FINAL REGISTRATION REPORT

Part B

Section 5

Analytical Methods

Detailed summary of the risk assessment

Product code: GLOB289H / SAP63H

Product name(s): Zeppos / Moxie

Chemical active substance(s):

Iodosulfuron-methyl-sodium, 6 g/kg
Mesosulfuron-methyl, 30 g/kg
Safener: Mefenpyr-diethyl, 90 g/kg

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

Applicant: Globachem N.V. / Ascenza Agro S.A.

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5 Analytical methods

This document summarises the information related to the analytical methods for the plant protection product Iodosulfuron-methyl-sodium + Mesosulfuron-methyl + Mefenpyr-diethyl (0.6+3+9)%WG (also referred to as SAP63H, GLOB289H, Iodosulfuron + Mesosulfuron (0.6% + 3%) WG and Zeppos in the dossier). The product contains two active substances iodosulfuron-methyl-sodium and mesosulfuron-methyl-sodium, and safener mefenpyr-diethyl.

Where appropriate, this document refers to the conclusions of the EU reviews of the active substances. This will be where:

- The active substance data is relied upon in the risk assessment of the formulation; or
- The EU review concluded that additional data/information should be considered at national registration.

Note: This Part B document only reviews data (Annex II and/or Annex III) and additional information that has not previously been considered within the EU review process, as part of the Annex I inclusion decision. New Annex II or Annex III data were included if they are considered essential for the evaluation and in this case a full study summary is provided. In the case where studies have been previously evaluated at European level, detailed summaries have not been provided.

The product Iodosulfuron-methyl-sodium + Mesosulfuron-methyl + Mefenpyr-diethyl (0.6% + 3% + 9%) WG was not the representative formulation during the Annex I inclusion of Iodosulfuron-methyl-sodium or Mesosulfuron-methyl and has thus not yet been evaluated.

Iodosulfuron-methyl-sodium

Iodosulfuron-methyl-sodium was included into Annex I of Directive 91/414/EEC in 2003 (Directive 2003/84/EC) and re-evaluated in accordance with Regulation (EC) No 1107/2009 and Commission Implementing Regulation (EU) No 844/2012, leading to the renewal of the approval of the active substance iodosulfuron-methyl-sodium (Commission Implementing Regulation (EU) 2017/407 of 8 March 2017, entry into force 1st of April 2017).

For the implementation of the Uniform Principles of Annex VI, the conclusions of the Renewal Report on iodosulfuron-methyl-sodium, as finalised in the Standing Committee on Plants, Animals, Food and Feed at its meeting on 7 December 2016 shall be taken into account.

In this overall assessment Member States should pay attention to:

- The protection of consumers,
- The protection of non-target terrestrial plants,
- The protection of aquatic plants

The Renewal Report (SANTE/2016/11167 Rev 3, 7/12/2016) for iodosulfuron-methyl-sodium provides a summary of the relevant scientific information from the EU review.

Mesosulfuron-methyl

Mesosulfuron-methyl was included in Annex I of Directive 91/414/EEC in 2003 (Directive 2003/119/EEC) and re-evaluated in accordance with Regulation (EC) No 1107/2009 and Commission Implementing Regulation (EU) No 844/2012, leading to the renewal of the approval of the active substance mesosulfuron-methyl (Commission Implementing Regulation (EU) 2017/755 of 28 April 2017, entry into force 1st of July 2017).

For the implementation of the Uniform Principles of Annex VI, the conclusions of the Renewal Report on mesosulfuron-methyl, as finalised in the Standing Committee on Plants, Animals, Food and Feed at is meeting on 23 March 2017 shall be taken into account.

In this overall assessment Member States should pay attention to:

- The protection of aquatic organisms and non-target terrestrial plants;
- The protection of groundwater

The Renewal Report (SANTE/11827/2016 Rev 2, 23/03/2017) for mesosulfuron-methyl provides a summary of the relevant scientific information from the EU review.

Safener mefenpyr-diethyl
Mefenpyr-diethyl is a safener used in combination with herbicides and was not reviewed under Directive 91/414/EEC or Regulation (EC) No 1107/2009. In order to facilitate the assessment of products containing mefenpyr-diethyl, France and Austria in a work-sharing project prepared an assessment report for this substance in the format of a DAR. France was responsible for the sections "Phys-Chem Properties" (B.1-B.5), Environmental Fate and Ecotoxicology (B.8-B.9) and Austria for sections Toxicology and Residue Data (B.6-B.7). A bilateral peer-review in the form of comments took place between the two rapporteurs; the respective reporting tables were made available to all MS. In September 2011 the assessment report was "peer-reviewed" (in an unscheduled procedure on voluntary basis) by all MS. The revised assessment report can be found on CIRCA (Archive individual substances – Mefenpyr-diethyl (safener)).
All exposure and risk assessments presented will be based on agreed endpoints, if not otherwise stated.

5.1 Conclusion and summary of assessment

Sufficiently sensitive and selective analytical methods are available for the active substances and relevant impurities in the plant protection product.

Sufficiently sensitive and selective analytical methods are available for all analytes included in the residue definitions.

Commodity/crop	Supported/ Not supported
Cereals	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 analytical method has been developed for the determination of the active substances iodosulfuron-methyl-sodium, mesosulfuron-methyl and mefenpyr-diethyl in GLOB289H.

This method has not previously been reviewed according to the Uniform Principles and is provided in support of this assessment.

Comments of zRMS:	Accepted

Reference:	KCP 5.1.1-01
Report	Iodosulfuron-methyl-sodium 0.6% + Mesosulfuron-methyl 3.0% + Mefenpyr-diethyl 9.0% WG (SAP63H) – Physical, chemical and technical properties of the plant protection product – Annex I: Mesosulfuron, Iodosulfuron and Mefenpyr method validation and quantification; Silva, S.; 2019; EF/298/19
Guideline(s):	Yes (SANCO 3030/99)

Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Mesosulfuron-methyl, Iodosulfuron-methyl-sodium and Mefenpyr-diethyl were quantified using a HPLC-DAD method developed and validated. The method is presented below.

Chromatographic conditions – HPLC-DAD for active substance quantification

Mobile phase:	40.0 % Water ultrapure : 60 % Acetonitrile
Flow Rate:	1.5 mL/min
Column:	Hypersl ODS-C18, 4mm x 250 mm, 5 µm
Column temperature :	30.0°C
Wavelength:	254 nm (Mesosulfuron and Iodosulfuron) 300 nm (Mefenpyr)
Injection Volume:	10 µL
Retention Time:	Iodosulfuron-methyl-sodium: Approximately 2.4 minutes Mesosulfuron-methyl: Approximately 1.9 minutes Mefenpyr-diethyl: Approximately 5.5 minutes
Run time	10 minutes

MS conditions for active substance identification

MS ionization mode :	ESI
Polarity:	Positive
Mass range :	100 *m/z* - 600 *m/z*
Dry gas temperature :	350°C
Dry gas flow :	12 L/min
Nebulizer pressure :	55 psi
HV capillary :	- 3500 V
HV end plate offset :	- 500 V

Standard solution preparation

Mesosulfuron

25 mg ± 2.5 mg of Mesosulfuron-methyl reference material was weighed into a 25 mL volumetric flask, dissolved and made to volume with acetonitrile (1.0 mg/mL).

Iodosulfuron

25 mg ± 2.5 mg of Iodosulfuron-methyl-sodium reference material was weighed into a 25 mL volumetric flask, dissolved and made to volume with acetonitrile (1.0 mg/mL).

Mefenpyr

50 mg ± 2.5 mg of Mefenpyr-diethyl reference material was weighed into a 25 mL volumetric flask, dissolved and made to volume with acetonitrile (2.0 mg/mL).

Final solution

A calibration solution was prepared by diluting 1.5 mL of Mesosulfuron standard stock solution, 0.3 mL of Iodosulfuron stock solution and 2.25 mL of Mefenpyr standard stock solution to a final volume of 10 mL in acetonitrile (0.15 $mg_{mesosulfuron}$/mL, 0.03 $mg_{iodosulfuon}$/mL and 0.45 $mg_{mefenpyr}$/mL).

Sample solution preparation

The test item was grinded carefully and 500 mg ± 10 mg was weighed into a volumetric flask, dissolved and made to volume with acetonitrile. The solution was placed into an ultra-sonic bath and filtered using a 0.2 µm disk filter (5.0 mg/mL). This sample was prepared in duplicate.

Validation - Results and discussions

The validation parameters for the iodosulfuron-methyl-sodium, mesosulfuron-methyl and mefenpyr-diethyl methodology have been met for this study under the SANCO/3030/99 rev. 4 guidelines. A summary of these results are presented below.

Table 5.2-1: Methods suitable for the determination of active substances iodosulfuron-methyl-sodium, mesosulfuron-methyl and mefenpyr-diethyl in plant protection product GLOB289H

	Iodosulfuron-methyl-sodium	**Mesosulfuron-methyl**	**Mefenpyr-diethyl**
Author(s), year	Silva S., 2019	Silva S., 2019	Silva S., 2019
Principle of method	HPLC-DAD-MS	HPLC-DAD-MS	HPLC-DAD-MS
Linearity	Five calibration solutions were prepared from the duplicate stock solutions by dilutions with acetonitrile. Linear between 0.005 and 0.060 mg/mL, 0.11% and 1.28% r = 0.9999 y = 8039.0415 x -1.5325	Five calibration solutions were prepared from the duplicate stock solutions by dilutions with acetonitrile. Linear between 0.010 and 0.300 mg/mL, 0.20% and 6.17% r = 0.9999 y = 10845.0170 x -14.6757	Five calibration solutions were prepared from the duplicate stock solutions by dilutions with acetonitrile. Linear between 0.0 and 1.0 mg/mL, 0.58% and 19.36% r = 0.9999 y = 10748.0113 x -14.0886
Precision – Repeatability Mean n = 5 (%RSD)	Five independent solutions of the same sampe were injected and the peak area and sample mass related. The method is repeatable with the sample mass - peak area ratio ranging from 0.4323 to 0.4361, a mean of 0.4337, a standard deviation of 0.0017 and a percentage relative standard deviation of 0.4.	Five independent solutions of the same sampe were injected and the peak area and sample mass related. The method is repeatable with the sample mass - peak area ratio ranging from 3.1413 to 3.1659, a mean of 3.1513, a standard deviation of 0.0095 and a percentage relative standard deviation of 0.3.	Five independent solutions of the same sampe were injected and the peak area and sample mass related. The method is repeatable with the sample mass - peak area ratio ranging from 9.5161 to 9.5863, a mean of 9.5444, a standard deviation of 0.0289 and a percentage relative standard deviation of 0.3.
Accuracy n = 5 (% Recovery)	Accuracy was evaluated at two levels. The content of five independent solutions prepared with blank formulation, Mesosulfuron-methyl technical material, Iodosulfuron-methyl-sodium technical material and Mefenpyr-diethyl technical material was determinded. First level: 0.100 mg/mL mesosulfuron, 0.015 mg/mL iodosulfuron and 0.300 mg/mL mefenpyr Second level: 0.150 mg/mL mesosulfuron, 0.030 mg/mL iodosulfuron and 0.450 mg/mL mefenpyr		
	The method is accurate with values of percentage recovery ranging from 99.13% to 100.87%, a mean of 100.23%, a standard deviation of 0.712 and a %RSD of 0.71 in the first level; and values of percentage recovery ranging from 99.15% to 100.31%, a mean of 99.74%, a standard deviation of 0.593 and a %RSD of 0.59 in the second level.	The method is accurate with values of percentage recovery ranging from 98.59% to 100.21%, a mean of 99.30%, a standard deviation of 0.626 and a %RSD of 0.63 in the first level; and values of percentage recovery ranging from 98.81% to 99.78%, a mean of 99.78%, a standard deviation of 0.392 and a %RSD of 0.40 in the second level.	The method is accurate with values of percentage recovery ranging from 98.88% to 100.65%, a mean of 99.47%, a standard deviation of 0.717 and a %RSD of 0.72 in the first level; and values of percentage recovery ranging from 98.34% to 100.03%, a mean of 99.17%, a standard deviation of 0.706 and a %RSD of 0.71 in the second level.
Interference/ Specificity	To evaluate the presence of compounds that might interfere with the quantification of Mesosulfuron, Iodosulfuron and Mefenpyr, blank solution, Mesosulfuron-methyl-sodium standard solution, Iodosulfuron-methyl-sodium standard solution, Mefenpyr-diethyl standard solution, sample solution and blank formulation solution were injected separately. As it can be seen in representative chromatograms there are no peaks that could interfere with active substance quantification.		
RMS Comment	Accepted The validation parameters for the Iodosulfuron-methyl-sodium, methodology have been met for this study under the SANCO/3030/99 rev.4 guide-	Accepted The validation parameters for the Mesosulfuron-methyl methodology have been met for this study under the SANCO/3030/99 rev.4 guidelines but	Accepted The validation parameters for Mefenpyr-diethyl methodology have been met for this study under the SANCO/3030/99 rev.4 guidelines but it should be

	Iodosulfuron-methyl-sodium	Mesosulfuron-methyl	Mefenpyr-diethyl
	lines, but it should be under rev. 5 because the test started at December 2019 After analysis, it was found that all results are within acceptable limits	it should be under rev. 5 because the test started at December 2019 After analysis, it was found that all results are within acceptable limits	under rev. 5 because the test started at December 2019 After analysis, it was found that all results are within acceptable limits

Conclusion

The validation parameters for the Iodosulfuron-methyl-sodium, Mesosulfuron-methyl and Mefenpyr-diethyl methodology have been met for this study under the SANCO/3030/99 rev.4 guidelines.

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

No relevant impurities are present in GLOB289H

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

Under current EU legislation methods on formulants are not required. However if a formulant is defined as relevant for toxicity (environment, health), then a method needs to be provided. There are however no formulants in GLOB289H that are defined as relevant for toxicity.

5.2.1.4 Applicability of existing CIPAC methods (KCP 5.1.1)

There are no CIPAC methods available for the determination of Iodosulfuron-methyl-sodium and Mesosulfuron-methyl.
A CIPAC method (651.229) for determination of Mefenpyr-diethyl exists:

- HPLC method (651.229/TC/M-) for the determination of mefenpyr-diethyl in TC
- HPLC method (651.229/WG/M-) for the determination of mefenpyr-diethyl in WG formulation.

The above methods were not validated for GLOB289H, although the method described and validated under KCP 5.1.1 inspires from the CIPAC method.

5.2.2 Methods for the determination of residues (KCP 5.1.2)

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

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

Component of residue definition: iodosulfuron-methyl-sodium				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Plant, high protein/high starch content (dry commodities; wheat straw) (Residues)	Primary	0.01 mg/kg	UPLC-TQ-S-micro	Arias A., 2019
Water (*daphnia*) (Ecotoxicology)	Primary	12.43 µg/L	RP-HPLC-MS/MS	Renner P., 2018a
Water (algae) (Ecotoxicology)	Primary	5.981 µg/L	RP-HPLC-MS/MS	Renner P., 2018b
Water (*lemna)* (Ecotoxicology)	Primary	0.047 µg/L	RP-HPLC-MS/MS	Renner P., 2018c
Water (*lemna)* (Ecotoxicology)	Primary	0.02 µg/L	RP-HPLC-MS/MS	Renner P, 2019a
Water (*lemna)* (Ecotoxicology)	Primary	0.02 µg/L	RP-HPLC-MS/MS	Renner P, 2019b

Component of residue definition: mesosulfuron-methyl				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Plant matrices (wheat grain and straw) (Residues)	Primary	0.01 mg/kg	UPLC-TQ-S-micro	Arias A., 2019
Water (*daphnia*) (Ecotoxicology)	Primary	64.79 µg/L	RP-HPLC-MS/MS	Renner P., 2018a
Water (algae) (Ecotoxicology)	Primary	31.19 µg/L	RP-HPLC-MS/MS	Renner P., 2018b
Water (*lemna)* (Ecotoxicology)	Primary	0.059 µg/L	RP-HPLC-MS/MS	Renner P., 2018c
Water (*lemna)* (Ecotoxicology)	Primary	0.047 µg/L	RP-HPLC-MS/MS	Renner P, 2019a
Water (*lemna)* (Ecotoxicology)	Primary	0.035 µg/L	RP-HPLC-MS/MS	Renner P, 2019b

Component of residue definition: mefenpyr-diethyl				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Plant, high protein/high starch content (dry commodities; wheat grain) (Residues)	Primary	0.04 mg/kg	UPLC-TQ-S-micro	Arias A., 2019
Plant, high protein/high starch content (dry commodities; wheat straw) (Residues)	Primary	0.01 mg/kg	UPLC-TQ-S-micro	Arias A., 2019
Component of residue definition: triazine amine (metabolite of iodosulfuron-methyl-sodium)				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Plant, high water content (barley forage) (Residues)	Primary	0.01 mg/kg	UPLC-TQ-S-micro	Gordo J., 2019
Plant, high protein/high starch content (dry commodities; barley hay, barley and wheat grain and straw) (Residues)	Primary	0.01 mg/kg	UPLC-TQ-S-micro	Gordo J., 2019

Table 5.2-3: Statement on extraction efficiency

	Method for products of plant origin
Required, available from:	In the new residue studies, the extraction method based on the Quechers method was validated against the extraction method applied in 14^{C}metabolism studies. The cross-validation studies are summarised in Appendix 2.

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 iodosulfuron-methyl-sodium (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 Renewal 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	Sum of iodosulfuron-methyl and its salts, expressed as iodosulfuron-methyl-sodium	0.01 mg/kg	EFSA Journal 2016;14(4):4453
Plant, high acid content		0.01 mg/kg	EFSA Journal 2016;14(4):4453
Plant, high protein/high starch content (dry commodities)		0.01 mg/kg	EFSA Journal 2016;14(4):4453
Plant, high oil content		0.01 mg/kg	EFSA Journal 2016;14(4):4453
Muscle	-	Not required	-
Milk		Not required	-
Eggs		Not required	-
Fat		Not required	-
Liver, kidney		Not required	-
Soil (Ecotoxicology)	Sum of iodosulfuron-methyl and its salts, expressed as iodosulfuron-methyl-sodium	0.021 µg/kg	EFSA Journal 2016;14(4):4453 NOEC < 0.032 g a.s./ha
Drinking water (Human toxicology)	Sum of iodosulfuron-methyl and its salts, expressed as iodosulfuron-methyl-sodium and metsulfuron-methyl (AE F075736)	0.1 µg/L	general limit for drinking water
Surface water (Ecotoxicology)		0.74 µg/L	EFSA Journal 2016;14(4):4453 ErC_{50} (*Lemna gibba)*
Air	Sum of iodosulfuron-methyl and its salts, expressed as iodosulfuron-methyl-sodium	15 µg/m^3	EFSA Journal 2016;14(4):4453 AOEL sys: 0.05 mg/kg bw/d
Tissue (meat or liver)	Sum of iodosulfuron-methyl and its salts, expressed as iodosulfuron-methyl-sodium	0.01 mg/kg	General limit for body tissue
Body fluids		0.05mg/L	General limit for body fluids

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 iodosulfuron-methyl-sodium 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: iodosulfuron-methyl-sodium				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
High water / Hight acid / High oil / high protein / high starch content	Primary	0.01 mg/kg	UPLC-TQ-S-micro	Morias, 2017a
	ILV	0.01 mg/kg	UPLC-MS/MS	Schlewitz, 2018

Table 5.3-3: Statement on extraction efficiency

	Method for products of plant origin
Required, available from:	Morias, 2017b
Not required, because:	-

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

No methods for the analysis of residues in food and feed of animal origin were submitted. No residue definition is proposed since no residues in food and feed of animal origin are anticipated.

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 iodosulfuron-methyl-sodium in soil is given in the following tables.

Table 5.3-4: Validated methods for soil

Component of residue definition: iodosulfuron-methyl-sodium			
Method type	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	0.01 mg/kg	UPLC-TQ-S-micro	Arias, 2017
Confirmatory	Not required (primary method is LC-MS/MS)		

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 iodosulfuron-methyl-soduim in surface and drinking water is given in the following tables.

Table 5.3-5: Validated methods for water

Component of residue definition: iodosulfuron-methyl-sodium				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Surface water	Primary	0.05 µg/L	HPLC-MS/MS	Gaffney V., 2017
	ILV	0.05 µg/L	HPLC-MS/MS	Schlewitz P., 2017
Drinking water	Primary	0.05 µg/L	HPLC-MS/MS	Gaffney V., 2017

Table 5.3-6: Validated methods for water

Component of residue definition: metsulfuron-methyl				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Surface water	Primary	0.05 µg/L	HPLC-MS/MS	Gaffney V., 2017
	ILV	0.05 µg/L	HPLC-MS/MS	Schlewitz P., 2017
Drinking water	Primary	0.05 µg/L	HPLC-MS/MS	Gaffney V., 2017

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 iodosulfuron-methyl-sodium in air is given in the following tables.

