue Identity

Quantification was performed by use of highly selective liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). Two selected ion mass transitions were evaluated in order to demonstrate that the method achieves a high level of selectivity. The retention times of analyte in extracts corresponds to that of the calibration standards with a tolerance of < ± 0.1 min. also confirmation ratios for Lambda-Cyhalothrin in all samples were within ± 30 % of the average found for the standards.
No significant interference above 30 % of LOQ was detected in any of the reagent blanks or control specimen extracts of potatoes matrix, so that a high level of selectivity was demonstrated and an additional confirmatory method is not necessary.

Matrix Effects
Matrix effects on the detection of Lambda-Cyhalothrin in extracts of potatoes were found to be insignificant (< 20 %). However, and without any significant impact on the results, matrix-matched standards were used for quantification.

Linearity
The linearity of the detector response was demonstrated by single determination of matrix-matched calibration standards at seven concentration levels ranging from 0.001 µg/mL to 0.1 µg/mL. This range corresponds to 0.002 mg/kg to 0.2 mg/kg for potatoes thus covers the range from no more than 30 % of the LOQ and at least + 20 % of the highest analyte concentration level detected in sample.
The calibration curves obtained for both ion mass transitions were linear with the coefficients of determination (R^2) greater than 0.99. Linear regression was performed with 1/x weighting. In the validation study linear regression was performed without weighting with force through zero. Using 1/x weighting in this study without forcing through zero has no significant impact to the obtained results.

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

Mobile phase A (10 mM ammonium formate buffer 4.0)
500 mL volumetric flask was half filled with water, 0.315 g of ammonium formate and approximately 113 µL of formic acid was added than solution was agitated gently until all ammonium formate was completely dissolved. Volumetric flask was filled up to the mark with water, closed tightly and mixed by inverting several times. Subsequently proceeded to solvent filtration apparatus equipped with a 0.22 µm Teflon filter. After filtration solvent was transferred to amber HPLC solvent reservoir.

Mobile phase B
Methanol.

Blank solution
10 mM ammonium formate buffer: acetonitrile 50:50 (v/v).

Extraction mixture
500 mL volumetric flask was half filled with acetonitrile, 5 mL of acetic acid and 5 mL of Chlorpyrifos D10 working internal standard solution (for preparation of procedural reagent blank sample extraction mixture didn't contain internal standard) was added than volumetric flask was filled up to the mark, closed tightly and mixed by inverting several times.

Sample extraction
10.00 ± 0.1g of homogenized matrix was weighed into a 50 mL Teflon® centrifuge tube. Sample weight was recorded. If necessary fortification of the concurrent recovery sample(s) by aliquoting the fortification standard onto the matrix was carried out at this step. The tube was shaken in a vortex mixer for 1 min. and allowed to stand for about 5 min. Using glass volumetric pipettes 5 mL of LC/MS grade water and 10 mL of extraction mixture (99/1, v/v acetonitrile: acetic acid: 0.00483 µg/ml Chlorpyrifos D10) was added. The Teflon® centrifuge tube was closed tightly and vortex thoroughly for 1 min.

Results and discussions

Table A 3: Recovery results from independent laboratory validation of Lambda-Cyhalothrin using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (n = 5)	Mean recovery (%)	RSD (%)	Comments
Potato	Lambda-Cyhalothrin	0.01	105	3.5	First mass transition
		0.1	107	5.1	
		0.01	104	1.8	Second mass transition
		0.1	107	5.3	

Table A 4: Characteristics for the analytical method used for independent laboratory validation of Lambda-cyhalothrin residues in potato

	Lambda-cyhalothrin
Specificity	For both ion mass transitions, the specimen showed no significant interference (above 30 % of the LOQ) at the retention time of the analyte. The method is specific.
Calibration (type, number of data points)	7 points 0.002 mg/kg to 0.2 mg/kg First mass transition y=1511152.666149x-221.148811 R^2=0.99735883 Second mass transition y=494982.7772805x-51.169893 R^2=0.99745238
Assessment of matrix effects is presented	Yes
Limit of determination/quantification	LOQ = 0.01 mg/kg LOD = 0.002 mg/kg

Conclusion

According to SANTE/2020/12830 Rev.1 the method was successfully validated and is suitable for determination of Lambda-cyhalothrin residues in potato.

A 2.1.2.1.1.3 Confirmatory method (if required)

No confirmatory method is required.

A 2.1.2.1.2 Analytical method 2

A 2.1.2.1.2.1 Method validation

Comments of zRMS:	Validation is accepted

Reference:	KCP 5.2.2
Report	Validation of the analytical procedure for the determination of Lambda Cyhalothrin in oranges by liquid chromatography. M. Pivato, 2016, Report No. 16.554813.0008
Guideline(s):	SANCO/3030/99 Rev. 4 SANCO/825/00 Rev. 8.1 SANCO/3029/99 Rev. 4 OECD-204/2014
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Short term study for the validation of analytical method, based on QuECheRs procedure for the determination of Lambda Cyhalothrin in oranges.

Mobile phase A (10 mM ammonium formate buffer 4.0)
About 0.63 g of ammonium formate were accurately weighed into a 100 mL volumetric flask and dissolved with milliQ water. 0.45 mL of formic acid 50% (v/v) were added and then the solution was diluted to volume with milliQ water.

Mobile phase B
Methanol.

Blank solution
10 mM ammonium formate buffer: acetonitrile 50:50 (v/v).

Extraction mixture
Extraction mixture
About 20 mL of glacial acetic acid and 1 mL of internal standard (Chlorpyrifos ethyl-D10) solution were introduced into a 2000 mL volumetric flask (glacial acetic acid final concentration: 1% v/v; internal standard final concentration about 100 ng/mL). The solution was filled up to the volume with acetonitrile.

Sample extraction
About 10 g of grounded oranges were introduced in a 50 mL plastic tube, 5 mL of milliQ water and 10 mL of extraction mixture were added. After vortexing for about 1 min, a pouch of sodium acetate and magnesium sulfate anhydrous was added to the sample and vortexed again for about 1 min. The tube was centrifuged at 4750 rpm for 5 min and purified. Test sample was prepared in triplicate.

Instrumental conditions:
- Column: Acquity UPLC BEH C18, 50 mm x 2.1 mm x 1.7 µm
- Mobile phase A: 10 mM ammonium formate buffer pH 4.0
- Mobile phase B: Methanol
- Flow: 0.2 ml/min
- Injection volume: 5 µl
- Detector: MS XEVO TQS (Waters-Micromass)
- Source: APCI
- Source temp.: 150 °C
- Probe temp.: 500 °C
- Cone gas: 150 l/h
- Desolvation gas: 800 l/h

- Run time: 13 minutes
- Run mode: MRM (First transition: 450 > 225 m/z; Second transition (confirmatory ion): 450 > 141 m/z)
- Elution: Gradient

Results and discussions

Table A 5: Recovery results from method validation of Lambda-Cyhalothrin using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
Orange	Lambda-cyhalothrin	0.01	96	3.8	First mass transition
		0.1	89	5.8	
		0.01	100	5.0	Second mass transition
		0.1	93	5.0	

Table A 6: Characteristics for the analytical method used for validation of Lambda-cyhalothrin residues in oranges

	Lambda-cyhalothrin
Specificity	For both ion mass transitions, the specimen showed no significant interference (above 30 % of the LOQ) at the retention time of the analyte. The method is specific.
Calibration (type, number of data points)	7 points 0.003 mg/kg to 0.320 mg/kg First mass transition y=306998x R^2=1.00 Second mass transition y=109013x R^2=1.00
Assessment of matrix effects is presented	Yes
Limit of determination/quantification	LOQ = 0.01 mg/kg LOD = 0.003 mg/kg

Conclusion

According to SANTE/2020/12830 Rev.1 the method was successfully validated and suitable for determination of residues of lambda cyhalothrin in oranges.

A 2.1.2.1.2.2 Independent laboratory validation

Comments of zRMS:	Validation is accepted

Reference: KCP 5.2.2.1

Report Independent Laboratory Validation of the Method for Determination of

	Lambda-Cyhalothrin in Oranges by Liquid Chromatography. P. Sikorski, 2017, Report No. ZBBZ-2016/03/DPL/3
Guideline(s):	SANCO/3030/99 Rev. 4 SANCO/825/00 Rev. 8.1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The objective of the study was to independently validate a method for the determination of Lambda-Cyhalothrin in oranges in accordance to the guidance document SANCO/825/00, rev. 8.1 of the European Commission. The validated limit of quantification is 0.01 mg/kg.
In brief, before initial extraction water was added than oranges sample was extracted with acidified acetonitrile. After addition of a buffer-salt mixture containing magnesium sulphate and sodium acetate the extract was shaken. After centrifugation, an aliquot of the acetonitrile phase was cleaned by primary secondary amine (PSA) and dehydrated by magnesium sulphate addition.

Selectivity and Confirmation of Residue Identity
Quantification was performed by use of highly selective liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). Two selected ion mass transitions were evaluated in order to demonstrate that the method achieves a high level of selectivity. The retention times of analyte in extracts corresponds to that of the calibration standards with a tolerance of < ± 0.1 min. also confirmation ratios for Lambda-Cyhalothrin in all samples were within ± 30 % of the average found for the standards.
No significant interference above 30 % of LOQ was detected in any of the reagent blanks or control specimen extracts of oranges matrix, so that a high level of selectivity was demonstrated and an additional confirmatory method is not necessary.

Matrix Effects
Matrix effects on the detection of Lambda-Cyhalothrin in extracts of oranges were found to be insignificant (< 20 %). However, and without any significant impact on the results, matrix-matched standards were used for quantification.

Linearity
The linearity of the detector response was demonstrated by single determination of matrix-matched calibration standards at seven concentration levels ranging from 0.001 µg/mL to 0.1 µg/mL. This range corresponds to 0.002 mg/kg to 0.2 mg/kg for oranges thus covers the range from no more than 30 % of the LOQ and at least + 20 % of the highest analyte concentration level detected in sample.
The calibration curves obtained for both ion mass transitions were linear with the coefficients of determination (R^2) greater than 0.99. Linear regression was performed with 1/x weighting. In the validation study linear regression was performed without weighting with force through zero. Using 1/x weighting in this study without forcing through zero has no significant impact to the obtained results.

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

Mobile phase A (10 mM ammonium formate buffer 4.0)
500 mL volumetric flask was half filled with water, 0.315 g of ammonium formate and approximately 113 µL of formic acid was added than solution was agitated gently until all ammonium formate was completely dissolved. Volumetric flask was filled up to the mark with water, closed tightly and mixed by inverting several times. Subsequently proceeded to solvent filtration apparatus equipped with a 0.22 µm Teflon filter. After filtration solvent was transferred to amber HPLC solvent reservoir.

Mobile phase B
Methanol.

Blank solution
10 mM ammonium formate buffer: acetonitrile 50:50 (v/v).

Extraction mixture
500 mL volumetric flask was half filled with acetonitrile, 5 mL of acetic acid and 5 mL of Chlorpyrifos D10 working internal standard solution (for preparation of procedural reagent blank sample extraction mixture didn't contain internal standard) was added than volumetric flask was filled up to the mark, closed tightly and mixed by inverting several times.

Sample extraction
10.00 ± 0.1g of homogenized matrix was weighed into a 50 mL Teflon® centrifuge tube. Sample weight was recorded. If necessary fortification of the concurrent recovery sample(s) by aliquoting the fortification standard onto the matrix was carried out at this step. The tube was shaken in a vortex mixer for 1 min. and allowed to stand for about 5 min. Using glass volumetric pipettes 5 mL of LC/MS grade water and 10 mL of extraction mixture (99/1, v/v acetonitrile: acetic acid: 0.00483 µg/ml Chlorpyrifos D10) was added. The Teflon® centrifuge tube was closed tightly and vortex thoroughly for 1 min.

