FINAL REGISTRATION REPORT

Part B

Section 5

Analytical Methods

Detailed summary of the risk assessment

Product code: SHA 4307 A

Product name: PRIMARY MX

Chemical active substances:

Rimsulfuron, 30 g/kg

Nicosulfuron, 120 g/kg

Mesotrione, 360 g/kg

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

Applicant: SHARDA Cropchem España S.L.

Submission date: February 2020

Update date**:** 03.2021 12.2022

MS Finalisation date: 08.2022; 12.2022; 03.2023

Version history

When	What
March 2021	Applicant update
August 2022	zRMS first evaluation
December 2022	Applicant update
December 2022	Final zRMS assessment
March 2023	Updated assessment in relation to comments

Table of Contents

5 Analytical methods

5.1 Conclusion and summary of assessment

Sufficiently sensitive and selective analytical methods are available for the active substances and relevant impurities.

Sufficiently sensitive and selective analytical methods are available for all analytes included in the residue definitions.
Noticed data gaps are (~~post registration requirement~~):

- ILV for Nicosulfuron for drinking water.
- ~~ILV for Rimsulfuron for drinking water~~

Following new methods were provided by the Applicant:

- Methods for high starch content (dry) matrix (primary and ILV);
- Primary and ILV methods for drinking water (Rimsulfuron).

Noticed data gaps are:
Rimsulfuron
The proposed ILV for dry matrices by Rubino, (2019) contains a different extraction step (no addition of water and sodium chloride) and an additional clean-up step (dispersive SPE with C18 material) compared to the proposed primary method by Markowicz (2019). According to table 3 of SANTE/2020/12830 this is considered as an unacceptable alteration of the ILV, resulting in its unacceptability. Therefore, ILV for dry matrices is required.
Nicosulfuron
ILV for drinking water is required

The above-mentioned data gaps should be completed before authorization.

Commodity/crop	Supported/ Not supported
Dry commodities / Maize	Supported

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

5.2.1 Analysis of the plant protection product (KCP 5.1.1)

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

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

Reference:	KCP 5.1.1
Report	Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WG: Analysis of active substance and physicochemical properties of initial preparation and preparation after accelerated storage procedure, Institute of Heavy Organic Synthesis, "Blachownia'' report no. 7/2018
Guideline(s):	SANCO/3030/99 rev4
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The analytical determination of Rimsulfuron, Nicosulfuron and Mesotrione content has been carried out by HPLC-UV. Quantitative analysis of Rimsulfuron, Nicosulfuron and Mesotrione was based on external calibration using standards.

Reagents:
- Water for HPLC; CAS: 7732-18-5
- Acetonitrile for HPLC; CAS: 75-08-8
- Phosphoric acid, 10% (v/v)

Quantitative analysis of Rimsulfuron, Nicosulfuron and Mesotrione was based on external calibration using standards as follow:
Rimsulfuron PESTANAL™ analytical standard, 99,9% (HPLC), Sigma-Aldrich, CAS:122931-48-0
Nicosulfuron PESTANAL™ analytical standard, 99,9% (HPLC), Sigma-Aldrich, CAS:111991-09-4
Mesotrione PESTANAL™ analytical standard, 99,8% (HPLC), Sigma-Aldrich, CAS:104206-82-8

Sample solution preparation: 9.0 mg of the test sample was diluted to 1 cm^3 with water and fill up to 10 cm^3 with acetonitrile. This solution was analyzed by HPLC-UV under stable chromatographic conditions.

Standard solutions preparations:
The standard solutions were prepared as follows:
mesotrione 9,9mg/10 ml ACN
nicosulfuron 8,6mg/10ml ACN
rimsulfuron 3,8mg/10ml ACN

Validation - Results and discussions

The analytical method for determination of Rimsulfuron, Nicosulfuron and Mesotrione active ingredient content in Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WG was validated. The validation covered the aspects namely: specificity, linearity, repeatability, intermediate precision and accuracy (% recovery).

Specificity

The combination of the HPLC column, mobile phase composition and the column temperature was designed to ensure separation of the active ingredient from any impurities that possibly could interfere with the determination of the active ingredient.
The selectivity of the HPLC method was assessed by examination of peak homogeneity and peak purity using diode array detector.

For Rimsulfuron, interferences from impurities constitute 0.12% of total peak area in test sample and 0.02% of total peak area in standard analyte sample. In both cases do not contribute 3% (according to SANCO regulations).
For Nicosulfuron, interferences from impurities constitute 0.04% of total peak area in test sample and 0.05% of total peak area in standard analyte sample. In both cases do not contribute 3% (according to SANCO regulations).
For Mesotrione, interferences from impurities constitute 0.10% of total peak area in test sample and 0.04% of total peak area in standard analyte sample. In both cases do not contribute 3% (according to SANCO regulations).

Precision and Repeatability – Horwitz equation

To determine the instrument reproducibility, the **Rimsulfuron** standard solution of 29.6 mg/L (what corresponding more or less to Rimsulfuron concentration in 9.0 mg weight amount of the sample) was injected 7 times using the same chromatographic conditions as during the test item measurements and calibration process.
The relative standard deviation for 7 replicates of standard solution was 0.17%. According to validation guidelines: for the content of analyte below 0.1% relative standard deviation should be ≤ 20 %. This condition is fulfilled.
For sample repeatability evaluation, five independent test item solutions prepared from five individual weights of the test item, were analyzed in duplicate using the same chromatographic conditions as during the calibration process.

To determine the instrument reproducibility, the **Nicosulfuron** standard solution of 118.8 mg/L (what corresponding more or less to Nicosulfuron concentration in 9.0 mg weight amount of the sample) was injected 7 times using the same chromatographic conditions as during the test item measurements and calibration process.
The relative standard deviation for 7 replicates of standard solution was 0.08%. According to validation guidelines: for the content of analyte below 0.1% relative standard deviation should be ≤ 20 %. This condition is fulfilled.
For sample repeatability evaluation, five independent test item solutions prepared from five individual weights of the test item, were analyzed in duplicate using the same chromatographic conditions as during the calibration process.

To determine the instrument reproducibility, the **Mesotrione** standard solution of 346.1 mg/L (what corresponding more or less to Mesotrione concentration in 9.0 mg weight amount of the sample) was injected 7 times using the same chromatographic conditions as during the test item measurements and calibration process.
The relative standard deviation for 7 replicates of standard solution was 0.28%. According to validation guidelines: for the content of analyte below 0.1% relative standard deviation should be ≤ 20 %. This condition is fulfilled.
For sample repeatability evaluation, five independent test item solutions prepared from five individual weights of the test item, were analyzed in duplicate using the same chromatographic conditions as during the calibration process.

Accuracy

For an accuracy estimation, three solutions with active substance concentration prepared from analytical standard in the blank formulation matrix were prepared in the range of 80 – 120% of analyte concentration in the tested sample.
Each solution was injected 7 times using the same chromatographic conditions as during the test item measurements and calibration process.

For **Rimsulfuron**, the relative standard deviation for 7 replicates in all three concentrations level did not

exceed 0.35% and % of Mean Recovery was 97.7; 101.0 and 99.7 for the range 80, 100 and 120% respectively. According to validation guidelines: for the content of active substance in a range 1-10%, the mean recovery should be 97 – 103%. This condition is fulfilled.

For **Nicosulfuron**, the relative standard deviation for 7 replicates in all three concentrations level did not exceed 0.38% and % of Mean Recovery was 98.2; 100.9 and 98.5 for the range 80, 100 and 120% respectively. According to validation guidelines: for the content of active substance above 10%, the mean recovery should be 98 – 102%. This condition is fulfilled.

For **Mesotrione**, the relative standard deviation for 7 replicates in all three concentrations level did not exceed 0.15% and % of Mean Recovery was 101.6; 101.6 and 101.7 for the range 80, 100 and 120% respectively. According to validation guidelines: for the content of active substance above 10%, the mean recovery should be 98 – 102%. This condition is fulfilled.

Linearity and range
For **Rimsulfuron** calibration 5 standard solutions were prepared in the concentration range about 80-120% of analyte content in tested sample. Each calibration solution was analyzed twice under stable chromatographic conditions.
Linearity range of Rimsulfuron was 23.7 – 35.5 mg/L. The achieved linear correlation coefficient (R) was 0.999 and it fulfils established condition >0.99.

For **Nicosulfuron** calibration 5 standard solutions were prepared in the concentration range about 80-120% of analyte content in tested sample. Each calibration solution was analyzed twice under stable chromatographic conditions.
Linearity range of Nicosulfuron was 95.0 – 142.5 mg/L. The achieved linear correlation coefficient (R) was 0.999 and it fulfils established condition >0.99.

For **Mesotrione** calibration 5 standard solutions were prepared in the concentration range about 80-120% of analyte content in tested sample. Each calibration solution was analyzed twice under stable chromatographic conditions.
Linearity range of Mesotrione was 276.9 – 415.4 mg/L. The achieved linear correlation coefficient (R) was 0.999 and it fulfils established condition >0.99.

Table 5.2-1: Methods suitable for the determination of Rimsulfuron, Nicosulfuron and Mesotrione in plant protection product Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WG

	Rimsulfuron	**Nicosulfuron**	**Mesotrione**
Author(s), year	Michalec-Minch, 2018		
Principle of method	The active ingredient content of Rimsulfuron, Nicosulfuron and Mesotrione is determined by high performance liquid chromatography and UV detection at 220 nm.		
Linearity (linear between mg/L / % range of the declared content) (correlation coefficient, expressed as r)	Linearity range: 23.7 – 35.5 mg/L Calibration equotion y = 0.3058x -0.1627 Correlation coefficient ~~R = 0.999~~ R= 0.998	Linearity range: 95.0 – 142.5 mg/l Calibration equotion y = 0.2978x -1.6402 Correlation coefficient R = 0.999	Linearity range: 276.9 – 415.4 mg/L Calibration equotion y = 0.4974x -0.4525 Correlation coefficient ~~R = 0.999~~ R= 0.998
Precision – Repeatability Mean n = 5 (%RSD)	RSD = 0.17% Horwitz equation RSD = 0.86% ~~RSD_R = 3.39%~~	RSD = 0.08% Horwitz equation RSD = 1.53% ~~RSD_R = 2.75%~~	RSD = 0.28% Horwitz equation RSD = 0.90% ~~RSD_R = 2.33%~~

		Rimsulfuron	Nicosulfuron	Mesotrione
		RSD_r = 2.27%	RSD_r = 1.84%	RSD_r = 1.56%
Accuracy n = 7 (% Recovery)	Analyte conc.80%	Recovery = 97.7%	Recovery = 98.2%	Recovery = 101.6%
	Analyte conc.100%	Recovery = 101.0%	Recovery = 100.9%	Recovery = 101.6%
	Analyte conc.120%	Recovery = 99.7%	Recovery = 98.5%	Recovery = 101.7%
Interference/ Specificity		No interference	No interference	No interference
Comment		Accepted	Accepted	Accepted

Conclusion

The analytical method (HPLC-UV) for the determination of Rimsulfuron, Nicosulfuron and Mesotrione in the formulation Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WG has been submitted.

The method for the determination of Rimsulfuron, Nicosulfuron and Mesotrione is acceptable and validated according the requirements SANCO 3030/99 rev.4, (because of the starting experimental phase on July 2018).

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

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

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

Reference:	KCP 5.1.1-01
Report	Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WG Analysis of relevant impurities content of initial preparation and preparation after accelerated storage procedure; Nowakowska-Bogdan E., 2020, Report No.: 163/2020
Guideline(s):	SANCO/3030/99
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

1-cyano-6-(methylsulfonyl)-7-nitro-9H-xanthen-9-one (R287431)
The LC/MS analysis was performed according to the internal procedure BA-AB/MS/SPO-1 ″General principles of analysis by coupled system of high performance liquid chromatography and mass spectrometry″ and research method no. BA-AB/MS/MB-12 ″The analytical method for determination of R287431 and R287432″.
Quantitative analysis of R287431 was based on externač calibration using certified analytical standard.

6-(methylsulfonyl)-9-oxo-9H-xanthene-1-carbonitrille (R287432)

The LC/MS analysis was performed according to the internal procedure BA-AB/MS/SPO-1 ″General principles of analysis by coupled system of high performance liquid chromatography and mass spectrometry″ and research method no. BA-AB/MS/MB-12 ″The analytical method for determination of R287431 and R287432″.
Quantitative analysis of R287432 was based on externač calibration using certified analytical standard.

LC/MS test result conclusion
For calibration 5 calibration solutions from analytical standard were prepared (with different concentration relevant impurity substance in methanol). Each calibration solution was analyzed twice under stable chromatographic conditions.
Appropriate amount of the test sample was diluted to 10 mL with 1 mL DFM and filled to volume with methanol.
Five independent test item solutions prepared from five individual weights of the test item (initial formulation) were analyzed in duplicate.
Two independent test item solutions prepared from two individual weights of the test item (formulation after accelerated storage) were analyzed in duplicate.
The solutions were analyzed by LC/MS under stable chromatographic conditions (the same as calibration conditions).
The results were express in % w/w (g/kg).

