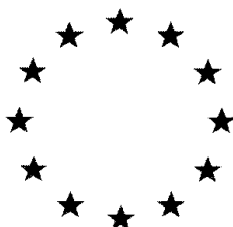


# **European Commission**



**VOLUME 3 – Annex B (PPP)**

**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

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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 Analysis of the plant protection product****(a) Methods for the determination of the active substance and/or variant in the plant protection product**

An analytical method was validated for the determination of the active substance, laminarin, in the product Vacciplant Fruits et Légumes. This study was not submitted previously in support of Laminarin inscription in Annex I of the Directive 91/414/EEC and is provided in this dossier.

Data point addressed:	CP 5.1.1/01
Author(s) (year):	Cage S. (2015)
Title:	Laminarin Formulation, Vacciplant Fruits et Légumes Method Validation for the Assay of Active Substance, Laminarin
Report number (Doc. No.):	Study No.DQU0005
Testing facility:	Huntingdon Life Sciences, Eye Research Centre, Eye, Suffolk, UK
Published:	No
Test guideline used:	SANCO/3030/99 revision 4
Deviations:	None
GLP:	Yes

**Executive Summary:**

A study was performed to validate a method of analysis for the determination of the content of the active substance, laminarin, in the formulation, Vacciplant Fruits et Légumes.

The study was conducted to comply with the requirements of Commission Regulation (EU) No. 284/2013 in accordance with Regulation (EC) No 1107/2009 and the requirements of SANCO/3030/99 revision 4.

The active substance in the test substance, Vacciplant Fruits et Légumes, was determined by an ion chromatography external standard method with amperometric detection. The analyte was quantified relative to bracketing standard solutions.

The method was validated with respect to specificity, linearity, precision, accuracy and range of method. The validation results are summarised as follows:

Parameter	Results
Specificity	No interference from co-formulants of blank formulation. Retention time match between active substance in product and in analytical standard.
Linearity	Calibration range: 19-130 mg/L (7 calibration standards) Correlation coefficient: 0.9966
Accuracy	Mean recovery 99.9% (n = 4 at nominal concentration)
Precision	RSD 1.1% (n = 10)
Reproducibility	RSD 0.8% (n = 10)
Method range	Mean recovery 104.8% (n = 2 with 75% sample weight) Mean recovery 96.6% (n = 2 with 125% sample weight)

**MATERIALS AND CONDITIONS:****1. Test Material:**

Description:

Lot/Batch #:

Purity:

Vacciplant Fruits et Légumes = Iodus 2

pale brown liquid

14041175

Nominal: Laminarin: 45 g/L

Analytical standard: Analytical: Laminarin: 43.7 g/L  
 Formulation blank: Laminarin batch SLBJ3234V, purity 92.1%w/w  
 Batch 10.06.14

## 2. Analytical conditions:

Instrument: Agilent 1100 Series Liquid Chromatograph with Chemstation software  
 Metrohm 896 Professional Detector

Column: Carbopac PA1 (25 cm)  
 Guard column: Carbopac PA1 (5 cm)  
 Column temperature: Ambient  
 Mobile phase composition: Mobile phase A - anhydrous sodium acetate (82g) was transferred to a 2 L volumetric flask and was dissolved and diluted to volume with purified water. An aliquot (8.2 mL) of 46 - 51% sodium hydroxide was added to the resulting solution.

Mobile phase B - an aliquot (8.2 mL) of 46 - 51% sodium hydroxide was transferred to a 2 L volumetric flask and diluted to volume with purified water.

Gradient:

Time (minutes)	% A	% B
0.0	30	70
1.9	60	40
3.9	100	0
15.0	100	0
15.01	30	70
25.0	30	70

Flow rate: 1 mL/min  
 Injection volume: 50 µL  
 Detector: amperometric mode

Step	Time (ms)	Potential (Volts)
0	400	+ 0.05
1	200	+ 0.75
2	400	- 0.05

Cycle duration: 1000 ms

## 3. Calibration standards

Laminarin analytical standard (approximately 20 mg at 92.1% purity) was accurately weighed into a 100 mL volumetric flask. Purified water was used to dissolve the sample and dilute the resulting solution to volume.

