

FINAL REGISTRATION REPORT

Part B

Section 5

Analytical Methods

Detailed summary of the risk assessment

Product code: SHA 126000 B

Product name(s): CLARA

Chemical active substance:

Chlormequat chloride, 720 g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(authorization)

Applicant: Sharda Cropchem Ltd.

Submission date: February 2022

Finalisation date: June 2023; October 2023

Version history

When	What
December 2022	Applicant update
June 2023	zRMS evaluated dRR submitted by Applicant.
October 2023	The Final Registration Report

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).....	4
5.2.1	Analysis of the plant protection product (KCP 5.1.1)	4
5.2.1.1	Determination of active substance and/or variant in the plant protection product (KCP 5.1.1).....	4
5.2.1.1	Description of analytical methods for the determination of relevant impurities (KCP 5.1.1).....	7
5.2.1.2	Description of analytical methods for the determination of formulants (KCP 5.1.1)	11
5.2.1.3	Applicability of existing CIPAC methods (KCP 5.1.1).....	11
5.2.2	Methods for the determination of residues (KCP 5.1.2).....	11
5.3	Methods for post-authorization control and monitoring purposes (KCP 5.2)	12
5.3.1	Analysis of the plant protection product (KCP 5.2)	12
5.3.2	Description of analytical methods for the determination of residues Chlormequat chloride (KCP 5.2)	12
5.3.2.1	Overview of residue definitions and levels for which compliance is required	12
5.3.2.2	Description of analytical methods for the determination of residues in plant matrices (KCP 5.2).....	13
5.3.2.3	Description of analytical methods for the determination of residues in animal matrices (KCP 5.2).....	14
5.3.2.4	Description of methods for the analysis of soil (KCP 5.2).....	15
5.3.2.5	Description of methods for the analysis of water (KCP 5.2).....	16
5.3.2.6	Description of methods for the analysis of air (KCP 5.2).....	16
5.3.2.7	Description of methods for the analysis of body fluids and tissues (KCP 5.2)	17
5.3.2.8	Other studies/ information	17
Appendix 1	Lists of data considered in support of the evaluation.....	18
Appendix 2	Detailed evaluation of submitted analytical methods	21
A 2.1	Analytical methods for chlormequat chloride.....	21
A 2.1.1	Methods used for the generation of pre-authorization data (KCP 5.1).....	21
A 2.1.2	Methods for post-authorization control and monitoring purposes (KCP 5.2)	24

5 Analytical methods

5.1 Conclusion and summary of assessment

The analytical methods for determination of active substance and relevant impurities in the formulation CLARA are submitted and validated and meets criteria of specificity, linearity and precision according to the requirements SANCO 3030/99 rev 5. The potential data gaps are listed below.

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:

- no data gaps

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

Noticed data gaps are:

- Data gap (minor): ILV method for water. This data gap can be supplemented after registration.

Commodity/crop	Supported/ Not supported
High starch content (Whinter wheat)	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 Chlormequat chloride, in plant protection product is provided as follows:

Comments of zRMS:	The analytical method HPLC-MS for determination of Chlormequat chloride in the formulation CLARA is validated and meets criteria of specificity, linearity and precision according to the requirements SANCO 3030/99 rev 5, therefore the method is acceptable.
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Reference:	KCP 5.1.1
Report	Chlormequat chloride 72% SL: Validation of the Analytical Method for the Determination of the Active Ingredient Content. M. Urbani, 2018, Report No. CH – 1027/2017
Guideline(s):	SANCO/3030/99 rev. 4
Deviations:	No
GLP:	Yes

Acceptability: Yes

Materials and methods

Specificity

The specificity test was conducted injecting, in the adjusted chromatographic conditions, a solvent wash (acetonitrile), Chlormequat chloride reference material, Chlormequat chloride test substance, Atrazine internal standard, Placebo and test item, then comparing the chromatograms in order to check possible cross contaminations. These solutions' concentrations were in the range of this method, but their exact values were not reported, since they were not used in calculations.

Linearity

Preparation of the stock and diluted reference material solution:

Using the analytical balance, a 1048.0 µg/mL stock standard solution was prepared, taking into account its 98.4 % purity, by weighing 21.3 mg of Chlormequat chloride reference material into a 20.00 mL volumetric flask and then dissolving to volume with acetonitrile. Using a volumetric pipette, a 10.5 µg/mL first diluted standard solution was prepared by transferring 1.00 mL of the stock standard solution into a 100.00 mL volumetric flask and making up to volume with acetonitrile. Using a volumetric pipette, a 523.98 ng/mL second diluted standard solution was prepared by transferring 1.00 mL of the first diluted standard solution into a 20.00 mL volumetric flask and making up to volume with acetonitrile.

Preparation of the stock and diluted internal standard solution:

Using the analytical balance, a 1185.0 µg/mL stock internal standard solution was prepared by weighing 23.7 mg of Atrazine internal standard into a 20.00 mL volumetric flask and then dissolving to volume with acetonitrile.