Reagents
- 1-cyano-6-(methylsulfonyl)-7-nitro-9H-xanthen-9-one (R287431), analytical standard 98.13%, Varanous, CAS: NA, Eypiry date: 02.10.2022 (Retest), VI/244/BA-AB
- 6-(methylsulfonyl)-9-oxo-9H-xanthene-1-carbonitrille (R287432), analytical standard 99.44%, Varanous, CAS: NA, Expiry date: 21.06.2022 (Retest), VI/243/BA-AB
- Water ultrapure from direct-q system; CAS: 7732-18-5
- Methanol LC/MS grade; CAS: 75-05-8
- Formic acid LC/MS grade, CAS: 64-18-6
- DFM

Solutions and Solvent Mixtures
Mobile Phase A: 0.1% (v/v) Formic acid in water
1000 mL volumetric flask was half filled with water and 1 mL of formic acid (HCOOH) was added. Volumetric flask was filled up to the mark with water, closed tightly and mixed by inverting several times. Solvent was transferred to amber HPLC solvent reservoir.

Mobile Phase B:
1000 mL volumetric flask was filled with acetonitrile. Solvent was transferred to amber HPLC solvent reservoir.

Preparation of Stock Standard Solutions
Stock standard solutions were used for preparation of calibration standard for the LC-MS/MS instrument and fortification sample for determination of method recoveries.
Using five-figure balance and appropriate amounts of neat standards (corrected for purity) were weighted into volumetric flask.
The standards were diluted to volume with indicated solvent, then mixed well to ensure complete dissolution. Stock standard solutions were stored in freezer when not in use to prevent decomposition and/or concentration of the standard.

Sample solution preparation
1-cyano-6-(methylsulfonyl)-7-nitro-9H-xanthen-9-one (R287431)
Amount of about 300 mg of initial formulation was diluted to 10 mL with 1 mL of DMF and filled to volume with methanol. Five independent test item solutions prepared from five individual weights (300.1; 301.0; 299.8; 300.3 and 299.1 mg) of the test item were analyzed 2 times under the same chromatographic conditions and used for calibration.

Amount of about 300 mg of formulation after accelerated storage procedure was diluted to 10 mL with 1 mL of methanol and filled to volume with methanol. Two independent test item solutions prepared from two individual weights (300.00 and 299,7 mg) of the test item were analyzed 2 times under the same chromatographic conditions as used for calibration.

6-(methylsulfonyl)-9-oxo-9H-xanthene-1-carbonitrille (R287432)

Amount of about 30 mg of initial formulation was diluted to 10 mL with methanol. The samples were diluted 1:10. Five independent test item solutions prepared from five individual weights (all 3.1 mg) of the test item were analyzed 2 times under the same chromatographic conditions and used for calibration.
Amount of about 30 mg of formulation after accelerated storage procedure was diluted to 10 mL with 1 mL of methanol and filled to volume with methanol. The samples were diluted 1:10. Two independent test item solutions prepared from two individual weights (both 3.0 mg) of the test item were analyzed 2 times under the same chromatographic conditions as used for calibration.

Chromatographic conditions

Column:	Synergi™, 4µ Fusion-RP 80A, 50x2.00 mm (BA-AB MS07)	
Column temperature:	30 °C	
Flow rate:	0.5 cm^3/mm	
Eluent:	60% acetonitrile : 40% H_2O + 0.1% formic acid	
Elution program:	isocratic	
Injection volume:	10 µL	
Run time:	4 minutes	
Retention time:	R287431	about 0.6 min
	R287432	about 0.5 min

Mass spectrometric conditions

MS System	Sciex Q TRAP 4000
Ionisation type	Electrospray
Polarity	Positive ion mode
Temperature	600 °C
Nebulizing gas, Gas1, psig	60
Nebulizing gas, Gas2, psig	50
Curtain Gas, psig	35
Ion Spray Voltage, V	5200
Scan type	MS/MS, multiple Reaction Monitoring (MRM)
Scan resolution	MS1/MS2 – UNIT (0.7 amu)

Analyte	Precursion ion, Da	Product ion, Da	DP, V	EP, V	CE, V	CXP, V
R287431	346.035	236	121	10	35	18
R287431	346.035	180.1	121	10	57	30
R287432	301.004	222	131	10	43	12
R287432	301.004	210	131	10	39	10

1,2-dichloroethane

The GC-MS analysis was performed according to the internal test procedure BA-AC/SPO-2 "Quantitative and qualitative determination of pesticides in plat protection formulations by GC/MS" and research metod No. BA- ACMB-22 "Relevant impurity - 1,2-dichlorethane".
Quantitative analysis of 1,2-dichloroethane (DCE) was based on external calibration using certified analytical standard.
For calibration 5 calibration solutions from analytical standard was prepared (with different concentration relevant impurity substance in methanol). Each calibration solution was analyzed twice under stable chromatographic conditions.
Appropriate amount of the test sample was diluted so 10 mL with methanol.
Five independent test item solutions prepared from five individual weights of the test item (initial formulation) was analyzed in duplicated.

Two independent test item solutions prepared fom two individual weights of the test item (formulation after accelerated storage) was analyzed in duplicate
These solutions were analyzed by GC/MS under stable chromatographic conditions (the same as calibration conditions.
The results were express in % w/v (g/L) and like % w/w (g/kg).

Reagents

- Methanol for HPLC, CAS: 64-56-1
- 1,2-dichloroethane Emplura®, Merck, purity: 99.9%, CAS: 107-06-2, Expiry date: 30/11/2021

Preparation of Standard Stock Solutions

Stock standard solution containing DCE was used for preparation of mixture of calibration standard for the GC.MS/MS instrument and fortification of sample for determination of method recoveries.
Using five-figure balance the appropriate amount of DCE neat standard (corrected for purity) were weighted into volumetric flask.
The standard was diluted to volume with methanol, than mixed well to insure complete dissolution. Standard stock solution was stored in freezer when not in use to prevent decomposition and/or concentration of the standard.

Sample solution preparation

Amount of about 500 mg of initial formulation was diluted to 10 mL with methanol. Five independent test item solutions prepared from five individual weights (488.9, 470.8, 501.3, 504.3 and 505.8 mg) of the test item were analyzed 2 times under the same chromatographic conditions and used for calibration.
Amount of about 500 mg of formulation after accelerated storage procedure was diluted to 10 mL with methanol.
Two independent test item solutions prepared from two individual weights (512.6 and 501.1 mg) of the test item were analyzed 2 times under the same chromatographic conditions as used for calibration.
All sample solution were analyzed by GC/MS under stable chromatographic conditions.

Description of the test method

Principle of Measurement

An aliquot of the sample solution was injected into the gas chromatograph coupled with tandem mass spectrometry (MS/MS). The MS/MS instrument was operated in the Multiple Reaction Monitoring Mode (MRM). The 1,2-dicloroethane (precursor ion/ions) generated in electron impact ionization source (EI) were isolated by the first quadrupole by their mass/charge (m/z) ratio and subjected to collision induced dissociation (CID) which occurs in collision cell (second quadrupole). The resulting fragment ions (product ions) were separated according to their m/z ratio in third quadrupole.
An aliquot of the sample solution was injected into the gas chromatograph coupled with tandem mass spectrometry (MS/MS).

Chromatographic conditions for formulations:

Column:	HP-5MS 30m × 250 ×µm × 0.25 µm
Pre-Column:	3m, FS, Deactivated 0.250 mm × 3 m
Carrier gas:	Helium
Inlet pressure:	20 psi (constant pressure mode) during run 2 psi (during blackflush)
Thermal program:	
Formulation:	50 °C for 2 min, 30 °C/ min to 180 °C, 30 °C/min to 280 °C isotherm of 11 minutes
Calibration:	50 °C for 2 min, 30 °C/min to 180 °C isotherm of 0 minutes
Injection volume:	2.5 µL
Inlet temperature:	180 °C
Injection mode:	pulsed splitless
Injection pulse pressure:	55 psi unit 0.2 min
Septum purge flow:	3 mL/min
Purge flow to split vent:	50 mL/ min at 0.22 min

Mass Spectrometric conditions:

- MS system: 7000 Quadripole MS/MS EI Agilent Technologies
- Source type: Electron Impact (EI)
- Polarity: Positive ion mode
- Transfer line temp.: 180 °C
- Source temp.: 280 °C
- Quadrupole temp.: Q1 and Q2 180 °C

MRM Mode conditions:

- Scan type: MS/MS, MRM (multiple reaction monitoring)
- Scan resolution: MS1/MS2 Wide-Wide
- Collision gas flows: N2 Collision gas 1.5 mL/min
  He Quench gas 2.25 mL/min

Compound	Retention time (min)	Precursor Ion	Fragment Ion	Collision Energy (eV)	Transition name
DCE	3.1 – 3.2	98	62	5	Quantification
		99.9	64	10	Confirmation

Validation - Results and discussions

Table 5.2-2: Methods suitable for the determination of the relevant impurities in plant protection product (PPP) Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WG

	R287431 Max. 2 mg/kg	R287432 Max. 2 g/kg	1,2-dichloroethane Max. 1 g/kg
Author(s), year	Ewa Nowakowska-Bogdan, 2020		
Principle of method	LC/MS	LC/MS	GC/MS
Linearity (linear between mg/L) (correlation coefficient, expressed as r)	Linearity range: 0.015 – 0.264 mg/L Calibration equotion Y = 7.59E + 04X – (-105) Correlation coefficient R = 0.9987 Calibration point = 5	Linearity range: 0.153 – 2.627 mg/L Calibration equotion Y = 7.72E + 05X – (-2.45E + 04) Correlation coefficient R = 0.9954 Calibration point = 5	Linearity range: .0105 – 0.350 mg/L Calibration equotion Y = 2326.4X + 13.576 Correlation coefficient R = 0.9998 Calibration point = 5
Precision – Repeatability Mean n = 5 (%RSD)	RSD (reproducibility) = 11.5% RSD (repeatability) = 7.42%	RSD (reproducibility) = 1.31% RSD (repeatability) = 3.68%	RSD (reproducibility) = 2.01% RSD (repeatability) = 9.25%
Accuracy n = 5 (% Recovery)	Analyte conc. LOQ Recovery = 90.2% Analyte conc. 10LOQ Recovery = 95.6%	Analyte conc. LOQ Recovery = 109.5% Analyte conc. 10LOQ Recovery = 109.9%	Analyte conc. LOQ Recovery = 84.60% Analyte conc. 10LOQ Recovery = 100.64%
Interference/ Specificity	Retention time of analyte = 0.6 min Retention time of calibration standard = 0.6	Retention time of analyte = 0.5 min Retention time of calibration standard = 0.5	Retention time of analyte = 3.14 min Retention time of calibration standard =

	R287431 **Max. 2 mg/kg**	**R287432** **Max. 2 g/kg**	**1,2-dichloroethane** **Max. 1 g/kg**
	min Confirmation ion ratio defferences = 5.2%	min Confirmation ion ratio defferences = 3.9%	3.15 min Confirmation ion ratio defferences = 3.5%
LOQ	0.015 mg/L (0,00005%)	0.153 mg/L (0,05%)	0.0336 mg/L (0,00007%)

Validation results: R287431
Quantification: 346.0 → 236.0
Confirmation: 346.0 → 180.1

Validation results: R287432
Quantification: 301.0 → 222.0
Confirmation: 301.0 → 210.1

Validation results: 1,2-dichloroethane
Quantification: 98.0 → 62.0
Confirmation: 99.9 → 64.0

Conclusion
For the experimental data obtained according to the internal test procedure, it can be concluded that: Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WG initial formulation and formulation after accelerated storage do not contain R287431, R287432 and 2,4-dichloroethane.

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

Not relevant.

5.2.1.5 Applicability of existing CIPAC methods (KCP 5.1.1)

A CIPAC method No. 716 is available for Rimsulfuron.
A CIPAC method No. 709 is available for Nicosulfuron.
A CIPAC method No. 625 is available for Mesotrione.

5.2.2 Methods for the determination of residues (KCP 5.1.2)

Please refer to post-registration methods.

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

5.3.1 Analysis of the plant protection product (KCP 5.2)

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

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

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

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

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

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Plant, high water content	Rimsulfuron	0.01 mg/kg	Regulation No. 617/2014
Plant, high acid content		0.01 mg/kg	Regulation No. 617/2014
Plant, high protein/high starch content (dry commodities)		0.01 mg/kg	Regulation No. 617/2014
Plant, high oil content		0.01 mg/kg	Regulation No. 617/2014
Plant, difficult matrices (hops, spices, tea)		0.05 mg/kg	Regulation No. 617/2014
Muscle	Rimsulfuron	0.02 mg/kg	Regulation No. 617/2014
Milk		0.02 mg/kg	Regulation No. 617/2014
Eggs		0.02 mg/kg	Regulation No. 617/2014
Fat		0.02 mg/kg	Regulation No. 617/2014
Liver, kidney		0.02 mg/kg	Regulation No. 617/2014
Soil (Ecotoxicology)	Rimsulfuron	0.05 mg/kg	common limit
Drinking water (Human toxicology)	Rimsulfuron	0.1 µg/L	general limit for drinking water
Surface water (Ecotoxicology)	Rimsulfuron	4.6 µg/L	Lowest EC_{50} from *L. minor* study
Air	Rimsulfuron	21 µg/m^3	AOEL sys: 0.07 mg/kg bw/d
Tissue (meat or liver)		not required	not classified as T / T+
Body fluids		not required	not classified as T / T+

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 Rimsulfuron in plant matrices is given in the following tables (please refer to the DAR of Rimsulfuron, July 2005).

