FINAL REGISTRATION REPORT

Part B

Section 5

Analytical Methods

Detailed summary of the risk assessment

Product code: T-75WG-OR2C

Product name(s): TOSCANA TOP 75 WG

Chemical active substance:

Tribenuron methyl, 750 g/kg

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(authorization)

Applicant: CIECH Sarzyna S.A.

Submission date: 03/2021

Finalisation date: 15/10/2021

Version history

When	What
December 2020	First submission of product authorization.
02/2021	Dossier sent for evaluation to Merit Mark (PL)
March 2021	Correction on first submission for product
08/2021	zRMS finalised evaluation
10/2021	Evaluation after commenting period - RR

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

5 Analytical methods

5.1 Conclusion and summary of assessment

Data gap:

ILV method for tribenuron methyl analysis in products of animal origin is required.

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.

Commodity/crop	Supported/ Not supported
Winter soft wheat	Supported
Spring soft wheat	Supported
Winter rye	Supported
Spring rye	Supported
Winter triticale	Supported
Spring triticale	Supported
Winter barley	Supported
Spring barley	Supported
Durum wheat	Supported
Spelt wheat	Supported
Einkorn wheat	Supported
Emmer wheat	Supported
Miscanthus sp.	Supported
Grasses grown for seeds	Supported

The product will be registered under trade name TOSCANA TOP 75 WG but in the pre-authorisation data it is presented as Tribenuron metylu 75 WG, Tribenuron metyl 75 WG, T-75WG-OR2-C, Tribenuron methyl 75 WG.

Applicant possess Letter of Access to alternative data package for active substance Tribenuron-methyl owned by Tribenuron TF. Applicant possess also Letter of Access to data for PPP owned by Proplan.

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 tribenuron-methyl in plant protection product is provided as follows:

Comments of zRMS:	This method is validated and can be applied for analysing tribenuron in the PPP.

Reference:	KCP 5.1.1
Report	Tribenuron metylu 75 WG, Method development and validation for determination of the content of active substance in the formulation, M. Xxxx, 2017, BA-23/17
Guideline(s):	Yes (SANCO/3030/99 rev.4)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The content of active substance in the examined specimen was determined by high performance liquid chromatography HPLC with UV/Vis detector using reversed phase column. External standard method was used.

Chromatographic conditions
- Column temperature: ambient
- Mobile phase: MeCN : 0.5 % H_3PO_4 (45:55, v/v)
- Flow rate: 1.0 mL/min
- Wavelength λ = 230 nm
- Volume of specimen injected: 20 µL

Validation - Results and discussions

Table 5.2-1: Methods suitable for the determination of tribenuron methyl in plant protection product TOSCANA TOP 75 WG

	Tribenuron methyl
Author(s), year	M.Xxxx, 2017
Principle of method	Principle of the method was determination of active substances by high performance liquid chromatography with UV/Vis detector.
Linearity (linear between mg/L / % range of the declared content) (correlation coefficient, expressed as r)	The linearity of the detector response was assessed using five standard solutions at the concentration range of tribenuron methyl from 0.0040 mg/mL to 0.0112 mg/mL, which corresponds to the concentration range of 67% to 187% of tribenuron methyl content in the preparation. Correlation coefficient should be R2 ≥ 0.99. The obtained result

	Tribenuron methyl
	is acceptable.
Precision – Repeatability Mean **n = 6** **(%RSD)**	% RSD = 1.31 acceptance criterion ≤ 1.40 % Horrat value: Hr = %RSD/%RSDr H_r = 0.94 acceptance criterion: Hr ≤ 1
Accuracy **n = 12** **(% Recovery)**	99.94 % acceptance criterion 100 ± 2%
Interference/ Specificity	No interference
Comment	-

Conclusion

The method has good precision, accuracy and linearity and fulfils requirements of SANCO/3030/99 rev.4 which guarantee correctness of Tribenuron-methyl determination in the preparation TOSCANA TOP 75 WG

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

TOSCANA TOP 75 WG does not contain impurities which are of toxicological, ecotoxicological or environmental concern which could be arisen in the manufacturing process or as a result of degradation during storage of the product. It is not necessary to submit the analytical methods for determination of above mentioned impurities.

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

The other formulants and also components of other formulants of TOSCANA TOP 75 WG are not of toxicological and/or ecotoxicological or environmental concern and therefore it is not necessary to submit the analytical methods for determination of other formulants or components of other formulants of above product.

5.2.1.4 Applicability of existing CIPAC methods (KCP 5.1.1)

Tribenuron-methyl: 546/TC/(M)/3, CIPAC Handbook K, p. 129, 2003

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 tribenuron methyl for the generation of pre-authorization data is given in the following table. For the detailed evaluation of new/ additional studies it is referred to Appendix 2.

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

Component of residue definition: tribenuron methyl				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Wheat (Residues)	Primary	LOQ = 0.01 mg/kg	HPLC with UV-Vis	Xxxx M., 2018, "Determination of the residues of tribenuron-methyl in grain and straw of wheat" Study code: C/04/17 Institute Of Industrial Organic Chemixxxx Branch Pszczyna Xxxx M., 2018, "Determination of the residues of MCPA and tribenuron-methyl in grain and straw of wheat" Study code: C/05/17 Institute Of Industrial Organic Chemixxxx Branch Pszczyna
Water and culture media (Ecotoxicology)	Primary	LOQ = 0.1 mg/kg	HPLC with UV-Vis	- Xxxx A., 2018, Tribenuron metyl 75 WG Terrestrial Plant Test: Vegetative Vigour Test, Study Code G/157/17 - Xxxx A., 2018, TRIBENURON METYL 75 WG Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test, Study Code G/156/17
		LOQ = 0.001 mg/L		-Xxxx E., 2017, Tribenuron metyl 75 WG *Daphnia magna*, Acute immobilisation Test; Study Code: W/265/17 - Xxxx E., 2018, Tribenuron metyl 75 WG *Pseudokirchinella subcapitata* SAG 61.81, Growth inhibtion Test; Study Code: W/266/17 - Xxxx E., 2018, Tribenuron metyl 75 WG *Lemna gibba* CPCC 310, Growth inhibtion Test; Study Code: W/268/17 - Xxxx E., 2018, Tribenuron metyl 75 WG *Navicula pelliculosa* SAG 1050-3, Growth inhibtion Test; Study Code: W/267/17
Sucrose Matrix (Ecotoxicology)		LOQ = 20.20 µg/ml		- Xxxx T., 2016, Chronic toxicity of PP-108 H (Tribenuron methyl 75 WG) on honeybees (Apis mellifera L.),

Component of residue definition: tribenuron methyl				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
				STUDY TRC15-250BA - Xxxx T., 2016, Toxicity of PP-108 H (Tribenuron methyl 75 WG) on honey bee larvae (Apis mellifera L.) after repeated exposure under laboratory conditions, STUDY TRC15-249BA
Diet Matrix (Ecotoxicology)		LOQ = 4.91 µg/ml		- Xxxx T., 2016, Chronic toxicity of PP-108 H (Tribenuron methyl 75 WG) on honeybees (Apis mellifera L.), STUDY TRC15-250BA - Xxxx T., 2016, Toxicity of PP-108 H (Tribenuron methyl 75 WG) on honey bee larvae (Apis mellifera L.) after repeated exposure under laboratory conditions, STUDY TRC15-249BA

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

5.3.1 Analysis of the plant protection product (KCP 5.2)

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

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

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

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

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

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Food/feed of plant origin (analytical technique and LOQ for methods for monitoring	Tribenuron-methyl	LOQ 0.01 mg/kg	COMMISSION REGULATION (EU) 2015/1040 of 30 June 2015

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
purposes)			
Food/feed of animal origin (analytical technique and LOQ for methods for monitoring purposes)		LOQ 0.01 mg/kg	COMMISSION REGULATION (EU) 2015/1040 of 30 June 2015
Soil (Ecotoxicology)	Tribenuron-methyl and IN-L5296	0.05 µg/kg or 1 µg/kg for Tribenuron-methyl and IN-L5296	(EFSA Journal 2017;15(7):4912): (Xxxx, S.J., Xxxx, J J., 2001), (xxxx, S., 2007), (xxx C., 2001)
Drinking water (Human toxicology)	Tribenuron-methyl	0.1 µg/L	general limit for drinking water
Surface water (Ecotoxicology)	Tribenuron-methyl	0.05 µg/L and 0.01 µg/L	(EFSA Journal 2017;15(7):4912): (Xxxx, M.R., Xxxx, J.J., 2001), (xxxxx, H., 2010)
Air	Tribenuron-methyl	1.5 µg/m^3	(EFSA Journal 2017;15(7):4912): (Xxxx T., Xxxx, S., 2000),
Tissue (meat or liver)	no data available	-	(EFSA Journal 2017;15(7):4912)
Body fluids			

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 tribenuron methyl in plant matrices is given in the following tables. For the detailed evaluation of new/ additional studies it is referred to Appendix 2.

zRMS comments:
According to the EFSA Journal 2017;15(7):4912 and Regulation (EU) 2015/1040 residue definition for analytical methods for residues is tribenuron methyl. The applicable MRL value for cereals is 0.01 mg/kg (Reg. (EU) 2015/1040).

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: Tribenuron methyl				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
~~Wheat~~	~~Primary~~	~~LOQ = 0.01 mg/kg~~	~~HPLC-UV~~	~~DAR 2003, Xxxx J.S, Xxxx W, 2000, DuPont-3595~~
~~Cereals~~	Primary	LOQ = 0.01	~~HPLC-UV~~	~~DAR 2003, Xxxx E., Xxxx., 2000,~~

Component of residue definition: Tribenuron methyl				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Food/feed of plant origin		mg/kg	LC-MS/MS	~~DuPont 2261 Revision No. 1~~ DUP: KCA 4.2/09 (Xxxx, R.M., Xxxx, J.J., 2013), LC-MS/MS, 0.01 mg/kg DUP (not acidic crops): KCA 4.2/15 (Xxxx, A.M., xxxx Jr., F.Q., 2005), KCA 4.2/02 (Xxxx, T., Xxxx, S., 2007a), and KCA 4.2/03 (Xxxx, T., Xxxx, S., 2007b), LC-MS/MS, 0.01 mg/kg TFF: KCA 4.2/01 (xxxxx, E., 2006), LC-MS/MS, 0.01 mg/kg Method equivalence for cereals demonstrated according to the data matching table prepared by Sweden
	ILV	LOQ = 0.01 mg/kg	~~HPLC-UV~~ LC-MS/MS	~~DAR, 2003, Xxxx B, 2001, DuPont-5587~~ Nie, B. 2015 Method equivalence for cereals demonstrated according to the data matching table prepared by Sweden
Tomato, orange, avocado, wheat straw, wheat grain and wheat whole plant	Primary	LOQ = 0.01 mg/kg	LC-MS/MS	Xxxx A., 2019, S18-07519
Tomato, wheat	ILV	LOQ = 0.01 mg/kg	HPLC-MS	Xxxx S., 2019, P 5114 G

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

Table 5.3-3: Statement on extraction efficiency

	Method for products of animal origin
Required, available from:	-
Not required, because:	In compliance with SANCO/825/00 rev. 8.1 the extraction procedure has to be adressed for all matrix groups for which residues ≥ LOQ are expected. For tribenuron-methyl non of residue value exceed LOQ.

Provided data and are considered to adequate.

zRMS comments:
zRMS agrees that extraction efficiency is not needed.

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 tribenuron-methyl in animal matrices is given in the following tables. For the detailed evaluation of new/ additional studies it is referred to Appendix 2.

zRMS comments:
According to the EFSA Journal 2017;15(7):4912 and Regulation (EU) 2015/1040 residue definition for analytical methods for residues is tribenuron methyl. The applicable MRL value for all products of animal origin is 0.01 mg/kg (Reg. (EU) 2015/1040).

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

Component of residue definition: tribenuron-methyl				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Milk, Eggs, Muscle, fat, liver, kidney	Primary	~~LOQ = 0.02 mg/kg~~ LOQ = 0.01 mg/kg	~~HPLC~~ LC-MS/MS	~~DAR 2003, Xxxx M.D., 1996, AMR 3698-95~~ EFSA Journal 2017;15(7):4912: DUP: KCA 4.2/07 (Xxxx, R.M., Xxxx, J.J., 2007), LC-MS/MS, 0.01 mg/kg in milk DUP: KCA 4.2/08 (Xxxx, R.M., Xxxx, J.J., 2007), LC-MS/MS, 0.01 mg/kg in eggs DUP: KCA 4.2/16 (Xxxx, A.M., Xxxx, M.E.Y., 2012), LCMS/MS, 0.01 mg/kg in animal tissues
	ILV	~~LOQ = 0.02 mg/kg~~ LOQ = 0.05 mg/kg	~~HPLC~~ LC-MS/MS	~~DAR 2003, Xxxx N.L., 2000, DuPont 4245~~ Xxxx, M., 2018, equivalent study to xxxx, N., 2010a; Xxxx 2016, equivalent study to Gant 2012 Equivalence demonstrated according to the data matching table prepared by Sweden
~~Milk, Eggs, Muscle, Cream, Liver~~	~~Primary~~	~~LOQ = 0.05 mg/kg~~	~~LC-MS~~	~~Xxxx D., 2016, DNA3620~~ ~~Xxxx D., 2019, DNA3620~~
	~~ILV~~	~~LOQ = 0.05 mg/kg~~	~~LC-MS~~	~~Xxxx M., Xxxx S., 2018, 123361101~~

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

Table 5.3-5: Statement on extraction efficiency

	Method for products of animal origin
Required, available from:	-
Not required, because:	In compliance with SANCO/825/00 rev. 8.1 the extraction procedure has to be adressed for all matrix groups for which residues ≥ LOQ are expected. For tribenuron-methyl non of residue value exceed LOQ.