Using a volumetric pipette, a 11.9 µg/mL first diluted internal standard solution was prepared by transferring 1.00 mL of the stock internal standard solution into a 100.00 mL volumetric flask and making up to volume with acetonitrile.

Using a volumetric pipette, a 592.50 ng/mL second diluted internal standard solution was prepared by transferring 1.00 mL of the first diluted internal standard solution into a 20.00 mL volumetric flask and making up to volume with acetonitrile.

Preparation of the working standard solutions:

Using volumetric flasks and volumetric pipettes, five working standard solutions for linear calibration were prepared as follows:

WSS 1. 0.50 mL of the second diluted standard solution and 1.00 mL of the second diluted internal standard solution were transferred into a 10.00 mL volumetric flask, making to volume with acetonitrile (working standard solution at 26.20 ng/mL and 59.25 ng/mL, respectively).

WSS 2. 0.75 mL of the second diluted standard solution and 1.00 mL of the second diluted internal standard solution were transferred into a 10.00 mL volumetric flask, making to volume with acetonitrile (working standard solution at 39.30 ng/mL and 59.25 ng/mL, respectively).

WSS 3. 1.00 mL of the second diluted standard solution and 1.00 mL of the second diluted internal standard solution were transferred into a 10.00 mL volumetric flask, making to volume with acetonitrile (working standard solution at 52.40 ng/mL and 59.25 ng/mL, respectively).

WSS 4. 1.25 mL of the second diluted standard solution and 1.00 mL of the second diluted internal standard solution were transferred into a 10.00 mL volumetric flask, making to volume with acetonitrile (working standard solution at 65.50 ng/mL and 59.25 ng/mL, respectively).

WSS 5. 1.50 mL of the second diluted standard solution and 1.00 mL of the second diluted internal standard solution were transferred into a 10.00 mL volumetric flask, making to volume with acetonitrile (working standard solution at 78.60 ng/mL and 59.25 ng/mL, respectively).

Repeatability (Precision)

Standard 1. Using the analytical balance, 22.4 mg of Chlormequat chloride reference material and 27.2

mg of Atrazine internal standard were weighed into a conical flask and dissolved with 25 mL of acetonitrile. The resulting solution was diluted 1:20000 with acetonitrile. Standard 2. Using the analytical balance, 23.5 mg of Chlormequat chloride reference material and 28.6 mg of Atrazine internal standard were weighed into a conical flask and dissolved with 25 mL of acetonitrile. The resulting solution was diluted 1:20000 with acetonitrile. Six solutions of the test item (labelled from A to F) were prepared and injected as described in Internal Analytical Method No. 1027/2017.

Recovery (Accuracy)

The test was performed by spiking six aliquots of the Placebo with the Chlormequat chloride test substance at three levels in duplicate, corresponding to additions of 75 %, 100 % and 125% of the nominal concentration of active ingredient.

Preparation of the standard solutions:

Standard 1. Using the analytical balance, 25.3 mg of Chlormequat chloride reference material and 24.0 mg of Atrazine internal standard were weighed into a conical flask and dissolved with 25 mL of acetonitrile. The resulting solution was diluted 1:20000 with acetonitrile.

Standard 2. Using the analytical balance, 26.5 mg of Chlormequat chloride reference material and 25.1 mg of Atrazine internal standard were weighed into a conical flask and dissolved with 25 mL of acetonitrile. The resulting solution was diluted 1:20000 with acetonitrile.

Fortified sample preparation and analysis:

Using the analytical balance, six Placebo aliquots and six internal standard aliquots were weighed into six conical flasks.

To obtain test item fortification at three levels, corresponding to nominal additions of 75 %, 100 % and 125 % of the active ingredient, about 60 mg, 80 mg and 100 mg nominal aliquots of the test substance were added using the analytical balance

Validation - Results and discussions

Table 5.2-1: Methods suitable for the determination of active substance Chlormequat chloride in plant protection product CLARA/SHA 126000 B

	Chlormequat chloride
Author(s), year	M. Urbani, 2018
Principle of method	HPLC
Linearity (linear between mg/L / % range of the declared content) (correlation coefficient, expressed as r)	5 points 26.20 ng/mL to 78.60 ng/mL 33% w/w to 90% w/w $y = 8607x + 48970$ $R^2 = 0.99355$
Precision – Repeatability Mean n = 6 (%RSD)	63.4 % w/w %RSD = 1.01 %RSD _R = 2.14 %RSD _r = 1.44 Hr = 0.70 ≤ 1
Accuracy n = 6-2 for each level (% Total Recovery)	Low level at 50% w/w – 99.2% Medium level at 60% w/w – 98.5% High level at 80% w/w – 98.2% Total mean recovery: 98.6%
Interference/ Specificity	No interference: The method is specific

Conclusion

According to SANCO3030/99 rev. 5 the method was successfully validated and is suitable for determination of active substance Chlormequat chloride in the test item Chlormequat chloride 72% SL.

