FINAL REGISTRATION REPORT

Part B

Section 5

Analytical Methods

Detailed summary of the risk assessment

Product code: DNT-162OD-R-CPd

Product name(s): EVRITELL 162 OD

Chemical active substances:

Dicamba, 110 g/L
Nicosulfuron, 40 g/L
Thifensulfuron-methyl, 12 g/L

Central

Zonal Rapporteur Member State: zRMS

CORE ASSESSMENT

Poland

(authorization)

Applicant: QEMETICA Agricultural Solutions Poland S.A. (formerly: CIECH Sarzyna S.A.).

Submission date: 01/2024, 03/2025, 08/2025

Finalisation date: 10/2024, 03/2025, 08/2025

Version history

When	What
January 2024	First submission to zRMS
October 2024	RMS assessment
March 2025	Update by the Applicant due to addition of monitoring methods
March 2025	Final assessment
August 2025	Update by the Applicant on the Evaluator's request
August 2025	Evaluation of applicant's additions

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5 Analytical methods

5.1 Conclusion and summary of assessment

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

Sufficiently sensitive and selective analytical methods are available for all analytes included in the residue definitions.
Data gap:

- Nicosulfuron - Table 5.3.11

Analytical methods for commodities with high water content, high acid content and high oil content are missing. ~~The applicant should complete the Table before registration~~.
August 2025
The applicant's addition was accepted. The table has been completed. These analytical methods were not submitted at the EU substance approval level. The methods should be supplemented after the renewal of the active substance. No additional data is required.

Commodity/crop	Supported/ Not supported
Maize	Supported

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

5.2.1 Analysis of the plant protection product (KCP 5.1.1)

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

An overview on the acceptable methods and possible data gaps for analysis of dicamba, nicosulfuron and thifensulfuron-methyl in plant protection product is provided as follows:

Comments of zRMS:	The proposed analytical method is suitable for the determination of dicamba, nicosulfuron and thifensulfuron-methyl in the plant protection product Evritell 162OD (DNT-162OD-R-CPd). The proposed analytical method has been fully validated in terms of specificity, linearity, accuracy and precision. Proposed method fulfils the requirements of SANCO/3030/99 rev.5 guidance. The validation of the analytical method has been accepted.

Reference:	KCP 5.1.1
Report	DNT-162OD-R-CPd, Determination of physicochemical properties, STAB.23-03/INIT, ~~2022~~ 2023, K. Posłuszna
Guideline(s):	Yes (SANCO/3030/99 rev.5)
Deviations:	No
GLP:	Yes

Acceptability: Yes

Materials and methods

The method for dicamba, nicosulfuron and thifensulfuron-methyl content determination was developed and validated by using RP-HPLC-UV-DAD method with external standard calibration.

HPLC chromatographic conditions

Apparatus	Nexera-i Liquid Chromatograph
Detector	Diode array detector
Column	Kinetex 5 µm C18 110 Å, 150 × 4.6 mm, Phenomenex precolumn C18 (AJ0-4287), Phenomenex
Wavelength [nm]	230
Oven temperature [°C]	35
Flow rate [mL/min]	1.0
Mobile phase	A: 0.1% (v/v) ~~H3PO4~~ H_3PO_4 (aq) B: acetonitrile Isocratic: 65% A+35% B (v/v)
Injection volume [µl]	2
Analyte elution time [min]	nicosulfuron: 3.1 ± 0.1 min thifensulfuron-methyl: 4.5 ± 0.1 min dicamba: 5.5 ± 0.1 min

Validation - Results and discussions

Table 5.2-1: Methods suitable for the determination of active substances dicamba, nicosulfuron and thifensulfuron-methyl in plant protection product DNT-162OD-R-CPd

	dicamba	nicosulfuron	thifensulfuron-methyl
Author(s), year	K. Posłuszna, ~~2022~~ 2023		
Principle of method	The method for dicamba, nicosulfuron and thifensulfuron-methyl content determination was developed and validated by using RP-HPLC-UV-DAD method with external standard calibration.		
Linearity (linear between mg/L / % range of the declared content) (correlation coefficient, expressed as r)	Method linearity was determined with a calibration curve. The calibration curve was prepared using 5 levels, plotting individual concentrations against corresponding peak area		
	y = 4154 × x + 136682 R^2 = 0.9990 643 – 1112 µg/mL (81 – 139 g/kg)	y = 4627 × x + 8794 R^2 = 0.9996 228 – 417 µg/mL (28 – 52 g/kg)	y = 5366 × x – 6036 R^2 = 0.9994 68 – 125 µg/mL (8.5 – 16 g/kg)
Precision – Repeatability Mean n = 6 (%RSD)	RSD [%] = 0.47 RSDr [%] = 1.87 Hr = 0.25	RSD [%] = 0.44 RSDr [%] = 2.18 Hr = 0.20	RSD [%] = 0.32 RSDr [%] = 2.62 Hr = 0.12
System Precision	RSD [%] = 0.14 RSDr [%] = 1.87 Hr = 0.07	RSD [%] = 0.22 RSDr [%] = 2.18 Hr = 0.10	RSD [%] = 0.22 RSDr [%] = 2.62 Hr = 0.08

Accuracy **n = 6** **(% Recovery)**	Total Recovery [%] = 99.01 RSD %= 0.60 Criterion % = 97-103	Total Recovery [%] = 97.15 RSD %= 0.54 Criterion % = 90-110	Total Recovery [%] = 98.72 RSD %= 0.59 Criterion % = 90-110
Interference/ Specificity	The specificity was demonstrated by the absence of interferences superior to 3% at the retention time of the analytes. Overlaid chromatograms of: solvents (reference item solvent as acetonitrile and mobile phase as test item solvent), blank formulation of DNT-162OD-R-CPd (placebo), reference items solutions of dicamba, nicosulfuron and thifensulfuron-methyl and test item presented in the report. Additionally, UV spectra, UV purity spectra and 3D-UV spectra of dicamba, nicosulfuron and thifensulfuron-methyl in reference items and in DNT-162OD-R-CPd formulation are shown for comparison.		
Comment	-	-	-

Conclusion

Basing on the presented spectra the method can be considered specific under the analytical conditions as required by SANCO/3030/99 rev.5.

The assessed recovery is within the criteria of acceptance required by SANCO/3030/99 rev.5 (22/03/2019).

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

There are no impurities of toxicological and/or ecotoxicological or environmental concern in the active substances, even if present or could theoretically be formed in technical active substance at < 1 g/kg.

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

Not applicable

5.2.1.4 Applicability of existing CIPAC methods (KCP 5.1.1)

Dicamba: CIPAC 85/SL/M/2, Handbook K, p. 35 2003
Dicamba is dissolved in methanol and determined by high performance liquid chromatography on a reversed phase column (RP18) using UV detection and external standardization.
The method is usable for TC, SL and WG formulations.
Nicosulfuron: 709/TC/M/3, CIPAC/4903
Nicosulfuron is determined by high performance liquid chromatography using a Zorbax® SB column, UV detection at 245 nm and internal standardisation (3-methyl-1,1-diphenylurea). The active ingredient content is quantified using a calibration curve.
The method is usable for TC and WG-formulations, method extension, OD.
Thifensulfuron-methyl: CIPAC 452/TC/M-, CIPAC Handbook K, p. 115, 2003
Thifensulfuron-methyl is determined by reversed phase high performance liquid chromatography using a C18 column, UV detection at 280 nm and external standardisation. The content of active ingredient is quantified using a calibration curve.
The method is usable for TC and WG-formulations.

5.2.2 Methods for the determination of residues (KCP 5.1.2)

An overview on the acceptable methods and possible data gaps for analysis of residues of Dicamba, Nicosulfuron, Thifensulfuron-methyl for the generation of pre-authorization data is given in the following table. For the detailed evaluation of studies included in the table below it is referred to Appendix 1.

Table 5.2-2: Validated methods for the generation of pre-authorization data

Component of residue definition: **Dicamba:** Dicamba and 5-OH-dicamba, free and conjugated expressed as dicamba **Nicosulfuron:** Nicosulfuron **Thifensulfuron-methyl:** For oilseeds and cereals (weed-control use): Thifensulfuron-methyl and provisionally triazine amine (IN-A4098) For animal feed items (grass / alfalfa): Sum of thifensulfuron-methyl and thifensulfuron acid (IN-L9225), expressed as thifensulfuron-methyl and provisionally triazine amine (IN-A4098)				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Dicamba				
Winter wheat grain (Residues, dry commodities)	Primary	0,01 mg/kg	UPLC-MS/MS	V.Faessel, 2023 Report no: R C2140
Winter wheat straw (Residues, dry commodities)	Primary	0,01 mg/kg	LC-MS/MS	V.Lefebvre, 2023 Report no: R C2136
Maize grain (Residues, dry commodities)	Primary	0,01 mg/kg	LC-MS/MS	V. Faessel, 2023 Report no: R C2146
Nicosulfuron, Thifensulfuron-methyl				
Maize grain (Residues, dry commodities)	Primary	0,01 mg/kg	LC-MS/MS	V.Faessel, 2023 Report no: R C2160
Maize straw (Residues, dry commodities)	Primary	0,01 mg/kg	LC-MS/MS	E.Thomas – Delille, 2023 Report no: R C2156
Dicamba, Nicosulfuron, Thifensulfuron-methyl				
Sucrose solution (Ecotoxicology, Honeybees)	Primary	29 µg/L	UHPLC-MS	P. Parma, 2022 EMI/4/62/2022
Water (Ecotoxicology, Honeybees)	Primary	29 µg/L	UHPLC-MS	P. Parma, 2022 EMI/4/63/2022
50% sucrose solution in water (Ecotoxicology, Bumblebee)	Primary	10 mg/L	HPLC-DAD	A.Wojciech, 2023 B-56-23
1% Triton (R) X-100 water solution (Ecotoxicology,	Primary	10 mg/L	HPLC-DAD	A.Wojciech, 2023 B-57-23

Component of residue definition:

Dicamba: Dicamba and 5-OH-dicamba, free and conjugated expressed as dicamba

Nicosulfuron: Nicosulfuron

Thifensulfuron-methyl: For oilseeds and cereals (weed-control use): Thifensulfuron-methyl and provisionally triazine amine (IN-A4098)

For animal feed items (grass / alfalfa): Sum of thifensulfuron-methyl and thifensulfuron acid (IN-L9225), expressed as thifensulfuron-methyl and provisionally triazine amine (IN-A4098)

Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
Bumblebee)				
Water (Ecotoxicology, aquatic invertebrates, Daphnia magna)	Primary	29 µg/L	UHPLC-MS/MS	S. Szlauer, 2022 EMI/4/70/2022
Water (Ecotoxicology, green alga, Pseudokirchneriella subcapitata)	Primary	29 µg/L	UHPLC-MS/MS	S. Szlauer, 2022 EMI/4/71/2022
Water (Ecotoxicology, green alga, Navicula pelliculosa)	Primary	0.05mg/L	HPLC-DAD	Z. Kacperek-Karetta, 2023 W-11-23
Water (Ecotoxicology, Lemna gibba)	Primary	Dilution method Dicamba 10.0 µg/L Nicosulfuron 1.0 µg/L thifensulfuron-methyl 1.0 µg/L SPE method Dicamba 0.4µg/L Nicosulfuron 0.04 µg/L thifensulfuron-methyl 0.04 µg/L	HPLC with MS/MS detection	Z. Kacperek-Karetta, 2023 W-12-23
Water (Ecotoxicology, Vegetative Vigour Test)	Primary	Dilution method LOQ= 2.0 mg/L Solid Phase Extraction method 0.005 mg/L	HPLC with DAD detection.	P. Pieczka, 2023 G-33-23
Water (Ecotoxicology, Terrestrial Plant Test:)	Primary	Dilution method LOQ= 2.0 mg/L Solid Phase Extraction	HPLC with DAD detection.	P. Pieczka, 2024 G-34-23

Component of residue definition: **Dicamba:** Dicamba and 5-OH-dicamba, free and conjugated expressed as dicamba **Nicosulfuron:** Nicosulfuron **Thifensulfuron-methyl:** For oilseeds and cereals (weed-control use): Thifensulfuron-methyl and provisionally triazine amine (IN-A4098) For animal feed items (grass / alfalfa): Sum of thifensulfuron-methyl and thifensulfuron acid (IN-L9225), expressed as thifensulfuron-methyl and provisionally triazine amine (IN-A4098)				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
		method 0.005 mg/L		

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

5.3.1 Analysis of the plant protection product (KCP 5.2)

Analytical methods for the determination of the active substance and relevant impurities in the plant protection product are already submitted in point 5.2.1.

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

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

Compared to the residue definition proposed in the Draft Assessment Report (incl. its addenda) the current legal residue definition is not identical.
Residue definition (EFSA Journal 2011;9(1):1965):
Plant matrices, animal matrices: Dicamba and its salts and conjugated dicamba expressed as dicamba
Soil, Water: Dicamba, DCSA and their salts
Air: Dicamba

zRMS: Compared to the residue definition proposed in the DAR (incl. its addenda) the current legal residue definition is not identical. For food of plant and animal origin, the residue definition is proposed as follows: dicamba and its salts and conjugated dicamba expressed as dicamba (EFSA Journal 2011;9(1):1965). The currently applicable definition for monitoring is: Dicamba (Reg. (EU) 2015/845).
The analytical methods presented in the Table 5.3-2 have been accepted at EU level. Therefore, presented in this table methods are accepted by zRMS.
According to the EFSA Journal 2013;11(11):3470:
The method REM 193.01 is claimed to analyse dicamba and its conjugates, according to the enforcement residue definition proposed in the conclusion of the peer review. However, further information was requested to confirm whether the hydrolysis step is efficient in releasing dicamba conjugates (EFSA, 2011). This data requirement is not relevant for the current application as the current residue definition under Regulation (EC) No 396/2005 is limited to dicamba only

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	Dicamba ~~and its salts and conjugated dicamba expressed as dicamba~~	0.05 mg/kg	Regulation (EU) 2015/845
Plant, high acid content		0.05 mg/kg	
Plant, high protein/high starch content (dry commodities)		MRL maize 0.5 mg/kg	
Plant, high oil content		0.05 mg/kg	
Plant, difficult matrices (hops, spices, tea)		Not required for the intended use	
Muscle	Dicamba ~~and its salts and conjugated dicamba expressed as dicamba~~	0.02 mg/kg	Regulation (EU) 2015/845
Milk		0.2 mg/kg	
Eggs		0.05 mg/kg	
Fat		0.04 mg/kg	
Liver, kidney		0.07 mg/kg	
Soil (Ecotoxicology)	Dicamba, DCSA and their salts	0.016 mg/kg	based on ER50 for *beta vulgaris* of 24.4 g a.s./ha EFSA Conclusion on pesticide peer review, EFSA Journal 2011;9(1):1965;
Drinking water (Human toxicology)	Dicamba, DCSA and their salts	0.1 µg/L	general limit for drinking water
Surface water (Ecotoxicology)	Dicamba, DCSA and their salts	450 µg/L (dicamba) 11900 µg/L (DCSA)	E_bC_{50} *Myriophyllum spicatum* E_bC_{50} *Lemna gibba* EFSA Journal 2011;9(1):1965;
Air	Dicamba	90 µg/m3	AOEL sys: 0.3 mg/kg bw/d EFSA Journal 2011;9(1):1965
Tissue (meat or liver)	Dicamba*	0.01 mg/kg	not classified as T / T+, but required according to Reg. (EU) No. 283/2013 SANTE/2020/12830, Rev.2
Body fluids		0.01 mg/L	not classified as T / T+, but required according to Reg. (EU) No. 283/2013 SANTE/2020/12830, Rev.2

* LoEP, DRAR 2018

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

An overview on the acceptable methods and possible data gaps for analysis of dicamba in plant matrices is

given in the following tables.

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: dicamba				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
High water content	Primary&Confirmatory	0.01 mg/kg	GC-MS	Maffezoni M., 2004, method no. REM 193.01, EU agreed
	ILV	0.01 mg/kg	GC-MS	Steinhauer, 2004, EU agreed
High acid content	Primary&Confirmatory	0.01 mg/kg	GC-MS	Maffezoni M., 2004, method no. REM 193.01,EU agreed
	ILV	-	-	not required
High oil content	Primary&Confirmatory	0.01 mg/kg	GC-MS	Maffezoni M., 2004, method no. REM 193.01,EU agreed
	ILV	0.01 mg/kg	GC-MS	not required
High protein/high starch content (dry)	Primary&Confirmatory	0.01 mg/kg	GC-MS	Maffezoni M., 2004, method no. REM 193.01,EU agreed
	ILV	0.01 mg/kg	GC-MS	Steinhauer, 2004, EU agreed

Table 5.3-3: Statement on extraction efficiency

	Method for products of plant origin
Not required, because:	The extraction efficiency of the monitoring method for the determination of residues of Dicamba in plant matrices was not considered during the active substance approval (EFSA Journal 2011;9(1):1965. Dicamba was assessed using the data requirements under Reg (EU) 544/2011, therefore data to address the extraction efficiency will be addressed at 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 dicamba in animal matrices is given in the following tables. For the detailed evaluation of studies it is referred to Appendix 1.

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

Component of residue definition: dicamba				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Milk	Primary &Confirmatory	0.01 mg/kg	LC-MS/MS	Martinez, 2021 CH – 0672/2021

Component of residue definition: dicamba				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
	ILV	0.01 mg/kg	LC-MS/MS	Longhi, 2021 GLP-STUDY-21-118 Appendix
Eggs	Primary &Confirmatory	0.01 mg/kg	LC-MS/MS	Longhi, 2022 GLP-STUDY-22-1 Appendix
	ILV	0.01 mg/kg	LC-MS/MS	Rigamonti, E., 2022 CH – 0992/2021 Appendix
Muscle	Primary &Confirmatory	0.01 mg/kg	LC-MS/MS	Martinez, 2021 CH – 0670/2021 Appendix
	ILV	0.01 mg/kg	LC-MS/MS	Longhi, 2021 GLP-STUDY-21-116 Appendix
Fat	Primary &Confirmatory	0.01 mg/kg	LC-MS/MS	Martinez, 2021 CH-0667/2021 Appendix
	ILV	0.01 mg/kg	LC-MS/MS	Longhi, 2021 GLP-STUDY-21-115 Appendix
Kidney	Primary &Confirmatory	0.01 mg/kg	LC-MS/MS	Martinez, 2021 CH – 0671/2021 Appendix
	ILV	0.01 mg/kg	LC-MS/MS	Longhi, 2021 GLP-STUDY-21-117 Appendix

For any special comments or remarkable points concerning the analytical methods for the determination of residues in animal matrices, please refer to Appendix 1.

Table 5.3-5: Statement on extraction efficiency

	Method for products of animal origin
Required, available from:	It is referred to the point KCP 5.2_03.

For the detailed evaluation of studies on extraction efficiency please refer to Appendix 1.

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 dicamba in soil is given in the following tables.

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

Component of residue definition: dicamba, DCSA and their salts			
Method type	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	0.01 mg/kg	GC-MS	Gasser, 2000a, 2000b , EU agreed

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

An overview on the acceptable methods and possible data gaps for analysis of dicamba in surface and drinking water is given in the following tables. For the detailed valuation of new studies it is referred to Appendix 1.

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

Component of residue definition: dicamba, DCSA and their salts				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Drinking water	Primary&Confirmatory	0.10 µg/L	LC-MS/MS	Martinez, 2020 CH – 0472/2020 Appendix
	ILV	0.10 µg/L	LC-MS/MS	Sala, 2020 GLP-STUDY-20-62 Appendix
Surface water	Primary	0.10 µg/L	GC-MS	Gasser, 2000c, 2000d, EU agreed

For any special comments or remarkable points concerning the analytical methods for water please refer to Appendix 1.

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

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

Table 5.3-8: Validated methods for air (if appropriate)

Component of residue definition: dicamba			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	21 µg/m^3	HPLC-UV	Kettner & Karapally, 1993, EU agreed

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 dicamba in body fluids and tissues is given in the following table. For the detailed evaluation of new studies it is referred to Appendix 1.

Table 5.3-9: Methods for body fluids

Component of residue definition: dicamba			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary& Confirmatory	0.01 mg/L	LC-MS/MS	Longhi, D., 2023, it is reffered to Appendix 2

Description of methods for the analysis of tissues it is referred to point 5.3.2.3.
For any special comments or remarkable points concerning the analytical methods for body fluids and tissues please refer to Appendix 1.

5.3.2.8 Other studies/ information

Not applicable

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

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

Compared to the residue definition proposed in the Draft Assessment Report (incl. its addenda) and EFSA's conclusion on the peer review, the current legal residue definition is identical.
Residue definition based on *EFSA Scientific Report* (2007) 120, 1-91, Conclusion on the peer review of nicosulfuron.

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	Reg. (EU) No 617/2014
Plant, high acid content		0.01 mg/kg	
Plant, high protein/high starch content (dry commodities)		0.01 mg/kg	
Plant, high oil content		0.01 mg/kg	
Plant, difficult matrices (hops, spices, tea)		Not required for the intended use	
Muscle	nicosulfuron	0.02 mg/kg	Reg. (EU) No 617/2014
Milk		0.02 mg/kg	
Eggs		0.02 mg/kg	

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Fat		0.04 mg/kg	
Liver		0.05 mg/kg	
Kidney		0.02 mg/kg	
Soil (Ecotoxicology)	nicosulfuron	0.001 mg/kg	phytotoxic effects on succeeding crops, EFSA Scientific Report 120 (2007) 1-91, section 3.1.2
Drinking water (Human toxicology)	nicosulfuron	0.1 µg/L	general limit for drinking water
Surface water (Ecotoxicology)	nicosulfuron	1.7 µg/L	E_bC_{50} *Lemna gibba* EFSA Scientific Report 120 (2007) 1-91, section 5.2
Air	nicosulfuron	240 µg/m3	AOEL sys: 0.8 mg/kg bw/d; EFSA Scientific Report 120 (2007) 1-91, list of endpoints,
Tissue (meat or liver)	NA	0.01 mg/kg	not classified as T / T+, but required according to Reg. (EU) No. 283/2013 SANTE/2020/12830, Rev.2
Body fluids		0.01 mg/L	not classified as T / T+, but required according to Reg. (EU) No. 283/2013 SANTE/2020/12830, Rev.2

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. Presented analytical methods are refer to the crops applied for (dry commodities). These are considered sufficient to cover the proposed uses of product on maize.

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	Primary	No data	No data	These analytical methods were not submitted at the EU substance approval level. Methods are not related to the intended GAP, therefore were not presented.
	ILV	No data	No data	
	Confirmatory (if required)	No data	No data	

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 acid content	Primary	No data	No data	These analytical methods were not submitted at the EU substance approval level. Methods are not related to the intended GAP, therefore were not presented.
	ILV	No data	No data	
	Confirmatory (if required)	No data	No data	
High oil content	Primary	No data	No data	These analytical methods were not submitted at the EU substance approval level. Methods are not related to the intended GAP, therefore were not presented.
	ILV	No data	No data	
	Confirmatory (if required)	No data	No data	
High protein/high starch content (dry)	Primary	0.01 mg/kg	LC-MS/MS	Wolf, S., 2000, 793596, EU agreed
	ILV	0.01 mg/kg	LC-MS/MS	Ginzburg, N., 2000, A-22-00-04,EU agreed
	Confirmatory (if required)	No required	No required	No required

zRMS: Analytical methods for commodities with high water content, high acid content and high oil content are missing (data gap). ~~The applicant should complete the Table before registration.~~

August 2025
The applicant's addition was accepted. The table has been completed. These analytical methods were not submitted at the EU substance approval level. The methods should be supplemented after the renewal of the active substance. No additional data is required.

Table 5.3-12: Statement on extraction efficiency

	Method for products of plant origin
Not required, because:	The extraction efficiency of the monitoring method for the determination of residues of Nicosulfuron in plant matrices was not considered during the active substance approval (DAR, 2007). Nicosulfuron was assessed using the data requirements under Reg (EU) 544/2011, therefore data to address the extraction efficiency will be addressed at renewal of the active substance.

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

According to EFSA Scientific Report (2007) 120, 1-91, Conclusion on the peer review of Nicosulfuron an analytical method for monitoring residues in food of animal origin is not required due to the fact that no residue definition is proposed. Data to address the analytical methods for the determination of residues in animal matrices will be addressed at renewal of the active substance.
New studies are not provided

Table 5.3-13: Validated methods for food and feed of animal origin (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**
Milk	Primary &Confirmatory	LOQ = 0.01 mg/kg	LC-MS/MS	Blumberg, O., 2024, S23-107311 Appendix
	ILV	LOQ = 0.01 mg/kg	LC-MS/MS	Jooss, S., 2024 S23-107248 Appendix
Eggs	Primary &Confirmatory	LOQ = 0.01 mg/kg	LC-MS/MS	Blumberg, O., 2024, S23-107311 Appendix
	ILV	LOQ = 0.01 mg/kg	LC-MS/MS	Jooss, S., 2024 S23-107248 Appendix
Muscle	Primary &Confirmatory	LOQ = 0.01 mg/kg	LC-MS/MS	Blumberg, O., 2024, S23-107311 Appendix
	ILV	LOQ = 0.01 mg/kg	LC-MS/MS	Jooss, S., 2024 S23-107248 Appendix
Fat	Primary &Confirmatory	LOQ = 0.01 mg/kg	LC-MS/MS	Blumberg, O., 2024, S23-107311 Appendix
	ILV	LOQ = 0.01 mg/kg	LC-MS/MS	Jooss, S., 2024 S23-107248 Appendix
Kidney, liver	Primary &Confirmatory	LOQ = 0.01 mg/kg	LC-MS/MS	Blumberg, O., 2024, S23-107311 Appendix
	ILV	LOQ = 0.01 mg/kg	LC-MS/MS	Jooss, S., 2024 S23-107248 Appendix

Table 5.3-14: Statement on extraction efficiency

	Method for products of animal origin
Not required, because:	Residues in food of animal origin are not expected

For the detailed evaluation of studies on extraction efficiency please refer to Appendix 1.