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

Component of residue definition: Rimsulfuron				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
High water content	Primary	0.05 mg/kg 0.05 mg/kg	HPLC-UV HPLC-UV	LaRochelle, 1989 Amoo, 1996
	ILV	0.05 mg/kg	HPLC-UV	Clayton, 2001
	Confirmatory (if required)	0.01 mg/kg	LC-MS/MS	Fulton, 2001
High acid content	Primary	-	-	-
	ILV	-	-	-
	Confirmatory (if required)	-	-	-
High oil content	Primary	-	-	-
	ILV	-	-	-
	Confirmatory (if required)	-	-	-
High protein/high starch content (dry)	Primary	0.05 mg/kg 0.05 mg/kg	HPLC-UV HPLC-UV	LaRochelle, 1989 Amoo, 1996
	ILV	0.05 mg/kg	HPLC-UV	Clayton, 2001
	Confirmatory (if required)	0.01mg/kg	LC-MS/MS	Fulton, 2001
	Primary	0.01 mg/kg	LC-MS/MS	KCP 5.2.1. A. Markowicz, 2019 Report No. 19/FSL/15/1A
	~~ILV~~	~~0.01 mg/kg~~	~~LC-MS~~	~~KCP 5.2.1.1~~ ~~M. Rubino, 2019~~ ~~Report No. 19.500341.0001~~
	Confirmatory (if required)	-	-	The applied LC-MS/MS is highly selective method and 2 transitions were monitored, therefore no other confirmatory method is required.

Table 5.3-3: Statement on extraction efficiency

	Method for products of plant origin
Required, available from:	Extraction efficiency was not investigated in this study as a solvent system similar to that used in metabolism studies reported in the DAR from the previous EU Review for rimsulfuron was used for extraction (Volume 3, Annex B, B.7, July 2005. Brown and Young (1989) AMR 1222-88 (maize); Brown (1990) AMR

	Method for products of plant origin
	1444-89 (potato) and Zhang et al. (1996) AMR 3520-95 (tomato foliage)).
Not required, because:	-

Study Comments: IIIA 5.3.2.2	EFSA Journal 2018;16(5):5258: *Rimsulfuron residue can be monitored in food and feed of plant origin by high-performance liquid chromatography with tandem mass spectrometry (HPLC–MS/MS) with a limit of quantification (LOQ) of0.01 mg/kg in all commodity groups. Rimsulfuron residue in dry and high water content commodities can be determined also by the quick, easy, cheap, effective and safe method (QuEChERS) using HPLC–MS/MS with a LOQ of 0.01 mg/kg.* *It should be noted that data gaps for methods for monitoring of rimsulfuron residue in: plant commodities with high acid and high oil content… for Task Force were identified.*
Agreed end-point: IIIA 5.3.2.2	Proposed use is supported by the available data.

zRMS: New primary and ILV methods were provided by the Applicant (maize). ~~These methods are acceptable.~~ Primary method is acceptable.
The LOQ of the methods were defined as the lowest analyte concentration at which the methodology had been successfully validated. Thus, an LOQ of 0.01 mg/kg was confirmed for Rimsulfuron in maize matrices.

The proposed ILV for dry matrices by Rubino, (2019) contains a different extraction step (no addition of water and sodium chloride) and an additional clean-up step (dispersive SPE with C18 material) compared to the proposed primary method by Markowicz (2019). According to table 3 of SANTE/2020/12830 this is considered as an unacceptable alteration of the ILV, resulting in its unacceptability. Therefore, ILV for dry matrices is required.

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

Not relevant, no residue definition is proposed.

Study Comments: IIIA 5.3.2.3	The explanation provided by the applicant is accepted.
Agreed end-point: IIIA 5.3.2.3	As no residue definition was proposed, the analytical method for the determination of residues of Rimsulfuron in animal matrices is not required.

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 Rimsulfuron in soil is given in the following tables (please refer to the DAR of Rimsulfuron, July 2005).

Table 5.3-4: Validated methods for soil (if appropriate)

Component of residue definition: Rimsulfuron, IN-70912, IN-70941, IN-J0290 and IN-E9260			
Method type	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary (Rimsulfuron)	0.2 µg/kg 0.05 µg/kg	LC-MS/MS LC-MS/MS	Connolly, 2001 (This method is specific, validated on two mass transitions, so confirmatory is not required) Hill and Stry, 2001 (This method is specific, validated on two mass transitions, so confirmatory is not required)
Confirmatory (Rimsulfuron)	-	-	-
Primary (IN-70942, IN-70941, IN-J0290, IN-E9260 metabolites)	0.2 µg/kg	LC-MS/MS	Connolly, 2001 (This method is specific, validated on two mass transitions, so confirmatory is not required)
Confirmatory (IN-70942, IN-70941, IN-J0290, IN-E9260 metabolites)	-	-	-

*IN-70942, IN-70941, IN-J0290, IN-E9260 metabolites are not component of residue definition.

Study Comments: IIIA 5.3.2.4	Adequate method exists to monitor Rimsulfuron residues in soil. The analytical methods presented by the applicant are active substances data, which were reviewed in the Assessment Report for Rimsulfuron and were considered adequate. Additional methods for the purpose of the evaluation are not required.
Agreed end-point: IIIA 5.3.2.4	Residues of Rimsulfuron in soil: LC-MS/MS LOQ = 0.2 µg/kg

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

An overview on the acceptable methods and possible data gaps for analysis of Rimsulfuron in surface and drinking water is given in the following tables (please refer to the DAR of Rimsulfuron, July 2005).

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

Component of residue definition: Rimsulfuron				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Drinking water	Primary	0.1 µg/L 0.05 µg/L	HPLC-UV LC-MS/MS	Powley and de Bernard, 1996 Devine and Jin, 2001 (This method is

Component of residue definition: Rimsulfuron				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
				specific, validated on two mass transitions, so confirmatory is not required)
	ILV	-	-	According to SANCO/825/00 rev. 8.1, ILV is not required.
	Confirmatory	0.1 µg/L	LC-MS/MS	Jin, 2001
Drinking water	Primary	0.05 µg/L	LC/MS	KCP 5.2.2 M. Rubino, 2019 Report No. 19.500341.0007
	ILV	0.05 µg/L	LC-MS/MS	KCP 5.2.2.1 M. Zarębska, 2020 Report No. 30/2020
	Confirmatory	-	-	The applied LC-MS is highly selective method and 2 transitions were monitored, therefore no other confirmatory method is required.
Surface water	Primary	0.1µg/L 0.05 µg/L	HPLC-UV LC-MS/MS	Powley and de Bernard, 1996 Devine and Jin, 2001 (This method is specific, validated on two mass transitions, so confirmatory is not required)
	Confirmatory	0.1 µg/L	LC-MS/MS	Jin, 2001

Study Comments: IIIA 5.3.2.5	Adequate method exists to monitor Rimsulfuron residues in surface water. The analytical methods presented by the applicant are active substances data, which were reviewed in the Assessment Report for Rimsulfuron and were considered adequate. With regard to the drinking water: ILV for drinking water is required according to Regulation (EU) 284/2013, legally binding in contrast to guideline. EFSA Journal 2018;16(5):5258: *Rimsulfuron residue in water can be monitored by QuEChERS HPLC–MS/MS or single HPLC–MS/MS with LOQs 0.05 µg/L and 0.1 µg/L, respectively.*
Agreed end-point: IIIA 5.3.2.5	Residues of Rimsulfuron in surface water: HPLC-UV LOQ = 0.1 µg/L LC-MS/MS LOQ = 0.05 µg/L ILV for drinking water: data gap.

zRMS: New primary and ILV methods were provided by the Applicant. These methods are acceptable. According to SANTE/2020/12830 Rev.1 the method was sufficiently validated and it is suitable for determination of Rimsulfuron in drinking water. ILV is acceptable. Limit of quantification is 0.05 µg/L.

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 Rimsulfuron in air is given in the following tables (please refer to the DAR of Rimsulfuron, July 2005).

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

Component of residue definition: Rimsulfuron			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	3 µg/m^3 air	LC-MS/MS	Bacher, 2001 (This method is specific, validated on two mass transitions, so confirmatory is not required)
Confirmatory	-	-	-

Study Comments: IIIA 5.3.2.6	Adequate LC-MS/MS method exists to monitor Rimsulfuron residues in air. The analytical methods presented by the applicant are active substances data, which were reviewed in the Assessment Report for Rimsulfuron and were considered adequate. Additional methods for the purpose of the evaluation are not required.
Agreed end-point: IIIA 5.3.2.6	Residues of Rimsulfuron in air: LC-MS/MS LOQ = 3 µg/m^3

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

Not relevant, the active substance Rimsulfuron is not classified as toxic or very toxic, no residue method for body fluids and tissues is required.

5.3.2.8 Other studies/ information

No new or additional studies have been submitted.

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

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

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

Table 5.3-7: Relevant residue definitions for monitoring/enforcement and levels for which compliance is required

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Plant, high water content	Nicosulfuron	0.01 mg/kg	Regulation No. 617/2014
Plant, high acid content		0.01 mg/kg	Regulation No. 617/2014
Plant, high protein/high starch content (dry commodities)		0.01 mg/kg	Regulation No. 617/2014
Plant, high oil content		0.01 mg/kg	Regulation No. 617/2014
Plant, difficult matrices (hops, spices, tea)		0.05 mg/kg	Regulation No. 617/2014
Muscle	Nicosulfuron	0.02 mg/kg	Regulation No. 617/2014
Milk		0.02 mg/kg	Regulation No. 617/2014
Eggs		0.02 mg/kg	Regulation No. 617/2014
Fat		0.02 mg/kg	Regulation No. 617/2014
Liver, kidney		0.02 mg/kg	Regulation No. 617/2014
Soil (Ecotoxicology)	Nicosulfuron	0.05 mg/kg	common limit
Drinking water (Human toxicology)	Nicosulfuron	0.1 µg/L	general limit for drinking water
Surface water (Ecotoxicology)	Nicosulfuron	1.7 µg/L	Lowest EC_{50} from *Lemna gibba* study
Air	Nicosulfuron	240 µg/m^3	AOEL sys: 0.8 mg/kg bw/d
Tissue (meat or liver)		Not required	not classified as T / T+
Body fluids		Not required	not classified as T / T+

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

An overview on the acceptable methods and possible data gaps for analysis of Nicosulfuron in plant matrices is given in the following tables (please refer to the DAR of Nicosulfuron, June 2006 and Addendum to the DAR, July 2007).

Table 5.3-8: Validated methods for food and feed of plant origin (required for all matrix types, "difficult" matrix only when indicated by intended GAP)

Component of residue definition: Nicosulfuron				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
High water content	Primary (Nicosulfuron)	0.01 mg/kg 0.01 mg/kg	HPLC-UV HPLC-MS/MS	Huber, 1996a Wolf, 2000

Component of residue definition: Nicosulfuron				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
	ILV (Nicosulfuron)	0.01 mg/kg	HPLC-MS/MS	Ginzburg, 2000
	Confirmatory (if required) (Nicosulfuron)	0.05mg/kg	GC/MS, LC-MS	Mirbach, 1998
	Primary (ADMP metabolite)*	0.04 mg/kg	HPLC-UV	Huber, 1996a
	ILV (ADMP metabolite)*	-	-	-
	Confirmatory (if required) (ADMP metabolite)*	-	-	-
	Primary (ASDM metabolite)*	0.06 mg/kg	HPLC-UV	Huber, 1996a
	ILV (ASDM metabolite)*	-	-	-
	Confirmatory (if required) (ASDM metabolite)*	-	-	-
High acid content	Primary	-	-	-
	ILV	-	-	-
	Confirmatory (if required)	-	-	-
High oil content	Primary	-	-	-
	ILV	-	-	-
	Confirmatory (if required)	-	-	-
High protein/high starch content (dry)	Primary (Nicosulfuron)	0.02 mg/kg 0.01 mg/kg	HPLC-UV HPLC-MS/MS	Huber, 1996a Wolf, 2000
	ILV (Nicosulfuron)	0.01 mg/kg	HPLC-MS/MS	Ginzburg, 2000
	Confirmatory (if required) (Nicosulfuron)	0.025mg/kg	GC/MS, LC-MS	Mirbach, 1998
	Primary (ADMP metabolite)*	0.04 mg/kg	HPLC-UV	Huber, 1996a
	ILV (ADMP metabolite)*	-	-	-
	Confirmatory (if required) (ADMP metabolite)*	-	-	-
	Primary	0.02 mg/kg	HPLC-UV	Huber, 1996a

Component of residue definition: Nicosulfuron				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
	(ASDM metabolite)*			
	ILV (ASDM metabolite)*	-	-	-
	Confirmatory (if required) (ASDM metabolite)*	-	-	-
Difficult (if required, depends on intended use)	Primary	-	-	-
	ILV	-	-	-
	Confirmatory (if required)	-	-	-

* ADMP and ASDM are not components of residue definition.

