

# **REGISTRATION REPORT**

## **Part B**

### **Section 5**

#### **Analytical Methods**

Detailed summary of the risk assessment

Product code: MEZI 100 SC

Product name(s): Rumezo Twist 100 SC,  
Malton Twist 100 SC

Chemical active substance(s):

Mesotrione, 100 g/L

Central Zone

Zonal Rapporteur Member State: Poland

#### **CORE ASSESSMENT**

(authorization)

Applicant: Innvigo Sp. z o.o.

Submission date: December 2023, **October 2024**

**zRMS Assessment: 24/07/2024**

**Following commenting period: 21/10/2024**

## Version history

When	What
July 2024	zRMS assessment
October 2024	Following commenting period
October 2024	Applicant update

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Callisto 100 SC is the original product to which Innvigo Sp. z o.o. would like to refer. 10 years for registration data of Calisto 100 SC was expired in Poland. Thus, the data protection of studies provided in registration report of Calisto 100 SC has expired. Innvigo Sp. z o.o. refers to above mentioned studies within this document

## 5 Analytical methods

### 5.1 Conclusion and summary of assessment

Sufficiently sensitive and selective analytical methods are available for the active substance(s) and relevant impurities in the plant protection product.

Noticed data gaps are:

- no data gaps

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)

Comments of zRMS:	The analytical method code: BF- 49/20 was fully validated in term of specificity, linearity, repeatability, accuracy according to SANCO/3030/99 rev.5. The results of analytical method validation confirm that this method is suitable for analysis the content of the active substance (mesotrione). The method is successfully validated and accepted.
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An overview on the acceptable methods and possible data gaps for analysis of Mesotrione and in plant protection product is provided as follows:

Reference:	KCP 5.1.1/01
Report	CHR/H/MEZZO 100 SC Determination of physicochemical properties of the preparation, Study code: BF- 49/20
Guideline(s):	SANCO/3030/99 rev.5, 22 March 2019 guideline
Deviations:	No
GLP:	Yes
Acceptability:	

## Materials and methods

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

Method validation for determination of the Mesotrione content was determined using HPLC chromatography with DAD detection using external standard method (MT/BA-36/20).

Validation parameters

- Specificity
- Linearity
- Precision (repeatability)
- Recovery (total)

## Validation - Results and discussions

**Table 5.2-1: Methods suitable for the determination of Mesotrione, in plant protection product CHR/H/MEZI 100 SC**

	Mesotrione				
<b>Author(s), year</b>	Arévalo E., 2021				
<b>Principle of method</b>	HPLC with DAD				
	<b>Acceptance criteria</b>	<b>Obtained result</b>			
<b>Linearity (linear between mg/L / % range of the declared content) (correlation coefficient, expressed as r)</b>	$R^2 \geq 0.99$	The linearity of the detector response was assessed using five standard solutions at the concentration range of Mesotrione 60.93 mg/l to 142.17 mg/l, which corresponds to the concentration range of 61% to 143% of Mesotrione content in the preparation. All solutions were analyzed twice. Correlation coefficient should be $R^2 \geq 0.99$ . The obtained result ( $R^2=0.9996$ ) is acceptable.			
<b>Precision – Repeatability Mean n = 6 (%RSD)</b>	$RSDr \leq 1.90\%$	The method repeatability was assessed on the basis of six independent determinations of active substance content in CHR/H/MEZZO 100 SC preparation. Relative standard deviation ( <b>RSD =1.19%</b> ) of determination of Mesotrione fulfils acceptance criterion. RSD for substance at the concentration of ~ 10 % should be less than or equal to 1.90 %.			
		<b>Chromatogram name</b>	<b>Specimen weight [mg]</b>	<b>Concentration C [mg/l]</b>	<b>Peak area</b>
		p1	100.21	1002.10	1638413
		p2	99.39	993.90	1650490
		p3	96.61	966.10	1571761
		p4	98.79	987.90	1625123
		p5	96.52	965.20	1621921
					<b>Result [%]</b>
					9.19
					9.33
					9.14
					9.24
					9.44

Mesotrione												
		p6		101.47	1014.70		1685597	9.33				
		Average						9.280				
		SD						0.110				
		RSD [%]						1.19				
	Hr ≤ 1	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, <b>Hr is 0.62</b> and fulfils acceptance criterion Hr ≤ 1										
Recovery	97-103	Recovery of active substance determination in CHR/H/MEZZO 100 SC was assessed by total recovery value at two levels of concentration. 0.15 ml of the Mesotrione standard solution at concentration of 2.031 mg/ml and 0.25 ml of sample solution at concentration of 10.057 mg/ml were added to the each of the first six 5 ml flasks and acetonitrile were added up to the volume. To another six flasks 0.20 ml of Mesotrione standard solution at the same concentration and 0.25 ml of sample solution at the same concentration 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 ( <b>y=18174x- 35763</b> )										
			Chromatogram name	C from sample [mg/l]	C added [mg/l]	Sum	Peak area	C determined [mg/l]	Recovery	Average	SD	RSD [%]
		level I	odz1-1	46.66	60.93	107.59	1966899	110.19	102.42	100.11	1.79	1.78
			odz1-2	46.66	60.93	107.59	1928868	108.10	100.47			
			odz1-3	46.66	60.93	107.59	1944925	108.98	101.29			
			odz1-4	46.66	60.93	107.59	1863354	104.50	97.12			
			odz1-5	46.66	60.93	107.59	1939296	108.67	101.01			
			odz1-6	46.66	60.93	107.59	1926313	107.96	100.34			
		level II	odz2-1	46.66	81.24	127.90	2357192	131.67	102.95	101.57	2.19	2.23
			odz2-2	46.66	81.24	127.90	2238322	125.13	97.83			
odz2-3	46.66		81.24	127.90	2351486	131.36	102.70					
odz2-4	46.66		81.24	127.90	2288679	127.90	100.00					
odz2-5	46.66		81.24	127.90	2369288	132.33	103.47					
odz2-6	46.66		81.24	127.90	2346337	131.07	102.48					
Average							101.01					
For the main ingredient at concentration of >10 % the average recovery value should be 100 ± 3%. The <b>obtained result of 101.01%</b> is acceptable.												
Interference/ Specificity	The chromatograms of solvent, standard solution and the examined specimen solution were performed and superimposed. There are no interferences between the analyte and other components of the specimen											
Comment	-											

## Conclusions

Specificity: There are no interferences between the analyte and other components of the specimen.

Linearity: The obtained result is acceptable.

Precision (repeatability): Horrat value is 0.62 and fulfils acceptance criterion  $Hr \leq 1$

Recovery: The obtained result of 101.01% is acceptable.

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

Comments of zRMS:	<p>According to the applicant information the study SD-977/2 was formerly recognized as acceptable.</p> <p>The validation study has been positively evaluated in Part B Section 5 of the Registration Report from 30/04/2020 for to renewal of mesotrione at the EU level of product Calisto 100 SC.</p> <p>The following conclusion was made:</p> <p><i>„The LC-MS analytical method (SD-977/2) for the determination of R287431 in A12739A formulated materials, has been successfully validated in terms of specificity, recovery, linearity, precision and accuracy according to SANCO/3030/99/rev.4.</i></p> <p><i>The validated LOQ in formulation is not low enough to support the specification for the relevant impurity. Based on the specification of the impurity and the content of the active substance in A12739A, a <math>LOQ \leq 0.2 \mu\text{g/g}</math> in the formulation should be validated.</i></p> <p><i>Taking in to account that the relevant impurity R287431 (6-methanesulfonyl-7-nitro-9-oxo-9H-xanthene-1-carbonitrile) may be formed in trace amounts during the chemical synthesis of mesotrione technical material and cannot be formed from mesotrione or from other formulation components of A12739A and the fact that according to SANCO/3030/99 rev.5 2017 in certain cases when content of the active substance in the plant protection product can be too low in order to determine a relevant impurity at the level derived from the maximum content in the technical active substance, in this case, the validation must be performed at the lowest possible concentration. For R287431 impurity it is demonstrated that it is not technically possible to reach the theoretically required LOQ (with chromatogram or some experiment data). The argumentation provided by the applicant, demonstrating that the LOQ is reached properly can be considered as sufficient and the proposed method can be considered as acceptable..”</i></p>
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Based on following analytical studies from Callisto 100 SC relevant impurities are determined.

Mesotrione relevant impurities:

- R287431 (6-methanesulfonyl-7-nitro-9-oxo-9H-xanthene-1-carbonitrile)
- R287432 (6-methanesulfonyl-9-oxo-9H-xanthene-1-carbonitrile)
- 1,2-dichloroethane

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

#### **Impurity: R287431 (6-methanesulfonyl-7-nitro-9-oxo-9H-xanthene-1-carbonitrile)**

Reference:	KCP 5.1.1/02
Report	Analytical method SD-977/2. Hager M., 2011, Report No. 10427012
Guideline(s):	None stated
Deviations:	None stated

GLP: No

Acceptability:

Reference: KCP 5.1.1/03

Report Validation of analytical method SD-977/2 – R287431 in A14203B, A13789C, A14351BX, A12909A, A15189G, A12738A and A18219B. Hager M., 2011a, Report No. 10427878

Guideline(s): SANCO 3030/99 rev. 4

Deviations: None stated

GLP: Yes

Acceptability:

Reference: KCP 5.1.1/04

Report A12739A - Statement on Validation of Analytical Method SD-977/2 for Determination of R287431 (Xan-1) in Formulation A12739A (MESOTRI-ONE SC 100). Hager M., 2015, Report No. 300035703

Guideline(s): SANCO 3030/99 rev. 4

Deviations: None stated

GLP: No

Acceptability:

Reference: KCP 5.1.1/05

Report A12739A - Response to the Danish Regulatory Authority Concerning Relevant Impurity R287431, Baker, S. et al, 2018, Syngenta File N.o. A12739A\_11278

Guideline(s): SANCO 3030/99 rev. 4

Deviations: None stated

GLP: No

Acceptability:



## Materials and methods

The relevant impurity R287431 in A12739A is separated by HPLC on a BEH C18 column (100 mm x 2.1 mm). For elution a gradient of 5 mM ammonium formate in water and 1% formic acid in acetonitrile was used, followed by spectrophotometric detection at 270 nm (DAD). Identification was by ion trap mass spectroscopy with a source temperature of 150°C. Parent ion was m/z 344 and daughter ion at 207 m/z. Quantification is by comparison of peak areas ratios to those of a reference solution.

(Hager M., 2011))

## Validation - Results and discussions

**Table 5.2-2: Validation of Method SD-977/2 for the determination of R287431 in A12738A**

	<b>R287431-maximum content in A12738A 2mg/kg in mesotrione</b>
<b>Author(s), year</b>	Hager M., 2011a
<b>Principle of method</b>	High Performance Liquid Chromatography/Mass Spectroscopy (HPLC/MS)
<b>Linearity</b> <b>n=5</b> <b>(linear 50 between 150% of the target concentration)</b> <b>(2µg/g) of R287431</b>	r = 0.99 y = 17683 x + 19.88
<b>Precision – Repeatability Mean</b> <b>n = 5</b> <b>(%RSD)</b>	Srel (%RSD): 7.3 % mean concentration: 0.019 µg/ml
<b>Accuracy</b> <b>n = 3</b> determined between 70 and 130% of the target concentration (2µg/g) of R287431 <b>(% Recovery)</b>	mean recovery: 100 %
<b>Interference/ Specificity</b>	No significant interference was observed. The analytical method is able to separate the impurity R287431 from the formulation blank and solvent with no significant co-elution.
<b>LOQ</b>	The validation data prove that the limit of quantification for R287431 is established at concentration of 1 µg/g.
<b>Comment</b>	The method is suitable for the specific, accurate and precise determination of the relevant impurity R287431 in A12738A.

**Table 5.2-3: Validation of Method SD-977/2 for the determination of R287431 in A12739A**

	<b>R287431-maximum content in A12739A 2mg/kg in mesotrione</b>
<b>Author(s), year</b>	Hager M., 2015
<b>Principle of method</b>	High Performance Liquid Chromatography/Mass Spectroscopy (HPLC/MS)
<b>Linearity</b> <b>n=5</b> <b>(linear 50 between 150% of the target concentration)</b>	r = 0.99 y = 17683 x + 19.88

	<b>R287431-maximum content in A12739A 2mg/kg in mesotrione</b>
<b>(2µg/g) of R287431</b>	
<b>Precision – Repeatability Mean</b> <b>n = 7</b> <b>(%RSD)</b>	S <sub>rel</sub> (%RSD): 7.83 % mean concentration: 0.0177 µg/ml
<b>Accuracy</b> <b>n = 3</b> determined between 70 and 130% of the target concentration (2µg/g) of R287431 <b>(% Recovery)</b>	mean recovery: 94 %
<b>Interference/ Specificity</b>	No significant interference was observed. The analytical method is able to separate the impurity R287431 from the formulation blank and solvent with no significant co-elution.
<b>LOQ</b>	The validation data prove that the limit of quantification for R287431 is established at concentration of 1 µg/g.
<b>Comment</b>	The method is suitable for the specific, accurate and precise determination of the relevant impurity R287431 in A12738A.

Due to the extremely low concentration of R287431 in technical mesotrione and even lower concentration in formulated materials, quantification at such low concentrations is extremely challenging, even based on the latest technology available in mass spectrometry. Current LC/MS inlet designs and ionisation techniques result in a limit of detection (LOD) of approximately 0.2µg/g for R287431. Therefore, attempting quantifiable levels below 1µg/g is unreliable. Fundamentally, this is a consequence of the chemical structure of R287431; the molecule is difficult to ionise due to its chemical structure (ionisation is required to perform mass spectrometry). So the LC/MS method effectively only has access to a very small fraction of the injected quantity of R287431 to detect and quantify. As a result the sensitivity of the method is significantly reduced and this is the key technical reasoning behind the LOQ being set at 1 µg/g.

## Conclusion

In summary, Syngenta believes that the method (SD-977/2) is not sufficiently robust to be able to define a sub-ppm LOQ. Quantification at such low concentrations is extremely challenging, even based on the latest technology available in mass spectroscopy. Current LC/MS inlet designs and ionisation techniques result in a limit of detection (LOD) of approximately 0.2 µg/g for R287431. Therefore attempting measureable levels much below 1 µg/g would be unreliable.

Furthermore, Syngenta guarantees that R287431 will always be below 2ppm in technical mesotrione and that R287431 cannot form in mesotrione formulations. Therefore, R287431 will always be below 2ppm in technical material and even lower by the appropriate dilution factor in formulated mesotrione. This means that R287431 will always be lower than 0.002g/kg (2ppm) in A12739A which was established as the concentration of no (eco)toxicological concern during the EU review of mesotrione.

Therefore, Syngenta believes that an LOQ of 1ppm for the determination of R287431 in formulated mesotrione is appropriate to ensure compliance and safety with respect to A12739A and this position is validated by the current analytical method guidance SANCO/3030/99 rev.5 2015.

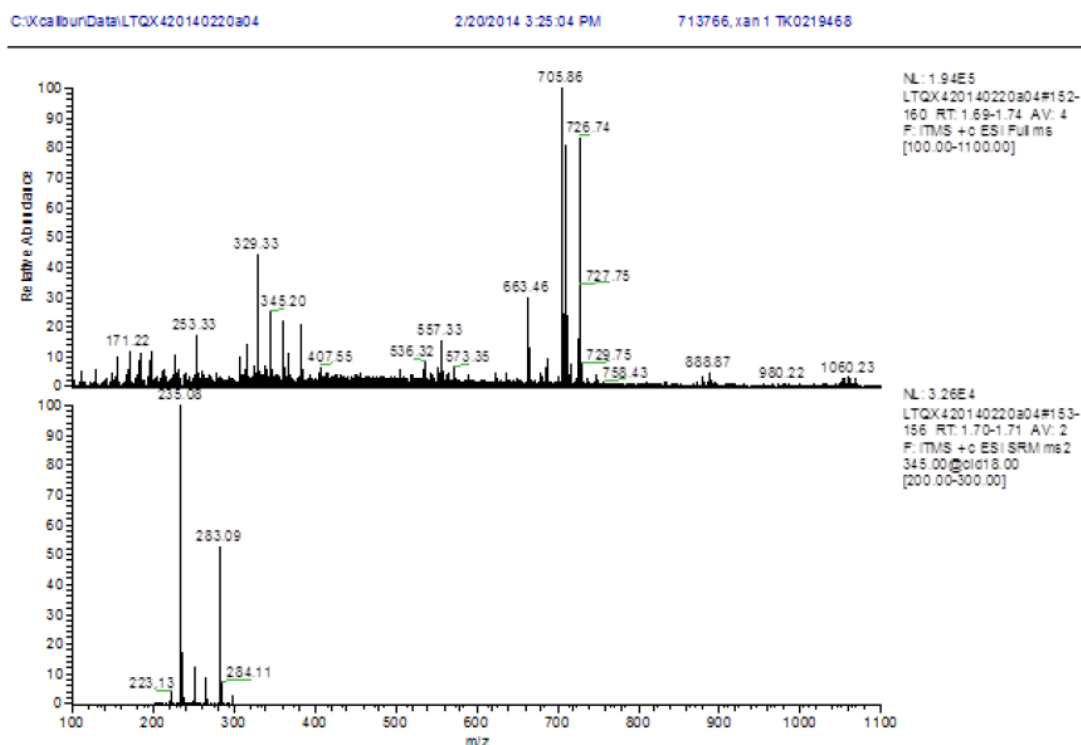
## Confirmation of Identity of R287431

According to SANCO/3030/99: *Confirmatory techniques are required to support identification of the a.s. and significant and/or relevant impurities, when the primary method of determination is not GC-MS or another highly specific method such as HPLC-UV DAD.*

The analytical method SD-977/1 determines the relevant impurity R287431 in mesotrione containing formulations using multiple-point external standard calibration based on liquid chromatography with mass spectroscopy detection (LC/MS). The detection is by MS/MS (tandem mass spectrometry) for a specific multiple reaction monitoring (SRM) of an analyte, therefore, high specificity for R287431 is inherent in the method and confirmation of identity of R287431 is inherent in the method.

