REGISTRATION REPORT

Part B

Section 5

Analytical Methods

Detailed summary of the risk assessment

Product code: CHR/H/MEZO 30 OD

Product name(s): Vidal 30 OD, Pacyfik 30 OD

Chemical active substance:

Mesosulfuron-methyl, 30g/L

Central Zone

Zonal Rapporteur Member State: PL

CORE ASSESSMENT

(authorization)

Applicant: Innvigo Sp. z o.o.

Submission date: December 2023

zRMS Assessment: 26/07/2024

MS Finalisation date: 19/11/2024

Version history

When	What
July 2024	zRMS Assessment
November 2024	Following commenting period

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5 Analytical methods

5.1 Conclusion and summary of assessment

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

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

- none

Commodity/crop	Supported/ Not supported
Winter wheat	Supported
Spelt	Supported

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

5.2.1 Analysis of the plant protection product (KCP 5.1.1)

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

Comments of zRMS:	Described method validation ICB/79/2022 for the determination of mesosulfuron-methyl and mefenpyr-diethyl in Mesosulfuron 30 OD (CHR/H/MEZO 30 OD) was validated in accordance with SANCO/3030/99 rev.5 22/03/19 and GLP. The method is acceptable and suitable for the determination of the active substance.

Reference:	KCP 5.1.1
Report	Validation of analytical method for Mesosulfuron 30 OD (CHR/H/MEZO 30 OD) for determination of mesosulfuron-methyl and mefenpyr-diethyl.; Study Code: ICB/79/2022, Iwona Knapik, 2023
Guideline(s):	Revision of 30 April 2013 draft, 4 November 2013; Guidance document for single laboratory validation of quantitative analytical methods used in support of pre- and post-registration data requirements for plant protection and bio-cidal products, - The Joint FAO/IAEA Division of Nuclear Techniques in Food and Agricul-ture July 2009; Quality Control of Pesticide Products,

	- SANCO/3030/99 rev.5 22/03/19 Technical Active Substance and Plant protection products: Guidance for generating and reporting methods of analysis in support of pre- and post-registration data requirements for Annex (Section 4) of Regulation (EU) No 283/2013 and Annex (Section 5) of Regulation (EU) No 284/2013
Deviations:	No
GLP:	Yes
Acceptability:	

Materials and methods

For tests, which are in these document there was prepared:

- For calibration: 2 standards stock solution in acetonitrile (1 for Mesosulfuron-methyl- 10.5 mg and 1 for Mefenpyr- diethyl- 10.7mg) and 10 working standard solutions in acetonitrile (5 for Mesosulfuron-methyl and 5 for mefenpyr- diethyl) with increasing standard solution volumes.
- For determination recovery: 2 standards stocks (1 for Mesosulfuron-methyl- 31 mg and 1 for mefenpyr- diethyl- 45.2 mg).

For determination: concentration of Mesosulfuron-methyl and Mefenpyr-diethyl, recovery, linearity specificity in test item used liquid chromatography with diode array detection (HPLC-DAD). In this chromatography there were 2 chromatographic systems: primary and secondary.

Validation parameters were determined in according to the requirements in Guidance document for single laboratory validation of quantitative analytical methods used in support of pre- and post-registration data requirements for plant protection and biocidal products, . Revision of 30 April 2013 draft, 4 November 2013 and SANCO/3030/99 rev.5 22/03/19 Technical Active Substance and Plant protection products: Guidance for generating and reporting methods of analysis in support of pre- and post-registration data requirements for Annex (Section 4) of Regulation (EU) No 283/2013 and Annex (Section 5) of Regulation (EU) No 284/2013

Validation - Results and discussions

Table 5.2-1: Methods suitable for the determination of active substance Mesosulfuron-methyl and safener Mefenpyr-diethyl in plant protection products Vidal 30 OD, Pacyfik 30 OD/ CHR/H/MEZO 30 OD.

	Mesosulfuron-methyl (active substance)	**Mefenpyr-diethyl (safener)**
Author(s), year	Iwona Knapik, 2023	
Principle of method	HPLC-DAD	
Linearity (linear between mg/L / % range of the declared content) (correlation coefficient, expressed as r)	Primary chromatographic system: R2=0.9998570	Primary chromatographic system: R2=0.9999384
	Secondary chromatographic system: R2=0.9999142	Secondary chromatographic system: R2=0.9998822

	Mesosulfuron-methyl (active substance)	**Mefenpyr-diethyl (safener)**
Precision – Repeatability Mean n = 5 (%RSD)	Primary chromatographic system: 0.98	Primary chromatographic system: ~~0.28~~ 2.98
	Secondary chromatographic system: 1.71%	Secondary chromatographic system: 1.30%
Accuracy n = 5 (% Average recovery)	Primary chromatographic system: 102.4%	Primary chromatographic system: 88.7%
	Secondary chromatographic system: 100.9%	Secondary chromatographic system: 93.0%
Interference/ Specificity	Analysis showed no overlapping of determined ingredients signal with the signals of matrix components under method conditions, hence method specificity criterion is fulfilled.	
Comment	-	-

Conclusion

This method is specific because there were no overlapping peaks of determined ingredients signal with the signals of matrix components. Other parameters: linearity, precision, accuracy are: in the acceptance range and complies with EU requirements and SANCO/3030/99 rev.5 22/03/19.

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

According to EFSA Journal 2016;14(10):4584 Mesosulfuron-methyl hasn't any relevant impurities

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

Please go to the point 5.2.1.1.

5.2.1.4 Applicability of existing CIPAC methods (KCP 5.1.1)

Analytical methods for determination of Mesosulfuron-methyl and Mefenpyr-diethyl impurities and relevance of CIPAC methods in CHR/H/MEZO 30 OD were not evaluated as part of the EU review. Therefore, all relevant data are provided and are considered adequate

5.2.2 Methods for the determination of residues (KCP 5.1.2)

Available data

The following studies: Stuke S., Ballmann C, (2013), MR-13/007; Stuke S., (2015) MR-15/036; Schmeer K., Philipowski C., (2011) 01208/M001; Freitag T., (2013) M-310074-03-1; Reichert, N. (2009) IF-100/21283-00; Krebber R., Braune M., (2013) MR13/085; were presented in core assessment and latest supplements of registration report Part B, Section 5: Analytical Methods of Atlantis 12 OD revised in

03/2020.We are obliged to relied upon following studies taking account that according to Regulation (EC) No 1107/2009 Article 59 Data protection: The period of data protection is 30 months if study was necessary for the renewal or review of an authorisation. Product Atlantis 12 OD was renewed in 24.08.2020 under MRiRW decision R – 555/2020d and data presented was necessary for authorisation renewal. According to Official Journal of the European Union C 229/2 Period of protection is 30 months from date of first renewal of authorisation of product containing that active substance in each Member State where the data is necessary for the renewal of authorisation, therefore no new study was provided.

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 LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Plants, plant products,... (Residues)	Primary	0.01 mg/kg	LC-MS/MS	Stuke, S.; Ballmann, C, 2013
	Confirmatory (if required)	0.01 mg/kg	LC-MS/MS	Stuke, S.; 2015
Animal products, food of animal origin,... (Residues)	Primary	0.01 mg/kg	HPLC-MS/MS	Schmeer, K., Philipowski, C., 2011
	Confirmatory (if required)	Not required		
Soil, water, sediment,... (Environmental fate)	Primary	0.1 mg/kg	LC-MS/MS	Freitag, T.; 2013
	Confirmatory (if required)	Not required		
Air,... (Exposure)	Primary	12 µg/m^3	HPLC-UV	Reichert N., 2009
	Confirmatory (if required)	Not required		
Soil, water,... (Ecotoxicology)	Primary	0.1 µg/kg	LC/MS-MS	Freitag T.; 2013
	Confirmatory (if required)	Not required		
Water, buffer solutions,... (Properties)	Primary	0.05 µg/L	HPLC-MS/MS	Krebber R.; Braune M.; 2013
	Confirmatory (if required)	Not required		

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

Data which used to create Appendix 1 are sufficient for post-authorizations methods. All of these data are described in EU approved documents for :DAR, Mesosulfuron - Volume 3-Annex B.5 (AS)
Methods are described and presented in Table 5.2-3 in point KCP 5.1.2

5.3.1 Analysis of the plant protection product (KCP 5.2)

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

5.3.2 Description of analytical methods for the determination of residues of Mesosulfuron-methyl (KCP 5.2)

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

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

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

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Plant, high water content	mesosulfuron-methyl	0.01mg/kg	Reg. (EU) No 289/2014
Plant, high acid content		0.01mg/kg	Reg. (EU) No 289/2014
Plant, high protein/high starch content (dry commodities)		0.01mg/kg	Reg. (EU) No 289/2014
Plant, high oil content		0.01 mg/kg	Reg. (EU) No 289/2014
Plant, difficult matrices (hops, spices, tea)		0.05 mg/kg	Reg. (EU) No 289/2014
Muscle	mesosulfuron methyl	0.02 mg/kg	Reg. (EU) No 289/2014
Milk		0.02 mg/kg	Reg. (EU) No 289/2014
Eggs		0.02 mg/kg	Reg. (EU) No 289/2014
Fat		0.02 mg/kg	Reg. (EU) No 289/2014
Liver, kidney		0.02 mg/kg	Reg. (EU) No 289/2014
Soil (Ecotoxicology)	mesosulfuron methyl	0.1 µg/kg	common limit
Drinking water (Human toxicology)	mesosulfuron methyl	0.1 µg/L	general limit for drinking water
Surface water (Ecotoxicology)	mesosulfuron methyl	NOEC =1.8 mg a.s./L mm (nom)	EFSA Journal 2016;14(10):4584
Air	mesosulfuron methyl	12 µg/m^3	AOEL sys: 0.13 mg/kg bw/d
Tissue (meat or liver)	mesosulfuron methyl	0.01 mg/kg	notclassified as T / T+
Body fluids		According to EFSA Journal 2016;14(10):4584: Data required. Method is being developed and will be available at the end	notclassified as T / T+

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
		of 2016. For tissues, analytical methods (Schmeer, K., Philipowski, C., 2010 amended in 2011 and ILV Derek Netzband, 2010) have been validated for foodstuff of animal origin (muscle, liver, kidney).	

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

Available data

The following studies: Stuke S., Ballmann C, (2013), MR-13/007; Konrad S., (2013) 2013/0060/01; were presented in core assessment and latest supplements of registration report Part B, Section 5: Analytical Methods of Atlantis 12 OD revised in 03/2020.We are obliged to relied upon following studies taking account that according to Regulation (EC) No 1107/2009 Article 59 Data protection: The period of data protection is 30 months if study was necessary for the renewal or review of an authorisation. Product Atlantis 12 OD was renewed in 24.08.2020 under MRiRW decision R – 555/2020d and data presented was necessary for authorisation renewal. According to Official Journal of the European Union C 229/2 Period of protection is 30 months from date of first renewal of authorisation of product containing that active substance in each Member State where the data is necessary for the renewal of authorisation, therefore no new study was provided.

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

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

Component of residue definition: Mesosulfuron-methyl				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
High water content	Primary	0.01 mg/kg	LC-MS/MS	Stuke S.; Ballmann C, 2013
	ILV	0.01mg/kg	LC-MS/MS	Konrad S.; 2013
	Confirmatory (if required)	Not required		
High acid content	Primary	0.01 mg/kg	LC-MS/MS	Stuke S.; Ballmann C, 2013
	ILV	0.01 mg/kg	LC-MS/MS	Konrad, S.; 2013
	Confirmatory (if required)	Not required		

Component of residue definition: Mesosulfuron-methyl				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
High oil content	Primary	0.01 mg/kg	LC-MS/MS	Stuke S.; Ballmann C, 2013
	ILV	0.01 mg/kg	LC-MS/MS	Konrad S.; 2013
	Confirmatory (if required)	Not required		
High protein/high starch content (dry)	Primary	0.01 mg/kg	LC-MS/MS	Stuke S.; Ballmann C, 2013
	ILV	0.01 mg/kg	LC-MS/MS	Konrad S.; 2013
	Confirmatory (if required)	Not required		
Difficult (if required, depends on intended use)	Primary	Not required		
	ILV			
	Confirmatory (if required)			

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

Table 5.3-3: Statement on extraction efficiency

	Method for products of plant origin
Required, available from:	RAR MESOSULFURON METHYL, Volume 3 Annex B5 (AS)
Not required, because:	-

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

Available data

The following studies: Schmeer K., Philipowski C., (2011) 01208/M001, Netzband D., 2015 (RAMML014-01); were presented in core assessment and latest supplements of registration report Part B, Section 5: Analytical Methods of Atlantis 12 OD revised in 03/2020.We are obliged to relied upon following studies taking account that according to Regulation (EC) No 1107/2009 Article 59 Data protection: The period of data protection is 30 months if study was necessary for the renewal or review of an authorisation. Product Atlantis 12 OD was renewed in 24.08.2020 under MRiRW decision R – 555/2020d and data presented was necessary for authorisation renewal. According to Official Journal of the European Union C 229/2 Period of protection is 30 months from date of first renewal of authorisation of product containing that active substance in each Member State where the data is necessary for the renewal of authorisation, therefore no new study was provided.