Table 5.3-7: Validated methods for air

Component of residue definition: iodosulfuron-methyl			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	1.6 µg/m^3	HPLC-UV	EFSA 2016; 14(10):4584 (Reichert, 2000 – 2009)

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 iodosulfuron-methyl-sodium in body fluids and tissues is given in the following table. For the detailed evaluation of new studies it is referred to Appendix 2.

Table 5.3-8: Methods for body fluids and tissues

Component of residue definition: Iodosulfuron-methyl-sodium			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	0.01 mg/kg body tissue / 0.05mg/L body fluids	LC-MS/MS	Andrews G., Bills K., 2019

Component of residue definition: Iodosulfuron-methyl-sodium			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Confirmatory	/	/	/

5.3.2.8 Other studies/ information

/

5.3.3 Description of analytical methods for the determination of residues of mesosulfuron-methyl (KCP 5.2)

5.3.3.1 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 5.3-9: 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	Mesosulfuron-methyl	0.01 mg/kg	EFSA Journal 2016;14(10):4584
Plant, high acid content		0.01 mg/kg	EFSA Journal 2016;14(10):4584
Plant, high protein/high starch content (dry commodities)		0.01 mg/kg	EFSA Journal 2016;14(10):4584
Plant, high oil content		0.01 mg/kg	EFSA Journal 2016;14(10):4584
Muscle	Mesosulfuron-methyl	0.02 *mg/kg	EFSA Journal 2016;14(10):4584
Milk		0.02* mg/kg	EFSA Journal 2016;14(10):4584
Eggs		0.02* mg/kg	EFSA Journal 2016;14(10):4584
Fat		0.02* mg/kg	EFSA Journal 2016;14(10):4584
Liver, kidney		0.02* mg/kg	EFSA Journal 2016;14(10):4584
Soil (Ecotoxicology)	Mesosulfuron-methyl	0.05 mg/kg	EFSA Journal 2016;14(10):4584 NOEC = 0.125 g/kg
Drinking water (Human toxicology)	Mesosulfuron-methyl	0.1 µg/L	general limit for drinking water

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Surface water (Ecotoxicology)	Mesosulfuron-methyl	0.39 µg/L	EFSA Journal 2016;14(10):4584 PNEC
Air	Mesosulfuron-methyl	12 µg/m^3	AOEL sys: 0.13 mg/kg bw/d
Tissue (meat or liver)	Mesosulfuron-methyl	0.01 mg/kg	General limit for tissues
Body fluids		0.05 mg/kg	General limit for blood

5.3.3.2 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 mesosulfuron-methyl in plant matrices is given in the following tables. For the detailed evaluation of new studies it is referred to Appendix 2.

Table 5.3-10: 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: mesosulfuron-methyl				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
High water content	Primary	0.01 mg/kg	HPLC-MS/MS	Gordo J., 2018a
	ILV	0.01 mg/kg	HPLC-MS/MS	Wößner A., 2018a
High acid content	Primary	0.01 mg/kg	HPLC-MS/MS	Gordo J., 2018a
	ILV	0.01 mg/kg	HPLC-MS/MS	Wößner A., 2018a
High oil content	Primary	0.01 mg/kg	HPLC-MS/MS	Gordo J., 2018a
	ILV	0.01 mg/kg	HPLC-MS/MS	Wößner A., 2018a
High protein/high starch content (dry)	Primary	0.01 mg/kg	HPLC-MS/MS	Gordo J., 2018a
	ILV	0.01 mg/kg	HPLC-MS/MS	Wößner A., 2018a

Table 5.3-11: Statement on extraction efficiency

	Method for products of plant origin
Required, available from:	EFSA Journal 2016;14(10):4584
Not required, because:	-

Extraction efficiency has been demonstrated for high water content crops and straw. For dry commodities, residues are very low.

5.3.3.3 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 mesosulfuron-methyl in animal matrices is given in the following tables.

Table 5.3-12: Validated methods for food and feed of animal origin

Component of residue definition: mesosulfuron-methyl				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Milk	Primary	0.01 mg/kg	HPLC-MS/MS	EFSA Journal 2016;14(10):4584 (Schmeer, 2010)
	ILV	0.01 mg/kg	HPLC-MS/MS	Arias A., 2018
Eggs	Primary	0.01 mg/kg	HPLC-MS/MS	EFSA Journal 2016;14(10):4584 (Schmeer, 2010)
	ILV	0.01 mg/kg	HPLC-MS/MS	Arias A., 2018
Muscle	Primary	0.01 mg/kg	HPLC-MS/MS	EFSA Journal 2016;14(10):4584 (Schmeer, 2010)
	ILV	0.01 mg/kg	HPLC-MS/MS	Arias A., 2018
Fat	Primary	0.01 mg/kg	HPLC-MS/MS	EFSA Journal 2016;14(10):4584 (Schmeer, 2010)
	ILV	0.01 mg/kg	HPLC-MS/MS	Arias A., 2018
Kidney, liver	Primary	0.01 mg/kg	HPLC-MS/MS	EFSA Journal 2016;14(10):4584 (Schmeer, 2010)
	ILV	0.01 mg/kg	HPLC-MS/MS	Arias A., 2018

Table 5.3-13: Statement on extraction efficiency

	Method for products of animal origin
Required, available from:	-
Not required, because:	Extraction efficiency is only necessary for pesticides showing significant residues, i.e. residues at or above the limit of quantification (LOQ) of the analytical method. This is not the case in food and feed of animal origin

5.3.3.4 Description of methods for the analysis of soil (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of mesosulfuron-methyl in soil is given in the following tables.

Table 5.3-14: Validated methods for soil

Component of residue definition: mesosulfuron-methyl			
Method type	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	0.1 µg/kg	UPLC-MS/MS	Gordo J., 2018b

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

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

Table 5.3-15: Validated methods for water (if appropriate)

Component of residue definition: mesosulfuron-methyl				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Drinking and surface water	Primary	0.05 µg/L	LC-MS/MS	Gaffney V., 2018
	ILV	0.05 µg/L	LC-MS/MS	Wößner A., 2018b

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

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

Table 5.3-16: Validated methods for air

Component of residue definition: mesosulfuron-methyl			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	12 µg/m^3	HPLC-UV	EFSA 2016; 14(10):4584 (Reichert, 2000)

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

In the EFSA review of mesosulfuron-methyl (EFSA, 2016), a data gap was identified for an analytical method for body fluids. For tissues, analytical methods (Schmeer, K., Phillipowski, C., 2010 – amended in 2011 and ILV Derek Netzband, 2010) have been validated for foodstuff of animal origin (muscle, liver, kidney). An overview on the acceptable methods and possible data gaps for analysis of mesosulfuron-methyl in body fluids is given in the following table. For methods of analysis in body tissues, reference is made to Table 5.3-12. For the detailed evaluation of new studies it is referred to Appendix 2.

Table 5.3-17: Methods for body fluids and tissues

Component of residue definition: Mesosulfuron-methyl			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	0.01 mg/kg body tissue 0.05mg/L body fluids	HPLC-MS/MS	*Tissues*: EFSA Journal 2016;14(10):4584 (Schmeer, 2010) *Fluids*: Knop M., 2018 and Andrews G., Pearson J., 2018 (ILV)
Confirmatory	-	-	-

For any special comments or remarkable points concerning the analytical methods for body fluids and tissues please refer to Appendix 2.

5.3.3.8 Other studies/ information

/

5.3.4 Description of analytical methods for the determination of residues of mefenpyr-diethyl (KCP 5.2)

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

The following table summarised the agreed European residue definitions.

	EU residue definition	**Source**
Plant residue definition for monitoring	Cereal grain: mefenpyr-diethyl (AE F107892) and metabolite AE F094270, expressed as parent AE F107892. Shoot and straw: mefenpyr-diethyl (AE F107892) and its metabolites AE F113225, AE F094270 and AE F109453 expressed as mefenpyr-diethyl	DAR Addendum, October 2011
Plant residue definition for risk assessment	Cereal grain: mefenpyr-diethyl (AE F107892) and metabolite AE F094270, expressed as parent AE F107892. Shoot and straw: mefenpyr-diethyl (AE F107892) and its metabolites AE F113225, AE F094270 and AE F109453 expressed as mefenpyr-diethyl	DAR Addendum, October 2011
Animal residue definition for monitoring	Mefenpyr-diethyl (AE F107892) and metabolite AE F113225 expressed as mefenpyr-diethyl.	DAR Addendum, October 2011
Animal residue definition for risk assessment	Mefenpyr-diethyl (AE F107892) and metabolite AE F113225 expressed as mefenpyr-diethyl	DAR Addendum, October 2011

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

Table 5.3-18: 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	Mefenpyr-diethyl	0.01 mg/kg	DAR 2011 LOQ
Plant, high acid content			
Plant, high protein/high starch content (dry commodities)			
Plant, high oil content			
Muscle	Mefenpyr-diethyl	0.01 mg/kg	DAR 2011 LOQ
Milk		0.01 mg/kg	DAR 2011 LOQ
Eggs		0.01 mg/kg	DAR 2011 LOQ
Fat		0.01 mg/kg	DAR 2011 LOQ
Liver, kidney		0.01 mg/kg	DAR 2011 LOQ
Soil (Ecotoxicology)	Mefenpyr-diethyl	0.05 mg/kg	common limit
Drinking water (Human toxicology)	Mefenpyr-diethyl	0.1 µg/L	general limit for drinking water
Surface water (Ecotoxicology)	Mefenpyr-diethyl	7600 µg/L	DAR 2011 NOEC (*Lemna gibba)*
Air	Mefenpyr-diethyl	8 µg/m^3	DAR 2011 AOEL sys: 0.1 mg/kg bw/d
Tissue (meat or liver)	Mefenpyr-diethyl	Not requred	Not classified as T / T+
Body fluids		Not required	Not classified as T / T+

5.3.4.2 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 mefenpyr-diethyl in plant matrices is given in the following tables.

Table 5.3-19: 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: mefenpyr-diethyl				
Matrix	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Cereal grain	Primary	0.01 mg/kg	LC-MS/MS	DAR 2011

Component of residue definition: mefenpyr-diethyl				
Matrix	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
	ILV	0.01 mg/kg	LC-MS/MS	DAR 2011
Cereal green material	Primary	0.1 mg/kg	LC-MS/MS	DAR 2011 (Billian & Wolters, 2007)
Ceral straw	Primary	0.05 mg/kg	LC-MS/MS	DAR 2011 (Billian & Wolters, 2007)

Table 5.3-20: Statement on extraction efficiency

	Method for products of plant origin
Required, available from:	-
Not required, because:	Extraction efficiency is only necessary for pesticides showing significant residues, i.e. residues at or above the limit of quantification (LOQ) of the analytical method. This is not the case in food and feed of plant origin

5.3.4.3 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 mefenpyr-diethyl in animal matrices is given in the following tables.

Table 5.3-21: Validated methods for food and feed of animal origin (if appropriate)

Component of residue definition: mefenpyr-diethyl				
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	DAR 2011 (Zimmer & Stucke, 2007)
	ILV	0.01 mg/kg	LC-MS/MS	DAR 2011 (Tzepka & Rotzoll, 2007)
Eggs	Primary	0.01 mg/kg	LC-MS/MS	DAR 2011 (Brillian & Druskus, 2010)
Muscle	Primary	0.01 mg/kg	LC-MS/MS	DAR 2011 (Zimmer & Stucke, 2007)
	ILV	0.01 mg/kg	LC-MS/MS	DAR 2011 (Tzepka & Rotzoll, 2007)
Fat	Primary	0.01 mg/kg	LC-MS/MS	DAR 2011 (Zimmer & Stucke, 2007)
	ILV	0.01 mg/kg	LC-MS/MS	DAR 2011 (Tzepka & Rotzoll, 2007)
Kidney, liver	Primary	0.01 mg/kg	LC-MS/MS	DAR 2011 (Zimmer & Stucke, 2007)

Component of residue definition: mefenpyr-diethyl				
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	DAR 2011 (Tzepka & Rotzoll, 2007)

Table 5.3-22: Statement on extraction efficiency

	Method for products of animal origin
Required, available from:	-
Not required, because:	Extraction efficiency is only necessary for pesticides showing significant residues, i.e. residues at or above the limit of quantification (LOQ) of the analytical method. This is not the case in food and feed of animal origin

5.3.4.4 Description of methods for the analysis of soil (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of mefenpyr-diethyl in soil is given in the following tables.

Table 5.3-23: Validated methods for soil (if appropriate)

Component of residue definition: mefenpyr-diethyl (AE F113225) (AE F094270)			
Method type	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	5 µg/kg	HPLC-MS/MS	Freitag Th. (2013)

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

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

Table 5.3-24: Validated methods for water (if appropriate)

Component of residue definition: mefenpyr-diethyl and metabolites AE F113225, AE F094270					
Matrix type	**Analytes**	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Drinking and surface water	Mefenpyr-diethyl	Primary	0.05 µg/L	HPLC-MS/MS	Krebber R. & Braune M. (2013)
	AE F113225 AE F094270		0.1 µg/L	HPLC-MS/MS	

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

An overview on the acceptable methods and possible data gaps for analysis of mefenpyr-diethyl in air is given in the following table.

Table 5.3-25: Validated methods for air

Component of residue definition: mefenpyr-diethyl			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	8 µg/m^3	HPLC-UV	DAR, 2011

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

Not required as mefenpyr-diethyl is not toxic.

5.3.4.8 Other studies/ information

In several ecotoxicological studies summarised in section B9 of the dRR (toxicity to aquatic organisms honeybees), analytical methods were used for the detection of the active substance iodosulfuron-methyl-sodium and mesosulfuron-methyl in the different test mediums. The analytical part of these studies is summarised in Appendix 2.

Appendix 1 Lists of data considered in support of the evaluation

Tables considered not relevant can be deleted as appropriate.

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

List of data submitted by the applicant and relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 5.1.1	Silva S.	2019	Iodosulfuron-methyl-sodium 0.6% + Mesosulfuron-methyl 3.0% + Mefenpyr-diethyl 9.0% WG (SAP63H) – Physical, chemical and technical properties of the plant protection product. EF/298/19 Ascenza Agro, S.A. GLP Unpublished	N	Globachem N.V. Ascenza Agro S.A.
KCP 5.1.2-01	Arias A.	2019	Validation of the analytical method for the determination of mesosulfuron-methyl, mefenpyr-dietyl and its metabolites (AE F113225 and AE F094270) in wheat (grain and straw) and iodosulfuron-methyl in straw. VAL25/18 Ascenza Laboratorio de Residuos GLP Unpublished	N	Globachem N.V. Ascenza Agro S.A.
KCP 5.1.2-02	Gordo J.	2018	Validation of the analytical method for the determination of triazine amine (AE F059411) residues in barley, wheat, lettuce and radish. VAL02/18 SAPEC Agro - Laboratorio de Residuos GLP Unpublished	N	Globachem N.V. Ascenza Agro S.A
KCP 5.1.2-03	Morias F.F.	2018	Cross validation of an extraction method based on Quechers Method vs. an extraction method applied in	N	Globachem

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
			14C-metabolism studies for the determination of iodosulfuron-methyl in wheat (green material). VAL48/17 SAPEC Agro - Laboratorio de Residuos GLP Unpublished		N.V. Ascenza Agro S.A.
KCP 5.1.2-04	Arias A.	2018a	Cross validation of an extraction method based on Quechers Method vs. an extraction method applied in 14C-metabolism studies for the determination of mesosulfuron-methyl in wheat (green material). VAL19/18 Ascenza Laboratorio de Residuos GLP Unpublished	N	Globachem N.V. Ascenza Agro S.A.
KCP 5.1.2-05 (submitted as KCP 10.2-01)	Renner, P.	2018a	Acute toxicity of GLOB289H to Daphnia magna in a 48-hour static test. 18 48 ADL 0008 BioChem Agrar GLP Unpublished	N	Globachem N.V. Ascenza Agro S.A.
KCP 5.1.2-06 (submitted as KCP 10.2-02)	Renner, P.	2018b	Effects of GLOB289H on *Pseudokirchneriella subcapitata* in an algal growth inhibition test. 18 48 AAL 0019 BioChem Agrar GLP Unpublished	N	Globachem N.V. Ascenza Agro S.A.
KCP 5.1.2-07 (submitted as KCP 10.2-03)	Renner, P.	2018c	Effects of GLOB289H on *Lemna gibba* in a growth inhibition test under semi-static conditions – Appendix 3: Analytical phase report 18 48 ALE 0006 BioChem Agrar GLP Unpublished	N	Globachem N.V. Ascenza Agro S.A.
KCP 5.1.2-08 (submitted as KCP 10.2-04)	Renner P.	2019a	Effects of GLOB289H and Actirob B on *Lenma gibba* in a growth inhibition test under semi-static test conditions – Appendix 3: Analytical phase report 19 48 ALE 0004	N	Globachem N.V. Ascenza Agro

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
			BioChem Agrar GLP Unpublished		S.A.
KCP 5.1.2-09 (submitted as KCP 10.2-05)	Renner P.	2019b	Effects of Iodosulfuron-methyl-sodium + Mesosulfuron-methyl + mefenpyr-diethyl (6+30+90) g/kg WG (SAP63H) and the adjuvant (Pottok) on *Lemna gibba* in a growth inhibition test under semi-static test conditions – Appendix 3: Analytical phase report 19 48 ALE 0007 BioChem Agrar GLP Unpublished	N	Globachem N.V. Ascenza Agro S.A.
KCP 5.1.2-10 (submitted as KCP 10.3.1.2-01)	Kleebaum K.	2018	GLOB289H – Repeated exposure of honey bee (*Apis mellifera* L.) larvae under laboratory conditions (*in vitro)* 17 48 BLC 0089 BioChem Agrar GLP Unpublished	N	Globachem N.V. Ascenza Agro S.A.
KCP 5.1.2-11 (submitted as KCP 10.3.1.2-02)	Ruhland S.	2018	Chronic toxicity of Iodosulfuron-methyl-sodium + Mesosulfuron-methyl + Mefenpyr-diethyl (0.6 + 3 + 9)% WG to the honey bee *Apis mellifera* L. under laboratory conditions 17 48 BAC 0055 BioChem Agrar GLP Unpublished	N	Globachem N.V. Ascenza Agro S.A.
KCP 5.2-01 (submitted as KCA 4.2-13/14/15/16/19)	Morias F.F.	2017	Validation of the analytical method for the determination of iodosulfuron-methyl in several plant matrices. VAL 19/17 Ascenza Agro, S.A. GLP Unpublished	N	Globachem N.V. Ascenza Agro S.A.
KCP 5.2-02 (submitted as KCA 4.2-	Schlewitz P.	2018	Independent laboratory validation of the determination of iodosulfuron-methyl in several plant matrices R B8064 Anadiag	N	Globachem N.V. Ascenza Agro

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
17/18/20)			GLP Unpublished		S.A.
KCP 5.2-03 (submitted as KCP 4.2-24)	Arias A.	2017	Validation of an analytical method for the determination of iodosulfuron-methyl-sodium in soils VAL21/17 Ascenza Agro, S.A. GLP Unpublished	N	Globachem N.V. Ascenza Agro S.A.
KCP 5.2-04 (submitted as KCA 4.4.2-22)	Gaffney V.	2017	Validation of an analytical method for the determination of iodosulfuron-methyl-sodium and metsulfuron-methyl in surface and drinking water VAL20/17 Ascenza Agro, S.A. GLP Unpublished	N	Globachem N.V. Ascenza Agro S.A.
KCP 5.2-05 (submitted as KCA 4.2-23)	Schlewitz P.	2017	Independent Laboratory Validation of the determination of iodosulfuron-methyl-sodium and metsulfuron-methyl in surface water. R B7267 Anadiag GLP Unpublished	N	Globachem N.V. Ascenza Agro S.A.
KCP 5.2-06 (submitted as KCA 4.2-19/26)	Gordo J.	2018b	Validation of an analytical method for the determination of mesosulfuron-methyl in plant matrices. VAL59/17 Ascenza Agro, S.A. GLP Unpublished	N	Globachem N.V. Ascenza Agro S.A.
KCP 5.2-07 (submitted as KCA 4.2-20)	Wößner A	2018a	Independent laboratory validation of mesosulfuron-methyl in plant matrices S17-07888 Eurofins GLP Unpublished	N	Globachem N.V. Ascenza Agro S.A.

