European Commission

Combined Draft Renewal Assessment Report prepared according to Regulation (EC) N° 1107/2009
and
Proposal for Harmonised Classification and Labelling (CLH Report) according to Regulation (EC) N° 1272/2008

HEPTAMALOXYLOGLUCAN

Volume 3 – B.5 (AS)

Rapporteur Member State: France
Co-Rapporteur Member State: Spain

Version History

When	What
2020-09	Initial RAR

Introduction
The applicant Elicityl prepared a draft renewal assessment report to support the renewal of inclusion of the active substance Heptamaloxyloglucan.

Heptamaloxyloglucan was included in Annex I of Directive 91/414/EEC under Commission Directive 2010/14/EU, which entered into force on 01 June 2010. According to Regulation (EU) No 540/2011, heptamaloxyloglucan is deemed to have been approved under Regulation (EC) No 1107/2009. An extension of approval has been granted by Regulation (EU) 2017/1527 until 31/05/2021.

The Annex I Inclusion Directive for Heptamaloxyloglucan (2010/14/EU) provides specific provisions under Part B which need to be considered by the applicant in the preparation of their submission and by the MS prior to granting an authorisation:
For the implementation of the uniform principles of Annex VI, the conclusions of the review report on heptamaloxyloglucan (SANCO/10502/09 – final, 27/11/2009), and in particular Appendices I and II thereof, as finalised in the Standing Committee on the Food Chain and Animal Health on 27/11/2009 shall be taken into account.

Heptamaloxyloglucan is included in AIR 4 program (SANTE-2016-10616–rev 9, June 2018). The rapporteur Member State is France and the co-rapporteur Member State is Spain (Commission Implementing Regulation (EU) 2016/183 of 11 February 2016).

The draft renewal assessment report will be prepared according to Commission Regulation No.844/2012.

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B.5. METHODS OF ANALYSIS

B.5.1. METHODS USED FOR THE GENERATION OF PRE-AUTHORISATION DATA

B.5.1.1. Methods for the analysis of the active substance as manufactured

B.5.1.1.1 Determination of the pure active substance in the active substance as manufactured

The analytical method for the determination of the active substance in the technical material EL101GV has been validated by two studies: Ricau 2005 and Groult 2006.

Report: Ricau H. 2005
Validation of the analytical method on the technical test item Heptamaloxyloglucan
Defitraces, 69126 Brindas, France
Unpublished
Study report 05-905012-007
Guidelines: EC Principles of Good Laboratory Practice
GLP: Yes

Reference: KCA 4.1.1/01
Report: Ricau H. 2007
Validation of the analytical method on the technical test item Heptamaloxyloglucan
Defitraces, 69126 Brindas, France
Unpublished
Addendum of April 2nd, 2007 to the Study report No.05-905012-007
Guidelines: EC Principles of Good Laboratory Practice
GLP: Yes

Report: Groult R. 2006
Validation of the analytical method for the determination of the active substance in technical material
CIT, 27005 Evreux, France
Unpublished
Study report 30987 final report + Amendment
Guidelines: SANCO 3030/99 rev. 4.
GLP: No, but in the spirit of Good Laboratory Practice

Principle of the method
The content of the active substance heptamaloxyloglucan in the technical active substance is determined by dissolving samples in water followed by analysis with HPAEC-PAD (High Performance Anion Exchange Chromatography - Pulsed Amperometric Detection). Heptamaloxyloglucan content is determined by comparing peak area with that of a standard of the pure substance.

Material and conditions
The analytical conditions of this method are detailed in Table below:

Table CA 4.1.1-1: Analytical conditions for phosphonate ions method

HPAEC-PAD parameters and materials			
Instrument	GP50 Gradient pump - ED50A Electrochemical detector with amperometric cell – Injector AS50 (Dionex)		
Column	Carbopac PA 100, 250 x 4 mm, 8.5 µm (Carbopac PA 100 Guard, 50 x 4 mm)		
Flow rate	1.0 mL/min		
Injection volume	20 µL		
Mobile phase	A: NaOH 100 mM B: NaOH 100 mM / sodium acetate 500 mM		
Gradient	Time (min)	A (% v/v)	B (% v/v)
	0	100	0
	20	100	0
	50	87.5	12.5
	50.1	0	100
	60	0	100
	60.1	100	0
	75	100	0
Retention time	Heptamaloxyloglucan: about 35 min		
Run time	75 min		

Standards

Chemical name	Chemical structure	Batch No.	Purity
Heptamaloxyloglucan	$C_{40}H_{70}O_{33}$	ALD0405	993.7 g/kg

Preparation of technical sample solution
Technical Heptamaloxyloglucan sample: 11.6 mg dissolved in 50 mL of water; then diluted 5 mL in 20 mL with water.

