FINAL REGISTRATION REPORT

Part B

Section 5

Analytical Methods

Detailed summary of the risk assessment

Product code: MEZOT 100 SC

Product name(s): Mezot 100 SC

Chemical active substance:

Mesotrione, 100 g/L

Central

Zonal Rapporteur Member State: POLAND

CORE ASSESSMENT

(authorization)

Applicant: Elvita Sp. z o.o.

Submission date: 28/01/2021

MS Finalisation date: 08/2023**;** 12/2023

Version history

When	What
02.02.2023	Point 5.2.1.1 – Completion of data and information.
02.02.2023	Point 5.2.2 – Correction of data and information.
02.02.2023	Point 5.3.2.2 – 5.3.2.3 – Correction of data and information.
02.02.2023	Appendix 1 – Completion of data and information.
08.2023	ZRMs evaluated dRR submitted by Applicant
12.2023	The final Registration Report

Table of Contents

This document reviews the environmental fate and behaviour for the product Mezot 100 SC containing Mesotrione as active substance which was included into Annex I of Directive 91/414 (current legislation – regulation 540/2011) by Directive 2003/68/EC (July, 11th 2003) and renewed by Regulation (EU) 2017/725 of April, 24th 2017.
A full risk assessment according to Uniform Principles is provided which demonstrates that the product is safe for the environment.

Where appropriate this document refers to the conclusions of the EU review of Mesotrione. This will be where:

- the protection of operators,
- the protection of groundwater in vulnerable regions,
- the protection of mammals, aquatic and non-target plants..

Note: this Part B document only reviews data (Annex II or Annex III) and additional information that has not previously been considered within the EU review process, as part of the Annex I inclusion decision. New annex II data must only be included if they are considered essential for the evaluation and in this case a full study summary must be provided.

Mezot 100 SC as formulation has not been previously evaluated in Poland according to Uniform Principles.

EFSA Journal 2016;14(3):4419 conclusion on the peer review of the pesticide risk assessment of the active substance Mesotrione are considered to provide the relevant review information or a reference to where such information can be found.

The Annex I Directive 2003/68/EC (July, 11th 2003) and Regulation (EU) 2017/725 of April, 24th 2017 provide specific provisions under Part B which need to be considered by the applicant in the preparation of their submission and by the MS prior to granting an authorisation.

For the implementation of the uniform principles of Annex VI, the conclusions of the review report on the Mesotrione and in particular Appendices thereof, as finalised in the Standing Committee on the Food Chain and Animal Health on 23 March 2017 (SANTE/11654/2016) shall be taken into account.

5 Analytical methods

5.1 Conclusion and summary of assessment

Sufficiently sensitive and selective analytical methods are available for determination of the active substance and relevant impurities in the plant protection product Mezot 100 SC.

Analytical method HPLC-DAD for determination of a.s. Mesotrione and relevant impurity R287432 has been validated and meet criteria of specificity, linearity, precision and accuracy according to the requirement SANCO 3030/99 rev. 5, therefore it is acceptable.

HS-GC-FID method for determination of the relevant impurity R287431 has been validated and meet criteria of specificity, linearity, precision and accuracy according to the requirement SANCO 3030/99 rev. 5. Method is considered acceptable.

UHPLC-MS/MS method for determination of the relevant impurity 1,2-dichloroethane has been validated and meet criteria of specificity, linearity, precision and accuracy according to the requirement SANCO 3030/99 rev. 5. Method is considered acceptable.

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 relevant impurities in the plant protection product (KCP 5.1.1)

An overview on the acceptable methods and possible data gaps for analysis of Mesotrione, and ~~Mesotrione~~ relevant impurities: R287432 and 1,2-dichloroethane in plant protection product is provided as follows:

Reference:	5.2.1.1/01, Wołoszynowska M., Chałas A., 2019.
Report	Mezot 100 SC; Validation of the method for determination of the active substance and two relevant impurities content in the formulation.
Guideline(s):	SANCO/3030/99 rev. ~~4~~ 5
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Description of the method – active substance and 6-(methylsulfonyl)-9-oxo-9H-xanthene-1-carbonitrile (R287432).

Method validation for determination of the Mesotrione and 6-(methylsulfonyl)-9-oxo-9Hxanthene-1-carbonitrile (R287432) content was determined using HPLC chromatography with DAD detection using external standard method.

Reagents and standard:

Analytical standard:

- Mesotrione, IPO 941, Series No. 1A/18, purity 99,8%
- 6-(Methylsulfonyl)-9-oxo-xanthene-1-carbonitrile, GalChimia, batch no ga0051168, purity 99.2%

Reagents and equipment:

Apparatus and materials:

- Shimadzu liquid chromatograph equipped with DAD detector
- Luna Omega C18 column (5µm), 250 x 4.6 mm
- Analytical balance RADWAG AS82/220 X2, accuracy 0.01 mg
- Glass pipettes
- Glass graduated flasks
- Ultrasonic bath
- Automatic pipette
- Typical laboratory equipment

Reagents:

- Deionized water, ultra-pure, Millipore
- Acetonitrile for HPLC – Super gradient, POCh
- o-phosphoric acid 85%, Chempur

Chromatographic conditions:

- Column temperature: 35 ºC
- Mobile phase: A: acetonitrile (40%)
  B: 0.5% H3PO4 (60%)
- Flow rate: 1.0 ml/min
- Wavelength $\lambda = 238$ nm
- Volume of sample solution injected: 10 µl

Under above conditions retention time of Mesotrione was 10.3 min ± 0.3 min. and 6-(methylsulfonyl)-9-oxo-9H-xanthene-1-carbonitrile (R287432) was 9.0 min ± 0.3 min. The total time of analysis was 25 min.

Solutions:

Preparation of Mesotrione solution - standards solution:
About 10 mg of mesotrione standard was weighed (with the accuracy of 0.01 mg) into 10 ml flask with a screw cap and acetonitrile was added up to the volume. The flask was put into the ultrasonic bath for 5 min.

Calibration curve:
Into five 10 ml volumetric flasks the following amounts of standard were pipetted:

Solution No.	1	2	3	4	5
Mesotrione	0.10 ml	0.14 ml	0.18 ml	0.20 ml	0.25 ml

Acetonitrile was added to the mark and the solutions were stirred.

Specimen solution:
About 20 mg of examined specimen was weighed (with the accuracy of 0.01 mg) into a 10 ml flask with a screw cap and acetonitrile was added up to the volume. The flask was put into the ultrasonic bath for 5 min. After cooling solution was diluted 10-times. Six samples were prepared to assess the repeatability.

Preparation of solutions (6-(methylsulfonyl)-9-oxo-9H-xanthene-1-carbonitrile) - standards solution:
About 6 mg of 6-(methylsulfonyl)-9-oxo-9H-xanthene-1-carbonitrile standard was weighed (with the accuracy of 0.01 mg) into 10 ml flask with a screw cap and acetonitrile was added up to the volume. The flask was put into the ultrasonic bath for 5 min. After cooling solution

was diluted 10-times

Calibration curve:
Into one 100 ml, one 50 ml and four 10 ml volumetric flasks the following amounts of standard were pipetted:

Solution No.	1	2	3	4	5	6
6-(methylsulfonyl)-9-oxo-9Hxanthene-1-carbonitrile (R287432)	0.30 ml	0.20 ml	0.10 ml	0.20 ml	0.30 ml	0.50 ml

Acetonitrile was added to the mark and the solutions were stirred.

Specimen solution:
About 300 mg of examined specimen was weighed (with the accuracy of 0.01 mg) into a 10 ml flask with a screw cap and acetonitrile was added up to the volume. The flask was put into the ultrasonic bath for 5 min.
Procedure:
Chromatographic system was conditioned before analysis. Standards solution had been introduced into the column several times until obtained peak areas were different no more than 3%. The standard solution and the specimen solution were introduced into the chromatographic column.

Description of the method – 1,2-dichloroethane.

Method validation 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.

Reagents and standard - analytical standard:
- 1,2-dichlorethane, 99.8 %, IPO, Series No. 3A/17

Reagents and equipment:
Apparatus and materials:
- VARIAN CP-3800 Gas Chromatograph with FID
- Teledyne Tekmar HT-3 Headspace Autosampler
- Rxi®-1301Sil MS capillary column, 30 m × 0.25 mm × 1.0 µm (RESTEK)
- Analytical balance Mettler Toledo XS205 Dual Range, Total recovery 0.01 mg
- Glass pipettes
- Glass graduated flasks
- Automatic pipettes
- 20 ml headspace vials with alumina caps and Teflon-silicone septa
- Laboratory crimper
- Autosampler vials
- Typical laboratory equipment

Reagents:
- Deionized water, ultra-pure, Millipore
- Dimethyl sulfoxide (DMSO), for headspace analysis (99.99 %), VWR Chemicals

Chromatographic conditions:
- Oven: 45 ºC (1 min), 10 ºC/min → 110 ºC, 20 ºC /min → 250 ºC
- Carrier gas: Helium
- Flow: 2 ml/min
- Inlet temperature: 250 ºC
- 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 ºC
- Transfer line temperature: 120 ºC
- Platen/sample temperature: 100 ºC
- Sample equilibration time: 20 min
- Mixing time: 5 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 4.11 ± 0.03 min.
The total time of analysis is 14.50 min.