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.

Table 5.3-15: 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	LC-MS/MS	Wais, A., 2000, 770117, EU agreed

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. For the detailed valuation of new studies it is referred to Appendix 1.

Table 5.3-16 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&Confirmatory	0.05 µg/L	LC-MS/MS	Wolf, S., 2007, B25773, EU agreed
	ILV	0.05 µg/L	LC-MS/MS	Rudzinski, K., 2020, 20/FSL/04, ASB2020-13621 Appendix 2
Surface water	Primary&Confirmatory	0.05 µg/L	LC-MS/MS	Wolf, S., 2007, B25773, EU agreed

For any special comments or remarkable points concerning the analytical methods please refer to Appendix 1.

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.

Table 5.3-17: 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/m3	LC-UV	Schulz, M. and Ulrich-Mitzel A., 1995b, Report

Component of residue definition: nicosulfuron			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
			no. 385470, EU agreed

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

~~According to EFSA Scientific Report (2007) 120, 1-91, Conclusion on the peer review of Nicosulfuron an analytical method for monitoring residues in body fluids is not required due to the fact that no residue definition is proposed. Data to address the analytical methods for the determination of residues in body fluids and tissue will be addressed at renewal of the active substance.~~
A new analytical methods for the determination of residues in body fluids has been submitted. For method in tissues please refer to animal matrices methods.

Table 5.3-18: Validated methods for body fluids

Component of residue definition: nicosulfuron			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary, confirmatory	LOQ = 0.01 mg/L	LC-MS/MS	Blumberg, O., 2024, S23-107311 Appendix

5.3.4 Description of analytical methods for the determination of residues of Thifensulfuron-methyl (KCP 5.2)

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

Compared to the residue definition proposed in the Draft Assessment Report (incl. its addenda) the current legal residue definition is identical.
Plant matrices: Thifensulfuron-methyl (parent only)
Although currently no EU MRLs are set for feed commodities, for possible future applicability it is proposed: For Animal feed items (grass / alfalfa): Sum of thifensulfuron-methyl and thifensulfuron acid (IN-L9225), expressed as thifensulfuron-methyl
Animal matrices: Thifensulfuron-methyl (parent only)

Table 5.3-19: 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	Thifensulfuron-methyl	0.01 mg/kg	Reg. (EU) No 617/2014

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Plant, high acid content		0.01 mg/kg	
Plant, high protein/high starch content (dry commodities)		0.01 mg/kg	
Plant, high oil content		0.01 mg/kg	
Plant, difficult matrices (hops, spices, tea)		Not required for the intended use	
Muscle	Thifensulfuron-methyl	0.01 mg/kg	Reg. (EU) No 617/2014
Milk		0.01 mg/kg	
Eggs		0.01 mg/kg	
Fat		0.01 mg/kg	
Liver, kidney		0.01 mg/kg	
Soil (Ecotoxicology)	Thifensulfuron-methyl	0.05 mg/kg	common limit
Drinking water (Human toxicology)	Thifensulfuron-methyl	0.1 µg/L	general limit for drinking water
Surface water (Ecotoxicology)	Thifensulfuron-methyl	E_yC_{50} = 0.00066 mg a.s./L E_yC_{50} = 0.0012 mg a.s./L E_rC_{50} = 0.0011 mg a.s./L	Duckweed (*Lemna gibba*) EFSA Journal 2015;13(7):4201
Air	Thifensulfuron-methyl	21 µg/m3	AOEL: 0.07 mg/kg bw per day EFSA Journal 2015;13(7):4201
Tissue (meat or liver)	Thifensulfuron-methyl	0.01 mg/kg	not classified as T / T+, but required according to Reg. (EU) No. 283/2013 SANTE/2020/12830, Rev.2
Body fluids		0.01 mg/L	not classified as T / T+, but required according to Reg. (EU) No. 283/2013 SANTE/2020/12830, Rev.2

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

Details on analytical methods for the active substance thifensulfuron methyl are derived from the respective EFSA conclusions for this active as indicated below.
EFSA Journal 2015;13(7):4201. Conclusion on the peer review of the pesticide risk assessment of the active substance thifensulfuron-methyl.

Table 5.3-20: 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: thifensulfuron methyl				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
High protein/high starch content (dry)	Primary&Confirmatory	0.01 mg/kg	LC-MS/MS	Devine, T.J., Nanita, S.C.,2007 DuPont-13412, Supplement No. 1 EU agreed
High oil content	Primary&Confirmatory	0.01 mg/kg	LC/MS/MS	Pentz A.M., Bramble F .Q., 2005 DuPont-13412, Revision No. 1 EU agreed
	ILV	0.01 mg/kg	LC/MS/MS	Charles, E., Doran, A.M., 2004 DuPont-13398 EU agreed
Oil, water, acidic and dry crops	Primary&Confirmatory	0.01 mg/kg	LC/MS/MS	Henze, R.M., Stry, J.J. 2014 DuPont-13412, Supplement No. 4, Revision No. 1 EU agreed
	ILV	0.01 mg/kg	LC/MS/MS	Plastridge, B., 2006 DuPont-17207, Revision No. 1 EU agreed
Dry commodities	Primary&Confirmatory	0.01 mg/kg	LC/MS/MS	Amoo, J.S., Jones, W. 2001 DuPont-5367 EU agreed
Acidic comodties	Primary&Confirmatory	0.01 mg/kg	LC/MS/MS	Brookey, F.M., Westberg, G.L. 2007 DuPont-5367, Supplement No. 1 EU agreed
Dry commodities	Primary&Confirmatory	0.01 mg/kg	LC/MS/MS	Seck C. 2008 Report No. QG/07/021 EU agreed
	ILV	0.01 mg/kg	LC/MS/MS	Watson, G 2010 Report No. S09-02905 EU agreed

Table 5.3-21: Statement on extraction efficiency

	Method for products of plant origin
Not required, because:	According to SANTE/2017/10632 Rev. 5, the evaluation of the extraction efficiency is only necessary for pesticides showing significant residues, i.e. residues at or above the limit of quantification (LOQ) of the analytical method. Residue test results for thifensulfuron-methyl: grain, straw NDR*: (below the LOD ; LOD = 0.003 mg/kg) * no detectable residues

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

Details on analytical methods for the active substance thifensulfuron methyl are derived from the respective EFSA conclusions for this active as indicated below.
EFSA Journal 2015;13(7):4201. Conclusion on the peer review of the pesticide risk assessment of the active substance thifensulfuron-methyl.
Confirmatory data_March 2019, Thifensulfuron-methyl - Volume 2, Annex A : List of Tests and Studies

Table 5.3-22: Validated methods for food and feed of animal origin (if appropriate)

Component of residue definition: thifensulfuron-methyl				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Milk	Primary &Confirmatory	0.02 mg/kg	LC-UV	de Bernard, P.A., Powley, C.R. 1993, AMR 2715-93 EU agreed
	ILV	0.02 mg/kg	LC-UV	Schwartz, N.L. 2010c DuPont-30911, EU agreed
Eggs	Primary &Confirmatory	0.01 mg/kg	LC-MS/MS	Henze, R.M., Stry, J.J., 2007a DuPont-24187 EU agreed
	ILV	0.01 mg/kg	LC-MS/MS	Schwartz, N.L. 2010b DuPont-30910 EU agreed
Muscle	Primary &Confirmatory	0.02 mg/kg	LC-UV	de Bernard, P.A., Powley, C.R. 1993, AMR 2715-93 EU agreed
	ILV	0.02 mg/kg	LC-UV	Schwartz, N.L. 2010c DuPont-30911 EU agreed
Liver	Primary &Confirmatory	0.02 mg/kg	LC-UV	de Bernard, P.A., Powley, C.R. 1993, AMR 2715-93 EU agreed
	ILV	0.02 mg/kg	LC-UV	Schwartz, N.L. 2010c DuPont-30911 EU agreed
bovine meat (beef), liver, kidney and fat	Primary &Confirmatory	0.01 mg/kg	LC/MS/MS	Pentz, A. M., Cabusas, M.E. 2012, DuPont-30449 Pentz, A.M., Cabusas, M.E.Y. 2014, DuPont-30449, Supplement No. 1 EU agreed
	ILV	0.01 mg/kg.	LC/MS/MS	Gant, A. G. 2012, DuPont-30450 EU agreed

zRMS: EFSA Journal 2015;13(7):4201: *A method of analysis for products of animal origin is not required as no MRLs are proposed. A method of analysis for body fluids and tissues is not required.*
No data gap regarding to LOQ is identified.

Table 5.3-23: Statement on extraction efficiency

	Method for products of animal origin
Not required, because:	Residues in food of animal origin are not expected

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

Details on analytical methods for the active substance thifensulfuron methyl are derived from the respective EFSA conclusions for this active as indicated below.
EFSA Journal 2015;13(7):4201. Conclusion on the peer review of the pesticide risk assessment of the active substance thifensulfuron-methyl.

Table 5.3-22: Validated methods for soil (if appropriate)

Component of residue definition: thifensulfuron methyl			
Method type	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary&Confirmatory	0.05 mg/kg	LC-MS/MS	Hill and Stry, 2001 DuPont-5082, Stry, 2013 DuPont-5082, Supplement No. 1 EU agreed
Primary&Confirmatory	0.005 mg/kg	LC-MS/MS	Sadgrove, L., 2012a Report No. PII0022 and amendment 1 EU agreed

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

Details on analytical methods for the active substance thifensulfuron methyl are derived from the respective EFSA conclusions for this active as indicated below.
EFSA Journal 2015;13(7):4201. Conclusion on the peer review of the pesticide risk assessment of the active substance thifensulfuron-methyl.

Table 5.3-24: Validated methods for water (if appropriate)

Component of residue definition: thifensulfuron methyl				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Water (tap, well, pond, river, ocean)	Primary&Confirma-tory	0.05 µg/L	LC/MS/MS	Devine and Jin, 2004 DuPont-5491 Pentz and Cabusas, 2014 DuPont-5491,Supplement No. 1 EU agreed
Surface water	Primary&Confirma-tory	0.01 µg/L	LC/MS/MS	Sadgrove, L., 2012 No. PII0024 and amendment 1 EU agreed
Drinking Water, Well Water, Surface water	Primary&Confirma-tory	0.00010 mg/kg	LC/MS/MS	Henze, R.M., Stry, J.J DuPont-35704, 2013 EU agreed
Drinking Wa-ter, Well Wa-ter, Surface wa-ter	ILV	0.10 µg/kg	LC/MS/MS	Mason, B.J. DuPont-36531, 2013 EU agreed

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

Details on analytical methods for the active substance thifensulfuron methyl are derived from the respective EFSA conclusions for this active as indicated below.
EFSA Journal 2015;13(7):4201. Conclusion on the peer review of the pesticide risk assessment of the active substance thifensulfuron-methyl.

Table 5.3-25: Validated methods for air (if appropriate)

Component of residue definition: thifensulfuron-methyl			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	2.8 µg/m3	LC/MS/MS	Bacher, R. 2001, DuPont-4560, EU agreed
Primary	21 µg/m3	LC/MS/MS	Sadgrove, L., 2012c, Report No. PII0023 and amendment 1 EU agreed

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

An overview on the acceptable methods and possible data gaps for analysis of thifensulfuron-methyl in body fluids is given in the following tables.

For method for the analysis of tissue please refer to the point 5.3.4.3.

Table 5.3-26: Methods for body fluids

Component of residue definition: thifensulfuron-methyl			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary& Confirmatory	Plasma 1.0 µg/kg Urine 3.0 µg/kg	LC/MS/MS	Henze, R. M., Stry J. J. 2016, DuPont-47394 EU agreed

Appendix 1 Lists of data considered in support of the evaluation

References

Author(s)	Year	Title
EFSA	2010	Conclusion on the peer review of the pesticide risk assessment of the active substance dicamba EFSA Journal 2011;9(1):1965, 1-52
EFSA	2007	Conclusion regarding the peer review of the pesticide risk assessment of the active substance nicosulfuron EFSA Scientific Report (2007) 120, 1-91
EFSA	2015	Conclusion on the peer review of the pesticide risk assessment of the active substance thifensulfuron-methyl. EFSA Journal 2015;13(7):4201
UK	2018	Revised renewal assessment report on thifensulfuron-methyl, confirmatory data, December 2018, revised March 2019.
-	2001	Review report for the active substance thifensulfuron-methyl SANCO/7577/VI/97-final

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	K. Posłuszna	~~2022~~ 2023	DNT-162OD-R-CPd, Determination of physicochemical properties STAB.23-03/INIT CIECH Agro GLP ul. Chemików 1 31-310 Nowa Sarzyna Poland GLP Unpublished	N	CIECH Sarzyna S.A.
KCP 5.1.2_01	V. FAESSEL	2023	Validation of the Analytical Method for the Analysis of Dicamba (sum of Dicamba, 5-OH-dicamba and their conjugates expressed as Dicamba) and Fenoxaprop-p-ethyl and all metabolites which may be converted to 6-chloro-2,3-dihydrobenzoxazol-2-one (expressed as Fenoxaprop-p-ethyl) in Winter Wheat grain. R C2140 ANADIAG 16 rue Ampère 67500 HAGUENAU – FRANCE GLP Unpublished	N	CIECH Sarzyna S.A.
KCP 5.1.2_02	C. Lefebvre	2023	Determination of MCPA, Dicamba and Fenoxaprop-P-Ethyl Residues in winter wheat following one foliar application with MDF-368EW-R-CPd under field conditions in Northern Europe in 2022 R C2136 ANADIAG 16 rue Ampère 67500 HAGUENAU – FRANCE GLP Unpublished	N	CIECH Sarzyna S.A.

KCP 5.1.2_03	E. Schneider	2023	Determination of MCPA and Dicamba Residues in Maize following foliar application with C-340SL-Roz-C under Field Conditions in Northern Europe in 2022 R C2146 ANADIAG 16 rue Ampère 67500 HAGUENAU – FRANCE GLP Unpublished	N	CIECH Sarzyna S.A.
KCP 5.1.2_04	V. FAESSEL	2023	Validation of the Analytical Method for the Analysis of Nicosulfuron, Thifensulfuron-methyl and Triazine amine IN-A4098 in Maize grain R C2160 ANADIAG 16 rue Ampère 67500 HAGUENAU – FRANCE GLP Unpublished	N	CIECH Sarzyna S.A.
KCP 5.1.2_05	E. Thomas - Delille	2023	Determination of Dicamba, Nicosulfuron and Thifensulfuron-methyl Residues in Maize Following Foliar application with DNT-162OD-R-CPd under Field Conditions in Northern Europe in 2022 C2156 ANADIAG 16 rue Ampère 67500 HAGUENAU – FRANCE GLP Unpublished	N	CIECH Sarzyna S.A.
KCP 5.1.2_06	P. Parma	2022	Honeybees *(Apis mellifera L.),* Chronic Oral Toxicity Test EMI/4/62/2022 Ecomelius Institute Sp. z o. o. Kalinowa 2, Zaborze 43-520 Chybie, Poland GLP Unpublished	N	CIECH Sarzyna S.A.

KCP 5.1.2_07	P. Parma	2022	Honeybees *(Apis mellifera L.)* Larval Toxicity Test, Repeated Exposure EMI/4/63/2022 Ecomelius Institute Sp. z o. o. Kalinowa 2, Zaborze 43-520 Chybie, Poland GLP Unpublished	N	CIECH Sarzyna S.A.
KCP 5.1.2_08	S. Szlauer	2022	Daphnia sp., Acute Immobilisation Test EMI/4/70/2022 Ecomelius Institute Sp. z o. o. Kalinowa 2, Zaborze 43-520 Chybie, Poland GLP Unpublished	N	CIECH Sarzyna S.A.
KCP 5.1.2_09	S. Szlauer	2022	Freshwater Alga and Cyanobacteria, Growth Inhibition Test EMI/4/71/2022 Ecomelius Institute Sp. z o. o. Kalinowa 2, Zaborze 43-520 Chybie, Poland GLP Unpublished	N	CIECH Sarzyna S.A.
KCP 5.1.2_10	Z. Kacperek-Karetta	2023	DNT-162OD-R-CPd Navicula pelliculosa SAG 1050-3, Growth inhibition test W-11-23 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Ecotoxicology Research Group Doswiadczalna 27, 43-200 Pszczyna, Poland GLP Unpublished	N	CIECH Sarzyna S.A.

KCP 5.1.2_11	Z. Kacperek-Karetta	2023	DNT-162OD-R-CPd W-12-23 Lemna gibba CPCC 310, Growth inhibition test Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Ecotoxicology Research Group Doświadczalna 27, 43-200 Pszczyna, Poland GLP Unpublished		CIECH Sarzyna S.A.
KCP 5.1.2_12	A.Wojciech	2023	DNT-162OD-R-CPd Bumblebees (Bombus spp.), Acute Oral Toxicity Test STUDY CODE: B-56-23 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Ecotoxicology Research Group Doświadczalna 27, 43-200 Pszczyna, Poland GLP Unpublished	N	CIECH Sarzyna S.A.
KCP 5.1.2_13	A.Wojciech	2023	DNT-162OD-R-CPd Bumblebees (Bombus spp.), Acute Contact Toxicity Test STUDY CODE: B-57-23 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Ecotoxicology Research Group Doświadczalna 27, 43-200 Pszczyna, Poland GLP Unpublished	N	CIECH Sarzyna S.A.

KCP 5.1.2_14	P. Pieczka	2023	DNT-162OD-R-CPd Terrestial Plant Test: Vegetative Vigour Test STUDY CODE: G-33-23 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Ecotoxicology Research Group Doświadczalna 27, 43-200 Pszczyna, Poland GLP Unpublished	N	CIECH Sarzyna S.A.
KCP 5.1.2_15	P. Pieczka	2024	DNT-162OD-R-CPd Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test STUDY CODE: G-34-23 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Ecotoxicology Research Group Doświadczalna 27, 43-200 Pszczyna, Poland GLP Unpublished	N	CIECH Sarzyna S.A.
KCP 5.2_01	Martinez, M. P.	2021	Validation of the analytical method for the determination of Dicamba in bovine fat CH-0667/2021 ChemService S.r.l. Controlli e Ricerche GLP Studies Department Via Fratelli Beltrami, 15, 20026 Novate Milanese (MI) Italy GLP: Yes Unpublished	N	CIECH Sarzyna S.A.

KCP 5.2_02	Longhi D.	2021	Independent laboratory validation of the analytical method 0667/2021 for the determination of Dicamba in Bovine Fat samples GLP-STUDY-21-115 LabAnalysis s.r.l. GLP Studies Department Via Europa 5 27041 Casanova Lonati (PV) Italy GLP: Yes Unpublished	N	CIECH Sarzyna S.A.
KCP 5.2_03	Martinez M. P.	2023	Integration to the Analytical Methods Validations for the Determination of Dicamba in animal matrices CH – 1124-2022 ChemService S.r.l. Controlli e Ricerche GLP Studies Department Via Fratelli Beltrami, 15, 20026 Novate Milanese (MI) Italy GLP: Yes Unpublished	N	CIECH Sarzyna S.A.
KCP 5.2_04	Martinez, M. P	2021	Validation of the Analytical Method for the Determination of Dicamba in Bovine muscle CH – 0670/2021 ChemService S.r.l. Controlli e Ricerche GLP Studies Department Via Fratelli Beltrami, 15 20026 Novate Milanese (MI) Italy GLP: Yes Unpublished	N	CIECH Sarzyna S.A.

KCP 5.2_05	Longhi D.	2021	Independent laboratory validation of the analytical method 0670/2021 for the determination of Dicamba in Bovine Muscle samples GLP-STUDY-21-116 LabAnalysis s.r.l. GLP Studies Department Via Europa 5 27041 Casanova Lonati (PV) Italy GLP: Yes Unpublished	N	CIECH Sarzyna S.A.
KCP 5.2_06	Martinez, M. P	2021	Validation of the Analytical Method for the Determination of Dicamba in Bovine kidney CH – 0671/2021 ChemService S.r.l. Controlli e Ricerche GLP Studies Department Via Fratelli Beltrami, 15 20026 Novate Milanese (MI) Italy GLP: Yes Unpublished	N	CIECH Sarzyna S.A.
KCP 5.2_07	Longhi D.	2021	Independent laboratory validation of the analytical method 0671/2021 for the determination of Dicamba in Bovine Kidney samples GLP-STUDY-21-117 LabAnalysis s.r.l. GLP Studies Department Via Europa 5 27041 Casanova Lonati (PV) Italy GLP: Yes Unpublished	N	CIECH Sarzyna S.A.

KCP 5.2_08	Martinez, M. P	2021	Validation of the Analytical Method for the Determination of Dicamba in Bovine milk CH – 0672/2021 ChemService S.r.l. Controlli e Ricerche GLP Studies Department Via Fratelli Beltrami, 15 20026 Novate Milanese (MI) Italy GLP: Yes Unpublished	N	CIECH Sarzyna S.A.
KCP 5.2_09	Longhi D.	2021	Independent laboratory validation of the analytical method 0672/2021 for the determination of Dicamba in Bovine Milk samples GLP-STUDY-21-118 LabAnalysis s.r.l. GLP Studies Department Via Europa 5 27041 Casanova Lonati (PV) Italy GLP: Yes Unpublished	N	CIECH Sarzyna S.A.
KCP 5.2_10	Longhi D.	2022	Validation of an analytical method for the quantification of Dicamba in poultry eggs GLP-STUDY-22-1 LabAnalysis s.r.l. GLP Studies Department Via Europa 5 27041 Casanova Lonati (PV) Italy GLP: Yes Unpublished	N	CIECH Sarzyna S.A.

KCP 5.2_11	E. Rigamonti	2022	Independent Laboratory Validation (ILV) of the Analytical Method for the Determination of Dicamba in Poultry Eggs CH – 0992/2021 ChemService S.r.l. Controlli e Ricerche GLP Studies Department Via Fratelli Beltrami, 15, 20026 Novate Milanese (MI) Italy GLP: Yes Unpublished	N	CIECH Sarzyna S.A.
KCP 5.2_12	Martinez, M. P	2020	Validation of the Analytical Method for the Determination of Dicamba, Dicamba-5-Hydroxy and Dicamba-desmethyl in Drinking Water CH – 0472/2020 ChemService S.r.l. Controlli e Ricerche GLP Studies Department Via Fratelli Beltrami, 15, 20026 Novate Milanese (MI) Italy GLP: Yes Unpublished	N	CIECH Sarzyna S.A.
KCP 5.2_13	A. Sala	2020	Independent Laboratory Validation (ILV) of the analytical method for the Determination of Dicamba, Dicamba-5-hydroxy and Dicamba-desmethyl in Drinking Water GLP-STUDY-20-62 LabAnalysis s.r.l. GLP Studies Department Via Europa 5, 27041 Casanova Lonati (PV), Italy GLP: Yes Unpublished	N	CIECH Sarzyna S.A.
KCP 5.2_14	D. Longhi	2023	Validation of an analytical method for the quantification of Dicamba in bovine urine LBN-0004-2023 Labanalysis s.r.l. GLP Studies Department Via Europa 5. 27041 Casanova Lonati (PV) Italy GLP: Yes Unpublished	N	CIECH Sarzyna S.A.

KCP 5.2_15	K. Rudziński	2020	VALIDATION OF A METHOD FOR DETERMINATION OF NICOSULFURON IN DRINKING WATER BY LIQUID CHROMATOGRAPHY 20/FSL/04 Food Safety Laboratory Research Institute of Horticulture 13B Pomologiczna Street 96-100 Skierniewice GLP: Yes Unpublished	N	CIECH Sarzyna S.A.
KCP 5.2_16	Blumberg Olga	2024	Validation of an Analytical Method for Nicosulfuron in Food of Animal Origin and Body Fluids S23-107311 Eurofins Agroscience Services EcoChem GmbH GLP Unpublished	N	CIECH Sarzyna S.A.
KCP 5.2_17	Jooss Sandro	2024	Independent Laboratory Validation of an Analytical Method for Determination of Nicosulfuron in Food of Animal Origin and Body Fluids S23-107248 Eurofins Agroscience Services EAG Laboratories GmbH GLP Unpublished	N	CIECH Sarzyna S.A.

