FINAL REGISTRATION REPORT

Part B

Section 5

Analytical Methods

Detailed summary of the risk assessment

Product code: MT-565SG-OR2-C

Product name(s): HAKSAR TOP 565 SG

Chemical active substance(s):

MCPA, 550 g/kg
Tribenuron methyl, 15 g/kg

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(authorization)

Applicant: CIECH Sarzyna S.A.

Submission date: 01/2021

MS Finalisation date: 06/12/2021

Version history

When	What
January 2021	First submission of product authorization.
02/2021	Dossier sent for evaluation to Merit Mark (PL)
August 2021	Correction on first submission for product
08/2021	zRMS finalised evaluation
December 2021	Final RR

Table of Contents

zRMS comments:
This report has been completed by the applicant.
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 all analytes included in the residue definitions.

Commodity/crop	Supported/ Not supported
Winter wheat	Supported
Winter rye	Supported
Winter triticale	Supported
Winter barley	Supported
Spring wheat	Supported
Spring barley	Supported
Oat	Supported

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

5.2.1 Analysis of the plant protection product (KCP 5.1.1)

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

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

Comments of zRMS:	The method is accepted and may be applied for analysing MCPA and Tribenuron methyl in the PPP.

Reference:	KCP 5.1.1
Report	MCPA + Tribenuron metyl 565 SG Method development and validation for the determination of active substances and free phenols content in the formulation, M. Wołoszynowska, 2017, BA-24/17, Institute of Industrial Organic Chemistry
Guideline(s):	Yes (SANCO/3030/99)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The aim of the study was to validate an analytical method for determination of active substances MCPA and Tribenuron-methyl in HAKSAR TOP 565 SG in accordance with actual EU requirements.

The content of MCPA and Tribenuron methyl in the examined specimen was determined by high performance liquid chromatography HPLC with UV/Vis detector using reversed phase column.
The determination was carried out under following chromatographic conditions:

- Column temperature: 30ºC
- Mobile phase: MeCN : 0.5 % H_3PO_4 (40:60, v/v)
- Flow rate: 1.2 mL/min
- Wavelength λ = 230 nm
- Volume of sample solution injected: 10 µL

Validation - Results and discussions

Table 5.2-1: Methods suitable for the determination of active substances MCPA and Tribenuron-methyl in plant protection product HAKSAR TOP 565 SG

	MCPA	Tribenuron-methyl
Author(s), year	M. Wołoszynowska, 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 mixtures of standard solutions at the concentration range of MCPA from 0.6073 mg/mL to 1.5182 mg/mL, which corresponds to the concentration range from 53% to 133% of MCPA content in the preparation. Correlation coefficient is equal r = 0.9980 (acceptance criterion $R^2 \geq 0.99$) The obtained results are acceptable.	The linearity of the detector response was assessed using five mixtures of standard solutions at the concentration range of tribenuron methyl from 0.0163 mg/mL to 0.0435 mg/mL, which corresponds to the concentration range from 58% to 155% of tribenuron methyl content in the preparation Correlation coefficient is equal r = 0.9994 (acceptance criterion $R^2 \geq 0.99$) The obtained results are acceptable.
Precision – Repeatability Mean n = 6 (%RSD)	% RSD = 1,29 acceptance criterion ≤ 1,47 % Horrat value: Hr = %RSD/%RSDr H_r = 0.88 acceptance criterion: Hr ≤ 1	% RSD = 2,25 acceptance criterion ≤ 2,56 % Horrat value: Hr = %RSD/%RSDr H_r = 0.88 acceptance criterion: Hr ≤ 1
Accuracy n = 12 (% Recovery)	100.24 % acceptance criterion 100 ± 2%	100.12 % acceptance criterion 100 ± 3%
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 MCPA and Tribenuron-methyl determination in the preparation HAKSAR TOP 565 SG.

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

Free phenols are not relevant impurities although are listed by FAO Specifications for Plant Protection Products MCPA, Food and Agriculture Organization of the United Nations, Rome, 1994. The maximum limits permitted for free phenols are 10 g/kg of technical MCPA (calculating on 4-chloro-2-methylophenol). Thus analytical method for determination of free phenols is submitted

An overview on the acceptable methods and possible data gaps for analysis of relevant impurities in HAKSAR TOP 565 SG is provided as follows:

Comments of zRMS:	Based on inclusion regulation no 540/2011 MCPA has no relevant impurity. FREE phenols are part of the FAO/WHO specification. We do not require it to be provided. It meets the Sanco/3030/99 rev. 5 requirements anyway.

Reference:	KCP 5.1.1
Report	MCPA + Tribenuron metyl 565 SG Method development and validation for the determination of active substances and free phenols content in the formulation, M. Wołoszynowska, 2017, BA-24/17, Institute of Industrial Organic Chemistry
Guideline(s):	Yes (SANCO/3030/99)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The content of free phenols was determined by UV spectrophotometry. The absorbance of complex of chlorophenols with 4-aminoantipyrine in alkaline media in presence of potassium hexacyanoferrate (III) as oxidant was measured at wavelength 520 nm.

Apparatus and materials
- Spectrophotometer UV-1700 PharmaSpec, Shimadzu,
- Microburette: 1 mL, 2 mL,
- Burette 25 mL,
- Pipette 5 mL, 10 mL,
- Beaker 25 mL,
- Measuring cylinder 25 mL,
- Volumetric flasks 100 mL

Reagents
- 4-aminoantipyrine 98%, Aldrich, (0.2 % w/v water solution obtained from 2 % mother, solution. stored in dark bottle,
- Potassium hexacyanoferrate (III) $K_3[Fe(CN)_6]$, POCh (fresh aqueous solution 4 g/L),
- Ammonia pure per analysis, POCh (aqueous solution - 0.05 N),
- Ethanol 96% pure per analysis, POCh,
- Acetone pure per analysis, POCh,
- MCPA standard 99.8% %,
- 4-chloro-2-methylphenol standard 99%.

Validation - Results and discussions

Table 5.2-2: Methods suitable for the determination of the relevant impurities in plant protection product (PPP) HAKSAR TOP 565 SG

	Free phenols **Max 10 g/kg**
Author(s), year	M. Wołoszynowska, 2017
Principle of method	The content of free phenols was determined by UV spectrophotometry
Linearity (linear between mg/L) (correlation coefficient, expressed as r)	The standard five solutions were prepared. Correlation coefficient is equal r = 0.9919 (acceptance criterion $R^2 \geq 0.99$). The obtained result is acceptable
Precision – Repeatability Mean n = 5 (%RSD)	The method repeatability was assessed on the basis of five independent determinations of free phenols content in preparation. %RSD = 3.54 acceptance criterion ≤ 3.96 % Horrat value: Hr = %RSD/%RSDr $H_r = 0.89$ acceptance criterion: Hr ≤ 1
Accuracy n = 5 (% Recovery)	118.52 % acceptance criterion 100 ± 25%
Interference/ Specificity	No interference
Comment	

Conclusion

The methods for determination of active substances and free phenols in MCPA + Tribenuron metyl 565 SG preparation are specific. The validation parameters for linearity, instrument precision, repeatability and accuracy are within the acceptance range.

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 HAKSAR TOP 565 SG 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)

MCPA: 2/TC/M3/4.3, GLC method, CIPAC 1C, p.2138, or 2/TC/M3/4.4, HPLC as referee method, p.2139) and for free phenols (MT 69.2, CIPAC 1, p.1000) (Note 2).
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 MCPA and Tribenuron methyl for the generation of pre-authorization data is given in the following table. For the detailed evaluation of additional studies it is referred to Appendix 2.

Table 5.2-3: Validated methods for the generation of pre-authorization data

Component of residue definition: MCPA and 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 for MCPA = 0.0188 mg/kg LOQ for Tribenuron-methyl = 0.010 mg/kg	HPLC with LC-MS/MS	➢ M.Wójcik, Determination of the residues of MCPA and tribenuron-methyl in grain and straw of wheat, 2018, C/05/17
Water (Ecotoxicology)	Primary	LOQ for MCPA 0.028504 mg/L LOQ for Tribenuron-methyl = 0.057898 mg/L	HPLC with diode array detection UV-DAD	➢ M. Świstak, Validation of analytical method for the determination of active substance – MCPA and methyl tribenuron in aqueous solution of the test item, 2020, 0016/0100/FA
Water (Ecotoxicology)	Primary	LOQ = 0.001 mg/L for MCPA and Tribenuron-methyl	LC-UV-VIS	➢ Tina Turek MCPA+Tribenuron metyl 565 SG, *Daphnia magna*, Acute Immobilization Test, 2017, W/269/17 ➢ Tina Turek MCPA+Tribenuron metyl 565 SG, *Pseudokirchinella subcapitata*, Growth inhibition Test, 2018, W/270/17 ➢ Tina Turek MCPA+Tribenuron metyl 565 SG, *Navicula pelliculosa*, Growth Inhibition Test, 2017, W/271/17 ➢ Tina Turek MCPA+Tribenuron metyl 565 SG, *Lemna gibba*, Growth Inhibition Test, 2018, W/272/17 ➢ Anna Świerkot MCPA+Tribenuron metyl 565 SG, *Myriophyllum spicatum*, Toxicity Test, 2018, W/181/17 ➢ Paweł Bąk, MCPA+Tribenuron metyl 565 SG, *Daphnia magna*, Reproduction Test, 2018, W/36/18

Component of residue definition: MCPA and Tribenuron methyl				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
				➢ MCPA+Tribenuron metyl 565 SG, Terrestial Plant Test: Seedling Emergence and Seedling Growth Test, G/160/17, Aneta Gierbuszewska ➢ MCPA+Tribenuron metyl 565 SG, Terrestrial Plant Test: Vegetative Vigour Test, G/161/17, Weronika Dec
Sucrose solution (Ecotoxicology)	Primary	LOQ = 1 mg/kg for MCPA and for Tribenuron-methyl	HPLC with UV-Vis detection	➢ P. Parma, MCPA + Tribenuron Metyl 565 SG: Honeybees (Apis mellifera L.), Chronic Oral Toxic Test, 2019, B/26/18

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 MCPA (KCP 5.2)

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

Compared to the residue definition proposed in the Draft Assessment Report (incl. its addenda) the current legal residue definition is not identical.
Residue definition (previous): MCPA
Residue definition (existing): MCPA and MCPB (MCPA, MCPB including their salts, esters and conjugates expressed as MCPA) (Regulation n°491/2014)

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

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Plant, high protein/high starch content (dry commodities)	MCPA	LOQ = 0.07 mg/kg	0.2 mg/kg
Muscle	MCPA	LOD = 0.05 mg/kg	0.1 mg/kg

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Milk		LOD = 0.05 mg/kg	0.05 mg/kg
Soil (Ecotoxicology)	MCPA (and its metabolites)	50 mg/kg	AOEL sys: 0.04 mg/kg bw/d
Drinking water (Human toxicology)	MCPA (and its metabolites)	0.1 µg/L	general limit for drinking water
Surface water (Ecotoxicology)	MCPA (and its metabolites)	0.01 µg/L (2,4-D, 2,4-DB, MCPA, MCPB, Dichloroprop-P) 0.02 µg/L (Mecporop-P)	Watson G, 2014
Air	MCPA	0.6 µg/m^3	AOEL sys: 0.04 mg/kg bw/d
Tissue (meat or liver)	-	not required	notclassified as T / T+
Body fluids		not required	notclassified as T / T+

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

An overview on the acceptable methods and possible data gaps for analysis of MCPA in plant matrices is given in the following tables.

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

Component of residue definition: MCPA				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
High protein/high starch content (dry)	Primary	LOQ = 0.07 mg/kg	GC/ECD	Flynn S. G., 1979
Cirus fruit (orang), olives	Primary	0.01 mg/L	LC-MS/MS	Allen L., 2014
	ILV	0.01 mg/L	LC-MS/MS	Watson G., 2014

Table 5.3-3: Statement on extraction efficiency

	Method for products of plant 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 expectd. For MCPA non of residue value exceed LOQ

All analytical methods are active substance data and were evaluated during the EU review of MCPA. No additional studies have been performed.

These data have been provided and are considered to adequate.

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 MCPA in animal matrices is given in the following tables.

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

Component of residue definition: MCPA				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Milk, muscle	Primary	LOQ < 0.05 mg/kg	GC-MSD	DAR 2001/ EU agreed
Eggs, bovine fats, muscle and liver	Primary	0.01 mg/L	LC-MS/MS	Allen L., 2014
	ILV	0.01 mg/L	LC-MS/MS	Watson G., 2014

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 expectd. For MCPA non of residue value exceed LOQ.

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

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 MCPA in soil is given in the following tables.

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

Component of residue definition: MCPA (and two its metabolites 4-chloro-*o*-cresol and 5-chloro-3-methylcatechol)			
Method type	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	LOQ = 50 mg/kg	GC with EC detection	Sattar M. A. and J. Paasivirta, 1979

All analytical methods are active substance data and were evaluated during the EU review of MCPA. No additional studies have been performed.
These data have been provided 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 MCPA in surface and drinking water is given in the following tables.

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

Component of residue definition: 2,4-D, 2,4-DB, MCPA, MCPB, Dichloroprop-P)				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Surface water	Primary	0.01 µg/L	LC-MS/MS	Allen L, 2014
Drinking water	ILV	0.01 µg/L	LC-MS/MS	Weir A, 2014
And Mecoprop-P				
Surface water	Primary	0.02 µg/L	LC-MS/MS	Allen L, 2014
Drinking water	ILV	0.02 µg/L	LC-MS/MS	Weir A, 2014

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 MCPA in air is given in the following tables.

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

Component of residue definition: MCPA			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	0.6 µg/m^3	HPLC	Reichert N. 1994
Confirmatory	0.24 µg/m^3	HPLC/UV	Zangmeister W. 1995

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

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

Analytical methods for the determination of residues in body fluids and tissues are not required as MCPA is not classified as toxic or highly toxic

5.3.2.8 Other studies/ information

There are no additional European requirements for formulated products.

5.3.3 Description of analytical methods for the determination of residues of Tribenuron-methyl

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

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

Table 5.3-1: 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 purposes)	Tribenuron-methyl	LOQ 0.01 mg/kg	COMMISSION REGULATION (EU) 2015/1040 of 30 June 2015
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 0.01 µg/L for Tribenuron-methyl and IN-L5296	(EFSA Journal 2017;15(7):4912): (Hill, S.J., Stry, J J., 2001), (Lakaschus, S., 2007), (Pope 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): (Gagnon, M.R., Stry, J.J., 2001), (Weber, H., 2010)
Air	Tribenuron-methyl	1.5 µg/m^3	(EFSA Journal 2017;15(7):4912): (Class T., Hausmann, S., 2000),
Tissue (meat or liver)	Tribenuron-methyl	0.01 mg/kg in body tissues 1 µg/kg (plasma), 3 µg/kg (urine)	(EFSA Journal 2017;15(7):4912)
Body fluids			

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

An overview on the acceptable methods and possible data gaps for analysis of tribenuron methyl in plant matrices is given in the following tables.

Table 5.3-9: 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, Amoo J.S, Jones W, 2000, DuPont-3595
Cereals	Primary	LOQ = 0.01 mg/kg	HPLC-UV	DAR 2003, Zietz E., Jin L., 2000, DuPont-2261 Revision No. 1
	ILV	LOQ = 0.01 mg/kg	HPLC-UV	DAR, 2003, Clayton B, 2001, DuPont-5587
Tomato, orange, avocado, wheat straw, wheat grain and wheat whole plant	Primary	LOQ = 0.01 mg/kg	LC-MS/MS	Roth A., 2019, S18-07519
Tomato, wheat	ILV	LOQ = 0.01 mg/kg	HPLC-MS	Schmiedt S., 2019, P 5114 G

Table 5.3-10: 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.

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

Table 5.3-11: Validated methods for food and feed of animal origin (if appropriate)

An overview on the acceptable methods and possible data gaps for analysis of tribenuron-methyl in animal matrices is given in the following tables.

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	Primary	LOQ = 0.02 mg/kg	HPLC	DAR 2003, Williams M.D., 1996, AMR 3698-95
	ILV	LOQ = 0.02 mg/kg	HPLC	DAR 2003, Gagnon N.L., 2000, DuPont-4245
Milk, Eggs, Muscle, Cream, Liver	Primary	LOQ = 0.05 mg/kg	LC-MS	Norris D., 2016, DNA3620 Norris D., 2019, DNA3620
	ILV	LOQ = 0.05 mg/kg	LC-MS	Eichler M., Hermann 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 5.3-12: 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.

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

These data have been provided and are considered to adequate.

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

Table 5.3-13: 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, Hill S. J., Stry J. J., 2001, DuPont-5082
Primary	LOQ = 1.0 µg/kg	HPLC-MS/MS	DAR 2003, Gagnon M.R., Devine T.J., Cabusas 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	Kotthoff M., 2018, PRO-001/6-20/B Hennecke S., 2019, PRO-001/6-20/B

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

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

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	Gagnon, M. R., Stry, J.J., 2001, Stry, J.J., 2014
Drinking water	Primary	LOQ = 0.1 µg/L	LC-MS/MS	Hennecke S., 2018, PRO-001/6-22
Drinking water	ILV	LOQ = 0.1 µg/L	LC-MS/MS	Böhmer, 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.

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

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	Class, T. Hausmann, 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.
These data have been provided and are considered to adequate

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

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

Component of residue definition: tribneuron-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)	LC-MS/MS	Henze, R.M., Stry, J.J., 2016

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.

