

FINAL REGISTRATION REPORT

Part B

Section 5

Analytical Methods

Detailed summary of the risk assessment

Product code: JME-HER 12 OD

Product name(s): -

Chemical active substance:

iodosulfuron-methyl-sodium, 2 g/L

mesosulfuron methyl, 10 g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(authorization)

Applicant: Pestila Sp. z o.o.

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Version history

When	What
January 2024	Dossier sent for evaluation
04.2024	Update of dRR on evaluator's request
July 2024	zRMS finalised evaluation
October 2024	Final version prepared by zRMS after Commenting period

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zRMS comments:

The text highlighted in grey was provided by the evaluator.

5 Analytical methods

This document has been prepared by copying the risk assessments and summary of studies included in the Atlantis 12 OD renewal Registration Report (zRMS: Poland, MS finalisation: 12/02019). The information and studies used in this document are not protected in accordance with Art. 59 Reg. 1107/2009 and can be used for purpose of JME-HER 12 OD registration.

5.1 Conclusion and summary of assessment

Residues

The Applicant did not provide any new data. dRR is based on data evaluated in the Atlantis 12 OD renewal Registration Report (zRMS PL, 2020). The dRR Part B7 assessment for Atlantis 12 OD has been made available by the Ministry of Agriculture and Rural Development for review by zRMS.

The cGAPs assessed and accepted for Atlantis 12 OD covers the cGAPs proposed for JME-HER 12 OD.

Since the assessment for Atlantis 12 OD, there have been no changes to the residue definitions of both active substances. The MRL values have also not changed, the MRL values in accordance with Reg. (EU) 289/2014 for both active substances still apply. It should therefore be considered that the conclusion of the Atlantis 12 OD evaluation also applies to JME-HER 12 OD.

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

Noticed data gaps are:

none

Commodity/crop	Supported/ Not supported
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)

RMS comment on use of the art. 34 of the 1107/2009 to support JME-HER 12 OD registration in Poland

From physicochemical perspective JME-HER 12 OD is considered equivalent/ comparable to already registered Atlantis 12 OD in Poland. So, unprotected physicochemical data taken from Atlantis 12 OD can be used to support JME-HER 12 OD registration in Poland.

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

An overview on the acceptable methods and possible data gaps for analysis of active substances

mesosulfuron-methyl and iodosulfuron-methyl-sodium in plant protection product is provided as follows:

Comments of zRMS:	The method is accepted and can be applied for analysing mesosulfuron-methyl and iodosulfuron-methyl-sodium in the PPP.
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Reference:	5.1.1
Report	JME-HER 12 OD. Determination of physicochemical properties of the preparation in a COEX bottle, Ciach J., 2023, report No. 001/DPL/2023
Guideline(s):	Yes, 3030/99 rev.5
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Determination of mesosulfuron-methyl and iodosulfuron-methyl-sodium in JME-HER 12 OD was performed with LC liquid chromatograph equipped with DAD detector and external standard.

Equipment and chromatographic conditions for mesosulfuron-methyl and iodosulfuron-methyl-sodium analysis

- Agilent Technologies 1220 Infinity II LC Liquid chromatograph equipped with DAD detector,
- Analytical balance,
- Sartorius,
- Ultrasonic bath,
- Bandelin Sonorex Digiplus, WP/78;
- Water purification system, Millipore, WP/86;
- Magnetic stirrer,
- Steinberg Systems, WP/79, WP/80;
- Basic laboratory glassware and equipment.

The preparation of standard solution mesosulfuron-methyl and iodosulfuron-methyl-sodium analysis

Mesosulfuron-methyl

0.040±0.008 g of the mesosulfuron methyl standard was weighed with accuracy of ± 0.0002 g into the flask of 100 ml, Then the flask was made up to ¾ of the volume of the eluent A. It was left in an ultrasonic bath for 15 minutes and the solution was cooled to ambient temperature (18÷25°C) in a water bath for 30 min. The flask was made up to the volume of the eluent A and the content was mixed.

Iodosulfuron-methyl-sodium

0.040±0.008 g of the iodosulfuron-methyl-sodium standard was weighed with accuracy of ± 0.0002 g into the flask of 100 ml, Then the flask was made up to ¾ of the volume of the eluent A. It was left in an ultrasonic bath for 15 minutes and the solution was cooled to ambient temperature (18÷25°C) in a water bath for 30 min. The flask was made up to the volume of the eluent A and the content was mixed.

Calibration standard

25 ml of the mesosulfuron methyl standard stock solution and 5 ml of the iodosulfuron-methyl-sodium standard stock solution was added with a one-mark pipette into the flask of 100 ml. The flask was made up to the volume of the acetonitrile and the content was mixed.

The preparation of test item solution

1.0 g ± 0.2 g of the preparation was weighed with accuracy of ± 0.0002 g into the flask of 100 ml, 5 ml of the tetrahydrofuran was added and mixed until the preparation was completely dispersed. Then 5 ml of

the eluent A was added, the content was mixed and the flask was made up to $\frac{3}{4}$ of the volume of the acetonitrile. It was left in an ultrasonic bath for 15 minutes and the solution was cooled to ambient temperature ($18 \div 25^{\circ}\text{C}$) in a water bath for 30 min. The flask was made up to the volume of the acetonitrile, the content was mixed and filtered through a $0.22\ \mu\text{m}$ PTFE syringe filter prior to injection.

Validation - Results and discussions

Table 5.2-1: Methods suitable for the determination of active substances mesosulfuron-methyl and iodosulfuron-methyl-sodium in plant protection product JMe-HER 12 OD

	mesosulfuron-methyl	iodosulfuron-methyl-sodium
Author(s), year	Ciach J., 2023	
Principle of method	SANCO/3030/99 rev.5, 22 March 2019	
Linearity (linear between mg/L / % range of the declared content) (correlation coefficient, expressed as r)	The linearity of the analytical method was assessed using five mesosulfuron-methyl standard solutions in the concentration range from 3.281 % w/w to 16.403 % w/w. Correlation coefficient: $R^2 = 99981$ Required: $R^2 \geq 0.98$	The linearity of the analytical method was assessed using seven iodosulfuron-methyl-sodium standard solutions in the concentration range from 0.064 % w/w to 0.32 % w/w. Correlation coefficient: $R^2 = 99984$ 99984 Required: $R^2 \geq 0.98$
Precision – Repeatability Mean n = 6 (%RSD)	$H_r = 0.19$ Required: $H_r \leq 1$ $\text{RSD} = 0.51\%$ Required: $\text{RSD} \leq 2.66\%$	$H_r = 0.19$ Required: $H_r \leq 1$ $\text{RSD} = 0.65\%$ Required: $\text{RSD} \leq 3.39\%$
Accuracy n = 6 (% Recovery)	101.54% (range: 99.14% - 104.26%) Required: $90\% \div 110\%$	102.02% (range: 101.14% - 104.3%) Required: $80\% \div 120\%$
Interference/ Specificity	There are no any interferences coming from impurities for the peak of the target analyte – mesosulfuron-methyl.	There are no any interferences coming from impurities for the peak of the target analyte – iodosulfuron-methyl-sodium.
Comment	No comments.	No comments.

Conclusion

The LC liquid chromatograph equipped with DAD detector and external standard, used to quantify mesosulfuron-methyl and iodosulfuron-methyl-sodium in JME-HER 12 OD was fully validated. Method validation included linearity, non-analyte interference, precision, accuracy and specificity. All measured parameters meet the criteria given in SANCO/3030/99 rev.5, 22 March 2019.

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

Not relevant. The product JME-HER 12 OD does not contain relevant impurities.

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

5.1.1)

Not relevant for new registration according to art. 34 of Reg. 1107/2009 based on data which protection period has expired. For the purpose of evaluation of JME HER 12 OD please refer to Renewal RR for Atlantis 12 OD.

With respect to toxicological, eco-toxicological or environmental aspects the product does not contain any relevant formulants. Therefore, a special analytical method and validation is not needed.

5.2.1.4 Applicability of existing CIPAC methods (KCP 5.1.1)

Not relevant for new registration according to art. 34 of Reg. 1107/2009 based on data which protection period has expired. For the purpose of evaluation of JME HER 12 OD please refer to Renewal RR for Atlantis 12 OD.

There is no CIPAC method available for the determination of iodosulfuron-methyl-sodium or mesosulfuron-methyl.

A CIPAC method exists for the analysis of the safener mefenpyr-diethyl in formulations (CIPAC 651.229).

5.2.2 Methods for the determination of residues (KCP 5.1.2)

Not relevant for new registration according to art. 34 of Reg. 1107/2009 based on data which protection period has expired. For the purpose of evaluation of JME HER 12 OD please refer to Renewal RR for Atlantis 12 OD, which are, in applicant's opinion fulfil requirements of "Guidance Document on Pesticide Analytical Methods for Risk Assessment and Post approval Control and Monitoring Purposes" (SANTE/2020/12830, Rev.2, 14 February 2023).

Iodosulfuron-methyl-sodium

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.

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

Component of residue definition: iodosulfuron-methyl and its salts				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Method No / Author(s), year / Document No (report) / missing / EU agreed (Source)
Wheat grain (Residues)	Primary Method AL008/96-0	0.01 mg/kg	HPLC-UV	Wrede A., 1997; M-141767-02-1 DAR, Germany, 2000; EU agreed
	Amendment Method AL008/96-0	0.01 mg/kg	HPLC-UV	Wrede A., 1998; M-141767-02-1 DAR, Germany, 2000; EU agreed
	Confirmatory Method AL008/96-0	0.01 mg/kg	HPLC-UV	Wrede A., 1998; M-182662-01-1 DAR, Germany, 2000; EU agreed
	ILV	0.01 mg/kg	HPLC-UV	Taylor N., 1998;

Component of residue definition: iodosulfuron-methyl and its salts				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Method No / Author(s), year / Document No (report) / missing / EU agreed (Source)
	Method AL008/96-0			M-181323-02-1 DAR, Germany, 2000; EU agreed
Wheat straw (Residues)	Primary Method AL121/96-0	0.05 mg/kg	HPLC-UV	Wrede A., 1997; M-143342-01-1 DAR, Germany, 2000; EU agreed
Wheat shoot (Residues)	Primary Method AL120/96-0	0.05 mg/kg	HPLC-UV	Wrede A., 1997; M-142974-01-1 DAR, Germany, 2000; EU agreed
Wheat shoot and straw (Residues)	Radiovalidation of methods AL120/96-0 (shoot) and AL121/96-0 (straw)	0.05 mg/kg	HPLC-UV	Wrede A., 1998; M-182685-01-1 DAR, Germany, 2000 EU agreed
Cereal grain Cereal shoot Cereal straw	Primary Method EM F08/99 renamed 00815	0.01 mg/kg 0.05 mg/kg 0.05 mg/kg	LC-MS/MS	Wrede A., 2000; M-194528-01-1 DAR, Germany, 2000; EU agreed
Cereal grain Cereal shoot Cereal straw	Validation Method EM F08/99-0	0.01 mg/kg 0.05 mg/kg 0.05 mg/kg	LC-MS/MS	Wrede A., 2000; M-194531-01-1 DAR, Germany, 2000; EU agreed
Lemon Tomato Maize kernel	Validation of EM Method F08/99-0	0.01 mg/kg 0.01 mg/kg 0.01 mg/kg	LC-MS/MS	Wrede A., 2002; M-212674-01-1 RAR, Sweden, 2015; EU agreed
Wheat grain	ILV Method EM F08/99-0	0.01 mg/kg	LC-MS/MS	Ansprach T., 2001; M-200884-01-1 RAR, Sweden, 2015; EU agreed
Tomato Citrus	ILV Method EM F08/99-0	0.01 mg/kg	LC-MS/MS	Reichert N., Klimmek S., 2002; M-215456-01-1 RAR, Sweden, 2015; EU agreed
Cereal grain Cereal shoot Cereal straw Flax (grain, pomace,wet, oil) Flax (plant without root)	Primary Method 00815/M001 (modification of EM F08/99-0)	0.01 mg/kg 0.05 mg/kg 0.05 mg/kg 0.01 mg/kg 0.05 mg/kg	LC-MS/MS	Heinemann, O., 2004; M-226888-01-1 RAR Dec.2015 EU agreed
	Primary Method	0.05 mg/kg 0.01 mg/kg	LC-MS/MS	Stuke, S., 2017; M-503498-02-1;

Component of residue definition: iodosulfuron-methyl and its salts				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Method No / Author(s), year / Document No (report) / missing / EU agreed (Source)
	00815/M001 Additional validation in the residue study 13-2127	0.05 mg/kg		Not Peer reviewed; Appendix 2
	Primary Method 00815/M001 Additional validation in the residue study 13- 2129	0.05 mg/kg 0.01 mg/kg 0.05 mg/kg	LC-MS/MS	Stuke, S., 2017, M-506719-02-1 Not Peer reviewed Appendix 2
Cereal green material, Cereal grain, Cereal straw	Primary Method 00815/M001 Additional validation in the residue study 16-2030 (barley only)	0.05 mg/kg 0.01 mg/kg 0.05 mg/kg	LC-MS/MS	Mahlo, C.; Gabriel, E. ; Vagt, I.; Meyer, M.; 2017 M-612290-01-1 Not Peer reviewed; Appendix 2
Wheat grain Wheat green material Wheat straw Rape seed	Primary method 01376/M002	0.01 mg/kg	LC-MS/MS (2 MRM transitions validated)	Kaussmann M.; 2017, M-587949-01-1 Not peer reviewed Appendix 2
Wheat grain Wheat green material Wheat straw Barley grain Barley green material Barley straw	Primary method 01514	0.01 mg/kg (iodosulfuron- methyl-sodium metabolites: AE F059411 and AE 0031838)	LC-MS/MS (2MRM transitions validated)	Kaussmann M., 2017 M-583894-01-1 Not peer reviewed Appendix 2

Mesosulfuron-methyl

An overview on the acceptable methods and possible data gaps for analysis of residues of mesosulfuron-methyl for the generation of pre-authorization data is given in the following table.