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

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
DICAMBA					
KIIA 4.2.1.2/05 DAR, 2007	Maffezoni M.	2004	Dicamba (SAN 837): Validation of Residue Method REM 193.01 in Corn, Rape Seed, Pasture and Oranges Syngenta Crop Protection AG, Basel, Switzerland ADME - Bioanalyses, Vergèze, France, Report No SYN/DIC/03041 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**
			Not Published Syngenta File N° SAN837/6146		
KIIA 4.2.1.2/06 DAR, 2007 .	Steinhauer, S.	2004	Dicamba (SAN 837): Independent Laboratory Validation of Residue Method REM 193.01 for the determination of Dicamba (SAN837) and 5-OH Dicamba (NOA 405873) in Maize (Grain) and Pasture Syngenta Crop Protection AG, Basel, Switzerland Dr. Specht & Partner Chem. Laboratorien GmbH, Hamburg, Germany, Report No ADE-0402V Az. G04-0039 GLP Not Published Syngenta File N° SAN837/6260	N	SYNGENTA
KIIA 4.2.2 DAR, 2007	Gasser, A.	2000a	Determination of Parent Compound Dicamba and Metabolite Dichlorosalicylic Acid by Gas Chromatography (MSD) Novartis Crop Protection AG, Basel, Switzerland, Report No REM 193.02 GLP Not Published Syngenta File N° SAN837/5927	N	SYNGENTA
KIIA 4.2.2 DAR, 2007	Gasser, A.	2000b	Validation of Method REM 193.02 : Validation by Analysis of Soil Specimens Fortified with Dicamba (SAN 837) and its metabolite Dichlorosalicylic Acid (DCSA) and determination of Recoveries Novartis Crop Protection AG, Basel, Switzerland, Report No 301/00 GLP Not Published Syngenta File N° SAN837/5928	N	SYNGENTA
IIA 4.2.4 DAR, 2007	Kettner, R., Karapally, J.	1993	Determination of Dicamba in Air Novartis Crop Protection AG, Basel, Switzerland Sandoz AG, Basel, Switzerland, Report No 21401 GLP Not Published Syngenta File N° SAN837/5366	N	SYNGENTA
NICOSULFURON					

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
IIA, 4.2.1/ 05 DAR 2006	Wolf, St.	2000	Development and validation of the residue analytical method for SL-950 (nicosulfuron) in maize (corn and straw). ISK RCC Ltd, report no. 793596, 2000-12-04 GLP, unpublished	N	ISK
IIA, 4.2.1/ 06 DAR 2006	Ginzburg, N.	2000	Independent laboratory validation (ILV) of the residue analytical method for SL-950 (nicosulfuron) in maize (corn and straw). ISK Battelle, report no. A-22-00- 04, 2000-12-20 GLP, unpublished	N	ISK
IIA, 4.2.2/ 01 DAR 2006	Wais, A.	2000a	Validation of the residue analytical method for SL-950 (nicosulfuron) in soil. ISK RCC Ltd, report no. 770117, 2000-05-03 GLP, unpublished	N	ISK
IIA, 4.2.3 Final addendum to the DAR 2007	Wolf S.	2007	Development and validation of a residue analytical method for nicosulfuron in drinking water and surface water RCC Ltd Report: B25773 GLP: yes Published: No	N	Ishihara Sangyo Kaisha Ltd.
IIA, 4.2.2/ 01 Final addendum to the DAR 2007	Wais, A.	2000c	Validation of the residue analytical method for SL-950 (nicosulfuron) in air. ISK RCC Ltd, report no. 765358, 2000-05-17 GLP, unpublished	N	ISK
THIFENSULFURON-METHYL					

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
IIA, 4.7/01 RAR 2015	Bacher, R.	2001	Development and validation of analytical methods for the determination of seven sulfonylurea herbicides in air PTRL Europe DuPont-4560 GLP: Yes Published: No	N	DuPont
IIA, 4.7/01 RAR 2015	Sadgrove, L.	2012c	Thifensulfuron-methyl: Validation of Methodology for the Determination of Residues in Air. Huntingdon Life Sciences Ltd. EU TSM AIR 2 Task Force Report No. PII0023 and amendment 1 GLP, Unpublished	N	EU TSM AIR 2 Task Force
IIA, 4.5./01 RAR 2015	Devine, T.J., Jin, L.	2004	Analytical method for the determination and confirmation of 13 DuPont sulfonylurea herbicides in water using LC/MS/MS DuPont Stine-Haskell Research Center DuPont-5491, Revision No. 1 **GLP: No** Published: No	N	DuPont
IIA, 4.5/04 RAR 2015	Pentz, A.M., Cabusas, M.E.Y.	2014	Analytical method for the determination and confirmation of 13 DuPont sulfonylurea herbicides in water using LC/MS/MS DuPont Stine-Haskell Research Center DuPont-5491, Supplement No. 1 **GLP: No** Published: No	N	DuPont
IIA, 4.5/01 RAR 2015	Sadgrove, L.	2012b	Thifensulfuron-methyl and thifensulfuron-acid: Validation of Methodology for the Determination of Residues in Surface Water. Huntingdon Life Sciences Ltd. EU TSM AIR 2 Task Force Report No. PII0024 and amendment 1 GLP, Unpublished	N	EU TSM AIR 2 Task Force
IIA, 4.5./02	Henze, R.M., Stry, J.J	2013	Analytical method for the determination of thifensulfuron methyl in water using LC/MS/MS DuPont Stine-Haskell Research Center	N	DuPont

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**
RAR 2015			DuPont-35704 **GLP: No** Published: No		
IIA, 4.5/03 RAR 2015	Mason, B.J.	2013	Independent laboratory validation of DuPont-35704, "Analytical method for the determination of thifensulfuron methyl in water using LC/MS/MS" Morse Laboratories, Inc. DuPont-36531 GLP: Yes Published: No	N	DuPont
IIA, 4.4./02 RAR 2015	Hill, S.J., Stry, J.J.	2001	Analytical method for the determination of 13 DuPont sulfonylurea herbicides in soil using LC/MS/MS DuPont Stine-Haskell Research Center DuPont-5082, Revision No. 1 **GLP: No** Published: No	N	DuPont
IIA, 4.4/04 RAR 2015	Stry, J.J.	2013	Analytical method for the determination of 13 DuPont sulfonylurea herbicides in soil using LC/MS/MS DuPont Stine-Haskell Research Center DuPont-5082, Revision No. 1, Supplement No. 1 **GLP: No** Published: No	N	DuPont
IIA, 4.3/03 RAR 2015	Sadgrove, L.	2012a	Thifensulfuron-methyl and thifensulfuron-acid: Validation of Methodology for the Determination of Residues in Soil. Huntingdon Life Sciences Ltd. EU TSM AIR 2 Task Force Report No. PII0022 and amendment 1 GLP, Unpublished	N	EU TSM AIR 2 Task Force
IIA, 4.3/05 RAR 2015	Charles, E., Doran, A.M.	2004	Independent laboratory validation of analytical method DuPont-13412 for the determination of Thifensulfuron-methyl, ethametsulfuron methyl, rimsulfuron, tribenuron methyl and chlorimuron ethyl in olives and soybean seed using SPE purification and LC/MS/MS detection Inveresk Research International (IRI) Limited (Scotland) DuPont-13398 GLP: Yes	N	DuPont

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**
			Published: No		
IIA, 4.3/07 RAR 2015	Devine, T.J., Nanita, S.C.	2007	Multiresidue analytical method for the determination of sulfonyurea herbicides in oily, watery, acidic and dry crops using SPE purification and LC/MS/MS detection DuPont Stine-Haskell Research Center DuPont-13412, Supplement No. 1 **GLP: No** Published: No	N	DuPont
IIA, 4.3/15 RAR 2015	Pentz, A.M., Bramble, F.Q.	2015	Analytical method for the determination of nicosulfuron, Thifensulfuron-methyl, ethametsulfuron methyl, rimsulfuron, tribenuron methyl, and chlorimuron ethyl in oily crop matrices using SPE purification and LC/MS/MS detection DuPont Stine-Haskell Research Center DuPont-13412, Revision No. 1 **GLP: No** Published: No	N	DuPont
IIA 4.3/16 RAR 2015	Pentz, A.M., Bramble, F.Q., Stry, J.J.	2011	Analytical method for the determination of thifensulfuron methyl and metabolites in crops using LC/MS/MS DuPont Stine-Haskell Research Center DuPont-28527 **GLP: No** Published: No	N	DuPont
IIA 4.3/27 RAR 2015	Henze, R.M., Stry, J.J.	2014	Multiresidue analytical method for the determination of sulfonylurea herbicides in oily, watery, acidic and dry crops using SPE purification and LC/MS/MS detection. DuPont Stine-Haskell Research Center DuPont-13412, Supplement No. 4, Revision No. 1 **GLP: No** Published: No	N	DuPont
IIA, 4.3/01 RAR 2015	Seck, C.	2008	Determination of Residues of Metsulfuron-methyl and Thifensulfuron-methyl in Cereal - Method Validation. Battelle UK Ltd. Rotam Report No. QG/07/021	N	EU TSM AIR 2 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**
			GLP, Unpublished		
IIA, 4.3/02 RAR 2015	Watson, G.	2010	Independent Laboratory Validation of the analytical procedure detailed in the study performed under Rotam ID Number 74-07-47 'Determination of residues of metsulfuron-methyl and Thifensulfuron-methyl in cereal – method validation'. Eurofins Agroscience Services Rotam Report No. S09-02905 GLP, Unpublished	N	EU TSM AIR 2 Task Force
IIA, 4.3/23 Confirmatory information RAR 2019	Schwartz, N.L.	2010c	Independent laboratory validation of "Enforcement method for the determination of Thifensulfuron-methyl, metsulfuron methyl and chlorsulfuron in milk and animal tissues" ABC Laboratories, Inc. (Missouri) DuPont-30911 **GLP: Yes** Published: No	N	DuPont
IIA, 4.3/06 RAR 2015, originally evaluated in 2001	de Bernard, P.A., Powley, C.R.	1993	Enforcement method for the determination of Thifensulfuron-methyl, metsulfuron methyl, and chlorsulfuron in milk and animal tissues DuPont Experimental Station AMR 2715-93 **GLP: No** Published: No	N	DuPont
IIA, 4.3/11 Confirmatory information RAR 2019	Henze, R.M., Stry, J.J.	2007a	Analytical method for the determination of ten DuPont sulfonylurea herbicides in eggs using LC/MS/MS DuPont Stine-Haskell Research Center DuPont-24187 **GLP: No** Published: No	N	DuPont
IIA, 4.3/17 Confirmatory	Pentz, A.M., Cabusas, M.E.Y.	2014	Analytical method for the determination of DuPont sulfonylurea herbicides in animal matrices using HPLC/MS/MS DuPont-30449 Supplement No. 1 DuPont Stine-Haskell Research Center	N	DuPont

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**
information RAR 2019			**GLP: No** Published: No REGISTRATION REPORT Product code: GF-3969 (Poland - Dragster®) Part B, Section 5, Analytical Methods		
IIA, 4.3/22 Confirmatory information RAR 2019	Schwartz, N.L.	2010b	Independent laboratory validation of "Analytical method for the determination of 13 DuPont sulfonylurea herbicides in skim milk, whole milk, and cream using LC/MS/MS" and "Analytical method for the determination of ten DuPont sulfonylurea herbicides in eggs using LC/MS/MS" ABC Laboratories, Inc. (Missouri) DuPont-30910 GLP: Yes Published: No	N	DuPont
IIA, 4.3/21 Confirmatory information RAR 2019	Schwartz, N.L.	2010a	Independent laboratory validation of "Analytical method for the determination of 13 DuPont sulfonylurea herbicides in skim milk, whole milk, and cream using LC/MS/MS" and "Analytical method for the determination of ten DuPont sulfonylurea herbicides in eggs using LC/MS/MS" pertaining to: Independent laboratory validation of "Analytical method for the determination of 13 DuPont sulfonylurea herbicides in skim milk, whole milk, and cream using LC/MS/MS" ABC Laboratories, Inc. (Missouri) DuPont-30909 GLP: Yes Published: No	N	DuPont
IIA, 4.3/08 Confirmatory information RAR 2019	Gant, A.G.	2012	Independent laboratory validation of DuPont-30449, "Analytical method for the determination of DuPont sulfonylurea herbicides in animal matrices using HPLC/MS/MS" ABC Laboratories, Inc. DuPont-30450 GLP: Yes Published: No	N	DuPont
CP 5.2 Product code: GF-3969;	Henze, R.M., Stry, J.J.	2016a	Analytical method for the determination of chlorsulfuron, metsulfuron methyl, thifensulfuron methyl and tribenuron methyl in plasma and urine by LC/MS/MS DuPont Stine-Haskell Research Center DuPont-47394 **Not GLP**	N	DuPont

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
Dragster®			Unpublished		

List of data submitted by the applicant and not relied on

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

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

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

Appendix 1 Detailed evaluation of submitted analytical methods

A 1.1 Methods used for the generation of pre-authorization data (KCP 5.1)

A 1.1.1 Description of analytical methods for the determination of residues in plant matrices (KCP 5.1)

A 1.1.1.1 Analytical method 1

Comments of zRMS:	Method is accepted

Reference:	KCP 5.1.2_01
Report	Validation of the Analytical Method for the Analysis of Dicamba (sum of Dicamba, 5-OH-dicamba and their conjugates expressed as Dicamba) and Fenoxaprop-p-ethyl and all metabolites which may be converted to 6-chloro-2,3-dihydrobenzoxazol-2-one (expressed as Fenoxaprop-p-ethyl) in Winter Wheat grain. R C2140, 2023, V. FAESSEL
Guideline(s):	Yes (SANTE/2020/12830, Rev.1)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

The validation report is linked to the residue studies, point KCA 6.3_01 in Section B7.

Materials and methods
The method under discussion describes the determination of Dicamba (sum of Dicamba, 5-OH-dicamba and their conjugates expressed as Dicamba) in Winter Wheat grain. The method was validated at 0.01 mg/kg for each analyte.

Samples were hydrolyzed at 90°C for 1.5 hours with hydrochloric acid (1M). Residues were extracted by shaking with acetonitrile, MgSO4 and NaCl. After centrifugation, the acetonitrile extract was cleaned up by solid phase extraction (SPE) using Envi-Carb cartridges. Extract was evaporated near to dryness and reconstituted in H2O/ACN 80:20 prior to quantification using liquid chromatography with tandem mass spectrometric detection (UPLC-MS/MS).

Chromatographic conditions

Apparatus	Analytical conditions LC-MS/MS - 6500
Column	ACQUITY HSS T3, 2.1 x 100 mm
Mobile phase	A = H_20 ultra-pure + 0.2% acetic acid B = Acetonitrile
Column temperature	40°C
Detector, IONISATION mode	ES
TRANSITIONS	Dicamba: 174.9 > 144.4 (quantitation) 174.9 > 34.6 (qualification)

	5-OH-dicamba: 235.0 > 140.0 (quantitation) 235.0 > 155.0 (qualification)
Retention time	Dicamba: ≈ 5.6 min. 5-OH-dicamba: ≈ 4.3 min
Injected volume	20 µL

Results and discussions

For the primary method, recovery and repeatability (as precision, % RSD) data complied with the requirements of the SANTE/2020/12830, Rev.1 guideline as mean recoveries were within the range 60-120% with RSD less than 30% for spiked samples at 0.01 mg/kg and within the range 70-120% with RSD less than 20% for spiked samples at 0.10 mg/kg.

For the confirmatory method, recovery and repeatability (as precision, % RSD) data complied with the requirements of the SANTE/2020/12830, Rev.1 guideline as mean recoveries were within the range 60-120% and RSD were less than 30% for spiked samples at the LOQ level.

Table A 1: Recovery results from method validation of dicamba, 5-OH-dicamba using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
Primary 174.9 > 144.4					
winter wheat grain	Dicamba	0.01	82.6	7.3	-
		0.10	97.2	5.9	-
Primary 235.0 > 140.0					
winter wheat grain	5-OH-dicamba	0.01	75.6	6.8	-
		0.10	108.6	4.5	-
Primary 174.9 > 144.4					
winter wheat grain	Dicamba glycoside	0.01	86.3	8.3	-
		0.10	88.7	6.8	-
Primary 235.0 > 140.0					
winter wheat grain	5-OH-dicamba glycoside	0.01	79.1	15.9	-
		0.10	84.9	4.7	-
Confirmatory method 174.9 > 34.6					
winter wheat grain	Dicamba	0.01	85.6	6.1	-
Confirmatory method 235.0 > 155.0					
winter wheat grain	5-OH-dicamba	0.01	103.9	4.6	-
Confirmatory method 174.9 > 34.6					
winter wheat grain	Dicamba glycoside	0.01	74.8	25.2	-
Confirmatory method 235.0 > 155.0					

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
winter wheat grain	5-OH-dicamba glycoside	0.01	73.1	9.8	-

Table A 2: Characteristics for the analytical method used for validation of dicamba, 5-OH-dicamba residues in winter wheat grain

	dicamba	5-OH-dicamba
Specificity	Representative, clearly labelled chromatograms of standard at the lowest calibrated level, untreated sample and sample fortified at the lowest fortification level for each analyte and for both primary and confirmatory methods were provided to prove selectivity of the method. Mass spectra were provided to justify the selection of ions used for determination. Untreated samples (non-fortified samples) were determined from the matrice used in fortification experiments and were not higher than 30% of the LOQ for both primary and confirmatory methods.	
	Primary transition m/z 174.9 > 144.4 Confirmatory transition m/z 174.9 > 34.6	Primary transition m/z 235.0 > 140.0 Confirmatory transition m/z 235.0 > 155.0
Calibration (type, number of data points)	n=5 The linear correlation coefficients were > 0.990, showing good linearity with regression residuals randomly distributed for both primary and confirmatory methods.	
Calibration range	1.7 ng/mL to 120 ng/mL (corresponding to 0.002 to 0.12 mg/kg for Dicamba and 5-OH-dicamba and 0.003 to 0.20 mg/kg for Dicamba glycoside and 5-OH-dicamba glycoside)	
Assessment of matrix effects is presented	Assessment of matrix effects was performed by comparing the analyte response of at least one individual standard prepared in solvent to at least one prepared in blank matrix for both primary and confirmatory methods for Winter Wheat grain. Matrix effects (enhancement or suppression) on the instrument response were considered significant. Consequently, matrix-matched calibration solutions were used for calibration.	
	Primary method: -47.4% Confirmatory method: -48.1%	Primary method: -79.1% Confirmatory method: -80.2%
Stability results for extracts	The stability of extracts during frozen or refrigerated storage was investigated. Dicamba and 5-OH-dicamba residues were stable in winter wheat grain extracts for at least 17 days of refrigerated storage. The stability of matrix-matched standard solutions during refrigerated storage was investigated. Dicamba and 5-OH-dicamba were stable in winter wheat grain matrix-matched calibration solutions for at least 16 days of refrigerated storage.	
Limit of determination/quantification	LOQ = 0.01 mg/kg LOD - 1.7 ng/mL for Dicamba and 5-OH-dicamba in Winter Wheat grain (corresponding to 0.002 mg/kg for Dicamba and 5-OH-dicamba and 0.003 mg/kg for Dicamba glycoside and 5-OH-dicamba glycoside).	

Conclusion

The analytical method was validated according to SANTE/2020/12830, Rev.1. The validation meets all

requirements of the guideline.
The applicability of the method to wheat straw was demonstrated by concurrent recoveries in study number C2136 described under the point KCP 5.1.2_02.

A 1.1.1.2 Analytical method 2

Comments of zRMS:	Method is accepted

Reference:	KCP 5.1.2_02
Report	Determination of MCPA, Dicamba and Fenoxaprop-P-Ethyl Residues in winter wheat following one foliar application with MDF-368EW-R-CPd under field conditions in Northern Europe in 2022, R C2136, C.Lefebvre
Guideline(s):	Yes (SANTE/2020/12830, Rev.1)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Validation of the method on wheat grain was presented in study No. C2140 (submitted under the point KCP 5.1.2_01). The above report – R C2136 - is presented only to demonstrate concurrent (procedural) recoveries for wheat straw.

Table A 3: Concurrent (procedural) recoveries of Dicamba and 5-OH-dicamba

Matrix	Analyte	Fortification level (mg/kg) (*n* = 3)	Mean recovery (%)	RSD (%)	Comments
Straw	Dicamba	0.01	72.3	18.1	-
		0.10	75.8	4.9	-
Straw	5-OH-dicamba	0.01	80.9	8.6	-
		0.10	84.0	1.4	-
Straw	Dicamba glycoside	0.01	73.4	28.9	-
		0.10	75.3	5.3	-
Straw	5-OH-dicamba glycoside	0.01	63.6	6.3	-
		0.10	78.6	2.8	-

Recovery and repeatability (as precision, % RSD) data complied with the requirements of the SANTE/2020/12830, guideline as mean recoveries were within the range 60-120% with RSD less than 30% for spiked samples at 0.01 mg/kg and within the range 70-120% with RSD less than 20% for spiked samples at 0.10 mg/kg.

A 1.1.1.3 Analytical method 3

Comments of zRMS:	Method is accepted

Reference:	KCP 5.1.2_03
Report	Determination of MCPA and Dicamba Residues in Maize Following Foliar application with C-340SL-Roz-C under Field Conditions in Northern Europe in 2022, R C2146, E. Schneider
Guideline(s):	Yes (SANTE/2020/12830, Rev.1)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Validation of the method on wheat grain was presented in study No. C2140 (submitted under the point KCP 5.1.2_01). The above report – R C2146 - is presented only to demonstrate concurrent (procedural) recoveries for maize grain and straw.

Table A 4: Concurrent (procedural) recoveries of Dicamba and 5-OH-dicamba

Matrix	Analyte	Fortification level (mg/kg) (*n* = 3)	Mean recovery (%)	RSD (%)	Comments
Maize Grain	Dicamba	0.01	96.0	6.9	-
		0.10	83.8	4.9	-
Maize Straw		0.01	83.4	22.6	-
		0.10	82.8	8.5	-
Maize Grain	5-OH-dicamba	0.01	85.0	10.5	-
		0.10	81.3	4.8	-
Maize Straw		0.01	95.9	11.0	-
		0.10	91.2	8.0	-
Maize Grain	Dicamba glycoside	0.01	68.1	11.2	-
		0.10	86.7	6.6	-
Maize Straw		0.01	106.6	11.0	-
		0.10	79.6	3.4	-
Maize Grain	5-OH-dicamba glycoside	0.01	84.6	3.4	-
		0.10	82.5	8.7	-
Mazie Straw		0.01	65.9	4.8	-
		0.10	82.3	3.6	-

For grain and straw, recovery and repeatability (as precision, % RSD) data complied with the requirements of the SANTE/2020/12830, Rev.1 guideline as mean recoveries were within the range 60-120% with RSD less than 30% for spiked samples at 0.01 mg/kg and within the range 70-120% with RSD less than 20% for spiked samples at 0.10 mg/kg.

A 1.1.1.4 Analytical method 4

Comments of zRMS:	Method is accepted

Reference:	KCP 5.1.2_04
Report	Validation of the Analytical Method for the Analysis of Nicosulfuron, Thifensulfuron-methyl and Triazine amine IN-A4098 in Maize grain, R C2160, 2023, V.Faessel
Guideline(s):	Yes (SANTE/2020/12830, Rev.1)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The objective of the study was to validate the analytical method for the analysis of nicosulfuron, thifensulfuron-methyl and triazine amine IN-A4098 in maize grain (dry commodities).
Samples were analysed using an Anadiag's in-house method developed from the method "Foods of plant origin - Multimethod for the determination of pesticide residues using GC- and LC-based analysis following acetonitrile extraction/partitioning and clean-up by dispersive SPE - Modular QuEChERSmethod" NF EN 15662:2018-05 (recorded in ANADIAG SOP MC 641).
Samples are extracted by homogenisation with pH buffer at 7 and acetonitrile. After addition of magnesium sulfate and sodium chloride, the mixture is shaken intensively and centrifuged for phase separation. An aliquot of the organic phase is analyzed by LC-MS/MS.

Chromatographic conditions

Apparatus	LC-MS/MS - 6500
Column	HSS T3, 2.1 x 100 mm
Mobile phase	A = Ultra-pure H2O + 0.1% formic acid B = Acetonitrile + 0.1% formic acid
Column temperature	+40°C
Detector, IONISATION mode	ES
Retention time	Nicosulfuron : ≈ 6.0 min. Thifensulfuron-methyl : ≈ 6.1 min. Triazine amine IN-A4098 : ≈ 3.4 min.
Injected volume	4 µL

Results and discussions

For the primary method, recovery and repeatability (as precision, % RSD) data complied with the requirements of the SANTE/2020/12830, Rev.1 guideline as mean recoveries were within the range 60-120% with RSD less than 30% for spiked samples at 0.01 mg/kg and within the range 70-120% with RSD less than 20% for spiked samples at 0.10 mg/kg.
For the confirmatory method, recovery and repeatability (as precision, % RSD) data complied with the requirements of the SANTE/2020/12830, Rev.1 guideline as mean recoveries were within the range 60-120% and RSD were less than 30% for spiked samples at the LOQ level.

Table A 5: Recovery results from method validation of Nicosulfuron, Thifensulfuron-methyl and Triazine amine IN-A4098 using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
Primary method					
Maize Grain	Nicosulfuron	0.01	84.3	1.7	-
		0.10	91.5	2.5	-
Maize Grain	Thifensulfuron methyl	0.01	95.0	5.0	-
		0.10	98.7	1.9	-
Maize Grain	Triazine amine IN-A4098	0.01	82.8	6.1	-
		0.10	80.3	2.1	-
Confirmatory method					
Maize Grain	Nicosulfuron	0.01	80.4	3.0	-
Maize Grain	Thifensulfuron methyl	0.01	95.3	1.8	-
Maize Grain	Triazine amine IN-A4098	0.01	82.8	6.4	-

Table A 6: Characteristics for the analytical method used for validation Nicosulfuron, Thifensulfuron-methyl and Triazine amine IN-A4098 residues in maize grain

	Nicosulfuron	Thifensulfuron-methyl and Triazine amine IN-A4098
Specificity	Representative, clearly labelled chromatograms of standard at the lowest calibrated level, untreated sample and sample fortified at the lowest fortification level for each analyte/matrix combination and for both primary and confirmatory methods were provided in appendix IV to prove selectivity of the method. Mass spectra were provided in appendix III to justify the selection of ions used for determination. Untreated samples (non-fortified samples) were determined from the matrix used in fortification experiments and were not higher than 30% of the LOQ for both primary and confirmatory methods.	
	Primary transition m/z 411.0 > 182.0 Confirmatory transition m/z 411.0 > 213.0	Primary transition Thifensulfuron-methyl m/z 388.0 > 140.9 Triazine amine IN-A4098 m/z 141.0 > 42.0 Confirmatory transition Thifensulfuron-methyl m/z 388.0 > 166.8 Triazine amine IN-A4098 m/z 141.0 > 56.9
Calibration (type, number of data points)	The linear correlation coefficients were > 0.990, showing good linearity with regression residuals randomly distributed for both primary and confirmatory methods.	