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 5.2-08	Andrews G. Bills K.	2019	Method validation – Analytical method for the determination of iodosulfuron-methyl in body fluid and tissue FH/19/002 Batelle UK GLP Unpublished	N	Globachem N.V. Ascenza Agro S.A.
KCP 5.2-09	Knop M.	2018	Validation of the analytical method for the determination of mesosulfuron-methyl in body fluids and animal matrices. S17-7891 Eurofins GLP Unpublished	N	Globachem N.V. Ascenza Agro S.A.
KCP 5.2-10	Andrews G. Pearson J.	2018	Independent method validation – determination of residues of mesosulfuron-methyl in body fluid FH/18/004 Batelle GLP Unpublished	N	Globachem N.V. Ascenza Agro S.A.
KCP 5.2-11 (submitted as KCA 4.2-21)	Gordo J.	2018c	Validation of an analytical method for the determination of mesosulfuron-methyl in soils VAL60/17 Ascenza Agro, S.A. GLP Unpublished	N	Globachem N.V. Ascenza Agro S.A.
KCP 5.2-12 (submitted as KCA 4.2-22)	Gaffney V.	2018	Validation of an analytical method for the determination of mesosulfuron-methyl in surface and drinking water. VAL61/17 Ascenza Agro, S.A. GLP Unpublished	N	Globachem N.V. Ascenza Agro S.A.
KCP 5.2-13 (submitted as	Wößner A.	2018b	Independent Laboratory Validation of mesosulfuron-methyl in water. S17-07890	N	Globachem N.V.

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCA 4.2-23)			Eurofins GLP Unpublished		Ascenza Agro S.A.
KCP 5.2-14 (submitted as KCA 4.2-27)	Gordo J.	2018b	Validation of an analytical method for the determination of Mesosulfuron-methyl in food of animal origin, ILV. VAL62/17 Ascenza Agro, S.A. GLP Unpublished	N	Globachem N.V. Ascenza Agro S.A.

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

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

The following tables are to be completed by MS

List of data submitted by the applicant and not relied on

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

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

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

Appendix 2 Detailed evaluation of submitted analytical methods

A 2.1 Analytical methods for iodosulfuron-methyl-sodium

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

Reference is made to 5.2.1. New/additional studies are summarized below.

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

A 2.1.1.1.1 Analytical method and validation

Comments of zRMS:	Method is accepted
Reference:	KCP 5.1.2-01
Report	Validation of the analytical method for the determination of mesosulfuron-methyl, mefenpyr-diethyl and its metabolites (AE F113225 and AE F094270) in wheat (grain and straw) and iodosulfuron-methyl in straw, Arias A., 2019, VAL25/18.
Guideline(s):	SANCO/3029/99 rev. 4 (11/07/2000)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

For the determination of residues of each analyte in wheat straw and grain, samples were analysed using methods based on QuEchERS Method (EN 15662:2008) and following the respective internal instructions.
Full validation was performed with five recovery tests at each fortification level for all matrices.

The method validation was in accordance with the criteria set on SANCO/3029/99 rev. 4 and SANCO/825/00 rev. 8.1.

Principle of the methods
The extraction solvent for grain and for straw was acetonitrile and acidic acetonitrile respectively, the analytical portion was weighted, water was added and a clean-up step was performed. Final extracts haven been prepared according to internal instructions.
The analysis were carried out by UPLC-TQ-S-micro. The conditions are summarized below:

Analytical column – ACQUITY UPLC HSS T3 1.8µm from Waters, 2.1 x 100 mm

UPLC pump gradient timetable

Time	A%	B%	Flow	Curve
0.00	90.0	10.0	0.300	Initial
5.00	10.0	90.0	0.300	6
6.00	10.0	90.0	0.300	6
6.10	90.0	10.0	0.300	6
7.00	90.0	10.0	0.300	6

A H_2O in 0.1% formic acid
B ACN in 0.1% formic acid
Time (minutes)
Flow (mL/min)

Autosampler temperature: 20°C
Injection volume: 20 µL
Column temperature: 40°C
Electrospray polarity: Positive
Nebulization, dessolvation and cone gas: nitrogen
Collision gas: argon

Mesosulfuron-methyl
Cone voltage: 2V
MRM1 collision energy (504.0 > 182.1): 22eV
MRM2 collision energy (504.0 > 139.1): 54eV
Dwell: 0.010 (s)
Typical retention time: 4.7 min (with tolerance of ± 0.2 min in each analytical batch)
Typical MRM transition ratio: 1.3 (with tolerance of ± 30% in each analytical batch)

Mefenpyr-diethyl
Cone voltage: 22V
MRM1 collision energy (373.0 > 160.0): 30eV
MRM2 collision energy (373.0 > 132.9): 52eV
Dwell: 0.020 (s)
Typical retention time: 6.5 min (with tolerance of ± 0.2 min in each analytical batch)
Typical MRM transition ratio: 1.3 (with tolerance of ± 30% in each analytical batch)

AE F113225
Cone voltage: 18V
MRM1 collision energy (344.9 > 160.2): 26eV
MRM2 collision energy (344.9 > 253.0): 22eV
Dwell: 0.001 (s)
Typical retention time: 5.5 min (with tolerance of ± 0.2 min in each analytical batch)
Typical MRM transition ratio: 1.3 (with tolerance of ± 30% in each analytical batch)

AE F094270
Cone voltage: 20V
MRM1 collision energy (271.1 > 189.1): 34eV
MRM2 collision energy (271.1 > 163.0): 32eV
Dwell: 0.030 (s)
Typical retention time: 4.8 min (with tolerance of ± 0.2 min in each analytical batch)
Typical MRM transition ratio: 2.8 (with tolerance of ± 30% in each analytical batch)

Iodosulfuron-methyl-sodium
Cone voltage: 26V
MRM1 collision energy (508.0 > 167.0): 20eV
MRM2 collision energy (508.0 > 141.0): 18eV
Dwell: 0.050 (s)
Typical retention time: 5.2 min (with tolerance of ± 0.2 min in each analytical batch)
Typical MRM transition ratio: 1.7 (with tolerance of ± 30% in each analytical batch)

Methods validation

The data obtained during the methods validation demonstrate that they are fit for purpose.
The adequacy of the methods in the study matrices was demonstrated.
The MRM1 transitions (see table below) were selected for quantification.
Therefore, the validation parameters of the methods were obtained using data acquired with that MRM transition.
Moreover, in accordance with the requirements stated in SANCO/825/00 rev. 8.1, in the report data obtained with MRM2 transition can be found.
MRM1 and MRM2 transitions are described in the following table.

Analyte	MRM1 transition	MRM2 transition
mesosulfuron-methyl	504.0 > 182.1	504.0 > 139.1
mefenpyr-diethyl	373.0 > 160.0	373.0 > 132.9
AE F113225	344.9 > 160.2	344.9 > 253.0
AE F094270	271.1 > 189.1	271.1 > 163.0
iodosulfuron-methyl	508.0 > 167.0	508.0 > 141.0

Table 7 - MRM1 and MRM2 transitions

Linearity of the analytical methods

The linearity of calibration curves with matrix-matched solutions was shown by correlation coefficients above 0.99. On the table below is described the injected and validated calibration ranges for each matrix.

Specimen	Analyte	Injected calibration range (1) (ng/µL)	Injected calibration range (1) (mg/kg)	Validated calibration range (1) (ng/µL)	Validated calibration range (1) (mg/kg)
Grain	mesosulfuron-methyl	0.00075 - 0.050	0.003 - 0.20	0.00075 - 0.050	0.003 - 0.20
	mefenpyr-diethyl	0.0003 - 0.020	0.0012 - 0.080	0.0003 - 0.020	0.0012 - 0.080
	AE F113225	0.00225 - 0.15	0.009 - 0.60	0.00225 - 0.1125	0.009 - 0.45
	AE F094270	0.00075 - 0.050	0.003 - 0.20	0.00075 - 0.050	0.003 - 0.20
Straw	mesosulfuron-methyl	0.0015 - 0.10	0.003 - 0.20	0.0015 - 0.075	0.003 - 0.15
	mefenpyr-diethyl	0.0015 - 0.10	0.003 - 0.20	0.0015 - 0.10	0.003 - 0.20
	AE F113225	0.01125 - 0.75	0.0225 - 1.5	0.01125 - 0.75	0.0225 - 1.5
	AE F094270	0.001125 - 0.075	0.00225 - 0.15	0.001125 - 0.075	0.00225 - 0.15
	iodosulfuron-methyl	0.0015 - 0.10	0.003 - 0.20	0.0015 - 0.075	0.003 - 0.15

(1) - at least five calibration levels have been injected with matrix matched standard solutions in each specimen extract

* - see details in point 4.9

Table 8 - Calibration ranges

Specimen	Analyte	MRM transition	Correlation coefficient (r) Calibration curve
Grain	Mesosulfuron-methyl	504.0 > 182.1	r = 0.999951 y= 6.57335e+007 x + 22769 n=6
		504.0 > 139.1	r = 0.99889 y = 2E+07x + 9381.2 n=7
	Mefenpyr-diethyl	373.0 > 160.0	r = 0.993807 y= 1.84631e+007 x- 2515.01 n=7
		373.0 > 132.9	r = 0.99190 y = 1E+07x – 1899.6 n=7
	AE F113225	344.9 > 160.2	r = 0.999231 y = 2.68518e+006 x + 1398.89 n=7
		344.9 > 253.0	r = 0.99959 y = 2E+0.6x + 965.46 n=7

	AE F094270	271.1 > 189.1	r = 0.999988 y = 9.0322e+006 x - 294.332 n=7
		271.1 > 163.0	r = 0.99996 y = 3E+06 x – 335 n=7
Straw	Mesosulfuron-methyl	504.0 > 182.1	r = 0.997702 y= 1.22362e+007x + 12780.7 n=6
		504.0 > 139.1	r = 0.99684 y= 3E + 0.6x + 3340.5 n=7
	Mefenpyr-diethyl	373.0 > 160.0	r = 0.998964 y = 602178 x + 418.61 n=7
		373.0 > 132.9	r = 0.99893 y = 446783 x + 338.55 n=7
	AE F113225	344.9 > 160.2	r = 0.999702 y =83907.6 x + 442.964 n=7
		344.9 > 253.0	r = 0.99981 y = 64637x + 261.06 n=7
	AE F094270	271.1 > 189.1	r = 0.999422 y = 526931 x + 88.3657
		271.1 > 163.0	r = 0.99980 y = 19648x – 12.074 n=7
	Iodosulfuron-methyl-sodium	508.0 > 167.0	r = 0.997789 y = 6.17277e+006 x + 6105.19 n=7
		508.0 > 141.0	r = 0.99903 y = 4E+06x + 992.64 n=7

Limit of quantification, LOQ

The LOQ set for all specimen/analyte is indicated in the table below.

Specimen	Analyte	LOQ[1]	LOQ[2]
Grain	mesosulfuron-methyl	0.010	-
	mefenpyr-diethyl	0.004	0.050
	AE F113225	0.030	
	AE F094270	0.010	
Straw	mesosulfuron-methyl	0.010	-
	mefenpyr-diethyl	0.010	0.10
	AE F113225	0.075	
	AE F094270	0.0075	
	iodosulfuron-methyl	0.010	-

1- per analyte

2- mefenpyr-diethyl, as the sum of mefenpyr-diethyl, AE F113225 and AE F094270 expressed in mefenpyr-diethyl

LOQ - Limit of quantification (mg/kg)

Table 9 - Limit of quantification

Limit of detection, LOD

The limit of detection of the methods, for each specimen/analyte is 30% of the respective LOQ.

Accuracy and precision

The accuracy of the methods based on recovery studies done at LOQ and 10 x LOQ was in accordance

with the criteria set.
Also, based on recovery studies done at the fortification levels described above, the relative standard deviation, RSD, achieved was in accordance with the requirements in the reference documents (see table below).

Summary of recovery studies for MRM1 transition. LOQ – limit of quantification; Method – results taking into account the performance at the two studied fortification levels

Specimen	Analyte	Sample code	LOQ	Mean recovery (%)			Relative standard deviation (%)		
				LOQ	10xLOQ	Method	LOQ	10xLOQ	Method
Grain	mesosulfuron-methyl	1267/VAL25/18	0.010	77.6	70.2	73.9	6.8	1.6	7.2
	mefenpyr-diethyl		0.004	109.1	75.1	92.1	3.6	2.4	19.7
	AE F11325		0.030	82.0	96.8	89.4	5.0	6.4	10.3
	AE F094270		0.010	86.7	96.8	91.8	3.2	6.0	7.5
Straw	mesosulfuron-methyl	790/VAL25/18	0.010	96.3	103.2	99.7	3.1	10.2	8.2
	mefenpyr-diethyl		0.010	89.9	97.9	93.9	5.3	5.0	6.6
	AE F113225		0.075	71.7	84.0	77.8	7.0	15.7	14.7
	AE F094270		0.0075	93.8	102.6	98.2	8.1	3.1	7.3
	iodosulfuron-methyl		0.010	87.8	96.2	92.0	2.4	7.1	7.1

Summary of recovery studies for MRM2 transition. LOQ – limit of quantification; Method – results taking into account the performance at the two studied fortification levels

Specimen	Analyte	Sample code	LOQ	Mean recovery (%)			Relative standard deviation (%)		
				LOQ	10xLOQ	Method	LOQ	10xLOQ	Method
Grain	mesosulfuron-methyl	1267/VAL25/18	0.010	71.4	72.0	71.7	7.8	2.6	5.5
	mefenpyr-diethyl		0.004	110.1	76.2	93.2	4.8	3.5	19.6
	AE F11325		0.030	87.6	97.5	92.6	5.6	4.2	7.2
	AE F094270		0.010	86.9	95.9	91.4	4.4	6.8	7.6
Straw	mesosulfuron-methyl	790/VAL25/18	0.010	92.0	100.0	96.4	3.1	10.2	8.8
	mefenpyr-diethyl		0.010	89.8	95.9	92.8	1.9	5.3	5.2
	AE F113225		0.075	83.2	81.2	82.2	17.2	10.6	13.6
	AE F094270		0.075	88.6	102.8	95.7	2.9	3.2	8.4
	iodosulfuron-methyl		0.010	93.6	89.6	91.6	2.8	8.0	6.0

Specificity / Selectivity
For the instrumental conditions used, the methods have shown to be able to identify and quantify the analytes in all matrices tested.

Matrix effects
All extracts shown significant matrix effects in UPLC-TQ-S-micro. Matrix-matched solutions were used for all matrices.

Stability of extracts and working solutions
For mesosulfuron-methyl and mefenpyr-diethyl analysis in wheat grain extracts, it was demonstrated that the extracts are stable for 6 days, when stored at a temperature ≤ -18°C. Other extracts were injected within 24 hours after extraction, therefore no stability was assessed.
The stability of the fortification and calibration solutions was not assessed as these were used within 24 hours after preparation.

Comments of zRMS:	Method is accepted

Reference:	KCP 5.1.2-02
Report	Validation of the analytical method for the determination of triazine amine

	(AE F059411) residues in barley, wheat, lettuce and radish, Gordo J., 2018, VAL02/18.
Guideline(s):	SANCO/3029/99 rev. 4 (11/07/2000) and SANCO/825/00
Deviations:	No
GLP:	Yes
Acceptability:	yes

For the determination of residues of triazine amine in several plant matrices, samples were analysed using methods based on acidified Quechers method and/or following the respective internal instructions.
Full validation was performed with five recovery tests at each fortification level for all matrices.
The method validation was in accordance with the criteria set in SANCO/3029/99 rev. 4 and SANCO/825/00 rev. 8.1.

Principle of the method
The extraction solvent for all the matrices was acidified acetonitrile and depending on the matrix to analyse, the analytical portion was weighted, water was added and clean up steps were performed.
The analyses were carried out by UPLC-TQ-S-micro.
The method is based on acidified Quechers method.

UPLC-TQ-S-micro conditions
Inlet File 1
Analytical column – ACQUITY UPLC BEH HILIC 1.7 µm from Waters, 2.1 x 100 mm

UPLC pump gradient timetable for triazine amine analysis

Time	A%	B%	Flow	Curve
0.00	20.0	80.0	0.600	Initial
1.00	20.0	80.0	0.600	6
1.20	75.0	25.0	0.600	6
2.00	75.0	25.0	0.600	6
2.20	20.0	80.0	0.600	6
3.00	20.0	80.0	0.600	6

A H_2O in 0.1% formic acid
B ACN in 0.1% formic acid
Time (minutes)
Flow (mL/min)

Autosampler temperature: 10°C
Injection volume: 0.5 µL (for forage, leaves with tops and grain) or 1 µL (for the other matrices)
Column temperature: 30°C
Electrospray Polarity: positive
Nebulization, Dessolvatation and cone gas: nitrogen
Collision gas: argon
Cone voltage: 5 V
MRM1 collision energy (141.0 > 56.8): 15 eV
MRM2 collision energy (141.0 > 42.8): 15 eV
Dwell: 0.10 (s)
Typical retention time: 0.60 min (with tolerance of ± 0.2 min in each analytical batch)
Typical MRM transition ratio: 3.5 (with tolerance of ± 30% in each analytical batch)

Inlet File 2
Analytical column – ACQUITY UPLC HSS T3 1.8 µm from Waters, 2.1 x 100 mm

UPLC pump gradient timetable for triazine amine analysis

Time	A%	B%	Flow	Curve
0.00	100.0	0.0	0.400	Initial
1.00	100.0	0.0	0.400	6
2.00	0.0	100.0	0.400	6
2.50	0.0	100.0	0.400	6
3.00	100.0	0.0	0.400	6
3.50	100.0	0.0	0.400	6

A H_2O in 0.1% formic acid
B ACN in 0.1% formic acid
Time (minutes)
Flow (mL/min)

Autosampler temperature: 20°C
Injection volume: 5 µL
Column temperature: 40°C
Electrospray Polarity: positive
Nebulization, Dessolvatation and cone gas: nitrogen
Collision gas: argon
Cone voltage: 5 V
MRM1 collision energy (141.0 > 56.8): 15 eV
MRM2 collision energy (141.0 > 42.8): 15 eV
Dwell: 0.10 (s)
Typical retention time: 0.71 min (with tolerance of ± 0.2 min in each analytical batch)
Typical MRM transition ratio: 2.6 (with tolerance of ± 30% in each analytical batch)

Method validation

The data obtained during the method validation and presented in the report, demonstrate that they are fit for purpose.
The adequacy of the methods in the studied matrices was demonstrated.
The MRM1 transition 141.0 > 56.8 was selected for quantification.
Therefore, the validation parameters of the methods were obtained using data acquired with that MRM transition.
Moreover, in accordance with the requirements stated in SANCO/825/00 rev. 8.1, data obtained with MRM2 transition, 141.0 > 42.8 can be found in the report.

Linearity of the analytical method

The linearity of calibration curves with matrix-matched solutions were shown by correlation coefficient above 0.99.
In the table below is described the injected and validated calibration ranges for each matrix.

