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CERTIFICATE OF ANALYSIS

(For research use only)

GENERAL DATA			
Article Number	09.01.26		
Article description	*Alternaria alternata* medium (CBS 103.33)*		
Storage	-20 °C		
Production Date	December 10, 2011		
Charge Number	18L17		
RAW MATERIAL / PRODUCTION PROCES			
Cultivation location	Groningen, The Netherlands		
Cultivation method	Shaken Culture in Erlenmeyer Flask at 25°C		
Source of moulds	Centraal Bureau voor Schimmelcultures (CBS)		
Inoculation	Source described, identified, selected by Centraal Bureau voor Schimmelcultures (CBS)*		
Medium	Czapek Dox Broth + 0.1% yeast extract		
POST HARVEST PROCESSING OF MOLDS			
Harvesting of mould	Mycelium/spores filtrated		
Drying	The filtrated mycelium has been freeze dried		
IDENTITY / ANALYSES			
Identity	Result	Specifications	Reference
Species	Ok	*Alternaria alternata*	Database CBS*
Character material	Ok	Brown/grey powder	Visual determination
Allergenic pattern (mg/g) Alt a1	3.0	1 – 10	ELISA
Protein content (mg/g)	41 204	10 – 150 150 – 300	Bradford assay BCA assay
Protein pattern	Clear bands	Clear bands	SDS PAGE gel; CBB & silver staining
Blotting	Clear bands	Clear bands	Blotting Assay
Sugar content (mg/g)	103	75 – 200	Phenol-sulfuric acid method
Bioburden (Cfu/g) SDA TSA	75×10^3 27×10^3	SDA $< 1 \times 10^6$ TSA $< 1 \times 10^6$	Ph. Eur.
Water content (%)	9.1	< 15	Karl Fischer determination
Mycotoxin	No visible peaks	No visible peaks	HPLC

* CBS, Westerdijk Fungal Biodiversity Institute, Utrecht

April 30, 2019

Head of Production

HQA

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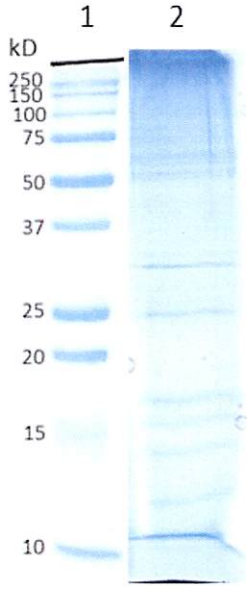
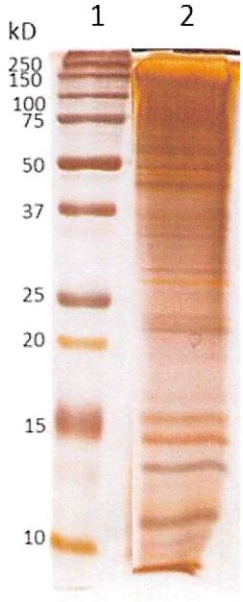
SDS-PAGE analysis

CBB Stain:



Silver Stain:



1: Marker, **2**: **18L17**

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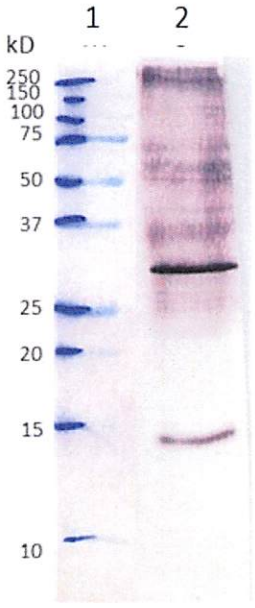
Immunodetection:



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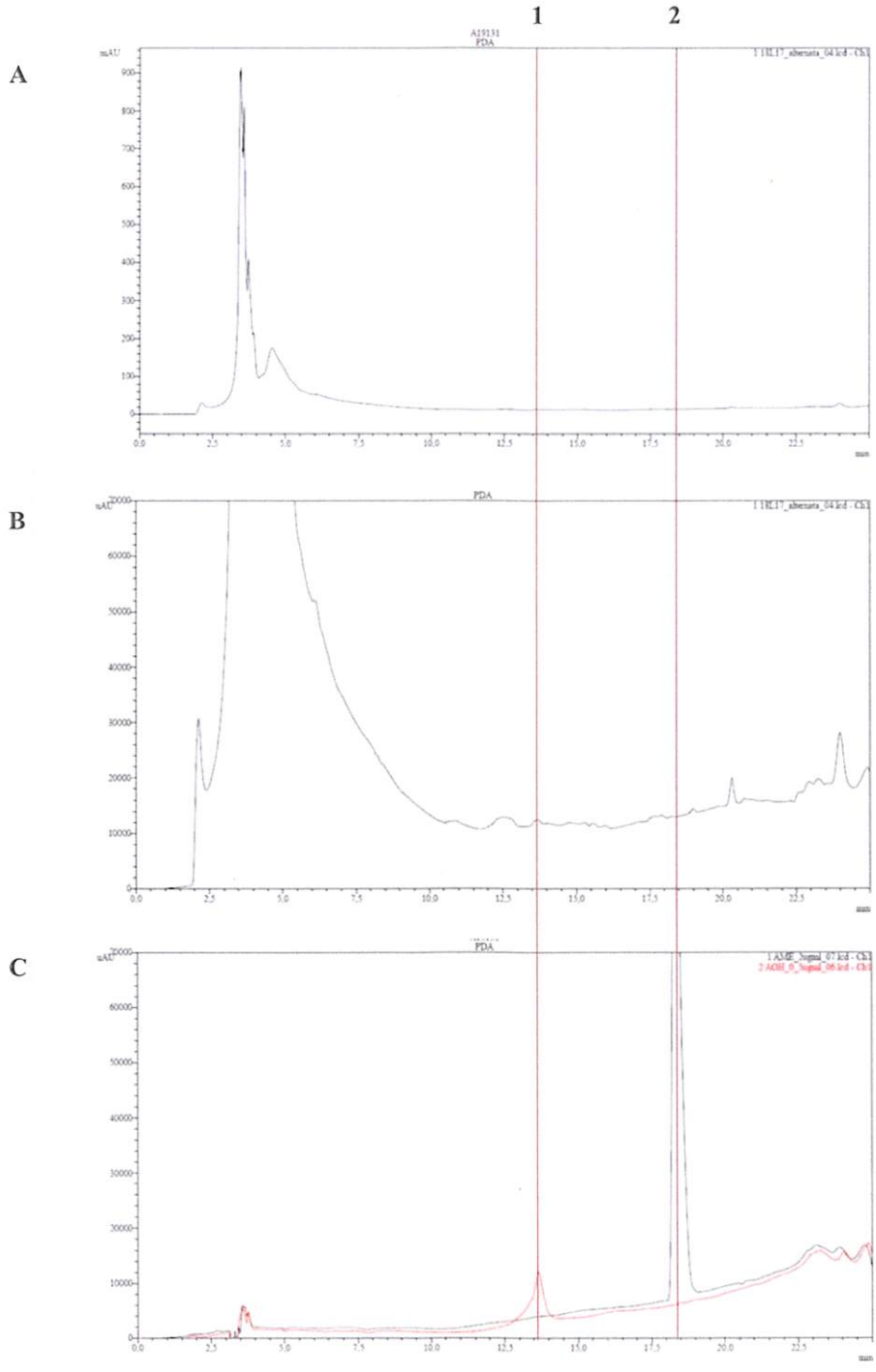
Methanol extraction: A) *Alternaria alternate* medium, B) *Alternaria alternate* medium, zoomed, C) mycotoxins AOH (1) and AME (2), 13.5 and 18.3 minutes, 7.5 pg AOH and 30 pg, respectively.



A 5% extract was applied on a Inertsil 5µm ODS-2, 250 x 4.6 mm C18 –column. The analysis was performed using a binary gradient system. Solvent A is composed of 0.02% aqueous formic acid (pH 3) whereas methanol is used as solvent B. The total flow-rate was 0.8 ml/min. The detection wavelength was set at 256 nm, and 50µl of sample was injected. Three analysis were performed *Alternaria alternata* extract FD, AOH and AME. Standard solutions of 0.5 µg/ml AOH and 2 µg/ml of AME were prepared by diluting the mycotoxins in acetonitrile – water (3:1, v/v).