FINAL REGISTRATION REPORT

Part B

Section 5

Analytical Methods

Detailed summary of the risk assessment

Product code: SHA 2619 A

Product name(s): KONARK

Chemical active substances:

Flufenacet, 60 g/L
Pendimethalin, 300 g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

Applicant: Sharda Cropchem España S.L.

Submission date: March 2021

Update date: September 2021

Finalisation date: October 2021, May 2023

Version history

When	What
September 2021	Updated by the Applicant
October 2021	Updated by Applicant
October 2021	zRMS evaluation
April 2023	Updated by Applicant
May 2023	zRMS revision after commenting phase – final version of the RR

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5 Analytical methods

5.1 Conclusion and summary of assessment

Sufficiently sensitive and selective analytical methods are available for the active substances Flufenacet and Pendimethalin and relevant impurities (1,2-dichloroethane and N-Nitroso compounds) in the plant protection product SHA 2619 A (KONARK). Methods have been validated according to the SANCO/3030/99 rev.5.

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

- ~~None~~
- A method, including confirmation, for the determination of flufenacet + FOE thiadone in tissues is required according to Regulation (EU) 284/2013 (post-registration requirement).

Commodity/crop	Supported/ Not supported
Winter wheat	Supported
Winter barley	Supported
Winter rye	Supported
Triticale	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 Flufenacet and Pendimethalin in plant protection product is provided as follows:

Comments of zRMS:	The analytical method proposed is suitable for the determination of Flufenacet and Pendimethalin in the formulation SHA 2619 A (KONARK). Method has been validated in terms of specificity, linearity, precision and accuracy and fulfills requirements of the guideline SANCO/3030/99 rev.5. The method has been accepted

Reference:	KCP 5.1.1
Report	Accelerated storage stability study of flufenacet 6% + pendimethalin 30% EC. B. Rajasekhar, 2020. Report No. 7713/2020
Guideline(s):	Yes (SANCO 3030/99 rev. 5)
Deviations:	No

GLP: Yes

Acceptability: Yes

Materials and methods

Test item:

Name:	Flufenacet 6% + Pendimethalin 30% EC
Active substances:	Flufenacet Pendimethalin
CAS No:	142459-58-3 40487-42-1
Batch No:	SCL-39855
Date of expiry:	28.08.2021

Equipment: GC Chromatography, Shimadzu GC-2010, Flame Ionized Detector (FID)

Preparation of sample solution
An aliquot of 0.999 ml sample stock solution was taken into 10 ml volumetric flask, diluted with acetonitrile and made up to the mark with the acetonitrile. The concentration was equivalent to 100 mg/L. the prepared solution was used for specificity determination.

The specificity of GC-FID method for flufenacet+pendimethalin was determined by injecting the standard and sample solutions along with control blank. There was no interreference observed with the peak of interest. Hence the method was considered to be specific for the analysis of Flufenacet 6% + Pendimethalin 30% EC.

Validation - Results and discussions

Table 5.2-1: Methods suitable for the determination of active substances in plant protection product Flufenacet 6% + pendimethalin 30% SC

	Flufenacet	**Pendimethalin**
Author(s), year	B. Rajasekhar, 2020. Report No. 7713/2020	B. Rajasekhar, 2020. Report No. 7713/2020
Principle of method	GC-FID	GC-FID
Linearity (linear between mg/L / % range of the declared content) (correlation coefficient, expressed as r)	0.1– 0.6 mg/L (*n*=5) y = 6466.3x – 63.2 r = 0.9972 R^2 = 0.9945	~~0.1 – 0.6~~ 1.00 – 3.00 mg/L (*n*=5) y = 7507.8x – 3059.3 r = 0.9936 R^2 = ~~0.9945~~ 0.9873
Precision – Repeatability Mean n = 5 (%RSD)	RSD: ~~0.422~~ 0.475 % RSDr (Horwitz): 2.04 % Hr <1 (0.23)	RSD: 1.430 % RSDr (Horwitz): ~~0.89~~ 1.6 % Hr <1 (0.89)
Accuracy (marginal recovery) Blank fortification levels n = 5 (% Recovery)	Low level (2% w/w): 99.03% High level (8% w/w): 98.15%	Low level (20% w/w): 99.70% High level (30% w/w): 100.28%
Interference/ Specificity	Chromatograms of blank	Chromatograms of blank

	Flufenacet	Pendimethalin
	(acetonitrile) was ~~in~~ submitted. According to provided chromatograms, there were no interferences, method is specific	(acetonitrile) was ~~in~~ submitted. According to provided chromatograms, there were no interferences, method is specific
Comment	-	-

Conclusion

From the results of the analytical method validation, it is concluded that the analytical method is specific, sensitive, precise, and accurate for the determination of flufenacet and pendimethalin in Flufenacet 6% + pendimethalin 30% SC. The results of validation criteria are within the specified limits of SANCO/3030/99 rev.5 guideline.

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

~~Not relevant~~

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

Comments of zRMS:	Analytical methods proposed are suitable for the determination of relevant impurities (1,2-dichloroethane and N-Nitroso compounds) in the formulation SHA 2619 A (KONARK). Methods have been validated in terms of specificity, linearity, precision and accuracy and fulfill requirements of the guideline SANCO/3030/99 rev.5. Accepted

Reference:	KCP 5.1.1-2
Report	Accelerated storage stability test by heating at elevated temperature of Flufenacet 6% + Pendimethalin 30% SC. S. D. Revankar, 2021, Report No. G21362
Guideline(s):	SANCO/3030/99 rev. 5
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Test item:	
Name:	Flufenacet 6% + Pendimethalin 30% EC
Active substances:	Flufenacet Pendimethalin
CAS No:	142459-58-3 40487-42-1
Batch No:	SCL-44652
Date of expiry:	09.02.2023

Equipment:

- Tandem Mass Spectrometer API-3200 connected to Shimadzu Prominance High Performance Liquid Chromatograph (HPLC) equipped with Variable Wavelength Detector (VWD) and PC based data system
- Gas Chromatograph equipped with HS-20 Head space sampler/Autosampler, FID and PC based data system: Schimadzu GCMS/Lab solution Gas Chromatograph
- Gas Chromatograph (GC) equipped with Autosampler, TQ-8040 Mass Detector and PC based data system: Schimadzu GCMS/Lab solution Gas Chrromatograph

N- Nitroso Pendimethalin

The content of N-Nitroso Pendimethalin in the test item was determined by using an in-house developed and validated analytical LC-MS/MS method.

Specifity and Selectivity

The Specifity and selectivity of the method was established by injecting the diluent (acetonitrile) along with the impurity working standard solutions to the LC-MS/MS. The chromatograms of the diluent were checked for the absence of any interference at the retention time of the impurities.

Detector Linearity and Range

A known aliquot from each of the N-Nitroso pendimethalin reference standard sub stock solutions prepared was transferred into a volumetric flask and diluted with diluent (acetonitrile) to get five different working standard solutions for detector linearity check. Equal volumes each of the impurity working standard solutions were injected in triplicate into LC-MS/MS and the peak areas of N-Nitroso-Pendimethalin were recorded for each injection.

Precision

About 0.03 g of the test item, Flufenacet 6% + Pendimethalin 30% SC was weighed in five replications to separate 10 mL volumetric flasks and the contents of the flask were dissolved in diluent (Acetonitrile by sonicateing for 2 minutes. Later, the volume was made up to the mark with Acetonitrile, the solution was shaken thoroughly, filered and used for the analysis of impurities content by injecting to LC-MS/MS.

Accuracy

About 0.03 g of test item Flufenacet 6% + Pendimethalin 30% EC was weighed into separate 10 mL volumetric flasks (three for each of the three fortification levels) and the contents of the volumetric flasks were dissolved in diluent (Acetonitrile) by sonicating for 2 minutes. Later, the test item solutions were fortified with N-Nitroso Pendimethalin reference standard stocl solution. Further, the volume was made up to the mark with Acetonitrile, the solutions were shaken thoroughly, filtered and used for analysis of impurity content by injecting to LC-MS/MS.

1,2-Dichloroethane

The content of 1,2-Dichloroethane in the test item was determined using an in-house developed and validated analytical method using head space GC with FID detector.

Specifity

The specifity of the method was established by injecting the diluent along with the 1,2-Dichloroethane working standard solution to the GC-HS. The chromatogram of the diluent was checked for the absence of any interference at the retention time of the 1,2-Dichloroethane.

Detector linearity

A known aliquot from the impurity reference standard stock solution prepared was transferred into a volumetric flask and diluted with diluent to get five different working standard solutions for detector linearity check. Equal volumes of the working standard solutions were injected in triplicate into head space GC-FID.

Precision

About 0.1 of the test item, Flufenacet 6% + Pendimethalin 30% SC was weighed in five replications into

separate head space vials and an aliquot of 1.0 mL of diluent (N-Methyl-2-pyrrolidine~~o~~ine) was added. Further the vials were sealed with rubber septa and aluminum cap. The solution was used for the analysis of impurity content by injecting to head space GC-FID.

Accuracy

About 0.1 g of the test item Flufenacet 6% + Pendimethalin 30% EC was weighed into separate head space vials (three for each of three fortification levels). Further, fortified with 1.0 mL of 1,2-Dichloroethane standard solution of different concentration for different fortification levels. Further the vials were sealed with rubber septa and aluminium cap and used for analysis of 1,2-Dichloroethane content by injecting to head space GC-FID.

N-Nitrosodimethylamine, N-Nitrosomethylethylamine, N-Nitrosodiethylamine, N-Nitrosopyrrolidine, N-Nitrosodipropylamine, N-Nitrosopiperidine, N-Nitrosodibutylamine

The content of N-Nitrosoamine impurities in the test item was determined by using an in-house developed and validated analytical GC-MS/MS method.

Specifity and selectivity

The specifity and selectivity of the method was established by injecting diluent along with the impurity working standard solution to the GC-MS/MS. The chromatograms of the diluent were checked for the absence of any interference at the retention time of the impurities.

Detector Linearity

A known aliquot from each of the impurity reference standard stock solution was transferred into a volumetric flask and diluted with Acetonitrile to get five different working standard solutions for detector linearity check. Equal volumes each of the impurity working standard solutions were injected in triplicate into GC-MS/MS and the peak areas of impurity were recorded for each injection.

Precision

About 0.1 g of the test item, Flufenacet 6% + Pendimethalin 30% EC was weighed in five replications to separate 5 mL volumetric flasks and the contents of the flask were dissolved in Acetonitrile. Later, the volume wass made up to the mark with diluent, the solution was shaken thoroughly and used for the analysis of impurity content by injecting to GC-MS/MS.

Validation - Results and discussions

Table 5.2-2: Methods suitable for the determination of the relevant impurities in plant protection product (PPP) KONARK/SHA 2619 A

	N-Nitroso Pendimethalin (Imp 1)	**1,2-Dichloro-ethane (Imp 9)**	**N-Nitrosodimethyl-amine (Imp 2)**	**N-Nitrosomethylethyla-mine (Imp 3)**	**N-Nitrosodiethyl-amine (Imp 4)**
Author(s), year	S. D. Revankar, 2021				
Principle of method	~~HP~~LC-MS/MS	GC-FID	GC-MS/MS	GC-MS/MS	GC-MS/MS
Linearity (linear between mg/L) (correlation coefficient, expressed as	5 points 0.00521 to 0.20832 µg/mL (1.732-69.440 µg/g) y=1489096x+6176	5 points ~~10.39~~ 1.039 to 41.558 µg/mL (10.39 – 415.58 µg/g) y=307.9x-52.5 R=0.9990	5 points 0.0201 to 0.2510 µg/mL (0.9 – 9 µg/g) y=334165x+1505 R=0.9996	5 points 0.0201 to 0.2511 µg/mL (0.9 – 9 µg/g) y=370502x+1328 R=0.9995	5 points 0.0201 to 0.2508 µg/mL (0.9 – 9 µg/g) y=291975x+1067

	N-Nitroso Pendimethalin (Imp 1)	**1,2-Dichloro-ethane (Imp 9)**	**N-Nitrosodimethyl-amine (Imp 2)**	**N-Nitrosomethylethyla-mine (Imp 3)**	**N-Nitrosodiethyl-amine (Imp 4)**
r)	R=0.9998				R=0.9995
Mean Con-centration Precision – Repeatabil-ity ~~Mean~~ n = 5 (%RSD)	3.955 µg/g RSD = 5.158% RSD_R=13.009% RSDr=8.7158% Hr=0.5918<1	98.688 µg/g RSD = 2.160 % RSD_R=8.016% RSDr=5.371% Hr=0.4022<1	2.406 µg/g RSD = 2.660% RSD_R=14.020% RSDr=9.393% Hr=0.2832<1	2.458 µg/g RSD = 3.580% RSD_R=13.974% RSDr=9.363% Hr=0.~~2~~3824<1	2.476 µg/g RSD = 2.464% RSD_R=13.959% RSDr=9.352% Hr=0.2635<1
Accuracy 3 concentra-tions n = 3 for each level (%Recov-ery)	Marginal recovery Low (1.6 µg/g): 96.36% Medium (13 µg/g): 96.74% High (26 µg/g): 107.35% Mean recovery = 100.151 ± 6.271%	Marginal recovery Low (20 µg/g): 115.28% Medium 105 µg/g): 100.69% High (202 µg/g): 102.36% Mean recovery = 106.110 ± 7.247%	Total recovery Low (0.9 µg/g): 100.11% Medium (2.3 µg/g): 94.91% High (4.5 µg/g): 96.70% Mean recovery = 97.242 ± 3.169%	Total recovery Low (0.9 µg/g):: 101.91% Medium (2.4 µg/g): 98.79% High (4.8 µg/g): 99.68% Mean recovery = 100.128 ± 2.227%	Total recovery Low (0.9 µg/g):: 104.72% Medium (2.4 µg/g): 100.51% High (4.8 µg/g): 100.13% Mean recovery = 101.79 ± 3.054%
Interference/ Specificity	According to blank chromatogram (acetonitrile), there were no interference. The method is specific	According to blank chromatogram, there were no interference. The method is specific	According to blank chromatogram, there were no interference. The method is specific	According to blank chromatogram, there were no interference. The method is specific	According to blank chromatogram, there were no interference. The method is specific
LOQ	LOD = 0.225 µg/g LOQ = 1.667 µg/g (the lowest level of fortification)	LOD = 2.943 µg/g LOQ = ~~10.40~~ 20 µg/g (the lowest level of fortification)	LOD = 0.092 µg/g LOQ = 0.971 µg/g (the lowest level of fortification)	LOD = 0.015 µg/g LOQ = 0.972 µg/g (the lowest level of fortification)	LOD = 0.004 µg/g LOQ = 0.970 µg/g (the lowest level of fortification)
Comment	-	-	-	-	-

	N-Nitrosopyrrolid~~o~~ine (Imp 5)	**N-Nitrosodipropylamine (Imp 6)**	**N-Nitrosopiperidine (Imp 7)**	**N-Nitrosodibutylamine (Imp 8)**
Author(s), year	S. D. Revankar, 2021			
Principle of method	GC-MS/MS	GC-MS/MS	GC-MS/MS	GC-MS/MS
Linearity (linear be-tween mg/L)	5 points 0.0201 to 0.2508 µg/mL (0.9 – 9 µg/g)	5 points 0.0201 to 0.2509 µg/mL (0.9 – 9 µg/g)	5 points 0.0201 to 0.2508 µg/mL (0.9 – 9 µg/g)	5 points 0.0201 to 0.2509 µg/mL (0.9 – 9 µg/g)

	N-Nitrosopyrrolidoine (Imp 5)	N-Nitrosodipropylamine (Imp 6)	N-Nitrosopiperidine (Imp 7)	N-Nitrosodibutylamine (Imp 8)
(correlation coefficient, expressed as r)	y=184180x+490 R=0.9995	y=283845x+98 R=0.9974	y=234217x+835 R=0.9995	y=203239x+995 R=0.9996
Mean Concentration Precision – Repeatability Mean n = 5 (%RSD)	2.624 µg/g RSD = 3.392% RSD_R=13.8374% RSDr=9.271% Hr=0.3659<1	3.805 µg/g RSD = 2.7733% RSD_R=13.086% RSDr=78.768% Hr=0.315702<1	3.805 µg/g RSD = 3.222% RSD_R=13.876% RSDr=9.297% Hr=0.3466<1	2.576 µg/g RSD = 4.030% RSD_R=13.699% RSDr=9.178% Hr=0.4391<1
Accuracy 3 concentrations n = 3 for each level (% Recovery)	Total recovery Low (0.9 µg/g): 107.03% Medium (2.4 µg/g): 101.04% High (4.8 µg/g): 103.55% Mean recovery = 103.875 ± 3.63%	Total recovery Low (0.9 µg/g): 109.37% Medium (2.4 µg/g): 103.97% High (4.8 µg/g): 116.46% Mean recovery = 109.933 ± 6.390%	Total recovery Low (0.9 µg/g): 109.05% Medium (2.4 µg/g): 101.69% High (4.8 µg/g): 101.19% Mean recovery = 103.978 ± 4.576%	Total recovery Low (0.9 µg/g): 112.17% Medium (2.4 µg/g): 83.18% High (4.8 µg/g): 91.00% Mean recovery = 95.451 ± 13.063%
Interference/ Specificity	According to blank chromatogram, there were no interference. The method is specific	According to blank chromatogram, there were no interference. The method is specific	According to blank chromatogram, there were no interference. The method is specific	According to blank chromatogram, there were no interference. The method is specific
LOQ	LOD = 0.009 µg/g LOQ = 0.970 µg/g (the lowest level of fortification)	LOD = 0.010 µg/g LOQ = 0.971 µg/g (the lowest level of fortification)	LOD = 0.005 µg/g LOQ = 0.970 µg/g (the lowest level of fortification)	LOD = 0.216 µg/g LOQ = 0.971 µg/g (the lowest level of fortification)
Comment	-	-	-	-

Conclusion

According to SANCO/3030/99 rev. 5 the method was successfully validated and is suitable for determination of relevant impurities (N-Nitroso Pendimethalin, 1,2-dichloroethane, N-Nitrosodimethylamine, N-Nitrosomethylethylamine, N-Nitrosodiethylamine, N-Nitrossopyrrolidoine, N-Nitrosodipropylamine, N-Nitrosopiperidine, N-Nitrosodibutylamine) content in the test item KONARK/SHA 2619 A.