Analyte	Crop	Specimen	Chromatographic conditions*	Sample Code	Injected calibration range (1) (ng/µL)	Injected calibration range (1) (mg/kg)	Validated calibration range (1) (ng/µL)	Validated calibration range (1) (mg/kg)
Triazine amine	Barley	Forage	Inlet File 1	301/VAL02/18	0.0015 - 0.030	0.003 - 0.060	0.0015 - 0.030	0.003 - 0.060
		Hay		369/VAL02/18	0.00075 - 0.025	0.003 - 0.10	0.00075 - 0.015	0.003 - 0.060
		Grain		79/VAL02/18	0.00075 - 0.025	0.003 - 0.10	0.00075 - 0.015	0.003 - 0.060
		Straw		125/VAL02/18	0.00075 - 0.025	0.003 - 0.10	0.00075 - 0.025	0.003 - 0.10
	Wheat	Grain		78/VAL02/18	0.00075 - 0.025	0.003 - 0.10	0.00075 - 0.025	0.003 - 0.10
		Straw		126/VAL02/18	0.00075 - 0.025	0.003 - 0.10	0.00075 - 0.025	0.003 - 0.10
	Lettuce		Inlet File 1	93/VAL02/18	0.0015 - 0.10	0.003 - 0.20	0.0015 - 0.075	0.003 - 0.15
			Inlet File 2		0.0015 - 0.10	0.003 - 0.20	0.0015 - 0.10	0.003 - 0.20
	Radish	Leaves with tops	Inlet File 1	141/VAL02/18	0.0015 - 0.030	0.003 - 0.060	0.0015 - 0.030	0.003 - 0.060
			Inlet File 2	70/VAL02/18_f	0.0015 - 0.10	0.003 - 0.20	0.0015 - 0.10	0.003 - 0.20
		Roots	Inlet File 1	70/VAL02/18_r	0.0015 - 0.10	0.003 - 0.20	0.0015 - 0.10	0.003 - 0.20
			Inlet File 2		0.0015 - 0.10	0.003 - 0.20	0.0015 - 0.10	0.003 - 0.20

(1) - at least five calibration levels have been injected with matrix matched standard solutions in each specimen extract

* - see details in point 4.9

Table 3 - Calibration ranges

Calibration details MRM1 transition – 141.0 > 56.8

Analyte	Crop	Specimen	Chromatographic conditions	Correlation coefficient (r) Calibration curve
Triazine-amine	Radish	Roots	Inlet File 1	r = 0.999746 y = 835492x + 650.846 n= 7
			Inlet File 2	r = 0.999505 y = 761811x -221.988 n= 7
		Leaves with tops	Inlet File 1	r = 0.999012 y = 826438x – 798.096 n= 6
			Inlet File 2	r = 0.998855 y = 246195x – 119.531 n= 7
	Lettuce		Inlet File 1	r = 0.9997474 y = 901863x -664.436 n=7
			Inlet File 2	r = 0.998294 y = 1.46843E006x + 598.655 n= 6
	Wheat	Grain		r = 0.999890 y = 1.21832E006x – 77.9036 n=7
		Straw		r = 0.999638 y = 1.88896E006x – 177.238 n= 7
	Barley	Straw		r = 0.999860 y = 1.01847E006x – 260.272 n=7
		Grain		r = 0.997008 y = 1.55814E006x – 467.505 n= 6
		Forage		r = 0.999186 y = 592236x + 528.511 n= 7
		Hay		r = 0.994375 y = 1.62056E006x – 571.384 n= 6

Calibration details MRM2 transition – 141.0 > 42.8

Analyte	Crop	Specimen	Chromatographic conditions	Correlation coefficient (r) Calibration curve
Triazine-amine	Radish	Roots	Inlet File 1	r = 0.99988 y = 244531x + 199.61 n=7
			Inlet File 2	r = 0.99970 y = 30335x – 144.59 n=7
		Leaves with tops	Inlet File 1	r = 0.99903

				y = 238189x – 274.16 n= 6
			Inlet File 2	r = 0.99925 y = 98225x + 49.282 n= 7
	Lettuce		Inlet File 1	r = 0.99954 y = 25335x – 207.64 n= 7
			Inlet File 2	r = 0.99840 y = 603897x + 176.72 n=6
	Wheat	Grain		r = 0.99980 y = 357923x – 34.587 n=7
		Straw		r = 0.99975 y = 563249x – 11.553 n=7
	Barley	Straw		r = 0.99897 y = 306145x – 89.614 n=7
		Grain		r = 0.99723 y = 465326x – 239.35 n= 6
		Forage		r = 0.99889 y = 170031x + 99.169 n=7
		Hay		r = 0.99065 y = 469371x – 260.84 n=7

Limit of quantification, LOQ
The LOQ for triazine amine was set at 0.010 mg/kg, for all matrices.

Limit of detection, LOD
The limit of detection of the method, defined as 30% of LOQ is 0.003 mg/kg, for all matrices.

Accuracy and precision
The accuracy of the methods based on recovery studies done at LOQ and 10 x LOQ was in accordance with the criteria set. Also based on recovery studies done at the fortification levels described above, the relative standard deviation, RSD, achieved was in accordance with the requirements in the reference documents (see table below).

Summary of recovery studies of triazine amine for MRM1 transition. LOQ – limit of quantification (0.010 mg/kg); Method – results taking into account the performance at the two studied fortification levels

Analyte	Crop	Specimen	Chromatographic conditions	Sample code	LOQ (mg/kg)	Mean recovery (%)			Relative standard deviation (%)		
						LOQ	10xLOQ	Method	LOQ	10xLOQ	Method
2.0Triazine amine4.0	Barley	Forage	Inlet File 1	301/VAL02/18	0.010	99.5	100.9	100.2	3.8	3.2	3.4
		Hay		369/VAL02/18		93.4	82.1	87.8	8.4	1.3	9.1
		Grain		79/VAL02/18		74.9	100.2	87.6	5.1	5.8	16.1
		Straw		125/VAL02/18		101.0	98.6	99.8	6.4	3.2	5.0
	Wheat	Grain		78/VAL02/18		74.5	76.3	75.4	4.0	6.1	5.0
		Straw		126/VAL02/18		95.5	88.7	92.1	2.2	4.7	5.2
	Lettuce		Inlet File 1	93/VAL02/18		94.5	74.7	84.6	2.0	1.3	12.4
			Inlet File 2			93.8	83.7	83.7	5.1	8.1	6.4
	Radish	Leaves with tops	Inlet File 1	141/VAL02/18		93.8	88.6	91.2	4.7	2.1	4.6
			Inlet File 2	70/VAL02/18_f		110.1	86.5	98.3	7.2	14.8	16.2
		Roots	Inlet File 1	70/VAL02/18_r		80.7	81.3	81.0	8.8	1.6	5.9
			Inlet File 2			105.6	84.3	94.9	10.2	3.8	14.2

Summary of recovery studies of triazine amine for MRM2 transition. LOQ – limit of quantification (0.010 mg/kg); Method – results taking into account the performance at the two studied fortification levels

Analyte	Crop	Specimen	Chromatographic conditions	Sample code	LOQ (mg/kg)	Mean recovery (%)			Relative standard deviation (%)		
						LOQ	10xLOQ	Method	LOQ	10xLOQ	Method
Triazine amine	Barley	Forage	Inlet File 1	301/VAL02/18	0.010	98.7	100.3	99.5	1.7	7.1	5.0
		Hay		369/VAL02/18		96.7	87.6	92.1	7.4	3.5	7.7
		Grain		79/VAL02/18		77.8	107.8	92.8	5.1	5.2	17.7
		Straw		125/VAL02/18		97.8	97.7	97.7	11.9	3.1	8.2
	Wheat	Grain		78/VAL02/18		75.7	75.1	75.4	4.7	6.8	5.5
		Straw		126/VAL02/18		92.7	68.9	89.8	3.7	5.6	5.6
	Lettuce		Inlet File 1	93/VAL02/18		96.8	76.8	86.8	2.8	1.2	12.3
			Inlet File 2			85.4	83.3	84.3	5.9	8.2	6.8
	Radish	Leaves with tops	Inlet File 1	141/VAL02/18		95.2	90.1	92.6	3.8	2.7	4.3
			Inlet File 2	70/VAL02/18_f		109.6	86.7	98.2	11.6	14.0	17.2
		Roots	Inlet File 1	70/VAL02/18_r		81.1	81.0	81.0	7.5	3.2	5.4

			Inlet File 2			105.5	84.7	95.1	10.6	3.7	14.1

Specificity/selectivity
For all instrumental conditions used, the method has shown to be able to identify and quantify triazine amine in all matrices tested.

Matrix effects
Several matrices showed significant matrix effect in UPLC-MS/MS. To quantify the spiked samples, matrix-matched solutions were used for all matrices.

Stability of extracts and working solutions
Some extracts were injected within 24 hours after extraction, therefore no stability was assessed. For others, the stability is showed in the following table.

Analyte	Crop	Specimen	Chromatographic conditions*	Extracts Stability **
Triazine amine	Barley	Forage	Inlet File 1	-
		Hay		-
		Grain		2
		Straw		2
	Wheat	Grain		-
		Straw		2
	Lettuce		Inlet File 1	-
			Inlet File 2	8
	Radish	Leaves with tops	Inlet File 1	-
			Inlet File 2	4
		Roots	Inlet File 1	-
			Inlet File 2	7

* - see details in point 4.9

**- days between extraction date and analysis date

Table 4 - Calibration ranges

The stability of stock solutions (in H20/01M hydrochloric acid) was demonstrated for at least 24 days when stored at ≤ - 18°C during GLP study VAL03/18 and, concerning fortification solutions, the stability when prepared in acetonitrile and stored in refrigerated conditions (≤ 6°C) was demonstrated for at least 8 days.
The stability of the calibration solutions was not assessed as these were prepared on a daily basis.

A 2.1.1.1.1.1 Extraction efficiency

Comments of zRMS:	Method is accepted
Reference:	KCP 5.2.1-03
Report	Cross validation of an extraction method based on Quechers method vs; an extraction method applied in 14C-metabolism studies for the determination of iodosulfuron-methyl in wheat (green material), Morais F.F., 2018, VAL48/17.
Guideline(s):	SANCO/3029/99 rev. 4 and SANCO/825/00

Deviations:	No
GLP:	Yes
Acceptability:	Yes

The objective of the current study was to perform a cross validation between a method based on the QuEChERS method and the extraction conditions used in the 14C-metabolism studies, for the determination of iodosulfuron-methyl in wheat (green material).
This evaluation was performed by replicate extractions of incurred samples using both methods. Furthermore, a method validation was also done for both methodologies. These validations were performed according to SANCO/3029/99 rev. 4

One sample of untreated wheat green material and one with incurred residues of iodosulfuron-methyl were generated during the residue study (ANADIAG study B8008 under the direction of Corinne Ertus) and were sent to LabResíduos for the evaluation of the extraction efficiency of iodosulfuron-methyl residues.
The homogenization step has been done by ANADIAG. Frozen specimens were delivered in good conditions to LabResíduos.
This analytical phase was conducted as an independent study from the field phase. Analytical methods validation were performed within the scope of this study. The extraction procedures for the determination of iodosulfuron-methyl residues were based on the extraction conditions used in the 14C-metabolism studies and on QuEChERS method. The performance achieved for both methods was fit for purpose (see below). The results obtained were in accordance with the requirements set on SANCO/3029/99 and also on SANCO/825/00.

Analyte	Specimen	Extraction methodology	Equipment	Calibration range[1] (mg/kg)	MRM1	MRM2	LOQ (mg/kg)	LOD (mg/kg)	Type of validation	Accuracy[2] (%)			Precision[2] (%)			Study Code
										LOQ	10xLOQ	Method[3]	LOQ	10xLOQ	Method[3]	
Iodosulfuron-methyl	Wheat green material	14C-metabolism study	UPLC-TQ-S-micro 1	0.0006 - 0.03	508.0 > 167.0	508.0 > 141.0	0.002	0.0006	full	109.5	104.0	106.7	3.1	3.7	4.2	VAL48/17
Iodosulfuron-methyl	Wheat green material	Quechers	UPLC-TQ-S-micro 1	0.0006 - 0.04	508.0 > 167.0	508.0 > 141.0	0.002	0.0006	full	97.1	100.9	99.0	1.6	2.7	2.9	VAL48/17

(1) - at least five calibration levels have been injected with matrix matched standard solutions
(2) - data for MRM1 transition based on five replicates at each fortification level
(3) - results taking into account the performance at the two studied fortification levels
LOQ - limit of quantification; LOD - limit of detection
Table 4 - Summary of validation data

The extraction efficiency was sufficiently proven, by comparing the residue amounts quantified in the incurred sample, using the method based on the extraction conditions applied during the 14Cmetabolism studies and on QuEChERS method. The incurred sample was analysed in triplicate for both methods and the results obtained for the two methods differs only by 0.5 % (criteria 30 %). The results of the extraction efficiency are compiled in the table below.

Anadiag Sample Code	LabResíduos Sample Code	Plot	Days	Iodosulfuron-methyl (mg/kg) *		Extraction Efficiency (%)
				14C-metabolism	QuEChERS	
B8008 PL1/D7/G/A	246/VAL48/17/18	T	8 DAA	0.00377	0.00379	100.5

T - treated plot; **DAA** - days after application
* - Mean values obtained from three replicate extractions for each method. The rounded value, according to internal standard operating procedure PT15 - *Expressão de Resultados* , is 0.004 mg/kg for both methods.
Table 1 - Summary of results

The storage time of each sample is reported below.

Field Trial Code	Field Code	Laboratory Code	Sample Reception	Extraction*	Storage Time** (Days)
B8008 PL1	B8008 PL1/U7/G/A	245/VAL48/17/18	2018-05-30	2018-06-05	6
				2018-06-06	7
	B8008 PL1/D7/G/A	246/VAL48/17/18	2018-05-30	2018-06-05	6
				2018-06-06	7

* - The first date for each sample refers to the extraction with 14C-metabolism studies method and the second date refers to Quechers extraction
** - Days between the extraction date and the sample reception date

Table 2 - Summary of samples storage

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

No new or additional studies have been submitted.

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

No new or additional studies have been submitted

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

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

No new or additional studies have been submitted

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

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

A 2.1.1.7.1 RP-HPLC with MS-MS detection

Comments of zRMS:	Method is accepted

Reference:	KCP 5.1.2-05 (filed as KCP 10.2-01)
Report	Acute toxicity of GLOB289H to *Daphnia magna* in a 48-hour static test. Renner P., 2018, 18 48 ADL 0008 – Appendix 3: Analytical phase report
Guideline(s):	SANCO/3029/99 rev. 4
Deviations:	No
GLP:	Yes

Acceptability: Yes

Principle of the method

The purpose of the analytical phase of the study was to determine the concentration of the active ingredient iodosulfuron-methyl sodium and mesosulfuron-methyl in the test solution. The determination was conducted by an in-house developmed method using reversed phase – high performance liquid chromatography (RP-HPLC) with mass-spectrometric (MS-MS) detection.

Materials and methods

Chromatography system:	Shimadzu LC-8040 with triple quadrupole mass spectrometric detector
Pumps :	Shimadzu, LC-20ADXR
Degasser:	Shimadzu, DGU-A3R
Autosampler:	Shimadzu, SIL-20ACXR
Column oven:	Shimadzu, CTO-20A
MS-detector:	Shimadzu, LCMS-8040
Controller:	Shimadzu, CBM-20A
Data-system:	Shimadzu, LabSolutions Version 5.86

Conditions

Mobile phase:	A: Water containing 0.1% formic acid and 5 mM ammonium formate	
	B: Methanol containing 0.1% formic acid	
Flow rate:	0.350 mL/min.	
Column:	Zorbax Eclipse Plus C18, 50*2.1 mm, 1.8 µm	
Column temperature:	40°C	
Injection volume:	10 µL	
Gradient:	0.00 min	5% B
	1.50 min	50% B
	4.50 min	50% B
	6.50 min	100% B
	7.00 min	100% B
	7.01 min	5%
	9.00 min	Stop
Run time:	9.00 min (include 2 min postrun time)	
Detection:	ESI positive, MRM	
	Iodosulfuron-methyl sodium: m/z 508.1 → 167.1, 508.1 → 141.1	
Retention times:	4.8 min. for iodosulfuron-methyl sodium	
	3.9 min. for mesosulfuron-methyl	

Method validation uses the following standards of iodosulfuron-methyl sodium and mesosulfuron-methyl:

Iodosulfuron-methyl sodium:

Source:	HPC Standards GmbH
Batch:	779188
Expiry date:	1 March 2022
Purity:	98.6%

Mesosulfuron-methyl:

Source:	HPC Standards GmbH
Batch:	779265
Expiry date:	1 March 2022
Purity:	97.0%

Validation

Validation blank samples had peak areas of less than 30% of the lowest validated concentration. No interfering peaks were detected.
The specificity of the method was assured by MS/MS-detection and the absence of interfering peaks. The recovery and precision data show that the influences of test medium were within the limits of the guidance document SANCO/3029/99; therefore, all criteria were fulfilled:

	Iodosulfuron-methyl sodium	**Mesosulfuron-methyl**
Accuracy / Repeatability	The accuracy of the method was tested by spiking test medium with test item at 12.43 and 443.8 µg/L. Five determinations were made at each level to define the RSD. Mean recovery high: 109% of nominal; RSD: 1.1% Mean recovery low: 107% of nominal; RSD: 0.3%	The accuracy of the method was tested by spiking test medium with test item at 64.79 and 2314 µg/L. Five determinations were made at each level to define the RSD. Mean recovery high: 109% of nominal; RSD: 0.3% Mean recovery low: 97% of nominal; RSD: 1.9%
Linearity	Calibration functions were linear in the range of 8.115 to 38.64 µg/L. Correlation coefficient > 0.99	Calibration functions were linear in the range of 42.51 to 202.4 µg/L. Correlation coefficient > 0.99
LOQ	12.43 µg/L	64.79 µg/L

Conclusion

The method is able to determine both iodosulfuron-methyl sodium and mesosulfuron-methyl in *Daphnia* medium at a LOQ of respectively 12.43 µg/L and 64.79 µg/L for iodosulfuron-methyl sodium and mesosulfuron-methyl.

Comments of zRMS:	Method is accepted

Reference:	KCP 5.1.2-06 (filed as KCP 10.2-02)
Report	Effects of GLOB289H on *Pseudokirchneriella subcapitata* in an algal growth inhibition test. Renner P., 2018, 18 48 AAL 0019 – Appendix 4: Analytical phase report
Guideline(s):	SANCO/3029/99 rev. 4
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Principle of the method

The purpose of the analytical phase of the study was to determine the concentration of the active ingredient iodosulfuron-methyl sodium and mesosulfuron-methyl in the test solution. The determination was conducted by an in-house developed method using reversed phase – high performance liquid chromatography (RP-HPLC) with mass-spectrometric (MS-MS) detection.

Materials and methods

Chromatography system: Shimadzu LC-8040 with triple quadrupole mass spectrometric detector

Pumps :	Shimadzu, LC-20ADXR
Degasser:	Shimadzu, DGU-A3R
Autosampler:	Shimadzu, SIL-20ACXR
Column oven:	Shimadzu, CTO-20A
MS-detector:	Shimadzu, LCMS-8040
Controller:	Shimadzu, CBM-20A
Data-system:	Shimadzu, LabSolutions Version 5.86

Conditions

Mobile phase:	A: Water containing 0.1% formic acid and 5 mM ammonium formate	
	B: Methanol containing 0.1% formic acid	
Flow rate:	0.350 mL/min.	
Column:	Zorbax Eclipse Plus C18, 50*2.1 mm, 1.8 µm	
Column temperature:	40°C	
Injection volume:	10 µL	
Gradient:	0.00 min	5% B
	1.50 min	50% B
	4.50 min	50% B
	6.50 min	100% B
	7.00 min	100% B
	7.01 min	5%
	9.00 min	Stop
Run time:	9.00 min (include 2 min postrun time)	
Detection:	ESI positive, MRM	
	Iodosulfuron-methyl sodium: m/z 508.1 → 167.1, 508.1 → 141.1	
	Mesosulfuron-methyl: m/z 504.3 → 182.1, 504.3 → 306.0	
Retention times:	4.8 min. for iodosulfuron-methyl sodium	
	3.9 min. for mesosulfuron-methyl	

Method validation uses the following standards of iodosulfuron-methyl sodium and mesosulfuron-methyl:

Iodosulfuron-methyl sodium:

Source:	HPC Standards GmbH
Batch:	779188
Expiry date:	1 March 2022
Purity:	98.6%

Mesosulfuron-methyl:

Source:	HPC Standards GmbH
Batch:	779265
Expiry date:	1 March 2022
Purity:	97.0%

Validation

Validation blank samples had peak areas of less than 30% of the lowest validated concentration. No interfering peaks were detected.

The specificity of the method was assured by MS/MS-detection and the absence of interfering peaks. The recovery and precision data show that the influences of test medium were within the limits of the guidance document SANCO/3029/99; therefore, all criteria were fulfilled:

	Iodosulfuron-methyl sodium	**Mesosulfuron-methyl**
Accuracy / Repeatability	The accuracy of the method was tested by spiking test medium with test item at 5.981 and 239.2 µg/L. Five determinations	The accuracy of the method was tested by spiking test medium with test item at 31.19 and 1247 µg/L. Five determinations were

	were made at each level to define the RSD. Mean recovery high: 110% of nominal; RSD: 0.4% Mean recovery low: 104% of nominal; RSD: 2.3%	made at each level to define the RSD. Mean recovery high: 108% of nominal; RSD: 1.7% Mean recovery low: 103% of nominal; RSD: 2.3%
Linearity	Calibration functions were linear in the range of 4.340 to 36.17 µg/L. Correlation coefficient > 0.99	Calibration functions were linear in the range of 23.08 to 192.4 µg/L. Correlation coefficient > 0.99
LOQ	5.981 µg/L	31.19 µg/L

Conclusion

The method is able to determine both iodosulfuron-methyl sodium and mesosulfuron-methyl in Algal medium at a LOQ of respectively 5.981 µg/L and 31.19 µg/L for iodosulfuron-methyl sodium and mesosulfuron-methyl.

Comments of zRMS:	Method is accepted

Reference:	KCP 5.1.2-07 (filed as KCP 10.2-03)
Report	Effects of GLOB289H on *Lemna gibba* in a growth inhibition test under semi-static conditions. Renner P., 2018, 18 48 ALE 0006 – Appendix 3: Analytical phase report
Guideline(s):	SANCO/3029/99 rev. 4
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Principle of the method

The purpose of the analytical phase of the study was to determine the concentration of the active ingredient iodosulfuron-methyl sodium and mesosulfuron-methyl in the test solution. The determination was conducted by an in-house developed method using reversed phase – high performance liquid chromatography (RP-HPLC) with mass-spectrometric (MS-MS) detection.

