FINAL REGISTRATION REPORT

Part B

Section 5

Analytical Methods

Detailed summary of the risk assessment

Product code: **MEZOFLOR 103 SC**

Product names: **MEZOFLOR 103 SC / FLOCORN 103 SC**

Chemical active substances:

Mesotrione, 100 g/L
Florasulam, 3 g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(authorization)

Applicant: **Synthos Agro Sp. z o.o.**

Submission date: 07/2023

Finalisation date: 12/2023, 12/2024

Version history

When	What
07/2023	Initial dRR
04/2024	Update of KCP 5.1.2 (analitical methods for ecotoxicology studies)
12/2023	zRMS assessment of dRR
12/2024	The final Registration Report

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5 Analytical methods

5.1 Conclusion and summary of assessment

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
Maize	Supported

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

5.2.1 Analysis of the plant protection product (KCP 5.1.1)

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

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

Comments of zRMS:	The proposed analytical method is suitable for the simultaneous determination of active substances mesotrione and florasulam in plant protection product MEZOFLOR 103 SC. The proposed analytical method has been fully validated in terms of specificity, linearity, repeatability, and accuracy. The proposed method fulfils the requirements of SANCO/3030/99 rev.5 guidance. The validation of the analytical method has been accepted.

Reference:	KCP 2.1 – KCP 2.11
Report	MEZOFLOR 103 SC, PART I: Determination of physicochemical properties of the initial preparation, after accelerated and low temperature storage, Enzo Arévalo, Warsaw 2021, BF-20/21
Guideline(s):	Yes, SANCO 3030/99 rev.5
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Test Item: MEZOFLOR 103 SC
Batch number: SNS-H-05-18
Producer: Synthos Agro

Production date: 01.2021
Expiration date: 01.2023

Method validation for determination of mesotrione and florasulam content was performed using high performance liquid chromatography (HPLC) with DAD detector and external standard method.

Reagents, standards and equipment:
Analytical standards:
Mesotrione, IPO 941, Series No. 1A/18, purity 99.8% .
Florasulam, IPO 940, Series No. 1A/18, purity 99.7%.
Reagents:
Deionized water, ultra-pure, Millipore
Acetonitrile for HPLC – Super gradient, POCh
o-phosphoric acid 85%, Chempur
Equipment:
Shimadzu liquid chromatograph equipped with DAD detector,
Column: Kinetex EVO, 250 × 4.6 mm, 5 µm,
Analytical balance RADWAG AS82/220 X2, accuracy 0.01 mg,
Typical laboratory equipment.

Chromatographic conditions:
Column temperature: 30 ºC
Mobile phase: acetonitrile (MeCN) : 0.1% aqueous solution of H_3PO_4 (28% + 72%; v/v)
0 - 10 min: 28% MeCN : 72% 0.1% H_3PO_4
10.01 - 18 min: 80% MeCN : 20% 0.1% H_3PO_4
18.01 - 30 min: 28% MeCN : 72% 0.1% H_3PO_4
Flow rate: 1.4 ml/min
Wavelength. λ = 220 nm for mesotrione and λ = 259 nm for florasulam
Volume of sample injected: 5 µl
Under above conditions retention time of mesotrione was 9.7 min ± 0.3 min and for florasulam was 11.5 min ± 0.3 min.

Preparation of solutions
Standard solution - mesotrione
15.37 mg of standard was weighed (with the accuracy of 0.01 mg) into 10 ml flask and acetonitrile was added up to the volume. The flask was put into the ultrasonic bath for 5 min. After cooling, the standard solution was appropriately diluted, mixed with florasulam standard solution and analysed.
Standard solution - florasulam
20.20 mg of standard was weighed (with the accuracy of 0.01 mg) into 10 ml flask and acetonitrile was added up to the volume. The flask was put into the ultrasonic bath for 5 min. After cooling, the standard solution was appropriately diluted, mixed with mesotrione standard solution and analysed.
Specimen solution
About 100 mg of preparation was weighed (with the accuracy of 0.01 mg) into a 50 ml flask, 2 ml of water was added and acetonitrile was added up to the volume. The flask was put into the ultrasonic bath for 5 min. After cooling, the solution was analysed.

Validation - Results and discussions

Table 5.2-1: Methods suitable for the determination of active substances mesotrione and florasulam in plant protection product MEZOFLOR 103 SC

	mesotrione	florasulam
Author(s), year	Enzo Arévalo, 2021	
Principle of method	The method for determination of mesotrione and florasulam was carried out using reversed phase high performance liquid chromatography (RP-HPLC) with DAD detection using external standard. Chromatographic conditions: Column temperature: 30 ºC Mobile phase: acetonitrile (MeCN) : 0.1% aqueous solution of H_3PO_4 (28% + 72%; v/v) 0 - 10 min: 28% MeCN : 72% 0.1% H_3PO_4 10.01 - 18 min: 80% MeCN : 20% 0.1% H_3PO_4 18.01 - 30 min: 28% MeCN: 72% 0.1% H_3PO_4 Flow rate: 1.4 ml/min Wavelength. λ = 220 nm for mesotrione and λ = 259 nm for florasulam Volume of sample injected: 5 µl Under above conditions retention time of mesotrione was 9.7 min ± 0.3 min. and for florasulam was 11.5 min ± 0.3 min.	
Linearity (linear between mg/L / % range of the declared content) (correlation coefficient, expressed as r)	The linearity of the detector response was assessed using five mixtures of standards solutions at the concentration range from 0.0767 mg/ml to 0.3068 mg/ml for mesotrione which corresponds to the concentration range from 38.7% to 154.7% of determined mesotrione content in the preparation. All solutions were analysed twice. Y = 13469527.2569 * X + 34864.8003 R^2 = 0.9997 r = 0.99985	The linearity of the detector response was assessed using five mixtures of standards solutions at the concentration range from 0.0020 mg/ml to 0.0101 mg/ml for florasulam which corresponds to the concentration range from 34.6% to 173.2% of determined florasulam content in the preparation. All solutions were analysed twice. Y = 6297307.7649 * X + 839.1000 R^2 = 0.9986 r = 0.9993
Precision – Repeatability Mean n = 6 (%RSD)	RSD = 0.67 % H_r=0.35 The repeatability of the method was assessed on the basis of six determinations of mesotrione content in the examined material. The precision of the method was expressed as the relative standard deviation (RSD). Acceptable relative standard deviation for active substance -mesotrione (C ~9.6 %) according SANCO/3030/99 (Horwitz coefficient) is RSDr ≥ ≤ 1.91 %. The obtained result 0.67 % and the Horrat value Hr = 0.35 is acceptable.	RSD = 2.24 % H_r=0.69 The repeatability of the method was assessed on the basis of six determinations of florasulam content in the examined material. The precision of the method was expressed as the relative standard deviation (RSD). Acceptable relative standard deviation for active substance - florasulam (C ~0.28 %) according SANCO/3030/99 (Horwitz coefficient) is RSDr ≥ ≤ 3.25 %. The obtained result 2.24 % nd the Horrat value Hr = 0.69 is acceptable.
Accuracy n = 12 (% Recovery)	Recovery 102.28 % The accuracy of the active ingredient determination in MEZOFLOR 103 SC was estimated by the recovery measurement. Known amount of the mesotrione and florasulam standard	Recovery 103.44 % The accuracy of the active ingredient determination in MEZOFLOR 103 SC was estimated by the recovery measurement. Known amount of the mesotrione and florasulam standard

	mesotrione	**florasulam**
	mixture were added to twelve weightings of the placebo. The accuracy was determined by spiking performed at two levels of concentrations of the active ingredients. The average recovery value for the active substance mesotrione (1-10 %) should be 100 ± 10 %. The obtained result 102.28 % is acceptable.	mixture were added to twelve weightings of the placebo. The accuracy was determined by spiking performed at two levels of concentrations of the active ingredients. The average recovery value for the active substance florasulam (0.1-1 %) should be 100 ± 20 %. The obtained result 103.44 % is acceptable.
Interference/ Specificity	Chromatograms of mobile phase, placebo, standards mixture solution, examined sample were performed and superimposed. On the chromatogram of placebo there were no peaks interfering with the determined compounds.	Chromatograms of mobile phase, placebo, standards mixture solution, examined sample were performed and superimposed. On the chromatogram of placebo there were no peaks interfering with the determined compounds.
Comment	The validation parameters are within the acceptance range and fulfil EU requirements given in SANCO /3030 /99 rev.5.	The validation parameters are within the acceptance range and fulfil EU requirements given in SANCO /3030 /99 rev.5.

Conclusion

It was confirmed that chromatographic methods of determination of the active compounds (mesotrione and florasulam) are specific. No interference was observed. The validation parameters are within the acceptance range and fulfil EU requirements given in SANCO /3030 /99 rev.5.

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

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

Comments of zRMS:	The analytical methods for the determination of relevant impurities (IMP1, IMP2, 2,6-DFA and 1,2-dichloroethane) in plant protection product MEZOFLOR 103 SC are suitable for the determination of the content of each of the relevant impurity in the presence of each other, active substance and other components. The methods have been fully validated. The validation parameters of proposed analytical methods – interference, specificity, linearity, recovery, repeatability, and LOQ values fulfil the requirements of SANCO/3030/99 rev. 5 guidance. The validation of the analytical methods has been accepted.

Reference:	KCP 2.1 – KCP 2.11
Report	MEZOFLOR 103 SC, PART I: Determination of physicochemical properties of the initial preparation, after accelerated and low temperature storage, Enzo Arévalo, Warsaw 2021, BF-20/21
Guideline(s):	Yes, SANCO 3030/99 rev.5
Deviations:	No

GLP: Yes

Acceptability: Yes

Materials and methods

Test Item: MEZOFLOR 103 SC
Batch number: SNS-H-05-18
Producer: Synthos Agro
Production date: 01.2021
Expiration date: 01.2023

Determination of 1-cyano-6-(methylsulfonyl)-7-nitro-9H-xanthen-9-one (IMP 1)

The following standard was used as reference item:

- 1-cyano-6-(methylsulfonyl)-7-nitro-9H-xanthen-9-one (IMP 1), 97.0%, EPP Ltd., Series No. EPP/JMK 443

Reagents and equipment:

- Sciex QTRAP 4500 mass spectrometer with UHPLC
- Column: Luna Omega Polar PS C18, 100 × 2,1 mm, Phenomenex
- Analytical balance RADWAG AS82/220 X2, accuracy 0.01 mg
- Typical laboratory equipment

Reagents:

- Deionized water, ultra-pure, Millipore
- Acetonitrile hypergrade for LC-MS, Supelco
- Formic acid > 95%, Sigma-Aldrich
- Ammonium formate ≥ 99.995%, Sigma-Aldrich

Analytical method:

The method for determination of 1-cyano-6-(methylsulfonyl)-7-nitro-9H-xanthen-9-one was performed using UHPLC chromatography with MS/MS detection using external standard method.

Chromatographic conditions:

- Column temperature: 30 ^{o}C
- Mobile phase: 5 mmol aqueous solution of ammonium formate + 0.1% aqueous solution of formic acid + 5 mmol acetonitrile solution of ammonium formate + 0.1% acetonitrile solution of formic acid;
- Flow rate: 0.4 ml/min (gradient flow)
- Volume of sample injected: 10 µl

A triple quadrupole MS equipped with a positive electrospray ionization source was used in the MRM mode. The equipment was set with a drying gas flow, nebulizer pressure, gas temperature. A spray voltage of 5500 V was used for MS.

The MS conditions of MRM for IMP 1 was:

- Precursor ion m/z: 362.0
- Product ions m/z: 234.9* and 206.1 (* used for calculation)
- Collision energy (CE) (eV): 36 and 68 respectively to product ion
- Delustering potential (DP): 28

Under above conditions retention time of IMP 1 was 6.30 min ± 0.20 min.

Preparation of the specimen solution
About 200 mg of examined specimen was weighted into a 10 ml volumetric flask, then acetonitrile was added up to the mark. The content was mixed and the flask was put into the ultrasonic bath for 2 minutes. After cooling, the solution was analysed.

Determination of 6-(methylsulfonyl)-9-oxo-9H-xanthene-1-carbonitrile (IMP 2)
The following standard was used as reference item:

- 6-(methylsulfonyl)-9-oxo-9H-xanthene-1-carbonitrile (IMP 2), 99.74%, Galchimia,, Series No. ga0060004ba

Reagents and equipment:

- Sciex QTRAP 4500 mass spectrometer with UHPLC
- Column: Luna Omega Polar PS C18, 100 × 2,1 mm, Phenomenex
- Analytical balance RADWAG AS82/220 X2, accuracy 0.01 mg
- Typical laboratory equipment

Reagents:

- Deionized water, ultra-pure, Millipore
- Acetonitrile hypergrade for LC-MS, Supelco
- Formic acid > 95%, Sigma-Aldrich
- Ammonium formate ≥ 99.995%, Sigma-Aldrich

Analytical method:
The method for determination of 6-(methylsulfonyl)-9-oxo-9H-xanthene-1-carbonitrile was performed using UHPLC chromatography with MS/MS detection using external standard method.
Chromatographic conditions:

- Column temperature: 30 ^{o}C
- Mobile phase: 5 mmol aqueous solution of ammonium formate + 0.1% aqueous solution of formic acid + 5 mmol acetonitrile solution of ammonium formate + 0.1% acetonitrile solution of formic acid
- Flow rate: 0.4 ml/min; (gradient flow)
- Volume of sample injected: 10 µl

A triple quadrupole MS equipped with a positive electrospray ionization source was used in the MRM mode. The equipment was set with a drying gas flow, nebulizer pressure, gas temperature. A spray voltage of 5500 V was used for MS.
The MS conditions of MRM for IMP 2 was:

- Precursor ion m/z: 317.0
- Product ions m/z: 221.0* and 209.1 (* used for calculation)
- Collision energy (CE) (eV): 47 and 43 respectively to product ion
- Delustering potential (DP): 25

Under above conditions retention time of IMP 2 was 6.10 min ± 0.20 min.