Results and discussions

Table A 7: Recovery results from independent laboratory validation of Lambda-Cyhalothrin using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (n = 5)	Mean recovery (%)	RSD (%)	Comments
Orange	Lambda-Cyhalothrin	0.01	95	3.7	First mass transition
		0.1	106	4.3	
		0.01	96	3.1	Second mass transition
		0.1	105	4.4	

Table A 8: Characteristics for the analytical method used for independent laboratory validation of lambda-cyhalothrin residues in oranges

	Lambda-cyhalothrin
Specificity	For both ion mass transitions, the specimen showed no significant interference (above 30 % of the LOQ) at the retention time of the analyte. The method is specific.
Calibration (type, number of data points)	7 points 0.002 mg/kg to 0.2 mg/kg First mass transition y=1668884.318626x+10.916729 R^2=0.99346098 Second mass transition y=549412.134703x+147.446110 R^2=0.99383607
Assessment of matrix effects is presented	Yes
Limit of determination/quantification	LOQ = 0.01 mg/kg

	Lambda-cyhalothrin
	LOD = 0.002 mg/kg

Conclusion

According to SANTE/2020/12830 Rev.1the method was successfully validated and is suitable for determination of Lambda-cyhalothrin residues in oranges.

A 2.1.2.1.2.3 Confirmatory method (if required)

No confirmatory method is required.

A 2.1.2.1.3 Analytical method 3

A 2.1.2.1.3.1 Method validation

Comments of zRMS:	Method is accepted

Reference:	KCP 5.2.3
Report	Validation of the analytical procedure for the determination of Lambda cyhalothrin in Rape seeds by liquid chromatography. M. Pivato, 2016, Report No. 16.554813.0005
Guideline(s):	SANCO/3030/99 Rev. 4 SANCO/825/00 Rev. 8.1 SANCO/3029/99 Rev. 4 OECD-204/2014
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Short term study for the validation of analytical method, based on QuECheRs procedure for the determination of Lambda Cyhalothrin in Rape seeds.

Mobile phase A (10 mM ammonium formate buffer 4.0)
About 0.63 g of ammonium formate were accurately weighed into a 1000 mL volumetric flask and dissolved with milliQ water. 0.45 mL of formic acid 50% (v/v) were added and then the solution was diluted to volume with milliQ water.

Mobile phase B
Methanol.

Blank solution
10 mM ammonium formate buffer: acetonitrile 50:50 (v/v).

Extraction mixture
About 20 mL of glacial acetic acid and 1 mL of internal standard (Chlorpyrifos ethyl-D10) solution were introduced into a 2000 mL volumetric flask (glacial acetic acid final concentration: 1% v/v; internal standard final concentration about 100 ng/mL). The solution was filled up to the volume with acetonitrile.

Sample extraction
About 5 g of grinded rape seeds were introduced in a 50 mL plastic tube, 7.5 mL of milliQ water were added in order to dissolve the matrix and then, 10 mL of extraction mixture were added to the sample. After vortexing for about 1 min, apouch of sodium acetate and magnesium sulfate anhydrous was added to the sample and vortexed again for about 1 min. The tube was centrifuged at 4750 rpm for 5 min and kept at about -20°C for about 2 hours. Then, the tube was centrifuged for 3 minutes at 4750 rpm and purified. Test sample was prepared in triplicate.

Instrumental conditions:
- Column: Acquity UPLC BEH C18, 50 mm x 2.1 mm x 1.7 µm
- Mobile phase A: 10 mM ammonium formate buffer pH 4.0
- Mobile phase B: Methanol
- Flow: 0.2 ml/min
- Injection volume: 5 µl
- Detector: MS XEVO TQS (Waters-Micromass)
- Source: APCI
- Source temp.: 150 °C
- Probe temp.: 500 °C
- Cone gas: 150 l/h
- Desolvation gas: 800 l/h
- Run time: 13 minutes
- Run mode: MRM (First transition: 450 > 225 m/z; Second transition (confirmatory ion): 450 > 141 m/z)
- Elution: Gradient

Results and discussions

Table A 9: Recovery results from method validation of Lambda-Cyhalothrin using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
Rape seeds	Lambda-cyhalothrin	0.01	99	3.1	First mass transition
		0.1	80	1.3	
		0.01	101	6.2	Second mass transition
		0.1	81	3.3	

Table A 10: Characteristics for the analytical method used for validation of Lambda-cyhalothrin residues in Rape seeds

	Lambda-cyhalothrin
Specificity	For both ion mass transitions, the specimen showed no significant interference (above 30 % of the LOQ) at the retention time of the analyte. The method is specific.
Calibration (type, number of data points)	6 points

	Lambda-cyhalothrin
Specificity	For both ion mass transitions, the specimen showed no significant interference (above 30 % of the LOQ) at the retention time of the analyte. The method is specific.
	0.003 mg/kg to 0.407 mg/kg First mass transition y=225499x R^2=1.00 Second mass transition y=82660x R^2=1.00
Assessment of matrix effects is presented	Yes
Limit of determination/quantification	LOQ = 0.01 mg/kg LOD = 0.003 mg/kg

Conclusion

According to SANTE/2020/12830 Rev.1 the method was successfully validated and suitable for determination of residues of lambda cyhalothrin in Rape seeds.

A 2.1.2.1.3.2 Independent laboratory validation

Comments of zRMS:	Validation is accepted

Reference:	KCP 5.2.3.1
Report	Independent Laboratory Validation of the Method for Determination of Lambda-Cyhalothrin in Rape Seeds by Liquid Chromatography. P. Sikorski, 2017, Report No. ZBBZ-2016/03/DPL/2
Guideline(s):	SANCO/3030/99 Rev. 4 SANCO/825/00 Rev. 8.1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The objective of the study was to independently validate a method for the determination of Lambda-Cyhalothrin in rape seeds in accordance to the guidance document SANCO/825/00, rev. 8.1 of the European Commission. The validated limit of quantification is 0.01 mg/kg.
In brief, before initial extraction water was added than rape seeds sample was extracted with acidified acetonitrile. After addition of a buffer-salt mixture containing magnesium sulphate and sodium acetate the extract was shaken. After centrifugation and freezing out step, an aliquot of the acetonitrile phase was cleaned by primary secondary amine (PSA) and dehydrated by magnesium sulphate addition.

Selectivity and Confirmation of Residue Identity
Quantification was performed by use of highly selective liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). Two selected ion mass transitions were evaluated in order to demon-

strate that the method achieves a high level of selectivity. The retention times of analyte in extracts corresponds to that of the calibration standards with a tolerance of < ± 0.1 min. also confirmation ratios for Lambda-Cyhalothrin in all samples were within ± 30 % of the average found for the standards.
No significant interference above 30 % of LOQ was detected in any of the reagent blanks or control specimen extracts of rape seeds matrix, so that a high level of selectivity was demonstrated and an additional confirmatory method is not necessary.

Matrix Effects
Matrix effects on the detection of Lambda-Cyhalothrin in extracts of rape seeds were found to be significant (> 20 %). Thus matrix-matched standards were used for quantification.

Linearity
The linearity of the detector response was demonstrated by single determination of matrix-matched calibration standards at seven concentration levels ranging from 0.0005 µg/mL to 0.05 µg/mL. This range corresponds to 0.002 mg/kg to 0.2 mg/kg for rape seeds thus covers the range from no more than 30 % of the LOQ and at least + 20 % of the highest analyte concentration level detected in sample.
The calibration curves obtained for both ion mass transitions were linear with the coefficients of determination (R^2) greater than 0.99. Linear regression was performed with 1/x weighting. In the validation study linear regression was performed without weighting with force through zero. Using 1/x weighting in this study without forcing through zero has no significant impact to the obtained results.

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

Mobile phase A (10 mM ammonium formate buffer 4.0)
500 mL volumetric flask was half filled with water, 0.315 g of ammonium formate and approximately 113 µL of formic acid was added than solution was agitated gently until all ammonium formate was completely dissolved. Volumetric flask was filled up to the mark with water, closed tightly and mixed by inverting several times. Subsequently proceeded to solvent filtration apparatus equipped with a 0.22 µm Teflon filter. After filtration solvent was transferred to amber HPLC solvent reservoir.

Mobile phase B
Methanol.

Blank solution
10 mM ammonium formate buffer: acetonitrile 50:50 (v/v).

Extraction mixture
500 mL volumetric flask was half filled with acetonitrile, 5 mL of acetic acid and 5 mL of Chlorpyrifos D10 working internal standard solution (for preparation of procedural reagent blank sample extraction mixture didn't contain internal standard) was added than volumetric flask was filled up to the mark, closed tightly and mixed by inverting several times.

Sample extraction
5.00 ± 0.1g of homogenized matrix was weighed into a 50 mL Teflon® centrifuge tube. Sample weight was recorded. If necessary fortification of the concurrent recovery sample(s) by aliquoting the fortification standard onto the matrix was carried out at this step. The tube was shaken in a vortex mixer for 1 min. and allowed to stand for about 5 min. Using glass volumetric pipettes 7.5 mL of LC/MS grade water and 10 mL of extraction mixture (99/1, v/v acetonitrile: acetic acid: 0.00483 µg/ml Chlorpyrifos D10) was added. The Teflon® centrifuge tube was closed tightly and vortex thoroughly for 1 min.

Results and discussions

Table A 11: Recovery results from independent laboratory validation of Lambda-Cyhalothrin using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (n = 5)	Mean recovery (%)	RSD (%)	Comments
Rape seed	Lambda-Cyhalothrin	0.01	76	4.6	First mass transition
		0.1	77	5.5	
		0.01	78	7.7	Second mass transition
		0.1	76	5.4	

Table A 12: Characteristics for the analytical method used for independent laboratory validation of Lambda-cyhalothrin residues in rape seed

	Lambda-cyhalothrin
Specificity	For both ion mass transitions, the specimen showed no significant interference (above 30 % of the LOQ) at the retention time of the analyte. The method is specific.
Calibration (type, number of data points)	7 points 0.002 mg/kg to 0.2 mg/kg First mass transition y=10200006.230561+193.655590 R^2=0.0.99837379 Second mass transition y=340396.960079x+105.434696 R^2=0.99832022
Assessment of matrix effects is presented	Yes
Limit of determination/quantification	LOQ = 0.01 mg/kg LOD = 0.002 mg/kg

Conclusion

According to SANTE/2020/12830 Rev.1 the method was successfully validated and is suitable for determination of Lambda-cyhalothrin residues in rape seed.

A 2.1.2.1.3.3 Confirmatory method (if required)

No confirmatory method is required.

A 2.1.2.1.4 Analytical method 4

A 2.1.2.1.4.1 Method validation

Comments of zRMS:	Validation is accepted

Reference:	KCP 5.2.4
Report	Validation of the analytical procedure for the determination of lambda cyhalothrin in wheat by liquid chromatography. M. Pivato, 2016, Report No. 16.554813.0007
Guideline(s):	SANCO/3030/99 Rev. 4 SANCO/825/00 Rev. 8.1 SANCO/3029/99 Rev. 4 OECD-204/2014
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Short term study for the validation of analytical method, based on QuECheRs procedure for the determination of Lambda Cyhalothrin in wheat.

Mobile phase A (10 mM ammonium formate buffer 4.0)
About 0.63 g of ammonium formate were accurately weighed into a 1000 mL volumetric flask and dissolved with milliQ water. 0.45 mL of formic acid 50% (v/v) were added and then the solution was diluted to volume with milliQ water.

Mobile phase B
Methanol.

Blank solution
10 mM ammonium formate buffer: acetonitrile 50:50 (v/v).

Extraction mixture
About 20 mL of glacial acetic acid and 1 mL of internal standard (Chlorpyrifos ethyl-D10) solution were introduced into a 2000 mL volumetric flask (glacial acetic acid final concentration: 1% v/v; internal standard final concentration about 100 ng/mL). The solution was filled up to the volume with acetonitrile.

Sample extraction
About 5 g of wheat were introduced in a 50 mL plastic tube, 7.5 mL of milliQ water were added in order to dissolve the matrix and then, 10 mL of extraction mixture were added to the sample. After vortexing for about 1 min, apouch of sodium acetate and magnesium sulfate anhydrous was added to the sample and vortexed again for about 1 min. The tube was centrifuged at 4750 rpm for 5 min and kept at about -20°C for about 2 hours. Then, the tube was centrifuged for 3 minutes at 4750 rpm and purified. Test sample was prepared in triplicate.

