

FINAL REGISTRATION REPORT

Part B

Section 5

Analytical Methods

Detailed summary of the risk assessment

Product code: SHA 2600 E

Product name(s): PENSHUI

Chemical active substances:

Pendimethalin, 455 g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

Applicant: Sharda Cropchem España S.L.

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Version history

When	What
January 2021	Applicant update
February 2021	Assessment
February 2022	Applicant update
April 2022	RMS Assessment after commenting and Applicant's update

Table of Contents

5	Analytical methods.....	4
5.1	Conclusion and summary of assessment.....	4
5.2	Methods used for the generation of pre-authorization data (KCP 5.1).....	5
5.2.1	Analysis of the plant protection product (KCP 5.1.1)	5
5.2.1.1	Determination of active substance and/or variant in the plant protection product (KCP 5.1.1).....	5
5.2.1.2	Description of analytical methods for the determination of relevant impurities (KCP 5.1.1).....	11
5.2.1.3	Description of analytical methods for the determination of formulants (KCP 5.1.1)	18
5.2.1.4	Applicability of existing CIPAC methods (KCP 5.1.1).....	18
5.2.2	Methods for the determination of residues (KCP 5.1.2).....	18
5.3	Methods for post-authorization control and monitoring purposes (KCP 5.2)	18
5.3.1	Analysis of the plant protection product (KCP 5.2)	18
5.3.2	Description of analytical methods for the determination of residues of pendimethalin (KCP 5.2)	19
5.3.2.1	Overview of residue definitions and levels for which compliance is required	19
5.3.2.2	Description of analytical methods for the determination of residues in plant matrices (KCP 5.2).....	19
5.3.2.3	Description of analytical methods for the determination of residues in animal matrices (KCP 5.2).....	21
5.3.2.4	Description of methods for the analysis of soil (KCP 5.2).....	21
5.3.2.5	Description of methods for the analysis of water (KCP 5.2).....	22
5.3.2.6	Description of methods for the analysis of air (KCP 5.2).....	23
5.3.2.7	Description of methods for the analysis of body fluids and tissues (KCP 5.2)	23
5.3.2.8	Other studies/ information	24
Appendix 1	Lists of data considered in support of the evaluation.....	25
Appendix 2	Detailed evaluation of submitted analytical methods	30
A 2.1	Analytical methods for pendimethalin.....	30
A 2.1.1	Methods used for the generation of pre-authorization data (KCP 5.1).....	30
A 2.1.2	Methods for post-authorization control and monitoring purposes (KCP 5.2)	30

5 Analytical methods

5.1 Conclusion and summary of assessment

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

Noticed data gaps are:

- None

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

Noticed data gaps are:

- none
-

Commodity/crop	Supported/ Not supported
Wheat	Supported
Barley	Supported
Rye	Supported
Oats	Supported
Triticale	Supported
Maize	Supported
Apple	Supported
Pear	Supported
Peach	Supported
Apricot	Supported
Plum	Supported
Nectarine	Supported
Cherry	Supported
Sunflower	Supported
Soybean	Supported
Onion	Supported
Garlic	Supported
Shallot	Supported
Spring onion	Supported
Bean	Supported
Pea	Supported

Commodity/crop	Supported/ Not supported
Broad bean	Supported
Field bean	Supported
Carrot	Supported
Parsley	Supported
Lupine	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 pendimethalin in plant protection product is provided as follows:

Comments of zRMS:	<p>The proposed analytical method is suitable for the determination of active substances pendimethalin in plant protection product PENSHUI.</p> <p>The proposed analytical method has been fully validated in terms of specificity, linearity, repeatability, and accuracy. Proposed method fulfils the requirements of SANCO/3030/99 rev.4 and SANCO/3030/99 rev.5 guidance.</p> <p>The validation of the analytical method has been accepted.</p>
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Reference: KCP 5.1.1

Report Validation of analytical method for determination of active ingredient content of pendimethalin 455 g/L CS. XXX, 2017. Document no.: 228-2-12-16345

Guideline(s): Yes
 SANCO/3030/99 rev. 4

Deviations: No

GLP: Yes

Acceptability: Yes

Materials and methods

Principle of the method:

Active ingredient pendimethalin was detected by ~~GC/FID analysis~~ high performance liquid chromatography on a reversed phase column using internal standard.

Material:

Test item:

Name: Pendimethalin 455 g/L CS

Active ingredient content: 455 g/L
Batch: SCL-40026
Preparation date: April 10, 2016
Expiry date: April 09, 2018

Reference item:

Name: Pendimethalin
CAS number: 40487-42-1
Purity: 98.8%
Batch: SZBD302XV
Preparation date: October 29, 2013
Expiry date: October 29, 2018

Blank formulation:

Name: Pendimethalin 455 g/L CS Blank Formulation
Batch: SCL - 40146
Preparation date: June 27, 2016
Expiry date: June 26, 2018

The active ingredient concentration of pendimethalin 455 g/L CS is determined by high performance liquid chromatography on a reversed phase column using Acetonitrile (90%): MilliQ Water (10%), v/v as mobile phase and UV detection at 240 nm.

Validation - Results and discussions

Table 5.2-1: Methods suitable for the determination of pendimethalin in plant protection product pendimethalin 455 g/L CS

	Pendimethalin 455 g/L CS
Author(s), year	XXX, 2017
Principle of method	HPLC-UV
Linearity (linear between mg/L / % range of the declared content) (correlation coefficient, expressed as r)	54.32 to 200.33 (mg/L) Correlation Coefficient 0.999
Precision – Repeatability Mean (%RSD)	The repeatability was assessed by analysing five samples on two different days. RSD=0.03% Acceptable RSDr (Horwith) = 1.54% Horrat value = 0.019
Accuracy (% Recovery)	The accuracy was assessed by analysing three samples on three fortification levels. Marginal recovery=98.99%
Interference/ Specificity	No interference, the 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 analysis of pendimethalin in Pendimethalin 455 g/L CS. The results of validation criteria are within the specified limits of SANCO/3030/99 rev. 4 and rev. 5, Dir. 91/414/EEC (2000), U.S. EPA, (OCSPP) 830.1800 and ABNT NBR 14029 guidelines.

Comments of zRMS:	The proposed analytical method is suitable for the determination of active substances pendimethalin in plant protection product PENSUI/SHA 2600 E. The proposed analytical method has been fully validated in terms of specificity, linearity, repeatability, and accuracy. Proposed method fulfils the requirements of SANCO/3030/99 rev.5 guidance. The validation of the analytical method has been accepted.
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Reference: KCP 5.1.1-3

Report Pendimethalin 45.5% CS Analysis of active substance content and physico-chemical properties of initial preparation and preparation after accelerated and freeze/thaw storage procedures. B. XXX, 2021, Report No. 47/2021

Guideline(s): Yes
SANCO/3030/99 rev. 5

Deviations: No

GLP: Yes

Acceptability: Yes

Materials and methods

The test was carried out in Liquid Chromatography Laboratory.

In order to determine the concentration of active substance in the test samples the HPLC-UV method was used.

Test item:

Name: Pendimethalin 455 g/L CS
Active ingredient content: 456 g/L
Batch: SCL- 32690
Preparation date: 10/04/2021
Expiry date: 09/04/2023

Reference item:

Name: Pendimethalin analytical standard
CAS number: 40487-42-1
Purity: 98.7%
Batch: BCBX2084
Preparation date:
Expiry date: Jun/23

Blank formulation:

Name: Pendimethalin 45.5 % CS Blank formulation
Batch: SCL-77564
Preparation date: 15/03/2021
Expiry date: 14/03/2023

Standard solution preparation:

Pendimethalin: 14,9 mg of the standard was diluted to 10cm³ with acetonitrile.

Sample solution preparation:

Initial preparation: five independent weights (about 11 mg) of the test sample was diluted with 1cm³ water and 9cm³ acetonitrile. These solutions were analyzed by HPLC-UV under stable chromatographic conditions.

Blank sample solution preparation:

41,3 mg of the blank formulation sample was diluted with 1cm³ water and 9cm³ acetonitrile.