Aliquots (2, 4, 6, 8, 10, 12 and 14 mL) of the stock solution were diluted to 20 mL with purified water to produce a series of calibration standards covering the approximate range of 19 to 130 mg/L.

An intermediate point from the series of calibration standards was used as a bracketing standard sample during the assay of the test substance samples.

## 4. Samples preparation

Samples (approximately 1.2 g) of the test substance were accurately weighed into separate 100 mL volumetric flasks. Purified water was added to each sample and the mixtures were diluted to volume. The contents of each flask were heated at 60°C for 2 hours and shaken vigorously to ensure homogeneity.

Each homogenised sample was then added to a reservoir on top of a 600 mg SAX Maxiclean (Alltech) cartridge and a 600 mg SCX Maxiclean (Alltech) cartridge connected in series (the

cartridges had previously been washed with water (10 mL)). The first 10 mL of sample passing through the cartridges was discarded and then a sample was collected. A portion (4 mL) of the filtrate was diluted to 25 mL with purified water. The final solutions were analysed by the ion chromatography method relative to the bracketing standard solution.

### 5. Treatment of data

On analysis of Vacciplant Fruits et Légumes by the HPLC analytical method, the laminarin content of each sample was calculated by reference to the bracketing standards. The concentration of laminarin in the analysed solution (C) was calculated from standards introduced before and after samples (bracketing standards) by the following equation:

$$C \text{ (mg/L)} = \frac{\text{sample peak area} \times \text{standard concentration (mg/L)}}{\text{mean peak area of bracketing standards}}$$

The contents of laminarin in the test samples were then calculated from the following equation:

$$\text{Content (\% w/w)} = \frac{C \text{ (mg/L)} \times \text{dilution factor} \times 0.1}{\text{sample weight (mg)}} \times 100$$

where the dilution factor was 6.25 and 0.1 is the factor accounting for the initial volume of the samples diluted in water.

The final contents were then converted from units of % w/w to units of g/L according to the equation:

$$\text{Content (g/L)} = \text{content (\% w/w)} \times \text{relative density} \times 10$$

where the relative density of the product was 1.047 (determined in study DQU0006, CP 2.1/01, 2015a).

## RESULTS AND DISCUSSION

### 1. Specificity:

A sample of blank formulation was analysed. The resulting profile clearly illustrated that the material did not contain components which would cause interferences in the analysis of the active substance. The identity of the active substance peak was confirmed by comparison of the retention time with that of the analytical standard.

### 2. Linearity

Seven calibration solutions in the range 19 to 130 mg/L for the active substance were prepared in pure water. These were then chromatographed using the conditions described. A calibration graph of concentration versus peak area was prepared and the data were regressed linearly.

Standard concentration (mg/L)	Peak area
130.1	40812
111.5	35937
92.93	30953
74.34	25941
55.76	20620
37.17	14095
18.59	7168.9

Linear regression:  $y = 310.9x + 1714$ ,  $r^2 = 0.9932$ ,  $r = 0.9966$

### 3. Accuracy

The accuracy of the analytical procedure was assessed by analysing reconstituted samples. Four reconstituted samples were prepared by mixing portions of laminarin analytical standard (approximately 0.05 g) with portions of the blank formulation (approximately 1.15 g) in 100 mL

volumetric flasks. Each sample was then prepared by the procedure described for the test substance sample preparation and analysed by the ion chromatography method.

The accuracy of the assay procedure was determined by calculating the recoveries from the assay of the four reconstituted samples.

$$\% \text{ recovery} = \frac{\text{content determined}}{\text{theoretical content}} \times 100$$

Sample	Theoretical content (% w/w)	Analysed content (% w/w)	Recovery (%)
A	3.866	3.900	100.9
B	4.156	4.148	99.8
C	4.142	4.111	99.3
D	4.123	4.110	99.7
Mean			99.9

The mean recovery is well within the range 97-103% (for preparations containing 1 - 10% nominal active substance).