Preparation of the specimen solution:
About 200 mg of examined specimen was weighted into a 10 ml volumetric flask, then acetonitrile was added up to the mark. The content was mixed and the flask was put into the ultrasonic bath for 2 minutes. After cooling, the solution was analysed.

Determination of 2,6-difluoroaniline (2,6-DFA)

The following standard was used as reference item:

- 2,6-difluoroaniline (2,6-DFA), 99.90%, Sigma-Aldrich, Series No. STBJ0362

Reagents and equipment:

- Sciex QTRAP 4500 mass spectrometer with UHPLC
- Column: Luna Omega Polar PS C18, 100 × 2,1 mm, Phenomenex
- Analytical balance RADWAG AS82/220 X2, accuracy 0.01 mg
- Typical laboratory equipment

Reagents:

- Deionized water, ultra-pure, Millipore
- Acetonitrile hypergrade for LC-MS, Supelco
- Formic acid > 95%, Sigma-Aldrich
- Ammonium formate ≥ 99.995%, Sigma-Aldrich

Analytical method:

The method for determination of 2,6-DFA was performed using UHPLC chromatography with MS/MS detection using external standard method.

Chromatographic conditions:

- Column temperature: 30 ºC
- Mobile phase: 5 mmol aqueous solution of ammonium formate + 0.1% aqueous solution of formic acid + 5 mmol acetonitrile solution of ammonium formate + 0.1% acetonitrile solution of formic acid.
- Flow rate: 0.4 ml/min (gradient flow)
- Volume of sample injected: 10 µl

The MS conditions of MRM for 2,6-DFA was:

- Precursor ion m/z: 130.1
- Product ions m/z: 110.1* and 90.1 (* used for calculation)
- Collision energy (CE) (eV): 20 and 22 respectively to product ion
- Delustering potential (DP): 20

Under above conditions retention time of 2,6-DFA was 5.98 min ± 0.20 min.

Preparation of the specimen solution

About 200 mg of examined specimen was weighted into a 10 ml volumetric flask, then acetonitrile was added up to the mark. The content was mixed and the flask was put into the ultrasonic bath for 2 minutes. After cooling, the solution was analysed.

Determination of 1,2-dichloroethane

The content of 1,2-dichloroethane in the examined preparation was determined using headspace analysis in combination with gas chromatography and flame ionization detection (HS-GC-FID) using external standard method.

Chromatographic conditions:

- Oven: 45 ˚C (1 min), 10 ˚C/min → 110 ˚C (0 min), 20 ˚C /min → 250 ˚C
- Carrier gas: Helium
- Flow: 2 ml/min
- Inlet temperature: 250 ˚C
- Detector temperature: 300 ºC

- Split ratio: 1:20

Auxiliary gases flow:

- nitrogen: 25 ml/min
- hydrogen: 30 ml/min
- air: 300 ml/min

Headspace Autosampler conditions:

- Valve oven temperature: 110 ˚C
- Transfer line temperature: 120 ˚C
- Platen/sample temperature: 80 ˚C
- Sample equilibration time: 10 min
- Mixing time: 2 min (Level 5)
- Pressurization: 7 psig
- Loop fill pressure: 5 psig
- Loop volume: 1 ml
- Injection time: 1 min

Under the above conditions retention time of 1,2-dichloroethane was 3.99 ± 0.05 min and the total time of analysis is 14.5 min.

Preparation of the specimen solution:

Specimen solution

About 200 mg of the examined preparations were placed in four headspace vials and then 2 ml DMSO was added. The vials were tightly closed and analysed.

Table 5.2-2: Methods suitable for the determination of the relevant impurities in plant protection product (PPP) MEZOFLOR 103 SC

	1-cyano-6-(methylsulfonyl)-7-nitro-9H-xanthen-9-one (IMP 1)	6-(methylsulfonyl)-9-oxo-9H-xanthene-1-carbonitrile (IMP 2)	2,6-difluoroaniline (2,6-DFA)	1,2-dichloroethane
Author(s), year	Enzo Arévalo, 2021			
Principle of method	The content of IMP 1 in the examined sample was determined using UHPLC chromatography with MS/MS detection using external standard method.	The method for determination of IMP2 was performed using UHPLC chromatography with MS/MS detection using external standard method.	The method for determination of 2,6-DFA was performed using UHPLC chromatography with MS/MS detection using external standard method.	The method for determination of the 1,2-dichloroethane content was performed by headspace analysis in combination with gas chromatography and flame ionization detection (HS-GC-FID) using external standard method.
Linearity (linear between mg/L) (correlation coefficient, expressed as r)	The linearity of the detector response was assessed using eight standards solutions at the concentration range from 0.00043 µg/ml to 0.02561 µg/ml for IMP1 which	The linearity of the detector response was assessed using six standards solutions at the concentration range from 0.00015 µg/ml to 0.29204 µg/ml for IMP2 which	The linearity of the detector response was assessed using six standards solutions at the concentration range from 0.00039 µg/ml to 0.3873 µg/ml for 2,6-DFA which	The linearity of the detector response was assessed using six standards solutions at the concentration range from 0.002 mg/ml to 0.02 mg/ml for 1,2-dichloroethane which

	corresponds to the concentration range from 10.7% to 640.2% of maximum acceptable limit (FAO) for IMP1 content in the preparation. All solutions were analysed at least twice. R^2 = 0.9991 r = 0.9995	corresponds to the concentration range from 0.04% to 73.01% of maximum acceptable limit (FAO) for IMP2 content in the preparation. All solutions were analysed at least twice. R^2 = 1.0000 r = 1.0000	corresponds to the concentration range from 0.3% to 322.7% of maximum acceptable limit (FAO) for 2,6-DFA content in the preparation. All solutions were analysed at least twice. R^2 = 0.9998 r = 0.9999	corresponds to the concentration range from 10% to 120% of maximum acceptable limit (FAO) for 1,2-dichloroethane content in the preparation. R^2 = 0.9998 r = 0.9999
Precision – Repeatability Mean n = 6 (%RSD)	RSD = 5.34 % H_r=0.28 The method repeatability was assessed on the basis of six independent determinations of IMP1 content in MEZOFLOR 103 SC preparation. In none of the examined samples IMP1 was detected above the LOQ. Therefore, for the determination of repeatability six portions of placebo fotified with IMP1 at LOQ level (0.00043 µg/ml) were analyzed. The precision of the method was expressed as the relative standard deviation (RSD). Acceptable relative standard deviation for IMP1 (C ~0.000002 %) is RSDr ≧ ≤ 19.32 %. The obtained result 5.34 % is acceptable.	RSD = 2.13 % H_r=0.13 The method repeatability was assessed on the basis of six independent determinations of IMP2 content in MEZOFLOR 103 SC preparation. The precision of the method was expressed as the relative standard deviation (RSD). Acceptable relative standard deviation for IMP2 (C ~0.0000055 %) is RSDr ≧ ≤ 16.61 %. The obtained result 2.13 % is acceptable.	RSD = 4.85 % H_r=0.25 The method repeatability was assessed on the basis of six independent determinations of 2,6-DFA content in MEZOFLOR 103 SC preparation. In none of the examined samples 2,6-DFA was detected above the LOQ. Therefore, for the determination of repeatability six portions of placebo fotified with 2,6-DFA at LOQ level (0.00039 µg/ml) were analyzed. The precision of the method was expressed as the relative standard deviation (RSD). Acceptable relative standard deviation for 2,6-DFA (C ~0.000002 %) is RSDr ≧ ≤ 19.32 %. The obtained result 4.85 % is acceptable.	RSD = 3.18 % H_r=0.42 The method repeatability was assessed on the basis of six independent determinations of 1,2-dichloroethane content in MEZOFLOR 103 SC preparation. In none of the examined samples 1,2-dichloroethane was detected above the LOQ. Therefore, for the determination of repeatability six portions of placebo fotified with 1,2-dichloroethane at LOQ level (0.0020 mg/ml) were analyzed. The precision of the method was expressed as the relative standard deviation (RSD). Acceptable relative standard deviation for 1,2-dichloroethane (C ~0.001 %) is RSDr ≧ ≤ 7.58 %. The obtained result 3.18 % is acceptable.
Accuracy n = 12 (% Recovery)	Recovery (total): 100.44 %; level I = 96.05%, RSD = 5.34%; level II = 104.83%, RSD = 2.02%. The accuracy of the IMP1 determination in MEZOFLOR 103 SC was estimated by the recovery measurement. Known amount of the IMP1 standard mixture	Recovery (total): 95.08 %; level I = 90.91%, RSD = 1.34%; level II = 99.26%, RSD = 3.41%. The accuracy of the IMP2 determination in MEZOFLOR 103 SC was estimated by the recovery measurement. Known amount of the IMP2 standard mixture	Recovery (total): 98.82 %; level I = 93.47%, RSD = 4.85%; level II = 104.18%, RSD = 8.33%. The accuracy of the 2,6-DFA determination in MEZOFLOR 103 SC was estimated by the recovery measurement. Known amount of the 2,6-DFA	Recovery (total): 96.4 %, RSD = 3.16% The accuracy of the 1,2-dichloroethane determination in MEZOFLOR 103 SC was estimated by the recovery measurement. Known amount of the 1,2-dichloroethane standard mixture were added to twelve

	were added to twelve weightings of the placebo. The accuracy was determined by spiking performed at two levels of concentrations of IMP1. The average recovery value for IMP1 (< 0.01%) should be 100 ± 30 %. The obtained result 100.44 % is acceptable.	were added to twelve weightings of the placebo. The accuracy was determined by spiking performed at two levels of concentrations of IMP2. The average recovery value for IMP2 (< 0.01%) should be 100 ± 30 %. The obtained result 95.08 % is acceptable.	standard mixture were added to twelve weightings of the placebo. The accuracy was determined by spiking performed at two levels of concentrations of 2,6-DFA. The average recovery value for 2,6-DFA (< 0.01%) should be 100 ± 30 %. The obtained result 98.82 % is acceptable.	weightings of the placebo. The accuracy was determined by spiking performed at two levels of concentrations of 1,2-dichloroethane. The average recovery value for 1,2-dichloroethane (< 0.01%) should be 100 ± 30 %. The obtained result 96.4 % is acceptable.
Interference/ Specificity	Chromatograms of standard solution, sample solution, placebo, placebo solution fortified at LOQ level, solvent were performed and superimposed. There are no interferences between the analyte and other components of the specimen. Total ion chromatogram and MS/MS spectra were included.	Chromatograms of standard solution, sample solution, placebo, placebo solution fortified at LOQ level, solvent were performed and superimposed. There are no interferences between the analyte and other components of the specimen. Total ion chromatogram and MS/MS spectra were included.	Chromatograms of standard solution, sample solution, placebo, placebo solution fortified at LOQ level, solvent were performed and superimposed. There are no interferences between the analyte and other components of the specimen. Total ion chromatogram and MS/MS spectra were included.	Chromatograms of standard solution in DMSO, sample solution in DMSO, placebo in DMSO, solvent (DMSO) were performed and superimposed. There are no interferences between the analyte and other components of the specimen.
LOQ	LOQ of IMP1 in MEZOFLOR 103 SC preparation was defined as the lowest concentration of injected standard that gave precise and accurate measurements. LOQ is 0.00043 µg/ml what corresponds to 0.000021 g/kg (0.021 ppm) of MEZOFLOR 103 SC, so 0.000214 g/kg of mesotrione.	LOQ of IMP2 in MEZOFLOR 103 SC preparation was defined as the lowest concentration of injected standard that gave precise and accurate measurements. LOQ is 0.00015 µg/ml what corresponds to 0.000007 g/kg (0.007 ppm) of MEZOFLOR 103 SC, so 0.000074 g/kg of mesotrione.	LOQ of 2,6-DFA in MEZOFLOR 103 SC preparation was defined as the lowest concentration of injected standard that gave precise and accurate measurements. LOQ is 0.00039 µg/ml what corresponds to 0.000019 g/kg (0.019 ppm) of MEZOFLOR 103 SC, so ~~0.000197~~ 0.0063 g/kg of florasulam	LOQ of 1,2-dichloroethane in MEZOFLOR 103 SC preparation was defined as the lowest quantity of injected standard that gave precise and accurate measurements. LOQ is 0.002 mg/ml what corresponds to 0.01 g/kg (0.001%) of MEZOFLOR 103 SC, so ~~0.001~~ 0.1 g/kg of mesotrione
Comment	The determined validation parameters such as specificity, linearity, limit of quantification (LOQ), repeatability (precision) and accuracy are compliant with EU requirements given in SANCO/3030/99 rev.5.	The determined validation parameters such as specificity, linearity, limit of quantification (LOQ), repeatability (precision) and accuracy are compliant with EU requirements given in SANCO/3030/99 rev.5.	The determined validation parameters such as specificity, linearity, limit of quantification (LOQ), repeatability (precision) and accuracy are compliant with EU requirements given in SANCO/3030/99 rev.5.	The determined validation parameters such as specificity, linearity, limit of quantification (LOQ), repeatability (precision) and accuracy are compliant with EU requirements given in SANCO/3030/99 rev.5.

Conclusion

It was confirmed that chromatographic methods of determination of the relevant impurities (IMP1, IMP2, 2,6-DFA and 1,2-dichloroethane) are specific. No interference was observed. The validation parameters (linearity, LOQ, repeatability and accuracy) are within the acceptance range and fulfil EU requirements given in SANCO /3030 /99 rev. 5.

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

With respect to toxicological, eco-toxicological or environmental aspects MEZOFLOR 103 SC does not contain any relevant formulants. Therefore, a special analytical method and validation is not needed.

5.2.1.4 Applicability of existing CIPAC methods (KCP 5.1.1)

There are no CIPAC method for determination mesotrione and florasulam in the product of SC formulation.

