FINAL REGISTRATION REPORT

Part B

Section 5

Analytical Methods

Detailed summary of the risk assessment

Product code: SAE053H/01

Product name(s): KAGURA/GENKI

Chemical active substances:

Mesotrione, 80 g/L
Nicosulfuron, 30 g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

Document number - SAEDoc-00016 CEU

(authorization)

Applicant: Sumi Agro Europe Limited

Submission date: November 2019, Revised July 2021

MS Finalisation date: 18/02/2022

Version history

When	What
November 2019	dRR submitted by applicant
August 2020	Dossier sent for evaluation to Merit Mark (PL)
July 2021	Version 1, updated dRR Horwitz ratio added Analytical methods information on three additional ecotoxicology studies added. One of these (Lemna) was accidentally missed out from the original submission. The other two studies (Wolffia and Spirodela) have reported recently and were not included in the original Article 33 Application.
October 2021	zRMS finalised evaluation
January 2022	Final version prepared by zRMS after Commenting period
February 2022	Final version prepared by zRMS after Commenting period

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zRMS comments:
The text highlighted in grey was provided by the evaluator.

5 Analytical methods

This document summarizes the analytical methods on the plant protection product SAE053H/01, an oil dispersion (OD) containing 80 g/L mesotrione and 30 g/L nicosulfuron for use in maize in Central Zone according to article 33 of the Regulation 1107/2009.
This application follows the data requirements for the active substances laid down in Regulation (EC) No. 283/2013 or 544/2011 and the data requirements for the plant protection product laid down in Regulation (EC) No. 284/2013.

5.1 Conclusion and summary of assessment

Sufficiently sensitive and selective analytical methods are available for the active substances and relevant impurities in the plant protection product.
Noticed data gaps are:

- None.

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

- None.

Commodity/crop	Supported/ Not supported
Maize	Supported

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

5.2.1 Analysis of the plant protection product (KCP 5.1.1)

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

An overview on the acceptable methods for analysis of mesotrione and nicosulfuron in plant protection product SAE053H/01, an oil dispersion (OD) containing 80 g/L mesotrione and 30 g/L nicosulfuron, is provided as follows:

Comments of zRMS:	This method is accepted and may be used for analyzing mesotrione and nicosulfuron in the PPP.

Reference:	**5.2.1.1/01 (KCP 5.1.1/01)**
Report	Walker A.F., 2016 Validation of Analytical Method JP6001-1 for the determination of Mesotrione and Nicosulfuron Content in Product SAE053H/01 (80/30 OD) Report No.: JP16001-1
Method	JP16001-1
Guideline(s):	SANCO/3030/99 rev. 4
Deviations:	None
GLP:	Yes
Acceptability:	Yes

Materials and methods

The HPLC method for the determination of mesotrione and nicosulfuron content in product SAE053H/01 (80/30 Mesotrione/Nicosulfuron OD) was validated. The determination of mesotrione and nicosulfuron was performed after dilution with acetonitrile by HPLC analysis with UV detection at 255 nm (nicosulfuron) and 290 nm (mesotrione). Quantitation was performed by external standard calibration.

Validation - Results and discussions

Table 5.2-1: Methods suitable for the determination of active substances mesotrione and nicosulfuron in plant protection product SAE053H/01

	Nicosulfuron	**Mesotrione**
Author(s), year	Walker, A.F. 2016	
Principle of method	HPLC analysis with UV detection	
Linearity (linear between mg/L / % range of the declared content) (correlation coefficient, expressed as r)	The linearity of response for the determination of actives in the formulation was determined by the preparation of five (5) reference item solutions over approx. 50-150% of the nominal analytical concentration (0.15 mg/ mL Nicosulfuron and 0.4 mg/ mL Mesotrione). The solutions were injected in duplicate. Calibration curves are presented in the report.	
	Range of concentration levels: 0.075 mg/mL – 0.214 mg/mL The calibration was found to be linear and the results meet the acceptance criteria of r ≥0.99. Y = 9977.4 * X + 3.32, r = 1.0000	Range of concentration levels: 0.214 mg/mL – 0.612 mg/mL The calibration was found to be linear and the results meet the acceptance criteria of r ≥0.99. Y = 8897.6 * X + 16.74, r = 1.0000

	Nicosulfuron	Mesotrione
Precision – Repeatability Mean n = 6, (%RSD)	Mean 30.3 g/L, RSD ±0.43%	Mean 81.3 g/L, RSD ±0.42%
Accuracy n = 6 (% Recovery)	The blank formulation was "spiked" at approximately 75, 100 and 125% of the nominal nicosulfuron and mesotrione concentration (2.01, 2.73, 3.53 % w/w nicosulfuron and 5.74, 7.81, 10.1 %w/w mesotrione). Each fortification was undertaken in duplicate prior to the addition of the extraction solvent. Each fortification level was taken through the sample preparation procedure for method JP16001-1 and quantified in order to calculate % recovery.	
	2.01% w/w: 99.9% 2.73% w/w: 99.9% 3.53% w/w: 100%	5.74% w/w: 101% 7.81% w/w: 101% 10.1% w/w: 102%
Interference/ Specificity	No significant interferences were present in the region of the nicosulfuron peak (2.5 - 3.5 min.) or mesotrione peak (4.5 – 6.0 min.) in the reagent blank or the formulation blank chromatogram. No significant interferences were present in the region of the nicosulfuron peak from the mesotrione reference item and no significant interferences were present in the region of the mesotrione peak from the nicosulfuron reference item.	
Peak Identity	Peak apex UV spectra for nicosulfuron and mesotrione in the reference item chromatograms gave a qualitative match to those generated for the corresponding peaks in the test item chromatogram. Peak apex mass spectra for nicosulfuron and mesotrione in the test item chromatogram gave comparable profiles to those recorded for the reference items. The mass spectra are consistent with the nicosulfuron and mesotrione structures.	
Solution Stability	Both calibration and formulation solutions are stable for at least 48 hours at 5°C	
Comment	Acceptable	Acceptable

Conclusion

The HPLC method JP16001-1 for the determination of mesotrione and nicosulfuron content in product SAE053H/01 (80/30 Mesotrione/Nicosulfuron OD) was validated according to SANCO/3029/99 rev. 4.

Walker A.F. (2016)

The new current requirement of the guideline SANCO/3030/99 rev.5, Horwitz ratio (Hr), is calculated below in order to fulfil the requirements of the new version of the guideline:

H_r = 0.221 for Mesotrione, acceptable criteria for the precision.
H_r = 0.186 for Nicosulfuron, acceptable criteria for the precision.

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

An overview on the acceptable methods and possible data gaps for analysis of relevant impurities in the plant protection product SAE053H/01, an oil dispersion (OD) containing 80 g/L mesotrione and 30 g/L nicosulfuron, is provided as follows.

R1 = Impurity R287431 (6-(Methylsulfonyl)-7-nitro-9-oxo-9H-xanthene-1-carbonitrile)
R2 = impurity R287432 (6-(Methylsulfonyl)-9-oxo-9H-xanthene-1-carbonitrile)

The relevant mesotrione impurities R1 and R2 are present after the manufacturing process of technical mesotrione. Therefore analytical method validation for the determination of the impurities R1 and R2 in the plant protection product SAE053H/01 were performed and the summary of these methods are presented below. For more details, see Appendix 2.

1,2 Dichloroethane (DCE) is present as a residual solvent used in the manufacturing process of technical mesotrione, however, the level of this substance will not increase during storage of the final OD-formulation, since there are no chemical or physical processes through which DCE can be formed from the molecule mesotrione. As 1,2 Dichloroethane is considered to be of toxicological concern, a method for its determination in the SAE053H/01 was performed and the summary of this method is presented below.

During the manufacturing process of nicosulfuron no relevant impurities are produced, therefore no analytical method validation is presented.

Comments of zRMS:	These methods are accepted and may be used for analysing impyrities R1 and R2 in the PPP.

Reference:	**5.2.1.1/02 (KCP 5.1.1/02)**
Report	Wronska L., 2016 Validation of Analytical Method JP6001-5 for the determination of Impurities R1 and R2 in Product SAE053H/01 (80/30 Mesotrione/Nicosulfuron OD) Report No.: JP160015
Method	JP16001-5
Guideline(s):	SANCO/3030/99 rev. 4
Deviations:	None
GLP:	Yes
Acceptability:	Yes

Materials and methods

The HPLC method for the determination of mesotrione and nicosulfuron content in product SAE053H/01 (80/30 Mesotrione/Nicosulfuron OD) has been validated. The determination of mesotrione and nicosulfuron was performed after dilution with acetonitrile by HPLC analysis with UV detection and external standard calibration.

R1 = Impurity R287431 (6-(Methylsulfonyl)-7-nitro-9-oxo-9H-xanthene-1-carbonitrile)
R2 = impurity R287432 (6-(Methylsulfonyl)-9-oxo-9H-xanthene-1-carbonitrile)

The method has shown acceptable performance for determining impurity R2 in terms of specificity, linearity, accuracy (recovery), precision (repeatability) and the LOQ of the method was determined to be 0.0092% w/w of impurity R2 relative to the formulation. This equates to an LOQ of 0.11 % of the mesotrione in the formulation, where mesotrione was determined as 8.33 % w/w in SAE053H/01.

In addition, due to the interference detected with the R1 impurity peak and the low levels of R1 required, a limit test method was verified for mesotrione impurity R1. The method is capable of detecting R1 at a level of above 1 µg/g in formulation SAE053H/01. This is a qualitative method which comprises of the analysing the formulation and the formulation spiked with R1 at a known concentration. The chromatograms are then overlaid and a pass/fail criteria applied if the peak area of the unspiked sample is greater (fail) or less (pass) than that of the spiked sample.

Validation - Results and discussions

Table 5.2-2: Method suitable for the determination of the relevant impurity R2 in plant protection product SAE053H/01

	Impurity R2 (R287432 (6-(Methylsulfonyl)-9-oxo-9H-xanthene-1-carbonitrile))
Author(s), year	Wronska, L., 2016
Principle of method	HPLC analysis with UV detection
Linearity (linear between mg/L / % range of the declared content) (correlation coefficient, expressed as r)	The linearity of the detector response of R2 was demonstrated by duplicate determination of solvent calibration standards at five (5) concentration levels ranging from 2 µg/mL to 50 µg/mL. This range corresponds to 40 µg/g to 1000 µg/g (0.004 % to 0.1 %) in the formulation. The approximate equivalent to mesotrione is 0.05% - 1.20 % (for 8.33 % w/w of mesotrione in the formulation). The calibration was found to be linear with a correlation coefficient (r) of 0.9999. These results meet the acceptance criteria of r ≥0.99. Y = 15.3805 * X + 2.6304, r = 0.9999
Precision – Repeatability Mean n = 2-6 (%RSD)	6 independent samples of the test item were analysed and no R2 impurity was detected in the precision samples (the mean content is reported as <LOQ). Since R2 impurity was not found in the test item samples, the relative standard deviation (RSD) of 6 replicates of recovery solutions spiked at the LOQ level was measured and found to be: Repeatability: 0.039 % (RSD) at 0.0092% (measured LOQ) %RSD < %RSD (Horwitz) (5.4%) (1)
Accuracy n = 6 (% Recovery)	Overall Mean Recovery = 102.6%: n=6 replicates at 0.008% (nominal LOQ) n=2 duplicate at 0.016% n=2 duplicate at 0.024% The overall recovery was in the range of 75 - 125 % and thus complies with the standard acceptance criteria of the guidance document SANCO/3030/99 rev 4 for a preparation containing <0.1 % impurity.
Specificity (degree of interference)	Interferences from co-formulants: No interfering peaks were detected from co-formulants or reagents in chromatograms of the blank formulation, mesotrione (TGAI) and nicosulfuron (TGAI), reference standards, and the solvent blank, in the region of the R2 impurity peak. The R2 reference standard and test item both gave peaks at retention times

<table>
<tr><td></td><td>Impurity R2 (R287432 (6-(Methylsulfonyl)-9-oxo-9H-xanthene-1-carbonitrile))</td></tr>
<tr><td></td><td>(± 0.02 min) of 29.6 minutes.</td></tr>
<tr><td>Specificity (identification)</td><td>Peak Identity:
The retention time and UV-DAD of the impurity chromatographic peak obtained for the test item/spiked test item were recorded and compared to those recorded for the corresponding peak generated for the certified reference item.

The retention time recorded for the R2 impurity peak in the test item matched that recorded for the reference standard. UV recorded for the R2 impurity peak in the spiked test item matched those recorded for the reference standard.

As R2 impurity was not present in the test item at a high enough level to generate spectral data, it was spiked at approximately 0.1% of the formulation.</td></tr>
<tr><td>LOQ</td><td>The limit of quantitation (LOQ) is defined as the lowest concentration tested, at which an acceptable mean recovery and % RSD are obtained and has been determined from the Accuracy data (% recovery from formulation).
<table>
<tr><td>Specification Limit</td><td>Sample</td><td>% w/w R2 impurity (recovered)</td><td>% Recovery</td></tr>
<tr><td rowspan="6">0.5 x</td><td>0.008% Recovery 1</td><td>0.0090579</td><td>98.3</td></tr>
<tr><td>0.008% Recovery 2</td><td>0.0091683</td><td>99.5</td></tr>
<tr><td>0.008% Recovery 3</td><td>0.0096701</td><td>105.9</td></tr>
<tr><td>0.008% Recovery 4</td><td>0.0092941</td><td>102.4</td></tr>
<tr><td>0.008% Recovery 5</td><td>0.0094363</td><td>102.1</td></tr>
<tr><td>0.008% Recovery 6</td><td>0.0085963</td><td>101.2</td></tr>
<tr><td colspan="2">Mean:</td><td>0.0092038</td><td>101.6</td></tr>
<tr><td colspan="2">Std Dev:</td><td>0.00036639</td><td></td></tr>
<tr><td colspan="2">% RSD:</td><td>0.039808</td><td></td></tr>
<tr><td colspan="2">Acceptable RSDr:</td><td>5.4</td><td></td></tr>
</table>
The LOQ of the method is 0.0092% w/w of impurity R2 relative to the formulation. This equates to an LOQ of 0.11 % of the Mesotrione in the formulation, where Mesotrione was determined as 8.33 % w/w in SAE053H/01.

The acceptability of recovery for a preparation containing <0.1% impurity is 75-125%; the acceptability of the %RSD using the modified Horwitz equation for a preparation containing 0.009% analyte is ≤5.4.</td></tr>
<tr><td>Comment</td><td>Acceptable</td></tr>
</table>

(1) %RSD (Horwitz) = $2^{(1-0.5 \log C)}$*0.67

Table 5.2-3: Method suitable for the determination of the relevant impurity R1 in plant protection product SAE053H/01

	Impurity R1 (R287431 (6-(Methylsulfonyl)-7-nitro-9-oxo-9H-xanthene-1-carbonitrile))
Author(s), year	Wronska, L., 2016

	Impurity R1 (R287431 (6-(Methylsulfonyl)-7-nitro-9-oxo-9H-xanthene-1-carbonitrile))
Principle of method	HPLC analysis with UV detection
Linearity (linear between mg/L / % range of the declared content) (correlation coefficient, expressed as r)	The linearity of the detector response of R1 was demonstrated by duplicate determination of solvent calibration standards at three (3) concentration levels ranging from 0.25 µg/mL to 1.25 µg/mL. This range corresponds to 1 µg/g to 5 µg/g (0.0001 % to 0.0005 %) in the formulation. The approximate equivalent to mesotrione is 0.001% - 0.006 % (for 8.33 % w/w of mesotrione in the formulation). The calibration was found to be linear with a correlation coefficient (r) of 0.99981. These results meet the acceptance criteria of r ≥0.99. Linear regression was performed without weighting. Y = 183.01907 * X + (-2.87492), r = 0.99981
Precision – Repeatability Mean (%RSD)	Not determined, as a limit test method was used due to the interference observed with the R1 impurity peak.
Accuracy (% Recovery)	Not determined, as a limit test method was used due to the interference observed with the R1 impurity peak.
Specificity (degree of interference)	Interferences from co-formulants: There was no interference in the region of the R1 impurity peak in the chromatograms of the solvent blank, or mesotrione TGAI solutions and small interference in the blank formulation. There was an interference seen in the formulation solution and nicosulfuron TGAI solution which interfered with the R1 impurity peak. It was not possible to resolve the R1 and the impurity from one another using various chromatographic methods. The R1 reference standard gave a peak at retention time of 43.16 minutes. The impurity seen in the formulation solution had a retention time of 43.18 minutes. The UV spectra confirm the peak observed in the formulation solution is not the R1 impurity. The UV spectrum recorded for the R1 impurity peak in the spiked test item provided a good match that recorded for the corresponding peak in the reference standard.
Specificity (identification)	Peak Identity: R1 impurity was not detected in the test item and therefore a UV spectra could not be generated; a test item solution spiked with the impurity was used for spectral identification. The retention time and UV-DAD of the impurity chromatographic peak obtained for the test item/spiked test item were recorded and compared to those recorded for the corresponding peak generated for the certified reference item. It was noted during the analysis that the retention times did shift due to high concentration of sample injected.
Specificity (Limit Test)	Due to the interference detected and the low levels of R1 required, a limit spike test was deemed the most suitable. This is a qualitative method which comprises of the analysing the formulation and the formulation spiked with R1 at a known concentration. The chromatograms are then overlaid and a pass/fail criteria applied if the peak area of the unspiked sample is greater (fail) or less (pass) than that of the spiked sample. In order to demonstrate the method can be used as a limit test for impurity

	Impurity R1 (R287431 (6-(Methylsulfonyl)-7-nitro-9-oxo-9H-xanthene-1-carbonitrile))
	R1 above 1 µg/g in the formulation, the following solutions were prepared, in duplicate, according to the method. - Test item - Test item spiked with 1 µg/g R1 (0.0001 %) - Test item spiked with 2 µg/g R1 (0.0002 %) The limit test was evaluated by overlaying chromatograms to show the increase in R1 response in the spiked test item chromatograms compared to the chromatograms for the test item unspiked. From the overlay, it is possible to state that the R1 in the formulation sample is less than 0.0001% or 1 µg/g in the formulation; equivalent to 0.0012% of Mesotrione in the formulation.
LOQ	Not determined, as a limit test method was used due to the interference observed with the R1 impurity peak.
Comment	Acceptable

Conclusion

The HPLC Analytical Method JP16001-5 for the determination of mesotrione impurity R2 (R287432) in formulation SAE053H/01 has been validated to SANCO/3029/99 rev. 4 regulations.
The limit test method for impurity R1 (R287431) has been shown to be fit for purpose for detecting R1 in formulation SAE053H/01 above a level of 0.0001% (1 µg/g) R1 impurity relative to the formulation.

A GC-FID method used to quantify 1,2 Dichloroethane in Product SAE053H/01 was also fully validated and is presented below. For more details, see Appendix 2.

Comments of zRMS:	The method is accepted for analysing 1,2 Dichloroethane in the PPP.

Reference:	**5.2.1.1/03 (KCP 5.1.1/03)**
Report	Wronska, L. (2017) Validation of Analytical Method JP6001-6 for the determination of Impurity 1,2 Dichloroethane in Product SAE053H/01 (80/30 Mesotrione/Nicosulfuron OD) Report No.: JP160016
Method	JP16001-5
Guideline(s):	SANCO/3030/99 rev. 4
Deviations:	None
GLP:	Yes
Acceptability:	Yes

Materials and methods

The GC-FID method of analysis JP16001-6 for the determination of the mesotrione impurity 1,2 Dichloroethane (DCE) in the formulation SAE053H/01 (80/30 Mesotrione/Nicosulfuron OD) has been validated. A test item solution spiked with the impurity was used for spectral identification and quantification was done by GC-FID. The LOQ of the method is 0.004% w/w of impurity 1,2, Dichloroethane relative to

the formulation. This equates to an LOQ of 0.05 % of the mesotrione in the formulation, where mesotrione was determined as 8.33 % w/w in SAE053H/01.

Validation - Results and discussions

Table 5.2-4: Method suitable for the determination of the relevant impurity 1,2 Dichloroethane in plant protection product SAE053H/01

	Impurity 1,2 Dichloroethane
Author(s), year	Wronska, L., 2017
Principle of method	GC analysis with FID detection
Linearity (linear between mg/L / % range of the declared content) (correlation coefficient, expressed as r)	The linearity of the detector response of DCE was demonstrated by duplicate determination of solvent calibration standards at five (5) concentration levels ranging from 1 µg/mL to 10 µg/mL. This range corresponds to 20 µg/g to 200 µg/g (0.002 % to 0.02 %) in the formulation. The approximate equivalent to mesotrione is 0.024% - 0.240 % (for 8.33 % w/w of mesotrione in the formulation). The calibration was found to be linear with a correlation coefficient (r) of 0.99982. These results meet the acceptance criteria of r ≥0.99. Y = 144947.44 * X + (-39374.15), r = 0.99982
Precision – Repeatability Mean n = 6 (%RSD)	6 independent samples of the test item were analysed and the mean content found to be 0.0008% w/w of DCE in formulation, which is equivalent to <LOQ to Mesotrione. Since 1,2-Dichloroethane was found as <LOQ in the test item samples, the relative standard deviation (RSD) of 6 replicates of recovery solutions spiked at the LOQ level was measured, and found to be: Repeatability: 6.96 % (RSD) at 0.004% (LOQ) The calculated %RSD was ~7%. The modified Horwitz equation is derived empirically and is not suitable for all situations. A 7% RSD on a %w/w value of 0.004 equates to a range of ±0.00028%. The modified Horwitz %RSD limit is 6.2% (1) which would equate to 0.00025%. The difference between the two values at that level is not significant. The RSD% is deemed acceptable for the concentration levels assessed
Accuracy n = 2-6 (% Recovery)	Overall Mean Recovery = 99.0%: n=6 replicates at 0.004% (LOQ) n=2 duplicate at 0.008% (content of DCE measured in the sample) n=2 duplicate at 0.012% The overall recovery was in the range of 80 - 120 % and thus complies with the standard acceptance criteria of the guidance document SANCO/3030/99 rev 4 for a preparation containing 0.1 - 1 % impurity.
Specificity (degree of interference)	Interferences from co-formulants: No interfering peaks were detected in the reagent blank or co-formulant Nicosulfuron, in the region of the DCE impurity peak at ~2.27 minutes. Small peak detected to the right of the main analyte peak in the formulation blank and small peak detected in the formulation and in co-formulant mesotrione; likely to be DCE.
Specificity (identification)	Peak Identity: The DCE impurity was detected in the test item but at such a low level that a meaningful GC-MS spectrum could not be generated. A test item solution

<table>
<tr><td></td><td>Impurity 1,2 Dichloroethane</td></tr>
<tr><td></td><td>spiked with the impurity was used for spectral identification.

The retention time recorded for the DCE impurity peak in the spiked test item matched that recorded for the reference standard in GC-FID.
GC-MS spectra for the DCE impurity peak in the spiked test item matched those recorded for the reference standard.
Ions observed:
DCE Standard: m/z 49, 62, 98
DCE Spiked Sample: m/z 49, 62, 98</td></tr>
<tr><td>LOQ</td><td>The limit of quantitation (LOQ) is defined as the lowest concentration tested, at which an acceptable mean recovery and % RSD are obtained and has been determined from the Accuracy data (% recovery from formulation).

<table>
<tr><td>Specification Limit</td><td>Sample</td><td>% w/w DCE (recovered)</td><td>% Recovery</td></tr>
<tr><td rowspan="6">0.5 x</td><td>0.004% Recovery 1</td><td>0.0035</td><td>91.2</td></tr>
<tr><td>0.004% Recovery 2</td><td>0.0043</td><td>111.2</td></tr>
<tr><td>0.004% Recovery 3</td><td>0.0038</td><td>97.4</td></tr>
<tr><td>0.004% Recovery 4</td><td>0.0037</td><td>95.5</td></tr>
<tr><td>0.004% Recovery 5</td><td>0.0037</td><td>96.7</td></tr>
<tr><td>0.004% Recovery 6</td><td>0.0038</td><td>99.4</td></tr>
<tr><td colspan="2">Mean:</td><td>0.0038</td><td>98.6</td></tr>
<tr><td colspan="2">Std Dev:</td><td>0.000246</td><td></td></tr>
<tr><td colspan="2">% RSD:</td><td>6.96</td><td></td></tr>
<tr><td colspan="2">Acceptable RSDr:</td><td>6.20</td><td></td></tr>
</table>

The LOQ of the method is 0.004% w/w of impurity DCE relative to the formulation. This equates to an LOQ of 0.05 % of the Mesotrione in the formulation, where Mesotrione was determined as 8.33 % w/w in SAE053H/01.

The acceptability of recovery for a preparation containing 0.1-1% impurity is 80-120% and the acceptability of the %RSD using the modified Horwitz equation for a preparation containing 0.004% analyte is ≤6.2.

The calculated %RSD was ~7%. The modified Horwitz equation is derived empirically and is not suitable for all situations. A 7% RSD on a %w/w value of 0.004 equates to a range of ±0.00028%. The modified Horwitz %RSD limit is 6.2% which would equate to 0.00025%.

The difference between the two values at that level is not significant. The %RSD is deemed acceptable for the concentration levels assessed.</td></tr>
<tr><td>Comment</td><td>Acceptable</td></tr>
</table>

(1) %RSD (Horwitz) = $2^{(1-0.5 \log C)}$*0.67

Conclusion

The GC-FID method JP16001-6 for the determination of mesotrione impurity 1,2 Dichloroethane in formulation SAE053H/01 has been validated to SANCO/3029/99 rev. 4 regulations.

Wronska, L. (2017)

The new current requirement of the guideline SANCO/3030/99 rev.5, Horwitz ratio (Hr), is calculated below in order to fulfil the requirements of the new version of the guideline:

H_r = 1.12 for 1,2-dichloroethane at LOQ level, acceptable criteria for the precision with previous explanation.

According to current guideline SANCO/3030/99 rev. 5, the Horrat value between 1 and 2 is acceptable in case of a suggested explanation.

As it has explained above, the range for the real deviation and the calculated deviation are very close. At this level to determination (LOQ = 0.004 %) a small difference between the values can promote a big deviation. The difference between the two values at this level is not significant.

Additionally, if the 110% recovery (0.0043 % w/w DCE) is treated as an outlier and discarded, and only the remaining 5 values of recovery are considered, the standard deviation becomes 0.00011 and therefore the RSD value drops to 3.94. In this situation, the Horrat value obtained is 0.63 and the validated method to determine the quantity of the 1,2-dichloroethane is acceptable.

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

This is not an EC data requirement / not required by Regulation EC 1107/2009.

5.2.1.4 Applicability of existing CIPAC methods (KCP 5.1.1)

The plant protection product SAE053H/01 contains two active substances, mesotrione and nicosulfuron. For these substance the following CIPAC methods are published:

CIPAC method [625] is available for the analysis of mesotrione but no publication is available.

CIPAC method [709] is published for the analysis of nicosulfuron in OD formulation types. The extension of the scope (CIPAC/4903) of CIPAC method 709/TC/M/3 for the determination of the nicosulfuron content of oil-based suspension concentrate formulations (OD) was accepted as a full CIPAC method.

5.2.2 Methods for the determination of residues (KCP 5.1.2)

Mesotrione / Pre-registration methods

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

Table 5.2-5: Validated methods for the generation of pre-authorization data - Mesotrione

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**
Method validation in Maize (whole plant, grain and rest of plant) (Residues)	Primary	0.01 mg/kg	Multi-residue QuEChERS method HPLC-MS/MS m/z 338 → 291 m/z 338 → 212	KCP 5.1.2/01 Schernikau N., Colorado C.S., 2016 Report No. S15-04204 See Appendix 2
	Confirmatory (if required)	0.01 mg/kg	See above (Schernikau N., Colorado C.S., 2016)	
Maize (whole plant, grain and rest of plant) (Residues)	Primary	0.01 mg/kg	Multi-residue QuEChERS method HPLC-MS/MS m/z 338 → 291	KCP 5.1.2/02 (also filed under KCP 8.3.1/01) Semrau J., 2017 Report No. S15-03081 See Appendix 2
	Confirmatory (if required)	-	Not required, highly specific detection system was used (MS)	
Maize (seedlings) (Residues)	Primary	0.01 mg/kg	Multi-residue QuEChERS method HPLC-MS/MS m/z 338 → 291 m/z 338 → 212	KCP 5.1.2/03 (also filed under KCP 8.10) Bakker F., 2016 Report No. JS001LRM See Appendix 2
	Confirmatory (if required)	0.01 mg/kg	See above (Schernikau N., Colorado C.S., 2016)	
Decline residue in maize seedling (residue)	Primary	0.01 mg/kg whole plant w/o roots	Multi-residue QuEChERS method HPLC-MS/MS m/z 338 → 291	KCP 5.1.2/04 (also filed under KCP 8.10) van de Sandt, H.J., 2019 Report No. S17-05218 See Appendix 2
	Confirmatory (if required)	-	Not required, highly specific detection system was used (MS)	
Animal products, food of animal origin (Residues)	Primary	-	No additional data.	
	Confirmatory (if required)	-	No additional data.	
Soil, water, sediment,... (Environmental fate)	Primary	-	No additional data.	
	Confirmatory (if required)	-	No additional data.	
Soil, water,...	Primary	-	No additional data.	

Component of residue definition: mesotrione				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
(Efficacy)	Confirmatory (if required)	-	No additional data.	
Feed, body fluids,... (Toxicology)	Primary	-	HPLC-MS/MS m/z 338→291	KCP 5.2/03 Giesau A. and Grewe D., 2016 Report No. S16-04653 (JSC-1604V) See Appendix 2
	Confirmatory (if required)	-	HPLC-MS/MS m/z 338→212	
Body fluids, air,.... (Exposure)	Primary	-	No additional data.	
	Confirmatory (if required)	-	No additional data.	
Fish acute Toxicity Study OECD 203 (Ecotoxicology)	Primary	LOQ = 0.3 mg/L test item corresponds to 0.0250 mg/L of mesotrione	HPLC-MS/MS m/z 337.85→291.00	KCP 5.1.2/05 (also filed under KCP 10.2.1/01) xxx., 2016 Report No. S16-03041 See Appendix 2
	Confirmatory (if required)	-	Not required, highly specific detection system was used (MS/MS)	
Daphnia acute Toxicity Study OECD 202 (Ecotoxicology)	Primary	LOQ = 0.3 mg/L of test item corresponds to 0.0250 mg/L of mesotrione	HPLC-MS/MS m/z 337.8→290.9	KCP 5.1.2/06 (also filed under KCP 10.2.1/02) Zawadsky C., 2016 Report No. S16-03042 See Appendix 2
	Confirmatory (if required)	-	Not required, highly specific detection system was used (MS/MS)	
Algae Toxicity Study OECD 201 (Ecotoxicology)	Primary	LOQ = 0.05 mg/L of test item corresponds to 0.00417 mg/L of mesotrione	HPLC-MS/MS m/z 337.8→290.9	KCP 5.1.2/07 (also filed under KCP 10.2.1/03) Falk S., 2016a Report No. S16-03039 See Appendix 2
	Confirmatory (if required)	-	Not required, highly specific detection system was used (MS/MS)	
Algae Toxicity Study OECD 201 (Ecotoxicology)	Primary	LOQ = 0.3 mg/L of test item corresponds to 0.0250 mg/L of mesotrione	HPLC-MS/MS m/z 337.8→290.9	KCP 5.1.2/08 (also filed under KCP 10.2.1/04) Falk S., 2016b Report No. S16-03040 See Appendix 2
	Confirmatory (if required)	-	Not required, highly specific detection system was used (MS/MS)	

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**
Lemna acute Toxicity Study OECD 221 (Ecotoxicology)	Primary	LOQ = 3.0 µg/L of test item corresponds to 0.250 µg/L of mesotrione	HPLC-MS/MS m/z 337.85→291.00	KCP 5.1.2/09 (also filed under KCP 10.2.1/05) Lang C., 2016b Report No. S16-03044 See Appendix 2
	Confirmatory (if required)	-	Not required, highly specific detection system was used (MS/MS)	
Lemna acute Toxicity Study OECD 221 (Ecotoxicology)	Primary	LOQ = 0.0698 µg/L of Mesotrione	HPLC-MS/MS m/z 338.0→291.0	KCP 5.1.2/16 (also filed under KCP 10.2.1/07) Bertrand, C., 2019 Report No. S19-03470 See Appendix 2
	Confirmatory (if required)	-	Not required, highly specific detection system was used (MS/MS)	
Spirodela polyrhiza Toxicity Study OECD 221 (Ecotoxicology)	Primary		HPLC-MS/MS m/z 338.2→291.1	KCP 5.1.2/17 (also filed under KCP 10.2.1/08) Christmann, R., 2021a Report No. 218-32 See Appendix 2
	Confirmatory (if required)	-	Not required, highly specific detection system was used (MS/MS)	
Wolffia arrhiza Toxicity Study OECD 221 (Ecotoxicology)	Primary		HPLC-MS/MS m/z 338.2→291.1	KCP 5.1.2/18 (also filed under KCP 10.2.1/09) Christmann, R., 2021b Report No. 218-32 See Appendix 2
	Confirmatory (if required)	-	Not required, highly specific detection system was used (MS/MS)	
Water-sediment Myriophyllum Toxicity Study OECD 239 (Ecotoxicology)	Primary	LOQ (test medium) = 0.003 mg/L of test item corresponds to 0.000250 mg/L of mesotrione LOQ (sediment) = 0.001 mg/kg of mesotrione	HPLC-MS/MS m/z 337.85→291.00	KCP 5.1.2/10 (also filed under KCP 10.2.1/06) Gonsior G., 2016 Report No. S16-03045 See Appendix 2
	Confirmatory (if required)	-	Not required, highly specific detection system was used (MS/MS)	
Daphnia Toxicity Study (reproduction)	Primary	LOQ = 0.003 mg/L of test item corresponds to 0.000250 mg/L of mesotrione	HPLC-MS/MS m/z 337.85→291.00	KCP 5.1.2/11 (also filed under KCP 10.2.2/01) Lang C., 2016a

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**
OECD 211 (Ecotoxicology)				Report No. S16-03043 See Appendix 2
	Confirmatory (if required)	-	Not required, highly specific detection system was used (MS/MS)	
Honey Bee Toxicity Study (Feeding solutions) (Ecotoxicology)	Primary	LOQ = 25.0 mg/L of test item corresponds to 2.08 mg/L of mesotrione	HPLC-MS/MS m/z 338→291	KCP 5.1.2/12 (also filed under KCP 10.3.1.1/01) Molitor A.M., 2016b Report No. S16-02518 See Appendix 2
	Confirmatory (if required)	-	Not required, highly specific detection system was used (MS/MS)	
Honey Bee Larval Toxicity Study (diet samples) (Ecotoxicology)	Primary	LOQ = 120 mg/kg of test item corresponds to 10 mg/kg of mesotrione	HPLC-MS/MS m/z 338→212	KCP 5.1.2/13 (also filed under KCP 10.3.1.3/01) Vergé E. and Wagner J., 2016 Report No. S16-02503 See Appendix 2
	Confirmatory (if required)	-	Not required, highly specific detection system was used (MS/MS)	
Terrestrial Plant Study OECD 208 (spray solutions) (Ecotoxicology)	Primary	LOQ = 700 mg/L test item corresponds to 58.4 mg/L of mesotrione	HPLC-PDA	KCP 5.1.2/14 (also filed under 10.6.2/01) Gröning C., 2017a Report No. S16-02421 See Appendix 2
	Confirmatory (if required)	-	Not required, specific detection system was used (HPLC-PDA)	
Vegetative Vigour Study OECD 227 (spray solutions) (Ecotoxicology)	Primary	LOQ = 0.005 g/L test item corresponds to 0.000417 g/L of mesotrione	HPLC-MS/MS m/z 338→291	KCP 5.1.2/15 (also filed under KCP 10.6.2/02) Gröning C., 2017b Report No. S16-02422 See Appendix 2
	Confirmatory (if required)	-	Not required, highly specific detection system was used (MS/MS)	
Water, buffer solutions,... (Properties)	Primary	-	No additional data.	
	Confirmatory (if required)	-	No additional data.	

Nicosulfuron / Pre-registration methods

An overview on the acceptable methods and possible data gaps for analysis of residues of nicosulfuron for the generation of pre-authorization data is given in the following table. For the detailed evaluation of

new studies refer to Appendix 2.

Table 5.2-6: Validated methods for the generation of pre-authorization data - Nicosulfuron

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**
Plants, plant products,... (Residues)	Primary	-	No additional data.	
	Confirmatory (if required)	-	No additional data.	
Animal products, food of animal origin,… (Residues)	Primary	-	No additional data.	
	Confirmatory (if required)	-	No additional data.	
Soil, water, sediment,... (Environmental fate)	Primary	-	No additional data.	
	Confirmatory (if required)	-	No additional data.	
Soil, water,... (Efficacy)	Primary	-	No additional data.	
	Confirmatory (if required)	-	No additional data.	
Feed, body fluids,... (Toxicology)	Primary	-	No additional data.	
	Confirmatory (if required)	-	No additional data. -	
Body fluids, air,.... (Exposure)	Primary	-	No additional data.	
	Confirmatory (if required)	-	No additional data.	
Fish acute Toxicity Study OECD 203 (Ecotoxicology)	Primary	LOQ = 0.3 mg/L test item corresponds to 0.00939 mg/L of nicosulfuron	HPLC-MS/MS m/z 411.12→182.10	KCP 5.1.2/05 (also filed under KCP 10.2.1/01) xxx, 2016 Report No. S16-03041 See Appendix 2
	Confirmatory (if required)		Not required, highly specific detection system was used (MS/MS)	
Daphnia acute Toxicity Study OECD 202 (Ecotoxicology)	Primary	LOQ = 0.3 mg/L of test item corresponds to 0.00939 mg/L of nicosulfuron	HPLC-MS/MS m/z 411.1→181.8	KCP 5.1.2/06 (also filed under KCP 10.2.1/02) Zawadsky C., 2016 Report No. S16-03042 See Appendix 2
	Confirmatory (if required)		Not required, highly specific detection system was used (MS/MS)	
Algae Toxicity Study OECD 201	Primary	LOQ = 0.05 mg/L of test item corresponds to 0.00157 mg/L of nicosulfuron	HPLC-MS/MS m/z 411.1→181.8	KCP 5.1.2/07 (also filed under KCP 10.2.1/03) Falk S., 2016a Report No. S16-03039 See Appendix 2

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**
(Ecotoxicology)	Confirmatory (if required)		Not required, highly specific detection system was used (MS/MS)	
Algae Toxicity Study OECD 201 (Ecotoxicology)	Primary	LOQ = 0.3 mg/L of test item corresponds to 0.00939 mg/L of nicosulfuron	HPLC-MS/MS m/z 411.1→181.8	KCP 5.1.2/08 (also filed under KCP 10.2.1/04) Falk S., 2016b Report No. S16-03040 See Appendix 2
	Confirmatory (if required)		Not required, highly specific detection system was used (MS/MS)	
Lemna acute Toxicity Study OECD 221 (Ecotoxicology)	Primary	LOQ = 3.0 µg/L of test item corresponds to 0.0939 µg/L of nicosulfuron	HPLC-MS/MS m/z 411.12→182.10	KCP 5.1.2/09 (also filed under KCP 10.2.1/05) Lang C., 2016b Report No. S16-03044 See Appendix 2
	Confirmatory (if required)		Not required, highly specific detection system was used (MS/MS)	
Water-sediment Myriophyllum Toxicity Study OECD 239 (Ecotoxicology)	Primary	LOQ (test medium) = 0.003 mg/L of test item corresponds to 0.0000939 mg/L of nicosulfuron LOQ (sediment) = 0.001 mg/kg of nicosulfuron	HPLC-MS/MS m/z 411.12→182.10	KCP 5.1.2/10 (also filed under KCP 10.2.1/06) Gonsior G., 2016 Report No. S16-03045 See Appendix 2
	Confirmatory (if required)		Not required, highly specific detection system was used (MS/MS)	
Daphnia Toxicity Study (reproduction) OECD 211 (Ecotoxicology)	Primary	LOQ = 0.003 mg/L of test item corresponds to 0.0000939 mg/L of nicosulfuron	HPLC-MS/MS m/z 411.12→182.10	KCP 5.1.2/11 (also filed under KCP 10.2.2/01) Lang C., 2016a Report No. S16-03043 See Appendix 2
	Confirmatory (if required)		Not required, highly specific detection system was used (MS/MS)	
Honey Bee Toxicity Study (Feeding solutions) (Ecotoxicology)	Primary	LOQ = 25.0 mg/L of test item corresponds to 0.783 mg/L of nicosulfuron	HPLC-MS/MS m/z 411→182	KCP 5.1.2/12 (also filed under KCP 10.3.1.1/01) Molitor A.M., 2016b Report No. S16-02518 See Appendix 2
	Confirmatory (if required)		Not required, highly specific detection system was used (MS/MS)	
Honey Bee Larval Toxicity Study (diet samples)	Primary	LOQ = 120 mg/kg of test item corresponds to 3.76 mg/kg of nicosulfuron	HPLC-MS/MS m/z 411→182	KCP 5.1.2/13 (also filed under KCP 10.3.1.3/01) Vergé E. and Wagner J., 2016

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**
(Ecotoxicology)				Report No. S16-02503 See Appendix 2
	Confirmatory (if required)		Not required, highly specific detection system was used (MS/MS)	
Terrestrial Plant Study OECD 208 (spray solutions) (Ecotoxicology)	Primary	LOQ = 700 mg/L test item corresponds to 21.9 mg/L of nicosulfuron	HPLC-PDA	KCP 5.1.2/14 (also filed under 10.6.2/01) Gröning C., 2017a Report No. S16-02421 See Appendix 2
	Confirmatory (if required)		Not required, specific detection system was used (HPLC-PDA)	
Vegetative Vigour Study OECD 227 (spray solutions) (Ecotoxicology)	Primary	LOQ = 0.005 g/L test item corresponds to 0.000157 g/L of nicosulfuron	HPLC-MS/MS m/z 411→182	KCP 5.1.2/15 (also filed under KCP 10.6.2/02) Gröning C., 2017b Report No. S16-02422 See Appendix 2
	Confirmatory (if required)		Not required, highly specific detection system was used (MS/MS)	
Water, buffer solutions,... (Properties)	Primary	-	No additional data.	
	Confirmatory (if required)	-	No additional data.	