Calibration range	The analytical calibration consisted of matrix-matched calibration solutions of nicosulfuron, thifensulfuron-methyl and triazine amine IN-A4098, at least at 5 concentration levels, ranged from 0.75 ng/mL to 30 ng/mL (corresponding to 0.003 to 0.12 mg/kg). The calibration covered two orders of magnitude and ranged from 30% of the LOQ to 20% above the highest level. Standard concentrations were distributed evenly over the full calibration range.	
Assessment of matrix effects is presented	Assessment of matrix effects was performed by comparing the analyte response of at least one individual standard prepared in solvent to at least one prepared in blank matrix for both primary and confirmatory methods. Matrix effects (enhancement or suppression) on the instrument response were considered significant for nicosulfuron and thifensulfuron-methyl (confirmatory method). Consequently, matrix-matched calibration solutions were used for calibration.	
	Primary method 12.5% Confirmatory method 20.4%	Primary method Thifensulfuron-methyl -7.5% Triazine amine IN-A4098 -8.7% Confirmatory method Thifensulfuron-methyl 23.0% Triazine amine IN-A4098 -7.2%
Stability results for extracts	Spiked samples at 10xLOQ level were stored frozen after samples extraction and analysed against freshly prepared standards to check the stability of the final extracts. Nicosulfuron, thifensulfuron-methyl and triazine amine IN-A4098 residues were stable in maize grain extracts for at least 5 days of frozen storage.	
Storage stability of matrix-matched standard solutions	Nicosulfuron, thifensulfuron-methyl and triazine amine IN-A4098 residues were stable in maize grain matrix matched calibration solutions for at least 5 days of frozen storage.	
Limit of determination/quantification	The LOQ was 0.01 mg/kg for nicosulfuron, thifensulfuron-methyl and triazine amine IN-A4098 in maize grain. The LOD was 0.75 ng/mL for nicosulfuron, thifensulfuron-methyl and triazine amine IN-A4098 in maize grain (corresponding to 0.003 mg/kg).	

Conclusion

The analytical method was validated according to SANTE/2020/12830, Rev.1. The validation meets all requirements of the guideline.

A 1.1.1.5 Analytical method 5

Comments of zRMS:	Method is accepted

Reference:	KCP 5.1.2_05
Report	Determination of Dicamba, Nicosulfuron and Thifensulfuron-methyl Residues in Maize Following Foliar application with DNT-162OD-R-CPd under Field Conditions in Northern Europe in 2022, R C2156, 2023, E.Thomas-Delille
Guideline(s):	Yes (SANTE/2020/12830, Rev.1)
Deviations:	No

GLP: Yes

Acceptability: Yes

Validation of the method on wheat grain was presented in study No. C2140 (submitted under the point KCP 5.1.2_01). The above report – R C2156 - is presented only to demonstrate concurrent (procedural) recoveries for maize straw.

Table A 7: Concurrent (procedural) recoveries of of Nicosulfuron, Thifensulfuron-methyl and Triazine amine IN-A4098

Matrix	Analyte	Fortification level (mg/kg) (*n* = 3)	Mean recovery (%)	RSD (%)	Comments
Maize Straw	Nicosulfuron	0.01	73.4	0.1	-
		0.10	70.1	1.6	-
Maize Straw	Thifensulfuron-methyl	0.01	90.8	3.8	-
		0.10	85.3	1.5	-
Maize Straw	IN-A4098	0.01	81.3	0.9	-
		0.10	80.2	1.6	-

Recovery and repeatability (as precision, % RSD) data complied with the requirements of the SANTE/2020/12830, Rev.1 guideline* as mean recoveries were within the range 60-120% with RSD less than 30% for spiked samples at 0.01 mg/kg and within the range 70-120% with RSD less than 20% for spiked samples at 0.10 mg/kg.

A 1.1.2 Description of analytical methods for the determination residues in support of ecotoxicology studies (KCP 5.1)

A 1.1.2.1 Analytical method used for determination residues in Honeybee Chronic Oral Toxicity Test

Comments of zRMS:	Method is accepted

Reference: KCP 5.1.2_06

Report: Honeybees (Apis mellifera L.), Chronic Oral Toxicity Test, EMI/4/62/2022, 2022, P. Parma

Guideline(s): Yes (SANTE/2020/12830, Rev.1)

Deviations: No

GLP: Yes

Acceptability: Yes

The validation method is linked to the ecotoxicological study – Honeybee, Chronic Oral Toxicity Test , point KCP 10.3.1/03 in Section B9.

Materials and methods

The concentration of Dicamba, Nicosulfuron, Thifensulfuron-methyl in 50% sucrose solution was determined using a validated ultrahigh performance liquid chromatographic method with mass spectrometer detection.

Chromatographic conditions

UHPLC-MS/MS	Agilent Infinity 1290, HiP Sampler G4226A, Binary Pump G4220A, Column Comp. G1316C, 6460 Triple Quad Mass Spectrometer (Ion Source AJS ESI)
Column	Guard Column Zorbax SB-C18 2,1×5 mm, 1,8 µm , Column Zorbax SB-C18 RRHT 2,1×50 mm, 1,8 µm, 600 bar
Injection	10 µL
Elution	Isocratic
Mobile phases	60%: water + formic acid 0.5%; 40%: methanol + formic acid 0.05%
Flow	0.4 mL/min
Column temperature	50 °C

Results and discussions

Table A 8: Recovery results from method validation of Dicamba, Nicosulfuron, Thifensulfuron-methyl using the analytical method

Precision and accuracy were determined at 2 concentration levels: 29 µg/L (LOQ) and 290 µg/L (10 × LOQ) of the Test Item DNT-162OD-R-CPd.

Matrix	Analyte	Fortification level (µg/L) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
Sucrose solution	Dicamba	LOQ	111.4	1.8	-
		10xLOQ	92.4	0.6	-
Sucrose solution	Nicosulfuron	LOQ	115.6	1.1	-
		10xLOQ	115.0	5.5	-
Sucrose solution	Thifensulfuron methyl	LOQ	98.6	1.7	-
		10xLOQ	99.8	2.5	-

Table A 9: Characteristics for the analytical method used for validation Dicamba, Nicosulfuron, Thifensulfuron-methyl used for ecotoxicological study

	Dicamba	Nicosulfuron	Thifensulfuron-methyl
Specificity	Specificity was determined on the basis of chromatograms obtained from matrix blank sample and the fortified sample (at LOQ level).		
	The analysis showed that signal of Dicamba was not overlapping with the matrix signal of the control samples under the	The analysis showed that signal of Nicosulfuron was not overlapping with the matrix signal of the control samples under the	The analysis showed that signal of Thifensulfuron-methyl was not overlapping with the matrix signal of the

	experimental conditions. To confirm specificity of analytical method two ion transitions were recorded: Target: 218.9→ 175 Qualifier: 220.9→ 177	experimental conditions. To confirm specificity of analytical method two ion transitions were recorded: Target: 411.1→ 181.9 Qualifier: 411.1→ 139.0	control samples under the experimental conditions. To confirm specificity of analytical method two ion transitions were recorded: Target: 388→ 167.2 Qualifier: 388→ 126.1
Calibration (type, number of data points)	Calibration curve equation: Area = 69.509 C + 40.075 Coefficient of determination R^2: 0.9981	Calibration curve equation: Area = 5908 C + 1617 Coefficient of determination R^2: 0.9969	Calibration curve equation: Area = 1395 C + 106 Coefficient of determination R^2: 0.9964
Calibration range	Linearity was determined by preparing a series of standard solutions of Dicamba in water at the concentrations: 1.0; 3.3; 10.0; 20.0; 33.0; 45.0; 60.0; 80.0 and 100.0 µg/L.	Linearity was determined by preparing a series of standard solutions of Nicosulfuron in water at the concentrations: 0.34; 1.13; 3.4; 6.9; 11.3; 15.4; 20.6; 27.4 and 34.0 µg/L.	Linearity was determined by preparing a series of standard solutions of Thifensulfuron-methyl in water at the concentrations: 0.1; 0.345; 1.0; 2.1; 3.45; 4.7; 6.3; 8.4 and 10.0 µg/L.
Sample stability	Sample of the Test Item in sucrose solution 50% has been stored in ambient temperature for 7 days.		
Assessment of matrix effects is presented	Assessment of matrix effects were performed by comparing the analytes response of the standard solution in ultrapure water containing 33 µg/L of Dicamba to spiked matrix blank ultrapure 50% sucrose solution solution with DNT-162OD-R-CPd at a concentration of 290.1 µg/L. -0.5%	Assessment of matrix effects were performed by comparing the analytes response of the standard solution in ultrapure water containing 11.3 µg/L of Nicosulfuron to spiked matrix blank 50% sucrose solution with DNT-162OD-R-CPd at a concentration of 290.1 µg/L 2.1%	Assessment of matrix effects were performed by comparing the analytes response of the standard solution in ultrapure water containing 3.45 µg/L of Thifensulfuron-methyl to spiked matrix blank 50% sucrose solution with DNT-162OD-R-CPd at a concentration of 290 µg/L. -6.4%
	The matrix effect did not exceed ±20% so it is not considered significant.		
Limit of determination/quantification	LOQ: 29 µg/L for DNT-162OD-R-CPd		
	LOQ = 3.3 µg/L LOD = 1 µg/L	LOQ = 1.13 µg/L LOD = 0.34 µg/L	LOQ = 0.345 µg/L LOD = 0.1 µg/L

Conclusion

The analytical method was validated according to SANTE/2020/12830, Rev.1. The validation meets all requirements of the guideline.

A 1.1.2.2 Analytical method used for determination residues in Honeybees Larval Toxicity Test, Repeated Exposure

Comments of zRMS:	Method is accepted

Reference:	KCP 5.1.2_07
Report	Honeybees (Apis mellifera L.) Larval Toxicity Test, Repeated Exposure, EMI/4/63/2022, P.Parma, 2022
Guideline(s):	Yes (SANTE/2020/12830, Rev.1)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

The validation method is linked to the ecotoxicological study – Honeybees Larval Toxicity Test, Repeated Exposure, point KCP 10.3.1/04 in Section B9.

Materials and methods

The concentration of Dicamba, Nicosulfuron, Thifensulfuron-methyl in water was determined using a validated ultrahigh performance liquid chromatographic method with mass spectrometer detection.

Chromatographic conditions

UHPLC-MS/MS	Agilent Infinity 1290, HiP Sampler G4226A, Binary Pump G4220A, Column Comp. G1316C, 6460 Triple Quad Mass Spectrometer (Ion Source AJS ESI).
Column	Guard Column Zorbax SB-C18 2,1×5 mm, 1,8 µm , Column Zorbax SB-C18 RRHT 2,1×50 mm, 1,8 µm, 600 bar
Injection	10 µL
Elution	Isocratic
Mobile phases	60%: water + formic acid 0.5%; 40%: methanol + formic acid 0.05%
Flow	0.4 mL/min
Column temperature	50 °C

Results and discussions

Table A 10: Recovery results from method validation of Dicamba, Nicosulfuron, Thifensulfuron-methyl using the analytical method

Precision and accuracy were determined at 2 concentration levels: 29 µg/L (LOQ) and 290 µg/L (10 × LOQ) of the Test Item DNT-162OD-R-CPd.

Matrix	Analyte	Fortification level (µg/L) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
water	Dicamba	LOQ	110.0	1.3	-
		10xLOQ	96.4	4.6	-
water	Nicosulfuron	LOQ	119.0	1.7	-
		10xLOQ	111.0	1.3	-
water	Thifensulfuron methyl	LOQ	98.7	0.5	-
		10xLOQ	102.5	0.6	-

Table A 11: Characteristics for the analytical method used for validation Dicamba, Nicosulfuron, Thifensulfuron-methyl used for ecotoxicological study

	Dicamba	Nicosulfuron	Thifensulfuron-methyl
Specificity	Specificity was determined on the basis of chromatograms obtained from matrix blank sample and the fortified sample (at LOQ level).		
	The analysis showed that signal of Dicamba was not overlapping with the matrix signal of the control samples under the experimental conditions. To confirm specificity of analytical method two ion transitions were recorded: Target: 218.9→ 175 Qualifier: 220.9→ 177	The analysis showed that signal of Nicosulfuron was not overlapping with the matrix signal of the control samples under the experimental conditions. To confirm specificity of analytical method two ion transitions were recorded: Target: 411.1→ 181.9 Qualifier: 411.1→ 139.0	The analysis showed that signal of Thifensulfuron-methyl was not overlapping with the matrix signal of the control samples under the experimental conditions. To confirm specificity of analytical method two ion transitions were recorded: Target: 388→ 167.2 Qualifier: 388→ 126.1
Calibration (type, number of data points)	Calibration curve equation: Area = 69.509 C + 40.075 Coefficient of determination R^2: 0.9981	Calibration curve equation: Area = 5908 C + 1617 Coefficient of determination R^2: 0.9969	Calibration curve equation: Area = 1395 C + 106 Coefficient of determination R^2: 0.9964
Calibration range	Linearity was determined by preparing a series of standard solutions of Dicamba in water at the concentrations: 1.0; 3.3; 10.0; 20.0; 33.0; 45.0; 60.0; 80.0 and 100.0 µg/L.	Linearity was determined by preparing a series of standard solutions of Nicosulfuron in water at the concentrations: 0.34; 1.13; 3.4; 6.9; 11.3; 15.4; 20.6; 27.4 and 34.0 µg/L.	Linearity was determined by preparing a series of standard solutions of Thifensulfuron-methyl in water at the concentrations: 0.1; 0.345; 1.0; 2.1; 3.45; 4.7; 6.3; 8.4 and 10.0 µg/L.
Sample stability	Sample of the Test Item in water, has been stored in ambient temperature for 7 days. Then samples were analysed by LC-MS/MS. Recovery of Dicamba, Nicosulfuron, Thifensulfuron-methyl meet the acceptance criteria 70-120% so analyte in water is stable for at least for 7 days.		
Assessment of matrix effects is presented	Assessment of matrix effects were performed by comparing the analytes response of the standard solution in ultrapure water containing 33 µg/L of Dicamba to spiked matrix blank ultrapure water with DNT-162OD-R-CPd at a concentration of 290.1 µg/L 0.5%	Assessment of matrix effects were performed by comparing the analytes response of the standard solution in ultrapure water containing 11.3 µg/L of Nicosulfuron to spiked matrix blank ultrapure water with DNT-162OD-R-CPd at a concentration of 290.1 µg/L 5.4%	Assessment of matrix effects were performed by comparing the analytes response of the standard solution in ultrapure water containing 3.45 µg/L of Thifensulfuron-methyl to spiked matrix blank ultrapure water with DNT-162OD-R-CPd at a concentration of 290 µg/L. 5.6%
	The matrix effect did not exceed ±20% so it is not considered significant.		
Limit of determination/quantification	LOQ: 29 µg/L for DNT-162OD-R-CPd		
	LOQ = 3.3 µg/L LOD = 1 µg/L	LOQ = 1.13 µg/L LOD = 0.34 µg/L	LOQ = 0.345 µg/L LOD = 0.1 µg/L

Conclusion

The analytical method was validated according to SANTE/2020/12830, Rev.1. The validation meets all requirements of the guideline.

A 1.1.2.3 Analytical method used for determination residues in Bumblebees *(Bombus spp.)*, Acute Oral Toxicity Test

Comments of zRMS:	Method is accepted

Reference:	KCP 5.1.2_12
Report	DNT-162OD-R-CPd, Bumblebees (Bombus spp.), Acute Oral Toxicity Test, B-56-23, A. Wojciech, 2023
Guideline(s):	Yes (SANTE/2020/12830, Rev.2)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

The validation method is linked to the ecotoxicological study – Bumblebees *(Bombus spp.),* Acute Oral Toxicity Test , point in KCP 10.3.1_05 Section B9.

Materials and methods

The concentration of active substances of test item was chemically determined using the validated high performance liquid chromatographic method with DAD detection.

Chromatographic conditions

Chromatographic System	High Performance Liquid Chromatography (HPLC)
Column	Luna 5 µm C18 100Å 250 mm×4.6 mm
Owen temperature	35°C
Injection	20 µL
Mobile phase	acetonitryl HPLC:ortho-phosphoric acid solution 0.05 % (45:55. v/v)
Flow	1 mL/min
Wave length	223 nm for dicamba and thifensulfuron-methyl 238 nm for nicosulfuron
Retention time	approx.: 8.15 minute for dicamba approx.: 6.52 minute for thifensulfuron-methyl approx.: 4.89 minute for nicosulfuron

Results and discussions

Table A 12: Recovery results from method validation of Dicamba, Nicosulfuron, Thifen-sulfuron-methyl using the analytical method

Precision of a particular methods is defined as its repeatability (RSD – relative standard deviation [%]). The repeatability for detected substance analyzed are presented in table below. The RSD is ≤ 20% per each level.

Matrix	Analyte	Fortification level (mg/L) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
50% sucrose solution in water	Dicamba	10	94.0	0.2	-
		100	96.4	3.9	-
50% sucrose solution in water	Nicosulfuron	10	94.0	0.5	-
		100	98.2	0.2	-
50% sucrose solution in water	Thifensulfuron methyl	10	93.9	0.4	-
		100	98.1	0.3	-

Table A 13: Characteristics for the analytical method used for validation Dicamba, Nicosulfuron, Thifensulfuron-methyl used for ecotoxicological study

	Dicamba	Nicosulfuron	Thifensulfuron-methyl
Specificity	The analytical method specificity was estimated on the basis of the analysis of the chromatograms obtained for the control, and fortified samples of matrix. Considering the results of the analysis, no signal of detected substance was overlapping with matrix signal of the control samples in the experiments conditions.		
Calibration (type, number of data points)	Linear N=5	Linear N=5	Linear N=5
Calibration range	0.2 – 20.0 mg/L r^2= 0.9997175	0.2 – 20.0 mg/L r^2= 0.9997128	0.2 – 20.0 mg/L r^2= 0.9996955
Assessment of matrix effects is presented	Since potential matrix effects were compensated by using matrix matched calibration standards (same matrix load), no instrument recovery samples were prepared and analysed in addition.		
Limit of determination/quantification	LOQ = 10 mg analyte /L LOD = 2 mg analyte /L	LOQ = 10 mg analyte /L LOD = 2 mg analyte /L	LOQ = 10 mg analyte /L LOD = 2 mg analyte /L
Stock solution	The results for stability were obtained after 0, 26, 48, 59, 91, 125, 157, 172 and 182 days of storage at cool temperature i.e. from 20C to 80C. Compared to the mean recoveries measured at 0 day, significant decline was not observed after 182 days.	The results for stability were obtained after 0, 5, 21 and 41 days of storage at cool temperature i.e. from 20C to 80C. Compared to the mean recoveries measured at 0 day, significant decline was not observed after 41 days.	The results for stability were obtained after 0, 3, 9, 14, 42, 66 and 139 days of storage at cool temperature i.e. from 20C to 80C. Compared to the mean recoveries measured at 0 day, significant decline was not observed after 139 days.

Conclusion

The analytical method was validated according to SANTE/2020/12830, Rev.2. The validation meets all requirements of the guideline.

A 1.1.2.4 Analytical method used for determination residues in Bumblebees *(Bombus spp.),* Acute Contact Toxicity Test

Comments of zRMS:	Method is accepted

Reference:	KCP 5.1.2_13
Report	DNT-162OD-R-CPd, Bumblebees (Bombus spp.), Acute Contact Toxicity Test, B-57-23, 2023, A. Wojciech
Guideline(s):	Yes (SANTE/2020/12830, Rev.2)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

The validation method is linked to the ecotoxicological study – Bumblebees *(Bombus spp.),* Acute Contact Toxicity Test , point in KCP 10.3.1_06 Section B9.

Materials and methods
The concentration of active substances of test item was chemically determined using the validated high performance liquid chromatographic method with DAD detection.

Chromatographic conditions

Chromatographic System	High Performance Liquid Chromatography (HPLC)
Column	Luna 5 µm C18 100Å 250 mm×4.6 mm
Owen temperature	35°C
Injection	20 µL
Mobile phase	acetonitryl HPLC:ortho-phosphoric acid solution 0.05 % (45:55. v/v)
Flow	1 mL/min
Wave length	223 nm for dicamba and thifensulfuron-methyl 238 nm for nicosulfuron
Retention time	approx.: 8.15 minute for dicamba approx.: 6.52 minute for thifensulfuron-methyl approx.: 4.89 minute for nicosulfuron

Results and discussions

Table A 14: Recovery results from method validation of Dicamba, Nicosulfuron, Thifensulfuron-methyl using the analytical method

Precision of a particular methods is defined as its repeatability (RSD – relative standard deviation [%]). The repeatability for detected substance analyzed are presented in table below. The RSD is ≤ 20% per each level.

Matrix	Analyte	Fortification level (mg/L) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
1% Triton (R) X-100 water solution	Dicamba	10	102.8	2.6	-
		100	96.9	2.4	-

Matrix	Analyte	Fortification level (mg/L) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
1% Triton (R) X-100 water solution	Nicosulfuron	10	100.6	2.8	-
		100	90.7	4.8	-
1% Triton (R) X-100 water solution	Thifensulfuron methyl	10	95.6	4.8	-
		100	93.9	1.8	-

Table A 15: Characteristics for the analytical method used for validation Dicamba, Nicosulfuron, Thifensulfuron-methyl used for ecotoxicological study

	Dicamba	Nicosulfuron	Thifensulfuron-methyl
Specificity	The analytical method specificity was estimated on the basis of the analysis of the chromatograms obtained for the control, and fortified samples of matrix. Considering the results of the analysis, no signal of detected substance was overlapping with matrix signal of the control samples in the experiments conditions.		
Calibration (type, number of data points)	Linear N=5	Linear N=5	Linear N=5
Calibration range	0.2 – 20.0 mg/L r^2= 0.9998293 Equivalent calibration range of linearity [mg/L Triton (R) X-1001% solution] 2 – 200	0.2 – 20.0 mg/L r^2= 0.9996983 Equivalent calibration range of linearity [mg/L Triton (R) X-1001% solution] 2 – 200	0.2 – 20.0 mg/L r^2= 0.9997428 Equivalent calibration range of linearity [mg/L Triton (R) X-1001% solution] 2 – 200
Assessment of matrix effects is presented	Since potential matrix effects were compensated by using matrix matched calibration standards (same matrix load), no instrument recovery samples were prepared and analysed in addition.		
Limit of determination/quantification	LOQ = 10 mg analyte /L LOD = 2 mg analyte /L	LOQ = 10 mg analyte /L LOD = 2 mg analyte /L	LOQ = 10 mg analyte /L LOD = 2 mg analyte /L
Stock solution	The results for stability were obtained after 0, 26, 48, 59, 91, 125, 157, 172 and 182 days of storage at cool temperature i.e. from 20C to 80C. Compared to the mean recoveries measured at 0 day, significant decline was not observed after 182 days.	The results for stability were obtained after 0, 5, 21 and 41 days of storage at cool temperature i.e. from 20C to 80C. Compared to the mean recoveries measured at 0 day, significant decline was not observed after 41 days.	The results for stability were obtained after 0, 3, 9, 14, 42, 66 and 139 days of storage at cool temperature i.e. from 20C to 80C. Compared to the mean recoveries measured at 0 day, significant decline was not observed after 139 days.

Conclusion

The analytical method was validated according to SANTE/2020/12830, Rev.2. The validation meets all requirements of the guideline.

A 1.1.2.5 Analytical method used for determination residues in Daphnia sp., Acute Immobilisation Test

Comments of zRMS:	Method is accepted

Reference:	KCP 5.1.2_08
Report	Daphnia sp., Acute Immobilisation Test, EMI/4/70/2022, S. Szlauer, 2022
Guideline(s):	Yes (SANTE/2020/12830, Rev.1)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

The validation method is linked to the ecotoxicological study – Daphnia sp., Acute Immobilisation Test, point KCP 10.2.1/02 in Section B9.

Materials and methods
The concentration of Dicamba, Nicosulfuron, Thifensulfuron-methyl in Eledent medium was determined using a validated ultrahigh performance liquid chromatographic method with mass spectrometer detection.

Chromatographic conditions

UHPLC-MS/MS	Agilent Infinity 1290, HiP Sampler G4226A, Binary Pump G4220A, Column Comp. G1316C, 6460 Triple Quad Mass Spectrometer (Ion Source AJS ESI).
Column	Guard Column Zorbax SB-C18 2,1×5 mm, 1,8 µm , Column Zorbax SB-C18 RRHT 2,1×50 mm, 1,8 µm, 600 bar
Injection	10 µL
Elution	Isocratic
Mobile phases	60%: water + formic acid 0.5%; 40%: methanol + formic acid 0.05%
Flow	0.4 mL/min
Column temperature	50 °C

Results and discussions

Table A 16: Recovery results from method validation of Dicamba, Nicosulfuron, Thifen-sulfuron-methyl using the analytical method

Precision and accuracy were determined at 2 concentration levels: 29 µg/L (LOQ) and 290 µg/L (10 × LOQ) of the Test Item DNT-162OD-R-CPd.