5.2.2 Methods for the determination of residues (KCP 5.1.2)

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

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

Component of residue definition: mesotrione				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Soil (ecotoxicology)	Primary	0.20 mg/kg	HPLC-DAD	Paweł Pieczka, 2021a
	Confirmatory (if required)	Not required due to specific method to the analytes. According to SANTE/2020/12830 rev.1		
Soil (ecotoxicology)	Primary	0.2 mg/kg	HPLC-DAD	Magdalena Czarnynoga, 2024 Małgorzata Górska, 2024
	Confirmatory (if required)	Not required due to specific method to the analytes. According to SANTE/2020/12830 rev.2		
Water (ecotoxicology)	Primary	0.20 mg/L	HPLC-DAD	Paweł Pieczka, 2021b
	Confirmatory (if required)	Not required due to specific method to the analytes. According to SANTE/2020/12830 rev.1		
Water (ecotoxicology)	Primary	0.20 mg/L	HPLC-DAD	Magdalena Wołany, 2021
	Confirmatory (if required)	Not required due to specific method to the analytes. According to SANTE/2020/12830 rev.1		
Water (ecotoxicology)	Primary	0.20 mg/L	HPLC-DAD	Małgorzata Czarnecka, 2021
	Confirmatory (if required)	Not required due to specific method to the analytes. According to SANTE/2020/12830 rev.1		

Water (ecotoxicology)	Primary	0.20 mg/L	HPLC-DAD	Ewa Nierzędska, 2021
	Confirmatory (if required)	Not required due to specific method to the analytes. According to SANTE/2020/12830 rev.1		
Water (Honeybees Tests)	Primary	3.03943 mg/L	HPLC-DAD	Marcin Świstak, 2021
	Confirmatory (if required)	Not required due to specific method to the analytes. According to SANTE/2020/12830 rev.1		
Water (Bumblebees Tests)	Primary	0.2 mg/L	HPLC-DAD	Elżbieta Kulec-Płoszczyca, 2021c
	Confirmatory (if required)	Not required due to specific method to the analytes. According to SANTE/2020/12830 rev.1		
Sucrose solution (Honeybees Tests)	Primary	1.0 mg/kg	HPLC-DAD	Elżbieta Kulec-Płoszczyca, 2021a
	Confirmatory (if required)	Not required due to specific method to the analytes. According to SANTE/2020/12830 rev.1		
Sucrose solution (Bumblebees Tests)	Primary	1.0 mg/kg	HPLC-DAD	Elżbieta Kulec-Płoszczyca, 2021b
	Confirmatory (if required)	Not required due to specific method to the analytes. According to SANTE/2020/12830 rev.1		
Component of residue definition: florasulam				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Soil (ecotoxicology)	Primary	0.20 mg/kg	HPLC-DAD	Paweł Pieczka, 2021a
	Confirmatory (if required)	Not required due to specific method to the analytes. According to SANTE/2020/12830 rev.1		
Soil (ecotoxicology)	Primary	0.2 mg/kg	HPLC-DAD	Magdalena Czarnynoga, 2024 Małgorzata Górska, 2024
	Confirmatory (if required)	Not required due to specific method to the analytes. According to SANTE/2020/12830 rev.2		
Water (ecotoxicology)	Primary	0.001 mg/L	HPLC-DAD	Paweł Pieczka, 2021b
	Confirmatory (if required)	Not required due to specific method to the analytes. According to SANTE/2020/12830 rev.1		
Water (ecotoxicology)	Primary	0.001 mg/L	HPLC-DAD	Magdalena Wołany, 2021
	Confirmatory (if required)	Not required due to specific method to the analytes. According to SANTE/2020/12830 rev.1		
Water (ecotoxicology)	Primary	0.20 mg/L	HPLC-DAD	Małgorzata Czarnecka, 2021
	Confirmatory (if required)	Not required due to specific method to the analytes. According to SANTE/2020/12830 rev.1		
Water (ecotoxicology)	Primary	0.20 mg/L	HPLC-DAD	Ewa Nierzędska, 2021
	Confirmatory (if required)	Not required due to specific method to the analytes. According to SANTE/2020/12830 rev.1		
Water (Honey bee Tests)	Primary	0.11341 mg/L	HPLC-DAD	Marcin Świstak, 2021
	Confirmatory (if required)	Not required due to specific method to the analytes. According to SANTE/2020/12830 rev.1		

Water (Bumblebees Tests)	Primary	0.2 mg/L	HPLC-DAD	Elżbieta Kulec-Płoszczyca, 2021c
	Confirmatory (if required)	Not required due to specific method to the analytes. According to SANTE/2020/12830 rev.1		
Sucrose solution (Honeybees Tests)	Primary	1.0 mg/kg	HPLC-DAD	Elżbieta Kulec-Płoszczyca, 2021a
	Confirmatory (if required)	Not required due to specific method to the analytes. According to SANTE/2020/12830 rev.1		
Sucrose solution (Bumblebees Tests)	Primary	1.0 mg/kg	HPLC-DAD	Elżbieta Kulec-Płoszczyca, 2021b
	Confirmatory (if required)	Not required due to specific method to the analytes. According to SANTE/2020/12830 rev.1		

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

5.3.1 Analysis of the plant protection product (KCP 5.2)

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

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

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

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

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

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Plant, high water content	Mesotrione	0.01 mg/kg	Reg. (EU) 2017/626 Reg. (EU) 2024/1077
Plant, high acid content		0.01 mg/kg	Reg. (EU) 2017/626 Reg. (EU) 2024/1077
Plant, high protein/high starch content (dry commodities)		0.01 mg/kg	Reg. (EU) 2017/626 Reg. (EU) 2024/1077
Plant, high oil content		0.01 mg/kg	Reg. (EU) 2017/626 Reg. (EU) 2024/1077

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Plant, difficult matrices (hops, spices, tea)		0.05 mg/kg	Reg. (EU) 2017/626 Reg. (EU) 2024/1077
Muscle	Not required (provisional)	0.01 mg/kg	Reg. (EU) 2017/626 Reg. (EU) 2024/1077
Milk		0.01 mg/kg	Reg. (EU) 2017/626 Reg. (EU) 2024/1077
Eggs		0.01 mg/kg	Reg. (EU) 2017/626 Reg. (EU) 2024/1077
Fat		0.01 mg/kg	Reg. (EU) 2017/626 Reg. (EU) 2024/1077
Liver, kidney		0.01 mg/kg	Reg. (EU) 2017/626 Reg. (EU) 2024/1077
Soil (Ecotoxicology)	Mesotrione, MNBA, AMBA	Mesotrione: 0.96 mg/kg 0.002 mg/kg MNBA: 1.13 m/kg 0.002 mg/kg AMBA: 1.13 mg/kg 0.002 mg/kg	~~Mesotrione: NOEC for earthworm reproduction; Pawel Pieczka, 2021 G-63-20~~ ~~No significant adverse effect was observed during tests on soil microbial; EFSA Journal 2016;14(3):4419~~ ~~No significant adverse effect was observed during tests on soil microbial~~; EFSA Journal 2016;14(3):4419
Drinking water (Human toxicology)	Mesotrione, MNBA, AMBA	0.1 µg/L 0.05 µg/L	general limit for drinking water EFSA Journal 2016;14(3):4419
Surface water (Ecotoxicology)	Mesotrione, MNBA, AMBA	Mesotrione: 7.7 µg/L MNBA: 42000 µg/L AMBA: 14000 µg/L	ErC50 for growth inhibition of aquatic macrophytes; EFSA Journal 2016;14(3):4419 ErC50 for growth inhibition of algae; EFSA Journal 2016;14(3):4419 ErC50 for growth inhibition of algae; EFSA Journal 2016;14(3):4419
Air	Mesotrione	LOQ= 0.45 µg/m^3; EFSA Journal 2016;14(3):4419	AOEL inhal: 0.005 mg/kg bw/d; EFSA Journal 2016;14(3):4419
Tissue (meat or liver)	No residue relevant	0.01 mg/kg	Default LOQ SANTE/2020/12830, Rev.1
Body fluids		0.01 mg/kg	Default LOQ

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
			SANTE/2020/12830, Rev.1

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

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

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

Component of residue definition: mesotrione				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
High water content	Primary	0.01 mg/kg	LC-MS/MS	Crook S., 2002, included in RAR for mesotrione, Vol. 3, part B.5
High protein/high starch content (dry)		0.01 mg/kg	LC-MS/MS	Watson G., 2013a, included in RAR for mesotrione, Vol. 3, part B.5
	ILV	0.01 mg/kg	LC-MS/MS	Tessier V., 2013, included in RAR for mesotrione, Vol. 3, part B.5
	Confirmatory (if required)	Confirmatory method is not required, LC-MS/MS is considered as highly specific method, according to SANTE 12830/2020		
High acid content	Primary	0.01 mg/kg	LC-MS/MS	Watson G., 2013a, included in RAR for mesotrione, Vol. 3, part B.5
	Confirmatory (if required)	Confirmatory method is not required, LC-MS/MS is considered as highly specific method, according to SANTE 12830/2020		
High oil content	Primary	0.01 mg/kg	LC-MS/MS	Watson G., 2013a, included in RAR for mesotrione, Vol. 3, part B.5
	Confirmatory (if required)	Confirmatory method is not required, LC-MS/MS is considered as highly specific method, according to SANTE 12830/2020		

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:	-

	Method for products of plant origin
Not required, because:	The residue level in metabolism studies does not occur at level ≥ 0.01 mg/kg. According to SANTE 2017/10632 extraction efficiency is not needed.

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

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

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

Component of residue definition: mesotrione				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Milk	Primary	0.01 mg/kg	LC-MS/MS	Watson G., 2013b, included in RAR for mesotrione, Vol. 3, part B.5
	ILV	0.01 mg/kg	LC-MS/MS	Bernal J., 2013, included in RAR for mesotrione, Vol. 3, part B.5
	Confirmatory (if required)	The use of LC-MS/MS is considered a sufficiently specific method therefore a supporting confirmatory method is not considered to be required.		
Eggs	Primary	0.01 mg/kg	LC-MS/MS	Watson G., 2013b, included in RAR for mesotrione, Vol. 3, part B.5
	ILV	0.01 mg/kg	LC-MS/MS	Bernal J., 2013, included in RAR for mesotrione, Vol. 3, part B.5
	Confirmatory (if required)	The use of LC-MS/MS is considered a sufficiently specific method therefore a supporting confirmatory method is not considered to be required.		
Muscle	Primary	0.01 mg/kg	LC-MS/MS	Watson G., 2013b, included in RAR for mesotrione, Vol. 3, part B.5
	Confirmatory (if required)	The use of LC-MS/MS is considered a sufficiently specific method therefore a supporting confirmatory method is not considered to be required.		
Fat	Primary	0.01 mg/kg	LC-MS/MS	Watson G., 2013b, included in RAR for mesotrione, Vol. 3, part B.5
	Confirmatory (if required)	The use of LC-MS/MS is considered a sufficiently specific method therefore a supporting confirmatory method is not considered to be required.		
Kidney, liver	Primary	0.01 mg/kg	LC-MS/MS	Watson G., 2013b, included in RAR for mesotrione, Vol. 3, part B.5
	ILV	0.01 mg/kg (liver)	LC-MS/MS	Bernal J., 2013, included in RAR for mesotrione, Vol. 3, part B.5
	Confirmatory (if required)	The use of LC-MS/MS is considered a sufficiently specific method therefore a supporting confirmatory method is not considered to be required.		

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

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

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

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

Component of residue definition: Mesotrione and its metabolites AMBA and MNBA			
Method type	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	0.02 mg/kg	LC-MS/MS	Jutsum L., Williams R., 2013, RAR for mesotrione, Vol. 3, part B.5
	0.002 mg/kg	LC-MS/MS	Jutsum L., 2013, RAR for mesotrione, Vol. 3, part B.5
Confirmatory	Not required according to SANTE 12830/2020 – LC-MS/MS is considered as a highly specific method		

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

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

An overview on the acceptable methods and possible data gaps for analysis of mesotrione in surface and drinking water is given in the following tables. For the detailed valuation of new / additional studies it is referred to Appendix 2.

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

Component of residue definition: mesotrione and its metabolites MNBA and AMBA				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Drinking water	Primary	0.05 µg/L (for mesotrione, AMBA and MNBA)	LC-MS/MS	Jutsum L., Chamkesam N., 2013, RAR for mesotrione, Vol. 3, part B.5
		0.05 µg/L	LC-MS/MS	Jutsum L., 2013a, RAR for mesotrione, Vol. 3, part B.5
	ILV	0.05 µg/L	LC-MS/MS	Wiesner F., Breyer N., 2013, RAR for mesotrione, Vol. 3, part B.5
	Confirmatory	Not required according to SANTE 12830/2020 – LC-MS/MS is considered as a highly specific method.		
Surface water	Primary	0.05 µg/L (for	LC-MS/MS	Jutsum L., Chamkesam N.,

Component of residue definition: mesotrione and its metabolites MNBA and AMBA				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
		mesotrione, AMBA and MNBA) 0.05 µg/L	 LC-MS/MS	2013, RAR for mesotrione, Vol. 3, part B.5 Jutsum L., 2013a, RAR for mesotrione, Vol. 3, part B.5
	Confirmatory	Not required according to SANTE 12830/2020 – LC-MS/MS is considered as a highly specific method		

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

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

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

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

Component of residue definition: mesotrione			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	0.45 µg/m^3 0.45 µg/m^3	LC-MS/MS LC-MS/MS	Jutsum L., 2013b, RAR to mesotrione, Vol.3, part B.5 Jutsum L., 2013c, RAR to mesotrione, Vol.3, part B.5
Confirmatory	Not required – LC-MS/MS is recognized as highly specific method according to SANTE 12830/2020		

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

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

An overview on the acceptable methods and possible data gaps for analysis of mesotrione in body fluids and tissues is given in the following table. For the detailed evaluation of new / additional studies it is referred to Appendix 2.

Table 5.3-6: Methods for body fluids and tissues (if appropriate)

Component of residue definition: mesotrione			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	0.01 mg/L	LC-MS/MS	Watson G., 2013b, RAR RAR to mesotrione, Vol.3, part B.5
Confirmatory	The use of LC-MS/MS is considered a sufficiently specific method therefore a supporting confirmatory method is not considered to be required.		

