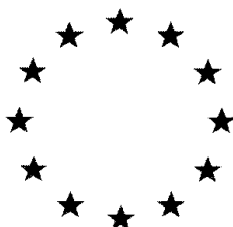


# **European Commission**



**VOLUME 3 – Annex B (AS)**

**Laminarin**

**B.5 Methods of analysis**

**Rapporteur Member State: The Netherlands**

**April 2016**

**Draft Re-Assessment Report and Proposed decision of the Netherlands  
prepared in the context of the possible renewal of laminarin under Regulation  
(EC) 1107/2009**

## Version history page

Date	Version history
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**TABLE OF CONTENTS – VOLUME 3 B.5**

B.5	Methods of analysis .....	4
B.5.1	Methods used for the generation of pre-approval data .....	4
B.5.2	Methods for post-approval control and monitoring purposes .....	7
B.5.3	References relied on .....	8

## **B.5 Methods of analysis**

### **B.5.1 Methods used for the generation of pre-approval data**

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

**(a) Determination of the pure active substance in the active substance as manufactured and specified in the dossier submitted in support of approval under Regulation (EC) No 1107/2009**

##### **New data**

Please refer to Volume 4 (confidential data). Please note that a batch analysis is still ongoing and is not yet included in the draft RAR.

##### **Existing data (volume 3, B5 of the DAR, May 2003)**

- Method ME-0211 A – Laminarin : measuring by ionic chromatography, amperometric detection (Duval, 2001)

##### GLP :

No GLP-compliance stated

##### Principle of the method :

The purity of technical Laminarin is determined by High Performance Ionic Chromatography (HPIC) of the glucose produced after total acid hydrolysis.

An equal volume of sample solution (1 mg/mL technical Laminarin in water) and of 1 M HCl is mixed and heated at 100°C for 3 h. After cooling at room temperature, the hydrolysed solution is diluted 1/20 with phosphate buffer (pH 6.86) and the glucose obtained is determined by HPIC (PA1 + PA1 guard) using a Dionex DX300 system equipped with a GP40 quaternary gradient pump and an ED40 electrochemical detector in integrated amperometry mode. Quantification by external standardization (use of Laminarin standard (Sigma) for correction).

##### Findings :

*Specificity – interferences* demonstrated by confirmatory method (HPLC)

:  
- HPIC-result : 86.96%  
- HPLC-result : 86.76%

*Linearity :* linearity range : 0.1 – 30 mg/L (n = 10)  
 $r^2 = 0.9997$

y (area) = 7836.2x – 963.17

*Accuracy* recovery = 96.37%

:

*Repeatability* RSD = 1.30% (n = 3)

:

*Limit of quantification (LOQ) :* 1.5 g/kg

##### Conclusions :

HPIC-method ME-0211 A is suitable for the determination of Laminarin in technical Laminarin. This method was used for the 5-batch analysis study (ME-0211 A is identical to ME-0207 B, as far as Laminarin determination is concerned) and it will be used for the final purity control of technical Laminarin at the end of the industrial process.

- SEP/00-067 : Analysis of Laminarin in water samples after a hydrosolubility test (CEE A6) (Quintelas, 2001a)

##### GLP :

GLP-compliance stated

##### Principle of the method :

Laminarin is determined after acid hydrolysis of the polysaccharide into glucose. The glucose is quantified as its trimethylsilyl derivative by GC using FID. Quantification by external standardization; Laminarin is expressed as its monomeric unit glucose.

Equal volumes of 10x diluted Laminarin sample solution and of 1M HCl are mixed and heated at 100°C for 3 h. After cooling at room temperature, the hydrolysed solution is diluted 10x with water,

after which 2 mL of this solution is mixed with an equal volume of L-rhamnose solution (5000 mg/L) and circa 25 mL of acetonitrile. After drying, the D-glucose and L-rhamnose mixture is dissolved in 10 mL of dry pyridine and derivatised using Sil A solution (made with 18 mL of dry pyridine, 2 mL of trimethylchlorosilane and 6 mL of hexamethyldisilazane). The trimethylsilyl derivatives of D-glucose and L-rhamnose are analysed as their anomeric forms by GC (SPB-35; 0.5  $\mu$ m) using FID; the quantification of trimethylsilyl L-rhamnose being used as an internal reference to follow the reproducibility of the derivatisation step.

#### Findings :

*Specificity – interferences* chromatograms of reference solution, spiked samples and samples are shown

*Linearity :* linearity range : 0.1 – 200 mg/L (n = 13)

$$r^2 = 0.9998$$

$$C = 4.758 \times 10^{-5} S (\text{area}) - 0.2923$$

<i>Accuracy</i>	<u>Spiking concentration (g/L)</u>	<u>Recovery (%)</u>
:	0.1	85
	1.0	96
	100	99

*Repeatability* RSD = 2.3% (n = 5)

:

*Limit of quantification (LOQ) :* 0.1 g/L of Laminarin expressed as glucose

#### Conclusions :

The GC-FID method, which has been used in several physicochemical and ecotoxicological studies on the a.s. and the preparation, has been validated for that purpose.