Matrix	Analyte	Fortification level (µg/L) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
Eledent medium	Dicamba	LOQ	110.5	1.5	-
		10xLOQ	95.7	5.5	-
Eledent medium	Nicosulfuron	LOQ	105.9	5.3	-
		10xLOQ	115.6	0.7	-

Matrix	Analyte	Fortification level (µg/L) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
Eledent medium	Thifensulfuron methyl	LOQ	98.3	1.1	-
		10xLOQ	100.0	3.6	-

Table A 17: Characteristics for the analytical method used for validation Dicamba, Nicosulfuron, Thifensulfuron-methyl used for ecotoxicological study

	Dicamba	Nicosulfuron	Thifensulfuron-methyl
Specificity	Specificity was determined on the basis of chromatograms obtained from matrix blank sample and the fortified sample (at LOQ level).		
	The analysis showed that signal of Dicamba was not overlapping with the matrix signal of the control samples under the experimental conditions. To confirm specificity of analytical method two ion transitions were recorded: Target: 218.9→ 175 Qualifier: 220.9→ 177	The analysis showed that signal of Nicosulfuron was not overlapping with the matrix signal of the control samples under the experimental conditions. To confirm specificity of analytical method two ion transitions were recorded: Target: 411.1→ 181.9 Qualifier: 411.1→ 139.0	The analysis showed that signal of Thifensulfuron-methyl was not overlapping with the matrix signal of the control samples under the experimental conditions. To confirm specificity of analytical method two ion transitions were recorded: Target: 388→ 167.2 Qualifier: 388→ 126.1
Calibration (type, number of data points)	Calibration curve equation: Area = 69.509 C + 40.075 Coefficient of determination R^2: 0.9981	Calibration curve equation: Area = 5908 C + 1617 Coefficient of determination R^2: 0.9969	Calibration curve equation: Area = 1395 C + 106 Coefficient of determination R^2: 0.9964
Calibration range	Linearity was determined by preparing a series of standard solutions of Dicamba in water at the concentrations: 1.0; 3.3; 10.0; 20.0; 33.0; 45.0; 60.0; 80.0 and 100.0 µg/L.	Linearity was determined by preparing a series of standard solutions of Nicosulfuron in water at the concentrations: 0.34; 1.13; 3.4; 6.9; 11.3; 15.4; 20.6; 27.4 and 34.0 µg/L.	Linearity was determined by preparing a series of standard solutions of Thifensulfuron-methyl in water at the concentrations: 0.1; 0.345; 1.0; 2.1; 3.45; 4.7; 6.3; 8.4 and 10.0 µg/L.
Sample stability	Sample of the Test Item in Eledent medium, has been stored in ambient temperature for 7 days. Then samples were analysed by LC-MS/MS. Recovery of Dicamba, Nicosulfuron, Thifensulfuron-methyl meet the acceptance criteria 70-120% so analyte in Eledent medium is stable for at least for 7 days.		
Assessment of matrix effects is presented	Assessment of matrix effects were performed by comparing the analytes response of the standard solution in ultrapure water containing 33 µg/L of Dicamba to spiked matrix Eledent medium with DNT-162OD-R-CPd at a concentration of 290.1 µg/L	Assessment of matrix effects were performed by comparing the analytes response of the standard solution in ultrapure water containing 11.3 µg/L of Nicosulfuron to spiked matrix Eledent medium with DNT-162OD-R-CPd at a concentration of 290.1 µg/L	Assessment of matrix effects were performed by comparing the analytes response of the standard solution in ultrapure water containing 3.45 µg/L of Thifensulfuron-methyl to spiked Eledent medium with DNT-162OD-R-CPd at a concentration of 290 µg/L.

	1.6%	-6.4%	-3.7%
	The matrix effect did not exceed ±20% so it is not considered significant.		
Limit of determination/quantification	LOQ: 29 µg/L for DNT-162OD-R-CPd		
	LOQ = 3.3 µg/L LOD = 1 µg/L	LOQ = 1.13 µg/L LOD = 0.34 µg/L	LOQ = 0.345 µg/L LOD = 0.1 µg/L

Conclusion

The analytical method was validated according to SANTE/2020/12830, Rev.1. The validation meets all requirements of the guideline.

A 1.1.2.6 Analytical method used for determination residues in Freshwater Alga and Cyanobacteria, Growth Inhibition Test

Comments of zRMS:	Method is accepted

Reference:	KCP 5.1.2_09
Report	Freshwater Alga and Cyanobacteria,, EMI/4/71/2022, S. Szlauer, 2022
Guideline(s):	Yes (SANTE/2020/12830, Rev.1)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

The validation method is linked to the ecotoxicological study – Freshwater Alga and Cyanobacteria, Growth Inhibition Test - point KCP 10.2.1/03 in Section B9.

Materials and methods

The concentration of Dicamba, Nicosulfuron, Thifensulfuron-methyl in AAP medium was determined using a validated ultrahigh performance liquid chromatographic method with mass spectrometer detection.

Chromatographic conditions

UHPLC-MS/MS	Agilent Infinity 1290, HiP Sampler G4226A, Binary Pump G4220A, Column Comp. G1316C, 6460 Triple Quad Mass Spectrometer (Ion Source AJS ESI).
Column	Guard Column Zorbax SB-C18 2,1×5 mm, 1,8 µm , Column Zorbax SB-C18 RRHT 2,1×50 mm, 1,8 µm, 600 bar
Injection	10 µL
Elution	Isocratic
Mobile phases	60%: water + formic acid 0.5%; 40%: methanol + formic acid 0.05%
Flow	0.4 mL/min
Column temperature	50 °C

Results and discussions

Table A 18: Recovery results from method validation of Dicamba, Nicosulfuron, Thifensulfuron-methyl using the analytical method

Precision and accuracy were determined at 2 concentration levels: 29 µg/L (LOQ) and 290 µg/L (10 × LOQ) of the Test Item DNT-162OD-R-CPd.

Matrix	Analyte	Fortification level (µg/L) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
AAP medium	Dicamba	LOQ	110.3	1.1	-
		10xLOQ	96.4	5.1	-
AAP medium	Nicosulfuron	LOQ	97.4.	0.7	-
		10xLOQ	108.9	1.7	-
AAP medium	Thifensulfuron methyl	LOQ	98.0	1.4	-
		10xLOQ	98.7	1.8	-

Table A 19: Characteristics for the analytical method used for validation Dicamba, Nicosulfuron, Thifensulfuron-methyl used for ecotoxicological study

	Dicamba	Nicosulfuron	Thifensulfuron-methyl
Specificity	Specificity was determined on the basis of chromatograms obtained from matrix blank sample and the fortified sample (at LOQ level).		
	The analysis showed that signal of Dicamba was not overlapping with the matrix signal of the control samples under the experimental conditions. To confirm specificity of analytical method two ion transitions were recorded: Target: 218.9→ 175 Qualifier: 220.9→ 177	The analysis showed that signal of Nicosulfuron was not overlapping with the matrix signal of the control samples under the experimental conditions. To confirm specificity of analytical method two ion transitions were recorded: Target: 411.1→ 181.9 Qualifier: 411.1→ 139.0	The analysis showed that signal of Thifensulfuron-methyl was not overlapping with the matrix signal of the control samples under the experimental conditions. To confirm specificity of analytical method two ion transitions were recorded: Target: 388→ 167.2 Qualifier: 388→ 126.1
Calibration (type, number of data points)	Calibration curve equation: Area = 69.509 C + 40.075 Coefficient of determination R^2: 0.9981	Calibration curve equation: Area = 5908 C + 1617 Coefficient of determination R^2: 0.9969	Calibration curve equation: Area = 1395 C + 106 Coefficient of determination R^2: 0.9964
Calibration range	Linearity was determined by preparing a series of standard solutions of Dicamba in water at the concentrations: 1.0; 3.3; 10.0; 20.0; 33.0; 45.0; 60.0; 80.0 and 100.0 µg/L.	Linearity was determined by preparing a series of standard solutions of Nicosulfuron in water at the concentrations: 0.34; 1.13; 3.4; 6.9; 11.3; 15.4; 20.6; 27.4 and 34.0 µg/L.	Linearity was determined by preparing a series of standard solutions of Thifensulfuron-methyl in water at the concentrations: 0.1; 0.345; 1.0; 2.1; 3.45; 4.7; 6.3; 8.4 and 10.0 µg/L.
Sample stability	Sample of the Test Item in AAP medium, has been stored in ambient temperature for 7 days. Then samples were analysed by LC-MS/MS. Recovery of Dicamba (after 7 days), Nicosulfuron (after 8 days), Thifensulfuron-		

	methyl (7 days) meet the acceptance criteria 70-120% so analyte in AAP is stable for at least for 7 days.		
Assessment of matrix effects is presented	Assessment of matrix effects were performed by comparing the analytes response of the standard solution in ultrapure water containing 33 µg/L of Dicamba to spiked matrix blank AAP medium with DNT-162OD-R-CPd at a concentration of 290.1 µg/L 1.9%	Assessment of matrix effects were performed by comparing the analytes response of the standard solution in ultrapure water containing 11.3 µg/L of Nicosulfuron to spiked matrix AAP medium with DNT-162OD-R-CPd at a concentration of 290.1 µg/L -6.4%	Assessment of matrix effects were performed by comparing the analytes response of the standard solution in ultrapure water containing 3.45 µg/L of Thifensulfuron-methyl to spiked AAP medium with DNT-162OD-R-CPd at a concentration of 290 µg/L. -5.4%
	The matrix effect did not exceed ±20% so it is not considered significant.		
Limit of determination/quantification	LOQ: 29 µg/L for DNT-162OD-R-CPd		
	LOQ = 3.3 µg/L LOD = 1 µg/L	LOQ = 1.13 µg/L LOD = 0.34 µg/L	LOQ = 0.345 µg/L LOD = 0.1 µg/L

Conclusion

The analytical method was validated according to SANTE/2020/12830, Rev.1. The validation meets all requirements of the guideline.

A 1.1.2.7 Analytical method used for determination residues in Navicula pelliculosa SAG 1050-3, Growth inhibition test

Comments of zRMS:	Method is accepted

Reference:	KCP 5.1.2_10
Report	Navicula pelliculosa SAG 1050-3, Growth inhibition test, W-11-23, Z.Kacperek-Karetta, 2023
Guideline(s):	Yes (SANTE/2020/12830, Rev.2)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

The validation method is linked to the ecotoxicological study – Navicula pelliculosa SAG 1050-3, Growth inhibition test - point KCP 10.2.1/01 in Section B9.

Materials and methods

The concentration of active substances of test item was chemically determined using the validated high performance liquid chromatographic method with DAD detection.

Chromatographic conditions

UHPLC-MS/MS Shimadzu, Prominence-i (Shimadzu Corporation Japan)

Column	Luna 5 µm C18 (2)100Å 250 mm×4.6 mm
Injection	35 µL
Mobile phases	acetonitryl HPLC : ortho-phosphoric acid solution 0.05 % (45 : 55. v/v)
Flow	1 mL/min
Column temperature	35°C
Wave length	223 nm for dicamba and thifensulfuron-methyl 238 nm for nicosulfuron
Retention time	approx.: 8.15 minute for dicamba approx.: 6.52 minute for thifensulfuron-methyl approx.: 4.89 minute for nicosulfuron

Results and discussions

Table A 20: Recovery results from method validation of Dicamba, Nicosulfuron, Thifensulfuron-methyl using the analytical method

Matrix	Analyte	Fortification level (mg/L) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
AAP+Si medium	Dicamba	LOQ	90.0	2.2	-
		10xLOQ	99.4	1.4	-
AAP+Si medium	Nicosulfuron	LOQ	96.0	0.0	-
		10xLOQ	101.2	1.4	-
AAP+Si medium	Thifensulfuron methyl	LOQ	100.0	2.0	-
		10xLOQ	103.8	1.5	-

Table A 21: Characteristics for the analytical method used for validation Dicamba, Nicosulfuron, Thifensulfuron-methyl used for ecotoxicological study

	Dicamba	Nicosulfuron	Thifensulfuron-methyl
Specificity	The analytical method specificity was estimated on the basis of the analysis of the chromatograms obtained for the control, and fortified samples of matrix. Considering the results of the analysis, no signal of detected substance was overlapping with matrix signal of the control samples in the experiments conditions. Therefore, the specificity of the method was demonstrated.		
Calibration (type, number of data points)	The equation of the calibration line is presented as the linear equation; y = ax + b (a – slope, b – intercept).		
	Slope 104543 Intercept -28.3014 Coefficient r^2 0.9991448	Slope 73668.3 Intercept 303.245 Coefficient r^2 0.9991586	Slope 105378 Intercept 366.121 Coefficient r^2 0.9984630
Calibration range	Range of linearity of calibration curve: 0.01 – 5.0mg/L Equivalent calibration range of linearity [mg/L AAP+Si medium]: 0.01 – 5.0	Range of linearity of calibration curve: 0.01 – 5.0mg/L Equivalent calibration range of linearity [mg/L AAP+Si medium]: 0.01 – 5.0	Range of linearity of calibration curve: 0.01 – 5.0mg/L Equivalent calibration range of linearity [mg/L AAP+Si medium]: 0.01 – 5.0

Stock solution stability	The results for stability were obtained after 0, 26, 48, 59, 91, 125, 157, 172 and 182 days of storage at cool temperature i.e. from 20°C to 80°C. Compared to the mean recoveries measured at 0 day. significant decline was not observed after 182 days.	The results for stability were obtained after 0, 5, 21 and 41 days of storage at cool temperaturę, i.e. from 20°C to 80°C. Compared to the mean recoveries measured at 0 day, significant decline was not observed after 41 days.	The results for stability were obtained after 0, 3, 9, 14, 42, 66 and 139 days of storage at cool te i.e. from 20°C to 80°C. Compared to the mean recoveries measured at 0 day, significant decline was not observed after 139 days.mperature
	Since potential matrix effects were compensated by using matrix matched calibration standards (same matrix load), no instrument recovery samples were prepared and analysed in addition.		
Limit of determination/quantification	LOQ= 0.05mg/L LOD= 0.01mg/L		

Conclusion

The analytical method was validated according to SANTE/2020/12830, Rev.2. The validation meets all requirements of the guideline.

A 1.1.2.8 Analytical method used for determination residues in Lemna gibba CPCC 310, Growth inhibition test

Comments of zRMS:	Method is accepted

Reference:	KCP 5.1.2_11
Report	Lemna gibba CPCC 310, Growth inhibition test,W-12-23, Z.Kacperek-Karetta, 2023
Guideline(s):	Yes (SANTE/2020/12830, Rev.2)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

The validation method is linked to the ecotoxicological study – Lemna gibba CPCC 310, Growth inhibition test - point KCP 10.2.1/04 in Section B9.

Materials and methods

The determination was accomplished by the high performance liquid chromatography (HPLC) with MS/MS detection.

Chromatographic conditions, Dicamba

HPLC-MS/MS	Shimadzu Nexera XR
Column	Luna Omega 3µm PS C18 100Å, l=100 mm, Ø=2.1 mm
Injection	20µL
Mobile phases	Water : Formic acid (1000 : 1, v/v) Acetonitrile
Flow	0.6 mL/min

Column temperature	40°C
Transitions	218.90 --> 175.101 220.90 --> 176.90
Retention time	approx. 2.1 min

Chromatographic conditions, Nicosulfuron and Thifensulfuron-methyl

HPLC-MS/MS	Shimadzu Nexera XR
Column	Luna Omega 3µm PS C18 100Å, l=100 mm, Ø=2.1 mm
Injection	20 µL
Mobile phases	Water : Formic acid (1000 : 1, v/v) Acetonitrile
Flow	0.4 mL/min
Column temperature	40°C
Transitions	Nicosulfuron 411.1 --> 182.11 411.1 --> 213.12 Thifensulfuron-methyl 388.0 --> 176.11 388.2 --> 141.12
Retention time	Nicosulfuron approx. 3.6 min Thifensulfuron-methyl approx. 3.7 min

Results and discussions

Table A 22: Recovery results from method validation of Dicamba, Nicosulfuron, Thifensulfuron-methyl using the analytical method

Matrix	Analyte	Fortification level (mg/L) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
dilution method					
20X AAP medium	Dicamba	LOQ	92.3	7.9	-
		10xLOQ	110.0	1.8	-
SPE method					
20X AAP medium	Dicamba	LOQ	70.8	1.1	
		10xLOQ	72.5	1.4	
dilution method					
20X AAP medium	Nicosulfuron	LOQ	85.0	2.2	-
		10xLOQ	105.0	3.8	-
SPE method					
20X AAP medium	Nicosulfuron	LOQ	90.8	9.1	
		10xLOQ	93.5	3.7	
dilution method					
20X AAP medium	Thifensulfuron methyl	LOQ	103.0	4.9	-
		10xLOQ	102.0	11.8	-

Matrix	Analyte	Fortification level (mg/L) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
SPE method					
20X AAP medium	Thifensulfuron methyl	LOQ	84.3	5.9	
		10xLOQ	82.8	5.4	

Table A 23: Characteristics for the analytical method used for validation Dicamba, Nicosulfuron, Thifensulfuron-methyl used for ecotoxicological study

	Dicamba	Nicosulfuron	Thifensulfuron-methyl
Specificity	The analytical methods specificity were estimated on the basic of the analysis of the chromatograms obtained for the control matrix, and fortified samples of matrix. Considering the results of the analysis, no signal of detected substance was overlapping with matrix signal of the control samples in the experimental conditions. Therefore, the specificity of the method was demonstrated		
Calibration (type, number of data points)	The standard curves of dicamba, nicosulfuron and thifensulfuron-methyl (peak area versus quantity of the standard) are linear. n=5 The equations of the calibration lines are presented as the linear equation; y = ax + b (a – slope, b – intercept).		
	dilution method		
	Slope 18618.5 Intercept 13059.4	Slope 708852 Intercept 128888	Slope 612123 Intercept 70582.8
	SPE method		
	Slope 21791.9 Intercept -1023.46	Slope 469611 Intercept 21748.0	Slope 465844 Intercept 5767.51
Calibration range	range of linearity of calibration curve [ng/mL] dilution method 1.0 – 100.0, R^2 = 0.9996841 SPE method 1.0 – 100.0, R^2 = 0.9997150	range of linearity of calibration curve [ng/mL] dilution method 0.1 – 10.0 R^2 = 0.9995937 SPE method 0.05 – 5.0 R^2 = 0.9969953	range of linearity of calibration curve [ng/mL] dilution method 0.1 – 10.0 R^2= 0.9995891 SPE method 0.05 – 5.0 R^2= 0.9975623
Stock solution stability	The results for stability were obtained after 0, 26, 48, 59, 91, 125, 157, 172 and 182 days of storage at cool temperature i.e. from 2ºC to 8ºC. Compared to the mean recoveries measured at 0 day. significant decline was not observed after 182 days.	The results for stability were obtained after 0, 5, 21 and 41 days of storage at cool temperature i.e. from 2ºC to 8ºC. Compared to the mean recoveries measured at 0 day. significant decline was not observed after 41 days.	The results for stability were obtained after 0, 3, 9, 14, 42, 66 and 139 days of storage at cool temperature i.e. from 2ºC to 8ºC. Compared to the mean recoveries measured at 0 day significant decline was not observed after 139 days.
Matrix effect	Since potential matrix effects were compensated by using matrix matched calibration standards (same matrix load), no instrument recovery samples were prepared and analysed in addition.		
Limit of	LOQ	LOQ	LOQ

determination/quantification	dilution method 10.0 µg/L SPE method 0.4 µg/L LOD dilution method 2.0 µg/L SPE method 0.2 µg/L	dilution method 1.0 µg/L SPE method 0.04 µg/L LOD dilution method 2.0 µg/L SPE method 0.01 µg/L	dilution method 1.0 µg/L SPE method 0.04 µg/L LOD dilution method 0.2 µg/L SPE method 0.01 µg/L

Conclusion

The analytical method was validated according to SANTE/2020/12830, Rev.2. The validation meets all requirements of the guideline.

A 1.1.2.9 Analytical method used for determination residues in Terrestial Plant Test: Vegetative Vigour Test

Comments of zRMS:	Method is accepted

Reference:	KCP 5.1.2_14
Report	DNT-162OD-R-CPd Terrestial Plant Test: Vegetative Vigour Test, G-33-23, P. Pieczka, 2023
Guideline(s):	Yes (SANTE/2020/12830, Rev.2)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

The validation method is linked to the ecotoxicological study – Terrestial Plant Test: Vegetative Vigour Test - point KCP 10.6.2_02 in Section B9.

Materials and methods

The analytical method was developed for the determination of active substances of test item in matrix. The determination was accomplished by the high performance liquid chromatography (HPLC) with DAD detection. The validated analytical method was performed according to SANTE/2020/12830. Rev. 2

Chromatographic conditions

Chromatographic System	High Performance Liquid Chromatography (HPLC)
Chromatograph	Shimadzu, Prominence-i (Shimadzu Corporation Japan)
Analytical Column	Luna 5 µm C18 100Å 250 mm×4.6 mm
Oven temperature	35°C
Injection Volume	20 µL (dilution analytical method) 30 µL (SPE method)
Mobile Phase	acetonitrile HPLC : ortho-phosphoric acid solution 0.05 % (45 : 55. v/v)
Flow Rate	1 mL/min
Wave length	223 nm for dicamba and thifensulfuron-methyl 238 nm for nicosulfuron
Retention time	approx.: 8.15 minute for dicamba approx.: 4.89 minute for nicosulfuron approx.: 6.52 minute for thifensulfuron-methyl
Detection System	Diode Array Detector

Results and discussions

The accuracy of the method is reported as mean recovery ± relative standard deviation. Recovery was reported as mean recovery ± relative standard deviation (RSD). Mean recoveries for each level is in the range 70-120%.

Table A 24: Recovery results from method validation of Dicamba, Nicosulfuron, Thifensulfuron-methyl using the analytical method

Matrix	Analyte	Fortification level (mg/L) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
Dilution method					
water	Dicamba	2	105.5	1.0	-
		20	101.5	0.2	-
water	Nicosulfuron	2	105.7	1.4	-
		20	100.0	0.3	-
water	Thifensulfuron methyl	2	106.4	1.1	-
		20	98.5	0.2	-
SPE method					
water	Dicamba	0.005	83.2	8.4	-
		0.05	102.2	7.0	-
water	Nicosulfuron	0.005	102.8	13.8	-
		0.05	90.2	0.9	-
water	Thifensulfuron methyl	0.005	119.4	8.4	-
		0.05	91.6	2.6	-

Table A 25: Characteristics for the analytical method used for validation Dicamba, Nicosulfuron, Thifensulfuron-methyl used for ecotoxicological study

	Dicamba	Nicosulfuron	Thifensulfuron-methyl
Specificity	The analytical method specificity was estimated on the basis of the analysis of the chromatograms obtained for the control, and fortified samples of matrix. Considering the results of the analysis, no signal of detected substances were overlapping with matrix signal of the control samples in the experiments conditions. Therefore, the specificity of the method was demonstrated.		
Precision	Precision of a particular methods is defined as its repeatability (RSD – relative standard deviation [%]). The RSD is ≤ 20% per each level.		
Calibration (type, number of data points)	dilution analytical method		
	The standard curves of dicamba, nicosulfuron, thifensulfurn-methyl (peak area versus quantity of the standard) are linear.		
	r2= 0.9999367	r2= 0.9999324	r2= 0.9999517

	SPE method		
	r2= 0.9998101	r2= 0.9999487	r2= 0.9996366
Calibration range	dilution analytical method		
	range of linearity of calibration curve [mg/L] 0.2 – 20 equivalent calibration range of linearity [mg/L water] 0.4 – 40	range of linearity of calibration curve [mg/L] 0.2– 20 equivalent calibration range of linearity [mg/L water] 0.4 – 40	range of linearity of calibration curve [mg/L] 0.2– 20 equivalent calibration range of linearity [mg/L water] 0.4 – 40
	SPE method		
	range of linearity of calibration curve [mg/L] 0.02-5.0 equivalent calibration range of linearity [mg/L water] 0.001 – 0.25	range of linearity of calibration curve [mg/L] 0.02-5.0 equivalent calibration range of linearity [mg/L water] 0.001 – 0.25	range of linearity of calibration curve [mg/L] 0.02-5.0 equivalent calibration range of linearity [mg/L water] 0.001 – 0.25
Stock solution stability	The results for stability were obtained after 0, 26, 48, 59, 91, 125, 157, 172 and 182 days of storage at cool temperature i.e. from 2°C to 8°C. Compared to the mean recoveries measured at 0 day. significant decline was not observed after 182 days.	The results for stability were obtained after 0, 5, 21 and 41 days of storage at cool temperature i.e. from 2°C to 8°C. Compared to the mean recoveries measured at 0 day. significant decline was not observed after 41 days.	The results for stability were obtained after 0, 3, 9, 14, 42, 66 and 139 days of storage at cool temperature i.e. from 2°C to 8°C. Compared to the mean recoveries measured at 0 day. significant decline was not observed after 139 days.
Matrix effect	dilution analytical method		
	Since potential matrix effects were compensated by using matrix matched calibration standards (same matrix load), no instrument recovery samples were prepared and analysed in addition.		
	SPE method		
	Matrix effect for each method was checked during the method validation. Assessment of matrix effects was performed by comparing the standard preparing in solution to standard preparing in blank matrix at appropriate concentration.		
	5.2	6.0	-0.9
Limit of determination/quantification	Dilution method LOQ= 2.0 mg/L, LOD= 0.4 mg/L Solid Phase Extraction method (SPE method) LOQ= 0.005 mg/L, LOD= 0.001 mg/L		

Conclusion

The analytical method was validated according to SANTE/2020/12830, Rev.2. The validation meets all requirements of the guideline.

A 1.1.2.10 Analytical method used for determination residues in Terrestial Plant Test: Seedling Emergence and Seedling Growth Test

Comments of zRMS:	Method is accepted

Reference:	KCP 5.1.2_15
Report	DNT-162OD-R-CPd Terrestial Plant Test: Seedling Emergence and Seedling Growth Test, G-34-23, P. Pieczka, 2024
Guideline(s):	Yes (SANTE/2020/12830, Rev.2)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

The validation method is linked to the ecotoxicological study – Terrestial Plant Test: Seedling Emergence and Seedling Growth TestTest - KCP 10.6.2_01 in Section B9.

Materials and methods

The analytical method was developed for the determination of active substances of test item in matrix. The determination was accomplished by the high performance liquid chromatography (HPLC) with DAD detection. The validated analytical method was performed according to SANTE/2020/12830. Rev. 2

Chromatographic conditions

Chromatographic System	High Performance Liquid Chromatography (HPLC)
Chromatograph	Shimadzu, Prominence-i (Shimadzu Corporation Japan)
Analytical Column	Luna 5 µm C18 100Å 250 mm×4.6 mm
Oven temperature	35°C
Injection Volume	20 µL (dilution analytical method) 30 µL (SPE method)
Mobile Phase	acetonitrile HPLC: ortho-phosphoric acid solution 0.05 % (45:55. v/v)
Flow Rate	1 mL/min
Wave length	223 nm for dicamba and thifensulfuron-methyl 238 nm for nicosulfuron
Retention time	approx.: 8.15 minute for dicamba approx.: 4.89 minute for nicosulfuron approx.: 6.52 minute for thifensulfuron-methyl
Detection System	Diode Array Detector

Results and discussions

The accuracy of the method is reported as mean recovery ± relative standard deviation. Recovery was reported as mean recovery ± relative standard deviation (RSD). Mean recoveries for each level is in the range 70-120%.