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

Table 5.3-4: Validated methods for food and feed of animal origin (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**
Milk	Primary	0.01 mg/kg	LC-MS/MS	Schmeer K., Philipowski C., 2011
	ILV	0.01 mg/kg	LC-MS/MS	Netzband D., 2015
	Confirmatory (if required)	Not required		
Eggs	Primary	0.01mg/kg	LC-MS/MS	Schmeer, K., Philipowski, C., 2011
	ILV	0.01mg/kg	LC-MS/MS	Netzband, 2015
	Confirmatory (if required)	Not required		
Muscle	Primary	0.01 mg/kg	LC-MS/MS	Schmeer K., Philipowski C., 2011
	ILV	0.01 mg/kg	LC-MS/MS	Netzband D., 2015
	Confirmatory (if required)	Not required		
Fat	Primary	0.01 mg/kg	LC-MS/MS	Schmeer K., Philipowski C., 2011
	ILV	0.01 mg/kg	LC-MS/MS	Netzband D., 2015
	Confirmatory (if required)	Not required		
Kidney, liver	Primary	0.01 mg/kg	LC-MS/MS	Schmeer K., Philipowski C., 2011
	ILV	0.01mg/kg	LC-MS/MS	Netzband D., 2015
	Confirmatory (if required)	Not required		

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 5.3-5: Statement on extraction efficiency

	Method for products of animal origin
Required, available from:	RAR September 2016 MESOSULFURON METHYL, Volume 3 Annex B5 (AS)
Not required, because:	-

For the detailed evaluation of (additional) studies on extraction efficiency please refer to Appendix 2.

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

Available data:

The following study: Freitag T., (2013) M-310074-03-1 was presented in core assessment and latest supplements of registration report Part B, Section 5: Analytical Methods of Atlantis 12 OD revised in 03/2020.We are obliged to relied upon following studies taking account that according to Regulation (EC) No 1107/2009 Article 59 Data protection: The period of data protection is 30 months if study was necessary for the renewal or review of an authorisation. Product Atlantis 12 OD was renewed in 24.08.2020 under MRiRW decision R – 555/2020d and data presented was necessary for authorisation renewal. According to Official Journal of the European Union C 229/2 Period of protection is 30 months from date of first renewal of authorisation of product containing that active substance in each Member State where the data is necessary for the renewal of authorisation, therefore no new study was provided.

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

Table 5.3-6: 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	0.1 µg/kg	LC-MS/MS	Freitag T.; 2013
Confirmatory	Not required		

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)

Available data

The following studies: Krebber R., Braune M., (2013) MR13/085; Stanislowski, T. (2013) P3117 G were presented in core assessment and latest supplements of registration report Part B, Section 5: Analytical Methods of Atlantis 12 OD revised in 03/2020.We are obliged to relied upon following studies taking account that according to Regulation (EC) No 1107/2009 Article 59 Data protection: The period of data protection is 30 months if study was necessary for the renewal or review of an authorisation. Product Atlantis 12 OD was renewed in 24.08.2020 under MRiRW decision R – 555/2020d and data presented was necessary for authorisation renewal. According to Official Journal of the European Union C 229/2 Period of protection is 30 months from date of first renewal of authorisation of product containing that active substance in each Member State where the data is necessary for the renewal of authorisation, therefore no new study was provided.

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 5.3-7: 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	0.05 µg/L	LC-MS/MS	Krebber R.; Braune M.; 2013
	ILV	0.05 µg/L	LC-MS/MS	Stanislowski, T.; 2013
	Confirmatory	Not required		
Surface water	Primary	0.05 µg/L	LC-MS/MS	Krebber R.; Braune M.; 2013
	Confirmatory	Not required		

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

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

Available data

The following study; Reichert, N. (2009) IF-100/21283-00; was presented in core assessment and latest supplements of registration report Part B, Section 5: Analytical Methods of Atlantis 12 OD re-vised in 03/2020.We are obliged to relied upon following studies taking account that according to Regulation (EC) No 1107/2009 Article 59 Data protection: The period of data protection is 30 months if study was necessary for the renewal or review of an authorisation. Product Atlantis 12 OD was renewed in 24.08.2020 under MRiRW decision R – 555/2020d and data presented was necessary for authorisation renewal. According to Official Journal of the European Union C 229/2 Period of protection is 30 months from date of first renewal of authorisation of product containing that active substance in each Member State where the data is necessary for the renewal of authorisation, therefore no new study was provided.

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

Table 5.3-8: 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	12 µg/m^3	HPLC-UV	Reichert N., 2009
Confirmatory	Not required		

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)

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.

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

Available data

The following studies: Schmeer K., Philipowski C., (2011) 01208/M001, were presented in core assessment and latest supplements of registration report Part B, Section 5: Analytical Methods of Atlantis 12 OD revised in 03/2020.We are obliged to relied upon following studies taking account that according to Regulation (EC) No 1107/2009 Article 59 Data protection: The period of data protection is 30 months if study was necessary for the renewal or review of an authorisation. Product Atlantis 12 OD was renewed in 24.08.2020 under MRiRW decision R – 555/2020d and data presented was necessary for authorisation renewal. According to Official Journal of the European Union C 229/2 Period of protection is 30 months from date of first renewal of authorisation of product containing that active substance in each Member State where the data is necessary for the renewal of authorisation, therefore no new study was provided.

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

According to EFSA Journal 2016;14(10):4584: Data required. Method is being developed and will be available at the end of 2016. For tissues, analytical methods (Schmeer, K., Philipowski, C., 2010 amended in 2011 and ILV Derek Netzband, 2010) have been validated for foodstuff of animal origin (muscle, liver, kidney).

Component of residue definition: Mesosulfuron-methyl			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	0.01 mg/kg	HPLC-MS/MS	Schmeer K., Philipowski C., 2011- for tissue
Confirmatory	Not required		

5.3.2.8 Other studies/ information (KCP 5.3)

- **Method of validation which was used in ecotoxicological study of bees**

Autor	Fulczyk A.
Title of the study in which validation method was used	Mesosulfuron 30 OD [CHR/H/MEZO 30 OD] Honeybees (*Apis mellifera* L.), Chronic Oral Toxicity Test
Study code	B-71-20
Guidelines:	OECD Guideline No. 245 (2017)
GLP	Yes
Deviations	No
Acceptability	
Duplications (if vertebrate study):	No
Validation method	HPLC with DAD
Detected substances	Mesosulfuron-methyl Mefenpyr-diethyl

For detailed information about this methods please go to the point of Appendix 2

- **Method of validation which was used in ecotoxicological study of honey bee larval.**

Autor	Woźniak A.
Title of the study in which validation method was used	Honey bee larval toxicity test following repeated exposure of the test item Mesosulfuron 30 OD (CHR/H/MEZO 30 OD)
Study code	0038/0104/E
Guidelines:	OECD GD 239 ENV/JM/MONO(2016)34
GLP	Yes
Deviations	Yes
Acceptability	
Duplications (if vertebrate study):	No
Validation method	HPLC with UV-DAD
Detected substances	Mesosulfuron-methyl Mefenpyr-diethyl

For detailed information about this methods please go to the point of Appendix 2

- **Method of validation which was used in ecotoxicological study of aquatic organism *Daphnia magna***

Autor	Brzozowska-Wojoczek K.
Title of the study in which validation method was used	Mesosulfuron 30 OD [CHR/H/MEZO 30 OD] *Daphnia magna*, Acute Immobilisation Test
Study code	W-61-20
Guidelines:	OECD Guideline No. 202 (2004)/EU method C.2.
GLP	Yes
Deviations	No
Acceptability	
Duplications (if vertebrate study):	No
Validation method	HPLC with DAD
Detected substances	Mesosulfuron-methyl Mefenpyr-diethyl

For detailed information about this methods please go to the point of Appendix 2

- **Method of validation which was used in ecotoxicological study of aquatic organism *Lemma gibba***

Autor	Brzozowska-Wojoczek K.
Title of the study in which validation method was used	Mesosulfuron 30 OD [CHR/H/MEZO 30 OD] *Lemna gibba* CPCC 310, Growth inhibition test
Study code	W-62-20
Guidelines:	OECD Guideline No. 221 (2006)/ EU Method C.26.
GLP	Yes
Deviations	No
Acceptability	
Duplications (if vertebrate study):	No
Validation method	HPLC with DAD
Detected substances	Mesosulfuron-methyl Mefenpyr-diethyl

For detailed information about this methods please go to the point of Appendix 2

- **Method of validation which was used in ecotoxicological study of aquatic organism *Anabaena flos-aquae* UTEX B 1444**

Autor	Brzozowska-Wojoczek K.
Title of the study in which validation method was used	Mesosulfuron 30 OD [CHR/H/MEZO 30 OD] *Anabaena flos-aquae* UTEX B 1444 Growth inhibition test
Study code	W-64-20
Guidelines:	OECD Guideline No. 201 (2006)/EU method C.3.
GLP	Yes
Deviations	No
Acceptability	
Duplications (if vertebrate study):	No
Validation method	HPLC with DAD
Detected substances	Mesosulfuron-methyl Mefenpyr-diethyl

For detailed information about this methods please go to the point of Appendix 2

- **Method of validation which was used in ecotoxicological study of Collembolan (*Folsomia candida*)**

Autor	Pieczka P.
Title of the study in which validation method was used	Mesosulfuron 30 OD (CHR/H/MEZO 30 OD) Collembolan (*Folsomia candida*) Reproduction Test
Study code	Study code: G-46-20
Guidelines:	OECD Guideline No. 232 (2016)
GLP	Yes
Deviations	Yes
Acceptability	
Duplications (if vertebrate study):	No
Validation method	HPLC with DAD
Detected substances	Mesosulfuron-methyl Mefenpyr-diethyl

For detailed information about this methods please go to the point of Appendix 2

- **Method of validation which was used in ecotoxicological study of (*Hypoaspis (Geolaelaps) aculeifer*)**

Autor	Wróbel A
Title of the study in which validation method was used	Mesosulfuron 30 OD (CHR/H/MEZO 30 OD) Predatory mite (*Hypoaspis (Geolaelaps) aculeifer*) reproduction test in soil
Study code	Study code: G-47-20
Guidelines:	OECD Guideline No. 226 (2016)
GLP	Yes
Deviations	Yes
Acceptability	
Duplications (if vertebrate study):	No
Validation method	HPLC with DAD
Detected substances	Mesosulfuron-methyl Mefenpyr-diethyl

For detailed information about this methods please go to the point of Appendix 2

- **Method of validation which was used in ecotoxicological study of Terrestial Plant Test: Vegetative Vigour Test**

Autor	Gierbuszewska A.
Title of the study in which validation method was used	Mesosulfuron 30 OD (CHR/H/MEZO 30 OD) Terrestial Plant Test: Vegetative Vigour Test
Study code	G-49-20
Guidelines:	OECD Guideline No. 227 (2006)
GLP	Yes
Deviations	Yes
Acceptability	
Duplications (if vertebrate study):	No
Validation method	HPLC with DAD
Detected substances	Mesosulfuron-methyl Mefenpyr-diethyl

For detailed information about this methods please go to the point of Appendix 2

Appendix 1 Lists of data considered in support of the evaluation

Tables considered not relevant can be deleted as appropriate.

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

List of data submitted by the applicant and relied on

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Verte-brate study Y/N**	Owner
KCP 5.1.1	Knapik I..	2023	Validation of analytical method for Mesosulfuron 30 OD (CHR/H/MEZO 30 OD) for determination of mesosulfuron-methyl and mefenpyr-diethyl ICB Pharma, Jaworzno, Poland Study code: ICB/79/2022 GLP: yes unpublished	N	PUH Chemirol
KCP 5.3	Fulczyk A.,	2023	Mesosulfuron 30 OD (CHR/H/MEZO 30 OD) Honeybees *(Apis mellifera* L.), Chronic Oral Toxicity Test Study code B-71-20 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna Ecotoxicology Research Group GLP Unpublished	N	PUH „Chemirol” Sp. z o. o.
KCP 5.3	Woźniak A.	2023	Honey bee larval toxicity test following repeated exposure of the test item Mesosulfuron 30 OD (CHR/H/MEZO 30 OD) Study code 0038/0104/E	N	PUH „Chemirol” Sp. z o. o.