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

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

Comments of zRMS:	The analytical method GC-FID for determination of relevant impurity Vinyl chloride in the formulation CLARA is validated and meets criteria of specificity, linearity and precision according to the requirements SANCO 3030/99 rev 5, therefore the method is acceptable.
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Reference:	KCP 5.1.1-2
Report	Chlormequat chloride 72% SL: Validation of the Analytical Method for the Determination of Vinyl chloride Relevant Impurity Content, M. Urbani, 2018, Report No. CH – 1029/2017
Guideline(s):	SANCO/3030/99 rev. 4
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

- Vinyl chloride

Specificity

The specificity test was conducted injecting, in the adjusted chromatographic conditions a solvent wash (water), Vinyl chloride reference material, test item and fortified test item solution at high level and then comparing the chromatograms in order to check possible cross contaminations. These solutions' concentrations were in the range of this method, but their exact values were not reported, since they were not used in calculations.

Linearity

Preparation of the diluted standard solutions:

Diluted standard solution for WSS 1

Using a volumetric pipette, a 20.37 µg/mL diluted standard solution for WSS 1 was prepared by transferring 0.25 mL of the stock standard solution (at 2036.5 µg/mL) into a 25.00 mL volumetric flask and making up to volume with methanol.

Diluted standard solution for WSS 2

Using a volumetric pipette, a 50.91 µg/mL diluted standard solution for WSS 2 was prepared by transferring 0.25 mL of the stock standard solution (at 2036.5 µg/mL) into a 10.00 mL volumetric flask and making up to volume with methanol.

Diluted standard solution for WSS 3

Using a volumetric pipette, a 101.83 µg/mL diluted standard solution for WSS 3 was prepared by transferring 0.50 mL of the stock standard solution (at 2036.5 µg/mL) into a 10.00 mL volumetric flask and making up to volume with methanol.

Diluted standard solution for WSS 4

Using a volumetric pipette, a 203.65 µg/mL diluted standard solution for WSS 4 was prepared by transferring 1.00 mL of the stock standard solution (at 2036.5 µg/mL) into a 10.00 mL volumetric flask and making up to volume with methanol.

Diluted standard solution for WSS 5

Using a volumetric pipette, a 407.30 µg/mL diluted standard solution for WSS 5 was prepared by transferring 2.00 mL of the stock standard solution (at 2036.5 µg/mL) into a 10.00 mL volumetric flask and making up to volume with methanol.

Preparation of the working standard solutions:

Using volumetric flasks and volumetric syringes or pipettes, five working standard solutions for linear calibration were prepared as follows.

WSS 1. 0.05 mL of the diluted standard solution for WSS 1 were transferred into a 10.00 mL volumetric flask, making to volume with water (working standard solution at 1.02 µg).

WSS 2. 0.05 mL of the second diluted standard solution for WSS 2 were transferred into a 10.00 mL volumetric flask, making to volume with (working standard solution at 2.55 µg).

WSS 3. 0.05 mL of the second diluted standard solution for WSS 3 were transferred into a 10.00 mL volumetric flask, making to volume with water (working standard solution at 5.09 µg).

WSS 4. 0.05 mL of the second diluted standard solution for WSS 4 were transferred into a 10.00 mL volumetric flask, making to volume with water (working standard solution at 10.18 µg).

WSS 5. 0.05 mL of the second diluted standard solution for WSS 5 were transferred into a 10.00 mL volumetric flask, making to volume with water (working standard solution at 20.37 µg).

Accuracy and Precision

Recovery tests were performed by spiking the test item six times at two fortification levels.

Preparation of the diluted fortification standard solutions:

First diluted standard solution for spike low

Using a volumetric pipette, a 152.74 µg/mL first diluted standard solution for Spike Low was prepared by transferring 3.00 mL of the stock standard solution (at 2036.5 µg/mL) into a 40.00 mL volumetric flask and making up to volume with methanol.

Second diluted standard solution for spike low

Using a volumetric pipette, a 30.55 µg/mL second diluted standard solution for Spike Low was prepared by transferring 2.00 mL of the first diluted standard solution for Spike Low into a 10.00 mL volumetric flask and making up to volume with methanol.

First diluted standard solution for spike high

Using a volumetric pipette, a 203.65 µg/mL first diluted standard solution for spike high was prepared by transferring 1.00 mL of the stock standard solution (at 2036.5 µg/mL) into a 10.00 mL volumetric flask and making up to volume with methanol.

Low fortification level (nominal at 0.15 mg/kg)

Using the analytical balance, seven 10 g aliquots of the test item were weighed into seven 20 mL head-space vials. One of them was the control solution. Using a volumetric pipette, 0.05 mL of the second diluted standard solution for spike low were added to the other six flasks.

High fortification level (nominal at 1.00 mg/kg)

Using the analytical balance, seven 10 g aliquots of the test item were weighed into seven 20 mL head-space vials. One of them was the control solution. Using a volumetric pipette, 0.05 mL of the first diluted standard solution for spike high were added to the other six flasks.