Table A 26: Recovery results from method validation of Dicamba, Nicosulfuron, Thifensulfuron-methyl using the analytical method

Matrix	Analyte	Fortification level (mg/L) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
Dilution method					
water	Dicamba	2	105.5	1.0	-
		20	101.5	0.2	-
water	Nicosulfuron	2	105.7	1.4	-
		20	100.0	0.3	-
water	Thifensulfuron methyl	2	106.4	1.1	-
		20	98.5	0.2	-
SPE method					
water	Dicamba	0.005	83.2	8.4	-
		0.05	102.2	7.0	-
water	Nicosulfuron	0.005	102.8	13.8	-
		0.05	90.2	0.9	-
water	Thifensulfuron methyl	0.005	119.4	8.4	-
		0.05	91.6	2.6	-

Table A 27: Characteristics for the analytical method used for validation Dicamba, Nicosulfuron, Thifensulfuron-methyl used for ecotoxicological study

	Dicamba	Nicosulfuron	Thifensulfuron-methyl
Specificity	The analytical method specificity was estimated on the basis of the analysis of the chromatograms obtained for the control, and fortified samples of matrix. Considering the results of the analysis, no signal of detected substances were overlapping with matrix signal of the control samples in the experiments conditions. Therefore, the specificity of the method was demonstrated.		
Precision	Precision of a particular methods is defined as its repeatability (RSD – relative standard deviation [%]). The RSD is ≤ 20% per each level.		
Calibration (type, number of data points)	dilution analytical method		
	The standard curves of dicamba, nicosulfuron, thifensulfurn-methyl (peak area versus quantity of the standard) are linear.		
	r2= 0.9999367	r2= 0.9999324	r2= 0.9999517
	SPE method		
	r2= 0.9998101	r2= 0.9999487	r2= 0.9996366
Calibration range	dilution analytical method		
	range of linearity of calibration curve [mg/L] 0.2– 20 equivalent calibration range of linearity [mg/L water] 0.4 – 40	range of linearity of calibration curve [mg/L] 0.2– 20 equivalent calibration range of linearity [mg/L water] 0.4 – 40	range of linearity of calibration curve [mg/L] 0.2– 20 equivalent calibration range of linearity [mg/L water] 0.4 – 40
	SPE method		

	range of linearity of calibration curve [mg/L] 0.02-5.0 equivalent calibration range of linearity [mg/L water] 0.001 – 0.25	range of linearity of calibration curve [mg/L] 0.02-5.0 equivalent calibration range of linearity [mg/L water] 0.001 – 0.25	range of linearity of calibration curve [mg/L] 0.02-5.0 equivalent calibration range of linearity [mg/L water] 0.001 – 0.25
Stock solution stability	The results for stability were obtained after 0, 26, 48, 59, 91, 125, 157, 172 and 182 days of storage at cool temperature i.e. from 2°C to 8°C. Compared to the mean recoveries measured at 0 day. significant decline was not observed after 182 days.	The results for stability were obtained after 0, 5, 21 and 41 days of storage at cool temperature i.e. from 2°C to 8°C. Compared to the mean recoveries measured at 0 day. significant decline was not observed after 41 days.	The results for stability were obtained after 0, 3, 9, 14, 42, 66 and 139 days of storage at cool temperature i.e. from 2°C to 8°C. Compared to the mean recoveries measured at 0 day. significant decline was not observed after 139 days.
Matrix effect	dilution analytical method		
	Since potential matrix effects were compensated by using matrix matched calibration standards (same matrix load), no instrument recovery samples were prepared and analysed in addition.		
	SPE method		
	Matrix effect for each method was checked during the method validation. Assessment of matrix effects was performed by comparing the standard preparing in solution to standard preparing in blank matrix at appropriate concentration.		
	5.2	6.0	-0.9
Limit of determination/quantification	Dilution method LOQ= 2.0 mg/L, LOD= 0.4 mg/L Solid Phase Extraction method (SPE method) LOQ= 0.005 mg/L, LOD= 0.001 mg/L		

Conclusion

The analytical method was validated according to SANTE/2020/12830, Rev.2. The validation meets all requirements of the guideline.

A 1.2 Methods for post-authorization control and monitoring purposes, Dicamba (KCP 5.2)

A 1.2.1 Description of analytical methods for the determination of residues in plant matrices (KCP 5.2)

No new or additional studies have been submitted

A 1.2.2 Description of analytical methods for the determination of residues in animal matrices (KCP 5.2)

A 1.2.2.1 Description of analytical methods for the determination of residues in Bovine Fat (KCP 5.2)

A 1.2.2.1.1 Method validation

Comments of zRMS:	Method is accepted

Reference:	KCP 5.2_01
Report	Validation of the Analytical Method for the Determination, CH – 0667/2021, M. Pardo Martinez, 2021
Guideline(s):	Yes, SANTE/2020/12830 rev. 1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The method was demonstrated to be highly specific for the determination Dicamba residues in Bovine Fat matrix samples by virtue of the HPLC/MS/MS technique.
The procedure of extraction used is a QuEChERS technique that involves an extraction phase with solvent.

Chromatographic conditions

HPLC Column	Luna Omega Polar C18, 5 µm, 150 x 4.6 mm i.d.
Phenomenex or equivalent	
Interface	Electron spray ionization (ESI), negative polarity
Detector	MS Triple quadrupole (MRM mode)
Column temperature	30°C
Eluent D	Water with 0.2% formic acid
Eluent C	Methanol with 0.2% formic acid
Gradient	from D:C: 70:30 to D:C: 5:95 in 15 minutes
	D:C: 5:95 for 5 minutes
Eluent flow	0.4 mL/min
Volume of injection	20 µL
R. T. Dicamba	about 16.1 minutes
Mass scan parameters	
Ion mode	ESI -
Dry gas temperature	350°C
Dry gas flow	10 L/min
Precursor ion	175 m/z [M less COOH]-
Product ion	35.1 m/z with collision energy of 5 V (quantifier)
	145.0 m/z with collision energy of 0 V (qualifier)

Results and discussions

Table A 28: Recovery results from method validation of dicamba using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (n = 5)	Mean recovery (%)	RSD (%)	Comments
quantifier, 175 *m/z* > 35.1 *m/z*					

Matrix	Analyte	Fortification level (mg/kg) (n = 5)	Mean recovery (%)	RSD (%)	Comments
Bovine Fat	dicamba	0.010	103.4	3.88	-
		0.100	88.5	11.63	-
Product ion 145.0 *m/z*					
Bovine Fat	dicamba	0.010	107.4	6.07	-
		0.100	86.5	10.81	-

Table A 29: Characteristics for the analytical method used for validation of dicamba residues in bovine fat

	Dicamba
Specificity	The analytical method, using the HPLC/MS/MS instrument with quantification by external standard, was shown to be specific for Dicamba residues in Bovine Fat samples since no residues of the analyte were detected in any blank samples at or above the Limit of Detection.
Confirmatory	Since the analysis by HPLC using an external standard and MS triple quadrupole detector (HPLC/MS/MS) in MRM mode is highly specific and gave both quantification and identification data, a confirmatory test using another instrumental technique was not necessary. Precursor ion (m/z) 175.0 Product ion (m/z) Quantifier 35.1 Qualifier 145.0
Calibration (type, number of data points)	Linear N=5 Quantifier: Y = 50X + 29 Qualifier: Y = 26X + 1
Calibration range	(mg/kg) 0.002 – 0.197
Assessment of matrix effects is presented	A significant matrix effects (ion suppression/enhancement) for Dicamba in the Bovine Fat matrix was found (higher than 20%). Therefore, matrix-matched calibration standards were used throughout the entire study.
Limit of determination/quantification	LOQ= 0.010mg/kg LOD= 0.002mg/kg

Conclusion

According the SANTE/2020/12830 rev. 1 (2021) guideline's requirement, the mean recovery values must be in the range 70 to 120 %, with an RSD% lower than 20%.
Since all recovery values for the analyte at both fortification levels (L.O.Q and 10 x L.O.Q.) resulted to be in the correct range, this criterion was fulfilled and therefore, the analytical method can be considered suitable to quantify Dicamba in Bovine Fat samples.

A 1.2.2.1.2 Independent laboratory validation

Comments of zRMS:	Method is accepted

Reference:	KCP 5.2_02
Report	Independent laboratory validation of the analytical method 0667/2021 for the determination of Dicamba in Bovine Fat samples, GLP-STUDY-21-115, D. Longhi, 2021
Guideline(s):	Yes, SANTE/2020/12830 rev.1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The objective of this study was the independent laboratory validation (ILV) of the analytical method 0667/2021 (Test Facility Chemservice s.r.l.) to determine Dicamba residues in bovine fat samples.
The analytical method was based on an extraction of the analyte from the sample using the QuEChERS method and on the analysis of the extract with a HPLC-MS/MS. The analytical method was validated in compliance with SANTE/2020/12830 rev.1 (2021) guideline in the study:
- CH – 0667/2021, Test Facility: Chemservice s.r.l., Study Director: Mercedes Pardo Martinez.

Chromatographic conditions

Instrument	HPLC Agilent 1290 Infinity II coupled with a triple quadrupole mass spectrometer Agilent 6470A
Column	Phenomenex LUNA C18 (2), 4.6 x 150 mm, 5 µm
Column temperature	30°C
Flow	0.4 mL/min
Injection volume	20 µL
Mobile phase A	Water with 0.2% formic acid (prepared dissolving 4 mL of formic acid in 2 L of LC-MS grade water)
Mobile phase B	Methanol with 0.2% formic acid (prepared dissolving 4 mL of formic acid in 2 L of LC-MS grade methanol)
Source type:	ESI
Gas temperature:	350°C
Gas flow (L/min):	10
MRM monitored transitions	Quantifier, Precursor ion (m/z) 175, Product ion (m/z) 35.1 Qualifier, Precursor ion (m/z) 175, Product ion (m/z) 145.0

Results and discussions

Table A 30: Recovery results from independent laboratory validation of dicamba using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (n = 5)	Mean recovery (%)	RSD (%)	Comments
Primary (175.0/35.1)					
Bovine fat	dicamba	0.0100	102.5	2.7	-
		0.100	93.5	1.8	-
Confirmatory (175.0/145.0)					
Bovine fat	dicamba	0.0100	104.1	3.4	-
		0.100	92.5	0.84	-

Table A 31: Characteristics for the analytical method used for independent laboratory validation of dicamba residues in bovine fat

	dicamba
Specificity	Verified: no interferences found in untreated samples in amounts higher than 30% of the LOQ (< LOD)
Calibration (type, number of data points)	The regression residuals plots show that residuals are randomly distributed, hence demonstrating applicability of the linear calibration. Correlation coefficients R^2 are ≥ 0.99. N=5
Calibration range	Range: 2.00 – 200 µg/L 0.00200 – 0.200 mg/kg (from 20 % of LOQ to 100 % above 10xLOQ)
Assessment of matrix effects is presented	Calibration matrix-matched
Limit of determination/quantification	LOQ =0.010 mg/kg LOD= 0.100 mg/kg)

Conclusion

The ILV was carried out according to SANTE/2020/12830 rev.1 (2021) validating the parameters of linearity, recovery, precision, LOQ and selectivity/specificity. The results meet all the requirements of the guideline.

A 1.2.2.1.3 Extraction efficiency

Comments of zRMS:	Method is accepted

Reference:	KCP 5.2_03
Report	Integration to the Analytical Methods Validations for the Determination of Dicamba in animal matrices, M.P.Martinez, CH – 1124-2022, 2023

Guideline(s):	Yes, SANTE/2020/12830 rev.1, SANTE 2017/10632 rev. 3
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The aim of this study is to integrate, to the GLP Studies CH-0667/2021, CH-0670/2021, CH- 0671/2021 and CH-0672/2021, the extraction efficiency by a cross-validation with the procedures used in metabolism studies reported in the Draft Renewal Assessment Report prepared according to the Commission Regulation (EU) N° 1107/2009 for Dicamba (Volume 3 – B.5 (AS).

The quantification for the extracted samples using the method reported in the studies CH-0667/2021, CH-0670/2021, CH-0671/2021 and CH-0672/2021 were performed using a single point standard prepared in solvent since no matrix effect was verified in the validated methods.
The quantification for the extracted samples using the method reported in the metabolism studies were performed using a single point standard prepared in a matrix-matched solutions obtained by the same extraction method at the theoretical solution concentration.

Chromatographic conditions

HPLC column	Phenomenex or equivalent Luna Omega Polar C18, 5 µm, 150 x 4.6 mm i.d.
Column temperature	30°C
Eluent D	Water with 0.2% formic acid
Eluent C	Methanol with 0.2% formic acid
Eluent flow	0.4 mL/min
Volume of injection	20 µL
Dicamba ret. time	about 16.1 minutes
Source parameters	
Ion mode	AJS ESI -
Gas temperature	350°C
Dry gas flow	10 L/min.

Results

Matrix	Ion (m/z)	VALIDATED METHOD Results	METABOLISM METHOD Results	DIFFERENCE (%) (limit: +/- 30%) (Metabolism method used as reference)
Bovine fat	Quantifier m/z 35.1	0.93	1.04	-10.2
	Qualifier m/z 145.0	0.90	1.01	-10.9
Bovine muscle	Quantifier m/z 35.1	0.78	0.95	-18.0
	Qualifier m/z 145.0	0.77	0.95	-19.6
Bovine kidney	Quantifier m/z 35.1	0.72	0.92	-21.7
	Qualifier m/z 145.0	0.73	0.92	-20.6
Bovine milk	Quantifier m/z 35.1	0.80	1.05	-24.3
	Qualifier m/z 145.0	0.77	1.00	-22.3

The difference of the amounts of analyte found in the sample with the validated analytical method and those found applying the extraction procedure of the metabolism study is less than 30%: the extraction efficiency can be considered proven.

A 1.2.2.2 Description of analytical methods for the determination of residues in Bovine Fat (KCP 5.2)

A 1.2.2.2.1 Method validation

Comments of zRMS:	Method is accepted

Reference:	KCP 5.2_04
Report	Validation of the Analytical Method for the Determination of Dicamba in Bovine muscle, CH – 0670/2021, M. Pardo Martinez, 2021
Guideline(s):	Yes, SANTE/2020/12830 rev. 1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The method was demonstrated to be highly specific for the determination Dicamba residues in Bovine muscle matrix samples by virtue of the HPLC/MS/MS technique.
The procedure of extraction used is a QuEChERS technique that involves an extraction phase with solvent and a clean-up phase to purify the extract before the injection at HPLC/MS/MS,

Chromatographic conditions

HPLC Column	Luna Omega Polar C18, 5 µm, 150 x 4.6 mm i.d.
Phenomenex or equivalent	
Interface	Electron spray ionization (ESI), negative polarity
Detector	MS Triple quadrupole (MRM mode)
Column temperature	30°C
Eluent D	Water with 0.2% formic acid
Eluent C	Methanol with 0.2% formic acid
Gradient	from D:C: 70:30 to D:C: 5:95 in 15 minutes D:C: 5:95 for 5 minutes
Eluent flow	0.4 mL/min
Volume of injection	20 µL
R. T. Dicamba	about 16.1 minutes
Mass scan parameters	
Ion mode	ESI -
Dry gas temperature	350°C
Dry gas flow	10 L/min
Precursor ion	175 m/z [M less COOH]-
Product ion	35.1 m/z with collision energy of 5 V (quantifier) 145.0 m/z with collision energy of 0 V (qualifier)

Results and discussions

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

Matrix	Analyte	Fortification level (mg/kg) (n = 5)	Mean recovery (%)	RSD (%)	Comments
quantifier, 175 *m/z* > 35.1 *m/z*					
Bovine muscle	dicamba	0.010	74.7	2.87	-
		0.100	79.0	2.11	-
Product ion 145.0 *m/z*					
Bovine muscle	dicamba	0.010	74.9	1.75	-
		0.100	86.1	1.82	-

Table A 33: Characteristics for the analytical method used for validation of dicamba residues in bovine muscle

	Dicamba
Specificity	The analytical method, using the HPLC/MS/MS instrument with quantification by external standard, was shown to be specific for Dicamba residues in Bovine muscle samples since no residues of the analyte were detected in any blank samples at or above the Limit of Detection.
Confirmatory	Since the analysis by HPLC using an external standard and MS triple quadrupole detector (HPLC/MS/MS) in MRM mode is highly specific and gave both quantification and identification data, a confirmatory test using another instrumental technique was not necessary. Precursor ion (m/z) 175.0 Product ion (m/z) Quantifier 35.1 Qualifier 145.0
Calibration (type, number of data points)	Linear N=5 No significant memory peak was detected in the wash matrix injected after the highest working standard solution and the range tested for Dicamba was found to be linear (each correlation coefficient > 0.99).
Calibration range	(mg/kg) 0.002 – 0.207
Assessment of matrix effects is presented	No significant matrix effects (ion suppression/enhancement) for Dicamba in the Bovine muscle matrix were found (higher than 20%). Since no significant matrix effect was found, the calibration standards could be prepared both in solvent (acetonitrile) or in matrix (extracted bovine muscle). A matrix-matched calibration standards were used throughout the entire study.
Limit of determination/quantification	LOQ= 0.010 mg/kg LOD= 0.002 mg/kg

Conclusion

According the SANTE/2020/12830 rev. 1 (2021) guideline's requirement, the mean recovery values must be in the range 70 to 120 %, with an RSD% lower than 20%.

Since all recovery values for the analyte at both fortification levels (L.O.Q and 10 x L.O.Q.) resulted to be in the correct range, this criterion was fulfilled and therefore, the analytical method can be considered suitable to quantify Dicamba in bovine muscle samples.

A 1.2.2.2.2 Independent laboratory validation

Comments of zRMS:	Method is accepted

Reference:	KCP 5.2_05
Report	Independent laboratory validation of the analytical method 0670/2021 for the determination of Dicamba in Bovine Muscle samples, GLP-STUDY-21-116, D. Longhi, 2021
Guideline(s):	Yes, SANTE/2020/12830 rev. 1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The objective of this study was the independent laboratory validation (ILV) of the analytical method 0670/2021 (Test Facility Chemservice s.r.l.) to determine Dicamba residues in bovine muscle samples.
The analytical method is based on an extraction of the analyte from the sample using the QuEChERS method, followed by a clean-up with a SPE and on the analysis of the extract with a HPLC-MS/MS. The analytical method was validated in compliance with SANTE/2020/12830 rev.1 (2021) guideline in the study:
- CH – 0670/2021, Test Facility: Chemservice s.r.l., Study Director: Mercedes Pardo Martinez.

Chromatographic conditions

Instrument	HPLC Agilent 1290 Infinity II coupled with a triple quadrupole mass spectrometer Agilent 6470A
Column	Phenomenex LUNA C18 (2), 4.6 x 150 mm, 5 µm
Column temperature	30°C
Flow	0.4 mL/min
Injection volume	20 µL
Mobile phase A	Water with 0.2% formic acid (prepared dissolving 4 mL of formic acid in 2 L of LC-MS grade water)
Mobile phase B	Methanol with 0.2% formic acid (prepared dissolving 4 mL of formic acid in 2 L of LC-MS grade methanol)
Source type:	ESI
Gas temperature:	350°C
Gas flow (L/min):	10
MRM monitored transitions	Quantifier, Precursor ion (m/z) 175, Product ion (m/z) 35.1 Qualifier, Precursor ion (m/z) 175, Product ion (m/z) 145.0

Results and discussions

Table A 34: Recovery results from independent laboratory validation of dicamba using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (n = 5)	Mean recovery (%)	RSD (%)	Comments
Primary (175.0/35.1)					
Bovine muscle	dicamba	0.0100	100.9	14.0	-
		0.100	96.3	12.2	-
Confirmatory (175.0/145.0)					
Bovine muscle	dicamba	0.0100	108.3	5.4	-
		0.100	107.8	5.2	-

Table A 35: Characteristics for the analytical method used for independent laboratory validation of dicamba residues in bovine muscle

	dicamba
Specificity	Verified: no interferences found in untreated samples in amounts higher than 30% of the LOQ (< LOD)
Calibration (type, number of data points)	The regression residuals plots show that residuals are randomly distributed, hence demonstrating applicability of the linear calibration. Correlation coefficients R^2 are ≥ 0.99. N=5
Calibration range	Range: 2.00 – 200 µg/L 0.00200 – 0.200 mg/kg (from 20 % of LOQ to 100 % above 10xLOQ)
Assessment of matrix effects is presented	Calibration matrix-matched
Limit of determination/quantification	LOQ =0.010 mg/kg LOD= 0.100 mg/kg)

Conclusion

The ILV was carried out according to SANTE/2020/12830 rev.1 (2021) validating the parameters of linearity, recovery, precision, LOQ and selectivity/specificity. The results meet all the requirements of the guideline.

A 1.2.2.2.3 Extraction efficiency

It is referred to Point A.2.2.1.3.

A 1.2.2.3 Description of analytical methods for the determination of residues in Bovine kidney (KCP 5.2)

A 1.2.2.3.1 Method validation

Comments of zRMS:	Method is accepted

Reference:	KCP 5.2_06
Report	Validation of the Analytical Method for the Determination of Dicamba in Bovine kidney, CH – 0671/2021, M.P.Martinez, 2021
Guideline(s):	Yes, SANTE/2020/12830 rev. 1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The method was demonstrated to be highly specific for the determination Dicamba residues in Bovine kidney matrix samples by virtue of the HPLC/MS/MS technique.
The procedure of extraction used is a QuEChERS technique that involves an extraction phase with solvent and a clean-up phase to purify the extract before the injection at HPLC/MS/MS,

Chromatographic conditions

HPLC Column	Luna Omega Polar C18, 5 µm, 150 x 4.6 mm i.d.
Phenomenex or equivalent	
Interface	Electron spray ionization (ESI), negative polarity
Detector	MS Triple quadrupole (MRM mode)
Column temperature	30°C
Eluent D	Water with 0.2% formic acid
Eluent C	Methanol with 0.2% formic acid
Gradient	from D:C: 70:30 to D:C: 5:95 in 15 minutes D:C: 5:95 for 5 minutes
Eluent flow	0.4 mL/min
Volume of injection	20 µL
R. T. Dicamba	about 16.1 minutes
Mass scan parameters	
Ion mode	ESI -
Dry gas temperature	350°C
Dry gas flow	10 L/min
Precursor ion	175 m/z [M less COOH]-
Product ion	35.1 m/z with collision energy of 5 V (quantifier) 145.0 m/z with collision energy of 0 V (qualifier)

Results and discussions

Table A 36: Recovery results from method validation of dicamba using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (n = 5)	Mean recovery (%)	RSD (%)	Comments
Quantifier, 175 *m/z* > 35.1 *m/z*					
Bovine kideny	dicamba	0.010	99.8	4.82	-
		0.100	73.8	6.61	-
Qualifier 175 *m/z* >145.0 *m/z*					
Bovine kidney	dicamba	0.010	95.8	14.68	-
		0.100	78.4	2.09	-

Table A 37: Characteristics for the analytical method used for validation of dicamba residues in bovine muscle

	Dicamba
Specificity	The analytical method, using the HPLC/MS/MS instrument with quantification by external standard, was shown to be specific for Dicamba residues in Bovine kidney samples since no residues of the analyte were detected in any blank samples at or above the Limit of Detection.
Confirmatory	Since the analysis by HPLC using an external standard and MS triple quadrupole detector (HPLC/MS/MS) in MRM mode is highly specific and gave both quantification and identification data, a confirmatory test using another instrumental technique was not necessary. Precursor ion (m/z) 175.0 Product ion (m/z) Quantifier 35.1 Qualifier 145.0
Calibration (type, number of data points)	Linear N=5 No significant memory peak was detected in the wash matrix injected after the highest working standard solution and the range tested for Dicamba was found to be linear (each correlation coefficient > 0.99).
Calibration range	(mg/kg) 0.002 – 0.201
Assessment of matrix effects is presented	No significant matrix effects (ion suppression/enhancement) for Dicamba in the Bovine kidney matrix were found (higher than 20%). Since no significant matrix effect was found, the calibration standards could be prepared both in solvent (acetonitrile) or in matrix (extracted Bovine kidney). A matrix-matched calibration standards were used throughout the entire study.
Limit of determination/quantification	LOQ= 0.010 mg/kg LOD= 0.002 mg/kg

Conclusion

According the SANTE/2020/12830 rev. 1 (2021) guideline's requirement, the mean recovery values must be in the range 70 to 120 %, with an RSD% lower than 20%.
Since all recovery values for the analyte at both fortification levels (L.O.Q and 10 x L.O.Q.) resulted to be in the correct range, this criterion was fulfilled and therefore, the analytical method can be considered suitable to quantify Dicamba in bovine kidney samples.

A 1.2.2.3.2 Independent laboratory validation

Comments of zRMS:	Method is accepted

Reference:	KCP 5.2_07
Report	Independent laboratory validation of the analytical method 0671/2021 for the determination of Dicamba in Bovine Kidney samples,GLP-STUDY-21-117, D. Longhi, 2021
Guideline(s):	Yes, SANTE/2020/12830 rev. 1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The objective of this study was the independent laboratory validation (ILV) of the analytical method 0671/2021 (Test Facility Chemservice s.r.l.) to determine Dicamba residues in bovine kidney samples.
The analytical method is based on a extraction of the analyte from the sample using acetonitrile, followed by the analysis of the extract with a HPLC-MS/MS. The analytical method was validated in compliance with SANTE/2020/12830 rev.1 (2021) guideline in the study:
- CH – 0671/2021, Test Facility: Chemservice s.r.l., Study Director: Mercedes Pardo Martinez.