Provided data and are considered to adequate.

zRMS comments:
zRMS agrees that extraction efficiency is not needed. The studies for products of animal origin submitted by the Applicant were not accepted. The applicable MRL value for all products of animal origin is 0.01 mg/kg. Therefore LOQ at the level 0.05 mg/kg reported in provided studies is too high. However, studies of tribenuron methyl in products of animal origin assessed at Community level are no longer protected and are therefore sufficient. Taking into account that the ILV study evaluated at Community level is still covered by data protection and that the ILV study submitted by the Applicant was not accepted, it is necessary to fill this data gap.

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 tribenuron methyl in soil is given in the following tables. For the detailed evaluation of new/ additional studies it is referred to Appendix 2.

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

Component of residue definition: Tribenuron methyl (and its metabolites IN-R9805, IN-L5296, IN-A4098 and IN-00581)			
Method type	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	LOQ = 0.05 µg/kg.	LC/MS/MS	DAR 2003, Xxxx S. J., Xxxx J. J., 2001, DuPont-5082
Primary	LOQ = 1.0 µg/kg	HPLC-MS/MS	DAR 2003, Xxxx M.R., Xxxx T.J., Xxxx M.E.Y., 2001, DuPont-5838
Primary	LOQ = 0.06 µg/kg dw in soil for tribenuron methyl LOQ = 1.2 µg/kg dw in soil for metabolites	LC-MS/MS	Xxxx M., 2018, PRO-001/6-20/B Xxxx S., 2019, PRO-001/6-20/B

For any special comments or remarkable points concerning the analytical methods for soil please refer to Appendix 2.
Provided data and are considered to adequate.

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 tribenuron methyl in surface and drinking water is given in the following tables. For the detailed evaluation of new/ additional studies it is referred to Appendix 2.

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

Component of residue definition: tribenuron methyl and its metabolites (IN-L5296, IN-A4098, IN-D5119)				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Water	Primary	LOQ = 0.05 µg/L LOQ = 0.1 µg/L	LC/MS/MS	Xxxx, M. R., Xxxx, J.J., 2001, Xxxx, J.J., 2014
Drinking water	Primary	LOQ = 0.1 µg/L	LC-MS/MS	Xxxx S., 2018, PRO-001/6-22
Drinking water	ILV	LOQ = 0.1 µg/L	LC-MS/MS	Xxxx, 2018, PRO-001/6-22/a

For any special comments or remarkable points concerning the analytical methods for water please refer to Appendix 2.
Provided data and are considered to adequate.

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 tribenuron methyl in air is given in the following tables.

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

Component of residue definition: tribenuron methyl			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	LOQ = 1.5 µg/m^3	HPLC-UV MS/MS	Xxxx, T. Xxxx, S., 2000

All analytical methods are active substance data and were evaluated during the EU review of tribenuron methyl. No additional studies have been performed.
Provided data and are considered to adequate.

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 tribenuron methyl in body fluids and tissue is given in the following tables.

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

Component of residue definition: tribenuron methyl			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	LOQ 1 µg/kg (plasma) LOQ 3 µg/kg (urine) LOQ = 0.01 mg/kg (body tissues)	LC-MS/MS LC-MS/MS	Xxxx, R.M., Xxxx, J.J., 2016 Xxxx, A.M., Xxxx, M.E.Y., 2012

All analytical methods are active substance data and were evaluated during the EU review of tribenuron

methyl. No additional studies have been performed.
Provided data and are considered to adequate.

5.3.2.8 Other studies/ information

There are no additional European requirements for formulated products.

Appendix 1 Lists of data considered in support of the evaluation

List of data submitted by the applicant and relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 5.1.1	M. Xxxx	2017	Tribenuron metylu 75 WG, Method development and validation for determination of the content of active substance in the formulation, BA-23/17 INSTITUTE OF INDUSTRIAL ORGANIC CHEMIXXXX GLP Unpublished	N	CIECH Sarzyna S.A.
KCP 5.1.2/01	M. Xxxx	2018	Determination of the residues of tribenuron-methyl in grain and straw of wheat (T-75WG-OR2-C) C/04/17 Institute of Industrial Organic Chemixxxx Branch Pszczyna, Department of Ecotoxicology, Pszczyna, Poland GLP Unpublished	N	CIECH Sarzyna S.A.
KCP 5.1.2/02	M. Xxxx	2018	Determination of the residues of MCPA and tribenuron-methyl in grain and straw of wheat C/05/17 Institute of Industrial Organic Chemixxxx Branch Pszczyna, Department of Ecotoxicology, Pszczyna, Poland GLP Unpublished	N	CIECH Sarzyna S.A.
KCP 5.1.2/03	Xxxx E.	2017	Tribenuron methyl 75 WG, *Daphnia magna*, Acute immobilisation Test W/265/17 Institute of Industrial Organic Chemixxxx Branch Pszczyna, Department of Ecotoxicology, Pszczyna, Poland GLP Unpublished	N	CIECH Sarzyna S.A.
KCP 5.1.2/04	Xxxx E.	2018	Tribenuron methyl 75 WG, *Pseudokirchinella subcapitata* SAG 61.81, Growth inhibtion Test; W/266/17 Institute of Industrial Organic Chemixxxx Branch Pszczyna, Department of Ecotoxicology, Pszczyna, Poland GLP Unpublished	N	CIECH Sarzyna S.A.
KCP 5.1.2/05	Xxxx E.	2018	Tribenuron methyl 75 WG, *Lemna gibba* CPCC 310, Growth inhibtion Test W/268/17	N	CIECH Sarzyna S.A.

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
			Institute of Industrial Organic Chemixxxx Branch Pszczyna, Department of Ecotoxicology, Pszczyna, Poland GLP Unpublished		
KCP 5.1.2/06	Xxxx E.	2018	Tribenuron methyl 75 WG, *Navicula pelliculosa* SAG 1050-3, Growth inhibtion Test W/267/17 Institute of Industrial Organic Chemixxxx Branch Pszczyna, Department of Ecotoxicology, Pszczyna, Poland GLP Unpublished	N	CIECH Sarzyna S.A.
KCP 5.1.2/07	Xxxx A.	2018	Tribenuron metyl 75 WG Terrestrial Plant Test: Vegetative Vigour Test G/157/17 Institute of Industrial Organic Chemixxxx Branch Pszczyna, Department of Ecotoxicology, Pszczyna, Poland GLP Unpublished	N	CIECH Sarzyna S.A.
KCP 5.1.2/08	Xxxx A.	2018	Tribenuron metyl 75 WG Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test G/156/17 Institute of Industrial Organic Chemixxxx Branch Pszczyna, Department of Ecotoxicology, Pszczyna, Poland GLP Unpublished	N	CIECH Sarzyna S.A.
KCP 5.1.2/09	Xxxx T.	2016	Chronic toxicity of PP-108 H (Tribenuron methyl 75 WG) on honeybees (Apis mellifera L.) TRIALCAMP S.L.U. STUDY TRC15-250BA GLP Unpublished	N	PROPLAN, Plant Protection Company S.L.
KCP 5.1.2/10	Xxxx T.	2016	Toxicity of PP-108 H (Tribenuron methyl 75 WG) on honey bee larvae (Apis mellifera L.) after repeated exposure unde laboratory conditions TRIALCAMP S.L.U. STUDY TRC15-249BA GLP Unpublished	N	PROPLAN, Plant Protection Company S.L.
KCP 5.2/01	Xxxx A.	2019	Development and Validation of an Analytical Method for Determination of Tribenuron-methyl in Plant Matrices Eurofins S18-07519	N	TF PROPLAN-SARABIA

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		
KCP 5.2/02	Xxxx S.	2019	Independent Laboratory validation of a Multi-Residue Method QuEChERS for the determination of Tribenuron-methyl in Two Matrices of Plant Origin Eurofins P5114 G GLP Unpublished	N	TF PROPLAN-SARABIA
KCP 5.2/03	Xxxx D.	2016	Validation of the Methods of Analysis used for the Determination of Metsulfuron-Methyl, Thifensulfuron-Methyl and Tribenuron-Methyl in various matrices, in Compliance with Good Laboratory Practice and referencing SANCO/3029/99 Analytical Laboratories Ltd DNA3620 GLP Unpublished	N	TF PROPLAN-SARABIA
KCP 5.2/04	Xxxx D	2019	Addendum 1 Issued 21st February 2019 Validation of the Methods of Analysis used for the Determination of Metsulfuron-Methyl, Thifensulfuron-Methyl and Tribenuron-Methyl in various matrices, in Compliance with Good Laboratory Practice and referencing SANCO/3029/99 Analytical Laboratories Ltd DNA3620 GLP Unpublished	N	TF PROPLAN-SARABIA
KCP 5.2/05	Xxxx M., Xxxx S.	2018	Metsulfuron-methyl and Tribenuron methyl: Independent Laboratory Validation of an Analytical Method for the determination in Animal Matrices Ibacon GmbH 123361101 GLP Unpublished	N	TF PROPLAN-SARABIA
KCP 5.2/06	Xxxx M.	2018	Validation of an analytical method for the determination of Tribenuron methyl and its metabolites in soil according to SANCO/825/00 rev. 8.1 Fraunhofer PRO-001/6-20/B	N	TF PROPLAN-SARABIA

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		
KCP 5.2/07	Xxxx S.	2019	Amendment Report. Validation of an analytical method for the determination of Tribenuron methyl and its metabolites in soil according to SANCO/3029/99 rev. 4 and SANCO/825/00 rev. 8.1 Fraunhofer PRO-001/6-20/B GLP Unpublished	N	TF PROPLAN-SARABIA
KCP 5.2/08	Xxxx S.	2018	Validation of an analytical method for the determination of Tribenuron methyl and its metabolites in drinking water according to SANCO/825/00 rev. 8.1 PRO-001/6-22 Fraunhofer Istitute for Molecular Biology and Applied Ecology IME GLP Unpublished	N	TF PROPLAN-SARABIA
KCP 5.2/09	Xxxx W.	2018	Independent Laboratory Validation (ILV) of an analytical method for the determination of Tribenuron methyl and three of its metabolites in drinking water according to SANCO/825/00 rev. 8.1 PRO-001/6-22/a Fraunhofer Istitute for Molecular Biology and Applied Ecology IME GLP Unpublished	N	TF PROPLAN-SARABIA

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

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 5.2/01	Xxxx J.S, Xxxx W.	2000	Analytical enforcement method for the determination of Tribenuron methyl in cereals (grain, forage and	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
			straw) using column-switching liquid chromatography with ultraviolet detection DuPont Experimental Station DuPont-3595 non GLP Unpublished		
KCP 5.2/02	Xxxx E., Xxxx.	2000	Combined decline and magnitude of residue of Tribenuron methyl in cereals (spring barley, spring wheat, winter wheat) following application of Tribenuron methyl 75WG-Europe, season 1999 Institute Fresenius Chemische und Biologische/GmbH DuPont-2261 Revision No.1 GLP Unpublished	N	DuPont
KCP 5.2/03	Xxxx B	2001	Independent laboratory validation for the “Analytical enforcement method for the quantitation of Tribenuron methyl in wheat grain, straw, forage by HPLC column-switching with UV detection” (DuPont-3595) as described by Institute Fresenius in DuPont-2261 Revision No.1 project report EN-CAS Analytical Laboratories DuPont-5587 GLP Unpublished	N	DuPont
KCP 5.2/04	Xxxx N.L	2000	Independent laboratory validation and confirmatory methodology of DuPont method report number AMR 3698-95 “Analytical method for the determination of Tribenuron methyl (DPX-L5300) in whole milk, eggs and animal tissues (beef and poultry muscle) by HPLC” Dupont Stine-Haskell Research Center DuPont-4245 GLP Unpublished	N	DuPont
KCP 5.2/05	Xxxx M.D.	1996	Analytical method for the determination of Tribenuron methyl (DPX-L5300) in whole milk, eggs and animal tissues (beef and poultry muscle) by HPLC ABC Laboratories, Inc. AMR 3698-95 non 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
			Unpublished		
KCP 5.2/06	Xxxx N.L	2000	Independent laboratory validation and confirmatory methodology of DuPont method report number AMR 3698-95 “Analytical method for the determination of Tribenuron methyl (DPX-L5300) in whole milk, eggs and animal tissues (beef and poultry muscle) by HPLC” Dupont Stine-Haskell Research Center DuPont-4245 GLP Unpublished	N	DuPont
KCP 5.2/07	Xxxx, S. J., Xxxx, J. J.	2001	Analytical method for the determination of 13 DuPont sulfonylurea herbicides in soil using LC/MS/MS DuPont-5082, Revision No. 1 GLP/GEP: no, Unpublished	N	DuPont
KCP 5.2/08	Xxxx M. R., Xxxx T.J., Xxxx M.E.Y	2001	Analytical method for the determination of Tribenuron methyl metabolites IN-L5296, IN-A4098 and IN-00581 in soil using HPLC-MS/MS Dupont Stine-Haskell Research Center DuPont-5838 non GLP Unpublished	N	DuPont
KCP 5.2/09	Xxxx M. R., Xxxx J.J.	2001	Analytical method for the determination of Tribenuron methyl and metabolites IN-L5296, IN-A4098, IN-D5119, and IN 00581 in water using LC/MS/MS DuPont-5856; GLP/GEP: no, Unpublished	N	DuPont
KCP 5.2/10	Xxxx J.J./	2014	Analytical method for the determination of tribenuron methyl and metabolites IN-L5296, IN-A4098, IN-D5119, and IN-00581 in water using LC/MS/MS DuPont-5856, Supplement No. 1; GLP/GEP: no, Unpublished	N	DuPont
KCP 5.2/11	Xxxx T., Xxxx S.	2000	Analytical method and confirmatory method for the determination of tribenuron methyl in air DuPont-4108	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**
			PTRL Europe GLP Unpublished		
KCP 5.2/12	Xxxx, R. M., Xxxx, J. J.	2016	Analytical method for the determination of chlorsulfuron, metsulfuron methyl, thifensulfuron methyl and tribenuron methyl in plasma and urine by LC/MS/MS Dupont-47394 DuPont Stine-Haskell Research Center GLP: no Unpublished	N	DuPont