Table 5.2-3: Validated methods for the generation of pre-authorization data

Component of residue definition: mesosulfuron-methyl				
Matrix type	Method type / Method No	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
Wheat grain Wheat straw Wheat shoot	Primary DGM F02/99-0	0.01mg/kg 0.05 mg/kg 0.05 mg/kg	LC-MS/MS	Wrede, A., 1999, M-191437-01-1 DAR Dec.2001

Component of residue definition: mesosulfuron-methyl				
Matrix type	Method type / Method No	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
(Residues)				EU agreed
Cereal grain Cereal shoot Cereal straw	Primary EM F08/99 renamed 00815	0.01mg/kg 0.05 mg/kg 0.05 mg/kg	LC-MS/MS	Wrede, A., 2000, M-194528-01-1 RAR Dec.2015 EU agreed
Cereal grain Cereal shoot Cereal straw	Validation of EM F08/99-0 renamed 00815	0.01 mg/kg 0.05 mg/kg 0.05 mg/kg	LC-MS/MS	Wrede, A., 2000, M-194531-01-1 RAR Dec.2015 EU agreed
Lemon Tomato Maize kernel	Validation of EM F08/99-0 renamed 00815	0.01 mg/kg	LC-MS/MS	Wrede, A., 2002, M-212674-01 -1 RAR Dec.2015 EU agreed
Wheat grain	ILV of EM F08/99-0 renamed 00815	0.01 mg/kg	LC-MS/MS	Reichert, N., 2000, M-198857-01-1 RAR Dec.2015 EU agreed
Tomato Citrus	ILV of EM F08/99-0 renamed 00815	0.01 mg/kg	LC-MS/MS	Reichert, N., Klimmek, S., 2002; M-215456-01-1 RAR Dec.2015 EU agreed
Cereal grain Cereal shoot Cereal straw Flax (grain, pomace,wet, oil) Flax (plant without root)	Primary Method 00815/M001 (modification of EM F08/99-0)	0.01 mg/kg 0.05 mg/kg 0.05 mg/kg 0.01 mg/kg 0.05 mg/kg	LC-MS/MS	Heinemann, O., 2004; M-226888-01-1 RAR Dec.2015 EU agreed
Cereal green material, Cereal grain, Cereal straw	Primary Method 00815/M001 Additional validation in the residue study 13-2127	0.05 mg/kg 0.01 mg/kg 0.05 mg/kg	LC-MS/MS	Stuke, S., 2017 M-503498-03-1 Not Peer reviewed; Appendix 2
	Primary Method 00815/M001 Additional validation in the residue study 13-2129	0.05 mg/kg 0.01 mg/kg 0.05 mg/kg	LC-MS/MS	Stuke, S., 2017 M-506719-02-1; Not Peer reviewed; Appendix 2

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

Not relevant for new registration according to art. 34 of Reg. 1107/2009 based on data which protection period has expired. For the purpose of evaluation of JME HER 12 OD please refer to Renewal RR for

Atlantis 12 OD.

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.

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

Not relevant for new registration according to art. 34 of Reg. 1107/2009 based on data which protection period has expired. For the purpose of evaluation of JME HER 12 OD please refer to Renewal RR for Atlantis 12 OD.

Compared to the residue definition proposed in the Draft Assessment Report 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	0.01* mg/kg / LOQ	Reg. (EU) 289/2014
Plant, high acid content		0.01* mg/kg / LOQ	Reg. (EU) 289/2014
Plant, high protein/high starch content (dry commodities)		0.01* mg/kg / LOQ	EFSA Journal, 2012; 10(11):2974 Reg. (EU) 289/2014
Plant, high oil content		0.02* mg/kg / LOQ	Reg. (EU) 289/2014
Plant, difficult matrices (hops, spices, tea)		0.05* mg/kg / LOQ	Reg. (EU) 289/2014
Muscle	Not defined (EFSA, 2016)	0.02* mg/kg / LOQ	Reg. (EU) 289/2014
Milk			
Eggs			
Fat			
Liver, kidney			
Soil (Ecotoxicology)	Sum of iodosulfuron-methyl and its salts, expressed as iodosulfuron-methyl	0.01 µg/kg / LOQ	
Drinking water (Human toxicology)	Sum of iodosulfuron-methyl and its salts, expressed as iodosulfuron-methyl and metsulfuron-methyl (AE F075736)	0.05 µg/L / LOQ (both analytes)	general limit for drinking water: 0.1 µg/L

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Surface water (Ecotoxicology)	Sum of iodosulfuron-methyl and its salts, expressed as iodosulfuron-methyl and metsulfuron-methyl (AE F075736)	0.05 µg/L / LOQ (both analytes)	
Air	Sum of iodosulfuron-methyl and its salts, expressed as iodosulfuron-methyl	1.05 µg/m ³ / LOQ 1.6 µg/m ³ / LOQ	
Body fluids	Sum of iodosulfuron-methyl and its salts, expressed as iodosulfuron-methyl	Not set / LOQ 0.05 mg/L 0.01 mg/L	Not classified as T / T+ SANTE/2020/12830 rev. 2

* indicates lower limit of analytical quantification

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

Not relevant for new registration according to art. 34 of Reg. 1107/2009 based on data which protection period has expired. For the purpose of evaluation of JME-HER 12 OD please refer to the methods describes in Renewal RR for Atlantis 12 OD.

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. For the detailed evaluation of new/additional studies, refer to Appendix 2.

Table 5.3-2: Validated methods for food and feed of plant origin

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, Document No (report) / missing / EU agreed
High water content (sugar beet leaf)	Primary Method 01360	0.01 mg/kg	LC-MS/MS (2 MRM validated)	Stuke, S., Ballmann, C., 2013; M-455564-01-1 EU reviewed, EFSA, 2016
High acid content (lemon fruit)	ILV Method 01360	0.01 mg/kg	LC-MS/MS (2 MRM validated)	Konrad, S., 2013; M-470160-01-1 EU reviewed, EFSA, 2016
High oil content (oilseed rape) High protein/high starch content (dry) (wheat straw/sugar beet body)	Confirmatory method is not required. The primary method was confirmed by validation of two different mass transitions.			
High starch content (dry) (wheat grain)	Primary Method 01360/M001	0.01 mg/kg	LC-MS/MS (2 MRM validated)	Stuke, S., 2015; M-537921-01-1; Appendix 2

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, Document No (report) / missing / EU agreed
	ILV - not required as available for the 4 other matrix types			
	Confirmatory method is not required. The primary method was confirmed by validation of two different mass transitions.			

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

Table 5.3-4: Statement on extraction efficiency

	Method for products of plant origin
Available from:	Stuke, S., 2015, M-525863-01-1, RAR, Sweden, Dec.2015, EU agreed
Not required, because:	As residues are not expected to be \geq LOQ in cereal grain, extraction efficiency is not required according to SANCO/825/00 rev. 8.1.

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

Not relevant for new registration according to art. 34 of Reg. 1107/2009 based on data which protection period has expired. For the purpose of evaluation of JME HER 12 OD please refer to Renewal RR for Atlantis 12 OD.

An overview on the acceptable methods for analysis of iodosulfuron-methyl-sodium in animal matrices is given in the following tables. In matrices of animal origin, no residue definition was proposed during the Annex I Renewal since no residues are anticipated. Although not required for iodosulfuron-methyl-sodium by the current EU regulations, some analytical methods for matrices of animal origin were developed for sulfonylureas.

Table 5.3-3: Validated methods for food and feed of animal origin (if appropriate)

Component of residue definition: iodosulfuron-methyl-sodium*				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Method No / Author(s), year / missing
Milk, eggs, muscle, fat, kidney, liver	Primary Method 01208/M001	0.01 mg/kg	LC-MS/MS (2 MRM validated)	Pross, S.; M-389788-04-1; Appendix 2
	ILV Method 01208/M001	0.01 mg/kg	LC-MS/MS (2 MRM validated)	Moore, S., 2010; M-398300-02-1; Appendix 2

* refers to the analyte relevant to the method as no residue definition is proposed for matrices of animal origin.

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

Table 5.3-4: Statement on extraction efficiency

	Method for products of animal origin
Required, available from:	-
Not required, because:	Methods for products of animal origin are not required (no residue definition). Residues are not expected to be \geq LOQ in animal matrices, so extraction efficiency is not required.

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

Not relevant for new registration according to art. 34 of Reg. 1107/2009 based on data which protection period has expired. For the purpose of evaluation of JME HER 12 OD please refer to Renewal RR for Atlantis 12 OD.

An overview on the acceptable methods and possible data gaps for analysis of iodosulfuron-methyl-sodium in soil is given in the following tables. For the detailed evaluation of new/additional studies, refer to Appendix 2.

Table 5.3-5: Validated methods for soil (if appropriate)

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 µg/kg	HPLC-MS/MS	Freitag, T., Wolters, A., 2013, M-310074-03-1 RAR, France, 2016; EU agreed

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

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

Not relevant for new registration according to art. 34 of Reg. 1107/2009 based on data which protection period has expired. For the purpose of evaluation of JME HER 12 OD please refer to Renewal RR for Atlantis 12 OD.

An overview on the acceptable methods and possible data gaps for analysis of iodosulfuron-methyl-sodium and its metabolite metsulfuron-methyl (AE F075736) in surface and drinking water is given in the following tables. For the detailed valuation of new/additional studies, refer to Appendix 2

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

Component of residue definition: iodosulfuron-methyl-sodium (and metabolite metsulfuron-methyl (AE F075736))				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Drinking water	Primary	0.05 µg/L (for both	HPLC-MS/MS	Krebber, R., Braune, M., 2013, M-466732-01-1

Component of residue definition: iodosulfuron-methyl-sodium (and metabolite metsulfuron-methyl (AE F075736))				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
		substances)		RAR, Sweden, 2015; EU agreed
	ILV to M-466732-01-1	0.05 µg/L (for both substances)	DI-HPLC-MS/MS	Stanislawski, 2013, M-470714-02-1 RAR, Sweden, 2015; EU agreed
Surface water	Primary	0.05 µg/L (for both substances)	HPLC-MS/MS	Krebber, R., Braune, M., 2013,
	ILV to M-466732-01-1	0.05 µg/L (for both substances)	DI-HPLC-MS/MS	Stanislawski, 2013

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

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

Not relevant for new registration according to art. 34 of Reg. 1107/2009 based on data which protection period has expired. For the purpose of evaluation of JME HER 12 OD please refer to Renewal RR for Atlantis 12 OD.

An overview on the acceptable methods and possible data gaps for analysis of iodosulfuron-methyl-sodium in air is given in the following tables. For the detailed evaluation of new/additional studies please refer to Appendix 2.

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

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	1.05 µg/m ³	GC-ECD	Monograph (22 May 2000), M-181311-03-1, EU agreed
Primary	1.6 µg/m ³	LC-UV	Addendum to Monograph (18 June 2001), EU agreed

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

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

Not relevant for new registration according to art. 34 of Reg. 1107/2009 based on data which protection period has expired. For the purpose of evaluation of JME HER 12 OD please refer to Renewal RR for Atlantis 12 OD.

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/additional studies, refer to Appendix 2.

Table 5.3-8: Methods for body fluids and tissues (if appropriate)

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 Method 01478	50 µg/L	HPLC-MS/MS (2 transitions)	Kaussmann, M.; 2016; M-551992-01-1; Appendix 2
Confirmatory	Not required		

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

5.3.2.8 Other studies/ information

Not relevant.

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

Not relevant for new registration according to art. 34 of Reg. 1107/2009 based on data which protection period has expired. For the purpose of evaluation of JME HER 12 OD please refer to Renewal RR for Atlantis 12 OD.

Compared to the residue definition proposed in the 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	Reg. (EU) 289/2014
Plant, high acid content		0.01 * mg/kg	Reg. (EU) 289/2014
Plant, high protein/high starch content (dry commodities)		0.01 * mg/kg	EFSA Journal, 2012; 10(11):2974 Reg. (EU) 289/2014
Plant, high oil content		0.02 * mg/kg / LOQ	Reg. (EU) 289/2014
Plant, difficult matrices (hops, spices, tea)		0.05 * mg/kg / LOQ	Reg. (EU) 289/2014
Muscle	Mesosulfuron-methyl	0.02 * mg/kg / LOQ	Reg. (EU) 289/2014
Milk			
Eggs			
Fat			
Liver, kidney			
Soil (Ecotoxicology)	Mesosulfuron-methyl	0.01 µg/kg / LOQ	
Drinking water (Human toxicology)	Mesosulfuron-methyl	0.05 µg/L / LOQ	general limit for drinking water: 0.1 µg/L
Surface water (Ecotoxicology)	Mesosulfuron-methyl	0.05 µg/L / LOQ	
Air	Mesosulfuron-methyl	12 µg/m ³ / LOQ	
Body fluids	Mesosulfuron-methyl	Not set / LOQ 0.05 mg/L	Not classified as T / T+

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

Not relevant for new registration according to art. 34 of Reg. 1107/2009 based on data which protection period has expired. For the purpose of evaluation of JME HER 12 OD please refer to the methods

describes in Renewal RR for Atlantis 12 OD.

An overview on the acceptable methods and possible data gaps for analysis of residues of mesosulfuron-methyl for the generation of pre-authorization data is given in the following table. For the detailed evaluation of new/additional studies, refer to Appendix 2.

Table 5.3-10: Validated methods for food and feed of plant 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, Document No (report) / missing / EU agreed
High water content	Primary EM 01360	0.01 mg/kg	LC-MS/MS	Stuke, S., Ballmann, C., 2013, M-455564-01-1 EU agreed RAR 2016
	ILV	0.01 mg/kg	LC-MS/MS	Konrad, S., 2013, M-470160-01-1 EU agreed RAR 2016
High acid content	Primary EM 01360	0.01 mg/kg	LC-MS/MS	Stuke, S., Ballmann, C., 2013, M-455564-01-1 EU agreed RAR 2016
	ILV	0.01 mg/kg	LC-MS/MS	Konrad, S., 2013, M-470160-01-1 EU agreed RAR 2016
High oil content	Primary EM 01360	0.01 mg/kg	LC-MS/MS	Stuke, S., Ballmann, C., 2013, M-455564-01-1 EU agreed RAR 2016
	ILV	0.01 mg/kg	LC-MS/MS	Konrad, S., 2013, M-470160-01-1 EU agreed RAR 2016
High protein/high starch content (dry): straw	Primary EM 01360	0.01 mg/kg	LC-MS/MS	Stuke, S., Ballmann, C., 2013, M-455564-01-1 EU agreed RAR 2016
	ILV	0.01 mg/kg	LC-MS/MS	Konrad, S., 2013, M-470160-01-1 EU agreed RAR 2016
Dry commodities: cereal grain	Primary EM 01360/M001 (MR-15/090)	0.01 mg/kg	LC-MS/MS	Stuke, S., 2015, M-537921-01-1 not Peer reviewed Appendix 2

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

Table 5.3-11: Statement on extraction efficiency

	Method for products of plant origin
Available from:	Stuke, S., 2015, M-525863-01-1, RAR, Sweden, Dec.2015, EU agreed
Not required, because:	As residues are not expected to be \geq LOQ in cereal grain, extraction efficiency is not required according to SANCO/825/00 rev. 8.1.

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

Not relevant for new registration according to art. 34 of Reg. 1107/2009 based on data which protection period has expired. For the purpose of evaluation of JME HER 12 OD please refer to Renewal RR for Atlantis 12 OD.

An overview on the acceptable methods for analysis of mesosulfuron-methyl in animal matrices is given in the following table. In matrices of animal origin, no residue definition was proposed during the Annex I Renewal since no residues are anticipated. Although not required for iodosulfuron-methyl-sodium by the current EU regulations, some analytical methods for matrices of animal origin were developed for sulfonylureas.