Chromatographic conditions

Instrument	HPLC Agilent 1290 Infinity II coupled with a triple quadrupole mass spectrometer Agilent 6470A
Column	Phenomenex LUNA C18 (2), 4.6 x 150 mm, 5 µm
Column temperature	30°C
Flow	0.4 mL/min
Injection volume	20 µL
Mobile phase A	Water with 0.2% formic acid (prepared dissolving 4 mL of formic acid in 2 L of LC-MS grade water)
Mobile phase B	Methanol with 0.2% formic acid (prepared dissolving 4 mL of formic acid in 2 L of LC-MS grade methanol)
Source type:	ESI
Gas temperature:	350°C
Gas flow (L/min):	10
MRM monitored transitions	Quantifier, Precursor ion (m/z) 175, Product ion (m/z) 35.1 Qualifier, Precursor ion (m/z) 175, Product ion (m/z) 145.0

Results and discussions

Table A 38: Recovery results from independent laboratory validation of dicamba using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (n = 5)	Mean recovery (%)	RSD (%)	Comments
Primary (175.0/35.1)					
Bovine kideny	dicamba	0.0100	90.8	9.8	-
		0.100	76.1	5.4	-
Confirmatory (175.0/145.0)					
Bovine kideny	dicamba	0.0100	97.7	9.7	-
		0.100	76.5	3.3	-

Table A 39: Characteristics for the analytical method used for independent laboratory validation of dicamba residues in bovine kideny

	dicamba
Specificity	Verified: no interferences found in untreated samples in amounts higher than 30% of the LOQ (< LOD)
Calibration (type, number of data points)	The regression residuals plots show that residuals are randomly distributed, hence demonstrating applicability of the linear calibration. Correlation coefficients R^2 are ≥ 0.99. N=5
Calibration range	Range: 2.00 – 200 µg/L 0.00200 – 0.200 mg/kg (from 20 % of LOQ to 100 % above 10xLOQ)
Assessment of matrix effects is presented	Calibration matrix-matched
Limit of determination/quantification	LOQ =0.010 mg/kg LOD= 0.100 mg/kg)

Conclusion

The ILV was carried out according to SANTE/2020/12830 rev.1 (2021) validating the parameters of linearity, recovery, precision, LOQ and selectivity/specificity. The results meet all the requirements of the guideline.

A 1.2.2.3.3 Extraction efficiency

It is referred to Point A.2.2.1.3.

A 1.2.2.4 Description of analytical methods for the determination of residues in Bovine milk (KCP 5.2)

A 1.2.2.4.1 Method validation

Comments of zRMS:	Method is accepted

Reference:	KCP 5.2_08
Report	Validation of the Analytical Method for the Determination of Dicamba in Bovine milk, CH – 0672/2021, M.P.Martinez, 2021
Guideline(s):	Yes, SANTE/2020/12830 rev. 1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The method was demonstrated to be highly specific for the determination Dicamba residues in Bovine milk matrix samples by virtue of the HPLC/MS/MS technique.

Chromatographic conditions

HPLC Column	Luna Omega Polar C18, 5 µm, 150 x 4.6 mm i.d.
Phenomenex or equivalent	
Interface	Electron spray ionization (ESI), negative polarity
Detector	MS Triple quadrupole (MRM mode)
Column temperature	30°C
Eluent D	Water with 0.2% formic acid
Eluent C	Methanol with 0.2% formic acid
Gradient	from D:C: 70:30 to D:C: 5:95 in 15 minutes D:C: 5:95 for 5 minutes
Eluent flow	0.4 mL/min
Volume of injection	20 µL
R. T. Dicamba	about 16.1 minutes
Mass scan parameters	
Ion mode	ESI -
Dry gas temperature	350°C
Dry gas flow	10 L/min
Precursor ion	175 m/z [M less COOH]-
Product ion	35.1 m/z with collision energy of 5 V (quantifier) 145.0 m/z with collision energy of 0 V (qualifier)

Results and discussions

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

Matrix	Analyte	Fortification level (mg/kg) (n = 5)	Mean recovery (%)	RSD (%)	Comments
Quantifier, 175 *m/z* > 35.1 *m/z*					
Bovine milk	dicamba	0.010	113.5	1.58	-

Matrix	Analyte	Fortification level (mg/kg) (n = 5)	Mean recovery (%)	RSD (%)	Comments
		0.100	79.7	2.06	-
Qualifier 175 *m/z* >145.0 *m/z*					
Bovine milk	dicamba	0.010	113.0	2.33	-
		0.100	81.6	3.88	-

Table A 41: Characteristics for the analytical method used for validation of dicamba residues in bovine milk

	Dicamba
Specificity	The analytical method, using the HPLC/MS/MS instrument with quantification by external standard, was shown to be specific for Dicamba residues in Bovine milk samples since no residues of the analyte were detected in any blank samples at or above the Limit of Detection.
Confirmatory	Since the analysis by HPLC using an external standard and MS triple quadrupole detector (HPLC/MS/MS) in MRM mode is highly specific and gave both quantification and identification data, a confirmatory test using another instrumental technique was not necessary. Precursor ion (m/z) 175.0 Product ion (m/z) Quantifier 35.1 Qualifier 145.0
Calibration (type, number of data points)	Linear N=5 No significant memory peak was detected in the wash matrix injected after the highest working standard solution and the range tested for Dicamba was found to be linear (each correlation coefficient > 0.99).
Calibration range	(mg/kg) 0.002 – 0.199
Assessment of matrix effects is presented	No significant matrix effects (ion suppression/enhancement) for Dicamba in the Bovine milk matrix were found (higher than 20%). Since no significant matrix effect was found, the calibration standards could be prepared both in solvent (ethyl acetate) or in matrix (extracted Bovine milk).
Limit of determination/quantification	LOQ= 0.010 mg/kg LOD= 0.002 mg/kg

Conclusion

According the SANTE/2020/12830 rev. 1 (2021) guideline's requirement, the mean recovery values must be in the range 70 to 120 %, with an RSD% lower than 20%.
Since all recovery values for the analyte at both fortification levels (L.O.Q and 10 x L.O.Q.) resulted to be in the correct range, this criterion was fulfilled and therefore, the analytical method can be considered suitable to quantify Dicamba in bovine milk samples.

A 1.2.2.4.2 Independent laboratory validation

Comments of zRMS:	Method is accepted

Reference:	KCP 5.2_09
Report	Independent laboratory validation of the analytical method 0672/2021 for the determination of Dicamba in Bovine Milk samples, GLP-STUDY-21-118, D. Longhi, 2021
Guideline(s):	Yes, SANTE/2020/12830 rev. 1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The objective of this study was the independent laboratory validation (ILV) of the analytical method 0672/2021 (Test Facility Chemservice s.r.l.) to determine Dicamba residues in bovine milk samples.
The analytical method is based on a extraction of the analyte from the sample using ethyl acetate, followed by a concentration of the extract and the analysis of the extract with a HPLC-MS/MS. The analytical method was validated in compliance with SANTE/2020/12830 rev.1 (2021) guideline in the study:
- CH – 0672/2021, Test Facility: Chemservice s.r.l., Study Director: Mercedes Pardo Martinez.

Chromatographic conditions

Instrument	HPLC Agilent 1290 Infinity II coupled with a triple quadrupole mass spectrometer Agilent 6470A
Column	Phenomenex LUNA C18 (2), 4.6 x 150 mm, 5 µm
Column temperature	30°C
Flow	0.4 mL/min
Injection volume	20 µL
Mobile phase A	Water with 0.2% formic acid (prepared dissolving 4 mL of formic acid in 2 L of LC-MS grade water)
Mobile phase B	Methanol with 0.2% formic acid (prepared dissolving 4 mL of formic acid in 2 L of LC-MS grade methanol)
Source type:	ESI
Gas temperature:	350°C
Gas flow (L/min):	10
MRM monitored transitions	Quantifier, Precursor ion (m/z) 175, Product ion (m/z) 35.1 Qualifier, Precursor ion (m/z) 175, Product ion (m/z) 145.0

Results and discussions

Table A 42: Recovery results from independent laboratory validation of dicamba using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (n = 5)	Mean recovery (%)	RSD (%)	Comments
Primary (175.0/35.1)					
Bovine milk	dicamba	0.0100	84.1	4.9	-
		0.100	83.2	3.0	-
Confirmatory (175.0/145.0)					
Bovine milk	dicamba	0.0100	88.3	6.9	-
		0.100	84.1	2.1	-

Table A 43: Characteristics for the analytical method used for independent laboratory validation of dicamba residues in bovine milk

	dicamba
Specificity	Verified: no interferences found in untreated samples in amounts higher than 30% of the LOQ (< LOD)
Calibration (type, number of data points)	The regression residuals plots show that residuals are randomly distributed, hence demonstrating applicability of the linear calibration. Correlation coefficients R^2 are ≥ 0.99. N=5
Calibration range	Range: 2.00 – 200 µg/L 0.00200 – 0.200 mg/kg (from 20 % of LOQ to 100 % above 10xLOQ)
Assessment of matrix effects is presented	Calibration matrix-matched
Limit of determination/quantification	LOQ =0.010 mg/kg LOD= 0.100 mg/kg)

Conclusion

The ILV was carried out according to SANTE/2020/12830 rev.1 (2021) validating the parameters of linearity, recovery, precision, LOQ and selectivity/specificity. The results meet all the requirements of the guideline.

A 1.2.2.4.3 Extraction efficiency

It is referred to Point A.2.2.1.3.

A 1.2.2.5 Description of analytical methods for the determination of residues in poultry eggs (KCP 5.2)

A 1.2.2.5.1 Method validation

Comments of zRMS:	Method is accepted

Reference:	KCP 5.2_10
Report	Validation of an analytical method for the quantification of Dicamba in poultry eggs, D. Longhi, GLP-STUDY-22-1, 2022
Guideline(s):	Yes, SANTE/2020/12830 rev. 1; SANTE 2017/10632 rev.3
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The analytical method for the determination of total Dicamba in poultry eggs samples was validated under GLP compliance according to SANTE/2020/12830 Rev.1.
The analytical method was based on the EURL acidified-QuEChERS (A-QueChERS) method. The method for Dicamba consisted of an extraction and partition of the analyte from the matrix and of an instrumental determination using a HPLC-MS/MS (high-performance liquid chromatography + triple-quadrupole mass spectrometry).

Chromatographic conditions

Instrument	Agilent HPLC 1290 Infinity II + Agilent MS spectrometer 6470A Triple Quad.	
HPLC Column	Waters Acquity UPLC HSS PFP 1.8µm, 2.1 x 150 mm.	
Column temperature	40°C	
Mobile phase A	LC-MS grade water with 0.5 % of formic acid	
Mobile phase B	LC-MS grade acetonitrile	
Eluent flow	0.4 mL/min	
Volume of injection	2 µL	
Retention time (approx, min)	3.68	
Mass scan parameters		
Ion mode	ESI -	
Dry gas temperature	225°C	
Dry gas flow	8 L/min	
Precursor ion	Primary 219 *m/z*	
		Confirmatory 221 *m/z*
Product ion	Primary 175 *m/z*	
		Confirmatory 177 *m/z*

Results and discussions

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

Matrix	Analyte	Fortification level (mg/kg) (n = 5)	Mean recovery (%)	RSD (%)	Comments
Primary (219/175)					
Poultry eggs	dicamba	0.010	97.2	3.3	-
		0.100	96.1	2.0	-
Confirmatory (221/177)					
Poultry eggs	dicamba	0.010	94.6	2.6	-
		0.100	94.0	1.4	-

Table A 45: Characteristics for the analytical method used for validation of dicamba residues in poultry eggs

	Dicamba
Specificity	Verified for each matrix: no interferences found untreated samples in amounts higher than the 30% of the LOQ (< LOD)
Confirmatory	Confirmation achieved by simultaneous determination of a confirmatory SRM transition. Calibration data, recovery and precision in compliance with the requirements
Calibration (type, number of data points)	The regression residuals plots show that residuals are randomly distributed, hence demonstrating the linear calibration. R^2 primary transition (219 → 175) 0.9986 R^2 confirmatory transition (221 → 177) 0.9976
Calibration range	1.50 – 100 µg/L (0.003 – 0.2 mg/kg)
Assessment of matrix effects is presented	+ 31% (significant) Calibration matrix-matched was used.
Limit of determination/quantification	LOQ= 0.01 mg/kg LOD= 0.003 mg/kg (30% of LOQ)
Stability of the analyte in the samples extract	Verified for 4 days at 5 ± 3°C in the dark (poultry eggs: 90.7%)
Stability of the analyte in the standard solution	Verified for 13 days at 5 ± 3°C in the dark (stock solution in acetonitrile): the difference from the stored and a fresh solution was 1.8%
Extraction efficiency	Verified by cross-validation with a method used in metabolism study. Differences in the analyte extraction: +7.2%

Conclusion

According the SANTE/2020/12830 rev. 1 (2021) guideline's requirement, the mean recovery values must be in the range 70 to 120 %, with an RSD% lower than 20%.
Since all recovery values for the analyte at both fortification levels (L.O.Q and 10 x L.O.Q.) resulted to be in the correct range, this criterion was fulfilled and therefore, the analytical method can be considered suitable to quantify Dicamba in bovine milk samples.

A 1.2.2.5.2 Extraction Efficiency

The evaluation of the extraction efficiency was done in compliance with SANTE 2017/10632 rev.3, applying a cross validation approach.
The extraction efficiency was evaluated comparing the amount extracted from an homogenized egg sample containing Dicamba using the extraction procedures of the following analytical methods:
- M1 = AM1-GLP-STUDY-22-1, the analytical method under validation, whose extraction efficiency must be proven.
- M2 = an extraction procedure with proven extraction efficiency (demonstrated using radio-labelled chemicals) coming from the following metabolism study, described in the Draft Renewal Assessment Report (RAR) "DICAMBA Volume 3 – B.7 (A)" (2020/12) (document provided by the Sponsor):
o Section: B.7.2.2./02
o Title: "Dicamba: Metabolism in Laying Hens (1994)"
o GLP : yes.

	METHOD UNDER VALIDATION (AM-GLP-STUDY-22-1)	METABOLISM METHOD (M2)	
Analyte	Sample code: EGG-M1-SPK Result (mg/kg)	Sample code: EGG-M2-SPK Result (mg/kg)	DIFFERENCE (%) (limit: +/- 30%) (Metabolism method used as reference)
Dicamba	1.08	1.01	+ 7.2

The difference of the amounts of analytes found in the sample with the analytical method under validation and those found applying the extraction procedure of the metabolism study is less than 30%: the extraction efficiency can be considered proven.

A 1.2.2.5.3 Independent laboratory validation

Comments of zRMS:	Method is accepted

Reference:	KCP 5.2_11
Report	Independent Laboratory Validation (ILV) of the Analytical Method for the Determination of Dicamba in Poultry Eggs, CH – 0992/2021, E. Rigamonti, 2022
Guideline(s):	Yes, SANTE/2020/12830 rev. 1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The Test Facility perform an Independent Laboratory Validation (ILV) of the analytical method adjusted and validated in GLP study Code GLP-STUDY-22-1 by LabAnalysis s.r.l. for the determination of Dicamba residue in poultry eggs.

Chromatographic conditions

Column	ACQUITY HSS FPF, 1.8 µm, 150 x 2.1 mm i.d.
Interface	Agilent Jet Stream (AJS) Electron spray ionization (ESI), negative polarity
Detector	MS Triple quadrupole (MRM mode)
Column temperature	25°C
Flow	0.400 mL/min
Injection volume	2.0 µL
Eluent A	Water with 0.5% formic acid
Eluent B	Acetonitrile
R. T. Dicamba	about 3.8 minutes
Source type:	AJS ESI -
Gas temperature:	225°C
Gas flow (L/min):	8 L/min

Results and discussions

Table A 46: Recovery results from independent laboratory validation of dicamba using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (n = 5)	Mean recovery (%)	RSD (%)	Comments
Product ion 175.0					
poultry eggs	dicamba	0.0100	108.8	0.81	-
		0.100	101.9	3.83	-
Product ion 177.0					
poultry eggs	dicamba	0.0100	107.7	2.28	-
		0.100	102.6	3.92	-

Table A 47: Characteristics for the analytical method used for independent laboratory validation of dicamba residues in poultry eggs

	dicamba
Specificity	The analytical method, using the HPLC/MS/MS instrument with quantification by external standard, was shown to be specific for Dicamba residues in poultry eggs samples since no residues of the analyte were detected in any blank samples at or above the Limit of Detection.
Confirmatory	Since the analysis by HPLC using an external standard and MS triple quadrupole detector (HPLC/MS/MS) in MRM mode is highly specific and gave both quantification and identification data, a confirmatory test using another instrumental technique was not necessary.
Calibration (type, number of data points)	N=5 No significant memory peak was detected in the wash matrix injected after the highest working standard solution and the range tested for Dicamba was found to be linear (each correlation coefficient > 0.99).

	dicamba
Calibration range	0.003 – 0.2 mg/kg
Assessment of matrix effects is presented	A significant matrix effects (ion suppression/enhancement) for Dicamba in the poultry eggs matrix was found (higher than 20%). Therefore, matrix-matched calibration standards were used throughout the entire study.
Limit of determination/quantification	LOQ= 0.010 mg/kg LOD= 0.003 mg/kg

Conclusion

The ILV was carried out according to SANTE/2020/12830 rev.1 (2021) validating the parameters of linearity, recovery, precision, LOQ and selectivity/specificity. The results meet all the requirements of the guideline.

A 1.2.2.6 Description of Methods for the Analysis of Soil (KCP 5.2)

No new or additional studies have been submitted

A 1.2.2.7 Description of Methods for the Analysis of Water (KCP 5.2)

A 1.2.2.7.1 Method validation

Comments of zRMS:	Method is accepted

Reference:	KCP 5.2_12
Report	Validation of the Analytical Method for the Determination of Dicamba, Dicamba-5-Hydroxy and Dicamba-desmethyl in Drinking Water, CH – 0472/2020, M. P. Martinez, 2020
Guideline(s):	SANCO/3029/99 rev. 4 and SANCO/825/00 rev. 8.1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The Test Facility conducted a study to adjust and validate the analytical method for the determination of Dicamba, Dicamba-5-Hydroxy and Dicamba-desmethyl residues in drinking water samples.

Chromatographic conditions

HPLC column	Agilent or equivalent Zorbax Eclipse plus C18, 1.8 µm, 150 x 2.1 mm i.d.
Interface	Agilent Jet Stream (AJS) Electron spray ionization (ESI), negative polarity
Detector	MS Triple quadrupole (MRM mode)

Column temperature	25°C
Eluent A	Water with 0.2% formic acid
Eluent B	Methanol with 0.2% formic acid
Eluent (isocratic)	A/B = 40 /60 % v/v
Eluent flow	0.3 mL/min
Volume of injection	20 µL
Dicamba ret. time	about 3.2 minutes
Dicamba-5-Hydroxy ret. time	about 2.0 minutes
Dicamba-desmethyl ret. time	about 2.9 minutes
Source parameters	
Ion mode	AJS ESI -
Gas temperature	120°C
Dry gas flow	12 L/min.

Results and discussions

Table A 48: Recovery results from validation of dicamba using the analytical method

Matrix	Analyte	Fortification level (µg/L) (n = 5)	Mean recovery (%)	RSD (%)	Comments
Product ion 175					
Drinking water	dicamba	0.10	75.2	2.99	-
		1.00	106.0	0.93	-
Product ion 177					
Drinking water	dicamba	0.10	101.8	0.54	-
		1.00	104.4	6.85	-
Product ion 155					
Drinking water	Dicamba-5-Hydroxy	0.10	74.3	4.48	-
		1.00	98.1	7.62	-
Product ion 190.9					
Drinking water	Dicamba-5-Hydroxy	0.10	87.2	2.23	-
		1.00	94.2	8.18	-
Product ion 160.9					
Drinking water	Dicamba-desmethyl	0.10	104.7	5.34	-
		1.00	92.8	4.79	-
Product ion 124.7					
Drinking water	Dicamba-desmethyl	0.10	80.9	6.25	-
		1.00	95.1	3.64	-

Table A 49: Characteristics for the analytical method used for validation of dicamba residues in drinking water

	dicamba
Specificity	The analytical method, using the UHPLC/MS/MS instrument with quantification by external standard, was shown to be specific for Dicamba, Dicamba-5-Hydroxy and Dicamba-desmethyl residues in drinking water samples.
Confirmatory	Since the analysis by UHPLC using an external standard and MS triple quadrupole detector (HPLC/MS/MS) in MRM mode is highly specific and gave both quantification and identification data, a confirmatory test using another instrumental technique was not necessary.
Calibration (type, number of data points)	N=5
Calibration range	<table><tr><th>Impurity</th><th>Injected range (ng/mL)</th><th>Linearity Range (µg/L) (1)</th></tr><tr><td>Dicamba</td><td>0.03 – 4.98</td><td>0.03 – 4.98</td></tr><tr><td>Dicamba-5-Hydroxy</td><td>0.03 – 4.97</td><td>0.03 – 4.97</td></tr><tr><td>Dicamba-desmethyl</td><td>0.03 – 4.74</td><td>0.03 – 4.74</td></tr></table> No significant memory peak was detected in the wash solvent injected after the highest working standard solution and the range tested for Dicamba, Dicamba-5-Hydroxy and Dicamba-desmethyl was found to be linear (each correlation coefficient > 0.99).
Assessment of matrix effects is presented	Yes No significant matrix effects for Dicamba, Dicamba-5-Hydroxy and Dicamba-desmethyl in the drinking water matrix was found (lower than 20%).
Limit of determination/quantification	LOQ= 0.10 µg/L LOD= 0.015 µg/L

Conclusion

The validation was carried out according to SANCO/3029/99 rev. 4 and SANCO/825/00 rev. 8.1 validating the parameters of linearity, recovery, precision, LOQ and selectivity/specificity. The results meet all the requirements of the guideline.

A 1.2.2.7.2 Independent laboratory validation

Comments of zRMS:	Method is accepted

Reference:	KCP 5.2_13
Report	Independent Laboratory Validation (ILV) of the analytical method for the Determination of Dicamba, Dicamba-5-hydroxy and Dicamba-desmethyl in Drinking Water, GLP-STUDY-20-62, A. Sala, 2022
Guideline(s):	SANCO/3029/99 rev. 4 and SANCO/825/00 rev. 8.1

Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The objective of this study was to perform an Independent Laboratory Validation of an analytical method to determine Dicamba, Dicamba-5-Hydroxy and Dicamba-desmethyl residues in drinking water samples. The analytical method was firstly validated in the study coded CH – 0472/2020: "Validation of the Analytical Method for the Determination of Dicamba, Dicamba-5-Hydroxy and Dicamba-desmethyl in Drinking Water". Test Facility ChemService S.r.l. Controlli e Ricerche - Study Director Mercedes Pardo Martinez - August 12, 2020. The analytical method was validated according to SANCO/825/00 rev.8.1, SANCO/3029/99 rev. 4 and OECD (2007): "Guidance Document on Pesticide Residue Analytical Methods - ENV/JM/MONO(2007)17" guidelines.
The aim of this study was to perform an Independent Laboratory Validation (ILV) to confirm the primary validation of the analytical method.

Chromatographic conditions

HPLC-MS/MS	Agilent HPLC 1290 Infinity II + Agilent MS spectrometer 6470A Triple Quad
Column	Agilent Zorbax RRHD Eclipse Plus C18/ 1.8 µm 3x150 mm
Column temperature	20°C
Mobile phase A	LC-MS grade water + 0.2% formic acid
Mobile phase B	LC-MS grade methanol + 0.2% formic acid
Eluent (isocratic)	Isocratic 40/60 % v/v
Eluent flow	0.300 mL/min
Volume of injection	20.0 µL
Dicamba ret. time	3.2 min
Dicamba-5-Hydroxy ret. time	2.0 min
Dicamba-desmethyl ret. time	2.9 min
Source parameters	ESI-
Gas temperature	190°C
Dry gas flow	10

Results and discussions

Table A 50: Recovery results from independent laboratory validation of dicamba using the analytical method

Matrix	Analyte	Fortification level (µg/L) (n = 5)	Mean recovery (%)	RSD (%)	Comments
219/175 (Quantifier)					
Drinking water	dicamba	0.10	102.8	3.80	-
		1.00	104.5	2.02	-
221/177 (Qualifier)					
Drinking water	dicamba	0.10	103.4	6.28	-
		1.00	104.5	2.89	-

Matrix	Analyte	Fortification level (µg/L) (n = 5)	Mean recovery (%)	RSD (%)	Comments
235/155 (Quantifier)					
Drinking water	Dicamba-5-Hydroxy	0.10	104.3	4.32	-
		1.00	106.1	0.82	-
235/191 (Qualifier)					
Drinking water	Dicamba-5-Hydroxy	0.10	103.2	1.93	-
		1.00	103.3	0.83	-
205/161 (Quantifier)					
Drinking water	Dicamba-desmethyl	0.10	106.3	2.40	-
		1.00	101.8	1.32	-
205/124.7 (Qualifier)					
Drinking water	Dicamba-desmethyl	0.10	106.3	1.87	-
		1.00	100.0	2.47	-

Table A 51: Characteristics for the analytical method used for independent laboratory validation of dicamba residues in drinking water

	dicamba
Specificity	The method was found to be selective for the determination of all three analytes in drinking water. In the analysis of drinking water blank samples (unspiked samples) no detectable peaks of all 3 analytes (Dicamba, Dicamba-5-hydroxy, Dicamba-desmethyl) were measured for both quantifier and qualifier MRM transitions. This result is in compliance with the guideline requirements (blank values must be not higher than 30% of LOQ).
Confirmatory	The confirmatory analysis was carried out simultaneously with the primary detection acquiring a second MRM MS/MS transition for each analyte. Linearity, accuracy, precision and blank values for each analyte were calculated also with the second transition (qualifier - confirmatory transition).
Calibration (type, number of data points)	The linearity was tested with a 5 point calibration curve (each analyte). The linearity range tested (each analyte) brackets the range 30% LOQ - 20% above the highest analyte amount to be quantified (corresponding to 10xLOQ).
Calibration range	Drinking water (matrix matched standard) Impurity: Dicamba, Dicamba-5-Hydroxy, Dicamba-desmethyl — Injected range: 0.03-5.0 µg/L
Assessment of matrix effects is presented	The same calibration curve for each analyte/transition was prepared and analysed using both matrix matched (drinking water) and solvent

	dicamba
	(LC-MS grade water) standard solutions. The matrix effect resulted lower than ±20% in most cases asn slightly higher than ±20% therefore as stated on SANCO guidelines matrix matched standard solution must be used.
Limit of determination/quantification	LOQ= 0.10 µg/L LOD= 0.015 µg/L

Conclusion

The Independent Laboratory Validation (ILV) carried out in this study confirms the results obtained in the primary validation study: Study coded CH – 0472/2020: "Validation of the Analytical Method for the Determination of Dicamba, Dicamba-5-Hydroxy and Dicamba-desmethyl in Drinking Water". Test Facility ChemService S.r.l. Controlli e Ricerche - Study Director Mercedes Pardo Martinez - August 12, 2020.
The method applied can be considered validated in compliance with SANCO/825/00 rev.8.1, SANCO/3029/99 rev. 4 and OECD (2007): "Guidance Document on Pesticide Residue Analytical Methods - ENV/JM/MONO(2007)17" guidelines for the quantification of Dicamba, Dicamba-5-hydroxy and Dicamba-desmethyl in Drinking Water samples.