Materials and methods

Chromatography system:	Shimadzu LC-8040 with triple quadrupole mass spectrometric detector
Pumps :	Shimadzu, LC-20ADXR
Degasser:	Shimadzu, DGU-A3R
Autosampler:	Shimadzu, SIL-20ACXR
Column oven:	Shimadzu, CTO-20A
MS-detector:	Shimadzu, LCMS-8040
Controller:	Shimadzu, CBM-20A
Data-system:	Shimadzu, LabSolutions Version 5.86

Conditions

Mobile phase:	A: Water containing 0.1% formic acid and 5 mM ammonium formate B: Methanol containing 0.1% formic acid

Flow rate:	0.350 mL/min.	
Column:	Zorbax Eclipse Plus C18, 50*2.1 mm, 1.8 µm	
Column temperature:	40°C	
Injection volume:	50 µL	
Gradient:	0.00 min	5% B
	1.50 min	55% B
	4.50 min	55% B
	6.50 min	100% B
	7.00 min	100% B
	7.01 min	5% B
	10.00 min	Stop
Run time:	10.00 min	
Detection:	ESI positive, MRM	
	Iodosulfuron-methyl sodium: m/z 508.1 → 167.1, 508.1 → 141.1	
	Mesosulfuron-methyl: m/z 504.3 → 182.1, 504.3 → 306.0	
Retention times:	5.0 min. for iodosulfuron-methyl sodium	
	4.0 - 4.1 min. for mesosulfuron-methyl	

Method validation uses the following standards of iodosulfuron-methyl sodium and mesosulfuron-methyl:
Iodosulfuron-methyl sodium:

Source:	HPC Standards GmbH
Batch:	779188
Expiry date:	1 March 2022
Purity:	98.6%

Mesosulfuron-methyl:

Source:	HPC Standards GmbH
Batch:	779265
Expiry date:	1 March 2022
Purity:	97.0%

Validation

Validation blank samples had peak areas of less than 30% of the lowest validated concentration. No interfering peaks were detected.
The specificity of the method was assured by MS/MS-detection and the absence of interfering peaks. The recovery and precision data show that the influences of test medium were within the limits of the guidance document SANCO/3029/99; therefore, all criteria were fulfilled:

	Iodosulfuron-methyl sodium	**Mesosulfuron-methyl**
Accuracy / Repeatability	The accuracy of the method was tested by spiking test medium with test item at 11.48 ng/L and 478.5 ng/L. The used analytical method did not allow quantification below the EC_{50}, therefore an additional validation concentration of 47.85 ng/L was analysed. Five determinations were made at each level to define the RSD. Mean recovery high: 109% of nominal; RSD: 0.5 % Mean recovery medium: 84% of nominal; RSD: 5.8%	The accuracy of the method was tested by spiking test medium with test item at 59.88 ng/L and 2495 ng/L. Because of the low concentrations, an additional validation concentration of 249.5 ng/L was analysed. Five determinations were made at each level to define the RSD. Mean recovery high: 109% of nominal; RSD: 1.1 % Mean recovery medium: 102% of nominal; RSD: 2.3% Mean recovery low: 91% of nominal; RSD: 1.7

	Mean recovery low: -	
Linearity	Calibration functions were linear in the range of 36.39 to 234.7 ng/L. Correlation coefficient > 0.99	Calibration functions were linear in the range of 42.77 to 1222 ng/L. Correlation coefficient > 0.99
LOQ	47.85 ng/L	59.88 ng/L

Conclusion

The method is able to determine both iodosulfuron-methyl sodium and mesosulfuron-methyl in lemna medium at a LOQ of respectively 47.85 ng/L and 59.88 ng/L for iodosulfuron-methyl sodium and mesosulfuron-methyl.

Comments of zRMS:	Method is accepted

Reference:	KCP 5.1.2-08 (filed as KCP 10.2-04)
Report	Effects of GLOB289H and Actirob B on *Lemna gibba* in a growth inhibition test under semi-static conditions. Renner P., 2019, 19 48 ALE 0004 – Appendix 3: Analytical phase report
Guideline(s):	SANCO/3029/99 rev. 4
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Principle of the method

Błąd! Nie można odnaleźć źródła odwołania.

Materials and methods

Chromatography system:	Shimadzu HPLC-20 system with Shimadzu 8040 triple quadrupole mass spectrometric detector
Pumps :	Shimadzu, LC-20ADXR
Degasser:	Shimadzu, DGU-A3R
Autosampler:	Shimadzu, SIL-20ACXR
Column oven:	Shimadzu, CTO-20A
MS-detector:	Shimadzu, LCMS-8040
Controller:	Shimadzu, CBM-20A
Data-system:	Shimadzu, LabSolutions Version 5.86

Conditions

Mobile phase:	A: Water containing 0.1% formic acid and 5 mM ammonium formate B: Methanol containing 0.1% formic acid	
Flow rate:	0.350 mL/min.	
Column:	Zorbax Eclipse Plus C18, 50*2.1 mm, 1.8 µm	
Column temperature:	40°C	
Injection volume:	10 µL	
Gradient:	0.00 min	5% B
	1.50 min	55% B
	4.50 min	55% B
	6.50 min	100% B
	7.00 min	100% B

	7.01 min	5% B
	9.00 min	Stop
Run time:	9.00 min (include 2 min postrun time)	
Detection:	ESI positive, MRM	
	Iodosulfuron-methyl-sodium: m/z 508.1 → 167.1, 508.1 → 141.1	
	Mesosulfuron-methyl: m/z 504.3 → 182.1, 504.3 → 306.0	
Retention times:	4.8 min. for iodosulfuron-methyl sodium	
	3.9 min. for mesosulfuron-methyl	

Validation

For Iodosulfuron-methyl-sodium, the method was validated with test medium spiked with test item at 114% of the lowest nominal test concentration (0.020 µg/L) and at 124% of the highest nominal test concentration (0.35 µg/L, corresponding to 0.17 µg/L in diluted samples).
For mesosulfuron-methyl, the method was validated with test medium spiked with test item at 50% of the lowest nominal test concentrations (0.047% µg/L) and 124% of the highest nominal test concentrations (1.86 µg/L, corresponding to 0.93 µg/L in diluted samples).

Results:
The following validation results were obtained.

Table 26: Summary validation results for *Błąd! Nie można odnaleźć źródła odwołania.*

Level	*n*	*Nominal conc. of a.i. [µg/L]*	*Mean analysed conc. of a.i. [µg/L]*	*REC [%]*	*RSD [%]*
Low	5	0.0200	0.0219	110	10.6
High	5	0.347	0.363	105	3.29
Blank	2	0.00	n.d.	-	-

Błąd! Nie można odnaleźć źródła odwołania.

Table 27: Summary validation results for *Błąd! Nie można odnaleźć źródła odwołania.*

Level	*n*	*Nominal conc. of a.i. [µg/L]*	*Mean analysed conc. of a.i. [µg/L]*	*REC [%]*	*RSD [%]*
Low	5	0.0470	0.0487	103	9.88
High	5	1.86	1.75	94	3.40
Blank	2	0.00	n.d.	-	-

n.d.: not detected or <30% LOQ, LOQ: *Błąd! Nie można odnaleźć źródła odwołania.*

Błąd! Nie można odnaleźć źródła odwołania. Limit of Quantification (LOQ)

Błąd! Nie można odnaleźć źródła odwołania.

Results of the specimen measurements

The following results were obtained.

Table 28: Summary specimen results for ***Błąd! Nie można odnaleźć źródła odwołania.*** **Błąd! Nie można odnaleźć źródła odwołania.** **Błąd! Nie można odnaleźć źródła odwołania.**

Table 29: Summary specimen results for ***Błąd! Nie można odnaleźć źródła odwołania.*** **Błąd! Nie można odnaleźć źródła odwołania.**
Błąd! Nie można odnaleźć źródła odwołania.

Conclusion

The method is able to determine both iodosulfuron-methyl sodium and mesosulfuron-methyl in lemna medium at a LOQ of respectively 0.020 µg/L and 0.047 µg/L for iodosulfuron-methyl sodium and mesosulfuron-methyl.

Comments of zRMS:	Method is accepted

Reference:	KCP 5.1.2-09 (filed as KCP 10.2-05)
Report	Effects of Iodosulfuron-methyl-sodium + Mesosulfuron-methyl + Mefenpyr-diethyl (6 + 30 + 90) g/kg WG (SAP63H) and the adjuvant (Pottok) on *Lemna gibba* in a growth inhibition test under semi-static test conditions, Renner P., 2019, 19 48 ALE 0007 – Appendix 3: Analytical phase report
Guideline(s):	SANCO/3029/99 rev. 4
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Principle of the method

Błąd! Nie można odnaleźć źródła odwołania.

Test item was re-certified during the course of the analytical phase. All nominal values refer to the re-certified content of iodosulfuron-methyl-sodium.

Materials and methods

Chromatography system:	Shimadzu HPLC-20 system with Shimadzu 8040 triple quadrupole mass spectrometric detector
Pumps :	Shimadzu, LC-20ADXR
Degasser:	Shimadzu, DGU-A3R
Autosampler:	Shimadzu, SIL-20ACXR
Column oven:	Shimadzu, CTO-20A
MS-detector:	Shimadzu, LCMS-8040
Controller:	Shimadzu, CBM-20A
Data-system:	Shimadzu, LabSolutions Version 5.86

Conditions

Mobile phase:	A: Water containing 0.1% formic acid and 5 mM ammonium formate B: Methanol containing 0.1% formic acid	
Flow rate:	0.350 mL/min.	
Column:	Zorbax Eclipse Plus C18, 50*2.1 mm, 1.8 µm	
Column temperature:	40°C	
Injection volume:	10 µL	
Gradient:	0.00 min	5% B
	1.50 min	55% B
	4.50 min	55% B

	6.50 min	100% B
	7.00 min	100% B
	7.01 min	5% B
	9.00 min	Stop
Run time:	9.00 min (include 2 min postrun time)	
Detection:	ESI positive, MRM	
	Iodosulfuron-methyl-sodium: m/z 508.1 → 167.1, 508.1 → 141.1	
	Mesosulfuron-methyl: m/z 504.3 → 182.1, 504.3 → 306.0	
Retention times:	5.1 min. for iodosulfuron-methyl sodium	
	4.0 min. for mesosulfuron-methyl	

Validation

The following validation results were obtained.

Table 1: Summary validation results for iodosulfuron-methyl sodium

Level	*n*	*Nominal conc. of a.i. [µg/L]*	*Mean analysed conc. of a.i. [µg/L]*	*REC [%]*	*RSD [%]*
Low	5	0.0190	0.0191	101	14.0
High	5	0.355	0.303	85	4.84
Blank	2	0.00	n.d.	-	-

n.d. = not detected or <LOQ; LOQ: 0.0190 µg/L

Table 2: Summary validation results for mesosulfuron-methyl

Level	*n*	*Nominal conc. of a.i. [µg/L]*	*Mean analysed conc. of a.i. [µg/L]*	*REC [%]*	*RSD [%]*
Low	5	0.0349	0.0353	103	5.77
High	5	1.31	1.29	98	10.12
Blank	2	0.00	n.d.	-	-

n.d.: not detected or <30% LOQ, LOQ: 0.0349 µg/L

Błąd! Nie można odnaleźć źródła odwołania. Limit of Quantification (LOQ)

Błąd! Nie można odnaleźć źródła odwołania.

Results of the specimen measurements

The following results were obtained.

Table 3: Summary retain specimen results for iodosulfuron-methyl sodium

Treatment group	Iodosulfuron-methyl sodium				
	Nominal conc. of a.i. [µg/L]	Analysed conc. of a.i. [µg/L]	REC [%]	Analysed conc. of a.i. [µg/L]	REC [%]
		fresh		spent	
		day 0		day 2	
1	0.00	n.d.	-	n.d.	-
2	0.00	n.d.	-	n.d.	-
3	0.0183	0.0190	104	0.0158	86
4	0.0369	0.0315	85	0.0422	114
5	0.0733	0.0768	105	0.0756	103
6	0.147	0.121	82	0.131	89
7	0.294	0.247	84	0.247	84
		day 2		day 5	
1	0.00	n.d.	-	n.d.	-
2	0.00	n.d.	-	n.d.	-
3	0.0183	0.0151	82	0.0166	91
4	0.0369	0.0346	94	0.0396	108
5	0.0733	0.0664	91	0.0652	89
6	0.147	0.121	82	0.126	86
7	0.294	0.241	82	0.244	83
		day 5		day 7	
1	0.00	n.d.	-	n.d.	-
2	0.00	n.d.	-	n.d.	-
3	0.0183	0.0151	82	0.0158	86
4	0.0369	0.0409	111	0.0384	104
5	0.0733	0.0641	87	0.0658	90
6	0.147	0.133	90	0.123	84
7	0.294	0.274	93	0.252	86

n.d. = not detected or <30% LOQ; LOQ: 0.0190 µg/L

Błąd! Nie można odnaleźć źródła odwołania.

Table 4: Summary retain specimen results for mesosulfuron-methyl

Treatment group	Mesosulfuron-methyl				
	Nominal conc. of a.i. [µg/L]	Analysed conc. of a.i. [µg/L]	REC [%]	Analysed conc. of a.i. [µg/L]	REC [%]
		fresh		spent	
		day 0		day 2	
1	0.00	n.d.	-	n.d.	-
2	0.00	n.d.	-	n.d.	-
3	0.07	0.0685	101	0.0673	99
4	0.14	0.149	109	0.154	113
5	0.27	0.288	106	0.286	105
6	0.54	0.608	112	0.640	118
7	1.09	1.23	113	1.22	112
		day 2		day 5	
1	0.00	n.d.	-	n.d.	-
2	0.00	n.d.	-	n.d.	-
3	0.07	0.0685	101	0.0661	98
4	0.14	0.146	107	0.140	102
5	0.27	0.297	110	0.300	111
6	0.54	0.638	117	0.603	111
7	1.09	1.20	110	1.24	114
		day 5		day 7	
1	0.00	n.d.	-	n.d.	-
2	0.00	n.d.	-	n.d.	-
3	0.07	0.0626	92	0.0732	108
4	0.14	0.151	111	0.159	116
5	0.27	0.302	111	0.307	113
6	0.54	0.601	110	0.636	117
7	1.09	1.29	118	1.17	108

n.d.: not detected or < 30% LOQ; LOQ: 0.0349 µg/L

Błąd! Nie można odnaleźć źródła odwołania.

Conclusion

The method is able to determine both iodosulfuron-methyl sodium and mesosulfuron-methyl in lemna medium at a LOQ of respectively 0.019 µg/L and 0.035 µg/L for iodosulfuron-methyl sodium and mesosulfuron-methyl.

A 2.1.1.7.2 HPLC with DAD detection

Comments of zRMS:	Method is accepted

Reference:	KCP 5.1.2-10 (submitted as KCP 10.3.1.2-01)
Report	GLOB289H – Repeated exposure of honey bee (*Apis mellifera*) larvae under laboratory conditions (*in vitro*) – Verification of the concentration of the active ingredients in the test item stock solutions, Kleebaum K., 2018, 17 48 BLC 0089.
Guideline(s):	SANCO/3029/99 rev. 4 (11/07/2000)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Principle of the method
The determination of the active ingredient in acetone was conducted by an in-house developed method using HPLC with DAD-detection.

HPLC-DAD conditions:

Instrument: Shimadzu LC-20 HPLC system equipped with a diode-array detector
Column: Sep Serv Ultrsep, ES Pharm RP18, 200 mm x 2.0 mm 5µm
Mobile phase: A: Water with 0.1 % (v/v) phosphoric acid
B: Acetonitrile with 0.1 % (v/v) phosphoric acid

Time (minutes)	Eluent B (% v/v)
0.00	25%
6.00	95%
10.00	95%
10.01	25%
13.00	Stop

Flow rate: 0.25 mL/min
Column temperature: 50 °C
Wavelength: UV-detection at 235 nm for mesosulfuron-methyl
UV-detection at 226 nm for iodosulfuron-methyl-sodium
Retention time: Approximately 6.0 min for both iodosulfuron-methyl-sodium and mesosulfuron-methyl.

Validation

The method was validated with test matrix spike with test item at approximately 50% of the nominal test concentration (3.060 mg/L of mesosulfuron-methyl and 0.587 mg/L of iodosulfuron-methyl-sodium) and at the nominal test concentration (358.4 mg/L of mesosulfuron-methyl and 68.73 mg/L of iodosulfuron-methyl-sodium).

Summary of the validation results:

Validation	Number of	Nominal conc. of	Nominal conc. of	Mean measured	Recalibration factor	Dilution factor	Mean analysed	Mean recovery	RSD [%]

	replicates	a.i. [mg/L]	a.i. regarding DF [mg/L]	conc. of a.i. [mg/L]	(RCF)	(DF)	conc. of a.i. [mg/L]	[% of nominal]	
Iodosulfuron-methyl-sodium									
Validation low conc.	5	0.587	0.293	0.309	0.990	2	0.612	104	1.8
Validation high conc.	5	68.73	0.598	0.607	0.990	114.9	69.07	100	1.5
Mesosulfuron-methyl									
Validation low conc.	5	3.060	1.530	1.683	0.988	2	3.325	109	1.1
Validation high conc.	5	358.4	3.118	3.392	0.993	114.9	387.0	108	0.8

Validation blank samples had peak areas of less than 30% of the lowest validated concentration. No interfering peaks were detected.

The specificity of the method was assured by the following method: UV spectra from 200 to 300 nm were continuously recorded by the diode-array detector. Spectra of the peaks were compared to those of the reference. Similar spectra with approximately equal absorption maxima, a constant chromatographic retention time and no interfering peaks were observed.

All validity criteria of the guidance document SANCO/3029/99 are fulfilled:

Criteria:	
Linearity:	Linearity was tested for 5 points at a concentration range of at least ± 20% of a.i. in the analytical solution, with correlation coefficient of > 0.99.
Specificity/ interferences :	LOQ - blank values did not exceed 30% of the lowest validated concentration
Repeatability (precision):	Repeatability with 5 replicates for each level with the RSD (relative standard deviation) was < 20% per level.
Accuracy :	Accuracy was tested by spiking sample matrix with test item at 2 concentrations levels. Mean recoveries for each level were in the range 70-110%,
Conclusion:	The method is suitable for determination of the Iodosulfuron-methyl-sodium and mesosulfuron-methyl content.

Comments of zRMS:	Method is accepted

Reference:	KCP 5.1.2-11 (submitted as KCP 10.3.1-2-02)
Report	Chronic toxicity of iodosulfuron-methyl-sodium +mesosulfuron-methyl + mefenpyr-diethyl (0.6+3+9)% WG to the honey bee *Apis mellifera L.* under laboratory conditions – Verification of the concentration of the active ingredients in the test item feeding solution, Ruhland S., 2018, 17 48 BAC 0055.
Guideline(s):	SANCO/3029/99 rev. 4 (11/07/2000)
Deviations:	No

GLP: Yes

Acceptability: Yes

Materials and methods

Principle of the method

The determination of the active ingredient in the feeding solutions was conducted by an in-house developed method using HPLC with DAD-detection.

HPLC-DAD conditions:

Instrument: Shimadzu LC-20 HPLC system equipped with a diode-array detector
Column: Sep Serv Ultrsep, ES Pharm RP18, 200 mm x 2.0 mm 5µm
Mobile phase: A: Water with 0.1 % (v/v) phosphoric acid
B: Acetonitrile with 0.1 % (v/v) phosphoric acid

Time (minutes)	Eluent B (% v/v)
0.00	25%
6.00	95%
10.00	95%
10.01	25%
13.00	Stop

Flow rate: 0.25 mL/min
Wavelength: UV-detection at 235 nm for mesosulfuron-methyl
UV-detection at 226 nm for iodosulfuron-methyl-sodium
Retention time: Approximately 6.0 min for mesosulfuron-methyl and 6.6 min for iodosulfuron-methyl-sodium.

Validation

The method was validated with test matrix spike with test item at approximately 50% of the nominal test concentration (22.87 mg/L of mesosulfuron-methyl and 4.386 mg/L of iodosulfuron-methyl-sodium) and at the nominal test concentration (742.5 mg/L of mesosulfuron-methyl and 142.4 mg/L of iodosulfuron-methyl-sodium).

Summary of the validation results:

Validation	Number of replicates	Nominal conc. of a.i. [mg/L]	Nominal conc. of a.i. regarding DF [mg/L]	Mean measured conc. of a.i. [mg/L]	Recalibration factor (RCF)	Dilution factor (DF)	Mean analysed conc. of a.i. [mg/L]	Mean recovery [% of nominal]	RSD [%]
Iodosulfuron-methyl-sodium									
Validation low conc.	5	4.386	0.351	0.384	0.991	12.5	4.751	108	1.4
Validation high conc.	5	142.4	0.712	0.791	0.987	200	156.2	110	1.4
Mesosulfuron-methyl									
Validation low conc.	5	22.87	1.830	1.904	0.995	12.5	23.68	104	1.2
Validation high conc.	5	742.5	3.713	4.128	0.992	200	818.6	110	0.8

Validation blank samples had peak areas of less than 30% of the lowest validated concentration. No interfering peaks were detected.