Table 5.3-12: Validated methods for food and feed of animal origin (if appropriate)

Component of residue definition: mesosulfuron-methyl				
Matrix type	Method type / Method No / Document No (report)	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / Document No (report) / missing
Milk	Primary 01208/M001	0.01 mg/kg	LC-MS/MS	Schmeer, K., 2011, M-389788-03-1 EU agreed RAR 2016 Amended in 2018 by Pross, S. (M-389788-04-1), no impact on the evaluation
	ILV	0.01 mg/kg	LC-MS/MS	Moore, S., 2010, M-398300-01-1 EU agreed RAR 2016
Eggs	Primary 01208/M001	0.01 mg/kg	LC-MS/MS	Schmeer, K., 2011, M-389788-03-1 EU agreed RAR 2016 Amended in 2018 by Pross, S. (M-389788-04-1), no impact on the evaluation
	ILV M-398300-01-1	0.01 mg/kg	LC-MS/MS	Moore, S., 2010, M-398300-01-1 EU agreed RAR 2016
Muscle	Primary 01208/M001	0.01 mg/kg	LC-MS/MS	Schmeer, K., 2011, M-389788-03-1 EU agreed RAR 2016 Amended in 2018 by Pross, S. (M-389788-04-1), no impact on the evaluation
	ILV	0.01 mg/kg	LC-MS/MS	Moore, S., 2010, M-398300-01-1 EU agreed RAR 2016
Fat	Primary 01208/M001	0.01 mg/kg	LC-MS/MS	Schmeer, K., 2011, M-389788-03-1 EU agreed RAR 2016 Amended in 2018 by Pross, S. (M-389788-04-1), no impact on the evaluation

Component of residue definition: mesosulfuron-methyl				
Matrix type	Method type / Method No / Document No (report)	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / Document No (report) / missing
	ILV	0.01 mg/kg	LC-MS/MS	Moore, S., 2010, M-398300-01-1 EU agreed RAR 2016
Kidney, liver	Primary 01208/M001	0.01 mg/kg	LC-MS/MS	Schmeer, K., 2011, M-389788-03-1 EU agreed RAR 2016 Amended in 2018 by Pross, S. (M-389788-04-1), no impact on the evaluation
	ILV M-398300-01-1	0.01 mg/kg	LC-MS/MS	Moore, S., 2010, M-398300-01-1 EU agreed RAR 2016

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

Table 5.3-13: Statement on extraction efficiency

	Method for products of animal origin
Required, available from:	-
Not required, because:	Methods for products of animal origin are not required (no residue definition). Residues are not expected to be \geq LOQ in animal matrices, so extraction efficiency is not required.

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

Not relevant for new registration according to art. 34 of Reg. 1107/2009 based on data which protection period has expired. For the purpose of evaluation of JME HER 12 OD please refer to Renewal RR for Atlantis 12 OD.

An overview on the acceptable methods and possible data gaps for analysis of mesosulfuron-methyl in soil is given in the following tables. For the detailed evaluation of new/ additional studies it is referred to Appendix 2.

Table 5.3-14: Validated methods for soil (if appropriate)

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 Method 01115	0.1 µg/kg	HPLC-MS/MS	Freitag, T., Wolters, A., 2013, M-310074-03-1 RAR, France, 2016; EU agreed

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

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

Not relevant for new registration according to art. 34 of Reg. 1107/2009 based on data which protection period has expired. For the purpose of evaluation of JME HER 12 OD please refer to Renewal RR for Atlantis 12 OD.

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. For the detailed valuation of new/ additional studies it is referred to Appendix 2.

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 water	Primary Method 01387	0.05 µg/L	HPLC-MS/MS	Krebber, R., Braune, M., 2013, M-466732-01-1 RAR; France, 2016 EU agreed
	ILV to M-466732-01-1	0.05 µg/L	DI-HPLC-MS/MS	Stanislawski, 2013, M-470714-02-1 RAR; France, 2016 EU agreed
Surface water	Primary	0.05 µg/L	HPLC-MS/MS	Krebber, R., Braune, M., 2013, RAR; France, 2016 EU agreed
	ILV to M-466732-01-1	0.05 µg/L	DI-HPLC-MS/MS	Stanislawski, 2013, RAR; France, 2016 EU agreed

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

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

Not relevant for new registration according to art. 34 of Reg. 1107/2009 based on data which protection period has expired. For the purpose of evaluation of JME HER 12 OD please refer to Renewal RR for Atlantis 12 OD.

An overview on the acceptable methods and possible data gaps for analysis of mesosulfuron-methyl in air is given in the following tables. For the detailed evaluation of new/ additional studies please refer to Appendix 2.

Table 5.3-16: Validated methods for air (if appropriate)

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	1.6 µg/m ³	LC-UV	Addendum to Monograph (18 June 2001), , EU agreed

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

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

Not relevant for new registration according to art. 34 of Reg. 1107/2009 based on data which protection period has expired. For the purpose of evaluation of JME-HER 12 OD please refer to Renewal RR for Atlantis 12 OD.

An overview on the acceptable methods and possible data gaps for analysis of mesosulfuron-methyl in body fluids and tissues is given in the following table. For the detailed evaluation of new/additional 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 Method 01478	50 µg/L	HPLC-MS/MS (2 transitions)	Kaussmann, M.; 2016, M-551992-01-1 Not peer reviewed Appendix 2
Confirmatory	Not required		

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

Not relevant.

Appendix 1 Lists of data considered in support of the evaluation

List of data submitted by the applicant and relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 5.1.1	Ciach J.	2023	JME-HER 12 OD. Determination of physicochemical properties of the preparation in a COEX bottle. Report No: 001/DPL/2023 Source: Pestila Sp. z o. o. GLP: Yes Published: No	N	Pestila*

* Pestila spółka z ograniczoną odpowiedzialnością

List of unprotected data referred to by the applicant and relied on, but already evaluated

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 5.1.2 /01	Stuke, S.; Kerkerling, S.	2018	Amendment no. 2: Determination of the residues of BYH 18636 , iodosulfuron-methyl-sodium, mefenpyr-diethyl and mesosulfuron-methyl in/on winter wheat after spraying of IMS & MSM & MPR WG 12.6 and thienencarbazone-methyl WG 70 in the field in Germany, Spain and Portugal Report No.: 13-2127, Edition Number: M-503498-03-1 Bayer AG, Crop Science Division, Monheim, Germany ... amended: 2018-08-31 GLP/GEP: Yes unpublished	No	Bayer
KCP 5.1.2 /02	Stuke, S.; Kerkerling, S.	2018	Amendment no. 2: Determination of the residues of mefenpyr-diethyl, BYH 18636, iodosulfuron-methylsodium and mesosulfuron-methyl in/on winter wheat after spraying of IMS & MSM & MPR WG 15	No	Bayer

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
			and thien carbazole-methyl WG 70 in the field in Belgium, the Netherlands and Italy Report No.: 13-2129, Edition Number: M-506719-03-1 Bayer AG, Crop Science Division, Monheim, Germany ... amended: 2018-08-31 GLP/GEP: Yes unpublished		
KCP 5.1.2 /03	Mahlo, C.; Gabriel, E.; Vagt, I.; Meyer, M.	2017	Determination of the residues of amidosulfuron and iodosulfuron-methyl-sodium in/on wheat and barley after spray application of AMS & IMS & MPR OD 375 in Germany, Denmark, Poland and the United Kingdom Report No.: 16-2030, Edition Number: M-612290-01-1 SGS Institut Fresenius GmbH, Taunusstein, Germany GLP/GEP: Yes unpublished	No	Bayer
KCP 5.1.2 /04	Kaussmann, M.	2017	Modification M002 of the residue analytical method 01376 for the determination of foramsulfuron, iodosulfuron-methyl, metsulfuron-methyl and AE F153745 in/on plant material by HPLC-MS/MS Report No.: 01376/M002, Edition Number: M-587949-01-1 Bayer AG, Crop Science Division, Monheim, Germany GLP/GEP: Yes unpublished	No	Bayer
KCP 5.1.2 /05	Kaussmann, M.	2017	Analytical method 01514 for the determination of AE F092944, AE F059411 and AE 0031838 in/on plant by HPLC-MS/MS Report No.: P602166508, Edition Number: M-583894-01-1 Bayer AG, Crop Science Division, Monheim, Germany GLP/GEP: Yes unpublished	No	Bayer

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

Please note that all data mentioned as part of DAR, RAR, or EFSA journals are considered as relied on.

Iodosulfuron-Methyl-Sodium

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 4.1.1 /01	Griggs, G. M.	1997	Analytical method Determination in technical materials and process matrices by high performance liquid chromatography AE F114844 and AE F115008 Code: AE F115008 AgrEvo UK Crop Protection Ltd., Hauxton, United Kingdom Bayer CropScience, Report No.: A59463, Edition Number: M-143135-01-1 Date: 1997-08-20 GLP/GEP: no, unpublished	N	Bayer
KCA 4.1.1 /04	Griggs, G. M.	1996	Validation of the analytical method for the determination of Hoe 114844 and Hoe 115008 as Hoe 114844 in technical materials and process matrices by high performance liquid chromatography (reference AM 7600/01/01) AgrEvo UK Crop Protection Ltd., Chesterford Park, United Kingdom Bayer CropScience, Report No.: C001149, Edition Number: M-181871-01-1 Date: 1996-05-09 GLP/GEP: no, unpublished	N	Bayer
KCA 4.1.1 /06	Selzer, J.; Eichelmann, C.	2012	Determination of AE F114844 (Iodosulfuron-methyl) and AE F115008 (Iodosulfuron-methyl sodium) in technical grade and pure active substance by high performance liquid chromatography (HPLC) Bayer CropScience, Report No.: AM033623FP1, Edition Number: M-434444-01-1 Date: 2012-07-10 GLP/GEP: no, unpublished	N	Bayer
KCA 4.1.1 /07	Selzer, J.; Eichelmann, C.	2012	Validation of HPLC-analytical method AM033612FP1 Determination of AE F114844 (Iodosulfuron-methyl) and AE F115008 (Iodosulfuron-methyl sodium) in technical grade and pure active substance by high performance liquid chromatography (HPLC) Bayer CropScience, Report No.: PA12/052,	N	Bayer

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
			<i>Edition Number: M-434439-01-1</i> <i>Date: 2012-07-09</i> <i>GLP/GEP: yes, unpublished</i>		
KCA 4.1.1/08	Griggs, G. M.; Bowen, T. J.	1999	<i>The validation of the analytical method AM 7600/01/01 for the determination of AE F114844 and AE F115008 as AE F114844 in technical materials and process matrices by high performance liquid chromatography Code: AE F115008</i> <i>AgrEvo UK Crop Protection Ltd., Hauxton, United Kingdom</i> <i>Bayer CropScience,</i> <i>Report No.: C002587,</i> <i>Report includes Trial Nos.: AM7600/01/01</i> <i>Edition Number: M-184764-01-1</i> <i>EPA MRID No.: 45108707</i> <i>Date: 1999-07-13</i> <i>GLP/GEP: yes, unpublished</i>	N	Bayer
KCA 4.1.2/01	Wrede, A.	1997	<i>Analytical method, validation Determination of residues in straw by HPLC Code: AE F115008</i> <i>Hoechst Schering AgrEvo GmbH, Frankfurt am Main, Germany</i> <i>Bayer CropScience,</i> <i>Report No.: A59678,</i> <i>Report includes Trial Nos.: CR96/027</i> <i>Edition Number: M-143342-01-1</i> <i>Date: 1997-12-02</i> <i>GLP/GEP: yes, unpublished</i>	N	Bayer
KCA 4.1.2/02	Wrede, A.	1997	<i>Analytical method and validation Determination of residues in shoot by HPLC Code: AE F115008</i> <i>Hoechst Schering AgrEvo GmbH, Frankfurt am Main, Germany</i> <i>Bayer CropScience,</i> <i>Report No.: A59290,</i> <i>Report includes Trial Nos.: CR96/028</i> <i>Edition Number: M-142974-01-1</i>	N	Bayer

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
			<i>Date: 1997-09-04</i> <i>GLP/GEP: yes, unpublished</i>		
KCA 4.1.2 /03	Wrede, A.	1997	Aged residue in wheat straw and shoot. Radio validation of the residue analytical method AL121/96-0 (straw) and AL120/96-0 (shoot) Code: AE F115008 Hoechst Schering AgrEvo GmbH, Frankfurt am Main, Germany Bayer CropScience, Report No.: C001460, Edition Number: M-182685-01-1 Date: 1998-11-09 GLP/GEP: yes, unpublished	N	Bayer
KCA 4.1.2 /04	Wrede, A.	1997	Aged residue of AE F115008 and its metabolite AE F075736 in soil. - Radio validation of the residue analytical method DGM F 06/97 - 0 - Code: AE F115008 Hoechst Schering AgrEvo GmbH, Frankfurt am Main, Germany Bayer CropScience, Report No.: C001581, Edition Number: M-182982-01-1 EPA MRID No.: 45108718 Date: 1998-11-26 GLP/GEP: yes, unpublished	N	Bayer
KCA 4.2 /01	Wrede, A.	1997	Analytical method, validation Determination of residues in grain by HPLC Code: AE F115008 Hoechst Schering AgrEvo GmbH, Frankfurt am Main, Germany Bayer CropScience, Report No.: A58043, Report includes Trial Nos.: CR96/019 Edition Number: M-141767-02-1 Date: 1997-04-28 ...Amended: 1998-06-29 GLP/GEP: yes, unpublished	N	Bayer
KCA 4.2	Wrede, A.	1998	Validation of the analytical method AL - 008/96 -0 for the determination of residues of AE F115008 in	N	Bayer

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
/02			grain by HPLC with two different HPLC columns Code: AE F115008 Hoechst Schering AgrEvo GmbH, Frankfurt am Main, Germany Bayer CropScience, Report No.: C001452, Edition Number: M-182662-01-1 Date: 1998-11-05 GLP/GEP: yes, unpublished		
KCA 4.2 /03	Taylor, N. W.	1998	Independent laboratory validation (ILV) of the Analytical Method (AL-008/96-0) for the determination of AE F115008 residues in cereal grain - Final Report AE F115008 analytical grade Code: AE F115008 AgrEvo UK Crop Protection Ltd., Chesterford Park, United Kingdom Bayer CropScience, Report No.: C000836, Report includes Trial Nos.: 200/07/001 Edition Number: M-181323-01-1 Date: 1998-09-28 GLP/GEP: yes, unpublished	N	Bayer
KCA 4.2 /04	Stan, H. J.; Schwarzer, F.; Brockmeyer, R.	1992	Practical application of methods for the analysis of sulfonylurea herbicides and their metabolites in foodstuffs Techn. Universitaet Berlin, DEU; Institut fuer Lebensmittelchemie Report No.: C005929, Edition Number: M-131964-01-2 Date: 1992-04-30 GLP/GEP: no, unpublished	N	Bayer
KCA 4.2 /05	Wrede, A.	1998	Multi-residue method for the determination of AE F115008 in grain (statement) Code: AE F115008 Hoechst Schering AgrEvo GmbH, Frankfurt am Main, Germany Bayer CropScience, Report No.: C001451, Edition Number: M-182660-01-1 Date: 1998-11-06 GLP/GEP: no, unpublished	N	Bayer

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 /06	Wrede, A.	1998	Analytical method and validation for the determination of residues of AE F115008 and its metabolite AE F075736 in soil using HPLC (method and validation) Code: AE F115008 Hoechst Schering AgrEvo GmbH, Frankfurt am Main, Germany Bayer CropScience, Report No.: A67461, Report includes Trial Nos.: CR97/032 Edition Number: M-147842-01-1 EPA MRID No.: 45108717 Date: 1998-07-14 GLP/GEP: yes, unpublished	N	Bayer
KCA 4.2 /06	Wrede, A.	2000	Enforcement Method for Soil by LC-MS/MS Metsulfuron-methyl (AE F075736) Iodosulfuron-methyl-sodium (AE F115008) Hoechst Schering AgrEvo GmbH, Frankfurt am Main, Germany Bayer CropScience, Report No.: C006394, Edition Number: M-193807-01-1 EPA MRID No.: 45108502 Date: 2000-02-04 GLP/GEP: no, unpublished	N	Bayer
KCA 4.2/08	Wrede, A.	2000	Validation of the enforcement method EM F13/99-0 in soil by LC-MS/MS - Metsulfuron-methyl - Iodosulfuron-methyl-sodium - Code: AE F075736, AE F115008 Hoechst Schering AgrEvo GmbH, Frankfurt am Main, Germany Bayer CropScience, Report No.: C006396, Edition Number: M-193814-01-1 EPA MRID No.: 45108720 Date: 2000-02-04 GLP/GEP: yes, unpublished	N	Bayer
KCA 4.2/09	Wrede, A.	1998	Analytical method and validation for the determination of residues of AE F115008 and its metabolite AE F075736 in water using HPLC Code: AE F115008	N	Bayer