- **1-2-DCE**

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

Comments of zRMS:	The analytical method GC-FID for determination of relevant impurity 1,2-Dichloroethane in the formulation CLARA is validated and meets criteria of specificity, linearity and precision according to the requirements SANCO 3030/99 rev 5, therefore the method is acceptable.
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Reference:	KCP 5.1.1-3
Report	Chlormequat chloride 72% SL: Validation of the Analytical Method for the Determination of 1,2-Dichloroethane (1,2-DCE) Relevant Impurity Content, M. Urbani, 2018, Report No. CH – 1028/2017
Guideline(s):	SANCO/3030/99 rev. 45
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Specificity

The specificity test was conducted injecting, in the adjusted chromatographic conditions a solvent wash (water), 1,2-Dichloroethane (1,2-DCE) reference material (at about 1.0 µg/mL), test item solution (at about 5 mg/mL) and fortified test item solution (at high level) and then comparing the chromatograms in order to check possible cross contaminations

Linearity

Preparation of the stock and diluted standard solutions:

Using the analytical balance, a 1028.0 µg/mL stock standard solution was prepared, taking into account its 100.0 % purity, by weighing 51.4 mg of 1,2-Dichloroethane (1,2-DCE) reference material into a 50.00 mL volumetric flask and then dissolving to volume with methanol.

Using a volumetric pipette, a 102.80 µg/mL first diluted standard solution was prepared by transferring 10.00 mL of the stock standard solution into a 100.00 mL volumetric flask and making up to volume with methanol.

Using a volumetric pipette, a 10.28 µg/mL second diluted standard solution was prepared by transferring 20.00 mL of the first diluted standard solution into a 200.00 mL volumetric flask and making up to volume with methanol.

Preparation of the working standard solutions:

Using volumetric flasks and volumetric syringes or pipettes, five working standard solutions for linear calibration were prepared as follows.

WSS 1. 1.00 mL of the second diluted standard solution were transferred into a 200.00 mL volumetric flask, making to volume with water (working standard solution at 0.05 µg/mL).

WSS 2. 0.50 mL of the second diluted standard solution were transferred into a 20.00 mL volumetric flask, making to volume with water (working standard solution at 0.26 µg/mL).

WSS 3. 20.00 mL of the second diluted standard solution was transferred into a 200.00 mL volumetric flask, making to volume with water (working standard solution at 1.03 µg/mL).
 WSS 4. 5.00 mL of the second diluted standard solution were transferred into a 20.00 mL volumetric flask, making to volume with water (working standard solution at 2.57 µg/mL).
 WSS 5. 100.00 mL of the second diluted standard solution were transferred into a 200.00 mL volumetric flask, making to volume with water (working standard solution at 5.14 µg/mL).

Accuracy and Precision

Preparation of the stock and diluted fortification standard solutions:

Using the analytical balance, a 956.0 µg/mL stock fortification standard solution was prepared, taking into account its 100.0 % purity, by weighing 23.9 mg of 1,2-DCE reference material into a 25.00 mL volumetric flask and then dissolving to volume with methanol.

Using a volumetric pipette, a 9.56 µg/mL first diluted fortification standard solution was prepared by transferring 0.50 mL of the stock standard solution into a 50.00 mL volumetric flask and making up to volume with methanol.

Using a volumetric pipette, a 0.96 µg/mL second diluted fortification standard solution was prepared by transferring 5.00 mL of the first diluted standard solution into a 50.00 mL volumetric flask and making up to volume with methanol.

Preparation of the test item stock solution:

Using the analytical balance, a test item stock solution was prepared weighing 1.0208 g of test item in a 100.00 mL volumetric flask and making to volume with water.

Low fortification level (at 0.013 g/kg, 13.11 µg/g)

Using a volumetric pipette, seven 5.00 mL aliquots of the test item stock solution were transferred into seven 10.00 mL volumetric flasks. One of them, the control solution, was made to volume with water. Using a volumetric pipette, 0.70 mL of the second diluted fortification standard solution was added to the other six flasks, making to volume with water.

High fortification level (at 0.13 g/kg, 131.11 µg/g)

Using a volumetric pipette, seven 5.00 mL aliquots of the test item stock solution were transferred into seven 10.00 mL volumetric flasks. One of them, the control solution, was made to volume with water. Using a volumetric pipette, 0.70 mL of the first diluted fortification standard solution was added to the other six flasks, making to volume with water.

Validation - Results and discussions

Table 5.2-2: Methods suitable for the determination of the relevant impurities in plant protection product (PPP) CLARA/ SHA 126000 B

	1,2-dichloroethane	Chloroethene (vinyl chloride)
Author(s), year	M.Urbani, 2018	
Principle of method	GC-FID	
Linearity (linear between mg/L) (correlation coefficient, expressed as r)	5 points, 0.05 µg/mL – 5.14 µg/mL 0.01 g/kg – 1 g/kg y = 461353x + 24895 R ² = 0.99812	5 points, 1.02 µg/mL – 20.37 µg/mL 0.1 mg/kg - 2.00 mg/kg y = 42837x + 6831 R ² = 0.99953
Precision – Repeatability Mean n = 6 for each level (%RSD)	Low level (0.013 g/kg): %RSD = 6.61 %RSD_R = 10.88 %RSD _f = 7.29	Low level (0.15 mg/kg): %RSD = 7.42 %RSD_R = 21.29 %RSD _f = 14.26

