FINAL REGISTRATION REPORT

Part B

Section 5

Analytical Methods

Detailed summary of the risk assessment

Product code: SHA6100A

Product name: ALIVE

Chemical active substance:

Propaquizafop, 100 g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

Applicant: Sharda Cropchem España S.L.

Submission date: October 2020

Finalisation date: June 2021 March 2022

Version history

When	What
October 2020	Applicant version
December 2021	ZRMS evaluated the dRR
January 2022	Applicant update
March 2022	Assessment of the updated dRR

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5 Analytical methods

5.1 Conclusion and summary of assessment

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

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

- None

Sufficiently sensitive and selective analytical methods are available for all analytes included in the residue definitions except for acid matrices.
Applicant refers to the unprotected data RR Agil 100 EC (Registration No. R-208/2014).
Noticed data gaps are:

- Plant matrices:

1. Method for high acid content matrices should be provided by the applicant.
2. ILV and confirmatory methods for plant matrices should be provided with currently required LOQs (Reg. (EU) 2019/973).
3. Extraction efficiency for plant matrices methods need to be demonstrated at least in one crop/matrix

- Animal matrices:

1. ILV and confirmatory methods should be provided by the applicant. Extraction efficiency need to be demonstrated.

- Appendix 1. *List of data submitted or referred to by the applicant and relied on, but already evaluated at EU peer review* should be supplemented.

The Applicant has completed the dRR (January 2022)
The additions have been accepted and are sufficient.
Noticed data gaps are:
none

Commodity/crop	**Supported/ Not supported**
Sugar beet	Supported
Winter oilseed rape	Supported
Potato	Supported
Onion	Supported
Bean	Supported
Green peas, peas for dry seeds	Supported
Cabbage	Supported
Carrot, parsley	Supported
Strawberry	~~Not supported~~ Supported
Spring oilseed rape	Supported
Opium poppy, common flax, linen flax	Supported
Broccoli, brussels sprouts	Supported

Commodity/crop	Supported/ Not supported
Broad beans, faba bean, field peas, white lupine, yellow lupine, narrow-leaved lupine	Supported
Root celery, parsnipi, swede	Supported
Garlic, shallot	Supported
Fodder beet, beetroot	Supported
Jerusalem artichoke, horseradish, black radish, japanese radish, radish, salsify, white turnip, black turnip	Supported
Alfalfa, yellow alfalfa, black medic, red clover, white clover, crimson clover, common sainfoin, vetch, little white bird's-foot, letnitl, white melilot, yellow melilot, grass pea,	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 Propaquizafop in Propaquizafop 10% EC is provided as follows:

Comments of zRMS:	The analytical method meets the criteria of specificity, linearity, precision and accuracy. The method is acceptable and is suitable for determination of propaquizafop in plant protection product Alive.

Reference:	KCP 5.1.1
Report	Propaquizafop 10% EC: Analysis of active substances content and physico-chemical properties of initial preparation and preparation after accelerated storage procedure, Report no. 100/2017-BA-AD, Kedzierzyn-Kozle, 2017
Guideline(s):	Yes (SANCO/3030/99 rev. 5)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Equipment
Gas chromatograph Agilent Technologies 7890A with MSD detector type 7000 GC/MS Triple Quad and computer program "MassHunger"
Chromatographic column HP-5MS UI; 30 m x 0.25 mm i.d. 0.25 µm film
Analytical balance SHIMADZU AUW 220D
Aromatic pipette LLG Labware MA695682 10-100µL, MA 904590 100-1000 µL

Laboratory glasswere (A class)

Reagents
Acetonitrile for HPLC, CAS: 75-05-8

Chromatographic conditions
Column: HP-5MS; 30 m x 0.25 mm i.d. 0.25 µm film
Injector: 300°C
Column flow: 1 mL/min
Temp program: 70°C for 1 min, then 30°C/min to 290°C, hold 3 min
Injection volume: 1µL
Interface temp: 290°C
Ion source temp.: 230°C

Quantitative analysis of Propaquizafop was based on external calibration using standards as follow: Propaquizafop, Pestanal analytical standard 99.4% (HPLC), Sigma Aldrich, CAS: 111479-05-1.

Solution was analyzed by GC/MS under stable chromatographic conditions.

Specificity:
The selectivity of the GC/MS method was assessed by examination of peak homogeneity and peak purity. For this purpose, for active substance: one measurement for the standard solution and one measurement for tested sample was made.

Precision (Reproducibility and repeatability):
Propaquizalofop analytical standard solution of 345 mg/L was injected 7 times under the same chromatographic conditions as during the test item measurements and calibration process.

Accuracy:
Three solutions with concentration of Propaquizafop (analytical standard) in blank formulation in range 80%, 100% and 120% level of the analyte in the test sample were prepared. Each solution was injected 7 times using the same chromatographic conditions as during the test item measurements and calibration process.

Linearity and range:
Five standard solutions were prepared in the concentration range about 80- 120% of analyte content in tested sample. Each calibration solution was analyzed twice under stable chromatographic conditions.

Validation - Results and discussions

Table 5.2-1: Methods suitable for the determination of Propaquizafop in plant protection product Propaquizafop 10% EC

	Propaquizafop
Author(s), year	Kedzierzyn-Kozle, 2017
Principle of method	GC/MS
Linearity (linear between mg/L / % range of the declared content) (correlation coefficient, expressed as r)	277-417 mg/L R^2=0.9999 y=243208x-876812

	Propaquizafop
Precision – Repeatability Mean n = 7 (%RSD)	0.04%
Accuracy n = 7 (% Recovery)	8 %w/v: 100.1% 10 %w/v: 99.8% 12 %w/v: 99.2%
Interference/ Specificity	Interferences from impurities constitute didn't exceed 0,71% of total peak area in test sample and 0.3% of total peak area in analytical standard. In both cases do not contribute acceptable 3%.
Comment	-

Conclusion

The GC/MS analytical method for the determination of propaquizafop content in the test item was fully validated, according to SANCO/3030/99 rev. 5 guidance document.

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

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

Comments of zRMS:	The analytical method meets the criteria of specificity, linearity, precision and accuracy. The method is acceptable and is suitable for determination of relevant impurity toluene in plant protection product Alive.

Reference:	KCP 5.1.1
Report	Propaquizafop 10% EC: Analysis of active substances content and physico-chemical properties of initial preparation and preparation after accelerated storage procedure, Report no. 100/2017-BA-AD, Kedzierzyn-Kozle, 2017
Guideline(s):	Yes (SANCO/3030/99 rev. 5)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Equipment
Gas chromatograph Agilent Technologies 7890A with MSD detector type 7000 GC/MS Triple Quad and computer program "MassHunger"

Chromatographic column HP-5MS UI; 30 m x 0.25 mm i.d. 0.25 µm film
Analytical balance SHIMADZU AUW 220D
Aromatic pipette LLG Labware MA695682 10-100µL, MA 904590 100-1000 µL
Laboratory glasswere (A class)

Reagents
Acetonitrile for HPLC, CAS: 75-05-8

Chromatographic conditions
Column: HP-5MS; 30 m x 0.25 mm i.d. 0.25 µm film
Injector: 300°C
Column flow: 1 mL/min
Temp program: 50°C for 1 min, then 30°C/min to 290°C, hold 3 min
Injection volume: 1µL
Interface temp: 290°C
Ion source temp.: 230°C

Quantitative analysis of Toluene was based on external calibration using standards as follow: Toluene Pure P.A. 99.9% (GC), Avantors Performance Materials, CAS: 108-88-3

Solution was analyzed by GC/MS under stable chromatographic conditions.

Specificity:
The selectivity of the GC/MS method was assessed by examination of peak homogeneity and peak purity. For this purpose, for active substance: one measurement for the standard solution and one measurement for tested sample was made.

Precision (reproducibility and repeatability):
Toluene standard solution of 4.6 mg/L what is in approximately corresponding to Toluene concentration in 166.5 mg weight amount of the sample in 10 mL acetonitrile, was injected 7 times using the same chromatographic conditions as during the test item measurements and calibration process.

Accuracy:
Three solutions with concentration of Toluene (analytical standard) in blank formulation in range 80%, 100% and 120% level of the analyte in the test sample were prepared. Each solution was injected 7 times using the same chromatographic conditions as during the test item measurements and calibration process.

Linearity and range:
Five standard solutions were prepared in the concentration range about 80-120% analyte content in tested sample. Each calibration solution was analyzed twice under stable chromatographic conditions.

Validation - Results and discussions

Table 5.2-2: Methods suitable for the determination of the relevant impurities in plant protection product (PPP) ALIVE/SHA6100A

	Toluene **max. content in PPP: 0.5 g/kg**
Author(s), year	Kedzierzyn-Kozle, 2017
Principle of method	GC/MS
Linearity (linear between	3.7 – 5.4 mg/L R^2=0.9991

	Toluene **max. content in PPP: 0.5 g/kg**
mg/L) **(correlation coefficient, expressed as r)**	y=617423x-20875
Precision – Repeatability Mean **n = 7** **(%RSD)**	0.16%
Accuracy **n = 7** **(% Recovery)**	8 %w/v: 99.4% 10 %w/v: 99.7% 12 %w/v: 99.1%
Interference/ Specificity	Interferences from impurities constitute didn't exceed 0.42% of total peak area in test sample and 1.3% of total peak area in analytical standard. In both cases do not contribute acceptable 3%.
LOQ	3.71 mg/L
Comment	-

Conclusion

The GC/MS analytical method for the determination of toluene content in the test item was fully validated.

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. 713 is available for Propaquizafop.

5.2.2 Methods for the determination of residues (KCP 5.1.2)

Please refer to post-registration methods.

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

5.3.1 Analysis of the plant protection product (KCP 5.2)

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

5.3.2 Description of analytical methods for the determination of residues of Propaquizafop (KCP 5.2)

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

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

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

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Plant, high water content	Quizalofop (sum of quizalofop, its salts, its esters (including propaquizafop) and its conjugates, expressed as quizalofop (any ratio of constituent isomers))	0.01 mg/kg	Reg. (EU) 2019/973
Plant, high acid content		0.02 mg/kg	Reg. (EU) 2019/973
Plant, high protein/high starch content (dry commodities)		0.01 mg/kg	Reg. (EU) 2019/973
Plant, high oil content		0.01 mg/kg	Reg. (EU) 2019/973
Plant, difficult matrices (hops, spices, tea)		0.05 mg/kg	Reg. (EU) 2019/973
Muscle	Quizalofop (sum of quizalofop, its salts, its esters (including propaquizafop) and its conjugates, expressed as quizalofop (any ratio of constituent isomers))	0.02 mg/kg	Reg. (EU) 2019/973
Milk		0.015 mg/kg	Reg. (EU) 2019/973
Eggs		0.01 mg/kg	Reg. (EU) 2019/973
Fat		0.02 mg/kg	Reg. (EU) 2019/973
Liver, kidney		0.03 mg/kg	Reg. (EU) 2019/973
Soil (Ecotoxicology)	Propaquizafop	0.05 mg/kg	common limit
Drinking water (Human toxicology)	Propaquizafop	0.1 µg/L	general limit for drinking water
Surface water (Ecotoxicology)	Propaquizafop	19 µg/L	Lowest NOEC form aquatic toxicity study on *Oncorhynchus mykiss*
Air	Propaquizafop	12 µg/m^3	AOEL sys: 0.04 mg/kg bw/d
Tissue (meat or liver)	-	Not required	Not classified as T / T+
Body fluids		Not required	Not classified as T / T+

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

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

Evaluator's comments:
Sufficiently validated in matrices with high water, high oil content and in dry matrices methods at the

validated limit of quantification (LOQ) of 0.005 mg/kg are available. An independent validation (ILV) of this method was performed in high water, high acid, high oil content matrices and in dry matrices at the LOQ of 0.01 mg/kg. Extraction efficiency and hydrolysis step need to be demonstrated at least in one crop/matrix (EFSA, 2017).

Applicant refers to the unprotected data RR Agil 100 EC (Registration No. R-208/2014):
., 2010 method was validated using apple, tomato, oilseed rape and wheat grain matrices.
This method meets the requirements intended for monitoring purposes. LOQ = 0.005 mg/kg.
., 2004 method was validated using lupin and soybean seeds matrices.
This method meets the requirements intended for monitoring purposes. LOQ = 0.01 mg/kg.

Data gaps:
Method for high acid content matrices should be provided by the applicant.
ILV and confirmatory methods for plant matrices should be provided with currently required LOQs (Reg. (EU) 2019/973).
Extraction efficiency for plant matrices methods need to be demonstrated at least in one crop/matrix

See point 5.1
The Applicant has completed the dRR (January 2022)
The additions have been accepted and are sufficient.

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: Sum of propaquizafop and quizalofop, expressed as quizalofop				
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	0.2 mg/kg 0.02 mg/kg 0.04 mg/kg	GC-ECD GC-MS LC-MS/MS GC-NPD GC-NPD	DAR,2005
	ILV	0.02 mg/kg	LC-MS/MS	DAR,2005
	Confirmatory (if required)	0.02 mg/kg	LC-MS/MS	DAR,2005
High acid content	Primary	-	-	-
	ILV	-	-	-
	Confirmatory (if required)	-	-	-
High oil content	Primary	0.02 mg/kg 0.04 mg/kg	GC-MS LC-MS/MS GC-NPD GC-NPD	DAR,2005
	ILV	0.02 mg/kg	GC-MS	
	Confirmatory (if required)	0.02 mg/kg	GC-MS	
High protein/high	Primary	0.1 - 0.2 mg/kg 0.02 mg/kg	GC-ECD GC-MS	DAR,2005

Component of residue definition: Sum of propaquizafop and quizalofop, expressed as quizalofop				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
starch content (dry)		0.04 mg/kg 0.02 mg/kg	GC-NPD GC-NPD LC-MS/MS	
	ILV	0.02 mg/kg	LC-MS/MS	
	Confirmatory (if required)	0.02 mg/kg	LC-MS/MS	

Table 5.3-3: Statement on extraction efficiency

	Method for products of plant origin
Required, available from:	Results of extraction efficiencies using the different methods and identification of extracted radioactivity are presented in soybean and cotton during the inclusion of active ingredient (DAR 2006)
Not required, because:	Not provided during the EU review

Following same residue definition for propaquizafop and quizalofop-p-ethyl, Quizalofop (sum of quizalofop, its salts, its esters (including propaquizafop) and its conjugates, expressed as quizalofop (any ration of constituent isomers)) Regulation (EU) 2019/973, applicant hereby presents own analytical methods prepared for quizalofop-p-ethly in order to cover the same residue definition required for propaquizafop in product Propaquizafop 10% EC:

Table 5.3-4: 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: Quizalofop (sum of quizalofop, its salts, its esters (including propaquizafop) and its conjugates, expressed as quizalofop (any ratio of constituent isomers))				
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	0.005 mg/kg 0.01 µg/kg	HPLC-MS/MS HPLC-MS/MS	Düsterloh K., 2008 Bedoret T., 2013b
	ILV	0.005 mg/kg	HPLC-MS/MS	Lentheric I, 2008
	Confirmatory (if required)	-	-	Provided on second mass transition.
High acid content	Primary	0.01 mg/kg 0.005 mg/kg	HPLC-MS/MS LC-MS	Bedoret, T., 2013c Pivato M., 2017 Meseguer, C., 2018 (KCP 5.2.1/07)
	ILV	0.01 mg/kg	HPLC-MS/MS	Paszek, G., 2021 (KCP 5.2.1/19)*

Component of residue definition: Quizalofop (sum of quizalofop, its salts, its esters (including propaquizafop) and its conjugates, expressed as quizalofop (any ratio of constituent isomers))				
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)	-	-	Provided on second mass transition.
High oil content	Primary	0.005 mg/kg 0.01 mg/kg 0.01 µg/kg	HPLC-MS/MS LC-MS/MS HPLC-MS/MS	Pigeon O, 2009 Düsterloh K., 2008 Bedoret T., 2013a
	ILV	0.01 mg/kg	LC-MS/MS	Düsterloh K., 2008
	Confirmatory (if required)	-	-	Provided on second mass transition.
High protein/high starch content (dry)	Primary	0.01 µg/kg 0.005 mg/kg (dry pea) 0.005 mg/kg (fresh pea)	HPLC-MS/MS LC-MS LC-MS	Bedoret T., 2013b Pivato M., 2017 Pivot M., 2017
	ILV	-	-	-
	Confirmatory (if required)	-	-	-

*If the primary method is identical for all matrix group, it is sufficient to perform the ILV for commodities of two of these groups, one of them with high water content (SANTE/2020/12830). Considering this approach, the study Paszek, G., 2021 would be considered also ILV of Meseguer, C., 2018 in all group matrices (high water, high oil, high starch/protein, and high acid).

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

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 Propaquizafop in animal matrices is given in the following tables. Since there will be no requirement to set RMLs in products of animal origin, a post registration method is not considered necessary for Propaquizafop under the proposed GAP. However, one study was submitted in a DAR and is presented below.

Evaluator's comments:
HPLC-FLD (common moiety method), 0.01 mg/kg (milk and eggs) 0.02 mg/kg (tissues). ILV and confirmatory methods available. Extraction efficiency need to be demonstrated. (EFSA, 2017).

Applicant refers to the unprotected data RR Agil 100 EC (Registration No. R-208/2014).
Data gaps:
ILV and confirmatory methods should be provided by the applicant. Extraction efficiency need to be demonstrated.

See point 5.1
The Applicant has completed the dRR (January 2022)
The additions have been accepted and are sufficient.

Table 5.3-5: Validated methods for food and feed of animal origin (if appropriate)

Component of residue definition: Propaquizafop (defined as propaquizafop, propaquizafop acid and hydroxy ether metabolites)				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Milk	Primary	0.005 mg/L	GC-NPD	DAR,2005
	ILV	-	-	-
	Confirmatory (if required)	-	-	-
Eggs	Primary	0.01 mg/kg	GC-NPD	/ DAR,2005
	ILV	-	-	-
	Confirmatory (if required)	-	-	-
Muscle	Primary	0.01 mg/kg	GC-NPD	DAR,2005
	ILV	-	-	-
	Confirmatory (if required)	-	-	-
Fat	Primary	0.01 mg/kg	GC-NPD	DAR,2005
	ILV	-	-	-
	Confirmatory (if required)	-	-	-
Kidney, liver	Primary	0.01 mg/kg	GC-NPD	DAR,2005
	ILV	-	-	-
	Confirmatory (if required)	-	-	-

Table 5.3-6: Statement on extraction efficiency

	Method for products of animal origin
Required, available from:	-
Not required, because:	Not provided during the EU review

Animal metabolism studies presented during the Annex I inclusion process of Propaquizafop demonstrate that Propaquizafop is rapidly metabolised and excreted from the body and therefore it is unlikely that there will be significant accumulation of residues in animal tissues and animal products. Since there will be no requirement to set MRLs in products of animal origin a post registration method is not considered necessary for propaquizafop under the proposed GAP. Therefore, no extraction efficiency is needed.

Following same residue definition for propaquizafop and quizalofop-p-ethyl, Quizalofop (sum of quizalofop, its salts, its esters (including propaquizafop) and its conjugates, expressed as quizalofop (any ration of constituent isomers)) Regulation (EU) 2019/973, applicant hereby presents own analytical methods prepared for quizalofop-p-ethly in order to cover the same residue definition required for propaquizafop in product Propaquizafop 10% EC:

Table 5.3-7: Validated methods for food and feed of animal origin (if appropriate)

Component of residue definition: quizalofop-p-ethyl and quizalofop-P				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Milk	Primary	0.005 mg/kg	LC-MS	Pivato, M., 2016
	ILV	-	-	-
	Confirmatory (if required)	-	-	Provided on second mass transition.
Eggs	Primary	0.005 mg/kg	LC-MS	Pivato, M., 2016
	ILV	-	-	-
	Confirmatory (if required)	-	-	Provided on second mass transition.
Muscle	Primary	0.005 mg/kg	LC-MS	Pivato, M., 2016
	ILV	0.005 mg/kg	LC-MS	Markowicz, A, 2020
	Confirmatory (if required)	-	-	Provided on second mass transition.
Fat	Primary	0.005 mg/kg	LC-MS	Pivato, M., 2016
	ILV	0.005 mg/kg	LC-MS	Markowicz, A, 2020
	Confirmatory (if required)	-	-	Provided on second mass transition.
Kidney, liver	Primary	0.005 mg/kg	LC-MS	Pivato, M., 2016
	ILV	-	-	-
	Confirmatory (if required)	-	-	Provided on second mass transition.

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

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 Propaquizafop in soil is given in the following tables.

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

Component of residue definition: Propaquizafop			
Method type	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	0.04 mg/kg 0.02 mg/kg 0.02 mg/kg 0.01 mg/kg	GC-NPD GC-NPD GC-MS HPLC-UV	DAR,2005
Confirmatory	0.01 mg/kg	HPLC-UV	/ DAR,2005

For any special comments or remarkable points concerning the analytical methods for soil 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 Propaquizafop in surface and drinking water is given in the following tables.

Evaluator's comments:
Applicant refers to the unprotected data RR Agil 100 EC (Registration No. R-208/2014).

The method is validated, analytical method meets the requirements intended for monitoring purposes. LOQ: 0.1 µg/L

See point 5.1
The Applicant has completed the dRR (January 2022)
The additions have been accepted and are sufficient.