Solutions:
Preparation of solutions:
About 100 mg of 1,2-dichloroethane was weighed (with the accuracy of 0.01 mg) into 10 ml flask with a screw cap and DMSO was added up to the volume.
The stock solutions of 1,2-dichloroethane:

Substance	Stock solution No.	Mass [mg]	Purity [%]	Dilution [mL]	Concentration [mg/mL]
1,2-dichloroetane	1	99.9	99.8	10	9.9700
	2	100.06	99.8	10	9.9860

Working solution:
To determine limit of quantification (LOQ) and method linearity working standard solutions of 1,2-dichloroethane in DMSO were prepared:

Stock solution No.	Concentration of the stock solution (A1 and A-2)	Dilution [mL/mL]	Concentration 1 (B-1 and B-2) [mg/mL]	Dilution [mL/mL]	Concentration 2 (C-1 and C-2) [mg/mL])	Dilution [mL/mL]	Concentration 3 (D-1 and D-2) [mg/mL])
1	9.9700	1/10	0.9970	1/10	0.0997	1.0/10	0.00997
2	9.9860	1/10	0.9986	1/10	0.0999	1.0/10	0.00999

Because content of 1,2-dichloroethane in the Mezot 100 SC preparation was below LOQ level, analytical results were compared to results obtained for placebo of Mezot 100 SC preparation fortified with 1,2-dichloroethane at LOQ level.

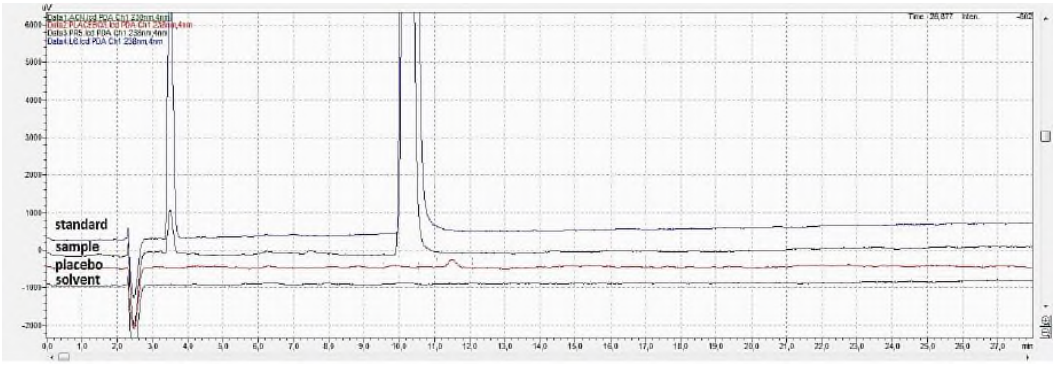
Calculations.
Chromatographic system was conditioned before analysis. Standard solution had been introduced into the column several times until obtained peak areas were different no more than 3%. The standard solution and the specimen solution were introduced into the chromatographic column.
The content of analyzed substance was calculated according to the equation:

$f = (Awz) / (Cwz)$
$X\ \% = (Apr * 100) / (Cpr * f)$
where:
f - average calculating factor
Apr – peak area of analysed substance on the specimen solution chromatogram
Awz – peak area of analysed substance on the standard solution chromatogram
Cwz – concentration of the analysed substance standard
Cpr – concentration of the examined specimen

All chromatograms (peak area) were integrated manually.

Method validation for determination of active substance.
Validation parameters:
- Specificity
- Linearity
- Precision (repeatability)
- Recovery (total)
- Accuracy

Specificity:
The chromatograms of solvent, standard solution, placebo solution and the examined specimen solution were performed and superimposed:



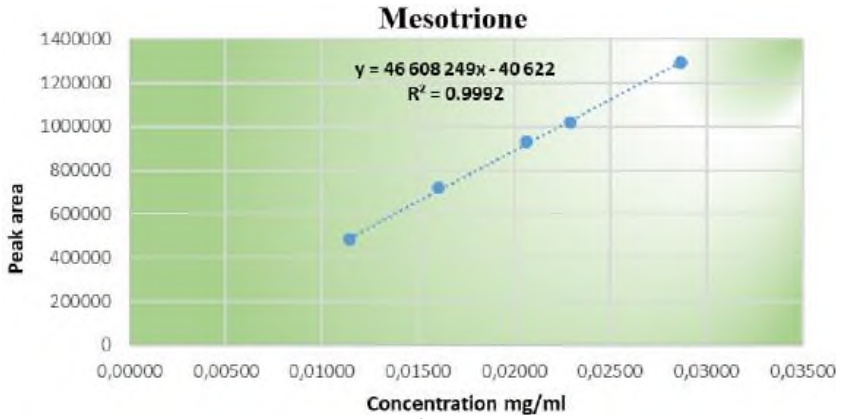
Linearity:

The linearity of the detector response was assessed using five standard solutions at the concentration range of Mesotrione from 0.01147 mg/ml to 0.02867 mg/ml, which corresponds to the concentration range of 57% to 142% of Mesotrione content in the preparation. All solutions were analyzed twice.

Mesotrione – detector response:

Concentration of Mesotrione* [mg/ml]	Peak area	Average
0.01147	485197	485375
	485553	
0.01605	719799	718290
	716781	
0.02064	926654	928577
	930499	
0.02293	1018426	1020152
	1021878	
0.02867	1291458	1294277
	1297096	

* after taking into account the purity of standard



Correlation coefficient should be $R^2 \geq 0.99$. The obtained result is acceptable.

Precision (repeatability):

The method repeatability was assessed on the basis of six independent determinations of active substance content in MEZOT 100 SC preparation.

Repeatability of Mesotrione determination:

Chromatogram name	Specimen weight [mg]	Concentration [mg/l]	Peak area	Result [%]
P1	22.51	0.225	949115	9.51
P2	22.28	0.223	918376	9.34
P3	18.68	0.187	795302	9.60
P4	22.03	0.220	944491	9.67
P5	19.09	0.191	817111	9.65
P6	18.35	0.184	761695	9.36
			Average	**9.523**
			SD	0.145
			RSD [%]	**1.52**

Relative standard deviation of determination of Mesotrione fulfils acceptance criterion.
RSD for substance at the concentration of ~ 10 % should be less than or equal to 1.90 %.
Horrat value calculated with the equation:
Hr = %RSD / % RSDr
where,
%RSD is obtained repeatability
%RSDr is expected repeatability obtained with modified Horwitz equation,
is 0.80 and fulfils acceptance criterion Hr ≤ 1
According to these data confidence interval was evaluated:

$$\bar{x} = \frac{t \times S_D}{\sqrt{n}}$$

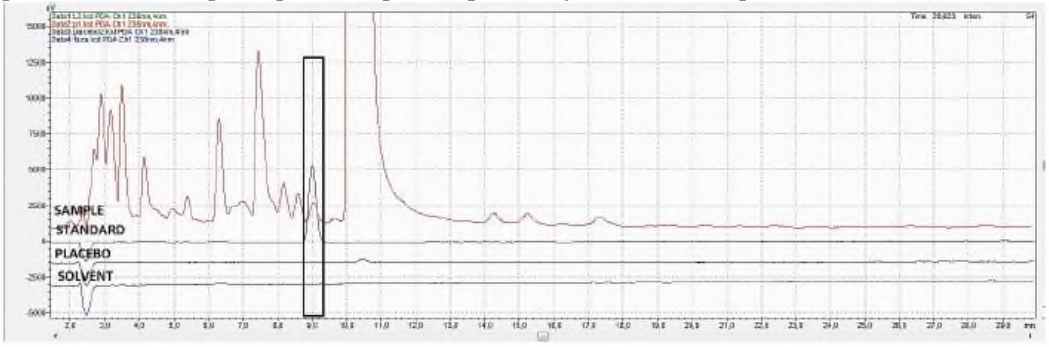
t – t-Student distribution value for n-1 degrees of freedom
S_D- standard deviation
n- number of samples in each series

$$\bar{x} = \frac{2.571 \times S_D}{\sqrt{6}}$$

$X_{Mesotrione}$ = 0.11%

Recovery:
Recovery of active substance determination in MEZOT 100 SC preparation was assessed by total recovery value at two levels of concentration. 0.20 ml of the Mesotrione standard solution at concentration of 0.479 mg/ml and 10 mg of placebo were added to the each of the first six 10 ml flasks and acetonitrile were added up to the volume. To another six 10 ml flasks 0.13 ml of Mesotrione standard solution at the same concentration) and 10 mg of placebo were added and acetonitrile was added up to the volume. The flasks were put into the ultrasonic bath for 5 min. The concentration of analyte in each solution was calculated from the equation of the calibration curve. Obtained final concentrations were examined and the
theoretical and calculated contents were compared.
For the main ingredient at concentration of >10 % the average recovery value should be
100 ± 3%. The obtained result of 99.48% is acceptable.

Method validation for determination of 6-(methylsulfonyl)-9-oxo-9Hxanthene-1-carbonitrile R287432.
Specificity:
The chromatograms of solvent, standard solution, placebo solution and the examined specimen solution were performed and superimposed to prove specificity of the developed method.



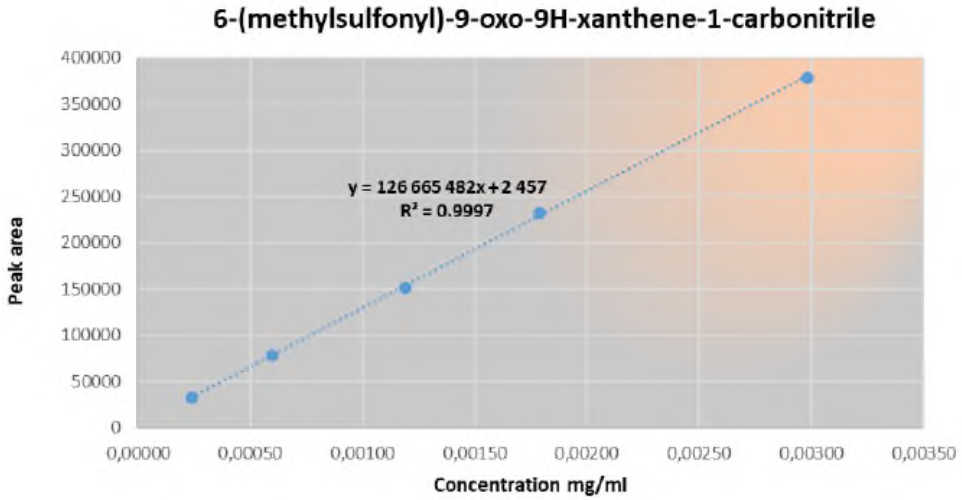
To confirm the identity of 6-(methylsulfonyl)-9-oxo-9H-xanthene-1-carbonitrile, the specificity was developed in the second system (Fig.6) under the following conditions:

- Column: Gemini NX-C18, 250 x 4.6 mm, 5µm
- Column temperature: 25 ºC
- Flow rate: 0.8 mL/min
- Wavelength λ = 238 nm
- Volume of sample solution injected: 15 µL
- Mobile phase: A: acetonitrile (55%) + B: 1 mmol ammonium acetate in H2O (45%)

Linearity:
The linearity of the detector response was assessed using five standard solutions at the concentration range of 6-(methylsulfonyl)-9-oxo-9H-xanthene-1-carbonitrile from 0.000060 mg/ml to 0.00298 mg/ml, which corresponds to the concentration range of 4% to 225% of 6-(methylsulfonyl)-9-oxo-9H-xanthene-1-carbonitrile maximum content of the relevant impurity in the preparation. All solutions were analysed twice.