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

Mesotrione

With regard to the monitoring enforcement methods (post-registration methods) for mesotrione, reference is made to the analytical methods evaluated on EU level during the AIR process.
For summary, reference is made to the matching studies developed by SAE and Albaugh Europe Sárl, to match the protected methods available on EU level presented in the RAR (2015) for AIR renewal. Sumi Agro Europe Ltd. (the applicant) has access to the equivalent data package. The equivalence has been approved by CRD (UK). Besides, own Sumi Agro Europe Ltd. studies on the active substance are available where necessary. These data are considered to provide the relevant dossier information on the active substance.

Nicosulfuron

With regard to monitoring enforcement methods (post-registration methods) for nicosulfuron, reference is made to the unprotected analytical methods available on EU level and to the analytical methods evaluated on EU level during the AIR process.

Reference is made to the data compiled in the Draft Assessment Report of nicosulfuron from June 2006 and in the AIR renewal dossier from June 2016. Sumi Agro Europe Ltd. have letters of access to use the Nicosulfuron studies evaluated at EU level and which are referenced in this Application.

No additional / new methods are presented in this dossier.
These data are considered to provide the relevant dossier information on the active substance.

5.3.1 Analysis of the plant protection product (KCP 5.2)

Please refer to the analytical methods for the determination of the active substance and relevant impurities in the plant protection product as provided in chapter 5.2.1.

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

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

For this application, it is referred to the following EU concluded residue definitions:

Matrix	Residue Definition	Reference
Plant commodities	mesotrione	EFSA Journal 2016;14(3):4419
Animal origin	Not required as no MRLs were set (provisional)	EFSA Journal 2016;14(3):4419
Soil	mesotrione and metabolite A (open)	EFSA Journal 2016;14(3):4419
Surface water	mesotrione and metabolite A (open)	EFSA Journal 2016;14(3):4419
Drinking / ground water	mesotrione and metabolite A (open)	EFSA Journal 2016;14(3):4419
Air	mesotrione	EFSA Journal 2016;14(3):4419
Body fluids / tissues	mesotrione	EFSA Journal 2016;14(3):4419

Metabolite A = non-identified metabolite

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

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Plant, high water content	mesotrione	0.01 mg/kg	Com. Reg. 2017/626

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Plant, high acid content		0.01 mg/kg	Com. Reg. 2017/626
Plant, high protein/high starch content (dry commodities)		0.01 mg/kg	Com. Reg. 2017/626
Plant, high oil content		0.01 mg/kg	Com. Reg. 2017/626
Plant, difficult matrices (hops, spices, tea)		0.05 mg/kg	Com. Reg. 2017/626
Muscle	mesotrione	0.01 mg/kg	Com. Reg. 2017/626
Milk		0.01 mg/kg	Com. Reg. 2017/626
Eggs		0.01 mg/kg	Com. Reg. 2017/626
Fat		0.01 mg/kg	Com. Reg. 2017/626
Liver, kidney		0.01 mg/kg	Com. Reg. 2017/626
Soil (Ecotoxicology)	mesotrione and metabolite A (open)	0.05 mg/kg	SANCO/825/00 rev. 8.1
Drinking water (Human toxicology)	mesotrione and metabolite A (open)	0.1 µg/L	General limit for drinking water
Surface water (Ecotoxicology)	mesotrione and metabolite A (open)	0.0077 mg/L	lowest NOEC/EC 50 from aquatic toxicity study E_bC50 (Chronic *lemna gibba*) (EFSA scientific report 2016)
Air	mesotrione	1.5 µg/m^3	SANCO/825/00 rev. 8.1, based on AOEL sys/AOEL inhal: 0.005 mg/kg bw/d (EFSA scientific report 2016)
Tissue (meat or liver)	mesotrione	0.1 mg/kg	not classified as T / T+
Body fluids		0.05 mg/L	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 for analysis of mesotrione in plant matrices is given in the following tables. Sumi Agro Europe Ltd. (the applicant) has developed together with Albaugh Europe Sárl his own methods for plant residue analysis to match the protected methods available on EU level presented in the RAR (2015) for AIR renewal. Access to those equivalent studies is available (Albaugh/Sumi Agro Europe Ltd.) and their equivalence has been approved by CRD (UK). No additional / new methods are presented in this dossier.

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

Component of residue definition: Mesotrione				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
High water content (Maize	Primary	0.01 mg/kg	QuEChERS HPLC-MS/MS	Watson G., 2013a, RAR 2015

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**
forage), High acid content (Whole orange), High oil content (Oilseed rape seed), High protein/high starch content (dry) (Maize kernel)			m/z 338 → 291 m/z 338 → 212	
	ILV (High water content and High protein/ high starch content (dry))	0.01 mg/kg	QuEChERS HPLC-MS/MS m/z 338 → 291 m/z 338 → 212	Tessier V., 2013 RAR 2015
	Confirmatory (if required)	0.01 mg/kg	see above (Watson G., 2013a)	

The following equivalent study reports are available as matching studies to the validation method from Watson G. (2013a) presented in the RAR 2015:

- Report No. S15-04204 by Schernikau N., Colorado, C.S. (2016)
- Report No. S16-04650 by Giesau A., Bruhn F. (2016)
- Report No. S17-00739 by Giesau A., Schneider B., Giesler W. (2017)

The following equivalent study reports are available as matching studies to the ILV method from Tessier V. (2013) presented in the RAR 2015:

- Report No. S15-04205 by Meseguer C. (2016)
- Report No. S16-05123 by Lesot C. (2017)
- Report No. S16-06606 by Lesot C. (2017)

Table 5.3-3: Statement on extraction efficiency

	Method for products of plant origin
Not required	Reference is made to the EU evaluation and the data being reviewed under AIR renewal of the active substance.

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

An overview on the acceptable methods and possible data gaps for analysis of mesotrione in animal matrices is given in the following tables. Beside the protected methods available on EU level (RAR 2015), the applicant has developed his own methods for animal matrices analysis. Evaluation of new/additional studies is detailed in Appendix 2.

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

Component of residue definition: Mesotrione				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Milk, eggs, muscle (meat), fat, liver and	Primary	0.01 mg/kg	QuEChERS HPLC-MS/MS	Watson G., 2013b RAR 2015

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**
kidney			m/z 338 → 291 m/z 338 → 212	
	ILV	0.01 mg/kg	QuEChERS HPLC-MS/MS m/z 338 → 291 m/z 338 → 212	Bernal J., 2013 RAR 2015
	Confirmatory (if required)	0.01 mg/kg	see above (Watson G., 2013b)	
Milk, eggs, muscle (meat), fat and liver	Primary	0.01 mg/kg	HPLC-MS/MS Mesotrione*: m/z 338 → 291 m/z 338 → 212 MNBA metabolite: m/z 244 → 200 m/z 244 → 142 AMBA metabolite: m/z 214 → 155 m/z 214 → 91	KCP 5.2/01 Schernikau N. and Colorado C.S., 2017 Report No. S17-04087 (JSC-1702V) See Appendix 2
	ILV	0.01 mg/kg	HPLC-MS/MS Mesotrione*: m/z 338 → 291 m/z 338 → 212 MNBA metabolite: m/z 244 → 200 m/z 244 → 142 AMBA metabolite: m/z 214 → 155 m/z 214 → 91	KCP 5.2/02 Lesot C., 2017 Report No. S17-04125 See Appendix 2
	Confirmatory (if required)	0.01 mg/kg	see above (Schernikau N. and Colorado C.S., 2017)	

* Although metabolites AMBA and MNBA are not part of the residue definition, they were analysed in this method

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

Table 5.3-5: Statement on extraction efficiency

	Method for products of animal origin
Not required	Reference is made to the EU evaluation and the data being reviewed under AIR renewal of the active substance.

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

An overview on the acceptable methods and possible data gaps for analysis of mesotrione in soil is given in the following tables. Sumi Agro Europe Ltd. (the applicant) has access to an equivalent data package (developed jointly by SAE and Albaugh Europe Sárl), which matches the protected methods available on EU level presented in the RAR (2015) for AIR renewal. The equivalence has been approved by CRD (UK). No additional / new methods are presented in this dossier.

Table 5.3-6: Validated methods for soil (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**
Soil	Primary	0.002 mg/kg	HPLC-MS/MS Mesotrione: m/z 338 → 291 m/z 338 → 212 AMBA metabolite: m/z 214 → 170 m/z 214 → 64 MNBA metabolite: m/z 244 → 200 m/z 244 → 170	Jutsum L., 2013a RAR 2015
	Confirmatory (if required)	0.002 mg/kg	see above (Jutsum L., 2013)	

* Although metabolites AMBA and MNBA are not part of the residue definition, they were analysed in this method

The equivalent study report S16-04651 by Giesau A., Schneider B. (2016) is available as a matching study to the validation method from Jutsum L. (2013a) presented in the RAR 2015.

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

An overview on the acceptable methods for analysis of mesotrione in surface and drinking water is given in the following tables. Sumi Agro Europe Ltd. (the applicant) has access to an equivalent data package (developed jointly by SAE and Albaugh Europe Sárl),which matches the protected methods available on EU level presented in the RAR (2015) for AIR renewal. The equivalence has been approved by CRD (UK). No additional / new methods are presented in this dossier.

Table 5.3-7: 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 and Surface water	Primary	0.05 µg/L	HPLC-MS/MS Mesotrione*: m/z 338 → 291 m/z 338 → 212 AMBA metabolite: m/z 214 → 170	Jutsum L., 2013b RAR 2015

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**
			m/z 214 → 155 MNBA metabolite: m/z 244 → 142 m/z 244 → 170	
	ILV	0.05 µg/L	HPLC-MS/MS Mesotrione*: m/z 338 → 291 m/z 338 → 212 AMBA metabolite: m/z 214 → 170 m/z 214 → 155 MNBA metabolite: m/z 244 → 142 m/z 244 → 170	Wiesner F., Breyer N., 2013 RAR 2015
	Confirmatory	0.05 µg/L	see above (Jutsum L, 2013a)	

* Although metabolites AMBA and MNBA are not part of the residue definition, they were analysed in these methods

The equivalent study reports S16-04652 by Giesau A., Schneider B. (2016) and its ILV S16-05124 by Driss F. (2017) are available as matching studies to the validation method from Jutsum L. (2013b) and its ILV from Wiesner F., Breyer N. (2013) presented in the RAR 2015.

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

An overview on the acceptable methods for analysis of mesotrione in air is given in the following tables. Sumi Agro Europe Ltd. (the applicant) has access to an equivalent data package (developed jointly by SAE and Albaugh Europe Sárl),which matches the protected methods available on EU level presented in the RAR (2015) for AIR renewal. The equivalence has been approved by CRD (UK). No additional / new methods are presented in this dossier.

Table 5.3-8: Validated methods for air (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**
Air	Primary	0.45 µg/m^3	HPLC-MS/MS m/z 338 → 291 m/z 338 → 212	Jutsum L., 2013c RAR 2015
	Confirmatory	0.45 µg/m^3	see above (Jutsum L., 2013b)	

The equivalent study report S16-04896 by Driss F. (2016) is available as a matching study to the validation method from Jutsum L. (2013c) presented in the RAR 2015.

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

An overview on the acceptable methods and possible data gaps for analysis of mesotrione in body fluids and tissues is given in the following table. Beside the protected methods available on EU level (RAR 2015), the applicant has developed his own methods for plant residue analysis. Evaluation of new/additional studies is detailed in Appendix 2.

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

Component of residue definition: Mesotrione				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Body tissues (Muscle (meat), liver, kidney) Body fluids (blood)	Primary	0.01 mg/kg	HPLC-MS/MS m/z 338 → 291 m/z 338 → 212	Watson G., 2013b RAR 2015
	Confirmatory	0.01 mg/kg	see above (Watson G, 2013b)	
Body tissues (liver) Body fluids (blood)	Primary	0.01 mg/kg	HPLC-MS/MS Mesotrione*: m/z 338 → 291 m/z 338 → 212 MNBA metabolite: m/z 244 → 142 m/z 244 → 170 AMBA metabolite: m/z 214 → 91 m/z 214 → 79	KCP 5.2/03 Giesau A. and Grewe D., 2016 Report No. S16-04653 (JSC-1604V) See Appendix 2
	Confirmatory	0.01 mg/kg	see above (Giesau A. and Grewe D., 2016)	

* Although metabolites AMBA and MNBA are not part of the residue definition, they were analysed in this method

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

5.3.2.8 Other studies/ information

No additional / other information.

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

For this application, it is referred to the following EU concluded residue definitions:

Matrix	Residue Definition	Reference
Plant commodities	nicosulfuron	EFSA Scientific Report (2007) 120, 1-91

Animal origin	Unable to propose, however not required for representative use	EFSA Scientific Report (2007) 120, 1-91
Soil	nicosulfuron	EFSA Scientific Report (2007) 120, 1-91
Surface water	nicosulfuron	EFSA Scientific Report (2007) 120, 1-91
Drinking / ground water	nicosulfuron	EFSA Scientific Report (2007) 120, 1-91
Air	nicosulfuron	EFSA Scientific Report (2007) 120, 1-91
Body fluids / tissues	Not required	EFSA Scientific Report (2007) 120, 1-91

Table 5.3-10: 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	Com. Reg. 617/2014
Plant, high acid content		0.01 mg/kg	Com. Reg. 617/2014
Plant, high protein/high starch content (dry commodities)		0.01 mg/kg	Com. Reg. 617/2014
Plant, high oil content		0.02 mg/kg	Com. Reg. 617/2014
Plant, difficult matrices (hops, spices, tea)		0.05 mg/kg	Com. Reg. 617/2014
Muscle	nicosulfuron	0.02 mg/kg	Com. Reg. 617/2014
Milk		0.02 mg/kg	Com. Reg. 617/2014
Eggs		0.02 mg/kg	Com. Reg. 617/2014
Fat		0.02 mg/kg	Com. Reg. 617/2014
Liver, kidney		0.02 mg/kg	Com. Reg. 617/2014
Soil (Ecotoxicology)	nicosulfuron	0.05 mg/kg	SANCO/825/00 rev. 8.1
Drinking water (Human toxicology)	nicosulfuron	0.1 µg/L	General limit for drinking water
Surface water (Ecotoxicology)	nicosulfuron	0.0017 mg/L	lowest NOEC/EC 50 from aquatic toxicity study EC50 (Acute *lemna gibba*) (EFSA scientific report 2007)
Air	nicosulfuron	240 µg/m^3	SANCO/825/00 rev. 8.1, based on AOEL sys/AOEL inhal: 0.8 mg/kg bw/d (EFSA scientific report 2007)
Tissue (meat or liver)	nicosulfuron	0.1 mg/kg	Not classified as T / T+
Body fluids		0.05 mg/L	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. Beside the unprotected methods available on EU level, Sumi Agro Europe Ltd. (the applicant) has a letter of access to use the protected studies evaluated at EU level which are owned by Ishihara Sangyo Kaisha Ltd./Nicosulfuron TaskForce (or for which to ISK or SAE have Letters of access). No additional / new methods are presented in this dossier.

Access to ISK studies also includes two expert's statements by Wais A., 2004 and by Ginzburg N., 2007 (presented in the AIR dossier 2016) which were written to provide evidence that the multi-residues method, DF S-19, published by an international official standardisation body, cannot be used for the determination of nicosulfuron in plant material (inadequate recovery).

Table 5.3-11: 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 (maize forage), High protein/high starch content (dry) (maize corn)	Primary	0.01 mg/kg	HPLC-MS/MS m/z 411.2 → 182.1 m/z 411.2 → 213.0	Gemrot F., 2013 AIR dossier 2016
	ILV	0.01 mg/kg	HPLC-MS/MS m/z 411 → 182 m/z 411 → 213	Richter S., 2013 AIR dossier 2016
	Confirmatory (if required)	0.01 mg/kg	see above (Gemrot, 2013)	
High water content (cherry), High protein/high starch content (dry) (corn), High acid content (lemon) High oil content (soybean seed)	Primary	0.01 mg/kg	HPLC-MS/MS m/z 411 → 182 m/z 411 → 213	Cabusas, M. E. Y. and Pentz A., 2012 (Rev. 2) and McInerney K. 2016b AIR dossier 2016
High water content (corn silage), High protein/high starch content (dry) (corn grain)	ILV	0.01 mg/kg	HPLC-MS/MS m/z 411 → 182 m/z 411 → 213	Ducat, N. and Pigeon, O. 2004 AIR dossier 2016
High water content (cherry), High protein/high starch content (dry) (corn), High acid content (lemon) High oil content (soybean seed)	Confirmatory (if required)	0.01 mg/kg	see above (Cabusas, M. E. Y. and Pentz A., 2012 (Rev. 2) and McInerney K. 2016b)	

Table 5.3-12: Statement on extraction efficiency

	Method for products of plant origin
Not required	Reference is made to the EU evaluation and the data being reviewed under AIR renewal of the active substance.

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

Following the EFSA Conclusion on nicosulfuron (EFSA Scientific Report (2007), 120) and as presented in the EU dossier for AIR renewal of nicosulfuron, an analytical method for food of animal origin is not required due to the fact that no residue definition is proposed. This has been re-enforced in the EFSA Reasoned opinion on the review of the existing MRLs for nicosulfuron (EFSA Journal 2012; 10(12):3048), where it says:
"…, considering that there is no significant intake of residues by livestock, no residue definition and no MRLs are proposed for commodities of animal origin (section 3.2). Therefore, an analytical method for enforcement of residues in food of animal origin is not necessary."
Considering that there is still no significant intake of residues by livestock, no residue definition and no MRLs are proposed for commodities of animal origin, therefore an analytical method for enforcement of residues in food of animal origin is still not necessary.
No additional / new methods are presented in this dossier.

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. Beside the unprotected methods available on EU level, Sumi Agro Europe Ltd. (the applicant) has a letter of access to use the protected studies evaluated at EU level which are owned by Ishihara Sangyo Kaisha Ltd/Nicosulfuron TaskForce (or for which to ISK or SAE have Letters of access). No additional / new methods are presented in this dossier.

Table 5.3-13: 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	0.05 µg/kg	HPLC-MS/MS m/z 411.1 → 181.8	Wais A., 2000a DAR 2006
Confirmatory	0.005 mg/kg	HPLC-UV	Hubber H.P., 1996b DAR 2006

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. Beside the unprotected methods available on EU level, Sumi Agro Europe Ltd. (the applicant) has a letter of access to use the protected studies evaluated at EU level which are owned by Ishihara Sangyo Kaisha Ltd./Nicosulfuron TaskForce (or for which to ISK or SAE have Letters of access). No additional / new methods are presented in this dossier.

Table 5.3-14: 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	Primary	0.05 µg/L	HPLC-UV	Schulz and Ullrich-Mitzel, 1995a DAR 2006
Surface water	Primary	0.05 µg/L	HPLC-UV	Wais, 2000b DAR 2006
Drinking and Surface water	Primary	0.05 µg/L	HPLC-MS/MS m/z 411.2 → 182.2 m/z 411.2 → 106.1	Wolf, 2007 AIR dossier 2016
	Confirmatory	0.05 µg/L	See above (Wolf, 2007)	
Drinking water	ILV	0.05 µg/L	HPLC-MS/MS m/z 411 → 182 m/z 411 → 213	Heillaut C., 2008 and McInerney K., 2016c AIR dossier 2016

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. Beside the unprotected methods available on EU level, Sumi Agro Europe Ltd. (the applicant) has a letter of access to use the protected studies evaluated at EU level which are owned by Ishihara Sangyo Kaisha Ltd./Nicosulfuron TaskForce (or for which to ISK or SAE have Letters of access). No additional / new methods are presented in this dossier.

Table 5.3-15: 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	HPLC-UV	Wais A., 2000c DAR 2006
Confirmatory	1.2 µg/m^3	HPLC-UV	Schulz M., Ullrich-Mitzel A., 1995b DAR 2006

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

Nicosulfuron is not classified as toxic (T) or very toxic (T+). However, according to Regulation 283/2013, a validated method of nicosulfuron residue in body fluid and tissues has to be provided.
An overview on the acceptable methods and possible data gaps for analysis of nicosulfuron in body fluids and tissues is given in the following table. Beside the unprotected methods available on EU level, Sumi Agro Europe Ltd. (the applicant) has a letter of access to use the protected studies evaluated at EU level which are owned by Ishihara Sangyo Kaisha Ltd./Nicosulfuron TaskForce (or for which to ISK or SAE have Letters of access). No additional / new methods are presented in this dossier.

Table 5.3-16: Methods for body fluids and tissues (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**
Body tissues	Primary	-	Not required as no residue definition is proposed for animal matrices*	
	Confirmatory	-	Not required as no residue definition is proposed for animal matrices*	
Body fluids (plasma)	Primary	0.05 mg/L	HPLC-MS/MS m/z 411 → 182 m/z 411 → 106	McInerney K., 2016d AIR dossier 2016
	Confirmatory	0.05 mg/L	see above (McInerney K., 2016d)	

* See chapter 5.3.3.3 for details

5.3.3.8 Other studies/ information

No other studies / information.

Appendix 1 Lists of data considered in support of the evaluation

List of data submitted by the applicant and relied on

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Vertebrate study Y/N**	Owner
KCP 5.1.1/01	Walker, A.F.	2016	Validation of Analytical Method JP6001-1 for the determination of Mesotrione and Nicosulfuron Content in Product SAE053H/01 (80/30 OD) Report No.: JP160011 Batelle UK Ltd. GLP Unpublished	N	Sumi Agro Europe Ltd.
KCP 5.1.1/02	Wronska, L.	2016	Validation of Analytical Method JP6001-5 for the determination of Impurities R1 and R2 in Product SAE053H/01 (80/30 Mesotrione/Nicosulfuron OD) Report No.: JP160015 Batelle UK Ltd. GLP Unpublished	N	Sumi Agro Europe Ltd.
KCP 5.1.1/03	Wronska, L.	2017	Validation of Analytical Method JP6001-6 for the determination of Impurity 1,2 Dichloroethane in Product SAE053H/01 (80/30 Mesotrione/Nicosulfuron OD) Report No.: JP160016 Batelle UK Ltd. GLP Unpublished	N	Sumi Agro Europe Ltd.
KCP 5.1.2/01	Schernikau N. and Colorado C.S.	2016	Validation of the Analytical Method QuEChERS for the Determination of Mesotrione in Maize Matrices Report No. S15-04204 Eurofins Agroscience Services Chem GmbH GLP Unpublished	N	Sumi Agro Europe Ltd.
KCP 5.1.2/02	Semrau J.	2017	Determination of residues of mesotrione after one application of Mesotrione 80 g/L + Nicosulfuron 30 g/L OD in maize at 4 sites in Northern Europe 2015	N	Sumi Agro Europe Ltd.

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
(also filed under KCP 8.3.1/01)			Report No. S15-03081 Eurofins Agroscience Services GmbH GLP Unpublished		
KCP 5.1.2/03 (also filed under KCP 8.10)	Bakker F.	2016	Magnitude of Mesotrione Residues in Maize Plants following one application in Southern and Northern Europe in 2016 Report No. JS001LRM Eurofins - MITOX GLP Unpublished	N	Sumi Agro Europe Ltd. and Albaugh Europe Sarl
KCP 5.1.2/04 (also filed under KCP 8.10)	van de Sandt, H.J.	2019	Decline of mesotrione residues in maize plants following one application in The Netherlands – 2017 Report No. S17-05218 GLP Unpublished	N	Albaugh Europe Sàrl
KCP 5.1.2/05 (also filed under KCP 10.2.1/01)	xxxx	2016	SAE053H/01: Toxicity to the Rainbow Trout Oncorhynchus mykiss under Laboratory Conditions (Acute Toxicity Test –Static) Report No. S16-03041 xxx GLP Unpublished	Y	Sumi Agro Europe Ltd.
KCP 5.1.2/06 (also filed under KCP 10.2.1/02)	Zawadsky C.	2016	SAE053H/01: Toxicity to the Water Flea Daphnia magna Straus under Laboratory Conditions (Acute Immobilisation Test – Static) Report No. S16-03042 Eurofins Agroscience Services EcoChem GmbH / Eurofins Agroscience Services Ecotox GmbH GLP Unpublished	N	Sumi Agro Europe Ltd.
KCP 5.1.2/07 (also filed	Falk S.	2016a	SAE053H/01: Toxicity to the Single Cell Green Alga Pseudokirchneriella subcapitata Hindák under Laboratory Conditions Report No. S16-03039	N	Sumi Agro Europe Ltd.

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**
under KCP 10.2.1/03)			Eurofins Agroscience Services EcoChem GmbH / Eurofins Agroscience Services Ecotox GmbH GLP Unpublished		
KCP 5.1.2/08 (also filed under KCP 10.2.1/04)	Falk S.	2016b	SAE053H/01: Toxicity to the Diatom Navicula pelliculosa under Laboratory Conditions Report No. S16-03040 Eurofins Agroscience Services EcoChem GmbH / Eurofins Agroscience Services Ecotox GmbH GLP Unpublished	N	Sumi Agro Europe Ltd.
KCP 5.1.2/09 (also filed under KCP 10.2.1/05)	Lang C.	2016b	SAE053H/01: Toxicity to the Duckweed Lemna gibba under Laboratory Conditions (Acute Test – Semi-static) Report No. S16-03044 Eurofins Agroscience Services EcoChem GmbH / Eurofins Agroscience Services Ecotox GmbH GLP Unpublished	N	Sumi Agro Europe Ltd.
KCP 5.1.2/10 (also filed under KCP 10.2.1/06)	Gonsior G.	2016	SAE053H/01: Growth Inhibition of Myriophyllum spicatum in a Water/Sediment System Report No. S16-03045 Eurofins Agroscience Services EcoChem GmbH / Eurofins Agroscience Services Ecotox GmbH GLP Unpublished	N	Sumi Agro Europe Ltd.
KCP 5.1.2/11 (also filed under KCP 10.2.2/01)	Lang C.	2016a	SAE053H/01: Toxicity to the Water Flea Daphnia magna Straus under Laboratory Conditions (Reproduction Test) Report No. S16-03043 Eurofins Agroscience Services EcoChem GmbH / Eurofins Agroscience Services Ecotox GmbH GLP Unpublished	N	Sumi Agro Europe Ltd.
KCP 5.1.2/12 (also filed under KCP	Molitor A.M.	2016	SAE053H/01- Assessment of Effects on the Adult Honey Bee, Apis mellifera L., in a 10 Day Chronic Feeding Test under Laboratory Conditions Report No. S16-02518 Eurofins Agroscience Services EcoChem GmbH / Eurofins Agroscience Services Ecotox GmbH	N	Sumi Agro Europe Ltd.

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
10.3.1.1/01)			GLP Unpublished		
KCP 5.1.2/13 (also filed under KCP 10.3.1.3/01)	Vergé E. and Wagner J.	2016	SAE053H/01 - Honey Bee (Apis mellifera L.) Larval Toxicity Test (Repeated Exposure) Report No. S16-02503 Eurofins Agroscience Services EcoChem GmbH / Eurofins Agroscience Services Ecotox GmbH GLP Unpublished	N	Sumi Agro Europe Ltd.
KCP 5.1.2/14 (also filed under 10.6.2/01)	Gröning C.	2017a	SAE053H/01 – Effects on the Seedling Emergence of Ten Non-Target Terrestrial Plant Species under Greenhouse Conditions Report No. S16-02421 Eurofins Agroscience Services EcoChem GmbH / Eurofins Agroscience Services Ecotox GmbH GLP Unpublished	N	Sumi Agro Europe Ltd.
KCP 5.1.2/15 (also filed under KCP 10.6.2/02)	Gröning C.	2017b	SAE053H/01: Effects on the Vegetative Vigour of Ten Non-Target Terrestrial Plant Species under Greenhouse Conditions Report No. S16-02422 Eurofins Agroscience Services EcoChem GmbH / Eurofins Agroscience Services Ecotox GmbH GLP Unpublished	N	Sumi Agro Europe Ltd.
KCP 5.1.2/16 (also filed under KCP 10.2.1/07)	Bertrand, C.	2019	Mesotrione technical: Toxicity to the duckweed Lemna gibba under laboratory conditions (acute test – semi static) Report No. S19-03470 Eurofins Agroscience Services EcoChem GmbH / Eurofins Agroscience Services Ecotox GmbH, Niefern-Öschelbronn, Germany GLP Unpublished	N	Sumi Agro Europe & Albaugh Europe Sàrl
KCP 5.1.2/17 (also filed under KCP	Christmann, R.	2021a	Mesotrione: Toxicity to the aquatic plant *Spirodela polyrhiza* in a growth inhibition test Report No.: 218-31 Institut für Gewässerschutz MESOCOSM GmbH, Homberg, Germany GLP	N	Sumi Agro Europe & Albaugh

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**
10.2.1/08)			Unpublished		Europe Sàrl
KCP 5.1.2/18 (also filed under KCP 10.2.1/09)	Christmann, R.	2021b	Mesotrione: Toxicity to the aquatic plant *Wolffia arrhiza* in a growth inhibition test Report No.: 218-32 Institut für Gewässerschutz MESOCOSM GmbH, Homberg, Germany GLP Unpublished	N	Sumi Agro Europe & Albaugh Europe Sàrl
KCP 5.2/01	Schernikau N., Colorado C.S.	2017	Validation of an Analytical Method for the Determination of Mesotrione and its Metabolites MNBA and AMBA in Animal Matrices Report No. S17-04087 (JSC-1702V) Eurofins Agroscience Services Chem GmbH GLP Unpublished	N	Sumi Agro Europe Ltd. and Albaugh Europe Sarl
KCP 5.2/02	Lesot C.	2017	Independent Laboratory Validation of a Method for the Determination of Residues of Mesotrione and its Metabolites MNBA and AMBA in Animal Matrices Report No. S17-04125 Eurofins Agroscience Services Chem SAS GLP Unpublished	N	Sumi Agro Europe Ltd. and Albaugh Europe Sarl
KCP 5.2/03	Giesau A., Grewe D.	2016	Validation of an Analytical Method for the Determination of Mesotrione and its Metabolites MNBA and AMBA in Body Fluid and Tissues Report No. S16-04653 (JSC-1604V) Eurofins Agroscience Services Chem GmbH GLP Unpublished	N	Sumi Agro Europe Ltd. and Albaugh Europe Sarl

List of data submitted or referred to Mesotrione 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
CA 5.2, RAR 2015	Watson G.	2013a	Mesotrione - Validation of the QuEChERS Method for the Determination of Residues of mesotrione in Crop Matrices by LC-MS/MS Report No. S12-03251, Syngenta File No ZA1296_10090 Eurofins Agroscience Services Ltd GLP Unpublished	N	Syngenta
CA 5.2, RAR 2015	Tessier V.	2013	Mesotrione - Independent Laboratory Validation of the QuEChERS Method for the Determination of Residues of Mesotrione in Crop Matrices by LC-MS/MS Report No. S12-04607, Syngenta File No ZA1296_10129 Eurofins Agroscience Services Chem SAS GLP Unpublished	N	Syngenta
CA 5.2, RAR 2015	Watson G.	2013b	Mesotrione - Validation of the QuEChERS Method for the Determination of Residues of mesotrione in Animal Matrices by LC-MS/MS Report No. S12-03250, Syngenta File No ZA1296_10093 Eurofins Agroscience Services Ltd GLP Unpublished	N	Syngenta
CA 5.2, RAR 2015	Bernal J.	2013	Mesotrione - Independent Laboratory Validation of the QuEChERS Method for the Determination of Residues of Mesotrione in Animal matrices by LC-MS/MS Report No. S12-04608, Syngenta File No ZA1296_10130 Eurofins Agroscience Services Chem SAS GLP Unpublished	N	Syngenta
CA 5.2, RAR 2015	Jutsum L.	2013a	Jutsum L. 2013 Mesotrione – Validation of Draft Residue Method GRM007.10A for the Determination of Mesotrione and its Metabolites AMBA and MNBA in Soil Report No. CEMR-5657-REG, Syngenta File No ZA1296_10088 CEMAS GLP	N	Syngenta

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Vertebrate study Y/N**	**Owner**
			Unpublished		
CA 5.2, RAR 2015	Jutsum L.	2013b	Mesotrione - Validation of Draft Residue Method GRM007.09A for the Determination of Mesotrione and its metabolites AMBA and MNBA in Water Report No. CEMR-5658-REG, Syngenta File No ZA1296_10087 CEMAS GLP Unpublished	N	Syngenta
CA 5.2, RAR 2015	Wiesner F., Breyer N.	2013	Independent Laboratory Validation of Analytical Method GRM007.09A for the Determination of Residues of Mesotrione and its Metabolites in AMBA and MNBA Water Report No. S13-04185, Syngenta File No ZA1296_10174 Eurofins Agroscience Services Chem GmbH GLP Unpublished	N	Syngenta
CA 5.2, RAR 2015	Jutsum L.	2013c	Mesotrione - Validation of Residue Method GRM007.08A for the Determination of Mesotrione in Air Report No. CEMR-5403-REG, Syngenta File No ZA1296_10084 CEMAS GLP Unpublished	N	Syngenta
CA 4.2 from data matching	Schernikau N., Colorado, C.S.	2016	Validation of the analytical method QuEChERS for the determination of mesotrione in maize matrices Report No. S15-04204 Eurofins Agroscience Services Chem GmbH GLP Unpublished	N	Sumi Agro Europe Ltd.
CA 4.2 from data matching	Giesau A., Bruhn F.	2016	Validation of an analytical method for the determination of mesotrione and its metabolite MNBA in different matrices of plant origin Report No. S16-04650 Eurofins Agroscience Services Chem GmbH GLP Unpublished	N	Sumi Agro Europe Ltd. and Albaugh Europe Sárl

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
CA 4.2 from data matching	Giesau A., Schneider B., Giesler W.	2017	Validation of an analytical method for the determination of mesotrione metabolite MNBA in different matrices of plant origin Report No. S17-00739 Eurofins Agroscience Services Chem GmbH GLP Unpublished	N	Sumi Agro Europe Ltd. and Albaugh Europe Sárl
CA 4.2 from data matching	Meseguer C.	2016	Independent Laboratory Validation of QuEChERS method for the determination of residues of mesotrione in maize matrices (whole plant, grain, rest of plant) Report No. S15-04205 Eurofins Agroscience Services Chem SAS GLP Unpublished	N	Sumi Agro Europe Ltd.
CA 4.2 from data matching	Lesot C.	2017	Independent Laboratory validation of the analytical method for the determination of mesotrione and its metabolites MNBA in different matrices of plant origin Report No. S16-05123 Eurofins Agroscience Services Chem SAS GLP Unpublished	N	Sumi Agro Europe Ltd. and Albaugh Europe Sárl
CA 4.2 from data matching	Lesot C.	2017	Independent Laboratory validation of the analytical method for the determination of mesotrione metabolite MNBA in different matrices of plant origin Report No. S16-06606 Eurofins Agroscience Services Chem SAS GLP Unpublished	N	Sumi Agro Europe Ltd. and Albaugh Europe Sárl
CA 4.2 from data matching	Giesau A., Schneider B.	2016	Validation of an analytical method for the determination of mesotrione and its metabolites MNBA and AMBA in/on soil Report No. S16-04651 Eurofins Agroscience Services Chem GmbH GLP Unpublished	N	Sumi Agro Europe Ltd. and Albaugh Europe Sárl

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
CA 4.2 from data matching	Giesau A., Schneider B.	2016	Validation of an analytical method for the determination of mesotrione and its metabolites MNBA and AMBA in surface and drinking water Report No. S16-04652 Eurofins Agroscience Services Chem GmbH GLP Unpublished	N	Sumi Agro Europe Ltd. and Albaugh Europe Sárl
CA 4.2 from data matching	Driss F.	2017	Mesotrione – Independent laboratory validation of the analytical method for the determination of mesotrione and its metabolites MNBA and AMBA in drinking water Report No. S16-05124 Eurofins Agroscience Services Chem SAS GLP Unpublished	N	Sumi Agro Europe Ltd. and Albaugh Europe Sárl

List of data submitted or referred to Nicosulfuron 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
CA 4.1.2/32, AIR dossier 2016	Gemrot F.	2013	Nicosulfuron – Method Validation Study for the determination of Nicosulfuron Residues in Maize forage and grain Report: S13-00195 Eurofins Agroscience Services Chem SAS GLP Unpublished	N	Ishihara Sangyo Kaisha Ltd.
CA 4.2/05, AIR	Richter S.	2013	Independent Laboratory Validation (ILV) of a Residue Analytical Method for the Determination of Nicosulfuron in Maize Grain and Forage	N	Ishihara Sangyo

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
dossier 2016			Report: PTRL Europe ID P 2975 G PTRL Europe GLP Unpublished		Kaisha Ltd.
CA 4.2/06, AIR dossier 2016	Cabusas, M. E. Y. and Pentz A.	2012	Analytical Methods for the Determination of Nicosulfuron (DPXV9360) and its Metabolite IN-V9367 in Animal Matrices by HPLC/ESI-MS/MS Report: DuPont-17927, Revision No. 2 E.I. du Pont de Nemours and Company GLP Unpublished	N	E.I. du Pont de Nemours and Company
CA 4.2/07, AIR dossier 2016	McInerney K.	2016b	Validation report DuPont-11776 RV2: Extension of the Linearity Range for Nicosulfuron in Oily and Acidic Crop Report: 100077587-03 Battelle Norwell GLP Unpublished	N	Nicosulfuron Task Force
CA 4.2/08, AIR dossier 2016	Ducat, N. and Pigeon, O.	2004	Independent Laboratory validation of DuPont-11776, “Analytical Enforcement Method for the Determination of Nicosulfuron in Corn Matrices using HPLC/ESI-MS/MS Report: DuPont-12347 Centre wallon de Recherches Agronomiques (CRA-W) GLP Unpublished	N	E.I. du Pont de Nemours and Company
CA 4.1.2/01, DAR 2006	Wais A.	2000a	Validation of the residue analytical method for SL-950 (nicosulfuron) in soil Report: 770117 RCC Ltd GLP Unpublished	N	Ishihara Sangyo Kaisha Ltd.
CA 4.1.2/02, DAR 2006	Hubber H.P.	1996b	Compilation of Analytical methods for SL-950 and its metabolites ADMP and ASDM in soil (various types) from existing validated residue analyses Report: 614340	N	Ishihara Sangyo Kaisha Ltd.