Table 5.3-9: Validated methods for water (if appropriate)

Component of residue definition: Propaquizafop				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Drinking water	Primary	0.1 µg/L 0.05 µg/L 0.05 µg/L 0.1 µg/L	GC-NPD GC-NPD HPLC-UV HPLC-UV	DAR,2005
	ILV	-	-	-
	Confirmatory	0.1 µg/L	HPLC-UV	DAR,2005
Surface water	Primary	0.1 µg/L	HPLC-UV	DAR,2005
	Confirmatory	0.1 µg/L	HPLC-UV	DAR,2005

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 Propaquizafop in air is given in the following tables.

Table 5.3-10: Validated methods for air (if appropriate)

Component of residue definition: Propaquizafop			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	5 µg/m^3 0.45 µg/m^3	HPLC-UV HPLC-UV	DAR,2005
Confirmatory	0.45 µg/m^3	HPLC-UV	/ DAR,2005

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

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

Not relevant, as Propaquizafop is not classified as toxic or very toxic.

5.3.2.8 Other studies/ information

No new or additional studies have been submitted.

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.	Barbara Krzysiak-Warzała	2017	Propaquizafop 10% EC: Analysis of active substances content and physicochemical properites of initial preparation and preparation after accelerated storage procedure ISCO, Report No 100/2017/BA-AD GLP Unpublished	N	Sharda Cropchem Limited
KCP 5.2.1-01	Düsterloh K.	2008	Development and Validation of a residue analytical method for the determination of Quizalofop-P-Ethyl and its metabolites (all expressed as Quizalofop-P-Ethyl equivalents) in sugar beet tops and roots. RCC Ltd, Switzerland, RCC Study number B72922, GLP, Unpublished	N	SHARDA Worldwide Exports Pvt. Ltd
KCP 5.2.1-01	Lentheric I.	2008	ILV (Independent Laboratory Validation) of a Residue Analytical Method for the Determination of Quizalofop-P-Ethyl and its Metabolites (all expressed as Quizalofop-P-Ethyl equivalents) in Sugar Beet Tops and Roots Harlan Laboratories S.A. Study S16134 GLP, Unpublished	N	SHARDA Worldwide Exports Pvt. Ltd
KCP 5.2.1-06	Bedoret, T.	2013c	Residue of quizalofop-ethyl, quizalofop and quizalofop conjugate at harvest following one application of SHAQPE120 in grapevine in open field conditions. France, Spain and Italy, seasone 2012 Redebel	N	Sharda Cropchem Ltd.

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			Report no.: SHA-G140TO142-12 GLP Unpublished		
KCP 5.2.1-02	Pigeon, O.	2009	Residue of quizalofop-p-ethyl (and its metabolites) in sunflower in open field conditions at harvest or at intervals following one application of quizalofop-p-ethyl 5% EC. Greece, Italy, Spain and Southern France – Saison 2008 REdebel Report no.: B21852 GLP Unpublished	N	Sharda Cropchem Ltd.
KCP 5.2.1-03	Düsterloh, K.	2008	Validtion of residue analytical method for the determiatnio of quizalofop-p-ethyl technical 95% and its metabolites (all expressed as quizalofop-pethyl equivalents) in oil seed rape Harlan Report no.: B91618 GLP Unpublished	N	Sharda Cropchem Ltd.
KCP 5.2.1-05	Bedoret, T.	2013b	Residue of quizalofop-ethyl, quizalofop and quizalofop conjugate at intervals or at harvest following one application of SHAQPE120 in peas in open field conditions. France, Spain and Italy, Season 2012 Redebel Report no.: SHA-G103TO110-12 GLP Unpublished	N	Sharda Cropchem Ltd.
KCP 5.2.1-04	Bedoret, T.	2013a	Residue of quizalofop-ethyl, quizalofop and quizalofop conjugate at intervals following one appliation of SHAQPE50 or SHAQPE120 in winter oilseed rape in open field conditions. France, Season 2011-2012 Redebel Report no.: SHA-G101TO102-12 GLP Unpublished	N	Sharda Cropchem Ltd.
KCP 5.2.1-07	Carole Meseguer	2018	Validation of the common moiety Method for the Determination of Quizalofop, Quizalofop-P-ester(s) and Quizalofop conjugate(s) expressed as quizalofop (sum of isomers) in various crops types.	N	Sharda Cropchem

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
			Study code S17-06616 GLP Unpublished		Limited
KCP 5.2.1-08	Pivato M.	2017	Validation of the analytical procedure for the determination of quizalofop free acid and quizalofop-P-ethyl after hydrolysis in grape vine by liquid chromatography Chelabs Study no FR 16.563341.0012 GLP Unpublished	N	Sharda
KCP 5.2.1-09	Pivato M.	2017	Validation of the analytical procedure for the determination of quizalofop free acid and quizalofop-P-ethyl after hydrolysis in fresh pea by liquid chromatography Chelabs Study no FR 16.563341.0011 GLP Unpublished	N	Sharda
KCP 5.2.1-10	Pivato M.	2017	Validation of the analytical procedure for the determination of quizalofop free acid and quizalofop-P-ethyl after hydrolysis in dried peas by liquid chromatography Chelabs Study no FR 16.563341.0010 GLP Unpublished	N	Sharda
KCP 5.2.1-11	Pivato, M.	2016	Validation of the analytical procedure for the determination of quizalofop free acid and quizalofop-P-ethyl after hydrolysis in milk by liquid chromatography Chelab Report no.: 16.563341.0001 GLP Unpublished	N	Sharda Cropchem Ltd.
KCP 5.2.1-12	Pivato, M.	2016	Validation of the analytical procedure for the determination of quizalofop free acid and quizalofop-P-ethyl after hydrolysis in eggs by liquid chromatography Chelab	N	Sharda Cropchem Ltd.

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			Report no.: 16.563341.0003 GLP Unpublished		
KCP 5.2.1-13	Pivato, M.	2016	Validation of the analytical procedure for the determination of quizalofop free acid and quizalofop-P-ethyl after hydrolysis in meat by liquid chromatography Chelab Report no.: 16.563341.0004 GLP Unpublished	N	Sharda Cropchem Ltd.
KCP 5.2.1-14	Pivato, M.	2016	Validation of the analytical procedure for the determination of quizalofop free acid and quizalofop-P-ethyl after hydrolysis in fat by liquid chromatography Chelab Report no.: 16.563341.0002 GLP Unpublished	N	Sharda Cropchem Ltd.
KCP 5.2.1-15	Pivato, M.	2016	Validation of the analytical procedure for the determination of quizalofop free acid and quizalofop-P-ethyl after hydrolysis in kidney by liquid chromatography Chelab Report no.: 16.563341.0006 GLP Unpublished	N	Sharda Cropchem Ltd.
KCP 5.2.1-16	Pivato, M.	2016	Validation of the analytical procedure for the determination of quizalofop free acid and quizalofop-P-ethyl after hydrolysis in liver by liquid chromatography Chelab Report no.: 16.563341.0005 GLP Unpublished	N	Sharda Cropchem Ltd.
KCP 5.2.1-17	Markowicz, A.	2020	Independent laboratory validation of a method for the determination of quizalofop free acid and quizalofop-p-ethyl after hydrolysis in meat (poultry) by liquid chromatography. Food Safety Laboratory	N	Sharda Cropchem Ltd.

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			Report no.: ZBBZ-2016/09/DPL/2A GLP Unpublished		
KCP 5.2.1-18	Markowicz, A.	2019	Independent laboratory validation of a method for determination of quizalofop free acid and quizalofop-p-etthyl after hydrolysis in fat by liquid chromatography. Food Safety Laboratory Report no.: ZBBZ-2016/09/DPL/1A GLP Unpublished	N	Sharda Cropchem Ltd.
KCP 5.2.1-19	Paszek, G.	2021	Validation of an analytical method for the determination of residues of propaquizafop, quizalofop-ester, quizalofop and quizalofop conjugate in olive, tomato and orange. SGS Poland Report no.: VAL/11/2020 GLP Unpublished	N	Sharda Cropchem Ltd.

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
IIIA 5.3.1/01		2010	Validation of a residue analytical method for the determination of propaquizafop and its metabolite quizalofop-P (expressed as quizalofop) in apple, tomato, oilseed rape and wheat grain	Y	Quena Plant Protection N.V.

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
			Harlan Laboratories Ltd., Itingen, Switzerland Quena Plant Protection N.V. Report No.: 90012134 GLP, Unpublished		
IIIA 5.3.1/02		2004	Validation study of the analytical method for the determination of propaquizafop in lupin and soybean Anadiag, Haguenau, France Quena Plant Protection N.V. Report No.: 90011535 GLP, Unpublished	Y	Quena Plant Protection N.V.
IIIA 5.6/01		2009	Validation of a residue analytical method for the determination of propaquizafop and its metabolite quizalofop-P in surface water Harlan Laboratories Ltd., Itingen, Switzerland Quena Plant Protection N.V. Report No.: 90011787 GLP, Unpublished	Y	Quena Plant Protection N.V.
IIA, 4.2,1/01		1987	CGA233380, Analytical determination of Ro 17-3664/000 and its metabolites Ro 17-3102 and Ro 16-1981 in agricultural products and soil samples. Dr. R. Maag Ltd., Company Report No. 041-6954 (art. 90003669) No GLP, Unpublished	Y	Quena
IIA, 4.2.1/07		1996	Validation of Method REM 163.04, by fortification of untreated pea seed with CGA 233380, CGA 287422, CGA 129674 and CGA 290291 Ciba Agriculture Company Report no. HR0495ER (art. 90003682) GLP, Unpublished	Y	Quena
IIA,		2003a	Development and Validation of a residue analytical method for propaquizafop and propaquizafop-acid in	Y	Quena

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
4.2.1/09			sunflower seed, wheat grain, tomato and apple, Quena Plant Protection N.V. Company Report no. 845105 (art. 90005421) GLP, Unpublished		
IIA, 4.2.1/02		1993	Determination of total residues of parent compound and metabolites CGA 287422 and CGA 129674 as CGA 289746 by gas chromatography (GC), Plant materials, soil ciba geigy LTd. Company report no. REM 163-04 (art. 90003672) GLP, Unpublished	Y	Quena
IIA, 4.2.2/02		1995c	Determination of residue of parent compound as CGA 289746 by high performance liquid chromatography (HPLC), plant materials, soil. Ciba Geigy Ltd. Company report no. REM 163.07 (art. 90003681) GLP unpublished	Y	Quena
IIA, 4.2.1/11		2003	1st Amendment of final report ILV of the analytical method for the determination of propaquizafop and propaquizfaop-acid in Wheat (Frain) and Tomao. Quena Plant protection N.V. Company report no. IF-03/00061233 (art. 90005492) GLP, Unpublished	Y	Quena
IIA, 4.2.1/04		1995a	Validation by Analysis of fortified specimens and determination of recoveries. Validation of Method REM 163.04 Ciba Geigy Ltd. Company report no. special study 113/95 (art. 90003678) GLP, Unpublished	Y	Quena
IIA, 4.2.1/08		2003a	Development and validation of a residue analytical method for propaquizafop and propaquizafop-acid in potato (tubers), sugar beet (roots and tops with leaves), soya (seeds, straw and whole plant) and sunflower (seeds and whole plants) Quena Plant Protection N.V. Company Report no. 845104 (art. 90005414) GLP, Unpublished	Y	Quena

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
IIA, 4.2.1/03		1994a	Validation by analysis of fortified specimens and determination of recoveries Ciba Geigy Ltd. Company report no. Special study 119/94 (art. 90003675) GLP, Unpublished	Y	Quena
IIA, 4.2.1/10		1992	CGA233380, determination of total residues of parent compound and metabolites CGA 287422 and CGA 129674 as CGA289746 in animal tissues. Ciba-Geigy Ltd. Company report no. REM 163.02 (art. 90003671) Not GLP, Unpublished	Y	Quena
IIA, 4.2.1/03		2003b	Development and Validation of a residue analytical method for propaquizafop and propaquizafop-acid in soil. Quena plant protection N.V. Company report no. 845103 (art. 90005283) GLP, Unpublished	Y	Quena
IIA, 4.2.1/04		2003c	Development and validation of a residue analytical method for propaquizafop and propaquizafop-acid in soil Quena Plant Protection N.V. Company Report No. 846117 (art. 90005475) GLP, Unpublished	Y	Quena
IIA, 4.2.3/01		1992	Determination of residues of parent compound and metabolite CGA 287422 as CGA 289746 by gas chromatography (GC), Ciba-Geigy Ltd. Company report No. REM 163.01 (art. 90003670) Not GLP, Unpublished	Y	Quena
IIA, 4.2.3/02		1994b	Validation by analysis of fortified specimens and determination of recoveries Ciba Geigy Ltd. Company report no. Special study 133/94 (art. 90003674) GLP, Unpublished	Y	Quena
IIA, 4.2.3/03		1995d	Determination of parent compound by high performance liquid chromatography (HPLC) Ciba Geigy Ltd. Company report no. REM 163.05 (art. 90003679) GLP, Unpublished	Y	Quena

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
IIA, 4.2.3/04		1999a	Determination of Parent Compound and metabolite CGA 287422 by High performance liquid chromatography (HPLC) Novartis Crop Protection AG Company Report No. REM 163.08 (art. 90003684) GLP, Unpublished	Y	Quena
IIA, 4.2.3/05		1999b	Validation of Method REM 163.08 by analysis of fortified water specimens for propaquizafop (CGA 233380) and its metabolite CGA 287422 and Evaluation of recoveries Novartis Crop Protection AG Company report no. special study 321/99 (art. 90003685) GLP, Unpublished	Y	Quena
IIA, 4.2.4/01		1994c	Sampling of air and determination of residues of parent compound by high performance liquid chromatography, air Ciba-Geigy Ltd. Company Report No. REM 163.03 (art. 90003673) GLP, Unpublished	Y	Quena
IIA, 4.2.4/02		2003b	Developent and validation of a residue analytical method for propaquizafop and propaquizafop-acid in air. Quena plant protection N.V. Company report no. 845106 (art. 90005357) GLP, Unpublished	Y	Quena

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**
KCP XX	Author	YYYY	Title Company Report N Source GLP/non GLP/GEP/non GEP Published/Unpublished	Y/N	Owner

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

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

Appendix 2 Detailed evaluation of submitted analytical methods

A 2.1 Analytical methods for Propaquizafop

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

No new or additional studies have been submitted

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

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

New data have been submitted.

A 2.1.2.1.1 Analytical method 1

A 2.1.2.1.1.1 Method validation

Comments of zRMS:	The method is accepted

Reference:	KCP 5.2.1-01
Report	Development and validation of a residue analytical method for the determination of quizalofop-p-ethyl and its metabolites (all expressed as quizalofop-p-ethyl equivalents) in sugar beet tops and roots, Düsterloh K., 2008, Report no. B72922
Guideline(s):	Yes (European Commision Directive 96/46/EC, July 16, 1996, SANCO/825/00 rev. 7,SANCO/3029/99 rev. 4)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

A non GLP assessment LC-MS/MS method was developed at RCC Ltd. After liquid extraction, liquid-liquid partition and methylation, concentrations of quizalofop-P-ethyl equivalents were determined as quizalofop-Methyl by LC-MS/MS measurement.

Results and discussions

Specificity

The retention times of quizalofop-ethyl signals in the specimen extracts match the retention time of the standard solution. Interferences were not observed.

Validation

Specificity: no interference with other substances was observed at the retention time of quizalofop-P-ethyl equivalents. Mass spectrometric determination is very specific.

Linearity: method has been shown to be linear over a working range from 0.75 to 15 ng/ml, using 7 different concentrations (N det = 1) with calibration curve in sugar beet tops y=1799.41x-305.26 and correlation coefficient r^2=0.9988 and calibration curve in sugar beet root y=1802.29x-231.95 with correlation coefficient r^2=0.9978 for primary method.

Confirmatory method: calibration curve in sugar beet tops y=9746.76x-1392.60 and correlation coefficient r^2=0.9972 and in sugar beet roots y=9738.10x-1779.65 with correlation coefficient r^2=0.9986.

Accuracy: The mean recovery stay between 70 and 110% of the nominal concentration. From 76% to 83% recovery at lower (0.005mg/kg) and higher (0.05mg/kg) fortification level in sugar beet tops and roots.

Precision: standard deviation ranges from 1 to 4%, therefore stays below the recommended 20%.

Table A 1: Recovery results from method validation of quizalofop-p-ethyl using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
Sugar beet tops	Quizalofop-p-ethyl	0.005	94	4	Primary method
	Quizalofop-p-ethyl	0.050	89	2	
Sugar beet roots	Quizalofop-p-ethyl	0.005	89	1	
	Quizalofop-p-ethyl	0.050	92	4	
Sugar beet tops	Quizalofop-p-ethyl	0.005	81	5	Confirmatory method
	Quizalofop-p-ethyl	0.050	76	4	
Sugar beet roots	Quizalofop-p-ethyl	0.005	83	3	
	Quizalofop-p-ethyl	0.050	83	5	

Table A 2: Characteristics for the analytical method used for validation of quizalofop-p-ethyl in sugar beet

	Quizalofop-p-ethyl
Specificity	Retention time of quizalofop-ethyl signals in the specimen extracts match the retention time of the standard solution. Interferences were not observed.
Calibration (type, number of data points)	Calibration curves were established by injecting MMS calibration solution, N=7
Calibration range	The analytical calibration should extended over a range appropriate to the lowest and highest nominal concentration the analyte ±at least 20%. Accepted calibration range in concentration units ranging from 0.75 ng/mL to 15 ng/mL. Sugar beet tops: y=1799.41x-305.26 R^2=0.9988 Sugar beet roots: y=1802.29x-231.95 R^2=0.9978 Corresponding calibration range in mass ratio units for the sample from 0.005 to 0.050 mg/kg
Assessment of matrix effects is presented	yes
Limit of determination/quantification	limit of quantification was 0.005 mg/kg for all matrices

Conclusion

This method has been successfully validated in terms of specificity, linearity, precision and repeatability. Therefore, it is concluded that this method is suitable for the determination of residue of quizalofop-p-ethyl-equivalent.

A 2.1.2.1.1.2 Independent laboratory validation

Comments of zRMS:	The method is accepted

Reference:	KCP 5.2.1-01
Report	ILV (Independent Laboratory Validation) of a residue analytical method for the Determination of Quizalofop-p-ethyl and its metabolites (all expressed as quizalofop-p-ethyl equivalents) in Sugar beet tops and roots, Lentheric, I., 2009, Report no. S16134
Guideline(s):	Yes (EC Directive 96/46/EC, SANCO/825/00 rev. 7, SANCO/3029/99 rev. 4)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

An ILV was conducted for an HPLC-MS/MS procedure designed for the accurate determination of Quizalofop-P-Ethyl and its metabolites in sugar beet tops and roots. The procedure was already validated at Harlan Laboratories LTD Study no. B72922.
A limit quantification (LOQ) of 0.005 mg/kg and a working range from 0.005 to 0.05 mg/kg were validated.
For Quizalofop-P-Ethyl equivalents the analytical method was successfully validated in sugar beet tops and roots by analyzing two blank control samples, five replicates fortified at LOQ (0.005 mg/kg) and five replicates fortified at 10xLOQ (0.05 mg/kg).
Acquisition and peak calculations and quantification of the analytical reference were performed with the software Analyst, version 1.4.2. using the regression models: y = b*x+a.
The calculation of results is based on peak area measurement and external calibration curve using calibration solutions in solvent matrix matched standard solutions. The calibration curve ranges from 0.75 ng/mL to 15 ng/mL.

Results and discussions

The validation acceptance criteria for Quizalofop-P-Ethyl equivalents were fulfilled and no interference signals were detected in blank control specimen.
LOQ
The limit of quantification is defined as the lowest fortification level with mean recoveries ranging from 70 – 110% at a relative standard deviation (RSD) of ≤20%. The criteria were fulfilled for Quizalofop-p-ethyl equivalents in sugar beet tops and roots at the level of 0.005 mg/kg.
LOD
The limit of detection was found to be 0.003 mg/kg for Quizalofop-p-ethyl equivalents. The LOD was estimated from the lowest calibration standard concentration (0.75 ng/mL) by calculating.
Linearity
Calibration curves were established by injection calibration solution of 7 levels ranging from 0.75 ng/mL to 15 ng/mL, with regression coefficient r≥0.990.

Representative calibration curve in sugar beet tops;
Primary method: y = 9.79e+003x + -3.73e + 003, r = 0.9997
Confirmatory method: y = 1.68e+003x + -671, r = 0.9988

Representative calibration curve in sugar beet roots;
Primary method: y = 8.07e + 003x + 203, r = 0.9992
Confirmatory method: y = 1.4e + 003x + -267, r = 0.9980

Specificity
The retention times of Quizalofop-p-Methyl signals in the specimen extracts match the retention time of the standard solution. Interferences were not observed.

Table A 3: Recovery results from independent laboratory validation of quizalofop-p-ethyl using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (n = 4, n=5)	Mean recovery (%)	RSD (%)	Comments
Sugar beet tops	Quizalofop-p-ethyl	0.005	103	6	
	Quizalofop-p-	0.050	84	17	

Matrix	Analyte	Fortification level (mg/kg) (n = 4, n=5)	Mean recovery (%)	RSD (%)	Comments
	ethyl				
Sugar beet roots	Quizalofop-p-ethyl	0.005	86	10	
	Quizalofop-p-ethyl	0.050	89	14	

Table A 4: Characteristics for the analytical method used for independent laboratory validation of quizalofop-p-ethyl residues in sugar beet roots and tops

	Quizalofop-p-ethyl
Specificity	The retention times of Quizalofop-p-Methyl signals in the specimen extracts match the retention time of the standard solution. Interferences were not observed.
Calibration (type, number of data points)	Calibration curves were established by injection calibration solution of 7 levels ranging from 0.75 ng/mL to 15 ng/mL, with regression coefficient r≥0.990.
Calibration range	The analytical calibration should extended over a range appropriate to the lowest and highest nominal concentration the analyte ±at least 20%. Accepted calibration range in concentration units ranging from 0.75 ng/mL to 15 ng/mL. Sugar beet tops: y=9.79e+003x+-3.73e+003 r=0.9997 Sugar beet roots: y=8.07e+003x+203 r=0.9992 Corresponding calibration range in mass ratio units for the sample from 0.005 to 0.050 mg/kg
Assessment of matrix effects is presented	Yes
Limit of determination/quantification	limit of quantification was 0.005 mg/kg for all matrices

Conclusion

To demonstrate the method to be highly specific a second transition was monitored. As no Quizalofop-P-Ethyl equivalents were recovered in the untreated control specimens the specificity of the method is confirmed.
The method is highly specific and appropriate for the determination of Quizalofop-P-Ethyl equivalents in sugar beet tops and roots.

