

REGISTRATION REPORT

Part B

Section 5

Analytical Methods

Detailed summary of the risk assessment

Product code: GLOB1912H

Product name: **Jura Max**

Chemical active substances:

Prosulfocarb, 667 g/L

Diflufenican, 14 g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

Applicant: Globachem NV

Submission date: November 2021

Evaluation date: August 2022

MS Finalisation date: December 2022

Version history

When	What
November 2021	Initial submission by applicant for approval of new product.
August 2022	Initial RR
December 2022	Revised version taking into account comments of cMSs and the applicant

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5 Analytical methods

5.1 Conclusion and summary of assessment

Sufficiently sensitive and selective analytical methods are **not** available for the active substance(s) and relevant impurities in the plant protection product.

Noticed data gaps are: **none**

- data-gap-1
- data-gap-2
- data-gap-3

Sufficiently sensitive and selective analytical methods are available for all analytes included in the residue definitions. The text of the applicant was not rewritten. The zRMS text, evaluating residue methods, is on grey background.

Noticed data gaps are: none in the context of the authorisation request.

Commodity/crop	Supported/ Not supported
Cereals, potato, oilseeds	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 prosulfocarb and diflufenican in plant protection product is provided as follows:

Comments of zRMS:	The method is validated and may be used for analysing prosulfocarb and diflufenican in the PPP. Furthermore, it is suitable to be used for analysing content of these active substances in water dilutions (the method used for testing effectiveness of cleaning procedures in the physicochemical section).
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Reference:	KCP 5.1.1
Report	Validation of the methods of determination of prosulfocarb and diflufenican in an EC formulation, in compliance with good laboratory practice. Sowle J., 2020, DNA5958
Guideline(s):	Yes, SANCO/3030/99 rev.5
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

A sample is diluted in acetonitrile and analysed by LC-QQQ.

LC-QQQ LC Conditions – MS Analysis

Instrument:	Agilent 1200 Series LC-QQQ
Mode:	Isocratic Reverse Phase
Column:	Grace Genesis C8, (250mm x 4.6mm)
Packing:	C8, 3µm
Eluent:	75% Acetonitrile + 25% Deionised Water with Trifluoro Acetic Acid at pH 2.6
Flow Rate:	1.0mL/min
Injection Volume:	2.0µL
Column Temperature:	25°C
Data Collection:	Mass Hunter
Retention Time:	Prosulfocarb: approximately 8.6 to 8.7 minutes Diflufenican: approximately 7.0 minutes

LC-QQQ MS Conditions – MS Analysis

Instrument:	Agilent 6470 Series QQQ Mass Spectrometer		
Ionisation:	Positive	Sheath Gas Temperature:	350°C
Gas Temperature:	250°C	Sheath Gas flow:	10L/min
Gas Flow:	8L/min	Capillary Voltage:	3500V
Nebulizer:	30psi	Nozzle Voltage:	500V

Prosulfocarb:

MRM Precursor Ion: 252.1m/z

MRM Precursor Ion (m/z)	MRM Product Ion (m/z)	Dwell Time (ms)	Fragmentor (V)	Collision Energy (V)	Accelerator Voltage (V)
252.1	128.1	200	121	12	5
252.1	91.1	200	121	36	5
252.1	65.1	200	121	68	5

Diflufenican:

MRM Precursor Ion: 395.1m/z


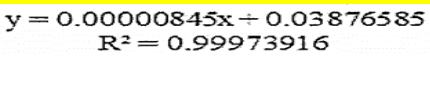
MRM Precursor Ion (m/z)	MRM Product Ion (m/z)	Dwell Time (ms)	Fragmentor (V)	Collision Energy (V)	Accelerator Voltage (V)
395.1	266.0	200	179	28	5
395.1	246.0	200	179	44	5
395.1	238.0	200	179	48	5

Data Acquisition: Mass Hunter

The standards are prepared in acetonitrile.

Validation - Results and discussions

Table 5.2-1: Methods suitable for the determination of the active substances prosulfocarb and diflufenican in plant protection product GLOB1912H

	Prosulfocarb	Diflufenican
Author(s), year	Sowle J., 2020	
Principle of method	LC-QQQ	
Linearity (linear between mg/L / % range of the declared content) (correlation coefficient, expressed as r)	0 - 10.0 mg/L r = 0.9998 – 1.0000 	0 - 10.0 mg/L r = 0.9997 
Precision – Repeatability Mean n = 6 (%RSD)	Mean: 678.4 ± 2.180 g/L %RSD: 0.321 HorRat=0.226	Mean: 14.36 ± 0.122 g/L %RSD: 0.850 HorRat=0.335
Accuracy n = 6 (% Recovery)	Mean: 98.04 ± 0.470% %RSD: 0.479	Mean: 99.12 ± 0.536% %RSD: 0.540
Interference/ Specificity	In the specificity chromatograms 1 mg/L prosulfocarb eluted at 8.6 min with a response of 5493382. Other significant peaks were accounted for by assaying samples of diflufenican, a solvent blank and a sample of the formulation blank. Background levels of prosulfocarb were detected in the solvent blank, formulation blank and diflufenican and represent concentrations of less than 3% relative to the active ingredient. This demonstrates that there were no analyte interferences.	In the specificity chromatograms 10 mg/L diflufenican eluted at 7.0 min with a response of 1157409. Other significant peaks were accounted for by assaying samples of prosulfocarb, a solvent blank and a sample of the formulation blank. There were no other peaks present in these chromatograms at the same elution time as diflufenican. This demonstrates that there were no analyte interferences.
Comment	-	

Conclusion

The validation parameters for these methodologies have been met for this study under the SANCO/3030/99 rev. 5 guidelines.

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

GLOB1912H does not contain relevant impurities.

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

GLOB1912H does not contain relevant formulants.

5.2.1.4 Applicability of existing CIPAC methods (KCP 5.1.1)

There are no CIPAC methods available for analysing one of these active substances in the presence of the other active substance.

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

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

Component of residue definition: Prosulfocarb				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
Plants, plant products,... (Residues)	Primary	0.01 mg/kg	GC-MSD (multi-residue)	Weber H, Pelz S., 2000/EU agreed
	Primary	0.01 mg/kg	LC-MS/MS	Jonchère F., 2010a*
	Primary	0.01 mg/kg	LC-MS/MS	Laguna O., 2021a*
	Confirmatory (if required)	-	Not required	-
Soil (Environmental fate)	Primary	0.02 mg/kg	GC-MSD	Crook, 2000/EU agreed
	Confirmatory (if required)	-	Not required	-
Water (Environmental fate)	Primary	0.1 µg/L	GC-MSD	Hargreaves, 1999/EU agreed
	Confirmatory (if required)	-	Not required	-
Air (Exposure)	Primary	0.00015 µg/m ³	GC-MSD	Kwaitkowski, 2002/EU agreed
	Confirmatory (if required)	-	Not required	-
Water (Ecotoxicology)	Primary	97.82 µg/L	HPLC-MS/MS	Juckeland D., 2021a
		3.354 µg/L	HPLC-MS/MS	Juckeland D., 2021b
		13.92 µg/L	HPLC-MS/MS	Juckeland D., 2021c
		1.467 µg/L	HPLC-MS/MS	Juckeland D., 2021d
	Confirmatory (if required)	-	Not required	-
Soil (Ecotoxicology)	Primary	0.1 mg/kg	HPLC-UV	Schulz L., 2015
		0.02 mg/L	HPLC-UV	Sacker D., 2008
Other (Ecotoxicology)	Primary	5.73 mg/kg	HPLC-MS/MS	Amsel K., 2021
		20441 mg/L	HPLC-MS/MS	Amsel K., 2021
		5.55 mg/kg	HPLC-MS/MS	Ruhland S., 2021
		4.64 mg/kg	HPLC-MS/MS	Schmidt K., 2021
		2858 mg/L	HPLC-MS/MS	Lewington-Gower M., 2021

Metabolites of prosulfocarb: Prosulfocarb sulfoxide				
Water (Ecotoxicology)	Primary	6.21 µg/L	HPLC-MS/MS and UV/VIS	Juckeland D., 2012a
		0.038 mg/L		Juckeland D., 2012b
		0.1002 mg/L		Juckeland D., 2012c
		0.1002 mg/L		Juckeland D., 2012d
		0.01076 mg/L	HPLC-UV/VIS	Juckeland D., 2012e
Component of residue definition: Diflufenican				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
Plants, plant products,... (Residues)	Primary	0.01 mg/kg	GC-ECD	Class T., 2001/EU agreed
	Confirmatory (if required)	0.01 mg/kg	GC-MS	Class T., 2001/EU agreed
	Primary	0.01 mg/kg	UPLC-MS/MS	Jonchère F., 2011*
	Primary	0.01 mg/kg	HPLC-MS/MS	Laguna O., 2021b*
	Primary	0.01 mg/kg	LC-MS/MS	Jonchère F., 2010b*
	Primary	0.01 mg/kg	LC-MS/MS	Laguna O., 2021c*
Animal products, food of animal origin,... (Residues)	Primary	0.01 mg/kg (milk) 0.02 mg/kg (tissue)	GC-MS	xxx., 1999/EU agreed
	Confirmatory (if required)	0.02 mg/kg	GC-ECD	xxx 1999/EU agreed
Soil (Environmental fate)	Primary	0.002 mg/kg	LC-MS/MS	Bacher R., 2002/EU agreed
	Confirmatory (if required)	-	Not required	-
Water (Environmental fate)	Primary	0.05 µg/L	LC-MS/MS	Bacher R., 2002/EU agreed
	Confirmatory (if required)	-	Not required	-
Air (Exposure)	Primary	0.4 µg/m³	LC-MS/MS	Bacher R., 2002/EU agreed
	Confirmatory (if required)	-	Not required	-
Water (Ecotoxicology)	Primary	2.064 µg/L	HPLC-MS/MS	Juckeland D., 2021a
		0.07079 µg/L	HPLC-MS/MS	Juckeland D., 2021b
		0.2939 µg/L	HPLC-MS/MS	Juckeland D., 2021c
		0.03097 µg/L	HPLC-MS/MS	Juckeland D., 2021d
	Confirmatory (if required)	-	Not required	-
Other (Ecotoxicology)	Primary	0.12 mg/kg	HPLC-MS/MS	Amsel K., 2021
		431.42 mg/L	HPLC-MS/MS	Amsel K., 2021
		0.12 mg/kg	HPLC-MS/MS	Ruhland S., 2021
		0.10 mg/kg	HPLC-MS/MS	Schmidt K., 2021

		605.3 mg/L	HPLC-MS/MS	Lewington-Gower M., 2021
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*Validation of the method used in the residue trials submitted in section B7.

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 prosulfocarb (KCP 5.2)

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

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

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

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Plant, high water content	Prosulfocarb	0.01 mg/kg	EFSA, 2007
Plant, high acid content		0.01 mg/kg	EFSA, 2007
Plant, high protein/high starch content (dry commodities)		0.01 mg/kg	EFSA, 2007
Plant, high oil content		0.01 mg/kg	EFSA, 2007
Plant, difficult matrices (hops, spices, tea)		0.01 mg/kg	EFSA, 2007
Muscle	Not required		EFSA, 2007
Milk			
Eggs			
Fat			
Liver, kidney			
Soil (Ecotoxicology)	Prosulfocarb	0.02 mg/kg	EFSA, 2007
Drinking water (Human toxicology)	Prosulfocarb	0.1 µg/L	general limit for drinking water
Surface water (Ecotoxicology)	Prosulfocarb	0.1 µg/L	EFSA, 2007
Air	Prosulfocarb	0.00015 mg/m ³	AOEL: 0.007 mg/kg bw/d

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Tissue (meat or liver)	-	Not required	Not classified as T / T+
Body fluids		Not required	Not classified as T / T+

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

An overview on the acceptable methods and possible data gaps for analysis of prosulfocarb 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: Prosulfocarb				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
High water content	Primary	0.01 mg/kg	GC-MSD (multi-residue)	Weber H, Pelz S., 2000/EU agreed
	ILV	0.01 mg/kg	GC-MSD (multi-residue)	Ryan J, Osborne V., 2000/EU agreed
	Confirmatory (if required)	-	Not required	-
High acid content	Primary	0.01 mg/kg	GC-MSD (multi-residue)	Weber H, Pelz S., 2000/EU agreed
	ILV	0.01 mg/kg	GC-MSD (multi-residue)	Ryan J, Osborne V., 2000/EU agreed
	Confirmatory (if required)	-	Not required	-
High oil content	Primary	0.01 mg/kg	GC-MSD (multi-residue)	Weber H, Pelz S., 2000/EU agreed
	ILV	0.01 mg/kg	GC-MSD (multi-residue)	Ryan J, Osborne V., 2000/EU agreed
	Confirmatory (if required)	-	Not required	-
High protein/high starch content (dry)	Primary	0.01 mg/kg	GC-MSD (multi-residue)	Weber H, Pelz S., 2000/EU agreed
	ILV	0.01 mg/kg	GC-MSD (multi-residue)	Ryan J, Osborne V., 2000/EU agreed
	Confirmatory (if required)	-	Not required	-
Difficult (if required, depends on intended use)	Not required			

Table 5.3-3: Statement on extraction efficiency

	Method for products of plant origin
Required, available from:	The DFG S19 multi-residue method uses mixtures of acetone and water as an extraction solvent. Acetone/water mixtures and acetonitrile/water mixtures are considered to be of similar polarity. Prosulfocarb is equally soluble in both solvent mixtures. The solvent system is considered to be similar to that used in the carrot metabolism study (Derz, 2015). The extraction system is therefore validated and fit-for-purpose.
Not required, because:	-

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

Not required.

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

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

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

Component of residue definition: Prosulfocarb			
Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Primary	0.02 mg/kg	GC-MSD	Crook, 2000/EU agreed
Confirmatory	-	Not required	-

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 prosulfocarb in surface and drinking water is given in the following tables.

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

Component of residue definition: Prosulfocarb				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Drinking water	Primary	0.1 µg/L	GC-MSD	Hargreaves, 1999/EU agreed
	ILV	missing		
	Confirmatory	-	Not required	-
Surface water	Primary	0.1 µg/L	GC-MSD	Hargreaves, 1999/EU agreed
	Confirmatory	-	Not required	-

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 prosulfocarb in air is given in the following tables.