Table 5.3-9: Statement on extraction efficiency

	Method for products of plant origin
Required, available from:	DAR, 2005
Not required, because:	-

Study Comments: IIIA 5.3.3.2	Adequate method exists to monitor Nicosulfuron residues in high protein/high starch content (dry). The analytical methods presented by the applicant are active substances data, which were reviewed in the Assessment Report for Nicosulfuron and were considered adequate. Additional methods for the purpose of the evaluation are not required.
Agreed end-point: IIIA 5.3.3.2	Residues of Nicosulfuron in commodities with high protein/high starch content (dry): HPLC-UV LOQ = 0.02 mg/kg HPLC-MS/MS LOQ = 0.01 mg/kg

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

An analytical method for food of animal origin is not required due to the fact that no residue definition is proposed (*EFSA Scientific Report (2007) 120, 1-91*).

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

An overview on the acceptable methods and possible data gaps for analysis of Nicosulfuron in soil is given in the following tables (please refer to the DAR of Nicosulfuron, June 2006 and Addendum to the DAR, July 2007).

Table 5.3-10: Validated methods for soil (if appropriate)

Component of residue definition: Nicosulfuron			
Method type	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary (Nicosulfuron)	0.005 mg/kg	HPLC-UV	Huber, 1996b
Confirmatory (Nicosulfuron)	0.05 µg/kg	LC-MS/MS	Wais, 2000a
Primary (ADMP and ASDM metabolites)	0.02 mg/kg	HPLC-UV	Huber, 1996b
Confirmatory (ADMP and ASDM metabolites)	-	-	-
Primary (AUSN and UCSN metabolites)	0.01 mg/kg	LC-MS/MS	Wolf, 2003 (This method is specific, validated on two mass transitions, so confirmatory is not required)
Confirmatory (AUSN and UCSN metabolites)	-	-	-

*ADMP, ASDM, AUSN and UCSN are not component of residue definition.

Study Comments: IIIA 5.3.3.4	Adequate method exists to monitor Nicosulfuron residues in soil. The analytical methods presented by the applicant are active substances data, which were reviewed in the Assessment Report for Nicosulfuron and were considered adequate. Additional methods for the purpose of the evaluation are not required.
Agreed end-point: IIIA 5.3.3.4	Residues of Nicosulfuron in soil: LC-MS/MS LOQ = 0.05 µg/kg

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

An overview on the acceptable methods and possible data gaps for analysis of Nicosulfuron in surface and drinking water is given in the following tables (please refer to the DAR of Nicosulfuron, June 2006 and Addendum to the DAR, July 2007).

Table 5.3-11: Validated methods for water (if appropriate)

Component of residue definition: Nicosulfuron				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Drinking water (Nicosulfuron)	Primary	0.05 µg/L 0.05 µg/L	HPLC-UV HPLC-MS/MS	Schulz and Ullrich-Mitzel, 1995a Wolf, 2007 (This method is specific, validated on two mass transitions, so confirmatory is not required)
	ILV	-	-	According to SANCO/825/00 rev. 8.1,

Component of residue definition: Nicosulfuron				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
				ILV is not required.
	Confirmatory	0.05 µg/L	LC-DAD	Wais, 2000b
Drinking water (ADMP, ASDM and AUSN metabolites)*	Primary	0.05 µg/L	HPLC-UV	Wais and Ullrich-Mitzel, 1997
	ILV	-	-	According to SANCO/825/00 rev. 8.1, ILV is not required.
	Confirmatory	-	-	-
Surface water (Nicosulfuron)	Primary	0.05 µg/L	HPLC-MS/MS	Wolf, 2007 (This method is specific, validated on two mass transitions, so confirmatory is not required)
	Confirmatory	0.05 µg/L	HPLC-DAD	Wais, 2000b

*ADMP, ASDM and AUSN are not component of residue definition.

Study Comments: IIIA 5.3.3.5	Adequate method exists to monitor Nicosulfuron residues in surface water. The analytical methods presented by the applicant are active substances data, which were reviewed in the Assessment Report for Nicosulfuron and were considered adequate. With regard to the drinking water: ILV for drinking water is required according to Regulation (EU) 284/2013, legally binding in contrast to guideline. Data gap for ILV for drinking water is stated.
Agreed end-point: IIIA 5.3.3.5	Residues of Nicosulfuron in surface water: HPLC-UV LOQ = 0.05 µg/L LC-DAD LOQ = 0.05 µg/L Residues of Nicosulfuron in drinking water: Data gap on ILV for drinking water.

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

An overview on the acceptable methods and possible data gaps for analysis of Nicosulfuron in air is given in the following tables (please refer to the DAR of Nicosulfuron, June 2006 and Addendum to the DAR, July 2007).

Table 5.3-12: Validated methods for air (if appropriate)

Component of residue definition: Nicosulfuron			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	1.2 µg/m^3 air 1.2 µg/m^3 air	HPLC-UV HPLC-UV	Schulz and Ullrich-Mitzel, 1995b Wais, 2000c
Confirmatory	-	-	Sufficient confirmatory methods are available for the determination in soil or water therefore confirmatory methods for the determination of residues in air are not required.

Study Comments: IIIA 5.3.3.6	Adequate method exists to monitor Nicosulfuron residues in air. The analytical methods presented by the applicant are active substances data, which were reviewed in the Assessment Report for Nicosulfuron and were considered adequate. Additional methods for the purpose of the evaluation are not required.
Agreed end-point: IIIA 5.3.3.6	Residues of Nicosulfuron in air: HPLC-UV LOQ = 1.2 µg/m^3

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

As Nicosulfuron is classified as neither toxic nor highly toxic, methods for therapeutic analysis are not required (*DAR, 2005*).

5.3.3.8 Other studies/ information

No new or additional studies have been submitted.

5.3.4 Description of analytical methods for the determination of residues of Mesotrione (KCP 5.2)

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

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

Table 5.3-13: Relevant residue definitions for monitoring/enforcement and levels for which compliance is required

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Plant, high water content	Mesotrione	0.01 mg/kg	Regulation No. 626/2017
Plant, high acid content		0.01 mg/kg	Regulation No. 626/2017
Plant, high protein/high starch content (dry commodities)		0.01 mg/kg	Regulation No. 626/2017
Plant, high oil content		0.01 mg/kg	Regulation No. 626/2017
Plant, difficult matrices (hops, spices, tea)		0.05 mg/kg	Regulation No. 626/2017
Muscle	Mesotrione	0.01 mg/kg	Regulation No. 626/2017
Milk		0.01 mg/kg	Regulation No. 626/2017
Eggs		0.01 mg/kg	Regulation No. 626/2017
Fat		0.01 mg/kg	Regulation No. 626/2017
Liver, kidney		0.01 mg/kg	Regulation No. 626/2017
Soil (Ecotoxicology)	Mesotrione MNBA AMBA	0.002 mg/kg 0.002 mg/kg 0.002 mg/kg	EFSA Journal 2016;14(3):4419
Drinking water (Human toxicology)	Mesotrione MNBA AMBA	0.05 µg/L 0.05 µg/L 0.05 µg/L	EFSA Journal 2016;14(3):4419
Surface water (Ecotoxicology)	Mesotrione MNBA AMBA	0.05 µg/L 0.05 µg/L 0.05 µg/L	EFSA Journal 2016;14(3):4419
Air	Mesotrione	0.45 µg/m^3	EFSA Journal 2016;14(3):4419
Tissue (meat or liver)	Mesotrione	not required	not classified as T / T+
Body fluids		not required	not classified as T / T+

5.3.4.2 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 5.3-14: Validated methods for food and feed of plant origin (required for all matrix types, "difficult" matrix only when indicated by intended GAP)

Component of residue definition: Mesotrione				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
High water content (Maize: forage and silage)	Primary (Mesotrione)	0.01 mg/kg	~~ChEChERS~~ QuEChERS LC-MS/MS	EU agreed EFSA Journal 2016; 14 (3) : 4419 Watson G., 2013a (RAR 2015)
	ILV (Mesotrione)	0.01 mg/kg	LC-MS/MS	EU agreed EFSA Journal 2016; 14 (3) : 4419 Tessier V., 2013 (RAR 2015)
	Confirmatory (if required) (Mesotrione)	-	-	Not required, the method is highly specific
High acid content (Orange)	Primary	0.01 mg/kg	~~ChEChERS~~ QuEChERS LC-MS/MS	EU agreed EFSA Journal 2016; 14 (3) : 4419 Watson G., 2013a (RAR 2015)
	ILV	0.01 mg/kg	LC-MS/MS	EU agreed EFSA Journal 2016; 14 (3) : 4419
	Confirmatory (if required)	-	-	Not required, the method is highly specific
High oil content (Oilseed rape)	Primary	0.01 mg/kg	~~ChEChERS~~ QuEChERS	EU agreed EFSA Journal 2016; 14 (3) : 4419 Watson G., 2013a (RAR 2015)
	ILV	0.01 mg/kg	LC-MS/MS	EU agreed EFSA Journal 2016; 14 (3) : 4419
	Confirmatory (if required)	-	-	Not required, the method is highly specific
High protein/high starch content (dry)(Maize: grain)	Primary (Mesotrione)	0.01 mg/kg	ChEChERS LC-MS/MS	EU agreed EFSA Journal 2016; 14 (3) : 4419 Watson G., 2013a (RAR 2015)
	ILV (Mesotrione)	0.01 mg/kg	LC-MS/MS	EFSA Journal 2016; 14 (3) : 4419 Tessier V., 2013 (RAR 2015)
	Confirmatory (if required) (Mesotrione)	-	-	Not required, the method is highly specific
Difficult (if required, depends on intended use)	Primary	-	-	-
	ILV	-	-	-
	Confirmatory (if required)	-	-	-

* MNBA is not component of residue definition

Table 5.3-15: Statement on extraction efficiency

	Method for products of plant origin
Required, available from:	-
Not required, because:	Not required residues ≥ LOQ are not expected.

Study Comments: IIIA 5.3.4.2	Adequate method exists to monitor Mesotrione residues in high protein/high starch content (dry). The analytical methods presented by the applicant are active substances data, which were reviewed in the Assessment Report for Mesotrione and were considered adequate. Data matching for the studies relied upon in the EU review for Mesotrione has been completed under PL evaluation therefore no new methods of analysis for Mesotrione in crops are required.
Agreed end-point: IIIA 5.3.4.2	Residues of Mesotrione in commodities with high protein/high starch content (dry): QuEChERS LC-MS/MS LOQ = 0.01 mg/kg

5.3.4.3 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 5.3-16: Validated methods for food and feed of animal origin (if appropriate)

Component of residue definition: Mesotrione				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Milk	Primary	0.01 mg/kg	QuEChERS LC-MS/MS	EU agreed EFSA Journal 2016; 14 (3) : 4419 Watson G., 2013b (RAR 2015)
	ILV	0.01 mg/kg	QuEChERS LC-MS/MS	Bernal J., 2013 (RAR 2015)
	Confirmatory (if required)	-	-	Not required, the method is highly specific
Eggs	Primary	0.01 mg/kg	QuEChERS LC-MS/MS	EU agreed EFSA Journal 2016; 14 (3) : 4419 Watson G., 2013b (RAR 2015)
	ILV	0.01 mg/kg	QuEChERS LC-MS/MS	Bernal J., 2013 (RAR 2015)
	Confirmatory (if required)	-	-	Not required, the method is highly specific
Muscle	Primary	0.01 mg/kg	QuEChERS LC-MS/MS	EU agreed EFSA Journal 2016; 14 (3) : 4419 Watson G., 2013b (RAR 2015)
	ILV	-	-	-

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**
	Confirmatory (if required)	-	-	Not required, the method is highly specific
Fat	Primary	0.01 mg/kg	QuEChERS LC-MS/MS	EU agreed EFSA Journal 2016; 14 (3) : 4419 Watson G., 2013b (RAR 2015)
	ILV	-	-	-
	Confirmatory (if required)	-	-	Not required, the method is highly specific
Kidney, liver	Primary	0.01 mg/kg	QuEChERS LC-MS/MS	EU agreed EFSA Journal 2016; 14 (3) : 4419 Watson G., 2013b (RAR 2015)
	ILV	0.01 mg/kg	QuEChERS LC-MS/MS	Bernal J., 2013 (RAR 2015)
	Confirmatory (if required)	-	-	Not required, the method is highly specific

Table 5.3-17: Statement on extraction efficiency

	Method for products of animal origin
Required, available from:	-
Not required, because:	Not required residues ≥ LOQ are not expected.