The specificity of the method was assured by the following method: UV spectra from 200 to 300 nm were continuously recorded by the diode-array detector. Spectra of the peaks were compared to those of the reference. Similar spectra with approximately equal absorption maxima, a constant chromatographic retention time and no interfering peaks were observed.

All validity criteria of the guidance document SANCO/3029/99 are fulfilled:

Criteria:	
Linearity:	Linearity was tested for 5 points at a concentration range of at least ± 20% of a.i. in the analytical solution, with correlation coefficient of > 0.99.
Specificity/ interferences :	LOQ - blank values did not exceed 30% of the lowest validated concentration
Repeatability (precision):	Repeatability with 5 replicates for each level with the RSD (relative standard deviation) was < 20% per level.
Accuracy :	Accuracy was tested by spiking sample matrix with test item at 2 concentrations levels. Mean recoveries for each level were in the range 70-110%,
Conclusion:	The method is suitable for determination of the Iodosulfuron-methyl-sodium and mesosulfuron-methyl content.

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

A 2.1.3 Description of analytical methods for the determination of residues in plant matrices (KCP 5.2)

Comments of zRMS:	Method is accepted

Reference:	KCP 5.2-01 (submitted as KCA 4.2-13/14/15/16/19)
Report	Validation of the analytical method for the determination of iodosulfuron-methyl in several plant matrices. Morais, F., 2017. VAL 19/17.
Guideline(s):	SANCO/825/00 rev. 8.1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Principle of the method
The extraction solvent for all matrices was acetonitrile and depending on the matrix to analyse, the analytical portion was weighted, water was added and clean up steps were performed. Final extracts have been prepared according to PT10 – Método Quechers.
The analyses were carried out by UPLC-TQ-S-micro.

UPLC-TQ-S conditions:

Instrument:	UPLC-TQ-S-micro-1 - Triple quadrupole mass spectrometer from Waters Xevo TQ-S-micro, equipped with ESI probe, Waters Acquity UPLC H Class separations module, rotary vane pump SV40 BI FC 960331V3010 and a MassLynx software.
Column:	ACQUITY UPLC HSS T3 1.8 µm from Waters, 2.1 x 100 mm
Mobile phase:	A: H_2O in 0.1% formic acid C: MeOH in 0.1% formic acid

Time (minutes)	A%	C%	Flow (mL/min)	Curve
0.00	90.0	10.0	0.300	Initial
1.00	10.0	90.0	0.300	6
4.00	10.0	90.0	0.300	6
4.10	90.0	10.0	0.300	6
5.00	90.0	10.0	0.300	6

Autosampler temp:	15°C
Injection volume:	0.5 µL
Column temperature:	30 °C
Electrospray polarity:	Positive
Nebulization, dessolvation and cone gase:	nitrogen
Collision gas:	argon
Cone voltage:	26 V
MRM1 collision energy (508.0 > 167.0):	20 eV
MRM2 collision energy (508.0 > 141.0):	18 eV
Dwell:	0.025 (s)

Retention time: 3.2 min (with tolerance of ± 0.2 min in each analytical batch)
MRM transition ratio: 1.8 (with tolerance of ± 30% in each analytical batch)

Validation

The data obtained during the methods validation demonstrate that they are fit for purpose. The adequacy of the methods in the studies matrices was demonstrated. The MRM1 transition 508.0 > 167.0 was selected for quantification. Therefore, the validation parameters of the methods were obtained using data acquired with that MRM transition. In accordance with the requirements stated in SANCO/825/00 rev. 8.1 the data obtained with MRM2 transition, 508.0 > 141.0, can be found in Appendix VI of the report.

Linearity

The linearity of calibration curves with matrix-matched solutions was shown by correlation coefficients above 0.99. On the table below is described the calibration ranges for each matrix.

Specimen	**Calibration range[(1)] (ng/µL)**	**Calibration range[(1)] (mg/kg)**
Apple	0.0015 – 0.075	0.003 – 0.15
Grapes	0.0015 – 0.10	0.003 – 0.20
Rapeseed	0.00075 – 0.05	0.003 – 0.20
Wheat (grain)	0.00075 – 0.03	0.003 – 0.12

[(1)] at least five calibration levels have been injected with matrix matched standard solutions in each specimen extract

LOQ

The LOQ for iodosulfuron-methyl was set at 0.010 mg/kg, for all matrices

LOD

The limit of detection of the method, defined as 30% of LOQ is 0.003 mg/kg, for all matrices.

Accuracy and precision

The accuracy of the methods based on recovery studies done at LOQ and 10x LOQ was in accordance with the criteria set.

The accuracy of the methods is expressed as the mean recovery for each matrix studied. The precision of the methods is expressed by the relative standard deviation, RSD, calculated based on the dispersion of all the recovery data. The results are presented in the table below:

Analyte	Specimen	Sample Code	LOQ (mg/kg)	Mean Recovery (%)			Relative Standard Deviation (%)		
				LOQ	10xLOQ	Method	LOQ	10xLOQ	Method
Iodosulfuron-methyl	Apple	436/VAL19/17	0.010	99.6	82.8	91.2	2.9	2.9	10.1
	Grapes	197/VAL19/17	0.010	106.2	92.6	99.4	1.9	1.0	7.4
	Rapeseed	282/VAL19/17	0.010	85.8	72.9	79.4	4.2	3.0	9.3
	Wheat (grain)	437/VAL19/17	0.010	109.0	88.6	98.8	1.5	3.2	11.1

Specificity / selectivity

For the instrumental conditions used, the methods have shown to be able to identify and quantify iodosulfuron-methyl in all matrices tested.

The MRM transition 508.0 < 1567.0 was used for quantification. The ratio between the MRM transitions 508.0 < 167.0 and 508.0 < 141.0 was used for confirmation of the identity. The methods were able to determine iodosulfuron-methyl in all samples used for recovery tests during this study. This was checked by analysing blank and spiked specimens to verify the absence of interfering signals. The signal from the blank samples was lower than the LOD.

Matrix effects
The response of a calibration solution prepared in each matrix was compared to the response of the same calibration solution in solvent. The results are presented in the table below.

Analyte	Specimen	Matrix Effect (%)
		MRM1 Transition
Iodosulfuron-methyl	Apple	21.5
	Grapes	18.7
	Rapeseed	-15.7
	Wheat (grain)	28.7

Apple and wheat grain extracts have shown significant matrix effects in UPLC-TQ-S-micro, whereas grapes and rapeseed haven't shown significant matrix effects. To quantify the spiked samples, matrix-matched solutions were used for all matrices.

Comments of zRMS:	Method is accepted
Reference:	KCP 5.2-02 (submitted as KCA 4.2-17/18/20)
Report	Independent laboratory validation of the determination of iodosulfuron-methyl in several plant matrices. Schlewitz, P. 2018. R B8064.
Guideline(s):	SANCO/825/00 rev. 8.1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The method under discussion describes the determination of residues of iodosulfuron-methyl in apple, grape, rapeseed and wheat grain. The method was validated at 0.010 mg/kg in apple, grape, rapeseed and wheat grain.
The following points were examined during the study:

Linearity
The linearity of the method was studied between 1.5 ng/mL and 100.4 ng/mL for apple and grape and between 0.8 ng/mL and 50.2 ng/mL for rapeseed and wheat grain (corresponding to 0.003 to 0.2 in mg/kg). The linear correlation coefficients were typically > 0.990, showing a good linearity.

Sensitivity
The limit of quantification (LOQ) is the lowest validated level where a mean recovery within the range 70-110% with a RSD less than 20% could be obtained.
The LOQ was set at 0.010 mg/kg in apple, grape, rapeseed and wheat grain.

Summary of recoveries:

Analyte	Matrix	Fortification level (mg/kg)	Mean recovery Percentage (%)	Standard deviation (SD) (%)	Relative standard deviation (RSD) (%)	Number of fortified samples (n)
Iodosulfuron-methyl	Apple	0.010	106.7%	2.3%	2.1%	5
		0.10	107.9%	2.2%	2.0%	5
		All levels	107.3%	2.2%	2.0%	10
Iodosulfuron-methyl	Grape	0.010	97.4%	1.7%	1.7%	5
		0.10	103.9%	2.8%	2.7%	5
		All levels	100.7%	4.0%	4.0%	10
Iodosulfuron-methyl	Rapeseed	0.010	88.9%	2.8%	3.2%	5
		0.10	94.4%	2.0%	2.1%	5
		All levels	91.7%	3.7%	4.0%	10
Iodosulfuron-methyl	Wheat grain	0.010	105.4%	2.9%	2.7%	5
		0.10	109.5%	2.0%	1.9%	5
		All levels	107.5%	3.2%	3.0%	10

Accuary

The accuracy of the method was assessed on the basis of the determined recovery rates.

	Iodosulfuron-methyl		Iodosulfuron-methyl	
Matrix	Apple		Grape	
Fortification level (mg/kg)	0.010	0.10	0.010	0.10
Single recovery rates	103.4% to 109.2%	105.3% to 110.6%	95.8% to 99.6%	101.1% to 106.9%
Mean recoveries per fortification level	106.7%	107.9%	97.4%	103.9%

	Iodosulfuron-methyl		Iodosulfuron-methyl	
Matrix	Rapeseed		Wheat grain	
Fortification level (mg/kg)	0.010	0.10	0.010	0.10
Single recovery rates	84.0% to 91.0%	91.2% to 96.1%	102.6% to 108.6%	107.3% to 112.5%
Mean recoveries per fortification level	88.9%	94.4%	105.4%	109.5%

Average recoveries at each spiking level are in the range 70-110%, showing a good accuracy of the method.

Precision and repeatability

Repeatability tests (5 recoveries at each fortification level) were performed at the LOQ level and at 10 x LOQ for apple, grape, rapeseed and wheat grain.

	Iodosulfuron-methyl		Iodosulfuron-methyl	
Matrix	Apple		Grape	
Fortification level (mg/kg)	0.010	0.10	0.010	0.10
RSD for each fortification level	2.1%	2.0%	1.7%	2.7%

	Iodosulfuron-methyl		Iodosulfuron-methyl	
Matrix	Rapeseed		Wheat grain	
Fortification level (mg/kg)	0.010	0.10	0.010	0.10
RSD for each fortification level	3.2%	2.1%	2.7%	1.9%

All RSD determined are less than 20%, the method therefore fulfils the requirements of residue analytical methods.

Specificity

The method is able to determine iodosulfuron-methyl in apple, grape, rapeseed and wheat grain. This was checked by analysing control and spiked specimens to verify the absence of interfering peaks. No interfering peaks were present at > 30% of the LOQ.
The analyses were carried out by LC-MS/MS, monitoring two transitions. The method was considered highly specific, thus the use of an alternative method was not necessary.

Confirmatory method
Repeatability tests (5 recoveries) were performed at the LOQ level and at 10 x LOQ level for apple, grape, rapeseed and wheat grain for the qualification transition.
Summary of recoveries:

Analyte	Matrix	Fortification level (mg/kg)	Mean recovery Percentage (%)	Standard deviation (SD) (%)	Relative standard deviation (RSD) (%)	Number of fortified samples (n)
Iodosulfuron-methyl	Apple	0.010	109.9%	3.2%	2.9%	5
		0.10	108.3%	2.6%	2.4%	5
Iodosulfuron-methyl	Grape	0.010	107.8%	3.5%	3.2%	5
		0.10	102.8%	3.6%	3.5%	5
Iodosulfuron-methyl	Rapeseed	0.010	98.8%	7.2%	7.3%	5
		0.10	95.3%	2.3%	2.4%	5
Iodosulfuron-methyl	Wheat grain	0.010	108.8%	3.1%	2.9%	5
		0.10	107.4%	3.7%	3.4%	5

Recoveries and precision data for the qualifier transition comply with the requirements of SANCO/3029/99 rev. 4 as mean recoveries at each spiking level are within the range 70-110% and RSD is less than 20%.

A 2.1.3.1 Description of analytical methods for the determination of residues in animal matrices (KCP 5.2)

No new or additional studies have been submitted

A 2.1.3.2 Description of Methods for the Analysis of Soil (KCP 5.2)

Comments of zRMS:	Method is accepted

Reference:	KCP 5.2-03 (submitted as KCP 4.2-24)
Report	Validation of an analytical method for the determination of iodosulfuron-methyl-sodium in soils. Arias A., 2017. VAL21/17.
Guideline(s):	SANCO/825/00 rev. 8.1 SANCO/3029/99 rev. 4
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Analytical method
Extraction: extraction solvent acetonitrile was added to the sample for extraction and shaken on a vortex. A mixture of magnesium sulphate, sodium chloride and buffering citrate salts was added to the extract

and shaken. After centrifugation, the extract was isolated for UPLC-TS-S-micro analysis.

Method conditions:

Analytical column: ACQUITY UPLC HSS T3 1.8µm from Waters, 2.1 x 100 mm

Gradient:

Time	A %	C %	Flow	Curve
0.00	90.0	10.0	0.300	Initial
1.00	10.0	90.0	0.300	6
4.00	10.0	90.0	0.300	6
4.10	90.0	10.0	0.300	6
5.00	90.0	10.0	0.300	6

A H_2O in 0.1% formic acid
C MeOH in 0.1% formic acid

Autosampler temp: 15°C
Injection volume: 10 µL
Column temp: 30°C
Electrospray polarity: positive
Nebulization, dessolvatation and cone gas: nitrogen
Collision gas: argon
Cone voltage: 26 V
MRM1 collision energy (508.0 > 167.0): 20 eV
MRM2 collision energy (508.0 > 141.0): 18 eV
Dwell: 0.025 (s)
Typical Retention time: 3.3 min (with tolerance of ± 0.2 min. in each analytical batch)
Typical MRM Transition Ratio: 2.0 (with tolerance of ± 30% in each analytical batch)

Method validation

The developed method was validated in terms of linearity, specificity, accuracy and precision. The LOQ was established at 0.010 µg/kg. Matrix effects were also investigated.

Validation data:	
Linearity:	The linearity was investigated with matrix matched solutions in the range of 0.000024 ng/µL to 0.0008 ng/µL (0.003 µg/kg to 0.10 µg/kg) for soil 6S and from 0.000024 ng/µL to 0.0016 ng/µL (0.003 µg/kg to 0.20 µg/kg) for soil 2.2. Correlation coefficients greater than 0.99 were achieved demonstrating acceptable linearity.
Specificity/ interferences :	Specificity was confirmed by the use of LC-MS/MS which is a highly specific technique. Two mass ions were analysed. One reagent blank and at least one control sample was analysed to demonstrate that no interferences greater than 30% of the LOQ were present at the retention time of the analytes.
Repeatability (precision):	The precision of the method was determined by measuring the relative standard deviation at each fortification level from at least five replicates of untreated control samples spiked with each analyte at the method LOQ and 10 x LOQ. Satisfactory precision data were achieved for both transitions from replicate determinations at each fortification level. The relative standard deviation (RSD) obtained at each fortification level was in the acceptable range of ≤ 20 %, demonstrating precision (repeatability) of the method.
Accuracy:	The accuracy was determined from the analysis of at least five replicates of fortified control samples at 0.010 µg/kg and 0.10 µg/kg. The mean recoveries were within the acceptable range of 70 to 110%, demonstrating the analytical accuracy of the method.
Matrix effects:	The matrix effects were determined by comparing the peak area of a solvent stand-

	ard solution to the peak area of a matrix-matched standard solution prepared at equivalent concentrations to the LOQ and 10 x LOQ of the sample method. Significant matrix effects were observed, therefore matrix matched samples were used.

A 2.1.3.3 Description of Methods for the Analysis of Water (KCP 5.2)

Comments of zRMS:	Method is accepted

Reference: KCP 5.2-04 (submitted as KCA 4.4.2-22)

Report: Validation of an analytical method for the determination of iodosulfuron-methyl-sodium and metsulfuron-methyl in surface and drinking water. Gaffney V., 2017. VAL20/17.

Guideline(s): SANCO/825/00 rev. 8.1

Deviations: No

GLP: Yes

Acceptability: Yes

Analytical method

Analytical column: ACQUITY UPLC HSS T3 1.8µm from Waters, 2.1 x 100 mm

Gradient:

Time	A %	B %	Flow	Curve
0.00	90.0	10.0	0.400	Initial
1.00	90.0	10.0	0.400	6
2.00	0.0	100.0	0.400	6
3.00	0.0	100.0	0.400	6
4.00	90.0	10.0	0.400	6
5.00	90.0	10.0	0.400	6

A H_2O in 0.1% formic acid
B ACN in 0.1% formic acid

Autosampler temp: 15°C
Injection volume: 50 µL
Column temp: 40°C
Electrospray polarity: positive
Nebulization, dessolvatation and cone gas: nitrogen
Collision gas: argon

Metsulfuron-methyl

Cone voltage: 40 V
MRM1 collision energy (382.0 > 167.0): 14 eV
MRM2 collision energy (382.0 > 141.0): 14 eV
Dwell: 0.025 (s)
Typical Retention time: 3.1 min (with tolerance of ± 0.2 min. in each analytical batch)
Typical MRM Transition Ratio: 1.6 (with tolerance of ± 30% in each analytical batch)

Iodosulfuron-methyl-sodium

Cone voltage: 26 V
MRM1 collision energy (508.0 > 167.0): 20 eV
MRM2 collision energy (508.0 > 141.0): 18 eV
Dwell: 0.025 (s)
Typical Retention time: 3.3 min (with tolerance of ± 0.2 min. in each analytical batch)
Typical MRM Transition Ratio: 1.2 (with tolerance of ± 30% in each analytical batch)

Method validation

The developed method was validated in terms of linearity, specificity, accuracy and precision. The LOQ was established at 0.050 µg/L. Matrix effects were also investigated.

Validation data:	
Linearity:	The linearity was investigated with matrix matched solutions in the range of 0.015 µg/L to 1.0 µg/L. Correlation coefficients greater than 0.99 were achieved demonstrating acceptable linearity.
Specificity/ interferences :	Specificity was confirmed by the use of LC-MS/MS which is a highly specific technique. Two mass ions were analyzed. One reagent blank and at least one control sample was analyzed to demonstrate that no interferences greater than 30% of the LOQ were present at the retention time of the analytes.
Repeatability (precision):	The precision of the method was determined by measuring the relative standard deviation at each fortification level from at least five replicates of untreated control samples spiked with each analyte at the method LOQ and 10 x LOQ. Satisfactory precision data were achieved for both transitions from replicate determinations at each fortification level. The relative standard deviation (RSD) obtained at each fortification level was in the acceptable range of ≤ 20 %, demonstrating precision (repeatability) of the method.
Matrix effects:	The matrix effects were determined by comparing the peak area of a solvent standard solution to the peak area of a matrix-matched standard solution prepared at equivalent concentrations to the LOQ and 10 x LOQ of the sample method. Significant matrix effects were observed, therefore matrix matched samples were used.