5.3.3.8 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	Małgorzata Wołoszynowska	2017	MCPA + Tribenuron metyl 565 SG Method development and validation for the determination of active substances and free phenols content in the formulation BA-24/17 INSTITUTE OF INDUSTRIAL ORGANIC CHEMISTRY GLP: yes Unpublished	N	CIECH Sarzyna S.A.
KCP 5.1.2/01	Marcin Świstak	2020	Validation of analytical method for the determination of active substances – MCPA and methyl tribenuron in aqueous of the test item, 0016/0100/FA, SORBOLAB Research Laboratory GLP: Yes Unpublished	N	CIECH Sarzyna S.A.
KCP 5.1.2/02	Tina Turek	2017	MCPA+Tribenuron metyl 565 SG: *Daphnia magna*, Acute Immobilization Test, Institute of Industrial Organic Chemistry Branch Pszczyna W/269/17 GLP: Yes Unpublished	N	CIECH Sarzyna S.A.
KCP 5.1.2/03	Tina Turek	2018	MCPA+Tribenuron metyl 565 SG: *Pseudokirchinella subcapitata*, Growth inhibition Test, Institute of Industrial Organic Chemistry Branch Pszczyna W/270/17 GLP: Yes Unpublished	N	CIECH Sarzyna S.A.
KCP 5.1.2/04	Tina Turek	2017	MCPA+Tribenuron metyl 565 SG: *Navicula pelliculosa*, Growth Inhibition Test, Institute of Industrial Organic Chemistry Branch Pszczyna W/271/17	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**
			GLP: Yes Unpublished		
KCP 5.1.2/05	Tina Turek	2018	MCPA+Tribenuron metyl 565 SG: *Lemna gibba*, Growth Inhibition Test, Institute of Industrial Organic Chemistry Branch Pszczyna W/272/17 GLP: Yes Unpublished	N	CIECH Sarzyna S.A.
KCP 5.1.2/06	Anna Świerkot	2018	MCPA+Tribenuron metyl 565 SG: *Myriophyllum spicatum*, Toxicity Test, Institute of Industrial Organic Chemistry Branch Pszczyna W/181/17 GLP: Yes Unpublished	N	CIECH Sarzyna S.A.
KCP 5.1.2/07	Paweł Bąk	2018	MCPA+Tribenuron metyl 565 SG: *Daphnia magna*, Reproduction Test,, Institute of Industrial Organic Chemistry Branch Pszczyna W/36/18 GLP: Yes Unpublished	N	CIECH Sarzyna S.A.
KCP 5.1.2/08	Aneta Gierbuszewska	2018	MCPA + TRIBENURON METYL 565 SG Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test G/160/17 GLP: Yes Unpublished	N	CIECH Sarzyna S.A.
KCP 5.1.2/09	Weronika Dec	2018	MCPA + TRIBENURON METYL 565 SG Terrestrial Plant Test: Vegetative Vigour Test G/161/17 GLP: Yes Unpublished	N	CIECH Sarzyna S.A.
KCP 5.1.2/10	P. Parma	2019	MCPA + Tribenuron Metyl 565 SG: Honeybees (*Apis mellifera L.*), Chronic Oral Toxic Test, 2019, B/26/18 GLP: Yes	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
			Unpublished		
KCP 5.1.2/11	M. Wójcik	2018	Determination of the residues of MCPA and tribenuron-methyl in grain and straw of wheat C/05/17 INSTITUTE OF INDUSTRIAL ORGANIC CHEMISTRY BRANCH PSZCZYNA GLP Unpublished	N	CIECH Sarzyna S.A.
KCP 5.1.2/12	J. Johannes	2020	Validation of an Analytical Method using LC-MS-MS for the determination of MCPA in the matrix "Wash solution for foliar dislodging experiments" 19112203G926, "Preparation and shipment of Field Fortification Standard solutions of MCPA", 19112203G405 LAUS GmbH GLP Unpublished	N	CIECH Sarzyna S.A.
KCP 5.2/01	L. Allen	2014	Analytical Method for the Determination of Phenoxy Acids and Their Corresponding 2 Ethyl-hexyl esters and glycine conjugates in cereal grain, straw and foliage, bovine muscle, fat, liver, kidney and milk, poultry eggs, citrus fruit and olives and phenoxy acids and their corresponding 2 ethyl-hexyl esters in surface water, soil and air (Universal Method) CEMAS Study #CAM-0004/003 GLP Unpublished	N	MCPA TASK FORCE
KCP 5.2/02	A. Weir	2014	Phenoxy Herbicides - Independent Laboratory Validation of the Analytical Method CAM-0004/003 for the Determination of Phenoxy Acids and Their Corresponding 2 Ethyl-Hexyl Esters in Drinking Water by LC-MS/MS (Universal Method) Eurofins Agroscience Services Chem Ltd Study #S14-01199 GLP Unpublished	N	MCPA TASK FORCE
KCP 5.2/03	G. Watson	2014	Phenoxy Herbicides - Independent Laboratory Validation of the Analytical Method CAM-0004/002 for the Determination of Phenoxy Acids and Their Corresponding 2 Ethyl-Hexyl Esters and Glycine Conjugates in six matrices by LC-MS/MS (Universal Method)	N	MCPA TASK FORCE

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			Eurofins Agroscience Services Chem Ltd Study #S14-00286 GLP Unpublished		
KCP 5.2/04	Roth A.	2019	Development and Validation of an Analytical Method for Determination of Tribenuron-methyl in Plant Matrices Eurofins S18-07519 GLP Unpublished	N	TF PROPLAN-SARABIA
KCP 5.2/05	Schmiedt 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 5.2/06	Norris 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 5.2/07	Norris 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

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 5.2/08	Eichler M., Hermann 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 5.2/09	Kotthoff 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 GLP Unpublished	N	TF PROPLAN-SARABIA
KCP 5.2/10	Hennecke 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 5.2/11	Hennecke 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 5.2/12	Böhmer 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
KIIA 4.3	S. G. Flynn	1979	Determination of 2-methyl-4-chlorophenoxy acetic acid (MCPA) residues in spring barley grain and straw, oat grain and straw, oat grain and straw, grass and hay. Generated by: BASF AG/TPH Submitted by: MCPA Dossier, Preparation Working Group, File No: 60288	N	-
KIIA 4.4	M. A. Sattar J. Paasivirta	1979	Simultaneous determination of MCPA and its metabolites in soil by Gas chromatography Generated by: Published literature, Submitted by: MCPA Dossier, Preparation Working Group	N	MCPA TASK FORCE
KIIA 4.7	N. Reichert	1994	Development and validation of a method for the determination of 2,4-D MCPA. Dichloprop-P and Mecoprop-P in air Submitted by: MCPA Dossier, Preparation Working Group File No: RCC 439705	N	MCPA TASK FORCE
KIIA 4.2.4	W. Zangmeister	1995	Recovery of 2,4-DK, MCPA-DMA, Mecoprop-p-DMA and Dichloroprop-P-DMA after elution from TENAX – supplement to analytical method RCC project 439 705. GLP	N	AHM, BAS, DAS, ROP, NUF
KCP 5.2/01	Amoo J.S, Jones W.	2000	Analytical enforcement method for the determination of Tribenuron methyl in cereals (grain, forage and straw) using column-switching liquid chromatography with ultraviolet detection DuPont Experimental Station DuPont-3595 non GLP Unpublished	N	DuPont
KCP 5.2/02	Zietz E., Jin L.	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	N	DuPont

			DuPont-2261 Revision No.1 GLP Unpublished		
KCP 5.2/03	Clayton 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	Gagnon 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	Williams 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 Unpublished	N	DuPont
KCP 5.2/06	Gagnon 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	Hill, S. J., Stry, 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	Gagnon M. R., Devine T.J., Cabusas 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	N	DuPont

			Unpublished		
KCP 5.2/09	Gagnon M. R., Stry 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	Stry 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	Class T., Hausmann S.	2000	Analytical method and confirmatory method for the determination of tribenuron methyl in air DuPont-4108 PTRL Europe GLP Unpublished	N	DuPont
KCP 5.2/12	Henze, R. M., Stry, 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 MCPA and 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 active substance and/or variant in the plant protection product (KCP 5.1.1)

A 2.1.1.1.1 Description of analytical methods for the determination of active substances and free phenols content in the product (KCP 5.1.1)

A 2.1.1.1.1.1 Method validation

Comments of zRMS:	Accepted

Reference:	KCP 5.1.1
Report	M. Wołoszynowska, MCPA + Tribenuron metyl 565 SG, Method development and validation for the determination of active substances and free phenols content in the formulation, 2017, Report no: BA-24/17
Guideline(s):	Yes (SANCO/3030/99 rev. 4)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Determination of active substances content

For other materials, methods and liquid chromatography conditions See point. 5.2.1.1

Materials and methods

The validated analytical method is specific. External standard method was used. There are no interferences between the analytes and other components of the specimen. The method has good precision, accuracy and linearity and fulfils requirements of SANCO/3030/99 rev.4.

Apparatus and materials

• Shimadzu liquid chromatograph equipped with UV/Vis detector, a thermostated column oven and autosampler
• Gemini NX-C18 column (5µm), 150 x 4.6 mm
The retention time of MCPA was ~ 6.5 min and the retention time of was tribenuron methyl ~ 9.1 min. The total time of analysis was 25 min.
Standard solution – appropriate amounts of active substances standards were weighed (with the accuracy of 0.01 mg) into two 10 mL flask with a screw cap and acetonitrile was added up to the volume. Specimen solution – about 20 mg of examined specimen was weighed (with the accuracy of 0.01 mg) into a 10 mL flask with a screw cap, 1 mL of water and 5 mL of acetonitrile was added. Chromatographic system was conditioned before analysis.

Results and discussions

For the results and discussions see point 5.2.1.1

Determination of free phenols content

For other materials, methods and liquid chromatography conditions See point 5.2.1.2

Materials and methods

Solution A
10.08 mg of 4C2MP standard was weighed into the 25 mL beaker (to the nearest 0.0002 g), dissolved in acetone (1 mL), transferred quantitatively into 100 mL volumetric flask and diluted to the mark with distilled water. The solution was diluted 10-times with water (1 mL A1 = 10 μg of phenol).

Solution B
125.06 mg of MCPA standard was weighed into the 250 mL beaker (to the nearest 0.0002 g), dissolved in ethanol (12.5 mL), transferred quantitatively into 250 mL volumetric flask. Ammonia solution 0.05 N (9 mL) was added and mixture was diluted up to the mark with distilled water.

• Determination of free phenols

About 50 mg of MCPA sample was weighed with accuracy of 0.0002 g into the 50 mL beaker. The sample was transferred quantitatively into 100 ml volumetric flask, dissolved in hot water (10 mL), ammonia solution (9 mL) was also added and mixture was diluted to 100 mL with distilled water. 10 mL of this solution was transferred by pipette into measuring cylinder. Afterwards ammonia (5 mL). 4-aminoantipyrine (5 mL) and potassium hexacyanoferrate (III) (5 mL) solutions were added. Mixture was shaken for 1 minute after addition of any component. The blank sample was prepared in parallel. The absorbance of solution was measured at wavelength λ = 520 nm. The measurement was performed between 5 and 10 minutes after addition of potassium hexacyanoferrate (III).

Results and discussions

For the results and discussions see point 5.2.1.2

A 2.1.1.2 Description of analytical methods for the determination of MCPA and Tribenuron methyl in ecotoxicological testing (KCP 5.1.2)

A 2.1.1.2.1 Determination of residues in water used in support of ecotoxicological studies. Study 1

A.2.1.1.2.1.1 Method validation

Comments of zRMS:	Analytical part of the method is not accepted. Results of recovery for MCPA were presented in the study for higher concentration (0.617 mg/L for MCPA) than proposed LOQ (0.028 mg/L). For tribenuron methyl proposed LOQ is 0.0578 mg/kg but recovery data are presented for 0.01739 mg/kg and 1.24201 mg/kg. It is not clear where the results indicated in the table A 15 come from. They are not included in the study report.

Reference:	KCP 5.1.2/01
Report	Validation of analytical method for the determination of active substances – MCPA and methyl tribenuron in aqueous of the test item, M. Świstak, 0016/0100/FA, SORBOLAB Research Laboratory
Guideline(s):	Yes. According to the SANCO/3029/99 rev. 4
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Determination of active substances – MCPA and methyl tribenuron in deionized water was performed by high performance liquid chromatography with diode array detection UV-DAD based on signal from the active substances. Identification of active substances in test item was made by comparing the retention time of the active substances and sample of test item.

Apparatus and materials

- analytical balance Radwag XA 82_220.4Y.A,
- high performance liquid chromatography Shimadzu Prominence series LC-20 with PDA detector,
- volumetric flask class A,
- adjustable automatic pipettes: Transferpette 5 mL, Acura Manual 826 XS, Transferpette 10 µL,
- deionizer Solpure 78,
- system for obtaining ultrapure water Millipore Synergy UV,
- ultrasonic washer Sonic-10,
- syringes and syringe filters 0.22 µm

Reagents

- acetonitrile HPLC grade, POCH,
- methanol HPLC grade, Honeywell,
- MCPA standard, IPO Warszawa,
- methyl tribenuron standard, IPO Warszawa, lot number 2A/17,
- deionized water,
- ultrapure water,
- orthophosphoric acid 85%, p.a., Chempur

Results and discussions

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

Matrix	Analyte	Fortification level (mg/L) n=5	Mean recovery (%)	RSD (%)	Comments
Deionized water	MCPA	0.001	97.7	0.05	-
		1.000	103.3	1.39	
Deionized water	Tribenuron methyl	0.001	93.9	2.25	-
		1.000	101.5	3.14	

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 16: Characteristics for the analytical method used for validation of MCPA and tribenuron methyl residues in water

	MCPA	Tribenuron methyl
Specifity	The analytical method specificity was estimated on the basis of the analysis of the chromatograms obtained for the control and fortified samples. Considering the results of the analysis, no signal of detected substance was overlapping with the matrix signal of the control samples under the experimental conditions. Therefore, the specificity of the methods were demonstrated.	
Calibration (type, number of data points)	Curve Type: Linear y = +54968.9x+3123.86 – MCPA n=5	Curve Type: Linear y = +29692.1x+816.633 – Tribenuron methyl n=5
Calibration range	calibration curve: 1.92988 mg/L – 30.87812 mg/L – MCPA The calibrations were found to be linear with correlation coefficients (r) greater than 0.99. These results meet the acceptance criteria of r ≥ 0.99	calibration curve: 0.10091 mg/L – 1.61462 mg/L – Tribenuron-methyl The calibrations were found to be linear with correlation coefficients (r) greater than 0.99. These results meet the acceptance criteria of r ≥ 0.99
Assessment of matrix effects is presented	no	no
Limit of determination/quantification	The Limit of Quantification (LOQ) analyzed in water medium is 0.028504 mg/L (MCPA) and the Limit of Detection (LOD) is 0.009406 mg/L for MCPA	The Limit of Quantification (LOQ) analyzed in water medium is 0.057898 mg/L (tribenuron methyl) Limit of Detection (LOD) is 0.019106 mg/L for Tribenuron methyl

Conclusion

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

A 2.1.1.2.2. Determination of residues in water used in support of ecotoxicological studies. Study 2

A.2.1.1.2.2.1. 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 0.0005 mg/L for MCPA and tribenuron methyl. 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% for both analysed compounds.
Reference:	KCP 5.1.2/02
Report	MCPA + TRIBENURON METYL 565 SG, *Daphnia magna*, Acute Immobilisation Test, Tina Turek, MSc, 2017, STUDY CODE: W/269/17, Institute of Industrial Organic Chemistry, Branch Pszczyna

Guideline(s): Yes. According to the OECD Guideline No. 202 (2004)

Deviations: No

GLP: Yes

Acceptability: Yes

Materials and methods

For study report please see section B9

Sample preparation:

Each sample in a volume between 10 - 100 mL (i.e. control sample, test sample, sample fortified with standard) was shaken twice with 25 mL of dichloromethane. The organic phases were filtered through anhydrous sodium sulphate (VI). The combined extracts were evaporated to dryness using vacuum rotary evaporator at 450C.The dry residue was dissolved in acetonitrile and 20 µL was injected to chromatographic column.

Determination:

The aim of the analytical part of the definitive test was to determine the test item concentrations with a validated liquid chromatographic method with UV-Vis. Samples of each test item concentration and the control collected at exposure initiation and termination were analyzed.

Liquid chromatography conditions:

- 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: 220 nm – MCPA
  231 nm – tribenuron methyl
- Flow rate: 1.0 mL/min
- Injected volume: 20µL

Results and discussions

Table A 1: Recovery results from method validation of MCPA and tribenuron methyl using the analytical method

Matrix	Analyte	Fortification level (mg/L)	Mean recovery (%)	RSD (%)	Comments
Elendt M7 medium	MCPA	0.001	103.6	8.4	-
		1.000	90.4	4.4	
	Tribenuron methyl	0.001	100.1	5.4	-
		1.000	84.4	1.9	

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 2: Characteristics for the analytical method used for validation of MCPA and tribenuron methyl residues in water

	MCPA and tribenuron methyl
Specifity	The analytical method specificity was estimated on the basis of the analysis of the chromatograms obtained for the control and fortified samples. Considering the results of the analysis, no signal of detected substance was overlapping with the matrix signal of the control samples under the experimental conditions. Therefore, the specificity of the method was demonstrated.
Calibration (type, number of data points)	Curve Type: Linear y = +3.587880e+005x - MCPA y = +6.009879e+005x – Tribenuron methyl
Calibration range	The calibration was performed using calibration solutions (7 concentrations) within the range of 0.05 to 20.0 µg/mL The a regression coefficient (r2) was 0.999299(MCPA) and 0.999695 (tribenuron methyl). The range of linearity of the analytical is from 0.05 µg/mL to 20.0 µg/mL. The calibrations were found to be linear with correlation coefficients (r) greater than 0.99. These results meet the acceptance criteria of $r \geq 0.99$
Assessment of matrix effects is presented	no
Limit of determination/quantification	The Limit of Quantification (LOQ) analyzed in Elendt M7 medium is 0.001 mg/L and the Limit of Detection (LOD) is 0.0005 mg/L for both MCPA and Tribenuron methyl

Conclusion

The analytical method therefore meets the requirements of guideline SANCO/3029/99 rev. 4 and SANCO825/00, rev. 8.1.

A.2.1.1.2.3 Determination of residues in water used in support of ecotoxicological studies. Study 3

A.2.1.1.2.3.1. 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 0.0005 mg/L for MCPA and tribenuron methyl. 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% for both compounds.
Reference:	KCP 5.1.2/03
Report	MCPA + TRIBENURON METYL 565 SG, *Pseudokirchineriella subcapitata SAG 61.81*, Growth Inhibition Test, Tina Turek, MSc, 2018, STUDY CODE: W/270/17, Institute of Industrial Organic Chemistry, Branch Pszczyna
Guideline(s):	Yes. According to the OECD Guideline No. 201 (2006)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

For materials, methods and liquid chromatography conditions See point. A.2.1.1.2.2.1

For study report please see section B9

Results and discussions

Table A 6: Recovery results from method validation of MCPA and tribenuron methyl using the analytical method

Matrix	Analyte	Fortification level n=5	Mean recovery (%)	RSD (%)	Comments
APP medium	MCPA	0.001	98.4	4.9	-
		1.000	85.8	6.3	
	Tribenuron methyl	0.001	94.8	8.5	-
		1.000	85.1	4.0	

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 7: Characteristics for the analytical method used for validation of MCPA and tribenuron methyl residues in water

	MCPA and tribenuron methyl
Specifity	The analytical method specificity was estimated on the basis of the analysis of the chromatograms obtained for the control and fortified samples. Considering the results of the analysis, no signal of detected substance was overlapping with the matrix signal of the control samples under the experimental conditions. Therefore, the specificity of the method was demonstrated.
Calibration (type, number of data points)	Curve Type: Linear y = +3.587880e+005x - MCPA y = +6.009879e+005x – Tribenuron methyl
Calibration range	The calibration was performed using calibration solutions (7 concentrations) within the range of 0.05 to 20.0 µg/mL The a regression coefficient (r2) was 0.999299(MCPA) and 0.999695 (tribenuron methyl). The range of linearity of the analytical is from 0.05 µg/mL to 20.0 µg/mL. The calibrations were found to be linear with correlation coefficients (r) greater than 0.99. 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) analyzed in APP medium is 0.001 mg/L and the Limit of Detection (LOD) is 0.0005 mg/L for both MCPA and Tribenuron methyl

Conclusion

The analytical method therefore meets the requirements of guideline SANCO/3029/99 rev. 4 and SANCO825/00, rev. 8.1.

A.2.1.1.2.4 Determination of residues in water used in support of ecotoxicological studies. Study 4

A.2.1.1.2.4.1. 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) 0.0005 mg/L for MCPA and tribenuron methyl. 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% for both analysed compounds.
Reference:	KCP 5.1.2/04
Report	MCPA + TRIBENURON METYL 565 SG , *Naviculla pelliculosa*, Growth Inhibition Test, Tina Turek, MSc, 2017, STUDY CODE: W/271/17, Institute of Industrial Organic Chemistry, Branch Pszczyna
Guideline(s):	Yes. According to the OECD Guideline No. 201 (2006)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

For materials, methods and liquid chromatography conditions See point. A.2.1.1.2.2.1

For study report please see section B9

Results and discussions

Table A 3: Recovery results from method validation of MCPA and tribenuron methyl using the analytical method

Matrix	Analyte	Fortification level (mg/L) n=5	Mean recovery (%)	RSD (%)	Comments
APP-Si medium	MCPA	0.001	104.6	6.0	-
		1.000	92.5	4.2	
	Tribenuron methyl	0.001	91.6	7.9	-
		1.000	89.4	2.8	

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 MCPA and tribenuron methyl residues in water

	MCPA and tribenuron methyl
Specifity	The analytical method specificity was estimated on the basis of the analysis of the chromatograms obtained for the control and fortified samples.

