FINAL REGISTRATION REPORT

Part B

Section 5

Analytical Methods

Detailed summary of the risk assessment

Product code: SHA 126085 A

Product name: MEPCY

Chemical active substances:

Chlormequat chloride, 345 g/L

Mepiquat chloride, 115 g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

Applicant: Sharda Cropchem Ltd.

Submission date: February 2022

Update: December 2022, September 2023

Finalisation date: May 2023, August 2023, January 2024 August 2024

Version history

When	What
December 2022	Applicant update
May 2023	ZRMS assessment of dRR
August 2023	Final version of RR
September 2023	Applicant update
January 2024	zRMS assessment after Applicant's update (analytical methods for RI)
August 2024	ZRMS update

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5 Analytical methods

5.1 Conclusion and summary of assessment

Sufficiently sensitive and selective analytical methods are ~~not~~ available for the active substance(s) and relevant impurities in the plant protection product.
Noticed data gaps are:

- None ~~development and validation of an analytical method for the determination of relevant impurities 1,2-dichloroethane and chloroethene (study is ongoing and the results will be available in August 2023)~~

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

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

Mepiquat chloride

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

Missing data should be completed when the plant protection product is reassessed.

Commodity/crop	Supported/ Not supported
High starch content (winter 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 and Mepiquat chloride in plant protection product is provided as follows:

Comments of zRMS:	The proposed analytical method is suitable for the simultaneous determination of active substances chlormequat chloride and mepiquat chloride in plant protection product Mepcy (Chlormequat 345 g/L + Mepiquat 115 g/L SL). The proposed analytical method has been fully validated in terms of specificity, linearity, repeatability, and accuracy. The proposed method fulfils the requirements of SANCO/3030/99 rev.4 and SANCO/3030/99 rev.5 guidance. The validation of the analytical method has been accepted.

Reference:	KCP 5.1.1
Report	Physical-chemical characterization and accelerated storage stability (2 weeks/54±2°C) of test item Chlormequat 345 g/L + Mepiquat 115 g/L SL. S. Aversa, 2018, Report No. BT022/18
Guideline(s):	SANCO/3030/99 rev. 4

Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

An HPLC-MS/MS analytical method for Chlormequat chloride and Mepiquat chloride determination was validated according to SANCO/3030/99 Rev. 4 Guidance Document.

Apparatus

Liquid Chromatograph:	Agilent HPLC-MS/MS System: HPLC 1200 series with 6410 Triple Quad Spectrometer
Column:	Phenomenex Luna 3 µm CN 100 Å – 75 x 4.6 mm

Experimental Conditions

Eluent A:	H2O acidified with 0.1 % formic acid
Eluent B:	Acetonitrile
Ratio A/B:	30 % / 70 %
Diluent:	Acetonitrile
Flow rate:	0.500 mL/min
Injected Volume:	1 µL
Column Temperature:	35 °C
Run Time:	10 minutes
Retention Times:	Chlormequat chloride 6.5 min approx Mepiquat chloride 6.9 min approx

Linearity test

10.3 mg of Mepiquat chloride analytical standard were weighted in a 10 mL graduated flask, dissolved and made up to volume with ultrapure water. This Stock Solution was named SS1-Mep and had a Mepiquat chloride concentration of 1014.550 mg/L. 10.1 mg of Chlormequat chloride analytical standard were weighted in a 10 mL graduated flask, dissolved and made up to volume with ultrapure water. This Stock Solution was named SS1-Chlor and had a Chlormequat chloride concentration of 993.840 mg/L. 0.10 mL of SS1-Mep and 0.10 mL of SS1-Chlor were transferred in a 10 mL graduated flask and made up to volume with acetonitrile, to have the SS2-Mix solution (Mepiquat chloride 10145.500 µg/L and Chlormequat chloride 9938.400 µg/L).
1.0 mL of SS2-Mix were transferred in a 10 mL graduated flask and made up to volume with acetonitrile, to have the SS3-Mix solution (Mepiquat chloride 1014.550 µg/L and Chlormequat chloride 993.840 µg/L). The SS3-Mix solution was diluted with acetonitrile to obtain the LIN1 - LIN 5 solutions of chlormequat chloride concentrations from 9.938 to 99.384 µg/L and mepiquat chloride concentrations from 10.146 to 101.455 µg/L.

Accuracy test

The accuracy was estimated by the standard addition method. The blank formulation (BT code 325/17/C) was spiked with an aliquot of analytical standard solution at two fortification levels.
For the accuracy test, two stock solution of Chlormequat and Mepiquat were prepared. 51.0 mg of Mepiquat chloride analytical standard were weighted in a 10 mL graduated flask, dissolved and made up to volume with ultrapure water. This Stock Solution was named SS1Mep-Rec and had a Mepiquat chloride concentration of 5023.500 mg/L.
152.7 mg of Chlormequat chloride analytical standard were weighted in a 10 mL graduated flask, dissolved and made up to volume with ultrapure water. This Stock Solution was named SS1Chlo-Rec and had a Chlormequat chloride concentration of 15025.680 mg/L.

Recovery low level (Chlormequat chloride about 21% w/w and Mepiquat chloride about 7% w/w)

About 10 mg of blank formulation were weighted in a 100 mL graduated flask, spiked with 0.2 mL of SS1Mep-Rec and 0.2 mL of SS1Chlo-Rec standard solution, dispersed and made up to volume with ultrapure water.
The solution was diluted 0.15 mL to 10 mL with acetonitrile and again 1 mL to 10 mL with acetonitrile and analysed with single injection
Five replicates were prepared and analysed by single injection. Solution code: REC LOW 01, REC LOW 02, REC LOW 03, REC LOW 04, REC LOW 05.