	1,2-dichloroethane	Chloroethene (vinyl chloride)
	Hr = $0.91 \leq 1$ High level (0.13 g/kg): %RSD = 3.56 %RSD_R = 7.69 %RSD _r = 5.15 Hr = $0.69 \leq 1$	Hr = $0.52 \leq 1$ High level (1.02 mg/kg): %RSD = 4.94 %RSD_R = 15.95 %RSD _r = 10.69 Hr = $0.46 \leq 1$
Accuracy n = 6 for each level (% Total Recovery)	Low level (0.013 g/kg): 95.6% High level (0.13 g/kg): 98.4%	Low level (0.15 mg/kg): 96.6% High level (1.02 mg/kg): 89.5%
Interference/ Specificity	No interference, the method is specific	No interference, the method is specific
LOQ	0.014 g/kg	0.15 mg/kg

Conclusion

According to SANCO3030/99 rev. 5 the method was successfully validated and is suitable for determination of relevant impurities 1,2-dichloroethane and chloroethene (vinyl chloride) in the test item Chlormequat chloride 72% SL.

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

Not relevant

5.2.1.3 Applicability of existing CIPAC methods (KCP 5.1.1)

Chlormequat chloride CIPAC No. 143.302
 Chlormequat CIPAC No. 143

5.2.2 Methods for the determination of residues (KCP 5.1.2)

An overview on the acceptable methods and possible data gaps for analysis of residues of chlormequat chloride for the generation of pre-authorization data is given in the following table. For the detailed evaluation of new studies it is referred to Appendix 2.

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

Component of residue definition: chlormequat chloride				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
Winter wheat (whole plant, grain, straw)	Primary	0.01 mg/kg	LC-MS/MS	D. Gąsczyk, 2021, Report No.: PW-2021-05 and admendment No. 1
(Residues)	Confirmatory (if required)	-	-	LC-MS/MS is highly selective method, therefore no confirmatory method is required

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 Chlormequat chloride (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	Sum of chlormequat and its salts expressed as chlormequat chloride	0.01 mg/kg	Reg. (EU) 2020/1565 Reg. (EU) 2022/1290
Plant, high acid content		0.01 mg/kg	Reg. (EU) 2020/1565 Reg. (EU) 2022/1290
Plant, high protein/high starch content (dry commodities)		0.01 mg/kg	Reg. (EU) 2020/1565 Reg. (EU) 2022/1290
Plant, high oil content		0.01 mg/kg	Reg. (EU) 2020/1565 Reg. (EU) 2022/1290
Plant, difficult matrices (hops, spices, tea)		0.05 mg/kg	Reg. (EU) 2020/1565 Reg. (EU) 2022/1290
Muscle	Sum of chlormequat and its salts expressed as chlormequat chloride	0.05 mg/kg	Reg. (EU) 2020/1565 Reg. (EU) 2022/1290
Milk		0.05 mg/kg	Reg. (EU) 2020/1565 Reg. (EU) 2022/1290
Eggs		0.15 mg/kg	Reg. (EU) 2020/1565 Reg. (EU) 2022/1290
Fat		0.15 mg/kg	Reg. (EU) 2020/1565 Reg. (EU) 2022/1290
Liver, kidney		0.15 mg/kg	Reg. (EU) 2020/1565 Reg. (EU) 2022/1290
Soil (Ecotoxicology)	Sum of chlormequat and its salts expressed as chlormequat chloride	0.05 mg/kg	common limit
Drinking water (Human toxicology)	Sum of chlormequat and its salts expressed as	0.1 µg/L	general limit for drinking water

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
	chlormequat chloride		
Surface water (Ecotoxicology)	Sum of chlormequat and its salts expressed as chlormequat chloride	2.4 mg/L	Reproduction, NOEC (<i>Daphnia magna</i>)
Air	Sum of chlormequat and its salts expressed as chlormequat chloride	12 µg/m ³	AOEL sys 0.04 mg/kg bw/d
Tissue (meat or liver)	Not defined	Not required	notclassified as T / T+
Body fluids		Not required	notclassified as T / T+

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

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

zRMS:

Presented methods are cover the proposed use (wheat).

Since the MRL value for wheat is 7 mg/kg, the presented analytical methods, although their limit of determination is higher than 0.01 mg/kg, are acceptable for this application.

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

Component of residue definition: Sum of chlormequat and its salts expressed as chlormequat chloride				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
High protein/high starch content (dry)	Primary	0.05 mg/kg	LC-MS/MS	Kerl, W., Mackenroth, C., 2006, Report No.: 168367 DAR, UK, 2007, Part B5 EU agreed
	ILV	0.05 mg/kg	LC-MS/MS	Richter, M., 2006, Report No.: 247717 DAR, UK, 2007, Part B5 EU agreed
	Confirmatory (if required)	-	-	LC-MS/MS is highly selective method, therefore no confirmatory method is required

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

Table 5.3-3: Statement on extraction efficiency

	Method for products of plant origin
Required, available from:	Please refer to the DAR, UK, 2007, part B7

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

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

zRMS:

Presented methods are acceptable.