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

5.3.2.7 Other studies/ information

5.3.3 Description of analytical methods for the determination of residues of florasulam (KCP 5.2)

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

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

Table 5.3-7: 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	Florasulam	LOQ = 0.01 mg/kg	Reg. (EU) 2022/1363
Plant, high acid content		LOQ = 0.01 mg/kg	Reg. (EU) 2022/1363
Plant, high protein/high starch content (dry commodities)		LOQ = 0.01 mg/kg	Reg. (EU) 2022/1363
Plant, high oil content		LOQ = 0.01 mg/kg	Reg. (EU) 2022/1363
Plant, difficult matrices (hops, spices, tea)		LOQ = 0.05 mg/kg	Reg. (EU) 2022/1363
Muscle	Florasulam	LOQ = 0.01 mg/kg	Reg. (EU) 2022/1363
Milk		LOQ = 0.01 mg/kg	Reg. (EU) 2022/1363
Eggs		LOQ = 0.01 mg/kg	Reg. (EU) 2022/1363
Fat		LOQ = 0.01 mg/kg	Reg. (EU) 2022/1363
Liver, kidney		LOQ = 0.01 mg/kg	Reg. (EU) 2022/1363
Soil (Ecotoxicology)	Florasulam	LOQ = 0.05 µg/kg	EFSA Journal 2015;13(1): 3984

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Drinking water (Human toxicology)	Florasulam	LOQ = 0.05 µg/L	EFSA Journal 2015;13(1): 3984
Surface water (Ecotoxicology)	Florasulam	LOQ = 0.05 µg/L	EFSA Journal 2015;13(1): 3984
Air	Florasulam	LOQ = 1.30 mg/m^3	EFSA Journal 2015;13(1): 3984
Tissue (meat or liver)	Florasulam	0.01 mg/kg	Default LOQ SANTE/2020/12830, Rev.1
Body fluids			

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

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

Table 5.3-8: 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: florasulam				
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	Rodrigues Junior, A., 2011, RAR of florasulam, Volume 3, Annex B.5 point B.5.2.1.1
	ILV	0.01 mg/kg	LC-MS/MS	Bacher, R., 2011, RAR of florasulam, Volume 3, Annex B.5 point B.5.2.1.1b
	Confirmatory (if required)	LC-MS/MS is known to be highly specific, no confirmatory method is required		
High acid content	Primary	0.01 mg/kg	LC-MS/MS	Rodrigues Junior A., 2011, RAR of florasulam, Volume 3, Annex B.5 point B.5.2.1.1
	ILV	0.01 mg/kg	LC-MS/MS	Bacher, R., 2011, RAR of florasulam, Volume 3, Annex B.5 point B.5.2.1.1b
	Confirmatory (if required)	LC-MS/MS is known to be highly specific, no confirmatory method is required		
High oil content	Primary	0.01 mg/kg	LC-MS/MS	Rodrigues Junior A., 2011, RAR of florasulam, Volume 3, Annex B.5 point B.5.2.1.1
	ILV	According to SANTE 12830/2020 ILV method is sufficient for two matrices, if a primary method is identical for all matrices performed.		
	Confirmatory	LC-MS/MS is known to be highly specific, no confirmatory method is		

Component of residue definition: florasulam				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
	(if required)	required		
High protein/high starch content (dry)	Primary	0.01 mg/kg	LC-MS/MS	Rodrigues Junior A., 2011, RAR of florasulam, Volume 3, Annex B.5 point B.5.2.1.1
	ILV	According to SANTE 12830/2020 ILV method is sufficient for two matrices, if a primary method is identical for all matrices performed.		
	Confirmatory (if required)	LC-MS/MS is known to be highly specific, no confirmatory method is required		
Difficult (if required, depends on intended use)	Primary	not required (no intended use in difficult matrices)		
	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 5.3-9: Statement on extraction efficiency

	Method for products of plant origin
Required, available from:	Rodrigues Junior, 2011
Not required, because:	-

For the detailed evaluation of (additional) studies on extraction efficiency, it is referred to Appendix 2.

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

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

Table 5.3-10: Validated methods for food and feed of animal origin (if appropriate)

Component of residue definition: florasulam				
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	Bacher, R., 2011, RAR of florasulam, Volume 3, Annex B.5 point B.5.2.2.1
	ILV	0.01 mg/kg	LC-MS/MS	Robaugh David A., RAR of florasulam, Volume 3, Annex B.5 point B.5.2.2.1b

Component of residue definition: florasulam				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
	Confirmatory (if required)	LC-MS/MS is known to be highly specific, no confirmatory method is required		
Eggs	Primary	0.01 mg/kg	LC-MS/MS	Bacher, R., 2011, RAR of florasulam, Volume 3, Annex B.5 point B.5.2.2.1
	ILV	0.01 mg/kg	LC-MS/MS	Robaugh David A., RAR of florasulam, Volume 3, Annex B.5 point B.5.2.2.1b
	Confirmatory (if required)	LC-MS/MS is known to be highly specific, no confirmatory method is required		
Muscle	Primary	0.01 mg/kg	LC-MS/MS	Bacher, R., 2011, RAR of florasulam, Volume 3, Annex B.5 point B.5.2.2.1
	ILV	0.01 mg/kg	LC-MS/MS	Robaugh David A., RAR of florasulam, Volume 3, Annex B.5 point B.5.2.2.1b
	Confirmatory (if required)	0.01 mg/kg	LC-MS/MS	Lidner M., RAR of florasulam, Volume 3, Annex B.5 point B.5.2.2.1c
Fat	Primary	0.01 mg/kg	LC-MS/MS	Robaugh David A., RAR of florasulam, Volume 3, Annex B.5 point B.5.2.2.1b
Kidney, liver	Primary	0.01 mg/kg	LC-MS/MS	Bacher, R., 2011, RAR of florasulam, Volume 3, Annex B.5 point B.5.2.2.1
	ILV	0.01 mg/kg	LC-MS/MS	Robaugh David A., RAR of florasulam, Volume 3, Annex B.5 point B.5.2.2.1b
	Confirmatory (if required)	LC-MS/MS is known to be highly specific, no confirmatory method is 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.

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

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

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

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

Component of residue definition: florasulam			
Method type	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	0.05 µg/kg	LC-MS/MS	Bacher R., RAR of florasulam, Volume 3, Annex B.5 point B.5.3.1.1a
Confirmatory	LC-MS/MS is known to be highly specific, no confirmatory method is required		

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

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

An overview on the acceptable methods and possible data gaps for analysis of florasulam in surface and drinking water is given in the following tables. For the detailed valuation of new/additional studies it is referred to Appendix 2.

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

Component of residue definition: florasulam				
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	Class, T., 2011, RAR of florasulam, Volume 3, Annex B.5 point B.5.3.2.1a
	ILV	0.05 µg/L	LC-MS/MS	Souza N., 2011, , RAR of florasulam, Volume 3, Annex B.5 point B.5.3.2.1b
	Confirmatory	LC-MS/MS is known to be highly specific, no confirmatory method is required		
Surface water	Primary	0.05 µg/L	LC-MS/MS	Class, T., 2011, RAR of florasulam, Volume 3, Annex B.5 point B.5.3.2.1a
	Confirmatory	LC-MS/MS is known to be highly specific, no confirmatory method is required		

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

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

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

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

Component of residue definition: florasulam			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	1.5 µg/m^3	LC-MS/MS	Class, T., 2011, RAR of florasulam, Volume 3, Annex B.5 point B.5.3.3.1a
Confirmatory	LC-MS/MS is known to be highly specific, no confirmatory method is required		

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

Table 5.3-8: Statement on extraction efficiency

	Method for products of animal origin
Required, available from:	Class T., 2011
Not required, because:	-

5.3.3.7 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 florasulam in body fluids and tissues is given in the following table. For the detailed evaluation of new/additional studies it is referred to Appendix 2.

Table 5.3-11: Methods for body fluids and tissues (if appropriate)

Component of residue definition: florasulam			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	0.05 mg/L	LC-MS/MS	Class, T., Göcer, M., 2011, RAR of florasulam, Volume 3, Annex B.5 point B.5.4.2a
Confirmatory	LC-MS/MS is known to be highly specific, no confirmatory method is required		

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

5.3.3.8 Other studies/ information

No other studies or information are submitted.

Appendix 1 Lists of data considered in support of the evaluation

List of data submitted by the applicant and relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 5.1.1	Enzo Arévalo	2021	MEZOFLOR 103 SC Determination of phsicochemical properties of the initial preparation, after accelerated and low temperature storage Study code number: BF – 20/21 Łukasiewicz – Institute of Industrial Organic Chemistry Warsaw, 2021 GLP Unpublished	N	Synthos Agro Sp. z o.o.
KCP 5.1.2	Małgorzata Czarnecka	2021	*Daphnia magna*, Acute immobilisation Test Study code number: W-56-20 Łukasiewicz Research Network - Institute of Industrial Organic Chemistry Pszczyna, 2021 GLP Unpublished	N	Synthos Agro Sp. z o.o.
KCP 5.1.2	Elżbieta Kulec-Płoszczyca	2021a	Honeybees, (*Apis mellifera* L.) Chronic Oral Toxicity Test Study code number B-16-21 Łukasiewicz Research Network - Institute of Industrial Organic Chemistry Pszczyna, 2021 GLP Unpublished	N	Synthos Agro Sp. z o.o.
KCP 5.1.2	Elżbieta Kulec-Płoszczyca	2021b	Bumblebees, (*Bombus spp.*) Acute Oral Toxicity Test Study code number B-19-21 Łukasiewicz Research Network - Institute of Industrial Organic Chemistry Pszczyna, 2021	N	Synthos Agro 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	Vertebrate study Y/N	Owner
			GLP Unpublished		
KCP 5.1.2	Elżbieta Kulec-Płoszczyca	2021c	Bumblebees, (*Bombus spp.*) Acute Contact Toxicity Test Study code number B-20-21 Łukasiewicz Research Network - Institute of Industrial Organic Chemistry Pszczyna, 2021 GLP Unpublished	N	Synthos Agro Sp. z o.o.
KCP 5.1.2	Ewa Nierzędska	2021	*Raphidocelis subcapitata* SAG 61.81 (formerly *Pseudokirchneriella subcapitata*), Growth inhibition test Study code number B-57-20 Łukasiewicz Research Network - Institute of Industrial Organic Chemistry Pszczyna, 2021 GLP Unpublished	N	Synthos Agro Sp. z o.o.
KCP 5.1.2	Paweł Pieczka	2021a	Earthworm reproduction test (*Eisenia andrei*) Study code number G-63-20 Łukasiewicz Research Network - Institute of Industrial Organic Chemistry Pszczyna, 2021 GLP Unpublished	N	Synthos Agro Sp. z o.o.
KCP 5.1.2	Paweł Pieczka	2021b	Terrestial Plant Test: Vegetative Vigour Test Study code number G-65-20 Łukasiewicz Research Network - Institute of Industrial Organic Chemistry Pszczyna, 2021 GLP Unpublished	N	Synthos Agro Sp. z o.o.
KCP 5.1.2	Marcin Świstak	2021	Validation of analytical method for the determination of active substances – mesotrione and florasulam in aqueous solution of the test item MEZOFLOR 103 SC Study code number: 0030/0016/FA Sorbolab Sp. z o.o.	N	Synthos Agro 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	Vertebrate study Y/N	Owner
			Poznań, 2021 GLP Unpublished		
KCP 5.1.2	Magdalena Wołany	2021	Terrestial Plant Test: Seedling Emergence and Seedling Growth Test Study code number G-66-20 Łukasiewicz Research Network - Institute of Industrial Organic Chemistry Pszczyna, 2021 GLP Unpublished	N	Synthos Agro Sp. z o.o.
KCP 5.1.2	Magdalena Czarnynoga	2024	MEZOFLOR 103 SC Collembolan (*Folsomia candida*) Reproduction Test Study code number G-44-24 Łukasiewicz Research Network - Institute of Industrial Organic Chemistry Pszczyna, 2024 GLP Unpublished	N	Synthos Agro Sp. z o.o.
KCP 5.1.2	Małgorzata Górska	2024	MEZOFLOR 103 SC Predatory mite (*Hypoaspis (Geolaelaps) aculeifer*) reproduction test in soil Study code number G-45-24 Łukasiewicz Research Network - Institute of Industrial Organic Chemistry Pszczyna, 2024 GLP Unpublished	N	Synthos Agro 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 5.2	Bacher, R.	2011a	Florasulam: Independent Laboratory Validation of a Residue Method for the Determination of florasulam in Agricultural Commodities Dow AgroSciences, Report no. 110536 GLP, unpublished	N	Dow AgroSciences
KCP 5.2	Bacher, R.	2011b	Method Validation Study for the Determination of Residues of Florasulam and its 5-OH Metabilite in Soil by Liquid Chromatography with Tandem Mass Spectrometry Dow AgroSciences, Report no. 110537 GLP, unpublished	N	Dow AgroSciences
KCP 5.2	Bacher, R.	2011c	Method Validation Study for the determination of Residues of Florasulam in Foodstuffs of Animal Origin by Liquid Chromatography with Tandem Mass Spectrometry Dow AgroSciences, Report no. 110540 GLP, unpublished	N	Dow AgroSciences
KCP 5.2	Class, T.	2011a	Method Validation Study for the Determination of Residues of Florasulam and its 5-OH Metabolite in Surface Water, Ground Water and Drinking Water by Liquid Chromatography with Tandem Mass Spectrometry Dow AgroSciences, Report no. 110538 GLP, unpublished	N	Dow AgroSciences
KCP 5.2	Watson G.	2013b	Validation of the QuEChERS Method for the Determination of Residues of mesotrione in Animal Matrices by LC-MS/MS Syngenta Eurofins Agroscience Services Ltd, Wilson, UK, S12-03250 GLP not published Syngenta File No ZA1296_10093	N	Syngenta
KCP 5.2	Bernal J.	2013	Mesotrione - Independent Laboratory Validation of the QuEChERS Method for the Determination of Residues of Mesotrione in Animal matrices by LC-MS/MS Syngenta Eurofins Agroscience Services Chem SAS, Vergèze, France, S12-04608 GLP not published	N	Syngenta