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

Not relevant.

5.2.1.4 Applicability of existing CIPAC methods (KCP 5.1.1)

A CIPAC method No 588 is available for Flufenacet and a CIPAC method No 357 is available for Pendimethalin.

5.2.2 Methods for the determination of residues (KCP 5.1.2)

Please refer to post-registration methods for both Flufenacet and Pendimethalin active substances.

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

5.3.1 Analysis of the plant protection product (KCP 5.2)

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

5.3.2 Description of analytical methods for the determination of residues of Flufenacet (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	Flufenacet (sum of all compounds containing the N fluorophenyl-N-isopropyl moiety expressed as flufenacet equivalent)	0.05 mg/kg	Reg. (EU) No 1127/2014
Plant, high acid content		0.05 mg/kg	Reg. (EU) No 1127/2014
Plant, high protein/high starch content (dry commodities)		0.05 mg/kg	Reg. (EU) No 1127/2014
Plant, high oil content		0.05 mg/kg	Reg. (EU) No 1127/2014
Plant, difficult matrices (hops, spices, tea)		0.05 mg/kg	Reg. (EU) No 1127/2014
Muscle	Flufenacet (sum of all compounds containing the N fluorophenyl-N-isopropyl moiety expressed as flufenacet equivalent)	0.05 mg/kg	Reg. (EU) No 1127/2014
Milk		0.05 mg/kg	Reg. (EU) No 1127/2014
Eggs		0.05 mg/kg	Reg. (EU) No 1127/2014
Fat		0.05 mg/kg	Reg. (EU) No 1127/2014
Liver, kidney		0.02 mg/kg	Reg. (EU) No 1127/2014
Soil (Ecotoxicology)	Flufenacet (sum of all compounds containing the N fluorophenyl-N-isopropyl moiety expressed as flufenacet equivalent)	0.05 mg/kg	common limit
Drinking water (Human toxicology)	Flufenacet (sum of all compounds containing the N fluorophenyl-N-isopropyl moiety expressed as flufenacet equivalent)	0.1 µg/L	general limit for drinking water

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Surface water (Ecotoxicology)	Flufenacet (sum of all compounds containing the N fluorophenyl-N-isopropyl moiety expressed as flufenacet equivalent)	0.00204 mg/L	From lowest EbC50, 72 h; *Selenastrum capricornutum;* Review report Flufenacet 7469/VI/98-Final, 3 July 2003
Air	Flufenacet	5.1 µg/m^3	AOEL sys: 0.017 mg/kg bw/d
Tissue (meat or liver)	Not required	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 Flufenacet 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: sum of all compounds containing the N fluorophenyl-N-isopropyl moiety expressed as flufenacet				
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	0.05 mg/kg (corn forage-green material)	GC-MS *Method 00346	Gould T.J., Lemke V.J. 1995; An analytical method for the determination of FOE 5043 residues in plant matrices; Report No: 106406 DAR of flufenacet 1997/EU Accepted Seym M. 1995; Analytical method for the determination of the total residues of FOE 5043 in plant material; Doc No MR-981/95;DAR of flufenacet 1997/EU Accepted
	ILV	0.05 mg/kg (corn forage-green material, spinach)	GC-MS *ILV of Method 00346	Seym M. (1994): Independent laboratory validation of the residue analytical method for FOE 5043 residues in plant. Doc No: 106907; DAR of flufenacet 1997/EU Accepted
	Confirmatory (if required)	-	-	-
High acid content	Primary	-	-	-
	ILV	-	-	-

Component of residue definition: sum of all compounds containing the N fluorophenyl-N-isopropyl moiety expressed as flufenacet				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
	Confirmatory (if required)	-	-	-
High oil content	Primary	0.05 mg/kg (soya bean, sunflower)	GC-MS *Method 00346	Gould T.J., Lemke V.J. 1995; An analytical method for the determination of FOE 5043 residues in plant matrices; Report No: 106406 DAR of flufenacet 1997/EU Accepted Seym M. 1995; Analytical method for the determination of the total residues of FOE 5043 in plant material; Doc No MR-981/95;DAR of flufenacet 1997/EU Accepted
	ILV			
	Confirmatory (if required)	-	-	-
High protein/high starch content (dry)	Primary	0.05 mg/kg (cereal grain, maize) 0.1 mg/kg (straw)	GC-MS *Method 00346	Gould T.J., Lemke V.J. 1995; An analytical method for the determination of FOE 5043 residues in plant matrices; Report No: 106406 DAR of flufenacet 1997/EU Accepted Seym M. 1995; Analytical method for the determination of the total residues of FOE 5043 in plant material; Doc No MR-981/95;DAR of flufenacet 1997/EU Accepted
	Primary	0.01 mg/kg	HPLC/MS/MS	KCP 5.3.2.2.1 E. Rigamonti, 2019 Report No. CH - 0753/2019
	ILV			
	Confirmatory (if required)	-	-	-
Difficult (if required, depends on intended use)	Primary	-		-
	ILV	-	-	-
	Confirmatory (if required)	-	-	-

*Method 00346, Analytes: FOE, FOE oxalate, FOE sulfonic acid, FOE thioglycolate sulfoxide; Determined as common moiety: 4-fluoro-N-methylethyl benzenamin trifluoroacetamide

Table 5.3-3: Statement on extraction efficiency

	Method for products of plant origin
Required, available from:	-
Not required, because:	Not provided during the EU review

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

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

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

Component of residue definition: sum of all compounds containing the N fluorophenyl-N-isopropyl moiety expressed as flufenacet				
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 *Method 0048	Gould T.J., Lemke V.J., Zoloty K.L., 1995, Report no. 106773; An analytical method for the determination of FOE 5043 residues in animal matrices /DAR of flufenacet 1997/EU Accepted
	ILV	-	-	-
	Confirmatory (if required)	-	-	-
Eggs	Primary	0.05 mg/kg	GC-MS *Method 00418/M001	Seym M. (1995): Modification M001 for eggs. Doc No: MR-118/95;DAR of flufenacet 1997/EU Accepted
	ILV	-	-	-
	Confirmatory (if required)	-	-	-
Muscle	Primary	0.05 mg/kg	GC-MS *Method 0048	Gould T.J., Lemke V.J., Zoloty K.L., 1995, Report no. 106773; An analytical method for the determination of FOE 5043 residues in animal matrices /DAR of flufenacet 1997/EU Accepted
	ILV	-	-	-
	Confirmatory (if required)	-	-	-
Fat	Primary	0.05 mg/kg	GC-MS *Method 0048	Gould T.J., Lemke V.J., Zoloty K.L., 1995, Report no. 106773; An analytical method for the

Component of residue definition: sum of all compounds containing the N fluorophenyl-N-isopropyl moiety expressed as flufenacet				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
				determination of FOE 5043 residues in animal matrices /DAR of flufenacet 1997/EU Accepted
	ILV	-	-	-
	Confirmatory (if required)	-		-
Kidney, liver	Primary	0.02 mg/kg liver 0.05 mg/kg kidney	GC-MS *Method 0048	Gould T.J., Lemke V.J., Zoloty K.L., 1995, Report no. 106773; An analytical method for the determination of FOE 5043 residues in animal matrices /DAR of flufenacet 1997/EU Accepted
	ILV	0.05 mg/kg (liver)	GC-MS *ILV of Method 0048	Bajzik M.E. 1995: Independent laboratory validation of the analytical method for the determination of FOE 5043 residues in a/nimal matrices. Doc No: 106913/ DAR of flufenacet 1997/EU Accepted
	Confirmatory (if required)	-	-	-

*Method 00418, ILV of 00418 and Method 00418/M001 (Modification for eggs); Analytes: FOE, FOE oxalate, FOE sulfonic acid, FOE thioglycolate sulfoxide; Residues of FOE 5043 were determined as common moiety: 4-fluoro-N-methylethyl benzamine trifluoroacetamide

Table 5.3-5: Statement on extraction efficiency

	Method for products of animal origin
Required, available from:	-
Not required, because:	Not provided during the EU review

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 Flufenacet in soil is given in the following tables.

Component of residue definition: FOE 5043 (flufenacet)			
Method type	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	0.01 mg/kg (soil)	HPLC-MS-MS Method 00359	Bachlecher G. and Allmendinger H.,

Component of residue definition: FOE 5043 (flufenacet)			
Method type	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
			1994;Validated method for the determination of FOE 5043 and its metabolites FOE 5043 alcohol, FOE 5043 oxalate and FOE 5043 sulfonic acid in soil using HPLC-MS-MS; Doc No: RA-399/94/DAR of flufenacet 1997, EU Accepted
Confirmatory	-	-	-

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

Component of residue definition: FOE 5043 alcohol			
Method type	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	0.01 mg/kg (soil)	HPLC-MS-MS Method 00359	Bachlecher G. and Allmendinger H., 1994;Validated method for the determination of FOE 5043 and its metabolites FOE 5043 alcohol, FOE 5043 oxalate and FOE 5043 sulfonic acid in soil using HPLC-MS-MS; Doc No: RA-399/94/DAR of flufenacet 1997, EU Accepted
Confirmatory	-	-	-

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

Component of residue definition: FOE 5043 oxalate			
Method type	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	0.01 mg/kg (soil)	HPLC-MS-MS Method 00359	Bachlecher G. and Allmendinger H., 1994;Validated method for the determination of FOE 5043 and its metabolites FOE 5043 alcohol, FOE 5043 oxalate and FOE 5043 sulfonic acid in soil using HPLC-MS-MS; Doc No: RA-399/94/DAR of flufenacet 1997, EU Accepted
Confirmatory	-	-	-

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

Component of residue definition: FOE 5043 sulfonic acid			
Method type	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	0.01 mg/kg (soil)	HPLC-MS-MS Method 00359	Bachlecher G. and Allmendinger H., 1994;Validated method for the determination of FOE 5043 and its metabolites FOE 5043 alcohol, FOE 5043 oxalate and FOE 5043 sulfonic acid in soil using HPLC-MS-MS; Doc No: RA-399/94/DAR of flufenacet 1997, EU Accepted
Confirmatory	-	-	-

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

Table 5.3-9: Validated methods for water (if appropriate)

Component of residue definition: FOE 5043 (flufenacet)				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**

Water (surface and drinking)	Primary	**0.04 µg/L** (FOE 5043 & FOE 5043 alcohol) 0.02 µg/L (FOE 5043 solfunic acid) 0.05 µg/L (FOE 5043 oxalate) 0.08µg/L (FOE 5043 thiadone)	HPLC - ESI/MS/MS Method AMFOE	Bethern R.A., Peterson R.G., Leimkuhler W., Mattern G.C.,1995: Determination of FOE 5043 and the alcohol, oxalate, thaidone and sulfonic acid metabolites in groundwater by high performance liquid chromatography, electrospray tandem mass spectrometry (LC-ESI/MS/MS). Doc No 107138/ DAR of flufenacet 1997
	ILV	0.1 µg/L	HPLC - ESI/MS/MS	Bruns G. and Hoshowski J., 1995
	Confirmatory	-	-	-
Drinking water	Primary	0.05 µg/L	GC-ECD Method No 00412	DAR of flufenacet 1997 Konig T. 1996: Method for the determination of FOE 5043 in drinking water by gas chromatography. Doc No: MR-894/95
	Confirmatory	-	-	-

Table 5.3-10: Validated methods for water (if appropriate)

Component of residue definition: FOE 5043 sulfonic acid				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Water (surface and drinking)	Primary	0.04 µg/L (FOE 5043 & FOE 5043 alcohol) **0.02 µg/L** (FOE 5043 solfunic acid) 0.05 µg/L (FOE 5043 oxalate) 0.08µg/L (FOE 5043 thiadone)0.02 µg/L (water)	HPLC - ESI/MS/MS Method AMFOE	Bethern R.A., Peterson R.G., Leimkuhler W., Mattern G.C.,1995: Determination of FOE 5043 and the alcohol, oxalate, thaidone and sulfonic acid metabolites in groundwater by high performance liquid chromatography, electrospray tandem mass spectrometry (LC-ESI/MS/MS). Doc No 107138/ DAR of flufenacet 1997
	ILV	-	-	-
	Confirmatory	-	-	-

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 Flufenacet in air is given in the following tables.

Table 5.3-11: Validated methods for air (if appropriate)

Component of residue definition: flufenacet			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	0.0022 mg/m^3	HPLC – UV at 230 nm Method 00410	Reigner K., 1995:Method for the determination of FOE 5043 in air. Doc No: MR-798/95/ DAR of flufenacet 1997, EU Accepted
Confirmatory	-	-	-

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

Since Flufenacet is not classified as Toxic or Very Toxic active substance, no method for the analysis of body fluids and tissues was provided during the EU review process.

zRMS:
A method, including confirmation, for the determination of flufenacet + FOE thiadone in tissues is required according to Regulation (EU) 284/2013. According to the applicant's declaration, the method is already contracted. The requirement can be completed after registration.

5.3.2.8 Other studies/ information

Not relevant.

5.3.3 Description of analytical methods for the determination of residues of Pendimethalin (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 5.3-12: 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	Pendimethalin	0.05 mg/kg	Reg. (EU) 2019/1791
Plant, high acid content		0.05 mg/kg	Reg. (EU) 2019/1791

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Plant, high protein/high starch content (dry commodities)		0.05 mg/kg	Reg. (EU) 2019/1791
Plant, high oil content		0.05 mg/kg	Reg. (EU) 2019/1791
Plant, difficult matrices (hops, spices, tea)		0.05 mg/kg	Reg. (EU) 2019/1791
Muscle	Pendimethalin	0.01 mg/kg	Reg. (EU) 2019/1791
Milk		0.02 mg/kg	Reg. (EU) 2019/1791
Eggs		0.01 mg/kg	Reg. (EU) 2019/1791
Fat		0.01 mg/kg	Reg. (EU) 2019/1791
Liver, kidney		0.01 mg/kg	Reg. (EU) 2019/1791
Soil (Ecotoxicology)	Pendimethalin	0.05 mg/kg	Lowest NOEC from aquatic toxicity study (Long term toxicity fish – *Pimephales promelas)*
Drinking water (Human toxicology)	Pendimethalin	0.1 µg/L	general limit for drinking water
Surface water (Ecotoxicology)	Pendimethalin	0.006 mg/L	
Air	Pendimethalin	51.0 µg/m^3	AOEL: 0.17 mg/kg bw/d
Tissue (meat or liver)	Pendimethalin	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 pendimethalin in plant matrices is given in the following tables.

Table 5.3-13: 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: pendimethalin				
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 0.01 mg/kg	HPLC-MS/MS HPLC-MS/MS	RAR 2015, EFSA 2016 Weber H., 2011 KCP 5.3.3.2.4 xxx Report No. 16.586423.0002
	ILV	0.01 mg/kg 0.01 mg/kg	HPLC-MS/MS HPLC-MS/MS	RAR 2015, EFSA 2016 Scherthan D., 2012 KCP 5.3.3.2.7

Component of residue definition: pendimethalin				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
				J. Kicińska, 2017 Report No. ZBBZ-2016/12/DPL/4DE
	Confirmatory (if required)	-	-	Not required.
High acid content	Primary	0.01 mg/kg 0.01 mg/kg	HPLC-MS/MS HPLC-MS/MS	RAR 2015, EFSA 2016 Weber H., 2011 KCP 5.3.3.2.5 xxx Report No. 16.566423.0003
	ILV	0.01 mg/kg 0.01 mg/kg	HPLC-MS/MS HPLC-MS/MS	RAR 2015, EFSA 2016 Scherthan D., 2012 KCP 5.3.3.2.9 xxx 2014 YV/13/035
	Confirmatory (if required)	-	-	Not required.
High oil content	Primary	0.01 mg/kg 0.01 mg/kg	HPLC-MS/MS HPLC-MS/MS	RAR 2015, EFSA 2016 Weber H., 2011 KCP 5.3.3.2.6 xxx N. 16.566423.0001)
	ILV	0.01 mg/kg 0.01 mg/kg	HPLC-MS/MS HPLC-MS/MS	RAR 2015, EFSA 2016 Scherthan D., 2012 KCP 5.3.3.2.8 J. Kicińska, 2018 Report No. ZBBZ-2016/12/DPL/8PL
	Confirmatory (if required)	-	-	Not required.
High protein/high starch content (dry)	Primary	0.01 mg/kg	HPLC-MS/MS	RAR 2015, EFSA 2016 Weber H., 2011
	ILV	0.01 mg/kg	HPLC-MS/MS	RAR 2015, EFSA 2016 Scherthan D., 2012
	Primary	0.01 mg/kg	HPLC-MS/MS	KCP 5.3.3.2.1 xxx Report No. 16.566423.0005
	ILV	0.01 mg/kg	HPLC-MS/MS	KCP 5.3.3.2.2 J. Kicińska, 2017 Report No. ZBBZ-2016/69/DPL/1ES
	Primary (cereals straw)	0.01 mg/kg	HPLC-MS/MS	KCP 5.3.3.2.3 xxx Report No. 16.566423.0006
	Confirmatory (if required)	-	-	Not required.

Table 5.3-14: Statement on extraction efficiency

	Method for products of plant origin
Required, available from:	RAR, 2015
Not required, because:	-

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

Based on EFSA Journal 2016;14(3):4420:
Not required since no MRLS are proposed.

zRMS:
Since MRLs are set for animal matrices (see Regulation No. (EU) 2019/1791), the methods are required (primary and ILV). Applicant updated section with necessary methods for animal matrices.

An overview on the acceptable methods and possible data gaps for analysis of Pendimethalin in animal matrices is given in the following tables. For the detailed evaluation of new/ additional studies it is referred to Appendix 2.