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
			RCC Umweltchemie AG GLP Unpublished		
CA 4.1.2/11, DAR 2006	Schulz M. and Ullrich-Mitzel A.	1995a	SL-950: Validation of analytical method for determination of residues in drinking water Report: 604383 RCC Umweltchemie AG GLP Unpublished	N	Ishihara Sangyo Kaisha Ltd.
CA 4.1.2/13, DAR 2006	Wais A.	2000b	Development and validation of the residue analytical method for SL-950 (nicosulfuron) in surface water Report: 770128 RCC Ltd GLP Unpublished	N	Ishihara Sangyo Kaisha Ltd.
CA 4.1.2/14, AIR dossier 2016	Wolf S.	2007	Development and validation of a residue analytical method for nicosulfuron in drinking water and surface water Report: B25773 RCC Ltd GLP Unpublished	N	Ishihara Sangyo Kaisha Ltd.
CA 4.2/11, AIR dossier 2016	Heillaut C.	2008	Nicosulfuron – Independent Laboratory Validation of an Analytical Method for the Determination of Nicosulfuron Residues in Drinking Water Report: NUF/NIC/08002 Eurofins ADME Bioanalyses GLP Unpublished	N	Nufarm S.A.S
CA 4.2/12, AIR dossier 2016	McInerney K.	2016c	ILV report NUF-NIC-08002: Extension of the linearity range for nicosulfuron in drinking water Report: 100077587-02 Battelle Norwell GLP Unpublished	N	Nicosulfuron Task Force

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
CA 4.1.2/25, DAR 2006	Wais A.	2000c	Validation of the residue analytical method for SL-950 (Nicosulfuron) in air Report: 765358 RCC Ltd GLP Unpublished	N	Ishihara Sangyo Kaisha Ltd.
CA 4.1.2/24, DAR 2006	Schulz M. and Ullrich-Mitzel A.	1995b	Analytical method for the determination of SL-950 in air Report: 385470 RCC Umweltchemie AG GLP Unpublished	N	Ishihara Sangyo Kaisha Ltd.
CA 4.2/15, AIR dossier 2016	McInerney K.	2016d	Method Validation for the Determination of Nicosulfuron in Mouse Plasma Report: 100077587-04 Battelle Norwell GLP Unpublished	N	Nicosulfuron Task Force

Appendix 2 Detailed evaluation of submitted analytical methods

A 2.1 Analytical methods for mesotrione and nicosulfuron

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

A 2.1.1.1 Description of analytical methods for the determination of mesotrione and nicosulfuron and relevant impurities in plant protection product SAE053H/01 (KCP 5.1)

A 2.1.1.1.1 Analytical method for determination of mesotrione and nicosulfuron in the preparation

A 2.1.1.1.1.1 Method validation

Comments of zRMS:	This method is accepted and may be used for analyzing Mesotrione and Nicosulfuron in the PPP.

Reference:	**2.1.1.1.1.1/01 (KCP 5.1.1/01)**
Report	Walker, A.F. (2016) Validation of Analytical Method JP6001-1 for the determination of Mesotrione and Nicosulfuron Content in Product SAE053H/01 (80/30 OD) Report No.: JP160011
Guideline(s):	SANCO/3030/99 rev. 4
Deviations:	None
GLP:	Yes
Acceptability:	Yes

Materials and methods

The HPLC method for the determination of mesotrione and nicosulfuron content in product SAE053H/01 (80/30 Mesotrione/Nicosulfuron OD) was validated. The determination of mesotrione and nicosulfuron was performed after dilution with acetonitrile by HPLC analysis with UV detection at 255 nm (nicosulfuron) and 290 nm (mesotrione). Quantitation was performed by external standard calibration.

Sample preparation

Formulation:
In duplicate, approximately 0.25g (0.1mg accuracy) of mesotrione/nicosulfuron (80/30 OD) was weight into two 50 mL volumetric flasks. Approximately 40 mL of acetonitrile was added and sonicated for 15 min. with occasional vigorous agitation. The sample was equilibrated to room temperature and then diluted to volume with acetonitrile. The extract was filtered through a 0.45 µm PTFE syringe filter and analysed by HPLC with UV-detection.

Spray Tank Dilution (range: 1L product/400 L water to 1.5 L product/200 L water):
In duplicate, 20 mL of homogenised spray dilution was pipetted into a 50 mL volumetric flask. 25 mL of acetonitrile was added and sonicated with occasional vigorous agitation. The sample was equilibrated to room temperature and afterwards diluted to volume with acetonitrile. The extract was filtered through a 0.45 µm PTFE syringe filter and analysed by HPLC with UV-detection.

Chromatographic conditions

HPLC system	Equipped with loop injector, variable UV detector, and electronic integrator.			
Column:	Zorbax XDB-C18 150 x 4.6mm 3.5µm s/n: USWA018805			
Column temperature:	30 °C			
Injection Volume:	5 µL			
Mobile phase:	Eluent A: 10:90v/v MeCN:H_2O + 0.1% Acetic Acid Eluent B: 90:10v/v MeCN:H_2O + 0.1% Acetic Acid			
Gradient:	Time [min]	% Eluent A	% Eluent B	Flow [mL/min]
	0.00	65	35	1
	5.00	65	35	1
	10.00	10	90	1
	15.00	10	90	1
	15.01	65	35	1
	20.00	65	35	1
Wavelength Program	Initial 255nm BW 4nm Ref 360 BW 60nm. 4.00 min. 290nm BW 4nm Ref 360nm BW 60nm 4.01 Balance			
Runtime:	20 min			
Retention time(s)	Nicosulfuron: approx. 2.98 min Mesotrione: approx. 5.17 min			

Results and discussions

Table A 1: Accuracy data of nicosulfuron and mesotrione in product

Matrix	Fortification level (% w/w)	Recovery (%)	Replicates	Mean Recovery (%)	Overall Mean Recovery (%)
Nicosulfuron					
Formulation (SAE053H/01)	2.01 (75% of nominal)	99.6, 100.2	2	99.9	100.0
	2.73 (100% of nominal)	100.1, 99.7	2	99.9	
	3.53 (125% of nominal)	100.0, 100.2	2	100.1	
Mesotrione					
Formulation (SAE053H/01)	5.74 (75% of nominal)	101.2, 101.6	2	101.4	101.5
	7.81	101.7, 101.2	2	101.4	

Matrix	Fortification level (% w/w)	Recovery (%)	Replicates	Mean Recovery (%)	Overall Mean Recovery (%)
Nicosulfuron					
	(100% of nominal)				
	10.1 (125% of nominal)	101.6, 101.8	2	101.7	

The mean recoveries were in the range of 97 - 103 % and thus comply with the standard acceptance criteria of the guidance document SANCO/3030/99 rev 4 for a preparation containing 1 - 10 % active.

Table A 2: Precision data of nicosulfuron and mesotrione in product

Matrix	% w/w	g/L (relative density = 0.983)	Mean Recovery (g/L)	RSD (%)
Nicosulfuron				
Formulation (SAE053H/01)	3.0963 3.0696 3.0778 3.0768 3.0676 3.0988	30.436 30.174 30.255 30.245 30.155 30.462	30.3	± 0.43
Mesotrione				
Formulation (SAE053H/01)	8.3151 8.2516 8.2552 8.2429 8.2318 8.3074	81.738 81.113 81.149 81.027 80.918 81.662	81.3	± 0.42

The calculated % RSD values are within the maximum requirements of SANCO/3030/99 rev.4 (11/07/00) based on the modified Horwitz equation which are ≤ 2.26% for Nicosulfuron (3.08% w/w) and ≤ 1.95% for Mesotrione (8.27% w/w).

Table A 3: Characteristics for the analytical method used for residue determination of nicosulfuron and mesotrione in product

	Nicosulfuron	Mesotrione
Specificity (degree of interference)	Interferences from co-formulants: No significant interferences were present in the region of the nicosulfuron peak (2.5 - 3.5 min.) or mesotrione peak (4.5 – 6.0 min.) in the reagent blank or the formulation blank chromatogram. No significant interferences were present in the region of the nicosulfuron peak from the mesotrione reference item and no significant interferences were present in the region of the mesotrione peak from the nicosulfuron reference item.	
Specificity (identification)	Peak Identity: Peak apex UV spectra for nicosulfuron and mesotrione in the reference item chromatograms gave a qualitative match to those generated for the corresponding peaks in the test item chromatogram. Peak apex mass spectra for nicosulfuron and mesotrione in the test item chroma-	

	Nicosulfuron	Mesotrione
	togram gave comparable profiles to those recorded for the reference items. The mass spectra are consistent with the nicosulfuron and mesotrione structures.	
Calibration (type, number of data points) **Calibration range** **Formulation**	The linearity of response for the determination of actives in the formulation was determined by the preparation of five (5) reference item solutions over approx. 50-150% of the nominal analytical concentration (0.15 mg/ mL nicosulfuron and 0.4 mg/ mL mesotrione). These were injected in duplicate and the nicosulfuron and mesotrione peak area for each obtained.	
	Range of concentration levels: 0.075 mg/mL – 0.214 mg/mL The calibration was found to be linear and the results meet the acceptance criteria of r ≥0.99. Y = 9977.4 * X + 3.32, r= 1.0000	Range of concentration levels: 0.214 mg/mL – 0.612 mg/mL The calibration was found to be linear and the results meet the acceptance criteria of r ≥0.99. Y = 8897.6 * X + 16.74 r= 1.0000
Calibration (type, number of data points) **Calibration range** **Spray Dilution**	Linearity of concentration against peak area was determined. The upper and lower spray tank dilutions for product SAE053H/01 are 1.5 litre product in 200 L water and 1 litre product in 400 L water respectively. The nominal analytical concentration range for the upper and lower spray dilutions is 0.03-0.09 mg/mL nicosulfuron and 0.08-0.24 mg/mL mesotrione. The linearity of response for both actives was determined by the preparation of five reference item solutions over the analytical concentration range 0.015-0.15 mg/mL nicosulfuron and 0.04-0.40 mg/mL mesotrione which covers the range 50-150% of the analytical concentrations. These were injected in duplicate and the nicosulfuron and mesotrione peak area for each obtained. Linearity of concentration against peak area was determined.	
	Range of concentration levels: 0.015 mg/mL – 0.148 mg/mL The calibration was found to be linear and the results meet the acceptance criteria of r ≥0.99. Y = 10050.6 * X + (-1.49), r= 1.0000	Range of concentration levels: 0.039 mg/mL – 0.395 mg/mL The calibration was found to be linear and the results meet the acceptance criteria of r ≥0.99. Y = 8963.9 * X + (-2.93), r= 1.0000
Calibration (type, number of data points) **Calibration range** **Tank Cleaning**	After a simulated tank cleaning, a 10 mL aliquot of acetonitrile is used to extract residual active which is then analysed against a multilevel calibration covering the range 1-500 ppm (µg/mL) The linearity of response for both actives was determined by the preparation of five reference item solutions over the analytical concentration range approx. 1-500 µg/mL for each active. These were injected in duplicate and the nicosulfuron and mesotrione peak area for each obtained. Linearity of concentration against peak area was determined.	
	Range of concentration levels: 0.99 µg/mL – 496 µg/mL The calibration was found to be linear and the results meet the acceptance criteria of r ≥0.99. Y = 10.0 * X + (4.76), r= 1.0000	Range of concentration levels: 0.99 µg/mL – 495 µg/mL The calibration was found to be linear and the results meet the acceptance criteria of r ≥0.99. Y = 9.0 * X + (3.76), r= 1.0000
Solution Stability	Both calibration and formulation solutions are stable for at least 48 hours at 5°C	

Conclusion

The HPLC method of analysis JP16001-1 for the determination of mesotrione and nicosulfuron content in product SAE053H/01 (80/30 Mesotrione/Nicosulfuron OD) has been validated.to SANCO/3029/99 rev. 4 regulations.

Walker, A.F. (2016)

A 2.1.1.1.2 Analytical method for determination of relevant impurities R1 and R2 in the preparation

A 2.1.1.1.2.1 Method validation

Comments of zRMS:	These methods are accepted and may be used for analysing impyrities R1 and R2 in the PPP.

Reference:	**2.1.1.1.2.1/01 (KCP 5.1.1/02)**
Report	Wronska, L. (2016) Validation of Analytical Method JP6001-5 for the determination of Impurities R1 and R2 in Product SAE053H/01 (80/30 Mesotrione/Nicosulfuron OD) Report No.: JP160015
Guideline(s):	SANCO/3030/99 rev. 4
Deviations:	None
GLP:	Yes
Acceptability:	Yes

Materials and methods

R1 = Impurity R287431 (6-(Methylsulfonyl)-7-nitro-9-oxo-9H-xanthene-1-carbonitrile)
R2 = impurity R287432 (6-(Methylsulfonyl)-9-oxo-9H-xanthene-1-carbonitrile)

The HPLC method for the determination of mesotrione and nicosulfuron content in product SAE053H/01 (80/30 Mesotrione/Nicosulfuron OD) has been validated. The determination of mesotrione and nicosulfuron was performed after dilution with acetonitrile by HPLC analysis with UV detection and external standard calibration.

The method has shown acceptable performance for determining impurity R2 in terms of specificity, linearity, accuracy (recovery), precision (repeatability) and the LOQ of the method was determined to be 0.0092% w/w of impurity R2 relative to the formulation. This equates to an LOQ of 0.11 % of the mesotrione in the formulation, where mesotrione was determined as 8.33 % w/w in SAE053H/01.

In addition, due to the interference detected with the R1 impurity peak and the low levels of R1 required, a limit test method was verified for mesotrione impurity R1. The method is capable of detecting R1 at a level of above 1 µg/g in formulation SAE053H/01. This is a qualitative method which comprises of the analysing the formulation and the formulation spiked with R1 at a known concentration. The chromatograms are then overlaid and a pass/fail criteria applied if the peak area of the unspiked sample is greater (fail) or less (pass) than that of the spiked sample.

Sample preparation

<u>R2 impurity</u>

In duplicate, approximately 1000 mg of the test item was weighed (0.1mg accuracy) into two separate 20 mL volumetric flasks. About 15mL of acetonitrile was added, shaken vigorously and sonicated for 20 minutes to dissolve. After equilibration to room temperature the sample was filled up to volume with acetonitrile and transferred to vials prior to analysis.

R1 impurity

In duplicate, 5000 mg to within ±10 mg (0.1mg accuracy) of the test item was weighted into a 20 mL volumetric flask. 5 mL of dioxane was added to wash any residue down the sides of the flask. Into one of the flasks, 1 mL of Stock A1was added. This flask was label as spiked sample 1 µg/g. The second flask was labelled was test item. dioxane was added to within 1 cm of the mark, shaken vigorously and sonicated for 30 minutes. The sample was equilibrated to room temperature and filled up to volume with dioxane. The flask was shaken well and transferred into a 50 mL centrifuge tube and centrifuged for 3 minutes at 3000 rpm. The sample of the supernatant was filled into a vial and analysed by HPLC-DAD.

HPLC conditions – determination of R2 impurity

HPLC system	Agilent HP 1100 UV-DAD HPLC			
Column:	Kinetex Biphenyl, 150 x 4.6mm, 5µm or equivalent			
Column temperature:	35 °C			
Injection Volume:	5 µL			
Mobile phase:	Eluent A: Water + 0.1 Acetic Acid Eluent B: Acetonitrile + 0.1% Acetic Acid			
Gradient:	Time [min]	% Eluent A	% Eluent B	Flow [mL/min]
	0.0.	80	20	1.0
	20.0	80	20	1.0
	40.0	35	65	1.0
	40.1	0	100	1.0
	40.2	0	100	1.5
	45.0	0	100	1.5
	45.1	0	100	1.0
	46.0	80	20	1.0
	50.0	80	20	1.0
Retention time(s)	R2: approx. 29.6 min			
Detector wavelength	254 nm			

HPLC conditions – determination of R1 impurity

HPLC system	Agilent HP 1290 UHPLC			
Column:	Ace C18-AR, 150 x 4.6mm, 3µm or equivalent C18 guard column, 3 mm or equivalent			
Column temperature:	40 °C			
Injection Volume:	10 µL			
Mobile phase:	Eluent A: Water + 0.05% Formic Acid Eluent B: Methanol + 0.05% Formic Acid			
Gradient:	Time [min]	% Eluent A	% Eluent B	Flow [mL/min]
	0.0	90	10	0.25
	2.0	90	10	0.25
	40.0	5	95	0.25

	44.0	5	95	0.25
	45.0	5	95	0.50
	50.0	5	95	0.50
	50.1	90	10	0.50
	60.0	90	10	0.50
	61.0	90	10	0.25
Retention time(s)	R1: approx. 61 min			
Detector wavelength	225 nm			

Results and discussions

Table A 4: Accuracy data of impurity R2 in product

Matrix	**Fortification level (µg/g) ***	**Recovery (%)**	**Replicates**	**Overall Mean Recovery (%)**
Formulation (SAE053H/01)	80 (0.5 x)	98.3, 99.5, 105.9, 102.4, 102.1, 102.2	6	102.6
	160 (1 x)	102.6, 104.7	2	
	240 (1.5 x)	106.2, 103.7	2	

***** The formulation was "spiked" with R2 impurity reference item at different concentrations. All values are relative to the formulation. Each fortification level was quantified according to the analytical method in order to calculate % recovery compared to the theoretical. The mean level of R2 impurity determined for precision in the test item is taken into account before calculating % recovery.

The overall recovery was in the range of 75 - 125 % and thus complies with the standard acceptance criteria of the guidance document SANCO/3030/99 rev 4 for a preparation containing < 0.1 % impurity.

Table A 5: Precision data of impurity R2 in product

Matrix	**% w/w determined**	**Mean Recovery (mg/L)**	**RSD (%)**
Formulation (SAE053H/01)	6x n.d.	< LOQ	-

Data was generated to demonstrate the repeatability of the method (six replicate analyses of the test item). No R2 impurity was detected in the precision samples. The mean is reported as <LOQ (0.0092% of impurity 2 in formulation).

Table A 6: Characteristics for the analytical method used for residue determination of impurity R2 in product

	Impurity R2
Specificity (degree of interference)	Interferences from co-formulants: No interfering peaks were detected from co-formulants or reagents in chromatograms of the blank formulation, mesotrione (TGAI) and nicosulfuron (TGAI), reference standards, and the solvent blank, in the region of the R2 impurity peak. The R2 reference standard and test item both gave peaks at retention times (± 0.02 min) of 29.6 minutes.
Specificity (identification)	Peak Identity: The retention time and UV-DAD of the impurity chromatographic peak obtained for the test item/spiked test item were recorded and compared to those recorded for the corresponding peak generated for the certified reference item.

<table>
<tr><td></td><td>Impurity R2</td></tr>
<tr><td></td><td>The retention time recorded for the R2 impurity peak in the test item matched that recorded for the reference standard. UV recorded for the R2 impurity peak in the spiked test item matched those recorded for the reference standard.

As R2 impurity was not present in the test item at a high enough level to generate spectral data, it was spiked at approximately 0.1% of the formulation.</td></tr>
<tr><td>Calibration (type, number of data points)
Calibration range</td><td>The linearity of the detector response of R2 was demonstrated by duplicate determination of solvent calibration standards at five (5) concentration levels ranging from 2 µg/mL to 50 µg/mL. This range corresponds to 40 µg/g to 1000 µg/g (0.004 % to 0.1 %) in the formulation. The approximate equivalent to mesotrione is 0.05% - 1.20 % (for 8.33 % w/w of mesotrione in the formulation.

The calibration was found to be linear with a correlation coefficients (r) of 0.9999. These results meet the acceptance criteria of r ≥0.99.
Y = 15.3805 * X + 2.6304, r= 0.9999</td></tr>
<tr><td>Limit of determination / quantification</td><td>The limit of quantitation (LOQ) is defined as the lowest concentration tested, at which an acceptable mean recovery and % RSD are obtained and has been determined from the Accuracy data (% recovery from formulation).
<table>
<tr><td>Specification Limit</td><td>Sample</td><td>% w/w R2 impurity (recovered)</td><td>% Recovery</td></tr>
<tr><td rowspan="6">0.5 x</td><td>0.008% Recovery 1</td><td>0.0090579</td><td>98.3</td></tr>
<tr><td>0.008% Recovery 2</td><td>0.0091683</td><td>99.5</td></tr>
<tr><td>0.008% Recovery 3</td><td>0.0096701</td><td>105.9</td></tr>
<tr><td>0.008% Recovery 4</td><td>0.0092941</td><td>102.4</td></tr>
<tr><td>0.008% Recovery 5</td><td>0.0094363</td><td>102.1</td></tr>
<tr><td>0.008% Recovery 6</td><td>0.0085963</td><td>101.2</td></tr>
<tr><td colspan="2">Mean:</td><td>0.0092038</td><td>101.6</td></tr>
<tr><td colspan="2">Std Dev:</td><td>0.00036639</td><td></td></tr>
<tr><td colspan="2">% RSD:</td><td>0.039808</td><td></td></tr>
<tr><td colspan="2">Acceptable RSDr:</td><td>5.4</td><td></td></tr>
</table>
The LOQ of the method is 0.0092% w/w of impurity R2 relative to the formulation. This equates to an LOQ of 0.11 % of the Mesotrione in the formulation, where Mesotrione was determined as 8.33 % w/w in SAE053H/01.

The acceptability of recovery for a preparation containing <0.1% impurity is 75-125%; the acceptability of the %RSD using the modified Horwitz equation for a preparation containing 0.009% analyte is ≤5.4.</td></tr>
</table>

Table A 7: Characteristics for the analytical method used for residue determination of impurity R1 in product

<table>
<tr><td></td><td>Impurity R1</td></tr>
<tr><td>Specificity
(degree of interference)</td><td>Interferences from co-formulants:
There was no interference in the region of the R1 impurity peak in the chromatograms of the solvent blank, or mesotrione TGAI solutions and small interference in the blank formulation.
There was an interference seen in the formulation solution and nicosulfuron TGAI solution which interfered with the R1 impurity peak. It was not possible to resolve the R1 and the impurity from one another using various chromatographic methods.

The R1 reference standard gave a peak at retention time of 43.16 minutes. The</td></tr>
</table>

	Impurity R1
	impurity seen in the formulation solution had a retention time of 43.18 minutes. The UV spectra confirm the peak observed in the formulation solution is not the R1 impurity. The UV spectrum recorded for the R1 impurity peak in the spiked test item provided a good match that recorded for the corresponding peak in the reference standard.
Specificity (identification)	Peak Identity: R1 impurity was not detected in the test item and therefore a UV spectra could not be generated; a test item solution spiked with the impurity was used for spectral identification. The retention time and UV-DAD of the impurity chromatographic peak obtained for the test item/spiked test item were recorded and compared to those recorded for the corresponding peak generated for the certified reference item. It was noted during the analysis that the retention times did shift due to high concentration of sample injected.
Specificity (Limit Test)	Due to the interference detected and the low levels of R1 required, a limit spike test was deemed the most suitable. This is a qualitative method which comprises of the analysing the formulation and the formulation spiked with R1 at a known concentration. The chromatograms are then overlaid and a pass/fail criteria applied if the peak area of the unspiked sample is greater (fail) or less (pass) than that of the spiked sample. In order to demonstrate the method can be used as a limit test for impurity R1 above 1 µg/g in the formulation, the following solutions were prepared, in duplicate, according to the method. - Test item - Test item spiked with 1 µg/g R1 (0.0001 %) - Test item spiked with 2 µg/g R1 (0.0002 %) The limit test was evaluated by overlaying chromatograms to show the increase in R1 response in the spiked test item chromatograms compared to the chromatograms for the test item unspiked. From the overlay, it is possible to state that the R1 in the formulation sample is less than 0.0001% or 1 µg/g in the formulation; equivalent to 0.0012% of Mesotrione in the formulation.
Calibration (type, number of data points) **Calibration range**	The linearity of the detector response of R1 was demonstrated by duplicate determination of solvent calibration standards at three (3) concentration levels ranging from 0.25 µg/mL to 1.25 µg/mL. This range corresponds to 1 µg/g to 5 µg/g (0.0001 % to 0.0005 %) in the formulation. The approximate equivalent to mesotrione is 0.001% - 0.006 % (for 8.33 % w/w of mesotrione in the formulation. The calibration was found to be linear with a correlation coefficients (r) of 0.99981. These results meet the acceptance criteria of r ≥0.99. Linear regression was performed without weighting. Y = 183.01907 * X + (-2.87492), r= 0.99981

Conclusion

The HPLC Analytical Method JP16001-5 for the determination of mesotrione impurity R2 in formulation SAE053H/01 has been validated to SANCO/3029/99 rev. 4 regulations.

The limit test method for impurity R1 has been shown to be fit for purpose for detecting R1 in formulation SAE053H/01 above a level of 0.0001% (1 µg/g) R1 impurity relative to the formulation.

Wronska, L. (2016)

A 2.1.1.1.3 Analytical method for determination of relevant impurity 1,2 Dichloroethane in the preparation

A 2.1.1.1.3.1 Method validation

Comments of zRMS:	The method is accepted for analysing 1,2 Dichloroethane in the PPP.

Reference: **2.1.1.1.3.1/01 (KCP 5.1.1/03)**

Report: Wronska, L. (2017)
Validation of Analytical Method JP6001-6 for the determination of Impurity 1,2 Dichloroethane in Product SAE053H/01 (80/30 Mesotrione/Nicosulfuron OD)
Report No.: JP160016

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

Deviations: None

GLP: Yes

Acceptability: Yes

Principle of the method

The GC-FID method of analysis JP16001-6 for the determination of the mesotrione impurity 1,2 Dichloroethane (DCE) in the formulation SAE053H/01 (80/30 Mesotrione/Nicosulfuron OD) has been validated. A test item solution spiked with the impurity was used for spectral identification and quantification was by GC-FID. The LOQ of the method is 0.004% w/w of impurity 1,2, Dichloroethane relative to the formulation. This equates to an LOQ of 0.05 % of the mesotrione in the formulation, where mesotrione was determined as 8.33 % w/w in SAE053H/01.

Sample preparation

In duplicate, approximately 2500 mg of the test item was weighed (0.1 mg accuracy) into two separate 50 mL volumetric flasks. 50 mL of acetone was added and well shaken. Filter with 0.45 µm filter prior to analysis.

GC-FID conditions – determination of 1,2 Dichloroethane impurity

GC system	Agilent HP7890A GC / HP5975C MSD Equipped with variable injector, FID detector, and electronic integrator.			
Column:	ZB-5, 30 m x 0.25 mm x 0.25 µm or equivalent			
Oven Program:	Starting Temp. (°C)	Ramp °C/min	Final Temp. (°C)	Hold Time (min)
	40	-	-	4
	-	30	300	7.3
Run time:	Approx. 20 mins			
Mode	Split / Constant Flow			
Flow:	Helium 1.3 mL/min			
Split:	7:1			
Injector Temperature:	240 °C			
Injector Volume:	2 µL			

Detector Temperature:	300 °C
H_2 Flow:	30 mL/min
Air Flow:	300 mL/min
Make up Flow (N_2)	40 mL/min

Results and discussions

Table A 8: Accuracy data of impurity 1,2 Dichloroethane in product

Matrix	Fortification level (µg/g) *	Recovery (%)	Replicates	Overall Mean Recovery (%)
Formulation (SAE053H/01)	40 (0.5 x)	91.2, 111.2, 97.4, 95.5, 96.7, 99.4	6	99.0
	80 (1 x)	100.9, 98.5	2	
	120 (1.5 x)	100.0, 99.2	2	

* The formulation was "spiked" with 1,2 Dichloroethane impurity reference item at different concentrations. All values are relative to the formulation. Each fortification level was quantified according to the analytical method in order to calculate % recovery compared to the theoretical. The mean level of 1,2 Dichloroethane impurity determined for precision in the test item is taken into account before calculating % recovery.

The overall recovery was in the range of 80 - 120 % and thus complies with the standard acceptance criteria of the guidance document SANCO/3030/99 rev 4 for a preparation containing 0.1 - 1 % impurity.

Table A 9: Precision data of impurity 1,2, Dichloroethane in product

Matrix	% w/w determined	Mean Recovery (mg/L)	RSD (%)
Formulation (SAE053H/01)	6x < LOQ	< LOQ	-

Table A 10: Characteristics for the analytical method used for residue determination of impurity 1,2 Dichloroethane in product

	Impurity 1,2 Dichloroethane
Specificity (degree of interference)	Interferences from co-formulants: No interfering peaks were detected in the reagent blank or co-formulant Nicosulfuron, in the region of the DCE impurity peak at ~2.27 minutes. Small peak detected to the right of the main analyte peak in the formulation blank and small peak detected in the formulation and in co-formulant mesotrione; likely to be DCE.
Specificity (identification)	Peak Identity: The DCE impurity was detected in the test item but at such a low level that a meaningful GC-MS spectrum could not be generated. A test item solution spiked with the impurity was used for spectral identification. The retention time recorded for the DCE impurity peak in the spiked test item matched that recorded for the reference standard in GC-FID. GC-MS spectra for the DCE impurity peak in the spiked test item matched those recorded for the reference standard. Ions observed: DCE Standard: m/z 49, 62, 98

<table>
<tr><td></td><td>Impurity 1,2 Dichloroethane</td></tr>
<tr><td></td><td>DCE Spiked Sample: m/z 49, 62, 98</td></tr>
<tr><td>Calibration (type, number of data points)
Calibration range</td><td>The linearity of the detector response of DCE was demonstrated by duplicate determination of solvent calibration standards at five (5) concentration levels ranging from 1 µg/mL to 10 µg/mL. This range corresponds to 20 µg/g to 200 µg/g (0.002 % to 0.02 %) in the formulation. The approximate equivalent to mesotrione is 0.024% - 0.240 % (for 8.33 % w/w of mesotrione in the formulation).
The calibration was found to be linear with a correlation coefficients (r) of 0.99982. These results meet the acceptance criteria of r ≥0.99.
Y = 144947.44 * X + (-39374.15), r= 0.99982</td></tr>
<tr><td>Limit of determination / quantification</td><td>The limit of quantitation (LOQ) is defined as the lowest concentration tested, at which an acceptable mean recovery and % RSD are obtained and has been determined from the Accuracy data (% recovery from formulation).

<table>
<tr><td>Specification Limit</td><td>Sample</td><td>% w/w DCE (recovered)</td><td>% Recovery</td></tr>
<tr><td rowspan="6">0.5 x</td><td>0.004% Recovery 1</td><td>0.0035</td><td>91.2</td></tr>
<tr><td>0.004% Recovery 2</td><td>0.0043</td><td>111.2</td></tr>
<tr><td>0.004% Recovery 3</td><td>0.0038</td><td>97.4</td></tr>
<tr><td>0.004% Recovery 4</td><td>0.0037</td><td>95.5</td></tr>
<tr><td>0.004% Recovery 5</td><td>0.0037</td><td>96.7</td></tr>
<tr><td>0.004% Recovery 6</td><td>0.0038</td><td>99.4</td></tr>
<tr><td colspan="2">Mean:</td><td>0.0038</td><td>98.6</td></tr>
<tr><td colspan="2">Std Dev:</td><td>0.000246</td><td></td></tr>
<tr><td colspan="2">% RSD:</td><td>6.96</td><td></td></tr>
<tr><td colspan="2">Acceptable RSDr:</td><td>6.20</td><td></td></tr>
</table>

The LOQ of the method is 0.004% w/w of impurity DCE relative to the formulation. This equates to an LOQ of 0.05 % of the Mesotrione in the formulation, where Mesotrione was determined as 8.33 % w/w in SAE053H/01.

The acceptability of recovery for a preparation containing 0.1-1% impurity is 80-120%; the acceptability of the %RSD using the modified Horwitz equation for a preparation containing 0.004% analyte is ≤6.2.

The calculated %RSD was ~7%. The modified Horwitz equation is derived empirically and is not suitable for all situations. A 7% RSD on a %w/w value of 0.004 equates to a range of ±0.00028%. The modified Horwitz %RSD limit is 6.2% which would equate to 0.00025%.

The difference between the two values at that level is not significant. The RSD% is deemed acceptable for the concentration levels assessed.</td></tr>
</table>

Conclusion

The GC-FID method JP16001-6 for the determination of mesotrione impurity 1,2 Dichloroethane in formulation SAE053H/01 has been validated to SANCO/3029/99 rev. 4 regulations.

Wronska, L. (2017)

A 2.1.1.2 Description of analytical methods for the determination of mesotrione and nicosulfuron residues in crops (Residues) (KCP 5.1.2)

A 2.1.1.2.1 Analytical method 1

A 2.1.1.2.1.1 Method validation

The validation of the analytical method used for the determination of mesotrione in maize matrix was performed by Schernikau N. and Colorado C.S., 2016 in study S15-04204. This validated method was later used in study S15-03081 for the determination of mesotrione residues in maize matrix. (see KCP 5.1.2/01 and KCP 5.1.2/02).

Comments of zRMS:	The analytical method codified as S15-04204 was fully validated in term of specificity, linearity, repeatability, accuracy and LOQ according to SANCO 3029/99 rev. 4.The validation was performed quantifying mesotrione in maize matrices (whole plant, grain and rest of plant) by LC-MS/MS after extraction prepared using the multi-residue QuEChERS method. Two transitions were monitored: 338 m/z - 291 m/z (for quantification) and 338 m/z – 212 m/z (for confirmation). The limit of quantification (LOQ) is successfully established at 0.01 mg/kg in all matrices of maize for both ion mass transition. The limit of detection (LOD) for all matrices was set at 0.003 mg/kg (30% of the LOQ). The method linearity was evaluated at 8 levels, ranging from 0.15 ng/ml to 10 ng/ml, corresponds to 0.003 mg/kg to 0.2 mg/kg. Recovery analysis was performed for samples spiked with mesotrione at LOQ (0.01 mg/kg) and 10xLOQ (0.1 mg/kg) for both ion mass transition, 5 replicate analysis were performed for each level. Mean recovery is between 70%-110% with RSD <20% in all cases, in accordance with acceptance criteria. The method is accepted and suitable for determination of mesotrione in maize grain, whole plant and rest of plant.

Reference:	**2.1.1.2.1.1/01 (KCP 5.1.2/01)**
Report	Schernikau N. and Colorado C.S., 2016 Validation of the Analytical Method QuEChERS for the Determination of Mesotrione in Maize Matrices Report No. S15-04204
Guideline(s):	SANCO/825/00 rev. 8.1
Deviations:	No
GLP	Yes
Acceptability:	Yes

Materials and Methods

A residue analytical method for the determination of mesotrione in maize matrices (whole plant, grain and rest of plant) in analogy to the multi-residue QuEChERS method was validated. In brief, samples of maize (whole plant, grain and rest of plant) were extracted with acetonitrile after addition of water. After addition of a buffer salt mixture, containing magnesium sulphate, sodium chloride and sodium citrate, the extract was shaken. After centrifugation, an aliquot of the acetonitrile phase was cleaned by dispersive solid phase extraction, using primary secondary amine (PSA). The quantification was performed by high-

ly selective LC-MS/MS detection using external calibration. The limit of quantification (LOQ) was set to 0.01 mg/kg for all matrices.

Sample preparation for all matrices
Specimens (maize grain, rest of plant and whole plant) were thoroughly homogenized in a cutter with dry ice. The homogenised specimens were stored at ≤ -18°C until start of analysis.

Specimen amount taken for analysis was 5 g for all matrices.

Specimen preparation
Extraction and Liquid/Liquid Partition of maize (whole plant, grain and rest of plant)

5.0 g of the homogenized specimen of maize (whole plant, grain and rest of plant) was weighed into a 50 ml centrifuge tube. 6 ml of water was added to each matrix. Exactly 10.0 ml of acetonitrile was added to each tube, the tube was capped and shaken vigorously by hand for at least one minute. Thereafter, 4.0 g of magnesium sulfate, 1.0 g of sodium chloride, 1.0 g of trisodium citrate dihydrate and 0.5 g of disodium hydrogen citrate sesquihydrate were added and the centrifuge tube was shaken about one minute. afterwards, the sample tubes were centrifuged.

Extract Clean-up and Reconstitution for Analysis
After centrifugation an aliquot of 1.5 ml of the cleaned-up acetonitrile phase was pipetted into a 2 mL BEKOlut tube (BEKOlut PSA-kit 01) which already contains 150 mg magnesium sulfate and 25 mg PSA. The tube was shaken vigorously in a vortex mixer for 30 seconds followed by centrifugation for 3 minutes. Before analysis, 100 µL of the extract of maize (whole plant, grain and rest of plant) was mixed with 900 µL of acetonitrile / water (1/1, v/v) to achieve a final volume of 1.0 ml. The final sample was analysed by LC-MS/MS.

Equipment for mesotrione determination

<table>
<tr><td>HPLC system</td><td>Series 1200 HPLC (Agilent Technologies)</td></tr>
<tr><td>Column:</td><td>Agilent Eclipse XDB C18, 50 x 4.6 mm, 1.8µm particle size</td></tr>
<tr><td>Column temperature:</td><td>45°C</td></tr>
<tr><td>Injection Volume:</td><td>10 µL</td></tr>
<tr><td>Mobile phases:</td><td>Eluent A: Acetonitrile
Eluent B: Water + 0.2 % (v/v) Formic acid</td></tr>
<tr><td>Gradient:</td><td>
<table>
<tr><td>Time [min]</td><td>% Eluent A</td><td>% Eluent B</td><td>Flow [µL/min]</td></tr>
<tr><td>0.00</td><td>0</td><td>100</td><td>800</td></tr>
<tr><td>4.00</td><td>95</td><td>5</td><td>800</td></tr>
<tr><td>5.00</td><td>95</td><td>5</td><td>800</td></tr>
<tr><td>5.10</td><td>0</td><td>100</td><td>800</td></tr>
<tr><td>6.50</td><td>0</td><td>100</td><td>800</td></tr>
</table>
</td></tr>
<tr><td>Divert valve:</td><td>0.0 min to 2.8 min to waste; 2.8 min to 4.2 min to MS</td></tr>
<tr><td>Retention time:</td><td>3.4 min</td></tr>
<tr><td colspan="2">Mass spectrometric conditions</td></tr>
<tr><td>MS system:</td><td>API 4000™ LC/MS/MS System, AB Sciex</td></tr>
<tr><td>Ionisation type:</td><td>Electrospray (ESI, Turboion Spray)</td></tr>
<tr><td>Polarity:</td><td>Negative ion mode</td></tr>
<tr><td>Scan type:</td><td>MS/MS, Multiple Reaction Monitoring (MRM)</td></tr>
<tr><td>Analyte monitored</td><td>Mesotrione</td></tr>
<tr><td>Ion mass transition</td><td>338 → 291 # collision energy = - 14V</td></tr>
</table>

monitored (m/z)	338 → 212 collision energy = - 44V

proposed as quantification transition but any of the ion mass transitions listed can be used for quantification.