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Verte-brate study Y/N**	Owner
			SORBOLAB Research Laboratory LLC GLP: yes unpublished		
KCP 5.3	Brzozowska-Wojoczek K.	2023	Mesosulfuron 30 OD [CHR/H/MEZO 30 OD] *Daphnia magna*, Acute Immobilisation Test Study code W-61-20 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna Ecotoxicology Research Group GLP Unpublished	N	PUH „Chemirol” Sp. z o. o.
KCP 5.3	Brzozowska-Wojoczek K.	2023	Mesosulfuron 30 OD [CHR/H/MEZO 30 OD] *Lemna gibba* CPCC 310, Growth inhibition test Study code W-62-20 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna Ecotoxicology Research Group GLP Unpublished	N	PUH „Chemirol” Sp. z o. o.
KCP 5.3	Brzozowska-Wojoczek K.	2023	*Anabaena flos-aquae* UTEX B 1444 Growth inhibition test Study code W-64-20 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna Ecotoxicology Research Group GLP Unpublished	N	PUH „Chemirol” Sp. z o. o.
KCP 5.3	Pieczka P.	2023	Mesosulfuron 30 OD (CHR/H/MEZO 30 OD) Collembolan (*Folsomia candida*) Reproduction Test Study code: G-46-20 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna Ecotoxicology Research Group GLP Unpublished	N	PUH „Chemirol” Sp. z o. o.
KCP 5.3	Wróbel A.	2023	Mesosulfuron 30 OD (CHR/H/MEZO 30 OD) Predatory mite (*Hypoaspis (Geolaelaps) aculeifer*)	N	PUH

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Verte-brate study Y/N**	**Owner**
			reproduction test in soil Study code: G-47-20 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna Ecotoxicology Research Group GLP: yes unpublished		„Chemirol” Sp. z o. o.
KCP 5.3	Gierbuszewska A.	2023	Mesosulfuron 30 OD (CHR/H/MEZO 30 OD) Terrestial Plant Test: Vegetative Vigour Test Study code: G-49-20 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna Ecotoxicology Research Group GLP: yes unpublished	N	PUH „Chemirol” Sp. z o. o.

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

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Vertebrate study Y/N**	**Owner**
KCP 5.1.2 KCP 5.2	Stuke S.; Ballmann C.	2013	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 No.: MR-13/007 GLP yes, unpublished	N	Bayer CropSci ence
KCP 5.1.2	Stuke S.	2015	Cross validation of enforcement method 01360 for the determination of sulfonylureas	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
			vs. extraction procedure applied in 14C-metabolism studies using incurred residues in plant matrices analysed by HPLC-MS/MS Report No.: MR-15/036 GLP yes, unpublished		CropScience
KCP 5.1.2 KCP 5.2	Schmeer K., Philipowski C.	2011	Modification M001 of the residue analytical method 01208 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 No.: 01208/M001, Date: 2010-09-06 ...**Amended: 2011-01-03** GLP yes, unpublished	N	Bayer CropScience
KCP 5.1.2 KCP 5.2	Freitag T.,	2013	Amendment no. 0001 to report no.: MR-08/138 - Analytical Method 01115 for the determination of residues of amidosulfuron,iodosulfuro-methyl-sodium, metsulfuron-methyl, mesosulfuron-methyl and foramsulfuron in soil by HPLC-MS/MS Report No.: M-310074-03-1, Date: 2008-10-27 **...Amended: 2013-08-08** GLP: yes, unpublished	N	Bayer CropScience
KCP 5.1.2 KCP 5.2	Reichert N.	2009	Development and validation of an analytical method for the determination of AE F130060 in air Institut Fresenius Chem.und Biolog. Lab. AG, Taunusstein, Germany Report No.: IF-100/21283-00, Date: 2000-11-22 ...**Amended: 2009-06-19** GLP: yes,	N	Bayer CropScience

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			Unpublished		
KCP 5.1.2 KCP 5.2	Krebber R.; Braune M.	2013	Analytical method 01387 for the determination of various pesticides in drinking and surface water by HPLC-MS/MS Report No.: MR-13/085, GLP: yes, unpublished	N	Bayer CropScience
KCP 5.2	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 Report No.: 2013/0060/01 Currenta GmbH & Co. OHG, Leverkusen, Germany GLP: yes, unpublished	N	Bayer CropScience
KCP 5.2	Netzband D.	2015	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 No.: RAMML014-01 Date: 2010-12-22 ..**Amended: 2015-11-03** Bayer CropScience LP, Stilwell, KS, USA GLP yes unpublished	N	Bayer CropScience
KCP 5.2	Stanislowski 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 Report No: P3117 G, PTRL Europe, Ulm, Germany,	N	Bayer CropScience

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
			GLP: yes Unpublished		

The following tables are to be completed by MS

List of data submitted by the applicant and not relied on

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

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

Appendix 2 Detailed evaluation of submitted analytical methods

A 2.1 Analytical methods for Mesosulfuron-methyl

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

No new or additional studies have been submitted

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

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

Not required.

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

A 2.1.2.7 Other Studies/ Information (KCP 5.3)

A 2.1.2.7.1 Methods of validation of analytical method for mesosulfuron-methyl and mefenpyr-diethyl (KCP 5.3)

A 2.1.2.7.1.1 Method of validation which was used in ecotoxicological study of bees

Comments of zRMS:	Described method validation B-71-20 for the determination of mesosulfuron-methyl and mefenpyr-diethyl in sucrose solution was validated in accordance with SANTE/2020/12830, Rev. 1 and GLP. The method is acceptable and suitable for the determination of the active substance.

Reference:	KCP 5.3
Report	Mesosulfuron 30 OD (CHR/H/MEZO 30 OD) Honeybees (*Apis mellifera* L.), Chronic Oral Toxicity Test, Fulczyk A., 2023, Study code: B-71-20
Guideline(s):	OECD Guideline No. 245 (2017); SANTE/2020/12830, Rev. 1
Deviations:	No
GLP:	Yes
Acceptability:	
Duplication (if vertebrate study)	No

The analytical method was developed for the determination of mesosulfuron-methyl and mefenpyr-diethyl in sucrose solution. The range of linearity of the analytical graph, the regression residual (di), selectivity and specificity, precision, matrix effect, accuracy, stability stock solution, limit of quantification and detection were determined. The determination was accomplished by the high performance liquid chromatography (HPLC) with DAD detection. The validated analytical method was performed according to SANTE/2020/12830, Rev. 1 and Standard Operating Procedure SOP/C/9.

The concentrations of active substances of test item were chemically determined using the validated high performance liquid chromatographic method with DAD detection Test item concentration of 666.7 mg/kg and the control were analysed.

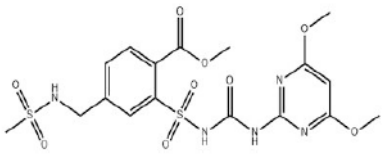
1. Detected substance

Standard of mesosulfuron-methyl from Sigma-Aldrich was used to method validation.
Standard of mefenpyr-diethyl from Sigma-Aldrich was used to method validation.

Mesosulfuron-methyl

IUPAC Name:	methyl2-[(4,6-dimethoxypyrimidin-2-ylcarbamoyl)sulfamoyl]-a-(methanesulfonamido)-p-toluate
Molecular Formula:	$C_{17}H_{21}N_5O_9S_2$
CAS No.:	208465-21-8
Purity:	98.4%
Series number:	BCBV9244
Expiry date:	October 2022
Manufacturing:	Sigma-Aldrich
Molecular weight:	503.51 g/Mol

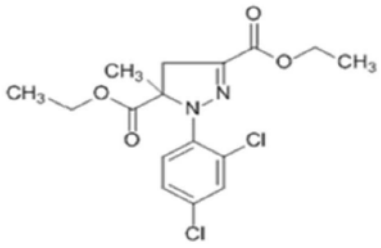
Structural formula:



Mefenpyr-diethyl

IUPAC Name:	diethyl(RS)-1-(2,4-dichlorophenyl)-4,5-dihydro-5-methyl-1H-pyrazole-3,5-dicarboxylate
Molecular Formula:	$C_{16}H_{18}Cl_2N_2O_4$
CAS No.:	135590-91-9
Purity:	98.8%
Series number:	BCBX4666
Expiry date:	May 2023
Manufacturing:	Sigma-Aldrich
Molecular weight:	373.23 g/Mol

Structural formula:



2. Method validation

Tabl. 1 Parameters of the validation

<table>
<tr><th colspan="2">Linearity</th></tr>
<tr><td>Range of the calibration curve</td><td>0.2 mg/L to 20.0 mg/kg in sucrose solution</td></tr>
<tr><td>Tested working solutions</td><td>Mesosulfuron-methyl, Mefenpyr diethyl</td></tr>
<tr><td>Concentrations of working solutions</td><td>0.2, 0.5, 1.0, 2.0, 5.0, 10, 20 µg/mL</td></tr>
<tr><td>Linear equation</td><td>y = ax + b
(a – slope, b - intercept)
Range of the
linear was given in µg/mL equivalent to mg/L.

<table>
<tr><th>Analyte</th><th>Slope</th><th>Intercept</th><th>Coefficient</th></tr>
<tr><td>Mesosulfuron-methyl</td><td>88571.7</td><td>1360.71</td><td>0.9995577</td></tr>
<tr><td>Mefenpyr-diethyl</td><td>85661.2</td><td>-268.789</td><td>0.9999925</td></tr>
</table>
</td></tr>
<tr><th colspan="2">Selectivity and specificity</th></tr>
</table>

Tested samples	Controlled matrix, fortified matrix
Method	Analysis of the chromatograms
results	No signal of detected substance were overlapping with matrix signal of the control samples in the experimental conditions

Precision

Repeatability for detected substance in sucrose solutions	Mesosulfuron-methyl: 4.2% - 4.2% Mefenpyr-diethyl: 1.2% - 2.8%
RSD – relative standard deviation [%]	≤ 20% per each level

Accurancy

Mean recovery ± relative standard deviation	70 – 120%.

Recovery

Matrix	**Detected substance**	**Fortification Level [mg/kg]**	**Number of Replicate**	**Mean Recovery [%]**	**RSD [%]**
Sucrose solution	Mesosulfuron-methyl	0.5	5	99.4	4.2
		5	5	93.7	4.2
	Mefenpyr-diethyl	0.5	5	113.2	1.2
		5.0	5	96.3	2.8

Matrix effect

$$\text{Matrix effects [\%]} = 100 \times \frac{\text{peak area (matrix)}}{\text{peak area (solvent)}} - 100$$

The matrix effect is not exceed ± 20%.

Matrix	**Detected substance**	**Concentration mg/L**	**Matrix effect [%]**
Sucrose solution	Mesosulfuron-methyl	0.5	-6.1
	Mefenpyr-diethyl	0.5	3.2

Limit of Quantification and Limit of Detection

LOQ	**Equivalent calibration level**	**LOD**	**Equivalent calibration level**
0.5 mg mesosulfuron-methyl/kg	0.5 µg/ml	0.2 mg mesosulfuron-methyl/kg	0.2 µg/ml
0.5 mg mefenpyr-diethyl	0.5 µg/ml	0.2 mg mefenpyr-diethyl/kg	0.2 µg/ml

3. Calculation

The concentration (P) of mesosulfuron-methyl and mefenpyr-diethyl in sucrose solution for fortified and test samples was calculated as follows:

$$P(mg/kg)^* = c \cdot Vk / mp * w$$

where:

- c is the concentration of the detected compound in the final solution determined on the basis of the calibration curve (µg/mL)
- V_k is the final volume of the solution (mL)
- m_p is the weight of the sample (g)
- w is a conversion factor (w/w= 1000/1000=1)

Example of Calculation – fortified samples

The concentration (P) [mg/kg] of mefenpyr-diethyl in fortified sample 5.0 mg/kg was calculated as follow:
The following values were used in this calculation:

Lab sample description	**sucrose 5 mg-kg validation method_013**
concentration (c) [mg/L]	4.8071
final volume of the solution (V_k) [mL]	1
weight of the sample (m_p) [g]	1
conversion factor (w)	1000/1000=1

Concentration was calculated as follow:

$$P(mg/kg)^* = c \cdot V_k / m_p * w$$

$$P=4.8071*1/1*1=4.807 \text{ mg/kg}$$

Example of Calculation – test samples

The concentration (P) [mg/kg] of mefenpyr-diethyl in test sample 666.7 mg/kg was calculated as follow:
The following values were used in this calculation:

Lab sample description	**666.7 mg-kg day 0_Definitive test B-71-20_005**
concentration (c) [mg/L]	6.0541
final volume of the solution (V_k) [mL]	10
weight of the sample (m_p) [g]	1
conversion factor (w)	1

Concentration was calculated as follow:

$$P(mg/kg)^* = c \cdot V_k / m_p * w$$

$$P=6.0541*10/1*1=60.541 \text{ mg/kg}$$

**Numerical values in this report are frequently rounded to a smaller degree of precision (number of digits) than were used in the actual calculation to increase readability and to indicate the approximate precision of the reported results. Minor differences in the results obtained with such "rounded" values in comparison to those obtained with higher precision values are well within the limits of the experimental accuracy and therefore of no practical concern.*

A 2.1.2.7.1.2 Method of validation which was used in ecotoxicological study of Honey bee larval

Comments of zRMS:	Described method validation 0038/0104/E for the determination of mesosulfuron-methyl and mefenpyr-diethyl in deionized water was validated in accordance with SANTE/2020/12830, Rev. 1 and GLP. The method is acceptable and suitable for the determination of the active substance.

Reference:	KCP 5.3
Report	Honey bee larval toxicity test following repeated exposure of the test item Mesosulfuron 30 OD (CHR/H/MEZO 30 OD), Woźniak A., 2023, Study code: 0038/0104/E
Guideline(s):	OECD GD 239 ENV/JM/MONO(2016)34; SANTE/2020/12830, rev.1
Deviations:	Yes *Deviations from Guideline / Standard Experimental Procedure / Study plan* During the, range-finding, definitive and reference test changes in temperature and humidity took place. They resulted from everyday activities and observations and were recorded and corrected on an ongoing basis. These were short-term changes which did not affect the condition of the test system. The above deviations did not affect the test result. The study met the validity criteria.
GLP:	Yes
Acceptability:	
Duplication (if vertebrate study)	No

Determination of active substance (mesosulfuron-methyl) and safener (mefenpyr diethyl) of the test item in deionized water will be performed by high performance liquid chromatography with UV-DAD detection according to experimental procedure. During the validations of the analytical methods the following parameters: selectivity, matrix effects, linearity, accuracy, precision (repeatability), limit of detection and limit of quantification were determined.