Chromatographic conditions:

column: SynergiTM, 4µm Hydro-RP 80Å, 250x4.6 mm
column temperature: 35°C
flow rate: 1,4 cm³/min
eluent: 90% acetonitrile : 10% H₂O
elution program: isocratic
injection volume: 5µL
detector: UV 240 nm
run time: 9 minutes

Calibration curve

For Pendimethalin calibration 5 standard solutions were prepared in the concentration range about 80-120% of analyte content in tested sample. Each calibration solution was analyzed twice under stable chromatographic conditions. Linearity range of Pendimethalin was 346,6 – 519,8 mg/L

Specificity

The combination of the HPLC column, mobile phase composition and the column temperature was designed to ensure separation of the active ingredient from any impurities that possibly could interfere with the determination of the active ingredient.

The selectivity of the HPLC method was assessed by examination of peak homogeneity and peak purity using diode array detector.

Precision

For sample repeatability evaluation, five independent test item solutions prepared from five individual weights of the test item (initial preparation), were analyzed in duplicate using the same chromatographic conditions as during the calibration process.

Accuracy

For an accuracy estimation, two solutions with active substance concentration prepared from analytical standard in the blank formulation matrix were prepared in the range of 90 % and 110% of analyte concentration in the tested solutions.

Each solution was injected 7 times using the same chromatographic conditions as during the test item measurements and calibration process

Validation - Results and discussions

Table 5.2-2: Methods suitable for the determination of active substances Pendimethalin in plant protection product PENSUI/SHA 2600 E

	Pendimethalin
Author(s), year	B. XXX, 2021
Principle of method	HPLC-UV
Linearity (linear between mg/L / % range of the declared content) (correlation coefficient, expressed as r)	347 – 520 mg/L $y=0.2825x-3.9096$ $R=0.9995$ 0.9991
Precision – Repeatability Mean n = 5 (in duplicate)	RSD = 0.41% RSD _R =2.25%

	Pendimethalin
(%RSD)	RSD _r =1.51% Hr=0.27<1
Accuracy n = 7 (% Recovery)	Total recovery: 90%: 101.2% 110%: 101.1%
Interference/ Specificity	No interference. The method is specific

Conclusion

According to SANCO/3030/99 rev. 5 the method was successfully validated and is suitable for determination of Pendimethalin in the test item.

Comments of zRMS:	<p>The proposed analytical method is suitable for the determination of the free and encapsulated pendimethalin content in plant protection product PENSHUI/SHA 2600 E.</p> <p>The proposed analytical method has been fully validated in terms of specificity, linearity, repeatability, and accuracy. Proposed method fulfils the requirements of SANCO/3030/99 rev.5 guidance.</p> <p>The validation of the analytical method has been accepted.</p>
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Reference: KCP 5.1.1-3

Report Pendimethalin 45.5% CS Analysis of active substance content and physico-chemical properties of initial preparation and preparation after accelerated and freeze/thaw storage procedures. B. XXX, 2021, Report No. 47/2021

Guideline(s): Yes
SANCO/3030/99 rev. 5

Deviations: No

GLP: Yes

Acceptability: Yes

Free and encapsulated active substance content

The test consist of few following stages:

Extraction of free Pendimethalin

Extraction was carried out at temperature between 20 °C and 25 °C.

Sample containing 70 – 80 mg of Pendimethalin (about 0,2 g) was putted into glass bottle. The volume of formulation was calculated (by using the sample density 1,1802 g/mL). To this sufficient distilled water was added to give a total volume of 6 ml, and then 50 ml of hexane was added. The bottle was closed and placed horizontally on the roller. The bottle was rolled with a rotational speed of 70 ± 10 rpm for a period of 5 minutes (± 10 s). After that clear hexane extract was taken to quantitative determination of free Pendimethalin content. Five independent extracts were prepared.

Free Pendimethalin content determination

The HPLC analysis was performed according to the internal test procedure BA-AB/SPO-3 “Pesticide analysis in the formulations of plant protection products” and research method No. BA-AB/MB-28 “Pendimethalin” validated before used.

Quantitative analysis of Pendimethalin was based on external calibration using certified analytical standard.

For calibration 5 – 7 calibration solutions from analytical standard were prepared (with different concentration active substance in suitable solvent). Each calibration solution was analyzed twice under stable chromatographic conditions.

Test sample extracts were analyzed in directly form by HPLC-UV under stable chromatographic conditions (the same as calibration conditions). Two measurements of content of Pendimethalin in the each obtained extract were made. The results was express in mg/ml.

Standard solution preparation:

Pendimethalin: 14,9 mg of the standard was diluted to 10cm³ with hexane. 1 cm³ of this solution was diluted to 10cm³ with hexane.

Sample solution preparation:

Five independent test sample, directly form, were analyzed by HPLC-UV under stable chromatographic conditions.

Chromatographic conditions:

column:	SynergiTM, 4µm Hydro-RP 80Å, 250x4.6 mm
column temperature:	35oC
flow rate:	1,4 cm ³ /min
eluent: 80% acetonitrile:	20% H ₂ O
elution program:	isocratic
injection volume:	5µL
detector:	UV 240 nm
run time:	9 minutes

Specificity

The combination of the HPLC column, mobile phase composition and the column temperature was designed to ensure separation of the active ingredient from any impurities that possibly could interfere with the determination of the active ingredient.

The selectivity of the HPLC method was assessed by examination of peak homogeneity and peak purity using diode array detector.

Precision

For sample repeatability evaluation, five independent test item solutions (initial preparation) were injected twice using the same chromatographic conditions as during the calibration process.

Accuracy

For an accuracy estimation, two solutions with a concentration of analyte in the range of 90% and 110% level in the test item were prepared from test item extract spiked by analytical standard.

Each solution was injected 7 times using the same chromatographic conditions as during the test item measurements and calibration process.

Linearity

For Pendimethalin calibration 5 standard solutions were prepared in the concentration range about 80-120% of analyte content in tested sample. Each calibration solution was analyzed twice under stable chromatographic conditions. Linearity range of Pendimethalin was 4,44 – 6,66 mg/l

Validation - Results and discussions

Table 5.2-3: Methods suitable for the determination of free active substances Pendimthalin in plant protection product PENSHUI/SHA 2600 E

	Free Pendimethalin
Author(s), year	B. XXX, 2021

	Free Pendimethalin
Principle of method	HPLC-UV
Linearity (linear between mg/L / % range of the declared content) (correlation coefficient, expressed as r)	4.44-6.66 mg/L $y=0.2867x-0.033$ $R=0.9978$ 0.9955
Precision – Repeatability Mean n = 5 (in duplicate) (%RSD)	RSD = 5.56% $RSD_R=12.34\%$ $RSD_r=8.27\%$ $Hr=0.67<1$
Accuracy n = 7 (% Recovery)	Total recovery: 90%: 127.9% 110%: 127.0%
Interference/ Specificity	No interference. The method is specific

Conclusion

According to SANCO/3030/99 rev. 5 the method was successfully validated and is suitable for determination of free and encapsulated content of Pendimethalin in the test item.

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

Comments of zRMS:	<p>The proposed analytical methods are suitable for the determination of relevant impurities 1,2-dichlorethane and N-nitrosopendimethalin in plant protection product PENSHUI.</p> <p>The proposed analytical methods have been fully validated in terms of specificity, linearity, repeatability, and accuracy. The LOQs were determined.</p> <p>Proposed methods fulfil the requirements of SANCO/3030/99 rev.4 and SANCO/3030/99 rev.5 guidance.</p> <p>The validation of the analytical methods has been accepted.</p>
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Reference: KCP 5.1.2

Report Pendimethalin 455 g/L CS. Methods validation for determination of the relevant impurities of pendimethalin. A. XXX, 2017. Document no.: BA-45/17

Guideline(s): Yes
 SANCO/3030/99 rev. 4

Deviations: No

GLP: Yes

Acceptability: Yes

Materials and methods

Material:

Test item:

Name: Pendimethalin 455 g/L CS

Active ingredient content: 455 g/L
Batch: SCL-40026
Preparation date: April 10, 2016
Expiry date: April 09, 2018

Name: 1,2-Dichloroethane
IUPAC name: 1,2-Dichloroethane
Chemical formula: $C_2H_4Cl_2$
CAS No: [107-06-2]
Molar mass: 98.95 g/mol

Reference item

1,2-dichloroethane, 99.9 %, Sigma-Aldrich, Lot#BCBS5198V
N-nitrosopendimethalin, 99.2 \pm 0.1 % (m/m), IPO, Series No. 3A/16

1,2-dichloroethane

The method is based on determination of 1,2-dichloroethane by headspace analysis in combination with gas chromatography and flame ionization detection (HS-GC-FID) using external standard method with application of placebo of PENDIMETHALIN 455 g/l CS preparation as a matrix to which known amount of 1,2-dichloroethane was added to determine the individual validation parameters.