	MCPA and tribenuron methyl
	Considering the results of the analysis, no signal of detected substance was overlapping with the matrix signal of the control samples under the experimental conditions. Therefore, the specificity of the method was demonstrated.
Calibration (type, number of data points)	Curve Type: Linear y = +3.587880e+005x - MCPA y = +6.009879e+005x – Tribenuron methyl
Calibration range	The calibration was performed using calibration solutions (7 concentrations) within the range of 0.05 to 20.0 µg/mL The a regression coefficient (r2) was 0.999299(MCPA) and 0.999695 (tribenuron methyl). The range of linearity of the analytical is from 0.05 µg/mL to 20.0 µg/mL. The calibrations were found to be linear with correlation coefficients (r) greater than 0.99. These results meet the acceptance criteria of $r \geq 0.99$
Assessment of matrix effects is presented	no
Limit of determination/quantification	The Limit of Quantification (LOQ) analyzed in APP-Si medium is 0.001 mg/L and the Limit of Detection (LOD) is 0.0005 mg/L for both MCPA and Tribenuron methyl

Conclusion

The analytical method therefore meets the requirements of guideline SANCO/3029/99 rev. 4 and SANCO825/00, rev. 8.1.

A.2.1.1.2.5 Determination of residues in water used in support of ecotoxicological studies. Study 5

A.2.1.1.2.5.1. Method Validation

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 for MCPA and tribenuron methyl. 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% for both analysed compounds.
Reference:	KCP 5.1.2/05
Report	MCPA + TRIBENURON METYL 565 SG, *Lemna gibba CPCC 310*, Growth Inhibition Test, Tina Turek, MSc, 2018, STUDY CODE: W/272/17, Institute of Industrial Organic Chemistry, Branch Pszczyna
Guideline(s):	Yes. According to the OECD Guideline No. 201 (2006)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

For materials, methods and liquid chromatography conditions See point. A.2.1.1.2.2.1

For study report please see section B9

Results and discussions

Table A 9: Recovery results from method validation of MCPA and tribenuron methyl using the analytical method

Matrix	Analyte	Fortification level (mg/L) n=5	Mean recovery (%)	RSD (%)	Comments
20x APP medium	MCPA	0.001	103.8	7.6	-
		1.000	87.7	2.8	
	Tribenuron methyl	0.001	101.2	8.3	-
		1.000	86.2	4.4	

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 MCPA and tribenuron methyl residues in water

	MCPA + Tribenuron methyl
Specifity	The analytical method specificity was estimated on the basis of the analysis of the chromatograms obtained for the control and fortified samples. Considering the results of the analysis, no signal of detected substance was overlapping with the matrix signal of the control samples under the experimental conditions. Therefore, the specificity of the method was demonstrated.
Calibration (type, number of data points)	Curve Type: Linear y = +3.587880e+005x - MCPA y = +6.009879e+005x - Tribenuron methyl
Calibration range	The calibration was performed using calibration solutions (7 concentrations) within the range of 0.05 to 20.0 µg/mL The a regression coefficient (r2) was 0.999299(MCPA) and 0.999695 (tribenuron methyl). The range of linearity of the analytical is from 0.05 µg/mL to 20.0 µg/mL. The calibrations were found to be linear with correlation coefficients (r) greater than 0.99. 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) analyzed in ~~APP Si~~ 20xAAP medium is 0.001 mg/L and the Limit of Detection (LOD) is 0.0005~~3~~ mg/L for both MCPA and Tribenuron methyl

Conclusion

The analytical method therefore meets the requirements of guideline SANCO/3029/99 rev. 4 and SANCO825/00, rev. 8.1.

A.2.1.1.2.6 Determination of residues in water used in support of ecotoxicological studies. Study 6

A.2.1.1.2.6.1. Method Validation

Method Validation

Comments of zRMS:	Analytical part of the method is accepted. The limit of quantification in water sediment equal 0.5 mg/kg and the limit of detection 0.15 mg/kg for MCPA and tribenuron methyl. The limit of quantification (LOQ) analyzed in Smart and Barko medium is 0.001 mg/L for MCPA and tribenuron methyl. The mean recoveries were in the range 70-120% with RSD <20% for both analysed compounds in all matrices.
Reference:	KCP 5.1.2/06
Report	MCPA + TRIBENURON METYL 565 SG, Water-sediment *Myriophyllum spicatum*, Toxicity Test, Tina Turek, MSc, 2018, STUDY CODE: W/181/17, Institute of Industrial Organic Chemistry, Branch Pszczyna
Guideline(s):	Yes. According to the OECD Guideline No. 201 (2006)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

For materials, methods and liquid chromatography conditions See point. A.2.1.1.2.2.1

For study report please see section B9

Aqueous phase, sediment pore water - See point. A.2.1.1.2

Sediment
First, 5 mL of acetonitrile, was added into 10 g of sediment sample, shaken for 2 minute, and sonicated for 10 minutes. The sample was centrifuged and filtered through filter paper. Then extraction was repeated with 5 mL of acetonitrile. Finally, 20 µL of the extract was introduced into a chromatographic column. The sample was diluted with acetonitrile (if necessary).

Preparation of Fortified Samples
For validation experiments, 100 mL or 50 mL of untreated Smart and Barko medium were spiked with appropriate volumes of fortification solutions.
For validation experiments, 10 g of untreated sediment were spiked with appropriate volumes of fortification solutions.
Fortified samples were concentrated before chromatographic analysis. This was done to ensure the result fits within the range of the respective standard curve.

Results and discussions

Table A 11: Recovery results from method validation of MCPA and tribenuron methyl using the analytical method

Matrix	Analyte	Fortification level n=5	Mean recovery (%)	RSD (%)	Comments
Sediment	MCPA	0.5 mg/kg	95.1	6.5	-
		5 mg/kg	99.5	6.3	
Smart and Barko		0.001 mg/L	102.1	6.6	-
		1.000 mg/L	81.3	2.2	
Sediment	Tribenuron methyl	0.5 mg/kg	77.6	11.2	-
		5 mg/kg	73.7	7.2	
Smart and Barko		0.001 mg/L	102.5	5.2	-
		1.000 mg/L	76.7	4.3	

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 MCPA and tribenuron methyl residues in water

	MCPA + Tribenuron methyl
Specifity	The analytical method specificity was estimated on the basis of the analysis of the chromatograms obtained for the control and fortified samples. Considering the results of the analysis, no signal of detected substance was overlapping with the matrix signal of the control samples under the experimental conditions. Therefore, the specificity of the method was demonstrated.
Calibration (type, number of data points)	Curve Type: Linear y = 3.913649e+005x – MCPA y = 7.318332e+005x – Tribenuron methyl
Calibration range	The calibration was performed using calibration solutions (7 concentrations) within the range of 0.05 to 20.0 µg/mL The a regression coefficient (r2) was0.999304 (MCPA)and 0.999477 (tribenuron methyl). The range of linearity of the analytical is from 0.05 µg/mL to 20.0 µg/mL. The calibrations were found to be linear with correlation coefficients (r) greater than 0.99. 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) analyzed in Smart and Barko medium is 0.001 mg/L for MCPAAND tribenuron methyl. The Limit of Detection (LOD) is 0.0003 mg/L for both active substances. The Limit of Quantification (LOQ) analyzed in sediment contpartment is 0.5 mg.kg for MCPAand tribenuron methyl and the Limit of Detection (LOD) is 0.15 mg/kg for both of active substances.

Conclusion

The analytical method therefore meets the requirements of guideline SANCO/3029/99 rev. 4 and SANCO825/00, rev. 8.1.

A.2.1.1.2.7 Determination of residues in water used in support of ecotoxicological studies. Study 7

A.2.1.1.2.7.1. Method Validation

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 0.0005mg/L for MCPA and tribenuron methyl. 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% for both analysed compounds.

Reference:	KCP 5.1.2/07
Report	MCPA + TRIBENURON METYL 565 SG *Daphnia magna*, Reproduction Test, Paweł Bąk, MSc, 2018, STUDY CODE: W/36/18, Institute of Industrial Organic Chemistry, Branch Pszczyna
Guideline(s):	Yes. According to the OECD Guideline No. 211 (2012)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

For materials, methods and liquid chromatography conditions See point. A.2.1.1.2.2.1

For study report please see section B9

Results and discussions

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

Matrix	Analyte	Fortification level (mg/L) n=5	Mean recovery (%)	RSD (%)	Comments
Elendt M7	MCPA	0.001	103.6	8.4	-
		1.000	90.4	4.4	
	Tribenuron methyl	0.001	100.1	5.4	-
		1.000	84.4	1.9	

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 MCPA residues in water

	MCPA + Tribenuron methyl
Specifity	The analytical method specificity was estimated on the basis of the analysis of the chromatograms obtained for the control and fortified samples. Considering the results of the analysis, no signal of detected substance was overlapping with the matrix signal of the control samples under the experimental conditions. Therefore, the specificity of the method was demonstrated.
Calibration (type, number of data points)	Curve Type: Linear y = +3.587880e+005x - MCPA y = +6.009879e+005x – Tribenuron methyl
Calibration range	The calibration was performed using calibration solutions (7 concentrations) within the range of 0.05 to 20.0 µg/mL The a regression coefficient (r2) was 0.999299(MCPA) and 0.999695 (tribenuron methyl). The range of linearity of the analytical is from 0.05 µg/mL to 20.0 µg/mL. The calibrations were found to be linear with correlation coefficients (r) greater than 0.99. These results meet the acceptance criteria of $r \geq 0.99$
Assessment of matrix effects is presented	no
Limit of determination/quantification	The Limit of Quantification (LOQ) analyzed in Elendt 7 medium is 0.001 mg/L and the Limit of Detection (LOD) is 0.0005 mg/L for both MCPA and Tribenuron methyl

Conclusion

The analytical method therefore meets the requirements of guideline SANCO/3029/99 rev. 4 and SANCO825/00, rev. 8.1.

A.2.1.1.2.8 Determination of residues in water used in support of ecotoxicological studies. Study 8

A.2.1.1.2.8.1. Method Validation

Comments of zRMS:	Analytical part of the method is accepted with the limit of quantification in deionized water equal 0.001 mg/L and the limit of detection 0.0005 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 5.1.2/08
Report	MCPA + TRIBENURON METYL 565 SG Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test, A. Gierbuszewska, 2018, G/160/17, Institute of Industrial Organic Chemistry, Branch Pszczyna
Guideline(s):	Yes. According to the OECD Guideline No. 227 (2006)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

For study report please see section B9

Sample preparation:

First 0.1 mL of ortho-phosphoric acid was added to each sample in a volume between 10 - 100 mL. Next the sample was shaken twice with 25 mL of dichloromethane. The organic phases were filtered through anhydrous sodium sulphate (VI). The combined extracts were evaporated to dryness using vacuum rotary evaporator at 45°C. The dry residue was dissolved in acetonitrile and injected to the chromatographic column in a volume of 20.0 µL.

Determination:

The concentration of MCPA and tribenuron methyl in water was determined using the validated high performance liquid chromatographic method with UV-Vis detection.

The following liquid chromatography parameters were used:

column: Luna 5µ C18 100A, l = 250 mm, f = 4,6 mm
mobile phase: acetonitrile : 0.05% solution of orthophosphoric (V) acid (60 : 40, v/v),
wave length: 220 nm – MCPA; 231 nm – tribenuron methyl
flow rate 1.0 mL/min.

Results and discussions

Table A 14: Recovery results from method validation of MCPA and tribenuron methyl using the analytical method

Matrix	Analyte	Fortification level (mg/L)	Mean recovery (%)	RSD (%)	Comments
Deionized water	MCPA	0.001	100.2	4.5	-
		1.000	99.3	3.6	
	Tribenuron methyl	0.001	101.4	7.5	-
		1.000	98.5	5.6	

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 15: Characteristics for the analytical method used for validation of MCPA and tribenuron methyl residues in water

	MCPA and tribenuron methyl
Specifity	The analytical method specificity was estimated 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 substances was overlapping with the matrix signal of the control samples under the experimental conditions. Therefore, the specificity of the method was demonstrated.

	MCPA and tribenuron methyl
Calibration (type, number of data points)	Curve Type: Linear y = +3.587880e+005x – MCPA y = +6.009879e+005x – Tribenuron methyl
Calibration range	The calibration was performed using calibration solutions (7 concentrations) within the range of 0.05 to 20.0 µg/mL The a regression coefficient (r2) was 0.999299(MCPA) and 0.999695 (tribenuron methyl). The range of linearity of the analytical is from 0.05 µg/mL to 20.0 µg/mL. The calibrations were found to be linear with correlation coefficients (r) greater than 0.99. These results meet the acceptance criteria of $r \geq 0.99$
Assessment of matrix effects is presented	no
Limit of determination/quantification	The Limit of Quantification (LOQ) for for both MCPA and Tribenuron methyl is 0.001 mg/L and the Limit of Detection (LOD) is 0.0005 mg/L for both MCPA and Tribenuron methyl

A.2.1.1.2.9 Determination of residues in water used in support of ecotoxicological studies. Study 9

A.2.1.1.2.9.1. Method Validation

Comments of zRMS:	The Applicant has not provided any data in this dossier. Not accepted.
Reference:	KCP 5.1.2/09
Report	MCPA + TRIBENURON METYL 565 SG Terrestial Plant Test: Vegetative Vigour Test, W. Dec, 2018, G/161/17, Institute of Industrial Organic Chemistry, Branch Pszczyna
Guideline(s):	Yes. According to the OECD Guideline No. 245 (2017)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

For study report please see section B9

A.2.1.1.2.10 Determination of residues in sucrose solution used in support of ecotoxicological studies. Study 10

A.2.1.1.2.10.1. Method Validation

Comments of zRMS:	Analytical part of the study is insufficiently described. Not accepted.
Reference:	KCP 5.1.2/10

Report	MCPA + Tribenuron Metyl 565 SG: Honeybees (*Apis mellifera L.*), Chronic Oral Toxic Test, 2019, B/26/18, P. Parma, Institute of Industrial Organic Chemistry, Branch Pszczyna
Guideline(s):	Yes (SANCO/3029/99 rev.4)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

For study report please see section B9

Sample preparation:

A sucrose solution sample of 1 g was weighted, then 2 mL 0.05% solution of ortho-phosphoric acid was added to the sample, and next the volume was made up to 10 ml mixture of acetonitrile: 0.05% solution of ortho-phosphoric acid (80:20 v/v). Finally, 20 µL of the solution was introduced into a chromatographic column. The sample was diluted with mixture of acetonitrile : 0.05% solution of ortho-phosphoric acid (80:20, v/v).

Determination:

The concentration of MCPA and tribenuron methyl in water was determined using the validated high performance liquid chromatographic method with UV-Vis detection.

The following liquid chromatography parameters were used:

chromatograph: Varian Performance Liquid Chromatography (HPLC)
column: Luna 5µ C18 100A, l = 250 mm, f = 4,6 mm
mobile phase: acetonitrile : 0.05% solution of orthophosphoric (V) acid (60 : 40, v/v),
wave length: 220 nm – MCPA; 231 nm – tribenuron methyl
flow rate: 1.0 mL/min.
injection volume: 20 µl
wave length: 220 nm – MCPA; 231 nm – tribenuron methyl

The analytical method was developed for the determination of MCPA + Tribenuron methyl 565 SG in sucrose solution. The range of linearity of the analytical graphs, specificity, precision, recovery, and limits of quantification and detection of analytes were determined. The method has a LOQ of 1 mg/kg and LOD = 0.3 mg/kg for tribenuron methyl in sucrose solution.

For more details please refer to the report.

A 2.1.1.3 Description of analytical methods for the determination of residues in plant matrices (KCP 5.2)

A 2.1.1.4 Determination of the residues of MCPA and tribenuron-methyl in grain and straw of wheat

A 2.1.1.4.1.1 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 MCPA and MCPB (MCPA and MCPB expressed as MCPA) was 0.0188 mg/kg grains and straw of wheat. The limit of detection (LOD) was 0.005 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.

The recovery results presented in the Table A 1. are improper. According to the report of the study results are presented below:

Table 4. Recovery and precision for MCPA and MCPB (MCPA and MCPB expressed as MCPA) in grains of wheat, obtained during model analyses

Fortification level [mg/kg]	*Determined concentration [mg/kg]*	*Average [mg/kg]*	*Recovery [%]*	*Relative standard deviation RSD [%]*
0.0000	< LOD < LOD	< LOD	-	-
0.0188	0.0168 0.0169 0.0164 0.0169 0.0170	0.0168	89.3	1.3
0.188	0.1732 0.1689 0.1700 0.1714 0.1696	0.1706	90.8	1.0

LOD = 0.0050 mg/kg

Table 6. Recovery and precision for MCPA and MCPB (MCPA and MCPB expressed as MCPA) in straw of wheat, obtained during model analyses.

Fortification level [mg/kg]	*Determined concentration [mg/kg]*	*Average [mg/kg]*	*Recovery [%]*	*Relative standard deviation RSD [%]*
0.000	< LOD < LOD	< LOD	-	-
0.010	0.0159 0.0169 0.0161 0.0170 0.0160	0.0164	87.1	3.1
0.100	0.1661 0.1650 0.1748 0.1700 0.1647	0.1681	89.4	2.6

LOD = 0.0050 mg/kg

Table 8. Recovery and precision for tribenuron-methyl in grains of wheat, obtained during model analyses.

Fortification level [mg/kg]	*Determined concentration [mg/kg]*	*Average [mg/kg]*	*Recovery [%]*	*Relative standard deviation RSD [%]*
0.000	< LOD < LOD	< LOD	-	-
0.010	0.0098 0.0093 0.0094 0.0086 0.0093	0.0093	92.9	4.5
0.100	0.0907 0.0948 0.0953 0.0893 0.0883	0.0917	91.7	3.5

LOD = 0.003 mg/kg

Table 10. Recovery and precision for tribenuron-methyl in straw of wheat, obtained dur model analyses.

Fortification level [mg/kg]	*Determined concentration [mg/kg]*	*Average [mg/kg]*	*Recovery [%]*	*Relative standard deviation RSD [%]*
0.000	< LOD < LOD	< LOD	-	-
0.010	0.0084 0.0087 0.0095 0.0087 0.0088	0.0088	88.3	4.9
0.100	0.0892 0.0864 0.0858 0.0899 0.0862	0.0875	87.5	2.2

LOD = 0.0030 mg/kg

Table 13. Results of the determination of the residues of MCPA in grains of wheat

Trial	*Sample code/ Specimen ID*	*Plot*	*Determined concentration MCPA and MCPB expressed as MCPA 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	0.0136 0.0135 0.0140	0.0137 (< LOQ)
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

LOQ = 0.0188 mg/kg
LOD = 0.005 mg/kg

Table 14. Results of the determination of the residues of MCPA in straw of wheat

Trial	*Sample code/ Specimen ID*	*Plot*	*Determined concentration MCPA and MCPB expressed as MCPA 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	0.0650 0.0649 0.0666	0.0655
S17-04789-02 (UK)	S17-04789-02-002A	U1	< LOD < LOD < LOD	< LOD
S17-04789-02 (UK)	S17-04789-02-004A	2	0.0321 0.0321 0.0327	0.0322
S17-04789-03 (Hungary)	S17-04789-03-002A	U1	< LOD < LOD < LOD	< LOD
S17-04789-03 (Hungary)	S17-04789-03-004A	2	0.1589 0.1570 0.1574	0.1578
SRPL-057-428HR (Poland)	SRPL-057-428HR-02	Plot 1	< LOD < LOD < LOD	< LOD
SRPL-057-428HR (Poland)	SRPL-057-428HR-04	Plot 2	0.0312 0.0318 0.0317	0.0316

LOQ = 0.0188 mg/kg
LOD = 0.005 mg/kg

Table 15. 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

LOD = 0.003 mg/kg

Table 16. 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

LOD = 0.0030 mg/kg

In the grain of wheat sample treated with MT-565SG-OR2-C, Trial S17-04789-03 (Hungary) Sample code S17-04789-03-003A Plot 2, 0.0137 mg MCPA and MCPB expressed as MCPA /kg was determined, The residues of MCPA and MCPB (MCPA and MCPB expressed as MCPA) in the other grain of wheat samples are below the limit of detection, i.e. 0.005 mg/kg. Hence, they are below

	the maximum residue limit, i.e. is 0.2 mg/kg of grain of wheat for MCPA and MCPB (MCPA, MCPB including their salts, esters and conjugates expressed as MCPA). 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.