1.Detected substance

Mesosulfuron-methyl standard (HPC Standards, lot number 794926)

Mefenpyr-diethyl standard (SUPELCO, lot number BCCG1824)

2. Method validation

Tabl. 1: Analytical method validation results

Parameter	Required criterion	The result					
		Mesosulfuron-methyl			Mefenpyr-diethyl		
Selectivity	At the place originating from active substance signal, there is no signals originating from other substances of area exceeding 30% of active substance area in the test item solution at the level (LOQ). The UV spectrum of active substance in the standard solution and the test item solution is comparable.	At the place originating from active substance signal, there is no signals originating from other substances of area exceeding 30% of active substance area in the test item solution at the level (LOQ). the UV spectrum of active substance in the standard solution and the test item solution is comparable.			At the place originating from active substance signal, there is no signals originating from other substances of area exceeding 30% of active substance area in the test item solution at the level (LOQ). the UV spectrum of active substance in the standard solution and the test item solution is comparable.		
Matrix effects [%]	±20	3.04			-1.04		
Linearity	r ≥ 0.99 Random distribution of regression residuals	r = 0.999 (0.219 mg/L – 14.004 mg/L) A random distribution of regression residues was obtained			r = 0.999 (0.731 mg/L – 46.791 mg/L) A random distribution of regression residues was obtained		
Accuracy [%]	70-120	level I*	116.84	108.05	level I**	102.80	100.83
		level II*	99.26		level II**	98.85	
Precision [% RSD]	≤20	level I***	0.350		level I****	0.600	
		level II***	0.100		level II****	0.150	
Limit of detection [mg/L]	≤0.219 (Mesosulfuron-methyl) ≤0.733 (Mefenpyr-diethyl)	0.219			0.731		
Limit of quantification [mg/L]	-	0.730			2.444		

* Nominal concentration: level I = 0.730 mg/L, level II = 7.297 mg/L.
** Nominal concentration: level I = 2.444 mg/L, level II = 24.439 mg/L.
*** Mean determined concentration: level I = 0.853 mg/L, level II = 7.243 mg/L.
**** Mean determined concentration: level I = 2.512 mg/L, level II = 24.157 mg/L.

A 2.1.2.7.1.3 Method of validation which was used in ecotoxicological study of aquatic organism *Daphnia magna*

Comments of zRMS:	Described method validation W-61-20 for the determination of mesosulfuron-methyl and mefenpyr-diethyl in medium was validated in accordance with SANTE/2020/12830, Rev. 1 and GLP. The method is acceptable and suitable for the determination of the concentrations of mesosulfuron-methyl and mefenphyr-dietyl.

Reference:	KCP 5.3
Report	Mesosulfuron 30 OD [CHR/H/MEZO 30 OD] *Daphnia magna*, Acute Immobilisation Test, Brzozowska-Wojoczek K. Study code: W-61-20,
Guideline(s):	OECD Guideline No. 202 (2004)/EU method C.2.; SANTE/2020/12830, Rev. 1
Deviations:	No
GLP:	Yes
Acceptability:	
Duplication (if vertebrate study)	No

The concentration of active substance of test item was chemically determined using the validated high performance liquid chromatographic method with DAD detection. The analytical method was developed for the determination of active substance of test item in matrix. The range of linearity of the analytical graph, the regression residual (di), selectivity and specificity, precision, matrix effect, accuracy, and limit of quantification and detection were determined.
The determination was accomplished by the high performance liquid chromatography (HPLC) with DAD detection. The validated analytical method was performed according to SANTE/2020/12830, Rev. 1 and Standard Operating Procedure.

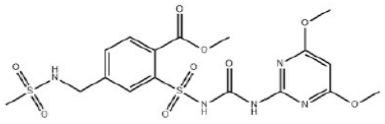
1. Detected substances

Standard of mesosulfuron-methyl from Sigma-Aldrich was used to method validation. Standard of mefenpyr-diethyl from Sigma-Aldrich was used to method validation.

Mesosulfuron-methyl

IUPAC Name:	methyl2-[(4,6-dimethoxypyrimidin-2-ylcarbamoyl)sulfamoyl]-a-(methanesulfonamido)-p-toluate
Molecular Formula:	$C_{17}H_{21}N_5O_9S_2$
CAS No.:	208465-21-8
Purity:	98.4%
Series number:	BCBV9244
Expiry date:	October 2022
Manufacturing:	Sigma-Aldrich
Molecular weight:	503.51 g/Mol

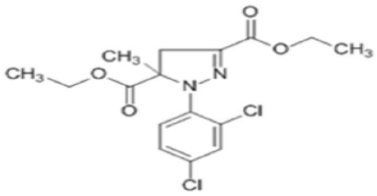
Structural formula:



Mefenpyr-diethyl

IUPAC Name:	diethyl(RS)-1-(2,4-dichlorophenyl)-4,5-dihydro-5-methyl-1H-pyrazole-3,5-dicarboxylate
Molecular Formula:	$C_{16}H_{18}Cl_2N_2O_4$
CAS No.:	135590-91-9
Purity:	98.8%
Series number:	BCBX4666
Expiry date:	May 2023
Manufacturing:	Sigma-Aldrich
Molecular weight:	373.23 g/Mol

Structural formula:



2. Method validation

Tabl 1. Parameters of the validation

<table>
<tr><th colspan="2">Linearity</th></tr>
<tr><td>Calibration curve</td><td>0.02 mg/L to 1.0 mg/L.</td></tr>
<tr><td>Range of the calibration curve</td><td>
<table>
<tr><th colspan="3">Dilution method</th></tr>
<tr><th>Analyte</th><th>range of linearity of calibration curve [mg/L]</th><th>equivalent calibration range of linearity [mg/L Elendt M7 medium]</th></tr>
<tr><td>Mesosulfuron-methyl</td><td>0.02-1.0</td><td>0.04-2.0</td></tr>
<tr><td>Mefenpyr-diethyl</td><td>0.02-1.0</td><td>0.04-2.0</td></tr>
<tr><td></td><td></td><td></td></tr>
</table>
<table>
<tr><th colspan="3">Method SPE</th></tr>
<tr><th>Analyte</th><th>range of linearity of calibration curve [mg/L]</th><th>equivalent calibration range of linearity [mg/L Elendt M7 medium]</th></tr>
<tr><td>Mesosulfuron-methyl</td><td>0.02-1.0</td><td>0.002-1.0</td></tr>
<tr><td>Mefenpyr-diethyl</td><td>0.02-1.0</td><td>0.002-1.0</td></tr>
</table>
</td></tr>
<tr><td>Tested working solutions</td><td>Mesosulfuron-methyl, Mefenpyr diethyl</td></tr>
</table>

<table>
<tr><td>Concentrations of working solutions</td><td>0.02, 0.05, 0.1, 0.2, 0.5, 1.0 µg/mL</td></tr>
<tr><td>Linear equation</td><td>y = ax + b (a – slope, b - intercept)
The linear coefficient r2 must be higher than 0.99.
Range of the linear was given in µg/mL equivalent to mg/L.

<table><tr><th>Analyte</th><th>Slope</th><th>Intercept</th><th>Coefficient</th></tr><tr><td>Mesosulfuron-methyl</td><td>87247.1</td><td>-161.142</td><td>0.9996951</td></tr><tr><td>Mefenpyr-diethyl</td><td>86283.7</td><td>-14.6681</td><td>0.9998535</td></tr></table></td></tr>
<tr><td>Calibration curve</td><td>0.2 mg/L to 20.0 mg/L.</td></tr>
<tr><td>Range of the calibration curve</td><td><table><tr><th colspan="3">Dilution method</th></tr><tr><th>Analyte</th><th>range of linearity of calibration curve [mg/L]</th><th>equivalent calibration range of linearity [mg/L Elendt M7 medium]</th></tr><tr><td>Mesosulfuron-methyl</td><td>0.2-20</td><td>0.4-40</td></tr><tr><td>Mefenpyr-diethyl</td><td>0.2-20</td><td>0.4-40</td></tr><tr><td></td><td></td><td></td></tr></table>
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<tr><td>Tested working solutions</td><td>Mesosulfuron-methyl, Mefenpyr diethyl</td></tr>
<tr><td>Concentrations of working solutions</td><td>0.02, 0.05, 0.1, 0.2, 0.5, 1.0 µg/mL</td></tr>
<tr><td>Linear equation</td><td>y = ax + b (a – slope, b - intercept)
The linear coefficient r2 must be higher than 0.99.
Range of the linear was given in µg/mL equivalent to mg/L.

<table><tr><th>Analyte</th><th>Slope</th><th>Intercept</th><th>Coefficient</th></tr><tr><td>Mesosulfuron-methyl</td><td>88571.7</td><td>1360.71</td><td>0.9995577</td></tr><tr><td>Mefenpyr-diethyl</td><td>85661.2</td><td>-268.789</td><td>0.9999925</td></tr></table></td></tr>
<tr><td colspan="2"><u>Selectivity and specificity</u></td></tr>
<tr><td>Tested samples</td><td>Controlled matrix, fortified matrix</td></tr>
<tr><td>Method</td><td>Analysis of the chromatograms</td></tr>
<tr><td>Results</td><td>No signal of detected substances were overlapping with matrix signal of the control samples in the experimental conditions</td></tr>
<tr><td colspan="2"><u>Precision</u></td></tr>
<tr><td>Repeatability for detected substance</td><td>repeatability for detected substances analyzed in Elendt M7 medium are presented in the table which is in part Accurancy</td></tr>
</table>

RSD – relative standard deviation [%]	≤ 20% per each level
Accurancy	
Mean recovery ± relative standard deviation	70 – 120%.
Recovery	

Matrix	**Detected substance**	**Fortification Level [mg/kg]**	**Number of Replicate**	**Mean Recovery [%]**	**RSD [%]**
Dilution method					
Elendt M7 medium	Mesosulfuron-methyl	0.1	5	118.0	7.6
		1.0	5	102.1	0.2
	Mefenpyr-diethyl	0.1	5	102.0	1.0
		1.0	5	103.8	0.2
Method SPE					
Elendt M7 medium	Mesosulfuron-methyl	0.01	5	99.0	1.0
		0.1	5	101.0	0.2
	Mefenpyr-diethyl	0.01	5	94.0	1.1
		0.1	5	98.2	0.1

Matrix effects

$$\text{Matrix effects [\%]} = 100 \times \frac{\text{peak area (matrix)}}{\text{peak area (solvent)}} - 100$$

The matrix effect is not exceed ± 20%.

Dilution method			
Matrix	**Detected substance**	**Concentration mg/L**	**Matrix effect [%]**
Elendt M7 medium	Mesosulfuron-methyl	0.05	11.8
	Mefenpyr-diethyl	0.05	3.0
Method SPE			
Matrix	**Detected substance**	**Concentration mg/L**	**Matrix effect [%]**
Elendt M7 medium	Mesosulfuron-methyl	0.1	1.3
	Mefenpyr-diethyl	0.1	0.9

Limit of Quantification (LOQ) and Limit of Detection (LOD)

LOQ	Equivalent calibration level	LOD	Equivalent calibration level
Dilution method			
0.1 mg mesosulfuronme-thyl/kg	0.05 µg/ml	0.04 mg mesosulfuron-methyl/kg	0.02 µg/ml
0.1 mg mefenpyr-diethyl	0.05 µg/ml	0.04 mg mefenpyr-di-ethyl/kg	0.02 µg/ml
Method SPE			
0.01 mg mesosulfuronme-thyl/kg	0.1 µg/ml	0.002 mg mesosulfuron-methyl/kg	0.02 µg/ml
0.01 mg mefenpyr-diethyl	0.1 µg/ml	0.002 mg mefenpyr-di-ethyl/kg	0.02 µg/ml

Stock solution stability

Mesosulfuron-methyl				
Days of storage	**Average con-scentration [mg/L]**	**RSD [%]**	**Recovery [%]**	**Decrease in relation to the initial concentration [%]**
0	996.8	0.1	99.7	-
1	973.7	0.1	97.4	2.3
6	1006.1	0.1	100.6	-0.9
20	1087.2	0.1	108.7	-9.1
32	1041.5	0.1	104.2	-4.5
47	1083.1	0.1	108.3	-8.7
109	1040.9	0.1	104.1	-4.4

Mefenpyr diethyl				
Days of storage	**Average con-scentration [mg/L]**	**RSD [%]**	**Recovery [%]**	**Decrease in relation to the initial concentration [%]**
0	1000.4	0.1	100.0	-
1	1004.5	0.1	100.5	-0.4
6	1005.7	0.0	100.6	-0.5
20	1092.4	0.1	109.2	-9.2
32	1038.0	0.1	103.8	-3.8
47	1074.6	0.1	107.5	-7.4
109	1097.6	0.7	109.8	-9.4