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
			Hoechst Schering AgrEvo GmbH, Frankfurt am Main, Germany Bayer CropScience, Report No.: C000710, Report includes Trial Nos.: CR98/002 Edition Number: M-181134-01-1 EPA MRID No.: 45108721 Date: 1998-09-22 GLP/GEP: no, unpublished		
KCA 4.2/10	Wrede, A.	2000	Enforcement method of iodosulfuron-methyl-sodium and its metabolite metsulfuron-methyl in surface water by HPLC incl. validation. Extension of the enforcement method EM F 01/98-0 for iodosulfuron-methyl-sodium in drinking water to its metabolite metsulfuron-methyl incl. validation Hoechst Schering AgrEvo GmbH, Frankfurt am Main, Germany Bayer CropScience, Report No.: C006395, Report includes Trial Nos.: CR99/029 Edition Number: M-193810-01-1 EPA MRID No.: 45108722 Date: 2000-02-08 GLP/GEP: no, unpublished	N	Bayer
KCA 4.2/12	Reichert, N.	2000	Development and validation of an analytical method for the determination of iodosulfuron methyl sodium in air Institut Fresenius Chem.und Biolog. Lab. AG, Taunusstein, Germany Bayer CropScience, Report No.: IF-100/21283-00, Edition Number: M-199299-02-1 Date: 2000-11-22 ...Amended: 2009-06-19 GLP/GEP: yes, unpublished	N	Bayer
KCA	Wrede, A.	2000	Enforcement Method for Cereal Grain, Straw and Shoot by LC-MS/MS Amidosulfuron (AE F075032)	N	Bayer

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
4.2/13			Metsulfuron-methyl (AE F075736) Iodosulfuron-methyl-sodium (AE F115008) AE F130060 AE F130360 Hoechst Schering AgrEvo GmbH, Frankfurt am Main, Germany Bayer CropScience, Report No.: C006734, Edition Number: M-194528-01-1 Date: 2000-03-02 GLP/GEP: no, unpublished		
KCA 4.2/14	Wrede, A.	2000	Validation of the Enforcement Method EM F08/99-0 of cereal grain, straw and shoot by LC-MS/MS - Amidosulfuron (AE F075032) - Metsulfuron-methyl (AE F075736) Iodosulfuron-methyl-sodium (AE F115008) - AE F130060 - AE F 130360 Hoechst Schering AgrEvo GmbH, Frankfurt am Main, Germany Bayer CropScience, Report No.: C006735, Edition Number: M-194531-01-1 Date: 2000-03-02 GLP/GEP: yes, unpublished	N	Bayer
KCA 4.2/15	Wrede, A.	2002	Validation of the enforcement method EM F08/99-0 for lemon, tomato and maize kernel by LC-MS/MS - Amidosulfuron (AE F075032) - Iodosulfuron-methyl-sodium (AE F115008) - Mesosulfuron-methyl (AE F130060) - Foramsulfuron (AE F130360) Aventis CropScience GmbH, Frankfurt am Main, Germany Bayer CropScience, Report No.: C022220, Edition Number: M-212674-01-1 Date: 2002-04-30 GLP/GEP: yes, unpublished	N	Bayer
KCA 4.2 /16	Heinemann, O.	2004	Modification M001 to method 00815 for the determination of residues of amidosulfuron, iodosulfuron- methyl-sodium including metabolite metsulfuron-methyl, foramsulfuron and mesosulfuron-methyl in/on flax and wheat matrices by HPLC-MS/MS Bayer CropScience, Report No.: 00815/M001, Report includes Trial Nos.:	N	Bayer

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
			<i>P602033000</i> <i>Edition Number: M-226888-01-1</i> <i>Date: 2004-01-30</i> <i>GLP/GEP: yes, unpublished</i> <i>...also filed: KCA 6.1 /06</i>		
<i>KCA 4.2/17</i>	<i>Anspach, T.</i>	<i>2001</i>	<i>Independent laboratory validation of the enforcement method for the determination of residues of iodosulfuron-methyl-sodium (AE F115008) and amidosulfuron (AE F075032) in wheat (grain)</i> <i>Dr. Specht & Partner, Chemische Laboratorien GmbH, Germany</i> <i>Bayer CropScience,</i> <i>Report No.: C015636,</i> <i>Edition Number: M-200884-01-1</i> <i>Date: 2001-08-28</i> <i>GLP/GEP: yes, unpublished</i>	<i>N</i>	<i>Bayer</i>
<i>KCA 4.2 /18</i>	<i>Reichert, N.; Klimmek, S.</i>	<i>2002</i>	<i>Independent laboratory validation of the analytical method EM F08/99-0 for the residue analysis of Amidosulfuron (AE F075032), Iodosulfuron-methyl-sodium (AE F115008), Mesosulfuron-methyl (AE F130060), Foramsulfuron (AE F130360) in tomato and citrus</i> <i>Institut Fresenius Chem.und Biolog. Lab. AG, Taunusstein, Germany</i> <i>Bayer CropScience,</i> <i>Report No.: C023679,</i> <i>Edition Number: M-215456-01-1</i> <i>Date: 2002-06-06</i> <i>GLP/GEP: yes, unpublished</i>	<i>N</i>	<i>Bayer</i>
<i>KCA 4.2 /19</i>	<i>Stuke, S.; Ballmann, C.</i>	<i>2013</i>	<i>Analytical method 01360 for the determination of amidosulfuron, metsulfuron-methyl, iodosulfuron-methyl-sodium, mesosulfuron-methyl, and foramsulfuron in samples from plant origin by HPLC-MS/MS</i> <i>Bayer CropScience,</i> <i>Report No.: MR-13/007,</i> <i>Edition Number: M-455564-01-1</i> <i>Method Report No.: MR-13/007</i> <i>Date: 2013-05-28</i> <i>GLP/GEP: yes, unpublished</i> <i>...also filed: KCA 6.1 /07</i>	<i>N</i>	<i>Bayer</i>

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	Stuke, S.	2015	Cross validation of enforcement method 01360 for the determination of sulfonylureas vs. extraction procedure applied in 14C-metabolism studies using incurred residues in plant matrices analysed by HPLC-MS/MS	N	Bayer
KCA 4.2/20	Konrad, S.	2013	Independent lab validation of BCS method 01360 for the determination of residues of amidosulfuron, metsulfuron-methyl, iodosulfuron-methyl-sodium, mesosulfuron-methyl and foramsulfuron in samples from plant origin by HPLC-MS/MS Currenta GmbH & Co. OHG, Leverkusen, Germany BCS, Report No.: 2013/0060/01, Edition Number: M-470160-01-1 Date: 2013-10-18 GLP/GEP: yes, unpublished ...also filed: KCA 6.1 /08	N	Bayer
KCA 4.2/22	Krebber, R.; Braune, M.	2013	Analytical method 01387 for the determination of various pesticides in drinking and surface water by HPLC-MS/MS Bayer CropScience, Report No.: MR-13/085, Edition Number: M-466732-01-1 Method Report No.: MR-13/085 Date: 2013-10-09 GLP/GEP: yes, unpublished	N	Bayer
KCA 4.2/23	Stanislawski, T.	2013	Independent laboratory validation of BCS analytical methods 01333 and 01387 for determination of various pesticides in surface water by Di-HPLC-MS/MS PTRL Europe, Ulm, Germany Bayer CropScience, Report No.: P3117 G, Edition Number: M-470714-02-1 Date: 2013-12-13 GLP/GEP: yes, unpublished	N	Bayer
KCA	Freitag, T.	2013	Amendment no. 0001 to report no.: MR-08/138 - Analytical Method 01115 for the determination of	N	Bayer

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
4.2/24			residues of amidosulfuron, iodosulfuron-methyl-sodium, metsulfuron-methyl, mesosulfuron-methyl and foramsulfuron in soil by HPLC-MS/MS Bayer CropScience, Report No.: M-310074-03-1, Edition Number: M-310074-03-1 Method Report No.: MR-08/138 Date: 2008-10-27 (original report by Freitag & Wolters) ...Amended: 2013-08-08 GLP/GEP: yes, unpublished		

Mesosulfuron Methyl

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
/	/	/	Analytical method for the determination of AE F130060 in dog diet by High Performance liquid chromatography (HPLC) Report 98.0289 Document M-147837-01-1 GLP	N	Bayer
KCA 5.3_01	B A Mallyon	2000	Analytical method for the determination of test material in diet by HPLC RESID/93/34 M-198511-01-1 GLP	N	Bayer
/	/	/	Hoe 130060 Determination of concentrations in dietary formulations by high performance liquid chromatography Report 96.0191	N	Bayer

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
			M-143016-01-1 GLP		
KCA 5.6.2_04	Hofmann Th	2000	AE F130060; substance technical Code: AE F130060 00 IC95 0001 Rabbit oral developmental toxicity (teratogenicity) study Report 98.0254 M-181336-02-1 GLP	N	Bayer
KCA 5.1.1 /10	Solà, J.	2013	[Pyrimidyl-2-14C]mesosulfuron-methyl: Metabolic stability and profiling in liver microsomes from rats and humans for Inter-Species Comparison Harlan Laboratories S.A. Centro Industrial Santiga, c/Argenters, Mogoda, Spain Bayer CropScience, Report No.: EnSa-13-0829, Edition Number: M-470477-01-1 Date: 2013-11-15 GLP/GEP: yes, unpublished	N	Bayer
KCA 5.2.6 /02	Leidenfrost, P.	2003	1st amendment to report no.: AT00537 of July 10.2003 - Study for the skin sensitization effect in guinea pigs (Guinea pig maximization test according to Magnusson and Kligman) Bayer Pharma AG, Wuppertal, Germany Bayer CropScience, Report No.: T3072716, Edition Number: M-235831-02-1 Date: 2003-07-10 ...Amended: 2016-02-29 GLP/GEP: yes, unpublished	N	Bayer
KCA 5.2.7 /01	Heppenheimer, A.	2014	Mesosulfuron-methyl (AE F130060) technical: Cytotoxicity assay in vitro with BALB/c3T3 c31 cells: Neutral Red (NR) test during simultaneous irradiation with artificial sunlight Harlan Cytotest Cell Research GmbH (Harlan CCR), Rossdorf, Germany Bayer CropScience, Report No.: 1592100, GLP/GEP: yes, unpublished	N	Bayer

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 5.4.1 /05	Sokolowski, A.	2016	Mesosulfuron-methyl (AE F130060): Salmonella typhimurium reverse mutation assay Envigo CRS GmbH, Rossdorf, Germany Bayer CropScience, Report No.: 1744700, Edition Number: M-547488-01-1 Date: 2016-02-12 GLP/GEP: yes, unpublished	N	Bayer
KCA 5.8.1 /01	Sokolowski, A.	2012	Salmonella typhimurium reverse mutation assay with AE F147447 Harlan CCR, Rossdorf, Germany Bayer CropScience, Report No.: 1462101, GLP/GEP: yes, unpublished	N	Bayer
KCA 5.8.1 /02	Bohnenberger, S.	2015	Report amendment - In vitro chromosome aberration test in Chinese hamster V79 cells with AE F147447 Harlan Cytotest Cell Research GmbH (Harlan CCR), Rossdorf, Germany Bayer CropScience, Report No.: 1462102, GLP/GEP: yes, unpublished	N	Bayer
KCA 5.8.1 /03	Wollny, H. E.	2012	Gene mutation assay in Chinese hamster V79 cells in vitro (V79 / HPRT) - AE F147447 Harlan Cytotest Cell Research GmbH (Harlan CCR), Rossdorf, Germany Bayer CropScience, Report No.: 1462103, GLP/GEP: yes, unpublished	N	Bayer
KCA 5.8.1 /04	Sokolowski, A.	2012	Salmonella typhimurium reverse mutation assay with AE F160460 Harlan CCR, Rossdorf, Germany Bayer CropScience, Report No.: 1462301, Edition GLP/GEP: yes, unpublished	N	Bayer
KCA 5.8.1 /05	Bohnenberger, S.	2015	Report amendment - In vitro chromosome aberration test in Chinese hamster V79 cells with AE F160460 Harlan Cytotest Cell Research GmbH (Harlan CCR), Rossdorf, Germany Bayer CropScience,	N	Bayer

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			Report No.: 1462302, GLP/GEP: yes, unpublished		
KCA 5.8.1 /06	Wollny, H. E.	2015	Report amendment no. 1 - Gene mutation assay in Chinese hamster V79 cells in vitro (V79 / HPRT) - AE F160460 Harlan Cytotest Cell Research GmbH (Harlan CCR), Rossdorf, Germany Bayer CropScience, Report No.: 1462303, GLP/GEP: yes, unpublished	N	Bayer
KCA 5.8.1 /07	Sokolowski, A.	2012	Salmonella typhimurium reverse mutation assay with BCS-CV14885 Harlan Cytotest Cell Research GmbH (Harlan CCR), Rossdorf, Germany Bayer CropScience, Report No.: 1490201, GLP/GEP: yes, unpublished	N	Bayer
KCA 5.8.1 /08	Bohnenberger, S.	2015	Report amendment - In vitro chromosome aberration test in Chinese hamster V79 cells with BCS-CV14885 Harlan Cytotest Cell Research GmbH (Harlan CCR), Rossdorf, Germany Bayer CropScience, Report No.: 1490202, GLP/GEP: yes, unpublished	N	Bayer
KCA 5.8.1 /09	Wollny, H. E.	2015	Report amendment no. 1 - Gene mutation assay in Chinese hamster V79 cells in vitro (V79/HPRT) - BCS-CV14885 Harlan Cytotest Cell Research GmbH (Harlan CCR), Rossdorf, Germany Bayer CropScience, Report No.: 1490203, GLP/GEP: yes, unpublished	N	Bayer
1	1	1	Analytical method for the determination of AE F130060 in dog diet by High Performance liquid chromatography (HPLC) Report 98.0289 Document M-147837-01-1 GLP	N	Bayer

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 the active substance iodosulfuron-methyl-sodium

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

New analytical method for determination of active substance and its relevant metabolites in plant protection product have been submitted and summarised in points 5.2.1.1. and 5.2.1.2. Submitted methods can be used for post-authorisation monitoring.