Results and discussions

Table A 11: Recovery results from method validation of mesotrione in maize matrices

Matrix	Fortification level (mg/kg)	Recovery (%)	Mean recovery (%)	RSD (%)	Replicates	Overall Mean Recovery (%)	Overall RSD (%)
Ion Mass Transition 338 → 291 m/z (quantification)							
Maize (whole plant)	0.01	83, 81, 81, 83, 82	82	1.2	5	79	4.9
	0.1	71, 77, 77, 76,81	76	4.7	5		
Maize (grain)	0.01	68, 70, 72, 74,74	72	3.6	5	71	5.2
	0.1	77, 72, 73,67,65	71	6.8	5		
Maize (rest of plant)	0.01	81,82,85,85, 90	85	4.1	5	84	6.3
	0.1	78, 76, 82, 93, 88	83	8.5	5		
Ion Mass Transition 388 → 212 m/z (Confirmation)							
Maize (whole plant)	0.01	78, 79,83, 76,78	79	3.3	5	78	3.6
	0.1	72, 77, 76,77,79	76	3.4	5		
Maize (grain)	0.01	72, 75, 76,65, 81	74	8.0	5	72	6.7
	0.1	72, 71, 72,66,68	70	3.8	5		
Maize (rest of plant)	0.01	76, 82, 82, 73, 88	80	7.3	5	81	7.1
	0.1	77,80, 79,92,85	83	7.3	5		

RSD = Relative Standard Deviation

All mean recovery values at the fortification levels of 0.01 mg/kg and 0.1 mg/kg for both ion mass transitions comply with the standard acceptance criteria of SANCO/825/00 rev 8.1 since mean recoveries were in the range of 70 - 110 % with a relative standard deviation of ≤ 20 % for all matrices.

Table A 12: Characteristics for the analytical method used for residue determination of mesotrione in matrices of plant origin

	Mesotrione
Specificity / Selectivity	A highly specific detection system was used (LC-MS/MS) and two mass transitions were monitored. For both ion mass transitions, the specimens showed no significant interference (above 30 % of LOQ) at the retention time of the analytes in all studied plant matrices, therefore showing that the method is highly specific. Blank correction was not necessary. Exemplary chromatograms for each matrix representing control specimens, the lowest calibration level, specimens fortified at the LOQ and specimens fortified at 10 x LOQ are included in the report together with a mass scan and product ion spectrum.

	Mesotrione
Calibration (type, number of data points) **Calibration range**	The linearity of the detector response of mesotrione in maize (whole plant, grain and rest of plant) was demonstrated by single determination of solvent calibration standards at eight (8) concentration levels ranging from 0.15 ng/ml to 10 ng/ml. This range corresponds to a fortification level of 0.003 mg/kg to 0.2 mg/kg and thus covers the range from no more than 30 % of the LOQ and at least+ 20 % of the highest analyte concentration detected in a sample. The calibration curves obtained for both ion mass transitions in maize (whole plant, grain and rest of plant) were linear with coefficients of determination (r^2) greater than 0.99. Linear regression was performed with 1/x- weighting. Mesotrione whole plant: 338 → 291 m/z: Y = 9673.49 x – 268.36, R^2= 0.9997 338 → 212 m/z: Y = 2091.58 x – 31.67, R^2 = 0.9987 Mesotrione maize grain: 338 → 291 m/z: Y = 10589.40 x – 411.85, R^2= 0.9991 338 → 212 m/z: Y = 2250.18 x – 84.2386, R^2 = 0.9968 Mesotrione maize (rest of plant): 338 → 291 m/z: Y = 11794.27 x – 189.48, R^2= 0.9995 338 → 212 m/z: Y = 2567.65 x – 13.2340, R^2 = 0.9998
Assessment of matrix effects is presented	Matrix effects on the detection of mesotrione in extracts of maize (whole plant, grain and rest of plant) were found to be insignificant (< 20 %). Therefore, solvent standards were used for quantification for these matrices.
Limit of determination / quantification	The limit of quantification (LOQ) is defined as the lowest validated level and was successfully established at 0.01 mg/kg. The limit of detection (LOD) was set at 0.003 mg/kg, which is 30% of the LOQ.
Extract Stability	Mesotrione was found to be stable in final extracts of each matrix for a maximum of 7 days when stored at 5 °C ± 4 °C

Conclusion

The method was successfully validated for mesotrione and maize (whole plant, grain and rest of plant) at the tested LOQ of 0.01 mg/kg according to the guidance document SANCO/825/00 rev 8.1.

Schernikau N., Colorado C.S., 2016

A 2.1.1.2.1.2 Method validation

Comments of zRMS:	The analytical method in study codified as No. S15-03081was not fully validated according to SANCO 3029/99 rev. 4 (only procedural recoveries were investigated instead of 5 samples at LOQ and 10xLOQ), hovewer the same method codified as S15-03081 was already fully validated before in the same laboratory under S15-04204 (KCP 5.1.2/01). Presented data are sufficient to demonstrate that the method is adeccuate for the determination of residues of mesotrione in maize matrices (whole plant, grain and rest of plant). The LC-MS/MS after extraction prepared using the multi-residue QuEChERS method was used. Two transitions were monitored: 338 m/z - 291 m/z (for quantification) and 338 m/z – 212 m/z (for confirmation). The limit of quantification (LOQ) was established at 0.01 mg/kg in all matrices of maize for both ion mass transition. The limit of detection (LOD) for all matrices was set at 0.003 mg/kg (30% of the LOQ). The method linearity was evaluated at 7 levels, ranging from 0.15 ng/ml to 10 ng/ml, corresponds to 0.003 mg/kg to 0.2 mg/kg. Fortifications were performed at the level of

	0.01 mg/kg, 0.10 mg/kg, 1.0 mg/kg (for maize grain and rest of plant) and 8.0 mg/kg (for maize whole plant). Mean recovery is between 70%-110% with RSD <20% in all cases, in accordance with acceptance criteria. The method is accepted.

Reference:	**2.1.1.2.1.2/01 (KCP 5.1.2/02 also filed under KCP 8.3.1/01)**
Report	Semrau J., 2017 Determination of residues of mesotrione after one application of Mesotrione 80 g/L + Nicosulfuron 30 g/L OD in maize at 4 sites in Northern Europe 2015 Report No. S15-03081
Guideline(s):	SANCO/3029/00 rev. 4
Deviations:	No
GLP	Yes
Acceptability:	Yes

Materials and methods

A residue analytical method for mesotrione in maize matrices (grain, rest of plant and whole plant) in analogy to the multiresidue method QuEChERS was used. The method was previously validated in study S15-04204 by Schernikau, N. and Colorado S.C., 2016 ("Validation of the Analytical Method QuEChERS for the Determination of Mesotrione in Maize Matrices") (see KCP 5.1.2/01). The method involves extraction of mesotrione with buffered acetonitrile / water, followed by appropriate dilution of the extract with acetonitrile and subsequent liquid chromatography separation coupled with tandem mass spectrometric detection (LC-MS/MS). The limit of quantification (LOQ) was set to 0.01 mg/kg for all matrices.

Within this study, procedural recoveries were made to verify the validity of the used method.

Sample preparation for all matrices

Sample preparation is identical as described under KCP 5.1.2/01.

Specimen preparation

Specimen preparation is identical as described under KCP 5.1.2/01.

Equipment for mesotrione determination for all matrices

HPLC system	Agilent Series 1200 HPLC			
Column:	Agilent Eclipse XDB-C18, 50 mm × 4.6 mm, 1.8 µm			
Column temperature:	45 °C			
Injection Volume:	10 µL			
Mobile phase:	Eluent A: Acetonitrile Eluent B: Water + 0.2 % formic acid			
Gradient:	Time [min]	% Eluent A	% Eluent B	Flow [µL/min]
	0	0	100	800
	4	95	5	800
	5	95	5	800
	5.1	0	100	800
	6.5	0	100	800

Retention time(s)	approx. 3.7 min
Mass spectrometric conditions	
MS system	API 4000 Mass Spectrometer
Ionisation type:	Electro spray ionization (ESI)
Polarity:	Negative ion mode
Scan type:	MS/MS, Multiple Reaction Monitoring (MRM)
Analyte monitored:	Mesotrione
Ion mass transition monitored (m/z):	338 → 291 # collision energy = - 14V 338 → 212 collision energy = - 44V

proposed as quantification transition but any of the ion mass transitions listed can be used for quantification.

Results and discussions

Table A 13: Accuracy and Precision data of mesotrione in maize matrices

Matrix	Fortification level (mg/kg)	Recovery (%)	Overall Mean Recovery (%)	Overall RSD (%)
Ion Mass Transition 338 → 291 m/z (Quantification)				
Maize (whole plant)	0.01 (LOQ)	70	75	8.5
	0.10	76		
	1.0	84		
	8.0	71		
Maize (grain)	0.01 (LOQ)	71	77	12
	0.10	72		
	1.0	87		
Maize (rest of plant)	0.01 (LOQ)	71	77	7.8
	0.10	83		
	1.0	78		

Single recoveries as well as the mean recovery values at each fortification level (for all matrices) were in the range of 70-110%. The overall mean recovery and the overall relative standard deviation (RSD) was ≤ 20% in all matrix types. Recoveries as well as RSD comply with the standard acceptance criteria of the guidance documents.

Table A 14: Characteristics for the analytical method used for residue determination of mesotrione in matrices of plant origin

	Mesotrione
Specificity	Quantification was performed by use of LC-MS/MS detection. Two (2) mass transitions were evaluated in order to demonstrate that the method achieves a high level of specificity. The retention times of mesotrione signals in the specimen extracts match the retention time of the standard solution. No interferences above 30 % of the LOQ at the retention time of approx. 3.7 minutes (all matrices) were detected in the untreated control samples. Conclusively the method is specific for the determination of mesotrione in maize matrices (grain and whole

	Mesotrione
	plant).
Calibration (type, number of data points) **Calibration range**	The linearity of the detector response was demonstrated by single determination of matrix-matched and solvent calibration standards at a minimum of seven (7) concentration levels ranging from 0.15 ng/L to 10 ng/L. This range corresponds to 0.003 mg/kg to 0.20 mg/kg and thus covers the range of no more than 30% of the LOQ and at least + 20 % of the highest analyte concentration detected in any (diluted) specimen extract. These margins cover the required minimum range. Matrix-matched standards were used for all matrices. The calibration was found to be linear in the concentration range of 0.15 ng/L to 10 ng/L with correlation coefficients (r) ranging from 0.9991 to 0.9999. These results meet the acceptance criteria of r ≥0.99. Linear regression was performed without weighting. Mesotrione in maize (grain): 338 → 291 m/z: Y = 9840.7195 x - 111.6797, r= 0.9999 Mesotrione in maize (rest of plant): 338 → 291 m/z: Y = 10536.5063 x + 25.9976, r= 0.9991 Mesotrione in maize (whole plant): 338 → 291 m/z: Y = 12220.0554 x - 508.6863, r= 0.9997
Assessment of matrix effects is presented	Mean matrix effects were ≤ ± 20% and therefore considered to be insignificant for all matrices. Nevertheless, matrix-matched standards were used for all matrices.
Limit of determination / quantification	The limit of quantification (LOQ) is defined as the lowest fortification level with mean recoveries ranging from 70% to 110% at a relative standard deviation (RSD) of ≤20%. These criteria were fulfilled for mesotrione in maize matrices with an LOQ of 0.01 mg/kg. The limit of detection (LOD) was found to be 0.003 mg/kg for mesotrione. The LOD was defined as 30% of the limit of quantification for residues in control samples (i.e. 0.003 mg/kg)
Extract Stability	-

Conclusion

The applicability of the method for the analysis of residues of mesotrione in maize matrices was tested. The procedural recovery data presented demonstrate that the method permits the determination of residues of mesotrione in maize matrices.

Semrau, J., 2017

A 2.1.1.2.1.3 Method validation

Comments of zRMS:	The analytical method in the study codified as JS001LRM was fully validated in term of specificity, linearity, repeatability, accuracy and LOQ according to SANCO 3029/99 rev. 4.The validation was performed quantifying mesotrione in maize seedlings by LC-MS/MS after extraction prepared using the multi-residue QuEChERS method. Two transitions were monitored: 338 m/z - 291 m/z (for quantification) and 338 m/z – 212 m/z (for confirmation). The limit of quantification (LOQ) is successfully established at 0.01 mg/kg for both ion mass transition. The limit of detection (LOD) was set at 0.003 mg/kg (30% of the LOQ). The method linearity was evaluated at 8 levels, ranging from 0.15 ng/ml to 25 ng/ml, corresponds to 0.003 mg/kg to 0.5 mg/kg. Recovery analysis was performed for

	samples spiked with mesotrione at LOQ (0.01 mg/kg) and 10xLOQ (0.1 mg/kg) for both ion mass transition, 5 replicate analysis were performed for each level. Mean recovery is between 70%-110% with RSD <20% in all cases, in accordance with acceptance criteria. Based on recovery results it is possible to asses that spiked sample extracts show good stability over analysis time. The method is accepted and suitable for determination of mesotrione in maize seedlings.

Reference:	**2.1.1.2.1.3/01 (KCP 5.1.2/03 also filed under KCP 8.10/01)**
Report	Bakker F., 2016 Magnitude of Mesotrione Residues in Maize Plants following one application in Southern and Northern Europe in 2016 Study Code: JS001LRM Analytical Phase Code: S16-00945-L1 (GAB-1650)
Guideline(s):	SANCO/3029/99, rev. 4
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

A residue analytical method for mesotrione in maize matrices (maize seedlings) based on the multi residue method QuEChERS (EN15662) was validated. The method involves extraction of mesotrione with buffered acetonitrile / water and, if required, followed by appropriate dilution of the extract with acetonitrile and subsequent liquid chromatography separation coupled with tandem mass spectrometric detection (LC-MS/MS). The limit of quantification (LOQ) was set to 0.01 mg/kg.

Sample preparation for all matrices
Specimens (maize seedlings) were thoroughly homogenized in a cutter with dry ice. The homogenised specimens were stored at ≤ -18°C until start of analysis.

Extraction of mesotrione for all matrices
5 g of the homogenized specimen of maize seedling was weighed into a 50 mL centrifuge tube and 7 mL of water were added with at least 20 minute soaking time at room temperature. Subsequently exactly 10 mL (V_{EX}) of acetonitrile were added. The centrifuge tube was capped and shaken for 5 minutes on a shaker. Thereafter, QuEChERS EN15662 salt-mixture (4.0 g of magnesium sulphate, 1.0 g of sodium chloride, 1.0 g of trisodium citrate dehydrate and 0.5 g of disodium hydrogen citrate sesquihydrate) was added. The centrifuge tube was capped again and immediately shaken by hand and centrifuged for 2 minutes. An aliquot of 100 µL (V_{Ali}) of the extract of maize seedlings was diluted with 900 µL of acetonitrile/water (1/1, v/v) and transferred into a HPLC-vial. The final extracts are stored at 1°C to 10°C in the dark until analysis by HPLC-MS/MS.

If necessary, final specimen extracts were diluted with acetonitrile/water (1/1, v/v) to be within the calibration range.

Equipment for mesotrione determination for all matrices

HPLC system	1200 Infinity Binary LC System, Agilent Technologies (HPLC, ≤ 600 bar)
Column:	ZORBAX Eclipse XDB-C18, 50 mm × 4.6 mm, 1.8 µm
Column temperature:	45 °C
Injection Volume:	15 µL

Mobile phase:	Eluent A: Acetonitrile Eluent B: Water containing 0.2 % (v/v) formic acid			
Gradient:	Time [min]	% Eluent A	% Eluent B	Flow [µL/min]
	0.0.	0	100	800
	4.0	95	5	800
	5.0	95	5	800
	5.1	0	100	800
	6.5	0	100	800
Retention time(s)	approx. 3.3 min			
Mass spectrometric conditions				
MS system	API 4000 System, SCIEX (Triple quadrupole mass spectrometer)			
Ionisation type:	Electrospray ionization (ESI, TurboIon Spray)			
Polarity:	Negative ion mode			
Scan type:	MS/MS, Multiple Reaction Monitoring (MRM)			
Analyte monitored:	Mesotrione			
Ion mass transition monitored (m/z):	338 → 291 # collision energy = - 14V 338 → 212 collision energy = - 44V			

proposed as quantification transition but any of the ion mass transitions listed can be used for quantification.

Results and discussions

Table A 15: Accuracy and Precision data of mesotrione in matrices of plant origin (Method validation)

Matrix	Fortification level (mg/kg)	Recovery (%)	Mean recovery (%)	RSD (%)	Replicates	Overall Mean Recovery (%)	Overall RSD (%)
Ion Mass Transition 338 → 291 m/z (Quantification)							
Maize (seedlings)	0.01 (LOQ)	83, 87, 88, 94, 80	86	6.2	5	90	6.2
	0.1	94, 94, 97, 93, 94	94	1.6	5		
Ion Mass Transition 338 → 212 m/z (Confirmation)							
Maize (seedlings)	0.01 (LOQ)	89, 83, 78, 82, 89	84	5.7	5	89	7.0
	0.1	94, 93, 94, 97, 93	94	1.7	5		

The mean recovery at each fortification level was in the range of 70 - 110 % with a relative standard deviation of ≤ 20 % for both mass transitions and thus complies with the standard acceptance criteria of the guidance document SANCO/3029/99 rev 4.

Table A 16: Accuracy and Precision data of mesotrione in maize matrix (Procedural Recoveries)

Matrix	Fortification level (mg/kg)	Recovery (%)	Mean recovery (%)	RSD (%)	Replicates	Overall Mean Recovery (%)	Overall RSD (%)
Ion Mass Transition 338 → 291 m/z (Quantification)							
Maize (seedlings)	0.01 (LOQ)	77, 77, 72, 74, 70, 73, 82, 72, 83, 80, 72, 71, 91, 111	79	14	14	81	13
	0.1	84, 86, 85, 83, 79, 82, 76, 70, 86, 85, 73, 73, 101	82	9.8	13		
	40	104	104	-	1		

Single recoveries were in the range of 70 -110 % each, while the mean recoveries at each fortification level were in the range of 70 - 110 %. Wherever applicable (n ≥ 3), the relative standard deviation was ≤ 20 % for each level.

Table A 17: Characteristics for the analytical method used for residue determination of mesotrione in maize matrix

	Mesotrione
Specificity	Quantification was performed by use of LC-MS/MS detection. Two (2) mass transitions were evaluated in order to demonstrate that the method achieves a high level of specificity. The retention times of mesotrione signals in the specimen extracts match the retention time of the standard solution. No interferences above 30 % of the LOQ at the retention time of approx. 3.3 minutes were detected in the untreated control samples. Conclusively the method is specific for the determination of mesotrione in maize seedlings.
Calibration (type, number of data points) **Calibration range**	The linearity of the detector response was demonstrated by single determination of solvent calibration standards at eight (8) concentration levels ranging from 0.15 ng/L to 25 ng/L. This range corresponds to 0.003 mg/kg to 0.50 mg/kg and thus covers the range of no more than 30% of the LOQ and at least + 20 % of the highest analyte concentration detected in any (diluted) specimen extract. These margins cover the required minimum range. The calibration was found to be linear in the concentration range of 0.15 ng/L to 25 ng/L with correlation coefficients (r) of 0.9999. These results meet the acceptance criteria of r ≥0.99. Linear regression was performed without weighting. Mesotrione in maize (seedlings): 338 → 291 m/z: Y = 19131.1866 x - 378.0856, r= 0.9999 338 → 212 m/z: Y = 3910.3908 x - 392.0428, r= 0.9999
Assessment of matrix effects is presented	Mean matrix effects were ≤ ± 20% and therefore considered to be insignificant for all matrices. Therefore, solvent standards were used.
Limit of determination / quantification	The limit of quantification (LOQ) is defined as the lowest fortification level with mean recoveries ranging from 70% to 110% at a relative standard deviation (RSD) of ≤20%. These criteria were fulfilled for mesotrione in maize matrices with an LOQ of 0.01 mg/kg. The limit of detection (LOD) was found to be 0.003 mg/kg for mesotrione. The LOD was defined as 30% of the limit of quantification for residues in control samples (i.e. 0.003 mg/kg)
Extract Stability	The maximum storage interval of final sample extracts at 1°C to 10°C from extraction to injection to LC-MS/MS was 5 days. Extract stability was proven by the corresponding procedural recovery samples,

	Mesotrione
	which were stored under the same conditions together with the extracts of the specimens for residue analysis.

Conclusion

The method was successfully validated for determination of mesotrione in maize seedlings with an LOQ of 0.01 mg/kg according to the guidance document SANCO/3029/99 rev. 4.
With regard to selectivity, accuracy and precision, the analytical method was applied successfully for each analytical set when analysing the specimens of the study.

Bakker F., 2016

A 2.1.1.2.1.4 Method validation

Comments of zRMS:	The analytical method in the study codified as S17-05218 is identical as described in study No JS00LRM (KCP 5.1.2/03 also filed under KCP 8.10) - fully validated in term of specificity, linearity, repeatability, accuracy and LOQ according to SANCO 3029/99 rev. 4.The validation was performed quantifying mesotrione in maize seedlings by LC-MS/MS after extraction prepared using the multi-residue QuEChERS method. Two transitions were monitored: 338 m/z - 291 m/z (for quantification) and 338 m/z – 212 m/z (for confirmation). The limit of quantification (LOQ) is successfully established at 0.01 mg/kg for both ion mass transition. The limit of detection (LOD) was set at 0.003 mg/kg (30% of the LOQ). The method linearity was evaluated at 8 levels, ranging from 0.15 ng/ml to 25 ng/ml, corresponds to 0.003 mg/kg to 0.5 mg/kg. Procedural recovery was performed. For samples spiked with mesotrione at LOQ (0.01 mg/kg) and 10xLOQ (0.1 mg/kg) per analytical set of each respective matrix. Mean recovery is between 70%-110% with RSD <20% in all cases. The method is accepted.

Reference:	**2.1.1.2.1.4/01 (KCP 5.1.2/04 also filed under KCP 8.10/02)**
Report	van de Sandt, H.J., 2019 Decline of mesotrione residues in maize plants following one application in The Netherlands – 2017 Study Code: S17-05218 Analytical Phase Code: S17-05218-L1 (BRE-1704)
Guideline(s):	SANCO/3029/99, rev. 4
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

A residue analytical method for mesotrione in maize matrices (maize seedlings) based on the multi residue method QuEChERS (EN15662) was validated in study JS001LRM (see KCP 5.1.2/03).

The method involves extraction of mesotrione with buffered acetonitrile / water and, if required, followed by appropriate dilution of the extract with acetonitrile and subsequent liquid chromatography separation coupled with tandem mass spectrometric detection (LC-MS/MS). The limit of quantification (LOQ) was set to 0.01 mg/kg.

Sample preparation for all matrices

Whole seedlings (without roots) were thoroughly homogenized in a cutter with dry ice. The homogenised specimens were stored at ≤ -18°C until start of analysis.
For maize seedlings, the maximum storage interval at -18°C from sampling to extraction was 82 days. The maximum storage interval of final sample extracts at 1 °C to 10°C from extraction to until injection to HPLC-MS/MS was 8 days.

Extraction of mesotrione for all matrices

5 g of the homogenized specimen of maize seedling was weighed into a 50 mL centrifuge tube and 7 mL of water were added with at least 20 minute soaking time at room temperature. Subsequently exactly 10 mL (V_{EX}) of acetonitrile were added. The centrifuge tube was capped and shaken for 5 minutes on a shaker. Thereafter, QuEChERS EN15662 salt-mixture (4.0 g of magnesium sulphate, 1.0 g of sodium chloride, 1.0 g of trisodium citrate dehydrate and 0.5 g of disodium hydrogen citrate sesquihydrate) was added. The centrifuge tube was capped again and immediately shaken by hand and centrifuged for 2 minutes. An aliquot of 100 µL (V_{Ali}) of the extract of maize seedlings was diluted with 900 µL of acetonitrile/water (1/1, v/v) and transferred into a HPLC-vial. The final extracts are stored at 1°C to 10°C in the dark until analysis by HPLC-MS/MS.

If necessary, final specimen extracts were diluted with acetonitrile/water (1/1, v/v) to be within the calibration range.

Equipment for mesotrione determination for all matrices

HPLC system	1200 Infinity Binary LC System, Agilent Technologies (HPLC, ≤ 600 bar)			
Column:	ZORBAX Eclipse XDB-C18, 50 mm × 4.6 mm, 1.8 µm			
Column temperature:	45 °C			
Injection Volume:	15 µL			
Mobile phase:	Eluent A: Acetonitrile Eluent B: Water containing 0.2 % (v/v) formic acid			
Gradient:	Time [min]	% Eluent A	% Eluent B	Flow [µL/min]
	0.0.	0	100	800
	4.0	95	5	800
	5.0	95	5	800
	5.1	0	100	800
	6.5	0	100	800
Retention time(s)	approx. 3.3 min			
Mass spectrometric conditions				
MS system	API 4000 System, SCIEX (Triple quadrupole mass spectrometer)			
Ionisation type:	Electrospray ionization (ESI, TurboIon Spray)			
Polarity:	Negative ion mode			
Scan type:	MS/MS, Multiple Reaction Monitoring (MRM)			
Analyte monitored:	Mesotrione			
Ion mass transition monitored (m/z):	338 → 291 # collision energy = - 14V 338 → 212 collision energy = - 44V			

proposed as quantification transition but any of the ion mass transitions listed can be used for quantification.

Results and discussions

Table A 18: Procedural recovery data of mesotrione in maize specimen (whole plant w/o roots)

Matrix	Fortification level (mg/kg)	Recovery (%)	Mean recovery (%)	RSD (%)	Replicates	Overall Mean Recovery (%)	Overall RSD (%)
Ion Mass Transition 338 → 291 m/z (Quantification)							
Maize (whole plant w/o roots)	0.01 (LOQ)	75, 72, 99, 103, 66, 64, 85, 89	82	18	8	89	14
	0.1	95, 93, 91, 87, 93, 95, 102, 95	94	4.5	8		
	40	102	102	-	1		

Single recoveries were in the range of 60 – 120 % each, while the mean recoveries at each fortification level were in the range of 70 - 110 %. Wherever applicable n ≥ 3), the relative standard deviation was ≤ 20% for each level for mesotrione in maize seedlings.

Table A 19: Characteristics for the analytical method used for residue determination of mesotrione in maize matrix

	Mesotrione
Specificity	HPLC-MS/MS a highly specific analytical system was used. Quantification was performed by use of LC-MS/MS detection. One (1) mass transitions was evaluated. A second mass transition was monitored for confirmation of peak identity but was not used for quantification of specimen. At least one control sample per each matrix type and analytical set was analysed to investigate the residue level and the check any background interferences at the expected retention time. No interferences above 30 % of the LOQ at the retention time were detected in the untreated control samples.
Calibration (type, number of data points) **Calibration range**	The linearity of the detector response was demonstrated by single determination of solvent calibration standards at eight (8) concentration levels ranging from 0.15 ng/L to 25 ng/L. This range corresponds to 0.003 mg/kg to 0.50 mg/kg and thus covers the range of no more than 30% of the LOQ and at least + 20 % of the highest analyte concentration detected in any (diluted) specimen extract. These margins cover the required minimum range. The calibration was found to be linear in the concentration range of 0.15 ng/L to 25 ng/L with correlation coefficients (r) of 0.999. These results meet the acceptance criteria of r ≥0.99. Linear regression was performed without weighting. Detailed calibration curves and data are presented in the report.
Assessment of matrix effects is presented	Mean matrix effects were ≤ ± 20% and therefore considered to be insignificant for all matrices. Therefore, solvent standards were used.
Limit of determination / quantification	The limit of quantification (LOQ) is defined as the lowest fortification level with mean recoveries ranging from 70% to 110% at a relative standard deviation (RSD) of ≤20%. These criteria were fulfilled for mesotrione in maize matrices with an LOQ of 0.01 mg/kg. The limit of detection (LOD) was found to be 0.003 mg/kg for mesotrione. The LOD was defined as 30% of the limit of quantification for residues in control samples (i.e. 0.003 mg/kg)
Extract Stability	For maize seedlings, the maximum storage interval at -18°C from sampling to extraction was 82 days. The maximum storage interval of final sample extracts at 1 °C to 10°C from extraction to until injection to HPLC-MS/MS was 8 days. The

	Mesotrione
	stability was proven by the procedural recovery samples, which stored under the same conditions.

Conclusion

In regard to selectivity, accuracy and precision, the analytical method was applied successfully for each analytical set when analysing the specimen of the study.

Van de Sandt, H.J., 2019

A 2.1.1.3 Description of analytical methods for the determination of mesotrione and nicosulfuron residues in aquatic media (Ecotoxicology) (KCP 5.1.2)

Different analytical methods to determine concentrations of mesotrione and nicosulfuron in aquatic media has been developed and validated in the following study reports by

A 2.1.1.3.1 Analytical methods

- xxx ., 2016, Report No. S16-03041 (SAE053H/01: Toxicity to Rainbow trout– static) (see KCP 5.1.2/05)
- Zawadsky C., 2016, Report No. S16-03042 (SAE053H/01: Toxicity to *Daphnia magna*– static) (see KCP 5.1.2/06)
- Falk S., 2016a, Report No. S16-03039 (SAE053H/01: Toxicity to Alga) (see KCP 5.1.2/07)
- Falk S., 2016b, Report No. S16-03040 (SAE053H/01: Toxicity to Diatom) (see KCP 5.1.2/08)
- Lang C., 2016b, Report No. S16-03044 (SAE053H/01: Toxicity to *Lemna gibba*– Semi-static) (see KCP 5.1.2/09)
- Bertrand, C., 2019, Report No. S19-03470 (Mesotrione TC: Toxicity to *Lemna gibba*– Semi-static) (see KCP 5.1.2/16)
- Christmann, R., 2021a, Report No. 218-31 (Mesotrione TC: Toxicity to Spirodela polyrhiza, Growth Inhibition Test) (see KCP 5.1.2/17)
- Christmann, R., 2021b, Report No. 218-32 (Mesotrione TC: Toxicity to *Wolffia arrhiza*, Growth Inhibition Test) (see KCP 5.1.2/18)
- Gonsior G., 2016, Report No. S16-03045 (SAE053H/01: Growth Inhibition of *Myriophyllum spicatum*) (see KCP 5.1.2/10)
- Lang C., 2016a, Report No. S16-03043 (SAE053H/01: Toxicity to *Daphnia magna* – Reproduction Test) (see KCP 5.1.2/11)
- Molitor A.M., 2016b, Report No. S16-02518 (SAE053H/01: Honey Bee Toxicity Test) (see KCP 5.1.2/12)
- Vergé E. and Wagner J., 2016, Report No. S16-02503 (SAE053H/01: Larval Toxicity Test) (see KCP 5.1.2/13)
- Gröning C., 2017a, Report No. S16-02421 (SAE053H/01: Seedling Emergence) (see KCP 5.1.2/14)
- Gröning C., 2017b, Report No. S16-02422 (SAE053H/01: Vegetative Vigour) (see KCP 5.1.2/15)

A 2.1.1.3.2 Analytical method - Mesotrione and nicosulfuron residues in OECD 203 test medium

A 2.1.1.3.2.1 Method validation

Comments of zRMS:	The HPLC-MS/MS method used in the study S16-03041 for determination of mesotrione and nicosulfuron is acceptable validated according to SANCO/3029/99 rev. 4.

Reference:	**2.1.1.3.2.1/01 (KCP 5.1.2/05 also filed under KCP 10.2.1/01)**
Report	xxx., 2016 SAE053H/01: Toxicity to the Rainbow Trout *Oncorhynchus mykiss* under Laboratory Conditions (Acute Toxicity Test –Static) Report No. S16-03041
Guideline(s):	SANCO/3029/99 rev. 4
Deviations:	No
GLP	Yes
Acceptability:	Yes

Materials and methods

An analytical method for mesotrione and nicosulfuron was validated in OECD 203 test medium. The method involves dilution of test medium samples by a factor of 2 with acetonitrile, followed by further dilution with acetonitrile/water (1:1, v/v) if necessary, prior to analysis by liquid chromatography coupled with a tandem mass spectrometric detection (HPLC-MS/MS) using external calibration. The limit of quantification (LOQ) of 0.3 mg/L of test item was validated, corresponding to 0.0250 mg/L of mesotrione and 0.00939 mg/L of nicosulfuron.

Specimen preparation

After sampling, the test medium samples (0.5 ml) were mixed with 0.5 mL acetonitrile and stored deep-frozen (≤ - 18 °C) until analysis. In the analytical laboratory, the samples were thawed to ambient temperature and shaken well using a Vortex-Mixer. If necessary, the samples were further diluted with acetonitrile/water (1:1, v/v) prior to analysis by HPLC-MS/MS.
Recovery samples were prepared by fortification of untreated test medium with the test item. 0.5 mL of recovery samples were mixed with 0.5 mL acetonitrile and shaken well using a Vortex-Mixer. If necessary, the samples were further diluted with acetonitrile/water (1:1, v/v) prior to analysis by HPLC-MS/MS.

Equipment for mesotrione and nicosulfuron determination

LC-MS/MS system	Shimadzu LC-30 AD HPLC pump with autosampler SIL-30ACMP coupled with SCIEX API 5500 MS/MS system
Analytical column	Phenomenex Luna 5µ Phenyl-Hexyl, 150 mm x 2 mm i.d., 5 µm mean particle size (No. 00F-4257-B0) with 4 mm guard column
Column temperature	30°C
Injection volume	10 µL
Flow rate	0.500 mL/min
Mobile phase	A: Water + 0.5 % (v/v) formic acid B: Methanol + 0.5 % (v/v) formic acid

Time (min)	% A	% B

0.10	90	10
4.00	5	95
6.00	5	95
6.10	90	10
8.00	90	10

Retention time	Approx. 4.3 min (nicosulfuron) and 4.3 min (mesotrione)
Ion mode	ESI, Positive/negative ion switching mode
Ion mass transition monitored (m/z)	411.12→ 182.10 (quantitation) with collision energy (CE)= 27V
for nicosulfuron	411.12 → 213.00 (confirmation) with collision energy (CE)= 27V
Ion mass transition monitored (m/z)	337.85→ 291.00 (quantitation) with collision energy (CE)= -14V
for mesotrione	337.85 → 212.00 (confirmation) with collision energy (CE)= -42V

Results and discussions

Table A 20: Recovery results from method validation of mesotrione using the analytical method

Matrix	Analyte	Test item fortification level (mg/L) (*n* = x)	Analyte fortification level (mg/L) (*n* = x)	Recovery (%)	Mean recovery ± RSD (%)	Comments
Test medium	mesotrione	0.3 (n = 5)	0.0250 (n = 5)	69, 72, 70, 72, 72	71 ± 2	Acceptable
Test medium	mesotrione	100 (n = 5)	8.33 (n = 5)	80, 75, 77, 77, 77	77 ± 2	Acceptable

RSD = relative standard deviation

Mean recoveries at each fortification level were between 70 and 110% and the overall RSD was below 20%, in compliance with SANCO/3029/99 rev. 4 requirements.

Table A 21: Recovery results from method validation of nicosulfuron using the analytical method

Matrix	Analyte	Test item fortification level (mg/L) (*n* = x)	Analyte fortification level (mg/L) (*n* = x)	Recovery (%)	Mean recovery ± RSD (%)	Comments
Test medium	nicosulfuron	0.3 (n = 5)	0.00939 (n = 5)	73, 73, 71, 72, 72	72 ± 1	Acceptable
Test medium	nicosulfuron	100 (n = 5)	3.13 (n = 5)	80, 77, 79, 79, 77	78 ± 2	Acceptable

RSD = relative standard deviation

Mean recoveries at each fortification level were between 70 and 110% and the overall RSD was below 20%, in compliance with SANCO/3029/99 rev. 4 requirements.

Table A 22: Characteristics for the analytical method used for validation of Mesotrione and Nicosulfuron residues in test medium

	Mesotrione	**Nicosulfuron**
Specificity	A highly specific detection system was used (MS/MS). The retention time of each analyte in the sample extracts matches the retention time in the calibration solution. No significant interference >30% LOQ occurred at the retention time of each analyte in any of the control samples. The analytical method can therefore be regarded as highly specific and selective for mesotrione and nicosulfuron	
Calibration (type, number of data points) **Calibration range**	Dilutions for calibration were prepared in water/acetonitrile (1:1, v/v). The linearity of the detector was demonstrated by determinations of 8 calibration standards ranging from 1.0 ng/mL to 70 ng/mL, covering the working range of no more than 30 % of the LOQ to at least + 20 % of the highest analyte concentration level in a (diluted) sample. The detector response for analysis was linear for mesotrione and second order for nicosulfuron, with correlation coefficients $r \geq 0.995$:	
	$y = 1.34e^{+005} x + 9.76e^{+004}$, r = 0.9979 (1/x weighting)	$y = -8.98e^{+003} x^{\wedge 2} + 1.45e^{+006} x + 4.05e^{+005}$, r = 0.9999
Assessment of matrix effects is presented	Not tested, sample solutions and calibration solutions were prepared in the sample solvent system. Solvent standards were used for analysis of test medium samples.	
Storage stability of samples	The maximum storage period from sampling to analysis was 29 days within this study. Residues are regarded as stable if the samples are stored deep-frozen for up to 30 days between sampling and analysis (EU COM 7032/VI/95 and OPPTS 860.1380). Therefore, the storage stability of Mesotrione and Nicosulfuron was not verified.	
Limit of determination/ quantification	The limit of quantification (LOQ) is defined as the lowest validated level and was successfully established at 0.3 mg/L of test item (0.0250 mg/L of mesotrione). The limit of detection (LOD) was defined as 30 % of LOQ (0.00750 mg/L of mesotrione).	The limit of quantification (LOQ) is defined as the lowest validated level and was successfully established at 0.3 mg/L of test item 0.00939 mg/L of nicosulfuron). The limit of detection (LOD) was defined as 30 % of LOQ (0.00282 mg/L of nicosulfuron).

Conclusion

The method was found to be valid for the determination of mesotrione and nicosulfuron in OECD 203 test medium used in ecotoxicology studies, at fortification levels of 0.3 mg/L and 100 mg/L of the test item, in accordance to SANCO/3029/99 rev. 4 requirements. The limit of quantification (LOQ) was successfully established at 0.3 mg/L of test item (0.0250 mg/L of mesotrione and 0.00939 mg/L of nicosulfuron).

xxx., 2016

A 2.1.1.3.3 Analytical method - Mesotrione and nicosulfuron residues in OECD 202 test medium

A 2.1.1.3.3.1 Method validation

Comments of zRMS:	The HPLC-MS/MS method used in the study S16-03042 for determination of mesotrione and nicosulfuron is acceptable validated according to SANCO/3029/99 rev. 4.