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
			Syngenta File No ZA1296_10130		
KCP 5.2	Class, T.	2011b	The Development and Validation of a Method for the Analysis of Florasulam in Air Dow AgroSciences, Report no. 110282 GLP, unpublished	N	Dow AgroSciences
KCP 5.2	Class, T., Göcer, M.	2011	Florasulam: Development of an Analytical Method for the determination of Florasulam in Body Fluid(s) Dow AgroSciences, Report no. 110283 GLP, unpublished	N	Dow AgroSciences
KCP 5.2	Crook S.	2002	Mesotrione: Residue Analytical Method for the Determination of Residues of Mesotrione and 4-(Methylsulfonyl)-2-Nitrobenzoic Acid (MNBA) in Crop Samples Syngenta Crop Protection	N	Syngenta Crop Protection
KCP 5.2	Jutsum L.,	2013	Mesotrione – Validation of Draft Residue Method GRM007.10A for the Determination of Mesotrione and its Metabolites AMBA and MNBA in Soil Syngenta Crop Protection GLP, unpublished		Syngenta Crop Protection
KCP 5.2	Jutsum L.	2013a	Mesotrione – Validation of Draft Residue Method for the Determination of Mesotrione and its metabolites AMBA and MNBA in Water CEMAS, North Ascot, UK Syngenta File No ZA 1296_10087	N	Syngenta Crop Protection
KCP 5.2	Jutsum L.,	2013c	Mesotrione – Validation of Residue Method GRM007.08A for the Dertermination of Mesotrione in Air Syngenta Crop Protection GLP, Unpublished	N	Syngenta Crop Protection
KCP 5.2	Jutsum L., Chamkesam N.	2013	Mesotrione – Analytical Method GRM007.09A for the determination of mesotrione and its Metabolites AMBA and MNBA in water CEMAS, North Ascot, UK Syngenta File No ZA 1296_10091	N	Syngenta Crop Protection
KCP 5.2	Jutsum L., Williams R.	2013	Mesotrione – Analytical Method GRM007.10A for the determination of Mesotrione and its Metabolites AMBA and MNBA in Soil CEMAS, Noth Ascot UK	N	Syngenta Crop Protection

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
			Syngenta File ZA1296_10092		
KCP 5.2	Lindner M.,	2011	Examination of the applicability of the Modular Analytical Method L 00.00-34 for the determination of Residues of Florasulam Dow AgroSciences, Report no. 110671 GLP, unpublished	N	Dow AgroSciences
KCP 5.2	Robaugh, D.A.	2011	Independent Laboratory Validation Study for the determination of Residues of Florasulam in Bovine and Poultry Tissues by Liquid Chromatography with Tandem Mass Spectrometry Dow AgroSciences, Report no. 110541 GLP, unpublished	N	Dow AgroSciences
KCP 5.2	Rodrigues Junior, A.	2011	Residue Method Validation for the Determination of Florasulam in Agricultural Commodities Dow AgroSciences, Report no. 110535 GLP, unpublished	N	Dow AgroSciences
KCP 5.2	Souza, N.	2011	Independent Laboratory Validation of Dow AgroSciences LLC Method – Determination of Residues of Florasulam and its 5-OH Metabolite in Drinking Water, Ground Water and Surface Water by Liquid Chromatography with Tandem Mass Spectrometric Detection Dow AgroSciences, Report no. 110539 GLP, unpublished	N	Dow AgroSciences
KCP 5.2	Tessier V.	2013	Mesotrione – Independent Laboratory Validation of the QuEChERS Method for the Determination of Residues of Mesotrione in Crop Matrices by LC-MS/MS Syngenta Crop Protection GLP, unpublished	N	Syngenta Crop Protection
KCP 5.2	Watson G.	2013	Mesotrione – Validation of Syngenta Method RAM 366/01 for the Determination od Residues of Mesotrione and MNBA in Crop Matrices by LC-MS/MS Syngenta Crop Protection GLP, unpublished	N	Syngenta Crop Protection
KCP 5.2	Wiesner F., Breyer N.	2013	Mesotrione – Independent Laboratory Validation of Analytical Method GRM007.09A for the Determination of Residues of Mesotrione and its Metabolites in AMBA and MNBA in Water Syngenta Crop Protection GLP, unpublished	N	Syngenta Crop Protection

Appendix 2 Detailed evaluation of submitted analytical methods

A 2.1 Analytical methods for mesotrione and florasulam

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)

No new or additional studies have been submitted.

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)

A 2.1.2.3.1 Analytical method 1

A 2.1.2.3.1.1 Method validation

zRMS:
This method was provided only for pre-registration purposes.
The method is accepted for this purpose.

Reference:	KCP 5.2
Report	MEZOFLOR 103 SC Earthworm reproduction test (*Eisenia Andrei*) Paweł Pieczka, Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, G-63-20
Guideline(s):	SANTE 12830/2020
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The concentrations of active substances of the test item has been chemically determined using the high performance liquid method with DAD detection.

Sample preparation for the chemical determinations:
First, 5 ml of 0.05% ortho-phosphoric acid solution was added to 10 g of artificial soil sample and shaken with 40 ml of ethyl acetate for 60 minutes by using laboratory shaker. The extract phase was filtered through anhydrous sodium sulphate and evaporated. The dry residues was resolved in 5 ml of mixture of acetonitrile for HPLC and 0.05 % ortho-phosphoric acid solution (50:50 v/v) and the sample were diluted with the mixture of acetonitrile for HPLC and 0.05% ortho-phosphoric acid solution (50:50 v/v), if necessary.

Reagents and solvents:

- Ethyl acetate, pure p.a., POCH, Batch no: 1135/06/20, exp. 06.2025 and POCH batch no. 1183/11/20, exp. 11.2025
- Anhydrous sodium sulphate, pure p.a., J.T.Baker, 1831301832, exp. 11.2023 and Acros Organic, A0410956, exp. 08.2024
- Water deionized, fresh prepared before analysis
- Acetonitrile for HPLC, J.T. Baker 1728501868, exp. 10.2022
- Ortho-phosphoric acid, 85% pure p.a., POCH 1077/05/17, exp. 05.2021 and SUPELCO Z0721828108, exp. 31.07.2023
- Mesotrione standard, IPO Warsaw, Series no. 1A/18, exp. 12/2021
- Florasulam standard, IPO Warsaw, Series no. 1A/18, exp. 12/2021

Equipment:

- Volumetric flasks, various volumes, Glassco (Germany)
- Pipettes, various volumes, Brand (Germany)
- Automatic pipettes, various volumes, Eppendorf (Germany)
- Balance, WPS 510/C, ZMP Radwag (Poland)
- Rotary vacuum evaporator with water bath, RV 10 digital, HB 10 digital, IKA-WERKE (Germany)
- Rotary vacuum evaporator with water bath, RV 05 basic, HB 4 basic, IKA-WERKE (Germany)
- Laboratory shaker, WL-2000, J.W. Electronic (Poland)
- Chromatograph, Prominence, Shimadzu Corp. (Japan)

HPLC - DAD parameters:

- Chromatographic system: High Performance Liquid Chromatography (HPLC)
- Chromatograph: Prominence (Shimadzu Corp. Japan)
- Detection System: Diode Array Detector (DAD)
- Analytical column: Agilent Eclipse 5µ XDB-C8, I-150 mm, ϕ – 4.6 mm
- Oven temperature: 35◦C
- Injection volume: 20 µl
- Mobile phase: acetonitrile for HPLC: 0.05 % ortho-phosphoric acid (38:62, v/v)
- Flow rate: 0.5 ml/ min
- Wave length: 220 nm

Results and discussions

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

Matrix	Analyte	Fortification level (mg/kg) (n = 10)	Mean recovery (%)	RSD (%)	Comments
soil	mesotrione	control	-	-	-
		0.20 mg/kg	82.9 %	2.1 %	
		2.0 mg/kg	72.9 %	0.9 %	

Table A 2: Recovery results from method validation of florasulam using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (n = 10)	Mean recovery (%)	RSD (%)	Comments
soil	florasulam	control	-	-	-
		0.20 mg/kg	100.9 %	1.4 %	
		2.0 mg/kg	99.0 %	0.5 %	

Table A 3: Characteristics for the analytical method used for validation of mesotrione and florasulam in soil

	mesotrione	florasulam
Specificity	The analytical method specificity was estimated on the basis of the analysis of the chromatograms obtained for the control samples and fortified samples. Considering the results of the analysis, no signal of detected substances was overlapping with the matrix signal of the control samples under the experimental conditions. Therefore, the specificity of the method was demonstrated.	
Calibration (type, number of data points)	The first calibration curve: Working solutions of mesotrione at concentrations of 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, 5.0 µg/ml. The calibration curve was linear and expressed by an equation: y= 171180 x + 589.241 Correlation coefficient r^2=0.9999143 Regression residual (d_i) is randomly distributed The second calibration curve: Working solutions of mesotrione at concentrations of 0.2, 0.5, 1.0, 5.0, 10.0, 20.0 µg/ml. The calibration curve was linear and expressed by an equation: y= 170364 x + 690.575 Correlation coefficient r^2=0.9999663 Regression residual (d_i) is	The first calibration curve: Working solutions of florasulam at concentrations of 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, 5.0 µg/ml. The calibration curve was linear and expressed by an equation: y= 86519.5 x – 176.366 Correlation coefficient r^2=0.9998583 Regression residual (d_i) is randomly distributed The second calibration curve: Working solutions of florasulam at concentrations of 0.2, 0.5, 1.0, 5.0, 10.0, 20.0 µg/ml The calibration curve was linear and expressed by an equation: y= 85683.4 x + 1007.88 Correlation coefficient r^2=0.9999816 Regression residual (d_i) is randomly

	mesotrione	**florasulam**
	randomly distributed	distributed
Calibration range	The range of linearity of the first calibration curve is from 0.05 µg/mL to 5.0 µg/mL, which is equivalent to 0.025 mg mesotrione /kg artifical soil – 2.5 mg mesotrione / kg artifical soil . The range of linearity of the second calibration curve is from 0.2 µg/mL to 20.0 µg/mL, which is equivalent to 0.1 mg mesotrione /kg artifical soil – 10 mg mesotrione / kg artifical soil.	The range of linearity of the first calibration curve is from 0.05 µg/mL to 5.0 µg/mL, which is equivalent to 0.025 mg florasulam /kg artifical soil – 2.5 mg florasulam / kg artifical soil . The range of linearity of the second calibration curve is from 0.2 µg/mL to 20.0 µg/mL, which is equivalent to 0.1 mg florasulam /kg artifical soil – 10 mg florasulam / kg artifical soil.
Assessment of matrix effects is presented	Matrix effect was determined for mesotrione is 4.6 % and not exceed ± 20 %	Matrix effect for florasulam is 1.7 % and not exceed ± 20 %
Limit of determination/quantification	LOQ was estimated as the lowest concentration of a detected substance at which an acceptable mean recovery is obtained (normally 70- 120 % with RSD of preferably ≤ 20 %) LOQ is 0.2 mg for mesotrione and equivalent to the calibration level at concentration 0.4 µg mesotrione /mL LOD is defined as the lowest detectable concentration or amount of an analyte in a sample. LOD is 0.025 mg mesotrione /kg artifical soil and equivalent to the lowest calibration standard, i.e. 0.05 µg mesotrione/ mL	LOQ was estimated as the lowest concentration of a detected substance at which an acceptable mean recovery is obtained (normally 70- 120 % with RSD of preferably ≤ 20 %) LOQ is 0.2 mg for florasulam and equivalent to the calibration level at concentration 0.4 µg florasulam /mL LOD is defined as the lowest detectable concentration or amount of an analyte in a sample. LOD is 0.025 mg florasulam /kg artifical soil and equivalent to the lowest calibration standard, i.e. 0.05 µg florasulam/ mL

Conclusion

The linearity of response of the analytical method, its specificity, precision, recovery, limit of quantification and detection were assessed in the process of the analytical method validation. The validated analytical methods was performed according to SANTE/12830/2020 and fulfil its requirements.

A 2.1.2.3.2 Analytical method 2

A 2.1.2.3.2.1 Method validation

zRMS:
This method was provided only for pre-registration purposes.
The method is accepted for this purpose.

Report	Validation included in reports: MEZOFLOR 103 SC Collembolan (*Folsomia candida*) Reproduction Test, Magdalena Czarnynoga, Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, G-44-24, 2024 MEZOFLOR 103 SC Predatory mite (*Hypoaspis (Geolaelaps) aculeifer*) reproduction test in soil, Małgorzata Górska, Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, G-45-24, 2024
Guideline(s):	SANTE 12830/2020 Rev. 2
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The concentrations of florasulam and mesotrione were chemically determined using the validated high performance liquid chromatographic method with DAD detection.

Sample preparation for the chemical determinations:
10 g of artificial soil sample was weighted and 5 mL of 0.05% ortho-phosphoric acid solution in deionized water (v/v) was added. The sample was shaken with 40 mL of ethyl acetate for 60 minute by using laboratory shaker. Next the organic phase was filtered through sodium sulfate anhydrous. The eluate was evaporated to dryness using vacuum rotary evaporator. The dry residue was dissolved in 5 mL of mixture of acetonitrile for HPLC and 0.05% ortho-phosphoric acid solution in deionized water (v/v) (50:50; v/v). The sample was diluted with mixture of acetonitrile for HPLC and 0.05% ortho-phosphoric acid solution in deionized water (v/v) (50:50; v/v), if necessary. An aliquot of the final volume was transferred into a HPLC vial for further quantification using HPLC-DAD.