Comments of zRMS:	Method is accepted

Reference:	KCP 5.2-05 (submitted as KCA 4.2-23)
Report	Independent Laboratory Validation of the determination of iodosulfuron-methyl-sodium and metsulfuron-methyl in surface water. Schlewitz P., 2017. R B7267.
Guideline(s):	SANCO/825/00 rev. 8.1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Analytical method

Apparatus	**Xevo - LC-MS/MS**

Column

Description	BEH HSS T3	Supplier	WATERS	Particles	1.7 µm
Internal diam. x length	100 x 2.1 mm	Supplier reference	186003539	Temperature	40 °C
Development Column ANADIAG Number	237	Stationary Phase	HSS T3	Comment	

Mobile phase

A =	H_2O + 0.1 % Formic Acid
B =	Acetonitrile + 0.1 % Formic Acid

Sample temperature	15 ° C

Elution

Elution	Time	Flow	Composition (%)		Curve	Elution	Time	Flow	Composition (%)		Curve
	min	mL/min	A	B	(type)		min	mL/min	A	B	(type)
Pg1	Initial	0.400	90.0	10.0	Initial	Pg5	4.00	0.400	90.0	10.0	6
Pg2	1.00	0.400	90.0	10.0	6	Pg6	5.00	0.400	90.0	10.0	6
Pg3	2.00	0.400	0.0	100.0	6						
Pg4	3.00	0.400	0.0	100.0	6						

6: linear

Detector

IONISATION mode*	ES X	APCI
Polarity*	Pos X	Neg
Capillary (kV)	3.00	
Desolvation temperature (°C)	650 °C	

*make a cross in the right choice

Active ingredient(s)	Cone voltage (V)	Collision voltage (V)	Dwell time (ms)	TRANSITION 1 Parent > Daughter	TRANSITION 2 Parent > Daughter	RT (min.)
Metsulfuron-methyl	20	15	65	382.1 > 167.1 *	-	2.6
	20	15	65	-	382.1 > 141.1	
Iodosulfuron-methyl-sodium	20	20	65	508.0 > 167.1 *	-	2.8
	20	20	65	-	508.0 > 141.1	

** Transition used for quantification*

Date of application of analytical conditions: 03/10/2017

Study	B7267	Column ANADIAG number	237
Matrix	Surface water	Retention time	Iodosulfuron-methyl-sodium: ≈ 2.8 min. Metsulfuron-methyl: ≈ 2.6 min.
Sample temperature	+15°C	Injected volume	40 µL

Method validation

The method under discussion describes the determination of residues of iodosulfuron-methyl-sodium and metsulfuron-methyl in surface water. The method was validated at 0.05 µg/L in surface water.
The following points were examined during the study:

Validation data: *Linearity:*	The linearity of the method was studied between 0.015 ng/mL and 1 ng/mL of iodosulfuron-methyl-sodium and metsulfuron-methyl in matrix-matched calibration

	solutions (corresponding to 0.015 to 1 in µg/L). The linear correlation coefficients were > 0.990, showing a good linearity
Specificity/ interferences :	The method is able to determine iodosulfuron-methyl-sodium and metsulfuron-methyl in surface water. This was checked by analysing control and spiked specimens to verify the absence of interfering peaks. No interfering peaks were present at > 30% of the LOQ. The analyses were carried out by LC-MS/MS, monitoring two transitions. The method was considered highly specific, thus the use of an alternative method was not necessary.
Repeatability (precision):	The precision of the method was determined by measuring the relative standard deviation at each fortification level from at least five replicates of untreated control samples spiked with each analyte at the method LOQ and 10 x LOQ. Satisfactory precision data were achieved for both transitions from replicate determinations at each fortification level. The relative standard deviation (RSD) obtained at each fortification level was in the acceptable range of ≤ 20 %, demonstrating precision (repeatability) of the method.
Accuracy:	The accuracy was determined from the analysis of at least five replicates of fortified control samples at 0.05 µg/L and 0.5 µg/L. The mean recoveries were within the acceptable range of 70 to 110%, demonstrating the analytical accuracy of the method.

A 2.1.3.4 Description of Methods for the Analysis of Air (KCP 5.2)

No new or additional studies have been submitted

A 2.1.3.5 Description of Methods for the Analysis of Body Fluids and Tissues (KCP 5.2)

Comments of zRMS:	Method is accepted

Reference:	KCP 5.2-08
Report	Method validation – Analytical method for the determination of Iodosulfuron Methyl in body fluid and tissue, Andrews G. & Bills K., 2019, FH/19/002.
Guideline(s):	SANCO/825/00 rev. 8.1 SANCO/3029/99 rev. 4
Deviations:	No
GLP:	Yes
Acceptability:	Yes

The objective of this study was to perform a validation for the determination of residues of Iodosulfuron-methyl-sodium in body fluids and tissues.

Materials and methods

Principle of the method

The determination of iodosulfuron-methyl-sodium in body fluid consisted of dilution in methanol:water (1:1 v/v) followed by determination by LC-MS/MS monitoring two ion mass transitions.
The determination of iodosulfuron-methyl-sodium in body tissues consisted of extraction of the samples based on the QuEChERS multi-residue method. Solvents extracts were then diluted in methanol:water (1:1 v/v). Final determination was by LC-MS/MS monitoring two ion mass transitions.

LC-MS/MS Conditions

Instrument: Agilent 1100 HPLC system with Applied Biosystems MDS Sciex API 5000
Column: Waters Xterra MS C8 50 x 2.1
Mobile phase: Gradient
Eluent: A: 0.1% formic acid in water
B: Methanol

Time (minutes)	Eluent A (% v/v)	Eluent B (% v/v)
0.0	90	10
0.1	90	10
3.5	10	90
4.0	10	90
5.0	90	10
6.0	90	10

Flow rate: 0.5 mL/min
Injection volume: 50 µL
Column temperature: 30 °C
Transitions: m/z 506.001 → 139.100
m/z 506.001 → 307.900
Retention time: approximately 3.6 min

Validation

The developed method was validated in terms of linearity, specificity, accuracy ad precision. The LOQ was established at 0.05 mg/L for urine and 0.01 mg/kg for liver. Matrix effects were also investigated. Samples were fortified as described in the following table:

Matrix	Untreated Control Replicates	Replicates at LOQ Fortification Level	Replicates at LOQ x 10 Fortification Level	Reagent Blank Replicates
Urine	2	5 at 0.05 mg/L	5 at 0.5 mg/L	1
Liver	2	5 at 0.01 mg/kg	5 at 0.1 mg/kg	1

Validation data:	
Linearity:	The linearity for iodosulfuron-methyl-sodium was investigated with matrix matched solutions prepared in the range of 0.2 to 12 ng/mL, corresponding to 0.011 to 0.630 mg/L in urine and 0.002 to 0.121 mg/kg in liver. Iodosulfuron-methyl in urine – transition 1: r= 0.9999; y = 48000x + 7840 Iodosulfuron-methyl in urine – transition 2: r= 1.0000; y = 66200x – 202 Iodosulfuron-methyl in liver – transition 1: r = 0.9999; y = 519000x + 99400 Iodosulfuron-methyl in liver – transition 2: r = 0.9999; y = 71200x + 12800

Correlation co-efficients, r, greater than 0.995 were achieved for each matrix using a linear regression with a weighting of 1/x, demonstrating acceptable linearity.

Specificity/ interferences :

Specificity was confirmed by the use of LC-MS/MS which is a highly specific technique. Two mass ions were analysed for each analyte.
One reagent blank and at least one control sample was analysed for each matrix to demonstrate that no interferences greater than 30% of the LOQ were present at the retention time of the analytes.

Repeatability (precision):

The precision of the method was determined by measuring the relative standard deviation at each fortification level from at least five replicates of untreated control samples spiked with each analyte at the method LOQ and 10 x LOQ.
Satisfactory precision data were achieved for both transitions for each matrix from replicate determinations at each fortification level. The relative standard deviation (RSD) obtained at each fortification level was in the acceptable range of ≤ 20 %, demonstrating precision (repeatability) of the method.

Accuracy:

The accuracy was determined from the analysis of at least five replicates of fortified control samples of urine and liver at the method LOQ and at 10 x LOQ. The mean recoveries were within the acceptable range of 70 to 110%, demonstrating the analytical accuracy of the method.

Matrix	**Fortification level**	**Transition**	**Recovery (%)**	**SD (%)**	**RSD (%)**
Urine	0.050 (LOQ)	506.001/139.1	100	1.2	1.2
		506.001/307.9	99	1.6	1.6
	0.5 (10xLOQ)	506.001/139.1	100	0.2	0.2
		506.001/307.9	100	0.9	0.9
Liver	0.010 (LOQ)	506.001/139.1	89	2.3	2.5
		506.001/307.9	87	3.7	4.2
	0.100 (10xLOQ)	506.001/139.1	88	1.0	1.1
		506.001/307.9	87	1.2	1.4

Matrix effects:

The matrix effects were determined for each analyte by comparing the peak area of a solvent standard solution to the peak area of a matrix-matched standard solution prepared at equivalent concentrations to the LOQ and 10 x LOQ of the sample method.
No significant matrix effects (<20%) were observed in either matrix.

Extract stability:

The stability of each analyte was investigated in solvent and in matrix.
Stability of the analytes in solvent stock was determined by comparing the peak area of the stored solutions in a refrigerator to the peak area of a freshly prepared solution at the same concentration.
Stability of the analytes in working solutions was determined by comparing the peak area of the stored solutions in a refrigerator to the peak area of a freshly prepared solution at the same concentration.
Stability of the analytes in matrix was determined by re-quantifying an LOQ extract stored in a refrigerator against a fresh set of calibration solutions.
Results:
Iodosulfuron in methanol: stable up to 35 days when stored in a refrigerator.

	Iodosulfuron in methanol:water (1:1% v/v): stable after 17d storage in a refrigerator. Iodosulfuron in urine extracts: stable after 14d storage in refrigerator Iodosulfuron in liver extracts: stable after 3d storage in refrigerator
Conclusion:	The analytical method for the determination of Iodosulfuron-methyl-sodium in the body fluids and tissue samples tested was found to be satisfactory in terms of linearity, specificity, accuracy and precision and therefore fulfills the requirements according to guidance document SANCO/825/00 rev. 8.1 of 16 November 2010.

A 2.1.3.6 A.2.A.9 Other Studies/ Information

No new or additional studies have been submitted

A 2.2 Analytical methods for mesosulfuron-methyl

A 2.2.1 Methods used for the generation of pre-authorization data (KCP 5.1)

Reference is made to 5.2.1. New/additional studies are summarized below.

A 2.2.1.1 Description of analytical methods for the determination of residues in plant matrices (KCP 5.2)

A 2.2.1.1.1 Analytical method and validation

Reference is made to the methods described in A 2.1.1.1.1.

A 2.2.1.1.1.1 Extraction efficiency

Comments of zRMS:	Method is accepted
Reference:	KCP 5.1.2-04
Report	Cross validation of an extraction method based on Quechers method vs. an extraction method applied in 14C-metabolism studies in the determination of mesosulfuron-methyl in wheat (green material, Arias A., 2018, VAL19/18
Guideline(s):	SANCO/3029/99 rev. 4 (11/07/2000) and SANCO/825/00
Deviations:	No
GLP:	Yes
Acceptability:	Yes

The objective of the study was to perform a cross validation between a method based on the QuEChERS method and the extraction conditions used in the 14C-metabolism studies, for the determination of mesosulfuron-methyl in wheat (green material).
This evaluation was performed by replicate extractions of incurred samples using both methods. Furthermore, a method validation was also done for both methodologies. These validations were

performed according to SANCO/3029/99 rev. 4 and SANCO/825/00 rev. 8.1.

One sample of untreated wheat green material and one with incurred residues of mesosulfuron-methyl were generated during the residue study (ANADIAG study B8019 under the direction of Agnès) and were sent to LabResíduos for the evaluation of the extraction efficiency of mesosulfuron-methyl residues. The homogenization step has been done by ANADIAG. Frozen specimens were delivered in good conditions to LabResíduos.
Analytical methods validation were performed within the scope of this study. The extraction procedures for the determination of mesosulfuron-methyl residues were based on the extraction conditions used in the 14C-metabolism studies and on QuEChERS method. The performance achieved for both methods was fit for purpose (see Table below).

Analyte	Specimen	Extraction methodology	Equipment	Calibration range[1] (mg/kg)	MRM1	MRM2	LOQ (mg/kg)	LOD (mg/kg)	Type of validation	Accuracy[2] (%)			Precision[2] (%)			Study Code
										LOQ	10xLOQ	Method[3]	LOQ	10xLOQ	Method[3]	
Mesosulfuron-methyl	Wheat green material	14C-metabolism study	LC-MS/MS 1	0.003 - 0.20	504.3 > 182.3	504.3 > 139.3	0.010	0.003	full	99.8	93.0	96.4	1.6	0.72	3.9	VAL19/18
Mesosulfuron-methyl	Wheat green material	Quechers	LC-MS/MS 1	0.003 - 0.20	504.3 > 182.3	504.3 > 139.3	0.010	0.003	full	99.6	101.5	100.6	2.8	1.3	2.3	VAL19/18

(1) - at least five calibration levels have been injected with matrix matched standard solutions
(2) - data based on five replicates at each fortification level
(3) - results taking into account the performance at the two studied fortification levels
LOQ - limit of quantification; LOD - limit of detection
Table 4 - Summary of validation data

The results obtained were in accordance with the requirements set on SANCO/3029/99 and also on SANCO/825/00.
The extraction efficiency was sufficiently proven, by comparing the residue amounts quantified in the incurred sample, using the method based on the extraction conditions applied during the 14Cmetabolism studies and on QuEChERS method. The incurred sample was analysed in triplicate for both methods and the results obtained for the two methods differs by 9.1 % (criteria 30 %).
The results of the extraction efficiency are compiled in the table below.

Anadiag Sample Code	LabResíduos Sample Code	Plot	Days	Mesosulfuron-methyl (mg/kg) *		Extraction Efficiency (%)
				14C-metabolism	QuEChERS	
B8019 TL1/T7/B/A	138/VAL19/18	T	7 DAA	0.033	0.036	109.1

T - treated plot; **DAA** - days after application
* - Mean values obtained from three replicate extractions for each method.
Table 1 - Summary of results

The storage time for each sample is reported below.

Field Trial Code	Field Code	Laboratory Code	Sample Reception	Extraction*	Storage Time** (Days)
B8019 TL1	B8019 TL1/U7/B/A	137/VAL19/18	2018-04-18	2018-05-02	14
				2018-04-30	12
	B8019 TL1/T7/B/A	138/VAL19/18	2018-04-18	2018-05-02	14
				2018-04-30	12

* - The first date for each sample refers to the extraction with 14C-metabolism studies method and the second date refers to Quechers extraction
** - Days between the extraction date and the sample reception date
Table 2 - Summary of samples storage

A 2.2.1.2 Description of analytical methods for the determination of residues in animal matrices (KCP 5.2)

No new or additional studies have been submitted.

A 2.2.1.3 Description of Methods for the Analysis of Soil (KCP 5.2)

No new or additional studies have been submitted.

A 2.2.1.4 Description of Methods for the Analysis of Water (KCP 5.2)

Reference is made to the studies summarised for iodosulfuron-methyl-sodium under A 2.1.1.4.1.

A 2.2.1.5 Description of Methods for the Analysis of Air (KCP 5.2)

No new or additional studies have been submitted.

A 2.2.1.6 Description of Methods for the Analysis of Body Fluids and Tissues (KCP 5.2)

No new or additional studies have been submitted.

A 2.2.1.7 A.2.A.9 Other Studies/ Information

Reference is made to the studies submitted under A 2.1.1.7.

A 2.2.2 Methods for post-authorization control and monitoring purposes (KCP 5.2)

A 2.2.2.1 Description of analytical methods for the determination of residues in plant matrices (KCP 5.2)

Comments of zRMS:	Method is accepted

Reference:	KCP 5.2-06 (submitted as KCA 4.2-19/26)
Report	Validation of an analytical method for the determination of mesosulfuron-methyl in plant matrices. Gordo J., 2018. VAL59/17.
Guideline(s):	SANCO/825/00 rev. 8.1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Analytical method

Analytical column:	ACQUITY UPLC HSS T3 1.8µm from Waters, 2.1 x 100 mm

Gradient:

Time	A %	C %	Flow	Curve
0.00	90.0	10.0	0.300	Initial
1.00	10.0	90.0	0.300	6
4.00	10.0	90.0	0.300	6
4.10	90.0	10.0	0.300	6
5.00	90.0	10.0	0.300	6

A H_2O in 0.1% formic acid
B MeOH in 0.1% formic acid

Autosampler temp: 15°C
Injection volume: 1 µL
Column temp: 30°C
Electrospray polarity: positive
Nebulization, dessolvatation and cone gas: nitrogen
Collision gas: argon
Cone voltage: 2 V
MRM1 collision energy (504.0 > 182.1): 22 eV
MRM2 collision energy (504.0 > 139.1): 54 eV
Dwell: 0.025 (s)
Typical Retention time: 3.1 min (with tolerance of ± 0.2 min. in each analytical batch)
Typical MRM Transition Ratio: 4.2 (with tolerance of ± 30% in each analytical batch)

Method validation

The developed method was validated in terms of linearity, specificity, accuracy and precision. The LOQ was established at 0.010 mg/kg. Matrix effects were also investigated.

Hier

Validation data:

Linearity:

The linearity was investigated with matrix matched solutions in the ranges presented in the table below.

Specimen	Calibration range (1) (ng/µL)	Calibration range (1) (mg/kg)
Apple	0.0015 - 0.10	0.003 - 0.20
Grapes	0.0015 - 0.10	0.003 - 0.20
Rapeseed	0.00075 - 0.0375	0.003 - 0.15
Wheat (grain)	0.00075 - 0.050	0.003 - 0.20
Wheat (straw)	0.00075 - 0.0375	0.003 - 0.15

Correlation coefficients greater than 0.99 were achieved demonstrating acceptable linearity.

Specificity/ interferences :

Specificity was confirmed by the use of LC-MS/MS which is a highly specific technique. Two mass ions were analyzed.
One reagent blank and at least one control sample was analyzed to demonstrate that no interferences greater than 30% of the LOQ were present at the retention time of the analytes.

Repeatability (precision):

The precision of the method was determined by measuring the relative standard deviation at each fortification level from at least five replicates of untreated control samples spiked with each analyte at the method LOQ and 10 x LOQ.
Satisfactory precision data were achieved for both transitions from replicate determinations at each fortification level. The relative standard deviation (RSD) obtained at each fortification level was in the acceptable range of ≤ 20 %, demonstrating pre-

cision (repeatability) of the method.

Accuracy

The accuracy was determined from the analysis of at least five replicates of fortified control samples of urine and liver at the method LOQ and at 10 x LOQ. The mean recoveries were within the acceptable range of 70 to 110%, demonstrating the analytical accuracy of the method.

Analyte	Specimen	Sample Code	LOQ (mg/kg)	Mean Recovery (%)		
				LOQ	10xLOQ	Method
Mesosulfuron-methyl	Apple	1070/VAL59/17	0.010	104.9	91.4	98.2
	Grapes	1069/VAL59/17	0.010	95.0	99.1	97.1
	Rapeseed	1150/VAL59/17	0.010	108.6	96.1	102.3
	Wheat (grain)	437/VAL59/17	0.010	105.2	108.2	106.7
	Wheat (straw)	201/VAL59/17	0.010	105.6	93.4	99.5

Matrix effects:

The matrix effects were determined by comparing the peak area of a solvent standard solution to the peak area of a matrix-matched standard solution prepared at equivalent concentrations to the LOQ and 10 x LOQ of the sample method.
Significant matrix effects were observed for grape and wheat straw, whereas apple, rapeseed and wheat grain haven't shown significant matrix effects.

Comments of zRMS:	Method is accepted

Reference:	KCP 5.2-07 (submitted as KCA 4.2-20)
Report	Independent laboratory validation of mesosulfuron-methyl in plant matrices. Wößner A., 2018. S17-07888.
Guideline(s):	SANCO/825/00 rev. 8.1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Method validation

The developed method was validated in terms of linearity, specificity, accuracy and precision. The LOQ was established at 0.010 mg/kg. Matrix effects were also investigated.

Hier

Validation data:

Linearity:

The linearity was investigated with matrix matched calibration standards at a minimum of seven concentrations ranging from 0.75 ng/mL to 50 ng/mL (wheat grain, wheat straw and rapeseed) or 1.5 ng/mL to 100 ng/mL (apple and grapes). The range corresponds to 0.003 mg/kg to 0.2 mg/kg and thus covers the range from no more than 30% of the LOQ and at least + 20% of the highest analyte concentration level detected in a sample extract.
The calibration curves obtained for both mass transitions and all matrices were linear since coefficients of determination correlation coefficients (R) were ≥ 0.995. Linear regression was performed with 1/x-weighting. Linear regression was performed with 1/x-weighting.

Specificity/ interferences :

Specificity was confirmed by the use of LC-MS/MS which is a highly specific technique. Two mass ions were analyzed.

	One reagent blank and at least one control sample was analyzed to demonstrate that no interferences greater than 30% of the LOQ were present at the retention time of the analytes.
Repeatability (precision):	The precision of the method was determined by measuring the relative standard deviation at each fortification level from at least five replicates of untreated control samples spiked with each analyte at the method LOQ and 10 x LOQ. Satisfactory precision data were achieved for both transitions from replicate determinations at each fortification level. The relative standard deviation (RSD) obtained at each fortification level was in the acceptable range of ≤ 20 %, demonstrating precision (repeatability) of the method.
Accuracy	The accuracy was determined from the analysis of at least five replicates of fortified control samples of urine and liver at the method LOQ and at 10 x LOQ. The mean recoveries were within the acceptable range of 70 to 110%, demonstrating the analytical accuracy of the method.
Matrix effects:	The matrix effects were determined by comparing the peak area of a solvent standard solution to the peak area of a matrix-matched standard solution prepared at equivalent concentrations to the LOQ and 10 x LOQ of the sample method. Matrix suppression or enhancement was < 20 % for rape seed and wheat straw and thus deemed to be insignificant. However, matrix-matched standards were used for quantification throughout the study. Matrix effects were ≥ ± 20 % and deemed to be significant for apple, grapes and wheat grain. Therefore, matrix-matched standards were used for quantification throughout the study.