Preparation of standard solutions
Stock standard solution: 10 mg of Heptamaloxyloglucan standard dissolved in 50 mL of ultra-pure water. The stock solution is diluted 4 times with water (reference solution).
Other calibration standards are prepared using 7.5 mg and 12.5 mg of Heptamaloxyloglucan standard dissolved in 200 mL water.

Quantification:
Based on mean response factor of bracketing reference solutions.
Summary of the validation of the methods:

Specificity:
Specificity was studied by analysis of the blank solvent (analytical solvent = water), the heptamaloxyloglucan reference solution and the technical heptamaloxyloglucan test solution. The specificity was assessed by checking for any interference in the chromatograms.
Heptamaloxyloglucan was not detected in blank solvent. In the technical heptamaloxyloglucan, several small impurity peaks were observed and a main peak at the retention time of Heptamaloxyloglucan; none of those impurity peaks interferes with heptamaloxyloglucan peak.
Therefore, the analytical method showed a good specificity for analysis of heptamaloxyloglucan in technical grade.

Linearity:
Ricau H. 2005: To define the linearity of the detector answer for heptamaloxyloglucan, three concentrations taken between 75% and 125% (from 0.037 mg/mL to 0.062 mg/mL) of the heptamaloxyloglucan reference item were analysed twice.
Groult R. 2006: To define the linearity of the detector answer for heptamaloxyloglucan, five concentrations taken between 20% and 200% (from 10 mg/L to 100 mg/L) of the heptamaloxyloglucan reference item were analysed.
The response of the detector during the analysis of heptamaloxyloglucan was linear within the both calibration range. The correlation coefficients r were > 0.99 showing a good linearity.

Accuracy: Accuracy is not required for technical active substance.
Ricau H. 2005: Accuracy was determined by analysis twice two test item samples. The concentrations found during the accuracy study *versus* the concentration found during the precision study were compared.

Content (% m/m)	Number of samples (n)	Recovery Range (%)	Mean Recovery (%)	RSD (%)
87	4	99 – 99.6	99.4	1.36

The accuracy results of heptamaloxyloglucan were in conformity with the Guidelines requirements for formulations containing an active substance higher than 10% w/w. Indeed, the mean recovery results should be in the range 98% - 102% and it was experimentally equal to 99.4%.
Mean recovery rate = 99.4% (n = 4).

Precision:
Ricau H. 2005: The precision was determined by analyzing twice 5 independent preparations of test item using the same equipment. Then, the average value of the content, the standard deviation and the Relative Standard Deviation (R.S.D.) were calculated.
The concentration of heptamaloxyloglucan in the test item was equal to 87.0% w/w or 870 g/kg.
The precision was acceptable as the R.S.D. was lower than the result of the modified Horwitz equation: 1.36% < 1.37% (C = 0.87).
Groult R. 2006: The precision was determined by analysing 6 independent preparations of test item at one concentration level (50 mg/L) using the same equipment. Then, the standard deviation and the Relative Standard Deviation (R.S.D.) were calculated with the peak area. The precision was acceptable as the R.S.D. (on peak area) was lower than the result of the modified Horwitz equation: 0.8% < 1.39% (C = 0.782).

LOD/LOQ determination

LOQ is not required for active substance determination in the technical material, however as the same method was used for the determination of significant impurities in the technical material, this information has been required by RMS.

Ricau H. 2007: The method of definition of the Limit of Quantification and the Limit of Detection is based on visual evaluation by establishing the minimum level at which the analyte can be quantified or detected.
The Limit of Quantification of heptamaloxyloglucan with this method is defined as 10 times the noise signal.
The Limit of Detection of heptamaloxyloglucan with this method is defined as 3 times the noise signal.
LOQ = 32 µg/L (0.07% of nominal concentration of heptamaloxyloglucan 49 mg/L – REF01).
LOD = 10 µg/L (0.02% of nominal concentration of heptamaloxyloglucan 49 mg/L – REF01).

This is not the definition of LOQ according to SANCO/3030/99 rev.4, however as LOQ is not a requirement for the determination of active substance in the technical material, it is considered acceptable not to have any validated LOQ for this analytical method.

Conclusions:
The method for the determination of heptamaloxyloglucan in the technical active substance was fully validated. The linearity, specificity and repeatability were checked and found acceptable. According to the literature (Analusis magazine, 2000, 2, n°1; Dionex technical note 20), HPAEC-PAD (High Performance Anion Exchange Chromatography - Pulsed Amperometric Detection) can be considered highly specific for the determination of carbohydrates. The RMS proposal is to considered this study acceptable. Therefore, no confirmatory method is required.

CIPAC Methods:
No CIPAC methods exist for the analysis of heptamaloxyloglucan in either technical or formulated material.

B.5.1.1.2 Methods for the determination of significant and/or relevant impurities and additives in the active substance as manufactured

The technical active substance contains no additives, therefore no analytical method is required.