Reference:	KCP 5.1.2/10
Report	MT-565SG-OR2-C determination of the residues of MCPA and tribenuron-methyl in grain and straw of wheat, C/05/17, M. Wójcik, 2018
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 involved the determination of the residues of MCPA and MCPB (MCPA and MCPB expressed as MCPA) and tribenuron-methyl in grains and straw of wheat treated with MT-565SG-OR2-C.
MCPA, MCPB and tribenuron-methyl was detected using liquid chromatographic method (HPLC) with LC-MS/MS.

Table A 6: Recovery results from method validation of analyte using the analytical method

~~Matrix~~	~~Analyte~~	~~Fortification level (mg/kg) (n = 2)~~	~~Mean recovery (%)~~	~~RSD (%)~~	~~Comments~~
~~Grains of wheat~~	~~MCPA and MCPB (expressed as MCPA)~~	~~0.0188~~ ~~0.188~~	~~100~~	~~2.4~~	~~-~~
	~~Tribenuron-methyl~~	~~0.0188~~ ~~0.188~~	~~97~~	~~2.1~~	~~-~~
~~Straw of wheat~~	~~MCPA and MCPB (expressed as MCPA)~~	~~0.0188~~ ~~0.188~~	~~100~~	~~2.5~~	~~-~~
	~~Tribenuron-methyl~~	~~0.0188~~ ~~0.188~~	~~94~~	~~2.1~~	~~-~~

Table A 7: Characteristics for the analytical method used for validation of MCPA, MCPB and tribenuron-methyl residues in plant matrix

	MCPA and MCPB	Tribenuron-methyl
Specificity	The specificity of the analytical method was determined on the basis of the analysis of chromatograms for control and fortified grains and straw of wheat samples.	The specificity of the analytical method was determined on the basis of the analysis of chromatograms for control and fortified grains and straw of wheat samples.
Calibration (type, number of data points)	These results meet the acceptance criteria of $r \geq 0.99$ (n=7)	The regression coefficient (r^2) was 0.9998284. These results meet the acceptance criteria of $r \geq 0.99$ (n=7)
Calibration range	The standard curves representing the relationship between the peak area and the concentration of MCPA and MCPB were linear. The range of linearity of the analytical graphs varied from 2.0 ng/mL to 200.0 ng/mL.	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 1.0 ng/mL to 100.0 ng/mL.
Assessment of matrix effects is presented	yes	yes
Limit of determination/quantification	The limit of quantification (LOQ) for MCPA and MCPB expressed as MCPA was 0.0188 mg/kg grains and straw of wheat. The limit of detection (LOD) was 0.005 mg/kg test item.	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 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.
The method was validated in accordance with EC Guidance Document SANCO/3029/99 rev. 4 and SANCO/825/00 rev 8.1

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 Method validation

Comments of zRMS:	The study has not been evaluated for plant matrices. The results presented below do not apply to the uses proposed in the GAP for HAKSAR TOP 565 SG.

Reference: KCP 5.2

Report: Analytical Method for the Determination of Phenoxy Acids and Their Corresponding 2 Ethyl-hexyl esters and glycine conjugates in cereal grain, straw and foliage, bovine muscle, fat, liver, kidney and milk, poultry eggs,

citrus fruit and olives and phenoxy acids and their corresponding 2 ethyl-hexyl esters in surface water, soil and air (Universal Method)
CEMAS Study #CAM-0004/003, L. Allen, 2014

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

Deviations: No

GLP: Yes

Acceptability: Yes

For details, materials and methods, chromatographic conditions please see A 2.1.2.6.1 and the study report.

Results and discussions

Recovery of 2,4-D in Olives

Matrix		Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)
Quantitation Transition m/z 218.8 → 161.0						
Olives	Acid	0.01*	91, 88, 85, 99, 95	5	92	6.1
		0.1	98, 103, 97, 104, 94	5	99	4.2
		Overall		10	95	6.4
	2EH Ester	0.01*	92, 85, 97, 93, 94	5	92	4.8
		0.1	112, 108, 98, 97, 102	5	103	6.3
		Overall		10	98	8.1
	Glycine conjugate	0.01*	101, 99, 68, 105, 102	5	95	16.1
		0.1	111, 107, 107, 93, 102	5	104	6.7
		Overall		10	100	12.2
Confirmatory Transition m/z 220.8 → 162.9						
Olives	Acid	0.01*	92, 84, 92, 90, 84	5	88	4.6
		0.1	93, 101, 91, 97, 89	5	94	5.1
		Overall		10	91	5.7
	2EH Ester	0.01*	89, 99, 97, 102, 93	5	96	5.3
		0.1	106, 108, 95, 91, 103	5	101	7.3
		Overall		10	98	6.5
	Glycine conjugate	0.01*	98, 98, 71, 102, 103	5	94	14.1
		0.1	109, 110, 110, 93, 100	5	104	7.3
		Overall		10	99	11.6

Residue in the control specimens and reagent blank was less than 30 % of the LOQ.
*Limit of Quantitation (LOQ)

Recovery of 2,4-DB in Olives

Matrix		Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)	Range (%)
Quantitation Transition m/z 247.0 → 161.0							
Olives	Acid	0.01*	80, 87, 89, 88, 81	5	85	4.9	80-89
		0.1	106, 90, 108, 103, 98	5	101	7.1	90-108
		Overall		10	93	10.9	80-108
	2EH Ester	0.01*	93, 102, 103, 79, 92	5	94	10.3	79-103
		0.1	94, 90, 98, 97, 78	5	91	8.9	78-98
		Overall		10	93	9.2	78-103
	Glycine conjugate	0.01*	101, 74, 59, 74, 79	5	77	19.6	59-101
		0.1	72, 88, 80, 78, 75	5	79	7.7	72-88
		Overall		10	78	14.0	59-101
Confirmatory Transition m/z 249.0 → 163.0							
Olives	Acid	0.01*	72, 97, 76, 75, 103	5	85	16.9	72-103
		0.1	101, 87, 109, 102, 95	5	99	8.4	87-109
		Overall		10	92	14.5	72-109
	2EH Ester	0.01*	105, 93, 114, 89, 95	5	99	10.2	89-114
		0.1	95, 89, 101, 100, 84	5	94	7.7	84-101
		Overall		10	97	9.1	84-114
	Glycine conjugate	0.01*	89, 84, 68, 78, 77	5	79	10.0	68-89
		0.1	66, 89, 75, 73, 75	5	76	11.1	66-89
		Overall		10	77	10.2	66-89

Residue in the control specimens and reagent blank was less than 30 % of the LOQ.
*Limit of Quantitation (LOQ)

Recovery of MCPA in Olives

Matrix		Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)
Quantitation Transition m/z 199.0 → 140.9						
Olives	Acid	0.01*	104, 107, 120, 114, 116	5	112	5.9
		0.1	113, 105, 113, 111, 110	5	110	3.0
		Overall		10	111	4.5
	2EH Ester	0.01*	118, 109, 114, 95, 109	5	109	8.0
		0.1	96, 96, 114, 112, 96	5	103	9.1
		Overall		10	106	8.6
	Glycine conjugate	0.01*	128, 108, 81, 117, 117	5	110	16.1
		0.1	112, 127, 117, 120, 114	5	118	5.0
		Overall		10	114	11.5
Confirmatory Transition m/z 200.9 → 142.9						
Olives	Acid	0.01*	98, 105, 107, 112, 108	5	106	4.9
		0.1	120, 107, 124, 107, 112	5	114	6.8
		Overall		10	110	6.8
	2EH Ester	0.01*	118, 107, 105, 96, 109	5	107	7.4
		0.1	100, 100, 110, 118, 94	5	104	9.1
		Overall		10	106	7.9
	Glycine conjugate	0.01*	127, 115, 75, 116, 123	5	111	18.7
		0.1	110, 126, 114, 112, 109	5	114	6.0
		Overall		10	113	13.1

Residue in the control specimens and reagent blank was less than 30 % of the LOQ.
*Limit of Quantitation (LOQ)

Recovery of MCPB in Olives

Matrix		Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)
Quantitation Transition m/z 227.0 → 140.9						
Olives	Acid	0.01*	79, 69, 86, 79, 74	5	77	8.2
		0.1	92, 93, 96, 98, 88	5	93	4.1
		Overall		10	85	11.4
	2EH Ester	0.01*	85, 79, 90, 86, 84	5	85	4.7
		0.1	115, 100, 85, 86, 89	5	95	13.3
		Overall		10	90	11.5
	Glycine conjugate	0.01*	82, 77, 64, 68, 81	5	74	10.8
		0.1	81, 78, 82, 74, 75	5	78	4.5
		Overall		10	76	8.1
Confirmatory Transition m/z 229.0 → 142.9						
Olives	Acid	0.01*	88, 75, 76, 89, 68	5	79	11.4
		0.1	95, 95, 94, 91, 87	5	92	3.7
		Overall		10	86	11.1
	2EH Ester	0.01*	78, 79, 74, 88, 78	5	79	6.5
		0.1	112, 92, 87, 89, 91	5	94	10.8
		Overall		10	87	12.5
	Glycine conjugate	0.01*	82, 78, 62, 76, 81	5	76	10.7
		0.1	78, 80, 83, 72, 73	5	77	6.0
		Overall		10	77	8.2

Residue in the control specimens and reagent blank was less than 30 % of the LOQ.
*Limit of Quantitation (LOQ)

Recovery of Mecoprop-P in Olives

Matrix		Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)
Quantitation Transition m/z 227.0 → 140.9						
Olives	Acid	0.01*	79, 69, 86, 79, 74	5	77	8.2
		0.1	92, 93, 96, 98, 88	5	93	4.1
		Overall		10	85	11.4
	2EH Ester	0.01*	85, 79, 90, 86, 84	5	85	4.7
		0.1	115, 100, 85, 86, 89	5	95	13.3
		Overall		10	90	11.5
	Glycine conjugate	0.01*	82, 77, 64, 68, 81	5	74	10.8
		0.1	81, 78, 82, 74, 75	5	78	4.5
		Overall		10	76	8.1
Confirmatory Transition m/z 229.0 → 142.9						
Olives	Acid	0.01*	88, 75, 76, 89, 68	5	79	11.4
		0.1	95, 95, 94, 91, 87	5	92	3.7
		Overall		10	86	11.1
	2EH Ester	0.01*	78, 79, 74, 88, 78	5	79	6.5
		0.1	112, 92, 87, 89, 91	5	94	10.8
		Overall		10	87	12.5
	Glycine conjugate	0.01*	82, 78, 62, 76, 81	5	76	10.7
		0.1	78, 80, 83, 72, 73	5	77	6.0
		Overall		10	77	8.2

Residue in the control specimens and reagent blank was less than 30 % of the LOQ.
*Limit of Quantitation (LOQ)

Recovery of Mecoprop-P in Olives

Matrix		Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)
Quantitation Transition m/z 212.9 → 140.9						
Olives	Acid	0.01*	106, 96, 102, 103, 101	5	102	3.6
		0.1	99, 100, 108, 99, 101	5	101	3.7
		Overall		10	102	3.5
	2EH Ester	0.01*	87, 93, 91, 87, 86	5	89	3.4
		0.1	101, 95, 87, 86, 86	5	91	7.4
		Overall		10	90	5.6
	Glycine conjugate	0.01*	106, 103, 96, 101, 102	5	102	3.6
		0.1	114, 109, 106, 100, 101	5	106	5.5
		Overall		10	104	4.9
Confirmatory Transition m/z 215.0 → 142.9						
Olives	Acid	0.01*	96, 94, 101, 98, 94	5	97	3.1
		0.1	99, 101, 104, 102, 96	5	100	3.0
		Overall		10	99	3.5
	2EH Ester	0.01*	89, 89, 82, 96, 79	5	87	7.7
		0.1	108, 99, 85, 89, 89	5	94	10.0
		Overall		10	91	9.4
	Glycine conjugate	0.01*	107, 104, 96, 109, 102	5	104	4.9
		0.1	109, 109, 105, 101, 98	5	104	4.7
		Overall		10	104	4.5

Residue in the control specimens and reagent blank was less than 30 % of the LOQ.
*Limit of Quantitation (LOQ)

Recovery of Dichloroprop-P in Olives

Matrix		Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)
Quantitation Transition m/z 232.9 → 160.8						
Olives	Acid	0.01*	84, 94, 96, 109, 89	5	94	10.0
		0.1	104, 105, 108, 110, 99	5	105	4.0
		Overall		10	100	8.9
	2EH Ester	0.01*	104, 93, 104, 103, 98	5	100	4.8
		0.1	103, 106, 97, 103, 99	5	102	3.5
		Overall		10	101	4.0
	Glycine conjugate	0.01*	90, 90, 70, 96, 91	5	87	11.5
		0.1	96, 100, 97, 91, 90	5	95	4.4
		Overall		10	91	9.0
Confirmatory Transition m/z 234.9 → 162.8						
Olives	Acid	0.01*	107, 103, 100, 102, 104	5	103	2.5
		0.1	106, 107, 112, 105, 102	5	106	3.4
		Overall		10	105	3.3
	2EH Ester	0.01*	105, 96, 101, 97, 100	5	100	3.6
		0.1	104, 102, 95, 95, 96	5	98	4.3
		Overall		10	99	3.8
	Glycine conjugate	0.01*	87, 82, 66, 87, 88	5	82	11.3
		0.1	89, 97, 92, 86, 90	5	91	4.5
		Overall		10	86	9.5

Residue in the control specimens and reagent blank was less than 30 % of the LOQ.
*Limit of Quantitation (LOQ)

Recovery of 2,4-D in Orange (Whole)

Matrix		Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)
Quantitation Transition m/z 218.8 → 161.0						
Orange (Whole)	Acid	0.01*	92, 90, 93, 91, 99	5	93	3.8
		0.1	89, 91, 96, 91, 97	5	93	3.8
		Overall		10	93	3.6
	2EH Ester	0.01*	90, 92, 98, 90, 88	5	92	4.2
		0.1	90, 91, 95, 96, 93	5	93	2.7
		Overall		10	92	3.4
	Glycine conjugate	0.01*	87, 94, 91, 85, 90	5	89	3.9
		0.1	97, 91, 92, 91, 92	5	93	2.7
		Overall		10	91	3.7
Confirmatory Transition m/z 220.8 → 162.9						
Orange (Whole)	Acid	0.01*	96, 88, 92, 91, 95	5	92	3.5
		0.1	89, 93, 98, 92, 97	5	94	3.9
		Overall		10	93	3.6
	2EH Ester	0.01*	97, 93, 95, 96, 101	5	96	3.1
		0.1	91, 93, 91, 95, 93	5	93	1.8
		Overall		10	95	3.2
	Glycine conjugate	0.01*	85, 85, 86, 88, 92	5	87	3.4
		0.1	94, 92, 90, 91, 88	5	91	2.5
		Overall		10	89	3.6

Residue in the control specimens and reagent blank was less than 30 % of the LOQ.
*Limit of Quantitation (LOQ)

Recovery of 2,4-DB in Orange (Whole)

Matrix		Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)
Quantitation Transition m/z 247.0 → 161.0						
Orange (Whole)	Acid	0.01*	96, 80, 77, 85, 89	5	85	8.8
		0.1	89, 98, 94, 94, 101	5	95	4.8
		Overall		10	90	8.6
	2EH Ester	0.01*	94, 101, 93, 90, 81	5	92	7.9
		0.1	96, 91, 99, 95, 91	5	94	3.6
		Overall		10	93	5.9
	Glycine conjugate	0.01*	109, 113, 108, 98, 97	5	105	6.8
		0.1	86, 97, 91, 100, 96	5	94	5.9
		Overall		10	100	8.4
Confirmatory Transition m/z 249.0 → 163.0						
Orange (Whole)	Acid	0.01*	110, 112, 109, 101, 87	5	104	9.8
		0.1	93, 90, 95, 94, 100	5	94	3.9
		Overall		10	99	8.9
	2EH Ester	0.01*	103, 77, 72, 102, 74	5	86	18.1
		0.1	93, 95, 106, 94, 93	5	96	5.8
		Overall		10	91	13.6
	Glycine conjugate	0.01*	95, 95, 95, 110, 110	5	101	8.1
		0.1	91, 100, 89, 98, 100	5	96	5.5
		Overall		10	98	7.2

Residue in the control specimens and reagent blank was less than 30 % of the LOQ.
*Limit of Quantitation (LOQ)

Recovery of MCPA in Orange (Whole)

Matrix		Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)
Quantitation Transition m/z 199.0 → 140.9						
Orange (Whole)	Acid	0.01*	91, 90, 94, 87, 95	5	91	3.5
		0.1	95, 99, 98, 98, 98	5	98	1.6
		Overall		10	95	4.3
	2EH Ester	0.01*	94, 90, 91, 93, 95	5	93	2.2
		0.1	98, 102, 100, 104, 99	5	101	2.4
		Overall		10	97	4.9
	Glycine conjugate	0.01*	89, 91, 89, 87, 93	5	90	2.5
		0.1	95, 96, 96, 102, 95	5	97	3.0
		Overall		10	93	4.8
Confirmatory Transition m/z 200.9 → 142.9						
Orange (Whole)	Acid	0.01*	95, 94, 93, 93, 88	5	93	2.9
		0.1	90, 97, 94, 98, 96	5	95	3.3
		Overall		10	94	3.2
	2EH Ester	0.01*	103, 113, 80, 96, 109	5	100	13.0
		0.1	97, 102, 100, 100, 99	5	100	1.8
		Overall		10	100	8.8
	Glycine conjugate	0.01*	83, 100, 83, 97, 88	5	90	8.8
		0.1	97, 96, 94, 100, 94	5	96	2.6
		Overall		10	93	6.8

Residue in the control specimens and reagent blank was less than 30 % of the LOQ.
*Limit of Quantitation (LOQ)

Recovery of MCPB in Orange (Whole)

Matrix		Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)
Quantitation Transition m/z 199.0 → 140.9						
Orange (Whole)	Acid	0.01*	91, 90, 94, 87, 95	5	91	3.5
		0.1	95, 99, 98, 98, 98	5	98	1.6
		Overall		10	95	4.3
	2EH Ester	0.01*	94, 90, 91, 93, 95	5	93	2.2
		0.1	98, 102, 100, 104, 99	5	101	2.4
		Overall		10	97	4.9
	Glycine conjugate	0.01*	89, 91, 89, 87, 93	5	90	2.5
		0.1	95, 96, 96, 102, 95	5	97	3.0
		Overall		10	93	4.8
Confirmatory Transition m/z 200.9 → 142.9						
Orange (Whole)	Acid	0.01*	95, 94, 93, 93, 88	5	93	2.9
		0.1	90, 97, 94, 98, 96	5	95	3.3
		Overall		10	94	3.2
	2EH Ester	0.01*	103, 113, 80, 96, 109	5	100	13.0
		0.1	97, 102, 100, 100, 99	5	100	1.8
		Overall		10	100	8.8
	Glycine conjugate	0.01*	83, 100, 83, 97, 88	5	90	8.8
		0.1	97, 96, 94, 100, 94	5	96	2.6
		Overall		10	93	6.8