A 2.1.2.1.2 Analytical method 2

A 2.1.2.1.2.1 Method validation

Comments of zRMS:	The method is accepted

Reference:	KCP 5.2.1-02
Report	Residues of quizalofop-p-ethyl (and its metabolites) in sunflower in open field conditions at harvest or at intervals following one application of quizalofop-p-ethyl, Pigeon O., 2009, Report no. 21852
Guideline(s):	Yes (96/46/EC Directive of July 16, 1996 modifying the 91/414/EEC/Directive, 96/68/EC Directive of October 21, 1996 modifying the 91/414/EEC Directive, SANCO/3029/99, 2000, SANCO/825/00, 2004, SANCO 2007/3131, 2007, Regulation (EC) No 396/2005)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Quizalofop-P-ethyl and quizalofop-P residues are extracted from sunflower (whole plant and seeds) with an acetone / water (80/20, v/v) solution. The samples are extracted using an Ultra Turrax blender for 2 minutes. After filtration and partial evaporation, the extract is cleaned by Solid Supported Liquid / Liquid Extraction (SSLLE) on a diatomaceous earth cartridge. The final extract is analysed by Ultra Performance Liquid Chromatography with Tandem Mass Spectrometry Detection (UPLC-MS/MS) for determination of quizalofop-P-ethyl and quizalofop-P using the external standard calibration.

Results and discussions

Quizalofop-p-ethyl and quizalofop-P residues are extracted from sunflower (whole plant and seeds) with an acetone/water (80/20, v/v) solution. The samples are extracted using an Ultra Turrax blender for 2 minutes. After filtration and partial evaporation, the extract is cleaned by Solid Supported Liquid/Liquid Extraction on a diatomaceous earth cartridge. The final extract is analysed by Ultra Performance Liquid Chromatography with Tandem Mass Spectrometry Detection (UPLC-MS/MS) for determination of quizalofop-p-ethyl and quizalofop-P using the external standard calibration.

Table A 5: Recovery results from method validation of quizalofop-p-ethyl and quizalofop-p using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
Sunflower (whole plant)	Quizalofop-p-ethyl	0.005	83±14%	14.1%	
	Quizalofop-p-ethyl	0.05	70±14%	15.6%	
Sunflower (seed)	Quizalofop-p-ethyl	0.005	77±6%	6.6%	
	Quizalofop-p-ethyl	0.05	94±8%	7.1%	
Sunflower (whole plant)	Quizalofop-p	0.005	71±10%	10.9%	
	Quizalofop-p	0.05	78±6%	6.7%	
Sunflower	Quizalofop-p	0.005	93±10%	8.9%	

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
(seed)	Quizalofop-p	0.05	99±5%	3.7%	

Table A 6: Characteristics for the analytical method used for validation of quizalofop-p-ethyl residues in sunflower

	Quizalofop-p-ethyl	Quizalofop-p
Specificity	No interference likely to affect the chromatographic peak of quizalofop-p-ethyl (<30%LOQ)	No interference likely to affect the chromatographic peak of quizalofop-p (<30%LOQ)
Calibration (type, number of data points)	Linearity was demonstrated over a range of conc. From 0.001 µg/mL to 0.2 µg/mL expressed as quizalofop-p-ethyl and from 0.001 µg/mL to 0.2 µg/mL expressed as quizalofop-P by measuring detector response versus quizalofop-p-ethyl or quizalofop-P concentration.	Linearity was demonstrated over a range of conc. From 0.001 µg/mL to 0.2 µg/mL expressed as quizalofop-p-ethyl and from 0.001 µg/mL to 0.2 µg/mL expressed as quizalofop-P by measuring detector response versus quizalofop-p-ethyl or quizalofop-P concentration.
Calibration range	Curve (8 points) : the response of quizalofop-P-ethyl standard solution in whole plant matrix is linear in the range 0.001 – 0.2 µg/mL. **r^2 = 0.9992** Curve (7 points) : the response of quizalofop-P-ethyl standard solution in seeds matrix is linear in the range 0.001 – 0.1 µg/mL. **r^2 = 0.9995**	Curve (8 points) : the response of quizalofop-P standard solution in top and leaves matrix is linear in the range 0.001 – 0.2 µg/mL. **r^2 = 0.9996** Curve (8 points) : the response of quizalofop-P standard solution in seeds matrix is linear in the range 0.001 – 0.2 µg/mL. **r^2 = 0.9985**
Assessment of matrix effects is presented	Yes	Yes
Limit of quantification	0.005 mg/kg	0.005 mg/kg

Conclusion

This method has been successfully validated in terms of specificity, linearity, precision and repeatability. Therefore, it is concluded that this method is suitable for the determination of residue of quizalofop-p-ethyl-equivalent.

A 2.1.2.1.3 Analytical method 3

A 2.1.2.1.3.1 Method validation

Comments of zRMS:	The method is accepted

Reference:	KCP 5.2.1-03
Report	Development and validation of residue analytical method for the determination of quizalofop-p-ethyl and its metabolites (all expressed as quizalofop-p-ethyl equivalents) in oil seed rape, Düsterloh K., 2008, Report no. B91618
Guideline(s):	Yes (SANCO/825/00 rev. 7, SANCO/3029/99 rev. 4)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The method for the determination of Quizalofop-P-Ethyl equivalents in oil seed rape seeds was formally validated at Harlan Laboratories Ltd. under GLP compliance, according to international guideline (SANCO) using liquid extraction and a LC coupled with MS/MS detection with a limit of quantification (LOQ) of 0.005 mg/kg.

Results and discussions

For Quizalofop-P-Ethyl equivalents the analytical method was successfully validated in oil seed rape seeds by analyzing two blank control samples, five replicates fortified at LOQ (0.01 mg/kg) and five replicates fortified at 10xLOQ (0.10 mg/kg).
The average recovery rate of 107%, at the lower fortification level and 105%, at the higher fortification level, with relative standard deviations of ≤5% without interference signals in the control specimens meets the validation acceptance criteria.
Specificity: The retention times of Quizalofop-methyl signals in the specimen extracts match the retention time of the standard solution. Interferences were not observed.
In conclusion the method is sufficiently specific for the determination of Quizalofop-P-Ethyl equivalents as Quizalofop-Methyl in oil seed rape
Linearity: from 0.8 ng/mL to 15 ng/mL with a r2 ≥0,990.
Accuracy: The validation acceptance criteria for Quizalofop-P-Ethyl equivalents were fulfilled by average recovery rates ranging from 95% to 118% with RDS ≤7%, and no interference signals were detected in blank control specimen.
Precision: Relative standard deviations (RSD) of ≤5%, without interference, therefore stay below the recommended 20%.

Table A 7: Recovery results from method validation of quizalofop-p-ethyl using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (n = 4, n = 5)	Mean recovery (%)	RSD (%)	Comments
Oilseed rape seeds	Quizalofop-p-ethyl	0.010	107%	5%	Primary method
	Quizalofop-p-ethyl	0.100	105%	5%	

Matrix	Analyte	Fortification level (mg/kg) (n = 4, n = 5)	Mean recovery (%)	RSD (%)	Comments
Oilseed rape seeds	Quizalofop-p-ethyl	0.010	107%	7%	Confirmatory method
	Quizalofop-p-ethyl	0.100	106%	7%	

Table A 8: Characteristics for the analytical method used for validation of quizalofop-p-ethyl residues in oilseed rape seeds

	Quizalofop-p-ethyl
Specificity	The retention times of Quizalofop-methyl signals in the specimen exptracts match the retention time of the standard solution. Interferences were not observerd. In conclusion the method is sufficiently specific for the determination of quizalofop-p-ethyl equivalents as quizalofop-methyl in oilseed rape. To demonstrate the method to be highly specific a second transition was monitored. As no Quizalofop-p-ethyl equivalents were recovered in the untreated control specimens the specificity of the method is confirmed.
Calibration (type, number of data points)	For analysis of Quizalofop-p-ethyl as quizalofop-methyl, calibration curves were established by injecting calibration solution of 6 levels ranging from 0.8 ng/mL to 15 ng/mL.
Calibration range	Calibration functions were calculated by linear regression showing regression coefficients r≥0.990. Y = 5214.8x-293.7 R = 0.9985 Confirmatory: Calibration functions were calculated by linear regression showing regression coefficients r≥0.990. y= 1702.7x- 54.867 r = 0.9975
Assessment of matrix effects is presented	Yes
Limit of quantification	0.01 mg/kg

Conclusion

Harlan Laboratories Ltd. performed the validation of the residue analytical method for the determination of Quizalofop-P-Ethyl equivalents in oil seed rape seeds.

The limit of quantification was established at 0.005 mg/kg. It was proven that the extraction method fulfils the reproducibility requirements as defined in the EU Directive 91/414/EEC Annex II (Part A, Section 4.2) and EC Guidance document on Residue Analytical Method (SANCO/825/00 rev. 7 17/03/04) and is, therefore, applicable as enforcement method.

A 2.1.2.1.4 Analytical method 4

A 2.1.2.1.4.1 Method validation

Comments of zRMS:	The method is accepted

Reference:	KCP 5.2.1-04
Report	Residue of quizalofop-ethyl, quizalofop and quizalofop conjugate at intervals following one application of SHAQPE50 or SHAQPE120 in winter oil seed rape in open field conditions. France, Season 2011-2012, Bedoret T., 2013a, Report no. SHA-G101TO102-12
Guideline(s):	Yes (SANCO/825/00 rev. 8.1, 2010, SANCO/3029/99 rev. 4, 2000, OECD 2007 Guidance Document No. 39 and No. 72)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Specimen were mechanically blended in acetone/water (80/20 v/v), acidified to hydrolyse conjugates and cleaned by solid supported liquid-liquid extraction. The final extract was analysed by HPLC equipped with MS triple quadrupole detector. Three ions (one primary and two confirmatory/qualifier ions) were monitored for Quizalofop-p-ethyl and Quizalofop.

Results and discussions

Recovery Findings
Recovery of Quizalofop-p-ethyl and Quizalofop through the analytical procedure was assessed by fortifying 5 aliquots of each matrix at the LOQ of 0.01 µg/kg and 5 aliquots at 50 x LOQ (0.5 µg/kg).
The mean recovery were 56 % (RSD 11.1%) and 64 % (RSD 7.5 %) at fortification of 0.01 and 0.5 mg/kg respectively for Quizalofop-p-ethyl and 87 % (RSD 4.0 %) and 79 % (RSD 8.1 %) at the same fortification levels for Quizalofop.

Considering possible conversion of Quizalofop-p-ethyl to Quizalofop and further degradation of Quizalofop during hydrolysis the total amount of Quizalofop expressed as sum of Quizalofop-p-ethyl, quizalofop and Quizalofop conjugate (sum of all isomers) is deemed to be appropriate for demonstrating acceptable recoveries.

The mean recovery for Quizalofop expressed as sum of Quizalofop-p-ethyl, Quizalofop and Quizalofop conjugate in oilseed rape (grain) meets the EU requirements (SANCO/12495/2011) as the recovery fell into the range of 70 – 120 % with a relative standard deviation of less than 20 %.

Specificity
The method includes three MS transitions (1 primary and 2 confirmatory). No significant interferences arising from the matrices, reagents or solvents tested have been observed at the retention time of interest for Quizalofop-p-ethyl and Quizalofop. As the method provided quantification and identification a confirmatory method was not necessary.

Linearity
The linearity of the HPLC-MS detector responses was confirmed by generating calibration curves.
The linearity of the detector response was assessed by analysis of 8 standard solutions covering the working range of 0.01 – 5 µg/L for Quizalofop-p-ethyl and Quizalofop. Four series of injections were carried out. The correlation coefficients (r^2) of all calibration plots were found to be ≥ 0.99 for Quizalifop-p-ethyl and Quizalofop in oily matrix.

Accuracy
Acceptable mean recoveries between 70% and 120% with a relative standard deviation lower than 20% were found for the ions m/z 373, 299 and 91 for Quizalofop-p-ethyl and m/z 345, 299 163 for Quizalofop in oily matrix.

Repeatability
The relative standard deviations of the 6 consecutive measurements of Quizalofop-p-ethyl and Quizalofop in oilseed rape (grain) were less than 20 % at a fortification level of 0.5 µg/ml. The overall relative standard deviation ranged from 4.2 – 7.3 %. Hence, the method is considered to have acceptable repeatability.

Limit of Quantification
The limit of quantification (LOQ) of the method is defined as the lowest fortification level in the oily matrix at which the methodology has been validated and for which a mean recovery of 70% – 110% with a relative standard deviation (RSD) of ≤ 20% has been obtained.
A limit of quantification of 0.01 µg/kg was confirmed for total Quizalofop-p-ethyl and Quizalofop in oilseed grain.

Table A 9: Recovery results from method validation of quizalofop-p-ethyl using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
Oilseed rape (grain)	Quizalofop	0.02	72%	4.6%	
	Quizalofop	1.0	72%	4.1%	

Table A 10: Characteristics for the analytical method used for validation of quizalofop-p-ethyl residues in oilseed rape (grain)

	Quizalofop-p-ethyl and quizalofop
Specificity	The analysis of blanks and untreated samples in comparison with the analysis of standard solutions and spiked samples showed the absence of compound interfering with the determination of quizalofop-ethyl and quizalofop (<30% LOQ). Moreover the relative intensities of the detected m/z ions in the spiked samples corresponds to those of the standard solutions at comparable concentrations.
Calibration (type, number of data points)	The linearity of the detector response was assessed by analysis of 8 standard solutions covering the working range of 0.01 – 5 µg/L for Quizalofop-p-ethyl and Quizalofop expressed as quizalofop equivalent by measuring the detector response versus quizalofop-ethyl and quizalofop conc.. Four series of injections were carried out. The correlation coefficients (r^2) of all calibration plots were found to be ≥ 0.99 for Quizalifop-p-ethyl and Quizalofop in oily matrix.
Calibration range	Calibration curve of quizalofop-ethyl by LC-MS/MS: Y=40808x + 3899.2 R^2 = 0.9951 Calibration curve of quizalofop by LC-MS/MS Y=9 853.4985 + 252.3685 R^2 = 0.9980

	Quizalofop-p-ethyl and quizalofop
Specificity	The analysis of blanks and untreated samples in comparison with the analysis of standard solutions and spiked samples showed the absence of compound interfering with the determination of quizalofop-ethyl and quizalofop (<30% LOQ). Moreover the relative intensities of the detected m/z ions in the spiked samples corresponds to those of the standard solutions at comparable concentrations.
Assessment of matrix effects is presented	yes
Limit of quantification	0.01 mg/kg

Conclusion

Determination of Quizalofop expressed as Quizalofop-p-ethyl, Quizalofop and Quizalofop conjugate in oilseed rape grain by HPLC-MS/MS has been successfully validated and an LOQ of 0.01 µg/kg was established fpr both analytes. Results obtained were within the guideline requirements (mean recovery 70 – 120 %, RSD <20%).

A 2.1.2.1.5 Analytical method 5

A 2.1.2.1.5.1 Method validation

Comments of zRMS:	The method is accepted

Reference:	KCP 5.2.1-05
Report	Residue of quizalofop-ethyl, quizalofop and quizalofop conjugate at intervals or at harvest following one application of SHAQPE120 in peas in open field conditions. France, Spain and Italy, season 2012, Bedoret T., 2013b, Report no. SHA-G103TO110-12
Guideline(s):	Yes (SANCO/825/00 rev. 8.1, SANCO/3029/99 rev. 4, Guide to Codex Alimentarius recommendations concerning pesticide residue. FAO, Rome, 1993 and 2000)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

This analytical method and validation were not evaluated during the original Annex I submission. The analytical method presented here is suitable for the determination of Quizalofop-p-ethyl and Quizalofop in pea (whole plant) and pea (dry seed) as representative matrices for commodities of high water and high starch content, respectively. The validation was carried out in conjunction with the analysis of residues. The analytical phase report is attached to the residue report as Appendix.
Specimen were mechanically blended in acetone/water (80/20 v/v), acidified to hydrolyse conjugates and cleaned by solid supported liquid-liquid extraction. The final extract was analysed by HPLC equipped

with MS triple quadrupole detector. Three ions (one primary and two confirmatory/qualifier ions) were monitored for Quizalofop-p-ethyl and Quizalofop.

Results and discussions

Recovery of Quizalofop-p-ethyl and Quizalofop through the analytical procedure was assessed by fortifying 5 aliquots of each matrix at the LOQ of 0.01 µg/kg and 5 aliquots at 50 x LOQ (0.5 µg/kg).
In pea (whole plant) the mean recovery were 66 % (RSD 9.0%) and 80 % (RSD 4.7 %) at fortification of 0.01 and 0.5 mg/kg respectively for Quizalofop-p-ethyl and 74 % (RSD 10.9 %) and 89 % (RSD 4.2 %) at the same fortification levels for Quizalofop.
In pea (dry seed) the mean recovery were 76 % (RSD 13.4 %) and 113 % (RSD 9.2 %) at fortification of 0.01 and 0.5 mg/kg respectively for Quizalofop-p-ethyl and 75 % (RSD 7.5 %) and 78 % (RSD 18.3 %) at the same fortification levels for Quizalofop.

Considering possible conversion of Quizalofop-p-ethyl to Quizalofop and further degradation of Quizalofop during hydrolysis the total amount of Quizalofop expressed as sum of Quizalofop-p-ethyl, quizalofop and Quizalofop conjugate (sum of all isomers) is deemed to be appropriate for demonstrating acceptable recoveries.

The mean recovery for quizalofop-p-ethyl expressed as sum of Quizalofop-p-ethyl, Quizalofop and Quizalofop conjugate in pea (whole plant) and pea (dry seed) meets the EU requirements (SANCO/12495/2011) as the data fell into the range of 70 – 120 % with a relative standard deviation of less than 20 %.

Specificity
The method includes three MS transitions (1 primary and 2 confirmatory). No significant interferences arising from the matrices, reagents or solvents tested have been observed at the retention time of interest for Quizalofop-p-ethyl and Quizalofop. As the method provided quantification and identification a confirmatory method was not necessary.

Linearity
The linearity of the HPLC-MS detector responses was confirmed by generating calibration curves.
The linearity of the detector response was assessed by analysis of 8 standard solutions covering the working range of 0.01 – 5 µg/L for Quizalofop-p-ethyl and Quizalofop. Four series of injections were carried out. The correlation coefficients (r) of all calibration plots were found to be ≥ 0.99 for Quizalifop-p-ethyl and Quizalofop in pea (whole plant) and pea (dry seed).

Accuracy
Acceptable mean recoveries between 70% and 120% with a relative standard deviation lower than 20% were found for the ions m/z 373, 299 and 91 for quizalofop-p-ethyl and m/z 345, 299 163 for quizalofop in both matrices.

Repeatability
The relative standard deviations of the measurements of Quizalofop-p-ethyl and Quizalofop in the pea (whole plant) and pea (dry seed) were less than 20% at fortification level of 0.5 µg/ml in either matrix.
The overall relative standard deviation (n = 6) ranged from 5.0 – 7.2 % and therefore the method is considered to have acceptable repeatability.

Limit of Quantification
The limit of quantification (LOQ) of the method is defined as the lowest fortification level in the matrix at which the methodology has been validated and for which a mean recovery of
70% – 110% with a relative standard deviation (RSD) of ≤ 20% has been obtained.
A limit of quantification of 0.01 µg/kg was confirmed for total Quizalofop-p-ethyl and Quizalofop in pea (whole plant) and pea (dry seed).