As additional proof of identity i.e. Mass Spectra generated during the characterization of the reference material R2827431, Batch713766 (Study TK0219468) are given below.

**Fig 2. Mass Spectra for R287431 Analytical Standard, Batch 713766 (Study TK0219468)**



## Conclusion

The method is suitable for the specific, accurate and precise determination of the relevant impurity R287431 in A12739A.

- **Impurity: R287432 (6-methanesulfonyl-9-oxo-9H-xanthene-1-carbonitrile)**

Comments of zRMS:	<p>According to the applicant information the study SD-1990/1 was formerly recognized as acceptable.</p> <p>The validation study has been positively evaluated in Part B Section 5 of the Registration Report from 30/04/2020 for to renewal of mesotrione at the EU level of product Calisto 100 SC.</p> <p>The following conclusion was made:</p> <p>„ The LC-MS analytical method (SD-1990/1) for the determination of R287432 in mesotrione containing formulations, has been successfully validated in terms of specificity, recovery, linearity, precision and accuracy according to SAN-CO/3030/99/rev.4. Full validation of the method SD-1990/1 has been conducted. The method has been shown to be specific for the determination of R287432 in product A12739A and no significant interference was observed. Based on the results for repeatability, recovery, linearity and specificity, precision and accuracy of the method are established. Therefore, the method is suitable for the</p>
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	<i>specific, accurate and precise determination of R287432 in product A12739A."</i>
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Reference: KCP 5.1.1/06

Report ZA1296 - SD-1990/1 - Determination of R287432 in Mesotrione Related Formulations by Liquid Chromatography/Mass Spectrometry (LC/MS). Huang S., 2016 Report No. 300068727

Guideline(s): None stated

Deviations: None stated

GLP: No

Acceptability:

Reference: KCP 5.1.1/07

Report Validation of analytical method SD-1990/1, Huang S., 2016a, Report No. USGR160250

Guideline(s): SANCO 3030/99 rev. 4

Deviations: None stated

GLP: Yes

Acceptability:

Reference: KCP 5.1.1/08

Report A12739A - Statement on Validation of Analytical Method SD-1990/1 for Determination of R287432 in Formulation A12739A (ZA1296 SC (100)), Huang S., 2016b Report No. 300072558

Guideline(s): SANCO 3030/99 rev. 4

Deviations: None stated

GLP: No

Acceptability:

## Materials and methods

The relevant impurity R287432 in A12739A is separated by HPLC on a fused silica BEH-C18 column (100 mm x 2.1 mm) at 40°C. For elution a gradient of 0.1% formic acid in water and 0.1% formic acid in acetonitrile was used. Detection and identification was by ion trap mass spectroscopy after vaporizing the sample at 450°C under nitrogen. Parent ion was m/z 300 and MRM product ions are 209 m/z and 221 m/z with the latter being used for quantification.

Quantification is by comparison of peak areas ratios to those of a reference solution.

(Huang S., 2016)

## Validation - Results and discussions

Full validation of the method SD-1990/1 has been conducted. The method has been shown to be specific for the determination of R287432 in product A12739A and no significant interference was observed. Based on the results for repeatability, recovery, linearity and specificity, precision and accuracy of the method are established. Therefore, the method is suitable for the specific, accurate and precise determination of R287432 in product A12739A. A summary of the validation data is given in Tables 5.2-4 and 5.2-5.

(Huang S., 2016a & b)

**Table 5.2-4: Validation of Method SD-1990/1 for the determination of R287432 in A13789C**

	<b>R287432-maximum content in A13789C 0.075g/L, (2g/kg in technical mesotrione)</b>
<b>Author(s), year</b>	Huang S., 2016a (Unpublished Report No. USGR160250)
<b>Principle of method</b>	Liquid Chromatography/Mass Spectroscopy (LC/MS)
<b>Linearity</b> <b>N=6</b> <b>(linear range 10-200 µg/g in formulation)</b>	$r = 0.9998$ $y = 0.9991x + 0.0005$
<b>Precision – Repeatability Mean</b> <b>n = 6</b> <b>(%RSD)</b>	Srel (%RSD): 1.5% mean concentration: 40 µg/g in formulation
<b>Accuracy</b> <b>n = 4</b> <b>(% Recovery)</b>	mean recovery: 100 % (determined between 10 and 200µg/g of R287432 in formulation)
<b>Interference/ Specificity</b>	No significant interference was observed. The analytical method is able to separate the impurity R287432 from the formulation blank with no significant co-elution
<b>LOQ</b>	The validation data prove that the limit of quantification for R287432 is established at concentration of 10ug/g.
<b>Comment</b>	The method is suitable for the specific, accurate and precise determination of the relevant impurity R287432 in formulation.

**Table 5.2-5: Validation of Method SD-1990/1 for the determination of R287432 in A12739A**

	<b>R287432-maximum content in A12739A 2g/kg in mesotrione</b>
<b>Author(s), year</b>	Huang S., 2016b (Unpublished Report No. 300072559)
<b>Principle of method</b>	Liquid Chromatography/Mass Spectroscopy (LC/MS)
<b>Linearity</b> <b>n = 6</b> <b>linear range 10 to 200 µg/g in A13726E</b>	$r = 0.9999$ $y = 0.9962x + 0.0034$
<b>Precision – Repeatability Mean</b> <b>n = 6</b>	Srel (%RSD): 2.2% mean concentration: 40 µg/g
<b>Accuracy</b> <b>(% Recovery)</b>	mean recovery: 102.5 % (determined at 40 µg/g of R287432 in A13726E)

	<b>R287432-maximum content in A12739A 2g/kg in mes-otrione</b>
<b>Interference/ Specificity</b>	No significant interference was observed. The analytical method is able to separate the impurity R287432 from the formulation blank with no significant co-elution.
<b>LOQ</b>	The validation data prove that the limit of quantification for R287432 is established at concentration of 10 µg/g.
<b>Comment</b>	The method is suitable for the specific, accurate and precise determination of the relevant impurity R287432 in A12739A.

### Special note in relation to LOQ:

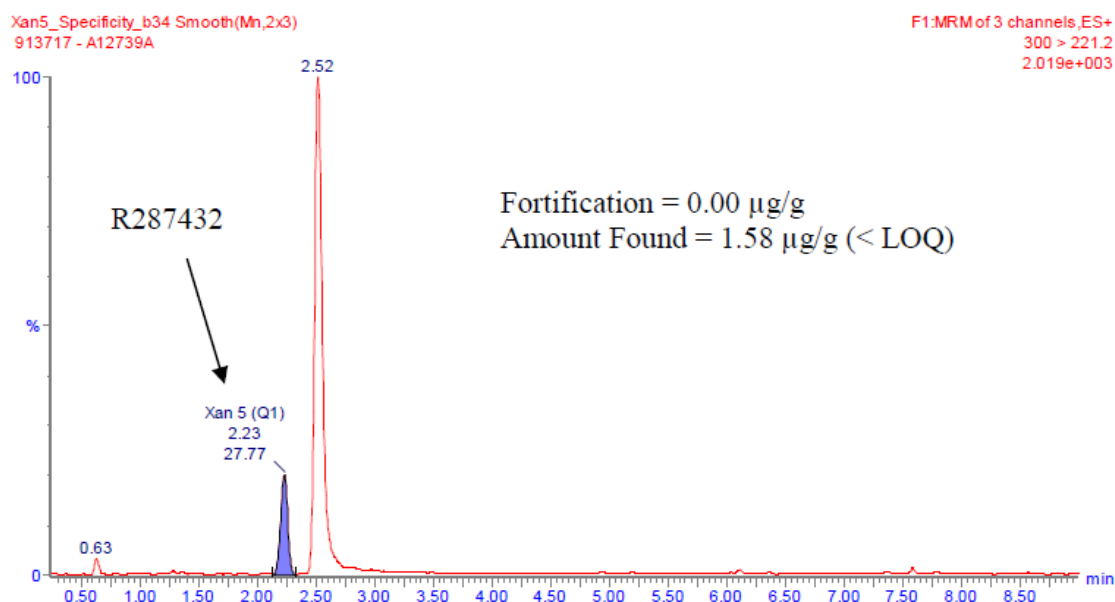
According to SANCO/3030/99 rev.5 2015:

However, in certain cases the content of the active substance in the plant protection product can be too low in order to determine a relevant impurity at the level derived from the maximum content in the technical active substance. In this case, the validation must be performed at the lowest possible concentration, and by means of chromatograms it must be demonstrated that the content of the analyte is below the LOQ.

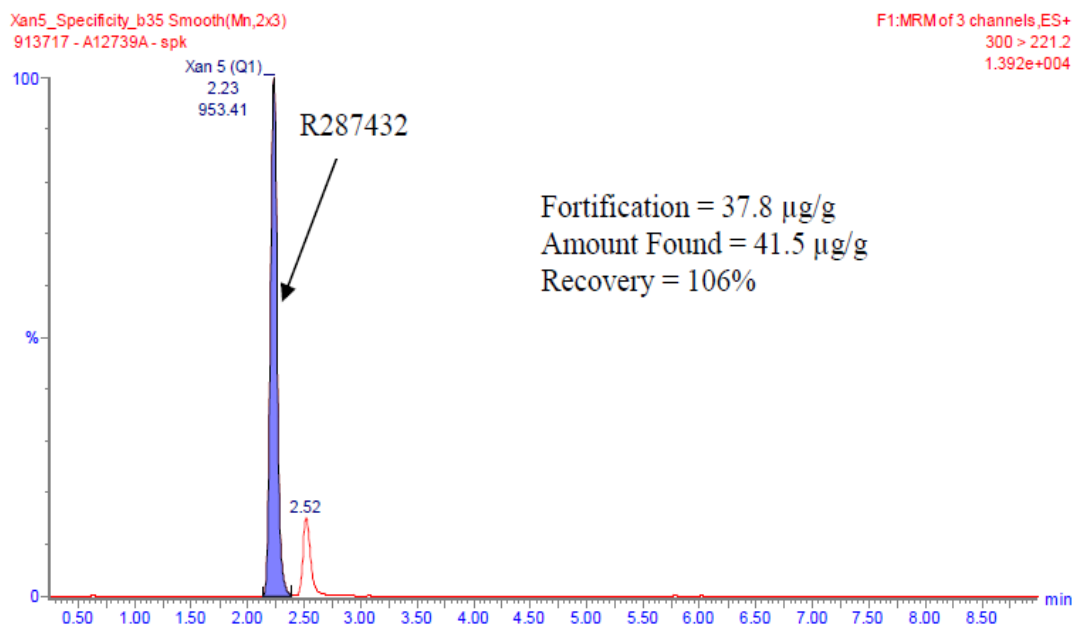
**Fig 3. Chromatogram to demonstrate that R287432 is below the LOQ of 10 µg/g (10 ppm) in A12739A**

The following chromatograms demonstrate that the concentration of R287432 in an unfortified batch of A12739A is below the LOQ (10 µg/g or 10 ppm).

A12739A (ID 913717) at ~5.0 mg/mL fortified with R287432 at ~ 0.00 µg/mL



A12739A (ID 913717) at ~5.0 mg/mL fortified with R287432 at ~0.20 µg/mL



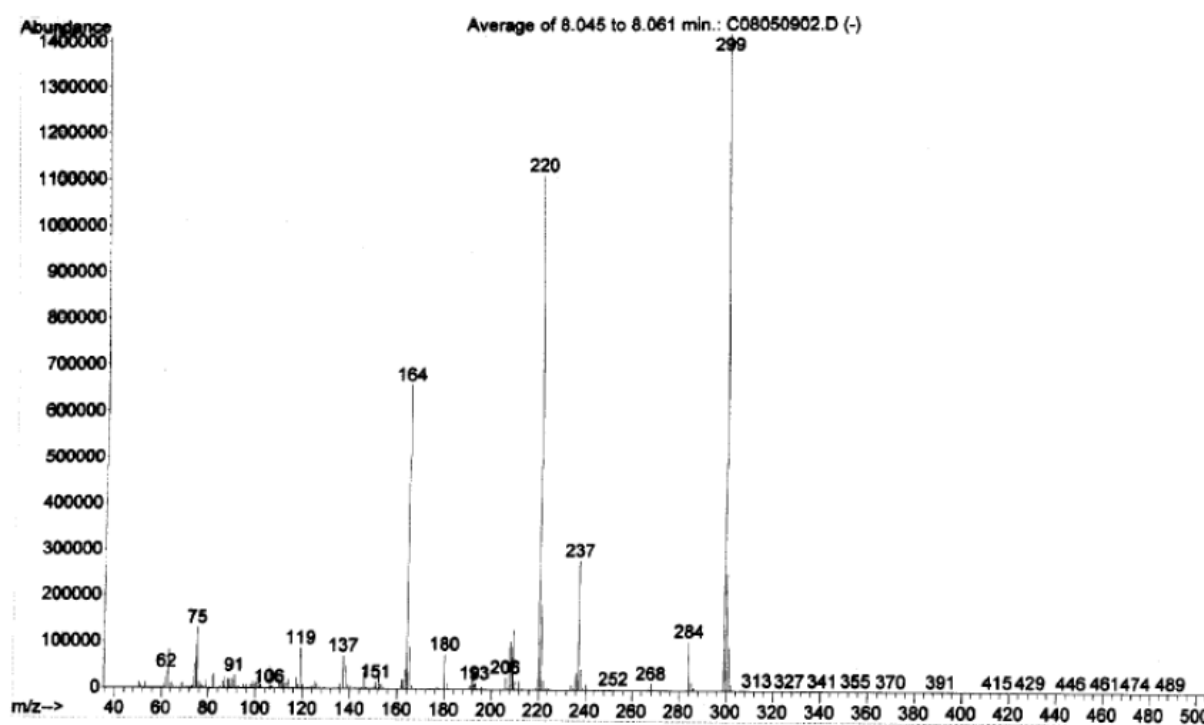
### Confirmation of Identity of R287432

According to SANCO/3030/99: *Confirmatory techniques are required to support identification of the a.s. and significant and/or relevant impurities, when the primary method of determination is not GC-MS or another highly specific method such as HPLC-UV DAD.*

The analytical method SD-1990/1 determines the relevant impurity R287432 in mesotrione containing formulations using multiple-point external standard calibration based on liquid chromatography with mass spectroscopy detection (LC/MS). The detection is by MS/MS (tandem mass spectrometry) for specific multiple reaction monitoring (MRM) of an analyte, therefore, high specificity for R287432 is inherent in the method and confirmation of identity of R287431 is inherent in the method.

As additional proof of identity i.e. Mass Spectra generated during the characterization of the reference material R2827432, Batch 53688 (Study T001406-08) are given below.

**Fig 4. Mass Spectra for R287432 Analytical Standard, Batch 53688 (Study T001406-08)**



## Conclusion

The method is suitable for the specific, accurate and precise determination of the relevant impurity R287432 in A12739A.