Residue in the control specimens and reagent blank was less than 30 % of the LOQ.
*Limit of Quantitation (LOQ)

Recovery of Mecoprop-P in Orange (Whole)

Matrix		Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)
Quantitation Transition m/z 199.0 → 140.9						
Orange (Whole)	Acid	0.01*	91, 90, 94, 87, 95	5	91	3.5
		0.1	95, 99, 98, 98, 98	5	98	1.6
		Overall		10	95	4.3
	2EH Ester	0.01*	94, 90, 91, 93, 95	5	93	2.2
		0.1	98, 102, 100, 104, 99	5	101	2.4
		Overall		10	97	4.9
	Glycine conjugate	0.01*	89, 91, 89, 87, 93	5	90	2.5
		0.1	95, 96, 96, 102, 95	5	97	3.0
		Overall		10	93	4.8
Confirmatory Transition m/z 200.9 → 142.9						
Orange (Whole)	Acid	0.01*	95, 94, 93, 93, 88	5	93	2.9
		0.1	90, 97, 94, 98, 96	5	95	3.3
		Overall		10	94	3.2
	2EH Ester	0.01*	103, 113, 80, 96, 109	5	100	13.0
		0.1	97, 102, 100, 100, 99	5	100	1.8
		Overall		10	100	8.8
	Glycine conjugate	0.01*	83, 100, 83, 97, 88	5	90	8.8
		0.1	97, 96, 94, 100, 94	5	96	2.6
		Overall		10	93	6.8

Residue in the control specimens and reagent blank was less than 30 % of the LOQ.
*Limit of Quantitation (LOQ)

Recovery of Mecoprop-P in Orange (Whole)

Matrix		Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)
Quantitation Transition m/z 212.9 → 140.9						
Orange (Whole)	Acid	0.01*	91, 85, 95, 90, 98	5	92	5.4
		0.1	97, 97, 100, 99, 100	5	99	1.5
		Overall		10	95	5.2
	2EH Ester	0.01*	99, 93, 91, 89, 90	5	92	4.3
		0.1	94, 96, 102, 99, 96	5	97	3.2
		Overall		10	95	4.5
	Glycine conjugate	0.01*	98, 96, 94, 96, 98	5	96	1.7
		0.1	99, 97, 98, 99, 99	5	98	0.9
		Overall		10	97	1.7
Confirmatory Transition m/z 215.0 → 142.9						
Orange (Whole)	Acid	0.01*	95, 91, 91, 96, 100	5	95	4.0
		0.1	93, 96, 98, 96, 96	5	96	1.9
		Overall		10	95	3.0
	2EH Ester	0.01*	103, 100, 91, 88, 99	5	96	6.6
		0.1	93, 93, 98, 101, 97	5	96	3.6
		Overall		10	96	5.0
	Glycine conjugate	0.01*	100, 95, 99, 89, 96	5	96	4.5
		0.1	99, 95, 95, 102, 96	5	97	3.1
		Overall		10	97	3.8

Residue in the control specimens and reagent blank was less than 30 % of the LOQ.
*Limit of Quantitation (LOQ)

Recovery of Dichloroprop-P in Orange (Whole)

Matrix		Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)
Quantitation Transition m/z 232.9 → 160.8						
Orange (Whole)	Acid	0.01*	98, 89, 92, 92, 98	5	94	4.3
		0.1	93, 97, 98, 96, 99	5	97	2.4
		Overall		10	95	3.6
	2EH Ester	0.01*	101, 94, 93, 91, 104	5	97	5.8
		0.1	100, 100, 102, 107, 104	5	103	2.9
		Overall		10	100	5.3
	Glycine conjugate	0.01*	96, 93, 93, 88, 95	5	93	3.3
		0.1	90, 90, 92, 93, 86	5	90	3.0
		Overall		10	92	3.4
Confirmatory Transition m/z 234.9 → 162.8						
Orange (Whole)	Acid	0.01*	92, 89, 93, 87, 90	5	90	2.6
		0.1	96, 97, 95, 99, 100	5	97	2.1
		Overall		10	94	4.6
	2EH Ester	0.01*	100, 94, 92, 92, 100	5	96	4.3
		0.1	98, 99, 104, 105, 101	5	101	3.0
		Overall		10	99	4.6
	Glycine conjugate	0.01*	91, 86, 89, 96, 86	5	90	4.6
		0.1	91, 91, 91, 91, 89	5	91	1.0
		Overall		10	90	3.2

Residue in the control specimens and reagent blank was less than 30 % of the LOQ.
*Limit of Quantitation (LOQ)

Table A 3: Characteristics for the analytical method used for validation of MCPA and its metabolites residues in plant matrix

	MCPA and its metabolites
Specificity	The analytical method developed for the determination of phenoxy acids in surface water has been shown to be highly specific due to the instrumentation used (LC-MS/MS) and the detection of two characteristic isotopic mass transitions. There were no components present in the control that interfered with the analysis at levels above 30% of the limit of quantitation.
Calibration (type, number of data points)	The LC-MS/MS responses were shown to be linear throughout the entire study (correlation coefficients (r) > 0.99), for more details and every calibration graph please refer to the study report.
Calibration range	The calibration was performed using calibration solutions (9 concentrations), for more details and every calibration graph please refer to the study report.
Assessment of matrix effects is presented	yes
Limit of determination/quantification	The LOQ is 0.01 mg/kg

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 orange and olives with the tested LOQ of 0.01 mg/kg.

A 2.1.2.1.2 Independent laboratory validation

Comments of zRMS:	There is no adequate description of the study to allow for assessment. Not accepted.

Reference:	KCP 5.2
Report	Phenoxy Herbicides - Independent Laboratory Validation of the Analytical Method CAM-0004/002 for the Determination of Phenoxy Acids and Their Corresponding 2 Ethyl-Hexyl Esters and Glycine Conjugates in six matrices by LC-MS/MS (Universal Method) Eurofins Agroscience Services Chem Ltd Study #S14-00286, G. Watson, 2014
Guideline(s):	Yes (SANCO/825/00, rev. 8.1)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The purpose of the study was to carry out an Independent Laboratory Validation (ILV) of the analytical method for the determination of the phenoxy acid 2,4-D, 2,4-DB, MCPA, MCPB, Mecoprop-P and Dichloroprop-P (as the total phenoxy acid, when present in the matrix as the acid or ester) in muscle and fat, liver, eggs, citrus fruit, olives a limit of Quantitation (LOQ) of 0.01 mg/kg.
In brief, the method involves hydrolysis of samples overnight in a strong solution of sodium hydroxide to convert the ethyl-hexyl esters and glycine conjugates back to the parent acid for quantification.
The hydrolysed samples are acidified and analytes extracted into acetonitrile using QuEChERS. An aliquot of the acetonitrile is diluted acidified water before analysis by LC-MS/MS.

The method was successfully validated at the second attempt for all matrixes.
The ILV was carried out in two separate batches each consisting of a reagent blank, 2 control specimens, 5 recoveries fortified at the LOQ and 5 recoveries fortified at x10 LOQ for Mecoprop-P).One batch was fortified with the acid and one batch was fortified with corresponding 2 ethyl-hexyl ester and one batch was fortified with the corresponding glycine conjugate. In each case residues were quantified as the parent acid residue. Where recoveries were fortified with the 2 ethyl-hexyl ester or glycine conjugate, they were fortified in an equimolar concentration to the intended acid concentration (acid equivalent).

For more details please refer to the report.

Characteristics for the analytical method used for independent laboratory validation of MCPA and its metabolites residues in drinking water

	MCPA and its metabolites
Specificity	A reagent blank and two control specimens were extracted and analysed to investigate the presence of analyte residue and/or background interference at the analyte retention time. For both mass transistions, no significant interferences above 30% of the LOQ were detected at the retention time of 2,4-D, 2,4-DB, MCPA, MCPB, Mecoprop-P or Dichloroprop-P in bovine muscle and fat, liver, poultry eggs, citrus fruit, olives each mass transistion is specific to each analyte.
Calibration (type, number of data points)	The linearity of the detector was checked by single determination of calibration standards at 9 concentration levels ranging from 0.6 ng/mL to 200 ng/mL. This calibrationrange is equivalent to 30% of the LOQ up to 1.0 µg/L, The calibration curves obtained for both primary and confirmatory transitions for each analyte combination were linear with

	MCPA and its metabolites
	coefficient correlation grater than 0.990.
Calibration range	An appropriate calibration curve was prepared by plotting the peak area ratio (analyte peak area / internal standard peak area) versus concentration (ng/mL). Using 1/x weighted linear regression, the equation of the line and correletion coefficients greater than 0.990.
Assessment of matrix effects is presented	yes
Limit of determination/quantification	The LOQ in bovine muscle and fat, liver, poultry eggs, citrus fruit, olives is 0.01 mg/kg.

Conclusions

The analytical method showed good accuracy and precision for 2,4-D, 2,4-DB, MCPA, MCPB and Dichloroprop-P in bovine muscle and fat, liver, poultry eggs, citrus fruit, olives in the range 60-120% with the RSD of < 30% for the LOQ level.
The method is acceptable as ILV for the primary method, was successfully validated.

A 2.1.2.1.3 Confirmatory method

No confirmatory method is required.

A 2.1.2.2.1 Method validation

Comments of zRMS:	The study is accepted. The method is adequate for the determination of tribenuron-methyl in tomato, avocado, orange and wheat (straw, grain and whole plant) with the LOQ of 0.01 mg/kg. All mean recovery values at fortification levels of 0.01 mg/kg (n=5) and 0.1 mg/kg (n=5) for two mass transitions are within 70 – 110 % with RSD < 20 % and thereby comply with the standard acceptance criteria of the guidance document SANCO/825/00, rev. 8.1.

Reference:	KCP 5.2
Report	Development and Validation of an Analytical Method for Determination of Tribenuron-methyl in Plant Matrices,Roth 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 2.50 / 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 8 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	

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
		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 5 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 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 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.

	Quantification	Qualification
Tomato	y = 1.07e+006 x + 1.19e+004	y = 7.32e+005 x + 6.52e+003
Orange	y = 1e+006 x + 8.55e+003	y = 6.73e+005 x + 5.07e+003
Avocado	y = 7.74e+005 x + 3.06e+003	y = 5.19e+005 x + 516

	Wheat straw	y = 2.88e+005 x + 2.04e+003	y = 1.92e+005 x + 2.71e+003
	Wheat grain	y = 1.01e+006 x + 3.96e+003	y = 6.87e+005 x + 563
	Wheat whole plant	y = 7.09e+005 x + 2.72e+003	y = 4.84e+005 x + -2.67e+003
Calibration range	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		
Assessment of matrix effects is presented	yes		
Limit of determination/quantification	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.		

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 2.1.2.2.2. Independent laboratory validation

Comments of zRMS:	The method was successfully and independently validated for the determination of tribenuron-methyl in tomato fruit and wheat grain with LOQ of 0.01 mg/kg according to the guidance document SANCO/825/00, rev. 8.1.

Reference:	KCP 5.2
Report	Independent Laboratory validation of a Multi-Residue MEthod QuEChERS for the Determination of Tribenuron-methyl in Two Matrices of Plant Origin, Schmiedt 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 6 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 7 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: Quantification; m/z 396→155: Tomato: y = 1.31e+006 x – 2.5e+004; Wheat: y = 1.77e+006 x – 2.13e+004 Confirmation; m/z 396→181: Tomato: y = 5.77e+005 x – 1.15e+004; Wheat: 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
Limit of determination/quantification	LOQ of 0.01 mg/kg was confirmed for tribenuron-methyl in tomato fruit and wheat grain.

	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.
	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 2.1.2.2.3 Confirmatory method

No confirmatory method is required

A 2.1.2.3.1 Description of analytical methods for the determination of MCPA and its metabolites in animal matrices (KCP 5.2)

Comments of zRMS:	The study is accepted. The study describes the procedure for the determination of the total phenoxy acid present in cereal grain, straw, foliage, bovine muscle, fat, liver, kidney, milk, poultry eggs, citrus fruit and olives, whether in the form of the acid, ester (eg. ethyl-hexyl) or conjugate (eg. glycine) and the total phenoxy acid present in surface water, soil and air, whether in the form of the acid or ester (eg. ethyl-hexyl). During the extraction procedure samples are hydrolysed to convert the ethyl-hexyl esters and glycine conjugates back to the parent acid for quantitation. The analysis is performed using a hydrolysis reaction, QuEChERS extraction and determination by LC-MS/MS detection. The LOQ for all analytes in cereal grain, straw, foliage, bovine muscle, fat, liver, kidney, milk, poultry eggs, citrus fruit, olives and soil is 0.01 mg/kg, the LOQ for all analytes in air is 0.05 µg/tube, the LOQ for 2,4-D, 2,4-DB, MCPA, MCPB and Dichloroprop-P in surface water is 0.01 µg/L and the LOQ for Mecoprop-P in surface water is 0.02 µg/L. Samples are hydrolysed overnight in a strong aqueous solution of sodium hydroxide to convert the ethyl-hexyl esters and glycine conjugates back to the parent acid for quantitation. The hydrolysed samples are acidified and, with the exception of the water extraction procedure where QuEChERS is not required, analytes extracted into acetonitrile using QuEChERS before being concentrated for analysis. The reverse phase LC-MS/MS setup uses a monolithic column and flow split to optimise sensitivity. This analytical method was successfully validated to a limit of quantitation (LOQ) of 0.01 mg/kg for all analytes in cereal grain, straw, foliage, bovine muscle, fat,

	liver, kidney, milk, poultry eggs, citrus fruit, olives and soil, an LOQ of 0.05 µg/tube for all analytes in air, an LOQ of 0.01 µg/mL for 2,4-D, 2,4-DB, MCPA, MCPB and Dichloroprop-P in surface water and an LOQ of 0.02 µg/mL for Mecoprop-P in surface water under the CEMAS GLP Studies CEMS-6228 (for cereal grain, straw and foliage), CEMS-6229 (for bovine muscle, fat, liver, kidney, milk, poultry eggs, citrus fruit and olives) and CEMS-6230 (for surface water, soil and air). All of the validation criteria set out in the guidelines were met. No GLP compliance is claimed for this analytical method. This method has been validated under the CEMAS GLP Studies CEMS-6228, CEMS-6229 and CEMS-6230.

Reference:	KCP 5.2
Report	Analytical Method for the Determination of Phenoxy Acids and Their Corresponding 2 Ethyl-hexyl esters and glycine conjugates in cereal grain, straw and foliage, bovine muscle, fat, liver, kidney and milk, poultry eggs, citrus fruit and olives and phenoxy acids and their corresponding 2 ethyl-hexyl esters in surface water, soil and air (Universal Method) CEMAS Study #CAM-0004/003, L. Allen, 2014
Guideline(s):	Yes (SANCO/825/00, rev. 8.1); (SANCO 3029/99, rev.4)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

For details, materials and methods, chromatographic conditions please see A 2.1.2.6.1 and the study report.

Results and discussions

Recovery of 2,4-D in Eggs (Poultry)

Matrix		Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)
Quantitation Transition m/z 218.8 → 161.0						
Eggs (Poultry)	Acid	0.01*	96, 99, 93, 96, 86	5	94	5.3
		0.1	96, 99, 87, 89, 96	5	93	5.5
		Overall		10	94	5.1
	2EH Ester	0.01*	83, 91, 85, 84, 89	5	86	4.0
		0.1	88, 85, 93, 88, 87	5	88	3.3
		Overall		10	87	3.6
	Glycine conjugate	0.01*	71, 75, 77, 72, 80	5	75	4.9
		0.1	80, 85, 81, 84, 85	5	83	2.8
		Overall		10	79	6.5
Confirmatory Transition m/z 220.8 → 162.9						
Eggs (Poultry)	Acid	0.01*	93, 101, 89, 87, 95	5	93	5.9
		0.1	93, 97, 89, 91, 96	5	93	3.6
		Overall		10	93	4.6
	2EH Ester	0.01*	85, 90, 76, 81, 82	5	83	6.2
		0.1	88, 85, 93, 90, 84	5	88	4.2
		Overall		10	85	5.9
	Glycine conjugate	0.01*	78, 77, 85, 80, 80	5	80	3.9
		0.1	81, 85, 82, 82, 82	5	82	1.8
		Overall		10	81	3.2

Residue in the control specimens and reagent blank was less than 30 % of the LOQ.
*Limit of Quantitation (LOQ)

Recovery of 2,4-DB in Eggs (Poultry)

Matrix		Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)
Quantitation Transition m/z 247.0 → 161.0						
Eggs (Poultry)	Acid	0.01*	93, 89, 89, 93, 74	5	88	9.0
		0.1	99, 106, 95, 98, 91	5	98	5.7
		Overall		10	93	9.0
	2EH Ester	0.01*	93, 102, 82, 85, 78	5	88	10.9
		0.1	99, 92, 95, 97, 105	5	98	5.0
		Overall		10	93	9.4
	Glycine conjugate	0.01*	81, 93, 80, 90, 77	5	84	8.2
		0.1	83, 82, 80, 83, 75	5	81	4.2
		Overall		10	82	6.6
Confirmatory Transition m/z 249.0 → 163.0						
Eggs (Poultry)	Acid	0.01*	85, 98, 82, 100, 82	5	90	10.2
		0.1	99, 101, 93, 98, 96	5	97	3.1
		Overall		10	94	8.2
	2EH Ester	0.01*	77, 100, 95, 98, 85	5	91	10.7
		0.1	89, 94, 89, 89, 98	5	92	4.5
		Overall		10	91	7.7
	Glycine conjugate	0.01*	73, 93, 95, 79, 81	5	84	11.2
		0.1	86, 85, 82, 82, 74	5	82	5.8
		Overall		10	83	8.6

Residue in the control specimens and reagent blank was less than 30 % of the LOQ.
*Limit of Quantitation (LOQ)

Recovery of MCPA in Eggs (Poultry)

Matrix		Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)
Quantitation Transition m/z 199.0 → 140.9						
Eggs (Poultry)	Acid	0.01*	101, 87, 96, 102, 89	5	95	7.2
		0.1	100, 111, 100, 100, 94	5	101	6.1
		Overall		10	98	7.0
	2EH Ester	0.01*	100, 98, 93, 85, 88	5	93	6.9
		0.1	104, 103, 105, 103, 103	5	104	0.9
		Overall		10	98	7.3
	Glycine conjugate	0.01*	89, 95, 92, 93, 98	5	93	3.6
		0.1	94, 94, 96, 93, 95	5	94	1.2
		Overall		10	94	2.6
Confirmatory Transition m/z 200.9 → 142.9						
Eggs (Poultry)	Acid	0.01*	106, 95, 107, 104, 86	5	100	9.0
		0.1	93, 108, 97, 98, 91	5	97	6.8
		Overall		10	99	7.6
	2EH Ester	0.01*	94, 93, 85, 87, 88	5	89	4.4
		0.1	101, 99, 104, 103, 99	5	101	2.3
		Overall		10	95	7.3
	Glycine conjugate	0.01*	91, 95, 94, 98, 89	5	93	3.8
		0.1	96, 96, 92, 92, 94	5	94	2.1
		Overall		10	94	2.9

Residue in the control specimens and reagent blank was less than 30 % of the LOQ.
*Limit of Quantitation (LOQ)

Recovery of MCPB in Eggs (Poultry)

Matrix		Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)
Quantitation Transition m/z 227.0 → 140.9						
Eggs (Poultry)	Acid	0.01*	92, 101, 90, 103, 94	5	96	5.9
		0.1	94, 96, 88, 89, 91	5	92	3.7
		Overall		10	94	5.3
	2EH Ester	0.01*	87, 87, 85, 83, 96	5	88	5.7
		0.1	89, 88, 92, 85, 95	5	90	4.3
		Overall		10	89	4.9
	Glycine conjugate	0.01*	71, 79, 78, 79, 74	5	76	4.7
		0.1	81, 75, 77, 71, 78	5	76	4.9
		Overall		10	76	4.5
Confirmatory Transition m/z 229.0 → 142.9						
Eggs (Poultry)	Acid	0.01*	96, 84, 92, 92, 94	5	92	5.0
		0.1	90, 97, 87, 92, 92	5	92	4.0
		Overall		10	92	4.3
	2EH Ester	0.01*	89, 67, 71, 81, 86	5	79	12.1
		0.1	95, 91, 97, 89, 92	5	93	3.4
		Overall		10	86	11.6
	Glycine conjugate	0.01*	61, 76, 59, 68, 65	5	66**	10.2
		0.1	82, 75, 79, 73, 79	5	78	4.6
		Overall		10	72	11.2

Residue in the control specimens and reagent blank was less than 30 % of the LOQ.