Recovery high level (Chlormequat chloride about 43% w/w and Mepiquat chloride about 14.5% w/w)
About 10 mg of blank formulation were weighted in a 100 mL graduated flask, spiked with 0.7 mL of SS1Mep-Rec and 0.7 mL of SS1Chlo-Rec standard solution, dispersed and made up to volume with ultrapure water. The solution was diluted 0.7 mL to 10 mL with acetonitrile and again 0.1 mL to 10 mL with acetonitrile and analysed with single injection Five replicates were prepared and analysed by single injection. Solution code: REC HIGH 01, REC HIGH 02, REC HIGH 03, REC HIGH 04, REC HIGH 05.

Interference check
Blank formulation (BT code 325/17/C) was injected during the sequence in order to evaluate the presence of interference at the retention time of the analytes. About 100 mg were weighted in a 100 mL graduated flask, dissolved, prepared and diluted as described in "*precision test*". Solutions code: BLANK 01 – BLANK 02.
Blank formulation (BT code 325/17/C) was spiked with 3.3 mL of SS1Chlo-Rec in order to evaluate the presence of interference at the retention time of Mepiquat. About 100 mg were weighted in a 100 mL graduated flask, dissolved, prepared and diluted as described in "*precision test*". Solutions code: BLANK CHLO 01 – BLANK CHLO 02.
Blank formulation (BT code 325/17/C) was spiked with 2.4 mL of SS1Mep-Rec in order to evaluate the presence of interference at the retention time of Chlormequat. About 100 mg were weighted in a 100 mL graduated flask, dissolved, prepared and diluted as described in "*precision test*". Solutions code: BLANK MEP 01 – BLANK MEP 02.

Precision test
About 100 mg of the fresh test item (BT code 324/17/C1) were weighted in a 100 mL graduated flask, dispersed and made up to volume with ultrapure water.
The solution was diluted 0.25 mL to 10 mL with acetonitrile and again 0.1 mL to 10 mL with acetonitrile and analysed with single injection (code PREC 01 – PREC 02 – PREC 03 – PREC 04 – PREC 05).

Validation - Results and discussions

Table 5.2-1: Methods suitable for the determination of Chlormequat chloride and Mepiquat chloride in plant protection product Chlormequat 34.5% + Mepiquat 11.5% SL

	Chlormequat chloride	**Mepiquat chloride**
Author(s), year	S. Aversa, 2018	
Principle of method	HPLC-MS/MS	
Linearity (linear between mg/L / % range of the declared content) (correlation coefficient, expressed as r)	5 points 99.384, 74.538, 49.692, 24.846, 9.938 µg/L <u>First mass transition</u> R^2 = 0.9991 y=225.708810x – 495.897924 <u>Second mass transition</u> R^2 = 0.9990 y=56.076039x – 103.137826	5 points 101.455, 76.091, 50.728, 25.364, 10.146 µg/L <u>First mass transition</u> R^2 = 0.9993 y=179.362204x – 315.448262 <u>Second mass transition</u> R^2 = 0.9988 y=147.440060x – 162.124351

	Chlormequat chloride	Mepiquat chloride
Precision – Repeatability Mean **n = 5** **(%RSD)**	First mass transition %RSD = 0.60 $\%RSD_R$ = 2.38 %RSDr = 1.59 Hr = 0.377 ≤ 1 Second mass transition %RSD = 1.23 $\%RSD_R$ = 2.38 %RSDr = 1.59 Hr = 0.774 ≤ 1	First mass transition %RSD = 1.52 $\%RSD_R$ = 2.78 %RSDr = 1.87 Hr = 0.813 ≤ 1 Second mass transition %RSD = 1.67 $\%RSD_R$ = 2.77 %RSDr = 1.86 Hr = 0.898 ≤ 1
Accuracy (standard addition method) **n = 5** **(% Recovery)**	**Low level 21% w/w** First mass transition 100.65% Second mass transition 99.83% **High level 43% w/w** First mass transition 101.72% Second mass transition 101.40%	**Low level 7% w/w** First mass transition 101.34% Second mass transition 102.56% **High level 14.5% w/w** First mass transition 100.06% Second mass transition 100.55%
Interference/ Specificity	No interference	No interference

Conclusion

The method was successfully validated and is suitable to determine the content of chlormequat chloride and mepiquat chloride in the test item Chlormequat 34.5% + Mepiqat 11.5% SL.

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

~~Not relevant.~~

According to Commission Implementing Regulation (EU) No 540/2011 and SANCO/175/08 final rev 2 (29 May 2015), for the active substance chlormequat chloride the manufacturing impurities 1,2-dichloroethane and chloroethene (vinylchloride) are of toxicological concern and must not exceed respectively 0.1 g/kg and 0.0005 g/kg in the technical material (based on the dry chlormequat chloride content).

According to Commission Implementing Regulation (EU) No 540/2011 and SANCO/106/08 rev 2 (20 May 2008), for the active substance mepiquat chloride no impurities are of toxicological concern.

~~A study on the development and validation of an analytical method for the determination of relevant impurities 1,2-dichloroethane and chloroethene is ongoing and the results will be available in August 2023.~~

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 methods for the determination of relevant impurities (1,2-dichloroethane and vinyl chloride) in plant protection product Mepcy are suitable for the determination of the content of each of the relevant impurity in the presence of each other, active substance and other components.

	The methods have been fully validated. The validation parameters of proposed analytical methods – interference, specificity, linearity, recovery, repeatability, and LOQ values fulfil the requirements of SANCO/3030/99 rev. 5 guidance. The validation of the analytical methods has been accepted.