Study Comments: IIIA 5.3.4.3	Adequate method exists to monitor Mesotrione residues in animal matrices. The analytical methods presented by the applicant are active substances data, which were reviewed in the Assessment Report for Mesotrione and were considered adequate. Data matching for the studies relied upon in the EU review for Mesotrione has been completed under PL evaluation therefore no new methods of analysis for Mesotrione in animal matrices are required.
Agreed end-point: IIIA 5.3.4.3	Residues of Mesotrione in all animal matrices: QuEChERS LC-MS/MS LOQ = 0.01 mg/kg

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

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

Table 5.3-18: Validated methods for soil (if appropriate)

Component of residue definition: Mesotrione			
Method type	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary (Mesotrione)	0.002 mg/kg	LC-MS/MS	EU agreed EFSA Journal 2016; 14 (3) : 4419 Jutsum L, Williams R.W., 2012, (Jutsum L, 2013 (RAR 2015)
Confirmatory (Mesotrione)	-	-	Not required, the method is highly specific
Primary (MNBA metabolite)	0.002 mg/kg	LC-MS/MS	EU agreed EFSA Journal 2016; 14 (3) : 4419 Jutsum L, Williams R.W., 2012, (Jutsum L, 2013 (RAR 2015)
Confirmatory (MNBA metabolite)	-	-	Not required, the method is highly specific
Primary (AMBA metabolite)	0.002 mg/kg	LC-MS/MS	EU agreed EFSA Journal 2016; 14 (3) : 4419 Jutsum L, Williams R.W., 2012, (Jutsum L, 2013 (RAR 2015)
Confirmatory (AMBA metabolite)	-	-	Not required, the method is highly specific

*MNBA is not component of residue definition.

Study Comments: IIIA 5.3.4.4	Adequate method exists to monitor Mesotrione residues as well as the metabolites AMBA and MNBA in soil. The analytical methods presented by the applicant are active substances data, which were reviewed in the Assessment Report for Mesotrione and were considered adequate. Data matching for the studies relied upon in the EU review for Mesotrione has been completed under PL evaluation therefore no new methods of analysis for Mesotrione and its metabolites in soil are required.
Agreed end-point: IIIA 5.3.4.4	Residues of Mesotrione in soil: LC-MS/MS LOQ = 0.002 mg/kg AMBA in soil: LC-MS/MS LOQ = 0.002 mg/kg MNBA in soil: LC-MS/MS LOQ = 0.002 mg/kg

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

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

Table 5.3-19: Validated methods for water (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**
Drinking water (Mesotrione)	Primary	0.05 µg/L	LC-MS/MS	EU agreed EFSA Journal 2016; 14 (3) : 4419 Jutsum L, Chamkesam N, 2013, Jutsum L, 2013a, (RAR 2015)
	ILV	0.05 µg/L	LC-MS/MS	EU agreed EFSA Journal 2016; 14 (3) : 4419 Wiesner F, Breyer N, 2013 (RAR 2015)
	Confirmatory	-	-	Not required, the method is highly specific
Drinking water (AMBA metabolite)	Primary	0.05 µg/L	LC-MS/MS	EU agreed EFSA Journal 2016; 14 (3) : 4419 Jutsum L, Chamkesam N, 2013, Jutsum L, 2013a, (RAR 2015)
	ILV	0.05µg/L	LC-MS/MS	EU agreed EFSA Journal 2016; 14 (3) : 4419 Wiesner F, Breyer N, 2013 (RAR 2015)
	Confirmatory	-	-	Not required, the method is highly specific
Drinking water (Mesotrione + MNBA metabolite)	Primary	0.05 µg/L	LC-MS/MS	EU agreed EFSA Journal 2016; 14 (3) : 4419 Jutsum L, Chamkesam N, 2013, Jutsum L, 2013a, (RAR 2015)
	ILV	0.05µg/L	LC-MS/MS	EU agreed EFSA Journal 2016; 14 (3) : 4419 Wiesner F, Breyer N, 2013 (RAR 2015)
	Confirmatory	-	-	Not required, the method is highly specific
Surface water (Mesotrione)	Primary	0.05 µg/L	LC-MS/MS	EU agreed EFSA Journal 2016; 14 (3) : 4419 Jutsum L, Chamkesam N, 2013, Jutsum L, 2013a, (RAR 2015)
	Confirmatory	-	-	Not required, the method is highly specific
Surface water (MNBA metabolite)	Primary	0.05 µg/L	LC-MS/MS	EU agreed EFSA Journal 2016; 14 (3) : 4419 Jutsum L, Chamkesam N, 2013, Jutsum L, 2013a, (RAR 2015)
	Confirmatory	-	-	Not required, the method is highly specific
Surface water (AMBA metabolite)*	Primary	0.05 µg/L	LC-MS/MS	EU agreed EFSA Journal 2016; 14 (3) : 4419 Jutsum L, Chamkesam N, 2013, Jutsum L, 2013a, (RAR 2015)
	Confirmatory	-	-	Not required, the method is highly specific

*MNBA is not component of residue definition.

Study Comments: IIIA 5.3.4.5	Adequate method exists to monitor Mesotrione residues as well as the metabolites AMBA and MNBA in drinking and surface water. The analytical methods presented by the applicant are active substances data, which were reviewed in the Assessment Report for Mesotrione and were considered adequate. Data matching for the studies relied upon in the EU review for Mesotrione has been completed under PL evaluation therefore no new methods of analysis for Mesotrione and its metabolites in water are required.
Agreed end-point: IIIA 5.3.4.5	Residues of Mesotrione in water: LC-MS/MS LOQ = 0.05 µg/L AMBA in water:

	LC-MS/MS LOQ = 0.05 µg/L MNBA in water: LC-MS/MS LOQ = 0.05 µg/L

5.3.4.6 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 5.3-20: Validated methods for air (if appropriate)

Component of residue definition: Mesotrione			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	0.45 µg/m^3	LC-MS/MS	EU agreed EFSA Journal 2016; 14 (3) : 4419 Jutsum L, 2013b, Jutsum L, 2013c (RAR 2015)
Confirmatory	-	-	Not required, the method is highly specific

Study Comments: IIIA 5.3.4.6	Adequate method exists to monitor Mesotrione residues in air. The analytical methods presented by the applicant are active substances data, which were reviewed in the Assessment Report for Mesotrione and were considered adequate. Data matching for the studies relied upon in the EU review for Mesotrione has been completed under PL evaluation therefore no new methods of analysis for Mesotrione in air are required.
Agreed end-point: IIIA 5.3.4.6	Residues of Mesotrione in air: LC-MS/MS LOQ = 0.45 µg/m^3

5.3.4.7 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 tables.

Table 5.3-21: Methods for body fluids and tissues (if appropriate)

Component of residue definition: Mesotrione			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	0.01 mg/kg in blood	LC-MS/MS	EU agreed EFSA Journal 2016; 14 (3) : 4419 Watson G., 2013b (RAR 2015)
Confirmatory	-	-	Not required, the method is highly

Component of residue definition: Mesotrione			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
			specific

Study Comments: IIIA 5.3.4.7	Adequate method exists to monitor Mesotrione residues in body fluids and tissues. The analytical methods presented by the applicant are active substances data, which were reviewed in the Assessment Report for Mesotrione and were considered adequate. Data matching for the studies relied upon in the EU review for Mesotrione has been completed under PL evaluation therefore no new methods of analysis for Mesotrione in body fluids and tissues are required.
Agreed end-point: IIIA 5.3.4.7	Residues of Mesotrione in blood: QuEChERS LC-MS/MS LOQ = 0.01 mg/kg

5.3.4.8 Other studies/ information

No new or additional studies have been submitted.

Appendix 1 Lists of data considered in support of the evaluation

Tables considered not relevant can be deleted as appropriate.

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

List of data submitted by the applicant and relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 5.1.1	Michalec-Minch	2018	Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WG: Analysis of active substance and physicochemical properties of initial preparation and preparation after accelerated storage procedure Institute of Heavy Organic Synthesis „Blachownia'' report no. 7/2018 GLP; unpublished	N	Sharda Cropchem Limited
KCP 5.1.1-01	Ewa Nowakowska-Bogdan	2020	Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WG Analysis of relevant impurities content of initial preparation and preparation after accelerated storage procedure Report No.: 163/2020 GLP Unpublished	N	Sharda Cropchem Limited
KCP 5.2.1	Anna Markowicz	2019	Validation of the method for determination of Rimsulfuron in maize by liquid chromatograpchy Food Safety Labolatory Research Institute of Horticulture, Report No. 19/FSL/15/1A GLP Unpublished	N	Sharda Cropchem Limited
KCP 5.2.1.1	Manuel Rubino	2019	Validation of the analytical procedure for the determination of residues of Rimsulfuron (CAS: 122931-48-0) in maize by LC-MS CHELAB, Report No. 19.500341.0001 GLP Unpublished	N	Sharda Cropchem Limited

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 5.2.2	Manuel Rubino	2019	Validation of the analytical procedure for the determination of residues of Rimsulfuron (CAS: 122931-48-0) in drinking water by LC-MS CHELAB, Report No. 19.500341.0007 GLP Unpublished	N	Sharda Cropchem Limited
KCP 5.2.2.1	Magdalena Zarębska	2020	Independent Labolatory Validation of the analytical procedure for the determination of residues of Rimsulfuron (CAS: 122931-48-0) in drinking water by liquid chromatography Institute of Heavy Organic Synthesis “Blachownia”, Report No. 30/2020 GLP Unpublished	N	Sharda Cropchem Limited

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

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

The following tables are to be completed by MS

List of data submitted by the applicant and not relied on

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

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

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

Appendix 2 Detailed evaluation of submitted analytical methods

A 2.1 Analytical methods for Rimsulfuron

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

No new or additional studies have been submitted

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

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

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

A 2.1.2.1.1 Analytical method 1

A 2.1.2.1.1.1 Method validation

Comments of zRMS:	The method is acceptable.

Reference:	KCP 5.2.1
Report	Validation of the method for determination of Rimsulfuron in maize by liquid chromatograpchy, Anna Markowicz, 2019, Report No. 19/FSL/15/1A
Guideline(s):	SANCO/3029/99 Rev. 4 SANCO/825/00 Rev. 8.1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Untreated maize samples were storage in the Test Facility in a freezer (≤ -20 °C) in the dark.

Reference substance:
- Rimsulfuron, CAS 122931-48-0, Batch G753032 purchased from Dr. Ehrenstorfer, Purity 96.56%

Reagents:
- Triphenyl phosphate TPP, CAS 115-86-6, Batch 00020650-0138 purchased from ChromaDex, Purity 99.9%;
- Acetonitrile LC/MS grade, CAS 75-05-8, No. 1.00029.2500 from Merck;
- Ammonium formate LC/MS grade, CAS 540-69-2, No. 14266-25G from Fluka;
- ESI-L Low Concentration Tuning Mix, No. G1969-85000 from Agilent;

- Formic acid LC/MS grade, CAS 64-18-6, No. 56302-50ML-F from Fluka;
- Magnesium sulfate anhydrous, CAS 7487-88-9, No. 208094 from Sigma-Aldrich;
- Sodium chloride, CAS 7647-14-5, No. S9888 from Sigma-Aldrich;
- Sodium hydrogen citrate sesquihydrate, CAS 6132-05-4, No. 359084 from Sigma-Aldrich;
- Sodium citrate tribasic dihydrate, CAS 6132-04-3, No. S4641 from Sigma-Aldrich;
- Water LC/MS grade from Merck (Direct-Q).

Materials and apparatus:

- Analytical balance, Sartorius CPA225D-0EC, d=0.01 mg (100 g);
- Analytical balance, Sartorius CPA224S-0CE, d=0.1 mg (220 g);
- Centrifuge Tubes, Thermo Scientific, 50 mL Teflon® FEP;
- Common laboratory glassware;
- Eppendorf® centrifuge tubes, FL Medical, Round-bottom polypropylene Safe-lock, micro-centrifuge tubes (volume 2 ml);
- Freezer, Whirlpool AFG 651-B;
- Freezer, MPM MPM-270-SK-03;
- Freezer, Gorenje F6181AX;
- HPLC Autosampler vials, Anchem Amber glass, 1.5 mL short thread vial;
- Laboratory centrifuge, MPW MPW-350;
- Laboratory centrifuge, Eppendorf Mini Spin;
- Laboratory balance, Radwag PS 1200/C/2, d=0.01g (1200 g);
- Laboratory balance, Radwag WPT 5C;
- Laboratory mill, Robot Coupe R5 Plus;
- Mobile Phase Filtration Apparatus, Sartorius Stedim, Glass filtration system;
- Pipettors, Brand, Set of adjustable pipettors, checked for accuracy and precision, and capable of delivering volumes ranging from 10-1000 µL (10-100 µL, 20-200 µL, 100-1000 µL);
- PTFE Syringe Filter, Alfatec Hydrophilic PTFE, 0.22 µm, 17 mm;
- PTFE Membrane Filter, Membrane Solutions Hydrophilic PTFE, 0.22 µm, 47 mm;
- Pipettes, Glass, Class A, volumetric, various sizes (0.5 ml, 1 mL, 2.5 ml, 5 mL, 10 mL;
- QuEChERS Hand Motion Shaker, Eberbach EL680.Q;
- Refrigerator, Whirlpool, ARZ-845/H;
- Syringe, B. Braun Medical, All plastic barrel, plunger and tip (2 mL);
- Vacuum Pump, KNF Laboport N820.3FT.18;
- Volumetric flasks, Glass, Class A, various sizes (10 mL, 50 mL, 100 mL);
- Vortex Mixer, IKA MS 3 Digital.