Instrumental conditions:

- Column: Acquity UPLC BEH C18, 50 mm x 2.1 mm x 1.7 µm
- Mobile phase A: 10 mM ammonium formate buffer pH 4.0
- Mobile phase B: Methanol
- Flow: 0.2 ml/min
- Injection volume: 5 µl
- Detector: MS XEVO TQS (Waters-Micromass)
- Source: APCI
- Source temp.: 150 °C
- Probe temp.: 500 °C
- Cone gas: 150 l/h

- Desolvation gas: 800 l/h
- Run time: 13 minutes
- Run mode: MRM (First transition: 450 > 225 m/z; Second transition (confirmatory ion): 450 > 141 m/z)
- Elution: Gradient

Results and discussions

Table A 53: Recovery results from method validation of Lambda Cyhalothrin using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
Wheat	Lambda-cyhalothrin	0.01	97	1.2	First mass transition
		0.1	82	0.7	
		0.01	95	8.6	Second mass transition
		0.1	86	1.0	

Table A 14: Characteristics for the analytical method used for validation of Lambda-cyhalothrin residues in wheat

	Lambda-cyhalothrin
Specificity	For both ion mass transitions, the specimen showed no significant interference (above 30 % of the LOQ) at the retention time of the analyte. The method is specific.
Calibration (type, number of data points)	6 points 0.003 mg/kg to 0.43 mg/kg First mass transition y=259036x R^2=1.00 Second mass transition y=88876x R^2=1.00
Assessment of matrix effects is presented	Yes
Limit of determination/quantification	LOQ = 0.01 mg/kg LOD = 0.003 mg/kg

Conclusion

According to SANTE/2020/12830 Rev.1 the method was successfully validated and suitable for determination of residues of lambda cyhalothrin in wheat.

A 2.1.2.1.4.2 Independent laboratory validation

Comments of zRMS:	Validation is accepted

Reference: KCP 5.2.4.1

Report	Independent Laboratory Validation of the Method for Determination of Lambda-Cyhalothrin in Wheat by Liquid Chromatography. P. Sikorski, 2017, Report No. ZBBZ-2016/03/DPL/4
Guideline(s):	SANCO/3030/99 Rev. 4 SANCO/825/00 Rev. 8.1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The objective of the study was to independently validate a method for the determination of Lambda-Cyhalothrin in wheat in accordance to the guidance document SANCO/825/00, rev. 8.1 of the European Commission. The validated limit of quantification is 0.01 mg/kg.
In brief, before initial extraction water was added than wheat sample was extracted with acidified acetonitrile. After addition of a buffer-salt mixture containing magnesium sulphate and sodium acetate the extract was shaken. After centrifugation and freezing out step, an aliquot of the acetonitrile phase was cleaned by primary secondary amine (PSA) and dehydrated by magnesium sulphate addition.

Selectivity and Confirmation of Residue Identity
Quantification was performed by use of highly selective liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). Two selected ion mass transitions were evaluated in order to demonstrate that the method achieves a high level of selectivity. The retention times of analyte in extracts corresponds to that of the calibration standards with a tolerance of < ± 0.1 min. also confirmation ratios for Lambda-Cyhalothrin in all samples were within ± 30 % of the average found for the standards.
No significant interference above 30 % of LOQ was detected in any of the reagent blanks or control specimen extracts of wheat matrix, so that a high level of selectivity was demonstrated and an additional confirmatory method is not necessary.

Matrix Effects
Matrix effects on the detection of Lambda-Cyhalothrin in extracts of wheat were found to be significant (> 20 %). Thus matrix-matched standards were used for quantification.

Linearity
The linearity of the detector response was demonstrated by single determination of matrix-matched calibration standards at seven concentration levels ranging from 0.0005 µg/mL to 0.05 µg/mL. This range corresponds to 0.002 mg/kg to 0.2 mg/kg for wheat thus covers the range from no more than 30 % of the LOQ and at least + 20 % of the highest analyte concentration level detected in sample.
The calibration curves obtained for both ion mass transitions were linear with the coefficients of determination (R^2) greater than 0.99. Linear regression was performed with 1/x weighting. In the validation study linear regression was performed without weighting with force through zero. Using 1/x weighting in this study without forcing through zero has no significant impact to the obtained results.

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

Mobile phase A (10 mM ammonium formate buffer 4.0)
500 mL volumetric flask was half filled with water, 0.315 g of ammonium formate and approximately 113 µL of formic acid was added than solution was agitated gently until all ammonium formate was completely dissolved. Volumetric flask was filled up to the mark with water, closed tightly and mixed by inverting several times. Subsequently proceeded to solvent filtration apparatus equipped with a 0.22 µm

Teflon filter. After filtration solvent was transferred to amber HPLC solvent reservoir.

Mobile phase B
Methanol.

Blank solution
10 mM ammonium formate buffer: acetonitrile 50:50 (v/v).

Extraction mixture
500 mL volumetric flask was half filled with acetonitrile, 5 mL of acetic acid and 5 mL of Chlorpyrifos D10 working internal standard solution (for preparation of procedural reagent blank sample extraction mixture didn't contain internal standard) was added than volumetric flask was filled up to the mark, closed tightly and mixed by inverting several times.

Sample extraction
5.00 ± 0.1g of homogenized matrix was weighed into a 50 mL Teflon® centrifuge tube. Sample weight was recorded. If necessary fortification of the concurrent recovery sample(s) by aliquoting the fortification standard onto the matrix was carried out at this step. The tube was shaken in a vortex mixer for 1 min. and allowed to stand for about 5 min. Using glass volumetric pipettes 7.5 mL of LC/MS grade water and 10 mL of extraction mixture (99/1, v/v acetonitrile: acetic acid: 0.00483 µg/ml Chlorpyrifos D10) was added. The Teflon® centrifuge tube was closed tightly and vortex thoroughly for 1 min.

Results and discussions

Table A 15: Recovery results from independent laboratory validation of Lambda-cyhalothrin using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (n = 5)	Mean recovery (%)	RSD (%)	Comments
Wheat	Lambda-cyhalothrin	0.01	100	4.1	First mass transition
		0.1	106	4.0	
		0.01	108	7.6	Second mass transition
		0.1	105	3.5	

Table A 16: Characteristics for the analytical method used for independent laboratory validation of Lambda-cyhalothrin residues in wheat

	Lambda-cyhalothrin
Specificity	For both ion mass transitions, the specimen showed no significant interference (above 30 % of the LOQ) at the retention time of the analyte. The method is specific.
Calibration (type, number of data points)	7 points 0.002 mg/kg to 0.2 mg/kg First mass transition y=645115.505451x+75.057489 R^2=0.99967008 Second mass transition y=216112.042961+13.700847 R^2=0.99841651
Assessment of matrix effects is presented	Yes

	Lambda-cyhalothrin
Limit of determination/quantification	LOQ = 0.01 mg/kg LOD = 0.002 mg/kg

Conclusion

According to SANTE/2020/12830 Rev.1 the method was successfully validated and is suitable for determination of Lambda-cyhalothrin residues in wheat.

A 2.1.2.1.4.3 Confirmatory method (if required)

No confirmatory method is required.

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

A 2.1.2.2.1 Analytical method 1

A 2.1.2.2.1.1 Method validation

Comments of zRMS:	Validation is accepted

Reference:	KCP 5.2.5
Report	Validation of the analytical procedure for the determination of lambda cyhalothrin in milk by liquid chromatography. M. Pivato, 2016, Report No. 16.554813.0009
Guideline(s):	SANCO/3030/99 Rev. 4 SANCO/825/00 Rev. 8.1 SANCO/3029/99 Rev. 4 OECD-204/2014
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Short term study for the validation of analytical method, based on QuECheRs procedure for the determination of Lambda Cyhalothrin in milk.

Mobile phase A (10 mM ammonium formate buffer 4.0)
About 0.63 g of ammonium formate were accurately weighed into a 1000 mL volumetric flask and dissolved with milliQ water. 0.45 mL of formic acid 50% (v/v) were added and then the solution was diluted to volume with milliQ water.

Mobile phase B

Methanol.

Blank solution
10 mM ammonium formate buffer: acetonitrile 50:50 (v/v).

Extraction mixture
About 20 mL of glacial acetic acid and 1 mL of internal standard (Chlorpyrifos ethyl-D10) solution were introduced into a 2000 mL volumetric flask (glacial acetic acid final concentration: 1% v/v; internal standard final concentration about 100 ng/mL). The solution was filled up to the volume with acetonitrile.

Sample extraction
About 10 g of milk were introduced into a 50 mL plastic tube, 5 mL of milliQ water and 10 mL of extraction mixture were added to the sample. After vortexing for about 1 min, a pouch of sodium acetate and magnesium sulfate anhydrous was added to the sample and vortexed again for about 1 min. The tube was centrifuged at 4750 rpm for 5 min and purified. Test sample was prepared in triplicate.

Instrumental conditions:
- Column: Acquity UPLC BEH C18, 50 mm x 2.1 mm x 1.7 µm
- Mobile phase A: 10 mM ammonium formate buffer pH 4.0
- Mobile phase B: Methanol
- Flow: 0.2 ml/min
- Injection volume: 5 µl
- Detector: MS XEVO TQS (Waters-Micromass)
- Source: APCI
- Source temp.: 150 °C
- Probe temp.: 500 °C
- Cone gas: 150 l/h
- Desolvation gas: 800 l/h
- Run time: 13 minutes
- Run mode: MRM (First transition: 450 > 225 m/z; Second transition (confirmatory ion): 450 > 141 m/z)
- Elution: Gradient

Results and discussions

Table A 67: Recovery results from method validation of Lambda-cyhalothrin using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
Milk	Lambda-cyhalothrin	0.01	89	9.0	First mass transition
		0.1	86	5.5	
		0.01	93	11.3	Second mass transition
		0.1	89	7.3	

Table A 18: Characteristics for the analytical method used for validation of Lambda-cyhalothrin residues in milk

	Lambda-cyhalothrin
Specificity	For both ion mass transitions, the specimen showed no significant interference (above 30 % of the LOQ) at the retention time of the analyte. The method is specific.
Calibration (type, number of data points)	7 points 0.003 mg/kg to 0.341 mg/kg First mass transition y=245280x R^2=1.00 Second mass transition y=87412x R^2=1.00
Assessment of matrix effects is presented	Yes
Limit of determination/quantification	LOQ = 0.01 mg/kg LOD = 0.003 mg/kg

Conclusion

According to SANTE/2020/12830 Rev.1 the method was successfully validated and suitable for determination of residues of lambda cyhalothrin in milk.

A 2.1.2.2.1.2 Independent laboratory validation

Comments of zRMS:	Validation is accepted

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Reference:	KCP 5.2.5.1
Report	Independent Laboratory Validation of the Method for Determination of Lambda-Cyhalothrin in Milk by Liquid Chromatography. P. Sikorski, 2017, Report No. ZBBZ-2016/02/DPL/1
Guideline(s):	SANCO/3030/99 Rev. 4 SANCO/825/00 Rev. 8.1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The objective of the study was to independently validate a method for the determination of Lambda-Cyhalothrin in milk in accordance to the guidance document SANCO/825/00, rev. 8.1 of the European Commission. The validated limit of quantification is 0.01 mg/kg.
In brief, before initial extraction water was added than milk sample was extracted with acidified acetonitrile. After addition of a buffer-salt mixture containing magnesium sulphate and sodium acetate the extract was shaken. After centrifugation, an aliquot of the acetonitrile phase was cleaned by primary secondary amine (PSA) and dehydrated by magnesium sulphate addition.

Selectivity and Confirmation of Residue Identity
Quantification was performed by use of highly selective liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). Two selected ion mass transitions were evaluated in order to demonstrate that the method achieves a high level of selectivity. The retention times of analyte in extracts corresponds to that of the calibration standards with a tolerance of < ± 0.1 min. also confirmation ratios for Lambda-Cyhalothrin in all samples were within ± 30 % of the average found for the standards.
No significant interference above 30 % of LOQ was detected in any of the reagent blanks or control specimen extracts of milk matrix, so that a high level of selectivity was demonstrated and an additional confirmatory method is not necessary.

Matrix Effects
Matrix effects on the detection of Lambda-Cyhalothrin in extracts of milk were found to be insignificant (≤ 20 %). However, and without any significant impact on the results, matrix-matched standards were used for quantification.