Recovery of Mecoprop-P in Eggs (Poultry)

Matrix		Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)
Quantitation Transition m/z 212.9 → 140.9						
Eggs (Poultry)	Acid	0.01*	87, 87, 82, 94, 87	5	87	4.9
		0.1	98, 106, 95, 95, 100	5	99	4.6
		Overall		10	93	7.9
	2EH Ester	0.01*	93, 99, 89, 92, 95	5	94	4.0
		0.1	93, 93, 97, 92, 98	5	95	2.9
		Overall		10	94	3.3
	Glycine conjugate	0.01*	90, 84, 89, 88, 91	5	88	3.1
		0.1	91, 94, 86, 92, 94	5	91	3.6
		Overall		10	90	3.6
Confirmatory Transition m/z 215.0 → 142.9						
Eggs (Poultry)	Acid	0.01*	92, 86, 90, 98, 83	5	90	6.4
		0.1	97, 105, 92, 93, 100	5	97	5.5
		Overall		10	94	7.0
	2EH Ester	0.01*	90, 92, 92, 89, 82	5	89	4.6
		0.1	93, 96, 97, 91, 98	5	95	3.1
		Overall		10	92	5.0
	Glycine conjugate	0.01*	93, 96, 94, 98, 98	5	96	2.4
		0.1	94, 93, 92, 90, 91	5	92	1.7
		Overall		10	94	2.9

Residue in the control specimens and reagent blank was less than 30 % of the LOQ.
*Limit of Quantitation (LOQ)

Recovery of Dichloroprop-P in Eggs (Poultry)

Matrix		Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)
Quantitation Transition m/z 232.9 → 160.8						
Eggs (Poultry)	Acid	0.01*	85, 94, 92, 97, 86	5	91	5.7
		0.1	100, 107, 96, 98, 104	5	101	4.4
		Overall		10	96	7.3
	2EH Ester	0.01*	92, 98, 87, 90, 89	5	91	4.6
		0.1	101, 98, 102, 97, 101	5	100	2.2
		Overall		10	96	5.8
	Glycine conjugate	0.01*	81, 82, 80, 75, 80	5	80	3.4
		0.1	85, 87, 80, 83, 86	5	84	3.3
		Overall		10	82	4.3
Confirmatory Transition m/z 234.9 → 162.8						
Eggs (Poultry)	Acid	0.01*	90, 89, 100, 101, 86	5	93	7.3
		0.1	98, 105, 94, 96, 100	5	99	4.3
		Overall		10	96	6.3
	2EH Ester	0.01*	93, 87, 90, 89, 83	5	88	4.2
		0.1	95, 95, 102, 94, 95	5	96	3.4
		Overall		10	92	5.7
	Glycine conjugate	0.01*	78, 81, 86, 75, 75	5	79	5.9
		0.1	84, 87, 81, 82, 89	5	85	4.0
		Overall		10	82	4.8

Residue in the control specimens and reagent blank was less than 30 % of the LOQ.
*Limit of Quantitation (LOQ)

Recovery of 2,4-D in Fat (Bovine)

Matrix		Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)	Range (%)
Quantitation Transition m/z 218.8 → 161.0							
Fat (Bovine)	Acid	0.01*	111, 103, 104, 122, 111	5	110	6.9	103-122
		0.1	100, 106, 200**, 102, 100	4	102	2.8	100-106
		Overall		9	107	6.7	100-122
	2EH Ester	0.01*	69, 102, 101, 101, 100	5	95	15.1	69-102
		0.1	106, 113, 108, 111, 104	5	108	3.4	104-113
		Overall		10	102	12.1	69-113
	Glycine conjugate	0.01*	102, 109, 98, 108, 94	5	102	6.3	94-109
		0.1	106, 106, 107, 101, 113	5	107	4.0	101-113
		Overall		10	104	5.4	94-113
Confirmatory Transition m/z 220.8 → 162.9							
Fat (Bovine)	Acid	0.01*	121, 104, 106, 113, 113	5	111	6.0	104-121
		0.1	96, 107, 220**, 108, 110	4	105	6.0	96-110
		Overall		9	109	6.4	96-121
	2EH Ester	0.01*	61, 100, 102, 102, 102	5	99	4.8	61-102
		0.1	107, 115, 108, 111, 105	5	109	3.6	105-115
		Overall		10	104	6.3	61-115
	Glycine conjugate	0.01*	90, 92, 94, 89, 88	5	91	2.7	88-94
		0.1	103, 108, 107, 102, 103	5	105	2.6	102-108
		Overall		10	98	8.0	88-108

Residue in the control specimens and reagent blank was less than 30 % of the LOQ.
*Limit of Quantitation (LOQ)
**200 and 220 % recovery values have been shown to be outliers by the Grubbs statistical test and so are not included in the mean and RSD calculations. Caused by fortifying the sample vial twice in error.

Recovery of 2,4-DB in Fat (Bovine)

Matrix		Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)	Range (%)
Quantitation Transition m/z 247.0 → 161.0							
Fat (Bovine)	Acid	0.01*	83, 93, 91, 90, 89	5	89	4.2	83-93
		0.1	87, 80, 178**, 86, 81	4	84	4.2	80-87
		Overall		9	87	5.3	80-93
	2EH Ester	0.01*	92, 94, 96, 85, 95	5	92	4.8	85-96
		0.1	93, 109, 100, 106, 98	5	101	6.3	93-109
		Overall		10	97	7.2	85-109
	Glycine conjugate	0.01*	80, 98, 78, 62, 55	5	75	22.5	55-98
		0.1	74, 76, 72, 85, 65	5	74	9.7	65-85
		Overall		10	75	16.4	55-98
Confirmatory Transition m/z 249.0 → 163.0							
Fat (Bovine)	Acid	0.01*	93, 75, 109, 90, 106	5	95	14.4	75-109
		0.1	88, 88, 177**, 86, 80	4	86	4.4	80-88
		Overall		9	91	12.2	75-109
	2EH Ester	0.01*	107, 79, 90, 94, 83	5	91	12.0	79-107
		0.1	95, 102, 96, 106, 92	5	98	5.8	92-106
		Overall		10	94	9.6	79-107
	Glycine conjugate	0.01*	89, 101, 96, 83, 70	5	88	13.7	70-101
		0.1	65, 78, 72, 76, 67	5	72	7.8	65-78
		Overall		10	80	15.4	65-101

Residue in the control specimens and reagent blank was less than 30 % of the LOQ.
*Limit of Quantitation (LOQ)
**178 and 177 % recovery values have been shown to be outliers by the Grubbs statistical test and so are not included in the mean and RSD calculations. Caused by fortifying the sample vial twice in error.

Recovery of MCPA in Fat (Bovine)

Matrix		Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)	Range (%)
Quantitation Transition m/z 199.0 → 140.9							
Fat (Bovine)	Acid	0.01*	117, 107, 102, 117, 118	5	112	6.5	102-118
		0.1	101, 102, 224**, 104, 105	4	103	1.8	101-105
		Overall		9	108	6.6	101-118
	2EH Ester	0.01*	85, 115, 116, 110, 115	5	108	12.2	85-116
		0.1	115, 125, 117, 123, 113	5	119	4.4	113-125
		Overall		10	113	9.6	85-125
	Glycine conjugate	0.01*	122, 126, 113, 111, 124	5	119	5.7	111-126
		0.1	122, 112, 128, 130, 115	5	121	6.5	112-130
		Overall		10	120	5.8	111-130
Confirmatory Transition m/z 200.9 → 142.9							
Fat (Bovine)	Acid	0.01*	103, 106, 97, 114, 115	5	107	7.1	97-115
		0.1	103, 102, 213**, 108, 107	4	105	2.8	102-108
		Overall		9	106	5.4	97-115
	2EH Ester	0.01*	80, 112, 104, 107, 108	5	102	12.5	80-112
		0.1	116, 124, 117, 129, 113	5	120	5.5	116-129
		Overall		10	111	12.0	80-129
	Glycine conjugate	0.01*	108, 118, 134, 122, 116	5	120	8.0	108-134
		0.1	113, 111, 121, 123, 120	5	118	4.5	111-123
		Overall		10	119	6.2	108-134

Residue in the control specimens and reagent blank was less than 30 % of the LOQ.
*Limit of Quantitation (LOQ)
**224 and 213 % recovery values have been shown to be outliers by the Grubbs statistical test and so are not included in the mean and RSD calculations. Caused by fortifying the sample vial twice in error.

Recovery of MCPB in Fat (Bovine)

Matrix		Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)	Range (%)
Quantitation Transition m/z 227.0 → 140.9							
Fat (Bovine)	Acid	0.01*	101, 78, 104, 85, 107	5	95	13.4	78-107
		0.1	80, 76, 167**, 80, 83	4	80	3.6	76-83
		Overall		9	88	13.8	76-107
	2EH Ester	0.01*	83, 91, 95, 99, 91	5	92	6.5	83-99
		0.1	94, 104, 99, 95,93	5	97	4.7	93-104
		Overall		10	94	6.0	83-104
	Glycine conjugate	0.01*	70, 71, 81, 60, 60	5	68	12.8	60-81
		0.1	64, 67, 59, 73, 60	5	65	8.8	59-73
		Overall		10	66	10.7	59-81
Confirmatory Transition m/z 229.0 → 142.9							
Fat (Bovine)	Acid	0.01*	100, 94, 96, 94, 98	5	96	2.7	94-100
		0.1	83, 83, 168**, 77, 80	4	81	3.6	77-83
		Overall		9	89	9.7	77-100
	2EH Ester	0.01*	91, 101, 91, 95, 90	5	94	4.9	90-101
		0.1	96, 105, 93, 102, 93	5	98	5.6	93-105
		Overall		10	96	5.5	90-105
	Glycine conjugate	0.01*	55, 75, 43, 48, 58	5	56	21.9	43-75
		0.1	63, 75, 58, 70, 62	5	66	10.4	58-75
		Overall		10	61	17.6	43-75

Residue in the control specimens and reagent blank was less than 30 % of the LOQ.
*Limit of Quantitation (LOQ)
**167 and 168 % recovery values have been shown to be outliers by the Grubbs statistical test and so are not included in the mean and RSD calculations. Caused by fortifying the sample vial twice in error.

Recovery of Mecoprop-P in Fat (Bovine)

Matrix		Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)	Range (%)
Quantitation Transition m/z 212.9 → 140.9							
Fat (Bovine)	Acid	0.01*	100, 90, 106, 107, 101	5	101	6.7	90-107
		0.1	101, 99, 197**, 97, 99	4	99	1.6	97-101
		Overall		9	100	5.0	90-107
	2EH Ester	0.01*	87, 103, 106, 99, 100	5	99	7.3	87-106
		0.1	100, 112, 109, 109, 99	5	106	5.6	99-112
		Overall		10	102	7.0	87-112
	Glycine conjugate	0.01*	100, 108, 110, 97, 100	5	103	5.5	97-110
		0.1	105, 99, 105, 109, 95	5	103	5.4	95-109
		Overall		10	103	5.1	95-110
Confirmatory Transition m/z 215.0 → 142.9							
Fat (Bovine)	Acid	0.01*	107, 105, 88, 113, 113	5	105	9.7	88-113
		0.1	96, 94, 204**, 99, 97	4	97	2.2	94-99
		Overall		9	101	8.6	88-113
	2EH Ester	0.01*	87, 94, 100, 104, 98	5	97	6.7	87-104
		0.1	102, 107, 102, 107, 104	5	104	2.4	102-107
		Overall		10	101	6.2	82-107
	Glycine conjugate	0.01*	92, 117, 101, 106, 98	5	103	9.2	92-117
		0.1	101, 99, 105, 109, 91	5	101	6.7	91-109
		Overall		10	102	7.6	91-117

Residue in the control specimens and reagent blank was less than 30 % of the LOQ.
*Limit of Quantitation (LOQ)
**197 and 204 % recovery values have been shown to be outliers by the Grubbs statistical test and so are not included in the mean and RSD calculations. Caused by fortifying the sample vial twice in error.

Recovery of Dichloroprop-P in Fat (Bovine)

Matrix		Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)	Range (%)
Quantitation Transition m/z 232.9 → 160.8							
Fat (Bovine)	Acid	0.01*	98, 97, 93, 109, 117	5	103	9.6	93-117
		0.1	92, 99, 194**, 96, 96	4	96	3.0	92-99
		Overall		9	100	8.2	92-117
	2EH Ester	0.01*	91, 99, 101, 98, 105	5	99	5.2	91-105
		0.1	112, 117, 116, 124, 111	5	116	4.4	111-124
		Overall		10	107	9.6	91-124
	Glycine conjugate	0.01*	97, 96, 93, 98, 88	5	94	4.3	88-98
		0.1	93, 86, 94, 99, 84	5	91	6.7	84-99
		Overall		10	93	5.6	84-99
Confirmatory Transition m/z 234.9 → 162.8							
Fat (Bovine)	Acid	0.01*	104, 96, 88, 90, 105	5	97	8.1	88-105
		0.1	99, 95, 209**, 96, 101	4	98	2.8	95-101
		Overall		9	97	6.0	88-105
	2EH Ester	0.01*	87, 116, 109, 104, 109	5	105	10.4	87-116
		0.1	105, 118, 113 ,118, 109	5	113	5.0	105-118
		Overall		10	109	8.4	87-118
	Glycine conjugate	0.01*	93, 97, 90, 91, 82	5	91	6.1	82-97
		0.1	91, 89, 94, 102, 94	5	92	7.3	89-102
		Overall		10	91	6.4	82-102

Residue in the control specimens and reagent blank was less than 30 % of the LOQ.
*Limit of Quantitation (LOQ)
**194 and 209 % recovery values have been shown to be outliers by the Grubbs statistical test and so are not included in the mean and RSD calculations. Caused by fortifying the sample vial twice in error.

Recovery of 2,4-D in Liver (Bovine)

Matrix		Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)
Quantitation Transition m/z 218.8 → 161.0						
Liver (Bovine)	Acid	0.01*	99, 94, 75, 96, 86	5	90	10.7
		0.1	97, 93, 95, 97, 99	5	96	2.4
		Overall		10	93	7.9
	2EH Ester	0.01*	79, 93, 93, 92, 87	5	89	6.8
		0.1	95, 97, 94, 98, 95	5	96	1.7
		Overall		10	92	6.0
	Glycine conjugate	0.01*	85, 102, 78, 90, 84	5	88	10.3
		0.1	93, 92, 97, 92, 92	5	93	2.3
		Overall		10	91	7.5
Confirmatory Transition m/z 220.8 → 162.9						
Liver (Bovine)	Acid	0.01*	95, 95, 82, 91, 86	5	90	6.4
		0.1	98, 96, 95, 99, 100	5	98	2.1
		Overall		10	94	6.2
	2EH Ester	0.01*	81, 94, 109, 87, 94	5	93	11.3
		0.1	99, 96, 100, 96, 97	5	98	1.9
		Overall		10	95	7.9
	Glycine conjugate	0.01*	94, 89, 80, 78, 79	5	84	8.5
		0.1	90, 89, 91, 86, 89	5	89	2.1
		Overall		10	87	6.4

Residue in the control specimens and reagent blank was less than 30 % of the LOQ.
*Limit of Quantitation (LOQ)

Recovery of 2,4-DB in Liver (Bovine)

Matrix		Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)
Quantitation Transition m/z 247.0 → 161.0						
Liver (Bovine)	Acid	0.01*	85, 95, 87, 87, 90	5	89	4.4
		0.1	105, 94, 98, 98, 107	5	100	5.4
		Overall		10	95	8.0
	2EH Ester	0.01*	92, 100, 106, 103, 104	5	101	5.4
		0.1	99, 97, 97, 104, 101	5	100	3.0
		Overall		10	100	4.2
	Glycine conjugate	0.01*	97, 85, 96, 99, 88	5	93	6.6
		0.1	86, 90, 81, 92, 84	5	87	5.1
		Overall		10	90	6.8
Confirmatory Transition m/z 249.0 → 163.0						
Liver (Bovine)	Acid	0.01*	85, 84, 72, 82, 81	5	81	6.4
		0.1	101, 90, 90, 93, 110	5	97	8.9
		Overall		10	89	12.1
	2EH Ester	0.01*	95, 99, 92, 96, 121	5	101	11.6
		0.1	104, 98, 96, 103, 97	5	100	3.7
		Overall		10	100	8.2
	Glycine conjugate	0.01*	82, 85, 89, 120, 81	5	91	17.8
		0.1	89, 94, 85, 95, 82	5	89	6.3
		Overall		10	90	12.8

Residue in the control specimens and reagent blank was less than 30 % of the LOQ.
*Limit of Quantitation (LOQ)

Recovery of MCPA in Liver (Bovine)

Matrix		Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)
Quantitation Transition m/z 199.0 → 140.9						
Liver (Bovine)	Acid	0.01*	89, 88, 71, 93, 91	5	86	10.2
		0.1	99, 96, 101, 95, 110	5	100	6.0
		Overall		10	93	10.9
	2EH Ester	0.01*	82, 87, 97, 93, 93	5	90	6.5
		0.1	99, 98, 98, 100, 99	5	99	0.8
		Overall		10	95	6.3
	Glycine conjugate	0.01*	89, 85, 81, 93, 77	5	85	7.4
		0.1	92, 97, 97, 93, 94	5	95	2.4
		Overall		10	90	7.5
Confirmatory Transition m/z 200.9 → 142.9						
Liver (Bovine)	Acid	0.01*	86, 94, 71, 92, 102	5	89	13.0
		0.1	98, 97, 106, 95, 105	5	100	5.0
		Overall		10	95	10.9
	2EH Ester	0.01*	71, 86, 90, 96, 92	5	87	11.1
		0.1	100, 94, 95, 101, 96	5	97	3.2
		Overall		10	92	9.4
	Glycine conjugate	0.01*	86, 89, 86, 93, 80	5	87	5.5
		0.1	89, 92, 90, 93, 93	5	91	2.0
		Overall		10	89	4.7

Residue in the control specimens and reagent blank was less than 30 % of the LOQ.
*Limit of Quantitation (LOQ)

Recovery of MCPB in Liver (Bovine)