Reference:	KCP 5.1.1-2
Report	Accelerated storage stability test of Chlormequat 34.5% + Mepiquat 11.5% SL, K.S. Kishora, 2023, Report No.: AG-G0028
Guideline(s):	Yes SANCO/3030/99 rev. 5
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Test Item information

Test Item	:	Chlormequat chloride 34.5% + Mepiquat chloride 11.5% SL
CAS No.	:	Chlormequat chloride: 999-81-5 Mepiquat chloride: 24307-26-4
Chemical Name (IU-PAC)	:	Chlormequat chloride: 2-chloroethyl trimethyl ammonium chloride Mepiquat chloride: 1,1-dimethylpiperidinium chloride
Physical Appearance	:	Slightly yellow liquid
Purity as per Certificate of Analysis	:	Chlormequat chloride: 34.56 % w/w Mepiquat chloride: 11.52 % w/w
Batch No.	:	SCL-20220
Manufactured Date	:	23/04/2022
Expiry Date	:	22/04/2024

Reference standard

Analytical/Impurity standard	Batch/Lot Number	Purity (%)	Expiry/Retest date	Source
1,2-Dichloroethane	G401409	99.96	Sep 2027	Spectrochem Pvt. Ltd.
Vinyl chloride	LRAD3587	99.9	Sep 2025	Supleco

Method Validation and Analysis for Impurity-I (1,2-Dichloroethane)

Chromatographic Conditions:

The estimation of active ingredient content in the samples were carried out by means of GC operated under the following conditions.

Instrument	:	Gas chromatograph equipped with Flame Ionization Detector and PC based data system

Column	:	Rxi-624 Sil MS, 30 m long, 0.32 mm internal diameter, 1.8 µm film thickness
Split ratio	:	2:1
Detector	:	250°C
Column Oven	:	Initial: 50°C hold for 5 min. Ramp 1: 10°C/min. to 150°C, hold for 0 min. Ramp 2: 30°C/min. to 240°C, hold for 5 min.

Condition of Head Space Sampler

Oven Temperature	:	95 °C
Sample line Temperature	:	105 °C
Transfer line Temperature	:	115°C
Sample equilibration Time	:	10 min
GC Cycle Time	:	30 min
Load Time	:	0.5 min
Inject Time	:	0.5 min
Pressurisation Time	:	0.5 min

Specificity
The specificity of the method for active ingredient was studied by injecting individual solutions (single preparation) of working standard solution of each impurity and analytical blank (diluent) to the GC-HS operated under the conditions of the method being validated.

Linearity
For detector linearity, five prepared standard solutions of different concentrations were injected to GC-HS in triplicate. A graph of instrument response versus concentration was plotted and linear regression coefficient (r) and coefficient of determination r^2 was established.

LOD and LOQ
The Impurity standard solution of limit of detection (LOD) was prepared by diluting the DLC-1 standard solution (conc = 0.126 µg/mL). The minimum quantity of Impurity detected with signal to noise ratio of ≥ 3, was considered as limit of detection (LOD) of equipment.

The lowest fortified concentration (%) of the Impurity in the test item, at which method was validated with acceptable results was considered as limit of quantitation (LOQ) of the method for analysis of Impurity in test item.

Precision
Accurately 0.1 g of the test item in five replications was weighed into a GC headspace vial and 1 mL of diluent (N-methyl-2-pyrrolidinone) was added into the vial. These head space vials were sealed with aluminum cap and rubber septa using crimper. These vials were analyzed for the content of 1,2-DCE using GC-HS.

Accuracy
The test item was fortified at minimum of 2 different concentration levels (five replications at each level) with reference standard of impurity.
About 0.1 g of test item was weighed in five replications at each of two fortification levels into GC head space vial and were fortified with 1.0 mL of impurity reference standard mix solution. Later, the headspace vials were sealed using rubber septa and aluminum cap using crimper. The solutions were injecting to GC.

Method Validation and Analysis for Impurity-II (Vinyl chloride):

Chromatographic Conditions

The estimation of active ingredient content in the samples were carried out by means of GC operated under the following conditions.

Instrument	:	Gas chromatograph equipped with Flame Ionization Detector and PC based data system
Column	:	Rxi-624 Sil MS, 30 m long, 0.32 mm internal diameter, 1.8 µm film thickness
Split ratio	:	10:1
Detector	:	280°C
Column Oven	:	Initial: 30°C hold for 3 min. Ramp 1: 30°C/min. to 200°C, hold for 1 min.

Condition of Head Space Sampler

Oven Temperature	:	80°C
Sample line Temperature	:	90°C
Transfer line Temperature	:	100°C
Sample equilibration Time	:	10 min
GC Cycle Time	:	22 min
Load Time	:	0.5 min
Inject Time	:	0.5 min
Pressurisation Time	:	1.0 min

MS Conditions

Acq. Mode	:	MRM
Detector Gain	:	+0.50kV
Ion Source Temp.	:	200°C
Interface Temp.	:	220°C
Solvent Cut Time	:	0.70 min

Specificity

The specificity of the method for active ingredient was studied by injecting individual solutions (single preparation) of working standard solution of each impurity and analytical blank (diluent) to the GC-MS operated under the conditions of the method being validated.

Linearity

For detector linearity, five prepared standard solutions of different concentrations were injected to GC-MS in triplicate. A graph of instrument response versus concentration was plotted and linear regression coefficient (r) and coefficient of determination r^2 was established.

LOD and LOQ

The Impurity standard solution of limit of detection (LOD) was prepared by diluting the DLC-1 standard solution (conc = 0.0031 µg/mL). The minimum quantity of Impurity detected with signal to noise ratio of ≥ 3, was considered as limit of detection (LOD) of equipment.

The lowest fortified concentration (%) of the Impurity in the test item, at which method was validated with acceptable results was considered as limit of quantitation (LOQ) of the method for analysis of Impurity in test item.