Reagents and solvents:

- 0.05% ortho-phosphoric acid solution in deionized water (v/v),
- mixture of acetonitrile for HPLC and 0.05% ortho-phosphoric acid solution in deionized water (50:50, v/v)-D1,
- standard solution of 1 mg/mL of florasulam in acetonitrile for HPLC,
- working solution of florasulam at concentrations 10 and 100 µg/mL in acetonitrile for HPLC,
- working solution of florasulam at concentrations 10 µg/mL in mixture of acetonitrile for HPLC and 0.05% ortho-phosphoric acid solution in deionized wate (50:50, v/v),
- standard solution of 1 mg/mL of mesotrione in acetonitrile for HPLC,
- working solution of mesotrione at concentrations 10 and 100 µg/mL in acetonitrile for HPLC,
- working solution of mesotrione at concentrations 10 µg/mL in mixture of acetonitrile for HPLC and 0.05% ortho-phosphoric acid solution in deionized wate (50:50, v/v),
- fortification common solution of florasulam and mesotrione at concentration 1 and 10 µg/mL in acetonitrile for HPLC,
- working common solutions of florasulam and mesotrione at concentration 10.0, 5.0, 2.0, 1.0, 0.5, 0.2, 0.1, 0.05 µg/mL in mixture of acetonitrile for HPLC and 0.05% ortho-phosphoric acid solution in deionized wate (50:50, v/v).

Equipment:

- Volumetric flasks, various volumes, Glassco (Germany)
- Variable volume single-channel pipettes, various volumes, Eppendorf AG (Germany)
- Laboratory shaker, WL-2000, J.W.Electronic (Poland)
- Autosampler vials with PTFE/silicone septa and screw caps, Clear glass, 2 mL, Alwsci Technologies (China)
- Analytical Balance, Adventurer Pro AV 114CM, Ohaus Corporation (USA)
- Rotary vacuum evaporator with water bath, RV 05 basic, HB 4 basic, IKA - WERKE (Germany)
- Rotary vacuum evaporator with water bath, RV 10 digital, HB 10 digital, IKA - WERKE (Germany)
- Balance, PS 600.X2, RADWAG (Poland)

HPLC - DAD parameters:

- Chromatographic system: High Performance Liquid Chromatography (HPLC)
- Chromatograph: Prominence (Shimadzu Corp. Japan)
- Detection System: Diode Array Detector (DAD)
- Analytical column: Luna Omega 5µm PS C18 100Å 150x4.6 mm
- Oven temperature: 35°C
- Injection volume: 20 µl
- Mobile phase: acetonitryl HPLC : ortho-phosphoric acid solution 0.05 %, (33 : 67, v/v)
- Flow rate: 0.50 ml/ min
- Wave length: 220 nm

Results and discussions

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

Matrix	Analyte	Fortification level (mg/kg) (n = 10)	Mean recovery (%)	RSD (%)	Comments
Artificial soil	mesotrione	control	-	-	-
		0.2	89.5 %	1.7 %	
		2.0	96.2 %	3.2 %	

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

Matrix	Analyte	Fortification level (mg/kg) (n = 10)	Mean recovery (%)	RSD (%)	Comments
Artificial soil	florasulam	control	-	-	-
		0.2	102 %	3.4 %	
		2.0	101 %	2.4 %	

Table A 6: Characteristics for the analytical method used for validation of mesotrione and florasulam in sucrose solution

<table>
<tr><th></th><th>mesotrione</th><th>florasulam</th></tr>
<tr><td>Specificity</td><td colspan="2">The analytical method specificity was estimated on the basis of the analysis of the chromatograms obtained for the control, and fortified samples of matrix. Considering the results of the analysis, no signals of detected substances were overlapping with matrix signal of the control samples in the experiments conditions. Therefore, the specificity of the method was demonstrated. Furthermore, any interference are directly apparent and would be observed in the chromatograms of the matrix control sample.</td></tr>
<tr><td>Calibration (type, number of data points)</td><td colspan="2">Working solutions of florasulam and mesotrione at the concentrations of 0.05, 0.1, 0.5, 1.0 and 5.0 mg/mL were injected successively to the chromatographic column and the chromatograms were recorded.

The equations of the calibration line were presented as the 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.

<table>
<tr><th>Analyte</th><th>Slope</th><th>Intercept</th><th>Coefficient</th></tr>
<tr><td>florasulam</td><td>80512.1</td><td>–807.750</td><td>0.9997112</td></tr>
<tr><td>mesotrione</td><td>167064</td><td>-908.918</td><td>0.9998598</td></tr>
</table></td></tr>
<tr><td>Calibration range</td><td>The range of linearity of the analytical graph is from 0.05 mg/mL to 5.0 mg/mL. The range of calibration curve of mesotrione is equivalent to range from 0.5 mg/L to 50.0 mg/L in artificial soil.</td><td>The range of linearity of the analytical graph is from 0.05 mg/mL to 5.0 mg/mL. The range of calibration curve of florasulam is equivalent to range from 0.5 mg/L to 50.0 mg/L in artificial soil.</td></tr>
<tr><td>Assessment of matrix effects is presented</td><td>Matrix effect for mesotrione was -0.9 % and not exceed ± 20 %.</td><td>Matrix for florasulam was 6.3 % and not exceed ± 20 %.</td></tr>
<tr><td>Limit of determination/quantification</td><td colspan="2">Limit of Quantification was estimated as the lowest concentration of a detected substance at which an acceptable mean recovery is obtained (normally 70 – 120% with a relative standard deviation of preferably ≤ 20%).

Limit of quantification (LOQ) and a limit of detection (LOD) are presented in the table below:

<table>
<tr><th>Analyte</th><th>LOQ [mg analyte/kg]</th><th>Equivalent calibration level [mg/L]</th><th>LOD [mg analyte/kg]</th><th>Equivalent calibration level [mg/L]</th></tr>
<tr><td>florasulam</td><td>0.2</td><td>0.4</td><td>0.025</td><td>0.05</td></tr>
<tr><td>mesotrione</td><td>0.2</td><td>0.4</td><td>0.025</td><td>0.05</td></tr>
</table></td></tr>
</table>

Conclusion

The linearity of response of the analytical method, its specificity, precision, recovery, limit of quantification and detection were assessed in the process of the analytical method validation. The validated analytical methods was performed according to SANTE/12830/2020 Rev. 2 and fulfil its requirements.

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

Analytical Method 1:

zRMS:
This method was provided only for pre-registration purposes. HPLC
The method is accepted for this purpose.

Report	Validation included in a reports: MEZOFLOR 103 SC *Daphnia magna* , Acute Immobilisation Test Małgorzata Czarnecka, Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, W-56-20 MEZOFLOR 103 SC Bumblebees (Bombus spp.), Acute Contact Toxicity Test, Kulec – Płoszczyca, 2021c, B-20-21
Guideline(s):	SANTE 12830/2020
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The concentrations of active substances of the test item has been chemically determined using the validated high performance liquid method with DAD detection.

Sample preparation for the chemical determinations:
Acetonitrile for HPLC in a volume of 5 mL of was added to sample in a volume of 5 mL and mixed. An aliquot of the final volume was transferred into a HPLC vial for further quantification using HPLC-DAD

Reagents and solvents:

- Water deionized, fresh prepared before analysis
- Acetonitrile for HPLC, J.T. Baker 1728501868, exp. 10.2022
- Ortho-phosphoric acid, 85% pure p.a., POCH 1077/05/17, exp. 05.2021
- Mesotrione standard, IPO Warsaw, Series no. 1A/18, exp. 12/2021
- Florasulam standard, IPO Warsaw, Series no. 1A/18, exp. 12/2021

Equipment:

- Volumetric flasks, various volumes, Glassco (Germany)
- Pipettes, various volumes, Brand (Germany)
- Automatic pipettes, various volumes, Eppendorf (Germany)
- Balance, Adventurer Pro AV, OHaus Corp. (USA)
- Chromatograph, Prominence, Shimadzu Corp. (Japan)

HPLC - DAD parameters:

- Chromatographic system: High Performance Liquid Chromatography (HPLC)
- Chromatograph: Prominence (Shimadzu Corp. Japan)
- Detection System: Diode Array Detector (DAD)
- Analytical column: Agilent Eclipse 5µ XDB-C8, I-150 mm, ϕ – 4.6 mm
- Oven temperature: 35◦C
- Injection volume: 20 µl

- Mobile phase: acetonitrile for HPLC: 0.05 % ortho-phosphoric acid (38:62, v/v)
- Flow rate: 0.5 ml/ min
- Wave length: 220 nm

Results and discussions

Table A ~~4~~7: Recovery results from method validation of mesotrione using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (n = 10)	Mean recovery (%)	RSD (%)	Comments
water	mesotrione	control	-	-	-
		0.20 mg/kg	97.4 %	1.3 %	
		2.0 mg/kg	97.5 %	0.3 %	

Table A ~~5~~8: Recovery results from method validation of florasulam using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (n = 10)	Mean recovery (%)	RSD (%)	Comments
water	florasulam	control	-	-	-
		0.20 mg/kg	98.6 %	2.3 %	
		2.0 mg/kg	98.5 %	0.2 %	

Table A ~~6~~9: Characteristics for the analytical method used for validation of mesotrione and florasulam in water

	mesotrione	florasulam
Specificity	The analytical method specificity was estimated on the basis of the analysis of the chromatograms obtained for the control samples and fortified samples. Considering the results of the analysis, no signal of detected substances was overlapping with the matrix signal of the control samples under the experimental conditions. Therefore, the specificity of the method was demonstrated.	
Calibration (type, number of data points)	The first calibration curve: Working solutions of mesotrione at concentrations of 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, 5.0 µg/ml. The calibration curve was linear and expressed by an equation: y= 171180 x + 589.241 Correlation coefficient r^2=0.9999143 Regression residual (d_i) is randomly distributed The second calibration curve: Working solutions of mesotrione at concentrations of 0.2, 0.5, 1.0, 2.0, 5.0, 10.0, 20.0 µg/ml.	The first calibration curve: Working solutions of florasulam at concentrations of 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, 5.0 µg/ml. The calibration curve was linear and expressed by an equation: y= 86519.5 x – 176.366 Correlation coefficient r^2=0.9998583 Regression residual (d_i) is randomly distributed The second calibration curve: Working solutions of florasulam at concentrations of 0.2, 0.5, 1.0, 2.0, 5.0, 10.0, 20.0 µg/ml

	mesotrione	**florasulam**
	The calibration curve was linear and expressed by an equation: y= 170364 x + 690.575 Correlation coefficient r^2=0.9999663 Regression residual (d_i) is randomly distributed	The calibration curve was linear and expressed by an equation: y= 85683.4 x + 1007.88 Correlation coefficient r^2=0.9999816 Regression residual (d_i) is randomly distributed
Calibration range	The range of linearity of the first calibration curve is from 0.05 µg/mL to 5.0 µg/mL, which is equivalent to 0.1 mg mesotrione /L of water – 10.0 mg mesotrione / L of water . The range of linearity of the second calibration curve is from 0.2 µg/mL to 20.0 µg/mL, which is equivalent to 0.4 mg mesotrione / L of water – 40 mg mesotrione / L of water.	The range of linearity of the first calibration curve is from 0.05 µg/mL to 5.0 µg/mL, which is equivalent to 0.1 mg florasulam / L of water – 10.0 mg florasulam / L of water . The range of linearity of the second calibration curve is from 0.2 µg/mL to 20.0 µg/mL, which is equivalent to 0.4 mg florasulam / L of water – 40 mg florasulam / L of water.
Assessment of matrix effects is presented	Matrix effect was determined for each research independently. It was calculated for mesotrione: -5.0 % (Czarnecka, 2021) and -2.4 % (Kulec-Płoszczyca, 2021) and not exceed ± 20 %.	Matrix effect was determined for each research independently. It was calculated for florasulam: -3.4 % (Czarnecka, 2021) and -1.4 % (Kulec-Płoszczyca, 2021) and not exceed ± 20 %.
Limit of determination/quantification	LOQ was estimated as the lowest concentration of a detected substance at which an acceptable mean recovery is obtained (normally 70- 120 % with RSD of preferably ≤ 20 %) LOQ is 0.2 mg for mesotrione and equivalent to the calibration level at concentration 0.1 µg mesotrione /mL LOD is defined as the lowest detectable concentration or amount of an analyte in a sample. LOD is 0.1 mg mesotrione /L of water and equivalent to the lowest calibration standard, i.e. 0.05 µg mesotrione/ mL	LOQ was estimated as the lowest concentration of a detected substance at which an acceptable mean recovery is obtained (normally 70- 120 % with RSD of preferably ≤ 20 %) LOQ is 0.2 mg for florasulam and equivalent to the calibration level at concentration 0.1 µg florasulam /mL LOD is defined as the lowest detectable concentration or amount of an analyte in a sample. LOD is 0.1 mg florasulam /L of water and equivalent to the lowest calibration standard, i.e. 0.05 µg florasulam/ mL

Conclusion

The linearity of response of the analytical method, its specificity, precision, recovery, limit of quantification and detection were assessed in the process of the analytical method validation. The validated analytical methods was performed according to SANTE/12830/2020 and fulfil its requirements.

Analytical Method 2:

zRMS:
This method was provided only for pre-registration purposes. HPLC
The method is accepted for this purpose.

Report	Validation included in a report: MEZOFLOR 103 SC *Raphidocelis subcapitata* SAG 61.81, (formerly *Pseudokirchneriella subcapitata*), Growth inhibition Test Ewa Nierzedska, Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, W-57-20
Guideline(s):	SANTE 12830/2020
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The concentrations of active substances of the test item has been chemically determined using the validated high performance liquid method with DAD detection.

Sample preparation for the chemical determinations:
Each sample of 100 mL volume was acidified by hydrochloric acid to pH ≈ 2 and applied to ENVI-18 (3 mL, 500 mg) column conditioned previously by sequential washing twice with 5 mL of acetone, twice with 5 mL of methanol, twice with 5 mL of deionised water pH≈2. Following the sample introduction the column was dried under vacuum for 5 minutes. The analytes were eluted with twice 5 mL methanol and twice 5 mL of acetone. The eluate was evaporated to dryness using vacuum rotary evaporator. The dry residue was redissolved in mixture acetonitrile for HPLC : 0.05% orthophosphoric acid (50:50; v/v). An aliquot of the final volume was transferred into a HPLC vial for further quantification using HPLC-DAD.