Matrix		Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)
Quantitation Transition m/z 227.0 → 140.9						
Liver (Bovine)	Acid	0.01*	103, 96, 81, 93, 94	5	93	8.5
		0.1	101, 98, 91, 105, 99	5	99	5.2
		Overall		10	96	7.2
	2EH Ester	0.01*	93, 102, 113, 96, 99	5	101	7.7
		0.1	97, 113, 106, 112, 104	5	106	6.1
		Overall		10	104	7.1
	Glycine conjugate	0.01*	92, 95, 81, 91, 85	5	89	6.4
		0.1	89, 77, 86, 87, 83	5	84	5.5
		Overall		10	87	6.3
Confirmatory Transition m/z 229.0 → 142.9						
Liver (Bovine)	Acid	0.01*	109, 92, 81, 106, 97	5	97	11.6
		0.1	104, 90, 96, 99, 96	5	97	5.3
		Overall		10	97	8.5
	2EH Ester	0.01*	80, 75, 88, 84, 86	5	83	6.3
		0.1	102, 103, 105, 102, 106	5	104	1.8
		Overall		10	93	12.5
	Glycine conjugate	0.01*	86, 93, 94, 65, 74	5	82	15.3
		0.1	89, 95, 87, 87, 87	5	89	3.9
		Overall		10	86	10.9

Residue in the control specimens and reagent blank was less than 30 % of the LOQ.
*Limit of Quantitation (LOQ)

Recovery of Mecoprop-P in Liver (Bovine)

Matrix		Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)
Quantitation Transition m/z 212.9 → 140.9						
Liver (Bovine)	Acid	0.01*	91, 90, 69, 91, 98	5	88	12.5
		0.1	102, 95, 100, 99, 111	5	101	5.9
		Overall		10	95	11.6
	2EH Ester	0.01*	81, 94, 97, 100, 101	5	95	8.5
		0.1	102, 101, 101, 101, 99	5	101	1.1
		Overall		10	98	6.5
	Glycine conjugate	0.01*	95, 98, 87, 92, 74	5	89	10.6
		0.1	95, 98, 96, 103, 100	5	98	3.3
		Overall		10	94	8.8
Confirmatory Transition m/z 215.0 → 142.9						
Liver (Bovine)	Acid	0.01*	89, 94, 70, 95, 87	5	87	11.6
		0.1	98, 92, 98, 97, 109	5	99	6.3
		Overall		10	93	10.8
	2EH Ester	0.01*	92, 97, 104, 111, 109	5	103	7.8
		0.1	102, 98, 103, 100, 102	5	101	2.0
		Overall		10	102	5.5
	Glycine conjugate	0.01*	97, 96, 90, 93, 73	5	90	10.9
		0.1	90, 97, 94, 95, 97	5	95	3.0
		Overall		10	92	7.9

Residue in the control specimens and reagent blank was less than 30 % of the LOQ.
*Limit of Quantitation (LOQ)

Recovery of 2,4-D in Muscle (Bovine)

Matrix		Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)
Quantitation Transition m/z 218.8 → 161.0						
Muscle (Bovine)	Acid	0.01*	88, 81, 76, 87, 86	5	84	6.0
		0.1	74, 77, 87, 85, 87	5	82	7.4
		Overall		10	83	6.4
	2EH Ester	0.01*	88, 84, 86, 87, 82	5	85	2.8
		0.1	90, 87, 93, 91, 91	5	90	2.4
		Overall		10	88	3.9
	Glycine conjugate	0.01*	79, 80, 80, 83, 84	5	81	2.7
		0.1	78, 86, 80, 82, 80	5	81	3.7
		Overall		10	81	3.1
Confirmatory Transition m/z 220.8 → 162.9						
Muscle (Bovine)	Acid	0.01*	81, 88, 76, 87, 82	5	83	5.9
		0.1	74, 77, 88, 81, 86	5	81	7.3
		Overall		10	82	6.3
	2EH Ester	0.01*	84, 79, 87, 83, 92	5	85	5.7
		0.1	88, 88, 90, 88, 90	5	89	1.2
		Overall		10	87	4.5
	Glycine conjugate	0.01*	86, 83, 87, 85, 84	5	85	1.9
		0.1	87, 87, 82, 85, 84	5	85	2.5
		Overall		10	85	2.1

Residue in the control specimens and reagent blank was less than 30 % of the LOQ.
*Limit of Quantitation (LOQ)

Recovery of 2,4-DB in Muscle (Bovine)

Matrix		Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)
Quantitation Transition m/z 247.0 → 161.0						
Muscle (Bovine)	Acid	0.01*	84, 93, 78, 91, 91	5	87	7.2
		0.1	80, 82, 92, 90, 96	5	88	7.7
		Overall		10	88	7.0
	2EH Ester	0.01*	70, 86, 90, 90, 81	5	83	10.0
		0.1	82, 74, 83, 84, 80	5	81	4.9
		Overall		10	82	7.7
	Glycine conjugate	0.01*	90, 88, 98, 100, 90	5	93	5.8
		0.1	90, 87, 94, 87, 95	5	91	4.2
		Overall		10	92	5.0
Confirmatory Transition m/z 249.0 → 163.0						
Muscle (Bovine)	Acid	0.01*	70, 95, 67, 90, 84	5	81	15.1
		0.1	82, 84, 95, 96, 93	5	90	7.2
		Overall		10	86	12.1
	2EH Ester	0.01*	75, 77, 78, 92, 81	5	81	8.4
		0.1	82, 81, 79, 87, 83	5	82	3.6
		Overall		10	82	6.1
	Glycine conjugate	0.01*	93, 92, 94, 82, 95	5	91	5.8
		0.1	86, 92, 97, 90, 92	5	91	4.3
		Overall		10	91	4.8

Residue in the control specimens and reagent blank was less than 30 % of the LOQ.
*Limit of Quantitation (LOQ)

Recovery of MCPA in Muscle (Bovine)

Matrix		Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)
Quantitation Transition m/z 199.0 → 140.9						
Muscle (Bovine)	Acid	0.01*	83, 81, 77, 79, 81	5	80	2.8
		0.1	76, 81, 87, 93, 90	5	85	8.1
		Overall		10	83	6.7
	2EH Ester	0.01*	81, 84, 89, 88, 82	5	85	4.2
		0.1	91, 92, 91, 91, 89	5	91	1.2
		Overall		10	88	4.6
	Glycine conjugate	0.01*	88, 89, 87, 90, 84	5	88	2.6
		0.1	83, 89, 88, 87, 93	5	88	4.1
		Overall		10	88	3.3
Confirmatory Transition m/z 200.9 → 142.9						
Muscle (Bovine)	Acid	0.01*	87, 81, 77, 90, 92	5	85	7.3
		0.1	78, 81, 86, 95, 93	5	87	8.5
		Overall		10	86	7.5
	2EH Ester	0.01*	80, 87, 92, 90, 88	5	87	5.2
		0.1	89, 87, 89, 89, 88	5	88	1.0
		Overall		10	88	3.6
	Glycine conjugate	0.01*	80, 98, 91, 92, 87	5	90	7.4
		0.1	85, 91, 85, 87, 87	5	87	2.8
		Overall		10	88	5.6

Residue in the control specimens and reagent blank was less than 30 % of the LOQ.
*Limit of Quantitation (LOQ)

Recovery of MCPB in Muscle (Bovine)

Matrix		Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)
Quantitation Transition m/z 227.0 → 140.9						
Muscle (Bovine)	Acid	0.01*	92, 92, 84, 95, 94	5	91	4.7
		0.1	79, 85, 94, 90, 90	5	88	6.6
		Overall		10	90	5.8
	2EH Ester	0.01*	78, 88, 81, 74, 87	5	82	7.3
		0.1	85, 78, 81, 85, 82	5	82	3.6
		Overall		10	82	5.4
	Glycine conjugate	0.01*	79, 99, 88, 85, 90	5	88	8.3
		0.1	84, 88, 88, 86, 84	5	86	2.3
		Overall		10	87	6.0
Confirmatory Transition m/z 229.0 → 142.9						
Muscle (Bovine)	Acid	0.01*	91, 95, 81, 111, 88	5	93	12.0
		0.1	82, 86, 94, 89, 93	5	89	5.6
		Overall		10	91	9.3
	2EH Ester	0.01*	84, 85, 78, 95, 78	5	84	8.3
		0.1	81, 77, 76, 79, 83	5	79	3.6
		Overall		10	82	6.9
	Glycine conjugate	0.01*	77, 104, 82, 67, 89	5	84	16.5
		0.1	98, 81, 90, 88, 86	5	89	7.0
		Overall		10	86	12.1

Residue in the control specimens and reagent blank was less than 30 % of the LOQ.
*Limit of Quantitation (LOQ)

Recovery of Mecoprop-P in Muscle (Bovine)

Matrix		Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)
Quantitation Transition m/z 212.9 → 140.9						
Muscle (Bovine)	Acid	0.01*	86, 89, 81, 87, 89	5	86	3.8
		0.1	78, 79, 91, 93, 93	5	87	8.8
		Overall		10	87	6.4
	2EH Ester	0.01*	75, 79, 82, 80, 82	5	80	3.6
		0.1	84, 83, 85, 90, 84	5	85	3.3
		Overall		10	82	4.8
	Glycine conjugate	0.01*	92, 100, 96, 95, 89	5	94	4.4
		0.1	88, 92, 91, 94, 91	5	91	2.4
		Overall		10	93	3.8
Confirmatory Transition m/z 215.0 → 142.9						
Muscle (Bovine)	Acid	0.01*	83, 81, 76, 81, 86	5	81	4.5
		0.1	78, 79, 87, 92, 93	5	86	8.2
		Overall		10	84	6.9
	2EH Ester	0.01*	72, 83, 82, 82, 79	5	80	5.7
		0.1	85, 84, 83, 86, 80	5	84	2.8
		Overall		10	82	4.9
	Glycine conjugate	0.01*	91, 98, 97, 92, 85	5	93	5.6
		0.1	99, 88, 94, 87, 90	5	92	5.4
		Overall		10	92	5.2

Residue in the control specimens and reagent blank was less than 30 % of the LOQ.
*Limit of Quantitation (LOQ)

Recovery of Dichloroprop-P in Muscle (Bovine)

Matrix		Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)
Quantitation Transition m/z 232.9 → 160.8						
Muscle (Bovine)	Acid	0.01*	86, 83, 77, 87, 84	5	83	4.7
		0.1	78, 85, 91, 91, 95	5	88	7.5
		Overall		10	86	6.6
	2EH Ester	0.01*	88, 83, 82, 83, 79	5	83	3.9
		0.1	87, 87, 86, 89, 88	5	87	1.3
		Overall		10	85	3.8
	Glycine conjugate	0.01*	84, 87, 74, 81, 81	5	81	5.9
		0.1	79, 82, 84, 80, 81	5	81	2.4
		Overall		10	81	4.3
Confirmatory Transition m/z 234.9 → 162.8						
Muscle (Bovine)	Acid	0.01*	85, 92, 74, 78, 81	5	82	8.4
		0.1	76, 76, 90, 91, 94	5	85	10.2
		Overall		10	84	9.1
	2EH Ester	0.01*	80, 85, 88, 81, 87	5	84	4.2
		0.1	90, 85, 82, 89, 86	5	86	3.7
		Overall		10	85	4.0
	Glycine conjugate	0.01*	96, 88, 84, 84, 80	5	86	7.0
		0.1	83, 83, 83, 82, 87	5	84	2.3
		Overall		10	85	5.3

Residue in the control specimens and reagent blank was less than 30 % of the LOQ.
*Limit of Quantitation (LOQ)

A 8: Characteristics for the analytical method used for validation of MCPA and its metabolites residues in bovine muscle and fat, liver, poultry eggs.

	MCPA and its metabolites
Specificity	The analytical method developed for the determination of phenoxy acids in surface water has been shown to be highly specific due to the instrumentation used (LC-MS/MS) and the detection of two characteristic isotopic mass transitions. There were no components present in the control that interfered with the analysis at levels above 30% of the limit of quantitation.
Calibration (type, number of data points)	The LC-MS/MS responses were shown to be linear throughout the entire study (correlation coefficients (r) > 0.99), for more details and every calibration graph please refer to the study report.
Calibration range	The calibration was performed using calibration solutions (9 concentrations), for more details and every calibration graph please refer to the study report.
Assessment of matrix effects is presented	yes
Limit of determination/quantification	The LOQ for all analytes in bovine muscle and fat, liver, poultry eggs, citrus fruit, olives is 0.01 mg/L

Conclusion

Analytical method for the determination of MCPA and its metabolites in bovine muscle and fat, liver,

poultry eggs was developed and successfully validated according to the EC guidance document SANCO/825/00 Rev. 8.1 with regard to LOQ, recovery, precision, specificity, linearity, matrix effects and stability of solutions and extracts.

A 2.1.2.3.2. Independent laboratory validation

Comments of zRMS:	There is no adequate description of the study to allow for assessment. Not accepted.

Reference:	KCP 5.2
Report	Phenoxy Herbicides - Independent Laboratory Validation of the Analytical Method CAM-0004/002 for the Determination of Phenoxy Acids and Their Corresponding 2 Ethyl-Hexyl Esters and Glycine Conjugates in six matrices by LC-MS/MS (Universal Method) Eurofins Agroscience Services Chem Ltd Study #S14-00286, G. Watson, 2014
Guideline(s):	Yes (SANCO/825/00, rev. 8.1)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

For details, materials and methods, chromatographic conditions please refer to A 2.1.2.1.2 and see the study report.

A 2.1.2.3.3 Confirmatory method

No confirmatory method is required

A 2.1.2.4.1 Determination of tribenuron methyl in animal matrices

A 2.1.2.4.1.1. Method validation

Comments of zRMS:	Not accepted. The applicable MRLs in animal products are 0.01 mg/kg.

Reference:	KCP 5.2
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, Norris 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 9 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 10 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 2.2.2.4.2 Independent laboratory validation

Comments of zRMS:	Not accepted. The applicable MRLs in animal products are 0.01 mg/kg.

Reference:	KCP 5.2
Report	Metsulfuron-methyl and Tribenuron methyl: Independent Laboratory Validation of an Analytical Method for the Determination in Animal Matrices, Eichler M, Hermann 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 11 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	0.05	104	2	

Matrix	Analyte	Fortification level (mg/kg) (n = 6)	Mean recovery (%)	RSD (%)	Comments
	methyl	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 12 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 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.

	Tribenuron methyl
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 2.1.2.4.3 Confirmatory method

No confirmatory method is required

A 2.1.2.5.1 Description of Methods for the Analysis of Soil (KCP 5.2)

A 2.1.2.5.1.1 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 soil according to SANCO/3029/99 rev. 4, Kotthoff 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, Hennecke 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 13 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	
Soil	IN-00581	1.2	106.1	4.4	
		12.2	110.9	1.6	
Confirmatory mass transition					
Soil	Tribenuron	0.06	110.0	7.5	

Matrix	Analyte	Fortification level (mg/kg) (n = 5)	Mean recovery (%)	RSD (%)	Comments
	methyl	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 14 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 + 12973.4*x + 768.295	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
Calibration range	Soil samples were fortified with tribenuron methyl to 10	Correlation coefficient: r= 0.997060	Coefficient of determination: r^2= 0.998770	Coefficient of determination: r^2= 0.998288	Correlation coefficient: r= 0.998288

	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	Additional correlation coefficient r= 0.997077 These results meet the acceptance criteria of r ≥ 0.99	These results meet the acceptance criteria of r ≥ 0.99	These results meet the acceptance criteria of r ≥ 0.99	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 2.1.2.5.2 Confirmatory method

No confirmatory method is required.

A 2.1.2.6.1 Description of Methods for the Analysis of Water (KCP 5.2)

A 2.1.2.6.1.1 Method validation

Comments of zRMS:	The study is accepted.

Reference:	KCP 5.2
Report	Analytical Method for the Determination of Phenoxy Acids and Their Corresponding 2 Ethyl-hexyl esters and glycine conjugates in cereal grain, straw and foliage, bovine muscle, fat, liver, kidney and milk, poultry eggs, citrus fruit and olives and phenoxy acids and their corresponding 2 ethyl-hexyl esters in surface water, soil and air (Universal Method), L. Allen, CEMAS Study #CAM-0004/003
Guideline(s):	Yes (SANCO/825/00 rev. 8.1); (SANCO/3029/99 rev. 4)
Deviations:	No
GLP:	Yes

Acceptability: Yes

Materials and methods

This analytical method describes the procedure for the determination of the total phenoxy acid present in cereal grain, straw, foliage, bovine muscle, fat, liver, kidney, milk, poultry eggs, citrus fruit and olives, whether in the form of the acid, ester (eg. ethyl-hexyl) or conjugate (eg. glycine) and the total phenoxy acid present in surface water, soil and air, whether in the form of the acid or ester (eg. ethyl-hexyl).
During the extraction procedure samples are hydrolysed to convert the ethyl-hexyl esters and glycine conjugates back to the parent acid for quantitation.
The analysis is performed using a hydrolysis reaction, QuEChERS extraction and determination by LC-MS/MS detection. The LOQ for the 2,4-D, 2,4-DB, MCPA, MCPB and Dichloroprop-P in surface water is 0.01 µg/L and the LOQ for Mecoprop-P in surface water is 0.02 µg/L

Preparation of Reagents and Solutions

• Sodium Hydroxide (47%)/Water (15/85, v/v):
To 425 mL deionised water add 75 mL of 47% sodium hydroxide solution and allow to mix. Store in a glass bottle.
• Sodium Hydroxide (47%)/Water (30/70, v/v):
To 350 mL deionised water add 150 mL of 47% sodium hydroxide solution and allow to mix. Store in a glass bottle.
• 15N Sulphuric acid:

To 148 mL HPLC grade water carefully add 102 mL of concentrated sulphuric acid (S.G 1.84 = 36.76N). Allow to mix and transfer to a 250 mL glass bottle. Store at 4ºC.
• 1M Monochloroacetic acid:
Weigh 47.25 g of acid into a plastic weighing boat and transfer to a 500 mL glass bottle, dissolve in 500 mL of HPLC grade water.
• 0.2 % formic acid in Water:
To 500 mL of HPLC grade water add 1 mL of formic acid solution and allow to mix. Store in a glass bottle.
• 1% ammonia in acetonitrile:
Add 5 mL of a 20% ammonia solution to 95 mL of acetonitrile and allow to mix. Store in a glass bottle.

Reagents:

- Acetonitrile (HPLC grade),
- Ultra-pure water (HPLC grade),
- Methanol (HPLC grade),
- Hexane (HPLC grade),
- Formic acid (HPLC grade),
- Sulphuric acid SG 1.84 (HPLC grade),
- Sodium hydroxide 47%,
- Monochloroacetic acid,
- QuEChERS Salts, EN 15662 (Part 5982-7650),
- Anhydrous Magnesium Sulphate (bulk sorbent),
- Graphitized Carbon Black (carbon SPE Bulk sorbent),
- Aluminium Oxide (100-250 mesh chromatography reagent)

Internal Standard
- (2,4,6-trimethyl-phenoxy)acetic acid (2,4,6-TMAA),
- (4-chloro-3,5-dimethylphenoxy)acetic acid (4-CDMAA)

Instrumental parameters:

HPLC system	Symbiosis Pharma Liquid Chromatography System AB Sciex 4000 triple quad MS System AB Sciex Analyst 1.4.2 data system
Column	Onyx C18 monolithic column, 3.0 x 100 mm
Column temperature	Ambient
Injection volume	40 µL
Flow rate	1 mL/min; split 1:4 to the mass spectrometer
Mobile phase A:	HPLC water + 0.1 % formic acid
Mobile phase B:	Methanol + 0.1 % formic acid
Gradient mode	

Time [min:secs]	A [%]	B [%]
0:01	55	45
0:03	55	45
6:00	25	75
6:01	5	95
7:15	5	95
7:16	55	45
9:00	55	45

Results and discussions

Table A 95 Recovery results from method validation of 2,4-D in Surface Water

Matrix	Analyte	Fortification level (mg/kg) (n = x)	Mean recovery (%)	RSD (%)	Comments
Quantitation Transition m/z 218.8 -> 161.0					
Surface water	Acid	0.01*	93	3.8	
		1.0	105	4.7	
		Overall	99	7.7	
Surface water	2EH Ester	0.01*	113	9.3	
		1.0	110	3.6	
		Overall	111	6.9	
Confirmatory Transition m/z 220.8 -> 162.9					
Surface water	Acid	0.01*	95	7.0	
		1.0	108	6.4	
		Overall	102	9.2	
Surface water	2EH Ester	0.01*	113	8.7	
		1.0	110	3.8	
		Overall	112	6.6.	