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

Component of residue definition: Sum of chlormequat and its salts expressed as chlormequat chloride				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Milk	Primary	0.01 mg/kg	LC-MS/MS	Schulz H.,Meyer M., 2007a, Addendum to the DAR, UK, 2014 EU agreed
	ILV	0.01 mg/kg	LC-MS/MS	Weber H., 2010a, Addendum to the DAR, UK, 2014 EU agreed
	Confirmatory (if required)	-	-	LC-MS/MS is highly selective mtehod, therefore no confirmatory method is required
Eggs	Primary	0.01 mg/kg	LC-MS/MS	Schulz H.,Meyer M., 2007a, Addendum to the DAR, UK, 2014 EU agreed
	ILV	0.01 mg/kg	LC-MS/MS	Weber H., 2010a, Addendum to the DAR, UK, 2014 EU agreed
	Confirmatory (if required)	-	-	LC-MS/MS is highly selective mtehod, therefore no confirmatory method is required
Muscle	Primary	0.01 mg/kg	LC-MS/MS	Schulz H.,Meyer M., 2007a Addendum to the DAR, UK, 2014 EU agreed
	ILV	0.01 mg/kg	LC-MS/MS	Weber H., 2010a, Addendum to the DAR, UK, 2014 EU agreed
	Confirmatory (if required)	-	-	LC-MS/MS is highly selective mtehod, therefore no confirmatory method is required
Fat	Primary	0.01 mg/kg	LC-MS/MS	Schulz H.,Meyer M., 2007a Addendum to the DAR, UK, 2014

Component of residue definition: Sum of chlormequat and its salts expressed as chlormequat chloride				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
				EU agreed
	ILV	0.01 mg/kg	LC-MS/MS	Weber H., 2010a, Addendum to the DAR, UK, 2014 EU agreed
	Confirmatory (if required)	-	-	LC-MS/MS is highly selective method, therefore no confirmatory method is required
Kidney, liver	Primary	Liver: 0.05 mg/kg Kidney: 0.01 mg/kg	LC-MS/MS	Schulz H., Meyer M., 2007a Addendum to the DAR, UK, 2014 EU agreed
	ILV	Liver: 0.05 mg/kg Kidney: 0.01 mg/kg	LC-MS/MS	Weber H., 2010a, Addendum to the DAR, UK, 2014 EU agreed
	Confirmatory (if required)	-	-	LC-MS/MS is highly selective method, therefore no confirmatory method is required

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

Table 5.3-5: Statement on extraction efficiency

	Method for products of animal origin
Not required, because:	Residues above LOQ are not expected

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 Chlormequat chloride in soil is given in the following tables.

zRMS:

Presented methods are acceptable.

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

Component of residue definition: Sum of chlormequat and its salts expressed as chlormequat chloride			
Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Primary	0.01 mg/kg	IC-SCD (ion chromatography with suppressed conductivity detection)	Grote, C, 2003, Report No.: 2001/1014998 DAR, UK, 2007, Part B1- B5, EU agreed
Confirmatory	0.01 mg/kg	HPLC-MS-MS	

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 Chlormequat chloride in surface and drinking water is given in the following tables.

zRMS:

Data gap: ILV method for water. This data gap can be supplemented after registration.

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

Component of residue definition: Sum of chlormequat and its salts expressed as chlormequat chloride				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Drinking water	Primary	0.05 µg/L	LC-MS/MS	Schulz H., Meyer M., 2007b, Report No.: IF-07/00871633 Addendum to the DAR, UK, 2014 EU agreed
	ILV	-	-	Not provided in EU peer review
	Confirmatory	-	-	LC-MS/MS is highly selective method, therefore no confirmatory method is required
Surface water	Primary	0.05 µg/L	LC-MS/MS	Schulz H., Meyer M., 2007b, Addendum to the DAR, UK, 2014 EU agreed
	Confirmatory	-	-	LC-MS/MS is highly selective method, therefore no confirmatory method is required

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

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 Chlormequat chloride in air is given in the following tables.

zRMS:

Presented method is acceptable.

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

Component of residue definition: Sum of chlormequat and its salts expressed as chlormequat chloride			
Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Primary	0.0014 mg/m ³	IC-SCD (ion chromatography with suppressed conductivity detection)	Zangmeister, W., 2001 (amended 2003), Report No.: 2001/1008954 DAR, UK, 2007, Part B1-B5, EU agreed
Confirmatory	-	-	Not provided in EU peer review

For any special comments or remarkable points concerning the analytical methods for air it is referred to Appendix 2.

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

Not required as Chlormequat chloride is not classified as toxic or highly toxic (EFSA Scientific Report (2008) 179, 1-77)

zRMS:

According to Reg. (EU) No 283/2013, these methods are required.

During the peer review of the active substance, no residue definition was set for body fluid and tissues.

Since no definition has been established, the method is not required.