Reference:	**2.1.1.3.3.1/01 (KCP 5.1.2/06 also filed under KCP 10.2.1/02)**
Report	Zawadsky C., 2016 SAE053H/01: Toxicity to the Water Flea *Daphnia magna Straus* under Laboratory Conditions (Acute Immobilisation Test – Static) Report No. S16-03042
Guideline(s):	SANCO/3029/99 rev. 4
Deviations:	No
GLP	Yes
Acceptability:	Yes

Materials and methods

An analytical method for mesotrione and nicosulfuron was validated in OECD 202 test medium. The method involves dilution of test medium samples by a factor of 2 with acetonitrile, followed by further dilution with acetonitrile/water (1:1, v/v) or with acetonitrile/test medium (1:1, v/v) if necessary, prior to analysis by liquid chromatography coupled with a tandem mass spectrometric detection (HPLC-MS/MS) using external calibration. The limit of quantification (LOQ) of 0.3 mg/L of test item was validated, corresponding to 0.0250 mg/L of mesotrione and 0.00939 mg/L of nicosulfuron.

Specimen preparation

After sampling, the test medium samples (10 mL) were stored deep-frozen (≤ - 18 °C) until analysis. In the analytical laboratory, the samples were thawed to ambient temperature, 10 mL of acetonitrile were added and shaken well using a Vortex-Mixer. If necessary, the samples were further diluted with acetonitrile/water (1:1, v/v) for analysis of mesotrione samples or with acetonitrile/test medium (1:1, v/v) for analysis of nicosulfuron samples by HPLC-MS/MS. Recovery samples were prepared by fortification of untreated test medium with the test item. 10 mL of recovery samples were mixed with 10 mL acetonitrile and shaken well using a Vortex-Mixer. If necessary, the samples were further diluted with acetonitrile/water (1:1, v/v) for analysis of mesotrione samples or with acetonitrile/test medium (1:1, v/v) for analysis of nicosulfuron by HPLC-MS/MS.

Equipment for mesotrione determination

LC-MS/MS system	Thermo Surveyor MS pump with Thermo Surveyor autosampler coupled with Thermo TSQ Quantum triple quadrupole system
Analytical column	Phenomenex Luna 5µ Phenyl-Hexyl, 150 mm x 2 mm i.d., 5 µm mean particle size (No. 00F-4257-B0) with 4 mm guard column
Column temperature	40°C
Injection volume	5 µL
Flow rate	0.500 mL/min
Mobile phase	A: Water

B: Methanol
C: 1.0 % (v/v) formic acid in water

Time (min)	% A	% B	% C
0.0	78	20	2
3.00	5	93	2
5.00	5	93	2
5.01	78	20	2
8.00	78	20	2

Split before MS: Approx. 1:5
Retention time: Approx. 4.0 min (mesotrione)
Ion mode: ESI, Negative ion mode
Ion mass transition monitored (m/z) for mesotrione: 337.8→ 290.9 (quantitation) with collision energy (CE)= -14V
337.8 → 211.9 (confirmation) with collision energy (CE)= -34V

Equipment for nicosulfuron determination

LC-MS/MS system: Thermo Surveyor MS pump with Thermo Surveyor autosampler coupled with Thermo TSQ Quantum triple quadrupole system
Analytical column: Phenomenex Luna 5µ Phenyl-Hexyl, 150 mm x 2 mm i.d., 5 µm mean particle size (No. 00F-4257-B0) with 4 mm guard column
Column temperature: 40°C
Injection volume: 10 µL
Flow rate: 0.500 mL/min
Mobile phase: A: Water
B: Methanol
C: 1.0 % (v/v) formic acid in water

Time (min)	% A	% B	% C
0.0	78	20	2
3.00	5	93	2
5.00	5	93	2
5.01	78	20	2
8.00	78	20	2

Split before MS: Approx. 1:5
Retention time: Approx. 3.9 min (nicosulfuron)
Ion mode: ESI, Positive ion mode
Ion mass transition monitored (m/z) for nicosulfuron: 411.1→ 181.8 (quantitation) with collision energy (CE)= 20V
411.1→ 212.9 (confirmation) with collision energy (CE)= 17V

Results and discussions

Table A 23: Recovery results from method validation of mesotrione using the analytical method

Matrix	**Analyte**	**Test item fortification level (mg/L) (*n* = x)**	**Analyte forti-fication level (mg/L) (*n* = x)**	**Recovery (%)**	**Mean recovery ± RSD (%)**	**Comments**
Test medium	mesotrione	0.3	0.0250	102, 95, 86, 89,	92 ± 7	Acceptable

Matrix	Analyte	Test item fortification level (mg/L) (*n* = x)	Analyte fortification level (mg/L) (*n* = x)	Recovery (%)	Mean recovery ± RSD (%)	Comments
		(n = 5)	(n = 5)	90		
Test medium	mesotrione	15 (n = 5)	1.25 (n = 5)	90, 88, 86, 82, 79	85 ± 5	Acceptable

RSD = relative standard deviation

Mean recoveries at each fortification level were between 70 and 110% and the overall RSD was below 20%, in compliance with SANCO/3029/99 rev. 4 requirements.

Table A 24: Recovery results from method validation of nicosulfuron using the analytical method

Matrix	Analyte	Test item fortification level (mg/L) (*n* = x)	Analyte fortification level (mg/L) (*n* = x)	Recovery (%)	Mean recovery ± RSD (%)	Comments
Test medium	nicosulfuron	0.3 (n = 5)	0.00939 (n = 5)	77, 79, 73, 78, 75	76 ± 3	Acceptable
Test medium	nicosulfuron	15 (n = 5)	0.470 (n = 5)	71, 74, 83, 77, 76	76 ± 6	Acceptable

RSD = relative standard deviation

Mean recoveries at each fortification level were between 70 and 110% and the overall RSD was below 20%, in compliance with SANCO/3029/99 rev. 4 requirements.

Table A 25: Characteristics for the analytical method used for validation of Mesotrione and Nicosulfuron residues in test medium

	Mesotrione	Nicosulfuron
Specificity	A highly specific detection system was used (MS/MS). The retention time of each analyte in the sample extracts matches the retention time in the calibration solution. No significant interference >30% LOQ occurred at the retention time of each analyte in any of the control samples. The analytical method can therefore be regarded as highly specific and selective for mesotrione and nicosulfuron	
Calibration (type, number of data points) **Calibration range**	Dilutions for calibration were prepared in water/acetonitrile (1:1, v/v). The linearity of the detector was demonstrated by determinations of 9 calibration standards ranging from 0.5 ng/mL to 100 ng/mL, covering the working range of no more than 30 % of the LOQ to at least + 20 % of the highest analyte concentration level in a (diluted) sample. The detector response for analysis was linear for mesotrione, with correlation coefficients r ≥ 0.995:	Dilutions for calibration were prepared in acetonitrile/test medium (1:1, v/v). The linearity of the detector was demonstrated by determinations of 6 matrix matched standards ranging from 0.5 ng/mL to 30 ng/mL, covering the working range of no more than 30 % of the LOQ to at least + 20 % of the highest analyte concentration level in a (diluted) sample. The detector response for analysis was of second order for nicosulfuron, with correlation coefficients r ≥ 0.995:
	y = -966.468+4502.16 x, R^2 = 0.9997 (1/x weighting)	y = -314.195 x^2 + 69244.9 x + 2907.66, R^2 = 0.9998

	Mesotrione	Nicosulfuron
Assessment of matrix effects is presented	Not tested, sample solutions and calibration solutions were prepared in the sample solvent system. Solvent standards were used for analysis of test medium samples for mesotrione and matrix matched standards were used for analysis of test medium samples for nicosulfuron.	
Storage stability of samples	The maximum storage period from sampling to analysis was 29 days within this study. Residues are regarded as stable if the samples are stored deep-frozen for up to 30 days between sampling and analysis (EU COM 7032/VI/95 and OPPTS 860.1380). Therefore, the storage stability of Mesotrione and Nicosulfuron was not verified.	
Limit of determination/ quantification	The limit of quantification (LOQ) is defined as the lowest validated level and was successfully established at 0.3 mg/L of test item (0.0250 mg/L of mesotrione). The limit of detection (LOD) was defined as 30 % of LOQ (0.00750 mg/L of mesotrione).	The limit of quantification (LOQ) is defined as the lowest validated level and was successfully established at 0.3 mg/L of test item 0.00939 mg/L of nicosulfuron). The limit of detection (LOD) was defined as 30 % of LOQ (0.00282 mg/L of nicosulfuron).

Conclusion

The method was found to be valid for the determination of mesotrione and nicosulfuron in OECD 202 test medium used in ecotoxicology studies, at fortification levels of 0.3 mg/L and 15 mg/L of the test item, in accordance to SANCO/3029/99 rev. 4 requirements. The limit of quantification (LOQ) was successfully established at 0.3 mg/L of test item (0.0250 mg/L of mesotrione and 0.00939 mg/L of nicosulfuron).

Zawadsky C., 2016

A 2.1.1.3.4 Analytical method - Mesotrione and nicosulfuron residues in OECD 201 test medium

A 2.1.1.3.4.1 Method validation

Comments of zRMS:	The HPLC-MS/MS method used in the study S16-03039 for determination of mesotrione and nicosulfuron is acceptable validated according to SANCO/3029/99 rev. 4.

Reference:	**2.1.1.3.4.1/01 (KCP 5.1.2/07 also filed under KCP 10.2.1/03)**
Report	Falk S., 2016a SAE053H/01: Toxicity to the Single Cell Green Alga *Pseudokirchneriella subcapitata* Hindák under Laboratory Conditions Report No. S16-03039
Guideline(s):	SANCO/3029/99 rev. 4
Deviations:	No
GLP	Yes
Acceptability:	Yes

Materials and methods

An analytical method for mesotrione and nicosulfuron was validated in OECD 201 test medium. The

method involves dilution of test medium samples by a factor of 2 with acetonitrile, followed by further dilution with acetonitrile/water (1:1, v/v) if necessary, prior to analysis by liquid chromatography coupled with a tandem mass spectrometric detection (HPLC-MS/MS) using external calibration. The limit of quantification (LOQ) of 0.05 mg/L of test item was validated, corresponding to 0.00417 mg/L of mesotrione and 0.00157 mg/L of nicosulfuron.

Specimen preparation

After sampling, the test medium samples (0.5 mL) were diluted with 0.5 mL of acetonitrile and stored deep-frozen (≤ - 18 °C) until analysis. In the analytical laboratory, the samples were thawed to ambient temperature and shaken well using a Vortex-Mixer. If necessary, the samples were further diluted with acetonitrile/water (1:1, v/v) prior to analysis by HPLC-MS/MS.
Recovery samples were prepared by fortification of untreated test medium with the test item. 0.5 mL of each recovery sample were mixed with 0.5 mL acetonitrile and shaken well using a Vortex-Mixer. If necessary, the samples were further diluted with acetonitrile/water (1:1, v/v) prior to analysis by HPLC-MS/MS.

Equipment for mesotrione determination

LC-MS/MS system	Thermo Surveyor MS pump with Thermo Surveyor autosampler coupled with Thermo TSQ Quantum triple quadrupole system
Analytical column	Phenomenex Luna 5µ Phenyl-Hexyl, 150 mm x 2 mm i.d., 5 µm mean particle size (No. 00F-4257-B0) with 4 mm guard column
Column temperature	40°C
Injection volume	5 µL
Flow rate	0.500 mL/min
Mobile phase	A: Water B: Methanol C: 1.0 % (v/v) formic acid in water

Time (min)	% A	% B	% C
0.0	78	20	2
3.00	5	93	2
5.00	5	93	2
5.01	78	20	2
8.00	78	20	2

Split before MS	Approx. 1:5
Retention time	Approx. 4.0 min (mesotrione)
Ion mode	ESI, Negative ion mode
Ion mass transition monitored (m/z)	337.8→ 290.9 (quantitation) with collision energy (CE)= -14V
for mesotrione	337.8 → 211.9 (confirmation) with collision energy (CE)= -34V

Equipment for nicosulfuron determination

LC-MS/MS system	Thermo Surveyor MS pump with Thermo Surveyor autosampler coupled with Thermo TSQ Quantum triple quadrupole system
Analytical column	Phenomenex Luna 5µ Phenyl-Hexyl, 150 mm x 2 mm i.d., 5 µm mean particle size (No. 00F-4257-B0) with 4 mm guard column
Column temperature	40°C
Injection volume	10 µL
Flow rate	0.500 mL/min
Mobile phase	A: Water B: Methanol

C: 1.0 % (v/v) formic acid in water

Time (min)	% A	% B	% C
0.0	78	20	2
3.00	5	93	2
5.00	5	93	2
5.01	78	20	2
8.00	78	20	2

Split before MS	Approx. 1:5
Retention time	Approx. 4.0 min (nicosulfuron)
Ion mode	ESI, Positive ion mode
Ion mass transition monitored (m/z)	411.1→ 181.8 (quantitation) with collision energy (CE)= 20V
for nicosulfuron	411.1→ 212.9 (confirmation) with collision energy (CE)= 17V

Results and discussions

Table A 26: Recovery results from method validation of mesotrione using the analytical method

Matrix	**Analyte**	**Test item fortification level (mg/L) (*n* = x)**	**Analyte fortification level (mg/L) (*n* = x)**	**Recovery (%)**	**Mean recovery ± RSD (%)**	**Comments**
Test medium	mesotrione	0.05 (n = 5)	0.00417 (n = 5)	96, 85, 87, 82, 86	87 ± 6	Acceptable
Test medium	mesotrione	70 (n = 5)	5.84 (n = 5)	81, 81, 105, 96, 86	90 ± 12	Acceptable

RSD = relative standard deviation

Mean recoveries at each fortification level were between 70 and 110% and the overall RSD was below 20%, in compliance with SANCO/3029/99 rev. 4 requirements.

Table A 27: Recovery results from method validation of nicosulfuron using the analytical method

Matrix	**Analyte**	**Test item fortification level (mg/L) (*n* = x)**	**Analyte fortification level (mg/L) (*n* = x)**	**Recovery (%)**	**Mean recovery ± RSD (%)**	**Comments**
Test medium	nicosulfuron	0.05 (n = 5)	0.00157 (n = 5)	106, 89, 101, 88, 94	96 ± 8	Acceptable
Test medium	nicosulfuron	70 (n = 5)	2.19 (n = 5)	103, 104, 106, 102, 95	102 ± 4	Acceptable

RSD = relative standard deviation

Mean recoveries at each fortification level were between 70 and 110% and the overall RSD was below 20%, in compliance with SANCO/3029/99 rev. 4 requirements.

Table A 28: Characteristics for the analytical method used for validation of Mesotrione and Nicosulfuron residues in test medium

	Mesotrione	**Nicosulfuron**
Specificity	A highly specific detection system was used (MS/MS). The retention time of each analyte in the sample extracts matches the retention time in the calibration solution. No significant interference >30% LOQ occurred at the retention time of each analyte in any of the control samples. The analytical method can therefore be regarded as highly specific and selective for mesotrione and nicosulfuron	
Calibration (type, number of data points) **Calibration range**	Dilutions for calibration were prepared in water/acetonitrile (1:1, v/v). The linearity of the detector was demonstrated by determinations of 9 calibration standards ranging from 0.5 ng/mL to 100 ng/mL, covering the working range of no more than 30 % of the LOQ to at least + 20 % of the highest analyte concentration level in a (diluted) sample. The detector response for analysis was linear for mesotrione, with correlation coefficients r ≥ 0.995:	Dilutions for calibration were prepared in water/acetonitrile (1:1, v/v). The linearity of the detector was demonstrated by determinations of 8 calibration standards ranging from 0.2 ng/mL to 30 ng/mL, covering the working range of no more than 30 % of the LOQ to at least + 20 % of the highest analyte concentration level in a (diluted) sample. The detector response for analysis was linear for nicosulfuron, with correlation coefficients r ≥ 0.995:
	y = -16.5507+3344.98 x, R^2 = 0.9993 (1/x weighting)	y = -1432.19+52364 x, R^2 = 0.9998 (1/x weighting)
Assessment of matrix effects is presented	Not tested, sample solutions and calibration solutions were prepared in the sample solvent system. Solvent standards were used for analysis of test medium samples.	
Storage stability of samples	The maximum storage period from sampling to analysis was 29 days within this study. Residues are regarded as stable if the samples are stored deep-frozen for up to 30 days between sampling and analysis (EU COM 7032/VI/95 and OPPTS 860.1380). Therefore, the storage stability of Mesotrione and Nicosulfuron was not verified.	
Limit of determination/ quantification	The limit of quantification (LOQ) is defined as the lowest validated level and was successfully established at 0.05 mg/L of test item (0.00417 mg/L of mesotrione). The limit of detection (LOD) was defined as 30 % of LOQ (0.00125 mg/L of mesotrione).	The limit of quantification (LOQ) is defined as the lowest validated level and was successfully established at 0.05 mg/L of test item (0.00157 mg/L of nicosulfuron. The limit of detection (LOD) was defined as 30 % of LOQ (0.000471 mg/L of nicosulfuron).

Conclusion

The method was found to be valid for the determination of mesotrione and nicosulfuron in OECD 201 test medium used in ecotoxicology studies, at fortification levels of 0.05 mg/L and 70 mg/L of the test item, in accordance to SANCO/3029/99 rev. 4 requirements. The limit of quantification (LOQ) was successfully established at 0.05 mg/L of test item (0.00417 mg/L of mesotrione and 0.00157 mg/L of nicosulfuron).

Falk S., 2016a

A 2.1.1.3.5 Analytical method - Mesotrione and nicosulfuron residues in OECD 201 test medium

A 2.1.1.3.5.1 Method validation

Comments of zRMS:	The HPLC-MS/MS method used in the study S16-03040 for determination of mesotrione and nicosulfuron is acceptable validated according to SANCO/3029/99 rev. 4.

Reference:	**2.1.1.3.5.1/01 (KCP 5.1.2/08 (also filed under KCP 10.2.1/04)**
Report	Falk S., 2016b SAE053H/01: Toxicity to the Diatom *Navicula pelliculosa* under Laboratory Conditions Report No. S16-03040
Guideline(s):	SANCO/3029/99 rev. 4
Deviations:	No
GLP	Yes
Acceptability:	Yes

Materials and methods

An analytical method for mesotrione and nicosulfuron was validated in OECD 201 test medium. The method involves dilution of test medium samples by a factor of 2 with acetonitrile, followed by further dilution with acetonitrile/water (1:1, v/v) if necessary, prior to analysis by liquid chromatography coupled with a tandem mass spectrometric detection (HPLC-MS/MS) using external calibration. The limit of quantification (LOQ) of 0.3 mg/L of test item was validated, corresponding to 0.0250 mg/L of mesotrione and 0.00939 mg/L of nicosulfuron.

Specimen preparation

After sampling, the test medium samples (0.5 mL) were diluted with 0.5 mL of acetonitrile and stored deep-frozen (≤ - 18 °C) until analysis. In the analytical laboratory, the samples were thawed to ambient temperature and shaken well using a Vortex-Mixer. If necessary, the samples were further diluted with acetonitrile/water (1:1, v/v) prior to analysis by HPLC-MS/MS.
Recovery samples were prepared by fortification of untreated test medium with the test item. 0.5 mL of each recovery samples were mixed with 0.5 mL acetonitrile and shaken well using a Vortex-Mixer. If necessary, the samples were further diluted with acetonitrile/water (1:1, v/v) prior to analysis by HPLC-MS/MS.

Equipment for mesotrione determination

LC-MS/MS system	Thermo Surveyor MS pump with Thermo Surveyor autosampler coupled with Thermo TSQ Quantum triple quadrupole system
Analytical column	Phenomenex Luna 5µ Phenyl-Hexyl, 150 mm x 2 mm i.d., 5 µm mean particle size (No. 00F-4257-B0) with 4 mm guard column
Column temperature	40°C
Injection volume	5 µL
Flow rate	0.500 mL/min
Mobile phase	A: Water

B: Methanol
C: 1.0 % (v/v) formic acid in water

Time (min)	% A	% B	% C
0.0	78	20	2
3.00	5	93	2
5.00	5	93	2
5.01	78	20	2
8.00	78	20	2

Split before MS: Approx. 1:5
Retention time: Approx. 4.0 min (mesotrione)
Ion mode: ESI, Negative ion mode
Ion mass transition monitored (m/z) for mesotrione: 337.8→ 290.9 (quantitation) with collision energy (CE)= -14V
337.8 → 211.9 (confirmation) with collision energy (CE)= -34V

Equipment for nicosulfuron determination

LC-MS/MS system: Thermo Surveyor MS pump with Thermo Surveyor autosampler coupled with Thermo TSQ Quantum triple quadrupole system
Analytical column: Phenomenex Luna 5µ Phenyl-Hexyl, 150 mm x 2 mm i.d., 5 µm mean particle size (No. 00F-4257-B0) with 4 mm guard column
Column temperature: 40°C
Injection volume: 10 µL
Flow rate: 0.500 mL/min
Mobile phase: A: Water
B: Methanol
C: 1.0 % (v/v) formic acid in water

Time (min)	% A	% B	% C
0.0	78	20	2
3.00	5	93	2
5.00	5	93	2
5.01	78	20	2
8.00	78	20	2

Split before MS: Approx. 1:5
Retention time: Approx. 4.0 min (nicosulfuron)
Ion mode: ESI, Positive ion mode
Ion mass transition monitored (m/z) for nicosulfuron: 411.1→ 181.8 (quantitation) with collision energy (CE)= 20V
411.1→ 212.9 (confirmation) with collision energy (CE)= 17V

Results and discussions

Table A 29: Recovery results from method validation of mesotrione using the analytical method

Matrix	**Analyte**	**Test item fortification level (mg/L) (*n* = x)**	**Analyte forti-fication level (mg/L) (*n* = x)**	**Recovery (%)**	**Mean recovery ± RSD (%)**	**Comments**
Test medium	mesotrione	0.3	0.0250	100, 105, 100,	99 ± 4	Acceptable

Matrix	Analyte	Test item fortification level (mg/L) (*n* = x)	Analyte fortification level (mg/L) (*n* = x)	Recovery (%)	Mean recovery ± RSD (%)	Comments
		(n = 5)	(n = 5)	96, 95		
Test medium	mesotrione	150 (n = 5)	12.5 (n = 5)	94, 93, 92, 92, 90	92 ± 2	Acceptable

RSD = relative standard deviation

Mean recoveries at each fortification level were between 70 and 110% and the overall RSD was below 20%, in compliance with SANCO/3029/99 rev. 4 requirements.

Table A 30: Recovery results from method validation of nicosulfuron using the analytical method

Matrix	Analyte	Test item fortification level (mg/L) (*n* = x)	Analyte fortification level (mg/L) (*n* = x)	Recovery (%)	Mean recovery ± RSD (%)	Comments
Test medium	nicosulfuron	0.3 (n = 5)	0.00939 (n = 5)	94, 98, 92, 92, 91	93 ± 3	Acceptable
Test medium	nicosulfuron	150 (n = 5)	4.70 (n = 5)	91, 94, 90, 91, 90	91 ± 2	Acceptable

RSD = relative standard deviation

Mean recoveries at each fortification level were between 70 and 110% and the overall RSD was below 20%, in compliance with SANCO/3029/99 rev. 4 requirements.

Table A 31: Characteristics for the analytical method used for validation of Mesotrione and Nicosulfuron residues in test medium

	Mesotrione	Nicosulfuron
Specificity	A highly specific detection system was used (MS/MS). The retention time of each analyte in the sample extracts matches the retention time in the calibration solution. No significant interference >30% LOQ occurred at the retention time of each analyte in any of the control samples. The analytical method can therefore be regarded as highly specific and selective for mesotrione and nicosulfuron	
Calibration (type, number of data points) **Calibration range**	Dilutions for calibration were prepared in water/acetonitrile (1:1, v/v). The linearity of the detector was demonstrated by determinations of 9 calibration standards ranging from 0.5 ng/mL to 100 ng/mL, covering the working range of no more than 30 % of the LOQ to at least + 20 % of the highest analyte concentration level in a (diluted) sample. The detector response for analysis was linear for mesotrione, with correlation coefficients r ≥ 0.995:	Dilutions for calibration were prepared in water/acetonitrile (1:1, v/v). The linearity of the detector was demonstrated by determinations of 8 calibration standards ranging from 0.2 ng/mL to 30 ng/mL, covering the working range of no more than 30 % of the LOQ to at least + 20 % of the highest analyte concentration level in a (diluted) sample. The detector response for analysis was of second order for nicosulfuron, with correlation coefficients r ≥ 0.995:

	Mesotrione	**Nicosulfuron**
	y = -296.406 + 4209.65x, R^2 = 0.9986 (1/x weighting)	y = -1048.56+50892.4x – 146.696x^2, R^2 = 0.9994 (1/x weighting)
Assessment of matrix effects is presented	Not tested, sample solutions and calibration solutions were prepared in the sample solvent system. Solvent standards were used for analysis of test medium samples.	
Storage stability of samples	The maximum storage period from sampling to analysis was 29 days within this study. Residues are regarded as stable if the samples are stored deep-frozen for up to 30 days between sampling and analysis (EU COM 7032/VI/95 and OPPTS 860.1380). Therefore, the storage stability of Mesotrione and Nicosulfuron was not verified.	
Limit of determination/ quantification	The limit of quantification (LOQ) is defined as the lowest validated level and was successfully established at 0.3 mg/L of test item (0.0250 mg/L of mesotrione). The limit of detection (LOD) was defined as 30 % of LOQ (0.00750 mg/L of mesotrione).	The limit of quantification (LOQ) is defined as the lowest validated level and was successfully established at 0.3 mg/L of test item (0.00939 mg/L of nicosulfuron). The limit of detection (LOD) was defined as 30 % of LOQ (0.00282 mg/L of nicosulfuron).

Conclusion

The method was found to be valid for the determination of mesotrione and nicosulfuron in OECD 201 test medium used in ecotoxicology studies, at fortification levels of 0.3 mg/L and 150 mg/L of the test item, in accordance to SANCO/3029/99 rev. 4 requirements. The limit of quantification (LOQ) was successfully established at 0.3 mg/L of test item (0.0250 mg/L of mesotrione and 0.00939 mg/L of nicosulfuron).

Falk S., 2016b

A 2.1.1.3.6 Analytical method - Mesotrione and nicosulfuron residues in OECD 221 test medium

A 2.1.1.3.6.1 Method validation

Comments of zRMS:	The HPLC-MS/MS method used in the study S16-03044 for determination of mesotrione and nicosulfuron is acceptable validated according to SANCO/3029/99 rev. 4.

Reference:	**2.1.1.3.6.1/01 (KCP 5.1.2/09 also filed under KCP 10.2.1/05)**
Report	Lang C., 2016b SAE053H/01: Toxicity to the Duckweed *Lemna gibba* under Laboratory Conditions (Acute Test – Semi-static) Report No. S16-03044
Guideline(s):	SANCO/3029/99 rev. 4
Deviations:	No
GLP	Yes
Acceptability:	Yes

Materials and methods

An analytical method for mesotrione and nicosulfuron was validated in OECD 221 test medium. The method involves dilution of test medium samples by a factor of 2 with acetonitrile, followed by further dilution with acetonitrile/test medium (1:1, v/v) if necessary, prior to analysis by liquid chromatography coupled with a tandem mass spectrometric detection (HPLC-MS/MS) using external calibration. The limit of quantification (LOQ) of 3.0 µg/L of test item was validated, corresponding to 0.250 µg/L of mesotrione and 0.0939 µg/L of nicosulfuron.

Specimen preparation

After sampling, the test medium samples (10 mL) were stored deep-frozen (≤ - 18 °C) until analysis. In the analytical laboratory, the samples were thawed to ambient temperature and mixed with 10 mL of acetonitrile and shaken well using a Vortex-Mixer. If necessary, the samples were further diluted with acetonitrile/test medium (1:1, v/v) prior to analysis by HPLC-MS/MS.
Recovery samples were prepared by fortification of untreated test medium with the test item. For the 3 µg/L recovery samples: 10 mL of recovery samples were mixed with 10 mL acetonitrile and shaken well using a Vortex-Mixer. Aliquots were transferred into HPLC glass vials. For the 150 µg/L recovery: 0.5 mL of recovery samples were mixed with 0.5 mL acetonitrile in HPLC glass vials and shaken well using a Vortex-Mixer. If necessary, the samples were further diluted with acetonitrile/test medium (1:1, v/v) prior to analysis by HPLC-MS/MS.

Equipment for mesotrione and nicosulfuron determination

LC-MS/MS system	Shimadzu LC-30 AD HPLC pump with autosampler SIL-30ACMP coupled with SCIEX API 5500 MS/MS system
Analytical column	Phenomenex Luna 5µ Phenyl-Hexyl, 150 mm x 2 mm i.d., 5 µm mean particle size (No. 00F-4257-B0) with 4 mm guard column
Column temperature	30°C
Injection volume	40 µL
Flow rate	0.500 mL/min
Mobile phase	A: Water + 0.5 % (v/v) formic acid B: Methanol + 0.5 % (v/v) formic acid

Time (min)	% A	% B
0.10	90	10
4.00	5	95
5.00	5	95
5.10	90	10
7.00	90	10

Retention time	Approx. 4.5 min (nicosulfuron) and 4.5 min (mesotrione)
Ion mode	ESI, Positive/negative ion switching mode
Ion mass transition monitored (m/z)	411.12→ 182.10 (quantitation) with collision energy (CE)= 27V
for nicosulfuron	411.12 → 213.00 (confirmation) with collision energy (CE)= 27V
Ion mass transition monitored (m/z)	337.85→ 291.00 (quantitation) with collision energy (CE)= -14V
for mesotrione	337.85 → 212.00 (confirmation) with collision energy (CE)= -42V

Results and discussions

Table A 32: Recovery results from method validation of mesotrione using the analytical method

Matrix	Analyte	Test item fortification level (µg/L) (*n* = x)	Analyte fortification level (µg/L) (*n* = x)	Recovery (%)	Mean recovery ± RSD (%)	Comments
Test medium	mesotrione	3.0 (n = 5)	0.250 (n = 5)	80, 84, 82, 80, 80	81 ± 2	Acceptable
Test medium	mesotrione	150 (n = 5)	12.5 (n = 5)	88, 88, 88, 88, 89	88 ± 1	Acceptable

RSD = relative standard deviation

Mean recoveries at each fortification level were between 70 and 110% and the overall RSD was below 20%, in compliance with SANCO/3029/99 rev. 4 requirements.

Table A 33: Recovery results from method validation of nicosulfuron using the analytical method

Matrix	Analyte	Test item fortification level (µg/L) (*n* = x)	Analyte fortification level (µg/L) (*n* = x)	Recovery (%)	Mean recovery ± RSD (%)	Comments
Test medium	nicosulfuron	3.0 (n = 5)	0.0939 (n = 5)	106, 102, 103, 106, 109	105 ± 3	Acceptable
Test medium	nicosulfuron	150 (n = 5)	4.70 (n = 5)	110, 110, 110, 110, 110	110 ± 0	Acceptable

RSD = relative standard deviation

Mean recoveries at each fortification level were between 70 and 110% and the overall RSD was below 20%, in compliance with SANCO/3029/99 rev. 4 requirements.

In addition the following procedural recoveries were analysed:

Table A 34: Procedural Recovery results of mesotrione using the analytical method

Matrix	Analyte	Test item fortification level (µg/L) (*n* = x)	Analyte fortification level (µg/L) (*n* = x)	Recovery (%)	Mean recovery	Comments
Test medium	mesotrione	3.0 (n = 2)	0.250 (n = 2)	108, 103	106	Acceptable

RSD = relative standard deviation

Table A 35: Procedural Recovery results of nicosulfuron using the analytical method

Matrix	Analyte	Test item fortification level (µg/L) (*n* = x)	Analyte fortification level (µg/L) (*n* = x)	Recovery (%)	Mean recovery	Comments

Matrix	Analyte	Test item fortification level (µg/L) (*n* = x)	Analyte fortification level (µg/L) (*n* = x)	Recovery (%)	Mean recovery	Comments
Test medium	nicosulfuron	3.0 (n = 2)	0.0939 (n = 2)	109, 109	109	Acceptable

RSD = relative standard deviation

Table A 36: Characteristics for the analytical method used for validation of Mesotrione and Nicosulfuron residues in test medium

	Mesotrione	Nicosulfuron
Specificity	A highly specific detection system was used (MS/MS). The retention time of each analyte in the sample extracts matches the retention time in the calibration solution. No significant interference >30% LOQ occurred at the retention time of each analyte in any of the control samples. The analytical method can therefore be regarded as highly specific and selective for mesotrione and nicosulfuron	
Calibration (type, number of data points) **Calibration range**	Dilutions for calibration were prepared in acetonitrile/test medium (1:1). The linearity of the detector was demonstrated by determinations of 7 matrix matched standards ranging from 0.01 ng/mL to 3.0 ng/mL, covering the working range of no more than 30 % of the LOQ to at least + 20 % of the highest analyte concentration level in a (diluted) sample. The detector response for analysis was linear for mesotrione and nicosulfuron, with correlation coefficients r ≥ 0.995:	
	$y = 4.71e^{+005} x + 1.9e^{+003}$, r = 0.9996 (1/x weighting)	$y = 1.72e^{+006} x + 931$ r = 0.9999 (1/x weighting)
Assessment of matrix effects is presented	Not tested, sample solutions and calibration solutions were prepared in the sample solvent system. Matrix matched standards were used for analysis of test medium samples.	
Storage stability of samples	Mesotrione and nicosulfuron were found to be stable when stored for 45 days under deep-frozen conditions (≤ - 18°C) in test medium. This covers the maximum storage period from sampling to analysis within this study.	
Limit of determination/ quantification	The limit of quantification (LOQ) is defined as the lowest validated level and was successfully established at 3.0 µg/L of test item (0.250 µg/L of mesotrione). The limit of detection (LOD) was defined as 30 % of LOQ (0.0750 µg/L of mesotrione).	The limit of quantification (LOQ) is defined as the lowest validated level and was successfully established at 3.0 µg/L of test item (0.0939 µg/L of nicosulfuron). The limit of detection (LOD) was defined as 30 % of LOQ (0.0282 µg/L of nicosulfuron).

Conclusion

The method was found to be valid for the determination of mesotrione and nicosulfuron in OECD 221 test medium used in ecotoxicology studies, at fortification levels of 3.0 µg/L and 150 µg/L of the test item, in accordance to SANCO/3029/99 rev. 4 requirements. The limit of quantification (LOQ) was successfully established at 3.0 µg/L of test item (0.250 µg/L of mesotrione and 0.0939 µg/L of nicosulfuron).

Lang C., 2016b

A 2.1.1.3.6.2

Comments of zRMS:	The HPLC-MS/MS method used in the study S19-03470 for determination of mesotrione is acceptable validated according to SANCO/3029/99 rev. 4.

Reference:	**2.1.1.3.6.2/01 (KCP 5.1.2/16 also filed under KCP 10.2.1/07)**
Report	Bertrand, C., 2019 Mesotrione Technical: Toxicity to the Duckweed *Lemna gibba* under Laboratory Conditions (Acute Test – Semi-static) Report No. S19-03470
Guideline(s):	SANCO/3029/99 rev. 4 and OECD 221
Deviations:	No
GLP	Yes
Acceptability:	Yes

Materials and methods

An analytical method for determination of Mesotrione was validated in OECD 221 test medium, in order to evaluate a potential inhibition of growth rate and yield.
Sample analysis was performed by direct injection of test medium samples after dilution with acetonitrile and quantification by HPLC-MS/MS detection. The limit of quantification (LOQ) of the analytical method is 0.0698 µg/L of Mesotrione.

Specimen preparation

After sampling, the test medium samples (10 mL) were stored deep-frozen (≤ - 18 °C) until analysis. In the analytical laboratory, the samples were thawed to ambient temperature and mixed with 2 mL of acetonitrile and shaken well using a Vortex-Mixer. If necessary, the samples were further diluted with acetonitrile/test medium (20:80, v/v) prior to analysis by HPLC-MS/MS.
Recovery samples were prepared by fortification of untreated test medium with the test item. 10 mL of recovery samples were mixed with 2 mL acetonitrile and shaken well using a Vortex-Mixer. If necessary, the samples were further diluted with acetonitrile/test medium (20:80, v/v) prior to analysis by HPLC-MS/MS.

Equipment for Mesotrione determination

LC-MS/MS system	Agilent 1290 Infinity II HPLC system			
Analytical column	Agilent Poroshell 120Phenyl-Hexyl, 100 mm × 3 mm i.d., 2.7 µm mean particle size (No. 695975-312) with 2.1 mm guard column			
Column temperature	40 °C			
Injection volume	40 µL			
Flow rate	0.500 mL/min			
Mobile phase	A: Water + 0.5 % (v/v) formic acid			
	B: Methanol + 0.5 % (v/v) formic acid			
Gradient	Time [min]	% A	% B	
	0.00	90	10	
	2.50	40	60	
	3.00	5	95	
	4.50	5	95	

	4.60	90	10	
	6.50	90	10	
Retention time	Approx. 4.5 min (Mesotrione)			
MS system detector	SCIEX API 6500^{+}			
Ion mode	ESI, Negative ion mode			
Ion mass transition monitored (m/z) for Mesotrione	338.0 → 291.0 (quantitation) with collision energy (CE) = -12 V 338.0 → 212.0 (confirmation) with collision energy (CE) = -42 V			

Results and discussions

Table A 37: Recovery results from method validation of Mesotrione using the analytical method

Matrix	Analyte	Test item fortification level [µg/L]	Recovery [%]	Replicates	Mean recovery ± RSD [%]	Comments
Test medium	Mesotrione	0.0698	88, 93, 89, 97, 98	5	93 ± 5	Acceptable
Test medium	Mesotrione	975	85, 87, 87, 101, 102	5	92 ± 9	Acceptable

RSD = relative standard deviation

Mean recoveries at each fortification level were between 70 and 110 % and the overall RSD was below 20 %, in compliance with SANCO/3029/99 rev. 4 requirements.

In addition the following procedural recoveries were analysed:

Table A 38: Procedural Recovery results of Mesotrione using the analytical method

Matrix	Analyte	Test item fortification level [µg/L]	Recovery [%]	Replicates	Mean recovery ± RSD [%]	Comments
Test medium	Mesotrione	0.0698	96, 92, 95	3	94 ± 2	Acceptable
Test medium	Mesotrione	975	108, 109, 107	3	108 ± 1	Acceptable

RSD = relative standard deviation

Table A 39: Characteristics for the analytical method used for validation of Mesotrione residues in test medium

	Mesotrione

	Mesotrione
Specificity	One analyte was determined in the final sample extracts by use of LC-MS/MS detection. One MS/MS mass transition was evaluated. A second mass transition was monitored for confirmation of peak identity but was not used for quantification of samples. Untreated test medium samples were analysed according to the method to investigate the presence of residue and/or background interference at the retention time of Mesotrione. The samples showed no significant interference (above 30 % of LOQ) at the retention time of the analyte in test medium, therefore showing that the method is highly specific
Calibration (type, number of data points) **Calibration range**	The linearity of the detector was demonstrated by determinations of 7 matrix matched standards ranging from 0.01 ng/mL to 0.2 ng/mL, covering the working range of no more than 30 % of the LOQ to at least + 20 % of the highest analyte concentration level in a (diluted) sample. The detector response for analysis was linear for Mesotrione, with correlation coefficients $r \geq 0.995$: $y = 1.57 \cdot 10^6 x + 1.03 \cdot 10^3$, $r = 0.9992$ (1/x weighting)
Assessment of matrix effects is presented	Not tested, sample solutions and calibration solutions were prepared in the sample solvent system.
Storage stability of samples	The maximum storage period from sampling to analysis was 7 days within this study. Residues are regarded as stable if samples are stored deep-frozen up to 30 days between sampling and analysis. Therefore, the storage stability of Mesotrione was not verified.
Limit of determination/ quantification	The limit of quantification (LOQ) is defined as the lowest validated level and was successfully established at 0.0698 µg/L The limit of detection (LOD) was defined as 30 % of LOQ (0.0209 µg/L of Mesotrione).