Precision
Accurately 0.1 g of the test item in five replications was weighed into a GC headspace vial and 1 mL of diluent (Milli-Q Water) was added into the vial. These head space vials were sealed with aluminum cap and rubber septa using crimper. These vials were analyzed for the content of Vinyl chloride using GC-MS.

Accuracy
The test item was fortified at minimum of 2 different concentration levels (five replications at each level) with reference standard of impurity.
About 0.1 g of test item was weighed in five replications at each of two fortification levels into GC head space vial and were fortified with 1.0 mL of impurity reference standard mix solution. Later, the head-space vials were sealed using rubber septa and aluminum cap using crimper. The solutions were injecting to GC-MS.

Validation - Results and discussions

Table 5.2-2: Methods suitable for the determination of the relevant impurities in plant protection product (PPP) MIRA (SHA 126085 A)

	1,2-Dichloroethane	**Vinyl chloride**
Author(s), year	K.S. Kishora, 2023	
Principle of method	GC-HS	GC-MS
Linearity (linear between mg/L) (correlation coefficient, expressed as r)	5 points Conc. Range = 1.007 to 16.106 µg/mL (0.0010 to 0.0161%) Y = 2774 × + 159 r = 0.99950	5 points Conc. Range = 0.025 to 0.400 µg/mL (0.000025 to 0.00040 %) Y= 12409 × + 587 r = 0.99948
Precision – Repeatability Mean n = 5 (%RSD)	Mean conc. of impurity-I = 0.00241 %w/w %RSD = 2.075 $\%RSD_R$ = 9.91 %RSDr = 6.64 Hr = 0.313 ≤ 1	Mean conc. of impurity-II = 0.0001008 %w/w %RSD = 1.190 $\%RSD_R$ = 15.98 %RSDr = 10.705 Hr = 0.111 ≤ 1
Accuracy n = 5 at each of 2 levels (% Recovery)	Conc. of Impurity-I Standard Stock Solution used for Fortification: 1.51 µg/mL: Accuracy as recvery (%): 94.905 ± 4.500 (s.d.) Conc. of Impurity-I Standard Stock Solution used for Fortification: 8.06 µg/mL: Accuracy as recovery (%): 90.299 ± 4.075 (s.d.) Overall mean recovery (%): 92.602 ±4.720	Conc. of Impurity-I Standard Stock Solution used for Fortification: 0.075 µg/mL: Accuracy as recvery (%): 100.079 ± 3.920 (s.d.) Conc. of Impurity-I Standard Stock Solution used for Fortification: 0.220 µg/mL: Accuracy as recovery (%): 97.220 ± 8.937 (s.d.) Overall mean recovery (%): 98.650 ± 6.678
Interference/ Specificity	No interference, the method is specific The chromatograms of blank, standard, and test item were included.	No interference, the method is specific The chromatograms of blank, standard, and test item were included.
LOQ	0.00143 % w/w	~~0.00006401~~ 0.00006348 % w/w

	1,2-Dichloroethane	Vinyl chloride
Comment	-	-

Conclusion

The method was successfully validated according to the GD SANCO/3030/99 rev. 5 and is suitable to determine content of relevant impurities – 1,2-dichloroethane and Vinyl chloride in the test item Chlormequat 34.5% + Mepiqat 11.5% SL.

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

Not relevant.

5.2.1.4 Applicability of existing CIPAC methods (KCP 5.1.1)

A CIPAC method No. 143.302 is available for Chlormequat chloride.
A CIPAC method No. 143 is available for Chlormequat.
A CIPAC method No. 440 is available for Mepiquat.
A CIPAC method No. 440.302 is available for Mepiquat chloride.

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) (Residues)	Primary	0.01 mg/kg	LC-MS/MS	D. Gąszczyk, 2021, Report No.: PW-2021-05 and admendment No. 1
	Confirmatory (if required)	-	-	LC-MS/MS is highly selective mtehod, therefore no confirmatory method is required
Component of residue definition: mepiquat chloride				
Wheat (whole plant, grain, straw) (Residues)	Primary	0.01 mg/kg	LC-MS/MS	K. Zagibajło, 2022, Report No.: 21/FSL/07/V
	Confirmatory (if required)	-	-	LC-MS/MS is highly selective mtehod, 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 of 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		0.5 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 Chlormequat chloride	0.1 µg/L	general limit for drinking water

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Surface water (Ecotoxicology)	Sum of Chlormequat and its salts expressed as Chlormequat chloride	2400 µg/L	Lowest NOEC from *Daphnia magna*
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 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)

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.

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

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

Component of residue definition: Sum of 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 mtehod, therefore no confirmatory method is required

Table 5.3-3: Statement on extraction efficiency

	Method for products of plant origin
Required, available from:	DAR Chlormequat-chloride – Volume 3, Annex B.5: Methods of analysis (April 2007)
Not required, because:	-

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

zRMS:
Presented methods are acceptable.

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

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

Component of residue definition: Sum of 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 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

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**
				method is required
Kidney, liver	Primary	0.05 mg/kg liver 0.01 mg/kg kidney	LC-MS/MS	Schulz H.,Meyer M., 2007a Addendum to the DAR, UK, 2014 EU agreed
	ILV	0.05 mg/kg liver 0.01 mg/kg kidney	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

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)

zRMS:
Presented methods are acceptable.

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

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 supressed conductivity detection)	C. Grote, 2003 Report No. 2001/1014998 DAR, UK, 2007 EU agreed
Confirmatory	0.01 mg/kg	HPLC-MS/MS	C. Grote, 2003 Report No. 2001/1014998 DAR, UK, 2007 EU agreed

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

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

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.