Preparation of standard solutions

About 100 mg of 1,2-dichloroethane standard was weighed with accuracy of 0.01 mg into the 10 mL volumetric flask. DMSO was added to 10 mL and content was mixed (solution A - ~10mg/mL). The solution A was diluted 10-times with DMSO (solution B - ~1 mg/mL). Solution B was diluted: 10-times, 20-times, 40-times, 50-times, 80-times, 100-times and 200-times giving solutions of 1,2-dichloroethane from C-1 to C-7 with concentrations from 0.1000 to 0.0050 mg/mL

Preparation of the test item solutions

About 200 mg of PENDIMETHALIN 455 g/l CS preparation was weighted in headspace vial and then 1 mL DMSO and 6 mL deionized water were added. The vials were tightly closed and headspace of the solution was analyzed.

N-nitrosopendimethalin

The method is based on determination of N-nitrosopendimethalin using reversed phase high performance liquid chromatography (RP-HPLC) with UV detection at wavelength 206 nm using external standard method.

Preparation of standard solutions

About 5 mg of N-nitrosopendimethalin standard was weighted into a 10 mL volumetric flask and dissolved in acetonitrile. Acetonitrile was added to 10 mL and the content was mixed. The solution was diluted 250 times with acetonitrile.
Acetonitrile was used as solvent.

Preparation of the test item solutions

About 40 mg of PENDIMETHALIN 455 g/l CS preparation was weighted into a 10 mL volumetric flask, 2.0 mL of water was added and the content was mixed. Then about 7 mL of acetonitrile was added and the flask was put into an ultrasonic bath for 10 minutes. The solution was adjusted to room temperature. Acetonitrile was added up to 10 mL and the content was mixed.

Validation - Results and discussions

Table 5.2-4: Methods suitable for the determination of the relevant impurities in plant protection product (PPP) pendimethalin 455 g/L CS

	1,2-dichloroethane	N-nitrosopendimethalin
Author(s), year	A. XXX, 2017	A. XXX, 2017
Principle of method	HS-GC-FID	HPLC with UV-DAD
Linearity (linear between mg/L) (correlation coefficient, expressed as r)	0.0050 - 0.1000 mg/mL R=0.9999	0.039 – 0.088 µg/mL R=0.9933
Precision – Repeatability Mean (%RSD)	RSD = 1.06% Acceptable RSDr (Horwith) = 6.60% Horrat value=0.16	RSD = 3.38% Acceptable RSDr (Horwith) = 7.58% Horrat value=0.45
Accuracy (% Recovery)	The accuracy was assessed by analysing six samples on two fortification levels Marginal recovery=99.2%	The accuracy was assessed by analysing six samples on two fortification levels Marginal recovery=97.6%
Interference/ Specificity	No interference, the method is specific	No interference, the method is specific
LOQ	0.025 g/kg	0.010 g/kg
Comment	–	–

Conclusion

The content of the analyzed relevant impurities, 1,2-dichloroethane and N-nitrosopendimethalin in PENDIMETHALIN 455 g/l CS preparation, determined by developed and validated method were below limit of quantification (LOQ), it means below 0.025 g/kg for 1,2-dichloroethane and below 0.010 g/kg for N-nitrosopendimethalin, therefore below permitted level (1,2-dichloroethane \leq 0.386 g/kg, N-nitrosopendimethalin \leq 0.017 g/kg)

Comments of zRMS:	The proposed analytical methods are suitable for the determination of relevant impurities 1,2-dichloroethane, N-nitrosopendimethalin, and N-Nitrosoamines in the plant protection product PENSHUI. The proposed analytical methods have been fully validated in terms of specificity, linearity, repeatability, and accuracy. The LOQs were determined. Proposed methods fulfil the requirements of SANCO/3030/99 rev.5 guidance. The validation of the analytical methods has been accepted.
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Reference:	KCP 5.1.1-2
Report	Accelerated storage stability test by heating at elevated temperature of Pendimethalin 455 g/L CS. S. Revankar, 2021, Report No. G21360
Guideline(s):	SANCO/3030/99 rev. 5
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

N-Nitroso Pendimethalin

The method is based on determination of N-Nitroso Pendimethalin by LC-MS/MS method using external standard.

Specificity

The specificity and selectivity of the method was established by injecting the diluent 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.

Linearity

A known aliquot from each of the N-Nitroso pendimethalin reference standard stock solutions prepared was transferred into a volumetric flask and diluted with diluent 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-NitrosoPendimethalin were recorded for each injection.

Precision

About 0.6 g of the test item, Pendimethalin 455 g/L CS was weighed in five replications to separate 100 mL volumetric flasks and the contents of the flask were dissolved in diluent. Later, the volume was made up to the mark with diluent, the solution was shaken thoroughly and used for the analysis of impurity content by injecting to the LC-MS/MS.

Accuracy

About 0.6 g of test item, Pendimethalin 455 g/L CS was weighed into separate 100 mL volumetric flasks (three for each of the three fortification levels) and the contents of the volumetric flasks were dissolved in diluent. Later, the test item solutions were fortified with N-Nitroso Pendimethalin reference standard stock solution. Further, the volume was made up to the mark with diluent, the solutions were shaken thoroughly and used for analysis of impurity content by injecting to LC-MS/MS.

Chromatographic conditions

Instrument: API 3200 LC-MS/MS system with Shimadzu front end HPLC equipped with PC based data system
Column: Waters Sunfire C18, 150x4.6 mm, particle size 3.5 µm
Mobile phase A: 0.1% formic acid in Milli-Q water
Mobile phase B: Acetonitrile:Methanol (50:50% v/v)
Gradient:

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0.01	50	50
10.00	10	90
15.00	10	90
15.10	50	50
20.00	50	50

Flow rate: 1.0 mL/min with splitter
Sample temperature: 15°C
Mass spectrometer: API 3200
Ion Source: ESI
Polarity: positive
Mode: MRM
Ion monitored: Q1: 311.1 and Q3: 211.1
Volume of injection: 50 µL
Run time: 20min
Retention time: 10.1 min.

1,2-Dichloroethane

The method is based on determination of 1,2-dichloroethane by headspace analysis in combination with gas chromatography and flame ionization detection (HS-GC-FID) using external standard.

Specificity

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

Linearity

A known aliquot from the impurity reference standard stock solution prepared was transferred in to a volumetric flask and diluted with diluent as detailed below to get five different working standard solutions for detector linearity check.

Precision

About 0.1 g of the test item, Pendimethalin 455 g/L CS was weighed in five replications in to separate head space vials and an aliquot of 1.0 mL of diluent 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 GC-HS-FID.

Accuracy

About 0.1 g of the test item Pendimethalin 455 g/L CS was weighed into separate head space vials (three for each of the 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 GC-FID

Chromatographic conditions

Instrument: Gas Chromatograph equipped with HS auto sampler, FID Detector and PC based data system
Column: DB-624, 30 m long, 0.32 mm internal diameter, 1.8 µm film thickness
Split ratio: 25:1
Column Oven: Initial: 40°C hold for 5min
Ramp 2: 7°C/min to 100°C, hold for 0 min
Ramp 3: 60°C/min to 250°C, hold for 5min
Helium: 2.0 mL/min

N-Nitrosoamines

The method is based on determination of N-Nitrosoamines by GC-MS/MS method using external standard.