Linearity
The linearity of the detector response was demonstrated by single determination of matrix-matched calibration standards at seven concentration levels ranging from 0.001 µg/mL to 0.1 µg/mL. This range corresponds to 0.002 mg/kg to 0.2 mg/kg for milk thus covers the range from no more than 30 % of the LOQ and at least + 20 % of the highest analyte concentration level detected in sample.
The calibration curves obtained for both ion mass transitions were linear with the coefficients of determination (R^2) greater than 0.99. Linear regression was performed with 1/x weighting. In the validation study linear regression was performed without weighting with force through zero. Using 1/x weighting in this study without forcing through zero has no significant impact to the obtained results.

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

Mobile phase A (10 mM ammonium formate buffer 4.0)
500 mL volumetric flask was half filled with water, 0.315 g of ammonium formate and approximately 113 µL of formic acid was added than solution was agitated gently until all ammonium formate was completely dissolved. Volumetric flask was filled up to the mark with water, closed tightly and mixed by inverting several times. Subsequently proceeded to solvent filtration apparatus equipped with a 0.22 µm Teflon filter. After filtration solvent was transferred to amber HPLC solvent reservoir.

Mobile phase B
Methanol.

Blank solution
10 mM ammonium formate buffer: acetonitrile 50:50 (v/v).

Extraction mixture
500 mL volumetric flask was half filled with acetonitrile, 5 mL of acetic acid and 5 mL of Chlorpyrifos D10 working internal standard solution (for preparation of procedural reagent blank sample extraction mixture didn't contain internal standard) was added than volumetric flask was filled up to the mark, closed tightly and mixed by inverting several times.

Sample extraction
Immediately after shaking 10.00± 0.1g of specimen material were weighed into a 50 mL Teflon centrifuge tube. Sample weight was recorded. If necessary fortification of the concurrent recovery sample(s) by aliquoting the fortification standard onto the matrix was carried out at this step. The tube was shaken in a vortex mixer for 1 min. and allowed to stand for about 5 min. Using glass volumetric pipettes 5 mL of LC/MS grade water and 10 mL of extraction mixture (99/1, v/v acetonitrile: acetic acid: 0.00483 µg/ml Chlorpyrifos D10) was added. The Teflon® centrifuge tube was closed tightly and vortex thoroughly for

1 min.

Results and discussions

Table A 19: Recovery results from independent laboratory validation of Lambda-cyhalothrin using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (n = 5)	Mean recovery (%)	RSD (%)	Comments
Milk	Lambda-cyhalothrin	0.01	93	2.5	First mass transition
		0.1	96	2.3	
		0.01	94	6.0	Second mass transition
		0.1	96	4.3	

Table A 20: Characteristics for the analytical method used for independent laboratory validation of Lambda-cyhalothrin residues in milk

	Lambda-cyhalothrin
Specificity	For both ion mass transitions, the specimen showed no significant interference (above 30 % of the LOQ) at the retention time of the analyte. The method is specific.
Calibration (type, number of data points)	7 points 0.002 mg/kg to 0.2 mg/kg First mass transition y=1693410.68995x-113.518359 R^2=0.99837263 Second mass transition y=207714.936946x+14.642774 R^2=0.99758636
Assessment of matrix effects is presented	Yes
Limit of determination/quantification	LOQ = 0.01 mg/kg LOD = 0.002 mg/kg

Conclusion

According to SANTE/2020/12830 Rev.1 the method was successfully validated and is suitable for determination of Lambda-cyhalothrin residues in milk.

A 2.1.2.2.1.3 Confirmatory method (if required)

No confirmatory method is required.

A 2.1.2.2.2 Analytical method 2

A 2.1.2.2.2.1 Method validation

Comments of zRMS:	Method is accepted

Reference:	KCP 5.2.6
Report	Validation of the analytical procedure for the determination of lambda cyhalothrin in eggs by liquid chromatography. M. Pivato, 2016, Report No. 16.554813.0011
Guideline(s):	SANCO/3030/99 Rev. 4 SANCO/825/00 Rev. 8.1 SANCO/3029/99 Rev. 4 OECD-204/2014
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Short term study for the validation of analytical method, based on QuECheRs procedure for the determination of Lambda Cyhalothrin in eggs.

Mobile phase A (10 mM ammonium formate buffer 4.0)
About 0.63 g of ammonium formate were accurately weighed into a 1000 mL volumetric flask and dissolved with milliQ water. 0.45 mL of formic acid 50% (v/v) were added and then the solution was diluted to volume with milliQ water.

Mobile phase B
Methanol.

Blank solution
10 mM ammonium formate buffer: acetonitrile 50:50 (v/v).

Extraction mixture
About 20 mL of glacial acetic acid and 1 mL of internal standard (Chlorpyrifos ethyl-D10) solution were introduced into a 2000 mL volumetric flask (glacial acetic acid final concentration: 1% v/v; internal standard final concentration about 100 ng/mL). The solution was filled up to the volume with acetonitrile.

Sample extraction
About 5 g of grinded eggs were introduced in a 50 mL plastic tube, 7.5 mL of milliQ water were added in order to dissolve the matrix and then, 10 mL of extraction mixture were added to the sample. After vortexing for about 1 min, a pouch of sodium acetate and magnesium sulfate anhydrous was added to the sample and vortexed again for about 1 min. The tube was centrifuged at 4750 rpm for 5 min and kept at about -20°C for about 2 hours. Then, the tube was centrifuged for 3 minutes at 4750 rpm and purified. Test sample was prepared in triplicate.

Instrumental conditions:
- Column: Acquity UPLC BEH C18, 50 mm x 2.1 mm x 1.7 µm
- Mobile phase A: 10 mM ammonium formate buffer pH 4.0
- Mobile phase B: Methanol
- Flow: 0.2 ml/min
- Injection volume: 5 µl
- Detector: MS XEVO TQS (Waters-Micromass)
- Source: APCI

- Source temp.: 150 °C
- Probe temp.: 500 °C
- Cone gas: 150 l/h
- Desolvation gas: 800 l/h
- Run time: 13 minutes
- Run mode: MRM (First transition: 450 > 225 m/z; Second transition (confirmatory ion): 450 > 141 m/z)
- Elution: Gradient

Results and discussions

Table A 21: Recovery results from method validation of Lambda-cyhalothrin using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
Eggs	Lambda-cyhalothrin	0.01	99	7.0	First mass transition
		0.1	82	1.7	
		0.01	98	8.4	Second mass transition
		0.1	81	2.1	

Table A 22: Characteristics for the analytical method used for validation of Lambda-cyhalothrin residues in eggs

	Lambda-cyhalothrin
Specificity	For both ion mass transitions, the specimen showed no significant interference (above 30 % of the LOQ) at the retention time of the analyte. The method is specific.
Calibration (type, number of data points)	6 points 0.003 mg/kg to 0.428 mg/kg First mass transition y=240614x R^2=1.00 Second mass transition y=88020x R^2=1.00
Assessment of matrix effects is presented	Yes
Limit of determination/quantification	LOQ = 0.01 mg/kg LOD = 0.003 mg/kg

Conclusion

According to SANTE/2020/12830 Rev.1 the method was successfully validated and suitable for determination of residues of lambda cyhalothrin in eggs.

A 2.1.2.2.2.2 Independent laboratory validation

Comments of zRMS:	Validation is accepted

Reference:	KCP 5.2.6.1
Report	Independent Laboratory Validation of the Method for Determination of Lambda-Cyhalothrin in Eggs by Liquid Chromatography. P. Sikorski, 2017, Report No. ZBBZ-2016/02/DPL/4
Guideline(s):	SANCO/3030/99 Rev. 4 SANCO/825/00 Rev. 8.1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The objective of the study was to independently validate a method for the determination of Lambda-Cyhalothrin in eggs in accordance to the guidance document SANCO/825/00, rev. 8.1 of the European Commission. The validated limit of quantification is 0.01 mg/kg.
In brief, before initial extraction water was added than eggs sample was extracted with acidified acetonitrile. After addition of a buffer-salt mixture containing magnesium sulphate and sodium acetate the extract was shaken. After centrifugation and freezing out step, an aliquot of the acetonitrile phase was cleaned by primary secondary amine (PSA) and dehydrated by magnesium sulphate addition.

Selectivity and Confirmation of Residue Identity
Quantification was performed by use of highly selective liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). Two selected ion mass transitions were evaluated in order to demonstrate that the method achieves a high level of selectivity. The retention times of analyte in extracts corresponds to that of the calibration standards with a tolerance of < ± 0.1 min. also confirmation ratios for Lambda-Cyhalothrin in all samples were within ± 30 % of the average found for the standards.
No significant interference above 30 % of LOQ was detected in any of the reagent blanks or control specimen extracts of eggs matrix, so that a high level of selectivity was demonstrated and an additional confirmatory method is not necessary.

Matrix Effects
Matrix effects on the detection of Lambda-Cyhalothrin in extracts of eggs were found to be insignificant (≤ 20 %). However, and without any significant impact on the results, matrix-matched standards were used for quantification.

Linearity
The linearity of the detector response was demonstrated by single determination of matrix-matched calibration standards at seven concentration levels ranging from 0.0005 µg/mL to 0.05 µg/mL. This range corresponds to 0.002 mg/kg to 0.2 mg/kg for eggs thus covers the range from no more than 30 % of the LOQ and at least + 20 % of the highest analyte concentration level detected in sample.
The calibration curves obtained for both ion mass transitions were linear with the coefficients of determination (R^2) greater than 0.99. Linear regression was performed with 1/x weighting. In the validation study linear regression was performed without weighting with force through zero. Using 1/x weighting in this study without forcing through zero has no significant impact to the obtained results.

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

Mobile phase A (10 mM ammonium formate buffer 4.0)

500 mL volumetric flask was half filled with water, 0.315 g of ammonium formate and approximately 113 µL of formic acid was added than solution was agitated gently until all ammonium formate was completely dissolved. Volumetric flask was filled up to the mark with water, closed tightly and mixed by inverting several times. Subsequently proceeded to solvent filtration apparatus equipped with a 0.22 µm Teflon filter. After filtration solvent was transferred to amber HPLC solvent reservoir.

Mobile phase B
Methanol.

Blank solution
10 mM ammonium formate buffer: acetonitrile 50:50 (v/v).

Extraction mixture
500 mL volumetric flask was half filled with acetonitrile, 5 mL of acetic acid and 5 mL of Chlorpyrifos D10 working internal standard solution (for preparation of procedural reagent blank sample extraction mixture didn't contain internal standard) was added than volumetric flask was filled up to the mark, closed tightly and mixed by inverting several times.

Sample extraction
5.00 ± 0.1g of homogenized matrix was weighed into a 50 mL Teflon® centrifuge tube. Sample weight was recorded. Sample weight was recorded. If necessary fortification of the concurrent recovery sample(s) by aliquoting the fortification standard onto the matrix was carried out at this step. The tube was shaken in a vortex mixer for 1 min. and allowed to stand for about 5 min. Using glass volumetric pipettes 7.5 mL of LC/MS grade water and 10 mL of extraction mixture (99/1, v/v acetonitrile: acetic acid: 0.00483 µg/ml Chlorpyrifos D10) was added. The Teflon® centrifuge tube was closed tightly and vortex thoroughly for 1 min.

Results and discussions

Table A 23: Recovery results from independent laboratory validation of Lambda-cyhalothrin using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (n = 5)	Mean recovery (%)	RSD (%)	Comments
Eggs	Lambda-cyhalothrin	0.01	82	1.7	First mass transition
		0.1	89	4.0	
		0.01	82	6.8	Second mass transition
		0.1	88	4.9	

Table A 27: Characteristics for the analytical method used for independent laboratory validation of Lambda-cyhalothrin residues in eggs

	Lambda-cyhalothrin
Specificity	For both ion mass transitions, the specimen showed no significant interference (above 30 % of the LOQ) at the retention time of the analyte. The method is specific.
Calibration (type, number of data points)	7 points 0.002 mg/kg to 0.2 mg/kg First mass transition y=1702714.376870x-82.612193 R^2=0.99715299

	Lambda-cyhalothrin
	Second mass transition y=214065.456871x-14.617513 R^2=0.99599540
Assessment of matrix effects is presented	Yes
Limit of determination/quantification	LOQ = 0.01 mg/kg LOD = 0.002 mg/kg

Conclusion

According to SANTE/2020/12830 Rev.1 the method was successfully validated and is suitable for determination of Lambda-cyhalothrin residues in eggs.