Table 5.3-15: Validated methods for food and feed of animal origin (if appropriate)

Component of residue definition: Pendimethalin				
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	LC- MS/MS	KCP 5.3.3.3.1 K. xxx 2022, Report No.: 9421/2021
	Confirmatory (if required)	0.01 mg/kg	LC- MS/MS	KCP 5.3.3.3.1 K. xxx, 2022, Report No.: 9421/2021
Eggs	Primary	0.01 mg/kg	LC- MS/MS	KCP 5.3.3.3.1 K. xxx, 2022, Report No.: 9421/2021
	Confirmatory (if required)	0.01 mg/kg	LC- MS/MS	KCP 5.3.3.3.1 K. xxx, 2022, Report No.: 9421/2021
Muscle	Primary	0.01 mg/kg	LC- MS/MS	KCP 5.3.3.3.1 K. xxx, 2022, Report No.: 9421/2021
	Confirmatory (if required)	0.01 mg/kg	LC- MS/MS	KCP 5.3.3.3.1 K. xxx, 2022, Report No.: 9421/2021
Fat	Primary	0.01 mg/kg	LC- MS/MS	KCP 5.3.3.3.1 K. xxx, 2022, Report No.: 9421/2021
	Confirmatory	0.01 mg/kg	LC- MS/MS	KCP 5.3.3.3.1

Component of residue definition: Pendimethalin				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
	(if required)			K. xxx, 2022, Report No.: 9421/2021
Kidney, liver	Primary (liver)	0.1 mg/kg	LC-MS/MS	KCP 5.3.3.3.2 xxx 16.566423.004
	ILV	0.01 mg/kg	LC-MS/MS	KCP 5.3.3.3.4 M.J. Benotti, 2015, Report No.: 100041492A
	Confirmatory (if required)	0.1 mg/kg	LC-MS/MS	KCP 5.3.3.3.2 xxx
	Primary (kidney)	0.01 mg/kg	LC-MS/MS	KCP 5.3.3.3.3 xxx Report No.: YV/13/033
	ILV	0.01 mg/kg	LC-MS/MS	KCP 5.3.3.3.4 xxx.: 100041492A
	Confirmatory (if required)	0.01 mg/kg	LC-MS/MS	KCP 5.3.3.3.3 xxx Report No.: YV/13/033

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

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 pendimethalin in soil is given in the following tables.

Table 5.3-16: Validated methods for soil (if appropriate)

Component of residue definition: pendimethalin			
Method type	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	0.01 mg/kg	HPLC-MS/MS	RAR 2015, EFSA 2016 Heinz N.,2013; Class T., 2013
	0.01 mg/kg	LC-MS/MS	KCP 5.3.3.4.1 (xxx Study N. 16.566423.0008)
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 pendimethalin in surface and drinking water is given in the following tables.

Table 5.3-17: Validated methods for water (if appropriate)

Component of residue definition: pendimethalin				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Drinking water	Primary	0.02 µg/L 0.1 µg/L	HPLC-MS/MS GC/MS	RAR 2015, EFSA 2016 Heinz N., 2013 KCP 5.3.3.5.1 (xxx Study N. YV/13/034)
	ILV	0.02 µg/L 0.1 µg/L	HPLC-MS/MS GC/MS	RAR 2015, EFSA 2016 Wiesner F., Breyer N., 2013 KCP 5.3.3.5.2 (K. Mclnerney 2016; Study N. 100041492B)
	Confirmatory	-	-	Not required.
Surface water	Primary	0.02 µg/L 0.02 µg/L	HPLC-MS/MS HPLC-MS/MS	RAR 2015, EFSA 2016 Heinz N., 2013 KCP 5.3.3.5.3 xxx Report No. 16.566423.0010
	Confirmatory	-	-	Not required.

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 pendimethalin in air is given in the following tables.

Table 5.3-18: Validated methods for air (if appropriate)

Component of residue definition: pendimethalin			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	~~4 µg/m^3~~ 70 µg/m^3 4 µg/m^3	LC-MS/MS LC-MS/MS	RAR 2015, EFSA 2016 Penning H., 2013 KCP 5.3.3.6.1 (xxx 16.566423.00012)
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 pendimethalin 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 5.3-19: Methods for body fluids and tissues (if appropriate)

Component of residue definition: pendimethalin			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary (blood)	0.05 mg/l	LC-MS/MS	KCP 5.3.3.7.1 (xxx Study N. 16.566423.0007)
Confirmatory	-	-	Not required.
Primary (liver)	0.01 mg/kg	LC-MS/MS	KCP 5.3.3.7.2 (xxx Study N. 16.566423.0004)
Confirmatory	-	-	Not required.

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

5.3.3.8 Other studies/ information

Not relevant

Appendix 1 Lists of data considered in support of the evaluation

Tables considered not relevant can be deleted as appropriate.

MS to blacken authors of vertebrate studies in the version made available to third parties/public.

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	B. Rajasekhar	2020 2021	Accelerated storage stability study of flufenacet 6% + pendimethalin 30% EC. Report No. 7713/2020 GLP Unpublished Amendment to Study Report	N	Sharda
KCP 5.1.1-2	S. D. Revankar	2021	Accelerated storage stability test by heating at elevated temperature of Flufenacet 6% + Pendimethalin 30% SC. Report No. G21362 GLP Unpublished	N	Sharda
KCP 5.3.2.2.1	E. Rigamonti	2019	Validation of the Analytical Method for the Determination of Flufenacet and metabolites (Flufenacet Cysteine conjugate (M23), Flufenacet OA, Flufenacet sulfonic acid (M2), Flufenacet thioglycolate sulfoxide (TGS)) Residues in Barley grain Matrix. E. Rigamonti, 2019 Report No. CH - 0753/2019. GLP Unpublished	N	Sharda
KCP 5.3.3.2.1	M. L. Greco	2017	Validation of the analytical procedure for the determination of pendimethalin (CAS: 40487-42-1), in wheat grains by liquid chromatography. M. L. Greco, 2017 Report No. 16.566423.0005 GLP Unpublished	N	Sharda

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 5.3.3.2.2	Joanna Kicinska	2019	Determination of the residues of pendimethalin applied as “pendimethalin 330 g/l” in barley at one site in Spain, 2016. J. Kicińska, 2017 Report No. ZBBZ-2016/69/DPL/1ES GLP Unpublished	N	Sharda
KCP 5.3.3.2.3	M. L. Greco	2017	Validation of the analytical procedure for the determination of pendimethalin (CAS: 40487-42-1), in wheat straw by liquid chromatography. M. L. Greco, 2017, Report No. 16.566423.0006 GLP Unpublished	N	Sharda
KCP 5.3.3.2.4	M. L. Greco	2017	Validation of the analytical procedure for the determination of pendimethalin (CAS: 40487-42-1) in potato by liquid chromatography. M. L. Greco, 2017, Chelab Report No. 16.586423.0002 GLP Unpublished	KCP 5.3.2.2.1	M. L. Greco
KCP 5.3.3.2.5	M. L. Greco	2017	Validation of the analytical procedure for the determination of Pendimethalin (CAS: 40487-42-1) in oranges by liquid chromatography. M. L. Greco, 2017, Chelab Report No. 16.566423.0003 GLP Unpublished	KCP 5.3.2.2.2	M. L. Greco
KCP 5.3.3.2.6	Maria Laura Greco	2014	Validation of the analytical procedure for the determination of pendimethalin (CAS: 40487-42-1), in soy beans by liquid chromatography. Study No. 16.566423.0001 GLP Unpublished	KCP 5.3.2.2.3	Maria Laura Greco

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 5.3.3.2.7	J. Kicińska	2017	Determination of residues of Pendimethalin applied as “Pendimethalin 330 g/L” in potato at one site in Germany, 2016. J. Kicińska, 2017, Food Safety Laboratory Report No. ZBBZ-2016/12/DPL/4DE GLP Unpublished	N	Sharda Cropchem Ltd
KCP 5.3.3.2.8	J. Kicińska	2018	Determination of residues of Pendimethalin applied as “Penddimethalin 330 g/L EC” in soybean at one site in Poland, 2016. Food Safety Laboratory Report No. ZBBZ-2016/12/DPL/8PL GLP Unpublished	N	Sharda Cropchem Ltd
KCP 5.3.3.2.9	Andrews, G.M. and Pitman H.	2014	Independent Laboratory Validation: determination of pendimethalin residues in crop (dry, high water, high acid and high oil content) [Method EN15662:2008]. Battelle Report No YV/13/035 GLP Unpublished	N	Sharda Cropchem Ltd
KCP 5.3.3.3.1	xxx	2022	Validation of the Analytical Methodology used for the determination of pendimethalin in Animal Matirx (Meat, Fat, Milk & Eggs) Report No.: 9421/2021 GLP Unpublished	Y	Sharda Cropchem Ltd
KCP 5.3.3.3.2	xxx	2017	Validation of the analyctical procedure for the determination of Pendimethalin (CAS: 40487-42-1) in liver by liquid chromatography Report No.: 16.566423.0004 GLP Unpublished	Y	Sharda Cropchem Ltd

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 5.3.3.3.3	xxx	2014	Method Validation: Determination of Residues of Pendimethalin in Products of Animal Origin, Report No.: YV/13/033 GLP Unpublished	Y	Sharda Cropchem Ltd
KCP 5.3.3.3.4	xxx	2015	Independent Laboratory validation (ILV) of Analytical Method: YV/13/033: Method Validation: Determination of Residues of pendimethalin in Products of Animal Origin, Report No.: 100041492A GLP Unpublished	Y	Sharda Cropchem Ltd
KCP 5.3.3.4.1	Maria Laura Greco	2017	Validation of the analytical procedure for the determination of pendimethalin (CAS: 40487-42-1), loamy sand soil by liquid chromatography. Study No. 16.566423.0008 GLP Unpublished	N	Sharda Cropchem Ltd
KCP 5.3.3.5.1	G Andrews	2015	Method Validation: Determination of Residues of Pendimethalin in Drinking Water Samples Study No. YV/13/034 GLP Unpublished	N	Sharda Cropchem Ltd
KCP 5.3.3.5.2	K. Mclnerney	2016	Independent Laboratory Validation (ILV) of Analytical Method for the Determination of Residues of Pendimethalin in Drinking Water Study No. 100041492B GLP Unpublished	N	Sharda Cropchem Ltd
KCP 5.3.3.5.3	Maria Laura Greco	2017	Validation of the analytical procedure for the determination of Pendimethalin (CAS: 40487-42-1), in surface water by liquid chromatography. Report No. 16.566423.0010 GLP Unpublished	N	Sharda Cropchem Ltd

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 5.3.3.6.1	Maria Laura Greco	2017	Validation of the analytical procedure for the determination of pendimethalin (CAS: 40487-42-1), in air by liquid chromatography. Study No. 16.566423.0012 GLP Unpublished	N	Sharda Cropchem Ltd
KCP 5.3.3.7.1	xxx	2017	Validation of the analyticayl procedure for the determination of pendimethalin (CAS: 40487-42-1) in blood by liquid chromatography FR 16.566423.0007 GLP Unpublished	Y	Sharda
KCP 5.3.3.7.2	xxx	2017	Validation of the analyticayl procedure for the determination of pendimethalin (CAS: 40487-42-1) in liver by liquid chromatography FR 16.566423.0004 GLP Unpublished	Y	Sharda

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

The following tables are to be completed by MS

List of data submitted by the applicant and not relied on

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

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

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

Appendix 2 Detailed evaluation of submitted analytical methods

A 2.1 Analytical methods for Flufenacet

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

No new or additional studies have been submitted

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

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

A 2.1.2.1.1 Analytical method 1

A 2.1.2.1.1.1 Method validation

Comments of zRMS:	Method is accepted

Reference:	KCP 5.3.2.2.1
Report	Validation of the Analytical Method for the Determination of Flufenacet and metabolites (Flufenacet Cysteine conjugate (M23), Flufenacet OA, Flufenacet sulfonic acid (M2), Flufenacet thioglycolate sulfoxide (TGS)) Residues in Barley grain Matrix. E. Rigamonti, 2019, Report No. CH - 0753/2019.
Guideline(s):	Yes SANCO/3029/99 rev. 4 SANCO/825/00 rev. 8.1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Principle of the method
The determination of the Flufenacet and metabolites (Flufenacet Cysteine conjugate (M23), Flufenacet OA, Flufenacet sulfonic acid (M2), Flufenacet thioglycolate sulfoxide (TGS)) residues content in the matrix is performed by UHPLC using an external standard and triple quadrupole detector in the MRM mode.
Its quantification is achieved by comparing the reference material peak area versus the peak area in matrix samples.

Equipment
- UHPLC/MS/MS, equipped with binary pump, autosampler and coupled with a Triple Quadrupole Mass Detector JS ESI interfaces, Mass Hunter software for data processing
- Analytical balance, 0.1 mg precision
- Refrigerator
- Freezer
- Ultrasonic bath
- Centrifuge
- Volumetric glassware: pipettes, flasks, measuring cylinders
- Usual laboratory glassware.

Reagents
- Water, LC-MS grade
- Methanol, LC-MS grade
- Formic acid, high purity for mass spectroscopy

Reference Materials
- Flufenacet analytical standard.
- Flufenacet Cysteine conjugate (M23) (CC) analytical standard.
- Flufenacet OA analytical standard.
- Flufenacet sulfonic acid (M2) (SA) analytical standard.
- Flufenacet thioglycolate sulfoxide (TGS) analytical standard.

Preparation of Eluent (water with 0.1% formic acid)
Into a 1 L conical flask, place 0.95 L of water LC-MS grade, add 1.00 mL of formic acid and then make to 1 L with water.
Preparation of Eluent (methanol with 0.1% formic acid)
Into a 1 L conical flask, place 0.95 L of methanol LC-MS grade, add 1.00 mL of formic acid and then make to 1 L with methanol.

Preparation of the matrix solutions
Using an analytical balance, weight an aliquot of ca. 0.5 g of the Barley grain into a 15 mL centrifuge test tube. Using a volumetric pipette, add an aliquot of 5.00 mL of methanol. Shake the sample using vortex for 5 minutes and for a further 30 minutes into a mechanical shaker. After that time had elapsed, filter the solution using plastic syringe and PTFE filter by 0.20 µm. Inject an aliquot of 1 µL of the final solution into the UHPLC and determine the peak area.

Preparation of the Low fortification level (at about 0.01 mg/kg).
Using an analytical balance, weight an aliquot of ca. 0.5 g of the Barley grain into a 15 mL centrifuge test tube. Using a volumetric pipette, add an aliquot of 5.00 mL of LFRMS. Shake the sample using vortex for 5 minutes and for a further 30 minutes into a mechanical shaker. After that time had elapsed, filter the solution using plastic syringe and PTFE filter by 0.20 µm. Inject an aliquot of 1 µL of the final solution into the UHPLC and determine the peak area.

Preparation of the Low fortification level (at about 0.10 mg/kg).
Using an analytical balance, weight an aliquot of ca. 0.5 g of the Barley grain into a 15 mL centrifuge test tube. Using a volumetric pipette, add an aliquot of 5.00 mL of HFRMS. Shake the sample using vortex for 5 minutes and for a further 30 minutes into a mechanical shaker. After that time had elapsed, filter the solution using plastic syringe and PTFE filter by 0.20 µm. Inject an aliquot of 1 µL of the final solution into the UHPLC and determine the peak area.

Results and discussions

Table A 1: Recovery results from method validation of Flufenacet and metabolites (Flufenacet Cysteine conjugate (M23), Flufenacet OA, Flufenacet sulfonic acid (M2), Flufenacet thioglycolate sulfoxide (TGS) using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (*n* =5)	Mean recovery (%)	RSD (%)	Comments
Barley grain	Flufenacet OA	0.01	105.7	3.20	Quantifier (m/z 137.9)
		0.1	76.9	2.54	
		0.01	106.4	2.86	1st Qualifier (m/z 109.9)
Barley grain	Flufenacet SA	0.01	90.3	5.91	Quantifier (m/z 111.9)
		0.1	75.6	2.61	
		0.01	107.7	2.41	1st Qualifier (m/z 233.9)
		0.01	91.6	13.60	2nd Qualifier (m/z 215.9)
Barley grain	Flufenacet TGS	0.01	97.6	4.29	Quantifier (m/z 110.9)
		0.1	74.9	1.85	
		0.01	107.9	0.45	1st Qualifier (m/z 241.9)
		0.01	106.9	0.90	2nd Qualifier (m/z 284.0
Barley grain	Flufenacet CC	0.01	106.9	2.58	Quantifier (m/z 225.9)
		0.1	76.0	1.16	
		0.01	103.8	1.13	1st Qualifier (m/z 141.9)
		0.01	107.4	2.13	2nd Qualifier (m/z 111.9)
Barley grain	Flufenacet	0.01	101.1	2.39	Quantifier (m/z 151.9)
		0.1	78.5	1.83	
		0.01	99.8	3.55	1st Qualifier (m/z 194)
		0.01	104.2	0.93	2nd Qualifier (m/z 124)

Table A 2: Characteristics for the analytical method used for validation of Flufenacet and metabolites (Flufenacet Cysteine conjugate (M23), Flufenacet OA, Flufenacet sulfonic acid (M2), Flufenacet thioglycolate sulfoxide (TGS) residues in barley grain

	Flufenacet and metabolites (Flufenacet Cysteine conjugate (M23), Flufenacet OA, Flufenacet sulfonic acid (M2), Flufenacet thioglycolate sulfoxide (TGS)
Specificity	No significant peaks (≤30% LOQ) are detected at RT of the target analyte in the Blank and Test Solution with respect to the Spiked Test Solution for both transitions. The method is specific.
Calibration (type, number of data points)	5 points Concentrations range (ng/mL) Flufenacet OA (0.30 – 99.30) Flufenacet SA (0.30 – 99.91) Flufenacet TGS (0.29 – 97.00)

	Flufenacet CC (0.30 – 99.69) Flufenacet (0.30 – 99.40)
Assessment of matrix effects is presented	Yes (the matrix effects for all analyte residue in Barley grain matrix were found not significant (lower than 20%))
Limit of determination/quantification	LOQ = 0.01 mg/kg LOD = 0.002 mg/kg

Conclusion

According to SANCO/3029/99 and SANCO/825/00 the method was successfully validated and is suitable for determination of residues of Flufenacet and metabolites (Flufenacet Cysteine conjugate (M23), Flufenacet OA, Flufenacet sulfonic acid (M2), Flufenacet thioglycolate sulfoxide (TGS) residues in barley grain.

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 A.2.A.9 Other Studies/ Information

No new or additional studies have been submitted

A 2.2 Analytical methods for Pendimethalin

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

No new or additional studies have been submitted

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 2.2.2.1.1 Analytical method 1

A 2.2.2.1.1.1 Method validation

Comments of zRMS:	Metod is accepted

Reference:	KCP 5.3.3.2.1
Report	Validation of the analytical procedure for the determination of pendimethalin (CAS: 40487-42-1), in wheat grains by liquid chromatography. xxx Report No. 16.566423.0005
Guideline(s):	Yes SANCO/3029/99 rev. 4 SANCO/825/00 rev. 8.1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Short term study for the validation of an analytical method for the determination of Pendimethalin (CAS: 40487-42-1) in wheat grain.