Reagents and solvents:
- Water deionized, fresh prepared before analysis
- Acetonitrile for HPLC, J.T. Baker 1728501868, exp. 10.2022
- Ortho-phosphoric acid, 85% pure p.a., POCH 1077/05/17, exp. 05.2021
- Mesotrione standard, IPO Warsaw, Series no. 1A/18, exp. 12/2021
- Florasulam standard, IPO Warsaw, Series no. 1A/18, exp. 12/2021
- Acetone, pure p.a., POCH, 1062/0620, exp. 06.2024
- Methanol, pure p.a., POCH, 1261/01/20, exp. 01.2025
- SUPELCLEAN ENVI-18 SPE, 3mL, 500 mg, SUPELCO, 12546401, exp. 19.02.2026
- Hydrochloric acid, ACS reagent 37 %, Sigma-Aldrich, STBJ 1296, exp. 03.2021

Equipment:
- Volumetric flasks, various volumes, Glassco (Germany)
- Pipettes, various volumes, Brand (Germany)
- Automatic pipettes, various volumes, Eppendorf (Germany)
- Balance, Adventurer Pro AV, Ohaus Corp. (USA)
- Chromatograph, Prominence, Shimadzu Corp. (Japan)
- Rotary vacuum evaporator with water medium bath, RV 05 basic, HB 4 basic, IKA-WERKE (Germany)

- SPE vacuum manifold, Visiprep, Supelco (USA)
- SPE cartridges, Supelclean ENVI-18, Supelco (USA)
- Rotary vacuum evaporator with water medium bath, RV 10 digital, HB 10 digital, IKA – WERKE (Germany)

HPLC - DAD parameters:

- Chromatographic system: High Performance Liquid Chromatography (HPLC)
- Chromatograph: Prominence (Shimadzu Corp. Japan)
- Detection System: Diode Array Detector (DAD)
- Analytical column: Agilent Eclipse 5µ XDB-C8, I-150 mm, ϕ – 4.6 mm
- Oven temperature: 35◦C
- Injection volume: 20 µl
- Mobile phase: acetonitrile for HPLC: 0.05 % ortho-phosphoric acid (38:62, v/v)
- Flow rate: 0.5 ml/ min
- Wave length: 220 nm

Results and discussions

Table A ~~7~~10: Recovery results from method validation of mesotrione using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (n = 10)	Mean recovery (%)	RSD (%)	Comments
water	mesotrione	control	-	-	-
		0.01 mg/L	81.0 %	3.4 %	
		0.10 mg/L	85.4 %	1.8 %	

Table A ~~8~~11: Recovery results from method validation of florasulam using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (n = 15)	Mean recovery (%)	RSD (%)	Comments
water	florasulam	control	-	-	-
		0.001 mg/L	104.0 %	4.8 %	
		0.01 mg/L	100.0 %	0.9 %	
		0.1 mg/L	98.2 %	0.8 %	

Table A ~~9~~12: Characteristics for the analytical method used for validation of mesotrione and florasulam in water

	mesotrione	florasulam
Specificity	The analytical method specificity was estimated on the basis of the analysis of the chromatograms obtained for the control samples and fortified samples. Considering the results of the analysis, no signal of detected substances was overlapping with the matrix signal of the control samples under the experimental conditions. Therefore, the specificity of the method was demonstrated.	

	mesotrione	**florasulam**
Calibration (type, number of data points)	The first calibration curve: Working solutions of mesotrione at concentrations of 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, 5.0 µg/ml. The calibration curve was linear and expressed by an equation: y= 171180 x + 589.241 Correlation coefficient r^2=0.9999143 Regression residual (d_i) is randomly distributed The second calibration curve: Working solutions of mesotrione at concentrations of 0.2, 0.5, 1.0, 2.0, 5.0, 10.0, 20.0 µg/ml. The calibration curve was linear and expressed by an equation: y= 170364 x + 690.575 Correlation coefficient r^2=0.9999663 Regression residual (d_i) is randomly distributed	The first calibration curve: Working solutions of florasulam at concentrations of 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, 5.0 µg/ml. The calibration curve was linear and expressed by an equation: y= 86519.5 x – 176.366 Correlation coefficient r^2=0.9998583 Regression residual (d_i) is randomly distributed The second calibration curve: Working solutions of florasulam at concentrations of 0.2, 0.5, 1.0, 2.0, 5.0, 10.0, 20.0 µg/ml The calibration curve was linear and expressed by an equation: y= 85683.4 x + 1007.88 Correlation coefficient r^2=0.9999816 Regression residual (d_i) is randomly distributed
Calibration range	The range of linearity of the first calibration curve is from 0.05 µg/mL to 5.0 µg/mL, which is equivalent to 0.001 mg mesotrione /L of water – 0.1 mg mesotrione / L of water . The range of linearity of the second calibration curve is from 0.2 µg/mL to 20.0 µg/mL, which is equivalent to 0.004 mg mesotrione / L of water – 0.4 mg mesotrione / L of water.	The range of linearity of the first calibration curve is from 0.05 µg/mL to 5.0 µg/mL, which is equivalent to 0.0005 mg florasulam / L of water – 0.1 mg florasulam / L of water . The range of linearity of the second calibration curve is from 0.2 µg/mL to 20.0 µg/mL, which is equivalent to 0.002 mg florasulam / L of water – 0.2 mg florasulam / L of water.
Assessment of matrix effects is presented	Matrix effect was for mesotrione: -0.8 % and not exceed ± 20 %.	Matrix effect was for florasulam: -1.0 % and not exceed ± 20 %.
Limit of determination/quantification	LOQ was estimated as the lowest concentration of a detected substnce at which an acceptable mean recovery is obtained (normally 70- 120 % with RSD of preferably ≤ 20 %) LOQ is 0.01 mg mesotrione / L and equivalent to the calibration level at concentration 0.5 µg mesotrione /mL LOD is defined as the lowest detectable concentration or amount of an analyte in a sample. LOD is 0.001 mg mesotrione /L of water and equivalent to the lowest calibration standard, i.e. 0.05 µg mesotrione/ mL	LOQ was estimated as the lowest concentration of a detected substnce at which an acceptable mean recovery is obtained (normally 70-120 % with RSD of preferably ≤ 20 %) LOQ is 0.001 mg for florasulam and equivalent to the calibration level at concentration 0.1 µg florasulam /mL LOD is defined as the lowest detectable concentration or amount of an analyte in a sample. LOD is 0.0005 mg florasulam /L of water and equivalent to the lowest calibration standard, i.e. 0.05 µg florasulam/ mL

Conclusion

The linearity of response of the analytical method, its specificity, precision, recovery, limit of quantification and detection were assessed in the process of the analytical method validation. The validated analytical methods was performed according to SANTE/12830/2020 and fulfil its requirements.

Analytical Method 3:

zRMS:
This method was provided only for pre-registration purposes. HPLC
The method is accepted for this purpose.

Report	Validation included in reports: MEZOFLOR 103 SC Terrestial Plant Test: Vegetative Vigour Test Paweł Pieczka, Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, G-65-20 MEZOFLOR 103 SC, Terrestial Plant Test: Seedling Emergence and Seedling Growth Test, Magdalena Wołany 2021, G-66-20
Guideline(s):	SANTE 12830/2020
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The concentrations of active substances of the test item has been chemically determined using the validated high performance liquid method with DAD detection.
The two analytical methods were developed for the determination of mesotrione and florasulam in water. Prior to analysis, the samples were concentrated by SPE method or diluted. The SPE method has a LOQ 0.001 mg florasulam / Lwater and a LOD 0.0005 mg florasulam /L water.
The dilution method has a LOQ 0.2 mg mesotrione and florasulam/L water and a LOD 0.1 mg mesotrione and florasulam / L water.

Sample preparation for the chemical determinations:
Solid phase extraction method (SPE method):
Each sample of 100 mL volume was acidified by hydrochloric acid to pH ≈ 2 and applied to ENVI-18 (3 mL, 500 mg) column conditioned previously by sequential washing twice with 5 mL of acetone, twice with 5 mL of methanol, twice with 5 mL of deionised water pH≈2. Following the sample introduction the column was dried under vacuum for 5 minutes. The analytes were eluted with twice 5 mL methanol and twice 5 mL of acetone. The eluate was evaporated to dryness using vacuum rotary evaporator. The dry residue was redissolved in mixture acetonitrile for HPLC : 0.05% orthophosphoric acid (50:50; v/v). An aliquot of the final volume was transferred into a HPLC vial for further quantification using HPLC-DAD.
Dilution method:
Acetonitrile for HPLC a volume of 5 mL was added to sample a volume of 5mL and mixed. An aliquot of the final volume was transferred into a HPLC vial for further quantification using HPLC -DAD.

Reagents and solvents:

- Water deionized, fresh prepared before analysis

- Acetonitrile for HPLC, J.T. Baker 1728501868, exp. 10.2022
- Ortho-phosphoric acid, 85% pure p.a., POCH 1077/05/17, exp. 05.2021
- Mesotrione standard, IPO Warsaw, Series no. 1A/18, exp. 12/2021
- Florasulam standard, IPO Warsaw, Series no. 1A/18, exp. 12/2021
- Acetone, pure p.a., POCH, 1062/0620, exp. 06.2024
- Methanol, pure p.a., POCH, 1261/01/20, exp. 01.2025
- SUPELCLEAN ENVI-18 SPE, 3mL, 500 mg, SUPELCO, 12546401, exp. 19.02.2026
- Hydrochloric acid, ACS reagent 37 %, Sigma-Aldrich, STBJ 1296, exp. 03.2021

Equipment:

- Volumetric flasks, various volumes, Glassco (Germany)
- Pipettes, various volumes, Brand (Germany)
- Automatic pipettes, various volumes, Eppendorf (Germany)
- Balance, Adventurer Pro AV, OHaus Corp. (USA)
- Chromatograph, Prominence, Shimadzu Corp. (Japan)
- Rotary vacuum evaporator with water medium bath, RV 05 basic, HB 4 basic, IKA-WERKE (Germany)
- SPE vacuum manifold, Visiprep, Supelco (USA)
- SPE cartridges, Supelclean ENVI-18, Supelco (USA)
- Rotary vacuum evaporator with water medium bath, RV 10 digital, HB 10 digital, IKA – WERKE (Germany)

HPLC - DAD parameters:

- Chromatographic system: High Performance Liquid Chromatography (HPLC)
- Chromatograph: Prominence (Shimadzu Corp. Japan)
- Detection System: Diode Array Detector (DAD)
- Analytical column: Agilent Eclipse 5µ XDB-C8, I-150 mm, ϕ – 4.6 mm
- Oven temperature: 35°C
- Injection volume: 20 µl
- Mobile phase: acetonitrile for HPLC: 0.05 % ortho-phosphoric acid (38:62, v/v)
- Flow rate: 0.5 ml/ min
- Wave length: 220 nm

Results and discussions

Table A ~~10~~13: Recovery results from method validation of mesotrione using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (n = 10)	Mean recovery (%)	RSD (%)	Comments
water	mesotrione	control	-	-	-
		0.2 mg/L	97.4 %	1.3 %	
		2.0 mg/L	97.5 %	0.3 %	

Table A ~~11~~14: Recovery results from method validation of florasulam using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (n = 15)	Mean recovery (%)	RSD (%)	Comments
water	florasulam	control	-	-	-
		0.001 mg/L	104.0 %	4.8 %	
		0.01 mg/L	100.0 %	0.9 %	
		0.1 mg/L	98.2 %	0.8 %	

Table A ~~12~~15: Characteristics for the analytical method used for validation of mesotrione and florasulam in water

	mesotrione	florasulam
Specificity	The analytical method specificity was estimated on the basis of the analysis of the chromatograms obtained for the control samples and fortified samples. Considering the results of the analysis, no signal of detected substances was overlapping with the matrix signal of the control samples under the experimental conditions. Therefore, the specificity of the method was demonstrated.	
Calibration (type, number of data points)	The first calibration curve: Working solutions of mesotrione at concentrations of 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, 5.0 µg/ml. The calibration curve was linear and expressed by an equation: y= 171180 x + 589.241 Correlation coefficient r^2=0.9999143 Regression residual (d_i) is randomly distributed The second calibration curve: Working solutions of mesotrione at concentrations of 0.2, 0.5, 1.0, 2.0, 5.0, 10.0, 20.0 µg/ml. The calibration curve was linear and expressed by an equation: y= 170364 x + 690.575 Correlation coefficient r^2=0.9999663 Regression residual (d_i) is randomly distributed	The first calibration curve: Working solutions of florasulam at concentrations of 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, 5.0 µg/ml. The calibration curve was linear and expressed by an equation: y= 86519.5 x – 176.366 Correlation coefficient r^2=0.9998583 Regression residual (d_i) is randomly distributed The second calibration curve: Working solutions of florasulam at concentrations of 0.2, 0.5, 1.0, 2.0, 5.0, 10.0, 20.0 µg/ml The calibration curve was linear and expressed by an equation: y= 85683.4 x + 1007.88 Correlation coefficient r^2=0.9999816 Regression residual (d_i) is randomly distributed
Calibration range	The range of linearity of the first calibration curve is from 0.05 µg/mL to 5.0 µg/mL, which is equivalent to 0.001 mg mesotrione /L of water – 0.1 mg mesotrione / L of water . The range of linearity of the second calibration curve is from 0.2 µg/mL to 20.0 µg/mL, which is	The range of linearity of the first calibration curve is from 0.05 µg/mL to 5.0 µg/mL, which is equivalent to 0.0005 mg florasulam / L of water – 0.1 mg florasulam / L of water . The range of linearity of the second calibration curve is from 0.2 µg/mL to 20.0 µg/mL, which is equivalent

	mesotrione	**florasulam**
	equivalent to 0.4 mg mesotrione / L of water – 40 mg mesotrione / L of water.	to 0.4 mg florasulam / L of water – 40 mg florasulam / L of water.
Assessment of matrix effects is presented	Matrix effect was for mesotrione: -2.4 % and not exceed ± 20 %.	Matrix effect was for florasulam: -1.0 % (SPE) method and -1.4 % (dilution method) and not exceed ± 20 %.
Limit of determination/quantification	LOQ was estimated as the lowest concentration of a detected substance at which an acceptable mean recovery is obtained (normally 70- 120 % with RSD of preferably ≤ 20 %) The LOQ is 0.2 mg mesotrione / L and equivalent to the calibration level at concentration 0.1 µg mesotrione /mL LOD is defined as the lowest detectable concentration or amount of an analyte in a sample. LOD is 0.1 mg mesotrione /L of water and equivalent to the lowest calibration standard, i.e. 0.05 µg mesotrione/ mL	LOQ was estimated as the lowest concentration of a detected substance at which an acceptable mean recovery is obtained (normally 70- 120 % with RSD of preferably ≤ 20 %) The LOQ for SPE method is 0.001 mg florasulam /L water and equivalent to the calibration level at concentration 0.1 µg florasulam /mL. LOQ for dilution method is 0.2 mg for florasulam and equivalent to the calibration level at concentration 0.1 µg florasulam /mL LOD is defined as the lowest detectable concentration or amount of an analyte in a sample. LOD for SPE method is 0.0005 mg florasulam /L of water and equivalent to the lowest calibration standard, i.e. 0.05 µg florasulam/ mL. LOD for dilution method is 0.1 mg florasulam / L water and equivalent to the lowest calibration standard, i.e. 0.05 µg florasulam/ mL.