Appendix 2 Detailed evaluation of submitted analytical methods

A 2.1 Analytical methods for tribenuron methyl

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

A 2.1.1.1 Determination of tribenuron methyl residues in wheat

A 2.1.1.1.1 Method validation

Comments of zRMS:	The study is accepted. The method was validated in accordance with EC Guidance Document SANCO/3029/99 rev. 4 and SANCO/825/00 rev 8.1. The limit of quantification (LOQ) for tribenuron-methyl was 0.010 mg/kg grains and straw of wheat. The limit of detection (LOD) was 0.003 mg/kg. The recovery of the method and confirmatory method aimed at determining the residues of tribenuron-methyl in grains and straw of wheat was estimated for three fortification levels, i.e. 0.01, 0.10, and 1.00 mg/kg. The mean extraction recovery levels for tribenuron-methyl in grains of wheat were 92.7%, 87.6% and 86.5%. The mean extraction recovery levels for tribenuron-methyl in straw of wheat were 94.2%, 87.2% and 84.7%. In the confirmatory method the mean extraction recovery levels for tribenuron-methyl in grains of wheat were 94.1%, 91.3% and 88.1%. In the confirmatory method the mean extraction recovery levels for tribenuron-methyl in straw of wheat were 94.1%, 91.0% and 90.6%. The precision for tribenuron-methyl in grains of wheat was between 2.3% - 4.5%. The precision for tribenuron-methyl in straw of wheat was between 3.8% - 4.3%. In the confirmatory method the precision for tribenuron-methyl in grains of wheat was between 2.2% - 4.2%. In the confirmatory method the precision for tribenuron-methyl in straw of wheat was between 2.0% - 5.6%. The mean recovery results of the quality control samples of tribenuron-methyl was 97% for grain of wheat and 98% for straw of wheat. In confirmatory method the mean recovery results of the quality control samples of tribenuron-methyl was 97% for grain of wheat and 98% for straw of wheat. Table 1. Results of the determination of the residues of tribenuron-methyl in grains of wheat

Trial	Sample code/ Specimen ID	Plot	Determined concentration mg/kg	
			in replicates	average
S17-04788-01 (Germany)	S17-04788-01-001A	U1	< LOD < LOD < LOD	< LOD
S17-04788-01 (Germany)	S17-04788-01-003A	2	< LOD < LOD < LOD	< LOD
S17-04788-03 (Hungary)	S17-04788-03-001A	U1	< LOD < LOD < LOD	< LOD
S17-04788-03 (Hungary)	S17-04788-03-003A	2	< LOD < LOD < LOD	< LOD
SRPL-056-428FR (Poland)	SRPL-056-428FR-01	Plot 1	< LOD < LOD < LOD	< LOD
SRPL-056-428FR (Poland)	SRPL-056-428FR-03	Plot 2	< LOD < LOD < LOD	< LOD

Table 2. Results of the determination of the residues of tribenuron-methyl in straw of wheat

Trial	Sample code/ Specimen ID	Plot	Determined concentration mg/kg	
			in replicates	average
S17-04788-01 (Germany)	S17-04788-01-002A	U1	< LOD < LOD < LOD	< LOD
S17-04788-01 (Germany)	S17-04788-01-004A	2	< LOD < LOD < LOD	< LOD
S17-04788-03 (Hungary)	S17-04788-03-002A	U1	< LOD < LOD < LOD	< LOD
S17-04788-03 (Hungary)	S17-04788-03-004A	2	< LOD < LOD < LOD	< LOD
SRPL-056-428FR (Poland)	SRPL-056-428FR-02	Plot 1	< LOD < LOD < LOD	< LOD
SRPL-056-428FR (Poland)	SRPL-056-428FR-04	Plot 2	< LOD < LOD < LOD	< LOD

Reference: KCP 5.1.2/01

Report	Determination of the residues of tribenuron-methyl in grain and straw of wheat (T-75WG-OR2-C), M. Xxxx, 2018, Study Code: C/04/17, Institute of Industrial Organic Chemixxxx Branch Pszczyna, Department of Ecotoxicology, Pszczyna, Poland
Guideline(s):	Yes (SANCO/825/00, rev. 8.1., SANCO/3029/99, rev. 4)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The study involved the determination of the residues of tribenuron-methyl in grains and straw of wheat treated with T-75WG-OR2-C. Tribenuron-methyl was detected using liquid chromatographic method (HPLC) with UV-Vis.
To develop and validate the analytical method and confirmatory method used samples of grain and straw of wheat, delivered by the Sponsor. They were not treated with any preparations containing tribenuron-methyl. The range of linearity of the analytical graph, specificity, precision, recovery, matrix effects and limits of quantification and detection of the detected compound were determined. The range of linearity of the analytical graphs of tribenuron-methyl varied from 0.05 µg//mL to 10.0 µg/mL. The recovery
of the method and confirmatory method aimed at determining the residues of tribenuron-methyl in grains and straw of wheat was estimated for three fortification levels, i.e. 0.01, 0.10, and 1.00 mg/kg.
The limit of quantification (LOQ) for tribenuron-methyl was 0.010 mg/kg grains and straw of wheat. The limit of detection (LOD) was 0.003 mg/kg.

Table A 1: Recovery results from method validation tribenuron-methyl using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Recovery (%)	RSD (%)	Mean Instrument Recovery (%)	Mean RSD (%)	Comments
Grains of wheat	Tribenuron-methyl	0.01*	92.7	4.5	97	1.8	-
		0.100	87.6	2.3			-
		1.000	86.5	3.1			
Straw of wheat		0.01*	94.2	3.9	98	1.9	-
		0.100	87.2	4.3			-
		1.000	84.7	3.8			

*-Limit of quantification, defined by the lowest validated fortification level

Table A 2 Characteristics for the analytical method used for validation of Tribenuron-Methyl residues in grains and straw of wheat

	Tribenuron-Methyl
Specificity	The specificity of the analytical and confirmatory method was determined on the basis of the analysis of chromatograms for control and fortified grains and straw of wheat samples. When considering the results of the analysis, it was found out that no signals of tribenuron-methyl were overlapping with the matrix signals of the control samples under the experimental conditions. Therefore, the specificity of the method was demonstrated.

	Tribenuron-Methyl
Specificity	The specificity of the analytical and confirmatory method was determined on the basis of the analysis of chromatograms for control and fortified grains and straw of wheat samples. When considering the results of the analysis, it was found out that no signals of tribenuron-methyl were overlapping with the matrix signals of the control samples under the experimental conditions. Therefore, the specificity of the method was demonstrated.
Calibration (type, number of data points)	The standard curve representing the relationship between the peak area and the concentration of tribenuron-methyl was linear. The range of linearity of the analytical graphs varied from 0.05 µg/mL to 10.0 µg/mL. The calibration curve for analytical method is described as y = 719230.1x – 12796.89 (a – slope, b – constant term). The regression coefficient (r^2) was 0.998297. The calibration curve for confirmatory method is described as y = 723243.7x – 5477.912 (a – slope, b – constant term). The regression coefficient (r^2) was 0.999921.
Assessment of matrix effects is presented	Yes
Limit of determination/quantification	The limit of quantification is the lowest concentration of tribenuron-methyl at which the acceptable mean recovery is obtained (i.e. 70 – 110%; RSD ≤ 20%). The limit of detection is the lowest concentration of tribenuron-methyl that can be reliably differentiated from the background noise. The limit of quantification (LOQ) for tribenuron-methyl was 0.01 mg/kg grains and straw of wheat. The limit of detection (LOD) was 0.003 mg/kg test item.

Conclusion

The study involved the determination of the residues of tribenuron-methyl in grains and straw of wheat treated with T-75 WG-OR2-C. The range of linearity of the analytical graph, specificity, precision, recovery, matrix effects and limits of quantification and detection of the detected compound were determined. The method was validated in accordance with EC Guidance Document SANCO/3029/99 rev. 4 and SANCO/825/00 rev 8.1.

The range of linearity of the analytical graphs of tribenuron-methyl varied from 0.05 µg/mL to 10.0 µg//mL.
The recovery of the method and confirmatory method aimed at determining the residues of tribenuron-methyl in grains and straw of wheat was estimated for three fortification levels, i.e. 0.01, 0.10, and 1.00 mg/kg. The mean recovery results of the quality control samples of tribenuron-methyl was 97% for grain of wheat and 98% for straw of wheat. The limit of quantification (LOQ) for tribenuron-methyl was 0.010 mg/kg grains and straw of wheat.
The limit of detection (LOD) was 0.003 mg/kg.

The levels of residues of tribenuron-methyl in all grain and straw of wheat samples are below the limit of detection, i.e. 0.003 mg/kg. Hence, they are below the maximum residue limit, i.e. 0.01 mg/kg of grains of wheat for tribenuron-methyl.

A 2.1.1.1.2 Confirmatory method

No confirmatory method is required.
According to SANCO/3029/99 rev. 4 11/07/00 p. 3.2 additional confirmatory analysis will not be required where primary residue method is shown to be specific to the analyte of interest.

The method which was used to determination of residues in plant sample is specific for tribenuron methyl. For details please see point A 2.1.1.1.1

A 2.1.1.2 Determination of tribenuron methyl residues in wheat

A 2.1.1.2.1 Method validation

Comments of zRMS:	The study is accepted. The method was validated in accordance with EC Guidance Document SANCO/3029/99 rev. 4 [4] and SANCO/825/00 rev 8.1. The recovery of the method aimed at determining the residues of tribenuron-methyl in grains and straw of wheat was estimated for two fortification levels, i.e. 0.01 and 0.10 mg/kg. The mean extraction recovery levels for tribenuron-methyl in grains of wheat were 92.9% and 91.7%. The mean extraction recovery levels for tribenuron-methyl in straw of wheat were 88.3% and 87.5%. The precision for tribenuron-methyl in grains of wheat was between 3.5% - 4.5%. The precision for tribenuron-methyl in straw of wheat was between 2.2% - 4.9%. The mean recovery results of the quality control samples of tribenuron-methyl was 97% for grain of wheat and 94% for straw of wheat. The limit of quantification (LOQ) for tribenuron-methyl was 0.010 mg/kg grains and straw of wheat. The limit of detection (LOD) was 0.003 mg/kg. The levels of residues of tribenuron-methyl in all grain and straw of wheat samples are below the limit of detection, i.e. 0.003 mg/kg. Hence, they are below the maximum residue limit, i.e. 0.01 mg/kg of grains of wheat for tribenuron-methyl. Table 1. Results of the determination of the residues of tribenuron-methyl in grains of wheat.

Trial	Sample code/ Specimen ID	Plot	Determined concentration mg/kg	
			in replicates	average
S17-04789-01 (Germany)	S17-04789-01-001A	U1	< LOD < LOD < LOD	< LOD
S17-04789-01 (Germany)	S17-04789-01-003A	2	< LOD < LOD < LOD	< LOD
S17-04789-02 (UK)	S17-04789-02-001A	U1	< LOD < LOD < LOD	< LOD
S17-04789-02 (UK)	S17-04789-02-003A	2	< LOD < LOD < LOD	< LOD
S17-04789-03 (Hungary)	S17-04789-03-001A	U1	< LOD < LOD < LOD	< LOD
S17-04789-03 (Hungary)	S17-04789-03-003A	2	< LOD < LOD < LOD	< LOD
SRPL-057-428HR (Poland)	SRPL-057-428HR-01	Plot 1	< LOD < LOD < LOD	< LOD
SRPL-057-428HR (Poland)	SRPL-057-428HR-03	Plot 2	< LOD < LOD < LOD	< LOD

Table 2. Results of the determination of the residues of tribenuron-methyl in straw of wheat.

Trial	Sample code/ Specimen ID	Plot	Determined concentration mg/kg	
			in replicates	average
S17-04789-01 (Germany)	S17-04789-01-002A	U1	< LOD < LOD < LOD	< LOD
S17-04789-01 (Germany)	S17-04789-01-004A	2	< LOD < LOD < LOD	< LOD
S17-04789-02 (UK)	S17-04789-02-002A	U1	< LOD < LOD < LOD	< LOD
S17-04789-02 (UK)	S17-04789-02-004A	2	< LOD < LOD < LOD	< LOD
S17-04789-03 (Hungary)	S17-04789-03-002A	U1	< LOD < LOD < LOD	< LOD
S17-04789-03 (Hungary)	S17-04789-03-004A	2	< LOD < LOD < LOD	< LOD
SRPL-057-428HR (Poland)	SRPL-057-428HR-02	Plot 1	< LOD < LOD < LOD	< LOD
SRPL-057-428HR (Poland)	SRPL-057-428HR-04	Plot 2	< LOD < LOD < LOD	< LOD

Reference: KCP 5.1.2/02

Report: Determination of the residues of MCPA and tribenuron-methyl in grain and straw of wheat, M. Xxxx, 2018, Study Code: C/05/17, Institute of Industrial Organic Chemixxxx Branch Pszczyna, Department of Ecotoxicology, Pszczyna, Poland

Guideline(s): Yes (SANCO/825/00, rev. 8.1., SANCO/3029/99, rev. 4)

Deviations: No

GLP: Yes

Acceptability: Yes

Materials and methods

The study involved the determination of the residues of tribenuron-methyl in grains and straw of wheat treated with MT-565SG-OR2-C (containing 15 g/kg of tribenuron methyl). Tribenuron-methyl was detected using liquid chromatographic method (HPLC) with LC-MS/MS.
To develop and validate the analytical method and confirmatory method used samples of grain and straw of wheat, delivered by the Sponsor. They were not treated with any preparations containing tribenuron-methyl. The range of linearity of the analytical graph, specificity, precision, recovery, matrix effects and

limits of quantification and detection of the detected compound were determined. The range of linearity of the analytical graphs of tribenuron-methyl varied from 1.0 ng/mL to 100.0 ng/mL. The recovery of the method aimed at determining the residues of tribenuron-methyl in grains and straw of wheat was estimated for two fortification levels, i.e. 0.01 and 0.10 mg/kg.
The limit of quantification (LOQ) for tribenuron-methyl was 0.010 mg/kg grains and straw of wheat.
The limit of detection (LOD) was 0.003 mg/kg.