Specificity

Specificity and selectivity of the method was established by injecting duplicate preparations of 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.

Linearity

A known aliquot from each of the impurity reference standard stock solutions prepared was transferred in to 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 GC-MS/MS. The peak areas of impurity were recorded for each injection.

Precision

The N-Nitrosoamine impurities were not detected in the test item. To calculate the precision of the method, the test item was fortified with known amount of N-Nitrosoamine impurities. About 0.1 g of the test item, Pendimethalin 455 g/L CS was weighed in five replications to separate 5 mL volumetric flasks and an aliquot of 0.25 mL of N-Nitrosoamine impurity mix standard solution of about 2µg/mL was added, and the contents of the flask were dissolved in diluent. Later, the volume was made up to the mark with diluent, the solution was shaken thoroughly, filtered and used for the analysis of impurity content by injecting to GC-MS/MS.

Accuracy

About 0.1 g of test item, Pendimethalin 455 g/L CS was weighed into separate 5 mL volumetric flasks (Three for each of the three fortification levels) and the contents of the volumetric flasks were dissolved in diluent. Later the test item solutions were fortified with N-Nitrosoamine impurities reference standard stock solution. Later, the volume was made up to the mark with diluent, the solutions were shaken thoroughly, filtered and used for analysis of impurity content by injecting to GC-MS/MS.

Chromatographic conditions

Instrument: Shimadzu Gas Chromatography for mass spectrometer equipped with HS-20 autosampler, GCMS-TQ8050 and PC based data system with Shimadzu GC-MS/MS software.

GC conditions

Column: DB-35MS, 30 m long, 0.25 mm internal diameter and 0.25 µm film thickness
Split ratio: 5:1
Helium: 1.0 mL/min
Injection volume: 1.0 µL
Run time: 21 min.
Column oven: initial: 80°C hold for 1.0 min
Ramp 1: 15°C/min to 150°C, hold for 0.0 min.
Ramp2: 25°C/min to 300°C, hold for 5 min.
Ion source temp: 230°C
Interface temp: 300°C
Acquisition mode: MRM

Validation - Results and discussions

Table 5.2-5: Methods suitable for the determination of the relevant impurities in plant protection product (PPP) PENSUI/SHA 2600 E

	N-Nitrosopendimethalin	1,2-dichloroethane	N-Nitrosodimethylamine	N-Nitrosomethylethylamine
Author(s), year	S. Revankar, 2021			
Principle of method	LC-MS/MS	GC-FID	GC-MS/MS	GC-MS/MS
Linearity (linear between mg/L) (correlation coefficient, expressed as r)	5 points 0.00605 to 0.24205 µg/mL First mass transition $y=346748x-41$ $R=0.9998$ Second mass transition $y=82890.15201x-$	5 points 0.504 to 20.158 µg/mL First mass transition $y=1684.36x-216.46$ $R=0.9999$	5 points 0.0201 to 0.2510 µg/mL First mass transition $y=1505x+334165$ $R=0.9996$ Second mass transition $y=114988$	5 points 0.0201 to 0.2511 µg/mL First mass transition $y=1328x+370502$ $R=0.9995$ Second mass transition $y=182860.6884x+484.$

	N- Nitrosopendimethalin	1,2-dichloroethane	N- Nitrosodimethylamine	N- Nitrosomethylethylamine
	51.713 R=0.9995		114989x+154.45 R=0.9996	3878 R=0.9997
Precision – Repeatability Mean n = 5 (%RSD)	First mass transition %RSD=3.458 %RSD _R =12.26 %RSD _r =8.21 Hr=0.42<1 Second mass transition %RSD=5.357 %RSD _R =12.30 %RSD _r =8.24 Hr=0.67<1	First mass transition %RSD=2.113 %RSD _R =9.78 %RSD _r =6.55 Hr=0.32<1	First mass transition %RSD=2.103 %RSD _R =14.12 %RSD _r =9.46 Hr=0.22<1 Second mass transition %RSD=4.141 %RSD _R =14.12 %RSD _r =9.46 Hr=0.44<1	First mass transition %RSD=1.670 %RSD _R =14.12 %RSD _r =9.46 Hr=0.18<1 Second mass transition %RSD=2.509 %RSD _R =13.93 %RSD _r =9.33 Hr=0.27<1
Accuracy 3 concentrations n = 3 (% Recovery)	First mass transition Total mean recovery = 88.932 ± 5.95% Second mass transition 86.390 ± 4.655%	Total mean recovery = 107.337 ± 8.684%	First mass transition Total mean recovery = 103.786 ± 8.035% Second mass transition 99.298 ± 5.062%	First mass transition Total mean recovery = 108.136 ± 5.552% Second mass transition 99.412 ± 3.08%
Interference/ Specificity	No interference. The method is specific.	No interference. The method is specific.	No interference. The method is specific.	No interference. The method is specific.
LOQ	0.006 µg/mL	0.504 µg/mL	0.967 mg/kg	0.968 mg/kg
Comment	-	-	-	-

	N- Nitrosodiethylamine	N- Nitrosopyrrolidine	N- Nitrosodipropylamine	N- Nitrosopiperidine	N- Nitrosodibutylamine
Author(s), year	S. Revankar, 2021				
Principle of method	GC-MS/MS	GC-MS/MS	GC-MS/MS	GC-MS/MS	GC-MS/MS
Linearity (linear between mg/L) (correlation coefficient, expressed as r)	5 points 0.0201 to 0.2508 µg/mL First mass transition y=1067x+291975 R=0.9995 Second mass transition y=113220.3120x+354.6928 R=0.9995	5 points 0.0201 to 0.2508 µg/mL First mass transition y=184180x+490 R=0.9995 Second mass transition y=175963.8595x+552.1607 R=0.9995	5 points 0.0201 to 0.25109 µg/mL First mass transition y=283845x+98 R=0.9974 Second mass transition y=166593.1973x+444.3638 R=0.9987	5 points 0.0201 to 0.2508 µg/mL First mass transition y=234217x+835 R=0.9995 Second mass transition y=124810.5807x+239.8151 R=0.9996	5 points 0.0201 to 0.2508 µg/mL First mass transition y=203337x+994 R=0.9996 Second mass transition y=131830.7235x-146.0902 R=0.9994
Precision – Repeatability Mean n = 5	First mass transition %RSD=1.650 %RSD _R =14.12 %RSD _r =9.46	First mass transition %RSD=2.223 %RSD _R =14.03 %RSD _r =9.40	First mass transition %RSD=6.205 %RSD _R =13.74 %RSD _r =9.21	First mass transition %RSD=1.014 %RSD _R =13.93 %RSD _r =9.33	First mass transition %RSD=3.494 %RSD _R =14.03 %RSD _r =9.40

(%RSD)	Hr=0.17<1 Second mass transition %RSD=1.330 %RSDR=14.12 %RSDr=9.46 Hr=0.14<1	Hr=0.24<1 Second mass transition %RSD=4.497 %RSDR=14.03 %RSDr=9.40 Hr=0.36<1	Hr=0.67<1 Second mass transition %RSD=3.771 %RSDR=13.93 %RSDr=9.33 Hr=0.40<1	Hr=0.11<1 Second mass transition %RSD=2.632 %RSDR=13.93 %RSDr=9.33 Hr=0.28<1	Hr=0.37<1 Second mass transition %RSD=7.982 %RSDR=14.12 %RSDr=9.46 Hr=0.84<1
Accuracy 3 concentrations = 3 (% Recovery)	First mass transition Total mean recovery = 111.631 ± 5.661% Second mass transition 100.268 ± 5.82%	First mass transition Total mean recovery = 108.209 ± 6.712% Second mass transition 107.164 ± 4.565%	First mass transition Total mean recovery = 110.973 ± 3.196% Second mass transition 99.629 ± 12.29%	First mass transition Total mean recovery = 111.379 ± 6.018% Second mass transition 107.499 ± 5.42%	First mass transition Total mean recovery = 103.855 ± 5.261% Second mass transition 110.780 ± 4.191%
Interference/ Specificity	No interference. The method is specific.	No interference. The method is specific.	No interference. The method is specific.	No interference. The method is specific.	No interference. The method is specific.
LOQ	0.966 mg/kg	0.966 mg/kg	0.967 mg/kg	0.966 mg/kg	0.967 mg/kg
Comment	-	-	-	-	-

Conclusion

According to SANCO/3030/99 rev. 5 the method was successfully validated and is suitable for determination of Relevant impurities (1,2-dichloroethane, N-Nitroso compounds) of pendimethalin in the test item PENSHUI/SHA 2600 E

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)

CIPAC method No 357 is available.