Linearity of 6-(methylsulfonyl)-9-oxo-9H-xanthene-1-carbonitrile – detector response:

Concentration of 6-(methylsulfonyl)-9-oxo-9H-xanthene-1-carbonitrile [mg/ml]	**Peak area**	**Average**
0.000060	9613	9639
	9466	
	9761	
	9812	
	9487	
	9695	
0.00024	31620	31898
	32175	
0.00060	78597	78786
	78974	
0.00119	150763	151053
	151342	
0.00179	232972	232805
	232637	
0.00298	379120	378638
	378156	



Correlation coefficient should be $R^2 \geq 0.99$. The obtained result is acceptable.

Limit of quantification:
The limit of quantification (LOQ) was defined as the lowest concentration ie. 0.00050 mg/ml (0.000199 g/kg of 6-(methylsulfonyl)-9-oxo-9H-xanthene-1-carbonitrile content in the preparation and 0.0209 g/kg for mesotrione) for. Limit of detection (LOD) is LOQ/2 i.e. about 0.000099 g/kg.

RMS comments:
According to SANCO/3030/99 rev. 5 the LOQ for relevant impurity should be defined as the lowest concentration tested, at which an acceptable recovery and an acceptable precision (repeatability), is obtained. Therefore, for impurity R287432 the LOQ should be established at 0.0008 % (precision and recovery see summary table).

Precision (repeatability):
The method repeatability was assessed on the basis of six independent determinations of 6-(methylsulfonyl)-9-oxo-9H-xanthene-1-carbonitrile content in MEZOT 100 SC preparation.
Repeatability of 6-(methylsulfonyl)-9-oxo-9H-xanthene-1-carbonitrile determination:

Chromatogram name	Specimen weight [mg]	Concentration [mg/l]	Peak area	Result [%]
P1	300.57	30.057	19141	0.00044
P2	301.25	30.125	19372	0.00044
P3	301.29	30.129	19181	0.00044
P4	300.93	30.093	19159	0.00044
P5	300.99	30.099	18904	0.00043
P6	300.75	30.075	18912	0.00043
			Average	**0.00044**
			SD	0.000004
			RSD [%]	**1.03**

Relative standard deviation of determination of 6-(methylsulfonyl)-9-oxo-9H-xanthene-1-carbonitrile fulfils acceptance criterion. RSD for substance at the concentration of ~ 0,0004 % should be less than or equal to 8.58 %.
Horrat value calculated with the equation:
Hr = %RSD / % RSDr
where,
%RSD is obtained repeatability
%RSDr is expected repeatability obtained with modified Horwitz equation,
is 0.12 and fulfils acceptance criterion Hr ≤ 1

According to these data confidence interval was evaluated:

$$\bar{x} = \frac{t \times S_D}{\sqrt{n}}$$

t – t-Student distribution value for n-1 degrees of freedom
S_D- standard deviation
n- number of samples in each series

$$\bar{x} = \frac{2.571 \times S_D}{\sqrt{6}}$$

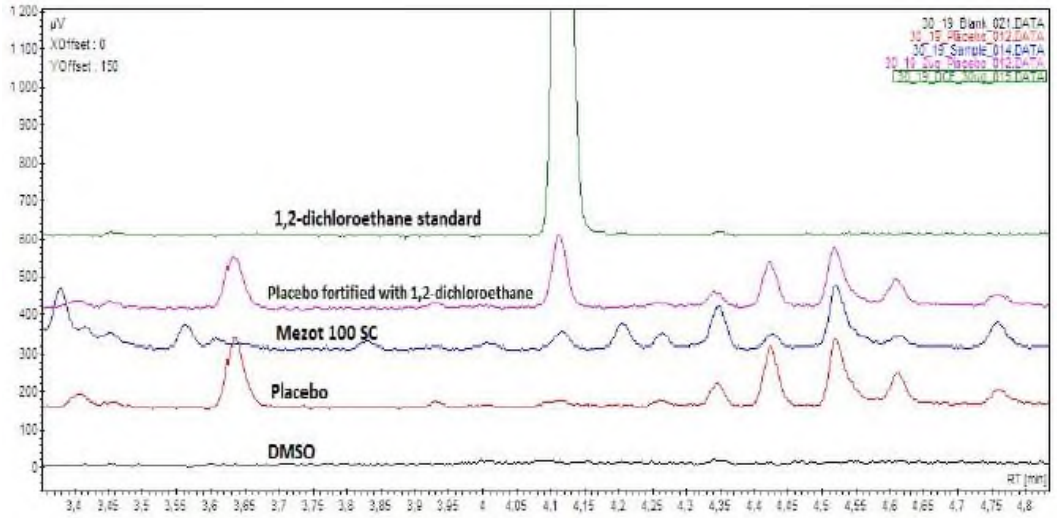
$X_{Mesotrione}$ = 0.000005%

Recovery:
Recovery of the method for determination of 6-(methylsulfonyl)-9-oxo-9H-xanthene-1-carbonitrile in MEZOT 100 SC preparation was assessed by recovery value at two levels of concentration. Each of twelve 10 ml volumetric flasks were charged with approximately 250 mg of placebo and weighed. About 0.40 ml of the 6-(methylsulfonyl)-9-oxo-9H-xanthene-1-carbonitrile standard solution at concentration of 0.00596 mg/ml was added to the each of the first six flasks and acetonitrile was added up to the volume. To each of the remaining six flasks 0.50 ml of the 6-(methylsulfonyl)-9-oxo-9H-xanthene-1-carbonitrile standard solution at the concentration of 0.05535 mg/ml was added and acetonitrile was added up to the volume. The flasks were put into the ultrasonic bath for 5 min. The concentration of analyte in each solution was calculated from the equation of the calibration curve. Obtained final concentrations were examined and the nominal and calculated contents were compared.
For the relevant impurity at concentration < 0.01% the average recovery value should be 100 ± 30%. The obtained result of 102.46% is acceptable.

Method validation for determination of the 1.2-dichloroethane.

Specificity:
To prove specificity of the developed method the headspace chromatograms of: DMSO (sample solvent, 2 mL), placebo solution in DMSO (300 mg+2mL), sample solution (MEZOT 100 SC) in DMSO (300 mg+2mL), standard solution of 1,2-dichloroethane in DMSO (2 mL) and placebo solution in DMSO (300 mg+2mL) fortified with 1,2-dichloroethane at LOQ level were performed and superimposed.
There are no interferences between the analyte and other components of the specimen.

Superimposed chromatograms – specificity examination:

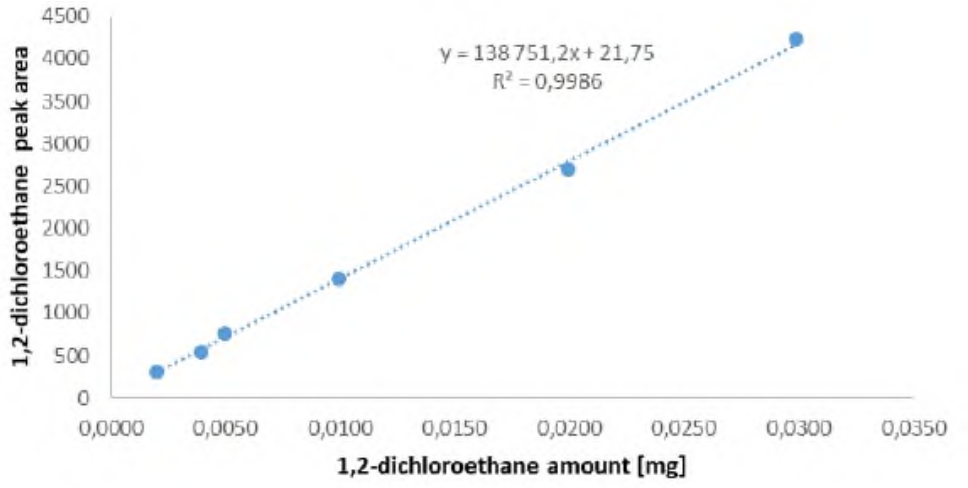


Linearity:

Detector response linearity was examined using 6 solutions in the range of 1,2-dichloroethane content from 0.002 to 0.0299 mg what for 300 mg of preparation sample constitutes from around 7 % to 110 % of maximum allowed content of 1,2-dichloroethane in MEZOT 100 SC preparation (m/m). Three calibration solutions (0.0299 mg, 0.0100 mg and 0.0040 mg) were prepared from Stock solution No.1 (B-1 and C-1), another three calibration solutions (0.0200 mg, 0.0050 mg and 0.002 mg) were prepared from Stock solution No.2 (C-2 and D-2). Because matrix components of the preparation had an effect on partition of 1,2-dichloroethane between liquid and gaseous phases, the calibration curve was determined using placebo of MEZOT 100 SC preparation fortified with known amount of 1,2-dichloroethane standard.
For this purpose to about 300 mg of placebo placed in headspace vials appropriate volume of DMSO and working standard solutions were added. The vials were tightly closed and then analyzed. Headspace of each of the above mentioned solutions was analyzed once (one replicate), except the lowest level which was analyzed six times (six replicates) to determine limit of quantification (LOQ) of the method and accuracy of determination of 1,2-dichloroethane.