Table A 11: Recovery results from method validation of quizalofop-ethyl and quizalofop using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
Pea (whole plant)	Quizalofop-ethyl	0.01	66	9	
	Quizalofop-ethyl	0.5	80	4.7	
Pea (dry seed)	Quizalofop-ethyl	0.01	76	13.4	
	Quizalofop-ethyl	0.5	113	9.2	
Pea (whole plant)	Quizalofop	0.01	74	10.9	
	Quizalofop	0.5	89	4.2	
Pea (dry seed)	Quizalofop	0.01	75	7.5	
	quizalofop	0.5	78	18.3	
Pea (whole plant)	Total quizalofop	0.02	70	9.5	
	Total quizalofop	1.0	85	3.5	
Pea (dry seed)	Total quizalofop	0.02	75	8.7	
	Total quizalofop	1.0	95	12.4	

Table A 12: Characteristics for the analytical method used for validation of quizalofop and quizalofop-ethyl, expressed as quizalofop residues in pea (whole plant and dry seed)

	Quizalofop-ethyl	Quizalofop
Specificity	The analysis of blanks and untreated samples in comparison with the analysis of standard solutions and spiked samples showed the absence of compound interfering with the determination of quizalofop-ethyl and quizalofop (<30% LOQ)	
Calibration (type, number of data points)	The linearity was demonstrated over a range of concentrations from 0.01 µg/mL to 5 µg/mL for quizalofop and quizalofop-ethyl, expressed as quizalfoop equivalent by measuring the detector response versus quizalofop-ethyl and quizalofop concentratrion for a series of 8 standard solutions.	
Calibration range	Pea whole plant (from 0.01 µg/mL to 5 µg/mL): y=24721x+1213.3 R^2=0.9989 Pea dry seed (from 0.01 µg/mL to 2 µg/mL): y=25397.7480x-245.7611 R^2=0.9950	Pea whole plant(from 0.01 µg/mL to 5 µg/mL): y=4547.4x+229.75 R^2=0.9995 Pea dry seed (from 0.01 µg/mL to 5 µg/mL): Y=5697x+464.55 R^2=0.9932
Assessment of matrix effects is presented	yes	yes
Limit of quantification	0.01 µg/kg	0.01 µg/kg

Conclusion

Determination of Quizalofop expressed as Quizalofop-p-ethyl, Quizalofop and Quizalofop conjugate in pea (whole plant) and pea (dry seed) by HPLC-MS/MS has been successfully validated and a LOQ of 0.01 µg/kg was established. Results obtained were within the guideline requirements (mean recovery 70 – 120 %, RSD <20%).

A 2.1.2.1.6 Analytical method 6

A 2.1.2.1.6.1 Method validation

Comments of zRMS:	The method is accepted

Reference:	KCP 5.2.1-06
Report	Residue of quizalofop-p-ethyl, quizalofop and quizalofop conjugate at harvest following one application of SHAQPE 120 in grapevine in open field conditions, France, Italy, Spain Season 2012, Bedoret T., 2013c, Report no. SHA-G103TO142-12
Guideline(s):	Yes (EU Guideline SANCO/825/00 rev. 8.1, 2010, SANCO/3029/99 rev.4, 2000, OECD (2007) Guidance Document on Pesticide Residue Analytical Methods. Series on Pesticides No. 39 and No. 72)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

This analytical method and validation were not evaluated during the original Annex I submission. The analytical method presented here is suitable for the determination of Quizalofop-p-ethyl and Quizalofop in grape as representative matrices for commodities of high acid content. The validation was carried out in conjunction with the analysis of residues. The analytical phase report is attached to the residue report as Appendix.
Specimen were mechanically blended in acetone/water (80/20 v/v), acidified to hydrolyse conjugates and cleaned by solid supported liquid-liquid extraction. The final extract was analysed by HPLC equipped with MS triple quadrupole detector. Three ions (one primary and two confirmatory/qualifier ions) were monitored for Quizalofop-p-ethyl and Quizalofop.

Results and discussions

Recovery of Quizalofop-p-ethyl and Quizalofop through the analytical procedure was assessed by fortifying 5 aliquots of each matrix at the LOQ of 0.01 µg/kg and 5 aliquots at 50 x LOQ (0.5 µg/kg).
In grape the mean recovery were 87 % (RSD 2.6 %) and 95 % (RSD 5.3 %) at fortification of 0.01 and 0.5 mg/kg respectively for Quizalofop-p-ethyl and 76 % (RSD 6.7 %) and 87 % (RSD 2.6 %) at the same fortification levels for Quizalofop.

Considering possible conversion of Quizalofop-p-ethyl to Quizalofop and further degradation of Quizalofop during hydrolysis the total amount of Quizalofop expressed as sum of Quizalofop-p-ethyl, quizalofop

and Quizalofop conjugate (sum of all isomers) is deemed to be appropriate for demonstrating acceptable recoveries.

The mean recovery for Quizalofop expressed as sum of Quizalofop-p-ethyl, Quizalofop and Quizalofop conjugate in grape meets the EU requirements (SANCO/12495/2011) as the recovery fell into the range of 70 – 120 % with a relative standard deviation of less than 20 %.

Specificity
The method includes three MS transitions (1 primary and 2 confirmatory). No significant interferences arising from the matrices, reagents or solvents tested have been observed at the retention time of interest for Quizalofop-p-ethyl and Quizalofop. As the method provided quantification and identification a confirmatory method was not necessary.

Linearity
The linearity of the HPLC-MS detector responses was confirmed by generating calibration curves.
The linearity of the detector response was assessed by analysis of 8 standard solutions covering the working range of 0.01 – 5 µg/L for Quizalofop-p-ethyl and Quizalofop. Four series of injections were carried out. The correlation coefficients (r^2) of all calibration plots were found to be ≥ 0.99 for Quizalifop-p-ethyl and Quizalofop in grapes.

Accuracy
Acceptable mean recoveries between 70% and 120% with a relative standard deviation lower than 20% were found for the ions m/z 373, 299 and 91 for quizalofop-p-ethyl and m/z 345, 299 163 for quizalofop in grape matrix.

Repeatability
The relative standard deviations of the 6 consecutive measurements of Quizalofop-p-ethyl and Quizalofop in grape were less than 20 % at a fortification level of 0.5 µg/ml. The overall relative standard deviation ranged from 3.5 – 5.9 %. Hence, the method is considered to have acceptable repeatability.

Limit of Quantification
The limit of quantification (LOQ) of the method is defined as the lowest fortification level in the grape matrix at which the methodology has been validated and for which a mean recovery of
70% – 110% with a relative standard deviation (RSD) of ≤ 20% has been obtained.
A limit of quantification of 0.01 µg/kg was confirmed for total Quizalofop-p-ethyl and Quizalofop in grape.

Table A 13: Recovery results from method validation of quizalofop-ethyl and quizalofop using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
grape	Quizalofop-ethyl	0.01	87	2.6	
	Quizalofop-ethyl	0.5	95	5.3	
grape	quizalofop	0.01	76	6.7	
	quizalofop	0.5	87	2.6	
grape	Total quizalofop	0.02	81	2.8	
	Total quizalofop	1.0	91	3.5	

Table A 14: Characteristics for the analytical method used for validation of quizalofop-pethyl residues in grapes

	Quizalofop-ethyl	quizalofop
Specificity	The analysis of blanks and untreated samples in comparison with the analysis of standard solutions and spiked samples slowed the absence of compound interfering with the determination of quizalofop-ethyl and quizalofop (<30% LOQ).	
Calibration (type, number of data points)	The linearity was demonstraged over a range of concentrations from 0.01 µg/mL for quizalofop, expressed as quizalofop equivalent by measuring the deterctor response versus quizalofop-ethyl and quizalofop concentration for a series of 8 or 9 standard solution in grape matrix of known concentration.	
Calibration range	grape (from 0.01 µg/mL to 5 µg/mL): y=17876x+1603.4 R^2=0.9956	grape (from 0.01 µg/mL to 2 µg/mL): y=3829.7338x+36.2732 R^2=0.9994
Assessment of matrix effects is presented	yes	yes
Limit of quantification	0.01 µg/kg	0.01 µg/kg

Conclusion

Determination of Quizalofop expressed as Quizalofop-p-ethyl, Quizalofop and Quizalofop conjugate in grape by HPLC-MS/MS has been successfully validated and an LOQ of 0.01 µg/kg was established. Results obtained were within the guideline requirements (mean recovery 70 – 120 %, RSD <20%).

A 2.1.2.1.1 Analytical method 7

A 2.1.2.1.1.1 Method validation

Comments of zRMS:	The method is accepted

Reference:	KCP 5.2.1-07
Report	Validation of the common moiety Method for the Determination of Quizalofop, Quizalofop-P-ester(s) and Quizalofop conjugate(s) expressed as quizalofop (sum of isomers) in various crops types. Carole Meseguer, 2018. Study code S17-06616
Guideline(s):	Yes SANCO/825/00, rev. 8.1 SANCO/3029/99, rev. 4
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Study Objective
The objective of the study was to validate an analytical method for the determination of quizalofop, quizalofop-P-ethyl, quizalofop-P-tefuryl, propaquizafop and quizalofop- P-acid glucose conjugate expressed as quizalofop (sum isomers) in cucumber (high water content), grape (high acid content), carrot (high starch content), rice (high starch content) and peas (high protein content) matrices according to the guidance documents SANCO/825/00, rev. 8.1 and SANCO/3029/99, rev. 4 with an intended limit of quantification (LOQ) of 0.01 mg/kg for each analyte.
Analytical Procedure
In summary, residues of quizalofop, quizalofop-P-ethyl, quizalofop-P-tefuryl, propaquizafop and quizalofop- P-acid glucose conjugate were extracted with acetone/water (80/20), followed by a basic hydrolysis with KOH. During basic hydrolysis, all quizalofop esters and conjugates are hydrolysed into quizalofop. The extracts were then diluted with ultra-pure water prior to quantification of quizalofop by LC-MS/MS.

Extraction Procedure for cucumber, carrot and grape

Extraction
- 20 mL of acetone/ultra-pure water (80/20) (v/v) was added and mixed using flatbed shaker for 10 minutes at 150 rpm.
- The sample was centrifuged at 4000 rpm for 3 minutes.

Hydrolysis
Note: from this step, the extraction procedure was not stopped until the last step to avoid degradation of quizalofop
- 10 mL of the supernatant was transferred into a 15 mL polypropylene tube.
- 1000 µL of KOH 5M was added.
- The sample was shaken using flatbed shaker for 20 minutes at 150 rpm.
- The sample was centrifuged at 4000 rpm for 3 minutes.

Dilution
- 1 mL of the sample (supernatant) was transferred into a 15 mL polypropylene tube.
- 500 µL of ultra-pure-water + 2% formic acid was added.
- 8.5 mL of ultra-pure-water was added.
- The pH was checked to be between 2 and 7 with pH paper
- The sample was vortexed for 30 seconds.
- The sample was transferred into a vial for LC-MS/MS injection.

Extraction Procedure for rice

Soaking
- 5 mL of ultra-pure water + 2% formic acid was added.
- The sample was vortexed and left in contact for at least 5 minutes.

Extraction
- 20 mL of acetone/ultra-pure water (80/20) (v/v) was added
- The sample was homogenised using ultra-turrax for 1 minute at 7000 rpm
- The sample was mixed using flatbed shaker for 30 minutes at 150 rpm.
- The sample was centrifuged at 4000 rpm for 3 minutes.
- The supernatant was transferred into another centrifuge tube (50mL size) and the pellet was kept for the next step
- 20 mL of acetone/ultra-pure water (80/20) (v/v) was added in the centrifuge tube containing the pellet
- The sample was mixed using flatbed shaker for 30 minutes at 150 rpm.
- The sample was centrifuged at 4000 rpm for 3 minutes.
- The supernatant was combined with the previous obtained supernatant (the extract sample volume was about 40mL)

Hydrolysis
Note: from this step, the extraction procedure was not stopped until the last step to avoid degradation of quizalofop
- 10 mL of the supernatant was transferred into a 15 mL polypropylene tube.
- 1000 µL of KOH 5M was added.
- The sample was shaken using flatbed shaker for 20 minutes at 150 rpm.
- The sample was centrifuged at 4000 rpm for 3 minutes.

Dilution
- 1 mL of the sample (supernatant) was transferred into a 15 mL polypropylene tube.
- 500 µL of ultra-pure-water + 2% formic acid was added.
- 8.5 mL of ultra-pure-water was added.
- The pH was checked to be between 2 and 7 with pH paper
- The sample was vortexed for 30 seconds.
- The sample was transferred into a vial for LC-MS/MS injection.

Results and discussions

Selectivity
Quantification was performed by use of LC-MS/MS detection. Two (2) mass transitions were evaluated for quizalofop in order to demonstrate that the method achieves a high level of selectivity. No significant interference above 30 % of LOQ was detected in any of the reagent blanks or the control sample extracts of each matrix, so that a high level of selectivity was demonstrated.
Matrix Effects
Matrix effects on the detection of quizalofop in extracts of cucumber, grape, carrot, rice and peas matrices were found to be insignificant (< 20 %). However, matrix-matched standards were used for quantification.
Linearity
The linearity of the detector response was demonstrated by single determination of matrix-matched calibration standards for quizalofop at a minimum of seven (7) concentration levels ranging from 0.05 ng/mL to 10 ng/mL for all matrices except rice and from 0.02 ng/mL to 10 ng/mL for rice. This range corresponds to 0.0022 mg/kg to 0.44 mg/kg for all matrices except rice and 0.00176 mg/kg to 0.88 mg/kg for rice and thus covers the range from no more than 30 % of the LOQ and at least + 20 % of the highest analyte concentration level detected in a (diluted) sample extract.
The calibration curves obtained for both mass transitions and all matrices were linear since coefficients of determination (R^2) were ≥ 0.990. Linear regression was performed with 1/x-weighting.
Quantification
Quantification of quizalofop was performed by using linear regression with additional drift verification by injecting bracketing standards.
Accuracy and Precision
Accuracy was determined by fortification of control samples with individual solutions containing a known amount of quizalofop or quizalofop-P-ethyl or quizalofop-P-tefuryl or propaquizafop orquizalofop- P-acid glucose conjugate. Five fortifications were performed at 0.01 mg/kg (LOQ) and 5 fortifications at 0.1 mg/kg (10 x LOQ) for each analyte. The procedural recoveries determinations were performed by quantitation of quizalofop upon applying the test method.
Precision was determined by repeatability (relative standard deviation).
All mean recovery values at individual fortification levels of 0.01 mg/kg and 0.1 mg/kg of quizalofop, quizalofop-P-ethyl, quizalofop-P-tefuryl, propaquizafop and quizalofop- P-acid glucose conjugate for the five matrices (rice, peas, carrot, cucumber and grapes) comply with the standard acceptance criteria of the guidance document SANCO/825/00, rev. 8.1 and SANCO/3029/99 rev. 4.
Limit of Quantification (LOQ) and Limit of Detection (LOD)
The LOQ is the lowest validated fortification level for each analyte and was thus successfully established at 0.01 mg/kg for each analyte in cucumber, grape, carrot, rice and peas matrices for the two (2) mass transitions.
The LOD was set at 0.003 mg/kg for each analyte, which is 20% of the LOQ.

Stability of Stock Solutions
Quizalofop, quizalofop-P-ethyl, propaquizafop and quizalofop- P-acid glucose conjugate were found to be stable for at least 31 days when prepared in methanol at 250 µg/mL and stored at a target temperature set at 4°C for quizalofop, quizalofop-P-ehtyl and propaquizafop and -20°C for quizalofop- P-acid glucose conjugate in the dark.
As the reference item for quizalofop-p-tefuryl is a solution, the stability data of the stock solution was already available on the certificate of analysis.
Stability of Fortification Solutions
Quizalofop, quizalofop-P-ethyl, propaquizafop, quizalofop-p-tefuryl and quizalofop- P-acid glucose conjugate was found to be stable for at least 28 days when prepared in methanol/ultra-pure water (50/50 ; v/v) and stored at a target temperature set at 4°C in the dark.
Extract Stability
Quizalofop, as a hydrolysis product of quizalofop-P-ethyl, propaquizafop, quizalofop-p-tefuryl and quizalofop- P-

acid glucose conjugate was found to be stable in final extracts of all matrices for at least 7 days when stored at a target temperature set at 4°C in the dark.

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

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
Mass Transition 345.1→272.1 m/z (Quantification)					
Cucumber	Quizalofop	0.01	98	9	
		0.1	80	12	
Grape		0.01	991	3	
		0.1	91	10	
Carrot		0.01	78	8	
		0.1	77	3	
Rice		0.01	87	3	
		0.1	92	3	
Peas		0.01	81	5	
		0.1	86	5	
Mass Transition 345.1→244.0 m/z (Confirmation)					
Cucumber	Quizalofop	0.01	86	8	
		0.1	81	4	
Grape		0.01	86	7	
		0.1	94	8	
Carrot		0.01	78	7	
		0.1	79	1	
Rice		0.01	96	4	
		0.1	93	4	
Peas		0.01	90	5	
		0.1	85	2	

Table A 2: Recovery results from method validation of quizalofop-P-ethyl using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
Mass Transition 345.1→272.1 m/z (Quantification)					
Cucumber	Quizalofop-P-ethyl expressed as quizalofop	0.0092	98	7	
		0.092	85	6	
Grape		0.0092	84	9	
		0.092	97	9	
Carrot		0.0092	82	6	

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
		0.092	81	8	
Rice		0.0092	94	7	
		0.092	86	3	
Peas		0.0092	91	10	
		0.092	91	4	
Mass Transition 345.1→244.0 m/z (Confirmation)					
Cucumber	Quizalofop-P-ethyl expressed as quizalofop	0.0092	96	7	
		0.092	83	5	
Grape		0.0092	71	9	
		0.092	101	3	
Carrot		0.0092	88	10	
		0.092	82	5	
Rice		0.0092	94	4	
		0.092	87	3	
Peas		0.0092	99	16	
		0.092	90	1	

Table A 3: Recovery results from method validation of quizalofop-P- tefuryl using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
Mass Transition 345.1→272.1 m/z (Quantification)					
Cucumber	Quizalofop-P-tefuryl expressed as quizalofop	0.008	78	6	
		0.08	75	2	
Grape		0.008	90	4	
		0.08	82	3	
Carrot		0.008	96	6	
		0.08	89	9	
Rice		0.008	85	8	
		0.08	85	2	
Peas		0.008	91	14	
		0.08	86	5	
Mass Transition 345.1→244.0 m/z (Confirmation)					
Cucumber	Quizalofop-P-tefuryl expressed as quizalofop	0.008	75	11	
		0.08	77	3	
Grape		0.008	86	4	
		0.08	82	4	

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
Carrot		0.008	93	4	
		0.08	96	4	
Rice		0.008	90	2	
		0.08	86	3	
Peas		0.008	81	10	
		0.08	83	5	

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

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
Mass Transition 345.1→272.1 m/z (Quantification)					
Cucumber	Propaquizafop expressed as quizalofop	0.0078	80	6	
		0.078	75	1	
Grape		0.0078	88	5	
		0.078	77	11	
Carrot		0.0078	95	6	
		0.078	91	10	
Rice		0.0078	91	4	
		0.078	88	4	
Peas		0.0078	86	4	
		0.078	86	2	
Mass Transition 345.1→244.0 m/z (Confirmation)					
Cucumber	Propaquizafop expressed as quizalofop	0.0078	77	10	
		0.078	76	2	
Grape		0.0078	92	20	
		0.078	83	2	
Carrot		0.0078	89	5	
		0.078	92	9	
Rice		0.0078	92	9	
		0.078	90	3	
Peas		0.0078	78	7	
		0.078	81	12	

Table A 5: Recovery results from method validation of Quizalofop- P-acid glucose conjugate using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
Mass Transition 345.1→272.1 m/z (Quantification)					
Cucumber	Quizalofop- P-acid glucose conjugate expressed as quizalofop	0.0068	76	7	
		0.068	70	2	
Grape		0.0068	78	6	
		0.068	75	4	
Carrot		0.0068	77	8	
		0.068	81	2	
Rice		0.0068	96	9	
		0.068	89	2	
Peas		0.0068	87	10	
		0.068	90	1	
Mass Transition 345.1→244.0 m/z (Confirmation)					
Cucumber	Quizalofop- P-acid glucose conjugate expressed as quizalofop	0.0068	72	4	
		0.068	70	3	
Grape		0.0068	78	14	
		0.068	72	9	
Carrot		0.0068	79	6	
		0.068	82	2	
Rice		0.0068	95	9	
		0.068	90	3	
Peas		0.0068	79	5	
		0.068	87	4	

Table A 16: Characteristics for the analytical method

	Quizalofop
Specificity	The analysis of blanks and untreated samples in comparison with the analysis of standard solutions and spiked samples showed the absence of compound interfering with the determination of quizalofop-ethyl and quizalofop (<30% LOQ)
Calibration (type, number of data points)	The linearity of the detector response was demonstrated by single determination of matrix-matched calibration standards for quizalofop at a minimum of seven (7) concentration levels ranging from 0.05 ng/mL to 10 ng/mL for all matrices except rice and from 0.02 ng/mL to 10 ng/mL for rice. This range corresponds to 0.0022 mg/kg to 0.44 mg/kg for all matrices except rice and 0.00176 mg/kg to 0.88 mg/kg for rice and thus covers the range from no more than 30 % of the LOQ and at

	least + 20 % of the highest analyte concentration level detected in a sample extract. The calibration curves obtained for both ion mass transitions and all matrices were linear since coefficients of determination (R^2) were ≥ 0.990. Linear regression was performed with 1/x-weighting.
Assessment of matrix effects is presented	yes
Limit of quantification	0.01 mg/kg

Conclusion

The method was found to be valid according to the guidance documents SANCO/825/00, rev 8.1 and SANCO/3029/99/00, rev. 4 for the determination of quizalofop, quizalofop-P-ethyl, quizalofop-P-tefuryl, propaquizafop and quizalofop- P-acid glucose conjugate in cucumber, grape, carrot, rice and peas matrices with the tested LOQ of 0.01 mg/kg.

A 2.1.2.1.2 Analytical method 8

A 2.1.2.1.2.1 Method validation

Comments of zRMS:	The method is accepted

Reference:	5.2.1-08
Report	Pivato M., (2017), 'Validation of the analytical procedure for the determination of quizalofop free acid and quizalofop-P-ethyl after hydrolysis in grape vine by liquid chromatography' Study no FR 16.563341.0012, Chelab – Italy
Guideline(s):	European commission Directive 96/46/EC, July 16, 1996 European commission, Residues: Guidance for generating and reporting methods of analysis in support of pre-registration data requirements for Annex II (part A, Section 4)and Annex III (part A, Section 5) of Directive 91/414, SANCO/3029/99 rev.4, July11, 2000 – Working document European commission, Guidance document on pesticide residue analytical methods, SANCO/825/00 rev. 7, March 17, 2004
Deviations:	None
GLP:	Yes
Acceptability:	Yes

Materials and methods

Material

Test item

Since the purpose of this study is development and validation of a method for the quantification of quizalofop free acid and quizalofop-p-ethyl in grapevine specimens, the test item of the study is constituted by the test system fortified with reference substances.