A 2.1.2.2.2.3 Confirmatory method (if required)

No confirmatory method is required.

A 2.1.2.2.3 Analytical method 3

A 2.1.2.2.3.1 Method validation

Comments of zRMS:	Validation is accepted

Reference:	KCP 5.2.7
Report	Validation of the analytical procedure for the determination of lambda cyhalothrin in meat by liquid chromatography. xxxxxxxxxx, 2016, Report No. 16.554813.0012
Guideline(s):	SANCO/3030/99 Rev. 4 SANCO/825/00 Rev. 8.1 SANCO/3029/99 Rev. 4 OECD-204/2014
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Short term study for the validation of analytical method, based on QuECheRs procedure for the determination of Lambda Cyhalothrin in meat.

Mobile phase A (10 mM ammonium formate buffer 4.0)
About 0.63 g of ammonium formate were accurately weighed into a 1000 mL volumetric flask and dissolved with milliQ water. 0.45 mL of formic acid 50% (v/v) were added and then the solution was diluted to volume with milliQ water.

Mobile phase B

Methanol.

Blank solution
10 mM ammonium formate buffer: acetonitrile 50:50 (v/v).

Extraction mixture
About 20 mL of glacial acetic acid and 1 mL of internal standard (Chlorpyrifos ethyl-D10) solution were introduced into a 2000 mL volumetric flask (glacial acetic acid final concentration: 1% v/v; internal standard final concentration about 100 ng/mL). The solution was filled up to the volume with acetonitrile.

Sample extraction
About 5 g of grinded meat were introduced in a 50 mL plastic tube, 7.5 mL of milliQ water were added in order to dissolve the matrix and then, 10 mL of extraction mixture were added to the sample. After vortexing for about 1 min, a pouch of sodium acetate and magnesium sulfate anhydrous was added to the sample and vortexed again for about 1 min. The tube was centrifuged at 4750 rpm for 5 min and kept at about -20°C for about 2 hours. Then, the tube was centrifuged for 3 minutes at 4750 rpm and purified. Test sample was prepared in triplicate.

Instrumental conditions:
- Column: Acquity UPLC BEH C18, 50 mm x 2.1 mm x 1.7 µm
- Mobile phase A: 10 mM ammonium formate buffer pH 4.0
- Mobile phase B: Methanol
- Flow: 0.2 ml/min
- Injection volume: 5 µl
- Detector: MS XEVO TQS (Waters-Micromass)
- Source: APCI
- Source temp.: 150 °C
- Probe temp.: 500 °C
- Cone gas: 150 l/h
- Desolvation gas: 800 l/h
- Run time: 13 minutes
- Run mode: MRM (First transition: 450 > 225 m/z; Second transition (confirmatory ion): 450 > 141 m/z)
- Elution: Gradient

Results and discussions

Table A 25: Recovery results from method validation of Lambda-cyhalothrin using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
Meat	Lambda-cyhalothrin	0.01	102	4.6	First mass transition
		0.1	90	4.0	
		0.01	106	2.5	Second mass transition
		0.1	90	5.6	

Table A 26: Characteristics for the analytical method used for validation of Lambda-cyhalothrin residues in meat

	Lambda-cyhalothrin
Specificity	For both ion mass transitions, the specimen showed no significant interference (above 30 % of the LOQ) at the retention time of the analyte. The method is specific.
Calibration (type, number of data points)	6 points 0.003 mg/kg to 0.426 mg/kg First mass transition y=218205x R^2=1.00 Second mass transition y=78858x R^2=1.00
Assessment of matrix effects is presented	Yes
Limit of determination/quantification	LOQ = 0.01 mg/kg LOD = 0.003 mg/kg

Conclusion

According to SANTE/2020/12830 Rev.1 the method was successfully validated and suitable for determination of residues of lambda cyhalothrin in meat.

A 2.1.2.2.3.2 Independent laboratory validation

Comments of zRMS:	Validation is accepted

Reference: KCP 5.2.7.1

Report: Independent Laboratory Validation of the Method for Determination of Lambda-Cyhalothrin in Meat by Liquid Chromatography. xxxxxxxxxxx, 2017, Report No. ZBBZ-2016/02/DPL/3

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

Deviations: No

GLP: Yes

Acceptability: Yes

Materials and methods

The objective of the study was to independently validate a method for the determination of Lambda-Cyhalothrin in meat in accordance to the guidance document SANCO/825/00, rev. 8.1 of the European Commission. The validated limit of quantification is 0.01 mg/kg.
In brief, before initial extraction water was added than meat sample was extracted with acidified acetonitrile. After addition of a buffer-salt mixture containing magnesium sulphate and sodium acetate the extract was shaken. After centrifugation and freezing out step, an aliquot of the acetonitrile phase was cleaned by primary secondary amine (PSA) and dehydrated by magnesium sulphate addition.

Selectivity and Confirmation of Residue Identity
Quantification was performed by use of highly selective liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). Two selected ion mass transitions were evaluated in order to demonstrate that the method achieves a high level of selectivity. The retention times of analyte in extracts corresponds to that of the calibration standards with a tolerance of < ± 0.1 min. also confirmation ratios for Lambda-Cyhalothrin in all samples were within ± 30 % of the average found for the standards.
No significant interference above 30 % of LOQ was detected in any of the reagent blanks or control specimen extracts of meat matrix, so that a high level of selectivity was demonstrated and an additional confirmatory method is not necessary.

Matrix Effects
Matrix effects on the detection of Lambda-Cyhalothrin in extracts of meat were found to be insignificant (≤ 20 %). However, and without any significant impact on the results, matrix-matched standards were used for quantification.

Linearity
The linearity of the detector response was demonstrated by single determination of matrix-matched calibration standards at seven concentration levels ranging from 0.0005 µg/mL to 0.05 µg/mL. This range corresponds to 0.002 mg/kg to 0.2 mg/kg for meat thus covers the range from no more than 30 % of the LOQ and at least + 20 % of the highest analyte concentration level detected in sample.
The calibration curves obtained for both ion mass transitions were linear with the coefficients of determination (R^2) greater than 0.99. Linear regression was performed with 1/x weighting. In the validation study linear regression was performed without weighting with force through zero. Using 1/x weighting in this study without forcing through zero has no significant impact to the obtained results.

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

Mobile phase A (10 mM ammonium formate buffer 4.0)
500 mL volumetric flask was half filled with water, 0.315 g of ammonium formate and approximately 113 µL of formic acid was added than solution was agitated gently until all ammonium formate was completely dissolved. Volumetric flask was filled up to the mark with water, closed tightly and mixed by inverting several times. Subsequently proceeded to solvent filtration apparatus equipped with a 0.22 µm Teflon filter. After filtration solvent was transferred to amber HPLC solvent reservoir.

Mobile phase B
Methanol.

Blank solution
10 mM ammonium formate buffer: acetonitrile 50:50 (v/v).

Extraction mixture
500 mL volumetric flask was half filled with acetonitrile, 5 mL of acetic acid and 5 mL of Chlorpyrifos D10 working internal standard solution (for preparation of procedural reagent blank sample extraction mixture didn't contain internal standard) was added than volumetric flask was filled up to the mark, closed tightly and mixed by inverting several times.

Sample extraction
5.00 ± 0.1g of homogenized matrix was weighed into a 50 mL Teflon® centrifuge tube. Sample weight was recorded. If necessary fortification of the concurrent recovery sample(s) by aliquoting the fortification standard onto the matrix was carried out at this step. The tube was shaken in a vortex mixer for 1 min. and allowed to stand for about 5 min. Using glass volumetric pipettes 7.5 mL of LC/MS grade water and 10 mL of extraction mixture (99/1, v/v acetonitrile: acetic acid: 0.00483 µg/ml Chlorpyrifos D10) was added. The Teflon® centrifuge tube was closed tightly and vortex thoroughly for 1 min.

Results and discussions

Table A 27: Recovery results from independent laboratory validation of Lambda-cyhalothrin using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (n = 5)	Mean recovery (%)	RSD (%)	Comments
Meat	Lambda-Cyhalothrin	0.01	96	4.1	First mass transition
		0.1	97	4.9	
		0.01	97	9.2	Second mass transition
		0.1	97	7.2	

Table A 28: Characteristics for the analytical method used for independent laboratory validation of Lambda-cyhalothrin residues in meat

	Lambda-cyhalothrin
Specificity	For both ion mass transitions, the specimen showed no significant interference (above 30 % of the LOQ) at the retention time of the analyte. The method is specific.
Calibration (type, number of data points)	7 points 0.002 mg/kg to 0.2 mg/kg First mass transition y=1894439.739648x-113.099633 R^2=0.99827415 Second mass transition y=238447.381291x+8.486392 R^2=0.99740602
Assessment of matrix effects is presented	Yes
Limit of determination/quantification	LOQ = 0.01 mg/kg LOD = 0.002 mg/kg

Conclusion

According to SANTE/2020/12830 Rev.1 the method was successfully validated and is suitable for determination of Lambda-cyhalothrin residues in meat.

A 2.1.2.2.3.3 Confirmatory method (if required)

No confirmatory method is required.

A 2.1.2.2.4 Analytical method 4

A 2.1.2.2.4.1 Method validation

Comments of zRMS:	Validation is accepted

Reference:	KCP 5.2.8
Report	Validation of the analytical procedure for the determination of lambda cyhalothrin in fat by liquid chromatography. xxxxxxxxxxxxxx, 2016, Report No. 16.554813.0010
Guideline(s):	SANCO/3030/99 Rev. 4 SANCO/825/00 Rev. 8.1 SANCO/3029/99 Rev. 4 OECD-204/2014
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Short term study for the validation of analytical method, based on QuECheRs procedure for the determination of Lambda Cyhalothrin in fat.

Mobile phase A (10 mM ammonium formate buffer 4.0)
About 0.63 g of ammonium formate were accurately weighed into a 1000 mL volumetric flask and dissolved with milliQ water. 0.45 mL of formic acid 50% (v/v) were added and then the solution was diluted to volume with milliQ water.

Mobile phase B
Methanol.

Blank solution
10 mM ammonium formate buffer: acetonitrile 50:50 (v/v).

Extraction mixture
About 20 mL of glacial acetic acid and 1 mL of internal standard (Chlorpyrifos ethyl-D10) solution were introduced into a 2000 mL volumetric flask (glacial acetic acid final concentration: 1% v/v; internal standard final concentration about 100 ng/mL). The solution was filled up to the volume with acetonitrile.

Sample extraction
About 3 g of sample were introduced in a 50 mL plastic tube, 9 mL of extraction mixture were added to the sample. After vortexing for about 2 min, the sample was centrifuged at 4750 rpm for about 5 minutes. About 8 mL of supernatant were transferred into a 10 mL tube and purified. Test sample was prepared in triplicate.

Instrumental conditions:
- Column: Acquity UPLC BEH C18, 50 mm x 2.1 mm x 1.7 µm
- Mobile phase A: 10 mM ammonium formate buffer pH 4.0
- Mobile phase B: Methanol
- Flow: 0.2 ml/min
- Injection volume: 5 µl
- Detector: MS XEVO TQS (Waters-Micromass)
- Source: APCI
- Source temp.: 150 °C
- Probe temp.: 500 °C
- Cone gas: 150 l/h
- Desolvation gas: 800 l/h
- Run time: 13 minutes

- Run mode: MRM (First transition: 450 > 225 m/z; Second transition (confirmatory ion): 450 > 141 m/z)
- Elution: Gradient

Results and discussions

Table A 29: Recovery results from method validation of Lambda-Cyhalothrin using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
Fat	Lambda-cyhalothrin	0.01	94	7.6	First mass transition
		0.1	81	4.9	
		0.01	101	6.5	Second mass transition
		0.1	84	4.7	

Table A 30: Characteristics for the analytical method used for validation of Lambda-cyhalothrin residues in fat

	Lambda-cyhalothrin
Specificity	For both ion mass transitions, the specimen showed no significant interference (above 30 % of the LOQ) at the retention time of the analyte. The method is specific.
Calibration (type, number of data points)	6 points 0.003 mg/kg to 0.552 mg/kg First mass transition y=171274x R^2=1.00 Second mass transition y=64144x R^2=1.00
Assessment of matrix effects is presented	Yes
Limit of determination/quantification	LOQ = 0.01 mg/kg LOD = 0.003 mg/kg

Conclusion

According to SANTE/2020/12830 Rev.1 the method was successfully validated and suitable for determination of residues of lambda cyhalothrin in fat.