Chromatographic conditions:

- HPLC system: Series 1260 Infinity II (Agilent Technologies), Binary Pump (G7111B), Mul-tisampler (G7167A), Thermostatted Column Compartment MCT(G7116A);
- Pre-Column: Agilent 1290 Infinity In-Line Filter (PN: 5067-4368) with 0.3µm frit ring installed (PN: 5023-0271);
- Column: Agilent Poroshell 120 (PN: 699975-302) EC-C18 2.7µm 3.0 x 50 mm
- Column oven temperature: 40 °C;
- Injection volume: 10 µL;
- Autosampler temperature: 10 °C;
- Flow: 0.4 mL/min;
- Mobile phase A: 5 mM Ammonium Formate with 0.01% (v/v) Formic Acid in Water;
- Mobile Phase B: 5 mM Ammonium Formate with 0.01% (v/v) Formic Acid in ACN:H2O 95:5 (v/v);
- Gradient:

Time (min)	Mobile phase A%	Mobile phase B%
0.00	90	10
1.00	90	10
10.00	10	90
14.00	10	90

- Post time: 4 min;
- Total analysis time: 18 min;
- Retention time (approx.): 6.5 min for Rimsulfuron;
- MS system: Agilent Technologies 6470 Triple Quad LC/MS
- Ionization type: Electrospray (ESI, Agilent Jet Stream, G1958-65138)
- Polarity: Positive ion mode;
- Drying gas temperature: 225 °C;
- Drying gas flow: 8 (l/min);
- Sheath gas heater: 350 °C;
- Sheath gas flow: 11 (l/min);
- Nebulizer pressure: 40 (psi);
- Capillary voltage: 5000 (V);
- Nozzle voltage: 500 (V);
- Scan type: MS/MS, Multiple Reaction Monitoring (MRM);
- Time segments:

Index	Start Time (min)	Divert Valve	Polarity	Delta EMV
1	0	To Waste	Negative	0
2	5	To MS	Positive	200
3	11	To Waste	Positive	0

- Scan resolution (FWHM):

MS1- Unit (0.7 amu)	MS2- Unit (0.7 amu)

- Ion mass transition:

Ion mass transition monitored m/z			m/z	Collision energy (V)
Rimsulfuron	432.1	Trans 1:	182.0	21
		Trans 2:	325.0	13
TPP	327.1	Trans 1:	77.0	49
		Trans 2:	152.0	45

Solutions preparation:

- Mobile Phase A: 5 mM ammonium formate 0.01% Formic acid:

500 mL volumetric flask was half filled with water, 0.157 g of ammonium formate (NH4HCO2) and 50 µl of formic acid (HCOOH) was added than solution was agitated gently until all ammonium formate was completely dissolved. Volumetric flask was filled up to the mark with water, closed tightly and mixed by inverting several times. Subsequently proceeded to solvent filtration apparatus equipped with a 0.22 µm Teflon filter. After filtration solvent was transferred to amber HPLC solvent reservoir.

- Mobile Phase B: 5mM Ammonium formate 0.01% formic acid in ACN: H2O, 95:5 (v/v):

500 mL mixing cylinder was filled with 475 mL of acetonitrile. Then, 25 mL volumetric flask was half filled with water, 0.157 g of ammonium formate (NH4HCO2) and 50 µl of formic acid (HCOOH) was added than solution was agitated gently until all ammonium formate was completely dissolved. Volumetric flask was filled up to the mark with water, closed tightly and mixed by inverting several times. Content of volumetric flask was transferred to mixing cylinder. Cylinder was closed and mixed by inverting several times. Subsequently proceeded to solvent filtration apparatus equipped with 0.22 µm Teflon filter. After filtration solvent was transferred to HPLC solvent reservoir.

- Extraction mixture: Acetonitrile (+1 Vol% formic acid):

500 mL volumetric flask was half filled with acetonitrile, 5 mL of formic acid (HCOOH) was added and the solution was agitated gently. Volumetric flask was filled up to the mark with acetonitrile, closed tightly and mixed by.

- Working and stability testing standard solutions:

Using balance reading to five decimals places the appropriate amount of reference item neat standard (corrected for purity) was weighted into appropriate volumetric flask such that when diluted in acetonitrile it yield standard solution of reference item containing 1 mg/mL. Then such prepared stock was used to prepare the standards solutions by diluting with appropriate volume of acetonitrile to obtain the indicated concentrations.

- Solvent and matrix-matched calibration solutions for rimsulfuron:

The respective final sample extracts of control (untreated) samples are fortified with working solution of

Rimsulfuron. Exemplary pipetting scheme for the preparation of solvent and matrix matched calibrations at 0.001 µg/mL and 0.01 µg/mL is presented below.

Additions		Solvent based		Matrix-matched	
Calibration levels (µg/mL)		0.001	0.01	0.001	0.01
Volume of sample extract (µL)		-	-	200	200
Volume of Solvent A (µL)		700	700	700	700
Volume of Acetonitrile (µL)		200	200	-	-
Volume of TPP (µL)		50	50	50	50
Pesticide working solutions	0.02 (µg/mL)	50	-	50	-
	0.2 (µg/mL)	-	50	-	50
Total volume (µL)		1000	1000	1000	1000

Sample preparation:

- Sample extraction

5.00 g ± 0.05 g of homogenized matrix was weighed into a 50 mL Teflon centrifuge tube. Sample weight was recorded. If necessary, fortification of the concurrent recovery sample(s) by aliquoting the fortification standard onto the matrix was carried out at this step. The tube was shaken in a vortex mixer for 1 min and allowed to stand for about 5 min. Using glass volumetric pipette 10 mL of water was added. Using glass volumetric pipette 10 mL of acidified acetonitrile (1% formic acid v/v) was added. The Teflon centrifuge tube was closed tightly and shaken thoroughly for 2 min.

- Liquid-Liquid Partition

A salt mixture (4 g ± 0.2 g of magnesium sulfate anhydrous and 1 g ± 0.05 g of sodium chloride) was added and the centrifuge tube was closed and shaken for 1 min. The extract was centrifuged at 8100 rpm for 5 min.

- Sample Dilution

An aliquot of 0.2 mL of was transferred to new Eppendorf safe-lock tube and subsequently diluted with 0.7 mL of solvent A, 0.05 mL of acetonitrile and 0.05 mL of TPP (0.5 µg/mL). Content was mix gently and filtered through the 0.22 µm Teflon filter attached to a syringe direct into amber HPLC vial. Vial was labelled so that it may be identified.

Results and discussions

Specificity:

The method is specific for the determination of Rimsulfuron by virtue of the chromatographic separation and selective detection system used. Reagents blank and control specimens were extracted and analyzed according to the method to investigate the presence of residue and/or background interference at the retention time of the analyte. For both ion mass transition 1 and 2, the specimen showed no significant interference (≤ 30% LOQ) at the retention time of the analyte.

Linearity:

The linearity of the detector response for Rimsulfuron was demonstrated by single determination of matrix-matched calibration standards at nine concentration levels ranging from 0.0002 µg/mL to 0.1 µg/mL for maize whole plant and grain. This range correspond from 0.002 mg/kg to 1 mg/kg thus covering the range from no more than 30 % of the LOQ and at least + 20 % of the highest analyte concentration level detected in samples.

Accuracy and Precision:

Five recovery determinations were performed at the LOQ (0.01 mg/kg) and at the 10 x LOQ (0.1 mg/kg) for maize grain and at the LOQ (0.01 mg/kg) and at the 50 x LOQ (0.5 mg/kg) for maize whole plant respectively. Analysis was performed by extraction and single injection.

The mean recovery values at the fortification levels of 0.01 mg/kg, 0.1 mg/kg and 0.5 mg/kg for both ion mass transitions were all in the range 70 – 110 % and thus comply with the standard acceptance criteria.

All precision values at the fortification levels of 0.01 mg/kg, 0.1 mg/kg and 0.5 mg/kg for both ion mass transitions were < 20%.

Limit of Quantification (LOQ):
The LOQ of the method was defined as the lowest analyte concentration at which the methodology had been successfully validated. Thus, an LOQ of 0.01 mg/kg was confirmed for Rimsulfuron in maize matrices.

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

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
Maize whole plant	Rimsulfuron	0.01	Trans 1: 88 Trans 2: 88	Trans 1: 3.5 Trans 2: 3.5	-
Maize whole plant	Rimsulfuron	0.5	Trans 1: 92 Trans 2: 93	Trans 1: 4.3 Trans 2: 4.1	-
Maize grain	Rimsulfuron	0.01	Trans 1: 75 Trans 2: 76	Trans 1: 4.0 Trans 2: 6.2	-
Maize grain	Rimsulfuron	0.1	Trans 1: 83 Trans 2: 83	Trans 1: 3.6 Trans 2: 3.5	-

Table A 2: Characteristics for the analytical method used for validation of Rimsulfuron residues in maize

	Rimsulfuron
Specificity	The method is specific. Reagents blank and control specimens were extracted and analyzed according to the method to investigate the presence of residue and/or background interference at the retention time of the analyte. For both ion mass transitions, the specimen showed no significant interference (above 30 % of the LOQ) at the retention time of the analyte.
Calibration (type, number of data points)	It was evalueated 9 different levels of concentration covering the range from no more than 30 % of the LOQ and at least + 20 % of the highest analyte concentration level detected in samples. Regresion plot of Transition 1 for maize whole plant: $y=0.576358x + 0.0002272284$, $R^2=0.99972503$ Regresion plot of Transition 2 for maize whole plant: $y=0.244676x + 0.0001974397$, $R^2=0.99968640$ Regresion plot of Transition 1 for maize grain: $y=0.531089x + 0.0007327460$, $R^2=0.99875093$ Regresion plot of Transition 2 for maize grain: $y=0.225681x + 0.0004165412$, $R^2=0.99832024$
Calibration range	Accepted calibration range in concentration units from 0.002 mg/kg to 1 mg/kg. Corresponding calibration range in mass ratio units for the sample was 0.01 mg/kg.
Assessment of matrix effects is presented	Yes
Limit of determination/quantification	Limit of determination was 0.002 mg/kg; Limit of quantification was 0.01 mg/kg.

Conclusion

According to SANTE/2020/12830 Rev.1 the method was sufficiently validated and it is suitable for determination of Rimsulfuron in maize.

A 2.1.2.1.1.2 Independent laboratory validation

Comments of zRMS:	The method is acceptable as ILV.

Reference:	KCP 5.2.1.1
Report	Validation of the analytical procedure for the determination of residues of Rimsulfuron (CAS: 122931-48-0) in maize by LC-MS, Manuel Rubino, 2019, Report No. 19.500341.0001
Guideline(s):	SANCO/3029/99 Rev. 4 SANCO/825/00 Rev. 8.1 OECD-204/2014
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Maize specimens were supplied by the Test Facility and the absence of Rimsulfuron was ensured before using the test item. The test substance was grinded and stored in a freezer before use (about -20°C).

Reference substance:
- Rimsulfuron, CAS 122931-48-0, Batch 774850 purchased from HPC Standards GmbH, Purity 99.4 %

Reagents:
- MilliQ water, SRA 787;
- Acetonitrile, TI-0038770 purchased from VWR;
- Ammonium formate, TI-0037141 purchased from Sigma Aldrich;
- Bakerbond Octadecyl (C18) 40 µm, TI0017316 purchased from J.T.Baker;
- Formic acid (99-100%), TI-0028375 purchased from VWR;
- Magnesium sulfate anhydrous, TI-0023069 purchased from Sigma Aldrich.

Materials and apparatus:
- Common analytical glassware;
- Fridge, SRA 7;
- Analytical balance (±0,1 mg), SRA 602;
- Analytical balance (±0,01 mg), SRA 768;
- Vortex;
- Ultrasonic bath, SRA 469;
- Centrifuge, SRA 55;
- Thermostatic bath equipped with N_2 flow, SRA 66;
- Syringe filter 0.45 µm RC-membrane;
- MS XEVO TQS (Waters-Micromass), SRA 470;
- Acquity UPLC BEH C18, 50 mm x 2.1 mm x 1.7 µm (ID: LC 23).