- 1,2-dichloroethane

Comments of zRMS:	<p>According to the applicant information the study SD-1990/1 was formerly recognized as acceptable.</p> <p>The validation study has been positively evaluated in Part B Section 5 of the Registration Report from 30/04/2020 for to renewal of mesotrione at the EU level of product Calisto 100 SC.</p> <p>The following conclusion was made:</p> <p><i>„The HS/GC/FID analytical method (SD-1973/1) for the determination of 1,2-dichloroethane (DCE) in mesotrione containing formulations, has been successfully validated in terms of specificity, recovery, linearity, precision and accuracy according to SANCO/3030/99/rev.4. Full validation of the method SD-1973/1 has been conducted. The method has been shown to be specific for the determination of 1,2-dichloroethane (DCE) in product A12739A and no significant interference was observed. Based on the results for repeatability, recovery, linearity and specificity, precision and accuracy of the method are established. Therefore, the method is suitable for the specific, accurate and precise determination of 1,2-dichloroethane (DCE) in product A12739A. The identity of 1,2-dichloroethane (DCE) has been confirmed.”</i></p>
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Reference: KCP 5.1.1/09

Report Analytical Method SD-1973/1 Determination of Impurity 1,2-dichloroethane (DCE) in formulation by headspace gas chromatography. Zhang Y. et al., 2016, Report No. 300066025

Guideline(s): None stated

Deviations: None stated

GLP: No

Acceptability:

Reference: KCP 5.1.1/10

Report Validation of analytical method SD-1973/1. Meyerhoffer W., 2016, Report No. USGR160249

Guideline(s): SANCO 3030/99 rev. 4

Deviations: None stated

GLP: Yes

Acceptability:

Reference: KCP 5.1.1/11

Report A12739A - Statement on Validation of Analytical Method SD-1973/1 for Determination of 1,2-Dichloroethane (DCE) in Formulation A12739A (ZA1296 SC (100)). Meyerhoffer W. , 2016a, Report No. 300072402

Guideline(s): SANCO 3030/99 rev. 4

Deviations: None stated

GLP: No

Acceptability:

### Materials and methods

The relevant impurity 1,2-dichloroethane (DCE) is determined in mesotrione containing formulations by gas chromatography on a fused silica DB-1 column (30m, 0.25 mm i.d., temperature program 40°C up to 230°C), using helium as a carrier gas and flame ionisation detection (FID). Quantification is by comparison of peak areas ratios to those of a reference solution.

(Zhang Y. *et al.*, 2016)

### Validation of method SD-1973/1 - Results and discussions

Full validation of the method SD-1973/1 has been conducted. The method has been shown to be specific for the determination of 1,2-dichloroethane (DCE) in product A12739A and no significant interference was observed. Based on the results for repeatability, recovery, linearity and specificity, precision and accuracy of the method are established. Therefore, the method is suitable for the specific, accurate and precise determination of 1,2-dichloroethane (DCE) in product A12739A. A summary of the validation data is given in Tables 5.2-6 and 5.2-7.

(Meyerhoffer W., 2016 & 2016a)

**Table 5.2-6: Validation of Method SD-1973/1 for the determination of DCE in A13789C**

	<b>DCE: maximum content in A13789C 0.0375g/L, (1g/kg in technical mesotrione)</b>
<b>Author(s), year</b>	Meyerhoffer W. 2016 (Unpublished Report No. USGR160249)
<b>Principle of method</b>	Headspace gas chromatography with flame ionization detection (HS/GC/FID)
<b>Linearity</b> <b>n = 5</b> <b>linear range 5 - 20 µg/ml in formulation</b>	$r = 0.9997$ $y = 0.5084x + 0.0408$
<b>Precision – Repeatability Mean</b> <b>n = 6</b> <b>(%RSD)</b>	Srel (%RSD): 3.9% mean concentration: 10.19 µg/ml in formulation
<b>Accuracy</b> <b>n = 5</b> <b>(% Recovery)</b>	mean recovery: 100 % (determined at 5, 8 10, 16 and 20 µg/ml of DCE in formulation)
<b>Interference/ Specificity</b>	No significant interference was observed. The analytical method is able to separate the impurity DCE from the formulation blank with no significant co-elution.
<b>LOQ</b>	The validation data demonstrate that the limit of quantification for DCE is established at concentration of 4ug/ml.
<b>Comment</b>	The method is suitable for the specific, accurate and precise determination of the relevant impurity DCE in formulation.

**Table 5.2-7: Validation of Method SD-1973/1 for the determination of DCE in A12739A**

	<b>DCE: maximum content in A12739A 0.1g/L, (1g/kg in technical mesotrione)</b>
<b>Author(s), year</b>	Meyerhoffer W. 2016a (Unpublished Report No. 300072402)
<b>Principle of method</b>	Headspace gas chromatography with flame ionization detection (HS/GC/FID)
<b>Linearity</b> <b>n = 5</b> <b>linear range 5 - 20 µg/ml in A13726E</b>	$r = 0.9993$ $y = 0.0068x + 0.0004$
<b>Precision – Repeatability Mean</b> <b>n = 6</b>	Srel (%RSD): 3.9% <sup>1</sup> mean concentration: 10 µg/g in formulation
<b>Accuracy</b> <b>n = 5</b> <b>(% Recovery)</b>	mean recovery: 99.7 % (determined at 5, 8 10, 16 and 20 µg/ml of DCE in formulation)
<b>Interference/ Specificity</b>	No significant interference was observed. The analytical

	<b>DCE: maximum content in A12739A 0.1g/L, (1g/kg in technical mesotrione)</b>
	method is able to separate the impurity DCE from the formulation blank with no significant co-elution
<b>LOQ</b>	The validation data demonstrate that the limit of quantification for DCE is established at concentration of 4ug/ml.
<b>Comment</b>	The method is suitable for the specific, accurate and precise determination of the relevant impurity DCE in A12739A.

1 precision is quoted for the repeatability data generated in study USGR160249 stated in Table 5.2-5, this is justified based on the fact that headspace analysis involves the analysis of pure DCE, in the vapour phase, and therefore the precision data generated relates to the method only and is independent of the formulation matrix.

**Special note in relation to LOQ:**

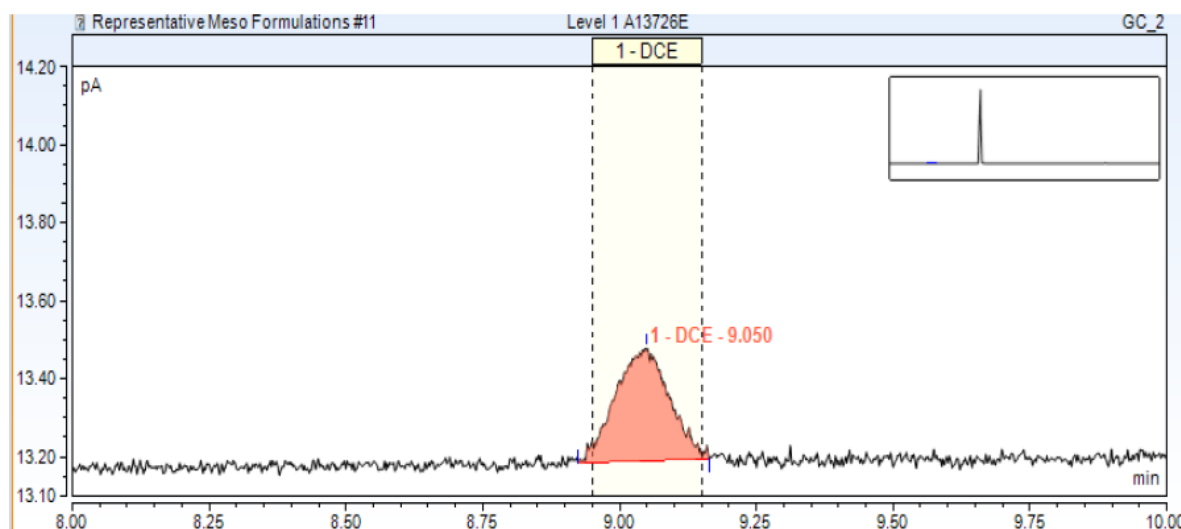
According to SANCO/3030/99 rev.5 2015:

*However, in certain cases the content of the active substance in the plant protection product can be too low in order to determine a relevant impurity at the level derived from the maximum content in the technical active substance. In this case, the validation must be performed at the lowest possible concentration, and by means of chromatograms it must be demonstrated that the content of the analyte is below the LOQ.*

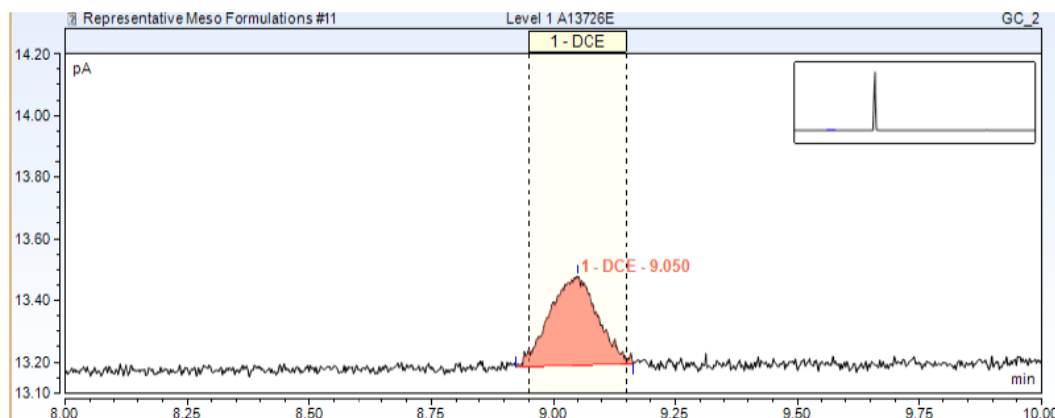
**Fig 5. Chromatogram to demonstrate that DCE is below the LOQ of 4 µg/ml (4 ppm) in A12739A**

The following chromatograms demonstrate that the concentration of DCE in an unfortified batch of A12739A is below the LOQ (4 µg/ml or 4 ppm).

### Chromatogram of A12739A Formulation Spiked at 5.01 µg/mL DCE



### Chromatogram of A12739A Formulation Batch 913717



### Confirmation of Identity of 1,2-dichloroethane (DCE)

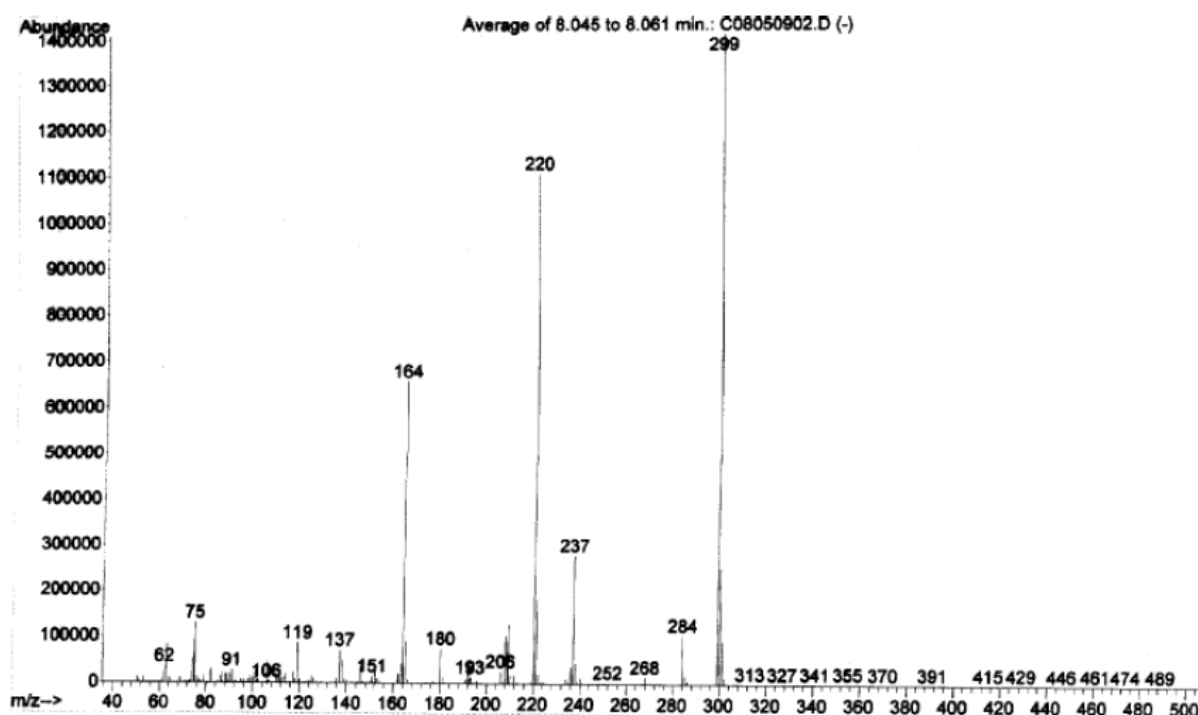
According to SANCO/3030/99:

*Confirmatory techniques are required to support identification of the a.s. and significant and/or relevant impurities, when the primary method of determination is not GC-MS or another highly specific method such as HPLC-UV DAD.*

The analytical method SD-1973/1 determines relevant impurity 1,2-dichloroethane (DCE) in mesotrione containing formulations using a multiple point, standard addition calibration based on headspace gas chromatography with flame ionization detection (HS/GC/FID). The analytical technique of headspace analysis requires that the analyte is evaporated from the matrix and is therefore by definition it is presented to the detector as pure phase and therefore the formulation matrix has no inherent influence on the specificity of the method. Furthermore, standard addition is used in analytical method SD-1973/1 which ensures that identity is unequivocally confirmed with each analysis as the analyte samples are fortified with certified pure analytical standard of 1,2-dichloroethane. Therefore, confirmation of identity of 1,2-dichloroethane is inherent in the method.

As additional proof of identity i.e. Mass Spectra generated during the characterization of the reference material DCE, Batch 53688 (Study T001406-08) are given below.

**Fig 4. Mass Spectra for DCE Analytical Standard, Batch 53688 (Study T001406-08)**



## Conclusion

The method is suitable for the specific, accurate and precise determination of the relevant impurity 1,2-dichloroethane (DCE) in A12739A.

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

There are no relevant formulants in MEZI 100 SC therefore no methods are required.

### 5.2.1.4 Applicability of existing CIPAC methods (KCP 5.1.1)

There is no CIPAC method available for the determination of Mesotrione in SC formulations.

## 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 tables.

**Table 5.2-8: Validated methods for the generation of pre-authorization data for Mesotrione in plant and animal products in support of environmental fate studies**

Component of residue definition: Mesotrione, MNBA and AMBA				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
Soil	TMR0661B	0.005 mg/kg	HPLC-FSD	Method: Alferness (1996) Report TMR0661B EU agreed (RAR Mesotrione, 2015, 2015a)

**Table 5.2-9: Validated methods for the generation of pre-authorization data for Mesotrione in plant and animal products in support of residue studies**

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 protein/high starch content (dry) <i>Maize forage, maize grain</i>	TMR0643B	0.01 mg/kg	HPLC-FL	Method:  *Alferness, 1996 Report: TMR0643B
High water content <i>Maize fodder</i>				Validation: *Bolygo, 1996 Report: RJ0689B  EU agreed (RAR Mesotrione, 2015, 2015a)
High protein/high starch content (dry) <i>Maize grain, maize silage, maize stover</i>	RAM 366/01	0.01 mg/kg	HPLC-MS/MS (2 transitions)	Method: Crook, 2002 Report: RAM 366/01  Validation: Hill, 2004 Report: RJ3253B Bruns <i>et al.</i> 2001 Report: ZA1296/0656  Bruns <i>et al.</i> 2001 Report: ZA1296/0656  EU agreed (RAR Mesotrione, 2015, 2015a)
High water content <i>Maize whole plant,</i>				
High water content <i>Oilseed rape whole plant</i>		0.01 mg/kg		Validation: Malet & Allard, 2010 Report: RXCO00307 EU agreed (RAR Mesotrione, 2015, 2015a)
High oil content <i>Oilseed rape seed</i>				
High oil content Linseed seed		0.01 mg/kg		Validation: Simon, 2004a

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 oil content Poppyseed seed		0.01 mg/kg		Report: gpp067003 EU agreed (RAR Mesotrione, 2015, 2015a)
High protein/high starch content (dry) <i>Maize grain (whole)</i>	GRM007.11A (update to RAM 366/01)	0.01 mg/kg	HPLC-MS/MS (2 transitions)	Method: Watson & Crook, 2013 Report: GRM007.11A
High water content <i>Maize forage</i>				Validation:  Watson, 2013 Report: S12-03629
High acid content Whole orange				Amic, 2013 Report: S13-02460
High oil content <i>Oilseed rape seed</i>				EU agreed (RAR Mesotrione, 2015, 2015a)

**Table 5.2-10: Validated methods for the generation of pre-authorization data for Mesotrione in plant matrices in support of ecotoxicological studies**

Component of residue definition: Mesotrione and MNBA				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
Plants	GRM007.11A	0.01 mg/kg	HPLC-MS/MS (2 transitions)	Method: North, L., 2016 Report: S15-02057

**Table 5.2-11: Validated methods for the generation of pre-authorization data for Mesotrione in water in support of ecotoxicological studies**

Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
Component of residue definition: Mesotrione, AMBA and MNBA				
Water	S12-03986	5 µg/L	HPLC-MS/MS	Method: Zawadsky, 2013 Report S12-03986 EU agreed (RAR Mesotrione, 2015, 2015a)
Component of residue definition: Mesotrione and MNBA				
Water	6179-04	0.05 µg/L	HPLC-MS/MS	Method: Chamkasem, 2004 Report T006179-04

				EU agreed (RAR Mesotri- one, 2015, 2015a)
<b>Component of residue definition: Mesotrione</b>				
Water	ZA1296/2049	0.838 mg/L	HPLC-UV	Method: Volz, 2005 Re- port ZA1296/2049 EU agreed (RAR Mesotri- one, 2015, 2015a)
Water	RJ3714B	0.05 µg/L	HPLC-MS/MS	Method: Ricketts & Lan- gridge, 2005 Report RJ3714B EU agreed (RAR Mesotri- one, 2015, 2015a)
Water	105731240A	1 µg/L	HPLC-MS/MS	Method: Hengsberger & Wydra, 2015 Report 105731240
Water	105732240A	1 µg/L	HPLC-MS/MS	Method: Kosak & Wydra, 2016 Report 1105732240
Water	S16-06273	0.4 µg/L	HPLC-MS/MS	Method: Gonsior, 2017 Report S16-06273
<b>Component of residue definition: SYN546974</b>				
Water	D77394	0.0497 mg/L	HPLC-UV	Method: Liedtke, 2013 Report D77394 EU agreed (RAR Mesotri- one, 2015, 2015a)
<b>Component of residue definition: AMBA</b>				
Water	D55614	0.171mg/L	HPLC-UV	Method: Liedtke, 2013a Report D55614 EU agreed (RAR Mesotri- one, 2015, 2015a)
<b>Component of residue definition: MNBA</b>				
Water	D55614	0.161 mg/L	HPLC-UV	Method: Liedtke, 2013b Report D55592 EU agreed (RAR Mesotri- one, 2015, 2015a)



**Table 5.2-12 Validated methods for the generation of pre-authorization data for Mesotrione in body fluids, air and any additional matrices used in support of operator, worker, resident and bystander exposure studies.**

Method type	Matrix type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
<b>Component of residue definition: MNBA</b>				
Aqueous carboxymethylcellulose	BFI0148	1 mg/L	HPLC-UV	Method Bachelor B., 2014 Report 11070
Aqueous carboxymethylcellulose	BFI0147	1 mg/L	HPLC-UV	Method Faulkner &Heap, 2013 Report BFI0147
Aqueous carboxymethylcellulose	BFI0148	1 mg/L	HPLC-UV	Method Faulkner & Heap, 2013a Report BFI0148
Aqueous carboxymethylcellulose	BFI0148	1 mg/L	HPLC-UV	Method Faulkner & Heap, 2013b Report BFI0149
<b>Component of residue definition: AMBA</b>				
Blood and plasma of rats	BFI068MS for blood and BFI074MS for plasma	Not given	HPLC-MS/MS	Method Chubb D., 2016 Report BFI0533

### **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)**

Data provided on Annex I inclusion is sufficient for post-authorizations methods. All data is described in EU approved documents for :

- Methods are described and presented in Table 5.2-8- 5.2-12 in point KCP 5.1.2.