Conclusion

The method was found to be valid for the determination of Mesotrione in OECD 221 test medium used in ecotoxicology studies, at fortification levels of 0.0698 µg/L and 975 µg/L of the test item, in accordance to SANCO/3029/99 rev. 4 requirements. The limit of quantification (LOQ) was successfully established at 0.0698 µg/L of Mesotrione.

Bertrand, C. (2019)

A 2.1.1.3.6.3 Method validation

Comments of zRMS:	The HPLC-MS/MS method used in the study report 218-31 for determination of mesotrione is acceptable validated according to SANCO/3029/99 rev. 4.

Reference:	**2.1.1.3.6.3/01 (KCP 5.1.2/17 also filed under KCP 10.2.1/08)**
Report	Christmann, R., 2021 Mesotrione: Toxicity to the Aquatic Plant *Spirodela polyrhiza* in a Growth Inhibition Test Report No. 218-31

Guideline(s): SANCO/3029/99 rev. 4

Deviations: No

GLP Yes

Acceptability: Yes

Materials and methods

An analytical method for Mesotrione was validated in OECD 221 test medium, and the purpose of the study phase was to quantify the concentration of the test item Mesotrione in fresh and aged aqueous samples to support ecotoxicological study. The limit of quantification (LOQ) is established at 0.18 µg test item/L.

Specimen preparation

- Stock solution: the test item was used to prepare a stock solution. 25.47 mg and 25.10 mg of the test item were suspended in 1 L pure water (10 min. ultrasonication, 40 min. stirring) to obtain a stock solution of approx. 25 mg test item/L.
- Standard solutions: appropriate amounts of the stock solution were diluted with test water to obtain standard solutions in the range from 0.1 – 10 µg item/L. The solutions were then acidified to a concentration of 0.1 % acetic acid.
- Fortified samples: approx. 25 mg of the test item were suspended in 1 L pure water (10 min. ultrasonication and up to 80 min. stirring) to obtain a stock solution of approx. 25 mg test item/L. Four independent stock solutions were prepared. Appropriate amounts of the stock solutions were diluted with test water to obtain fortified samples at a level of 0.18, 0.40 and 120 µg test item/L. the solutions were then acidified to a concentration of 0.1 % acetic acid.
- Biological treatment samples and control samples: the samples were allowed to thaw to room temperature. They were then shaken well and treated with ultrasound for 1 minute to obtain homogeneous samples. The samples were already acidified to 0.1 % acetic acid directly after sampling. The samples were diluted further with test water + 0.1% acetic acid to match the calibration range, if necessary.
- Fortified samples and analytical blank control samples: the samples were shaken well. They were diluted further with test water + 0.1 5 acetic acid to match the calibration range, if necessary.

Equipment for Mesotrione determination

LC-MS/MS system	Agilent series 1200 pump and autosampler			
Analytical column	PLRP-S 100A, 50 mm × 4.6 mm i.d., 5 µm			
Column temperature	35 °C			
Injection volume	40 µL			
Flow rate	0.800 mL/min			
Mobile phase	A: Acetonitrile			
	B: HPLC water + 0.1 % acetic acid			
Gradient	Time [min]	% A	% B	
	0.0	2	98	
	0.1	2	98	
	4.1	50	50	
	6.0	75	25	
	7.0	95	5	
	8.0	95	5	

	8.2	5	95	
	10.0	5	95	
Retention time	Approx. 6.3 min (Mesotrione)			
MS system detector	SCIEX API 4000			
Ion mode	ESI, Negative ion mode			
Ion mass transition monitored (m/z) for Mesotrione	338.2 → 291.1 (quantitation) with collision energy (CE) = -12 V 338.2 → 212.1 (confirmation) with collision energy (CE) = -42 V			

Results and discussions

Table A 40: Recovery results from method validation of Mesotrione using the analytical method

Matrix	Analyte	Test item fortification level [µg/L]	Recovery [%]	Replicates	Mean recovery ± RSD [%]	Comments
Test medium	Mesotrione	0.18	111, 109, 101, 100, 110	5	106 ± 5	Acceptable
Test medium	Mesotrione	0.40	103, 109, 101, 97, 99	5	102 ± 5	Acceptable
Test medium	Mesotrione	120	95, 97, 100, 98, 98	5	102 ± 2	Acceptable

RSD = relative standard deviation

Mean recoveries at each fortification level were between 70 and 110 % and the overall RSD was below 20 %, in compliance with SANCO/3029/99 rev. 4 requirements.

Table A 41: Characteristics for the analytical method used for validation of Mesotrione residues in test medium

	Mesotrione
Specificity	A highly specific detection system was used (MS/MS). The retention time of each analyte in the sample extracts matches the retention time in the calibration solution. No significant interference >30% LOQ occurred at the retention time of each analyte in any of the control samples. The analytical method can therefore be regarded as highly specific and selective for Mesotrione.
Calibration (type, number of data points) **Calibration range**	The linearity of the detector was demonstrated by determinations of 9 matrix matched standards ranging from 0.1 µg test item/L to 10.19 µg test item/L, covering the working range of no more than 30 % of the LOQ to at least + 20 % of the highest analyte concentration level in a (diluted) sample. The detector response for analysis was linear for Mesotrione, with correlation coefficients $r \geq 0.995$: y = 15425 x – 189, r = 0.9999 (1/x weighting)
Assessment of matrix	Not tested, sample solutions and calibration solutions were prepared in the sample solvent system. Matrix matched standards were used for analysis of test medium sam-

	Mesotrione
effects is presented	ples.
Limit of determination/ quantification	The limit of quantification (LOQ) is defined as the lowest validated level and was successfully established at 0.18 µg test item/L. The limit of detection (LOD) was defined as the lowest concentration having a peak height equivalent to or better than three times the baseline noise (LOD = 0.05 µg test item/L).

Conclusion

The method was found to be valid for the determination of Mesotrione in OECD 221 test medium used in ecotoxicology studies, at fortification levels of 0.18 µg test item /L, 0.4 µg test item/L and 120 µg test item/L of the test item, in accordance to SANCO/3029/99 rev. 4 requirements. The limit of quantification (LOQ) was successfully established at 0.18 µg test item/L of Mesotrione.

Christmann, R. (2021a)

A 2.1.1.3.6.4 Method validation

Comments of zRMS:	The HPLC-MS/MS method used in the study S16-03044 for determination of mesotrione and nicosulfuron is acceptable validated according to SANCO/3029/99 rev. 4.

Reference:	**2.1.1.3.6.4/01 (KCP 5.1.2/18 also filed under KCP 10.2.1/09)**
Report	Christmann, R., 2021 Mesotrione: Toxicity to the Aquatic Plant *Wolffia arrhiza* in a Growth Inhibition Test Report No. 218-32
Guideline(s):	SANCO/3029/99 rev. 4
Deviations:	No
GLP	Yes
Acceptability:	Yes

Materials and methods

An analytical method for Mesotrione was validated in OECD 221 test medium, and the purpose of the study phase was to quantify the concentration of the test item Mesotrione in fresh and aged aqueous samples to support ecotoxicological study. The limit of quantification (LOQ) is established at 0.18 µg test item/L.

Specimen preparation

- Stock solution: the test item was used to prepare a stock solution. 25.14 mg of the test item were suspended in 1 L pure water (10 min. ultrasonication, 50 min. stirring) to obtain a stock solution of approx. 25 mg test item/L.
- Standard solutions: appropriate amounts of the stock solution were diluted with test water to obtain standard solutions in the range from 0.1 – 10 µg item/L. The solutions were then acidified to a concentration of 0.1 % acetic acid.

- Fortified samples: approx. 25 mg of the test item were suspended in 1 L pure water (10 min. ultrasonication and up to 130 min. stirring) to obtain a stock solution of approx. 25 mg test item/L. Four independent stock solutions were prepared. Appropriate amounts of the stock solutions were diluted with test water to obtain fortified samples at a level of 0.18, 0.40 and 120 µg test item/L. the solutions were then acidified to a concentration of 0.1 % acetic acid.
- Biological treatment samples and control samples: the samples were allowed to thaw to room temperature. They were then shaken well and treated with ultrasound for 1 minute to obtain homogeneous samples. The samples were already acidified to 0.1 % acetic acid directly after sampling. The samples were diluted further with test water + 0.1% acetic acid to match the calibration range, if necessary.
- Fortified samples and analytical blank control samples: the samples were shaken well. They were diluted further with test water + 0.1 5 acetic acid to match the calibration range, if necessary.

Equipment for Mesotrione determination

LC-MS/MS system	Agilent series 1200 pump and autosampler			
Analytical column	PLRP-S 100A, 50 mm × 4.6 mm i.d., 5 µm			
Column temperature	35 °C			
Injection volume	40 µL			
Flow rate	0.800 mL/min			
Mobile phase	A: Acetonitrile			
	B: HPLC water + 0.1 % acetic acid			
Gradient	Time [min]	% A	% B	
	0.0	2	98	
	0.1	2	98	
	4.1	50	50	
	6.0	75	25	
	7.0	95	5	
	8.0	95	5	
	8.2	5	95	
	10.0	5	95	
Retention time	Approx. 6.14 min (Mesotrione)			
Run time	10 min			
MS system detector	SCIEX API 4000			
Ion mode	ESI, Negative ion mode			
Ion mass transition monitored (m/z) for Mesotrione	338.2 → 291.1 (quantitation) with collision energy (CE) = -12 V 338.2 → 212.1 (confirmation) with collision energy (CE) = -42 V			

Results and discussions

Table A 37: Recovery results from method validation of Mesotrione using the analytical method

Matrix	Analyte	Test item fortification level [µg/L]	Recovery [%]	Replicates	Mean recovery ± RSD [%]	Comments

Matrix	Analyte	Test item fortification level [µg/L]	Recovery [%]	Replicates	Mean recovery ± RSD [%]	Comments
Test medium	Mesotrione	0.18	95, 88, 94, 81, 90	5	91 ± 3	Acceptable
Test medium	Mesotrione	0.40	92, 93, 101, 108, 105	5	100 ± 7	Acceptable
Test medium	Mesotrione	120	104, 106, 107, 110, 112	5	108 ± 3	Acceptable

RSD = relative standard deviation

Mean recoveries at each fortification level were between 70 and 110 % and the overall RSD was below 20 %, in compliance with SANCO/3029/99 rev. 4 requirements.

Table A 43: Characteristics for the analytical method used for validation of Mesotrione residues in test medium

	Mesotrione
Specificity	A highly specific detection system was used (MS/MS). The retention time of each analyte in the sample extracts matches the retention time in the calibration solution. No significant interference >30% LOQ occurred at the retention time of each analyte in any of the control samples. The analytical method can therefore be regarded as highly specific and selective for Mesotrione.
Calibration (type, number of data points) **Calibration range**	The linearity of the detector was demonstrated by determinations of 9 matrix matched standards ranging from 0.1 µg test item/L to 10 µg test item/L, covering the working range of no more than 30 % of the LOQ to at least + 20 % of the highest analyte concentration level in a (diluted) sample. The detector response for analysis was linear for Mesotrione, with correlation coefficients $r \geq 0.995$: y = 54470 x + 1131, r = 1.0000 (1/x weighting)
Assessment of matrix effects is presented	Not tested, sample solutions and calibration solutions were prepared in the sample solvent system. Matrix matched standards were used for analysis of test medium samples.
Limit of determination/ quantification	The limit of quantification (LOQ) is defined as the lowest validated level and was successfully established at 0.18 µg test item/L. The limit of detection (LOD) was defined as the lowest concentration having a peak height equivalent to or better than three times the baseline noise (LOD = 0.04 µg test item/L).

Conclusion

The method was found to be valid for the determination of Mesotrione in OECD 221 test medium used in ecotoxicology studies, at fortification levels of 0.18 µg test item /L, 0.4 µg test item/L and 120 µg test item/L of the test item, in accordance to SANCO/3029/99 rev. 4 requirements. The limit of quantification (LOQ) was successfully established at 0.18 µg test item/L of Mesotrione.

Christmann, R. (2021b)

A 2.1.1.3.7 Analytical method - Mesotrione and nicosulfuron residues in OECD 239 water-sediment system

A 2.1.1.3.7.1 Method validation

Comments of zRMS:	The HPLC-MS/MS method used in the study S16-03045 for determination of mesotrione and nicosulfuron is acceptable validated according to SANCO/3029/99 rev. 4.

Reference:	**2.1.1.3.7.1/01 (KCP 5.1.2/10 also filed under KCP 10.2.1/06)**
Report	Gonsior G., 2016 SAE053H/01: Growth Inhibition of *Myriophyllum spicatum* in a Water/Sediment System Report No. S16-03045
Guideline(s):	SANCO/3029/99 rev. 4
Deviations:	No
GLP	Yes
Acceptability:	Yes

Materials and methods

An analytical method for mesotrione and nicosulfuron was validated in OECD 239 water-sediment system, composed of SMART AND BARKO test medium (overlaying water) and artificial sterilised sediment. For test medium analysis, the method involves dilution of test medium samples by a factor of 2 with acetonitrile, followed by further dilution with acetonitrile/test medium (1:1, v/v) if necessary, prior to analysis by liquid chromatography coupled with a tandem mass spectrometric detection (HPLC-MS/MS) using external calibration. For sediment analysis, the method involves extraction of sediment samples with acetonitrile/water (1:1, v/v), followed by further dilution with sediment blank extract if necessary, prior to analysis by liquid chromatography coupled with a tandem mass spectrometric detection (HPLC-MS/MS) using external calibration. The limit of quantification (LOQ) of 0.003 mg/L of test item was validated for medium analysis, corresponding to 0.000250 mg/L of mesotrione and 0.0000939 mg/L of nicosulfuron. An LOQ of 0.001 mg/kg of mesotrione and nicosulfuron was confirmed in sediment.

Specimen preparation for Analysis of Test medium samples
After sampling, the test medium samples (10 mL) were stored deep-frozen (≤ - 18 °C) until analysis. In the analytical laboratory, the samples were thawed to ambient temperature and mixed with 10 mL of acetonitrile and shaken well using a Vortex-Mixer. If necessary, the samples were further diluted with acetonitrile/test medium (1:1, v/v) prior to analysis by HPLC-MS/MS.
Recovery samples were prepared by fortification of untreated test medium with the test item. 10 mL of recovery samples were mixed with 10 mL acetonitrile and shaken well using a Vortex-Mixer. Aliquots were transferred into HPLC vials. If necessary, the samples were further diluted with acetonitrile/test medium (1:1, v/v) prior to analysis by HPLC-MS/MS.

Specimen preparation for Analysis of Sediment samples
Sediment samples were stored deep-frozen (≤ - 18 °C) after sampling. At the day of analysis, the samples were thawed to ambient temperature and mixed manually. A 10 g aliquot was transferred into a 250 mL glass bottle and extracted with 100 mL acetonitrile/water (1:1, v/v) on a horizontal flatbed shaker for 2 hours. After sedimentation, an aliquot of the extract was filtered over a 0.45 µm single-use syringe filter.

Further dilution was performed with sediment blank extract for analysis by HPLC/MS-MS, if necessary. Recovery samples were prepared by fortification of untreated sediment with the analytical standards or a mixture of the analytical standards and analysed as described above.

Equipment for mesotrione and nicosulfuron determination

LC-MS/MS system	Shimadzu LC-30 AD HPLC pump with autosampler SIL-30ACMP coupled with SCIEX API 5500 MS/MS system
Analytical column	Phenomenex Luna 5µ Phenyl-Hexyl, 150 mm x 2 mm i.d., 5 µm mean particle size (No. 00F-4257-B0) with 4 mm guard column
Column temperature	30°C
Injection volume	40 µL
Flow rate	0.500 mL/min
Mobile phase	A: Water + 0.5 % (v/v) formic acid B: Methanol + 0.5 % (v/v) formic acid

Time (min)	% A	% B
0.10	90	10
4.00	5	95
5.00	5	95
5.10	90	10
7.00	90	10

Retention time	Approx. 4.3 min (nicosulfuron) and 4.3 min (mesotrione)
Ion mode	ESI, Positive/negative ion switching mode
Ion mass transition monitored (m/z)	411.12→ 182.10 (quantitation) with collision energy (CE)= 27V
for nicosulfuron	411.12 → 213.00 (confirmation) with collision energy (CE)= 27V
Ion mass transition monitored (m/z)	337.85→ 291.00 (quantitation) with collision energy (CE)= -14V
for mesotrione	337.85 → 212.00 (confirmation) with collision energy (CE)= -42V

Results and discussions

Table A 38: Recovery results from method validation of mesotrione in Test medium using the analytical method

Matrix	**Analyte**	**Test item fortification level (mg/L) (*n* = x)**	**Analyte fortification level (mg/L) (*n* = x)**	**Recovery (%)**	**Mean recovery ± RSD (%)**	**Comments**
Test medium	mesotrione	0.003 (n = 5)	0.000250 (n = 5)	98, 98, 96, 97, 98	97 ± 1	Acceptable
Test medium	mesotrione	1.3 (n = 5)	0.108 (n = 5)	103, 106, 101, 108, 103	104 ± 3	Acceptable

RSD = relative standard deviation

Mean recoveries at each fortification level were between 70 and 110% and the overall RSD was below 20%, in compliance with SANCO/3029/99 rev. 4 requirements.

Table A 39: Recovery results from method validation of nicosulfuron in Test medium using the analytical method

Matrix	Analyte	Test item fortification level (mg/L) (*n* = x)	Analyte fortification level (mg/L) (*n* = x)	Recovery (%)	Mean recovery ± RSD (%)	Comments
Test medium	nicosulfuron	0.003 (n = 5)	0.0000939 (n = 5)	94, 97, 96, 94, 93	95 ± 2	Acceptable
Test medium	nicosulfuron	1.3 (n = 5)	0.0470 (n = 5)	97, 100, 103, 102, 99	100 ± 2	Acceptable

RSD = relative standard deviation

Mean recoveries at each fortification level were between 70 and 110% and the overall RSD was below 20%, in compliance with SANCO/3029/99 rev. 4 requirements.

Table A 40: Recovery results from method validation of mesotrione in Sediment using the analytical method

Matrix	Analyte	Analyte fortification level (mg/kg) (*n* = x)	Recovery (%)	Mean recovery ± RSD (%)	Comments
Sediment	mesotrione	0.001 (n = 5)	111, 105, 105, 110, 110	108 ± 3	Acceptable
Sediment	mesotrione	15 (n = 5)	107, 104, 98, 107, 109	105 ± 4	Acceptable

RSD = relative standard deviation

Mean recoveries at each fortification level were between 70 and 110% and the overall RSD was below 20%, in compliance with SANCO/3029/99 rev. 4 requirements.

Table A 41: Recovery results from method validation of nicosulfuron in Sediment using the analytical method

Matrix	Analyte	Analyte fortification level (mg/kg) (*n* = x)	Recovery (%)	Mean recovery ± RSD (%)	Comments
Sediment	nicosulfuron	0.001 (n = 5)	111, 101, 102, 99, 104	103 ± 4	Acceptable
Sediment	nicosulfuron	15 (n = 5)	100, 106, 95, 109, 102	102 ± 5	Acceptable

RSD = relative standard deviation

Mean recoveries at each fortification level were between 70 and 110% and the overall RSD was below 20%, in compliance with SANCO/3029/99 rev. 4 requirements.

Table A 42: Characteristics for the analytical method used for validation of Mesotrione and Nicosulfuron residues in test medium and sediment samples

	Mesotrione	**Nicosulfuron**
Specificity	A highly specific detection system was used (MS/MS). The retention time of each analyte in the sample extracts matches the retention time in the calibration solution. No significant interference >30% LOQ occurred at the retention time of each analyte in any of the control samples. The analytical method can therefore be regarded as highly specific and selective for mesotrione and nicosulfuron	
Calibration (type, number of data points) **Calibration range**	Dilutions for calibration of HPLC-MS/MS analysis of nicosulfuron and mesotrione in test medium were prepared in acetonitrile/test medium (1:1). Dilutions for calibration of HPLC-MS/MS analysis of nicosulfuron and mesotrione in sediment were prepared in sediment blank extract The linearity of the detector was demonstrated by determinations of 7 matrix matched standards ranging from 0.01 ng/mL to 3.0 ng/mL in test medium and 0.020 ng/mL to 3 ng/mL in sediment, covering the working range of no more than 30 % of the LOQ to at least + 20 % of the highest analyte concentration level in a (diluted) sample. The detector response for analysis was linear for mesotrione and nicosulfuron, with correlation coefficients $r \geq 0.995$:	
	<u>Test medium:</u> $y = 5.71e^{+005} x + 2.2e^{+003}$, r = 0.9991 (1/x weighting) <u>Sediment:</u> $y = 3.89e^{+005} x + 1.12e^{+003}$, r = 0.9995 (1/x weighting)	<u>Test medium:</u> $y = 2.35e^{+006} x + 3.28e^{+003}$ r = 0.9998 (1/x weighting) <u>Sediment:</u> $y = 2.62e^{+006} x + -4.62e^{+003}$, r = 0.9998 (1/x weighting)
Assessment of matrix effects is presented	Not tested, sample solutions and calibration solutions were prepared in the sample solvent system. Matrix matched standards were used for analysis of test medium samples.	
Storage stability of samples	The maximum storage period from sampling to analysis was 41 days for test medium samples and 27 days for sediment samples within this study. Residues are regarded as stable if the samples are stored deep-frozen for up to 30 days between sampling and analysis (EU COM 7032/VI/95 and OPPTS 860.1380). Therefore, the storage stability of mesotrione and nicosulfuron in test medium was verified. Mesotrione and nicosulfuron were found to be stable when stored for 41 days under deep-frozen conditions (≤ - 18°C) in test medium. This covers the maximum storage period from sampling to analysis within this study.	
Limit of determination/ quantification	The limit of quantification (LOQ) is defined as the lowest validated level and was successfully established at 0.003 mg/L of test item (0.000250 mg/L of mesotrione). An LOQ of 0.001 mg/kg of mesotrione was confirmed in sediment. The limit of detection (LOD) was defined as 30 % of LOQ (0.0000750 mg/L of mesotrione in test medium and 0.000300 mg/kg for mesotrione in sediment).	The limit of quantification (LOQ) is defined as the lowest validated level and was successfully established at 0.003 mg/L of test item (0.0000939 mg/L of nicosulfuron). An LOQ of 0.001 mg/kg of nicosulfuron was confirmed in sediment. The limit of detection (LOD) was defined as 30 % of LOQ (0.0000282 mg/L of nicosulfuron in test medium and 0.000300 mg/kg for nicosulfuron in sediment).

Conclusion

The method was found to be valid for the determination of mesotrione and nicosulfuron in OECD 239 test medium and sediment samples used in ecotoxicology studies, at fortification levels of 0.003 mg/L and 1.3 mg/L of the test item in test medium, and at fortification levels of 0.001 mg/kg of test item and 15 mg/kg

of test item in sediment, in accordance to SANCO/3029/99 rev. 4 requirements. The limit of quantification (LOQ) was successfully established at 0.003 mg/L of test item (0.000250 mg/L of mesotrione and 0.0000939 mg/L of nicosulfuron) in test medium, and at 0.001 mg/kg of mesotrione and nicosulfuron in sediment.

Gonsior G., 2016

A 2.1.1.3.8 Analytical method - Mesotrione and nicosulfuron residues in OECD 211 test medium

A 2.1.1.3.8.1 Method validation

Comments of zRMS:	The HPLC-MS/MS method used in the study S16-03043 for determination of mesotrione and nicosulfuron is acceptable validated according to SANCO/3029/99 rev. 4.

Reference:	**2.1.1.3.8.1/01 (KCP 5.1.2/11 also filed under KCP 10.2.2/01)**
Report	Lang C., 2016a SAE053H/01: Toxicity to the Water Flea *Daphnia magna* Straus under Laboratory Conditions (Reproduction Test) Report No. S16-03043
Guideline(s):	SANCO/3029/99 rev. 4
Deviations:	No
GLP	Yes
Acceptability:	Yes

Materials and methods

An analytical method for mesotrione and nicosulfuron was validated in OECD 211 test medium. The method involves dilution of test medium samples by a factor of 2 with acetonitrile, followed by further dilution with acetonitrile/test medium (1:1, v/v) if necessary, prior to analysis by liquid chromatography coupled with a tandem mass spectrometric detection (HPLC-MS/MS) using external calibration. The limit of quantification (LOQ) of 0.003 mg/L of test item was validated, corresponding to 0.000250 mg/L of mesotrione and 0.0000939 mg/L of nicosulfuron.

Specimen preparation

After sampling, the test medium samples (10 ml) were stored deep-frozen (≤ - 18 °C) until analysis. In the analytical laboratory, the samples were thawed to ambient temperature, mixed with 10 mL acetonitrile and shaken well using a Vortex-Mixer. The samples were further diluted with acetonitrile/test medium (1:1, v/v) prior to analysis by HPLC-MS/MS.

Recovery samples were prepared by fortification of untreated test medium with the test item. For 10 mg/L recovery samples 0.5 mL of recovery samples were mixed with 0.5 mL acetonitrile and shaken well using a Vortex-Mixer. If necessary, the samples were further diluted with acetonitrile/test medium (1:1, v/v) prior to analysis by HPLC-MS/MS. For 0.003 mg/L recovery samples 10 mL of test medium were transferred into a glass vial. After fortification, 10 mL recovery sample were diluted with 10 mL acetonitrile and shaken on a Vortex-Mixer. Aliquots were transferred into HPLC glass vials prior to analysis by HPLC-MS/MS.

Equipment for mesotrione and nicosulfuron determination

LC-MS/MS system	Shimadzu LC-30 AD HPLC pump with autosampler SIL-30ACMP coupled with SCIEX API 5500 MS/MS system
Analytical column	Phenomenex Luna 5µ Phenyl-Hexyl, 150 mm x 2 mm i.d., 5 µm mean particle size (No. 00F-4257-B0) with 4 mm guard column
Column temperature	30°C
Injection volume	40 µL
Flow rate	0.500 mL/min
Mobile phase	A: Water + 0.5 % (v/v) formic acid B: Methanol + 0.5 % (v/v) formic acid

Time (min)	% A	% B
0.10	90	10
4.00	5	95
5.00	5	95
5.10	90	10
7.00	90	10

Retention time	Approx. 4.3 min (nicosulfuron) and 4.3 min (mesotrione)
Ion mode	ESI, Positive/negative ion switching mode
Ion mass transition monitored (m/z)	411.12→ 182.10 (quantitation) with collision energy (CE)= 27V
for nicosulfuron	411.12 → 213.00 (confirmation) with collision energy (CE)= 27V
Ion mass transition monitored (m/z)	337.85→ 291.00 (quantitation) with collision energy (CE)= -14V
for mesotrione	337.85 → 212.00 (confirmation) with collision energy (CE)= -42V

Results and discussions

Table A 43: Recovery results from method validation of mesotrione using the analytical method

Matrix	**Analyte**	**Test item fortification level (mg/L) (*n* = x)**	**Analyte fortification level (mg/L) (*n* = x)**	**Recovery (%)**	**Mean recovery ± RSD (%)**	**Comments**
Test medium	mesotrione	0.003 (n = 5)	0.000250 (n = 5)	84, 84, 84, 81, 81	83 ± 2	Acceptable
Test medium	mesotrione	10 (n = 5)	0.833 (n = 5)	84, 87, 86, 85, 89	86 ± 2	Acceptable

RSD = relative standard deviation

Mean recoveries at each fortification level were between 70 and 110% and the overall RSD was below 20%, in compliance with SANCO/3029/99 rev. 4 requirements.

Table A 44: Recovery results from method validation of nicosulfuron using the analytical method

Matrix	**Analyte**	**Test item fortification level (mg/L) (*n* = x)**	**Analyte fortification level (mg/L) (*n* = x)**	**Recovery (%)**	**Mean recovery ± RSD (%)**	**Comments**
Test medium	nicosulfuron	0.003 (n = 5)	0.0000939 (n = 5)	100, 103, 101, 106, 101	102 ± 1	Acceptable

Matrix	Analyte	Test item fortification level (mg/L) (*n* = x)	Analyte fortification level (mg/L) (*n* = x)	Recovery (%)	Mean recovery ± RSD (%)	Comments
Test medium	nicosulfuron	10 (n = 5)	0.313 (n = 5)	109, 111, 112, 109, 111	110 ± 1	Acceptable

RSD = relative standard deviation

Mean recoveries at each fortification level were between 70 and 110% and the overall RSD was below 20%, in compliance with SANCO/3029/99 rev. 4 requirements.

Table A 45: Characteristics for the analytical method used for validation of Mesotrione and Nicosulfuron residues in test medium

	Mesotrione	Nicosulfuron
Specificity	A highly specific detection system was used (MS/MS). The retention time of each analyte in the sample extracts matches the retention time in the calibration solution. No significant interference >30% LOQ occurred at the retention time of each analyte in any of the control samples. The analytical method can therefore be regarded as highly specific and selective for mesotrione and nicosulfuron	
Calibration (type, number of data points) **Calibration range**	Dilutions for calibration were prepared in acetonitrile/test medium (1:1, v/v). The linearity of the detector was demonstrated by determinations of 8 matrix-matched calibration standards ranging from 0.01 ng/mL to 5 ng/mL, covering the working range of no more than 30 % of the LOQ to at least + 20 % of the highest analyte concentration level in a (diluted) sample. The detector response for analysis was linear for mesotrione and nicosulfuron, with correlation coefficients r ≥ 0.995:	
	$y = 4.52e^{+005} x + 3.81e^{+003}$ r = 0.9994 (1/x weighting)	$y = 1.38e^{+006} x + 1.2e^{+003}$, r = 0.9999 (1/x weighting)
Assessment of matrix effects is presented	Not tested, sample solutions and calibration solutions were prepared in the sample solvent system. Matrix matched standards were used for analysis of test medium samples.	
Storage stability of samples	The maximum storage period from sampling to analysis was 29 days within this study. Residues are regarded as stable if the samples are stored deep-frozen for up to 30 days between sampling and analysis (EU COM 7032/VI/95 and OPPTS 860.1380). Therefore, the storage stability of Mesotrione and Nicosulfuron was not verified.	
Limit of determination/ quantification	The limit of quantification (LOQ) is defined as the lowest validated level and was successfully established at 0.003 mg/L of test item (0.000250 mg/L of mesotrione). The limit of detection (LOD) was defined as 30 % of LOQ (0.0000750 mg/L of mesotrione).	The limit of quantification (LOQ) is defined as the lowest validated level and was successfully established at 0.003 mg/L of test item 0.0000939 mg/L of nicosulfuron). The limit of detection (LOD) was defined as 30 % of LOQ (0.0000282 mg/L of nicosulfuron).

Conclusion

The method was found to be valid for the determination of mesotrione and nicosulfuron in OECD 211 test medium used in ecotoxicology studies, at fortification levels of 0.003 mg/L and 10 mg/L of the test item, in accordance to SANCO/3029/99 rev. 4 requirements. The limit of quantification (LOQ) was successfully established at 0.003 mg/L of test item (0.000250 mg/L of mesotrione and 0.0000939 mg/L of nicosulfuron).

Lang C., 2016a

A 2.1.1.3.9 Analytical method - Mesotrione and nicosulfuron residues in honey bee feeding solutions

A 2.1.1.3.9.1 Method validation

Comments of zRMS:	The HPLC-MS/MS method used in the study S16-02518 for determination of mesotrione and nicosulfuron is acceptable validated according to SANCO/3029/99 rev. 4.

Reference:	**2.1.1.3.9.1/01 (KCP 5.1.2/12 also filed under KCP 10.3.1.1/01)**
Report	Molitor A.M., 2016 SAE053H/01- Assessment of Effects on the Adult Honey Bee, *Apis mellifera* L., in a 10 Day Chronic Feeding Test under Laboratory Conditions Report No. S16-02518
Guideline(s):	SANCO/3029/99 rev. 4
Deviations:	No
GLP	Yes
Acceptability:	Yes

Materials and methods

An analytical method for mesotrione and nicosulfuron was validated in honey bee feeding solutions (50 % (w/v) aqueous sucrose solutions). The method involves dilution of test medium samples with acetonitrile/water (1:1, v/v) if necessary, prior to analysis by liquid chromatography coupled with a tandem mass spectrometric detection (HPLC-MS/MS) using external calibration. The limit of quantification (LOQ) of 25.0 mg/L of test item was validated, corresponding to 2.08 mg/L of mesotrione and 0.783 mg/L of nicosulfuron.

Specimen preparation

After sampling, the analytical samples were stored deep-frozen (≤ - 18 °C) until analysis. In the analytical laboratory, the samples were thawed to ambient temperature and shaken well using a Vortex-Mixer. If necessary, the samples were further diluted with acetonitrile/water (1:1, v/v) prior to analysis by HPLC-MS/MS.

Recovery samples were prepared by fortifying untreated 50 % (w/v) aqueous sucrose solution with the test item. If necessary, the samples were further diluted with acetonitrile/water (1:1, v/v) prior to analysis by HPLC-MS/MS.

Equipment for mesotrione and nicosulfuron determination

LC-MS/MS system	Thermo Surveyor MS pump with Thermo Surveyor autosampler coupled with Thermo TSQ Quantum triple quadrupole system
Analytical column	Phenomenex Luna 5µ Phenyl-Hexyl, 150 mm x 2 mm i.d., 5 µm mean particle size (No. 00F-4257-B0) with 4 mm guard column
Column temperature	40°C
Injection volume	10 µL

Flow rate 0.500 mL/min

Mobile phase A: Water

B: Methanol

C: 1.0 % (v/v) formic acid in water

Time (min)	% A	% B	% C
0.00	78	20	2
3.00	5	93	2
5.00	5	93	2
5.01	78	20	2
8.00	78	20	2

Split before MS Approx. 1:5

Retention time Approx. 3.9 min (for mesotrione and nicosulfuron)

Ion mode ESI, Positive ion mode (nicosulfuron) Negative ion mode (mesotrione)

Ion mass transition monitored (m/z) for nicosulfuron: 411→ 182 (quantitation) with collision energy (CE)= 20V; 411 → 213 (confirmation) with collision energy (CE)= 17V

Ion mass transition monitored (m/z) for mesotrione: 338→ 291 (quantitation) with collision energy (CE)= -34V; 338→ 212 (confirmation) with collision energy (CE)= -14V

Results and discussions

Table A 46: Recovery results from method validation of mesotrione using the analytical method

Matrix	**Analyte**	**Test item fortification level (mg/L) (*n* = x)**	**Analyte fortification level (mg/L) (*n* = x)**	**Recovery (%)**	**Mean recovery ± RSD (%)**	**Comments**
Test medium	mesotrione	25 (n = 5)	2.08 (n = 5)	84, 86, 87, 96, 95	90 ± 6	Acceptable
Test medium	mesotrione	6500 (n = 5)	561, 535, 543, 553, 541 (n = 5)	101, 108, 114, 107, 108	108 ± 4	Acceptable

RSD = relative standard deviation

Mean recoveries at each fortification level were between 70 and 110% and the overall RSD was below 20%, in compliance with SANCO/3029/99 rev. 4 requirements.

Table A 47: Recovery results from method validation of nicosulfuron using the analytical method

Matrix	**Analyte**	**Test item fortification level (mg/L) (*n* = x)**	**Analyte fortification level (mg/L) (*n* = x)**	**Recovery (%)**	**Mean recovery ± RSD (%)**	**Comments**
Test medium	nicosulfuron	25 (n = 5)	0.783 (n = 5)	83, 81, 82, 86, 85	83 ± 2	Acceptable
Test medium	nicosulfuron	6500 (n = 5)	211, 201, 204, 208, 203 (n = 5)	103, 101, 97, 94, 97	98 ± 4	Acceptable

RSD = relative standard deviation

Mean recoveries at each fortification level were between 70 and 110% and the overall RSD was below 20%, in compliance with SANCO/3029/99 rev. 4 requirements.

Table A 48: Characteristics for the analytical method used for validation of Mesotrione and Nicosulfuron residues in test medium

	Mesotrione	**Nicosulfuron**
Specificity	A highly specific detection system was used (MS/MS). The retention time of each analyte in the sample extracts matches the retention time in the calibration solution. No significant interference >30% LOQ occurred at the retention time of each analyte in any of the control samples. The analytical method can therefore be regarded as highly specific and selective for mesotrione and nicosulfuron	
Calibration (type, number of data points) **Calibration range**	Dilutions for calibration were prepared in water/acetonitrile (1:1, v/v). The linearity of the detector was demonstrated by determinations of 6 calibration standards ranging from 10 ng/mL to 100 ng/mL, covering the working range of no more than 30 % of the LOQ to at least + 20 % of the highest analyte concentration level in a (diluted) sample. The detector response for analysis was linear for mesotrione and nicosulfuron, with correlation coefficients r ≥ 0.995:	
	y = 959.182+7002.94 x, R^2 = 0.9945 (1/x weighting)	y = 17311.2+40926.8x, R^2 = 0.9999 (1/x weighting)
Assessment of matrix effects is presented	Not tested, sample solutions and calibration solutions were prepared in the sample solvent system. Solvent standards were used for analysis of test medium samples.	
Storage stability of samples	The maximum storage period from sampling to analysis was 21 days within this study. Residues are regarded as stable if the samples are stored deep-frozen for up to 30 days between sampling and analysis (EU COM 7032/VI/95 and OPPTS 860.1380). Therefore, the storage stability of Mesotrione and Nicosulfuron was not verified.	
Limit of determination/ quantification	The limit of quantification (LOQ) is defined as the lowest validated level and was successfully established at 25.0 mg/L of test item (2.08 mg/L of mesotrione). The limit of detection (LOD) was defined as 30 % of LOQ (0.624 mg/L of mesotrione).	The limit of quantification (LOQ) is defined as the lowest validated level and was successfully established at 25.0 mg/L of test item (0.783 mg/L of nicosulfuron). The limit of detection (LOD) was defined as 30 % of LOQ (0.235 mg/L of nicosulfuron).

Conclusion

The method was found to be valid for the determination of mesotrione and nicosulfuron in honey bee feeding solutions (50 % (w/v) aqueous sucrose solutions) used in ecotoxicology studies, at fortification levels of 25 mg/L and 6500 mg/L of the test item, in accordance to SANCO/3029/99 rev. 4 requirements. The limit of quantification (LOQ) was successfully established at 25.0 mg/L of test item (2.08 mg/L of mesotrione and 0.783 mg/L of nicosulfuron).