Reference item - Quizalofop-p-ethyl

Name:	Quizalofop-p-ethyl
IUPAC name	ethyl-2-[4-((6-chloro-quinoxalinyl)oxy) phenoxy]-propionate
Batch number:	SZBF181XV
CAS No:	100646-51-3
Formula	$C_{19}H_{17}ClN_2O_4$
Molar weight	372.8 g/mol
Purity	98.4%

Reference item - Quizalofop free acid

Name:	Quizalofop free acid
IUPAC name	2-[4-((6-chloro-2-quinoxalinyl)oxy)phenoxy]-propionic acid
Batch number:	21122
CAS No:	76578-12-6
Formula	$C_{17}H_{13}ClN_2O_4$
Molar weight	344.75 g/mol
Purity	98.6%

Test system

Grapevine

Grapevine specimens were supplied by the test facility and absence of quizalofop free acid and quizalofop-p-ethyl was ensured before using the test item.

Method

Principle of the method

After sample extraction, quizalofop-p-ethyl were determined by LC/MS

Sample preparation and extraction

1. Extraction

About 5 g of grinded grapevine were introduced into a digester tube and 7.5 mL of milliQ water were added in order to hydrtae the matrix and then 10 mL of extraction mixture (acetonitrile) were added to the sample. After vortexing for about 1 minute, 2 mL of 1 N NaOH were added and pH checked. The tube was vortexed again for about 1 minute ad incubated into a digester plate at 75°C for 1 hour. After cooling to room temperature, the solution was transferred into a 50 mL plastic falcon and pH was slowly lowered to about 1 with 37% HCl. About 6 g of magnesium sulphate anhydrous were added to the sample and vortexed again for about 1 minute. The tube was centrifuged at 4750 rpm for 5 minutes and kept at about -20°C for about 2 hours. Then, the tube was centrifuged again for 5 minutes at 4750 rpm at 15°C and purified. Test sample was prepared in triplicate (one for matrix evaluation and two for test sample determination)

2. Sample purification

Each obtained supernatant was split in two tubes for quizalofop-p-ethyl and quizalofop free acid purification.

- Positive purification quizalofop-p-ethyl: 3 mL of supernatant obtained from sample extraction were transferred into a 10 mL plastic tube, containing about 450 mg of magnesium sulphaye anhydrous and 150 mg of PSA resin

- Negative purification quizalofop free acid: 3 mL of supernatant obtained from sample extraction were transferred into a 10 mL plastic tube, containing about 450 mg of magnesium sulphate anhydrous and 150 mg of C18 resin

Each tube was vortexed for about 1 minute and centrifuged at 4000 rpm for 5 min. The supernatants were recovered and diluted 1:2 with 10 mM ammonium formate buffer pH before injection.

Preparation of the solutions

Reference standard solution A (SRSS-A) - 22 mg of quizalofop-p-ethyl were accurately weighed into a 100 mL volumetric flask and diluted to volume with acetonitrile. The final concentration was 216 mg/mL

Reference standard solution B (SRSS-B) - 7 mL of quizalofop free acid solution were introduced into a 10 mL volumetric flask and diluted to volume with acetonitrile. The final concentration was 200 mg/mL

Intermediate reference standard B (IRS-B) – 1 mL of SRSS-B was introduced into a 10 mL volumetric flask and diluted to volume with acetonitrile. 1 mL of this solution was introduced into a 20 mL volumetric flask and diluted to volume with acetonitrile. The final concentration was 1 mg/mL

Spiking solution 10x A of quizalofop-p-ethyl – 1 mL of SRSS-A was introduced into a 10 mL volumetric flask and diluted to volume with acetonitrile. Then 1 mL of this solution was introduced into 20 mL volumetric flask and diluted to volume with acetonitrile. The final concentration was 1 mg/L

Spiking solution LOQ A of quizalofop-p-ethyl – 1 mL of spiking solution 10x A was introduced into a 10 mL volumetric flask and diluted to volume with acetonitrile. The final concentration was 0.1 mg/L

Intermediate reference standard mix (IRSM) - 1 mL of spiking solution 10xA and 1 mL of IRS-B were introduced into a 10 mL volumetric flask and diluted to volume with acetonitrile. The final concentration was 0.1 mg/L for each analyte

Linearity solutions – Five solutions were prepared (L1 to L5) of different concentration for quizalofop-p-ethyl (0.0003 to 0.0433 mg/L) and quizalofop free acid (0.0003 to 0.0380 mg/L)

Preparation of reference solutions for matrix effect calculation

0.5 mL of each supernatant deriving from quizalofop-p-ethyl and quizalofop free acid purification were transferred into a 10 mL tube and dried by N2 flux. The dried samples were then resuspended with 1 mL of L3 solution

Spiked sample at LOQ level

About 5 g of grinded grapevine were introduced into a digester tube. 0.25 mL of spiking solution LOQ A and 7.5 mL of milliQ water were added in order to hydrate the matrix and then 10 mL of extraction mixture (acetonitrile) were added to the sample. After vortexing for about 1 minute, 2 mL of 1 N NaOH were added and pH checked. The tube was vortexed again for about 1 minute ad incubated into a digester plate at 75°C for 1 hour. After cooling to room temperature, the solution was transferred into a 50 mL plastic falcon and pH was slowly lowered to about 1 with 37% HCl. About 6 g of magnesium sulphate anhydrous were added to the sample and vortexed again for about 1 minute. The tube was centrifuged at 4750

rpm for 5 minutes and kept at about -20°C for about 2 hours. Then, the tube was centrifuged again for 5 minutes at 4750 rpm at 15°C and purified. Test sample was prepared in quintuplicate

Spiked sample at 10xLOQ level

About 5 g of grinded grapevine were introduced into a digester tube. 0.25 mL of spiking solution 10xA and 7.5 mL of milliQ water were added in order to hydrate the matrix and then 10 mL of extraction mixture (acetonitrile) were added to the sample. After vortexing for about 1 minute, 2 mL of 1 N NaOH were added and pH checked. The tube was vortexed again for about 1 minute ad incubated into a digester plate at 75°C for 1 hour. After cooling to room temperature, the solution was transferred into a 50 mL plastic falcon and pH was slowly lowered to about 1 with 37% HCl. About 6 g of magnesium sulphate anhydrous were added to the sample and vortexed again for about 1 minute. The tube was centrifuged at 4750 rpm for 5 minutes and kept at about -20°C for about 2 hours. Then, the tube was centrifuged again for 5 minutes at 4750 rpm at 15°C and purified. Test sample was prepared in quintuplicate.

LC/MS conditions:

Column	Acquity UPLC BEH C18, 50 mm x 2.1 mm x 1.7 µm (LC 23)							
Mobile phase	A – 10 mM ammonium formate buffer pH 4 B – Methanol							
Gradient program	**Time (min)**	**0**	**0.3**	**4.5**	**6.3**	**6.4**	**6.9**	**7.0**	**8.0**
	A (%)	65	65	0	0	100	100	65	65
	B (%)	35	35	100	100	0	0	35	35
Volume of injection:	1 µL							
Detector:	MS XEVO TQS (Waters – micromass), SRA 470							
Source	Electron spray ionization							
Gas flow	10 L/min							
Gas temperature	400°C							
Run mode	Multiple reaction monitoring							
Run time	8 minutes							
Nebulizer	6 psi							

Results and discussions

Specificity

Blank solution, reference solution at LOQ level, test solution and spiked test solution (at LOQ level) were injected for specificity evaluation. The method is capable of determining the analytes in the presence of sample matrix. No significant peaks are detected at retention time of the target analytes in blank and test solution with respect to spiked test solution for both transitions 1 and 2.

Linearity

The method linearity was evaluated at 5 different levels of concentration ranging from 30% LOQ (0.0015 mg/kg) to about 30xLOQ (0.15 mg/kg).

Results	**Slope**	**Intercept**	**Co-efficient of determination**
Transition 1 for quizalofop-p-ethyl	108225959	0	1.00

Transition 2 for quizalofop-p-ethyl	30920896	0	1.00
Transition 1 for quizalofop free acid	1016439	0	1.00
Transition 2 for quizalofop free acid	113475	0	1.00

Repeatability

Repeatability was performed on aliquots of sample spiked with quizalofop-p-ethyl at LOQ (0.005 mg/kg) and 10xLOQ (0.05 mg/kg) and quantified as quizalofop free acid after hydrolysis reaction. Five replicate analysis were performed for each spiking level. Overall % recovery results comply with acceptance criteria.

Results	Average content (% w/w)	Standard deviation	% RSD	Criteria	Conformity
Quizalofop-p-ethyl					
Transition 1	109.0	0.7	1	%RSD ≤ 20	Yes
Transition 2	108.6	1.7	2		Yes
Quizalofop free acid					
Transition 1	105.2	2.9	3	%RSD ≤ 20	Yes
Transition 2	109.4	0.9	1		Yes

Repeatability precision of the overall percent recovery:

Result	Average content (% w/w)	Standard deviation	% RSD	Criteria	Conformity
Transition 1	107	3	3	%RSD ≤ 20	Yes
Transition 2	109	1	1		Yes

Accuracy

The accuracy of the method fulfils the requirements for residue analytical methods which demand that the mean recoveries per fortification level should be in the range 70-110%. The recovery was found to be 107% (Trans 1) and 109% (Trans 2) recovery, in accordance with acceptance criteria.

Limit of quantification

The limit of quantification was found to be 0.005 mg/kg.

Table A 3: Recovery results from method validation of quizalofop-p-ethyl using the analytical method

Matrix	Analyte	Fortification level (mg/kg)	Mean recovery (%)	RSD (%)	Comments
Grapevine	Quizalofop-p-ethyl	0.005 (Transistion 1)	109.0	1	
		0.005 (Transistion 2)	108.6	2	
		0.05 (Transistion 1)	105.2	3	
		0.05 (Transistion 2)	109.4	1	

Table A 4: Characteristics for the analytical method used for validation of active substance quizalofop-p-ethyl residues in plant matrices (grape vine)

	Quizalofop-p-ethyl
Specificity	No significant peaks are detected at retention time of the target analytes. The method is considered to be specific.
Calibration (type, number of data points)	5 data points

	Quizalofop-p-ethyl
	Transition 1 for quizalofop-p-ethyl Slope: 108225959 Intercept: 0 R^2: 1.00 Transition 2 for quizalofop-p-ethyl Slope: 30920896 Inercept: 0 R^2: 1.00 Transition 1 for quizalofop free acid Slope: 1016439 Intercept: 0 R^2: 1.00 Transition 2 for quizalofop free acid Slope: 113475 Intercept: 0 R^2: 1.00
Calibration range	Quizalofop-p-ethyl: 0.0003 – 0.0433 mg/L Quizalofop free acid: 0.0003 – 0.0380 mg/L
Assessment of matrix effects is presented	Yes
Limit of determination/quantification	LOQ – 0.005 mg/kg

Conclusion

This method has been successfully validated in terms of specificity, linearity, accuracy, repetability and limit of quantification. Therefore, it is concluded that this method is suitable for the determination of residue of quizalofop-p-ethyl-equivalent in grapevine

A 2.1.2.1.3 Analytical method 9

A 2.1.2.1.3.1 Method validation

Comments of zRMS:	The method is accepted

Reference:	5.2.1-09
Report	Pivato M., (2016), 'Validation of the analytical procedure for the determination of quizalofop free acid and quizalofop-P-ethyl after hydrolysis in fresh peas by liquid chromatography' Study no FR16.563341.0011, Chelab – Italy
Guideline(s):	European commission Directive 96/46/EC, July 16, 1996 European commission, Residues: Guidance for generating and reporting methods of analysis in support of pre-registration data requirements for Annex II (part A, Section 4)and Annex III (part A, Section 5) of Directive 91/414, SANCO/3029/99 rev.4, July11, 2000 – Working document European commission, Guidance document on pesticide residue analytical methods, SANCO/825/00 rev. 7, March 17, 2004
Deviations:	None
GLP:	Yes
Acceptability:	Yes

Materials and methods

Material

Test item

Since the purpose of this study is development and validation of a method for the quantification of quizalofop free acid and quizalofop-p-ethyl in fresh pea specimens, the test item of the study is constituted by the test system fortified with reference substances.

Reference item - Quizalofop-p-ethyl

Name:	Quizalofop-p-ethyl
IUPAC name	ethyl-2-[4-((6-chloro-quinoxalinyl)oxy) phenoxy]-propionate
Batch number:	SZBF181XV
CAS No:	100646-51-3
Formula	$C_{19}H_{17}ClN_2O_4$
Molar weight	372.8 g/mol
Purity	98.4%

Reference item - Quizalofop free acid

Name:	Quizalofop free acid
IUPAC name	2-[4-((6-chloro-2-quinoxalinyl)oxy)phenoxy]-propionic acid
Batch number:	21122
CAS No:	76578-12-6
Formula	$C_{17}H_{13}ClN_2O_4$
Molar weight	344.75 g/mol
Purity	98.6%

Test system

Fresh pea

Fresh pea specimens were supplied by the test facility and absence of quizalofop free acid and quizalofop-p-ethyl was ensured before using the test item.

Method

Principle of the method

After sample extraction, quizalofop-p-ethyl were determined by LC/MS

Sample preparation and extraction

1. Extraction

About 5 g of grinded fresh pea were introduced into a digester tube and 7.5 mL of milliQ water were added in order to hydrtae the matrix and then 10 mL of extraction mixture (acetonitrile) were added to the sample. After vortexing for about 1 minute, 2 mL of 1 N NaOH were added and pH checked. The tube was vortexed again for about 1 minute ad incubated into a digester plate at 75°C for 1 hour. After cooling to room temperature, the solution was transferred into a 50 mL plastic falcon and pH was slowly lowered to about 1 with 37% HCl. About 6 g of magnesium sulphate anhydrous were added to the sample and vortexed again for about 1 minute. The tube was centrifuged at 4750 rpm for 5 minutes and kept at about -20°C for about 2 hours. Then, the tube was centrifuged again for 5 minutes at 4750 rpm at 15°C and purified. Test sample was prepared in triplicate (one for matrix evaluation and two for test sample determination)

2. Sample purification

Each obtained supernatant was split in two tubes for quizalofop-p-ethyl and quizalofop free acid purification.

- Positive purification quizalofop-p-ethyl: 3 mL of supernatant obtained from sample extraction were transferred into a 10 mL plastic tube, containing about 450 mg of magnesium sulphaye anhydrous and 150 mg of PSA resin

- Negative purification quizalofop free acid: 3 mL of supernatant obtained from sample extraction were transferred into a 10 mL plastic tube, containing about 450 mg of magnesium sulphate anhydrous and 150 mg of C18 resin

Each tube was vortexed for about 1 minute and centrifuged at 4000 rpm for 5 min. The supernatants were recovered and diluted 1:2 with 10 mM ammonium formate buffer pH before injection.

Preparation of the solutions

Reference standard solution A (SRSS-A) – 22.5 mg of quizalofop-p-ethyl were accurately weighed into a 100 mL volumetric flask and diluted to volume with acetonitrile. The final concentration was 200 mg/mL

Reference standard solution B (SRSS-B) - 7 mL of quizalofop free acid solution were introduced into a 10 mL volumetric flask and diluted to volume with acetonitrile. The final concentration was 200 mg/mL

Intermediate reference standard B (IRS-B) – 1 mL of SRSS-B was introduced into a 10 mL volumetric flask and diluted to volume with acetonitrile. 1 mL of this solution was introduced into a 20 mL volumetric flask and diluted to volume with acetonitrile. The final concentration was 1 mg/mL

Spiking solution 10x A of quizalofop-p-ethyl – 1 mL of SRSS-A was introduced into a 10 mL volumetric flask and diluted to volume with acetonitrile. Then 1 mL of this solution was introduced into 20 mL volumetric flask and diluted to volume with acetonitrile. The final concentration was 1 mg/L

Spiking solution LOQ A of quizalofop-p-ethyl – 1 mL of spiking solution 10x A was introduced into a 10 mL volumetric flask and diluted to volume with acetonitrile. The final concentration was 0.1 mg/L

Intermediate reference standard mix (IRSM) - 1 mL of spiking solution 10xA and 1 mL of IRS-B were introduced into a 10 mL volumetric flask and diluted to volume with acetonitrile. The final concentration was 0.1 mg/L for each analyte

Linearity solutions – Five solutions were prepared (L1 to L5) of different concentration for quizalofop-p-ethyl (0.0003 to 0.0443 mg/L) and quizalofop free acid (0.0003 to 0.0380 mg/L)

Preparation of reference solutions for matrix effect calculation

0.5 mL of each supernatant deriving from quizalofop-p-ethyl and quizalofop free acid purification were transferred into a 10 mL tube and dried by N2 flux. The dried samples were then resuspended with 1 mL of L3 solution

Spiked sample at LOQ level

About 5 g of grinded fresh pea were introduced into a digester tube. 0.25 mL of spiking solution LOQ A and 7.5 mL of milliQ water were added in order to hydrate the matrix and then 10 mL of extraction mixture (acetonitrile) were added to the sample. After vortexing for about 1 minute, 2 mL of 1 N NaOH were

added and pH checked. The tube was vortexed again for about 1 minute ad incubated into a digester plate at 75°C for 1 hour. After cooling to room temperature, the solution was transferred into a 50 mL plastic falcon and pH was slowly lowered to about 1 with 37% HCl. About 6 g of magnesium sulphate anhydrous were added to the sample and vortexed again for about 1 minute. The tube was centrifuged at 4750 rpm for 5 minutes and kept at about -20°C for about 2 hours. Then, the tube was centrifuged again for 5 minutes at 4750 rpm at 15°C and purified. Test sample was prepared in quintuplicate

Spiked sample at 10xLOQ level

About 5 g of grinded fresh pea were introduced into a digester tube. 0.25 mL of spiking solution 10xA and 7.5 mL of milliQ water were added in order to hydrate the matrix and then 10 mL of extraction mixture (acetonitrile) were added to the sample. After vortexing for about 1 minute, 2 mL of 1 N NaOH were added and pH checked. The tube was vortexed again for about 1 minute ad incubated into a digester plate at 75°C for 1 hour. After cooling to room temperature, the solution was transferred into a 50 mL plastic falcon and pH was slowly lowered to about 1 with 37% HCl. About 6 g of magnesium sulphate anhydrous were added to the sample and vortexed again for about 1 minute. The tube was centrifuged at 4750 rpm for 5 minutes and kept at about -20°C for about 2 hours. Then, the tube was centrifuged again for 5 minutes at 4750 rpm at 15°C and purified. Test sample was prepared in quintuplicate.

LC/MS conditions:

Column	Acquity UPLC BEH C18, 50 mm x 2.1 mm x 1.7 µm (LC 23)							
Mobile phase	A – 10 mM ammonium formate buffer pH 4 B – Methanol							
Gradient program	**Time (min)**	**0**	**0.3**	**4.5**	**6.3**	**6.4**	**6.9**	**7.0**	**8.0**
	A (%)	65	65	0	0	100	100	65	65
	B (%)	35	35	100	100	0	0	35	35
Volume of injection:	1 µL							
Detector:	MS XEVO TQS (Waters – micromass), SRA 470							
Source	Electron spray ionization							
Gas flow	10 L/min							
Gas temperature	400°C							
Run mode	Multiple reaction monitoring							
Run time	8 minutes							
Nebulizer	6 psi							

Results and discussions

Specificity

Blank solution, reference solution at LOQ level, test solution and spiked test solution (at LOQ level) were injected for specificity evaluation. The method is capable of determining the analytes in the presence of sample matrix. No significant peaks are detected at retention time of the target analytes in blank and test solution with respect to spiked test solution for both transitions 1 and 2.

Linearity

The method linearity was evaluated at 5 different levels of concentration ranging from 30% LOQ (0.0015 mg/kg) to about 30xLOQ (0.15 mg/kg). Solutions were prepared and analysed by LC-MS

Results	Slope	Intercept	Co-efficient of determination
Transition 1 for quizalofop-p-ethyl	9605740	0	1.00
Transition 2 for quizalofop-p-ethyl	27922930	0	1.00
Transition 1 for quizalofop free acid	841357	0	1.00
Transition 2 for quizalofop free acid	97696	0	1.00

Repeatability

Repeatability was performed on aliquots of sample spiked with quizalofop-p-ethyl at LOQ (0.005 mg/kg) and 10xLOQ (0.05 mg/kg) and quantified as quizalofop free acid after hydrolysis reaction. Five replicate analysis were performed for each spiking level. Overall % recovery results comply with acceptance criteria.

Results	Average content (% w/w)	Standard deviation	% RSD	Criteria	Conformity
Quizalofop-p-ethyl					
Transition 1	108.8	1.6	2	%RSD ≤ 20	Yes
Transition 2	104.2	5.3	5		Yes
Quizalofop free acid					
Transition 1	109.0	1.7	2	%RSD ≤ 20	Yes
Transition 2	107.4	2.3	2		Yes

Repeatability precision of overall percent recovery:

Result	Average content (% w/w)	Standard deviation	% RSD	Criteria	Conformity
Transition 1	109	2	1	%RSD ≤ 20	Yes
Transition 2	106	4	4		Yes

Accuracy

The accuracy of the method fulfils the requirements for residue analytical methods which demand that the mean recoveries per fortification level should be in the range 70-110%. The recovery was found to be 109% (Trans 1) and 106% (Trans 2) recovery, in accordance with acceptance criteria.