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

Component of residue definition: Prosulfocarb			
Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Primary	0.00015 µg/m ³	GC-MSD	Kwaitkowski, 2002/EU agreed
Confirmatory	-	Not required	-

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

Prosulfocarb and prosulfocarb metabolites are not defined as “relevant for monitoring” and are not categorised as toxic or very toxic in any recognised classification system. Consequently, analytical methods for post-approval control of residues in body fluids and tissues are not required.

5.3.2.8 Other studies/ information

No other studies were submitted.

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

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

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

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

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Plant, high water content	Diflufenican	0.01 mg/kg	EFSA, 2007
Plant, high acid content		0.01 mg/kg	EFSA, 2007
Plant, high protein/high starch content (dry commodities)		0.01 mg/kg	EFSA, 2007
Plant, high oil content		0.01 mg/kg	EFSA, 2007
Plant, difficult matrices (hops, spices, tea)		0.01 mg/kg	EFSA, 2007
Muscle	Diflufenican	0.02 mg/kg	EFSA, 2007

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Milk		0.01 mg/kg	EFSA, 2007
Eggs		0.02 mg/kg	EFSA, 2007
Fat		0.02 mg/kg	EFSA, 2007
Liver, kidney		0.02 mg/kg	EFSA, 2007
Soil (Ecotoxicology)	Diflufenican	0.002 mg/kg	EFSA, 2007
Drinking water (Human toxicology)	Diflufenican	0.1 µg/L	general limit for drinking water
Surface water (Ecotoxicology)	Diflufenican	0.05 µg/L	EFSA, 2007
Air	Diflufenican	0.4 µg/m ³	AOEL sys/AOEL inhal: 0.11 mg/kg bw/d
Tissue (meat or liver)	-	Not required	Not classified as T / T+
Body fluids		Not required	Not classified as T / T+

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

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

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

Component of residue definition: Diflufenican				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
High water content	Primary	0.01 mg/kg	GC-ECD	Bacher R., 2002/EU agreed
	ILV	0.01 mg/kg	GC-ECD	Thom M., 2003/EU agreed
	Confirmatory (if required)	0.01 mg/kg	GC-MS	Bacher R., 2002/EU agreed
High acid content	Primary	0.01 mg/kg	GC-ECD	Bacher R., 2002/EU agreed
	ILV	0.01 mg/kg	GC-ECD	Thom M., 2003/EU agreed
	Confirmatory (if required)	0.01 mg/kg	GC-MS	Bacher R., 2002/EU agreed
High oil content	Primary	0.01 mg/kg	GC-ECD	Bacher R., 2002/EU agreed
	ILV	0.01 mg/kg	GC-ECD	Thom M., 2003/EU agreed
	Confirmatory (if required)	0.01 mg/kg	GC-MS	Bacher R., 2002/EU agreed
High protein/high	Primary	0.01 mg/kg	GC-ECD	Class T., 2001/EU agreed
	ILV	0.01 mg/kg	GC-ECD	Klumpp M., 2001/EU agreed

Component of residue definition: Diflufenican				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
starch content (dry)	Confirmatory (if required)	0.01 mg/kg	GC-MS	Class T., 2001/EU agreed
Difficult (if required, depends on intended use)	Primary	Not required		
	ILV			
	Confirmatory (if required)			

Table 5.3-9: Statement on extraction efficiency

	Method for products of plant origin
Required, available from:	As the same solvent, acetonitrile, is used in both methods, the method extraction can be considered comparable to the extraction in the metabolism study.
Not required, because:	-

5.3.3.3 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 diflufenican in animal matrices is given in the following tables.

Table 5.3-10: Validated methods for food and feed of animal origin (if appropriate)

Component of residue definition: Diflufenican				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Milk	Primary	0.01 mg/kg	GC-MS	xxx 1999/EU agreed
	ILV	0.01 mg/kg	GC-MS	xxx 2002/EU agreed
	Confirmatory (if required)	0.01 mg/kg	GC-ECD	xxx 1999/EU agreed
Eggs	Primary	0.02 mg/kg	GC-MS	xxx 1999/EU agreed
	ILV	0.01 mg/kg	GC-MS	xxx., 2002/EU agreed
	Confirmatory (if required)	0.02 mg/kg	GC-ECD	xxx 1999/EU agreed
Muscle	Primary	0.02 mg/kg	GC-MS	xxx 1999/EU agreed
	ILV	0.01 mg/kg	GC-MS	xxx 2002/EU agreed
	Confirmatory (if required)	0.02 mg/kg	GC-ECD	xxx 1999/EU agreed
Fat	Primary	0.02 mg/kg	GC-MS	xxx1999/EU agreed

Component of residue definition: Diflufenican				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Kidney, liver	ILV	0.01 mg/kg	GC-MS	xxx., 2002/EU agreed
	Confirmatory (if required)	0.02 mg/kg	GC-ECD	xxx., 1999/EU agreed
	Primary	0.02 mg/kg	GC-MS	xxx., 1999/EU agreed
	ILV	0.01 mg/kg	GC-MS	xxx., 2002/EU agreed
	Confirmatory (if required)	0.02 mg/kg	GC-ECD	xxx., 1999/EU agreed

Table 5.3-11: Statement on extraction efficiency

	Method for products of animal origin
Required, available from:	As the same solvent, acetonitrile, is used in both methods, the method extraction can be considered comparable to the extraction in the metabolism study.
Not required, because:	-

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

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

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

Component of residue definition: Diflufenican			
Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Primary	0.002 mg/kg	LC-MS/MS	Bacher R., 2002/EU agreed
Confirmatory	-	Not required	-

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

An overview on the acceptable methods and possible data gaps for analysis of diflufenican in surface and drinking water is given in the following tables. For the detailed valuation of new/ additional studies it is referred to Appendix 2.

Table 5.3-13: Validated methods for water (if appropriate)

Component of residue definition: diflufenican				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Drinking water	Primary	0.05 µg/L	LC-MS/MS	Bacher R., 2002/EU agreed

Component of residue definition: diflufenican				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
	Primary	0.05 µg/L	LC-MS/MS	Turnbull G., 2008
	ILV	0.05 µg/L	LC-MS/MS	Figueiredo H., 2016
	Confirmatory	-	Not required	-
Surface water	Primary	0.05 µg/L	LC-MS/MS	Bacher R., 2002/EU agreed
	Confirmatory	-	Not required	-

For any special comments or remarkable points concerning the analytical methods for water please refer to Appendix 2.

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

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

Table 5.3-14: Validated methods for air (if appropriate)

Component of residue definition: diflufenican			
Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Primary	0.4 µg/m ³	LC-MS/MS	Bacher R., 2002/EU agreed
Confirmatory	-	Not required	-

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

An overview on the acceptable methods and possible data gaps for analysis of diflufenican in body fluids and tissues is given in the following table. For the detailed evaluation of new/ additional studies it is referred to Appendix 2.

Table 5.3-15: Methods for body fluids and tissues (if appropriate)

Component of residue definition: diflufenican			
Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Primary	0.01 mg/kg / 0.05 mg/L	LC-MS/MS	xxx 2015
Confirmatory	-	Not required	-

5.3.3.8 Other studies/ information

No other studies were submitted.

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	Sowle J.	2020	Validation of the Methods of Determination of Prosulfocarb and Diflufenican in an EC Formulation, in Compliance with Good Laboratory Practice DNA5958 David Norris Analytical Laboratories Ltd. GLP Unpublished	N	Globachem NV
KCP 5.1.2 <i>Submitted as KCP 10.2.1</i>	Juckeland D.	2021a	Acute toxicity of GLOB1817H to <i>Daphnia magna</i> in a 48-hour semi-static test 20 48 ADL 0015 Biochem Agrar GmbH GLP Unpublished	N	Globachem NV
KCP 5.1.2 <i>Submitted as KCP 10.2.1</i>	Juckeland D.	2021b	Effects of GLOB1817H on <i>Pseudokirchneriella subcapitata</i> in an algal growth inhibition test 20 48 AAL 0019 Biochem Agrar GmbH GLP Unpublished	N	Globachem NV
KCP 5.1.2 <i>Submitted as KCP 10.2.1</i>	Juckeland D.	2021c	Effects of GLOB1817H on <i>Lemna gibba</i> in a growth inhibition test under semi-static test conditions 20 48 ALE 0017 Biochem Agrar GmbH GLP Unpublished	N	Globachem NV
KCP 5.1.2 <i>Submitted as KCP</i>	Juckeland D.	2021d	Effect of GLOB1817H on <i>Myriophyllum spicatum</i> in a semi-static water-sediment system 20 48 AMS 0010 Biochem Agrar GmbH	N	Globachem NV

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
10.2.1			GLP Unpublished		
KCP 5.1.2 Submitted as KCA 8.2.6.1	Juckeland, D.	2012a	Effects of Prosulfocarb sulfoxide on <i>Chlamydomonas reinhardtii</i> in an algal growth inhibition test 12 10 48 057 W Biochem Agrar GmbH GLP Unpublished	N	Globachem NV
KCP 5.1.2 Submitted as KCA 8.2.6.1	Juckeland, D.	2012b	Effects of Prosulfocarb sulfoxide on <i>Chlorella vulgaris</i> in an algal growth inhibition test 12 10 48 059 W Biochem Agrar GmbH GLP Unpublished	N	Globachem NV
KCP 5.1.2 Submitted as KCA 8.2.6.2	Juckeland, D.	2012c	Effects of Prosulfocarb sulfoxide on <i>Anabaena flos-aquae</i> in an algal growth inhibition test 12 10 48 058 W Biochem Agrar GmbH GLP Unpublished	N	Globachem NV
KCP 5.1.2 Submitted as KCA 8.2.6.2	Juckeland, D.	2012d	Effects of Prosulfocarb sulfoxide on <i>Navicula pelliculosa</i> in an algal growth inhibition test 12 10 48 053 W Biochem Agrar GmbH GLP Unpublished	N	Globachem NV
KCP 5.1.2 Submitted as KCA 8.2.6.2	Juckeland, D.	2012e	Effects of Prosulfocarb sulfoxide on <i>Skeletonema costatum</i> in an algal growth inhibition test 12 10 48 060 W Biochem Agrar GmbH GLP Unpublished	N	Globachem NV
KCP 5.1.2 Submitted	Amsel, K.	2021	Acute toxicity of GLOB1817H to the bumblebee <i>Bombus terrestris</i> L. under laboratory conditions 20 48 BBA 0029	N	Globachem NV

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
as KCP 10.3.1.1.1			Biochem Agrar GmbH GLP Unpublished		
KCP 5.1.2 Submitted as KCP 10.3.1.2	Ruhland, S.	2021	Chronic toxicity of GLOB1817H to the honey bee <i>Apis mellifera</i> L. under laboratory conditions 20 48 BAC 0071 Biochem Agrar GmbH GLP Unpublished	N	Globachem NV
KCP 5.1.2 Submitted as KCP 10.3.1.3	Schmidt, K.	2021	GLOB1817H – Repeated exposure of the honey bee (<i>Apis mellifera</i> L.) larvae under laboratory conditions 20 48 BLC 0052 Biochem Agrar GmbH GLP Unpublished	N	Globachem NV
KCP 5.1.2 Submitted as KCP 10.4.1.2	Schulz, L.	2015	Effects of prosulfocarb 800 g/L EC on earthworms under field conditions. Biochem Agrar Report Number 14 10 48 008 F GLP Unpublished	N	Globachem NV
KCP 5.1.2 Submitted as KCA 8.1.3	Sacker, D.	2008	The bioaccumulation potential of prosulfocarb in earthworm (<i>Eisenia foetida foetida</i>). ENV8333/040822 Chemex Environmental International Ltd GLP Unpublished	N	Globachem NV
KCP 5.1.2 Submitted as KCP 10.6	Lewington-Gower, M.	2021	GLOB1817H: terrestrial plant test: vegetative vigour test STC/20/E1409 Stockbridge Technology Center Ltd GLP Unpublished	N	Globachem NV

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 5.1.2	Jonchère F.	2010a	Validation of the Analytical Method for the Determination of Prosulfocarb Residues in Potato Tubers, Sunflower Seeds and Winter Wheat Whole Plant + Amendment 1 to final report No R A9085 (2014) A9085 Anadiag GLP Unpublished	N	Globachem NV
KCP 5.1.2	Laguna O.	2021a	Validation of analytical method for the determination of residues of prosulfocarb in sunflower seeds. E21024 Laboratoire Phytocontrol GLP Unpublished	N	Globachem NV
KCP 5.1.2	Jonchère F.	2011	Validation of the analytical method for the determination of diflufenican residues in potato (tubers) B0133 Anadiag GLP Unpublished	N	Globachem NV
KCP 5.1.2	Laguna O.	2021b	Diflufenican – Validation of analytical methods for the determination of diflufenican and its metabolites on potato tubers E21003 Laboratoire Phytocontrol GLP Unpublished	N	Globachem NV
KCP 5.1.2	Jonchère F.	2010b	Validation of the analytical method for the determination of diflufenican residues in oilseed rape seeds. A9259 Anadiag GLP Unpublished	N	Globachem NV
KCP 5.1.2	Laguna O.	2021c	Validation of analytical method for the determination of residues of diflufenican and its metabolites and conjugates in sunflower seeds. E21023	N	Globachem NV

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
			Laboratoire Phytocontrol GLP Unpublished		
KCP 5.2	Turnbull G.	2008	Development and validation of a method for the determination of diflufenican and two metabolites in surface water and drinking water. PGD-307 Central Science Laboratory GLP Unpublished	N	Sapac Group & Globachem NV & Punjab Chemicals and Crop Protection Ltd.
KCP 5.2	Figueiredo H.	2016	Validation of an analytical method for the determination of diflufenican in drinking water, ILV. VAL10/16 Laboratório de residuos Sapac Agro S.A. GLP Unpublished	N	Sapac Agro S.A. & Globachem NV
KCP 5.2	xxx	2015	Validation of the analytical method for the analysis of diflufenican in fat and blood. B6276 Anadiag GLP Unpublished	N	Sapac Agro S.A. & Globachem NV

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
None					

Appendix 2 Detailed evaluation of submitted analytical methods

A 2.1 Analytical methods for prosulfocarb

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

Reference is made to 5.2.1 for a summary of the methods used to determine the active substances in the formulated product.