Detector response - 1,2-dichloroethane determined with addition of placebo of MEZOT 100 SC preparation:

Level	Working solution	Volume of working solution [mL]	Volume of DMSO [mL]	Standard amount [mg]	Standard content (for 300 mg sample)	Standard peak area
1	D-2	0.200	1.800	0.0020	0.0067	310.4 318.5 323.6 298.0 300.2 306.7 **Mean: 309.6** **RSD: 3.25 %**
2	C-1	0.040	1.960	0.0040	0.0133	**551.4**
3	C-2	0.050	1.950	0.0050	0.0166	**760.7**
4	C-1	0.100	1.900	0.0100	0.0332	**1412.2**
5	C-2	0.200	1.800	0.0200	0.0666	**2694.6**
6	B-1	0.030	1.970	0.0299	0.0997	**4229.8**



Correlation coefficient should be R2 ≥ 0.99. The obtained result is acceptable.

Limit of quantification:
The limit of quantification (LOQ) was defined as the lowest quantity of injected 1,2-dichloroethane standard that gave precise and accurate measurements and is expressed as the lowest 1,2-dichloroethane amount used for calibration curve (Table 12. Limit of quantification is 0.0020 mg what corresponds to 0.0007 % (0.067 g/kg)of 1,2-dichloroethane content in the preparation.

Precision (repeatability):
The method repeatability was assessed on the basis of six independent determinations of 1,2-dichloroethane content in MEZOT 100 SC preparation. About 300 mg of the examined preparation were placed in headspace vials and then 2 mL DMSO was added. The vials were tightly closed and analyzed. In none of the examined samples 1,2-dichloroethane was detected above LOQ. Therefore for the determination of repeatability six portions about 300 mg of placebo fortified with 1,2-dichloroethane at LOQ level (0.0020 mg) were analyzed. To six portions about 300 mg of placebo 0.200 mL of working standard solution D-2 and 1.800 mL DMSO were added.

Repeatability determination - 1,2-dichloroethane:

No.	Amount of added 1.2-dichloroethane [mg]	1.2-dichloroethane peak area
1	0.0020	310.4
2	0.0020	318.5
3	0.0020	323.6
4	0.0020	298.0
5	0.0020	300.2
6	0.0020	306.7
	Mean	**309.6**
	SD	**10.1**
	RSD %	**3.25**

Relative standard deviation of determination of 1,2-dichloroethane fulfils acceptance criterion. RSD for substance at the concentration of ~ 0,0007 % should be less than or equal to 8,00 %.
Horrat value calculated with the equation:
Hr = %RSD / % RSDr
where,
%RSD is obtained repeatability
%RSDr is expected repeatability obtained with modified Horwitz equation,
is 0.41 and fulfils acceptance criterion Hr ≤ 1

According to these data confidence interval was evaluated:

$$\bar{x} = \frac{t \times S_D}{\sqrt{n}}$$

t – t-Student distribution value for n-1 degrees of freedom
S_D- standard deviation
n- number of samples in each series

$$\bar{x} = \frac{2.571 \times S_D}{\sqrt{6}}$$

$X_{Mesotrione}$ = 0.0003%

Recovery:
Recovery of the method for 1,2-dichloroethane determination in MEZOT 100 SC preparation was assessed at two levels of concentration.
Twelve portions about 300 mg of placebo were placed in headspace vials. To six of them 0.200 mL of working standard solution D-2 (Table 3, Table 14) and 1.800 mL DMSO were added and to other six 0.100 mL of working standard solution C-1 (Table 3, Table 14) and 1.900 mL DMSO were added. Tightly closed vials were analyzed and the content of 1,2-dichloroethane and recovery were calculated using the calibration curve determined with addition of placebo of MEZOT 100 SC preparation.
For the impurities at concentration of ≥0.01% the average recovery value should be 100 ± 30%. The obtained result of 103.4% is acceptable.

Conclusion:
The validation parameters (specificity, linearity, instrument precision, repeatability and accuracy) are within the acceptance range and fulfil EU requirements given in SANCO /3030 /99 rev.~~4.~~ 5

Summary of validation parameters for a.s. Mesotrione

	Mesotrione
Author(s), year	Wołoszynowska M., Chałas A., 2019
Principle of method	HPLC - DAD
Linearity (linear between mg/L / % range of the declared content) (correlation coefficient, expressed as r)	0.01147 mg/ml to 0.02867 mg/mL (5% – 13%) n = 5 R^2 = 0.9992 y = 46608249x – 40622
Precision – Repeatability Mean n = 6 (%RSD)	Mean conc.: 9.523 % RSD = 1.52 % RSDr = 1.91 % Hr value <1
Accuracy n = 6 (each level) (% Recovery)	Level I: 0.0186 mg/L (8%): 98.51 % Level II: 0.0210 mg/L (10%): 100.44 % 99.48%
Interference/ Specificity	No interferences between the analyte and other components of the specimen, method is specific
Comment	Accepted

Summary of validation parameters for relevant impurities: 1.2-dichloroethane and 6-(methylsulfonyl)-9-oxo-9Hxanthene-1-carbonitrile (R287432)

	1.2-dichloroethane (max 0.1 g/L in PPP)	**R287432 (max 0.2 g/L in PPP)**
Author(s), year	Wołoszynowska M., Chałas A., 2019	
Principle of method	HPLC - DAD	
Linearity (linear between mg/L / % range of the declared content) (correlation coefficient, expressed as r)	0.002 mg to 0.0299 mg (0.0007% to 0.01%) n = 6 R^2 = 0.9986 y = 138751.2x + 21.75	0.000060 mg/mL to 0.00298 mg/mL (0.0022% to 0.0087%) n = 5 R^2 = 0.9997 y = 126665482x + 2437
Precision – Repeatability Mean n = 6 (%RSD)	Mean conc.: 0.0007 % RSD = 3.25 % RSDr = 8.00 % Hr value <1	Mean conc.: 0.00044 % RSD = 1.03 % RSDr = 8.58 % Hr value <1
Accuracy n = 6 (% Recovery)	Total recovery Level I: 0.0007%: 104.02 % Level II: 0.0035% mg: 102.84 % 103.4 %	Total recovery Level I: 0.0002385 mg/mL (0.0008%): 100.41 % Level II: 0.0027677 mg/mL(0.008%): 104.51 % 102.46 %
Interference/ Specificity	No interferences between each analyte and other components of the specimen.	
LOQ	0.0020 mg (0.0007%)	0.0008 % lower level of recovery RSD = 0.83% (n=6) RSDr = 7.84% Hr value < 1
Comment	Accepted	

Reference:	5.2.1.1/02, Sowik I., 2012.
Report	MEZOT 100 SC Determination of physicochemical properties.
Guideline(s):	SANCO/3030/99 rev. 5
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Notes	The aim of the study was to determine pH for undiluted formulation and content of relevant impurity of mesotrione: 1-cyano-6-(methylsulfonyl)-7-nitro-9H-xanthen-9-one (IMP 1) in MEZOT 100 SC preparation at initial stage and after accelerated storage

Validation of the methods

The method validation for determination of relevant impurity in **MEZOT 100 SC** preparation was performed in **MEZOT 100 SC** preparation were in Analytical Research Laboratory of the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry (Łukasiewicz-IPO) in Warsaw according to EU requirements described in SANCO/3030/99 rev.5. 22 March 2019 guideline.

Method validation for determination of the **(IMP 1)** 1-cyano-6-(methylsulfonyl)-7-nitro-9H-xanthen- 9-one R287431 content was performed using ultra high-performance liquid chromatography (UHPLC) with MS/MS detection and external standard method.

The following validation parameters were determined: specificity. linearity. precision (repeatability). limit of quantification and recovery.

Summary of validation parameters for 1-cyano-6-(methylsulfonyl)-7-nitro-9H-xanthen-9-one (R287431),

~~Parameter~~	~~Acceptance criteria~~	~~Obtained result~~
~~Specificity~~	~~Fulfilled~~	
~~Linearity~~	~~$R^2 \geq 0.99$~~	~~$R^2 = 0.9998$~~
~~LOQ~~	~~0.00000105% (0.00000105 g/kg of the preparation)~~	
~~Precision (repeatability) [%]~~	~~$RSD_r \leq 6.78$~~	~~1.68%~~
	~~$Hr \leq 1$~~	~~0.25~~
~~Recovery [%]~~	~~70 – 130~~	~~103.3~~

	R287431 (max 0.0002 g/L in PPP)
Author(s), year	Sowik I., 2012
Principle of method	UHPLC - MS/MS
Linearity (linear between mg/L / % range of the declared content) (correlation coefficient, expressed as r)	0.0105 to 0.4182 mg/L (0.00000105 to 0.00004182 %) n = 6 R^2 = 0.9998 y = 1110082.9216x – 4259.7405
Precision – Repeatability Mean n = 6 (%RSD)	Mean conc.: 0.00000209 % RSD = 1.68 % RSDr = 19.19 % Hr value <1
Accuracy n = 6 (% Recovery)	I Level: 0.0209 mg/L (0.00000209%): 109.86 % II Level: 0.1255 mg/L (0.00001255%): 96.7 % 103.3 %
Interference/ Specificity	No interferences between each analyte and other components of the specimen
LOQ	0.00000209%-Lower recovery level
Comment	Accepted

Analytical method

The method validation was carried out in pursuance of actual EU requirements SANCO/3030/99 rev.5 (22/03/19) in Analytical Laboratory of Łukasiewicz Research Network – Institute of Industrial Organic Chemistry which possess a Good Laboratory Practice compliance certificate.