Limit of quantification

The limit of quantification was found to be 0.005 mg/kg.

Table A 5: Recovery results from method validation of quizalofop-p-ethyl using the analytical method

Matrix	Analyte	Fortification level (mg/kg)	Mean recovery (%)	RSD (%)	Comments
Fresh pea	Quizalofop-p-ethyl	0.005 (Transistion 1)	108.8	2	
		0.005 (Transistion 2)	104.2	5	
		0.05 (Transistion 1)	109.0	2	
		0.05 (Transistion 2)	107.4	2	

Table A 6: Characteristics for the analytical method used for validation of ative substance quizalofop-p-ethyl residues in plant matrices (peas)

	Quizalofop-p-ethyl
Specificity	No significant peaks are detected at retention time of the target analytes. The method is considered to be specific.
Calibration (type, number of data points)	5 data points Transition 1 for quizalofop-p-ethyl Slope: 9605740 Inercept: 0 R^2: 1.00 Transition 2 for quizalofop-p-ethyl Slope: 27922930 Intercept: 0 R^2: 1.00 Transition 1 for quizalofop free acid Slope: 841357 Intercept: 0 R^2: 1.00 Transition 2 for quizalofop free acid Slope: 97696 Intercept: 0 R^2: 1.00
Calibration range	Quizalofop-p-ethyl: 0.0003 – 0.0433 mg/L Quizalofop free acid: 0.0003 – 0.0380 mg/L
Assessment of matrix effects is presented	Yes
Limit of determination/quantification	LOQ – 0.005 mg/kg

Conclusion

This method has been successfully validated in terms of specificity, linearity, accuracy, repeatability and limit of quantification. Therefore, it is concluded that this method is suitable for the determination of residue of quizalofop-p-ethyl-equivalent in fresh pea.

A 2.1.2.1.4 Analytical method 10

A 2.1.2.1.4.1 Method validation

Comments of zRMS:	The method is accepted

Reference:	5.2.1-10
Report	Pivato M., (2016), 'Validation of the analytical procedure for the determination of quizalofop free acid and quizalofop-P-ethyl after hydrolysis in dry peas by liquid chromatography' Study no FR16.563341.0010, Chelab – Italy
Guideline(s):	European commission Directive 96/46/EC, July 16, 1996 European commission, Residues: Guidance for generating and reporting methods of analysis in support of pre-registration data requirements for Annex II (part A, Section 4)and Annex III (part A, Section 5) of Directive 91/414, SANCO/3029/99 rev.4, July11, 2000 – Working document European commission, Guidance document on pesticide residue analytical methods, SANCO/825/00 rev. 7, March 17, 2004
Deviations:	None
GLP:	Yes
Acceptability:	Yes

Materials and methods

Material

Test item

Since the purpose of this study is development and validation of a method for the quantification of quizalofop free acid and quizalofop-p-ethyl in dry pea specimens, the test item of the study is constituted by the test system fortified with reference substances.

Reference item - Quizalofop-p-ethyl

Name:	Quizalofop-p-ethyl
IUPAC name	ethyl-2-[4-((6-chloro-quinoxalinyl)oxy) phenoxy]-propionate
Batch number:	SZBF181XV
CAS No:	100646-51-3
Formula	$C_{19}H_{17}ClN_2O_4$
Molar weight	372.8 g/mol
Purity	98.4%

Reference item - Quizalofop free acid

Name:	Quizalofop free acid
IUPAC name	2-[4-((6-chloro-2-quinoxalinyl)oxy)phenoxy]-propionic acid
Batch number:	21122
CAS No:	76578-12-6
Formula	$C_{17}H_{13}ClN_2O_4$
Molar weight	344.75 g/mol
Purity	98.6%

Test system

Dry peas

Dry pea specimens were supplied by the test facility and absence of quizalofop free acid and quizalofop-p-ethyl was ensured before using the test item.

Method

Principle of the method

After sample extraction, quizalofop-p-ethyl were determined by LC/MS

Sample preparation and extraction

1. Extraction

About 5 g of grinded dry pea were introduced into a digester tube and 7.5 mL of milliQ water were added in order to hydrtae the matrix and then 10 mL of extraction mixture (acetonitrile) were added to the sample. After vortexing for about 1 minute, 2 mL of 1 N NaOH were added and pH checked. The tube was vortexed again for about 1 minute ad incubated into a digester plate at 75°C for 1 hour. After cooling to room temperature, the solution was transferred into a 50 mL plastic falcon and pH was slowly lowered to about 1 with 37% HCl. About 6 g of magnesium sulphate anhydrous were added to the sample and vortexed again for about 1 minute. The tube was centrifuged at 4750 rpm for 5 minutes and kept at about -20°C for about 2 hours. Then, the tube was centrifuged again for 5 minutes at 4750 rpm at 15°C and puri-

fied. Test sample was prepared in triplicate (one for matrix evaluation and two for test sample determination)

2. Sample purification

Each obtained supernatant was split in two tubes for quizalofop-p-ethyl and quizalofop free acid purification.

- Positive purification quizalofop-p-ethyl: 3 mL of supernatant obtained from sample extraction were transferred into a 10 mL plastic tube, containing about 450 mg of magnesium sulphaye anhydrous and 150 mg of PSA resin

- Negative purification quizalofop free acid: 3 mL of supernatant obtained from sample extraction were transferred into a 10 mL plastic tube, containing about 450 mg of magnesium sulphate anhydrous and 150 mg of C18 resin

Each tube was vortexed for about 1 minute and centrifuged at 4000 rpm for 5 min. The supernatants were recovered and diluted 1:2 with 10 mM ammonium formate buffer pH before injection.

Preparation of the solutions

Reference standard solution A (SRSS-A) – 22.33 mg of quizalofop-p-ethyl were accurately weighed into a 100 mL volumetric flask and diluted to volume with acetonitrile. The final concentration was 220 mg/mL

Reference standard solution B (SRSS-B) - 7 mL of quizalofop free acid solution were introduced into a 10 mL volumetric flask and diluted to volume with acetonitrile. The final concentration was 200 mg/mL

Intermediate reference standard B (IRS-B) – 1 mL of SRSS-B was introduced into a 10 mL volumetric flask and diluted to volume with acetonitrile. 1 mL of this solution was introduced into a 20 mL volumetric flask and diluted to volume with acetonitrile. The final concentration was 1 mg/mL

Spiking solution 10x A of quizalofop-p-ethyl – 1 mL of SRSS-A was introduced into a 10 mL volumetric flask and diluted to volume with acetonitrile. Then 1 mL of this solution was introduced into 20 mL volumetric flask and diluted to volume with acetonitrile. The final concentration was 1 mg/L

Spiking solution LOQ A of quizalofop-p-ethyl – 1 mL of spiking solution 10x A was introduced into a 10 mL volumetric flask and diluted to volume with acetonitrile. The final concentration was 0.1 mg/L

Intermediate reference standard mix (IRSM) - 1 mL of spiking solution 10xA and 1 mL of IRS-B were introduced into a 10 mL volumetric flask and diluted to volume with acetonitrile. The final concentration was 0.1 mg/L for each analyte

Linearity solutions – Five solutions were prepared (L1 to L5) of different concentration for quizalofop-p-ethyl (0.0003 to 0.0439 mg/L) and quizalofop free acid (0.0003 to 0.0380 mg/L)

Preparation of reference solutions for matrix effect calculation

0.5 mL of each supernatant deriving from quizalofop-p-ethyl and quizalofop free acid purification were transferred into a 10 mL tube and dried by N2 flux. The dried samples were then resuspended with 1 mL of L3 solution

Spiked sample at LOQ level

About 5 g of grinded dry pea were introduced into a digester tube. 0.25 mL of spiking solution LOQ A and 7.5 mL of milliQ water were added in order to hydrate the matrix and then 10 mL of extraction mixture (acetonitrile) were added to the sample. After vortexing for about 1 minute, 2 mL of 1 N NaOH were added and pH checked. The tube was vortexed again for about 1 minute ad incubated into a digester plate at 75°C for 1 hour. After cooling to room temperature, the solution was transferred into a 50 mL plastic falcon and pH was slowly lowered to about 1 with 37% HCl. About 6 g of magnesium sulphate anhydrous were added to the sample and vortexed again for about 1 minute. The tube was centrifuged at 4750 rpm for 5 minutes and kept at about -20°C for about 2 hours. Then, the tube was centrifuged again for 5 minutes at 4750 rpm at 15°C and purified. Test sample was prepared in quintuplicate

Spiked sample at 10xLOQ level

About 5 g of grinded dry pea were introduced into a digester tube. 0.25 mL of spiking solution 10xA and 7.5 mL of milliQ water were added in order to hydrate the matrix and then 10 mL of extraction mixture (acetonitrile) were added to the sample. After vortexing for about 1 minute, 2 mL of 1 N NaOH were added and pH checked. The tube was vortexed again for about 1 minute ad incubated into a digester plate at 75°C for 1 hour. After cooling to room temperature, the solution was transferred into a 50 mL plastic falcon and pH was slowly lowered to about 1 with 37% HCl. About 6 g of magnesium sulphate anhydrous were added to the sample and vortexed again for about 1 minute. The tube was centrifuged at 4750 rpm for 5 minutes and kept at about -20°C for about 2 hours. Then, the tube was centrifuged again for 5 minutes at 4750 rpm at 15°C and purified. Test sample was prepared in quintuplicate.

LC/MS conditions:

Column	Acquity UPLC BEH C18, 50 mm x 2.1 mm x 1.7 µm (LC 23)							
Mobile phase	A – 10 mM ammonium formate buffer pH 4 B – Methanol							
Gradient program	**Time (min)**	**0**	**0.3**	**4.5**	**6.3**	**6.4**	**6.9**	**7.0**	**8.0**
	A (%)	65	65	0	0	100	100	65	65
	B (%)	35	35	100	100	0	0	35	35
Volume of injection:	1 µL							
Detector:	MS XEVO TQS (Waters – micromass), SRA 470							
Source	Electron spray ionization							
Gas flow	10 L/min							
Gas temperature	400°C							
Run mode	Multiple reaction monitoring							
Run time	8 minutes							
Nebulizer	6 psi							

Results and discussions

Specificity

Blank solution, reference solution at LOQ level, test solution and spiked test solution (at LOQ level) were injected for specificity evaluation. The method is capable of determining the analytes in the presence of sample matrix. No significant peaks are detected at retention time of the target analytes in blank and test solution with respect to spiked test solution for both transitions 1 and 2.

Linearity

The method linearity was evaluated at 5 different levels of concentration ranging from 30% LOQ (0.0015 mg/kg) to about 30xLOQ (0.15 mg/kg). Solutions were prepared and analysed by LC-MS

Results	Slope	Intercept	Co-efficient of determination
Transition 1 for quizalofop-p-ethyl	102187272	0	1.00
Transition 2 for quizalofop-p-ethyl	26127047	0	1.00
Transition 1 for quizalofop free acid	767516	0	1.00
Transition 2 for quizalofop free acid	86825	0	1.00

Repeatability

Repeatability was performed on aliquots of sample spiked with quizalofop-p-ethyl at LOQ (0.005 mg/kg) and 10xLOQ (0.05 mg/kg) and quantified as quizalofop free acid after hydrolysis reaction. Five replicate analysis were performed for each spiking level. Overall % recovery results comply with acceptance criteria.

Results	Average content (% w/w)	Standard deviation	% RSD	Criteria	Conformity
Quizalofop-p-ethyl					
Transition 1	109	1.4	1	%RSD ≤ 20	Yes
Transition 2	102.6	5.7	6		Yes
Quizalofop free acid					
Transition 1	103.4	2.3	2	%RSD ≤ 20	Yes
Transition 2	103.6	4.8	5		Yes

Repeatability precision of overall percent recovery:

Result	Average content (% w/w)	Standard deviation	% RSD	Criteria	Conformity
Transition 1	106	3	3	%RSD ≤ 20	Yes
Transition 2	103	5	5		Yes

Accuracy

The accuracy of the method fulfils the requirements for residue analytical methods which demand that the mean recoveries per fortification level should be in the range 70-110%. The recovery was found to be 106% (Trans 1) and 103% (Trans 2) recovery, in accordance with acceptance criteria.

Limit of quantification

The limit of quantification was found to be 0.005 mg/kg.

Table A 7: Recovery results from method validation of quizalofop-p-ethyl using the analytical method

Matrix	Analyte	Fortification level (mg/kg)	Mean recovery (%)	RSD (%)	Comments
Dry peas	Quizalofop-p-ethyl	0.005 (Transistion 1)	109.0	1	
		0.005 (Transistion 2)	102.6	6	
		0.05 (Transistion 1)	103.4	2	
		0.05 (Transistion 2)	103.6	5	

Table A 8: Characteristics for the analytical method used for validation of ative substance quizalofop-p-ethyl residues in plant matrices (sugar beet)

	Quizalofop-p-ethyl
Specificity	No interference
Calibration (type, number of data points)	5 data points Transition 1 for quizalofop-p-ethyl Slope: 102187272 Intecept: 0 R^2: 1.00 Transition 2 for quizalofop-p-ethyl Slope: 26127047 Intercept: 0 R^2: 1.00

	Quizalofop-p-ethyl
	Transition 1 for quizalofop free acid Slope: 767516 Intercept: 0 R^2: 1.00 Transition 2 for quizalofop free acid Slope: 86825 Intercept: 0 R^2: 1.00
Calibration range	Quizalofop-p-ethyl: 0.0003 – 0.0439 mg/L Quizalofop free acid: 0.0003 – 0.0380 mg/L
Assessment of matrix effects is presented	Yes
Limit of determination/quantification	LOQ – 0.005 mg/kg

Conclusion

This method has been successfully validated in terms of specificity, linearity, accuracy, repeatability and limit of quantification. Therefore, it is concluded that this method is suitable for the determination of residue of quizalofop-p-ethyl-equivalent in dry pea.

A 2.1.2.1.5 Analytical method 11

A 2.1.2.1.5.1 Method validation

Comments of zRMS:	The method is accepted

Reference:	KCP 5.2.1-19
Report	Validation of an analytical method for the determination of residues of propaquizafop, quizalofop-ester, quizalofop and quizalofop conjugates in olive, tomato and orange. Paszek, 2021. Study code VAL/11/2020
Guideline(s):	Yes SANTE/2020/12830
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Study Objective
The objective of the study was to validate an analytical method for the determination of quizalofop, quizalofop-P-ethyl, quizalofop-P-tefuryl, propaquizafop and quizalofop- P-acid glucose conjugate expressed as quizalofop (sum isomers) in tomato (high water content), orange (high acid content) and olive (high oil content) matrices according to the guidance documents SANTE/2020/12830 with an intended limit of quantification (LOQ) of 0.01 mg/kg for each analyte.
Analytical Procedure
In summary, residues of quizalofop, quizalofop-P-ethyl, quizalofop-P-tefuryl, propaquizafop and quizalofop- P-acid glucose conjugate were extracted with acetone/water, followed by a basic hydrolysis with KOH. During basic hydrolysis, all quizalofop esters and conjugates are hydrolysed into quizalofop. The extracts were then diluted with ultra-pure water prior to quantification of quizalofop by LC-MS/MS.

Results and discussions

Selectivity
Quantification was performed by use of LC-MS/MS detection. Two (2) mass transitions were evaluated for quizalofop in order to demonstrate that the method achieves a high level of selectivity. No significant interference above 30 % of LOQ was detected in any of the reagent blanks or the control sample extracts of each matrix, so that a high level of selectivity was demonstrated.
Matrix Effects
Matrix effects on the detection of quizalofop in extracts of cucumber, grape, carrot, rice and peas matrices were found to be insignificant (< 20 %). However, matrix-matched standards were used for quantification.
Linearity
The linearity of the detector response was demonstrated by single determination of matrix-matched calibration standards for quizalofop at a minimum of six concentration levels ranging from 0.05 ng/mL to 10 ng/mL for all matrices.
Quantification
Quantification of quizalofop was performed by using linear regression.
Accuracy and Precision
Accuracy was determined by fortification of control samples with individual solutions containing a known amount of quizalofop or quizalofop-P-ethyl or quizalofop-P-tefuryl or propaquizafop orquizalofop- P-acid glucose conjugate. Five fortifications were performed at 0.01 mg/kg (LOQ) and 5 fortifications at 0.1 mg/kg (10 x LOQ) for each analyte. The procedural recoveries determinations were performed by quantitation of quizalofop upon applying the test method.
Precision was determined by repeatability (relative standard deviation).
Limit of Quantification (LOQ) and Limit of Detection (LOD)
The LOQ is the lowest validated fortification level for each analyte and was thus successfully established at 0.01 mg/kg for each analyte in matrices for the two (2) mass transitions.
The LOD was set at 0.003 mg/kg for each analyte, which is 20% of the LOQ.
Stability of Stock Solutions
Quizalofop, quizalofop-P-ethyl, propaquizafop and quizalofop- P-acid glucose conjugate were found to be stable for at least 24 h during sequence analysis.

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

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
Mass Transition 343.4→270.9 m/z (Quantification)					
Olive	Quizalofop	0.01	109	14.8	
		0.1	105	12.3	
Tomato		0.01	118	1.2	
		0.1	116	1.9	
Lemon pulp		0.01	78	6.9	
		0.1	78	3.9	
Mass Transition 343.4→235 m/z (Confirmation)					
olive	Quizalofop	0.01	103	16.7	
		0.1	109	10.6	
Tomato		0.01	113	1.7	
		0.1	114	1.9	
Lemon pulp		0.01	77	6.3	
		0.1	83	2.6	

Table A 2: Recovery results from method validation of quizalofop-P-ethyl using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
Mass Transition 343.4→270.9 m/z (Quantification)					
olive	Quizalofop-P-ethyl expressed as quizalofop	0.01	113	7.1	
		0.1	86	12.1	
Tomato		0.01	113	4.3	
		0.1	113	1.0	
Lemon pulp		0.01	79	3.2	
		0.1	75	1.4	
Mass Transition 343.4→235 m/z (Confirmation)					
Olive	Quizalofop-P-ethyl expressed as quizalofop	0.01	109	11.8	
		0.1	101	12.7	
Tomato		0.01	112	4.6	
		0.1	113	2.9	
Lemon pulp		0.01	78	10.2	
		0.1	75	0.7	

Table A 3: Recovery results from method validation of quizalofop-P- tefuryl using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
Mass Transition 343.4→270.9 m/z (Quantification)					
Olive	Quizalofop-P-tefuryl expressed as quizalofop	0.01	106	12.4	
		0.1	936	13.3	
Tomato		0.01	117	2.0	
		0.1	116	1.5	
Lemon pulp		0.01	84	3.6	
		0.1	78	3.0	
Mass Transition 343.4→235 m/z (Confirmation)					
Olive	Quizalofop-P-tefuryl expressed as quizalofop	0.01	111	3.7	
		0.1	108	8.7	
Tomato		0.01	115	5.0	
		0.1	113	2.6	
Lemon pulp		0.01	82	13.2	
		0.1	88	2.9	

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

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
Mass Transition 343.4→270.9 m/z (Quantification)					
Olive	Propaquizafop expressed as quizalofop	0.01	102	12.1	
		0.1	87	17.6	
Tomato		0.01	110	3.9	
		0.1	100	0.9	
Lemon pulp		0.01	83	3.9	
		0.1	77	5.0	
Mass Transition 343.4→235 m/z (Confirmation)					
Olive	Propaquizafop expressed as quizalofop	0.01	108	8.8	
		0.1	104	12.3	
Tomato		0.01	114	2.2	
		0.1	113	3.1	
Lemon pulp		0.01	86	3.9	
		0.1	89	4.6	

Table A 5: Recovery results from method validation of Quizalofop- P-acid glucose conjugate using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
Mass Transition 343.4→270.9 m/z (Quantification)					
Olive	Quizalofop- P-acid glucose conjugate expressed as quizalofop	0.01	104	11.6	
		0.1	90	15.1	
Tomato		0.01	107	4.5	
		0.1	91	3.9	
Lemon pulp		0.01	80	3.2	
		0.1	76	3.3	
Mass Transition 343.4→235 m/z (Confirmation)					
Olive	Quizalofop- P-acid glucose conjugate expressed as quizalofop	0.01	109	4.5	
		0.1	108	14.4	
Tomato		0.01	114	5.3	
		0.1	102	5.7	
Lemon pulp		0.01	89	3.9	
		0.1	83	3.3	

Table A 18: Characteristics for the analytical method

	Quizalofop
Specificity	The analysis of blanks and untreated samples in comparison with the analysis of standard solutions and spiked samples showed the absence of compound interfering with the determination of quizalofop-ethyl and quizalofop (<30% LOQ)
Calibration (type, number of data points)	The linearity of the detector response was demonstrated by single determination of matrix-matched calibration standards for quizalofop at a minimum of six concentration levels ranging from 0.05 ng/mL to 10 ng/mL for all matrices. This range corresponds to 0.0022 mg/kg to 0.44 mg/kg for all matrices and thus covers the range from no more than 30 % of the LOQ and at least + 20 % of the highest analyte concentration level detected in a sample extract. The calibration curves obtained for both ion mass transitions and all matrices were linear since coefficients of determination (R^2) were ≥ 0.990.
Assessment of matrix effects is presented	yes
Limit of quantification	0.01 mg/kg

Conclusion

The method was found to be valid according to the guidance document SANTE/2020/12830 for the determination of quizalofop, quizalofop-P-ethyl, quizalofop-P-tefuryl, propaquizafop and quizalofop- P-acid glucose conjugate in water, oil an acid plant matrices with the tested LOQ of 0.01 mg/kg.
This study can be considered as ILV of analytical method reported as KCP 5.2.1/07 (Study code S17-06616) because same analytical procedure was followed in matrices.