A 2.1.1.1.1 Analytical method for prosulfocarb in cereals, potato and sunflower

A 2.1.1.1.1.1 Method validation

Comments of zRMS:	<p>The validation in potato tubers, sunflowers and winter wheat at 0,01 mg/kg has been accepted.</p> <p>Analysis of Prosulfocarb in LC/MS-MS, with monitoring of two transitions can be considered highly specific. The use of confirmation method is not necessary. On 2 validated levels a mean recovery is within the range 70-110 % with a RSD less than 20 %.</p> <p>This study was already evaluated in PL.</p>
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Reference:	KCP 5.1.2
Report	Validation of the Analytical Method for the Determination of Prosulfocarb Residues in Potato Tubers, Sunflower Seeds and Winter Wheat Whole Plant + Amendment 1 to final report No. R A9085 (2014), Jonchère F, 2010a, A9085.
Guideline(s):	Yes, SANCO/825/00 rev. 8.1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and Methods

The method is based on the following reference: “Foods of plant origin - Determination of pesticide residues using GC-MS and/or LC-MS/MS following acetonitrile extraction/partitioning and clean-up by dispersive SPE - QuEChERS-method.”

Residues are extracted with acetonitrile/acetic acid 99.9 : 0.1 % in the presence of magnesium sulfate and sodium chloride. After centrifugation the extract is purified with magnesium sulfate and PSA. The internal standard (triphenylphosphate in acetonitrile) and formic acid are added to the extract before analysis by liquid chromatography using a MS/MS detector with the following conditions:

Apparatus	UPLC /MS /MS
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Column

Description	BEH C18	Supplier	WATERS	Particles	1.7 µm
Internal diam. x length	2.1*100 mm	Supplier reference	186002352	Temperature	40 °C
Development Column ANADIAG Number	130	Stationary Phase	C18	Comment	-

Mobile phase

A =	Methanol HPLC/ H2O HPLC 20:80 + 5 mM ammonium acetate	C =	-
B =	Methanol HPLC/ H2O HPLC 90:10 + 5 mM ammonium acetate	D =	-

Sample temperature	15 °C
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Elution

Elution	Time min	Flow ml/min	Composition (%)				Curve (type)
			A	B	C	D	
Pg1	0.00	0.40	100	0	-	-	1
Pg2	4.00	0.40	0	100	-	-	1
Pg3	6.40	0.40	0	100	-	-	1
Pg4	6.50	0.40	100	0	-	-	1
Pg5	8.00	0.40	100	0	-	-	1

Detector

IONISATION mode*	ES x	APCI
Polarity*	Pos x	Neg

*make a cross in the right choice

Active ingredient(s)	Cone voltage	Collision Voltage	Dwell time (ms)	TRANSITION 1	TRANSITION 2	RT (min.)
				Parent > Daughter	Parent > Daughter	
Prosulfocarb	20	15	50	252.1 > 91.2	-	≈ 4.8
	25	10		-	252.1 > 128.3	≈ 4.8
Triphenyl phosphate	40	27		327.0 > 215.0	-	≈ 4.5

Results and Discussion

The results of the validation of the 252.1 > 91.2 transition are given below and show that the method meets the requirements of the SANCO 825/00 rev. 8.1:

- Linearity:

The linearity of the method was studied in matrix-matched calibration solutions between 3 ng/mL to 120 ng/mL of Prosulfocarb for potato tubers and sunflower seeds, and between 2 ng/mL to 60 ng/mL of Prosulfocarb for winter wheat whole plant. The linear correlation coefficients were typically > 0.990, showing a good linearity.

- Sensitivity:

The limit of quantification (LOQ) is the lowest validated level where a mean recovery within the range 70-110 % with a RSD less than 20 % could be obtained.

The LOQ was set at 0.01 mg/kg in potato tubers, sunflower seeds and winter wheat whole plant.

- Precision:

Repeatability tests (5 recoveries at each fortification level) were performed at LOQ level and at 10 x LOQ for each matrix.

	Prosulfocarb
RSD for each fortification level	1.2 to 9.3 %
Overall RSD per sample material	2.0 to 10.8 %

All RSD determined were less than 20%, the method therefore fulfills the requirements of residue analytical methods.

- Recovery/accuracy:

The recovery results are presented hereunder. The accuracy of the method fulfills the requirements for residue analytical methods which demand that the mean recoveries per fortification level should be in the range 70-110 %.

Matrix	Active ingredient	Fortification level (mg/kg)	Mean recovery (%)	Standard deviation (SD) (%)	Relative standard deviation (RSD) (%)	Number of fortified samples (n)
Potato tubers	Prosulfocarb	0.01	88.2	8.2	9.3	5
		0.10	101.3	7.6	7.5	5
		All levels	94.7	10.2	10.8	10
Sunflower seeds	Prosulfocarb	0.01	91.4	1.1	1.2	5
		0.10	88.7	1.1	1.3	5
		All levels	90.0	1.8	2.0	10
Winter wheat whole plant	Prosulfocarb	0.01	107.3	1.6	1.5	5
		0.10	105.3	3.9	3.7	5
		All levels	106.3	3.0	2.8	10

- Specificity:

Analysis of Prosulfocarb in LC/MS-MS, with monitoring of two transitions is considered as specific, thus the use of an alternative method was not necessary. However, to meet the requirements of the SANCO 825/00 rev. 8.1, the validation data of a second transition are provided below.

Table A 1: Validation data for a second transition at 0.01 ppm (252.1 > 128.3)

Crop/Matrix	Study	Analytical method
Winter wheat whole plant	Validation	Prosulfocarb in the untreated (n=2): <LOD Linearity: $R^2 = 0.996$ Recovery whole plant (n=5): 99.7-108.1 (mean = 104.6%) Precision whole plant (n=5): 3.3%
Sunflower seeds	Validation	Prosulfocarb in the untreated (n=2): <LOD Linearity: $R^2 = 0.996$ Recovery sunflower (n=5): 84.3-92.2% (mean = 89%) Precision sunflower: RSD (n=5): 3.3%
Potato tubers	Validation	Prosulfocarb in the untreated (n=2): <LOD Linearity: $R^2 = 0.996$ Recovery potato (n=5): 71.4-91.3% (mean = 86.2%) Precision potato: RSD (n=5): 9.7%

Conclusion

The method meets the requirement of the SANCO 825/00 rev. 8.1 and can be used to reliably and accurately determine prosulfocarb in potato tubers, sunflowers and winter wheat to a limit of quantification of 0.01

mg/kg.

Comments of zRMS:	<p>The method has been accepted.</p> <p>The purpose of this study was to validate the analytical method for the determination of Prosulfocarb in sunflower seeds.</p> <p>For Prosulfocarb, one reagent blank sample, 2 control samples, 5 samples fortified at the limit of quantification (LOQ) (<i>i.e.</i> 0.01 mg/kg) and 5 samples fortified at 10 times the LOQ (<i>i.e.</i> 0.1 mg/kg) were analysed in sunflower seeds.</p> <p>Acceptable mean recoveries between 70% and 120%, with a relative standard deviation less than 20%, were found for both primary and confirmatory transitions for Prosulfocarb in sunflower seeds.</p> <p>Significant matrix effect (suppression or enhancement; $> \pm 20\%$) on the detector response were observed in all matrices for Prosulfocarb.</p> <p>Therefore, matrix-matched standards were used for calibration and quantification for all matrices and for both mass transitions (primary and confirmatory modes). The response of the LC-MS/MS detector was shown to be linear for Prosulfocarb for each mass transition over a concentration range of 0.3 to 15 ng/mL (equivalent to 0.003 to 0.15 mg/kg) for matrix-matched standards in all matrices. Visual inspection also showed that the regression residuals were randomly distributed for each calibration curve for each analyte, and hence linear calibration was demonstrated.</p> <p>The limit of detection (LOD) was determined for Prosulfocarb for both the primary and confirmatory transitions and was found to be equivalent to less than 0.003 mg/kg (30% of the LOQ) in all matrices.</p> <p>The limit of quantification (LOQ) of the analytical method for Prosulfocarb was established at 0.01 mg/kg in all matrices.</p> <p>The stability of Prosulfocarb in final sample extracts stored in amber glass vials at 4°C, was assessed. Sample extracts were re-analysed after 7 days of storage, and their recoveries were compared against freshly prepared calibration standards (matrix-matched). Prosulfocarb residues were stable in the final extracts when stored at a target temperature of 4°C for 7 days in sunflower seed extracts.</p> <p>Residues of Prosulfocarb measured in the control samples were below 30% of the limit of quantification (LOQ; 0.01 mg/kg) in all of the control and reagent blank samples used in this study. This demonstrates that no interferences were present at the retention time of Prosulfocarb in the test systems. This is in accordance with the level specified in SANTE/2020/12830 Rev.1.</p> <p>The repeatability and specificity of the method have been demonstrated and the analytical method is therefore considered valid for the determination of residues of Prosulfocarb in sunflower seeds at the LOQ of 0.01 mg/kg over concentration ranges typical of those for which the method will be used.</p> <p>Since two characteristic mass transitions are used to monitor Prosulfocarb, the method achieves a high level of specificity and no further confirmation on a different detector was necessary. The method has been validated according to the EU guidelines SANTE/2020/12830, Rev. 1.</p>
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Reference:	KCP 5.1.2
Report	Validation of analytical method for the determination of residues of prosulfocarb in sunflower seeds, Laguna O., 2021a, E21024.
Guideline(s):	Yes, SANTE/2020/12830 Rev. 1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Sunflower seeds will be homogenized using solid carbon dioxide to maintain the frozen state. Residues of prosulfocarb are extracted by agitation with acetonitrile after addition of water in presence of magnesium sulfate, sodium chloride, sodium citrate dibasic sesquihydrate and sodium citrate tribasic dihydrate. The mixture is then frozen at -20°C during 2 h. After centrifugation, an aliquot is diluted in acetonitrile prior to quantification by highly selective LC-MS/MS.

Standards were prepared in acetonitrile.

Results and discussions

Table A 2: Recovery results from method validation of prosulfocarb using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (n = x)	Mean recovery (%)	RSD (%)
Sunflower seeds	Prosulfocarb	LOQ (5)	74.1	2.6
		10 x LOQ (5)	71.1	2.0
		All levels (10)	72.6	3.1

Table A 3: Characteristics for the analytical method used for validation of residues of prosulfocarb in sunflower seeds

	Prosulfocarb
Specificity	blank value < 30 % LOQ
Calibration (type, number of data points)	Linear R ² > 0.990 8 data points
Calibration range	0.15 – 15 ng/mL
Assessment of matrix effects is presented	yes
Limit of determination/quantification	0.003 mg/kg / 0.01 mg/kg

Conclusion

The method was sufficiently validated according to SANTE/2020/12830 Rev. 1 and can be used to reliably and accurately determine prosulfocarb in sunflower seeds to a limit of quantification of 0.01 mg/kg.

A 2.1.1.1.2 Analytical methods in water used in aquatic toxicity studies

A 2.1.1.1.2.1 Method validation

Comments of zRMS:	The method is considered acceptable. The method validation for prosulfocarb, diflufenican and halauxifen-methyl in water meets the requirements. All validation parameters are in required range. This study was already evaluated in PL.
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Reference:	KCP 5.1.2 (submitted as KCP 10.2.1)
Report	Acute toxicity of GLOB1817H to <i>Daphnia magna</i> in a 48 hour semi-static test, Juckeland D., 2021a, 20 48 ADL 0015
Guideline(s):	Yes, OECD 202 (2004)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The samples were stabilised with an equal amount of 2-propanol after sampling and stored frozen. For analysis the samples were thawed at room temperature and homogenised by shaking. Aliquots were diluted with deionised and diluent in autosampler vials. No further extraction/purification/enrichment step was performed. The vials were placed in the cooled autosampler and the diluted aliquots were injected directly into the HPLC-system.

All standards were prepared in methanol.

Results and discussions

Table A 4: Recovery results from method validation of prosulfocarb, diflufenican and halauxifen-methyl using the analytical method

Matrix	Analyte	Fortification level (µg/L) (n = x)	Mean recovery (%)	RSD (%)
Reconstituted water	Prosulfocarb	97.82 (5)	100.2	1.4
		1304 (5)	101.2	0.9
	Diflufenican	2.064 (5)	90.6	1.1
		27.53 (5)	93.2	0.8
	Halauxifen-methyl	0.1923 (5)	87.8	2.4
		2.565 (5)	90.4	0.9

Table A 5: Characteristics for the analytical method used for validation of prosulfocarb, diflufenican and halauxifen-methyl residues in reconstituted water

	Prosulfocarb	Diflufenican	Halauxifen-methyl
Specificity	blank value < 30 % LOQ	blank value < 30 % LOQ	blank value < 30 % LOQ
Calibration (type, number of data points)	Cubic Y = -1.325X ³ + 343.5X ² + 117602X + 181208 r ² = 0.99990 number of data points: 7	Linear Y = 865847X + 9018 r ² = 0.99968 number of data points: 7	Quadratic Y = -1986110X ² + 5179720X - 6440 r ² = 0.99973 number of data points: 7
Calibration range	11.59 – 165.5 µg/L	0.2443 – 3.490 µg/L	0.02280 – 0.3258 µg/L
Assessment of matrix effects is presented	yes	yes	yes
Limit of	LOQ = 97.82 µg/L / LOD = 29.34 µg/L	2.064 µg/L / 0.6193 µg/L	0.1923 µg/L / 0.05770 µg/L

	Prosulfocarb	Diufenenican	Halaxifen-methyl
Specificity	blank value < 30 % LOQ	blank value < 30 % LOQ	blank value < 30 % LOQ
determination/quantification			

Conclusion

The method was sufficiently validated according to SANTE/2020/12830, Rev.1 and can be used for analytical determination of prosulfocarb, diufenenican and halaxifen-methyl in reconstituted water.