The technical active substance may contain a mycotoxin, patulin, considering its manufacturing process. It is considered a relevant impurity and its maximum content in the technical substance is 50 µg/kg. The analytical methods for the determination of patulin in the technical active substance and the starting material from which it is manufactured were derived from the international analytical method ISO 8128-1:1993 (Apple juice, apple juice concentrates and drinks containing apple juice – Determination of patulin content – Part 1: Method using high performance liquid chromatography). Further information is available is Vol. 4.

The methods of determination of significant impurities is described in Vol. 4.

B.5.1.2. Methods for risk assessment

Heptamaloxyloglucan is a branched xyloglucan molecule extracted from apples without additive nor chemical product. It is composed of 7 hexose residues: glucopyranosyl, fucopyranosyl, xylopyranosyl and galactopyranosyl. The terminal glucose residue is reduced as a glucitol residue. All these hexose and hexol residues are natural components of the apple and of other dicotyledone plants, where they are major constituents of cellulose and hemicellulose molecules, which are themselves the principal components of cell walls.

It is reasonable to consider that these different natural substances are rapidly degraded by soil macro- and micro-organisms as a natural component of humus. This degradation leads to simple components, also present in the natural environment.

Therefore, analytical methods for risk assessment could not be validated properly in any case, especially for this natural substance for which a natural background would have to be taken into account.

B.5.2. METHODS FOR POST-APPROVAL CONTROL AND MONITORING PURPOSES

Heptamaloxyloglucan is a branched xyloglucan molecule extracted from apples without additive nor chemical product. It is composed of 7 hexose residues: glucopyranosyl, fucopyranosyl, xylopyranosyl and galactopyranosyl. The terminal glucose residue is reduced as a glucitol residue. All these hexose and hexol residues are natural components of the apple and of other dicotyledone plants, where they are major constituents of cellulose and hemicellulose molecules, which are themselves the principal components of cell walls.
It is reasonable to consider that these different natural substances are rapidly degraded by soil macro- and micro-organisms as a natural component of humus. This degradation leads to simple components also present in the natural environment.

Thus, as heptamaloxyloglucan is a naturally occurring non-toxic active substance, and as no MRL are set in plants, no analytical methods are required in plants, soil, water and air, according to guideline SANCO 825/00 rev. 9.1. No methods are required for residues in animal and human body fluids and tissues, as the active substance heptamaloxyloglucan is naturally present in plants, and that there is no definition of residues in these matrices.

Also, for plants, heptamaloxyloglucan is applied on vines at stages BBCH 07-16 when no edible part of the crop is formed and the compound is not systemic.

In addition, as no MRL are set in plant and animal matrices, the extraction efficiency of analytical methods shall not be evaluated.

For the different compartments of the environment calculated PECs are below the trigger values for LOQs:
Soil: PECs < 0.01 mg/kg
Water: PECsw < 0.1 µg/L

Therefore analytical methods for monitoring purposes could in any case not be validated properly, especially for this natural substance for which a natural background would have to be taken into account.

B.5.3. REFERENCES RELIED ON

Data Point	**Author(s)**	**Year**	**Title** **Report No.** **Document No.** **Source (where different from company)** **GLP/ Officially recognised testing facilities[2,3]** **Published or not**	**Vertebrate study Y/N**	**Data protection claimed Y/N**	**Justification if data protection is claimed**	**Owner**	**Previously used[1]** **Y/N** **If yes, for which data point?**
KCA 4.1/01	Ricau, H.	2006	Validation of the analytical method on the technical test item Heptamaloxyloglucan Defitraces, 69126 Brindas, France Study report 05-905012-007 Yes unpublished	N	N	-	Elicityl	Y (DAR, 2007)
KCA 4.1/02	Groud, R.	2005	Validation of the analytical method for the determination of the active substance in technical material CIT, 27005 Evreux, France Study report 30987 No, but study conducted in the spirit of GLP unpublished	N	N	-	Elicityl	Y (DAR, 2007)
KCA 4.1/02	Groud, R	2006	Validation of the analytical method for the determination of the active substance in technical material Amendment n°1	N	N	-	Elicityl	Y (DAR, 2007)

			CIT, 27005 Evreux, France Study report 30987 No, but study conducted in the spirit of GLP unpublished					
B.5.1.1.2	Anonymous	1993	ISO 8128-1:1993 Apple juice, apple juice concentrates and drinks containing apple juice — Determination of patulin content — Part 1: Method using high-performance liquid chromatography NA Published	N	N	-	NA	Y (Addendum of DAR, 2009)

[1] In order to facilitate the compilation of the final list of the tests and studies relied upon and the corresponding data protection, indicate whether the study was used in the previous DAR/RAR or, when the information is available, whether the study was already submitted in the framework of national authorisations.
[2] See Art.3 of Annex of Regulation No 283/2013 and 284/2013
[3] The RMS shall check that the GLP statement has been properly signed in the study report, that the study results are properly reported in accordance with GLP standards and following the relevant guidance by OECD on the review of the GLP status of non-clinical safety data (currently under development).