Table A 3 Recovery results from method validation tribenuron-methyl using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Recovery (%)	RSD (%)	Mean Instrument Recovery (%)	Mean RSD (%)	Comments
Grains of wheat	Tribenuron-methyl	0.01	92.9	4.5	97	2.1	-
		0.100	91.7	3.5			-
Straw of wheat		0.01	88.3	4.9	98	2.1	-
		0.100	87.5	2.2			-

Table A 4 Characteristics for the analytical method used for validation of Tribenuron-Methyl residues in grains and straw of wheat

	Tribenuron-Methyl
Specificity	The specificity of the analytical and confirmatory method was determined on the basis of the analysis of chromatograms for control and fortified grains and straw of wheat samples. When considering the results of the analysis, it was found out that no signals of tribenuron-methyl were overlapping with the matrix signals of the control samples under the experimental conditions. Therefore, the specificity of the method was demonstrated.
Calibration (type, number of data points)	The standard curve representing the relationship between the peak area and the concentration of tribenuron-methyl was linear. The range of linearity of the analytical graphs varied from 0.05 µg/mL to 10.0 µg/mL. The calibration curve for analytical method is described as y = 89881.8x + 725.183 (a – slope, b – constant term). The regression coefficient (r_2) was 0.9998284
Assessment of matrix effects is presented	Yes
Limit of determination/quantification	The limit of quantification is the lowest concentration of tribenuron-methyl at which the acceptable mean recovery is obtained (i.e. 70 – 110%; RSD ≤ 20%). The limit of detection is the lowest concentration of tribenuron-methyl that can be reliably differentiated from the background noise. The limit of quantification (LOQ) for tribenuron-methyl was 0.01 mg/kg grains and straw of wheat. The limit of detection (LOD) was 0.003 mg/kg test item.

Conclusion

The study involved the determination of the residues of tribenuron-methyl in grains and straw of wheat treated with MT-565SG-OR2-C (containing 15 g/kg of tribenuron methyl). The range of linearity of the analytical graph, specificity, precision, recovery, matrix effects and limits of quantification and detection of the detected compound were determined. The method was validated in accordance with EC Guidance Document SANCO/3029/99 rev. 4 and SANCO/825/00 rev 8.1.

The range of linearity of the analytical graphs of tribenuron-methyl varied from 1.0 ng/mL to 100.0

ng/mL. The recovery of the method aimed at determining the residues of tribenuron-methyl in grains and straw of wheat was estimated for two fortification levels, i.e. 0.01 and 0.10 mg/kg.
The mean recovery results of the quality control samples of tribenuron-methyl were 97% for grains of wheat and 94% for straw of wheat.
The limit of quantification (LOQ) for tribenuron-methyl was 0.01 mg/kg grains and straw of wheat. The limit of detection (LOD) was 0.003 mg/kg test item.

The levels of residues of tribenuron-methyl in all grain and straw of wheat samples are below the limit of detection, i.e. 0.003 mg/kg. Hence, they are below the maximum residue limit, i.e. 0.01 mg/kg of grains of wheat for tribenuron-methyl.

A 2.1.1.2.2 Confirmatory method

No confirmatory method is required.
According to SANCO/3029/99 rev. 4 11/07/00 p. 3.2 additional confirmatory analysis will not be required where primary residue method is shown to be specific to the analyte of interest.
The method which was used to determination of residues in plant sample is specific for tribenuron methyl. For details please see point A 2.1.1.2.1.

A 2.1.1.3 Determination of residues in culture medium used in support of ecotoxicological studies. Study 1

A.2.1.1.3.1 Method validation

Comments of zRMS:	Analytical part of the method is accepted with the limit of quantification in Elendt M7 medium equal 0.001 mg/L and the limit of detection 0.0003 mg/L. The mean recoveries at the levels 0.001 mg/L (n=5) and 1.0 mg/L (n=5) were in the range 70-120% with RSD <20%.

Reference:	KCP 5.1.2/03
Report	Tribenuron metyl 75 WG, *Daphnia magna* Acute Immobilization Test, E. Xxxx, Msc Eng 2017, W/265/17
Guideline(s):	Yes (OECD Guidline No. 202 (2004)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

For study report see section B9.

Materials and methods

The following liquid chromatography parameters were used:

column	Luna 5µ C18 100A, l = 250 mm, ϕ = 4.6 mm
mobile phase	acetonitrile : 0.05% solution of orthophosphoric (V) acid (60 : 40, v/v),
wave length	231 nm
flow rate	1.0 mL/min.
injected volume	20 µL

Sample preparation:

Each sample of 10 to 100 mL volume (i.e. control sample, test sample, sample fortified with standard) was collected. The sample was applied to ENVI-18 (3 mL, 500 mg) column conditioned previously by sequential washing twice with 5 mL of acetonitrile, twice with 5 mL of deionized water.
Following the sample introduction the column was dried for 5 minutes. The part of sample with affinity to the column was eluted with 12 mL of acetonitrile. Eluate was evaporated to dryness using vacuum rotary evaporator (at 35°C). The dry residue was dissolved in acetonitrile and 20 µL was applied to the chromatographic column.

Determination:

The concentrations of tribenuron methyl were chemically determined using a validated liquid chromatographic method with UV-Vis detection. Samples of each test item concentration and the control collected at exposure initiation and termination were analyzed.

Results and discussions

Table A 5 Recovery level and precision of tribenuron methyl in fortified samples of Elendt M7 medium (n=5)

Nominal concentration [mg/L]	Determined concentration of tribenuron methyl in replicates [mg/L]					Average [mg/L]	Recovery [%]	SD [mg/L]	RSD [%]
	1	2	3	4	5				
control	0.00000	0.00000	--	--	--	0.00000	--	0.00000	--
0.001	0.00099	0.00104	0.00096	0.00102	0.00105	0.00101	101.3	0.00004	3.8
1.000	1.034	1.046	1.057	0.965	1.066	1.034	103.4	0.040	3.9

LoQ = 0.001 mg/L
LoD = 0.0003 mg/L
SD – standard deviation
RSD – relative standard deviation

Results of the recovery experiments indicate that the recovery efficiency and repeatability were within acceptable limits of 70% - 120% for mean recovery and ≤ 20% RSD.

Table A 6 Characteristics for the analytical method used for validation of tribenuron methyl in Elendt M7 medium

	tribenuron methyl
Specifity	The specificity of analytical method was determined on the basis of the analysis of the chromatograms obtained for the control (Elendt M7 medium)

	tribenuron methyl
	and fortified samples. Considering the results of the analysis, no signal of detected substance was overlapping with matrix signals under the experimental conditions. Therefore, the specificity of the method was demonstrated.
Calibration (type, number of data points)	Curve Type: Linear y = +6.009879e+005x
Calibration range	The working solutions of tribenuron methyl at the concentrations of 0.05, 0.1, 0.5, 1.0, 5.0, 10.0 and 20.0 mg/mLwere injected successively to the chromatographic column and the chromatograms were recorded. The standard curve was linear with the regression coefficient (r2) is 0.999695. The range of linearity is from 0.05 µg/mL to 20.0 µg/mL.
Assessment of matrix effects is presented	no
Limit of determination/quantification	The Limit of Quantification (LoQ) in Elendt M7 medium is 0.001 mg/L and the Limit of Detection (LoD) is 0.0003 mg/L.

Conclusion

The analytical method therefore meets the requirements of guideline SANCO/3029/99 rev. 4

A 2.1.1.4 Determination of residues in culture medium used in support of ecotoxicological studies. Study 2

A.2.1.1.4.1 Method validation

Comments of zRMS:	Analytical part of the method is accepted with the limit of quantification in AAP medium equal 0.001 mg/L and the limit of detection 0.0003 mg/L. The mean recoveries at the levels 0.001 mg/L (n=5) and 1.0 mg/L (n=5) were in the range 70-120% with RSD <20%.

Reference:	KCP 5.1.2/04
Report	Tribenuron metyl 75 WG, *Pseudokirchneriella subcapitata* SAG 61.81, Growth Inhibition Test, Ewa Xxxx, 2018, W/266/17
Guideline(s):	Yes (OECD Guidline No. 201 (2006)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

For material, methods and sample preparation see point A 2.1.1.3.1

For study report see section B9

Results and discussions

Table A 7: Recovery level and precision of tribenuron methyl in fortified samples of AAP medium (n=5)

Nominal concentration [mg/L]	Determined concentration of tribenuron methyl in replicates [mg/L]					Average [mg/L]	Recovery [%]	SD [mg/L]	RSD [%]
	1	2	3	4	5				
Control	0.0000	0.0000	--	--	--	0.0000	--	0.0000	--
0.001	0.00087	0.00093	0.00080	0.00082	0.00093	0.00087	86.9	0.00006	6.9
1.000	1.065	1.035	0.875	0.870	1.025	0.974	97.4	0.094	9.6

LoQ = 0.001 mg/L
LoD = 0.0003 mg/L
SD – standard deviation
RSD – relative standard deviation

Results of the recovery experiments indicate that the recovery efficiency and repeatability were within acceptable limits of 70% - 120% for mean recovery and ≤ 20% RSD.

Table A 8 Characteristics for the analytical method used for validation of tribenuron methyl in AAP medium

	tribenuron methyl
Specifity	The specificity of analytical method was determined on the basis of the analysis of the chromatograms obtained for the control (AAP medium) and fortified samples. Considering the results of the analysis, no signal of detected substance was overlapping with matrix signals under the experimental conditions. Therefore, the specificity of the method was demonstrated.
Calibration (type, number of data points)	Curve Type: Linear y = +6.009879e+005x
Calibration range	The working solutions of tribenuron methyl at the concentrations of 0.05, 0.1, 0.5, 1.0, 5.0, 10.0 and 20.0 mg/mLwere injected successively to the chromatographic column and the chromatograms were recorded. The standard curve was linear with the regression coefficient (r2) is 0.999695. The range of linearity is from 0.05 µg/mL to 20.0 µg/mL.
Assessment of matrix effects is presented	no
Limit of determination/quantification	The Limit of Quantification (LoQ) in AAP medium is 0.001 mg/L and the Limit of Detection (LoD) is 0.0003 mg/L.

A 2.1.1.5 Determination of residues in culture medium used in support of ecotoxicological studies. Study 3

A.2.1.1.5.1 Method validation

Comments of zRMS:	Analytical part of the method is accepted with the limit of quantification in AAP medium equal 0.001 mg/L and the limit of detection 0.0003 mg/L. The mean recoveries at the levels 0.001 mg/L (n=5) and 1.0 mg/L (n=5) were in the range

	70-120% with RSD <20%.

Reference:	KCP 5.1.2/05
Report	Tribenuron metyl 75 WG, *Lemna gibba* L. CPCC 310, Growth Inhibition Test, Ewa Xxxx, 2018, W/268/17
Guideline(s):	Yes (OECD Guidline No. 221 (2006)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

For material, methods and sample preparation see point A 2.1.1.3.1

For study report see section B9

Results and discussions

Table A 9 Recovery level and precision of tribenuron methyl in fortified samples of AAP medium (n=5)

Nominal concentration [mg/L]	Determined concentration of tribenuron methyl in replicates [mg/L]					Average [mg/L]	Recovery [%]	SD [mg/L]	RSD [%]
	1	2	3	4	5				
Control	0.00000	0.00000	--	--	--	0.00000	--	0.00000	--
0.001	0.00117	0.00104	0.00094	0.00111	0.00097	0.00105	104.6	0.0009	8.8
1.000	1.096	1.015	1.057	1.053	1.028	1.050	105.0	0.031	3.0

LoQ = 0.001 mg/L
LoD = 0.0003 mg/L
SD – standard deviation
RSD – relative standard deviation

Results of the recovery experiments indicate that the recovery efficiency and repeatability were within acceptable limits of 70% - 120% for mean recovery and ≤ 20% RSD.

Table A 10 Characteristics for the analytical method used for validation of tribenuron methyl in AAP medium

	tribenuron methyl
Specifity	The specificity of analytical method was determined on the basis of the analysis of the chromatograms obtained for the control (AAP medium) and fortified samples. Considering the results of the analysis, no signal of detected substance was overlapping with matrix signals under the experimental conditions. Therefore, the specificity of the method was demonstrated.
Calibration (type, number of data points)	Curve Type: Linear y = +6.009879e+005x
Calibration range	The working solutions of tribenuron methyl at the concentrations of 0.05, 0.1, 0.5, 1.0, 5.0, 10.0 and 20.0 mg/mLwere injected successively to the chromatographic column and the chromatograms were recorded. The standard curve was linear with the regression coefficient (r2)

	tribenuron methyl
	is 0.999695. The range of linearity is from 0.05 µg/mL to 20.0 µg/mL.
Assessment of matrix effects is presented	no
Limit of determination/quantification	The Limit of Quantification (LoQ) in AAP medium is 0.001 mg/L and the Limit of Detection (LoD) is 0.0003 mg/L.

A 2.1.1.6 Determination of residues in culture medium used in support of ecotoxicological studies. Study 4

A.2.1.1.6.1 Method validation

Comments of zRMS:	Analytical part of the method is accepted with the limit of quantification in AAP-Si medium equal 0.001 mg/L and the limit of detection (LoD) 0.0003 mg/L. The mean recoveries at the levels 0.001 mg/L (n=5) and 1.0 mg/L (n=5) were in the range 70-120% with RSD <20%.