#### 4. Precision (repeatability) and reproducibility

To determine the precision of the assay procedure used for the determination of the active substance, laminarin, the batch of test substance was assayed ten times over two days (five times on each day) using fresh bracketing standard solutions on each occasion.

Sample	Analysed content (% w/w)	Mean	SD	RSD
A (day 1)	4.015	4.066	0.037	0.90
B (day 1)	4.099			
C (day 1)	4.076			
D (day 1)	4.098			
E (day 1)	4.043			
F (day 2)	3.969	4.036	0.049	1.22
G (day 2)	4.070			
H (day 2)	4.094			
I (day 2)	4.011			
J (day 2)	4.037			
Global mean	4.051			
Global SD	0.044			
Global RSD	1.08			

The mean content was found to be 4.05% w/w corresponding to 42.4 g/L (relative density of 1.047 – study No.DQU0006, CP 2.1/01, 2015a).

In addition, a single sample was assayed ten times in order to determine the reproducibility of the chromatographic system.

The reproducibility of the chromatographic system with respect to the active substance was then taken as the relative standard deviation (RSD) of the content of the analyte found in the single sample of Vacciplant Fruits et Légumes.

Sample	Analysed content (% w/w)
1	4.049
2	4.077
3	4.108
4	4.109
5	4.040
6	4.031
7	4.017
8	4.030
9	4.036
10	4.048
Mean	4.055

SD	0.033
RSD	0.80

All of the relative standard deviation data for both the repeatability and reproducibility assessments fall within the acceptable RSD range of 2.2% (modified Horwitz equation).

### 5. Range of the method

Four samples of Vacciplant Fruits et Légumes were weighed out and analysed, however, on this occasion the weights corresponded approximately to 75% and 125% of the nominal sample weights (1.2 g) taken for the assay and were analysed in order to demonstrate the linearity of the assay method.

The content of active substance found in the samples prepared at 75% and 125% of the nominal sample weights were expressed as percentage of the mean concentration of the ten samples from the repeatability experiment (4.05% w/w laminarin).

Sample	Theoretical content (% w/w)	Analysed content (% w/w)	Recovery (%)	Mean
75% A	4.05	4.282	105.7	104.8
75% B		4.207	103.8	
125%A		3.945	97.4	96.6
125%B		3.881	95.8	
Mean			99.9	

## CONCLUSION

The method (IC with amperometric detection) was validated with respect to specificity, linearity, precision and accuracy. Method validation complies with the requirements of SANCO/3030/99 revision 4.

### (b) Methods for determination of relevant impurities identified in the technical material or which may be formed during manufacture of the plant protection product or from degradation of the plant protection product during storage

Methods are not required for impurities as no toxicologically, ecotoxicologically or environmentally relevant impurities will be formed in the technical material, or during manufacture of the plant protection product or from degradation of the plant protection product during storage.

### (c) Methods for the determination of relevant co-formulants or components of co-formulants, where required by the national competent authorities

No method is required for co-formulants or components of co-formulants.

#### B.5.1.2 Methods for the determination of residues

Please refer to volume 3CA B-5.

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

Please refer to volume 3CA B-5.

#### B.5.3 References relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company)	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner



			<b>GLP or GEP status Published or not</b>				
Cage S.	CP 5.1.1/01	2015	Laminarin Formulation, Vacciplant Fruits et Légumes, Method Validation for the Assay of Active Substance, Laminarin Huntingdon Life Sciences Eye Research Centre Eye, Suffolk, UK Study No.DQU0005 - report GLP - Unpublished	N	Y	A 10-year data protection period is claimed as this study : - is necessary for the registration of Vacciplant Fruits et Légumes - has not been submitted in the past - has been made in accordance with the GLP principles	Laboratoires Goëmar S.A.S.