Comments of zRMS:	<p>The method has been accepted.</p> <p>The purpose of the method was the determination of prosulfocarb, diufenenican and halaxifen-methyl in aquatic matrix.</p> <p>RP-LC-MS/MS technique was employed. The validation parameters were in required range. The specificity of the method was assured by MS/MS detection and the absence of interfering peaks.</p> <p>This study was already evaluated in PL.</p>
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Reference:	KCP 5.1.2 (submitted as KCP 10.2.1)
Report	Effects of GLOB1817H on <i>Pseudokirchneriella subcapitata</i> in an algal growth inhibition test, Juckeland D., 2021b, 20 48 AAL 0019
Guideline(s):	Yes, OECD 201 (2011)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The samples were stabilised with an equal amount of 2-propanol after sampling and stored frozen. For analysis the samples were thawed at room temperature and homogenised by shaking. Aliquots were diluted with deionised water and/or diluent in autosampler vials. No further extraction/purification/enrichment step was performed. The vials were placed in the cooled autosampler and the diluted aliquots were injected directly into the HPLC-system.

All standards were prepared in methanol.

Results and discussions

Table A 6: Recovery results from method validation of prosulfocarb, diufenenican and halaxifen-methyl using the analytical method

Matrix	Analyte	Fortification level (µg/L) (n = x)	Mean recovery (%)	RSD (%)
Reconstituted water	Prosulfocarb	3.354 (5)	103.2	1.2
		74.53 (5)	101.5	0.5
	Diufenenican	0.07079 (5)	100.9	9.3
		1.573 (5)	93.3	0.6

Matrix	Analyte	Fortification level (µg/L) (n = x)	Mean recovery (%)	RSD (%)
	Halauxifen-methyl	0.006595 (5)	88.2	1.9
		0.1466 (5)	88.5	1.2

Table A 7: Characteristics for the analytical method used for validation of prosulfocarb, diflufenican and halauxifen-methyl residues in OECD medium

	Prosulfocarb	Diflufenican	Halauxifen-methyl
Specificity	blank value < 30 % LOQ	blank value < 30 % LOQ	blank value < 30 % LOQ
Calibration (type, number of data points)	Linear Y = 176690X + 12164 r ² = 0.99993 number of data points: 8	Linear Y = 3664590X + 10807 r ² = 0.99982 number of data points: 8	Linear Y = 5986500X + 1397 r ² = 0.99996 number of data points: 8
Calibration range	0.7425 – 27.50 µg/L	0.01565 – 0.5798 µg/L	0.001461 – 0.05412 µg/L
Assessment of matrix effects is presented	yes	yes	yes
Limit of determination/quantification	1.006 µg/L / 3.354 µg/L	0.02124 µg/L / 0.07079 µg/L	0.001979 µg/L / 0.006595 µg/L

Conclusion

The method was sufficiently validated according to SANTE/2020/12830, Rev.1 and can be used for analytical determination of prosulfocarb, diflufenican and halauxifen-methyl in OECD medium.

Comments of zRMS:	<p>The method is considered acceptable.</p> <p>The purpose of the method was the determination of prosulfocarb, diflufenican and halauxifen-methyl in test solutions.</p> <p>RP-LC-MS/MS technique was applied. The validation parameters were in required range. The specificity of the method was assured by MS/MS detection and the absence of interfering peaks.</p> <p>This study was already evaluated in PL.</p>
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Reference:	KCP 5.1.2 (submitted as KCP 10.2.1)
Report	Effects of GLOB1817H on <i>Lemna gibba</i> in a growth inhibition test under semi-static conditions, Juckeland D., 2021c, 20 48 ALE 0017
Guideline(s):	Yes, OECD 221 (2006)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The samples were stabilised with an equal amount of 2-propanol after sampling and stored frozen. For analysis the samples were thawed at room temperature and homogenised by shaking. Aliquots were diluted with diluent and/or deionised water in autosampler vials. No further extraction/purification/enrichment

step was performed. The vials were placed in the cooled autosampler and the diluted aliquots were injected directly into the HPLC-system.

All standards were prepared in methanol.

Results and discussions

Table A 8: Recovery results from method validation of prosulfocarb, diflufenican and halauxifen-methyl using the analytical method

Matrix	Analyte	Fortification level (µg/L) (n = x)	Mean recovery (%)	RSD (%)
20x AAP medium	Prosulfocarb	13.92 (5)	108.9	1.4
		480.2 (5)	106.3	0.7
	Diflufenican	0.2939 (5)	95.9	1.6
		10.13 (5)	96.5	0.6
	Halauxifen-methyl	0.02738 (5)	95.5	2.1
		0.9442 (5)	94.4	2.4

Table A 9: Characteristics for the analytical method used for validation of prosulfocarb, diflufenican and halauxifen-methyl residues in 20x AAP medium

	Prosulfocarb	Diflufenican	Halauxifen-methyl
Specificity	blank value < 30 % LOQ	blank value < 30 % LOQ	blank value < 30 % LOQ
Calibration (type, number of data points)	Linear Y = 154140X + 28666 r ² = 0.99988 number of data points: 7	Linear Y = 402287X + 10120 r ² = 0.99877 number of data points: 7	Linear Y = 4031440X + 3651 r ² = 0.99930 number of data points: 7
Calibration range	3.053 – 64.95 µg/L	0.06436 – 1.369 µg/L	0.006008 – 0.1278 µg/L
Assessment of matrix effects is presented	yes	yes	yes
Limit of determination/quantification	LOQ = 13.92 µg/L / LOD = 4.177 µg/L	0.2939 µg/L / 0.08817 µg/L	0.02738 µg/L / 0.008215 µg/L

Conclusion

The method was sufficiently validated according to SANTE/2020/12830, Rev.1 and can be used for analytical determination of prosulfocarb, diflufenican and halauxifen-methyl in 20x AAP medium.

Comments of zRMS:	<p>The method is considered acceptable.</p> <p>The purpose of the method was the verification of the concentrations of prosulfocarb, diflufenican and halauxifen-methyl in the test solutions. RP-LC-MS/MS technique was applied. The validation parameters were in required range. The specificity of the method was assured by MS/MS detection and the absence of interfering peaks.</p> <p>This study was already evaluated in PL.</p>
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Reference:	KCP 5.1.2 (submitted as KCP 10.2.1)
Report	Effects of GLOB1817H on <i>Myriophyllum spicatum</i> in a semi-static water-sediment system, Juckeland D., 2021d, 20 48 AMS 0010
Guideline(s):	Yes, OECD 239 (2014)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The samples were stabilised with an equal amount of 2-propanol after sampling and stored frozen. For analysis the samples were thawed at room temperature and homogenised by shaking. Aliquots were diluted with diluent and/or deionised water in autosampler vials. No further extraction/purification/enrichment step was performed. The vials were placed in the cooled autosampler and the diluted aliquots were injected directly into the HPLC-system.

All standards were prepared in methanol.

Results and discussions

Table A 10: Recovery results from method validation of prosulfocarb, diflufenican and halauxifen-methyl using the analytical method

Matrix	Analyte	Fortification level (µg/L) (n = x)	Mean recovery (%)	RSD (%)
Smart and Barko medium	Prosulfocarb	1.467 (5)	95.8	2.5
		1334 (5)	99.6	1.0
	Diflufenican	0.03097 (5)	88.4	3.3
		28.15 (5)	89.4	0.7
	Halauxifen-methyl	0.002885 (5)	83.2	6.3
		2.623 (5)	87.5	1.2

Table A 11: Characteristics for the analytical method used for validation of prosulfocarb, diflufenican and halauxifen-methyl residues in Smart and Barko medium

	Prosulfocarb	Diflufenican	Halauxifen-methyl
Specificity	blank value < 30 % LOQ	blank value < 30 % LOQ	blank value < 30 % LOQ
Calibration (type, number of data points)	Linear Y = 189008X + 23020 r ² = 0.99971 number of data points: 8	Quadratic Y = -681034X ² + 6168920X - 11553 r ² = 0.99997 number of data points: 8	Linear Y = 5904100X + 1487 r ² = 0.99987 number of data points: 8
Calibration range	0.3243 – 16.21 µg/L	0.006837 – 0.3419 µg/L	0.0006382 – 0.03191 µg/L
Assessment of matrix effects is presented	yes	yes	yes

	Prosulfocarb	Diflufenican	Halauxifen-methyl
Specificity	blank value < 30 % LOQ	blank value < 30 % LOQ	blank value < 30 % LOQ
Limit of determination/quantification	0.4402 µg/L / 1.467 µg/L	0.009291 µg/L / 0.03097 µg/L	0.008656 µg/L / 0.002885 µg/L

Conclusion

The method was sufficiently validated according to SANTE/2020/12830, Rev.1 and can be used for analytical determination of prosulfocarb, diflufenican and halauxifen-methyl in Smart and Barko medium.

Comments of zRMS:	<p>The method has been accepted.</p> <p>The purpose of the method was the determination of prosulfocarb sulfoxide in the test solutions. In the method RP-HPLC with MS and UV detection were applied. Only signals of the MS detector were used to calculate the results. The recoveries were within the required range. LOQ was set at 6,21 µg/L.</p> <p>UV detector data were reported as additional information.</p> <p>This study was already evaluated in PL.</p>
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Reference:	KCP 5.1.2 (Submitted as KCA 8.2.6.1)
Report	Effects of Prosulfocarb sulfoxide on <i>Chlamydomonas reinhardtii</i> in an algal growth inhibition test, Juckeland D., 2012a, 12 10 48 057 W
Guideline(s):	Yes, OECD 201
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The samples were thawed at room temperature, homogenized by shaking and aliquots filled into autosampler vials.

Standard solutions were dissolved in methanol and diluted in water.

All samples were analysed using HPLC-MS/MS (m/z 160.1) and UV/VIS (250 nm).

Results and discussions

Table A 12: Recovery results from method validation of prosulfocarb sulfoxide using the analytical method

Matrix	Analyte	Detection method	Fortification level (µg/L) (n = x)	Mean recovery (%)	RSD (%)
Test medium	Prosulfocarb sulfoxide	MS	6.21 (5)	97	2.5
			577.80 (5)	99	1.3
		UV	577.80 (5)	100	0.9
			UV detection could not be successfully validated, the sensitivity was too low. The results of the UV detector		

Matrix	Analyte	Detection method	Fortification level (µg/L) (n = x)	Mean recovery (%)	RSD (%)
			are reported as additional information.		

Table A 13: Characteristics for the analytical method used for validation of prosulfocarb sulfoxide residues in test medium

	Prosulfocarb sulfoxide
Specificity	blank value < 30 % LOQ
Calibration (type, number of data points)	UV: Linear $y = 81.6976x - 83.746$ $r^2 = 0.9999$ MS/MS: Quadratic $y = 2.918629x^2 + 4249.561x + 11575.14$ $r^2 = 0.9993$ number of data points: 7
Calibration range	4.97 – 597.306 µg/L
Assessment of matrix effects is presented	yes
Limit of quantification	6.21 µg/L

Conclusion

The method was sufficiently validated according to SANCO/3029/99 Rev. 4 and can be used for analytical determination of prosulfocarb sulfoxide in test medium.

Comments of zRMS:	The method has been accepted. The purpose of the method was the determination of prosulfocarb sulfoxide in the test solutions. In the method RP-HPLC with MS and UV detection were applied. The recoveries were within the required range. LOQ was set at 38,0 µg/L. This study was already evaluated in PL.
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Reference:	KCP 5.1.2 (Submitted as KCA 8.2.6.1)
Report	Effects of Prosulfocarb sulfoxide on <i>Chlorella vulgaris</i> in an algal growth inhibition test, Juckeland D., 2012b, 12 10 48 059 W
Guideline(s):	Yes, OECD 201
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The samples were thawed at room temperature, homogenized by shaking and aliquots filled into

autosampler vials.

Standard solutions were dissolved in methanol and diluted in water.

All samples were analysed using HPLC-MS/MS (m/z 160.1) and UV/VIS (250 nm).

Results and discussions

Table A 14: Recovery results from method validation of prosulfocarb sulfoxide using the analytical method

Matrix	Analyte	Detection method	Fortification level (µg/L) (n = x)	Mean recovery (%)	RSD (%)
Test medium	Prosulfocarb sulfoxide	MS	38.0 (5)	104	3.5
			2003 (5)	98	5.4
		UV	38.0 (5)	89	5.3
			2003 (5)	100	1.0

Table A 15: Characteristics for the analytical method used for validation of prosulfocarb sulfoxide residues in test medium

	Prosulfocarb sulfoxide
Specificity	blank value < 30 % LOQ
Calibration (type, number of data points)	UV: Linear $y = 44.3018x + 163.337$ $r^2 = 0.9999$ MS/MS: Quadratic $y = 0.2051259x^2 + 1214.308x + 14167.29$ $r^2 = 0.9994$ number of data points: 6
Calibration range	4 – 2407.5 µg/L
Assessment of matrix effects is presented	yes
Limit of quantification	38 µg/L

Conclusion

The method was sufficiently validated according to SANCO/3029/99 Rev. 4 and can be used for analytical determination of prosulfocarb sulfoxide in test medium.

Comments of zRMS:	The method has been accepted. The purpose of the method was the determination of prosulfocarb sulfoxide in the test solutions. In the method RP-HPLC with MS and UV detection were applied. The recoveries were within the required range. LOQ was set at 100,15 µg/L. This study was already evaluated in PL.
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Report	Effects of Prosulfocarb sulfoxide on <i>Anabaena flos-aquae</i> in an algal growth inhibition test, Juckeland D., 2012c, 12 10 48 058 W
Guideline(s):	Yes, OECD 201
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The samples were thawed at room temperature, homogenized by shaking and aliquots filled into autosampler vials.

Standard solutions were dissolved in methanol and diluted in water.

All samples were analysed using HPLC-MS/MS (m/z 160.1) and UV/VIS (250 nm).