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

~~No new or additional studies have been submitted.~~

A 2.1.1.1.1 Additional validation data of analytical method 00815/M001 (study 13- 2127 and 13-2129 Cereal)

A 2.1.1.1.1.1 Method validation

Comments of zRMS:	The study was evaluated and accepted in dRR Part B7 for Atlantis 12 OD.
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Reference:	KCP 5.1.2/01
Title:	<i>Amendment no. 2: Determination of the residues of BYH 18636 , iodosulfuron-methyl-sodium, mefenpyr-diethyl and mesosulfuron-methyl in/on winter wheat after spraying of IMS & MSM & MPR WG 12.6 and thiencazone-methyl WG 70 in the field in Germany, Spain and Portugal</i>
Report:	Stuke, S.; Kerkerling, S.; 2018; 13-2127; M-503498-03-1
Authority registration No:	
Guideline(s):	Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC EC Guidance working document 7029/VI/95 rev.5 (1997-07-22) OECD 509 Adopted 2009-09-07, OECD GUIDELINE FOR THE TESTING OF CHEMICALS, Crop Field US EPA OCSPP Guideline No. 860.1500
Deviations:	Yes, see Appendix 5
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

Reference:	KCP 5.1.2/02
Title:	Amendment no. 2: Determination of the residues of mefenpyr-diethyl, BYH 18636, iodosulfuron-methylsodium and mesosulfuron-methyl in/on winter wheat after spraying of IMS & MSM & MPR WG 15 and thiencazone-methyl WG 70 in the field in Belgium, the Netherlands and Italy
Report:	Stuke, S.; Kerkerling, S.; 2018; 13-2129; M-506719-03-1
Authority registration No:	
Guideline(s):	Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC EC Guidance working document 7029/VI/95 rev.5 (1997-07-22) OECD 509 Adopted 2009-09-07, OECD GUIDELINE FOR THE TESTING OF CHEMICALS, Crop Field Trial US EPA OCSPP Guideline No. 860.1500
Deviations:	None
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

Material and methods

Full validation data is documented with the method 00815/M001 itself for matrices flax linseed, wheat grain, straw and green material.

Further validation sets (3 repetitions each at two fortification levels LOQ and 10x LOQ) on wheat grain, straw and green material were conducted within the course of the studies 13-2127/13-2129. Method validations performed during the conduct of these studies are summarized in the tables below:

Results and discussions

Apparent residues in control samples were below 30% of the LOQ. Mean recoveries per fortification level for both analytes were in a range of 70 – 110 % with RSD < 20%.

Table A 1: Recovery data for iodosulfuron-methyl-sodium using the analytical method

Matrix	Fortification level (mg/kg)	n	Mean recovery (%)	RSD (%)*	Comments
iodosulfuron-methyl-sodium					
wheat / green material	0.05	3	99	5.0	Appendix 4 of the residue report 13-2127 and 13-2129
	0.50	3	95	4.0	
wheat / grain	0.01	3	105	5.8	Appendix 4 of the residue report 13-2127 and 13-2129
	0.10	3	100	2.1	
wheat / straw	0.05	3	101	0.6	Appendix 4 of the residue report 13-2127 and 13-2129
	0.50	3	96	2.6	

FL = Fortification level, RSD = Relative standard deviation, LOQ = Practical limit of quantification

Fortified with iodosulfuron-methyl-sodium, determined as AE F114844 and calculated as iodosulfuron-methyl-sodium

* Several RSD values shown within the analytical phase report are differing from RSD values illustrated within this table to some extent. The RSD values illustrated within this table are correct.

Table A 2: Characteristics for the analytical method used for validation of residues in Cereal (green material, grain and straw)

	iodosulfuron-methyl-sodium
Specificity	Mass spectra are provided in Appendix of the original EU reviewed method 00815/M001 blank values < 30% LOQ
Calibration (type, number of data points)	Calibration data presented and Calibration line presented for each analyte and each sample material number of data points: at least 5 R > 0.999
Calibration range	Matrix-matched standards from 0.1 to 25.0 ng/mL in green material, grain and straw. Corresponding calibration range in mass ratio units for the sample: * Green material and straw: 0.025 to 6.25 mg/kg. * Grain: 0.005 to 1.25 mg/kg
Assessment of matrix effects is presented	No. The quantification was done using external matrix-matched standards. It compensates for matrix effect.
Limit of determination/quantification	LOQ: green material → 0.05 mg/kg grain → 0.01 mg/kg straw → 0.05 mg/kg

Conclusion

All method validation data are in compliance with the guideline requirements for data collection methods.

The validation of method 00815/M001 can therefore be considered successful for the matrices green material, grain and straw.

The limits of quantitation (LOQ) for iodosulfuron-methyl-sodium are 0.01 mg/kg, for grain, and 0.05 mg/kg for green material and straw, corresponding to the lowest fortification level of successfully conducted recovery experiments.

A 2.1.1.1.2 Additional validation data of analytical method 00815/M001 (study 16- 2030 Cereal)

A 2.1.1.1.2.1 Method validation

Comments of zRMS:	The study was evaluated and accepted in dRR Part B7 for Atlantis 12 OD.
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Reference:	KCP 5.1.2/03
Title:	Determination of the residues of amidosulfuron and iodosulfuron-methyl-sodium in/on wheat and barley after spray application of AMS & IMS & MPR OD 375 in Germany, Denmark, Poland and the United Kingdom
Report:	Mahlo, C.; Gabriel, E.; Vagt, I.; Meyer, M.; 2017; 16-2030; M-612290-01-1
Authority registration No.:	
Guideline(s):	Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC OECD 509 Adopted 2009-09-07, OECD GUIDELINE FOR THE TESTING OF CHEMICALS, Crop Field Trial US EPA OCSPP Guideline No. 860.1500
Deviations:	Yes (see report)

GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

Material and methods

Full validation data are documented with the method 00815/M001 itself for cereal matrices (wheat grain, straw and green material) and oily matrices (flax seed and oil).

A limited set (one control sample, 3 repetitions each at two fortification levels) in barley grain, straw and green material were conducted within the course of the study 16-2030. Method validation data are summarized in the tables below.

Results and discussions

Apparent residues in control samples were below 30% of the LOQ. Mean recoveries per fortification level for both analytes were in a range of 70 – 110% with RSD <20%.

Table A 3: Recovery data for iodosulfuron-methyl-sodium using the analytical method

Matrix	Fortification level (mg/kg)	n	Mean recovery (%)	RSD (%)*	Comments
iodosulfuron-methyl-sodium					
Barley/green material	0.05	3	92	2.3	Appendix 7 of the residue study report (16-2030)
	0.50	3	91	0.6	
Barley, grain	0.01	3	78	5.8	Appendix 7 of the residue study report (16-2030)
	0.10	3	94	2.2	
Barley, straw	0.05	3	97	2.1	Appendix 7 of the residue study report (16-2030)
	0.50	3	96	2.2	

FL = Fortification level, RSD = Relative standard deviation, LOQ = Practical limit of quantification

Fortified with iodosulfuron-methyl-sodium, determined as AE F114844 and calculated as iodosulfuron-methyl-sodium

* Several RSD values shown within the analytical phase report are differing from RSD values illustrated within this table to some extent. The RSD values illustrated within this table are correct.

Table A 4: Characteristics for the analytical method used for validation of residues in barley (green material, grain and straw)

	iodosulfuron-methyl-sodium
Specificity	Mass spectra are provided in Appendix of the original EU reviewed method 00815/M001 blank values < 30% LOQ
Calibration (type, number of data points)	Calibration data presented and Calibration line presented for each analyte and each sample material number of data points: at least 5 R > 0.999
Calibration range	From 0.10 to 25 ng/mL for standards in green material, grain and straw matrix solution. Corresponding calibration range in mass ratio units for the sample: Green material and straw: 0.025 to 6.25 mg/kg Grain: 0.005 to 1.25 mg/kg
Assessment of matrix effects is presented	No. The quantification was done using external matrix-matched standards. It compensates for matrix effect.
Limit of determination/quantification	LOQ: 0.01 mg/kg in grain and 0.05 mg/kg in green material and straw

Conclusion

All method validation data are in compliance with the guideline requirements for data collection methods.

The validation of method 00815/M001 can therefore be considered successful for the matrices green material, grain and straw.

The limits of quantification (LOQ) for iodosulfuron-methyl-sodium are 0.01 mg/kg for grain and 0.05 mg/kg for green material and straw, corresponding to the lowest fortification level of successfully conducted recovery experiments.

A 2.1.1.1.3 Analytical method 01376/M002

A 2.1.1.1.3.1 Method validation

Comments of zRMS:	The study was evaluated and accepted in dRR Part B7 for Atlantis 12 OD.
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Reference:	KCP 5.1.2/04
Title:	Modification M002 of the residue analytical method 01376 for the determination of foramsulfuron, iodosulfuron-methyl, metsulfuron-methyl and AE F153745 in/on plant material by HPLC-MS/MS
Report:	Kaussmann, M.; 2017; 01376/M002; M-587949-01-1
Authority registration No:	
Guideline(s):	Regulation (EC) No 1107/2009 of the European Parliament and the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC European Commission Guidance Document for Generating and Reporting Methods of Analysis in Support of Pre-Registration data Requirements for Annex II (part A, Section 4) and Annex III (part A, section 5) of directive 91/414, SANCO/3029/99 rev. 4, 11/07/00 Guidance document on residue analytical methods, SANCO/825/00/rev. 8.1, European Commission, Directorate General Health and Consumer Protection 16/11/2010 US EPA Residue Chemistry Test Guideline OCSPP 860.1340: Residue Analytical Method OECD Guideline, ENV/JM/MONO (2007)17, Aug 13, 2007
Deviations:	--
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

Materials and methods

The analytical method 01376/M002 was developed for the determination of foramsulfuron, iodosulfuron-methyl, metsulfuron-methyl and AE F153745 (metabolite of foramsulfuron) residues in/on samples of plant origin wheat (grain, green material, straw) and rape (seed)). In this report only data for iodosulfuron-methyl will be demonstrated. Residues of iodosulfuron-methyl were extracted from sample materials with a mixture of acetonitrile/0.02M triethylamin in water (4:1, v:v). The extracts were submitted to LC-MS/MS analysis without any further clean-up. The residues were quantified using matrix-matched standards.

Two MRM transitions were monitored for iodosulfuron-methyl measured by MS/MS (operated in positive mode), namely:

m/z 508 \rightarrow 167 (for quantitation)

m/z 508 \rightarrow 141 (for confirmation)

The HPLC-MS/MS method is highly specific and an additional confirmatory method was not necessary. All solutions were prepared in a mixture of acetonitrile/0.02 M triethylamin in water (4:1, v:v).

Results and discussions

Iodosulfuron-methyl was stable in stock and standard solutions (acetonitrile/0.02 M triethylamin in water (4:1, v:v) as well as in the sample extracts during the course of the study for at least 7 days when stored at $4^{\circ}\text{C} \pm 3^{\circ}\text{C}$ in the dark (due to the structure of the analyte a longer stability can be expected).

Method validation data are summarised in the tables below.

All residues of iodosulfuron-methyl in untreated control samples were below 30% of the LOQ of 0.01 mg/kg.

For validation of the method, recovery experiments were performed by fortifying control samples of plant matrices with iodosulfuron-methyl at levels of the LOQ (0.01 mg/kg) and $10 \times \text{LOQ}$ (0.1 mg/kg).

Mean recoveries for iodosulfuron-methyl for both fortification levels and both mass transitions ranged between 76% and 103% (with the RSDs between 0.8% and 12.7%).

Table A 5: Recovery results from method validation of iodosulfuron-methyl using the analytical method 01376/M002

Matrix	Fortification level (mg/kg)	n	Mean recovery (%)	RSD (%)	Comments
Iodosulfuron-methyl (quantification) (m/z 508 → 167)					
Wheat, grain	0.01	5	101	3.6	-
	0.10	5	103	0.8	-
Wheat, green	0.01	5	92	2.0	-
	0.10	5	77	11.9	-
Wheat, straw	0.01	5	98	3.6	-
	0.10	5	96	1.4	-
Rape, seed	0.01	5	96	2.8	-
	0.10	5	101	1.8	-
Iodosulfuron-methyl (confirmation) (m/z 508 → 141)					
Wheat, grain	0.01	5	102	3.5	-
	0.10	5	99	1.6	-
Wheat, green	0.01	5	87	10.2	-
	0.10	5	76	12.7	-
Wheat, straw	0.01	5	89	3.4	-
	0.10	5	95	2.3	-
Rape, seed	0.01	5	96	2.8	-
	0.10	5	100	3.1	-

Table A 6: Characteristics for the analytical method used for validation of iodosulfuron-methyl residues wheat, grain, wheat, green, wheat, straw, and rape seed.

	Iodosulfuron-methyl
Specificity	Representative mass spectrum is provided Blank value < 30% LOQ for all matrices The HPLC-MS/MS method is highly specific and an additional confirmatory method is not necessary.
Calibration (type, number of data points)	Individual calibration data including calibration line equation presented in the study report; 1/x weighted linear regression model: 1 st MRM transition (m/z 508 → 167): R ≥ 0.9988 2 nd MRM transition (m/z 508 → 141): R ≥ 0.9993 Number of data points: 6
Calibration range	Accepted calibration range: For matrix-matched standards: 0.15 - 10 µg/L (corresponding to 0.003 – 0.2 mg/kg).
Assessment of matrix effects is presented	Yes. Matrix-matched standards were used for all matrices.

Limit of determination/quantification	The limit of quantitation (LOQ) for iodosulfuron-methyl was 0.01 mg/kg while the limit of detection (LOD) was 0.0007 to 0.0028 mg/kg for all matrices.
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Conclusion

The method 01376/M002 was successfully validated and meets all guideline criteria to determine residues of iodosulfuron-methyl in/on samples from plant origin (represented here by wheat (grain, green material, straw) and rape (seed)) at LOQ levels of 0.01 mg/kg.

A 2.1.1.1.4 Analytical method 01514

A 2.1.1.1.4.1 Method validation

Comments of zRMS:	The study was evaluated and accepted in dRR Part B7 for Atlantis 12 OD.	
Reference:	KCP 5.1.2/05	
Title:	Analytical method 01514 for the determination of AE F092944, AE F059411 and AE 0031838 in/on plant by HPLC-MS/MS	
Report:	Kaussmann, M.; 2017; P602166508; M-583894-01-1	
Authority registration No:		
Guideline(s):	Regulation (EC) No 1107/2009 of the European Parliament and the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC European Commission Guidance Document for Generating and Reporting Methods of Analysis in Support of Pre-Registration data Requirements for Annex II (part A, Section 4) and Annex III (part A, section 5) of directive 91/414, SANCO/3029/99 rev. 4, 11/07/00 Guidance document on residue analytical methods, SANCO/825/00/rev. 8.1, European Commission, Directorate General Health and Consumer Protection 16/11/2010 US EPA Residue Chemistry Test Guideline OCSPP 860.1340: Residue Analytical Method OECD Guideline, ENV/JM/MONO (2007)17, Aug 13, 2007	
Deviations:	none	
GLP/GEP:	yes	
Acceptability:	yes	
Duplication (if vertebrate study):		

Materials and methods

The analytical method 01514 was developed for the determination of AE F092944 (metabolite of Foramsulfuron), AE F059411 and AE 0031838 (metabolites of Iodosulfuron-methyl-sodium) residues in wheat (grain, green material, straw) and barley (grain, green material, straw). In this report only data for metabolites of iodosulfuron-methyl-sodium will be demonstrated. Residues of iodosulfuron-methyl were extracted from sample materials with a mixture of acetonitrile:water (1:1, v:v). For AE 0031838 the extracts were submitted to LC-MS/MS analysis without any further clean-up, for AE F059411 the extracts were cleaned up with a diatomaceous earth cartridge before analysis. The residues were quantified using matrix matched standards. Two MRM transitions were monitored for the metabolites of iodosulfuron-methyl-sodium by MS/MS (operated in positive mode), namely:

Analyte	1 st MRM transition (quantification)	2 nd MRM transition (confirmation)
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AE F059411	m/z 141 → 57	m/z 141 → 56
AE 0031838	m/z 143 → 86	m/z 143 → 101

The HPLC-MS/MS method is highly specific and an additional confirmatory method was not necessary. Stock solutions were prepared in acetonitrile and in a mixture of acetonitrile:water (1:1, v:v). Standard solutions (secondary standard) were prepared in a mixture of acetonitrile:water (1:1, v:v).

Results and discussions

Metabolites of iodosulfuron-methyl-sodium were stable in stock and standard solutions in a mixture of water:acetonitrile (1:1; v:v) for at least 10 days of storage in a refrigerator at $4^{\circ}\text{C} \pm 3^{\circ}\text{C}$ under dark conditions. Due to the structure of the analytes a much longer stability is expected. The analytes in extracts were stable for at least 7 days of storage in a refrigerator at $+4^{\circ}\text{C} \pm 3^{\circ}\text{C}$.

Method validation data are summarised in the tables below.

All residues of metabolites of iodosulfuron-methyl-methyl-sodium (AE F059411 and AE 0031838) in untreated control samples were below 30% of the LOQ of 0.01 mg/kg.

For validation of the method, recovery experiments were performed by fortifying control samples of plant matrices with iodosulfuron-methyl-sodium metabolites at levels of the LOQ (0.01 mg/kg) and $10 \times \text{LOQ}$ (0.1 mg/kg).

Mean recoveries for AE F059411 for both fortification levels and both mass transitions ranged between 81 and 98% (with the RSDs between 1.4% and 8.2%), whereas for AE 0031838 recoveries ranged between 74 and 103% (with the RSDs between 1.2 and 9.6%).

Table A 7: Recovery results from method validation of AE F059411 and AE 0031838 using the analytical method 01514

Matrix	Fortification level (mg/kg)	n	Mean recovery (%)	RSD (%)	Comments
AE F059411 (quantification) (m/z 141 → 57)					
Wheat, grain	0.01	5	97	2.4	-
	0.10	5	95	5.6	-
Wheat, green material	0.01	5	96	5.9	-
	0.10	5	92	2.8	-
Wheat, straw	0.01	5	81	4.7	-
	0.10	5	84	8.2	-
Barley, grain	0.01	5	93	3.9	-
	0.10	5	92	1.4	-
Barley, green material	0.01	5	89	2.9	-
	0.10	5	87	2.3	-
Barley, straw	0.01	5	93	3.3	-
	0.10	5	90	2.2	-
AE F059411 (confirmation) (m/z 141 → 56)					
Wheat, grain	0.01	5	93	4.1	-
	0.10	5	94	5.6	-
Wheat, green	0.01	5	98	4.1	-

Matrix	Fortification level (mg/kg)	n	Mean recovery (%)	RSD (%)	Comments
material	0.10	5	91	3.4	-
Wheat, straw	0.01	5	81	4.3	-
	0.10	5	84	7.8	-
Barley, grain	0.01	5	93	6.5	-
	0.10	5	91	2.3	-
Barley, green material	0.01	5	87	3.8	-
	0.10	5	86	1.9	-
Barley, straw	0.01	5	94	8.0	-
	0.10	5	91	3.4	-
AE 0031838 (quantification) (m/z 143 → 86)					
Wheat, grain	0.01	5	94	6.2	-
	0.10	5	97	3.0	-
Wheat, green material	0.01	5	103	1.8	-
	0.10	5	98	3.6	-
Wheat, straw	0.01	5	82	2.0	-
	0.10	5	75	4.1	-
Barley, grain	0.01	5	95	9.3	-
	0.10	5	92	7.1	-
Barley, green material	0.01	5	101	3.1	-
	0.10	5	93	1.4	-
Barley, straw	0.01	5	82	3.4	-
	0.10	5	75	3.9	-
AE 0031838 (confirmation) (m/z 143 → 101)					
Wheat, grain	0.01	5	92	3.9	-
	0.10	5	97	2.8	-
Wheat, green material	0.01	5	102	2.9	-
	0.10	5	98	3.2	-
Wheat, straw	0.01	5	78	5.6	-
	0.10	5	75	4.1	-
Barley, grain	0.01	5	98	9.6	-
	0.10	5	91	6.9	-
Barley, green material	0.01	5	103	5.7	-
	0.10	5	92	1.2	-

Matrix	Fortification level (mg/kg)	n	Mean recovery (%)	RSD (%)	Comments
Barley, straw	0.01	5	80	4.3	-
	0.10	5	74	5.4	-

Table A 8: Characteristics for the analytical method used for validation of AE F059411 and AE0031838 residues in wheat (grain, green material, straw) and barley (grain, green material, straw).

	AE F059411	AE0031838
Specificity	Representative mass spectrum is provided Blank value < 30% LOQ for all matrices The HPLC-MS/MS method is highly specific and an additional confirmatory method is not necessary.	
Calibration (type, number of data points)	Individual calibration data including calibration line equation presented in the study report; 1/x weighted linear regression model: 1 st MRM transition (mz 141 → 57): R: 0.9996 -0.9999 2 nd MRM transition (mz 141 → 56): R: 0.9994 -0.9998 Number of data points: 5	Individual calibration data including calibration line equation presented in the study report; 1/x weighted linear regression model: 1 st MRM transition (mz 143 → 86): R: 0.9981 -0.9992 2 nd MRM transition (mz 143 → 101): R: 0.9971 -0.9992 Number of data points: 5
Calibration range	Accepted calibration range: For matrix-matched standards: 0.25 - 10 µg/L corresponding to 0.005 mg/kg to 0.2 mg/kg.	
Assessment of matrix effects is presented	Yes. Matrix-matched standards were used for all matrices.	
Limit of determination/quantification	The limit of quantitation (LOQ) for iodosulfuron-methyl –sodium metabolites was 0.01 mg/kg while the limit of detection (LOD) was < 0.003 mg/kg for all matrices.	

Conclusion

The method 01514 was successfully validated and meets all guideline criteria to determine residues of metabolites of iodosulfuron-methyl-sodium (AE F059411 and AE 0031838) in/on samples from plant origin (represented here by wheat (grain, green material and straw) and barley (grain, green material, and straw) at LOQ levels of 0.01 mg/kg.

A 2.1.1.2 Description of analytical methods for the determination of residues in animal matrices (KCP 5.1)

No new or additional studies have been submitted.

A 2.1.1.3 Description of analytical methods for the determination of residues in support to environmental fate studies (KCP 5.1)

No new or additional studies have been submitted.

A 2.1.1.4 Description of analytical methods for the determination of residues in

support to toxicological studies (KCP 5.1)

No new or additional studies have been submitted.

A 2.1.1.5 Description of analytical methods for the determination of residues in support of operator, worker, resident and bystander exposure studies (KCP 5.1)

No new or additional studies have been submitted.

A 2.1.1.6 Description of analytical methods for the determination of residues in of ecotoxicology studies (KCP 5.1)

No new or additional studies have been submitted.

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

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

A 2.1.2.1.1 Analytical method 01360/M001

A 2.1.2.1.1.1 Method validation

Comments of zRMS: The study was evaluated and accepted in dRR Part B7 for Atlantis 12 OD.

Reference:	KCP 5.2.1/01
Title:	Modification 001 of analytical method 01360 for the determination of amidosulfuron, metsulfuron-methyl, iodosulfuron-methyl-sodium, mesosulfuron-methyl, and foramsulfuron in samples from plant origin by HPLC-MS/MS
Report:	Stuke, S.; 2015; MR-15/090; M-537921-01-1
Authority registration No:	
Guideline(s):	Regulation (EC) No 1107/2009 of the European Parliament and the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC Guidance document on residue analytical methods, SANCO/825/00/rev. 8.1, European Commission, Directorate General Health and Consumer Protection 16/11/2010 US EPA Residue Chemistry Test Guideline OCSPP 860.1340: Residue Analytical Method OECD Guideline, ENV/JM/MONO (2007) 17, Aug 13, 2007
Deviations:	not specified
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

Materials and methods

The analytical method 01360 was developed for the determination of amidosulfuron, metsulfuron-methyl, iodosulfuron-methyl, mesosulfuron-methyl and foramsulfuron residues in/on plant materials (represented here by sugar beet body (high starch content), sugar beet leaf (high water content), lemon fruit (high acid content), oilseed rape (high oil/fat content) and wheat straw (high protein content, dry commodity)). The modification 001 of method 01360 was validated for the additional sample material wheat grain (high starch content, dry commodity).

The sample preparation is based on the official QuEChERS method (unbuffered). Residues of these analytes were extracted from the plant samples with a mixture of acetonitrile:water (1:1, v:v), where the water content of the corresponding sample material is considered. Ten mL water was added to 5 g of the wheat grain samples to adjust the water content of the extraction solvent. The extraction was conducted using an automated over-head shaker (shaking time 15 minutes). Magnesium sulfate and sodium chloride were added and the samples were shaken again strongly for 1 minute. After centrifugation the supernatant was diluted with Milli-Q water/acetonitrile and triethylamine. After filtration an aliquot of the extract was injected into a high performance liquid chromatograph and analysed with reversed phased chromatography coupled with tandem mass spectrometry (LC-MS/MS) using electrospray ionisation (ESI). Residues were quantified against matrix-matched standards. In the following only results for iodosulfuron-methyl-sodium are presented as the most relevant.

Two MRM transitions were monitored for iodosulfuron-methyl-sodium (both in positive ionisation mode), namely:

m/z 508 \rightarrow 167 (1st MRM) quantitation

m/z 508 \rightarrow 141 (2nd MRM) confirmation

Stock solutions and secondary solutions were prepared in a mixture of acetonitrile:water (4:1; v:v) + 0.02 mol/L triethylamine.

Results and discussions

The stability in final plant extracts was checked for the tested sample material wheat grain over a period of 15 days. For iodosulfuron-methyl-sodium an increase of 30% in the sample extracts was observed. Therefore, it is suggested that samples are analysed as soon as possible after preparation. The blank values were below 30% of LOQ for iodosulfuron-methyl-sodium. Recovery experiments were conducted at the targeted LOQ (0.01 mg/kg) and at $10 \times$ LOQ (0.1 mg/kg). Five replicates per fortification level were analysed. The mean recoveries (for both fortification levels and the two MRM transitions) ranged between 99 and 109% with the RSDs of 4.1 – 5.7%. All results are summarised in the tables below.

Table A 9: Recovery results from method validation of iodosulfuron-methyl-sodium using the analytical method

Matrix	Fortification level (mg/kg)	n	Mean recovery (%)	RSD (%)	Comments
Iodosulfuron-methyl-sodium m/z 508 \rightarrow 167 (quantitation)					
Wheat grain	0.01	5	104	5.0	-
	0.10	5	109	4.9	-
Iodosulfuron-methyl-sodium m/z 508 \rightarrow 141 (confirmation)					
Wheat grain	0.01	5	99	5.7	-
	0.10	5	103	4.1	-

Table A 10: Characteristics for the analytical method used for validation of iodosulfuron-methyl-sodium residues in wheat grain

	Iodosulfuron-methyl-sodium
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Specificity	Representative mass spectrum provided in the study report Blank value < 30% of LOQ
Calibration (type, number of data points)	Individual calibration data including calibration line equation presented in the study report. 1/x weighted linear regression model: R: 0.9968-0.9969 Number of data points: 8
Calibration range	Accepted calibration range in concentration units: 0.2 - 50 µg/L corresponding to 0.004 – 1.0 mg/kg
Assessment of matrix effects is presented	Matrix-matched standards were used
Limit of determination/quantification	0.01 mg/kg

Conclusion

The modification M001 of method 01360 was successfully validated and meets all guideline criteria to determine iodosulfuron-methyl-sodium in cereal grain at the LOQ of 0.01 mg/kg. An ILV is not needed as it has been performed successfully for all other matrices of method 01360.

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

A 2.1.2.2.1 Method 01208/M001

A 2.1.2.2.1.1 Method validation

Comments of zRMS: The study was evaluated and accepted in dRR Part B7 for Atlantis 12 OD.

Reference:	KCP 5.2.2/01
Title:	Amendment no. 3 to final report - Modification M001 of the residue analytical method 01208 for the determination of amidosulfuron (AE F075032), metsulfuron-methyl (AE F075736)
Report:	Pross, S.; 2018; 01208/M001; M-389788-04-I
Authority registration No:	
Guideline(s):	91/414/EEC, 96/68/EC 91/414, SANCO/3029/99 SANCO/825/00 rev. 7, OPPTS 860.5340;
Deviations:	None
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

Materials and methods

The modification of the method 01208 was developed for the determination of amidosulfuron (AE F075032), metsulfuron-methyl (AE F075736), iodosulfuron-methyl-sodium (AE F115008), mesosulfuron-methyl (AE F130060), foramsulfuron (AE F130360) in animal tissues (represented here by kidney, liver, meat, fat, egg and milk).

The homogenised samples were extracted with acetone, centrifuged and the supernatant brought to volume. An aliquot was evaporated to dryness, taken up with acetonitrile:water (with 0.02 M

triethylamine) (2:8, v:v) and analysed by LC-MS/MS equipped with electrospray ionization mode (ESI). In the following only results for iodosulfuron are given as the most relevant.

Two MRM transitions were monitored (both in positive ionisation mode) for iodosulfuron-methyl-sodium, namely:

m/z 508 \rightarrow 167 (1st MRM) for quantitation

m/z 508 \rightarrow 141 (2nd MRM) for confirmation

Quantitation of iodosulfuron-methyl-sodium was performed with the use of matrix-matched standards. Stock solutions were prepared in a mixture of acetonitrile:water (0.02 M triethylamine) (4:1, v:v). Secondary solutions were prepared from the stock solutions by dilutions either with the mixture of acetonitrile:water (0.02 M triethylamine) (4:1, v:v) or with a mixture of acetonitrile:water (0.02 M triethylamine) (2:8, v:v).

Results and discussions

Iodosulfuron-methyl-sodium was stable in all sample extracts when stored at $4^{\circ}\text{C} \pm 3^{\circ}\text{C}$ for at least two weeks. The blank values were below 30% of LOQ for iodosulfuron-methyl-sodium. Recovery experiments were conducted at the targeted LOQ (0.01 mg/kg) and at $10 \times \text{LOQ}$ (0.1 mg/kg). Five replicates per fortification level were analysed. The mean recoveries (for both fortification levels and the two MRM transitions) ranged between 84 and 98% with the RSDs of 0.9 – 5.9%. All results are summarised in the tables below.