3.*Calculation*

The concentration (P) [mg/L] of active substances for fortified samples and test samples were calculated as follows:

$$P = c \cdot \frac{V_k}{V_p} \cdot w$$

where:
c is the concentration of the detected compound in the final solution determined on the basis of the calibration curve (mg/L)
V_k is the final volume of the solution (mL)
V_p is the volume of the sample (mL)
w is a conversion factor

Example of Calculation – fortified samples

The concentration of mefenpyr-diethyl (P) [mg/L] in fortified sample 0.1 mg/L for dilution method was calculated as follow:
The following values were used in this calculation:

Lab sample description	**Elendt M7 medium 0.1 mg-L_validation method dilution_006**
concentration (c) [mg/L]	0.0503
final volume of the solution (V_k) [mL]	4
volume of the sample (V_p) [mL]	2
conversion factor (w)	1/1=1

Concentration was calculated as follow:

$$P\ (mg/L) = c \cdot \frac{V_k}{V_p} \cdot w$$

$$P\ = 0.0503 \cdot \frac{4}{2} \cdot 1 = 0.101\ (mg/L)$$

Example of Calculation – test samples
The concentration of mesosulfuron-methyl (P) [mg/L] in test sample 200 mg/L was calculated as follow:
The following values were used in this calculation:

Lab sample description	**Daphnia 200 mg-L_dilution method_ Definitive test_W-61-20_0h_030**
concentration (c) [mg/L]	2.6840
final volume of the solution (V_k) [mL]	4
volume of the sample (V_p) [mL]	2
conversion factor (w)	1

Concentration was calculated as follow:

$$P\ (mg/L) = c \cdot \frac{V_k}{V_p} \cdot w$$

$$P\ = 2.6840\ \cdot \frac{4}{2} \cdot 1 = 5.368\ (mg/L)$$

Numerical values in this report are frequently rounded to a smaller degree of precision (number of digits) than were used in the actual calculation to increase readability and to indicate the approximate precision of the reported results. Minor differences in the results obtained with such "rounded" values in comparison to those obtained with higher precision values are well within the limits of the experimental accuracy and therefore of no practical concern.

A 2.1.2.7.1.4 Method of validation which was used in ecotoxicological study of aquatic organism *Lemma gibba*

Comments of zRMS:	Described method validation W-62-20 for the determination of mesosulfuron-methyl and mefenpyr-diethyl in medium was validated in accordance with SANTE/2020/12830, Rev. 1 and GLP. The method is acceptable and suitable for the determination of the concentrations of mesosulfuron-methyl and mefenphyr-dietyl.

Reference:	KCP 5.3
Report	Mesosulfuron 30 OD [CHR/H/MEZO 30 OD] *Lemna gibba* CPCC 310, Growth inhibition test, Brzozowska-Wojoczek K. Study code: W-62-20,
Guideline(s):	OECD Guideline No. 202 (2004)/EU method C.2.; SANTE/2020/12830, rev.1
Deviations:	No
GLP:	Yes
Acceptability:	
Duplication (if vertebrate study)	No

The analytical method was developed for the determination of mesosulfuron-methyl and mefenpyr-diethyl in water. The range of linearity of the analytical graph, the regression residual (di), selectivity and specificity, precision, matrix effect, accuracy, stability stock solution, limit of quantification and detection were determined.
The determination was accomplished by the high performance liquid chromatography (HPLC) with DAD detection. The validated analytical method was performed according to SANTE/2020/12830, Rev. 1 and Standard Operating Procedure.

1. Detected substances

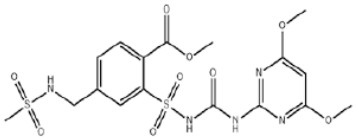
Standard of mesosulfuron-methyl from Sigma-Aldrich was used to method validation.

Standard of mefenpyr-diethyl from Sigma-Aldrich was used to method validation.

Mesosulfuron-methyl

IUPAC Name:	methyl2-[(4,6-dimethoxypyrimidin-2-ylcarbamoyl)sulfamoyl]-a-(methanesulfonamido)-p-toluate
Molecular Formula:	$C_{17}H_{21}N_5O_9S_2$
CAS No.:	208465-21-8
Purity:	98.4%
Series number:	BCBV9244
Expiry date:	October 2022
Manufacturing:	Sigma-Aldrich
Molecular weight:	503.51 g/Mol

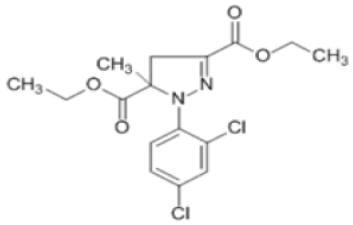
Structural formula:



Mefenpyr-diethyl

IUPAC Name:	diethyl(RS)-1-(2,4-dichlorophenyl)-4,5-dihydro-5-methyl-1H-pyrazole-3,5-dicarboxylate
Molecular Formula:	$C_{16}H_{18}Cl_2N_2O_4$
CAS No.:	135590-91-9
Purity:	98.8%
Series number:	BCBX4666
Expiry date:	May 2023
Manufacturing:	Sigma-Aldrich
Molecular weight:	373.23 g/Mol

Structural formula:



2. Method validation

Linearity	
Calibration curve	0.02 mg/L to 1.0 mg/L.

Range of the calibration curve

Dilution method		
Analyte	**range of linearity of calibration curve [mg/L]**	**equivalent calibration range of linearity [mg/Lwater]**
Mesosulfuron-methyl	0.02-1.0	0.04-2.0
Mefenpyr-diethyl	0.02-1.0	0.04-2.0

Method SPE		
Analyte	**range of linearity of calibration curve [mg/L]**	**equivalent calibration range of linearity [mg/L water]**
Mesosulfuron-methyl	0.02-1.0	0.0002-0.01
Mefenpyr-diethyl	0.02-1.0	0.0002-0.01

Tested working solutions: Mesosulfuron-methyl, Mefenpyr diethyl

Concentrations of working solutions: 0.02, 0.05, 0.1, 0.2, 0.5, 1.0 µg/mL

Linear equation

y = ax + b (a – slope, b - intercept)
The linear coefficient r2 must be higher than 0.99.
Range of the linear was given in µg/mL equivalent to mg/L.

Analyte	Slope	Intercept	Coefficient
Mesosulfuron-methyl	87247.1	-161.142	0.9996951
Mefenpyr-diethyl	86283.7	-14.6681	0.9998535

Calibration curve: 0.2 mg/L to 20.0 mg/L.

Range of the calibration curve

Dilution method		
Analyte	**range of linearity of calibration curve [mg/L]**	**equivalent calibration range of linearity [mg/Lwater]**
Mesosulfuron-methyl	0.2-20	0.4-40
Mefenpyr-diethyl	0.2-20	0.4-40

Method SPE		
Analyte	**range of linearity of calibration curve [mg/L]**	**equivalent calibration range of linearity [mg/L water]**
Mesosulfuron-methyl	0.2-20	0.002-0.2
Mefenpyr-diethyl	0.2-20	0.002-0.2

Tested working solutions: Mesosulfuron-methyl, Mefenpyr diethyl

<table>
<tr><td>Concentrations of working solutions</td><td>0.2, 0.5,1.0 , 2.0 , 5.0, 10, 20 µg/mL</td></tr>
<tr><td>Linear equation</td><td>y = ax + b (a – slope, b - intercept)
The linear coefficient r2 must be higher than 0.99.
Range of the linear was given in µg/mL equivalent to mg/L.

<table>
<tr><th>Analyte</th><th>Slope</th><th>Intercept</th><th>Coefficient</th></tr>
<tr><td>Mesosulfuron-methyl</td><td>88571.7</td><td>1360.71</td><td>0.9995577</td></tr>
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<tr><td>Tested samples</td><td>Controlled matrix, fortified matrix</td></tr>
<tr><td>Method</td><td>Analysis of the chromatograms</td></tr>
<tr><td>Results</td><td>No signal of detected substance were overlapping with matrix signal of the control samples in the experimental conditions</td></tr>
<tr><td colspan="2"><u>Precision</u></td></tr>
<tr><td>Repeatability for detected substance</td><td>repeatability for detected substances analyzed in water
are presented in the table which is in part Accurancy</td></tr>
<tr><td>RSD – relative standard deviation [%]</td><td>≤ 20% per each level</td></tr>
<tr><td colspan="2"><u>Accurancy</u></td></tr>
<tr><td>Mean recovery ± relative standard deviation</td><td>70 – 120%.</td></tr>
<tr><td>Recovery</td><td>
<table>
<tr><th>Matrix</th><th>Detected substance</th><th>Fortification Level [mg/kg]</th><th>Number of Replicate</th><th>Mean Recovery [%]</th><th>RSD [%]</th></tr>
<tr><td colspan="6">Dilution method</td></tr>
<tr><td rowspan="4">Water</td><td rowspan="2">Mesosulfuron-methyl</td><td>0.1</td><td>5</td><td>103.0</td><td>3.9</td></tr>
<tr><td>1.0</td><td>5</td><td>101.7</td><td>0.4</td></tr>
<tr><td rowspan="2">Mefenpyr-diethyl</td><td>0.1</td><td>5</td><td>94.0</td><td>2.1</td></tr>
<tr><td>1.0</td><td>5</td><td>103.3</td><td>0.2</td></tr>
<tr><td colspan="6">Method SPE</td></tr>
<tr><td rowspan="4">Water</td><td rowspan="2">Mesosulfuron-methyl</td><td>0.0005</td><td>5</td><td>90.0</td><td>13.3</td></tr>
<tr><td>0.005</td><td>5</td><td>106.4</td><td>0.6</td></tr>
<tr><td rowspan="2">Mefenpyr-diethyl</td><td>0.0005</td><td>5</td><td>102.0</td><td>1.0</td></tr>
<tr><td>0.005</td><td>5</td><td>96.6</td><td>0.2</td></tr>
</table></td></tr>
<tr><td colspan="2"><u>Matrix effects</u></td></tr>
</table>

$$\text{Matrix effects [\%]} = 100 \times \frac{\text{peak area (matrix)}}{\text{peak area (solvent)}} - 100$$

The matrix effect is not exceed ± 20%.

Dilution method			
Matrix	Detected substance	Concentration mg/L	Matrix effect [%]
Elendt M7 medium	Mesosulfuron-methyl	0.05	10.3
	Mefenpyr-diethyl	0.05	8.1
Method SPE			
Matrix	Detected substance	Concentration mg/L	Matrix effect [%]
Elendt M7 medium	Mesosulfuron-methyl	0.05	4.3
	Mefenpyr-diethyl	0.05	-3.5

Limit of Quantification (LOQ) and Limit of Detection (LOD)

LOQ	Equivalent calibration level	LOD	Equivalent calibration level
Dilution method			
0.1 mg mesosulfuronmethyl/L	0.05 µg/ml	0.04 mg mesosulfuron-methyl/kg	0.02 µg/ml
0.1 mg mefenpyr-diethyl/L	0.05 µg/ml	0.04 mg mefenpyr-diethyl/kg	0.02 µg/ml
Method SPE			
0.0005 mg mesosulfuron-methyl/L	0.05 µg/ml	0.0002 mg mesosulfuron-methyl/kg	0.02 µg/ml
0.0005 mg mefenpyr-diethyl/L	0.05 µg/ml	0.0002 mg mefenpyr-diethyl/kg	0.02 µg/ml

3.Calculation

The concentration (P) of mesosulfuron-methyl and mefenpyr-diethyl in 20xAAP medium for fortified and test samples was calculated as follows:

$$P = c \cdot \frac{V_k}{V_p} \cdot w$$

where:
c is the concentration of the detected compound in the final solution determined on the basis of the calibration curve (µg/mL)
Vk is the final volume of the solution (mL)
vp is the volume of the sample (mL)

w is a conversion factor (w/w= 1000/1000=1)

Example of Calculation – fortified samples

The concentration of mefenpyr-diethyl (P) [mg/L] in fortified sample 0.1 mg/L for dilution method was calculated as follow:

The following values were used in this calculation

Lab sample description	**water 0.1 mg-L_validation method _004**
concentration (c) [mg/L]	0.0478
final volume of the solution (V_k) [mL]	4
volume of the sample (V_p) [mL]	2
conversion factor (w)	1/1=1

Concentration was calculated as follow:

$$P\ (mg/L) = c \cdot \frac{V_k}{V_p} \cdot w$$

$$P\ = 0.0478 \cdot \frac{4}{2} \cdot 1 = 0.096\ (mg/L)$$

Example of Calculation – test samples

The concentration (P) [mg/L] of mefenpyr-diethyl in test sample 10 mg/L was calculated as follow:

The following values were used in this calculation:

Lab sample description	**10 mg-L fresh_dilution method_definitive test_Day 0_ W-62-20_032**
concentration (c) [mg/L]	0.4644
final volume of the solution (V_k) [mL]	4
volume of the sample (V_p) [mL]	2
conversion factor (w)	1

Concentration was calculated as follow:

$$P = c \cdot \frac{V_k}{V_p} \cdot W\ \text{(mg/L)}$$

P=0.4644*4/2*1=0.929 mg/L

**Numerical values in this report are frequently rounded to a smaller degree of precision (number of digits) than were used in the actual calculation to increase readability and to indicate the approximate precision of the reported results. Minor differences in the results obtained with such "rounded" values in comparison*

to those obtained with higher precision values are well within the limits of the experimental accuracy and therefore of no practical concern.