5.2.2 Methods for the determination of residues (KCP 5.1.2)

Please refer to the post-registration method.

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 pendimethalin (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	Pendimethalin	0.05 mg/kg	Reg. (EU) 2019/1791
Plant, high acid content		0.05 mg/kg	Reg. (EU) 2019/1791
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 – <i>Pimephales promelas</i>)
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+

5.3.2.2 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 pendimethalin 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: 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	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 5.3.2.2.1 XXX, 2017 Report No. 16.586423.0002
	ILV	0.01 mg/kg	HPLC-MS/MS	KCP 5.3.2.2.5 J. XXX, 2017 Report No. ZBBZ- 2016/12/DPL/4DE
	Confirmatory (if required)	-	-	Not required.
High acid content	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 5.3.2.2.2 XXX, 2017 Report No. 16.566423.0003
	Confirmatory (if required)	-	-	Not required.
High oil content	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 (soy bean)	0.01 mg/kg	LC-MS/MS	KCP 5.3.2.2.3 (XXX, 2017; Study N. 16.566423.0001)
	ILV	0.01 mg/kg	LC-MS/MS	KCP 5.3.2.2.6 J. XXX, 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 (cereals)	0.01 mg/kg	HPLC-MS/MS	KCP 5.3.2.2.4 XXX, 2017 Report No. 16.566423.0005

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
	Confirmatory (if required)	-	-	Not required.

Table 5.3-3: Statement on extraction efficiency

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

5.3.2.3 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.

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 pendimethalin in soil is given in the following tables.

Applicant wish to refer to the matching list:

KCP 5.3.3.4.1 (XXX, 2017; Study N. 16.566423.0008);

“Acceptable

- The HPLC-MS/MS analytical method with acetone/hexane extract ion of the soil with an LOQ of 0.001 mg/kg used in the GLP study by Greco, M.L. (sponsor Sharda) is fully validated according to SAN-CO/825/00 rev. 8.1. Therefore this study can be used for data matching for an monitoring/enforcement method for pendimethalin residues in soil.”

KCP 5.3.3.4.2 (XXX, 2017; Study N. 16.566423.0009)

“Acceptable

- The HPLC-MS/MS analytical method with acetone/hexane extract ion of the soil with an LOQ of 0.001 mg/kg used in the GLP study by Greco, M.L. (sponsor Sharda) is fully validated according to SAN-CO/825/00 rev. 8.1. Therefore this study can be used for data matching for an monitoring/enforcement method for pendimethalin residues in soil.”

Table 5.3-4: 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

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	LC-MS/MS	KCP 5.3.2.4.1 (XXX, 2017; Study N. 16.566423.0008)
Primary	0.01 mg/kg	LC-MS/MS	KCP 5.3.2.4.2 (XXX, 2017; Study N. 16.566423.0009)
Confirmatory	-	-	Not required

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

An overview on the acceptable methods and possible data gaps for analysis of pendimethalin in surface and drinking water is given in the following tables.

Applicant wish to refer to the matching list:

KCP 5.3.3.5.1 (G XXX 2015; Study N. YV/13/034)

“Acceptable

- The GC-MS analytical method with DCM extract ion of the drinking water with an LOQ of 0.1 µg/L used in the GLP study XXX, G. (sponsor Sharda) is fully validated according to SANCO/825/00 rev. 8.1. Therefore this study can be used for data matching for an monitoring/enforcement method for pendimethalin residues in drinking water.”

KCP 5.3.3.5.2 (K. McInerney 2016; Study N. 100041492B)

“Acceptable

- The ILV GC-MS analytical method with DCM extract ion of the drinking water with an LOQ of 0.1 µg/L used in the GLP study XXX, G. (sponsor Sharda) is fully validated according to SANCO/825/00 rev. 8.1. Therefore this study can be used for data matching for an monitoring/enforcement method for pendimethalin residues in drinking water.”

Table 5.3-5: 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	HPLC-MS/MS	RAR 2015, EFSA 2016 Heinz N., 2013
	ILV	0.02 µg/L	HPLC-MS/MS	RAR 2015, EFSA 2016 Wiesner F., Breyer N., 2013
	Primary	0.1 µg/L	GC-MS	KCP 5.3.2.5.1 (G XXX 2015; Study N. YV/13/034)
	ILV	0.1 µg/L	GC-MS	KCP 5.3.2.5.2 (K. McInerney 2016; Study N. 100041492B)
	Confirmatory	-	-	Not required.
Surface water	Primary	0.02 µg/L	HPLC-MS/MS	RAR 2015, EFSA 2016 Heinz N., 2013
	Primary	0.02 µg/L	HPLC-MS/MS	KCP 5.3.2.5.5 XXX, 2017

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
				Report No. 16.566423.0010
	Confirmatory	-	-	Not required.

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

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

Applicant wish to refer to the matching list:

KCP 5.3.3.6.1 (XXX, 2017; Study N. 16.566423.00012)

"Acceptable

- The HPLC-MS/MS analytical method with acetone/hexane extraction of the air (collected on a polyurethane plug) with an LOQ of 4 µg/m³ used in the GLP study Greco, M.L. (sponsor Sharda) is fully validated according to SANCO/825/00 rev. 8.1. Therefore this study can be used for data matching for an monitoring/enforcement method for pendimethalin residues in air."

Table 5.3-6: 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	LC-MS/MS	RAR 2015, EFSA 2016 Penning H., 2013
	4 µg/m ³	LC-MS/MS	KCP 5.3.2.6.1 (XXX, 2017; Study N. 16.566423.00012)
Confirmatory	-	-	Not required.

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

An overview on the acceptable methods and possible data gaps for analysis of 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 5.3-7: 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 5.3.1 (XXX, 2017; Study N. 16.566423.0007)

Component of residue definition: pendimethalin			
Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Confirmatory	-	-	Not required.
Primary (liver)	0.01 mg/kg	LC-MS/MS	KCP 5.3.2 (XXX, 2017; 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.