A 2.2.2.2 Description of analytical methods for the determination of residues in animal matrices (KCP 5.2)

Comments of zRMS:	Method is accepted
Reference:	KCP 5.2-14 (submitted as KCA 4.2-27)
Report	Validation of an analytical method for the determination of Mesosulfuron-methyl in food of animal origin, ILV. Arias A., 2018. VAL62/17
Guideline(s):	SANCO/825/00 rev. 8.1 SANCO/3029/99 rev. 4
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Analytical method

Analytical column: ACQUITY UPLC HSS T3 1.8µm from Waters, 2.1 x 100 mm

Gradient:

Time	A %	C %	Flow	Curve
0.00	70.0	30.0	0.400	Initial
0.50	70.0	30.0	0.400	6
3.00	5.0	95.0	0.400	6
4.25	5.0	95.0	0.400	6
4.30	70.0	30.0	0.400	6
6.00	70.0	30.0	0.400	6

A H_2O in 0.1% formic acid
B MeOH in 0.1% formic acid

Autosampler temp: 20°C
Injection volume: 10 µL
Column temp: 40°C
Electrospray polarity: positive
Nebulization, dessolvatation and cone gas: nitrogen
Collision gas: argon
Cone voltage: 2 V
MRM1 collision energy (504.0 > 182.1): 22 eV
MRM2 collision energy (504.0 > 139.1): 54 eV
Dwell: 0.025 (s)
Typical Retention time: 3.6 min (with tolerance of ± 0.2 min. in each analytical batch)
Typical MRM Transition Ratio: 4.0 (with tolerance of ± 30% in each analytical batch)

Method validation

The developed method was validated in terms of linearity, specificity, accuracy and precision. The LOQ was established at 0.010 mg/kg. Matrix effects were also investigated.

Validation data:

Linearity:

The linearity was investigated with calibration solutions in solvent. The linearity of the detector response was checked by injecting several standard solutions, covering a working range from 0.003 mg/kg to 0.60 mg/kg.
The correlation coefficients obtained were higher than 0.99.

Specificity/ interferences :

Specificity was confirmed by the use of LC-MS/MS which is a highly specific technique. Two mass ions were analyzed.
One reagent blank and at least one control sample was analyzed to demonstrate that no interferences greater than 30% of the LOQ were present at the retention time of the analytes.

Repeatability (precision):

The precision of the method was determined by measuring the relative standard deviation at each fortification level from at least five replicates of untreated control samples spiked with each analyte at the method LOQ and 10 x LOQ.
Satisfactory precision data were achieved for both transitions from replicate determinations at each fortification level. The relative standard deviation (RSD) obtained at each fortification level was in the acceptable range of ≤ 20 %, demonstrating precision (repeatability) of the method.

Accuracy

The accuracy was determined from the analysis of at least five replicates of fortified control samples of urine and liver at the method LOQ and at 10 x LOQ. The mean recoveries were within the acceptable range of 70 to 110%, demonstrating the analytical accuracy of the method.

Analyte	Specimen	Sample Code	LOQ (mg/kg)	Mean Recovery (%)		
				LOQ	10xLOQ	Method
Mesosulfuron-methyl	Apple	1070/VAL59/17	0.010	104.9	91.4	98.2
	Grapes	1069/VAL59/17	0.010	95.0	99.1	97.1
	Rapeseed	1150/VAL59/17	0.010	108.6	96.1	102.3
	Wheat (grain)	437/VAL59/17	0.010	105.2	108.2	106.7
	Wheat (straw)	201/VAL59/17	0.010	105.6	93.4	99.5

Matrix effects:

The matrix effects were determined by comparing the peak area of a solvent standard solution to the peak area of a matrix-matched standard solution prepared at equivalent concentrations to the LOQ and 10 x LOQ of the sample method.
Matrix effects were seen and with exception of fat, all matrices showed significant matrix effects.

A 2.2.2.3 Description of Methods for the Analysis of Soil (KCP 5.2)

Comments of zRMS:	Method is accepted

Reference: KCP 5.2-11 (submitted as KCA 4.2-21)

Report: Validation of an analytical method for the determination of mesosulfuron-methyl in soils, Gordo J., 2018, VAL60/17.

Guideline(s): SANCO/825/00 rev. 8.1
SANCO/3029/99 rev. 4

Deviations: No

GLP: Yes

Acceptability: Yes

Analytical method

Analytical column: ACQUITY UPLC HSS T3 1.8µm from Waters, 2.1 x 100 mm

Gradient:

Time	A %	B %	Flow	Curve
0.00	90.0	10.0	0.300	Initial
1.00	90.0	10.0	0.300	6
2.00	0.0	100.0	0.300	6
3.00	0.0	100.0	0.300	6
4.00	90.0	10.0	0.300	6
5.00	90.0	10.0	0.300	6

A H_2O in 0.1% formic acid
B ACN in 0.1% formic acid

Autosampler temp: 15°C
Injection volume: 10 µL
Column temp: 40°C
Electrospray polarity: positive
Nebulization, dessolvatation and cone gas: nitrogen
Collision gas: argon
Cone voltage: 2 V
MRM1 collision energy (504.0 > 182.1): 22 eV
MRM2 collision energy (504.0 > 193.1): 54 eV
Dwell: 0.025 (s)
Typical Retention time: 3.6 min (with tolerance of ± 0.2 min. in each analytical batch)
Typical MRM Transition Ratio: 4.1 (with tolerance of ± 30% in each analytical batch)

Method validation

The MRM1 transition 504.0 > 182.1 was selected for quantification. Therefore, the validation parameters of the methods were obtained using data acquired with that MRM transition. Moreover, in accordance with SANCO/825/00 rev. 8.1, data obtained with MRM2 transition, 504.0 > 139.1 can be found in the study report.

Linearity

The linearity of the calibration curves with matrix matched solutions were shown by correlation coefficient above 0.99.
The calibration range was from 0.00006 ng/µL to 0.004 ng/µL (0.03 µg/kg to 2 µg/kg) for soil 6S and from 0.00006 ng/µL to 0.003 ng/µL (0.03 µg/kg to 1.5 µg/kg) for soil 2.2.

LOQ

The LOQ for mesosulfuron-methyl was set at 0.10 µg/kg.

LOD

The limit of detection of the method, defined as 30% of LOQ is 0.03 µg/kg.

Accuracy and precision
For accuracy and precision, recovery tests were done at LOQ and 10x LOQ. The results are shown in the table below.

Analyte	Specimen	Sample Code	LOQ (µg/kg)	Mean Recovery (%)			Relative Standard Deviation (%)		
				LOQ	10xLOQ	Method	LOQ	10xLOQ	Method
mesosulfuron-methyl	Soil 2.2	58/VAL60/17/18	0.10	102.2	80.4	91.3	4.7	10.6	14.5
	Soil 6S	59/VAL60/17/18	0.10	98.0	79.2	88.6	9.7	4.7	13.5

The obtained values fulfil the requirements from SANCO/825/00 and SANCO/3029/99 set out for accuracy and precision of pesticide residue analytical method for soils.

Specificity/Selectivity
MRM transition 504.0 > 182.1 was used for quantification. The ratio between the MRM transition 504.0 > 182.1 and 504.0 > 139.1 was used for confirmation of the identity. The method is able to determine mesosulfuron-methyl in both samples used for recovery tests during this study. This was checked by analysing blank and spiked specimens to verify the absence of interfering signals.
The signal from blank samples was lower than LOD.

Matrix effects
The response of a calibration solution (0.002 ng/µL) prepared in each matrix was compared against the same calibration solution in solvent. Both matrices, soil 2.2 and soil 6S, showed significant matrix effects in UPLC-TQ-S-micro.

A 2.2.2.4 Description of Methods for the Analysis of Water (KCP 5.2)

Comments of zRMS:	Method is accepted

Reference:	KCP 5.2-12 (submitted as KCA 4.2-22)
Report	Validation of an analytical method for the determination of mesosulfuron-methyl in surface and drinking water. Gaffney V., 2018. VAL61/17.
Guideline(s):	SANCO/825/00 rev. 8.1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Analytical method

Analytical column: ACQUITY UPLC HSS T3 1.8µm from Waters, 2.1 x 100 mm

Gradient:

Time	A %	B %	Flow	Curve
0.00	90.0	10.0	0.300	Initial
1.00	90.0	10.0	0.300	6
2.00	0.0	100.0	0.300	6
3.00	0.0	100.0	0.300	6
4.00	90.0	10.0	0.300	6
5.00	90.0	10.0	0.300	6

A H_2O in 0.1% formic acid
B ACN in 0.1% formic acid

Autosampler temp: 15°C
Injection volume: 50 µL
Column temp: 40°C

Electrospray polarity: positive
Nebulization, dessolvatation and cone gas: nitrogen
Collision gas: argon
Cone voltage: 2 V
MRM1 collision energy (504.0 > 182.1): 22 eV
MRM2 collision energy (504.0 > 139.1): 54 eV
Dwell: 0.025 (s)
Typical Retention time: 3.6 min (with tolerance of ± 0.2 min. in each analytical batch)
Typical MRM Transition Ratio: 4.3 (with tolerance of ± 30% in each analytical batch)

Method validation
The developed method was validated in terms of linearity, specificity, accuracy and precision. The LOQ was established at 0.050 µg/L. Matrix effects were also investigated.

Validation data:	
Linearity:	The linearity was investigated with matrix matched solutions in the range of 0.015 µg/L to 0.75 µg/L. Correlation coefficients greater than 0.99 were achieved demonstrating acceptable linearity.
Specificity/ interferences :	Specificity was confirmed by the use of LC-MS/MS which is a highly specific technique. Two mass ions were analyzed. One reagent blank and at least one control sample was analyzed to demonstrate that no interferences greater than 30% of the LOQ were present at the retention time of the analytes.
Repeatability (precision):	The precision of the method was determined by measuring the relative standard deviation at each fortification level from at least five replicates of untreated control samples spiked with each analyte at the method LOQ and 10 x LOQ. Satisfactory precision data were achieved for both transitions from replicate determinations at each fortification level. The relative standard deviation (RSD) obtained at each fortification level was in the acceptable range of ≤ 20 %, demonstrating precision (repeatability) of the method.
Matrix effects:	The matrix effects were determined by comparing the peak area of a solvent standard solution to the peak area of a matrix-matched standard solution prepared at equivalent concentrations to the LOQ and 10 x LOQ of the sample method. No significant matrix effects were observed.

Comments of zRMS:	Method is accepted

Reference:	KCP 5.2-13 (submitted as KCA 4.2-23)
Report	Independent Laboratory Validation of mesosulfuron-methyl in water. Wößner A., 2018. S17-07890.
Guideline(s):	SANCO/825/00 rev. 8.1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Method validation
The method under discussion describes the determination of residues of mesosulfuron-methyl insurface water. The method was validated at 0.05 µg/L in surface water.

The following points were examined during the study:

Validation data:	
Linearity:	The linearity of the method was studied between 0.015 ng/mL and 1 ng/mL (for the validation) or 3 ng/mL (for matrix effect test). The linear correlation coefficients were > 0.995, showing a good linearity
Specificity/ interferences :	The method is able to determine iodosulfuron-methyl-sodium and metsulfuron-methyl in surface water. This was checked by analysing control and spiked specimens to verify the absence of interfering peaks. No interfering peaks were present at > 30% of the LOQ. The analyses were carried out by LC-MS/MS, monitoring two transitions. The method was considered highly specific, thus the use of an alternative method was not necessary.
Repeatability (precision):	The precision of the method was determined by measuring the relative standard deviation at each fortification level from at least five replicates of untreated control samples spiked with each analyte at the method LOQ and 10 x LOQ. Satisfactory precision data were achieved for both transitions from replicate determinations at each fortification level. The relative standard deviation (RSD) obtained at each fortification level was in the acceptable range of ≤ 20 %, demonstrating precision (repeatability) of the method.
Accuracy:	The accuracy was determined from the analysis of at least five replicates of fortified control samples at 0.05 µg/L and 0.5 µg/L. The mean recoveries were within the acceptable range of 70 to 110%, demonstrating the analytical accuracy of the method.
Matrix effects	Matrix effects on the detection of mesosulfuron-methyl in surface water were found to be significant (≥ 20%). Therefore, matrix matched standards were used for quantification.

A 2.2.2.5 Description of Methods for the Analysis of Air (KCP 5.2)

No new or additional studies have been submitted

A 2.2.2.6 Description of Methods for the Analysis of Body Fluids and Tissues (KCP 5.2)

A 2.2.2.6.1 Analytical method and validation

Comments of zRMS:	Method is accepted

Reference:	KCP 5.2-09
Report	Validation of an analytical method for the determination of mesosulfuron-methyl in body fluids and animal matrices, Knop M., 2018, S17-07891.
Guideline(s):	SANCO/825/00 rev. 8.1
Deviations:	No

GLP: Yes

Acceptability: Yes

The objective of this study was to develop and validate an analytical method for the determination of mesosulfuron-methyl in body fluids according to the guidance document SANCO/825/00 rev. 8.1 of the European Commission with a limit of quantification (LOQ) of 0.05 mg/L for body fluids.

Materials and methods

Principle of the method
In brief, samples of matrix were extracted with acetonitrile after addition of water. A salt mixture containing magnesium sulphate, sodium chloride and sodium citrate was added and the extract was shaken. After centrifugation, the samples were diluted. Quantification was performed by use of LC-MS/MS detection.

Body fluid (urine) samples are extracted by QuEChERS clean-up prior to quantitation performed using liquid chromatography with tandem mass spectrometric detection (LC-MS/MS) monitoring two ion transitions to satisfy the confirmatory analysis requirement. Subsamples of each of the matrices were fortified with known concentrations of mesosulfuron-methyl and then analysed according to the following regime:

- 2 subsamples of control matrix
- 5 subsamples of control matrix fortified at the LOQ (0.05 mg/L for body fluids)

These samples were then analysed using the analytical methodology, with each sample injected onto the chromatograph once.

LC-MS/MS Conditions

Instrument: 1290 Infinity II HPLC, Agilent Technologies
Pre-column: UHPLC guard column (AJ0-9000, Phenomenex) with 2.1 mm C18 cartridge (AJ0-8782, Phenomenex)
Column: Agilent ZORBAX Eclipse XDB-C18, 600bar, 50 mm x 4.6 mm, 1.8 µm, (Part No. 927975-902)
Mobile phase: Eluent A: Water containing 0.1 % (v/v) formic acid
Eluent B: Methanol containing 0.1 % (v/v) formic acid

Time (minutes)	Eluent A (% v/v)	Eluent B (% v/v)
0.0	70	30
0.5	70	30
3.0	5	95
4.25	5	95
4.30	70	30
6.0	70	30

Divert valve: 0.0 min to 3.0 min to waste; 3.0 min to 4.2 min to MS;
4.2 min to 6.0 min to waste
Flow rate: 0.7 mL/min
Injection volume: 10 µL
Column temperature: 40 °C
Transitions: m/z 504 → 182
m/z 504 → 139
Retention time: approximately 3.5 min

Validation
The developed method was validated in terms of linearity, specificity, accuracy ad precision. The LOQ was established at 0.05 mg/L. Matrix effects were also investigated.

Validation data:	

Linearity:

The linearity of the detector response was demonstrated by single determination of matrix-matched calibration standards at a minimum of eight concentration levels ranging from 0.075 ng/mL to 15 ng/mL. This range corresponds to a fortification level of 0.015 mg/L to 3.0 mg/L for urine and thus covers the range from no more than 30% of the LOQ and at least 20% of the highest analyte concentration detected in a sample extract.

mesosulfuron-methyl in urine, 504→182 *m/z*: r = 0.9999; y= 2.18E006x + 1.54E004
mesosulfuron-methyl in urine, 504→139 *m/z*: r = 0.9998; y= 3.5E005x + 1.42E003

The calibration curves obtained for both ion mass transitions were linear with correlation coefficients R ≥ 0.995. Linear regression was performed with 1/x-weighting.

Specificity/ interferences :

Specificity was confirmed by the use of LC-MS/MS which is a highly specific technique. Two mass ions were analysed for each analyte.
One reagent blank and two control samples were analysed for each matrix to demonstrate that no interferences greater than 30% of the LOQ were present at the retention time of the analytes.

Accuracy and Repeatability (precision):

Accuracy was determined by fortification of control samples with known amounts of the test reference item and subsequent determination of the recoveries when applying the extraction procedure. Precision was determined by repeatability (relative standard deviation).
5 recovery determinations were performed at 0.05 m/L. Analysis was performed by extraction and single injection.
Mean recoveries were in the range of 70-120% (89 and 90%) with relative standard deviations of ≤ 20% (2 and 3%).

Matrix	**Fortification level (mg/L)**	**Recovery (%)**	**Mean recovery (%)**	**Rel. std. dev. (%)**	**Replicates**
Mass Transition 504 → 182 *m/z* (quantification					
Urine	0.05	85, 91, 92, 89, 91	90	3	5
Mass transition 504 → 139 m/z (confirmation)					
Urine	0.05	85, 90, 90, 89, 89	89	2	5

Matrix effects:

The matrix effects were determined for each analyte by comparing the peak area of a solvent standard solution to the peak area of a matrix-matched standard solution prepared at identical nominal concentrations.
No significant matrix effects (<20%) were observed.

Extract stability:

Following the first analysis, the final extract of urine at the LOQ level together with 2 control sample extracts were stored at typically 1°C to 10°C in the dark for at least 11 days. After this period, the final extracts were re-analysed against freshly prepared calibration standards. One mass transition was evaluated.
The mean recovery of the freshly prepared standard was 90%, after storage the mean recovery decrease to 83%. As a conclusion, the mean recovery values of the re-analysed extracts were in the range of 70-120% and within 20% of the original result. Therefore, extracts are considered to be stable when stored at 1°C to 10°C for at least 11 days in the dark.

Conclusion:	The analytical method for the determination of Mesosulfuron-methyl in the body fluid sample tested was found to be satisfactory in terms of linearity, specificity, accuracy and precision and therefore fulfills the requirements according to guidance document SANCO/825/00 rev. 8.1 of 16 November 2010.

A 2.2.2.6.2 Independent Laboratory Validation

Reference:	KCP 5.2-10
Report	Independent method validation – determination of residues of mesosulfuron-methyl in body fluid, Andrews G. and Pearson J, 2018, FH/18/004.
Guideline(s):	SANCO/825/00 rev. 8.1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

The method described in Eurofins report No. S17-07891 was independently validated in terms of linarity, specificity, accuracy and precision using two ion mass transitions (one for quantification and a second for confirmation). The LOQ was established and matrix effects of the analyte were also investigated.

Samples were fortified as described in the following table:

Matrix Sample	Untreated Control	Replicates at LOQ Fortification Level	Reagent Blank
Urine	2	5 at 0.05 mg/L	1

Linearity
The linearity of the method was investigated by analysing matrix-matched calibration solutions containing mesosulfuron-methyl at eight different levels. These calibration standards covered a nominal range from 0.075 to 15 ng/mL in urine, corresponding to 0.015 to 3.0 mg/L in the sample extract.

Specificity
Specificity was confirmed by the use of LC-MS/MS which is a highly specific technique. One reagent blank and two untreated urine samples were analysed to demonstrate that no interferences greater than 30% of the LOQ were present at the retention time of the analyte.

Accuracy
The accuracy was determined from the analysis of five replicates of fortified control samples at the LOQ.

Precision
The precision of the method was determined by measuring the relative standard deviation at the fortification level from replicates of untreated urine samples spiked with mesosulfuron-methyl at the LOQ.

Confirmation
Two structurally significant ion mass transitions were monitored, one for quantification purposes and one for confirmation purposes. Accuracy and precision data for both transitions were reported.

Matrix effects
Matrix effects were assessed by comparing the response between a solvent calibration solution and matrix

matched calibration solutions prepared at the same concentrations. Matrix effects were determined at the LOQ level.

Conclusion
The method was successfully validated independently and is suitable for the determination of residues of mesosulfuron-methyl in urine over the concentration range tested.

A 2.2.2.7 A.2.A.9 Other Studies/ Information

No new or additional studies have been submitted

A 2.3 Analytical methods for mefenpyr-diethyl

A 2.3.1 Methods used for the generation of pre-authorization data (KCP 5.1)

Reference is made to 5.2.1.

A 2.3.2 Methods for post-authorization control and monitoring purposes (KCP 5.2)

A 2.3.2.1 Description of analytical methods for the determination of residues in plant matrices (KCP 5.2)

No new or additional studies have been submitted

A 2.3.2.2 Description of analytical methods for the determination of residues in animal matrices (KCP 5.2)

No new or additional studies have been submitted

A 2.3.2.3 Description of Methods for the Analysis of Soil (KCP 5.2)

No new or additional studies have been submitted

A 2.3.2.4 Description of Methods for the Analysis of Water (KCP 5.2)

No new or additional studies have been submitted

A 2.3.2.5 Description of Methods for the Analysis of Air (KCP 5.2)

No new or additional studies have been submitted

A 2.3.2.6 Description of Methods for the Analysis of Body Fluids and Tissues (KCP 5.2)

No new or additional studies have been submitted

A 2.3.2.7 A.2.A.9 Other Studies/ Information

No new or additional studies have been submitted