#### **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 cur-

rent 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 <i>Lettuce</i>	Mesotrione	0.01 mg/kg	Reg. (EU) 2017/626
Plant, high acid content <i>Orange</i>		0.01 mg/kg	Reg. (EU) 2017/626
Plant, high protein/high starch content (dry commodities) <i>Maize grain, dry broad bean</i>		0.01 mg/kg	Reg. (EU) 2017/626
Plant, high oil content <i>Sunflower seed</i>		0.01 mg/kg	Reg. (EU) 2017/626
Plant, difficult matrices (hops, spices, tea)		0.05 mg/kg	Reg. (EU) 2017/626
Muscle	Mesotrione	0.01 mg/kg	Reg. (EU) 2017/626
Milk		0.01 mg/kg	Reg. (EU) 2017/626
Eggs		0.01 mg/kg	Reg. (EU) 2017/626
Fat		0.01 mg/kg	Reg. (EU) 2017/626
Liver, kidney		0.01 mg/kg	Reg. (EU) 2017/626
Soil (Ecotoxicology)	Mesotrione	0.01 µg/kg	common limit
Drinking water (Human toxicology)	Mesotrione	0.1 µg/L	general limit for drinking water
Surface water (Ecotoxicology)	Mesotrione	3.1 µg/L	Endpoint covers geometric mean aquatic macrophytes based on ErC <sub>50</sub>
Air	Mesotrione	4.75 mg/L	Endpoint covers the acute inhalation study
Tissue (meat or liver)	Mesotrione	0.01 mg/kg	Validation: Watson, 2013b Report S12-03250 EU agreed (RAR Mesotrione, 2015, 2015a)
Body fluids		0.01 mg/kg	

a) No residue definition has been set for products of animal origin.

### 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 matrices is given in the following tables.

**Table 5.3-2: Validated methods for food and feed of plant 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 / EU agreed
High water content <i>Maize forage</i>	QuEChERS	0.01 mg/kg	LC-MS/MS (multi-residue)	QuEChERS Method and validation: Watson, 2013a Report: S12-03251  ILV: Tessier, 2013 Report: S12-04607 EU agreed (RAR Mesotrione, 2015, 2015a)
	ILV (QuEChERS)	0.01 mg/kg		
High acid content <i>Whole orange</i>	QuEChERS	0.01 mg/kg		
High oil content <i>Oilseed rape seed</i>	Primary	0.01 mg/kg		
	ILV	0.01 mg/kg		
High protein/high starch content (dry) Maize kernel	Primary	0.01 mg/kg		
	ILV (QuEChERS)	0.01 mg/kg		
High protein/high starch content (dry) Maize forage, maize grain	TMR0643B	0.01 mg/kg	HPLC-FL	Registration report of Callisto 100 SC:  Method: Alferness, 1996 Report: TMR0643B
High water content Maize fodder				Validation: Bolygo, 1996 Report: RJ2149B  EU agreed (UK, 2015, 2015a)

**Table 5.3-3: Statement on extraction efficiency**

	Method for products of plant origin
Required, available from:	<p>Studies on the metabolism of mesotrione in maize incorporate a number of different extraction steps, one of which is extraction with acetonitrile/water in a 1:1 ratio. Residues of mesotrione in grain were extremely low and therefore no data are available to address the extraction efficiency in grain however data are available from samples of maize fodder and forage leaf as these samples contained the majority of the radioactivity. These data indicate that the majority of the total radioactive residue obtained via solvent extraction from these matrices was extracted via use of acetonitrile/water and subsequent characterisation indicated that these extracts contained the residue of mesotrione. This would therefore indicate that the use of acetonitrile/water in a 1:1 v/v ratio is effective for extraction of residues of mesotrione.</p> <p>Wei &amp; Dohn, 1997 Report: RR 96-026B Tarr &amp; van Neste, 1997 Report: RR96-007B EU agreed (UK, 2015, 2015a)</p>
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 matrices is given in the following tables.

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

Component of residue definition: Mesotrione				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Milk	QuEChERS	0.01 mg/kg	LC-MS/MS (multi-residue)	QuEChERS Method and validation: Watson, 2013b Report: S12-03250  EU agreed (RAR Mesotrione, 2015, 2015a)  ILV: Bernal, 2013 Report: S12-04608  EU agreed (RAR Mesotrione , 2015, 2015a)
	ILV	0.01 mg/kg		
Eggs	QuEChERS	0.01 mg/kg		
	ILV	0.01 mg/kg		
Muscle	QuEChERS	0.01 mg/kg		
Fat	QuEChERS	0.01 mg/kg		
	ILV	0.01 mg/kg		
Kidney	QuEChERS	0.01 mg/kg		
Liver	QuEChERS	0.01 mg/kg		
	ILV	0.01 mg/kg		
Whole blood	QuEChERS	0.01 mg/kg		

(a) No residue definition has been set for products of animal origin.

**Table 5.3-5: Statement on extraction efficiency**

	Method for products of animal origin
Required, available from:	-
Not required, because:	No study on mesotrione metabolism and extraction efficiency in animal matrices is required since intake of mesotrione is not significant. Consequently, no MRLs have been set for products of animal origin and no monitoring method for residues in animal products is necessary.

### 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, MNBA, AMBA in soil is given in the following tables.

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

Component of residue definition: mesotrione, MNBA and AMBA			
Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
GRM007.10A	0.002 mg/kg	HPLC-MS/MS	Method: Jutsum & Williams, 2013 GRM007.10A

Component of residue definition: mesotrione, MNBA and AMBA			
Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
			Validation: Jutsum, 2013 CEMR-5657-REG  EU agreed (RAR Mesotrione, 2015, 2015a)
T001200-03	0.002 mg/kg	HPLC-MS/MS	Method: Williams, 2004 Report T001200-03

### 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, MNBA, AM-BA in surface and drinking water is given in the following tables.

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

Component of residue definition: mesotrione, 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	GRM007.09A	0.05 µg/L (surface water)	HPLC-MS/MS	Method: Jutsum & Chamkesam, 2013 Report GRM007.09A  Validation: Jutsum, 2013a Report CEMR-5658-REG  EU agreed (RAR Mesotrione, 2015, 2015a)
	ILV (GRM007.09A)	0.05 µg/L	HPLC-MS/MS	
Ground water	GRM007.09A	0.05 µg/L	HPLC-MS/MS	Method: Jutsum & Chamkesam, 2013 Report GRM007.09A  Validation: Jutsum, 2013a Report CEMR-5658-REG  EU agreed (RAR Mesotrione, 2015, 2015a)
Component of residue definition: Mesotrione and MNBA				
Drinking water	TMR0707B	0.05 µg/L	GC-MSD	Method: Meyers, 1997 Report TMR0707B EU Agreed (RAR Mesotrione, 2015, 2015a)

### 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 tables.

**Table 5.3-8: 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
RR 97-031B	0.01 mg/m <sup>3</sup>	HPLC-UV	Method: Leung, 1997 Report RR 97-031B  EU agreed (RAR Mesotrione, 2015, 2015a)
GRM007-08B	0.45 µg/m <sup>3</sup>	HPLC-MS/MS	Method: Jutsum, 2013b GRM007.08B Validation: Jutsum, 2013c CEMR-5403-REG  EU agreed (RAR Mesotrione, 2015, 2015a)

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 5.3-9: Methods for body fluids and tissues (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
Whole blood	QuEChERS Method	0.01 mg/kg	HPLC-MS/MS	Validation: Watson, 2013b Report S12-03250 EU agreed (UK, 2015, 2015a)

### 5.3.2.8 Other studies/ information (KCP 5.2)

#### • Method of validation: HPLC with UV-DAD

Reference	KCP 5.2/01
Autor/year	Zaworska K.,2023-Ciorga B.,2023

Title of the study in which validation method was used	Validation of analytical method for determination of active substance (mesotrione) of the test item MEZI 100 SC in 50% (w/v) sucrose solution
Study code	0038/0177/FA
Guidelines:	SANTE/2020/12830, rev 2
GLP	Yes
Deviations	Yes Deviation from the Study plan was found 1. Both the starting and completion dates were shifted to October from the initial term which was set for September in Study plan.  The deviation did not affect the reliability of the study results. No deviations from the SANTE/2020/12830 rev.2 guideline were found
Acceptability	
Duplications (if vertebrate study):	No
Validation method	HPLC with UV-DAD
Validation parameters	During the validation of the analytical method the following parameters: selectivity, matrix effects, linearity, accuracy, precision (repeatability), limit of detection and limit of quantification, stability of the standard in acetonitrile were determined.
Detected substances	Mesotrione
Matrix type	50% (w/v) sucrose solution

For the detailed information of this study it is referred to Appendix 2.

• **Method of validation: HPLC with PDA**

Reference	KCP 5.2/02
Autor/ year	Ciorga B.,2023
Title of the study in which validation method was used	Validation of analytical method for determination of active substance (mesotrione) of the test item MEZI 100 SC in deionized water
Study code	0038/0175/FA
Guidelines:	SANTE/2020/12830, rev 2
GLP	Yes
Deviations	Yes Deviation from the Study plan was found: 1. Both the starting and completion dates were shifted to October from the initial term which was set for September in Study plan.  The deviation did not affect the reliability of the study results. No deviations from the SANTE/2020/12830 rev.2 guideline were found.
Acceptability	
Duplications (if vertebrate study):	No
Validation method	HPLC with PDA
Validation parameters	During the validation of the analytical method the following parameters: selectivity, matrix effect, linearity, accuracy, precision (repeatability), limit of detection, limit of quantification and standard stability in acetonitrile were determined
Detected substances	Mesotrione
Matrix type	Dejonized water

For the detailed information of this study it is referred to Appendix 2.

## 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 5.1.1/01	Arévalo E.,	2021	CHR/H/MEZZO 100 SC Determination of physicochemical properties of the preparation Study code: BF-49/20 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry GLP Unpublished	N	„Chemiroł” Sp. z o.o.
KCP 5.2/01	Zaworska K., Ciorga B., 2023	2023	Validation of analytical method for determination of active substance (mesotrione) of the test item MEZI 100 SC in 50% (w/v) sucrose solution Study code: 0038/0177/FA SORBOLAB Research Laboratory LLC GLP: yes unpublished	N	„Chemiroł” Sp. z o.o.
KCP 5.2/02	Ciorga B.,	2023	Validation of analytical method for determination of active substance (mesotrione) of the test item MEZI 100 SC in deionized water Study code: 0038/0175/FA SORBOLAB Research Laboratory LLC GLP: yes unpublished	N	„Chemiroł” Sp. z o.o.



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

<b>Data point</b>	<b>Author(s)</b>	<b>Year</b>	<b>Title Company Report No. Source (where different from company) GLP or GEP status Published or not</b>	<b>Vertebrate study Y/N</b>	<b>Owner</b>
KCP 5.1.2	Alferness PL	1999	ZA1296: Liquid Chromatographic Determination with Fluorescence Detection of ZA1296 & 4-(Methylsulfonyl)-2-Nitrobenzoic Acid in Crops after Conversion to 2-Amino-4-(Methylsulfonyl)-Benzoic Acid - A Modification of TMR0643B Zeneca Agrochemicals, Jealott's Hill, United Kingdom, TMR0882B Not GLP, not published Syngenta File No ZA1296/0121	N	SYN
KCP 5.1.2 KCP 5.2	Bolygo, E.	1996	ZA 1296: Independent Laboratory Confirmation of an Analytical Method for Liquid Chromatographic Determination with Fluorescence Detection of ZA 1296 and 4-(methylsulfonyl)-2-nitrobenzoic acid in Crops after Conversion to 2-amino-4-(methylsulfonyl)-benzoic acid Zeneca Report No. RJ2149B	N	SYN
KCP 5.1.2	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 AG, Basel, Switzerland Syngenta – Jealott's Hill International, Bracknell, Berkshire, United Kingdom, RAM 366/01, 2704-01 Not GLP, not published Syngenta File No ZA1296/0752	N	SYN
KCP 5.1.2	Hill S.	2004	Report revision: Mesotrione and MNBA (Metabolite) : Validation of a Residue Analytical Method (RAM 366/01) for the Determination of the Residues in Maize Syngenta Crop Protection AG, Basel, Switzerland Syngenta - Jealott's Hill International, Bracknell, Berkshire, United Kingdom, RJ3253B GLP, not published Syngenta File No ZA1296/0655	N	SYN
KCP 5.1.2	Bruns G., McLean N., Nelson S.	2001	Independent Laboratory Validation of the Analytical Method, "Residue Analytical Method for the Determination of Residues of Mesotrione and 4-(Methylsulfonyl)-2-Nitrobenzoic acid (MNBA) in Crop Samples". Syngenta Crop Protection AG, Basel, Switzerland	N	SYN

<b>Data point</b>	<b>Author(s)</b>	<b>Year</b>	<b>Title Company Report No. Source (where different from company) GLP or GEP status Published or not</b>	<b>Vertebrate study Y/N</b>	<b>Owner</b>
			Enviro-Test Laboratories, Edmonton, Alberta, Canada, 01SYN83.REP GLP, not published Syngenta File No ZA1296/0656		
KCP 5.1.2	Watson G.	2013	Mesotrione - Validation of Syngenta Method RAM 366/01 for the Determination of Residues of Mesotrione and MNBA in Crop Matrices by LC-MS/MS Syngenta Eurofins Agroscience Services Ltd, Wilson, UK, S12-03629 GLP, not published Syngenta File No ZA1296_10101	N	SYN
KCP 5.1.2	Liedtke A.	2013	SYN546974 - Toxicity to the aquatic higher plant Lemna gibba in a 7-day growth inhibition test Syngenta Harlan Laboratories Ltd., Itingen, Switzerland, D77394 GLP, not published Syngenta File No SYN546974_10001	N	SYN
KCP 5.1.2	Liedtke A.	2013a	R44276 - Toxicity to the Aquatic Higher Plant Lemna gibba in a 7-Day Growth Inhibition Test Syngenta Harlan Laboratories Ltd., Itingen, Switzerland, D55614 GLP, not published Syngenta File No R044276_10001	N	SYN
KCP 5.1.2	Liedtke A.	2013b	R169649 - Toxicity to the Aquatic Higher Plant Lemna gibba in a 7-Day Growth Inhibition Test Syngenta Harlan Laboratories Ltd., Itingen, Switzerland, D55592 GLP, not published Syngenta File No CA3511_10001	N	SYN
KCP 5.1.2	Volz E.	2005	Mesotrione 100 SC Formulation (A12739A): Toxicity to Pseudokirchneriella subcapitata (formerly Selenas-trum capricornutum) in a 96-hour algal growth inhibition test	N	SYN