Molitor A.M., 2016

A 2.1.1.3.10 Analytical method - Mesotrione and nicosulfuron residues in larval diet samples

A 2.1.1.3.10.1 Method validation

Comments of zRMS:	The HPLC-MS/MS method used in the study S16-02503 for determination of mesotrione and nicosulfuron is acceptable validated according to SANCO/3029/99 rev. 4.

Reference:	**2.1.1.3.10.1/01 (KCP 5.1.2/13 also filed under KCP 10.3.1.3/01)**
Report	Vergé E. and Wagner J., 2016 SAE053H/01 - Honey Bee (*Apis mellifera* L.) Larval Toxicity Test (Repeated Exposure) Report No. S16-02503
Guideline(s):	SANCO/3029/99 rev. 4
Deviations:	No
GLP	Yes
Acceptability:	Yes

Materials and methods

An analytical method for mesotrione and nicosulfuron was validated in larval diet samples. The method involves extraction of test medium samples with acetonitrile/water (1:1, v/v) (+ 0.5 % 2.5 N sodium hydroxide solution for mesotrione analysis), followed by dilution with acetonitrile/water (1:1, v/v), and by further dilution with blank matrix extract if necessary, prior to analysis by liquid chromatography coupled with a tandem mass spectrometric detection (HPLC-MS/MS) using external calibration. The limit of quantification (LOQ) of 120 mg/kg of test item was validated, corresponding to 10 mg/kg of mesotrione and 3.76 mg/kg of nicosulfuron.

Specimen preparation for mesotrione:
The samples were extracted in the plastic tube with the necessary amount of acetonitrile/water (1:1, v/v) + 0.5 % 2.5 N sodium hydroxide solution (10 mL per 500 mg sample), shaken for 2x 30 sec and centrifuged (5 min, 4000rpm). The supernatant was diluted with acetonitrile/water (1:1, v/v) by a factor of 100. If necessary samples were further diluted with blank matrix extract to fit the calibration range prior to analysis by HPLC-MS/MS.
Recovery samples were prepared by fortification of untreated larval diet with the test item. About 500 mg of larval diet were weighed into 15 mL plastic tube. The necessary spiking volume adjusted to the sample weight was added and recovery samples were prepared as described above.
Matrix blank extract was prepared from untreated larval diet as described above and used for dilution of samples and preparation of calibration standards.

Specimen preparation for nicosulfuron:
The samples were extracted in the plastic tube with the necessary amount of acetonitrile/water (1:1, v/v) (10 mL per 500 mg sample), shaken for 2x 30 sec, approx. 1 g sodium chloride was added. Samples were shaken again for short and centrifuged (5 min, 4000rpm). The organic supernatant was diluted with acetonitrile/water (1:1, v/v) by a factor of 100. If necessary samples were further diluted with blank matrix extract to fit the calibration range prior to analysis by HPLCMS/MS.
Recovery samples were prepared by fortification of untreated larval diet with the test item. About 500 mg of larval diet were weighed into 15 mL plastic tube. The necessary spiking volume adjusted to the sample weight was added and recovery samples were prepared as described above.

Matrix blank extract was prepared from untreated larval diet as described above and used for dilution of samples and preparation of calibration standards.

Equipment for mesotrione and nicosulfuron determination

LC-MS/MS system	Thermo Surveyor MS pump with Thermo Surveyor autosampler coupled with Thermo TSQ Quantum triple quadrupole system
Analytical column	Phenomenex Luna 5µ Phenyl-Hexyl. No. 00F-4257-Bo, 150 mm x 2 mm, 5 µm; HPLC guard column (KJO-4282, Phenomenex) with 4 mm Fusion RP cartridge (AJO-7556, Phenomenex)
Column temperature	40°C
Injection volume	40 µL
Flow rate	0.500 mL/min
Mobile phase	A: Water B: Methanol C: 1.0 % (v/v) formic acid in water

Time (min)	% A	% B	% C
0.00	78	20	2
3.00	5	93	2
5.00	5	93	2
5.01	78	20	2
8.00	78	20	2

Split before MS	Approx. 1:5
Retention time	Approx. 4.0 min (for mesotrione and nicosulfuron)
Ion mode	ESI, Positive ion mode (nicosulfuron) Negative ion mode (mesotrione)
Ion mass transition monitored (m/z)	411→ 182 (quantitation) with collision energy (CE)= 20V
for nicosulfuron	411 → 213 (confirmation) with collision energy (CE)= 17V
Ion mass transition monitored (m/z)	338→ 212 (quantitation) with collision energy (CE)= -14V
for mesotrione	338→ 291 (confirmation) with collision energy (CE)= -34V

Results and discussions

Table A 49: Recovery results from method validation of mesotrione using the analytical method

Matrix	**Analyte**	**Test item fortification level (mg/kg) (*n* = x)**	**Analyte fortification level (mg/kg) (*n* = x)**	**Recovery (%)**	**Mean recovery ± RSD (%)**	**Comments**
Test medium	mesotrione	120 (n = 5)	10 (n = 5)	88, 99, 95, 95, 95	94 ± 4	Acceptable
Test medium	mesotrione	12000 (n = 5)	1000 (n = 5)	93, 96, 92, 96, 82	92 ± 6	Acceptable

RSD = relative standard deviation

Mean recoveries at each fortification level were between 70 and 110% and the overall RSD was below 20%, in compliance with SANCO/3029/99 rev. 4 requirements.

Table A 50: Recovery results from method validation of nicosulfuron using the analytical method

Matrix	Analyte	Test item fortification level (mg/kg) (*n* = x)	Analyte fortification level (mg/kg) (*n* = x)	Recovery (%)	Mean recovery ± RSD (%)	Comments
Test medium	nicosulfuron	120 (n = 5)	3.76 (n = 5)	84, 85, 86, 84, 94	87 ± 5	Acceptable
Test medium	nicosulfuron	12000 (n = 5)	376 (n = 5)	89, 92, 83, 86, 90	88 ± 4	Acceptable

RSD = relative standard deviation

Mean recoveries at each fortification level were between 70 and 110% and the overall RSD was below 20%, in compliance with SANCO/3029/99 rev. 4 requirements.

Table A 51: Characteristics for the analytical method used for validation of Mesotrione and Nicosulfuron residues in test medium

	Mesotrione	Nicosulfuron
Specificity	A highly specific detection system was used (MS/MS). The retention time of each analyte in the sample extracts matches the retention time in the calibration solution. No significant interference >30% LOQ occurred at the retention time of each analyte in any of the control samples. The analytical method can therefore be regarded as highly specific and selective for mesotrione and nicosulfuron	
Calibration (type, number of data points) **Calibration range**	Dilutions for calibration were prepared in matrix blank extract. The linearity of the detector was demonstrated by determinations of 8 matrix-matched calibration standards ranging from 0.5 ng/mL to 25 ng/mL, covering the working range of no more than 30 % of the LOQ to at least + 20 % of the highest analyte concentration level in a (diluted) sample. The detector response for analysis was of second order for mesotrione, with correlation coefficients $r \geq 0.995$:	Dilutions for calibration were prepared in matrix blank extract. The linearity of the detector was demonstrated by determinations of 8 matrix-matched calibration standards ranging from 0.3 ng/mL to 20 ng/mL, covering the working range of no more than 30 % of the LOQ to at least + 20 % of the highest analyte concentration level in a (diluted) sample. The detector response for analysis was linear for nicosulfuron, with correlation coefficients $r \geq 0.995$:
	$y = -2486.73+8636.1x-65.1977x^2$, $R^2 = 0.9991$	$y = 9801.12+190601x$, $R^2 = 0.9994$ (1/x weighting)
Assessment of matrix effects is presented	Not tested, sample solutions and calibration solutions were prepared in the sample solvent system. Matrix matched standards were used for analysis of test medium samples.	
Storage stability of samples	The maximum storage period from sampling to analysis was 29 days within this study. Residues are regarded as stable if the samples are stored deep-frozen for up to 30 days between sampling and analysis (EU COM 7032/VI/95 and OPPTS 860.1380). Therefore, the storage stability of Mesotrione and Nicosulfuron was not verified.	
Limit of determination/ quantification	The limit of quantification (LOQ) is defined as the lowest validated level and was successfully established at 120 mg/kg of test item (10 mg/kg of mesotrione).	The limit of quantification (LOQ) is defined as the lowest validated level and was successfully established at 120 mg/kg of test item 3.76 mg/kg of nicosulfuron).

	Mesotrione	**Nicosulfuron**
	The limit of detection (LOD) was defined as 30 % of LOQ (3.0 mg/kg of mesotrione).	The limit of detection (LOD) was defined as 30 % of LOQ (1.13 mg/kg of nicosulfuron).

Conclusion

The method was found to be valid for the determination of mesotrione and nicosulfuron in larval diet samples used in ecotoxicology studies, at fortification levels of 120 mg/kg and 12000 mg/kg of the test item, in accordance to SANCO/3029/99 rev. 4 requirements. The limit of quantification (LOQ) was successfully established at 120 mg/kg of test item (10 mg/kg of mesotrione and 3.76 mg/kg of nicosulfuron).

Vergé E. and Wagner J., 2016

A 2.1.1.3.11 Analytical method - Mesotrione and nicosulfuron residues in OECD 208 spray solutions

A 2.1.1.3.11.1 Method validation

Comments of zRMS:	The HPLC-PDA method used in the study S16-02421 for determination of mesotrione and nicosulfuron is acceptable validated according to SANCO/3029/99 rev. 4.

Reference:	**2.1.1.3.11.1/01 (KCP 5.1.2/14 also filed under 10.6.2/01)**
Report	Gröning C., 2017a SAE053H/01 – Effects on the Seedling Emergence of Ten Non-Target Terrestrial Plant Species under Greenhouse Conditions Report No. S16-02421
Guideline(s):	SANCO/3029/99 rev. 4
Deviations:	No
GLP	Yes
Acceptability:	Yes

Materials and methods

An analytical method for mesotrione and nicosulfuron was validated in OECD 208 spray solutions. The method involves dilution of test medium samples with acetonitrile/water (1:1, v/v), prior to analysis by HPLC-PDA using external calibration. The limit of quantification (LOQ) of 700 mg/L test item was validated, corresponding to 21.9 mg/L of nicosulfuron and 58.4 mg/L of mesotrione.

Specimen preparation:
After sampling, the samples were stored deep-frozen (≤ -18 °C) until analysis. At the analytical laboratory, the samples were thawed and shaken well using a Vortex mixer. Recovery samples were prepared by fortification of deionised water with the test item. The samples were diluted with acetonitrile/water (1/1, v/v) prior to analysis by HPLC-PDA.

Equipment for mesotrione and nicosulfuron determination

HPLC-PDA system	Agilent Technologies 1260 Infinity coupled with Agilent DAD G4212B Detector, with Dionex Chromeleon 7.2.4.8525 / 7.2.4.8179 Software

Analytical column	Gemini-NX 3 µ C18 110A, 150 x 4.6 mm, 3 µm (Part No.: 00F-4453-E0) with 3 mm guard column (Part No.: AJ0-8368)
Column temperature	40°C
Injection volume	15 µL
Flow rate	1.0 mL/min
Mobile phase	A: Acetonitrile B: Ultra-pure water + 0.1% phosphoric acid

Time (min)	% A	% B
0.00	50	50
8.00	60	40
9.00	90	10
12.00	90	10
13.00	50	50
17.00	50	50

Retention time	Approx. 2.1 min (nicosulfuron) and 3.0 min (mesotrione)
Detection wavelength	239 nm

Results and discussions

Table A 52: Recovery results from method validation of mesotrione using the analytical method

Matrix	Analyte	Test item fortification level (mg/L) (*n* = x)	Analyte fortification level (mg/L) (*n* = x)	Recovery (%)	Mean recovery ± RSD (%)	Comments
Deionised water	mesotrione	700 (n = 5)	58.4 (n = 5)	96, 98, 98, 98, 97	97 ± 1	Acceptable
Deionised water	mesotrione	10000 (n = 5)	834 (n = 5)	100, 102, 101, 99, 101	100 ± 1	Acceptable

RSD = relative standard deviation

Mean recoveries at each fortification level were between 70 and 110% and the overall RSD was below 20%, in compliance with SANCO/3029/99 rev. 4 requirements.

Table A 53: Recovery results from method validation of nicosulfuron using the analytical method

Matrix	Analyte	Test item fortification level (mg/L) (*n* = x)	Analyte fortification level (mg/L) (*n* = x)	Recovery (%)	Mean recovery ± RSD (%)	Comments
Deionised water	nicosulfuron	700 (n = 5)	21.9 (n = 5)	96, 97, 97, 96, 95	96 ± 1	Acceptable
Deionised water	nicosulfuron	10000 (n = 5)	313 (n = 5)	100, 101, 101, 100, 102	101 ± 1	Acceptable

RSD = relative standard deviation

Mean recoveries at each fortification level were between 70 and 110% and the overall RSD was below

20%, in compliance with SANCO/3029/99 rev. 4 requirements.

In addition the following procedural recoveries were analysed.

Table A 54: Procedural Recovery results of mesotrione using the analytical method

Matrix	Analyte	Test item fortification level (mg/L) (*n* = x)	Analyte fortification level (mg/L) (*n* = x)	Recovery (%)	Mean recovery ± RSD (%)	Comments
Deionised water	mesotrione	10000 (n = 6)	834 (n = 6)	100, 102, 99, 100, 100, 101	100 ± 1	Acceptable

RSD = relative standard deviation

Table A 55: Procedural Recovery results of nicosulfuron using the analytical method

Matrix	Analyte	Test item fortification level (mg/L) (*n* = x)	Analyte fortification level (mg/L) (*n* = x)	Recovery (%)	Mean recovery ± RSD (%)	Comments
Deionised water	nicosulfuron	10000 (n = 6)	313 (n = 6)	102, 103, 101, 103, 103, 104	103 ± 1	Acceptable

RSD = relative standard deviation

Table A 56: Characteristics for the analytical method used for validation of Mesotrione and Nicosulfuron residues in test medium

	Mesotrione	Nicosulfuron
Specificity	A specific detection system was used (HPLC-PDA). The retention time of each analyte in the sample extracts matches the retention time in the calibration solution. No significant interference >30% LOQ occurred at the retention time of each analyte in any of the control samples. The analytical method can therefore be regarded as specific and selective for mesotrione and nicosulfuron	
Calibration (type, number of data points)	Dilutions for calibration were prepared in acetonitrile/water (1/1, v/v). The linearity of the detector was demonstrated by determinations of 7 calibration standards ranging from 0.5 mg/L to 20 mg/L. The detector response for analysis was linear for mesotrione and nicosulfuron , with correlation coefficients r ≥ 0.995:	
Calibration range	y= 3.6130x + 0.0582, R^2 = 0.9999	y= 3.2115x + 0.0472, R^2 = 1.0000
Assessment of matrix effects is presented	Not tested, sample solutions and calibration solutions were prepared in the sample solvent system. Solvent standards were used for analysis of test medium samples.	
Storage stability of samples	The maximum storage period from sampling to analysis was 15 days within this study. Residues are regarded as stable if the samples are stored deep-frozen for up to 30 days between sampling and analysis (EU COM 7032/VI/95 and OPPTS 860.1380). Therefore, the storage stability of Mesotrione and Nicosulfuron was not verified.	
Limit of determination/ quantification	The limit of quantification (LOQ) is defined as the lowest validated level and was successfully established at 700 mg/L test item (58.4 mg/L of	The limit of quantification (LOQ) is defined as the lowest validated level and was successfully established at 700 mg/L test item (21.9 mg/L of

	Mesotrione	Nicosulfuron
	mesotrione). The limit of detection (LOD) was defined as 30 % of LOQ (17.5 mg/L of mesotrione).	nicosulfuron). The limit of detection (LOD) was defined as 30 % of LOQ (6.57 mg/L of nicosulfuron).

Conclusion

The method was found to be valid for the determination of mesotrione and nicosulfuron in OECD 227 and 208 spray solutions used in ecotoxicology studies, at fortification levels of 700 mg/L and 10000 mg/L of the test item, in accordance to SANCO/3029/99 rev. 4 requirements. The limit of quantification (LOQ) was successfully established at 700 mg/L test item (21.9 mg/L of nicosulfuron and 58.4 mg/L of mesotrione).

Gröning C., 2017a

A 2.1.1.3.12 Analytical method - Mesotrione and nicosulfuron residues in OECD 227 spray solutions

A 2.1.1.3.12.1 Method validation

Comments of zRMS:	The HPLC-MS/MS method used in the study S16-02422 for determination of mesotrione and nicosulfuron is acceptable validated according to SANCO/3029/99 rev. 4.

Reference:	**2.1.1.3.12.1/01 (KCP 5.1.2/15 also filed under KCP 10.6.2/02)**
Report	Gröning C., 2017b SAE053H/01: Effects on the Vegetative Vigour of Ten Non-Target Terrestrial Plant Species under Greenhouse Conditions Report No. S16-02422
Guideline(s):	SANCO/3029/99 rev. 4
Deviations:	No
GLP	Yes
Acceptability:	Yes

Materials and methods

An analytical method for mesotrione and nicosulfuron was validated in OECD 227 spray solutions. The method involves dilution of the test medium samples with acetonitrile followed by further dilution with acetonitrile/water (1:1, v/v), prior to analysis by liquid chromatography coupled with a tandem mass spectrometric detection (HPLC-MS/MS) using external calibration. The limit of quantification (LOQ) of 0.005 g/L test item was validated, corresponding to 0.000417 g/L of mesotrione and 0.000157 g/L of nicosulfuron.

Specimen preparation:
After sampling, the samples were stored deep-frozen (≤ -18 °C) until analysis. At the analytical laboratory, the samples were thawed to ambient temperature and shaken well using a Vortex mixer. The whole sample volume (10 mL) was quantitatively transferred into a 50 mL volumetric flask. The original sample vessel was rinsed in several steps with 25 mL acetonitrile and the acetonitrile was transferred into the 50 mL volumetric flask. The volumetric flask was filled up to mark with ultra-pure water. This procedure

resulted in a dilution factor of 5. The samples were further diluted with acetonitrile/water (1/1, v/v) prior to analysis by HPLC-MS/MS.
Recovery samples were prepared by fortification of tap water with the test item. After fortification, the whole recovery sample volume (10 mL) was quantitatively transferred into a 50 mL volumetric flask. The original sample vessel was rinsed in several steps with 25 mL acetonitrile and the acetonitrile was transferred into the 50 mL volumetric flask. The volumetric flask was filled up to mark with ultra-pure water. This procedure resulted in a dilution factor of 5. The samples were further diluted with acetonitrile/water (1/1, v/v) prior to analysis by HPLC-MS/MS.

Equipment for mesotrione and nicosulfuron determination

LC-MS/MS system	Shimadzu LC-30 AD HPLC pump with autosampler SIL-30ACMP coupled with SCIEX API 5500 MS/MS system
Analytical column	Phenomenex Luna 5µ Phenyl-Hexyl, 150 mm x 2 mm i.d., 5 µm mean particle size (No. 00F-4257-B0) with 4 mm guard column
Column temperature	30°C
Injection volume	10 µL
Flow rate	0.500 mL/min
Mobile phase	A: Water + 0.5 % (v/v) formic acid B: Methanol + 0.5 % (v/v) formic acid

Time (min)	% A	% B
0.01	90	10
4.00	5	95
5.00	5	95
5.10	90	10
7.00	90	10

Retention time	Approx. 4.2 min (nicosulfuron) and 4.2 min (mesotrione)
Ion mode	ESI, Positive ion mode (nicosulfuron) Negative ion mode (mesotrione)
Ion mass transition monitored (m/z)	411→ 182 (quantitation) with collision energy (CE)= 27V
for nicosulfuron	411 → 213 (confirmation) with collision energy (CE)= 27V
Ion mass transition monitored (m/z)	338→ 291 (quantitation) with collision energy (CE)= -14V
for mesotrione	338→ 212 (confirmation) with collision energy (CE)= -42V

Results and discussions

Table A 57: Recovery results from method validation of mesotrione using the analytical method

Matrix	**Analyte**	**Test item fortification level (g/L) (*n* = x)**	**Analyte fortification level (g/L) (*n* = x)**	**Recovery (%)**	**Mean recovery ± RSD (%)**	**Comments**
Tap water	mesotrione	0.005 (n = 5)	0.000417 (n = 5)	85, 82, 91, 100, 110	94 ± 12	Acceptable
Tap water	mesotrione	10.0 (n = 5)	0.872* (n = 5)	86, 86, 92, 93, 94	90 ± 4	Acceptable

RSD = relative standard deviation
* Mean out of 5 recovery samples

Mean recoveries at each fortification level were between 70 and 110% and the overall RSD was below 20%, in compliance with SANCO/3029/99 rev. 4 requirements.

Table A 58: Recovery results from method validation of nicosulfuron using the analytical method

Matrix	Analyte	Test item fortification level (g/L) (*n* = x)	Analyte fortification level (g/L) (*n* = x)	Recovery (%)	Mean recovery ± RSD (%)	Comments
Tap water	nicosulfuron	0.005 (n = 5)	0.000157 (n = 5)	100, 101, 104, 109, 109	105 ± 4	Acceptable
Tap water	nicosulfuron	10.0 (n = 5)	0.327* (n = 5)	87, 87, 95, 97, 97	93 ± 6	Acceptable

RSD = relative standard deviation
* Mean out of 5 recovery samples

Mean recoveries at each fortification level were between 70 and 110% and the overall RSD was below 20%, in compliance with SANCO/3029/99 rev. 4 requirements.

In addition the following procedural recoveries were analysed.

Table A 59: Procedural Recovery results of mesotrione using the analytical method

Matrix	Analyte	Test item fortification level (g/L) (*n* = x)	Analyte fortification level (g/L) (*n* = x)	Recovery (%)	Comments
Tap water	mesotrione	0.005 (n = 1)	0.000417 (n = 1)	109	Acceptable

RSD = relative standard deviation

Table A 606: Procedural Recovery results of nicosulfuron using the analytical method

Matrix	Analyte	Test item fortification level (g/L) (*n* = x)	Analyte fortification level (g/L) (*n* = x)	Recovery (%)	Comments
Tap water	nicosulfuron	0.005 (n = 1)	0.000157 (n = 1)	108	Acceptable

RSD = relative standard deviation

Table A 61: Characteristics for the analytical method used for validation of Mesotrione and Nicosulfuron residues in test medium

	Mesotrione	**Nicosulfuron**
Specificity	A highly specific detection system was used (MS/MS). The retention time of each analyte in the sample extracts matches the retention time in the calibration solution. No significant interference >30% LOQ occurred at the retention time of each analyte in any of the control samples. The analytical method can therefore be regarded as highly specific and selective for mesotrione and nicosulfuron	
Calibration (type, number of data points) **Calibration range**	Dilutions for calibration were prepared in acetonitrile/water (1/1, v/v). The linearity of the detector was demonstrated by determinations of 8 calibration standards ranging from 2.0 ng/mL to 65 ng/mL. The detector response for analysis was of second order for mesotrione and nicosulfuron , with correlation coefficients $r \geq 0.995$:	
	$y = -155\ x^2 + 1.06e^{+005}\ x + 4.99e^{+004}$, r = 0.9997 (1/x-weighting)	$y = -7.7e^{+003}\ x^2 + 1.22e^{+006}\ x + 2.67e^{+005}$, r = 0.9998 (1/x-weighting)
Assessment of matrix effects is presented	Not tested, sample solutions and calibration solutions were prepared in the sample solvent system. Solvent standards were used for analysis of test medium samples.	
Storage stability of samples	The maximum storage period from sampling to analysis was 28 days within this study. Residues are regarded as stable if the samples are stored deep-frozen for up to 30 days between sampling and analysis (EU COM 7032/VI/95 and OPPTS 860.1380). Therefore, the storage stability of Mesotrione and Nicosulfuron was not verified.	
Limit of determination/ quantification	The limit of quantification (LOQ) is defined as the lowest validated level and was successfully established at 0.005 g/L test item (0.000417 g/L of mesotrione). The limit of detection (LOD) was defined as 30 % of LOQ (0.000125 g/L of mesotrione).	The limit of quantification (LOQ) is defined as the lowest validated level and was successfully established at 0.005 g/L test item (0.000157 g/L of nicosulfuron). The limit of detection (LOD) was defined as 30 % of LOQ (0.0000471 g/L of nicosulfuron).

Conclusion

The method was found to be valid for the determination of mesotrione and nicosulfuron in OECD 227 spray solutions used in ecotoxicology studies, at fortification levels of 0.005 g/L and 10.0 g/L of the test item, in accordance to SANCO/3029/99 rev. 4 requirements. The limit of quantification (LOQ) was successfully established at 0.005 g/L test item (0.000417 g/L of mesotrione and 0.000157 g/L of nicosulfuron)

Gröning C., 2017b

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

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

No new or additional studies have been submitted.

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

A 2.1.2.2.1 Analytical method 1

An analytical method for the determination of the active substance mesotrione and its metabolites MNBA and AMBA in different animal matrices has been validated in milk, bovine meat, liver, fat and poultry egg by Schernikau N. and Colorado C.S. (2017) (see KCP 5.2/01). The method was independently validated by Lesot C.(2017) (KCP 5.2/02).

A 2.1.2.2.1.1 Method validation

Comments of zRMS:	The LC-MS/MS method used in the study S17-04087 for determination of mesotrione and and its metabolites MNBA and AMBA in animal matrices (milk, bovine meat, liver, fat and poultry egg) is acceptable validated according to SANCO/825/00 rev. 8.1. Two mass transitions (one for quantification and one for confirmation) for each analyte were evaluated in order to demonstrate that the method achieves a high level of selectivity. The LOQ for each analyte was successfully established at 0.01 mg/kg in animal matrices (milk, meat, egg, fat and liver) for the both mass transitions.The linearity of the detector response was demonstrated by single determination of matrix-matched and solvent calibration standards at five concentration levels ranging from 0.1 ng/mL to 20 ng/mL. This range corresponds to 0.002 mg/kg to 0.4 mg/kg of each analyte. All mean recovery values at fortification levels of 0.01 mg/kg and 0.1 mg/kg for both mass transitions for all compounds and in all analysed matrices comply with the standard acceptance criteria of the guidance document SANCO/825/00, rev. 8, recovery within 70-120% and RDS < 20%. Mesotrione and its metabolites MNBA and AMBA were found to be stable in final extracts of animal matrices (meat, egg, milk and liver) for at least 7 days when stored refrigerated (1 °C to 10 °C) in the dark. AMBA was found to be stable in final extracts of bovine fat for at least 9 days when stored refrigerated (1 °C to 10 °C) in the dark. Mesotrione and MNBA were found to be unstable in final extracts of bovine fat for 9 days when stored refrigerated (1 °C to 10 °C) in the dark. Samples of fat should be analysed as soon as possible (within 2 days).

Reference:	**2.1.2.2.1.1/01 (KCP 5.2/01)**
Report	Schernikau N. and Colorado C.S. (2017) Validation of an Analytical Method for the Determination of Mesotrione

	and its Metabolites MNBA and AMBA in Animal Matrices Report No. S17-04087 (JSC-1702V)
Guideline(s):	SANCO/825/00 rev. 8.1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

A residue analytical method for the determination of mesotrione and its metabolites MNBA and AMBA was validated in animal matrices (milk, bovine meat, liver, fat and poultry egg). The method involves extraction of mesotrione, MNBA and AMBA once with mixture of acetonitrile/water (2+1, v+v) and once with mixture of 0.05 M ammonium hydroxide/acetone (1+1, v+v), followed by cellulose filtration (all matrices) and SPE clean-up (all matrices except fat), prior to analysis by liquid chromatography separation coupled with tandem mass spectrometric detection (LC-MS/MS). The limit of quantification (LOQ) was set to 0.01 mg/kg.

Specimen preparation for Bovine Milk

Each 5.0 ± 0.05 g homogenized specimen of milk was weighed into a 50-mL Sarstedt centrifuge tube. 20 mL acetonitrile / water (2+1, v+v) was added and shaken well for 2 minutes by hand. Exactly 20 mL of mixture of 0.05 M ammonium hydroxide/acetone (1/1, v/v) was added, shaken vigorously by hand for at least 2 min and filled-up to 50 mL with 0.05 M ammonium hydroxide/acetone (1/1, v/v). The extract was centrifuged for 5 min at about 4000 rpm and the supernatant was cleaned up by filtration (cellulose) and diluted with 10 mL of water/formic acid (98/2, v/v) by a factor of 10. The pH value of the extracts was checked (should be less than 4).

Specimen preparation for Bovine Liver, Meat and Fat and Poultry Egg

Each 5.0 ± 0.05 g homogenized specimen of liver, meat, fat or egg was weighed into a 50-mL Sarstedt centrifuge tube. Exactly 20 mL of mixture of acetonitrile/water (2/1, v/v) was added, shaken vigorously by hand for at least 2 min and centrifuged for 5 min at about 3500 rpm. The supernatant was decanted into another tube. Exactly 20 mL of mixture of 0.05 M ammonium hydroxide/acetone (1/1, v/v) was added to the remaining pellet, shaken vigorously by hand for at least 2 min and centrifuged for 5 min at about 3500 rpm. Next, the supernatants were combined and filled-up to 50 mL with 0.05 M ammonium hydroxide/acetone (1/1, v/v). (For bovine fat: extract was filtered through a funnel filled with cotton wool.). The extract was centrifuged for 5 min at about 4000 rpm and the supernatant was cleaned-up by filtration (cellulose). Except for fat, the extract was diluted with 10 mL of water/formic acid (98/2, v/v) by a factor of 10 and the pH value of the extracts was checked (should be less than 4). For bovine fat, the extract was diluted with 0.1% acetic acid by a factor of 2.

Extract Clean-up and Reconstitution for Analysis for Bovine Milk, Meat and Liver and Poultry Egg

The sample extract was cleaned up using an Oasis HLB cartridge (60 mg, 3cc). The cartridge was conditioned with 2 mL of methanol followed by 2 mL of water. The whole diluted sample extract (ca. 11 mL) was transferred through the cartridge. The sample jar was rinsed twice with 2 mL of methanol/water (5/95, v/v). Each rinsing portion was transferred though the cartridge, discarding the eluates. The cartridge was soaked to dryness by using a vacuum for 10 minutes. The analytes were eluted with 4 mL of methanol followed by 2 mL of acetonitrile and collected in a 10-mL test tube. The sample extract was evaporated to dryness using a gentle stream of nitrogen and a water bath set at approx. 40 °C. The residue was taken up and solved in 2.0 mL of water containing 0.1 % acetic acid using an ultrasonic bath prior to analysis by LC-MS/MS.

Equipment for mesotrione, AMBA and MNBA determination

HPLC-MS/MS system	1200 Binary Rapid Resolution LC System, Agilent Technologies (HPLC, ≤ 600 bar) coupled with API 4000 System, SCIEX (Triple quadrupole mass spectrometer)

Column	ZORBAX Eclipse XDB-C18, 50 mm x 4.6 mm, 1.8 µm, Agilent, Art. No. 927975-902
Column oven temperature	45°C
Injection Volume	30 µL
Mobile phase	Eluent A: Acetonitrile Eluent B: Water containing 0.2 % (v/v) formic acid

Gradient

Time [min]	% Eluent A	% Eluent B	Flow [µL/min]
0.0	0	100	800
4.00	95	5	800
5.00	95	5	800
5.10	0	100	800
6.50	0	100	800

Retention time(s)	Approx. 2.0 min (MNBA); approx. 2.5 min (AMBA); approx. 3.4 min (Mesotrione)
Ionisation type	Electrospray ionization (ESI, TurboIon Spray)
Polarity	Negative ion mode
Scan type	MS/MS, Multiple Reaction Monitoring (MRM)
Ion mass transition monitored *(m/z)*for Mesotrione	338 → 291 (Quantification) with collision energy (CE)= -14V 338 → 212 (Confirmation) with collision energy (CE)= -44V
Ion mass transition monitored *(m/z)*for MNBA	244 → 200 (Quantification) with collision energy (CE)= -12V 244 → 142 (Confirmation) with collision energy (CE)= -30V
Ion mass transition monitored *(m/z)*for AMBA	214 → 155 (Quantification) with collision energy (CE)= -28V 214 → 91 (Confirmation) with collision energy (CE)= -40V

Results and discussions

Table A 62: Accuracy and precision data of mesotrione in matrices of animal origin

Matrix	Fortification level (mg/kg)	Recovery (%)	Mean recovery (%)	RSD (%)	Replicates	Overall Mean Recovery (%)	Overall RSD (%)
Ion Mass Transition 338 → 291 *m/z* (Quantification)							
Milk	0.01	103, 105, 104, 94, 117	105	7.8	5	101	6.6
	0.10	99, 94, 100, 98, 98	98	2.3	5		
Bovine meat	0.01	81, 79, 92, 85, 79	83	6.6	5	84	4.9
	0.10	83, 83, 81, 88, 84	84	3.1	5		
Poultry egg	0.01	81, 80, 87, 81, 84	83	3.5	5	81	5.2
	0.10	84, 73, 79, 82, 75	79	5.9	5		
Bovine fat	0.01	80, 91, 90, 76, 86	85	7.6	5	85	6.4
	0.10	88, 77, 88, 83, 88	85	5.7	5		
Bovine	0.01	80, 78, 81, 78, 82	80	2.2	5	82	4.1

Matrix	Fortification level (mg/kg)	Recovery (%)	Mean recovery (%)	RSD (%)	Replicates	Overall Mean Recovery (%)	Overall RSD (%)
liver	0.10	78, 88, 82, 82, 86	83	4.7	5		
Ion Mass Transition 338 → 212 m/z (Confirmation)							
Milk	0.01	102, 88, 100, 98, 101	98	5.8	5	98	5.7
	0.10	103, 104, 90, 93, 96	97	6.3	5		
Bovine meat	0.01	72, 67, 90, 70, 75	75	12	5	78	10
	0.10	81, 88, 73, 86, 81	82	7.1	5		
Poultry egg	0.01	92, 78, 90, 81, 76	83	8.6	5	82	7.5
	0.10	87, 73, 83, 84, 78	81	6.8	5		
Bovine fat	0.01	74, 87, 81, 77, 71	78	8.0	5	81	8.2
	0.10	84, 74, 87, 83, 91	84	7.5	5		
Bovine liver	0.01	82, 68, 85, 83, 70	78	10	5	83	10
	0.10	79, 93, 90, 83, 92	87	7.0	5		

RSD = relative standard deviation

Table A 63: Accuracy and precision data of MNBA in matrices of animal origin

Matrix	Fortification level (mg/kg)	Recovery (%)	Mean recovery (%)	RSD (%)	Replicates	Overall Mean Recovery (%)	Overall RSD (%)
Ion Mass Transition 244 → 200 *m/z* (Quantification)							
Milk	0.01	72, 86, 86, 82, 71	79	9.3	5	77	7.4
	0.10	74, 79, 74, 74, 73	75	3.2	5		
Bovine meat	0.01	107, 95, 85, 82, 92	92	11	5	91	7.8
	0.10	86, 89, 90, 96, 88	90	4.2	5		
Poultry egg	0.01	67, 78, 91, 82, 86	81	11	5	83	8.1
	0.10	88, 82, 85, 87, 82	85	3.3	5		
Bovine fat	0.01	76, 87, 87, 79, 86	83	6.2	5	83	8.2
	0.10	83, 74, 96, 76, 87	83	11	5		
Bovine liver	0.01	83, 89, 81, 90, 79	84	5.8	5	82	6.2
	0.10	75, 80, 87, 77, 79	80	5.7	5		
Ion Mass Transition 244 → 142 m/z (Confirmation)							

Matrix	Fortification level (mg/kg)	Recovery (%)	Mean recovery (%)	RSD (%)	Replicates	Overall Mean Recovery (%)	Overall RSD (%)
Milk	0.01	72, 64, 77, 70, 75	72	7.0	5	74	6.3
	0.10	75, 81, 77, 74, 72	76	4.5	5		
Bovine meat	0.01	80, 88, 74, 93, 83	84	8.7	5	89	8.7
	0.10	92, 94, 90, 99, 96	94	3.7	5		
Poultry egg	0.01	61, 79, 77, 89, 82	78	13	5	81	9.9
	0.10	87, 77, 86, 84, 83	83	4.7	5		
Bovine fat	0.01	78, 81, 77, 79, 91	81	7.0	5	85	7.5
	0.10	92, 85, 95, 86, 89	89	4.7	5		
Bovine liver	0.01	78, 93, 82, 74, 87	83	9.0	5	82	8.1
	0.10	76, 78, 91, 76, 81	80	7.8	5		

RSD = relative standard deviation

Table A 64: Accuracy and precision data of AMBA in matrices of animal origin

Matrix	Fortification level (mg/kg)	Recovery (%)	Mean recovery (%)	RSD (%)	Replicates	Overall Mean Recovery (%)	Overall RSD (%)
Ion Mass Transition 214 → 155 *m/z* (Quantification)							
Milk	0.01	93, 104, 93, 89, 100	96	6.3	5	96	4.5
	0.10	100, 95, 94, 95, 95	96	2.5	5		
Bovine meat	0.01	95, 100, 110, 105, 104	103	5.5	5	100	5.6
	0.10	97, 92, 99, 104, 95	97	4.6	5		
Poultry egg	0.01	96, 87, 98, 95, 77	91	9.6	5	94	8.4
	0.10	103, 87, 101, 98, 93	96	6.7	5		
Bovine fat	0.01	87, 103, 97, 92, 88	93	7.1	5	93	6.3
	0.10	95, 82, 95, 94, 92	92	6.0	5		
Bovine liver	0.01	84, 98, 87, 81, 72	84	11	5	84	7.8
	0.10	80, 84, 86, 85, 81	83	3.1	5		
Ion Mass Transition 214 → 91 m/z (Confirmation)							
Milk	0.01	99, 95, 103, 78, 95	94	10.1	5	94	7.2
	0.10	96, 94, 89, 96, 91	93	3.3	5		
Bovine	0.01	101, 94, 81, 98, 99	95	8.5	5	93	6.5

Matrix	Fortification level (mg/kg)	Recovery (%)	Mean recovery (%)	RSD (%)	Replicates	Overall Mean Recovery (%)	Overall RSD (%)
meat	0.10	88, 89, 92, 95, 89	91	3.2	5		
Poultry egg	0.01	95, 97, 85, 93, 81	90	7.6	5	93	8.2
	0.10	103, 84, 102, 98, 91	96	8.4	5		
Bovine fat	0.01	96, 97, 95, 97, 97	96	0.9	5	92	6.4
	0.10	88, 81, 87, 85, 94	87	5.5	5		
Bovine liver	0.01	88, 97, 80, 86, 83	87	7.4	5	86	6.3
	0.10	79, 89, 89, 85, 81	85	5.4	5		

RSD = relative standard deviation

All mean recovery values at fortification levels of 0.01 mg/kg and 0.1 mg/kg (for all matrices and analytes) for two (2) mass transitions comply with the standard acceptance criteria of the guidance documents.