Conclusion

The linearity of response of the analytical method, its specificity, precision, recovery, limit of quantification and detection were assessed in the process of the analytical method validation. The validated analytical methods was performed according to SANTE/12830/2020 and fulfil its requirements.

Analytical Method 4:

zRMS:
This method was provided only for pre-registration purposes. HPLC
The method is accepted for this purpose.

Report — Validation included in reports:
MEZOFLOR 103 SC Honeybees (*Aphis mellifera* L.) Chronic Oral Toxicity Test, Elżbieta Kulec - Płoszczyca, Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, B-16-21, 2021a
MEZOFLOR 103 SC Bumblebees (*Bombus spp.*), Acute Oral Toxici-

	ty Test, Kulec – Płoszczyca, 2021b, B-19-21
Guideline(s):	SANTE 12830/2020
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The concentrations of active substances of the test item has been chemically determined using the validated high performance liquid method with DAD detection.

Sample preparation for the chemical determinations:
First, 1 g sucrose sample was weighted into a volumetric flask with a capacity of 10 mL and added 6 mL of solution 0.05% ortho-phosphoric acid in deionized water (v/v). Next, the volume was made up to 10 mL with acetonitrile for HPLC. The eluate was diluted with mixture acetonitrile for HPLC and 0.05% ortho-phosphoric acid (50:50; v/v), if necessary. An aliquot of the final volume was transferred into a HPLC vial for further quantification using HPLC-DAD.

Reagents and solvents:
- Water deionized, fresh prepared before analysis
- Acetonitrile for HPLC, J.T. Baker 1728501868, exp. 10.2022, POCH 1024/07/21, exp. 07.2024
- Ortho-phosphoric acid, 85% pure p.a., POCH 1077/05/17, exp. 05.2021 and SUPELCO Z0721828108, exp. 31.07.2023
- Mesotrione standard, IPO Warsaw, Series no. 1A/18, exp. 12/2021
- Florasulam standard, IPO Warsaw, Series no. 1A/18, exp. 12/2021

Equipment:
- Volumetric flasks, various volumes, Glassco (Germany)
- Pipettes, various volumes, Brand (Germany)
- Automatic pipettes, various volumes, Eppendorf (Germany)
- Balance, Adventurer Pro AV, OHaus Corp. (USA)
- Chromatograph, Prominence, Shimadzu Corp. (Japan)
- Balance, WPS 510/C, Radwag (Poland)

HPLC - DAD parameters:
- Chromatographic system: High Performance Liquid Chromatography (HPLC)
- Chromatograph: Prominence (Shimadzu Corp. Japan)
- Detection System: Diode Array Detector (DAD)
- Analytical column: Agilent Eclipse 5µ XDB-C8, I-150 mm, ϕ – 4.6 mm
- Oven temperature: 35◦C
- Injection volume: 20 µl
- Mobile phase: acetonitrile for HPLC: 0.05 % ortho-phosphoric acid (38:62, v/v)
- Flow rate: 0.5 ml/ min
- Wave length: 220 nm

Results and discussions

Table A ~~13~~16: Recovery results from method validation of mesotrione using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (n = 10)	Mean recovery (%)	RSD (%)	Comments
Sucrose solution	mesotrione	control	-	-	-
		1.0 mg/kg	97.3 %	1.7 %	
		10.0 mg/kg	97.1 %	0.2 %	

Table A ~~14~~17: Recovery results from method validation of florasulam using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (n = 10)	Mean recovery (%)	RSD (%)	Comments
Sucrose solution	florasulam	control	-	-	-
		1.0 mg/kg	98.6 %	2.3 %	
		10.0 mg/kg	98.0 %	0.4 %	

Table A ~~15~~18: Characteristics for the analytical method used for validation of mesotrione and florasulam in sucrose solution

	mesotrione	florasulam
Specificity	The analytical method specificity was estimated on the basis of the analysis of the chromatograms obtained for the control samples and fortified samples. Considering the results of the analysis, no signal of detected substances was overlapping with the matrix signal of the control samples under the experimental conditions. Therefore, the specificity of the method was demonstrated.	
Calibration (type, number of data points)	The first calibration curve: Working solutions of mesotrione at concentrations of 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, 5.0 µg/ml. The calibration curve was linear and expressed by an equation: y= 171180 x + 589.241 Correlation coefficient r^2=0.9999143 Regression residual (d_i) is randomly distributed The second calibration curve: Working solutions of mesotrione at concentrations of 0.2, 0.5, 1.0, 2.0, 5.0, 10.0, 20.0 µg/ml. The calibration curve was linear and expressed by an equation: y= 170364 x + 690.575 Correlation coefficient r^2=0.9999663 Regression residual (d_i) is	The first calibration curve: Working solutions of florasulam at concentrations of 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, 5.0 µg/ml. The calibration curve was linear and expressed by an equation: y= 86519.5 x – 176.366 Correlation coefficient r^2=0.9998583 Regression residual (d_i) is randomly distributed The second calibration curve: Working solutions of florasulam at concentrations of 0.2, 0.5, 1.0, 2.0, 5.0, 10.0, 20.0 µg/ml The calibration curve was linear and expressed by an equation: y= 85683.4 x + 1007.88 Correlation coefficient r^2=0.9999816 Regression residual (d_i) is randomly

	mesotrione	**florasulam**
	randomly distributed	distributed
Calibration range	The range of linearity of the first calibration curve is from 0.05 µg/mL to 5.0 µg/mL, which is equivalent to 0.5 mg mesotrione /kg sucrose solution – 50 mg mesotrione / kg sucrose solution The range of linearity of the second calibration curve is from 0.2 µg/mL to 20.0 µg/mL, which is equivalent to 2.0 mg mesotrione /kg sucrose solution – 200 mg mesotrione / kg sucrose solution.	The range of linearity of the first calibration curve is from 0.05 µg/mL to 5.0 µg/mL, which is equivalent to 0.5 mg florasulam /kg sucrose solution – 50 mg florasulam / kg sucrose solution. The range of linearity of the second calibration curve is from 0.2 µg/mL to 20.0 µg/mL, which is equivalent to 2.0 mg florasulam /kg sucrose solution – 200 mg florasulam / kg sucrose solution.
Assessment of matrix effects is presented	Matrix effect for mesotrione was -2.7 % and not exceed ± 20 %.	Matrix for florasulam was 0.8 % and not exceed ± 20 %.
Limit of determination/quantification	LOQ was estimated as the lowest concentration of a detected substnce at which an acceptable mean recovery is obtained (normally 70- 120 % with RSD of preferably ≤ 20 %) LOQ is 1 mg for mesotrione / kg sucrose solution and equivalent to the calibration level at concentration 0.1 µg mesotrione /mL LOD is defined as the lowest detectable concentration or amount of an analyte in a sample. LOD is 0.5 mg mesotrione /kg sucrose solution and equivalent to the lowest calibration standard, i.e. 0.05 µg mesotrione/ mL	LOQ was estimated as the lowest concentration of a detected substnce at which an acceptable mean recovery is obtained (normally 70-120 % with RSD of preferably ≤ 20 %) LOQ is 1 mg for florasulam / kg sucrose solution and equivalent to the calibration level at concentration 0.1 µg florasulam /mL LOD is defined as the lowest detectable concentration or amount of an analyte in a sample. LOD is 0.5 mg florasulam /kg sucrose solution and equivalent to the lowest calibration standard, i.e. 0.05 µg florasulam/ mL

Conclusion

The linearity of response of the analytical method, its specificity, precision, recovery, limit of quantification and detection were assessed in the process of the analytical method validation. The validated analytical methods was performed according to SANTE/12830/2020 and fulfil its requirements.

Analytical Method 5:

zRMS:
This method was provided only for pre-registration purposes. HPLC
The method is accepted for this purpose.

Report	Validation of analytical method for the determination of active substances – mesotrione and florasulam in aqueous solution of the test item MEZOFLOR 103 SC Marcin Świstak, SORBOLAB Research Laboratory LLC, 0030/0016

Guideline(s):	SANTE 12830/2020
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Determination of active substances (mesotrione and florasulam) in aqueous solution of the test item was performed by high performance liquid chromatography with UV-DAD detection on the basis of signals from active substances. Identification of active substances was made by comparing the UV spectrums and retention times of standards solutions and solution of the test item.

Sample preparation for the chemical determinations:
48.80 mg of test item was weighed into a 10 mL graduated flask. The flask was filled up to the mark with deionized water and whole was mixed thoroughly. A test item solution with concentration of 4880 mg/L was obtained. The prepared solution was diluted with deionized water.

Reagents and solvents:

- acetonitrile, HPLC grade, POCH, lot number 1251/02/21
- methanol, HPLC grade, Avantor, lot number 1225/07/20
- orthophosphoric acid 85%, p.a, Chempur, lot number 19/04/08
- 0.1% (v/v) orthophosphoric acid ((prepared by adding 1.176 mL of orthophosphoric acid 85%, p.a. to 1000 mL volumetric flask filled with 900 mL of ultrapure water, and then filled up to the mark with ultrapure water)
- deionized water
- ultrapure water
- mesotrione standard, IPO Warszawa, lot number 1A/18
- florasulam standard, IPO Warszawa, lot number 1A/18

Equipment:

- high performance liquid chromatography Shimadzu Prominence series LC-20 with PDA detector
- analytical balance Radwag XA 82_220.4Y.A
- deionizer SolPure 78
- system for obtaining ultrapure water Millipore Synergy UV
- ultrasonic washer Sonic-10
- adjustable automatic pipettes: Transferpette S 1 mL, Transferpette S 5 mL, Transferpette S 10 mL, Transferpette S 10 µL, Transferpette S 200 µL
- volumetric flask class A
- syringes and syringe filters 0.22 µm

HPLC - DAD parameters:

- Column: Kinetex C18 5 µm 100 Å 150 x 4.60 mm
- Detection: 254 nm
- Injection volume: 100 µL
- Column thermostat temperature: 40°C
- Mobile phase: Acetonitrile : 0.1% H_3PO_4 (30:70)

- Flow of mobile phase: 2 mL/min

Results and discussions

Table A ~~16~~19: Recovery results from method validation of mesotrione using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (n = 10)	Mean recovery (%)	RSD (%)	Comments
water	mesotrione	control	-	-	-
		3.44128 mg/L	88.3 %	0.25 %	
		18.85632 mg/L	85.5 %	0.56 %	

Table A ~~17~~20: Recovery results from method validation of florasulam using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (n = 10)	Mean recovery (%)	RSD (%)	Comments
water	florasulam	control	-	-	-
		0.10331 mg/kg	109.8 %	2.33 %	
		0.56608 mg/kg	102.6 %	1.17 %	

Table A ~~18~~21: Characteristics for the analytical method used for validation of mesotrione and florasulam in water

	mesotrione	florasulam
Specificity	The analytical method specificity was estimated on the basis of the analysis of the chromatograms obtained for the control samples and fortified samples. Considering the results of the analysis, no signal of detected substances was overlapping with the matrix signal of the control samples under the experimental conditions. Therefore, the specificity of the method was demonstrated.	
Calibration (type, number of data points)	Working solutions of mesotrione at concentrations of 0.89245 mg/L, 4.95806 mg/L, 24.79032 mg/L (each in duplicate) were prepared. The calibration curve was linear and expressed by an equation: y= 141300 x - 5131.59 Correlation coefficient r=0.999 Regression residual (d_i) is randomly distributed	Working solutions of florasulam at concentrations of 0.02868 mg/L, 0.23898 mg/L, 2.00744 mg/L The calibration curve was linear and expressed by an equation: y= 65164.8 x – 299.476 Correlation coefficient r=0.999 Regression residual (d_i) is randomly distributed.
Calibration range	The calibration curve was linear in full range.	The calibration curve was linear in full range.
Assessment of matrix effects is presented	Matrix effect for mesotrione was -2.7 % and not exceed ± 20 %.	Matrix for florasulam was 0.8 % and not exceed ± 20 %.
Limit of determination/quantification	The limit of quantification is the average value of the concentration of the active substance in solutions	The limit of quantification is the average value of the concentration of the active substance in solutions

	mesotrione	florasulam
	of the test item at the lower level of the accuracy analysis. The limit of detection is the lowest point on the calibration curve, which is below 30% of LOQ value. Limit of detection (LOD) for mesotrione: 0.89245 mg/L Limit of quantification (LOQ) for mesotrione: 3.03943 mg/L	of the test item at the lower level of the accuracy analysis. The limit of detection is the lowest point on the calibration curve, which is below 30% of LOQ value. Limit of detection (LOD) for florasulam: 0.02868 mg/L Limit of quantification (LOQ) for mesotrione: 0.11341 mg/L

Conclusion

The linearity of response of the analytical method, its specificity, precision, recovery, limit of quantification and detection were assessed in the process of the analytical method validation. The validated analytical methods was performed according to SANTE/12830/2020 and fulfil its requirements.

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

No new or additional studies have been submitted.

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

No new or additional studies have been submitted.

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

No new or additional studies have been submitted.