5.3.2.8 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 5.1.1/01	XXX	2017	Validation of analytical method for determination of active ingredient content of pendimethalin 455 g/L CS. Document no.: 228-2-12-16345 Institute of Industrial Organic Chemistry GLP Unpublished	N	Sharda
KCP 5.1.2/01	A. XXX	2017	Pendimethalin 455 g/L CS. Methods validation for determination of the relevant impurities of pendimethalin. Document no.: BA-45/17 JAI Reesearch Foundation GLP Unpublished	N	Sharda
KCP 5.1.1-2 KCP 5.1.2/02	S. Revankar	2021	Accelerated storage stability test by heating at elevated temperature of Pendimethalin 455 g/L CS. Report No. G21360 Eurofins Advinus GLP Unpublished	N	Sharda
KCP 5.1.1-3	B. XXX	2021	Pendimethalin 45.5% CS Analysis of active substance content and physicochemical properties of preparation and preparation after accelerated and freeze/thaw storage procedures. Report No. 47/2021 Institute of Heavy Organic Synthesis "Blachownia"	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
			GLP Unpublished		
KCP 5.3.1	XXX	2017	Validation of the analytical procedure for the determination of pendimethalin (CAS: 40487-42-1), in blood by liquid chromatography. Study No. 16.566423.0007 GLP Unpublished	Y	Sharda
KCP 5.3.2	XXX	2017	Validation of the analytical procedure for the determination of pendimethalin (CAS: 40487-42-1), in liver by liquid chromatography. Study No. 16.566423.0004 GLP Unpublished	Y	Sharda
KCP 5.3.2.2.1	XXX	2017	Validation of the analytical procedure for the determination of pendimethalin (CAS: 40487-42-1) in potato by liquid chromatography. XXX, 2017. Chelab Report No. 16.586423.0002 GLP Unpublished	N	Sharda Cropchem Ltd
KCP 5.3.2.2.2	XXX	2017	Validation of the analytical procedure for the determination of Pendimethalin (CAS: 40487-42-1) in oranges by liquid chromatography. XXX, 2017. Chelab Report No. 16.566423.0003 GLP Unpublished	N	Sharda Cropchem Ltd
KCP 5.3.2.2.3	XXX	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	N	Sharda Cropchem Ltd
KCP	XXX	2017	Validation of the analytical procedure for the determination of pendimethalin (CAS: 40487-42-1), in	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
5.3.2.2.4			wheat grains by liquid chromatography. XXX, 2017. Chelab Report No. 16.566423.0005 GLP Unpublished		Cropchem Ltd
KCP 5.3.2.2.5	J. XXX	2017	Determination of residues of Pendimethalin applied as “Pendimethalin 330 g/L” in potato at one site in Germany, 2016. J. XXX, 2017. Food Safety Laboratory Report No. ZBBZ-2016/12/DPL/4DE GLP Unpublished	N	Sharda Cropchem Ltd
KCP 5.3.2.2.6	J. XXX	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 5.3.2.4.1	XXX	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 5.3.2.4.2	XXX	2017	Validation of the analytical procedure for the determination of pendimethalin (CAS: 40487-42-1), in sandy loam soil by liquid chromatography. Study No. 16.566423.0009 GLP Unpublished	N	Sharda Cropchem Ltd
KCP 5.3.2.5.1	G XXX	2015	Method Validation: Determination of Residues of Pendimethalin in Drinking Water Samples Study No. YV/13/034 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 5.3.2.5.2	K. McInerney	2016	Independent Laboratory Validation (ILV) of Analytical Method for the Determination of Residues of Pendimethalin in Drinking Water Study No. 1000414928 GLP Unpublished	N	Sharda Cropchem Ltd
KCP 5.3.2.5.5	XXX	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
KCP 5.3.2.6.1	XXX	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

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 pendimethalin

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)

No new or additional studies have been submitted

A 2.1.2.1.1 Analytical method 1

A 2.1.2.1.1.1 Method validation

Comments of zRMS:	Study is accepted
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Reference: KCP 5.3.2.2.1

Report Validation of the analytical procedure for the determination of pendimethalin (CAS: 40487-42-1) in potato by liquid chromatography. XXX, 2017, 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 volume with milliQ water. pH was 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 1: 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 2: Characteristics for the analytical method used for validation of pendimethalin residues in potato

	Pendimethalin
Specificity	No significant peaks ($\leq 30\%$ 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 0.4 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

	Pendimethalin
Specificity	No significant peaks ($\leq 30\%$ 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 rev. 4 the method was successfully validated and is suitable for determination of residues of pendimethalin in potato.

A 2.1.2.1.2 Analytical method 2

A 2.1.2.1.2.1 Method validation

Comments of zRMS:	Study is accepted
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Reference: KCP 5.3.2.2.2

Report Validation of the analytical procedure for the determination of Pendimethalin (CAS: 40487-42-1) in oranges by liquid chromatography. XXX, 2017, 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 volume with milliQ water. pH was 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 3: 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 4: Characteristics for the analytical method used for validation of pendimethalin residues in oranges

	Pendimethalin
Specificity	No significant peaks ($\leq 30\%$ 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
Limit of determination/quantification	LOQ = 0.01 mg/kg LOD = 0.003 mg/kg

Conclusion

According to SANCO/3029/99 rev. 4 the method was successfully validated and is suitable for determination of residues of pendimethalin in oranges.

A 2.1.2.1.1 Analytical method 3

A 2.1.2.1.1.1 Method validation

Comments of zRMS:	Study is accepted
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Reference: KCP 5.3.2.3.3

Report Validation of the analytical procedure for the determination of pendimethalin (CAS: 40487-42-1), in soy beans by liquid chromatography. XXX, 2017; 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
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 VWR;
- 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: 961.2, 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;
- 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 °C
- Nebulizer.: 6 bar
- Cone gas: 150 l/h
- Desolvation gas: 400 l/h
- 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-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).

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 not higher than 10%, in accordance to the acceptance criteria for SST.

MRM	Transition 1	Transition 2	Transition 1	Transition 2
Analytical session:	n°1		n°2	
determination	area	area	area	area
1	72845	8010	70475	7576
2	64317	6819	61727	6607
3	62536	7033	62190	6722
4	71852	7752	68209	7428
Average	67888	7404	65650	7083
Std. Dev.	5128	568	4366	490
RSD	8	8	7	7
% RSD ≤	10	10	10	10
Conformity	Yes	Yes	Yes	Yes

Specificity

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 ($\leq 30\%$ 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.

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 sam- ple	Trans 1 area	Trans 2 area
L1	1	0.4	50	0.001	0.003	20717	2283
L2	1	0.6	20	0.003	0.010	63785	6985
L3	1	3.0	10	0.030	0.099	645963	68291
L4	0.6	1	10	0.060	0.199	1297527	138194
L5	1.0	1	10	0.099	0.331	2052077	219730

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.

%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 0,01 mg/kg.

Table A 5: 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	

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

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

	pendimethalin
Specificity	No significant peaks ($\leq 30\%$ 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 SOPa-284-LABCHI-Rev.0 internally developed is suitable to qualitatively and quantitatively determine pendimethalin in soy bean specimens, according to SANCO 3029/99 Rev.4 and OECD-204/2014 guidelines and for the given concentration range.

A 2.1.2.1.2 Analytical method 4

A 2.1.2.1.2.1 Method validation

Comments of zRMS:	Study is accepted
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Reference: KCP 5.3.2.2.4

Report	Validation of the analytical procedure for the determination of pendimethalin (CAS: 40487-42-1), in wheat grains by liquid chromatography. XXX, 2017, 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 volume with milliQ water. pH was 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 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 grain	Pendimethalin	0.01	100.3	2	First mass transition
		0.1	100.2	2	

Matrix	Analyte	Fortification level (mg/kg) (n =5)	Mean recovery (%)	RSD (%)	Comments
		0.01	108.2	1	Second mass transition
		0.1	97.7	1	

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

	Pendimethalin
Specificity	No significant peaks ($\leq 30\%$ 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 0.0358 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 rev. 4 the method was successfully validated and is suitable for determination of residues of pendimethalin in wheat grain.

A 2.1.2.1.2.2 Independent laboratory validation

Comments of zRMS:	Study is accepted
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Reference:	KCP 5.3.2.2.5
Report	Determination of residues of Pendimethalin applied as "Pendimethalin 330 g/L" in potato at one site in Germany, 2016. J. XXX, 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 of Potato 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 $< \pm 0.1$ 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 $\mu\text{g/mL}$ to 0.025 $\mu\text{g/mL}$. 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.

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 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
		0.1	94	1.3	

Table A 10: Characteristics for the analytical method used for independent laboratory validation of Pendimethalin residues in potato

	Potato
Specificity	No significant peaks ($\leq 30\%$ 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 rev. 4 the method was successfully validated and is suitable for determination of residues of pendimethalin in potato.

A 2.1.2.1.2.3 Independent laboratory validation

Comments of zRMS:	Study is accepted
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Reference: KCP 5.3.2.2.6

Report: Determination of residues of Pendimethalin applied as "Pendimethalin 330 g/L EC" in soybean at one site in Poland, 2016. J. XXX, 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 $< \pm 0.1$ 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 $\mu\text{g/mL}$ to 0.025 $\mu\text{g/mL}$. 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.

5.00 g \pm 0.05 g of homogenized matrix was weighed into a 50 mL Teflon® centrifuge tube. Sample weight was recorded.

If necessary fortification of the concurrent recovery sample(s) by aliquoting the fortification standard onto the matrix was carried out at this step. The tube was shaken vigorously by QuEChERS Hand Motion Shaker for 1 min. and allowed to stand for about 5 min.