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

New data have been submitted.

A 2.1.2.2.1 Analytical method 1

A 2.1.2.2.1.1 Method validation

Comments of zRMS:	The method is accepted

Reference:	KCP 5.2.1-11
Report	Validation of the analytical procedure for the deremination of Quizalfoop free acid and Quizalofop-P-ethyl after hydrolysis in milk by liquid chromatography, Pivato, M., 2016, Report no. 16.563341.0001

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

Materials and methods

Short term study for the validation of an in-house analytical method, based on QuECheRs procedure and internally codified as SOPa-222-LABCHI-Rev.0 for determination of Quizalofop free acid and Quizalofop p-ethly after hydrolysis in milk. LOQ required and verified was 0.005 mg/kg.
The validation was performed quantifying Quizalofop Free Acid after hydrolysis reaction of Quizalofop-p-ethyl. For each analyte two SRM transitions were monitored:
Quizalofop-p-ethyl:

- Transition 1: 373 m/z (parent ion)>299 m/z (daughter ion);
- Transition 2: 373 m/z (parent ion)>91 m/z (daughter ion).

Quizalofop Free Acid:

- Transition 1: 343 m/z (parent ion)>271 m/z (daughter ion);
- Transition 2: 343 m/z (parent ion)>243 m/z (daughter ion).

Solutions were analysed by LC-MS.

Results and discussions

The validation data demonstrates that the analytical method is suitable to qualitatively and quantitatively determine Quizalofop Free Acid after hydrolysis reaction of Quizalofop-p-ethyl in milk specimens according to Sanco/3029/99 rev. 4 and OECD-204/2014 guidelines and for the given concentration range.

Table A 19: Recovery results from method validation of Quizalofop-p-ethyl and Quizalofop-P using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (n = 5)	Mean recovery (%)	RSD (%)	Comments
milk	Quizalofop-p-ethyl	0.005	0.8 (transition 1) 12.2 (transition 2)	-	
	Quizalofop-p-ethyl	0.050	0.3 (transition 1) 1.5 (transition 2)	-	
	Quizalofop free acid	0.005	107.8 (transition 1) 94.5 (transition 2)	-	
	Quizalofop free acid	0.050	103.1 (transition 1) 99.5 (transition 2)	-	
	Sum of quizalofop-p-ethyl and quizalofop free acid	0.005	108.6 (transition 1) 106.7 (transition 2)	1 (transition 1) 3 (transition 2)	
	Sum of quizalofop-p-ethyl and quizalofop free acid	0.050	108.1 (transition 1) 96.0 (transition 2)	1 (transition 1) 5 (transition 2)	

Table A 20: Characteristics for the analytical method used for validation of Quizalofop-p-ethlyl and Quizalofop free acid residues in milk

	Quizalofop-p-ethyl	Quizalofop Free Acid
Specificity	Blank and test solution not containing the anlyte: no significant peaks (≤30% LOQ) detected at RT of the target analyte and comparison with the Spiked Test Solution by visual examination.	
Calibration (type, number of data points)	The method linearity was evaluated at 5 different levels of concentration, ranging from about 30% LOQ (about 0.0015 mg/kg) to about 30xLOD (about 0.15 mg/kg). Solutions were analysed by LC-MS.	The method linearity was evaluated at 5 different levels of concentration, ranging from about 30% LOQ (about 0.0015 mg/kg) to about 30xLOD (about 0.15 mg/kg). Solutions were analysed by LC-MS.
Calibration range	Accepted calibration range: 0.0015 mg/kg – 0.15 mg/kg Corresponding calibration range: 0.0015 mg/kg – 0.1599 mg/kg Transition 1: y=57800308x R^2=1.00 Transition 2: y=19511711x R^2=0.99	Accepted calibration range: 0.0015 mg/kg – 0.15 mg/kg Corresponding calibration range: 0.0015 mg/kg – 0.1521 mg/kg Transition 1: y=712031x R^2=1.00 Transition 2: y=35982x R^2=1.00
Assessment of matrix effects is presented	yes	yes
Limit of quantification	0.005 mg/kg	0.005 mg/kg

Conclusion

The validation data demonstrate that the analytical method SOPa-222-LABCHI-Rev.0 internally developed is suitable to qualitatively and quantitatively determine Quizalofop Free Acid after hydrolysis reaction of Quizalofop-p-ethyl in milk specimens.

A 2.1.2.2.2 Analytical method 2

A 2.1.2.2.2.1 Method validation

Comments of zRMS:	The method is accepted

Reference:	KCP 5.2.1-12
Report	Validation of the analytical procedure for the determination of Quizalofop Free Acid and Quizalofop-p-ethyl after hydrolysis in eggs by liquid chromatography, Pivato, M., 2016, Report no. 16.563341.0003
Guideline(s):	Yes (SANCO/825/00 rev. 8.1, SANCO/3029/99 rev. 4 and OECD-204/2014)

Deviations: No

GLP: Yes

Acceptability: Yes

Materials and methods

Study purpose is short term study for the validation of an in-house analytical method, based on QuECheRs procedure and internally codified as SOPa-224-LABCHI-Rev.0, for the determination of Quizalofop Free Acid and Quizalofop-p-ethyl after hydrolysis in eggs. LOQ required and verified was 0.005 mg/kg.
The validation was performed quantifying Quizalofop Free Acid after hydrolysis reaction of Quizalofop-p-ethyl. For each analyte two SRM transitions were monitored:
Quizalofop-p-ethyl:

- Transition 1: 373 m/z (parent ion)>299 m/z (daughter ion);
- Transition 2: 373 m/z (parent ion)>91 m/z (daughter ion).

Quizalofop Free Acid:

- Transition 1: 343 m/z (parent ion)>271 m/z (daughter ion);
- Transition 2: 343 m/z (parent ion)>243 m/z (daughter ion).

Solutions were analysed by LC-MS.

Results and discussions

The validation data demonstrage that the analytical method SOPa-224-LABCHI-Rev.0 internally developed is suitable to qualitatively and quantitatively determine Quizalofop Free Acid after hydrolysis reaction of Quizalofop-p-ethyl in eggs specimens according to SANCO/3029/99 Rev. 4 and OECD-204/2014 guidelines and for the given concentration range.

Table A 21: Recovery results from method validation of Quizalofop-p-ethyl and Quizalofop-P using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (n = 5)	Mean recovery (%)	RSD (%)	Comments
eggs	Quizalofop-p-ethyl	0.005	2.9 (transition 1) 7.2 (transition 2)		
	Quizalofop-p-ethyl	0.050	0.5 (transition 1) 0.9 (transition 2)		
	Quizalofop free acid	0.005	106.5 (transition 1) 99.2 (transition 2)		
	Quizalofop free acid	0.050	107.6 (transition 1) 107.3 (transition 2)		
	Sum of quizalofop-p-ethyl and quizalofop free acid	0.005	109.6 (transition 1) 106.4 (transition 2)	1 (transition 1) 2 (transition 2)	
	Sum of quizalofop-p-	0.050	108.1 (transition 1)	1 (transition 1) 5 (transition 2)	

Matrix	Analyte	Fortification level (mg/kg) (n = 5)	Mean recovery (%)	RSD (%)	Comments
	ethyl and quizalofop free acid		96.0 (transition 2)		

Table A 22: Characteristics for the analytical method used for validation of quizalofop-p-ethyl and quizalofop free acid residues in eggs

	Quizalofop-P-ethyl	Quizalofop Free Acid
Specificity	No significant peaks (≤30%) are detected at RT of the target analytes in the Blank and Test Solution with respect to the Spiked Test Solution for both transition 1 and 2.	
Calibration (type, number of data points)	The method linearity was evaluated at 5 different levels of concentration, ranging from 30% LOQ (0.0015 mg/kg) to about 30xLOQ (0.15 mg/kg). Solutions were analysed by LC-MS.	The method linearity was evaluated at 5 different levels of concentration, ranging from 30% LOQ (0.0015 mg/kg) to about 30xLOQ (0.15 mg/kg). Solutions were analysed by LC-MS.
Calibration range	Accepted calibration range: 0.0015 mg/kg – 0.15 mg/kg Corresponding calibration range: 0.0012 mg/kg – 0.1598 mg/kg Transition 1: y=103819814x R^2=1.00 Transition 2: y=30904020x R^2=1.00	Accepted calibration range: 0.0015 mg/kg – 0.15 mg/kg Corresponding calibration range: 0.0012 mg/kg – 0.1521 mg/kg Transition 1: y=885533x R^2=1.00 Transition 2: y=101571x R^2=1.00
Assessment of matrix effects is presented	yes	yes
Limit of quantification	0.005 mg/kg	0.005 mg/kg

Conclusion

The validation data demonstrate that the analytical method SOPa-222-LABCHI-Rev.0 internally developed is suitable to qualitatively and quantitatively determine Quizalofop Free Acid after hydrolysis reaction of Quizalofop-p-ethyl in milk specimens.

A 2.1.2.2.3 Analytical method 3

A 2.1.2.2.3.1 Method validation

Comments of zRMS:	The method is accepted

Reference:	KCP 5.2.1-13
Report	Validation of the analytical procedure for the determination of Quizalofop free acid and Quizalofop-p-ethyl hydrolysis in meat (poultry) by liquid chromatography, Pivato, M., 2016, Report no. 16.563341.0004
Guideline(s):	Yes (SANCO/825/00 rev. 8.1, SANCO/3029/99 rev. 4 and OECD-204/2014)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Study purpose is short term study for the validation of an in-house analytical method, based on QuECheRs procedure and internally codified as SOPa-225-LABCHI-Rev.0 for the determination of Quizalofop Free Acid and Quizalofop-p-ethyl after hydrolysis in meat (poultry). Meat (poultry) samples were used as representative matrix. LOQ required and verified was 0.005 mg/kg.
The validation was performed quantifying Quizalofop Free Acid after hydrolysis reaction of Quizalofop-p-ethyl. For each analyte two SRM transitions were monitored:
Quizalofop-p-ethyl:

- Transition 1: 373 m/z (parent ion)>299 m/z (daughter ion);
- Transition 2: 373 m/z (parent ion)>91 m/z (daughter ion).

Quizalofop Free Acid:

- Transition 1: 343 m/z (parent ion)>271 m/z (daughter ion);
- Transition 2: 343 m/z (parent ion)>243 m/z (daughter ion).

Solutions were analysed by LC-MS.

Results and discussions

The validation data demonstrage that the analytical method SOPa-224-LABCHI-Rev.0 internally developed is suitable to qualitatively and quantitatively determine Quizalofop Free Acid after hydrolysis reaction of Quizalofop-p-ethyl in meat (poultry) specimens according to SANCO/3029/99 Rev. 4 and OECD-204/2014 guidelines and for the given concentration range.

Table A 23: Recovery results from method validation of Quizalofop-p-ethyl and Quizalofop free acid using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (n = 5)	Mean recovery (%)	RSD (%)	Comments
Meat (poultry)	Quizalofop-p-ethyl	0.005	4.5 (transition 1) 12.6 (transition 2)		
	Quizalofop-p-ethyl	0.050	1.9 (transition 1) 2.6 (transition 2)		
	Quizalofop free acid	0.005	104.6 (transition 1) 90.5 (transition 2)		
	Quizalofop free acid	0.050	107.9 (transition 1) 85.1 (transition 2)		
	Sum of	0.005	108.8 (transition 1)	2 (transition 1)	

Matrix	Analyte	Fortification level (mg/kg) (n = 5)	Mean recovery (%)	RSD (%)	Comments
	quizalofop-p-ethyl and quizalofop free acid		103.2 (transition 2)	6 (transition 2)	
	Sum of quizalofop-p-ethyl and quizalofop free acid	0.050	108.1 (transition 1) 96.0 (transition 2)	1 (transition 1) 5 (transition 2)	

Table A 24: Characteristics for the analytical method used for validation of Quizalofop-p-ethyl and Quizalofop free acid residues in meat (poultry)

	Quizalofop-p-ethyl	Quizalofop Free Acid
Specificity	No significant peaks (≤30%) are detected at RT of the target analytes in the Blank and Test Solution with respect to the Spiked Test Solution for both transition 1 and 2.	
Calibration (type, number of data points)	The method linearity was evaluated at 5 different levels of concentration, ranging from 30% LOQ (0.005 mg/kg) to about 30xLOQ (0.15 mg/kg). Solutions were analysed by LC-MS.	The method linearity was evaluated at 5 different levels of concentration, ranging from 30% LOQ (0.005 mg/kg) to about 30xLOQ (0.15 mg/kg). Solutions were analysed by LC-MS.
Calibration range	Accepted calibration range: 0.0015 mg/kg – 0.15 mg/kg Corresponding calibration range: 0.0012 mg/kg – 0.1596 mg/kg Transition 1: y=81990160x R^2=1.00 Transition 2: y=26399037x R^2=1.00	Accepted calibration range: 0.0015 mg/kg – 0.15 mg/kg Corresponding calibration range: 0.0012 mg/kg – 0.1521 mg/kg Transition 1: y=820398x R^2=1.00 Transition 2: y=66411x R^2=1.00
Assessment of matrix effects is presented	yes	yes
Limit of quantification	0.005 mg/kg	0.005 mg/kg

Conclusion

The validation data demonstrate that the analytical method SOPa-222-LABCHI-Rev.0 internally developed is suitable to qualitatively and quantitatively determine Quizalofop Free Acid after hydrolysis reaction of Quizalofop-p-ethyl in meat (poultry) specimens.

A 2.1.2.2.3.2 Independent laboratory validation

Comments of zRMS:	The method is accepted

Reference:	KCP 5.2.1-17
Report	Independent laboratory validation of a method for the determination of quizalofop free acid and quizalofop-p-ethyl after hydrolysis in meat (poultry) by liquid chromatography. A. Markowicz, 2020, Report No. ZBBZ-2016/09/DPL/2A
Guideline(s):	SANCO/825/00 rev. 8.1 SANCO/3029/99 rev. 4
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The objective of the study is an independent validation of the method for the determination of Quizalofop free acid and Quizalofop P-Ethyl after hydrolysis in meat (poultry) described in Final Report. N. 16.563341.0004 "Validation of the analytical procedure for the determination of Quizalofop free acid and Quizalofop P-Ethyl after hydrolysis in meat (poultry) by liquid chromatography" and in accordance to the guidance document SANCO/825/00, rev. 8.1 of the European Commission.
In brief, the meat sample was milled with the addition of dry ice until a homogeneous mixture was obtained before initial extraction, water was added then meat sample was extracted with acetonitrile. Then the sample was hydrolysis with NaOH to convert Quizalofop P-Ethyl to the parent acid (Quizalofop free acid). After addition of magnesium sulphate the extract was shaken. Following centrifugation, the extract was kept at about -20°C for about 2 hours. Then, an aliquot of the upper acetonitrile phase after centrifugation was cleaned by addition of primary secondary amine (PSA) and dehydrated by magnesium sulphate for Quizalofop P-Ethyl and was cleaned by addition of bakerbond-octadecyl (C18) and dehydrated by magnesium sulphate for Quizalofop free acid.

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 (for each analytes) were evaluated in order to demonstrate that the method achieves a high level of selectivity. The retention times of analytes in extracts correspond to that of the calibration standards with a tolerance of < ± 0.1 min. Also, confirmation ratios for Quizalofop free acid and Quizalofop P-Ethyl in all samples were within ± 30 % of the average found for the standards.
No significant interference above 30 % of LOQ was detected in any of the reagent blanks or control specimen extracts for meat matrix, so that a highly level of selectivity was demonstrated and an additional confirmatory method is not necessary.

Matrix Effects

The matrix effects are outside the range 0.8 and 1.2, matrix-matched standards were used for quantification for meat matrix.

Linearity

The correlation between the injected concentration of analytes standard and detector response was demonstrated to be linear by single determination of matrix-matched calibration standards at five concentration levels ranging from 0.0003 µg/mL to 0.04 µg/mL for Quizalofop free acid and Quizalofop P-Ethyl. This range corresponds from 0.0012 mg/kg to 0.16 mg/kg thus covers the range from no more than 30 % of the LOQ to at least 10 x LOQ.
The calibration curves obtained for both ions mass transitions of Quizalofop free acid and Quizalofop P-Ethyl were linear with the coefficients of correlation (R) greater than 0.99. Linear regression was performed with 1/x weighting.

Quantification
Quantification was performed by using weighted (1/x) linear regression as described in the section "Linearity". The validation was performed quantifying Quizalofop free acid after hydrolysis reaction of Quizalofop P-Ethyl.
System Suitability Test
System suitability test (SST) was performed to verify the performance of LC-MS/MS system and to ensure its adequacy for Quizalofop free acid and Quizalofop P-Ethyl determination in meat. %RSD of Quizalofop free acid and Quizalofop P-Ethyl peak areas for the first and second ions mass transition was found within the acceptable range (≤ 15%) indicating that the system was suitable for the intended analysis.
Stability of Analyte in the Final Dilution
Recoveries of the fortified samples within the acceptable range of 70-110% obtained with calibration solutions and the use of bracketing standards at LOQ level (SST samples) to insure integrity of the analytical sequence sufficiently demonstrate the stability of analytes in the final dilution.
Accuracy and Precision
Accuracy was determined by fortification of control sample with known amounts of Quizalofop P-Ethyl, subsequent determination of the recoveries when applying the extraction procedure and quantified as Quizalofop free acid. Precision was determined by repeatability (relative standard deviation – RSD). Five recovery determinations were performed at the LOQ (0.005 mg/kg) and at the 10xLOQ (0.05 mg/kg) for meat, respectively.
The mean recovery values at the fortification levels of 0.005 mg/kg and 0.05 mg/kg for both ions mass transitions of Quizalofop free acid were all in the range 70 – 110 % and thus comply with the standard acceptance criteria of the guidance documents SANCO/825/00 rev. 8.1. All precision values at the fortification levels of 0.005 mg/kg and 0.05 mg/kg for both ions mass transitions of Quizalofop free acid were < 20%.

Solutions and solvent mixtures
Mobile Phase A: 10 mM ammonium formate 0.022% formic acid
500 mL volumetric flask was half filled with water, 0.315 g of ammonium formate (NH_4HCO_2) and 110 µl of formic acid (HCOOH) 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
Approximately 500 mL of LC/MS grade methanol was transferred to HPLC solvent reservoir.

Sample preparation
Preparation of Sample Matrix
Portion of dry ice was added to a homogenizer apparatus (Robot Coupe). Subsequent appropriate amount of sample was added to the apparatus in small portions. Sample was blended after each addition until a homogeneous mixture was obtained.
Contents of the apparatus was poured into polyethylene bags and stored in a freezer until the last traces of dry ice have sublimed.

Extraction
5.00 g ± 0.05 g 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.
Using glass volumetric pipettes 7.5 mL of water and 10 mL of acetonitrile was added.
The Teflon® centrifuge tube was closed tightly and shaken vigorously by QuEChERS Hand Motion Shaker for 1 min.

Hydrolysis

Using glass volumetric pipettes 2 mL 1N NaOH were added and pH was checked
The tube was vortex for 1 min and incubated at 75°C for 1 hour
After 1 hour, the samples were cooled to room temperature
The pH was slowly lowered to about 1 wit 37% HCl

Liquid-liquid Partition
6 g ± 0.1 g of magnesium sulfate anhydrous was added and the centrifuge tube was closed and shaken vigorously by QuEChERS Hand Motion Shaker for 1 min.
The extract was centrifuged at >4750 rpm for 5 min.
The centrifuge tube kept at about -20°C for about 2 hours
The extract was centrifuged at >4750 rpm for 5 min again.

Sample Purification
The supernatant obtained from sample extraction was split in two tubes, one for Quizalofop free acid purification and one for Quizalofop P-Ethyl purification.
Transfer 3 mL of supernatant into 10 mL tube containing about 450 mg of magnesium sulfate and 150 mg of PSA for Quizalofop P-Ethyl purification
Transfer 3 mL of supernatant into 10 mL tube containing about 450 mg of magnesium sulfate and 150 mg of C18 for Quizalofop free acid purification
Shake the sample in the vortex for 1 minute
Centrifuge for 5 min at approx. 4000 rpm

Results and discussions

Table A 25: Recovery results from independent laboratory validation of Quizalofop free acid and Quizalofop-P-Ethyl using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (n = 5)	Mean recovery (%)	RSD (%)	Comments
Meat (poultry)	Quizalofop free acid	0.005	84	6.0	First mass transition
		0.05	83	13.8	
		0.005	87	3.4	Second mass transition
		0.05	84	14.5	

Table A 26: Characteristics for the analytical method used for independent laboratory validation of Quizalofop-p-ethyl and quizalofop free acid residues in muscle

	Quizalofop free acid	Quizalofop-ethyl
Specificity	For both ion mass transitions, the specimen showed no significant interference (above 30 % of the LOD) at the retention times of the analytes. The method is specific	For both ion mass transitions, the specimen showed no significant interference (above 30 % of the LOD) at the retention times of the analytes. The method is specific
Calibration (type, number of data points)	5 points 0.0012 to 0.16 mg/kg First mass transition y=1922345.619770x-	5 points 0.0012 to 0.16 mg/kg First mass transition y=43007391.324535x-

	Quizalofop free acid	Quizalofop-ethyl
	48.301539 R^2=0.99137182 Second mass transition y=582963.784388x-1.893647 R^2=0.99043740	4472.865516 R^2=0.99832271 Second mass transition y=9493216.911121x-1034.542454 R^2=0.99846520
Assessment of matrix effects is presented	Yes	Yes
Limit of determination/quantification	LOQ = 0.005 mg/kg LOD = 0.0012 mg/kg	LOQ = 0.005 mg/kg LOD = 0.0012 mg/kg

Conclusion

According to SANCO/3029/99 rev. 4 the method for determination of residues of quizalofop-p-ethyl and quizalofop free acid in meat matrices was independently validated.