Results and discussions

Table A 16: Recovery results from method validation of prosulfocarb sulfoxide using the analytical method

Matrix	Analyte	Detection method	Fortification level (µg/L) (n = x)	Mean recovery (%)	RSD (%)
Test medium	Prosulfocarb sulfoxide	MS	3100.15 (5)	101.34	1.9
			1637 (5)	98	3.2
			51039 (5)	92	2.5
		UV	3100.15 (5)	93	5.7
			1637 (5)	101	1.5
			51039 (5)	103	0.3

Table A 17: Characteristics for the analytical method used for validation of prosulfocarb sulfoxide residues in test medium

	Prosulfocarb sulfoxide
Specificity	blank value < 30 % LOQ
Calibration (type, number of data points)	UV: Linear $y = 18.4253x + 129.72$ $r^2 = 0.9999$ MS/MS: Quadratic $y = 0.1192590x^2 + 706.9643x + 32486.08$ $r^2 = 0.9995$ number of data points: 5
Calibration range	80.12 – 2003.04 µg/L
Assessment of matrix effects is presented	yes

	Prosulfocarb sulfoxide
Limit of quantification	100.15 µg/L

Conclusion

The method was sufficiently validated according to SANCO/3029/99 Rev. 4 and can be used for analytical determination of prosulfocarb sulfoxide in test medium.

Comments of zRMS:	<p>The method has been accepted.</p> <p>The purpose of the method was the determination of prosulfocarb sulfoxide in the test solutions. In the method RP-HPLC with MS and UV detection were applied. The recoveries were within the required range. LOQ was set at 100,2 µg/L. This study was already evaluated in PL.</p>
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Reference:	KCP 5.1.2 (Submitted as KCA 8.2.6.2)
Report	Effects of Prosulfocarb sulfoxide on <i>Navicula pelliculosa</i> in an algal growth inhibition test, Juckeland D., 2012d, 12 10 48 053 W
Guideline(s):	Yes, OECD 201
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The samples were thawed at room temperature, homogenized by shaking and aliquots filled into autosampler vials.

Standard solutions were dissolved in methanol and diluted in water.

All samples were analysed using HPLC-MS/MS (m/z 160.1) and UV/VIS (250 nm).

Results and discussions

Table A 18: Recovery results from method validation of prosulfocarb sulfoxide using the analytical method

Matrix	Analyte	Detection method	Fortification level (µg/L) (n = x)	Mean recovery (%)	RSD (%)
Test medium	Prosulfocarb sulfoxide	MS	100.2 (5)	95.9	4.2
			1541 (5)	91	1.3
			50076 (5)	90	2.7
		UV	100.2 (5)	99	3.1
			15417 (5)	101	1.8
			500769 (5)	97	2

Table A 19: Characteristics for the analytical method used for validation of prosulfocarb sulfoxide residues in test medium

	Prosulfocarb sulfoxide
Specificity	blank value < 30 % LOQ
Calibration (type, number of data points)	UV: Linear $y = 43.4484x - 290.748$ $r^2 = 0.9998$ MS/MS: Quadratic $y = 0.2507586x^2 + 1328.515x + 88046.21$ $r^2 = 0.9985$ number of data points: 5
Calibration range	78.1 – 2003.0 µg/L
Assessment of matrix effects is presented	yes
Limit of quantification	100.2 µg/L

Conclusion

The method was sufficiently validated according to SANCO/3029/99 Rev. 4 and can be used for analytical determination of prosulfocarb sulfoxide in test medium.

Comments of zRMS:	The method has been accepted. The purpose of the method was the determination of prosulfocarb sulfoxide in the test solutions. In the method RP-HPLC with MS and UV detection were applied. The recoveries were within the required range. LOQ was set at 10,76 µg/L. This study was already evaluated in PL.
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Reference:	KCP 5.1.2 (Submitted as KCA 8.2.6.2)
Report	Effects of Prosulfocarb sulfoxide on <i>Skeletonema costatum</i> in an algal growth inhibition test, Juckeland D., 2012e, 12 10 48 060 W
Guideline(s):	Yes, OECD 201
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The samples were thawed at room temperature, homogenized by shaking and aliquots filled into autosampler vials.

Standard solutions were dissolved in methanol and diluted in water.

All samples were analysed using HPLC-UV/VIS (250 nm).

Results and discussions

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

Matrix	Analyte	Detection method	Fortification level (µg/L) (n = x)	Mean recovery (%)	RSD (%)
Test medium	Prosulfocarb sulfoxide	UV	10.76 (5)	99	4.5
			606.7 (5)	109	1.7

Table A 21: Characteristics for the analytical method used for validation of prosulfocarb sulfoxide residues in test medium

	Prosulfocarb sulfoxide
Specificity	blank value < 30 % LOQ
Calibration (type, number of data points)	Linear y= 800.23x – 925.649 r ² = 0.9999 number of data points: 6
Calibration range	8.59 – 722.25 µg/L
Assessment of matrix effects is presented	yes
Limit of quantification	10.76 µg/L

Conclusion

The method was sufficiently validated according to SANCO/3029/99 Rev. 4 and can be used for analytical determination of prosulfocarb sulfoxide in test medium.

A 2.1.1.1.3 Analytical methods in soil used in ecotoxicological studies

A 2.1.1.1.3.1 Method validation

Comments of zRMS:	<p>The method has been accepted.</p> <p>The purpose of the method was to determine prosulfocarb in soil. The analytical method applied was based on the highly specific LC-MS/MS technique. Two transitions were applied. The method was validated in soil. The LOQ was set at 0.1 mg/kg dry soil.</p> <p>The mean recovery for soil was in the range of 70 to 110 % consistently with the requirements.</p> <p>This study was already evaluated in PL.</p>
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Reference:	KCP 5.1.2 (Submitted as KCP 10.4.1.2)
Report	Effects of Prosulfoarb 800 g/L EC on earthworms under field conditions, Schulz L., Biochem Agrar, 14 10 48 008 F
Guideline(s):	Yes, ISO 11268-3 (1999), Kula <i>et al.</i> , 2006 - Technical recommendations to ISO 11268-3
Deviations:	No
GLP:	Yes

Acceptability: Yes
Duplication /
(if vertebrate study)

Materials and methods

The soil dry weight was determined by heating soil aliquots to a temperature of 105 °C and keeping them at that temperature until the soil weight was constant. The soil moisture analysis was carried out once for each soil specimen. 10 g of soil were weighed into an evaporating dish and thoroughly blended with 5 g of sea sand. A 22 mL extraction cell was closed at one end of the tube with a screw cap. Two round filters were placed into the bottom of the extraction cell and covered with a 0.5 cm layer of sea sand. The specimen was transferred into the extraction cell using a powder funnel. The evaporating dish and the powder funnel were rinsed with sea sand which was subsequently added to the cell. At this stage, the standard solution was applied to the blended soil/sea sand mixtures destined for fortification. The content of the cell was pressed using a piston to secure a firm consistency and filled up with sea sand to about 1 mm below the top of the cell tube. It was covered with a round filter and screwed hand-tight with another screw cap. The extraction was carried out using ASE (accelerated solvent extraction). The eluate of the ASE cell was transferred into a 50 mL volumetric flask, then filled up to the mark with “dilution solution” (CH₃OH/H₂O/HCOOH; 70/30/0.1; v/v/v) and mixed. An aliquot of approximately 1 mL of this final solution was filtered through an 0.2 µm PTFE filter into an injection vial and analysed using LC-MS/MS. Extracts above the calibration range were diluted with methanol/ultra pure water; 1/1; v/v. Standard solution in methanol were diluted with water in range of 0.251 to 15.06 ng/mL

Results and discussions

Table A 22: Recovery results from method validation of prosulfocarb using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (n = x)	Mean recovery (%)	RSD (%)
Soil	Prosulfocarb	0.1 (5)	101	1.9
		1.0 (5)	103.9	1.3
		Overall (10)	102.4	2.1

Table A 23: Characteristics for the analytical method used for validation of prosulfocarb residues in soil

	Prosulfocarb
Specificity	blank value < 30 % LOQ
Calibration (type, number of data points)	Linear y=- 6516x + 446.3 r ² = 0.9999 number of data points: 8
Calibration range	0.251 – 15.06 ng/mL
Assessment of matrix effects is presented	yes
Limit of determination/quantification	≤ 30% of LOQ / 0.1 mg/kg

Conclusion

The method was sufficiently validated according to SANCO/3029/99, Rev.4 and can be used for analytical determination of prosulfocarb in soil.

Comments of zRMS:	<p>The method is acceptable.</p> <p>The purpose of HPLC method with UV detection was to determine prosulfocarb in the toxicity test exposure solutions. The method is considered suitable for bio-accumulation studies.</p> <p>This study was already evaluated in PL.</p>
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Reference:	KCP 5.1.2 (Submitted as KCA 8.1.3)
Report	The bioaccumulation potential of prosulfocarb in earthworm (<i>Eisenia foetida</i>), Sacker D., 2008, ENV8333/040822
Guideline(s):	Yes, OECD Guideline for Testing of Chemicals 207: Earthworm acute toxicity tests (1984), OECD Guideline for Testing of Chemicals 222: Earthworm Reproduction Test (<i>Eisenia fetida</i> / <i>Eisenia andrei</i>) (2004), OECD Guidelines for Testing of Chemicals 305, Bioconcentration: Flow-through Fish Test. (2006), OECD Guidelines for Testing of Chemicals, Bioaccumulation in sediment-dwelling Benthic Oligochaetes (Proposed December 2007)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Soil samples: extraction of the test sediment with dichloromethane/acetone in presence of sodium sulphate. Evaporation and redissolution in acetonitrile

Earthworm samples: the worms were ground and extracted with acetonitrile/acetone. Evaporation and redissolution in acetonitrile.

Standard solutions were diluted in acetonitrile.

All samples were analysed using HPLC-UV (220 nm).

Results and discussions

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

Matrix	Analyte	Fortification level (mg/L) (<i>n</i> = <i>x</i>)	Mean recovery (%)	RSD (%)
Soil	Prosulfocarb	2.5 (5)	99.9	1.18

Table A 25: Characteristics for the analytical method used for validation of prosulfocarb residues in soil

	Prosulfocarb
Specificity	blank value < 30 % LOQ
Calibration (type, number of data points)	Linear y = - 363269x + 5923.8 r ² = 1.0

	Prosulfocarb
Specificity	blank value < 30 % LOQ
	number of data points: 9
Calibration range	0.028 – 25 mg/L
Assessment of matrix effects is presented	yes
Limit of determination/quantification	0.01 mg/L / 0.02 mg/L

Conclusion

The method can be used for analytical determination of prosulfocarb in the earthworm bioaccumulation study.

A 2.1.1.1.4 Analytical methods used in other ecotoxicological studies

A 2.1.1.1.4.1 Method validation

Comments of zRMS:	<p>The method is acceptable.</p> <p>The purpose of this LC-MS/MS method was to determine prosulfocarb, diflufenican and halauxifen methyl in the test and feeding solutions in bumblebee toxicity tests. The validation parameters were within the required range. The method is suitable for the intended purposes.</p> <p>This study was already evaluated in PL.</p>
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Reference:	KCP 5.1.2 (submitted as KCP 10.3.1.1.1)
Report	Acute toxicity of GLOB1817H to the bumblebee <i>Bombus terrestris</i> L. under laboratory conditions, Amsel K., 2021, 20 48 BBA 0029
Guideline(s):	Yes, OECD 246, OECD 247
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Oral test

The samples were extracted prior the measurement. Therefore, 2.5 mL of acetonitrile were added to a sample aliquot of 0.400 ± 0.005 g. It was extracted by shaking on a FastPrep Instrument (3 cycles, 20 s at speed 5 m/s, 15 sec pause). Afterwards, the samples were centrifuged at 5000 rpm for 3 minutes and the extracts were diluted.

Contact test

The samples of were diluted in several steps prior to sample measurement.

All samples were analysed by high performance liquid chromatography with triple quadrupole mass spectrometric detection (LC-MS/MS)

All standards were prepared in methanol.

Results and discussions

Table A 26: Recovery results from method validation of prosulfocarb, diflufenican and halauxifen-methyl using the analytical method

Matrix	Analyte	Fortification level (n = x)	Mean recovery (%)	RSD (%)
50% (w/v) sucrose solution	Prosulfocarb	5.73 mg/kg (5)	89	2.5
		10238 mg/kg (5)	99	6.8
	Diflufenican	0.12 mg/kg (5)	95	2.3
		216.08 mg/kg (5)	110	7.4
	Halauxifen-methyl	0.01 mg/kg (5)	95	1.7
		20.13 mg/kg (5)	85	8.4
0.5% (v/v) TritonX in deionized water	Prosulfocarb	20441 mg/L (5)	99	1.2
		240479 mg/L (5)	100	1.8
	Diflufenican	431.42 mg/L (5)	86	2.2
		5076 mg/L (5)	92	1.7
	Halauxifen-methyl	40.19 mg/L (5)	84	1.3
		472.88 mg/L (5)	84	1.5

Table A 27: Characteristics for the analytical method used for validation of prosulfocarb, diflufenican and halauxifen-methyl residues in 50% (w/v) sucrose solution

	Prosulfocarb	Diflufenican	Halauxifen-methyl
Specificity	blank value < 30 % LOQ	blank value < 30 % LOQ	blank value < 30 % LOQ
Calibration (type, number of data points)	Quadratic Y = -12.3X ² +40803X+726353 r ² > 0.9998 number of data points: 7	Linear Y = 13789X+819.2 r ² > 0.9998 number of data points: 7	Linear Y = 41110X+552.7 r ² > 0.998 number of data points: 7
Calibration range	106.97 – 891.43 µg/L	2.19 – 18.27 µg/L	0.21 – 1.75 µg/L
Assessment of matrix effects is presented	yes	yes	yes
Limit of quantification/determination	5.73 mg/kg / 1.52 mg/kg	0.12 mg/kg / 0.03 mg/kg	0.01 mg/kg / 0.003 mg/kg

Table A 28: Characteristics for the analytical method used for validation of prosulfocarb, diflufenican and halauxifen-methyl residues in 0.5% (v/v) TritonX in deionized water

	Prosulfocarb	Diflufenican	Halauxifen-methyl
Specificity	blank value < 30 % LOQ	blank value < 30 % LOQ	blank value < 30 % LOQ
Calibration (type, number of data	Quadratic	Quadratic	Linear

	Prosulfocarb	Diflufenican	Halauixifen-methyl
Specificity	blank value < 30 % LOQ	blank value < 30 % LOQ	blank value < 30 % LOQ
points)	Y = - 16.4X ² +53926X+938388 r ² > 0.9997 number of data points: 7	Y = - 97.7X ² +19879X+6418 number of data points: 7	Y = 43728X+290.1 r ² > 0.9995 number of data points: 7
Calibration range	139.89 – 1165.71 µg/L	3.07 – 25.60 µg/L	0.27 – 2.29 µg/L
Assessment of matrix effects is presented	yes	yes	yes
Limit of quantification/determination	20441 mg/L / 5595 mg/L	431.42 mg/L / 122.89 mg/L	40.19 mg/L / 10.97 mg/L

Conclusion

The method was sufficiently validated according to SANTE/2020/12830, Rev.1 and can be used for analytical determination of prosulfocarb, diflufenican and halauixifen-methyl in 50% (w/v) sucrose solution and 0.5% (v/v) TritonX in deionized water.