Table A 11: Recovery results from method validation of iodosulfuron-methyl using the analytical method

Matrix	Fortification level (mg/kg)	n	Mean recovery (%)	RSD (%)	Comments
Iodosulfuron-methyl-sodium m/z 508 \rightarrow 167 (quantitation)					
Kidney	0.01	5	97	0.9	-
	0.1	5	97	1.6	-
Liver	0.01	5	97	3.1	-
	0.1	5	98	1.6	-
Meat	0.01	5	85	4.9	-
	0.1	5	91	4.8	-
Fat	0.01	5	93	3.3	-
	0.1	5	95	2.1	-
Milk	0.01	5	90	3.7	-
	0.1	5	94	2.1	-
Egg	0.01	5	87	4.0	-
	0.1	5	87	5.1	-
Iodosulfuron-methyl-sodium m/z 508 \rightarrow 141 (confirmation)					
Kidney	0.01	5	94	3.6	-
	0.1	5	90	2.5	-
Liver	0.01	5	92	2.8	-
	0.1	5	93	2.2	-
Meat	0.01	5	84	5.1	-

Matrix	Fortification level (mg/kg)	n	Mean recovery (%)	RSD (%)	Comments
	0.1	5	88	4.6	-
Fat	0.01	5	91	2.7	-
	0.1	5	92	1.3	-
Milk	0.01	5	95	5.9	-
	0.1	5	90	2.2	-
Egg	0.01	5	86	5.5	-
	0.1	5	87	4.5	-

Table A 12: Characteristics for the analytical method used for validation of iodosulfuron-methyl-sodium residues in kidney, liver, meat, fat, milk and egg.

	Iodosulfuron-methyl-sodium
Specificity	Mass spectrum provided in the original study report (01208). Blank value <30% of LOQ
Calibration (type, number of data points)	Individual calibration data presented in the study report including calibration line equation. 1/x weighted linear regression model: R: 0.9976 – 0.9994 (1 st MRM) R: 0.9982 – 0.9991 (2 nd MRM) Number of data points: 5
Calibration range	Accepted calibration range in concentration units: 0.6 – 50 µg/L corresponding to 0.006 – 0.50 mg/kg (fat) and 0.003 – 0.25 mg/kg (for all other matrices)
Assessment of matrix effects is presented	Matrix-matched standards were used
Limit of determination/quantification	0.01 mg/kg

Conclusion

The method 01208/M001 was successfully validated and meets all guideline criteria to determine residues of iodosulfuron-methyl-sodium in samples of animal origins (represented by kidney, liver, meat, fat, milk and egg) at the LOQ of 0.01 mg/kg.

A 2.1.2.2.1.2 Independent laboratory validation

Comments of zRMS: The study was evaluated and accepted in dRR Part B7 for Atlantis 12 OD.

Reference:	KCP 5.2.2/02
Title:	Independent laboratory validation of an analytical method 01208/M001 for the determination of amidosulfuron (AE F075032), metsulfuron-methyl (AE F075736), iodosulfuron-methyl-sodium (AE F115008), mesosulfuron-methyl (AE F130060), foramsulfuron (AE F130360) in animal tissues (meat, fat, liver, kidney), egg, and milk by HPLC-MS/MS
Report:	Netzband, D.; 2015; RAMML014-01; M-398300-02-1
Authority registration No:	
Guideline(s):	Residue Chemistry Test Guidelines: OPPTS 860.1340 Residue Analytical Method European Commission Guidelines: SANCO/825/00 rev. 7 Guidance Document on Residue Analytical Methods Residue Chemistry Guidelines:

	PMRA Regulatory Directive Dir98-02 DACO 7.2.3 Inter-laboratory Analytical Methodology Validation
Deviations:	not specified
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

Materials and methods

The purpose of this study was to perform an independent laboratory validation (ILV) of the method 01208/M001 for the determination of amidosulfuron (AE F075032), metsulfuronmethyl (AE F075736), iodosulfuron-methyl-sodium (AE F115008), mesosulfuron-methyl (AE F130060), foramsulfuron (AE F130360) in animal matrices (kidney, liver, meat, fat, milk and egg) by HPLC-MS/MS. In the following only results for iodosulfuron-methyl-sodium are demonstrated as the most relevant.

The extraction method of iodosulfuron-methyl sodium used for the animal matrices was in line with the one described in the original method/ primary method (01208/M001) except for fat sample, which was not evaporated to dryness and reconstituted in solvent as the other samples but partitioned between hexane and a mixture of acetonitrile:water. Determination and quantitation of the targeted analytes were conducted using high performance liquid chromatography (HPLC) employing electrospray ionisation mass spectrometric (ESI/MS/MS) detection.

The following MRM transitions were monitored using electrospray ionisation (ESI) in positive ionisation mode:

Iodosulfuron methyl-sodium:

m/z 508 \rightarrow 167 (1st MRM) quantitation

m/z 508 \rightarrow 141 (2nd MRM) confirmation

Quantitation of iodosulfuron-methyl-sodium was carried out using an external matrix-matched standard method.

Stock solutions were prepared in a mixture of acetonitrile:0.02M triethylamine in water (4:1, v:v) and secondary solutions were prepared by diluting the stock solutions with the same mixture or with a mixture of acetonitrile: 0.02M triethylamine in water (2:8, v:v).

Results and discussions

The analytes were stable in sample extracts for a test period of 13-14 days when stored at 4 °C \pm 3°C. The blank values were below 30% of the LOQ for iodosulfuron-methyl-sodium. Recovery experiments were conducted at the targeted LOQ (0.01 mg/kg) and at 10 \times LOQ (0.1 mg/kg). Five replicates per fortification level were analysed for each matrix. The mean recoveries (for both fortification levels and the two MRM transitions, results for the second attempt) ranged between 81 and 105% with the RSD of 1.1-11.0%. All results are summarised in the tables below.

Table A 13: Recovery results from method validation of iodosulfuron-methyl-sodium using the analytical method

Matrix	Fortification level (mg/kg)	n	Mean recovery (%)	RSD (%)	Comments
Iodosulfuron-methyl-sodium m/z 508 \rightarrow 167 (quantitation)					
Kidney	0.01	5	99	5.0	-
	0.1	5	98	2.8	-
Liver 1#	0.01	5	94	3.8	First attempt results

Matrix	Fortification level (mg/kg)	n	Mean recovery (%)	RSD (%)	Comments
	0.1	5	122	16	
Liver 2#	0.01	5	87	6.1	Second attempt results
	0.1	5	90	1.1	
Meat	0.01	5	85	5.5	-
	0.1	5	90	4.1	-
Fat 1#	0.01	5	101	4.5	First attempt results
	0.1	5	98	4.4	
Fat 2#	0.01	5	88	2.3	Second attempt results
	0.1	5	89	4.8	
Milk	0.01	5	90	3.0	-
	0.1	5	92	1.8	-
Egg	0.01	5	94	4.8	-
	0.1	5	95	1.6	-
Iodosulfuron-methyl-sodium m/z 508 → 141 (confirmation)					
Kidney	0.01	5	105	11.0	-
	0.1	5	100	5.1	-
Liver 1#	0.01	5	114	4.5	First attempt results
	0.1	5	117	16	
Liver 2#	0.01	5	84	4.1	Second attempt results
	0.1	5	90	4.3	-
Meat	0.01	5	81	9.5	-
	0.1	5	89	5.1	-
Fat 1#	0.01	5	106	10	First attempt results
	0.1	5	98	2.6	
Fat 2#	0.01	5	97	5.2	Second attempt results
	0.1	5	88	6.4	
Milk	0.01	5	93	8.9	-
	0.1	5	91	5.8	-
Egg	0.01	5	92	8.7	-
	0.1	5	94	5.4	-

Table A 14: Characteristics for the analytical method used for independent laboratory validation of iodosulfuron-methyl-sodium residues in kidney, liver, muscle, fat, milk and poultry egg

	Iodosulfuron-methyl-sodium
Specificity	Blank values <30% of LOQ
Calibration (type, number of data points)	Individual calibration data presented in the study report including calibration line equation. 1/x weighted linear regression model;

	Iodosulfuron-methyl-sodium
	R^2 : 0.9968 - 0.9991 (1 st MRM) R^2 : 0.9952 – 0.9987 (2 nd MRM) Number of data points: 6
Calibration range	Accepted calibration range in concentration units: 0.0006 – 0.05 µg/mL corresponding to 0.006 – 0.50 mg/kg (fat) and 0.003 – 0.25 mg/kg (for all other matrices)
Assessment of matrix effects is presented	Matrix-matched standards were used
Limit of determination/quantification	0.01 mg/kg

Conclusion

The method 01208/M001 was successfully validated by an independent laboratory and meets all guideline criteria to determine residues of iodosulfuron-methyl-sodium in/on samples from animal origins (represented here by kidney, liver, meat, fat, milk and poultry egg) at the LOQ of 0.01 mg/kg.

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

No new or additional studies have been submitted.

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

No new or additional studies have been submitted.

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

No new or additional studies have been submitted.

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

No new or additional studies have been submitted.

A 2.1.2.6.1 Analytical method 01478

A 2.1.2.6.1.1 Method validation

Comments of zRMS:	The study was evaluated and accepted in dRR Part B7 for Atlantis 12 OD.
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Reference:	KCP 5.2.3/01
Title:	Analytical method 01478 for the determination of various pesticides and selected pesticide metabolites in plasma by HPLC-MS/MS
Report:	Kaussmann, M.; 2016; 01478; M-551992-01-I
Authority registration No.:	
Guideline(s):	Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market Guidance Document on Residue Analytical Methods, SANCO/825/00 rev. 8.1 of November 16, 2010 European Commission Guidance Document for Generating and Reporting Methods of Analysis in Support of Pre-Registration data Requirements for Annex II (part A, section 4) and Annex III (part A, section 5) of directive 91/414, SANCO/3029/99 rev. 4, July 11, 2000
Deviations:	not specified
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

The method 01478 describes the determination of AE C415557 (RPA717879), isoxaflutole, isoxaflutole-diketonitrile, flurtamone, mesosulfuron-methyl, foramsulfuron and iodosulfuron-methyl in plasma at LOQ of 50 µg/L by HPLC-MS/MS and provides validation data for Multiple Reaction Monitoring (MRM) using electrospray ionization. Only iodosulfuron-methyl data will be presented here.

Materials and methods

Cattle Plasma samples were deproteinized by mixing with acetonitrile and subsequent centrifugation. An aliquot of the supernatant was subjected to HPLC-MS/MS analysis and the residues were quantified using matrix matched standards. Iodosulfuron-methyl was measured in positive ion mode.

Results and discussions

The limit of quantification for iodosulfuron-methyl in plasma was established at 50 µg/L, expressed as itself. The limit of detection (LOD) was estimated at 15 µg/L.

The high selectivity of the method resulted from the HPLC separation in combination with MS/MS detection. Two MRM transitions (m/z 508 → 167 and m/z 508 → 141) were monitored for iodosulfuron-methyl (mass transitions for quantitation and confirmation, respectively).

No signals/peaks interfering with the detection of the analyte were observed in solutions of untreated control specimens. Apparent concentrations in control samples of plasma were below 30% of the LOQ.

Due to the high sensitivity of the used detector, the detector response of iodosulfuron-methyl was quadratic (1/x weighted) for matrix matched standard solutions ranging from 1.5 µg/L to 75 µg/L. The detector response might be linear if other detectors are used.

The measured concentration is calculated by comparison of the analyte response to the respective calibration curve (1/x weighted). The correlation coefficients were ≥ 0.998 for both MRM transitions.

The analytes were stable in plasma for at least four days when stored in a freezer at $\leq -18^\circ\text{C}$. In addition the stability of the analytes in extracts was demonstrated for a period of four days when stored in a refrigerator at $\leq +6^\circ\text{C}$ under dark conditions.

Full validation data were generated for two MS/MS transitions. The first transition (m/z 508 → 167) is recommended for quantification and the second transition (m/z 508 → 141) may be used for confirmatory analyses. The two MRM transitions were successfully validated for plasma. Therefore, an additional confirmatory method is not necessary

The method was validated using a sample of cattle plasma. Fortification experiments were performed at the limit of quantitation and $10 \times \text{LOQ}$. There were 5 replicates at the limit of quantification (50 µg/L) and 5 replicates at the 10-fold limit of quantification (500 µg/L)

For each sample the recovery-rate was determined using the two different mass transitions. An overview of the results is given in Table below. The mean recoveries ranged between 105% and 109% with relative standard deviations less than 2.4%.

Table A 15: Validation of the method 01478 for the determination of iodosulfuron-methyl in plasma

Matrix	Fortification level (µg/L)	n	Mean recovery (%)	RSD (%)	Comments
Iodosulfuron-methyl-sodium m/z 508 → 167 (quantitation)					
Cattle plasma	50	5	109	1.4	-
	500	5	108	1.8	-
	overall	10	108	1.6	
Iodosulfuron-methyl-sodium m/z 508 → 141 (confirmation)					
Cattle plasma	50	5	105	2.4	-
	500	5	107	2.3	-
	overall	10	10.6	2.4	

RSD: relative standard deviation.

Table A 16: Characteristics for the analytical method used for validation of iodosulfuron-methyl residues in plasma matrix

Method 01478	iodosulfuron-methyl in plasma
Specificity	mass spectra provided in Appendix 4 of the report blank value <30% LOQ
Calibration (type, number of data points)	Calibration data presented in Appendix 6 Calibration line equation presented in Appendix 5 number of data points ≥ 5 (7 points) 1 st MRM: R = 0.9997 2 nd MRM: R = 0.9999
Calibration range	Due to the high sensitivity of the used detector, the detector response was quadratic (1/x weighted). Matrix-matched standard solutions ranged from 1.5 µg/L to 75 µg/L. The detector response might be linear if other detectors are used.
Assessment of matrix effects is presented	Yes. Matrix effects were not found. Nevertheless, quantification was performed against matrix matched standards
Limit of determination/quantification	LOQ= 50 µg/L

Conclusion

The method 01478 was developed for the determination of iodosulfuron-methyl in plasma. Quantification by means of LC-MS/MS with two MS/MS transitions ensures a high level of specificity. The results obtained during validation demonstrate accuracy and repeatability of the residue determination. The limit of quantification was established at 50 µg/L, expressed as iodosulfuron-methyl. The method validation data are in compliance with the guideline requirements for enforcement methods. An Independent laboratory validation is not required for body fluid methods of analysis.

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

No new or additional studies have been submitted.

A 2.2 Analytical methods for the active substance mesosulfuron methyl

New analytical method for determination of active substance and its relevant metabolites in plant protection product have been submitted and summarised in points 5.2.1.1. and 5.2.1.2. Submitted methods can be used for post-authorisation monitoring.

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

A 2.2.1.1 Description of analytical methods for the determination of residues in plant matrices (KCP 5.1)

A 2.2.1.1.1 Additional validation data of analytical method 00815/M001 (study 13-2127 and 13-2129 Cereal)

A 2.2.1.1.1.1 Method validation

Comments of zRMS: The study was evaluated and accepted in dRR Part B7 for Atlantis 12 OD.