Table A 65: Characteristics for the analytical method used for validation of mesotrione, MNBA and AMBA residues in matrices of animal origin

	Mesotrione, MNBA and AMBA
Specificity	Quantification was performed by use of LC-MS/MS detection. Two (2) mass transitions were evaluated in order to demonstrate that the method achieves a high level of specificity. The retention time of mesotrione and its metabolites MNBA and AMBA in the sample extracts matches the retention time in the calibration solutions. No interferences above 30 % of the LOQ at the retention time of the analytes were detected in the untreated control samples. Conclusively the method is specific for the determination of mesotrione and its metabolites MNBA and AMBA in animal matrices (milk, bovine meat, liver, fat and poultry egg).
Calibration (type, number of data points) **Calibration range**	Linearity of the detector response was demonstrated by single determination of minimum six solvent or matrix matched calibration standards ranging from 0.1 ng/mL to 20 ng/mL. This range corresponds to 0.002 mg/kg to 0.4 mg/kg and thus covers the range from no more than 30 % of the LOQ and at least + 20 % of the highest analyte concentration level detected in a sample extract. The calibrations were found linear with correlation coefficients r ≥0.99 (1/x-weighting): Mesotrione in bovine milk, meat: 338 → 291 *m/z*: Y = 30096.1626x -337.4107, r = 0.9994 338 → 212 *m/z*: Y = 6592.0994x + 84.1375, r = 0.9995 MNBA in bovine milk, meat: 244 → 200 *m/z*: Y = 13872.0475x + 148.4236, r = 0.9998 244 → 142 *m/z*: Y = 5693.3455x + 180.9806, r = 0.9994 AMPA in bovine milk, meat: 214 → 155 *m/z*: Y = 6553.8096x – 49.5789, r = 0.9993 214 → 91 *m/z*: Y = 6482.4738x – 51.0698, r = 0.9994 Mesotrione in poultry egg: 338 → 291 *m/z*: Y = 37935.3854x – 1093.6966, r = 0.9997 338 → 212 *m/z*: Y = 8553.7889x – 279.8933, r = 0.9996

	Mesotrione, MNBA and AMBA
	MNBA in poultry egg: 244 → 200 *m/z*: Y = 19184.3413x – 405.0823, r = 0.9994 244 → 142 *m/z*: Y = 8085.0359x + 176.5161, r = 0.9997 AMPA in poultry egg: 214 → 155 *m/z*: Y = 6325.7452x + 137.8905, r = 0.9995 214 → 91 *m/z*: Y = 6241.5937x + 107.4978, r = 0.9996 Mesotrione in bovine fat: 338 → 291 *m/z*: Y = 30832.3181x – 258.2113, r = 0.9993 338 → 212 *m/z*: Y = 6876.6104x + 52.9767, r = 0.9989 MNBA in bovine fat: 244 → 200 *m/z*: Y = 6085.3364x + 22.5737, r = 0.9974 244 → 142 *m/z*: Y = 2514.0442x + 93.9849, r = 0.9960 AMPA in bovine fat: 214 → 155 *m/z*: Y = 6628.8555x – 37.6887, r = 0.9999 214 → 91 *m/z*: Y = 6514.4451x – 133.6546, r = 0.9993 Mesotrione in bovine liver: 338 → 291 *m/z*: Y = 22885.9084x + 194.8477, r = 0.9996 338 → 212 *m/z*: Y = 4991.3643x + 28.1913, r = 0.9990 MNBA in bovine liver: 244 → 200 *m/z*: Y = 18291.0824x – 426.5398, r = 0.9991 244 → 142 *m/z*: Y = 7660.3976x – 172.7642, r = 0.9997 AMPA in bovine liver: 214 → 155 *m/z*: Y = 7554.7336x + 11.3260, r = 0.9998 214 → 91 *m/z*: Y = 7272.4202x + 67.3689, r = 0.9989
Assessment of matrix effects is presented	Matrix effects were < ± 20 % and deemed to be insignificant for detection of mesotrione in bovine milk, bovine meat and poultry egg, MNBA in bovine liver, bovine milk, bovine meat and poultry egg and AMBA in bovine liver, bovine milk and bovine meat. Therefore, solvent standards were used for quantification throughout the study. Matrix effects were ≥ ± 20 % and deemed to be significant for detection of AMBA in extracts of poultry egg, MNBA in bovine fat and mesotrione in bovine liver. Therefore, matrix-matched standards were used for quantification throughout the study. Matrix effects were < ± 20 % and deemed to be insignificant for detection of AMBA and mesotrione in extracts of bovine fat. However, at stability measurement matrix effects were observed. Therefore, solvent standards were used for quantification of validation samples and matrix-matched standards were used for stability measurements.
Limit of determination / quantification	The limit of quantification (LOQ) is defined as the lowest fortification level with mean recoveries ranging from 70 % to 110 % at a relative standard deviation (RSD) of ≤ 20%. These criteria were fulfilled for mesotrione, AMBA and MNBA in animal matrices (milk, bovine meat, liver, fat and poultry egg) with an LOQ of 0.01 mg/kg for two (2) mass transitions. The limit of detection (LOD) was defined in this study as 20% of the LOQ.
Extract Stability	Mesotrione, AMBA and MNBA were found to be stable in final extracts of meat, egg, milk and liver when stored at 1 °C to 10 °C for 7 (egg), 9 (liver) or 10 (milk/meat) days in the dark, except for AMBA in meat. AMBA was found to be unstable in final extracts of meat for 10 days when stored refrigerated (1 °C to 10 °C) in the dark. Mesotrione, AMBA and MNBA were found to be unstable in final extracts of

	Mesotrione, MNBA and AMBA
	bovine fat for 9 days when stored refrigerated (1 °C to 10 °C) in the dark. Samples of fat and meat should be analysed as soon as possible (within 2 days).

Conclusion

The method was found to be valid for the determination of mesotrione, AMBA and MNBA in animal matrices (milk, bovine meat, liver, fat and poultry egg) with a limit of quantification (LOQ) of 0.01 mg/kg in accordance to SANCO/825/00 rev. 8.1 requirements.

Schernikau N. and Colorado C.S. (2017)

A 2.1.2.2.1.2 Independent laboratory validation

Comments of zRMS:	The objective of the study was to independently validate an analytical method for the determination of mesotrione and its metabolites MNBA and AMBA in products of animal origin (milk, eggs, meat, fat and liver). Final sample extracts were analysed by LC-MS/MS. Two mass transitions (one for quantification and one for confirmation) for each analyte were evaluated. The LOQ for each analyte was successfully established at 0.01 mg/kg in all animal for the both mass transitions.The linearity of the detector response was demonstrated by single determination of matrix-matched and solvent calibration standards at 7 concentration levels ranging from 0.1 ng/mL to 20 ng/mL. This range corresponds to 0.002 mg/kg to 0.4 mg/kg of each analyte. All mean recovery values at fortification levels of 0.01 mg/kg and 0.1 mg/kg for both mass transitions for all compounds and in all analysed matrices comply with the standard acceptance criteria of the guidance document SANCO/825/00, rev. 8, recovery within 70-120% and RDS < 20%. The study is accepted.

Reference:	**2.1.2.2.1.2/01 (KCP 5.2/02)**
Report	Lesot C.(2017) Independent Laboratory Validation of a Method for the Determination of Residues of Mesotrione and its Metabolites MNBA and AMBA in Animal Matrices Report No. S17-04125
Guideline(s):	SANCO/825/00 rev. 8.1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The residue analytical method for mesotrione and its metabolites MNBA and AMBA in animal matrices validated by Schernikau N. and Colorado C.S. (2017), report No. S17-04087, was independently validated by Lesot C. (2017) in animal matrices (milk, bovine meat, liver, fat and poultry egg). The method involves extraction of mesotrione, MNBA and AMBA once with mixture of acetonitrile/water (2+1, v+v) and once with mixture of 0.05 M ammonium hydroxide/acetone (1+1, v+v), followed by cellulose filtration (all matrices) and SPE clean-up (all matrices except fat), prior to analysis by liquid chromatography separation coupled with tandem mass spectrometric detection (LC-MS/MS). The limit of quantification (LOQ) was set to 0.01 mg/kg.

Specimen preparation for Bovine Milk

Each 5.0 ± 0.05 g homogenized specimen of milk was weighed into a 50-mL Sarstedt centrifuge tube. 20 mL acetonitrile / water (2+1, v+v) was added and shaken well for 2 minutes by hand. Exactly 20 mL of mixture of 0.05 M ammonium hydroxide/acetone (1/1, v/v) was added, shaken vigorously by hand for at least 2 min and filled-up to 50 mL with 0.05 M ammonium hydroxide/acetone (1/1, v/v). The extract was centrifuged for 5 min at about 4000 rpm and the supernatant was cleaned up by filtration (cellulose) and diluted with 10 mL of water/formic acid (98/2, v/v) by a factor of 10. The pH value of the extracts was checked (should be less then 4).

Specimen preparation for Bovine Liver, Meat and Fat and Poultry Egg

Each 5.0 ± 0.05 g homogenized specimen of liver, meat, fat or egg was weighed into a 50-mL Sarstedt centrifuge tube. Exactly 20 mL of mixture of acetonitrile/water (2/1, v/v) was added, shaken vigorously by hand for at least 2 min and centrifuged for 5 min at about 3500 rpm. The supernatant was decanted into another tube. Exactly 20 mL of mixture of 0.05 M ammonium hydroxide/acetone (1/1, v/v) was added to the remaining pellet, shaken vigorously by hand for at least 2 min and centrifuged for 5 min at about 3500 rpm. Next, the supernatants were combined and filled-up to 50 mL with 0.05 M ammonium hydroxide/acetone (1/1, v/v). (For bovine fat: extract was filtered through a funnel filled with cotton wool.). The extract was centrifuged for 5 min at about 4000 rpm and the supernatant was cleaned-up by filtration (cellulose). Except for fat, the extract was diluted with 10 mL of water/formic acid (98/2, v/v) by a factor of 10 and the pH value of the extracts was checked (should be less than 4). For bovine fat, the extract was diluted with 0.1% acetic acid by a factor of 2.

Extract Clean-up and Reconstitution for Analysis for Bovine Milk, Meat and Liver and Poultry Egg

The sample extract was cleaned up using an Oasis HLB cartridge (60 mg, 3cc). The cartridge was conditioned with 2 mL of methanol followed by 2 mL of water. The whole diluted sample extract (ca. 11 mL) was transferred through the cartridge. The sample jar was rinsed twice with 2 mL of methanol/water (5/95, v/v). Each rinsing portion was transferred though the cartridge, discarding the eluates. The cartridge was soaked to dryness by using a vacuum for 10 minutes. The analytes were eluted with 4 mL of methanol followed by 2 mL of acetonitrile and collected in a 10-mL test tube. The sample extract was evaporated to dryness using a gentle stream of nitrogen and a water bath set at approx. 40 °C. The residue was taken up and solved in 2.0 mL of water containing 0.1 % acetic acid using an ultrasonic bath prior to analysis by LC-MS/MS.

Equipment for mesotrione, AMBA and MNBA determination

HPLC-MS/MS system	Shimadzu LC20ADXR + SIL20ACXR LC Pump + Autosampler and Shimadzu CTO-20AC Oven, coupled with API 5500 System, SCIEX (Triple quadrupole mass spectrometer)
Column	ZORBAX Eclipse XDB-C18, 50 mm x 4.6 mm, 1.8 µm
Column oven temperature	45°C
Injection Volume	10 or 20 µL
Mobile phase	Eluent A: Acetonitrile Eluent B: Water containing 0.2 % (v/v) formic acid

Gradient

Time [min]	% Eluent A	% Eluent B	Flow [µL/min]
0.0	0	100	800
4.00	95	5	800
5.00	95	5	800
5.10	0	100	800
6.50	0	100	800

Retention time(s)	Approx. 2.0 min (MNBA); approx. 2.5 min (AMBA); approx. 3.4 min (Mesotrione)
Ionisation type	Electrospray ionization (ESI, TurboIon Spray)
Polarity	Negative ion mode

Scan type	MS/MS, Multiple Reaction Monitoring (MRM)
Ion mass transition monitored *(m/z)*for Mesotrione	338 → 291 (Quantification) with collision energy (CE)= -14V 338 → 212 (Confirmation) with collision energy (CE)= -44V
Ion mass transition monitored *(m/z)*for MNBA	244 → 200 (Quantification) with collision energy (CE)= -12V 244 → 142 (Confirmation) with collision energy (CE)= -30V
Ion mass transition monitored *(m/z)*for AMBA	214 → 155 (Quantification) with collision energy (CE)= -28V 214 → 91 (Confirmation) with collision energy (CE)= -40V

Results and discussions

Table A 66: Accuracy and precision data of mesotrione in matrices of animal origin

Matrix	Fortification level (mg/kg)	Recovery (%)	Mean recovery (%)	RSD (%)	Replicates	Overall Mean Recovery (%)	Overall RSD (%)
Ion Mass Transition 338 → 291 *m/z* (Quantification)							
Milk	0.01	97, 88, 94, 88, 96	93	5	5	93	4
	0.10	97, 93, 92, 95, 88	93	4	5		
Bovine meat	0.01	99, 93, 90, 95, 93	94	4	5	95	4
	0.10	91, 103, 93, 93, 98	96	5	5		
Bovine fat	0.01	104, 107, 105, 108, 102	105	2	5	108	4
	0.10	113, 108, 114, 112, 111	112	2	5		
Bovine liver	0.01	87, 95, 103, 90, 92	93	7	5	90	7
	0.10	82, 81, 88, 85, 92	86	5	5		
Poultry egg	0.01	100, 92, 88, 99, 103	96	6	5	97	5
	0.10	102, 98, 97, 98, 97	98	2	5		
Ion Mass Transition 338 → 212 m/z (Confirmation)							
Milk	0.01	95, 95, 96, 94, 94	95	1	5	93	3
	0.10	97, 90, 90, 91, 92	92	3	5		
Bovine meat	0.01	101, 96, 95, 92, 94	96	4	5	95	3
	0.10	95, 90, 91, 96, 98	94	4	5		
Bovine fat	0.01	109, 100, 101, 101, 102	103	4	5	104	4
	0.10	102, 106, 99, 112, 110	106	5	5		
Bovine liver	0.01	87, 91, 107, 86, 94	93	9	5	91	9
	0.10	76, 90, 92, 94, 90	88	8	5		
Poultry egg	0.01	107, 94, 85, 95, 94	95	8	5	96	6

Matrix	Fortification level (mg/kg)	Recovery (%)	Mean recovery (%)	RSD (%)	Replicates	Overall Mean Recovery (%)	Overall RSD (%)
	0.10	98, 95, 95, 94, 98	96	2	5		

RSD = relative standard deviation

Table A 67: Accuracy and precision data of MNBA in matrices of animal origin

Matrix	Fortification level (mg/kg)	Recovery (%)	Mean recovery (%)	RSD (%)	Replicates	Overall Mean Recovery (%)	Overall RSD (%)
Ion Mass Transition 244 → 200 *m/z* (Quantification)							
Milk	0.01	68, 74, 69, 64, 79	71	8	5	71	6
	0.10	71, 75, 72, 68, 69	71	4	5		
Bovine meat	0.01	90, 77, 77, 67, 79	78	10	5	76	15
	0.10	64, 67, 63, 85, 95	75	19	5		
Bovine fat	0.01	86, 69, 95, 95	86	14	4*	86	11
	0.10	89, 76, 93, 94, 81	87	9	5		
Bovine liver	0.01	68, 79, 81, 70, 79	75	8	5	73	10
	0.10	77, 74, 59, 80, 66	71	12			
Poultry egg	0.01	90, 104, 94, 101, 78	93	11	5	82	20
	0.10	87, 83, 54, 65, 63	70	20	5		
Ion Mass Transition 244 → 142 m/z (Confirmation)							
Milk	0.01	70, 66, 76, 74, 76	72	6	5	72	6
	0.10	69, 70, 78, 68, 69	71	6	5		
Bovine meat	0.01	79, 74, 71, 76, 67	73	6	5	76	14
	0.10	65, 73, 64, 86, 100	78	20	5		
Bovine fat	0.01	76, 78, 97, 78	82	12	4*	84	10
	0.10	88, 73, 88, 95, 85	86	9	5		
Bovine liver	0.01	74, 68, 59, 82, 74	71	12	5	71	11
	0.10	75, 73, 60, 80, 66	71	11	5		
Poultry egg	0.01	81, 109, 79, 98, 103	94	14	5	86	16
	0.10	80, 81, 70, 75	77	7	4*		

RSD = relative standard deviation
* Outlier excluded by a Dixon test

Table A 68: Accuracy and precision data of AMBA in matrices of animal origin

Matrix	Fortification level (mg/kg)	Recovery (%)	Mean recovery (%)	RSD (%)	Replicates	Overall Mean Recovery (%)	Overall RSD (%)
Ion Mass Transition 214 → 155 *m/z* (Quantification)							
Milk	0.01	92, 91, 96, 81, 83	89	7	5	89	8
	0.10	96, 95, 92, 90, 76	90	9	5		
Bovine meat	0.01	90, 81, 90, 90, 87	88	4	5	88	4
	0.10	90, 82, 87, 87, 91	87	4	5		
Bovine fat	0.01	111, 126, 109, 117, 112	115	6	5	111	6
	0.10	99, 105, 109, 109, 113	107	5	5		
Bovine liver	0.01	89, 84, 82, 100, 83	88	8	5	90	7
	0.10	92, 89, 88, 101, 87	91	6	5		
Poultry egg	0.01	103, 86, 88, 91, 98	93	8	5	96	6
	0.10	103, 95, 98, 102, 96	99	4	5		
Ion Mass Transition 214 → 91 m/z (Confirmation)							
Milk	0.01	94, 102, 97, 92, 88	95	6	5	93	5
	0.10	95, 94, 93, 92, 85	92	4	5		
Bovine meat	0.01	87, 79, 79, 94, 82	84	8	5	87	7
	0.10	83, 91, 90, 92, 94	90	5	5		
Bovine fat	0.01	116, 125, 117, 119, 111	118	4	5	115	5
	0.10	114, 106, 106, 116, 116	112	5	5		
Bovine liver	0.01	85, 98, 92, 97, 91	93	6	5	91	6
	0.10	94, 84, 86, 97, 85	89	7	5		
Poultry egg	0.01	105, 94, 86, 98, 103	97	8	5	99	8
	0.10	102, 93, 112, 106, 94	101	8	5		

RSD = relative standard deviation

All mean recovery values at fortification levels of 0.01 mg/kg and 0.1 mg/kg (for all matrices and analytes) for two (2) mass transitions comply with the standard acceptance criteria of the guidance documents.

Table A 69: Characteristics for the ILV method used for validation of mesotrione, MNBA and AMBA residues in matrices of animal origin

	Mesotrione, MNBA and AMBA
Specificity	Quantification was performed by use of LC-MS/MS detection. Two (2) mass transitions were evaluated in order to demonstrate that the method achieves a

	Mesotrione, MNBA and AMBA
	high level of specificity. The retention time of mesotrione and its metabolites MNBA and AMBA in the sample extracts matches the retention time in the calibration solutions. No interferences above 30 % of the LOQ at the retention time of the analytes were detected in the untreated control samples. Conclusively the method is specific for the determination of mesotrione and its metabolites MNBA and AMBA in animal matrices (milk, bovine meat, liver, fat and poultry egg).
Calibration (type, number of data points) **Calibration range**	Linearity of the detector response was demonstrated by single determination of seven matrix matched calibration standards ranging from 0.1 ng/mL to 20 ng/mL. This range corresponds to 0.002 mg/kg to 0.4 mg/kg and thus covers the range from no more than 30 % of the LOQ and at least + 20 % of the highest analyte concentration level detected in a sample extract. The calibrations were found linear with correlation coefficients r ≥0.99 (1/x-weighting): Mesotrione in bovine meat: 338 → 291 *m/z*: Y = 127879x + 2526, r = 0.9997 338 → 212 *m/z*: Y = 25807x + 194, r = 0.9997 MNBA in bovine meat: 244 → 200 *m/z*: Y = 23448x + 587, r = 0.9982 244 → 142 *m/z*: Y = 12342x + 2082, r = 0.9900 AMPA in bovine meat: 214 → 155 *m/z*: Y = 21328x - 184, r = 0.9988 214 → 91 *m/z*: Y = 15622x + 288, r = 0.9992 Mesotrione in bovine milk: 338 → 291 *m/z*: Y = 99788x + 695, r = 0.9998 338 → 212 *m/z*: Y = 19633x + 0.2, r = 0.9993 MNBA in bovine milk: 244 → 200 *m/z*: Y = 30462x - 307, r = 0.9969 244 → 142 *m/z*: Y = 16295x + 151, r = 0.9991 AMPA in bovine milk: 214 → 155 *m/z*: Y = 17385x + 437, r = 0.9996 214 → 91 *m/z*: Y = 13650x + 113, r = 0.9992 Mesotrione in poultry egg: 338 → 291 *m/z*: Y = 106813x - 636, r = 0.9991 338 → 212 *m/z*: Y = 21469x - 272, r = 0.9988 MNBA in poultry egg: 244 → 200 *m/z*: Y = 32200x - 818, r = 0.9989 244 → 142 *m/z*: Y = 18154x - 346, r = 0.9967 AMPA in poultry egg: 214 → 155 *m/z*: Y = 17412x - 75, r = 0.99985 214 → 91 *m/z*: Y = 13176x + 60, r = 0.9999 Mesotrione in bovine fat: 338 → 291 *m/z*: Y = 227200x - 28, r = 0.9998 338 → 212 *m/z*: Y = 45890x - 498, r = 0.9999 MNBA in bovine fat: 244 → 200 *m/z*: Y = 40980x - 2655, r = 0.9991 244 → 142 *m/z*: Y = 21859x + 2836, r = 0.9966 AMPA in bovine fat:

	Mesotrione, MNBA and AMBA
	214 → 155 *m/z*: Y = 48314x - 58, r = 0.9990 214 → 91 *m/z*: Y = 37911x - 746, r = 0.9983 Mesotrione in bovine liver: 338 → 291 *m/z*: Y = 56646x - 841, r = 0.9998 338 → 212 *m/z*: Y = 10924x – 303, r = 0.9994 MNBA in bovine liver: 244 → 200 *m/z*: Y = 33616x + 287, r = 0.9997 244 → 142 *m/z*: Y = 18150x - 155, r = 0.9997 AMPA in bovine liver: 214 → 155 *m/z*: Y = 19385x - 34, r = 0.9994 214 → 91 *m/z*: Y = 14637x + 343, r = 0.9985
Assessment of matrix effects is presented	Matrix effects on the detection of mesotrione in extracts of milk, meat, fat and eggs, MNBA in extracts of milk, meat, liver, eggs and liver and AMBA in extracts of milk (quantification), meat, fat and liver were found to be insignificant (<20%). Matrix effects on the detection of AMBA in extracts of milk (confirmation) and mesotrione in extracts of liver was found to be significant (≥20%). However, matrix matched standards were used for quantification of all matrices.
Limit of determination / quantification	The limit of quantification (LOQ) is defined as the lowest fortification level with mean recoveries ranging from 70 % to 110 % at a relative standard deviation (RSD) of ≤ 20%. These criteria were fulfilled for mesotrione, AMBA and MNBA in animal matrices (milk, bovine meat, liver, fat and poultry egg) with an LOQ of 0.01 mg/kg for two (2) mass transitions. The limit of detection (LOD) was set at 0.003 mg/kg, which is 30 % of the LOQ.

Conclusion

The independently validated residue analytical method was found to be valid for the determination of mesotrione, AMBA and MNBA in animal matrices (milk, bovine meat, liver, fat and poultry egg) with a limit of quantification (LOQ) of 0.01 mg/kg in accordance to SANCO/825/00 rev. 8.1 requirements.

Lesot C.(2017)

A 2.1.2.2.1.3 Confirmatory method (if required)

The method of Schernikau N. and Colorado C.S. (2017), validated independently by Lesot C.(2017), includes an HPLC-MS/MS detection, which achieves a high level of specificity. Thus, an additional confirmatory method is not required.

zRMS comments:
No confirmatory method is required, since the used LC-MS/MS technique is highly specific.

A 2.1.2.2.1.4 Extraction efficiency

Not required, no residues ≥ LOQ in animal matrices are expected.

zRMS comments:
Not required. According to the SANCO/825/00 rev. 8.1 extraction efficiency is required only when residues ≥ LOQ are expected.

A 2.1.2.3 Description of analytical methods for the determination of mesotrione residues in soil (KCP 5.2)

No new or additional studies have been submitted

A 2.1.2.4 Description of analytical methods for the determination of mesotrione residues in water (KCP 5.2)

No new or additional studies have been submitted

A 2.1.2.5 Description of analytical methods for the determination of mesotrione residues in air (KCP 5.2)

No new or additional studies have been submitted

A 2.1.2.6 Description of analytical methods for the determination of mesotrione residues in body fluids and tissues (KCP 5.2)

A 2.1.2.6.1 Analytical method 1

An analytical method for the determination of the active substance mesotrione and its metabolites MNBA and AMBA in Body Fluids and Tissues has been validated in blood (bovine) and liver (bovine) by Giesau A. and Grewe D. (2016) (see KCP 5.2/03).

A 2.1.2.6.1.1 Method validation

Comments of zRMS:	The LC-MS/MS method used in the study S16-04653 for determination of mesotrione and and its metabolites MNBA and AMBA in body fluids and tissues (bovine blood and liver) is acceptable validated according to SANCO/825/00 rev. 8.1. Two mass transitions (one for quantification and one for confirmation) for each analyte were evaluated. The LOQ for each analyte was successfully established at 0.01 mg/kg in blood and liver for the both mass transitions. The linearity of the detector response was demonstrated by single determination of matrix-matched calibration standards at a minimum of eight (8) concentration levels ranging from 0.1 ng/mL to 20 ng/mL. This range corresponds to 0.002 mg/kg to 0.4 mg/kg in samples. All mean recovery values at fortification levels of 0.01 mg/kg and 0.1 mg/kg for both mass transitions for all compounds and in all analysed matrices comply with the standard acceptance criteria of the guidance document SANCO/825/00, rev. 8, recovery within 70-120% and RDS < 20%.

Reference:	**2.1.2.6.1.1/01 (KCP 5.2/03)**
Report	Giesau A. and Grewe D. (2016) Validation of an Analytical Method for the Determination of Mesotrione and its Metabolites MNBA and AMBA in Body Fluid and Tissues Report No. S16-04653 (JSC-1604V)
Guideline(s):	SANCO/825/00 rev. 8.1
Deviations:	No

GLP: Yes

Acceptability: Yes

Materials and methods

A residue analytical method for the determination of mesotrione and its metabolites MNBA and AMBA was validated in blood (bovine) and liver (bovine). The method involves extraction of mesotrione, MNBA and AMBA once with acetonitrile/water (2+1, v+v) and once with 0.05 M ammonium hydroxide/acetone (1/1, v/v) for samples of liver (bovine), or twice with 0.05 M ammonium hydroxide/acetone (1+1, v+v) for samples of blood (bovine), followed by cellulose filtration (all matrices) and SPE clean-up (all matrices), prior to analysis by liquid chromatography separation coupled with tandem mass spectrometric detection (LC-MS/MS). The limit of quantification (LOQ) was set to 0.01 mg/L for blood (bovine) and 0.01 mg/kg for liver (bovine).

Specimen preparation for Bovine Blood

Each 5.0 mL homogenized specimen of blood was weighed into a 50-mL Sarstedt centrifuge tube. 20 mL of 0.05 M ammonium hydroxide/acetone (1/1, v/v) was added and shaken vigorously by hand for at least 2 min and centrifuged for 5 min at about 3500 rpm. After repeating this procedure, the supernatants were mixed and filled-up to 50 mL with 0.05 M ammonium hydroxide/acetone (1/1, v/v). The extract was centrifuged for 5 min at about 4000 rpm and the supernatant was cleaned up by filtration (cellulose) and diluted with 10 mL of water/formic acid (98/2, v/v) by a factor of 10.

Specimen preparation for Bovine Liver

Each 5.0 ± 0.05 g homogenized specimen of liver was weighed into a 50-mL Sarstedt centrifuge tube. Exactly 20 mL acetonitrile/water (2/1, v/v) was added, shaken vigorously by hand for at least 2 min and centrifuged for 5 min at about 3500 rpm. The supernatant was decanted into another tube. Exactly 20 mL of 0.05 M ammonium hydroxide/acetone (1/1, v/v) was added to the remaining pellet, shaken vigorously by hand for at least 2 min and centrifuged for 5 min at about 3500 rpm. Next, the supernatants were combined and filled-up to 50 mL with 0.05 M ammonium hydroxide/acetone (1/1, v/v). The extract was centrifuged for 5 min at about 4000 rpm and the supernatant was cleaned-up by filtration (cellulose). The extract was diluted with water/formic acid (98/2, v/v) by a factor of 10.

Extract Clean-up and Reconstitution for Analysis for Bovine Liver and Blood

The sample extract was cleaned up using an Oasis HLB cartridge (60 mg, 3cc). The cartridge was conditioned with 2 mL of methanol followed by 2 mL of water. The whole diluted sample extract (ca. 11 mL) was transferred through the cartridge. The sample jar was rinsed twice with 2 mL of methanol/water (5/95, v/v). Each rinsing portion was transferred though the cartridge, discarding the eluates. The cartridge was soaked to dryness by using a vacuum for 10 minutes. The analytes were eluted with 2 mL of methanol followed by 2 mL of acetonitrile and collected in a 10-mL test tube. The sample extract was evaporated to dryness using a gentle stream of nitrogen and a water bath set at approx. 40 °C. The residue was taken up and solved in 2.0 mL of water containing 0.1 % acetic acid using an ultrasonic bath prior to analysis by LC-MS/MS.

Equipment for mesotrione, AMBA and MNBA determination

HPLC-MS/MS system	1260 Infinity Binary LC System, Agilent Technologies (HPLC, ≤ 600 bar) coupled with SCIEX TripleQuad 5000 System, SCIEX (Triple quadrupole mass spectrometer)
Column	Agilent PLRP-S (50 x 4.6 mm, 5 µm, 100A, Agilent, Art. No. PL1512-1500)
Column oven temperature	35°C
Injection Volume	100 µL
Mobile phase	Eluent A: Acetonitrile Eluent B: Water containing 0.1 % (v/v) acetic acid

Gradient

Time [min]	% Eluent A	% Eluent B	Flow [µL/min]

0.0	2	98	800
0.1	2	98	800
4.1	50	50	800
6.0	75	25	800
7.0	95	5	800
8.0	95	5	800
8.2	5	95	800
10	5	95	800

Retention time(s)	approx. 2.4 min (MNBA); approx. 3.6 min (AMBA); approx. 5.6 min (Mesotrione)
Ionisation type	Electrospray ionization (ESI, TurboIon Spray)
Polarity	Negative ion mode
Scan type	MS/MS, Multiple Reaction Monitoring (MRM)
Ion mass transition monitored *(m/z)*for Mesotrione	338 → 291 (Quantification) with collision energy (CE)= -10V 338 → 212 (Confirmation) with collision energy (CE)= -46V
Ion mass transition monitored *(m/z)*for MNBA	244 → 142 (Quantification) with collision energy (CE)= -28V 244 → 170 (Confirmation) with collision energy (CE)= -10V
Ion mass transition monitored *(m/z)*for AMBA	214 → 91 (Quantification) with collision energy (CE)= -20V 214 → 79 (Confirmation) with collision energy (CE)= -28V

Results and discussions

Table A 70: Accuracy and precision data of mesotrione in body fluids and tissues

Matrix	Fortification level (mg/L or mg/kg)	Recovery (%)	Mean recovery (%)	RSD (%)	Replicates	Overall Mean Recovery (%)	Overall RSD (%)
Ion Mass Transition 338 → 291 *m/z* (Quantification)							
Blood (bovine)	0.01	90, 93, 103, 100, 107	99	7.1	5	102	6.0
	0.10	104, 105, 104, 110, 100	105	3.4	5		
Liver (Bovine)	0.01	89, 87, 83, 73, 70	80	11	5	75	12
	0.10	72, 76, 72, 66, 62	70	8.0	5		
Ion Mass Transition 338 → 212 m/z (Confirmation)							
Blood (bovine)	0.01	83, 90, 101, 100, 104	96	9.2	5	98	7.3
	0.10	100, 100, 100, 108, 94	100	5.0	5		
Liver (Bovine)	0.01	86, 84, 89, 77, 69	81	9.9	5	75	12
	0.10	71, 77, 72, 66, 62	70	8.3	5		

RSD = relative standard deviation

Table A 71: Accuracy and precision data of MNBA in body fluids and tissues

Matrix	Fortification level (mg/L or mg/kg)	Recovery (%)	Mean recovery (%)	RSD (%)	Replicates	Overall Mean Recovery (%)	Overall RSD (%)
Ion Mass Transition 244 → 142 *m/z* (Quantification)							
Blood (bovine)	0.01	72, 76, 75, 80, 82	77	5.2	5	80	5.6
	0.10	78, 85, 84, 84, 83	83	3.4	5		
Liver (Bovine)	0.01	68, 67, 74, 71, 71	70	4.0	5	78	12
	0.10	82, 91, 91, 85, 82	86	5.3	5		
Ion Mass Transition 244 → 170 m/z (Confirmation)							
Blood (bovine)	0.01	72, 73, 72, 83, 76	75	6.2	5	78	5.8
	0.10	75, 83, 81, 82, 79	80	4.0	5		
Liver (Bovine)	0.01	68, 78, 75, 73, 71	73	5.2	5	77	8.6
	0.10	75, 89, 87, 81, 77	82	7.5	5		

RSD = relative standard deviation

Table A 72: Accuracy and precision data of AMBA in body fluids and tissues

Matrix	Fortification level (mg/L or mg/kg)	Recovery (%)	Mean recovery (%)	RSD (%)	Replicates	Overall Mean Recovery (%)	Overall RSD (%)
Ion Mass Transition 214 → 91 *m/z* (Quantification)							
Blood (bovine)	0.01	84, 88, 89, 93, 100	91	6.7	5	94	6.6
	0.10	96, 106, 95, 95, 96	98	4.8	5		
Liver (Bovine)	0.01	72, 76, 83, 78, 70	76	6.8	5	81	8.7
	0.10	85, 91, 90, 83, 82	86	4.7	5		
Ion Mass Transition 214 → 79 m/z (Confirmation)							
Blood (bovine)	0.01	85, 86, 95, 95, 99	92	6.7	5	94	5.8
	0.10	93, 103, 93, 91, 96	95	4.9	5		
Liver (Bovine)	0.01	75, 80, 79, 83, 69	77	7.0	5	78	6.0
	0.10	77, 83, 83, 79, 73	79	5.4	5		

RSD = relative standard deviation

All mean recovery values at fortification levels of 0.01 mg/kg and 0.1 mg/kg (for all matrices and analytes) for two (2) mass transitions comply with the standard acceptance criteria of the guidance documents.

Table A 73: Characteristics for the analytical method used for validation of mesotrione, MNBA and AMBA residues in body fluids and tissues

	Mesotrione, MNBA and AMBA
Specificity	Quantification was performed by use of LC-MS/MS detection. Two (2) mass transitions were evaluated in order to demonstrate that the method achieves a high level of specificity. The retention time of mesotrione and its metabolites MNBA and AMBA in the sample extracts matches the retention time in the calibration solutions. No interferences above 30 % of the LOQ at the retention time of the analytes were detected in the untreated control samples. Conclusively the method is specific for the determination of mesotrione and its metabolites MNBA and AMBA in blood (bovine) and liver (bovine).
Calibration (type, number of data points) **Calibration range**	Linearity of the detector response was demonstrated by single determination of minimum eight matrix matched calibration standards ranging from 0.1 ng/mL to 20 ng/mL. This range corresponds to 0.002 mg/kg to 0.4 mg/kg or 0.002 mg/L to 0.4 mg/L and thus covers the range from no more than 30 % of the LOQ and at least + 20 % of the highest analyte concentration level detected in a sample extract. The calibrations were found linear with correlation coefficients r ≥0.99 (1/x-weighting): Mesotrione in bovine blood: 338 → 291 *m/z*: Y = 800974x -16912, r = 0.9978 338 → 212 *m/z*: Y = 178044x -7902, r = 0.9992 MNBA in bovine blood: 244 → 142 *m/z*: Y = 256400x -541, r = 0.9994 244 → 170 *m/z*: Y = 36606x -767, r = 0.9994 AMPA in bovine blood: 214 → 91 *m/z*: Y = 276292x + 2352, r = 0.9994 214 → 79 *m/z*: Y = 146040x -840, r = 0.9998 Mesotrione in bovine liver: 338 → 291 *m/z*: Y = 219198x -4893, r = 0.9988 338 → 212 *m/z*: Y = 46919x -1284, r = 0.9991 MNBA in bovine liver: 244 → 142 *m/z*: Y = 98718x + 9494, r = 0.9951 244 → 170 *m/z*: Y = 12887x + 738, r = 0.9969 AMPA in bovine liver: 214 → 91 *m/z*: Y = 105796x + 6991, r = 0.9952 214 → 79 *m/z*: Y = 57504x +1912, r = 0.9975
Assessment of matrix effects is presented	Matrix effects on the detection of mesotrione in extracts of blood (bovine) and liver (bovine) and AMBA in extracts of liver (bovine) were found to be significant (≥ 20 %). Therefore, matrix-matched standards were used for quantification. Matrix effects on the detection of MNBA in extracts of blood (bovine) and liver (bovine) and AMBA in extracts of blood (bovine) were found to be insignificant (< 20 %). However, matrix-matched standards were used for quantification.
Limit of determination / quantification	The limit of quantification (LOQ) is defined as the lowest fortification level with mean recoveries ranging from 70 % to 110 % at a relative standard deviation (RSD) of ≤ 20%. These criteria were fulfilled for mesotrione, AMBA and MNBA in blood (bovine) and liver (bovine) with an LOQ of 0.01 mg/L for blood (bovine) and 0.01 mg/kg for liver (bovine) for two (2) mass transitions. The limit of detection (LOD) was defined in this study as 30% of the LOQ, which is 0.003 mg/L for blood (bovine) and 0.003 mg/kg in liver (bovine).
Extract Stability	Mesotrione, AMBA and MNBA were found to be stable in final extracts of blood (bovine) and liver (bovine) when stored at 1 °C to 10 °C for 10 days in the

	Mesotrione, MNBA and AMBA
	dark.
Standard Stability	Stock and fortification solutions prepared in acetonitrile were tested to be stable for at least 43 days when stored at 1 °C to 10 °C in the dark. Calibration solutions prepared in 0.1 % acetic acid in water were tested to be stable for 14 days when stored at 1 °C to 10 °C in the dark.

Conclusion

The method was found to be valid for the determination of mesotrione, AMBA and MNBA in blood (bovine) and liver (bovine) with a limit of quantification (LOQ) of 0.01 mg/L for blood (bovine) and 0.01 mg/kg for liver (bovine) in accordance to SANCO/825/00 rev. 8.1 requirements.

Giesau A. and Grewe D. (2016)

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

No new or additional studies have been submitted

A 2.1.3 Methods for post-authorization control and monitoring purposes for nicosulfuron (KCP 5.2)

A 2.1.3.1 Description of analytical methods for the determination of residues of nicosulfuron in plant matrices (KCP 5.2)

No new or additional studies have been submitted.

A 2.1.3.2 Description of analytical methods for the determination of residues of nicosulfuron in animal matrices (KCP 5.2)

No new or additional studies have been submitted.

A 2.1.3.3 Description of analytical methods for the determination of nicosulfuron residues in soil (KCP 5.2)

No new or additional studies have been submitted

A 2.1.3.4 Description of analytical methods for the determination of nicosulfuron residues in water (KCP 5.2)

No new or additional studies have been submitted

A 2.1.3.5 Description of analytical methods for the determination of nicosulfuron residues in air (KCP 5.2)

No new or additional studies have been submitted

A 2.1.3.6 Description of analytical methods for the determination of nicosulfuron residues in body fluids and tissues (KCP 5.2)

No new or additional studies have been submitted

A 2.1.3.7 Other Studies/ Information

No new or additional studies have been submitted