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/kg	LC-MS/MS	Schulz H.,Meyer M., 2007b, Report No.: IF-07/00871633 Addendum to the DAR, UK, 2014 EU agreed
	ILV	-	-	Not provided during EU review.
	Confirmatory	-	-	LC-MS/MS is a highly selective method therefore no other confirmatory method is required.
Surface water	Primary	0.05 µg/kg	LC-MS/MS	Schulz H.,Meyer M., 2007b, Addendum to the DAR, UK, 2014 EU agreed
	Confirmatory	-	-	LC-MS/MS is highly selective mtehod, therefore no confirmatory method is required

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

zRMS:
Presented method is acceptable.

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

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 µg/m^3	IC-SCD (ion chromatography with supressed conductivity detection)	Zangmeister, W., 2001 (amended 2003), Report No.: 2001/1008954 DAR, UK, 2007, EU agreed
Confirmatory	-	-	Not provided during EU review.

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

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.

Analytical methods for the determination of residues in body fluids and tissues are not required as Chlormequat is not classified as toxic or highly toxic (EFSA Scientific Report (2008) 179, 1-77).

5.3.2.8 Other studies/ information

No additional data provided.

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

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

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

Table 5.3-9: 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	The sum of mepiquat and its salts, expressed as Mepiquat chloride	0.02 mg/kg	Reg. (EU) No. 2021/976
Plant, high acid content		0.02 mg/kg	Reg. (EU) No. 2021/976
Plant, high protein/high starch content (dry commodities)		0.02 mg/kg	Reg. (EU) No. 2021/976
Plant, high oil content		0.02 mg/kg	Reg. (EU) No. 2021/976
Plant, difficult matrices (hops, spices, tea)		0.1 mg/kg	Reg. (EU) No. 2021/976
Muscle	The sum of mepiquat and its salts, expressed as Mepiquat chloride	0.05 mg/kg	Reg. (EU) No. 2021/976
Milk		0.07 mg/kg	Reg. (EU) No. 2021/976
Eggs		0.07 mg/kg	Reg. (EU) No. 2021/976
Fat		0.05 mg/kg	Reg. (EU) No. 2021/976
Liver, kidney		0.05 mg/kg	Reg. (EU) No. 2021/976
Soil (Ecotoxicology)	The sum of mepiquat and its salts expressed as Mepiquat chloride	0.05 mg/kg	common limit
Drinking water (Human toxicology)	The sum of mepiquat and its salts expressed as Mepiquat chloride	0.1 µg/L	general limit for drinking water
Surface water	The sum of mepiquat and	2600 µg/L	Lowest E_bC_{50} for *Lemna giba*

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
(Ecotoxicology)	its salts expressed as Mepiquat chloride		
Air	The sum of mepiquat and its salts expressed as Mepiquat chloride	90 µg/m^3	AOEL sys: 0.3 mg/kg bw/d
Tissue (meat or liver)		Not required.	Not classified as T / T+
Body fluids		Not required.	Not classified as T / T+

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

An overview on the acceptable methods and possible data gaps for analysis of Mepiquat 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 4 mg/kg, the presented analytical methods, although their limit of determination is higher than 0.02 mg/kg, are acceptable for this application.

Table 5.3-10: 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: The sum of Mepiquat and its salts, expressed as Mepiquat chloride				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
High water content	Primary	0.05 mg/kg	HPLC-MS/MS	Kerl W., 2003b, Report No.: 2003/1001371 A. Lehman, C. Mackenroth, 2003 Report No. 2003/1001373 Kerl W., Mackenroth, 2003b, Report No.: 2003/1001372 DAR, Belgium 2006 EU Agreed
	ILV	0.05 mg/kg	HPLC-MS/MS	H. Schultz, 2003b Report No. 2003/1001272 DAR, Belgium, 2006 EU agreed
	Confirmatory (if required)	-	-	LC/MS/MS is highly specific method and no confirmation is required.
High acid content	Primary	0.05 mg/kg	HPLC-MS/MS	Kerl W., 2003b, Report No.: 2003/1001371 A. Lehman, C. Mackenroth, 2003 Report No. 2003/1001373 Kerl W., Mackenroth, 2003b, Report No.: 2003/1001372

Component of residue definition: The sum of Mepiquat and its salts, expressed as Mepiquat chloride				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
				DAR, Belgium 2006 EU Agreed
	ILV	0.05 mg/kg	HPLC-MS/MS	H. Schultz, 2003b Report No. 2003/1001272 DAR, Belgium, 2006 EU agreed
	Confirmatory (if required)	-	-	LC/MS/MS is highly specific method and no confirmation is required.
High oil content	Primary	0.05 mg/kg	HPLC-MS/MS	Kerl W., 2003b, Report No.: 2003/1001371 A. Lehman, C. Mackenroth, 2003 Report No. 2003/1001373 Kerl W., Mackenroth, 2003b, Report No.: 2003/1001372 DAR, Belgium 2006 EU Agreed
	ILV	0.05 mg/kg	HPLC-MS/MS	H. Schultz, 2003b Report No. 2003/1001272 DAR, Belgium, 2006 EU agreed
	Confirmatory (if required)	-	-	LC/MS/MS is highly specific method and no confirmation is required.
High protein/high starch content (dry)	Primary	0.05 mg/kg	HPLC-MS/MS	Kerl W., 2003b, Report No.: 2003/1001371 A. Lehman, C. Mackenroth, 2003 Report No. 2003/1001373 Kerl W., Mackenroth, 2003b, Report No.: 2003/1001372 DAR, Belgium 2006 EU Agreed
	ILV	0.05 mg/kg	HPLC-MS/MS	H. Schultz, 2003b Report No. 2003/1001272 DAR, Belgium, 2006 EU agreed
	Confirmatory (if required)	-	-	LC/MS/MS is highly specific method and no confirmation is required.

Table 5.3-11: Statement on extraction efficiency

	Method for products of plant origin
Required, available from:	U. Rabe, H. Schleuter, 2003 Repor No. 1992/5069

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

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

zRMS:
Presented methods are acceptable.