A 2.1.2.2.4 Analytical method 4

A 2.1.2.2.4.1 Method validation

Comments of zRMS:	The method is accepted

Reference:	KCP 5.2.1-14
Report	Validation of the analytical procedure for the determination of Quizalofop Free Acid and Quizalofop-p-ethyl after hydrolysis in fat by Liquid Chromatography, Pivato, M., 2016, Report no. 16.563341.0002
Guideline(s):	Yes (SANCO/825/00 rev. 8.1, SANCO/3029/99 rev. 4 and OECD-204/2014)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Study purpose is short term study for the validation of an in-house analytical method, based on QuECheRs procedure and internally codified as SOPa-223-LABCHI-Rev.0, for the determination of Quizalofop Free Acid and Quizalofop-p-ethyl after hydrolysis in fat. Fat samples were used as representative matrix.
LOQ required and verified was 0.005 mg/kg.
The validation was performed quantifying Quizalofop Free Acid after hydrolysis reaction of Quizalofop-p-ethyl. For each analyte two SRM transitions were monitored:
Quizalofop-p-ethyl:

- Transition 1: 373 m/z (parent ion)>299 m/z (daughter ion);
- Transition 2: 373 m/z (parent ion)>91 m/z (daughter ion).

Quizalofop Free Acid:

- Transition 1: 343 m/z (parent ion)>271 m/z (daughter ion);
- Transition 2: 343 m/z (parent ion)>243 m/z (daughter ion).

Solutions were analysed by LC-MS.

Results and discussions

The validation data demonstrage that the analytical method SOPa-224-LABCHI-Rev.0 internally developed is suitable to qualitatively and quantitatively determine Quizalofop Free Acid after hydrolysis reaction of Quizalofop-p-ethyl in fat specimens according to SANCO/3029/99 Rev. 4 and OECD-204/2014 guidelines and for the given concentration range.

Table A 27: Recovery results from method validation of quizalofop-p-ethyl and quizalofop free acid using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (n = 5)	Mean recovery (%)	RSD (%)	Comments
Fat	Quizalofop-p-ethyl	0.005	7.3 (transition 1) 10.4 (transition 2)		
	Quizalofop-p-ethyl	0.050	2.7 (transition 1) 3.0 (transition 2)		
	Quizalofop free acid	0.005	97.9 (transition 1) 88.2 (transition 2)		
	Quizalofop free acid	0.050	98.7 (transition 1) 93.1 (transition 2)		
	Sum of quizalofop-p-ethyl and quizalofop free acid	0.005	105.2 (transition 1) 98.8 (transition 2)	3 (transition 1) 9 (transition 2)	
	Sum of quizalofop-p-ethyl and quizalofop free acid	0.050	101.4 (transition 1) 96.0 (transition 2)	2 (transition 1) 3 (transition 2)	

Table A 28: Characteristics for the analytical method used for validation of Quizalofop-p-ethly and Quizalofo Free Acid residues in fat

	Quizalofop-p-ethyl	Quizalofop Free Acid
Specificity	No significant peaks (≤30%) are detected at RT of the target analytes in the blank and test solution with respect to the spiked test solution for both transition 1 and 2.	
Calibration (type, number of data points)	The method linearity was evaluated at 5 different levels of concentration, ranging from 30% LOQ (0.0012 mg/kg) to about 30xLOQ (0.15 mg/kg). Solutions were analysed by LC-MS.	The method linearity was evaluated at 5 different levels of concentration, ranging from 30% LOQ (0.0012 mg/kg) to about 30xLOQ (0.15 mg/kg). Solutions were analysed by LC-MS.

	Quizalofop-p-ethyl	**Quizalofop Free Acid**
Calibration range	Accepted calibration range: 0.0012 mg/kg – 0.15 mg/kg Corresponding calibration range: 0.0013 mg/kg – 0.1759 mg/kg Transition 1: y=103389276x R^2=1.00 Transition 2: y=27757147x R^2=1.00	Accepted calibration range: 0.0012 mg/kg – 0.15 mg/kg Corresponding calibration range: 0.0012 mg/kg – 0.1518 mg/kg Transition 1: y=816664x R^2=1.00 Transition 2: y=92779x R^2=1.00
Assessment of matrix effects is presented	yes	yes
Limit of quantification	0.005 mg/kg	0.005 mg/kg

Conclusion

The validation data demonstrate that the analytical method SOPa-223-LABCHI-Rev.0 internally developed is suitable to qualitatively and quantitatively determine Quizalofop Free Acid after hydrolysis reaction of Quizalofop-p-ethyl in fat specimens.

A 2.1.2.2.4.2 Independent laboratory validation

Comments of zRMS:	The method is accepted

Reference:	KCP 5.2.1-18
Report	Independent laboratory validation of a method for determination of quizalofop free acid and quizalofop-p-etthyl after hydrolysis in fat by liquid chromatography. A. Markowicz, 2019, Report No. ZBBZ-2016/09/DPL/1A
Guideline(s):	SANCO/825/00 rev. 8.1 SANCO/3029/99 rev. 4
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The objective of the study is an independent validation of the method for the determination of Quizalofop free acid and Quizalofop P-Ethyl after hydrolysis in fat (bovine) described in Final Report. N. 16.563341.0002 “Validation of the analytical procedure for the determination of Quizalofop free acid and Quizalofop P-Ethyl after hydrolysis in fat by liquid chromatography” and in accordance to the guidance document SANCO/825/00, rev. 8.1 of the European Commission.
In brief, the fat (bovine) sample was milled with the addition of dry ice until a homogeneous mixture was obtained, then fat sample was extracted with acetonitrile. Then the sample was hydrolysis with NaOH to convert Quizalofop P-Ethyl to the parent acid (Quizalofop free acid). Following centrifugation, the extract was kept at about -20°C for about 2 hours. Then, an aliquot of the upper acetonitrile phase after centrifu-

gation was cleaned by addition of primary secondary amine (PSA), bakerbond-octadecyl (C18) and dehydrated by magnesium sulphate for Quizalofop P-Ethyl and was cleaned by addition of bakerbond-octadecyl (C18) and dehydrated by magnesium sulphate for Quizalofop free acid.

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 (for each analytes) were evaluated in order to demonstrate that the method achieves a high level of selectivity. The retention times of analytes in extracts correspond to that of the calibration standards with a tolerance of < ± 0.1 min. Also, confirmation ratios for Quizalofop free acid and Quizalofop P-Ethyl in all samples were within ± 30 % of the average found for the standards.

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

Matrix Effects

The matrix effects are outside the range 0.8 and 1.2, matrix-matched standards were used for quantification for fat matrix.

Linearity

The correlation between the injected concentration of analytes standard and detector response was demonstrated to be linear by single determination of matrix-matched calibration standards at five concentration levels ranging from 0.0003 µg/mL to 0.05 µg/mL for Quizalofop free acid and Quizalofop P-Ethyl. This range corresponds from 0.0012 mg/kg to 0.2 mg/kg thus covers the range from no more than 30 % of the LOQ to at least 10 x LOQ.

The calibration curves obtained for both ions mass transitions of Quizalofop free acid and Quizalofop P-Ethyl were linear with the coefficients of correlation (R) greater than 0.99. Linear regression was performed with 1/x weighting.

Quantification

Quantification was performed by using weighted (1/x) linear regression as described in the section "Linearity". The validation was performed quantifying Quizalofop free acid after hydrolysis reaction of Quizalofop P-Ethyl.

System Suitability Test

System suitability test (SST) was performed to verify the performance of LC-MS/MS system and to ensure its adequacy for Quizalofop free acid and Quizalofop P-Ethyl determination in fat. %RSD of Quizalofop free acid and Quizalofop P-Ethyl peak areas for the first and second ions mass transition was found within the acceptable range (≤ 15%) indicating that the system was suitable for the intended analysis.

Stability of Analyte in the Final Dilution

Recoveries of the fortified samples within the acceptable range of 70-110% obtained with calibration solutions and the use of bracketing standards at LOQ level (SST samples) to insure integrity of the analytical sequence sufficiently demonstrate the stability of analytes in the final dilution.

Accuracy and Precision

Accuracy was determined by fortification of control sample with known amounts of Quizalofop P-Ethyl, subsequent determination of the recoveries when applying the extraction procedure and quantified as Quizalofop free acid. Precision was determined by repeatability (relative standard deviation – RSD). Five recovery determinations were performed at the LOQ (0.005 mg/kg) and at the 10xLOQ (0.05 mg/kg) for fat, respectively.

Solutions and solvent mixtures

Mobile Phase A: 10 mM ammonium formate 0.022% formic acid

500 mL volumetric flask was half filled with water, 0.315 g of ammonium formate (NH_4HCO_2) and 110 µl of formic acid (HCOOH) 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
Approximately 500 mL of LC/MS grade methanol was transferred to HPLC solvent reservoir.

Sample preparation
Preparation of Sample Matrix
Portion of dry ice was added to a homogenizer apparatus (Robot Coupe). Subsequent appropriate amount of sample was added to the apparatus in small portions. Sample was blended after each addition until a homogeneous mixture was obtained.
Contents of the apparatus was poured into polyethylene bags and stored in a freezer until the last traces of dry ice have sublimed.

Extraction
3.00 g ± 0.05 g 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.
Using glass volumetric pipettes 9 mL of acetonitrile was added.
The Teflon® centrifuge tube was closed tightly and shaken vigorously by QuEChERS Hand Motion Shaker for 1 min.

Hydrolysis
Using glass volumetric pipettes 2 mL 1N NaOH were added and pH was checked
The tube was vortex for 1 min and incubated at 75°C for 1 hour
After 1 hour, the samples were cooled to room temperature
The pH was slowly lowered to about 1 with 37% HCl

Liquid-liquid Partition
The extract was centrifuged at >4750 rpm for 5 min.
The centrifuge tube kept at about -20°C for about 2 hours
The extract was centrifuged at >4750 rpm for 5 min again.

Sample Purification
The supernatant obtained from sample extraction was split in two tubes, one for Quizalofop free acid purification and one for Quizalofop P-Ethyl purification.
Transfer 4 mL of supernatant into 10 mL tube containing about 750 mg of magnesium sulfate and 140 mg of PSA for Quizalofop P-Ethyl purification
Transfer 3 mL of supernatant into 10 mL tube containing about 450 mg of magnesium sulfate and 150 mg of C18 for Quizalofop free acid purification
Shake the sample in the vortex for 1 minute
Centrifuge for 5 min at approx. 4000 rpm

Results and discussions

Table A 29: Recovery results from independent laboratory validation of Quizalofop free acid and Quizalofop-P-Ethyl using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (n = 5)	Mean recovery (%)	RSD (%)	Comments
Fat	Quizalofop free acid	0.005	101	2.1	First mass transition
		0.05	91	3.7	
		0.005	105	4.8	Second mass transition

Matrix	Analyte	Fortification level (mg/kg) (n = 5)	Mean recovery (%)	RSD (%)	Comments
		0.05	92	3.2	

Table A 30: Characteristics for the analytical method used for independent laboratory validation of Quizalofop-p-ethyl and quizalofop free acid residues in muscle

	Quizalofop free acid	Quizalofop-ethyl
Specificity	For both ion mass transitions, the specimen showed no significant interference (above 30 % of the LOD) at the retention times of the analytes. The method is specific	For both ion mass transitions, the specimen showed no significant interference (above 30 % of the LOD) at the retention times of the analytes. The method is specific
Calibration (type, number of data points)	6 points 0.0012 to 0.2 mg/kg First mass transition y=1791614.462936x-242.938250 R^2=0.99470171 Second mass transition y=546518.160850-6.899374 R^2=0.99563675	6 points 0.0012 to 0.2 mg/kg First mass transition y=10188804.873817x-107.532643 R^2=0.99676037 Second mass transition y=2258840.050477x-38.784626 R^2=0.99665939
Assessment of matrix effects is presented	Yes	Yes
Limit of determination/quantification	LOQ = 0.005 mg/kg LOD = 0.0012 mg/kg	LOQ = 0.005 mg/kg LOD = 0.0012 mg/kg

Conclusion

According to SANCO/3029/99 rev. 4 the method for determination of residues of quizalofop-p-ethyl and quizalofop free acid in fat matrices was independently validated.

A 2.1.2.2.5 Analytical method 5

A 2.1.2.2.5.1 Method validation

Comments of zRMS:	The method is accepted

Reference: KCP 5.2.1-15

Report: Validation of the analytical procedure for the determination of Quizalofop Free Acid and Quizalofop-p-ethyl after hydrolysis in kidney (bovine) by Liquid chromatography, Pivato, M., 2016, Report no. 16.563341.0006

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

Materials and methods

Study purpose is short term study for the validation of an in-house analytical method, based on QuECheRs procedure and internally codified as SOPa-223-LABCHI-Rev.0, for the determination of Quizalofop Free Acid and Quizalofop-p-ethyl after hydrolysis in kidneys (bovine). Kidneys samples were used as representative matrix.
LOQ required and verified was 0.005 mg/kg.
The validation was performed quantifying Quizalofop Free Acid after hydrolysis reaction of Quizalofop-p-ethyl. For each analyte two SRM transitions were monitored:
Quizalofop-p-ethyl:

- Transition 1: 373 m/z (parent ion)>299 m/z (daughter ion);
- Transition 2: 373 m/z (parent ion)>91 m/z (daughter ion).

Quizalofop Free Acid:

- Transition 1: 343 m/z (parent ion)>271 m/z (daughter ion);
- Transition 2: 343 m/z (parent ion)>243 m/z (daughter ion).

Solutions were analysed by LC-MS.

Results and discussions

The validation data demonstrage that the analytical method SOPa-224-LABCHI-Rev.0 internally developed is suitable to qualitatively and quantitatively determine Quizalofop Free Acid after hydrolysis reaction of Quizalofop-p-ethyl in kidneys (bovine) specimens according to SANCO/3029/99 Rev. 4 and OECD-204/2014 guidelines and for the given concentration range.

Table A 31: Recovery results from method validation of Quizalofop-p-ethyl and Quizalofop Free Acid using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (n = 5)	Mean recovery (%)	RSD (%)	Comments
Kidneys (bovine)	Quizalofop-p-ethyl	0.005	0.5 (transition 1) 4.5 (transition 2)		
	Quizalofop-p-ethyl	0.050	0.2 (transition 1) 0.5 (transition 2)		
	Quizalofop free acid	0.005	103.2 (transition 1) 101.0 (transition 2)		
	Quizalofop free acid	0.050	106.2 (transition 1) 108.8 (transition 2)		
	Sum of quizalofop-p-ethyl and quizalofop free acid	0.005	103.6 (transition 1) 105.2 (transition 2)	3 (transition 1) 2 (transition 2)	

Matrix	Analyte	Fortification level (mg/kg) (n = 5)	Mean recovery (%)	RSD (%)	Comments
	Sum of quizalofop-p-ethyl and quizalofop free acid	0.050	106.0 (transition 1) 109.4 (transition 2)	2 (transition 1) 1 (transition 2)	

Table A 32: Characteristics for the analytical method used for validation of Quizalofop-p-ethyl and Quizalofop Free Acid residues in kidneys (bovine)

	Quizalofop-p-ethyl	Quizalofop Free Acid
Specificity	No significant peaks (≤30%) are detected at RT of the target analytes in the Blank and Test Solution with respect to the Spiked Test Solution for both transition 1 and 2.	
Calibration (type, number of data points)	The method linearity was evaluated at 5 different levels of concentration, ranging from 30% LOQ (0.0015 mg/kg) to about 30xLOQ (0.15 mg/kg). Solutions were analysed by LC-MS.	The method linearity was evaluated at 5 different levels of concentration, ranging from 30% LOQ (0.0015 mg/kg) to about 30xLOQ (0.15 mg/kg). Solutions were analysed by LC-MS.
Calibration range	Accepted calibration range: 0.0015 mg/kg – 0.15 mg/kg Corresponding calibration range: 0.0012 mg/kg – 0.1597 mg/kg Transition 1: y=83460576x R^2=1.00 Transition 2: y=25714357x R^2=1.00	Accepted calibration range: 0.0015 mg/kg – 0.15 mg/kg Corresponding calibration range: 0.0012 mg/kg – 0.1521 mg/kg Transition 1: y=997051x R^2=1.00 Transition 2: y=114212x R^2=1.00
Assessment of matrix effects is presented	yes	yes
Limit of quantification	0.005 mg/kg	0.005 mg/kg

Conclusion

The validation data demonstrate that the analytical method SOPa-223-LABCHI-Rev.0 internally developed is suitable to qualitatively and quantitatively determine Quizalofop Free Acid after hydrolysis reaction of Quizalofop-p-ethyl in kidney (bovine) specimens.

A 2.1.2.2.6 Analytical method 6

A 2.1.2.2.6.1 Method validation

Comments of zRMS:	The method is accepted

Reference:	KCP 5.2.1-16
Report	Validation of the analytical procedure for the determination of Quizalofop Free Acid and Quizalofop-p-ethyl after hydrolysis in liver by liquid chromatography, Pivato, M., 2016, Report no. 16.563341.0005
Guideline(s):	Yes (SANCO/825/00 rev. 8.1, SANCO/3029/99 rev. 4 and OECD-204/2014)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Study purpose is short term study for the validation of an in-house analytical method, based on QuECheRs procedure and internally codified as SOPa-223-LABCHI-Rev.0, for the determination of Quizalofop Free Acid and Quizalofop-p-ethyl after hydrolysis in liver. Liver samples were used as representative matrix.
LOQ required and verified was 0.005 mg/kg.
The validation was performed quantifying Quizalofop Free Acid after hydrolysis reaction of Quizalofop-p-ethyl. For each analyte two SRM transitions were monitored:
Quizalofop-p-ethyl:

- Transition 1: 373 m/z (parent ion)>299 m/z (daughter ion);
- Transition 2: 373 m/z (parent ion)>91 m/z (daughter ion).

Quizalofop Free Acid:

- Transition 1: 343 m/z (parent ion)>271 m/z (daughter ion);
- Transition 2: 343 m/z (parent ion)>243 m/z (daughter ion).

Solutions were analysed by LC-MS.

Results and discussions

The validation data demonstrage that the analytical method SOPa-224-LABCHI-Rev.0 internally developed is suitable to qualitatively and quantitatively determine Quizalofop Free Acid after hydrolysis reaction of Quizalofop-p-ethyl in liver specimens according to SANCO/3029/99 Rev. 4 and OECD-204/2014 guidelines and for the given concentration range.

Table A 33: Recovery results from method validation of Quizalofop-p-ethyl and Quizalofop Free Acid using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (n = 5)	Mean recovery (%)	RSD (%)	Comments
liver	Quizalofop-p-ethyl	0.005	4.3 (transition 1) 5.9 (transition 2)		
	Quizalofop-p-ethyl	0.050	0.4 (transition 1) 0.6 (transition 2)		
	Quizalofop free acid	0.005	102.3 (transition 1) 101.7 (transition 2)		
	Quizalofop free acid	0.050	80.5 (transition 1) 90.8 (transition 2)		
	Sum of	0.005	106.6 (transition 1)	3 (transition 1)	

Matrix	Analyte	Fortification level (mg/kg) (n = 5)	Mean recovery (%)	RSD (%)	Comments
	quizalofop-p-ethyl and quizalofop free acid		107.8 (transition 2)	1 (transition 2)	
	Sum of quizalofop-p-ethyl and quizalofop free acid	0.050	80.6 (transition 1) 92.0 (transition 2)	11 (transition 1) 9 (transition 2)	

Table A 34: Characteristics for the analytical method used for validation of Quizalofop-p-ethyl and Quizalofop Free Acid residues in liver

	Quizalofop-P-ethyl	Quizalofop Free Acid
Specificity	No significant peaks (≤30%) are detected at RT or the target analytes in the Blant and Test solution with respect to the spiked test solution for both transitions 1 and 2.	
Calibration (type, number of data points)	The method linearity was evaluated at 5 different levels of concentration, ranging from 30% LOQ (0,0015 mg/kg) to about 30xLOQ (0.15 mg/kg). Solutions were analysed by LC-MS.	The method linearity was evaluated at 5 different levels of concentration, ranging from 30% LOQ (0,0015 mg/kg) to about 30xLOQ (0.15 mg/kg). Solutions were analysed by LC-MS.
Calibration range	Accepted calibration range: 0.0015 mg/kg – 0.15 mg/kg Corresponding calibration range: 0.0013 mg/kg – 0.1677 mg/kg Transition 1: y=119595477x R^2=1.00 Transition 2: y=29791588x R^2=1.00	Accepted calibration range: 0.0015 mg/kg – 0.15 mg/kg Corresponding calibration range: 0.0012 mg/kg – 0.1521 mg/kg Transition 1: y=939418x R^2=1.00 Transition 2: y=108122x R^2=1.00
Assessment of matrix effects is presented	yes	yes
Limit of quantification	0.005 mg/kg	0.005 mg/kg

Conclusion

The validation data demonstrate that the analytical method SOPa-223-LABCHI-Rev.0 internally developed is suitable to qualitatively and quantitatively determine Quizalofop Free Acid after hydrolysis reaction of Quizalofop-p-ethyl in liver specimens.

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted