FINAL REGISTRATION REPORT

Part B

Section 5

Analytical Methods

Detailed summary of the risk assessment

Product code: SHA 4300 A

Product name(s): MIGHTY

Chemical active substance:

Mesotrione, 100 g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

Applicant: Sharda Cropchem España S.L.

Submission date: December 2018

Update date: June 2020

MS Finalisation date: 04/06/2024

Version history

When	What
February 2020	Dossier sent for evaluation
June 2020	Applicant update
June 2020	zRMS finalised evaluation
April 2024	Final version prepared by zRMS after Commenting period
June 2024	Final version prepared by zRMS after the second commenting period

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5 Analytical methods

This documentation has been prepared by the Applicant. All comments and changes introduced by zRMS are marked in gray.

5.1 Conclusion and summary of assessment

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

Sufficiently sensitive and selective analytical methods are ~~not~~ available for all analytes included in the residue definitions.
Noticed data gaps are:
- no data gaps

Commodity/crop	Supported/ Not supported
Maize	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 Mesotrione in plant protection product is provided as follows:

Comments of zRMS:	The method is accepted for analysing mesotrione in the PPP

Reference:	KCP 5.1.1
Report	PHYSICAL AND CHEMICAL PROPERTIES AND ACCELERATED STORAGE STABILITY TEST FOR MESOTRIONE 10% SC (SUSPENSION CONCENTRATE, 10.2% W/W MESOTRIONE) - SPAIN 2015-. Mónica Berrios, 2016. Report: E-15-0004.
Guideline(s):	Yes (SANCO/3030/99 rev. 4)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The determination of Mesotrione content in Mesotrione 10% SC was determined using HPLC-DAD.

GC-MSD and FTIR analysis was used to confirm validation of HPLC-DAD method.

HPLC-DAD conditions:

Instrument:	HPLC Agilent HP 1100 System with quaternary pump, degassing unit, autosampler, thermostated column and diode array detectos.
Column:	XTerra RP_{18}. 5 µm, 250 mm, 4.6 mm
Eluent:	A: H_2O LC-MS; B: 1% v/v Phosphoric Acid in Methanol
Wavelength:	250 nm (Mesotrione), 242 nm (Internal Standard)

Flow Rate:	0.8 ml/min
Detector:	DAD
Injection Volume:	10 µl
Retention Time:	Approximately 2.6 minutes

Test Substance Information

Test substance:	Mesotrione 10% SC
Batch Nº:	SWEPL-41203
Declared concentration of Active Ingredient:	100 g/L, (10%)
Analyzed concentration of Active Ingredient (AGQ Labs):	98.4 g/L, (9.0% w/w%, 9.84% w/v)

Validation - Results and discussions

Specificity
Specificity was demonstrated using HPLC-DAD. Two solvent controls (methanol) were carried out to check the presence of interference. None of the samples showed interference >3% to the total peak area measured for the target analyte.

Linearity
The linearity was determined from a solvent calibration (methanol), with six calibration points. The HPLC-DAD response was linear in the range of standard injected from 25 to 400 mg/L. The correlation coefficient (R) was 0.9995.

Precision (Repeatability)
To show the system precision, the quantification point was injected by duplicate and the sample preparations were single injection.. The values ranged from a concentration of 8.82 to 9.21 %w/w, with a mean of 8.95 %w/w, a standard deviation of 0.15 %w/w and a precision of 1.69%.

Accuracy
Due to the samples were a simple solution of the preparation in a solvent, recovery is not required.

Table 5.2-1: Methods suitable for the determination of active substance in plant protection product Mesotrione 10% SC/SHA 4300 A

	Mesotrione
Author(s), year	Berrios, Mónica, 2016
Principle of method	HPLC-DAD
Linearity (linear between mg/L / % range of the declared content) (correlation coefficient, expressed as r)	Linear between 25 mg/mL and 400 mg/ml R = 0.9995
Precision – Repeatability Mean n = 5 (%RSD)	1.69
Accuracy n = XX (% Recovery)	Not required.
Interference/ Specificity	No interference, the method is specific

	Mesotrione
Comment	-

Conclusion

The method of analysis of Mesotrione in the test item has been conveniently validated. The method is suitable for determining Mesotrione in the test product Mesotrione 10% SC.

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

~~The study to determine the concentration of the relevant impurities in Mesotrione 10% SC is on-going.~~

Comments of zRMS:	The method meets the sanco/3030/99 rev.5 requirements and may be applied for analysing relevant impurities of Mesotrione - 1,2 dichloroethane, R-287432 and R-287431 in the PPP.

Reference:	KCP 5.1.1-02
Report	Determination of impurities and mesotrione in mesotrione 10% SC. Micaela Banos Gonzalez, Report: 18-4150-28
Guideline(s):	Yes, SANCO/3030/99 rev.4.
Deviations:	No
GLP:	Yes
Acceptability:	Yes/No/Supplementary

Materials and methods

Mestrione

Mesotrione is determined by reversed phase HPLC using a C18 HPLC column UV detection at 275 nm and external standardisation.

Preparation of test item solutions
Weigh (to the nearest 0.1 mg) 60-70 mg of test item into a 25 ml volumetric flask and up to volume with acetonitrile. Sonicate for 10 min and refrigerate. Filter thorough 0.45 µm filters. Weigh five independent samples of test item.

1,2 dichloroethane
1,2 dichloroethane was determined by gas chromatography using MSD (mass spectrometry detector) and internal standarisation.

Preparation of test item solutions
To homogenize the test item use a magnetic stirrer, add about 20 mL of test item into a beaker with a magnetic stir bar and keep stirring during the preparation of test item solutions.
Weigh (to the nearest 0.1 mg) 500-550 mg of the test item while being continuously stirred into a 10 mL volumetric flask, add 0.10 ml of internal standard Dil-IS. Bring to volume with methanol. Sonicate for 5 min. Filter thorough 0.45 µm filters before analysis.

R-287432
R-287432 is determined by LC using MSD (Triple Quadrupole detector) and internal standardisation.

Preparation of test item solutions
To homogenize test item use a magnetic stirrer, add about 20 mL of test item into a breaker with a magnetic stir bar and keep stirring during the preparation of test item solutions.
Weigh (to the nearest 0.1 mg) 110-140 mg of test item while being continuously stirred into a 10 mL volumetric flask, sonicate and filter thorough 0.45µm filters. Prepare a 1 to 10 dilution into a 10 mL volumetric flask containing 1-2 ml of acetonitrile, add 0.02 ml of internal standard Dil-IS. Up to volume with acetonitrile.

R-287431
R-287431 is determined by LC using MSD (Triple Quadrupole detector) and external standard.

Preparation of test item solutions
To homogenize test item use a magnetic stirrer, add about 20 mL of test item into a breaker with a magnetic stir bar and keep stirring during the preparation of test item solutions.
Weigh (to the nearest 0.1 mg) 980-1020 mg of test item while being continuously stirred into a 10 mL volumetric flask, sonicate and filter thorough 0.45µm filters. Prepare a 1 to 5 dilution into a 5 mL volumetric flask containing 1-2 ml of acetonitrile. Bring to volume with phase A, shake and filter thorough 0.45 µm filters.

Validation - Results and discussions

Table 5.2-2: Methods suitable for the determination of the relevant impurities in plant protection product (PPP) mesotrione 10% SC

	1,2 dichloroethane	**R-287432**	**R-287431**
Author(s), year	Micaela Banos Gonzalez, 2020	Micaela Banos Gonzalez, 2020	Micaela Banos Gonzalez, 2020
Principle of method	GC-MSD	LC-MSD	LC-MSD
Linearity n=6 (linear between mg/L) (correlation coefficient, expressed as r)	Range: 0.1999 – 7.4954 mg/L R = 0.99991	0.0101 – 0.2985 mg/L R = 0.99923	0.0010 – 0.0313 mg/L R = 0.99992
Precision – Repeatability Mean n = 10 (%RSD) (5 samples spiked at LOQ level and 5 samples spiked at 10x LOQ)	4 % (3% and 2%)	3 % (4% and 2%)	9 % (13% and 1%)
Accuracy n = 10 (% Recovery) (5 samples spiked at LOQ level and 5 samples spiked at 10x LOQ)	95 % (98 and 92%)	93 % (94% and 92%)	113 % (112% and 114%
Interference/ Specificity (following chromatograms are	No interference observed.	No interference observed.	No interference observed.

	1,2 dichloroethane	R-287432	R-287431
given – blanc of acetonitrile, reference standard solution, tested sample solution)			
LOQ	10 mg/kg (corresponding to 0.5 mg/l)	20 mg/kg (corresponding to 0.025 mg/l)	0.130 mg/kg (corresponding to 0.00261 mg/l)
Comment	-		

Conclusion

The method of analysis of mesotrione, 1,2 dichloroethane, R-287432, R-287431 in Mesotrione 10% SC has been conveniently validated.

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

Not relevant.

5.2.1.4 Applicability of existing CIPAC methods (KCP 5.1.1)

The CIPAC method No. 625 is available for Mesotrione active substance.

5.2.2 Methods for the determination of residues (KCP 5.1.2)

Please refer to the post-registration method.

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

5.3.1 Analysis of the plant protection product (KCP 5.2)

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

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

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

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

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

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Plant, high water content	Mesotrione	0.01 mg/kg	Reg. (EU) 2017/626
Plant, high acid content		0.01 mg/kg	Reg. (EU) 2017/626
Plant, high protein/high starch content (dry commodities)		0.01 mg/kg	Reg. (EU) 2017/626
Plant, high oil content		0.01 mg/kg	Reg. (EU) 2017/626
Plant, difficult matrices (hops, spices, tea)		0.05 mg/kg	Reg. (EU) 2017/626
Muscle	Mesotrione	0.01 mg/kg	Reg. (EU) 2017/626

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Milk		0.01 mg/kg	Reg. (EU) 2017/626
Eggs		0.01 mg/kg	Reg. (EU) 2017/626
Fat		0.01 mg/kg	Reg. (EU) 2017/626
Liver, kidney		0.01 mg/kg	Reg. (EU) 2017/626
Soil (Ecotoxicology)	Mesotrione and metabolite A (open)	0.05 mg/kg bw/d	Common limit
Drinking water (Human toxicology)	Mesotrione and metabolite A (open)	0.1 µg/L	General limit for drinking water
Surface water (Ecotoxicology)	Mesotrione and metabolite A (open)	7.7 µg/L	Lowest EC_{50} from aquatic toxicity study in EU agreed
Air	Mesotrione	4.5 µg/m^3	$AOEL_{inhalative}$: 0.015 mg/kg bw/d
Tissue (meat or liver)	Mesotrione	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 Mesotrione in plant matrices is given in the following tables.

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

Component of residue definition: Mesotrione				
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 (Maize: forage and silage)	Primary	0.01 mg/kg	LC-MS/MS	EU agreed EFSA journal 2016;14(3):4419
	ILV	0.01 mg/kg	LC-MS/MS	EU agreed EFSA journal 2016;14(3):4419
	Primary	0.01 mg/kg	LC-MS/MS	KCP 5.2.1 M. Rubino, 2018 Report No. 18.629767.0002
	ILV	0.01 mg/kg	LC-MS/MS	KCP 5.2.2 E. Signore, 2019 Report No. RAU-100-18
	Confirmatory (if required)	-	-	LC-MS/MS is highly specific method therefore no confirmatory method is required.
High acid content	Primary	-	-	Not required, the method is highly specific
	ILV	-	-	-
	Confirmatory (if required)	-	-	-
High oil content	Primary	-	-	-

Component of residue definition: Mesotrione				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
(Oilseed rape)	ILV	-	-	-
	Confirmatory (if required)	-	-	-
High protein/high starch content (dry)(Maize: grain)	Primary	0.01 mg/kg	LC-MS/MS	EU agreed EFSA journal 2016;14(3):4419
	ILV	0.01 mg/kg	LC-MS/MS	EU agreed EFSA journal 2016;14(3):4419
	Confirmatory (if required) (Mesotrione)	-	-	Not required, the method is highly specific
	Primary	0.01 mg/kg	LC-MS/MS	KCP 5.2.3 M. Rubino, 2018 Report No. 18.629767.0001
	ILV	0.01 mg/kg	LC-MS/MS	KCP 5.2.2 E. Signore, 2019 Report No. RAU-100-18
	Confirmatory (if required)	-	-	LC-MS/MS is highly specific method therefore no confirmatory method is required.
Difficult (if required, depends on intended use)	Primary	-	-	-
	ILV	-	-	-
	Confirmatory (if required)	-	-	-

* MNBA is not component of residue definition.

Table 5.3-3: Statement on extraction efficiency

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

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

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

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

Component of residue definition: Mesotrione				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Milk	Primary	0.01 mg/kg	LC-MS/MS	EU agreed EFSA journal 2016;14(3):4419
	ILV	0.01 mg/kg	LC-MS/MS	United Kingdom 2016

Component of residue definition: Mesotrione				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
	Confirmatory (if required)	-	-	Not required, the method is highly specific
	Primary	0.01 mg/kg	LC-MS/MS	KCP 5.2.4 M. Rubino, 2018 Report No. 18.629767.0004
	Confirmatory (if required)	-	-	LC-MS/MS is highly specific method therefore no confirmatory method is required.
Eggs	Primary	0.01 mg/kg	LC-MS/MS	EU agreed EFSA journal 2016;14(3):4419
	ILV	0.01 mg/kg	LC-MS/MS	United Kingdom 2016
	Confirmatory (if required)	-	-	Not required, the method is highly specific
	Primary	0.01 mg/kg	LC-MS/MS	KCP 5.2.5 M. Rubino, 2018 Report No. 18.629767.0005
	ILV	0.01 mg/kg	LC-MS/MS	KCP 5.2.9 E. Signore, 2019 Report No. RAU-008-19
	Confirmatory (if required)	-	-	LC-MS/MS is highly specific method therefore no confirmatory method is required.
Muscle	Primary	0.01 mg/kg	LC-MS/MS	EU agreed EFSA journal 2016;14(3):4419
	ILV	-	-	-
	Confirmatory (if required)	-	-	Not required, the method is highly specific
	Primary	0.01 mg/kg	LC-MS/MS	KCP 5.2.6 M. Rubino, 2018 Report No. 18.629767.0008
	ILV	0.01 mg/kg	LC-MS/MS	KCP 5.2.9 E. Signore, 2019 Report No. RAU-008-19
	Confirmatory (if required)	-	-	LC-MS/MS is highly specific method therefore no confirmatory method is required.
Fat	Primary	0.01 mg/kg	LC-MS/MS	EU agreed EFSA journal 2016;14(3):4419
	ILV	-	-	-
	Confirmatory (if required)	-	-	Not required, the method is highly specific
	Primary	0.01 mg/kg	LC-MS/MS	KCP 5.2.7 M. Rubino, 2018 Report No. 18.629767.0006

Component of residue definition: Mesotrione				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
	ILV	0.01 mg/kg	LC-MS/MS	KCP 5.2.9 E. Signore, 2019 Report No. RAU-008-19
	Confirmatory (if required)	-	-	LC-MS/MS is highly specific method therefore no confirmatory method is required.
Kidney, liver	Primary	0.01 mg/kg	LC-MS/MS	EU agreed EFSA journal 2016;14(3):4419
	ILV	0.01 mg/kg	LC-MS/MS	United Kingdom 2016
	Confirmatory (if required)	-	-	Not required, the method is highly specific
	Primary	0.01 mg/kg	LC-MS/MS	KCP 5.2.8 M. Rubino, 2018 Report No. 18.629767.0007
	ILV	0.01 mg/kg	LC-MS/MS	KCP 5.2.9 E. Signore, 2019 Report No. RAU-008-19
	Confirmatory (if required)	-	-	LC-MS/MS is highly specific method therefore no confirmatory method is required.

Table 5.3-5: Statement on extraction efficiency

	Method for products of animal origin
Required, available from:	-
Not required, because:	Not required residues ≥ LOQ are not expected.

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 Mesotrione in soil is given in the following tables.

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

Component of residue definition: Mesotrione (and metabolite MNBA or AMBA)			
Method type	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary (Mesotrione)	0.002 mg/kg	LC-MS/MS	EU agreed EFSA journal 2016;14(3):4419
Confirmatory (Mesotrione)	-	-	Not required, the method is highly specific
Primary (Mesotrione)	0.002 mg/kg	LC-MS/MS	KCP 5.2.10 M. Rubino, 2018 Report No. 18.629767.0009
Confirmatory (Mesotrione)	-	-	LC-MS/MS is highly specific method therefore no confirmatory method is required.

Component of residue definition: Mesotrione (and metabolite MNBA or AMBA)			
Method type	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary (MNBA metabolite)	0.002 mg/kg	LC-MS/MS	EU agreed EFSA journal 2016;14(3):4419
Confirmatory (MNBA metabolite)	-	-	Not required, the method is highly specific
Primary (MNBA metabolite)	0.002 mg/kg	LC-MS/MS	KCP 5.2.11 M. Rubino, 2018 Report No. 18.640093.0001
Confirmatory (MNBA metabolite)	-	-	LC-MS/MS is highly specific method therefore no confirmatory method is required.
Primary (AMBA metabolite)	0.002 mg/kg	LC-MS/MS	EU agreed EFSA journal 2016;14(3):4419
Confirmatory (AMBA metabolite)	-	-	Not required, the method is highly specific
Primary (AMBA metabolite)	0.002 mg/kg	LC-MS/MS	KCP 5.2.12 M. Rubino, 2018 Report No. 18.640093.0002
Confirmatory (AMBA metabolite)	-	-	LC-MS/MS is highly specific method therefore no confirmatory method is required.

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

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

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

Component of residue definition: Mesotrione (and metabolite MNBA or AMBA)				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Drinking water / ground water (Mesotrione)	Primary	0.05 µg/L	LC-MS/MS	EU agreed EFSA journal 2016;14(3):4419
	ILV	0.05 µg/L	LC-MS/MS	EU agreed EFSA journal 2016;14(3):4419
	Confirmatory	-	-	Not required, the method is highly specific
Drinking water / ground water (Mesotrione)	Primary	0.05 µg/L	LC-MS/MS	EU agreed EFSA journal 2016;14(3):4419
	ILV	0.05 µg/L	LC-MS/MS	EU agreed EFSA journal 2016;14(3):4419
	Confirmatory	-	-	Not required, the method is highly specific

Component of residue definition: Mesotrione (and metabolite MNBA or AMBA)				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Drinking water / ground water (Mesotrione)	Primary	0.05 µg/L	LC-MS/MS	EU agreed EFSA journal 2016;14(3):4419
	ILV	0.05 µg/L	LC-MS/MS	EU agreed EFSA journal 2016;14(3):4419
	Confirmatory	-	-	Not required, the method is highly specific
	Primary	0.05 µg/L	LC-MS/MS	KCP 5.2.13 M. Rubino, 2018 Report No. 18.629767.0015
	ILV	0.05 µg/L	LC-MS/MS	KCP 5.2.19 Z. Hordyjewicz-Baran, 2019 Report No. 163/2019
	Confirmatory	-	-	LC-MS/MS is highly specific method therefore no confirmatory method is required.
Ground water (MNBA metabolite)	Primary	0.05 µg/L	LC-MS/MS	KCP 5.2.15 M. Rubino, 2018 Report No. 18.629767.0017
	ILV	0.05 µg/L	LC-MS/MS	KCP 5.2.20 E. Signore, 2019 Report No. RAU-007-19
	Confirmatory	-	-	LC-MS/MS is highly specific method therefore no confirmatory method is required.
Ground water (AMBA metabolite)	Primary	0.05 µg/L	LC-MS/MS	KCP 5.2.16 M. Rubino, 2018 Report No. 18.629767.0016
	ILV	0.05 µg/L	LC-MS/MS	KCP 5.2.20 E. Signore, 2019 Report No. RAU-007-19
	Confirmatory	-	-	LC-MS/MS is highly specific method therefore no confirmatory method is required.
Surface water (Mesotrione)	Primary	0.05 µg/L	LC-MS/MS	EU agreed EFSA journal 2016;14(3):4419
	Confirmatory	-	-	Not required, the method is highly specific
	Primary	0.05 µg/L	LC-MS/MS	KCP 5.2.14 M. Rubino, 2018 Report No. 18.629767.0012
	Confirmatory	-	-	LC-MS/MS is highly specific method therefore no confirmatory method is required.

Component of residue definition: Mesotrione (and metabolite MNBA or AMBA)				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Surface water (MNBA metabolite)	Primary	0.05 µg/L	LC-MS/MS	EU agreed EFSA journal 2016;14(3):4419
	Confirmatory	-	-	Not required, the method is highly specific
	Primary	0.05 µg/L	LC-MS/MS	KCP 5.2.17 M. Rubino, 2018 Report No. 18.629767.0014
	Confirmatory	-	-	LC-MS/MS is highly specific method therefore no confirmatory method is required.
Surface water (AMBA metabolite)	Primary	0.05 µg/L	LC-MS/MS	EU agreed EFSA journal 2016;14(3):4419
	Confirmatory	-	-	Not required, the method is highly specific
	Primary	0.05 µg/L	LC-MS/MS	KCP 5.2.18 M. Rubino, 2018 Report No. 18.629767.0013
	Confirmatory	-	-	LC-MS/MS is highly specific method therefore no confirmatory method is required.

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

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

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

Component of residue definition: Mesotrione			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	0.45 µg/m^3 air	LC-MS/MS	EU agreed EFSA journal 2016;14(3):4419
Confirmatory	-	-	Not required, the method is highly specific
Primary	0.45 µg/m^3 air	LC-MS/MS	KCP 5.2.21 M. Rubino, 2018 Report No. 18.629767.0018
Confirmatory	-	-	LC-MS/MS is highly specific method therefore no confirmatory method is required.

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

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

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

Component of residue definition: Mesotrione			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	0.01 mg/kg / in blood	LC-MS/MS	EU agreed EFSA journal 2016;14(3):4419
Confirmatory	-	-	-
Primary	0.01 mg/kg / in blood	LC-MS/MS	KCP 5.2.22 M. Rubino, 2018 Report No. 18.629767.0003
Confirmatory	-	-	LC-MS/MS is highly specific method therefore no confirmatory method is required.

5.3.2.8 Other studies/ information

Not relevant, not required.

Appendix 1 Lists of data considered in support of the evaluation

List of data submitted by the applicant and relied on

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Vertebrate study Y/N**	Owner
KCP 5.1.1	Mónica Berrios	2016	PHYSICAL AND CHEMICAL PROPERTIES AND ACCELERATED STORAGE STABILITY TEST FOR MESOTRIONE 10% SC (SUSPENSION CONCENTRATE, 10.2% W/W MESOTRIONE) - SPAIN 2015- Labs & Technological Services AGQ, S.L., study number GLP/Unpublished	N	Sharda Cropchem Ltd.
KCP 5.1.1-02	Micaela Banos Gonzalez	2020	Determination of impurities and mesotrione in mesotrione 10% SC. Laboratorios Munuera Report: 18-4150-28 GLP Unpublished	N	Sharda Cropchem Ltd.
KCP 5.2.1	M. Rubino	2018	Validation of the analytical procedure for the determination of mesotrione (CAS: 104206-82-8) in forage by liquid chromatography. Chelab Report No. 18.629767.0002 GLP Unpublished	N	Sharda Cropchem Limited
KCP 5.2.2	M. Rubino	2018	Validation of the analytical procedure for the determination of mesotrione (CAS: 104206-82-8) in grain (maize) by liquid chromatography. Chelab Report No. 18.629767.0001 GLP Unpublished	N	Sharda Cropchem Limited
KCP 5.2.3	E. Signore	2019	Independent Laboratory Validation of analytical method for the determination of Mesotrione in Maize forage and grain validated in a study conducted by Chelab. Biospheres Report No. RAU-100-18 GLP	N	Sharda Cropchem Limited

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			Unpublished		
KCP 5.2.4	M. Rubino	2018	Validation of the analytical procedure for the determination of mesotrione (CAS: 104206-82-8) in milk by liquid chromatography. Chelab Report No. 18.629767.0004 GLP Unpublished	N	Sharda Cropchem Limited
KCP 5.2.5	M. Rubino	2018	Validation of the analytical procedure for the determination of mesotrione (CAS: 104206-82-8) in eggs by liquid chromatography. Chelab Report No. 18.629767.0005 GLP Unpublished	N	Sharda Cropchem Limited
KCP 5.2.6	M. Rubino	2018	Validation of the analytical procedure for the determination of mesotrione (CAS: 104206-82-8) in meat by liquid chromatography. Chelab Report No. 18.629767.0008 GLP Unpublished	N	Sharda Cropchem Limited
KCP 5.2.7	M. Rubino	2018	Validation of the analytical procedure for the determination of mesotrione (CAS: 104206-82-8) in fat by liquid chromatography. Chelab Report No. 18.629767.0006 GLP Unpublished	N	Sharda Cropchem Limited
KCP 5.2.8	M. Rubino	2018	Validation of the analytical procedure for the determination of mesotrione (CAS: 104206-82-8) in kidney by liquid chromatography. Chelab Report No. 18.629767.0007 GLP	N	Sharda Cropchem Limited

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			Unpublished		
KCP 5.2.9	E. Signore	2019	Independent Laboratory Validation of analytical methods for the determination of Mesotrione in animal matrices (eggs, fat, kidney and meat) validated in Studies conducted by Chelab. BioSpheres Report No. RAU 008-19 GLP Unpublished	N	Sharda Cropchem Limited
KCP 5.2.10	M. Rubino	2018	Validation of the analytical procedure for the determination of mesotrione (CAS: 104206-82-8) in soil by liquid chromatography. Chelab Report No. 18.629767.0009 GLP Unpublished	N	Sharda Cropchem Limited
KCP 5.2.11	M. Rubino	2018	Validation of the analytical procedure for the determination of MNBA (CAS: 110964-79-9) in soil by liquid chromatography. Chelab Report No. 18.640093.0001 GLP Unpublished	N	Sharda Cropchem Limited
KCP 5.2.12	M. Rubino	2018	Validation of the analytical procedure for the determination of AMBA (CAS: 393085-45-5) in soil by liquid chromatography. Chelab Report No. 18.640093.0002 GLP Unpublished	N	Sharda Cropchem Limited
KCP 5.2.13	M. Rubino	2018	Validation of the analytical procedure for the determination of mesotrione (CAS: 104206-82-8) in ground waterby liquid chromatography. Chelab Report No. 18.629767.0015 GLP	N	Sharda Cropchem Limited

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			Unpublished		
KCP 5.2.14	M. Rubino	2018	Validation of the analytical procedure for the determination of mesotrione (CAS: 104206-82-8) in surface water by liquid chromatography. Chelab Report No. 18.629767.0012 GLP Unpublished	N	Sharda Cropchem Limited
KCP 5.2.15	M. Rubino	2018	Validation of the analytical procedure for the determination of MNBA (CAS: 110964-79-9) in ground water by liquid chromatography. Chelab Report No. 18.629767.0017 GLP Unpublished	N	Sharda Cropchem Limited
KCP 5.2.16	M. Rubino	2018	Validation of the analytical procedure for the determination of AMBA (CAS: 393085-45-5) in ground water by liquid chromatography. Chelab Report No. 18.629767.0016 GLP Unpublished	N	Sharda Cropchem Limited
KCP 5.2.17	M. Rubino	2018	Validation of the analytical procedure for the determination of MNBA (CAS: 110964-79-9) in surface water by liquid chromatography. Chelab Report No. 18.629767.0014 GLP Unpublished	N	Sharda Cropchem Limited
KCP 5.2.18	M. Rubino	2018	Validation of the analytical procedure for the determination of AMBA (CAS: 393085-45-5) in surface water by liquid chromatography. Chelab Report No. 18.629767.0013 GLP	N	Sharda Cropchem Limited

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Vertebrate study** **Y/N**	**Owner**
			Unpublished		
KCP 5.2.19	Z. Hordyjewicz-Baran	2019	Independent Laboratory Validation of the analytical procedure for the determination of residues of Mesotrione (CAS 104206-82-8) in drinking water by Liquid Chromatography. Institute of Heavy Organic Synthesis Report No. 163/2019 GLP Unpublished	N	Sharda Cropchem Limited
KCP 5.2.20	E. Signore	2019	Independent Laboratory Validation of analytical method for the determination of Mesotrione metabolites (AMBA and MNBA) in drinking water validation a Study conducted by Chelab. Biospheres Report No. RAU-007-19 GLP Unpublished	N	Sharda Cropchem Limited
KCP 5.2.21	M. Rubino	2018	Validation of the analytical procedure for the determination of mesotrione (CAS: 104206-82-8) in air by LC-MS. Chelab Report No. 18.629767.0018 GLP Unpublished	N	Sharda Cropchem Limited
KCP 5.2.22	M. Rubino		Validation of the analytical procedure for the determination of mesotrione (CAS: 104206-82-8) in blood by liquid chromatography Chelab Report No. 18.629767.0003 GLP Unpublished	N	Sharda Cropchem Limited

Appendix 2 Detailed evaluation of submitted analytical methods

A 2.1 Analytical methods for Mesotrione

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

No new or additional studies have beed 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)

zRMS comments:
Residue definition for monitoring purposes for food of plant origin according to the EFSA Journal 2016;14(3):4419 and Reg. (EU) 2017/626 is mesotrione so analytical methods should include determination of this compound.

A 2.1.2.1.1 Analytical method 1

A 2.1.2.1.1.1 Method validation

Comments of zRMS:	The analytical method codified as SOPa-393-LABCHI-Rev.0 was fully validated in term of specificity, linearity, repeatability, accuracy and LOQ according to SANCO 3029/99 rev. 4.The validation was performed quantifying mesotrione in forage by LC-MS. Two SRM transitions were monitored: 338 m/z - 291 m/z (quantifier ion) and 338 m/z – 212 m/z (qualifier ion). The method linearity was evaluated at 5 levels, ranging from 30%LOQ (0.003 mg/kg) to 30xLOQ (0.3 mg/kg). Recovery analysis was performed for samples spiked with mesotrione at LOQ (0.01 mg/kg) and 10xLOQ (0.1 mg/kg), 5 replicate analysis were performed foe each level. Mean recovery is between 70%-110% with RSD <20% in all cases, in accordance with acceptance criteria. Based on recovery results it is possible to asses that spiked sample extracts show good stability over analysis time. The validation data demonstrate that the method is suitable to qualitatively and quantitatively determine mesotrione in forage. The method is accepted.

Reference:	KCP 5.2.1
Report	Validation of the analytical procedure for the determination of mesotrione (CAS: 104206-82-8) in forage by liquid chromatography. M. Rubino, 2018, Report No. 18.629767.0002
Guideline(s):	SANCO 3029/99 rev. 4
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Sample extraction:
About 5.00 g of grinded forage were weighed into a 50 ml falcon. 10 ml of extraction mixture were added to the sample. Vortexed for about 30 min in a mechanical stirrer, then about 4 g of magnesium sulphate anhydrous, 1 g of sodium chloride, 1 g of sodium citrate dehydrate and 0.5 g of disodium hydrogencitrate sesquidrate were added to the sample and vortexed with each addition for about 1 min. The tube was centrifuged at 4750 rpm for 5 min. The supernatant was transferred in a plastic tube and kept at about -20°C for abour 1 hour. Then, the tube was centrifuged at 3000 rpm for 5 min and it was proceed to

purification of the supernatant. 4 ml of supernatant were transferred into a 10 ml plastic tube, containing about 900 mg of magnesium sulphate anhydrous and 150 mg of C18 resin. Vortexed for about 1 min and centrifuged at 3000 rpm for 5 min. 0.5 ml of supernatant were transferred in 10 ml tube and were added 0.5 ml of mobile phase A. Vortexed for about 1 min, then transferred into an HPLC vial and injected. The sample was prepared in duplicate.

Results and discussions

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

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Mean recovery (%) (individual recovery values)	RSD (%)	Comments
Maize forage	Mesotrione	LOQ (0.01 mg/kg)	87 (89, 84, 86, 85, 91)	3.4	First mass transition
			84 (85, 80, 82, 91, 85)	4.9	Second mass transition
		10xLOQ (0.1 mg/kg)	95 (94, 95, 100, 91, 93)	3.4	First mass transition
			96 (97, 94, 98, 95, 97)	1.5	Second mass transition

Table A 2: Characteristics for the analytical method used for validation of mesotrione residues in maize forage

	Mesotrione
Specificity	The method is specific. No significant peaks (≤30% LOQ) are detected at RT of the target analyte in the Blank and Test Solution with respect to the Spiked Test Solution for both mass transitions.
Calibration (type, number of data points)	5 points 0.003 mg/kg to 0.3 mg/kg First mass transition y=599354x R^2 = 0.9946 Second mass transition y=144833x R^2 = 0.9963
Assessment of matrix effects is presented	yes
Limit of determination/quantification	LOQ = 0.01 mg/kg LOD = 0.003 mg/kg

Conclusion

The method was successfully validated and is suitable for determination of mesotrione in forage.

A 2.1.2.1.1.2 Independent laboratory validation

Comments of zRMS:	The study is an ILV of the analytical method for determination of mesotrione in maize forage and grain (study numbers 18.629767.0001 for grain and 18.629767.0002 for forage). Linearity, selectivity, specificity, accuracy (as recoveries), precision (as repeatability), limit of quantification (LOQ) and limit of detection (LOD) have been determined according to SANCO/825/00 rev.8.1 and

	SANCO/3029/99, rev. 4 (11/07/2000) guidance documents. The analysis was performed using HPLC coupled with triple quadrupole mass spectrometer (HPLC-MS/MS). Qualifier fragment ions was detected: -ion 291 (m/z) for quantification and qualifier ion 212 (m/z) for confirmation from precursor 338 (m/z) for forage. - ion 291 (m/z) for quantification and qualifier ion 249 (m/z) for confirmation from precursor 338 (m/z) for grain. The limit of quantification (LOQ) was 0.01 mg/kg for both matrices (with LOD 0.003 mg/kg). In order to demonstrate the validity of the analytical method, linearity was evaluated in each analytical sequence; selectivity, accuracy, precision, specificity, limit of quantification and limit of detection were evaluated on the following samples for both maize forage and grain:2 untreated samples, 5 untreated samples fortified at 0.01 mg/kg (LOQ), 5 untreated sample fortified at 0.10 mg/kg (10 x LOQ). Acceptability range for mean recovery: 70%-110% with RSD < 20%. The validation data demonstrate that the method is suitable to qualitatively and quantitatively determine mesotrione in maize forage and grain. The study is accepted.

Reference:	KCP ~~5.2.2~~ 5.2.3
Report	Independent Laboratory Validation of analytical method for the determination of Mesotrione in Maize forage and grain validated in a Study conducted by Chelab. E. Signore, 2019, Report No. RAU-100-18
Guideline(s):	SANCO 3029/99 rev. 4
Deviations:	No
GLP:	Yes
Acceptability:	Yes

About 5 g (± 0.1 g) of the grinded forage were weighed in a 50 mL plastic falcon (0.5 mL of SS- 10LOQ and 0.5 mL SS-LOQ were added) and 5 mL of milliQ water will be added to hydrate the matrix and after 10 mL of extra tion mixture were added (9.5 mL for the recoveries, considering 0.5 mL of spiking volume). After mechanical shaking the sample for 30 minutes, 4g of magnesium sulphate anhydrous, 1g of sodium chloride, 0.5g of sodium citrate dibasic sesquihydrate and 1g of sodium citrate tribasic dehydrate will be added to the mixture and the tube will be further shaken for 1 minute and centrifuged at 4750 RPM for 5 minutes. Before the purification the supernant will be trasferred in 10 mL centrifuge tube and kept at approximatively -20°C for 1 hour and and centrifuged at 3000 RPM for 5 minutes. 4 mL of the supernatant will be purified in a 10 mL plastic tube containing 900 mg of magnesium sulphate anhydrous and 150 mg of C18 resin by vortexing for 1 minute and centrifuging at 3000 RPM for 5 minutes. 0.5 mL of the supernatant will be transferred into 10 mL tube and will be added of 0.5 mL of mobile phase A. After vortexing for 1 minute finally the sample is transferred in a vial and analysed by HPLC-MS.

Results and discussions

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

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Mean recovery (%) (individual recovery values)	RSD (%)	Comments
Maize forage	Mesotrione	LOQ (0.01 mg/kg)	107.90 (109.65, 107.35, 107.88, 108.99, 105.65)	1.44	First mass transition (m/z 338 → 291)
			~~97.72~~ 110.51 (112.44, 111.03, 110.41, 110.41, 108.28)	~~2.68~~ 1.36	Second mass transition (m/z 338 → 212)

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Mean recovery (%) (individual recovery values)	RSD (%)	Comments
		10xLOQ (0.1 mg/kg)	~~110.51~~ 97.72 (100.27, 97.96, 100.25, 94.63, 95.48)	~~1.36~~ 2.68	First mass transition (m/z 338 → 291)
			98.94 (102.41, 98.91, 102.07, 94.26, 97.07)	3.47	Second mass transition (m/z 338 → 212)
Maize grain	Mesotrione	LOQ (0.01 mg/kg)	75.89 (74.61, 74.16, 76.13, 77.64, 76.92)	1.96	First mass transition (m/z 338 → 291)
			~~80.63~~ 75.21 (72.06, 75.33, 77.55, 80.57, 70.55)	~~3.88~~ 5.39	Second mass transition (m/z 338 → 249)
		10xLOQ (0.1 mg/kg)	~~75.21~~ 80.63 (80.43, 76.23, 80.40, 81.04, 85.04)	~~5.39~~ 3.88	First mass transition (m/z 338 → 291)
			79.87 (79.13, 75.7, 80.17, 79.52, 84.84)	4.10	Second mass transition (m/z 338 → 249)

Table A 4: Characteristics for the analytical method used for validation of mesotrione residues in maize forage and grain

	Mesotrione
Specificity	The method is specific. No significant peaks (≤30% LOQ) are detected at RT of the target analyte in the Blank and Test Solution with respect to the Spiked Test Solution for both mass transitions.
Calibration (type, number of data points)	5 points 0.0028 mg/kg to 0.32 mg/kg for maize forage 0.00283 mg/kg to 0.309 mg/kg for maize grain R > 0.99
Assessment of matrix effects is presented	yes
Limit of determination/quantification	LOQ = 0.01 mg/kg LOD = 0.003 mg/kg

Conclusion

The method was successfully validated and is suitable for determination of mesotrione in maize forage and grain.

A 2.1.2.1.1.3 Confirmatory method

LC-MS/MS is highly specific method therefore no confirmatory method is required.

A 2.1.2.1.1.4 Extraction efficiency

No new or additional studies have been submitted.

A 2.1.2.1.2 Analytical method 2

A 2.1.2.1.2.1 Method validation

Comments of zRMS:	The analytical method codified as SOPa-392-LABCHI-Rev.0 was fully validated in term of specificity, linearity, repeatability, accuracy and LOQ according to SANCO 3029/99 rev. 4 and SANCO/825/00 Rev. 8.1. The validation was performed quantifying mesotrione in maize grain by LC-MS. Two SRM transitions were monitored: 338 m/z - 291 m/z (quantifier ion) and 338 m/z – 249

	m/z (qualifier ion). The method linearity was evaluated at 5 levels, ranging from 30%LOQ (0.003 mg/kg) to 30xLOQ (0.3 mg/kg). Recovery analysis was performed for samples spiked with mesotrione at LOQ (0.01 mg/kg) and 10xLOQ (0.1 mg/kg), 5 replicate analysis were performed foe each level. Mean recovery is between 70%-110% with RSD <20% in all cases, in accordance with acceptance criteria. Based on recovery results it is possible to asses that spiked sample extracts show good stability over analysis time. The validation data demonstrate that the method is suitable to qualitatively and quantitatively determine mesotrione in maize grain. The method is accepted.

Reference:	KCP ~~5.2.3~~ 5.2.2
Report	Validation of the analytical procedure for the determination of mesotrione (CAS: 104206-82-8) in grain (maize) by liquid chromatography. M. Rubino, 2018, Report No. 18.629767.0001
Guideline(s):	SANCO 3029/99 rev. 4
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Sample extraction:
About 7.00 g of grinded maize were weighed into a 50 ml falcon. 5 ml of milliQ water were added in order to hydrate the matrix. 9 ml of extraction mixture were added to the sample. After vortexing for about 30 min in a mechanical stirrer, about 4 g of magnesium sulphate anhydrous, 1 g of sodium chloride, 1 g of sodium citrate dehydrate and 0.5 g of sodium hydrogencitrate sesqidrate were added to the samaple and vortexed with each addition for about 1 min. The tube was centrifuged at 4750 rpm for 5 min. The supernatant was transferred in a plastic tube and kept at about -20°C for about 1 hour. Then, the tube was crntrifuged at 3000 rpm for 5 min and it was proceeded to purification of the supernatant. 4 ml of supernatant were transferred into a 10 ml plastic tube, containing about 900 mg of magnesium sulphate anhydrous and 150 mg of C18 resin. Vortexed for about 1 min and centrifuged at 3000 rpm for 5 min. 0.5 ml of supernatant were transferred in 10 ml tube and were added 0.5 ml of mobile phase A. Vortexed for about 1min, then transferred into an HPLC vial and injected. The sample was prepared in duplicate.

Results and discussions

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

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Mean recovery (%) (individual recovery values)	RSD (%)	Comments
Maize grain	Mesotrione	LOQ (0.01 mg/kg)	~~98~~ 99 (100, 85, 102, 104, 106)	8.0	First mass transition
			~~98~~ 103 (99, 97, 109, 87, 125)	9.0	Second mass transition
		10xLOQ (0.1 mg/kg)	96.0 (94, 92, 94, 95, 104)	5.0	First mass transition
			97.0 (94, 93, 95, 96, 105)	5.0	Second mass transition

Table A 6: Characteristics for the analytical method used for validation of mesotrione residues in maize grain

	Mesotrione
Specificity	The method is specific. No significant peaks (≤30% LOQ) are detected at RT of the target analyte in the Blank and Test Solution with respect to the Spiked Test Solution for both mass transitions.
Calibration (type, number of data points)	5 points 0.003 mg/kg to 0.3 mg/kg First mass transition y=766206x R^2 = 0.9997 Second mass transition y=44600x R^2 = 0.9992
Assessment of matrix effects is presented	yes
Limit of determination/quantification	LOQ = 0.01 mg/kg LOD = 0.003 mg/kg

Conclusion

The method was successfully validated and is suitable for determination of mesotrione in maize grain.

A 2.1.2.1.2.2 Independent laboratory validation

Please refer to A 2.1.2.1.1.2.

A 2.1.2.1.2.3 Confirmatory method

LC-MS/MS is highly specific method therefore no confirmatory method is required.

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

zRMS comments:
Residue definition for monitoring purposes for food of animal origin according to the EFSA Journal 2016;14(3):4419 is not required (provisional, *residue monitoring method for food of animal origin is not required as no MRLs were set, however mesotrione can be determined in food and feed of animal origin by the QuEChERS method (LC-MS/MS) with LOQs of 0.01 mg/kg in all animal matrices*), according to the Reg. (EU) 2017/626 it is mesotrione so analytical methods (if available) should include determination of this compound.

A 2.1.2.2.1 Analytical method 1

A 2.1.2.2.1.1 Method validation

Comments of zRMS:	The analytical method was fully validated in term of specificity, linearity, repeatability, accuracy and LOQ according to SANCO 3029/99 rev. 4 and SANCO/825/00 Rev. 8.1. The validation was performed quantifying mesotrione in milk by LC-MS. LOQ corresponds to 0.01 mg/kg. Two SRM transitions were monitored: 338 m/z - 291 m/z (quantifier ion) and 338 m/z – 212 m/z (qualifier ion). The method linearity was evaluated at 5 levels, ranging from 30%LOQ (0.003 mg/kg) to 30xLOQ (0.3 mg/kg). Recovery analysis was performed for samples spiked with mesotrione at LOQ (0.01 mg/kg) and 10xLOQ (0.1 mg/kg), 5 replicate analysis were performed foe each level. Mean recovery is between 70%-110% with RSD <20% in all cases, in accordance with acceptance criteria. Based

	on recovery results it is possible to asses that spiked sample extracts show good stability over analysis time. The validation data demonstrate that the method is suitable to qualitatively and quantitatively determine mesotrione in milk. The method is successfully validated and accepted.

Reference:	KCP 5.2.4
Report	Validation of the analytical procedure for the determination of mesotrione (CAS: 104206-82-8) in milk by liquid chromatography. M. Rubino, 2018, Report No. 18.629767.0004
Guideline(s):	SANCO 3029/99 rev. 4
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Sample extraction:
About 5.00 g of milk were weighed into a 50 ml falcon. 10 ml of extraction mixture were added to the sample. Vortexed for about 30 min in a mechanical stirrer, then about 4 g of magnesium sulphate anhydrous, 1 g of sodium chloride, 1 g of sodium citrate dehydrate and 0.5 g of disodium hydrogencitrate sesquidrate were added to the sample and vortexed with each addition for about 1 min. The tube was centrifuged at 4750 rpm for 5 min. The supernatant was transferred in a plastic tube and kept at about -20°C for abour 1 hour. Then, the tube was centrifuged at 4750 rpm for 5 min and it was proceed to purification of the supernatant. 4 ml of supernatant were transferred into a 10 ml plastic tube, containing about 900 mg of magnesium sulphate anhydrous and 150 mg of C18 resin. Vortexed for about 1 min and centrifuged at 4750 rpm for 5 min. 0.5 ml of supernatant were transferred in 10 ml tube and were added 0.5 ml of mobile phase A. Vortexed for about 1 min, then transferred into an HPLC vial and injected. The sample was prepared in duplicate.

Results and discussions

Table A 7: Recovery results from method validation of mesotrione using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Mean recovery (%) (individual recovery values)	RSD (%)	Comments
Milk	Mesotrione	LOQ (0.01 mg/kg)	94 (97, 94, 92, 94, 94)	1.8	First mass transition
			95 (102, 90, 98, 97, 92)	5.0	Second mass transition
		10xLOQ (0.1 mg/kg)	96 (94, 97, 96, 99, 97)	1.6	First mass transition
			97 (93, 95, 96, 99, 100)	2.7	Second mass transition

Table A 8: Characteristics for the analytical method used for validation of mesotrione residues in milk

	Mesotrione
Specificity	The method is specific. No significant peaks (≤30% LOQ) are detected at RT of the target analyte in the Blank and Test Solution with respect to the Spiked Test Solution for both mass transitions.
Calibration (type, number of data points)	5 points 0.003 mg/kg to 0.3 mg/kg First mass transition y=828068x R^2 = 0.9963 Second mass transition y=200084x R^2 = 0.9962
Assessment of matrix effects is presented	yes
Limit of determination/quantification	LOQ = 0.01 mg/kg LOD = 0.003 mg/kg

Conclusion

The method was successfully validated and is suitable for determination of mesotrione in milk.

A 2.1.2.2.1.2 Independent laboratory validation.

No new or additional studies have been submitted.

A 2.1.2.2.1.3 Confirmatory method

LC-MS/MS is highly specific method therefore no confirmatory method is required.

A 2.1.2.2.1.4 Extraction efficiency

No new or additional studies have been submitted.

A 2.1.2.2.2 Analytical method 2

A 2.1.2.2.2.1 Method validation

Comments of zRMS:	The analytical method was fully validated in term of specificity, linearity, repeatability, accuracy and LOQ according to SANCO 3029/99 rev. 4 and SANCO/825/00 Rev. 8.1. The validation was performed quantifying mesotrione in eggs by LC-MS. LOQ corresponds to 0.01 mg/kg. Two SRM transitions were monitored: 338 m/z - 291 m/z (quantifier ion) and 338 m/z – 212 m/z (qualifier ion). The method linearity was evaluated at 5 levels, ranging from 30%LOQ (0.003 mg/kg, LOD) to 30xLOQ (0.3 mg/kg). Recovery analysis was performed for samples spiked with mesotrione at LOQ (0.01 mg/kg) and 10xLOQ (0.1 mg/kg), 5 replicate analysis were performed foe each level. Mean recovery is between 70%-110% with RSD <20% in all cases, in accordance with acceptance criteria. Based on recovery results it is possible to asses that spiked sample extracts show good stability over analysis time. The validation data demonstrate that the method is suitable to qualitatively and quantitatively determine mesotrione in eggs specimens. The method is successfully validated and accepted.

Reference: KCP 5.2.5

Report	Validation of the analytical procedure for the determination of mesotrione (CAS: 104206-82-8) in eggs by liquid chromatography. M. Rubino, 2018, Report No. 18.629767.0005
Guideline(s):	SANCO 3029/99 rev. 4
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Sample extraction:
About 5.00 g of eggs were weighed into a 50 ml falcon. 10 ml of extraction mixture were added to the sample. Vortexed for about 30 min in a mechanical stirrer, then about 4 g of magnesium sulphate anhydrous, 1 g of sodium chloride, 1 g of sodium citrate dehydrate and 0.5 g of disodium hydrogencitrate sesquidrate were added to the sample and vortexed with each addition for about 1 min. The tube was centrifuged at 4750 rpm for 5 min. The supernatant was transferred in a plastic tube and kept at about -20°C for abour 1 hour. Then, the tube was centrifuged at 4750 rpm for 5 min and it was proceed to purification of the supernatant. 4 ml of supernatant were transferred into a 10 ml plastic tube, containing about 900 mg of magnesium sulphate anhydrous and 150 mg of C18 resin. Vortexed for about 1 min and centrifuged at 4750 rpm for 5 min. 0.5 ml of supernatant were transferred in 10 ml tube and were added 0.5 ml of mobile phase A. Vortexed for about 1 min, then transferred into an HPLC vial and injected. The sample was prepared in duplicate.

Results and discussions

Table A 9: Recovery results from method validation of mesotrione using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Mean recovery (%) (individual recovery values)	RSD (%)	Comments
Eggs	Mesotrione	LOQ (0.01 mg/kg)	90 (92, 91, 91, 92, 87)	2.4	First mass transition
			89 (90, 87 84, 94, 93)	4.7	Second mass transition
		10xLOQ (0.1 mg/kg)	84 (81, 86, 87, 85, 82)	3.0	First mass transition
			85 (79, 90, 87, 87, 82)	5.1	Second mass transition

Table A 10: Characteristics for the analytical method used for validation of mesotrione residues in eggs

	Mesotrione
Specificity	The method is specific. No significant peaks (≤30% LOQ) are detected at RT of the target analyte in the Blank and Test Solution with respect to the Spiked Test Solution for both mass transitions.
Calibration (type, number of data points)	5 points 0.003 mg/kg to 0.3 mg/kg First mass transition y=875746x R^2 = 0.9962

	Mesotrione
Specificity	The method is specific. No significant peaks (≤30% LOQ) are detected at RT of the target analyte in the Blank and Test Solution with respect to the Spiked Test Solution for both mass transitions.
	Second mass transition y=207986x R^2 = 0.9944
Assessment of matrix effects is presented	yes
Limit of determination/quantification	LOQ = 0.01 mg/kg LOD = 0.003 mg/kg

Conclusion

The method was successfully validated and is suitable for determination of mesotrione in eggs.

A 2.1.2.2.2.2 Independent laboratory validation.

Please refer to A 2.1.2.2.5.2.

A 2.1.2.2.2.3 Confirmatory method

LC-MS/MS is highly specific method therefore no confirmatory method is required.

A 2.1.2.2.3 Analytical method 3

A 2.1.2.2.3.1 Method validation

Comments of zRMS:	The analytical method was fully validated in term of specificity, linearity, repeatability, accuracy and LOQ according to SANCO 3029/99 rev. 4 and SANCO/825/00 Rev. 8.1. The validation was performed quantifying mesotrione in meat by LC-MS. LOQ corresponds to 0.01 mg/kg. Two SRM transitions were monitored: 338 m/z - 291 m/z (quantifier ion) and 338 m/z – 212 m/z (qualifier ion). The method linearity was evaluated at 5 levels, ranging from 30%LOQ (0.003 mg/kg, LOD) to 30xLOQ (0.3 mg/kg). Recovery analysis was performed for samples spiked with mesotrione at LOQ (0.01 mg/kg) and 10xLOQ (0.1 mg/kg), 5 replicate analysis were performed for each level. Mean recovery is between 70%-110% with RSD <20% in all cases, in accordance with acceptance criteria. Based on recovery results it is possible to asses that spiked sample extracts show good stability over analysis time. The validation data demonstrate that the method is suitable to qualitatively and quantitatively determine mesotrione in meat specimens. The method is successfully validated and accepted.

Reference:	KCP 5.2.6
Report	Validation of the analytical procedure for the determination of mesotrione (CAS: 104206-82-8) in meat by liquid chromatography. M. Rubino, 2018, Report No. 18.629767.0008
Guideline(s):	SANCO 3029/99 rev. 4
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Sample extraction:
About 5.00 g of meat were weighed into a 50 ml falcon. 10 ml of extraction mixture were added to the sample. Vortexed for about 30 min in a mechanical stirrer, then about 4 g of magnesium sulphate anhydrous, 1 g of sodium chloride, 1 g of sodium citrate dehydrate and 0.5 g of disodium hydrogencitrate sesquidrate were added to the sample and vortexed with each addition for about 1 min. The tube was centrifuged at 4750 rpm for 5 min. The supernatant was transferred in a plastic tube and kept at about -20°C for abour 1 hour. Then, the tube was centrifuged at 4750 rpm for 5 min and it was proceed to purification of the supernatant. 4 ml of supernatant were transferred into a 10 ml plastic tube, containing about 900 mg of magnesium sulphate anhydrous and 150 mg of C18 resin. Vortexed for about 1 min and centrifuged at 4750 rpm for 5 min. 0.5 ml of supernatant were transferred in 10 ml tube and were added 0.5 ml of mobile phase A. Vortexed for about 1 min, then transferred into an HPLC vial and injected. The sample was prepared in duplicate.

Results and discussions

Table A 11: Recovery results from method validation of mesotrione using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Mean recovery (%) (individual recovery values)	RSD (%)	Comments
Meat	Mesotrione	LOQ (0.01 mg/kg)	90 (92, 84, 92, 86, 94)	5.2	First mass transition
			93 (95, 87, 96, 93, 95)	3.7	Second mass transition
		10xLOQ (0.1 mg/kg)	91 (91, 95, 89, 90, 90)	2.6	First mass transition
			97 (98, 101, 95, 94, 96)	2.7	Second mass transition

Table A 12: Characteristics for the analytical method used for validation of mesotrione residues in meat

	Mesotrione
Specificity	The method is specific. No significant peaks (≤30% LOQ) are detected at RT of the target analyte in the Blank and Test Solution with respect to the Spiked Test Solution for both mass transitions.
Calibration (type, number of data points)	5 points 0.003 mg/kg to 0.3 mg/kg First mass transition y=804636x R^2 = 0.9991 Second mass transition y=181961x R^2 = 0.9961
Assessment of matrix effects is presented	yes
Limit of determination/quantification	LOQ = 0.01 mg/kg LOD = 0.003 mg/kg

Conclusion

The method was successfully validated and is suitable for determination of mesotrione in muscle.

A 2.1.2.2.3.2 Independent laboratory validation.

Please refer to A 2.1.2.2.5.2.

A 2.1.2.2.3.3 Confirmatory method

LC-MS/MS is highly specific method therefore no confirmatory method is required.

A 2.1.2.2.4 Analytical method 4

A 2.1.2.2.4.1 Method validation

Comments of zRMS:	The analytical method was fully validated in term of specificity, linearity, repeatability, accuracy and LOQ according to SANCO 3029/99 rev. 4 and SANCO/825/00 Rev. 8.1. The validation was performed quantifying mesotrione in fat by LC-MS. LOQ corresponds to 0.01 mg/kg. Two SRM transitions were monitored: 338 m/z - 291 m/z (quantifier ion) and 338 m/z – 249 m/z (qualifier ion). The method linearity was evaluated at 5 levels, ranging from 30%LOQ (0.003 mg/kg, LOD) to 30xLOQ (0.3 mg/kg). Recovery analysis was performed for samples spiked with mesotrione at LOQ (0.01 mg/kg) and 10xLOQ (0.1 mg/kg), 5 replicate analysis were performed for each level. Mean recovery is between 70%-110% with RSD <20% in all cases, in accordance with acceptance criteria. Based on recovery results it is possible to asses that spiked sample extracts show good stability over analysis time. The validation data demonstrate that the method is suitable to qualitatively and quantitatively determine mesotrione in fat specimens. The method is successfully validated and accepted.

Reference:	KCP 5.2.7
Report	Validation of the analytical procedure for the determination of mesotrione (CAS: 104206-82-8) in fat by liquid chromatography. M. Rubino, 2018, Report No. 18.629767.0006
Guideline(s):	SANCO 3029/99 rev. 4
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Sample extraction:
About 5.00 g of kidney were weighed into a 50 ml falcon. 10 ml of extraction mixture were added to the sample. Vortexed for about 30 min in a mechanical stirrer, then about 4 g of magnesium sulphate anhydrous, 1 g of sodium chloride, 1 g of sodium citrate dehydrate and 0.5 g of disodium hydrogencitrate sesquidrate were added to the sample and vortexed with each addition for about 1 min. The tube was centrifuged at 4750 rpm for 5 min. The supernatant was transferred in a plastic tube and kept at about -20°C for abour 1 hour. Then, the tube was centrifuged at 3000 rpm for 5 min and it was proceed to purification of the supernatant. 4 ml of supernatant were transferred into a 10 ml plastic tube, containing about 900 mg of magnesium sulphate anhydrous and 150 mg of C18 resin. Vortexed for about 1 min and centrifuged at 3000 rpm for 5 min. 0.5 ml of supernatant were transferred in 10 ml tube and were added 0.5 ml of mobile phase A. Vortexed for about 1 min, then transferred into an HPLC vial and injected. The sample was prepared in duplicate.

Results and discussions

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

~~Matrix~~	~~Analyte~~	~~Fortification level (mg/kg) (n = 5)~~	~~Mean recovery (%)~~	~~RSD (%)~~	~~Comments~~
~~Kidney~~	~~Mesotrione~~	~~LOQ (0.01 mg/kg)~~	~~101 (~~	~~4.9~~	~~First mass transition~~
			~~97~~	~~4.5~~	~~Second mass transition~~
		~~10xLOQ (0.1 mg/kg)~~	~~94~~	~~2.0~~	~~First mass transition~~
			~~93~~	~~3.7~~	~~Second mass transition~~

zRMS comments:

The Applicant has attached the wrong table. The correct one has been presented below.

Matrix	Analyte	Fortification level (mg/kg) (n = 5)	Mean recovery (%) (individual recovery values)	RSD (%)	Comments
Fat	Mesotrione	LOQ (0.01 mg/kg)	99 (103, 93, 99, 97, 104)	5.0	First mass transition
			91 (91, 71, 94, 89, 109)	15.0	Second mass transition
		10xLOQ (0.1 mg/kg)	95.0 (83, 93, 97, 99, 93)	3.0	First mass transition
			94 (89, 92, 98, 93, 100)	5.0	Second mass transition

Table A 14: Characteristics for the analytical method used for validation of mesotrione residues in fat

	Mesotrione
Specificity	The method is specific. No significant peaks (≤30% LOQ) are detected at RT of the target analyte in the Blank and Test Solution with respect to the Spiked Test Solution for both mass transitions.
Calibration (type, number of data points)	5 points 0.003 mg/kg to 0.3 mg/kg First mass transition y=580530x R^2 = 0.9989 Second mass transition y=137782x R^2 = 0.9999
Assessment of matrix effects is presented	yes
Limit of determination/quantification	LOQ = 0.01 mg/kg LOD = 0.003 mg/kg

Conclusion

The method was successfully validated and is suitable for determination of mesotrione in ~~kidney~~ fat.

A 2.1.2.2.4.2 Independent laboratory validation.

Please refer to A 2.1.2.2.5.2.

A 2.1.2.2.4.3 Confirmatory method

LC-MS/MS is highly specific method therefore no confirmatory method is required.

A 2.1.2.2.5 Analytical method 5

A 2.1.2.2.5.1 Method validation

Comments of zRMS:	The analytical method was fully validated in term of specificity, linearity, repeatability, accuracy and LOQ according to SANCO 3029/99 rev. 4 and SANCO/825/00 Rev. 8.1. The validation was performed quantifying mesotrione in kidney by LC-MS. LOQ corresponds to 0.01 mg/kg. Two SRM transitions were monitored: 338 m/z - 291 m/z (quantifier ion) and 338 m/z – 212 m/z (qualifier ion). The method linearity was evaluated at 5 levels, ranging from 30%LOQ (0.003 mg/kg, LOD) to 30xLOQ (0.3 mg/kg). Recovery analysis was performed for samples spiked with mesotrione at LOQ (0.01 mg/kg) and 10xLOQ (0.1 mg/kg), 5 replicate analysis were performed for each level. Mean recovery is between 70%-110% with RSD <20% in all cases, in accordance with acceptance criteria. Based on recovery results it is possible to asses that spiked sample extracts show good stability over analysis time. The validation data demonstrate that the method is suitable to qualitatively and quantitatively determine mesotrione in kidney specimens. The method is successfully validated and accepted.

Reference:	KCP 5.2.8
Report	Validation of the analytical procedure for the determination of mesotrione (CAS: 104206-82-8) in kidney by liquid chromatography. M. Rubino, 2018, Report No. 18.629767.0007
Guideline(s):	SANCO 3029/99 rev. 4
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Sample extraction:
About 3.50 g of fat were weighed into a 50 ml falcon. 10 ml of extraction mixture were added to the sample. Vortexed for about 30 min in a mechanical stirrer. The tube was centrifuged at 4750 rpm for 5 min. The supernatant was transferred in a plastic tube and kept at about -20°C for about 1 hour. Then, the tube was centrifuged at 3000 rpm for 5 min 0.5 ml of supernatant were transferred in 10 ml tube and were added 0.5 ml of mobile phase A. Vortexed for about 1 min, then transferred into an HPLC vial and injected. The sample was prepared in duplicate.

Results and discussions

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

~~Matrix~~	~~Analyte~~	~~Fortification level (mg/kg) (*n* = 5)~~	~~Mean recovery (%)~~	~~RSD (%)~~	~~Comments~~
~~Fat~~	~~Mesotrione~~	~~LOQ (0.01 mg/kg)~~	~~99~~	~~5.0~~	~~First mass transition~~
			~~91~~	~~15.0~~	~~Second mass transition~~
		~~10xLOQ (0.1 mg/kg)~~	~~95.0~~	~~3.0~~	~~First mass transition~~
			~~94~~	~~5.0~~	~~Second mass transition~~

zRMS comments:

The Applicant has attached the wrong table. The correct one has been presented below.

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Mean recovery (%) (individual recovery values)	RSD (%)	Comments
Kidney	Mesotrione	LOQ (0.01 mg/kg)	101 (94, 105, 97, 103, 104)	4.9	First mass transition
			97 (92, 93, 100, 102, 98)	4.5	Second mass transition
		10xLOQ (0.1 mg/kg)	94 (94, 91, 96, 96, 93)	2.0	First mass transition
			93 (91, 88, 94, 96, 95)	3.7	Second mass transition

Table A 16: Characteristics for the analytical method used for validation of mesotrione residues in fat

	Mesotrione
Specificity	The method is specific. No significant peaks (≤30% LOQ) are detected at RT of the target analyte in the Blank and Test Solution with respect to the Spiked Test Solution for both mass transitions.
Calibration (type, number of data points)	5 points 0.003 mg/kg to 0.3 mg/kg First mass transition y=636108x R^2 = 0.9994 Second mass transition y=37190x R^2 = 0.9973
Assessment of matrix effects is presented	yes
Limit of determination/quantification	LOQ = 0.01 mg/kg LOD = 0.003 mg/kg

Conclusion

The method was successfully validated and is suitable for determination of mesotrione in fat.

A 2.1.2.2.5.2 Independent laboratory validation.

Comments of zRMS:	The study is an ILV of the analytical method for determination of mesotrione in animal matrices (study numbers 18.629767.0005-0008 for eggs, fat, kidney and meat). Linearity, selectivity, specificity, accuracy (as recoveries), precision (as repeatability), limit of quantification (LOQ) and limit of detection (LOD) have been determined according to SANCO/825/00 rev.8.1 and SANCO/3029/99, rev. 4 (11/07/2000) guidance documents. The analysis was performed using HPLC coupled with triple quadrupole mass spectrometer (HPLC-MS/MS). Qualifier fragment ions was detected: - ion 291 (m/z) for quantification and qualifier ion 212 (m/z) for confirmation of meat, eggs and kidney and 249 (m/z) for fat from precursor 338 (m/z) for all matrices. The limit of quantification (LOQ) was 0.01 mg/kg for all matrices (with LOD 0.003 mg/kg). In order to demonstrate the validity of the analytical method, linearity was evaluated in each analytical sequence; selectivity, accuracy, precision, specificity, limit of quantification and limit of detection were evaluated on the following samples for all animal matrices:2 untreated samples, 5 untreated samples fortified at 0.01 mg/kg (LOQ), 5 untreated sample fortified at 0.10 mg/kg (10 x LOQ). Acceptability range for mean recovery: 70%-110% with RSD < 20%. The validation data demonstrate that the method is suitable to qualitatively and quantitatively determine mesotrione in eggs, fat, kidney and meat specimens. The study is accepted.

Reference:	KCP 5.2.9
Report	Independent Laboratory Validation of analytical methods for the determination of Mesotrione in animal matrices (eggs, fat, kidney and meat) validated in Studies conducted by Chelab. E. Signore, 2019, Report No. RAU-008-19
Guideline(s):	SANCO 3029/99 rev. 4
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Results and discussions

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

~~Matrix~~	~~Analyte~~	~~Fortification level (mg/kg) (n = 10)~~	~~Mean recovery (%)~~	~~RSD (%)~~	~~Comments~~
~~Eggs~~	~~Mesotrione~~	~~LOQ (0.01 mg/kg)~~	~~72.37~~	~~6.91~~	~~First mass transition~~
			~~75.73~~	~~3.29~~	~~Second mass transition~~
		~~10xLOQ (0.1 mg/kg)~~	~~72.63~~	~~4.35~~	~~First mass transition~~
			~~73.05~~	~~3.97~~	~~Second mass transition~~
~~Fat~~		~~LOQ (0.01 mg/kg)~~	~~95.63~~	~~1.58~~	~~First mass transition~~
			~~94.13~~	~~4.0~~	~~Second mass transition~~
		~~10xLOQ (0.1~~	~~93.23~~	~~2.93~~	~~First mass transition~~

Matrix	Analyte	Fortification level (mg/kg) (*n* = 10)	Mean recovery (%)	RSD (%)	Comments
		mg/kg)	97.21	2.80	Second mass transition
Kidney		LOQ (0.01 mg/kg)	98.71	8.11	First mass transition
			105.61	15.68	Second mass transition
		10xLOQ (0.1 mg/kg)	96.54	3.26	First mass transition
			90.32	16.25	Second mass transition
Meat		LOQ (0.01 mg/kg)	108.65	1.99	First mass transition
			105.69	3.55	Second mass transition
		10xLOQ (0.1 mg/kg)	95.31	4.49	First mass transition
			96.07	4.01	Second mass transition

zRMS comments:

The Table A1 presented above contains erroneously swapped values. Data considered with the method description in document K is presented below:

Residues of Mesotrione on Eggs (291 m/z - quantifier ion)

Date of extraction	Date of analysis	Sample code	Fortification level (mg/Kg)	Recoveries (%)	Mean Recovery (%) and RSD (%)	Overall Mean Recovery (%) and RSD (%)
29/05/2019	29/05/2019	ME/EG/01/1R	0.01 (LOQ)	66.41	72.37 ± 6.91	72.50 ± 5.44
		ME/EG/01/3R		68.47		
		ME/EG/01/5R		76.83		
		ME/EG/01/7R		72.37		
		ME/EG/01/9R		77.79		
		ME/EG/01/2R	0.10 (10xLOQ)	71.91	72.63 ± 4.35	
		ME/EG/01/4R		74.06		
		ME/EG/01/6R		77.36		
		ME/EG/01/8R		69.66		
		ME/EG/01/10R		70.16		

Residues of Mesotrione on Eggs (212 m/z - qualifier ion)

Date of extraction	Date of analysis	Sample code	Fortification level (mg/Kg)	Recoveries (%)	Mean Recovery (%) and RSD (%)	Overall Mean Recovery (%) and RSD (%)
29/05/2019	29/05/2019	ME/EG/01/1R	0.01 (LOQ)	72.78	75.73 ± 3.20	74.39 ± 3.88
		ME/EG/01/3R		73.48		
		ME/EG/01/5R		77.80		
		ME/EG/01/7R		76.76		
		ME/EG/01/9R		77.83		
		ME/EG/01/2R	0.10 (10xLOQ)	72.59	73.05 ± 3.97	
		ME/EG/01/4R		72.39		
		ME/EG/01/6R		78.06		
		ME/EG/01/8R		70.71		
		ME/EG/01/10R		71.52		

Residues of Mesotrione on Meat (291 m/z - quantifier ion)

Date of extraction	Date of analysis	Sample code	Fortification level (mg/Kg)	Recoveries (%)	Mean Recovery (%) and RSD (%)	Overall Mean Recovery (%) and RSD (%)
30/05/2019	31/05/2019	ME/ME/01/1R	0.01 (LOQ)	111.19	108.65 ± 1.99	101.98 ± 7.57
		ME/ME/01/3R		109.72		
		ME/ME/01/5R		105.53		
		ME/ME/01/7R		107.59		
		ME/ME/01/9R		109.22		
		ME/ME/01/2R	0.10 (10xLOQ)	92.93	95.31 ± 4.49	
		ME/ME/01/4R		97.65		
		ME/ME/01/6R		99.65		
		ME/ME/01/8R		97.27		
		ME/ME/01/10R		89.04		

Residues of Mesotrione on Meat (212 m/z - qualifier ion)

Date of extraction	Date of analysis	Sample code	Fortification level (mg/Kg)	Recoveries (%)	Mean Recovery (%) and RSD (%)	Overall Mean Recovery (%) and RSD (%)
30/05/2019	31/05/2019	ME/ME/01/1R	0.01 (LOQ)	110.07	105.69 ± 3.55	100.88 ± 6.16
		ME/ME/01/3R		102.04		
		ME/ME/01/5R		108.04		
		ME/ME/01/7R		101.54		
		ME/ME/01/9R		106.77		
		ME/ME/01/2R	0.10 (10xLOQ)	93.56	96.07 ± 4.01	
		ME/ME/01/4R		98.27		
		ME/ME/01/6R		99.73		
		ME/ME/01/8R		98.23		
		ME/ME/01/10R		90.58		

Residues of Mesotrione on Kidney (291 m/z - quantifier ion)

Date of extraction	Date of analysis	Sample code	Fortification level (mg/Kg)	Recoveries (%)	Mean Recovery (%) and RSD (%)	Overall Mean Recovery (%) and RSD (%)
29/05/2019	29/05/2019	ME/KI/01/1R	0.01 (LOQ)	104.25	98.71 ± 8.11	97.63 ± 5.99
		ME/KI/01/3R		107.44		
		ME/KI/01/5R		101.28		
03/06/2019	03/06/2019	ME/KI/01/7R		89.50		
		ME/KI/01/9R		91.09		
29/05/2019	29/05/2019	ME/KI/01/2R	0.10 (10xLOQ)	98.80	96.54 ± 3.26	
		ME/KI/01/4R		99.39		
03/06/2019	03/06/2019	ME/KI/01/6R		91.60		
		ME/KI/01/8R		95.48		
		ME/KI/01/10R		97.42		

Residues of Mesotrione on Kidney (212 m/z - qualifier ion)

Date of extraction	Date of analysis	Sample code	Fortification level (mg/Kg)	Recoveries (%)	Mean Recovery (%) and RSD (%)	Overall Mean Recovery (%) and RSD (%)
29/05/2019	29/05/2019	ME/KI/01/1R	0.01 (LOQ)	118.87	105.61 ± 15.68	97.97 ± 17.16
		ME/KI/01/3R		117.88		
		ME/KI/01/5R		116.30		
03/06/2019	03/06/2019	ME/KI/01/7R		86.60		
		ME/KI/01/9R		88.42		
29/05/2019	29/05/2019	ME/KI/01/2R	0.10 (10xLOQ)	106.77	90.32 ± 16.25	
		ME/KI/01/4R		105.86		
03/06/2019	03/06/2019	ME/KI/01/6R		77.27		
		ME/KI/01/8R		80.94		
		ME/KI/01/10R		80.75		

Residues of Mesotrione on Fat (291 m/z - quantifier ion)

Date of extraction	Date of analysis	Sample code	Fortification level (mg/Kg)	Recoveries (%)	Mean Recovery (%) and RSD (%)	Overall Mean Recovery (%) and RSD (%)
31/05/2019	31/05/2019	ME/FA/01/1R	0.01 (LOQ)	96.17	95.63 ± 1.58	94.43 ± 2.58
		ME/FA/01/3R		93.83		
		ME/FA/01/5R		95.07		
		ME/FA/01/7R		97.89		
		ME/FA/01/9R		95.20		
		ME/FA/01/2R	0.10 (10xLOQ)	93.64	93.23 ± 2.93	
		ME/FA/01/4R		95.52		
		ME/FA/01/6R		94.22		
		ME/FA/01/8R		94.26		
		ME/FA/01/10R		88.50		

Residues of Mesotrione on Fat (249 m/z - qualifier ion)

Date of extraction	Date of analysis	Sample code	Fortification level (mg/Kg)	Recoveries (%)	Mean Recovery (%) and RSD (%)	Overall Mean Recovery (%) and RSD (%)
31/05/2019	31/05/2019	ME/FA/01/1R	0.01 (LOQ)	92.42	94.13 ± 4.00	95.67 ± 3.65
		ME/FA/01/3R		99.99		
		ME/FA/01/5R		90.78		
		ME/FA/01/7R		95.71		
		ME/FA/01/9R		91.76		
		ME/FA/01/2R	0.10 (10xLOQ)	96.34	97.21 ± 2.80	
		ME/FA/01/4R		101.54		
		ME/FA/01/6R		95.89		
		ME/FA/01/8R		97.90		
		ME/FA/01/10R		94.38		

Table A 18: Characteristics for the analytical method used for validation of mesotrione residues in maize forage and grain

	Mesotrione
Specificity	The method is specific. No significant peaks (≤30% LOQ) are detected at RT of the target analyte in the Blank and Test Solution with respect to the Spiked Test Solution for both mass transitions.
Calibration (type, number of data points)	5 points 0.0028 mg/kg to 0.3201 mg/kg meat, eggs and kidney 0.00286 mg/kg to 0.3144 mg/kg for fat R > 0.99
Assessment of matrix effects is presented	yes
Limit of determination/quantification	LOQ = 0.01 mg/kg LOD = 0.003 mg/kg

Conclusion

The method was successfully validated and is suitable for determination of mesotrione in meat, eggs, kidney and fat.

A 2.1.2.2.5.3 Confirmatory method

LC-MS/MS is highly specific method therefore no confirmatory method is required.

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

zRMS comments:
Residue definition for monitoring purposes for soil according to the EFSA Journal 2016;14(3):4419 is mesotrione and metabolite A (*residues of mesotrione and its metabolites AMBA and MNBA in soil can be monitored by LC-MS/MS with LOQs of 0.002 mg/kg for all three compounds*) so analytical methods should include determination of these compounds.

A 2.1.2.3.1 Analytical method 1

A 2.1.2.3.1.1 Method validation

Comments of zRMS:	The analytical method was fully validated in term of specificity, linearity, repeatability, accuracy and LOQ according to SANCO 3029/99 rev. 4 and SANCO/825/00 Rev. 8.1. The validation was performed quantifying mesotrione in soil by LC-MS. LOQ corresponds to 0.002 mg/kg. Two SRM transitions were monitored: 338 m/z - 291 m/z (quantifier ion) and 338 m/z – 212 m/z (qualifier ion). The method linearity was evaluated at 5 levels, ranging from 30%LOQ (0.0006 mg/kg, LOD) to 30xLOQ (0.06 mg/kg). Recovery analysis was performed for samples spiked with mesotrione at LOQ (0.002 mg/kg) and 10xLOQ (0.02 mg/kg), 5 replicate analysis were performed for each level. Mean recovery is between 70%-110% with RSD <20% in all cases, in accordance with acceptance criteria. Based on recovery results it is possible to asses that spiked sample extracts show good stability over analysis time. The validation data demonstrate that the method is suitable to qualitatively and quantitatively determine mesotrione in soil specimens. The method is successfully validated and accepted.

Reference:	KCP 5.2.10
Report	Validation of the analytical procedure for the determination of mesotrione (CAS: 104206-82-8) in soil by liquid chromatography. M. Rubino, 2018, Report No. 18.629767.0009
Guideline(s):	SANCO 3029/99 rev. 4
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Sample extraction:
About 20.0 g of soil were weighed into a 50 ml plastic falcon and 5 ml of milliQ water were added in order to hydrate the matrix. Then 9.5 ml of extraction mixture were added to the sample. Vortexed for about 30 min with a mechanical stirrer, about 4 g of magnesium sulfate anhydrous, 1 g of sodium chloride, 1 g of sodium citrate dehydrate and 0.5 g of disodium hydrogencitrate sesquidrate were added to the sample and vortexed with each addition for about 1 min. The tube was centrifuged at 4750 rpm for 5 min and transferred the supernatant in a plastic tube and kept at about -20°C for about 1 hour. Then, the

rube was centrifuged at 3000 rpm for 5 min and it was proceed to purification of the supernatant. 4 ml of supernatant were transferred into a 10 ml plastic tube, containing about 900 mg of magnesium sulphate anhydrous and 150 mg of C18 resin. Vortexed for about 1 min and centrifuged at 3000 rpm for 5 min. 0.5 ml of supernatant of purified sample were transferred into 5 different 10 ml tubes and dried by N_2 flux. The dried sample was the resuspended into 1 ml of linearity solution to have the calibration curve in matrix. Vortex for 1 min, transferred into an HPLC vial and inject.

Results and discussions

Table A 19: Recovery results from method validation of mesotrione using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Mean recovery (%) (individual recovery value)	RSD (%)	Comments
Soil	Mesotrione	LOQ (0.002 mg/kg)	~~106~~ 109 (114, 107, 109, 105, 109)	1.8	First mass transition
			107 (112, 109, 108, 106, 107)	1.1	Second mass transition
		10xLOQ (0.023 mg/kg)	100 (98, 98, 103, 99, 99)	2.2	First mass transition
			103 (103, 100, 103, 103, 103)	1.2	Second mass transition

Table A 20: Characteristics for the analytical method used for validation of mesotrione residues in soil

	Mesotrione
Specificity	The method is specific. No significant peaks (≤30% LOQ) are detected at RT of the target analyte in the Blank and Test Solution with respect to the Spiked Test Solution for both mass transitions.
Calibration (type, number of data points)	5 points 0.0006 mg/kg to 0.06 mg/kg First mass transition y=1561442x R^2 = 0.9980 Second mass transition y=355739x R^2 = 0.9980
Assessment of matrix effects is presented	yes
Limit of determination/quantification	LOQ = 0.002 mg/kg LOD = 0.0006 mg/kg

Conclusion

The method was successfully validated and is suitable for determination of mesotrione in soil.

A 2.1.2.3.1.2 Confirmatory method

LC-MS/MS is highly specific method therefore no confirmatory method is required.

A 2.1.2.3.2 Analytical method 2

A 2.1.2.3.2.1 Method validation

Comments of zRMS:	The analytical method was fully validated in term of specificity, linearity, repeatability, accuracy and LOQ according to SANCO 3029/99 rev. 4 and SANCO/825/00 Rev. 8.1. The validation was performed quantifying MNBA in soil by LC-MS. LOQ corresponds to 0.002 mg/kg. Two SRM transitions were monitored: 244 m/z - 200 m/z (quantifier ion) and 244 m/z – 170 m/z (qualifier ion). The method linearity was evaluated at 5 levels, ranging from 30%LOQ (0.0006 mg/kg, LOD) to 30xLOQ (0.06 mg/kg). Recovery analysis was performed for samples spiked with MNBA at LOQ (0.002 mg/kg) and 10xLOQ (0.02 mg/kg), 5 replicate analysis were performed for each level. Mean recovery is between 70%-110% with RSD <20% in all cases, in accordance with acceptance criteria. Based on recovery results it is possible to asses that spiked sample extracts show good stability over analysis time. The validation data demonstrate that the method is suitable to qualitatively and quantitatively determine MNBA in soil specimens. The method is successfully validated and accepted.

Reference:	KCP 5.2.11
Report	Validation of the analytical procedure for the determination of MNBA (CAS: 104206-82-8) in soil by liquid chromatography. M. Rubino, 2018, Report No. 18.640093.0001
Guideline(s):	SANCO 3029/99 rev. 4
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Sample extraction:
About 10.00 g of soil were weighed into a 50 ml plastic falcon and 5 ml of milliQ water were added in order to hydrate the matrix. Then 6 ml of extraction mixture were added to the sample. Vortexed for about 2min. Then, about 4 g of magnesium sulphate anhydrous were added to the sample and vortexed again about 2 min. The tube was centrifuged at 4750 rpm for 5 min and it was proceed to purification of the supernatant.
5 ml of supernatant were transferred into a 10 ml plastic tube, containing about 900 mg of magnesium sulphate anhydrous and 150 mg of C18 resin. Vortexed for about 1 min and centrifuged at 4750 rpm for 5 min. 2.5 ml of purified sample was transferred into 10 ml tube and dried by N_2 flux. The dried sample was resuspended with 0.25 ml of 10 mM ammonium formate buffer. Vortexed for about 1 min, then transferred into an HPLC vial and injected. The sample was prepared in duplicate.

Results and discussions

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

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Mean recovery (%) (individual recovery values)	RSD (%)	Comments
Soil	MNBA metabolite	LOQ (0.002 mg/kg)	84 (84, 73, 88, 73, 102)	14	First mass transition
			83 (77, 86, 83, 84, 86)	5.0	Second mass transition
		10xLOQ (0.02 mg/kg)	88 (83, 79, 84, 91, 103)	11	First mass transition
			90 (88, 83, 92, 93, 95)	5.0	Second mass transition

Table A 22: Characteristics for the analytical method used for validation of MNBA metabolite residues in soil

	MNBA metabolite
Specificity	The method is specific. No significant peaks (≤30% LOQ) are detected at RT of the target analyte in the Blank and Test Solution with respect to the Spiked Test Solution for both mass transitions.
Calibration (type, number of data points)	5 points 0.0006 mg/kg to 0.06 mg/kg First mass transition y=10014x R^2 = 0.9963 Second mass transition y=7829x R^2 = 0.9957
Assessment of matrix effects is presented	yes
Limit of determination/quantification	LOQ = 0.002 mg/kg LOD = 0.0006 mg/kg

Conclusion

The method was successfully validated and is suitable for determination of MNBA metabolite in soil.

A 2.1.2.3.2.2 Confirmatory method

LC-MS/MS is highly specific method therefore no confirmatory method is required.

A 2.1.2.3.3 Analytical method 3

A 2.1.2.3.3.1 Method validation

Comments of zRMS:	The analytical method was fully validated in term of specificity, linearity, repeatability, accuracy and LOQ according to SANCO 3029/99 rev. 4 and SANCO/825/00 Rev. 8.1. The validation was performed quantifying AMBA in soil by LC-MS/MS. LOQ corresponds to 0.002 mg/kg. Two SRM transitions were monitored: 216 m/z - 136 m/z (quantifier ion) and 216 m/z – 91 m/z (qualifier ion). The method linearity was evaluated at 5 levels, ranging from 30%LOQ (0.0006 mg/kg, LOD) to 30xLOQ (0.06 mg/kg). Recovery analysis was performed for samples spiked with AMBA at LOQ (0.002 mg/kg) and 10xLOQ (0.02

	mg/kg), 5 replicate analysis were performed for each level. Mean recovery is between 70%-110% with RSD <20% in all cases, in accordance with acceptance criteria. Based on recovery results it is possible to asses that spiked sample extracts show good stability over analysis time. The validation data demonstrate that the method is suitable to qualitatively and quantitatively determine AMBA in soil specimens. The method is successfully validated and accepted.

Reference:	KCP 5.2.12
Report	Validation of the analytical procedure for the determination of AMBA (CAS: 104206-82-8) in soil by liquid chromatography. M. Rubino, 2018, Report No. 18.640093.0002
Guideline(s):	SANCO 3029/99 rev. 4
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Sample extraction:
About 3.00 g of soil were weighed into a 50 ml plastic falcon and 5 ml of milliQ water were added in order to hydrate the matrix. Then 6 ml of extraction mixture were added to the sample. Vortexed for about 2min. Then, about 4 g of magnesium sulphate anhydrous were added to the sample and vortexed again about 2 min. The tube was centrifuged at 4750 rpm for 5 min and it was proceed to purification of the supernatant.
6 ml of supernatant were transferred into a 10 ml plastic tube, containing about 900 mg of magnesium sulphate anhydrous and 150 mg of C18 resin. Vortexed for about 1 min and centrifuged at 4750 rpm for 5 min. 2.5 ml of purified sample was transferred into 10 ml tube and dried by N_2 flux. The dried sample was resuspended with 0.5 ml of blank solution. Vortexed for about 1 min, then transferred into an HPLC vial and injected. The sample was prepared in duplicate.

Results and discussions

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

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Mean recovery (%) (Individual recovery values)	RSD (%)	Comments
Soil	AMBA metabolite	LOQ (0.002 mg/kg)	80 (80, 80, 80, 80, 81)	0.4	First mass transition
			94 (94, 92, 93, 97, 95)	1.8	Second mass transition
		10xLOQ (0.02 mg/kg)	91 (92, 71, 95, 99, 98)	12.4	First mass transition
			91 (93, 73, 97, 96, 95)	11.1	Second mass transition

Table A 24: Characteristics for the analytical method used for validation of AMBA metabolite residues in soil

	AMBA metabolite
Specificity	The method is specific. No significant peaks (≤30% LOQ) are detected at RT of the target analyte in the Blank and Test Solution with respect to the Spiked Test Solution for both mass transitions.
Calibration (type, number of data points)	5 points 0.0006 mg/kg to 0.06 mg/kg First mass transition y=4659481x R^2 = 0.9977 Second mass transition y=672120x R^2 = 0.9977
Assessment of matrix effects is presented	yes
Limit of determination/quantification	LOQ = 0.002 mg/kg LOD = 0.0006 mg/kg

Conclusion

The method was successfully validated and is suitable for determination of AMBA metabolite in soil.

A 2.1.2.3.3.2 Confirmatory method

LC-MS/MS is highly specific method therefore no confirmatory method is required.

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

zRMS comments:
Residue definition for monitoring purposes for water according to the EFSA Journal 2016;14(3):4419 is mesotrione and metabolite A (*appropriate LC-MS/MS method exists for monitoring residues of mesotrione and its metabolites AMBA and MNBA in ground water and surface water with a LOQ of 0.05 µg/L for all compounds*) so analytical methods should include determination of these compounds.

A 2.1.2.4.1 Analytical method 1

A 2.1.2.4.1.1 Method validation

Comments of zRMS:	The analytical method was fully validated in term of specificity, linearity, repeatability, accuracy and LOQ according to SANCO 3029/99 rev. 4 and SANCO/825/00 Rev. 8.1. The validation was performed quantifying mesotrione in ground water by LC-MS. LOQ corresponds to 0.05 µg/l. Two SRM transitions were monitored: 338 m/z - 291 m/z (quantifier ion) and 338 m/z – 212 m/z (qualifier ion). The method linearity was evaluated at 5 levels, ranging from 30%LOQ (0.015 µg/l, LOD) to 30xLOQ (1.5 µg/l). Recovery analysis was performed for samples spiked with mesotrione at LOQ (0.05 µg/l) and 10xLOQ (0.5 µg/l), 5 replicate analysis were performed for each level. Mean recovery is between 70%-110% with RSD <20% in all cases, in accordance with acceptance criteria. Based on recovery results it is possible to asses that spiked sample extracts show good stability over analysis time. The validation data demonstrate that the method is suitable to qualitatively and quantitatively determine mesotrione in ground water specimens. The method is successfully validated and accepted.

Reference:	KCP 5.2.13
Report	Validation of the analytical procedure for the determination of mesotrione (CAS: 104206-82-8) in ground water by liquid chromatography. M. Rubino, 2018, Report No. 18.629767.0015
Guideline(s):	SANCO 3029/99 rev. 4
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Sample extraction:
About 300 ml of ground water were transferred in a backer added about 0.2 ml of formic acid (pH 2-5). Then, it was percolate using a regular vacuum through a C18 cartridge (previously coordinated with 3 ml of methanol) with a flow rate of 2 drops/sec. The cartridge was washed with 3 ml of milliQ water and dried using centrifugation technique (2000 rpm, 2min.) The analyte was eluted with 2 ml of extraction mixture and the eluate recovered. This step was repeated a two more times and the three extracts were combined. 1.6 ml of eluted solution were dried under nitrogen flux and resuspended with 1 ml of blank solution. Vortexed for 1 min, transferred in vial and injected. Test sample was prepared in duplicate.

Results and discussions

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

Matrix	Analyte	Fortification level (µg/L) (*n* = 5)	Mean recovery (%) (individual recovery values)	RSD (%)	Comments
Ground water	Mesotrione	LOQ (0.05 µg/L)	83 (85, 82, 86, 82, 81)	3	First mass transition
			87 (88, 85, 86, 86, 89)	2	Second mass transition
		10xLOQ (0.5 µg/L)	88 (84, 88, 90, 85, 93)	4	First mass transition
			90 (90, 92, 93, 86, 88)	3	Second mass transition

Table A 26: Characteristics for the analytical method used for validation of mesotrione residues in ground water

	Mesotrione
Specificity	The method is specific. No significant peaks (≤30% LOQ) are detected at RT of the target analyte in the Blank and Test Solution with respect to the Spiked Test Solution for both mass transitions.
Calibration (type, number of data points)	5 points 0.015 µg/L to 1.5 µg/L First mass transition y=724830x R^2 = 0.9991 Second mass transition y=167049x

	Mesotrione
Specificity	The method is specific. No significant peaks (≤30% LOQ) are detected at RT of the target analyte in the Blank and Test Solution with respect to the Spiked Test Solution for both mass transitions.
	R^2 = 0.9993
Assessment of matrix effects is presented	yes
Limit of determination/quantification	LOQ = 0.05 µg/L

Conclusion

The method was successfully validated and is suitable for determination of mesotrione in ground water.

A 2.1.2.4.1.2 Independent laboratory validation.

Comments of zRMS:	The analytical method was fully validated in term of specificity, linearity, repeatability, accuracy and LOQ according to SANCO 3029/99 rev. 4 and SANCO/825/00 Rev. 8.1. The validation was performed quantifying mesotrione in drinking water by LC-MS/MS. LOQ corresponds to 0.05 µg/l. Two SRM transitions were monitored: 338 m/z - 291 m/z (quantifier ion) and 338 m/z – 212 m/z (qualifier ion). The method linearity was evaluated at 5 levels, ranging from 30%LOQ (0.015 µg/l) to 30xLOQ (1.5 µg/l). Recovery analysis was performed for samples spiked with mesotrione at LOQ (0.05 µg/l) and 10xLOQ (0.5 µg/l), 5 replicate analysis were performed for each level. Mean recovery is between 70%-110% with RSD <20% in all cases, in accordance with acceptance criteria. Based on recovery results it is possible to asses that spiked sample extracts show good stability over analysis time. The validation data demonstrate that the method is suitable to qualitatively and quantitatively determine mesotrione in drinking water specimens. The method is successfully validated and accepted.

Reference:	KCP 5.2.19
Report	Independent Laboratory Validation of the analytical procedure for the determination of residues of Mesotrione (CAS 104206-82-8) in drinking water by Liquid Chromatography. Z. Hordyjewicz-Baran, 2019. Report No. 163/2019
Guideline(s):	SANCO 3029/99 rev. 4
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Sample extraction

About 300 ml of drinking water was transferred in a backer added about 0.2 ml of formic acid (pH 2.5). Then, it was percolate using a regular vacuum through a C18 cartridge (previously conditionated with 3 ml of methanol) with a flow of 2 drops/sec. The cartridge was washed with 3 ml of milli-Q water and dried using centrifugation technique (2000 rpm, 2 min). The analyte was eluted with 2 ml of extraction mixture aand the elute recovered. This step was repeated a two more times and three extracts were combined. 1.6 ml of eluted solution was dried under nitrogen flux and resuspended with 1 ml of blank solution. Vortexed for 1 min and transferred in vial and injected. The sample was prepared in duplicate.

Results and discussions

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

Matrix	Analyte	Fortification level (mg/L) (*n* = 5)	Mean recovery (%) (individual recovery values)	RSD (%)	Comments
Drinking water	Mesotrione	LOQ (0.05 µg/L)	98 (92, 87, 103, 97, 109)	8.9	First mass transition
			~~104~~ 87 (79, 77, 87, 95, 96)	~~4.8~~ 9,7	Second mass transition
		10xLOQ (0.5 µg/L)	~~87~~ 104 (99, 101, 100, 108, 110)	~~9.7~~ 4,8	First mass transition
			103 (98, 101, 96, 110, 109)	6.2	Second mass transition

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

	Mesotrione
Specificity	The method is specific. No significant peaks (≤30% LOQ) are detected at RT of the target analyte in the Blank and Test Solution with respect to the Spiked Test Solution for both mass transitions.
Calibration (type, number of data points)	5 points 0.001 µg/L to 0.132 µg/L R = 0.9932 first mass transition R = 0.9912 second mass transition
Assessment of matrix effects is presented	yes
Limit of determination/quantification	LOQ = 0.05 µg/L LOD = 0.013 µg/L

Conclusion

The method was successfully validated and is suitable for determination of mesotrione in drinking water.

A 2.1.2.4.1.3 Confirmatory method

LC-MS/MS is highly specific method therefore no confirmatory method is required.

A 2.1.2.4.2 Analytical method 2

A 2.1.2.4.2.1 Method validation

Comments of zRMS:	The analytical method was fully validated in term of specificity, linearity, repeatability, accuracy and LOQ according to SANCO 3029/99 rev. 4 and SANCO/825/00 Rev. 8.1. The validation was performed quantifying mesotrione in surface water by LC-MS. LOQ corresponds to 0.05 µg/l. Two SRM transitions were monitored: 338 m/z - 291 m/z (quantifier ion) and 338 m/z – 212 m/z (qualifier ion). The method linearity was evaluated at 5 levels, ranging from 30%LOQ (0.015 µg/l) to 30xLOQ (1.5 µg/l). Recovery analysis was performed for samples spiked with mesotrione at LOQ (0.05 µg/l) and 10xLOQ (0.5 µg/l), 5 replicate analysis were performed for each level. Mean recovery is between 70%-110% with RSD <20% in all cases, in accordance with acceptance criteria. Based on recovery results it is possible to asses that spiked sample extracts show good stability over analysis time. The validation data demonstrate that the method is suitable to qualitatively and quantitatively determine mesotrione in surface water

	specimens. The method is successfully validated and accepted.

Reference:	KCP 5.2.14
Report	Validation of the analytical procedure for the determination of mesotrione (CAS: 104206-82-8) in surface water by liquid chromatography. M. Rubino, 2018, Report No. 18.629767.0012
Guideline(s):	SANCO 3029/99 rev. 4
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Sample extraction:
About 300 ml of surface water were transferred in a backer added about 0.2 ml of formic acid (pH 2-5). Then, it was percolate using a regular vacuum through a C18 cartridge (previously coordinated with 3 ml of methanol) with a flow rate of 2 drops/sec. The cartridge was washed with 3 ml of milliQ water and dried using centrifugation technique (2000 rpm, 2min.) The analyte was eluted with 2 ml of extraction mixture and the eluate recovered. This step was repeated a two more times and the three extracts were combined. 1.6 ml of eluted solution were dried under nitrogen flux and resuspended with 1 ml of blank solution. Vortexed for 1 min, transferred in vial and injected. Test sample was prepared in duplicate.

Results and discussions

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

Matrix	Analyte	Fortification level (µg/L) (*n* = 5)	Mean recovery (%) (individual recovery values)	RSD (%)	Comments
Surface water	Mesotrione	LOQ (0.05 µg/L)	96 (93, 95, 100, 95, 95)	3	First mass transition
			97 (94, 100, 95, 99, 90)	7	Second mass transition
		10xLOQ (0.5 µg/L)	104 (106, 105, 104, 100, 102)	2	First mass transition
			101 (105, 104, 103, 101, 92)	5	Second mass transition

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

	Mesotrione
Specificity	The method is specific. No significant peaks (≤30% LOQ) are detected at RT of the target analyte in the Blank and Test Solution with respect to the Spiked Test Solution for both mass transitions.
Calibration (type, number of data points)	5 points 0.015 µg/L to 1.5 µg/L

	Mesotrione
Specificity	The method is specific. No significant peaks (≤30% LOQ) are detected at RT of the target analyte in the Blank and Test Solution with respect to the Spiked Test Solution for both mass transitions.
	First mass transition y=1008280x R^2 = 0.9994 Second mass transition y=240110x R^2 = 0.9994
Assessment of matrix effects is presented	yes
Limit of determination/quantification	LOQ = 0.05 µg/L

Conclusion

The method was successfully validated and is suitable for determination of mesotrione in surface water.

A 2.1.2.4.2.2 Confirmatory method

LC-MS/MS is highly specific method therefore no confirmatory method is required.

A 2.1.2.4.3 Analytical method 3

A 2.1.2.4.3.1 Method validation

Comments of zRMS:	The analytical method was fully validated in term of specificity, linearity, repeatability, accuracy and LOQ according to SANCO 3029/99 rev. 4 and SANCO/825/00 Rev. 8.1. The validation was performed quantifying MNBA in ground water by LC-MS. LOQ corresponds to 0.05 µg/l. Two SRM transitions were monitored: 244 m/z - 200 m/z (quantifier ion) and 244 m/z – 170 m/z (qualifier ion). The method linearity was evaluated at 5 levels, ranging from 30%LOQ (0.015 µg/l) to 30xLOQ (1.5 µg/l). Recovery analysis was performed for samples spiked with MNBA at LOQ (0.05 µg/l) and 10xLOQ (0.5 µg/l), 5 replicate analysis were performed for each level. Mean recovery is between 70%-110% with RSD <20% in all cases, in accordance with acceptance criteria. Based on recovery results it is possible to asses that spiked sample extracts show good stability over analysis time. The validation data demonstrate that the method is suitable to qualitatively and quantitatively determine MNBA in ground water specimens. The method is successfully validated and accepted.

Reference:	KCP 5.2.15
Report	Validation of the analytical procedure for the determination of MNBA(CAS: 110964-79-9) in ground water by liquid chromatography. M. Rubino, 2018, Report No. 18.629767.0017
Guideline(s):	SANCO 3029/99 rev. 4
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Sample extraction:
About 200 ml of ground water were transferred in a backer, added about 5 ml of hydrochloric acid (pH-2). Then it was percolate using a regular vacuum through a MCX cartridge (previously conditionated with 2 ml of methanol and 2 ml of milliQ water w with a flow rate of 2 drops/sec. The cartridge was washed with 4 ml of 5% methanol in milliQ water and dried using eluted with 4 ml of extraction mixture and the eluate recovered. 3.5 ml of eluted solution were dried under nitrogen flux and resuspended with 0.35 ml of blank solution. Vortexed for 1 min, transferred in vial and injected.

Results and discussions

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

Matrix	Analyte	Fortification level (µg/L) (*n* = 5)	Mean recovery (%) (individual recovery values)	RSD (%)	Comments
Ground water	MNBA metabolite	LOQ (0.05 µg/L)	~~106~~ 100 (94, 99, 100, 103, 102)	5	First mass transition
			95 (97, 90, 98, 90, 99)	5	Second mass transition
		10xLOQ (0.5 µg/L)	94 (86, 91, 93, 99, 99)	8	First mass transition
			92 (88, 90, 92, 97, 92)	4	Second mass transition

Table A 32: Characteristics for the analytical method used for validation of MNBA metabolite residues in ground water

	MNBA metabolite
Specificity	The method is specific. No significant peaks (≤30% LOQ) are detected at RT of the target analyte in the Blank and Test Solution with respect to the Spiked Test Solution for both mass transitions.
Calibration (type, number of data points)	5 points 0.015 µg/L to 1.5 µg/L First mass transition y=57069x R^2 = 0.9998 Second mass transition y=37870x R^2 = 0.9994
Assessment of matrix effects is presented	yes
Limit of determination/quantification	LOQ = 0.05 µg/L

Conclusion

The method was successfully validated and is suitable for determination of MNBA metabolite in ground water.

A 2.1.2.4.3.2 Independent laboratory validation.

Comments of zRMS:	The study is an ILV of the analytical method for determination of mesotrione metabolites (AMBA and MNBA) in drinking water (study numbers

	18.629767.0016 for AMBA and 18.629767.0017 for MNBA). Linearity, selectivity, specificity, accuracy (as recoveries), precision (as repeatability), limit of quantification (LOQ) and limit of detection (LOD) have been determined according to SANCO/825/00 rev.8.1 and SANCO/3029/99, rev. 4 (11/07/2000) guidance documents. The analysis was performed using HPLC coupled with triple quadrupole mass spectrometer (HPLC-MS/MS). AMBA - (m/z 216 → 136 CE 20V) - quantifier ion AMBA - (m/z 216 → 91 CE 35V) - qualifier ion MNBA - (m/z 244 → 200 CE -13V) - quantifier ion MNBA - (m/z 244 → 170 CE -20V) - qualifier ion The limit of quantification (LOQ) was 0.05 µg/l for both metabolites. In order to demonstrate the validity of the analytical method, linearity was evaluated in each analytical sequence; selectivity, accuracy, precision, specificity, limit of quantification and limit of detection were evaluated on the following samples of drinking water for both AMBA and MNBA:2 untreated samples, 5 untreated samples fortified at 0.05 µg/l (LOQ), 5 untreated sample fortified at 0.50 µg/l (10 x LOQ). Acceptability range for mean recovery: 70%-110% with RSD < 20%. The validation data demonstrate that the method is suitable to qualitatively and quantitatively determine mesotrione metabolites, AMBA and MNBA in water. The study is accepted.

Reference:	KCP 5.2.20
Report	Independent Laboratory Validation of analytical method for the determination of Mesotrione metabolites (AMBA and MNBA) in drinking water validation a Study conducted by Chelab. E. Signore, 2019, Report No. RAU-007-19
Guideline(s):	SANCO 3029/99 rev. 4
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Sample extraction and purification

AMBA
About 3 mL of the water were transferred in a 15 mL plastic falcon and 0.15 of SS-LOQ for recoveries at LOQ level and 0.15 of SS-10LOQ for recoveries at 10LOQ level were added.
Afterwards, 2 mL of this solution were transferred into a 15 mL tube and dried by N_2 flux at about 45° C. The dried samples were resuspended with 0.25 mL of blank solution. The extract was vortexed for about 1 minute and transferred into a vial and injected.

MNBA
About 200 mL (± 0.10 mL) of the water were transferred in a backer and 0.4 of L2 solution for recoveries at LOQ level and 0.4 of L3 solution for recoveries at 10LOQ level were added. After 5 mL of hydrochloric acid were added (pH approx 2). Afterwards, it was percoleted using vacuum through MCX carteridge (previosly conditionate with 2 mL of methanol and 2 mL of milliQ water) with a flow rate of 2 drops/sec. The cartridge was washed with 4 mL of 5% methanol in milliQ water and dried under vaccum. This step was repeated a second time. The analyte was eluted with 4 mL of extraction mixture and the eluate recovered. 3.5 mL of eluted

solution were dried under nitrogen flux and resuspended with 0.35 mL of blank solution. Finally the sample was vortexed for 1 minute, transferred in a vial and injected.

Results and discussions

Table A 33: Recovery results from method validation of MNBA and AMBA metabolites using the analytical method

Matrix	Analyte	Fortification level (µg/L) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
Drinking water	MNBA metabolite	LOQ (0.05 µg/L)	105.22 (100.2, 97.89, 111.62, 106.53, 109.84)	5.68	First mass transition
			109.89 (106.44, 102.98, 114.99, 110.54, 114.51)	4.72	Second mass transition
		10xLOQ (0.5 µg/L)	86.60 (86.11, 86.15, 85.74, 86.87, 88.14)	1.10	First mass transition
			92.83 (91.54, 91.48, 91.28, 93.06, 96.77)	2.50	Second mass transition
	AMBA metabolite	LOQ (0.05 µg/L)	103.79 (110.17, 113.41, 98.06, 96.73, 100.57)	7.25	First mass transition
			92.91 (112.16, 98.55, 83.75, 77.71, 92.36)	14.42	Second mass transition
		10xLOQ (0.5 µg/L)	92.17 (93.91, 92.55, 92.71, 91.97, 89.70)	1.68	First mass transition
			72.99 (73.06, 78.24, 72.78, 73.12, 67.76)	5.08	Second mass transition

Table A 34: Characteristics for the analytical method used for validation of AMBA and MNBA metabolites residues in drinking water

	MNBA metabolite	AMBA metabolite
Specificity	The method is specific. No significant peaks (≤30% LOQ) are detected at RT of the target analyte in the Blank and Test Solution with respect to the Spiked Test Solution for both mass transitions.	
Calibration (type, number of data points)	5 points 0.0133 µg/L to 1.7703 µg/L R^2>0.99	5 points 0.0158 µg/L to 1.5750 µg/L R^2>0.99
Assessment of matrix effects is presented	yes	
Limit of determination/quantification	LOQ = 0.05 µg/L LOD = 0.013 µg/L	

Conclusion

The method was successfully validated and is suitable for determination of MNBA and AMBA metabolites in drinking water.

A 2.1.2.4.3.3 Confirmatory method

LC-MS/MS is highly specific method therefore no confirmatory method is required.

A 2.1.2.4.4 Analytical method 4

A 2.1.2.4.4.1 Method validation

Comments of zRMS:	The analytical method was fully validated in term of specificity, linearity, repeatability, accuracy and LOQ according to SANCO 3029/99 rev. 4 and SANCO/825/00 Rev. 8.1. The validation was performed quantifying AMBA in ground water by LC-MS. LOQ corresponds to 0.05 µg/l. Two SRM transitions were monitored: 216 m/z - 136 m/z (quantifier ion) and 216 m/z – 91 m/z (qualifier ion). The method linearity was evaluated at 5 levels, ranging from 30%LOQ (0.015 µg/l) to 30xLOQ (1.5 µg/l). Recovery analysis was performed for samples spiked with AMBA at LOQ (0.05 µg/l) and 10xLOQ (0.5 µg/l), 5 replicate analysis were performed for each level. Mean recovery is between 70%-110% with RSD <20% in all cases, in accordance with acceptance criteria. Based on recovery results it is possible to asses that spiked sample extracts show good stability over analysis time. The validation data demonstrate that the method is suitable to qualitatively and quantitatively determine AMBA in ground water specimens. The method is successfully validated and accepted.

Reference:	KCP 5.2.16
Report	Validation of the analytical procedure for the determination of AMBA (CAS: 393085-45-5) in ground water by liquid chromatography. M. Rubino, 2018, Report No. 18.629767.0016
Guideline(s):	SANCO 3029/99 rev. 4
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Sample extraction:
About 3 ml of ground water were transferred into a 10 ml plastic falcon. 2 ml of this solution was transferred into a 10 ml tube and dried by N_2 flux at about 45°C. The dried sample was resuspended with 0.25 ml of blank solution. Vortexed for about 1 min and transferred into an HPLC vial and injected. The sample was prepared in duplicate.