Instrumental conditions:
- Column: Acquity UPLC BEH C18, 50 mm x 2.1 mm x 1.7 µm (LC 23);
- Mobile Phase A: 10 mM ammonium formate buffer pH 4;
- Mobile Phase B: Acetonitrile;
- Flow: 0.2 mL/min;
- Retention time: ~5.10 minutes;

- Injection Volume: 10 µL;
- Detector: MS XEVO TQS (Waters-Micromass), SRA 470;
- Source: ESI+;
- Source temp.: 150 °C;
- Nebulizer: 6 bar;
- Cone gas: 150 L/h;
- Desolvation gas: 800 L/h;
- Run time: 13 minutes;
- Run mode: MRM (see table below):

	Precursor ion m/z		m/z	Collision energy
Rimsulfuron	432	Quantifier ion (trans 1):	182	20
		Qualifier ion (trans 2):	325	15

- Elution: Gradient

Time (min)	Mobile phase A%	Mobile phase B%
0.00	100	0
0.50	100	0
8.50	0	100
11.50	0	100
11.60	100	0
13.00	100	0

Solutions preparation:

- Mobile phase A (10 mM ammonium formate buffer pH 4):

About 0.62 g of ammonium formate were into a 1000 ml volumetric flask and dissolved with about 500 ml of milliQ water. 0.22 ml of formic acid were added and then the solution was diluted to volume with milliQ water. pH was 4.

- Mobile phase B:

Acetonitrile.

- Blank solution:

10 mM ammonium formate buffer pH 4: acetonitrile, 50:50 (v:v).

- Extraction phase (1% acid formic in acetonitrile:

In a 1000 ml volumetric flask containing about 50 ml of acetonitrile, about 10 ml of formic acid were introduced and then diluted to volume with acetonitrile.

- Stock Reference Standard Solution (SRSS):

10.52 mg (± 0.01 mg) of rimsulfuron were accurately weighed into a 25 m| volumetric flask and diluted to volume with acetonitrile. The final concentration was 418.28 mg/L.

- Intermediate Reference Solution A (IRS-A):

0.15 ml of SRSS were introduced into a 20 ml volumetric flask and diluted to volume with acetonitrile. The final concentration was 3.14 mg/L.

- Intermediate Reference Solution B (IRS-B):

1 ml of IRS-A was introduced into a 10 ml volumetric flask and diluted to volume with acetonitrile. The final concentration was 0.31 mg/L.

- Intermediate Reference Solution C (IRS-C):

1 ml of IRS-B was introduced into a 10 ml volumetric flask and diluted to volume with acetonitrile. The final concentration was 0.03 mg/L.

- Linearity solutions:

Linearity solutions were prepared in order to cover the range from about 30% LOQ (0.003 mg/kg) to about 30xLOQ (0.38 mg/kg) on the sample. LOQ corresponds to 0.01 mg/kg. Solutions were filled up to volume with blank solution. Solution corresponding to about LOQ in the sample, was injected in triplicate for system suitability evaluation; the other solutions were individually injected.

Sample extraction:

About 1 g (± 0.1 mg) of maize was weighed into a 50 ml falcon and 10 ml of extraction phase were added. After vortexing for about 1 min, the sample was introduced into the ultrasonic bath for 5 minutes. The tube was centrifuged at 4700 rpm for 5 min and then the purification step was carried out.

6 ml of supernatant were transferred into a 10 ml plastic tube, containing about 900 mg of magnesium sulphate anhydrous and 150 mg of C18 resin. Vortexed for about1 min and centrifuged at 4000 rpm for 5 min. 1 ml of supernatant was transferred into a 10 ml tube and dried by N_2 flux. The dried sample was then resuspended into 1 ml of blank solution. Vortexed for about 1 min, filtered, transferred into an HPLC vial and injected. The sample was prepared in duplicate.

Reference solution in matrix (for matrix effect calculation):
0.5 ml of supernatant of purified sample were transferred into a 10 ml tube and dried by N_2 flux. The dried sample was then resuspended into 0.5 ml of solution (reference standard at 10xLOQ), vortexed for about 1 min, filtered, transferred into an HPLC vial and injected.

Spiked Sample at LOQ level:
About 1 g (± 0.1 mg) of maize was weighed into a 50 ml falcon, added 0.30 ml of IRS-C solution and added 10 ml of extraction phase. After vortexing for about 1 min, the sample was introduced into the ultrasonic bath for 5 minutes. The tube was centrifuged at 4700 rpm for 5 min and then the purification step was carried out.
6 ml of supernatant were transferred into a 10 ml plastic tube, containing about 900 mg of magnesium sulphate anhydrous and 150 mg of C18 resin. Vortexed for about 1 min and centrifuged at 4000 rpm for 5 min. 1 ml of supernatant was transferred into a 10 ml tube and dried by N_2 flux. The dried sample was then resuspended into 1 ml of blank solution. Vortexed for about 1 min, filtered, transferred into an HPLC vial and inject The sample was prepared in quintuplicate.

Spiked Sample at 10xLOQ level:
About 1 g (± 0.1 mg) of maize was weighed into a 50 ml falcon, added 0.30 ml of IRS-B solution and added 10 ml of extraction phase. After vortexing for about 1 min, the sample was introduced into the ultrasonic bath for 5 minutes. The tube was centrifuged at 4700 rpm for 5 min and then the purification step was carried out.
6 ml of supernatant were transferred into a 10 ml plastic tube, containing about 900 mg of magnesium sulphate anhydrous and 150 mg of C18 resin. Vortexed for about 1 min and centrifuged at 4000 rpm for 5 min. 1 ml of supernatant was transferred into a 10 ml tube and dried by N_2 flux. The dried sample was then resuspended into 1 ml of blank solution. Vortexed for about 1 min, filtered, transferred into an HPLC vial and inject. The sample was prepared in quintuplicate.

Results and discussions

Specificity:
The method is capable to determine the analyte in the presence of the sample matrix. No significant peaks (≤ 30% LOQ) are detected at RT of the target analyte in the blank and test solution with respect to the spiked test solution for both transition 1 and 2.

Linearity:
The method linearity was evaluated at 5 different levels of concentration, ranging from at least 30% LOQ (0.003 mg/kg) to about 30xLOQ (0.38 mg/kg) of analyte on the sample.

Repeatability precision:
Repeatability evaluation was performed on aliquots of sample spiked with Rimsulfuron at LOQ (about 0.01 mg/kg), and 10xLOQ (about 0.10 mg/kg). 5 replicate analyses were performed for each spiking level.

Accuracy:
Recovery is included between 70% and 110% in all cases, in accordance with acceptance criteria.

Limit of Quantification (LOQ):
LOQ is the lowest concentration level where an acceptable degree of linearity, accuracy and precision is established. In this case LOQ corresponds to 0.01 mg/kg.

Table A 3: Recovery results from independent laboratory validation of Rimsulfuron using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (n = 5)	Mean recovery (%)	RSD (%)	Comments
Maize	Rimsulfuron	0.01	Trans 1: 106 Trans 2: 84	Trans 1: 3 Trans 2: 1	-
Maize	Rimsulfuron	0.10	Trans 1: 92 Trans 2: 83	Trans 1: 1 Trans 2: 2	-

Table A 4: Characteristics for the analytical method used for independent laboratory validation of Rimsulfuron residues in maize

	Rimsulfuron
Specificity	The method is capable to determine the analyte in the presence of the sample matrix. No significant peaks (≤30% LOQ) are detected at RT of the target analyte in the blank and test solution with respect to the spiked test solution for both Transition 1 and 2.
Calibration (type, number of data points)	It was evalueated 5 different levels of concentration starting from 30% of the LOQ to at least 120% of the expected highest concentration. Regresion plot of Transition 1: y=2128335x, R^2=0.9960 Regresion plot of Transition 2: y=781595x, R^2=0.9948
Calibration range	Accepted calibration range in concentration units from 0.003 mg/kg to 0.38 mg/kg. Corresponding calibration range in mass ratio units for the sample was 0.01 mg/kg.
Assessment of matrix effects is presented	Yes.
Limit of determination/quantification	Limit of quantification was 0.01 mg/kg.

Conclusion

According to SANTE/2020/12830 Rev.1 the method was sufficiently validated and it is acceptable as ILV for the primary method for determination of Rimsulfuron in maize.

A 2.1.2.1.1.3 Confirmatory method (if required)

No confirmatory method is required

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

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

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

A 2.1.2.4.1 Analytical method 1

A 2.1.2.4.1.1 Method validation

Comments of zRMS:	The method is acceptable.

Reference:	KCP 5.2.2
Report	Validation of the analytical procedure for the determination of residues of Rimsulfuron (CAS: 122931-48-0) in drinking water by LC-MS, Manuel Rubino, 2019, Report No. 19.500341.0007
Guideline(s):	SANCO/3029/99 Rev. 4 SANCO/825/00 Rev. 8.1 OECD-204/2014
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Drinking water specimens were supplied by the Test Facility and the absence of Rimsulfuron was ensured before using the test item. The test substance was stored in fridge at 4°C.

Reference substance:
- Rimsulfuron, CAS 122931-48-0, Batch 774850 purchased from HPC Standards GmbH, Purity 99.4 %

Reagents:
- MilliQ water, SRA 787;
- Methanol, TI-0038421 purchased from VWR;
- Acetonitrile, TI-0038183 purchased from Merck;
- Ammonium formate, TI-0037141 purchased from Sigma Aldrich;
- Formic acid, TI-0028375 purchased from VWR;
- Isolute column C18 500 mg/3 ml (C18), ID: TI-0Q28490, purchased from Biotage.

Materials and apparatus:
- Common analytical glassware;
- Fridge, SRA 7;
- Analytical balance (±0,01 mg), SRA 768;

- Vortex;
- Centrifuge, SRA 55;
- Thermostatic bath equipped with N_2 flow, SRA 66;
- MS XEVO TQS (Waters-Micromass), SRA 470;
- Acquity UPLC BEH C18, 50 mm x 2.1 mm x 1.7 µm (ID: LC 23).

Instrumental conditions:

- Column: Acquity UPLC BEH C18, 50 mm x 2.1 mm x 1.7 µm (LC 23);
- Mobile Phase A: 10 mM ammonium formate buffer pH 4;
- Mobile Phase B: methanol;
- Flow: 0.2 mL/min;
- Retention time: 6.40 minutes;
- Injection Volume: 5 µL;
- Detector: MS XEVO TQS (Waters-Micromass), SRA 470;
- Source: ESI+;
- Source temp.: 150 °C;
- Nebulizer: 6 bar;
- Cone gas: 150 L/h;
- Desolvation gas: 800 L/h;
- Run time: 13 minutes;
- Run mode: MRM (see table below):

Precursor ion m/z			m/z	Collision energy
Rimsulfuron	432	Quantifier ion (trans 1):	182	20
		Qualifier ion (trans 2):	325	15

- Elution: Gradient

Time (min)	Mobile phase A%	Mobile phase B%
0.00	100	0
0.30	100	0
8.50	0	100
11.50	0	100
11.60	100	0
13.00	100	0

Solutions preparation:

- Mobile phase A (10 mM ammonium formate buffer pH 4):

About 0.62 g of ammonium formate were accurately weighed (±0.01 g) into a 1000 ml volumetric flask and dissolved with about 500 ml of milliQ water. 0.22 ml of formic acid were added and then the solution was diluted to volume with milliQ water. pH was 4.

- Mobile phase B:

Methanol.

- Blank solution:

10 mM ammonium formate buffer pH 4: acetonitrile, 50:50 (v:v).

- Stock Reference Standard Solution (SRSS):

10.27 mg (± 0.10 mg) of rimsulfuron were accurately weighed into a 25 m| volumetric flask and diluted to volume with acetonitrile. The final concentration was 408.34 mg/L.

- Intermediate Reference Solution A (IRS-A):

0.15 ml of SRSS were introduced into a 20 ml volumetric flask and diluted to volume with acetonitrile. Then, 1 ml of this solution was introduced into a 10 ml of volumetric flask and diluted to volume with blank solution. The final concentration was 306.25 µg/L.

- Intermediate Reference Solution B (IRS-B):

1 ml of IRS-A was introduced into a 10 ml volumetric flask and diluted to volume with blank solution. The final concentration was 30.63 mg/L.

- Linearity solutions:

Linearity solutions were prepared in order to cover the range from about 30% LOQ (0.013 µg/l) to about 30xLOQ (1.53 µg/L) on the sample. LOQ corresponds to 0.05 µg/L. Solutions were filled up to volume

with blank solution. Solution corresponding to about LOQ in the sample, was injected in triplicate for system suitability evaluation; the other solutions were individually injected.

Sample extraction:
80.0 mL (±0.1 mL) of drinking water were introduced into a becker and added 0.5 ml of formic acid. Then, the water was percolated using a regular vacuum through a C18 cartridge (previously conditioned with 3 ml of methanol and 3 ml of milliQ water) with a flow rate of 2 drop/sec. Then, the cartridge was centrifuged (2000 rpm, 2 min) to eliminate all the water. The analyte was eluted with 4 ml of blank solution and recovered. Transferred into an HPLC vial and inject. The sample was prepared in duplicate.