A 2.1.2.7.1.5 Method of validation which was used in ecotoxicological study of aquatic organism *Anabaena flos-aquae* UTEX B 1444

Comments of zRMS:	Described method validation W-64-20 for the determination of mesosulfuron-methyl and mefenpyr-diethyl in medium was validated in accordance with SANTE/2020/12830, Rev. 1 and GLP. The method is acceptable and suitable for the determination of the concentrations of mesosulfuron-methyl and mefenphyr-dietyl.

Reference:	KCP 5.3
Report	Mesosulfuron 30 OD [CHR/H/MEZO 30 OD] *Anabaena flos-aquae* UTEX B 1444 Growth inhibition test. Brzozowska-Wojoczek K., 2023, Study code W-64-20
Guideline(s):	OECD Guideline No. 201 (2006)/EU method C.3.; SANTE/2020/12830, Rev. 1
Deviations:	No
GLP:	Yes
Acceptability:	
Duplication (if vertebrate study)	No

The concentration of active substance of test item was chemically determined using the validated high performance liquid chromatographic method with DAD detection. The analytical method was developed for the determination of active substance of test item in matrix. The range of linearity of the analytical graph, the regression residual (di), selectivity and specificity, precision, matrix effect, accuracy, and limit of quantification and detection were determined.

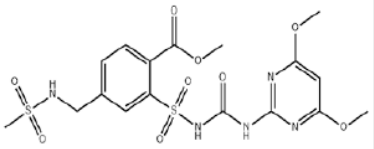
The determination was accomplished by the high performance liquid chromatography (HPLC) with DAD detection. The validated analytical method was performed according to SANTE/2020/12830, Rev. 1 and Standard Operating Procedure.

1. Detected substances
Standard of mesosulfuron-methyl from Sigma-Aldrich was used to method validation.
Standard of mefenpyr-diethyl from Sigma-Aldrich was used to method validation.

Mesosulfuron-methyl

IUPAC Name:	methyl2-[(4,6-dimethoxypyrimidin-2-ylcarbamoyl)sulfamoyl]-a-(methanesulfonamido)-p-toluate
Molecular Formula:	$C_{17}H_{21}N_5O_9S_2$
CAS No.:	208465-21-8
Purity:	98.4%
Series number:	BCBV9244
Expiry date:	October 2022
Manufacturing:	Sigma-Aldrich
Molecular weight:	503.51 g/Mol

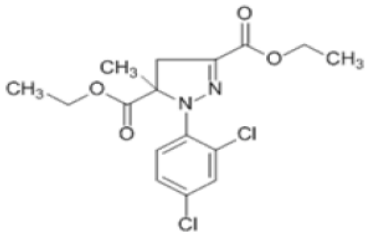
Structural formula:



Mefenpyr-diethyl

IUPAC Name:	diethyl(RS)-1-(2,4-dichlorophenyl)-4,5-dihydro-5-methyl-1H-pyrazole-3,5-dicarboxylate
Molecular Formula:	$C_{16}H_{18}Cl_2N_2O_4$
CAS No.:	135590-91-9
Purity:	98.8%
Series number:	BCBX4666
Expiry date:	May 2023
Manufacturing:	Sigma-Aldrich
Molecular weight:	373.23 g/Mol

Structural formula:



2. Method validation

Tabl. 1 Parameters of the validation

<table>
<tr><th colspan="2"><u>Linearity</u></th></tr>
<tr><td>Calibration curve</td><td>0.02 mg/L to 1.0 mg/L.</td></tr>
<tr><td>Range of the calibration curve</td><td>
<table>
<tr><th colspan="3">Dilution method</th></tr>
<tr><th>Analyte</th><th>range of linearity of calibration curve [mg/L]</th><th>equivalent calibration range of linearity [mg/Lwater]</th></tr>
<tr><td>Mesosulfuron-methyl</td><td>0.02-1.0</td><td>0.04-2.0</td></tr>
<tr><td>Mefenpyr-diethyl</td><td>0.02-1.0</td><td>0.04-2.0</td></tr>
<tr><td></td><td></td><td></td></tr>
</table>
<table>
<tr><th colspan="3">Method SPE</th></tr>
<tr><th>Analyte</th><th>range of linearity of calibration curve [mg/L]</th><th>equivalent calibration range of linearity [mg/L water]</th></tr>
<tr><td>Mesosulfuron-methyl</td><td>0.02-1.0</td><td>0.0002-0.01</td></tr>
<tr><td>Mefenpyr-diethyl</td><td>0.02-1.0</td><td>0.0002-0.01</td></tr>
</table>
</td></tr>
<tr><td>Tested working</td><td>Mesosulfuron-methyl, Mefenpyr diethyl</td></tr>
</table>

<table>
<tr><td>solutions</td><td></td></tr>
<tr><td>Concentrations of working solutions</td><td>0.02, 0.05, 0.1, 0.2, 0.5, 1.0 µg/mL</td></tr>
<tr><td>Linear equation</td><td>y = ax + b (a – slope, b - intercept)
The linear coefficient r2 must be higher than 0.99.
Range of the linear was given in µg/mL equivalent to mg/L.

<table><tr><th>Analyte</th><th>Slope</th><th>Intercept</th><th>Coefficient</th></tr><tr><td>Mesosulfuron-methyl</td><td>87247.1</td><td>-161.142</td><td>0.9996951</td></tr><tr><td>Mefenpyr-diethyl</td><td>86283.7</td><td>-14.6681</td><td>0.9998535</td></tr></table></td></tr>
<tr><td>Calibration curve</td><td>0.2 mg/L to 20.0 mg/L.</td></tr>
<tr><td>Range of the calibration curve</td><td><table><tr><th colspan="3">Dilution method</th></tr><tr><th>Analyte</th><th>range of linearity of calibration curve [mg/L]</th><th>equivalent calibration range of linearity [mg/Lwater]</th></tr><tr><td>Mesosulfuron-methyl</td><td>0.2-20</td><td>0.4-40</td></tr><tr><td>Mefenpyr-diethyl</td><td>0.2-20</td><td>0.4-40</td></tr><tr><td></td><td></td><td></td></tr></table>
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<tr><td>Tested working solutions</td><td>Mesosulfuron-methyl, Mefenpyr diethyl</td></tr>
<tr><td>Concentrations of working solutions</td><td>0.2, 0.5,1.0 , 2.0 , 5.0, 10, 20 µg/mL</td></tr>
<tr><td>Linear equation</td><td>y = ax + b (a – slope, b - intercept)
The linear coefficient r2 must be higher than 0.99.
Range of the linear was given in µg/mL equivalent to mg/L.

<table><tr><th>Analyte</th><th>Slope</th><th>Intercept</th><th>Coefficient</th></tr><tr><td>Mesosulfuron-methyl</td><td>88571.7</td><td>1360.71</td><td>0.9995577</td></tr><tr><td>Mefenpyr-diethyl</td><td>85661.2</td><td>-268.789</td><td>0.9999925</td></tr></table></td></tr>
<tr><td colspan="2"><u>Selectivity and specificity</u></td></tr>
<tr><td>Tested samples</td><td>Controlled matrix, fortified matrix</td></tr>
<tr><td>Method</td><td>Analysis of the chromatograms</td></tr>
<tr><td>Results</td><td>No signal of detected substance were overlapping with matrix signal of the control samples in the experimental conditions</td></tr>
<tr><td colspan="2"><u>Precision</u></td></tr>
<tr><td>Repeatability for detected substance</td><td>repeatability for detected substances analyzed in water
are presented in the table which is in part Accurancy</td></tr>
<tr><td>RSD – relative standard deviation [%]</td><td>≤ 20% per each level</td></tr>
<tr><td colspan="2"><u>Accurancy</u></td></tr>
</table>

Mean recovery ± relative standard deviation	70 – 120%.

Recovery

Matrix	Detected substance	Fortification Level [mg/kg]	Number of Replicate	Mean Recovery [%]	RSD [%]
Dilution method					
Water	Mesosulfuron-methyl	0.1	5	103.0	3.9
		1.0	5	101.7	0.4
	Mefenpyr-diethyl	0.1	5	94.0	2.1
		1.0	5	103.3	0.2
Method SPE					
Water	Mesosulfuron-methyl	0.0005	5	90.0	13.3
		0.005	5	106.4	0.6
	Mefenpyr-diethyl	0.0005	5	102.0	1.0
		0.005	5	96.6	0.2

Matrix effects

$$\text{Matrix effects [\%]} = 100 \times \frac{\text{peak area (matrix)}}{\text{peak area (solvent)}} - 100$$

The matrix effect is not exceed ± 20%.

Dilution method			
Matrix	**Detected substance**	**Concentration mg/L**	**Matrix effect [%]**
Water	Mesosulfuron-methyl	0.05	10.3
	Mefenpyr-diethyl	0.05	8.1
Method SPE			
Matrix	**Detected substance**	**Concentration mg/L**	**Matrix effect [%]**
Water	Mesosulfuron-methyl	0.05	4.3
	Mefenpyr-diethyl	0.05	-3.5

Limit of Quantification (LOQ) and Limit of Detection (LOD)

LOQ	Equivalent calibration level	LOD	Equivalent calibration level
Dilution method			
0.1 mg mesosulfuronmethyl/L	0.05 µg/ml	0.04 mg mesosulfuron-methyl/L	0.02 µg/ml
0.1 mg mefenpyr-diethyl/L	0.05 µg/ml	0.04 mg mefenpyr-diethyl/L	0.02 µg/ml
Method SPE			
0.0005 mg mesosulfuron-methyl/L	0.05 µg/ml	0.0002 mg mesosulfuron-methyl/L	0.02 µg/ml
0.0005 mg mefenpyr-diethyl/L	0.05 µg/ml	0.0002 mg mefenpyr-diethyl/L	0.02 µg/ml

3.Calculation

The concentration (P) [mg/L] of active substances for fortified samples and test samples were calculated as follows:

$$P = c \cdot \frac{V_k}{V_p} \cdot w$$

where:
c is the concentration of the detected compound in the final solution determined on the basis of the calibration curve (mg/L)
V_k is the final volume of the solution (mL)
V_p is the volume of the sample (mL)
w is a conversion factor

Example of Calculation – fortified samples
The concentration of mefenpyr-diethyl (P) [mg/L] in fortified sample 0.1 mg/L for dilution method was calculated as follow: The following values were used in this calculation:

Lab sample description	**water 0.1 mg-L_validation method _004**
concentration (c) [mg/L]	0.0478
final volume of the solution (V_k) [mL]	4
volume of the sample (V_p) [mL]	2
conversion factor (w)	1/1=1

Concentration was calculated as follow:

$$P\ (mg/L) = c \cdot \frac{V_k}{V_p} \cdot w$$

$$P\ = 0.0478 \cdot \frac{4}{2} \cdot 1 = 0.096\ (mg/L)$$

Example of Calculation – test samples
The concentration of mesosulfuron-methyl (P) [mg/L] in test sample 100 mg/L was calculated as follow: The following values were used in this calculation:

Lab sample description	**Anabaena 100 mg-L_dilution method_ Definitive test_W-64-20_0h_020**
concentration (c) [mg/L]	1.4050
final volume of the solution (V_k) [mL]	4
volume of the sample (V_p) [mL]	2
conversion factor (w)	1

Concentration was calculated as follow:

$$P\ (mg/L) = c \cdot \frac{V_k}{V_p} \cdot w$$

$$P\ = 1.4050\ \cdot \frac{4}{2} \cdot 1 = 2.810\ (mg/L)$$

Numerical values in this report are frequently rounded to a smaller degree of precision (number of digits) than were used in the actual calculation to increase readability and to indicate the approximate precision of the reported results. Minor differences in the results obtained with such "rounded" values in comparison to those obtained with higher precision values are well within the limits of the experimental accuracy and therefore of no practical concern

A 2.1.2.7.1.6 Method of validation which was used in ecotoxicological study of Collembolan (*Folsomia candida*)

Comments of zRMS:	Described method validation G-46-20 for the determination of mesosulfuron-methyl and mefenpyr-diethyl in artificial soil was validated in accordance with SANTE/2020/12830, Rev. 1 and GLP. The method is acceptable and suitable for the determination of the concentrations of mesosulfuron-methyl and mefenphyr-dietyl.

Reference:	KCP 5.3
Report	Mesosulfuron 30 OD (CHR/H/MEZO 30 OD) Collembolan (*Folsomia candida*) Reproduction Test. Pieczka P., 2023. Study code: G-46-20
Guideline(s):	OECD Guideline No. 232 (2016); SANTE/2020/12830, Rev. 1
Deviations:	Yes Deviations from the OECD Guideline No. 232 (2016): - culturing of collembolans takes place in plastic containers containing an artificial substrate consisting of plaster and charcoal in ratio 9:1 and not 10:1 or 8:1 as is mentioned in OECD Guideline No. 232 (2016) (3.3), - at the end of the test the soil moisture content was determined by drying small sample of the artificial soil in 105°C instead of weighing the test vessels

	as it is mentioned in OECD Guideline No. 232 (2016) (3.6.6). Deviation from the SOP/G/122: As it is indicated in the SOP/G/122, the amount of calcium carbonate to adjust the pH should be in the range from 0.02 to 0.04%. In the study, the needed amount of calcium carbonate was equal to 0.12%, therefore it is a deviation from the SOP/G/122. According to the OECD Guideline the amount of CaCO3 should be less than 1.0%. The applied quantity of the calcium carbonate in the study was in line with OECD assumptions. The deviations did not affect the results of the study.
GLP:	Yes
Acceptability:	
Duplication (if vertebrate study)	No

The analytical method was developed for the determination of active substances of test item in matrix. The range of linearity of the analytical graph, the regression residual (di), selectivity and specificity, precision, matrix effect, accuracy, stability stock solution, limit of quantification and detection were determined.
The determination was accomplished by the high performance liquid chromatography (HPLC) with DAD detection. The validated analytical method was performed according to SANTE/2020/12830, Rev. 1 and Standard Operating Procedure.