Analytical standard

- 1-cyano-6-(methylsulfonyl)-7-nitro-9H-xanthen-9-one **(IMP 1),** 96.0%. TRC Canada. Series No. 13 – GUY – 162 - 1 (*Annex* no. 1)

Reagents

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

Equipment

- Sciex QTRAP 4500 mass spectrometer with UHPLC

- Column: Kinetex Biphenyl C18. 100 × 2.1 mm. 2.6 µm
- Analytical balance Mettler Toledo At 261. accuracy of 0.01 mg
- Glass pipettes
- Glass graduated flasks
- Ultrasonic bath
- Typical laboratory equipment

Chromatographic conditions

Mobile phase	5 mmol aqueous solution of ammonium formate + 0.1% aqueous solution of formic acid (A) + 5 mmol acetonitrile solution of ammonium formate + 0.1% acetonitrile solution of formic acid (B) (A+B; v/v)		
Gradient:	Time [min.]	A [%]	B [%]
	0.01	90	10
	2.00	90	10
	5.00	10	90
	8.00	10	90
	10.00	90	10
	15.00	90	10
Flow rate	0.4 ml/min		
Column temperature	40 °C		
Volume of sample injected	5 µl		
Analysis time	15 min		

Parameters of the mass spectrometer and ion source

Parameters of the mass spectrometer	
Ionization mode	positive
CUR:	25.0
CAD:	10
IS:	5500
TEM:	500
GS1:	40
GS2:	50
EP:	10
Parameters of the ion source	
Precursor ion m/z	362.1 $[M+NH_4]^+$
Product ions m/z	206.2 and **235.1*** (* used for calculations)
CE (eV):	74 (206.2) i 44 (235.1)
DP:	25

Under above conditions retention time of **IMP 1** was 4.82 min ± 0.20 min.

Preparation of solutions

Standards solutions

10.89 mg of **IMP 1** standard was weighed (with the accuracy of 0.01 mg) into 25 ml flask and DMSO was added up to the volume. The solution was transferred quantitatively to a 100 ml flask and phase A was added up to the mark. The flask was put into the ultrasonic bath for 2 min. After cooling. 0.1 ml was taken from the solution into 5 ml flask and filled up to the mark with phase A.

Stock solution of IMP 1

Stock Solution	**Purity [%]**	**Mass [mg]**	**Dilution [ml]**	**Concentration [mg/ml]**
A1	96.00	10.89	100	**0.105**

IMP 1 working standard solution

Stock Solution	Concentration of stock solution [mg/ml]	IMP 1 working standard solution	Dilution [ml/ml]	Concentration [mg/ml]	Concentration [mg/l]
A1	0.105	**A2**	0.1 / 5.00	0.0021	**2.091**

Calibration curve

Into one 10 ml (point 1) and five 5 ml (point 2-6) volumetric flasks the following volume of standard solution (A2) were pipetted:

Standard solutions used for calibration curve

Solution number	1	2	3	4	5	6
A2 [ml]	0.05	0.05	0.10	0.50	0.60	1.00

Specimen solution

About 100 mg of preparation was weighed (with the accuracy of 0.01 mg) into a 10 ml flask. next diluent was added up to the volume. The flask was put into the ultrasonic bath for 2 min. After cooling. the solution was analyzed.

Diluent

Solution was prepared with acetonitrile and mobile phase A in the ratio 1:9.

Calculations

Chromatographic system was conditioned before analysis. Standards solutions had been introduced into the column several times until obtained peak areas were different no more than 3%. The standards solutions and the specimen solution were introduced into the chromatographic column.

Quantitation was done in Microsoft Excel with regression model:

$$y = a \cdot x + b$$

where:
y - peak area
a - slope
x - content of IMP 1 in injected solution [mg/l] b -
y- axis intercept

All chromatograms (peak area) were integrated manually.

Method validation

Validation parameters:

- Specificity
- Linearity
- Limit of quantification (LOQ)
- Precision (repeatability)
- Recovery (total)

Specificity

The chromatograms of solvent. standard solution. placebo solution and the examined specimen solution were performed and superimposed to prove specificity of the developed method. The obtained results are presented in ***Fig. 1***. **There are no interferences between the analyte and other components of the specimen.**

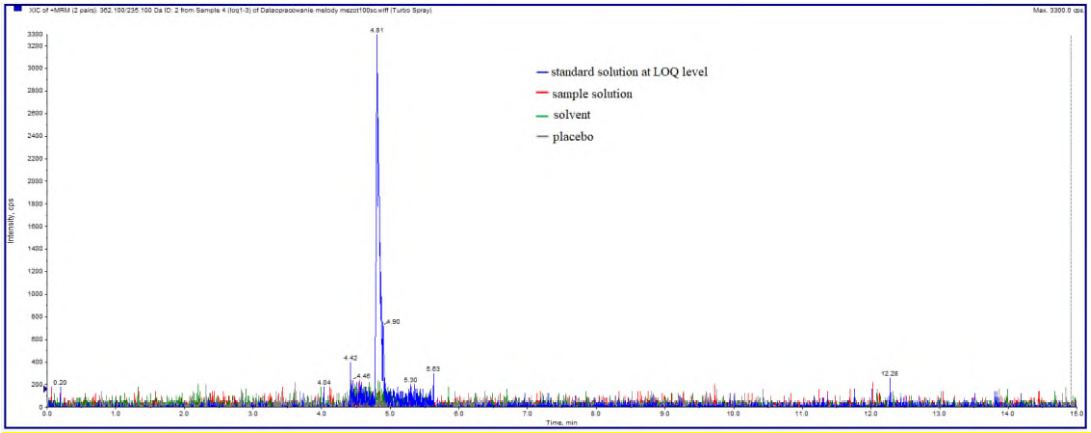


Fig. 1. Specificity of IMP 1. Overlaid chromatograms of: standard solution of IMP 1 in mobile phase A. MEZOT 100 SC preparation in mobile phase A. solvent (mobile phase A) and placebo of MEZOT 100 SC preparation in mobile phase A.

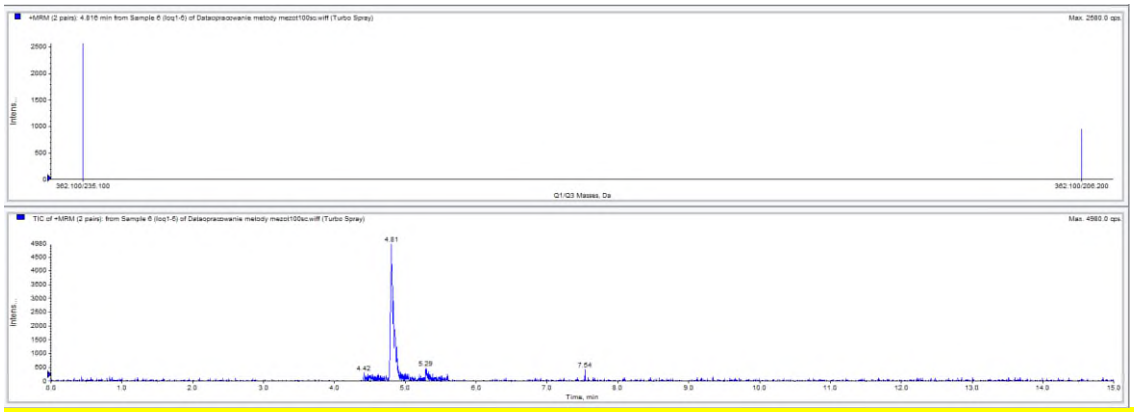


Fig. 2. MS/MS spectra of m/z 362.1 at retention time (RT) 4.82 min. and total ion chromatogram (TIC) of m/z 362.1 / 253.1 standard (at LOQ level)

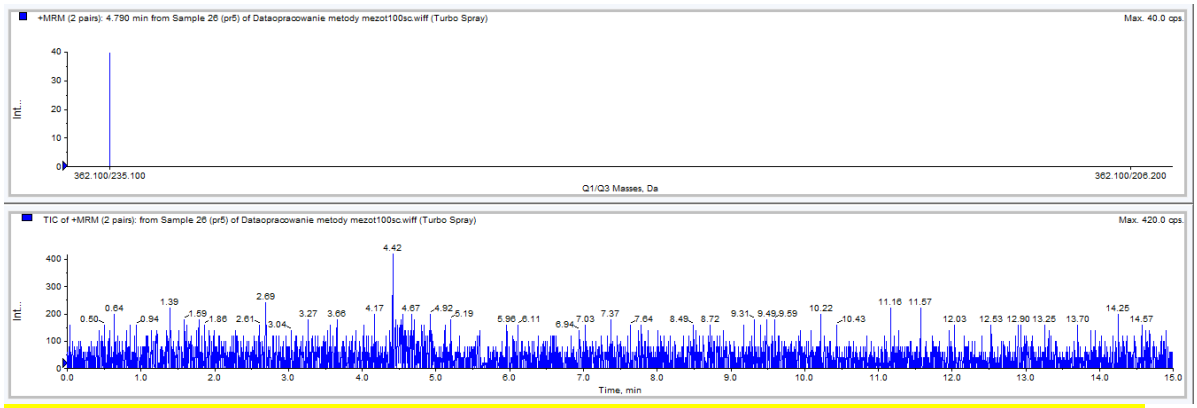


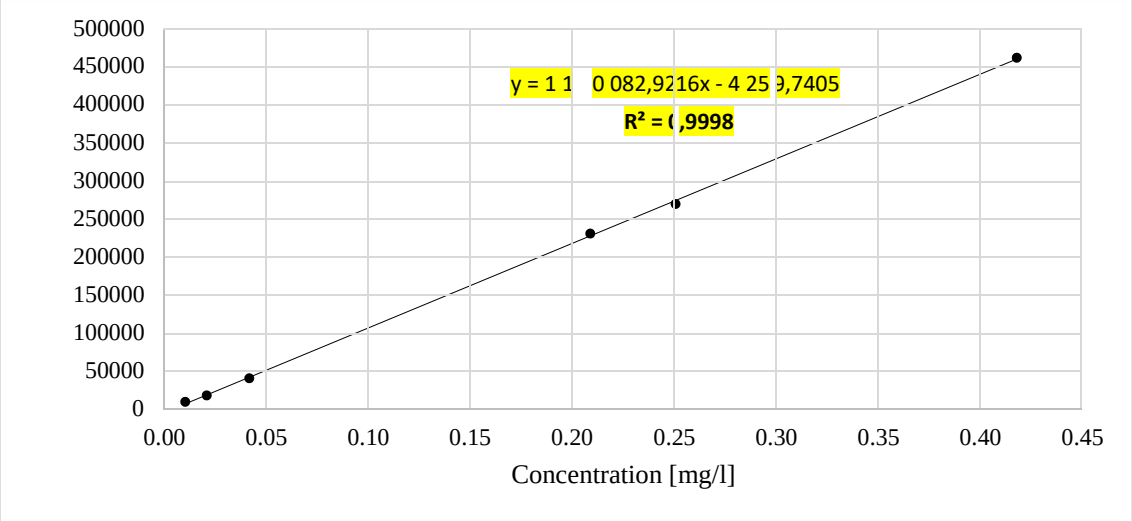
Fig. 3. MS/MS spectra of m/z 362.1 at retention time (RT) 4.82 min. and total ion chromatogram (TIC) of m/z 362.1 / 253.1 specimen.