Table 5.3-12: Validated methods for food and feed of animal origin (if appropriate)

Component of residue definition: The sum of Mepiquat and its salts, expressed as Mepiquat chloride				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Milk	Primary	0.05 mg/kg	IC	U. Schepers, 1990 Report No. 1990/0147; Horton W., Huber R., Schwemmer B., 1977, Report No.: 1977/5025 DAR, Belgium, 2006
	ILV	0.05 mg/kg	IC	D. J. Burkey, D. S. Malinsky, 1992 Report No. 1992/5130 DAR, Belgium, 2006
	Confirmatory (if required)	0.05 mg/kg	GC-NPD	W. Horton, R. Huber, B. Schwemmer, 1977 Report No. 1977/5025 and 1979/5040 DAR, Belgium, 2006
Eggs	Primary	0.05 mg/kg	IC	U. Schepers, 1990 Report No. 1990/0147; Horton W., Huber R., Schwemmer B., 1977, Report No.: 1977/5025 DAR, Belgium, 2006
	ILV	0.05 mg/kg	IC	D. J. Burkey, D. S. Malinsky, 1992 Report No. 1992/5130 DAR, Belgium, 2006
	Confirmatory (if required)	0.05 mg/kg	IC	W. Horton, R. Huber, B. Schwemmer, 1977 Report No. 1977/5025 and 1979/5040 DAR, Belgium, 2006 EU agreed
Muscle	Primary	0.05 mg/kg	IC	U. Schepers, 1990 Report No. 1990/0147; Horton W., Huber R., Schwemmer B., 1977, Report No.: 1977/5025 DAR, Belgium, 2006 EU agreed
	ILV	0.05 mg/kg	IC	D. J. Burkey, D. S. Malinsky, 1992 Report No. 1992/5130 DAR, Belgium, 2006

Component of residue definition: The sum of Mepiquat and its salts, expressed as Mepiquat chloride				
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)	0.05 mg/kg	GC-NPD	W. Horton, R. Huber, B. Schwemmer, 1977 Report No. 1977/5025 and 1979/5040 DAR, Belgium, 2006 EU agreed
Fat	Primary	0.05 mg/kg	IC	U. Schepers, 1990 Report No. 1990/0147; Horton W., Huber R., Schwemmer B., 1977, Report No.: 1977/5025 DAR, Belgium, 2006 EU agreed
	ILV	0.05 mg/kg	IC	D. J. Burkey, D. S. Malinsky, 1992 Report No. 1992/5130 DAR, Belgium, 2006 EU agreed
	Confirmatory (if required)	0.05 mg/kg	GC-NPD	W. Horton, R. Huber, B. Schwemmer, 1977 Report No. 1977/5025 and 1979/5040 DAR, Belgium, 2006 EU agreed
Kidney, liver	Primary	0.05 mg/kg	IC	U. Schepers, 1990 Report No. 1990/0147; Horton W., Huber R., Schwemmer B., 1977, Report No.: 1977/5025 DAR, Belgium, 2006 EU agreed
	ILV	0.05 mg/kg	IC	D. J. Burkey, D. S. Malinsky, 1992 Report No. 1992/5130 DAR, Belgium, 2006 EU agreed
	Confirmatory (if required)	0.05 mg/kg	GC-NPD	W. Horton, R. Huber, B. Schwemmer, 1977 Report No. 1977/5025 and 1979/5040 DAR, Belgium, 2006 EU agreed
	Primary	0.05 mg/kg	LC – MS/MS	H. Schultz, 2005 Report No. 2005/1026539 Addendum to the DAR, UK, 2008 EU Agreed
	ILV	0.05 mg/kg	LC – MS/MS	M. Richter, 2005 Report No. 2005/1026624 Addendum to the DAR, UK, 2008 EU Agreed

Component of residue definition: The sum of Mepiquat and its salts, expressed as Mepiquat chloride				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
	Confirmatory (if required)	-	-	LC/MS/MS is highly specific method and no confirmation 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 5.3-13: Statement on extraction efficiency

	Method for products of animal origin
Required, available from:	-
Not required, because:	Not presented in the DAR/EU review of Mepiquat.

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

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

zRMS:
Presented methods are acceptable.

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

Component of residue definition: The sum of Mepiquat and its salts expressed as Mepiquat 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	C. Grote, 2003a Report No. 2001/1014998 DAR, Belgium, 2006 EU agreed
Confirmatory	0.01 mg/kg	LC-MS/MS	C. Grote, 2003a Report No. 2001/1014998 DAR, Belgium, 2006 EU agreed

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

An overview on the acceptable methods and possible data gaps for analysis of Mepiquat 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 5.3-15: Validated methods for water (if appropriate)

Component of residue definition: The sum of Mepiquat and its salts expressed as Mepiquat 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	IC	C. Grote, 2003c Report No. 2001/1014999 DAR, Belgium, 2006 EU agreed
	ILV	-	-	Not provided during EU review.
	Confirmatory	0.05 µg/L	HPLC-MS/MS	C. Grote, 2003c Report No. 2001/1014999 DAR, Belgium, 2006 EU agreed
Surface water	Primary	0.05 µg/L	IC	C. Grote, 2003c Report No. 2001/1014999 DAR, Belgium, 2006 EU agreed
	Confirmatory	0.05 µg/L	HPLC-MS/MS	C. Grote, 2003c Report No. 2001/1014999 DAR, Belgium, 2006 EU agreed

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

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

zRMS:
Presented methods are acceptable.