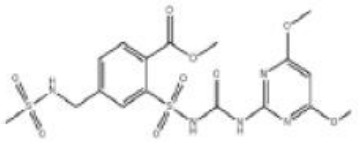
1. Detected substances

Standard of mesosulfuron-methyl from Sigma-Aldrich was used to method validation.
Standard of mefenpyr-diethyl from Sigma-Aldrich was used to method validation.

Mesosulfuron-methyl

IUPAC Name:	methyl2-[(4,6-dimethoxypyrimidin-2-ylcarbamoyl)sulfamoyl]-a-(methanesulfonamido)-p-toluate
Molecular Formula:	$C_{17}H_{21}N_5O_9S_2$
CAS No.:	208465-21-8
Purity:	98.4%
Series number:	BCBV9244
Expiry date:	October 2022
Manufacturing:	Sigma-Aldrich
Molecular weight:	503.51 g/Mol

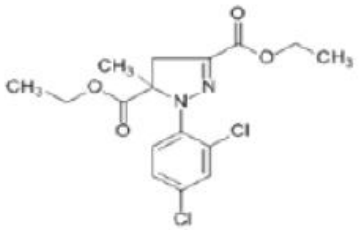
Structural formula:



Mefenpyr-diethyl

IUPAC Name:	diethyl(RS)-1-(2,4-dichlorophenyl)-4,5-dihydro-5-methyl-1H-pyrazole-3,5-dicarboxylate
Molecular Formula:	$C_{16}H_{18}Cl_2N_2O_4$
CAS No.:	135590-91-9
Purity:	98.8%
Series number:	BCBX4666
Expiry date:	May 2023
Manufacturing:	Sigma-Aldrich
Molecular weight:	373.23 g/Mol

Structural formula:



2. Method validation

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<tr><th>Analyte</th><th>range of linearity of calibration curve [mg/L]</th><th>equivalent calibration range of linearity [mg/kg artificial soil]</th></tr>
<tr><td>Mesosulfuron-methyl</td><td>0.02-1.0</td><td>0.02-1.0</td></tr>
<tr><td>Mefenpyr-diethyl</td><td>0.02-1.0</td><td>0.02-1.0</td></tr>
</table>
</td></tr>
<tr><td>Tested working solutions</td><td>Mesosulfuron-methyl, Mefenpyr diethyl</td></tr>
<tr><td>Concentrations of working solutions</td><td>0.02, 0.05, 0.1, 0.2, 0.5, 1.0 µg/mL</td></tr>
</table>

<table>
<tr><td>Linear equation</td><td>y = ax + b (a – slope, b - intercept)
The linear coefficient r2 must be higher than 0.99.
Range of the linear was given in µg/mL equivalent to mg/L.

<table>
<tr><th>Analyte</th><th>Analysis</th><th>Slope</th><th>Intercept</th><th>Coefficient</th></tr>
<tr><td>Mesosulfuron-methyl</td><td rowspan="2">Control LoQ Matrix effect</td><td>87247.1</td><td>-161.142</td><td>0.9996951</td></tr>
<tr><td>Mefenpyr-diethyl</td><td>86283.7</td><td>-14.6681</td><td>0.9998535</td></tr>
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<tr><td>Calibration curve</td><td>0.2 mg/L to 20.0 mg/L.</td></tr>
<tr><td>Range of the calibration curve</td><td>
<table>
<tr><th>Analyte</th><th>range of linearity of calibration curve [mg/L]</th><th>equivalent calibration range of linearity [mg/kg artificial soil]</th></tr>
<tr><td>Mesosulfuron-methyl</td><td>0.2-20</td><td>0.2-20</td></tr>
<tr><td>Mefenpyr-diethyl</td><td>0.2-20</td><td>0.2-20</td></tr>
</table></td></tr>
<tr><td>Tested working solutions</td><td>Mesosulfuron-methyl, Mefenpyr diethyl</td></tr>
<tr><td>Concentrations of working solutions</td><td>0.2, 0.5, 1, 2, 5, 10, 20 µg/mL</td></tr>
<tr><td>Linear equation</td><td>y = ax + b (a – slope, b - intercept)
The linear coefficient r2 must be higher than 0.99.
Range of the linear was given in µg/mL equivalent to mg/L.

<table>
<tr><th>Analyte</th><th>Analysis</th><th>Slope</th><th>Intercept</th><th>Coefficient</th></tr>
<tr><td>Mesosulfuron-methyl</td><td rowspan="2">10xLoQ Day 0, Day 14</td><td>88571.7</td><td>1360.71</td><td>0.9995577</td></tr>
<tr><td>Mefenpyr-diethyl</td><td>85661.2</td><td>-268.789</td><td>0.9999925</td></tr>
<tr><td>Mesosulfuron-methyl</td><td rowspan="2">Day 28</td><td>93277.5</td><td>213.134</td><td>0.9998846</td></tr>
<tr><td>Mefenpyr-diethyl</td><td>86912.9</td><td>-201.825</td><td>0.9998790</td></tr>
</table></td></tr>
<tr><td colspan="2"><u>Selectivity and specificity</u></td></tr>
<tr><td>Tested samples</td><td>Controlled matrix, fortified matrix</td></tr>
<tr><td>Method</td><td>Analysis of the chromatograms</td></tr>
<tr><td>Results</td><td>No signal of detected substances were overlapping with matrix signal of the control samples in the experimental conditions</td></tr>
<tr><td colspan="2"><u>Precision</u></td></tr>
<tr><td>Repeatability for detected substance</td><td>repeatability for detected substances analyzed in artificial soil are form 4.2-4.9% for Mesosulfuron-methyl and from 2.5-7.7% for mefenpyr-diethyl</td></tr>
<tr><td>RSD – relative standard deviation [%]</td><td>≤ 20% per each level</td></tr>
<tr><td colspan="2"><u>Accurancy</u></td></tr>
<tr><td>Mean recovery ± relative standard deviation</td><td>70 – 120%.</td></tr>
</table>

Recovery

Matrix	Detected substance	Fortification Level [mg/kg]	Number of Replicate	Mean Recovery [%]	RSD [%]
Artificial soil	Mesosulfuron-methyl	0.1	5	96.0	4.2
		1.0	5	75.8	4.9
	Mefenpyr-diethyl	0.1	5	104.0	7.7
		1.0	5	109.5	2.5

Matrix effects

$$\text{Matrix effects [\%]} = 100 \times \frac{\text{peak area (matrix)}}{\text{peak area (solvent)}} - 100$$

The matrix effect is not exceed ± 20%.

Matrix	Detected substance	Concentration mg/L	Matrix effect [%]
Artificial soil	Mesosulfuron-methyl	0.1	-18.1
	Mefenpyr-diethyl	0.1	6.9

Limit of Quantification (LOQ) and Limit of Detection (LOD)

LOQ	Equivalent calibration level	LOD	Equivalent calibration level
Method SPE			
0.01 mg mesosulfuronmethyl/kg	0.1 µg/ml	0.02 mg mesosulfuron-methyl/kg	0.02 µg/ml
0.01 mg mefenpyr-diethyl	0.1 µg/ml	0.02 mg mefenpyr-diethyl/kg	0.02 µg/ml

3. Calculations

The concentration (P1) [mg/kg] of active substance for fortified samples were calculated as follows (1):

$$P1 = c \cdot \frac{V_k}{m_p} \cdot w \qquad (1)$$

where:

c is the concentration of the detected compound in the final solution determined on the basis of the calibration curve (mg/L)

V_k is the final volume of the solution (mL)

m_p is the weight of the sample (g)

w is a conversion factor

The concentration (P) [mg/kg d.w.] of active substance for the test samples were calculated first using the formula (1) and then using the formula (2):

$$P = \frac{P1 \cdot 100\%}{M_{dw}} \qquad (2)$$

where:

P1 – concentration of active substance (mg/kg)

M_{dw} is the dry weight soil (%)

Example of Calculation – fortified samples

The concentration of mefenpyr-diethyl (P1) [mg/kg] in fortified sample 1.0 mg/kg was calculated as follow:

The following values were used in this calculation:

Lab sample description	Artificial soil 1 mg-kg validation method_009
concentration (c) [mg/L]	1.0861
final volume of the solution (V_k) [mL]	10
weight of the sample (m_p) [g]	10
conversion factor (w)	1000/1000=1

Concentration of mefenpyr-diethyl was calculated as follow:

$$P1\ (mg/kg) = c \cdot \frac{V_k}{m_p} \cdot w$$

$$P1 = 1.0861 \cdot \frac{10}{10} \cdot 1 = 1.086\ (mg/kg)$$

Example of Calculation – test samples

The concentration of mefenpyr-diethyl (P) [mg/$kg_{d.w.}$] in test sample at the concentration 1000 mg/kg collected at the beginning of the experiment was calculated as follow:

The following values were used in this calculation:

Lab sample description	Collembolan 1000 mg-kg d.w._Definitive test G-46-20_008
concentration (c) [mg/L]	6.6606
final volume of the solution (V_k) [mL]	110
weight of the sample (m_p) [g]	10
dry weight soil M_{dw} (%)	85.9
conversion factor (w)	1000/1000=1

Concentration of mefenpyr-diethyl was calculated as follow:

$$P1\ (mg/kg) = c \cdot \frac{V_k}{m_p} \cdot w$$

$$P1 = 6.6606 \cdot \frac{110}{10} \cdot 1 = 73.267\ (mg/kg)$$

$$P = \frac{73.267 \cdot 100\%}{85.9} = 85.293\ (mg/kg_{dw})$$

*Numerical values in this report are frequently rounded to a smaller degree of precision (number of digits) than were used in the actual calculation to increase readability and to indicate the approximate precision of the reported results. Minor differences in the results obtained with such "rounded" values in comparison to those obtained with higher precision values are well within the limits of the experimental accuracy and therefore of no practical concern.

A 2.1.2.7.1.7 Method of validation which was used in ecotoxicological study of Predatory mite (*Hypoaspis (Geolaelaps) aculeifer*)

Comments of zRMS:	Described method validation G-47-20 for the determination of mesosulfuron-methyl and mefenpyr-diethyl in artificial soil was validated in accordance with SANTE/2020/12830, Rev. 1 and GLP. The method is acceptable and suitable for the determination of the concentrations of mesosulfuron-methyl and mefenphyr-dietyl.

Reference:	KCP 5.3
Report	Predatory mite (*Hypoaspis (Geolaelaps) aculeifer*) reproduction test in soil. Wróbel A., 2023. Study code: G-47-20
Guideline(s):	OECD Guideline No. 226 (2016); SANTE/2020/12830, Rev. 1
Deviations:	Yes The study was performed according to OECD Guideline No. 226 (2016), Study Plan and the SOPs mentioned in chapter 8. *Deviations from the OECD Guideline No. 226 (2016):*

	There are three deviations from the OECD Guideline No. 226 (2016), however they did not affect the results: 1. According to the OECD Guideline No. 226 (2016) the water content of the artificial soil should be maintained throughout the test by weighing and if needed re-watering the vessels periodically. In the study to maintain proper moisture content, a small sample of soil was drying at 105°C and re-weighing at the beginning, after 7 days of the test and at the end of the test (Chapter 3.5.7). 2. Due to the use of the temperature extraction method, there was no need for euthanasia of the extracted organisms since the mites are fixed in a 70% ethanol solution (Chapter 3.5.8). 3. Due to the use of the temperature extraction method, it was not possible to record the symptoms with behavioral and morphology changes of the extracted predatory mites (Chapter 3.5.8). All above mentioned deviations did not influence the study course and results.
GLP:	Yes
Acceptability:	
Duplication (if vertebrate study)	No

The analytical method was developed for the determination of active substances of test item in matrix. The range of linearity of the analytical graph, the regression residual (di), selectivity and specificity, precision, matrix effect, accuracy, stability stock solution, limit of quantification and detection were determined.
The determination was accomplished by the high performance liquid chromatography (HPLC) with DAD detection. The validated analytical method was performed according to SANTE/2020/12830, Rev. 1 and Standard Operating Procedure.

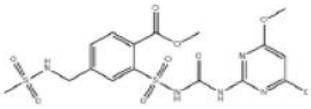
1. Detected substances

Standard of mesosulfuron-methyl from Sigma-Aldrich was used to method validation.
Standard of mefenpyr-diethyl from Sigma-Aldrich was used to method validation.