A 2.1.2.2.4.2 Independent laboratory validation

Comments of zRMS:	Validation is accepted

Reference: KCP 5.2.8.1

Report: Independent Laboratory Validation of the Method for Determination of Lambda-Cyhalothrin in Fat by Liquid Chromatography. xxxxxxxxxxx,

	2017, Report No. ZBBZ-2016/02/DPL/5
Guideline(s):	SANCO/3030/99 Rev. 4 SANCO/825/00 Rev. 8.1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The objective of the study was to independently validate a method for the determination of Lambda-Cyhalothrin in fat in accordance to the guidance document SANCO/825/00, rev. 8.1 of the European Commission. The validated limit of quantification is 0.01 mg/kg.
In brief, fat sample was extracted with acidified acetonitrile. After shaken and centrifugation, an aliquot of the acetonitrile phase was cleaned by primary secondary amine (PSA) and C18 resin and dehydrated by magnesium sulphate addition.

Selectivity and Confirmation of Residue Identity
Quantification was performed by use of highly selective liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). Two selected ion mass transitions were evaluated in order to demonstrate that the method achieves a high level of selectivity. The retention times of analyte in extracts corresponds to that of the calibration standards with a tolerance of $< \pm 0.1$ min. also confirmation ratios for Lambda-Cyhalothrin in all samples were within ± 30 % of the average found for the standards.
No significant interference above 30 % of LOQ was detected in any of the reagent blanks or control specimen extracts of fat matrix, so that a high level of selectivity was demonstrated and an additional confirmatory method is not necessary.

Matrix Effects
Matrix effects on the detection of Lambda-Cyhalothrin in extracts of fat were found to be insignificant (≤ 20 %). However, and without any significant impact on the results, matrix-matched standards were used for quantification.

Linearity
The linearity of the detector response was demonstrated by single determination of matrix-matched calibration standards at seven concentration levels ranging from 0.0005 µg/mL to 0.05 µg/mL. This range corresponds to 0.002 mg/kg to 0.2 mg/kg for fat thus covers the range from no more than 30 % of the LOQ and at least + 20 % of the highest analyte concentration level detected in sample.
The calibration curves obtained for both ion mass transitions were linear with the coefficients of determination (R^2) greater than 0.99. Linear regression was performed with 1/x weighting. In the validation study linear regression was performed without weighting with force through zero. Using 1/x weighting in this study without forcing through zero has no significant impact to the obtained results.

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

Mobile phase A (10 mM ammonium formate buffer 4.0)
About 0.63 g of ammonium formate were accurately weighed into a 1000 mL volumetric flask and dissolved with milliQ water. 0.45 mL of formic acid 50% (v/v) were added and then the solution was diluted to volume with milliQ water.

Mobile phase A (10 mM ammonium formate buffer 4.0)
500 mL volumetric flask was half filled with water, 0.315 g of ammonium formate and approximately 113 µL of formic acid was added than solution was agitated gently until all ammonium formate was com-

pletely dissolved. Volumetric flask was filled up to the mark with water, closed tightly and mixed by inverting several times. Subsequently proceeded to solvent filtration apparatus equipped with a 0.22 µm Teflon filter. After filtration solvent was transferred to amber HPLC solvent reservoir.

Mobile phase B
Methanol.

Blank solution
10 mM ammonium formate buffer: acetonitrile 50:50 (v/v).

Extraction mixture
500 mL volumetric flask was half filled with acetonitrile, 5 mL of acetic acid and 5 mL of Chlorpyrifos D10 working internal standard solution (for preparation of procedural reagent blank sample extraction mixture didn't contain internal standard) was added than volumetric flask was filled up to the mark, closed tightly and mixed by inverting several times.

Sample extraction
5.00 ± 0.1g of homogenized matrix was weighed into a 50 mL Teflon® centrifuge tube. Sample weight was recorded. If necessary fortification of the concurrent recovery sample(s) by aliquoting the fortification standard onto the matrix was carried out at this step. The tube was shaken in a vortex mixer for 1 min. and allowed to stand for about 5 min. Using glass volumetric pipettes 10 mL of extraction mixture (99/1, v/v acetonitrile: acetic acid: 0.00483 µg/ml Chlorpyrifos D10) was added. The Teflon® centrifuge tube was closed tightly and vortex thoroughly for 1 min.

Results and discussions

Table A 81: Recovery results from independent laboratory validation of Lambda-Cyhalothrin using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (n = 5)	Mean recovery (%)	RSD (%)	Comments
Fat	Lambda-Cyhalothrin	0.01	80	9.7	First mass transition
		0.1	75	9.0	
		0.01	78	19.5	Second mass transition
		0.1	75	9.7	

Table A 32: Characteristics for the analytical method used for independent laboratory validation of Lambda-cyhalothrin residues in fat

	Lambda-cyhalothrin
Specificity	For both ion mass transitions, the specimen showed no significant interference (above 30 % of the LOQ) at the retention time of the analyte. The method is specific.
Calibration (type, number of data points)	7 points 0.002 mg/kg to 0.2 mg/kg First mass transition y=1414745.295854+10.630967 R^2=0.99966319 Second mass transition y=178898.381186x+20.655028 R^2=0.99895530

	Lambda-cyhalothrin
Assessment of matrix effects is presented	Yes
Limit of determination/quantification	LOQ = 0.01 mg/kg LOD = 0.002 mg/kg

Conclusion

According to SANTE/2020/12830 Rev.1 the method was successfully validated and is suitable for determination of Lambda-cyhalothrin residues in fat.

A 2.1.2.2.4.3 Confirmatory method (if required)

No confirmatory method is required.

A 2.1.2.2.5 Analytical method 5

A 2.1.2.2.5.1 Method validation

Comments of zRMS:	Validation is accepted

Reference:	KCP 5.2.9
Report	Validation of the analytical procedure for the determination of lambda cyhalothrin in liver by liquid chromatography. xxxxxxxxxxxx, 2016, Report No. 16.554813.0013
Guideline(s):	SANCO/3030/99 Rev. 4 SANCO/825/00 Rev. 8.1 SANCO/3029/99 Rev. 4 OECD-204/2014
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Short term study for the validation of analytical method, based on QuECheRs procedure for the determination of Lambda Cyhalothrin in liver.

Mobile phase A (10 mM ammonium formate buffer 4.0)
About 0.63 g of ammonium formate were accurately weighed into a 1000 mL volumetric flask and dissolved with milliQ water. 0.45 mL of formic acid 50% (v/v) were added and then the solution was diluted to volume with milliQ water.

Mobile phase B
Methanol.

Blank solution

10 mM ammonium formate buffer: acetonitrile 50:50 (v/v).

Extraction mixture
About 20 mL of glacial acetic acid and 1 mL of internal standard (Chlorpyrifos ethyl-D10) solution were introduced into a 2000 mL volumetric flask (glacial acetic acid final concentration: 1% v/v; internal standard final concentration about 100 ng/mL). The solution was filled up to the volume with acetonitrile.

Sample extraction
About 5 g of grinded liver were introduced in a 50 mL plastic tube, 7.5 mL of milliQ water were added in order to dissolve the matrix and then, 10 mL of extraction mixture were added to the sample. After vortexing for about 1 min, a pouch of sodium acetate and magnesium sulfate anhydrous was added to the sample and vortexed again for about 1 min. The tube was centrifuged at 4750 rpm for 5 min and kept at about -20°C for about 2 hours. Then, the tube was centrifuged for 3 minutes at 4750 rpm and purified. Test sample was prepared in triplicate.

Instrumental conditions:
- Column: Acquity UPLC BEH C18, 50 mm x 2.1 mm x 1.7 µm
- Mobile phase A: 10 mM ammonium formate buffer pH 4.0
- Mobile phase B: Methanol
- Flow: 0.2 ml/min
- Injection volume: 5 µl
- Detector: MS XEVO TQS (Waters-Micromass)
- Source: APCI
- Source temp.: 150 °C
- Probe temp.: 500 °C
- Cone gas: 150 l/h
- Desolvation gas: 800 l/h
- Run time: 13 minutes
- Run mode: MRM (First transition: 450 > 225 m/z; Second transition (confirmatory ion): 450 > 141 m/z)
- Elution: Gradient

Results and discussions

Table A 33: Recovery results from method validation of Lambda-Cyhalothrin using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
Liver	Lambda-cyhalothrin	0.01	97	10.0	First mass transition
		0.1	90	6.1	
		0.01	99	10.2	Second mass transition
		0.1	88	6.2	

Table A 34: Characteristics for the analytical method used for validation of Lambda-cyhalothrin residues in liver

	Lambda-cyhalothrin
Specificity	For both ion mass transitions, the specimen showed no significant interference (above 30 % of the LOQ) at the retention time of the analyte. The method is specific.
Calibration (type, number of data points)	6 points 0.003 mg/kg to 0.424 mg/kg First mass transition y=222562x R^2=1.00 Second mass transition y=83722x R^2=1.00
Assessment of matrix effects is presented	Yes
Limit of determination/quantification	LOQ = 0.01 mg/kg LOD = 0.003 mg/kg

Conclusion

According to SANTE/2020/12830 Rev.1 the method was successfully validated and suitable for determination of residues of lambda cyhalothrin in liver.

A 2.1.2.2.5.2 Independent laboratory validation

Comments of zRMS:	Validation is accepted

Reference:	KCP 5.2.9.1
Report	Independent Laboratory Validation of the Method for Determination of Lambda-Cyhalothrin in Liver by Liquid Chromatography, xxxxxxxxxxxx, 2017, Report No. ZBBZ-2016/02/DPL/2
Guideline(s):	SANCO/3030/99 Rev. 4 SANCO/825/00 Rev. 8.1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

The objective of the study was to independently validate a method for the determination of Lambda-Cyhalothrin in liver in accordance to the guidance document SANCO/825/00, rev. 8.1 of the European Commission. The validated limit of quantification is 0.01 mg/kg.
In brief, before initial extraction water was added than liver sample was extracted with acidified acetonitrile. After addition of a buffer-salt mixture containing magnesium sulphate and sodium acetate the extract was shaken. After centrifugation and freezing out step, an aliquot of the acetonitrile phase was cleaned by primary secondary amine (PSA) and dehydrated by magnesium sulphate addition.

Selectivity and Confirmation of Residue Identity

Quantification was performed by use of highly selective liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). Two selected ion mass transitions were evaluated in order to demonstrate that the method achieves a high level of selectivity. The retention times of analyte in extracts corresponds to that of the calibration standards with a tolerance of < ± 0.1 min. also confirmation ratios for Lambda-Cyhalothrin in all samples were within ± 30 % of the average found for the standards.
No significant interference above 30 % of LOQ was detected in any of the reagent blanks or control specimen extracts of liver matrix, so that a high level of selectivity was demonstrated and an additional confirmatory method is not necessary.

Matrix Effects
Matrix effects on the detection of Lambda-Cyhalothrin in extracts of liver were found to be insignificant (≤ 20 %). However, and without any significant impact on the results, matrix-matched standards were used for quantification.

Linearity
The linearity of the detector response was demonstrated by single determination of matrix-matched calibration standards at seven concentration levels ranging from 0.0005 µg/mL to 0.05 µg/mL. This range corresponds to 0.002 mg/kg to 0.2 mg/kg for liver thus covers the range from no more than 30 % of the LOQ and at least + 20 % of the highest analyte concentration level detected in sample.
The calibration curves obtained for both ion mass transitions were linear with the coefficients of determination (R^2) greater than 0.99. Linear regression was performed with 1/x weighting. In the validation study linear regression was performed without weighting with force through zero. Using 1/x weighting in this study without forcing through zero has no significant impact to the obtained results.