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

Component of residue definition: The sum of Mepiquat and its salts expressed as Mepiquat chloride			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	0.016 µg/m^3	IC	W. Zengmeister, 2002, 2003 Report No. 2002/1000200 and No. 2003/1005457 DAR, Belgium, 2006 EU Agreed
Confirmatory	0.016 µg/m^3	HPLC-MS/MS	W. Zengmeister, 2002, 2003 Report No. 2002/1000200 and No. 2003/1005457 DAR, Belgium, 2006 EU Agreed

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

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

Not required as Mepiquat chloride is not classified as toxic or highly toxic (EFSA Scientific Report (2008) 146, 1-73)

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.

5.3.3.8 Other studies/ information

No additional data provided.

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	S. Aversa	2018	Physical-chemical characterization and accelerated storage stability (2 weeks/54±2°C) of test item Chlormequat 345 g/L + Mepiquat 115 g/L SL. Report No. BT022/18 GLP Unpublished	N	Sharda Cropchem Limited
KCP 5.1.1-2	K.S. Kishora	2023	Accelerated storage stability test of Chlormequat 34.5% + Mepiquat 11.5% SL, Report No.: AG-G0028 EUROFINS ADVINUS AGROSCIENCES SERVICES 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 Fertico Sp z o.o. GLP Unpublished	N	Sharda Cropchem Limited
KCP 5.1.3	K. Zagibajło	2022	Validation of the method for determination of mepiquat chloride in cotton and wheat by liquid chromatography Report No.: 21/FSL/07/V Food Safety Laboratory The National Institute of Horticultural Research GLP	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
			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

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 od 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	
0.2			20 from 10 µg/mL	470
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) pf 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 mtehod, 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) quantifer 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) quantifer 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) quantifer 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 - 1.0 µg/ml Corresponding calibration range in mass ratio units for the sample: 0.0001 mg/kg – 1.0 mg/kg	Accepted calibration range in concentration units: 0.0001 µg/ml - 1.0 µg/ml Corresponding calibration range in mass ratio units for the sample: 0.0001 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)

No new or additional studies have been submitted

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

A 2.1.2.7 Other Studies/ Information

No new or additional studies have been submitted

A 2.2 Analytical methods for Mepiquat chloride

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

No new or additional studies have been submitted

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

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

~~No new or additional studies have been submitted~~

A 2.2.2.1.1 Analytical method 1

A 2.2.2.1.1.1 Method validation

Comments of zRMS:	Method is acceptable

Reference:	KCP 5.1.3
Report	Validation of method for determination of Mepiquat chloride in cotton and wheat by Liquid Chromatography, K. Zagibajło, 2022, Report No.: 21/FSL/07/V
Guideline(s):	Yes EU Guidance Document SANTE/2020/12830, Rev.1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Preparation of samples for validation

Preparation of Sample Matrix

A. Portion of dry ice was added to a homogenizer apparatus (Robot Coupe). Subsequent appropriate amount of sample was added to the apparatus in small portions. Sample was blended after each addition until a homogeneous mixture was obtained.

B. The samples were stored at ≤-18°C in the dark until analysis.

Sample extraction

A. 5.00 g ± 0.05 g of homogenized wheat (plants, grain) matrices and 2.00 g ± 0.02 g of homogenized wheat (straw) matrix were weighed into a 50-mL Teflon® centrifuge tube. Sample weight was recorded.

B. 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 in a vortex mixer for 1 min.

C. In the case of wheat (straw, grain), 9 mL of water and 0.05 mL of internal standard of Mepiquat Iodide D3 (10 µg/mL) were added. Then, 10 mL of acidified methanol and extra 100 µL formic acid were added. The tube was closed and shaken. Then, 1 mL 10% aqueous EDTA solution was added. The tube was closed and shaken vigorously by a Hand Motion Shaker for 15 min.

D. In the case of wheat (plants), 10 mL of water and 0.05 ml of the internal standard of Mepiquat Iodide D3 (10 µg/mL) were added. Then, 10 mL of acidified methanol was added. The tube was closed and shaken vigorously by a Hand Motion Shaker for 15 min.

E. The sample was centrifuged using laboratory centrifuge for 10 min. at 11000 rpm at -10°C.

Sample dilution

A. An aliquot of 0.5 mL of the extract was transferred to Eppendorf tube and subsequently diluted with 0.5 mL of acetonitrile.

B. Content was mix gently and filtered through the 0.22 µm. Teflon filter direct into amber HPLC vial.

Final determination

A. Final determination was performed using LC-MS/MS.

Chromatographic Conditions

HPLC system	Series 1200/1260 HPLC (Agilent Technologies) Degasser (G1322A) Binary Pump (G1312B) High Performance Autosampler (G1367E) Thermostatted Column Compartment (G1316A)
Pre-Column	Agilent 1290 Infinity In-Line Filter (PN: 5067-4368) with 0.3µm frit ring installed (PN: 5023-0271)
Column	ZORBAX RRHD HILIC PLUS (P.N: 959758-901)

			2.1×100 mm, 1.8 µm	
Column oven temperature			40°C	
Injection volume			2µL	
Mobile phase			Eluent A: 20mM ammonium formate with 0.36% (v/v) Formic Acid in Water Eluent B: Acetonitrile	
Gradient	Time [min]	% Eluent A	% Eluent B	Flow [mL/min]
	0	3	97	0.3
	0.3	3	97	0.3
	4	30	70	0.3
	5	60	40	0.3
	7	60	40	0.3
	7.10	3	97	0.3
	15.0	3	97	0.3
Total analysis time	Stop time	15 min	Post time	3 min
Retention time (approx.)	6.5 min for Mepiquat		6.5 min for Mepiquat D3	

Parameters od MS-Triple Quadrupole Acquisition Method

Analyte	Ion Transitions	Collision Energy [V]	Cell Accelerator Voltage	Fragmentor
Mepiquat	114.2 → 98.2	28	4	88
	114.2 → 58.2	28		
Mepiquat D3	117.3 → 101.2	28	4	120
	117.3 → 61.2	28		