Mobile phase A (10 mM ammonium formate buffer pH 4)
About 0.62 g of ammonium formate were accurately weighed into a 1000 ml volumetric flask and dissolved with about 500 mL of milliQ water. 0.22 mL of formic acid were added and then the solution was diluted to bolume with milliQ water. pH wass 4

Mobile phase B
Methanol

Blank solution
10 mM ammonium formate buffer: acetonitrile, 50:50

Extraction mixture (5% formic acid acetonitrile)

In a 200 mL volumetric flask containing about 50 mL of acetonitrile, about 10 mL of acid formic were introduced and then diluted to volume with acetonitrile.

Sample extraction
About 5 g of wheat grains were introduced into a 50 mL plastic tube, 7.5 mL of milliQ water and 10 mL of extraction mixture were added to the sample. After vortexing for about 1 min, about 6 g of magnesium sulphate anhydrous and about 1.5 g of sodium acetate were added to the sample and vortexed again for about 1 min. The tube was centrifuged at 4750 rpm for 5 min and kept at about - 20°C for about 2 hours. Then, centrifuge the tube at 4750 rpm for 5 min and proceed to purification of the supernatant. 5 mL of supernatant were transferred into a 10 mL plastic tube, containing about 450 mg of magnesium sulphate anhydrous and 150 mg of PSA resin. Vortexed for about 1 min and centrifuged at 4750 rpm for 5 min. The supernatants of purified sample were recovered and transferred into an HPLC vial and inject. Test sample was prepared in duplicate.

Results and discussions

Table A 3: Recovery results from method validation of Pendimethalin using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (*n* =5)	Mean recovery (%)	RSD (%)	Comments
Wheat grain	Pendimethalin	0.01	100.3	2	First mass transition
		0.1	100.2	2	
		0.01	108.2	1	Second mass transition
		0.1	97.7	1	

Table A 4: Characteristics for the analytical method used for validation of pendimethalin residues in wheat grain

	Pendimethalin
Specificity	No significant peaks (≤30% LOQ) are detected at RT of the target analyte in the Blank and Test Solution with respect to the Spiked Test Solution for both transitions. The method is specific.
Calibration (type, number of data points)	5 points 0.002 to 0.0.358 mg/kg First mass transition y=9948520x R^2=0.9980 Second mass transition y=1071544 R^2=0.9981
Assessment of matrix effects is presented	Yes
Limit of determination/quantification	LOQ = 0.01 mg/kg LOD = 0.003 mg/kg

Conclusion

According to SANCO/3029/99 and SANCO/825 the method was successfully validated and is suitable for determination of residues of pendimethalin in wheat grain.

A 2.2.2.1.2 Analytical method 2

A 2.2.2.1.2.1 Method validation

Comments of zRMS:	Method is accepted

Reference:	KCP 5.3.3.2.2
Report	Determination of the residues of pendimethalin applied as "pendimethalin 330 g/l" in barley at one site in Spain, 2016. J. Kicińska, 2017, Report No. ZBBZ-2016/69/DPL/1ES
Guideline(s):	Yes SANCO/3029/99 rev. 4 SANCO/825/00 rev. 8.1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

To achieve the objective appropriate analytical method for determination of Pendimethalin was validated in accordance to the guidance documents SANCO/825/00, rev. 8.1. [1] and SANCO/3029/99, rev. 4 [2] of the European Commission and to meet residue regulatory requirements. The validated limit of quantification is 0.01 mg/kg.

The general principles of the analytical procedure were based on the Final Report N. 16.566423.005 [3] and Final Report N. 16.566423.006 [4]. In brief, samples of Barley were extracted with acidified acetonitrile. After addition of a buffer-salt mixture containing magnesium sulfate and sodium acetate the extract was shaken. After centrifugation, an aliquot of the upper acetonitrile phase was cleaned by primary secondary amine (PSA) and dehydrated by magnesium sulfate addition.

Results and discussions

Table A 5: Recovery results from method validation of Pendimethalin using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (*n* =5)	Mean recovery (%)	RSD (%)	Comments
Barley whole plants	Pendimethalin	0.01	84	8.0	First mass transition
		0.1	87	5.7	
		0.01	86	8.3	Second mass transition
		0.1	89	6.0	
Barley straw	Pendimethalin	0.01	93	7.1	First mass transition
		0.1	96	4.2	
		0.01	86	7.2	Second mass transition

Matrix	Analyte	Fortification level (mg/kg) (*n* =5)	Mean recovery (%)	RSD (%)	Comments
		0.1	97	4.0	
Barley grain	Pendimethalin	0.01	91	4.6	First mass transition
		0.1	100	5.2	
		0.01	99	3.3	Second mass transition
		0.1	102	5.4	

Table A 6: Characteristics for the analytical method used for validation of pendimethalin residues

	Pendimethalin
Specificity	No significant peaks (≤30% LOQ) are detected at RT of the target analyte in the Blank and Test Solution with respect to the Spiked Test Solution for both transitions. The method is specific.
Calibration (type, number of data points)	The correlation between the injected concentration of analyte standard and detector response was demonstrated to be linear by single determination of matrix-matched calibration standards at seven concentration levels ranging from 0.00025 µg/mL to 0.025 µg/mL for Barley (grain and plant) and eight concentration levels ranging from 0.0001 µg/mL to 0.025 µg/mL for Barley (straw). Those ranges correspond from 0.0025 mg/kg to 0.25 mg/kg for Barley (grain and plant) and from 0.002 mg/kg to 0.5 mg/kg for Pendimethalin (straw) thus covers the range from no more than 30 % of the LOQ and at least + 20 % of the highest analyte concentration level detected in samples.
Assessment of matrix effects is presented	Yes
Limit of determination/quantification	LOQ = 0.01 mg/kg LOD = 0.0025 mg/kg

Conclusion

According to SANCO/3029/99 and SANCO/825 the method was successfully validated and is suitable for determination of residues of pendimethalin in barley whole plants, straw and grain.

A 2.2.2.1.3 Analytical method 3

A 2.2.2.1.3.1 Method validation

Comments of zRMS:	Method is accepted

Reference: KCP 5.3.3.2.3

Report Validation of the analytical procedure for the determination of pendime-

	thalin (CAS: 40487-42-1), in wheat straw by liquid chromatography. xxx, Report No. 16.566423.0006
Guideline(s):	Yes SANCO/3029/99 rev. 4 SANCO/825/00 rev. 8.1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Short term study for the validation of an analytical method for the determination of Pendimethalin (CAS: 40487-42-1) in wheat straw.

Mobile phase A (10 mM ammonium formate buffer pH 4)
About 0.62 g of ammonium formate were accurately weighed into a 1000 ml volumetric flask and dissolved with about 500 mL of milliQ water. 0.22 mL of formic acid were added and then the solution was diluted to bolume with milliQ water. pH was 4

Mobile phase B
Methanol

Blank solution
Acetonitrile

Extraction mixture (5% formic acid acetonitrile)
In a 200 mL volumetric flask containing about 50 mL of acetonitrile, about 10 mL of acid formic were introduced and then diluted to volume with acetonitrile.

Sample extraction
About 3 g of grinded wheat straw were weighed into a 50 mL plastic tube and 12.5 mL of milliQ water were added to the sample. After vortexing for about 1 min, about 6 g of magnesium sulphate anhydrous and about 1.5 g of sodium acetate were added to the sample and vortexed again for about 1 min. The tube was centrifuged at 4750 rpm for 5 min and then, proceed to purification of the supernatant 5 mL of supernatant were transferred into a 10 mL plastic tube, containing about 450 mg of magnesium sulphate anhydrous and 150 mg of PSA resin. Vortexed for about 1 min and centrifuged at 4750 rpm for 5 min.
0.5 ml of supernatant of purified sample were transferred into 5 different 10 ml tubes and dried by N_2 flux. The dried sample was then resuspended into 0.5 ml of linearity solution (L1-L5) to have the calibration curve in matrix Vortex for 1 min, transferred into an HPLC vial and inject.

Results and discussions

Table A 7: Recovery results from method validation of Pendimethalin using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (*n* =5)	Mean recovery (%)	RSD (%)	Comments
Wheat straw	Pendimethalin	0.01	94.2	1.5	First mass transition
		0.1	86.0	2.9	
		0.01	108.4	0.3	Second mass transition

Matrix	Analyte	Fortification level (mg/kg) (*n* =5)	Mean recovery (%)	RSD (%)	Comments
		0.1	87.8	4.5	

Table A 8: Characteristics for the analytical method used for validation of pendimethalin residues

	Pendimethalin
Specificity	No significant peaks (≤30% LOQ) are detected at RT of the target analyte in the Blank and Test Solution with respect to the Spiked Test Solution for both transitions. The method is specific.
Calibration (type, number of data points)	5 points 0.003 to 0.398 mg/kg First mass transition R^2=0.9988 Second mass transition R^2=0.9982
Assessment of matrix effects is presented	Yes
Limit of determination/quantification	LOQ = 0.01 mg/kg LOD = 0.003 mg/kg

Conclusion

According to SANCO/3029/99 and SANCO/825/00 the method was successfully validated and is suitable for determination of residues of pendimethalin in wheat straw.

A 2.2.2.1.4 Analytical method 4

A 2.2.2.1.4.1 Method validation

Comments of zRMS:	Method is accepted

Reference:	KCP 5.3.3.2.4
Report	Validation of the analytical procedure for the determination of pendimethalin (CAS: 40487-42-1) in potato by liquid chromatography. xxx Report No. 16.586423.0002
Guideline(s):	Yes SANCO/3029/99 rev. 4 SANCO/825/00 rev. 8.1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Short term study for the validation of an analytical method for the determination of Pendimethalin (CAS: 40487-42-1) in potato.

Mobile phase A (10 mM ammonium formate buffer pH 4)
About 0.62 g of ammonium formate were accurately weighed into a 1000 ml volumetric flask and dissolved with about 500 mL of milliQ water. 0.22 mL of formic acid were added and then the solution was diluted to bolume with milliQ water. pH wass 4

Mobile phase B
Methanol

Blank solution
10 mM ammonium formate buffer: acetonitrile, 50:50

Extraction mixture (5% formic acid acetonitrile)
In a 200 mL volumetric flask containing about 50 mL of acetonitrile, about 10 mL of acid formic were introduced and then diluted to volume with acetonitrile.

Sample extraction
About 5 g of grinded potato were weighed into a 50 mL plastic falcon and 7.5 mL of milliQ water were added in order to hydrate the matrix. Then, 10 mL of extraction mixture were added to the sample. After vortexing for about 1 min, about 6 g of magnesium sulfate anhydrous and about 1.5 g of sodium acetate were added to the sample and vortexed again for about 1 min. The tube was centrifuged at 4750 rpm for 5 min and proceed to purification
5 mL of supernatant were transferred into a 10 mL plastic tube, containing about 450 mg of magnesium sulphate anhydrous and 150 mg of PSA resin. Vortexed for about 1 min and centrifuged at 4750 rpm for 5 min. The supernatants were recovered and transferred into an HPLC vial and injected. Test sample was prepared in triplicate.

Results and discussions

Table A 9: Recovery results from method validation of Pendimethalin using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (*n* =5)	Mean recovery (%)	RSD (%)	Comments
Potato	Pendimethalin	0.01	100.1	3	First mass transition
		0.1	97.5	2	
		0.01	96.3	3	Second mass transition
		0.1	97.5	2	

Table A 10: Characteristics for the analytical method used for validation of pendimethalin residues in potato

	Pendimethalin
Specificity	No significant peaks (≤30% LOQ) are detected at RT of the target analyte in the Blank and Test Solution with respect to the Spiked Test Solution for both transitions. The method is specific.
Calibration (type, number of data points)	5 points

	Pendimethalin
Specificity	No significant peaks (≤30% LOQ) are detected at RT of the target analyte in the Blank and Test Solution with respect to the Spiked Test Solution for both transitions. The method is specific.
	0.002 to 0.4 mg/kg First mass transition y=18900395x R^2=0.9959 Second mass transition y=2120709x R^2=0.9964
Assessment of matrix effects is presented	Yes
Limit of determination/quantification	LOQ = 0.01 mg/kg LOD = 0.003 mg/kg

Conclusion

According to SANCO/3029/99 and SANCO/825/00 the method was successfully validated and is suitable for determination of residues of pendimethalin in potato.

A 2.2.2.1.5 Analytical method 5

A 2.2.2.1.5.1 Method validation

Comments of zRMS:	Method is accepted

Reference:	KCP 5.3.3.2.5
Report	Validation of the analytical procedure for the determination of Pendimethalin (CAS: 40487-42-1) in oranges by liquid chromatography. xxx Report No. 16.566423.0003
Guideline(s):	Yes SANCO/3029/99 rev. 4 SANCO/825/00 rev. 8.1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Short term study for the validation of an analytical method for the determination of Pendimethalin (CAS: 40487-42-1) in oranges.

Mobile phase A (10 mM ammonium formate buffer pH 4)
About 0.62 g of ammonium formate were accurately weighed into a 1000 ml volumetric flask and dissolved with about 500 mL of milliQ water. 0.22 mL of formic acid were added and then the solution was

diluted to bolume with milliQ water. pH wass 4

Mobile phase B
Methanol

Blank solution
10 mM ammonium formate buffer: acetonitrile, 50:50

Extraction mixture (5% formic acid acetonitrile)
In a 200 mL volumetric flask containing about 50 mL of acetonitrile, about 10 mL of acid formic were introduced and then diluted to volume with acetonitrile.

Sample extraction
About 5 g of grinded oranges were weighed into a 50 mL plastic falcon and 7.5 mL of milliQ water were added in order to hydrate the matrix. Then, 10 mL of extraction mixture were added to the sample. After vortexing for about 1 min, about 6 g of magnesium sulfate anhydrous and about 1.5 g of sodium acetate were added to the sample and vortexed again for about 1 min. The tube was centrifuged at 4750 rpm for 5 min and proceed to purification
5 mL of supernatant were transferred into a 10 mL plastic tube, containing about 450 mg of magnesium sulphate anhydrous and 150 mg of PSA resin. Vortexed for about 1 min and centrifuged at 4750 rpm for 5 min. The supernatants were recovered and transferred into an HPLC vial and injected. Test sample was prepared in triplicate.

Results and discussions

Table A 11: Recovery results from method validation of Pendimethalin using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (*n* =5)	Mean recovery (%)	RSD (%)	Comments
Orange	Pendimethalin	0.01	101.9	2	First mass transition
		0.1	99.5	2	
		0.01	99.1	1	Second mass transition
		0.1	98.3	2	

Table A 12: Characteristics for the analytical method used for validation of pendimethalin residues in oranges

	Pendimethalin
Specificity	No significant peaks (≤30% LOQ) are detected at RT of the target analyte in the Blank and Test Solution with respect to the Spiked Test Solution for both transitions. The method is specific.
Calibration (type, number of data points)	5 points 0.002 to 0.398 mg/kg First mass transition y=35255318x R^2=0.9999 Second mass transition y=3842521x R^2=0.9999
Assessment of matrix effects is presented	Yes

	Pendimethalin
Specificity	No significant peaks (≤30% LOQ) are detected at RT of the target analyte in the Blank and Test Solution with respect to the Spiked Test Solution for both transitions. The method is specific.
Limit of determination/quantification	LOQ = 0.01 mg/kg LOD = 0.003 mg/kg

Conclusion

According to SANCO/3029/99 and SANCO/825/00 the method was successfully validated and is suitable for determination of residues of pendimethalin in oranges.

A 2.2.2.1.1 Analytical method 6

A 2.2.2.1.1.1 Method validation

Comments of zRMS:	Method is accepted

Reference:	KCP 5.3.3.3.6
Report	Validation of the analytical procedure for the determination of pendimethalin (CAS: 40487-42-1), in soy beans by liquid chromatography. xxx Report number: 16.566423.0001.
Guideline(s):	Yes (SANCO 3029/99 Rev.4 SANCO/825/00 Rev 8.1 and OECD-204/2014)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Reference Substance: Pendimethalin
Purity: 98,8%

Instrumental Conditions

• Column:	Acquity UPLC BEH C18, 50 mm x 2,1 mm x 1,7 µm (LC 23)
• Mobile Phase A:	10 mM ammonium formate buffer pH 4,0
• Mobile Phase B:	Methanol
• Flow:	0,2 ml/min
• Detector:	MS XEVO TQS (Waters-Micromass), SRA 470

	Precursor ion m/z		m/z	Collision energy
pendimethalin	282.15	Quantifier ion (trans 1)	212	10
		Qualifier ion (trans 2)	194	20

Results and discussions

Procedure
The analytical method, internally developed and codified as SOPa-284-LABCHI-Rev.0 was validated in terms of specificity, linearity, repeatability, accuracy and LOQ according to SANCO 3029/99 Rev.4 and OECD-204/2014 guidelines.
The validation was performed quantifying pendimethalin. Two SRM transitions were monitored pendimethalin:
• transition 1: 282.15 m/z (parent ion) > 212 m/z (daughter ion);
• transition 2: 282.15 m/z (parent ion) > 194 m/z (daughter ion).

Specify
Blank solution, Reference solution at LOQ level, Test Solution and Spiked Test Solution (at LOQ level) were injected for specificity evaluation.
Based on the chromatograms, the method is able to determine the analyte in the presence of the sample matrix.
No significant peaks (≤ 30% LOQ) are detected at RT of the target analyte in the Blank and Test Solution with respect to the Spiked Test Solution for both transition 1 and 2.

Linearity
The method linearity was evaluated at 5 different levels of concentration, ranging from at least 30% LOQ (0,003 mg/kg) to about 30xLOQ (0,3 mg/kg) of analyte on the sample.
Using the experimental data of area ratio (y) and the corresponding theoretical concentrations (x, in mg/l), the slope (b), the intercept (a) of the regression lines (y = a + bx) and determination coefficient R2 were calculated.

Linearity Parameters of transition 1: Coefficient of determination (R^2) – 0.9988
Linearity Parameters of transition 2: Coefficient of determination (R^2) – 0.9991

Repeatability precision
Repeatability evaluation was performed on aliquots of sample spiked with Pendimethalin at LOQ (about 0.003 mg/kg), and 10xLOQ (about 0.3 mg/kg). 5 replicate analyses were performed for each spiking level.