Reference:	KCP 5.1.2/06
Report	Tribenuron metyl 75 WG, *Navicula pelliculosa SAG 1050-3*, Growth Inhibition Test, Ewa Xxxx, 2018, W/267/17, Institute of Industrial Organic Chemixxxx Branch Pszczyna, Department of Ecotoxicology
Guideline(s):	Yes (OECD Guideline No. 201 (2006)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

For material, methods and sample preparation see point A 2.1.1.3.1

For study report see section B9

Results and discussions

Table A 11 Recovery level and precision of tribenuron methyl in fortified samples of AAP-Si medium (n=5)

Nominal concentration [mg/L]	Determined concentration of tribenuron methyl in replicates [mg/L]					Average [mg/L]	Recovery [%]	SD [mg/L]	RSD [%]
	1	2	3	4	5				
control	0.00000	0.00000	--	--	--	0.00000	--	0.00000	--
0.001	0.00103	0.00105	0.00100	0.00087	0.00101	0.00099	99.2	0.00007	7.3
1.000	1.046	0.978	1.075	1.025	0.980	1.020	102.0	0.042	4.1

LoQ = 0.001 mg/L
LoD = 0.0003 mg/L
SD – standard deviation
RSD – relative standard deviation

Results of the recovery experiments indicate that the recovery efficiency and repeatability were within acceptable limits of 70% - 120% for mean recovery and ≤ 20% RSD

Table A 12 Characteristics for the analytical method used for validation of tribenuron methyl in AAP-Si medium

	tribenuron methyl
Specifity	The specificity of analytical method was determined on the basis of the analysis of the chromatograms obtained for the control (AAP medium) and fortified samples. Considering the results of the analysis, no signal of detected substance was overlapping with matrix signals under the experimental conditions. Therefore, the specificity of the method was demonstrated.
Calibration (type, number of data points)	Curve Type: Linear y = +6.009879e+005x
Calibration range	The working solutions of tribenuron methyl at the concentrations of 0.05, 0.1, 0.5, 1.0, 5.0, 10.0 and 20.0 mg/mLwere injected successively to the chromatographic column and the chromatograms were recorded. The standard curve was linear with the regression coefficient (r2) is 0.999695. The range of linearity is from 0.05 µg/mL to 20.0 µg/mL.
Assessment of matrix effects is presented	no
Limit of determination/quantification	The Limit of Quantification (LoQ) in AAP medium is 0.001 mg/L and the Limit of Detection (LoD) is 0.0003 mg/L.

A 2.1.2.7 Determination of residues in deionized water used in support of eco-toxicological studies. Study 1

A 2.1.1.7.1 Method validation

Comments of zRMS:	Analytical part of the method is accepted with the limit of quantification in deionized water equal 0.1 mg/L and the limit of detection 0.05 mg/L. The mean

	recoveries at the levels 0.1 mg/L (n=5) and 10.0 mg/L (n=5) were in the range 70-120% with RSD <20%.

Reference: KCP 5.1.2/07

Report: Tribenuron metyl 75 WG Terrestrial Plant Test: Vegetative Vigour Test, Anna Xxxx, 2018, G/157/17

Guideline(s): Yes (OECD Guidline No. 227 (2006)

Deviations: No

GLP: Yes

Acceptability: Yes

Materials and methods

The concentration of Tribenuron methyl in water was determined using the validated high performance liquid chromatographic method with UV-Vis detection. To demonstrate the efficiency and repeatability of the analytical procedure by applying the analytical method, untreated deionized water samples fortified with Tribenuron methyl at concentration 0.1 mg/L (LOQ) were analysed on the day of analysis.

The following liquid chromatography parameters were used:

column	Luna 5µ C18 100A, l = 250 mm, ϕ = 4.6 mm
mobile phase	acetonitrile : 0.05% solution of orthophosphoric (V) acid (60 : 40, v/v),
wave length	231 nm
flow rate	1.0 mL/min.
injected volume	20 µL

Results and discussions

Table A 13 Recovery level and precision of tribenuron methyl in fortified samples (n=5)

Nominal concentration [mg/L]	Determined concentration of tribenuron methyl in replicates [mg/L]					Average [mg/L]	Recovery [%]	SD [mg/L]	RSD [%]
	1	2	3	4	5				
kontrola	0.000	0.000	--	--	--	0.000	--	0.000	--
0.1	0.090	0.094	0.084	0.099	0.094	0.092	92.4	0.006	6.2
10.0	9.938	9.574	9.207	9.104	8.019	9.169	91.7	0.722	7.9

LoQ = 0.1 mg/L
LoD = 0.05 mg/L

Results of the recovery experiments indicate that the recovery efficiency and repeatability were within acceptable limits of 70% - 120% for mean recovery and ≤ 20% RSD

Table A 14 Characteristics for the analytical method used for validation of tribenuron methyl in water

	tribenuron methyl
Specifity	The specificity of analytical method was determined on the basis of the analysis of the chromatograms obtained for the control deionized water samples and fortified samples. Considering the results of the analysis, no signal of detected substance was overlapping with matrix signals under the experimental conditions. Therefore, the specificity of the method was demonstrated.
Calibration (type, number of data points)	Curve Type: Linear y = +6.009879e+005x
Calibration range	The working solutions of tribenuron methyl at the concentrations of 0.05, 0.1, 0.5, 1.0, 5.0, 10.0 and 20.0 mg/mLwere injected successively to the chromatographic column and the chromatograms were recorded. The standard curve was linear with the regression coefficient (r2) is 0.999695. The range of linearity is from 0.05 µg/mL to 20.0 µg/mL.
Assessment of matrix effects is presented	no
Limit of determination/quantification	The Limit of Quantification (LoQ) in water is 0. 1 mg/L and the Limit of Detection (LoD) is 0.05 mg/L.

A 2.1.2.8 Determination of residues in deionized water used in support of eco-toxicological studies. Study 2

A 2.1.1.8.1 Method validation

Comments of zRMS:	Analytical part of the method is accepted with the limit of quantification in deionized water equal 0.1 mg/L and the limit of detection 0.05 mg/L. The mean recoveries at the levels 0.1 mg/L (n=5) and 10.0 mg/L (n=5) were in the range 70-120% with RSD <20%.
Reference:	KCP 5.1.2/08
Report	Tribenuron metyl 75 WG Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test, A. Xxxx, 2018, G/156/17
Guideline(s):	Yes (OECD Guidline No. 208 (2006)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

For material, methods, sample preparation and results and discussion see point A 2.1.1.7.1

A 2.1.1.9 Determination of residues in water, sucrose and diet matrix used in support of eco-toxicological studies.

A 2.1.1.9.1 Method validation

Comments of zRMS:	Analytical part of the method is accepted with the limit of quantification in: - water equal 20.20 µg/ml and the limit of detection 6.06 µg/ml. The mean recoveries at the levels 20.20 µg/ml (n=5) and 5 x LOQ = 99.99 µg/ml (n=5) were in the range 70-120% with RSD <20%; - sucrose equal 20.20 µg/ml and the limit of detection 6.06 µg/ml. The mean recoveries at the levels 20.20 µg/ml (n=5) and 5 x LOQ = 103.95 µg/ml (n=5) were in the range 70-120% with RSD <20%; - diet matrix equal 4.91 µg/ml and the limit of detection 1.47 µg/ml. The mean recoveries at the levels 4.91 µg/ml (n=5) and 20 x LOQ = 103.95 µg/ml (n=5) were in the range 70-120% with RSD <20%;

Reference:	KCP 5.1.2/09
Report	Chronic toxicity of PP-108 H (Tribenuron methyl 75 WG) on honeybees (Apis mellifera L.), Xxxx T., 2016, Study TRC15-250BA
Guideline(s):	Yes (SANCO/3029/99 Rev. 4)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Reference:	KCP 5.1.2/10
Report	Toxicity of PP-108 H (Tribenuron methyl 75 WG) on honeybees larvae (Apis mellifera L.) after repeated exposure under laboratory conditions, Xxxx T., 2016, Study TRC15-249BA
Guideline(s):	Yes (SANCO/3029/99 Rev. 4)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The aim of the test is to validate the analytical method for the quantification of the Tribenuron methyl content in stock solutions following the guideline SANCO 3029/99 rev 4. The technique applied for Tribenuron-methyl content determination was HPLC

Method conditions:

Column :	Zorbax eclipse plus (100 x 4.6 mm) agilent
Mobile Phase :	Acidic water (60 %) : Acetonitrile (40 %)
Flow :	2.0 ml/min
Wavelength :	254 nm
Time of analysis :	5 minutes
Oven Temperature :	40 şC
Injection :	25 µl

Treated solution sample was thawed at ambient temperature. The whole vial received was weighed, solvent was added and the solution was passed to the indicated volumetric flask. The received vial was

cleaned with solvent until final volume was achieved. The dried and empty vial was weighed.

Results and discussions

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

Matrix	Analyte	Fortification level (µg/ml) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
Water	Tribenuron methyl	20.20	95.7	0.86	
		99.99	97.1	1.51	
Sucrose	Tribenuron methyl	20.20	99.2	1.04	
		103.95	100.14	1.13	
Diet	Tribenuron methyl	4.91	95.0	2.26	
		103.95	100.75	0.79	

Table A 16: Characteristics for the analytical method used for validation of Tribenuron methyl residues in water matrix

	Tribenuron methyl
Specificity	Not overlapping of peaks is observed. Interference < 30 % of the LOQ
Calibration (type, number of data points)	Linear calibration function was obtained with correlation coefficient > 0.99. Linear Regression Equation: y = 62549x+5619.73
Calibration range	The calibration curve was calculated by one injection of six standards from 4.91 µg/ml to 201.96 µg/ml. The correlation coefficient was r=0.9999. These results meet the acceptance criteria of r ≥ 0.99
Assessment of matrix effects is presented	no
Limit of determination/quantification	LOQ = 20.20 µg/ml LOD = 6.06 µg/ml

Table A 17: Characteristics for the analytical method used for validation of Tribenuron methyl residues in sucrose matrix

	Tribenuron methyl
Specificity	Not overlapping of peaks is observed. Interference < 30 % of the LOQ
Calibration (type, number of data points)	Linear calibration function was obtained with correlation coefficient > 0.99. Linear Regression Equation: y = 61598.3x+9267.9
Calibration range	The calibration curve was calculated by one injection of six standards from 4.91 µg/ml to 201.96 µg/ml. The correlation coefficient was r=0.9997. These results meet

	Tribenuron methyl
Specificity	Not overlapping of peaks is observed. Interference < 30 % of the LOQ
	the acceptance criteria of r ≥ 0.99
Assessment of matrix effects is presented	no
Limit of determination/quantification	LOQ = 20.20 µg/ml LOD = 6.06 µg/ml

Table A 18: Characteristics for the analytical method used for validation of Tribenuron methyl residues in diet matrix

	Tribenuron methyl
Specificity	Not overlapping of peaks is observed. Interference < 30 % of the LOQ
Calibration (type, number of data points)	Linear calibration function was obtained with correlation coefficient > 0.99. Linear Regression Equation: y = 61862.6x+4989.5
Calibration range	The calibration curve was calculated by one injection of six standards from 1.96 µg/ml to 153.45 µg/ml. The correlation coefficient was r=1.0000. These results meet the acceptance criteria of r ≥ 0.99
Assessment of matrix effects is presented	no
Limit of determination/quantification	LOQ = 4.91 µg/ml LOD = 1.47 µg/ml

Conclusion

Results of the recovery experiments indicate that the recovery efficiency and repeatability were within acceptable limits of 70% - 110% for mean recovery and < 20% RSD.
A highly specific detection system was used.
The analytical method therefore meets the requirements of guideline SANCO/3029/99 rev. 4.

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

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

A 2.1.2.1.1 Determination of tribenuron methyl in plant matrices

A 2.1.2.1.1.1 Method validation

Comments of zRMS:	The study is accepted.

Reference: KCP 5.2/01

Report	Development and Validation of an Analytical Method for Determination of Tribenuron-methyl in Plant Matrices,Xxxx A., 2019, S18-07519
Guideline(s):	Yes (SANCO/825/00, rev. 8.1)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The objective of the study was to validate an analytical method for the determination of tribenuron-methyl in tomato, orange, avocado, wheat straw, wheat grain and wheat whole plant according to the guidance documents SANCO/825/00, rev. 8.1 with a limit of quantification (LOQ) of 0.01 mg/kg.

In brief, samples of tomato, orange, avocado, wheat straw, wheat grain and wheat whole plant were extracted with acetonitrile and, after addition of water, a salt mixture containing magnesium sulphate, sodium chloride and sodium citrate was added and the extract was shaken. After centrifugation an aliquot of the acetonitrile phase was cleaned by adding primary secondary amine (PSA).