Comments of zRMS:	<p>The method is acceptable.</p> <p>The purpose of the method was to determine prosulfocarb, diflufenican and halauixifen-methyl in the test and feeding solutions in honeybee toxicity tests. The determination of active ingredients in sucrose solution was conducted using reversed-phase high-performance liquid chromatography combined with mass spectrometry (RP-HPLC-MS/MS). The specificity of the method was assured by multiple reaction monitoring (MRM) - detection with at least 2 transitions and the absence of interfering peaks.</p> <p>The validation parameters were within the required range. The method is suitable for the intended purposes.</p> <p>This study was already evaluated in PL.</p>
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Reference:	KCP 5.1.2 (submitted as KCP 10.3.1.2)
Report	Chronic toxicity of GLOB1817H to the honeybee <i>Apis mellifera</i> L. under laboratory conditions, Ruhland S., 2021, 20 48 BAC 0071
Guideline(s):	Yes, OECD TG 245 (2017)
Deviations:	Yes, due to the higher abundance of the resulting ion, the mass transition m/z 345 → 250 was chosen for the quantification of halauixifen-methyl and m/z 345 → 285 was set for qualification. There is no impact on the analytical phase of the study.
GLP:	Yes
Acceptability:	Yes

Materials and methods

The samples were extracted prior the measurement. Therefore, 2.5 mL of acetonitrile were added to a sample aliquot of 0.500 ± 0.005 g. It was extracted by shaking on a FastPrep Instrument (3 cycles, 20 s at speed 5 m/s, 15 sec pause). Afterwards, the samples were centrifuged at 5000 rpm for 3 minutes and the extracts were diluted.

All samples were analysed by high performance liquid chromatography with triple quadrupole mass spectrometric detection (LC-MS/MS)

All standards were prepared in methanol.

Results and discussions

Table A 29: Recovery results from method validation of prosulfocarb, diflufenican and halauxifen-methyl using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (n = x)	Mean recovery (%)	RSD (%)
50% (w/v) sucrose solution containing 0.1% (w/v) xanthan	Prosulfocarb	5.55 (5)	94	0.5
		3085 (5)	104	1.1
	Diflufenican	0.12 (5)	85	0.9
		65.11 (5)	94	1.3
	Halauxifen-methyl	0.01 (5)	88	1.4
		6.07 (5)	88	1.0

Table A 30: Characteristics for the analytical method used for validation of prosulfocarb, diflufenican and halauxifen-methyl residues in 50% (w/v) sucrose solution containing 0.1% (w/v) xanthan

	Prosulfocarb	Diflufenican	Halauxifen-methyl
Specificity	blank value < 30 % LOQ	blank value < 30 % LOQ	blank value < 30 % LOQ
Calibration (type, number of data points)	Quadratic Y = -18.4X ² +52912X+392078 r ² > 0.99 number of data points: 6	Linear Y = 13307X+1354 r ² > 0.99 number of data points: 6	Linear Y = 37908X+1316 r ² > 0.99 number of data points: 6
Calibration range	111.19 – 654.07 µg/L	2.39 – 14.06 µg/L	0.22 – 1.29 µg/L
Assessment of matrix effects is presented	yes	yes	yes
Limit of quantification/determination	5.55 mg/kg / 1.55 mg/kg	0.12 mg/kg / 0.03 mg/kg	0.01 mg/kg / 0.003 mg/kg

Conclusion

The method was sufficiently validated according to SANTE/2020/12830, Rev.1 and can be used for analytical determination of prosulfocarb, diflufenican and halauxifen-methyl in 50% (w/v) sucrose solution containing 0.1% (w/v) xanthan.

Comments of zRMS:	The method is acceptable. The purpose of this LC-MS/MS method was to determine prosulfocarb, diflufenican and halauxifen methyl in the test item stock solutions of a honeybee larvae toxicity tests. The specificity of the method was assured by multiple reaction monitoring (MRM) - detection with at least 2 transitions and the absence of interfering peaks. The validation parameters of the method were within the required range. The method is suitable for the intended purposes.
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	This study was already evaluated in PL.
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Reference:	KCP 5.1.2 (submitted as KCP 10.3.1.3)
Report	GLOB1817H – Repeated exposure to the honeybee (<i>Apis mellifera</i> L.) larvae under laboratory conditions, Schmidt K., 2021, 20 48 BLC 0052
Guideline(s):	Yes, OECD 239 (2016)
Deviations:	Yes, because of a malfunction of the climatic chamber, the temperature and humidity were out of range on D8 for six hours. The temperature ranged in this time between 28.5 to 35.7°C (average 30.7°C) instead of 34.5 ± 0.5°C. The relative humidity ranged in this time between 18.9 to 97.1% (average 28.6% instead of 80 ± 5%). No impact is assumed as no effects on development of larvae in the untreated control were observed. Due to the higher abundance of the resulting ion, the mass transition m/z 345 → 250 was chosen for the quantification of halauxifen-methyl and m/z 345 → 285 was set for qualification. There is no impact on the analytical phase of the study.
GLP:	Yes
Acceptability:	Yes

Materials and methods

The samples were extracted prior the measurement. Therefore, 2.5 mL of acetonitrile were added to a sample aliquot of 0.500 ± 0.005 g. It was extracted by shaking on a FastPrep Instrument (3 cycles, 20 s at speed 5 m/s, 15 s pause). Afterwards, the samples were centrifuged at 5000 rpm for 3 minutes and the extracts were diluted.

All samples were analysed by high performance liquid chromatography with triple quadrupole mass spectrometric detection (LC-MS/MS)

All standards were prepared in methanol.

Results and discussions

Table A 31: Recovery results from method validation of prosulfocarb, diflufenican and halauxifen-methyl using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (n = x)	Mean recovery (%)	RSD (%)
Aqueous sugar solution	Prosulfocarb	4.64 (5)	88	2.0
		980.97 (5)	96	7.5
	Diflufenican	0.10 (5)	90	3.4
		20.70 (5)	99	6.7
	Halauxifen-methyl	0.01 (5)	87	0.8
		1.93 (5)	82	5.4

Table A 32: Characteristics for the analytical method used for validation of prosulfocarb, diflufenican and halauxifen-methyl residues in aqueous sugar solution

	Prosulfocarb	Diflufenican	Halauxifen-methyl
Specificity	blank value < 30 % LOQ	blank value < 30 % LOQ	blank value < 30 % LOQ
Calibration (type, number of data points)	Quadratic Y = -4.54X ² +24703X+92168 r ² > 0.99 number of data points: 8	Linear Y = 6423X+2313 r ² > 0.99 number of data points: 8	Linear Y = 26311X+590.5 r ² > 0.99 number of data points: 7
Calibration range	112.91 – 806.50 µg/L	2.47 – 17.67 µg/L	0.23 – 1.63 µg/L
Assessment of matrix effects is presented	yes	yes	yes
Limit of quantification/determination	4.64 mg/kg / 1.33 mg/kg	0.10 mg/kg / 0.03 mg/kg	0.01 mg/kg / 0.003 mg/kg

Conclusion

The method was sufficiently validated according to SANTE/2020/12830, Rev.1 and can be used for analytical determination of prosulfocarb, diflufenican and halauxifen-methyl in aqueous sugar solution.

Comments of zRMS:	The method is acceptable. The purpose of this HPLC-UV method was to determine prosulfocarb, diflufenican, halauxifen methyl and cloquintocet mexyl in the spray solution (water). The validation parameters of the method were within the required range. The method is suitable for the intended purposes. This study was already evaluated in PL.
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Reference:	KCP 5.1.2 (submitted as KCP 10.6)
Report	GLOB1817H: terrestrial plant test: vegetative vigour test, Lewington-Gower M., 2021, STC/20/E1409
Guideline(s):	Yes, OECD 227 (2006)
Deviations:	pH of the soils being 8.2, rather than 7-8 as stated in the study plan. Relative humidity falling below 45%, rather than 70% - 25% as stated in the study plan. These deviations were not to the detriment of the plants as photographs of the untreated plants taken at harvest show. These deviations will not impact on the validity of the study, as demonstrated by the control performance and the fact that the validity criteria for the study were met.
GLP:	Yes
Acceptability:	Yes

Materials and methods

The spray solution was diluted into the range of the calibration curve as follows:

The spray solution was sonicated for 20 minutes with intermittent shaking and stirred for 5 minutes prior to sampling. The stirring was continued as aliquots were removed for analysis.

Triplicate samples (10 mL) of the supplied spray solution were pipetted into separate 50 mL volumetric flasks and diluted to volume with acetonitrile.

Control samples were prepared by transferring aliquots (2 x 10 mL) of the supplied water to separate 50 mL volumetric flasks and diluted to volume with acetonitrile.

The final solutions were analysed by the HPLC method relative to a bracketing standard solution.

All standards were prepared in acetonitrile.

Results and discussions

Table A 33: Recovery results from method validation of prosulfocarb, diflufenican, halauxifen-methyl and cloquintocet-mexyl using the analytical method

Matrix	Analyte	Fortification level (mg/L) (n = x)	Mean recovery (%)	RSD (%)
Spray solution	Prosulfocarb	6193 (5)	99	0.3
		2858 (5)	100	0.5
	Diflufenican	130.7 (5)	87	0.3
		60.53 (5)	87	0.5
	Halauxifen-methyl	12.18 (5)	86	0.6
		5.640 (5)	86	1.2
	Cloquintocet-mexyl	12.42 (5)	83	1.2
		5.752 (5)	83	0.8

Table A 34: Characteristics for the analytical method used for validation of prosulfocarb, diflufenican, halauxifen-methyl and cloquintocet-mexyl residues in spray solution

	Prosulfocarb	Diflufenican	Halauxifen-methyl	Cloquintocet-mexyl
Specificity	blank value < 30 % LOQ	blank value < 30 % LOQ	blank value < 30 % LOQ	blank value < 30 % LOQ
Calibration (type, number of data points)	Linear $y = 5936x + 39680$ $r^2 = 1.000$ number of data points: 6	Linear $y = 35200x - 1010$ $r^2 = 1.000$ number of data points: 6	Linear $y = 133200x - 994.8$ $r^2 = 1.000$ number of data points: 6	Linear $y = 116100x + 96.88$ $r^2 = 1.000$ number of data points: 6
Calibration range	160.2 – 1602 mg/L	3.562 – 35.62 mg/L	0.3536 – 3.536 mg/L	0.3446 – 3.446 mg/L
Assessment of matrix effects is presented	NR	NR	NR	NR
Limit of quantification/determination	2858 mg/L / 840 mg/L	60.53 mg/L / 18 mg/L	5.640 mg/L / 1.7 mg/L	5.752 mg/L / 1.7 mg/L

Conclusion

The method was sufficiently validated according to SANCO/3029/99 rev.4 and can be used for analytical determination of prosulfocarb, diflufenican, halauxifen-methyl and cloquintocet-mexyl in spray solution.

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

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

A 2.1.2.7 Other Studies/ Information

No new or additional studies have been submitted.

A 2.2 Analytical methods for diflufenican

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

A 2.2.1.1.1 Analytical method for diflufenican in potato

A 2.2.1.1.1.1 Method validation

Comments of zRMS:

The method has been accepted.

The objective of the study was to validate the analytical LC-MS/MS method for the analysis of diflufenican in potato tubers.

The linear correlation coefficients were typically > 0.990, showing a good linearity.

The lowest validated level (LOQ) where a mean recovery in the range 70-110 % with a RSD less than 20 % was at 0.01 mg/kg for potato (tubers).

Analyte	Matrix	Fortification level (mg/kg)	Mean recovery Percentage (%)	Standard deviation (SD) (%)	Relative standard deviation (RSD) (%)	Number of fortified samples (n)
Diflufenican	Potato (tubers)	0.01	84.5%	6.7%	7.9%	5
		0.10	87.2%	6.5%	7.4%	5
		All levels	85.8%	6.4%	7.4%	10

The analyses were performed by UPLC-MS/MS, monitoring two transitions. The method was considered highly specific, thus the use of an alternative method was not necessary.

Reference:	KCP 5.1.2
Report	Validation of the analytical method for the determination of diflufenican residues in potato (tubers), Jonchère F., 2011, B0133.
Guideline(s):	Yes, SANCO/825/00 rev. 7, SANCO/3029/99 rev. 4
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The sample was cut into small pieces and homogenized. The sample was blended with dry ice and placed for at least 12 hours below -18°C. The amount required by the analytical method was weighed from this homogenous matrix.

Diflufenican residues are extracted with acetonitrile/acetic acid 99.9:0.1% in the presence of magnesium sulphate and sodium chloride. After centrifugation, the internal standard (triphenylphosphate) and formic acid are added to the extract before analysis by ULC using a MS/MS detector.

Standards were prepared in acetonitrile.

Results and discussions

Table A 35: Recovery results from method validation of diflufenican using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (n = x)	Mean recovery (%)	RSD (%)
Potato tubers	Diflufenican	LOQ (5)	84.5	7.9
		10 x LOQ (5)	87.2	7.4
		All levels (10)	85.8	6.4

Table A 36: Characteristics for the analytical method used for validation of diflufenican residues in potato tubers

	Diflufenican
Specificity	blank value < 30 % LOQ
Calibration (type, number of data points)	$y = 0.78288x - 0.010743$ $R^2 > 0.990$ 8 data points
Calibration range	3 – 120 ng/mL
Assessment of matrix effects is presented	Matrix-matched calibration solutions were used to avoid matrix effects.
Limit of determination/quantification	0.001 mg/kg / 0.01 mg/kg

Conclusion

The method was sufficiently validated and can be used to reliably and accurately determine diflufenican in potato tubers to a limit of quantification of 0.01 mg/kg.