A 1.2.2.8 Description of Methods for the Analysis of Air (KCP 5.2)

No new or additional studies have been submitted

A 1.2.2.9 Description of Methods for the Analysis of Body Fluids and Tissues (KCP 5.2)

A 1.2.2.9.1 Method validation

Comments of zRMS:	Method is accepted

Reference:	KCP 5.2_14
Report	Validation of an analytical method for the quantification of Dicamba in bovine urine, LBN-0004-2023, D. Longhi, 2023
Guideline(s):	SANTE/2020/12830, rev.1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The objective of this study was the validation of an analytical method to determine Dicamba in bovine urine.
The analytical determination was carried out using a HPLC-MS/MS method, validated in compliance with SANTE/2020/12830, rev.1 guideline. The analytical method was based on the EURL acidified-QuEChERS (A-QueChERS) method and consisted in an extraction and partition of the analyte in acetonitrile and in an instrumental determination using a HPLC-MS/MS (high-performance liquid chromatography + triple-

quadrupole mass spectrometry).

Chromatographic conditions

Instrument	Agilent HPLC 1290 Infinity II + Agilent MS spectrometer 6470A Triple Quad
Column	Waters Acquity UPLC HSS T3 1.8µm, 2.1 x 100 mm
Column temperature	40°C
Mobile phase A	LC-MS grade water with 0.5 % of formic acid
Mobile phase B	LC-MS grade acetonitrile
Eluent flow	0.4 mL/min
Volume of injection	2 µL
Dicamba ret. time	4.5min
Source parameters	electrospray ionisation (ESI) in negative ionization mode
Gas temperature	225°C
Dry gas flow	8

Results and discussions

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

Matrix	Analyte	Fortification level (mg/L) (n = 5)	Mean recovery (%)	RSD (%)	Comments
Primary (219/175)					
Bovine urine	dicamba	0.10	113.2	1.8	-
		1.00	103.6	5.6	-
Confirmatory (221/177)					
Bovine urine	dicamba	0.10	111.4	3.4	-
		1.00	104.7	6.1	-

Table A 53: Characteristics for the analytical method used for validation of dicamba residues in bovine urine

	dicamba
Specificity	Verified for each matrix: no interferences found untreated samples in amounts higher than the 30% of the LOQ (< LOD)
Confirmatory	Confirmation achieved by simultaneous determination of a confirmatory SRM transition. Calibration data, recovery and precision in compliance with the requirements
Calibration (type, number of data points)	N=5 The regression residuals plots show that residuals are randomly distributed, hence demonstrating the linear calibration.
Calibration range	1.50 – 100 µg/L
Assessment of matrix effects is presented	-21.6% (significant) Calibration matrix-matched

	dicamba
Limit of determination/quantification	verified at 0.01 mg/L recovery and repeatability data in compliance with the guideline Verified for each matrix at 0.003 mg/L (30% of LOQ) signal/noise ratio higher than 3
Stability of the analyte in the samples extract	Verified for 3 days at 5 ± 3°C in the dark (analyte amount after storage: 104.6%)
Stability of the analyte in the standard solution	Verified in the GLP study GLP-STUDY-22-1 for 13 days at 5 ± 3°C in the dark (stock solution in acetonitrile): the difference from the stored and a fresh solution was 1.8%

Conclusion

The analytical method for the determination of total Dicamba in bovine urine samples was validated under GLP compliance according to SANTE/2020/12830 Rev.1.

A 1.2.2.10 A.2.A.9 Other Studies/ Information

A 1.3 Methods for post-authorization control and monitoring purposes, Nicosulfuron (KCP 5.2)

A 1.3.1 Description of Methods for the Analysis of Water (KCP 5.2)

A 1.3.1.1 Independent laboratory validation

Comments of zRMS:	Method is accepted

Reference:	KCP 5.2_15
Report	VALIDATION OF A METHOD FOR DETERMINATION OF NICOSULFURON IN DRINKING WATER BY LIQUID CHROMATOGRAPHY, 20/FSL/04, K. Rudziński, 2020
Guideline(s):	SANCO/3029/99 rev. 4 and SANCO/825/00 rev. 8.1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The direct aqueous injection-liquid chromatography/tandem mass spectrometry (DAI-LC-MS/MS) method for the identification and quantification of Nicosulfuron in drinking water was used. In brief an aliquot of sample was cleaned up by filtration using syringe PVDF filter direct into HPLC vial.

Chromatographic conditions

HPLC system	Series 1200 HPLC
Column	Agilent ZORBAX Eclipse Plus C18, 2.1 x 100 mm, 1.8 µm
Pre-Column	Agilent 1290 Infinity In-Line Filter (PN: 5067-4368) with 0.3µm frit ring installed
Column temperature	45°C
Mobile phase	A: 5mM Ammonium Formate with 0.01% (v/v) Formic Acid in Water B: 5mM Ammonium Formate with 0.01% (v/v) Formic Acid in Acetonitrile: Water, 95/5
Eluent flow	0.3
Volume of injection	90 µL
Retention time	7.4 min for Nicosulfuron

Mass spectrometric conditions

MS system	Agilent Technologies 6410A Triple Quad LC/MS
Ionisation type	Electrospray
Polarity	
Polarity	Positive ion mode
Scan type	MS/MS, Multiple Reaction Monitoring (MRM)

Results and discussions

According to the guidance document SANCO/825/00, rev. 8.1 , point 6: "Analytical methods for residues in water" when the method of direct injection of water samples is used, recovery data cannot be calculated. Therefore, calibration and precision data are presented, only.

Table A 54: Summary of precision results for Nicosulfuron

Matrix	Analyte	Fortification level (µg/L) (n = 5)	Mean Results (µg/L)	RSD (%)	Comments
Quantification Ion Mass Transition 411.1→182.0					
Drinking water	nicosulfuron	0.05	0.055	1,8	-
		0.5	0.504	1,8	-
Confirmation Ion Mass Transition 411.1→213.0					
Drinking water	nicosulfuron	0.05	0.055	3,7	-
		0.5	0.501	2.8	-

Table A 55: Characteristics for the analytical method used for validation of nicosulfuron residues in drinking water

	nicosulfuron
Specificity Confirmatory	Quantification was performed by use of highly selective liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). Two selected ion mass transitions were evaluated in order to demonstrate that the method achieves a high level of selectivity. The retention times of target analyte in diluted samples corresponds to that of the calibration standards with a tolerance of < ± 0.1 min. Confirmation ion ratio for Nicosulfuron in all samples were within ± 30 % of the average found for the standards

	nicosulfuron
Calibration (type, number of data points)	N=5 The calibration curves obtained for both ion mass transitions of target analyte and matrices were linear with the coefficients of correlation (R) greater than 0.99. Linear regression was performed with 1/x weighting.
Calibration range	0.01 µg/L to 10 µg/L
Assessment of matrix effects is presented	Matrix effects on the detection of Nicosulfuron in extracts of drinking water were lower than 20% and thus considered insignificant, according to SANCO guidelines. Determination of analyte was performed using matrix-matched calibration standards.
Limit of determination/quantification	LOQ=0.05 µg/L LOQ=0.01 ug/L
Stability of Analyte in Diluted Sample	The stability of analyte in diluted samples was not tested specifically. Precision of the repeatable injected calibration solutions with the RSD ≤ 20% and the use of bracketing standards to insure integrity of the analytical sequence sufficiently demonstrate the stability.

Conclusion

The method was shown to be highly selective, as it includes two parent-daughter ion mass transitions for Nicosulfuron, and it yields accurate and repeatable results. The limit of quantification (LOQ) was established at 0.05 µg/L and the limit of detection (LOD) at 0.01 µg/L for Nicosulfuron in drinking water matrices.
It is concluded that method fulfils the requirements as defined in EC Guidance documents on residue analytical methods (SANCO/825/00, rev. 8.1. and SANCO/3029/99, rev. 4 and is, applicable as enforcement and data generation method for determination of Nicosulfuron in drinking water.

A 1.3.2 Description of analytical methods for the determination of residues in animal matrices (KCP 5.2)

A 1.3.2.1 Primary method validation (KCP 5.2)

Comments of zRMS:	Method is accepted

Reference:	KCP 5.2_16
Report	Validation of an Analytical Method for Nicosulfuron in Food of Animal Origin and Body Fluids, Olga Blumberg, 2024, S23-107311
Guideline(s):	Yes, SANTE/2020/12830 rev.2
Deviations:	No
GLP:	No
Acceptability:	Yes

The objective of this study was to develop and validate an analytical method for the determination of Nicosulfuron in different matrices of animal origin: Bovine Meat, Bovine Milk, Bovine Fat, Bovine Liver, Poultry's Egg, Honey in accordance with guidance SANTE/2020/12830, rev.2 for monitoring. The limit of quantification was 0.01 mg/kg.

Materials and methods
Validation of the method was based on SANTE/2020/12830 rev.2. Two different sample preparation approaches were applied, depending on the matrix type:

Procedure for Bovine Milk, Meat, Liver, Poultry's Eggs, Honey:
Extraction
1) Weigh a 1.00 g (± 0.01 g) of the homogenized matrix into a 50 mL centrifuge tube.
2) Fortify the samples at this point (if needed).
3) Add 9 mL of dem. water and shake vigorously by hand for 1 min.
4) Add 10 mL of acetonitrile and shake for 30 minutes at 300 rpm on a flatbed shaker.
5) Add Bekolut Citrat Kit 01 and shake vigorously for 1 min.(for milk and urine: shake on platform shaker for 10 mins)
6) Centrifuge the sample for 5 minutes at 4000 rpm.
7) Transfer 0.100 mL acetonitrile phase (upper layer) into a HPLC vial containing 0.900 mL of methanol/water (1/1, v/v) prior LC-MS/MS analysis. Vortex well.

Procedure for Bovine Fat
Extraction
1) Weigh a 1.00 g (± 0.01 g) of the homogenized bovine fat into a 50 mL centrifuge tube.
2) Melt the sample using the water bath at 55 °C (ca.20 min).
3) Fortify the samples at this point (if needed).
4) Add 9 mL of dem. water and shake vigorously by hand for 1 min.
5) Add 10 mL of acetonitrile and shake for 30 minutes at 300 rpm on a flatbed shaker.
6) Add Bekolut Citrat Kit 01 and shake vigorously for 1 min.
7) Centrifuge the sample for 5 minutes at 4000 rpm.
8) Transfer 9 mL of the acetonitrile phase into a 15 mL centrifuge tube.
9) Place the sample for at least 1.5 h into the freezer.
10) Centrifuge the sample for 3 minutes at 4000 rpm.
11) Transfer 0.100 mL acetonitrile phase into a HPLC vial containing 0.900 mL of methanol/water (1/1, v/v) prior LC-MS/MS analysis. Vortex well.

HPLC conditions

Column	Thermo HyPurity Aquastar, 150 mm×3 mm, 5 µm, with HPLC guard column (Phenomenex) with 4 mm Fusion RP cartridge	
Injection volume	20 µL	
Column oven temperature	40ºC	
Mobile phase flow	0.5 mL/min	
	Time [min]	**A [%]**
	0.0	80
	3.0	90
	5.0	10
	5.1	80
	7	80
Mobile phase	A: water+0.5% formic acid B: Methanol	
Retention time	4.47 min	

Mass spectrometric conditions

System	Triple quad

Ionisation	ESI +
Scan type	MS/MS (MRM)
Analyte monitored	411→182 (quantifier)
	411→213 (qualifier)

Results and discussions

Table A 56: Recovery results from method validation of nicosulfuron using the analytical method

Matrix	Analyte	Fortification level mg/kg) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
411→182					
Bovine Milk	nicosulfuron	0.01	86	2	LOQ
		0.1	92	2	10×LOQ
Bovine Meat		0.01	88	2	LOQ
		0.1	90	2	10×LOQ
Bovine Fat		0.01	87	2	LOQ
		0.1	89	3	10×LOQ
Bovine Liver		0.01	91	3	LOQ
		0.1	91	2	10×LOQ
Poultry's Egg		0.01	87	5	LOQ
		0.1	87	3	10×LOQ
Honey		0.01	90	3	LOQ
		0.1	91	3	10×LOQ
411→213					
Bovine Milk	nicosulfuron	0.01	81	2	LOQ
		0.1	92	2	10×LOQ
Bovine Meat		0.01	89	5	LOQ
		0.1	90	2	10×LOQ
Bovine Fat		0.01	86	6	LOQ
		0.1	89	4	10×LOQ
Bovine Liver		0.01	109	4	LOQ
		0.1	88	1	10×LOQ
Poultry's Egg		0.01	84	4	LOQ
		0.1	87	2	10×LOQ
Honey		0.01	94	8	LOQ
		0.1	91	3	10×LOQ

Table A 57: Characteristics of the analytical method for monitoring of nicosulfuron in animal matrices

Nicosulfuron **Bovine Meat, Bovine Milk, Bovine Fat, Bovine Liver, Poultry's Egg, Honey**	
Specificity	Demonstrated by validation of two (2) mass transitions for Nicosulfuron: *m/z* 411→182 and m/z 411→213 Analyte levels or chromatographic interferences of unknown compounds in a reagent blank and control sample extracts were below of what would correspond to an analyte level of 30 % of the LOQ.
Calibration (type, number of data points)	Linear regression with 1/x weighting. Regression residuals randomly distributed Coefficients of correlation coefficients R^2>0.99 Matrix-matched calibration standards A minimum of five (n=5) concentration levels
Calibration range	0.03 - 3.0 ng/mL → 0.003 – 0.3 mg/kg
Assessment of matrix effects is presented	Assessed. Significant for bovine fat, bovine liver and honey, however determination of analyte was performed using matrix-matched calibration standards.
Standard Solutions Stability	84 days in acetonitrile at 1-10 °C in the dark
Sample Extracts Stability	7 days at 1-10 °C in the dark
Limit of determination/quantification	LOQ= 0.01 mg/kg LOD= 0.003 mg/kg

Conclusions
It is concluded that the method fulfils requirements for monitoring methods on matrices of animal origin as defined in SANTE/2020/12830 rev.2.

A 1.3.2.2 Independent Laboratory Method Validation (KCP 5.2)

Comments of zRMS:	Method is accepted
Reference:	KCP 5.2_17
Report	Independent Laboratory Validation of an Analytical Method for Determination of Nicosulfuron in Food of Animal Origin and Body Fluids, Sandro Jooss, 2024, S23-107248
Guideline(s):	Yes, SANTE/2020/12830 rev.2
Deviations:	No
GLP:	No
Acceptability:	Yes

The objective of this study was to conduct independent laboratory validation for determination of Nicosulfuron in different matrices of animal origin: Bovine Meat, Bovine Milk, Bovine Fat, Honey in accordance with guidance SANTE/2020/12830, rev.2 for monitoring. The limit of quantification was 0.01 mg/kg.

Materials and methods
Validation of the method was based on SANTE/2020/12830 rev.2. Two different sample preparation approaches were applied, depending on the matrix type:

Procedure for Bovine Milk, Meat, Honey:

Extraction

1) Weigh a 1.00 g (± 0.01 g) of the homogenized matrix into a 50 mL centrifuge tube.
2) Fortify the samples at this point (if needed).
3) Add 9 mL of dem. water and shake vigorously by hand for 1 min.
4) Add 10 mL of acetonitrile and shake for 30 minutes at 300 rpm on a flatbed shaker.
5) Add Bekolut Citrat Kit 01 and shake vigorously for 1 min.(for milk and urine: shake on platform shaker for 10 mins)
6) Centrifuge the sample for 5 minutes at 4000 rpm.
7) Transfer 0.100 mL acetonitrile phase (upper layer) into a HPLC vial containing 0.900 mL of methanol/water (1/1, v/v) prior LC-MS/MS analysis. Vortex well.

Procedure for Bovine Fat

Extraction

1) Weigh a 1.00 g (± 0.01 g) of the homogenized bovine fat into a 50 mL centrifuge tube.
2) Melt the sample using the water bath at 55 °C (ca.20 min).
3) Fortify the samples at this point (if needed).
4) Add 9 mL of dem. water and shake vigorously by hand for 1 min.
5) Add 10 mL of acetonitrile and shake for 30 minutes at 300 rpm on a flatbed shaker.
6) Add Bekolut Citrat Kit 01 and shake vigorously for 1 min.
7) Centrifuge the sample for 5 minutes at 4000 rpm.
8) Transfer 9 mL of the acetonitrile phase into a 15 mL centrifuge tube.
9) Place the sample for at least 1.5 h into the freezer.
10) Centrifuge the sample for 3 minutes at 4000 rpm.
11) Transfer 0.100 mL acetonitrile phase into a HPLC vial containing 0.900 mL of methanol/water (1/1, v/v) prior LC-MS/MS analysis. Vortex well.

HPLC conditions

Column	Thermo HyPurity Aquastar, 150 mm×3 mm, 5 µm, with HPLC guard column (Phenomenex) C18 4×3mm	
Injection volume	20 µL	
Column oven temperature	40ºC	
Mobile phase flow	0.5 mL/min	
	Time [min]	**A [%]**
	0.0	80
	3.0	90
	5.0	10
	5.1	80
	7	80
Mobile phase	A: water+0.5% formic acid	
	B: Methanol	
Retention time	6.5 min	

Mass spectrometric conditions

System	Triple quad
Ionisation	ESI +
Scan type	MS/MS (MRM)
Analyte monitored	411→182 (quantifier)
	411→213 (qualifier)

Results and discussions

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

Matrix	Analyte	Fortification level mg/kg) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
411→182					
Bovine Milk	nicosulfuron	0.01	91.1	4.2	LOQ
		0.1	93.2	3.0	10×LOQ
Bovine Meat		0.01	86.0	1.7	LOQ
		0.1	86.4	6.1	10×LOQ
Bovine Fat		0.01	91.1	1.8	LOQ
		0.1	93.5	2.0	10×LOQ
Honey		0.01	85.2	1.0	LOQ
		0.1	89.0	2.7	10×LOQ
411→213					
Bovine Milk	nicosulfuron	0.01	91.6	3.0	LOQ
		0.1	93.0	3.2	10×LOQ
Bovine Meat		0.01	86.2	3.8	LOQ
		0.1	86.5	6.1	10×LOQ
Bovine Fat		0.01	93.3	1.7	LOQ
		0.1	93.2	2.2	10×LOQ
Honey		0.01	85.6	2.2	LOQ
		0.1	89.0	2.5	10×LOQ

Table A 59: Characteristics of the analytical method for monitoring of nicosulfuron in animal matrices

Nicosulfuron **Bovine Meat, Bovine Milk, Bovine Fat, Honey**	
Specificity	Demonstrated by validation of two (2) mass transitions for Nicosulfuron: *m/z* 411→182 and m/z 411→213 Analyte levels or chromatographic interferences of unknown compounds in a reagent blank and control sample extracts were below of what would correspond to an analyte level of 30 % of the LOQ.
Calibration (type, number of data points)	Linear regression with 1/x weighting. Regression residuals randomly distributed Coefficients of correlation coefficients R^2>0.99 Matrix-matched calibration standards A minimum of five (n=5) concentration levels
Calibration range	0.03 - 3.0 ng/mL → 0.003 – 0.3 mg/kg

Assessment of matrix effects is presented	Assessed. Non-significant, however determination of analyte was performed using matrix-matched calibration standards.
Standard Solutions Stability	84 days in acetonitrile at 1-10 °C in the dark
Sample Extracts Stability	7 days at 1-10 °C in the dark
Limit of determination/quantification	LOQ= 0.01 mg/kg LOD= 0.003 mg/kg

Conclusions
It is concluded that the ILV method fulfils requirements for monitoring methods on matrices of animal origin as defined in SANTE/2020/12830 rev.2.
Primary validation and independent laboratory validation were carried out at different locations and by different study personnel. No major addition or modification to the original method other than optimisation of instrumental parameters was done. No communication with the method developers or others familiar with the method was necessary to carry out the analysis.

A 1.3.3 Description of Methods for the Analysis of Body Fluids and Tissues (KCP 5.2)

A new study for body fluids monitoring method have been submitted:

Comments of zRMS:	Method is accepted
Reference:	KCP 5.2_16
Report	Validation of an Analytical Method for Nicosulfuron in Food of Animal Origin and Body Fluids, Olga Blumberg, 2024, S23-107311
Guideline(s):	Yes, SANTE/2020/12830 rev.2
Deviations:	No
GLP:	No
Acceptability:	Yes

The objective of this study was to develop and validate an analytical method for the determination of Nicosulfuron in body fluids (urine) in accordance with guidance SANTE/2020/12830, rev.2 for monitoring. The limit of quantification was 0.01 mg/L.

Materials and methods
Validation of the method was based on SANTE/2020/12830 rev.2.

Extraction
1) Transfer 1.00 mL (± 0.01 g) of the homogenized matrix into a 50 mL centrifuge tube.
2) Fortify the samples at this point (if needed).
3) Add 9 mL of dem. water and shake vigorously by hand for 1 min.
4) Add 10 mL of acetonitrile and shake for 30 minutes at 300 rpm on a flatbed shaker.
5) Add Bekolut Citrat Kit 01 and shake vigorously for 1 min.(for milk and urine: shake on platform shaker for 10 mins)
6) Centrifuge the sample for 5 minutes at 4000 rpm.
7) Transfer 0.100 mL acetonitrile phase (upper layer) into a HPLC vial containing 0.900 mL of methanol/water (1/1, v/v) prior LC-MS/MS analysis. Vortex well.

HPLC conditions

Column	Thermo HyPurity Aquastar, 150 mm×3 mm, 5 µm, with HPLC guard column (Phenomenex) with 4 mm Fusion RP cartridge
Injection volume	20 µL
Column oven temperature	40ºC
Mobile phase flow	0.5 mL/min

Time [min]	A [%]
0.0	80
3.0	90
5.0	10
5.1	80
7	80

Mobile phase	A: water+0.5% formic acid B: Methanol
Retention time	4.47 min

Mass spectrometric conditions

System	Triple quad
Ionisation	ESI +
Scan type	MS/MS (MRM)
Analyte monitored	411→182 (quantifier) 411→213 (qualifier)

Results and discussions

Table A 60: Recovery results from method validation of nicosulfuron using the analytical method

Matrix	Analyte	Fortification level mg/L) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
411→182					
Urine	nicosulfuron	0.01	102	4	LOQ
		0.1	98	3	10×LOQ
411→213					
Urine	nicosulfuron	0.01	99	4	LOQ
		0.1	98	3	10×LOQ

Table A 61: Characteristics of the analytical method for monitoring of nicosulfuron in body fluids

Nicosulfuron / Urine	
Specificity	Demonstrated by validation of two (2) mass transitions for Nicosulfuron: *m/z* 411→182 and m/z 411→213 Analyte levels or chromatographic interferences of unknown compounds in a reagent blank and control sample extracts

	were below of what would correspond to an analyte level of 30 % of the LOQ.
Calibration (type, number of data points)	Linear regression with 1/x weighting. Regression residuals randomly distributed Coefficients of correlation coefficients R^2>0.99 Matrix-matched calibration standards A minimum of five (n=5) concentration levels
Calibration range	0.03 - 3.0 ng/mL → 0.003 – 0.3 mg/L
Assessment of matrix effects is presented	Assessed. Non-significant, however determination of analyte was performed using matrix-matched calibration standards.
Standard Solutions Stability	84 days in acetonitrile at 1-10 °C in the dark
Sample Extracts Stability	7 days at 1-10 °C in the dark
Limit of determination/quantification	LOQ= 0.01 mg/L LOD= 0.003 mg/L

Conclusions
It is concluded that the method fulfils requirements for monitoring methods on matrices of animal origin as defined in SANTE/2020/12830 rev.2.

A 1.3.3.1 A.2.A.9 Other Studies/ Information

N/A

A 1.4 Methods for post-authorization control and monitoring purposes, Thifensulfuron-methyl (KCP 5.2)

No new or additional studies have been submitted.

A 1.4.1.1 A.2.A.9 Other Studies/ Information

N/A