Residue in the control specimens and reagent blank was less than 30 % of the LOQ.

***Limit of Quantitation (LOQ)**

Table A 16 Recovery results from method validation of 2,4-DB in Surface Water

Matrix	Analyte	Fortification level (mg/kg) (n = x)	Mean recovery (%)	RSD (%)	Comments
Quantitation Transition m/z 247.0 -> 161.0					
Surface water	Acid	0.01*	80	6.3	
		1.0	94	14.8	
		Overall	87	13.9	
Surface water	2EH Ester	0.01*	91	6.6	
		1.0	96	4.6	
		Overall	93	5.7	
Confirmatory Transition m/z 249.0 -> 163					
Surface water	Acid	0.01*	87	7.4	
		1.0	89	10.8	
		Overall	88	8.8	
Surface water	2EH Ester	0.01*	95	4.7	
		1.0	94	2.8	
		Overall	94	3.7	

Residue in the control specimens and reagent blank was less than 30 % of the LOQ.

***Limit of Quantitation (LOQ)**

Table A 17 Recovery results from method validation of MCPA in Surface Water

Matrix	Analyte	Fortification level (mg/kg) (n = x)	Mean recovery (%)	RSD (%)	Comments
Quantitation Transition m/z 199.0 -> 140.9					
Surface water	Acid	0.01*	106	4.4	
		1.0	117	11.7	
		Overall	111	10.0	
Surface water	2EH Ester	0.01*	117	8.1	
		1.0	118	5.7	
		Overall	117	6.6	
Confirmatory Transition m/z 200.9 -> 142.9					
Surface water	Acid	0.01*	98	5.6	
		1.0	114	9.2	
		Overall	106	10.7	
Surface water	2EH Ester	0.01*	114	9.8	
		1.0	121	7.3	

Matrix	Analyte	Fortification level (mg/kg) (n = x)	Mean recovery (%)	RSD (%)	Comments
		Overall	117	8.8	

Residue in the control specimens and reagent blank was less than 30 % of the LOQ.

***Limit of Quantitation (LOQ)**

Table A 18 Recovery results from method validation of MCPB in Surface Water

Matrix	Analyte	Fortification level (mg/kg) (n = x)	Mean recovery (%)	RSD (%)	Comments
Quantitation Transition m/z 227.0 -> 140.9					
Surface water	Acid	0.01*	78	12.9	
		1.0	102	9.0	
		Overall	90	17.3	
Surface water	2EH Ester	0.01*	98	8.7	
		1.0	106	4.2	
		Overall	102	7.5	
Confirmatory Transition m/z 229.0 -> 142.9					
Surface water	Acid	0.01*	80	6.8	
		1.0	101	2.5	
		Overall	91	13.0	
Surface water	2EH Ester	0.01*	100	5.9	
		1.0	107	1.0	
		Overall	103	5.3	

Residue in the control specimens and reagent blank was less than 30 % of the LOQ.

***Limit of Quantitation (LOQ)**

Table A 19 Recovery results from method validation of Mecoprop-P in Surface Water

Matrix	Analyte	Fortification level (mg/kg) (n = x)	Mean recovery (%)	RSD (%)	Comments
Quantitation Transition m/z 212.9 -> 140.9					
Surface water	Acid	0.01*	116	4.5	
		1.0	107	8.1	
		Overall	111	7.4	
Surface water	2EH Ester	0.01*	96	8.1	
		1.0	109	13.3	
		Overall	102	12.7	
Confirmatory Transition m/z 215.0 -> 142.9					
Surface water	Acid	0.01*	104	4.7	

Matrix	Analyte	Fortification level (mg/kg) (n = x)	Mean recovery (%)	RSD (%)	Comments
		1.0	111	14.5	
		Overall	107	11.0	
Surface water	2EH Ester	0.01*	88	9.0	
		1.0	104	7.3	
		Overall	96	11.3	

Residue in the control specimens and reagent blank was less than 30 % of the LOQ.

***Limit of Quantitation (LOQ)**

Table A 20 Recovery results from method validation of Dichloroprop-P in Surface Water

Matrix	Analyte	Fortification level (mg/kg) (n = x)	Mean recovery (%)	RSD (%)	Comments
Quantitation Transition m/z 232.9 -> 160.8					
Surface water	Acid	0.01*	89	6.5	
		1.0	105	10.0	
		Overall	97	11.9	
Surface water	2EH Ester	0.01*	99	10.3	
		1.0	93	5.4	
		Overall	96	8.4	
Confirmatory Transition m/z 234.9 -> 162.8					
Surface water	Acid	0.01*	86	3.6	
		1.0	105	11.4	
		Overall	95	13.6	
Surface water	2EH Ester	0.01*	99	11.1	
		1.0	100	11.8	
		Overall	99	10.8	

Residue in the control specimens and reagent blank was less than 30 % of the LOQ.

***Limit of Quantitation (LOQ)**

Table A 21 Characteristics for the analytical method used for validation of MCPA and its metabolites residues in surface water

	MCPA and its metabolites
Specificity	The analytical method developed for the determination of phenoxy acids in surface water has been shown to be highly specific due to the instrumentation used (LC-MS/MS) and the detection of two characteristic isotopic mass transitions. There were no components present in the control that interfered with the analysis at levels above 30% of the limit of quantitation.

	MCPA and its metabolites
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), for more details and every calibration graph please refer to the study report.
Calibration range	The calibration was performed using calibration solutions (9 concentrations), for more details and every calibration graph please refer to the study report.
Assessment of matrix effects is presented	yes
Limit of determination/quantification	The LOQ for the 2,4-D, 2,4-DB, MCPA, MCPB and Dichloroprop-P in surface water is 0.01 µg/L and the LOQ for Mecoprop-P in surface water is 0.02 µg/L .

Conclusion

analytical method for the determination of MCPA and their metabolites in surface 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 2.1.2.6.2 Independent laboratory validation

Comments of zRMS:	The study is accepted.

Reference:	KCP 5.2
Report	Phenoxy Herbicides - Independent Laboratory Validation of the Analytical Method CAM-0004/003 for the Determination of Phenoxy Acids and Their Corresponding 2 Ethyl-Hexyl Esters in Drinking Water by LC-MS/MS (Universal Method) Eurofins Agroscience Services Chem Ltd Study #S14-01199, A. Weir
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 carry out an Independent Laboratory Validation study (ILV) of the method CAM-0004/003 for the determination of the phenoxy acid 2,4-D, 2,4-DB, MCPA, MCPB, Mecoprop-P and Dichloroprop-P (as the total phenoxy acid, when present in the matrix as the acid or ester) in drinking water to a limit of Quantitation (LOQ) of 0.01 µg/L (0.02 µg/L for Mecoprop-P).
In brief, the method involves hydrolysis of samples overnight in a strong solution of sodium hydroxide to convert the ethyl-hexyl esters back to the present acid for quantification. The hydrolysed samples are acidified then purified/concentrated using a solid phase extraction step prior to quantification by LC-MS/MS.

The method was successfully validated at the second attempt for each analyte.
The ILV was carried out in two separate batches each consisting of a reagent blank, 2 control specimens, 5 recoveries fortified at the LOQ and 5 recoveries fortified at x10 LOQ (or x5 LOQ) for Mecoprop-

P).One batch was fortified with the acid and one batch was fortified with corresponding 2 ethyl-hexyl ester. In each case residues were quantified as the parent acid residue. Where recoveries were fortified with the 2 ethyl-hexyl ester, they were fortified in an equimolar concentration to the intended acid concentration (acid equivalent).

Results and discussions

Table A 22 Recovery results from independent laboratory validation of ~~MCPA~~ 2,4-D and its metabolites using the analytical method

Matrix	Analyte	Fortification level ~~(mg/kg)~~ (µg/L) (n = x)	Mean recovery (%)	RSD (%)	Comments
Quantitation Transition m/z 219 -> 161.0					
Drinking water*	Acid	0.01	90	7.9	
		1.0	94	2.5	
Drinking water*	2EH Ester	0.01	99	4.0	
		1.0	106	8.0	
Confirmatory Transition m/z 221 -> 163					
Drinking water*	Acid	0.01	90	6.1	
		1.0	93	2.5	
Drinking water*	2EH Ester	0.01	99	5.6	
		1.0	105	8.0	

Table A 23 Recovery results from method validation of 2,4-DB in Surface Water

Matrix	Analyte	Fortification level ~~(mg/kg)~~ (µg/L) (n = x)	Mean recovery (%)	RSD (%)	Comments
Quantitation Transition m/z 247.0 -> 161.0					
Drinking water*	Acid	0.01	80	7.2	
		1.0	94	3.8	
Drinking water*	2EH Ester	0.01	91	7.1	
		1.0	96	8.9	
Confirmatory Transition m/z 249.0 -> 163					
Drinking water*	Acid	0.01	87	6.8	
		1.0	89	2.4	
Drinking water*	2EH Ester	0.01	95	11.6	
		1.0	94	10.1	

Table A 24 Recovery results from method validation of MCPA in Surface Water

Matrix	Analyte	Fortification level ~~(mg/kg)~~ (µg/L) (n = x)	Mean recovery (%)	RSD (%)	Comments
Quantitation Transition m/z 199.0 -> 141					
Drinking water*	Acid	0.01	89	5.7	
		1.0	93	3.8	
Drinking water*	2EH Ester	0.01	95	7.7	
		1.0	103	7.6	
Confirmatory Transition m/z 201 -> 143					
Drinking water*	Acid	0.01	93	7.0	
		1.0	89	3.6	
Drinking water*	2EH Ester	0.01	97	6.8	
		1.0	99	5.8	

Table A 25 Recovery results from method validation of MCPB in Surface Water

Matrix	Analyte	Fortification level ~~(mg/kg)~~ (µg/L) (n = x)	Mean recovery (%)	RSD (%)	Comments
Quantitation Transition m/z 227.0 -> 141					
Drinking water*	Acid	0.01	87	4.1	
		1.0	96	2.1	
Drinking water*	2EH Ester	0.01	99	6.9	
		1.0	111	8.8	
Confirmatory Transition m/z 229.0 -> 143					
Drinking water*	Acid	0.01	85	6.6	
		1.0	97	1.9	
Drinking water*	2EH Ester	0.01	97	7.1	
		1.0	111	8.7	

Table A 26 Recovery results from method validation of Mecoprop-P in Surface Water

Matrix	Analyte	Fortification level ~~(mg/kg)~~ (µg/L) (n = x)	Mean recovery (%)	RSD (%)	Comments
Quantitation Transition m/z 213 -> 141					

Matrix	Analyte	Fortification level ~~(mg/kg)~~ (µg/L) (n = x)	Mean recovery (%)	RSD (%)	Comments
Drinking water*	Acid	0.01	85	5.7	
		1.0	91	3.1	
Drinking water*	2EH Ester	0.01	96	7.0	
		1.0	102	8.0	
Confirmatory Transition m/z 215.0 -> 143					
Drinking water*	Acid	0.01	84	5.8	
		1.0	90	3.6	
Drinking water*	2EH Ester	0.01	86	7.4	
		1.0	97	7.6	

Table A 27 Recovery results from method validation of Dichloroprop-P in Surface Water

Matrix	Analyte	Fortification level (~~(mg/kg)~~ (µg/L) (n = x)	Mean recovery (%)	RSD (%)	Comments
Quantitation Transition m/z 233 -> 161					
Drinking water*	Acid	0.01	84	5.5	
		1.0	90	3.5	
Drinking water*	2EH Ester	0.01	86	5.9	
		1.0	97	7.0	
Confirmatory Transition m/z 235 -> 163					
Drinking water*	Acid	0.01	84	6.0	
		1.0	89	3.0	
Drinking water*	2EH Ester	0.01	84	6.4	
		1.0	96	8.0	

Table A 28 Characteristics for the analytical method used for independent laboratory validation of MCPA and its metabolites residues in drinking water

	MCPA and its metabolites
Specificity	A reagent blank and two control specimens were extracted and analysed to investigate the presence of analyte residue and/or background interference at the analyte retention time. For both mass transistions, no significant interferences above 30% of the LOQ were detected at the retention time of 2,4-D, 2,4-DB, MCPA, MCPB, Mecoprop-P or Dichloroprop-P in drinking water demonstrating that each mass transistion is specific to each analyte.
Calibration (type, number of data points)	The linearity of the detector was checked by single determination of calibration standards at 9 concentration levels ranging from 0.6 ng/mL to 200 ng/mL. This calibrationrange is

	MCPA and its metabolites
	equivalent to 30% of the LOQ up to 1.0 µg/L, The calibration curves obtained for both primary and confirmatory transitions for each analyte combination were linear with coefficient correlation ® grater than 0.990.
Calibration range	An appropriate calibration curve was prepared by plotting the peak area ratio (analyte peak area / internal standard peak area) versus concentration (ng/mL). Using 1/x weighted linear regression, the equation of the line and correletion coefficients greater than 0.990.
Assessment of matrix effects is presented	yes
Limit of determination/quantification	The LOQ for the 2,4-D, 2,4-DB, MCPA, MCPB and Dichloroprop-P in drinking water is 0.01 µg/L and the LOQ for Mecoprop-P in drinking water is 0.02 µg/L .

Conclusion

Thee method was successfully validated at the second attempt for each analyte. At the first attempt residues above 30% LOQ were detected in the control matrix for some analytes therefore the validation was repeated using an alternative source of control matrix. The analytical method showed good accuracy and precision for 2,4-D, 2,4-DB, MCPA, MCPB and Dichloroprop-P in drinking water with mean recoveries in the range 70-120% with the RSD of < 20% for both fortification level.
The method is acceptable as ILV for the primary method.

A 2.1.2.6.3 Confirmatory method

No confirmatory method is required.

A 2.1.2.7.1 Determination of Tribenuron methyl and its metabolites in drinking water

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, Hennecke 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-class
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)~~ (µg/L) (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	
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	IN-00581	0.1	107.4	8.0	

Matrix	Analyte	Fortification level ~~(mg/kg)~~ (µg/L) (n = ~~x~~ 5)	Mean recovery (%)	RSD (%)	Comments
water		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 on a 1/x weighing.	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 on a 1/x weighing.	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 on a 1/x weighing.	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 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 2.1.2.7.2 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, Bohmer 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)~~ (µg/L) (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 +	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 +	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:

	Tribenuron methyl	IN-L5296	IN-A4098	IN-00581
		1972.08	134023 * x + 314.854	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 2.1.2.7.3 Confirmatory method

No confirmatory method is required.

A 2.1.2.8 Description of Methods for the Analysis of Air (KCP 5.2)

No new or additional studies have been submitted

A 2.1.2.9 Description of Methods for the Analysis of Body Fluids and Tissues (KCP 5.2)

No new or additional studies have been submitted

A 2.1.2.10 Other Studies/ Information

Method validation

Comments of zRMS:	The study is not accepted. The study is not sufficiently described by the Applicant, eg lack of information on fortification level.

Reference:	KCP 5.1.2/12
Report	Validation of an Analytical Method using LC-MS-MS for the determination of MCPA in the matrix "Wash solution for foliar dislodging experiments" based on SANCO/3029/99 rev. 4 and analysis of field samples, J. Johannes, 2020, study no. 19112203G926 "Preparation and shipment of Field Fortification Standard solutions of MCPA", J. Johannes, 2020, study no. 19112203G405,
Guideline(s):	Yes (SANCO/3029/99 rev. 4, SANCO/825/00, rev. 8.1)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

This study was performed in order to validate the analytical determinations of the test item MCPA in wash solution for foliar dislodging experiments, and analysis of samples provided by CropTrials GmbH, Ehlbeek 2, 30938 Burgwedel, Germany.

An LC/MS/MS method for the determination of MCPA was validated. The test item was separated with an ACE Excel SuperC18, 1.7 µm, 2.1x50 mm column and detected with ESI MS/MS.

Chromatographic conditions:

- Retention time: 0.76 – 0.78 min,
- Calibration solvent: Wash solution/acetonitrile 50/50 (% v/v),
- Eluent A: H_2O + 0.1 % HCOOH,
- Eluent B: Acetonitrile + 0.1 % HCOOH,
- Flow rate: 0.4 mL/min,
- Injection volume: 10 µL,
- Detection: ESI negative mode,
- Dwell time: 75 ms

Sample Preparation

1500 µL of each sample was diluted with an equal amount of acetonitrile (dilution factor 2) and filtrated over 0.45 µm PTFE membrane filters before measurement. Further dilutions to reach the calibrated range were performed using calibration solvent wash solution/acetonitrile 50/50 (% v/v).

- **Results and discussions**

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

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Average recovery at levels [%]	Method recovery [%]	Precision [% RSD]
Wash solution for foliar dislodging experiments	MCPA (Sample time: 0 DALA)	I	89.3	100	4.3
		II	89.8		6.6

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Average recovery at levels [%]	Method recovery [%]	Precision [% RSD]
Wash solution for foliar dislodging experiments	MCPA (Sample time: 21 DALA)	I	101.4	100	3.5
		II	97.8		6.3

Table A 20: Characteristics for the analytical method used for validation of determination of MCPA in the matrix

	MCPA
Specifity	The specificity was checked by injecting the blank solutions of wash solution after sample preparation. The condition "Area in blank < 30 % of reference value" was fulfilled.
Calibration (type, number of data points)	Function was linear in full range. A calibration curve was described by equation: f(x) = 256397.68027*x + 608.40103 Correlation coefficient was equal: r = 0.99997 Acceptance criterion was fulfilled: r ≥ 0.99.
Calibration range	For the calibration, the range of 0.2 – 5.5 µg/L was chosen.
Assessment of matrix effects is presented	yes
Limit of determination/quantification	Limit of detection is: 0.013735 mg/L, and limit of quantification: 0.2 µg/L

To assess the stability of the active substance MCPA in the dislodging solution during freezer storage, field fortification samples were prepared at the timings 0 DALA and 21 DALA.
On 02.02.2020, a control dislodging solution was prepared after collecting leaves from the trial site, according to the method described in chapter 7.4. This solution was stored deep-frozen (-18°C) until use. The needed amount was taken out of the freezer one day before use and was stored at ambient temperature until use. Standard solutions, containing defined concentrations of the active substance MCPA, were prepared under the responsibility of the analytical laboratory LAUS GmbH, Auf der Schafweide 20, 67489 Kirrweiler (Pfalz) Germany (Study No. 19112203G405).
The standard solutions were received at the test facility on 06.02.2020 after shipment on thermal packs. They were stored at the test facility in a refrigerator at temperatures between 1°C and 7°C.
The standard solutions were prepared in Acetonitrile in two concentrations: 1.0 µg MCPA/mL and 1000 µg MCPA/mL. 50 mL aliquots of this control dislodging solution were taken for each field fortification sample. The field fortification samples were made in triplicate at two fortification levels for MCPA:
50 µL of 1.0 µg MCPA/mL standard solution (1 µg/L, low level) and 50 µL of 1000 µg MCPA/mL standard solution (1000 µg/L, high level). A single control was also prepared which did not contain MCPA standard solution. The field fortification samples were labelled, wrapped in aluminium foil prior to being placed into polyethylene bags. The bags were again labelled. The samples were subsequently frozen.
All field fortification samples were prepared in duplicate ('ship' and 'retain' samples).

Conclusion

The analytical method therefore meets the requirements of guideline SANCO/3029/99 rev. 4 and SANCO/825/00, rev. 8.1.