Fortification level	Concentration ($\mu\text{g/mL}$)	Volume used (mL)
LOQ (0.01 mg/kg)	1	0.05
10 x LOQ (0.1 mg/kg)	10	0.05

Using glass volumetric pipettes 7.5 mL of water and 10 mL of acidified acetonitrile (+5 Vol % formic acid) were added.

The Teflon® centrifuge tube was closed tightly and shaken vigorously by QuEChERS Hand Motion Shaker for 1 min.

Results and discussions

Table A 11: 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 12: 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 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 rev. 4 the method was validated and is suitable for determination of Pendimethalin residues in soybean.

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 2.1.2.3.1 Analytical method 1

Comments of zRMS:	Study is accepted
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Reference:	KCP 5.3.2.4.1
Report	Validation of the analytical procedure for the determination of pendimethalin (CAS: 40487-42-1), loamy sand soil by liquid chromatography. XXX, 2017; 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 ($\pm 0,01$ g), SRA 49;
- Analytical balance ($\pm 0,01$ 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 °C
- Nebulizer.: 6 bar
- Cone gas: 150 l/h
- Desolvation gas: 400 l/h
- 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 not higher than 10%, in accordance to the acceptance criteria for SST.

MRM	Transition 1	Transition 2	Transition 1	Transition 2
Analytical session:	n°1		n°2	
determination	area	area	area	area
1	43207	4768	45644	4967
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

Specificity

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 ($\leq 30\%$ 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 sam- ple	Trans 1 area	Trans 2 area
L1	1	0.4	25	0.0016	0.0003	22834	2278
L2	1	0.5	10	0.005	0.001	43066	4368
L3	0.5	1	10	0.049	0.010	341773	37068
L4	1.0	1	10	0.099	0.020	672177	71987
L5	1.7	1	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 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	
Loamy sand soil	pendimethalin	0.001	103	104	2	4	

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

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

	pendimethalin
Specificity	No significant peaks ($\leq 30\%$ 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 2.1.2.3.1 Analytical method 2

Comments of zRMS:	Study is accepted
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Reference: KCP 5.3.2.4.2

Report: Validation of the analytical procedure for the determination of pendimethalin (CAS: 40487-42-1), sandy loam soil by liquid chromatography. XXX, 2017; Report number: 16.566423.0009.

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 STBG5687V 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 Sigma Aldrich
- PSA Resin 40 µm, ID: 306.6 - Varian
- 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;
- 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);

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
- 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 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 not higher than 10%, in accordance to the acceptance criteria for SST.

MRM	Transition 1	Transition 2	Transition 1	Transition 2
Analytical session:	n°1		n°2	
determination	area	area	area	area
1	13651	1404	15594	1664
2	13857	1398	15378	1701
3	13945	1508	15278	1728
4	15645	1606	15633	1654
Average	14275	1479	15471	1687
Std. Dev.	922	99	171	34
RSD	6	7	1	2
% RSD ≤	10	10	10	10
Conformity	Yes	Yes	Yes	Yes

Specificity

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 ($\leq 30\%$ 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 sam- ple	Trans 1 area	Trans 2 area
L1	1	0.4	25	0.002	0.0003	5517	595
L2	1	0.5	10	0.005	0.001	14100	1726
L3	0.5	1	10	0.050	0.010	145995	15575
L4	1.0	1	10	0.010	0.0199	294905	31818
L5	1.7	1	10	0.169	0.0339	524035	56616

Linearity Parameters of transition 1: Coefficient of determination (R^2) – 0.9991
 Linearity Parameters of transition 2: Coefficient of determination (R^2) – 0.9989

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 15: 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	
Sandy loam	pendimethalin	0.001	102	106	2	4	
Sandy loam	pendimethalin	0.010	103	104	1	1	

Table A 16: Characteristics for the analytical method used for validation of pendimethalin residues in soil

	pendimethalin
Specificity	No significant peaks ($\leq 30\%$ 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-292-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 2.1.2.4 Description of Methods for the Analysis of Water (KCP 5.2)

No new or additional studies have been submitted

A 2.1.2.4.1 Analytical method 1

Comments of zRMS:	Study is accepted
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Reference:	KCP 5.3.2.5.1
Report	Method Validation: Determination of Residues of Pendimethalin in Drinking Water Samples. G XXX, 2015 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 precision. 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 2.1.2.4.2 Analytical method 2

Comments of zRMS:	Study is accepted
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Reference: KCP 5.3.2.5.2

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	Analyte	Pendimethalin
	ug/L		
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	Analyte	Pendimethalin
	µg/L		
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 $\leq 20\%$.

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 2.1.2.4.3 Analytical method 3

A 2.1.2.4.3.1 Method validation

Comments of zRMS:	Study is accepted
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Reference: KCP 5.3.2.5.3

Report Validation of the analytical procedure for the determination of residues of Fluometuron (CAS: 2164-17-2) in ground water by LC-MS. M. Rubino, 2019, Report No. 19.515459.0001

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 an analytical method, for the determination of residues of Fluometuron in ground 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 volume with milliQ water. pH was 4

Mobile phase B

Methanol

Blank solution:

In a 200 mL volumetric flask was introduced 100 mL of mobile phase A and diluted to volume with acetonitrile.

Sample extraction

50 mL of filtered ground water were introduced with a 50 mL volumetric flask into a becker and then 2 drops of formic acid were added (pH about 4). The water was percolated using a regulated vacuum

through an Oasis HLB cartridge (previously conditioned with 4 mL of acetonitrile and 4 mL of milliQ water) with a flow rate of about 2 drops/sec. The analyte was eluted with about 4 mL of acetonitrile in a 10 mL tube. The eluate was dried under nitrogen flux and reconstituted with 0.5 mL of blank solution. Vortexed for about 30 sec, transferred into an HPLC vial and injected. The sample was prepared in duplicate.

Results and discussions

Table A 17: Recovery results from method validation of Pendimethalin using the analytical method

Matrix	Analyte	Fortification level (µg/L) (n = 5)	Mean recovery (%)	RSD (%)	Comments
Ground water	Fluometuron	0.05	95	1.0	First mass transition
		0.5	91	2.0	
		0.05	99	2.0	Second mass transition
		0.5	93	3.0	

Table A 18: Characteristics for the analytical method used for validation of Fluometuron residues in Ground water

	Fluometuron
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 0.012 to 1.708 µg/L First mass transition $y=4382529x$ $R^2=0.9999$ Second mass transition $y=570092x$ $R^2=1.000$
Assessment of matrix effects is presented	Yes
Limit of determination/quantification	LOQ = 0.5 µg/L LOD = 0.012 µg/L

Conclusion

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

A 2.1.2.4.4 Analytical method 4

A 2.1.2.4.4.1 Method validation

Comments of zRMS:	Study is accepted
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Reference:	KCP 5.3.2.5.4
Report	Validation of the analytical procedure for the determination of residues of Fluometuron (CAS: 2164-17-2) in surface water by LC-MS. M. Rubino, 2019, Report No. 19.515459.0001
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 an analytical method, for the determination of residues of Fluometuron 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 volume with milliQ water. pH was 4

Mobile phase B

Methanol

Blank solution:

In a 200 mL volumetric flask was introduced 100 mL of mobile phase A and diluted to volume with acetonitrile.

Sample extraction

50 mL of filtered ground water were introduced with a 50 mL volumetric flask into a becker and then 2 drops of formic acid were added (pH about 4). The water was percolated using a regulated vacuum through an Oasis HLB cartridge (previously conditioned with 4 mL of acetonitrile and 4 mL of milliQ water) with a flow rate of about 2 drops/sec. The analyte was eluted with about 4 mL of acetonitrile in a 10 mL tube. The eluate was dried under nitrogen flux and reconstituted with 0.5 mL of blank solution. Vortexed for about 30 sec, transferred into an HPLC vial and injected. The sample was prepared in duplicate.