Comments of zRMS:	<p>The method has been accepted.</p> <p>The purpose of this study was to validate the analytical method for the determination of Diflufenican and its metabolites Diflufenican Amide (AE 0542291), Diflufenican Acid (AE B107137) and glycerol conjugates of AE B107137 (BCS-CO86433 and BCS-CO86434) in potato specimens.</p> <p>For Diflufenican and its metabolites, one reagent blank sample, 2 control samples, 5 samples fortified at the limit of quantification (LOQ) (<i>i.e.</i> 0.01 mg/kg) and 5 samples fortified at 10 times the LOQ (<i>i.e.</i> 0.1 mg/kg) were analysed in potato tubers.</p> <p>Acceptable mean recoveries between 70% and 120%, with a relative standard deviation less than 20%, were found for both primary and confirmatory transitions for Diflufenican and its metabolites in potato tubers.</p> <p>Significant matrix effect (suppression or enhancement; $> \pm 20\%$) on the detector response were observed in all matrices for both Diflufenican and its metabolites. Therefore, matrix-matched standards were used for calibration and quantification for all matrices and for both mass transitions (primary and confirmatory modes). The response of the LC-MS/MS detector was shown to be linear for Diflufenican and its metabolites for each mass transition over a concentration range of 0.3 to 15 ng/mL (equivalent to 0.003 to 0.15 mg/kg) for matrix-matched standards in all matrices. Visual inspection also showed that the regression residuals were randomly distributed for each calibration curve for each analyte, and hence linear calibration was demonstrated.</p> <p>The limit of detection (LOD) was determined for Diflufenican and its metabolites for both the primary and confirmatory transitions and was found to be equivalent to less than 0.003 mg/kg (30% of the LOQ) in all matrices.</p> <p>The limit of quantification (LOQ) of the analytical method for Diflufenican and its metabolites was established at 0.01 mg/kg in all matrices.</p> <p>The stability of Diflufenican and its metabolites in final sample extracts stored in amber glass vials at 4°C, was assessed. Sample extracts were re-analysed after 7 days of storage, and their recoveries were compared against freshly prepared calibration standards (matrix-matched). Diflufenican and its metabolites residues were stable in the final extracts when stored at a target temperature of 4°C for 7 days in potato tubers extracts.</p> <p>Residues of Diflufenican and its metabolites measured in the control samples were below 30% of the limit of quantification (LOQ; 0.01 mg/kg) in all of the</p>
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	<p>control and reagent blank samples used in this study. This demonstrates that no interferences were present at the retention time of Diflufenican and its metabolites in the test systems. This is in accordance with the level specified in SANTE/2020/12830 Rev.1.</p> <p>The repeatability and specificity of the method have been demonstrated and the analytical method is therefore considered valid for the determination of residues of Diflufenican and its metabolites in potato tubers at the LOQ of 0.01 mg/kg over concentration ranges typical of those for which the method will be used.</p> <p>Since two characteristic mass transitions are used to monitor Diflufenican and its metabolites, the method achieves a high level of specificity and no further confirmation on a different detector was necessary.</p> <p>The method has been validated according to the EU guidelines SANTE/2020/12830, Rev. 1.</p>
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Reference:	KCP 5.1.2
Report	Diflufenican - Validation of analytical method for the determination of residues of diflufenican and its metabolites on potato tubers, Laguna O., 2021b, E21003.
Guideline(s):	Yes, SANTE/2020/12830 Rev. 1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Potato tubers were homogenized using dry ice. Residues of diflufenican and its metabolites are extracted with acetonitrile and acetonitrile/water (1:1 v/v) mixture. Extracts are filtrated and then hydrolysed with an aqueous NaOH solution (32%). Hydrolysed extracts are then two-fold concentrated. After filtration of the final solution with a 0.2 µm filter, the determination of diflufenican, diflufenican amide and total diflufenican acid is performed by high performance liquid chromatography with triple quadrupole mass spectrometric detection (HPLC-MS/MS).

Standards were prepared in acetonitrile.

Results and discussions

Table A 37: Recovery results from method validation of diflufenican and its metabolites using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (n = x)	Mean recovery (%)	RSD (%)
Potato tubers	Diflufenican	LOQ (5)	90.5	6.9
		10 x LOQ (5)	93.9	8.1
		All levels (10)	92.2	7.4
	Diflufenican Amide	LOQ (5)	80.1	7.0
		10 x LOQ (5)	83.8	4.4

Matrix	Analyte	Fortification level (mg/kg) (n = x)	Mean recovery (%)	RSD (%)
	Diflufenican Acid	All levels (10)	82.0	6.0
		LOQ (5)	78.0	3.8
		10 x LOQ (5)	88.5	5.8
		All levels (10)	83.3	8.1
	BCS-CO86434	LOQ (5)	93.1	8.8
		10 x LOQ (5)	99.8	3.6
		All levels (10)	96.5	7.2
	BCS-CO86433	LOQ (5)	79.8	11.1
		10 x LOQ (5)	92.7	2.3
		All levels (10)	86.3	10.5

Table A 38: Characteristics for the analytical method used for validation of residues of diflufenican and its metabolites in potato tubers

	Diflufenican	Diflufenican Amide	Diflufenican Acid
Specificity	blank value < 30 % LOQ		
Calibration (type, number of data points)	y = 98339.6x – 533.508 R2 > 0.99 7 data points	y = 9904.85x – 200.096 R2 > 0.99 7 data points	y = 43354.5x – 1937.95 R2 > 0.99 7 data points
Calibration range	0.3 – 15 ng/mL (equivalent to 0.003 – 0.15 mg/kg)		
Assessment of matrix effects is presented	Matrix-matched calibration solutions were used to avoid matrix effects.		
Limit of determination/quantification	0.003 mg/kg / 0.01 mg/kg		

Conclusion

The method was sufficiently validated according to SANTE/2020/12830 Rev. 1 and can be used to reliably and accurately determine diflufenican and its metabolites in potato tubers to a limit of quantification of 0.01 mg/kg.

A 2.2.1.1.2 Analytical method for diflufenican in sunflower

A 2.2.1.1.2.1 Method validation

Comments of zRMS:	The method has been accepted.					
	The objective of the study was to validate the analytical method for the analysis of diflufenican in oilseed rape seeds.					
	The linear determination coefficients were > 0.990, showing a good linearity. The limit of quantification (LOQ) is the lowest validated level where a mean recovery within the range 70-110 % with a RSD less than 20 % could be obtained.					
	The LOQ was set at 0.01 mg/kg in oilseed rape seeds. The method can determine diflufenican in presence of oilseed rape seeds. This was checked by analysing control and spiked specimens to verify the absence of interfering signals.					
	The analyses were carried out by LC-MS/MS, monitoring two transitions. The method was considered highly specific thus the use of an alternative method was not necessary.					
Analyte	Matrix	Fortification level (mg/kg)	Mean recovery Percentage (%)	Standard deviation (SD) (%)	Relative standard deviation (RSD) (%)	Number of fortified samples (n)
Diflufenican	Oilseed rape seeds	LOQ	77.6%	4.6%	5.9%	5
		10 x LOQ	73.7%	3.9%	5.3%	5
		All levels	75.6%	4.5%	6.0%	10

Reference:	KCP 5.1.2
Report	Validation of the analytical method for the determination of diflufenican residues in oilseed rape seeds, Jonchère F., 2010b, A9259.
Guideline(s):	Yes, SANCO/825/00 rev. 7, SANCO/3029/99 rev. 4
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Oilseed rape seeds were homogenized by mixing. A subspecimen of about 500 g was then blended with dry ice and placed for at least 12 hours below -18°C. The quantity required by the analytical method was weighed from this homogenous matrix.

Diflufenican residues are extracted with acetonitrile/acetic acid 99.9:0.1% in the presence of magnesium sulphate and sodium chloride. After centrifugation, the internal standard (triphenylphosphate in acetonitrile) and formic acid are added to the extract before analysis by liquid chromatography using a MS/MS detector.

Standards were prepared in acetonitrile.

Results and discussions

Table A 39: Recovery results from method validation of diflufenican using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (n = x)	Mean recovery (%)	RSD (%)
Oilseed rape	Diflufenican	LOQ	77.6	5.9

Matrix	Analyte	Fortification level (mg/kg) (n = x)	Mean recovery (%)	RSD (%)
seeds		10 x LOQ	73.7	5.3
		All levels	75.6	6.0

Table A 40: Characteristics for the analytical method used for validation of diflufenican residues in oilseed rape seeds

	Diflufenican
Specificity	blank value < 30 % LOQ
Calibration (type, number of data points)	y = 2.2067x - 4.8442E-03 R ² = 0.99598 7 data points
Calibration range	1.6 – 60.5 ng/mL (corresponding to 0.003 – 0.12 mg/kg)
Assessment of matrix effects is presented	Matrix-matched calibration solutions were used to avoid matrix effects.
Limit of determination/quantification	0.002 mg/kg / 0.01 mg/kg

Conclusion

The method was sufficiently validated and can be used to reliably and accurately determine diflufenican in oilseed rape seeds to a limit of quantification of 0.01 mg/kg.

Comments of zRMS:	<p>The method has been accepted.</p> <p>The purpose of this study was to validate the analytical method for the determination of Diflufenican and its metabolites Diflufenican Amide (AE 0542291), Diflufenican Acid (AE B107137) and glycerol conjugates of AE B107137 (BCS-CO86433 and BCS-CO86434) in sunflower seeds.</p> <p>For Diflufenican and its metabolites, one reagent blank sample, 2 control samples, 5 samples fortified at the limit of quantification (LOQ) (<i>i.e.</i> 0.01 mg/kg) and 5 samples fortified at 10 times the LOQ (<i>i.e.</i> 0.1 mg/kg) were analysed in sunflower seeds.</p> <p>Acceptable mean recoveries between 70% and 120%, with a relative standard deviation less than 20%, were found for both primary and confirmatory transitions for Diflufenican and its metabolites in sunflower seeds.</p> <p>Significant matrix effect (suppression or enhancement; > ±20%) on the detector response were observed in all matrices for both Diflufenican and its metabolites. Therefore, matrix-matched standards were used for calibration and quantification for all matrices and for both mass transitions (primary and confirmatory modes). The response of the LC-MS/MS detector was shown to be linear for Diflufenican and its metabolites for each mass transition over a concentration range of 0.3 to 15 ng/mL (equivalent to 0.003 to 0.15 mg/kg) for matrix-matched standards in all matrices. Visual inspection also showed that the regression residuals were randomly distributed for each calibration curve for each analyte, and hence linear calibration was demonstrated.</p> <p>The limit of detection (LOD) was determined for Diflufenican and its metabolites for both the primary and confirmatory transitions and was found to be equivalent to less than 0.003 mg/kg (30% of the LOQ) in all matrices.</p> <p>The limit of quantification (LOQ) of the analytical method for Diflufenican and its metabolites was established at 0.01 mg/kg in all matrices.</p> <p>The stability of Diflufenican and its metabolites in final sample extracts stored in</p>
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	<p>amber glass vials at 4°C, was assessed. Sample extracts were reanalyzed after 7 days of storage, and their recoveries were compared against freshly prepared calibration standards (matrix-matched). Diflufenican residues was stable when stored at a target temperature of 4°C for 7 days in sunflower seed final extracts. Diflufenican Amide and Diflufenican acid residues were not stable when stored at a target temperature of 4°C for 7 days in sunflower seed final extracts. Residues of Diflufenican and its metabolites measured in the control samples were below 30% of the limit of quantification (LOQ; 0.01 mg/kg) in all the control and reagent blank samples used in this study. This demonstrates that no interferences were present at the retention time of Diflufenican and its metabolites in the test systems. This is consistent with the level specified in SANTE/2020/12830 Rev.1.</p> <p>The repeatability and specificity of the method have been demonstrated and the analytical method is therefore considered valid for the determination of residues of Diflufenican and its metabolites in sunflower seeds at the LOQ of 0.01 mg/kg over concentration ranges typical of those for which the method will be used. Since two characteristic mass transitions are used to monitor Diflufenican and its metabolites, the method achieves a high level of specificity and no further confirmation on a different detector was necessary.</p> <p>The method has been validated according to the EU guidelines SANTE/2020/12830, Rev. 1.</p>
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Reference:	KCP 5.1.2
Report	Validation of analytical method for the determination of residues of diflufenican and its metabolites and conjugates in sunflower seeds, Laguna O., 2021c, E21023.
Guideline(s):	Yes, SANTE/2020/12830 Rev. 1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Sunflower seeds are homogenized using solid carbon dioxide to maintain the frozen state. Residues of diflufenican, diflufenican amide (AE 05422941) and total diflufenican acid (AE B107137) are extracted with acetonitrile and acetonitrile/water (1:1, v/v) through solid-liquid extraction from sunflower seeds. The extract is stored at 4°C for 3 h and lipids are then eliminated by centrifugation. Glycerol conjugates of AE B107137 (BCS-CO86433 and BCS-CO86434) are hydrolyzed into diflufenican acid with sodium hydroxide solution. After filtration, the final solution is diluted, filtered again through 0.2 µm pore size filter and analyzed by highly selective LC-MS/MS.

Standards were prepared in acetonitrile.

Results and discussions

Table A 41: Recovery results from method validation of diflufenican and its metabolites using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (n = x)	Mean recovery (%)	RSD (%)
Sunflower seeds	Diflufenican	LOQ (5)	83.6	7.2
		10 x LOQ (5)	81.9	8.2
		All levels (10)	82.7	7.4
	Diflufenican Amide	LOQ (5)	81.4	13.8
		10 x LOQ (5)	88.2	8.6
		All levels (10)	84.8	11.4
	Diflufenican Acid	LOQ (5)	85.6	17.4
		10 x LOQ (5)	74.2	7.4
		All levels (10)	79.9	15.2
	BCS-CO86433	LOQ (5)	114.0	3.2
		10 x LOQ (5)	118.2	6.2
		All levels (10)	116.1	5.1
	BCS-CO86434	LOQ (5)	88.6	3.7
		10 x LOQ (5)	86.6	5.8
		All levels (10)	87.6	4.7

Table A 42: Characteristics for the analytical method used for validation of residues of diflufenican and its metabolites in sunflower seeds

	Diflufenican	Diflufenican Amide	Diflufenican Acid
Specificity	blank value < 30 % LOQ		
Calibration (type, number of data points)	Linear R2 > 0.99 7 data points	Linear R2 > 0.99 7 data points	Linear R2 > 0.99 7 data points
Calibration range	0.3 – 15 ng/mL		
Assessment of matrix effects is presented	yes		
Limit of determination/quantification	0.003 mg/kg / 0.01 mg/kg		

Conclusion

The method was sufficiently validated according to SANTE/2020/12830 Rev. 1 and can be used to reliably and accurately determine diflufenican and its metabolites in sunflower seeds to a limit of quantification of 0.01 mg/kg.