<b>Data point</b>	<b>Author(s)</b>	<b>Year</b>	<b>Title Company Report No. Source (where different from company) GLP or GEP status Published or not</b>	<b>Vertebrate study Y/N</b>	<b>Owner</b>
			Syngenta Crop Protection AG, Basel, Switzerland RCC Ltd., Itingen, Switzerland, A18325 2031806 GLP, not published Syngenta File No ZA1296/2049		
KCP 5.2	Watson G., Crook S.	2013	Mesotrione - Analytical Method (GRM007.11A) for the Determination of Residues of Mesotrione and 4-(Methylsulfonyl)-2-Nitrobenzoic Acid (MNBA) in Crop Matrices by LC-MS/MS Syngenta Eurofins Agroscience Services Ltd, Wilson, UK, GRM007.11A Not GLP, not published Syngenta File No ZA1296_10102	N	SYN
KCP 5.1.2	Amic S.	2013	Mesotrione - Independent Laboratory Validation of Syngenta Method GRM007.11A for the Determination of Residues of Mesotrione and MNBA in Crop Matrices by LC-MS/MS Syngenta Eurofins Agroscience Services Chem SAS, Vergèze, France, S13-02460 GLP, not published Syngenta File No ZA1296_10120	N	SYN
KCP 5.1.2	Watson G.	2013a	Mesotrione - Validation of the QuEChERS Method for the Determination of Residues of mesotrione in Crop Matrices by LC-MS/MS Syngenta Eurofins Agroscience Services Ltd, Wilson, UK, S12-03251 GLP, not published Syngenta File No ZA1296_10090	N	SYN
KCP 5.1.2	Zawadsky C.	2013	Mesotrione SC (A12739A) - Assessment of Toxic Effects on the duckweed Lemna gibba in a 7 day Semi-Static Test and 14 day Recovery Period Syngenta Crop Protection AG, Basel, Switzerland Eurofins Agroscience Services EcoChem GmbH, N-Osch., Germany, S12-03986 GLP	N	SYN

<b>Data point</b>	<b>Author(s)</b>	<b>Year</b>	<b>Title Company Report No. Source (where different from company) GLP or GEP status Published or not</b>	<b>Vertebrate study Y/N</b>	<b>Owner</b>
			not published Syngenta File No A12739A_10273		
KCP 5.1.2	Chamkasem N.	2004	Analytical Method 6179-04 for the Determination of Mesotrione and its Degradates AMBA and MNBA in Water by Direct Injection High Performance Liquid Chromatography with Mass Spectrometric Detection Syngenta Crop Protection AG, Basel, Switzerland Syngenta Crop Protection, Inc., Greensboro, USA, T006179-04 GLP, not published Syngenta File No ZA1296/1508	N	SYN
KCP 5.1.2	Ricketts D., Langridge G.	2005	Mesotrione 100 g/L SC (A12739A): Acute toxicity to the Cladoceran Daphnia magna under static conditions Syngenta Crop Protection AG, Basel, Switzerland Syngenta - Jealott's Hill International, Bracknell, Berkshire, United Kingdom, RJ3714B GLP not published Syngenta File No ZA1296/2042		
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 Eurofins Agroscience Services Chem SAS, Vergèze, France, S12-04607 GLP, not published Syngenta File No ZA1296_10129	N	SYN
KCP 5.2	Jutsum L., Williams R.	2013	Mesotrione - Analytical Method GRM007.10A for the Determination of Mesotrione and its Metabolites AM-BA and MNBA in Soil Syngenta CEMAS, North Ascot, United Kingdom, GRM007.10A Not GLP, not published Syngenta File No ZA1296_10092	N	SYN
KCP 5.2	Jutsum L.,	2013	Mesotrione – Analytical Method GRM007.09A for the Determination of Mesotrione and its Metabolites AM-	N	SYN

<b>Data point</b>	<b>Author(s)</b>	<b>Year</b>	<b>Title Company Report No. Source (where different from company) GLP or GEP status Published or not</b>	<b>Vertebrate study Y/N</b>	<b>Owner</b>
	Chamkesam N.		BA and MNBA in Water Syngenta CEMAS, North Ascot, United Kingdom, GRM007.09A Not GLP, not published Syngenta File No ZA1296_10091		
KCP 5.2	Jutsum L.	2013a	Mesotrione - Validation of Draft Residue Method GRM007.09A for the Determination of Mesotrione and its metabolites AMBA and MNBA in Water Syngenta CEMAS, North Ascot, United Kingdom, CEMR-5658-REG GLP, not published Syngenta File No ZA1296_10087	N	SYN
KCP 5.2	Jutsum L.	2013b	Mesotrione - Residue Method GRM007.08B for the Determination of Mesotrione in Air Syngenta CEMAS, North Ascot, United Kingdom, GRM007.08B Not GLP, not published Syngenta File No ZA1296_10089	N	SYN
KCP 5.2	Jutsum L.	2013c	Mesotrione - Validation of Residue Method GRM007.08A for the Determination of Mesotrione in Air Syngenta CEMAS, North Ascot, United Kingdom, CEMR-5403-REG GLP, not published	N	SYN

**List of data submitted by the applicant and relied on, but evaluated before in Callisto 100 SC**

<b>Data point</b>	<b>Author(s)</b>	<b>Year</b>	<b>Title Company Report No. Source (where different from company) GLP or GEP status Published or not</b>	<b>Vertebrate study Y/N</b>	<b>Owner</b>
KCP 5.1.1	Hager M.	2011	R287431 - Analytical Method SD-977/2 Syngenta Crop Protection AG, Basel, Switzerland Syngenta File No R287431_10003, Report No. Syngenta Crop Protection, LLC, Greensboro, NC, USA, 10427012 Not GLP Unpublished	N	SYN
KCP 5.1.1	Hager M.	2011a	Validation of method SD-977/2 - R287431 in A14203B, A13789C, A14351BX, A12909Q, A15189G, A12738A, A15901A and A18219B Syngenta File No R287431_10001 Syngenta Crop Protection AG, Basel, Switzerland Syngenta Crop Protection, Inc., Greensboro, USA, 10427878 GLP Unpublished	N	SYN
KCP 5.1.1	Hager M.	2015	A12739A- Statement on Validation of Analytical Method SD-977/2 for Determination of R287431 (Xan-1) in Formulation A12739A (MESOTRIONE SC 100) Syngenta File No A12739A_11032 Syngenta Crop Protection AG, Basel, Switzerland Syngenta Crop Protection, LLC, Greensboro, NC, USA, 300035703 Not GLP Unpublished	N	SYN
KCP 5.1.1	Baker, S. <i>et al</i>	2018	A12739A- Response to the Danish Regulatory Authority Concerning Relevant Impurity R287431 Syngenta File No A12739A_11278 Syngenta Crop Protection, LLC, Greensboro, NC, USA, 300035703 Not GLP Unpublished	N	SYN
KCP 5.1.1	Huang S.	2016	ZA1296 - SD-1990/1 - Determination of R287432 in Mesotrione Related Formulations by Liquid Chromatography/Mass Spectrometry (LC/MS) Syngenta File No A13789C_50005 Syngenta Crop Protection AG, Basel, Switzerland	N	SYN

<b>Data point</b>	<b>Author(s)</b>	<b>Year</b>	<b>Title Company Report No. Source (where different from company) GLP or GEP status Published or not</b>	<b>Vertebrate study Y/N</b>	<b>Owner</b>
			Syngenta Crop Protection, LLC, Greensboro, NC, USA, 300068727 Not GLP Unpublished		
KCP 5.1.1	Huang S.	2016a	A13789C - Validation of Analytical Method SD-1990/1 Syngenta File No A13789C_50004 Syngenta Crop Protection AG, Basel, Switzerland Syngenta Crop Protection, LLC, Greensboro, NC, USA, USGR160250 GLP Unpublished	N	SYN
KCP 5.1.1	Huang S.	2016b	A12739A - Statement on Validation of Analytical Method SD-1990/1 for Determination of R287432 in Formulation A12739A (ZA1296 SC (100)) Syngenta File No A12739A_11095 Syngenta Crop Protection AG, Basel, Switzerland Syngenta Crop Protection, LLC, Greensboro, NC, USA, 300072558 Not GLP Unpublished	N	SYN
KCP 5.1.1	Meyerhoffer W., Zhang Y., Patterson J.	2016	ZA1296 - SD-1973/1 - Determination of Impurity DCE (1,2-dichloroethane) in Mesotrione Related Formulations by Headspace Gas Chromatography Syngenta File No A13789C_50002 Syngenta Crop Protection AG, Basel, Switzerland Syngenta Crop Protection, LLC, Greensboro, NC, USA, 300066025 Not GLP Unpublished	N	SYN
KCP 5.1.1	Meyerhoffer W.	2016	A13789C - Validation of Analytical Method SD-1973/1 Syngenta File No A13789C_50001 Syngenta Crop Protection AG, Basel, Switzerland Syngenta Crop Protection, LLC, Greensboro, NC, USA, USGR160249 GLP Unpublished	N	SYN

<b>Data point</b>	<b>Author(s)</b>	<b>Year</b>	<b>Title Company Report No. Source (where different from company) GLP or GEP status Published or not</b>	<b>Vertebrate study Y/N</b>	<b>Owner</b>
KCP 5.1.1	Meyerhoffer W.	2016a	A12739A - Statement on Validation of Analytical Method SD-1973/1 for Determination of 1,2-Dichloroethane (DCE) in Formulation A12739A (ZA1296 SC (100)) Syngenta File No A12739A_11096 Syngenta Crop Protection AG, Basel, Switzerland Syngenta Crop Protection, LLC, Greensboro, NC, USA, 300072402 Not GLP Unpublished	N	SYN
KCP 5.1.2	North L.	2016	Mesotrione - Foliage Decline with A12739A on Maize in Northern France and the United Kingdom in 2015 Syngenta File No A12739A_11065 Syngenta Crop Protection AG, Basel, Switzerland Eurofins Agrosience Services Ltd, Wilson, UK, S15-02057 GLP Unpublished	N	SYN
KCP 5.1.2	Hengsberger A., Wydra V.	2015	Mesotrione wet paste (ZA1296) - Toxicity to the aquatic plant Lemna gibba in a reciprocal growth inhibition test Syngenta Crop Protection AG, Basel, Switzerland IBACON GmbH, Rossdorf, Germany, 105731240 GLP Unpublished Syngenta File No ZA1296_10436	N	SYN
KCP 5.1.2.	Kosak L., Wydra V.	2016	Mesotrione wet paste (ZA1296) - Toxicity to the aquatic plant Lemna gibba in a semi-static growth inhibition test with a subsequent recovery period Syngenta Crop Protection AG, Basel, Switzerland IBACON GmbH, Rossdorf, Germany, 105732240 GLP Unpublished Syngenta File No ZA1296_10438	N	SYN
KCP 5.1.2.	Gonsior G.	2017	Mesotrione - Growth inhibition of Myriophyllum spicatum in a water/sediment system Syngenta Crop Protection AG, Basel, Switzerland	N	SYN



<b>Data point</b>	<b>Author(s)</b>	<b>Year</b>	<b>Title Company Report No. Source (where different from company) GLP or GEP status Published or not</b>	<b>Vertebrate study Y/N</b>	<b>Owner</b>
			Eurofins Agrosience Services EcoChem GmbH, N-Osch., Germany, S16-06273 GLP Unpublished Syngenta File No ZA1296_10504		
KCP 5.1.2.	Bachelor B.	2014	Analytical Method Transfer and Partial Validation for the Determination of CA3511 in Dosing Formulations Syngenta Crop Protection AG, Basel, Switzerland Syngenta File No CA3511_50013 Xenometrics, LLC, Stilwell, KS, USA, 11070 GLP Unpublished	N	SYN
KCP 5.1.2.	Faulkner L., Heap C.	2013	CA3511 - Feasibility of the Assay for the Determination of CA3511 in 1 % w/v Aqueous Carboxymethyl-cellulose Syngenta Crop Protection AG, Basel, Switzerland Sequani Limited, Ledbury, United Kingdom, BFI0147 Not GLP not published Syngenta File No CA3511_10006	N	SYN
KCP 5.1.2	Faulkner L., Heap C.	2013a	CA3511 - Validation of the Assay for the Determination of CA3511 in 1 % w/v Aqueous Carboxymethyl-cellulose Syngenta Crop Protection AG, Basel, Switzerland Sequani Limited, Ledbury, United Kingdom, BFI0148 GLP Unpublished Syngenta File No CA3511_10007	N	SYN

<b>Data point</b>	<b>Author(s)</b>	<b>Year</b>	<b>Title Company Report No. Source (where different from company) GLP or GEP status Published or not</b>	<b>Vertebrate study Y/N</b>	<b>Owner</b>
KCP 5.1.2	Faulkner L., Heap C.	2013b	CA3511 - Validation of the Formulation Procedure for CA3511 in 1 % w/v Aqueous Carboxymethylcellulose and Assessment of Formulation Stability Syngenta Crop Protection AG, Basel, Switzerland Sequani Limited, Ledbury, United Kingdom, BFI0149 GLP Unpublished Syngenta File No CA3511_10009	N	SYN
KCP 5.1.2.	Chubb D.	2016	AMBA - Single Dose Oral (Gavage) Proof of Exposure Study in the Rat Syngenta Crop Protection AG, Basel, Switzerland Sequani Limited, Ledbury, United Kingdom, BFI0533 GLP Unpublished Syngenta File No R044276_10012	Y	SYN
KCP 5.2.	Williams R.	2004	Analytical Method 1200-03 for the Determination of Mesotrione and its Metabolites AMBA and MNBA, in Soil, Using Liquid Chromatography - Electrospray Ionization Tandem Mass Spectrometry (Including Validation Data) Syngenta Crop Protection AG, Basel, Switzerland Syngenta Crop Protection, Inc., Greensboro, USA, T001200-03 GLP Unpublished	N	SYN

**List of data submitted by the applicant and not relied on**

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

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

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

Appendix 2 Detailed evaluation of submitted analytical methods

A 2.1 Analytical methods for the Mesotrione

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

A 2.1.1.1 Description of analytical methods for the determination of residues in support of environmental fate studies (KCP 5.1.2.1)

A new study has been conducted and reported below

A 2.1.1.1.1 Analytical method 1200-03

A 2.1.1.1.1.1 Method validation

Comments of zRMS:	According to the applicant information the study ZA1296/1567 was formerly recognized as acceptable. The validation study has been positively evaluated in Part B Section 5 of the Registration Report from 30/04/2020 for to renewal of mesotrione at the EU level of product Calisto 100 SC. The following conclusion was made: „ The analytical method 1200-03 (HPLC-MS/MS) for the determination of mesotrione and its metabolites AMBA and MNBA in soil was validated with regard to specificity, linearity, precision and accuracy according to the guideline SANCO/3029/99 rev. 4, 11/07/2000. The limit of quantification (LOQ) of the analytical method was 2.0 µg test item/kg. The study has been accepted.”
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Reference:	KCP 5.2
Report	Analytical Method 1200-03 for the Determination of mesotrione and its Metabolites AMBA and MNBA, in Soil, Using Liquid Chromatography–Electrospray Ionization Tandem Mass Spectrometry (Including Validation Data). Williams R. 2004 Syngenta File No ZA1296/1567
Guideline(s):	EPA Guideline No. 164-1
Deviations:	None
GLP:	Yes
Acceptability:	

Materials and methods

A. Materials

1. Standards

Reference item: Mesotrione  
CAS No.: 104206-82-8  
Purity: Not stated

Lot/batch No.: Not stated  
Expiry date: Not stated  
Standard for calibration: As above.

Reference item: MNBA  
CAS No.: 110964-79-9  
Purity: Not stated  
Lot/batch No.: Not stated  
Expiry date: Not stated  
Standard for calibration: As above.  
Reference item: AMBA  
CAS No.: 393085-45-5  
Purity: Not stated  
Lot/batch No.: Not stated  
Expiry date: Not stated  
Standard for calibration: As above.

2. Test item Not applicable

3. Test medium: Soil

## **B. Sample preparation and processing**

Soil samples (10 g) are extracted three times by shaking with solvent (once with 0.05M NH<sub>4</sub>OH, once with 50:50 (v/v) 0.05M NH<sub>4</sub>OH: acetone and finally with acetone) at room temperature. The extracts are combined and centrifuged to settle suspended solids. An aliquot of extract is taken and the organic solvent removed by evaporation (N-Evap). The samples are acidified and diluted with formic acid to precipitate soil acids. After centrifugation, an aliquot of the extract is transferred to an LC sample vial. Final determination is by high performance liquid chromatography with tandem mass spectrometric detection (HPLC/MS/MS). External standards in 10:90 (v/v) methanol:ultra-pure water are used for calibration.

Validation of the method was carried out for mesotrione, AMBA and MNBA in soil at fortification levels of 2.0 and 50 ppb (0.002 and 0.05 mg/kg).