Accuracy
%Recovery is included between 70% and 110% in all cases, in accordance with acceptance criteria. The extraction efficiency was evaluated by fortifying test system with reference item at LOQ and 10xLOQ and evaluating recovery%

Limit of Quantification (LOQ)
LOQ is the lowest concentration level where an acceptable degree of linearity, accuracy and precision is established. In this case LOQ corresponds to 0,01 mg/kg.

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

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Mean recovery (%)		RSD (%)		Comments
			Tran. 1	Tran. 2	Tran. 1	Tran. 2	
Soy bean	pendimethalin	0.01	92.4	96.5	3	4	
Soy bean	pendimethalin	0.10	88.8	88.0	2	2	

Table A 14: Characteristics for the analytical method used for validation of pendimethalin residues in soy bean

	pendimethalin
Specificity	No significant peaks (≤ 30% LOQ) are detected at RT of the target analyte in the Blank and Test Solution with respect to the Spiked Test Solution for both transition 1 and 2.
Calibration range	The method linearity was evaluated at 5 different levels of concentration, ranging from at least 30% LOQ (0,003 mg/kg) to about 30xLOQ (0,3 mg/kg) of analyte on the sample.
Assessment of matrix effects is presented	Yes
Limit of determination/quantification	LOQ is the lowest concentration level where an acceptable degree of linearity, accuracy and precision is established. In this case LOQ corresponds to 0,01 mg/kg.

Conclusion

The validation data demonstrate that the analytical method is suitable to qualitatively and quantitatively determine pendimethalin in soy bean specimens, according to SANCO 3029/99 and SANCO/825/00 guidelines and for the given concentration range.

A 2.2.2.1.2 Analytical method 7

A 2.2.2.1.2.1 Independent laboratory validation

Comments of zRMS:	Method is accepted

Reference:	KCP 5.3.3.2.7
Report	Determination of residues of Pendimethalin applied as “Pendimethalin 330 g/L” in potato at one site in Germany, 2016. J. Kicińska, 2017, Report No. ZBBZ-2016/12/DPL/4DE
Guideline(s):	SANCO/3029/99 rev. 4 SANCO/825/00 rev. 8.1
Deviations:	No

GLP: Yes

Acceptability: Yes

Materials and methods

The objective of this study was to determine the decline and the magnitude of residues of Pendimethalin in Potato samples taken from the field trial, after applications of Pendimethalin 330 g/L, under open field conditions. To achieve the objective appropriate analytical method for determination of Pendimethalin was validated in accordance to the guidance documents SANCO/825/00, rev. 8.1. and SANCO/3029/99, rev. 4 of the European Commission and to meet residue regulatory requirements. The validated limit of quantification is 0.01 mg/kg.

The general principles of the analytical procedure were based on the Final Report N. 16555423.002. In brief, samples were extracted with acidified acetonitrile. After addition of a buffer-salt mixture containing magnesium sulfate and sodium acetate the extract was shaken. After centrifugation, an aliquot of the upper acetonitrile phase was cleaned by primary secondary amine (PSA) and dehydrated by magnesium sulfate addition.

Selectivity and Confirmation of Residue Identity

Quantification was performed by use of highly selective liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). Two selected ion mass transitions were evaluated in order to demonstrate that the method achieves a high level of selectivity. The retention time of analyte in extracts correspond to that of the calibration standard with a tolerance of < ± 0.1 min. Also, confirmation ion ratios for Pendimethalin in all samples were within ± 30 % of the average found for the standards.

No significant interference above 30 % of LOQ was detected in any of the reagent blanks or control specimen extracts for Potato matrix, so that a high level of selectivity was demonstrated and an additional confirmatory method is not necessary.

Matrix Effects

Matrix effects on the detection of Pendimethalin in extracts of Potato were lesser than 20% and thus considered insignificant, according to SANCO/825/00, rev. 8.1. and SANCO/3029/99, rev. 4. Determination was performed using matrix-matched calibration standards.

Linearity

The correlation between the injected concentration of analyte standard and detector response was demonstrated to be linear by single determination of matrix-matched calibration standards at seven concentration levels ranging from 0.00025 µg/mL to 0.025 µg/mL. This range corresponds from 0.0025 mg/kg to 0.25 mg/kg for potato and thus covers the range from no more than 30 % of the LOQ and at least + 20 % of the highest analyte concentration level detected in samples.

The calibration curves obtained for both ion mass transitions of Pendimethalin were linear with the coefficients of correlation (R) greater than 0.99. Linear regression was performed with 1/x weighting.

Results and discussions

Table A 15: Recovery results from independent laboratory validation of Pendimethalin using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (n = 5)	Mean recovery (%)	RSD (%)	Comments
Potato	Pendimethalin	0.01	86	3.8	First mass transition
		0.1	95	1.5	
		0.01	90	6.7	Second mass transition

Matrix	Analyte	Fortification level (mg/kg) (n = 5)	Mean recovery (%)	RSD (%)	Comments
		0.1	94	1.3	

Table A 16: Characteristics for the analytical method used for independent laboratory validation of Pendimethalin residues in potato

	Potato
Specificity	No significant peaks (≤30% LOQ) are detected at RT of the target analyte in the Blank and Test Solution with respect to the Spiked Test Solution for both transitions. The method is specific.
Calibration (type, number of data points)	5 points 0.0025 to 0.25 mg/kg First mass transition y=6656250.770882x+557.271913 R^2=0.99961687 Second mass transition y=1101199.679722x+167.416310 R^2=0.99883311
Assessment of matrix effects is presented	Yes
Limit of determination/quantification	LOQ = 0.01 mg/kg LOD = 0.0025 mg/kg

Conclusion

According to SANCO/3029/99 and SANCO/825 the method was successfully validated and is suitable for determination of residues of pendimethalin in potato.

A 2.2.2.1.2.2 Independent laboratory validation

Comments of zRMS:	Method is accepted

Reference:	KCP 5.3.3.2.8
Report	Determination of residues of Pendimethalin applied as “Pendimethalin 330 g/L EC” in soybean at one site in Poland, 2016. J. Kicińska, 2018, Report No. ZBBZ-2016/12/12/DPL/8PL
Guideline(s):	Yes SANCO/3029/99 rev 4 SANCO/825/00 rev. 8.1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The objective of this study was to determine the residues of Pendimethalin in Soybean samples taken from the field trial, after applications of Pendimethalin 330 g/L EC, under open field conditions. To achieve the objective, appropriate analytical method for determination of Pendimethalin was validated in accordance to the guidance documents SANCO/825/00, rev. 8.1. and SANCO/3029/99, rev. 4 of the European Commission and to meet residue regulatory requirements. The validated limit of quantification is 0.01 mg/kg.

The general principles of the analytical procedure were based on the Final Report N. 16555423.0002 [3]. In brief, samples of Soybean were extracted with acidified acetonitrile. After addition of a buffer-salt mixture containing magnesium sulfate and sodium acetate the extract was shaken. After centrifugation, an aliquot of the upper acetonitrile phase was cleaned by primary secondary amine (PSA) and dehydrated by magnesium sulfate addition.

Selectivity and Confirmation of Residue Identity

Quantification was performed by use of highly selective liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). Two selected ion mass transitions were evaluated in order to demonstrate that the method achieves a high level of selectivity. The retention time of analyte in extracts correspond to that of the calibration standard with a tolerance of < ± 0.1 min. Also, confirmation ratios for Pendimethalin in all samples were within ± 30 % of the average found for the standards.

No significant interference above 30 % of LOQ was detected in any of the reagent blanks or control specimen extracts for Soybean matrix, so that a high level of selectivity was demonstrated and an additional confirmatory method is not necessary.

Matrix Effects

Matrix effects on the detection of Pendimethalin in extracts of Soybean were higher than 20% and thus considered significant, according to SANCO/825/00, rev. 8.1. and SANCO/3029/99, rev. 4. Determination was performed using matrix-matched calibration standards.

Linearity

The correlation between the injected concentration of analyte standard and detector response was demonstrated to be linear by single determination of matrix-matched calibration standards at seven concentration levels ranging from 0.00025 µg/mL to 0.025 µg/mL. This range corresponds from 0.0025 mg/kg to 0.25 mg/kg for Soybean and thus covers the range from no more than 30 % of the LOQ and at least + 20 % of the highest analyte concentration level detected in samples.

Results and discussions

Table A 17: Recovery results from independent laboratory validation of pendimethalin using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (n = 5)	Mean recovery (%)	RSD (%)	Comments
Soybean	Pendimethalin	0.01	74	5.7	First mass transition
		0.1	73	11.2	
		0.01	72	6.2	Second mass transition
		0.1	73	11.6	

Table A 18: Characteristics for the analytical method used for independent laboratory validation of Pendimethalin residues in soybean

	Pendimethalin
Specificity	No significant interference above 30 % of LOQ was detected in any of the reagent blanks or control

	Pendimethalin
	specimen extracts for Soybean matrix, so that a high level of selectivity was demonstrated and an additional confirmatory method is not necessary.
Calibration (type, number of data points)	7 points 0.00025 µg/mL to 0.025 µg/mL First mass transition y=12097357.433687x+673.276223 R=0.9963276476 Second mass transition y=1235894.921549x+239.521389 R=0.99604571
Assessment of matrix effects is presented	Yes
Limit of determination/quantification	LOQ = 0.01 mg/kg LOD = 0.0025 mg/kg

Conclusion

According to SANCO/3029/99 and SANCO/825/00 the method was validated and is suitable for determination of Pendimethalin residues in soybean.

A 2.2.2.1.2.3 Independent laboratory validation

Comments of zRMS:	Method is accepted

Reference:	KCP 5.3.3.2.9
Report	Independent Laboratory Validation: Determination of Pendimethalin Residues in Crop (Dry, High Water, High Acid and High Oil Content) [Method EN15662:2008). xxx. Report No YV/13/035.
Guideline(s):	Yes SANCO/3029/99 rev 4 SANCO/825/00 rev. 8.1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The objective of this study was to independently validate an existing analytical method for the determination of pendimethalin in barley grain, oilseed rape seeds, whole oranges and tomatoes according to Document SANCO/3029/99 rev.4 and Guidance Document SANCO/825/00 rev. 8.1.

The concentration of the analyte was determined by LC-MS/MS. The method involved the extraction with acetonitrile, followed by a clean-up using QuEChERS salts
The resulting solutions were then analysed using LC-MS/MS. During LC-MS/MS analysis, two transitions were monitored, the first transition for quantification and the second for confirmation.
The validated limit of quantification is 0.01 mg/kg.

Selectivity and Confirmation of Residue Identity

Quantification was performed by use of highly selective liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). Two selected ion mass transitions were evaluated in order to demonstrate that the method achieves a high level of selectivity. The retention time of analyte in extracts correspond to that of the calibration standard.
No significant interference above 30 % of LOQ was detected in any of the reagent blanks or control specimen extracts, so that a high level of selectivity was demonstrated and an additional confirmatory method is not necessary.

Linearity

The correlation between the injected concentration of analyte standard and detector response was demonstrated to be linear by single determination of matrix-matched calibration standards at six concentration levels ranging from 0.51 ng/mL to 25.52 ng/mL. This range corresponds from 0.0025 mg/kg to 0.25 mg/kg for matrix and thus covers the range from no more than 30 % of the LOQ and at least + 20 % of the highest analyte concentration level detected in samples.
Determination was performed using matrix-matched calibration standards.

Results and discussions

Table A 19: Recovery results from independent laboratory validation of pendimethalin using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (n = 5)	Mean recovery (%)	RSD (%)	Comments
Tomato	Pendimethalin	0.01	103	1.1	First mass transition (282-212 m/z)
		0.1	104	1.8	
		0.01	102	1.6	Second mass transition (282-194 m/z)
		0.1	106	2.2	
Barley grain	Pendimethalin	0.01	91	5.2	First mass transition (282-212 m/z)
		0.1	96	2.2	
		0.01	91	6.0	Second mass transition (282-194 m/z)
		0.1	96	2.1	
Oilseed rape seed	Pendimethalin	0.01	85	6.9	First mass transition (282-212 m/z)
		0.1	84	9.3	
		0.01	80	8.0	Second mass transition (282-194 m/z)
		0.1	87	7.6	
Orange	Pendimethalin	0.01	107	2.7	First mass transition
		0.1	96	12.7	
		0.01	105	2.3	Second mass transition
		0.1	96	12.3	

Table A 20: Characteristics for the analytical method used for independent laboratory validation of Pendimethalin residues

	Pendimethalin
Specificity	No significant interference above 30 % of LOQ was detected in any of the reagent blanks or control specimen extracts for all matrices, so that a high level of selectivity was demonstrated and an additional confirmatory method is not necessary.
Calibration (type, number of data points)	6 points 0.00025 µg/mL to 0.025 µg/mL y = 0.0107x + 0.00264 r = 0.9998 barley grain trans 1 y = 0.00146x + 0.000392 r = 0.9994 barley grain trans 2 y = 0.00891x + 0.000821 r = 0.9947 oilseed rape trans 1 y = 0.00122x + -3.82E-005 r = 0.9912 oilseed rape trans 2 y = 0.0242x + 0.000578 r = 0.9897 orange trans 1 y = 0.00328x + -3.59E-005 r = 0.9924 orange trans 2 y = 0.0144x + 0.00227 r = 0.9915 tomato trans 1 y = 0.00201x + 0.000206 r = 0.9911 tomato trans 2
Assessment of matrix effects is presented	Yes
Limit of determination/quantification	LOQ = 0.01 mg/kg LOD = 0.0025 mg/kg

Conclusion

According to SANCO/3029/99 and SANCO/825/00 the method was validated and is suitable for determination of Pendimethalin residues in orange, tomato, oilseed rape and barley grain.

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 2.2.2.2.1 Analytical method 1

A 2.2.2.2.1.1 Method validation

Comments of zRMS:	Method is accepted

Reference: KCP 5.3.3.3.1

Report: Validation of the Analytical Methodology used for the determination of pendimethalin in Animal Matirx (Meat, Fat, Milk & Eggs), xxx, 2022, Re-

	port No.: 9421/2021
Guideline(s):	Yes SANTE/2020/12830 rev.1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Reference standard

Product:	Pendimethanil
CAS No.:	40487-42-1
Formula:	$C_{13}H_{19}N_3O_4$
Lot Number:	G998095
Purity:	97.34%
Expiry date:	22/05/2025
Storage condition:	4°C±4°C
Supplier:	Dr. Ehrenstorfer

Chemical/Reagents

Acetonitrile (LC-MS grade, Lot No.: 0000252086, purity: ≥99.9%, expiry date: 08/01/2022, J.T. Baker)
Formic Acid (Analytical reagent, Lot No.: R290F18, purity: 98.0%, expiry date: 12/11/2022, Rankem)

Instrument conditions and quantification

Method validation of the Pendimethalin was carried out by following analytical method using HPLC coupled with LC-MS/MS and the respective instrument conditions are mentioned as following:

HPLC

Instrument name:	High performance liquid chromatograph (HPLC)
Make:	Shimadzu
Model:	LC-2030 Plus
Column name:	Shimadzu
Material:	3µm C_{18}
Dimensions:	4.6 x 150 mm
Injection volume:	0.9µL
Flow rate:	0.9 mL/min
Mobil phase-A:	Acetonitrile (70%)
Mobil phase-B:	0.1% Formic Acid (30%)
Mode of flow:	Isocratic
Column temperature:	40°C
Retention time:	3.3 min

LC-MS

Instrument name:	Liquid Chromatography Mass Spectrometer (LC-MS)
Make:	Shimadzu
Model:	LCMS-8045
Detector:	Mass Detector (triple-quadrupole)
Ionization mode:	Electro Spray Ionisation (ESI); + ve mode
Mass Monitoring Mode:	Multiple Reaction Monitoring (MRM)

Transitions monitored (Pendimethalin fragmentation mass spectrum, justifying fragments selection):
MRM1 – m/z: 282.20>212.10
MRM2 – m/z: 282.20>148.10

Quantification of Pendimethalin in Animal Matrices (Milk, Eggs, Fat, Muscle):

Preparation of Mobile phase-A:

An amount of 1000 mL LC-MS grade acetonitrile was taken into 1000 mL mobile phase bottle and sonicated.

Preparation of Mobile phase-B:

An aliquot of 1 mL Formic acid was taken into a 1000 mL mobile phase bottle and made upto the mark with 999 mL of water, than sonicated the mixture. This mobile phase was equivalent to 0.1% of Formic acid.

Specificity

Standard solution (0.01 mg/kg), control and sample solution (0.01 mg/L) was used for specificity to determine the mass of Pendimethalin and confirmed the retention time (RT) in LC-MS/MS.

Linearity

The linearity of method was established by injecting six different concentrations (0.002 – 0.12 mg/kg in matrix, equivalent to 0.002 – 0.14 mg/L in reference solution) of Pendimethalin standard in Milk, Eggs, by LC-MS/MS and plotting their respective standard concentrations (mg/L) against the respective peak areas.

Precision

An amount of 10g matrix was taken into separate 12 different conical flask, two conical flasks were used for control (non spiked sample solution) and 0.01 mL of specificity standard solution (10.21 mg/L for milk and eggs, 10.03 mg/L for fat, 10 mg/L for muscle) was added into 5 conical flasks (for LS1-LS5) and 0.1 mL (for muscle 1 mL) of specificity standard solution (10.21 mg/L for milk and eggs, 10.03 mg/L for fat, 10 mg/L for muscle) was added into other 5 conical flasks (HS1-HS5). Then 10 mL of acetonitrile was added to the each conical flasks and kept in mechanical shaker for 30 minutes. The solution was shaken by hand vigorously for 1 minute and centrifuged at 4000 rpm for 5 minutes. The solution was filtered through Whatman No. 1 filter paper. The filter was passed and collected through a solid phase extraction cartridge (SPE), packed with C_{18} packing materials separately. The extract was transferred into a volumetric flask and acidified with 80µL of 5% formic acid and the respective solutions along with control were injected into LC-MS/MS.

The precision evaluation, LS and HS samples used for accuracy test were monitored both ion MRM1 and MRM2 transitions.

Recovery

In each analytical run of precision, standard samples with five runs (LS) and five runs (HS) were freshly prepared to monitor both ion transitions.