Linearity

The linearity of the detector response was assessed using six standards solutions at the concentration range from 0.0105 mg/l to 0.4182 mg/l of **IMP 1** which corresponds to the concentration range of 0.00000105 to 0.00004182 % ~~5.23% to 209.09% of maximum acceptable limit (FAO) for **IMP 1** content in the preparation.~~ For this purpose, appropriate volumes of standard solution were added to the flasks and diluent was added up to the mark. Each of the solutions were analyzed twice (two replicates). except the lowest level which was analyzed six times (six replicates) to determine limit of quantification (LOQ) of the method.

Linearity of IMP 1 - detector response

Level	Sample name	Volume of standard solution A2 [ml]	Volume of flask [ml]	Concentration (obtained) [mg/l]	Peak area [-]	Average peak area [-]
LOQ	loq1-1	0.05	10	0.0105	9607.10	9634.59
	loq1-2				9512.99	
	loq1-3				9578.98	
	loq1-4				9625.62	
	loq1-5				9919.40	
	loq1-6				9563.44	
I	l1-1	0.05	5	0.0209	18229.2	18235.80
	l1-3				18242.4	
II	l2-2	0.10	5	0.0418	40909.4	40937.00
	l2-3				40964.6	
III	l3-1	0.50	5	0.2091	235833	230757.50
	l3-2				225682	
IV	l4-1	0.60	5	0.2509	273952	269499.00
	l4-2				265046	
V	l5-1	1.00	5	0.4182	478930	483552.00
	l5-2				488174	



Correlation coefficient should be $R^2 \geq 0.99$. **The obtained result is acceptable.**

Limit of quantification

Limit of quantification (LOQ) of **IMP 1** in **MEZOT 100 SC** preparation was defined as the lowest concentration of injected standard that gave precise and accurate measurements. Limit of quantification is 0.0105 mg/l what corresponds to 0.00000105 g/kg of **MEZOT 100 SC** preparation and 0.0000105 g/kg of mesotrione.

RMS comments:
According to SANCO/3030/99 rev. 5 the LOQ for relevant impurity should be defined as the lowest concentration tested, at which an acceptable recovery and an acceptable precision (repeatability), is obtained. Therefore, for impurity R287431 (IMP 1) the LOQ should be established at 0.00000209%.

Precision (repeatability)

The method repeatability was assessed on the basis of six independent determinations of **IMP 1** content in **MEZOT 100 SC** preparation.

Repeatability of IMP 1 determination

Chromatogram name	Specimen weight [mg]	Concentration [mg/ml]	Peak area [-]	Result [%]
pr1	107.95	10.80	-	<LOQ
pr2	98.13	9.81	-	<LOQ
pr3	110.20	11.02	-	<LOQ
pr4	108.29	10.83	-	<LOQ
pr5	109.77	10.98	-	<LOQ
pr6	125.00	12.50	-	<LOQ
			Average	-
			SD	-
			RSD [%]	-

In none of the examined samples **IMP 1** was detected above the LOQ. Therefore. for the determination of repeatability six portions of placebo were fortified with **IMP 1** at I level 0.2091 mg/l and analyzed. For this purpose, 0.50 ml of placebo solution at concentration 10.012 mg/ml (100.12 mg / 10 ml) was added into six 10 ml flasks and 0.10 ml of (**A2) IMP 1** standard solution was placed. Flasks were filled up to the volume with diluent.

Repeatability – placebo fortified with IMP 1.

No.	Concentration [mg/ml]	Peak area [-]	Concentration obtained [mg/l]
1	0.0209	21466.3	0.0232
2		21533.2	0.0232
3		21753.6	0.0234
4		21142.5	0.0229
5		20421.8	0.0222
6		21115.8	0.0229
		Average	**0.023**
		SD	0.0004
		RSD [%]	**1.68**

Relative standard deviation of determination of **IMP 1** fulfils acceptance criterion. RSD for substance at the concentration of ~~~ 0.000209 %~~ 0.00000209% should be less than or equal to ~~6.78~~ 19.19 %. Horrat value calculated with the equation:

$$Hr = \%RSD / \%RSDr$$

$$Hr = 1.68\% / \text{~~6.78~~}19.19\% = \mathbf{0.25} \leq \mathbf{1}$$

where: %RSD is obtained repeatability

%RSDr is expected repeatability obtained with modified Horwitz equation. is ~~**0.25**~~ **0.09 and fulfils acceptance criterion $Hr \leq 1$**.

Recovery

Recovery of the method for determination of **IMP 1** in **MEZOT 100 SC** preparation was assessed by at two levels of concentration.

Level I – 0.50 ml of placebo solution at concentration 10.012 mg/ml (100.12 mg / 10 ml) was added into six 5 ml flasks. next 0.05 ml of (**A2) IMP 1** solution was placed. and diluent was added up to the volume.

Level II – 0.50 ml of placebo solution at concentration 10.012 mg/ml (100.12 mg / 10 ml) was added into six 5 ml flasks and 0.30 ml of (**A2) IMP 1** solution was placed. and diluent was added up to the volume.

The flasks were put into the ultrasonic bath for 2 min. The concentration of analytes in each solution was calculated from the equation of the calibration curve. Obtained final concentrations were examined and the theoretical and calculated contents were compared.

Total recovery – IMP 1

Level	Sample name	IMP1 added [mg/l]	Peak area [-]	IMP1 determinated [mg/l]	Recovery [%]	Average [%]	SD	RSD [%]
I	odz2-1	**0.0209**	21466.3	0.0232	110.84	109.86	1.84	1.68
	odz2-2		21533.2	0.0232	111.13			
	odz2-3		21753.6	0.0234	112.08			
	odz2-4		21142.5	0.0229	109.44			
	odz2-5		20421.8	0.0222	106.34			
	odz2-6		21115.8	0.0229	109.33			
II	odz1-1	**0.1255**	130206	0.1211	96.56	96.70	1.05	1.08
	odz1-2		130945	0.1218	97.09			
	odz1-3		130222	0.1211	96.57			
	odz1-4		128521	0.1196	95.35			
	odz1-5		129375	0.1204	95.96			
	odz1-6		133198	0.1238	98.70			
				Average	**103.3**			

For the ingredients at concentration < 0.01% the average recovery value should be 100 □ 30%.
The obtained result of 103.3% is acceptable.

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

With respect to toxicological, eco-toxicological or environmental aspects Mezot 100 SC does not contain any relevant formulants. Therefore, a special analytical method and validation isn't needed.

5.2.1.3 Applicability of existing CIPAC methods (KCP 5.1.1)

CIPAC method no. 625 is available for Mesotrione.

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 preauthorization data is given in the following table. For the detailed evaluation of additional studies it is referred to Appendix 2.

Table 5.2-1: 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

Maize (plant, seed, silage, grain, stover, forage, kernel) Oilseed rape seed (Residues)	Primary	0.01 mg/kg	HPLC-MS/MS	EFSA, 2016 (Hill S., 2004; Bruns G. *et al*, 2001; Watson G., 2013)
Maize (forage, kernel) Oilseed rape seed (Residues)	Confirmation	0.01 mg/kg	HPLC-MS/MS	Watson G., 2013
Maize (whole plant and grain)	Primary	0.01 mg/kg	LC-MS/MS	Schneider E., 2016
	Confirmatory	0.01 mg/kg	LC-MS/MS	Schneider E., 2016

Component of residue definition: Mesotrione				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
Oilseed rape (whole plant)	Primary	0.01 mg/kg	LC-MS/MS	Faessel E., 2018
	Confirmatory	0.01 mg/kg	LC-MS/MS	Faessel E., 2018
Water: Mesotrione in Algal medium (Ecotoxicology)	Primary	0.0441 mg/L	HPLC	Beattie H., 2013
Water: Mesotrione in daphnia medium (Ecotoxicology)	Primary	0.01 mg/L	HPLC	Beattie H., 2013
Water: Mesotrione in lemna medium (Ecotoxicology)	Primary	0.01 mg/L	HPLC	Beattie H., 2014
Water: Mesotrione in fish medium (Ecotoxicology)	Primary	0.0125 mg/L	HPLC	Beattie H., 2013
Water: Mesotrione in 20xAAP Medium (Ecotoxicology)	Primary	0.02 mg/L	HPLC-UV	Kiss G., 2014

Component of residue definition: MNBA				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
Maize (plant, seed, silage, grain, stover, forage, kernel) Oilseed rape seed (Residues)	Primary	0.01 mg/kg	HPLC-MS/MS	EFSA, 2016 (Hill S., 2004; Bruns G. *et al*, 2001; Watson G., 2013)

Maize (forage, kernel) Oilseed rape seed (Residues)	Confirmation	0.01 mg/kg	HPLC-MS/MS	Watson G., 2013