Linearity

The correlation between the injected concentration ratios of analytes/internal standard and their detector response (peak area ratios) for Mepiquat expressed as Mepiquat Chloride was demonstrated to be linear by single determination of matrix-matched calibration standards at ten concentration levels ranging from 0.000125 µg/mL to 1.25 µg/mL for wheat (straw) and at ten concentrations levels ranging from 0.00025 µg/mL to 2.5 µg/mL for wheat (plants, grain). These ranges correspond from 0.0025 mg/kg to 25 mg/kg for Mepiquat Chloride (wheat straw) and from 0.002 mg/kg to 20 mg/kg for Mepiquat Chloride (wheat plants, grain) thus covering 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 Mepiquat expressed as Mepiquat Chloride for wheat (plants, straw, grain) were linear with the coefficients of correlation (R) greater than 0.99. Linear regression was performed with 1/x weighting.

Accuracy and Precision

Accuracy was determined by fortification of control samples with known amount of the reference item and subsequent determination of the recoveries when applying the extraction procedure. Precision was determined by repeatability (relative standard deviation – RSD).

The mean recovery values at the fortification levels of 0.01 mg/kg, 0.1 mg/kg for both ion mass transitions were all in the range 70 – 110 % and thus comply with the standard acceptance criteria of the guidance document SANTE/2020/12830, Rev.1. All precision values at the fortification levels of 0.01 mg/kg, 0.1 mg/kg for both ion mass transitions were < 20%.

Results and discussions

The method was shown to be highly selective, as it includes two parent-daughter ion mass transitions for Mepiquat expressed as Mepiquat Chloride and it yields accurate and repeatable results. The limit of quantification (LOQ) was established at 0.01 mg/kg for Mepiquat Chloride, interfering signal in control specimens were negligible, and thus the limit of detection (LOD) is 0.0025 mg/kg for Mepiquat Chloride wheat (straw) and 0.002 mg/kg for Mepiquat Chloride wheat (plant, grain).
It is concluded that method fulfils the requirements as defined in EC Guidance documents on residue analytical methods SANTE/2020/12830, Rev.1 and is, applicable as enforcement and data generation method for determination of Mepiquat Chloride in wheat (plants, seeds, grain).

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

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
Wheat whole plant	Mepiquat chloride	0.01	100	2.5	Mepiquat chloride (114.2 → 98.2) quantifer ion
		0.1	91	5.7	
		0.01	100	1.8	Mepiquat chloride (114.2 → 58.2) quantifer ion
		0.1	91	5.8	
Wheat straw		0.01	106	2.2	Mepiquat chloride (114.2 → 98.2) quantifer ion
		0.1	93	1.9	
		0.01	107	1.9	Mepiquat chloride (114.2 → 58.2) quantifer ion
		0.1	94	1.5	
Wheat grain		0.01	100	2.9	Mepiquat chloride (114.2 → 98.2) quantifer ion
		0.1	98	4.7	
		0.01	100	2.5	Mepiquat chloride (114.2 → 58.2) quantifer ion
		0.1	99	5.0	

Table A 4: Characteristics for the analytical method used for validation of Mepiquat chloride residues in winter wheat whole plant, grain and straw

	Mepiquat chloride first transition (114.2 → 98.2)	Mepiquat chloride second transition (114.2 → 58.2)
Specificity	No significant interference above 30 % of LOQ was detected in any of the reagent blank or control specimen extracts for wheat (plants, straw, grain) matrices, so that a highly level of selectivity was demonstrated and an additional confirmatory method is not necessary.	
Calibration (type, number of data points)	**Wheat whole plant:** y = 3.76759x – 0.035652 R^2 = 0.9997 10 points **Wheat grain:** y = 3.604171x – 0.020693 R^2 = 0.9999	**Wheat whole plant:** y = 3.740812x – 0.031393 R^2 = 0.9998 10 points **Wheat grain:** y = 3.551370x – 0.018326 R^2 = 0.9999

	Mepiquat chloride first transition (114.2 → 98.2)	**Mepiquat chloride second transition (114.2 → 58.2)**
	10 points **Wheat straw:** y = 3.722056x – 0.018746 R^2 = 0.9999 10 points	10 points **Wheat straw:** y = 3.656206x – 0.016878 R^2 = 0.9999 10 points
Calibration range	Accepted calibration range in concentration units: 0.000125 µg/ml - 1.25 µg/ml (straw) 0.00025 µg/ml – 2.5 µg/ml (plants and grain) Corresponding calibration range in mass ratio units for the sample: 0.0025 mg/kg – 25.0 mg/kg (straw) 0.002 mg/kg – 20.0 mg/kg (plants and grain)	Accepted calibration range in concentration units: 0.000125 µg/ml - 1.25 µg/ml (straw) 0.00025 µg/ml – 2.5 µg/ml (plants and grain) Corresponding calibration range in mass ratio units for the sample: 0.0025 mg/kg – 25.0 mg/kg (straw) 0.002 mg/kg – 20.0 mg/kg (plants and grain)
Assessment of matrix effects is presented	yes	yes
Limit of determination/quantification	LOD= 0.0025 mg/kg (straw) LOD = 0.002 mg/kg (plant and grain) LOQ= 0.01 mg/kg	LOD= 0.0025 mg/kg (straw) LOD = 0.002 mg/kg (plant and grain) LOQ= 0.01 mg/kg

Conclusion

According to SANTE/2020/12830, Rev. 1, the method for determination of Mepiquat chloride by Liquid Chromatography (LC-MS/MS) for wheat whole plant, grain and straw was successfully validated and is suitable.

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

A 2.2.2.7 Other Studies/ Information

No new or additional studies have been submitted