- FC98005 : Determination of pure Laminarin – Global method (Cruz, 1998)

- Validation of the method of the dosage of pure Laminarin : Global method – GLP study (Trebert, 2002)

#### GLP :

GLP-compliance stated for the validation study

Principle of the method : ME-0248 (Trad)

The purity of technical Laminarin is determined by High Performance Ionic Chromatography (HPIC) with amperometric detection, by direct injection (i.e. without preliminary hydrolysis).

The technical Laminarin sample is diluted with MilliQ-water, after which Laminarin is determined by HPIC (PA1 column + PA1 guard column) using a Dionex system equipped with a GP40 quaternary gradient pump and an ED40 electrochemical detector in integrated amperometry mode. Quantification by external standardization (reference item : Laminarin (Sigma)).

#### Findings :

*Specificity – interferences* typical chromatograms of blank, Laminarin standard, Laminarin sample and fortified Laminarin sample are shown, demonstrating specificity and absence of interferences

*Linearity :* linearity range : 10 – 50 mg/L (n = 5)

$$r^2 = 0.9996$$

$$y (\text{area}) = 2953325 x - 2176257$$

*Accuracy* mean recovery = 99.99% (n = 3; RSD = 0.2%)

:

*Repeatability* RSD = 1.27% (n = 5; mean content = 95.92%)

:

*Limit of quantification (LOQ) :* 10%

#### Conclusions :

Global HPIC-method ME-0248 (Trad) is suitable for determination of Laminarin content in technical Laminarin. It will be used to follow the purity of technical Laminarin during the industrial process, from the stage of extraction to the last stage of purification.

**(b) Determination of significant and relevant impurities and additives (such as stabilisers) in the active substance as manufactured****New data**

Please refer to volume 4 (confidential information).

**Existing data (volume 3, B5 of the DAR, May 2003)**

Methods for the determination of significant impurities 1-8 (content  $\geq 1$  g/kg) in technical Laminarin are described in Annex C, point C.1.2.4.1. Validation data for these methods are summarized in Table B.5.1.2-1.

None of the impurities present are of toxicological, ecotoxicological or environmental concern. There are no additives in technical Laminarin.

**Conclusions**

The submitted methods are suitable for determination of significant impurities (content  $\geq 1$  g/kg) in technical Laminarin.

**Table B.5.1.2-1 : Validation of methods for determination of significant impurities**

<b>Impurity N° (Method N°)</b>	<b>Specificity</b>	<b>Linearity*</b>	<b>Repeatability (% RSD)</b>	<b>Accuracy (% recovery)</b>	<b>LOQ (mg/kg)</b>
1 (ME-0207 B)	demonstrated (confirmation by HPLC)	0.1-10 mg/L (n = 6) $r^2 = 0.9981$ $y = 243.7x + 20.5$	5.28 (n=4)	99.16	13.3 g/kg
2 (ME-0204 B)	demonstrated (confirmation by ICP)	2-10 mg/L (n = 5) $r^2 = 0.9999$ $y = -0.00040x^2 + 0.01708x + 0.00018$	1.05 (n = 5)	107.5	0.44
3 (ME-0204 B)	demonstrated (confirmation by ICP)	1-5 mg/L (n = 5) $r^2 = 0.9996$ $y = -0.0014x^2 + 0.0426x + 0.0013$	1.18 (n = 5)	98.0	1.20
4 (ME-0204 B)	demonstrated (confirmation by ICP)	2-10 mg/L (n = 5) $r^2 = 0.9987$ $y = 5.10^{-5}x^2 + 0.0097x - 0.0011$	3.88 (n = 5)	82.6	12.0
5 (ME-0204 B)	demonstrated (confirmation by ICP)	2-6 mg/L (n = 3) $r^2 = 0.9993$ $y = -0.0017x^2 + 0.0356x + 0.0007$	1.00 (n = 5)	105.3	1.20
6 (ME-0201 A)	internationally accepted method	not relevant for this type of method	1.91 (n = 5)	95.3	0.0095 g/kg
7 (ME-0205 B)	demonstrated (confirmation by ICP)	0.5-20 mg/L (n = 6) $r^2 = 0.9990$ $y = 304.7670x - 93.9857$	3.77 (n = 5)	99.8	100
8 (ME-0202 B)	internationally accepted method	not relevant for this type of method	1.08 (n = 5)	87.4	0.1690 g/kg

\* for impurities 2-5 : calibration line equation is quadratic

#### **B.5.1.2 Methods for risk assessment**

##### **(a) Methods In soil, water, sediment, air and any additional matrices used in support of environmental fate studies**

No study has been conducted on the behaviour of Laminarin in soil or sediment (see MCA Section 7), therefore analytical methods are not necessary for these matrices.