Reference:	KCP 5.1.2/01
Title:	Amendment no. 2: Determination of the residues of BYH 18636 , iodosulfuron-methyl-sodium, mefenpyr-diethyl and mesosulfuron-methyl in/on winter wheat after spraying of IMS & MSM & MPR WG 12.6 and thiencazone-methyl WG 70 in the field in Germany, Spain and Portugal
Report:	Stuke, S.; Kerkerling, S.; 2018; 13-2127; M-503498-03-1
Authority registration No:	
Guideline(s):	Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC EC Guidance working document 7029/VI/95 rev.5 (1997-07-22) OECD 509 Adopted 2009-09-07, OECD GUIDELINE FOR THE TESTING OF CHEMICALS, Crop Field US EPA OCSPP Guideline No. 860.1500
Deviations:	Yes, see Appendix 5
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

Reference:	KCP 5.1.2/02
Title:	Amendment no. 2: Determination of the residues of mefenpyr-diethyl, BYH 18636, iodosulfuron-methylsodium and mesosulfuron-methyl in/on winter wheat after spraying of IMS & MSM & MPR WG 15 and thiencazone-methyl WG 70 in the field in Belgium, the Netherlands and Italy
Report:	Stuke, S.; Kerkerling, S.; 2018; 13-2129; M-506719-03-1
Authority registration No:	
Guideline(s):	Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC

	EC Guidance working document 7029/VI/95 rev.5 (1997-07-22) OECD 509 Adopted 2009-09-07, OECD GUIDELINE FOR THE TESTING OF CHEMICALS, Crop Field Trial US EPA OCSPP Guideline No. 860.1500
Deviations:	None
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

Materials and methods

Full validation data is documented with the method 00815/M001 itself for matrices flax linseed, wheat grain, straw and green material.

Further validation sets (3 repetitions each at two fortification levels LOQ and $10 \times \text{LOQ}$) on wheat grain, straw and green material were conducted within the course of the studies 13-2127/13-2129. Method validations performed during the conduct of these studies are summarized in the tables below:

Results and discussions

Apparent residues in control samples were below 30% of the LOQ. Mean recoveries per fortification level for both analytes were in a range of 70 – 110 % with RSD < 20%.

Table A 17: Recovery data for mesosulfuron-methyl-sodium using the analytical method

Matrix	Fortification level (mg/kg)	n	Mean recovery (%)	RSD (%)*	Comments
mesosulfuron-methyl					
wheat / green material	0.05	3	101	6.2	Appendix 4 of the residue report 13-2127 13-2129
	0.50	3	98	3.2	
wheat / grain	0.01	3	103	3.8	Appendix 4 of the residue report 13-2127 13-2129
	0.10	3	95	4.1	
wheat / straw	0.05	3	102	2.3	Appendix 4 of the residue report 13-2127 13-2129
	0.50	3	99	2.7	

FL = Fortification level, RSD = Relative standard deviation, LOQ = Practical limit of quantification

Fortified with mesosulfuron-methyl, determined as mesosulfuron-methyl and calculated as mesosulfuronmethyl

*Several RSD values shown within the analytical phase report are differing from RSD values illustrated within this table to some extent. The RSD values illustrated within this table are correct.

Table A 18: Characteristics for the analytical method used for validation of residues in Cereal (green material, grain and straw)

	mesosulfuron-methyl
Specificity	Mass spectra are provided in Appendix of the original EU reviewed method 00815/M001 blank values < 30% LOQ
Calibration (type, number of data points)	Calibration data presented and Calibration line presented for each analyte and each sample material number of data points: at least 5 R > 0.999
Calibration range	Matrix-matched standards from 0.1 to 25.0 ng/mL in green material, grain and straw. Corresponding calibration range in mass ratio units for the sample: * Green material and straw: 0.025 to 6.25 mg/kg. * Grain → 0.005 to 1.25 mg/kg
Assessment of matrix effects is presented	No. The quantification was done using external matrix-matched standards. It compensates for matrix effect.
Limit of determination/quantification	LOQ: green material → 0.05 mg/kg grain → 0.01 mg/kg straw → 0.05 mg/kg

Conclusion

All method validation data are in compliance with the guideline requirements for data collection methods.

The validation of method 00815/M001 can therefore be considered successful in wheat for the matrices green material, grain and straw.

The limits of quantitation (LOQ) for mesosulfuron-methyl are 0.01 mg/kg, for grain, and 0.05 mg/kg for green material and straw, corresponding to the lowest fortification level of successfully conducted recovery experiments.

A 2.2.1.2 Description of analytical methods for the determination of residues in animal matrices (KCP 5.1)

No new or additional studies have been submitted.

A 2.2.1.3 Description of analytical methods for the determination of residues in support to environmental fate studies (KCP 5.1)

No new or additional studies have been submitted.

A 2.2.1.4 Description of analytical methods for the determination of residues in support to toxicological studies (KCP 5.1)

No new or additional studies have been submitted.

A 2.2.1.5 Description of analytical methods for the determination of residues in

support of operator, worker, resident and bystander exposure studies (KCP 5.1)

No new or additional studies have been submitted.

A 2.2.1.6 Description of analytical methods for the determination of residues in of ecotoxicology studies (KCP 5.1)

No new or additional studies have been submitted.

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)

A 2.2.2.1.1 Analytical method 01360/M001

A 2.2.2.1.1.1 Method validation

Comments of zRMS: The study was evaluated and accepted in dRR Part B7 for Atlantis 12 OD.

Study Stuke, S.; 2015 was assessed and accepted in the RAR for mesosulfuron.

Reference:	KCP 5.2.1/01
Title:	Modification 001 of analytical method 01360 for the determination of amidosulfuron, metsulfuron-methyl, iodosulfuron-methyl-sodium, mesosulfuron-methyl, and foramsulfuron in samples from plant origin by HPLC-MS/MS
Report:	Stuke, S.; 2015; MR-15/090; M-537921-01-1
Authority registration No:	
Guideline(s):	Regulation (EC) No 1107/2009 of the European Parliament and the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC Guidance document on residue analytical methods, SANCO/825/00/rev. 8.1, European Commission, Directorate General Health and Consumer Protection 16/11/2010 US EPA Residue Chemistry Test Guideline OCSPP 860.1340: Residue Analytical Method OECD Guideline, ENV/JM/MONO (2007) 17, Aug 13, 2007
Deviations:	not specified
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

Materials and methods

The analytical method 01360 was developed for the determination of amidosulfuron, metsulfuron-methyl, iodosulfuron-methyl, mesosulfuron-methyl and foramsulfuron residues in/on plant materials. The 5 crop groups are represented by sugar beet body (high starch content), sugar beet leaf (high water content), lemon fruit (high acid content), oilseed rape (high oil/fat content) and wheat straw (high protein content,

dry commodities). The modification 001 of method 01360 was validated for the additional sample material wheat grain (dry commodities).

The sample preparation is based on the official QuEChERS method (unbuffered). Residues of these analytes were extracted from the plant samples with a mixture of acetonitrile:water (1:1, v:v), where the water content of the corresponding sample material is considered. 10 mL water was added to 5 g of the wheat grain samples to adjust the water content of the extraction solvent. The extraction was conducted using an automated over-head shaker (shaking time 15 minutes considering the latest recommendations by Hepperle/Anastassiades, publisher of the QuEChERS method at the LVUA Stuttgart, Germany). Magnesium sulfate and sodium chloride were added and the samples were shaken again strongly for 1 minute. After centrifugation the supernatant was diluted with Milli-Q water/acetonitrile and triethylamine. After filtration an aliquot of the extract was injected into a high performance liquid chromatograph and analyzed with reversed phased chromatography coupled with tandem mass spectrometry (LC-MS/MS). Residues were quantified against matrix-matched standards.

Two MRM transitions were monitored for mesosulfuron-methyl:

m/z 504 \rightarrow 182 for quantification

m/z 504 \rightarrow 83 for confirmation.

Therefore, the HPLC-MS/MS method is highly specific and an additional confirmatory method is not necessary.

The correlation between the injected amount of substance and the detector response was linear for matrix standards ranging from 0.2 $\mu\text{g/L}$ to 50 $\mu\text{g/L}$ for both MRM transitions. The correlation coefficients were > 0.99 .

Stock solutions were prepared in acetonitrile. Standard solutions for fortification and standard solutions used to run the linearity investigations were prepared in a mixture of acetonitrile:water (4:1; v:v) + 0.02 mol/L triethylamine.

The stability in final plant extracts was checked for wheat grain over a period of 15 days. The recoveries at day 0 (initial analysis) were compared to the recoveries analysed after storage of the final samples at $4^\circ\text{C} \pm 3^\circ\text{C}$ in the dark over 15 days. To check the stability freshly prepared matrix standards were analyzed together with the aged recovery samples. For mesosulfuron-methyl in wheat grain an increase of less than 20% was observed. These results can be due to different matrix effects in fresh matrix standards compared to the older recovery samples.

For mesosulfuron-methyl in wheat grain an increase of 16.9% ($n=5$) was observed.

Table 19: Stability of Mesosulfuron-methyl in Wheat Grain Extracts.

Sample Material	Fortification Level [mg/kg]		Recovery Rates [%]					Mean
Wheat Grain	0.1	Day 0 (initial analysis)	88	102	96	98	105	16.9
		15 days reanalyses	103	107	115	121	125	
		deviation day 0/15 days	17.0	4.9	19.8	23.5	19.0	

Mesosulfuron-methyl can be assumed as being stable in this matrix, most likely different matrix effects in aged samples and fresh matrix standards

Results and discussions

Apparent residues in control samples were below 30% of the LOQ. Recoveries were calculated with matrix-matched standards. Mean recoveries per fortification level were in a range of 70 – 110 % with $\text{RSD} < 20\%$.

The results of the method validation are summarized in the table below.

Table A 19: Recovery results from method validation of mesosulfuron-methyl using the analytical method

Matrix	Fortification level (mg/kg)	n	Mean recovery (%)	RSD (%)	Comments
mesosulfuron-methyl m/z 504 → 182 quantification					
Wheat grain	0.010	5	97	4.1	
	0.10	5	98	6.6	
	Overall	10	97	5.2	
mesosulfuron-methyl m/z 504 → 83 confirmation					
Wheat grain	0.010	5	93	6.2	
	0.10	5	96	5.7	
	Overall	10	94	5.7	

n: number of single results per fortification

RSD: Relative Standard Deviation

Table A 20: Characteristics for the analytical method used for validation of mesosulfuron-methyl residues in wheat grain

	mesosulfuron-methyl
Specificity	Mass spectra are provided in the method report (Appendix 5) blank values < 30% LOQ
Calibration (type, number of data points)	Calibration data presented Calibration line presented (linear regression) number of data points: 6 R > 0.99
Calibration range	0.2 to 50 µg/L, for standards in wheat, grain matrix solution Corresponding calibration range in mass ratio units for the sample: 0.002 to 0.5 mg/kg for wheat, grain
Assessment of matrix effects is presented	No. Matrix-matched standards were used to compensate the possible influence of the matrix.
Limit of determination/quantification	LOD calculated: 0.0014 mg/kg in wheat, grain LOQ: 0.01 mg/kg in wheat, grain

Conclusion

The modification M001 of method 01360 is acceptable and validated for the enforcement of mesosulfuron-methyl in cereal grain. It has been sufficiently validated.

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

No new or additional studies have been submitted

A 2.2.2.3 Description of Methods for the Analysis of Body Fluids and Tissues (KCP 5.2)

A 2.2.2.3.1 Analytical method 01478

A 2.2.2.3.1.1 Method validation

Comments of zRMS: The study was evaluated and accepted in dRR Part B7 for Atlantis 12 OD.

Reference:	KCP 5.2.3/01
Title:	Analytical method 01478 for the determination of various pesticides and selected pesticide metabolites in plasma by HPLC-MS/MS
Report:	Kaussmann, M.; 2016; 01478; M-551992-01-1
Authority registration No.:	
Guideline(s):	Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market Guidance Document on Residue Analytical Methods, SANCO/825/00 rev. 8.1 of November 16, 2010 European Commission Guidance Document for Generating and Reporting Methods of Analysis in Support of Pre-Registration data Requirements for Annex II (part A, section 4) and Annex III (part A, section 5) of directive 91/414, SANCO/3029/99 rev. 4, July 11, 2000
Deviations:	not specified
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

The method 01478 describes the determination of AE C415557 (RPA717879), isoxaflutole, isoxaflutole-diketonitrile, flurtamone, mesosulfuron-methyl, foramsulfuron and iodosulfuron-methyl in plasma at LOQ of 50 µg/L by HPLC-MS/MS and provides validation data for Multiple Reaction Monitoring (MRM) using electrospray ionization. Only mesosulfuron-methyl data will be presented here.

Materials and methods

Cattle Plasma samples were deproteinized by mixing with acetonitrile and subsequent centrifugation. An aliquot of the supernatant was subjected to HPLC-MS/MS analysis and the residues were quantified using matrix matched standards. Mesosulfuron -methyl was measured in positive ion mode.

Two MRM transitions were monitored for mesosulfuron-methyl:
m/z 504 → 182 for quantification and m/z 504 → 139 for confirmation. The two MRM transitions were successfully validated for plasma. Therefore, an additional confirmatory method is not necessary.

Due to the high sensitivity of the used detector the injected amount of mesosulfuron-methyl and the detector response was quadratic (1/x weighted) for matrix matched standard solutions ranging from 1.5 µg/L to 75 µg/L. The detector response might be linear if other detectors are used. The correlation coefficients were > 0.99 for both MRM transitions.

Stock solutions were prepared in acetonitrile. Secondary standard solutions (for fortification and calibration) were prepared from the stock solution by dilution with acetonitrile. Calibration solutions (matrix matched standards) were freshly prepared from the standard solutions by dilution with control plasma extracts.

Mesosulfuron-methyl was stable in plasma for at least four days when stored in a freezer at ≤ -18 °C. In addition the stability of mesosulfuron-methyl in extracts was demonstrated for a period of four days when stored in a refrigerator at ≤ +6 °C under dark conditions.

Results and discussions

Apparent residues in control samples were below 30% of the LOQ. Recoveries were calculated with matrix-matched standards. Mean recoveries per fortification level were in a range of 70 – 110 % with RSD < 20%.

The results of the method validation are summarized in the table below.

Table A 21: Recovery results from method validation of mesosulfuron-methyl using the analytical method

Matrix	Fortification level (µg/L)	n	Mean recovery (%)	RSD (%)	Comments
mesosulfuron-methyl m/z 504 → 182 quantification					
Cattle plasma	50	5	108	1.1	
	500	5	108	1.8	
	Overall	10	108	1.4	
mesosulfuron-methyl m/z 504 → 139 confirmation					
Cattle plasma	50	5	109	1.1	
	500	5	108	1.7	
	Overall	10	109	1.5	

n: number of single results per fortification

RSD: Relative Standard Deviation

Table A 22: Characteristics for the analytical method used for validation of mesosulfuron-methyl residues in wheat grain

	mesosulfuron-methyl
Specificity	Mass spectra are provided in the method report (Appendix 4) blank values < 30 % LOQ
Calibration (type, number of data points)	Calibration data presented Calibration line presented (quadratic regression) number of data points: 7 R > 0.99
Calibration range	1.5 µg/L to 75 µg/L, for matrix matched standards solutions
Assessment of matrix effects is presented	No. Matrix-matched standards were used to compensate the possible influence of the matrix.
Limit of determination/quantification	The limit of detection (LOD) was estimated as the lowest measured standard concentration in the matrix matched standard linearity. LOD estimated: 15 µg/L LOQ: 50 µg/L

Conclusion

The method 01478 was developed for the determination of mesosulfuron-methyl in plasma. Quantification by means of LC MS/MS with two MS/MS transitions ensures a high level of specificity. The results obtained during validation demonstrate accuracy and repeatability of the residue determination. The limit of quantification was established at 50 µg/L expressed as mesosulfuron-methyl. The method validation data are in compliance with the guideline requirements for enforcement methods. An Independent laboratory validation is not required for body fluid methods of analysis.