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
Maize	Mesotrione	0,01 mg/kg	COMMISSION REGULATION (EU) 2017/626 of 31 March 2017 amending Annexes II and III to Regulation (EC) No 396/2005 of the European Parliament and of the Council as regards maximum residue levels for acetamiprid, cyantraniliprole, cypermethrin, cyprodinil, difenoconazole, ethephon, fluopyram, flutriafol, fluxapyroxad, imazapic, imazapyr, lambda-cyhalothrin, mesotrione, profenofos, propiconazole, pyrimethanil, spirotetramat, tebuconazole, triazophos and trifloxystrobin in or on certain products.
Muscle		0,01 mg/kg	
Milk		0,01 mg/kg	
Eggs		0,01 mg/kg	
Fat		0,01 mg/kg	
Liver, kidney		0,01 mg/kg	
Soil (Ecotoxicology)	Mesotrione MNBA AMBA	~~0.015~~ 0.002 mg/kg ~~bw/day~~ 0.002 mg/kg 0.002 mg/kg	~~AOEL~~ EFSA Journal 2016;14(3):4419
Drinking water (Human toxicology)	Mesotrione MNBA AMBA	~~0.1~~ 0.05 µg/L 0.05 mg/L 0.05 mg/L	general limit for drinking water EFSA Journal 2016;14(3):4419
Surface water (Ecotoxicology)	Mesotrione MNBA AMBA	~~0,0077~~ 0.05 mg/L 0.05 mg/L 0.05 mg/L	~~DAR for Mesotrione~~ EFSA Journal 2016;14(3):4419
Air	Mesotrione	0,45 µg/m^3	DAR for Mesotrione (LOQ)

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Tissue (meat or liver)	Mesotrione	~~0,1~~ 0.01 mg/kg	~~SANCO/825/00 rev. 8.1~~ SANTE/2020/12830, Rev.1
Body fluids		~~0,05~~ 0.01 mg/L	~~SANCO/825/00 rev. 8.~~1 SANTE/2020/12830, Rev.1

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

An overview on the acceptable methods and possible data gaps for analysis of mesotrione in plant matri- ces is given in the following tables.

Table 5.3-2.1: 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 (and metabolite MNBA)				
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	QuEChERS, LC-MS/MS	EFSA, 2016 (Watson G., 2013)
	ILV	0.01 mg/kg	LC-MS/MS	EFSA, 2016 (Tessier V., 2012)
High acid content	Primary	0.01 mg/kg	QuEChERS, LC-MS/MS	EFSA, 2016 (Watson G., 2013)
High oil content	Primary	0.01 mg/kg	QuEChERS, LC-MS/MS	EFSA, 2016 (Watson G., 2013)
High pro- tein/high starch content (dry)	Primary	0.01 mg/kg	QuEChERS, LC-MS/MS	EFSA, 2016 (Watson G., 2013)
	ILV	0.01 mg/kg	LC-MS/MS	EFSA, 2016 (Tessier V., 2012)

Table 5.3-2.2: Statement on extraction efficiency

	Method for products of plant origin
Required, available from:	EFSA review of mesotrione (2016)
Not required, because:	-

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

An overview on the acceptable methods and possible data gaps for analysis of Mesotrione in animal ma- trices is given in the following table.

Table 5.3-3.1: Validated methods for food and feed of animal origin

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	QuEChERS (LC-MS/MS)	EFSA, 2016 (Watson G., 2013)
	ILV	0.01 mg/kg	QuEChERS (LC-MS/MS)	EFSA, 2016 (Bernal J., 2013)
Eggs	Primary	0.01 mg/kg	QuEChERS (LC-MS/MS)	EFSA, 2016 (Watson G., 2013)
	ILV	0.01 mg/kg	QuEChERS (LC-MS/MS)	EFSA, 2016 (Bernal J., 2013)
Muscle	Primary	0.01 mg/kg	QuEChERS (LC-MS/MS)	EFSA, 2016 (Watson G., 2013)
Fat	Primary	0.01 mg/kg	QuEChERS (LC-MS/MS)	EFSA, 2016 (Watson G., 2013)
Kidney, liver	Primary	0.01 mg/kg	QuEChERS (LC-MS/MS)	EFSA, 2016 (Watson G., 2013)
	ILV (liver)	0.01 mg/kg	QuEChERS (LC-MS/MS)	EFSA, 2016 (Bernal J., 2013)

Table 5.3-3.2: Statement on extraction efficiency

	Method for products of animal origin
Required, available from:	EFSA, 2016
Not required, because:	-

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

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

Table 5.3-4: Validated methods for soil

Component of residue definition: Mesotrione		
Method type	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**
Primary	0,005 mg/kg	HPLC -UV/GC
Confirmatory	HPLC-MS/MS is considered 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.

zRMS EU agreed EFSA Journal 2016, 14 (3) : 4419; Jutsum L, Williams R.W., 2012, (Jutsum L, 2013 (RAR 2015):

Residues of Mesotrione in soil:
LC-MS/MS
LOQ = 0.002 mg/kg
AMBA in soil:
LC-MS/MS
LOQ = 0.002 mg/kg
MNBA in soil:
LC-MS/MS
LOQ = 0.002 mg/kg

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

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

Table 5.3-5: Validated methods for water

Component of residue definition: Mesotrione			
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**
Drinking water; Surface water	Primary	0,05 µg/L	GC-MSD
	Confirmatory	HPLC/MS/MS is considered 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.

zRMS:
Adequate method exists to monitor Mesotrione residues as well as the metabolites AMBA and MNBA in drinking and surface water (Jutsum L. (2013a) (EFSA Journal 2016;14(3):4419)).
LC-MS/MS
LOQ = 0.05 µg/L
AMBA in water:
LC-MS/MS
LOQ = 0.05 µg/L
MNBA in water:
LC-MS/MS
LOQ = 0.05 µg/L
An ILV for drinking water is required according to Regulation (EU) No 284/2013.
Acceptable ILV method is available (EU agreed EFSA Journal 2016; 14 (3) : 4419; Wiesner F, Breyer N, 2013 (RAR 2015). LOQ = 0.05 µg/L for Mesotrione, AMBA and MNBA.

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

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

Table 5.3-2: Validated methods for air

Component of residue definition: Mesotrione		
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**
Primary	0,45 µg/m^3	HPLC/GC EU agreed EFSA Journal 2016; 14 (3) : 4419 Jutsum L, 2013b, Jutsum L, 2013c (RAR 2015)
Confirmatory	HPLC/MS/MS is considered 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.

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

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

Table 5.3-3: Methods for body fluids and tissues

Component of residue definition: Mesotrione		
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**
Primary	0,05 µg/mL	HPLC-UV
Confirmatory	0,05 µg/mL	LC-MS

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

zRMS:
Residues of Mesotrione in blood EU agreed EFSA Journal 2016; 14 (3) : 4419; Watson G., 2013b (RAR 2015):
QuEChERS
LC-MS/MS
LOQ = 0.01 mg/kg

5.3.2.8 Other studies/ information

No other studies or information.

Appendix 1 Lists of data considered in support of the evaluation

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 5.1.1/01	Wołoszynowska M., Chałas A.	2019	MEZOT 100 SC Method validation for determination of the active substance and two relevant impurities content in the formulation. Institute of Industrial Organic Chemistry, Warszawa GLP Unpublished	N	Elvita
KCP 5.1.1/02	Sowik I.	2022	MEZOT 100 SC Determination of physicochemical properties. Institute of Industrial Organic Chemistry, Warszawa BA-03/22 GLP Unpublished	N	Elvita
KCP 5.1.2	Niewelt S.	2019	Residues of Mesotrione on maize plants after spray application of Mezot 100 SC in early growth stages of maize in Poland – magnitude of residues and time course of residue decline – 2019. Analytical Phase. Report No.: DPL/122/2019 (19SGS10) SGS Polska Sp. z o.o., Warszawa GLP Unpublished	N	Elvita

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 5.3.2.2-01	Watson, G.	2013a	Mesotrione - Validation of the QuEChERS Method for the Determination of Residues of mesotrione in Crop Matrices by LC-MS/MS Report No. S12-03251 Syngenta File No ZA1296_10090 Eurofins Agroscience Services Ltd, Wilson, UK, GLP Unpublished	N	Syngenta
KCP 5.3.2.2-02	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 Report No.: S12-04607 Syngenta File No ZA1296_10129 Eurofins Agroscience Services Chem SAS, Vergèze, France GLP Unpublished	N	Syngenta
KCP 5.3.2.3-01	Watson, G.	2013b	Mesotrione - Validation of the QuEChERS Method for the Determination of Residues of mesotrione in Animal Matrices by LC-MS/MS Report No: S12-03250 Syngenta File No ZA1296_10093 Eurofins Agroscience Services Ltd, Wilson, UK GLP Unpublished	N	Syngenta
KCP 5.3.2.3-02	Bernal, J.	2013	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 Report No: S12-04608 Syngenta File No ZA1296_10130 Eurofins Agroscience Services Chem SAS, Vergèze, France GLP Unpublished	N	Syngenta
KCP 5.3.2.4-01	Jutsum L., Williams R.	2013	Analytical Method GRM007.10A for the Determination of Mesotrione and its Metabolites AMBA and MNBA in Soil Report No: GRM007.10A Syngenta File No ZA1296_10092 Syngenta CEMAS, North Ascot, United Kingdom, GRM007.10A Not GLP Unpublished	N	Syngenta