Mesosulfuron-methyl

IUPAC Name:	methyl2-[(4,6-dimethoxypyrimidin-2-ylcarbamoyl)sulfamoyl]-a-(methanesulfonamido)-p-toluate
Molecular Formula:	$C_{17}H_{21}N_5O_9S_2$
CAS No.:	208465-21-8
Purity:	98.4%
Series number:	BCBV9244
Expiry date:	October 2022
Manufacturing:	Sigma-Aldrich
Molecular weight:	503.51 g/Mol

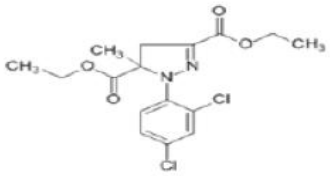
Structural formula:



Mefenpyr-diethyl

IUPAC Name:	diethyl(RS)-1-(2,4-dichlorophenyl)-4,5-dihydro-5-methyl-1H-pyrazole-3,5-dicarboxylate
Molecular Formula:	$C_{16}H_{18}Cl_2N_2O_4$
CAS No.:	135590-91-9
Purity:	98.8%
Series number:	BCBX4666
Expiry date:	May 2023
Manufacturing:	Sigma-Aldrich
Molecular weight:	373.23 g/Mol

Structural formula:



2. Method validation

Tabl. 1 Parameters of the validation

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<tr><td>Calibration curve</td><td>0.02 mg/L to 1.0 mg/L.</td></tr>
<tr><td>Range of the calibration curve</td><td>
<table>
<tr><th>Analyte</th><th>range of linearity of calibration curve [mg/L]</th><th>equivalent calibration range of linearity [mg/kg artificial soil]</th></tr>
<tr><td>Mesosulfuron-methyl</td><td>0.02-1.0</td><td>0.02-1.0</td></tr>
<tr><td>Mefenpyr-diethyl</td><td>0.02-1.0</td><td>0.02-1.0</td></tr>
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<tr><td>Repeatability for detected substance</td><td>repeatability for detected substance analyzed in artificial soil are from 4.2-4.9% for Mesosulfuron-methyl and from 2.5-7.7% for Mefenpyr-diethyl</td></tr>
<tr><td>RSD – relative standard deviation [%]</td><td>≤ 20% per each level</td></tr>
<tr><td colspan="2"><u>Accurancy</u></td></tr>
<tr><td>Mean recovery ± relative standard deviation</td><td>70 – 120%.</td></tr>
<tr><td>Recovery</td><td>
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<tr><th>Matrix</th><th>Detected substance</th><th>Fortification Level [mg/kg]</th><th>Number of Replicate</th><th>Mean Recovery [%]</th><th>RSD [%]</th></tr>
<tr><td rowspan="4">Artificial soil</td><td rowspan="2">Mesosulfuron-methyl</td><td>0.1</td><td>5</td><td>96.0</td><td>4.2</td></tr>
<tr><td>1.0</td><td>5</td><td>75.8</td><td>4.9</td></tr>
<tr><td rowspan="2">Mefenpyr-diethyl</td><td>0.1</td><td>5</td><td>104.0</td><td>7.7</td></tr>
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</table>

$$\text{Matrix effects [\%]} = 100 \times \frac{\text{peak area (matrix)}}{\text{peak area (solvent)}} - 100$$

The matrix effect is not exceed ± 20%.

Matrix	Detected substance	Concentration mg/L	Matrix effect [%]
Artificial soil	Mesosulfuron-methyl	0.1	-18.1
	Mefenpyr-diethyl	0.1	6.9

Limit of Quantification (LOQ) and Limit of Detection (LOD)

LOQ	Equivalent calibration level	LOD	Equivalent calibration level
0.01 mg Mesosulfuron-methyl/kg	0.1 µg/ml	0.02 mg Mesosulfuron-methyl/kg	0.02 µg/ml
0.01 mg Mefenpyr-diethyl	0.1 µg/ml	0.02 mg Mefenpyr-diethyl/kg	0.02 µg/ml

3. Calculations

The concentration (P1) [mg/kg] of active substance for fortified samples were calculated as follows (1):

$$P1 = c \cdot \frac{V_k}{m_p} \cdot w \qquad (1)$$

where:

c is the concentration of the detected compound in the final solution determined on the basis of the calibration curve (mg/L)

V_k is the final volume of the solution (mL)

m_p is the weight of the sample (g)

w is a conversion factor

The concentration (P) [mg/kg d.w.] of active substance for the test samples were calculated first using the formula (1) and then using the formula (2):

$$P = \frac{P1 \cdot 100\%}{M_{dw}} \qquad (2)$$

where:

P1 – concentration of active substance (mg/kg)

M_{dw} is the dry weight soil (%)

Example of Calculation – fortified samples

The concentration of mefenpyr-diethyl (P1) [mg/kg] in fortified sample 1.0 mg/kg was calculated as follow:

The following values were used in this calculation:

Lab sample description	Artificial soil 1 mg-kg validation method_009
concentration (c) [mg/L]	1.0861
final volume of the solution (V_k) [mL]	10
weight of the sample (m_p) [g]	10
conversion factor (w)	1000/1000=1

Concentration of mefenpyr-diethyl was calculated as follow:

$$P1\ (mg/kg) = c \cdot \frac{v_k}{m_p} \cdot w$$

$$P1\ = 1.0861 \cdot \frac{10}{10} \cdot 1 = 1.086\ (mg/kg)$$

Example of Calculation – test samples

The concentration of mefenpyr-diethyl (P) [mg/kg$_{d.w.}$] in test sample at the concentration 1000 mg/kg collected at the beginning of the experiment was calculated as follow:

The following values were used in this calculation:

Lab sample description	Hypoaspis 1000 mg-kg d.w._Definitive test G-47-20_007
concentration (c) [mg/L]	6.5579
final volume of the solution (V_k) [mL]	110
weight of the sample (m_p) [g]	10
dry weight soil M_{dw} (%)	83.6
conversion factor (w)	1000/1000=1

Concentration of mefenpyr-diethyl was calculated as follow:

$$P1\ (mg/kg) = c \cdot \frac{v_k}{m_p} \cdot w$$

$$P1 = 6.5579 \cdot \frac{110}{10} \cdot 1 = 72.137\ (mg/kg)$$

$$P = \frac{72.137 \cdot 100\%}{83.6} = 86.288\ (mg/kg_{dw})$$

*Numerical values in this report are frequently rounded to a smaller degree of precision (number of digits) than were used in the actual calculation to increase readability and to indicate the approximate precision of the reported results. Minor differences in the results obtained with such "rounded" values in comparison to those obtained with higher precision values are well within the limits of the experimental accuracy and therefore of no practical concern.

A 2.1.2.7.1.8 Method of validation which was used in ecotoxicological study of Terrestrial Plant Test: Vegetative Vigour Test.

Comments of zRMS:	Described method validation G-49-20 for the determination of mesosulfuron-methyl and mefenpyr-diethyl in water was validated in accordance with SANTE/2020/12830, Rev. 1 and GLP. The method is acceptable and suitable for the determination of the concentrations of mesosulfuron-methyl and mefenphyr-dietyl.

Reference:	KCP 5.3
Report	Mesosulfuron 30 OD (CHR/H/MEZO 30 OD) Terrestial Plant Test: Vegetative Vigour Test. Gierbuszewska A., 2023, Study code: G-49-20
Guideline(s):	OECD Guideline No. 227 (2006); SANTE/2020/12830, Rev. 1
Deviations:	Yes *Deviation from OECD Guideline No. 227:* According to OECD Guideline No. 227 (2006), the light intensity should be 350 ± 50µE/m2 /s. However, these values are recommended for tests conducted in greenhouses. The experiment was conducted in a test room, where only artificial lighting was used. The light intensity was between 69.9 – 260.5 µE/m2 /s. Good control plant vigour was observed. Therefore, it was concluded that the light intensity was suitable for plant growing. The deviation did not affect the results of the experiment.
GLP:	Yes
Acceptability:	
Duplication (if vertebrate study)	No

The analytical method was developed for the determination of active substances of test item in matrix. The range of linearity of the analytical graph, the regression residual (di), selectivity and specificity, precision, matrix effect, accuracy, stability stock solution, limit of quantification and detection were determined.
The determination was accomplished by the high performance liquid chromatography (HPLC) with DAD detection. The validated analytical method was performed according to SANTE/2020/12830, Rev. 1 and Standard Operating Procedure.

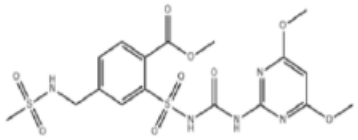
1. Detected substances

Standard of mesosulfuron-methyl from Sigma-Aldrich was used to method validation.
Standard of mefenpyr-diethyl from Sigma-Aldrich was used to method validation.

Mesosulfuron-methyl

IUPAC Name:	methyl2-[(4,6-dimethoxypyrimidin-2-ylcarbamoyl)sulfamoyl]-a-(methanesulfonamido)-p-toluate
Molecular Formula:	$C_{17}H_{21}N_5O_9S_2$
CAS No.:	208465-21-8
Purity:	98.4%
Series number:	BCBV9244
Expiry date:	October 2022
Manufacturing:	Sigma-Aldrich
Molecular weight:	503.51 g/Mol

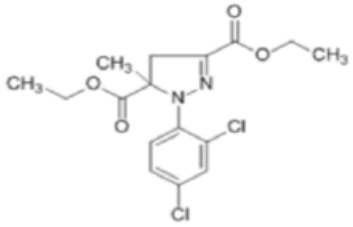
Structural formula:



Mefenpyr-diethyl

IUPAC Name:	diethyl(RS)-1-(2,4-dichlorophenyl)-4,5-dihydro-5-methyl-1H-pyrazole-3,5-dicarboxylate
Molecular Formula:	$C_{16}H_{18}Cl_2N_2O_4$
CAS No.:	135590-91-9
Purity:	98.8%
Series number:	BCBX4666
Expiry date:	May 2023
Manufacturing:	Sigma-Aldrich
Molecular weight:	373.23 g/Mol

Structural formula:



2. Method validation

Tabl. 1 Parameters of the validation

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Recovery

Matrix	Detected substance	Fortification Level [mg/kg]	Number of Replicate	Mean Recovery [%]	RSD [%]
Water	Mesosulfuron-methyl	0.1	5	103.0	3.9
		1.0	5	101.7	0.4
	Mefenpyr-diethyl	0.1	5	94.0	2.1
		1.0	5	103.3	0.2

Matrix effects

$$\text{Matrix effects [\%]} = 100 \times \frac{\text{peak area (matrix)}}{\text{peak area (solvent)}} - 100$$

The matrix effect is not exceed ± 20%.

Matrix	Detected substance	Concentration mg/L	Matrix effect [%]
Water	Mesosulfuron-methyl	0.05	10.3
	Mefenpyr-diethyl	0.05	8.1

Limit of Quantification (LOQ) and Limit of Detection (LOD)

LOQ	Equivalent calibration level	LOD	Equivalent calibration level
0.01 mg Mesosulfuron-methyl/L	~~0.1~~ 0.05 µg/ml	~~0.02~~ 0.04 mg Mesosulfuron-methyl/L	0.02 µg/ml
0.01 mg Mefenpyr-diethyl/L	~~0.1~~ 0.05 µg/ml	~~0.02~~ 0.04 Mefenpyr-diethyl/L	0.02 µg/ml

3.Calculations

The concentration (P) of mesosulfuron-methyl and mefenpyr-diethyl in water for fortified and test samples was calculated as follows (1):

$$P = c \cdot \frac{V_k}{V_p} \cdot w \quad (1)$$

where:

c is the concentration of the detected compound in the final solution determined on the basis of the calibration curve (µg/mL)

V_k is the final volume of the solution (mL)

v_p is the volume of the sample (mL)

w is a conversion factor (w/w= 1000/1000=1)

Example of Calculation – fortified samples

The concentration (P) [mg/L] of mefenpyr-diethyl in fortified sample 0.1 mg/L was calculated as follow:

The following values were used in this calculation:

Lab sample description	water 0.1 mg-L validation method_004
concentration (c) [mg/L]	0.0478
final volume of the solution (V_k) [mL]	4
volume of the sample (V_p) [mL]	2
conversion factor (w)	1/1=1

Concentration was calculated as follow:

$$P\ (mg/L) = c \cdot \frac{V_k}{V_p} \cdot w$$

P=0.0478*4/2*1= 0.096 mg/L

Example of Calculation – test samples

The concentration (P) [mg/L] of mesosulfuron-methyl in test sample 500 ml/ha was calculated as follow:

The following values were used in this calculation:

Lab sample description	500 ml-ha_definitive test G-49-20_031
concentration (c) [mg/L]	2.3973
final volume of the solution (V_k) [mL]	40
volume of the sample (V_p) [mL]	2
conversion factor (w)	1

Concentration was calculated as follow:

$$P = c \cdot \frac{V_k}{V_p} \cdot w$$

P=2.3973*40/2*1=47.946 mg/L

*Numerical values in this report are frequently rounded to a smaller degree of precision (number of digits) than were used in the actual calculation to increase readability and to indicate the approximate precision of the reported results. Minor differences in the results obtained with such "rounded" values in comparison to those obtained with higher precision values are well within the limits of the experimental accuracy and therefore of no practical concern.