The accuracy was determined by analyzing the spiked Pendimethalin sample solution at two concentration levels (LS and HS). The accuracy (%recovery) and precision (%RSD) was determined.

Results and discussions

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

Matrix	Analyte	Fortification level (mg/kg) (n = 5)	Mean recovery (%)	RSD (%)	Comments
Milk	Pendimethalin	0.01	97.79	1.691	MRM1
			98.87	0.670	MRM2
		0.1	99.04	0.550	MRM1

Matrix	Analyte	Fortification level (mg/kg) (n = 5)	Mean recovery (%)	RSD (%)	Comments
			98.07	0.948	MRM2

Table A 22: Characteristics for the analytical method used for validation of Pendimethalin residues in Milk

	Pendimethalin
Specificity	mass spectrum is provided blank value < 30 % LOQ
Calibration (type, number of data points)	6 points MRM1 y=279045x + 84.128 R= 0.9998 MRM2 y=170528x + 136.72 R= 0.9993
Calibration range	0.02 – 0.12 mg/kg in milk, equivalent to 0.002 – 0.14 mg/L in reference solution
Assessment of matrix effects is presented	Yes
Limit of determination/quantification	LOQ = 0.01 mg/kg

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

Matrix	Analyte	Fortification level (mg/kg) (n = 5)	Mean recovery (%)	RSD (%)	Comments
Muscle	Pendimethalin	0.01	97.60	1.663	MRM1
			98.98	0.287	MRM2
		0.1	96.68	2.183	MRM1
			99.66	0.158	MRM2

Table A 24: Characteristics for the analytical method used for validation of Pendimethalin residues in Muscle

	Pendimethalin
Specificity	mass spectrum is provided blank value < 30 % LOQ
Calibration (type, number of data points)	6 points MRM1 y=333832x – 60.501 R= 0.9997 MRM2 y=456200x + 112.45 R= 0.9999
Calibration range	0.03 – 0.12 mg/kg in muscle,

	Pendimethalin
	equivalent to 0.002 – 0.14 mg/L in reference solution
Assessment of matrix effects is presented	Yes
Limit of determination/quantification	LOQ = 0.01 mg/kg

Table A 25: Recovery results from method validation of Pendimethalin using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (n = 5)	Mean recovery (%)	RSD (%)	Comments
Fat	Pendimethalin	0.01	99.08	0.731	MRM1
			95.34	3.389	MRM2
		0.1	99.01	0.103	MRM1
			97.79	0.824	MRM2

Table A 26: Characteristics for the analytical method used for validation of Pendimethalin residues in Fat

	Pendimethalin
Specificity	mass spectrum is provided blank value < 30 % LOQ
Calibration (type, number of data points)	6 points MRM1 y=369089x – 61.877 R= 0.9998 MRM2 y=246807x + 14.984 R= 0.9999
Calibration range	0.002 – 0.12 mg/kg in fat, equivalent to 0.002 – 0.14 mg/L in reference solution
Assessment of matrix effects is presented	Yes
Limit of determination/quantification	LOQ = 0.01 mg/kg

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

Matrix	Analyte	Fortification level (mg/kg) (n = 5)	Mean recovery (%)	RSD (%)	Comments
Eggs	Pendimethalin	0.01	95.19	4.266	MRM1
			99.01	0.399	MRM2
		0.1	95.35	2.198	MRM1
			97.78	0.490	MRM2

Table A 28: Characteristics for the analytical method used for validation of Pendimethalin residues in Eggs

	Pendimethalin
Specificity	mass spectrum is provided blank value < 30 % LOQ
Calibration (type, number of data points)	6 points MRM1 y=315215x – 48.451 R= 0.9997 MRM2 y=158561x – 18.413 R= 0.9999
Calibration range	0.002 – 0.12 mg/kg in egg, equivalent to 0.002 – 0.14 mg/L in reference solution
Assessment of matrix effects is presented	Yes
Limit of determination/quantification	LOQ = 0.01 mg/kg

Conclusion

Method is successfully validated and is suitable for determination of Pendimethalin in Animal Matrices (Milk, Eggs, Fat and Muscle) according to SANTE/2020/12830 rev.1.

A 2.2.2.2.2 Analytical method 2

A 2.2.2.2.2.1 Method validation

Comments of zRMS:	Method is accepted

Reference:	KCP 5.3.3.3.2 and Amendment (KCP 5.3.3.3.2-1)
Report	Validation of the analytical procedure for the determination of Pendimethalin (CAS: 40487-42-1) in liver by liquid chromatography, xxx Report No.: 16.566423.0004
Guideline(s):	Yes SANCO/825/00 rev. 8.1 SANCO/3029/99 rev. 4
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Reference item
Reference substance: Pendimethalin

Batch:	SZBD302XV
CAS nr:	40487-42-1
Origin:	Sharda Cropchem Limited
Purity:	98.8%
Molecular weight:	281.31 g/mol
Molecular formula:	$C_{13}H_{19}N_3O_4$
Chemlab ID:	RS316512

Reagents

- miliQ water, SRA 35
- Metahol, batch 17A054007, purchased from VWR
- Acetonitrile, batch STBG5324V, purchased from Honeywell
- Magnesium sulfate anhydrous IS: 226.13, purchased from Sigma Aldrich
- Ammonium formate (LC-MS grade), ID: 237.7, purchased from Sigma Aldrich
- Sodium acetate ID: 701.3, purchased from Sigma Aldrich
- PSA Resin 40µm, ID: 306.6, purchased from Varian
- Formic acid, ID: 961.1, purchased from Suprapur
- Pendimethaliun reference Standard Solution, ID: 3422

Instrumental Conditions

Column:	Acquity UPLC BEH C18, 50 mm x2.1 mm x 1.7 µm (LC 23)
Mobile phase A:	10 mM ammonium formate buffer pH 4.0
Mobile phase B:	Methanol
Flow:	0.2 ml/min
Injection volume:	5µL
Detector:	MS XEVO TQS (waters-Micromass), SRA 470
Source:	ESI
Source temp:	150°C
Nebulizer:	6 bar
Cone gas:	150 l/h
Desolvation gas:	400 l/h
Run time:	13 min

MRM:
Pendimethalin 282.15 m/z (precursor ion), 212 m/z (quantifier ion, trans 1)
Pendimethalin 282.15 m/z (precursor ion), 194 m/z (qualifier ion, trans 2)

Specificity
Blank solution, reference solution at LOQ level, test solution and spiked test solution (at LOQ level) were injected for specificity evaluation. The method is capable to determine the analyte in the presence of the sample matrix. No significant peaks (≤30% LOQ) are detected at RT of the target analyte in the Blank and Test Solution with the respect to the Spiked Test Solution for both transition 1 and 2.

Linearity
The method linearity was evaluated at 5 different levels of concentration, ranging from at least 30% LOQ (0.03 mg/kg) to about 30xLOQ – 3.0 mg/kg of analyte on the sample. Solutions were analysed by LC-MS. Using the experimental data of area ratio (y) and the correspondent theoretical concentrations (x, in mg/L), the slope (b), the intercept (a) of the regression lines (y=a+bx) and determination coefficient R^2 were calculated.

Repeatability precision
Repeatability evaluation was performed on aliquots of sample spiked with Pendimethalin at LOQ (about 0.1 mg/kg) and 10xLOQ (about 1.0 mg/kg). Five replicate analyses were performed for each spiking level. %RSD at each fortified level was calculated for both transitions.

Accuracy
The accuracy of analytical method expresses the closeness of the consistency between the accepted true value and the value found. %Recovery is included between 70% and 110% in all cases, in accordance with acceptance criterial.

Limit of Quantification
LOQ is the lowest concentration level where an acceptable degree of linearity, accuracy and precision is established. In this case LOQ corresponds to 0.1 mg/kg.

Results and discussions

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

Matrix	Analyte	Fortification level (mg/kg) (n = 5)	Mean recovery (%)	RSD (%)	Comments
Liver	Pendimethalin	0.1	104.4	2	MRM1
		1.0	99.6	3	MRM1
		0.1	103.0	3	MRM2
		1.0	98.6	2	MRM2

Table A 30: Characteristics for the analytical method used for validation of Pendimethalin residues in liver

	Pendimethalin
Specificity	No significant peaks (≤30% LOQ) are detected at RT of the target analyte
Calibration (type, number of data points)	5 points MRM1: y=27432263x R^2= 0.9997 MRM2: Y=2944247x R^2= 0.9995
Calibration range	0.01 – 1.59 mg/L, corresponds to 0.02 – 3.18 mg/kg
Assessment of matrix effects is presented	yes
Limit of determination/quantification	LOQ = 0.1 mg/kg

Conclusion

Method is successfully validated and is suitable for determination of Pendimethalin in Liver according to SANTE/2020/12830 rev.1 with LOQ = 0.1 mg/kg.

A 2.2.2.2.3 Analytical method 3

A 2.2.2.2.3.1 Method validation

Comments of zRMS:	Method is accepted

Reference:	KCP 5.3.3.3.3
Report	Method Validation: Determination of Residues of Pendimethalin in Products of Animal Origin, xxx, Report No.: YV/13/033
Guideline(s):	Yes EU Guidance Document SANCO/825/00 rev. 8.1, 16/11/2010
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

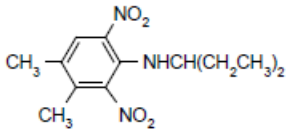
The objective of this study was to validate an analytical method for the determination of residues of pendimethalin in products of animal origin to fulfil the requirements according to Regulations (EU) 544/2011 and 545/2011 implementing Regulation (EC) 1107/2009, EU Guidance Document SANCO/825/00 rev. 8.1, 16/11/2010 and Directive 98/8/EC and Regulation (EU) 528/2012.

Kidney was the chosen product of animal origin to use to validate this method.

The analytical method for the determination of pendimethalin in kidney consisted of extraction with acetonitrile, followed by shaking and centrifugation of the sample. An aliquot was then diluted with water before analysis by LC-MS/MS. Calibration solutions for the determination of pendimethalin in products of animal origin were prepared in matrix.

Using the primary transition for quantification, the analytical method was successfully validated for use in terms of linearity, specificity, accuracy and precision. The secondary transition was determined to be suitable for confirmatory purposes.

Test item

Common name:	Pendimethalin
Chemical name (IUPAC):	*N*-(1-ethylpropyl)-2,6-dinitro-3,4-xylidine
Molecular Structure:	
CAS Number:	40487-42-1
Molecular formula:	$C_{13}H_{19}N_3O_4$
Molecular weight:	281.3
Source:	Sigma Aldrich
Batch number:	SZB8287XV
Expiry date:	13th October 2015
Purity:	98.8%

Storage conditions:	Ambient

LC-MS/MS Analysis

Liquid Chromatography			
Instrument:	Agilent 1290 Infinity HPLC System		
Column:	Phenomenex Kinetex XB-C18 5µ 100A 150 mm × 4.6 mm		
Column Temperature:	30 ºC		
Injection Volume:	75 µL		
Flow Rate:	1000 µL/min		
Mobile Phase A:	Water and 0.1% Formic Acid		
Mobile Phase B:	Acetonitrile and 0.1% Formic Acid		
	Time – minutes	**%A**	**%B**
	0.0	90	10
	0.5	90	10
	1.0	10	90
	6.5	10	90
	6.6	90	10
	9.0	90	10
Approximate Retention Time:	4.1 minutes		

Mass Spectrometry	
Instrument:	API 6500 LC-MS/MS, AB Sciex
Ion Source:	Electrospray (ESI, TurboIon Spray)
Polarity:	Positive
Collision Gas (CAD):	-2
Curtain Gas (CUR):	20
Ion Source Gas 1 (GS1):	50
Ion Source Gas 2 (GS2):	60
IonSpray Voltage (IS):	4000
Temperature (TEM):	300
Entrance Potential (EP):	10
Collision Cell Exit Potential (CXP):	14

MRM:
Pendimethalin 282.231 m/z (precursor ion), 212.0 m/z (quantifier ion, trans 1)
Pendimethalin 282.231 m/z (precursor ion), 193.9 m/z (qualifier ion, trans 2)

Specificity

No interference/contamination peak above 30% of the LOQ was detected at the retention time of pendimethalin in any control sample.

Linearity
For each analytical batch, at least five calibration standards were injected covering the range from 0.510 ng/mL to 25.515 ng/mL. Linear regression calculation was performed by the Analyst software, with 1/x weighting, using the concentration of the analyte versus the peak area. Correlation coefficients, r, were ≥0.99 demonstrating acceptable performance in terms of linearity.

Accuracy and precision
The recoveries were found to lie within the required range of 70-110% at all levels of fortification and the relative standard deviation was less than 20% which demonstrates acceptable accuracy and precision of the method. Repeatability evaluation was performed on aliquots of sample spiked with Pendimethalin at LOQ (about 0.01 mg/kg, n=5) and 10xLOQ (about 0.1 mg/kg, n=7)

LOD and LOQ
The limit of quantification (LOQ) was established at 0.01 mg/kg for pendimethalin in kidney.
The limit of detection (LOD) for the method was calculated to be 0.0004 mg/kg for pendimethalin.

Results and discussions

Table A 31: Recovery results from method validation of Pendimethalin using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (n = 5 for 0.01 mg/kg and n = 7 for 0.1 mg/kg)	Mean recovery (%)	RSD (%)	Comments
Kidney	Pendimetahlin	0.01	80	3.9	MRM1
		0.1	90	14.5	MRM1
		0.01	82	4.2	MRM2
		0.1	91	14.7	MRM2

Table A 32: Characteristics for the analytical method used for validation of Pendimethalin residues in kidney

	Pendimethalin
Specificity	No interference/contamination peak above 30% of the LOQ was detected at the retention time of pendimethalin in any control sample.
Calibration (type, number of data points)	7 points MRM1: Y=2.06e+005x+5.44e+004 R=0.9986 MRM2: Y=2.44e+004x+6.44e+003 R=0.9985
Calibration range	0.510- 25.515 ng/mL
Assessment of matrix effects is presented	yes

	Pendimethalin
Limit of determination/quantification	LOQ=0.01 mg/kg

Conclusion

The method was successfully validated and is suitable for the determination of residues of pendimethalin in kidney according to SANTE/12830/2020 rev.1. The LOQ of the method was validated at 0.01 mg/kg for pendimethalin in kidney during the validation study.

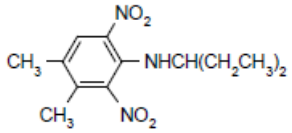
A 2.2.2.2.3.2 Independent laboratory validation

Comments of zRMS:	Method is accepted

Reference:	KCP 5.3.3.3.4
Report	Independent Laboratory validation (ILV) of Analytical Method: YV/13/033: Method Validation: Determination of Residues of pendimethalin in Products of Animal Origin, xxx Report No.: 100041492A
Guideline(s):	Yes SANCO/825/00 rev. 8.1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Analytical test and reference item

Common name:	Pendimethalin
IUPAC Name:	*N*-(1-ethylpropyl)-2,6-dinitro-3,4-xylidine
Structure:	
CAS No.:	40487-42-1
Empirical formula:	$C_{13}H_{19}N_3O_4$
Molecular Weight:	281.31 g/mol
Batch No.:	SZBD302XV
Expiry date:	29 October 2018
Purity:	98.8%

LC-MS/MS System

- Agilent 1260 HPLC System (vacuum solvent degasser, binary HPLC pump, column oven)
- CTC Analytics HTC-Pal Autosampler
- Applied Biosystems MDS Sciex API 5500 triple quadrupole LC/MS/MS system with Turbolonspray (ESI) source
- Phenomenex Kinetex XB-C18 Analytical Column: 150 x 4.6 mm (length x i.d.), 5µ particle size

Solvents and Chemicals

- MiliQ water (Battelle-Duxbury)
- Acetonitrile, HPLC Grade (Fisher Optima, Lot #132945 and Doe and Ingallas, Lot #0000050960)
- Formic acid (98-100%, Sigma Aldrich, Lot #SZBC1780V)

Extraction

1. Ten (10)g (*W*) of homogenized sample material was weighed in pre cleaned 125 mL Teflon centrifuge tubes.
2. Recovery control specimens were fortified with the required volume of the appropriate fortification solution.
a. Samples spiked at the LOQ were fortified with 100 µL of the 1.00 µg/mL fortification solution (Battelle ID: IA45)
b. Samples spiked at 10x the LOQ were fortified with 100 µL of the 10.0 µg/mL fortification solution (Battelle ID: IA44)
3. Twenty five (25.0) mL (V_{Ex}) of acetonitrile was added to each sample.
4. Each sample was shaken vigorously for 5 minutes on a shaker table.
5. Each sample was then sonicated for 5 minutes.
6. Each sample was centrifuged at 3000 RPM for 2 minutes.
7. A 0.2 mL aliquot of the supernatant was transferred to an HPLC injection vial and 0.8 mL of water was added.
8. Extracts were submitted for LC MS/MS analysis.

MRM:

Pendimethalin 282.2 m/z (precursor ion), 212.0 m/z (quantifier ion, trans 1)
Pendimethalin 282.2 m/z (precursor ion), 193.9 m/z (qualifier ion, trans 2)

Specificity

The highly specific LC MS/MS method utilizing two mass transitions was confirmed. The product ion spectra shown 212.0 m/z and 193.9 m/z are the dominant product ions for the 282.2 m/z precursor ion for the instrumental conditions used in this method.

Linearity

The project team was able to confirm that the LC MS/MS method afforded detection of the analyte at concentrations of 0.5 ng/mL with a 75 µL injection, providing sufficient sensitivity to quantify residues of the analyte in the final extracts. An instrument calibration for each transition was generated using seven concentrations of analyte in 1:4 sample matrix:water, ranging from 0.500 to 25.0 ng/mL and covering 30% to >3000% of LOQ.
Correlation coefficients (r) were 0.99 for each transition.
For each of the matrices, quantitative determination was carried out by external standardization using matrix matched calibration solutions.

Recovery and Repeatability

For each fortification level (0.01 mg/kg and 0.1 mg/kg), for both animal matrices and for both MS/MS transitions monitored, average recoveries at each fortification level and overall recoveries were in the acceptable range of 70 - 120%, and the relative standard deviations (RSD) at each fortification level were always 20%.