## **C. Analytical instrumentation and analysis**

### **1. HPLC parameters - mesotrione**

Instrumentation: Waters Alliance Model 2695  
Column: Polymer Laboratories PLRP-S, (50 x 4.6 mm)  
Column temp.: 30-35 °C  
Mobile phase: A: 0.1% Acetic acid in HPLC grade water  
B: 0.1% Acetic acid in acetonitrile

For AMBA and MNBA

Instrumentation: Waters Alliance Model 2695  
Column: Phenomenex Synergi Fusion-RP (75 x 4.6 mm)  
Column temp.: 30-35 °C  
Mobile phase: A: 0.1% Acetic acid in HPLC grade water  
B: 0.1% Acetic acid in acetonitrile

### **2. MS parameters**

Instrumentation: Micromass Quattro Ultima

Transitions monitored 1: m/z 338.2 → m/z 291.000 (Mesotrione)  
1: m/z 244.0 → m/z 199.800 (MNBA)  
1: m/z 214.0→ m/z 169.900 (AMBA)

D. Calibration

Principle: Six-point linear

Results and discussions

An HPLC-MS/MS method was used to determine concentrations of Mesotrione, AMBA and MNBA.

Table A 1: Procedural recovery data for mesotrione and MNBA Matrix

Matrix	Analyte	Fortification level (mg test item/kg)	Mean recovery (%)	RSD (%)	Comments
Soil	Mesotrione	2.0*	99	1.3	Range 97-100
		50.0	96	3.2	Range 92-98
	AMBA	2.0*	93	4.2	Range 88-97
		50.0	93	1.8	Range 91-94
	MNBA	2.0*	106	1.6	Range 104-108
		50.0	110	1.8	Range 107-112

\*Limit of quantification, defined as the lowest validated fortification level

Table A 2: Characteristics for the analytical method used for validation of mesotrione, AMBA and MNBA residues in Maize whole plant

Mesotrione, AMBA and MNBA	
Calibration (type, number of data points)	<p>A six-point linear calibration curve was used for target ana-lytes quantification and is presented in the study. The equation of the calibration curve is:</p> <p>Mesotrione : <math>y = 2147.83x + 341.076</math> <math>r = 0.9991</math></p> <p>MNBA : <math>y = 7809.13x + 1380.95</math> <math>r = 0.9994</math></p> <p>AMBA : <math>y = 1305.70x + 182.4770</math> <math>r = 0.9996</math></p>
Calibration range	2.0 - 50 µg reference items/L
Limit of quantification	LOQ: 2 µg test items/kg

Conclusion

The method was fully validated according to the requirements of EPA Guideline 164-1. Procedural recoveries are provided in the study summarised above and the original method is therefore acceptable for the determination of mesotrione in soil.

**A 2.1.1.2      Description of analytical methods for the determination of residues in support of efficacy studies**

No new or additional studies have been submitted.

**A 2.1.1.3      Description of analytical methods for the determination of residues in support of residues studies**

No new data have been submitted in the framework of this application.

**A 2.1.1.4      Description of analytical methods for the determination of residues in support of ecotoxicological studies**

New studies have been submitted and are described in detail below.

**A 2.1.1.4.1 Analytical method GRM007.11A**

Comments of zRMS:	According to the applicant information the study S15-02057 was formerly recognized as acceptable. The validation study has been positively evaluated in Part B Section 5 of the Registration Report from 30/04/2020 for to renewal of mesotrione at the EU level of product Calisto 100 SC. The following conclusion was made: <i>„ The analytical method GRM007.11A for the determination of mesotrione and its metabolites MNBA in maize has been demonstrated to be satisfactory in terms of specificity, linearity, precision and accuracy according to the guideline SANCO/3029/99 rev. 4, 11/07/2000.</i> <i>The limit of quantification (LOQ) of the analytical method was 0.01 mg test item/kg.</i> <i>The study has been accepted.”</i>
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Reference:	KCP 5.1.2
Report	Mesotrione – Foliage Decline Study with A12739A on Maize in Northern France and the United Kingdom in 2015. North. L., 2016. Report No. S15-02057
Guideline(s):	SANCO/3029/99 revision 4 (11 Jul 2000)
Deviations:	None
GLP:	Yes
Acceptability:	Yes

**Materials and methods**

**A. Materials**

1. Standards

Reference item: Mesotrione  
CAS No.: 104206-82-8

Purity: 99.5% (wt/wt)  
Lot/batch No.: 492970  
Expiry date: Feb 2016  
Standard for calibration: As above.  
Reference item: MNBA  
CAS No.: 434935-69-0  
Purity: 99.9% (wt/wt)  
Lot/batch No.: 454319  
Expiry date: Feb 2016  
Standard for calibration: As above.

## 2. Test item

Name: Mesotrione wet paste (ZA1296)  
Active ingredient: Mesotrione 99.3 (g/L)  
Batch No.: SAV5A15007  
Expiry data: March 2018

## 3. Test medium: Maize whole plant

### **B. Sample preparation and processing**

Samples are extracted with acetonitrile:water (50:50 v/v) after addition of sodium chloride. Aliquots are diluted with ultra-pure water. The fortified samples were homogenised, diluted with acetonitrile by a factor of two and analysed and concentrated using an Oasis® HLB solid phase extraction (SPE) cartridge on which residues of mesotrione and MNBA are retained. Mesotrione and MNBA are eluted using a solution of methanol containing 2% formic acid. Samples are evaporated under a stream of dry air and dissolved in ultra-pure water:methanol (90:10 v/v). Final determination is by high performance liquid chromatography with tandem mass spectrometric detection (HPLC-MS-MS).

### **C. Analytical instrumentation and analysis**

#### 1. HPLC parameters

Instrumentation: Agilent Series 1200  
Column: Synergi 4µ Polar RP 80A (50 × 20 mm)  
Column temp.: 20 °C  
Mobile phase: A: 0.1% Formic acid in ultra pure water  
B: 0.1% Formic acid in acetonitrile

#### 2. MS parameters

Instrumentation: API 5500 Mass spectrometer  
Transitions monitored 1: m/z 338.1 → m/z 291.000 (Mesotrione)  
1: m/z 224.0 → m/z 141.900 (MNBA)

### **D. Calibration**

Principle: Eight-point linear

### **Results and discussions**

An HPLC-MS/MS method was used to determine concentrations of Mesotrione and MNBA. The analytical method in support of this ecotoxicological study is GRM007.11.A. The method was already evaluated by the authority and published in the RAR UK, 2015 ; therefore in the study itself, only procedural recoveries and calibration data are provided and reference to the original method validation is made : Watson G, Crook S (2013). « Mesotrione - Analytical Method (GRM007.11A) for the Determination of Residues of Meso-trione and 4-(Methylsulfonyl)-2-Nitrobenzoic Acid (MNBA) in Crop Matrices by LC-MS/MS. Syngenta Method GRM007.11A ». The detector



response was linear within the range 0.003 – 0.02 µg/mL for both active substance and metabolite . The LOQ for the method was 0.01 mg test item/kg. Well-labelled chro-matograms are provided in the original study report.

**Table A 3: Procedural recovery data for mesotrione and MNBA**

Matrix	Analyte	Fortification level (mg test item/kg)	Mean recovery (%)	RSD (%)	Comments
Maize whole plant	Mesotrione	0.01(n=4)	81	11	-
		10 (n=3)			-
		15 (n=1)			-
Maize whole plants	MNBA	0.01 (n = 3)	78	11	
		10(n=2)			
		15(n=1)			

**Table A 4: Characteristics for the analytical method used for validation of mesotrione and MNBA residues in Maize whole plant**

Maize whole plant Mesotrione and MNBA	
Calibration (type, number of data points)	A eight-point linear calibration curve was used for target an-alytes quantification and is presented in the study. The equation of the calibration curve is: Mesotrione : $y = 360213682 x - 15714$ $r = 0.9961$ MNBA : $y = 40358946 x + 1139$ $r = 0.9999$
Calibration range	0.003 – 0.0200 µg reference items/mL
Limit of quantification	LOQ: 0.01 mg test items/kg

**Conclusion**

The method GRM007.11.A was fully validated according to the requirements of to SANCO/3029/99 rev. 4 in the original study Watson G, Crook S (2013). Procedural recoveries are provided in the study summarised above and the original method is therefore acceptable for the determination of mesotrione in plant matrices.

**A 2.1.1.4.2 Analytical method 105731240A**

Comments of zRMS:	According to the applicant information the study 105731240 was formerly recognized as acceptable. The validation study has been positively evaluated in Part B Section 5 of the Registration Report from 30/04/2020 for to renewal of mesotrione at the EU level of product Calisto 100 SC. The following conclusion was made: „ The analytical method 105731240A for the determination of mesotrione in test water supplemented with AAP medium was successfully validated according to SANCO/3029/99 rev. 4. The limit of quantification (LOQ) of the analytical method was 1 µg test item/L. The method is highly specific with two mass transitions monitored per analysis. The study has been accepted..”
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Reference:	KCP 5.1.2
Report	Mesotrione wet paste (ZA1296) - Toxicity to aquatic plant Lemna gibba in a reciprocal growth inhibition test. Hengsberger, A & Wydra, V. 2015. Report No. 105731240
Guideline(s):	SANCO/3029/99 rev. 4
Deviations:	No
GLP:	Yes
Acceptability:	

#### **A 2.1.1.4.2.1 Method validation**

##### **Materials and methods**

##### **A. Materials**

###### 1. Standards

Reference item: Mesotrione  
CAS No.: 104206-82-8  
Purity: 99.5% ± 0.5% (wt/wt)  
Lot/batch No.: 492970  
Expiry date: Recertification Feb 2016  
Standard for calibration: As above.

###### 2. Test item

Name: Mesotrione wet paste (ZA1296)  
Active ingredient: Mesotrione 86.1% (wt/wt)  
Batch No.: 631795 (SMO7F333)  
Expiry data: Recertification Feb 2016

###### 3. Test medium: Water (containing AAP-growth medium)

##### **B. Sample preparation and processing**

Stock solutions were prepared by diluting the test item in acetonitrile. Fortified samples were prepared by further diluting the stock solutions with the test medium to provide fortified samples at 1, 5 and 75 µg test item/L. The fortified samples were homogenised, diluted with acetonitrile by a factor of two and analysed by HPLC-MS/MS using the parameters detailed below.

##### **C. Analytical instrumentation and analysis**

###### 1. HPLC parameters

Instrumentation: Agilent Series 1200  
Column: Synergi 4µ Polar RP 80A (150 × 3 mm)  
Column temp.: 20 °C  
Mobile phase: A: 40% HPLC water containing 5mM ammonium acetate

B: 60% Methanol containing 5mM ammonium acetate

2. MS parameters

Instrumentation: API 4000 Mass spectrometer  
Interface: ESI  
Source polarity: positive  
Spray voltage: 4000 V  
Transitions monitored 1: m/z 357.071 → m/z 228.000 (quantification)  
2: m/z 357.071 → m/z 104.000 (confirmation)

**D. Calibration**

Principle: Seven-point linear

**Results and discussions**

An HPLC-MS/MS method was used to determine concentrations of Mesotrione in water supplemented with AAP-growth medium and validated according to SANCO/3029/99 rev. 4. The detector response was linear within the range 0.25 – 40 µg mesotrione/L. The method is highly specific with two mass transitions monitored per analysis. No interference was observed at the retention time of the target analyte. Target analyte concentrations in controls (blanks) were < 30% of the LOQ. The LOQ for the method was 1 µg test item/L. All recovery data meet the requirements of SANCO/3029/99 rev. 4 and are provided along with other validation data in the tables below. Well-labelled chromatograms are provided in the original study report.

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

Matrix	Analyte	Fortification level (µg test item/L)	Mean recovery (%)	RSD (%)	Comments
Water	Mesotrione	1 (n = 5)	107	5	-
		5 (n = 5)	114	2	-
		75 (n = 5)	105	2	-

**Table A 6: Characteristics for the analytical method used for validation of mesotrione residues in water supplemented with AAP -medium**

Mesotrione	
Specificity	Two mass transitions were monitored during each analysis. No interference observed in controls Concentration of mesotrione in controls (blanks) was < 30% LOQ.
Calibration (type, number of data points)	A seven-point linear calibration curve was used for target analyte quantification and is presented in the study. The equation of the calibration curve is: $y = 2597x - 474$ $r = > 0.999$
Calibration range	0.25 – 40 µg reference item/L
Limit of quantification Limit of detection	LOQ: 1 µg test item/L LOD: 0.15 µg test item/L

**Conclusion**

The method detailed above was fully validated according to the requirements of SANCO/3029/99 rev. 4. This method is therefore acceptable for the determination of mesotrione in water supplemented with AAP -medium.

#### A 2.1.1.4.3 Analytical method 105731240A

##### A 2.1.1.4.3.1 Method validation

Comments of zRMS:	<p>According to the applicant information the study 105732240 was formerly recognized as acceptable.</p> <p>The validation study has been positively evaluated in Part B Section 5 of the Registration Report from 30/04/2020 for to renewal of mesotrione at the EU level of product Calisto 100 SC.</p> <p>The following conclusion was made:</p> <p><i>„The analytical method 105732240A for the determination of mesotrione in test water supplemented with AAP medium was successfully validated according to SANCO/3029/99 rev. 4.</i></p> <p><i>The limit of quantification (LOQ) of the analytical method was 1 µg test item/L.</i></p> <p><i>The study has been accepted.”</i></p>
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In the following study the method validated above (105731240A) was used for analysis of Mesotrione in water.

Reference:	KCP 5.1.2
Report	Mesotrione wet paste (ZA1296) - Toxicity to aquatic plant Lemna gibba in a semi-static growth inhibition test with subsequent recovery period. Final report Amendment 2. Kosak, L & Wydra, V. 2016. Report No. 105732240
Guideline(s):	SANCO/3029/99 rev. 4
Deviations:	No
GLP:	Yes
Acceptability:	

#### Materials and methods

##### A. Materials

###### 1. Standards

Reference item: Mesotrione

CAS No.: 104206-82-8

Purity: 99.5% ± 0.5% (wt/wt)

Lot/batch No.: 492970

Expiry date: Recertification Feb 2016

Standard for calibration: As above.

###### 2. Test item

Name: Mesotrione wet paste (ZA1296)

Active ingredient: Mesotrione 86.1% (wt/wt)

Batch No.: 631795 (SMO7F333)

Expiry data: Recertification Feb 2016

###### 3. Test medium: Water (containing AAP-growth medium)

**B. Sample preparation and processing**

Stock solutions were prepared by diluting the test item in acetonitrile. Fortified samples were prepared by further diluting the stock solutions with the test medium to provide fortified samples at 1, 5 and 75µg test item/L. The fortified samples were homogenised, diluted with acetonitrile by a factor of two and analysed by HPLC-MS/MS using the parameters detailed below.

**C. Analytical instrumentation and analysis**

1. HPLC parameters

Instrumentation: Agilent Series 1200  
Column: Synergi 4µ Polar RP 80A (150 × 3 mm)  
Column temp.: 20 °C  
Mobile phase: A: 40% HPLC water containing 5mM ammonium acetate  
B: 60% Methanol containing 5mM ammonium acetate

2. MS parameters

Instrumentation: API 4000 Mass spectrometer  
Interface: ESI  
Source polarity: positive  
Spray voltage: 4000 V  
Transitions monitored 1: m/z 357.071 → m/z 228.000 (quantification)

**D. Calibration**

Principle: Nine-point linear

**Results and discussions**

An HPLC-MS/MS method was used to determine concentrations of Mesotrione in water supplemented with AAP-growth medium and validated according to SANCO/3029/99 rev. 4. The detector response was linear within the range 0.25 – 40 µg mesotrione/L. No interference was observed at the retention time of the target analyte. Target analyte concentrations in controls (blanks) were < 30% of the LOQ. The LOQ for the method was 1 µg test item/L. All recovery data meet the requirements of SANCO/3029/99 rev. 4 and are provided along with other validation data in the tables below. Well-labelled chromatograms are provided in the original study report.

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

Matrix	Analyte	Fortification level (µg test item/L)	Mean recovery (%)	RSD (%)	Comments
Water	Mesotrione	1 (n = 5)	107	5	-
		5 (n = 5)	105	6	-
		75 (n = 5)	104	5	-

**Table A 8: Characteristics for the analytical method used for validation of mesotrione residues in water supplemented with AAP -medium**

Mesotrione	
Specificity	Only one mass transition was measured in this report, but confirmation was done under: 105731240A No interference observed in controls Concentration of mesotrione in controls (blanks) was < 30% LOQ.
Calibration (type, number of data points)	A nine-point linear calibration curve was used for target an-alyte

	quantification and is presented in the study. The equation of the calibration curve is: $y = 2450x + 109$ $r = 1.00$
Calibration range	0.25 – 40 µg reference item/L
Limit of quantification Limit of detection	LOQ: 1 µg test item/L LOD: 0.15 µg test item/L

**Conclusion**

The method detailed above was fully validated according to the requirements of to SANCO/3029/99 rev. 4. This method is therefore acceptable for the determination of mesotrione in water supplemented with AAP -medium.