Analysis was performed under following chromatographic and mass spectrometric conditions:

HPLC system	1290 Infinity Binary LC System, Agilent Technologies (HPLC, ≤ 600 bar)					
Pre-column	UHPLC Guard Column (Phenomenex, Art. No. AJ0-9000) with 2.1 mm C18 Cartridge (Phenomenex, Art. No. AJ0-8782)					
Column	Kinetex® 2.6 um XB-C18 (100 mm x 4.6 mm, 2.6 µm, Phenomenex, Art. No. 00D-4496-E0)					
Column oven temperature	40 °C					
Injection volume	10 µL					
Mobile phases	Eluent A: Water containing 5 mM ammonium acetate and 0.1 % formic acid Eluent B: Methanol containing 5 mM ammonium acetate and 0.1 % formic acid					
Gradient	Time [min] / % Eluent A / % Eluent B / Flow [µL/min] 0.00 / 90 / 10 / 500 2.5 / 1 / 99 / 500 5.00 / 1 / 99 / 500 5.01 / 90 / 10 / 500 7.00 / 90 / 10 / 500					
Divert valve	0.0 min to 4.0 min to waste; 4.0 min to 5.2 min to MS; 5.2 min to 7.0 min to waste					
Retention time(s)	Tribenuron-methyl: approx. 4.3 min					
MS system	SCIEX TripleQuad API5000 System, SCIEX (Triple quadrupole mass spectrometer)					
Ionisation type	Electrospray ionisation (ESI)					
Polarity	Positive ion mode					
Scan type	MS/MS, Multiple Reaction Monitoring (MRM)					
Capillary voltage (IS)	4500 V		Ionspray turbo heater (TEM)		400 °C	
Curtain gas (CUR)	Nitrogen set at 40 (arbitrary units)		Gas flow 1 (GS1)		Nitrogen set at 40 (arbitrary units)	
Collision gas (CAD)	Nitrogen at 9 (arbitrary units)		Gas flow 2 (GS2)		Nitrogen set at 30 (arbitrary units)	
Analyte monitored	Mass transition monitored (m/z)	Declustering potential (DP) [V]	Entrance potential (EP) [V]	Collision energy (CE) [eV]	Cell exit potential (CXP) [V]	Dwell time [ms]
Tribenuron-methyl	396 → 155[#]	96	10	19	12	150

	396 → 181	96	10	27	16	150

proposed (and used) for quantification but both mass transitions listed above can be used for quantification

Results and discussions

Table A 19 Recovery results from method validation of tribenuron methyl using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
Mass Transition 396→155 m/z (Proposed for Quantification)					
Tomato	Tribenuron methyl	0.01	89	3	
		0.1	88	2	
Orange		0.01	92	4	
		0.1	93	7	
Avocado		0.01	102	8	
		0.1	88	7	
Wheat Straw		0.01	75	14	
		0.1	96	5	
Wheat Grain		0.01	78	3	
		0.1	77	5	
Wheat Whole Plant		0.01	88	9	
		0.1	83	10	
Mass Transition 396→181 m/z (Proposed for Confirmation)					
Tomato	Tribenuron methyl	0.01	88	3	
		0.1	88	2	
Orange		0.01	91	4	
		0.1	92	6	
Avocado		0.01	101	7	
		0.1	88	7	
Wheat Straw		0.01	75	12	
		0.1	96	6	
Wheat Grain		0.01	79	3	
		0.1	78	4	
Wheat Whole Plant		0.01	88	8	
		0.1	83	9	

Table A 20 Characteristics for the analytical method used for validation of tribenuron methyl residues in plant matrix

	Tribenuron methyl
Specificity	Quantification was performed by use of LC-MS/MS detection. Two (2) mass transitions were evaluated in order to demonstrate that the method

<table>
<tr><td></td><td>achieves a high level of selectivity. No significant interference above 30 % of LOQ was detected in any of the reagent blanks or the control sample extracts of each matrix, so that a high level of selectivity was demonstrated.</td></tr>
<tr><td>Calibration (type, number of data points)</td><td>The calibration curves obtained for all mass transitions and all matrices were linear with correlation coefficients (R) (≥ 0.995). Linear regression was performed with 1/x-weighting.

<table><tr><td></td><td>Quantification</td><td>Qualification</td></tr><tr><td>Tomato</td><td>y = 1.07e+006 x + 1.19e+004</td><td>y = 7.32e+005 x + 6.52e+003</td></tr><tr><td>Orange</td><td>y = 1e+006 x + 8.55e+003</td><td>y = 6.73e+005 x + 5.07e+003</td></tr><tr><td>Avocado</td><td>y = 7.74e+005 x + 3.06e+003</td><td>y = 5.19e+005 x + 516</td></tr><tr><td>Wheat straw</td><td>y = 2.88e+005 x + 2.04e+003</td><td>y = 1.92e+005 x + 2.71e+003</td></tr><tr><td>Wheat grain</td><td>y = 1.01e+006 x + 3.96e+003</td><td>y = 6.87e+005 x + 563</td></tr><tr><td>Wheat whole plant</td><td>y = 7.09e+005 x + 2.72e+003</td><td>y = 4.84e+005 x + -2.67e+003</td></tr></table></td></tr>
<tr><td>Calibration range</td><td>The linearity of the detector response was demonstrated by single determination of matrix-matched calibration standards at a minimum of six (6) concentration levels ranging from 0.03 ng/mL to 5.0 ng/mL for wheat straw and from 0.06 ng/mL to 5.0 ng/mL for all other matrices. This range corresponds to 0.003 mg/kg to 0.5 mg/kg for wheat straw and to 0.003 mg/kg to 0.25 mg/kg for all other matrices. The calibration curves obtained for all mass transitions and all matrices were linear with correlation coefficients (R)) (≥ 0.995). These results meet the acceptance criteria of r ≥ 0.99</td></tr>
<tr><td>Assessment of matrix effects is presented</td><td>yes</td></tr>
<tr><td>Limit of determination/quantification</td><td>The LOQ is the lowest validated fortification level for tribenuron-methyl and was thus successfully established at 0.01 mg/kg in wheat straw and wheat grain for the two (2) mass transitions. The LOD was set at 0.003 mg/kg for all matrices, which is 30 % of the LOQ.</td></tr>
</table>

Conclusion

The method was found to be valid according to the guidance documents SANCO/825/00, rev 8.1 for the determination of tribenuron-methyl in tomato, avocado, orange and wheat (straw, grain and whole plant) with the tested LOQ of 0.01 mg/kg.

A 2.1.2.1.1.2 Independent laboratory validation

Comments of zRMS:	The study is accepted.

Reference:	KCP 5.2/02
Report	Independent Laboratory validation of a Multi-Residue MEthod QuEChERS for the Determination of Tribenuron-methyl in Two Matrices of Plant Origin, Xxxx S., 2019, P 5114 G
Guideline(s):	Yes (SANCO/825/00, rev. 8.1)
Deviations:	No

GLP: Yes

Acceptability: Yes

Materials and methods

The objective of the study was to independently validate the multi-residue method QuEChERS as described in method validation report S18-07519 for the determination of Tribenuron-methyl exemplified in the matrices tomato (fruit) and wheat (grain) in accordance to the guidance document SANCO/825/00, rev. 8.1 of the European Commission. The limit of quantification was 0.01 mg/kg.

Method Reference(s)	Multi-residue method QuEChERS					
Extraction	Addition of water (if needed) and extraction with acetonitrile					
Liquid/Liquid Partition	Addition of magnesium sulphate, sodium chloride and sodium citrate followed by subsequent centrifugation					
Clean up / Purification	Purification of an aliquot of the acetonitrile extract by dispersive SPE with primary/secondary amine (PSA)					
Storage	Final sample extracts were stored at 1 °C to 10 °C (target) in the dark until analysis					
Detection	Liquid chromatography with tandem mass spectrometry (LC-MS/MS)					
Limit of Quantification (LOQ)	0.01 mg/kg					
Limit of Detection	30 % of the LOQ					
Chromatographic conditions						
HPLC system	Agilent Technologies 1290 Infinity Binary pump and degasser, HTC-xt eksigent PAL Autosampler, MayLab MistraSwitch column oven					
Pre-column	Phenomenex C_{18} 4x3 mm, Art. No. AJO-4287					
Column	Phenomenex Kinetex XB-C18 (100 mm x 4.6 mm, 2.6 µm, Art. No. 00D-4496-E0)					
Column oven temperature	40 °C					
Injection volume	10 µL					
Mobile phases	Eluent A: Water + 5mM ammonium acetate + 0.1 % formic acid Eluent B: Methanol + 5mM ammonium acetate + 0.1 % formic acid					
Gradient	Time [min] / % Eluent A / % Eluent B / Flow [µL/min] 0.0 / 90 / 10 / 500 2.5 / 1 / 99 / 500 5.0 / 1 / 99 / 500 5.1 / 90 / 10 / 500 7.0 / 90 / 10 / 500					
Divert valve	0.0 min to 1.0 min to waste; 1.0 min to 6.0 min to MS; 6.0 min to 7.0 min to waste					
Retention time	Tribenuron-methyl: approx. 4.3 min					
Mass spectrometric conditions						
MS system	SCIEX TripleQuad 5500 System, (Triple quadrupole mass spectrometer)					
Ionisation type	Electrospray ionisation (ESI, TurboIonSpray)					
Polarity	Positive ion mode					
Scan type	MS/MS, Multiple Reaction Monitoring (MRM)					
Capillary voltage (IS))	4500 V		Ionspray turbo heater (TEM)		400 °C	
Curtain gas (CUR)	Nitrogen set at 40 (arbitrary units)		Gas flow 1 (GS1)		Nitrogen set at 40 (arbitrary units)	
Collision gas (CAD)	Nitrogen set at 9 (arbitrary units)		Gas flow 2 (GS2)		Nitrogen set at 30 (arbitrary units)	
Analyte monitored	Mass transition monitored (m/z)	Declustering potential (DP) [V]	Entrance potential (EP) [V]	Collision energy (CE) [V]	Cell exit potential (CXP) [V]	Dwell time [ms]
Tribenuron-methyl	396 → 155[#]	96	10	19	12	150
	396 → 181	96	10	27	16	160

proposed (and/or used) for quantification but both of the mass transitions listed can be used for quantification

Results and discussions

Table A 21 Recovery results from method validation of Tribenuron methyl using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Mean recovery (%)	RSD (%)	Overall Mean Recover (%)	Overall RSD (%)	Comments
Mass Transition m/z 396→155 (Quantification)							
Tomato (whole fruit)	Tribenuron methyl	0.01	78	4	78	3	
		0.1	77	2			
Wheat (grain)	Tribenuron methyl	0.01	83	5	83	5	
		0.1	83	6			
Mass Transition m/z 396→181 (Confirmation)							
Tomato (whole fruit)	Tribenuron methyl	0.01	79	4	78	3	
		0.1	78	2			
Wheat (grain)	Tribenuron methyl	0.01	82	5	82	5	
		0.1	82	6			

Table A 22 Characteristics for the analytical method used for validation of tribenuron methyl residues in tomato and wheat

	Tribenuron methyl
Specificity	Quantification was performed by use of LC-MS/MS detection. Two (2) mass transitions were evaluated in order to demonstrate that the method achieves a high level of selectivity. No significant interference above 30 % of LOQ was detected in any of the reagent blanks or the control sample extracts of each matrix, so that a high level of selectivity was demonstrated.
Calibration (type, number of data points)	The linearity of the detector response was demonstrated by single determination of matrix-matched calibration standards at seven (7) concentration levels. Linear Regression Equation:

	Tomato	Wheat
Quantification; m/z 396→155	y = 1.31e+006 x – 2.5e+004	y = 1.77e+006 x – 2.13e+004
Confirmation; m/z 396→181	y = 5.77e+005 x – 1.15e+004	y = 7.91e+005 x – 6.86e+003

Calibration range	The linearity of the detector response was demonstrated by single determination of matrix-matched calibration standards at seven (7) concentration levels ranging from 0.060 ng/mL to 5.0 ng/mL. This range corresponds to 0.0030 mg/kg to 0.25 mg/kg and thus covers the range from no more than 30 % of the LOQ and at least + 20 % of the highest analyte concentration level detected in a sample extract. The calibration curves obtained for both mass transitions and all matrices were linear since correlation coefficients (R) were ≥ 0.99.
Assessment of matrix effects is presented	yes

	Tribenuron methyl
Specificity	Quantification was performed by use of LC-MS/MS detection. Two (2) mass transitions were evaluated in order to demonstrate that the method achieves a high level of selectivity. No significant interference above 30 % of LOQ was detected in any of the reagent blanks or the control sample extracts of each matrix, so that a high level of selectivity was demonstrated.
Limit of determination/quantification	LOQ of 0.01 mg/kg was confirmed for tribenuron-methyl in tomato fruit and wheat grain. The LOD was set at 30 % of the LOQ, which is 0.0030 mg/kg.

Conclusion

The method was successfully validated independently for the determination of tribenuron-methyl in tomato fruit and wheat grain from the tested LOQ of 0.01 mg/kg up to 0.1 mg/kg according to the guidance document SANCO/825/00, rev. 8.1.
No addition or modification to the original method other than optimization of instrumental parameters was done. Primary validation and independent laboratory validation were carried out at different locations, by different study personnel, and using different instrumentation and stocks of chemicals. No communication with the method developers or others familiar with the method was necessary to carry out the analysis.
The method is acceptable as ILV for the primary method.

A 2.1.2.1.1.3 Confirmatory method

No confirmatory method is required

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

A 2.1.2.2.1 Determination of tribenuron methyl in animal matrices

A 2.1.2.2.1.1 Method validation

Comments of zRMS:	The study is not accepted. The applicable MRL value for all products of animal origin is 0.01 mg/kg. Therefore LOQ at the level 0.05 mg/kg reported in this study is too high.

Reference:	KCP 5.2/03
Report	Validation of the Methods of Analysis used for the Determination of Metsulfuron-Methyl, Thifensulfuron-Methyl and Tribenuron-Methyl in various matrices, in Compliance with Good Laboratory Practice, and referencing SANCO/3029/99, Xxxx D., 2016, DNA3620 Addendum 1 Issued 21st February 2019
Guideline(s):	Yes (SANCO/3029/99 rev. 4, SANCO 825/00 rev. 8.1)
Deviations:	No
GLP:	Yes

Acceptability: Yes

Materials and methods

The study objective was to validate the method of analysis used for the determination of Tribenuron methyl in Eggs, Milk, Cream, Liver and Muscle in compliance with Good Laboratory Practice.