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

Mobile phase A (10 mM ammonium formate buffer 4.0)
500 mL volumetric flask was half filled with water, 0.315 g of ammonium formate and approximately 113 µL of formic acid was added than solution was agitated gently until all ammonium formate was completely dissolved. Volumetric flask was filled up to the mark with water, closed tightly and mixed by inverting several times. Subsequently proceeded to solvent filtration apparatus equipped with a 0.22 µm Teflon filter. After filtration solvent was transferred to amber HPLC solvent reservoir.

Mobile phase B
Methanol.

Blank solution
10 mM ammonium formate buffer: acetonitrile 50:50 (v/v).

Extraction mixture
500 mL volumetric flask was half filled with acetonitrile, 5 mL of acetic acid and 5 mL of Chlorpyrifos D10 working internal standard solution (for preparation of procedural reagent blank sample extraction mixture didn't contain internal standard) was added than volumetric flask was filled up to the mark, closed tightly and mixed by inverting several times.

Sample extraction
5.00 ± 0.1g of homogenized matrix was weighed into a 50 mL Teflon® centrifuge tube. Sample weight was recorded. If necessary fortification of the concurrent recovery sample(s) by aliquoting the fortification standard onto the matrix was carried out at this step. The tube was shaken in a vortex mixer for 1 min. and allowed to stand for about 5 min. Using glass volumetric pipettes 7.5 mL of LC/MS grade water and 10 mL of extraction mixture (99/1, v/v acetonitrile: acetic acid: 0.00483 µg/ml Chlorpyrifos D10) was added. The Teflon® centrifuge tube was closed tightly and vortex thoroughly for 1 min.

Results and discussions

Table A 35: Recovery results from independent laboratory validation of Lambda-Cyhalothrin using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (n = 5)	Mean recovery (%)	RSD (%)	Comments
Liver	Lambda-Cyhalothrin	0.01	91	4.4	First mass transition
		0.1	92	4.8	
		0.01	92	7.1	Second mass transition
		0.1	92	5.1	

Table A 36: Characteristics for the analytical method used for independent laboratory validation of Lambda-cyhalothrin residues in liver

	Lambda-cyhalothrin
Specificity	For both ion mass transitions, the specimen showed no significant interference (above 30 % of the LOQ) at the retention time of the analyte. The method is specific.
Calibration (type, number of data points)	7 points 0.002 mg/kg to 0.2 mg/kg First mass transition y=1551406.258527x+113.829499 R^2=0.99981235 Second mass transition y=192813.449156x+46.034619 R^2=0.99968822
Assessment of matrix effects is presented	Yes
Limit of determination/quantification	LOQ = 0.01 mg/kg LOD = 0.002 mg/kg

Conclusion

According to SANTE/2020/12830 Rev.1 the method was successfully validated and is suitable for determination of Lambda-cyhalothrin residues in liver.

A 2.1.2.2.5.3 Confirmatory method (if required)

No confirmatory method is required.

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

A 2.1.2.3.1 Analytical method 1

A 2.1.2.3.1.1 Method validation

Comments of zRMS:	Validation is accepted

Reference:	KCP 5.2.10
Report	Validation of the analytical procedure for the determination of lambda cyhalothrin in sand by liquid chromatography. M. Pivato, 2016, Report No. 16.554813.0003
Guideline(s):	SANCO/3030/99 Rev. 4 SANCO/825/00 Rev. 8.1 SANCO/3029/99 Rev. 4 OECD-204/2014
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Short term study for the validation of analytical method, based on QuECheRs procedure for the determination of Lambda Cyhalothrin in sand (from soil).

Mobile phase A (10 mM ammonium formate buffer 4.0)
About 0.63 g of ammonium formate were accurately weighed into a 1000 mL volumetric flask and dissolved with 500 mL of milliQ water. 0.45 mL of formic acid 50% (v/v) were added and then the solution was diluted to volume with milliQ water.

Mobile phase B
Methanol.

Blank solution
10 mM ammonium formate buffer: acetonitrile 50:50 (v/v).

Extraction mixture
About 20 mL of glacial acetic acid and 1 mL of internal standard (Chlorpyrifos ethyl-D10) solution were introduced into a 2000 mL volumetric flask (glacial acetic acid final concentration: 1% v/v; internal standard final concentration about 100 ng/mL). The solution was filled up to the volume with acetonitrile.

Sample extraction
About 10 g of sample were introduced in a 50 mL plastic tube, 5 mL of milliQ water and 10 mL of extraction mixture were added to the sample. After vortexing for about 1 min, a pouch of sodium acetate and magnesium sulfate anhydrous was added to the sample and vortexed again for about 1 min. The tube was centrifuged at 4750 rpm for 5 min and purified. Test sample was prepared in triplicate.

Instrumental conditions:
- Column: Acquity UPLC BEH C18, 50 mm x 2.1 mm x 1.7 µm
- Mobile phase A: 10 mM ammonium formate buffer pH 4.0
- Mobile phase B: Methanol
- Flow: 0.2 ml/min
- Injection volume: 5 µl
- Detector: MS XEVO TQS (Waters-Micromass)
- Source: APCI
- Source temp.: 150 °C
- Probe temp.: 500 °C

- Cone gas: 150 l/h
- Desolvation gas: 800 l/h
- Run time: 13 minutes
- Run mode: MRM (First transition: 450 > 225 m/z; Second transition (confirmatory ion): 450 > 141 m/z)
- Elution: Gradien

Results and discussions

Table A 37: Recovery results from method validation of Lambda-cyhalothrin using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
Sand	Lambda-cyhalothrin	0.01	100	7.7	First mass transition
		0.1	84	3.3	
		0.01	104	3.7	Second mass transition
		0.1	85	3.2	

Table A 38: Characteristics for the analytical method used for validation of Lambda-cyhalothrin residues in sand

	Lambda-cyhalothrin
Specificity	For both ion mass transitions, the specimen showed no significant interference (above 30 % of the LOQ) at the retention time of the analyte. The method is specific.
Calibration (type, number of data points)	6 points 0.003 mg/kg to 0.414 mg/kg First mass transition y=210048x R^2=1.00 Second mass transition y=74429x R^2=1.00
Assessment of matrix effects is presented	Yes
Limit of determination/quantification	LOQ = 0.01 mg/kg LOD = 0.003 mg/kg

Conclusion

According to SANTE/2020/12830 Rev.1 the method was successfully validated and suitable for determination of residues of lambda cyhalothrin in soil.

A 2.1.2.3.1.2 Confirmatory method (if required)

No confirmatory method is required.

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

A 2.1.2.4.1 Analytical method 2

A 2.1.2.4.1.1 Method validation

Comments of zRMS:	Validation is accepted

Reference:	KCP 5.2.11
Report	Validation of the analytical procedure for the determination of lambda cyhalothrin in drinking water by liquid chromatography. M. Pivato, 2016, Report No. 16.554813.0001
Guideline(s):	SANCO/3030/99 Rev. 4 SANCO/825/00 Rev. 8.1 SANCO/3029/99 Rev. 4 OECD-204/2014
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Short term study for the validation of analytical method, based on LC-MS/MS procedure for the determination of Lambda Cyhalothrin in drinking water.

Mobile phase A (10 mM ammonium formate buffer 4.0)
About 0.63 g of ammonium formate were accurately weighed into a 1000 mL volumetric flask and dissolved with 500 mL of milliQ water. 0.45 mL of formic acid 50% (v/v) were added and then the solution was diluted to volume with milliQ water.

Mobile phase B
Methanol.

Blank solution
10 mM ammonium formate buffer: acetonitrile 50:50 (v/v).

Extraction mixture
About 20 mL of glacial acetic acid and 1 mL of internal standard (Chlorpyrifos ethyl-D10) solution were introduced into a 2000 mL volumetric flask (glacial acetic acid final concentration: 1% v/v; internal standard final concentration about 100 ng/mL). The solution was filled up to the volume with acetonitrile.

Sample preparation
About 500 g of sample were exactly weighed into a 1000 mL beaker and transferred into a 500 mL stoppered separating funnel. 1 mL of extraction mixture and 80 mL of dichloromethane were added to the sample and the funnel was shaken three times by inversion. The stopper was opened in order to degas the solution and the organic phase was recovered into a 250 mL round-bottomed flask. 2 mL of 99% formic acid was added to the aqueous phase and after shaking 80 mL of diethyl ether were added. The aqueous phase was then recovered into a 1000 mL beaker and added of about 10 g of Na_2SO_4 anhydrous. Then, the aqueous phase was added again to the organic phase and discarded by liquid phase separation. The organic phase was recovered in the round-bottomed flask together with the dichloromethane solution and reduced to small volume by rotavapor. The test solution was transferred into a 10 mL glass tube and com-

pletely dried by N_2 flux bath. The dried sample was resuspended into 1 mL of blank solution, transferred into an HPLC vial and injected. Test sample was prepared in triplicate.

Instrumental conditions:

- Column: Acquity UPLC BEH C18, 50 mm x 2.1 mm x 1.7 µm
- Mobile phase A: 10 mM ammonium formate buffer pH 4.0
- Mobile phase B: Methanol
- Flow: 0.2 ml/min
- Injection volume: 5 µl
- Detector: MS XEVO TQS (Waters-Micromass)
- Source: APCI+
- Source temp.: 150 °C
- Probe temp.: 500 °C
- Cone gas: 150 l/h
- Desolvation gas: 800 l/h
- Run time: 13 minutes
- Run mode: MRM (First transition: 450 > 225 m/z; Second transition (confirmatory ion): 450 > 141 m/z)
- Elution: Gradien

Results and discussions

Table A 39: Recovery results from method validation of Lambda-cyhalothrin using the analytical method

Matrix	Analyte	Fortification level (mg/L) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
Drinking water	Lambda-cyhalothrin	0.0001	71	1.8	First mass transition
		0.001	71	1.1	
		0.0001	71	1.4	Second mass transition
		0.001	71	0.9	

Table A 40: Characteristics for the analytical method used for validation of Lambda-cyhalothrin residues in drinking water

	Lambda-cyhalothrin
Specificity	For both ion mass transitions, the specimen showed no significant interference (above 30 % of the LOQ) at the retention time of the analyte. The method is specific.
Calibration (type, number of data points)	6 points 0.00003 mg/L to 0.00414 mg/L First mass transition y=223316x R^2=1.00 Second mass transition y=82019x R^2=1.00
Assessment of matrix effects is presented	Yes
Limit of determination/quantification	LOQ = 0.0001 mg/L LOD = 0.00003 mg/L

Conclusion

According to SANTE/2020/12830 Rev.1 the method was successfully validated and suitable for determination of residues of lambda cyhalothrin in drinking water.

A 2.1.2.4.1.2 Confirmatory method (if required)

No confirmatory method is required.

A 2.1.2.4.2 Analytical method 2

A 2.1.2.4.2.1 Method validation

Comments of zRMS:	Validation is accepted

Reference:	KCP 5.2.12
Report	Validation of the analitycal procedure for the determination of lambda cyhalothrin in surface water by liquid chromatography. M. Pivato, 2016, Report No. 16.554813.0002
Guideline(s):	SANCO/3030/99 Rev. 4 SANCO/825/00 Rev. 8.1 SANCO/3029/99 Rev. 4 OECD-204/2014
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Short term study for the validation of analytical method, based on LC-MS/MS procedure for the determination of Lambda Cyhalothrin in surface water.

Reagents:

- milliQ water SRA 35
- Methanol
- Acetonitrile
- Glacial acetic acid
- Formic acid 50% v/v (LC-MS grade)
- Formic acid 99% v/v (LC-MS grade)
- Sodium sulfate anhydrous
- Diethyl ether
- Ammonium formate (LC-MS grade)
- Chlorpiriphos ehyl-D10
- Acetone
- Hexane
- Dichloromethane

Reference material:

- Lambda Cyhalothrin, Batch SZBB332XV, Sigma-Aldrich, Purity 97.8%, CAS 91465-08-6

Material and apparatus:

- Analytical balance (± 0.01 mg)
- Analytical balance (± 0.1 mg)
- Technical balance (± 0.01 g)
- Vortex
- Rotavapor
- Ultrasonic bath
- Thermostatic bath equipped with N_2 flow
- MS XEVO TQS (Waters-Micromass)
- Acquity UPLC BEH C18, 50 mm x 2.1 mm x 1.7 µm
- Analytical glassware

Mobile phase A (10 mM ammonium formate buffer 4.0)
About 0.63 g of ammonium formate were accurately weighed into a 1000 mL volumetric flask and dis-solved with 500 mL of milliQ water. 0.45 mL of formic acid 50% (v/v) were added and then the solu-tion was diluted to volume with milliQ water.