**A 2.1.1.4.4 Analytical method S16-06273**

**A 2.1.1.4.5 Method validation**

Comments of zRMS:	According to the applicant information the study S16-06273 was formerly recognized as acceptable. The validation study has been positively evaluated in Part B Section 5 of the Registration Report from 30/04/2020 for to renewal of mesotrione at the EU level of product Calisto 100 SC. The following conclusion was made: „ The analytical method S16-06273 for the determination of mesotrione in water supplemented with modified Andrews solution was successfully validated according to SANCO/3029/99 rev. 4. The limit of quantification (LOQ) of the analytical method was 0.4 µg test item/L. The study has been accepted. ”
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Reference:	KCP 5.2
Report	Mesotrione - Growth inhibition of Myrophyllyum spicatum in a water/sedi-ment system: Final Report Amendment 1. Gonsior, G. 2017. Report No. S16-06273
Guideline(s):	SANCO/3029/99 rev. 4
Deviations:	None
GLP:	Yes
Acceptability:	

**Materials and methods**

**A. Materials**

1. Standards

Reference item: Mesotrione technical  
CAS No.: 104206-82-8  
Purity: 84.6% (wt/wt)  
Lot/batch No.: 675385  
Expiry date: Recertification Feb 2019  
Standard for calibration: As above.

2. Test item As above

3. Test medium: Water supplemented with modified Andrews solution

**B. Sample preparation and processing**

Stock solutions were prepared by diluting the test item in methanol. Validation samples were prepared from diluted stock solutions to provide fortified samples at 0.4 and 800 µg test item/L and frozen below -18 °C until required for analysis. Frozen fortified (10 ml) samples were thawed, 10 ml acetonitrile were added to each sample and the samples were vortexed. If necessary, samples were further diluted with acetonitrile/water (1:1, v/v) and analysed by HPLC-MS/MS using the parameters detailed below.

**C. Analytical instrumentation and analysis**

1. HPLC parameters

Instrumentation: Shimadzu LC-30 AD

Column: Phenomenex Luna 5µ phenyl-hexyl, 150 mm × 2 mm i.d., 5 µm mean particle size and fitted with a 4 mm guard column.

Column temp.: 30 °C

Mobile phase: A: Water + 0.5% formic acid

B: Methanol + 0.5% formic acid

2. MS parameters

Instrumentation: Applied Biosystems API 5500 Mass spectrometer

Interface: ESI

Source polarity: negative

Spray voltage: -3500 V

Transitions monitored 1: m/z 337.845 → m/z 291.0 (quantification)

2: m/z 337.8451 → m/z 212.00 (confirmation)

**D. Calibration**

Principle: Eight-point curvilinear

**Results and discussions**

An HPLC-MS/MS method was used to determine concentrations of Mesotrione in water supplemented with modified Andrews solution and validated according to SANCO/3029/99 rev. 4. The method is highly specific with two mass transitions monitored per analysis. Residues were not detectable in untreated test medium controls (n = 2). The LOQ for the method was 0.4 µg test item/L. All recovery data meet the requirements of SANCO/3029/99 rev. 4 and are provided along with other validation data in the tables below. Well-labelled chromatograms are provided in the original study report.

**Table A 9: Recovery results from method validation of mesotrione using the analytical method**

Matrix	Analyte	Fortification level (µg/L)	Mean recovery (%)	RSD (%)	Comments
Water	Mesotrione	0.4 (n = 5)	93	3	-
		800(n = 5)	94	2	-

**Table A 10: Characteristics for the analytical method used for validation of mesotrione residues in water supplemented with modified Andrews solution**

Mesotrione	
Specificity	Two mass transitions were monitored during each analysis. No interference observed in controls Concentration of mesotrione in controls (blanks) was < 30% LOQ.
Calibration (type, number of data points)	An eight-point curvilinear calibration curve was used for target analyte quantification and is presented in the study. The equation of the calibration curve is: $y = y = -9.36e+004x^2 + 2.32e+006x + 3.74e+004$ $r = > 0.999$
Calibration range	0.05 – 10 ng mesotrione/L
Limit of quantification	LOQ: 0.4 µg test item/L

**Conclusion**

The method detailed above was fully validated according to the requirements of to SANCO/3029/99 rev. 4. This method is therefore acceptable for the determination of mesotrione in water supplemented with modified Andrews solution.

**A 2.1.1.5 Description of analytical methods for the determination of residues in support of physical and chemical properties tests (KCP 5.1.2.7)**

No new or additional studies have been submitted. Methods for post-authorization control and monitoring purposes (KCP 5.2)

**A 2.1.1.6 Description of analytical methods for the determination of residues in plant matrices (KCP 5.2.1)**

No new data have been submitted in the framework of this application.

**A 2.1.1.7 Description of analytical methods for the determination of residues in support of toxicological studies**

**A 2.1.1.7.1 Analytical method BFI0148**

**A 2.1.1.7.1.1 Method validation**

Comments of zRMS:	According to the applicant information the study 11070 was formerly recognized as acceptable. The validation study has been positively evaluated in Part B Section 5 of the Registration Report from 30/04/2020 for to renewal of mesotrione at the EU level of product Calisto 100 SC. The following conclusion was made: <i>„The analytical method HPLC-UV for the determination of 2-nitro-4-methylsulfonylbenzoic acid (MNBA) in aqueous carboxymethylcellulose was successfully validated according to SANCO/3029/99 rev. 4. The limit of quantification (LOQ) of the analytical method was 1 mg/mL. The study has been accepted.”</i>
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Reference:	KCP 5.1.2
Report	2-Nitro-4-Methylsulfonyl benzoic acid (CA3511) analytical method transfer and partial validation for the determination of CA3511 in dosing formulations. Bachelor, B., 2014. Study No.: 11070.
Guideline(s):	None stated in report
Deviations:	None
GLP:	Yes
Acceptability:	

## Materials and methods

### A. Materials

#### 1. Standards

Reference item: 2-nitro-4-methylsulfonylbenzoic acid (CA3511)

Synonym: MNBA

CAS No.: 110964-79-9

Purity: 99.8%

Lot/batch No.: SM03C0689

Expiry date: Recertification Date: May 2016

Standard for calibration: As above.

#### 2. Test item: 2-nitro-4-methylsulfonylbenzoic acid (CA3511)

Synonym: MNBA

#### 3. Test medium: Aqueous carboxymethylcellulose

### B. Sample preparation and processing

A solution of acetonitrile (ACN):Purified Water (50:50, v/v) was prepared for the partial method validation. Stock solutions of the test substance, were prepared at a concentration of 500 µg/ml of test item in acetoni-trile and then further diluted (with vehicle and diluent) to provide three fortification levels at 1.0, 35.0 and 120 mg/mL. Aliquots of the samples were individually transferred to separate HPLC vials and analysed using the instrumentation detailed below.

### C. Analytical instrumentation and analysis

#### 1. HPLC parameters

Instrumentation Agilent 1220 Infinity Gradient Compact LC

Column: Waters XBridge C8 (100 mm x 4.6 mm I.D., 5 µm particle size)

Mobile phase: A: Purified Water:Trifluoroacetic Acid (100:0.2, v/v)

B: Acetonitrile:Trifluoroacetic Acid (100:0.2, v/v)

#### 2. UV parameters

Detector UV fixed wavelength

Detector wave length: 270 nm

### D. Calibration

Principle: No information provided

Results and discussions

An HPLC-UV method was used to determine concentrations of MNBA (2-nitro-4-methylsulfonylbenzoic acid) in aqueous carboxymethylcellulose. The LOQ of the method is 1 mg mesotrione/mL. Mean recoveries and associated RSD's meet the requirements of SANCO/3029/99 rev. 4 and are provided along with other validation data in the tables below. Well-labelled chromatograms are provided in the original study report

Table A 11: Recovery results from method validation of MNBA using the analytical method

Matrix	Analyte	Fortification level (mg/ml)	Mean recovery (%)	RSD (%)	Comments
Aqueous carboxymethylcellulose	MNBA	1.00 (n=6)	102.0	0.352	-
		35.0 (n=6)	98.6	1.99	-
		120 (n=6)	108.8	0.984	

Table A 12 Recovery results from method validation of MNBA using the analytical method

2-nitro-4-methylsulfonylbenzoic acid	
Specificity	Analyte was not detected in any controls. (n=2)
Calibration (type, number of data points)	No information provided
Calibration range	No information provided
Limit of quantification (LOQ)	1 mg mesotrione/mL

Conclusion

The method detailed above was used to determine concentrations of MNBA in aqueous carboxymethyl-cellulose in support of a toxicological study. Mean recoveries and associated RSD's meet the requirements of SANCO/3029/99 rev. 4. The method used well-established analytical systems and techniques and should therefore be considered suitable for the determination of MNBA in aqueous carboxymethylcellulose.

A 2.1.1.7.2 Analytical method BFI0147

A 2.1.1.7.2.1 Method validation

A 2.1.1.7.3 Analytical method BFI0147

Comments of zRMS:	According to the applicant information the study BFI0147 was formerly recognized as acceptable. The validation study has been positively evaluated in Part B Section 5 of the Registration Report from 30/04/2020 for to renewal of mesotrione at the EU level of product Calisto 100 SC. The following conclusion was made: „ The analytical method HPLC-UV (BFI0147) for the determination of 2-nitro-4-methylsulfonyl benzoicacid (MNBA) in 1 % w/v aqueous carboxymethylcellulose is acceptable. Mean recoveries and associated RSD's meet the requirements of SANCO/3029/99 rev. 4 The limit of quantification (LOQ) of the analytical method was 1 mg/mL..”
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Reference:	
Report	CA3511 - Feasibility of the Assay for the Determination of CA3511 in 1 % w/v Aqueous Carboxymethylcellulose. Faulkner, L., Heap, C., 2013. Project No.: BFI0147.
Guideline(s):	None stated in report
Deviations:	None stated in report
GLP:	No
Acceptability:	

**Materials and methods**

**A. Materials**

1. Standards

Reference item: 2-nitro-4-methylsulfonylbenzoic acid (CA3511)  
Synonym: MNBA  
CAS No.: 110964-79-9  
Purity: 99.8  
Lot/batch No.: SMO3C0689  
Expiry date: End of May 2016  
Standard for calibration: As above.

2. Test item: 2-nitro-4-methylsulfonylbenzoic acid (CA3511)  
Synonym: MNBA

3. Test medium: Aqueous carboxymethylcellulose

**B. Sample preparation and processing**

Fortified samples were prepared at concentrations of 1 mg/mL, 10 mg/mL and 100 mg/mL CA3511 in vehicle. Samples were individually transferred to separate HPLC vials and analysed using the instrumentation below.

**C. Analytical instrumentation and analysis**

1. HPLC parameters

Instrumentation Sartorius HPLC  
Column: Hichrom HiRPB (150 mm x 4.6 mm I.D., 5 µm particle size)  
Mobile phase: 200 mL of acetonitrile and 800 mL of UHP water with 2 mL trifluoroacetic acid

2. UV parameters

Detector Sartorius UV-detector  
Detector wave length: 270 nm

**D. Calibration**

Principle: Six-poin linear

**Results and discussions**

An HPLC-UV method was used to determine concentrations of MNBA (2-nitro-4-methylsulfonylbenzoic acid) in aqueous carboxymethylcellulose. The LOQ of the method is 1 mg mesotrione/mL. Mean recoveries and associated RSD's meet the requirements of SANCO/3029/99 rev. 4 and are provided along with other validation data in the tables below. Chromatograms are provided in the original study report.

Table A 13: Recovery results from method validation of MNBA using the analytical method

Matrix	Analyte	Fortification level (mg/ml)	Mean recovery (%)	RSD (%)	Comments
Aqueous carboxymethylcellulose	MNBA	1.00 (n=3)	91	1.2	-
		10 (n=3)	100	7.7	-
		100(n=3)	100	1.6	

Table A 14: Characteristics for the analytical method used for validation of MNBA residues in water

2-nitro-4-methylsulfonylbenzoic acid	
Specificity	No co-chromatographic peaks, with the same retention time as CA3511, were detected in the diluted control samples or reagent blanks.
Calibration (type, number of data points)	Six-point linear used for target analyte quantification and is presented in the study. Individual calibration data are provided. The equation of the calibration curve is: $y = 3.935417e+003 - 2.876764e+003$ ; $r^2 = > 0.995$
Calibration range	5.465 - 15.71 µg/mL
Limit of quantification (LOQ)	1 mg/mL

Conclusion

The method detailed above was used to determine concentrations of MNBA in aqueous carboxymethyl-cellulose in support of a toxicological study. Mean recoveries and associated RSD's meet the requirements of SANCO/3029/99 rev. 4. The method used well-established analytical systems and techniques and should therefore be considered suitable for the determination of MNBA in aqueous carboxymethylcellulose.

A 2.1.1.7.4 Analytical method BFI0148

A 2.1.1.7.4.1 Method validation

Comments of zRMS:	According to the applicant information the study BFI0148 was formerly recognized as acceptable. The validation study has been positively evaluated in Part B Section 5 of the Registration Report from 30/04/2020 for to renewal of mesotrione at the EU level of product Calisto 100 SC. The following conclusion was made: <i>„The analytical method HPLC-UV (BFI0148) for the determination of 2-nitro-4-methylsulfonylbenzoic acid (MNBA) in 1 % w/v aqueous carboxymethylcellulose is acceptable. Mean recoveries and associated RSD's meet the requirements of SANCO/3029/99 rev. 4</i> <i>The limit of quantification (LOQ) of the analytical method was 1 mg/mL.”</i>
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Reference:	KCP 5.1.2
Report	CA3511 - Validation of the Assay for the Determination of CA3511 in 1 % w/v Aqueous Carboxymethylcellulose - Method Validation. Faulkner, L., Heap, C., 2013a. Project No.: BFI0148.
Guideline(s):	Not given
Deviations:	None reported
GLP:	Yes
Acceptability:	

**Materials and methods**

**A. Materials**

1. Standards

Reference item: 2-nitro-4-methylsulfonylbenzoic acid (CA3511)  
Synonym: MNBA  
CAS No.: 110964-79-9  
Purity: 99.8  
Lot/batch No.: SMO3C0689  
Expiry date: Re-certification date: End of May 2016

Standard for calibration: As above

2. Test item: 2-nitro-4-methylsulfonylbenzoic acid (CA3511)

Synonym: MNBA

3. Test medium: Aqueous carboxymethylcellulose

**B. Sample preparation and processing**

Fortified samples were prepared at concentrations of 1 mg/mL, 10 mg/mL and 100 mg/mL CA3511 in vehicle. Samples were individually transferred to separate HPLC vials and analysed using the instrumentation below.

**C. Analytical instrumentation and analysis**

1. HPLC parameters

Instrumentation Sartorius HPLC  
Column: Hichrom HiRPB (150 mm x 4.6 mm I.D., 5 µm particle size)  
Mobile phase: 200 mL of acetonitrile and 800 mL of UHP water with 2 mL trifluoroacetic acid

2. UV parameters

Detector Sartorius UV-detector  
Detector wave length: 270 nm

**D. Calibration**

Principle: Six-point linear

**Results and discussions**

An HPLC-UV method was used to determine concentrations of MNBA (2-nitro-4-methylsulfonylbenzoic acid) in aqueous carboxymethylcellulose. The LOQ of the method is 1 mg mestrone/mL. Mean recoveries and associated

RSD’s meet the requirements of SANCO/3029/99 rev. 4 and are provided along with other validation data in the tables below. Chromatograms are provided in the original study report.

Table A 15: Recovery results from method validation of MNBA using the analytical method

Matrix	Analyte	Fortification level (mg/ml)	Mean recovery (%)	RSD (%)	Comments
Aqueous carboxymethylcellulose	MNBA	1.00 (n=6)	100	0.5	-
		10(n=6)	97	0.7	-
		100(n=6)	97	0.5	

Table A 16: Characteristics for the analytical method used for validation of 2-nitro-4-methyl-sulfonylbenzoic acid residues in water

2-nitro-4-methylsulfonylbenzoic acid	
Specificity	No co-chromatographic peaks, with the same retention time as CA3511, were detected in the diluted control samples or reagent blanks.
Calibration (type, number of data points)	Six-point linear used for target analyte quantification and is presented in the study. Individual calibration data are provided. The equation of the calibration curve is: $y = 7.678763e+003 - 2.326498e+003;$ $r^2 = > 0.999$
Calibration range	5.190 - 15.83 µg/mL
Limit of quantification (LOQ)	1 mg/mL

Conclusion

The method detailed above was used to determine concentrations of MNBA in aqueous carboxymethyl-cellulose in support of a toxicological study. Mean recoveries and associated RSD’s meet the requirements of SANCO/3029/99 rev. 4. The method used well-established analytical systems and techniques and should therefore be considered suitable for the determination of MNBA in aqueous carboxymethylcellulose.