Instrument	Agilent QtoF 6530 connected to a 1260 Infinity LC
Mode	Isocratic Reverse Phase LC
Ionisation Mode	Jet Spray ESI Positive Ionisation
Column	Phenomenex Gemini C18 150 mm x 4.6 mm
Packing	C18 3µm 110 Å
Eluent	80% Acetonitrile with 0.05% Acetic Acid : 20% Water with 0.05% Acetic Acid
Wavelength	225 nm
Column Temperature	40°C
MS Scanning	50-1000 m/z
Flow Rate	0.6 ml/min
Injection Volume	10 µL
Data Collection	Mass Hunter
Retention Times	Approximately 3.5 to 4.1 minutes
MS Extracted Ions	Quantitation by Molecular Formula extraction $C_{15}H_{17}O_6N_5S_1$ Which relates to these ions 396.0972 m/z, 418.0792 m/z, 434.0531 m/z and 435.0057 m/z

Results and discussions

Table A 23 Recovery results from method validation of Tribenuron methyl using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (n = x)	Mean recovery (%)	RSD (%)	Comments
Eggs	Tribenuron methyl	5	90.52	1.982	
		0.5	88.16	2.001	
		0.1	89.04	3.217	
Milk	Tribenuron methyl	5	85.00	3.273	
		0.5	83.07	4.878	
		0.1	73.65	6.474	
Cream	Tribenuron methyl	5	93.84	2.851	
		0.5	95.94	3.408	
		0.1	95.66	3.919	
Liver	Tribenuron methyl	5	89.10	3.648	
		0.5	87.51	3.565	
		0.1	97.15	7.902	
Muscle	Tribenuron methyl	5	93.66	3.388	
		0.5	94.68	2.135	
		0.1	93.89	5.092	

Table A 24 Characteristics for the analytical method used for validation of Tribenuron methyl residues in Eggs, Milk, Cream, Liver, Muscle

<table>
<tr><th></th><th>Tribenuron methyl</th></tr>
<tr><td>Specificity</td><td>The compound was specifically extracted from the chromatogram using accurate high resolution mass spectrometry and there were no other peaks present at the same elution time as Tribenuron-Methyl
blank value < 30 % LOQ</td></tr>
<tr><td>Calibration (type, number of data points)</td><td>Linear calibration function was obtained with correlation coefficient > 0.99.

Linear Regression Equation:<table><tr><td>Eggs</td><td>y = 0.000048x – 1.203</td></tr><tr><td>Milk</td><td>y = 0.000076x + 2.224</td></tr><tr><td>Cream</td><td>y = 0.000021x – 0.849</td></tr><tr><td>Liver</td><td>y = 0.000087x – 0.413</td></tr><tr><td>Muscle</td><td>y = 0.000051x – 0.928</td></tr></table></td></tr>
<tr><td>Calibration range</td><td>For eggs, milk and liver the linearity was determined from sixteen injections of eight concentrations of standard ranging from a blank to 100µg/L Tribenuron Methyl. The samples were prepared for analysis at a sample concentration of 2 grams of Egg/2 grams of Milk/2 grams of Liver condensed to a final volume of 10 ml, with additional dilution or concentration as required to meet the linear range. This equates to a linear range of 10 mg/kg to 0.05 mg/kg in the matrix type samples.

For cream the linearity was determined from fourteen injections of seven concentrations of standard ranging from a blank to 75µg/L Tribenuron Methyl. The samples were prepared for analysis at a sample concentration of 2 grams of Cream condensed to a final volume of 10 ml, with additional dilution or concentration as required to meet the linear range. This equates to a linear range of 7.5 mg/kg to 0.05 mg/kg in the matrix type samples.

For muscle the linearity was determined from fourteen injections of seven concentrations of standard ranging from a blank to 100µg/L Tribenuron Methyl. The samples were prepared for analysis at a sample concentration of 2 grams of Muscle condensed to a final volume of 10 ml, with additional dilution or concentration as required to meet the linear range. This equates to a linear range of 10 mg/kg to 0.05 mg/kg in the matrix type samples.
Correlation coefficient

<table><tr><td>Eggs</td><td>0.9962</td></tr><tr><td>Milk</td><td>0.9914</td></tr><tr><td>Cream</td><td>0.9988</td></tr><tr><td>Liver</td><td>0.9999</td></tr><tr><td>Muscle</td><td>0.9990</td></tr></table>
These results meet the acceptance criteria of $r \geq 0.99$</td></tr>
<tr><td>Assessment of matrix effects is presented</td><td>no</td></tr>
<tr><td>Limit of determination/quantification</td><td>LOQ of 0.05 mg/kg was confirmed for tribenuron-methyl in animal matrices.</td></tr>
</table>

Conclusion

Results of the recovery experiments indicate that the recovery efficiency and repeatability were within

acceptable limits of 70% - 110% for mean recovery and < 20% RSD.
A highly specific detection system was used.
The analytical method therefore meets the requirements of guideline SANCO/3029/99 rev. 4 and SANCO/825/00, rev. 8.1.

A 2.1.2.2.1.2 Independent laboratory validation

Comments of zRMS:	The study is not accepted. The applicable MRL value for all products of animal origin is 0.01 mg/kg. Therefore LOQ at the level 0.05 mg/kg reported in this study is too high.

Reference:	KCP 5.2/05
Report	Metsulfuron-methyl and Tribenuron methyl: Independent Laboratory Validation of an Analytical Method for the Determination in Animal Matrices, Xxxx M, Xxxx S., 2018, 123361101
Guideline(s):	Yes (SANCO/825/00 rev. 8.1)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

An analytical method to determine Tribenuron methyl in animal matrices was presented above. The analytical method was modified as necessary to suit the purpose and the instrumentation available at the performing laboratory. The method was validated for two matrices of animal origin, cream and muscle tissue, with LOQ of 0.05 mg/kg.

LC	Agilent Series 1290 pump and autosampler
Mass Spectrometer	API 5500
Column	Gemini 3 µ C18 100A (150*3mm*3µm)
Mobile phase	A: HPLC-H_2O + 0.05 % acetic acid B: Acetonitrile + 0.05 % acetic acid
Gradient mode	0 -1 min : 40%A/60% B 2.5-5 min: 5%A/95% B 5.5-7 min: 40%A/60% B
Detector	MSD
Ion Source	5500
Flow Rate	0.7 ml/min
Injection Volume	10 µL
Mass Transitions	Quantifier (396 m/z > 155 m/z) Qualifier (396 m/z > 181 m/z)

Results and discussions

Table A 25 Recovery results from independent laboratory validation of tribenuron methyl using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (n = 6)	Mean recovery (%)	RSD (%)	Comments
Quantifier (396 m/z > 155 m/z)					
Muscle	Tribenuron methyl	0.05	104	2	
		0.1	103	8	
		0.5	94	16	
		5	94	6	
Qualifier (396 m/z > 181 m/z)					
Muscle	Tribenuron methyl	0.05	95	2	
		0.1	99	6	
		0.5	92	18	
		5	88	12	
Quantifier (396 m/z > 155 m/z)					
Cream	Tribenuron methyl	0.05	97	1	
		0.1	96	2	
		0.5	97	3	
		5	101	1	
Qualifier (396 m/z > 181 m/z)					
Cream	Tribenuron methyl	0.05	94	3	
		0.1	93	2	
		0.5	93	3	
		5	95	1	

Table A 26 Characteristics for the analytical method used for independent laboratory validation of Tribenuron metyl residues in animal matrices

	Tribenuron methyl
Specificity	A highly specific detection system was used The interference for the determination of the target analytes was not higher than 30 % of total mean peak area at LOQ level.
Calibration (type, number of data points)	Linear calibration function was obtained with correlation coefficient > 0.99. Typical Calibration Curves: Beef: y = 128387 * x - 8770 (quantitation mass) y = 122913 * x + 100320 (confirmation mass) Cream: y = 69812 * x – 13135 (quantitation mass) y = 50681 * x + 1009 (confirmation mass)
Calibration range	The calibration was performed using calibration solutions (7 concentrations for cream and 6 concentrations for muscle) within the range of 3 to 100 µg/mL for cream and 3.75 to 100 µg/mL for. Correlation

	Tribenuron methyl
	coefficient (r) of calibration curve was determined to be 0.9970 for muscle and 0.9999 for cream. These results meet the acceptance criteria of r ≥ 0.99.
Assessment of matrix effects is presented	no
Limit of determination/quantification	The limit of quantification (LOQ) was determined to be 0.05 mg/kg. The limit of detection (LOD) of the method was determined to be: 0.0001 mg/kg for muscle abd 0.00029 mg/kg for cream

Conclusion

The validity criteria linearity, accuracy, precision and repeatability were fulfilled for analysis of tribenuron methyl. The validated method is appropriate to determine the active ingredient at concentration levels between 0.05 g/kg and 5 g/kg in matrices of animal origin.
The method is acceptable as ILV for the primary method.

A 2.1.2.2.1.3 Confirmatory method

No confirmatory method is required

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

A 2.1.2.3.1 Determination of Tribenuron methyl and its metabolites in soil

A 2.1.2.3.1.1 Method validation

Comments of zRMS:	The study is accepted.

Reference:	KCP 5.2/06,07
Report	Validation of an analytical method for the determination of Tribenuron methyl and its metabolites in soil according to SANCO/3029/99 rev. 4, Xxxx M, 2018, PRO-001/6-20/B 1st Amendment to Report Validation of an analytical method for the determination of Tribenuron methyl and its metabolites in soil according to SANCO/3029/99 rev. 4 and SANCO/825/00 rev. 8.1, Xxxx S., 2019, PRO-001/6-20/B
Guideline(s):	Yes (SANCO/3029/99 rev. 4, SANCO 825/00 rev. 8.1)

Deviations: No

GLP: Yes

Acceptability: Yes

Materials and methods

The objective of the study was validation of analytical methods for the determination of the test items Tribenuron methyl and its metabolites IN-R9805, IN-L5296, IN-A4098 and IN-00581 in soil according to the guideline SANCO/3029/99 rev. 4 and SANCO/825/00 rev. 8.1. The analysis were performed by LC-MS/MS using positive and negative electrospray ionization (ESI+ and ESI-).

For analysis of the parent Tribenuron methyl the soil samples were extracted twice with acetonitrile. After ultrasonic treatment and centrifugation the extracts were combined and evaporated until dryness. The residues were resolved in 0.1% ammonia solution, centrifuged and analyzed by LC-MS/MS.
The metabolites IN-R9805, IN-L5296, IN-A4098 and IN-00581 were extracted from soil samples twice with acetonitrile + 25% aqueous ammonia solution (94+4, v/v) and twice with methanol. After ultrasonic treatment and centrifugation the extracts were combined and partly evaporated. Acetonitrile containing 0.1% formic acid was added and the extracts were cleaned by SPE using EnviCarb SPE cartriges and analyzed by LC-MS/MS.

Instrumental Parameters:

HPLC	UPLC Acquity, Waters
Mass spectrometer	TQS, Waters
Analytical column	250 x 2 mm Luna Phenyl-Hexyl, 5 µm, Phenomenex
Flow	0.35 ml/min
Injection volume	50 µL
Column temperature	Room temperature
Ionization mode	ESI+ for IN-R9805, IN-L5296, IN-A4098 ESI- for IN-00581

Results and discussions

Table A 27 Recovery results from method validation of Tribenuron methyl and its metabolites using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (n = 5)	Mean recovery (%)	RSD (%)	Comments
Primary mass transition					
Soil	Tribenuron methyl	0.06	98.1	8.3	
		0.61	86.4	10.6	
Soil	IN-R9805	1.2	108.5	9.2	
		12.2	106.2	4.1	
Soil	IN-L55296	1.2	94.4	5.6	
		12.2	99.1	6.9	
Soil	IN-A4098	1.2	90.6	4.6	
		12.2	100.3	6.7	

Matrix	Analyte	Fortification level (mg/kg) (n = 5)	Mean recovery (%)	RSD (%)	Comments
Soil	IN-00581	1.2	106.1	4.4	
		12.2	110.9	1.6	
Confirmatory mass transition					
Soil	Tribenuron methyl	0.06	110.0	7.5	
		0.61	85.0	11.5	
Soil	IN-R9805	1.2	103.6	8.4	
		12.2	105.1	3.1	
Soil	IN-L5296	1.2	86.5	7.3	
		12.2	95.6	7.1	
Soil	IN-A4098	1.2	90.2	7.7	
		12.2	101.0	6.3	
Soil	IN-00581	1.2	101.3	3.8	
		12.2	112.0	2.0	

Table A 28 Characteristics for the analytical method used for validation of Tribenuron methyl residues in soil

	Tribenuron methyl	IN-R9805	IN-L5296	IN-A4098	IN-00581
Specificity	The absence of interferences for the parent Tribenuron methyl is shown by comparison of the peak areas of both mass transitions of control and LOQ-level samples. Calculated ratio of about 1.4 %, respectively 14.5 %, are well below the '30 % of LOQ' limit value.	Nno interferences were detected at or near the retention times of the analytes in untreated controls. The '30 % of LOQ' criteria is met for the metabolites.			
Calibration (type, number of data points)	Linear calibration function was obtained with correlation coefficient > 0.997. Calibration curve: y= 452149*x+451.857 Addidional calibration functions: y=164657*x - 278.434	Linear calibration function was obtained with correlation coefficient > 0.997. Calibration curve: y= 155322*x+16141.2 Addidional calibration functions: y= 55397.2*x + 5409.01	Quadratic calibration function was obtained with coefficient of determination of > 0.998 Calibration curve: y= -5257.86*x^2+680364*x-5517.47 Addidional calibration functions: y= -1893.71*x^2 + 364218*x + 5088.07	Quadratic calibration function was obtained with coefficient of determination of > 0.998 Calibration curve: y= -158.534*x^2+30693.5*x+-147.202 Addidional calibration functions: y= -46.5694*x^2 +	Linear calibration function was obtained with correlation coefficient > 0.997. Calibration curve: y= 2575.01*x+490.273 Addidional calibration functions: y= 990.992*x + 337.264

				12973.4*x + 768.295	
Calibration range	Soil samples were fortified with tribenuron methyl to 10 concentrations levels in the range of 0.03 µg/kg dw to 5 µg/kg dw. Correlation coefficient: r= 0.999448 Additional correlation coefficient r= 0.998049 These results meet the acceptance criteria of r ≥ 0.99	Correlation coefficient: r= 0.997060 Additional correlation coefficient r= 0.997077 These results meet the acceptance criteria of r ≥ 0.99	Coefficient of determination: r^2= 0.998770 These results meet the acceptance criteria of r ≥ 0.99	Coefficient of determination: r^2= 0.998288 These results meet the acceptance criteria of r ≥ 0.99	Correlation coefficient: r= 0.998288 Additional correlation coefficient r= 0.998382 These results meet the acceptance criteria of r ≥ 0.99
Assessment of matrix effects is presented	yes				
Limit of determination/quantification	The limit of quanification of the analytical method was confirmed at 0.06 µg/kg dw in soil for tribenuron methyl	The limit of quanification of the analytical method was confirmed at 1.2 µg/kg dw in soil for metabolites.			