For the determination of the residues in water, the polymeric nature of the material makes the determination of low levels extremely difficult: during the 2001 studies on the effect of the substance on aquatic organisms, the method specially developed for this purpose hardly reached the level of 100 mg/L.

An analytical method is under development for the study on the effect of the substance on Lemna (see KCA 8.2.7). The analytical methodology used for this study will need to be summarised in this section.

Due to the very low volatility of the active substance, no method has been developed either for the determination of the residues in the air.

##### **(b) Methods in soil, water and any additional matrices used in support of efficacy studies**

No analytical method are deemed necessary to support efficacy studies for this dossier.

##### **(c) Methods in feed, body fluids and tissues, air and any additional matrices used in support of toxicological studies**

The substance being not classified as toxic, no method has been developed for determining the residues in these matrices.

##### **(d) Methods in body fluids, air and any additional matrices used in support of operator, worker, resident and bystander exposure studies**

No analytical method are deemed necessary to support operator, worker, resident and bystander exposure studies as none have been conducted for this dossier.

##### **(e) Methods in or on plants, plant products, processed food commodities, food of plant and animal origin, feed and any additional matrices used in support of residues studies**

Since Laminarin is exempted from the requirement of residues data, no analytical method has been specifically designed for these matrices.

##### **(f) Methods in soil, water, sediment, feed and any additional matrices used in support of ecotoxicology studies**

Please see Point (a) above.

##### **(g) Methods in water, buffer solutions, organic solvents and any additional matrices resulting from the physical and chemical properties tests**

No other methods have been conducted for the physical and chemical properties tests.

#### **B.5.2 Methods for post-approval control and monitoring purposes**

In the original DAR (May 2003), no residue definition was proposed. The substance is not classified as dangerous and had an MRL exemption. Breakdown products consist of oligosaccharides and glucose. Monitoring of the substance is not considered necessary.

**B.5.3 References relied on****Existing data**

<b>Point / Reference number</b>	<b>Author(s)</b>	<b>Year</b>	<b>Title Testing facility, Report n°, GLP or GEP Status published or not</b>	<b>Data Protection Claimed Y/N</b>	<b>Owner</b>
IIA 4.1/01	DUVAL P.	2001	Laminarin : measuring by ionic chromatography, amperometric detector SGS Laboratoire Crépin - Study N° ME-0211A Non-GLP, unpublished	Y	GOË
IIA 4.1/02 (location : IIA 2.6/01)	QUINTELAS G.	2001a	Analysis of Laminarin in water samples after a hydrosolubility test (EEC A6) SEPC – Study N°00-907005-008 GLP, unpublished	Y	GOË
IIA 4.1/03	CRUZ F.	1998	Determination of pure Laminarin : global method Laboratoires GOËMAR S.A. Study N° FC98005 Non-GLP, unpublished	Y	GOË
IIA 4.1/03d	TREBERT R.	2002	Validation of the method of the dosage of pure Laminarin : Global method – GLP study SGS Laboratoire Crépin – Report N° BPL 200107/1309 – rev.2 GLP, unpublished	Y	GOË
IIA 4.1/04	PORTAIL A.	2000a	Confidential information : <i>Please refer to Document J.</i> SGS Laboratoire Crépin -Study N° ME-0207B Non-GLP, unpublished	Y	GOË
IIA 4.1/05	DELACOURT H.M.	2000	Confidential information : <i>Please refer to Document J.</i> SGS Laboratoire Crépin -Study N° ME-0204B Non-GLP, unpublished	Y	GOË
IIA 4.1/06	TREBERT R.	2000a	Confidential information : <i>Please refer to Document J.</i> SGS Laboratoire Crépin -Study N° ME-0201A Non-GLP, unpublished	Y	GOË
IIA 4.1/07	PORTAIL A.	2000b	Confidential information : <i>Please refer to Document J.</i> SGS Laboratoire Crépin -Study N° ME-0205B Non-GLP, unpublished	Y	GOË



Point / Reference number	Author(s)	Year	Title Testing facility, Report n°, GLP or GEP Status published or not	Data Protection Claimed Y/N	Owner
IIA 4.1/08	TREBERT R.	2000b	Confidential information : <i>Please refer to Document J.</i> SGS Laboratoire Crépin -Study N° ME-0202B Non-GLP, unpublished	Y	GOË

### New data

No new data was provided. However, there are some studies ongoing that will need to be included in this section when they become available.