Mobile phase B
Methanol.

Blank solution
10 mM ammonium formate buffer: acetonitrile 50:50 (v/v).

Extraction mixture
About 20 mL of glacial acetic acid and 1 mL of internal standard (Chlorpyrifos ethyl-D10) solution were introduced into a 2000 mL volumetric flask (glacial acetic acid final concentration: 1% v/v; internal standard final concentration about 100 ng/mL). The solution was filled up to the volume with acetonitrile.

Sample preparation:
About 1000g (± 0.1g) of sample were exactly weighed into a 2000 ml beaker. 1 ml of extraction mixture (about 20 ml of glacial acetic acid and 1 ml of internal standard solution – Chlorpiriphos ethyl-D10 204.2 mg/l were introduced into a 2000 ml volumetric flask) and 160 ml of dichloromethane were added to the sample (surface water). The solution was shaken by a magnetic stirred and the organic phase was recov-ered into a 500 ml round bottomed flask by the help of a separating funnel. 4 ml of 99% formic acid were added to the aqueous phase and after shaking also 160 ml of diethyl ether were added. The aqueous phase (lower phase) was then recovered into a 1000 ml beaker and added of about 10 g (± 0.1g) of Na_2SO_4 an-hydrous. Then, the aqueous phase was added again to the organic phase and discarded by liquid phase separation by the help of separating funnel. The organic phase was recovered in the round-bottomed flask together with the dichloromethane solution and reduced to small volume (about 3 ml) by rotavapor. The test solution was transferred into a 10 ml glass tube and completely dried by N_2 flux bath. The dried sam-ple was resuspended into 160 µl of blank solution (10 mM ammonium formate buffer: acetonitrile 50:50 v/v), transferred into an HPLC vial and injected. Test sample was prepared in triplicate (one for matric effect evaluation and two for the test sample analysis).

Instrumental conditions:

- Column: Acquity UPLC BEH C18, 50 mm x 2.1 mm x 1.7 µm
- Mobile phase A: 10 mM ammonium formate buffer pH 4.0
- Mobile phase B: Methanol
- Flow: 0.2 ml/min
- Injection volume: 5 µl
- Detector: MS XEVO TQS (Waters-Micromass)

- Source: APCI+
- Source temp.: 150 °C
- Probe temp.: 500 °C
- Cone gas: 150 l/h
- Desolvation gas: 800 l/h
- Run time: 13 minutes
- Run mode: MRM (First transition: 450 >225 m/z; Second transition (confirmatory ion): 450 >141 m/z)
- Elution: Gradient

Results and discussions

Table A 41: Recovery results from method validation of lambda-cyhalothrin using the analytical method

Matrix	Analyte	Fortification level (µg/L) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
Surface water	Lambda cyhalothrin	0.0002	101	5	First mass transition
		0.002	70	1	
		0.0002	91	13	Second mass transition
		0.002	73	4	

Table A 42: Characteristics for the analytical method used for validation of lambda cyhalothrin residues in surface water

	Lambda cyhalothrin
Specificity	No significant peaks (≤ 30%) are detectted at RT of the target analyte in the blank and test solution with respect to the spiked test solution both for the transition 1 and transition 2.
Calibration (type, number of data points)	6 points 0.05 ng/L to 8 ng/L First mass transition: y=201656x R^2=1 Second mass transition: y=71123x R^2=1
Assessment of matrix effects is presented	Yes
Limit of determination/quantification	LOQ = 0.0002 µg/L LOD = 0.05 ng/L

Conclusion

The validation data demonstrate that the analytical method is suitable qualitatively and quantitatively determine Lambda Cyhalothrin in surface water specimens, according to SANTE 2020/12830 Rev.1 and OECD-204/2014 guidelines and for the given concentration range.

A 2.1.2.4.2.2 Confirmatory method

No confirmatory method is required since the validation data was achieved for two mass transitions.

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

A 2.1.2.5.1 Analytical method 1

A 2.1.2.5.1.1 Method validation

Comments of zRMS:	Validation is accepted

Reference:	KCP 5.2.13
Report	Validation of the analytical procedure for the determination of lambda cyhalothrin in ambient air by liquid chromatography. M. Pivato, 2016, Report No. 16.554813.0004
Guideline(s):	SANCO/3030/99 Rev. 4 SANCO/825/00 Rev. 8.1 SANCO/3029/99 Rev. 4 OECD-204/2014
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Short term study for the validation of analytical method, based on QuECheRs procedure for the determination of Lambda Cyhalothrin in air.

Mobile phase A (10 mM ammonium formate buffer 4.0)
About 0.63 g of ammonium formate were accurately weighed into a 1000 mL volumetric flask and dissolved with 500 mL of milliQ water. 0.45 mL of formic acid 50% (v/v) were added and then the solution was diluted to volume with milliQ water.

Mobile phase B
Methanol.

Blank solution
10 mM ammonium formate buffer: acetonitrile 50:50 (v/v).

Extraction mixture
About 20 mL of glacial acetic acid and 1 mL of internal standard (Chlorpyrifos ethyl-D10) solution were introduced into a 2000 mL volumetric flask (glacial acetic acid final concentration: 1% v/v; internal standard final concentration about 100 ng/mL). The solution was filled up to the volume with acetonitrile.

Sample preparation
A polyurethane plug was cleaned by acetone by soaking for about 6 hours and then dried in a heater at about 50°C. After drying, an incision was made on the plug and about 80 µL of extraction mixture were pipetted in it. Then, the plug was inserted into a glass syringe by connecting it to a pump. Air was collect-

ed for about 6 hours at a flux of 3 L/min by using a pump climatic chamber SRA 323 at 35°C and 80% of humidity. After sampling the plug was transferred into a 400 mL beaker and soaked into about 200 mL of acetone:hexane mixture (50:50). After sonication for about 20 minutes the solution was transferred into a 500 mL round bottomed flask. The volume was reduced to about 3 mL by rotavapor, the solution transferred into a 10 mL tube and completely dried by N_2 flux. The dried sample was resuspended into 0.75 mL of blank solution, filtered, transferred into an HPLC vial and injected. Sample was prepared in triplicate.

Instrumental conditions:

- Column: Acquity UPLC BEH C18, 50 mm x 2.1 mm x 1.7 µm
- Mobile phase A: 10 mM ammonium formate buffer pH 4.0
- Mobile phase B: Methanol
- Flow: 0.2 ml/min
- Injection volume: 5 µl
- Detector: MS XEVO TQS (Waters-Micromass)
- Source: APCI
- Source temp.: 150 °C
- Probe temp.: 500 °C
- Cone gas: 150 l/h
- Desolvation gas: 800 l/h
- Run time: 13 minutes
- Run mode: MRM (First transition: 450 > 225 m/z; Second transition (confirmatory ion): 450 > 141 m/z)
- Elution: Gradient

Results and discussions

Table A 43: Recovery results from method validation of Lambda-cyhalothrin using the analytical method

Matrix	Analyte	Fortification level (mg/L) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
Air	Lambda-cyhalothrin	0.00000008	108	1.0	First mass transition
		0.0000008	92	7.0	
		0.00000008	102	3.0	Second mass transition
		0.0000008	83	7.0	

Table A 44: Characteristics for the analytical method used for validation of Lambda-cyhalothrin residues in air

	Lambda-cyhalothrin
Specificity	For both ion mass transitions, the specimen showed no significant interference (above 30 % of the LOQ) at the retention time of the analyte. The method is specific.
Calibration (type, number of data points)	6 points 0.000000015 g/m^3 to 0.000002382 g/m^3 First mass transition y=159127x R^2=1.00 Second mass transition

	y=58533x R^2=1.00
Assessment of matrix effects is presented	Yes
Limit of determination/quantification	LOQ = 0.075 µg/m^3 LOD = 0.000000015 g/m^3

Conclusion

According to SANTE/2020/12830 Rev.1 the method was successfully validated and suitable for determination of residues of lambda cyhalothrin in air.

A 2.1.2.5.1.2 Confirmatory method (if required)

No confirmatory method is required.

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

A 2.1.2.6.1 Analytical method 1

A 2.1.2.6.1.1 Method validation

Comments of zRMS:	Validation is accepted

Reference:	KCP 5.2.14
Report	Validation of the analytical procedure for the determination of lambda cyhalothrin in blood by liquid chromatography. M. Pivato, 2016, Report No. 16.554813.0014
Guideline(s):	SANCO/3030/99 Rev. 4 SANCO/825/00 Rev. 8.1 SANCO/3029/99 Rev. 4 OECD-204/2014
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Short term study for the validation of analytical method, based on QuECheRs procedure for the determination of Lambda Cyhalothrin in blood.

Mobile phase A (10 mM ammonium formate buffer 4.0)
About 0.63 g of ammonium formate were accurately weighed into a 1000 mL volumetric flask and dissolved with 500 mL of milliQ water. 0.45 mL of formic acid 50% (v/v) were added and then the solution was diluted to volume with milliQ water.

Mobile phase B
Methanol.

Blank solution
10 mM ammonium formate buffer: acetonitrile 50:50 (v/v).

Extraction mixture
About 20 mL of glacial acetic acid and 1 mL of internal standard (Chlorpyrifos ethyl-D10) solution were introduced into a 2000 mL volumetric flask (glacial acetic acid final concentration: 1% v/v; internal standard final concentration about 100 ng/mL). The solution was filled up to the volume with acetonitrile.

Sample extraction
About 5 g of blood were introduced in a 50 mL plastic tube, 7.5 mL of milliQ water were added to the matrix and then, 10 mL of extraction mixture were added to the sample. After vortexing for about 1 min, a pouch of sodium acetate and magnesium sulfate anhydrous was added to the sample and vortexed again for about 1 min. The tube was centrifuged at 4750 rpm for 5 min and kept at about -20°C for about 2 hours. Then, the tube was centrifuged for 3 minutes at 4750 rpm and purified. Test sample was prepared in triplicate.

Instrumental conditions:
- Column: Acquity UPLC BEH C18, 50 mm x 2.1 mm x 1.7 µm
- Mobile phase A: 10 mM ammonium formate buffer pH 4.0
- Mobile phase B: Methanol
- Flow: 0.2 ml/min
- Injection volume: 5 µl
- Detector: MS XEVO TQS (Waters-Micromass)
- Source: APCI
- Source temp.: 150 °C
- Probe temp.: 500 °C
- Cone gas: 150 l/h
- Desolvation gas: 800 l/h
- Run time: 13 minutes
- Run mode: MRM (First transition: 450 > 225 m/z; Second transition (confirmatory ion): 450 > 141 m/z)
- Elution: Gradient

Results and discussions

Table A 45: Recovery results from method validation of Lambda-cyhalothrin using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
Blood	Lambda-cyhalothrin	0.05	105	2.7	First mass transition
		0.5	92	0.9	
		0.05	107	2.4	Second mass transition
		0.5	92	2.0	

Table A 45: Characteristics for the analytical method used for validation of Lambda-cyhalothrin residues in blood

	Lambda-cyhalothrin
Specificity	For both ion mass transitions, the specimen showed no significant interference (above 30 % of the LOQ) at the retention time of the analyte. The method is specific.
Calibration (type, number of data points)	6 points 0.015 mg/kg to 2.308 mg/kg First mass transition y=211715x R^2=1.00 Second mass transition y=75787x R^2=1.00
Assessment of matrix effects is presented	Yes
Limit of determination/quantification	LOQ = 0.05 mg/kg LOD = 0.015 mg/kg

Conclusion

According to SANTE/2020/12830 Rev.1 the method was successfully validated and suitable for determination of residues of lambda cyhalothrin in blood.

A 2.1.2.6.1.2 Confirmatory method (if required)

No confirmatory method is required.

A 2.1.2.7 Other Studies/ Information

No new or additional studies have been submitted.