Results and discussions

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

Matrix	Analyte	Fortification level (µg/L) (*n* = 5)	Mean recovery (%) (individual recovery values)	RSD (%)	Comments
Ground water	AMBA metabolite	LOQ (0.05 µg/L)	95 (98, 94, 99, 93, 93)	3	First mass transition
			106 (104, 109, 102, 109, 108)	3	Second mass transition
		10xLOQ (0.5 µg/L)	95 (96, 96, 95, 94, 97)	1	First mass transition
			97 (95, 101, 97, 94, 98)	3	Second mass transition

Table A 36: Characteristics for the analytical method used for validation of AMBA metabolite residues in ground water

	AMBA metabolite
Specificity	The method is specific. No significant peaks (≤30% LOQ) are detected at RT of the target analyte in the Blank and Test Solution with respect to the Spiked Test Solution for both mass transitions.
Calibration (type, number of data points)	5 points 0.015 µg/L to 1.5 µg/L First mass transition y=4841x R^2 = 0.9974 Second mass transition y=710x R^2 = 0.9939
Assessment of matrix effects is presented	yes
Limit of determination/quantification	LOQ = 0.05 µg/L

Conclusion

The method was successfully validated and is suitable for determination of AMBA metabolite in ground water.

A 2.1.2.4.4.2 Independent laboratory validation.

Please refer to A 2.1.2.4.3.2.

A 2.1.2.4.4.3 Confirmatory method

LC-MS/MS is highly specific method therefore no confirmatory method is required.

A 2.1.2.4.5 Analytical method 5

A 2.1.2.4.5.1 Method validation

Comments of zRMS:	The analytical method was fully validated in term of specificity, linearity, repeatability, accuracy and LOQ according to SANCO 3029/99 rev. 4 and SANCO/825/00 Rev. 8.1. The validation was performed quantifying MNBA in surface water by LC-MS. LOQ corresponds to 0.05 µg/l. Two SRM transitions were monitored: 244 m/z - 200 m/z (quantifier ion) and 244 m/z – 170 m/z (qualifier ion). The method linearity was evaluated at 5 levels, ranging from 30%LOQ (0.015 µg/l) to 30xLOQ (1.5 µg/l). Recovery analysis was performed for samples spiked with MNBA at LOQ (0.05 µg/l) and 10xLOQ (0.5 µg/l), 5 replicate analysis were performed for each level. Mean recovery is between 70%-110% with RSD <20% in all cases, in accordance with acceptance criteria. Based on recovery results it is possible to asses that spiked sample extracts show good stability over analysis time. The validation data demonstrate that the method is suitable to qualitatively and quantitatively determine MNBA in surface water specimens. The method is successfully validated and accepted.

Reference:	KCP 5.2.17
Report	Validation of the analytical procedure for the determination of MNBA (CAS: 110964-79-9) in surface water by liquid chromatography. M. Rubino, 2018, Report No. 18.629767.0014

Guideline(s):	SANCO 3029/99 rev. 4
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Sample extraction:
About 50 ml of surface water were transferred in a backer, added about 1.2 ml of hydrochloric acid (pH 2). Then, it was percolate using a regular vacuum through a MCX cartridge (previously conditionated with 2 ml of methanol and 2 ml of milliQ water) with a flow rate of 2 drops/sec. The cartridge was washed with 4 ml of 5% methanol in milliQ water and dried using centrifugation technique (2500 tpm, 5min), this step was repeated a second time. The analyte was eluted with 4 ml of extraction mixture and the eluate recovered. 3.5 ml of eluted solution were dried under nitrogen flux and resuspended with 0.25 ml of blank solution. Vortexed for 1 min. transferred in vial and injected. Test sample was prepared in duplicate.

Results and discussions

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

Matrix	Analyte	Fortification level (µg/L) (*n* = 5)	Mean recovery (%) (individual recovery values)	RSD (%)	Comments
Surrface water	MNBA metabolite	LOQ (0.05 µg/L)	102 (104, 107, 98, 96, 106)	5	First mass transition
			95 (105, 84, 89, 93, 104)	10	Second mass transition
		10xLOQ (0.5 µg/L)	81 (87, 84, 73, 73, 86)	9	First mass transition
			86 (89, 96, 80, 77, 90)	9	Second mass transition

Table A 38: Characteristics for the analytical method used for validation of MNBA metabolite residues in surface water

	MNBA metabolite
Specificity	The method is specific. No significant peaks (≤30% LOQ) are detected at RT of the target analyte in the Blank and Test Solution with respect to the Spiked Test Solution for both mass transitions.
Calibration (type, number of data points)	5 points 0.015 µg/L to 1.5 µg/L First mass transition y=35x R^2 = 0.9992 Second mass transition y=23x R^2 = 1.000
Assessment of matrix effects is presented	yes

	MNBA metabolite
Specificity	The method is specific. No significant peaks (≤30% LOQ) are detected at RT of the target analyte in the Blank and Test Solution with respect to the Spiked Test Solution for both mass transitions.
Limit of determination/quantification	LOQ = 0.05 µg/L

Conclusion

The method was successfully validated and is suitable for determination of MNBA metabolite in surface water.

A 2.1.2.4.5.2 Confirmatory method

LC-MS/MS is highly specific method therefore no confirmatory method is required.

A 2.1.2.4.6 Analytical method 6

A 2.1.2.4.6.1 Method validation

Comments of zRMS:	The analytical method was fully validated in term of specificity, linearity, repeatability, accuracy and LOQ according to SANCO 3029/99 rev. 4 and SANCO/825/00 Rev. 8.1. The validation was performed quantifying AMBA in surface water by LC-MS. LOQ corresponds to 0.05 µg/l. Two SRM transitions were monitored: 216 m/z - 136 m/z (quantifier ion) and 216 m/z – 91 m/z (qualifier ion). The method linearity was evaluated at 5 levels, ranging from 30%LOQ (0.015 µg/l) to 30xLOQ (1.5 µg/l). Recovery analysis was performed for samples spiked with AMBA at LOQ (0.05 µg/l) and 10xLOQ (0.5 µg/l), 5 replicate analysis were performed for each level. Mean recovery is between 70%-110% with RSD <20% in all cases, in accordance with acceptance criteria. Based on recovery results it is possible to asses that spiked sample extracts show good stability over analysis time. The validation data demonstrate that the method is suitable to qualitatively and quantitatively determine AMBA in surface water specimens. The method is successfully validated and accepted.

Reference:	KCP 5.2.18
Report	Validation of the analytical procedure for the determination of AMBA (CAS: 393085-45-5) in surface water by liquid chromatography. M. Rubino, 2018, Report No. 18.629767.0013
Guideline(s):	SANCO 3029/99 rev. 4
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Sample extraction:
About 3 ml of surface water were transferred into a 10 ml plastic falcon. 2 ml of this solution was transferred into a 10 ml tube and dried by nitrogen flux at about 45°C. The dried sample was resuspended with 0.25 ml of blank solution. Vortexed for about 1 min and transferred into an HPLC vial and injected. The sample was prepared in duplicate.

Results and discussions

Table A 39: Recovery results from method validation of AMBA metabolite using the analytical method

Matrix	Analyte	Fortification level (µg/L) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
Surrface water	AMBA metabolite	LOQ (0.05 µg/L)	99 (106, 99, 101, 98, 91)	6	First mass transition
			93 (94, 97, 94, 89, 90)	3	Second mass transition
		10xLOQ (0.5 µg/L)	90 (95, 88, 89, 89, 88)	3	First mass transition
			88 (90, 85, 88, 90, 85)	3	Second mass transition

Table A 40: Characteristics for the analytical method used for validation of AMBA metabolite residues in surface water

	AMBA metabolite
Specificity	The method is specific. No significant peaks (≤30% LOQ) are detected at RT of the target analyte in the Blank and Test Solution with respect to the Spiked Test Solution for both mass transitions.
Calibration (type, number of data points)	5 points 0.015 µg/L to 1.5 µg/L First mass transition y=4607x R^2 = 0.9998 Second mass transition y=678x R^2 = 0.9997
Assessment of matrix effects is presented	yes
Limit of determination/quantification	LOQ = 0.05 µg/L

Conclusion

The method was successfully validated and is suitable for determination of AMBA metabolite in surface water.

A 2.1.2.4.6.2 Confirmatory method

LC-MS/MS is highly specific method therefore no confirmatory method is required.

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

zRMS comments:
Residue definition for monitoring purposes for air according to the EFSA Journal 2016;14(3):4419 is mesotrione (*residues of mesotrione in air can be monitored by LC-MS/MS with a LOQ of 0.45 µg/m*^{3}) so analytical methods should include determination of this compound.

A 2.1.2.5.1 Analytical method 1

A 2.1.2.5.1.1 Method validation

Comments of zRMS:	The analytical method was fully validated in term of specificity, linearity, repeatability, accuracy and LOQ according to SANCO 3029/99 rev. 4 and SANCO/825/00 Rev. 8.1. The validation was performed quantifying mesotrione in air by LC-MS. LOQ corresponds to 0.45 µg/m^3. Two SRM transitions were monitored: 338 m/z - 291 m/z (quantifier ion) and 338 m/z – 212 m/z (qualifier ion). The method linearity was evaluated at 5 levels, ranging from 30%LOQ (0.135µg/m^3) to 30xLOQ (13.5 µg/m^3). Recovery analysis was performed for samples spiked with mesotrione at LOQ (0.45 µg/m^3) and 10xLOQ (4.5 µg/m^3), 5 replicate analysis were performed for each level. Mean recovery is between 70%-110% with RSD <20% in all cases, in accordance with acceptance criteria. Based on recovery results it is possible to asses that spiked sample extracts show good stability over analysis time. The validation data demonstrate that the method is suitable to qualitatively and quantitatively determine mesotrione in air specimens. The method is successfully validated and accepted.

Reference:	KCP 5.2.21
Report	Validation of the analytical procedure for the determination of mesotrione (CAS: 104206-82-8) in air by LC-MS. M. Rubino, 2018, Report No. 18.629767.0018
Guideline(s):	SANCO 3029/99 rev. 4
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Sample extraction:
A polyurethane (PUF) plug was soaked with acetone for about 6 hours and then dried in a heater at about 50°C. Then the plug was put into a glass syringe connected to a pump. Air was collected for about 6 hours with a flow of 3l/min (1080 l) by using a pump linked to the glass syringe conraining the plug. The collection system was put into the climatic chamber SRA 90 at about 35°C and 80% of humidity. After sampling the plug was transferred into a 100 ml round bottomed flask and soaked into 40 ml of extraction mixture. After sonication for about 40 minutes, 0.5 ml was transferred into a 10 ml glass tube and added 0.5 ml of mobile phase A. Vortexed and then transferred into an HPLC vial and injected. Test sample was prepared in duplicate.

Results and discussions

Table A 41: Recovery results from method validation of Mesotrione using the analytical method

Matrix	Analyte	Fortification level (µg/m^3) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
Air	Mesotrione	LOQ (0.4 µg/m^3)	80 (84, 81, 77, 79, 81)	3	First mass transition
			85 (88, 92, 80, 80, 85)	6	Second mass transition
		10xLOQ (4.0 µg/m^3)	99 (95, 102, 101, 98, 97)	3	First mass transition

Matrix	Analyte	Fortification level (µg/m³) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
			100 (94, 100, 105, 99, 101)	4	Second mass transition

Table A 42: Characteristics for the analytical method used for validation of Mesotrione residues in air

	Mesotrione
Specificity	The method is specific. No significant peaks (≤30% LOQ) are detected at RT of the target analyte in the Blank and Test Solution with respect to the Spiked Test Solution for both mass transitions.
Calibration (type, number of data points)	5 points 0.11 µg/m³ to 15.02 µg/m³ First mass transition y=514138x R^2 = 0.9956 Second mass transition y=121371x R^2 = 0.9966
Assessment of matrix effects is presented	yes
Limit of determination/quantification	LOQ = 0.45 µg/m³

Conclusion

The method was successfully validated and is suitable for determination of Mesotrione in air.

A 2.1.2.5.1.2 Confirmatory method

LC-MS/MS is highly specific method therefore no confirmatory method is required.

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

zRMS comments:
Residue definition for monitoring purposes for body fluids and tissues according to the EFSA Journal 2016;14(3):4419 is mesotrione (*the QuEChERS method (LC-MS/MS) can be used for monitoring mesotrione residues in blood with a LOQ of 0.01 mg/kg*) so analytical methods should include determination of this compound.

A 2.1.2.6.1 Analytical method 1

A 2.1.2.6.1.1 Method validation

Comments of zRMS:	The analytical method was fully validated in term of specificity, linearity, repeatability, accuracy and LOQ according to SANCO 3029/99 rev. 4 and SANCO/825/00 Rev. 8.1. The validation was performed quantifying mesotrione in blood by LC-MS. LOQ corresponds to 0.05 µg/l. Two SRM transitions were monitored: 338 m/z - 291 m/z (quantifier ion) and 338 m/z – 212 m/z (qualifier ion). The method linearity was evaluated at 5 levels, ranging from 30%LOQ (0.003 mg/l) to 30xLOQ (0.3 mg/l). Recovery analysis was performed for samples spiked with mesotrione at LOQ (0.01 mg/l) and 10xLOQ (0.09 mg/l), 5 replicate analysis were performed for each level. Mean recovery is between 70%-110% with RSD <20% in all cases, in accordance with acceptance criteria. Based on

	recovery results it is possible to asses that spiked sample extracts show good stability over analysis time. The validation data demonstrate that the method is suitable to qualitatively and quantitatively determine mesotrione in blood specimens. The method is successfully validated and accepted.

Reference:	KCP 5.2.22
Report	Validation of the analytical procedure for the determination of mesotrione (CAS: 104206-82-8) in blood by liquid chromatography. M. Rubino, 2018, Report No. 18.629767.0003
Guideline(s):	SANCO 3029/99 rev. 4
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Sample extraction:
About 5.00 ml of blood were transferred into a 50 ml falcon. 10 ml of extraction mixture were added to the sample. After vortexing for about 1 min, about 4 g of magnesium sulphate anhydrous, 1 g of sodium chloride, 1 g of sodium citrate dehydrate and 0.5 g of disodium hydrogencitrate sesquidrate were added to the sample and vortexed with each addition for about 1 min. The tube was centrifuged at 4750 rpm for 5min. The supernatant was transferred in a plastic tube and kept at about -20°C for about 1 hour. Then the tube was centrifuged at 4750 rpm for 5 min and it was proceed to purification of the supernatant. 4 ml of supernatant were transferred into a 10 ml plastic tube, containing about 900 mg of magnesium sulphate anhydrous and 150 mg of C18 resin. Vortexed for about 1 min and centrifuged at 3000 rpm for 5min. 0.5 ml of supernatant were transferred in 10 ml tube and were added 0.5 ml of mobile phase A. Vortexed for about 1min, then transferred into an HPLC vial and injected. The sample was prepared in duplicate.

Results and discussions

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

Matrix	Analyte	Fortification level (mg/L) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
Blood	Mesotrione	LOQ (0.01 mg/L)	83 (78, 87, 87, 84, 81)	4.6	First mass transition
			94 (84, 102, 106, 94, 85)	10.5	Second mass transition
		10xLOQ (0.1 mg/L)	89 (91, 90, 87, 86, 91)	2.6	First mass transition
			93 (97, 93, 91, 92, 94)	2.4	Second mass transition

Table A 44: Characteristics for the analytical method used for validation of Mesotrione residues in blood

	Mesotrione
Specificity	The method is specific. No significant peaks (≤30% LOQ) are detected at RT of the target analyte in the Blank and Test Solution with respect to the Spiked Test Solution for both mass transitions.
Calibration (type, number of data points)	5 points 0.03 mg/L to 0.325 mg/L First mass transition y=905386x R^2 = 0.9958 Second mass transition y=211378x R^2 = 0.9939
Assessment of matrix effects is presented	yes
Limit of quantification	LOQ = 0.01 mg/L

Conclusion

The method was successfully validated and is suitable for determination of Mesotrione in blood.

A 2.1.2.6.1.2 Confirmatory method

LC-MS/MS is highly specific method therefore no confirmatory method is required.

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

No new or additional studies have been submitted.