Results and discussions

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

Matrix	Analyte	Fortification level (µg/L) (n = 5)	Mean recovery (%)	RSD (%)	Comments
Ground water	Fluometuron	0.05	97	3.0	First mass transition
		0.5	93	4.0	

Matrix	Analyte	Fortification level (µg/L) (n = 5)	Mean recovery (%)	RSD (%)	Comments
		0.05	92	3.0	Second mass transition
		0.5	87	6.0	

Table A 20: Characteristics for the analytical method used for validation of Fluometuron residues in Ground water

	Fluometuron
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 0.012 to 1.708 µg/L First mass transition $y=5557953x$ $R^2=0.9999$ Second mass transition $y=586170x$ $R^2=0.9997$
Assessment of matrix effects is presented	Yes
Limit of determination/quantification	LOQ = 0.5 µg/L LOD = 0.012 µg/L

Conclusion

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

A 2.1.2.4.5 Analytical method 5

A 2.1.2.4.5.1 Method validation

Comments of zRMS:	Study is accepted
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Reference: KCP 5.3.2.5.5

Report Validation of the analytical procedure for the determination of Pendimethalin (CAS: 40487-42-1), in surface water by liquid chromatography. XXX, 2017, 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 volume 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 Na₂SO₄ 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 21: 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 22: 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

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

No new or additional studies have been submitted

A 2.1.2.5.1 Analytical method 1

Comments of zRMS:	Study is accepted
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Reference:	KCP 5.3.2.6.1
Report	Validation of the analytical procedure for the determination of pendimethalin (CAS: 40487-42-1) in air by liquid chromatography. XXX, 2017; 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 °C
- Nebulizer.: 6 bar
- Cone gas: 150 l/h
- Desolvation gas: 400 l/h
- 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 not higher than 10%, in accordance to the acceptance criteria for SST.

MRM	Transition 1	Transition 2	Transition 1	Transition 2
Analytical session:	n°1		n°2	
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

Specificity

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 ($\leq 30\%$ 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 R^2 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 sam- ple g/m ³	Trans 1 area	Trans 2 area
L1	1	0.2	100	0.002	0.0000009	113831	12905
L2	1	0.9	100	0.009	0.000004	451683	50638
L3	1.0	1	100	0.100	0.000046	4727300	565998
L4	1.0	1	50	0.199	0.000092	7797545	910245
L5	0.7	1	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/m³), and 10xLOQ (about 0.00004 g/m³). 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 23: 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 24: Characteristics for the analytical method used for validation of pendimethalin residues in air

	pendimethalin
Specificity	No significant peaks ($\leq 30\%$ 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.
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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 2.1.2.6 Description of Methods for the Analysis of Body Fluids and Tissues (KCP 5.2)

A 2.1.2.6.1 Analytical method 1

Comments of zRMS:	Study is accepted
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Reference:	KCP 5.3.1
Report	Validation of the analytical procedure for the determination of pendimethalin (CAS: 40487-42-1), in blood by liquid chromatography. XXX, 2017; Report number: 16.566423.0007.
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 17F074033 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 Sigma Aldrich
- PSA Resin 40 μm , ID: 306.6 - Varian
- Formic acid, ID: 961.1, Suprapur
- Pendimethalin Reference Standard Solution, ID: 3422, logbook n°1045 pag 1/20 (Conc. 496.95 mg/l)

Materials and Apparatus

- Common analytical glassware;
- Fridge, SRA 7;
- Technical balance ($\pm 0,01$ g), SRA 49;
- Analytical balance ($\pm 0,01$ mg), SRA 192;
- 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);

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
- 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-290-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 not higher than 10%, in accordance to the acceptance criteria for SST.

MRM	Transition 1	Transition 2	Transition 1	Transition 2
Analytical session:	n°1		n°2	
determination	area	area	area	area
1	429693	44406	432167	45411

MRM	Transition 1	Transition 2	Transition 1	Transition 2
2	407515	42262	414475	43688
3	401337	42091	412903	43007
4	447054	47757	453962	47245
Average	421400	44129	428377	44838
Std. Dev.	20994	2639	19163	1897
RSD	5	6	4	4
% RSD ≤	10	10	10	10
Conformity	Yes	Yes	Yes	Yes

Specificity

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 ($\leq 30\%$ 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 (0,015 mg/l) to about 30xLOQ (1.5 mg/l) 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 R^2 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	-	10	0.004	0.013	118417	12481
L2	1.5	-	10	0.015	0.050	404443	42116
L3	-	1.5	10	0.149	0.497	4228624	447504
L4	-	3.2	10	0.318	1.060	8100523	837765
L5	-	4.8	10	0.477	1.590	11979157	1344107

Linearity Parameters of transition 1: Coefficient of determination (R^2) – 0.9979

Linearity Parameters of transition 2: Coefficient of determination (R^2) – 0.9972

Repeatability precision

Repeatability evaluation was performed on aliquots of sample spiked with Pendimethalin at LOQ (about 0.015 mg/l), and 10xLOQ (about 1.5 mg/l). 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 25: 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	
blood	pendimethalin	0.05	98.6	93.2	1	1	
blood	pendimethalin	0.50	95.1	90.6	2	2	

Table A 26: Characteristics for the analytical method used for validation of pendimethalin residues in blood

	pendimethalin
Specificity	No significant peaks ($\leq 30\%$ 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 0,05 mg/l.
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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 soil specimens, according to SANCO 3029/99 Rev.4 and OECD-204/2014 guidelines and for the given concentration range.

A 2.1.2.6.2

Analytical method 2

Comments of zRMS:	Study is accepted
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Reference:	KCP 5.3.2
Report	Validation of the analytical procedure for the determination of pendimethalin (CAS: 40487-42-1), in liver by liquid chromatography. XXX, 2017; Report number: 16.566423.0004.
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 17F074033 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 Sigma Aldrich
- PSA Resin 40 µm, ID: 306.6 - Varian

- Formic acid, ID: 961.1, Suprapur
- Pendimethalin Reference Standard Solution, ID: 3422, logbook n°1045 pag 1/20 (Conc. 496.95 mg/l)

Materials and Apparatus

- Common analytical glassware;
- Fridge, SRA 7;
- Technical balance ($\pm 0,01$ g), SRA 49;
- Analytical balance ($\pm 0,01$ mg), SRA 192;
- 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 μ m (ID: LC 23);

Instrumental Conditions

- Column: Acquity UPLC BEH C18, 50 mm x 2,1 mm x 1,7 μ m (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 μ l
- 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
- 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-287-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 not higher than 10%, in accordance to the acceptance criteria for SST.

MRM	Transition 1	Transition 2	Transition 1	Transition 2
Analytical session:	n°1		n°2	
determination	area	area	area	area
1	126116	13212	129369	13981

MRM	Transition 1	Transition 2	Transition 1	Transition 2
2	125040	13288	125554	13191
3	122466	13268	128027	13565
Average	124541	13256	127650	13579
Std. Dev.	1876	39	1935	395
RSD	2	0	2	3
% RSD ≤	10	10	10	10
Conformity	Yes	Yes	Yes	Yes

Specificity

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 ($\leq 30\%$ 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.

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	-	0,6	50	0.01	0,02	33910	3936
L2	-	1,0	20	0.05	0,1	1678970	13256
L3	-	5,0	10	0.50	0,99	1299778	137815
L4	1,2	-	10	1.16	2,39	3298234	350532
L5	1,6	-	10	1.59	3,18	4362904	471392

Linearity Parameters of transition 1: Coefficient of determination (R²) – 0.9997

Linearity Parameters of transition 2: Coefficient of determination (R²) – 0.9995

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.

%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 0,01 mg/kg.

Table A 27: 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	
liver	pendimethalin	0.01	104.4	103.0	2	3	
liver	pendimethalin	0.10	99.6	98.6	3	2	

Table A 28: Characteristics for the analytical method used for validation of pendimethalin residues in liver

	pendimethalin
Specificity	No significant peaks ($\leq 30\%$ 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 0,01 mg/kg.
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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 soil specimens, according to SANCO 3029/99 Rev.4 and OECD-204/2014 guidelines and for the given concentration range.

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

No new or additional studies have been submitted