Reference solution in matrix (for matrix effect calculation):
80.0 ml (±0.1 ml) of drinking water were introduced into a becker and added 0.5 ml of formic acid. Then, the water was percolated using a regular vacuum through a C18 cartridge (previously conditioned with 3 ml of methanol and 3 ml of milliQ water) with a flow rate of 2 drop/sec. Then, the cartridge was centrifuged (2000 rpm, 2 min) to eliminate all the water. The analyte was eluted with 4 ml of blank solution and recovered. 0.5 ml of etuate were transferred in 10 ml of tube and dried by N_2 flux. The dried sample was resuspended with 0.5 ml of solution corresponded at 10xLOQ on the sample. Vortexed and transferred into an HPLC vial and injected.

Spiked Sample at LOQ level:
80.0 ml (±0.1 ml) of drinking water were introduced into a becker, added 0.15 ml of IRS-B and added 0.5 ml of formic acid. Then, the water was percolated using a regular vacuum through a C18 cartridge (previously conditioned with 3 ml of methanol and 3 ml of milliQ water) with a flow rate of 2 drop/sec. Then, the cartridge was centrifuged (2000 rpm, 2 min) to eliminate all the water. The analyte was eluted with 4 ml of blank solution and recovered. Transferred into an HPLC vial and injected. The sample was prepared in quintuplicate.

Spiked Sample at 10xLOQ level:
80.0 ml (±0.1 ml) of drinking water were introduced into a becker, added 0.15 ml of IRS-A and added 0.5 ml of formic acid. Then, the water was percolated using a regular vacuum through a C18 cartridge (previously conditioned with 3 ml of methanol and 3 ml of milliQ water) with a flow rate of 2 drop/sec. Then, the cartridge was centrifuged (2000 rpm, 2 min) to eliminate all the water. The analyte was eluted with 4 ml of blank solution and recovered. Transferred into an HPLC vial and injected, The sample was prepared in quintuplicate.

Results and discussions

Specificity:
The method is capable to determine the analyte in the presence of the sample matrix. No significant peaks (≤ 30% LOQ) are detected at RT of the target analyte in the blank and test solution with respect to the spiked test solution for both transition 1 and 2.

Linearity:
The method linearity was evaluated at 5 different levels of concentration, ranging from at least 30% LOQ (0.015 µg/L) to about 30xLOQ (1.53 µg/L) of analyte on the sample.

Repeatability precision:
Repeatability evaluation was performed on aliquots of sample spiked with Rimsulfuron at LOQ (about 0.05 µg/L), and 10xLOQ (about 0.50 µg/L). 5 replicate analyses were performed for each spiking level.

Accuracy:
Recovery is included between 70% and 110% in all cases, in accordance with acceptance criteria.

Limit of Quantification (LOQ):
LOQ is the lowest concentration level where an acceptable degree of linearity, accuracy and precision is

established. In this case LOQ corresponds to 0.05 µg/L.

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

Matrix	Analyte	Fortification level (µg/L) (n = 5)	Mean recovery (%)	RSD (%)	Comments
Drinking water	Rimsulfuron	0.05	Trans 1: 104 Trans 2: 105	Trans 1: 0.5 Trans 2: 3.8	-
Drinking water	Rimsulfuron	0.5	Trans 1: 105 Trans 2: 107	Trans 1: 1.1 Trans 2: 1.6	-

Table A 6: Characteristics for the analytical method used for validation of Rimsulfuron residues in drinking water

	Rimsulfuron
Specificity	The method is capable to determine the analyte in the presence of the sample matrix. No significant peaks (≤30% LOQ) are detected at RT of the target analyte in the blank and test solution with respect to the spiked test solution for both Transition 1 and 2.
Calibration (type, number of data points)	It was evalueated 5 different levels of concentration starting from 30% of the LOQ to at least 120% of the expected highest concentration. Regresion plot of Transition 1: $y=7904x$, $R^2=0.9994$ Regresion plot of Transition 2: $y=2157x$, $R^2=0.9953$
Calibration range	Accepted calibration range in concentration units from 0.015 µg/L to 1.53 µg/L. Corresponding calibration range in mass ratio units for the sample was 0.05 µg/L.
Assessment of matrix effects is presented	Yes
Limit of determination/quantification	Limit of determination was 0.015 µg/L. Limit of quantification was 0.05 µg/L.

Conclusion

According to SANTE/2020/12830 Rev.1 the method was sufficiently validated and it is suitable for determination of Rimsulfuron in drinking water.

A 2.1.2.4.1.2 Independent laboratory validation

Comments of zRMS:	The method is acceptable as ILV.

Reference:	KCP 5.2.2.1
Report	Independent Labolatory Validation of the analytical procedure for the determination of residues of Rimsulfuron (CAS: 122931-48-0) in drinking water by liquid chromatography, Magdalena Zarębska, 2020, Report No.

	30/2020
Guideline(s):	SANCO/3029/99 Rev. 4 SANCO/825/00 Rev. 8.1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The objective of this study was to perform Independent Laboratory Validation Study of the analytical method for the determination of Rimsulfuron residues in drinking water matrix. The analysis was performed on drinking water according the procedure delivered by the Sponsor "Validation of the analytical procedure for the determination of Rimsulfuron (CAS: 122931-48-0), in drinking water by LC/MS", M. Rubino, 19.500341.0007.

Reference substance:
- Rimsulfuron, CAS 122931-48-0, Batch BCBZ5003 purchased from Sigma Aldrich, Purity 98.3 %

Reagents:
- Water Direct Q3 UV remote, Merck Millipore, CAS 7732-18-5;
- Acetonitrile LC/MS grade, CAS 75-05-8;
- Methanol LC/MS grade, CAS 67-56-1;
- Ammonium formate, LC/MS grade; CAS 540-69-2;
- Formic acid LC/MS, CAS 64-18-6.

Materials and apparatus:
- liquid chromatograph Dionex UltiMate 3000 RS with computer program „CHROMELEON version 6.80";
- mass spectrometer Sciex Q TRAP 4000 with computer program "ANALYST version 1.5.1";
- chromatographic column Synergi Fusion RP, 4µm, 50x2mm;
- analytical balance type AG285, Mettler Toledo;
- automatic pipette Brand Transferpette S 10-100 µl;
- automatic pipette Brand Transferpette S 100-1000 µl;
- Chromabond C18 columns;
- A class laboratory glassware.

Chromatographic conditions:
- Column: Acquity UPLC BEH C18, 50 mmx2.1 mm, 1.7µm (LC 120) changed to Synergi Fusion RP, 4µm, 50x2mm BA-AB MS 07 - No impact;
- Detector: MS/MS;
- Solvent A: 10mM ammonium formate buffer pH 4;
- Solvent B: Methanol;
- Eluent flow: 0.2 mL/min changed to 0.6 mL/min - No impact;
- Column oven temperature: 25°C
- Retention time: ~6.4 minutes changed to ~3.8 minutes due to different column length - No impact;
- Injection volume: 5µl;
- Elution mode: Gradient

Time (min)	Mobile phase A%	Mobile phase B%
0.00	100	0
0.30	100	0
8.50	0	100
11.50	0	100

11.60	100	0
13.00	100	0
Changed to due to different column length – No impact		
0.00	100	0
0.10	100	0
3.00	0	100
4.00	0	100
4.10	100	0
5.00	100	0

- MS System: Sciex Q TRAP 4000

Precursor ion m/z			m/z
Rimsulfuron	432	Quantification (trans 1):	182
		Confirmation (trans 2):	325

- Ionisation type: Electrospray;
- Polarity: Positive ion mode;
- Temperature: 600°C;
- Nebulizing gas, Gas1: 60 psig;
- Drying gas, Gas2: 50 psig;
- Curtain Gas: 35 psig;
- Ion Spray Voltage: 5200 V;
- Scan type: MS/MS, Multiple Reaction Monitoring (MRM);
- Scan resolution: MS1/MS2 –UNIT (0.7 amu);
- Dwell time: 150 msec.

Results and discussions

Specificity:
For both ion mass transitions of Test Solution (unfortified sample) and Blank Solution, the value obtained was lower than 30% of value obtained by injection of standard solution 1.0µg/l (concentration of the standard solution corresponding to 0.05µg/l, LOQ) thus no significant interferences or contamination by Rimsulfuron were found on matrix blank sample.

Linearity:
The linearity of the detector response for Rimsulfuron was demonstrated by single injection of calibration standards at five concentration levels ranging from about 30% LOQ (0.31µg/l what corresponds to 0.015 µg/l) to about 30xLOQ (30.61µg/l what corresponds to 1.53µg/l) of analyte on the sample.

Precision (repeatability):
Repeatability evaluation was performed on aliquots of sample spiked with Rimsulfuron at LOQ (about 0.05 µg/L), and 10xLOQ (about 0.5 µg/L). 5 replicate analyses were performed for each spiking level. % RSD at each fortified level was calculated for both transitions.

Accuracy:
The mean recovery values at the fortification levels of LOQ (0.05 µg/L) and 10xLOQ (0.5 µg/L) for both ion mass transitions of Rimsulfuron were all in the range of 70% – 110% and thus comply with the standard acceptance criteria.

Limit of Quantification (LOQ):
The LOQ of the method at 0.05 µg/L was defined as the lowest analyte concentration at which the methodology was successfully validated.

Table A 7: Recovery results from independent laboratory validation of Rimsulfuron using the analytical method

Matrix	Analyte	Fortification level (µg/L) (n = 5)	Mean recovery (%)	RSD (%)	Comments
Drinking water	Rimsulfuron	0.05	Trans 1: 94 Trans 2: 94	Trans 1: 5.9 Trans 2: 5.0	-
Drinking water	Rimsulfuron	0.5	Trans 1: 101 Trans 2: 102	Trans 1: 1.9 Trans 2: 2.5	-

Table A 8: Characteristics for the analytical method used for independent laboratory validation of Rimsulfuron residues in drinking water

	Rimsulfuron
Specificity	The method is specific. No significant interferences or contaminations (≤30% LOQ) are detected at RT of the target analyte in the blank and test solution with respect to the spiked test solution for both Transition 1 and 2.
Calibration (type, number of data points)	It was evalueated 5 different levels of concentration starting from 30% of the LOQ to 30xLOQ. Regresion plot of Transition 1: $y=3.18e+004x+4.96e+004$, $R^2=0.9980$ Regresion plot of Transition 2: $y=1.85e+004x+2.89e+004$, $R^2=1$
Calibration range	Accepted calibration range in concentration units from 0.015 µg/L to 1.53 µg/L. Corresponding calibration range in mass ratio units for the sample was 0.05 µg/L.
Assessment of matrix effects is presented	Yes
Limit of determination/quantification	Limit of determination was 0.015 µg/L; Limit of quantification was 0.05 µg/L.

Conclusion

According to SANTE/2020/12830 Rev.1 the method was sufficiently validated and it is acceptable as ILV for the primary method for determination of Rimsulfuron in drinking water.

A 2.1.2.4.1.3 Confirmatory method (if required)

No confirmatory method is required

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

A 2.2 Analytical methods for Nicosulfuron

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

No new or additional studies have been submitted

A 2.2.2 Methods for post-authorization control and monitoring purposes (KCP 5.2)

A 2.2.2.1 Description of analytical methods for the determination of residues in plant matrices (KCP 5.2)

No new or additional studies have been submitted

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

No new or additional studies have been submitted

A 2.2.2.3 Description of Methods for the Analysis of Soil (KCP 5.2)

No new or additional studies have been submitted

A 2.2.2.4 Description of Methods for the Analysis of Water (KCP 5.2)

No new or additional studies have been submitted

A 2.2.2.5 Description of Methods for the Analysis of Air (KCP 5.2)

No new or additional studies have been submitted

A 2.2.2.6 Description of Methods for the Analysis of Body Fluids and Tissues (KCP 5.2)

No new or additional studies have been submitted

A 2.2.2.7 A.2.A.9 Other Studies/ Information

No new or additional studies have been submitted

A 2.3 Analytical methods for Mesotrione

A 2.3.1 Methods used for the generation of pre-authorization data (KCP 5.1)

No new or additional studies have been submitted

A 2.3.2 Methods for post-authorization control and monitoring purposes (KCP 5.2)

A 2.3.2.1 Description of analytical methods for the determination of residues in plant matrices (KCP 5.2)

No new or additional studies have been submitted

A 2.3.2.2 Description of analytical methods for the determination of residues in animal matrices (KCP 5.2)

No new or additional studies have been submitted

A 2.3.2.3 Description of Methods for the Analysis of Soil (KCP 5.2)

No new or additional studies have been submitted

A 2.3.2.4 Description of Methods for the Analysis of Water (KCP 5.2)

No new or additional studies have been submitted

A 2.3.2.5 Description of Methods for the Analysis of Air (KCP 5.2)

No new or additional studies have been submitted

A 2.3.2.6 Description of Methods for the Analysis of Body Fluids and Tissues (KCP 5.2)

No new or additional studies have been submitted

A 2.3.2.7 A.2.A.9 Other Studies/ Information

No new or additional studies have been submitted