Results and discussions

Table A 33: Recovery results from independent laboratory validation of Pendimethalin using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (n = 5 for 0.01 mg/kg and n = 7 for 0.1 mg/kg)	Mean recovery (%)	RSD (%)	Comments
Kidney	Pendimethalin	0.01	105%	3%	MRM1
		0.1	78%	5%	MRM1
		0.01	101%	7%	MRM2
		0.1	77%	4%	MRM2
Liver		0.01	81%	2%	MRM1
		0.1	83%	2%	MRM1
		0.01	81%	4%	MRM2
		0.1	83%	2%	MRM2

Table A 34: Characteristics for the analytical method used for independent laboratory validation of Pendimethalin residues in liver and kidney

	Pendimethalin
Specificity	No interference/contamination peak above 30% of the LOQ was detected at the retention time of pendimethalin in any control sample.
Calibration (type, number of data points)	7 points Kidney, MRM1: Y=3.58x+004x – 7.18e003 R=0.997 Kidney, MRM2: Y=4.75e+003x – 876 R=0.9995 Liver, MRM1: Y=3.6e+004x + 1.29e+003 R=-0.9999 Liver, MRM2: Y=4.79e+003x + 70.4 R=0.9999
Calibration range	0.500 ng/mL – 25.0 ng/mL
Assessment of matrix effects is presented	Yes
Limit of determination/quantification	LOQ = 0.01 mg/kg

Conclusion

The Independent laboratory Validation was successfully validated and is suitable for the determination of residues of pendimethalin in kidney and liver according to SANTE/12830/2020 rev.1. The LOQ of the method was validated at 0.01 mg/kg for pendimethalin in kidney and liver during the validation study.

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

New or additional studies have been submitted

A 2.2.2.3.1 Analytical method 1

Comments of zRMS:	Method is accepted

Reference:	KCP 5.3.3.4.1
Report	Validation of the analytical procedure for the determination of pendimethalin (CAS: 40487-42-1), loamy sand soil by liquid chromatography. xxx Report number: 16.566423.0008.
Guideline(s):	Yes (SANCO 3029/99 Rev.4 SANCO/825/00 Rev 8.1 and OECD-204/2014)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Reference Substance: Pendimethalin
IUPAC 3,4-Dimethyl-2,6-dinitro-N-pentan-3-yl-aniline
Batch: SZBD302XV
CAS nr.:40487-42-1
Origin: Sharda Cropchem Limited
Purity: 98,8%
Molecular Weight: 281,31 g/mol
Molecular Formula: C13H19N3O4
CHELAB ID: RS316512

Reagents
• milliQ water, SRA 35;
• Methanol, batch 17G174025 purchased from VWR;
• Acetonitrile, batch STBG5699V purchased from Honeywell;
• Ammonium formate (LC-MS grade), ID: 237.7 purchased from Sigma Aldrich
• Acetone, batch 17C294011 purchased from VWR;
• Hexane, batch 17C154010 purchased from VWR;
• Formic acid, ID: 961.1, Suprapur
• Pendimethalin Reference Standard Solution, ID: 3452, logbook n°1045 pag 11/20 (Conc. 497.95 mg/l)

Materials and Apparatus
• Common analytical glassware;
• Fridge, SRA 7;
• Technical balance (± 0,01 g), SRA 49;

• Analytical balance (± 0,01 mg), SRA 192;
• Vortex;
• Centrifuge, SRA 55;
• Thermostatic bath equipped with N2 flow, SRA 66;
• MS XEVO TQS (Waters-Micromass), SRA 470;
• Acquity UPLC BEH C18, 50 mm x 2,1 mm x 1,7 µm (ID: LC 23);

Instrumental Conditions

• Column: Acquity UPLC BEH C18, 50 mm x 2,1 mm x 1,7 µm (LC 23)
• Mobile Phase A: 10 mM ammonium formate buffer pH 4,0
• Mobile Phase B: Methanol
• Flow: 0,2 ml/min
• Injection Volume: 5 µl
• Detector: MS XEVO TQS (Waters-Micromass), SRA 470
• Source: ESI-
• Source temp.: 150 ºC
• Nebulizer.: 6 bar
• Cone gas: 150 l/h
• Desolvation gas: 400 l/ha
• Run time: 13 minutes
• Run mode: MRM (see table below)

	Precursor ion m/z		m/z	Collision energy
pendimethalin	282.15	Quantifier ion (trans 1)	212	10
		Qualifier ion (trans 2)	194	20

• Elution: Gradient

Time (min)	Mobile Phase A %	Mobile Phase B %
0	100	0
0.5	100	0
8.5	0	100
11.5	0	100
11.6	100	0
13	100	0

Results and discussions

Procedure

The analytical method, internally developed and codified as SOPa-291-LABCHI-Rev.0 was validated in terms of specificity, linearity, repeatability, accuracy and LOQ according to SANCO 3029/99 Rev.4 and OECD-204/2014 guidelines.

The validation was performed quantifying pendimethalin Two SRM transitions were monitored Fluometuron:

• transition 1: 282.15 m/z (parent ion) > 212 m/z (daughter ion);
• transition 2: 282.15 m/z (parent ion) > 194 m/z (daughter ion).

System Suitability Test (SST)

System suitability test (SST) was performed in order to verify the suitability of the system at the beginning of each analytical sequence.

For the purpose, the Reference Solution at a concentration corresponding to about LOQ in the sample, for pendimethalin was injected in triplicate at the beginning and single at the end of sequence. %RSD of area was calculated for the first and second transition. It was verified that it is to not higher than 10%, in accordance to the acceptance criteria for SST.

MRM	**Transition 1**	**Transition 2**	**Transition 1**	**Transition 2**
Analytical session:	nº1		nº2	
determination	area	area	area	area
1	43207	4768	45644	4967

MRM	Transition 1	Transition 2	Transition 1	Transition 2
2	40610	4535	42474	4726
3	40334	4639	44084	5216
4	48971	5339	43790	4858
Average	43281	4820	43998	4942
Std. Dev.	4009	359	1302	208
RSD	9	7	3	4
% RSD ≤	10	10	10	10
Conformity	Yes	Yes	Yes	Yes

Specifity
Blank solution, Reference solution at LOQ level, Test Solution and Spiked Test Solution (at LOQ level) were injected for specificity evaluation.
Based on the chromatograms, the method is able to determine the analyte in the presence of the sample matrix.
No significant peaks (≤ 30% LOQ) are detected at RT of the target analyte in the Blank and Test Solution with respect to the Spiked Test Solution for both transition 1 and 2.

Linearity
The method linearity was evaluated at 5 different levels of concentration, ranging from at least 30% LOQ (0,0003 mg/kg) to about 30xLOQ (0,03 mg/kg) of analyte on the sample.
Using the experimental data of area ratio (y) and the corresponding theoretical concentrations (x, in mg/l), the slope (b), the intercept (a) of the regression lines (y = a + bx) and determination coefficient R2 were calculated.

Results obtained and the statement of conformity to the acceptance criteria defined in the Study plan are listed below.

ID RS	IRS-A volume ml	IRS-B volume ml	RS final ml	RS final conc. mg/l	Conc. Vs sample	Trans 1 area	Trans 2 area
L1	-	0.4	25	0.0016	0.0003	22834	2278
L2	-	0.5	10	0.005	0.001	43066	4368
L3	0.5	-	10	0.049	0.010	341773	37068
L4	1.0	-	10	0.099	0.020	672177	71987
L5	1.7	-	10	0.169	0.034	1157845	121123

Linearity Parameters of transition 1: Coefficient of determination (R^2) – 0.9997
Linearity Parameters of transition 2: Coefficient of determination (R^2) – 0.9996

Repeatability precision
Repeatability evaluation was performed on aliquots of sample spiked with Pendimethalin at LOQ (about 0.001 ppm), and 10xLOQ (about 0.01 ppm). 5 replicate analyses were performed for each spiking level.

%RSD at each fortified level was calculated for both transitions.

$$\% \text{ Recovery} = \frac{\text{Measured Concentration}}{\text{Theoretical Concentration}} \times 100$$

All results comply with acceptance criteria defined in SANCO/3029/99 rev. 4 guidelines

Accuracy
The accuracy of the analytical method expresses the closeness of the consistency between the accepted true value and the value found.
%Recovery is included between 70% and 110% in all cases, in accordance with acceptance criteria. The extraction efficiency was evaluated by fortifying test system with reference item at LOQ and 10xLOQ and evaluating recovery%

Limit of Quantification (LOQ)
LOQ is the lowest concentration level where an acceptable degree of linearity, accuracy and precision is established. In this case LOQ corresponds to 0,001 mg/kg.

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

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Mean recovery (%)		RSD (%)		Comments
			Tran. 1	Tran. 2	Tran. 1	Tran. 2	
Loamy sand soil	pendimethalin	0.001	103	104	2	4	
Loamy sand soil	pendimethalin	0.010	80	84	6	6	

Table A 36: Characteristics for the analytical method used for validation of pendimethalin residues in soil

	pendimethalin
Specificity	No significant peaks (≤ 30% LOQ) are detected at RT of the target analyte in the Blank and Test Solution with respect to the Spiked Test Solution for both transition 1 and 2.
Calibration range	The method linearity was evaluated at 5 different levels of concentration, ranging from at least 30% LOQ to about 30xLOQ of analyte on the sample.
Assessment of matrix effects is presented	Yes

Limit of determination/quantification	LOQ is the lowest concentration level where an acceptable degree of linearity, accuracy and precision is established. In this case LOQ corresponds to 0,001 mg/kg.

Conclusion

The validation data demonstrate that the analytical method SOPa-291-LABCHI-Rev.0 internally developed is suitable to qualitatively and quantitatively determine pendimethalin in soil specimens, according to SANCO 3029/99 Rev.4 and OECD-204/2014 guidelines and for the given concentration range.

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

New or additional studies have been submitted

A 2.2.2.4.1 Analytical method 1

Comments of zRMS:	Method is accepted

Reference:	KCP 5.3.3.5.1
Report	Method Validation: Determination of Residues of Pendimethalin in Drinking Water Samples. xxx Report number: YV/13/034
Guideline(s):	Yes (SANCO/825/00 Rev 8.1)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Common name: Pendimethalin
Chemical name (IUPAC): *N*-(1-ethylpropyl)-2,6-dinitro-3,4-xylidine
CAS Number: 40487-42-1
Molecular structure:C13H19N3O4
Molecular weight: 281.3
Source: Sigma Aldrich
Batch number: SZB8287XV
Expiry date: 13th October 2015
Purity: 98.8%
Storage conditions: Ambient

Principle of the Method

The analytical method for the determination of pendimethalin in water samples consisted of a liquid liquid partition with dichloromethane (DCM) followed by evaporation of the organic layer.

The samples were then reconstituted with 1mL of toluene before analysis by GC-MS. Calibration solutions for the determination of pendimethalin in water were prepared in matrix

GC-MS Analysis
Method 1
Samples were analysed by Gas chromatography coupled with mass spectrometer (GC-MS).
Column: Agilent HP-5MS, 30 m x 0.25 mm (i.d.) x 0.25 µm (film thickness)
Injection: 2 µL, pulsed splitless (pulse pressure 50 psi for 0.5 minutes)
Temperature: 250°C.
Carrier: Helium, Constant Flow Mode, 1.0 mL/min
Oven: Initial temperature: 90°C, Hold time: 1 min
1. program rate: 65°C/min until 300°C, hold for 1.5 min
Mode of Ionisation: EI
Transfer Line: 250°C
MS Source: 230°C
MS Quad: 150°C
Detector: MSD SIM mode, Mass monitored: m/z 252
Electron multiplier voltage: about 1600 V
Under these chromatographic conditions, the retention time for Pendimethalin was found to be about 4.9 minutes.

Method 2
Samples were analysed by Gas chromatography coupled with mass spectrometer (GC-MS).
Column: Agilent Rxi-624 Sil MS, 30 m x 0.25 mm (i.d.) x 1.4 µm (film thickness)
Injection: 2 µL, pulsed splitless (pulse pressure 50 psi for 0.5 minutes)
Temperature: 250°C.
Carrier: Helium, Constant Flow Mode, 1.0 mL/min
Oven: Initial temperature: 90°C, Hold time: 1.0 min
1. program rate: 65°C/min until 300°C, hold for 3.0 min
Mode of Ionisation: EI
Transfer Line: 250°C
MS Source: 250°C
MS Quad: 150°C
Detector: MSD SIM mode, Mass monitored: m/z 252
Electron multiplier voltage: about 1600 V
Under these chromatographic conditions, the retention time for Pendimethalin was found to be about 5.4 minutes.

Summary
The objective of this study was to validate an analytical method for the determination of residues of pendimethalin in drinking water to fulfil the requirements according to Regulations (EU) 544/2011 and 545/2011 implementing Regulation (EC) 1107/2009, EU Guidance Document SANCO/825/00 rev. 8.1, 16/11/10 and Directive 98/8/EC and Regulation (EU) 528/2012.
The analytical method for the determination of pendimethalin in water samples consisted of a liquid liquid partition with dichloromethane (DCM) followed by evaporation of the organic layer. The samples were then reconstituted with 1mL of toluene before analysis by GC-MS. Calibration solutions for the determination of pendimethalin in water were prepared in matrix. One ion was used for quantification on column HP-5MS (30m x 0.25mm x 0.25µm) and the same ion was used for confirmation on a Rxi-624 Sil (30m x 0.25mm x 1.4 µm) column. The analytical method was successfully validated for use in terms of linearity, specificity, accuracy and preci-

sion. The analytical method was shown to be linear for pendimethalin over the concentration range 3 ng/mL to 120 ng/mL. The regression coefficient (r_2) was determined to be greater than 0.98. The limit of quantification (LOQ) was established at 0.1 µg/L for pendimethalin in drinking water.
No interference/contamination peak above 30% of the LOQ was detected at the retention time of pendimethalin in any control sample.
The average recoveries were found to lie within the required range of 70-110% at all levels of fortification and the relative standard deviation was less than 20% which demonstrates acceptable accuracy and precision of the method. The data is summarized below:

Analyte	GC-MS Analysis Method	Fortification Level (ug/L)	Number (n)	Mean Recovery (%)	R.S.D. (%)
Pendimethalin	1	0.1	5	86	16.4
	2	0.1	5	83	8.4
	1	1.0	5	95	13.5
	2	1.0	5	98	11.9

In conclusion, the method was successfully validated for the determination of residues of pendimethalin in drinking water with an LOQ of 0.1 µg/L.

A 2.2.2.4.2 Analytical method 2

Comments of zRMS:	Method is accepted

Reference:	KCP 5.3.3.5.2
Report	Independent Laboratory Validation (ILV) of Analytical Method for the Determination of Residues of Pendimethalin in Drinking Water. Kevin McInerney, 2016 Report number: 100041492B
Guideline(s):	Yes (SANCO/825/00 Rev 8.1)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Pendimethalin
IUPAC Name: N-(1-ethylpropyl)-2,6-dinitro-3,4-xylidene
CAS No.: 40487-42-1
Empirical formula: C13H19N3O4
Molecular weight: 281.31 g/mol
Batch No.: SZBD302XV
Expiry Date: 29 October 2018
Purity: 98.8%

Laboratory Equipment
Balances:
• Mettler MX5 balance, SN 1126452889 (used for preparation of the stock solution)
Extraction/Concentration:
• 250 ml separatory funnels (Labglass)
• Zymark TurboVap 2, SN TV9233N6327
• Pipette B1100300B

All reusable glassware was cleaned in a laboratory dishwasher, solvent rinsed, and air-dried before use. Consumable glassware (injection vials, glass pipettes) was baked at 400°C for at least 30 minutes before use.
GC-MS System
• Agilent 6890 Gas Chromatograph
• Agilent 5973N Mass Spectrometer
• Agilent 7683 Autosampler
• Agilent 7683 Injector
Solvents and Chemicals
• Milli-Q Water (Battelle-Norwell).
• Dichloromethane (Battelle reagent 151001-05)
• Toluene (Battelle reagent 150225-03)
• Drinking Water (Battelle reagent 151021-12)
• Sodium Sulfate
• Acetonitrile (Battelle reagent 150901-04)

Results and discussions

Method 1

Matrix	Fortification Level µg/L	Analyte	Pendimethalin
Drinking water	0.10	Avarage % Recovery	83
		% RSD	8.2
		n	5
	1.0	Avarage % Recovery	84
		% RSD	9.1
		n	5

Method 2

Matrix	Fortification Level µg/L	Analyte	Pendimethalin
Drinking water	0.10	Avarage % Recovery	81
		% RSD	6.2
		n	5
	1.0	Avarage % Recovery	82
		% RSD	7.3
		n	5

Specificity, Calibration, and Sensitivity

The project team was able to confirm that the GC-MS method afforded detection of the analyte at concentrations of 0.01 µg/L with a 2 µL injection, providing sufficient sensitivity to quantify residues of the analyte in the final extracts. Matrix matched instrument calibrations were generated using concentrations of analyte in toluene, ranging from 3.00 to 120.0 ng/mL. Calibration functions are shown in Figures 1 and 2. Correlation coefficients (r) were ≥ 0.99. Quantitative determination was carried out by external standardization using matrix-matched calibration solutions. As validated by this ILV, the method allows the determination of the analyte with a limit of quantification (LOQ) of 0.1 µg/L in drinking water. The limit of detection (LOD) of the method was set to 30 % of LOQ (i.e. to 0.003 µg/L).
Apparent residues or interferences in blank control specimens were not detected.

Recoveries and Repeatability
Average recoveries at each fortification level and overall recoveries were in the acceptable range of 70 - 120%, and the relative standard deviations (RSD) at each fortification level were always ≤20%.

Conclusions
The project team assessed the method described in the method validation and found it suitable for the determination of pendimethalin in drinking water. Minor deviations from the method were required to achieve proper instrument performance. The ILV was successfully performed to achieve a LOQ of 0.1 µg/L. The LOD was 0.003 µg/L for drinking water. This study complies with Commission Regulation (EU) No 544/2011 and 545/2011 implementing Regulation (EC) 1107/2009. EU Guidance Document SANCO/825/00 rev. 8.1, 16/11/2010 Directive 98/8/EC and Regulation (EU) 528/2012.