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	M. Urbani	2018	Chlormequat chloride 72% SL: Validation of the Analytical Method for the Determination of the Active Ingredient Content. Report No. CH – 1027/2017 ChemService S.r.l. Controlli e Ricerche GLP Unpublished	N	Sharda Cropchem Limited
KCP 5.1.1-02	M. Urbani	2018	Chlormequat chloride 72% SL: Validation of the Analytical Method for the Determination of Vinyl chloride Relevant Impurity Content Report No. CH – 1029/2017 ChemService S.r.l. Controlli e Ricerche GLP Unpublished	N	Sharda Cropchem Limited
KCP 5.1.1-03	M. Urbani	2018	Chlormequat chloride 72% SL: Validation of the Analytical Method for the Determination of 1,2-Dichloroethane (1,2-DCE) Relevant Impurity Content Report No. CH – 1028/2017 ChemService S.r.l. Controlli e Ricerche GLP Unpublished	N	Sharda Cropchem Limited
KCP 5.1.2	D. Gąszczyk	2021	Validation of method for determination of Chlormequat chloride by Liquid Chromatography (LC-MS/MS), Report No.: PW-2021-05 and amendment No. 1	N	Sharda Cropchem Limited

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
			Fertico Sp z o.o. GLP Unpublished		

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 chlormequat chloride

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

A 2.1.1.1 Description of analytical methods for the determination of residues in plant matrices (KCP 5.1.2)

A 2.1.1.1.1 Analytical method 1

A 2.1.1.1.1.1 Method validation

Comments of zRMS:	Method is acceptable
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Reference: KCP 5.1.2

Report Validation of method for determination of Chlormequat chloride by Liquid Chromatography (LC-MS/MS), Dorota Gąszczyk, 2021, Report No.: PW-2021-05, Amendment No. 1

Guideline(s): Yes
SANTE/2020/12830 rev. 1
SANTE/12682/2019

Deviations: No

GLP: Yes

Acceptability: Yes

Materials and methods

Preparation of samples for validation

Intreated homogenous matrix samples were weight at 5 g+/- 0.05g (winter wheat whole plant and grain) or 2 g +/- 0.05 g (winter wheat straw) into a 50 mL centrifuge tube. Spiking solution was added and then 10 ml of water and 1% HCOOH in methanol were added to receive final volume of 20 ml. The tube was closed and shaken vigorously by hand in room temperature for 1 min to 3 in. Then samples were shaken vigorously for 15 min using shaker and centrifuged for 10 min at 5500. After this time 0.5 mL of sample and 10 µL of Chlormequat chloride D4 were transferred into Eppendorf tube. Samples were diluted to the final volume of 1 mL by water. Additionally, winter wheat samples were centrifuged for 5 min at 9 rpm. Prepared samples were filtered with 0.22 µm PTFE into the injection vial for LC-MS/MS.

LC-MS/MS parameters

Solvent used for preparing samples: acetonitrile

Autosampler: with cooling (constant temperature 10°C)

Injection volume: 2µL

Injection mode: 200 µL/min

Chromatographic column: ZORBAX HILIC Plus with dimensions of 2.1 x 100 mm and gran diameter 3.5 µm, series number USCJP02725

Binary pump:

solvent A: 20mM ammonium formate, 0.4% formic acid in water,
 solvent B: acetonitrile with LC-MS purity,
 flow rate: 0.5 mL/min

Parameters of MS-Triple Quadrupole Acquisition Method

Analyte	Rt [min]	Ion Transitions	Collision Energy [V]	Cell Accelerator Voltage	Fragmentor	Polarity
Chlormequat chloride	4.48	122 → 63.1	22	4	127	Positive
		122 → 58.2	30			
Chlormequat chloride D4	4.48	126 → 67	20	4	75	Positive
		126 → 58	25			

Preparation of calibration curves

Calibration curves were performed on matrices – winter wheat whole plant, grain and straw. Eleven solutions of Chlormequat chloride were prepared. Winter wheat whole plant, grain and straw matrix extracts, 500 µL of matrix extracts, 10 µL of ISTD were transferred to the Eppendorf tubes. The appropriate volumes of Chlormequat chloride standard and water were added to the final volume of 1000 µL. In the next step Eppendorf tube were closed and shaken by hand. Prepared solutions were filtered with 0.22 µm PTFE into the injection vial for LC-MSMS. The details of preparation of calibration curves are presented below:

Concentration of prepared standard solution [µg/mL]	Matrix [µL]	Chlormequat chloride D4 (10 µg/mL) [µL]	Chlormequat chloride (ISTD) [µL]	Water [µL]	
0.0001	500	10	50 form 0.002 µg/mL	440	
0.0005			50 form 0.01 µg/mL		
0.001			50 form 0.02 µg/mL		
0.005			50 form 0.1 µg/mL		
0.01			50 form 0.2 µg/mL		
0.05			50 form 1 µg/mL		
0.1			50 form 2 µg/mL	470	
0.2			20 from 10 µg/mL		
0.4			40 from 10 µg/mL		450
0.6			60 from 10 µg/mL		430
1.0	100 from 10 µg/mL	390			

Accuracy and precision

The accuracy (as recoveries) and the precision (as repeatability) of method were calculated evaluating the results obtained from analysis of recovery tests carried out at spiking levels.