A 2.2.1.1.3 Analytical methods in water used in aquatic toxicity studies

Please refer to A 2.1.1.1.2.

A 2.2.1.1.4 Analytical methods used in other ecotoxicological studies

Please refer to A 2.1.1.1.4.

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

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

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

A 2.2.2.4.1 Analytical method for diflufenican in drinking water

A 2.2.2.4.1.1 Method validation

Comments of zRMS:	<p>The method has been accepted.</p> <p>The method is intended to determine diflufenican and metabolites AEB107137 (2-[3-(trifluoromethyl)phenoxy]nicotinic acid)) and AE0592370 (N-(2,4-difluorophenyl)-2-hydroxy-N-(3-trifluoromethylphenyl) nicotinamide)) in surface and drinking water by solid phase extraction (SPE) followed by LC-MS/MS. For each individual analyte, one ion transition was used for quantification and a further ion transition was also monitored for confirmation.</p> <p>The method was validated at 2 fortification levels for all analytes – at the LOQ of 0.05 µg/L and at 0.5 µg/L. The mean recovery at each fortification level was within the range 70 — 110% and corresponding RSD values were < 20%. No residues or interferences were detected in control surface water samples. The method is considered highly specific.</p> <p>This study was already evaluated in PL.</p>
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Reference:	KCP 5.2
Report	Development and validation of a method for the determination of diflufenican and two metabolites in surface water and drinking water, Turnbull G., 2008, PGD-307.
Guideline(s):	Yes, SANCO/825/00 rev. 7

Deviations: No
GLP: Yes
Acceptability: Yes

Materials and methods

Method Description

Concentrations of diflufenican and its metabolites AEB107137 and AE0592370 determined by liquid chromatography (LC) coupled with tandem mass spectrometric detection (LC-MS/MS).

Extraction

- Dispense 200ml of water sample and fortify recovery samples with diflufenican and metabolites according to the table below.

Fortification level (µg/L)	Volume (mL)	Concentration of diflufenican and metabolites in fortification solution (µg/ml)
0.05	0.2	0.05
0.5	0.2	0.5

- For SPE extraction, use vacuum where required. Condition an Oasis HLB 6cc, 500mg, LP cartridge with 5ml methanol followed by 5ml of water.
- Load the sample onto the cartridge and discard the eluate. Wash the cartridge with 4ml of 5% methanol in water and discard the eluate. For surface water samples, elute the cartridge with 10ml of methanol, collecting the eluate in an appropriate vessel. For drinking water samples, elute the cartridge with 12ml of methanol, collecting the eluate in an appropriate vessel.
- Evaporate the eluate to dryness at $\leq 35^{\circ}\text{C}$ in a TurboVap LV concentration workstation. Re-dissolve the residue (with the aid of ultra-sonication and a vortex mixer) in 1ml of methanol.
- To prepare the matrix-matched standards, evaporate portions of blank matrix, e.g. 200µL, to dryness at $\leq 35^{\circ}\text{C}$ in a TurboVap LV concentration workstation. Re-dissolve the residue (with the aid of ultra-sonication and vortex mixer) in the same volume, e.g. 200µL, of calibration standard.

Water characteristics

Test	Surface Water
Used for method validation of	diflufenican
Sampling point	River Derwent at Stamford bridge, East Riding of Yorkshire, United Kingdom
pH	7.8
Total hardness (as CaCO ₃)	246 mg/L
DOC (diluted organic carbon)	5.0 mg/L
Suspended solids	15 mg/L

Chromatographic conditions

HPLC

LC System	Agilent 1100 LC binary pump
LC Column	Waters, Atlantis dC18, 3 µm, 2.1 x 150 mm
LC Injection Volume	10 µL.

LC Method	Solvent A1: 10 mM ammonium acetate (surface water)			
	Solvent A2: 5 mM ammonium acetate + 0.01% acetic acid (drinking water, LOQ)			
	Solvent A3: 5 mM ammonium acetate + 0.1% formic acid (drinking water, 10xLOQ)			
	Solvent B: Methanol			
	Mobile Phase Composition:			
	Time (min)	Flow rate (mL/min)	% A	% B
	0.00	0.2	90	10
8	0.2	5	95	
23	0.2	5	95	
23.5	0.2	90	10	
32	0.2	90	10	
Retention Time	Around 15.4 minutes			

MS/MS

MS System	AB Sciex API 2000 mass spectrometer with TurboIonspray ESI source.
MS/MS Conditions for diflufenican:	MS/MS transition for quantification: 395.1 m/z > 266.2 m/z MS/MS transition for confirmation: 395.1 m/z > 245.8 m/z
MS/MS Conditions for AEB107137:	MS/MS transition for quantification: 284.1 m/z > 266.0 m/z MS/MS transition for confirmation: 284.1 m/z > 246.0 m/z
MS/MS Conditions for AE0592370:	MS/MS transition for quantification: 395.1 m/z > 122.0 m/z MS/MS transition for confirmation: 395.1 m/z > 140.0 m/z

Reagents and apparatus

Reagents	Apparatus
Water	Balance
Methanol	Microsyringe
Hydrochloric acid, 1M	Oasis HLB 6cc, 500mg, LP cartridges
5% methanol in water	SPE vacuum manifold and pump
Ammonium Acetate	TurboVap LV concentration workstation
Acetic Acid	Ultrasonice bath
Formic Acid	Vortex mixer
	Common laboratory glassware

Results and discussions

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

Matrix	Analyte	Fortification level (µg/L) (n = 5)	Mean recovery (%)	RSD (%)
Surface water	Diflufenican	0.05 0.5	84.2 71.4	11.5 14.4
Surface water	AEB1071137	0.05 0.5	103.6 102.3	2.5 6.3
Surface water	AE0592370	0.05 0.5	102.3 98.2	5.9 3.4
Drinking water	Diflufenican	0.05 0.5	75.8 71.9	8.6 4.6
Drinking water	AEB1071137	0.05 0.5	98.1 98.5	5.1 8.2
Drinking	AE0592370	0.05	100.5	6.8

Matrix	Analyte	Fortification level (µg/L) (n = 5)	Mean recovery (%)	RSD (%)
water		0.5	94.9	6.1

Table A 44: Characteristics for the analytical method used for validation of diflufenican residues in surface water and drinking water

	Diflufenican	AEB1071137	AE0592370
Specificity	LC-MS/MS is considered a specific technique as two different mass transitions were monitored; therefore additional methods to confirm the identity of the analytes are not considered necessary. Chromatograms have been provided which indicate no significant interference between the relevant peaks; diflufenican, AEB1071137 and AE0592370 and any of the water commodity matrices (>30% of the LOQ). Representative mass spectra for diflufenican have been provided that confirm the mass transitions are appropriate. The method is considered to have the required specificity.		
Calibration (type, number of data points)	Calibration was generated using standards prepared in blank matrix extracts (matrix matched standards). Linear calibration functions were calculated and plotted by regression analysis. Regression coefficients (r) were always > 0.99.		
Calibration range	The linear range is considered appropriate to the test sample concentrations used in this method. This includes 30% of the LOQ to 20% above the highest fortification level. The calibration range used for the low level validation samples was 0.002 to 0.1 µg/mL. The calibration range used for the high level validation samples was 0.002 to 0.2 µg/mL		
Assessment of matrix effects is presented	There were no significant matrix effects (<30%) observed for any matrices		
Limit of determination/quantification	0.05 µg/L		

Conclusion

The method for diflufenican and its metabolites AE B1071137 and AE 0592370 in surface water and drinking water was successfully validated and met the requirements of SANCO/825/00 rev. 7.

A 2.2.2.4.1.2 Independent laboratory validation

Comments of zRMS:	<p>The ILV for diflufenican has been accepted.</p> <p>The objective of the current study was an ILV of the analytical method validated in the previous study (A 2.2.2.4.1.1). Two MRM transitions were monitored for diflufenican. The recovery tests were done at two levels, LOQ and 10 times LOQ, with five replicates each, for each matrix. The validation parameters were within the required range.</p> <p>This study was already evaluated in PL.</p>
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Reference:

KCP 5.2

Report

Validation of an analytical method for the determination of diflufenican in drinking water, ILV, Figueiredo H., 2016, VAL10/16.

Guideline(s):	Yes, SANCO/825/00 rev 8.1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The primary method to determine diflufenican in matrices of water origin was independently validated by Laboratório de Resíduos, SAPEC AGRO, Portugal, a different test facility to the primary method. The method conditions and sample preparations are identical to those given in the primary method: PGD-307. Quantitation was performed using liquid chromatography electrospray ionization with tandem mass spectrometric detection (LC-MS/MS); the method monitored two ion transitions (quantitation transition m/z 395.3 \rightarrow 266.2 and confirmation transition m/z 395.3 \rightarrow 246.3 for diflufenican.

Results and discussions

Table A 45: Recovery results from independent laboratory validation of diflufenican using the analytical method

Matrix	Analyte	Fortification level ($\mu\text{g/L}$) (n = x)	Mean recovery (%)	RSD (%)
Drinking water	Diflufenican	0.050	78.8	6.7
		0.50	78.0	2.4

Table A 46: Characteristics for the analytical method used for independent laboratory validation of diflufenican residues in drinking water

	Diflufenican
Specificity	Chromatograms have been provided which indicate no significant interference between the relevant peak diflufenican and the matrix drinking water (>30% of the LOQ).
Calibration (type, number of data points)	The linearity of the detector response was checked by injecting several matrix matched standard solutions. The correlation coefficients obtained were higher than 0.99.
Calibration range	The validated calibration range for both levels was from 0.010 $\mu\text{g/L}$ to 1.0 $\mu\text{g/L}$ (0.002 $\mu\text{g/mL}$ to 0.20 $\mu\text{g/mL}$), for the drinking water matrix. The correlation coefficients obtained were higher than 0.99.
Assessment of matrix effects is presented	There were no significant matrix effects (<30%) observed for any matrices.
Limit of determination/quantification	0.05 $\mu\text{g/L}$

Conclusion

The method for diflufenican in drinking water is successfully validated according to SANCO/825/00 rev 8.1. The method is acceptable as ILV for the primary method.

A 2.2.2.4.1.3 Confirmatory method

No confirmatory method is required since LC-MS/MS is considered a specific technique as two different mass transitions were monitored.

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

No new or additional studies have been submitted

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

Comments of zRMS:

The method has been accepted.

The objective of the study was to validate the analytical method for the analysis of diflufenican in fat and blood.

The linear correlation coefficients were > 0.990, showing a good linearity. The limit of quantification (LOQ) is the lowest validated level where a mean recovery within the range 70-110% with a RSD less than 20% could be obtained. The LOQ was set at 0.01 mg/kg in fat and 0.05 mg/L in blood.

Analyte	Matrix	Fortification level	Mean recovery Percentage (%)	Standard deviation (SD) (%)	Relative standard deviation (RSD) (%)	Number of fortified samples (n)
Diflufenican	Fat	0.01 mg/kg	97.2%	4.6%	4.7%	5
		0.10 mg/kg	95.9%	4.0%	4.1%	5
		All levels	96.6%	4.1%	4.2%	10
Diflufenican	Blood	0.05 mg/L	106.8%	2.6%	2.4%	5

The method is able to determine diflufenican in presence of fat and blood. This was checked by analysing control and fortified specimens to verify the absence of interfering peaks. No interfering peaks were present at > 30% of the LOQ.

The determinations were carried out by LC-MS/MS, monitoring two transitions. The method was considered highly specific thus the use of an alternative method was not necessary.

Reference:	KCP 5.2
Report	Validation of the analytical method for the analysis of diflufenican in fat and blood, xxx, 2015, B6276.
Guideline(s):	Yes, ENV/JM/MONO(2007, ENV/MC/CHEM(98)17, SANCO/825/00 rev 8.1, SANCO/3029/99 rev. 4
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The method involves extraction with acetonitrile/acetic acid (99.9:0.1%) in the presence of magnesium sulphate and sodium chloride. The extract obtained is centrifuged prior to analysis by liquid chromatography (LC) coupled with tandem mass spectrometric detection (LC-MS/MS).

Results and discussions

Table A 47: Recovery results from independent laboratory validation of diflufenican using the analytical method

Matrix	Analyte	Fortification level (µg/L) (n = x)	Mean recovery (%)	RSD (%)
Fat	Diflufenican	0.01 (5)	97.2	4.7
		0.10 (5)	95.9	4.1
Blood		0.05 (5)*	106.8	2.4

*No validation data for an elevated concentration (10x LOQ) are required for body fluids and tissues.

Table A 48: Characteristics for the analytical method used for independent laboratory validation of diflufenican residues in fat and blood

	Diflufenican	
	Fat	Blood
Specificity	Chromatograms have been provided which indicate no significant interference between the relevant peak diflufenican and any of the animal commodity matrices (>30% of the LOQ).	
Calibration (type, number of data points)	Y = 1.6946E-01x + 2.4100E-04 7 data points R = 0.99947	Y = 1.4603E-01x + 6.6749E-03 7 data points R = 0.99971
Calibration range	0.6-24.2 ng/mL (0.003-0.12 mg/kg)	3.0-121.0 ng/mL (0.015-0.60 mg/L)
Assessment of matrix effects is presented	There were no significant matrix effects (<30%) observed for any matrices.	
Limit of determination/quantification	0.01 mg/kg	0.05 mg/L

Conclusion

The method for diflufenican in fat and blood is successfully validated according to SANCO/825/00 rev. 8.1.

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

No new or additional studies have been submitted