A 2.1.1.7.5 Analytical method BFI0148

A 2.1.1.7.5.1 Method validation

Comments of zRMS:	According to the applicant information the study BFI0149 was formerly recognized as acceptable. The validation study has been positively evaluated in Part B Section 5 of the Registration Report from 30/04/2020 for to renewal of mesotrione at the EU level of product Calisto 100 SC. The following conclusion was made: „ The analytical method HPLC-UV (BFI0148) for the determination of 2-nitro-4-methylsulfonylbenzoic acid (MNBA) in aqueous carboxymethylcellulose was validated according to SANCO/3029/99 rev. 4. The limit of quantification (LOQ) of the analytical method was 1 mg/mL. The study has been accepted.”
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Reference:	KCP 5.1.2
Report	CA3511 - Validation of the Formulation Procedure for CA3511 in 1 % w/v Aqueous Carboxymethylcellulose and Assessment of Formulation Stability - Method Validation. Faulkner, L., Heap, C., 2013b. Project No.: BFI0149.
Guideline(s):	Not given
Deviations:	None reported
GLP:	Yes
Acceptability:	

## Materials and methods

### A. Materials

#### 1. Standards

Reference item: 2-nitro-4-methylsulfonylbenzoic acid (CA3511)

Synonym: MNBA

CAS No.: Not stated

Purity: 99.8

Lot/batch No.: SMO3C0689

Expiry date: Re-certification date: End of May 2016

Standard for calibration: As above.

#### 2. Test item: 2-nitro-4-methylsulfonylbenzoic acid (CA3511)

Synonym: MNBA

#### 3. Test medium: Aqueous carboxymethylcellulose

### B. Sample preparation and processing

Triplicate samples were taken from the top, middle and bottom of each formulation, diluted to approximately 10 µg/mL with acetonitrile, and analysed using reversed phase HPLC with UV detection as detailed below.

### C. Analytical instrumentation and analysis

#### 1. HPLC parameters

Instrumentation Sartorius HPLC

Column: Hichrom HiRPB (150 mm x 4.6 mm I.D., 5 µm particle size)

Mobile phase: 200 mL of acetonitrile and 800 mL of UHP water with 2 mL trifluoroacetic acid

#### 2. UV parameters

Detector Sartorius UV-detector

Detector wave length: 270 nm

### D. Calibration

Principle: No further information provided

## Results and discussions

An HPLC-UV method was used to determine concentrations of MNBA (2-nitro-4-methylsulfonylbenzoic acid) in aqueous carboxymethylcellulose. The LOQ was found at 1 mg/mL. Mean recoveries and associated RSD's meet the requirements of SANCO/3029/99 rev. 4 and are provided along with other validation data in the tables below. Chromatograms are provided in the original study report

Table A 17: Recovery results from method validation of MNBA using the analytical method

Matrix	Analyte	Fortification level (mg /ml) nominal	Mean recovery (%)	RSD (%)	Comments
Aqueous carboxymethylcellulose	MNBA	1(n=9)	103	1.7	-
		10(n=9)	93	2.2	-
		100(n=9)	95	4.5	

Table A 18: Characteristics for the analytical method used for validation of 2-nitro-4-methyl-sulfonylbenzoic acid residues in water

2-nitro-4-methylsulfonylbenzoic acid	
Specificity	No information provided
Calibration (type, number of data points)	No information provided
Calibration range	No information provided
Limit of quantification (LOQ)	1 mg/mL

Conclusion

The method detailed above was used to determine concentrations of MNBA in aqueous carboxymethyl-cellulose in support of a toxicological study. Mean recoveries and associated RSD's meet the requirements of SANCO/3029/99 rev. 4. The method used well-established analytical systems and techniques and should therefore be considered suitable for the determination of MNBA in aqueous carboxymethylcellulose.

A 2.1.1.7.6 Analytical method BFI068MS and BFI074MS

A 2.1.1.7.6.1 Method validation

Comments of zRMS:	According to the applicant information the study BFI0533 was formerly recognized as acceptable. The validation study has been positively evaluated in Part B Section 5 of the Registration Report from 30/04/2020 for to renewal of mesotrione at the EU level of product Calisto 100 SC. The following conclusion was made: <i>„The analytical method HPLC-MS/MS for the determination of 2-amino-4-methylsulfonylbenzoic acid (AMBA) in blood and plasma of rats samples is considered acceptable with LOQ at 10 ng/mL.”</i>
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Reference:	KCP 5.1.2
Report	AMBA - Single dose oral (gavage) proof of exposure study in the rat. Chubb, D., 2016. Project No.: BFI0533.



Guideline(s):	Not given
Deviations:	None reported
GLP:	Yes
Acceptability:	

Reference:	KCP 5.1.2
Report	AMBA: Validation of a Bioanalytical Method for the Determination of AMBA in Rat Blood: Water [1:1 (v/v)] Method Validation Kosar F (2016) Project No BFI0495 (Syngenta File no R044276_10013)
Guideline(s):	Not given
Deviations:	None reported
GLP:	Yes
Acceptability:	

Reference:	KCP 5.1.2
Report	AMBA: Validation of a Bioanalytical Method for the Determination of AMBA in Rat Plasma Method Validation Kosar F (2016) Project No BFI0535 (Syngenta File no R044276_10014)
Guideline(s):	Not given
Deviations:	None reported
GLP:	Yes
Acceptability:	

## Materials and methods

### A. Materials

#### 1. Standards

Reference item: 2-amino-4-methylsulfonylbenzoic acid

Synonym: AMBA

Purity: 98.6%

Lot/batch No.: 924777

Expiry date: Re-certification date: End of March 2017

Standard for calibration: As above.

Internal standard: Tolbutamide

Purity: 100%

Lot/batch No.: SLBN2252V

Expiry date: 21 April 2020

#### 2. Test item: 2-amino-4-methylsulfonylbenzoic acid (CA3511)

Synonym: AMBA

3. Test medium: blood and plasma of rats

**B. Sample preparation and processing**

The test item was formulated on the day of dosing as a suspension in the vehicle, 1.0 % (w/v) carboxymethylcellulose with 0.1 % (v/v) Tween 80. Three male rats of the Crl:WI(Han) strain were allocated to the study and dosed once with 2000 mg/kg AMBA, by gavage, at a dose volume of 10 mL/kg body weight. Blood samples were taken on Day 1 at 1, 4 and 24 hours after dosing for proof of exposure.

Samples (Blood/Plasma) were diluted with water (1:1) and individually transferred to separate HPLC vials and analysed using the instrumentation below

**C. Analytical instrumentation and analysis**

1. HPLC parameters

Instrumentation Agilent HPLC pump  
Column: ACE Phenyl (50 mm x 2.1 mm I.D., 5 µm particle size)  
Mobile phase: A: 0.01 % Formic acid  
B: Methanol

2. MS parameters

Instrumentation: AB Sciex API4000 mass spectrometer  
Ionisation mode: Electro spray  
Heater gas Temp.: 500°C  
Transitions monitored AMBA: m/z 214 → m/z 470  
AMBA: m/z 214 → m/z 155  
AMBA: m/z 214 → m/z 79  
Tolbutamide: m/z 269 → m/z 170

**D. Calibration**

Principle: Eight-point

**Results and discussions**

An HPLC-MS/MS method was used to determine concentrations of AMBA (2-amino-4-methylsulfonylbenzoic acid) in blood and plasma of rats.

**Table A 19: Recovery results from method validation of AMBA using the analytical method**

Matrix	Analyte	Fortification level (mg/mL)	Mean recovery (%)	RSD (%)	Comments
Blood	MNBA	1(n=9)	103	1.7	-
		10(n=9)	93	2.2	-
Plasma	AMBA	100(n=9)	95	4.5	

**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 animal matrices (KCP 5.2)**

No new or additional studies have been submitted

**A 2.1.2.2 Description of Methods for the Analysis of Soil (KCP 5.2)**

No new or additional studies have been submitted

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

**A 2.1.2.5 Description of Methods for the Analysis of Body Fluids and Tissues (KCP 5.2)**

No new or additional studies have been submitted

**A 2.1.2.6 Other Studies/ Information (KCP 5.2)**

**A 2.1.2.6.1 Method of validation 1**

**A 2.1.2.6.1.1 Method validation: HPLC with UV-DAD**

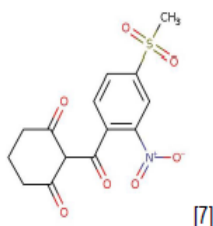
Comments of zRMS:	<p>The analytical method code: 0038/0177/FA was fully validated in term of specificity, linearity, repeatability, accuracy and LOQ according to SANTE/2020/12830, rev.2. The limit of detection (LOD) equals: 0.127 mg/L (which corresponds to 0.06 mg/kg of 50% (w/v) sucrose solution) and limit of quantification (LOQ) equals: 0.498 mg/L (which corresponds to 0.23 mg/kg of 50% (w/v) sucrose solution).</p> <p>The method linearity was evaluated at 5 levels.</p> <p>The results of analytical method validation confirm that this method is suitable for analysis the content of the active substance (mesotrione) of the test item in 50% (w/v) sucrose solution.</p> <p>The method is successfully validated and accepted.</p>
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Reference:	KCP 5.2
Report	Validation of analytical method for determination of active substance (mesotrione) of the test item MEZI 100 SC in 50% (w/v) sucrose solution <u>Zaworska K., 2023</u> Ciorga B.,2023, Study code: 0038/0177/FA
Guideline(s):	SANTE/2020/12830 rev.2
Deviations:	Yes Deviation from the Study plan was found 1. Both the starting and completion dates were shifted to October from the initial term which was set for September in Study plan.  The deviation did not affect the reliability of the study results. No deviations from the SANTE/2020/12830 rev.2 guideline were found.
GLP:	Yes
Acceptability:	

## Materials and methods

### • Material

Name	MEZI 100 SC
Type of packaging material	HDPE
Date of delivery	08.09.2023
Batch No.	CHR/12
Name of active substance	Mesotrione
IUPAC nomenclature of active substance	1,3-cyclohexanedione, 2-[4-(methylsulfonyl)-2-nitrobenzoyl] [7]
Molecular weight of active substance	339.32 g/mol [8]
Molecular formula of active substance	C <sub>14</sub> H <sub>13</sub> NO <sub>7</sub> S [7]
Structural formula of active substance	



Content of active substance	9.6% (w/w)
CAS of active substance	104206-82-8
Production date	09.2020
Expiry date	09.2024
Storage conditions	temperature: 10-30°C
Reference number	LBS/69/23
Certificate of analysis	Appendix 1

### • Methods

Validation of method was based on experimental procedure SPB-FA/11 and guideline SAN-TE/2020/12830, rev.2. During the validation of the analytical method the following parameters: selectivity, matrix effects, linearity, accuracy, precision (repeatability), limit of detection and limit of quantification, stability of the standard in acetonitrile were determined.

Determination of the active substance in 50% (w/v) sucrose solution was performed by high performance liquid chromatography with UV-DAD detection on the basis of signal from the active substance. Identification of active substance was made by comparing the UV spectra and retention time of the active substance standard solution and the test item solution.

## Results

Table 1. Validation parameters of analytical method for determination of active substance (mesotrione) of the test item MEZI 100 SC in 50% (w/v) sucrose solution

Parameter	Required criterion	The result			
Selectivity	at the place originating from active substance signal, there are no signals originating from other substances of area exceeding 30% of active substance area in the test item solution at the level (LOQ)	at the place originating from active substance signal, there are no signals originating from other substances of area exceeding 30% of active substance area in the test item solution at the level (LOQ)			
	the UV spectra of active substance in the standard solution and the test item solutions are comparable	the UV spectra of active substance in the standard solution and the test item solutions are comparable			
Linearity	$r \geq 0.99$  Random distribution of regression residuals	$r = 0.999$ (0.127 mg/L – 50.690 mg/L)*  A random distribution of regression residuals were obtained			
Matrix effects [%]	$\geq -20$ and $\leq 20$	-1.1			
Accuracy [%]	70-110	level I**	106.06	100.85	
		level II**	95.64		
Precision [% RSD]	$\leq 10$	level I***	0.57		
		level II***	0.33		
Limit of detection [mg/L]	$\leq 0.149$	0.127			
Limit of quantification [mg/L]	-	0.498			
Standard stability in acetonitrile**** [%]	$\leq 10$	0.2			

\* Range of linearity expressed in mg/kg of 50% (w/v) sucrose solution: 0.06 mg/kg – 25.35 mg/kg

\*\* Nominal concentration of the active substance in the test item solution in 50% (w/v) sucrose solution:

level I = 0.23 mg/kg (which corresponds to 0.498 mg/L), level II = 2.35 mg/kg (which corresponds to 5.092 mg/L).

\*\*\* Average determined concentration of the active substance in the test item solution in 50% (w/v) sucrose solution:

## Conclusion

The results of analytical method validation confirm that this method is suitable for analysis the content of the active substance (mesotrione) of the test item in 50% (w/v) sucrose solution.

### A 2.1.2.6.2 Method of validation 2

#### A 2.1.2.6.2.1 Method validation: HPLC with PDA

Comments of zRMS:	<p>The analytical method code: 0038/0175/FA was fully validated in term of specificity, linearity, repeatability, accuracy and LOQ according to SANTE/2020/12830, rev.2. The limit of detection (LOD) equals: 1.017 mg/L and limit of quantification (LOQ) equals: 4.812 mg/L.</p> <p>The method linearity was evaluated at 5 levels.</p> <p>The results of analytical method validation confirm that this method is suitable for analysis the content of the active substance (mesotrione) of the test item in deionized water.</p>
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	The method is successfully validated and accepted.
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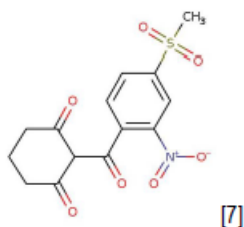
Reference:	KCP 5.2
Report	Validation of analytical method for determination of active substance (mesotrione) of the test item MEZI 100 SC in deionized water, Ciorga B., Study code: 0038/0175/FA
Guideline(s):	SANTE/2020/12830 rev.2
Deviations:	Yes Deviation from the Study plan was found: 1. Both the starting and completion dates were shifted to October from the initial term which was set for September in Study plan.  The deviation did not affect the reliability of the study results. No deviations from the SANTE/2020/12830 rev.2 guideline were found.
GLP:	Yes
Acceptability:	

## Materials and methods

### • Material

#### 2. Test item

Name	MEZI 100 SC
Type of packaging material	HDPE
Date of delivery	08.09.2023
Batch No.	CHR/12
Name of active substance	Mesotrione
IUPAC nomenclature of active substance	1,3-cyclohexanedione, 2-[4-(methylsulfonyl)-2-nitrobenzoyl] [7]
Molecular weight of active substance	339.32 g/mol [8]
Molecular formula of active substance	C <sub>14</sub> H <sub>13</sub> NO <sub>7</sub> S [7]
Structural formula of active substance	



Content of active substance	9.6% (w/w)
CAS of active substance	104206-82-8
Production date	09.2020
Expiry date	09.2024
Storage conditions	temperature: 10-30°C
Reference number	LBS/69/23
Certificate of analysis	Appendix 1

### • Methods

Validation of method was based on experimental procedure SPB-FA/11 and Guideline SAN-TE/2020/12830, rev. 2. During the validation of the analytical method the following parameters: selectivi-

ty, matrix effect, linearity, accuracy, precision (repeatability), limit of detection, limit of quantification and standard stability in acetonitrile were determined.

Determination of active substance of the test item in deionized water was performed by high performance liquid chromatography with PDA detection on the basis of signal from active substance. Identification of active substance was made by comparing the UV spectra and retention time of the active substance standard solution and the test item solution.

## Results

Table 1. Validation parameters of analytical method for determination of active substance (mesotrione) of the test item MEZI 100 SC in deionized water solution

Parameter	Required criterion	The result		
Selectivity	at the place originating from active substance signal, there are no signals originating from other substances of area exceeding 30% of active substance area in the test item solution at the level (LOQ)  the UV spectrum of active substance in the standard solution and the test item solution are comparable	at the place originating from active substance signal, there are no signals originating from other substances of area exceeding 30% of active substance area in the test item solution at the level (LOQ)  the UV spectrum of active substance in the standard solution and the test item solution are comparable		
Matrix effects [%]	≥-20 and ≤20	-0.1		
Linearity	$r \geq 0.99$  Random distribution of regression residuals	$r = 0.999$ (1.017 mg/L – 104.242 mg/L)  A random distribution of regression residues were obtained		
Accuracy [%]	70-120	level I*	100.49	102.91
		level II*	105.33	
Precision [% RSD]	≤20	level I**	0.60	
		level II**	1.08	
Limit of detection [mg/L]	≤1.444	1.017		
Limit of quantification [mg/L]	-	4.812		
Standard stability in acetonitrile*** [%]	≤10	0.2		

\* Nominal concentration: level I – 4.812 mg/L, level II – 48.125 mg/L.

\*\* Average determined concentration: level I – 4.835 mg/L, level II – 50.691 mg/L.

\*\*\* The active substance standard solutions which stability was confirmed, were used for analytical method validation (standard stability: 1 day).

## Conclusion

The results of analytical method validation confirm that this method is suitable for analysis the content of the active substance of the test item in deionized water