Conclusion

Results of the recovery experiments indicate that the recovery efficiency and repeatability were within acceptable limits of 70% - 110% for mean recovery and < 20% RSD.
A highly specific detection system was used.
The analytical method therefore meets the requirements of guideline SANCO/3029/99 rev. 4 and SANCO/825/00, rev. 8.1.

A 2.1.2.3.1.2 Confirmatory method

No confirmatory method is required.

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

A 2.1.2.4.1 Determination of Tribenuron methyl and its metabolites in drinking water

A 2.1.2.4.1.1 Method validation

Comments of zRMS:	The study is accepted.

Reference: KCP 5.2

Report: Validation of an analytical method for the determination of Tribenuron methyl and its metabolites in drinking water according to SANCO/825/00

rev. 8.1, Xxxx S., 2018, PRO-001/6-22

Guideline(s): Yes (SANCO/825/00 rev. 8.1)

Deviations: No

GLP: Yes

Acceptability: Yes

Materials and methods

The analytical method to quantitatively determine residues of tribenuron methyl and its metabolites IN-L5296, IN-A4098 and IN-00581 in drinking water was validated in this study according to SANCO/825/00 Rev. 8.1. Each analyte was directly determined by Liquid Chromatography (LC) coupled to tandem mass spectrometric detection (MS/MS).

Drinking water samples are fortified to 0.1 µg/L (LOQ-level) and 1.0 µg/L (10x-LOQ-level) with Tribenuron-methyl and each metabolite and analysed directly without further sample preparation.
Analysis is performed by LC-MS/MS using positive and negative electrospray ionization (ESI+ and ESI-). Quantification is performed by matrix calibration using external standardization.

Instrumental parameters:

HPLC system	Waters Acquity UPLC system, I-xxxx
Mass spectrometer	Waters LC-MS/MS system Xevo TQ-S (triple quadrupole system)
Column	Phenomenex HPLC 250 x 2 mm Luna Phenyl-Hexyl, 5 µm,
Column temperature	20 °C
Injection volume	50 µL
Flow rate	0.35 mL/min
Mobile phase A:	Water/MeOH (95+5, v/v) containing 0.1 % formic acid
Mobile phase B:	MeOH containing 0.1 % formic acid
Gradient mode	

Time [min]	A [%]	B [%]	Flow [ml/min]	Curve
Initial	100	0	0.35	-
10	0	100	0.35	6
13	0	100	0.35	1
16	100	0	0.35	1

Results and discussions

Table A 29 Recovery results from method validation of Tribenuron methyl and its metabolites using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (n = ~~x~~ 5)	Mean recovery (%)	RSD (%)	Comments
Primary Transition					
Drinking water	Tribenuron methyl	0.1	101.6	1.5	
		1.0	108.6	0.6	
Drinking water	IN-L5296	0.1	100.6	0.5	
		1.0	100.4	0.2	
Drinking water	IN-A4098	0.1	101.0	1.6	
		1.0	103.2	0.7	

Matrix	Analyte	Fortification level (mg/kg) (n = ~~x~~ 5)	Mean recovery (%)	RSD (%)	Comments
Drinking water	IN-00581	0.1	109.6	10.0	
		1.0	108.0	2.2	
Confirmatory Transition					
Drinking water	Tribenuron methyl	0.1	101.0	1.4	
		1.0	107.6	0.9	
Drinking water	IN-L5296	0.1	100.6	1.1	
		1.0	100.3	0.6	
Drinking water	IN-A4098	0.1	101.8	5.0	
		1.0	102.5	0.5	
Drinking water	IN-00581	0.1	107.4	8.0	
		1.0	109.9	1.1	

Table A 30 Characteristics for the analytical method used for validation of Tribenuron methyl residues in drinking water

	Tribenuron methyl	IN-L5296	IN-A4098	IN-00581
Specificity	In this study the lowest calibration levels of all test items were 0.03 µg/L which correspond to 30 % of LOQ. At this level no interferences above 30 % of the LOQ were detected at or near the retention times of the analytes in untreated controls for both primary and confirmatory transitions. This is shown by LC-MS/MS chromatograms of untreated fortification matrix blanks			
Calibration (type, number of data points)	The LC-MS/MS responses were shown to be linear throughout the entire study (correlation coefficients (r) > 0.998)			
	Calibration curve, primary: y= 355327 * x + 3948.89 Calibration curve, confirmatory: y= 119433 * x + 1202.99	Calibration curve, primary: y= 628968 * x + 9.14059 Calibration curve, confirmatory: y= 608422 * x + -456.619	Calibration curve, primary: y= 454444 * x + 1049.55 Calibration curve, confirmatory: y= 100076 * x + 16.2352	Calibration curve, primary: y= 2191.95 * x + -11.9843 Calibration curve, confirmatory: y= 1854.38 * x + -6.61945
Calibration range	The calibration was performed using calibration solutions (10 concentrations) within the range of 0.03 µg/L to 5 µg/L.			
	The linear correlation coefficients (r) for this data set were 0.998 for the m/z 396.2 -> 155.1 primary transition and 0.999 for the m/z 396.2 -> 181.1 confirmatory transition, based	The linear correlation coefficients (r) for this data set were 0.999 for the m/z 155.1 -> 57.2 primary transition and 0.999 for the m/z 155.1 -> 71.1 confirmatory transition, based	The linear correlation coefficients (r) for this data set were 0.999 for the m/z 141.1 -> 57.2 primary transition and 0.999 for the m/z 141.1 -> 250 confirmatory transition, based	The linear correlation coefficients (r) for this data set were 0.999 for the m/z 182.0 -> 106.1 primary transition and 0.999 for the m/z 182.0 -> 42.2 confirmatory transition, based

	Tribenuron methyl	IN-L5296	IN-A4098	IN-00581
	on a 1/x weighing.	on a 1/x weighing.	on a 1/x weighing.	on a 1/x weighing.
Assessment of matrix effects is presented	yes			
Limit of determination/quantification	The limits of quantification (LOQs) of the analytical method were confirmed at 0.1 µg/L in drinking water for Tribenuron methyl and each of its metabolites. According to SANCO/825/00 rev. 8.1 the Limit of Detection (LOD) was 0.03 µg/L for Tribenuron methyl and each of its metabolites.			

Conclusion

analytical method for the determination of the test items tribenuron methyl and its metabolites IN-L5296, IN-A4098 and IN-00581 in drinking water was developed and successfully validated according to the EC guidance document SANCO/825/00 Rev. 8.1 with regard to LOQ, recovery, precision, LOQ, specificity, linearity, matrix effects and stability of solutions and extracts.

A 2.1.2.4.1.2 Independent laboratory validation

Comments of zRMS:	The study is accepted.

Reference:	KCP 5.2
Report	Independent Laboratory Validation (ILV) of an analytical method for the determination of Tribenuron methyl and three of its metabolites in drinking water according to SANCO/825/00 rev.8.1, Xxxx W., 2018, PRO-001/6-22/a
Guideline(s):	Yes (SANCO/825/00 rev. 8.1)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The objective of the study was the performance of an Independent Laboratory Validation study (ILV) of the method 'Analytical method for the determination of Tribenuron methyl and its metabolites in drinking water' to quantitatively determine residues of Tribenuron methyl and its metabolites IN-L5296, IN-A4098 and IN-00581 in drinking water. The Limit of Quantitation (LOQ) for all analytes was 0.1 µg/L.
The quantitative measurements were carried out by liquid chromatography (LC) coupled to a triple quadrupole mass spectrometer (MS) using electrospray ionization positive (ESI+) for Tribenuron methyl, IN-L5296 and IN-A4098 and electrospray ionization negative (ESI-) for metabolite IN-00581. The MS was operated in the tandem mass spectrometry mode (MS/MS). Primary and confirmatory mass transitions were recorded and evaluated for all samples.

- The ILV study was performed according to the guideline SANCO/825/00 Rev.8.1
- The test was performed in the laboratory 'Organic trace analysis' of the test facility. This laboratory and its personnel were not involved in the development of the original method in the

laboratory 'Food and environmental analysis' nor in its subsequent use.

- The method applied in this ILV study reflects the original method and was kept as close to the original methods as possible. There were only minor differences with no impact on the quality and integrity of the study.
- The MS instruments used in the original method and in the ILV study were of similar type (Waters Xevo TQ-S and Xevo TQD Mass Spectrometer). Both are triple quadrupol mass selective detectors from the same manufacturer with only slight differences. Only slightly different MS parameters were necessary in the performance of the study.
- The MS ionization modes used were the same (electrospray positive (ESI+ for Tribenuron methyl, IN-L5296 and IN-A4098 and electrospray negative (ESI- for IN-00581).
- The LC instruments and LC-columns were of same type and the mobile phase gradient was the same. Due to lower sensitivity of the MS instrument in the ILV study a higher injection volume was used.
- According to SANCO 825 the stability of final extracts and of stock and working solutions was not be assessed in the ILV study.
- No other information was used and no further deviations occurred.

Results and discussions

Table A 31 Recovery results from independent laboratory validation of Tribenuron methyl and its metabolites using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (n = ~~x~~ 5)	Mean recovery (%)	RSD (%)	Comments
Primary Transition					
Drinking water	Tribenuron methyl	0.1	109.2	4.3	
		1.0	104.6	2.6	
Drinking water	IN-L5296	0.1	102.2	1.3	
		1.0	105.2	0.4	
Drinking water	IN-A4098	0.1	107.8	0.4	
		1.0	105.5	0.6	
Drinking water	IN-00581	0.1	86.4	3.3	
		1.0	100.9	1.5	
Confirmatory transition					
Drinking water	Tribenuron methyl	0.1	109.6	4.0	
		1.0	105.9	2.3	
Drinking water	IN-L5296	0.1	102.2	1.3	
		1.0	105.0	0.8	
Drinking water	IN-A4098	0.1	108.2	2.6	
		1.0	103.3	1.3	
Drinking water	IN-00581	0.1	84.2	5.1	
		1.0	102.7	2.6	

Table A 32 Characteristics for the analytical method used for independent laboratory validation of tribenuron methyl residues in drinking water

	Tribenuron methyl	IN-L5296	IN-A4098	IN-00581
Specificity	The specificity of the method is shown by LC-MS/MS chromatograms of untreated fortification matrix blanks. No interferences above 30 % of the LOQ were detected at or near the retention times of the analytes in untreated controls for both primary and confirmatory transitions.			
Calibration (type, number of data points)	The response of the LC-MS/MS was quadratic. Calibration curve, primary: y= -10164.7 * x^2 + 317230 * x + 1071.14 Calibration curve, confirmatory: y= -3649.3 * x^2 + 125652 * x + 518.135	The response of the LC-MS/MS was linear. Calibration curve, primary: y= 854954 * x + 3277.52 Calibration curve, confirmatory: y= 670518 * x + 1972.08	The response of the LC-MS/MS was quadratic. Calibration curve, primary: y= -24063.2 * x^2 + 620896 * x + 2031.51 Calibration curve, confirmatory: y= -5286.69 * x^2 + 134023 * x + 314.854	The response of the LC-MS/MS was quadratic. Calibration curve, primary: y= -117.211 * x^2 + 2328.23 * x + 72.7842 Calibration curve, confirmatory: y= -142.505 * x^2 + 2739.34 * x + 95.2036
Calibration range	The calibration accuracies for the linear and quadratic calibration functions are demonstrated by the high coefficients of determination (r^2) being close to 1 over the calibrated concentration (10) ranges from 0.03 µg/L to 5 µg/L. The coefficients of determination (r2) were > 0.999 for Tribenuron methyl, IN-L5296 and IN-A4098 and > 0.998 for IN-00581.			
Assessment of matrix effects is presented	no			
Limit of determination/quantification	The Limits of Quantification (LOQ = 0.1 µg/L) were the same as in the original method for all test items.			

Conclusion

The original analytical method for the determination of the test items Tribenuron methyl and its metabolites IN-L5296, IN-A4098 and IN-00581 in drinking water has been independently validated according to the EC guidance document SANCO/825/00 rev. 8.1.
For each test item two control samples and five fortified samples at the LOQ level of 0.1 µg/L and five fortified samples at the 10x LOQ level of 1.0 µg/L were analyzed in this study. The mean recovery was found to be acceptable (i.e. between 70 % and 120 %) with a relative standard deviation less than 20 % for each test item, each fortification level and for both mass transitions in the matrix drinking water.
No interferences above 30 % of the LOQ were found at or near the retention times of the analytes in untreated controls.
The method is acceptable as ILV for the primary method.

A 2.1.2.4.1.3 Confirmatory method

No confirmatory method is required.

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

No new or additional studies have been submitted

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

5.2)

No new or additional studies have been submitted

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

No new or additional studies have been submitted