KCP 5.3.2.4-02	Jutsum, L.	2013	Mesotrione – Validation of Draft Residue Method GRM007.10A for the Determination of Mesotrione and its Metabolites AMBA and MNBA in Soil Report No: CEMR-5657-REG Syngenta File No ZA1296_10088 CEMAS, North Ascot, United Kingdom GLP Unpublished	N	Syngenta
KCP 5.3.2.5-01	Jutsum L., Chamkesam N.	2013	Analytical Method GRM007.09A for the Determination of Mesotrione and its Metabolites AMBA and MNBA in Water Report No: GRM007.09A Syn- genta File No ZA1296_10092 CEMAS, North Ascot, United Kingdom Not GLP Unpublished	N	Syngenta
KCP 5.3.2.5-02	Jutsum L.	2013a	Validation of Draft Residue Method GRM007.09A for the Determination of Mesotrione and its metabolites AMBA and MNBA in Water Report No: CEMR-5658-REG Syngenta File No ZA1296_10087 CE- MAS, North Ascot, United Kingdom GLP Unpublished	N	Syngenta
KCP 5.3.2.5-03	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 Water Report No: S13-04185 Syngenta File No ZA1296_10174 Eurofins Agroscience Services Chem GmbH, Hamburg, Germany GLP Unpublished	N	Syngenta
KCP 5.3.2.6-01	Jutsum L.	2013b	Mesotrione - Residue Method GRM007.08B for the Determination of Mesotrione in Air Report GRM007.08B Syngenta File No ZA1296_10089 Syngenta CEMAS, North Ascot, United Kingdom Not GLP Unpublished	N	Syngenta

KCP 5.3.2.6-02	Jutsum L.	2013c	Mesotrione - Validation of Residue Method GRM007.08A for the Determination of Mesotrione in Air Report CEMR-5403-REG Syngenta File No ZA1296_10084 Syngenta CEMAS, North Ascot, United Kingdom GLP Unpublished	N	Syngenta

Appendix 2 Detailed evaluation of submitted analytical methods

A 2.1 Analytical methods for Mesotrione

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

No new study 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)

New study have been submitted.

A 2.1.2.1.1 Magnitude of the residue of Mesotrione in ~~Oil Seed Rape~~ maize.

A 2.1.2.1.1.1 Method validation

	This method was provided only for pre-registration purposes. HPLC-MS/MS method was used. Two mass transitions were used. The validation parameters: - Specificity Acceptable. No interferences at above 30% of the LOQ were detected at the retention time of the active substance in matrix blank samples - Linearity Matrix-matched calibration standards at seven concentration levels ranging from 0.5 ppg to 500 ppb of mesotrione. R^2 were greater than 0.990. - LOQ, LOD LOQ: 0.01 mg (lowest validated fortification level) LOD: 0.003 mg/kg Recovery (5 samples at the LOQ and 5 samples 10 x LOQ) is within 70-120% RSD is ≤20% Matrix-matched standards were used for quantification for all samples. Validation is acceptable.

Reference:	KCP 5.1.2, Niewelt, S., 2019
Report	Residues of Mesotrione on maize plants after spray application of Mezot 100 SC in early growth stages of maize in Poland – magnitude of residues and time course of residue decline – 2019. Study code: DPL/122/2019.
Guideline(s):	Yes – SANCO/3029/99 rev. 4.
Deviations:	No

GLP: Yes

Acceptability: Yes

Materials and methods

Reference items:
Mesotrione CAS No.: 104206-82-8; Supplier: Dr. Ehrenstorfer (Lot number G170639; expiry date 28.03.2021).

Sample types:
Maize (whole plants)
The field specimens arrived at the Test Site in good conditions, frozen and were stored in a freezer at ≤ -18 ^{0}C before analysis. All the samples were homogenized at Test Site, using a knife grinder (and with dry ice). The homogenized specimens were further stored at ≤ -18 ^{0}C (temperature range from -18.1 ^{0}C to -29.8 ^{0}C) until beginning of analysis.

Extraction:
5 g of the homogenized sample were weighed into a 50 mL centrifuge tube. 10 ml of acetonitrile and 10 ml of deionized water was added together with 50 µl of internal standard solution (1.3), and the mixture was shaken vigorously by hand for one minute. After addition of buffering salts (4 g anhydrous magnesium sulfate, 1 g sodium chloride, 1 g trisodium citrate dehydrate, 0.5 g disodium hydrogencitrate sesquihydrate), the mixture was shaken again intensively for 1 min, then centrifuged at 3000 rpm for 5 min for phase separation and finally subjected to a freezing process at ≤ -18 ^{0}C for 1,5 h. After that, 5 ml of the clean extract (organic phase) was transferred to a 10 ml vial. Following clean-up the extract was filtered through a membrane filter and the final extract was directly employed for LC-MS/MS analysis. Quantification was performed using an internal standard, which was added to the extract after the initial addition of acetonitrile.

Fortification and control samples:
For each analytical sequence two sample blank matrix, and two procedural recoveries at the level of LOQ and two at the level 10 x LOQ were prepared together with the study samples.
5 g of the homogenized untreated sample were weighed into a 50 ml centrifuge tube. Appropriate active substance standard solution was added, and the sample was extracted as described.

Preparation of fortification and control samples:

Fortification level	Amount of standard solution 1.1 added [µl]	Amount of standard solution 1.2 added [µl]
Matrix blank	-	-
PK 0,010 mg/kg	-	50,0
PK 0,10 mg/kg	50,0	-

Extraction of all field samples (treated and untreated), as well as control and fortified samples was performed on 12.07.2019 (trial III), 17.07.2019 (trial III, repeated and diluted treated samples), 23.07.2019 (trial IV), 25.07.2019 (trial I), 26.07.2019 (trial II) and after that the samples were directly employed for LC-MS/MS analysis, that was started on the same day.

Equipment and reagents:
- Electronic balance Class I
- Electronic balance Class II
- Freezers - storage of:
- analytical standards
- analytical samples before the analytical part
- analytical samples extracts until the end of the instrumental analysis
- archived analytical samples

- Cutter (knife grinder)
- Cereal grinder
- Centrifuge (about 3000 rpm)
- Polypropylene centrifuge tubes, with screw caps, 50 ml
- Plastic cups (stackable), 25 and 100 ml, for the storage of buffer-salt mixture portions and archived samples
- Volumetric flasks, 5 and 10 ml (class A)
- Automatic pipettes, 10 ml
- Laboratory syringes 10 µl, 50 µl, 100 µl, 250 µl, 500 µl and 1000 µl
- Vials with screw caps, amber glass, 10 ml
- Injection vials, 2,5 ml, amber glass, screw caps with septa butyl/PTFE
- Liquid Chromatography Instrument LC-MSMS Shimadzu 8050
- Deionized Water
- Acetonitrile, CH3CN, for GC applications (pesticide residues analysis)
- Methanol, CH3OH, HPLC grade
- Formic acid, HCOOH, 98 – 100%, for analysis
- Magnesium sulfate, MgSO4, anhydrous
- Sodium chloride, NaCl
- Disodium hydrogencitrate sesquihydrate, C6H6Na2O7·1,5H2O
- Trisodium citrate dihydrate, C5H5Na3O7·2H2O
- Buffer salt mixture containing:

- 4g ± 0,2g anhydrous magnesium sulfate
- 1g ± 0,05g sodium chloride
- 1g ± 0,05g trisodium citrate dehydrate
- 0,50g ± 0,03g disodium hydrogencitrate sesquihydrate

Preparation of solutions:
All solutions required to carry out of the study were prepared using glass graduated flasks, automatic pipette and/or laboratory syringes and an analytical balance.
Stock solutions of analytical standard were prepared by dissolving a weight of analytical standards in appropriate solvent.

Preparation of analytical standard stock solution:

Name of analytical standard	Amount [mg]	Flask volume [ml]	Final concentration [µg/ml]	Solvent
Mesotrione	10	10	1000	Acetonitrile
Mesotrione D4	5	10	500	Acetonitrile

The stock solutions were further diluted with appropriate solvent, in order to prepare the intermediate solutions to be used for preparation of fortification samples and calibration solutions.

Intermediate analytical standards solutions used in current study (prepared in acetonitrile):

Name of intermediate standard solution	Name of analytical standard	Volume of stock solution standard [µl]	Flask volume [ml]	Final concentration [µg/ml]
Intermediate solution (1.2)	Mesotrione	10	10	1
Intermediate solution (1.1)	Mesotrione	100	10	10
Intermediate solution (1.3)	Mesotrione D4	200	10	10

Calibration solutions:
Approximately 40 ml of maize (plant) matrix blank was prepared. The extracts were combined into one. Based on blank matrix (“matrix-matched”) working calibration solutions were prepared.

Approximately 4.5 ml of acetonitrile extracts were transferred to the 5 ml graduated flask. Then, 50 µl of internal standard solution (1.3) and a suitable amount of intermediate solution (1.1 or 1.2) were added. After adding the intermediate standard, the flasks were filled to the mark with acetonitrile.

Analysis:
The extracts were analyzed using liquid chromatography coupled with mass spectrometry, by single extraction and single injection to the detection system. Final extracts were employed for LC-MS/MS analysis directly after completion of the extraction procedure (on the same day). Data acquisition was carried out in the MRM mode. The analysis was performed using internal standard addition.
For each analyte, one mass transition was evaluated and used for quantification. Representative chromatograms are shown in this report. A second mass transition was monitored for confirmation of peak identity but was not used for quantification.

Results:

For each trial DT50 value was determined. For this purpose CAKE (Computer Assisted Kinetic Evaluation, version 3.3) program, following single first-order kinetics (SFO) was used.

Determined values of DT50:

Trial	DT_{50} [h]	DT_{50} [days]	Error [%]
I	21,6	0,90	6,97
II	6,28	0,26	17,9
III	7,55	0,31	15,3
IV	18,4	0,77	10,7

Conclusion

The validation parameters (specificity, linearity, accuracy, recovery and precision) are within the acceptance range and fulfil EU requirements given in SANCO /3029/99 rev.4.

A 2.1.2.1.1.2 Independent laboratory validation

Studies in independent laboratories have not been performed.

A 2.1.2.1.1.3 Confirmatory method

No confirmatory method is required.

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

No new or additional studies have been submitted.

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

No new or additional studies have been submitted.

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

No new or additional studies have been submitted.

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

No new or additional studies have been submitted.

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

No new or additional studies have been submitted.

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

No new or additional studies have been submitted.