Accuracy was determined based on the amplification of untreated matrices samples with known amounts of standards using solutions R1 (1 µg/mL) and R0 (10 µg/mL).

In amendment No.1 accuracy was determined based on the amplification of untreated matrices samples with known amounts of standards using solutions R (100 µg/mL) and R0 (10 µg/mL).

Repeatability is expressed as the relative standard deviation (RSD %) on the results from six replicates for all included analytes.

Six recovery determinations at LOQ=0.01 mg/kg and six recovery determinations at 10xLOQ were performed.

In amendment No.1 six recovery determinations at 200xLOQ and six recovery determinations at 500xLOQ were performed.

The mean recovery at each fortification level fits the range of 70-120%. Accuracy and precision were reported for both ion transitions, precision (RSD) does not exceed 20%.

Results and discussions

According to SANCO3030/99 rev. 5 the method for determination of Chlormequat chloride by Liquid Chromatography (LC-MS/MS) was successfully validated and is suitable. LC-MS/MS is highly selective method, therefore no confirmatory method is required.

Table A 1: Recovery results from method validation of Chlormequat chloride using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (n = 6)	Mean recovery (%)	RSD (%)	Comments
Winter wheat whole plant	Chlormequat chloride	0.01	72.49	0.72	Chlormequat chloride (122 → 58.2) quantifier ion
		0.1	75.01	0.54	
		2.0	83.38	2.73	
		5.0	85.85	1.04	
		0.01	72.66	1.42	Chlormequat chloride (122 → 63.1) qualifier ion
		0.1	75.27	0.67	
		2.0	82.69	2.84	
		5.0	85.33	1.14	
Winter wheat grain		0.01	83.48	1.61	Chlormequat chloride (122 → 58.2) quantifier ion
		0.1	87.23	0.66	
		2.0	99.67	6.94	
		5.0	101.27	5.01	
		0.01	81.69	3.00	Chlormequat chloride (122 → 63.1) qualifier ion
		0.1	87.28	0.93	
		2.0	98.00	6.91	
		5.0	100.59	5.11	
Winter wheat straw	0.01	86.96	2.73	Chlormequat chloride (122 → 58.2) quantifier ion	
	0.1	88.11	4.67		
	2.0	105.74	3.98		
	5.0	100.68	3.22		
	0.01	93.88	7.19	Chlormequat chloride (122 → 63.1) qualifier ion	
	0.1	88.15	4.72		
	2.0	102.22	4.03		
	5.0	96.18	3.35		

Table A 2: Characteristics for the analytical method used for validation of Chlormequat chloride residues in winter wheat whole plant, grain and straw

	Chlormequat chloride first transition (122.0 → 58.2)	Chlormequat chloride second transition (122.0 → 63.1)
Specificity	LC-MS/MS method is specific due to chromatographic separation and selective detection system. Method use the signal ratios of the two MRM pairs (quantifer nad qualifier ion)	
Calibration (type, number of data points)	Wheat whole plant: $y = 2.1395x - 4.6529E-4$ $R^2 = 0.9974$ 11 points Wheat grain: $y = 2.1436x - 8.5777E-4$ $R^2 = 0.9972$ 11 points Wheat straw: $y = 2.0949x + 0.0022$ $R^2 = 0.9968$ 11 points	Wheat whole plant: $y = 0.5766x - 1.1022E-4$ $R^2 = 0.9970$ 11 points Wheat grain: $y = 0.5815x - 2.695E-4$ $R^2 = 0.9966$ 11 points Wheat straw: $y = 0.5837x + 4.4170E-4$ $R^2 = 0.9932$ 11 points
Calibration range	Accepted calibration range in concentration units: 0.0001 µg/ml - 1000.0 µg/ml Corresponding calibration range in mass ratio units for the sample: 0.01 mg/kg – 1.0 mg/kg	Accepted calibration range in concentration units: 0.0001 µg/ml - 1000.0 µg/ml Corresponding calibration range in mass ratio units for the sample: 0.01 mg/kg – 1.0 mg/kg
Assessment of matrix effects is presented	yes	yes
Limit of determination/quantification	LOD= 0.00015 mg/kg LOQ= 0.01 mg/kg	LOD= 0.00015 mg/kg LOQ= 0.01 mg/kg

Conclusion

According to SANCO3030/99 rev. 5 the method for determination of Chlormequat chloride by Liquid Chromatography (LC-MS/MS) for wheat whole plant, grain and straw was successfully validated and is suitable.

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 animal matrices (KCP 5.2)

No new or additional studies have been submitted

A 2.1.2.2 Description of Methods for the Analysis of Soil (KCP 5.2)

No new or additional studies have been submitted

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

A 2.1.2.5 Description of Methods for the Analysis of Body Fluids and Tissues (KCP 5.2)

No new or additional studies have been submitted

A 2.1.2.6 Other Studies/ Information

No new or additional studies have been submitted