A 2.2.2.4.3 Analytical method 3

A 2.2.2.4.3.1 Method validation

Comments of zRMS:	Method is accepted

Reference:	KCP 5.3.3.5.3
Report	Validation of the analytical procedure for the determination of Pendimethalin (CAS: 40487-42-1), in surface water by liquid chromatography. xxx Report No. 16.566423.0010
Guideline(s):	SANCO/3029/99 Rev. 4 SANCO/825/00 Rev. 8.1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Short term study for the validation of analytical method, for the determination of Pendimethalin in surface water.

Preparation of solutions
Mobile phase A (10 mM ammonium formate buffer pH 4)
About 0.62 g of ammonium formate were accurately weighed into a 1000 mL volumetric flask and dissolved with about 500 mL of milliQ water. 0.22 mL of formic acid were added and then the solution was

diluted to bolume with milliQ water. pH was 4

Mobile phase B
Methanol

Blank solution:
Acetonitrile

Sample extraction
About 50 g of sample were exactly weighed into a 50 mL plastic tube. The solution was transferred into a 100 mL round bottomed flask by the help of a separating funnel and 8 mL of dichloromethane were added to the sample. The organic phase was recovered into a 20 mL glass tube. 0.2 mL of 99% formic acid were added to the aqueous phase and after shaking also 8 mL of diethyl ether were added. The aqueous phase was then recovered into a 100 mL beaker and added of about 1 g of Na_2SO_4 anhydrous. Then, the aqueous phase was added again to the organic phase and discarded by liquid phase separation by the help of a separating funnel. The organic phase was recovered in the glass tube together with the dichloromethane solution and dried by nitrogen flux bath. The dried sample was resuspended into 2 mL of blank solution, transferred into an HPLC vial and injected. Test sample was prepared in triplicate.

Results and discussions

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

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
Surface water	Pendimethalin	0.00002	87.7	1.0	First mass transition
		0.0002	87.9	1.0	
		0.00002	88.5	4.0	Second mass transition
		0.0002	88.2	2.0	

Table A 38: Characteristics for the analytical method used for validation of Pendimethalin residues in Surface water

	Pendimethalin
Specificity	No significant peaks are detected at RT of the target analyte in the Blank and Test Solution with respect to the Spiked Test Solution for both transition 1 and 2. The method is specific
Calibration (type, number of data points)	5 points 0.000006 to 0.0006 mg/kg First mass transition y=34234760x R^2=0.9998 Second mass transition y=34234760x R^2=0.9998
Assessment of matrix effects is presented	Yes
Limit of determination/quantification	LOQ = 0.00002 mg/kg LOD = 0.000006 mg/kg

Conclusion

According to SANCO/3029/99 Rev. 4 the method was validated and is suitable for determination of residues of Pendimethalin in surface water.

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

New or additional studies have been submitted

A 2.2.2.5.1 Analytical method 1

Comments of zRMS:	Method is accepted

Reference:	KCP 5.3.3.6.1
Report	Validation of the analytical procedure for the determination of pendimethalin (CAS: 40487-42-1) in air by liquid chromatography. xxx Report number: 16.566423.0012.
Guideline(s):	Yes (SANCO 3029/99 Rev.4 SANCO/825/00 Rev 8.1 and OECD-204/2014)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Reference Substance: Pendimethalin
IUPAC 3,4-Dimethyl-2,6-dinitro-N-pentan-3-yl-aniline
Batch: SZBD302XV
CAS nr.:40487-42-1
Origin: Sharda Cropchem Limited
Purity: 98,8%
Molecular Weight: 281,31 g/mol
Molecular Formula: C13H19N3O4
CHELAB ID: RS316512

Reagents
• milliQ water, SRA 35;
• Methanol, batch 17B074018 purchased from VWR;
• Acetonitrile, batch STBG5330V purchased from Honeywell;
• Acetone, batch 16K234011 – VWR
• Hexane, batch STBG4421 - VWR
• Ammonium formate (LC-MS grade), ID: 237.5 purchased from Sigma Aldrich
• Formic acid, batch BCBR6503V - Fluka
• Pendimethalin Reference Standard Solution, ID: 3452, logbook n°1045 pag 11/20 (Conc. 497.95 mg/l)

Materials and Apparatus
• Common analytical glassware;

• Raw Polyurethane Foam (PUF) plug, 22mm ODx 6.6 cm length;
• Technical balance (± 0,01 g), SRA 49;
• Analytical balance (± 0,01 mg), SRA 192;
• Vortex;
• Climatic chamber SRA 323;
• Thermostatic bath equipped with N2 flow, SRA 66;
• Sampler air;
• Ultrasonic bath, SRA 469
• MS XEVO TQS (Waters-Micromass), SRA 470;
• Acquity UPLC BEH C18, 50 mm x 2,1 mm x 1,7 µm (ID: LC 23);

Instrumental Conditions

• Column:	Acquity UPLC BEH C18, 50 mm x 2,1 mm x 1,7 µm (LC 23)
• Mobile Phase A:	10 mM ammonium formate buffer pH 4,0
• Mobile Phase B:	Methanol
• Flow:	0,2 ml/min
• Injection Volume:	5 µl
• Detector:	MS XEVO TQS (Waters-Micromass), SRA 470
• Source:	ESI-
• Source temp.:	150 ºC
• Nebulizer.:	6 bar
• Cone gas:	150 l/h
• Desolvation gas:	400 l/ha
• Run time:	13 minutes
• Run mode:	MRM (see table below)

Precursor ion m/z			m/z	Collision energy
pendimethalin	282.15	Quantifier ion (trans 1)	212	10
		Qualifier ion (trans 2)	194	20

• Elution: Gradient

Time (min)	Mobile Phase A %	Mobile Phase B %
0	100	0
0.5	100	0
8.5	0	100
11.5	0	100
11.6	100	0
13	100	0

Results and discussions

Procedure

The analytical method, internally developed and codified as SOPa-300-LABCHI-Rev.0 was validated in terms of specificity, linearity, repeatability, accuracy and LOQ according to SANCO 3029/99 Rev.4 and OECD-204/2014 guidelines.

The validation was performed quantifying pendimethalin Two SRM transitions were monitored pendimethalin:

• transition 1: 282.15 m/z (parent ion) > 212 m/z (daughter ion);
• transition 2: 282.15 m/z (parent ion) > 194 m/z (daughter ion).

System Suitability Test (SST)

System suitability test (SST) was performed in order to verify the suitability of the system at the beginning of each analytical sequence.

For the purpose, the Reference Solution at a concentration corresponding to about LOQ in the sample, for pendimethalin was injected in triplicate at the beginning and single at the end of sequence. %RSD of area was calculated for the first and second transition. It was verified that it is to not higher than 10%, in accordance to the acceptance criteria for SST.

MRM	**Transition 1**	**Transition 2**	**Transition 1**	**Transition 2**
Analytical session:	nº1		nº2	
determination	area	area	area	area
1	45171	51141	451400	51759

MRM	Transition 1	Transition 2	Transition 1	Transition 2
2	456907	51495	458337	51352
3	450941	50944	457126	52313
4	470504	53098	503188	56087
Average	458131	51670	467513	52878
Std. Dev.	8602	979	23975	2175
RSD	2	2	5	4
% RSD ≤	10	10	10	10
Conformity	Yes	Yes	Yes	Yes

Specifity
Blank solution, Reference solution at LOQ level, Test Solution and Spiked Test Solution (at LOQ level) were injected for specificity evaluation.
Based on the chromatograms, the method is able to determine the analyte in the presence of the sample matrix.
No significant peaks (≤ 30% LOQ) are detected at RT of the target analyte in the Blank and Test Solution with respect to the Spiked Test Solution for both transition 1 and 2.

Linearity
The method linearity was evaluated at 5 different levels of concentration, ranging from at least 30% LOQ (0,0012 ng/ml) to about 30xLOQ (0,12 ng/ml) of analyte on the sample.
Using the experimental data of area ratio (y) and the corresponding theoretical concentrations (x, in mg/l), the slope (b), the intercept (a) of the regression lines (y = a + bx) and determination coefficient R2 were calculated.

Results obtained and the statement of conformity to the acceptance criteria defined in the Study plan are listed below.

ID RS	IRS-A volume ml	IRS-B volume ml	RS final ml	RS final conc. mg/l	Conc. Vs sample g/m^3	Trans 1 area	Trans 2 area
L1	-	0.2	100	0.002	0.0000009	113831	12905
L2	-	0.9	100	0.009	0.000004	451683	50638
L3	1.0	-	100	0.100	0.000046	4727300	565998
L4	1.0	-	50	0.199	0.000092	7797545	910245
L5	0.7	-	25	0.279	0.000129	10600585	1306650

Linearity Parameters of transition 1: Coefficient of determination (R^2) – 0.9904
Linearity Parameters of transition 2: Coefficient of determination (R^2) – 0.9917

Repeatability precision
Repeatability evaluation was performed on aliquots of sample spiked with Pendimethalin at LOQ (about 0.000004 g/m^3), and 10xLOQ (about 0.00004 g/m^3). 5 replicate analyses were performed for each spiking level.

%RSD at each fortified level was calculated for both transitions.

$$\% \text{ Recovery} = \frac{\text{Measured Concentration}}{\text{Theoretical Concentration}} \times 100$$

All results comply with acceptance criteria defined in SANCO/3029/99 rev. 4 guidelines

Accuracy
The accuracy of the analytical method expresses the closeness of the consistency between the accepted true value and the value found.
%Recovery is included between 70% and 110% in all cases, in accordance with acceptance criteria. The extraction efficiency was evaluated by fortifying test system with reference item at LOQ and 10xLOQ and evaluating recovery%

Limit of Quantification (LOQ)
LOQ is the lowest concentration level where an acceptable degree of linearity, accuracy and precision is established. In this case LOQ corresponds to 4 ng/l.

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

Matrix	Analyte	Fortification level ng/l) (*n* = 5)	Mean recovery (%)		RSD (%)		Comments
			Tran. 1	Tran. 2	Tran. 1	Tran. 2	
air	pendimethalin	4	100.4	93.5	9	8	
air	pendimethalin	40	103.1	97.4	3	3	

Table A 40: Characteristics for the analytical method used for validation of pendimethalin residues in air

	pendimethalin
Specificity	No significant peaks (≤ 30% LOQ) are detected at RT of the target analyte in the Blank and Test Solution with respect to the Spiked Test Solution for both transition 1 and 2.
Calibration range	The method linearity was evaluated at 5 different levels of concentration, ranging from at least 30% LOQ to about 30xLOQ of analyte on the sample.
Assessment of matrix effects is presented	Yes

Limit of determination/quantification	LOQ is the lowest concentration level where an acceptable degree of linearity, accuracy and precision is established. In this case LOQ corresponds to 4 ng/l.

Conclusion

The validation data demonstrate that the analytical method SOPa-300-LABCHI-Rev.0 internally developed is suitable to qualitatively and quantitatively determine pendimethalin in soil specimens, according to SANCO 3029/99 Rev.4 and OECD-204/2014 guidelines and for the given concentration range.

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

A 2.2.2.6.1 Urine

Comments of zRMS:	Method is accepted

Reference:	KCP 5.3.3.7.1
Report	Validation of the analyticayl procedure for the determination of pendimethalin (CAS: 40487-42-1) in blood by liquid chromatography; xxx FR 16.566423.0007
Guideline(s):	Yes (SANCO/825/00 rev. 8.1, SANCO/3029/99 rev. 4 and OECD-204/2014)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

REFERENCE ITEMS
Reference Substance: Pendimethalin
IUPAC 3,4-Dimethyl-2,6-dinitro-N-pentan-3-yl-aniline
Batch: SZBD302XV
CAS nr.:40487-42-1
Origin: Sharda Cropchem Limited
Purity: 98,8%
Molecular Weight: 281,31 g/mol
Molecular Formula: C13H19N3O4
CHELAB ID: RS316512

Reagents
• milliQ water, SRA 35;
• Methanol, batch 17Z0666 purchased from VWR;

• Acetonitrile, batch STBG5324V purchased from Honeywell;
• Magnesium sulfate anhydrous ID: 226.13 purchased from Sigma Aldrich;
• Ammonium formate (LC-MS grade), ID: 237.7 purchased from Sigma Aldrich;
• Sodium acetate ID: 701.3 purchased from Sigma Aldrich;
• PSA Resin 40 µm, ID: 306.6 purchased from Varian;
• Formic acid, ID 961.1, purchased from Suprapur;
• Pendimethalin reference Standard solution, ID 3422 logbook n°1045, pag.1/20 (Conc.496,96 mg/l)

Materials and Apparatus
• Common analytical glassware;
• Fridge, SRA 7;
• Technical balance (± 0,01 g), SRA 49;
• Vortex;
• Grinder;
• Centrifuge, SRA 55;
• Thermostatic bath equipped with N2 flow, SRA 66;
• MS XEVO TQS (Waters-Micromass), SRA 470;
• Acquity UPLC BEH C18, 50 mm x 2,1 mm x 1,7 µm (ID: LC 23);
Instruments were calibrated before use.

Instrumental Conditions

Column:	Acquity UPLC BEH C18, 50 mm x 2,1 mm x 1,7 µm (LC 23)
Mobile Phase A:	10 mM ammonium formate buffer pH 4,0
Mobile Phase B:	Methanol
Flow:	0,2 ml/min
Injection Volume:	5 µl
Detector:	MS XEVO TQS (Waters-Micromass), SRA 470
Source:	ESI
Source temp.:	150 °C
Nebulizer.:	6 bar
Cone gas:	150 l/h
Desolvation gas:	400 l/h
Run time:	13 minutes

Results and discussions

Table A 41: Recovery results from method validation of pendimethalin using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (n = x)	Mean recovery (%)	RSD (%)	Comments
Transition 1					
blood	pendimethalin	0.01	98.6	1	
		0.1	95.1	2	
Transition 2					
blood	pendimethalin	0.01	93.2	1	
		0.1	90.6	2	

Table A 42: Characteristics for the analytical method used for validation of pendimethalin residues in urine

	pendimethalin
Specificity	No significant peaks (≤ 30% LOQ) are detected at RT of the target analyte in the Blank and Test Solution with respect to the Spiked Test Solution for both transition 1 and 2
Calibration range	0.013 – 1.590 mg/kg
Assessment of matrix effects is presented	Not relevant - linearity regression curve was prepared in the matrix, therefore it was not necessary to evaluate matrix effect
Limit of determination/quantification	0.05 mg/l

Conclusion

The validation data demonstrate that the analytical method SOPa-290-LABCHI-Rev.0 internally developed is suitable to qualitatively and quantitatively determine Pendimethalin in liver specimens, according to SANCO 3029/99 Rev.4 and OECD-204/2014 guidelines and for the given concentration range.

A 2.2.2.6.2 Liver

Comments of zRMS:	Method is accepted

Reference:	KCP 5.3.3.7.2
Report	Validation of the analyticayl procedure for the determination of pendimethalin (CAS: 40487-42-1) in liver by liquid chromatography; xxx FR 16.566423.0004
Guideline(s):	Yes (SANCO/825/00 rev. 8.1, SANCO/3029/99 rev. 4 and OECD-204/2014)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

REFERENCE ITEMS
Reference Substance: Pendimethalin
IUPAC 3,4-Dimethyl-2,6-dinitro-N-pentan-3-yl-aniline
Batch: SZBD302XV
CAS nr.:40487-42-1
Origin: Sharda Cropchem Limited
Purity: 98,8%
Molecular Weight: 281,31 g/mol
Molecular Formula: C13H19N3O4
CHELAB ID: RS316512

Reagents
• milliQ water, SRA 35;

• Methanol, batch 17A054007 purchased from VWR;
• Acetonitrile, batch STBG5324V purchased from Honeywell;
• Magnesium sulfate anhydrous ID: 226.13 purchased from Sigma Aldrich;
• Ammonium formate (LC-MS grade), ID: 237.7 purchased from Sigma Aldrich;
• Sodium acetate ID: 701.3 purchased from Sigma Aldrich;
• PSA Resin 40 µm, ID: 306.6 purchased from Varian;
• Formic acid, ID 961.1, purchased from Suprapur;
• Pendimethalin reference Standard solution, ID 3422 logbook n°1045, pag.1/20 (Conc.496,96 mg/l)

Materials and Apparatus
• Common analytical glassware;
• Analytical balance (± 0,01 mg), SRA 192;
• Fridge, SRA 7;
• Freezer with temperature ≤ - 16°C, SRA 25
• Technical balance (± 0,01 g), SRA 49;
• Vortex;
• Grinder;
• Centrifuge, SRA 55;
• Thermostatic bath equipped with N2 flow, SRA 66;
• MS XEVO TQS (Waters-Micromass), SRA 470;
• Acquity UPLC BEH C18, 50 mm x 2,1 mm x 1,7 µm (ID: LC 23);
Instruments were calibrated before use.

Instrumental Conditions

Column:	Acquity UPLC BEH C18, 50 mm x 2,1 mm x 1,7 µm (LC 23)
Mobile Phase A:	10 mM ammonium formate buffer pH 4,0
Mobile Phase B:	Methanol
Flow:	0,2 ml/min
Injection Volume:	5 µl
Detector:	MS XEVO TQS (Waters-Micromass), SRA 470
Source:	ESI
Source temp.:	150 °C
Nebulizer.:	6 bar
Cone gas:	150 l/h
Desolvation gas:	400 l/h
Run time:	13 minutes

Results and discussions

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

Matrix	Analyte	Fortification level (mg/kg) (n = x)	Mean recovery (%)	RSD (%)	Comments
Transition 1					
liver	pendimethalin	0.01	104.4	2	
		0.1	99.6	3	
Transition 2					
liver	pendimethalin	0.01	103.0	3	
		0.1	98.6	2	

Table A 44: Characteristics for the analytical method used for validation of pendimethalin residues in liver

	pendimethalin
Specificity	No significant peaks (≤ 30% LOQ) are detected at RT of the target analyte in the Blank and Test Solution with respect to the Spiked Test Solution for both transition 1 and 2
Calibration range	0.02 – 3.18 mg/kg
Assessment of matrix effects is presented	Not relevant - linearity regression curve was prepared in the matrix, therefore it was not necessary to evaluate matrix effect
Limit of determination/quantification	0.1 mg/kg

Conclusion

The validation data demonstrate that the analytical method SOPa-287-LABCHI-Rev.0 internally developed is suitable to qualitatively and quantitatively determine Pendimethalin in liver specimens, according to SANCO 3029/99 Rev.4 and OECD-204/2014 guidelines and for the given concentration range.

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

No new or additional studies have been submitted